Tetrahydroisoquinoline CXCR4 Antagonists Adopt a Hybrid Binding Mode within the Peptide Subpocket of the CXCR4 Receptor

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S Supporting Information

ABSTRACT: The rationale for the structural and mechanistic basis of a tetrahydroisoquinoline (THIQ) based series of CXCR4 antagonists is presented. Using the previously reported crystal structures which reveal two distinct binding sites of CXCR4 defined as the small molecule (IT1t or minor) binding pocket and peptide (CVX15 or major) binding pocket, we hypothesized our THIQ small molecule series could bind like either molecule in these respective receptor configurations (IT1t versus CVX15 based poses). To this end, a thorough investigation was performed through a combination of receptor mutation studies, medicinal chemistry, biological testing, conformational analysis, and flexible docking. Our findings showed that the CVX15 peptide-based CXCR4 receptor complexes (red pose) were consistently favored over the small molecule IT1t based CXCR4 receptor configurations (blue pose) to correctly explain the computational and mutational studies as well as key structural components of activity for these small molecules.

KEYWORDS: C-X-C-Chemokine Receptor 4, GPCR, stromal derived factor-1, CXCR4 antagonists, human immunodeficiency virus, receptor mutants, molecular modeling, conformational analysis, NAMFIS, congeneric series

The G protein-coupled C-X-C chemokine receptor 4 (CXCR4) has attracted significant attention over the past decade due to the roles it plays in both cancer progression and HIV infection.^{1,2} The receptor partners solely with the chemokine ligand CXCL12 (SDF-1) and is connected to a divergent set of signaling pathways ranging from chemotaxis and cell survival to gene transcription and proliferation.³ CXCR4 antagonists are heavily pursued for their potential to attenuate autoimmune diseases, cancer, and AIDS.^{4–6}

CXCR4 is composed of seven trans-membrane helices numbered sequentially from the N-terminus and was recently cocrystallized in unique conformations with the small molecule IT1t (1) and the cyclic peptide CVX15 (2, Chart 1).⁷ These structures revealed an extracellular binding region composed of two subpockets, which is consistent with the “two-site” binding epitope of most chemokine receptors.⁸ The minor subpocket of the CXCR4 receptor is composed of helices I, II, III, and VII, and contains the IT1t binding site. The major subpocket is composed of helices IV–VII and accommodates the larger CVX15 peptide. While the field has embraced the chemo-type pneumonic of assignment of peptide-based CXCR4 antagonists to the CVX15:CXCR4 complex and small molecules to the IT1t:CXCR4 complex, this assumption is not supported by receptor amino acid residue mutational studies with the biological peptides SDF-1 or HIV gp120, nor the small

Chart 1. Structures of Selected CXCR4 Antagonists

molecules AMD3100 (3) and AMD11070 (4, Chart 1).^{9–11} This led us to the emerging hypothesis that both crystal structures should be considered in studying molecular interactions with CXCR4 and that small molecules may adopt binding poses that are probable in either the IT1t:CXCR4 or CVX15:CXCR4 ligand–receptor configurations. Previously, we performed a flexible docking study on

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Table 1. Antagonist Activity of IT1t (1) and TIQ-15 (5) against Specified Amino Acid Residue-Alanine CXCR4 Receptor Mutants Measured Using the Gi BRET Assay

Compound	Antagonist IC ₅₀ ^a Values (μM) for Various CXCR4 Mutants by Residue and Mutant/Wild Type IC ₅₀ Ratios (<i>F</i> _{MUT} ^b)						
	WT	W94A	D97A	Y116A	D171A	D187A	E288A
IT1t (1)	0.326	6.37	18.9	7.19	0.223	0.430	0.967
±SEM ^a	±0.050	±2.36	±2.21	±1.35	±0.015	±0.168	±0.208
<i>F</i> _{MUT} ^b		20	58	22	1	1	3
TIQ-15 (5)	0.091	0.066	0.288	0.152	0.414	0.197	9.71
±SEM ^a	±0.012	±0.008	±0.021	±0.064	±0.038	±0.050	±3.02
<i>F</i> _{MUT} ^b		1	3	2	5	2	107

^aFrom three independent experiments (*n* = 3). ^b*F*_{MUT} is defined as MUT IC₅₀/WT IC₅₀.

AMD11070 utilizing existing mutation and bioactivity data with CXCR4 and suggested a binding pose more correlative with the CVX15: CXCR4 combination to support this hypothesis.¹² In addition to IT1t and AMD11070, several different classes of well-characterized CXCR4 antagonists and corresponding SAR data have also been published, including the tetrahydroisoquinoline (THIQ) based antagonist TIQ-15 (5, Chart 1) recently disclosed by our laboratory.¹³

Flexible docking can provide insights to molecular binding mechanisms and presents a complementary technique to X-ray crystallography. Computational approaches often lead to more accurate interpretations of protein–ligand interactions due to poorly refined ligand geometries derived during crystallography refinements. There are many cases where small molecules are depicted in erroneous conformations within the protein they bind most likely due to the much poorer resolution (RMSD > 3 Å) obtained during protein crystallography.¹⁴ Furthermore, a community-wide docking effort involving both IT1t and CXCR4 resulted in large RMSD values of positioning of the ligand within the receptor (>4 Å), a consistent omission of key residues involved in ligand binding (<45% of key residues identified), and alternative molecular orientations within the IT1t pocket.^{15,16} In this study, we attempt to better understand potential binding interactions for our THIQ based CXCR4 antagonists by a multidirectional and structure-guided investigation utilizing biological data, experimental conformational determinations using NMR (NAMFIS), medicinal chemistry, congeneric analysis, computational analysis using flexible docking and molecular dynamics for determining small molecule solution and receptor conformations, and protein–ligand interactions.

In order to provide a better understanding of the residues involved in the binding of TIQ-15 to CXCR4, mutational studies were undertaken. There are key differences between the CXCR4 receptor conformations for both the IT1t and CVX15 crystal structures including 32 residues that are significantly different in their positioning and ligand binding involvement.¹² Building on this observation, we focused on the ones that provided key interactions *via* salt bridges and/or hydrogen bonding. These “primary” residues within the CXCR4 receptor include the following: IT1t dependent (D97, E288) and CVX15 dependent (D171, D187, R188, D193, D262). Of this list, the key residues D97 and E288 are identified as critical for SDF-1 signaling, while D171 and D97 are the essential residues for HIV gp120 interactions.^{9,10} Therefore the following key residues of the receptor were selected for alanine substitutions: W94, D97, Y116, D171, D187, and E288. Those residues were chosen to quickly distinguish small molecule interactions with CXCR4 as they were found to be critical for both IT1t and AMD11070

binding.^{7,11} The six different mutants, W94A, D97A, Y116A, D171A, D187A, and E288A, were obtained by site-directed mutagenesis, and their cell surface expression as well as functional properties were assessed using flow cytometry (FACS) and bioluminescence resonance energy transfer (BRET) approaches, respectively. Analysis of cell surface expression by FACS validated that the different mutants displayed cell surface expression comparable to their wild-type counterpart (SI, Figure S1). The functionality of these various mutants was assessed by measuring activation of the Gi pathway after direct stimulation by SDF-1α. The decrease in the BRET signal between Gαi2-Rluc and GFP-Gγ1 was used as a read-out of Gi activation. All of the different mutants were able to engage the Gi pathway, with SDF-1α potency between 0.76 nM and 4.91 nM, confirming their functionality (SI, Figure S2).

As a next step, the activity of TIQ-15 (5) was assessed on the different mutants, using the Gi BRET assay (SI, Figure S3B). Given that this molecule is an antagonist of CXCR4, the ability of TIQ-15 to block SDF-1α mediated Gi activation was determined on the WT and the various mutants of the receptor (Table 1). Additionally, the antagonist IT1t (1) was used as a control to determine the relevance of the mutants to small molecule interactions observed in the IT1t: CXCR4 crystal structure (SI, Figure S3A). Most notably, there are no published mutational studies using IT1t and we wanted to evaluate correlations between the binding values and the interactions observed in the crystal structure. For IT1t (1), our studies successfully picked up the key residues identified from the IT1t: CXCR4 crystal structure as being sensitive to ligand binding (Table 1, W94, D97, Y116, and E288).⁷ Most notably, residue D97 provides the strongest response out of all the mutants (>33-fold decrease in activity). Residue W94 was identified as the second most significant residue in terms of mutant shift (20-fold decrease). The residue E288 ranks the fourth most important interaction behind Y116, although it corresponds to the second salt bridge observed in the crystal structure. The order of potency changes due to mutation follow the order D97 > W94 = Y116 > E288 with both the D171 and D187 alanine mutants having no effect on IT1t binding. This mutational data on IT1t for D97, W94, and E288 corresponds with ligand contact strengths from the X-ray crystal structure showing the same order of importance.¹⁵ The mutational studies and crystal structure both provide agreement that the D97 residue is the most important residue for IT1t binding.

In contrast, TIQ-15 (5) with these mutants expressed significantly different results (Table 1). Two of the five residues were found to be in common to IT1t (D97 and E288), but they were opposite in importance. For TIQ-15 the

order of effect on the magnitude of activity was found to be $E288 \gg D171 > D97$ while the other residue mutants (W94A, Y116A, and D187A) showed no significant change. Unlike in the case of IT1t, both residues W94 and Y116 were unchanged in mutant versus wild type results. The key differences are that TIQ-15 has a very strong interaction with E288, while for IT1t it is D97, indicating a shift in the loci of binding between the two molecules. Furthermore, the remaining three residues of importance are uncommon: for TIQ-15 it is D171 while for IT1t it is W94 and Y116. The dependence of TIQ-15 (**5**) on the residue D171 and given its importance in CVX15 binding led us to further question the binding mode of this molecule. Given these seemingly subtle but significant differences, we became interested in understanding how our THIQ analogs interact with the receptor through subsequent computational modeling and medicinal chemistry studies.

In light of the chemical structural differences between TIQ-15 and IT1t we began our independent computational studies by flexible docking of **5** into the IT1t:CXCR4 model which yielded a best pose where both heterocyclic rings occupied the minor binding pocket. Overlay of this pose with IT1t in the IT1t:CXCR4 crystal structure shows the binding loci of **5** within the receptor overlaps with the location of IT1t (Figure 1A) and forms similar interactions with the receptor. The THQ ring is buried in a hydrophobic region, π -stacks with Trp94, and overlaps in a location with one of the cyclohexyl rings from IT1t (Figure 2B). The molecule forms two notable

Figure 1. Binding modes of TIQ-15 (**5**) obtained from docking into the IT1t pocket of the IT1t:CXCR4 3ODU crystal structure (Panels A/B/C) and the CVX15 pocket of the peptide CVX15:CXCR4 3OE0 crystal structure (Panels D/E/F). (Panel A) Overlay of TIQ-15 and IT1t. (Panels B/E) 3-D representations of main contacts. (Panel D) Overlay of TIQ-15 and the Arg2-Nal3 residues of CVX15. (Panels C/F) 2-D representations of main contacts and all proximal residues of CXCR4 to TIQ-15.

Figure 2. In-solution conformers of TIQ-15 (**5**) generated by the NAMFIS technique compared to bioactive conformations predicted from docking into CXCR4 subpockets. (Panel A) Overlay of NAMFIS structures 1–6. (Panel B) Overlay of NAMFIS-5 (red) to the IT1t:CXCR4 bound conformation (green, RMSD = 3.9 Å). (Panel C) Overlay of NAMFIS-6 (red) to the CVX15:CXCR4 bound conformation (blue, RMSD = 2.5 Å).

salt bridges: (i) the nitrogen in the bottom THIQ ring forms an electrostatic interaction with Asp97; and (ii) the butyl amine forms an electrostatic interaction with Glu288 (Figure 1B). These three main interactions (Figure 1C, W94, D97, E288) for **5** are similar to those observed for IT1t in the crystal structure, indicating that our model recaptured similar interactions important to the CXCR4 cocrystallized ligand and shows that in this pose TIQ-15 binds much like IT1t.

Alternatively, performing flexible docking of **5** in the CVX15:CXCR4 grid yielded a different result.¹² First, in this pose the binding loci of **5** overlaps highly with the CVX15 residues Arg2-Nal3 in the CVX15:CXCR4 crystal structure (Figure 1D). The Arg2-Nal3 residues position in the D171 region and are key contributors to the binding of CVX15 to CXCR4, as supported by the X-ray electron density map showing the largest values for both the Arg2 and Nal3 residues on CVX15 (SI, Figure S4, Chart S1). For TIQ-15, the THIQ nitrogen from the bottom ring forms an electrostatic interaction with Glu288 and the butyl amine interacts with Asp171 (Figure 1E). Of note is the observation that the butyl amine side chain adopts a gauche conformation in order to make the interaction with Asp171, similar to the kinked conformation of the Arg2 side chain of the CVX15 peptide. The bottom benzyl moiety of the THIQ ring buries in a hydrophobic groove between the backbone of Arg188 and His113, while the top THQ moiety of **5** is buried in a hydrophobic pocket partially defined by Tyr190 and Val196 residues (Figure 1F), and also near the site of the naphthyl ring of the Nal3 residue in the CVX15 peptide crystal structure (Figure 1D). Another significant difference to this pose of TIQ-15 (**5**) shows that the nitrogen of the pyridine-THQ ring of **5** fills an identical role to the function of the Arg2-C(O)-NH-Nal3 amide carbonyl on the backbone of CVX15, in that both form hydrogen bonds to the Arg188 residue in the receptor.^{7,19}

The significant errors observed when deriving small molecule conformations within protein crystal structures prompted us to validate our computationally derived poses of TIQ-15 (**5**) within our two CXCR4 models.¹⁴ An important tool developed for real time analysis involves deriving solution structure conformations by the NMR-based NAMFIS method, which we used to compare the docked poses of **5** with experimentally derived solution conformers.¹⁷ In order to initiate this study, **5** was analyzed using various ¹H and ¹³C NMR methods. Twenty-two interproton distances measured from 2-D NOESY NMR spectra were used to deconvolute the approximately 3,000 *in silico* conformers (SI, Figure S5)

generated from MacroModel. The NAMFIS deconvolution of the solution structures resulted in a population of six conformers totaling 99+% of the Boltzmann distribution. The six NAMFIS-derived solution conformers (Figure 2A) were compared to the two CXCR4 binding poses of **5** derived from the two crystal structures (from Figures 1A/B and 1D/E). The best-fit NAMFIS conformer (NAMFIS-5, 27% population) to that found in the IT1t: CXCR4 model and the binding poses were overlaid by aligning both THIQ and THQ ring systems exhibiting an RMSD of 3.8 Å (Figure 2B), which coincidentally slightly exceeds the resolution of the IT1t: CXCR4 crystal structure. Conversely, the NAMFIS conformer (NAMFIS-6, 20% population) that was structurally similar to the conformer docked to the CVX15: CXCR4 crystal structure aligned well with a lower RMSD of 2.5 Å (Figure 2C). In support of the NAMFIS assignments, a single-point, constrained calculation of each conformation removed from the receptor (Figures 2B, 2C) at the B3LYP/6-31G** level of theory (implicit PBF solvent, Jaguar 2014) revealed that the conformation of **5** derived from the CVX15 crystal structure is 9.0 kJ/mol (2.2 kcal/mol) lower in energy than the IT1t-derived conformation. In relating this energy difference to a Boltzman population distribution, this would correspond to an approximate 30 to 1 ratio in favor of the CVX15 derived conformation.

Next, we tested the poses of **5** from the two CXCR4-based crystal structures (Figures 1A–F) by altering key positions of the small molecule. First, to probe the role of the pyridyl nitrogen in the hydrogen bond-based interaction in the CVX15 pose, **11** with a tetrahydronaphthyl ring in place of the tetrahydroisoquinoline was synthesized (Scheme 1). In similar fashion to the previously described route for TIQ-15, *tert*-butyl-(*R*)-3-formyl-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (**7**) and (*S*)-1-aminotetrahydronaphthylene (**6**) were converted to **11** in three straightforward steps. The compound was then tested in an antiviral MAGI assay with X4-Tropic HIV virus (HIV-1_{IIIB}). The HIV assay was utilized for the testing of

our compounds in these cases in part due to the sensitivity of gp120 to the D171 mutation whereas CXCL12 shows no such effect.⁹ It was found that **11** was much less active (>100-fold) than the parent **5** (IC₅₀ = 1.5 μM for **11** compared to 5 nM for **5**, Table 2). The stark difference in activity between **5** and **11** (greater than 2 log units) indicates that the THQ-ring nitrogen plays a major role in binding. This observation is further elucidated when docking **11** into the poses generated for **5**. When docking in the IT1t pose, the compound forms similar interactions as **5** with Asp97, Glu288, and Trp94. Most notably, the tetrahydronaphthyl moiety overlaps with the Trp94 residue in a similar fashion to the THQ portion of **5** via a π -stacking interaction, resulting in both complexes (Figures 1B/C) having similar energetics (Table 2, −79 versus −81 kcal/mol ΔG MMGBSA values). However, this is not the case for the CVX15 crystal structure generated poses as the tetrahydronaphthyl ring cannot form a hydrogen bond between the nitrogen atoms with the Arg188 residue when docking **11** into the CVX15: CXCR4 grid (Figures 1E/F). Furthermore, the energetics for **5** and **11** in the CVX15 binding pocket are sufficiently different (Table 2, −96 versus −86 kcal/mol MMGBSA) greatly favoring compound **5** by a significant amount (10 kcal/mol), while in the IT1t: CXCR4 pose (Figures 1B/C) the opposite is observed where **11** is favored over **5** by a modest gain in MMGBSA binding energy (2.4 kcal/mol; Table 2: −81 versus −79 kcal/mol). The energetic gain measured from the modeling poses of **5** versus **11** matches better with the difference in free energy of activity derived from the assay pIC₅₀ values for the CVX15 poses, which is most likely due to the role of hydrogen bonding of the THQ-pyridine nitrogen.¹⁸ Furthermore, a similar effect regarding the replacement of a pyridine nitrogen with a carbon atom (the pyridine to phenyl swap) was observed previously in the mono cyclam CXCR4 antagonists.¹⁹

Another difference between the IT1t and CVX15 derived poses of **5** involves the space around the bottom THIQ ring. In the IT1t based pose (Figure 1C), the bottom phenyl ring is facing toward solvent without interactions and flanked by hydrophilic residues. In the CVX15 pose (Figure 1F), the THIQ phenyl ring picks up aryl H-bond interactions with the His113 and Arg188 residues. We had previously shown that the SAR involving the THIQ ring with regard to the position of the phenyl ring and stereochemistry on the heterocyclic portion had led us to the discovery of **5**.¹³ An additional test of this region to elucidate the role of the phenyl ring was warranted, so we synthesized the simple piperidine-THIQ ring replacement **17** (Scheme 1). The route was similar to our previous chemistry in making TIQ-15 analogs.¹³ After testing this compound in the HIV-based assay, we found a drop of greater than 100-fold in activity when tested against HIV-1_{IIIB} in MAGI cells (IC₅₀ = 1.12 μM, Table 2), indicating a significant loss of interaction with the receptor and more consistent with the CVX15 pose. It is interesting to note that the replacement of the 1-Nal residue on the T140 cyclic peptide with alanine results in a similar loss in activity (>100-fold), with both cases pointing to the significance of the aromatic ring portion and that the THIQ ring may provide similar form and function to the 1-Nal residue in the CVX15 peptide.^{20–22} The energetics calculated for binding of this derivative (**17**) when compared to **5** showed a significant drop in energy was observed for the CVX15 poses (−22 kcal/mol; Table 2: −74 versus −96 kcal/mol) compared to the IT1t poses where a slight increase was observed favoring **17** (5 kcal/

Scheme 1^a

^aReagents: (i) **6** and **7**, NaHB(OAc)₃, 1,2-DCE; (ii) **8** and **9**, NaHB(OAc)₃, DCM; (iii) TFA, DCM; (iv) **12** and **13**, NaHB(OAc)₃, 1,2-DCE; (v) **14** and **15**, NaHB(OAc)₃, DCM; (vi) NH₂NH₂, MeOH; (vii) **7** and **12**, NaHB(OAc)₃, 1,2-DCE; (viii) **18** and **19a–c**, NaHB(OAc)₃, DCM.

Table 2. MAGI Assay Results and Docking Scores of THIQ Based CXCR4 Antagonists

Compound	MAGI HIV-1 _{IIIb}		CVX15: CXCR4Model		IT1t: CXCR4Model	
	IC ₅₀ (μM) ^{a,b}	pIC ₅₀	MM-GBSA ΔG Bind (kcal/mol)		MM-GBSA ΔG Bind (kcal/mol)	
5	0.005	2.30	−96.0		−78.7	
11	1.50	−0.18	−86.1		−81.1	
17	1.12	−0.05	−74.2		−83.8	
23	6.20	−0.79	−80.4		−72.8	
24	0.380	0.42	−87.5		−82.5	
25	0.170	0.77	−89.3		−80.5	
26	30.0	−1.48	−82.8		−70.9	
27	0.250	0.60	−87.1		−71.7	
28	30.0	−1.48	−83.9		−71.1	
29	1.40	−0.15	−87.6		−75.3	
30	0.450	0.35	−86.4		−65.5	

^aSingle experiment in triplicate. ^bCCRS antagonist TAK-779: IC₅₀ > 10 μM.

mol; Table 2: −84 versus −79 kcal/mol). The origins of these differences are easily explained by the hypothesized binding of 5 in the two grids (Figures 1C versus 1F). A lower binding energy would be expected with 17 if adopting an IT1t-like pose, as Figure 1C shows the removal of the phenyl ring should be favored due to proximity of hydrophilic residues and solvent exposure. This is further supported by the SAR in the IT1t series, where placement of phenyl rings on or around the region of the cyclohexyl rings resulted in much lower activity.²³ However, the CVX15 based pose in Figure 1F shows the aromatic ring interacts with His113 and is in proximity to Trp94, Leu120, and Cys186 which would favor an aromatic ring system.²¹ Therefore, the large drop in activity observed for 17 in the MAGI assay (>2 log units) matches better with the calculated energetics of the CVX15: CXCR4 pose than for the IT1t: CXCR4 generated grid.

Analysis by computational modeling can also help to explain the molecular mechanism of ligand binding as well as determine the structure–activity relationships by using a congeneric series of structures in a molecular mechanics approach.²⁴ In this case, we continued our analysis on the small molecule antagonist 5 and selected congeneric analogs for use in our modeling as validation of our docking studies. The congeneric analogs focusing on the THIQ ring were reported previously (26–30, Figure 3A), where changing the position of the phenyl ring and stereochemistry at the position adjacent to the THIQ nitrogen was found to have a profound effect on biological activity (Table 2).¹³ Our second variation

involved altering the butyl amine side chain, where we were interested in testing the effect of chain length for two through five carbon atoms (Figure 3A, 23–25) as we had successfully performed a docking analysis on a similar set of congeneric side chain analogs in the AMD11070 series.¹² These compounds were synthesized in a similar fashion to compound 5 with slight variations (Scheme 1) using mono-Boc-protected aliphatic aldehydes with the desired length of the side chain (19a–19c) from the previously described secondary amine 18.¹³ These side chain analogs were subsequently tested in the HIV-1_{IIIb}/MAGI assay (Table 2) to provide consistent data for our analysis. These congeneric sets (Figure 3A) were selected to test some of the concepts developed in the two bioactive poses of 5 in both the IT1t: CXCR4 and CVX15: CXCR4 grids (Figures 1A–F).

All compounds (23–30, Figure 3A) were docked into the IT1t: CXCR4 and CVX15: CXCR4 models and their Prime MM-GBSA scores were calculated and listed along with their pIC₅₀ values in the HIV-1_{IIIb} MAGI assay in Table 2. To determine which model provided better correlation, the congeneric analysis was performed by plotting the docking (MMGBSA) scores versus the intrinsic HIV biological activity (pIC₅₀ values).²⁷ The overall result was that scores from the CVX15: CXCR4 model better correlated with anti-HIV potency than for the IT1t: CXCR4 model (Figure 3B, R² = 0.82 versus R² = 0.22), similar to the result we had previously observed for the AMD11070 congeneric series analysis.¹²

In the side chain congeneric series (23–25), some reasons for the differences in prediction can be seen with individual examples. One such is the observation that the IT1t: CXCR4 model incorrectly predicted that the most potent chain length will be the compound with the 3 carbon chain (24) and predicted a 4 kcal/mol lower MM-GBSA energy versus 5 (Table 2, −83 versus −79 kcal/mol), while the CVX15: CXCR4 model provided energies for 5 and 24 that better reflected the observed biological activities (Table 2; 5 versus 380 nM; −96 versus −88 kcal/mol) with an 8 kcal/mol increase for 5. Based on the derived models (Figures 1A–C versus 1D–F), this difference stems mainly from the orientation between the amino chain and THIQ ring nitrogen atoms. In the IT1t pose, the THIQ ring and side chain position varies within the 110 to 150° range (Figure 1C). This allows the three-carbon chain of 24 to provide the best conformation to span the gap between the residues that make up the salt bridges, E288 and D97. In the CVX15 model (Figure 1F), the 180° difference in orientation between the amine side chain

Figure 3. (Panel A): Compounds used in congeneric docking comparisons. (Panel B): Correlations of HIV-1_{IIIb} bioactivities (pIC₅₀) with Prime MM-GBSA docking scores for the congeneric series of TIQ analogs in Panel A (5, 23–30) from data listed in Table 2 for the TIQ-15:IT1t: CXCR4 (red squares; R² = 0.22) and the peptide derived TIQ-15: CVX15: CXCR4 based models (blue circles; R² = 0.82).

and the THIQ ring nitrogen atoms places the 3 carbon length outside the optimal length to keep the salt bridge interactions between the E288 and D171 residues intact. Furthermore, the CVX15:CXCR4 derived model of **5** predicts that the bioactive pose will have a specific gauche interaction in the butyl amine side chain, supported by the in-solution NAMFIS conformers (Figures 2A–C) showing a dynamic conformation pool which correlates well with the observed bioactivities. These results especially highlight the superior performance of the CVX15 based model over the IT1t pneumatic in predicting biological activity in the side chain variant congeneric series.

In summary we have shown through several in depth studies that the tetrahydroisoquinoline based (TIQ-15) series of CXCR4 antagonists preferentially bind within the CVX15:CXCR4 versus the IT1t:CXCR4 based receptor complex. These include assays of CXCR4 receptor mutants, which indicate a difference between IT1t and TIQ-15, as well as docking and conformational studies of TIQ-15 and related congeneric series. We showed that each portion of the TIQ-15 molecule overlaps in function with the critical portions of each ligand in the docking studies (Figures 1A–F). First, the butyl amine side interacts via a salt bridge to D171 or E288. Second, the THQ “top ring” interacts via a nitrogen H-bond to Arg188 or π -stacking with W94. Third, the THIQ “lower ring” interacts via salt bridges to E288 or D97. This allowed us to compare IT1t and CVX15 emulating poses of TIQ-15 for the purposes of determining that the CVX15 pose better fit the mutational data. The intrinsic basis of our multipoint analysis provides unique insights into CXCR4 ligand binding while providing methodology for determining structure activity relationships, especially in lieu of having access to X-ray crystallography. The epiphany resulting from our study that not all small molecules bind in the same site of CXCR4 is also supported by other mutational studies.¹¹ This report should spur the re-examination and development of better ligand-CXCR4 binding models.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmchemlett.8b00441.

Figures S1–S5, Chart S1, NMR assignments for compound **5**, procedures for mutational studies, computational docking, NMR studies and NAMFIS analysis, the synthesis of **10**, **16**, and **22–24**, and the MAGI assay. (PDF)

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Author Contributions

LJW, JPS, BMK, and BDC devised the dual small molecule-peptide receptor ligand concept and performed the computational modeling and NMR studies. MH, AT, GMS, and BM devised and performed the mutational studies. YZ synthesized IT1t and TIQ-15. BK, ARP, and LJW synthesized **10**, **16**, and

23–25. LJW and DCL supervised the work. MH wrote up the mutational studies. LJW, BMK, and BDC contributed to the writing of the manuscript. All authors reviewed the manuscript and consented to its content.

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Notes

The authors declare the following competing financial interest(s): DCL and LJW are coinventors on Emory-owned Intellectual Property that includes CXCR4 antagonists.

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■ ABBREVIATIONS

CXCR4, C-X-C Chemokine receptor type 4; GPCR, G-Protein Coupled Receptor; THQ, 5,6,7,8-tetrahydroquinoline; THIQ, 1,2,3,4-tetrahydroisoquinoline; SDF-1, stromal derived growth factor-1; NAMFIS, NMR analysis of molecular flexibility in solution; RMSD, root mean squared deviation; BRET, bioluminescence resonance energy transfer; WT, wild type; HIV, human immunodeficiency virus; MAGI, multinuclear activator of a β -galactosidase indicator; HIV-1_{IIIB}, human immunodeficiency virus type 1-strain IIIB; 1,2-DCE, 1,2-dichloroethane; DCM, dichloromethane

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