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Synthesis and biological activity of N-substituted aminocarbonyl-1,3-dioxolanes as VLA-4 antagonists

Abdul Rehman [a,*], Ajay Soni [a], Keshav Naik [a], Sreeji Nair [a], Venkata P. Palle [a], Sunanda Dastidar [b], Abhijit Ray [b], M. S. Alam [c], Mohammad Salman [a], Ian A. Cliffe [a], Viswajanani Sattigeri [a]

[a] Department of Medicinal Chemistry, New Drug Discovery Research, Ranbaxy Research Laboratories, Plot 20, Sector 18, Gurgaon 122015, Haryana, India
[b] Department of Pharmacology, New Drug Discovery Research, Ranbaxy Research Laboratories, Plot 20, Sector 18, Gurgaon 122015, Haryana, India
[c] Department of Chemistry, Jamia Hamdard, New Delhi 110062, India

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ABSTRACT

A novel set of compounds with a 1,3-dioxolane ring which acts as a proline bioisostere have been successfully designed as VLA-4 receptor antagonists. Compounds (**18e**), (**28j**), and (**35g**) were shown to have high receptor affinities.

Very late antigen-4 (VLA-4, α4β1) is a member of the β1 subfamily of integrins and is expressed on all circulating leukocytes except platelets.[1,2] VLA-4 mediates both cell–cell and cell–matrix interactions by binding to cell surface ligands such as vascular cell adhesion molecule-1 (VCAM-1) and cell–matrix proteins such as fibronectin. These interactions are important for the activation, migration, and proliferation of leukocytes during normal and pathophysiological processes, and so the inhibition of VLA-4 is expected to be of therapeutic benefit in the treatment of a variety of inflammatory diseases.[3,4]

VLA-4 binds to a motif containing the sequence EILDVPST within VCAM-1, and peptidomimetic inhibitors of the critical leucine–aspartic acid–valine (LDV) sequence have been reported.[5] Non-peptidomimetic inhibitors have also been developed (Fig. 1) in

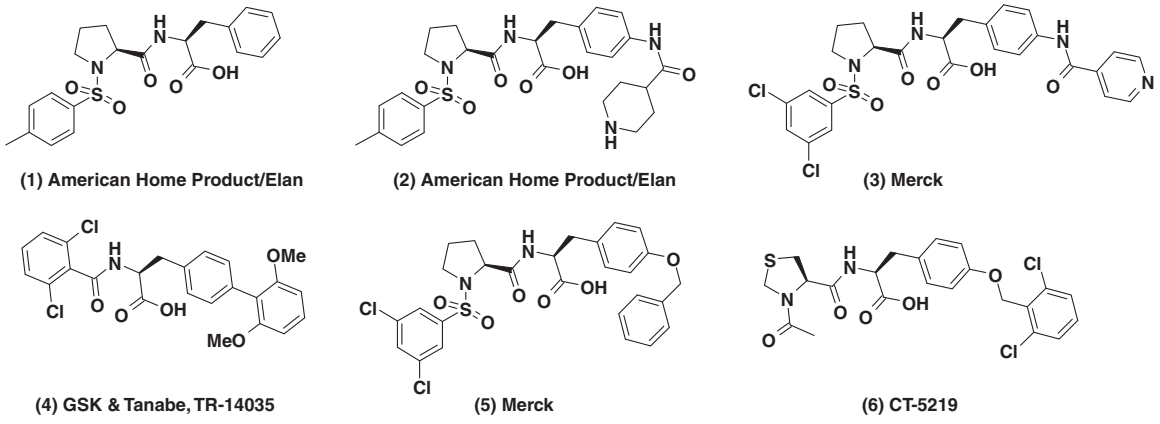

Figure 1. Potent VLA-4 inhibitors.

* Corresponding author. Tel.: +91 09999100317; fax: +91 124 2343545.
E-mail address: ab_rehman@rediffmail.com (A. Rehman).

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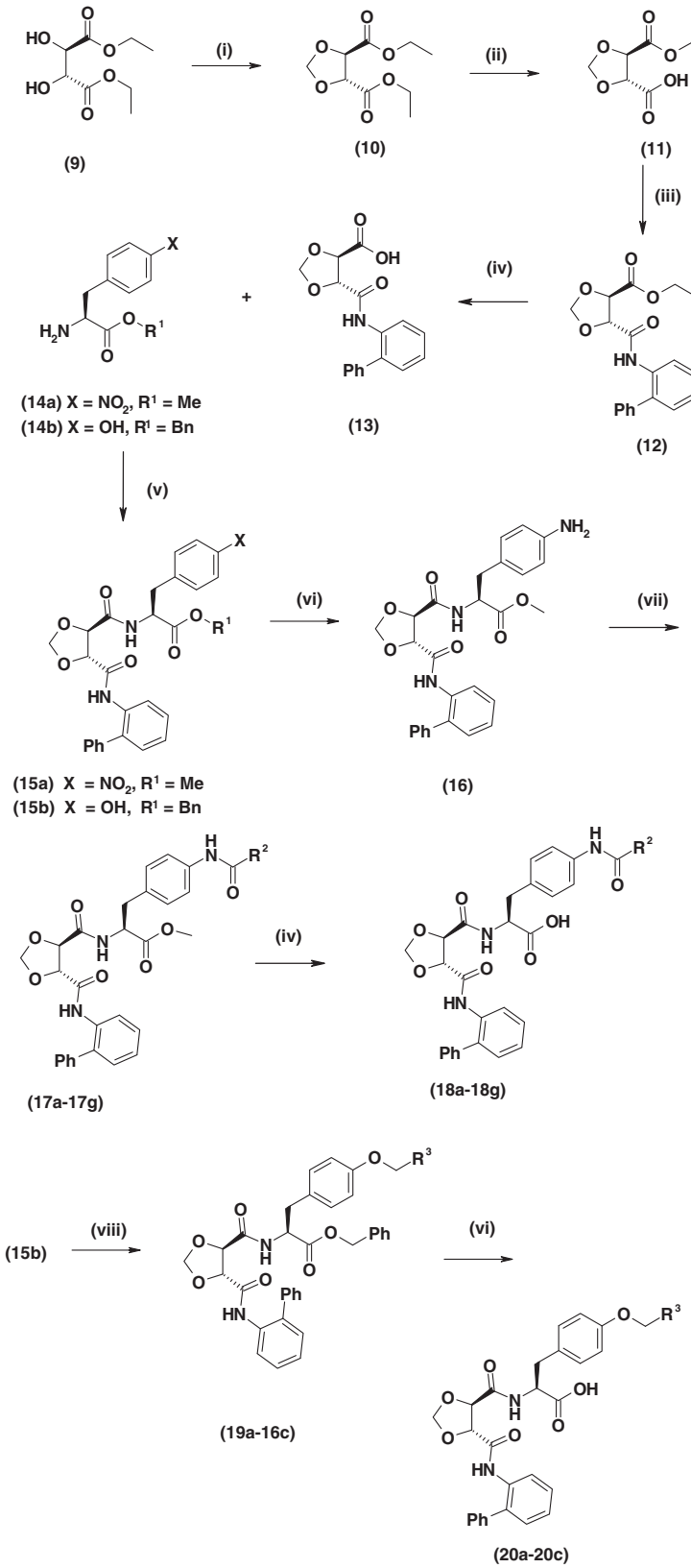
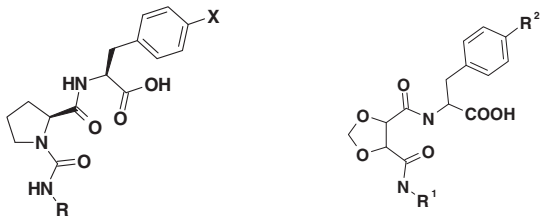


Scheme 1. Reagents: (i) dimethoxymethane, BF_3·EtO, isopropyl acetate, 100%; (ii) KOH, EtOH, 56%; (iii) 2-phenylaniline, EDC, NMM, HOBt, DMF, 67%; (iv) LiOH·H_2O, THF:MeOH:H_2O, 50–95%; (v) EDC, NMM, HOBt, DMF, 73%; (vi) 10% Pd/C, MeOH, 87%; (vii) R^2-COOH, EDC, NMM, HOBt, DMF, 45–75%; (viii) R^3-CH_2Br, K_2CO_3, acetone, 51–80%.

which an *N*-acylphenylalanine core appears to be critical for activity. Unlike the LDV-based mimics which in general are very selective for VLA-4, *N*-acylphenylalanine derivatives are dual inhibitors of VLA-4/VCAM and α4β7/MAdCAM (mucosal adhesion cell adhesion molecule) interactions. Elan has reported the SAR of compound (**1**) (CT-757) and shown that its oral bioavailability in rats



(7) (8)

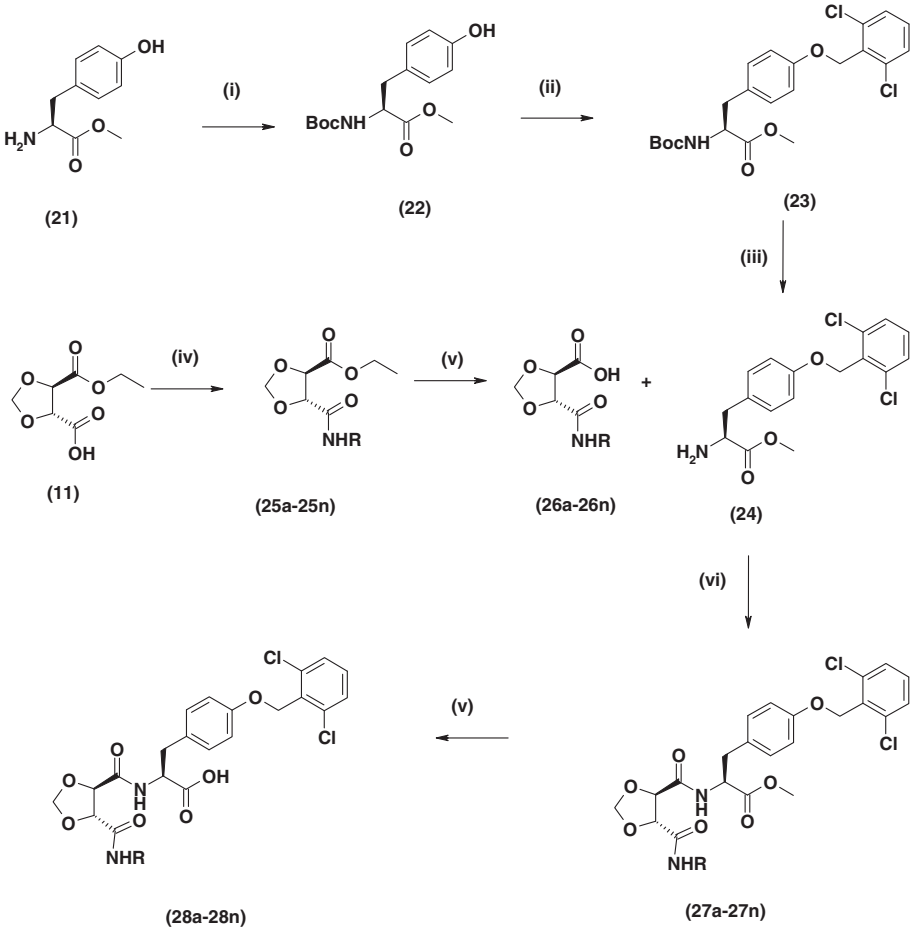
Figure 2. Proline-based and 1,3-dioxolane-based VLA-4 inhibitors.

was poor (10–15%) with most of the drug being eliminated rapidly in the bile.[6] Two potent analogs of compound (**1**) are the piperidine (**2**, IC_{50} = 40 nM) and pyridine (**3**, IC_{50} = 0.08 nM) which had less protein binding and lower bioavailability.[7,8] The biphenylalanine (**4**) (TR-14035) and the tyrosine analog (**5**) have also shown potent VLA-4 antagonistic activity[9] and Celltech has reported that the thiaproline (**6**, CT-5219) is a very potent VLA-4 antagonist (IC_{50} = 35 nM). This latter compound exhibited poor bioavailability in rats and sheep and showed rapid elimination, particularly in rats.[10,11]

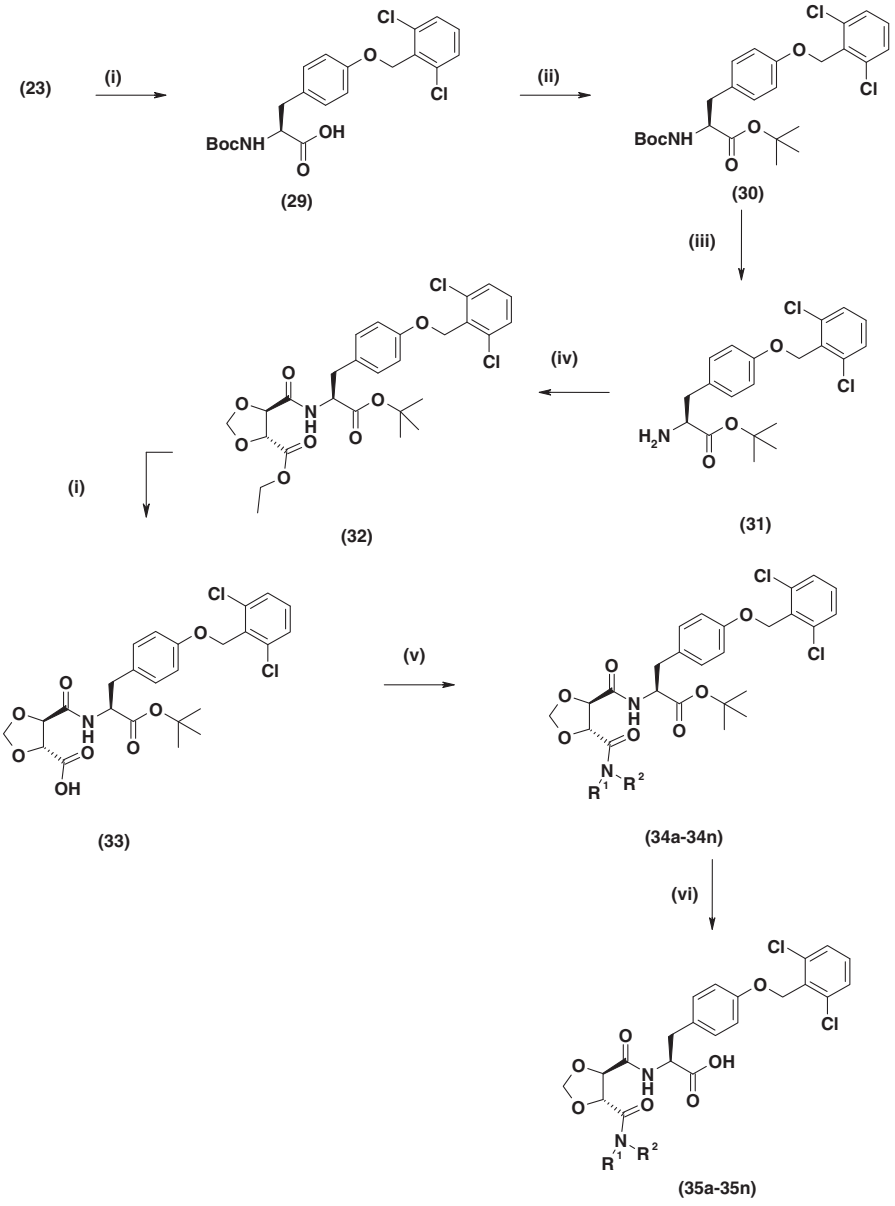
Literature suggests that derivatization of the phenylalanine portion of *N*-acylphenylalanine inhibitors has been a major focus of attempts to obtain optimal potency and selectivity for $\alpha_4\beta_1$. Earlier, we reported substituted proline ureas of general formula (**7**) as VLA-4 inhibitors (Fig. 2) in which the ureido group acted as a bioisostere of the sulfonamide group in compounds (**1–3**).[12] It was found that the compounds with highest binding affinities contained an *N*-(2-phenyl)phenylaminocarboxy group attached to the proline ring. We now report the results of further work wherein 1,3-dioxolane ring (**8**) was evaluated as bioisoster of proline in standard *N*-acylphenylalanine derivatives (**7**).

(+)-Diethyl-L-tartrate (**9**) was reacted with dimethoxymethane in the presence of borane trifluoride to form the 1,3-dioxolane derivative (**10**). Selective hydrolysis of a single ethyl ester group was performed under basic conditions and the resulting mono acid (**11**) was coupled with 2-phenylaniline using EDC and HOBt. The ester group of the amide (**12**) (Scheme 1) was hydrolyzed and the acid (**13**) was coupled with 4-nitro or 4-hydroxyphenyl-L-alanine methyl ester (**14a** or **14b**) to give compounds (**15a and 15b**). The nitro group of (**15a**) was reduced by catalytic hydrogenation and the aniline (**16**) coupled with various acids to give amides (**17a–17g**). Finally, the esters were hydrolyzed under basic conditions to produce the desired acids (**18a–18g**). To prepare ether derivatives, the phenolic group (**15b**) was alkylated with a range of benzyl bromides in the presence of potassium carbonate to furnish the desired esters (**19a–19c**) which were hydrolyzed under basic conditions to the acids (**20a–20c**).

In order to provide structure diversity in the acyl portion we prepared a number of amide derivatives by one of two different methods. In the first method (Scheme 2), the amino group of tyrosine (**21**) was Boc protected and the phenolic group of the product (**22**) alkylated using 2,6-dichlorobenzyl bromide and potassium carbonate to furnish ether (**23**). Deprotection of the Boc group under acidic conditions gave amine (**24**). Intermediate (**11**) was reacted with various amines to produce amides (**25a–25n**). Basic hydrolysis of the ester groups gave acids (**26a–26n**) which were



Scheme 2. Reagents: (i) Boc anhydride, Et_3N, DCM, 100%; (ii) 2,6-dichlorobenzylbromide, K_2CO_3, acetone, 74%; (iii) TFA, DCM, 100%; (iv) RNH_2, EDC, NMM, HOBt, DMF, 70–80%; (v) LiOH, THF, MeOH, H_2O, 70–80%, (vi) EDC, NMM, HOBt, DMF, 50–75%.



Scheme 3. Reagents and conditions: (i) LiOH, THF, MeOH, H_2O, 90%; (ii) *t*-BuOH, DMAP, rt, 30%; (iii) H_2SO_4, *t*-BuOAc, 78%; (iv) (**11**) EDC, NMM, HOBt, 68%; (v) R^1R^2NH, EDC, NMM, HOBt, DMF, 60–80% (**34a**–**34e**) or R^1R^2NH, ethylchloroformate, NMM, THF, 70–85% (**34f**–**34n**); (vi) TFA, DCM, 80–90%.

coupled with (**24**) to afford amides (**27a–27n**). Final hydrolysis gave the target compounds (**28a–28n**).

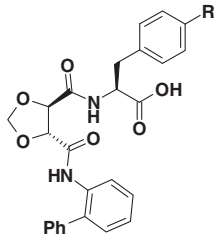
In the second method (Scheme 3), the intermediate (**23**) was hydrolyzed under basic conditions to afford the acid (**29**) which was protected as a *t*-butyl ester (**30**). Selective Boc deprotection gave amine (**31**) which was coupled with intermediate (**11**) to furnish the amide (**32**). The methyl ester group was hydrolyzed to afford the acid (**33**) which was coupled with a diverse set of amines to afford amides (**34a–34n**). Hydrolysis under acidic conditions then yielded the final compounds (**35a–35n**).

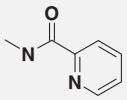
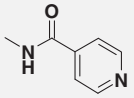
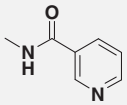
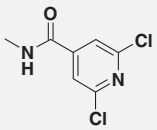
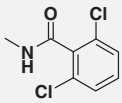
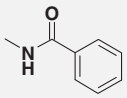
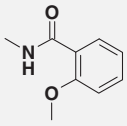
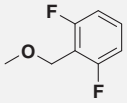
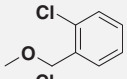
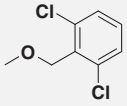
A diverse set of compounds incorporating a 1,3-dioxolane ring in place of the proline ring normally found in many VLA-4 antagonists were synthesized and evaluated as potential VLA-4 antagonists by measuring their ability to inhibit binding of VLA-4 to VCAM-1 in a U-937 (human T-cell line) cell adhesion assay.[13]

In the first part of the work, an exploration was undertaken of the effect on binding to the VCAM-1 receptor of compounds containing various substituents at the *para*-position of the phenyl ring of the phenylalanine group (Table 1). It was observed in the series of compounds (**18a–18g**) that 2,6-di-substitution of the phenylcarboxamido group gave a compound with higher binding affinity than the unsubstituted or mono-*ortho*-substituted compound (compare **18e** with **18g** and **18f**). The high binding affinity of the 2,6-dichloro derivative **18e** may arise from either an electron-withdrawing or steric effect. However, an examination of the results with the pyridylcarboxamido analogs (**18a–18d**) indicates that these compounds do not have particularly high binding affinities, thereby implying that the high binding affinity of compound **18e** arises from favorable steric and/or hydrophobic interactions at the receptor. A similar trend is observed in the series of derivatives in which an ether group is placed at the *para*-position of the phenylalanine group (**20a–20c**). The 2,6-dichlorophenylmethylether (**20c**) has a far higher receptor binding affinity than the 2-chlorophenylmethyl ether (**20b**) or the 2,6-difluorophenylmethylether (**20a**), suggesting once again that steric as opposed to electrostatic forces are at work.

In the second part of the work, the favored 2,6-dichlorophenylmethyl substituent was retained at the *para*-position of the

Table 1
Activity of 1,3-dioxolanes as VLA-4 antagonists



Compd	R	IC_{50} (μM)	*n*
18a		8.3	1
18b		2.7 ± 0.46	2
18c		9.5	1
18d		6.3	1
18e		0.1	1
18f		9.0	1
18g		6.3	1
20a		7.9	1
20b		8.7 ± 3.74	2
20c		0.5 ± 0.25	3

phenylalanine group and attention turned to substitution of the 1,3-dioxolane ring in a study of a range of N-substituted aminocarbonyl-1,3-dioxolanes (Table 2). Very little of a structure–activity trend was seen in the *N*-alkyl amides (**28a–28j, 35n**) with all compounds having IC_{50} values in the range of 1–7 μM with the exception of the *N*-cyclopropyl amide (**28j**) which had an IC_{50} value of 0.3 μM. In the set of *N*-aryl amides, *ortho*-substitution consistently gave compounds (**35a and 35b, 35d, 35f**) having higher binding affinities than unsubstituted (**35e**) or *para*-substituted (**35c**) derivatives. An additional beneficial effect on activity was not observed in compounds containing a second *ortho*-substituent (compare the *ortho*-substituted compounds, **35b** and **35a**, with the 2,6-disubstituted compounds, **35j** and **35i**, respectively). Electronic effects do

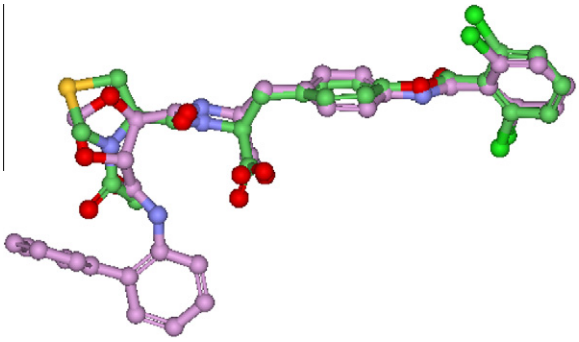


Figure 3a. Structural overlay of CT-5219 (green) with **18e**.

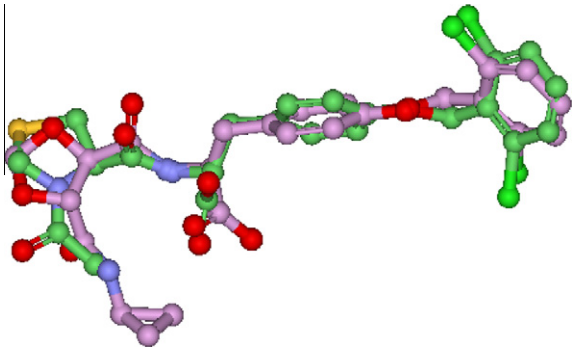


Figure 3b. Structural overlay of CT-5219 (green) with **28j**.

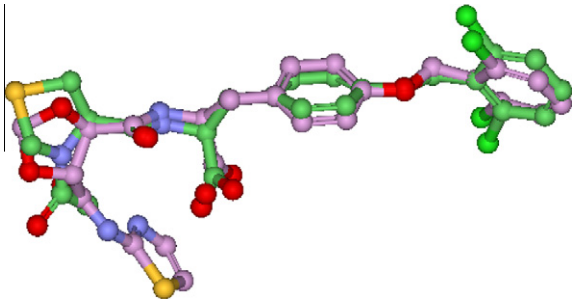
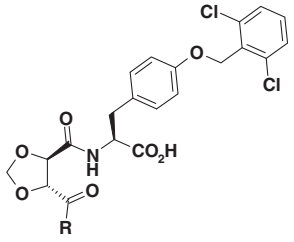


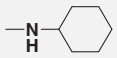
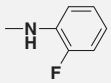
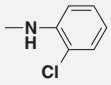
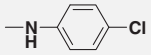
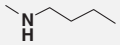
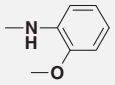
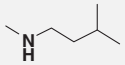
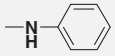
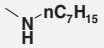
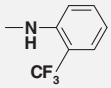
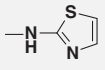
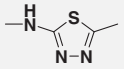
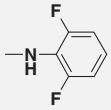
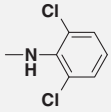
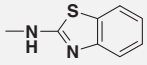
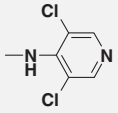
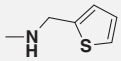
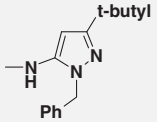
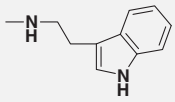
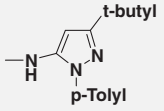
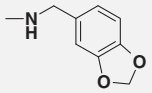
Figure 3c. Structural overlay of CT-5219 (green) with **35g**.

not appear to be playing a major role in this case because the 3,5-dichloropyrid-4-yl derivative (**35k**) had a similar binding affinity to the phenyl analog (**35j**). Compounds containing electron-rich aromatic systems such as thiazol-2-yl (**35g**), benzthiazol-2-yl (**28k**), thien-2-ylmethyl (**28l**), and indol-3-ylethyl (**28m**) all had sub-micromolar affinity which was reduced when the aromatic systems were substituted (**35l and 35m, 28n**). It appears, therefore, that a range of substituents are tolerated on the nitrogen atom of the aminocarbonyl-1,3-dioxolane, indicating perhaps that this part of the molecule plays a limited role in the binding of the ligands to the VCAM-1 receptor. When tested for metabolic stability in human liver microsomes (HLM) at 8 μM concentration for 1 h, compounds (**28j**) and (**35g**) were found to be stable 72% and 52%, respectively.

In conclusion, we have successfully demonstrated that the proline ring of the *N*-acylphenylalanine class of VLA-4 antagonists can be replaced by a range of N-substituted aminocarbonyl-1,3-dioxolanes. Three compounds with high affinity for the VLA-4 receptor, each having a 2,6-dichlorophenyl substituent attached by means of an amide or ether linkage to the *para* position of the phenylalanine group, were the *N*-(2-phenyl)phenyl amide (**18e**), *N*-cyclopro-

Table 2
Activity of 1,3-dioxolanes as VLA-4 antagonists



Compd	R	IC_{50} (μM)	*n*	Compd	R	IC_{50} (μM)	*n*
28a		1.4 ± 0.55	2	**35a**		0.6 ± 0.25	2
28b		1.2 ± 0.75	3	**35b**		0.7 ± 0.25	3
28c		2.1 ± 1.06	2	**35c**		2.8	1
28d		1.5 ± 0.40	2	**35d**		0.6 ± 0.2	3
28e		1.1 ± 0.45	3	**35e**		1.5 ± 0.7	2
28f		4.4 ± 3.4	2	**35f**		0.9 ± 0.5	3
28g		1.5	1	**35g**		0.2 ± 0.1	3
25h		6.6	1	**35h**		1.5	1
28i		1.2 ± 0.1	3	**35i**		0.5 ± 0.1	2
28j		0.3 ± 0.1	3	**35j**		0.7 ± 0.3	3
28k		0.6 ± 0.01	1	**35k**		0.4	1
28l		0.7 ± 0.3	3	**35l**		1	1
28m		0.6 ± 0.2	3	**35m**		2.6	1
28n		3 ± 0.3	2	**35n**		3.4	1

pyl amide (**28j**), and *N*-(thiazol-2-yl) amide (**35g**). Interestingly, overlay of the three compounds indicates that orientation of the functional groups of these compounds were similar to **CT-5219** in the energy minimized structure (Figs. 3a–c). Subsequent work on the further optimization of this series of compounds will be disclosed in due course.

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References and notes

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