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Article

Synthesis and Structure−Activity Relationship of Tetra-Substituted Cyclohexyl Diol Inhibitors of Proviral Insertion of Moloney Virus (PIM) Kinases

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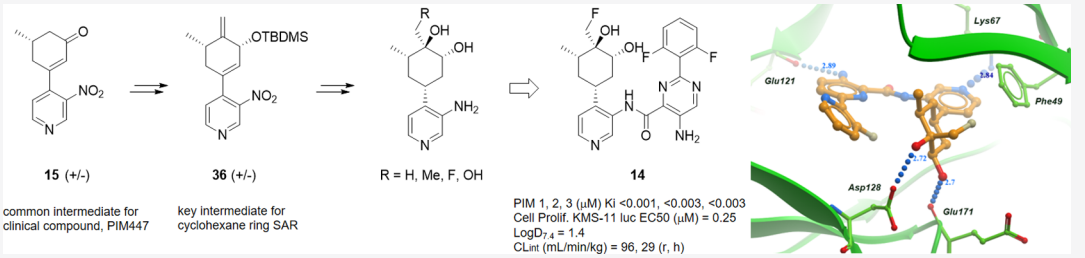

ABSTRACT: Overexpression of PIM 1, 2, and 3 kinases is frequently observed in many malignancies. Previously, we discovered a potent and selective pan-PIM kinase inhibitor, compound **2**, currently in phase I clinical trials. In this work, we were interested in replacing the amino group on the cyclohexane ring in compound **2** with a hydroxyl group. Structure-based drug design led to cellularly potent but metabolically unstable tetra-substituted cyclohexyl diols. Efforts on the reduction of Log D by introducing polar heterocycles improved metabolic stability. Incorporating fluorine to the tetra-substituted cyclohexyl diol moiety further reduced Log D, resulting in compound **14**, a cellularly potent tetra-substituted cyclohexyl diol inhibitor with moderate metabolic stability and good permeability. We also describe the development of efficient and scalable synthetic routes toward synthetically challenging tetra-substituted cyclohexyl diol compounds. In particular, intermediate **36** was identified as a versatile intermediate, enabling a large-scale synthesis of highly substituted cyclohexane derivatives.

INTRODUCTION

Proviral insertion of Moloney virus (PIM) kinases is a family of three serine/threonine kinases (PIM1, PIM2, and PIM3) initially identified as frequent sites of integration in viral-induced murine lymphomas.[1] In a normal setting, PIM expression is induced in response to growth factors and cytokines to promote the survival and proliferation of hematopoietic cells, and their activity is primarily regulated by the balance between synthesis and degradation.[2] However, the overexpression of PIMs *in vitro* or in transgenic mouse models has confirmed a role for this family in tumorigenesis of hematological tissues, either alone or in synergy with other oncogenes.[3−5] In humans, increased expression of PIM1 is reported in acute lymphoblastic leukemia, acute myeloid leukemia, and diffuse B-cell lymphoma, while high PIM2 expression is noted in multiple myeloma.[6] In addition, increased expression or dysfunction of all three PIMs has been reported in solid tumors including those of prostate, hepatocellular, colon, and gastric origin.[2,7−10]

Given the apparent functional redundancy of PIMs in oncogenesis and a differential expression across a wide array of cancers, a pan-PIM kinase inhibitor would be predicted to offer the greatest potential clinical utility.[11] In addition, elevated expression of PIM2 specifically has been described in multiple myeloma relative to other hematologic cancers, and this expression has been shown to be required for proliferation, presenting an effective pharmacological target for inhibition.[12] However, identification of pan-PIM inhibitors retaining potent PIM2 inhibitory capacity in myeloma cells is challenging given the 10−100 times lower K_m for ATP possessed by PIM2 relative to PIM1 and PIM3.[13−21]

In the course of our lead optimization efforts on the pyridylcarboxamide scaffold, we reported the discovery of compound **1**,[13] a potent pan-PIM inhibitor and *in vivo* tool compound, through the structure-guided optimization of a

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singleton HTS hit (Figure 1). Compound **1** demonstrated *in vivo* efficacy in multiple myeloma (KMS-11.luc) and acute

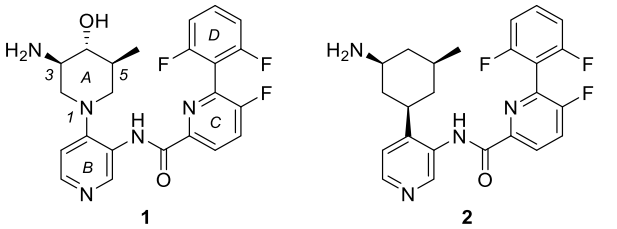

Figure 1. Novartis pan-PIM Inhibitors.

myeloid leukemia (EOL-1) xenograft mouse models. However, it showed high clearance in human liver microsomes, and the piperidine A-ring was identified as the major metabolic moiety by metabolic identification studies. Replacements of the piperidine A-ring with other rings such as heteroaryl[22] and cyclohexyl were explored, and cyclohexane rings improved metabolic stability in liver microsomes, resulting in the identification of an orally available, potent, and selective pan-PIM kinase inhibitor (PIM447), compound **2** (Figure 1).[23] We recently disclosed the phase I clinical trial data for PIM447 that displayed good tolerability and single agent efficacy in several patients with relapsed/refractory multiple myeloma.[24]

A key structural motif of compound **2** is the cyclohexyl amine moiety. The X-ray co-crystal structure in PIM1 showed that the amino group participated in polar H-bond interactions with the Asp128 side chain carboxylate and the Glu171 backbone carbonyl in the ribose patch, which are crucial for PIM potency.[23] In the early stage of the discovery of compound **2**, we were interested in replacing the amino group with non-basic isosteres such as a hydroxyl group as an alternative H-bond acceptor and donor. Without an ionizable group, such molecules could differentiate and display distinct overall drug properties.[25] Herein, we present the medicinal chemistry efforts on optimizing cyclohexyl diol inhibitors of pan-PIM kinases focusing on improving metabolic stability and cellular potency and describe the development of a diverse set of efficient synthetic routes to access synthetically challenging highly substituted cyclohexyl diol intermediates.

RESULTS AND DISCUSSION

Medicinal Chemistry. Our medicinal chemistry efforts of the cyclohexyl diol series began with replacement of the amino group of compound **2** with a hydroxyl group. We initially performed molecular docking studies of compound **3** with PIM1 kinase, which displayed a single H-bond interaction between the hydroxyl group and the Glu171 residue, unlike the amino group of compound **2** that makes two H-bond interactions with Glu171 and Asp128 residues, as shown in the X-ray co-crystal structure (Figure 2).

As predicted from the docking result, the potency of compound **3** decreased in both biochemical and cellular assays (EC_{50} = 3.41 μM, >20-fold decrease in potency in the KMS-11.luc assay, Table 1). Because the biochemical potency reached the lowest limit of detection in the series, the cellular potency was used as to guide SAR. The permeability profile of compound **3** was encouraging [Caco-2 assay: P_{app} (A−B) = 26.5 × 10^{-6} cm/s, ER = 0.9]; however, the increased lipophilicity (Log D = 4.6) resulted in high intrinsic clearance in liver microsomes across species (Table 1).[26] In the previous

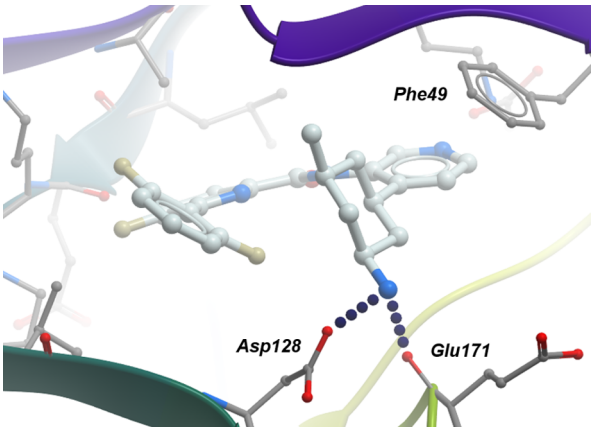

Figure 2. X-ray structure of compound **2** in PIM1 (PDB code: 5DWR).

piperidinyl amine series, a hydroxyl group adjacent to the amino group was successfully implemented to improve drug-like properties by lowering Log D.[13] Based on this knowledge, we synthesized the *trans*-diol analogue, compound **4**, with a reduced Log D value (Log D = 3.0). The introduction of the additional hydroxyl group favorably influenced microsomal intrinsic clearance while having no negative impact on Caco-2 permeability and efflux, and a slight gain in cellular potency (EC_{50} = 1.88 μM) was achieved relative to compound **3** (Table 1). Next, we employed a structure-based drug design approach to further improve on the potency of compound **4**. As previously reported for compounds **1** and **2**,[13,23] the methyl group at the 5-position fills a hydrophobic dimple in the glycine rich loop, resulting in the potency increase with minimal increase in lipophilicity. Therefore, we pursued the same strategy by examining the glycine rich loop region in the docking pose of compound **4**. It was anticipated that the axial methyl group at the 4-position would make a hydrophobic contact with the Phe49 residue of the glycine rich loop (Figure 3a). We synthesized compound **5** and observed an increase in the cellular potency by 6-fold (EC_{50} = 0.32 μM, Table 1) with minimal increase in lipophilicity (Log D = 3.2). Since compounds **4** and **5** have similar Caco-2 values, it was thought that the increased cellular potency mainly resulted from the increase in the binding affinity of compound **5** to the PIM kinases by incorporating the 4-methyl group. We obtained the X-ray co-crystal structure of compound **5** in PIM1 (Figure 3b), which demonstrated that the methyl group at the 4-carbinol position of the cyclohexane ring clearly made a hydrophobic interaction with Phe49 residue of the P-loop, as predicted by the docking model (Figure 3a). The X-ray structure also revealed that the 4-hydroxyl group participates in a hydrogen bond network with the Asp128 residue, an interaction that was not present in the X-ray structure of compound **1** illustrating the flexibility of the side chain of Asp128.

However, while compound **5** exhibited increased potency relative to compound **4**, it showed high *in vitro* and *in vivo* clearance[28] in rats (CL_{int} = 187 μL/min/mg and CL = 56 mL/min/kg, respectively), which correlated with the increased Log D (Log D for compound **5** = 3.2, Table 1). Having identified the 4-axial methyl group in the cyclohexyl diol A ring as a driver of potency, we next focused on improving metabolic stability of compound **5** by focusing on physicochemical properties, especially lowering Log D by introducing polar C and/or D rings. C ring heterocycle analogues of compound **5**

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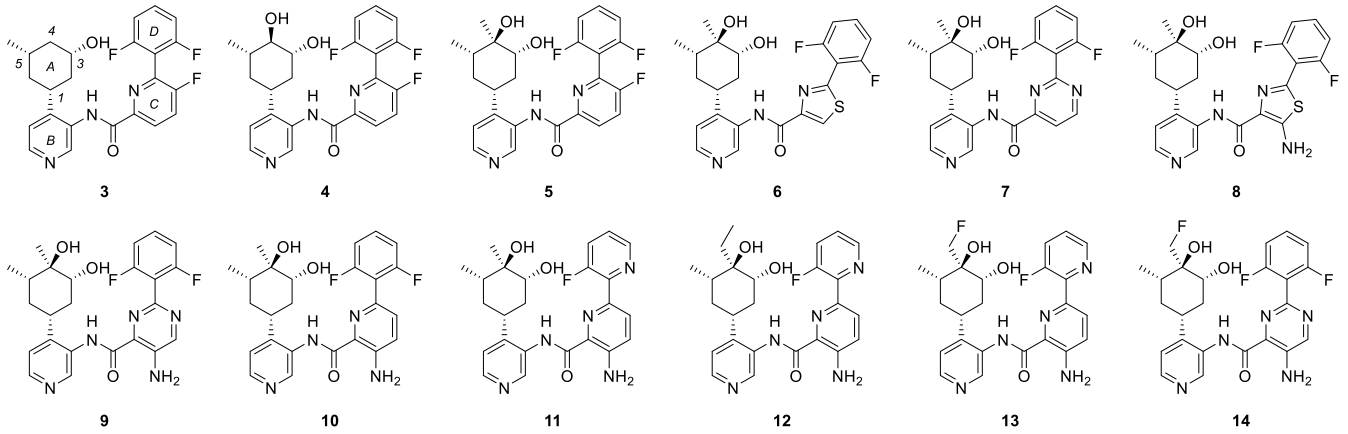
Table 1. Potency, Clearance, Permeability, and Physicochemical Property for PIM Compounds



Cpd no.	Ki (mM) PIM1	PIM2	PIM3	cell prolif KMS-11 luc EC_{50} (mM)	log $D_{7.4}$[a]	tPSA (Å)	CL_{int} (mL/min/kg) rat	human	ER_H[b] rat	human	Caco-2 P_{app}A−B (10^{-6} cm/s)	ER (P_{app}B−A/P_{app}A−B)[c]
1	<0.001	<0.003	<0.003	0.017	2.2	104	198	60	0.88	0.77	8.2	2.6
2	<0.001	<0.003	<0.003	0.17	1.9	81	28	13	0.51	0.42	8.5	2.6
3	<0.001	0.004	0.003	3.41	4.2	75	375	352	0.93	0.95	26.5	0.9
4	<0.001	<0.003	<0.003	1.88	3	95	107	61	0.8	0.77	28.8	1.1
5	<0.001	<0.003	<0.003	0.32	3.2	95	183	48	0.87	0.73	27.6	1.1
6	<0.001	0.003	<0.003	>10	2.5	95			N/D		16	1.8
7	0.005	0.013	0.004	>10	1.3	108			N/D		13.4	6.6
8	<0.001	<0.003	<0.003	1.06	2.6	121	77	44	0.74	0.71	28.1	1.4
9	<0.001	<0.003	<0.003	0.93	2.8	134	149	11	0.85	0.39	10.1	5.6
10	<0.001	<0.003	<0.003	0.45	3.7	121	75	67	0.73	0.79	23.5	0.9
11	0.001	0.003	0.004	3.24	2.3	134	35	30	0.56	0.62	6.6	6.9
12	0.001	0.011	0.005	1.71	3.2	134	133	62	0.83	0.78	30.3	1.1
13	<0.001	<0.003	<0.003	1.74	1.8	134	41	8	0.6	0.3	14.6	2.9
14	<0.001	<0.003	<0.003	0.25	1.4	134	96	29	0.78	0.62	28	1.7

[a]Measured Log D at pH 7.4. [b]ER_H (hepatic extraction ratio) = CL_h/Q_h (see the Experimental Section). [c]ER: Efflux ratio (P_{app}B−A/P_{app}A−B).

were first explored. Compounds **6** and **7**, thiazole, and pyrimidine C ring analogues were not potent in KMS-11.luc cellular assay, but the corresponding analogues having a hinge amino group on the C-ring, compounds **8** and **9**, showed around 1 μM in the cellular proliferation assay (Table 1). Interestingly, while the hinge amino group in thiazole and pyrimidine C rings provided a positive effect on cellular potency, pyridine C ring analogues showed the opposite trend; des-hinge amino compound **5** was slightly more potent than compound **10**. Our previous observation in other series was that the hinge amino group negatively affected permeability and/or efflux, but the cyclohexyl diol series did not show much difference on the Caco-2 values irrespective of the presence of hinge amino group. In addition, the introduction of a hinge amino group to the C ring did not affect liver microsomal clearance profiles, except for clearance in human liver microsomes for compound **9** (Table 1). We then investigated heterocyclic D rings such as thiazole, pyrazole, and pyridine in the presence of hinge amino C rings. Among them, compound **11**, a fluoropyridine D ring analogue, displayed moderate extraction ratio in rat and human liver microsomes in alignment with a lower Log D value (Log D for compound **11** = 2.3, Table 1). It was the first compound that showed a modest clearance in rat liver microsomes among cell active compounds in the series. A subsequent rat PK study of compound **11** showed good oral bioavailability (% F = 49) and moderate clearance (CL = 18 mL/min/kg). However, the cellular proliferation EC_{50} of compound **11** was not satisfactory (3.24 μM, Table 1), probably due to the reduced permeability and increased efflux [Caco-2 assay: P_{app} (A−B) = 6.61 × 10^{-6} cm/s, ER = 6.9] relative to compound **5** [Caco-2 assay: P_{app} (A−B) = 27.6 × 10^{-6} cm/s, ER = 1.1]. Since compound **11** has a hinge amino group that might deteriorate selectivity against other kinases, we assessed its kinase selectivity using an internal panel of 36 kinases (see Supporting Information). Gratifyingly, compound **11** was highly selective against 35 kinases (IC_{50}s > 10 μM), while maintaining >100-fold selectivity against GSK3β (IC_{50} = 0.54 μM).[29]

Having demonstrated that oral bioavailablity in the cyclohexyl diol series could be achieved, we turned our attention back to the diol functionality on the A-ring to further enhance the cellular potency. We hypothesized that incorporation of additional lipophilicity to the quaternary methyl group would increase membrane permeability by shielding the polar group[30,31] or *via* an intramolecular hydrogen bond.[32] Docking studies demonstrated that a methyl or fluorine would accommodate into the ligand pocket surface of the glycine rich residues.[33]

Therefore, we synthesized ethyl and fluoromethyl analogues of **11**, compounds **12** and **13**. Compared to compound **11** [EC_{50} = 3.24 μM, P_{app}(A−B) = 6.61, ER = 6.9, Table 1], both compounds improved the permeability and reduced the efflux

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J. Med. Chem. XXXX, XXX, XXX−XXX

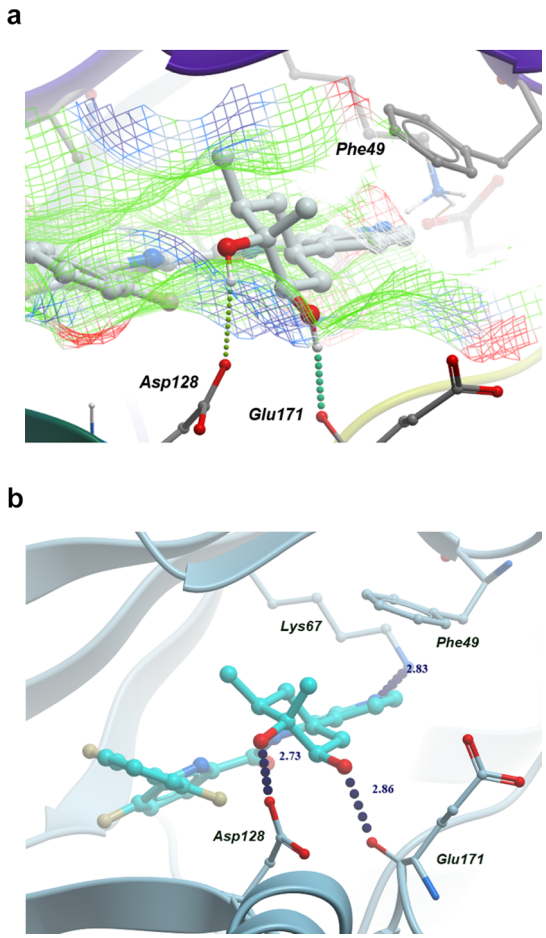

Figure 3. Docking model of compound **4** and the X-ray structure of compound **5** in PIM1. (a) Docking model of 4-methyl analogue of compound **4** in PIM1. (b) X-ray co-crystal structure of compound **5** in PIM1. The hydroxyl groups at the 3- and 4-positions on the cyclohexyl A-ring form H-bond interactions with the backbone residues Glu171 and Asp128, respectively. While the methyl group at the carbinol carbon made a hydrophobic interaction with Phe49, the methyl group at the 5-position occupies the hydrophobic dimple under the P-loop. The pyridine nitrogen at the B-ring interacts with the catalytic Lys67 residue (PDB code: 1oblv).[27]

ratio in Caco-2 permeability assay as hypothesized, but surprisingly, it did not significantly translate into the cellular potency (EC_{50} = 1.71 and 1.74 μM for **12** and **13**, Table 1). While the increased Log D value of compound **12** (Log D = 3.2) had a positive effect on the permeability in Caco-2 assay, it resulted in high microsomal clearances in rat and human liver microsomes. Compound **13** maintained the moderate intrinsic clearances that were explicable because it has a lower Log D value (1.8 *vs* 2.3) than compound **11**.

We obtained the X-ray co-crystal structure of compound **13** in PIM1 protein (Figure 4). The binding pose was almost identical to that of compound **5** with a newly formed hydrogen bond between Glu121 residue with the hinge amino group of compound **13**. As shown in compound **5**, the 4-hydroxyl group makes a hydrogen bond interaction with Asp128. The fluoro group in the fluoromethyl faces toward Phe49 residue, being in the gouche and anti-conformations with 3- and 4-hydroxyl groups, respectively. In the fluoropyridine D-ring, the pyridine nitrogen is located in the opposite side of the C ring pyridine nitrogen.

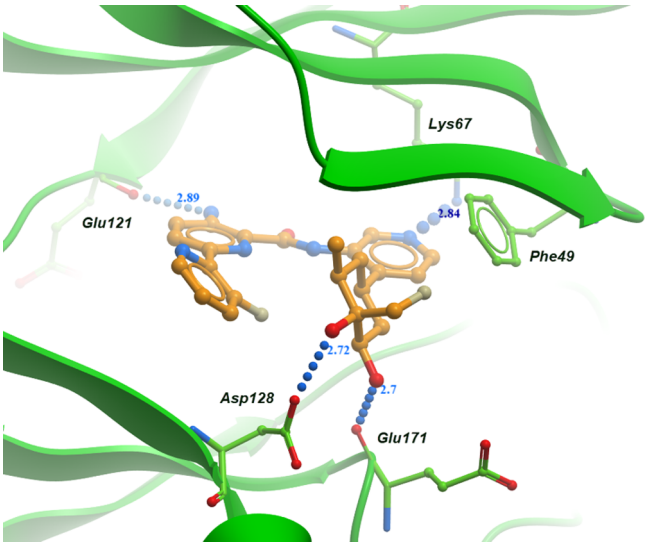

Figure 4. X-ray co-crystal structure of compound **13** in PIM1 (PDB code: 1ozrb).[34]

We continued to evaluate combinations of other C and/or D ring analogues of compound **13**. Among them, compound **14** with the aminopyrimidine C ring displayed a low Log D value (1.4, Table 1) and maintained good permeability and low efflux potential in Caco-2 assay that translated to improved cell proliferation activity (Table 1). Gratifyingly, compound **14** showed an EC_{50} of 0.25 μM in KMS-11.luc assay that was comparable to 0.18 μM for the clinical candidate, compound **2**. In comparison with its des-fluoro analogue, compound **9**, the fluorine effect[35] on compound **14** was more pronounced with respect to permeability and Log D. The permeability and efflux profiles were improved even with a lower Log D value by 1.4 unit (Log D for compound **14**: 1.4), which apparently resulted in the 3.7 fold increase in cellular potency, while still maintaining moderate intrinsic clearances. However, this fluoro addition reduced solubility (HT-Eq Sol. at pH 7.4: 6 μM for compound **14**; 19 μM for compound **9**) but improved the CYP inhibition profile of compound **14** (IC_{50}s of CYP450 3A4, 2C9, 2D6, and 1A2 isozymes: >30 μM for compound **14**; 26, 9.8, 9.9, and >30 μM, respectively, for compound **9**). Also, compound **14** demonstrated a superior permeability and efflux profile to the basic amine analogues, compounds **1** and **2** (Table 1). Due to the advancement of compound **2** (PIM447) to clinical stage, we made no further effort to pursue the cyclohexyl diol series. Overall, we demonstrated the improvement of permeability and microsomal stability by reducing Log D values through the structural modification of diol functionality and the introduction of polar C and/or D ring, resulting in the achievement of a cellularly potent pan-PIM inhibitor in the cyclohexyl diol series. In general, these cyclohexyl diol analogues did not show strong inhibitory activities against CYP450 enzymes and hERG potassium channel.[36]

CHEMISTRY

Our strategy for the synthesis of hydroxy- or di-hydroxy-substituted cyclohexyl analogues was to utilize (±)-5-methyl-3-(3-nitropyridin-4-yl)cyclohex-2-en-1-one **15**, a key intermediate for cyclohexyl amine A−B ring of compound **2** (Scheme 1),[23] to develop efficient and scalable syntheses to various tetra-substituted cyclohexyl diol intermediates. The final

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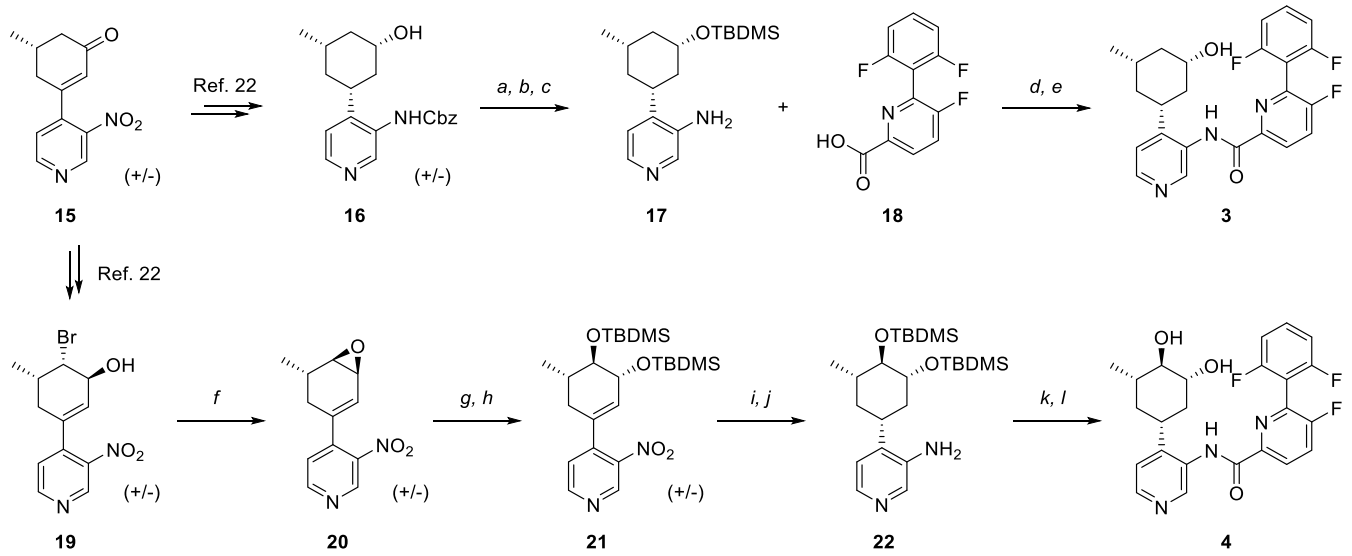
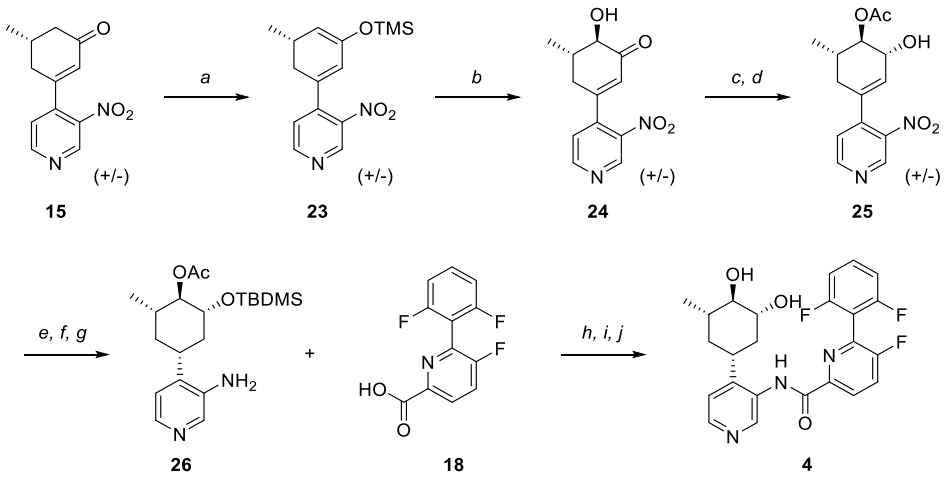
Scheme 1. Synthesis of Compounds 3 and 4[a]



[a]Reagents and conditions: (a) chiral separation; (b) TBDMSCl, imidazole, >99%; (c) H_2, Pd on carbon, EtOAc, MeOH, 2 h, 85%; (d) EDC, HOAt, DMF; (e) HCl, THF, MeOH, 37% (over 2 steps); (f) ^{t}BuOK, THF, 90%; (g) AcOH (cat.), MeCN, water, 33%; (h) TBDMSCl, imidazole, 50%; (i) H_2, Pd on carbon, EtOAc, EtOH, 50%; (j) chiral separation, 43%; (k) **18**, EDC, HOAt, DMF, >99%; (l) HCl, THF, MeOH, 57% (over 2 steps).

Scheme 2. Synthesis of Compound 4[a]



[a]Reagents and conditions: (a) LiHMDS, TMSCl, THF, 0 °C, 96%; (b) Oxone, $NaHCO_3$, acetone, water, EtOAc, 2 h, 61% (**24**/**24-cis** = 1.3:1); (c) acetic anhydride, pyridine, >99%; (d) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, THF, MeOH, 97%; (e) TBDMSCl, imidazole, DCM, 84%; (f) H_2, Pd on carbon, EtOAc, EtOH, 88%; (g) chiral separation, 45%; (h) EDC, HOAt, DMF; (i) HCl, THF, MeOH; (j) LiOH, THF, water, 17% (over 3 steps).

compounds were synthesized through amide coupling reaction between A−B rings and the corresponding C−D ring acids. When C−D ring acid intermediates were not accessible, C ring acids were used for amide coupling reaction with A−B rings followed by Suzuki coupling reaction to introduce the D ring. A racemic mixture of final compounds or intermediates was resolved by chiral HPLC. On the basis of X-ray co-crystal structural information and/or biochemical results, we assigned absolute stereochemistry for the final compounds.

The synthesis of compound **3** started from intermediate **16**. The desired enantiomer of **16**, benzyl (4-((1*R*,3*S*,5*S*)-3-hydroxy-5-methylcyclohexyl)pyridin-3-yl)carbamate, was protected with a TBDMS group followed by Cbz group deprotection. The resulting amino intermediate **17** underwent amide coupling with **18** and subsequent deprotection of the TBDMS group under acidic conditions to yield compound **3**.[37]

To synthesize the A-B ring diol intermediate **22**, the bromohydrin intermediate **19**[23] was subjected to basic conditions to produce an epoxide intermediate **20**. An acid-catalyzed epoxide ring opening reaction afforded a mixture of trans/cis cyclohexene diols in ∼3:2 ratio. After protecting the alcohols with TBDMS, a mixture of trans and cis isomers was separated by a series of purification methods: silica column chromatography and preparative reverse phase HPLC. The desired trans diastereomer **21** underwent palladium-catalyzed hydrogenation followed by chiral resolution to yield enantiomerically pure intermediate **22**. After amide coupling between **22** and **18** followed by desilylation compound **4** was obtained. However, the current method to intermediate **22**

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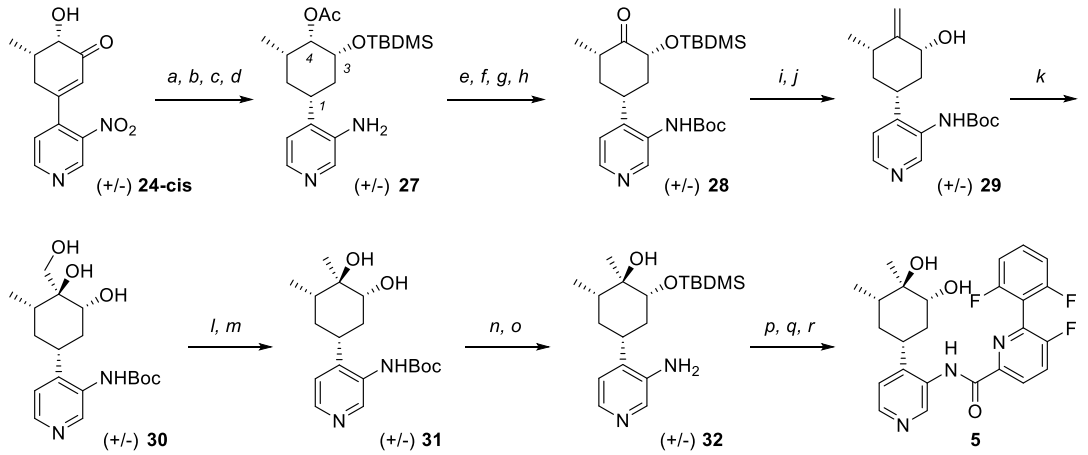
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 3. Synthesis of Compound 5[a]



[a]Reagents and conditions: (a) Ac_2O, pyridine, 83%; (b) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, THF, MeOH, 82%; (c) TBDMSCl, imidazole, DCM, 75%; (d) H_2, Pd on carbon, EtOAc, EtOH, >99%; (e) Boc_2O, DMAP, DCM, 65%; (f) LiOH, THF, 60 °C, 99%; (g) TBDMSCl, imidazole, DCM, 55%; (h) Dess-Martin reagent, DCM, 90%; (i) $TMSCH_2MgCl$, THF, 35 °C, 92%; (j) HCl (cat.), acetone, 97%; (k) OsO_4 (cat.), NMO, 77%; (l) MsCl, pyridine, DCM; (m) $LiAlH_4$, THF, 98% (over 2 steps); (n) TBDMSCl, imidazole, DCM, 70%; (o) TFA, DCM, >99%; (p) EDC, HOAt, DMF; (q) HCl, THF, MeOH, 45% (over 2 steps); (r) chiral separation, 39%.

gave a very poor yield (3% yield over 8 steps from **15**) that was not amenable for analogue and large scale. To improve the overall yield, we considered an orthogonal protection on the diol functionality strategy that would facilitate selective functionalization of A-ring diol. To execute this, we examined several different α-hydroxylation reactions of intermediate **15**. Among them, Rubottom oxidation of **23** successfully provided the desired α-hydroxy enone **24**. Whereas the reaction with mCPBA gave poor yield, *in situ*-generated DMDO[38] afforded good yield of a mixture of trans and cis hydroxy cyclohexenones (1.3:1 ratio) The facial selectivity was slightly favored toward the desired trans isomer **24**, which was readily separable by silica gel chromatography.

The trans isomer **24** was protected with an acetyl group, and the resulting acetate intermediate underwent $NaBH_4$ reduction in the presence of $CeCl_3$ which selectively yielded trans acetoxy cyclohexenol **25**. Subsequent TBDMS protection and palladium-catalyzed hydrogenation gave a racemic mixture of aniline intermediates that underwent chiral separation to give the desired aniline (**26**). Compound **26** was then coupled with intermediate **18** to yield compound **4** after the deprotection of TBDMS and acetyl groups. When compared to the previous route to **22**, this new synthetic route largely improved the yield of intermediate **26** with one less step (11% yield over 7 steps from **15**), enabling the scale-up of compound **4** for *in vivo* pharmacokinetic studies.

For the synthesis of compound **5** with a quaternary stereogenic center at the 4-position on the cyclohexane ring, we used intermediate **27** (Scheme 3) to modify the 4-position hydroxyl group selectively. Intermediate **27** was synthesized from the cis isomer of the Rubottom oxidation of **23** (**24-cis**), (51% yield over 4 steps, Scheme 2). The amino group of **27** was then protected with a Boc group to yield a mixture of mono- and bis-Boc protected products. Under basic conditions, the acetyl group underwent hydrolysis to give a 4-hydroxyl intermediate, which concomitantly removed one Boc group from the bis-Boc protected intermediate. The resulting 4-hydroxyl intermediate underwent 1,4 O → O silyl migration[39] leading to a mixture of 3- and 4-hydroxyl intermediates. The mixture was subsequently subjected to Dess−Martin oxidation reaction to afford a mixture of 3- and 4-cyclohexanone intermediates. The desired 4-cyclohexanone **28** was separated by silica column chromatography (20% yield over 4 steps from **27**, Scheme 3). To establish a tertiary alcohol at the 4-position, we tested the addition of MeMgBr to cyclohexanone **28** to examine axial/equatorial selectivity, which led to the predominant formation of undesired *cis*-cyclohexane diol resulting from the equatorial attack of the methyl group. On the basis of this outcome, we thought that an exo-methylene group at the 4-position would provide a handle to install hydroxyl group. The exo-methylene intermediate **29** was successfully synthesized through Peterson olefination reaction of intermediate **28**. While the epoxidation of **29** with mCPBA or DMDO mostly formed the N-oxide of **29** as a byproduct, osmium-catalyzed dihydroxylation provided the triol intermediate **30** in a highly stereoseletive manner.[40] The mesylation of the triol **30** selectively occurred at the primary hydroxy group, followed by $LiAlH_4$ reduction to yield the diol intermediate **31**. Sequential selective TBDMS protection and Boc de-protection provided intermediate **32**, which underwent amide coupling with **18**. After TBDMS deprotection, the coupling product was resolved by chiral column chromatography to yield compound **5**. The exo-methylene strategy successfully led to the desired *trans*-diol intermediate **32** having a quaternary carbon center, but the overall yield to **32** was only 4% over 18 steps from **15**.

To further improve the current synthetic route, we sought the possibility of early establishment of the exo-methylene moiety on intermediate **15**. We explored several different carbon-carbon bond formation reactions of **15**; however, reactions requiring a strong base, such as LiHMDS turned rapidly to a dark purple solution, which was supposedly due to the electron-withdrawing nitropyridine ring, leading to decomposition of the starting material. We found a suitable example for the introduction of an exo-methylene group to the α-position of cyclohexenone using Eschenmoser's salt.[41] In order to implement this strategy in our route, we prepared the silyl enol ether **23** from the common cyclohexenone intermediate **15**, which reacted with Eschenmoser's salt to give dimethylaminomethylation intermediate **33**. Subsequent

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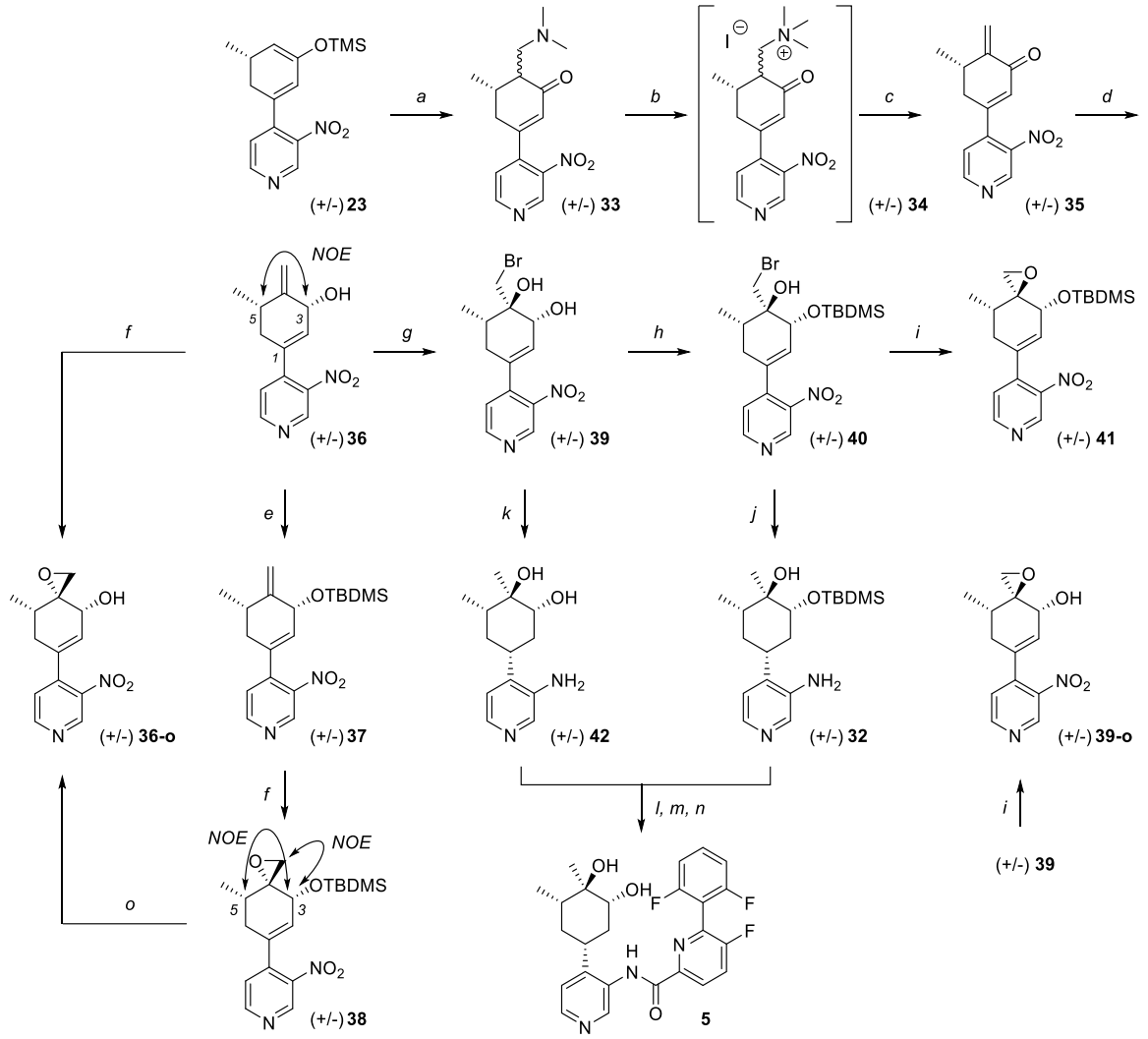
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 4. Synthesis of Exo-methylene Intermediate 36 and Compound 5[a]



[a]Reagents and conditions: (a) Eschenmoser's salt, DCM, 18 h, 99%; (b) Iodomethane, THF, 18 h; (c) $NaHCO_3$, 5 h, 63%; (d) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH, 72%; (e) TBDMSCl, imidazole, DCM, 86%; (f) mCPBA, $NaHCO_3$, DCM, 14 h, 90%; (g) NBS, THF, water, 3 h, 56%; (h) TBDMSCl, imidazole, DMF, 75%; (i) K_2CO_3, MeOH, water, 1 h, 97%; (j) H_2 (200 psi), $Pd(OH)_2$ on carbon, MeOH, 4 d; 88%; (k) H_2, Pd on carbon, pyridine, MeOH, 24 h; 70%; (l) **18**, EDC, HOAt, DMF, >99%; (m) HCl, THF, MeOH, 86% (over 2 steps); (n) chiral resolution, 31%; (o) TBAF, THF.

methylation gave a quaternary ammonium salt **34**, which underwent elimination under basic conditions to yield *α-exo*-methylene cyclohexenone **35**. For further functionalization of **35**, we performed selective reduction of the carbonyl group under Luche conditions, which successfully yielded *cis*-cyclohexenol intermediate **36** as the major isomer.

Our initial idea for creating a quarternary carbinol stereogenic center from the exo-methylene group of **36** was to employ an epoxidation/ring-opening strategy. To do this, after TBDMS group protection of **36**, the epoxidation of **37** was performed with mCPBA to afford epoxide intermediate **37** in good yield without forming a pyridin N-oxide byproduct that was observed in the epoxidation of NHBoc-pyridine compound **29** (Scheme 3). However, it turned out that the exo-methylene intermediate **37** underwent axial epoxidation, providing the epoxide **38** with unwanted regioselectivity that was confirmed by a ^{1}H NMR NOE study of **38**. This result was well supported by literature precedents[42] for similar exo-methylene cyclohexanes, which also described the equatorial epoxide formation through a halohydrin/ring closing reaction from exo-methylene cyclohexanes. Then, we carried out the bromohydrin formation by reacting intermediate **36** with N-bromosuccinimide in the presence of water, which resulted in the selective formation of a bromohydrin product **39** at the exo-methylene group with a decent facial selectivity toward the desired trans diol (~2.5:1). The stereochemistry of **39** was confirmed with the structure of the epoxide **41** that was synthesized from a ring closing reaction of TBDMS-protected bromohydrin **40** under basic conditions. The ^{1}H NMR spectra of **41** was clearly different from that of the epoxide **38** from mCPBA reaction.[43] Having established a trans relationship between the vicinal diol group of **39**, we next tried to find a global reduction condition for de-halogenation and hydrogenation of nitro and alkene groups. Under high pressure palladium-catalyzed hydrogenation conditions, we were able to obtain intermediate **32** in a decent yield with a good facial selectivity (~4:1). The amide coupling of **32** with **18** followed by deprotection of TBDMS group on under acidic conditions

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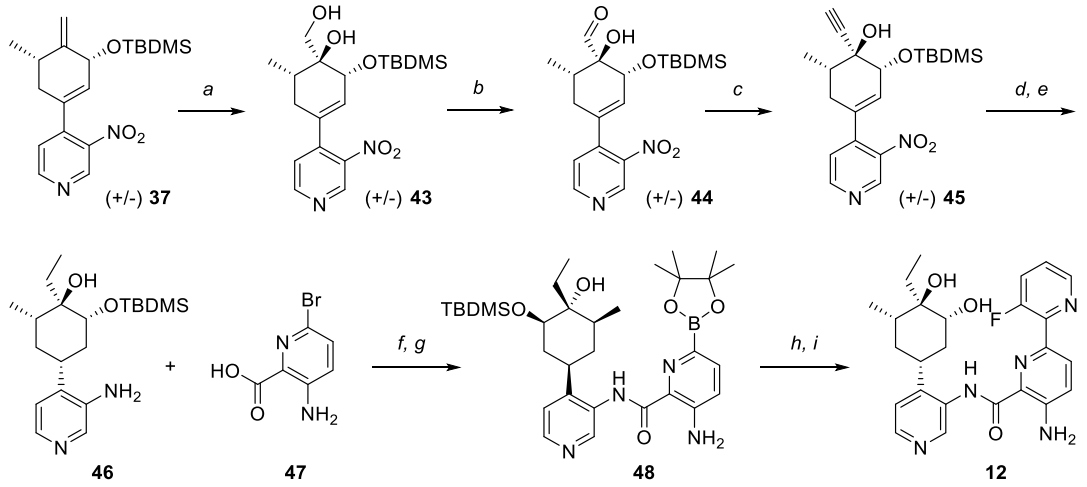
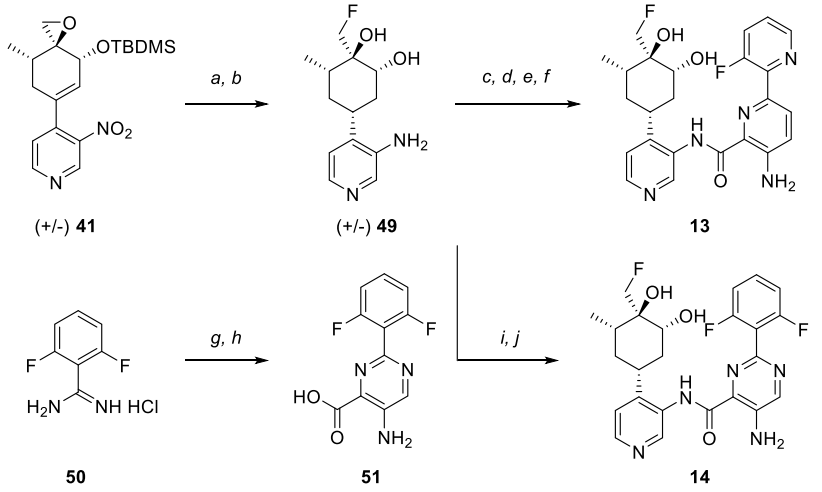
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 5. Synthesis of Compound 12[a]



[a]Reagents and conditions: (a) OsO_4, NMO, acetone, water, 1 h, 95%; (b) Dess−Martin periodinane, DCM, 72 h, 77%; (c) Bestmann−Ohira's reagent, MeOH, K_2CO_3, 1.5 h, 96%; (d) H_2, Pd on carbon, MeOH, 12 h, 33%; (e) chiral separation, 33%; (f) EDC, HOAt, DMF, 16 h; (g) 4,4,4′,4′,5,5,5′,5′-octamethyl-2,2′-bi(1,3,2-dioxaborolane), $Pd_2(dba)_3$, PCy_3, KOAc, dioxane, K_2CO_3, μW, 120 °C, 10 min; (h) 2-bromo-3-fluoropyridine, $PdCl_2(dppf)$, DME, Na_2CO_3, μW, 120 °C, 10 min; (i) HCl, THF, MeOH, 1 h, 30% (over 3 steps).

Scheme 6. Synthesis of Compounds 13 and 14[a]



[a]Reagents and conditions: (a) $Et_3N{\cdot}3HF$, 100 °C, 8 h, 46%; (b) H_2, Pd on carbon, MeOH, 12 h, 49%; (c) 3-amino-6-bromopicolinic acid **47**, EDC, HOAt, DMF, 16 h, 85%; (d) 4,4,4′,4′,5,5,5′,5′-octamethyl-2,2′-bi(1,3,2-dioxaborolane), $Pd_2(dba)_3$, PCy_3, KOAc, dioxane, K_2CO_3, μW, 120 °C, 10 min; (e) 2-bromo-3-fluoropyridine, $PdCl_2(dppf)$, DME, Na_2CO_3, μW, 120 °C, 10 min, 10% (over 3 steps); (f) chiral resolution, 24%; (g) mucobromic acid, NaOEt, EtOH, 40 °C, 2 h; (h) NH_4OH, $CuSO_4$, 110 °C, 1 h, 25% (over 2 steps); (i) **51**, EDC, HOAt, DMF, 12 h, 25%; (j) chiral resolution, 34%.

gave compound **5** after chiral resolution. When compared to the previous synthesis in Scheme 3 (4% yield over 17 steps from **15**), this new synthetic method to intermediate **32** made significant improvements in the overall yield as well as the number of reaction steps (15% yield over 7 steps from **15**). However, the hydrogenation reaction of **40** was very slow even under high pressure of hydrogen gas (200 psi). We reasoned that hydrogen bromide generated *in situ* may slow down the reactions. We continued to examine milder conditions using intermediate **39** and discovered that in the presence of pyridine, palladium-catalyzed hydrogenation went to completion even at room temperature and under atmospheric hydrogen pressure. Pearlman's catalyst was also replaced by Pd on carbon. The diol intermediate **42** was obtained in good yield with similar levels of selectivity (trans/cis = ∼4:1). We also found that chiral SFC could resolve the diol **42** in the absence of the TBDMS group, saving one additional step. The desired enantiomer was used for the synthesis of compound **5**. This newly developed synthetic route facilitated the scale-up of compound **5** for *in vivo* animal studies. Compounds **6** to **11** were synthesized using the desired diols of **32** or **42** and the corresponding carboxylic acids.

For the synthesis of compound **12**, intermediate **37** underwent Upjohn dihydroxylation to give a triol intermediate **43** with high levels of selectivity. Subsequent oxidation reaction yielded the aldehyde **44**, which was then reacted with Bestmann−Ohira's reagent for one carbon homologation.[44,45] The resulting alkyne **45** was subjected to a palladium-catalyzed hydrogenation followed by chiral resolution to afford the desired aniline intermediate **46**. The amide coupling of **46**

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J. Med. Chem. XXXX, XXX, XXX−XXX

with 3-amino-6-bromopicolinic acid **47** followed by boronic ester formation yielded intermediate **48**, which underwent Suzuki coupling reaction with 2-bromo-3-fluoropyridine to afford compound **12**.[46]

To synthesize fluoromethyl aniline **49**, the ring-opening reaction of epoxide intermediate **41** was performed in the presence of triethylamine trihydrofluoride (Scheme 6).[47−50] Subsequent Pd-catalyzed hydrogenation yielded intermediate **49**. Compound **14** was synthesized through amide coupling of **49** with pyrimidine acid intermediate **51** followed by chiral resolution. Compound **13** was prepared from **49** and 3-amino-6-bromopicolinic acid **47** (Scheme 6) using the same synthetic procedures used for the synthesis of compound **12** (Scheme 5). For the synthesis of intermediate **51**, intermediate **50** underwent a cyclization reaction with mucobromic acid to give 5-bromo-2-(2,6-difluorophenyl)pyrimidine-4-carboxylic acid, which turned to intermediate **51** through copper-catalyzed amination.

CONCLUSIONS

Our goal to identify a pan-PIM inhibitor bearing a cyclohexyl diol A-ring moiety was successfully achieved, leading to a cellularly potent compound **14** with moderate metabolic clearance profile. Structure-based design guided us to find compound **5** with the axial methyl group at the 4-position of the cyclohexyl A-ring that improved cellular potency but suffered from poor oral bioavailability and high *in vivo* clearance in rats. We focused on the modulation of physicochemical properities, especially, the reduction of Log D value by introducing more polar C and/or D ring to improve metabolic stability. In parallel, the introduction of a fluorine atom near the diol functionality to improve permeability resulted in a cellularly potent cyclohexyl diol compound **14** with moderate metabolic stability. For our structure−activity relationship (SAR) exploration, we established efficient and scalable synthetic routes to complex cyclohexyl diol ring systems that contain contiguous stereogenic centers including a tertiary alcohol. In particular, we identified (±)-cis-5-methyl-6-methylene-3-(3-nitropyridin-4-yl)cyclohex-2-en-1-ol **36** as a versatile intermediate for the synthesis of all the cyclohexyl analogues, which could be employed to synthesize a diverse set of cyclohexane ring systems.

EXPERIMENTAL SECTION

General. The compounds and/or intermediates were characterized by high performance liquid chromatography (HPLC) using a Waters Millenium chromatography system with a 2695 Separation Module (Milford, MA). The analytical columns were Alltima C18 reverse phase, 4.6 × 50 mm, flow 2.5 mL/min, from Alltech (Deerfield, IL). A gradient elution was used, typically starting with 5% acetonitrile/95% water and progressing to 100% acetonitrile over a period of 10 min. All solvents contained 0.1% TFA. Compounds were detected by ultraviolet light (UV) absorption at either 220 or 254 nm. HPLC solvents were from Burdick and Jackson (Muskegan, MI) or Fisher Scientific (Pittsburgh, PA). In some instances, purity was assessed by thin layer chromatography (TLC) using glass or plastic backed silica gel plates, such as, for example, Baker-Flex Silica Gel 1B2-F flexible sheets. TLC results were readily detected visually under ultraviolet light or by employing well known iodine vapor and other various staining techniques.

Mass spectrometric analysis was performed on one of two LCMS instruments: a Waters System (Alliance HT HPLC and a Micromass ZQ mass spectrometer; Column: Eclipse XDB-C18, 2.1 × 50 mm; solvent system: 5−95% (or 35−95%, or 65−95% or 95−95%) acetonitrile in water with 0.05% TFA; flow rate 0.8 mL/min; molecular weight range 200−1500; cone Voltage 20 V; column temperature 40 °C) or a Hewlett Packard System (Series 1100 HPLC; Column: Eclipse XDB-C18, 2.1 × 50 mm; solvent system: 1−95% acetonitrile in water with 0.05% TFA; flow rate 0.8 mL/min; molecular weight range 150−850; cone Voltage 50 V; column temperature 30 °C). All masses were reported as those of the protonated parent ions.

HRMS ESI-MS data were recorded using a Synapt G2 HDMS (TOF mass spectrometer, Waters) with electrospray ionization source. The resolution of the MS system was approximately 15,000. Leucine Enkephalin was used as lock mass (internal standards) infused from lockspray probe. The compound was infused into the mass spectrometer by UPLC (Acquity, Waters) from sample probe. The separation was performed on Acquity UPLC BEH-C18 1 × 50 mm column at 0.2 mL/min flow rate with the gradient from 5 to 95% in 3 min. Solvent A was water with 0.1% formic acid, and solvent B was acetonitrile with 0.1% formic acid. The mass accuracy of the system has been found to be <5 ppm with lock mass.

^{1}H Nuclear magnetic resonance (NMR) analysis was performed on some of the compounds with a Varian 300 or 400 MHz NMR and a Bruker 500 MHz NMR. The spectral reference was either TMS or the known chemical shift of the solvent.

Preparative separations were carried out using a Teledyne ISCO chromatography system, by flash column chromatography using silica gel (230−400 mesh) packing material, or by HPLC using a Waters 2767 Sample Manager, C18 reversed phase column, 30 × 50 mm, flow 75 mL/min. Typical solvents employed for the Teledyne ISCO chromatography system and flash column chromatography were dichloromethane, methanol, ethyl acetate, hexane, acetone, aqueous ammonia (or ammonium hydroxide), and triethylamine. Typical solvents employed for the reverse phase HPLC were varying concentrations of acetonitrile and water with 0.1% TFA. The purity of all compounds screened in the biological assays was examined by LC−MS analysis and was found to be ≥ 95%.

Synthesis of Compound 3. 4-((1*R*,3*S*,5*S*)-3-(*tert*-Butyldimethylsilyloxy)-5-methylcyclohexyl)pyridin-3-amine (**17**). (±)-benzyl 4-((1*R*,3*S*,5*S*)-3-hydroxy-5-methylcyclohexyl)pyridin-3-ylcarbamate **16** was resolved by chiral HPLC (4.5 mg/1 mL ethanol, heptane/isopropanol = 85:15, 1 mL/min, IA column). Benzyl 4-((1*R*,3*S*,5*S*)-3-hydroxy-5-methylcyclohexyl)pyridin-3-ylcarbamate was eluted at 8.526 min. To a solution of benzyl 4-((1*R*,3*S*,5*S*)-3-hydroxy-5-methylcyclohexyl)pyridin-3-ylcarbamate (423 mg, 1.243 mmol) in DMF (1.5 mL) was added TBDMSCl (243 mg, 1.615 mmol) and imidazole (186 mg, 2.73 mmol). The reaction mixture was stirred at room temperature overnight. After diluted with water, the reaction mixture was extracted with EtOAc (15 mL) three times. The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to yield crude benzyl 4-((1*R*,3*S*,5*S*)-3-(*tert*-butyldimethylsilyloxy)-5-methylcyclohexyl)pyridin-3-ylcarbamate (571 mg, >99% yield). LCMS (*m*/*z*): 455.4 $[M + H]^+$. To a solution of crude benzyl 4-((1*R*,3*S*,5*S*)-3-(*tert*-butyldimethylsilyloxy)-5-methylcyclohexyl)-pyridin-3-ylcarbamate (571 mg, 1.256 mmol) in EtOAc (3 mL) was added Pd on carbon (134 mg, 0.126 mmol) and MeOH (6 mL). The mixture was degassed with nitrogen stream for 10 min, purged with hydrogen gas, and equipped with hydrogen balloon. The reaction mixture was stirred for 3 h. The mixture was filtered through 1μm PTFE Acrodisc filter, eluting with EtOAc (30 mL). The filtrate was concentrated in vacuo to yield crude 4-((1*R*,3*S*,5*S*)-3-(*tert*-butyldimethylsilyloxy)-5-methylcyclohexyl)pyridin-3-amine **17** (403.4 mg, 98% yield). 6-(2,6-difluorophenyl)-5-fluoro-*N*-(4-((1*R*,3*S*,5*S*)-3-hydroxy-5-methylcyclohexyl)pyridin-3-yl)picolinamide (**3**). To a solution of 4-((1*R*,3*S*,5*S*)-3-(*tert*-butyldimethylsilyloxy)-5-methylcyclohexyl)pyridin-3-amine **17** (50 mg, 0.156 mmol) in DMF (1.5 mL) was added 6-(2,6-difluorophenyl)-5-fluoropicolinic acid **18** (47.4 mg, 0.187 mmol), HOAt (25.5 mg, 0.187 mmol), and EDC (35.9 mg, 0.187 mmol). The reaction mixture was stirred at room temperature overnight. LCMS showed the coupling reaction was completed. LCMS (*m*/*z*): 556.4 $[M + H]^+$. After partitioned between EtOAc (10 mL) and water (10 mL), the crude product was

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J. Med. Chem. XXXX, XXX, XXX−XXX

extracted with EtOAc. The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, filtered off, and concentrated in vacuo to yield crude *N*-(4-((1*R*,3*S*,5*S*)-3-(*tert*-butyldimethylsilyloxy)-5-methylcyclohexyl)pyridin-3-yl)-6-(2,6-difluorophenyl)-5-fluoropicolinamide. The crude product (87 mg, 0.157 mmol) was dissolved in THF (1 mL) and methanol (0.5 mL) followed by addition of 6 N HCl aqueous solution (0.048 mL, 1.57 mmol). The reaction mixture was stirred at room temperature for 2 h. After neutralized with saturated Na_2CO_3 aqueous solution, the mixture was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, filtered off, and concentrated in vacuo. The crude product was purified by reverse phase HPLC. After pure fractions were combined and free-based with saturated Na_2CO_3 aqueous solution, the crude product was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, filtered off, and concentrated in vacuo. After dissolving in water and acetonitrile (1:1, 2 mL) and adding 1 equivalent of 1 N HCl soltuion, the solution was lyophilized to yield 6-(2,6-difluorophenyl)-5-fluoro-*N*-(4-((1*R*,3*S*,5*S*)-3-hydroxy-5-methylcyclohexyl)pyridin-3-yl)picolinamide **3** as its HCl salt (60.9 mg, 0.137 mmol, 87% yield over 2 steps). LCMS (*m*/*z*): 442.2 $[M + H]^+$. HRMS (*m*/*z*): calcd for $C_{24}H_{23}N_3O_2F_3$ $[M + H]^+$, 442.1742; found, 442.1745. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 10.6 (s, 1H), 9.09 (s, 1H), 8.71 (d, *J* = 5.85 Hz, 1H), 8.38 (dd, *J* = 8.6, 3.92 Hz, 1H), 8.24 (t, *J* = 8.81 Hz, 1H), 7.78 (d, *J* = 6.24 Hz, 1H), 7.65−7.76 (m, 2H), 7.36 (t, *J* = 8.21 Hz, 2H), 3.41 (m, 1H), 2.95 (m, 1H), 1.75 (m, 2H), 1.61 (m, 1H), 1.4 (m, 1H), 1.31 (m, 1H), 0.99 (m, 1H), 0.89 (m, 1H), 0.86 (d, *J* = 6.63 Hz, 3H).

Synthesis of Compound 4 (Scheme 1). (±)-4-((1*S*,5*S*)-5-Methyl-7-oxabicyclo[4.1.0]hept-2-en-3-yl)-3-nitropyridine (**20**). To a solution of (±)-(1*S*,5*S*,6*S*)-6-bromo-5-methyl-3-(3-nitropyridin-4-yl)-cyclohex-2-enol **19** (1.05 g, 3.35 mmol) in THF (33.5 mL) was added potassium *tert*-butoxide (0.564 g, 5.03 mmol) at room temperature. The reaction mixture was stirred for 10 min. The reaction mixture was quenched with NH_4Cl solution and extracted with EtOAc by washing with water and brine. The organic layers were dried over anhydrous sodium sulfate, filtered off, and dried in vacuo. The crude product **20** (700 mg, 3.01 mmol, 90%) was used in the next step without further purification. R_f = 0.5 (50% EtOAc/hexanes). LCMS (*m*/*z*): 251.2 $[M + H]^+$ (detected as a diol). To a solution of crude (±)-4-((1*S*,5*S*)-5-methyl-7-oxabicyclo[4.1.0]hept-2-en-3-yl)-3-nitropyridine **20** (700 mg, 3.01 mmol) in CH_3CN (20.1 mL) and H_2O (10.1 mL) was added a catalytic amount of acetic acid (0.052 mL, 0.904 mmol) at room temperature. The reaction mixture was stirred for 16 h at room temperature. After quenched with $NaHCO_3$ solution, the reaction mixture was concentrated to remove the majority of CH_3CN and the residue was partitioned between EtOAc and water. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. A mixture of *trans*- and *cis*-(±)-6-methyl-4-(3-nitropyridin-4-yl)-cyclohex-3-ene-1,2-diols was obtained as a white solid in 33.1% yield (250 mg, 0.99 mmol) by flash column chromatography. R_f = 0.3 (100% EtOAc; diols were not separable on TLC). LCMS (*m*/*z*): 251.1 $[M + H]^+$. To a solution of a mixture of *trans*- and *cis*-(±)-6-methyl-4-(3-nitropyridin-4-yl)cyclohex-3-ene-1,2-diols (250 mg, 0.99 mmol) in DMF (3.33 mL, 0.3 M) was added TBDMSCl (1.05 g, 7.0 mmol), imidazole (612 mg, 9 mmol) at room temperature. The reaction mixture was stirred at room temperature overnight. After quenched with saturated $NaHCO_3$, the reaction mixture was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The trans and cis isomers were separated by ISCO (gradient eluting with EtOAc and hexanes) and subsequent preparative reverse phase HPLC (55−95% acetonitrile in water, then run 5−95% acetonitrile in water) to yield 130 mg of *cis*-(±)-4-((3*S*,4*R*,5*S*)-3,4-bis(*tert*-butyldimethylsilyloxy)-5-methylcyclohex-1-enyl)-3-nitropyridine (27.2% yield) and 240 mg of *trans*-(±)-4-((3*R*,4*R*,5*S*)-3,4-bis(*tert*-butyldimethylsilyloxy)-5-methylcyclohex-1-enyl)-3-nitropyridine **21** (50.2% yield). LCMS (*m*/*z*): 479.0 $[M + H]^+$, respectively. 4-((1*R*,3*R*,4*R*,5*S*)-3,4-Bis((*tert*-butyldimethylsilyl)-oxy)-5-methylcyclohexyl)pyridin-3-amine (**22**). To a solution of *trans*-(±)-4-((3*R*,4*R*,5*S*)-3,4-bis(*tert*-butyldimethylsilyloxy)-5-methylcyclohex-1-enyl)-3-nitropyridine **21** (550 mg, 1.149 mmol) in ethanol (5.7 mL) and EtOAc (5.7 mL) was added Pd on carbon (122 mg). After degassed for 15 min, the reaction mixture was charged with hydrogen at room temperature and stirred at room temperature overnight. The reaction mixture was filtered through Celite pad and washed with EtOAc. The filtrate was concentrated in vacuo. The crude product was purified by ISCO (heptane/EtOAc) to yield *trans*-(±)-4-((1*R*,3*R*,4*R*,5*S*)-3,4-bis(*tert*-butyldimethylsilyloxy)-5-methylcyclohexyl)pyridin-3-amine (260 mg, 58% yield). LCMS (*m*/*z*): 451.2 $[M + H]^+$. 260 mg of the pure racemate was resolved by chiral HPLC (IC column, 1 mL/min, 5% IPA in heptane). Peak 1: 4-((1*S*,3*S*,4*S*,5*R*)-3,4-bis(*tert*-butyldimethylsilyloxy)-5-methylcyclohexyl)pyridin-3-amine **22-ent** (114 mg, 99% ee, R_t = 6.1 min). LCMS (*m*/*z*): 451.2 $[M + H]^+$. Peak 2: 4-((1*R*,3*R*,4*R*,5*S*)-3,4-bis(*tert*-butyldimethylsilyloxy)-5-methylcyclohexyl)pyridin-3-amine **22** (112 mg, >99% ee, R_t = 7.83 min). LCMS (*m*/*z*): 451.2 $[M + H]^+$. 6-(2,6-difluorophenyl)-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5-methylcyclohexyl)pyridin-3-yl)-5-fluoropicolinamide (**4**). To a reaction vial with a stir bar was added 4-((1*R*,3*R*,4*R*,5*S*)-3,4-bis(*tert*-butyldimethylsilyloxy)-5-methylcyclohexyl)pyridin-3-amine **22** (17 mg, 0.038 mmol), 6-(2,6-difluorophenyl)-5-fluoropicolinic acid **18** (10.50 mg, 0.041 mmol), and HOAt (6.15 mg, 0.045 mmol) followed by DMF (0.4 mL). After the reaction mixture was homogeneously mixed, EDC (8.67 mg, 0.045 mmol) was added to the reaction mixture in a single portion. The reaction mixture was stirred at room temperature overnight. After quenched with water, the reaction mixture was extracted with EtOAc. The organic layers were washed with water, 1 M NaOH aqueous solution, and brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. Crude *N*-(4-((1*R*,3*R*,4*R*,5*S*)-3,4-bis(*tert*-butyldimethylsilyloxy)-5-methylcyclohexyl)pyridin-3-yl)-6-(2,6-difluorophenyl)-5-fluoropicolinamide was obtained in >99% yield (26 mg, 0.038 mmol). LCMS (*m*/*z*): 686.4 $[M + H]^+$. To a solution of crude *N*-(4-((1*R*,3*R*,4*R*,5*S*)-3,4-bis(*tert*-butyldimethylsilyloxy)-5-methylcyclohexyl)pyridin-3-yl)-6-(2,6-difluorophenyl)-5-fluoropicolinamide (26 mg, 0.038 mmol) in MeOH (126 μL) and THF (253 μL) was added HCl (120 μL, 0.360 mmol) at room temperature. The reaction mixture was stirred at room temperature for 1 h. After quenched with saturated $NaHCO_3$ solution, the reaction mixture was extracted with EtOAc. The organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. After preparative HPLC purification and lyophilzation, 6-(2,6-difluorophenyl)-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5-methylcyclohexyl)pyridin-3-yl)-5-fluoropicolinamide **4** was obtained as its TFA salt (12.4 mg, 0.021 mmol, 57% over 2 steps). LCMS (*m*/*z*): 458.3 $[M + H]^+$. HRMS (*m*/*z*): calcd for $C_{24}H_{23}N_3O_3F_3$ $[M + H]^+$, 458.1692; found, 458.1691. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 10.5 (s, 1H), 8.88 (s, 1H), 8.51 (d, *J* = 5.83 Hz, 1H), 8.31 (dd, *J* = 8.61, 3.91 Hz, 1H), 8.16 (t, *J* = 9.0 Hz, 1H), 7.65 (m, 2H), 7.29 (t, *J* = 8.22 Hz, 2H), 3.15 (ddd, *J* = 10.96, 8.61, 4.3 Hz, 1H), 2.94 (d, *J* = 12.3 Hz, 1H), 2.75 (t, *J* = 9.0 Hz, 1H), 1.81 (m, 1H), 1.58 (d, *J* = 12.3, 2.54 Hz, 1H), 1.46 (m, 1H), 1.23 (m, 2H), 0.84 (d, *J* = 6.26 Hz, 3H).

Synthesis of Compound 4 (Scheme 2). Trans-(±)-(5*S*,6*R*)-6-hydroxy-5-methyl-3-(3-nitropyridin-4-yl)cyclohex-2-enone (**23**). A solution of (±)-5-methyl-3-(3-nitropyridin-4-yl)cyclohex-2-enone **15** (3 g, 12.9 mmol) and TMSCl (1.65 mL, 12.9 mmol) in THF was added LiHMDS (1.0 M solution in THF, 13.56 mL, 13.56 mmoL) at 0 °C slowly over 1 h. The reaction mixture was warmed up to room temperature and stirred for 2 h. The reaction mixture was quenched with $NaHCO_3$ aqueous solution and removed THF *in vacuo*. The residue was extracted with EtOAc 3 times. The organic layers were washed with water and brine, dried over anhydrous K_2CO_3, filtered, and concentrated *in vacuo* to yield crude (±)-4-(5-methyl-3-(trimethylsilyloxy) cyclohexa-1,3-dienyl)-3-nitropyridine **23** (3.6 g, 11.83 mmol, 96% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 9.14−9.00 (m, 1H), 8.80−8.64 (m, 1H), 7.42−7.25 (m, 1H), 6.00−5.88 (m, 1H), 4.98 (br s, 1H), 2.86−2.53 (m, 1H), 2.51−2.29 (m, 1H), 2.27−2.03 (m, 1H), 1.21−1.03 (m, 3H), 0.36−0.15 (m, 9H). A

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J. Med. Chem. XXXX, XXX, XXX−XXX

solution of (±)-4-(5-methyl-3-(trimethylsilyloxy) cyclohexa-1,3-dienyl)-3-nitropyridine (3.6 g, 10.39 mmol), sodium bicarbonate (4.36 g, 51.9 mmol), acetone (7.63 mL, 104 mmol), water (51.9 mL), and ethyl acetate (51.9 mL) was vigorously stirred at 0 °C. To this, a solution of Oxone (6.39 g, 10.39 mmol) in water (45 mL was slowly added *via* dropping funnel for 1 h 30 min. After addition, the reaction mixture was allowed to stir at room temperature for 2 h. The pH of the mixture was adjusted to around 4 by adding 1 N HCl aqueous solution. After stirring for 30 min, the reaction mixture was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4 and filtered, and concentrated *in vacuo*. A mixture of crude trans and cis products was purified by ISCO (50% EtOAc in heptane). For *trans*-(±)-(5*S*,6*R*)-6-hydroxy-5-methyl-3-(3-nitropyridin-4-yl)cyclohex-2-enone **24** (890 mg, 34.5% yield), LCMS (*m*/*z*): 249 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ ppm 9.34 (s, 1H), 8.9 (d, J = 5.2 Hz, 1H), 7.28 (s, 1H), 6.09 (d, J = 2.4 Hz, 1H), 3.99 (d, J = 12.4 Hz, 1H), 3.68 (d, J = 2.0 Hz, 1H), 2.61−2.52 (m, 2H), 2.39 (m, 1H), 1.27 (d, J = 8.0 Hz, 3H). For *cis*-(±)-(5*S*,6*R*)-6-hydroxy-5-methyl-3-(3-nitropyridin-4-yl)cyclohex-2-enone **24-cis** (680 mg, 26.4% yield), LCMS (*m*/*z*): $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ ppm 9.32 (s, 1H), 8.9 (d, J = 4.0 Hz, 1H), 7.28 (s, 1H), 6.06 (d, J = 2.8 Hz, 1H), 4.53 (d, J = 12.0 Hz, 1H), 3.60 (m, 1H), 3.11 (m, 1H), 2.8 (m, 1H), 2.47 (d, J = 2.0 Hz, 1H), 1.09 (d, J = 8 Hz, 3H). (1*R*,2*R*,6*S*)-2-Hydroxy-6-methyl-4-(3-nitropyridin-4-yl)cyclohex-3-enyl acetate (**25**). To a solution of (±)-(5*S*,6*R*)-6-hydroxy-5-methyl-3-(3-nitropyridin-4-yl)-cyclohex-2-enone **24** (4.2 g, 16.9 mmol) in pyridine (56.4 mL) was slowly added acetic anhydride (3.19 mL, 33.8 mmol). The reaction mixture was stirred at room temperature overnight. After all volatile materials were removed in vacuo, the residue was diluted with EtOAc. The organic layers were washed with 1 N aqueous HCl solution and water, dried over anhydrous sodium sulfate, filtered off, and concentrated in vacuo. The crude product was purified by ISCO (gradient EtOAc in heptane) to yield (±)-(1*R*,6*S*)-6-methyl-4-(3-nitropyridin-4-yl)-2-oxocyclohex-3-enyl acetate (4.9 g, 16.9 mmol, >99% yield). LCMS (*m*/*z*): 291.1 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ ppm 9.35 (s, 1H), 8.9 (d, J = 4.0 Hz, 1H), 7.27 (s, 1H), 6.04 (s, 1H), 5.56 (d, J = 12.0 Hz, 1H), 2.65−2.55 (m, 3H), 2.25 (s, 3H), 1.16 (d, J = 4.0 Hz, 3H). To a suspension of (±)-(1*R*,6*S*)-6-methyl-4-(3-nitropyridin-4-yl)-2-oxocyclohex-3-enyl acetate (580 mg, 2 mmol) in THF (4 mL) and MeOH (6 mL) was added cerium(III) chloride heptahydrate (744 mg, 2 mmol). The reaction mixture was stirred for 2 h at room temperature. At 0 °C, $NaBH_4$ (76 mg, 2 mmol) was slowly added to the reaction mixture, which was then stirred at room temperature overnight. The reaction mixture was extracted with EtOAc (20 mL) twice. The combined organic layers were washed with water and $NaHCO_3$ solution and brine. The crude product was purified by ISCO (0−40% EtOAc in heptane) to yield (±)-(1*R*,2*R*,6*S*)-2-hydroxy-6-methyl-4-(3-nitropyridin-4-yl)cyclohex-3-enyl acetate **25** (567 mg, 1.94 mol, 97% yield). LCMS (*m*/*z*): 293.0 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ ppm 9.16 (s, 1H), 8.76 (d, J = 4.8 Hz, 1H), 7.27 (s, 1H), 6.04 (s, 1H), 5.3 (m, 1H on alkene), 4.75 (dd, J = 10.8, 7.2 Hz, 1H on acetoxy attached carbon), 4.38 (m, 1H on hydroxyl attached carbon), 2.35 (s, 1H), 2.21 (m, 2H), 2.19 (s, 3H), 1.16 (d, J = 6.0 Hz, 3H). To a solution of (1.34 g, 4.58 mmol) in DCM (9.17 mL) was added imidazole (0.468 g, 6.88 mmol) and TBDMSCl (0.829 g, 5.50 mmol). The reaction mixture was stirred at room temperature overnight. After diluted with water, the mixture was extracted with EtOAc (50 mL). The combined organic layers were washed with water and $NaHCO_3$ solution and brine. The crude product was purified by ISCO (0−40% EtOAc in heptane) to yield (±)-(1*R*,2*R*,6*S*)-2-(*tert*-butyldimethylsilyloxy)-6-methyl-4-(3-nitropyridin-4-yl)cyclohex-3-enyl acetate (1.56 g, 3.84 mmol, 84% yield). LCMS (*m*/*z*): 407.1 $[M + H]^+$. To a solution of (±)-(1*R*,2*R*,6*S*)-2-(*tert*-butyldimethylsilyloxy)-6-methyl-4-(3-nitropyridin-4-yl)cyclohex-3-enyl acetate (411 mg, 1.011 mmol) in ethanol (1.7 mL) and EtOAc (1.7 mL) was added Pd on carbon (108 mg). The reaction mixture was equipped with hydrogen gas balloon. The reaction mixture was stirred at room temperature overnight, filtered through Celite pad, and washed with EtOAc, and then, the filtrate was concentrated in vacuo. The crude product was purified by ISCO (gradient EtOAc in heptane) to yield (±)-(1*R*,2*R*,4*R*,6*S*)-4-(3-aminopyridin-4-yl)-2-((*tert*-butyldimethylsilyl)oxy)-6-methylcyclohexyl acetate **26-rac** (335 mg, 0.885 mmol, 88% yield). LCMS (*m*/*z*): 379.4 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ ppm 8.06 (s, 1H), 8.04 (d, J = 4.0 Hz, 1H), 6.99 (d, J = 8.0 Hz, 1H), 4.69 (t, J = 8.0 Hz, 1H), 3.7 (m, 1H), 3.61 (s, 1H), 2.65 (m, 1H), 2.11 (s, 3H), 2.05 (m, 3H), 1.82 (m, 1H), 0.95 (d, J = 8.0 Hz, 3H), 0.86 (s, 9H), 0.06 (s, 6H). 335 mg of **26-rac** was resolved by chiral HPLC (AS-H column, 1 mL/min, heptane/EtOH = 98:2). Peak 1: (1*S*,2*S*,4*S*,6*R*)-4-(3-aminopyridin-4-yl)-2-(*tert*-butyldimethylsilyloxy)-6-methylcyclohexyl acetate **26-ent** (115.7 mg, >99% ee, R_t = 6.72 min). LCMS (*m*/*z*): 379.0 $[M + H]^+$. Peak 2: (1*R*,2*R*,6*S*)-2-(*tert*-butyldimethylsilyloxy)-6-methyl-4-(3-nitropyridin-4-yl)cyclohex-3-enyl acetate **26** (154.7 mg, 99% ee, R_t = 11.31 min). LCMS (*m*/*z*): 379.0 $[M + H]^+$. 6-(2,6-Difluorophenyl)-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5-methylcyclohexyl)pyridin-3-yl)-5-fluoropicolinamide (**4**). To a reaction vial with a stir bar was added (1*R*,2*R*,6*S*)-2-(*tert*-butyldimethylsilyloxy)-6-methyl-4-(3-nitropyridin-4-yl)cyclohex-3-enyl acetate **26** (40 mg, 0.106 mmol), 6-(2,6-difluorophenyl)-5-fluoropicolinic acid **18** (29.4 mg, 0.116 mmol), and HOAt (18.7 mg, 0.137 mmol) followed by DMF (0.2 mL). After the reaction mixture was homogeneously mixed, EDC (30.4 mg, 0.158 mmol) was added to the reaction mixture in a single portion. The reaction mixture was stirred at room temperature for 18 h. After quenched with water, the reaction mixture was extracted with EtOAc. The organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo to afford crude (1*R*,2*R*,4*R*,6*S*)-2-(*tert*-butyldimethylsilyloxy)-4-(3-(6-(2,6-difluorophenyl)-5-fluoropicolinamido)pyridin-4-yl)-6-methylcyclohexyl acetate. LCMS (*m*/*z*): 614.3 $[M + H]^+$. The crude product was dissolved in THP (1 mL) and MeOH (0.5 mL) followed by 1 N HCl aqueous solution (0.5 mL). After stirring for 3 h, the mixture was neutralized with saturated $NaHCO_3$ solution and extracted with EtOAc. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified by reverse phase HPLC. The pure fractions were combined and lyophilized to yield (1*R*,2*R*,4*R*,6*S*)-4-(3-(6-(2,6-difluorophenyl)-5-fluoropicolinamido)pyridin-4-yl)-2-hydroxy-6-methylcyclohexyl acetate (19.4 mg, 0.039 mmol). LCMS (*m*/*z*): 500.2 $[M + H]^+$. The acetate product was dissolved in MeOH (0.5 mL), and THF (0.5 mL) was added 1 N LiOH solution (0.5 mL) at room temperature. The reaction mixture was stirred at room temperature overnight. After neutralized with 1 N HCl aqueous solution, the reaction mixture was extracted with EtOAc. The organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. After preparative HPLC purification and lyophilzation, 6-(2,6-difluorophenyl)-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-3,4-dihydroxy-5-methylcyclohexyl)pyridin-3-yl)-5-fluoropicolinamide **4** was obtained as its TFA salt (10 mg, 0.018 mmol, 17% over 3 steps). LCMS (*m*/*z*): 458.1 $[M + H]^+$.

Synthesis of Compound 5 (Scheme 3). *cis*-(±)-(1*S*,2*R*,4*R*,6*S*)-4-(3-Aminopyridin-4-yl)-2-(*tert*-butyldimethylsilyloxy)-6-methylcyclohexyl acetate (**27**). For step a, *cis*-(±)-(1*S*,6*S*)-6-methyl-4-(3-nitropyridin-4-yl)-2-oxocyclohex-3-enyl acetate was synthesized from *cis*-(±)-(5*S*,6*R*)-6-hydroxy-5-methyl-3-(3-nitropyridin-4-yl)cyclohex-2-enone (**24-cis**) according to step c in Scheme 2 (83% yield), LCMS (*m*/*z*): 291.1 $[M + H]^+$. For step b, *cis*-(±)-(1*S*,2*R*,6*S*)-2-hydroxy-6-methyl-4-(3-nitropyridin-4-yl)cyclohex-3-enyl acetate was synthesized according to step d in Scheme 2 (82% yield), LCMS (*m*/*z*): 293.0 $[M + H]^+$. For step c, *cis*-(±)-(1*S*,2*R*,6*S*)-2-(*tert*-butyldimethylsilyloxy)-6-methyl-4-(3-nitropyridin-4-yl)cyclohex-3-enyl acetate was synthesized according to step e in Scheme 2 (75% yield), LCMS (*m*/*z*): 407.2 $[M + H]^+$. For step d, *cis*-(±)-(1*S*,2*R*,4*R*,6*S*)-4-(3-aminopyridin-4-yl)-2-(*tert*-butyldimethylsilyloxy)-6-methylcyclohexyl acetate **27** was synthesized according to step f in Scheme 2 (>99% yield), LCMS (*m*/*z*): 379.4 $[M + H]^+$. (±)-*tert*-Butyl (4-((1*R*,3*R*,5*S*)-3-((*tert*-butyldimethylsilyl)oxy)-5-methyl-4-oxocyclohexyl)pyridin-3-yl)-carbamate (**28**). To a solution of (±)-(1*S*,2*R*,4*R*,6*S*)-4-(3-aminopyridin-4-yl)-2-(*tert*-butyldimethylsilyloxy)-6-methylcyclohexyl acetate (2.8 g, 7.40 mmol) in DCM (37.0 mL) was added DMAP

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J. Med. Chem. XXXX, XXX, XXX−XXX

(0.181 g, 1.48 mmol) and Boc_2O (4.29 mL, 18.49 mmol). The reaction mixture was stirred at room temperature over-the-weekend. After diluted with water, the mixture was extracted with DCM (10 mL). The combined organic layers were washed with water and $NaHCO_3$ solution and brine. The crude product was purified by ISCO (0−50% EtOAc in heptane) to yield (±)-(1*S*,2*R*,4*R*,6*S*)-4-(3-(bis(*tert*-butoxycarbonyl)amino)pyridin-4-yl)-2-(*tert*-butyldimethylsilyloxy)-6-methylcyclohexyl acetate (2.8 g, 4.84 mmol, 65% yield). LCMS (*m*/*z*): 579.3 $[M + H]^+$. To a solution of (±)-(1*S*,2*R*,4*R*,6*S*)-4-(3-(bis(*tert*-butoxycarbonyl)amino)pyridin-4-yl)-2-(*tert*-butyldimethylsilyloxy)-6-methylcyclohexyl acetate (2.8 g, 4.84 mmol) in THF (16.12 mL) was added LiOH (9.67 mL, 9.67 mmol). The reaction mixture was heated at 60 °C overnight. LCMS still showed the starting material remained. Next day, after adding 4 mL of LiOH more, the reaction was heated at 60 °C again overnight. LCMS showed the reaction went to completion, but two product peaks indicated that a half of TBDMS was migrated to de-acetylated alcohol (~1:1). After neutralized with 1 N HCl aqueous solution, the mixture was extracted with EtOAc. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified by ISCO (gradient EtOAc in heptane) to afford a mixture of (±)-*tert*-butyl 4-((1*R*,3*R*,4*S*,5*S*)-3-(*tert*-butyldimethylsilyloxy)-4-hydroxy-5-methylcyclohexyl)pyridin-3-ylcarbamate and (±)-*tert*-butyl 4-((1*R*,3*R*,4*S*,5*S*)-4-(*tert*-butyldimethylsilyloxy)-3-hydroxy-5-methylcyclohexyl)pyridin-3-ylcarbamate (2.1 g, 4.81 mmol, 99%). LCMS (*m*/*z*): 437.2 $[M + H]^+$, 0.98 min and 1.0 min. To a solution of a mixture of (±)-*tert*-butyl 4-((1*R*,3*R*,4*S*,5*S*)-3-(*tert*-butyldimethylsilyloxy)-4-hydroxy-5-methylcyclohexyl)pyridin-3-ylcarbamate and (±)-*tert*-butyl 4-((1*R*,3*R*,4*S*,5*S*)-4-(*tert*-butyldimethylsilyloxy)-3-hydroxy-5-methylcyclohexyl)pyridin-3-ylcarbamate (2.1 g, 4.81 mmol) in DCM (16 mL) was added Dess−Martin periodinane (2.55 g, 6.01 mmol) at room temperature. The reaction mixture was stirred at room temperature overnight. LCMS showed the reaction went to completion. After quenched with $NaHCO_3$ solution/$Na_2S_2O_3$ solution (8:1), the mixture was vigorously stirred for 2 h and extracted with EtOAc. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo. The desired cyclohexanone product was separated by ISCO (0−80% EtOAc in DCM) to yield (±)-*tert*-butyl 4-((1*R*,3*R*,5*S*)-3-(*tert*-butyldimethylsilyloxy)-5-methyl-4-oxocyclohexyl)pyridin-3-ylcarbamate (577 mg, 1.328 mmol, 55% yield). LCMS (*m*/*z*): 435.2 $[M + H]^+$, 0.96 min. The undesired cyclohexanone, (±)-*tert*-butyl 4-((1*R*,3*R*,4*S*,5*S*)-4-(*tert*-butyldimethylsilyloxy)-3-hydroxy-5-methylcyclohexyl)pyridin-3-ylcarbamate, was obtained in 34% yield (405 mg, 0.932 mmol). LCMS (*m*/*z*): 435.2 $[M + H]^+$, 1.01 min. (±)-*tert*-Butyl 4-((1*R*,3*R*,5*S*)-3-hydroxy-5-methyl-4-methylenecyclohexyl)pyridin-3-ylcarbamate (**29**). To a solution of (±)-*tert*-butyl 4-((1*R*,3*R*,5*S*)-3-(*tert*-butyldimethylsilyloxy)-5-methyl-4-oxocyclohexyl)pyridin-3-ylcarbamate (1.09 g, 2.508 mmol) in THF (12.5 mL) was added trimethylsilylmethylmagnesium chloride (25.1 mL, 25.1 mmol) at room temperature. The reaction mixture was heated to 35 °C overnight. After quenched with saturated NH_4Cl aqueous solution, the mixture was extracted with EtOAc. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified by ISCO (gradient EtOAc in heptane) to yield (±)-*tert*-butyl 4-((1*R*,3*R*,4*S*,5*S*)-3-(*tert*-butyldimethylsilyloxy)-4-hydroxy-5-methyl-4-((trimethylsilyl)methyl)cyclohexyl)pyridin-3-ylcarbamate (1.2 g, 2.3 mmol, 92%). LCMS (*m*/*z*): 523.2 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ ppm 8.6 (s, 1H), 8.38 (d, J = 4.0 Hz, 1H), 7.21 (d, J = 4.0 Hz, 1H), 6.11 (s, 1H), 4.69 (t, J = 8.0 Hz, 1H), 2.41 (m, 1H), 2.31 (s, 1H), 1.79 (m, 2H), 1.61 (m, 1H), 1.52 (s, 9H), 1.37 (m, 1H), 1.28 (m, 1H), 1.06 (d, J = 8.0 Hz, 3H), 0.9 (s, 9H), 0.88 (m, 2H), 0.13 (s, 4H), 0.09 (s, 9H). To a solution of (±)-*tert*-butyl 4-((1*R*,3*R*,4*S*,5*S*)-3-(*tert*-butyldimethylsilyloxy)-4-hydroxy-5-methyl-4-((trimethylsilyl)methyl)cyclohexyl)pyridin-3-ylcarbamate (1.2 g, 2.295 mmol) in acetone (7.65 mL) was added 0.191 mL of 1 N HCl aqueous solution. The mixture was stirred at room temperature for 20 min. After neutralized with saturated $NaHCO_3$ aqueous solution, the mixture was extracted with EtOAc. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo to yield crude (±)-*tert*-butyl 4-((1*R*,3*R*,5*S*)-3-hydroxy-5-methyl-4-methylenecyclohexyl)pyridin-3-ylcarbamate (710 mg, 2.23 mmol, 97%). LCMS (*m*/*z*): 319.0 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ ppm 8.69 (s, 1H), 8.36 (d, J = 8.0 Hz, 1H), 7.12 (d, J = 4.0 Hz, 1H), 6.2 (s, 1H), 5.15 (s, 1H), 4.88 (s, 1H), 4.23 (m, 1H), 3.08 (m, 1H), 2.27 (m, 2H), 1.91 (m, 2H), 1.79 (m, 2H), 1.65 (m, 1H), 1.52 (s, 9H), 1.18 (d, J = 8.0 Hz, 3H). (±)-*tert*-Butyl 4-((1*R*,3*R*,4*S*,5*S*)-3,4-dihydroxy-4-(hydroxymethyl)-5-methylcyclohexyl)pyridin-3-ylcarbamate (**30**). To a solution of (±)-*tert*-butyl 4-((1*R*,3*R*,5*S*)-3-hydroxy-5-methyl-4-methylenecyclohexyl)pyridin-3-ylcarbamate (490 mg, 1.539 mmol) in acetone (24.6 mL) and water (6.15 mL) was added 4-methylmorpholine 4-oxide (NMO) (541 mg, 4.62 mmol) followed by osmium tetroxide (4% in water) (0.097 mL, 0.308 mmol) at room temperature. The reaction was stirred overnight but did not attain completion. After adding the same amounts of the reagents, the reaction was stirred overnight once more. The reaction was quenched with saturated Na_2SO_3 aqueous solution (30 mL) and stirred for additional 1 h. After evaporation of acetone in vacuo, the mixture was extracted with EtOAc. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified by ISCO (gradient EtOAc in heptane) to yield (±±)-4-((1*R*,3*R*,4*S*,5*S*)-3,4-dihydroxy-4-(hydroxymethyl)-5-methylcyclohexyl)pyridin-3-ylcarbamate **30** (416 mg, 1.18 mmol, 77% yield). LCMS (*m*/*z*): 353.1 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ ppm 8.62 (s, 1H), 8.34 (d, J = 4.8 Hz, 1H), 7.15 (d, J = 5.2 Hz, 1H), 6.86 (s, 1H), 4.20 (d, J = 11.6 Hz, 1H), 3.82−3. 72 (m, 2H), 2.97 (m, 1H), 2.08 (m, 1H), 2.04 (m, 1H), 1.75 (m, 2H), 1.68 (m, 1H), 1.65 (m, 1H), 1.51 (s, 9H), 1.0 (d, J = 7.2 Hz, 3H). (±)-*tert*-Butyl 4-((1*R*,3*R*,4*R*,5*S*)-3,4-dihydroxy-4,5-dimethylcyclohexyl)pyridin-3-ylcarbamate (**31**). To a solution of (±)-*tert*-butyl 4-((1*R*,3*R*,4*S*,5*S*)-3,4-dihydroxy-4-(hydroxymethyl)-5-methylcyclohexyl)pyridin-3-ylcarbamate (84 mg, 0.238 mmol) in DCM (794 μL) was added pyridine (96 μL, 1.192 mmol). After cooling down to 0 °C, methanesulfornyl chloride (18.57 μL, 0.238 mmol) was added to the reaction mixture, which was stirred overnight. However, LCMS showed that a small amount of the product formed. One equivalent of methanesulfornyl chloride was added to the mixture, which was stirred for 2 h. LCMS showed no starting material remained. After quenched with water, the mixture was extracted with EtOAc. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo to yield (±)-((1*S*,2*R*,4*R*,6*S*)-4-(3-(*tert*-butoxycarbonylamino)pyridin-4-yl)-1,2-dihydroxy-6-methylcyclohexyl)methyl methanesulfonate. The crude product was used in the next step without further purification. LCMS (*m*/*z*): 337.1 $[M + H]^+$. To a solution of (±)-((1*S*,2*R*,4*R*,6*S*)-4-(3-(*tert*-butoxycarbonylamino)pyridin-4-yl)-1,2-dihydroxy-6-methylcyclohexyl)methyl methanesulfonate (85 mg, 0.197 mmol) in THF (1.974 mL) was slowly added $LiAlH_4$ (14.99 mg, 0.395 mmol) at room temperature. The reaction mixture was stirred at room temperature overnight. After quenched with water slowly, the reaction was extracted with EtOAc. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo to yield (±)-*tert*-butyl 4-((1*R*,3*R*,4*R*,5*S*)-3,4-dihydroxy-4,5-dimethylcyclohexyl)pyridin-3-ylcarbamate **31** (65 mg, 0.193 mmol, 98%). The crude product was used in the next step without further purification. (±)-(1*S*,2*R*,4*R*,6*S*)-4-(3-aminopyridin-4-yl)-2-(*tert*-butyldimethylsilyloxy)-1,6-dimethylcyclohexanol (**32**). To solution of (±)-*tert*-butyl 4-((1*R*,3*R*,4*R*,5*S*)-3,4-dihydroxy-4,5-dimethylcyclohexyl)pyridin-3-ylcarbamate (77 mg, 0.229 mmol) in DCM (0.458 mL) was added imidazole (23.37 mg, 0.34 mmol) and TBDMSCl (37.9 mg, 0.252 mmol). The reaction mixture was stirred at room temperature overnight. After quenched with water, the mixture was extracted with EtOAc. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified by ISCO (gradient EtOAc in heptane) to yield (±)-*tert*-butyl 4-((1*R*,3*R*,4*R*,5*S*)-3-(*tert*-butyldimethylsilyloxy)-4-hydroxy-4,5-dimethylcyclohexyl)pyridin-3-ylcarbamate (72 mg, 70% yield). LCMS (*m*/*z*): 451.4 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ ppm 8.67 (s, 1H), 8.37 (d, J = 5.2 Hz, 1H), 7.15 (d, J = 5.2 Hz, 1H), 6.12 (s, 1H), 3.61 (dd, J = 11.2 Hz, 4 Hz, 1H), 2.88 (m,

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J. Med. Chem. XXXX, XXX, XXX−XXX

1H), 2.1 (s, 1H), 1.89 (m, 1H), 1.76 (m, 1H), 1.68 (m, 1H), 1.52 (s, 9H), 1.23 (m, 2H), 1.14 (s, 3H), 1.01 (d, J = 7.2 Hz, 3H), 0.91 (s, 9H), 0.11 (s, 3H), 0.79 (s, 3H). To a solution of (±)-*tert*-butyl 4-((1*R*,3*R*,4*R*,5*S*)-3-(*tert*-butyldimethylsilyloxy)-4-hydroxy-4,5-dimethylcyclohexyl)pyridin-3-ylcarbamate (72 mg, 0.16 mmol) in DCM (1.2 mL) was added TFA (0.4 mL). The reaction mixture was stirred for 1 h 30 min. After diluted with toluene, the volatile materials were removed in vacuo, neutralized with saturated $NaHCO_3$ aqueous solution, and extracted with EtOAc. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo to yield crude (±)-(1*R*,2*R*,4*R*,6*S*)-4-(3-aminopyridin-4-yl)-2-(*tert*-butyldimethylsilyloxy)-1,6-dimethylcyclohexanol **32** (56 mg, 0.16 mmol, >99%). The crude product was used in the next step without further purification. LCMS (m/z): 351.3 $[M + H]^+$. 6-(2,6-Difluorophenyl)-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-3,4-dihydroxy-4,5-dimethylcyclohexyl)pyridin-3-yl)-5-fluoropicolinamide (**5**). To solution of (±)-(1*R*,2*R*,4*R*,6*S*)-4-(3-aminopyridin-4-yl)-2-(*tert*-butyldimethylsilyloxy)-1,6-dimethylcyclohexanol **32** (32 mg, 0.091 mmol) in DMF (114 μL) was added 6-(2,6-difluorophenyl)-5-fluoropicolinic acid **18** (25.4 mg, 0.1 mmol), HOAt (16.2 mg, 0.119 mmol) and EDC (26.2 mg, 0.137 mmol). The reaction mixture was stirred at room temperature for 18 h. After quenched with water, the mixture was extracted with EtOAc. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo. LCMS (m/z): 586.3 $[M + H]^+$. The crude product was dissolved in THF (1 mL) and MeOH (0.5 mL) followed by 3 N HCl aqueous solution (0.5 mL). The reaction mixture was stirred for 3 h. After neutralized with saturated $NaHCO_3$ aqueous solution, the mixture was extracted with EtOAc. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified by ISCO (0−10% MeOH in EtOAc) to yield 24 mg of (±)-6-(2,6-difluorophenyl)-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-3,4-dihydroxy-4,5-dimethylcyclohexyl)pyridin-3-yl)-5-fluoropicolinamide **5-rac** (45% yield over 2 steps). LCMS (m/z): 472.2 (MH^+). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.96 (br s, 1H), 9.34 (s, 1H), 8.44 (d, J = 4.0 Hz, 1H), 8.42 (m, 1H), 7.78 (t, J = 8.0 Hz, 1H), 7.52 (m, 1H), 7.19 (d, J = 4 Hz, 1H), 7.11 (t, J = 8.0 Hz, 1H), 3.57 (dd, J = 12 Hz, 4 Hz, 1H), 2.99 (m, 1H), 2.02 (m, 1H), 1.78−1.52 (m, 4H), 1.36−1.26 (m, 1H), 1.12 (s, 3H), 0.9 (d, J = 4 Hz, 3H). 24 mg of **5-rac** was resolved by chiral HPLC (AD-H column, heptane/EtOH = 75:25, 1 mL/min). Peak 1: 6-(2,6-difluorophenyl)-*N*-(4-((1*S*,3*S*,4*S*,5*R*)-3,4-dihydroxy-4,5-dimethylcyclohexyl)pyridin-3-yl)-5-fluoropicolinamide **5-ent** (9.7 mg, >99% ee, R_t = 4.45 min). LCMS (m/z): 472.2 $[M + H]^+$. Peak 2 6-(2,6-difluorophenyl)-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-3,4-dihydroxy-4,5-dimethylcyclohexyl)pyridin-3-yl)-5-fluoropicolinamide **5** (9.3 mg, >99% ee, R_t = 6.056 min). LCMS (m/z): 472.2 $[M + H]^+$. HRMS (m/z): calcd for $C_{25}H_{25}N_3O_3F_3$ $[M + H]^+$, 472.1848; found, 472.1850.

Synthesis of Compound 5 (Scheme 4). (±)-5-Methyl-6-methylene-3-(3-nitropyridin-4-yl)cyclohex-2-enone (**35**). To a solution of Eschenmoser's salt (19.33 g, 105 mmol) in DCM (140 mL) was added (±)-4-(5-methyl-3-(trimethylsilyloxy)cyclohexa-1, 3-dienyl)-3-nitropyridine **23** in DCM (100 mL) at 0 °C slowly over 60 min. The reaction mixture was allowed to warm up to room temperature and stirred for 18 h. After the reaction mixture was transferred to a larger vessel and diluted with DCM (100 mL), 1 M HCl aqueous solution (160 mL) was added to the reaction mixture, which was stirred for 20 min at 0 °C. After 2 N NaOH aqueous solution (80 mL) was slowly added at 0 °C, the reaction mixture was stirred for 1 h and pH of the mixture was adjusted to 12 by adding 3 N NaOH aqueous solution. After organic layers were separated, aqueous phase was extracted with DCM 3 times. The combined organic layers were dried over anhydrous Na_2SO_4, filtered off, and concentrated *in vacuo* to yield crude (±)-6-((dimethylamino)methyl)-5-methyl-3-(3-nitropyridin-4-yl)cyclohex-2-enone **33** (27.5 g, 95 mmol, 99% yield). LCMS (m/z): 290.0 $[M + H]^+$. To a solution of (±)-6-((dimethylamino) methyl)-5-methyl-3-(3-nitropyridin-4-yl) cyclohex-2-enone **33** (27.5 g, 95 mmol) in THF (190 mL) was added iodomethane (7.13 mL, 114 mmol) slowly at 0 °C. The reaction mixture was allowed to warm up to room temperature and stirred for 18 h to yield the trimethyl ammonium salt intermediate **34**. After saturated $NaHCO_3$ aqueous solution (35.9 g in water) was added, the reaction mixture was stirred at room temperature for 5 h, diluted with EtOAc, and stirred at room temperature for another 6 h. After the organic layers were separated, aqueous phase was extracted with EtOAc 3 times, the combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The crude product was purified by ISCO (gradient EtOAc in heptane) to afford (±)-5-methyl-6-methylene-3-(3-nitropyridin-4-yl)cyclohex-2-enone **35** (14.7 g, 60.2 mmol, 63%). LCMS (m/z): 245 $[M + H]^+$. ^{1}H NMR (400 M Hz, $CDCl_3$): δ ppm 9.33 (s, 1H), 8.88 (d, J = 5.1 Hz, 1H), 6.19 (m, 1H), 6.16 (s, 1H), 5.42 (s, 1H), 3.15 (m, 1H), 2.59 (dd, J = 17.4, 5.3 Hz, 1H), 2.43 (ddd, J = 7.3, 9.5, 2.2 Hz, 1H), 1.31 (d, J = 6.7 Hz, 3H). (±)-*cis*-5-Methyl-6-methylene-3-(3-nitropyridin-4-yl)cyclohex-2-enol (**36**). To a solution of (±)-5-methyl-6-methylene-3-(3-nitropyridin-4-yl)cyclohex-2-enone **35** (13.7 g, 56.1 mmol) in methanol (260 mL) was added cerium (III) chloride heptahydrate (23 g, 61.7 mmol). The reaction mixture was stirred at room temperature for 1 h. After cooled down to at 0 °C, $NaBH_4$ (2.1 g, 56.1 mmol) was added slowly and stirred for 30 min. After quenched with water, the volatile materials were removed *in vacuo*, and saturated $NaHCO_3$ aqueous solution was added into mixture with vigorous stirring. The reaction mixture was extracted with EtOAc, and the organic layers were washed with brine and dried over anhydrous sodium sulfate, filtered off, and concentrated *in vacuo*. The crude product was purified by ISCO (heptane/EtOAc, 80:20 to 20:80) to give (±)-*cis*-5-methyl-6-methylene-3-(3-nitropyridin-4-yl)-cyclohex-2-enol **36** as a yellow solid (10 g, 40.76 mmol, 72% yield). LCMS (m/z): 247 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 9.13 (s, 1H), 8.75 (d, J = 4.7 Hz, 1H), 7.26 (s, 1H), 5.72 (m, 1H), 5.25 (s, 1H), 5.03 (m, 1H), 4.86 (br s, 1H), 2.67 (m, 1H), 2.39 (dd, J = 16.6, 4.9 Hz, 1H), 2.11 (br s, 1H), 1.91 (m, 1H), 1.23 (d, J = 6.7 Hz, 3H). 1D NOE: selective irradiation of δ 4.86 ppm (H at 3-position) revealed the strongest NOE to δ 2.67 ppm (H at 5-position) (Scheme 4).

(±)-4-((3*R*,5*S*)-3-(*tert*-Butyldimethylsilyloxy)-5-methyl-4-methylenecyclohex-1-enyl)-3-nitropyridine (**37**). To solution of (±)-(1*R*,5*S*)-5-methyl-6-methylene-3-(3-nitropyridin-4-yl) cyclohex-2-enol **36** (5.0 g, 20.3 mol) in DCM (77 mL) was added imidazole (2.07 g, 30.5 mmol) and TBDMSCl (3.37 g, 22.33 mol). The reaction mixture was stirred for 18 h at room temperature. The volatile materials were removed *in vacuo*, and the residue was partitioned between EtOAc and water. The combined organic layers were washed with water and brine and dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The crude material was purified by flash column chromatography (gradient EtOAc in heptane) to yield (±)-4-((3*R*,5*S*)-3-(*tert*-butyldimethylsilyloxy)-5-methyl-4-methylenecyclohex-1-enyl)-3-nitropyridine **37** (6.3 g, 17.47 mmol, 86% yield). LCMS (m/z): 361.0 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 9.12 (s, 1H), 8.73 (d, J = 5.1 Hz, 1H), 7.27 (d, J = 5.1 Hz, 1H), 5.57 (t, J = 2.5 Hz, 1H), 5.24−5.20 (m, 1H), 4.98−4.94 (m, 1H), 4.84−4.92 (m, 1H), 2.57−2.72 (m, 1H), 2.37 (dd, J = 16.6, 5.3 Hz, 1H), 2.11−2.01 (m, 1H), 1.20 (d, J = 6.7 Hz, 3H), 0.94 (m, 9H), 0.15 (s, 3H), 0.12 (s, 3H). (±)-4-((3*R*,4*R*,8*S*)-4-(*tert*-Butyldimethylsilyloxy)-8-methyl-1-oxaspiro[2.5]oct-5-en-6-yl)-3-nitropyridine (**38**). To a solution of (±)-4-((3*R*,5*S*)-3-(*tert*-butyldimethylsilyloxy)-5-methyl-4-methylenecyclohex-1-enyl)-3-nitropyridine **37** (90 mg, 0.250 mmol) in DCM (832 μL) was added sodium bicarbonate (31.5 mg, 0.374 mmol). After cooling down to 0 °C, mCPBA (61.5 mg, 0.275 mmol) was added to the reaction mixture. The reaction was allowed to warm up to room temperature for 14 h. After quenched with $NaHCO_3$ solution, the reaction mixture was extracted with EtOAc. The organic layers were washed with saturated $NaHCO_3$ aquesous solution and brine, dried over anhydrous sodium sulfate, filtered off, and dried in vacuo. The crude product was purified by ISCO (gradient EtOAc in heptane) to yield (±)-4-((3*R*,4*R*,8*S*)-4-(*tert*-butyldimethylsilyloxy)-8-methyl-1-oxaspiro[2.5]oct-5-en-6-yl)-3-nitropyridine **38** (85 mg, 0.225 mmol, 90% yield). LCMS (m/z): 377.1, $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 9.15 (s, 1H), 8.77 (d, J = 4.0 Hz, 1H), 7.36 (d, J = 4.0 Hz, 1H), 5.65 (m, 1H), 4.53 (m, 1H), 2.95 (d, J = 8.0

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J. Med. Chem. XXXX, XXX, XXX−XXX

Hz, 1H), 2.84 (d, J = 4.0 Hz, 1H), 2.39−2.25 (m, 3H), 0.96 (d, J = 8.0 Hz, 3H), 0.9 (s, 9H), 0.1 (s, 3H), 0.08 (s, 3H). 1D NOE: selective irradiation of δ 4.83 ppm (H at 3-position) revealed the strong NOEs to δ 2.95 ppm (H at epoxide) and δ 2.34 ppm (H at position 5) (Scheme 4).

(±)-(3*R*,4*R*,8*S*)-8-Methyl-6-(3-nitropyridin-4-yl)-1-oxaspiro[2.5]-oct-5-en-4-ol **36-o** was synthesized from (±)-(1*R*,5*S*)-5-methyl-6-methylene-3-(3-nitropyridin-4-yl)cyclohex-2-enol **36** according to step f in Scheme 4 (96% yield). LCMS (m/z): 263.1 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 9.16 (s, 1H), 8.77 (d, J = 4.0 Hz, 1H), 7.29 (d, J = 4.0 Hz, 1H), 5.72 (m, 1H), 4.50 (m, 1H), 3.11 (d, J = 8.0 Hz, 1H), 2.97 (d, J = 4.0 Hz, 1H), 2.53 (m, 1H), 2.27 (m, 2H), 0.91 (d, J = 8.0 Hz, 3H). TBDMS protecting group of **38** was deprotected to give **36-o**, ^{1}H NMR spectra of which was matched with that of **36-o** from **36** (Scheme 4). (1*R*,2*R*,6*S*)-1-(bromomethyl)-2-((*tert*-butyldimethylsilyl)oxy)-6-methyl-4-(3-nitropyridin-4-yl)-cyclohex-3-en-1-ol (**40**). To a solution of (±)-(1*R*,5*S*)-5-methyl-6-methylene-3-(3-nitropyridin-4-yl)cyclohex-2-enol **36** (9 g, 36.5 mmol) in THF (91 mL) and water (91 mL) was slowly added *N*-bromosuccinimide (7.16 g, 40.2 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 3 h. After quenched with saturated sodium thiosulfite aqueous solution (300 mL), the reaction mixture was extracted with EtOAc. The organic layers were washed with $NaHCO_3$ solution, water and brine, dried over anhydrous sodium sulfate, filtered off, and concentrated in vacuo to give a mixture of diastereomers (~2.5:1). The crude product was purified by ISCO (0−5% MeOH/DCM, 330 g column) to yield (1*R*,2*R*,6*S*)-1-(bromomethyl)-6-methyl-4-(3-nitropyridin-4-yl)cyclohex-3-ene-1,2-diol **39** (7 g, 20.4 mmol, 56% yield). LCMS (m/z): 342.8, 344.9 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 9.13 (s, 1H), 8.77 (d, J = 4.0 Hz, 1H), 7.29 (d, J = 4.0 Hz, 1H), 5.73 (m, 1H), 4.28 (m, 1H), 4.06 (d, J = 8.0 Hz, 1H), 3.79 (d, J = 12.0 Hz, 1H), 2.75 (m, 2H), 2.42 (m, 1H), 2.27 (m, 1H), 2.12 (m, 1H), 1.19 (d, J = 8.0 Hz, 3H). To a solution of (±)-(1*R*,2*R*,6*S*)-1-(bromomethyl)-6-methyl-4-(3-nitropyridin-4-yl)cyclohex-3-ene-1,2-diol **39** (12 g, 35 mmol) in DMF (70 mL) was added TBDMSCl (7.91 g, 52.5 mmol), imidazole (4.76 g, 69.9 mmol) at room temperature. The reaction mixture was stirred for 24 h. After quenched with $NaHCO_3$, the reaction mixture was extracted with EtOAc 3 times. The organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The crude product was purified by ISCO (gradient EtOAc in heptane) to afford (±)-(1*R*,2*R*,6*S*)-1-(bromomethyl)-2-(*tert*-butyldimethylsilyloxy)-6-methyl-4-(3-nitropyridin-4-yl)cyclohex-3-enol **40** (12 g, 26.2 mmol, 75%). LCMS (m/z): 457, 459.0 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 9.11 (s, 1H), 8.75 (d, J = 5.1 Hz, 1H), 7.31−7.25 (m, 1H), 5.61 (br s, 1H), 4.15−4.08 (m, J = 3.5 Hz, 1H), 3.95 (d, J = 10.6 Hz, 1H), 3.76 (d, J = 10.2 Hz, 1H), 2.81 (dd, J = 17.6, 5.9 Hz, 1H), 2.35 (s, 1H), 2.32−2.23 (m, 1H), 2.06 (dd, J = 17.6, 3.5 Hz, 1H), 1.20 (d, J = 7.4 Hz, 3H), 0.83−0.97 (m, 9H), 0.13 (s, 3H), 0.08 (s, 3H). (±)-4-((3*S*,4*R*,8*S*)-4-(*tert*-butyldimethylsilyloxy)-8-methyl-1-oxaspiro[2.5]oct-5-en-6-yl)-3-nitropyridine (**41**). To a solution of (±)-(1*R*,2*R*,6*S*)-1-(bromomethyl)-2-(*tert*-butyldimethylsilyloxy)-6-methyl-4-(3-nitropyridin-4-yl)-cyclohex-3-enol **40** (3.0 g, 6.56 mmol) in MeOH (20 mL) and water (2 mL) was added potassium carbonate (1.36 g, 9.84 mmol). The reaction mixture was vigorously stirred for 1 h at room temperature. After the volatile material was evaporated, the reaction mixture was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered off, and concentrated *in vacuo* to yield crude (±)-4-((3*S*,4*R*,8*S*)-4-(*tert*-butyldimethylsilyloxy)-8-methyl-1-oxaspiro[2.5]oct-5-en-6-yl)-3-nitropyridine **41** (2.4 g, 6.4 mmol, 97% yield). LCMS (m/z): 377.1 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 9.14 (s, 1H), 8.76 (d, J = 4.0 Hz, 1H), 7.31 (d, J = 4.0 Hz, 1H), 5.59 (s, 1H), 4.49 (br s, 1H), 2.99 (d, J = 4.0 Hz, 1H), 2.73 (d, J = 4.0 Hz, 1H), 2.5 (m, 1H), 2.41 (m, 1H), 2.25 (m, 1H), 0.94 (d, J = 8.0 Hz, 1H), 0.89 (s, 9H), 0.08 (m, 6H). (±)-(3*S*,4*R*,8*S*)-8-methyl-6-(3-nitropyridin-4-yl)-1-oxaspiro[2.5]oct-5-en-4-ol **39-o** was synthesized from (±)-(1*R*,2*R*,6*S*)-1-(bromomethyl)-6-methyl-4-(3-nitropyridin-4-yl)-cyclohex-3-ene-1,2-diol **39** according to step i in Scheme 4 (53% yield). LCMS (m/z): 263.1 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 9.17 (s, 1H), 8.78 (d, J = 4.0 Hz, 1H), 7.31 (d, J = 4.0 Hz, 1H), 5.75 (m, 1H), 4.62 (m, 1H), 3.10 (d, J = 4.0 Hz, 1H), 2.83 (d, J = 4.0 Hz, 1H), 2.51 (m, 1H), 2.28 (m, 2H), 0.96 (d, J = 4.0 Hz, 3H).

(1*R*,2*R*,4*R*,6*S*)-4-(3-Aminopyridin-4-yl)-2-((*tert*-butyldimethylsilyl)oxy)-1,6-dimethylcyclohexan-1-ol (**32**). To a steel bomb reactor was added a solution of (±)-(1*R*,2*R*,6*S*)-1-(bromomethyl)-2-(*tert*-butyldimethylsilyloxy)-6-methyl-4-(3-nitropyridin-4-yl)cyclohex-3-enol **40** (3.7 g, 8.09 mmol) in methanol (27 mL). After degassed by nitrogen for 10 min followed by addition of 10% $Pd(OH)_2$ on carbon (2.8 g), the reaction mixture was charged with hydrogen to 200 psi and stirred at room temperature for 4 days. The reaction mixture was filtered through Celite pad, and the filtrate was neutralized with saturated $NaHCO_3$ aqueous solution. After volatile materials were removed in vacuo, the mixture was extracted with EtOAc. The organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered off, and concentrated in vacuo. The crude product was purified by ISCO (gradient EtOAc containing 2% TEA in DCM) to yield (±)-(1*R*,2*R*,4*R*,6*S*)-4-(3-aminopyridin-4-yl)-2-((*tert*-butyldimethylsilyl)oxy)-1,6-dimethylcyclohexanol **32** (2.5 g, 7.13 mmol, 88% yield). LCMS (m/z): 351.1 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.06 (s, 1H), 8.03 (m, 1H), 6.99 (m, 1H), 3.62 (m, 1H), 2.69 (m, 1H), 1.88 (m, 1H), 1.81−1.65 (m, 4H), 1.13 (s, 3H), 1.01 (d, J = 8.0 Hz, 3H), 0.89 (s, 9H), 0.11 (s, 3H), 0.03 (s, 3H). (±)-(1*R*,2*R*,4*R*,6*S*)-4-(3-aminopyridin-4-yl)-2-((*tert*-butyldimethylsilyl)oxy)-1,6-dimethylcyclohexanol **32** (850 mg, 2.42 mmol) was resolved by chiral HPLC (AD column, heptane/IPA = 95:5, 1 mL/min). Peak 1: (1*R*,2*R*,4*R*,6*S*)-4-(3-aminopyridin-4-yl)-2-((*tert*-butyldimethylsilyl)oxy)-1,6-dimethylcyclohexanol **32a** (340 mg, >99% ee, R_t = 2.74 min). LCMS (m/z): 351.1 $[M + H]^+$. Peak 2: (1*S*,2*S*,4*S*,6*R*)-4-(3-aminopyridin-4-yl)-2-((*tert*-butyldimethylsilyl)-oxy)-1,6-dimethylcyclohexanol **32b** (360 mg, 99% ee, R_t = 4.28 min). LCMS (m/z): 351.1 $[M + H]^+$. 6-(2,6-Difluorophenyl)-*N*-(4-((1*R*,3*R*,4*S*,5*S*)-3,4-dihydroxy-4,5-dimethylcyclohexyl)pyridin-3-yl)-5-fluoropicolinamide (**5**). To solution of (±)-(1*R*,2*R*,4*R*,6*S*)-4-(3-aminopyridin-4-yl)-2-((*tert*-butyldimethylsilyl)oxy)-1,6-dimethylcyclohexanol **32** (56 mg, 0.160 mmol) in DMF (200 μL) was added 6-(2,6-difluorophenyl)-5-fluoropicolinic acid (44.5 mg, 0.176 mmol), HOAt (28.3 mg, 0.208 mmol) and EDC (45.9 mg, 0.240 mmol). The reaction mixture was stirred at room temperature for 16 h. After the mixture was quenched with water and extracted with EtOAc, the combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered off, and concentrated in vacuo to yield crude *N*-(4-((1*R*,3*R*,4*S*,5*S*)-3-(*tert*-butyldimethylsilyloxy)-4-hydroxy-4,5-dimethylcyclohexyl)pyridin-3-yl)-6-(2,6-difluorophenyl)-5-fluoropicolinamide (93.5 mg, >99% yield). LCMS (m/z): 586.5 $[M + H]^+$. The crude product was dissolved in THF (2 mL) and MeOH (1 mL) followed by addition of 3 N HCl aqueous solution (0.5 mL). The reaction mixture was stirred for 3 h. After neutralized with saturated $NaHCO_3$ aqueous solution, the reaction mixture was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered off, and concentrated in vacuo. The crude produce was purified by ISCO (gradient EtOAc in DCM followed by 10% MeOH in EtOAc) to yield (±)-6-(2,6-difluorophenyl)-*N*-(4-((1*R*,3*R*,4*S*,5*S*)-3,4-dihydroxy-4,5-dimethylcyclohexyl)pyridin-3-yl)-5-fluoropicolinamide **5-rac** (65 mg, 0.137 mmol, 86% yield). LCMS (m/z):472.3 $[M + H]^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.90 (br s, 1H), 9.34 (s, 1H), 8.43 (d, J = 4 Hz, 1H), 8.41 (m, 1H), 7.78 (t, J = 8.4 Hz, 1H), 7.51 (m, 1H), 7.19 (d, J = 4 Hz, 1H), 7.1 (dd, J = 8.4, 7.6 Hz, 1H), 3.57 (m, 1H), 3.0 (m, 1H), 2.01 (m, 1H), 1.76−1.51 (m, 4H), 1.35−1.26 (m, 1H), 1.12 (s, 3H), 0.9 (d, J = 6.8 Hz, 3H). 65 mg of **5-rac** was resolved by chiral HPLC (AD-H column, heptane/EtOH = 75:25, 1 mL/min). Peak 1: 6-(2,6-difluorophenyl)-*N*-(4-((1*S*,3*S*,4*R*,5*R*)-3,4-dihydroxy-4,5-dimethylcyclohexyl)pyridin-3-yl)-5-fluoropicolinamide **5-ent** (26.8 mg, 98% ee, R_t = 3.8 min). LCMS (m/z): 472.0 $[M + H]^+$. Peak 2: 6-(2,6-difluorophenyl)-*N*-(4-((1*R*,3*R*,4*S*,5*S*)-3,4-dihydroxy-4,5-dimethylcyclohexyl)pyridin-3-yl)-5-fluoropicolinamide **5** (27 mg, 99% ee, R_t = 5.5 min). LCMS (m/z): 472.0 $[M + H]^+$. All analytical and biological data of compounds **5** and **5-ent** that were synthesized

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J. Med. Chem. XXXX, XXX, XXX−XXX

by procedures as described in Schemes 3 and 4 were matched. Compounds **5** and **5-ent** were synthesized respectively from two enantiomers **32a** and **32b** of **32** using the same procedures described above (Scheme 4). All the data of compound **5** from enantiomer **32a** were matched with those of compound **5** synthesized in Scheme 3. (1*R*,2*R*,4*R*,6*S*)-4-(3-aminopyridin-4-yl)-1,6-dimethylcyclohexane-1,2-diol (**42**). To a solution of (±)-(1*R*,2*R*,6*S*)-1-(bromomethyl)-6-methyl-4-(3-nitropyridin-4-yl)cyclohex-3-ene-1,2-diol **39** (2.3 g, 67 mmol) in ethanol (100 mL) was added Pd on carbon (content 5%, 4.28 g) and pyridine (5.42 mL, 59.4 mmol). The reaction vessel was degassed with nitrogen stream for 15 min, purged, and flushed with hydrogen gas three times. After equipped with hydrogen balloon, the reaction was stirred for 24 h. The reaction mixture was diluted with methanol and filtered. The residue was washed with 100 mL of methanol. The volatile materials were removed in vacuo. The crude product was purified by ISCO (95:5:0.5 DCM/MeOH/NH_4OH to 80:19:1 DCM/MeOH/NH_4OH over 15 min, 80 g silica cartridge) to give (±)-(1*R*,2*R*,4*R*,6*S*)-4-(3-aminopyridin-4-yl)-1,6-dimethylcyclohexane-1,2-diol **42** (1.1 g, 4.65 mmol, 70% yield). LCMS (*m*/*z*): 237.2 $[M + H]^+$. ^1H NMR (CD_3OD, 400 MHz): δ 7.92 (s, 1H), 7.77 (d, J = 4.8 Hz, 1H), 7.09 (d, J = 5.2 Hz, 1H), 4.8 (s, 4H), 3.64 (m, 1H), 2.91 (m, 1H), 1.95 (m, 1H), 1.8−1.62 (m, 4H), 1.31 (m, 1H), 1.12 (s, 3H), 0.98 (d, J = 6.8 Hz, 3H). 950 mg of (±)-(1*R*,2*R*,4*R*,6*S*)-4-(3-aminopyridin-4-yl)-1,6-dimethylcyclohexane-1,2-diol **42** was resolved by chiral HPLC (OJ-H column, heptane/IPA = 85:15, 1 mL/min). Peak 1: (1*R*,2*R*,4*R*,6*S*)-4-(3-aminopyridin-4-yl)-1,6-dimethylcyclohexane-1,2-diol **42a** (370 mg, >99% ee, R_t = 7.6 min). LCMS (*m*/*z*): 237.3 $[M + H]^+$. Peak 2: (1*S*,2*S*,4*S*,6*R*)-4-(3-aminopyridin-4-yl)-1,6-dimethylcyclohexane-1,2-diol **42b** (340 mg, >99% ee, R_t = 10.2 min). LCMS (*m*/*z*): 237.3 $[M + H]^+$. The amide coupling between **42a** and **18** yielded compound **5**. All analytical and biological data were matched with those for compound **5** obtained from intermediate **32**.

Synthesis of Compound 12. (±)-(1*S*,2*R*,6*S*)-2-(*tert*-Butyldimethylsilyloxy)-1-(hydroxymethyl)-6-methyl-4-(3-nitropyridin-4-yl)-cyclohex-3-enol (**43**). To a solution of (±)-4-((3*R*,5*S*)-3-(*tert*-butyldimethylsilyloxy)-5-methyl-4-methylenecyclohex-1-enyl)-3-nitropyridine **37** (6.3 g, 17.47 mmol) in acetone (132 mL) and water (43 mL) was added osmium tetroxide (10 mL, 2.5% in water) and NMO (13.06 g, 112 mmol). The reaction mixture was stirred at room temperature for 1 h. After quenched with $Na_2S_2O_3$ aqueous solution (21 g in 80 mL of water), the mixture was stirred for 1 h. After acetone was removed *in vacuo*, the reaction mixture was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered off, and concentrated *in vacuo*. The crude material was purified by ISCO (gradient EtOAc in heptane) to yield (±)-(1*S*,2*R*,6*S*)-2-(*tert*-butyldimethylsilyloxy)-1-(hydroxymethyl)-6-methyl-4-(3-nitropyridin-4-yl)cyclohex-3-enol **43** (7.0 g, 17.7 mmol, 95% yield). LCMS (*m*/*z*): 395.0 $[M + H]^+$. (±)-(1*R*,2*R*,6*S*)-2-(*tert*-butyldimethylsilyloxy)-1-hydroxy-6-methyl-4-(3-nitropyridin-4-yl)cyclohex-3-enecarbaldehyde (**44**). To a solution of (±)-(1*S*,2*R*,6*S*)-2-(*tert*-butyldimethylsilyloxy)-1-(hydroxymethyl)-6-methyl-4-(3-nitropyridin-4-yl)-cyclohex-3-enol **43** (3.4 g, 8.62 mmol) in DCM (28.7 mL, 0.3 M) was added Dess−Martin periodinane (4.02 g, 9.48 mmol). The reaction mixture was stirred at room temperature for 72 h. After quenched with $Na_2S_2O_3$ and $NaHCO_3$ solution (1:8) and vigorously stirred for 1 h, the reaction mixture was extracted with EtOAc, the organic layer was washed with water and brine, and dried by anhydrous sodium sulfate, filtered and concentrated *in vacuo*, and the crude product was purified by ISCO (0−40% EtOAc in heptane) to afford (±)-(1*R*,2*R*,6*S*)-2-(*tert*-butyldimethylsilyloxy)-1-hydroxy-6-methyl-4-(3-nitropyridin-4-yl)cyclohex-3-enecarbaldehyde **44** (2.6 g, 6.62 mmol, 77% yield). LCMS (*m*/*z*): 393.1 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ ppm 9.94−9.89 (m, 1H), 9.18 (s, 1H), 8.81 (d, J = 4.7 Hz, 1H), 7.32 (d, J = 5.1 Hz, 1H), 5.67 (s, 1H), 4.46−4.55 (m, 1H), 3.86−3.80 (s, 1H), 2.54 (d, J = 3.1 Hz, 1H), 2.49−2.32 (m, 2H), 0.97 (d, J = 6.7 Hz, 3H), 0.83 (s, 9H), 0.12−0.05 (m, 6H). (±)-(1*S*,2*R*,6*S*)-2-(*tert*-butyldimethylsilyloxy)-1-ethynyl-6-methyl-4-(3-nitropyridin-4-yl)cyclohex-3-enol (**45**). To a solution of (±)-(1*R*,2*R*,6*S*)-2-(*tert*-butyldimethylsilyloxy)-1-hydroxy-6-methyl-4-(3-nitropyridin-4-yl)cyclohex-3-enecarbaldehyde **44** (1.58 g, 4.03 mmol) in MeOH (130 mL) was added Bestmann−Ohira's reagent (1.55, 8.05 mmol) in MeOH (70 mL) followed by addition of potassium carbonate (2.78 g, 20.13 mmol) at room temperature. The reaction mixture was stirred for 1.5 h. After removing 90% of MeOH in vacuo, the mixture was partitioned with water and EtOAc, the organic layers were washed with saturated NH_4Cl solution and brine, dried with anhydrous sodium sulfate, filtered off, and concentrated. The crude compound was purified *via* ISCO (0−30% EtOAc in heptane) to yield (±)-(1*S*,2*R*,6*S*)-2-(*tert*-butyldimethylsilyloxy)-1-ethynyl-6-methyl-4-(3-nitropyridin-4-yl)cyclohex-3-enol **45** (1.5 g, 3.86 mmol, 96% yield). LCMS (*m*/*z*): 389.2 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ ppm, 9.12 (s, 1 H) 8.74 (d, J = 5.09 Hz, 1 H) 7.29 (d, J = 5.09 Hz, 1 H) 5.44 (s, 1 H) 4.33 (dt, J = 3.33, 1.86 Hz, 1 H) 2.66 (s, 1 H) 2.45 (s, 1 H) 2.38−2.30 (m, 2 H) 2.28−2.19 (m, 1 H) 1.17 (d, J = 6.26 Hz, 3 H) 0.93 (s, 9 H) 0.17−0.09 (m, 6 H). (1*R*,2*R*,4*R*,6*S*)-4-(3-Aminopyridin-4-yl)-2-(*tert*-butyldimethylsilyloxy)-1-ethyl-6-methylcyclohexanol (**46**). To a solution of (±)-(1*S*,2*R*,6*S*)-2-(*tert*-butyldimethylsilyloxy)-1-ethynyl-6-methyl-4-(3-nitropyridin-4-yl)cyclohex-3-enol **45** (1.1 g, 2.83 mmol) in MeOH (28 mL) was added 10% Pd on carbon (0.9 g). After degassed by nitrogen for 10 min and equipped with hydrogen balloon, the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was filtered through Celite and washed by MeOH and EtOAc. After the filtrate was concentrated in vacuo, the crude product was purified by ISCO (gradient EtOAc in DCM) to yield (±)-(1*R*,2*R*,4*R*,6*S*)-4-(3-aminopyridin-4-yl)-2-(*tert*-butyldimethylsilyloxy)-1-ethyl-6-methylcyclohexanol **46-rac** (336 mg, 0.922 mmol, 33% yield). LCMS (*m*/*z*): 365.2 $[M + H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ ppm 8.05 (s, 1H), 8.03 (m, 1H), 7.0 (m, 1H), 3.65 (m, 1H), 2.72 (m, 1H), 2.11−1.62 (m, 5H), 1.35 (m, 2H), 1.05 (d, J = 8.0 Hz, 3H), 0.91 (s, 9H), 0.89 (m, 3H), 0.12 (s, 3H), 0.05 (s, 3H). 336 mg of (±)-(1*R*,2*R*,4*R*,6*S*)-4-(3-aminopyridin-4-yl)-2-(*tert*-butyldimethylsilyloxy)-1-ethyl-6-methylcyclohexanol **46-rac** was resolved by chiral SFC (Chiralpak, 10 × 250, 15 mL/min, CO_2/EtOH + 0.1% DEA, 85/15. 40 °C). Peak 1: (1*S*,2*S*,4*S*,6*R*)-4-(3-aminopyridin-4-yl)-2-(*tert*-butyldimethylsilyloxy)-1-ethyl-6-methylcyclohexanol **46-ent** (97 mg, 99% ee, R_t = 1.49 min). LCMS (*m*/*z*): 365.1 $[M + H]^+$. Peak 2: (1*R*,2*R*,4*R*,6*S*)-4-(3-aminopyridin-4-yl)-2-(*tert*-butyldimethylsilyloxy)-1-ethyl-6-methylcyclohexanol **46** (112 mg, 99% ee, R_t = 1.91 min). LCMS (*m*/*z*): 365.1 $[M + H]^+$. 3-Amino-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-3-(*tert*-butyldimethylsilyloxy)-4-ethyl-4-hydroxy-5-methylcyclohexyl)-pyridin-3-yl)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-picolinamide (**48**). To solution of (1*R*,2*R*,4*R*,6*S*)-4-(3-aminopyridin-4-yl)-2-(*tert*-butyldimethylsilyloxy)-1-ethyl-6-methylcyclohexanol **46** (37 mg, 0.101 mmol) in DMF (127 μL) was added 3-amino-6-bromopicolinic acid **47** (102 mg, 0.471 mmol), HOAt (17.96 mg, 0.132 mmol), and EDC (29.2 mg, 0.152 mmol). The reaction mixture was stirred at room temperature for 16 h. After quenched with water, the reaction mixture was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered off, and concentrated *in vacuo* to yield crude 3-amino-6-bromo-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-3-(*tert*-butyldimethylsilyloxy)-4-ethyl-4-hydroxy-5-methylcyclohexyl)pyridin-3-yl)-picolinamide (56 mg, 99%). LCMS (*m*/*z*): 563.1, 565.1 $[M + H]^+$. To a microwave vessel was added 3-amino-6-bromo-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-3-(*tert*-butyldimethylsilyloxy)-4-ethyl-4-hydroxy-5-methylcyclohexyl)pyridin-3-yl)picolinamide (56 mg, 0.099 mmol), 4,4,4′,4′,5,5,5′,5′-octamethyl-2,2′-bi(1,3,2-dioxaborolane) (50.5 mg, 0.199 mmol), tricyclohexylphosphine (6.13 mg, 0.022 mmol), $Pd_2(dba)_3$ (10.0 mg, 10.9 μmol), and dioxane (497 μL). The reaction was degassed by nitrogen stream for 5 min followed by addition of potassium acetate (29.3 mg, 0.298 mmol). The reaction mixture was microwaved at 120 °C for 10 min. After diluted with EtOAc, the mixture was filtered through Celite pad. The volatile materials were removed in vacuo to yield crude 3-amino-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-3-(*tert*-butyldimethylsilyloxy)-4-ethyl-4-hydroxy-5-methylcyclohexyl)pyridin-3-yl)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)picolinamide **48** (60.7 mg, 0.099 mmol, >99%). LCMS (*m*/

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J. Med. Chem. XXXX, XXX, XXX−XXX

z): 529.2 $[M + H]^+$ for boronic acid. 5-Amino-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-4-ethyl-3,4-dihydroxy-5-methylcyclohexyl)pyridin-3-yl)-3′-fluoro-2,2′-bipyridine-6-carboxamide (**12**). To a microwave vessel, 3-amino-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-3-(*tert*-butyldimethylsilyloxy)-4-ethyl-4-hydroxy-5-methylcyclohexyl)pyridin-3-yl)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)picolinamide **48** (60.7 mg, 0.099 mmol), 2-bromo-3-fluoropyridine (26.2 mg, 0.149 mmol), $PdCl_2$(dppf) (10.91 mg, 0.015 mmol), DME (745 μL), and 2 M Na_2CO_3 solution (248 μL) were added. The reaction mixture was degassed by N_2 stream for 5 min. The reaction was microwaved at 120 °C for 10 min. The reaction mixture was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered off, and concentrated *in vacuo* to crude product. LCMS (m/z): 580.2 $[M + H]^+$. The crude product was dissolved in THF and MeOH (1:1, 1 mL) followed by addition of 3 N HCl (0.5 mL). The reaction mixture was stirred for 1 h. After added with saturated Na_2CO_3 aqueous solution until the mixture turned to pH 8, the reaction mixture was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered off, and concentrated *in vacuo*. The crude product was purified by prep HPLC. The combined pure fractions were lyophilized to yield 5-amino-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-4-ethyl-3,4-dihydroxy-5-methylcyclohexyl)pyridin-3-yl)-3′-fluoro-2,2′-bipyridine-6-carboxamide **12** as its TFA salt (17.4 mg, 30% yield over 3 steps). LCMS (m/z): 466.1 $[M + H]^+$. HRMS (m/z): calcd for $C_{25}H_{29}N_5O_3F$ $[M + H]^+$, 466.2254; found, 466.2251. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 10.52 (s, 1H), 9.35 (s, 1H), 8.51 (m, 2H), 8.11 (d, J = 8 Hz, 1H), 7.85 (d, J = 8 Hz, 1H), 7.78 (m, 1H), 7.51 (m, 1H), 7.43 (d, J = 8 Hz, 1H), 7.26 (br s, 2H), 3.12 (m, 1H), 1.76−1.55 (m, 5H), 1.47−1.34 (m, 2H), 0.92 (t, J = 8 Hz, 3H), 0.85 (d, J = 4 Hz, 3H).

Synthesis of Compound 13. (±)-(1*R*,2*R*,6*S*)-1-(Fluoromethyl)-6-methyl-4-(3-nitropyridin-4-yl)cyclohex-3-ene-1,2-diol (**49**). A solution of (±)-4-((3*S*,4*R*,8*S*)-4-(*tert*-butyldimethylsilyloxy)-8-methyl-1-oxaspiro[2.5]oct-5-en-6-yl)-3-nitropyridine **41** (520 mg, 1.381 mmol) in triethylamine trihydrofluoride (2.25 mL, 13.81 mmol) in a stainless steel reactor was heated at 100 °C for 8 h. After cooled down and quenched by saturated $NaHCO_3$ aqueous solution carefully, the reaction mixture was partitioned between EtOAc and water. The mixture was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered off, and concentrated in vacuo to yield (±)-(1*R*,2*R*,6*S*)-1-(fluoromethyl)-6-methyl-4-(3-nitropyridin-4-yl)cyclohex-3-ene-1,2-diol (180 mg, 0.638 mmol, 46% yield). LCMS (m/z): 283.0 $[M + H]^+$. A solution of (±)-(1*R*,2*R*,6*S*)-1-(fluoromethyl)-6-methyl-4-(3-nitropyridin-4-yl)cyclohex-3-ene-1,2-diol (180 mg, 0.638 mmol) in MeOH (5.3 mL) was degassed by nitrogen stream for 10 min followed by addition of 10% Pd on carbon (68 mg). The reaction mixture was stirred at room temperature for 12 h under hydrogen balloon. The reaction mixture was filtered through Celite pad and washed by MeOH and EtOAc, and the filtrate was concentrated in vacuo to give (±)-(1*R*,2*R*,4*R*,6*S*)-4-(3-aminopyridin-4-yl)-1-(fluoromethyl)-6-methylcyclohexane-1,2-diol **49** (79 mg, 0.311 mmol, 49% yield). LCMS (m/z): 255.0 $[M + H]^+$. 5-Amino-3′-fluoro-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-4-(fluoromethyl)-3,4-dihydroxy-5-methylcyclohexyl)-pyridin-3-yl)-[2,2′-bipyridine]-6-carboxamide (**13**). A solution of (±)-(1*R*,2*R*,4*R*,6*S*)-4-(3-aminopyridin-4-yl)-1-(fluoromethyl)-6-methylcyclohexane-1,2-diol **49** (79 mg, 0.311 mmol) and 3-amino-6-bromopicolinic acid **47** (101 mg, 0.466 mmol), HOAt (76 mg, 0.559 mmol) and EDC (107 mg, 0.559 mmol) in DMF (0.62 mL) was stirred for 12 h at room temperature. The reaction mixture was quenched with water and extracted with EtOAc. The organic layers were washed by saturated $NaHCO_3$ aqueous solution, water and brine, dried over anhydrous sodium sulfate, filtered off, and concentrated in vacuo to yield crude (±)-3-amino-6-bromo-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-4-(fluoromethyl)-3,4-dihydroxy-5-methylcyclohexyl)-pyridin-3-yl)picolinamide (120 mg, 0.265 mmol, 85% yield). The crude product was used in the next step without further purification. LCMS (m/z): 453, 455 $[M + H]^+$. To a microwave vessel was added (±)-3-amino-6-bromo-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-4-(fluoromethyl)-3,4-dihydroxy-5-methylcyclohexyl)pyridin-3-yl)picolinamide (120 mg, 0.265 mmol), 4,4,4′,4′,5,5,5′,5′-octamethyl-2,2′-bi(1,3,2-dioxaborolane) (134 mg, 0.529 mmol), tricyclohexylphoshpine (13.36 mg, 0.048 mmol), $Pd_2(dba)_3$ (19.39 mg, 0.021 mmol), and dioxane (0.88 mL). After the reaction was degassed by nitrogen stream for 5 min, potassium acetate (78 mg, 0.794 mmol) was added. The reaction mixture was microwaved at 120 °C for 10 min. LCMS (m/z): 419.0 $[M + H]^+$ for boronic acid. The reaction mixture was diluted with EtOAc, which was filtered through Celite pad. The volatile materials were removed to yield (±)-3-amino-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-4-(fluoromethyl)-3,4-dihydroxy-5-methylcyclohexyl)pyridin-3-yl)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)picolinamide (132 mg, 0.264 mmol, >99%), which was used in the next step without further purification. 5-Amino-3′-fluoro-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-4-(fluoromethyl)-3,4-dihydroxy-5-methylcyclohexyl)pyridin-3-yl)-[2,2′-bipyridine]-6-carboxamide (**13**). To solution of (±)-3-amino-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-4-(fluoromethyl)-3,4-dihydroxy-5-methylcyclohexyl)-pyridin-3-yl)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-picolinamide (132 mg, 0.264 mmol) in DME (2 mL) and 2 M Na_2CO_3 aqueous solution (0.66 mL) was added 2-bromo-3-fluoropyridine (55.7 mg, 0.317 mmol) and $PdCl_2$(dppf) (19.3 mg, 0.026 mmol). The reaction mixture was heated at microwave for 10 min at 120 °C. After diluted with EtOAc and water, the mixture was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered off, and concentrated in vacuo. The crude product was purified by prep HPLC, and pure fractions were free based followed by lyophilization to yield (±)-5-amino-3′-fluoro-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-4-(fluoromethyl)-3,4-dihydroxy-5-methylcyclohexyl)pyridin-3-yl)-2,2′-bipyridine-6-carboxamide (12.8 mg, 10% yield) **13-rac**. LCMS (m/z): 470.1 $[M + H]^+$. ^{1}H NMR (400MHz, $CDCl_3$): δ ppm 10.32 (s, 1H), 9.31 (s, 1H), 8.52 (d, J = 4.3 Hz, 1H), 8.40 (d, J = 5.1 Hz, 1H), 8.18 (d, J = 8.6 Hz, 1H), 7.54 (m, 1H), 7.32 (m, 1H), 7.21 (m, 2H), 6.26 (br s, 2H), 5.30 (s, 3H), 4.89−4.61 (m, 2H), 3.85 (d, J = 11.3 Hz, 1H), 3.21 (br s, 1H), 2.72 (br s, 1H), 2.29 (br s, 1H), 2.14 (m, 1H), 1.97−1.68 (m, 3H), 1.59 (m, 1H), 1.06 (d, J = 8.0 Hz, 3H). 12.8 mg of **13-rac** was resolved by chiral SFC (OJ column, 5 mL/min, EtOH + 0.1% DEA = 20%). Peak 1: 5-amino-3′-fluoro-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-4-(fluoromethyl)-3,4-dihydroxy-5-methylcyclohexyl)pyridin-3-yl)-2,2′-bipyridine-6-carboxamide **13** (3.1 mg, >99% ee, R_t = 1.52). LCMS (m/z): 470.1 $[M + H]^+$. HRMS (m/z): calcd for $C_{24}H_{26}N_5O_3F_2$ $[M + H]^+$, 470.2004; found, 470.2003. Peak 2: 5-amino-3′-fluoro-*N*-(4-((1*S*,3*S*,4*S*,5*R*)-4-(fluoromethyl)-3,4-dihydroxy-5-methylcyclohexyl)-pyridin-3-yl)-2,2′-bipyridine-6-carboxamide **13-ent** (3.6 mg, >99% ee, R_t = 1.92). LCMS (m/z): 470.1 $[M + H]^+$.

Synthesis of Compound 14. 5-Amino-2-(2,6-difluorophenyl)-pyrimidine-4-carboxylic acid (**46**). A solution of 2,6-difluorobenzimidamide hydrochloride **50** (3.8 g, 19.73 mmol) in anhydrous ethanol (63 mL) was cooled to 0 °C in an ice bath. Sodium ethoxide solution (16.14 mL, 41.2 mmol, 20%) was added slowly *via* syringe, the ice bath was removed, and the reaction mixture was warmed to room temperature and stirred for 1 h. A solution of mucobromic acid, (*E*)-2,3-dibromo-4-oxobut-2-enoic acid (3.54 g, 13.73 mmol), in ethanol (69 mL) was added dropwise *via* syringe, and the reaction was heated in a 40 °C oil bath for 2 h. The volatile materials were removed in vacuo and water (100 mL) and 6 N NaOH aqueous solution (50 mL) was added to dissolve the crude product. The basic aqueous solution was extracted with EtOAc. Then, the aqueous layer was acidified to pH 4.0 by adding 3 N HCl aqueous solution slowly and extracted with EtOAc. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered off, and concentrated in vacuo to afford crude 5-bromo-2-(2,6-difluorophenyl)pyrimidine-4-carboxylic acid (4.3 g, 13.7 mmol, >99% yield) as a reddish brown solid, which was used in the next step without purification. LCMS (m/z): 314.9, 316.9 $[M + H]^+$. To a steel bomb reactor was added 5-bromo-2-(2,6-difluorophenyl)-pyrimidine-4-carboxylic acid (4.3 g, 13.7 mmol) dissolved in 28% NH_4OH solution (10 mL) and copper (II) sulfate (0.219 g, 1.37 mmol). The reactor was placed in a preheated bath (110 °C) and stirred for 1 h. After cooling down, the reaction vessel was unsealed.

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J. Med. Chem. XXXX, XXX, XXX−XXX

The reaction mixture was transferred to Erlenmeyer flask by washing with water, which was acidified by pH 4.0 with 3 N HCl aqueous solution, and extracted with EtOAc. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered off, and concentrated in vacuo to yield a crude reddish solid (863 mg, 3.4 mmol, 25% yield over 2 steps). The crude product was used in the next step without further purification. LCMS (m/z): 252.0 $[M + H]^+$. 5-Amino-2-(2,6-difluorophenyl)-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-4-(fluoromethyl)-3,4-dihydroxy-5-methylcyclohexyl)-pyridin-3-yl)pyrimidine-4-carboxamide (**14**). To a solution of (±)-(1*R*,2*R*,4*R*,6*S*)-4-(3-aminopyridin-4-yl)-1-(fluoromethyl)-6-methylcyclohexane-1,2-diol **49** (50 mg, 0.197 mmol) in DMF (0.4 mL) was added 5-amino-2-(2,6-difluorophenyl)pyrimidine-4-carboxylic acid **51** (64.2 mg, 0.256 mmol), HOAt (40.1 mg, 0.295 mmol) and EDC (56.5 mg, 0.295 mmol). The reaction mixture was stirred for 12 h at room temperature. After partitioned between EtOAc and water, the mixture was extracted with EtOAc. The combined organic layers were washed by water and brine, dried over anhydrous sodium sulfate, filtered off, and concentrated in vacuo. The crude material was purified by reverse phase HPLC. The combined pure fractions were free-based and extracted with EtOAc. The organic layers were dried over anhydrous sodium sulfate, filtered off, and concentrated in vacuo to afford (±)-5-amino-2-(2,6-difluorophenyl)-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-4-(fluoromethyl)-3,4-dihydroxy-5-methylcyclohexyl)pyridin-3-yl)-pyrimidine-4-carboxamide **14-rac** (24 mg, 0.049 mmol, 25%). LCMS (m/z): 488.1 $[M + H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm: 10.08 (br s, 1H), 9.29 (s, 1H), 8.56 (s, 1H), 8.41 (d, J = 5.1 Hz, 1H), 7.35−7.45 (m, 1H), 7.20 (d, J = 5.1 Hz, 1H), 7.05 (t, J = 8.0 Hz, 2H), 6.12 (br s, 2H), 4.87−4.58 (m, 2H), 3.77 (d, J = 11.0 Hz, 1H), 3.07 (br s, 1H), 2.70 (br s, 1H), 2.44 (br s, 1H), 2.06 (m, 1H), 1.89−1.72 (m, 4H), 1.65−1.51 (m, 1H), 1.02 (d, J = 6.3 Hz, 3H). 20 mg of **14-rac** was resolved by chiral HPLC (AD-H column, EtOH/heptane = 15:85, 1 mL/min). Peak 1: 5-Amino-2-(2,6-difluorophenyl)-*N*-(4-((1*S*,3*S*,4*S*,5*R*)-4-(fluoromethyl)-3,4-dihydroxy-5-methylcyclohexyl)-pyridin-3-yl)pyrimidine-4-carboxamide **14-ent** (5.5 mg, >99% ee, R_t = 7.83 min), LCMS (m/z): 488.1 $[M + H]^+$. Peak 2: 5-amino-2-(2,6-difluorophenyl)-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-4-(fluoromethyl)-3,4-dihydroxy-5-methylcyclohexyl)pyridin-3-yl)pyrimidine-4-carboxamide **14** (6.7 mg, >99% ee, R_t = 9.28 min). LCMS (m/z): 488.1 $[M + H]^+$. HRMS (m/z): calcd for $C_{24}H_{25}N_3O_3F_3$ $[M + H]^+$, 488.1909; found, 488.1913.

Synthesis of Compounds 6, 7, 8, 9, and 10. According to steps l and m in Scheme 4, compounds **6**−**10** were synthesized using (1*R*,2*R*,4*R*,6*S*)-4-(3-aminopyridin-4-yl)-2-((*tert*-butyldimethylsilyl)-oxy)-1,6-dimethylcyclohexanol **32a** and the corresponding acids, 2-(2,6-difluorophenyl)thiazole-4-carboxylic acid, 2-(2,6-difluorophenyl)pyrimidine-4-carboxylic acid, 5-amino-2-(2,6-difluorophenyl)thiazole-4-carboxylic acid, 5-amino-2-(2,6-difluorophenyl)pyrimidine-4-carboxylic acid **51**, and 3-amino-6-(2,6-difluorophenyl)picolinic acid, respectively. The desired products were obtained as their TFA salts through reverse phase preparative HPLC. 2-(2,6-difluorophenyl)-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-3,4-dihydroxy-4,5-dimethylcyclohexyl)pyridin-3-yl)thiazole-4-carboxamide (**6**). LCMS (m/z): 460.0 $[M + H]^+$. HRMS (m/z): calcd for $C_{23}H_{24}N_3O_3F_2S$ $[M + H]^+$, 460.1506; found, 460.1505. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.2 (s, 1H), 8.67 (m, 2H), 8.48 (m, 1H), 7.68 (m, 2H), 7.36 (m, 2H), 3.31 (m, 1H), 3.04 (m, 1H), 1.75 (m, 1H), 1.58−1.17 (m, 4H), 0.96 (s, 3H), 0.85 (d, J = 6.8 Hz, 3H). 2-(2,6-Difluorophenyl)-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-3,4-dihydroxy-4,5-dimethylcyclohexyl)pyridin-3-yl)-pyrimidine-4-carboxamide (**7**). LCMS (m/z): 455.1 $[M + H]^+$. HRMS (m/z): calcd for $C_{24}H_{25}N_4O_3F_2$ $[M + H]^+$, 455.1895; found, 455.1894. 5-Amino-2-(2,6-difluorophenyl)-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-3,4-dihydroxy-4,5-dimethylcyclohexyl)pyridin-3-yl)thiazole-4-carboxamide (**8**). LCMS (m/z): 475.1 $[M + H]^+$. HRMS (m/z): calcd for $C_{23}H_{25}N_4O_3F_2S$ $[M + H]^+$, 475.1615; found, 475.1620. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.93 (s, 1H), 8.44 (d, J = 4.0 Hz, 1H), 7.63 (br s, 2H), 7.55 (m, 2H), 7.28 (m, 2H), 3.05 (m, 1H), 1.78 (m, 1H), 1.59−1.41 (m, 3H), 1.27 (m, 1H), 0.97 (s, 3H), 0.88 (d, J = 8.0 Hz, 3H). 5-Amino-2-(2,6-difluorophenyl)-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-3,4-dihydroxy-4,5-dimethylcyclohexyl)pyridin-3-yl)pyrimidine-4-carboxamide (**9**). LCMS (m/z): 470.2 $[M + H]^+$. HRMS (m/z): calcd for $C_{25}H_{27}N_4O_3F_2$ $[M + H]^+$, 470.2004; found, 470.2005. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.4 (s, 1H), 8.95 (s, 1H), 8.38 (d, J = 4.0 Hz, 1H), 8.14 (m, 2H), 7.82 (m, 1H), 7.52 (m, 1H), 6.88 (d, J = 12.0 Hz, 1H), 3.8 (s, 1H), 2.93 (m, 1H), 1.67 (m, 1H), 1.53−1.38 (m, 3H), 1.17 (m, 1H), 0.89 (s, 3H), 0.75 (d, J = 8.0 Hz, 3H). 3-Amino-6-(2,6-difluorophenyl)-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-3,4-dihydroxy-4,5-dimethylcyclohexyl)pyridin-3-yl)picolinamide (**10**). LCMS (m/z): 469.1 $[M + H]^+$. HRMS (m/z): calcd for $C_{25}H_{27}N_4O_3F_2$ $[M + H]^+$, 469.2051; found, 469.2056. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.3 (s, 1H), 9.14 (s, 1H), 8.45 (d, J = 4.0 Hz, 1H), 7.68 (d, J = 4.0 Hz, 1H), 7.58 (m, 1H), 7.45 (m, 1H), 7.20 (m, 1H), 3.0 (m, 1H), 1.72 (m, 1H), 1.54−1.35 (m, 3H), 1.24 (m, 1H), 0.94 (s, 3H), 0.77 (d, J = 4.0 Hz, 3H).

Synthesis of Compound 11. 5-Amino-*N*-(4-((1*R*,3*R*,4*R*,5*S*)-3,4-dihydroxy-4,5-dimethylcyclohexyl)pyridin-3-yl)-3′-fluoro-2,2′-bipyridine-6-carboxamide (**11**). According to steps f, g, h, and i in Scheme 5, compound **11** was synthesized using (1*R*,2*R*,4*R*,6*S*)-4-(3-aminopyridin-4-yl)-2-((*tert*-butyldimethylsilyl)oxy)-1,6-dimethylcyclohexanol **32a**. The desired product was obtained as its TFA salt through reverse phase preparative HPLC. LCMS (m/z): 452.2 $[M + H]^+$. HRMS (m/z): calcd for $C_{24}H_{27}N_5O_3F$ $[M + H]^+$, 452.2098; found, 452.2096. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.2 (s, 1H), 9.09 (s, 1H), 8.52 (m, 1H), 8.31 (d, J = 4.0 Hz, 1H), 8.11 (d, J = 8.0 Hz, 1H), 7.72 (m, 1H), 7.48 (m, 1H), 7.41 (m, 1H), 7.26 (br s, 1H), 4.48 (s, 2H), 4.07 (s, 1H), 2.97 (m, 1H), 1.75 (m, 1H), 1.68−1.41 (m, 3H), 1.28 (m, 1H), 0.94 (s, 3H), 0.84 (d, J = 8.0 Hz, 3H).

Microsomal Stability Testing. The test compounds were incubated at a concentration of 1 μM in the presence of a 0.5 mg/mL microsomal protein suspension, 1 mM UDPGA, 3 mM $MgCl_2$, 1 mM NADPH, and 25 μg alamethicin/mg microsomal protein. Over the time course of the incubation, duplicate samples were collected at intervals of 0, 5, 15, and 30 min for determination of metabolic rate and parent compound remaining at the end of 30 min incubation. Phenolphthalein was used as a reference control. Data were obtained utilizing semi-quantitative LC−MS/MS methods. Calculation of scaled intrinsic clearance: CL_{int} = 0.693 × [1/$t_{1/2}$ (min)] × (g of liver weight/kg of body weight) × (mL incubation/mg of microsomal protein) × (45 mg of microsomal protein/g of liver weight) with rat = 45 g of liver per kg of body weight and human = 25.7 g of liver per kg of body weight. Hepatic clearance was extrapolated from microsomal data using well-stirred liver model $CL_h = [Q_h \times (f_{ub}/f_{ui}) \times CL_{int}]/Q_h + (f_{ub}/f_{ui}) \times CL_{int}$, where assumption is f_{ub} (fraction unbound in blood) = f_{ui} (fraction unbound in incubation) and Q_h values of 55 mL/min/kg and 20.7 mL/min/kg were used for rat and human. Calculation of hepatic extraction ratio: ER_H = CL_h/Q_h.

PIM Enzymatic Assays. *Kinase-Glo Pim1, Pim2, Pim3 ATP depletion assays:* Pim1, Pim2 & Pim3 Kinase-Glo assays using ATP (at or below ATP Km) were used as previously described to determine the biochemical activity of compounds **1**−**7** and compounds **9**−**12**, as shown in Table 1.[13] *High ATP Pim1, Pim2, Pim3 AlphaScreen Assays:* Pim1, Pim2 & Pim3 AlphaScreen assays using high ATP (11−125 X ATP Km) were used as previously described to determine the biochemical activity of compounds **8**, **13**, and **14**, as shown in Table 1.[13]

Cell Lines and Reagents. The KMS-11.luc human multiple myeloma tumor cell line, a KMS-11 clone expressing firefly luciferase, was obtained from the University Health Network (UHN), Toronto, Ontario, Canada. KMS-11.luc was cultured in RPMI-1640 (ATCC, Manassas VA) supplemented with 10% FBS.

Cellular Proliferation Assays. Proliferation assays in KMS-11.luc were conducted as previously described with the concentration at which 50% maximal inhibition is reached reported as EC_{50}.[13]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.0c01279.

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J. Med. Chem. XXXX, XXX, XXX−XXX

Kinase selectivity profiles for compounds **5** and **11** and LCMS and HPLC spectra for representative compounds **5**, **11**, and **14** (PDF)

Molecular formula strings and statistical analysis of the associated biochemical data (CSV)

(XLSX)

Accession Codes

PDB codes of compounds **5** and **13** in PIM1 are 1oblv and 1ozrb, respectively. The atomic coordinates will be released upon article publication.

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All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

BOC, *tert*-butoxycarbonyl; Cbz, benzyloxycarbonyl; CL, clearance; Cl_{int}, intrinsic clearance; DCM, dichloromethane; DIEA, diisopropylethylamine; DMAP, dimethylaminopyridine; DMDO, dimethyldioxirane; DME, 1,2-dimethoxyethane; DMF, dimethylformamide; ee, enantiomeric excess; EDC, *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride; ER, efflux ratio; ER_H, extraction ratio; EtOAc, ethyl acetate; Log $D_{7.4}$, log of the octanol−water distribution coefficient at pH 7.4 of ionized and unionized compound; HOAt, 1-hydroxy-7-azabenzotriazole; HPLC, high performance liquid chromatography; HT-Eq Sol., high throughput equilibrium solubility; ISCO, Teledyne ISCO chromatography system; LCMS, liquid chromatography mass spectrometry; mCPBA, meta-chloroperoxybenzoic acid; NOE, nuclear overhauser effect; PSA, polar surface area; SFC, supercritical fluid chromatography; TBDMS, *tert*-butyldimethylsilyl; TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TLC, thin layer chromatography; TMS, trimethylsilyl

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