

Supporting Information (SI)

Function-oriented development of CXCR4 antagonists as selective Human Immunodeficiency Virus (HIV)-1 entry inhibitors

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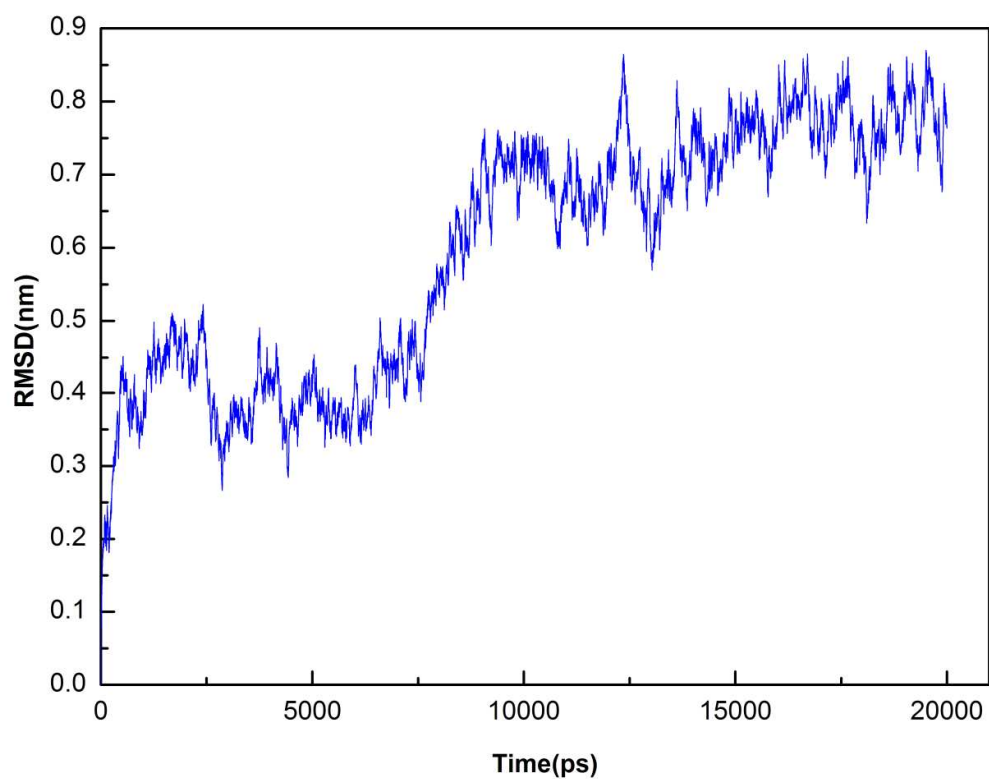


Figure S1. Molecular dynamics simulation (20 ns) of CXCR4 (PDB code 3OE0) in complex with compound **24**. The RMSD of the protein backbone of α C atom of amino acids during the simulation are shown in Figure S1.

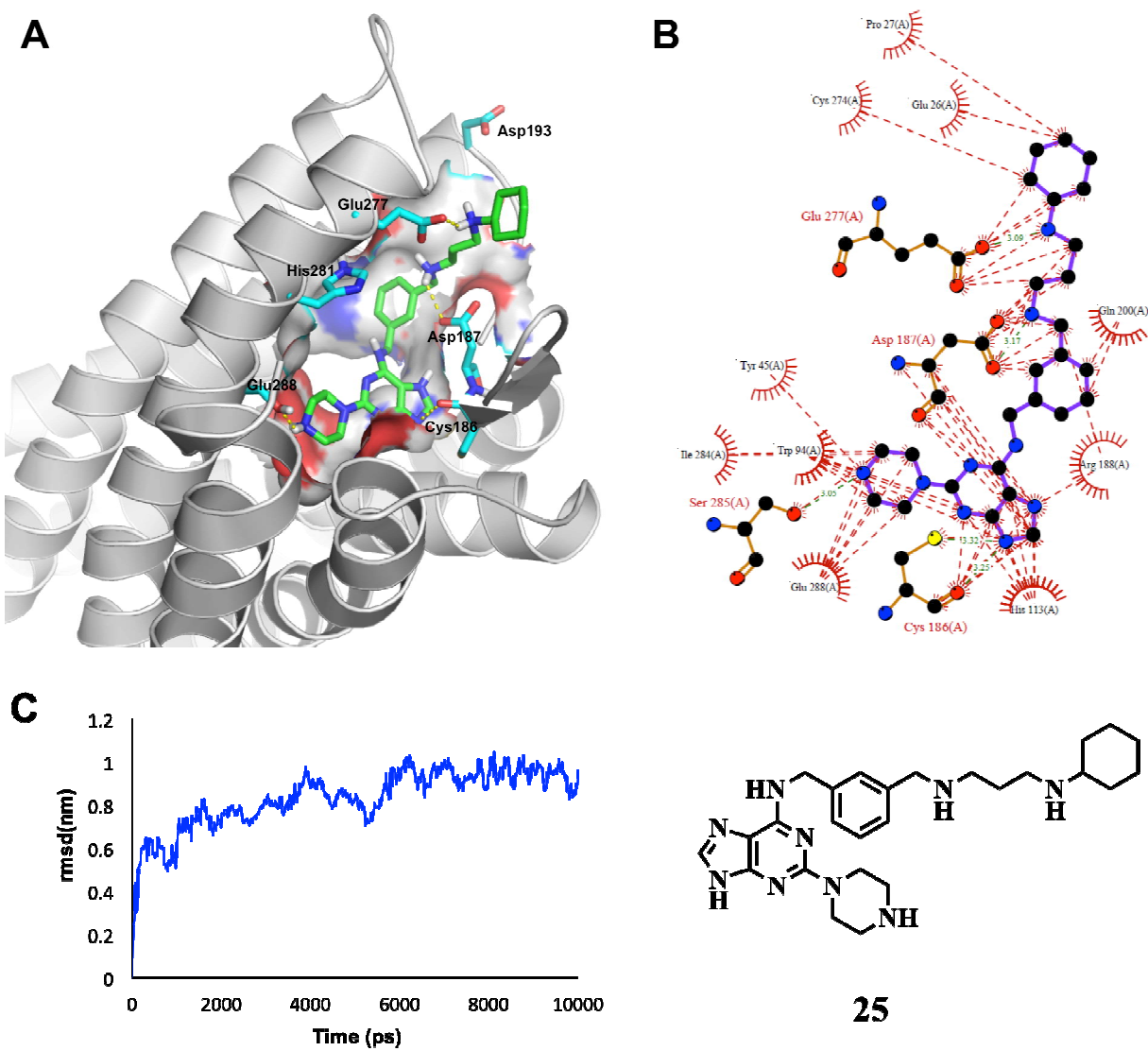


Figure S2. Molecular dynamics simulation (10ns) of CXCR4 (PDB code 3OE0) in complex with compound **25**. **A**. Structure of CXCR4-**25** complex after 10 ns of molecular dynamics (MD) simulations. The hydrogen bonding network (yellow lines) reveals strong hydrogen bondings around the compound **25** (green) with the residues of Cys186, Asp187, Glu277, and Glu288 (cyan). **B**. Ligplot diagram showing hydrophobic interactions between the indicated CXCR4 amino acid residues and compound **25** after 10 ns of MD simulation. The RMSD of the protein backbone of α C atom of amino acids during the simulation are shown in **C**.

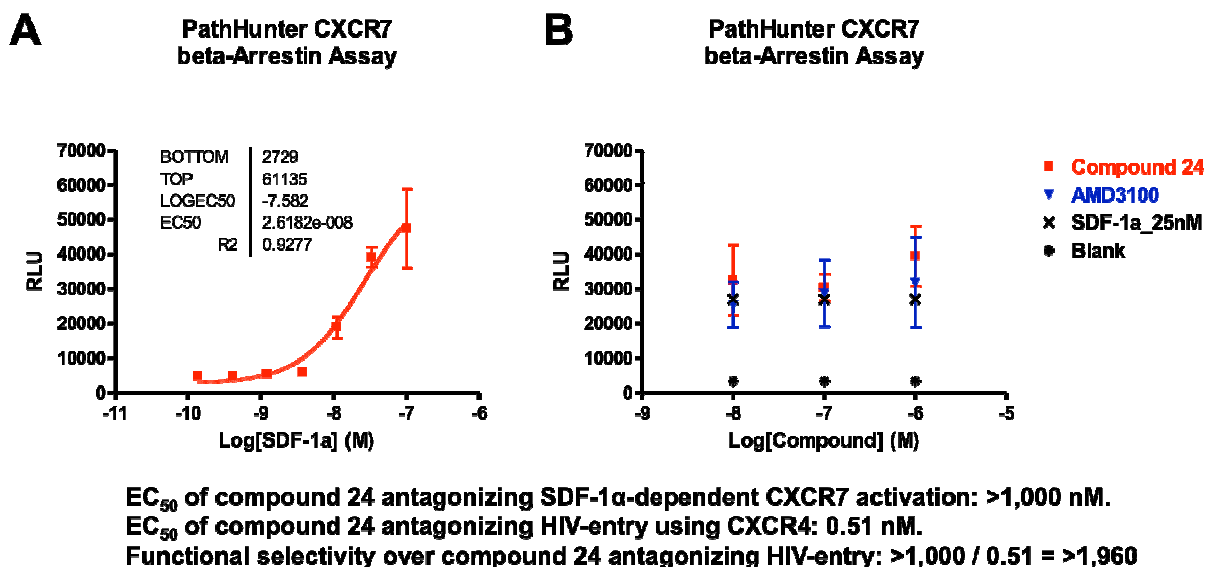


Figure S3. SDF-1 α -dependent β -arrestin assay of CXCR7. **A.** Determination of effective concentration (EC₅₀ = 26 nM) of SDF-1 α to activate β -arrestin signalling. **B.** Both compound **24** and **AMD3100** did not antagonize SDF-1 α -dependent CXCR7 β -arrestin signalling at various concentrations: 10nM, 100nM, and 1000nM (40X).

Radioligand Binding Assay and CXCR7 β -arrestin assay.

The CXCR7 β -arrestin assays were performed following the procedures by the DiscoverRX kit (PathHunter® eXpress β -Arrestin), which can be obtained at:

<http://www.discoverrx.com/products-applications/assay-ready-kits>

The binding assays of CXCR2, CCR2, CCR4, and CCR5 were performed by Eurofins Panlabs, Inc. The reagents, methods, and results are shown below:



Summary

STUDY OBJECTIVE

To evaluate, in Radioligand Binding assays, the activity of the test compounds.

METHODS

Methods employed in this study have been adapted from the scientific literature to maximize reliability and reproducibility. Reference standards were run as an integral part of each assay to ensure the validity of the results obtained. Assays were performed under conditions described in the accompanying "Methods" section of this report.

Where presented, IC_{50} values were determined by a non-linear, least squares regression analysis using MathIQ™ (ID Business Solutions Ltd., UK). Where inhibition constants (K_i) are presented, the K_i values were calculated using the equation of Cheng and Prusoff (Cheng, Y., Prusoff, W.H., Biochem. Pharmacol. 22:3099-3108, 1973) using the observed IC_{50} of the tested compound, the concentration of radioligand employed in the assay, and the historical values for the K_D of the ligand (obtained experimentally at Eurofins Panlabs, Inc.). Where presented, the Hill coefficient (n_H), defining the slope of the competitive binding curve, was calculated using MathIQ™. Hill coefficients significantly different than 1.0, may suggest that the binding displacement does not follow the laws of mass action with a single binding site. Where IC_{50} , K_i , and/or n_H data are presented without Standard Error of the Mean (SEM), data are insufficient to be quantitative, and the values presented (K_i , IC_{50} , n_H) should be interpreted with caution.

Summary of Significant Results

Biochemical assay results are presented as the percent inhibition of specific binding or activity throughout the report. All other results are expressed in terms of that assay's quantitation method.

- For primary assays, only the lowest concentration with a significant response judged by the assays' criteria, is shown in this summary.
- Where applicable, either the secondary assay results with the lowest dose/concentration meeting the significance criteria or, if inactive, the highest dose/concentration that did not meet the significance criteria is shown.
- Unless otherwise requested, primary screening in duplicate with quantitative data (e.g., $IC_{50} \pm SEM$, $K_i \pm SEM$ and n_H) are shown where applicable for individual requested assays. In screening packages, primary screening in duplicate with semi-quantitative data (e.g., estimated IC_{50} , K_i and n_H) are shown where applicable (concentration range of 4 log units); available secondary functional assays are carried out (30 mM) and MEC or MIC determined only if active in primary assays >50% at 1 log unit below initial test concentration.

Significant responses ($\geq 50\%$ inhibition or stimulation for Biochemical assays) were noted in the primary assays listed below:

Experimental Results

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.	IC ₅₀ *	K _i	n _H	R
Compound 24										
217550	Chemokine CCR2B	358624	hum	2	10 µM	10				
217660	Chemokine CCR4	358625	hum	2	10 µM	11				
217720	Chemokine CCR5	358626	hum	2	10 µM	15				
244500	Chemokine CXCR2 (IL-8R _B)	358418	hum	2	10 µM	16				
Compound 25										
217550	Chemokine CCR2B	358624	hum	2	10 µM	2				
217660	Chemokine CCR4	358625	hum	2	10 µM	-3				
217720	Chemokine CCR5	358626	hum	2	10 µM	14				
244500	Chemokine CXCR2 (IL-8R _B)	358418	hum	2	10 µM	12				

Note: Items meeting criteria for significance (≥50% stimulation or inhibition) are highlighted.
 * Batch: Represents compounds tested concurrently in the same assay(s).
 hum=Human

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Methods

■ 217550 Chemokine CCR2B

Source:	Human recombinant CHO-K1 cells	Ligand:	0.10 nM [¹²⁵ I] MCP-1
Vehicle:	1.00% DMSO	Non-Specific Ligand:	0.030 μM MCP-1
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	80% *
Incubation Buffer:	25 mM HEPES, pH 7.4, 1 mM CaCl ₂ , 0.5% BSA, 5 mM MgCl ₂	Quantitation Method:	Radioligand Binding
Kd:	0.063 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.25 pmole/mg Protein *

■ 217660 Chemokine CCR4

Source:	Human recombinant Chem-1 cells	Ligand:	0.050 nM [¹²⁵ I] TARC
Vehicle:	1.00% DMSO	Non-Specific Ligand:	0.10 μM TARC
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	70% *
Incubation Buffer:	50 mM HEPES, pH 7.4, 5 mM MgCl ₂ , 1 mM CaCl ₂ , 0.5% BSA	Quantitation Method:	Radioligand Binding
Kd:	0.34 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	1.50 pmole/mg Protein *

■ 217720 Chemokine CCR5

Source:	Human recombinant HEK-293T cells	Ligand:	0.060 nM [¹²⁵ I] MIP-1α
Vehicle:	1.00% DMSO	Non-Specific Ligand:	0.10 μM MIP-1β
Incubation Time/Temp:	2 hours @ 25°C	Specific Binding:	85% *
Incubation Buffer:	50 mM HEPES, pH 7.4, 1 mM CaCl ₂ , 0.2% BSA, 5 mM MgCl ₂	Quantitation Method:	Radioligand Binding
Kd:	0.068 nM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.48 pmole/mg Protein *

■ 244500 Chemokine CXCR2 (IL-8R_B)

Source:	Human recombinant CHO-K1 cells	Ligand:	15.0 pM [¹²⁵ I] IL-8
Vehicle:	1.00% DMSO	Non-Specific Ligand:	10 nM IL-8
Incubation Time/Temp:	60 minutes @ 25°C	Specific Binding:	80% *
Incubation Buffer:	25 mM HEPES, pH 7.4, 2 mM CaCl ₂ , 1 mM MgCl ₂ , 0.2% BSA	Quantitation Method:	Radioligand Binding
Kd:	12.0 pM *	Significance Criteria:	≥50% of max stimulation or inhibition
		Bmax:	0.28 pmole/mg Protein *

* Historical Values

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Reference Compounds

Cat #	Assay Name	Reference Compound	Historical			Concurrent	
			IC ₅₀ *	K _i	n _H	Batch *	IC ₅₀ *
217550	Chemokine CCR2B	MCP-1	0.077 nM	0.030 nM	0.90	358624	0.14 nM
217660	Chemokine CCR4	TARC	0.16 nM	0.14 nM	0.80	358625	0.23 nM
217720	Chemokine CCR5	MIP-1 β	0.092 nM	0.049 nM	0.70	358626	0.21 nM
244500	Chemokine CXCR2 (IL-8R _h)	IL-8	0.18 nM	0.080 nM	0.90	358418	0.25 nM

* Batch: Represents compounds tested concurrently in the same assay(s).

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General Procedure for the Synthesis of Compounds IIa-IIc. The general procedure is illustrated immediately below with compound **IIa** as a specific example.

6-Methoxy-quinazoline-2,4-diol (IIa). A mixture of amino acid (**Ia**) (2.01 g, 11.88 mmole) and urea (4.03g, 67.10 mmole) was heated at 200 °C in an oiled bath for 3 h and then cooled to 70 °C. The resulting mixture was added 1M NaOH(aq) (50 mL) and stirred for 1h, and then was acidified with 12N HCl (aq) (8 mL) at 70 °C. The suspension solution was stirred at 25 °C for 2 h and then filtered to get the cake, which was dried at 80 °C for 15h to give **Ia** (1.94 g, 85%) as a pale yellow solid. Compounds **IIb-IIc** were synthesized respectively from **Ib-Ic** with urea following a similar synthetic procedure for **IIa** as pale yellow solids.

General Procedure for the Synthesis of Compounds IVa-IVc. The general procedure is illustrated immediately below with compound **IVa** as a specific example.

4-(2-Chloro-6-methoxy-quinazolin-4-ylamino)-piperidine-1-carboxylic acid tert-butyl ester (IVa). To a magnetically stirred solution of 6-Methoxy-quinazoline-2,4-diol (**IIa**) (1.02 g, 5.31 mmol) in POCl₃ (7 mL) was added 2-ethylpyridine (1 mL) dropwise at 25 °C under an atmosphere of argon. The resulting mixture was heated to 110 °C for 4h and then cooled to room temperature. The reaction mixture was poured into ice–water and the aqueous layer was extracted with CH₂Cl₂ (2 × 100 mL). The combined organic extracts were washed with NaHCO₃, water and brine, dried over anhydrous Na₂SO₄, filtered to afford filtrate with compound **IIIa**. To a magnetically stirred filtrate of **IIIa** in DCM was added 4-amino-1-Boc-piperidine (1.03 g, 5.14 mmole) and TEA (0.91 g, 9.01 mmole) sequentially at 0 °C under an atmosphere of argon. The resulting mixture was warmed to room temperature for 15h and quenched with NH₄Cl(aq). The aqueous layer was extracted with CH₂Cl₂ (2 × 100 mL). The combined organic extracts were washed with water and brine, dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure to give a crude residue, which was purified by crystallization with n-hexane/ethyl acetate (1:1) as solvent system to afford compound **IVa** (1.23 g, 59% over two steps) as a pale yellow solid: mp 249.5–250.5 °C; ¹H-NMR (300 MHz, CDCl₃) δ 7.69 (d, *J* = 9.0 Hz, 1H), 7.37 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.94 (d, *J* = 2.4 Hz, 1H), 5.72 (m, 1H), 4.44 (m, 1H), 4.13 (m, 2H), 3.90 (s, 3H), 2.96 (m, 2H), 2.14 (m, 2H), 1.49 (m, 2H), 1.47 (s, 9H); ESMS *m/z*: 393.1 (M+1).

4-(2-Chloro-7-methoxy-quinazolin-4-ylamino)-piperidine-1-carboxylic acid tert-butyl ester (IVb). Compound **IVb** was synthesized from **IIb** (1.05 g, 5.46 mmol) with POCl₃ (7 mL) and 4-amino-1-Boc-piperidine (1.03 g, 5.14 mmole) following a similar synthetic procedure for **IVa** and obtained as a white solid (1.16 g, 54% over two steps): mp: 265.5–266.5 °C; ¹H NMR (300 MHz, CDCl₃) δ 7.58 (d, *J* = 8.7 Hz, 1H), 7.11 (d, *J* = 2.1 Hz, 1H), 7.04 (dd, *J* = 8.7, 2.1 Hz, 1H), 5.63 (m, 1H), 4.42 (m, 1H), 4.13 (m, 2H), 3.89 (s, 3H), 2.97 (m, 2H), 2.13 (m, 2H), 1.49 (m, 2H), 1.47 (s, 9H);

ESMS m/z : 393.1 (M+1).

4-(2-Chloro-6,7-dimethoxy-quinazolin-4-ylamino)-piperidine-1-carboxylic acid tert-butyl ester (IVc). Compound **IVc** was synthesized from **IIc** (1.05 g, 4.95 mmol) with POCl₃ (7 mL) and 4-amino-1-Boc-piperidine (1.01 g, 5.04 mmole) following a similar synthetic procedure for **IVa** and obtained as a white solid (1.21 g, 58% over two steps): mp 208.5–209.5 °C; ¹H NMR (300 MHz, CDCl₃) δ 6.99–6.98 (m, 2H), 5.83 (m, 1H), 4.43 (m, 1H), 4.06 (m, 2H), 3.99 (s, 3H), 3.96 (s, 3H), 2.94 (m, 2H), 2.12 (m, 2H), 1.49 (m, 2H), 1.46 (s, 9H); ESMS m/z : 423.1 (M+1).

General Procedure for the Synthesis of Compounds Va–Vc. The general procedure is illustrated immediately below with compound **Va** as a specific example.

4-{2-[4-({tert-Butoxycarbonyl-[3-(tert-butoxycarbonyl-cyclohexyl-amino)-propyl]-amino}-methyl)-benzylamino]-6-methoxy-quinazolin-4-ylamino}-piperidine-1-carboxylic acid tert-butyl ester (Va). A solution of **IVa** (0.4 g, 1.02 mmole) and side chain amine **D** (0.5 g, 1.05 mmole) in 1-pentanol (3 mL) was heated at 120 °C for 15 min by microwave radiation. After the completion of reaction, the solvent was removed under reduced pressure to give crude residue, which was purified with flash chromatography with *n*-hexane/ethyl acetate (1:9) to afford **Va** (0.53 g, 63%) as a sticky oil; ¹H NMR (300 MHz, CDCl₃) δ 7.42 (d, J = 9.0 Hz, 1H), 7.33 (d, J = 8.1 Hz, 2H), 7.22 (dd, J = 9.0, 2.4 Hz, 1H), 7.16 (m, 2H), 6.82 (d, J = 2.4 Hz, 1H), 5.38 (m, 1H), 4.63 (s, 2H), 4.39 (s, 2H), 4.21 (m, 1H), 4.09 (m, 2H), 3.84 (s, 3H), 3.11 (m, 2H), 2.99 (m, 2H), 2.86 (m, 2H), 2.03 (m, 2H), 1.78–1.47 (m, 9H), 1.46 (s, 18H), 1.41 (s, 9H), 1.27–1.22 (m, 5H), 1.02 (m, 1H); ESMS m/z : 832.5 (M+1).

4-{2-[4-({tert-Butoxycarbonyl-[3-(tert-butoxycarbonyl-cyclohexyl-amino)-propyl]-amino}-methyl)-benzylamino]-7-methoxy-quinazolin-4-ylamino}-piperidine-1-carboxylic acid tert-butyl ester (Vb). Compound **Vb** was synthesized from **IVb** (0.42 g, 1.07 mmol) and side chain amine **D** (0.51 g, 1.05 mmole) following a similar synthetic procedure for **Va** and obtained as a sticky oil (0.56 g, 63%); ¹H NMR (300 MHz, CDCl₃) δ 7.36 (d, J = 8.7 Hz, 1H), 7.33 (d, J = 7.8 Hz, 2H), 7.19 (m, 2H), 6.84 (d, J = 2.4 Hz, 1H), 6.70 (dd, J = 8.7, 2.4 Hz, 1H), 5.19 (m, 1H), 4.66 (d, J = 5.7 Hz, 2H), 4.40 (s, 2H), 4.21 (m, 1H), 4.10 (m, 2H), 3.87 (s, 3H), 3.12 (m, 2H), 3.00 (m, 2H), 2.87 (m, 2H), 2.04 (m, 2H), 1.78–1.47 (m, 9H), 1.46 (s, 18H), 1.42 (s, 9H), 1.28–1.21 (m, 5H), 1.03 (m, 1H); ESMS m/z : 832.5 (M+1).

4-{2-[4-({tert-Butoxycarbonyl-[3-(tert-butoxycarbonyl-cyclohexyl-amino)-propyl]-amino}-methyl)-benzylamino]-6,7-dimethoxy-quinazolin-4-ylamino}-piperidine-1-carboxylic acid tert-butyl ester (Vc). Compound **Vc** was synthesized from **IVc** (0.44 g, 1.04 mmol) and side chain amine **D** (0.51 g, 1.05 mmole) following a similar synthetic procedure for **Va** and obtained as a

sticky oil (0.53 g, 59%); ^1H NMR (300 MHz, CDCl_3) δ 7.31 (d, J = 7.8 Hz, 2H), 7.15 (d, J = 7.8 Hz, 2H), 6.88 (s, 1H), 6.86 (s, 1H), 5.54 (m, 1H), 4.63 (d, J = 4.8 Hz, 2H), 4.38 (s, 2H), 4.22 (m, 1H), 4.08 (m, 2H), 3.91 (s, 3H), 3.88 (s, 3H), 3.11 (m, 2H), 2.99 (m, 2H), 2.82 (m, 2H), 2.02 (m, 2H), 1.71–1.46 (m, 9H), 1.45 (s, 18H), 1.40 (s, 9H), 1.27–1.21 (m, 5H), 1.02 (m, 1H); ESMS m/z : 862.5 (M+1).

2,6-Dichloro-9-(tetrahydro-pyran-2-yl)-9H-purine (18-I). To a magnetically stirred solution of 2,6-dichloropurine (10 g, 52.9 mmole) in ethyl acetate (100 mL) was added *p*-toluenesulfonic acid monohydrate (0.08 g, 0.4 mmole), and the resultant mixture was heated to 50 °C under an atmosphere of nitrogen. To the stirred mixture was added 3,4-dihydro-2H-pyran (7.5 g, 89.2 mmole) over a period of 2 h. The mixture was stirred at 25 °C for 15 hours. The suspension mixture was filtrated by removing filtrate to give the crude solid, which was washed with n-hexane / ethyl acetate (1:1) to afford the product **18-I** (14.4 g, 100%) as a colorless solid. ^1H NMR (400 MHz, CDCl_3) δ 8.33 (s, 1H), 5.76 (dd, J = 10.8, 2.4 Hz, 1H), 4.20 (ddd, J = 12.0, 4.0, 2.0 Hz, 1H), 3.78 (ddd, J = 12.0, 11.6, 3.2 Hz, 1H), 2.19 (m, 1H), 2.04 (m, 1H), 1.98 (m, 1H), 1.82–1.65 (m, 10H).

{3-[tert-Butoxycarbonyl-(4-{2-chloro-9-(tetrahydro-pyran-2-yl)-9H-purin-6-ylamino]-methyl}-benzyl)-amino]-propyl}-cyclohexyl-carbamic acid tert-butyl ester (18-II). To a magnetically stirred solution of **18-I** (1.3 g, 4.76 mmole) in ethyl acetate (35 mL) under an atmosphere of nitrogen was added amine side chain **D** (2.3 g, 4.83 mmole) and TEA (1.5 g, 14.85 mmole). The mixture was heated to 50 °C for 4 h, and then quenched with NH_4Cl (aq) at 25 °C. The combined extracts were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated to give the crude residue. The residue was subjected to purification by flash chromatography on silica gel with MeOH / DCM (1:9) to afford the product **18-II** (2.1g, 62%) as a light yellow solid. ^1H NMR (400 MHz, CDCl_3) δ 7.91 (s, 1H), 7.31 (d, J = 8.0 Hz, 2H), 7.19 (m, 2H), 5.68 (dd, J = 10.8, 2.0 Hz, 1H), 4.78 (s, 2H), 4.41 (s, 2H), 4.13 (m, 1H), 3.78 (m, 1H), 3.22–2.92 (m, 4H), 2.14–2.01 (m, 2H), 1.92 (m, 1H), 1.80–1.59 (m, 10H), 1.53–1.24 (m, 23H), 1.02 (m, 1H).

{3-[tert-Butoxycarbonyl-(4-{2-piperazin-1-yl-9-(tetrahydro-pyran-2-yl)-9H-purin-6-ylamino]-methyl}-benzyl)-amino]-propyl}-cyclohexyl-carbamic acid tert-butyl ester (18-III). A solution of **18-II** (350 mg, 0.49 mmole) and piperazine (350 mg, 4.07 mmole) in 1-pentanol (2 mL) was heated at 100 °C for 15 hours. After the completion of reaction, the resulting mixture was concentrated under reduced pressure by removing 1-pentanol to give crude residue, which was purified with flash chromatography with MeOH / DCM (1:1) to afford the product **18-III** (240 mg, 64%). ^1H NMR (300 MHz, CDCl_3) δ 7.67 (s, 1H), 7.29 (m, 2H), 7.17 (m, 2H), 5.58 (m, 1H), 4.77 (s, 2H), 4.39 (s, 2H), 4.16 (m, 1H), 3.84 (br s, 4H), 3.75 (m, 1H), 3.22–2.93 (m, 8H), 2.14–1.94 (m, 3H), 1.80–1.57 (m, 10H), 1.53–1.24 (m, 23H), 1.02 (m, 1H).

[3-(tert-Butoxycarbonyl-cyclohexyl-amino)-propyl]-{4-[(2-chloro-9H-purin-6-ylamino)-methyl]-benzyl}-carbamic acid tert-butyl ester (19-I). To a magnetically stirred solution of 2,6-dichloropurine (0.5 g, 2.64 mmole) in *t*-BuOH (30 mL) under an atmosphere of nitrogen was added amine side chain **D** (1.51 g, 3.17 mmole) and TEA (0.5 g, 4.95 mmole). The mixture was heated to 50 °C for 4 h, and then quenched with NH₄Cl (aq). The combined extracts were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated to give the residue. The residue was subjected to purification by flash chromatography on silica gel with MeOH / DCM (1:9) to afford the product **19-I** (1.49 g, 89%) as white solid. ¹H NMR (300 MHz, CD₃OD) δ 7.98 (s, 1H), 7.35 (d, *J* = 7.8 Hz, 2H), 7.18 (d, *J* = 7.8 Hz, 2H), 4.64 (s, 2H), 4.36 (s, 2H), 3.12–2.88 (m, 4H), 1.63–1.54 (m, 7H), 1.53–1.24 (m, 23H), 1.02 (m, 1H).

[3-(tert-Butoxycarbonyl-cyclohexyl-amino)-propyl]-{4-[(2-chloro-9-methyl-9H-purin-6-ylamino)-methyl]-benzyl}-carbamic acid tert-butyl ester (19-II). **19-I** (300 mg, 0.48 mmole) and K₂CO₃ (72 mg, 0.52 mmole) were taken into DMF (6 mL) and dissolved completely into the DMF by heating it. After that the reaction mixture was allowed to cool at room temperature, then to this MeI (200 mg, 1.41 mmole) was added and the reaction mixture was stirred at rt for 6h, then reaction mixture was poured into water. The resulting mixture was extracted with ethyl acetate and the combined extracts were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated to afford the residue. The residue was subjected to purification by flash chromatography on silica gel with MeOH / DCM (1:9) to afford the product **19-II** (297 mg, 97%). ¹H NMR (300 MHz, CDCl₃) δ 7.57 (s, 1H), 7.29 (d, *J* = 7.8 Hz, 2H), 7.16 (m, 2H), 4.74 (s, 2H), 4.38 (s, 2H), 3.75 (s, 3H), 3.19–2.93 (m, 4H), 1.78–1.57 (m, 7H), 1.53–1.23 (m, 23H), 1.02 (m, 1H).

[3-(tert-Butoxycarbonyl-{4-[(9-methyl-2-piperazin-1-yl-9H-purin-6-ylamino)-methyl]-benzyl}-amino)-propyl]-cyclohexyl-carbamic acid tert-butyl ester (19-III). A solution of **19-II** (210 mg, 0.33 mmole) and piperazine (87 mg, 1.01 mmole) in ethylene glycol monomethyl ether (6 mL) was heated at 120 °C for 15 hours. After the reaction was complete, the reaction mixture was poured into water. The resulting mixture was extracted with ethyl acetate and the combined extracts were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated to afford the residue, which was purified with flash chromatography with MeOH / DCM (1:1) to afford the product **19-III** (156 mg, 69%). ¹H NMR (400 MHz, CDCl₃) δ 7.39 (s, 1H), 7.29 (d, *J* = 8.0 Hz, 2H), 7.16 (m, 2H), 4.73 (s, 2H), 4.38 (s, 2H), 3.85 (br s, 4H), 3.63 (s, 3H), 3.19–2.81 (m, 8H), 1.78–1.56 (m, 7H), 1.53–1.27 (m, 23H), 1.02 (m, 1H).

{3-[(4-Aminomethyl-benzyl)-tert-butoxycarbonyl-amino]-propyl}-cyclohexyl-carbamic acid tert-butyl ester (side chain D). Compound **side chain D** was synthesized from (4-Formyl-benzyl)-carbamic acid benzyl ester (2.05 g, 7.6 mmole) following a similar synthetic procedure in previous reference 18 and obtained as a oil (2.18 g, 65% over three steps); ¹H NMR

(300 MHz, CDCl₃) δ 7.27 (d, J = 7.8 Hz, 2H), 7.20 (m, 2H), 4.41 (s, 2H), 3.86 (s, 2H), 3.21–2.91 (m, 4H), 1.81–1.63 (br s, 2H), 1.78–1.58 (m, 7H), 1.56–1.41 (m, 18H), 1.37–1.22 (m, 5H), 1.04 (m, 1H); ESMS m/z : 476.3 (M+1).

{3-[(4-Aminomethyl-benzyl)-tert-butoxycarbonyl-amino]-propyl}-cycloheptyl-carbamic acid tert-butyl ester (side chain E). Compound **side chain E** was synthesized from (4-Formyl-benzyl)-carbamic acid benzyl ester (2.05 g, 7.6 mmole) following a similar synthetic procedure in previous reference 18 and obtained as a oil (2.24 g, 60% over three steps); ¹H NMR (300 MHz, CDCl₃) δ 7.41 (m, 2H), 7.22 (m, 2H), 4.42 (s, 2H), 3.86 (s, 2H), 3.21–2.94 (m, 4H), 1.78–1.49 (m, 13H), 1.48–1.46 (m, 20H); ESMS m/z : 490.3 (M+1).

(4-Aminomethyl-benzyl)-(3-piperidin-1-yl-propyl)-carbamic acid tert-butyl ester (side chain F). Compound **side chain F** was synthesized from 4-cyano-benzaldehyde (1.01 g, 7.6 mmole) following a similar synthetic procedure in previous reference 18 and obtained as a oil (1.70 g, 62% over three steps); ¹H NMR (400 MHz, CDCl₃) δ 7.27 (m, 2H), 7.18 (m, 2H), 4.42 (s, 2H), 3.84 (s, 2H), 3.21–3.06 (m, 2H), 2.40–2.22 (m, 6H), 2.01–1.83 (m, 2H), 1.78–1.53 (m, 6H), 1.47–1.43 (m, 9H); ESMS m/z : 362.3 (M+1).

