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Focused Library Approach for Identification of New *N*-Acylphenylalanines as VCAM/VLA-4 Antagonists

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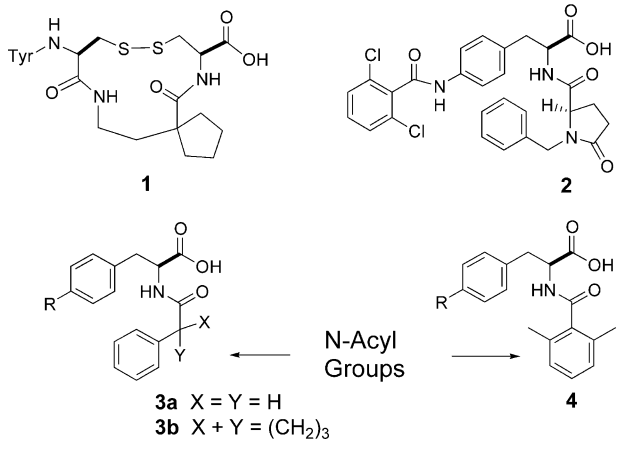
Abstract—A structure-based focused library approach was employed in an effort to identify more lipophilic replacements for the *N*-benzylpyroglutamyl group of the VCAM/VLA-4 antagonist **2**. This effort led to the discovery of two new classes of potent antagonists characterized by the *N*-(α-phenylcyclopentanoyl- and the *N*-(2,6-dimethylbenzoyl)-derivatives **60** and **64**. © 2002 Elsevier Science Ltd. All rights reserved.

The very late antigen 4 (VLA-4, $\alpha_4\beta_1$) is an integrin receptor expressed on many lymphocytes that mediates cell adhesion, infiltration and survival of these cell types, 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 have been intensively sought for the treatment of inflammatory diseases such as asthma.[1,2]



In previous papers,[3] we have described a conformational analysis and the potent VCAM/VLA-4 antagonist activity of cyclic peptide **1**. We have also reported on a class of antagonists characterized by **2** and developed the hypothesis that they mimic the cyclic peptide's interaction with the VLA-4 integrin.[4d]

Compounds related to **2** are rapidly cleared when administered iv to mice and these findings prompted us to employ a structure-based library design in an effort to identify a more lipophilic replacement of the *N*-benzylpyroglutamyl moiety. Herein, we report the implementation of such an approach that led to the identification of the new lead series characterized by **3** and **4**.

Recent reports on CH–π interactions suggested that both phenyl–phenyl and alkyl–phenyl interactions are dominated by dispersion forces where the surface contact between the CH and the aromatic ring is the major driving force.[5] Thus CH–π interactions are postulated to play an important role in protein folding and molecular recognition, as well as conformational pre-organization of small molecules. We designed compounds **3b** and **4** based on the hypothesis that an intramolecular CH-π interaction may facilitate the *N*-acylphenylalanines to adopt a gauche (−) conformation which mimics the core structure of potent peptide VCAM/VLA-4 inhibitors such as **1**. The published X-ray crystal structure of *N*-phenylacetyl-L-phenylalanine (**3a**) features a *gauche* (−) conformation of the phenylalanine and an edge-to-face interaction between the two benzene rings as shown in Figure 1.[6] The methylene hydrogens of **3a** were morphed into a cyclopentane ring and the resulting compound **3b** was minimized using the Sybyl molecular modeling program.[7]

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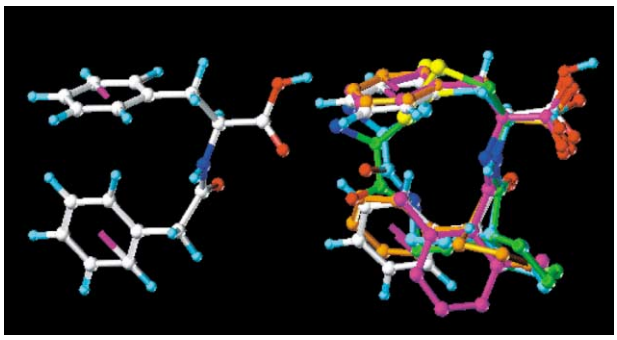


Figure 1. (left) X-ray structure of **3a** showing the CH–π interaction and (right) an overlay of NMR structures of **1** (in green and cyan), X-ray of **3a** and computer models of **3b** (in orange) and **4** (in magenta).

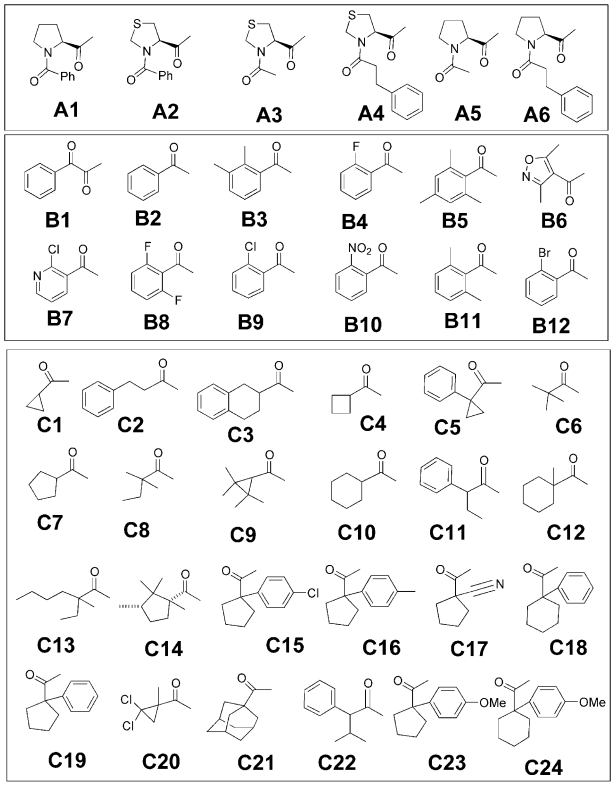
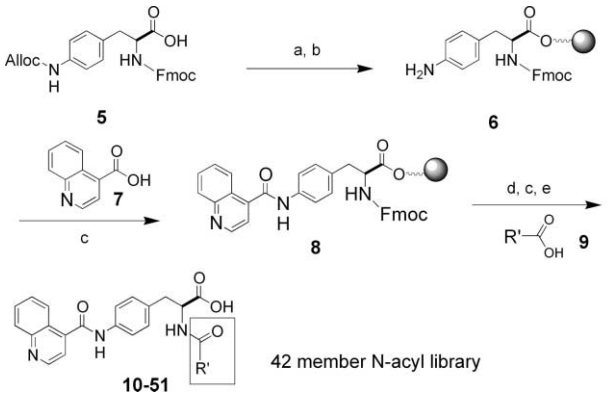


Figure 2. Building blocks of *N*-acyl focused library.

A preliminary study of *N*-aroyl-phenylalanines[4a] prompted us to consider replacement of the *N*-benzyl-pyroglutamyl moiety of **2** with a 2,6-dimethylbenzyl group as well to give compound **4**. Replacement of the benzyl group in **3a** with a 2,6-dimethylbenzoyl group and minimization gave the conformer of **4** shown in Figures 1 and 2. In this conformation one of the methyl groups of **4** is in close proximity to the benzene ring of L-phenylalanine and provides a CH–π interaction that could facilitate pre-organization of the molecule into the desired *gauche* (−) conformation. An overlay of the modeled conformations of **3b** and **4** with the NMR structure of the peptide core of **1** is shown in Figure 1. Compounds **3b** and **4** present the 5-membered ring and an *ortho*-methyl group to the putative receptor hydrophobic site occupied by the cyclopentyl group in compound **1**.



Scheme 1. (a) Wang resin, BOP, NMP; (b) $Pd(Ph_3P)_2Cl_2$. *N*-Bu_3SnH, HOAc, DCM, rt; (c) acid **7**, BOP, DIEA, NMP, rt; (d) 25% piperidine, NMP, rt; (e) 90% TFA, DCM, rt.

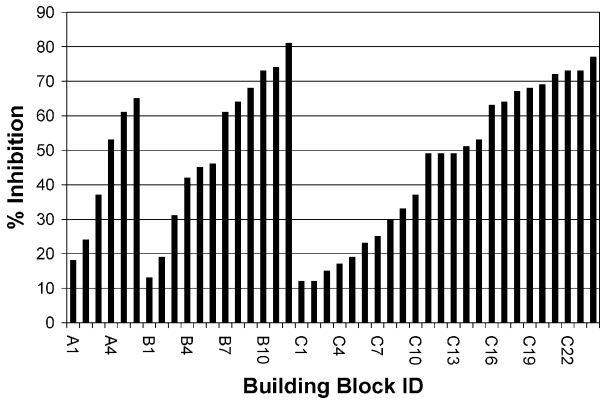


Chart 1. VCAM/VLA-4 inhibition screening at 10 nM in ELISA assay.

The *N*-acylphenylalanine focused library was designed to quickly explore the two new classes of *N*-acyl groups. As a reference set, we have also incorporated *N*-acyl-prolinyl building blocks (class A). Selection of the aroyl (class B) and the alkanoyl (class C) are based on the desire for rapid preliminary SAR development and availability of these building blocks from our in house collection (Fig. 2).

The synthesis of the focused library started with alloc- and Fmoc- orthogonally protected 4-aminophenylalanine (**5**) as shown in Scheme 1. Loading **5** to Wang resin using BOP reagent followed by removal of the alloc group gave compound **6**, which was coupled with 4-quinolinecarboxylic acid (**7**) to give **8**. Removal of the Fmoc followed by BOP mediated coupling of the three classes of building blocks (**9**) gave the focused library after cleavage from Wang resin using TFA in DCM. Library products were analyzed by HPLC using UV detection at a wavelength of 214 nM. Products with purity greater than 80% were screened in a VCAM/VLA-4 ELISA assay using VCAM-1 coated plates allowing VLA-4 derived from Ramos cells to compete with test compounds at 1 and 10 nM. The % inhibition of library members at 10 nM is shown in Chart 1. Compounds that showed greater than 50% inhibition at 10 nM were selected for purification and were further evaluated in the VCAM/VLA4 ELISA assay[4c] to determine the IC_{50} values as shown in Table 1.

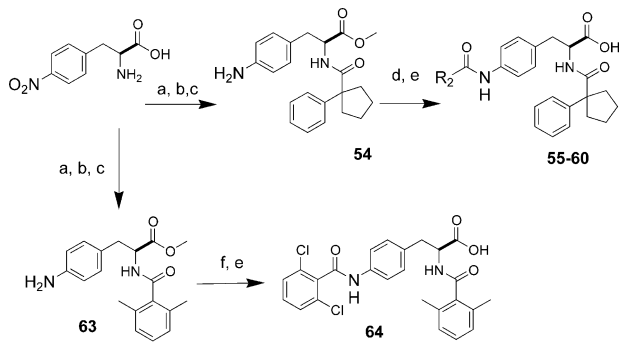
Results from screening at 10 nM showed a trend in the SAR within each building block class. As expected, compounds which gave >50% inhibition in the screen showed IC_{50} values lower than 10 nM. For analogues derived from building blocks of class A, *N*-acetyl- or substituted *N*-acetylprolinyl was better than *N*-benzoylprolinyl. In the case of analogues derived from building blocks of class B, it appears that an *ortho*-substitution is required for good activity and substitutions at both *ortho* positions are preferred. This result is in agreement with our model in which the aromatic ring of the *N*-aroyl group is preferentially aligned orthogonal to the plane of the amide bond in order to present an *ortho*-substituent as a receptor recognition element in the space occupied by the cyclopentyl group of cyclic peptide VCAM/VLA-4 antagonist **1**.

Among the compounds derived from building blocks in class C, several trends were observed. First, analogues derived from unsubstituted cyclic alkanoyl groups (building blocks: **C1**, **C4**, **C7**, and **C10**) were lower in potency compared with the corresponding α-substituted derivatives, for example, the α-phenyl species (building blocks: **C5**, **C18**, and **C19**). Secondly, in both groups, there was a tendency for increased potency associated with increased ring size. Thus compounds with three- and four-membered rings were relatively weak inhibitors compared to those incorporating five- and six-membered rings, which were essentially equipotent (building blocks: **C23**, and **C24**). Third, there appears to be a modest substitution effect in analogues derived from the α-(4-substituted-phenyl)cyclopentane carboxylic acids (**C15**, **C16**, **C19**, and **C23**), indicating a trend for increasing potency Cl, Me < H < MeO. We infer from these results that the five- and six-member cycloakanoyl rings mimic the hydrophobic cyclopentyl group of cyclic peptide **1**, while the α-substitutions facilitate pre-organization of the molecule into the preferred *gauche* (−) conformation through an aromatic-aromatic interaction. The hydrophobic group at the *N*-cap region seems essential for the potency and their spatial orientation is governed, at least in part, by the α-substituents of the class C analogues.

Table 1. VCAM/VLA4 inhibition activity of focused *N*-acyl library in ELISA assay

Compd	BB	% Inhib[a]	IC_{50} (nM)	Compd	BB	% Inhib[a]	IC_{50} (nM)
10	**A1**	18		**31**	**C4**	17	
11	**A2**	24		**32**	**C5**	19	
12	**A3**	37		**33**	**C6**	23	
13	**A4**	53	1.9	**34**	**C7**	25	
14	**A5**	61	2	**35**	**C8**	30	
15	**A6**	65	2.1	**36**	**C9**	33	
16	**B1**	13		**37**	**C10**	37	
17	**B2**	19		**38**	**C11**	49	5.8
18	**B3**	31		**39**	**C12**	49	7
19	**B4**	42		**40**	**C13**	49	8.2
20	**B5**	45		**41**	**C14**	51	2.3
21	**B6**	46		**42**	**C15**	53	6.4
22	**B7**	61	6.6	**43**	**C16**	63	9.1
23	**B8**	64	1.7	**44**	**C17**	64	7.6
24	**B9**	68	2.9	**45**	**C18**	67	5.6
25	**B10**	73	4.7	**46**	**C19**	68	3.4
26	**B11**	74	2.5	**47**	**C20**	69	3
27	**B12**	81	2.2	**48**	**C21**	72	2.4
28	**C1**	12		**49**	**C22**	73	4.6
29	**C2**	12		**50**	**C23**	73	1.4
30	**C3**	15		**51**	**C24**	77	1.2

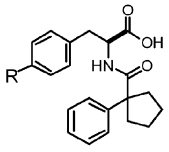
[a]% Inhibition at 10 nM of library compound in VCAM/VLA-4 ELISA assay.

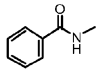
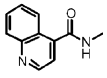
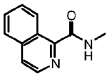
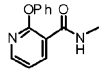
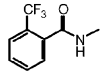
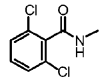


Scheme 2. (a) MeOH, HCl, rt, overnight; (b) HBTU, **B11** or **C19**, DIEA, DMF; (c) $SnCl_2$, DMF; (d) RCOOH, HBTU, DIEA, DMF; (e) 1 N NaOH, EtOH; (f) 2,6-dochlorobenzoyl chloride, DIEA.

Following up the results from this focused library, we decided to evaluate the effect of the substituent at the 4-position of *N*-acyl-L-phenylananines on the *N*-acyl analogues derived from α-phenylcyclopentyl-carboxylic acid (**C19**) and 2,6-dimethylbenzene carboxylic acid (**B11**). Their synthesis is shown in Scheme 2. The more potent compounds were further evaluated in a cell based assay in which the ability of compounds to inhibit the binding of fluorescently labeled Ramos cells to human VCAM coated 96-well plates was determined.[4c]

Table 2. VCAM/VLA4 inhibition activity of focused *N*-acyl library and follow up analogues in ELISA and Ramos cell based assay



Compd	R	ELISA (IC_{50}, nM)	Ramos (IC_{50}, nM)
2		0.37	12
55		23	
46		2.7	590
57		9.8	1300
58		6.1	2400
59		10	
60		1.7	98
64		1.2	81

The results summarized in Table 2 is in agreement with the trend observed previously among *N*-benzyl-pyroglutamyl derivatives of compound **2**.[4b] Excellent activity was seen with the 4-[(2,6-dichlorobenzoyl)-amino]- group in both series as evidenced by compounds **60** and **64**.

Recently, the Merck group reported a class of potent VLA4 inhibitors that contain class A type of building blocks in the *N*-acylphenylalanines. In that case, the most potent compounds were derived from the *N*-(3,5-dichlorobenzenesulfonyl)-L-Prolyl group.[8] It has also been reported that **A3** and **A5** analogues of 4-substituted-L-phenylalanine are potent VLA-4 inhibitors.[9] Most of these compounds suffered fast clearance in rodent PK studies as we have observed on the analogues of **2**.

In summary, we have identified two new classes of potent VCAM/VLA-4 antagonists, **3** and **4**, through a structure-based lead generation process. The results suggest that increased potency of the *N*-acylphenylalanine is associated with the capability of the *N*-acyl moiety to facilitate the pre-organization of the molecule and to present a receptor recognition element mimicking the cyclopentyl or thioprolyl group of the cyclic peptide VLA-4 antagonists.[3] This focused library approach provided a fast entry into new classes *N*-acylphenylalanine series and provides a good starting point for the further lead optimization of these classes of VCAM/VLA-4 antagonists. The VCAM/VLA-4 antagonist activity and the X-ray structures of the derivatives of **60** and **64** will be reported in due course to further support the SAR of these classes.[10]

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