

2,3-Diphenylpropionic acids as potent VLA-4 antagonists

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Abstract—The discovery and SAR of 2,3-diphenylpropionic acid derivatives as highly potent VLA-4 antagonists are described. One representative compound, **9cc** has inhibited intercellular adhesion by a VCAM-1/VLA-4 interaction with an IC₅₀ of 1.7 nM, and has good pharmacokinetics and oral bioavailability.

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The integrin very late antigen-4 (VLA-4) is a hetero dimeric adhesion molecule ($\alpha_4\beta_1$), expressed on the surface of leukocytes, including T-lymphocytes and eosinophils.¹ These leukocytes play key roles in inflammation and autoimmune diseases, and VLA-4 is involved in the cell adhesion, migration and activation of these cell types.² One of the ligands for VLA-4 is vascular cell adhesion molecule-1 (VCAM-1), which is expressed on activated endothelial cells at sites of inflammation, and the other is the connecting segment 1 (CS-1) domain of the extracellular matrix protein fibronectin.³ Interaction between VLA-4 and VCAM-1 initiates firm adhesion of the leukocytes to the vascular endothelium followed by extravasation into inflamed tissues.⁴ Antibodies to the α_4 subunit of VLA-4 are reported to be efficacious in inhibiting leukocyte infiltration and then preventing tissue damage in several animal models of inflammation. These experimental results jointly indicate that a small molecule capable of antagonizing the action of VLA-4 can be useful in the treatment of chronic inflammatory diseases such as asthma,⁵ multiple sclerosis,⁶ and rheumatoid arthritis.⁷ Therefore, we launched a program to create a small molecule VLA-4 antagonist aimed at the development of a new medicine for the previously mentioned diseases.⁸

Our anti-VLA-4 project initially started by working on peptidemimetic-based drug discovery, as we could have successfully utilized that technology in a previous study of growth hormone secretagogues.⁹ We could generate several lead compounds¹⁰ through the 3D-pharmacophore development and the 3D-database searching technique using various peptide-like VLA-4 antagonists¹¹ as initial informative sources, but most of which showed low oral bioavailabilities and unsuitable pharmacokinetic properties. Since our objective was to obtain orally active VLA-4 antagonists, we redirected our focus to phenylalanine derivatives such as **1** (TR-14035)¹² and **2**^{8g} shown in Figure 1.

In order to reduce the disadvantage intrinsically-linked to the peptidyl features of these compounds, we designed 2-(3-carboxylamino)phenyl-3-phenylpropionic acids **3** as a new template. We postulated that the benzene ring A would adequately serve as the peptide backbone of either **1** or **2**, and that the *tert*-amide group¹³ at the 3'-position would be able to work as the N-terminal variations. In addition to that, suitable substituent(s) could also be introduced as R¹ and/or R² to this benzene ring A for further manipulation. Taken together, we expected that the 2,3-diphenylpropionic acid derivatives would have a potent VLA-4 antagonistic activity and that a better oral bioavailability and metabolic stability as a result of its nonpeptidyl structure would be achieved.

Keywords: VLA-4; VCAM-1; 2,3-Diphenylpropionic acid.

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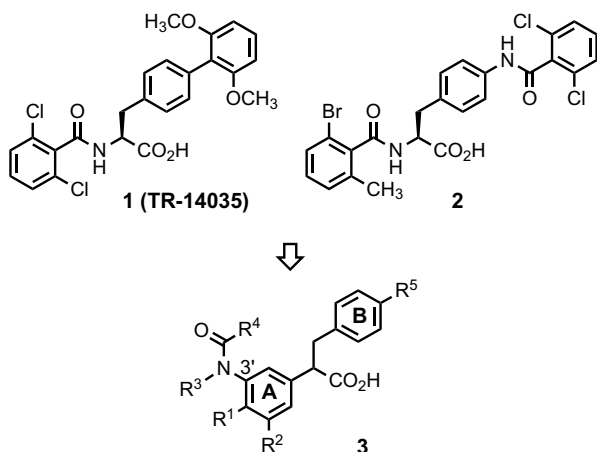
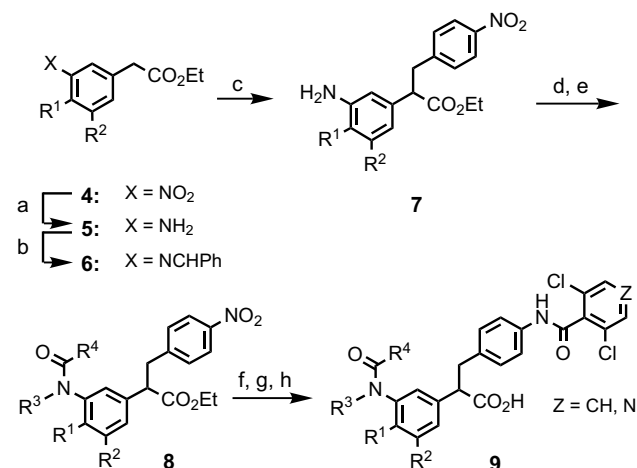


Figure 1.

The general method for the synthesis of the 2,3-diphenylpropionic acid derivatives is outlined in Scheme 1. Ethyl 3-nitrophenylacetate derivatives **4** were hydrogenated to the corresponding aniline derivatives **5**. Benzaldehyde imines **6**, prepared in an ordinary manner from **5**, were deprotonated at the α -position to the ethyl ester group by lithium diisopropylamide and then quenched with 4-nitrobenzyl bromide to give the benzylated imine intermediates. Subsequent hydrolysis of the imine moiety with aqueous hydrochloric acid provided the ethyl 2-(3-aminophenyl)-3-(4-nitrophenyl)acetate derivatives **7** in good yields. The free amino group in **7** was then converted to various kinds of *tert*-amide groups to afford **8** by a two-step sequence (reductive alkylation and acylation). Another nitro group located at the upper end of **8** was also converted to two aryl amide groups, i.e., 2,6-dichlorobenzamide and 3,5-dichloroisonicotinamide.¹⁴ Finally, hydrolysis of the ethyl esters with aqueous sodium hydroxide provided the target compounds **9**.



Scheme 1. (a) H_2 , 10% Pd-C, MeOH; (b) PhCHO, toluene, reflux; (c) lithium diisopropylamide, 4-nitrobenzylbromide, THF, $-78^\circ C \sim rt$ then HCl (aq); (d) aldehydes, NaBH(OAc)₃, MeOH; (e) R^4COCl , Et₃N, CHCl₃; (f) H_2 , 10% Pd-C, MeOH, AcOEt; (g) 2,6-dichlorobenzoylchloride or 3,5-dichloroisonicotinoyl chloride, Et₃N, CHCl₃; (h) NaOH (aq), THF, MeOH.

Based on the aforementioned procedure, we were able to synthesize many compounds with a great diversity of structures.

Compounds were assayed for their ability to inhibit the binding of VLA-4-expressing human leukemia cells (HL-60) to human VCAM-1 expressed on Chinese hamster ovary (CHO) cells.¹⁵ The IC₅₀ values of the relatively simple compounds with no substituents at the R¹ and R² positions are summarized in Table 1.

The comparison among **9a**, **9b**, and **9c** clearly showed that the R³ substituent was crucial for the VLA-4 antagonistic activities and that the isobutyl group, the bulkier one, was much better than the methyl group. The same tendency was also observed when the R⁴ group was changed from benzyl to cyclohexyl (**9d** and **9e**). Based on this significant achievement in which the 2,3-diphenylpropionic acid core unit without a peptide bond in it could definitely bestow the VLA-4 antagonist activities on the small molecules, a variety of functional groups were examined in order to optimize the R⁴ group. These results are shown in Table 2. It clearly

Table 1. Inhibitory activities of VLA-4 antagonists (1)

Compd	R ³	R ⁴	IC ₅₀ (nM)
9a	H	CH ₂ Ph	>100,000
9b	CH ₃	CH ₂ Ph	5000
9c	CH ₂ CH(CH ₃) ₂	CH ₂ Ph	620
9d	H	Cyclohexyl	>100,000
9e	CH ₂ CH(CH ₃) ₂	Cyclohexyl	930

Table 2. Inhibitory activities of VLA-4 antagonists (2)

Compd	R ³	R ⁴	IC ₅₀ (nM)
9f	CH ₂ CH(CH ₃) ₂	CH ₃	3300
9g	CH ₂ CH(CH ₃) ₂	CH ₂ CH ₃	940
9h	CH ₂ CH(CH ₃) ₂	CH(CH ₃) ₂	54
9i	CH ₂ CH(CH ₃) ₂	C(CH ₃) ₂	85
9j	CH ₂ CH(CH ₃) ₂	CH(CH ₂ CH ₃) ₂	48
9k	CH ₂ CH(CH ₃) ₂	CH ₂ C(CH ₃) ₃	650
9l	CH ₂ CH(CH ₃) ₂	Ph	1700
9m	CH ₂ CH(CH ₃) ₂	2-Thienyl	1700
9n	CH ₂ CH(CH ₃) ₂	C ₆ H ₄ (4-OCH ₃)	1100
9o	CH ₂ CH(CH ₃) ₂	2-Furyl	7000
9p	CH ₂ CH(CH ₃) ₂	<i>trans</i> -CH=CHPh	5300
9q	CH ₂ CH(CH ₃) ₂	OC(CH ₃) ₃	7100
9r	CH ₂ (CH ₂)CH ₃	C(CH ₃) ₃	1700
9s	CH ₂ Ph	C(CH ₃) ₃	580

indicated that the α -branched aliphatic alkyl groups (**9h–9j**) were perfectly used as the R^4 substituent. The aromatic ring with/without a hetero atom did not provide any advantage for on the VLA-4 antagonist activities. Carbamate instead of acylamide at the R^4 position represented by the comparison between **9k** and **9q** decreased the antagonistic activity. Along with the aforementioned structure–activity relationships, the comparison of the VLA-4 antagonist activities between **9j** in Table 2 and **9e** in Table 1 strongly suggested that the steric tolerance of the particular space occupied by the R^4 group was rather small as the electronic character of the 1-ethylpropyl group and cyclohexyl group is almost the same.

Now that we have obtained a suitable R^4 group, we attempted to further optimize the R^3 group by using the *tert*-butyl group as the fixed R^4 group. The *n*-pentyl group and benzyl group were examined as the R^3 group (**9r** and **9s**), but neither of them gave a good result. Though there might be a possibility of other kinds of R^3 groups working well, we fixed the *iso*-butyl group as the R^3 group for further exploration.

The introduction of substituent(s) at the R^1 and/or R^2 position(s) on the benzene ring depicted in Table 3 provided another important effect. The simple methoxy group dramatically increased the VLA-4 antagonist activity up to the IC_{50} value of 2.0 nM which is 40-fold more potent than the basic template **9i**. Generally, any substituents applied here at the R^1 position constantly increased the activities by more than ten times. Compound **9w** bearing an *n*-propoxy group revealed a subnanomolar activity for the first time in this series. These results suggested that the interaction between R^1 and the binding site on VLA-4 was quite important and that the particular space occupied by R^4 was rather large or enlarged by an induced-fit protein mobilization, otherwise it worked just as a supervisory group for the direction of the vicinal *tert*-amide. Though the R^2 group was also varied from hydrogen to some functional groups in

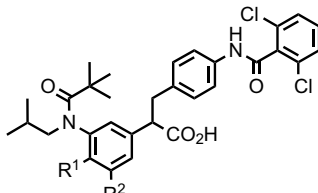
a limited way, it turned out that the existence of R^2 group also favorably affected the VLA-4 antagonist activities and that there would be great possibilities for the R^2 substituent with further activity enhancement.

Finally, we would like to briefly mention the R^5 group. 3,5-Dichloroisonicotinamide in place of 2,6-dichlorobenzamide generally gave a better result for the VLA-4 antagonistic activity in each case as shown in Table 4.^{8e,h,14}

The series of 2,3-diphenylpropionic acid derivatives we have explained in this letter had fairly good pharmacokinetics and oral bioavailabilities. The selected data of the representative compounds **9t** and **9cc** are shown in Table 5. Further optimization of the 2,3-diphenylpropionic acid derivatives and preclinical investigations of a group of selected candidates including **9cc** are proceeding and these results will be published in due course.

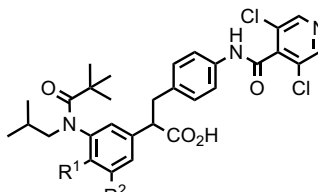
In conclusion, we launched the VLA-4 antagonist program based on the structure of the reported phenylalanine derivatives and created the proprietary core structure, 2,3-diphenylpropionic acid. After a process of trial and error, we discovered several very potent VLA-4 antagonists with subnanomolar activities. Since all of the compounds explained above are racemics, the optically pure forms would have the preferable characteristics.

Table 3. Inhibitory activities of VLA-4 antagonists (3)



Compd	R^1	R^2	IC_{50} (nM)
9t	OCH ₃	H	2.0
9u	OCH ₂ CH ₃	H	3.1
9v	OCH ₂ OCH ₃	H	3.8
9w	OCH ₂ CH ₂ CH ₃	H	0.1
9x	OCH(CH ₃) ₂	H	8.4
9y	OCH ₃	OCH ₃	16
9z	OH	H	11
9aa	CH ₂ CH ₃	H	4.9
9bb	H	CF ₃	23

Table 4. Inhibitory activities of VLA-4 antagonists (4)



Compd	R^1	R^2	IC_{50} (nM)
9cc	OCH ₃	H	1.7
9dd	OCH ₂ CH ₃	H	0.25
9ee	OCH ₂ CH ₂ CH ₃	H	0.65
9ff	OCH(CH ₃) ₂	H	1.1
9gg	CH ₂ CH ₃	H	0.1
9hh	H	CF ₃	0.4

Table 5. Pharmacological comparison among our compounds and representative VLA-4 antagonists

	1 (TR-14035)	2	9t	9cc
IC_{50} (nM)	61	17	2.0	1.7
Solubility in water (μ g/mL)	219	330	107	440
Protein binding (%) ^a	—	—	95.17	92.53
CLint (mL/min/mg) ^a	—	—	0.013	0.030
<i>F</i> (%) ^b	—	—	34	33

^a Rats.

^b SD rats, 10mg/kg, po.

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