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

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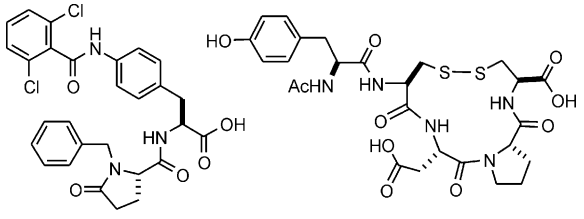
Abstract—A series of *N*-benzoyl-4-[(2,6-dichlorobenzoyl)amino]-L-phenylalanine derivatives was prepared in order to optimize the substitution on the *N*-benzoyl moiety for VCAM/VLA-4 antagonist activity. Disubstitution in the 2- and 6-positions is favored and a range of small alkyl and halogen are tolerated. A model of the bioactive conformation of these compounds is proposed. © 2002 Elsevier Science Ltd. All rights reserved.

The integrin 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] The variety of diseases potentially impacted has prompted an intense search for effective inhibitors of the VCAM/VLA-4 interaction.

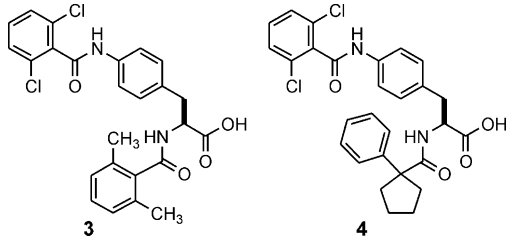
We have previously reported identification of the potent phenylalanine derivative **1** and crystal structure data supporting our proposal that *N*-benzylpyroglutamyl phenylalanine derivatives such as **1** mimic essential pharmacophoric features of a NMR derived three dimensional model of the cyclic peptide **2**.[3] Key common elements include the proline 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]

Pharmacokinetic studies of **1** in mice employing an ELISA based binding assay to determine blood levels indicate that this compound is cleared extremely rapidly ($t_{1/2}=15$ min) prompting us to explore a library approach to new *N*-acyl derivatives capable of conformationally mimicking the *N*-benzylpyroglutamyl moiety of **1**.



1 **2**

This work identified the *ortho*-substituted *N*-benzoyl analogue **3** and the *N*-[(1-phenyl)cycloalkyl]carbonyl derivative **4** as promising candidates for further exploration.[6] Herein, we describe the details of our structure–activity studies of *N*-benzoyl phenylalanines related to **3** and the X-ray crystal structure of the aniline



3 **4**

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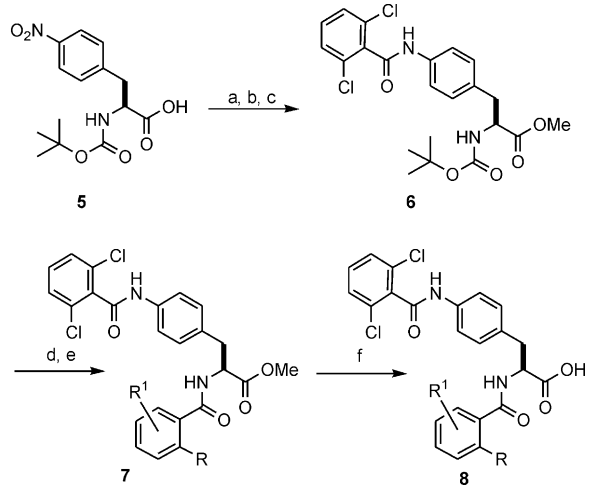
28, an intermediate in the scale up of one of the more potent members of this series, **8l**. This structure indicates that the molecule is able to adopt a compact, low energy, conformation that closely mimics the key pharmacophoric elements of the cyclic peptide **2**.

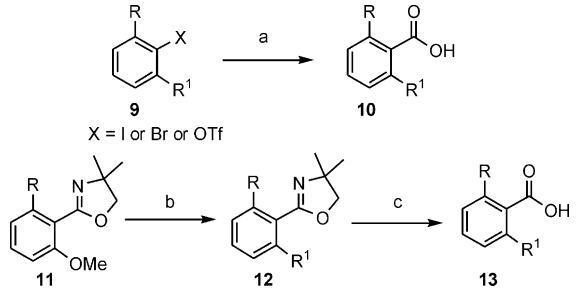
The new analogues were prepared as shown in Scheme 1 from Boc-4-nitrophenylalanine, **5**. *ortho*-Substituted benzoic acid derivatives that were not commercially available were prepared by conventional methods as shown in Scheme 2. For example, the *ortho*-disubstituted aryl bromides, iodides or triflates **9** were carbonylated in the presence of carbon monoxide, $Pd(OAc)_2$ and dppp in acetonitrile–water to obtain the corresponding acids **10**. Other approaches involved the treatment of the substituted 2-methoxyphenyloxazoline derivative, **11** with an alkyl Grignard reagent followed by hydrolysis of the oxazoline ring of **12** to give the desired acid **13**.[7] The benzoic acids required for the preparation of compounds **18** and **19** were prepared as shown in Scheme 3; (full experimental details are available in ref 8).

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).[8]

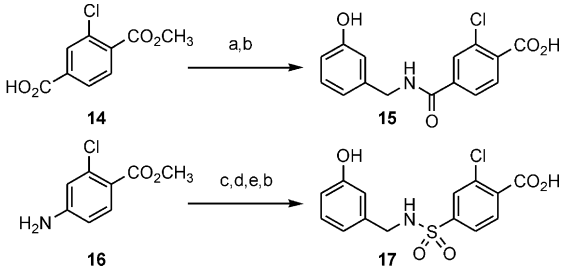
They were further evaluated in a cell based assay for their ability to block the interaction between fluorescently labeled Ramos cells, which express VLA-4, with VCAM coated microtiter plates. The ELISA assay does not discriminate well among compounds with IC_{50}s < 1.5 nM, we rely on the more stringent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



Scheme 1. (a) MeI, $NaHCO_3$, DMF, rt, 15 h, 98%; (b) Zn dust, NH_4Cl, MeOH, H_2O, 50 °C, 2 h, 98%; (c) 2,6-dichlorobenzoyl chloride, DIPEA, CH_2Cl_2, rt, 15 h, 99%; (d) 4.0 N HCl in dioxane, rt, 1 h, 97%; (e) aromatic acid chloride, DIPEA, CH_2Cl_2, rt, 15 h or aromatic acid, HBTU, DIPEA, DMF, rt, 15–48 h; (f) 1.0 N NaOH, EtOH, 45–50 °C, 4–15 h.

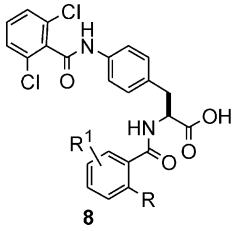


Scheme 2. (a) $Pd(OAc)_2$, dppp, CO, CH_3CN, H_2O, NEt_3, 83 °C, 15 h; (b) R^1MgCl, THF, −45 °C to rt, 15 h; (c) MeI, rt, 15 h then NaOH, MeOH, reflux, 15 h.



Scheme 3. (a) 3-Hydroxybenzylamine, HBTU, DIPEA, DMF; (b) 0.5 N NaOH, H_2O; (c) $NaNO_2$, concd HCl; (d) SO_2, HOAc, $CuCl_2$; (e) 3-Acetoxybenzylamine, NEt_3, CH_2Cl_2; (f) LiOH, 1:1 MeOH/THF.

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



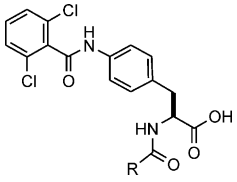
Compd	R	R^1	ELISA IC_{50} (nM)	Ramos IC_{50} (nM)
8a	Br	H	0.49	45
8b	OCH_3	H	8.6	6800
8c	$NHCH_3$	H	1.9	1000
8d	CF_3	H	16	650
8e	SCH_3	H	0.40	100
8f	SO_2CH_3	H	3.3	160
8g	$C(CH_3)_3$	H	1.9	74
3	CH_3	6-CH_3	1.3	84
8h	CH_3	6-C_2H_5	0.30	10
8i	CH_3	6-*n*-C_3H_7	0.42	17
8j	CH_3	6-*i*-C_3H_7	1.2	23
8k	CH_3	6-F	0.44	85
8l	CH_3	6-Cl	0.30	12
8m	CH_3	6-Br	0.20	9
8n	*i*-C_3H_7	6-*i*-C_3H_7	11	460
8o	F	6-F	1.2	67
8p	F	6-CF_3	0.40	85
8q	Cl	6-C(O)CH_3	1.6	49
8r	Cl	3-CH_3	1.4	1300
8s	Cl	3-Cl	2.2	1600
8t	Cl	5-CH_3	11	460
8u	Cl	5-CF_3	8.8	450
8v	Br	5-OCH_3	0.25	19
8w	Cl	4-OH	0.46	33
8x	Cl	4-OCH_3	1.9	350
8y	Cl	4-SO_2CH_3	0.25	9.5
8z	Cl	4-Br	1.60	1300

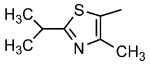
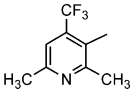
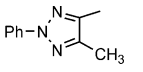
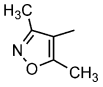
assay using Ramos cells to rank order potency. Of the mono *ortho*-substituted analogues, only the 2-bromo-, 2-methylthio- and 2-*tert*butyl- derivatives, **8a**, **8e** and **8g** were potent in both assays. In general, the best activity was seen among the 2,6-disubstituted analogues **3** and **8h–8q**. Among these, there is a wide tolerance for substitution by alkyl, from methyl to isopropyl and halogen, from fluorine to bromine. Only the 2,6-di-isopropyl derivative **8n** seems to exceed the favorable steric limitations of the binding site.

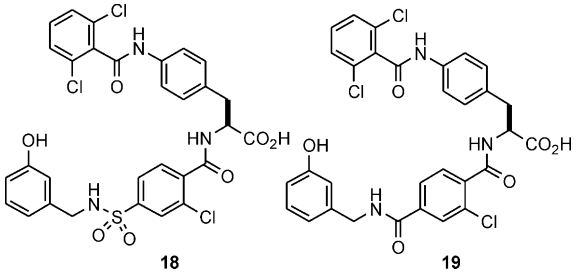
2,3-Disubstitution was unfavorable as evidenced by **8r** and **8s** whereas the activity of a limited number of 2,5-disubstituted examples is similar to that expected for the corresponding mono-*ortho* substituted analogues, suggesting that the substituents in the 5-position contribute little to binding.

The role of groups in the 4-position is somewhat enigmatic, particularly in light of the potent activity associated with **18** (IC_{50}s ELISA 0.50 nM, Ramos 110 nM) and **19** (IC_{50}s ELISA 0.53 nM, Ramos 76 nM), prepared as part of our LFA-ICAM inhibition program.[9] Given that the 2-chloro-4-hydroxy- (**8w**) and 2-chloro-4-methyl sulfonyl- (**8y**) derivatives are also potent inhibitors whereas the 2-chloro-4-methoxy- (**8x**) and 2-chloro-4-bromo- (**8z**) analogues are comparatively weak inhibitors, it is unlikely that the differences in potency are due to either steric or electronic effects. We thus conclude that in the bound conformation, the 4-position is unlikely to be in close contact with VLA-4 and that the differences we observe in potency may be due to solvation effects (Table 1).

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



Compd	R	ELISA IC_{50} (nM)	Ramos IC_{50} (nM)
20		1.5	890
21		0.56	69
22		2.0	180
23		0.89	23
24		—	200
25		2.0	190
26		25	1440
27		15	580



18 19

We also were interested in exploring *ortho*-substituted heteroaromatic rings in this series. The compounds in Table 2 were prepared from commercially available heterocycles using the general procedures indicated in Scheme 1. Whereas the 3-bromothiophen-2-yl analogue **20** was considerably less potent than the corresponding phenyl derivative **8a**, the 2,4-dimethyl-3-pyridinyl-compound **23** was slightly more potent than the corresponding phenyl derivative **3**. Substitution of the 4-methyl in **23** with a trifluoromethyl group (**24**) led to a decrease in activity. Of the other compounds in Table 2, only the thiazole **21** had good potency in both assay systems.

As stated in the introduction, we hypothesize that acyl and aroyl phenylalanines related to **1**, **3** and **4** are capable of conformationally mimicking the cyclic peptidic VCAM-VLA-4 inhibitor **2**. In further support of this notion, we obtained a crystal structure of the intermediate **28**, prepared during a scale up of **8l**. There are two molecules per unit cell which differ only by a 180° rotation of the 2-chloro-6-methylaryl ring about the amide bond. One of these is shown in Figure 1. As expected for a 2,6-disubstituted aroyl species, the plane of the aromatic ring is nearly orthogonal to that of the amide bond (87°). The X-ray structure reveals a compact, gauche (−) conformation of the phenylalanine with one of the *ortho*-substituents making a hydrophobic

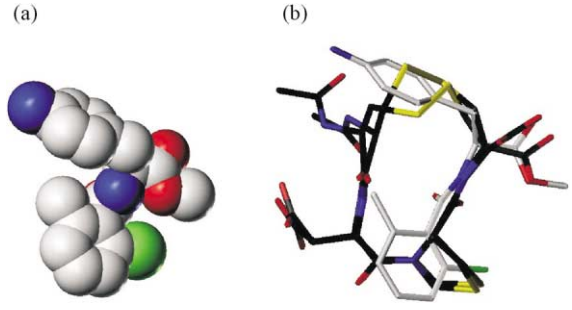
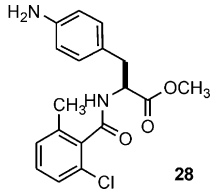


Figure 1. (a) Space filling representation of one conformer of the X-ray crystal structure of **28**. The second conformer differs only by a 180° rotation of the disubstituted aromatic ring with respect to the amide bond; (b) overlay of two NMR derived structures of the cyclic core of peptide **2** with **28**.

contact with the phenylalanine aromatic ring.[10] An overlay of one of these structures with the NMR derived conformational model of the cyclic peptide **2** is also shown in Figure 1 and is fully consistent with the concept that both classes of inhibitor bind to VLA-4 in a similar manner.



28

In the present work, 2,6-disubstitution is generally preferred although certain 2,4-disubstituted analogues are also highly potent. The requirement for at least one *ortho*-substituent suggests that a conformation in which the aromatic ring is rotated out of the plane of the amide bond is preferred. The lack of electronic effects in the present series and the comparable activity of cycloalkyl derivatives related to **4**[11] suggest that one of the groups in the *ortho* position makes a hydrophobic contact with VLA-4 and that this contact is important for efficient binding. Future papers in this series will document the pharmacokinetics and the in vivo pharmacology of members of this series.

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10. X-ray report: compound **28** was crystallized from ethyl acetate, mp: 100–102 °C, crystal size (mm): 0.17×0.22×0.86, space group: $P2_12_12_1$; Rw: 0.0604. The atomic coordinates were provided to CCD with deposition number CCDC185241.
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