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Synthetic study of VLA-4/VCAM-1 inhibitors: Synthesis and structure–activity relationship of piperazinylphenylalanine derivatives

Osamu Saku,* Kiminori Ohta, Eri Arai, Yuji Nomoto, Hiroko Miura, Hiroaki Nakamura, Eiichi Fuse and Yoshisuke Nakasato*

Pharmaceutical Research Center, Kyowa Hakko Kogyo Co., Ltd, 1188 Shimotogari, Nagaizumi-cho, Suntou-gun, Shizuoka 411-8731, Japan

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Abstract—To improve the poor pharmacokinetic characteristics of VLA-4 inhibitors, novel piperazinylphenylalanine derivatives were designed. This structure is expected to improve physicochemical properties by increasing overall basicity. By changing components at the 4-position of piperazine and the terminal group of the amido bond, **12t** was found to be the most potent of this series of compounds. In addition, dichlorobenzoyl derivative **12aa** exhibited better oral availability and showed efficacy in an in vivo model after oral administration.
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Adhesion with cells or extra-cellular matrices plays an important role in chemotaxis into inflammation sites, adhesion of leukocytes, and movement of stem cells at the time of ontogeny. In such processes, involvement of various adhesion molecules is evident, and integrin, an adhesion molecule that interacts with ligands expressed on cells, plays an important role in regulation of adhesion with cells or extra-cellular matrix.[1]

VLA-4 is a member of the integrin superfamily, composed of α4- and β1-heterodimers. VLA-4 is mainly expressed on eosinophils and lymphocytes. VCAM-1, expressed in vascular endothelial cells and fibronectins, ingredients of extra-cellular matrix, is the ligand for VLA-4. Binding of VLA-4 and VCAM-1 is one of the most important mechanisms for tight adhesion of leukocytes with vascular endothelial cells, and is strongly related to infiltration of inflammatory cells, such as lymphocytes and eosinophils, into inflammation sites. As VLA-4 also functions as a sub-signal in the activation and multiplication of T-cells, agents that inhibit adhesion of VLA-4 and VCAM-1 interaction are likely to be useful in the treatment of inflammatory disease. Anti-VLA-4 antibody has been demonstrated to be effective in various inflammation models,[2] with the humanized monoclonal antibody, Tysabri (natalizumab),[3] recently relaunched for the treatment of relapsing forms of multiple sclerosis. Therefore, non-peptide small molecule VLA-4 inhibitors are also an attractive target for the treatment of chronic inflammatory disease, with several reports on orally active inhibitors published in recent years.[4] Preclinical tests of peptide mimetic BIO-1272 (Biogen) derived from connecting segment 1 (CS-1) of fibronectins were performed for the first time as a small molecule inhibitor, showing a marked effect in inhibiting antigen induced respiratory hypersensitivity in sheep models of asthma.[5]

Although several phenylalanine derivatives with different chemotypes have been reported to exhibit oral bioavailability, there is still room for improvement in discovery and development of antagonists with improved pharmacokinetic properties. Therefore, improvement of the pharmacokinetic properties of these derivatives was attempted to identify novel inhibitors.

Previous studies of the SAR of phenylalanine derivatives suggest that the *para*-position of the phenyl group on phenylalanine is important for activity.[6] Several

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* Corresponding authors. Tel.: +81 55 989 2014; fax: +81 55 986 7430; e-mail: osamu.saku@kyowa.co.jp

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examples in published studies have directed their focus to phenylalanine derivatives in which the benzene ring is linked by amide or ether at the *para*-position (Fig. 1).[7,8] As part of this group's effort to develop novel VLA-4 inhibitors, replacement of the linkers with a piperazine ring was attempted, with the aim of producing a novel advantageous surrogate for these linkers. Introduction of two nitrogens reduces lipophilicity and is generally expected to contribute to improving the PK profile.

Piperazinylphenylalanine derivatives were prepared according to the methods shown in Scheme 1. In Method A, after deprotection of commercially available *N*-Boc tyrosine derivatives, amidation was performed with carboxylic acids to produce **3**. Subsequently **3** was converted to a triflated tyrosine **4**. A subsequent Pd-coupling reaction with piperazine **6** produced **9**, followed by hydrolysis to the acid **12**.[9] In Method B, triflate **5** was converted to piperazinylphenylalanine **7**, followed by deprotection and amidation to **9**. Carboxylic acid **12** was obtained by hydrolysis of **9**. Introduction of functional groups at the 4-position of piperazine was performed using Method C, which utilized **11** prepared by the deprotection of benzylpiperazine **7n** obtained in Method B. Although a racemic mixture was produced in the Pd-coupling reaction, optimized conditions using **13**[10] as the ligand in THF could be applied to avoid this problem.

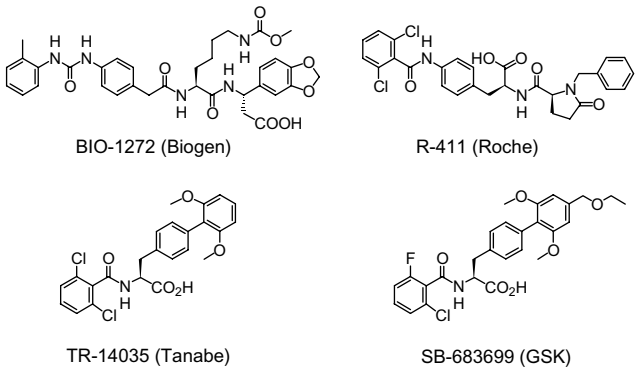
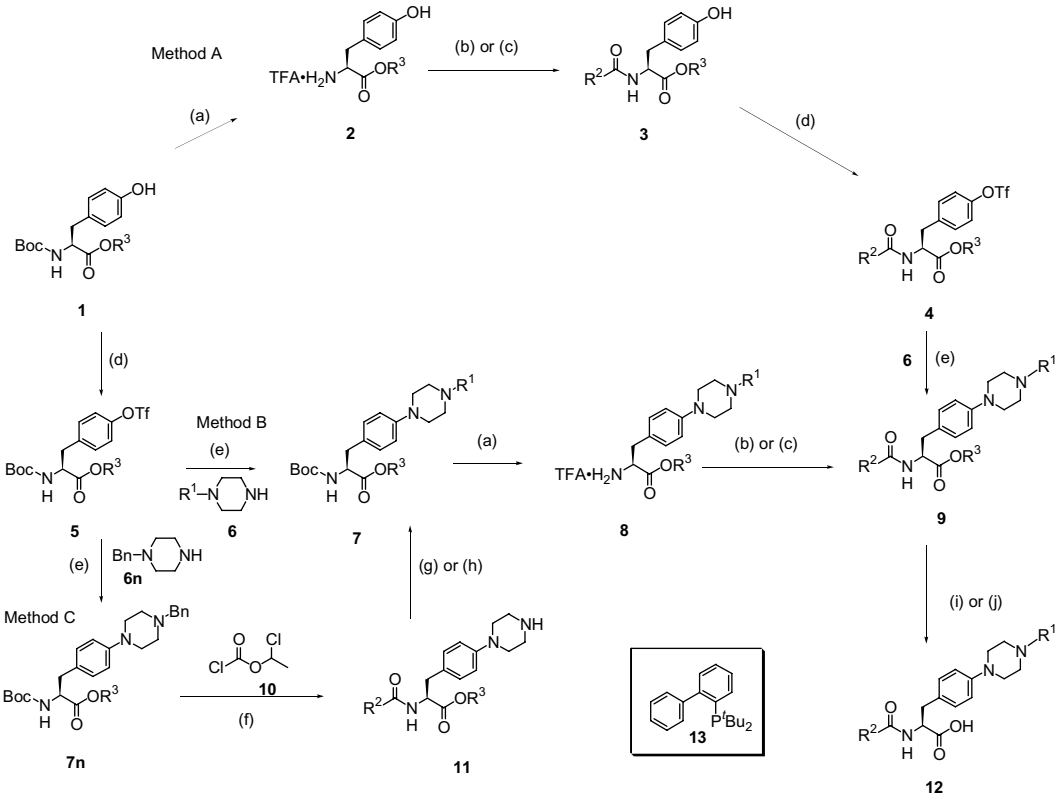


Figure 1. Structure of VLA-4 antagonists.

Compounds were assayed for VLA-4 antagonist activity using ELISA, in which VLA-4 derived from Jurkat cells was allowed to compete for bound human VCAM-1 in the presence of test compounds.

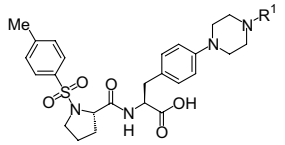
Compound **12a** substituted by tosylproline, demonstrated to improve potency in several derivatives, showed modest potency as shown in Table 1. Introduction of piperazine as a linking group was demonstrated to be tolerated. Subsequently, substitution at the 4-position of the piperazine ring was briefly explored.

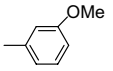
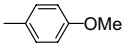
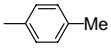
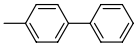
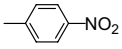
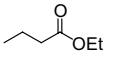
Comparison of all three positional isomers (**12b–d**) indicated that the position of substitute groups had little ef-



Scheme 1. Reagents and conditions: (a) TFA, CH_2Cl_2; (b) R^2COOH, WSC·HCl, HOBt·H_2O, Et_3N, DMF; (c) R^2COCl, $^iPr_2NEt$, CH_2Cl_2; (d) Tf_2O, pyridine, CH_2Cl_2; (e) **6**, **13**, $Pd(OAc)_2$, Cs_2CO_3, THF; (f) 1—**10**, CH_2Cl_2; 2—MeOH, reflux; (g) R^4COCl, Et_3N, $CHCl_3$; (h) ArX, $^iPr_2NEt$, DMF; (i) LiOH·H_2O, THF, H_2O; (j) TFA, CH_2Cl_2.

Table 1. Inhibitory effect of *N*-tosylproline derivatives

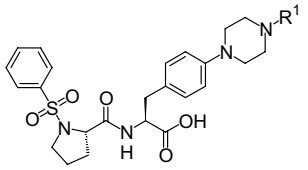


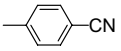
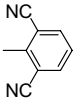
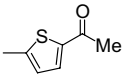
Compound	R^1	Jurkat/VCAM-1 IC_{50} (nmol/L)
12a		123 ± 5
12b		135 ± 4
12c		170 ± 35
12d		152 ± 30
12e		353 ± 78
12f		327 ± 59
12g		450 ± 6
12h		143 ± 18
12i		99 ± 8
12j		239 ± 85
12k		75 ± 10
12l		179 ± 40
12m	–Me	235 ± 20
12n		222 ± 19
12o		130 ± 42

IC_{50} values are means ± SEM of at least two separate assays with three replicates at each concentration.

fect on potency. However, a 3-fold decrease in potency was observed when a methyl or phenyl group was introduced (**12e**–**g**). *Para*-nitro (**12h**) and *ortho*-cyano (**12i**) analogs were equipotent to **12a**. Although the pyrimidine (**12j**) and pyridine (**12l**) analogs were slightly less potent than **12a**, 1,4-pyrazine analog **12k** exhibited greater activity (IC_{50} = 75 nmol/L). This finding indicates that the position of basic nitrogen in heterocyclic groups on piperazine is important for potency. An alkyl group on piperazine increases the basicity of nitrogen, and marked changes in properties were expected. However, *N*-methyl piperazine **12m** and *N*-benzyl piperazine **12n** were only 2-fold less potent than **12a**. The most potent analog in the *N*-alkyl piperazine derivatives was ester **12o**. From these results, it is concluded that this region has good tolerability.

Table 2. Inhibitory effect of *N*-phenylsulfonylproline derivatives



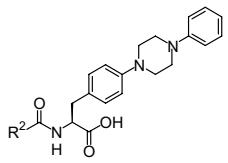
Compound	R^1	Jurkat/VCAM-1 IC_{50} (nmol/L)
12p		76 ± 18
12q		69 ± 12
12r		67 ± 29
12s		101 ± 9
12t		19 ± 2
12u		67 ± 10
12v		124 ± 17
12w		80 ± 6
12x		200 ± 12

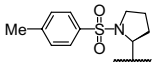
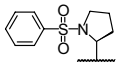
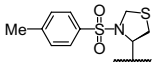
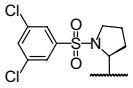
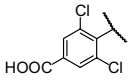
IC_{50} values are means ± SEM of at least two separate assays with three replicates at each concentration.

The phenylsulfonyl group appeared to be a suitable replacement for the tosyl group (**12p** vs **12a**). Thus, the effect of replacing this group is shown in Table 2. Electron-withdrawing groups on the phenyl group at the 4-position of piperazine, such as NO_2 (**12q**) in the *ortho*-position and CN (**12r**,**s**) in the *ortho*- or *para*-position, were better tolerated. Introduction of two cyano groups in the *ortho*-position (**12t**) produced a 4-fold improvement in potency over compound **12p**, and heterocyclic rings such as 2-thienyl (**12u**) and pyrazinyl (**12v**) were well tolerated. *N*-acyl piperazine (**12w**) was also tolerated, but a reduction in potency was observed for the urea analog (**12x**).

The left side of the molecule was examined next. Prompted by the finding that replacement of the tosyl group with a phenylsulfonyl group produced a 2-fold improvement in potency, this position was further characterized (Table 3). 3,5-Dichlorophenyl-substituted compound **12z** exhibited the most potent activity with an IC_{50} of 28 nmol/L, while introduction of the thiazolidine ring as a linker was detrimental (**12y**). Although benzoyl analogs showed a significantly reduced activity (**12aa**–**ac**), the dichloropyridine analog **12ad** displayed

Table 3. Inhibitory effect of prolinyl amide replacements



Compound	R^2	Jurkat/VCAM-1 IC_{50} (nmol/L)
12a		123 ± 5
12p		76 ± 18
12y		196 ± 30
12z		28 ± 3
12aa		1122 ± 250
12ab		6807 ± 1455
12ac		4318 ± 1976
12ad		510 ± 6
12ae		42 ± 10

IC_{50} values are means ± SEM of at least two separate assays with three replicates at each concentration.

approximately 2-fold higher potency compared to **12aa**. TR-14035 analogs[6] demonstrated that the carboxy analog **12ae**, with H-bond donors at the 4-position, exhibited much higher potency. In particular, potency of **12ae** was comparable to those of proline derivatives (IC_{50} = 42 nmol/L).

Initial pharmacokinetic screening studies were conducted to investigate oral bioavailability. PK profiles of proline derivatives in mice were characterized at a low plasma concentration. Several examinations indicated that the reason for low oral bioavailability of proline derivatives was a high biliary excretion rate. This efflux is likely due to a transporter-mediated process. Almost all of the compounds showed a high rate of excretion that exceeded 50% within 30 min. Consequently, structural modifications of proline derivatives did not improve oral bioavailability.

In contrast, the PK profiles of benzoyl derivatives in rats were excellent (Fig. 2). Oral bioavailability of compounds **12aa** and **12ad** was 86% and 32%, respectively (Table 4). Incorporation of the piperazine linker was demonstrated to be a useful approach. Furthermore, compound **12aa** reduced permeation of eosinophils to 40% in a pleurisy model induced by compound 48/80, a Ca^{2+}-dependent histamine releaser, in rats (100 mg/kg, po, t.i.d.). This inhibitory effect was nearly identical to that of prednisolone (Fig. 3).

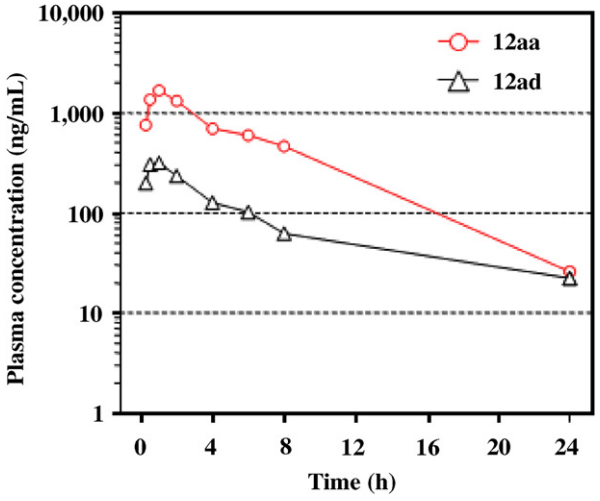


Figure 2. In vivo pharmacokinetic profile of compounds **12aa** and **12ad**. Compounds were orally administered to rats (10 mg/kg), then plasma samples were withdrawn at the time points indicated.

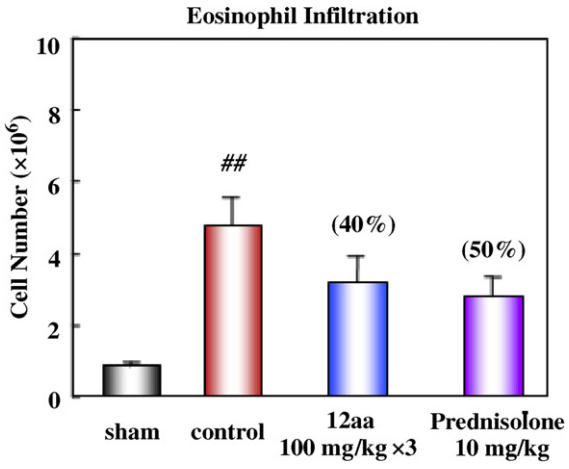


Figure 3. Effect of **12aa** on eosinophil infiltration into BAL fluid, 24 h after compound 48/80 challenge in a pleurisy model in rats.

Table 4. Pharmacokinetic profile for compounds **12aa** and **12ad**

Compound	$AUC_{0-\infty}$ (ng h/mL)[a] (iv)	Cl_p (L/h/kg)[a]	Vd_{ss} (L/kg)[a]	$t_{1/2\beta}$ (h)[a]	$AUC_{0-\infty}$ (ng h/mL)[b] (po)
12aa	1290	0.775	0.754	1.44	11,100
12ad	721	1.39	6.23	7.91	2290

[a] Study in fed male Sprague–Dawley rats (1 mg/kg in DMSO).
[b] Study in fed male Sprague–Dawley rats (10 mg/kg) as a suspension in 0.5% methylcellulose.

In summary, a series of potent piperazinylphenylalanine VLA-4 antagonists was prepared. Optimization at the 4-position of piperazine produced highly potent compound **12t** (IC_{50} = 19 nmol/L), while oral bioavailability was improved by alteration to the amide. Introduction of piperazine as a linking group resulted in a significant improvement in the PK profile. In addition, 2,6-dichlorobenzoyl derivative **12aa** demonstrated efficacy in an in vivo pleurisy model after oral administration.

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9. *Preparation of* **12i**: To a solution of *N*-(toluene-4-sulfonyl)-L-prolyl-L-tyrosine *tert*-butyl ester **3a**[11] (32.9 g, 67.3 mmol) in dichloromethane (200 mL), pyridine (16.0 mL, 198 mmol) and trifluoromethanesulfonic anhydride (14.6 mL, 86.8 mmol) were added. The reaction mixture was then stirred at room temperature for 4 h, acidified with 1 mol/L HCl, and then extracted with dichloromethane. The organic extract was washed successively with saturated $NaHCO_3$ solution and saturated NaCl solution, then dried over $MgSO_4$, filtered, and concentrated in vacuo to produce the crude product. Purification by silica gel chromatography (gradient: 25% EtOAc in hexane) produced *N*-(toluene-4-sulfonyl)-L-prolyl-*O*-(trifluoromethanesulfonyl)-L-tyrosine *tert*-butyl ester **4a** (36.5 g, 87%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): 1.40–1.56 (m, 3 H), 1.45 (s, 9H), 2.00–2.05 (m, 1H), 2.21 (s, 3H), 3.03–3.38 (m, 4H), 4.03–4.08 (m, 1H), 4.70–4.78 (m, 1H), 7.17 (d, J = 8.4 Hz, 2H), 7.28–7.36 (m, 5H), 7.84 (d, J = 8.3 Hz, 2H). **4a** (623 mg, 1.00 mmol) was dissolved in THF (2 mL) under Ar and 1-(2-cyanophenyl)piperazine (229 mg, 1.22 mmol), 2-(di-*tert*-butylphosphino)biphenyl (23.0 mg, 0.0771 mmol), palladium(II) acetate (11.0 mg, 0.0490 mmol), and cesium carbonate (462 mg, 1.42 mmol) were added, then the mixture was stirred at 60 °C for 4 h. Saturated NaCl solution was added and extracted with EtOAc. The organic extract was washed with saturated NaCl solution, then dried over $MgSO_4$, filtered, and concentrated in vacuo to produce the crude product. Purification by silica gel chromatography (gradient: 50% EtOAc in hexane) produced *N*-(toluene-4-sulfonyl)-L-prolyl-4-(4-(2-cyanophenyl)piperazine-1-yl)-L-phenylalanine *tert*-butyl ester **9i** (230 mg, 35%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): 1.42–1.47 (m, 4H), 1.48 (s, 9H), 2.43 (s, 3H), 2.98 (dd, J = 7.0, 14.0 Hz, 1H), 3.07–3.13 (m, 1H), 3.16 (dd, J = 5.7, 13.8 Hz, 1H), 3.35–3.36 (m, 9H), 4.07–4.09 (m, 1H), 4.65–4.72 (m, 1H), 6.89 (d, J = 8.4 Hz, 2H), 7.01–7.07 (m, 2H), 7.09 (d, J = 8.4 Hz, 2H), 7.30–7.31 (m, 1H), 7.32 (d, J = 8.4 Hz, 2H), 7.51 (t, J = 7.6 Hz, 1H), 7.58 (dd, J = 0.8, 8.1 Hz, 1H), 7.72 (d, J = 8.4 Hz, 2H). Trifluoroacetic acid (1.00 mL, 13.0 mmol) was added to a solution of **9i** (230 mg, 0.350 mol) in dichloromethane (5.0 mL) at room temperature under Ar. After 12 h, the mixture was concentrated, then dissolved in 1 mol/L NaOH solution. The mixture was acidified with acetic acid, then the precipitate was collected by filtration to produce *N*-(toluene-4-sulfonyl)-L-prolyl-4-(4-(2-cyanophenyl)piperazine-1-yl)-L-phenylalanine **12i** (149 mg, 71%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): 1.14–1.17 (m, 1H), 1.33–1.37 (m, 2H), 1.75–1.78 (m, 1H), 2.33 (s, 3H), 2.77–2.98 (m, 2H),3.25–3.28 (m, 10H), 4.05–4.10 (m, 1H), 4.64–4.74 (m, 1H), 6.81 (d, J = 7.6 Hz, 2H), 6.98–7.08 (m, 4H), 7.19 (d, J = 6.5 Hz, 2H), 7.49 (t, J = 8.1 Hz, 1H), 7.55 (d, J = 7.6 Hz, 2H), 7.69 (d, J = 7.3 Hz, 2H).
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