Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 4, pp. 429-432, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00036-X

PEPTIDE BASED INHIBITORS OF INTERLEUKIN-8: STRUCTURAL SIMPLIFICATION AND ENHANCED POTENCY

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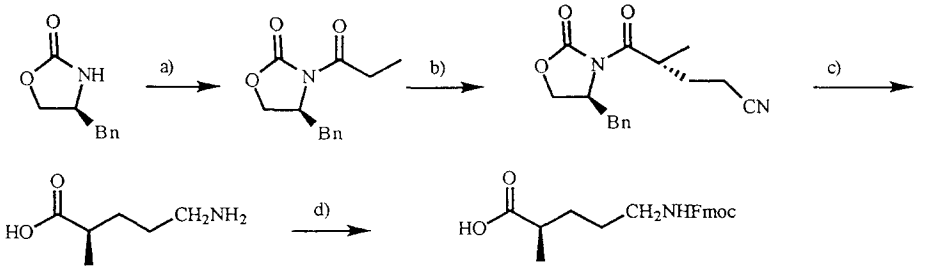
Abstract:
Important areas of a lead peptide inhibitor of IL-8 had been previously identified. Chemical modification led to the identification of key amide bonds which allowed the replacement of the central section of the peptide with modified amino acids and spacers. This approach led to inhibitors of lower molecular weight and of increased potency.

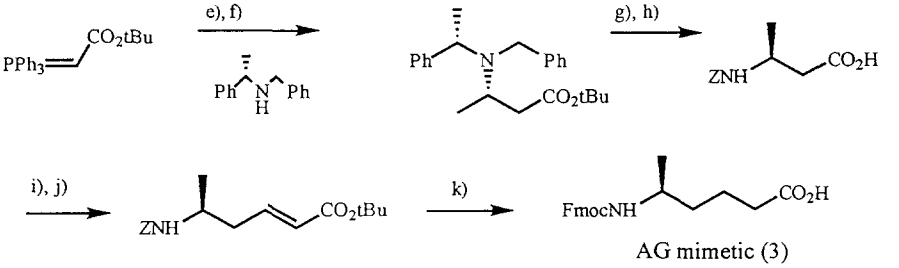
Interleukin-8 (IL-8) is a pro-inflammatory polypeptide chemokine produced by a number of cell types (e.g., T-lymphocytes, monocytes, endothelial cells, epithelial cells and neutrophils) in response to a variety of stimuli e.g., LPS, IL-1 and TNF.[1] Raised levels of IL-8 have been detected in several disease states[2] and experiments using monoclonal antibodies have indicated that inhibition of the action of this chemokine in some models of disease can lead to beneficial effects.[3] Previously,[4] we had identified a 21 aminoacid peptide **(1)**, K_i 13μM[5] as a lead for the inhibition of the interaction of IL-8 with its receptor.

In our previously reported studies[4] on the lead peptide **(1)** we identified several residues as particularly important for binding, together with a region not involved in a direct interaction with IL-8 (a putative spacer or template region) and showed a significant contribution to binding from the N-terminus of the peptide. In this communication we report our initial investigations to increase the potency of the lead peptide and to simplify its structure.

Although we had identified a central region of the peptide where sidechain interactions were unimportant, we had not been able to draw the same conclusions about potential backbone interactions. To gather precise information about these interactions required the replacement of the particular amide bonds of interest with appropriate linkers. Initial work focused on the central Thr-Gly-Met (TGM- henceforward one letter code will be used) region of the peptide, where it was already known from the Ala-scan that the sidechains could be replaced by methyl groups with no loss of activity. We first synthesised dipeptide mimetics **(2,3)** in which the amide bond was replaced by two methylenes and in which the sidechains were replaced by methyl groups (scheme 1).[6,7]



GA mimetic (2)



AG mimetic (3)

Reagents and Conditions

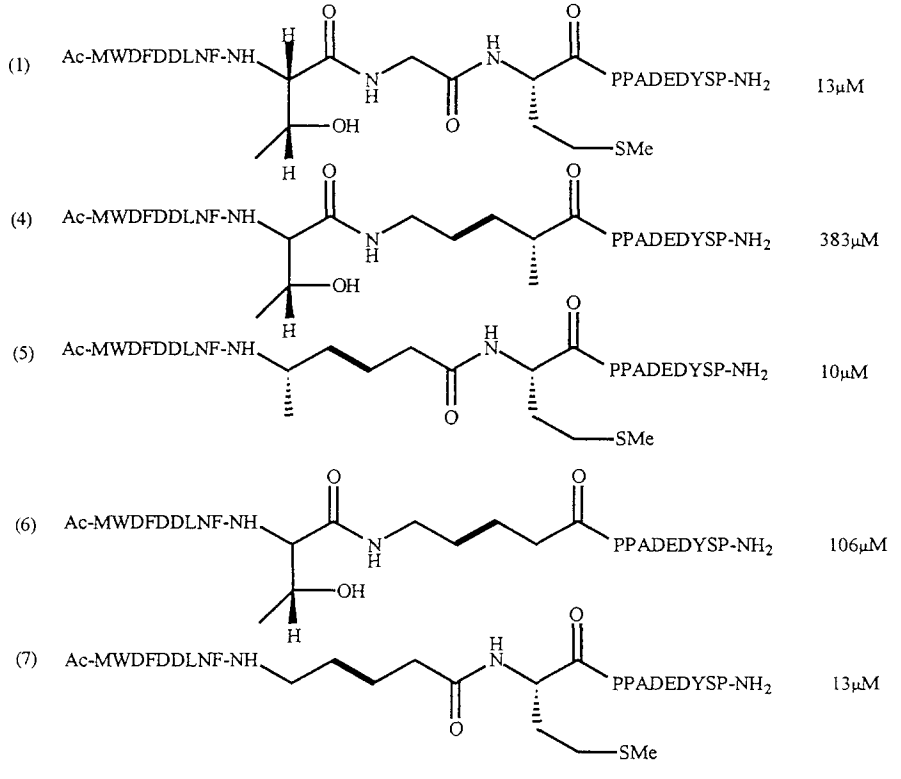
a) BuLi, EtCOCl, THF, -78°C-RT, 93%; b) acrylonitrile, $TiCl_3(OiPr)$, DIEA, 0°C, 94%;[6] c) aq LiOH, RT then H_2, PtO_2, aq MeOH, 92%; d) TMS-Cl, reflux then Fmoc-Cl, DIEA, 0°C, 38%;[8] e) MeCHO, Et_2O, 0°C;[7] f) BuLi, THF, -78-0°C, 68% over two steps; g) $Pd(OH)_2/C$, H_2, EtOH, 5atms, 50°C then TFA, CH_2Cl_2, RT, 77%; h) ZCl, dioxan, $NaHCO_3$, H_2O, RT, 98%; i) MeNH(OMe).HCl, EDCI, DMAP, Et_3N, CH_2Cl_2, RT, 63%;[9] j) 1M $LiAlH_4$, Et_2O, THF, 0°C-20°C; then (*t*-butoxycarbonylmethylene)triphenylphosphorane, 75%; k) H_2, Pd/C, EtOAc, then TFA, CH_2Cl_2, RT then, FmocOSu, Na_2CO_3, H_2O), RT, 79%.

Scheme 1

These mimetics were then incorporated into full length peptides **(4, 5)**.[10] A comparison of the biological activities of these two peptides, (Table 1 - dimethylene unit replacement for amide shown in bold), clearly shows the importance of the GM amide bond for good activity in strong contrast to the lack of importance of the TG amide bond. We then compared these results with those obtained for 5-aminovaleric acid spacers **(6, 7)** where the methyl side chain is absent. The similar potencies of both pairs of compounds implies that the entire side chains are unimportant for bioactivity, and that there is no entropic penalty in removing either methyl group.

Having demonstrated the lack of requirement for the TG amide bond, further replacement of the tetramethylene spacer by a more rigid spacer might be expected to increase potency on entropic grounds, provided that the rigid spacer allowed the required binding conformation to be attained. Gratifyingly the peptide incorporating the *p*-(aminomethyl)benzoic acid spacer **(10)** led to a 6 fold increase in potency.

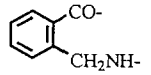
The lack of sensitivity to the positional isomerism of the (aminomethyl)benzoic acid moiety (activities of **(8)** and **(9)**) indicates that the bioactive conformation of the peptide is not extended in this area. This suggested that it might be possible to simplify the peptide still further by removing residues from this central section. Replacing the entire LNFTG unit with a spacer of variable length led to the discovery that the whole pentapeptide could be replaced by a simple methylene group with only 2.5-fold loss of activity **(11)**, whilst spacers of length varying from C3-C11 gave approximately equal potency as the lead **(12-16)**.

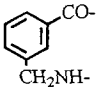


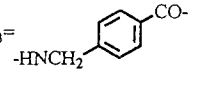
(8) Ac-MWDFDDLNFX_1MPPADEDYSP-NH_2 9.3μM

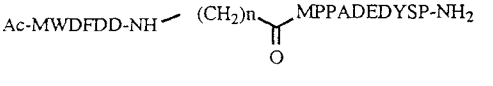
(9) Ac-MWDFDDLNFX_2MPPADEDYSP-NH_2 4.0μM

(10) Ac-MWDFDDLNFX_3MPPADEDYSP-NH_2 2.3μM

X_1= 

X_2= 

X_3= 



(11)	n=1	31 μM
(12)	3	14 μM
(13)	5	7 μM
(14)	7	12 μM
(15)	10	6 μM
(16)	11	4 μM

Table 1

In conclusion, we have confirmed our hypothesis that the central section of the peptide is merely acting as a spacer, and we have been able to reduce the molecular weight of the peptide significantly, replacing 5 amino acids by a simple spacer with no loss of activity. Furthermore we have been able to increase the potency of the peptide by the use of conformationally restrained spacers, leading to **(10)**, at 2.3 μM, the most potent inhibitor of IL-8 yet reported.

Acknowledgements: We thank Dr Steve Kirtland, Irene Cowan, Amanda Fallowfield and Gillian Bottomley for assay work. We are grateful to Dr Glyn Williams for useful discussions.

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10. Peptides were synthesised using a Milligen 9050 peptide synthesiser under continuous flow conditions employing Fmoc chemistry on PepsynK resin with AM linker. They were synthesised as N-terminal acetyl and C-terminal amides to prevent unwanted charged interactions at the termini. Resin cleavage conditions were TFA:phenol:EDT:anisole (95:2.5:2.5:2.5) 2h, RT. The resin was filtered and the solvent removed. The peptide was precipitated in ether, filtered and dried. Purification was by RP-HPLC on an Aquapore octyl column (20micron, 100mmx10mm).The elution gradient comprised 95%A-95%B over 15min where A=0.1%TFA in water and B=0.085%TFA in 70%acetonitrile. The peptides were checked for purity by analytical RP-HPLC and structures were validated by mass spectrometry, 1H NMR and amino acid analysis.

(*Received in Belgium* 9 *November* 1996; *accepted* 9 *January* 1997)