Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 6, pp. 711-714, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00090-5

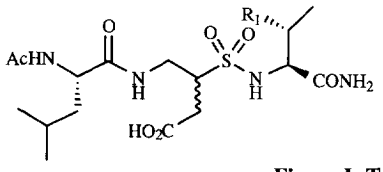
SULFONOPEPTIDE INHIBITORS OF LEUKOCYTE ADHESION

Kenneth G. Carson,* Charles F. Schwender, Hitesh N. Shroff,
Nancy A. Cochran, Debra L. Gallant, and Michael J. Briskin

LeukoSite, Inc., 215 First Street, Cambridge, MA 02142

Abstract: The amino acid sequence L-D-T-S-L has been shown to be a significant recognition motif involved in the interaction of integrin $\alpha_4\beta_7$ with its endothelial ligand, MAdCAM-1. Based on the known active peptide, a series of sulfonamide peptidomimetics was synthesized. Sulfonopeptide **2**, Ac-L-ΨD-V-NH_2, was shown to inhibit leukocyte adhesion. © 1997 Elsevier Science Ltd. All rights reserved.

Inflammation is characterized by infiltration of the affected tissue by leukocytes, such as lymphocytes, lymphoblasts, and mononuclear phagocytes. The remarkable selectivity by which leukocytes preferentially migrate to various tissues during both normal circulation and inflammation results from a series of adhesive and activating events involving multiple receptor-ligand interactions.[1,2] The process begins with a transient, rolling interaction mediated by the selectin receptor.[3] Next, activation is mediated by chemokine receptors, which trigger the firm adhesion of leukocytes to endothelium via leukocyte integrins and their endothelial ligands. Subsequent lymphocyte transendothelial migration from the circulation across the vascular endothelium is mediated by chemokine concentration gradients to the site of the inflammation.



1 Ac-L-ΨD-T-NH_2 $R_1 = OH$
2 Ac-L-ΨD-V-NH_2 $R_1 = CH_3$

Figure I: Targets

MAdCAM-1 (Mucosal Addressin Cell Adhesion Molecule-1), an immunoglobulin superfamily adhesion molecule for lymphocytes, is preferentially expressed in the gastrointestinal tract, specifically binds the lymphocyte $\alpha_4\beta_7$ integrin, and participates in the homing of these cells to these mucosal sites.[4,5] In a nonhuman primate model of inflammatory bowel disease (IBD), a blocking antibody to $\alpha_4\beta_7$ has demonstrated both a clinical and histologic improvement in inflammatory activity and disease.[6] Peptide epitope mapping and point mutation studies on MAdCAM-1 have shown that murine MAdCAM-1 requires the L-D-T-S-L peptide motif for binding to the $\alpha_4\beta_7$ integrin,[7,8] and that this essential motif is conserved in human MAdCAM-1 as well.[9]

We wish to report the synthesis of a new series of peptidomimetics based on the tripeptide lead Ac-L-D-T-NH_2. Following the approach of Liskamp,[10-13] we chose to replace the central aspartic acid residue with its corresponding β-aminosulfonamide analog. In replacing the central peptide residue, we extended the method of Liskamp to introduce a charged amino acid residue. In addition, a protecting group strategy was developed to allow the use of any amino acid at the C-terminus. The target compounds are shown in Figure I.

Synthesis

The synthetic scheme for compounds **1** and **2** is shown in Scheme I. Sulfonyl chloride **3**[14] was synthesized by a two-step route, without isolation of the intermediate. Taurine was first protected in aqueous tetrabutylammonium hydroxide, and the resulting Cbz-tau tetraalkylammonium salt was then extracted into CH_2Cl_2 and converted to the corresponding sulfonyl chloride using the method of Huang.[15] Sulfonyl chloride **3** was isolated in good yield (80% over two steps). Condensation of **3** with appropriately protected threonine or valine amides led to sulfonamides **4** and **5** in moderate yields. Compounds **4** and **5** were protected as methoxymethyl (MOM) ethers **6** and **7.** Liskamp has shown that protection of the sulfonamide nitrogen is necessary for α-alkylation to occur.[12] Deprotonation with excess (5-7 equiv) lithium diisopropylamide followed by alkylation with *t*-butyl bromoacetate led to protected peptides **8** and **9** in moderate yields. Alkylation proceeded without stereoinduction, resulting in a 1:1 mixture of diastereomers, as determined by ^1H NMR. No epimerization of the valine or threonine α-carbon was detected. The diastereomers were not separable by chromatography, and so were carried on to product as mixtures. Hydrogenolysis of the Cbz-group led to peptide amines **10** and **11.** Coupling with *N*-acetylleucine under standard peptide coupling conditions led to protected peptides **12** and **13.** Deprotection of **12** in TFA/CH_2Cl_2 led to incomplete removal of the MOM group, resulting in an oxazoline-containing product having a structure consistent with **14.** The use of TFA/CH_2Cl_2 with ethanedithiol as a formaldehyde trap allowed complete deprotection to sulfonopeptide **1.** Deprotection of **13** proceeded in satisfactory yield without scavenger to sulfonopeptide **2.**

Peptides were synthesized utilizing solid-phase peptide synthesis methodology,[8,16,17] with Fmoc/*t*-Bu chemistry on Rink Amide Am resin (50 mg, 0.5 mmol/g). Peptides synthesized were analyzed for purity by reverse phase HPLC and mass spectral analysis.

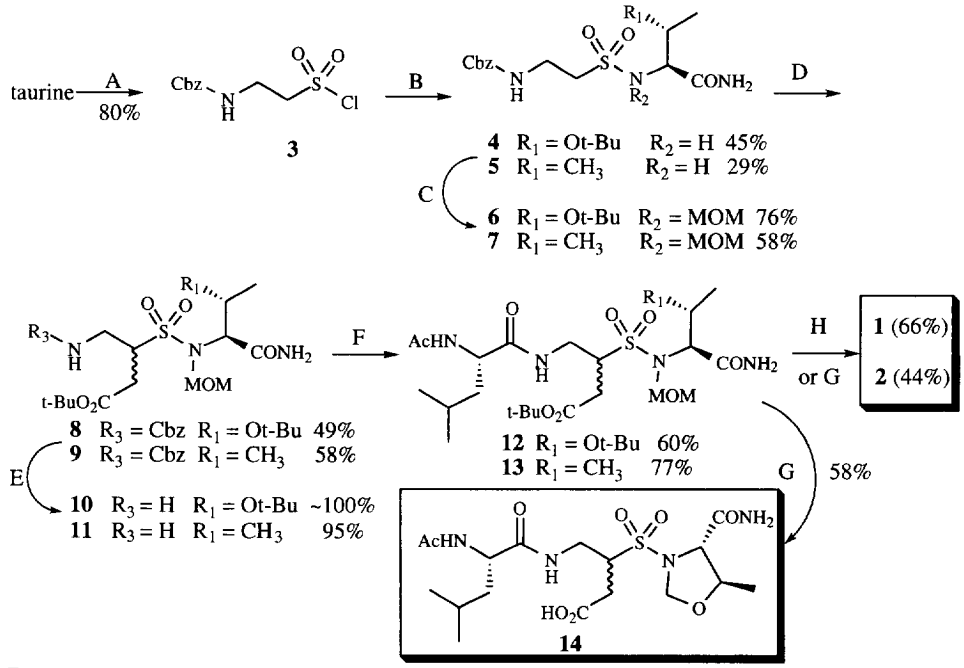
Cell Adhesion Assay

A cell adhesion assay involving the B-cell lymphoma, RPMI 8866 cells, and a soluble murine MAdCAM-1, produced in a baculovirus expression system, was used in a 96-well format.[7,8,18,19] RPMI 8866 cells have previously been shown to avidly bind murine MACAM-1.

RPMI 8866 cells were fluorescently labeled by preincubation with BCECF stain (Molecular Probes)

washed, and resuspended in assay buffer containing 2% fetal calf serum and 2 mM Mg^{+2}. Antibodies or compounds were tested in HBSS/2% FCS/25 mM HEPES buffer at 2.5×10^6 cells per mL. The typical assay consisted of a final volume of 200 µL containing 50 µL of cells at 1.25×10^5 cells per well. Adhesion assays for MAdCAM-1 were washed on an automatic plate washer using a wash buffer consisting of 50 mM Tris/150 mM NaCl/2 mM $MnCl_2$, pH 7.2, in a wash volume of 500 µL for 2 wash cycles. Assays were then read on an Idexx fluorescent plate reader at 485/535 ηm. Inhibition was determined by the number of cells adhering to the plates in the presence and absence of an inhibitor and IC_{50} values (the concentration of inhibitor required to prevent 50% of cells from adhering to MAdCAM-1 plates) were determined using KaleidaGraph (Adelbeck Software) and are reported as an average of multiple determinations.

SCHEME I



Reagents: (A) i. aq. *n*-Bu_4NOH, Cbz-Cl ii. $SOCl_2$ CH_2Cl_2 (B) valinamide or threoninamide-OtBu, Et_3N, CH_2Cl_2 (C) NaH, methyl chloromethyl ether, THF (D) LDA, *t*-butyl bromoacetate, THF, -78°C (E) H_2, 5% Pd/C (F) *N*-Acetylleucine, HBTU, DIEA, CH_2Cl_2 (G) TFA, CH_2Cl_2 (H) TFA, CH_2Cl_2, 1,2-ethanedithiol

Discussion

Table I shows the cell adhesion IC_{50} values for compounds **1** and **2** and the corresponding tripeptides **15** and **16**. Ac-L-ψD-T-NH_2 sulfonamide **1** did not inhibit the cell adhesion to MAdCAM, while the corresponding peptide **15** had a 276 µM IC_{50}. On the other hand, sulfonopeptide **2** was only slightly worse

than its peptide analog, compound **16**. Although the inhibition is weak in both cases, this does suggest that the sulfonopeptide can mimic the conformation of the natural peptide. It also should be noted that compound **2** was tested as a 1:1 diastereomeric mixture. To test for specificity, the compounds were tested in a fibronectin/K562 binding assay using the method of Pytela.[20] None of the compounds significantly inhibited the binding of K562 cells to fibronectin.

Table I. Inhibition of MAdCAM/$\alpha_4\beta_7$ Mediated Cell Adhesion

Compound	Structure	MAdCAM IC_{50} (μM)	
1	Ac-L-ψD-T-NH_2	>500	(n = 3)
2	Ac-L-ψD-V-NH_2	219	(n = 2)
15	Ac-L-D-T-NH_2	276	(n = 1)
16	Ac-L-D-V-NH_2	126	(n = 4)
17	Ac-L-D-T-S-L-NH_2	298	(n = 20)

MAdCAM IC_{50} is the μM concentration of inhibitor required to prevent 50% of cells from adhering to MAdCAM-1, where n represents the number of determinations of each value. ND = not determined.

References

1. Butcher, E. C. *Cell* **1991**, *67*, 1033.
2. Springer, T. A. *Cell* **1994**, *76*, 301.
3. Lawrence, M. B.; Springer, T. A. *Cell* **1991**, *65*, 859.
4. Briskin, M. J.; McEvoy, L. M.; Butcher, E. C. *Nature (London)* **1993**, *363*, 461.
5. Hamann, A.; Andrew, D. P.; Jablonski-Westrich, D.; Holzmann, B.; Butcher, E. C. *J. Immunol.* **1994**, *152*, 3282.
6. Hesterberg, P. E.; Winsor-Hines, D.; Briskin, M. J.; Soler-Ferran, D.; Merrill, C.; Mackay, C. R.; Newman, W.; Ringler, D. J. *Gastroenterol.* **1996**, *111*, 1373.
7. Briskin, M. J.; Rott, L.; Butcher, E. C. *J. Immunol.* **1996**, *156*, 719.
8. Shroff, H. N.; Schwender, C. F.; Dottavio, D.; Yang, L.; Briskin, M. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2495.
9. Shyjan, A.M.; Bertagnolli, M.; Kenney, C.J.; Briskin, M. J. *J. Immunol.* **1996**, *156*, 2851.
10. Moree, W. J.; van der Marel, G. A.; Liskamp, R. M. J. *Tetrahedron Lett.* **1992**, *33*, 6389.
11. Moree, W. J.; van Gent, L. C.; van der Marel, G. A.; Liskamp, R. M. J. *Tetrahedron* **1993**, *49*, 1133.
12. Moree, W. J.; van der Marel, G. A.; Liskamp, R. J. *J. Org. Chem.* **1995**, *60*, 5157.
13. Moree, W. J.; Schouten, A.; Kroon, J.; Liskamp, R. M. J. *Int. J. Peptide Protein Res.* **1995**, *45*, 501.
14. Bricas, E.; Kieffer, F.; Fromageot, C. *Biochim. Biophys. Acta* **1955**, *18*, 358.
15. Huang, J.; Widlanski, T.S. *Tetrahedron Lett.* **1992**, *33*, 2657.
16. Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *37*, 3404.
17. Gauspohl, H.; Boulin, C.; Kraft, M.; Frank, R. W. *Synthesis* **1992**, *5*, 315.
18. Earle, D. J.; Briskin, M. B.; Butcher, E. C.; Garcia-Pardo, A.; Lazarovits, A. I.; Tidswell, M. *J. Immunol.* **1994**, *153*, 517.
19. *Baculovirus Expression Vectors: A Laboratory Manual;* O'Reilly, D. R.; Miller, L. K.; Luckow, V. A., Eds. W. H. Freeman: New York, **1992**.
20. Pytela, R.; Pierschbacher, M. D.; Ruoslahti, E. *Cell* **1985a**, *40*, 191.

(*Received in USA* 6 *January* 1997; *accepted* 10 *February* 1997)