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N-Benzoyl Amino Acids as LFA-1/ICAM Inhibitors 1: Amino Acid Structure–Activity Relationship

Daniel J. Burdick,[a,*] Ken Paris,[a] Kenneth Weese,[a] Mark Stanley,[a] Maureen Beresini,[b] Kevin Clark,[b] Robert S. McDowell,[a,†] James C. Marsters, Jr.[a] and Thomas R. Gadek[a]

[a]*Department of Bioorganic Chemistry, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080, USA*
[b]*Bioanalytical Research and Development, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080, USA*

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Abstract—The association of ICAM-1 with LFA-1 plays a critical role in several autoimmune diseases. *N*-2-Bromobenzoyl L-tryptophan, compound **1**, was identified as an inhibitor to the formation of the LFA-1/ICAM complex. The SAR of the amino acid indicates that the carboxylic acid is required for inhibition and that L-histidine is the most favored amino acid.

Leukocyte function-associated antigen-1 (LFA-1, αLβ2, CD11a/CD18) is a member of the β2 integrin family.[1,2] Expressed exclusively on leukocytes, LFA-1 interacts with intracellular adhesion molecules 1, 2, and 3 (ICAM's -1, -2, -3) in a variety of cell adhesion events required for normal and pathologic functions of the immune system.[3–5] The interaction of LFA-1 with ICAM-1 has been implicated in numerous autoimmune diseases such as psoriasis, asthma, rheumatoid arthritis and graft rejection and it is clear that an antagonist to this interaction would play a critical therapeutic role in the treatment of these maladies.[6–8] Several monoclonal antibodies and small-molecule antagonists have been reported to address this medical need.[9–11]

We have recently reported our efforts to generate potent LFA-1/ICAM antagonists.[12,13] Mutagenesis of the first domain of ICAM-1 identified six residues, in particular glutamic acid 34 and tyrosine 66, that are essential for the interaction with LFA-1[14] and we have incorporated these critical amino acids in cyclic peptide antagonists. The screening hit *N*-2-bromobenzoyl L-tryptophan (**1**) (Fig. 1) was of interest not only because it was reported to competitively inhibit the binding of ICAM-1 to LFA-1 with an IC_{50} of 1.7 μM, but because it contained elements of the peptide structure–activity relationship (SAR).[12] This similarity to the protein and our cyclic peptides led us to evaluate the SAR of compound **1**.

*Corresponding author. Tel.: +1-650-225-1368; fax: +1-650-225-2061; e-mail: burdick.dan@gene.com
†Current address: Sunesis Pharmaceuticals, 341 Oyster Point Boulevard, South San Francisco, CA 94080, USA.

This communication will focus on the SAR of the amino acid moiety concentrating on the carboxylic acid and the amino acid side chain. A subsequent communication will address the SAR of the 2-bromobenzoyl moiety.

Two simple series of molecules were envisioned (Fig. 2) to evaluate the importance of the carboxy terminus. Series 1 (**2**–**5**) was designed to determine if the carboxylic acid could be replaced or modified. Series 2

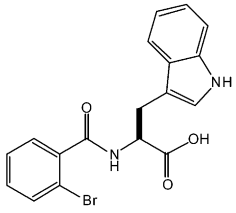

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Figure 1.

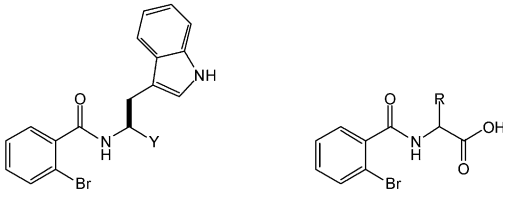

Figure 2.

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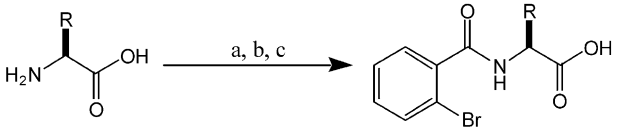
(**6**–**42**) was proposed to determine the effect of replacing the L-tryptophan with various tryptophan analogues, unnatural aromatic amino acids and naturally occurring L- and D-amino acids (R).

Compounds **3**–**13**, **35**–**39**, and **42** were synthesized by the route shown in Scheme 1 except where noted in the tables. Fischer esterification of the appropriate commercially available amino acid was done in methanol followed by an extractive workup. The amine was acylated with *o*-bromobenzoic acid using EDC, HOBT, and DIPEA in DMF. After purification by silica chromatography using 5% methanol in DCM as the eluent, the ester was removed with LiOH in THF/water. The crude product was purified by reverse phase HPLC, lyophilized and its molecular weight verified by electro spray mass spectroscopy.

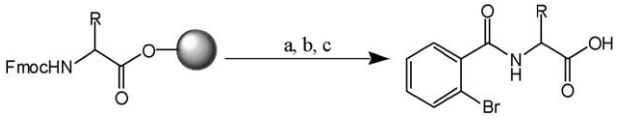
Compounds **1**, **2**, **14**–**34**, and **40**–**41** were synthesized by standard Fmoc solid-phase synthetic techniques (Scheme 2) on the appropriate commercially available Fmoc amino acid 4-hydroxymethylphenoxy resin (Wang resin).[15,16] Following removal of the Fmoc group with 20% piperidine in DMF and rinsing of the resin, *o*-bromobenzoic acid was coupled using HBTU, HOBT, and DIPEA in DMF. After drying the resin to a constant weight, it was treated with a solution of TFA containing 2.5% water and 2.5% anisole. Excess TFA was removed in vacuo and the cleaved molecule was extracted from the resin with a 10% solution of acetic acid in water. The crude product was purified by reverse-phase HPLC, lyophilized and its molecular weight verified by electro spray mass spectroscopy.

All compounds were evaluated in an ICAM-1/LFA-1 enzyme linked immunosorbant assay (ELISA). Potency is calculated as the concentration of inhibitor needed to inhibit 50% of the ICAM-1/LFA-1 complex formation (IC_{50}). Reported IC_{50}s are the arithmetic average of at least two assays.

Series 1 compounds were tested to determine the importance of the carboxylic acid of the inhibitors. It can be clearly seen in the data of Table 1, that the carboxylic acid is essential for inhibition. Converting the carboxylate to the primary amide **2** or to the ester **3** results in a > 580-fold loss in potency. The same effect is seen when the carboxylic acid is completely removed **4**. Even though hydroxamic acids are carboxylic acid surrogates, compound **5** showed only weak inhibition. These results agree with the protein mutagenesis data which showed that glutamic acid 34 was essential for binding.



Scheme 1. Reagents and conditions: (a) HCl (g), MeOH, 0 °C to rt, 18 h; (b) 2-bromobenzoic acid, EDC, HOBT, DIPEA, DMF, 2 h, rt; (c) LiOH, THF, water, 1 h, rt.



Scheme 2. Reagents and conditions: (a) 20% piperidine/DMF, rt; (b) 2-bromobenzoic acid, HBTU, HOBT, DIPEA, DMF, 2 h, rt; (c) triisopropylsilane, water, TFA, 1 h, rt.

Series 2 was proposed to determine the effect of modifying the tryptophan indole (Table 2), to evaluate the influence of the amino acid stereo chemistry and determine the contribution of the amino acid side chain (Table 3).

Table 1. ELISA assay results for compounds **1**–**5** of Series 1 (Fig. 2) demonstrating the effect of modifying the carboxylate

Compd	Y	IC_{50} (μM)	Scheme
1		1.7	2
2		> 1000	2[a]
3		> 1000	1[b]
4	H	> 1000	1[c]
5		140	1[d]

[a]Fmoc Rink resin used instead of Wang resin.
[b]Methyl ester not cleaved.
[c]Tryptamine coupled, then purified.
[d]Hydroxylamine coupled after methyl ester saponification, then purified.

Table 2. ELISA assay results for compounds **1** and **6**–**11** of Series 2 (Fig. 2) demonstrating the effect of substituents on the indole of tryptophan

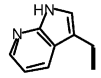
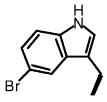
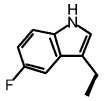
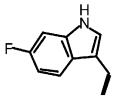
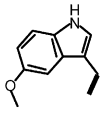
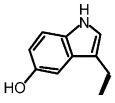
Compd	R	IC_{50} (μM)	Scheme
1	L-Trp	1.7	2
6		63.8	1
7		28.4	1
8		10.8	1
9		7.64	1
10		2.77	1
11		24.15	1

Table 3. ELISA assay results for compounds **1** and **12**–**42** of Series 2 (Fig. 2) demonstrating the effect of different natural and unnatural L- and D-amino acids

Compd	Amino acid	IC_{50} (μM)	Scheme
1	L-Trp	1.72	2
12	D-1-Nal	>200	1
13	D-2-Nal	242.5	1
14	D-Ala	57.45	2
15	D-Phe	280	2
16	D-Ser	72.6	2
17	D-Trp	76.55	2
18	L-Pro	>1000	2
19	L-Ala	6.64	2
20	L-Ile	106	2
21	L-Leu	54.25	2
22	L-Val	11.33	2
23	Gly	313.5	2
24	L-Arg	2.71	2
25	L-Lys	1.21	2
26	L-Asp	>200	2
27	L-Glu	30.1	2
28	L-Asn	1.08	2
29	L-Gln	1.77	2
30	L-Ser	6.15	2
31	L-Thr	3.84	2
32	L-Cys	4.26	2
33	L-Met	7.46	2
34	L-Phe	4.89	2
35	L-Hfe	5.09	1
36	L-1-Nal	15.95	1
37	L-2-Nal	24.4	1
38	L-Bip	50.5	1
39	L-Phe(4-CN)	36.8	1
40	L-Tyr	1.09	2
41	L-Thienylalanine	5.5	1
42	L-His	0.75	1

As shown in Table 2, any substitution on the indole of L-tryptophan (**1**) reduces the potency of the inhibitors. Replacing the carbon at the 7-position with a nitrogen (**6**) results in a 37-fold decrease in potency. Adding a halogen at the 5- (**7**, **8**) or 6- (**9**) position of the indole reduces potency 16.7-, 6.3- and 4.4-fold, respectively. A methoxy group at the 5-position (**10**) is 8.5 times more potent than a hydroxy substitution (**11**), but it is still 2-fold less potent than the unsubstituted case.

Table 3 shows various natural and unnatural amino acid changes. In all cases, it is clear that the D-amino acids tested (**12**–**17**) are less favored than the L-enantiomers (**1**, **19**, **30**, **34**, **36**, and **37**) regardless of the side chain. This preference may be due, in part, to the fact that the L-amino acids direct the carboxylic acid to a preferred point of contact while the D-amino acids do not. Of the L-amino acids, L-proline (**18**) was the poorest inhibitor. Possible explanations include: the pyrrolidine ring does not allow the carboxylic acid to interact with its point of contact; or the lack of amide bond hydrogen leads to lower affinity.

Compared to L-tryptophan (**1**), the alkyl amino acids were not as potent. L-Alanine (**19**) was 4-fold less potent than L-tryptophan, but it was 1.7- to 16-fold more potent than the branched amino acids (**20**–**22**). The basic side chains of L-arginine (**24**) and L-lysine (**25**) were of comparable potency to L-tryptophan. Conversely, the acidic side chains of L-aspartic acid (**26**) and L-glutamic acid (**27**) were not potent. Only when they were changed to their respective amide forms (**28** and **29**) did their potency improve.

The aromatic amino acid side chains were more potent as a group than the alkyl side chains and in some cases more potent than L-tryptophan. L-Phenylalanine (**34**) was 2.8 times less potent than L-tryptophan. Interestingly, lengthening the L-phenylalanine side chain by one methylene (**35**) did not change its potency. Extending the aromaticity with L-1-napthylalanine (**36**) and L-2-napthylalanine (**37**) decreased the potency compared to L-tryptophan 9.3- and 14.2- fold respectively. *para* Phenyl (**38**) and *para* cyano substitutions (**39**) resulted in a 29.4- and 21.4-fold decreases in potency. The *para* hydroxy substitution of L-tyrosine (**40**), however, showed a 1.6-fold increase in potency over L-tryptophan. Replacing the indole of L-tryptophan with thiophene (**41**) reduced the potency by 3.2-fold, but replacement with imidazole (**42**) improved inhibition 2.3-fold.

Based on these and previously reported results, we conclude that within the class of 2-bromobenzoyl amino acid antagonists the carboxylic acid is required for inhibition of the LFA-1/ICAM-1 complex.[12] This result follows the trend that was observed in the ICAM-1 mutagenesis, which showed that the carboxylic acid of the glutamic acid 34 side chain is critical for binding. The inhibitors prefer to have an L-amino acid to a D-amino acid regardless of the amino acid side chain. And finally, even though L-histidine proved to be the best amino acid side chain, L-tryptophan, L-asparagine, L-lysine and L-tyrosine were of comparable potency.

Low molecular weight inhibitors of large protein/protein interactions are one of the aims of the pharmaceutical industry. We have demonstrated that simple changes of amino acid side chains and stereochemistry can play a large role in the ability of these low molecular weight molecules to inhibit the formation of the ICAM-1/LFA-1 complex. A subsequent paper will explore the SAR of the 2-bromobenzoyl moiety.

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References and Notes

1. Hynes, R. O. *Cell* **1987**, *48*, 549.
2. Mazzone, A.; Ricevute, G. *Haematology* **1995**, *80*, 161.
3. Springer, T. A. *Nature* **1990**, *346*, 425.
4. Carlos, T. M.; Harlan, J. M. *Blood* **1994**, *84*, 2068.
5. Gahmberg, C. G.; Tolvanen, M.; Kotovuori, P. *Eur. J. Biochem*. **1997**, *245*, 215.
6. Bevilacqua, M. P.; Nelson, R. M.; Mannori, G. *Ann. Rev. Med*. **1994**, *45*, 361.
7. Cornejo, C. J.; Winn, R. K.; Harlan, J. M. *Adv. Pharmacol*. **1997**, *39*, 99.

8. Henricks, P. A.; Nijkamp, F. P. *Eur. J. Pharmacol.* **1998**, *344*, 1.
9. Liu, G. *Drugs Future* **2001**, *26*, 767.
10. Liu, G. *Expert Opin. Ther. Pat.* **2001**, *11*, 1383.
11. Yun, W.; Desai, B. B.; Du, Y.; Fotouhi, N.; Gillespie, P.; Guthrie, R. W.; Huang, K.; Kolinsky, K.; Li, S. H.; Mennona, F. A.; Perrota, A.; Pietranico-Cole, S. L.; Portland, L.; Vermeulen, J. *Abstracts of Papers*, 224 National Meeting of the American Chemical Society, Boston, MA; American Chemical Society: Washington, DC, 2002.
12. Gadek, T. R.; Burdick, D. J.; McDowell, R. S.; Stanley, M. S.; Marsters, J. C., Jr.; Paris, K. J.; Oare, D. A.; Reynolds, M. E.; Ladner, C.; Zioncheck, K. A.; Lee, W. P.; Gribling, P.; Dennis, M. S.; Skelton, N. J.; Tumas, D. B.; Clark, K. R.; Keating, S. M.; Beresini, M. H.; Tilley, J. W.; Presta, L. G.; Bodary, S. C. *Science* **2002**, *295*, 1086.
13. Burdick, D. J. In PCT Int. Appl. WO9949856, 1999.
14. Fisher, K. L.; Lu, J.; Riddle, L.; Kim, K. J.; Presta, L. G.; Bodary, S. C. *Mol. Biol. Cell* **1997**, *8*, 501.
15. Carpino, L.; Han, G. *J. Org. Chem.* **1981**, *37*, 3404.
16. Fields, G.; Noble, R. L. *Int. J. Peptide Protein Res.* **1990**, *35*, 161.