SUPPORTING INFORMATION

"Amino-Heterocycle Tetrahydroisoquinoline CXCR4 Antagonists with Improved ADME Profiles via Late-Stage Buchwald Couplings"

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I. Tables S1-S3 and Figures S1-S3.

Table S1. Physiochemical Parameters for 2-Aminoheterocycles in Table 1.

Compound	MW	tPSA	nRotBonds	clogP[a]	HBD	HBA
11070	349.48	66.01	8	3.01	3	4
TIQ-15	364.54	53.65	8	3.32	3	4
14	405.59	51.69	6	2.29	3	5
16	405.59	56.89	6	3.05	3	5
24	419.617	65.68	7	2.8	4	5
29	433.64	34.11	8	3.48	1	5
30	433.64	34.11	8	3.6	1	5
31	420.61	46.14	7	2.02	2	6
32	417.601	42.9	5	3.55	2	5
33	431.63	34.11	6	3.18	1	5
35	431.63	34.11	6	3.18	1	5
37	419.62	34.11	8	3.14	1	5

Table S2. Ligand Efficiency Parameters for Selected 2-Aminoheterocycles.

Compound	MW	pIC_{50}	BEI	clogP[a]	LipE
11070	349.48	8.29	2.37	3.01	5.28
TIQ-15	364.54	8.20	2.25	3.32	4.88
14	405.59	7.74	1.91	2.29	5.45
29	433.64	7.87	1.82	3.48	4.39
30	433.64	7.40	1.71	3.6	3.8
31	420.61	7.32	1.74	2.02	5.3
33	431.63	8.02	1.86	3.18	4.84
35	431.63	7.94	1.84	3.18	4.76
37	419.62	7.96	1.90	3.14	4.82

[a]Calculations using ACD labs software.

Table S3. $cLogD_{pH7.4}$ Values for 2-Aminoheterocycles in Table 1 using ChemAxon webware.

Compound	cLog D (pH 7.4)[a]
11070	-0.04
TIQ-15	-0.95[b]
14	-1.46
15	-1.46
16	-0.59
24	-1.04
29	0.63
30	-0.29
31	1.13
32	-1.89
33	-0.51
34	0.45
35	0.45
36	0.33
37	0.33

[a]Calculations using ChemAxon webware. [b]Experimental $eLogD_{pH7.4}$=-1.0.

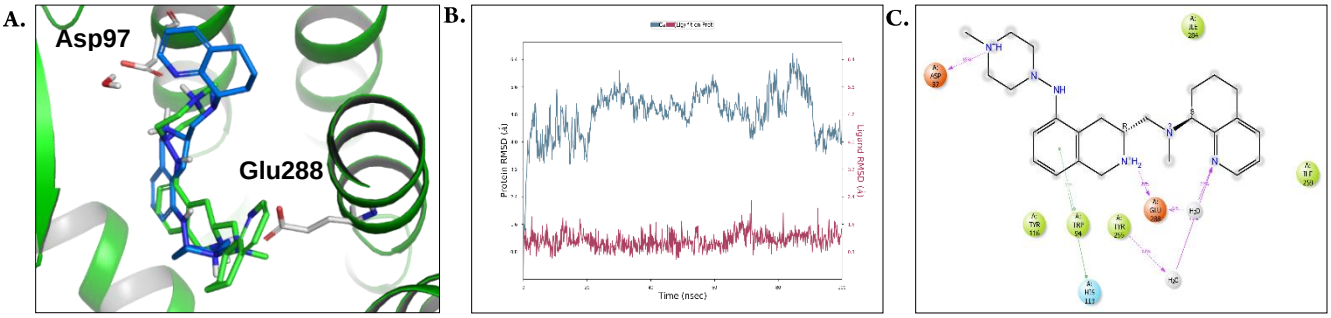


Figure S1: Panel 1: Pose 1 and Pose 2 overlay of compound **31**; Panel 2: RMSD of binding pose 1 of compound **31**; Panel 3: 2D-structure representation of major interactions of pose 1 of compound **31** over a 100 ns simulation.

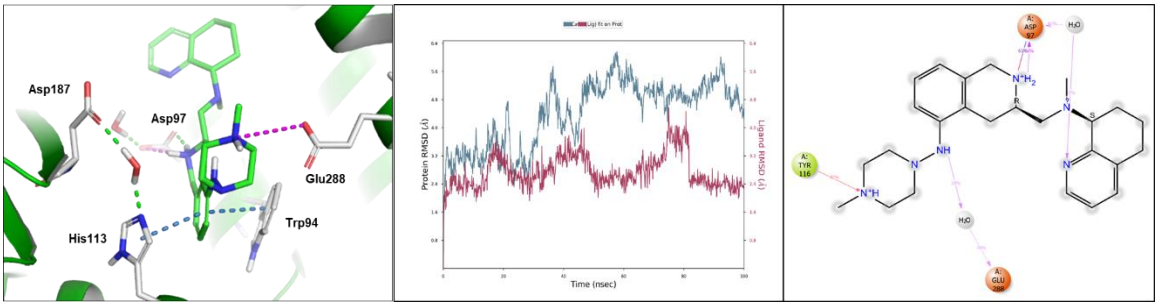


Figure S2. Panel 1: Initial pose of binding pose 2 of compound **31**; Panel 2: RMSD of compound **31** and the Cα protein backbone; Panel 3: 2D-major interaction (>30%) diagram of pose 2 of compound **31** throughout the 100 ns simulation.

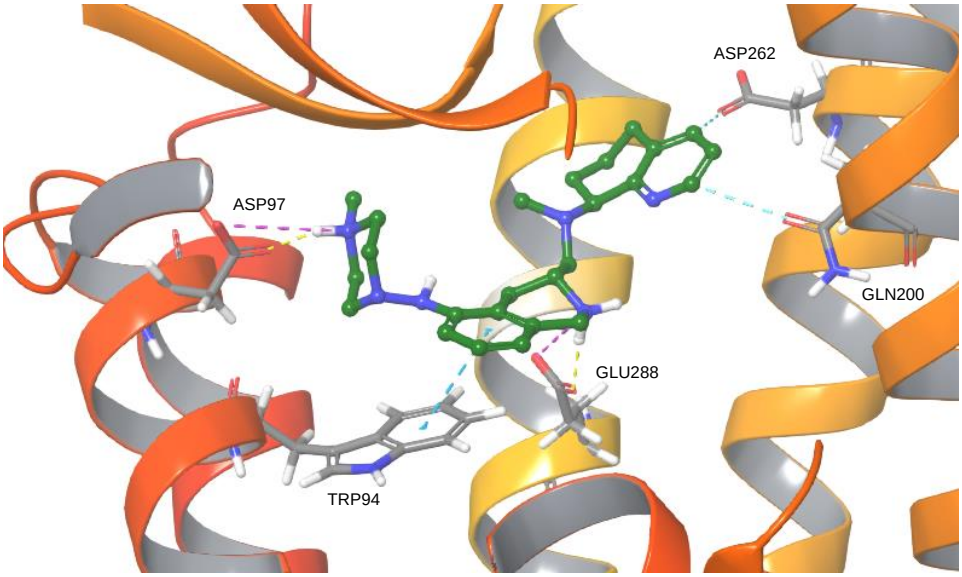


Figure S3. Pose of compound **31** in the CXCR4:IT1 grid (3ODU) without water molecules.

II. Biochemical and Assay Methods.

CXCR4 and Muscarinic Receptors Calcium Flux Assays. Exemplary compounds were tested for their ability to induce or inhibit calcium flux in CCRF-CEM cells. The experimental procedure and results are provided below. The exemplified biological assays were carried out with all compounds. Human T-lymphoblast cells (CCRF-CEM) expressing endogenous CXCR4 receptors and muscarinic acetylcholine receptors were grown in suspension culture and plated in clear bottom 384-well microplates (Greiner bio-one Cat# 789146) in assay buffer [Hank's Buffered Saline Solution (Gibco Cat# 14025-092) supplemented with 20 mM HEPES (Gibco Cat# 15630-080) and 0.1% fatty-acid free BSA (Sigma Cat# A9205)] at 40,000 cells per well. The cells were loaded with equal volume of calcium indicator dye (AAT Bioquest Inc, Cat# 34601) for 30 minutes at 37°C. The cells were then equilibrated to room temperature for 15 minutes before assay. Test compounds were solubilized and serially diluted in DMSO and then transferred to 384 well plates (Matrix Cat# 4307). The serially diluted compounds were further diluted to their working concentrations with the same assay buffer to 0.5% DMSO. They were added to the cells by the autosampler in the FDSS6000 (Hamamatsu) at final concentrations ranging from 25,000 nM to 0.423 nM. Activity of the compounds to induce calcium flux was monitored by FDSS in the "agonist mode" for 90 sec. For "antagonist mode" assessment, the cells are subsequently incubated for 25 minutes at room temperature. SDF-1α (R&D System Cat# 350-NS/CF) or acetylcholine was then added at a final concentration of 5 nM and 2,000 nM, respectively, to stimulate the cells. Inhibition of SDF-1α and acetylcholine-induced calcium flux was monitored by FDSS6000 for 90 seconds.

Activation data over a range of concentrations for each test compound was plotted as percent activation of the test compound (100% = maximum response triggered by a saturating concentration of SDF-1α, i.e., 160 nM). After correcting for background, the EC_{50} values were determined. The EC_{50} is defined as the concentration of test compound, which produces 50% of the maximal response and was quantified using the 4-parameter logistic equation to fit the data. Inhibition data for the test compound over a range of concentrations was plotted as percent inhibition of the test compound as compared to an internal control compound. The IC_{50} is defined as the concentration of test compound that inhibits 50% of the maximal response and was quantified using the 4-parameter logistic equation to fit the data.

None of the compounds tested demonstrated agonist activity in the calcium flux assays. All compounds demonstrated EC_{50} values >30 µM. In contrast, compounds demonstrated a range of potencies in inhibiting SDF-1 α -induced calcium flux.

PAMPA Assay. Compounds and controls were utilized as 10 mM stocks in 100% DMSO. Compounds were diluted 1:100 in pH 7.4 or pH 5.5 donor well buffer (pION CAT # 110151) providing a 100 µM assay solution in 1% DMSO. Compounds diluted in the donor well buffer was transferred to a Whatman Unifilter plate and filtered prior to dispensing 200 µL into the donor well of the assay plate (pION CAT #110163). The PAMPA membrane is formed by pipetting 4 µL of the lipid solution (pION CAT #110169) onto the filter plate (VWR CAT #13503). The membrane was then covered with 200 µL of acceptor well buffer at pH 7.4 (pION CAT #110139). The PAMPA assay plate (donor side and acceptor side) was combined and allowed to incubate at room temperature for 4 hours. The plate was disassembled and the spectrophotometer plates (VWR CAT #655801) were filled (150 µL/well). The donor, acceptor, reference, and blank plates were read in the SpectraMax UV plate reader. Data was captured by the pION software which analyzes the spectra and generated the Pc values.

Recombinant CYP2D6 Inhibition Assay. The CYP450 (2D6) inhibition assay utilizes enzymes from insect cells expressing human recombinant CYP450 (2D6) enzyme and fluorogenic probe (AMMC, 3-[2-(N,N-diethyl-N-methylamino)ethyl]-7-methoxy-4-methylcoumarin) that produces fluorescent metabolite; both reagents were obtained from Thermo Fisher Scientific/Discovery Labware (Woburn, MA). The assay was performed in a 1536-well microplates with a total volume of 5 µl. Automated liquid handling equipment (Thermo Multidrop Combi, LabCyte ECHO 550) was used in every step of compound preparation and for the addition of reagents. Each compound was tested in duplicate at 7 concentrations ranging from 1 nM to 20 µM; final concentration of DMSO in reactions was 0.2%. Positive controls were included in each experiment/run. Test compounds (10 nL/well) were first pre-incubated at 37°C for 30 min with 2.5 µL of prewarmed 2-fold-concentrated mixture of AMMC fluorogenic substrate (3 µM) and 12.5 nM rCYP2D6 enzyme in 100 mM potassium phosphate assay buffer pH 7.4. At the end of preincubation, the reactions were initiated by the addition of 2.5 µL of prewarmed 2-fold-concentrated NADPH-regenerating system (16.2 nM NADP) in the same assay buffer. Assay plates were then incubated at 37°C for 45 min. Immediately after the incubation was completed, the reactions were terminated by the addition of 3 µL of quench buffer (80% acetonitrile, 20% 0.5 M TRIS-base). Fluorescence intensity was measured using the Envision fluorescence plate reader (Perkin Elmer) at excitation and emission wavelengths of 405 and 460 nm, respectively, using a 430-nm cut-off filter. The end-point fluorescence readout was normalized to the fluorescence intensity of the reaction performed in the absence of the test substance (totals, 0% inhibition) and the mixture of reaction components in the presence of “Inhibitor Cocktail” (background, 100% inhibition). The IC_{50} value for each compound was derived from the fitted 20-point curve using a four-parameter logistic regression model.

Metabolic Stability. See experimental in: Kieltyka, K.; Zhang, J.; Li, S.; Vath, M.; Baglieri, C.; Ferraro, C.; Zvyaga, T. A.; Drexler, D. M.; Weller, H. N.; Shou, W. Z., A high-throughput bioanalytical platform using automated infusion for tandem mass spectrometric method optimization and its application in a metabolic stability screen. See: *Rapid Commun. Mass Spectrom.* **2009,** *23* (11), 1579-1591.

Chemicals and reagents

LC-MS grade acetonitrile (ACN) was purchased from Sigma Aldrich. Drug-free control blank mouse (K2EDTA) was obtained in-house by Sundia. Formic acid was purchased from Anpel Laboratory Technologies Co., Ltd. Propranolol were purchased from Sigma Aldrich. Stable internal standard (IS).

III. Pharmacokinetic studies in mice.

Male CD-1 mice (~ 30 g, total 12 mice with n=4/sampling time point) was obtained from Charles River Laboratories (Beijing, China). Mice was housed at the centralized animal facilities at the Sundia MediTech Company, Ltd (Shanghai, China) with a 12 h light-dark cycle. The housing temperature and relative humidity were controlled at 22°C and 55%, respectively. The animals had free access to water and food. However, they were fasted for 12 h (with water ad libitum) before the oral experiments. All animal experimental protocols were approved by the Institutional Animal Care and Use Committee of Sundia MediTech Company, Ltd, and all experiments were performed per the guidelines of this committee. An intravenous administration (IV) dose of 3mg/kg with a dosing volume of 5 mL/kg was administered via tail vein and an oral dose (PO) of 3,10, and 30 mg/kg with a dosing volume of 10 mL/kg was administered via gavage. The formulation used were 10%DMA+45% Kolliphor RH40+45%PBS for IV and 10%DMA+45%Kolliphor RH40+45%PBS for PO. All dosing solutions were freshly prepared on the day of administration. The blood samples (~ 15 μL) were collected in K_2EDTA tubes at 0, 0.1,0.25, 0.5, 1, 1.5, 2, 3, 4, 6, and 8 h for IV and 0, 0.5, 1, 2, 4, 6, 8, 10, 12, and 24h for PO after drug administration, and plasma (~ 7.5 μL) was harvested after centrifugation at 3000 rpm for 10 min. All plasma samples were stored at −80°C until analysis. The pharmacokinetic parameters were calculated using the non-compartmental approach (Model 200) of the pharmacokinetic software Phoenix WinNonlin, version 8.2. The area under the plasma concentration versus time curve (AUC) was calculated using the linear trapezoidal method. The slope of the apparent terminal phase was estimated by log linear regression using at least 3 data points and the terminal rate constant (λ) was derived from the slope. AUC0-∞ was estimated as the sum of the AUC0-t (where t is the time of the last measurable concentration) and Ct/λ. The apparent terminal half-life (t½) was calculated as 0.693/λ.

Preparation of calibration standards and quality control samples.
Stock solutions of compounds **2** and **31** in propranolol (IS) at 50ng/mL were prepared by dissolving each compound in DMSO and stored at -20°C. Calibration standards were prepared in drug-free blank mouse over a range of 1 – 2000 ng/mL, 2 – 4000 ng/mL and 2 – 2000 ng/mL respectively. The quality controls (QC) were prepared in blank mouse and rat plasma at 3, 30, 800 and 1600 ng/mL, 6, 30, 800, 1600 and 3200ng/mL and 6, 30, 800 and 1600ng/mL respectively.

Plasma sample extraction.
Calibration standards were freshly prepared on the day of sample analysis in control blank mouse plasma. All frozen PK study samples were thawed at room temperature prior to analysis. Once thawed, samples were thoroughly vortexed. In a 2-mL 96-well plate, a 21 μL aliquot of internal standard working solution (50ng/mL in ACN) was added to each well, then a 7 μL aliquot of standards, QCs and PK study samples was added. The EP tubes were capped, vortexed and centrifuged at 13000rpm for 10 min at 4°C. 20 μL of supernatant was transferred to a 96-well plate with 140μL pure water, vortexed and centrifuged at 13000rpm for 10 min at 4°C and 23μL, 23μL and 18 μL of supernatant were injected for UPLC-MS/MS analysis respectively. Samples were kept in the autosampler at 10°C during sample analysis.

UPLC–MS/MS conditions.
UPLC separation was carried out using a Shimadzu LC30AD system. The column was a Phenomenex Luna Omega Polar C18 1.6μm 30*2.1mm (1.6 μm, 2.1 × 30 mm) and was maintained at room temperature. The mobile phases were 0.1% formic acid in H2O (v/v) (A) and 0.1% formic acid in ACN (v/v) (B). The flow rate was 0.5 mL/min. The optimal UPLC elution gradient was: 0–0.4 min 5% B; 0.40 – 0.90 min 5 → 95% B; hold at 95%B for 0.60 min and 1.51–2.00 min 5% B. A AB SCIEX API4000 mass spectrometer was operated in positive atmospheric pressure chemical ionization source (+APCI) mode for assay development. The results were analyzed by 1/x2 weighted least-squares linear regression using Analyst (Applied Biosystems., Germany).

IV. Synthetic and Medicinal Chemistry Procedures: Preparation of Compounds 8-37.

General. All solvents and reagents were purchased from commercial suppliers and used without further purification. Analytical thin layer chromatography was carried out on silica pre-coated glass plates Merck KGaA (silica gel 60 F_{254}, 0.25 mm thickness) and visualized with UV light at 254 nm and/or with phosphomolybdic acid, idodine. Automated flash chromatography was performed on Teledyne ISCO CombiFlash R_f 200 system with RediSep R_f pre-packed silica cartridges (60 Å, 40-63 μm particle-size).

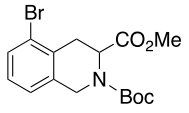
Concentration refers to rotary evaporation under reduced pressure.

^{1}H and ^{13}C NMR spectra were recorded on Varian INOVA or VNMR spectrometer operating at 400 MHz at ambient temperature with $CDCl_3$ or methanol-d_4 as solvents. Data for ^{1}H NMR were recorded as follows: δ chemical shift (ppm), multiplicity (s, singlet; d, doublet; dd = doublet of doublet; t, triplet; q, quartet; m, multiplet; br, broad; etc.), coupling constant (Hz), integration. Chemical shifts are reported in parts per million relative to internal reference $CDCl_3$ (^{1}H NMR: δ 7.26; ^{13}C NMR: δ 77.16), methanol-d_4 (^{1}H NMR, δ 4.87, 3.31; ^{13}C NMR, δ 49.00), and TMS (^{1}H NMR: δ 0.00).

Liquid chromatography/mass spectrometry (LCMS) data was obtained to verify molecular mass and analyze purity of products. The specifications of the LCMS instrument are the following: Agilent 1200 HPLC coupled to a 6120 quadrupole mass spectrometer (ESI-API), UV detection at 254 and 210 nm, Agilent Zorbax XDB-18 C_{18} column (50 mm x 4.6 mm, 3.5 µm), gradient mobile phase consisting of MeOH/water/0.1 % formic acid buffer, and a flow rate of 1.00 mL/min. The chemical purity of all final compounds was determined by LCMS and confirmed to be ≥ 95%.

Normal phase analytical chiral HPLC was performed on Agilent 1100 HPLC equipped with G1315B diode array detector using mixtures of hexanes/IPA and Daicel ChiralPak AD-H column (150 mm x 4.6 mm, 5 μM). Reverse phase HPLC was performed on the same instrument using mixtures of MeCN/H_2O and Daicel ChiralCel OD-RH column (150 mm x 4.6 mm, 5 μM). High resolution mass-spectra (HRMS) were acquired on a VG 70-S Nier Johnson or JEOL mass spectrometer.

Synthesis.



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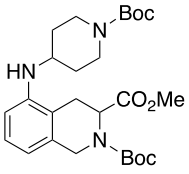
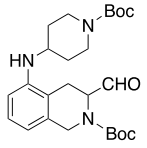
2-(tert-butyl) 3-methyl 5-bromo-3,4-dihydroisoquinoline-2,3(1H)-dicarboxylate (**8**). To a 250-mL round-bottom flask was charged with a racemic mixture of methyl 5-bromo-1,2,3,4-tetrahydroisoquinoline-3-carboxylate HCl salt (AstaTech, 3.0 g, 9.79 mmol), 1,4-dioxane (100 mL), and saturated sodium bicarbonate solution (100 mL). Di-tert-butyl dicarbonate (4.27 g, 19.6 mmol) was added at room temperature. The biphasic mixture was stirred for 90 minutes and then transferred to a separatory funnel and extracted twice with ethyl acetate. The combined organic extract was dried over anhydrous sodium sulfate and concentrated under reduced pressure to a crude material which was purified by CombiFlash system (40g silica column, 5 minutes hexanes → 30 minutes 0-20% EtOAc/hexanes) to afford the title compound as a clear gum (3.50 g, 96 % yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42 (t, J = 4.6 Hz, 1H), 7.18 - 6.89 (m, 2H), 5.20 (dd, J = 6.9, 2.6 Hz, 0.5H), 4.89 - 4.60 (m, 1.5H), 4.48 (dd, J = 29.2, 16.6 Hz, 1H), 3.65 (d, J = 10.9 Hz, 3H), 3.46 (ddd, J = 56.7, 16.5, 3.5 Hz, 1H), 3.08 (ddd, J = 48.9, 16.6, 6.7 Hz, 1H), 1.49 (d, J = 23.9 Hz, 9H). HRMS (*m/z*): calculated for $[C_{16}H_{20}BrNO_4 + H]^+$: 370.06540, found: 370.06521.



9a

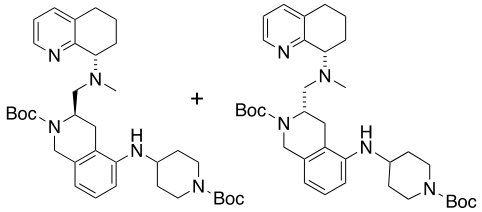
2-(tert-butyl) 3-methyl 5-((1-(tert-butoxycarbonyl)piperidin-4-yl)amino)-3,4-dihydroisoquinoline-2,3(1H)-dicarboxylate (**9a**). To an oven-dried Biotage 10-20 mL microwave vial equipped with a magnetic stir bar was charged with ester **8** (1.00 g, 2.70 mmol), tert-butyl 4-aminopiperidine-1-carboxylate (0.65 g, 3.25 mmol), $Pd_2(dba)_3$ (120 mg, 0.13 mmol), *rac*-BINAP (250 mg, 0.401 mmol), and cesium carbonate (1.23 g, 3.76 mmol). The vial was sealed with a Teflon-lined septum and flushed with argon for 5 minutes.

Degassed toluene (13.5 mL) was added, and the resulting mixture was heated at 100 °C for 48 hours. Upon the completion of the reaction as judged by TLC analysis, the mixture was allowed to cool to room temperature, filtered through a Celite pad, and concentrated to a crude material which was purified by CombiFlash system (40g silica column, 5 minutes hexanes → 30 minutes 0-30% EtOAc/hexanes) to afford the title compound as a yellow amorphous solid (1.31 g, 99% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.07 (t, *J* = 7.8 Hz, 1H), 6.61 – 6.42 (m, 2H), 5.26 (d, *J* = 5.1 Hz, 0.6H), 4.90 (s, 0.4H), 4.80 – 4.62 (m, 1H), 4.50 – 4.28 (m, 1H), 4.10 (bs, 1H), 3.65 (d, *J* = 9.1 Hz, 3H), 3.52 – 3.29 (m, 2H), 3.13 – 2.69 (m, 4H), 2.12 – 1.96 (m, 2H), 1.48 (d, 18H), 1.42 – 1.29 (m, 3H).



10a

tert-butyl 5-((1-(tert-butoxycarbonyl)piperidin-4-yl)amino)-3-formyl-3,4-dihydroisoquinoline-2(1H)-carboxylate (**10a**). To a 250-mL round-bottom flask containing a stir bar was charged with ester **9a** (1.31 g, 2.66 mmol) and anhydrous toluene (34 mL). Diisobutylaluminum hydride (1 M solution in toluene) (13.4 mL, 13.4 mmol) was added dropwise at -78 °C. After 2 h at -78 °C, reaction was quenched carefully with methanol then allowed to warm to 0 °C. A saturated solution of Rochelle salt was added, and the mixture was stirred at room temperature for an hour. The biphasic mixture was transferred to a separatory funnel. The aqueous layer was separated and extracted twice with ethyl acetate. The combined organic extract was dried over anhydrous sodium sulfate and concentrated under reduced pressure to afford the title compound as a crude material as a yellow foam which was used for the next step without purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.51 (s, 1H), 7.07 – 6.98 (m, 1H), 6.58 – 6.38 (m, 2H), 4.89 – 4.31 (m, 3H), 4.03 (bs, 1H), 3.61 – 3.20 (m, 2H), 3.07 – 2.57 (m, 4H), 2.20 – 1.90 (m, 2H), 1.68 – 1.21 (m, 21H).

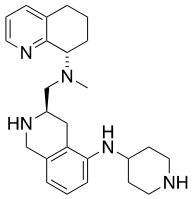


12a **13**

tert-butyl (R)-5-((1-(tert-butoxycarbonyl)piperidin-4-yl)amino)-3-((methyl((S)-5,6,7,8-tetrahydroquinolin-8-yl)amino)methyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (**12a**) *and tert-butyl (S)-5-((1-(tert-butoxycarbonyl)piperidin-4-yl)amino)-3-((methyl((S)-5,6,7,8-tetrahydroquinolin-8-yl)amino)methyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate* (**13**). To a 20-mL scintillation vial equipped with magnetic stir bar was charged with (S)-N-methyl-5,6,7,8-tetrahydroquinolin-8-amine **11** (650 mg, 4.00 mmol), sodium triacetoxyborohydride (1.02 g, 5.40 mmol), and 1,2-dichloroethane (10.4 mL). After stirring for 5 minutes, a solution of aldehyde **10a** (1.23 g, 2.68 mmol) in 1,2-dichloroethane (3 mL) was added dropwise. The resulting mixture was stirred at room temperature for 48-72 hours. Upon the completion of the reaction as judged by TLC and LCMS, the mixture was quenched by the addition of saturated $NaHCO_3$ solution. The biphasic mixture was transferred to a separatory funnel. The aqueous layer was separated and extracted with DCM (3 times). The combined organic extract was dried over anhydrous sodium sulfate and concentrated under reduced pressure to a crude diastereomeric mixture (1:1 *d.r.*) which was separated and purified by CombiFlash system (40g GOLD silica column, 5 minutes DCM → 30 minutes 0-10% MeOH/DCM) to afford title compounds as yellow foamy solid. Compound **12a** (310 mg, 19 % yield; Upper R_f) ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.27 (s, 1H), 7.20 (d, *J* = 7.4 Hz, 1H), 6.96 – 6.81 (m, 2H), 6.38 (d, *J* = 8.0 Hz, 1H), 6.33 – 6.21 (m, 1H), 4.76 – 4.46 (m, 2H), 4.02 (s, 2H), 3.87 – 3.72 (m, 2H), 3.54 (s, 1H), 3.44 – 3.33 (m, 1H), 2.95 – 2.81 (m, 2H), 2.78 – 2.63 (m, 2H), 2.61 – 2.43 (m, 4H), 2.35 (s, 3H), 2.07 – 1.85 (m, 4H), 1.80 – 1.66 (m, 1H), 1.64 – 1.25 (m, 21H). Compound **13** (246 mg, 15 % yield, Lower R_f): ^{1}H

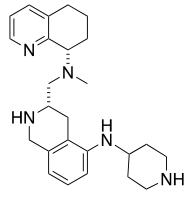
NMR (400 MHz, $CDCl_3$): δ 8.37 (s, 1H), 7.22 (s, 1H), 7.05 – 6.85 (m, 2H), 6.41 (d, *J* = 7.9 Hz, 2H), 4.75 – 4.42 (m, 2H), 4.18 (d, *J* = 17.0 Hz, 1H), 3.96 (s, 2H), 3.65 (s, 2H), 3.36 (s, 1H), 2.97 – 2.76 (m, 3H), 2.70 – 2.44 (m, 5H), 2.26 (s, 3H), 2.01 – 1.80 (m, 5H), 1.78 – 0.99 (m, 21H).

Global Boc Deprotection. To a 20-mL scintillation vial equipped with a magnetic stir bar was charged with the Boc-protected substrate (**12a** or **13**, 1 equiv) and DCM (0.13 M). Trifluoroacetic acid (36 equiv) was added dropwise, and the resulting mixture was stirred at room temperature overnight. Upon the completion of the reaction as judged by LCMS, the mixture was diluted with DCM, cooled in an ice-bath, and quenched by the addition of 3N NaOH until pH>12. The biphasic mixture was transferred to a separatory funnel. The aqueous layer was separated and extracted with DCM (3 times). The combined organic extract was dried over anhydrous sodium sulfate and concentrated under reduced pressure to a crude material which was purified by CombiFlash system using a gradient of solvent A (DCM) to solvent B (8:2:0.6 DCM/MeOH/NH_3 solution, 7N in MeOH) as eluent on a silica gel column to afford the final compound.



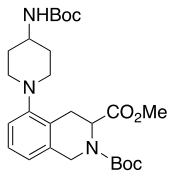
14

(S)-N-methyl-N-(((R)-5-(piperidin-4-ylamino)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**14**). Following general procedure A. Boc-protected substrate **12a** (0.31 g, 0.512 mmol) was diluted with TFA (1.4 mL) and DCM (4.0 mL). The crude material was purified by CombiFlash (12g silica column, 5 minutes A → 30 minutes 0-100% B) to afford the title compound as a light yellow foam (170.4 mg, 82% yield). ^{1}H NMR (400 MHz, methanol-d4): δ 8.45 (dd, J = 4.9, 1.6 Hz, 1H), 7.61 (dd, J = 7.8, 1.6 Hz, 1H), 7.28 (dd, J = 7.7, 4.8 Hz, 1H), 7.13 (t, J = 7.9 Hz, 1H), 6.72 (d, J = 8.2 Hz, 1H), 6.59 (d, J = 7.7 Hz, 1H), 4.98 (s, 3H), 4.37 (d, J = 3.2 Hz, 1H), 4.12 - 4.08 (m, 1H), 3.73 (dtd, J = 10.9, 5.6, 2.3 Hz, 2H), 3.44 (dt, J = 13.1, 4.0 Hz, 2H), 3.18 - 3.03 (m, 4H), 2.98 - 2.54 (m, 5H), 2.25 - 2.17 (m, 2H), 2.12 (s, 5H), 1.96 - 1.70 (m, 4H). ^{13}C NMR (101 MHz, methanol-d4): δ 157.1, 147.4, 145.7, 139.8, 136.8, 130.0, 128.8, 123.8, 117.7, 116.2, 111.1, 66.7, 59.6, 53.1, 48.2, 45.9, 44.2, 35.2, 30.0, 29.8, 26.4, 22.4, 22.4. HRMS (*m/z*): calculated for $[C_{25}H_{35}N_5 + H]^+$: 406.29707, found: 406.29646.



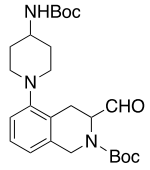
15

(S)-N-methyl-N-(((S)-5-(piperidin-4-ylamino)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**15**). Following general procedure A, Boc-protected substrate **13** (0.25 g, 0.41 mmol), was diluted with TFA (1.13 mL) and DCM (3.12 mL). The crude material was purified by CombiFlash (12g silica column, 5 minutes A → 30 minutes 0-100% B) to afford the title compound as an off-white foam (110.6 mg, 67 % yield). ^{1}H NMR (400 MHz, Chloroform-d): δ 8.41 (d, J = 4.7 Hz, 1H), 7.23 (d, J = 7.7 Hz, 1H), 7.01 - 6.81 (m, 2H), 6.33 (dd, J = 7.9, 4.5 Hz, 2H), 3.97 (d, J = 15.0 Hz, 1H), 3.93 - 3.83 (m, 2H), 3.43 - 3.20 (m, 4H), 2.94 (dtd, J = 23.6, 10.5, 9.3, 5.4 Hz, 3H), 2.74 - 2.55 (m, 5H), 2.39 (s, 3H), 2.19 - 1.73 (m, 8H), 1.61 (dp, J = 11.4, 4.2, 3.1 Hz, 1H), 1.30 - 1.16 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 156.8, 147.1, 144.1, 136.5, 136.2, 133.9, 125.9, 121.6, 118.8, 115.0, 107.7, 64.3, 59.1, 51.7, 49.4, 48.9, 44.8, 40.0, 33.2, 33.0, 29.0, 28.6, 22.8, 21.1. HRMS (*m/z*): calculated for $[C_{25}H_{35}N_5 + H]^+$: 406.29707, found: 406.29599.



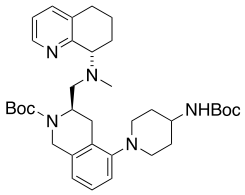
9b

2-(tert-butyl) 3-methyl 5-(4-((tert-butoxycarbonyl)amino)piperidin-1-yl)-3,4-dihydroisoquinoline-2,3(1H)-dicarboxylate (**9b**). *Rac*-2-tert-butyl 3-methyl 5-bromo-3,4-dihydroisoquinoline-2,3(1H)-dicarboxylate (**8**, 0.7 g, 1.891 mmol), tert-butyl piperidin-4-yl carbamate (0.454 g, 2.269 mmol), (+/-)-BINAP (0.177 g, 0.284 mmol), $Pd_2(dba)_3$ (0.087 g, 0.095 mmol), Cesium Carbonate (0.862 g, 2.65 mmol) were suspended in degassed toluene in a 20 ml microwave vial. The reaction was heated at 110°C for 3 days. The reaction suspension was filtered over celite and washed with EtOAc. The filtrate was evaporated and the residue was purified with column chromatography using Hexanes:EtOAc system to give 0.78 g (84% yield) of a yellow oil. ^{1}H NMR (399 MHz, $CDCl_3$) δ 7.21 – 7.09 (m, 1H), 6.96 – 6.78 (m, 2H), 4.84 – 4.21 (m, 2H), 4.23 – 3.79 (m, 1H), 3.65 (s, 1H), 3.60 (s, 3H), 3.36 – 2.46 (m, 8H), 2.29 – 1.69 (m, 3H), 1.46 (s, 9H), 1.44 (s, 9H).



10b

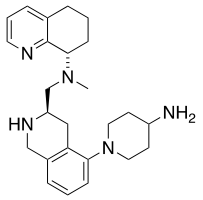
tert-butyl 5-(4-((tert-butoxycarbonyl)amino)piperidin-1-yl)-3-formyl-3,4-dihydroisoquinoline-2(1H)-carboxylate (**10b**). 2-tert-butyl 3-methyl 5-(4-((tert-butoxycarbonyl)amino)piperidin-1-yl)-3,4-dihydroisoquinoline-2,3(1H)-dicarboxylate (**9b**, 1.11 g, 2.267 mmoles) was dissolved in toluene and cooled to -78°C. Then added DIBAL-H (11.34 ml, 11.34 mmol) dropwise at -78°C. The reaction mixture was stirred at this temperature for 2 hours. Checked with TLC. All the starting material was consumed. quenched the reaction with 12 ml MeOH. Stirred at -78°C for 30 minutes then changed the bath to ice bath and let it warm to 0°C and added saturated Rachel`s salt solution. Stirred until the phases were clear (it takes at least 1-4 hour). Then stirred overnight with EtOAc. The organic layers were separated and the aqueous phase was extracted with EtOAc 2 times. Combined organic layer was dried over anhydrous $MgSO_4$, filtered off and evaporated. This material was used crude in the next step.



12b

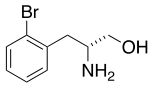
tert-butyl (R)-5-(4-((tert-butoxycarbonyl)amino)piperidin-1-yl)-3-((methyl((S)-5,6,7,8-tetrahydroquinolin-8-yl)amino)methyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (**12b**). (*S*)-N-methyl-5,6,7,8-tetrahydroquinolin-8-amine (**11**, 0.404 g, 2.489 mmol) was dissolved in 1,2-DCE and sodium triacetoxyborohydride (0.890 g, 4.07 mmol) was added and the mixture stirred for 5 minutes. Then the aldehyde tert-butyl 5-(4-((tert-butoxycarbonyl)amino)piperidin-1-yl)-3-formyl-3,4-dihydroisoquinoline-2(1H)-carboxylate (**10b**, 1.04 g, 2.263 mmol) was added and the reaction was stirred overnight. The reaction was quenched with saturated $NaHCO_3$(aq.) solution and extracted with DCM three times. The combined organic layers were dried over anhydrous $MgSO_4$, filtered and evaporated. The residue was purified with column chromatography using a EtOAc/Hexanes gradiant. ^{1}H NMR (399 MHz, $CDCl_3$): δ 8.26 (s, 1H), 7.32 (d, J = 7.8 Hz, 1H), 7.06 (t, J = 7.7 Hz, 1H), 6.99 (s, 1H), 6.83 (d, J = 7.8 Hz, 1H), 6.66

(s, 1H), 4.57 (d, J = 17.0 Hz, 1H), 4.46 (d, J = 41.2 Hz, 3H), 3.87 (d, J = 17.0 Hz, 1H), 3.57 (d, J = 7.3 Hz, 2H), 3.20 - 2.74 (m, 4H), 2.64 (dd, J = 13.8, 7.1 Hz, 3H), 2.27 (s, 3H), 2.08 (d, J = 11.3 Hz, 1H), 1.86 (d, J = 6.5 Hz, 2H), 1.60 (s, 3H), 1.49 (s, 9H), 1.47 (s, 9H).



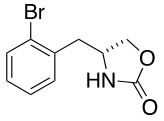
16

(S)-N-(((R)-5-(4-aminopiperidin-1-yl)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N-methyl-5,6,7,8-tetrahydroquinolin-8-amine (**16**). (*R*)-*tert*-butyl 5-(4-((tert-butoxycarbonyl)amino)piperidin-1-yl)-3-((methyl((*S*)-5,6,7,8-tetrahydroquinolin-8-yl)amino)methyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (**12b**, 0.515 g, 0.850 mmol) was dissolved in DCM and added TFA (1.310 ml, 17.00 mmol) and stirred overnight. The mixture was basified with 6M NaOH to pH >12 and then extracted with DCM several times. The combined organic layers were dried over $MgSO_4$, filtered and evaporated. The residue was purified with column chromatography starting with DCM and increased the polarity to DCM:MeOH:NH_3 (9:1:0.2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (dd, J = 4.9, 1.7 Hz, 1H), 7.38 - 7.31 (m, 1H), 7.12 - 7.03 (m, 2H), 6.86 (dd, J = 7.9, 1.2 Hz, 1H), 6.75 (dd, J = 7.8, 1.2 Hz, 1H), 4.13 (d, J = 15.5 Hz, 1H), 4.04 - 3.92 (m, 2H), 3.47 - 3.22 (m, 4H), 3.13 - 2.93 (m, 2H), 2.90 - 2.81 (m, 4H), 2.68 (dt, J = 17.0, 5.2 Hz, 2H), 2.43 (s, 3H), 2.30 (dd, J = 17.6, 10.9 Hz, 1H), 2.03 - 1.81 (m, 4H), 1.71 (dddd, J = 18.6, 13.3, 6.7, 3.4 Hz, 1H), 1.61 - 1.44 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) d 157.84, 151.83, 146.65, 136.98, 134.01, 126.15, 121.69, 121.33, 116.99, 109.99, 77.34, 77.03, 76.71, 64.49, 59.60, 51.74, 50.65, 48.65, 36.18, 36.03, 29.38, 29.22, 25.30, 21.33; HRMS (m/z): calculated for $C_{25}H_{36}N_5$ ($M+H^+$) 406.29652, found 406.29628.



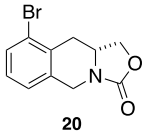
18

(R)-2-Amino-3-(2-bromophenyl)propan-1-ol (**18**). A 500 mL three-neck flask equipped with a magnetic stir bar, rubber septa and addition funnel was charged with *D*-bromo-phenylalanine (**17**, 20.0 g, 81.9 mmol, 1.00 eq) and the system was set under Ar. Then 200 mL of THF (not dried) was added at room temperature, followed by $NaBH_4$ (6.20 g 164 mmol, 2.00 eq) in one portion. Some exothermic gas expulsion was observed. After stirring at room temperature for 15 min, a solution of iodine (20.8 g, 81.9 mmol, 1.00 eq, grinded with mortar and pestle) dissolved in 100 mL THF was added dropwise over 90 min. The resulting reaction was slightly exothermic, and therefore a water bath was used to maintain room temperature. The resulting suspension turned slightly pink over time. After stirring at room temperature for 2 hrs, 16 mL of MeOH was added (not too much bubbling) and the stirring was continued for 10 min. Then 80 mL of aqueous NaOH (20% by wt.) was added and the stirring was continued for 20 min until the biphasic mixture became clear. For large scale reaction, it might be best to stir longer or warm to 60°C. Then 200 mL of DCM was added and the top layer was separated and washed with brine. The aqueous phase was extracted with DCM and the organic phase was washed with brine. The combined organics were dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to yield a sticky white solid. Some starting material remained, indicating that a slightly larger excess of $NaBH_4$ should be beneficial. To remove the starting material, the crude product was dissolved in DCM, THF and 2 M aqueous NaOH solution and stirred for 12 hrs at room temperature. After this time, the product was extracted twice with DCM and dried over anhydrous sodium sulfate. The resulting product was recrystallized from toluene, filtered, and washed with diethyl ether to afford the desired product as a white solid (**18**, 12.6 g, 67% yield).



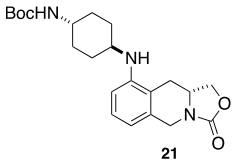
19

(R)-4-(2-Bromobenzyl)oxazolidin-2-one (**19**). A 100 mL flask equipped with a stir bar and rotary evaporator trap was charged with (*R*)-2-amino-3-(2-bromophenyl)propan-1-ol (**18**, 7.27 g, 31.6 mmol, 1.00 eq), diethyl carbonate (7.66 mL, 63.3 mmol, 2.00 eq) and potassium carbonate (0.440 g, 3.16 mmol, 0.10 eq). The resulting reaction mixture was heated to 135°C and stirred for 3 hrs while distilling off EtOH. Then saturated aqueous $NaHCO_3$ solution was added, and the resulting aqueous layer was extracted with 3 times with DCM. Combined organic layers were dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The crude product was purified by crystallization from diethyl ether, which was induced by scratching the glass wall. The product was filtered and washed with MTBE affording the desired product as white crystals (5.63 g, 70% yield). See Nomoto, *et al*., Preparation of 4-Heterocyclyl-pyrimidine Compounds as Pest Control Agents, WO 2011/016530 A1.



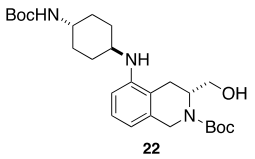
20

(R)-9-Bromo-1,5,10,10a-tetrahydro-3H-oxazolo[3,4-b]isoquinolin-3-one (**20**). A 50 mL round bottom flask equipped with a stir bar was charged with 3.76 g of the oxazolidinone **19** (14.7 mmol, 1 equiv), 0.485 g of paraformaldehyde (16.2 mmol, 1.1 equiv), 14.3 mL of acetic acid and 4.8 mL of sulfuric acid (acids must be premixed). After stirring at rt for 12 h, the reaction mixture was poured portion wise into sat. Na_2CO_3 solution, extracted with CH_2Cl_2 (3x) and dried over Na_2SO_4. The crude product was purified on silica gel column using 0 to 10 % EA in CH_2Cl_2 as eluent. The product was allowed to crystallize out of CH_2Cl_2 solution affording 3.04 g (77 %) of the product **20** as white needles. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ: 7.51-7.47 (m, 1H), 7.14-7.11 (m, 2H), 4.84 (A of AB, J_{AB} = 17.1 Hz, 1H), 4.62 (dd, J = 8.7, 7.9 Hz, 1H), 4.36 (B of AB, J_{AB} = 17.1, 1H), 4.22 (dd, J = 8.7, 4.8 Hz, 1H), 3.96 (ddt, J = 10.8, 7.8, 4.6 Hz, 1H), 3.25 (A of ABX, J_{AB} = 16.4 Hz, J_{AX} = 4.2 Hz, 1H), 2.67 (B of ABX, J_{AB} = 16.4 Hz, J_{BX} = 11.1 Hz, 1H).



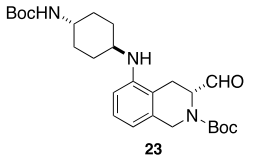
21

tert-butyl ((1R,4r)-4-(((R)-3-oxo-1,5,10,10a-tetrahydro-3H-oxazolo[3,4-b]isoquinolin-9-yl)amino)cyclohexyl)carbamate (**21**). A 10-20 mL μW tube equipped with a stir bar was charged with 500 mg of the bromide **20** (1.87 mmol, 1 equiv), 480 mg of trans-1,4-(N-Boc)-cyclohexyl-diamine (2.24 mmol, 1.2 equiv), 186 mg of (+/-)-BINAP (0.298 mmol, 0.16 equiv), 1.22 g of Cs_2CO_3 (3.73 mmol, 2 equiv) and 85.0 mg of $Pd_2(dba)_3$ (0.093 mmol, 0.05 equiv) and the system was set under Ar atmosphere by flashing through Ar for 1 h. Then 12.4 mL of dioxane (degassed by bubbling through Ar for 1 h) was added. After stirring at 140 °C for 3 h in the μW reactor (normal power), EA was added and the suspension was filtered through celite plug. The crude product was purified on silica gel column using 0-100 % EA in hexanes as eluent affording 227 mg (30%) of the product **21** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ: 7.12 (t, J = 7.9 Hz, 1H), 6.54 (d, J = 3.8 Hz, 1H), 6.52 (d, J = 4.3 Hz, 1H), 4.77 (A of AB, J_{AB} = 16.7 Hz, 1H), 4.61 (t, J = 8.1 Hz, 1H), 4.42 (d, J = 7.7 Hz, 1H), 4.33 (B of AB, J_{AB} = 16.9 Hz, 1H), 4.19 (dd, J = 8.7, 5.0 Hz, 1H), 3.97 (ddd, J = 12.6, 9.8, 4.8 Hz, 1H), 3.49 (br s, 1H), 3.25 (br s, 2H), 2.68 (A of ABX, J_{AB} = 14.9 Hz, J_{AX} = 4.6 Hz, 1H), 2.41 (B of ABX, J_{AB} = 15.0 Hz, J_{BX} = 10.6 Hz, 1H), 2.21-2.03 (m, 4H), 1.45 (s, 9H), 1.31-1.20 (m, 4H).



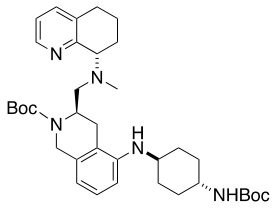
22

tert-butyl (R)-5-(((1r,4R)-4-((tert-butoxycarbonyl)amino)cyclohexyl)amino)-3-(hydroxymethyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (**22**). A 10-20 mL μW tube equipped with a stir bar was charged with 0.330 g of the oxazilidinone **21** (0.822 mmol, 1 equiv), 3.0 mL of 3 M NaOH solution (9.00 mmol, 11 equiv) and 7.0 mL of EtOH. After stirring at 110 °C for 1.5 h in a μW reactor (normal power), the reaction mixture was transferd to 50 mL round bottom flask, cooled to 0 °C and charged with 0.538 g of Boc_2O (2.47 mmol, 3 equiv) dissolved in 6.6 mL of 1,4-dioxane and 3.3 mL of water. After stirring at room temperature for 48 h, the reaction mixture was neutralized by addition of sat. NH_4Cl solution and diluted HCl, extracted with CH_2Cl_2 (3x) and dried over Na_2SO_4. The crude product was purified on silica gel column (40 g) using 0-100 % EA in hexanes as eluent to afford 0.208 g (53 %) of the product **22** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ: 7.07 (t, *J* = 7.8 Hz, 1H), 6.49 (d, *J* = 8.4 Hz, 2H), 4.71 (br s, 1H), 4.63-4.55 (m, 1H), 4.46-4.36 (m, 1H), 4.31-4.18 (m, 1H), 3.66-3.17 (m, 3H), 2.63 (A of ABX, J_{AB} = 15.9 Hz, J_{AX} = 6.5 Hz, 1H), 2.45 (B of ABX, J_{AB} = 15.9 Hz, J_{BX} = 2.6 Hz, 1H), 2.20-2.12 (m, 2H), 2.11.-2.03 (m, 2H), 1.60 (br s, 3H), 1.48 (s, 9H), 1.45 (s, 9H), 1.31-1.17 (m, 2H).



23

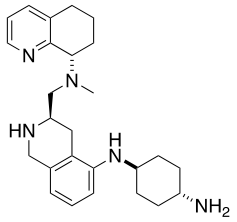
tert-butyl (R)-5-(((1r,4R)-4-((tert-butoxycarbonyl)amino)cyclohexyl)amino)-3-formyl-3,4-dihydroisoquinoline-2(1H)-carboxylate (**23**). A 50 mL Schlenk tube equipped with a magnetic stir bar and septum was charged with 0.208 g of the alcohol **22** (0.437 mmol, 1 equiv), 0.323 mL of triethyl amine (2.32 mmol, 5.3 equiv) and 1.33 mL of CH_2Cl_2. After the reaction mixture was cooled to 0 °C, 0.278 g of SO_3*Py (1.75 mmol, 4 equiv) dissolved in 1.33 mL of DMSO was added dropwise and the reaction mixture was allowed to warm up to rt in 5 h. The reaction mixture was quenched by addition of sat. $NaHCO_3$ solution, extracted with CH_2Cl_2 (3x) and dried over Na_2SO_4. The crude product was purified on silica gel column using 0 to 10 % EA in CH_2Cl_2 as eluent affording 150 mg (72 %) of the product **23** as a slightly yellow oil.



12c

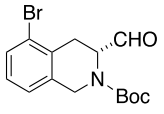
tert-butyl (R)-5-(((1r,4R)-4-((tert-butoxycarbonyl)amino)cyclohexyl)amino)-3-((methyl((S)-5,6,7,8-tetrahydroquinolin-8-yl)amino)methyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (**12c**). A 20 mL vial equipped with a stir bar was charged with 0.150 g of the aldehyde **23** (0.317 mmol, 1 equiv), 0.067 g of the amine **11** (0.412 mmol, 1.3 equiv) and 3.2 mL of 1,2-DCE. Then 0.101 g of STAB (0.475 mmol, 1.5 equiv) was added and the suspension was stirred at rt for 3 h. The reaction mixture was quenched by addition of sat. $NaHCO_3$ solution, extracted with CH_2Cl_2 (3x) and dried over Na_2SO_4. The crude product was purified on silica gel column using 0 to 100 % EA in hexanes as eluent affording 131 mg (67 %) of the product **12c** as a slightly yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ: 8.33 (br s, 1H), 7.24 (d, *J* = 7.7 Hz, 1H), 6.98-6.89 (m, 2H), 6.38 (d, *J* = 8.1 Hz, 1H), 6.31 (br s, 1H), 4.78-4.43 (m, 2H), 3.86 (d, *J* = 17.0 Hz, 1H), 3.79 (dd, *J* = 8.8, 4.9 Hz, 1H), 3.49 (br s, 2H), 3.27-3.15 (m, 1H), 2.75 (A of AB, J_{AB} = 15.8

Hz, 1H), 2.76-2.68 (m, 1H), 2.60 (dt, *J* = 16.8, 5.0 Hz, 1H), 2.55-2.45 (m, 2H), 2.39 (s, 3H), 2.23-2.16 (m, 1H), 2.14-2.03 (m, 3H), 1.99-1.88 (m, 2H), 1.80-1.69 (m, 1H), 1.67-1.55 (m, 1H), 1.46 (s, 9H), 1.44 (s, 9H), 1.33-1.17 (m, 4H); LC-MS (ESI-API, 254 nm) 75-95% MeOH in H_2O (0.1% HCO_2H), 6 min, 1.00 mL/min, C18 (Agilent Zorbax XDB-18, 50 mm x 4.6 mm, 3.5 µm), *m/z* = 620.3 (M + H), t = 0.730 min.



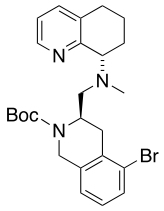
24

(1r,4R)-N[1]-((R)-3-((methyl((S)-5,6,7,8-tetrahydroquinolin-8-yl)amino)methyl)-1,2,3,4-tetrahydroisoquinolin-5-yl)cyclohexane-1,4-diamine (**24**). A 20 mL vial equipped with a stir bar was charged with 131 mg of the amine **12c** (0.211 mmol, 1 equiv) dissolved in 2.1 mL of CH_2Cl_2. Then 488 µL of TFA (6.34 mmol, 30 equiv) was added. After stirring at rt for 20 h, the reaction mixture was quenched by addition of 1 N KOH solution, extracted with CH_2Cl_2 (3x) and dried over Na_2SO_4. The crude material was purified on silica gel column using 0 to 60 % Solvent 2 in CH_2Cl_2 (solvent 2 = 70 % CH_2Cl_2, 30 % MeOH, 3 % NH_4OH) as eluent affording 77 mg (87 %) of the product **24** as a slightly yellow powder. ^{1}H NMR (600 MHz, $CDCl_3$, ppm) δ: 8.47 (d, *J* = 4.7 Hz, 1H), 7.34 (d, *J* = 7.7 Hz, 1H), 7.06 (dd, *J* = 7.7, 4.7 Hz, 1H), 7.00 (t, *J* = 7.8 Hz, 1H), 6.45 (d, *J* = 8.1 Hz, 1H), 6.39 (d, *J* = 7.6 Hz, 1H), 4.00 (dd, *J* = 8.9, 6.4 Hz, 1H), 3.94 (A of AB, J_{AB} = 15.3 Hz, 1H), 3.89 (B of AB, J_{AB} = 15.3 Hz, 1H), 3.33-3.22 (m, 2H), 2.91 (br s, 1H), 2.82-2.64 (m, 4H), 2.54 (s, 3H), 2.35 (dd, *J* = 15.5, 4.2 Hz, 1H), 2.17-1.64 (m, 9H), 1.30-1.14 (m, 4H). ^{13}C NMR (400 MHz, $CDCl_3$, ppm) δ: 157.70, 146.80, 144.74, 136.52, 136.25, 133.88, 126.03, 121.43, 118.60, 114.61, 107.61, 64.13, 59.67, 51.57, 51.18, 50.21, 48.95, 41.37, 35.35, 35.31, 32.18, 32.11, 29.19, 28.82, 24.84, 21.24; LC-MS (ESI-API, 254 nm) 75-95% MeOH in H_2O (0.1% HCO_2H), 3 min, 1.00 mL/min, C18 (Agilent Zorbax XDB-18, 50 mm x 4.6 mm, 3.5 µm), *m/z* = 420.2 (M + H), 210.6 (M/2 + H), t = 0.495 min.; HRMS (ESI+) calcd for $C_{26}H_{38}N_5$ ($[M+H]^+$): 420.3122. Found: 420.3123.



26

tert-butyl (R)-5-bromo-3-formyl-3,4-dihydroisoquinoline-2(1H)-carboxylate (**26**). To a 1000 mL round-bottom flask containing a stir bar was charged with (*R*)-2-tert-butyl 3-methyl 5-bromo-3,4-dihydroisoquinoline-2,3(1*H*)-dicarboxylate (**25**, 10 g, 27.0 mmol) which was prepared via literature method by Beadle et al (PCT Int. Appl., 2014193781, 04 Dec 2014) and anhydrous toluene (270 mL). Diisobutylaluminum hydride (1 M solution in toluene) (81 mL, 81 mmol) was added dropwise at -78 °C. After 2 h at -78 °C, reaction was quenched carefully with methanol then allowed to warm to 0 °C. A saturated solution of Rochelle salt was added, and the mixture was stirred at room temperature for an hour. The biphasic mixture was transferred to a separatory funnel. The aqueous layer was separated and extracted twice with ethyl acetate. The combined organic extract was dried over anhydrous sodium sulfate and concentrated under reduced pressure to afford the title compound which was used for the next step without purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.52 (d, J = 5.5 Hz, 1H), 7.53 - 7.36 (m, 1H), 7.18 - 6.92 (m, 2H), 4.96 (dd, J = 7.3, 3.2 Hz, 0.5H), 4.72 (dd, J = 16.7, 6.8 Hz, 1H), 4.66 - 4.37 (m, 1.5H), 3.58 - 3.28 (m, 1H), 3.05 (ddd, J = 33.6, 16.6, 6.7 Hz, 1H), 1.49 (d, J = 22.7 Hz, 9H).



27

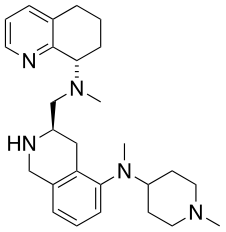
tert-butyl (R)-5-bromo-3-((methyl((S)-5,6,7,8-tetrahydroquinolin-8-yl)amino)methyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (**27**). To a 500-mL round-bottom flask equipped with magnetic stir bar was charged with (*S*)-N-methyl-5,6,7,8-tetrahydroquinolin-8-amine (**11**, 6.57 g, 40.5 mmol), sodium triacetoxyborohydride (10.31 g, 48.6 mmol), and 1,2-dichloroethane (100 mL). After stirring for 5 minutes, a solution of aldehyde **26** (9.19 g, 27.0 mmol) in 1,2-dichloroethane (35 mL) was added dropwise. The resulting mixture was stirred at room temperature for 48 hours. Upon the completion of the reaction as judged by TLC and LCMS, the mixture was quenched by addition of saturated $NaHCO_3$ solution. The biphasic mixture was transferred to a separatory funnel. The aqueous layer was separated and extracted with DCM (3 times). The combined organic extract was dried over anhydrous sodium sulfate and concentrated under reduced pressure to a crude material which was purified by CombiFlash system (220 g GOLD silica column, 5 minutes DCM → 30 minutes 0-5% MeOH/DCM) to afford the title compound as a colorless amorphous solid (10.51 g, 21.61 mmol, 80 % yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30 (s, 1H), 7.35 (d, J = 7.8 Hz, 1H), 7.28 (s, 1H), 6.94 (t, J = 7.4 Hz, 2H), 6.87 (s, 1H), 4.83 - 4.30 (m, 2H), 3.96 - 3.60 (m, 2H), 3.14 (d, J = 17.0 Hz, 1H), 2.84 - 2.70 (m, 2H), 2.70 - 2.48 (m, 2H), 2.36 (s, 4H), 2.09 - 1.72 (m, 3H), 1.62 (s, 1H), 1.47 (s, 9H). HRMS (*m/z*): calculated for $[C_{25}H_{32}BrN_3O_2 + H]^+$: 486.17561, found: 486.17731.

General procedure A for Late-Satge Buchwald-Hartwig coupling

Step 1: To an oven-dried Biotage 5-10 mL microwave vial equipped with a magnetic stir bar was charged with bromo compound **27** (1 equiv), $Pd_2(dba)_3$ (0.05 equiv, 5 mol %), *rac*-BINAP (0.15 equiv, 15 mol %), cesium carbonate (1.4 equiv), and solid amines (1.2 equiv). The vial was sealed with a Teflon-lined septum and purged with argon for 5 minutes. Degassed toluene (0.2 M) and liquid amines (1.2 equiv) were added successively via a syringe, and the vessel was degassed with argon for another 5 minutes. The resulting mixture was heated at 120 °C for 24 hours. Upon the completion of the reaction as judged by TLC and LCMS, the mixture was allowed to cool to room temperature, filtered through a Celite pad, and concentrated to a crude material which was purified by CombiFlash system using a gradient of solvent A (DCM) to solvent B (MeOH) as eluent on a RediSep Rf GOLD silica column to afford the Boc-protected product.

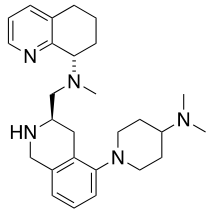
Step 2: To a 20-mL scintillation vial equipped with a magnetic stir bar was charged with the Boc-protected substrate from Step 1 (1 equiv) and DCM (0.13 M). Trifluoroacetic acid (36 equiv) was added dropwise, and the resulting mixture was stirred at room temperature overnight. Upon the completion of the reaction as judged by LCMS, the mixture was diluted with DCM, cooled in an ice-bath, and quenched by the addition of 3N NaOH until pH>12. The biphasic mixture was transferred to a separatory funnel. The aqueous layer was separated and extracted with DCM (3 times). The combined organic extract was dried over anhydrous sodium sulfate and concentrated under reduced pressure to a crude material which was purified by CombiFlash system using a gradient of solvent A (DCM) to solvent B (8:2:0.6 DCM/MeOH/NH_3 solution, 7N in MeOH) as eluent on a silica gel column to afford the final compound.

Compounds **29**-**37** were obtained using this general procedure.



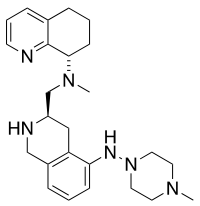
29

(S)-N-methyl-N-(((R)-5-(methyl(1-methylpiperidin-4-yl)amino)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**29**). Following general procedure A. Step 1: bromo-compound **27** (0.25 g, 0.514 mmol), 1,4-N,N'-dimethylpiperidin-4-amine (**28d**, liquid) (0.079 g, 0.617 mmol), $Pd_2(dba)_3$ (0.024 g, 0.026 mmol), rac-BINAP (0.048 g, 0.077 mmol), cesium carbonate (0.234 g, 0.720 mmol), toluene (2.57 mL). Purification by CombiFlash (5 minutes DCM then 30 min 0-80% 9:1:0.2 DCM/MeOH/NH_3 solution, 7N in MeOH) provided the Boc-protected product as yellow foamy solid (0.219 g, 0.411 mmol, 80 % yield). Step 2: Boc-substrate (**12d**, 0.2196 g, 0.411 mmol), TFA (1.141 mL, 14.81 mmol), and DCM (3.16 mL). The crude material was purified by CombiFlash (12g column, 5 minutes A then 30 minutes 0-50% B) to afford the title compound as a light yellow foam (162.9 mg, 91 % yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.44 (dd, J = 4.8, 1.7 Hz, 1H), 7.38 - 7.32 (m, 1H), 7.10 - 7.03 (m, 2H), 6.93 (dd, J = 7.9, 1.2 Hz, 1H), 6.76 (dd, J = 7.6, 1.1 Hz, 1H), 4.13 (d, J = 15.4 Hz, 1H), 4.03 - 3.93 (m, 2H), 2.88 - 2.65 (m, 9H), 2.59 (s, 3H), 2.45 (s, 3H), 2.24 (s, 3H), 2.08 - 1.53 (m, 12H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 157.6, 151.3, 146.6, 136.6, 136.1, 133.6, 130.9, 125.4, 121.4, 121.4, 119.5, 64.3, 59.7, 58.8, 55.2, 54.9, 51.5, 48.2, 45.9, 40.5, 36.0, 30.0, 29.9, 29.0, 27.2, 25.1, 21.1. HRMS (*m/z*): calculated for $[C27H39N5 + H]^+$: 434.32837, found: 434.32803.



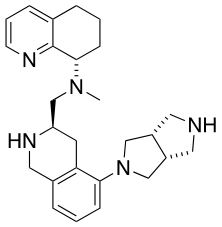
30

(S)-N-(((R)-5-(4-(dimethylamino)piperidin-1-yl)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N-methyl-5,6,7,8-tetrahydroquinolin-8-amine (**30**). Following general procedure A. Step 1: bromo-compound **27** (0.25 g, 0.514 mmol), N,N-dimethylpiperidin-4-amine (**28e**, liquid) (0.079 g, 0.617 mmol), $Pd_2(dba)_3$ (0.024 g, 0.026 mmol), rac-BINAP (0.048 g, 0.077 mmol), cesium carbonate (0.234 g, 0.720 mmol), toluene (2.57 mL). Purification by CombiFlash (5 minutes DCM then 30 min 0-80% 9:1:0.2 DCM/MeOH/NH_3 solution, 7N in MeOH) provided the Boc-protected product as yellow foamy solid (0.1616 g, 0.303 mmol, 59 % yield). Step 2: Boc-substrate (**12e**, 0.1616 g, 0.303 mmol), TFA (0.840 mL, 10.90 mmol), and DCM (2.329 mL). The crude material was purified by CombiFlash (12g column, 5 minutes A then 30 minutes 0-60% B) to afford title compound as a white foam (116.6 mg, 89 % yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.44 (dd, J = 4.8, 1.7 Hz, 1H), 7.39 - 7.33 (m, 1H), 7.12 - 7.06 (m, 2H), 6.86 (dd, J = 8.0, 1.1 Hz, 1H), 6.75 (dd, J = 7.6, 1.1 Hz, 1H), 4.17 (d, J = 15.5 Hz, 1H), 4.00 (d, J = 15.8 Hz, 2H), 3.16 - 3.05 (m, 2H), 2.90 - 2.65 (m, 7H), 2.44 (s, 3H), 2.33 (s, 6H), 2.29 - 2.14 (m, 2H), 2.09 - 1.83 (m, 6H), 1.79 - 1.54 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 157.8, 151.7, 146.7, 136.7, 136.0, 133.8, 129.6, 125.9, 121.5, 121.3, 116.5, 64.5, 62.1, 59.8, 52.2, 51.5, 51.1, 48.2, 41.7, 40.6, 29.7, 29.3, 29.2, 28.9, 25.4, 21.2. HRMS (*m/z*): calculated for $[C27H39N5 + H]^+$: 434.32837, found: 434.32751.



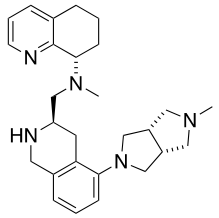
31

(S)-N-methyl-N-(((R)-5-((4-methylpiperazin-1-yl)amino)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**31**). Following general procedure A. Step 1: bromo-compound **27** (0.25 g, 0.514 mmol), 4-methylpiperazin-1-amine (**28f**, liquid) (0.071 g, 0.617 mmol), $Pd_2(dba)_3$ (0.024 g, 0.026 mmol), rac-BINAP (0.048 g, 0.077 mmol), cesium carbonate (0.234 g, 0.720 mmol), toluene (2.57 mL). Purification by CombiFlash (5 minutes DCM then 30 min 0-80% 9:1:0.2 DCM/MeOH/NH_3 solution, 7N in MeOH) provided the Boc-protected product as an orange foamy solid (0.0618 g, 0.119 mmol, 23.09 % yield). Step 2: Boc-substrate (**12f**, 0.0618 g, 0.119 mmol), TFA (0.329 mL, 4.27 mmol), and DCM (0.913 mL). The crude material was purified by CombiFlash (12g column, 5 minutes A then 30 minutes 0-60% B) to afford the title compound as a light orange foam (40.7 mg, 82% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.45 (dd, J = 4.7, 1.7 Hz, 1H), 7.37 - 7.29 (m, 1H), 7.10 - 6.93 (m, 3H), 6.47 (dd, J = 6.3, 2.6 Hz, 1H), 4.24 (s, 1H), 4.06 - 3.86 (m, 3H), 3.06 - 2.33 (m, 17H), 2.30 (s, 3H), 2.10 - 1.84 (m, 4H), 1.68 (dddt, J = 16.1, 11.2, 8.0, 3.0 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 157.8, 146.9, 144.7, 136.8, 134.1, 126.4, 121.7, 118.6, 117.0, 110.5, 64.3, 59.4, 55.8, 55.3, 51.7, 48.4, 45.9, 40.9, 29.3, 28.3, 25.0, 21.4. HRMS (*m/z*): calculated for $[C_{25}H_{36}N_6 + H]^+$: 421.30797, found: 421.30811.



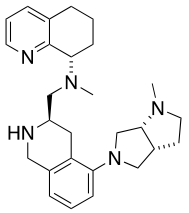
32

(S)-N-(((R)-5-((3aR,6aS)-hexahydropyrrolo[3,4-c]pyrrol-2(1H)-yl)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N-methyl-5,6,7,8-tetrahydroquinolin-8-amine (**32**). Following general procedure A. Step 1: bromo-compound **27** (0.25 g, 0.514 mmol), tert-butyl hexahydropyrrolo[3,4-c]pyrrole-2(1H)-carboxylate (solid) (**28g**, 0.131 g, 0.617 mmol), $Pd_2(dba)_3$ (0.024 g, 0.026 mmol), rac-BINAP (0.048 g, 0.077 mmol), cesium carbonate (0.234 g, 0.720 mmol), toluene (2.57 mL). Purification by CombiFlash (5 minutes DCM then 30 min 0-10% MeOH/DCM) provided the Boc-protected product as an orange foamy solid (0.190 g, 0.308 mmol, 60 % yield). Step 2: Boc-substrate (**12g**, 0.116 g, 0.188 mmol), TFA (0.521 mL, 6.77 mmol), and DCM (1.45 mL). The crude material was purified by CombiFlash (12g column, 5 minutes A then 30 minutes 0-100% B) to afford the title compound as an off-white solid (50 mg, 63 % yield). ^{1}H NMR (399 MHz, CD_3OD): δ 8.41 (d, J = 5.0 Hz, 1H), 7.57 (d, J = 7.9 Hz, 1H), 7.29 - 7.14 (m, 2H), 7.02 (d, J = 8.0 Hz, 1H), 6.97 - 6.85 (m, 1H), 4.31 (t, J = 8.4 Hz, 2H), 4.02 (dd, J = 10.2, 5.5 Hz, 1H), 3.50 - 3.41 (m, 2H), 3.29 - 2.57 (m, 17H), 2.15 - 2.00 (m, 5H), 1.91 (q, J = 11.7, 11.1 Hz, 1H), 1.71 (d, J = 13.7 Hz, 1H). ^{13}C NMR (101 MHz, methanol-d4): δ 193.6, 157.3, 148.5, 147.5, 139.6, 136.6, 132.3, 128.4, 128.1, 123.6, 122.7, 118.7, 79.6, 66.8, 60.0, 57.8, 57.0, 53.2, 53.0, 46.2, 43.0, 42.4, 35.6, 30.0, 28.5, 22.6, 22.3. HRMS (*m/z*): calculated for $[C26H35N5 + H]^+$: 418.29707, found: 418.29653.



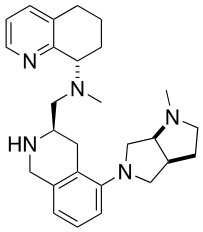
33

(S)-N-methyl-N-(((R)-5-((3aR,6aS)-5-methylhexahydropyrrolo[3,4-c]pyrrol-2(1H)-yl)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**33**). Following general procedure A. Step 1: bromo-compound **27** (0.25 g, 0.514 mmol), (3aR,6aS)-2-methyloctahydropyrrolo[3,4-c]pyrrole (liquid) (**28h**, 0.078 g, 0.617 mmol), $Pd_2(dba)_3$ (0.024 g, 0.026 mmol), *rac*-BINAP (0.048 g, 0.077 mmol), cesium carbonate (0.234 g, 0.720 mmol), toluene (2.57 mL). Purification by CombiFlash (5 minutes DCM then 30 min 0-100% 9:1:0.2 DCM/MeOH/NH_3 solution, 7N in MeOH) provided the Boc-protected product as a yellow foamy solid (0.2334 g, 0.439 mmol, 85 % yield). Step 2: Boc-substrate (**12h**, 0.2334 g, 0.439 mmol), TFA (1.217 mL, 15.80 mmol), and DCM (3.38 mL). The crude material was purified by CombiFlash (12g column, 5 minutes A then 30 minutes 0-80% B) to afford the title compound as a white foam (167 mg, 88 % yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.44 (dd, J = 4.8, 1.7 Hz, 1H), 7.36 (dd, J = 7.7, 1.7 Hz, 1H), 7.10 - 7.04 (m, 2H), 6.83 (dd, J = 8.1, 1.1 Hz, 1H), 6.71 (dd, J = 7.6, 1.1 Hz, 1H), 4.16 (d, J = 15.5 Hz, 1H), 4.04 - 3.98 (m, 2H), 3.28 (dd, J = 9.2, 6.5 Hz, 1H), 3.11 (dd, J = 9.2, 2.5 Hz, 1H), 2.89 - 2.63 (m, 13H), 2.44 (s, 3H), 2.34 (d, J = 8.1 Hz, 5H), 2.26 (dd, J = 8.8, 4.9 Hz, 1H), 2.08 - 1.91 (m, 3H), 1.79 - 1.65 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 157.7, 147.7, 146.6, 136.6, 136.0, 133.6, 128.4, 125.7, 121.4, 120.5, 115.4, 64.4, 62.2, 62.0, 60.0, 56.1, 55.7, 51.4, 48.4, 42.4, 41.9, 41.7, 40.5, 31.0, 29.0, 25.3, 21.0. HRMS (*m/z*): calculated for $[C27H37N5 + H]^+$: 432.31272, found: 432.31279.



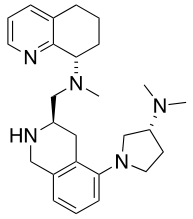
34

(S)-N-methyl-N-(((R)-5-((3aR,6aR)-1-methylhexahydropyrrolo[3,4-b]pyrrol-5(1H)-yl)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**34**). Following general procedure A. Step 1: bromo-compound **27** (0.25 g, 0.514 mmol), (3aR,6aR)-1-methyloctahydropyrrolo[3,4-b]pyrrole (liquid) (**28i**, 0.078 g, 0.617 mmol), $Pd_2(dba)_3$ (0.024 g, 0.026 mmol), *rac*-BINAP (0.048 g, 0.077 mmol), Cesium Carbonate (0.234 g, 0.720 mmol), toluene (2.57 mL). Purification by CombiFlash (5 minutes DCM then 30 min 0-60% 9:1:0.2 DCM/MeOH/NH_3 solution, 7N in MeOH) provided the Boc-protected product as a yellow foamy solid (0.2218 g, 0.417 mmol, 81 % yield). Step 2: Boc-substrate (**12i**, 0.2218 g, 0.417 mmol), TFA (1.157 mL, 15.02 mmol), and DCM (3.21 mL). The crude material was purified by CombiFlash (12g column, 5 minutes A then 30 minutes 0-50% B) to afford the title compound as a white foam (165 mg, 91 % yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.42 (ddd, J = 9.6, 4.8, 1.7 Hz, 1H), 7.37 (ddd, J = 7.7, 1.8, 0.9 Hz, 1H), 7.10 - 7.03 (m, 2H), 6.79 (t, J = 7.6 Hz, 1H), 6.69 (ddd, J = 7.6, 4.2, 1.1 Hz, 1H), 4.22 (dd, J = 29.2, 15.7 Hz, 1H), 4.13 - 3.97 (m, 2H), 3.31 (dq, J = 7.4, 2.5 Hz, 1H), 3.07 - 2.63 (m, 13H), 2.39 (dd, J = 9.4, 3.8 Hz, 6H), 2.13 - 1.86 (m, 5H), 1.85 - 1.61 (m, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 157.7, 148.6, 147.3, 146.7, 137.7, 136.8, 134.4, 127.0, 122.3, 120.0, 116.3, 69.6, 64.7, 57.8, 57.4, 55.7, 54.6, 53.5, 52.1, 42.6, 42.0, 41.3, 31.8, 29.8, 29.2, 25.1, 21.5. HRMS (*m/z*): calculated for $[C27H37N5 + H]^+$: 432.31272, found: 432.31329.



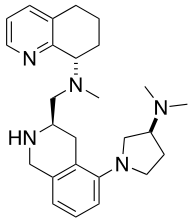
35

(S)-N-methyl-N-(((R)-5-((3aS,6aS)-1-methylhexahydropyrrolo[3,4-b]pyrrol-5(1H)-yl)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-5,6,7,8-tetrahydroquinolin-8-amine (**35**). Following general procedure A. Step 1: bromo-compound **27** (0.25 g, 0.514 mmol), (3aS,6aS)-1-methyloctahydropyrrolo[3,4-b]pyrrole (liquid) (**28j**, 0.078 g, 0.617 mmol), $Pd_2(dba)_3$ (0.024 g, 0.026 mmol), rac-BINAP (0.048 g, 0.077 mmol), cesium carbonate (0.234 g, 0.720 mmol), toluene (2.57 mL). Purification by CombiFlash (5 minutes DCM then 30 min 0-60% 9:1:0.2 DCM/MeOH/NH_3 solution, 7N in MeOH) provided the Boc-protected product as a yellow foamy solid (0.2236 g, 0.421 mmol, 82 % yield). Step 2: Boc-substrate (**12j**, 0.2236 g, 0.421 mmol), TFA (1.166 mL, 15.14 mmol), and DCM (3.23 mL). The crude material was purified by CombiFlash (12g column, 5 minutes A then 30 minutes 0-50% B) to afford the title compound as a white foam (170 mg, 94 % yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.40 (dd, J = 4.8, 1.6 Hz, 1H), 7.38 (dd, J = 7.7, 1.7 Hz, 1H), 7.10 - 7.05 (m, 2H), 6.81 - 6.77 (m, 1H), 6.72 - 6.66 (m, 1H), 4.29 (d, J = 15.6 Hz, 1H), 4.13 - 4.00 (m, 2H), 3.34 - 3.30 (m, 1H), 3.08 - 3.02 (m, 3H), 2.96 - 2.67 (m, 10H), 2.40 (d, J = 3.5 Hz, 3H), 2.36 (s, 3H), 2.11 - 1.92 (m, 5H), 1.86 - 1.65 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 157.8, 148.1, 146.7, 136.7, 135.8, 133.7, 128.0, 125.7, 121.5, 120.2, 114.7, 68.8, 64.4, 59.9, 57.4, 57.4, 55.6, 51.5, 48.5, 42.6, 41.6, 41.1, 32.0, 31.4, 29.1, 25.0, 21.2. HRMS (*m/z*): calculated for $[C_{27}H_{37}N_5 + H]^+$: 432.31272, found: 432.31243.



36

(S)-N-(((R)-5-((S)-3-(dimethylamino)pyrrolidin-1-yl)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N-methyl-5,6,7,8-tetrahydroquinolin-8-amine (**36**). Following general procedure A. Step 1: bromo-compound **27** (0.25 g, 0.514 mmol), (R)-N,N-dimethylpyrrolidin-3-amine (liquid) (**28k**, 0.070 g, 0.617 mmol), $Pd_2(dba)_3$ (0.024 g, 0.026 mmol), rac-BINAP (0.048 g, 0.077 mmol), cesium carbonate (0.234 g, 0.720 mmol), toluene (2.57 mL). Purification by CombiFlash (5 minutes DCM then 30 min 0-60% 9:1:0.2 DCM/MeOH/NH_3 solution, 7N in MeOH) provided the Boc-protected product as a yellow foamy solid (0.2286 g, 0.440 mmol, 86 % yield). Step 2: Boc-substrate (**12k**, 0.2286 g, 0.440 mmol), TFA (1.220 mL, 15.83 mmol), and DCM (3.38 mL). The crude material was purified by CombiFlash (12g column, 5 minutes A then 30 minutes 0-50% B) to afford the title compound as a light yellow foam (148 mg, 80 % yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.45 (dd, J = 4.7, 1.7 Hz, 1H), 7.38 - 7.33 (m, 1H), 7.10 - 7.02 (m, 2H), 6.81 (dd, J = 8.0, 1.1 Hz, 1H), 6.67 (dd, J = 7.6, 1.1 Hz, 1H), 4.12 (d, J = 15.5 Hz, 1H), 4.04 - 3.94 (m, 2H), 3.38 - 3.27 (m, 2H), 3.01 - 2.75 (m, 7H), 2.71 - 2.62 (m, 2H), 2.44 (s, 3H), 2.25 (s, 7H), 2.12 - 1.79 (m, 6H), 1.76 - 1.65 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 157.6, 148.6, 146.5, 136.6, 135.5, 133.7, 127.3, 125.8, 121.4, 119.9, 114.6, 65.0, 64.4, 59.3, 55.2, 51.3, 50.3, 47.7, 43.5, 40.1, 30.6, 29.0, 29.0, 25.0, 21.1. HRMS (*m/z*): calculated for [C26H37N5 + H]$^+$: 420.31272, found: 420.31278.



36

(S)-N-(((R)-5-((R)-3-(dimethylamino)pyrrolidin-1-yl)-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl)-N-methyl-5,6,7,8-tetrahydroquinolin-8-amine (**37**). Following general procedure A. Step 1: bromo-compound **27** (0.25 g, 0.514 mmol), (R)-N,N-dimethylpyrrolidin-3-amine (liquid) (**28l**, 0.070 g, 0.617 mmol), $Pd_2(dba)_3$ (0.024 g, 0.026 mmol), rac-BINAP (0.048 g, 0.077 mmol), cesium carbonate (0.234 g, 0.720 mmol), toluene (2.57 mL). Purification by CombiFlash (5 minutes DCM then 30 min 0-60% 9:1:0.2 DCM/MeOH/NH_3 solution, 7N in MeOH) provided the Boc-protected product as a yellow foamy solid (0.1907 g, 0.367 mmol, 71 % yield). Step 2: Boc-substrate (**12l**, 0.1907 g, 0.367 mmol), TFA (1.018 mL, 13.21 mmol), and DCM (2.82 mL). The crude material was purified by CombiFlash (12g column, 5 minutes A then 30 minutes 0-50% B) to afford the title compound as a light-yellow foam (136 mg, 88 % yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.43 (dd, J = 4.7, 1.7 Hz, 1H), 7.37 - 7.33 (m, 1H), 7.08 - 7.03 (m, 2H), 6.74 (d, J = 8.0 Hz, 1H), 6.67 - 6.62 (m, 1H), 4.21 (d, J = 15.5 Hz, 1H), 4.07 - 3.98 (m, 2H), 3.50 - 3.44 (m, 1H), 3.30 (d, J = 8.5 Hz, 1H), 3.08 - 3.04 (m, 1H), 2.99 - 2.94 (m, 1H), 2.85 - 2.60 (m, 8H), 2.43 (s, 3H), 2.27 (s, 6H), 2.14 - 1.69 (m, 7H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 157.6, 148.9, 146.7, 136.6, 136.1, 133.7, 126.4, 125.6, 121.4, 119.1, 113.0, 65.6, 64.4, 59.7, 56.1, 51.6, 50.1, 48.5, 44.3, 40.8, 32.1, 30.3, 29.2, 24.9, 21.3. HRMS (*m/z*): calculated for $[C_{26}H_{37}N_5 + H]^+$: 420.31272, found: 420.31315.

V. Computational Docking Procedures.

Protein Preparation

Similar to our previous study, we examined the 1T1t:CXCR4 X-ray crystal structure of CXCR4(PDB:3ODU).[1] Protein structures were prepared using Schrödinger Small Molecule Drug Discovery Suite. All water molecules (except 1629, 1646, and 1720) from the X-ray crystal structures were removed, and mutated residues in X-ray crystal structures were changed back to the wild type.[2] We added missing side chains and removed any extraneous ligands from the X-ray crystal structures of CXCR4.

Ligand Preparation

Two-dimensional (2D) structures of CXCR4 antagonists were sketched in the 2D sketcher module of Maestro and energy minimized using the ligprep protocol of the schrödinger suite 2021-2.[3,4] All calculations were completed using Optimized Potentials for Liquid Simulations 4 (OPLS4) force field in the gas phase.[4]

Induced-Fit Docking

Prepared ligands were docked within the 1T1t binding pocket using the Induced Fit Docking algorithm of Schrodinger 2021-2 in order to allow for optimization of the side chain amino acids near the docked ligand.[5] (Flexible ligand and side-chain sampling using the Glide software was applied within 5Å)[6] A total of five distinct binding poses (with RMSD ≥ 0.5 Å relative to the other poses) were generated for each ligand. Selection of the best pose for each ligand was made on the basis of computed values of Emodel score, GlideScore, and thorough visual inspection of the predicted binding modes in which favorable interactions with key residues were considered, as reported earlier.[2]

Binding pose Metadynamics

Binding-pose metadynamics is an enhanced sampling protocol, in which the molecule is forced to move about the binding pocket. If the molecule has high mobility over the course of the sampling this denotes instability of the binding pose suggesting it is likely not the active conformer.[7] Metadynamic simulations usually require manual setting of important parameters such as the collective variable (CV) and hill height.[8] (Hill height and width were set to 0.05 kcal/mol (about 1/10 of the characteristic thermal energy of the system, k_BT) and 0.02 Å, respectively) As a part of the binding pose metadynamics (BPMD) module in Maestro v.2021-2,

binding site residues are aligned to the first frame of the metadynamics trajectory before calculating the heavy atom root-mean-square deviation (rmsd) to the ligand conformation in the first frame.[7] In the preparatory phase of the metadynamics run, the protein-ligand complex was solvated in a box of SPC/E water molecules then subsequently minimized and restrained to allow the system to slowly reach the desired temperature of 300 K as well as releasing any bad contacts and/or strain in the initial starting structure. The final snapshot of the short unbiased metadynamics simulation of 0.5 ns is then used as the reference for the following metadynamics production phase. The production phase then consisted of 10 independent metadynamic simulations of 10 ns using the CV as the measure of the rmsd of the ligand heavy atoms relative to their starting position. The PoseScore output is indicative of the average RMSD difference from the starting pose, denoting overall stability of the binding pose. While Persistance Score (PersScore) measures hydrogen bond persistence from the starting pose to the last 2ns of the metadynamic run. (E.g., PersScore=0.8=80% of interactions were maintained)

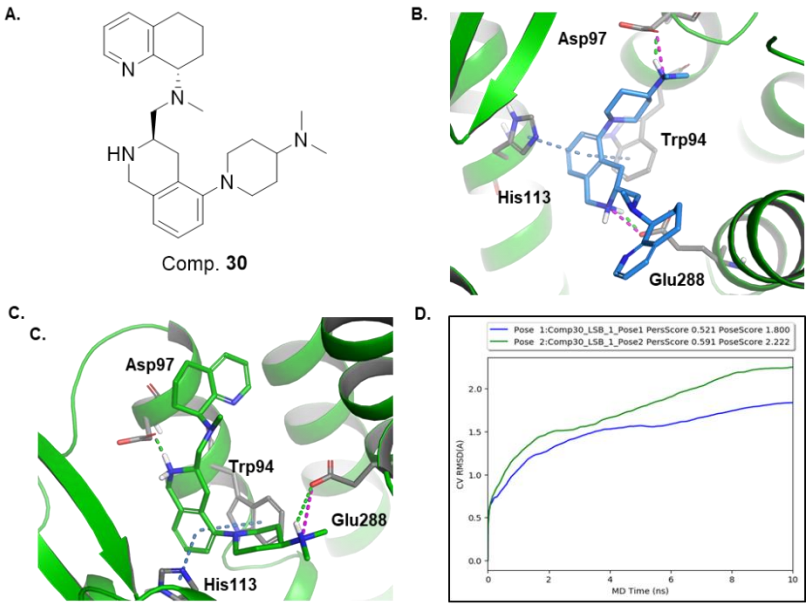
Molecular Dynamic Simulations

To further assess the stability of the complexes MD simulations were carried out using the Desmond module of Schrodinger suite 2021-2 similar to previous publications.[9–11] Binding pose 1 and 2 of compound **31** bound CXCR4 (PDB:3ODU) were embedded in a POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) bilayer and solvated with an 11 Å TIP3P water buffer using the OPLS4 (optimized potentials for liquid simulations) force field implemented in Desmond, Schrodinger.[12] The system was neutralized by adding chloride ions as needed and 0.15 M NaCl was added to the system and equilibrated using the built-in Desmond relaxation protocol for the NPT ensemble. **(Box 1)** The NPT ensemble with a temperature of 300 K and 1.01325 bar was applied in all the simulations with a production run length of 100 ns. The OPLS4 force field parameters were used in all simulations and long-range electrostatic interactions were calculated using the particle mesh Ewald method. Pressure and temperature were controlled via Langevin coupling scheme for the entirety of the 100 ns production run. A cutoff radius for Coulomb interactions was 9.0 Å was used and non-bonded forces were calculated using the RESPA integrator and the trajectories were saved at 13.3 ps intervals for analysis. Model system behavior between the ligand and protein was evaluated using the Simulation Interaction Diagram tool in the Desmond MD suite. The stability of the MD simulations was monitored via RMSD of the ligand and protein atom positions in time.

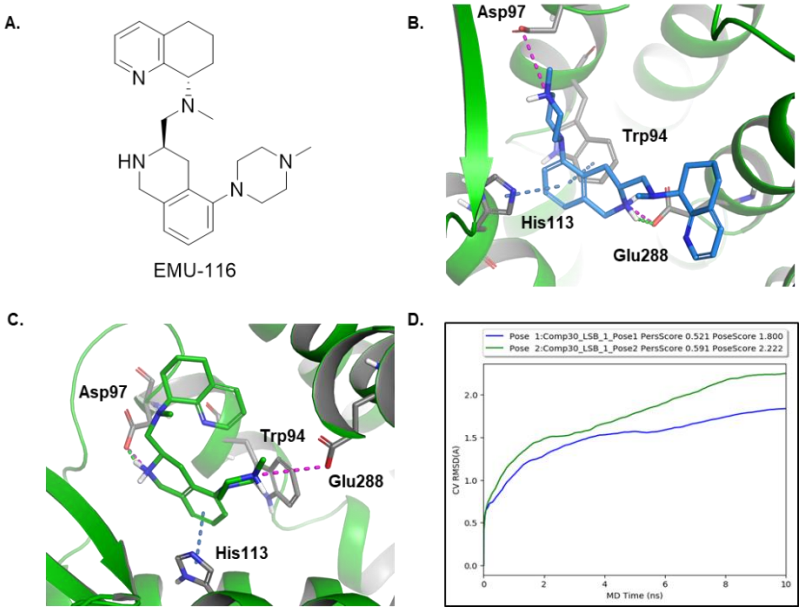
Box 1: Summary of Desmond Relaxation Protocol Stages

stage 1 – task
stage 2 - simulate, Brownian Dynamics NVT, T = 10 K, small timesteps, and restraints on solute heavy atoms, 100ps
stage 3 - simulate, 100 K, H2O Barrier, Browninan NPT, membrane restrained in z, protein restrained
stage 4 - simulate, 100 K, H2O Barrier, NPgT, membrane restrained in z, protein restrained
stage 5 - simulate, NPgT, Heating from 100 -> 300 K, H2O Barrier and gradual release of restrain
stage 6 - simulate, NVT production remove all restraints
stage 7 - simulate, NVT production remove all restraints
stage 8 - simulate
stage 9 - pl_analysis
(9 stages in total)

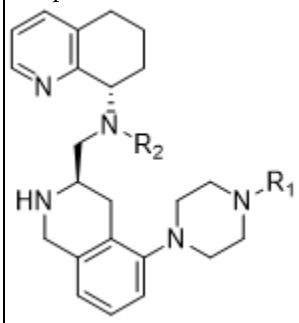
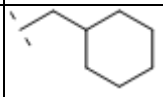
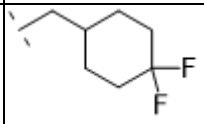
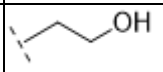
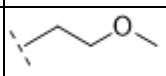
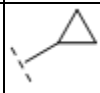
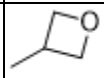
VI. Additional Computational Studies



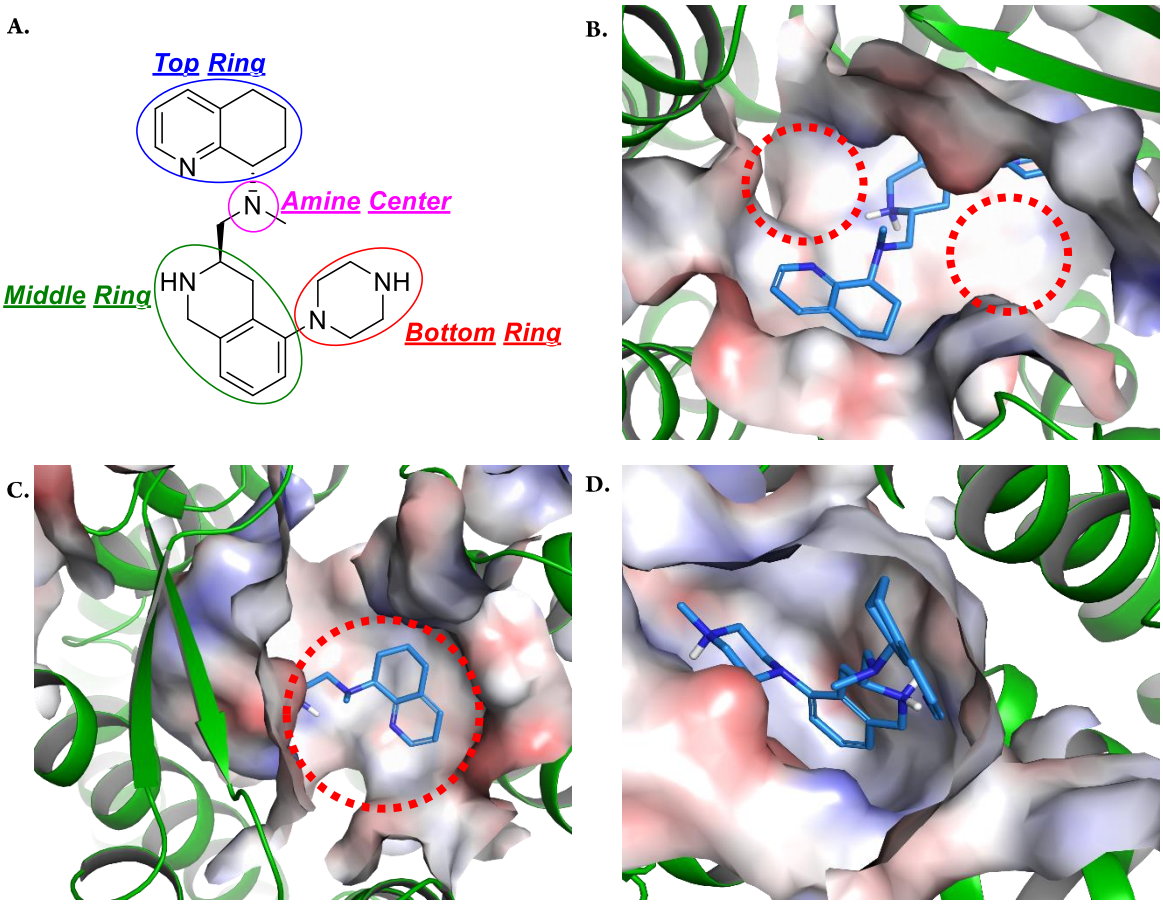
Supplementary Figure S4, **A:** Compound **30** 2D-structure representation, Compound **30 B:** Binding pose 1, **C:** Binding pose 2, **D:** BPMD result.



Supplementary Figure S5, **A: EMU-116** 2D-structure representation, **EMU-116 B:** Binding pose 1, **C:** Binding pose 2, **D:** BPMD result.

Supplementary Table 4: Previously synthesized molecules experimental CXCR Ca^{2+}Flux[1]			
Compound 	R_1	R_2	CXCR4 Ca^{2+} flux (nM)
EMU-116			29.6±15.7
A	-H		364±257
B	-H		715±182
C	-H		498±198
D	-H		195±4.10
E	-H		154±69.4
F			13.8±5.45
G			16.9±9.51
H			23.5±9.16
I			381±355

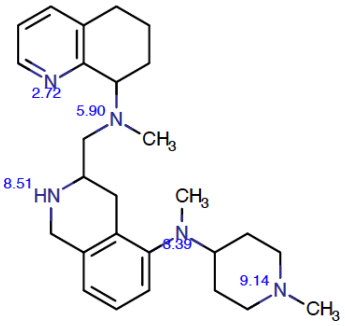
Based upon our metadynamic data, we next aimed to identify if previously synthesized analogs may concur that binding mode 1 is correct. Examination of the binding pocket around EMU-116 elucidated space is available around the head ring and amine center of the middle ring implying some movement, based upon substituent placement, could be tolerated. As evidenced by our previous experimental results, some steric bulk on the amine center could be tolerated, but too much significantly altered activity, likely due to a loss of important interactions with Glu288. **(Supplementary figure 5A-B, Supplementary table 1 compound A-E)** Alternatively, the bottom ring fits tightly within the binding pocket, suggesting larger substitutions would not be accommodated, again agreeing with previous data. **(Supplementary figure 5D and Supplementary table 1, compounds F-I)** Taken together, the computational and experimental data seemingly agree with one another, providing further justification that binding pose 1 is likely the correct binding pose for this type of compound.



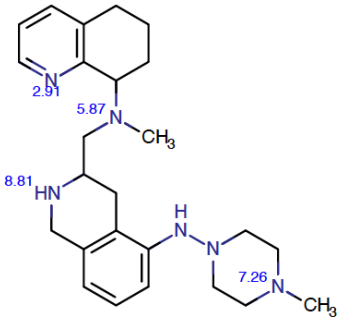
Supplementary Figure S5. **A:** 2D representation of EMU-116, **B:** Surface representation of area around the middle ring of Pose 1 of **EMU-116**, **C:** Surface representation of area around the top ring of Pose 1 of **EMU-116, D:** Surface representation of area around the bottom ring of Pose 1 of **EMU-116.**

VII. Calculated pKa Values for Compounds 29 and 31.

Calculations performed using Chemicalize™ software from ChemAxon.



Compound **29**



Compound **31**