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N-Acyl-L-phenylalanine Derivatives as Potent VLA-4 Antagonists that Mimic a Cyclic Peptide Conformation

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Abstract—A series of *N*-benzylpyroglutamyl-L-phenylalanine derivatives bearing carbamoyl substituents in the 3- or 4-positions was prepared and assayed for inhibition of the interaction between VCAM and VLA-4. Potent inhibition was observed in a number of analogues with substitution in the 4-position favored over the 3-position. A crystal structure of the key intermediate **25** indicates that it accesses a low energy conformation which closely matches key pharmacophores of a structurally characterized cyclic peptide.

The very late antigen 4 (VLA-4, $\alpha_4\beta_1$) is an integrin receptor expressed on many lymphocytes and mediates cell adhesion, infiltration and the co-stimulation response, when it interacts with its counter ligands, such as VCAM-1 and fibronectin. VLA-4 antagonists that inhibit the interaction of VCAM-1/VLA-4 are being intensively sought for the treatment of inflammatory diseases such as asthma (Fig. 1).[1–3]

Studies on cyclic peptide based VLA-4 antagonists,[4,5] such as **1** (IC_{50} 1.8 nM in an ELISA assay), suggested that a tyrosine residue at the N-terminus was beneficial to the activity of this class. We have previously described our efforts to scan a variety of *N*-(benzylpyroglutamyl)phenylalanines for their ability to inhibit the VCAM–VLA-4 interaction.[6,7] The most potent compounds to emerge from this work were the 4-acylaminophenylalanine derivatives typified by **2** and **3** with IC_{50}s of 0.37 and 6.5 nM, respectively. As a result of this work, we proposed that members of *N*-Acyl-Phe series may mimic the cyclic peptide inhibitors interaction with VLA-4. In the present paper, we provide additional support for this hypothesis and extend our SAR studies to the corresponding 3- and 4-aminocarbonyl-phenylalanines **4**.

Previous studies[6,7] on the SAR of cyclic peptides related to **1** indicated that the carboxylate group at the C-terminus, the proline ring and elements of the disulfide bridge were key pharmacophores. In addition, the potency is dependent on the selection of an appropriate N-terminal reside, such as a tyrosine residue.

NMR studies of cyclic peptides related to **1**[4,5] suggested a consistent picture of a relatively planar ring structure of the cyclic peptide core with the two ring amide bonds perpendicular to the ring and pointing in opposite directions from one another to minimize the overall dipole interaction. The precise orientation of the critical distal N-terminal group is ill defined. In the course of this work, we obtained a crystal structure of the *N*-(benzylpyroglutamyl)Phe derivative **25** that represents a low energy gauche (−) conformation with the two benzene rings in close proximity. Overlaying of the X-ray

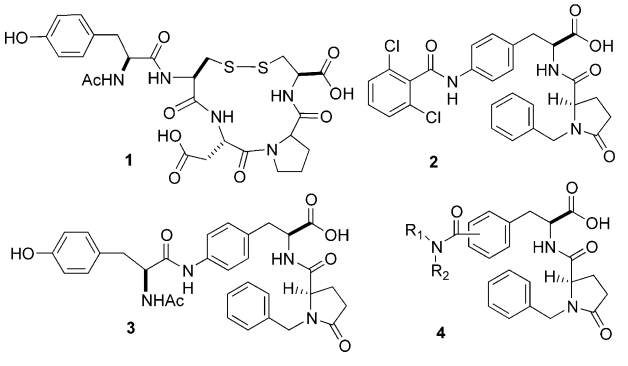

Figure 1. *N*-Acy-Phe and cyclic peptide class of VLA-4 antagonists.

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crystal structure of **25** with two most populated families of NMR structures of the cyclic peptide core suggests that these two classes of VLA-4 antagonist share key pharmacophores, including the 5-membered pyrrolidine ring and the C-terminal carboxylic acid, while the aromatic ring of **25** occupies the region of the disulfide bridge of the cyclic peptide (Fig. 2).

In this model, the C–H bonds in the 3- and 4-positions of benzene ring of the phenylalanine of **25** represent vectors which point toward the region occupied by the structurally ill defined tyrosine of cyclic peptide **1**. With this understanding, we chose to explore groups in either position which are capable of mimicking the function of the tyrosine of **1**.

For the preparation of 4-aminocarbonyl analogues of phenylalanine, L-tyrosine triflate methyl ester was acylated with *N*-benzylpyroglutamic acid **6** to give the triflate **7**, which was carbonylated with carbon monoxide at 50 psi in the presence of a palladium catalyst to give the intermediate acid **8** (Scheme 1). Coupling of **8** with amines using HBTU followed by hydrolysis provided compounds **9**–**13**. Alternatively, reaction of compound **7** with Boc-piperazine under the carbonylation conditions[8] followed by removal of the Boc group gave intermediate piperazine amide **14**, which was in turn acylated by a variety of acids and sulfonyl chlorides. The acylation products were subsequently hydrolyzed under basic conditions to give final products **15**–**21**.

The 3-substituted analogues were prepared through an enantioselective hydrogenation of the dehydro-phenylalanine derivative **23** as shown in Scheme 2. The *S*-stereochemistry of **24** was assigned based on the literature precedent[9] and was confirmed by X-ray crystallography[10] of the alcohol **25**. Jones oxidation of **25** followed by coupling with tyrosine diethyl amide and basic hydrolysis gave tyrosine analogue **26**. Similarly, acylation of intermediate **27** followed by hydrolysis of methyl ester gave 3-acylpiperazine analogue **28**. The sulfonamides **29** and **30** were synthesized by reaction of **27** with the sulfonyl chlorides followed by hydrolysis of the methyl esters.

The *N*-Acyl-Phe analogues were evaluated for their ability to inhibit the VCAM–VLA4 interaction in both an ELISA and Ramos cell based format.[11] The results, shown in Table 1, indicate that 4-tyrosinyl derivative **9** is nearly equal potent to peptide **1** in both the ELISA and Ramos cell based assays. Comparing the potencies of **9** and **3** suggests that it is relative insensitive to the directionality of the amide bond connecting the tyrosine and the phenylalanine. Compounds **10** and **11**, derived from benzyl amine, were less active than compound **9**. Activity of aniline derivative was low and was improved by incorporation of an acetyl group at the *ortho*-position. Incorporation of acylpiperazines at 4 position gave low nanomolar analogues, which were 4-fold more potent than the corresponding sulphonylpiperazine analogues. The most potent acylpiperazine analogues, such as compound **17** and **19**, are as active as **1** and **9**. It is noteworthy that compound **12** is almost 10-fold less potent than its reversed amide analogue,[7a] indicating a

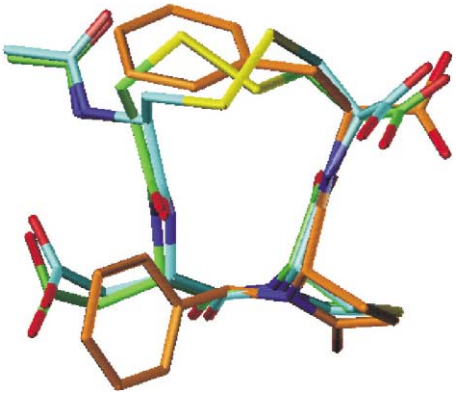
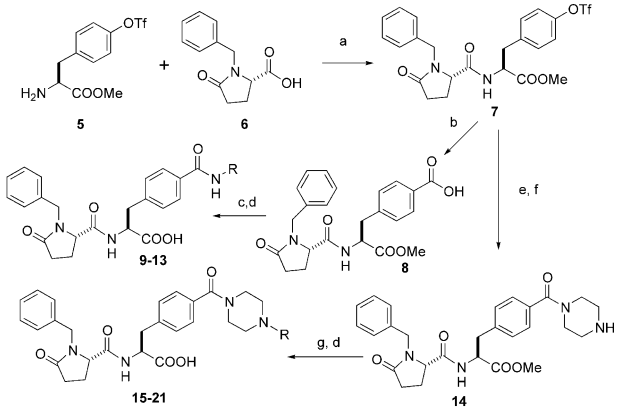
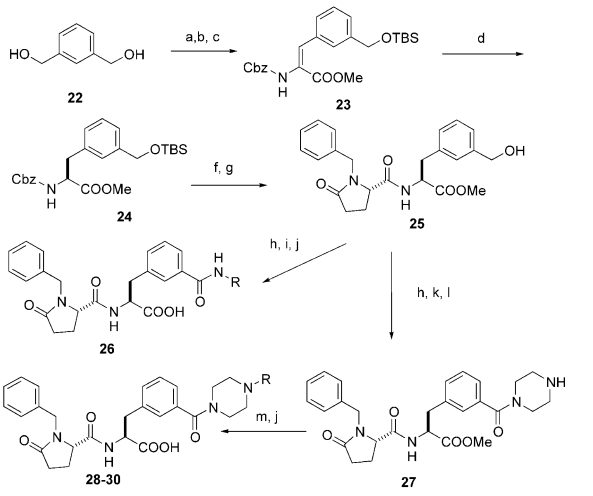


Figure 2. Overlay of X-ray of **25** (*m*-hydroxymethyl not shown) in gold with cyclic peptide core in green and cyan.

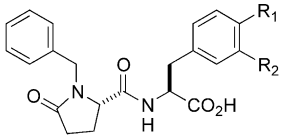


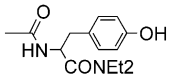
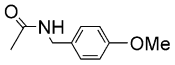
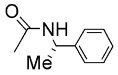
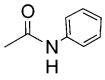
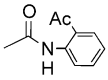
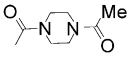
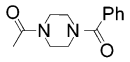
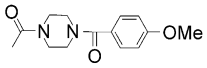
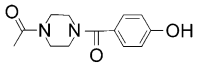
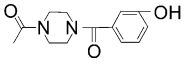
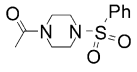
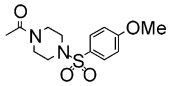
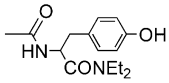
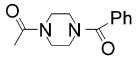
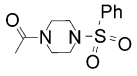
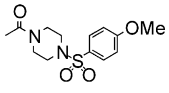
Scheme 1. Synthesis of 4-aminocarbonylphenylalanine analogues: (a) HBTU, DIEPA DMF, rt; (b) $PdAc_2$, dppp, DMSO, CO, Et_3N, H_2O, 60 °C; (c) NH_2R, HBTU, DIEPA, DMF, rt; (d) NaOH, EtOH, H_2O, rt; (e) $Pd(OAc)_2$, dppp, *N*-Boopiperazine CO, Et_3N, 60 °C; (f) 4 N HCl in cloxane; (g) benzoic acids, HBTU, DIPEA, rt; or benzenesulfonyl chloride, DCM, DIPEA, rt.



Scheme 2. Synthesis of 3-aminocarbonylphenylalanine analogues. (a) NaH, THF, TBSCI, 53%; (b) MnO_2, DCM, 79%; (c) Cbz-trimethylphosphonoglycine, TMG, DCM, 85%; (d) Duphas, MeOH, H_2, 98%; (e) Pd-C, H_2, MeOH; (f) *N*-Benzylpyroglu-OH, HBTU, DIEA, 62%; (g) HF, MeCN, 62% from **24**; (h) Jones reagent, acetone; (i) NH_2R, HBTU, DIEA, DMF, 72% from **25**; (j) 1 N NaOH, EtOH/H_2O; (k) N-Boc-piperazine, HBTU, DIPEA, DMF, rt; (l) 4 N HCl in dioxane; (m) acids, HBTU or sulfonyl chloride, DIPEA.

Table 1. Inhibitory activity of VCAM–VLA-4 interaction in ELISA and Ramos cell-based assay



Compd	R_1	R_2	**ELISA** IC_{50} (μM)	**Ramos** IC_{50} (μM)
1			0.002	0.200
2			0.00037	0.012
3			0.0065	2.1
9		H	0.005	0.550
10		H	0.061	
11		H	0.027	
12		H	0.057	
13		H	0.004	0.940
15		H	0.007	5.50
16		H	0.003	0.960
17		H	0.004	0.320
18		H	0.003	1.7
19		H	0.003	0.470
20		H	0.011	2.2
21		H	0.012	9.53
26	H		0.240	24.4
28	H		0.120	
29	H		0.060	
30			0.073	7.8

more favorable interaction of 4-aroylaminophenylalanine, such as **2**, with the VLA-4 receptor via the amide carbonyl group. Reports from this laboratory on a class of imide analogues[7b] further suggested the involvement of the amide carbonyl in the receptor recognition.

In summary, the potent VLA-4 antagonist activity of *N*-(benzylpyroglutamyl)phenylalanines together with the X-ray crystal structure of **25** suggested that this class of compound can effectively pre-organize into the bioactive conformation presented by the cyclic peptide such as **1**. A remarkable finding from this work is that a wide variety of sub-structure motifs are tolerated on the aromatic ring of the *N*-Acyl-Phe class of VCAM/VLA-4 antagonists. However, the exact nature of interaction with integrin receptor may vary between chemical classes. The results further suggested that members of *N*-Acyl-Phe class mimic the pharmacophores presented by the cyclic peptide core structure. Substitution on the phenylalanine interacts with binding sites different from that occupied by the tyrosine of **1**. Comparison of pairs of 3- versus 4-substituted analogues **9** versus **26**, **16** versus **28**, **20** versus **29** and **21** versus **30** firmly imply that substitutions in the 4-position of *N*-Acyl-Phe is favored over that in the 3-positions.

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8. Unpublished results. General procedure for carbonylation was described as following: To a pressure bottle (250 mL) were added compound **7** (5.28 g, 10 mmol), $Pd(AcO)_2$ (0.23 g, 0.1 mmol), *N*-[(dimethylethoxy)-carbonyl]piperazine (7.45 g, 40 mmol), 1,3-bis(diphenyl-phospino)propane (0.42 g, 0.10 mmol) in a mixture of DMSO (50 mL). The bottle was pressured with CO to 40 psi and the pressure released. After four cycles of pressure/release, the bottle was charged with 40 psi CO and stirred at 80 °C for 3 h. After cooling to room temperature, the mixture was diluted with 400 mL of ethyl acetate and washed with water (2×50 mL), 1N HCl (2×50 mL), saturated $NaHCO_3$ (50 mL) and saturated brine (50 mL). The solvent was then removed and the residue was filtered through silica gel eluting with ethyl acetate to give 4-[[4-[(dimethylethoxy)carbonyl]-1-piperazinyl]carbonyl]-*N*-[5-oxo-1-(phenylmethyl)-L-prolyl]-L-phenylalanine methyl ester (3.8 g, 64.5%), HRMS: obs mass, 593.2994. Calcd mass, 593.2975.
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10. X-ray report: compound **25** was crystalized from ethyl acetate, mp 125–125 °C, [α]: + 71.2 (*c* 1, MeOH), crystal size (mm): 0.15×0.25×0.45, space group: P1, Rw: 0.048. The atomic coordinates are provided to CCD with deposition number CCDC169413.
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