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N-Cycloalkanoyl-L-Phenylalanine Derivatives as VCAM/VLA-4 Antagonists

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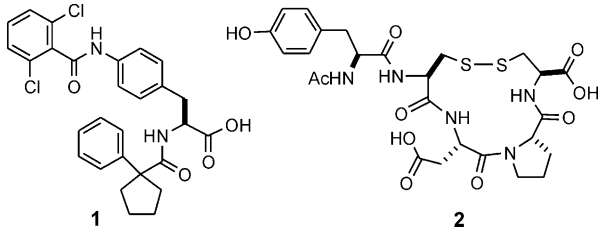
Abstract—A systematic structure–activity relationship investigation of the lead compound **1** resulted the identification of several *N*-[(substituted alkyl)cycloalkanoyl]-4-[((2,6-dichlorophenyl)carbonyl)amino]-L-phenylalanine derivatives as potent VCAM/VLA-4 antagonists. The data are consistent with a model of these compounds in which these alkanoylphenylalanines reside in a compact gauche (−) bioactive conformation. © 2002 Elsevier Science Ltd. All rights reserved.

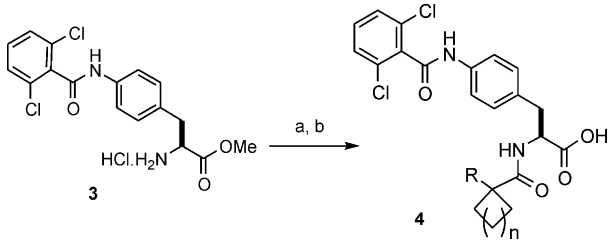
The integrin receptor VLA-4 ($\alpha_4\beta_1$) is expressed on a variety of leucocytes including B-cells, T-cells, basophils and eosinophils and is involved in the recruitment, activation and survival of these cell types.[1] Data supporting a role for VLA-4 in a number of inflammatory diseases including asthma, rheumatoid arthritis, inflammatory bowel diseases, multiple sclerosis, atherosclerosis, and diabetes have emerged and is summarized in recent reviews.[2] This putative role of VLA-4 in disease has prompted an intense search for effective inhibitors of the VCAM–VLA-4 interaction.

We have previously reported identification of the potent α-branched cycloalkanoyl phenylalanine derivative **1**, which was designed on the premise that it mimicked essential pharmacophoric features of a NMR derived three dimensional model of the cyclic peptide **2**.[3] Key common elements of the two classes of inhibitors include the five-membered ring and the carboxylic acid with the phenylalanine aromatic ring of **1** occupying the same region of space as the disulfide moiety of **2**.[4,5]

We now report a detailed investigation of the structure–activity relationship of cycloalkanoyl derivatives related to **1** as well as the X-ray crystal structure of the methyl ester of **16** that is fully consistent with our bioactive conformational hypothesis.

The new analogues were generally prepared from the L-phenylalanine derivative **3**[6] in two straight forward steps as shown in Scheme 1. The desired 1-(substituted alkyl)cycloalkanecarboxylic acids were generally prepared by alkylation of the anion derived from methyl cycloalkanecarboxylic acid esters as depicted in Scheme 2. The *p*- or *m*-(1-methyltetrazol-5-yl)benzyl chlorides were prepared in three steps from the corresponding





Scheme 1. (a) 1-(Substituted alkyl)cycloalkanecarboxylic acid, HBTU, DIPEA, DMF, rt, 15–48 h; (b) 1.0 N NaOH, EtOH, 50–55 °C, 15 h.

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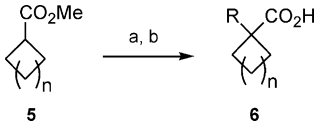


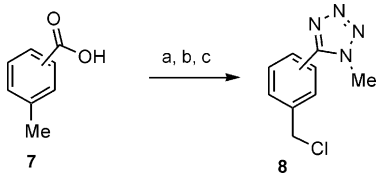
Scheme 2. (a) LDA, THF, −70 °C, RI, and warm to rt, 15 h; (b) 1.0 N NaOH, MeOH, THF, reflux, 15 h.

toluic acid as illustrated in Scheme 3. The methylthioalkyl-, methylsulfonylalkyl- (**10**) and azidoalkyl (**11**) cycloalkane carboxylic acids were obtained from a common bromoalkyl cycloalkane carboxylate intermediate **9** as shown in Scheme 4.[7]

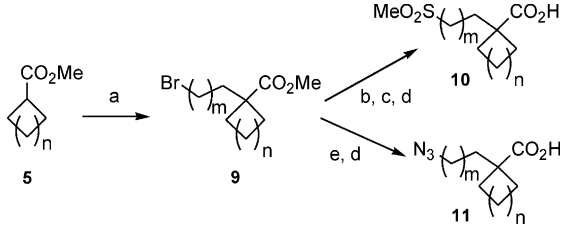
In the case of amines, after coupling of azidoalkyl cycloalkane carboxylic acids, **11** with **3**, the azides were converted to the corresponding amines using 1.0 M trimethylphosphine in toluene followed by quenching with water.[7] Subsequent amine acylation followed standard protocols to give the compounds in Table 3.

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]

They were further evaluated in a cell based assay for their ability to block the interaction between fluorescently labeled Ramos cells, which express VLA-4, with VCAM coated microtiter plates. The ELISA assay does not discriminate well among compounds with IC_{50}s < 1.5 nM, and we rely on the more stringent cell based assay using Ramos cells to rank order potency.



Scheme 3. (a) NCS, benzoylperoxide, chlorobenzene, 95 °C, 8 h; (b) $SOCl_2$, toluene, 85–90 °C, 15 h, then $MeNH_2HCl$, DIPEA, CH_2Cl_2, 0 °C and warm to rt, 1 h; (c) $SOCl_2$, toluene, 85–90 °C, 15 h, then NaN_3, TMSCl, CH_3CN, 0 °C to rt, 15 h.



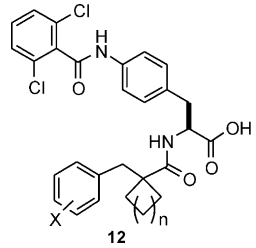
Scheme 4. (a) LDA, THF, −70 °C, 1,ω-dibromoalkane and allow to warm to rt, 15 h; (b) NaSMe, DMF, rt, 15 h; (c) H_2O_2, AcOH, 70 °C, 15 h; (d) 1.0 N NaOH, MeOH, THF, reflux, 15 h; (e) NaN_3, DMF, 50 °C, 15 h.

In an effort to improve on the potency of compounds related to **1**, a series of homologous 1-benzyl cycloalkanoyl derivatives was prepared (Table 1). Although, the unsubstituted benzyl derivative **12a** was relatively ineffective, addition of a substituent in the 3- or, preferably, the 4-position lead to a significant improvement in potency. In contrast to the case of direct analogues of **1**,[5] comparison of the 4-methoxy analogous **12b** and **12h** and the tetrazoles **12i**–**12k** suggest that four- and five-membered cycloalkyl rings are preferred over the corresponding six-membered rings. Although, we were able to improve the potency of the benzyl derivatives from triple digit to the low double digit nanomolar range, their molecular weight became of concern and we chose to investigate simpler analogues that might achieve the same conformational preference.

This thinking led us to prepare the alkyl and substituted alkyl derivatives shown in Tables 2 and 3. The data in Table 2 suggest the following: (a) a variety of functional groups including methoxy, hydroxy, cyano, methylthio, and methylsulfonyl are well tolerated, (b) in general, 2-carbon alkyl chain analogues have highest affinity with the exception of methylsulfonyl analogues where a four-carbon chain is preferred (**29** and **30**), (c) comparison of the methoxyethyl analogues **16** and **17** and the methylsulfones **29** and **30** indicate again that there is little difference in activity between the cyclobutyl and cyclopentyl derivatives.

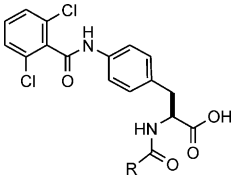
Most of the aminoalkyl derivatives (Table 3) have moderate to excellent activity in both assays. Particularly noteworthy are the acetamide (**34**) and the urea **41**.

Table 1. VCAM/VLA-4 binding inhibition of *N*-[(1-benzyl)cycloalkanoyl]-4-[((2,6-dichlorophenyl)carbonyl)amino]-L-phenylalanine derivatives



Compd	X	*n*	ELISA IC_{50} (nM)	Ramos IC_{50} (nM)
1	—	—	1.7	99
12a	H	2	6.6	340
12b	4-OCH_3	2	0.6	37
12c	4-CN	2	0.5	38
12d	3,4-$(OCH_3)_2$	2	0.8	95
12e	3-OCH_3	2	1.1	170
12f	3-(1-methyl-tetrazol-5-yl)	2	0.4	100
12g	2-Cl	2	1.6	350
12h	4-OCH_3	3	2.5	250
12i	4-(1-Methyl-tetrazol-5-yl)	1	0.42	27
12j	4-(1-Methyl-tetrazol-5-yl)	2	0.47	19
12k	4-(1-Methyl-tetrazol-5-yl)	3	1.3	61

Table 2. VCAM/VLA-4 binding inhibition of *N*-[(1-alkyl)cycloalkanoyl]-4-[((2,6-dichlorophenyl)carbonyl)amino]-L-phenylalanine derivatives



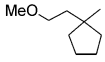
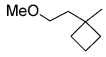
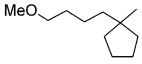
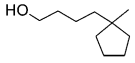
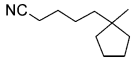
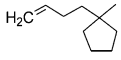
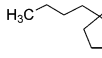
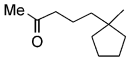
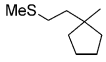
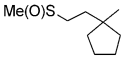
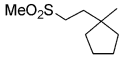
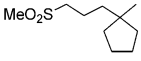
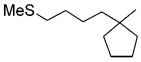
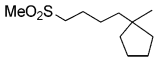
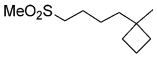
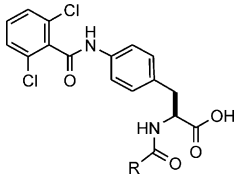
Compd	R	ELISA IC_{50} (nM)	Ramos IC_{50} (nM)
13		1.0	134
14		1.1	96
15		1.5	104
16		0.26	12
17		0.61	17
18		0.32	29
19		0.50	27
20		0.52	39
21		0.55	64
22		1.1	150
23		7.8	350
24		0.29	62
25		0.21	25
26		0.8	75
27		1.8	528
28		1.1	94
29		0.15	8
30		0.1	6

Table 3. VCAM/VLA-4 binding inhibition of *N*-[(1-(aminoalkyl))cycloalkanoyl]-4-[((2,6-dichlorophenyl)carbonyl)amino]-L-phenylalanine derivatives



Compd	R	ELISA IC_{50} (nM)	Ramos IC_{50} (nM)
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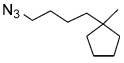
32		1.64	142
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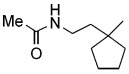
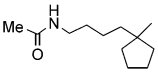
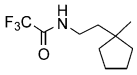
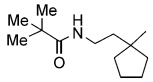
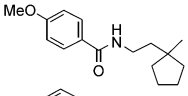
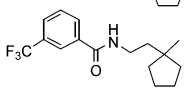
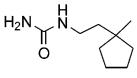
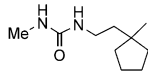
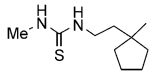
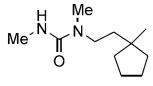
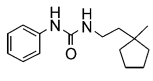
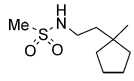
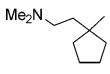
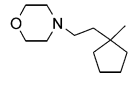
34		0.37	8
35		3.6	128
36		0.64	47
37		1.15	28
38		40	635
39		14	1034
40		0.46	36
41		0.1	11
42		0.14	22
43		1.15	19
44		5.8	914
45		0.33	27
46		3.4	89
47		2.4	151

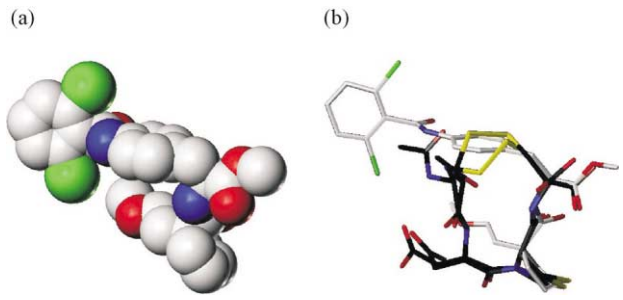


Figure 1. (a) Spacefill representation of the crystal structure of the methyl ester of **16** (**48**); (b) overlay of the two most populated conformations of cyclic peptide **2** as determined by NMR (black) with **48** (grey).

Increases in the size of alkanoyl group led to a decrease in potency as evidenced by examples **36**–**37** and **42**–**43**. The presence of an aryl group either as part of an amide (**38**–**39**) or urea (**44**) is detrimental. Among the amides, acetamido ethyl (**34**) is preferrable to acetamido butyl (**35**) and the presence of a basic nitrogen atom in the side chain is unfavorable (**33**, **46**, and **47**).

As previously noted, we believe that the cycloalkanoyl phenylalanine derivatives related to **1** and **16** are capable of conformationally mimicking the cyclic peptidic VCAM-VLA-4 inhibitor **2**. In support of this notion, the crystal structure of methyl ester of **16** (**48**) was obtained. There are two molecules per unit cell which differ only by an approximately 180° rotation of the cyclopentyl ring about the amide bond. One of these is shown in Figure 1a.[8] As expected, the crystal structure reveals a low energy, compact, gauche (−) conformation for the phenylalanine. In one conformation the cyclopentyl ring occupies the same position as proline ring of cyclic peptide **2** and the 2-methoxy ethyl side chain makes a hydrophobic contact with phenylalanine aromatic ring. An overlay of this conformer with the NMR derived conformational model of the cyclic peptide **2** is shown in Figure 1b.

In summary, within a series of *N*-(α-substituted cycloalkanoyl)phenylalanine derivatives, a variety of ring sizes and functional groups are tolerated in the side chain. Furthermore, our finding that α-substituents ranging from phenyl and benzyl to ω-substituted alkyl suggests that these substituents may not be directly involved in the mediating receptor interaction, but may play an important role in defining the preferred conformation of the ligand through intramolecular interactions. Differences in the solvation of these functional groups may also play an important role and account for some of the more subtle potency differences between individual members of the class. We made similar observations and conclusions during the course of our work on *N*-aroyl phenylalanine derivatives.[6] During the course of this work, we succeed in identifying compact and practical replacements for the *N*-benzylpyroglutamyl group without loss of potency in our in vitro assays.

References and Notes

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8. X-ray: the methyl ester of **16** was crystallized from ethyl acetate, mp 220–222 °C, crystal size (mm) 0.11×0.13×0.18, space group $P22_12_1$, R = 0.0669, Rw = 0.0783. The atomic coordinates were provided to the CCD with deposition number CCDC185240.