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N-Benzylpyroglutamyl-L-phenylalanine Derivatives as VCAM/VLA-4 Antagonists

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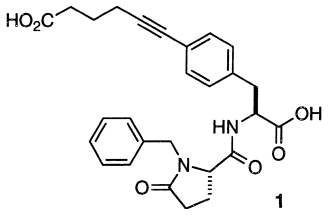
Abstract—A series of *N*-(*N*-benzylpyroglutamyl)-4-substituted-L-phenylalanine derivatives was prepared as VLA-4/VCAM antagonists. Analogues substituted by electron deficient benzoylamino groups bearing bulky *ortho* substituents had low-nM potency in an ELISA assay and low-μM activity in a cell based assay.

Vascular cell adhesion molecule-1 (VCAM-1), a member of the immunoglobulin (Ig) supergene family, 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 many lymphocytes including 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] Peptides derived from the connecting segment 1 (CS1) sequence of fibronectin have also been shown to block VCAM/VLA-4 interactions and to block allergen induced airway responses in a sheep model of asthma.[2,3] Thus we are interested in discovering orally active VCAM/VLA-4 antagonists which might be useful for the treatment of asthma or rheumatoid arthritis.

We previously reported our finding that certain *N*-acyl-L-phenylalanine derivatives are effective inhibitors of the VCAM/VLA-4 interaction.[4] In this work, *N*-benzylpyroglutamic acid emerged as a particularly favorable *N*-acyl group as demonstrated by the activities of **1** and **2** in an ELISA based VCAM/VLA-4 binding assay (20 and 46 nM, respectively). In this paper, we focus attention on the role of the phenylalanine ring and its preferred substitution. The goal of this effort was to scan a variety of substitution patterns with the intent of exploiting promising candidate substitutions using high throughput chemistry.



Derivatives **5**–**14** were prepared by acylation of the appropriate amino acid methyl esters with *N*-benzylpyroglutamic acid **3** and ester hydrolysis. The (2:1 *cis:trans*) 4-methylcyclohexylalanine and *trans*-4-*tert*-butylcyclohexylalanines required for **13** and **14** were available from glycine imine alkylation of the appropriate cyclohexylmethyl iodides.[5] The 4-(phenylethyl)phenylalanine **15** was obtained from catalytic hydrogenation of the acetylene **12**. In order to investigate the potential role of heteroatom linked substituents, we prepared the 4-nitrobenzylether **17** via a Mitsunobu reaction (Scheme 2). Stannous chloride mediated reduction of the 4-nitro derivative **18** gave the aniline **19** which was readily acylated with carboxylic acids or sulfonyl chlorides followed by ester hydrolysis to give the amides **20**–**23** and the sulfonamides **24** and **25** as shown in Scheme 3.

To prepare homologous analogues, 4-(hydoxymethyl)-*S*-phenylalanine **29** was synthesized by enantioselective hydrogenation of the dehydrophenylalanine derivative **28** as shown in Scheme 4. The *S*-stereochemistry was

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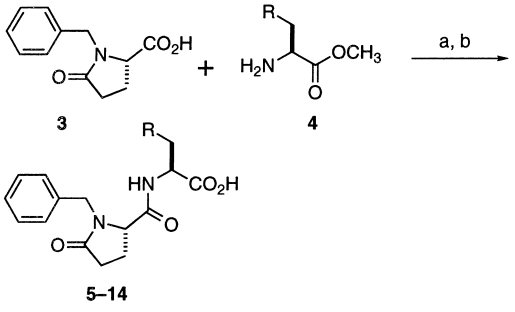
assigned based on the literature precedent[6] and by analogy with the corresponding 3-hydroxymethyl series in which the stereochemical outcome of the reduction was verified by crystallography. The 4-nitrophenyl ether **35** was prepared via a Mitsunobu reaction between the alcohol **29** and 4-nitrophenol and the styrene derivative **36** was available from a 3-step sequence involving Swern oxidation of **29** followed by a Wittig reaction and ester hydrolysis as outlined in Scheme 5.

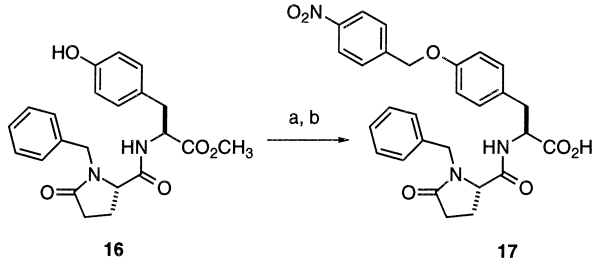
Results and Discussion

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 serial dilutions of test compound. VLA-4 bound to VCAM-1 was detected by a complex of anti-β1 antibody and HRP-conjugated anti-mouse IgG: chromogenic substrate (K-Blue).[7] From the data summarized in Table 1, it is evident from the lack of activity of **13** and **14,** the benzene ring of the phenylalanines contributes significantly to potency. Among the phenylalanine derivatives, comparison of **5–8** with **12**, **15**, **17** and **35**, suggests that activity generally increases with the size of the substituent in the 4-position. By far the most interesting group of compounds to emerge from this effort are the acylamino derivatives **20–23**, each of which have single digit nM potency in the VCAM/VLA-4 ELISA assay. Furthermore the benzoylamino analogues **20** and **21** are also the only compounds of this series to block the interaction between fluorescently labeled Ramos cells, which express VLA-4, with VCAM coated microtiter plates at submicromolar concentrations.

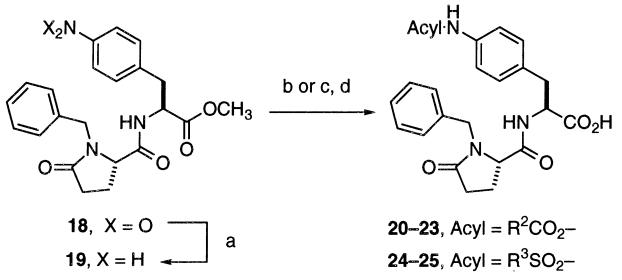
As a consequence of this initial effort, we chose to expand the series of benzoylamino derivatives by the



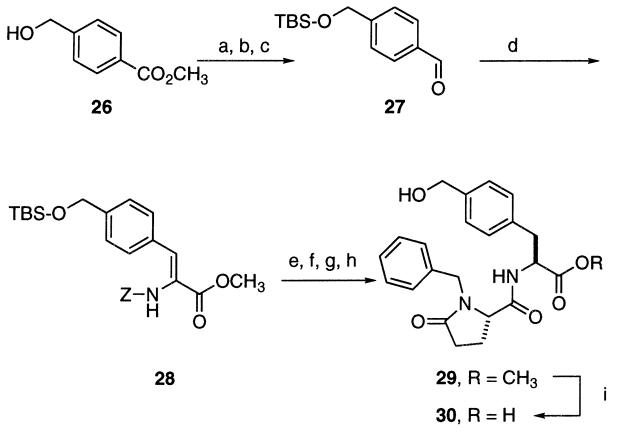
Scheme 1. (a) HBTU, DIPEA, DMF, 25 °C, (b) NaOH, THF, MeOH, 25 °C.



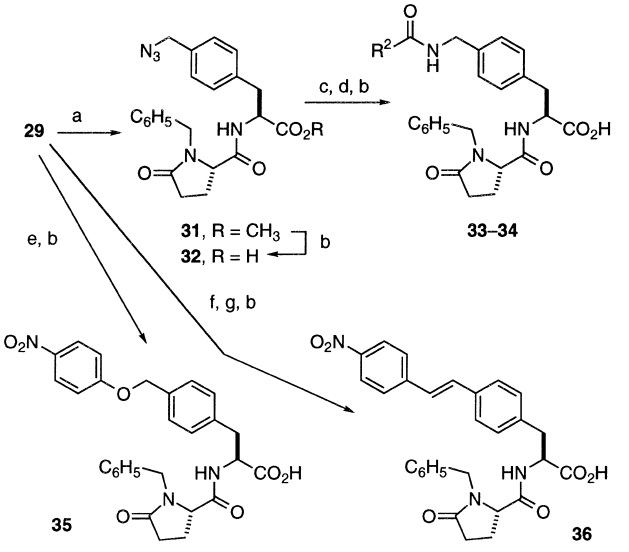
Scheme 2. (a) 4-Nitrobenzylether, DEAD, ϕ_3P, CH_2C1_2, (b) NaOH, EtOH.



Scheme 3. (a) $SnC1_2$, EtOH, reflux, 50 min (75%), (b) R^2CO_2H, HBTU, DIPEA, DMF, (c) R^3SO_2C1, pyridine, rt, d. NaOH, EtOH, rt.

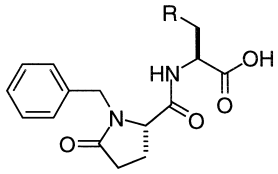


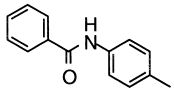
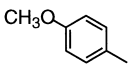
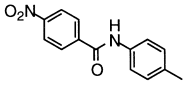
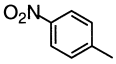
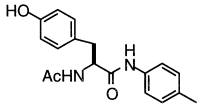
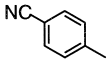
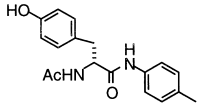
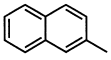
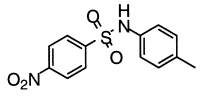
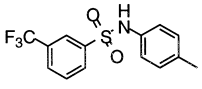
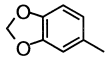
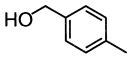
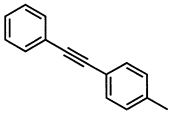
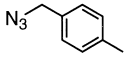
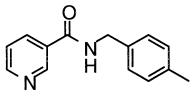
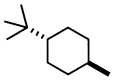
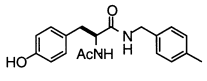
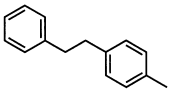
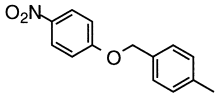
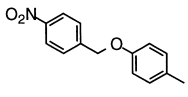
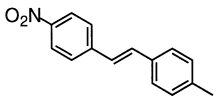
Scheme 4. (a) TBDMS-C1, imidazole (92%), (b) LAH, Et_20 (92%). (c) Excess MnO_2, 1:1 CH_2C1_2:hexane, 6 h (86%). (d) Trimethyl Z-phosphonogycine, tetramethylguanidine, −40 °C (85%), (e) Rh-DuPhos, H_2, MeOH, 50 psi, rt (97%), (f) HCO_2NH_4, MeOH, Pd(C), (g) *N*-Bzl-pyroglutamic acid, HBTU, DIPEA, DMF (78%, 2 steps), (h) 2 N HC1, MeOH (95%), (i) NaOH, EtOH, rt.



Scheme 5. (a) DPPA, DBU, (b) NaOH, EtOH, (c) H_2, Pd(C), EtOH, (d) R^2CO_2H, HBTU, DIPEA, DMF, (e) DEAD, ϕ_3P, 4-nitrophenol, (f) DMSO, oxalyl chloride, NEt_3, (g) 4-nitrobenzylphosphonium bromide, NEt_3, DMF.

Table 1. VCAM/VLA-4 binding inhibition of *N*-benzylpyroglutamyl-L-phenylalanine derivatives

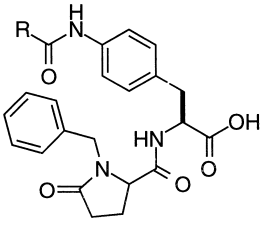


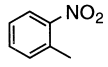
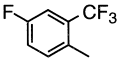
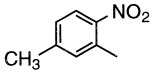
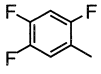
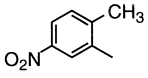
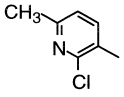
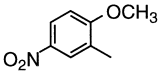
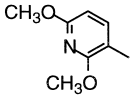
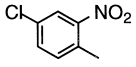
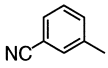
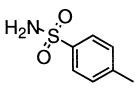
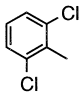
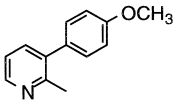
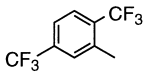
Compound	R	ELISA IC_{50} nM	Compound	R	ELISA IC_{50} nM	Ramos cell IC_{50} nM
5		460	**20**		6.3	310
6		160	**21**		2.2	560
7		560	**22**		8.2	2100
8		1000	**23**		5.3	1900
9		59	**24**		17	16,000
10		230	**25**		20	26.000
11		220	**30**		152	—
12		53	**32**		74	—
13		42,500	**33**		53	—
14		558% @ 250,000	**34**		20	—
15		38	**35**		32	—
17		18	**36**		90	—

solid-phase synthesis of approximately 100 analogues. These compounds were initially screened at concentrations of 10 and 100 nM and only those combining $\geq$60% inhibition of binding at 10 nM with $\geq$90% inhibition at 100 nM were purified or resynthesized for IC_{50} determination. Compounds thus characterized are shown in Table 2. A number of these compounds have low-nM IC_{50}s in the ELISA assay and low nM activity in the Ramos cell assay as well. Features common to the most potent compounds include electron deficient aromatic rings and at least one bulky *ortho* substituent. The data suggest that the presence of these elements is more important than their identity. For example the 2-nitro- (**37**), the 2-methoxy-5-nitro- (**40**) and the 2,6-dichloro- (44) analogues all have similar profiles.

One remarkable observation from this work is that virtually all members of the *N*-benzylpyroglutamyl-4-substituted-L-phenylalanine class investigated effectively inhibit the VCAM/VLA-4 interaction in our ELISA assay with submicromolar IC_{50}s. The preferred substitution is an electron deficient, *ortho*-substituted benzoylamino- moiety in the 4-position. We speculate that the VLA-4 recognition elements present in these molecules include the π-electron systems of both the benzoyl and the phenylalanine aromatic rings as well as a hydrogen bonding interaction with the carboxamido group in the 4-position. The requirement for high potency becomes evident when one considers the cell binding results, in which it is necessary to block many VCAM-VLA-4 interactions simultaneously along the

Table 2. Inhibition of VCAM/VLA-4 binding induced by *N*-benzylglutamyl-4-acylamino-L-phenylalanines



Compound	R	ELISA IC_{50} nM	Ramos cell IC_{50} nM	Compound	R	ELISA IC_{50} nM	Ramos cell IC_{50} nM
37		0.87	33	**46**		0.75	28
38		0.74	77	**47**		1.6	127
39		0.26	11	**48**		2.4	33
40		0.82	28	**49**		2.4	102
41		0.37	7	**50**		4.0	1850
42		4.6	260	**51**		3.6	260
43		3.5	1060	**52**		1.4	80
44		0.37	12	**53**		0.88	29
45		1.1	54				

Ramos cell-microtiter plate boundary. We have employed compounds from this work, particularly the 2,6-dichlorobenzoyl analogue **44** to initiate studies of their potential role as therapeutics in vivo.

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