Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 725–727

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

N-Acyl Phenylalanine Analogues as Potent Small Molecule VLA-4 Antagonists

Li Chen, Jefferson W. Tilley,* Tai-Nan Huang, Dorota Miklowski, Richard Trilles, Robert W. Guthrie, Kin Luk, Angela Hanglow, Karen Rowan, Virginia Schwinge and Barry Wolitzky

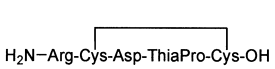
Roche Research Center, Hoffmann-La Roche, Inc., 340 Kingsland Street, Nutley, New Jersey 07110, USA

Received 4 October 1999; accepted 1 February 2000

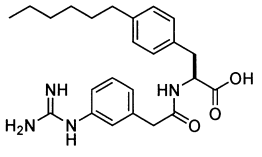
Abstract—We have identified a series of low molecular weight ($M_r < 500$) *N*-acylphenylalanines that are effective inhibitors of the VCAM-VLA-4 interaction. Investigation of the SAR of the *N*-acyl moiety led to the identification of *N*-benzylpyroglutamyl derivatives as being particularly potent.

Vascular cell adhesion molecule-1 (VCAM-1) is expressed on activated, but not resting, endothelium. The principal receptor for VCAM-1, the integrin very late antigen 4 (VLA-4, $\alpha_4\beta_1$), is expressed on circulating eosinophils, basophils, and monocytes, but not neutrophils. Antibodies to either protein are effective at inhibiting leukocyte infiltration and preventing tissue damage in several animal models of inflammation.[1,2] Peptide mimetics derived from the connecting segment 1 (CS1) sequence of fibronectin (Fn) have also been shown to block VCAM/VLA-4 interactions and to block allergen induced airway responses in a sheep model of asthma.[3,4] Thus, we are interested in discovering orally active VCAM/VLA-4 antagonists that might be useful for the treatment of asthma. In the current paper, we report our initial observations on the discovery of *N*-acyl-L-phenylalanine derivatives that effectively inhibit the VLA-4/VCAM interaction.

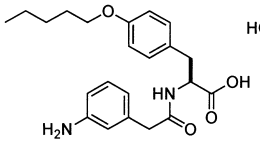
An earlier report indicated that the cyclic peptide **I** inhibits VLA-4 dependent cell adhesion to the alternatively spliced CS-1 region of Fn at low micromolar concentrations.[5] In the process of seeking related, non-peptide VLA-4 antagonists, we searched the Roche corporate database for compounds possessing a guanidine and a carboxylic acid separated by a spacer. Among the leads identified was the *N*-Acyl phenylalanine **II**, previously prepared as part of an IL-2 receptor antagonist program, which had an IC_{50} of 7 μM in the VLA-4/VCAM-1 assay. Initial SAR developed using analogues available from the IL-2 program indicated that compounds **III** and **IV**, lacking a guanidino moiety, inhibited VCAM/VLA-4 binding with enhanced potency,



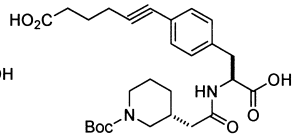
I



II, IC_{50} 7,000 nM



III, IC_{50} 4,500 nM



IV, IC_{50} 640 nM

*Corresponding author. Tel.: +1-973-235-4660; fax: +1-973-235-7122; e-mail: jefferson.tilley@roche.com

0960-894X/00/$ - see front matter
PII: S0960-894X(00)00088-3

suggesting that lead optimization should begin with investigation of the *N*-acyl moiety.

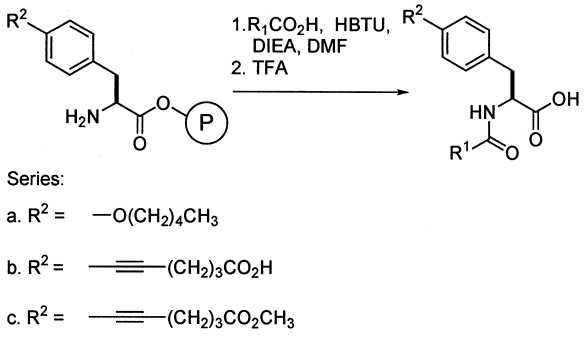
Thus, we chose to incorporate building blocks selected from a set of readily available low molecular weight carboxylic acids as shown in Scheme 1. The *N*-Fmoc-4-substituted phenylalanines, selected from intermediates available from our IL-2 program, were coupled to Wang resin under standard conditions. After removal of the protecting group, the carboxylic acids were coupled to the free amino group of the phenylalanine derivatives under standard conditions, followed by acid cleavage to provide 68 analogues of *N*-acylphenylalanine (**1**–**68**).

Compounds were assayed for VLA-4 antagonist activity using a solid-phase, dual antibody ELISA in which VLA-4 derived from Ramos cells was allowed to compete for bound recombinant human VCAM in the presence of 10 and 50 μM of test compound. The binding of VLA-4 to VCAM-1 was detected by a complex of anti-β1 antibody and HRP-conjugated anti-mouse IgG chromogenic substrate (K-Blue).[6] The % inhibition of library molecules at 10 μM is summarized in Chart 1. Compounds which had an average % inhibition of greater than 80% at 10 μM and $M_r < 500$ were selected for resynthesis and IC_{50} determination.

Results shown in Table 1 indicate that both *N*-aroyl (**4** and **49**) and *N*-alkanoyl (**33**, **47** and **60**) analogues of phenylalanine showed mid-nanomolar inhibition and were more potent than the lead compounds **II**–**IV**. Additional examples of each of these *N*-acyl derivatives, compounds **69**–**73** were then added to the library.

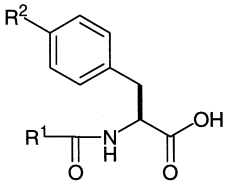
The most potent compound from the initial library was **33**, which was derived from *N*-benzylpyroglutamic acid, with an IC_{50} of 46 nM. Combination of this acyl group with the 4-hexynoate substituent gave **70** with an IC_{50} of 20 nM.

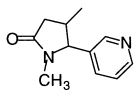
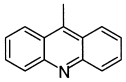
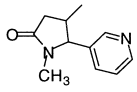
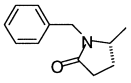
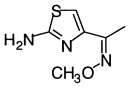
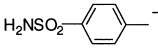
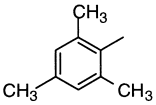
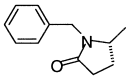
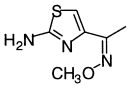
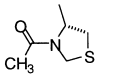
Incorporation of an *N*-acetylthiaprolinyl group into phenylalanine gave compound **73** with an IC_{50} of 112 nM. We speculate that this compound mimics the **Cys∗-XXX-ThiaPro-Cys∗-COOH** portion of the cyclic peptide **I** and interacts with VLA-4 through a motif mimicking the EI**LDV**P VLA-4 recognition sequence in the CS-1 alternatively spliced region of Fn and the Q**IDS**P sequence[7] in VCAM-1. Indeed, compound **73** also inhibited Ramos (VLA-4 expressing) cell adhesion to CS-1 peptide coated plates with an IC_{50} of 4,200 nM, which is comparable to the value reported for **I**.[5] We have subsequently gathered considerable data in support of this supposition that we will detail in future papers in this series.



Scheme 1.

Table 1. Inhibition activity of VLA-4 antagonists



Compound	R_1	R_2	VCAM/VLA-4 ELISA	
			% Inb (10 μM)	IC_{50} (μM)
3		—O(CH2)4CH3	81	1.7
4		—O(CH2)4CH3	92	0.59
8		—O(CH2)4CH3	87	3.8
22		—O(CH2)4CH3	90	0.87
33		—O(CH2)4CH3	97	0.046
47		—≡—(CH2)3CO2CH3	99	0.135
49		—≡—(CH2)3CO2CH3	94	0.317
60		—≡—(CH2)3CO2H	80	0.187
69		—O(CH2)4CH3		0.245
70		—≡—(CH2)3CO2H		0.020
71		—O(CH2)4CH3		0.060
72		—O(CH2)4CH3		0.210
73		—O(CH2)4CH3		0.112

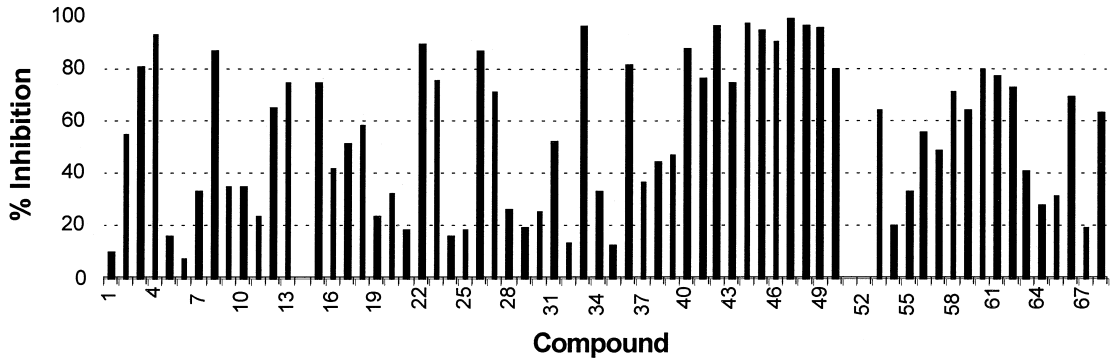


Chart 1. % Inhibition of VCAM/VLA-4 binding at 10 μM.

In summary, we have identified a class of low double digit nanomolar small molecule VLA-4 antagonists that contain a core structure of a *N*-acyl phenylalanine and have determined that from a series of 71 *N*-acyl groups, *N*-benzylpyroglutamyl- gives the highest potency. Thus, we have chosen this substituent for our further lead optimization studies in which we examine the role of substituents in the 3- and 4-postions of the benzene ring of phenylalanine. The outcome of these studies will also be reported in the near future.

References

1. Elices, M. In *Cell Adhesion Molecules and Matrix Proteins: Role in Health and Diseases*; Mousa, S. A., Ed.; Springer-Verlag: Berlin 1998; pp 133–147.
2. Elices, M. *Curr. Opin. Antiinflammat. and Immunomodulat. Investigat. Drugs* **1999**, *1*, 14.
3. Lin, K.-C.; Ateeq, H. S.; Hsiung, S. H.; Chong, L. T.; Zimmerman, C. N.; Castro, A.; Lee, W.-C.; Hammond, C. E.; Kalkunte, S.; Chen, L.-L.; Pepinsky, R. B.; Leone, D. R.; Sprague, A. G.; Abraham, W. M.; Gill, A.; Lobb, R. A.; Adams, S. P. *J. Med. Chem.* **1999**, *42*, 920.
4. Lin, K.-C.; Castro, A. C. *Curr. Opin. Chem. Biol.* **1998**, *2*, 453.
5. Nowlin, D. M.; Goresan, F.; Moscinski, M.; Chiang, S.-L.; Lobl, T.; Cardarelli, P. M. *J. Biol. Chem.* **1993**, *268*, 20352.
6. Chen, L.; Guthrie, R. W.; Huang, T.-N.; Hull, K. G.; Sidduri, A.; Tilley, J. W. WO 9910312, 1999.
7. Jones, E. Y.; Harlos, K.; Bottomley, M. J.; Robinson, R. C.; Driscoll, P. C.; Edwards, R. M.; Clements, J. M.; Dudgeon, T. J.; Stuart, D. I. *Nature* **1995**, *373*, 539.