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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 IC_{50} of 1.7 nM, and has good pharmacokinetics and oral bioavailability.

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 compounds[10] through the 3D-pharmacophore development and the 3D-database searching technique using various peptide-like VLA-4 antagonists[11] 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-carbonylamino)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 group[13] 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 R^1 and/or R^2 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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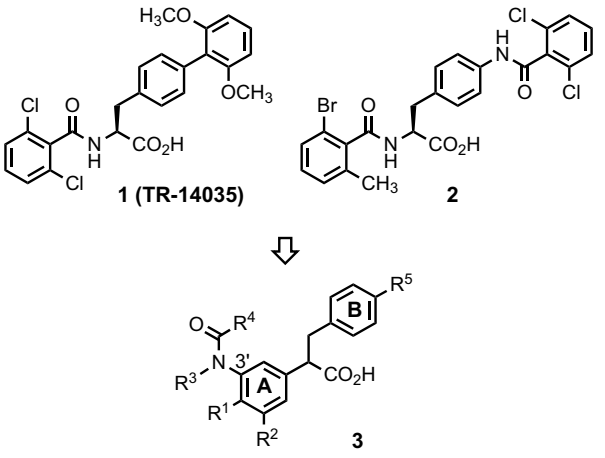
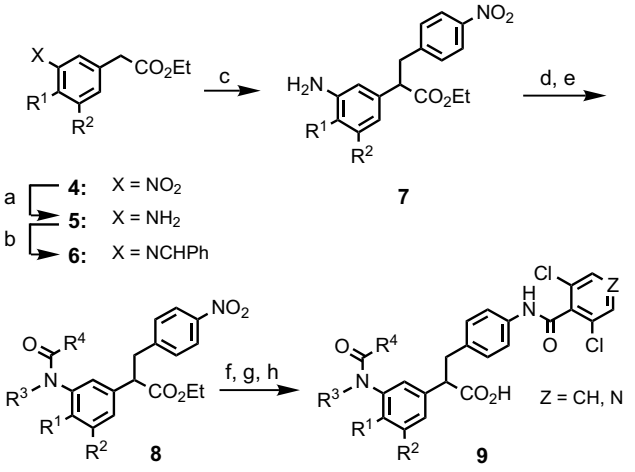


Figure 1.

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 aroyl 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**.



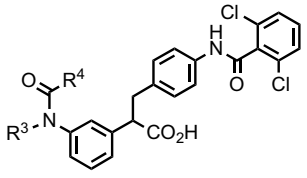
Scheme 1. (a) H_2, 10% Pd–C, MeOH; (b) PhCHO, toluene, reflux; (c) lithium diisopropylamide, 4-nitrobenzylbromide, THF, −78 °C ∼ rt then HCl (aq); (d) aldehydes, $NaBH(OAc)_3$, MeOH; (e) R^4COCl, Et_3N, $CHCl_3$; (f) H_2, 10% Pd–C, MeOH, AcOEt; (g) 2,6-dichlorobenzoylchloride or 3,5-dichloroisonicotinoyl chloride, Et_3N, $CHCl_3$; (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 IC_{50} values of the relatively simple compounds with no substituents at the R^1 and R^2 positions are summarized in Table 1.

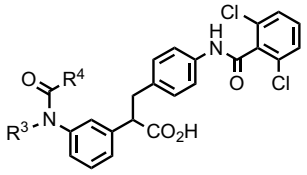
The comparison among **9a**, **9b**, and **9c** clearly showed that the R^3 substituent was crucial for the VLA-4 antagonistic activities and that the isobutyl group, the bulkier one, was much better than the methyl group. The same tendency was also observed when the R^4 group was changed from benzyl 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 R^4 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_2Ph	>100,000
9b	CH_3	CH_2Ph	5000
9c	$CH_2CH(CH_3)_2$	CH_2Ph	620
9d	H	Cyclohexyl	>100,000
9e	$CH_2CH(CH_3)_2$	Cyclohexyl	930

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



Compd	R^3	R^4	IC_{50} (nM)
9f	$CH_2CH(CH_3)_2$	CH_3	3300
9g	$CH_2CH(CH_3)_2$	CH_2CH_3	940
9h	$CH_2CH(CH_3)_2$	$CH(CH_3)_2$	54
9i	$CH_2CH(CH_3)_2$	$C(CH_3)_2$	85
9j	$CH_2CH(CH_3)_2$	$CH(CH_2CH_3)_2$	48
9k	$CH_2CH(CH_3)_2$	$CH_2C(CH_3)_3$	650
9l	$CH_2CH(CH_3)_2$	Ph	1700
9m	$CH_2CH(CH_3)_2$	2-Thienyl	1700
9n	$CH_2CH(CH_3)_2$	C_6H_4(4-OCH_3)	1100
9o	$CH_2CH(CH_3)_2$	2-Furyl	7000
9p	$CH_2CH(CH_3)_2$	*trans*-CH=CHPh	5300
9q	$CH_2CH(CH_3)_2$	$OC(CH_3)_3$	7100
9r	$CH_2(CH_2)CH_3$	$C(CH_3)_3$	1700
9s	CH_2Ph	$C(CH_3)_3$	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.

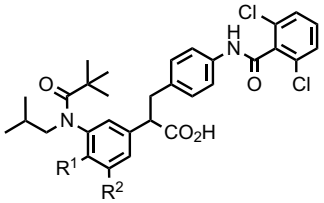
The introduction of substituent(s) at the R^1 and/or R^2 position(s) on the benzene ring depicted in Table 3 provided another important effect. The simple methoxy group dramatically increased the VLA-4 antagonist activity up to the IC_{50} value of 2.0 nM which is 40-fold more potent than the basic template **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.

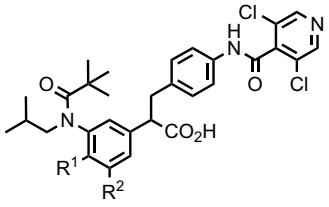
In conclusion, we launched the VLA-4 antagonist program based on the structure of the reported phenylalanine derivatives and created the proprietary core structure, 2,3-diphenylpropionic acid. After a process of trial and error, we discovered several very potent VLA-4 antagonists with subnanomolar activities. 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_2CH_3	H	3.1
9v	OCH_2OCH_3	H	3.8
9w	$OCH_2CH_2CH_3$	H	0.1
9x	$OCH(CH_3)_2$	H	8.4
9y	OCH_3	OCH_3	16
9z	OH	H	11
9aa	CH_2CH_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_2CH_3	H	0.25
9ee	$OCH_2CH_2CH_3$	H	0.65
9ff	$OCH(CH_3)_2$	H	1.1
9gg	CH_2CH_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 (μg/mL)	219	330	107	440
Protein binding (%)[a]	—	—	95.17	92.53
CLint (mL/min/mg)[a]	—	—	0.013	0.030
F (%)[b]	—	—	34	33

[a] Rats.
[b] SD rats, 10 mg/kg, po.

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