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N-Benzoyl amino acids as ICAM/LFA-1 inhibitors. Part 2: Structure–activity relationship of the benzoyl moiety

Daniel J. Burdick,[a,*] James C. Marsters, Jr.,[a] Ignacio Aliagas-Martin,[a] Mark Stanley,[a] Maureen Beresini,[b] Kevin Clark,[b] Robert S. McDowell[a,†] and Thomas R. Gadek[a]

[a]*Department of Medicinal 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—*o*-Bromobenzoyl L-tryptophan **1** inhibits the association of LFA-1 with ICAM-1 with an IC_{50} of 1.7 μM. Evaluation of the structure–activity relationship of the benzoyl moiety shows that 2,6-di-substitutions greatly enhance potency of this class of inhibitors. Electronegative substitutions that favor a 90° angle between the benzoyl ring and the amide bond yield the most potent compounds. There is a strong correlation between the potency of the compounds and the difference between the ab initio energy at 90° and the global minima energy for given compounds. Combining the favored benzoyl substitutions with L-histidine and L-asparagine resulted in a 15-fold increase in potency over compound **1**.

The interaction of leukocyte function-associated antigen-1 (LFA-1, αLβ2, CD11a/CD18) with intercellular adhesion molecule 1 (ICAM-1) has been implicated in numerous autoimmune diseases such as psoriasis, asthma, rheumatoid arthritis, and graft rejection.[1–3] In the past several years, there have been reports of several monoclonal antibodies and small-molecule antagonists to this interaction.[4–7] We have previously described the structure–activity relationship (SAR) of the amino acid portion of compound **1**, and in this communication we will turn our attention to the SAR of the benzoyl moiety[8] (Fig. 1).

We initially generated a set of compounds to evaluate the optimal distance of the benzoyl ring from the amino acid. Extending the ring out from the amide bond one to five methylene units diminished potency. Therefore, we decided to concentrate on improving the inhibitors through modifications of the benzoyl ring itself. Four classes of acids were coupled to L-tryptophan: monosubstituted benzoic acids; monosubstituted heterocyclic aromatic acids; saturated cyclic acids, and di-substituted benzoic acids. All compounds were evaluated in an ICAM-1/LFA-1 enzyme linked immunosorbant assay (ELISA).[4] 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} values are the arithmetic average of at least two assays.

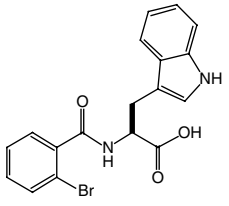

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Figure 1.

Compounds **1**–**58** were synthesized using standard Fmoc solid phase synthetic techniques (Scheme 1) on commercially available N^{α}-Fmoc-N^{in}-*tert*-butyloxycarbonyl-L-tryptophan 4-hydroxymethylphenoxy resin (Wang resin).[9,10] Following removal of the Fmoc group with 20% piperidine in DMF and rinsing of the resin, the amine was acylated with the appropriate commercially available acid using HBTU, HOBT, and DIPEA in DMF. After drying the resin to a constant weight, it was then treated with a solution of TFA containing 2.5% water and 2.5% anisole. Excess TFA was removed

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* 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.

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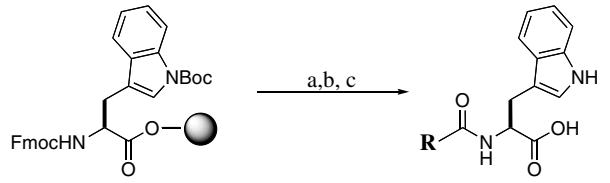


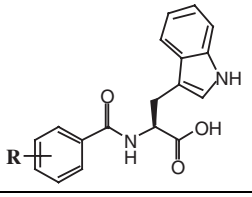
Scheme 1. Reagents and conditions: (a) 20% piperidine/DMF, rt; (b) appropriate acid ($RCOO_2H$), HBTU, HOBT, DIPEA, DMF, 2 h, rt; (c) triisopropylsilane, water, TFA, 1 h, rt.

in vacuo and the cleaved molecule was extracted away 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. Compounds **59**–**67** where synthesized in a similar manner with the exception that N^{α}-Fmoc-L-asparagine Wang resin and N^{α}-Fmoc-N^{im}-*tert*-butyloxycarbonyl-L-histidine Wang resin were used in place of N^{α}-Fmoc-N^{in}-*tert*-butyloxycarbonyl-L-tryptophan Wang resin where appropriate.

ELISA assay results for the monosubstituted benzoyl analogues are shown in Table 1. Two observations can be made from this data. First, compounds with halogen (**1**, **3**–**5**) or methyl (**6**) substitutions at the *ortho* position are more potent than the same substitution at either the *meta* or *para* position. For example, *N*-*ortho*-chlorobenzoyl-L-tryptophan (**4**) is almost 200 times more potent than *N*-*meta*-chlorobenzoyl-L-tryptophan (**11**) and more than 100 times more potent than *N*-*para*-chlorobenzoyl-L-tryptophan (**18**). In general, potency for regional isomers of a given substituent rank *ortho* > *para* > *meta*.

The second observation has to do with the potency of the *ortho* substituted analogues. Substitutions that help force the amide bond out of the plane of the benzoyl ring lead to better inhibitors than those that enforce co-planarity. This influence on potency appears to be a combination of steric and electronic effects. For example, the medium sized hydroxy (**8**) and methoxy substitutions are approximately 300- and 100-fold, respectively, less potent than the bromo substitution (**1**) and they are approximately 4- and 2-fold, respectively, less potent than the un-substituted benzoyl ring (**2**) itself. The alkoxy groups stabilize the co-planarity of the ring and amide bond with a large electronic resonance effect, as well as, internal hydrogen bonding with the amide bond NH hydrogen. The methyl substitution (**6**) has a larger steric effect and a smaller electronic resonance effect than the alkoxy groups. This could help force the amide bond to twist out of the plane of the benzoyl ring. The methyl inhibitor (**6**) is 2.3 times less potent than the bromo analogue (**1**) and 32 times more potent than the un-substituted analogue (**2**).

Table 1. ELISA assay results for monosubstituted *N*-benzoyl-L-tryptophan analogues



R	*ortho*		*meta*		*para*	
	Compd	IC_{50} (μM)	Compd	IC_{50} (μM)	Compd	IC_{50} (μM)
H	**2**	127				
F	**3**	7.4	**10**	148	**17**	136
Cl	**4**	1.5	**11**	295	**18**	169
Br	**1**	1.72	**12**	>100	**19**	61
I	**5**	8.9	**13**	>100	**20**	92
Me	**6**	4	**14**	295	**21**	129
Ph	**7**	>100			**22**	>100
OMe	**8**	225	**15**	438	**23**	178
OH	**9**	>500	**16**	>100	**24**	>100

Halogen substitutions (**1**, **3**–**5**) constitute the best overall groups of monosubstituted benzoyl inhibitors. With the exception of the fluoro analogue (**3**), the halogens all have a similar steric and electronic resonance effect, and in general their potency increases as they become smaller and more electronegative.[11] The chloro analogue (**4**) is slightly more potent than the bromo analogue (**1**), but it is six times more potent than the iodo compound (**5**). The fluoro analogue (**3**) is the anomaly. Its ability to hydrogen bond with the amide NH can favor a co-planar relationship between the amide bond and the phenyl ring and seems to overcome any steric effect.[12]

Figure 2 shows a plot of the strain energy at 90° (ΔE_{strain}) of model *N*-methyl *ortho*-monosubstituted-benzamides (H, Me, F, Cl, Br, OMe) versus the log of the IC_{50} value for the analogous N-*ortho*-substituted benzoyl L-tryptophan inhibitors (**1**–**6**, **8**) for which there was quantitative data.[13] ΔE_{strain} values estimate the energy needed to rotate the substituted amide from its global minima to a preferred angle of 90° in the bound state. With the exception of the fluoro-substituted analogue, there is a strong linear correlation found between the calculated strain energies and the experimental binding energies suggesting that $\Delta\Delta G$ is directly correlated to ΔE_{strain}. The calculated ΔE_{strain} value for the

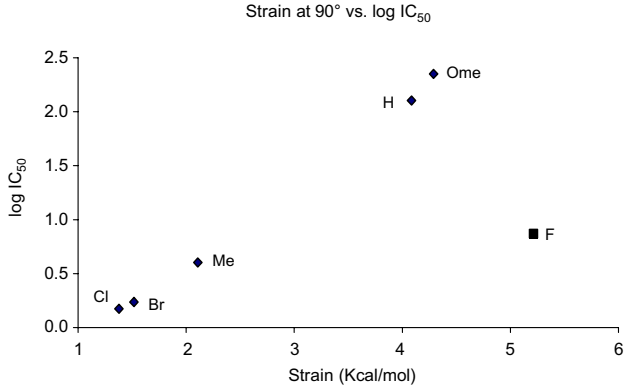
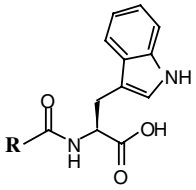


Figure 2. Plot of ΔE_{strain} versus log IC_{50} for monosubstituted *ortho*-benzoyl L-tryptophan inhibitors. There is a strong correlation between ΔE_{strain} and potency of the inhibitors. The fluoro analogue does not follow the trend due to enhanced hydrogen bond capability.

N-methyl *ortho*-hydroxybenzamide is 9.75 kcal/mol, which predicts that the analogous hydroxy compound (**9**) would be a very poor inhibitor. This prediction is borne out in the experimental data; compound **9** has an $IC_{50} > 500\,\mu M$. These calculations support the hypothesis that substitutions that help force the amide bond out of the plane of the benzoyl ring are better inhibitors than those that favor co-planarity.

Table 2 shows assay data for the aromatic heterocyclic and saturated analogues. The aromatic heterocyclic analogues follow the same trend established with the monosubstituted benzoyl analogues. The three pyridyl compounds (**25**–**27**) show inhibition similar to the analogous benzoyl compound (**2**). Compound **28**, which has a bromine *meta* to the carbonyl, is a poor inhibitor; while compounds with a chlorine *ortho* to the carbonyl (**29**, **30**) are much more potent. The saturated ring analogues (**32**–**36**) do not work as well as the aromatic compounds. However, the same '*ortho* effect' can be seen. Adding a methyl at position-2 (**33**) increases potency by twofold over cyclohexane ring (**32**) alone.

Table 2. ELISA results for heterocyclic and saturated L-tryptophan analogues



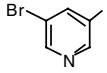
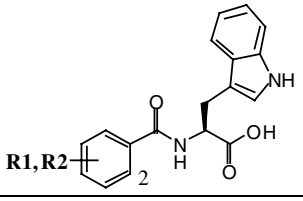
Compound	R	IC_{50} (μM)
25		>100
26		112
27		92
28		>200
29		32.6
30		1.2
31		>1000
32		61
33		28
34		380
35		>1000
36		>1000
37		>1000

Table 3 shows ELISA assay results for di-substituted *N*-benzoyl L-tryptophan analogues. As with the other series, the position and type of substitution has a great influence on the compounds potency. For instance, the 3,5-substituted di-fluoro (**38**) and di-chloro (**39**) analogues, as well as, the 3,4-di-chloro (**40**) analogue are poor inhibitors. However, as with the monobenzoyl inhibitors, an *ortho* substitution greatly enhances the inhibitors potency. The combination of an *ortho* halogen with a *meta* substituent (**41**–**47**) or a *para* substituent (**48**–**50**) yield inhibitors that are much more potent than the corresponding mono *meta* (**10**–**12**, **15**) or *para* analogues (**17**, **18**). As noted previously, if the *ortho* substituent is a hydroxy (**51**, **52**) the potency of the analogue decreases greatly.

The most dramatic increase in potency occurs when the amide carbonyl is flanked by two *ortho* substituents. When both *ortho* substitutions are the same, the greatest improvement is seen with the least potent

Table 3. ELISA results for di-substituted *N*-benzoyl-L-tryptophan analogues



Compound	2	3	4	5	6	IC_{50} (μM)
38		F		F		89.7
39		Cl		Cl		200
40		Cl	Cl			170.5
41	F	F				21.6
42	F			F		11.5
43	Cl	Cl				6.3
44	Cl			Cl		6.6
45	Cl			Br		17.9
46	Br			OMe		9
47	Br			Br		24.3
48	Cl		Cl			8.4
49	Cl		NO_2			5.2
50	Cl		NH_2			2
51	OH			Br		>500
52	OH			Br		>500
53	F				F	0.46
54	Cl				Cl	0.6
55	Me				Me	1.1
56	Cl				Me	0.64
57	F				Br	0.43
58	F				Cl	0.25

2,6-Di-substitutions greatly enhance potency in this series of inhibitors.

monosubstituents. For example, the 2,6-di-fluoro compound (**53**) is 16-fold more potent than the *ortho* fluoro compound (**3**); where as the 2,6-di-chloro compound (**54**) is only 2.5-fold more potent than compound **4**. Combinations of different *ortho* substituents have a greater effect. *N*-2-Chloro-6-methyl-benzoyl L-tryptophan (**56**) shows a moderate increase in potency over *N*-2-methyl-benzoyl L-tryptophan (**6**). The 2-bromo-6-fluoro (**57**) and 2-chloro-6-fluoro (**58**) substitutions constitute a 17- and 30-fold, respectively, improvement in potency over compound **3**. Compound **58** is seven times more potent than our original lead (**1**).

Figure 3 shows a plot of ΔE_{strain} of model *N*-methyl 2,6-di-substituted benzamides versus the log of the IC_{50} value for the analogous inhibitors (**53**–**58**). Two observations can be made from this graph. First, not all 2,6-di-substitutions result in a ΔE_{strain} of 0. The calculated ΔE_{strain} for the di-fluoro analogue (**53**) is 0.56, which is about 10-fold lower than the monofluoro analogue. This result suggests that there is a minimal steric size requirement for at least one of the *ortho* substituents to achieve a ΔE_{strain} of 0. Second, compounds with ΔE_{strain} values of 0 (**54**–**58**) do not have the same potency. Activity, once again, appears to be a combination of steric effect and electronegativity. For example, compound **55** has a ΔE_{strain} of 0 due to the large steric contributions of the methyl groups, but its potency is not that much better than the original lead (**1**). Compounds **54**, **56**–**58** have at least one substituent that contributes a large steric component and at least one substituent that contributes a large electronegative component. Interestingly, as the combination of substituents moves from less electronegative to more electronegative, the potency of the inhibitors increases.

We previously reported the SAR of the amino acid portion of the *N*-benzoyl L-tryptophan antagonists.[8] Combining the results of this work with the current benzoyl SAR evaluation, we were able to produce compounds with further enhanced potency. These results are displayed in Table 4. The trend found in the 2,6-di-substituted benzoyl L-tryptophan series holds for both the L-asparagine series and the L-histidine series.

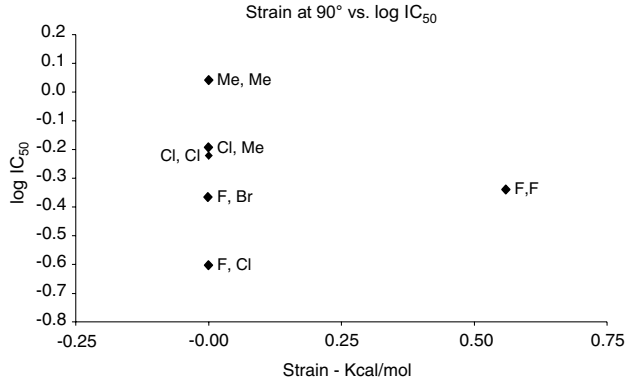
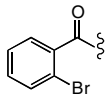
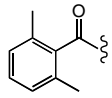
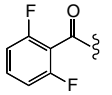
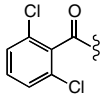
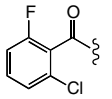


Figure 3. Plot of ΔE_{strain} versus $\log IC_{50}$ for 2,6-di-substituted *N*-benzoyl-L-tryptophan analogues. Potency for compounds that have a 90° angle between the amide bond and benzoyl ring increases as the *ortho* substituents become more electronegative.

Table 4. Comparison of the various 2,6-di-substituted benzoyl moieties in 3*N*-benzoyl-L-amino acid series

	L-Trp (compound) IC_{50}	L-Asn (compound) IC_{50}	L-His (compound) IC_{50}
	(**4**) 1.7	(**59**) 1.08	(**60**) 0.75
	(**55**) 1.1	—	(**61**) 1.77
	(**53**) 0.46	(**62**) 0.23	(**63**) 0.2
	(**54**) 0.6	(**64**) 0.17	(**65**) 0.16
	(**58**) 0.25	(**66**) 0.11	(**67**) 0.13

However, the enhancement in potency is not additive. In the case of L-tryptophan and L-histidine series there is a 6-fold improvement in potency moving from a 2-bromo benzoyl ring to a 2-chloro-6-fluoro benzoyl ring, while this same change in the L-asparagine series results in a 10-fold increase in potency.

Based on these results, we conclude that within the class of *N*-benzoyl L-tryptophan antagonists, 2,6-di-substitutions on the benzoyl ring are most favored for inhibition of the LFA-1/ICAM-1 complex; in particular, *N*-2-chloro-6-fluoro benzoyl L-tryptophan shows the highest potency of the analogues tested. Combining the best elements of the amino acid SAR and the benzoyl SAR results in two compounds that are 16-fold more potent than the original screening hit. A subsequent paper will address finding an additional interaction with the purpose of further increasing potency.

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References and notes

1. Bevilacqua, M. P.; Nelson, R. M.; Mannori, G. *Ann. Rev. Med.* **1994**, *45*, 361–378.
2. Cornejo, C. J.; Winn, R. K.; Harlan, J. M. *Adv. Pharmacol.* **1997**, *39*, 99–142.
3. Henricks, P. A.; Nijkamp, F. P. *Eur. J. Pharmacol.* **1998**, *344*, 1–13.
4. 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*, 1086–1089.
5. Liu, G. *Drugs Future* **2001**, *26*, 767.
6. Liu, G. *Expert Opin. Ther. Pat.* **2001**, *11*, 1383.
7. Yun, W.; Desai, B. B.; Du, Y.; Fotouhi, N.; Gillespie, P.; Guthrie, R. W.; Huang, K.; Kolinsky, K.; Li, S. H.; Mennona, F. A.; Perrotta, 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.
8. Burdick, D. J.; Paris, K.; Weese, K.; Stanley, M.; Beresini, M.; Clark, K.; McDowell, R. S.; Marsters, J. C., Jr.; Gadek, T. R. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1015–1018.
9. Carpino, L.; Han, G. *J. Org. Chem.* **1981**, *37*, 3404–3409.
10. Fields, G.; Noble, R. L. *Int. J. Pept. Protein res.* **1990**, *35*, 161–214.
11. March, J. Advanced Organic Chemistry, 4th ed., 1992, Chapter 9.
12. Howard, J. A. K.; Hoy, V. J.; O'Hagan, D.; Smith, G. T. *Tetrahedron* **1996**, *52*, 12613–12622.
13. Calculations were performed on a model set of *N*-methyl *ortho*-monosubstituted and 2,6-di-substituted benzamides for which binding data is available for analogous compounds. An ab initio approach was used to determine the strain energy when the torsional angle between the carbonyl and phenyl ring planes (O═C─C_1─C_2─X) is at 90°. The calculations were performed in vacuum using the Gaussian 98 program at the HF/6-31G* level of theory. Torsional barrier profiles were determined by optimizing the geometries at fixed values of the torsion O═C─C_1─C_2─X at 15° increments from 0° to 180° from which the ab initio energies (E_{ab}) were obtained. The global minima energies (E_{min}) were also calculated for each molecule. For a given molecule, the strain energy at 90° (ΔE_{strain}) is defined as the difference between the ab initio energy at 90° and the global minima energy ($E_{ab90} - E_{min}$). The ΔE_{strain} is part of the internal conformational energy of the molecule and does not include any solvation effects that would be difficult to estimate given the unknown nature of the ligand–protein interface.