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Article

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Novel Piperidinyl-Azetidines as Potent and Selective CCR4 Antagonists Elicit Antitumor Response as Single Agent and in Combination with Checkpoint Inhibitors

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ABSTRACT

The C-C chemokine receptor 4 (CCR4) is broadly expressed on regulatory T cells (T_{reg}) as well as other circulating and tissue-resident T cells. T_{reg} can be recruited to the tumor microenvironment (TME) through the C-C chemokines CCL17 and CCL22. T_{reg} accumulation in the TME has been shown to dampen anti-tumor immune response and is thought to be an important driver in tumor immune evasion. Preclinical and clinical data suggest that reducing the T_{reg} population in the TME can potentiate the anti-tumor immune response of checkpoint inhibitors. We have developed small molecule antagonists of CCR4, featuring a novel piperidinyl-azetidine motif, that inhibit the recruitment of T_{reg} into the TME, and elicit anti-tumor responses as a single agent or in combination with immune checkpoint blockade. The discovery of these potent, selective, and orally bioavailable CCR4 antagonists, and their activity in *in vitro* and *in vivo* models, is described herein.

INTRODUCTION

In many cancers, recruitment of $CD4^+$ $FOXP3^+$ regulatory T cells (T_{reg}) into the tumor microenvironment (TME) reduces the anti-tumor immune response and is thought to be a key driver in tumor immune evasion.[1-4] The accumulation of T_{reg} in the TME has been associated with poor patient prognosis in various cancers.[5-10] Preclinical and clinical data suggest that reduction of T_{reg} populations in the TME can enhance anti-tumor immune responses of checkpoint therapy.[11-13]

The C-C chemokine receptor 4 (CCR4) is a G protein-coupled receptor (GPCR) highly expressed on T_{reg}, in addition to other circulating and tissue-resident T cells, including T helper type 2 (Th2) cells and skin-homing T cells.[14, 15] CCR4 is the receptor of the C-C chemokines CCL17 (also known as thymus- and activation-regulated chemokine or TARC) and CCL22 (also called macrophage-derived chemokine or MDC). Gradients of these chemokines direct cell trafficking and homing through chemotaxis.[6, 15-17] More recently, chemokine-like factor 1 (CKLF1) has also been shown to induce chemotaxis via CCR4.[18, 19] It is recognized that CCR4 plays a key role in stimulating T_{reg}

trafficking into the TME of many tumors due to high expression levels of CCL17 and CCL22.[6, 20, 21]

Recognizing the important role of CCR4 in cell migration and homing on specific T cell subpopulations has made this receptor an attractive target for therapeutic intervention. Over the last decade, several small molecule CCR4 antagonists have been reported. These antagonists can be grouped into two classes based on their proposed binding sites.[22-24] Class I antagonists (**1**-**4a-b**,[25-28] Figure 1), which feature a heterocyclic core decorated with a lipophilic substituent and an amine-containing side chain, bind extracellularly at the allosteric antagonist binding site I. Class II antagonists (**5**-**6a-b**,[29, 30] Figure 1), containing a heterocyclic core substituted with a sulfonamide side chain and a lipophilic motif, bind intracellularly at the allosteric antagonist binding site II.

Previously reported CCR4 antagonists were developed targeting a subset of Th2 cells for the treatment of inflammation, allergic disorders, and autoimmune disease.[31, 32] Among them, GSK2239633 (**5**,[29] Figure 1), a class II antagonist developed by GlaxoSmithKline, was evaluated in a 2011 Phase I clinical trial in healthy volunteers for

the treatment of asthma. Low blood exposure resulted in low target engagement and prevented further clinical development.[33] AztraZeneca has also developed a series of CCR4 class II antagonists and have identified AZD-1678 and AZD-2098 as preclinical candidates (**6a**, **6b**, Figure 1),[30, 34] although no clinical development has been reported.

The CCR4 receptor has also been targeted with depleting monoclonal antibodies. Mogamulizumab (KW0761), a humanized defucosylated anti-CCR4 depleting antibody developed by Kyowa Hakko Kirin, was approved in Japan in 2012 for the treatment of relapsed/refractory $CCR4^+$ adult T-cell leukemia/lymphoma (ATCLL), and in 2014 for relapsed/refractory $CCR4^+$ cutaneous T cell lymphoma (CTCL).[15] In August of 2018, the FDA approved mogamulizumab to treat two types of CTCL, Sézary disease and mycosis fungoides.[35] However, systemic depletion of CCR4 containing cells in healthy tissues appears to lead to serious skin-related side effects,[36, 37] including cases of Stevens-Johnson Syndrome (SJS). This is presumably due to depletion of T_{reg} in the skin, leading to drastic changes in the ratio of T effector cells to T_{reg} and thus triggering an autoimmune response. Small-molecule reversible inhibition of CCR4-mediated cell migration and

homing, without depleting specific subpopulations of T cells, could offer a safer approach. We have demonstrated in a preclinical model that T_{reg} numbers in the skin were not affected, suggesting they are neither depleted nor their migration into healthy skin is prevented by CCR4 receptor blockade with small molecule antagonists.[38]

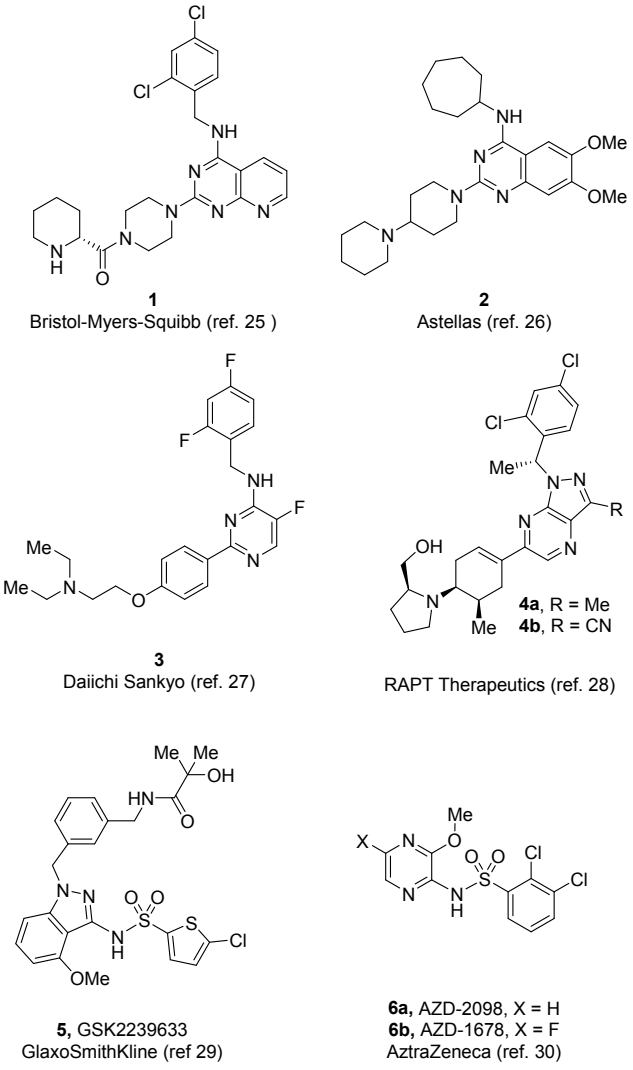


Figure 1. Representative Class I and Class II CCR4 antagonists.

We have recently reported the discovery of novel small-molecule antagonists that inhibit the trafficking of T_{reg} into the TME. These class I antagonists featured a novel pyrazolopyrazine core with a unique cyclohexenyl side chain (**4a-b**, Figure 1).[28] Although **4b** inhibited the migration of T_{reg} in both *in vitro* and *in vivo* models, it suffered from high clearance in rats and also required high doses to achieve significant reduction of T_{reg} migration in mouse models. While **4b** was a suitable tool compound for proof of concept experiments, we wanted to advance our program by discovering a more simplified side chain. These new antagonists should maintain potency and selectivity for CCR4 while also encompassing improved ADME properties required for oral dosing. Our search led us to the discovery of a novel piperidinyl-azetidine motif, which, in combination with our pyrazolopyrazine cores, yielded highly potent, selective, and orally bioavailable antagonists of CCR4. Herein we highlight the SAR of this novel series as well as their activity in several *in vitro* and *in vivo* models.

RESULTS AND DISCUSSION

Structure–Activity Relationships. We have previously reported a pharmacophore map that we built using the structures of published class I CCR4 antagonists (Figure 2A). This model was further refined using our published SAR in combination with computational studies using a known CCR4 homology model.[28,39] In this model, a highly optimized right hand phenyl group bearing two halogen substituents in positions 2 and 4 is connected to an aromatic core (usually heterocyclic). The core is linked to an amine-containing side chain. The size and spatial orientation of the linker critically dictates positioning of the basic nitrogen, which is key for activity. Using this pharmacophore model, compounds **4a-b** were designed and demonstrated high potency against CCR4.[28] We further applied this model to the design of a simplified side chain with the goal of reducing stereochemical complexity in our antagonists.

We turned our attention to smaller ring systems and were attracted to the simplicity of symmetrically substituted four membered rings. Our previously reported SAR,[28] as well as other published data,[25-27] suggested that the connecting atom from the side chain to the core should be a nitrogen or a sp^2 carbon in order to keep the most favorable spatial

orientation. Thus, we set out to explore the nitrogen containing azetidine ring. Our refined pharmacophore map incorporating azetidine as a linker is shown in Figure 2B.

Using a simple 6-4 ring system in which a piperidine ring is connected to the azetidine, we walked the nitrogen around the piperidine ring to identify the ideal positioning of the basic nitrogen (Figure 2C). Compound **7**, with the nitrogen directly connected to the azetidine ring, was found to be inactive in a calcium flux assay. Increasing the distance of the basic nitrogen to the azetidinyl nitrogen, as seen in compound **8**, resulted in modest but encouraging potency with an IC_{50} of 1.37 μM. We moved the basic nitrogen one atom further and tested the 3-(piperidin-3-yl)azetidine motif. We were pleased to find compound **9** had an IC_{50} of 596 nM in the calcium flux assay as a 1:1 mixture of diastereomers. Finally, we prepared the 3-(piperidin-4-yl)azetidine analog **10** that had the basic nitrogen furthest from the linker nitrogen and found that this analog was inactive.

Next, we set out to explore the chemical space around the novel 3-(piperidin-3-yl)azetidine side chain. This included the role substitutions on the basic nitrogen, and the

absolute stereochemistry of the piperidine ring, would play on potency of these antagonists. We found that polar groups linked by one, two, or three carbons to the piperidinyl nitrogen dramatically improved the potency of these CCR4 antagonists, as exemplified by sulfonamide **16**, which possessed an IC_{50} of 121 nM prepared as a 1:1 mixture of diastereomers from racemic amine **11** (Scheme 1). At this point, we decided to separate the diastereomers by chiral preparative HPLC and found that antagonist **18** bearing the (*R*) configuration was 5-fold more potent than the corresponding diastereomer **17** with (*S*) configuration.

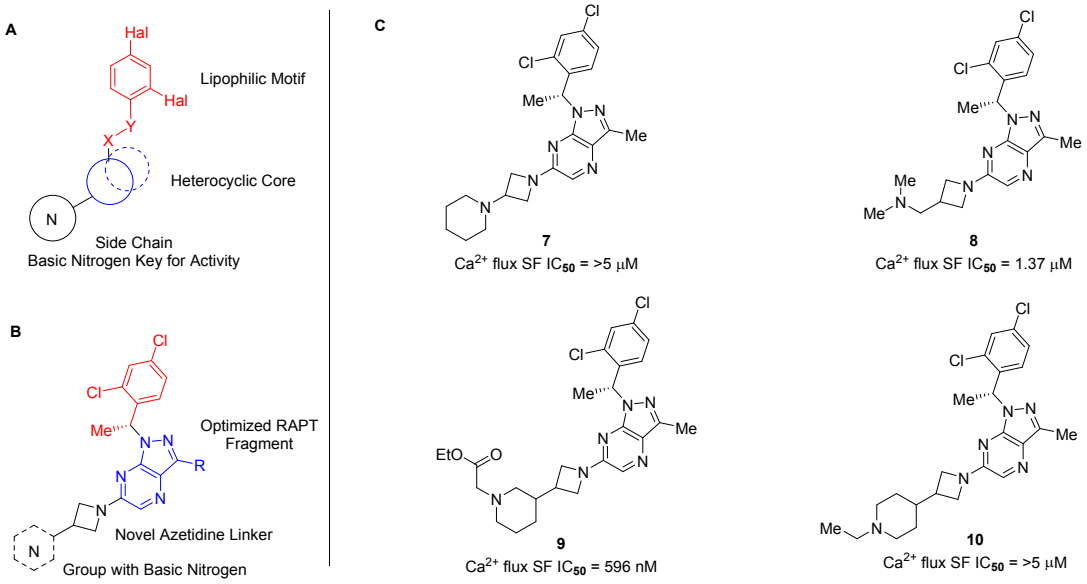
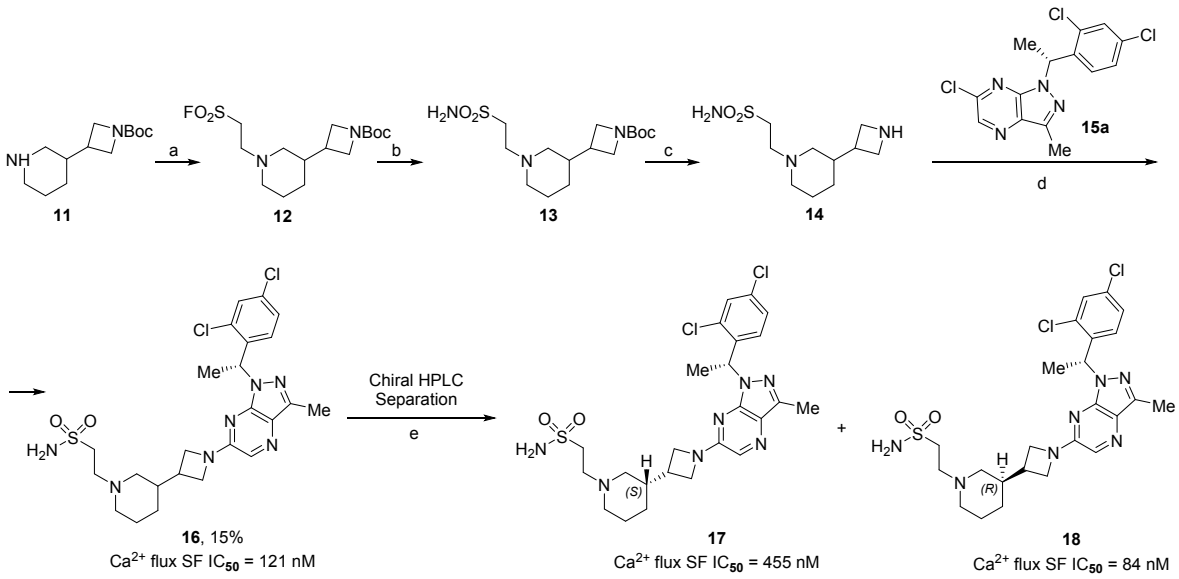


Figure 2. A. Pharmacophore map of previously reported CCR4 antagonists. B. Current RAPT Pharmacophore model. C. Design of novel piperidinyl-azetidine series using a pharmacophore map for class I CCR4 antagonists: topological and spatial orientation of the basic nitrogen is crucial for maintaining potency.

Scheme 1. Synthesis of **16** and activities of the single diastereomers **17** and **18**.[a]



[a]Reagents and conditions: (a) ethenesulfonyl fluoride, DCM. (b) MeOH, NH_4OH, 70℃. (c) 4N HCl in dioxane, DCM. (d) (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine, DMSO, *N,N*-diisopropylethylamine, 80 ℃, 4 hrs. (d) Separation of diastereomers on chiral HPLC.

The absolute stereochemistry of compound **18** was unambiguously confirmed by single crystal X-ray diffraction of a key intermediate (see supporting information for details).

These findings prompted an extensive SAR campaign to improve the potency of this series in the primary calcium flux assay, and subsequently, to evaluate the ability of

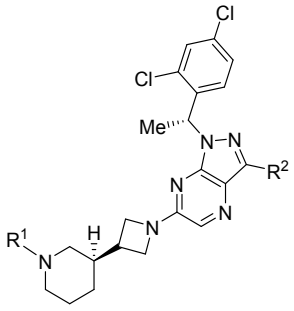
these antagonists to inhibit the migration of cells expressing CCR4 in the presence of a CCL22 gradient. We have previously reported a 96-well medium-throughput chemotaxis (CTX) assay using CCR4-expressing CCRF-CEM cells.[28] Antagonists that inhibited calcium flux in the double-digit nanomolar range were also run in a physiologically more relevant CTX assay. This platform is considered the gold standard assay for interrogating in vitro cellular migration. Since the assay is run in 100% human serum (HS), the reported potencies reflect the protein binding of the compounds. Although calcium flux allowed us to triage compounds based on their CCR4 affinity, a significant shift in CTX potency was often observed, likely due to plasma protein binding. Importantly, this platform can be extended to other relevant cell types, including primary immune cells (T_{reg} and Th2 cells).

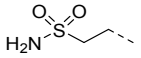
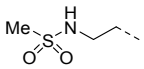
The results are summarized in Table 1. As mentioned above, sulfonamide **18** was highly potent in the calcium flux assay, however this compound suffered a large shift in the CTX assay. Reversing the terminal sulfonamide was tolerated and antagonists (**19** and **20**) displayed increased potency in the primary calcium flux assay (29 and 31 nM, respectively) with only a 4-5-fold shift in the cell migration assay. 2-carbon linked sulfone

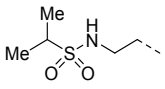
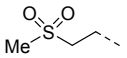
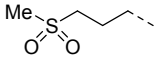
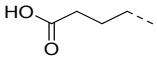
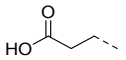
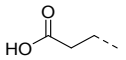
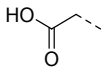
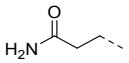
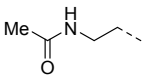
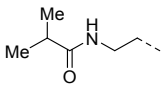
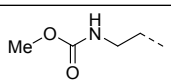
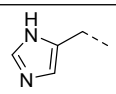
21 was equipotent to sulfonamide **18** and suffered a similar CTX shift. While the 3-carbon linked sulfone **22** was 3-fold more potent in the calcium flux assay (22 nM), it had a 10-fold shift in the CTX assay. Replacing the sulfonamide functional group for a 3-carbon linked carboxylic acid **23** displayed similar inhibition in the calcium flux and similar shift in the CTX assay, as compared to the parent sulfonamide. The 2-carbon linked carboxylic acid **24** had a similar potency in the calcium flux assay but was 2-fold more potent in the CTX assay. Interestingly, replacing the C3-methyl in the pyrazolopyrazine core for a C3-cyano pyrazolopyrazine core yielded carboxylic acid **25** that was 2-fold more potent in the calcium flux assay than the C3-methyl analog, but with similar CTX potencies. Decreasing the length of the linker by one carbon was tolerated and provided antagonist **26** that displayed increased activity in the primary calcium flux assay (36 nM) but also showed a large shift in the CTX assay. Terminal amides were also active CCR4 antagonists as seen in amide **27**, which was 42 nM in the calcium flux assay and 242 nM in the cell migration assay. Reversed amides **28** and **29** were found to be marginally more potent CCR4 antagonists. Amide **28** had an IC_{50} of 6 nM in the calcium flux assay and 40 nM in the

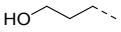
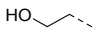
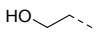
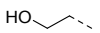
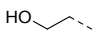
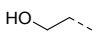
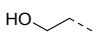
CTX assay, while the more lipophilic amide **29** was slightly less potent. Methyl carbamate **30** was also highly potent in the calcium flux assay (23 nM) but suffered a 5-fold shift in the chemotaxis assay. Introduction of a small heterocycle was also tolerated. Imidazole **31**, connected by a 1-carbon linker attached to the C3-cyano pyrazolopyrazine core, possessed a calcium flux potency of 21 nM and CTX potency of 97 nM.

Table 1. SAR of the Piperidinyl Side Chain



cmpd	R^1	R^2	Ca^{2+} flux IC_{50} (nM)[a]	CTX IC_{50} (nM)[b]
18		Me	84	717
19		Me	29	136

20		Me	31	115
21		Me	77	575
22		Me	22	259
23		Me	69	559
24		Me	91	370
25		CN	47	409
26		CN	36	775
27		Me	42	247
28		Me	6	40
29		Me	18	59
30		Me	23	133
31		CN	21	97
32		CN	1470	N.D.

33		CN	28	523
34		Me	505	N.D.
35		CN	370	1500
36		Me	37	109
37		Me	24	74
38		CN	22	50
39		$CONH_2$	8	36
40		CO_2H	>5000	N.D.
41		CONHMe	25	446
42		$CONMe_2$	9	379

[a]Assay ran in absence of serum. [b]Assay ran in 100% human serum. N.D. Not determined.

Terminal nitriles produced less potent CCR4 antagonists. Antagonist **32**, bearing a terminal nitrile connected to the piperidine through a one carbon linker, was 1470 nM in the calcium flux assay. Extending the linker by one carbon improved the potency of analog **33**, but it had a large shift in the chemotaxis assay. Similarly, introducing a terminal

trifluoromethyl with a 2-carbon tether was detrimental for potency, as compound **34** was 505 nM in the calcium flux assay and its analog **35** on the C3-cyano pyrazolopyrazine core had similar activity. Simple alcohols were also studied and yielded highly active CCR4 antagonists. The 3-carbon linked alcohol **36** was moderately less potent than its 2-carbon analog **37.** Antagonist **37** showed an IC_{50} of 24 nM in the inhibition of calcium flux and 74 nM in the chemotaxis assay. Replacing the C3-methyl for a C3-cyano on the core yielded compound **38** that was highly potent in both CCR4 functional assays. In the primary calcium flux assay, compound **38** had an IC_{50} of 22 nM and 50 nM in the CTX cell migration assay. We then explored other substitutions at the C3-position of the pyrazolopyrazine core. Placing terminal amides at the C3-position was productive and generated potent CCR4 antagonist **39**. Compound **39**, bearing an unsubstituted amide at the C3 position, displayed an IC_{50} 0f 8 nM in the calcium flux assay and 36 nM in the chemotaxis assay. The C3-carboxylic acid substituted pyrazolopyrazine **40** was found to be inactive. Monomethylated amide **41** was 3-fold less potent than the unsubstituted analog **39** and had a large shift in the migration assay. Finally, adding a second methyl

group on the terminal amide **42** restored potency in the calcium flux assay (9 nM) but this substituted amide suffered from a large shift in the chemotaxis assay.

We then evaluated the *in vitro* metabolic stability and pharmacokinetic properties of several of these potent CCR4 antagonists in human and rat hepatocytes as well as rat *in vivo* PK (Table 2). Sulfonamide **19** was moderately stable in a human hepatocyte assay with 85% remaining, while in rat hepatocytes only 53% remained after incubating for 60 minutes. The lower rat *in vitro* metabolic stability of **19** was also reflected in a rat *in vivo* PK study, which showed medium clearance and low bioavailability. The reverse amide **28** displayed good stability in both rat and human hepatocytes. In rat PK, **28** had relatively low clearance (11 mL/min/kg), however the bioavailability of this antagonist was only 13%. The *in vitro* metabolic stability of the 2-carbon linked alcohol **37** was also assessed and revealed that this antagonist was stable in human hepatocytes (87% remaining) and moderately stable in the rat hepatocyte assay (62% remaining). Consistent with these findings, the rat *in vivo* PK of compound **37** was remarkably improved, with moderate clearance (32.3 mL/min/kg) and 61% bioavailability.

Table 2. *In Vitro* Metabolic Stability and Rat *in Vivo* PK of Selected CCR4 Antagonist

cmpd	Hepatocytes[a] % remr/h	Rat IV CL (mL/min/kg)[b]	Rat %F[c]
19	53/85	41.6	11
28	68/94	11	13
37	62/87	32.3	61
38	73/91	47.6	49
39	95/95	190.8	0

[a]% remaining after incubating for 60 min . [b]Dose of 0.5 mg/kg IV. [c]Dose of 2 mg/kg PO.

Compound **38** had slightly improved metabolic stability in the *in vitro* rat and human hepatocyte assay as compared to its analog **37**. Compound **38** possessed medium clearance (47.6 mL/min/kg) in a rat *in vivo* PK experiment and was 49% bioavailable upon oral dosing. Amide **39** was remarkably stable in both in human and rat hepatocytes (95%

and 95% remaining, correspondingly) but had extremely high clearance in a rat *in vivo* PK study (190.8 mL/min/kg) and was not bioavailable.

Compounds **37** and **38** were evaluated for inhibition of the five most common isoforms of cytochrome P450 and found that both possessed clean CYP450 profiles (Table 3). Furthermore, compounds **37** and **38** showed no activity in a CYP450 induction assay. However, in a CYP450 Time-Dependent Inhibition assay (CYP450 TDI) for the 3A4 isoform, there was a 2.6-fold shift in its IC_{50} when compound **37** was incubated in the presence NADPH as compared to when it was incubated in the absence of the cofactor. Compound **38** did not display a significant shift in its IC_{50} when it was incubated in the presence NADPH as compared to incubation in the absence of the cofactor. We speculate that metabolism at the C3-methyl in the pyrazolopyrazine core might be responsible for the CYP450 TDI.

Table 3. *In Vitro* CYP Profile of Compounds **37** and **38**

Assay	Cmpd 37	Cmpd 38

CYP450 Inhibition (IC_{50}) for isoforms 1A2, 2C9, 2C19, 2D6 and 3A4 [a]	> 40 μM	> 40 μM
CYP Induction [a]	Negative	Negative
CYP450 TDI IC_{50} shift for isoform 3A4[a]	2.6-fold	1.2-fold

[a]See supporting information for details.

Based on these results, we further profiled compound **38** in several *in vitro* and *in vivo* experiments. A mouse *in vivo* PK study revealed that compound **38** had low clearance (10.2 mL/min/kg), medium volume of distribution (5.2 L/kg), a half-life of 6.9 h, and good bioavailability (%F = 29) that led to high exposure after oral dosing (Table 4).

When CCL17 was used as a chemoattractant instead of CCL22 the CTX IC_{50} value was 64 nM. Compound **38** was also evaluated for its ability to inhibit the CCR4-mediated migration of the more physiologically relevant induced mouse and human regulatory T cells (iT_{reg}) in an *in vitro* chemotaxis assay (Table 4). Compound **38** inhibited the migration of mouse iT_{reg} cells with an IC_{50} of 39 nM, while the IC_{50} in human iT_{reg} cells was 33 nM.

Table 4. Mouse PK, i*n Vitro* iTreg Chemotaxis and Selectivity Profile of Antagonist **38**

Assay	Result
mouse *in vivo* PK	
Cl (mL/min/kg)[a]	10.2
Vss (L/kg)[a]	5.2
$T_{1/2}$ (hr)[a]	6.9
PO $AUC_{0\text{-last}}$ (ng *hr /mL)[b]	751
F (%)[b]	29
CTX IC_{50} (nM)	50 / 64[c]
miTreg CTX IC_{50} (nM)[d]	39
hiTreg CTX IC_{50} (nM)[e]	33

[a]Dose of 0.5 mg/kg IV. [b]Dose of 2 mg/kg PO [c]CCL17 was used as a chemoattractant [d]Assay run in 100% mouse serum. [e]Assay run in 100% human serum.

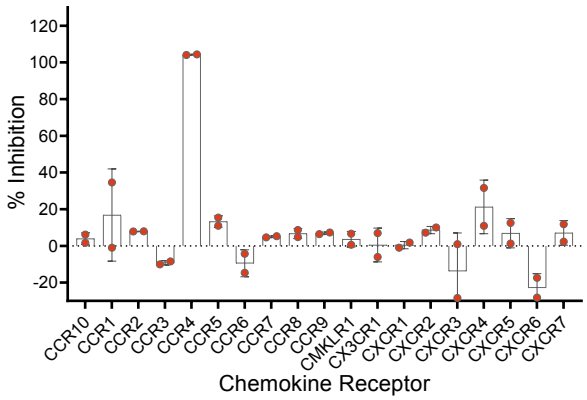


Figure 4. Selectivity of CCR4 antagonist **38** at 500 nM against a panel of human chemokine receptors in a beta arrestin recruitment assay.

The selectivity of CCR4 antagonist **38** against other prevalent chemokine receptors was also assessed and it was found that compound **38** was highly selective for CCR4, as it did not inhibit other chemokine receptors from the panel at a concentration of 500 nM (Figure 4).

***In Vivo* T_{reg} Migration Studies in Mouse**. Having established that antagonist **38** potently and selectively inhibited the migration of T_{reg} cells in *in vitro* models, we then evaluated the ability of **38** to inhibit the migration of T_{reg} into the TME using a previously reported *in*

vivo murine pancreatic ductal adenocarcinoma model (Pan02).[40,41] Compound **38** significantly inhibited the migration of adoptively transferred GFP$^+$ iT$_{reg}$ to the TME in a dose-dependent manner, as compared to the control group (Figure 5).[42] Importantly, treatment with compound **38** did not show any significant reduction in the number of GFP$^+$ or endogenous T$_{reg}$ in the spleen, nor other healthy tissues, including the skin and lung.[38]

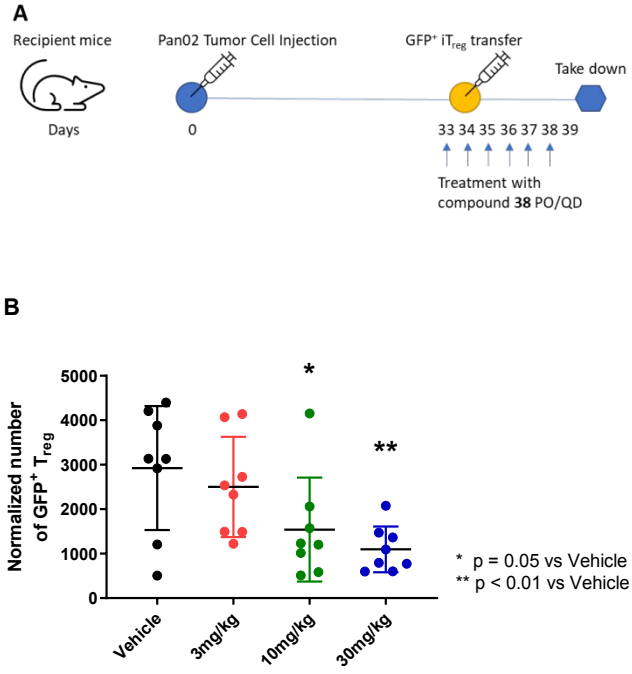


Figure 5. Treatment with Compound **38** reduces T$_{reg}$ migration into the tumor. (A) Schematic outline of treatment and tumor model that is used in this study. (B) Number of GFP$^+$ T$_{reg}$ in tumor. For statistical analysis, the two-tailed t-test was used.

Anti-tumor efficacy of compound 38 in a Pan02-OVA and CT26 tumor models as a single agent and in combination with checkpoint inhibitors. After confirming that CCR4 antagonist 38 effectively inhibited the migration of regulatory T cells into the TME in an *in vivo* model, we sought to explore the extent of anti-tumor efficacy that could be achieved through CCR4 antagonism. To that end, we used a Pan02-OVA tumor model (see supporting information), to examine the anti-tumor efficacy of antagonist 38 as a single agent and in combination with an anti-CTLA-4 checkpoint inhibitor with therapeutic dosing of both agents (Figure 6). Antagonist 38 significantly reduced the tumor growth as compared to the vehicle control group and was comparable to the group that received the anti-CTLA-4 antibody. Importantly, when CCR4 antagonist 38 was combined with anti-CTLA-4, a further reduction on the tumor growth was observed.

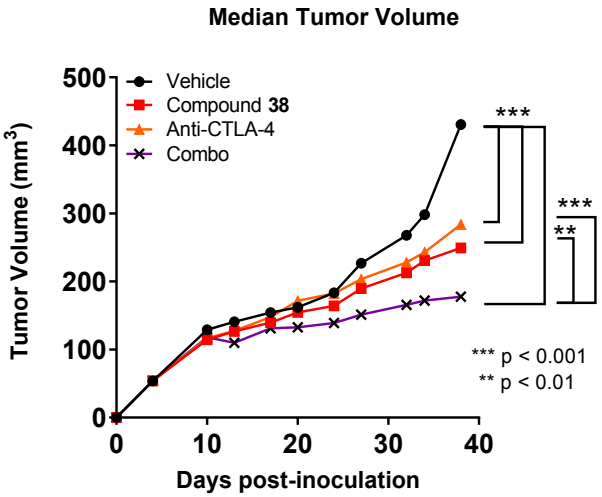


Figure 6. Anti-tumor efficacy of compound **38** in a Pan02-OVA model with 50 mg/kg PO QD dosing regimen. See supporting information for details. For statistical analysis, the two-tailed t-test was used.

Next, to evaluate synergy with another widely used checkpoint inhibitor, an anti-PD-L1 antibody, we designed a therapeutic combination study in the CT26 mouse model (see supporting information). In this study (Figure 7) we observed significant tumor growth inhibition in animals who received combination treatment compared to the single agent treatment groups. This suggests that inhibiting T_{reg} migration may enhance an immune-mediated anti-tumor response by removing the inhibitory effect of T_{reg} in TME.

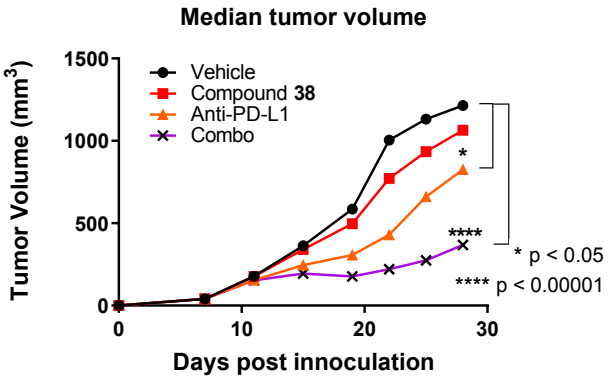


Figure 7. Anti-tumor efficacy of compound **38** in a CT26 model with 50 mg/kg PO QD dosing regimen. See supporting information for details. For statistical analysis, the two-tailed t-test was used.

Pharmacokinetic profiling of compound 38 in dog and cynomolgus monkey. Considering the promising *in vitro* and *in vivo* pharmacology profile of antagonist **38**, we sought to fully profile its pharmacokinetic properties in higher species (see Supporting Information). In higher species the *in vivo* clearance of compound **38** was 3-fold lower than predicted by the *in vitro* assay. In dog, compound **38** had low clearance (7.3 mL/min/kg), a half-life of 12.7 hr, and was 44% bioavailable. Similarly, in cynomolgus monkey, compound **38** had

low clearance (3.7 mL/min/kg), a long terminal half-life (10.7 hr), and good bioavailability (%F = 41).

Synthetic Chemistry. CCR4 antagonists **9** and **16-42** were synthesized through a straightforward sequence starting with introduction of an alkyl group to amine **11** (racemic or enantioenriched), followed by deprotection of the Boc group, and completed by nucleophilic aromatic substitution with an appropriately substituted pyrazolopyrazine intermediate **15a-d**.[28] In general, introduction of an alkyl group was achieved via reductive amination, alkylation with an alkyl halide, or Michael addition (General Procedures A-C in Experimental Section).

Compounds **7-10** were made via nucleophilic aromatic substitution reaction between intermediate **15a** and the corresponding amines, which were either commercially available or accessed via reductive amination as described in Scheme 2.

Compounds **9**, **19**, **20**, **22**, **24**, **27**, **28-31**, **36**, **37** and **39** were synthesized using racemic amine **11** and diastereomers were separated by chiral HPLC or SFC. Chiral resolution of racemic **11** was accomplished by fractional crystallization with *R*-mandelic acid. X-ray crystallography

determined that the piperidinyl stereocenter of **11** used to prepare the more active diastereomers possessed the *R* configuration. Stereochemistry of the piperidinyl stereocenter of the more active diastereomers isolated by SFC (compounds **19**, **28** and **37**) was unambiguously confirmed by synthesis using enantiopure **(*R*)-11** and comparing NMR spectra of the compounds prepared from racemic and enantioenriched **11**. On this basis, the absolute stereochemistry of the piperidinyl group of the more active isomers obtained by chromatographic separation was assigned as *R*. Compounds **19**, **21**, **23**, **25**, **26**, **28**, **32-35**, **37**, **38** and **40-42** were prepared from resolved **(*R*)-11**.

Sulfonylated and acylated compounds **19**, **20**, **28-30** were prepared by reductive amination with 2-(N-phthalimidyl)acetaldehyde aldehyde. Standard liberation of the protected amine with hydrazine hydrate, followed by acylation or sulfonylation with an appropriate reagent provided access to intermediate **48**, which was then Boc deprotected and used as is in the nucleophilic aromatic displacement with **15a**. (Scheme 3). Similar to previously described reactions, the yields for the final coupling were moderate to low (15-35%).

Compounds **21**, **14**, **15**, **17**, **33** were accessed using mild conditions for Michael addition of amine **11** to the corresponding electrophile in dichloromethane or DMF. These reactions went to complete conversion at room temperature and the products were used in the subsequent steps without purification (Scheme 4). In the case of compound **36**, Michael addition of methyl acrylate to amine **11** was followed by reduction of the ester using $LiBH_4$. Standard deprotection and S_NAr yielded the desired product (Scheme 5).

Reductive amination was used to synthesize compounds **23**, **26**, **31**, **36**-**42** (Scheme 6). Compounds **37**-**42** were obtained from the same hydroxyethyl intermediate **54** (R_1 = CH_2OTBS, **57**), which was prepared via reductive amination with TBS-protected hydroxyacetaldehyde. Acidic conditions for Boc removal also simultaneously deprotected the TBS group and the crude material was used in the S_NAr, with intermediate **15**, to access final compounds **37**, **38** (explicit synthesis of this key compound is shown in Scheme 8). Amides **40**-**42** were obtained from the intermediate compound **55** by hydrolysis of the ester on the pyrazolopyrimidine core (compound **40**) and T3P-mediated coupling with the methylamine (**41**) or dimethylamine (**42**).

Finally, simple alkylation with alkyl bromide along with a catalytic amount of sodium or potassium iodide was used to access intermediate **56**, which provided access to compounds **22**, **24**, **25**, and **32** via the same deprotection/coupling sequence (Scheme 7).

Due to low reactivity of the methyl substituted compound **15a** the yields of the aromatic nucleophilic substitution step were underwhelming (8-70%), with most of the

examples falling into the 20-30% yield range. Pyrazolopyrazines with electron-withdrawing substituents, such as CN (**15b**), CO_2Et (**15c**), or $CONH_2$ (**15d**), provided higher yields in the coupling step with yields ranging from 32-99%.

CONCLUSIONS

We have discovered a series of potent, selective, and orally bioavailable small molecule CCR4 antagonists containing a novel piperidinyl-azetidine functionality. By inhibiting CCR4-induced chemotaxis, these inhibitors block recruitment of T_{reg} to the TME. Compound **38** displayed good *in vitro* and *in vivo* ADME properties. After oral dosing, compound **38** significantly inhibited the migration of T_{reg} into the TME in a Pan02 tumor model in a dose dependent manner. Importantly, antagonist **38** did not affect the trafficking of T_{reg} to other healthy tissues.[38] We also demonstrated significant anti-tumor efficacy as a single agent in a Pan02-OVA tumor model, comparable to anti-CTLA-4 checkpoint inhibition. Furthermore, the combination of tool compound **38** with the anti-CTLA-4 and anti-PD-L1 antibodies enhanced the anti-tumor response. Together, these findings demonstrate the potential of CCR4 inhibition to potentiate standard-of-care

immunotherapy checkpoint inhibitors. These studies with tool compound **38** led to further work culminating in the clinical candidate FLX475 (structure not shown), currently in clinical trials against several cancers both as a single agent and in combination with the approved checkpoint inhibitor pembrolizumab.

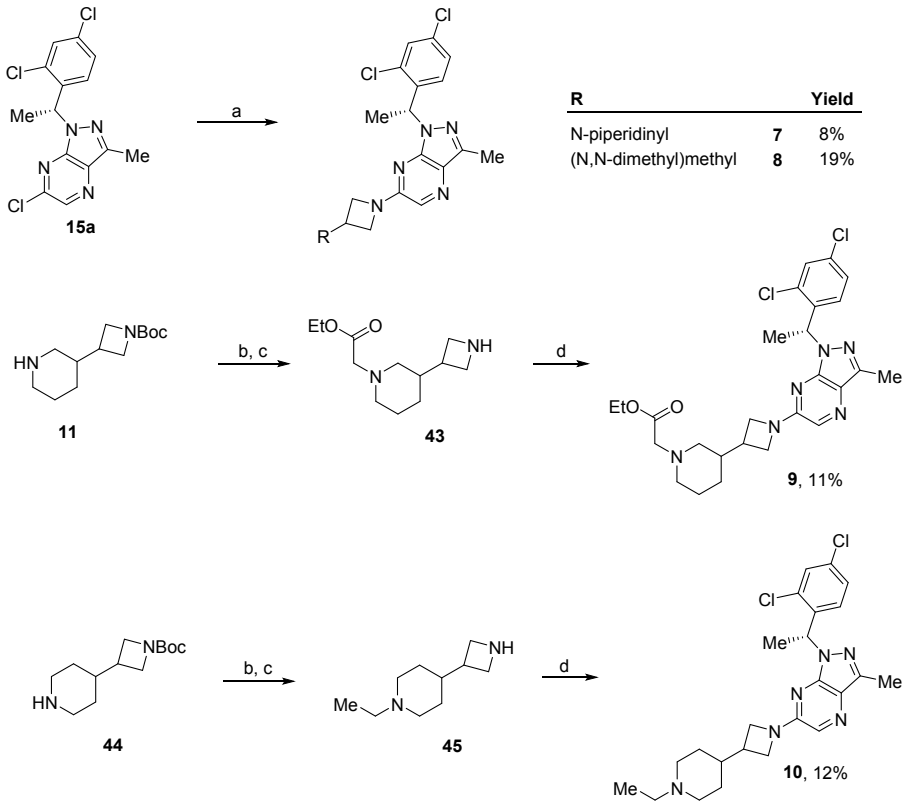
EXPERIMENTAL METHODS

General Methods for Chemistry. All commercial reagents and solvents were used as received unless otherwise noted. An inert atmosphere of nitrogen was used for reactions involving air of moisture sensitive reagents. Analytical thin layer chromatography (TLC) was performed on precoated glass backed plates (silica gel $60F_{254}$; 2.5 x 7.5 cm, 0.25mm thickness, EMD Millipore 1.15341.0001) visualized using combinations of UV visualization, *p*-anisaldehyde, potassium permanganate, and iodine staining. Silica gel column chromatography was performed using Teledyne ISCO RediSep Rf normal phase (35−70 μm) silica gel columns on a Teledyne ISCO CombiFlash Rf or CombiFlash Rf+ purification system (detection at 254 nm). Reversed phase preparative HPLC was carried out using a Gemini-NX-C18 column (10 μm, 250 x 30 mm, Phenomenex, Torrance, CA)

eluting with a linear gradient from 5 to 100% acetonitrile in water containing 0.1% trifluoroacetic acid over 30 minutes on a Teledyne ISCO EZ Prep, Teledyne ISCO ACCQPrep HP125, or Agilent 1200 Series purification system. Analytical reversed phase HPLC was performed using a Gemini-NX-C18 column (5 μm, 250 x 4.6 mm, Phenomenex, Torrance, CA) eluting with acetonitrile in water with 0.1% trifluoroacetic acid on an Agilent 1200 Series purification system (detection at 254 nm). Proton NMR spectra were recorded on a Varian Oxford 400 MHz spectrometer and carbon NMR spectra were recorded at 100 MHz. Chemical shifts (δ) are reported in parts per million (ppm) and referenced to tetramethylsilane (δ = 0 ppm). Coupling constants (*J*) are reported in Hertz (Hz), abbreviations for signal coupling are as follows: s = singlet, d = doublet, dd = double doublets, t = triplet, q = quartet, m = multiplet, bs = broad singlet. Analytical LC-MS was performed using a ZORBAX SB-C18 column (1.8 μm, 2.1 x 50 mm, 600 bar, Agilent, Santa Clara, CA) eluting with a linear gradient from 0% to 100% B over 2 min and then 100% B for 3 min (A = 5% acetonitrile in water with 0.1% formic acid, B = 5% water in acetonitrile with 0.1% formic acid, flow rate 0.4 mL/min) using an Agilent 1260

Infinity II LC System (detection at 254 nm) equipped with an Agilent 6120 Quadrupole LC-MS in electrospray ionization mode (ESI+). The purity of all compounds used in bioassays was determined by this method to be >95% pure.

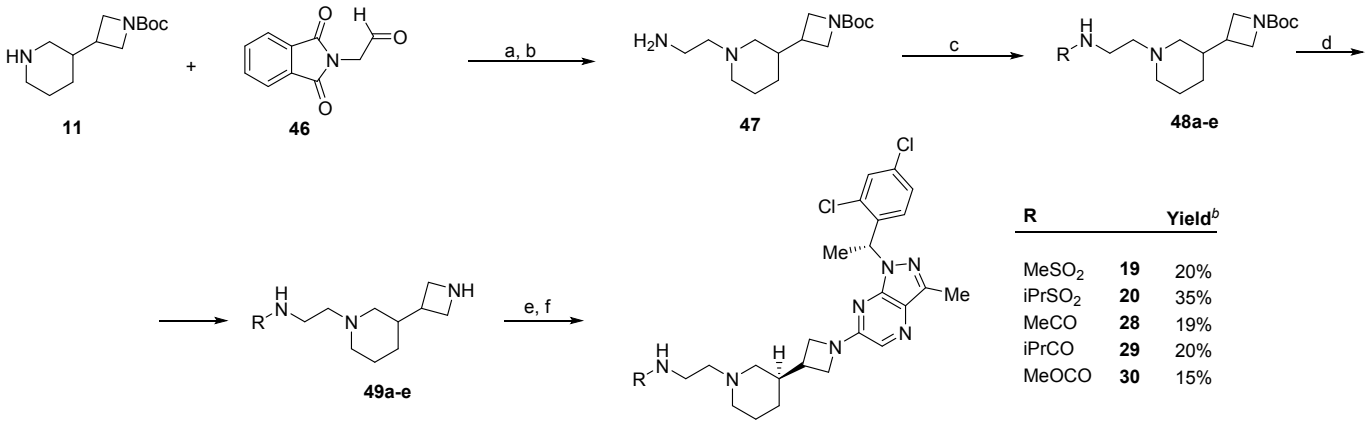
Scheme 2. Synthesis of Piperidinyl-Azetidine CCR4 Antagonists **7**-**10**.[a]



[a]Reagents and conditions: (a) 1-(azetidin-3-yl)piperidine or 1-(azetidin-3-yl)-N,N-dimethylmethanamine, *N,N*-diisopropylethylamine, DMSO, 80 ℃, 12 h. (b) ethyl

glyoxylate or acetaldehyde, $NaBH(OAc)_3$, 1,2-DCE, rt. (c) HCl, 1,4-dioxane, DCM, rt. (d) *N,N*-diisopropylethylamine, **15a**, DMF, 80 ℃, 12 h.

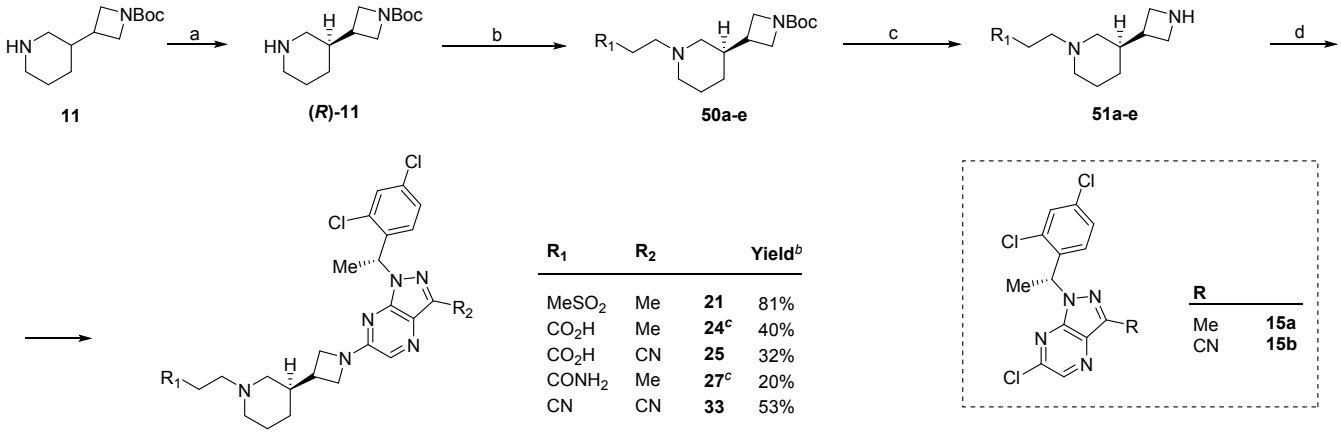
Scheme 3. Synthesis of Piperidinyl-Azetidine CCR4 Antagonists **19**, **20**, **28**-**30**.[a]



R		Yield[b]
$MeSO_2$	**19**	20%
$iPrSO_2$	**20**	35%
MeCO	**28**	19%
iPrCO	**29**	20%
MeOCO	**30**	15%

[a]Reagents and conditions: (a) $NaHB(OAC)_3$, 1,2-DCE, rt. (b) $N_2H_4xH_2O$, EtOH, rt. (c) RSO_2Cl or RCOCl, DCM, *N,N*-diisopropylethylamine. (d) HCl, 1,4-dioxane, DCM, rt. (e) **15a**, *N,N*-diisopropylethylamine, DMF, 80℃. (f) Separation of diastereomers by Chiral HPLC or SFC. [b]Yield for the last two steps based on maximum single diastereomer recovery.

Scheme 4. Synthesis of Piperidinyl-Azetidine CCR4 Antagonists **21**, **24**, **25**, **27**, and **33**.[a]

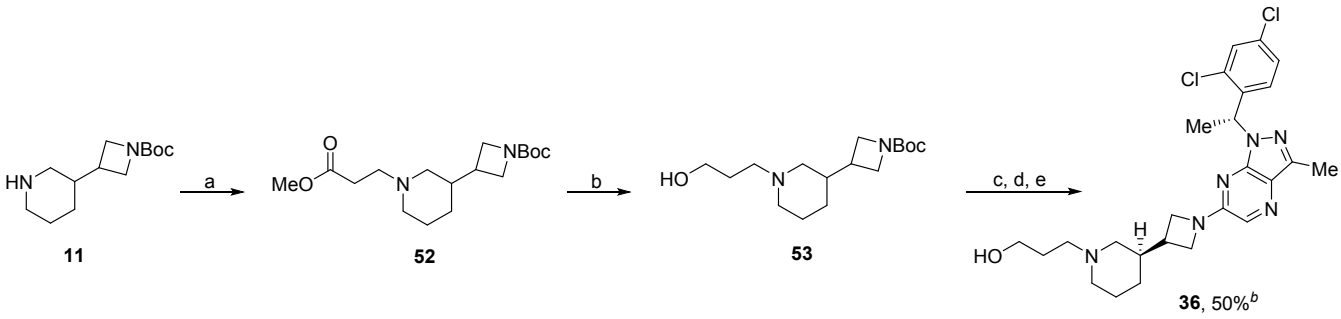


R_1	R_2		Yield[b]
$MeSO_2$	Me	**21**	81%
CO_2H	Me	**24**[c]	40%
CO_2H	CN	**25**	32%
$CONH_2$	Me	**27**[c]	20%
CN	CN	**33**	53%

R	
Me	**15a**
CN	**15b**

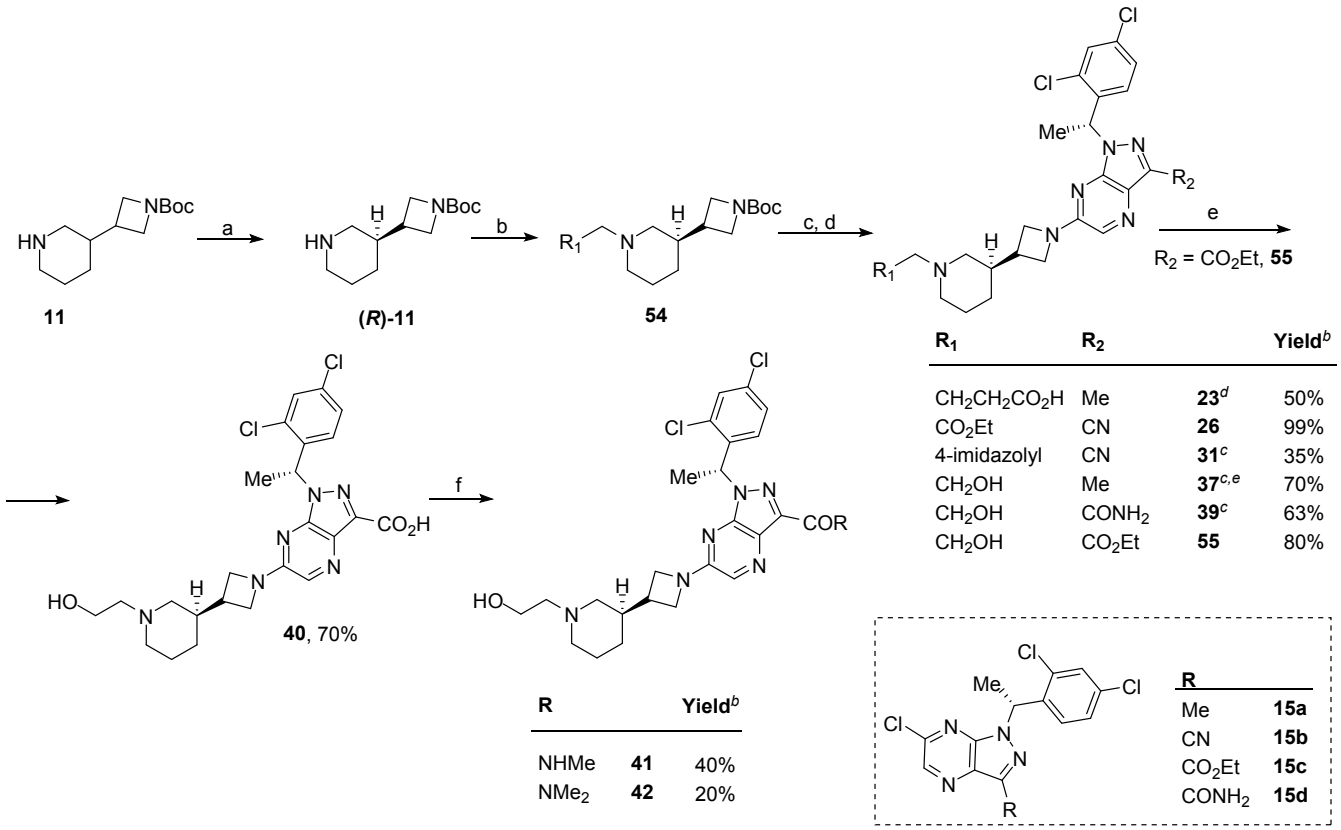
[a]Reagents and conditions: (a) *L*-(+)-mandelic acid, MTBE, 60 °C, 40%. (b) Michael acceptor, DCM, rt. (c) HCl, 1,4-dioxane, DCM, rt. (d) **15a or 15b**, *N,N*-diisopropylethylamine, DMF, 80℃. [b]Yield for the last two steps based on maximum single diastereomer recovery. [C]Compound was made from racemic amine and diastereomers were separated by chiral HPLC or SFC.

Scheme 5. Synthesis of Piperidinyl-Azetidine CCR4 Antagonist **36**.[a]



[a]Reagents and conditions: (a) methylacrylate, DMF, 50 ℃, 3h. (b) LiBH4, THF, MeOH, 50 ℃, 24 h. (c) HCl, 1,4-dioxane, DCM, rt. (d) **15a**, *N,N*-diisopropylethylamine, DMF, 80℃. (e) Separation of diastereomers by Chiral HPLC using OZ-H, 20% ethanol/80% heptanes with 0.1% diethylamine. [b]Yield for the last two steps based on maximum single diastereomer recovery.

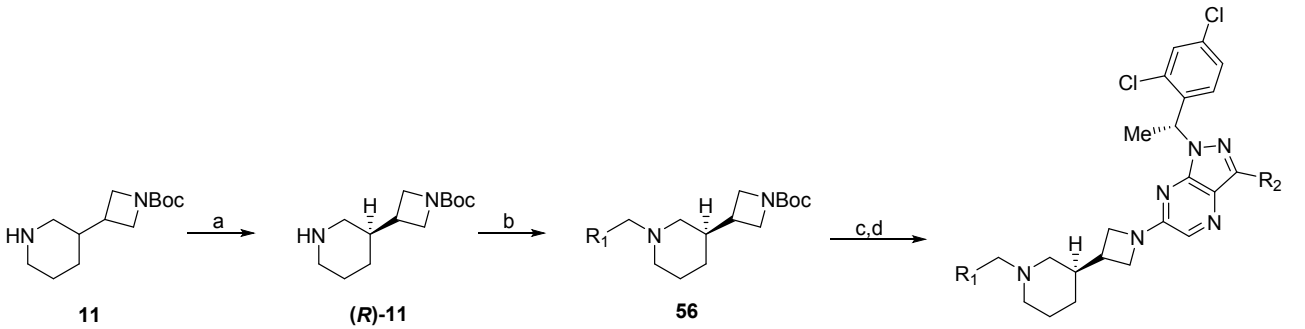
Scheme 6. Synthesis of Piperidinyl-Azetidine CCR4 Antagonists **23**, **26**, **31**, **37**-**39**, **40**-**42**.[a]



[a]Reagents and conditions: (a) *L*-(+)-mandelic acid, MTBE, 60 °C, 40%. (b) aldehyde, $NaHB(OAc)_3$, 1,2-DCE, rt, (c) HCl, 1,4-dioxane, DCM, rt. (d) **15,** *N,N*-diisopropylethylamine, DMF, 80℃. (e) LiOH, MeOH, water, rt. (f) $MeNH_2$ or Me_2NH, T3P, EtOAc. [b]Yield for the last two steps based on maximum single diastereomer recovery. [c]Compound was made from racemic amine and diastereomers were separated by chiral HPLC or SFC. [d]Yield is shown for methyl ester, which was hydrolyzed using LiOH in

MeOH/water with 90% yield to afford **23**. [e]This compound was prepared using both racemic amine **11** and enantioenriched (*R*)-**11**, confirming stereochemistry of the product.

Scheme 7. Synthesis of Piperidinyl-Azetidine CCR4 Antagonists **22**, **32**, **34**, **35**.

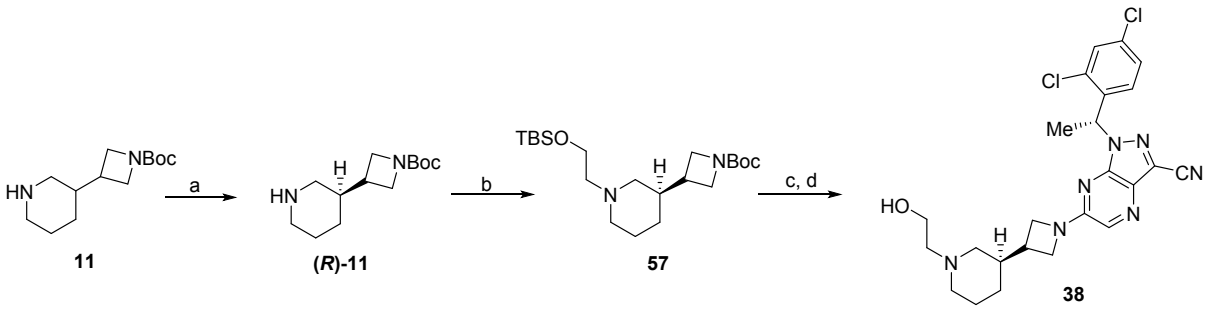


11 (***R***)-**11** **56**

R_1	R_2		Yield[b]
$CH_2CH_2SO_2Me$	Me	**22**[c]	25%
CN	CN	**32**	59%
CH_2CF_3	Me	**34**	10%
CH_2CF_3	CN	**35**	78%

[a]Reagents and conditions: (a) *L*-(+)-mandelic acid, MTBE, 60 °C, 40%. (b) R_1CH_2X (X=Br or I), acetonitrile or 1,4-dioxane, potassium or sodium carbonate, 75-90 ℃, 12 h. (c) HCl, 1,4-dioxane, DCM, rt. (d) **15a** or **15b**, *N,N*-diisopropylethylamine, DMF, 80℃. [b]Yield for the last two steps based on maximum single diastereomer recovery. [c]Compound was made from racemic amine and diastereomers were separated by chiral HPLC or SFC.

Scheme 8. Synthesis of Compound **38**[a]



[a]Reagents and conditions: (a) *L*-(+)-mandelic acid, MTBE, 60 °C, 40%. (b) $NaBH(OAc)_3$, 1,2-DCE, 22 °C, 98%. (c) HCl, 1,4-dioxane, DCM 22 °C, 99%. (d) **15b**, *N,N*-diisopropylethylamine, DCM, 22 °C, 83%.

General Procedure A for alkylation of *tert*-butyl 3-(piperidin-3-yl)azetidine-1-carboxylate by Reductive Amination. Commercially available *tert*-butyl 3-(piperidin-3-yl)azetidine-1-carboxylate **11** (1.0 equiv) was dissolved in 1,2-dichloroethane (0.2 M) and then the appropriate aldehyde or ketone (1.0-2.0 equiv) was added all at once. The mixture was stirred for 5 minutes at room temperature and then sodium triacetoxyborohydride (2.0 equiv) was added. The mixture was stirred at room temperature and conversion was monitored by LC-MS. Upon completion, the reaction was quenched with saturated

aqueous sodium bicarbonate solution and extracted with dichloromethane. The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure to afford the desired product, which was purified by silica gel chromatography, preparative reverse phase HPLC, or used without further purification.

General Procedure B for alkylation of *tert*-butyl 3-(piperidin-3-yl)azetidine-1-carboxylate. Commercially available *tert*-butyl 3-(piperidin-3-yl)azetidine-1-carboxylate **11** (1.0 equiv) was dissolved in the appropriate dry solvent (0.2 M) and an alkyl halide (1 equiv), sodium iodide (0.1 equiv) and sodium carbonate (2 equiv) were added to the solution, and then the mixture was heated to 75 °C under a nitrogen atmosphere and the reaction was monitored by LC-MS; upon completion, the reaction mixture was diluted with water (20 mL), extracted with ethyl acetate (3X), the combined organic fractions were dried over sodium sulfate, filtered and concentrated under reduced pressure; the residue was purified by silica gel chromatography using a gradient of methanol in dichloromethane as eluent.

General Procedure C for Michael addition with *tert*-butyl 3-(piperidin-3-yl)azetidine-1-carboxylate.

Commercially available *tert*-butyl 3-(piperidin-3-yl)azetidine-1-carboxylate **11** (1.0 equiv) was dissolved in the appropriate dry solvent (0.2 M) and either (a) a Michael acceptor (1 equiv) was added to the solution at once and the reaction mixture was stirred at room temperature or heated to 50 °C until complete conversion was achieved (monitored by LC-MS); upon completion, the volatiles were removed under reduced pressure and the residue was purified by silica gel chromatography using a gradient of methanol in dichloromethane as eluent.

General Procedure D for deprotection of 1-alkyl *tert*-butyl 3-(piperidin-3-yl)azetidine-1-carboxylate. 1-Alkyl *tert*-butyl 3-(piperidin-3-yl)azetidine-1-carboxylate **11** was dissolved in dichloromethane (0.2 M) and then a solution of HCl (3 equiv, 4 M in 1,4-dioxane) or trifluoroacetic acid (5 equiv) was added. The mixture was stirred at room temperature and the reaction was monitored by LC-MS; upon completion, the volatiles were removed

under reduced pressure and the crude product was used as is without any further purification.

General Procedure E for nucleophilic aromatic substitution (S_NAr) with 1-alkyl 3-(azetidin-3-yl)-piperidine. 1-Alkyl 3-(azetidin-3-yl)-piperidine **11** (1.1 equiv) and the appropriate heteroaryl chloride (1.0 equiv) were dissolved in dimethylformamide, dimethyl sulfoxide, or dichloromethane and *N,N*-diisopropylethylamine (2.0 to 3.0 equiv) was added. The reaction was heated to 40 – 100 °C. The reaction was monitored by LC-MS. Upon completion, the mixture was cooled to room temperature and purified by silica gel chromatography or reversed phase preparative HPLC.

(*R*)-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-(3-(piperidin-1-yl)azetidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine (7). Step 1: The reductive amination was performed according to general procedure A using *tert*-butyl 3-oxoazetidine-1-carboxylate (1.0 g, 5.84 mmol), piperidine (0.58 mL, 5.84 mmol) and sodium triacetoxyborohydride (3.7 g, 17.52 mmol) in 1,2-dichloroethane (15 mL) at 23 °C for 2 h. The reaction was quenched with saturated aqueous sodium bicarbonate solution and extracted with dichloromethane. The combined

organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure and the residue was purified by silica gel chromatography using a gradient of methanol in dichloromethane (0 to 5 %) and afforded *tert*-butyl 3-(piperidin-1-yl)azetidine-1-carboxylate (57% yield) . LRMS-ESI$^+$: *m/z* calcd for $C_{13}H_{25}N_2O_2$ $[M+H]^+$ = 241.19; found, 241.2.

Step 2: The Boc deprotection was performed according to general procedure D. *Tert*-butyl 3-(piperidin-1-yl)azetidine-1-carboxylate (800 mg, 3.33 mmol) was dissolved in dichloromethane (16 mL) and then trifluoroacetic acid (1.27 mL, 16.65 mmol) was added. The mixture was stirred at 23 °C for 12 h and then concentrated under reduced pressure to afford 1-(azetidin-3-yl)piperidine 2,2,2-trifluoroacetate, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_8H_{17}N_2$ $[M+H]^+$ = 141.14; found, 141.2.

Step 3: The S_NAr reaction was performed according to general procedure E. 1-(Azetidin-3-yl)piperidine 2,2,2-trifluoroacetate (185 mg, 0.73 mmol) was dissolved in dimethyl sulfoxide (2 mL), then (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-

pyrazolo[3,4-*b*]pyrazine **15a** (225 mg, 0.66 mmol) and *N*,*N*-diisopropylethylamine (0.5 mL, 2.92 mmol) were added. The mixture was heated to 80 °C for 12 h, then cooled to 23 °C. The mixture was purified directly by reversed 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 (*R*)-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-(3-(piperidin-1-yl)azetidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine 2,2,2-trifluoroacetate (**7**, 8% yield). ^{1}H NMR (400 MHz, CD_3OD; TFA salt) δ 7.85 (s, 1H), 7.44 (d, *J* = 2.1 Hz, 1H), 7.35 (d, *J* = 8.5 Hz, 1H), 7.27 (dd, *J* = 8.5, 2.1 Hz,1H), 6.31 (q, *J* = 7.0 Hz, 1H), 4.56 – 4.46 (m, 2H), 4.42 – 4.33 (m, 2H), 4.29 – 4.20 (m, 1H), 3.72 – 3.49 (m, 2H), 3.00 – 2.82 (m, 2H), 2.52 (s, 3H), 2.08 – 1.95 (m, 3H), 1.87 (d, *J* = 7.1 Hz, 3H), 1.86 – 1.65 (m, 2H), 1.63 – 1.48 (m, 1H). LRMS-ESI$^+$: *m/z* calcd for $C_{22}H_{27}Cl_2N_6$ $[M+H]^+$ = 445.17; found, 445.0.

(*R*)-1-(1-(1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)-*N*,*N*-dimethylmethanamine (8). The S_NAr reaction was performed according to general procedure E. Commercially available 1-(azetidin-3-yl)-*N*,*N*-

dimethylmethanamine dihydrochloride (60 mg, 0.32 mmol) was dissolved in dimethyl sulfoxide (1 mL), then (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine **15a** (100 mg, 0.29 mmol) and *N*,*N*-diisopropylethylamine (0.25 mL, 1.45 mmol) were added. The mixture was heated to 80 °C for 12 h, then cooled to 23 °C. The mixture was purified directly by reversed 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 (*R*)-1-(1-(1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)-*N*,*N*-dimethylmethanamine 2,2,2-trifluoroacetate (**8**, 19% yield). ^{1}H NMR (400 MHz, $CDCl_3$; TFA salt) δ 8.14 (bs, 1H), 7.69 (s, 1H), 7.37 (d, *J* = 8.5 Hz, 1H), 7.34 (d, *J* = 2.1 Hz, 1H), 7.15 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.31 (q, *J* = 7.1 Hz, 1H), 4.44 – 4.35 (m, 2H), 3.98 – 3.89 (m, 2H), 3.42 (d, *J* = 6.9 Hz, 2H), 3.40 – 3.30 (m, 1H), 2.89 (s, 6H), 2.57 (s, 3H), 1.88 (d, *J* = 7.1 Hz, 3H). LRMS-ESI$^+$: *m/z* calcd for $C_{20}H_{25}Cl_2N_6$ $[M+H]^+$ = 419.15; found, 419.0.

Ethyl 2-(3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)acetate (9). Step 1: The reductive amination was performed according to general procedure A using *tert*-butyl 3-(piperidin-3-yl)azetidine-1-carboxylate **11** (200 mg, 0.83 mmol), ethyl glyoxalate (0.17 mL, 0.83 mmol, 50% in toluene) and sodium triacetoxyborohydride (528 mg, 2.49 mmol) in 1,2-dichloroethane (3 mL) at 23 °C for 12 h. The reaction was quenched with saturated aqueous sodium bicarbonate solution and extracted with dichloromethane. The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure and the residue was purified by silica gel chromatography using a gradient of methanol in dichloromethane (0 to 10 %) and afforded *tert*-butyl 3-(1-(2-ethoxy-2-oxoethyl)piperidin-3-yl)azetidine-1-carboxylate (96% yield). LRMS-ESI$^+$: *m/z* calcd for $C_{17}H_{31}N_2O_2$ $[M+H]^+$ = 327.23; found, 327.2.

Step 2: The Boc deprotection was performed according to general procedure D. *Tert*-butyl 3-(1-(2-ethoxy-2-oxoethyl)piperidin-3-yl)azetidine-1-carboxylate (261 mg, 0.80 mmol) was dissolved in dichloromethane (4 mL) and then HCl (0.62 mL, 2.49 mmol, 4 N

in 1,4-dioxane) was added. The mixture was stirred at 23 °C for 4 h and then concentrated under reduced pressure to afford ethyl 2-(3-(azetidin-3-yl)piperidin-1-yl)acetate hydrochloride, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{12}H_{23}N_2O_2$ $[M+H]^+$ = 227.18; found, 227.1.

Step 3: The S_NAr reaction was performed according to general procedure E. Ethyl 2-(3-(azetidin-3-yl)piperidin-1-yl)acetate hydrochloride (210 mg, 0.80 mmol) was dissolved in dimethyl sulfoxide (2 mL), then (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine **15a** (273 mg, 0.80 mmol) and *N*,*N*-diisopropylethylamine (0.56 mL, 3.2 mmol) were added. The mixture was heated to 80 °C for 4 h, then cooled to 23 °C. The mixture was purified directly by reversed 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 ethyl 2-(3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)acetate 2,2,2-trifluoroacetate (**9**, 11% yield). ^{1}H NMR (400 MHz, $CDCl_3$; TFA salt) δ 10.43 (bs, 1H), 7.66 (s, 1H), 7.37 (d, *J* = 8.5 Hz, 1H),

7.34 (d, *J* = 2.2 Hz, 1H), 7.14 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.31 (q, *J* = 7.0 Hz, 1H), 4.24 (q, *J* = 7.2 Hz, 2H), 4.22 – 4.15 (m, 1H), 3.98 – 3.84 (m, 4H), 3.68 – 3.53 (m, 2H), 3.21 – 3.03 (m, 1H), 2.94 – 2.74 (m, 1H), 2.68 – 2.58 (m, 1H), 2.56 (s, 3H), 2.43 – 2.28 (m, 1H), 2.13 – 1.91 (m, 5H), 1.88 (d, *J* = 7.1 Hz, 3H), 1.29 (t, *J* = 7.1 Hz, 3H), 1.22 – 1.04 (m, 1H). LRMS-ESI$^+$: *m/z* calcd for $C_{26}H_{33}Cl_2N_6O_2$ $[M+H]^+$ = 531.20; found, 531.1.

(*R*)-1-(1-(2,4-dichlorophenyl)ethyl)-6-(3-(1-ethylpiperidin-4-yl)azetidin-1-yl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (10). Step 1: The reductive amination was performed according to general procedure A using *tert*-butyl 3-(piperidin-4-yl)azetidine-1-carboxylate **11** (200 mg, 0.83 mmol), acetaldehyde (46 μL, 1.66 mmol) and sodium triacetoxyborohydride (353 mg, 1.66 mmol) in 1,2-dichloroethane (4 mL) at 23 °C for 24 h. The reaction was quenched with saturated aqueous sodium bicarbonate solution and extracted with dichloromethane. The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure and afforded *tert*-butyl 3-(1-ethylpiperidin-4-yl)azetidine-1-carboxylate, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{15}H_{29}N_2O_2$ $[M+H]^+$ = 269.22; found, 269.2.

Step 2: The Boc deprotection was performed according to general procedure D. The crude from step 1 was dissolved in dichloromethane (4 mL) and then HCl (0.62 mL, 2.49 mmol, 4 N in 1,4-dioxane) was added. The mixture was stirred at 23 °C for 1 h and then concentrated under reduced pressure to afford 4-(azetidin-3-yl)-1-ethylpiperidine hydrochloride, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{10}H_{21}N_2$ $[M+H]^+$ = 169.17; found, 169.2.

Step 3: The S_NAr reaction was performed according to general procedure E. The crude 4-(azetidin-3-yl)-1-ethylpiperidine hydrochloride from step 2 was dissolved in dimethylformamide (5 mL), then (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine **15a** (283 mg, 0.83 mmol) and *N*,*N*-diisopropylethylamine (0.27 mL, 1.66 mmol) were added. The mixture was heated to 80 °C for 12 h, then cooled to 23 °C and concentrated under reduced pressure. The residue was purified by reversed 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 (*R*)-1-(1-(2,4-dichlorophenyl)ethyl)-6-(3-(1-

ethylpiperidin-4-yl)azetidin-1-yl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine 2,2,2-trifluoroacetate (**10**, 12% yield). ^{1}H NMR (400 MHz, CD_3CN; TFA salt) δ 7.75 (s, 1H), 7.49 (d, *J* = 2.2 Hz, 1H), 7.43 (d, *J* = 8.5 Hz, 1H), 7.28 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.27 (q, *J* = 7.0 Hz, 1H), 4.82 (bs, 1H), 4.27 – 4.16 (m, 2H), 3.92 – 3.81 (m, 2H), 3.64 – 3.50 (m, 1H), 3.49 – 3.31 (m, 1H), 3.22 – 2.76 (m, 3H), 2.66 – 2.53 (m, 1H), 2.47 (s, 3H), 2.03 – 1.95 (m, 7H), 1.86 (d, *J* = 7.0 Hz, 3H), 1.66 – 1.42 (m, 2H). LRMS-ESI$^+$: *m/z* calcd for $C_{24}H_{31}Cl_2N_6$ $[M+H]^+$ = 473.20; found, 473.2.

2-(3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)ethane-1-sulfonamide (16). Step 1: The alkylation was performed according to general procedure B(a) using *tert*-butyl 3-(piperidin-3-yl)azetidine-1-carboxylate **11** (200 mg, 0.83 mmol) and ethenesulfonyl fluoride (76 μL, 0.91 mmol) in dichloromethane (2 mL) at 23 °C for 10 min, then the volatiles were removed under reduced pressure and the residue was dissolved in methanol (2 mL) and then concentrated ammonium hydroxide (1 mL) was added. The mixture was heated to 70 °C for 30 min, then cooled to 23 °C and concentrated under reduced pressure to afford

tert-butyl 3-(1-(2-sulfamoylethyl)piperidin-3-yl)azetidine-1-carboxylate 13, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{15}H_{30}N_3O_4S$ $[M+H]^+$ = 348.20; found, 348.1.

Step 2: The Boc deprotection was performed according to general procedure D. The crude *tert*-butyl 3-(1-(2-sulfamoylethyl)piperidin-3-yl)azetidine-1-carboxylate **13** from step 1 was dissolved in dichloromethane (4 mL) and then HCl (0.62 mL, 2.49 mmol, 4 N in 1,4-dioxane) was added. The mixture was stirred at 23 °C for 1 h and then concentrated under reduced pressure to afford 2-(3-(azetidin-3-yl)piperidin-1-yl)ethane-1-sulfonamide hydrochloride **14**, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{10}H_{22}N_3O_2S$ $[M+H]^+$ = 248.14; found, 248.2.

Step 3: The S_NAr reaction was performed according to general procedure E. The crude 2-(3-(azetidin-3-yl)piperidin-1-yl)ethane-1-sulfonamide hydrochloride **14** from step 2 was dissolved in dimethyl sulfoxide (2 mL), then (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine **15a** (219 mg, 0.64 mmol) and *N*,*N*-diisopropylethylamine (0.45 mL, 2.56 mmol) were added. The mixture was heated to 80

°C for 4 h, then cooled to 23 °C. The mixture was purified directly by reversed 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 ethyl 2-(3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)acetate 2,2,2-trifluoroacetate as a 1:1 mixture of diastereomers (**16**, 15% yield). LRMS-ESI$^+$: *m/z* calcd for $C_{24}H_{32}Cl_2N_7O_2S$ $[M+H]^+$ = 552.17; found, 552.2.

2-((*S*)-3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)ethane-1-sulfonamide (17). The mixture of diastereomers **16** was basified using a basification column (PL-HCO_3MP SPE, 500 mg per 5 mL tube, Agilent) using dichloromethane and methanol (4:1) as eluent and concentrated under reduced pressure. The residue was separated by preparative chiral HPLC using a CHIRALPAK® IF column (Daicel Corporation, West Chester, PA) and 45% isopropanol in heptanes (0.1% diethylamine) as eluent and afforded 2-((*S*)-3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-

yl)ethane-1-sulfonamide (**17**) as the first eluting diastereomer: ^{1}H NMR (400 MHz, CD_3OD, free base): δ 7.70 (s, 1H), 7.43 (d, *J* = 2.1 Hz, 1H), 7.36 (d, *J* = 8.5 Hz, 1H), 7.24 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.28 (q, *J* = 7.1 Hz, 1H), 4.25 – 4.16 (m, 2H), 3.94 – 3.88 (m, 2H), 3.31 (bs, 1H), 3.28 (bs, 1H), 2.94 – 2.81 (m, 4H), 2.64 – 2.51 (m, 1H), 2.49 (s, 3H), 2.12 – 2.01 (m, 1H), 1.86 (d, *J* = 7.1 Hz, 3H), 1.84 – 1.69 (m, 4H), 1.64 – 1.50 (m, 1H), 1.15 (d, *J* = 6.2 Hz, 2H), 1.01 – 0.86 (m, 1H). LRMS-ESI$^+$: *m/z* calcd for $C_{24}H_{32}Cl_2N_7O_2S$ $[M+H]^+$ = 552.17; found, 552.2.

2-((*R*)-3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)ethane-1-sulfonamide (18). The mixture of diastereomers **16** was basified using a basification column (PL-HCO_3MP SPE, 500 mg per 5 mL tube, Agilent) using dichloromethane and methanol (4:1) as eluent and concentrated under reduced pressure. The residue was separated by preparative chiral HPLC using a CHIRALPAK® IF column (Daicel Corporation, West Chester, PA) and 45% isopropanol in heptanes (0.1% diethylamine) as eluent and afforded 2-((*R*)-3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-

yl)ethane-1-sulfonamide (**18**) as the second eluting diastereomer: ^{1}H NMR (400 MHz, CD_3OD, free base): δ 7.70 (s, 1H), 7.43 (d, *J* = 2.1 Hz, 1H), 7.37 (d, *J* = 8.5 Hz, 1H), 7.24 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.28 (q, *J* = 7.1 Hz, 1H), 4.27 – 4.14 (m, 2H), 3.97 – 3.85 (m, 2H), 3.29 (bs, 1H), 3.27 (bs, 1H), 2.95 – 2.80 (m, 3H), 2.64 – 2.52 (m, 1H), 2.49 (s, 3H), 2.12 – 2.01 (m, 1H), 1.86 (d, *J* = 7.1 Hz, 3H), 1.83 – 1.70 (m, 5H), 1.65 – 1.51 (m, 1H), 1.15 (d, *J* = 6.2 Hz, 2H), 1.00 – 0.86 (m, 1H). LRMS-ESI$^+$: *m/z* calcd for $C_{24}H_{32}Cl_2N_7O_2S$ $[M+H]^+$ = 552.17; found, 552.2.

***N*-(2-((*R*)-3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)ethyl)methanesulfonamide (19).**

Step 1: The reductive amination was performed according to general procedure A using either *tert*-butyl 3-(piperidin-3-yl)azetidine-1-carboxylate **11** or enantioenriched (*R*)-**11** (300 mg, 1.25 mmol), 3-(1,3-dioxoisoindolin-2-yl)propanal **46** (236 mg, 1.25 mmol, 50% in toluene) and sodium triacetoxyborohydride (529 mg, 2.5 mmol) in 1,2-dichloroethane (5 mL) at 23 °C for 15 min. The reaction was quenched with saturated aqueous sodium bicarbonate solution and extracted with dichloromethane. The combined organic layers

were dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was dissolved in methanol (5 mL) and then hydrazine hydrate (0.24 mL, 5.0 mmol) was added. The mixture was stirred at 23 °C for 18 h and then mixture was diluted with water and extracted with dichloromethane. The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude *tert*-butyl 3-[1-(2-aminoethyl)-3-piperidyl]azetidine-1-carboxylate **47** (120 mg, 0.423 mmol) was dissolved in dichloromethane (2 mL) and then with triethylamine (0.18 mL, 1.27 mmol) and methanesulfonyl chloride (39 μL, 0.508 mmol) were added. The mixture was stirred at 23 °C for 30 min, then the mixture was quenched with aqueous sodium carbonate (10 mL, 1M) and extracted with ethyl acetate (3 x 10 mL). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressured and afforded *tert*-butyl 3-(1-(2-(methylsulfonamido)ethyl)piperidin-3-yl)azetidine-1-carboxylate, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{16}H_{32}N_3O_4S$ $[M+H]^+$ = 362.21; found, 362.1.

Step 2: The Boc deprotection was performed according to general procedure D. The crude *tert*-butyl 3-(1-(2-(methylsulfonamido)ethyl)piperidin-3-yl)azetidine-1-carboxylate from step 1 was dissolved in dichloromethane (4 mL) and then HCl (0.50 mL, 2.0 mmol, 4 N in 1,4-dioxane) was added. The mixture was stirred at 23 °C for 1 h and then concentrated under reduced pressure to afford *N*-(2-(3-(azetidin-3-yl)piperidin-1-yl)ethyl)methanesulfonamide hydrochloride, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{11}H_{24}N_3O_2S$ $[M+H]^+$ = 262.16; found, 262.1.

Step 3: The S_NAr reaction was performed according to general procedure E. The crude *N*-(2-(3-(azetidin-3-yl)piperidin-1-yl)ethyl)methanesulfonamide hydrochloride from step 2 was dissolved in dimethylformamide (1 mL), then (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine **15a** (72 mg, 0.21 mmol) and *N*,*N*-diisopropylethylamine (0.18 mL, 1.05 mmol) were added. The mixture was heated to 80 °C for 4 h, then cooled to 23 °C. The mixture was purified directly by reversed 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 *N*-(2-(3-(1-(1-((R)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1H-pyrazolo[3,4-b]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)ethyl)methanesulfonamide 2,2,2-trifluoroacetate as a 1:1 mixture of diastereomers (if racemic **11** was used). The title compound was separated from its diastereomer by SFC using an AS-H column (2 x 25 cm) and eluting with 30% ethanol (0.1% diethylamine) in CO_2, 100 bar, to give the free base of the title compound as the second eluting isomer and converted to the corresponding HCl salt by dissolution in ethanol, cooling to 0 °C, and addition of 1 equiv. of 0.01M HCl in ethanol and afforded 13 mg (20% yield) of *N*-(2-((*R*)-3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)ethyl)methanesulfonamide (**19**). ^{1}H NMR (400 MHz, CD_3OD; HCl Salt) δ 7.76 (s, 1H), 7.45 (d, *J* = 2.1 Hz, 1H), 7.37 (d, *J* = 8.5 Hz, 1H), 7.27 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.29 (q, *J* = 7.1 Hz, 1H), 4.34 – 4.21 (m, 2H), 4.04 – 3.96 (m, 2H), 3.82 – 3.41 (m, 4H), 3.29 – 3.26 (m, 1H), 3.03 (s, 3H), 3.02 – 2.92 (m, 2H), 2.76 – 2.60 (m, 2H), 2.50 (s, 3H), 2.23 – 2.10 (m, 1H), 2.08 – 1.89 (m, 3H), 1.87 (d, *J* = 7.1 Hz,

3H), 1.25 – 1.18 (m, 1H). LRMS-ESI$^+$: *m/z* calcd for $C_{25}H_{34}Cl_2N_7O_2S$ $[M+H]^+$ = 566.19; found, 566.0.

***N*-(2-((*R*)-3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)ethyl)propane-2-sulfonamide (20).** A mixture of the title compound and its diastereomer was prepared as follows:

Step 1: The reductive amination was performed according to general procedure A using *tert*-butyl 3-(piperidin-3-yl)azetidine-1-carboxylate **11** (300 mg, 1.25 mmol), 3-(1,3-dioxoisoindolin-2-yl)propanal **46** (236 mg, 1.25 mmol, 50% in toluene) and sodium triacetoxyborohydride (529 mg, 2.5 mmol) in 1,2-dichloroethane (5 mL) at 23 °C for 15 min. The reaction was quenched with saturated aqueous sodium bicarbonate solution and extracted with dichloromethane. The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was dissolved in methanol (5 mL) and then hydrazine hydrate (0.24 mL, 5.0 mmol) was added. The mixture was stirred at 23 °C for 18 h and then mixture was diluted with water and extracted with dichloromethane. The combined organic layers were dried over sodium

sulfate, filtered and concentrated under reduced pressure. The crude tert-butyl 3-[1-(2-aminoethyl)-3-piperidyl]azetidine-1-carboxylate **47** (120 mg, 0.423 mmol) was dissolved in dichloromethane (2 mL) and then with triethylamine (0.18 mL, 1.27 mmol) and 2-propanesulfonyl chloride (57 μL, 0.508 mmol) were added. The mixture was stirred at 23 °C for 30 min, then the mixture was quenched with aqueous sodium carbonate (10 mL, 1M) and extracted with ethyl acetate (3 x 10 mL). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressured and afforded *tert*-butyl 3-(1-(2-((1-methylethyl)sulfonamido)ethyl)piperidin-3-yl)azetidine-1-carboxylate, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{18}H_{36}N_3O_4S$ $[M+H]^+$ = 390.24; found, 390.1.

Step 2: The Boc deprotection was performed according to general procedure D. *tert*-butyl 3-(1-(2-((1-methylethyl)sulfonamido)ethyl)piperidin-3-yl)azetidine-1-carboxylate from step 1 was dissolved in dichloromethane (4 mL) and then HCl (0.50 mL, 2.0 mmol, 4 N in 1,4-dioxane) was added. The mixture was stirred at 23 °C for 1 h and then concentrated under reduced pressure to afford *N*-(2-(3-(azetidin-3-yl)piperidin-1-

yl)ethyl)propane-2-sulfonamide hydrochloride, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{13}H_{28}N_3O_2S$ $[M+H]^+$ = 290.19; found, 290.1.

Step 3: The S_NAr reaction was performed according to general procedure E. The crude *N*-(2-(3-(azetidin-3-yl)piperidin-1-yl)ethyl)methanesulfonamide hydrochloride from step 2 was dissolved in dimethylformamide (1 mL), then (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine **15a** (72 mg, 0.21 mmol) and *N*,*N*-diisopropylethylamine (0.18 mL, 1.05 mmol) were added. The mixture was heated to 80 °C for 4 h, then cooled to 23 °C. The mixture was purified directly by reversed 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 *N*-(2-(3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)ethyl)propane-2-sulfonamide 2,2,2-trifluoroacetate as a 1:1 mixture of diastereomers. The title compound was separated from its diastereomer by SFC using an AS-H column (2 x 25 cm) and

eluting with 25% isopropanol (0.1% diethylamine) in CO_2, 100 bar, to give the free base of the title compound as the second eluting isomer and converted to the corresponding HCl salt by dissolution in ethanol, cooling to 0 °C, and addition of 1 equiv. of 0.01M HCl in ethanol and afforded 23 mg of *N*-(2-((*R*)-3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)ethyl)propane-2-sulfonamide (**20**, 35% yield). ^{1}H NMR (400 MHz, CD_3OD; HCl Salt) δ 7.74 (s, 1H), 7.44 (d, *J* = 2.1 Hz, 1H), 7.37 (d, *J* = 8.5 Hz, 1H), 7.26 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.29 (q, *J* = 7.1 Hz, 1H), 4.33 – 4.22 (m, 2H), 4.04 – 3.96 (m, 2H), 3.77 – 3.70 (m, 1H), 3.68 – 3.59 (m, 1H), 3.56 – 3.45 (m, 2H), 3.37 – 3.32 (m, 1H), 3.27 – 3.23 (m, 2H), 3.01 – 2.91 (m, 2H), 2.74 – 2.62 (m, 2H), 2.50 (s, 3H), 2.19 – 2.10 (m, 1H), 2.08 – 1.88 (m, 2H), 1.86 (d, *J* = 7.1 Hz, 3H), 1.37 (d, *J* = 6.8 Hz, 3H), 1.35 (d, *J* = 6.8 Hz, 3H), 1.24 – 1.18 (m, 1H). LRMS-ESI$^+$: *m/z* calcd for $C_{27}H_{38}Cl_2N_7O_2S$ $[M+H]^+$ = 594.22; found, 594.0.

1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-(3-((*R*)-1-(2-(methylsulfonyl)ethyl)piperidin-3-yl)azetidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine (21). Step 1: The alkylation was performed according to general procedure C using *tert*-butyl (*R*)-3-

(piperidin-3-yl)azetidine-1-carboxylate (*R*)-11 (1.15 g, 4.79 mmol) and methyl vinyl sulfone (0.42 mL, 4.79 mmol) in dichloromethane (24 mL) at 23 °C for 30 min, then the volatiles were removed under reduced pressure to afford *tert*-butyl (*R*)-3-(1-(2-(methylsulfonyl)ethyl)piperidin-3-yl)azetidine-1-carboxylate, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{16}H_{31}N_2O_4S$ $[M+H]^+$ = 347.20; found, 347.1.

Step 2: The Boc deprotection was performed according to general procedure D. The crude *tert*-butyl (*R*)-3-(1-(2-(methylsulfonyl)ethyl)piperidin-3-yl)azetidine-1-carboxylate from step 1 was dissolved in dichloromethane (24 mL) and then HCl (5.95 mL, 23.8 mmol, 4 N in 1,4-dioxane) was added. The mixture was stirred at 23 °C for 30 min and then concentrated under reduced pressure to afford (*R*)-3-(azetidin-3-yl)-1-(2-(methylsulfonyl)ethyl)piperidine hydrochloride, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{11}H_{23}N_2O_2S$ $[M+H]^+$ = 247.15; found, 247.1.

Step 3: The S_NAr reaction was performed according to general procedure E. The crude (*R*)-3-(azetidin-3-yl)-1-(2-(methylsulfonyl)ethyl)piperidine hydrochloride (137 mg, 0.483 mmol) from step 2 was dissolved in dimethylformamide (2 mL), then (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine (150 mg, 0.439 mmol) and *N*,*N*-diisopropylethylamine (0.23 mL, 1.32 mmol) were added. The mixture was heated to 80 °C for 2 h, then cooled to 23 °C. The mixture was purified directly by reversed 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 209 mg of 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-(3-((*R*)-1-(2-(methylsulfonyl)ethyl)piperidin-3-yl)azetidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine (**21**, 81% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (s, 1H), 7.39 (d, *J* = 8.5 Hz, 1H), 7.35 (d, *J* = 2.2 Hz, 1H), 7.16 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.32 (q, *J* = 7.0 Hz, 1H), 4.24 (q, *J* = 8.7 Hz, 2H), 4.00 – 3.88 (m, 2H), 3.74 – 3.60 (m, 3H), 3.61 – 3.51 (m, 2H), 3.03 (s, 3H), 2.87 – 2.69 (m, 1H), 2.56 (s, 3H), 2.50 (d, *J* = 17.2 Hz, 1H), 2.31 –

2.18 (m, 1H), 2.07 – 1.91 (m, 5H), 1.89 (d, *J* = 7.1 Hz, 3H), 1.27 – 1.05 (m, 1H). LRMS-ESI$^+$: *m/z* calcd for $C_{25}H_{33}Cl_2N_6O_2S$ $[M+H]^+$ = 551.18; found, 551.0.

1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-(3-((*R*)-1-(3-(methylsulfonyl)propyl)piperidin-3-yl)azetidin-1-yl)-1*H*-pyrazolo[3,4-b]pyrazine (22). A mixture of the title compound and its diastereomer was prepared as follows:

Step 1: The alkylation was performed according to general procedure B using *tert*-butyl 3-(piperidin-3-yl)azetidine-1-carboxylate **11** (775 mg, 3.22 mmol), 1-bromo-3-methylsulfonyl-propane (648 mg, 3.22 mmol), sodium iodide (48 mg, 0.3200 mmol), and sodium carbonate (683 mg, 6.45 mmol) in 1,4-dioxane (10 mL) at 75 °C for 12 h. The reaction mixture was diluted with water (25 mL), extracted with ethyl acetate (3 X 30mL), the combined organic fractions were dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography using a gradient of methanol in dichloromethane (0 to 10 %) and afforded *tert*-butyl 3-(1-(3-(methylsulfonyl)propyl)piperidin-3-yl)azetidine-1-carboxylate (780 mg) as a colorless oil. LRMS-ESI$^+$: m/z calcd for $C_{17}H_{33}N_2O_4S$ $[M+H]^+$ = 361.22; found, 361.1.

Step 2: The Boc deprotection was performed according to general procedure D. *tert*-Butyl 3-(1-(3-(methylsulfonyl)propyl)piperidin-3-yl)azetidine-1-carboxylate (760 mg, 2.11 mmol) from step 1 was dissolved in methanol (1 mL) and then HCl (2 mL, 8 mmol, 4 N in 1,4-dioxane) was added. The mixture was stirred at 23 °C for 60 min and then concentrated under reduced pressure to afford 3-(azetidin-3-yl)-1-(3-(methylsulfonyl)propyl)piperidine hydrochloride, which was used in the next step without further purification. LRMS-ESI$^+$: m/z calcd for $C_{12}H_{25}N_2O_2S$ $[M+H]^+$ = 261.16; found, 261.1.

Step 3: The S_NAr reaction was performed according to general procedure E. The crude 3-(azetidin-3-yl)-1-(3-(methylsulfonyl)propyl)piperidine hydrochloride (250 mg, 0.75 mmol) from step 2 was dissolved in dimethylformamide (2 mL), then (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine **15a** (256 mg, 0.75 mmol) and *N*,*N*-diisopropylethylamine (0.65 mL, 3.75 mmol) were added. The mixture was heated to 80 °C for 2 h, then cooled to 23 °C. The mixture was purified directly by reversed 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 contain-ing 0.1% TFA, gradient elution over 30 minutes to afford 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-(3-(1-(3-(methylsulfonyl)propyl)piperidin-3-yl)azetidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine 2,2,2-trifluoroacetate as a 1:1 mixture of diastereomer. The title compound was separated from its diastereomer by SFC using an IC column (2 x 25 cm) and eluting with 40% ethanol (0.1% diethylamine) in CO2, 100 bar, to give the free base of the title compound as the second eluting isomer and converted to the corresponding HCl salt by dissolution in ethanol, cooling to 0 °C, and addition of 1 equiv. of 0.01M HCl in ethanol and afforded 56 mg 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-(3-((*R*)-1-(3-(methylsulfonyl)propyl)piperidin-3-yl)azetidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine (**22**, 25% yield). ^{1}H NMR (400 MHz, CD_3OD, HCl Salt) δ 7.74 (s, 1H), 7.44 (d, *J* = 2.1 Hz, 1H), 7.36 (d, *J* = 8.5 Hz, 1H), 7.26 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.29 (q, *J* = 7.0 Hz, 1H), 4.32 – 4.21 (m, 2H), 3.97 (dd, *J* = 9.0, 5.7 Hz, 2H), 3.50 – 3.37 (m, 2H), 3.25 (t, *J* = 7.4 Hz, 2H), 3.20 – 3.10 (m, 2H), 3.03 (s, 3H), 2.83 – 2.51 (m, 3H), 2.50 (s, 3H), 2.32 – 2.17 (m, 2H), 2.13

– 1.90 (m, 3H), 1.86 (d, *J* = 7.1 Hz, 3H), 1.83 – 1.67 (m, 1H), 1.25 – 1.11 (m, 1H). LRMS-ESI$^+$: m/z calcd for $C_{26}H_{35}Cl_2N_6O_2S$ $[M+H]^+$ = 565.19; found, 565.0.

4-((*R*)-3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)butanoic acid (23). Step 1: The alkylation was performed according to general procedure A(a) using *tert*-butyl (*R*)-3-(piperidin-3-yl)azetidine-1-carboxylate (*R*)-11 (200 mg, 0.83 mmol) and methyl 4-oxobutanoate (96 mg, 0.83 mmol) in 1,2-dichloroethane (5 mL) at 23 °C for 1 hr. Upon completion, the reaction was quenched with saturated aqueous sodium bicarbonate solution and extracted with dichloromethane. The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure to afford the desired product, which was purified by silica gel chromatography using 0 to 5% methanol/dichloromethane. Then the solvent was removed under reduced pressure to afford 234 mg (83%) tert-butyl (*R*)-3-(1-(4-methoxy-4-oxobutyl)piperidin-3-yl)azetidine-1-carboxylate, which was used in the next step. LRMS-ESI$^+$: *m/z* calcd for $C_{18}H_{32}N_2O_4$ $[M+H]^+$ = 341.24; found, 341.15.

Step 2: The Boc deprotection was performed according to general procedure D, where 234 mg (0.69 mmol) of *tert*-butyl (*R*)-3-(1-(4-methoxy-4-oxobutyl)piperidin-3-yl)azetidine-1-carboxylate from step 1 was dissolved in dichloromethane (5 mL) and then HCl (5 mL, 4 N in 1,4-dioxane) was added. The mixture was stirred at 23 °C for 30 min and then concentrated under reduced pressure to afford 190 mg methyl (*R*)-4-(3-(azetidin-3-yl)piperidin-1-yl)butanoate hydrochloride, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{13}H_{24}N_2O_2$ $[M+H]^+$ = 241.19; found, 241.14.

Step 3: The S_NAr reaction was performed according to general procedure E. The crude (*R*)-4-(3-(azetidin-3-yl)piperidin-1-yl)butanoate hydrochloride (85 mg, 0.31 mmol) from step 2 was dissolved in anhydrous dimethylsulfoxide (2 mL), then (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine **15a** (106 mg, 0.31 mmol) and *N*,*N*-diisopropylethylamine (0.22 mL, 1.24 mmol) were added. The mixture was heated to 80 °C for 16 h, then cooled to 23 °C. The mixture was purified directly by reversed 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 methyl 4-((*R*)-3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)butanoic acid (84 mg, 50% yield).

Step 4: Methyl 4-((*R*)-3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)butanoic acid (84mg, 0.15 mmol) was dissolved in 2 mL of methanol and lithium hydroxide solution in water (18mg, 0.75 mmol in 0.5 mL of water) was added. The mixture was stirred for 16 hours at 23 °C and then then all solvent was removed in vacuo to dryness. Mixture of 2mL of acetonitrile and 1mL of 3M HCl was added to the residue and the solution was directly injected on reversed 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. 4-((*R*)-3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)butanoic acid (**23**) was obtained in 90% yield (71mg). ^{1}H NMR (400 MHz, Acetonitrile-d_3, TFA salt) δ 8.03 (bs, 1H), 7.73 (s, 1H), 7.47 (d, 1H, J = 2.2Hz), 7.41 (d, 1H, J = 8.5Hz), 7.26 (dd, 1H, J = 2.2Hz, J = 8.5Hz), 6.25 (q, 1H, J = 7.1Hz), 4.19 (td, 2H, J = 8.5Hz, J = 15.4Hz), 3.90 (dt, 2H, J = 5.7Hz, J = 9.3Hz), 3.50 (dd, 2H, J = 12.4Hz, J = 24.1Hz), 3.12 – 3.04 (m, 2H), 2.84 – 2.72 (m, 1H), 2.66 – 2.49 (m, 2H), 2.45 (s, 3H), 2.44 (t, 2H, J = 6.8Hz), 2.16 – 2.04 (m, 1H), 2.03 – 1.68 (m, 8H), 1.13 (dq, 1H, J = 4.0Hz, J = 12.9Hz) ppm. LRMS-ESI$^+$: *m/z* calcd for $C_{26}H_{32}Cl_2N_6O_2$ $[M+H]^+$ = 531.20; found, 531.1.

3-((*R*)-3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)propanoic acid (24). A mixture of the title compound and its diastereomer was prepared as follows:

Step 1: The alkylation was performed according to general procedure C using *tert*-butyl 3-(piperidin-3-yl)azetidine-1-carboxylate **11** (420 mg, 1.75 mmol) and *tert*-butyl acrylate (0.77 mL, 5.25 mmol) in dimethylformamide (2 mL) at 50 °C for 3 h, then the volatiles were removed under reduced pressure to afford *tert*-butyl 3-(1-(3-(*tert*-butoxy)-3-oxopropyl)piperidin-3-yl)azetidine-1-carboxylate, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{20}H_{37}N_2O_4$ $[M+H]^+$ = 369.28; found, 369.2.

Step 2: The Boc deprotection was performed according to general procedure D. The crude *tert*-butyl 3-(1-(3-(*tert*-butoxy)-3-oxopropyl)piperidin-3-yl)azetidine-1-carboxylate from step 1 was dissolved in dichloromethane (5 mL) and then HCl (6 mL, 24 mmol, 4 N in 1,4-dioxane) was added. The mixture was stirred at 23 °C for 3 h and then concentrated under reduced pressure to afford 3-(3-(azetidin-3-yl)piperidin-1-yl)propanoic acid

hydrochloride, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{11}H_{21}N_2O_2$ $[M+H]^+$ = 213.16; found, 213.2.

Step 3: The S_NAr reaction was performed according to general procedure E. The crude 3-(3-(azetidin-3-yl)piperidin-1-yl)propanoic acid hydrochloride from step 2 was dissolved in dimethyl sulfoxide (4 mL), then (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine **15a** (526 mg, 1.54 mmol) and *N*,*N*-diisopropylethylamine (1.1 mL, 6.16 mmol) were added. The mixture was heated to 80 °C for 12 h, then cooled to 23 °C. The mixture was purified directly by reversed 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 3-(3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)propanoic acid 2,2,2-trifluoroacetate as a 1:1 mixture of. The title compound was separated from its diastereomer by SFC using an AD-H column (2 x 25 cm) and eluting with 40% isopropanol (0.1% diethylamine) in CO_2, 100 bar, to afford 159 mg of 3-((*R*)-3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-

pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)propanoic acid (**24**, 40% yield) as the first eluting isomer. ^{1}H NMR (400 MHz, CD_3OD; free base) δ 7.73 (s, 1H), 7.44 (d, *J* = 2.1 Hz, 1H), 7.36 (d, *J* = 8.5 Hz, 1H), 7.25 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.28 (q, *J* = 7.1 Hz, 1H), 4.25 (q, *J* = 9.0 Hz, 2H), 4.01 – 3.91 (m, 2H), 3.42 – 3.33 (m, 2H), 3.20 – 3.09 (m, 2H), 2.76 – 2.60 (m, 2H), 2.53 (t, *J* = 6.9 Hz, 2H), 2.49 (s, 3H), 2.11 – 1.98 (m, 1H), 1.99 – 1.88 (m, 3H), 1.86 (d, *J* = 7.1 Hz, 3H), 1.81 – 1.66 (m, 1H), 1.24 – 1.09 (m, 1H). LRMS-ESI$^+$: *m/z* calcd for $C_{25}H_{31}Cl_2N_6O_2$ $[M+H]^+$ = 517.19; found, 517.1.

3-((*R*)-3-(1-(3-cyano-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)propanoic acid (25). Step 1: The alkylation was performed according to general procedure C using *tert*-butyl 3-[(3*R*)-3-piperidyl]azetidine-1-carboxylate **(*R*)-11** (3 g, 7.64 mmol) and ethyl acrylate (2.5 mL, 22.9 mmol) in dimethylformamide (10 mL) at 65 °C for 3 h, then the volatiles were removed under reduced pressure to afford *tert*-butyl 3-[(3*R*)-1-(3-ethoxy-3-oxo-propyl)-3-piperidyl]azetidine-1-carboxylate, which was used in the next step without further purification. LRMS-ESI$^+$: m/z calcd for $C_{18}H_{33}N_2O_4$ $[M+H]^+$ = 341.24; found, 341.1.

Step 2: The Boc deprotection was performed according to general procedure D. *tert*-Butyl 3-[(3*R*)-1-(3-ethoxy-3-oxo-propyl)-3-piperidyl]azetidine-1-carboxylate (200 mg, 0.587 mmol) from step 1 was dissolved in methanol (3 mL) and then HCl (5 mL, 20 mmol, 4 N in 1,4-dioxane) was added. The mixture was stirred at 23 °C for 3 h and then concentrated under reduced pressure. The residue was dissolved in a mixture of THF:water:ethanol (3:1:1, 5 mL), then lithium hydroxide was added (94 mg, 4.11 mmol) and the mixture was stirred at 23 °C for 24 h, then acidified to pH 4 with HCl (4 N in 1,4-dioxane). The volatiles were removed under reduced pressure to afford 3-[(3*R*)-3-(azetidin-3-yl)-1-piperidyl]propanoic acid hydrochloride, which was used in the next step without further purification. LRMS-ESI$^+$: m/z calcd for $C_{11}H_{21}N_2O_2$ $[M+H]^+$ = 213.16; found, 213.2.

Step 3: The S_NAr reaction was performed according to general procedure E. The crude 3-[(3*R*)-3-(azetidin-3-yl)-1-piperidyl]propanoic acid hydrochloride from step 2 was dissolved in dimethyl sulfoxide (1.5 mL), then 6-chloro-1-[(1*R*)-1-(2,4-dichlorophenyl)ethyl]pyrazolo[3,4-*b*]pyrazine-3-carbonitrile **15b** (207 mg, 0.587 mmol)

and *N*,*N*-diisopropylethylamine (0.307 mL, 1.76 mmol) were added. The mixture was heated to 50 °C for 12 h, then cooled to 23 °C. The mixture was purified directly by reversed 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 169 mg of 3-((*R*)-3-(1-(3-cyano-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)propanoic acid (**25**, 32% yield). ^{1}H NMR (400 MHz, Acetone-d_6, TFA Salt) δ 11.39 (bs, 1H), 8.00 (s, 1H), 7.51 (d, *J* = 2.1 Hz, 1H), 7.49 (d, *J* = 8.5 Hz, 1H), 7.38 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.46 (q, *J* = 7.0 Hz, 1H), 4.49 – 4.26 (m, 2H), 4.22 – 4.10 (m, 2H), 3.84 – 3.63 (m, 2H), 3.56 – 3.40 (m, 2H), 3.12 – 2.94 (m, 2H), 2.90 – 2.69 (m, 2H), 2.49 – 2.29 (m, 1H), 2.04 – 1.94 (m, 4H), 1.92 (d, *J* = 7.1 Hz, 3H), 1.29 (s, 1H). LRMS-ESI$^+$: m/z calcd for $C_{25}H_{28}Cl_2N_7O_2$ $[M+H]^+$ = 528.17; found, 528.0.

2-((*R*)-3-(1-(3-cyano-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)acetic acid (26). Step 1: The reductive amination was performed according to general procedure A using *tert*-butyl 3-[(3*R*)-3-piperidyl]azetidine-

1-carboxylate **(*R*)-11** (1.72 g, 7.16 mmol), ethyl glyoxalate (1.35 mL, 8.59 mmol, 50% in toluene) and sodium triacetoxyborohydride (3.79 g, 17.9 mmol) in 1,2-dichloroethane (35 mL) at 23 °C for 1 h. The reaction was quenched with saturated aqueous sodium bicarbonate solution and extracted with dichloromethane. The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure and afforded *tert*-butyl (*R*)-3-(1-(2-ethoxy-2-oxoethyl)piperidin-3-yl)azetidine-1-carboxylate (99 % yield). The crude was used in the next step without further purification LRMS-ESI$^+$: m/z calcd for $C_{17}H_{31}N_2O_2$ $[M+H]^+$ = 327.23; found, 327.2.

Step 2: The Boc deprotection was performed according to general procedure D. *tert*-butyl (*R*)-3-(1-(2-ethoxy-2-oxoethyl)piperidin-3-yl)azetidine-1-carboxylate (200 mg, 0.613 mmol) from step 1 was dissolved in dichloromethane (3 mL) and then HCl (0.76 mL, 3.06 mmol, 4 N in 1,4-dioxane) was added. The mixture was stirred at 23 °C for 4 h and then concentrated under reduced pressure. The residue was dissolved in a mixture of 1,4-dioxane:water (3:1, 4 mL), then lithium hydroxide was added (44 mg, 1.84 mmol) and the mixture was stirred at 50 °C for 1 h, then acidified to pH 4 with HCl (4 N in 1,4-dioxane).

The volatiles were removed under reduced pressure to afford (*R*)-2-(3-(azetidin-3-yl)piperidin-1-yl)acetic acid hydrochloride, which was used in the next step without further purification. LRMS-ESI$^+$: m/z calcd for $C_{12}H_{23}N_2O_2$ $[M+H]^+$ = 199.14; found, 199.1.

Step 3: The S_NAr reaction was performed according to general procedure E. (*R*)-2-(3-(azetidin-3-yl)piperidin-1-yl)acetic acid hydrochloride (129 mg, 0.55 mmol) was dissolved in dichloromethane (2.6 mL), then 6-chloro-1-[(1*R*)-1-(2,4-dichlorophenyl)ethyl]pyrazolo[3,4-*b*]pyrazine-3-carbonitrile **15b** (178 mg, 0.51 mmol) and *N*,*N*-diisopropylethylamine (0.26 mL, 1.52 mmol) were added. The mixture was stirred at 23 °C for 2 h, then concentrated under reduced pressure. The mixture was purified directly by reversed 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 2-((*R*)-3-(1-(3-cyano-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)acetic acid (**26**, 99% yield). The title compound was converted to the corresponding besylate salt by dissolution in dichloromethane, cooling to 0 °C, and addition of 1 equiv.

of benzenesulfonic acid in dichloromethane followed by concentration under reduced pressure. 1H NMR (400 MHz, CD_3OD, Besylate salt) δ 7.95 (s, 1H), 7.87 – 7.80 (m, 2H), 7.48 (d, *J* = 2.1 Hz, 1H), 7.44 – 7.39 (m, 3H), 7.37 (d, *J* = 8.5 Hz, 1H), 7.32 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.46 (q, *J* = 7.0 Hz, 1H), 4.40 – 4.19 (m, 2H), 4.04 (s, 2H), 4.03 – 3.95 (m, 2H), 3.76 – 3.50 (m, 2H), 3.10 – 2.94 (m, 1H), 2.90 – 2.75 (m, 1H), 2.75 – 2.61 (m, 1H), 2.30 – 2.13 (m, 1H), 2.08 – 1.93 (m, 2H), 1.91 (d, *J* = 7.0 Hz, 3H), 1.88 – 1.78 (m, 1H), 1.33 – 1.09 (m, 1H). LRMS-ESI$^+$: m/z calcd for $C_{24}H_{26}Cl_2N_7O_2$ $[M+H]^+$ = 514.15; found, 514.0.

3-((*R*)-3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)propanamide (27). A mixture of the title compound and its diastereomer was prepared as follows:

Step 1: The alkylation was performed according to general procedure C using *tert*-butyl 3-(piperidin-3-yl)azetidine-1-carboxylate **11** (200 mg, 0.83 mmol) and *tert*-butyl acrylamide (148 mg, 2.08 mmol) in dimethylformamide (1 mL) at 50 °C for 3 h, then the volatiles were removed under reduced pressure and the residue was purified by silica gel

chromatography using a gradient of methanol in dichloromethane (0 to 20 %) and afforded *tert*-butyl 3-(1-(3-amino-3-oxopropyl)piperidin-3-yl)azetidine-1-carboxylate (96 % yiled) . LRMS-ESI$^+$: *m/z* calcd for $C_{16}H_{30}N_3O_3$ $[M+H]^+$ = 312.23; found, 312.2.

Step 2: The Boc deprotection was performed according to general procedure D. *Tert*-butyl 3-(1-(3-amino-3-oxopropyl)piperidin-3-yl)azetidine-1-carboxylate (249 mg, 0.8 mmol) from step 1 was dissolved in dichloromethane (4 mL) and then trifluoroacetic acid (1 mL, 13.07 mmol) was added. The mixture was stirred at 23 °C for 8 h and then concentrated under reduced pressure to afford 3-(3-(azetidin-3-yl)piperidin-1-yl)propanamide 2,2,2-trifluoroacetate, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{11}H_{22}N_3O$ $[M+H]^+$ = 212.18; found, 212.2.

Step 3: The S_NAr reaction was performed according to general procedure E. The crude 3-(3-(azetidin-3-yl)piperidin-1-yl)propanamide 2,2,2-trifluoroacetate from step 2 was dissolved in dimethyl sulfoxide (2 mL), then (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine **15a** (273 mg, 0.80 mmol) and *N,N*-diisopropylethylamine (0.56 mL, 3.2 mmol) were added. The mixture was heated to 80 °C

for 12 h, then cooled to 23 °C. The mixture was purified directly by reversed 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 3-(3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)propanamide 2,2,2-trifluoroacetate as a 1:1 mixture of diastereomers. The mixture of diastereomers was basified using a basification column (PL-HCO3MP SPE, 500 mg per 5 mL tube, Agilent) using dichloromethane and methanol (4:1) as eluent and concentrated under reduced pressure. The residue was separated by preparative chiral HPLC using a CHIRALPAK® ID column (Daicel Corporation, West Chester, PA) and 20% ethanol in heptanes (0.1% diethylamine) as eluent and afforded 42 mg of 3-((*R*)-3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)propenamide (**27,** 20% yield) as the second eluting isomer. ^{1}H NMR (400 MHz, $CDCl_3$; free base) δ 8.09 (bs, 1H), 7.64 (s, 1H), 7.37 (d, *J* = 8.4 Hz, 1H), 7.34 (d, *J* = 2.1 Hz, 1H), 7.12 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.31 (q, *J* = 7.1 Hz, 1H), 5.54 (bs, 1H), 4.25 – 4.11 (m, 2H),

3.91 – 3.77 (m, 2H), 2.89 (t, *J* = 12.3 Hz, 2H), 2.68 – 2.56 (m, 3H), 2.56 (s, 3H), 2.46 – 2.37 (m, 2H), 2.08 – 1.95 (m, 1H), 1.88 (d, *J* = 7.1 Hz, 3H), 1.86 – 1.65 (m, 4H), 1.63 – 1.49 (m, 1H), 1.03 – 0.85 (m, 1H). LRMS-ESI$^+$: *m/z* calcd for $C_{25}H_{32}Cl_2N_7O$ $[M+H]^+$ = 516.20; found, 516.0.

_N_-(2-((_R_)-3-(1-(1-((_R_)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1_H_-pyrazolo[3,4-_b_]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)ethyl)acetamide (28).

Step 1: The reductive amination was performed according to general procedure A using *tert*-butyl 3-(piperidin-3-yl)azetidine-1-carboxylate **11** or enantioenriched **(*R*)-11** (300 mg, 1.25 mmol), 3-(1,3-dioxoisoindolin-2-yl)propanal (236 mg, 1.25 mmol, 50% in toluene) and sodium triacetoxyborohydride (529 mg, 2.5 mmol) in 1,2-dichloroethane (5 mL) at 23 °C for 15 min. The reaction was quenched with saturated aqueous sodium bicarbonate solution and extracted with dichloromethane. The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was dissolved in methanol (5 mL) and then hydrazine hydrate (0.24 mL, 5.0 mmol) was added. The mixture was stirred at 23 °C for 18 h and then mixture was diluted with water and

extracted with dichloromethane. The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude *tert*-butyl 3-[1-(2-aminoethyl)-3-piperidyl]azetidine-1-carboxylate (120 mg, 0.423 mmol) was dissolved in dichloromethane (2 mL) and then with triethylamine (0.18 mL, 1.27 mmol) and acetyl chloride (36 μL, 0.508 mmol) were added. The mixture was stirred at 23 °C for 30 min, then quenched with aqueous sodium carbonate (10 mL, 1M) and extracted with ethyl acetate (3 x 10 mL). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressured and afforded *tert*-butyl 3-(1-(2-acetamidoethyl)piperidin-3-yl)azetidine-1-carboxylate, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{17}H_{32}N_3O_3$ $[M+H]^+$ = 326.24; found, 326.2.

Step 2: The Boc deprotection was performed according to general procedure D. The crude *tert*-butyl 3-(1-(2-acetamidoethyl)piperidin-3-yl)azetidine-1-carboxylate from step 1 was dissolved in dichloromethane (4 mL) and then HCl (0.50 mL, 2.0 mmol, 4 N in 1,4-dioxane) was added. The mixture was stirred at 23 °C for 1 h and then concentrated under

reduced pressure to afford *N*-(2-(3-(azetidin-3-yl)piperidin-1-yl)ethyl)acetamide hydrochloride, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{12}H_{24}N_3O$ $[M+H]^+$ = 226.19; found, 226.1.

Step 3: The S_NAr reaction was performed according to general procedure E. The crude *N*-(2-(3-(azetidin-3-yl)piperidin-1-yl)ethyl)acetamide hydrochloride from step 2 was dissolved in dimethylformamide (1 mL), then (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine **15a** (72 mg, 0.21 mmol) and *N*,*N*-diisopropylethylamine (0.18 mL, 1.05 mmol) were added. The mixture was heated to 80 °C for 4 h, then cooled to 23 °C. The mixture was purified directly by reversed 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 *N*-(2-(3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1H-pyrazolo[3,4-b]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)ethyl)acetamide 2,2,2-trifluoroacetate as a 1:1 mixture of diastereomers (if racemic **11** was used). The title compound was separated from its diastereomer by SFC using an AD-H column (2 x 25

cm) and eluting with 25% ethanol (0.1% diethylamine) in CO_2, 100 bar, to give the free base of the title compound as the first eluting isomer and converted to the corresponding HCl salt by dissolution in ethanol, cooling to 0 °C, and addition of 1 equiv. of 0.01M HCl in ethanol and afforded 11 mg of *N*-(2-((*R*)-3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)ethyl)acetamide (**28**, 19% yield). ^{1}H NMR (400 MHz, CD_3OD; HCl Salt) δ 7.75 (s, 1H), 7.44 (d, *J* = 2.1 Hz, 1H), 7.36 (d, *J* = 8.5 Hz, 1H), 7.26 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.29 (q, *J* = 7.1 Hz, 1H), 4.27 (q, *J* = 9.0 Hz, 2H), 4.06 – 3.94 (m, 2H), 3.79 – 3.61 (m, 2H), 3.58 – 3.48 (m, 1H), 3.25 (t, *J* = 5.8 Hz, 2H), 2.99 – 2.86 (m, 1H), 2.72 – 2.62 (m, 2H), 2.50 (s, 3H), 2.15 – 1.85 (m, 4H), 1.99 (s, 3H), 1.86 (d, *J* = 7.1 Hz, 3H), 1.85 – 1.77 (m, 1H), 1.27 – 1.19 (m, 1H). LRMS-ESI$^+$: *m/z* calcd for $C_{26}H_{34}Cl_2N_7O$ $[M+H]^+$ = 530.22; found, 530.1.

N-(2-((*R*)-3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)ethyl)isobutyramide (29). A mixture of the title compound and its diastereomer was prepared as follows:

Step 1: The reductive amination was performed according to general procedure A using *tert*-butyl 3-(piperidin-3-yl)azetidine-1-carboxylate **11** (300 mg, 1.25 mmol), 3-(1,3-dioxoisoindolin-2-yl)propanal (236 mg, 1.25 mmol, 50% in toluene) and sodium triacetoxyborohydride (529 mg, 2.5 mmol) in 1,2-dichloroethane (5 mL) at 23 °C for 15 min. The reaction was quenched with saturated aqueous sodium bicarbonate solution and extracted with dichloromethane. The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was dissolved in methanol (5 mL) and then hydrazine hydrate (0.24 mL, 5.0 mmol) was added. The mixture was stirred at 23 °C for 18 h and then mixture was diluted with water and extracted with dichloromethane. The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude *tert*-butyl 3-[1-(2-aminoethyl)-3-piperidyl]azetidine-1-carboxylate (120 mg, 0.423 mmol) was dissolved in dimethylformamide (2 mL) and then with triethylamine (0.18 mL, 1.27 mmol), isobutyric acid (46 μL, 0.508 mmol) and HATU (193 mg, 0.508 mmol) were added. The mixture was stirred at 23 °C for 30 min, then quenched with aqueous sodium carbonate (10 mL, 1M)

and extracted with ethyl acetate (3 x 10 mL). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressured and afforded *tert*-butyl 3-(1-(2-isobutyramidoethyl)piperidin-3-yl)azetidine-1-carboxylate, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{19}H_{36}N_3O_3$ $[M+H]^+$ = 354.28; found, 354.2.

Step 2: The Boc deprotection was performed according to general procedure D. The crude *tert*-butyl 3-(1-(2-isobutyramidoethyl)piperidin-3-yl)azetidine-1-carboxylate from step 1 was dissolved in dichloromethane (4 mL) and then HCl (0.50 mL, 2.0 mmol, 4 N in 1,4-dioxane) was added. The mixture was stirred at 23 °C for 1 h and then concentrated under reduced pressure to afford *N*-(2-(3-(azetidin-3-yl)piperidin-1-yl)ethyl)isobutyramide hydrochloride, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{14}H_{28}N_3O$ $[M+H]^+$ = 254.22; found, 254.1.

Step 3: The S_NAr reaction was performed according to general procedure E. The crude *N*-(2-(3-(azetidin-3-yl)piperidin-1-yl)ethyl)isobutyramide hydrochloride from step 2 was dissolved in dimethylformamide (1 mL), then (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-

3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine **15a** (72 mg, 0.21 mmol) and *N,N*-diisopropylethylamine (0.18 mL, 1.05 mmol) were added. The mixture was heated to 80 °C for 4 h, then cooled to 23 °C. The mixture was purified directly by reversed 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 *N*-(2-(3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)ethyl)isobutyramide 2,2,2-trifluoroacetate as a 1:1 mixture of diastereomers. The title compound was separated from its diastereomer by SFC using an AD-H column (2 x 25 cm) and eluting with 25% ethanol (0.1% diethylamine) in CO_2, 100 bar, to give the free base of the title compound as the first eluting isomer and converted to the corresponding HCl salt by dissolution in ethanol, cooling to 0 °C, and addition of 1 equiv. of 0.01M HCl in ethanol and afforded 12 mg (20% yield) of *N*-(2-((*R*)-3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)ethyl)isobutyramide (**29**). ^{1}H NMR (400 MHz, CD_3OD; HCl Salt) δ 7.75 (s, 1H), 7.44 (d, *J* = 2.1 Hz, 1H), 7.35

(d, *J* = 8.4 Hz, 1H), 7.25 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.29 (q, *J* = 7.1 Hz, 1H), 4.33 – 4.21 (m, 2H), 4.05 – 3.92 (m, 2H), 3.73 – 3.55 (m, 4H), 3.28 – 3.20 (m, 2H), 3.01 – 2.87 (m, 1H), 2.76 – 2.62 (m, 2H), 2.50 (s, 3H), 2.22 – 1.88 (m, 5H), 1.86 (d, *J* = 7.1 Hz, 3H), 1.32 – 1.18 (m, 1H), 1.13 (d, *J* = 6.9 Hz, 6H). LRMS-ESI$^+$: *m/z* calcd for $C_{28}H_{38}Cl_2N_7O$ $[M+H]^+$ = 558.25; found, 558.1.

Methyl (2-((*R*)-3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)ethyl)carbamate (30). A mixture of the title compound and its diastereomer was prepared as follows:

Step 1: The reductive amination was performed according to general procedure A using *tert*-butyl 3-(piperidin-3-yl)azetidine-1-carboxylate **11** (300 mg, 1.25 mmol), 3-(1,3-dioxoisoindolin-2-yl)propanal (236 mg, 1.25 mmol, 50% in toluene) and sodium triacetoxyborohydride (529 mg, 2.5 mmol) in 1,2-dichloroethane (5 mL) at 23 °C for 15 min. The reaction was quenched with saturated aqueous sodium bicarbonate solution and extracted with dichloromethane. The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was

dissolved in methanol (5 mL) and then hydrazine hydrate (0.24 mL, 5.0 mmol) was added. The mixture was stirred at 23 °C for 18 h and then mixture was diluted with water and extracted with dichloromethane. The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude *tert*-butyl 3-[1-(2-aminoethyl)-3-piperidyl]azetidine-1-carboxylate (120 mg, 0.423 mmol) was dissolved in dichloromethane (2 mL) and then with triethylamine (0.18 mL, 1.27 mmol) and methyl chloroformate (39 μL, 0.508 mmol) were added. The mixture was stirred at 23 °C for 30 min, then quenched with aqueous sodium carbonate (10 mL, 1M) and extracted with ethyl acetate (3 x 10 mL). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressured and afforded *tert*-butyl 3-(1-(2-((methoxycarbonyl)amino)ethyl)piperidin-3-yl)azetidine-1-carboxylate, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{17}H_{32}N_3O_4$ $[M+H]^+$ = 342.24; found, 342.1.

Step 2: The Boc deprotection was performed according to general procedure D. The crude *tert*-butyl 3-(1-(2-((methoxycarbonyl)amino)ethyl)piperidin-3-yl)azetidine-1-

carboxylate from step 1 was dissolved in dichloromethane (4 mL) and then HCl (0.50 mL, 2.0 mmol, 4 N in 1,4-dioxane) was added. The mixture was stirred at 23 °C for 1 h and then concentrated under reduced pressure to afford methyl (2-(3-(azetidin-3-yl)piperidin-1-yl)ethyl)carbamate hydrochloride, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{12}H_{24}N_3O_2$ $[M+H]^+$ = 242.19; found, 242.2.

Step 3: The S_NAr reaction was performed according to general procedure E. The crude methyl (2-(3-(azetidin-3-yl)piperidin-1-yl)ethyl)carbamate hydrochloride from step 2 was dissolved in dimethylformamide (1 mL), then (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine **15a** (72 mg, 0.21 mmol) and *N*,*N*-diisopropylethylamine (0.18 mL, 1.05 mmol) were added. The mixture was heated to 80 °C for 4 h, then cooled to 23 °C. The mixture was purified directly by reversed 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 methyl (2-(3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-

yl)ethyl)carbamate 2,2,2-trifluoroacetate as a 1:1 mixture of diastereomers. The title compound was separated from its diastereomer by SFC using an AD-H column (2 x 25 cm) and eluting with 35% isopropanol (0.1% diethylamine) in CO_2, 100 bar, to give the free base of the title compound as the first eluting isomer and converted to the corresponding HCl salt by dissolution in ethanol, cooling to 0 °C, and addition of 1 equiv. of 0.01M HCl in ethanol and afforded 9 mg of methyl (2-((*R*)-3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)ethyl)carbamate (**30**, 55% yield). ^{1}H NMR (400 MHz, CD_3OD; HCl Salt) δ 7.74 (s, 1H), 7.44 (d, *J* = 2.1 Hz, 1H), 7.36 (d, *J* = 8.5 Hz, 1H), 7.25 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.29 (q, *J* = 7.1 Hz, 1H), 4.33 – 4.22 (m, 2H), 4.03 – 3.94 (m, 2H), 3.67 (s, 3H), 3.64 – 3.42 (m, 2H), 3.19 (d, *J* = 11.5 Hz, 3H), 2.86 (s, 1H), 2.72 – 2.55 (m, 2H), 2.50 (s, 3H), 2.16 – 1.90 (m, 4H), 1.86 (d, *J* = 7.1 Hz, 3H), 1.85 – 1.75 (m, 1H), 1.34 – 1.20 (m, 1H). LRMS-ESI$^+$: *m/z* calcd for $C_{26}H_{34}Cl_2N_7O$ $[M+H]^+$ = 546.22; found, 546.1.

6-(3-((*R*)-1-((1*H*-imidazol-5-yl)methyl)piperidin-3-yl)azetidin-1-yl)-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (31). A mixture of the title compound and its diastereomer was prepared as follows:

Step 1: The reductive amination was performed according to general procedure A using *tert*-butyl 3-(piperidin-3-yl)azetidine-1-carboxylate **11** (200 mg, 0.83 mmol), 1H-imidazole-5-carbaldehyde (120 mg, 1.25 mmol) and sodium triacetoxyborohydride (265 mg, 1.25 mmol) in 1,2-dichloroethane (2 mL) at 23 °C for 1 h. The reaction was quenched with saturated aqueous sodium bicarbonate solution and extracted with dichloromethane. The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure and afforded *tert*-butyl 3-(1-((1H-imidazol-5-yl)methyl)piperidin-3-yl)azetidine-1-carboxylate. The crude was used in the next step without further purification LRMS-ESI$^+$: m/z calcd for $C_{17}H_{29}N_4O_2$ $[M+H]^+$ = 321.23; found, 321.1.

Step 2: The Boc deprotection was performed according to general procedure D. The crude *tert*-butyl 3-(1-((1*H*-imidazol-5-yl)methyl)piperidin-3-yl)azetidine-1-carboxylate from step 1 was dissolved in methanol (1 mL) and then HCl (2 mL, 4 mmol, 4 N in 1,4-

dioxane) was added. The mixture was stirred at 23 °C for 4 h and then concentrated under reduced pressure to afford 1-((1*H*-imidazol-5-yl)methyl)-3-(azetidin-3-yl)piperidine dihydrochloride, which was used in the next step without further purification. LRMS-ESI$^+$: m/z calcd for $C_{12}H_{21}N_4$ $[M+H]^+$ = 221.1; found, 221.1.

Step 3: The S_NAr reaction was performed according to general procedure E. 1-((1*H*-imidazol-5-yl)methyl)-3-(azetidin-3-yl)piperidine dihydrochloride (243 mg, 0.83 mmol) was dissolved in dichloromethane (2 mL), then 6-chloro-1-[(1*R*)-1-(2,4-dichlorophenyl)ethyl]pyrazolo[3,4-*b*]pyrazine-3-carbonitrile **15b** (293 mg, 0.83 mmol) and *N*,*N*-diisopropylethylamine (0.5 mL, 2.91 mmol) were added. The mixture was stirred at 23 °C for 2 h, then concentrated under reduced pressure. The mixture was purified directly by reversed 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 contain-ing 0.1% TFA, gradient elution over 30 minutes to afford 6-(3-(1-((1H-imidazol-5-yl)methyl)piperidin-3-yl)azetidin-1-yl)-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile 2,2,2-trifluoroacetate as a 1:1 mixture of

diastereomers. The title compound was separated from its diastereomer by SFC using an AD-H column (2 x 25 cm) and eluting with 30% isopropanol (0.1% diethylamine) in CO2, 100 bar, to give 76 mg 6-(3-((*R*)-1-((1*H*-imidazol-5-yl)methyl)piperidin-3-yl)azetidin-1-yl)-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**31**, 35% yield) as the second eluting isomer. ^{1}H NMR (400 MHz, CD_3OD, Free base) δ 7.93 (s, 1H), 7.63 (d, *J* = 1.2 Hz, 1H), 7.48 (d, *J* = 2.1 Hz, 1H), 7.38 (d, *J* = 8.5 Hz, 1H), 7.31 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.00 (s, 1H), 6.45 (q, *J* = 7.0 Hz, 1H), 4.31 – 4.17 (m, 2H), 4.00 – 3.84 (m, 2H), 3.57 (s, 2H), 2.96 – 2.84 (m, 2H), 2.66 – 2.53 (m, 1H), 2.11 – 2.01 (m, 1H), 1.90 (d, *J* = 7.0 Hz, 3H), 1.86 – 1.69 (m, 4H), 1.65 – 1.51 (m, 1H), 0.96 – 0.83 (m, 1H). LRMS-ESI$^+$: m/z calcd for $C_{26}H_{28}Cl_2N_9$ $[M+H]^+$ = 536.18; found, 536.0.

6-(3-((*R*)-1-(cyanomethyl)piperidin-3-yl)azetidin-1-yl)-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1H-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (32). Step 1: The alkylation was performed according to general procedure B using *tert*-butyl (*R*)-3-(piperidin-3-yl)azetidine-1-carboxylate **(*R*)-11** (275 mg, 1.14 mmol), 2-bromoacetonitrile (192 mg, 1.6 mmol), potassium iodide (4 mg, 0.02 mmol), and potassium carbonate (316 mg, 2.29 mmol) in

acetonitrile (4.4 mL) at 90 °C for 12 h. The reaction mixture was diluted with water (20 mL), extracted with ethyl acetate (3X), the combined organic fractions were dried over sodium sulfate, filtered and concentrated under reduced pressure and *tert*-butyl (*R*)-3-(1-(cyanomethyl)piperidin-3-yl)azetidine-1-carboxylate was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{15}H_{25}N_3O_2$ $[M+H]^+$ = 280.20; found, 280.2.

Step 2: The Boc deprotection was performed according to general procedure D. *Tert*-butyl (*R*)-3-(1-(cyanomethyl)piperidin-3-yl)azetidine-1-carboxylate (300 mg, 1.07 mmol) from step 1 was dissolved in dichloromethane (10 mL) and then HCl (2 mL, 8 mmol, 4 N in 1,4-dioxane) was added. The mixture was stirred at 23 °C for 3 h and then concentrated under reduced pressure to afford (*R*)-2-(3-(azetidin-3-yl)piperidin-1-yl)acetonitrile hydrochloride, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{10}H_{17}N_3$ $[M+H]^+$ = 180.15; found, 180.2.

Step 3: The S_NAr reaction was performed according to general procedure E. The crude (*R*)-2-(3-(azetidin-3-yl)piperidin-1-yl)acetonitrile hydrochloride from step 2 was dissolved in dichloromethane (1.2 mL), then (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-

pyrazolo[3,4-*b*]pyrazine-3-carbonitrile **15b** (120 mg, 0.34 mmol) and *N,N*-diisopropylethylamine (0.30 mL, 1.70 mmol) were added. The mixture was stirred at 23 °C for 12 h. The reaction was diluted with dichloromethane and washed with saturated aqueous sodium bicarbonate solution. The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure and the residue was purified by reversed 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 97 mg of 6-(3-((*R*)-1-(cyanomethyl)piperidin-3-yl)azetidin-1-yl)-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile 2,2,2-trifluoroacetate (**32**, 59% yield). ^{1}H NMR (400 MHz, CD_3OD; TFA Salt) δ 7.95 (s, 1H), 7.48 (d, *J* = 2.1 Hz, 1H), 7.37 (d, *J* = 8.5 Hz, 1H), 7.31 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.45 (q, *J* = 7.0 Hz, 1H), 4.36 – 4.24 (m, 2H), 4.04 – 3.95 (m, 4H), 3.15 (d, *J* = 11.3 Hz, 2H), 2.76 – 2.66 (m, 1H), 2.67 – 2.58 (m, 1H), 2.35 (t, *J* = 11.0 Hz, 1H), 2.05 – 1.97 (m, 2H), 1.95 – 1.84 (m, 1H), 1.90 (d, *J* = 7.0 Hz, 3H), 1.79 –

1.62 (m, 1H), 1.16 – 0.98 (m, 1H). LRMS-ESI$^+$: *m/z* calcd for $C_{24}H_{24}Cl_2N_8$ $[M+H]^+$ = 495.16; found, 495.2.

6-(3-((*R*)-1-(2-cyanoethyl)piperidin-3-yl)azetidin-1-yl)-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (33). Step 1: The alkylation was performed according to general procedure C using *tert*-butyl (*R*)-3-(piperidin-3-yl)azetidine-1-carboxylate **(*R*)-11** (115 mg, 0.48 mmol) and acrylonitrile (0.03 mL, 0.48 mmol) in dichloromethane (2 mL) at 23 °C for 16 h, then the volatiles were removed under reduced pressure and the *tert*-butyl (*R*)-3-(1-(2-cyanoethyl)piperidin-3-yl)azetidine-1-carboxylate was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{16}H_{27}N_3O_2$ $[M+H]^+$ = 294.22; found, 294.2.

Step 2: The Boc deprotection was performed according to general procedure D. T*ert*-butyl (*R*)-3-(1-(2-cyanoethyl)piperidin-3-yl)azetidine-1-carboxylate (130 mg, 0.44 mmol) from step 1 was dissolved in dichloromethane (2 mL) and then HCl (1 mL, 4 mmol, 4 N in 1,4-dioxane) was added. The mixture was stirred at 23 °C for 2 h and then concentrated under reduced pressure to afford (*R*)-3-(3-(azetidin-3-yl)piperidin-1-yl)propanenitrile

hydrochloride, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{11}H_{19}N_3$ $[M+H]^+$ = 194.17; found, 194.2.

Step 3: The S_NAr reaction was performed according to general procedure E. The crude (*R*)-3-(3-(azetidin-3-yl)piperidin-1-yl)propanenitrile hydrochloride from step 2 was dissolved in dichloromethane (1.2 mL), then (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile **15b** (80 mg, 0.23 mmol) and *N,N*-diisopropylethylamine (0.20 mL, 1.13 mmol) were added. The mixture was stirred at 23 °C for 12 h. The reaction was diluted with dichloromethane and washed with saturated aqueous sodium bicarbonate solution. The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography using a gradient of methanol in dichloromethane (0 to 10 %) to afford 73mg of 6-(3-((*R*)-1-(2-cyanoethyl)piperidin-3-yl)azetidin-1-yl)-1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-1H-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**33**, 63% yield). ^{1}H NMR (400 MHz, CD_3OD; BsOH Salt) δ 8.20 (s, 1H), 8.12 – 8.04 (m, 2H), 7.74 (d, *J* = 2.1 Hz, 1H), 7.66 – 7.60 (m, 4H), 7.57 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.71 (q, *J* = 7.1 Hz, 1H), 4.64

– 4.48 (m, 2H), 4.36 – 4.20 (m, 2H), 3.97 – 3.82 (m, 2H), 3.78 (t, *J* = 7.2 Hz, 2H), 3.35 (t, *J* = 7.2 Hz, 2H), 3.30 – 3.19 (m, 2H), 3.02 (t, *J* = 12.0 Hz, 1H), 2.98 – 2.86 (m, 1H), 2.46 – 2.27 (m, 2H), 2.16 (d, *J* = 7.1 Hz, 3H), 2.25 – 2.02 (m, 1H), 1.59 – 1.38 (m, 1H). LRMS-ESI$^+$: *m/z* calcd for $C_{22}H_{26}Cl_2N_8$ $[M+H]^+$ = 509.17; found, 509.2.

1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-(3-((*R*)-1-(3,3,3-trifluoropropyl)piperidin-3-yl)azetidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine (34). Step 1: The alkylation was performed according to general procedure B using *tert*-butyl (*R*)-3-(piperidin-3-yl)azetidine-1-carboxylate **(*R*)-11** (1.1 g, 4.58 mmol) and 3-iodo-1,1,1-trifluoropropane (0.64 mL, 5.49 mmol), potassium iodide (15 mg, 0.02 mmol), and potassium carbonate (1.27 g, 9.15 mmol) in acetonitrile (22 mL) at 80 °C for 12 h. The reaction mixture was diluted with water (20 mL), extracted with ethyl acetate (3X), the combined organic fractions were dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was dissolved in dichloromethane and filtered through a silica gel plug using 50% ethyl acetate in hexanes and concentrated yielding *tert*-butyl (*R*)-3-(1-(3,3,3-trifluoropropyl)piperidin-3-yl)azetidine-1-carboxylate (90% yield) which was used in the

next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{16}H_{27}F_3N_2O_2$ $[M+H]^+$ = 337.21; found, 337.2.

Step 2: The Boc deprotection was performed according to general procedure D. *Tert*-butyl (*R*)-3-(1-(3,3,3-trifluoropropyl)piperidin-3-yl)azetidine-1-carboxylate (1.40 g, 4.16 mmol) from step 1 was dissolved in dichloromethane (28 mL) and then HCl (5.2 mL, 20.8 mmol, 4 N in 1,4-dioxane) was added. The mixture was stirred at 23 °C for 5 h and then concentrated under reduced pressure to afford (*R*)-3-(azetidin-3-yl)-1-(3,3,3-trifluoropropyl)piperidine hydrochloride, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{11}H_{19}F_3N_2$ $[M+H]^+$ = 237.16; found, 237.2.

Step 3: The S_NAr reaction was performed according to general procedure E. The crude (*R*)-3-(azetidin-3-yl)-1-(3,3,3-trifluoropropyl)piperidine hydrochloride (96 mg, 0.35 mmol) from step 2 was dissolved in dichloromethane (1.4 mL), then (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine **15a** (100 mg, 0.29 mmol) and *N*,*N*-diisopropylethylamine (0.15 mL, 0.88 mmol) were added. The mixture was stirred at 23 °C for 4 h. The reaction was diluted with dichloromethane and washed with saturated

aqueous sodium bicarbonate solution. The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography using a gradient of ethyl acetate in hexanes (0 to 75 %) to afford 15 mg of 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-6-(3-((*R*)-1-(3,3,3-trifluoropropyl)piperidin-3-yl)azetidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine (**34**, 10% yield). ^{1}H NMR (400 MHz, $CDCl_3$; free base) δ 7.64 (s, 1H), 7.38 (d, *J* = 8.5 Hz, 1H), 7.35 (d, *J* = 2.2 Hz, 1H), 7.14 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.31 (q, *J* = 7.1 Hz, 1H), 4.19 (td, *J* = 8.4, 4.2 Hz, 2H), 3.90 – 3.82 (m, 2H), 2.87 – 2.72 (m, 2H), 2.64 – 2.57 (m, 2H), 2.57, s, 3H), 2.39 – 2.23 (m, 2H), 2.06 = 1.94 (m, 1H), 1.89 (d, *J* = 7.1 Hz, 3H), 1.86 – 1.65 (m, 5H), 1.63 – 1.50 (m, 1H), 0.99 – 0.82 (m, 1H). LRMS-ESI$^+$: *m/z* calcd for $C_{25}H_{29}Cl_2F_3N_6$ $[M+H]^+$ = 541.19; found, 541.2.

1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(3-((*R*)-1-(3,3,3-trifluoropropyl)piperidin-3-yl)azetidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (35). The S_NAr reaction was performed according to general procedure E. (*R*)-3-(azetidin-3-yl)-1-(3,3,3-trifluoropropyl)piperidine 56 (R_1 = CH_2CF_3) hydrochloride (93 mg, 0.34 mmol) was

dissolved in dichloromethane (1.4 mL), then (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile **15b** (100 mg, 0.28 mmol) and *N,N*-diisopropylethylamine (0.15 mL, 0.88 mmol) were added. The mixture was stirred at 23 °C for 12 h. The reaction was diluted with dichloromethane and washed with saturated aqueous sodium bicarbonate solution. The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography using a gradient of methanol in dichloromethane (0 to 10 %) to afford 115 mg of 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(3-((*R*)-1-(3,3,3-trifluoropropyl)piperidin-3-yl)azetidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (35, 78% yield). ^{1}H NMR (400 MHz, $CDCl_3$; free base) δ 7.81 (s, 1H), 7.37 (d, *J* = 2.1 Hz, 1H), 7.36 (d, *J* = 8.5 Hz, 1H), 7.19 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.45 (q, *J* = 7.1 Hz, 1H), 4.30 – 4.18 (m, 2H), 3.96 – 3.85 (m, 2H), 2.84 – 2.72 (m, 2H), 2.71 – 2.51 (m, 2H), 2.39 – 2.20 (m, 2H), 2.06 – 1.97 (m, 1H), 1.89 (d, *J* = 7.1 Hz, 3H), 1.87 – 1.67 (m, 5H), 1.65 – 1.46 (m, 1H), 1.00 – 0.80 (m, 1H). LRMS-ESI$^+$: *m/z* calcd for $C_{25}H_{26}Cl_2F_3N_7$ $[M+H]^+$ = 552.17; found, 552.2.

3-((*R*)-3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)propan-1-ol (36). A mixture of the title compound and its diastereomer was prepared as follows:

Step 1: The alkylation was performed according to general procedure B(a) using *tert*-butyl 3-(piperidin-3-yl)azetidine-1-carboxylate **11** (350 mg, 1.46 mmol) and methyl acrylate (0.4 mL, 4.38 mmol) in dimethylformamide (3 mL) at 50 °C for 3 h, then the volatiles were removed under reduced pressure. The residue was dissolved in tetrahydrofuran (10 mL) and then lithium borohydride (128 mg, 5.84 mmol) and methanol (280 μL, 8.76 mmol) were added. The mixture was stirred at 50 °C for 24 h, then quenched with aqueous ammonium chloride (10 mL, 1M) and extracted with ethyl acetate (3 x 10 mL). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressured. The residue was purified by silica gel chromatography using a gradient of methanol in dichloromethane (0 to 10 %) and *tert*-butyl 3-(1-(3-hydroxypropyl)piperidin-3-yl)azetidine-1-carboxylate (44% yield). LRMS-ESI$^+$: *m/z* calcd for $C_{16}H_{31}N_2O_3$ $[M+H]^+$ = 299.23; found, 299.2.

Step 2: The Boc deprotection was performed according to general procedure D. *Tert*-butyl 3-(1-(3-hydroxypropyl)piperidin-3-yl)azetidine-1-carboxylate (190 mg, 0.64 mmol) from step 1 was dissolved in dichloromethane (5 mL) and then HCl (2 mL, 8 mmol, 4 N in 1,4-dioxane) was added. The mixture was stirred at 23 °C for 30 min and then concentrated under reduced pressure to afford 3-(3-(azetidin-3-yl)piperidin-1-yl)propan-1-ol hydrochloride, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{11}H_{23}N_2O$ $[M+H]^+$ = 199.18; found, 199.2.

Step 3: The S_NAr reaction was performed according to general procedure E. The crude 3-(3-(azetidin-3-yl)piperidin-1-yl)propan-1-ol hydrochloride from step 2 was dissolved in dimethyl sulfoxide (4 mL), then (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine **15a** (189 mg, 0.55 mmol) and *N,N*-diisopropylethylamine (0.38 mL, 2.20 mmol) were added. The mixture was heated to 80 °C for 12 h, then cooled to 23 °C. The mixture was purified directly by reversed 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 3-(3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)propan-1-ol 2,2,2-trifluoroacetate as a 1:1 mixture of diastereomers. The title compound was separated from its diastereomer by preparative chiral HPLC using a CHIRALCEL® OZ-H column (Daicel Corporation, West Chester, PA) and 20% ethanol in heptanes (0.1% diethylamine) as eluent and afforded 69 mg of 3-((*R*)-3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)propan-1-ol (**36**, 50% yield) as the first eluting isomer. ^{1}H NMR (400 MHz, $CDCl_3$; free base) δ 7.64 (s, 1H), 7.37 (d, *J* = 8.5 Hz, 1H), 7.34 (d, *J* = 2.1 Hz, 1H), 7.13 (dd, *J* = 8.6, 2.2 Hz, 1H), 6.31 (q, *J* = 7.1 Hz, 1H), 4.19 (t, *J* = 8.4 Hz, 2H), 3.91 (dd, *J* = 8.6, 5.9 Hz, 1H), 3.85 (dd, *J* = 8.5, 5.9 Hz, 1H), 3.80 (t, *J* = 5.2 Hz, 2H), 3.07 – 2.90 (m, 3H), 2.62 (t, *J* = 5.6 Hz, 2H), 2.56 (s, 3H), 2.55 – 2.51 (m, 1H), 2.08 – 1.93 (m, 1H), 1.89 (d, *J* = 7.1 Hz, 3H), 1.87 – 1.66 (m, 5H), 1.65 – 1.50 (m, 1H), 1.02 – 0.86 (m, 1H). LRMS-ESI$^+$: *m/z* calcd for $C_{25}H_{33}Cl_2N_6O$ $[M+H]^+$ = 503.21; found, 503.0.

2-((*R*)-3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)ethan-1-ol (37).

Step 1: The reductive amination was performed according to general procedure A using *tert*-butyl 3-(piperidin-3-yl)azetidine-1-carboxylate **11** or enantioenriched **(*R*)-11** (200 mg, 0.83 mmol), 2-((*tert*-butyldimethylsilyl)oxy)acetaldehyde (145 mg, 0.83 mmol) and sodium triacetoxyborohydride (352 mg, 1.66 mmol) in 1,2-dichloroethane (5 mL) at 23 °C for 2 h. The reaction was quenched with saturated aqueous sodium bicarbonate solution and extracted with dichloromethane. The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure and the residue was purified by silica gel chromatography using a gradient of methanol in dichloromethane (0 to 10 %) and afforded *tert*-butyl 3-(1-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)piperidin-3-yl)azetidine-1-carboxylate (88 % yield). LRMS-ESI$^+$: *m/z* calcd for $C_{21}H_{43}N_2O_3Si$ $[M+H]^+$ = 399.30; found, 399.2.

Step 2: The Boc deprotection was performed according to general procedure D. *Tert*-butyl 3-(1-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)piperidin-3-yl)azetidine-1-carboxylate (290 mg, 0.73 mmol) from step 1 was dissolved in dichloromethane (5 mL) and then HCl (3 mL, 12 mmol, 4 N in 1,4-dioxane) was added. The mixture was stirred at 23 °C for 90 min

and then concentrated under reduced pressure to afford 2-(3-(azetidin-3-yl)piperidin-1-yl)ethan-1-ol hydrochloride, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{10}H_{21}N_2O$ $[M+H]^+$ = 185.17; found, 185.2.

Step 3: The S_NAr reaction was performed according to general procedure E. The crude 2-(3-(azetidin-3-yl)piperidin-1-yl)ethan-1-ol hydrochloride from step 2 was dissolved in dimethylformamide (2 mL), then (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine **15a** (137 mg, 0.40 mmol) and *N*,*N*-diisopropylethylamine (0.36 mL, 2.05 mmol) were added. The mixture was heated to 80 °C for 3 h, then cooled to 23 °C and concentrated under reduced pressure. The residue was purified by silica gel chromatography using a gradient of methanol in dichloromethane (0 to 20 %) and afforded 2-(3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)ethan-1-ol as a 1:1 mixture of diastereomers (if racemic **11** was used). The title compound was separated from its diastereomer by SFC using an AD-H column (2 x 25 cm) and eluting with 40% isopropanol (0.1% diethylamine) in CO_2, 100 bar and afforded 68mg of 2-((*R*)-3-(1-(1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-3-methyl-

1*H*-pyrazolo[3,4-*b*]pyrazin-6-yl)azetidin-3-yl)piperidin-1-yl)ethan-1-ol (**37**, 70% yield) as the first eluting isomer. ^{1}H NMR (400 MHz, $CDCl_3$; free base) δ 7.64 (s, 1H), 7.38 (d, *J* = 8.5 Hz, 1H), 7.35 (d, *J* = 2.1 Hz, 1H), 7.14 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.32 (q, *J* = 7.1 Hz, 1H), 4.23 – 4.14 (m, 2H), 3.92 – 3.83 (m, 2H), 3.67 – 3.58 (m, 2H), 2.87 (t, *J* = 10.5 Hz, 2H), 2.66 – 2.58 (m, 1H), 2.57 (s, 3H), 2.34 – 2.21 (m, 2H), 2.09 (t, *J* = 11.2 Hz, 1H), 1.89 (d, *J* = 7.1 Hz, 3H), 1.87 – 1.69 (m, 4H), 1.69 – 1.53 (m, 1H), 1.03 – 0.89 (m, 1H). LRMS-ESI$^+$: *m/z* calcd for $C_{24}H_{31}Cl_2N_6O$ $[M+H]^+$ = 489.19; found, 489.1.

1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(3-((*R*)-1-(2-hydroxyethyl)piperidin-3-yl)azetidin-1-yl)-N-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (38). Step 1: The reductive amination was performed according to general procedure A using *tert*-butyl (*R*)-3-(piperidin-3-yl)azetidine-1-carboxylate (*R*)-11 (8.25 g, 34.3 mmol), 2-((*tert*-butyldimethylsilyl)oxy)acetaldehyde (5.98 g, 34.3 mmol) and sodium triacetoxyborohydride (18.17 g, 85.75 mmol) in 1,2-dichloroethane (172 mL) at 23 °C for 2 h. The reaction was quenched with saturated aqueous sodium bicarbonate solution and extracted with dichloromethane. The combined organic layers were dried over sodium

sulfate, filtered and concentrated under reduced pressure and afforded *tert*-butyl (*R*)-3-(1-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)piperidin-3-yl)azetidine-1-carboxylate, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{21}H_{43}N_2O_3Si$ $[M+H]^+$ = 399.30; found, 399.2.

Step 2: The Boc deprotection was performed according to general procedure D. The crude *tert*-butyl (*R*)-3-(1-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)piperidin-3-yl)azetidine-1-carboxylate from step 1 was dissolved in dichloromethane (68.6 mL) and then HCl (42.88 mL, 171.5 mmol, 4 N in 1,4-dioxane) was added. The mixture was stirred at 23 °C for 2 h and then concentrated under reduced pressure to afford (*R*)-2-(3-(azetidin-3-yl)piperidin-1-yl)ethan-1-ol hydrochloride, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{10}H_{21}N_2O$ $[M+H]^+$ = 185.17; found, 185.2.

Step 3: The S_NAr reaction was performed according to general procedure E. (*R*)-2-(3-(azetidin-3-yl)piperidin-1-yl)ethan-1-ol hydrochloride (7.57 g, 34.3 mmol) from step 2 was dissolved dichloromethane (62 mL), then (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-*N*-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile **15b** (43, 11 g, 31.2 mmol) and *N*,*N*-

diisopropylethylamine (16 mL, 93.6 mmol) were added. The mixture was stirred at 23 °C for 2 h, then diluted with dichloromethane (100 mL), washed with a saturated aqueous solution of sodium carbonate (100 mL). The aqueous fraction was extracted with dichloromethane, the combined organic fractions were dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography using a gradient of methanol in dichloromethane (0 to 20 %) and afforded 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(3-((*R*)-1-(2-hydroxyethyl)piperidin-3-yl)azetidin-1-yl)-*N*-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carbonitrile (**38**, 83% yield) and converted to the corresponding HCl salt by dissolution in ethanol, cooling to 0 °C, and addition of 1 equiv. of 0.01M HCl in ethanol. ^{1}H NMR (400 MHz, CD_3OD; HCl Salt) δ 7.97 (s, 1H), 7.49 (d, *J* = 2.1 Hz, 1H), 7.38 (d, *J* = 8.5 Hz, 1H), 7.32 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.46 (q, *J* = 7.1 Hz, 1H), 4.40 – 4.27 (m, 2H), 4.06 (dd, *J* = 9.2, 5.7 Hz, 2H), 3.93 (t, *J* = 5.2 Hz, 2H), 3.71 – 3.61 (m, 2H), 3.30 – 3.25 (m, 2H), 3.02 – 2.91 (m, 1H), 2.79 – 2.65 (m, 2H), 2.26 – 2.14 (m, 1H), 2.10 – 1.95 (m, 2H), 1.92 (d, *J* = 7.1 Hz, 3H), 1.90 – 1.80 (m, 1H), 1.31 – 1.20 (m, 1H). ^{13}C NMR (101 MHz; CD_3OD; HCl salt) δ 153.7, 143.0, 137.0,

134.1, 133.1, 133.0, 129.0, 128.9, 127.5, 125.1, 117.4, 112.2, 66.7, 59.1, 55.0, 54.9, 53.3, 53.1, 52.7, 38.0, 32.9, 24.7, 22.3, 18.6. LRMS-ESI$^+$: *m/z* calcd for $C_{24}H_{28}Cl_2N_7O$ $[M+H]^+$ = 500.17; found, 500.0.

1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(3-((*R*)-1-(2-hydroxyethyl)piperidin-3-yl)azetidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxamide (39). A mixture of the title compound and its diastereomer was prepared as follows:

Step 1: The reductive amination was performed according to general procedure A using *tert*-butyl 3-(piperidin-3-yl)azetidine-1-carboxylate **11** (200 mg, 0.83 mmol), 2-((*tert*-butyldimethylsilyl)oxy)acetaldehyde (145 mg, 0.83 mmol) and sodium triacetoxyborohydride (352 mg, 1.66 mmol) in 1,2-dichloroethane (5 mL) at 23 °C for 2 h. The reaction was quenched with saturated aqueous sodium bicarbonate solution and extracted with dichloromethane. The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure and the residue was purified by silica gel chromatography using a gradient of methanol in dichloromethane (0 to 10 %) and afforded *tert*-butyl 3-(1-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)piperidin-3-yl)azetidine-

1-carboxylate (88 % yiled). LRMS-ESI$^+$: *m/z* calcd for $C_{21}H_{43}N_2O_3Si$ $[M+H]^+$ = 399.30; found, 399.2.

Step 2: The Boc deprotection was performed according to general procedure D. *Tert*-butyl 3-(1-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)piperidin-3-yl)azetidine-1-carboxylate (290 mg, 0.73 mmol) from step 1 was dissolved in dichloromethane (5 mL) and then HCl (3 mL, 12 mmol, 4 N in 1,4-dioxane) was added. The mixture was stirred at 23 °C for 90 min and then concentrated under reduced pressure to afford 2-(3-(azetidin-3-yl)piperidin-1-yl)ethan-1-ol hydrochloride, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{10}H_{21}N_2O$ $[M+H]^+$ = 185.17; found, 185.2.

Step 3: The S_NAr reaction was performed according to general procedure E. The crude 2-(3-(azetidin-3-yl)piperidin-1-yl)ethan-1-ol hydrochloride from step 2 was dissolved in dimethylformamide (2 mL), then (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxamide[28] **15d** (148 mg, 0.40 mmol) and *N*,*N*-diisopropylethylamine (0.36 mL, 2.05 mmol) were added. The mixture was heated to 80 °C for 3 h, then cooled to 23 °C and concentrated under reduced pressure. The residue

was purified by silica gel chromatography using a gradient of methanol in dichloromethane (0 to 20 %) and afforded 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(3-(1-(2-hydroxyethyl)piperidin-3-yl)azetidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxamide as a 1:1 mixture of diastereomers. The title compound was separated from its diastereomer by SFC using an AD-H column (2 x 25 cm) and eluting with 40% ethanol (0.1% diethylamine) in CO_2, 100 bar and afforded 65 mg of 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(3-((*R*)-1-(2-hydroxyethyl)piperidin-3-yl)azetidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxamide (**39**, 63% yield) as the first eluting isomer. ^{1}H NMR (400 MHz, CD_3OD; free base) δ 7.88 (s, 1H), 7.46 (d, *J* = 2.1 Hz, 1H), 7.43 (d, *J* = 8.0 Hz, 1H), 7.27 (dd, *J* = 8.0, 2.1 Hz, 1H), 6.44 (q, *J* = 7.0 Hz, 1H), 4.24 (q, *J* = 9.0 Hz, 2H), 3.99 – 3.88 (m, 2H), 3.71 (t, *J* = 6.0 Hz, 2H), 3.04 – 2.92 (m, 2H), 2.67 – 2.54 (m, 3H), 2.18 – 2.06 (m, 1H), 1.93 (d, *J* = 7.1 Hz, 3H), 1.90 – 1.71 (m, 4H), 1.70 – 1.54 (m, 1H), 1.02 – 0.83 (m, 1H). LRMS-ESI$^+$: *m/z* calcd for $C_{24}H_{30}Cl_2N_7O_2$ $[M+H]^+$ = 518.18; found, 518.1.

1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(3-((*R*)-1-(2-hydroxyethyl)piperidin-3-yl)azetidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxylic acid (40). Step 1: The reductive amination

was performed according to general procedure A using *tert*-butyl (*R*)-3-(piperidin-3-yl)azetidine-1-carboxylate **(*R*)-11** (5.6 g, 23.3 mmol), 2-((*tert*-butyldimethylsilyl)oxy)acetaldehyde (5.42 g, 28.0 mmol) and sodium triacetoxyborohydride (12.3 g, 58.3 mmol) in 1,2-dichloroethane (116 mL) at 23 °C for 2 h. The reaction was quenched with saturated aqueous sodium bicarbonate solution and extracted with dichloromethane. The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure and afforded *tert*-butyl (*R*)-3-(1-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)piperidin-3-yl)azetidine-1-carboxylate, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{21}H_{43}N_2O_3Si$ $[M+H]^+$ = 399.30; found, 399.2.

Step 2: The Boc deprotection was performed according to general procedure D. *Tert*-butyl (*R*)-3-(1-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)piperidin-3-yl)azetidine-1-carboxylate (1.0 g, 2.51 mmol) from step 1 was dissolved in dichloromethane (5 mL) and then HCl (3.14 mL, 12.5 mmol, 4 N in 1,4-dioxane) was added. The mixture was stirred at 23 °C for 90 min and then concentrated under reduced pressure to afford (*R*)-2-(3-(azetidin-3-

yl)piperidin-1-yl)ethan-1-ol hydrochloride, which was used in the next step without further purification. LRMS-ESI$^+$: *m/z* calcd for $C_{10}H_{21}N_2O$ $[M+H]^+$ = 185.17; found, 185.2.

Step 3: The S_NAr reaction was performed according to general procedure E. The crude 2-(3-(azetidin-3-yl)piperidin-1-yl)ethan-1-ol hydrochloride from step 2 was dissolved in dichloromethane (8.3 mL), then ethyl (*R*)-6-chloro-1-(1-(2,4-dichlorophenyl)ethyl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxylate[28] **15c** (500 mg, 1.3 mmol) and *N,N*-diisopropylethylamine (1.1 mL, 6.3 mmol) were added. The mixture was stirred at 23 °C for 4 h, then concentrated under reduced pressure. The residue was purified by reversed 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 ethyl 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(3-((*R*)-1-(2-hydroxyethyl)piperidin-3-yl)azetidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxylate trifluoroacetate (**55**, 80% yield). LRMS-ESI$^+$: *m/z* calcd for $C_{24}H_{31}Cl_2N_6O$ $[M+H]^+$ = 547.20; found, 547.2.

Step 4: Lithium hydroxide monohydrate (210 mg, 5.0 mmol) was added to a solution of ethyl 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(3-((*R*)-1-(2-hydroxyethyl)piperidin-3-yl)azetidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxylate (548 mg, 1.0 mmol) in THF (2 mL) and water (2.0 mL). The reaction was heated at 50 °C for 2 h. After cooling to room temperature, the mixture was concentrated, and the residue was purified by reversed 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 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(3-((*R*)-1-(2-hydroxyethyl)piperidin-3-yl)azetidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxylic acid (**40**, 70% yield). ^{1}H NMR (400 MHz, CD_3OD; free base) δ 7.94 (s, 1H), 7.48 (d, 1H, *J* = 2.1 Hz), 7.40 (d, 1H, *J* = 8.2 Hz), 7.29 (dd, 1H, *J* = 2.1 Hz, *J* = 8.5 Hz), 6.48 (q, 1H, *J* = 7.0 Hz), 2.48 – 4.26 (m, 2H), 4.07 – 3.97 (m, 2H), 3.93 – 3.88 (m, 2H), 3.70 – 3.67 (m, 2H), 3.30 – 3.23 (m, 2H), 2.98 – 2.88 (m, 1H), 2.75 – 2.65 (m, 2H), 2.20 – 1.80 (m, 7H), 1.31 – 1.18 (m, 1H). LRMS-ESI$^+$: *m/z* calcd for $C_{24}H_{28}Cl_2N_6O_3$ $[M+H]^+$ = 519.16; found, 519.1.

1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(3-((*R*)-1-(2-hydroxyethyl)piperidin-3-yl)azetidin-1-yl)-N-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxamide (41). A solution of propylphosphonic anhydride (0.93 mL, 0.16 mmol, 50 wt% in ethyl acetate) was added to a mixture of 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(3-((*R*)-1-(2-hydroxyethyl)piperidin-3-yl)azetidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxylic acid 40 (68 mg, 0.13 mmol) and methylamine (325 mL, 0.65 mmol, 2M in THF) in ethyl acetate (1 mL) and dimethylformamide (0.5 mL). The reaction mixture was stirred at 50 °C for 16 hours and then quenched with 2 mL of a 1:1 mixture of water and brine then extracted with ethyl acetate. The organic layer was dried with sodium sulfate, filtered, concentrated, and the residue was purified by silica gel chromatography using a gradient of methanol in dichloromethane (0 to 20 %) to afford 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(3-((*R*)-1-(2-hydroxyethyl)piperidin-3-yl)azetidin-1-yl)-*N*-methyl-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxamide (41, 40% yield). ^{1}H NMR (400 MHz, CD_3OD; free base) δ 7.85 (s, 1H), 7.44 (d, *J* = 2.1 Hz, 1H), 7.43 (d, *J* = 8.5 Hz, 1H), 7.25 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.42 (q, *J* = 7.0 Hz, 1H), 4.23 (q, *J* = 8.8 Hz, 2H), 3.99 – 3.87 (m, 2H), 3.73 (t, *J* = 5.9 Hz, 2H), 3.10 –

3.03 (m, 2H), 3.01 (s, 3H), 2.68 (t, *J* = 5.9 Hz, 2H), 2.65 – 2.54 (m, 1H), 2.27 – 2.16 (m, 1H), 1.92 (d, *J* = 7.1 Hz, 3H), 1.90 – 1.73 (m, 4H), 1.73 – 1.58 (m, 1H), 1.05 – 0.91 (m, 1H). LRMS-ESI$^+$: *m/z* calcd for $C_{25}H_{32}Cl_2N_7O_2$ $[M+H]^+$ = 532.20; found, 532.2.

1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(3-((*R*)-1-(2-hydroxyethyl)piperidin-3-yl)azetidin-1-yl)-*N,N*-dimethyl-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxamide (42).

A solution of propylphosphonic anhydride (0.93 mL, 0.16 mmol, 50 wt% in ethyl acetate) was added to a mixture of 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(3-((*R*)-1-(2-hydroxyethyl)piperidin-3-yl)azetidin-1-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxylic acid **40** (68 mg, 0.13 mmol) and dimethylamine (325 mL, 0.65 mmol, 2M in THF) in ethyl acetate (1 mL) and dimethylformamide (0.5 mL). The reaction mixture was stirred at 50 °C for 16 hours and then quenched with 2 mL of a 1:1 mixture of water and brine then extracted with ethyl acetate. The organic layer was dried with sodium sulfate, filtered, concentrated, and the residue was purified by silica gel chromatography using a gradient of methanol in dichloromethane (0 to 20 %) to afford 1-((*R*)-1-(2,4-dichlorophenyl)ethyl)-6-(3-((*R*)-1-(2-hydroxyethyl)piperidin-3-yl)azetidin-1-yl)-*N,N*-dimethyl-1*H*-pyrazolo[3,4-*b*]pyrazine-3-carboxamide (**42**, 20% yield). ^{1}H NMR (400 MHz, CD_3OD; free base) δ 7.87 (s, 1H), 7.47 (d, 1H, *J* = 2.1 Hz), 7.43 (d, 1H, *J* = 8.5 Hz), 7.28 (dd, 1H, *J* = 8.5, 2.1 Hz),

6.42 (1H, q, *J* = 7.1 Hz), 4.32 – 4.22 (m, 2H), 4.03 – 3.93 (m, 2H), 3.76 (t, 2H, *J* = 5.8 Hz), 3.18 (s, 6H), 3.11 – 3.17 (m, 2H), 2.82 – 2.74 (m, 2H), 2.68 – 2.58 (m, 1H), 2.38 – 2.28 (m, 1H), 2.12 – 1.78 (m, 8H), 1.76 – 1.63 (m, 1H), 1.09 – 0.97 (m 1H). LRMS-ESI$^+$: *m/z* calcd for $C_{26}H_{34}Cl_2N_7O_2$ $[M+H]^+$ = 546.21; found, 546.2.

Chiral resolution of *tert*-butyl (*R*)-3-(piperidin-3-yl)azetidine-1-carboxylate ((*R*)-11).

Tert-butyl 3-(3-piperidyl)azetidine-1-carboxylate (77.0 g, 320 mmol) **11** was dissolved in *tert*-butyl methyl ether (1,700 mL) in a 5-L three-necked flask equipped with a mechanical stirring and a water condenser. When the mixture was stirred at reflux, *L*-(+)-Mandelic acid (24.38 g, 160 mmol) was added. The resulting mixture stirred at reflux for 15 min and then cooled to 23 °C over 12 h. The white solid was collected by filtration and rinsed with *tert*-butyl methyl ether (500 mL). The white solid was resuspended in *tert*-butyl methyl ether (1700 mL) and stirred at reflux for 30 min. The solid was collected by filtration and washed with *tert*-butyl methyl ether (500 mL). The process was repeated once more to afford a white solid (62.8 g, 95% ee by chiral HPLC on a CHIRALPAK® IC-3 column, 4.6 x 250 mm, 3 uM, eluting with 50% heptane, 50% isopropanol at 1 mL/min, detecting

at 210 nM, Rt = 8.8-9 min). The white solid was further purified by re-crystallization from chloroform/*tert*-butyl methyl ether (900 mL/900 mL). The salt was dissolved in chloroform (900 mL) and warmed to a slight boil, then *tert*-butyl methyl ether (900 mL) was slowly added. It was a clear solution when it was hot. The solution was cooled to 23 °C over 12 h, and crystals appeared. The crystals were isolated by filtration and further dried under high vacuum for 4 h to afford 64.9 g (>99% ee) of the mandelate salt with 1 equiv. of chloroform as confirmed by ^{1}H NMR (in CD_3OD) and by X-ray crystallography of a suitable single crystal (Cambridge Crystallographic Data Centre Deposition Number 1991309). The salt was free based by dissolving in dichloromethane (0.5 M) and washed with aqueous sodium hydroxide (1 M, 2 equiv.). The organic fraction was dried over sodium sulfate, filtered, and concentrated under reduced pressure to afford *tert*-butyl (*R*)-3-(piperidin-3-yl)azetidine-1-carboxylate **(*R*)-11** which was used in the next step without further purification.

Calcium Flux Assay. Chem-5 hCCR4 cells (EMD Millipore, HTS009C) were cultured under standard conditions and frozen in 1 mL per vial aliquots of $10x10^6$ cells/mL. The

day prior to testing, a vial was thawed rapidly and pipetted into 9 mL of media (DMEM + 10% FBS + 1% Pen-Strep + 1% *L*-glutamine). Cells were harvested by centrifugation and re-suspended in 25 mL of fresh culture medium. An aliquot (25 µL) of the cell suspension was seeded into 384 well plates (Corning CellBind Plates, Cat# 4579) resulting in a cell density of 10,000 cells per well. The plates were centrifuged at 300 x G for 10 seconds and incubated overnight at 37 °C and 5% CO_2. The next day, the media was removed and 25 µL of serum free media (DMEM + 1% Pen-Strep + 1% *L*-glutamine) was added. The cells were incubated for an additional 2 hours and 25 µL of assay buffer (Hank's balanced salt solution + 20 mM HEPES, pH 7.4) containing FLIPR Calcium 6 dye (Molecular Devices, #R8191) and probenecid (2.5 mM, Life Technologies, P36400) was added to each well and incubated at 37 °C and 5% CO_2 for 2 hours. The plate was normalized to 0.5% DMSO per well using an HP D300e and incubated at 37 °C and 5% CO_2 for 1 hour. Human CCL22 (Peprotech, #300-36A) was diluted to 500 nM (5x final concentration) with chemokine buffer (HBSS, 20 mM HEPES, 0.1% BSA) and serially diluted 1:2, 16 points with the 16th point containing no ligand. The 16-point hCCL22 serial

dilutions were transferred to a 384 well polypropylene plate (Corning Polypropylene Plates, Cat# Corning 3657). The assay plates and polypropylene plate containing working solutions of hCCL22 were transferred to a FlexStation3 plate reader (Molecular Devices) at 37 °C for 5 minutes. Fluorescence recordings (485 nm excitation/525 nm emission) were performed at 2.5 second intervals. After 16 seconds, 12.5 µL of hCCL22 solution was added to each well and reads were continuously performed for 45 seconds at 2.5 second intervals. The minimum fluorescence (F_{min}) was calculated by averaging the reads prior to ligand addition and the change in fluorescence (ΔF) is calculated by subtracting the F_{min} from the average of the reads following ligand addition. The response $\Delta F/F_{min}$ was plotted as a function of the hCCL22 concentration starting at 100 nM and the IC_{50} and IC_{80} values are derived by non-linear regression analysis using a 4-parameter fit in PRISM software. Values presented are the average of n = 2 or more determinations, where the value of each determination is within 3-fold difference of each other.

Chemotaxis (CTX) Assay. The assay was performed using the ChemoTX (NeuroProbe; Cat# 106-5) migration system with a 5 µm pore size polycarbonate trach-etch (PCTE)

membrane. CCRF-CEM (ATCC, Cat# CCL-119), in vitro generated mouse iT_{reg}, or in vitro generated human iT_{reg} cells were suspended in human serum at 2 million cells/mL. The test compound was added to the cell/serum mixture at a final DMSO concentration of 0.25% (v/v), using an HP D300e digital dispenser followed by a 30 min compound pre-incubation period. Separately, recombinant human CCL22 (Peprotech; #300-36A), recombinant human CCL17 (Peprotech; # 300-30), or mouse CCL22 (Peprotech; #250-23) was diluted to 0.9 nM in 1 x HBSS with 0.1% BSA and an aliquot (29 µL) of this ligand solution was placed in the lower wells of the ChemoTX plate. The PCTE membrane was placed onto the plate, and 50 µL of the cell/compound mixture was transferred onto each well of the membrane. The chemotaxis plate was incubated at 37 °C, 100% humidity, and 5% CO_2 for 60 minutes, after which the polycarbonate membranes were removed, and 15 µL of the ATP-binding agent CellTiter-Glo (Promega; G7571) was added to the lower wells. The amount of luminescence, corresponding to the number of migrated cells, was measured using an EnVision plate reader (PerkinElmer; Waltham, MA). The number of migrated cells in the presence of the ligand alone was set to 100% migration and the

numbers of migrated cells in the presence of ligands were expressed as the corresponding percentage. The results were analyzed by fitting the experimental curves (% migrated cells vs compound concentration) by non-linear regression using a four-parameter fit using the GraphPad PRISM software. Values presented are the average of n = 2 or more determinations, where the value of each determination is within 3-fold difference of each other.

ASSOCIATED CONTENT

Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

Assay conditions; hepatocyte stability assay; *in vitro* CYP450 assay; *in vivo* pharmacology studies, X-ray structure of synthetic intermediate of **38** (PDF)

Molecular Formula Strings (XLSX)

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Notes

The authors declare the following competing financial interests: All authors of this manuscript are/were employees of RAPT Therapeutics.

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ABBREVIATIONS

CCR4, CC chemokine receptor 4; CCL17, CC chemokine ligand 17; CCL22, CC chemokine ligand 22; T_{reg}, regulatory T cells; IO, immuno-oncology; T_{eff}, effector T cells; TME, tumor microenvironment; Th2, T helper type 2 cell; GPCR, G protein-coupled

receptor; CTCL, Cutaneous T-Cell Lymphoma; ADCC, antibody-dependent cell mediated cytotoxicity; CTX, chemotaxis; CTLA-4, cytotoxic T-lymphocyte-associated protein 4.

ANCILLARY INFORMATION

Single crystal X-ray structure of (*R*)-**11** (Cambridge Crystallographic Data Centre Deposition Number 1991309). Authors will release the atomic coordinates upon article publication.

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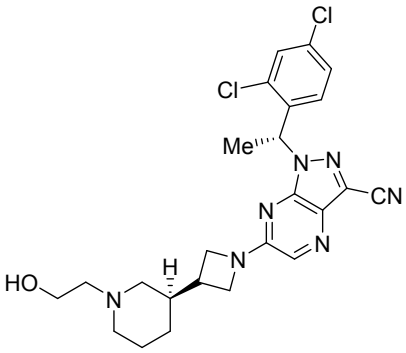
42. Compound **38** is referred to as **CCR4-351** in reference 38.

Table of Contents graphic.



38

CTX IC_{50} = 50 nM

Rat %F = 49