Proceedings of the 23rd American Peptide Symposium
Michal Lebl (Editor)
American Peptide Society, 2013

Synthetic Agonists for the CXCR4 Receptor: SAR, Signaling Pathways and Peptidomimetic Transition

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Introduction

CXCR4 a G-protein coupled receptor is an important pharmaceutical target in a variety of diseases including HIV and many forms of cancer. CXCR4 is therefore an important pharmaceutical target but besides the cognate agonist SDF-1 only synthetic antagonists/inverse agonists are targeting CXCR4 with pertinent affinities. CXCR4 has important household functions; those antagonists lead to the emergence of significant adverse drug effects. We recently describe nanomolar CXCR4 agonists [1] where the SDF-1 *N*-terminus was grafting to the inverse agonist T140 [2] (Figure 1). In order to translate these peptidic compounds into peptidomimetic agonists, permitting eventual pharmaceutical applications, the present contribution describes the SAR of the grafted SDF-N-terminus in order to pinpoint the agonist-antagonist transition and to determine the peptidomimetic transition strategies.

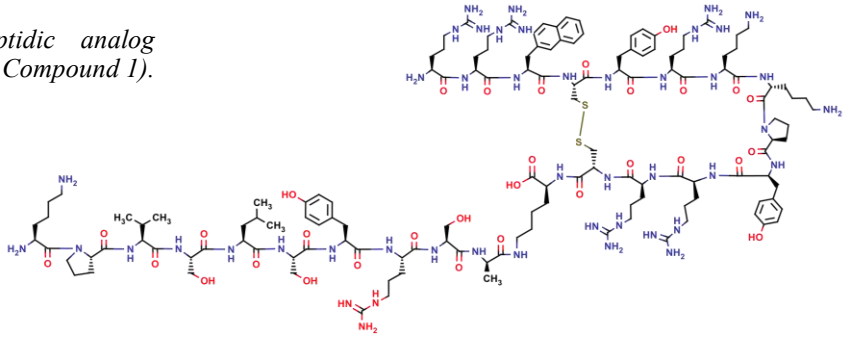

Fig. 1. Peptidic analog structure (i.e. Compound 1).

Results and Discussion

Peptides were synthesized using the Fmoc-based solid-phase strategy; as a first step the synthesis of the (Lys14 [ε-DDE]) T140 was completed and SDF-1 *N*-terminus grafted in position 14. Peptides were cleaved, purified, analyzed by HPLC indicating a purity greater than 97%, and molecular weights were confirmed by LC/MS. Peptide affinities were determined on CXCR4 binding assays (data not shown). Chemotactic activities were evaluated on Transwell migration assays on pre-B lymphocytes (REH cells). Signaling pathway Gi/o was evaluated with EPAC BRET assay on cAMP inhibition (Cotransfected HEK293 with HA-CXCR4 and EPAC Biosensor).

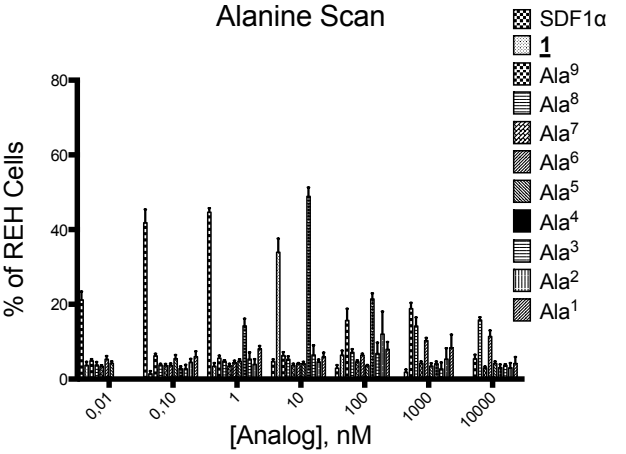

Fig. 2. Migration profiles of the Alanine Scan modified peptides.

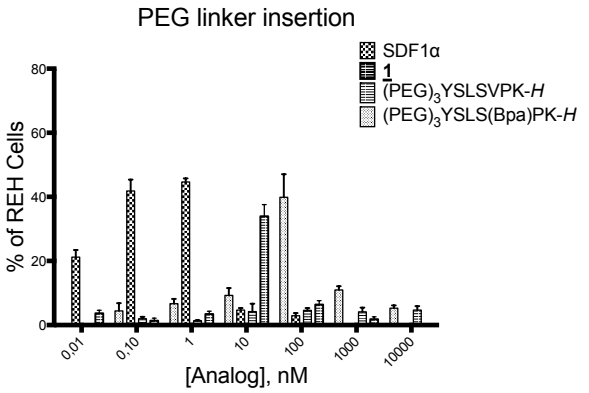

Fig. 3. Migration curves of PEG linker modified analogs.

The relative importance of each *N*-terminal SDF-1 residues was evaluated by an Alanine scan and chemotactic activity screened by Transwell assays. Position 1, 2, 3 and 7 seems to be critical for the conservation of the chemotactic activity (Figure 2): all these alanine containing compounds were in fact competitive antagonists (data not shown). Position 1 and 2 are reputed to be essential for biological activity [3] therefore position 3 was studied. Screen in position 3 shown that hydrophobic amino acids with the ability to make π-stacking in position 3 like Trp, 2-Nal and Bpa (*p*-Benzoyl-phenylalanine) gave potent chemotactic chimeras with a maximum of migration at 10nM and efficacy comparable to SDF-1 (Max. of migration at 1nM: 53%). Tert-Leucine replacement gave partial chemotactic agonist.

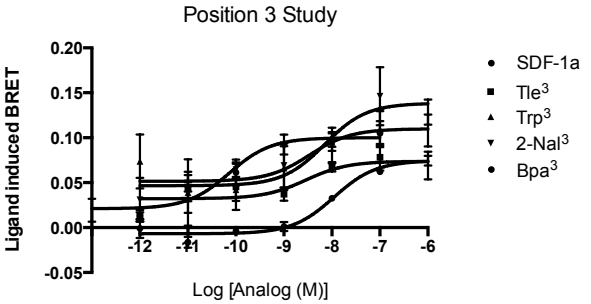

Fig. 4. AMPc inhibition concentration-response curves of position 3 modified peptides.

In order to progressively transform into peptidomimetic sequence in position 8 to 10 of SDF-1, *N*-terminus was replaced by a PEG linker. PEG insertion combined with hydrophobic modification in position 3 (i.e. Bpa) gave potent chemotactic agonist (Figure 3).

CXCR4 signals through Gi/o - therefore we evaluated the profile of our ligands using EPAC BRET assays [4]. All full chemotactic agonists were full agonists on the Gi/o pathway whereas partial agonists behaved like partial agonists (Figure 4).

Through this study, we have highlighted several important points for the conservation of the agonistic nature of our chimeras. The Alanine scan emphasizes the importance of residues like Lys^1, Pro^2, Val^3 and Tyr^7 and thus restricts the transition to peptidomimetic structures.

The position 3 of our chimeras seems to tolerate hydrophobic structural changes with the ability to make π-stacking. Hydrophobic amino acid in position 3 seems to positively modulate the agonistic behavior. Excessive flexibility appears to place the side chain into a less favorable conformation than the one required to induce the activation of the CXCR4 receptor. Nevertheless, some structural modifications (i.e. PEG) in position 8 to 10 combined with highly hydrophobic modification (i.e. BenzoylPhenylAlanine) in position 3 give potent CXCR4 agonist. Chemotaxis seems to correlate with the Gi/o pathway.

Acknowledgments

We would like to thank the *Conseil Régional de la Martinique* for studentship to Christine Mona. Christine Mona as a recipient of a APS Travel Award. Supported by Canadian Institutes of Health Research.

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