Design, Synthesis, and Biological Evaluation of Novel CXCR4 Inverse Agonists

Supporting Information

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Supporting information

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#	Structure	MW	Calculated (M+H ⁺)	Found (M+H ⁺)	HPLC Purity
IT1t		406.6515	407.2297	407.2296	95%
MEX4		421.6661	422.2407	422.2409	95%
GEX4	N HN HN HN HN HN HN HN HN HN HN HN HN HN	463.7061	464.2625	464.2630	95%
#	Structure	MW	Calculated (M+2H ⁺)/2	Found (M+2H ⁺)/2	HPLC Purity
16	$(\mathbf{A}_{12} \mathbf{N}_{12} N$	1510.9110	755.9229	755.9256	95%
17	$ \begin{array}{c} & H \\ & & H \\ $	1510.9110	755.9229	755.9259	95%
18	$\begin{array}{c} \begin{array}{c} \begin{array}{c} \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\$	1342.6749	671.8598	671.8612	95%

Table S1. Table of HRMS and Purity



Fig. S1 Effect of CXCl12, IT1t, MEX4, and GEX4 on HEK293 cells expressing the human HA-tagged CXCR4 receptor. A. Concentration-response curves for the G α i pathway and B. Concentration-response curves for β -arrestin-2 recruitment.



Fig. S2 Competition binding assay with ¹²⁵I-CXCL12 of CXCL12, compound **16**, and compound **17** on HEK293 cells stably expressing the human HA-tagged CXCR4 receptor.



Fig. S3 Concentration-response curves for the Gαi pathway. Effect of CXCL12, compound **16**, and compound **17** on HEK293 cells expressing the human HA-tagged CXCR4 receptor.



[Analog], nM

Fig. S4 Chemotaxis assay. Effect of CXCL12, compound **16**, and compound **17** on migration of pre-B lymphocytes using Transwell migration assays.



Fig. S5 Competition binding assay with ¹²⁵I-CXCL12 of CXCL12 and compound **18** on HEK293 cells stably expressing the human HA-tagged CXCR4 receptor.



Fig. S6 Concentration-response curves for the Gαi pathway. Effect of CXCL12 and compound **18** on HEK293 cells expressing the human HA-tagged CXCR4 receptor.



Fig. S7 Chemotaxis assay. Effect of CXCL12 and compound **18** on migration of pre-B lymphocytes using Transwell migration assays.



Fig. S8 Concentration-response curves for the G α i pathway in the pA2 experimental paradigm. Effect of fixed concentrations of compound 18 on concentration-response curves of CXCL12 on HEK293 cells expressing the human HA-tagged CXCR4 receptor.



Fig. S9 Concentration-response curves for the $G\alpha_i$ pathway. Effect of CXCL12, T140 and, compounds 16, 17, and 18, on HEK293 cells expressing the constitutively active mutant of the human HA-tagged CXCR4 receptor CXCR4-N119S).

IT1t¹H NMR



IT1t ¹³C NMR





Compound 1¹³C NMR





Compound 2¹³C NMR





Compound 3¹³C NMR



Compound 4¹H NMR



Compound 4¹³C NMR





Compound 6¹³C NMR





Compound 8¹³C NMR





Compound 9¹³C NMR





Compound **10**¹³C NMR









Compound **11** ¹³C NMR



Compound 11 2D COSY NMR



Compound **12** ¹H NMR



Compound **12**¹³C NMR



Compound **13** ¹H NMR



Compound 13 ¹³C NMR





Compound 14¹³C NMR





Compound **15**¹³C NMR





IT1t - LC/MS















Compound 8 – LC/MS



Compound 9 – LC/MS



Compound 10 – LC/MS





Compound 12 – LC/MS









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