

# Understanding the Interactions Between CXCR4 and AMD11070, a First-in-Class Small Molecule Antagonist of the HIV Coreceptor

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## Abstract

**Background:** AMD11070 is a first-in-class, bioavailable small molecule antagonist against the HIV coreceptor CXCR4. An ongoing safety and dose-finding study has demonstrated the activity of AMD11070 in HIV-infected patients harboring X4-tropic virus. This study aims to understand the interactions of AMD11070 with CXCR4 at the molecular level.

**Methods:** Site-directed mutagenesis was employed to generate mutated forms of the hCXCR4 receptor that carry single amino acid substitutions. The mutated hCXCR4 receptors were expressed transiently in a canine thymus cell line (Cf2Th). Calcium mobilization and <sup>125</sup>I-SDF-1α homologous competition binding experiments were used to characterize the SDF-1α signaling response and binding affinity respectively of the mutated hCXCR4. In addition, <sup>125</sup>I SDF-1α competition binding experiments were performed using these recombinant Cf2Th cell lines to examine how the single site mutations affected the binding of AMD11070. Molecular modeling was used to illustrate the experimental results.

**Results:** Fourteen single site mutants that span across the entire putative small molecule antagonist binding site of CXCR4 receptor were generated. The SDF-1α EC<sub>50</sub>s in calcium flux signaling and IC<sub>50</sub>s in homologous competition binding of these mutants were within 3 to 4-fold as compared to those of the wild type receptor. The mutations affected the binding of AMD11070 at various degrees. Among the hCXCR4 mutants studied, 4 of them (W94A, D97N, D171N and E288A) decreased the potency of AMD11070 binding by >100 fold, while 2 of them (Y45A and D262N) decreased the potency by 10-50 fold. This study also identified an amino acid residue (D97) on the receptor that interacts specifically with AMD11070, but not its predecessor AMD3100. Comparing with previously published data, these findings suggested that AMD11070 and AMD3100 share partially overlapping binding sites.

**Conclusions:** Binding studies using single site mutants of hCXCR4 showed that AMD11070 interacts in a similar, but distinct mode from its bicyclam predecessor AMD3100. This study suggests that it is possible to develop antiretroviral CXCR4 antagonists that have different binding mechanisms to the HIV coreceptor. These mechanistic studies might prove to be useful for the development of other structurally diverse CXCR4 antagonists with improved clinical pharmacology and safety profiles.

## Introduction

HIV entry into cells requires the binding of the viral envelope protein to the cellular receptor CD4 and a coreceptor, either the CCR5 or CXCR4 chemokine receptor. Viruses that utilize CXCR4 as an entry coreceptor are mainly found in patients with advanced disease and are associated with disease progression. Both CCR5 and CXCR4 have been validated as potential therapeutic targets for the development of a new class of HIV entry inhibitors. While 3 of the CCR5 inhibitors in development have been advanced to phase III clinical studies, the clinical development of CXCR4 inhibitors is less advanced.

AMD11070 is a selective, reversible and orally bioavailable inhibitor of CXCR4. It has been shown to inhibit HIV entry in PBMC and other cell lines *in vitro*. It is a first-in-class orally bioavailable CXCR4 inhibitor that entered clinical development. Recent phase Ib/IIa clinical studies have indicated the effectiveness of AMD11070 in reducing the X4-using viral load in HIV-infected patients.

CXCR4 is a chemokine receptor and belongs to the class A family of seven transmembrane (7 TM) G-protein coupled receptors (GPCR), a class which also includes a significant portion of proven drug targets (5). SDF-1 (also known as CXCL12) is the only known ligand that binds to CXCR4.

This study investigated the interactions of AMD11070 with the CXCR4 receptor. Site-directed mutagenesis was used to generate single site mutants of the human CXCR4 receptor. These mutants were then used to examine how single amino acid changes affect the binding of AMD11070 in a SDF-1α competition binding assay. Based on the findings, molecular modeling was employed to illustrate the possible molecular interactions between AMD11070 and the receptor. Understanding these interactions will likely assist in the development of future generation therapeutics with different resistance profiles and improved pharmacological and safety properties.

Figure 1  
AMD11070 Inhibition of HIV Entry

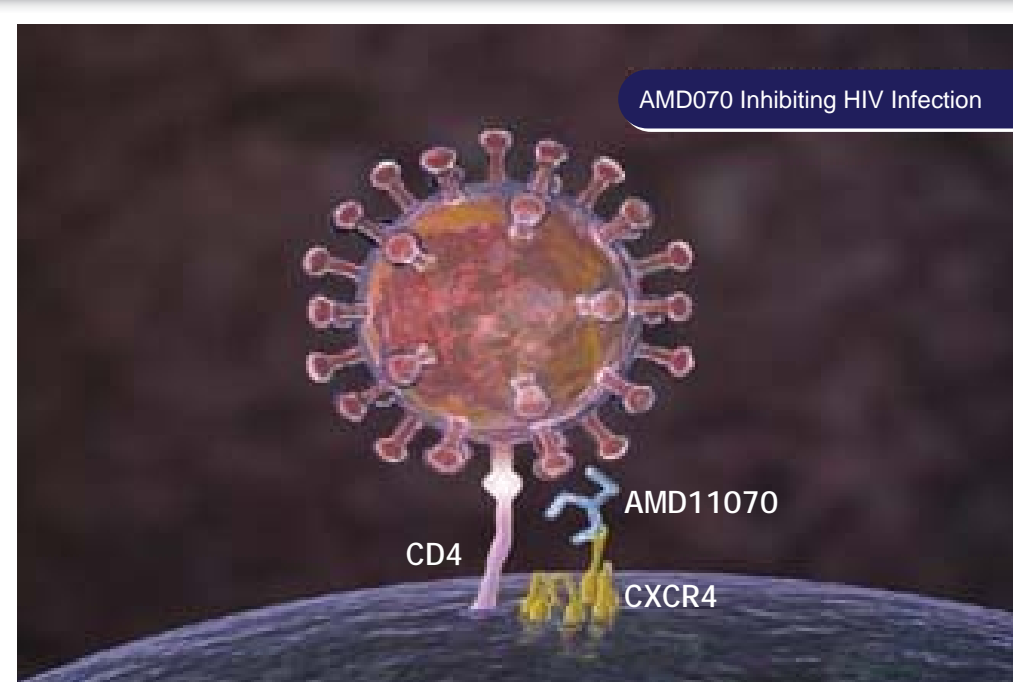


Figure 2  
Chemical Structure of AMD11070

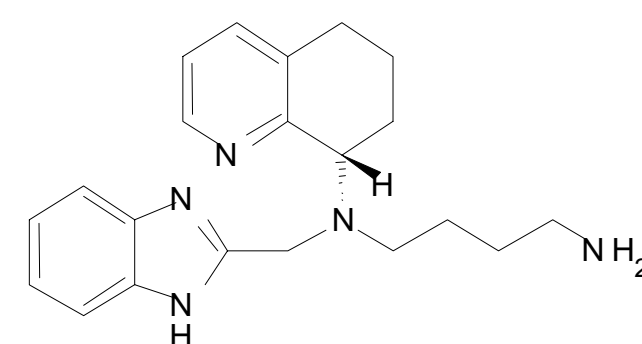
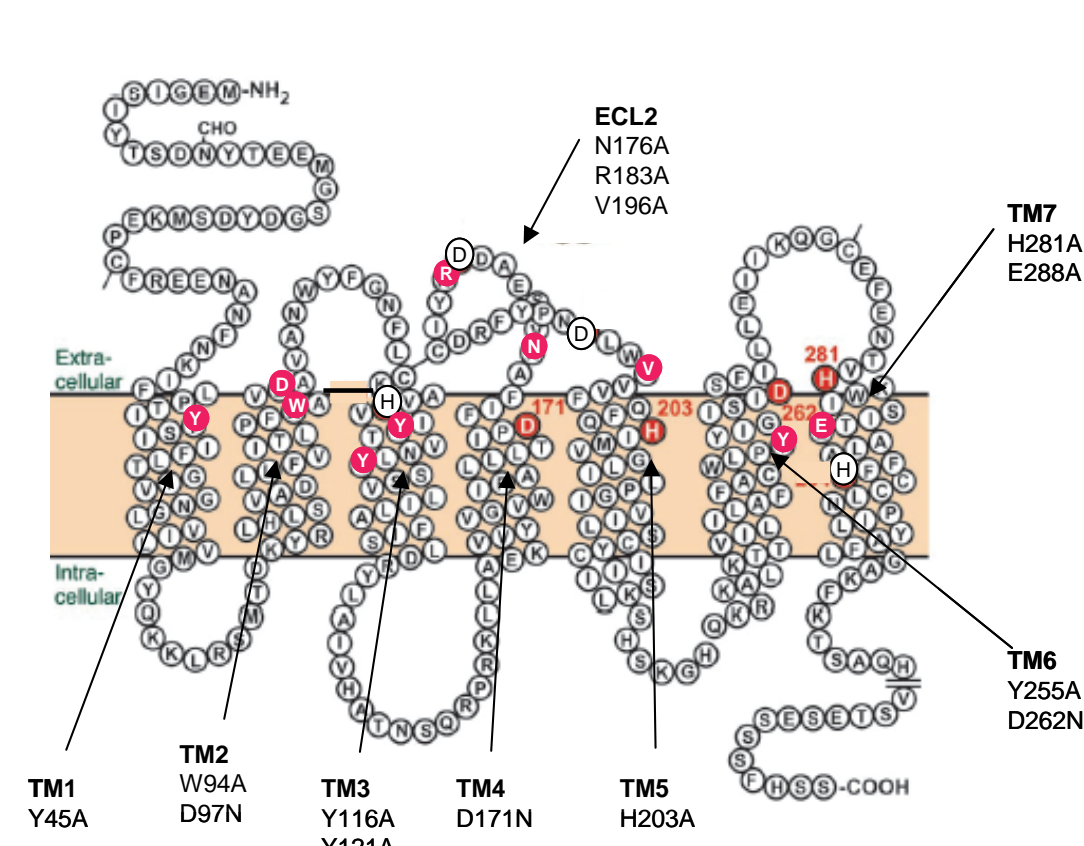


Figure 3  
Serpentine diagram of the CXCR4 receptor



The amino acids changed in the single site mutations are indicated by the red circles. Fourteen single site mutants of CXCR4 that span across the entire putative small molecule binding site of the receptor were generated by site-directed mutagenesis

Table 1  
Characterization of hCXCR4 mutants by <sup>125</sup>I-SDF-1α binding and SDF-1α induced calcium flux signaling in transfected Cf2Th cells

	<sup>125</sup> I-SDF-1α binding pIC <sub>50</sub>	SDF-1α calcium flux signaling pEC <sub>50</sub>
Wild Type	7.45	7.47
Y45A	7.64	7.47
W94A	7.26	7.35
D97N	7.32	NR
Y116A	7.47	NR
Y121A	7.06	7.00
D171N	7.13	6.86
N176A	7.82	7.23
R183A	7.79	7.37
V196A	7.64	6.86
H203A	7.37	7.58
Y255A	7.93	7.38
D262N	7.10	6.94
H281A	8.16	6.87
E288A	7.36	NR

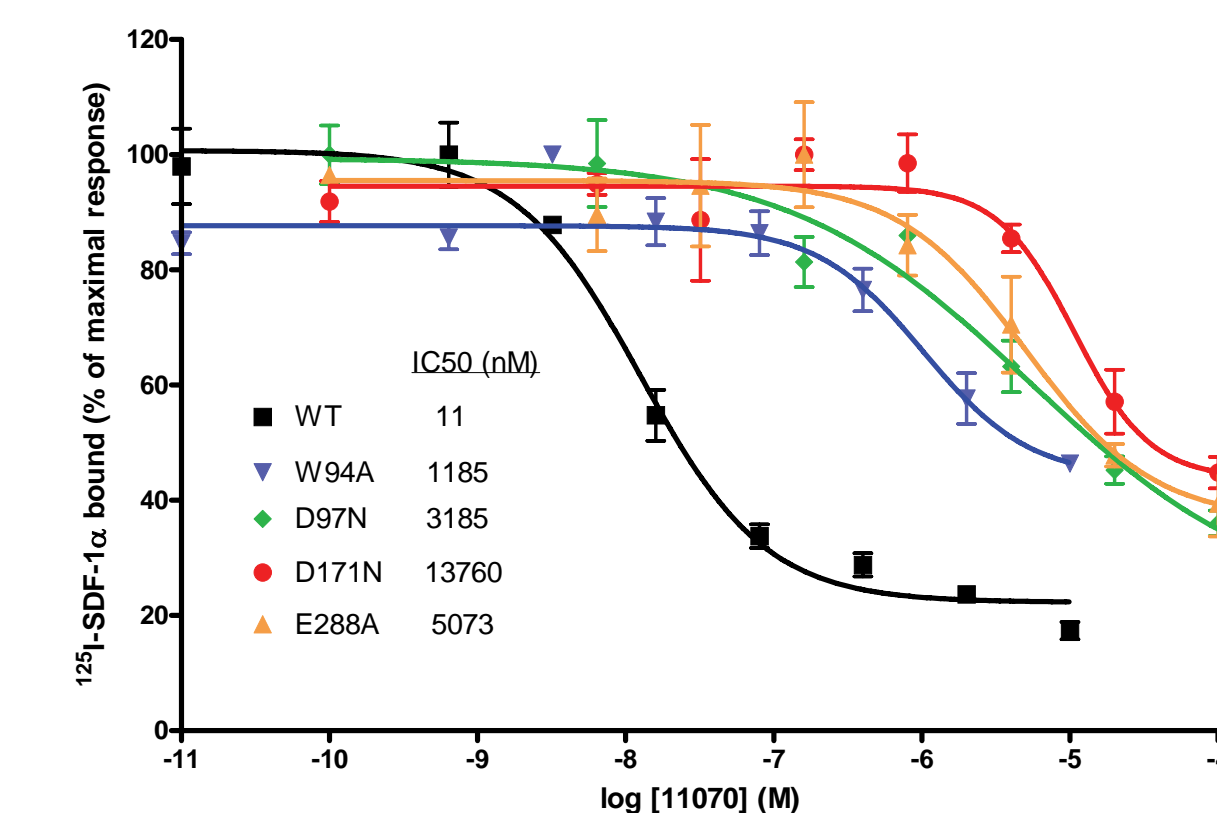
\*NR, no response  
Most of the mutants have similar to wild type affinity for the natural ligand, SDF-1α.

Table 2  
Effects of CXCR4 single site mutations on the AMD11070 Inhibition of <sup>125</sup>I-SDF-1α binding. Results are expressed as pIC<sub>50</sub>s

Site of mutation	Location in CXCR4	<sup>125</sup> I-SDF-1α pIC <sub>50</sub> of AMD11070 [Fold increase compared to wild type]
Wild Type		7.96 [1]
Y45A	TM1*	6.36 [39.8]
W94A	TMII	5.93 [107.5]
D97N	TMII	5.50 [289.0]
Y116A	TMIII	7.41 [3.5]
Y121A	TMIII	7.72 [1.7]
D171N	TMIV	4.86 [1248.6]
N176A	ECL2*	8.08 [0.8]
R183A	ECL2	7.81 [0.14]
V196A	ECL2	7.55 [2.5]
H203A	TMV	8.03 [0.9]
Y255A	TMVI	7.89 [1.2]
D262N	TMVI	6.86 [12.5]
H281A	TMVII	7.62 [2.2]
E288A	TMVII	5.29 [460.3]

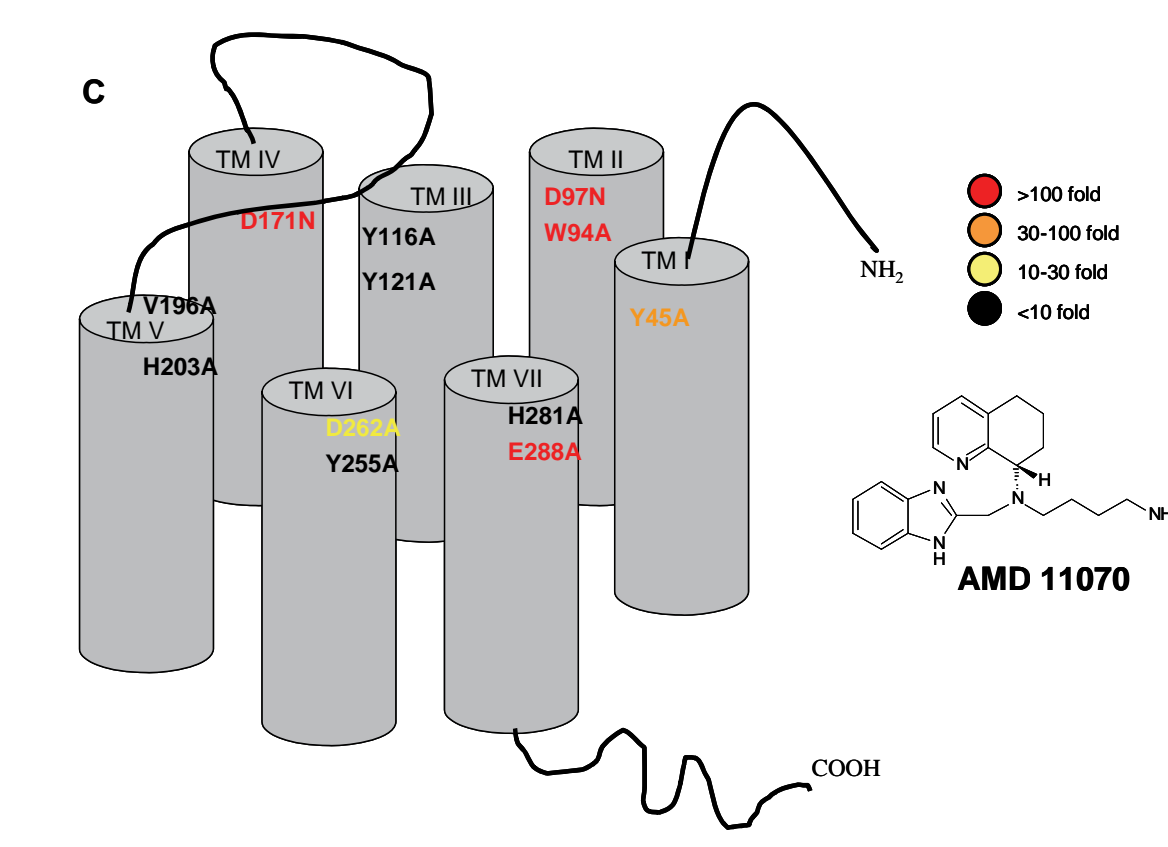
TM, transmembrane; ECL, extracellular loop  
The mutations affected AMD11070 binding at various degrees.

Figure 4  
The Effect of W94A, D97N, D171N and E288A mutations on the binding of AMD11070



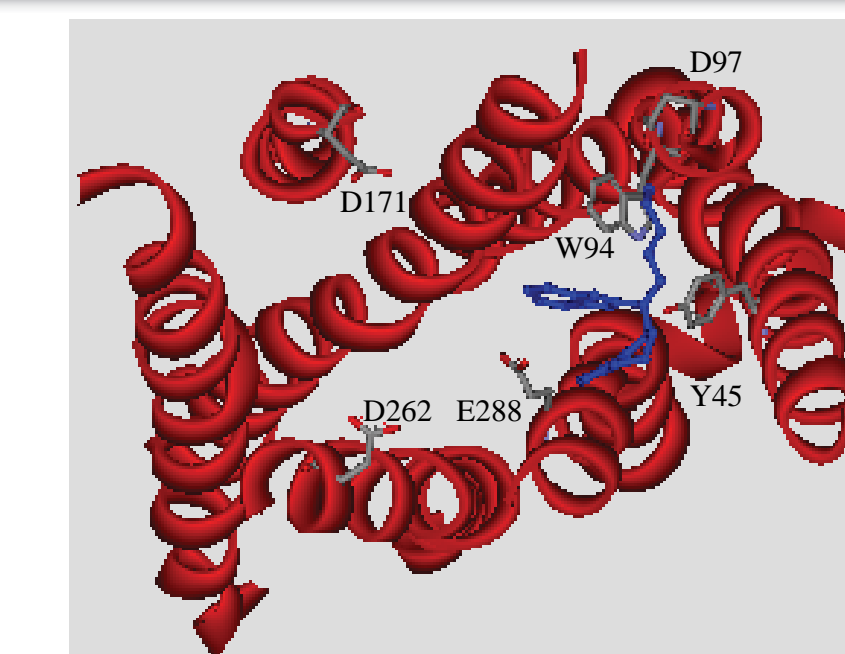
Competition binding curves of AMD11070 on wild type and mutated hCXCR4 receptors. <sup>125</sup>I-SDF-1α competition binding studies against hCXCR4 were performed in either CCRF-CEM cells or Cf2Th cells transiently transfected with hCXCR4 cDNA. Mutations at W94, D97, D171 and E288 had the biggest impact on AMD11070 binding.

Figure 5  
The Effect of W94A, D97N, D171N and E288A mutations on the binding of AMD11070



Schematic diagram of the CXCR4 receptor depicting the effect of mutations on the interactions with AMD11070. For clarity reason, all the intracellular and extracellular loops except for ECL2 are omitted. In addition, the mutations in ECL2, which did not affect the binding of any inhibitors, were not indicated. The levels of effect of each mutation on the IC<sub>50</sub> of AMD11070 in <sup>125</sup>I-SDF-1α competition binding are indicated by the font colour: red, >100 fold increase in IC<sub>50</sub>; orange, 30-100 fold increase in IC<sub>50</sub>; yellow, 10-30 fold increase in IC<sub>50</sub>; black, <10 fold increase in IC<sub>50</sub>.

Figure 6  
Docking of AMD11070 to the CXCR4 Receptor



One of the potential binding modes of AMD11070 showing the unique interactions with D97. Hydrogens are omitted for clarity.

## Summary of Results

- Fourteen single site mutants that span across the entire putative small molecule binding site of CXCR4 were generated.
- Most of the mutants had very similar to wild type affinity for the natural ligand SDF-1α in calcium flux signaling and SDF-1α binding assays.
- The mutations affected the binding of AMD11070 at various degrees. Mutations at W94, D97, D171 and E288 had the biggest impact. Mutations located in the second extracellular loop did not affect AMD11070 binding.
- The impact of D97N mutation is unique to AMD11070
- AMD11070 can potentially bind to CXCR4 in different binding modes

## Discussion

Our study investigated the interactions AMD11070 with CXCR4. We showed that AMD11070 interacts with overlapping, but not identical residues, to its predecessor AMD3100 in the binding pocket of the receptor<sup>1</sup>. Our data suggested that, like the other documented CCR5 small molecule inhibitors, AMD11070 mediates its effect by binding to the transmembrane regions of the receptor, which sequentially causes a conformational change that leads to the disruption of receptor function<sup>2,3</sup>. In addition, the identification of the unique interaction with the aspartic acid at position 97 (D97) has extended the size of the inhibitor binding pocket in the receptor. This new information might prove to be useful for the development of future generations of CXCR4 inhibitors with improved clinical pharmacology and safety profiles.

AMD11070 was recently placed on clinical hold by the FDA due to liver histology changes observed in longer term toxicity experiments. These findings are currently under investigation.

### REFERENCES

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