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Imide and Lactam Derivatives of *N*-Benzylpyroglutamyl-L-phenylalanine as VCAM/VLA-4 Antagonists

Jefferson W. Tilley,* Gerry Kaplan, Karen Rowan, Virginia Schwinge and Barry Wolitzky

Roche Research Center, Hoffmann-La Roche Inc., 340 Kingsland Street, Nutley, NJ 07110, USA

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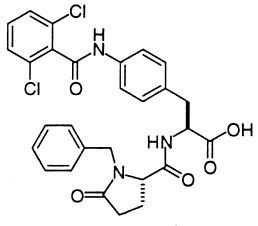
Abstract—A series of imides and lactams derived from 4-amino-*N*-benzylpyroglutamyl-L-phenylalanine was prepared and evaluated for activity as VCAM/VLA-4 antagonists. Imides were more potent than the corresponding lactams; several had subnanomolar IC_{50}s in an ELISA based assay and were also highly effective at blocking VLA-4 expressing Ramos cell binding to VCAM coated plates.

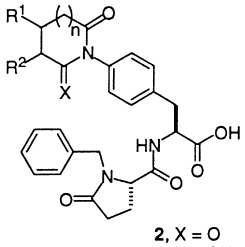
The integrin VLA-4, α4-β1, is expressed on a variety of lympocytes including B-cells, T-cells, basophils and eosinophils and is involved in modulating trafficking, activation and survival of these cell types.[1] Because of its potentially key role in regulating the inflammatory process, there is currently a great deal of interest in discovering compounds capable of interfering with the interaction of VLA-4 with its cognate receptor, VCAM. Such compounds are being considered for clinical trials in asthma,[2] multiple sclerosis,[3] and rheumatoid arthritis.[4]

We have previously reported the identification of a series of *N*-benzylpyroglutamyl 4-substituted-L-phenylalanine analogues that showed good potency as VCAM/VLA-4 antagonists in both ELISA and cell based assay formats.[5] Among the most potent compounds were those substituted by a benzoylamino group which incorporated both an *ortho* substituent and at least one electron withdrawing group such as **1**. As part of an effort to improve the pharmacokinetics of this class of molecule, we sought to investigate whether elimination of the *para*-amide NH- through incorporation of the nitrogen atom into a cyclic imide or lactam ring to give **2** or **3**, respectively, would be feasible.

While we recognized that the requirement for *ortho*-substituents in compounds related to **1** suggested that the π-system of the aromatic ring and the amide carbonyl would prefer to be out of conjugation, rather than co-planar as demanded by the proposed cyclic structures, our limited understanding of the details of the SAR of phenylalanine derived VCAM/VLA-4 antagonists prompted us to carry out the investigation described below. In this work we show that appropriate imides **2** are nearly equipotent with the amide **1** whereas the corresponding lactam derivatives **3** are several-fold less potent.



1



2, X = O
3, X = CH_2

The preparation of the imide derivatives is shown in Scheme 1 starting from Nα-Boc-4-Fmoc-aminophenylalanine **4**. Benzyl ester formation, cleavage of the Boc group, coupling with *N*-benzyl pyroglutamic acid and cleavage of the Fmoc group proceeded without incident to give the amine **5** despite the low solubility of the Fmoc containing intermediates. Formation of the imides was generally effected by treatment of **5** with an excess of the appropriate cyclic anhydride in the presence of excess carbonyl diimidazole. After stirring overnight, the reaction mixtures were concentrated, the residues dissolved in acetic acid and applied directly to a C-18 RP-HPLC column eluting with acetonitrile–water.

*Corresponding author. Tel.: +1-973-235-4660; fax: +1-973-235-6084; e-mail: jefferson.tilley@roche.com

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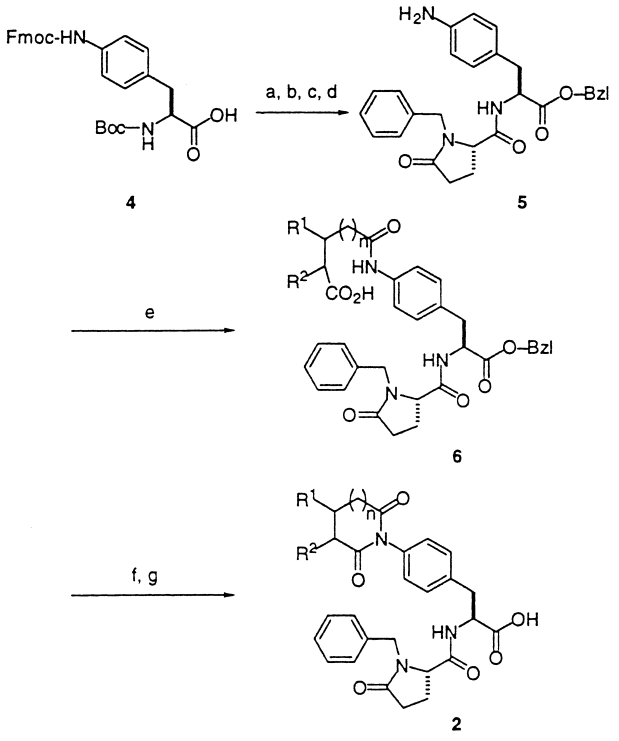
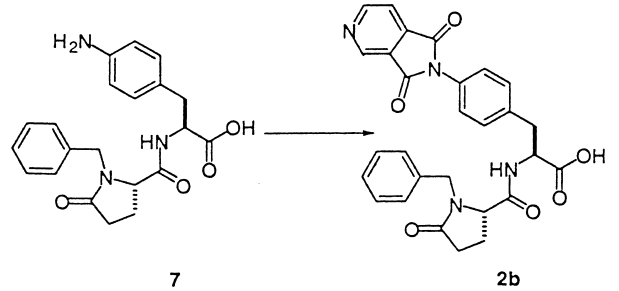


Scheme 1. (a) Benzyl-Br, $KHCO_3$, DMF, 18 h, 84%; (b) 4 N HCl, dioxane, 98%; (c) *N*-benzyl-L-pyroglutamic acid, HBTU, DIPEA, DMF, 95%; (d) diethylamine, DMF, 64%; (e) cyclic anhydride (3.4 equiv), carbonyl diimidazole (3 equiv), CH_2Cl_2; (f) carbonyl diimidazole, CH_2Cl_2 (if necessary); (g) H_2, Pd(C).

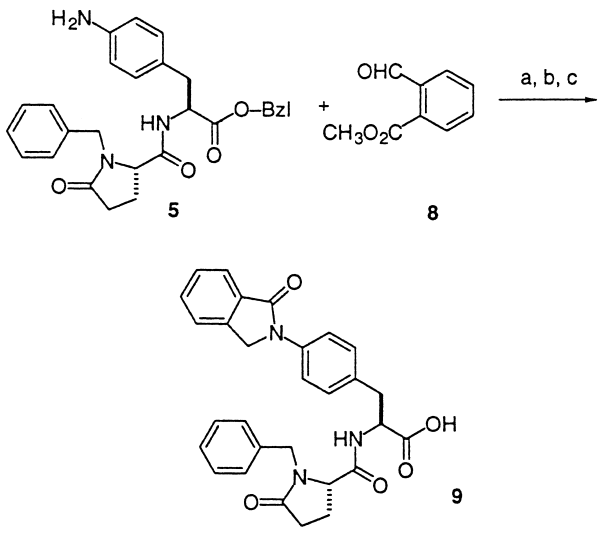
In the case of **2d** and **2f**, we obtained the intermediate amido acid **6** from this process and the carbonyl diimidazole treatment was repeated.

Finally, the benzyl ester was cleaved by catalytic hydrogenation. Typically, yields for the 2- to 3-step process were around 50%. For the preparation of the pyridinyl derivative **2b**, this process resulted in the partial hydrogenation of the pyridine ring. As an alternative, imide formation was carried out directly from the amino acid **7** by treatment with an excess of pyridine 3,4-dicarboxylic acid anhydride followed by carbonyl diimidazole in DMF. For the 4-hydroxyphenyl succinimide **2e**, we started with acetoxyphenyl succinic anhydride and the acetoxy group was lost during purification. In the case of the isobutyl succinimide **2h**, we started with the corresponding methylpropenylsuccinnic anhydride and saturated the double bond during ester cleavage.

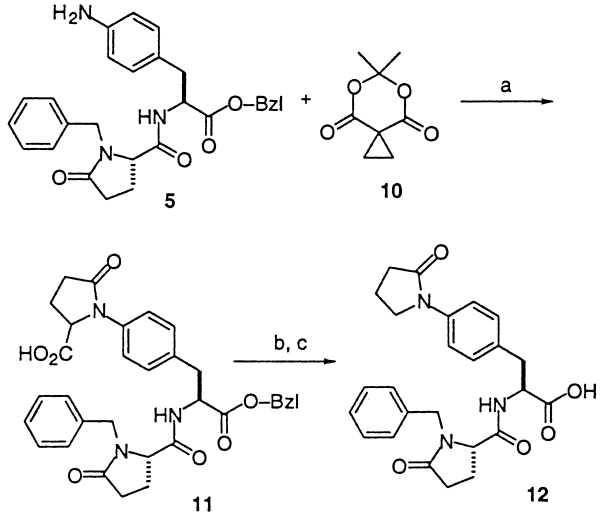


Scheme 2. (a) Pyridine 3,4-dicarboxylic acid anhydride, THF; (b) carbonyl diimidazole, DMF; (c) water.

The phthalide **9** was readily prepared by reductive amination of the amine **5** with 2-carbomethoxybenzaldehyde followed by heating the intermediate amino ester in acetic acid to effect ring closure and catalytic hydrogenolysis of the benzyl group as shown in Scheme 3. Refluxing a methylene chloride solution of the amine **5** and the ester **10** led through a nucleophilic ring opening and subsequent acylation to the carboxylic acid **11** (81%). Hydrogenolysis followed by thermal decarboxylation in DMSO at 100 °C then afforded the lactam **12** in 62% yield for the two steps (Scheme 4).



Scheme 3. (a) NaHBCN, HOAc, 4 A mol sieve, EtOH; (b) HOAc, reflux; (c) H_2, Pd(C).



Scheme 4. (a) CH_2Cl_2, reflux, 4 h (81%); (b) H_2, Pd(C) (77%); (c) DMSO, 100 °C, 12 h (81%).

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).[6] The data listed in Table 1 indicate that several compounds were potent inhibitors of the VCAM/VLA-4 interaction. In particular, the phthalimide **2a**, and the succinimide derivatives **2h** and **2j** each had subnanomolar activity and were only slightly less potent than **1**.

These compounds were further evaluated for their ability to block the interaction between fluorescently labeled Ramos cells, which express VLA-4, with VCAM coated microtiter plates. The rank order of potencies is the same as obtained in the ELISA assay and is consistent with our previous observation[5] that only compounds with subnanomolar potency in our ELISA assay show double digit nanomolar potency in the more demanding cell based assay. Again, the three most potent compounds were **2a**, **2h**, and **2j** and were only 2- to 5-fold less potent than **1**.

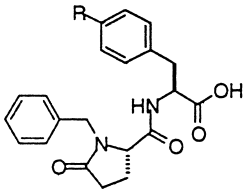
For comparison, the lactam derivatives **3a** and **3i** were prepared. As the data in Table 1 indicate, each was approximately 5-fold less potent than the corresponding imide in the ELISA assay. The more potent of the two (**3a**) was also evaluated in the Ramos cell assay and, as expected, found to have only weak activity.

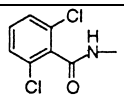
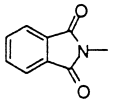
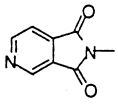
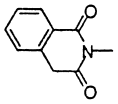
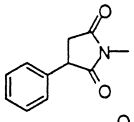
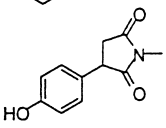
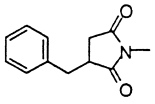
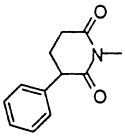
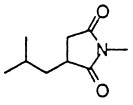
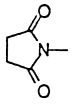
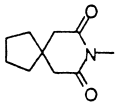
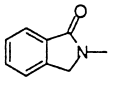
The relatively potent activity observed with members of the imide class is somewhat surprising since they are unable to adopt the same conformation as members of the prototype amide series such as **1**. As previously noted, activity in the later is favored by the presence of at least one *ortho*-substituent on an aromatic ring bearing at least one electron withdrawing group. The presence of the *ortho*-substituent on amides related to **1** should favor a conformation in which the aromatic π-system and the amide carbonyl are not fully conjugated. This is in marked contrast to the planar phthalimide **2a**. It is possible that the presence of the second carbonyl of the imide causes the entire phthalimido group to rotate with respect to the phenylalanine aromatic ring thus providing a comparable positioning of the phthalimido π-system to the aromatic π-system of **1**. In this case, the phthalide **3a** lacking the buttressing effect of the second carbonyl would be expected to be less active as observed.

While such arguments could account for the results obtained with the phthalimide **2a**, they do not explain the activity of the aliphatic imides, particularly **2i** and **2j**, which lack a comparable π-system. We previously speculated that the amide carbonyl of **1** may be acting as a hydrogen bond acceptor.[5] It is possible that the second carbonyl group of the imides is also involved in a binding or favorable solvent interaction thus compensating for the absence of a favorably oriented aromatic π-system.

It is also possible that subtle adjustments in the protein quaternary structure together with differences in the precise orientation of the ligand result in a different binding mode for members of the imide class of VCAM-4/VLA-4 antagonists. While further effort will be required in order to more fully understand the SAR of

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



No	R	ELISA (IC_{50} nM)	Ramos Cell (IC_{50} nM)
1		0.37	12
2a		0.52	20
2b		4.3	650
2c		0.98	107
2d[a]		1.7	92
2e[a]		2.6	420
2f[a]		0.94	123
2g[a]		0.99	184
2h[a]		0.76	41
2i		3.2	286
2j		0.54	57
3a		2.7	500
3i		16	–

[a]Mixture of *R*- and *S*-forms at the side chain chiral center.

the imides, the present work suggests that they offer an interesting new opportunity for the discovery of novel VCAM-4/VLA-4 antagonists.

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