Optimization of vCCL2 based CXCR4 inhibitors by Phage Display and Rational Design

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BACKGROUND

The viral broad spectrum chemokine vCCL2 binds to the chemokine receptors CXCR4 and CCR5 and inhibits HIV-1 entry protecting host cells against infection [1]. Peptide derived from vCCL2 N-terminus ($vCCL2_{1-21}$) displays modest CXCR4 and CXCR7 binding affinity and antiviral properties compared to the parental chemokine but mutations within its CC motif has been shown to enhance its antiviral properties, for review see [2]. **In this study, we exploited these observations to develop highly potent and selective CXCR4 antagonists.**

AIM

The aim of the current study was to develop a new class of short and selective CXCR4 inhibitors inspired by the binding mode of natural chemokines (Mimokines). The N-terminus of vCCL2 ($vCCL2_{(1-21)}$), a viral chemokine displaying antagonist properties on CXCR4, was used as scaffold to introduce controlled randomizations and to create phage library of peptide variants.

METHODS

A Mimokine phage display library, in which $C_{11}C_{12}$ amino acids within the N-terminus $vCCL2_{(1-21)}$ peptide were fully randomized (LGASWHRPDK**XX**LGYQKRPLP), was engineered by direct cloning of degenerated oligonucleotides into a filamentous phage vector. The isolation of Mimokines specific to CXCR4 was then performed by phage display biopanning on CXCR4 displayed at the surface of proteoliposomes.

RESULTS

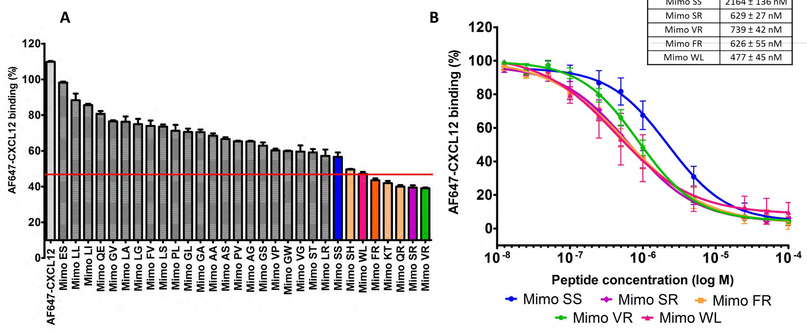
Screening of Mimokine phage library on CXCR4 magnetic proteoliposomes -

- Identification of 30 Mimokines
- In contrast to the N-terminus $vCCL2_{(1-21)}$ peptide, in which the $C_{11}C_{12}$ residues allow dimer formation, selected Mimokines are exclusively monomeric
- Bivalent ligands have been suggested to be stronger binders than their monomeric counterparts, we engineered and used a N-terminus $vCCL2_{(1-21)}$ $C_{11}S$-$C_{12}S$ mutant as monomeric control for the further characterization of the selected Mimokines

CXCR4 binding affinities of monomeric Mimokines -

Selected Mimokines were evaluated for their ability to bind to CXCR4 in competitive assay using a fluorescently labelled CXCL12 (AF647-CXCL12)

- At a final concentration of 1 µM, all selected Mimokines competed with AF647-CXCL12 for CXCR4 binding (Figure 1A).
- Mimokines VR, SR, FR and WL were the most potent inhibitors displaying 3- to 4-fold higher affinity for CXCR4 than Mimokine SS (Figure 1B).



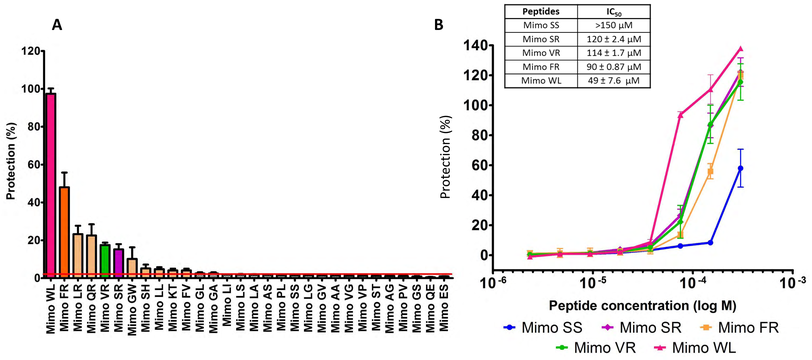
Peptides	IC_{50}
Mimo SS	2164 ± 136 nM
Mimo SR	629 ± 27 nM
Mimo VR	739 ± 42 nM
Mimo FR	626 ± 55 nM
Mimo WL	477 ± 45 nM

Figure 1. CXCR4 binding affinities of monomeric Mimokines. Inhibition of Alexa Fluor® 647-labeled CXCL12 binding (100 ng/mL) to Cf2Th–CXCR4 cells by (A) 1 µM final of selected Mimokines or (B) increasing concentrations of Mimokines (300 µM to 10 nM).

Antiviral properties of monomeric Mimokines -

Selected Mimokines were also tested for their ability to inhibit the infection of MT-4 cells with the laboratory-adapted CXCR4-using (X4) HIV-1 strain IIIB.

- At a final concentration of 100 µM, only Mimokine WL fully protected cells from virus cytopathic effect while Mimokine FR, LR, QR, VR and SR displayed 50, 22, 21, 18 and 17% protection, respectively (Figure 2A).
- Mimokine SS displayed a 60% protection at 300 µM while Mimokines VR, SR and FR showed an IC_{50} value of 114, 120 and 90 µM, respectively (Figure 2B).
- Mimokine WL displayed the most potent antiviral properties exhibiting an IC50 value of 49 µM (Figure 2B).



Peptides	IC_{50}
Mimo SS	>150 µM
Mimo SR	120 ± 2.4 µM
Mimo VR	114 ± 1.7 µM
Mimo FR	90 ± 0.87 µM
Mimo WL	49 ± 7.6 µM

Figure 2. Antiviral properties of monomeric Mimokines. Protection of MT-4 cells conferred by (A) 100 µM final of selected Mimokines or (B) increasing concentrations of Mimokines (300 µM to 10 nM) against the HXB2 virus (X4 tropic).

Molecular modelling and docking into CXCR4 3D structure -

- Higher affinity of Mimokines FR, SR and VR for CXCR4 might be due the formation of an additional salt bridge between the Arginine 12 of the Mimokine and the sulfated tyrosine 21 of the receptor (Figure 3).

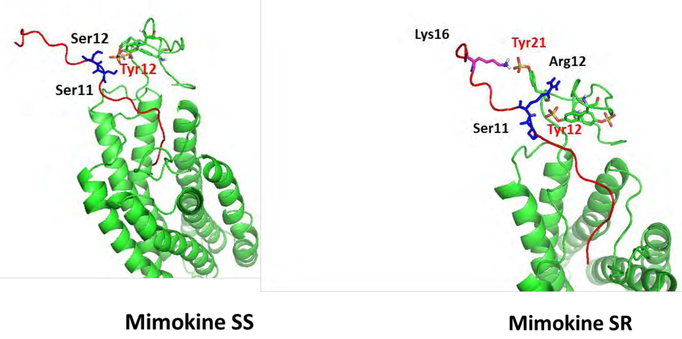
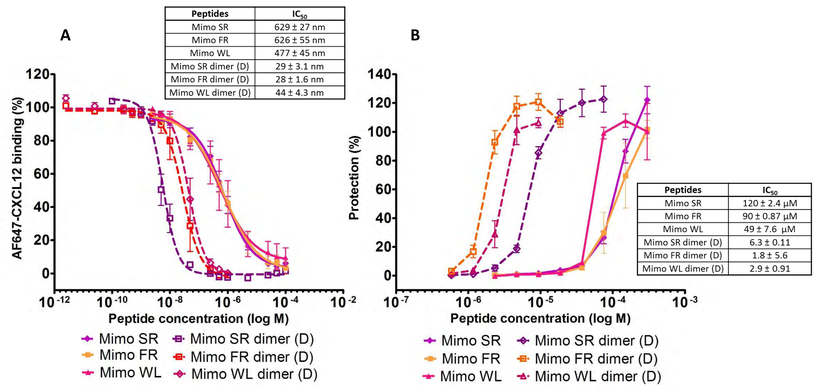


Figure 3. Molecular modelling and docking of Mimokine SR into CXCR4 3D structure.

Development of Dimeric and D-amino acids Mimokine analogues -

Taking advantage of the higher binding affinity and potency observed for bivalent and D-amino acids ligands, we further enhanced the potency of the selected Mimokines by combining dimerization and D-amino acids substitution.

- Bivalent Mimokines SR, VR, FR and WL in D-format exhibited 10 to 20-fold increase in CXCR4 binding affinity (Figure 4A) and 20 to 100-fold increase in antiviral activities when compared to their respective monomeric L-counterparts (Figure 4B).



Peptides	IC_{50}
Mimo SR	629 ± 27 nm
Mimo FR	626 ± 55 nm
Mimo WL	477 ± 45 nm
Mimo SR dimer (D)	29 ± 3.1 nm
Mimo FR dimer (D)	28 ± 1.6 nm
Mimo WL dimer (D)	44 ± 4.3 nm

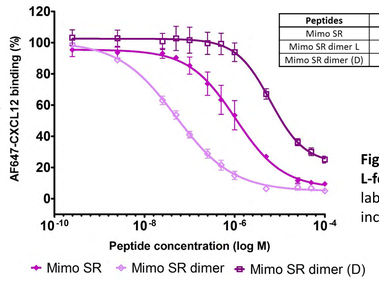
Peptides	IC_{50}
Mimo SR	120 ± 2.4 µM
Mimo FR	90 ± 0.87 µM
Mimo WL	49 ± 7.6 µM
Mimo SR dimer (D)	6.3 ± 0.11
Mimo FR dimer (D)	1.8 ± 5.6
Mimo WL dimer (D)	2.9 ± 0.91

Figure 4. CXCR4 binding affinities and antiviral properties of Mimokines in monomeric L-format and Dimeric D-format. (A) Inhibition of Alexa Fluor® 647-labeled CXCL12 binding (100 ng/mL) to Cf2Th–CXCR4 cells by increasing concentrations of monomeric L and Dimeric D Mimokines (300 µM to 10 nM). (B) Protection of MT-4 cells conferred by increasing concentrations of monomeric L and Dimeric D Mimokines (300 µM to 10 nM) against the HXB2 virus (X4 tropic).

CXCR7 binding affinity of Mimokine SR analogues -

As all GPCR are not permissive to chirality change, we wanted to determine whether our L- and D-format Dimeric Mimokine SR analogs could still bind to CXCR7.

- Dimerization of Mimokine SR ameliorated by a factor 16 its affinity for CXCR7 (Figure 5).
- Bivalent Mimokines SR in D-format exhibited a 108-fold reduction in binding CXCR7 than the Dimeric L-analog (Figure 5).



Peptides	IC_{50}
Mimo SR	1056 ± 136 nM
Mimo SR dimer L	49 ± 26 nM
Mimo SR dimer (D)	6222 ± 246 nM

Figure 5. CXCR7 binding affinities of Mimokine SR in monomeric L-format and dimeric D-format. Inhibition of Alexa Fluor® 647-labeled CXCL12 binding (100 ng/mL) to CXCR7 U87 cells by increasing concentrations of Mimokines (300 µM to 0,25 nM).

CONCLUSIONS

Optimized bivalent D-Mimokines displayed 10 to 20-fold enhanced CXCR4 affinity and 20 to 100-fold improved antiviral properties when compared to the initial monomeric L-Mimokines.

These bivalent D-Mimokines showed a reduced affinity for CXCR7 and may therefore serve as lead compounds for the further development of more selective CXCR4 HIV-1 inhibitors.

REFERENCES

[1] Boshoff, C et al., 1997. Angiogenic and HIV-inhibitory functions of KSHV-encoded chemokines. Science 278: 290–294.

[2] Chevigné et al., 2011. Engineering and screening the N-terminus of chemokines for drug discovery. Biochem Pharmacol 82(10):1438-56.

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