Bioorganic & Medicinal Chemistry 16 (2008) 9991–10000

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Identification of 4-[1-[3-chloro-4-[*N*'-(5-fluoro-2-methylphenyl)ureido]phenylacetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]benzoic acid as a potent, orally active VLA-4 antagonist

Fumihito Muro [a,*], Shin Iimura [a], Yoshiyuki Yoneda [a], Jun Chiba [a], Toshiyuki Watanabe [b], Masaki Setoguchi [a], Yutaka Iigou [c], Gensuke Takayama [c], Mika Yokoyama [c], Tohru Takashi [d], Atsushi Nakayama [a], Nobuo Machinaga [a]

[a] *Medicinal Chemistry Research Laboratories II, Daiichi Sankyo Co., Ltd, 1-16-13, Kitakasai, Edogawa-ku, Tokyo 134-8630, Japan*
[b] *Medicinal Chemistry Research Laboratories I, Daiichi Sankyo Co., Ltd, 1-2-58, Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan*
[c] *Biological Research Laboratories III, Daiichi Sankyo, Co., Ltd, 1-16-13, Kitakasai, Edogawa-ku, Tokyo 134-8630, Japan*
[d] *Biological Research Laboratories IV, Daiichi Sankyo, Co., Ltd, 1-16-13, Kitakasai, Edogawa-ku, Tokyo 134-8630, Japan*

ARTICLE INFO

Article history:
Received 26 September 2008
Revised 8 October 2008
Accepted 9 October 2008
Available online 12 October 2008

Keywords:
VLA-4
Integrin
Oral availability

ABSTRACT

Optimization of benzoic acid derivatives by introducing substituents into the diphenyl urea moiety led to the identification of compound **20l** as a potent VLA-4 antagonist. Compound **20l** inhibited eosinophil infiltration into bronchial alveolar lavage fluid in a murine asthma model by oral dosing and its efficacy was comparable to *anti*-mouse α4 antibody (R1-2). Furthermore, this compound significantly blocked bronchial hyper-responsiveness in the model.

1. Introduction

The integrin VLA-4 (very late antigen 4; α4β1; CD49d/CD29) is a non-covalently bound heterodimeric glycoprotein receptor expressed on the cell surface of most leukocytes.[1] It binds to vascular cell adhesion molecule-1 (VCAM-1) expressed on cytokine-stimulated endothelial cells and the alternatively spliced connecting segment-1 domain of fibronectin.[2,3] Through the VLA-4/ligand interaction, VLA-4 plays a critical role in the migration process of immune cells across the vascular endothelium. It also plays an important role in the activation, proliferation, and differentiation process of the immune cells within the parenchyma. *anti*-VLA-4 antibodies and small molecular VLA-4 antagonists[4] have been reported to inhibit leukocyte infiltration into extravascular tissue and thus prevent tissue damage in inflammatory animal models of asthma,[5] multiple sclerosis (MS),[6] rheumatoid arthritis,[7] and inflammatory bowel disease (IBD).[8] A humanized monoclonal *anti*-α4 antibody, natalizumab[9] (Elan Pharmaceutical Inc. and Biogen Idec Inc.), has demonstrated promising results in patients with MS and Crohn's disease in clinical trials and the FDA has approved the use of the monoclonal antibody in these populations. Therefore, the development of small molecular VLA-4 antagonists with an acceptable oral pharmacokinetic profile is viewed as a reasonable approach to a novel *anti*-inflammatory therapeutic intervention. At present, there are small molecular VLA-4 antagonists undergoing clinical trials for the treatment of MS and Crohn's disease,[10] but none of them has yet to reach the marketplace.

We have recently reported the identification of benzoic acid derivative **1**[11] (Fig. 1) as a potent VLA-4 antagonist which shows efficacy in a rat pleurisy model by oral dosing at 10 mg/kg, bid. In the course of this study, we have made it clear that introducing a halogen atom (Cl or Br) into the 3-position of the central benzene ring in the diphenyl urea moiety as in **1** leads to a significant improvement of plasma clearance and oral bioavailability in rodents and dogs. With compound **1** showing moderate oral availability and in vivo efficacy, our next efforts focused on further fine tuning of the substituents in the diphenyl urea moiety in **1**. In this study, after evaluation of VLA-4 inhibitory activity of the compounds we synthesized, we determined their distribution coefficients (log*D*) and their membrane permeability with MDCK monolayer cells, and estimated their serum concentration by the bio-assay method reported previously.[11] Regarding the representative compound **20l**, we evaluated its efficacy in asthma animal models and determined its pharmacokinetic parameters in rats and dogs.

* Corresponding author. Tel +81 3 3680 0151; fax: +81 3 5696 8609.
E-mail address: muro.fumihito.iy@daiichisankyo.co.jp (F. Muro).

0968-0896/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2008.10.020

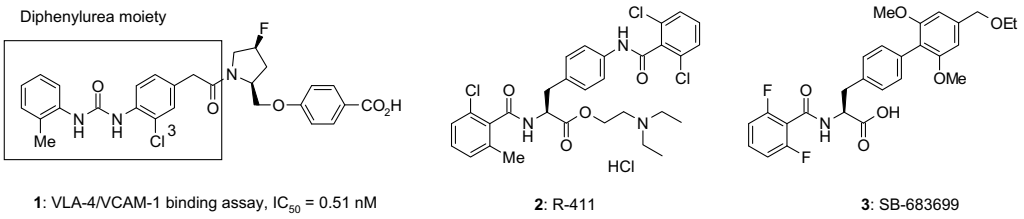


Figure 1. VLA-4 antagonists.

Herein, we report the synthesis, structure–activity relationships, and physicochemical properties of a series of benzoic acid derivatives as well as their biological evaluation.

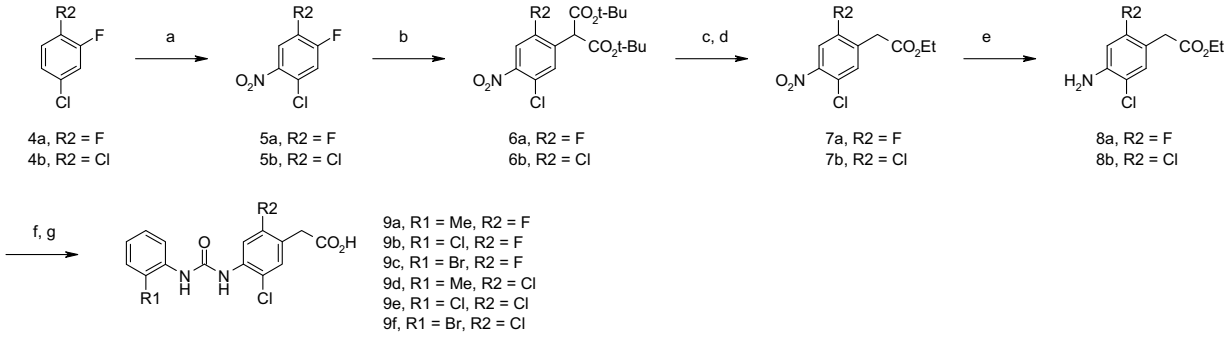
2. Chemistry

4-(*N*′-Phenylureido)phenylacetic acid intermediates **9a**–**f**, **13a**–**c**, and **18a**–**c** required for the synthesis of benzoic acid derivatives **20a**–**l** were prepared according to the procedures shown in Scheme 1–3.

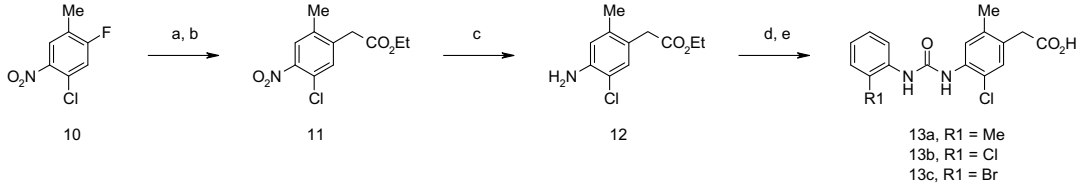
Commercially available halobenzene derivatives **4a**–**b** were converted to the nitrobenzenes **5a**–**b** via the procedure found in the literature[12] using a mixture of nitric and sulfuric acids. Nucleophilic displacement of **5a**–**b** with di-*tert*-butyl malonate in the presence of NaH gave the di-*tert*-butyl malonates **6a**–**b**. Following acidic hydrolysis (concd HCl/AcOH) of **6a**–**b** and subsequent decarboxylaton, the resultant carboxylic acid group was esterified to give the ethyl esters **7a**–**b**. The nitro group of **7a**–**b** was reduced using iron powder to afford **8a**–**b**. The anilines **8a**–**b** were treated with commercially available isocyanates, followed by basic hydrolysis to provide the 4-(*N*′-phenylureido)phenylacetic acid **9a**–**f** (Scheme 1).

In an analogous manner, nucleophilic displacement of commercially available nitrobenzene **10** with *tert*-butyl ethyl malonate, acidic hydrolysis and decarboxylation (TFA/CH_2Cl_2) gave **11**. After reduction of the nitro group of **11** with $SnCl_2$, the resultant aniline **12** was treated with commercially available isocyanates, followed by basic hydrolysis to afford the 4-(*N*′-phenylureido)phenylacetic acid **13a**–**c** (Scheme 2).

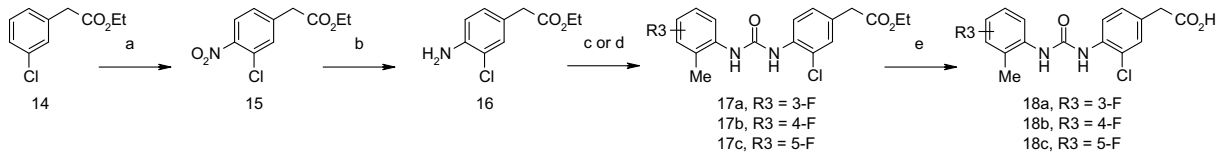
Ethyl 3-chloro-4-aminophenylacetate **16** was prepared from commercially available ethyl 3-chlorophenylacetate **14** according to the reported procedure.[13] Reaction of the aniline **16** with triphosgene resulted in the corresponding phenylcarbamoyl chloride, which was treated with 3-fluoro-2-methylaniline or 4-fluoro-2-methylaniline to afford **17a**–**b**. On the other hand, urea **17c** was prepared by treatment of **16** with 2-methyl-5-fluorophenyl isocy-



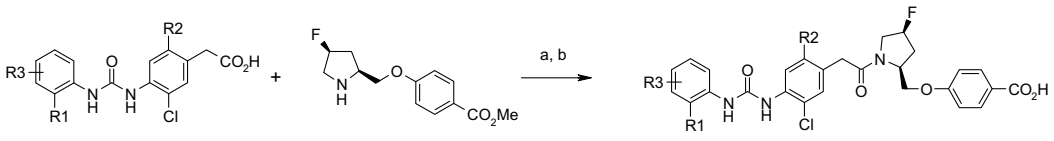
Scheme 1. Reagents and conditions: (a) 60% HNO_3, concd H_2SO_4; (b) di-*tert*-butyl malonate, NaH, DMF; (c) AcOH, conc. HCl, 110 °C; (d) concd H_2SO_4, EtOH, reflux; (e) Fe, AcOH, NaOAc, EtOH–H_2O; (f) 2-(R_1)-phenyl isocyanate, Et_3N, DMF; (g) 1 N NaOH, THF–MeOH.



Scheme 2. Reagents and conditions: (a) *tert*-butyl ethyl malonate, NaH, DMF; (b) TFA, CH_2Cl_2 reflux; (c) $SnCl_2$, EtOH, reflux; (d) 2-(R_1)-phenyl isocyanate, Et_3N, DMF; (e) 1 N NaOH, THF–MeOH.



Scheme 3. Reagents and conditions: (a) 60% HNO_3, concd H_2SO_4; (b) $SnCl_2$, EtOH, reflux; (c) 3-fluoro-2-methylaniline or 4-fluoro-2-methylaniline, triphosgene, pyridine, CH_2Cl_2; (d) 5-fluoro-2-methylphenyl isocyanate, Et_3N, THF; (e) 1N NaOH, THF–MeOH.



9a, R1 = Me, R2 = F, R3 = H
9b, R1 = Cl, R2 = F, R3 = H
9c, R1 = Br, R2 = F, R3 = H
9d, R1 = Me, R2 = Cl, R3 = H
9e, R1 = Cl, R2 = Cl, R3 = H
9f, R1 = Br, R2 = Cl, R3 = H
13a, R1 = Me, R2 = Me, R3 = H
13b, R1 = Cl, R2 = Me, R3 = H
13c, R1 = Br, R2 = Me, R3 = H
18a, R1 = Me, R2 = H, R3 = 3-F
18b, R1 = Me, R2 = H, R3 = 4-F
18c, R1 = Me, R2 = H, R3 = 5-F

19

20a, R1 = Me, R2 = F, R3 = H
20b, R1 = Me, R2 = Cl, R3 = H
20c, R1 = Me, R2 = Me, R3 = H
20d, R1 = Cl, R2 = F, R3 = H
20e, R1 = Cl, R2 =Cl, R3 = H
20f, R1 = Cl, R2 = Me, R3 = H
20g, R1 = Br, R2 = F, R3 = H
20h, R1 = Br, R2 = Cl, R3 = H
20i, R1 = Br, R2 = Me, R3 = H
20j, R1 = Me, R2 = H, R3 = 3-F
20k, R1 = Me, R2 = H, R3 = 4-F
20l, R1 = Me, R2 = H, R3 = 5-F

Scheme 4. Reagents and conditions: (a) EDC·HCl, HOBt, DMAP, DMF; (b) 0.25 N NaOH, THF–MeOH.

anate. Subsequently, compounds **17a–c** were hydrolyzed under basic conditions to afford the 4-(*N*′-phenylureido)phenylacetic acid **18a–c** (Scheme 3).

Benzoic acids **20a–l** were prepared according to the general procedure shown in Scheme 4. Thus, the 4-(*N*′-phenylureido)phenylacetic acid **9a–f**, **13a–c**, and **18a–c** were condensed with methyl 4-[(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]benzoate **19**[14] using EDC·HCl and HOBt followed by basic hydrolysis to furnish the target compounds **20a–l**.

3. Results and discussion

3.1. In vitro activity

The benzoic acid derivatives (**20a–l**) were evaluated for their VLA-4 inhibitory activities in a receptor binding assay (VLA-4 overexpressed CHO cells/Human VCAM-1 labeled with Europium). At the same time, we also evaluated the inhibitory activities of these compounds in the presence of 3% human serum albumin (HSA) to predict the in vivo efficacy. These results are summarized in Tables 1 and 2.

In order to determine the favorable pattern of substituents at the C-2 position (R_2) on the central benzene and the C-2 position (R_1) on the terminal benzene in the diphenylurea moiety for activity, we examined a methyl group and halogen atoms (F, Cl, and Br) as the substituents, based on the early SAR we obtained in this series of studies (Table 1).[11]

Introduction of a fluorine atom (**20a**), a chlorine atom (**20b**) and a methyl group (**20c**) into R_2 was tolerated in inhibitory activity as compared with compound **1**. In the case of R_2 = F, replacement of the methyl group (R_1) in **20a** with a chlorine atom (**20d**) and a bromine atom (**20g**) was also tolerated. Additionally, in the case of R_2 = Cl or Me, a similar trend was observed.

However, all the compounds showed a 1/20- to 1/200-fold decrease in activity in the presence of 3% HSA as compared with those in the absence of HSA. In the case of R_2 = Cl, replacement of the methyl group (R_1) in **20b** with a chlorine atom (**20e**) and a bromine atom (**20h**) caused significant loss of potency (IC_{50} (+3% HSA) = 63 nM for **20b**, 630 nM for **20e**, 1462 nM for 20 h). From those results, we considered that the loss of activity would be attributed to enhancement of the protein binding by the increased lipophilicity of the substituents.

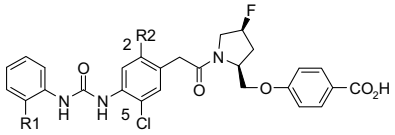
We next investigated the effect of a fluorine atom on the terminal benzene on inhibitory activity. In this modification, we fixed R_1 as the methyl group such as in **1** on the basis of the aforementioned higher potency (Table 2).

As a result, the introduction of a fluorine atom at the C-4 position (**20k**) and C-5 position (**20l**) except for the C-3 position (**20j**) was well tolerated. Similarly, the inhibitory activities of these compounds in the presence of 3% HSA decreased by 1/20–1/120. However, compounds **20a** and **20l** showed acceptable potency even in the presence of 3% HSA (IC_{50} = 44 nM for **20a**, 31 nM for **20l**).

For the purpose of predicting the oral absorption of all the compounds described above, we evaluated them for membrane permeability with MDCK monolayer cells and determined the distribution coefficient [log*D*, *n*-octanol-PBS (pH 7.4)] to assess their lipophilicity. Furthermore, we also estimated their oral absorption by the bio-assay method, in which we calculated the estimated serum concentration by measuring the activity in the serum 15 min after oral dosing at 10 mg/kg in mice. These results are summarized in Table 3.

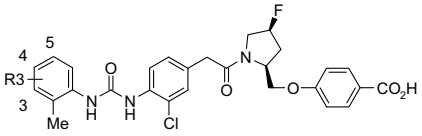
As expected, the substituents (Me and halogens) at those positions (R_1, R_2, and R_3) effectively increased the log*D* value as com-

Table 1
Inhibitory activity of VLA-4 antagonists



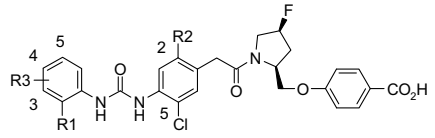
Compound	R1	R2	IC_{50} (nM)	IC_{50} (+3% HSA) (nM)	Ratio = IC_{50} (+3% HSA)/IC_{50}
1	Me	H	0.51	7.2	14.1
20a	Me	F	1.8	44	24.4
20b	Me	Cl	2.2	63	28.6
20c	Me	Me	1.9	36	18.9
20d	Cl	F	2.9	332	114.5
20e	Cl	Cl	3.4	630	185.3
20f	Cl	Me	3.5	394	112.6
20g	Br	F	4.3	688	160.0
20h	Br	Cl	7.5	1462	194.9
20i	Br	Me	4.8	629	131.0

Table 2
Inhibitory activity of VLA-4 antagonists



Compound	R3	IC_{50}(nM)	IC_{50} (+3% HSA) (nM)	Ratio = IC_{50} (+3% HSA)/IC_{50}
1	H	0.51	7.2	14.1
20j	3-F	12	745	62.1
20k	4-F	2.8	330	117.9
20l	5-F	1.6	31	19.4

Table 3
MDCK permeability, distribution coefficient, and serum concentration of VLA-4 antagonists



Compound	R1	R2	R3	MDCK Papp ($\times 10^{-6}$ cm/s)	log *D*	Serum concentration (ng/ml)
1	Me	H	H	2.7	1.6	3659
20a	Me	F	H	2.8	2.1	3077
20b	Me	Cl	H	5.4	2.7	165
20c	Me	Me	H	1.8	1.9	190
20d	Cl	F	H	5.8	3	3130
20e	Cl	Cl	H	8.9	3.2	1464
20f	Cl	Me	H	3.5	2.5	526
20g	Br	F	H	6.5	2.9	4241
20h	Br	Cl	H	< 6.0	3.2	3022
20i	Br	Me	H	4.7	2.6	265
20j	Me	H	3-F	2.4	2.4	1510
20k	Me	H	4-F	1.2	2.1	2274
20l	Me	H	5-F	2.6	2.4	4814

All the compounds were orally administered at 10 mg/kg and the serum concentration was measured after 15 min.

pared to **1**, resulting in an enhancement of the membrane permeability. Among them, compounds **20g** and **20l** exhibited relatively high estimated serum concentration with values of 4241 and 4814 ng/ml, respectively. On the other hand, the introduction of a methyl group into R_2 particularly showed a significant decrease in the estimated serum concentration (190 ng/ml for **20c**, 526 ng/ml for **20f**, and 265 ng/ml for **20i**). We previously reported that the nature of the substituent at the C-3 position in the central benzene as in **1** affected the plasma clearance in rodents.[11] In this case, we consider that the methyl group R_2 in common among those three compounds might have a negative effect on the plasma clearance.

3.2. In vivo efficacy

On the basis of the in vitro activity and oral absorption estimated by bio-assay, we selected compound **20l** and conducted biological evaluation.

At first, we evaluated anti-inflammatory effect of the selected compound **20l** in an *Ascaris*-antigen induced murine asthma model by measuring the level of eosinophils in bronchial alveolar lavage (BAL) fluid after antigen challenge (Fig. 2). As a result, we found that compound **20l** inhibited eosinophil infiltration into BAL fluid in a dose-dependent manner, and its efficacy was comparable to that of the *anti*-mouse α4 antibody[15] (R1-2) used as the positive control in this experiment.

Next, compound **20l** was also evaluated for its efficacy on bronchial hyper-responsiveness (BHR) at 48 h after an antigen challenge in a murine asthma model. Compound **20l** was administered orally at 15 or 75 mg/kg bid and the result is presented in Figure 3. It was found that compound **20l** significantly inhibited the increase of bronchial responsiveness to acetylcholine chloride in a dose-dependent manner. On the other hand, lead compound **1** was not effective in this asthma model even at an oral dose of 100 mg/kg bid (data not shown).

3.3. Pharmacokinetic properties

The encouraging result of compound **20l** in the murine asthma model prompted us to conduct a pharmacokinetic study on rats and dogs. Eventually, we found that compound **20l** exhibited moderate plasma clearance (CL = 6.3 ml/min/kg for rats, 5.2 ml/min/kg for dogs) and bioavailability (F = 23 for rats, 38% for dogs), as shown in Table 4.

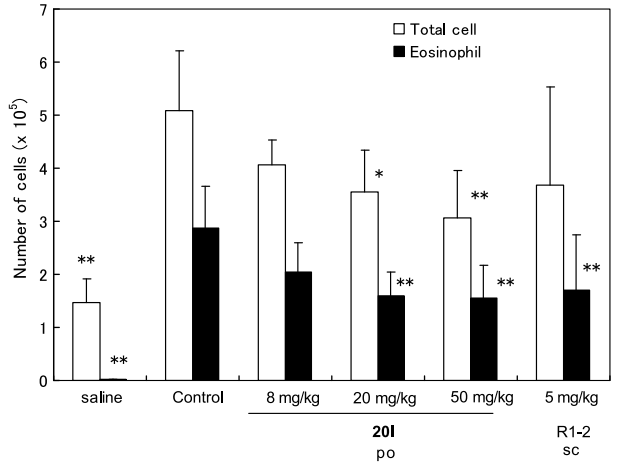


Figure 2. Effect of compound **20l** on the leukocyte infiltration in the BAL fluid 48h after antigen challenge in *Ascuaris suum* sensitized mice. Compound **20l** was given to mice tid for 2 days. The *anti*-mouse α4 antibody (R1-2) was given to mice sid for 2 days. Each column represents mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$: significantly different from the control by a Dunnett's multiple comparison test.

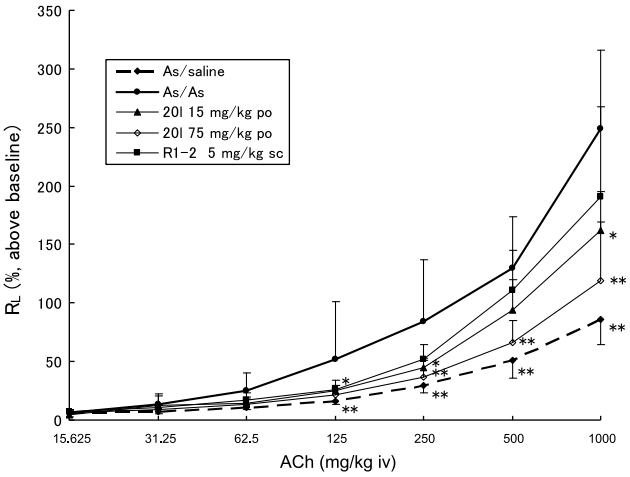


Figure 3. Effect of compound **20l** on antigen induced airway hyper-responsiveness to acetylcholine chloride (ACh) 48 h after antigen challenge in *Ascaris suum* sensitized mice. Compound **20l** was given to mice bid for 2 days. The *anti*-mouse α4 antibody (R1-2) was given to mice sid for 2 days. Results are expressed as means ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$: significantly different from the control (As/As) by a Dunnett's multiple comparison test.

4. Conclusion

For the purpose of the optimization of lead compound **1**, fine tuning on the substituent in the diphenyl urea moiety was carried out, culminating in the identification of compound **20l** with potent in vitro activity and an acceptable oral pharmacokinetic profile in rats and dogs. Compound **20l** inhibited eosinophil infiltration into BAL fluid in an *Ascaris*-antigen sensitized murine asthma model in a dose-dependent manner by oral dosing and the efficacy was comparable to that of the *anti*-mouse α4 antibody (R1-2). Compound **20l** also blocked the increase of bronchial responsiveness to acetylcholine chloride in the model. Further optimization studies for improvement of the pharmacokinetic profiles of compound **20l** will be reported in due course.

Table 4
Pharmacokinetic properties of **20l** in rats and dogs

Species	F (%)	PO			IV			
		AUC (ng h/ml)	C_{max} (ng/ml)	$T_{1/2}$ (h)	AUC (ng h/ml)	CL (ml/min/kg)	Vd_{ss} (l/kg)	$T_{1/2}$ (h)
Rat [a]	23	1438	1678	3.4	3128	6.3	0.17	1.6
Dog [b]	38	2389	674	5.0	6308	5.2	0.26	2.3

[a] Dose: po at 2 mg/kg; iv at 1 mg/kg (n = 4).
[b] Dose: po at 1 mg/kg; iv at 1 mg/kg (n = 3).

5. Experimental

5.1. General

Column chromatography was performed with Merck silica gel 60 (particle size 0.060–0.200 or 0.040–0.063). Flash column chromatography was performed with Biotage FLASH Si and YAMAZEN Hi-Flash packed columns. Thin-layer chromatography (TLC) was performed on Merck pre-coated TLC glass sheets with silica gel 60 F254. ^{1}H NMR spectra were recorded on a JEOL JNM-EX-400 spectrometer, and chemical shifts are given in ppm (δ) from tetramethylsilane as an internal standard. Spectral splitting patterns are designated as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; m, multiple. IR spectra were recorded on a HORIBA FT-720 spectrometer. ESI mass spectra were recorded on a SCIEX API-150EX spectrometer; FAB mass spectra were recorded on a JEOL JMS-HX110 spectrometer. Elemental analysis were determined by a Perkin-Elmer CHNS/O 2400II, Leco CHNS-932 and YOKOKAWA analysis IC7000RS.

5.2. General procedure A: preparation of 5-chloro-2-fluoro-4-[*N*′-(2-methylphenyl)ureido]phenylacetic acid (9a)

5.2.1. 1-Chloro-4,5-difluoro-2-nitrobenzene (5a)

To a cooled (0 °C), stirred solution of 4-chloro-1,2-difluorobenzene (2.00 g, 13.5 mmol) in concd H_2SO_4 (3.59 ml, 67.3 mmol) was added 60% HNO_3 (1.02 ml, 13.5 mmol), and the reaction mixture was stirred at room temperature for 4 h. The mixture was poured into ice-H_2O and extracted with EtOAc. The combined extracts were washed with satd $NaHCO_3$, brine, dried over Na_2SO_4, and concentrated in vacuo to give the title compound (2.54 g, 97%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 7.44 (1H, dd, J = 9.4, 7.1 Hz), 7.89 (1H, dd, J = 9.2, 7.8 Hz).

5.2.2. Di-*tert*-butyl (5-chloro-2-fluoro-4-nitrophenyl)malonate (6a)

To a cooled (0 °C), stirred suspension of NaH (60% in oil, 39.48 g, 0.987 mol) in DMF (500 ml) was added dropwise di-*tert*-butyl malonate (73.7 ml, 0.329 mol) and the mixture was stirred at 0 °C for 30 min. To the mixture was added 1-chloro-4,5-difluoro-2-nitrobenzene (63.70 g, 0.329 mol) in DMF (350 ml) and the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was quenched by the addition of 1 N HCl, and extracted with EtOAc. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo to give the title compound (131.8 g, 100%) as a brown oil. ^{1}H NMR ($CDCl_3$) δ 1.49 (18H, s), 4.79 (1H, s), 7.67 (1H, d, J = 8.6 Hz), 7.77 (1H, d, J = 6.4 Hz).

5.2.3. Ethyl 5-chloro-2-fluoro-4-nitrophenylacetate (7a)

To a stirred solution of di-*tert*-butyl (5-chloro-2-fluoro-4-nitrophenyl)malonate (128.2 g, 0.329 mol) in acetic acid (660 ml) was added concd HCl (330 ml) at 0 °C. The resulting mixture was stirred at 110 °C for 3 h. After removal of the solvent, the residue was dissolved into EtOH (350 ml). To the mixture was added concd H_2SO_4 (5 ml) and the mixture was stirred at 80 °C for 2 h. After removal of the solvent, the residue was diluted with satd $NaHCO_3$, and extracted with EtOAc. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified using a middle pressure chromatography system (YAMAZEN FR50N, *n*-hexane/EtOAc (9:1, v/v), φ 80 × 300 mm) to give the title compound (63.8 g, 74%) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.28 (3H, t, J = 7.1 Hz), 3.71 (2H, s), 4.21 (2H, q, J = 7.1 Hz), 7.51 (1H, d, J = 6.6 Hz), 7.68 (1H, d, J = 8.3 Hz); MS (ESI), m/z 262 (M^++1)

5.2.4. Ethyl 4-amino-5-chloro-2-fluorophenylacetate (8a)

To a stirred suspension of ethyl 5-chloro-2-fluoro-4-nitrophenylacetate (58.1 g, 0.222 mol) in EtOH–H_2O (1.5 l, 1:4, v/v) was added iron powder (40.4 g, 92% purity, 0.666 mol), NaOAc (16.6 g, 0.202 mol) and acetic acid (82.4 ml, 1.44 mol) and the resulting mixture was heated under reflux for 3 h. After being cooled to room temperature, the mixture was filtered through a Celite pad and the solvent was concentrated to a small volume. The mixture was extracted with EtOAc. The combined extracts were washed with satd $NaHCO_3$, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified using a middle pressure chromatography system (YAMAZEN FR50N, *n*-hexane/EtOAc (9:1, v/v) φ 80 × 300 mm) to give the title compound (47.0 g, 91%) as a light brown oil. ^{1}H NMR ($CDCl_3$) δ: 1.26 (3H, t, J = 7.1 Hz), 3.50 (2H, s), 4.10 (2H, broad s), 4.16 (2H, q, J = 7.1 Hz), 6.47 (1H, d, J = 10.8 Hz), 7.12 (1H, d, J = 7.4 Hz); MS (ESI), m/z 232 (M^++1).

5.2.5. 5-Chloro-2-fluoro-4-[*N*′-(2-methylphenyl)ureido]phenylacetic acid (9a)

To a stirred solution of ethyl 4-amino-5-chloro-2-fluorophenylacetate (695 mg, 3.0 mmol) in DMF (8 ml) was added triethylamine (0.418 ml, 3.0 mmol) and 2-methylphenyl isocyanate (0.362 ml, 3.0 mmol) at room temperature. The reaction mixture was stirred at 80 °C for 18 h. The mixture was concentrated to a small volume and the resulting solid was collected by suction and washed with *n*-hexane to give ethyl 5-chloro-2-fluoro-4-[*N*′-(2-methylphenyl)ureido]phenylacetate. To a stirred solution of 5-chloro-2-fluoro-4-[*N*′-(2-methylphenyl)ureido]phenylacetate in THF/MeOH (14 ml, 1:1, v/v) was added 1 N NaOH (7.0 ml, 7.00 mmol) and the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was poured into 1 N HCl and the resulting precipitate was collected by suction. The solid was recrystallized from *n*-hexane–$CHCl_3$ to give the title compound (720 mg, 71%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.26 (3 H, s), 3.59 (2H, s), 6.92–7.01 (2H, m), 7.11–7.20 (2H, m), 7.49–7.51 (1H, m), 7.73–7.81 (2H, m), 8.03 (1H, d, J = 12.5 Hz); MS (ESI), m/z, 337 (M^++1), 335 (M^+−1).

Compounds **9b**–**f** were prepared according to general procedure A.

5.3. 5-Chloro-4-[*N*′-(2-chlorophenyl)ureido]-2-fluorophenylacetic acid (9b)

Yield 74% (two steps). Colorless solid. ^{1}H NMR (DMSO-d_6) δ 3.60 (2H, s), 7.04–7.15 (2H, m), 7.29–7.33 (2H, m), 7.46–7.51 (2H, m), 7.95–8.00 (1H, m), 8.06–8.08 (1H, m); MS (ESI), m/z 357 (M^++1), 355 (M^+−1).

5.4. 4-[*N*′-(2-Bromophenyl)ureido]-5-chloro-2-fluorophenylacetic acid (9c)

Yield 66% (two steps). Colorless solid. ^{1}H NMR (DMSO-d_6) δ 3.60 (2H, s), 6.99–7.05 (2H, m), 7.32–7.38 (2H, m), 7.61–7.65 (2H, m), 7.91–7.99 (2H, m); MS (ESI), m/z 401 (M^++1).

5.5. 1,4-Dichloro-2-fluoro-5-nitrobenzene (5b)

Yield 91%. Yellow solid. ^{1}H NMR ($CDCl_3$) δ 7.40 (1H, d, J = 7.8 Hz), 8.09 (1H, d, J = 6.9 Hz).

5.6. Di-*tert*-butyl (2,5-dichloro-4-nitrophenyl)malonate (6b)

Yield 92%. Yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.50 (18H, s), 5.01 (1H, s), 7.77 (1H, s), 7.97 (1H, s); MS (ESI), m/z 428 (M^++Na).

5.7. Ethyl 2,5-dichloro-4-nitrophenylacetate (7b)

Yield 96%. Yellow solid. ^{1}H NMR ($CDCl_3$) δ 1.28 (3H, t, J = 7.1 Hz), 3.80 (2H, s), 4.21 (2H, q, J = 7.1 Hz), 7.53 (1H, s), 7.98 (1H, s); MS (ESI), m/z 278 (M^++1)

5.8. Ethyl 4-amino-2,5-dichlorophenylacetate (8b)

Yield 92%. Yellow solid. ^{1}H NMR ($CDCl_3$) δ 1.26 (3H, t, J = 7.1 Hz), 3.61 (2H, s), 4.07 (2H, broad s), 4.17 (2H, q, J = 7.1 Hz), 6.80 (1H, s), 7.16 (1H, s); MS (ESI), m/z 248 (M^++1).

5.9. 2,5-Dichloro-4-[*N*′-(2-methylphenyl)ureido]phenylacetic acid (9d)

Yield 72% (two steps). Colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.26 (3H, s), 3.67 (2H, s), 6.92–7.01 (2H, m), 7.11–7.20 (4H, m), 7.73–7.81 (2H, m); MS (ESI), m/z 353 (M^++1).

5.10. 4-[*N*′-(2-chlorophenyl)ureido]-2,5-dichlorophenylacetic acid (9e)

Yield 75% (two steps). Colorless solid. ^{1}H NMR (DMSO-d_6) δ 3.68 (2H, s), 7.04–7.10 (2H, m), 7.28–7.33 (2H, m), 7.46–7.49 (2H, m), 8.06 (2H, d, J = 7.1 Hz).

5.11. 4-[*N*′-(2-bromophenyl)ureido]-2,5-dichlorophenylacetic acid (9f)

Yield 80% (two steps). Colorless solid. ^{1}H NMR (DMSO-d_6) δ 3.68 (2H, s), 6.99–7.05 (2H, m), 7.32–7.37 (2H, m), 7.61–7.64 (2H, m), 7.92 (2H, dd, J = 1.5, 8.3 Hz); MS (ESI), m/z 417 (M^++1).

5.12. Ethyl 5-chloro-2-methyl-4-nitrophenylacetate (11)

To a stirred solution of *tert*-butyl ethyl malonate (3.86 g, 20.5 mmol) in DMF (200 ml) was added NaH (60% in oil, 2.46 g, 61.5 mmol) at room temperature and the mixture was stirred for 20 min. To the mixture was added dropwise 2-chloro-4-fluoro-5-methylnitrobenzene (3.89 g, 20.5 mmol) in DMF (50 ml) and the reaction mixture was stirred at room temperature for 3 h. The mixture was quenched by the addition of H_2O and extracted with EtOAc. The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was dissolved into CH_2Cl_2 (20 ml). To the solution was added TFA (20 ml) at room temperature and the reaction mixture was heated under reflux for 18 h. The reaction mixture was concentrated in vacuo and co-evaporated with toluene (2× 20 ml). The residue was purified using a middle pressure chromatography system (YAMAZEN YFLC-5404-FC, *n*-hexane/EtOAc 10:0 to 1:1, v/v, ϕ 50 × 300 mm) to give the title compound (5.29 g, 73%) as a yellow oil; ^{1}H NMR (CD_3OD) δ 1.30 (3H, t, J = 7.3 Hz), 2.35 (3H, s), 3.70 (2H, s), 4.20 (2H, q, J = 7.3 Hz), 7.40 (1H, s,), 7.72 (1H, s).

5.13. Ethyl 4-amino-5-chloro-2-methylphenylacetate (12)

To a stirred solution of ethyl 5-chloro-2-methyl-4-nitrophenylacetate (3.85 g, 14.9 mmol) in EtOH (100 ml) was added $SnCl_2$ (10.11 g, 44.8 mmol) and the reaction mixture was heated under reflux for 18 h. After removal of the solvent, the residue was diluted with $CHCl_3$ (200 ml) and poured into 4 N NaOH at 0 °C. The mixture was extracted with $CHCl_3$. The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified using a middle pressure chromatography system (YAMAZEN YFLC-5404, linear gradient of *n*-hexane/EtOAc from 9:1 to 1:1, v/v, φ 50 × 500 mm) to give the title compound (2.08 g, 61%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.24 (3H, t, J = 7.8 Hz), 2.20 (3H, s), 3.49 (2H, s), 3.91 (2H, broad s), 4.12 (2H, q, J = 7.8 Hz), 6.58 (1H, s), 7.05 (1H, s); MS (ESI), m/z 228 (M^++1).

Compounds **13a–c** were prepared according to general procedure A.

5.14. 5-Chloro-4-[*N*′-(2-methylphenyl)ureido]-2-methylphenylacetic acid (13a)

Yield 79% (two steps). Colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.19 (3H, s), 2.26 (3H, s), 3.55 (2H, s), 6.92–6.98 (1H, m), 7.11–7.19 (3H, m), 7.29 (1H, s), 7.76–7.81 (1H, m), 7.92 (1H, s), 8.54–8.57 (1H, m), 12.36 (1H, broad s); MS (ESI), m/z 331 (M^+-1).

5.15. 5-Chloro-4-[*N*′-(2-chlorophenyl)ureido]-2-methylphenylacetic acid (13b)

Yield 100% (two steps). Colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.19 (3H, s), 3.57 (2H, s), 7.03–7.08 (2H, m), 7.28–7.32 (2H, m), 7.45–7.48 (2H, m), 8.05–8.09 (2H, m), 12.39 (1H, broad s); MS (ESI), m/z 353 (M^++1), 351 (M^+-1).

5.16. 4-[*N*′-(2-Bromophenyl)ureido]-5-chloro-2-methylphenylacetic acid (13c)

Yield 100% (two steps). Colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.20 (3H, s), 3.57 (2H, s), 6.99–7.02 (2H, m), 7.32–7.35 (2H, m), 7.61–7.63 (2H, m), 7.90–7.96 (2H, m); MS (ESI), m/z 397(M^++1).

5.17. Ethyl 3-chloro-4-nitrophenylacetate (15)

To a cooled (0 °C), stirred solution of ethyl 3-chlorophenylacetate (141.2 g, 0.765 mol) in concd H_2SO_4 (300 ml) was added 60% HNO_3 (58.2 ml, 0.765 mol) and the reaction mixture was stirred at 5 °C for 22 h. The mixture was poured into ice-H_2O and extracted with EtOAc. The combined extracts were washed with H_2O, brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel with *n*-hexane/EtOAc (20:1– 5:1, v/v) as an eluent to give the title compound (71.6 g, 38%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.28 (3H, t, J = 7.2 Hz), 3.67 (2H, s), 4.19 (2H, q, J = 7.2 Hz), 7.34 (1H, dd, J = 1.6, 8.4 Hz), 7.50 (1H, d, J = 1.6 Hz), 7.87 (1H, d, J = 8.4 Hz).

5.18. Ethyl 4-amino-3-chlorophenylacetate (16)

To a stirred solution of ethyl 3-chloro-4-nitrophenylacetate (52.55 g, 0.216 mol) in EtOH (1000 ml) was added $SnCl_2{\cdot}2H_2O$ (147.0 g, 0.647 mol) and the reaction mixture was heated under re-

flux for 3 h. After removal of the solvent, the residue was diluted with $CHCl_3$. To the mixture was added 4 N NaOH (324 ml, 1.296 mol) at 0 °C and the resulting mixture was stirred at 0 °C for 30 min. The mixture was filtered through a Celite pad and the filtrate was extracted with $CHCl_3$. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel with $CHCl_3$–EtOAc (40:1–30:1, v/v) as an eluent to give the title compound (27.77 g, 60%) as a reddish brown oil. ^{1}H NMR ($CDCl_3$) δ 1.25 (3H, t, J = 7.2 Hz), 3.47 (2H, s), 4.01 (2H, broad s), 4.14 (2H, q, J = 7.2 Hz), 6.71 (1H, d, J = 8.4 Hz), 6.98 (1H, dd, J = 1.6, 8.0 Hz), 7.17 (1H, d, J = 2.0 Hz).

5.19. 3-Chloro-4-[*N*′-(3-fluoro-2-methylphenyl)ureido]phenylacetic acid (18a)

To a stirred solution of ethyl 3-chloro-4-aminophenylacetate (2.31 g, 10.8 mmol) in CH_2Cl_2 (60 ml) was added pyridine (6.0 ml, 74.2 mmol) and triphosgene (1.18 g, 3.97 mmol). After being stirred at room temperature for 3 h, 3-fluoro-2-methylaniline (1.23 ml, 10.8 mmol) was added to the mixture and the resulting mixture was stirred at room temperature for 20 h. After removal of the solvent, the mixture was triturated with *n*-hexane–THF and the resulting precipitate was collected by suction to give ethyl 3-chloro-4-[*N*′-(3-fluoro-2-methylphenyl)ureido]phenylacetate (**17a**) as a crude solid. To a stirred solution of ethyl 3-chloro-4-[*N*′-(3-fluoro-2-methylphenyl)ureido]phenylacetate (**17a**, 10.8 mmol) in THF–MeOH (50 ml, 4:1, v/v) was added 1 N NaOH (20 ml, 20.0 mmol) and the reaction mixture was stirred at room temperature for 17 h. After removal of the solvent, the mixture was acidified by the addition of 1 N HCl and the resulting precipitate was collected by suction and dried under vacuum to give the title compound (2.70 g, 74% for two steps) as a light brown solid. ^{1}H NMR (DMSO-d_6) δ 2.16 (3H, d, J = 1.7 Hz), 3.43 (2H, s), 6.86 (1H, t, J = 8.5 Hz), 7.12 (1H, d, J = 1.7 Hz), 7.15 (2H, s), 7.92 (1H, s), 7.94 (1H, s), 8.80 (1H, s), 8.91 (1H, s).

5.20. 3-Chloro-4-[*N*′-(4-fluoro-2-methylphenyl)ureido]phenylacetic acid (18b)

Yield 55% (two steps). Reddish purple solid. ^{1}H NMR (DMSO-d_6) δ 2.26 (3H, s), 3.54 (2H, s), 6.98 (1H, dt, J = 2.9, 8.8 Hz), 7.07 (1H, dd, J = 2.7, 9.5 Hz), 7.16 (1H, dd, J = 2.0, 8.5 Hz), 7.37 (1H, d, J = 2.0 Hz), 7.68–7.71 (1H, m), 8.02 (1H, d, J = 8.5 Hz), 8.58 (2H, d, J = 8.8 Hz), 12.38 (1H, broad s).

5.21. 3-Chloro-4-[*N*′-(5-fluoro-2-methylphenyl)ureido]phenylacetic acid (18c)

To a stirred solution of ethyl 4-amino-3-chlorophenylacetate (14.1 g, 66.0 mmol) and Et_3N (9.22 ml, 66.2 mmol) in THF (200 ml) was added 5-fluoro-2-methylphenyl isocyanate (10.0 g, 66.2 mmol) and the reaction mixture was stirred at room temperature for 17 h. The mixture was concentrated to a small volume and the resulting precipitate was collected by suction. The solid was washed with *n*-hexane and dried under vacuum to give ethyl 3-chloro-4-[*N*′-(5-fluoro-2-methylphenyl)ureido]phenylacetate (**17c**, 9.95 g, 41%) as a colorless solid. To a stirred solution of ethyl 3-chloro-4-[*N*′-(5-fluoro-2-methylphenyl)-ureido]phenylacetate (**17c**, 9.95 g, 27.3 mmol) in THF-MeOH (200 ml, 1:1, v/v) was added 1 N NaOH (100 ml, 100 mmol) and the reaction mixture was stirred at room temperature for 2 h. After being concentrated to a small volume, the mixture was acidified by the addition of 1 N HCl and the resulting precipitate was collected by suction. The solid was washed with H_2O and dried under a vacuum to give the title compound (8.81 g, 96%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.24 (3H, s), 3.45 (2H, s), 6.74 (1H, dt, J = 2.4, 8.4 Hz), 7.13–7.19 (2H, m), 7.34 (1H, d, J = 1.2 Hz), 7.79 (1H, dd, J = 2.4, 12.0 Hz), 7.92 (1H, d, J = 8.4 Hz), 8.82 (1H, s), 8.93 (1H, s).

5.22. General procedure B: preparation of 4-[1-[3-Chloro-4-[*N*′-(5-fluoro-2-methylphenyl)ureido]phenylacetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]benzoic acid (20l)

To a stirred solution of methyl 4-[1-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]benzoate (**19**, 200 mg, 0.79 mmol) and 3-chloro-4-[*N*′-(5-fluoro-2-methylphenyl)ureido]phenylacetic acid (**18c**, 266 mg, 0.79 mmol) in DMF (7 ml) was added EDC·HCl (229 mg, 1.19 mmol), HOBt (5 mg, 0.04 mmol) and DMAP (5 mg, 0.04 mmol). The reaction mixture was stirred at room temperature for 20 h. The mixture was diluted with H_2O and extracted with EtOAc. The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel with $CHCl_3$-MeOH (10:1, v/v) as an eluent to give methyl 4-[1-[3-chloro-4-[*N*′-(5-fluoro-2-methylphenyl)ureido]phenylacetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]benzoate (375 mg, 83%) as a pale yellow amorphous solid. IR (KBr) 1716, 1604, 1531 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.08–2.25 (1H, m), 2.15 (3H, s), 2.52–2.60 (1H, m), 3.51–3.61 (2H, m), 3.72–3.81 (1H, m), 3.86 (3H, s), 3.93–4.06 (2H, m), 4.48 (1H, dd, J = 3.6, 8.8 Hz), 4.56–4.64 (1H, m), 5.26–5.40 (1H, m), 6.66–6.71 (1H, m), 6.89–6.95 (2H, m), 7.01–7.05 (2H, m), 7.12–7.16 (1H, m), 7.59 (1H, dd, J = 2.4, 10.8 Hz), 7.72 (1H, broad s), 7.92–8.02 (4H, m); MS (ESI), m/z 572 (M^++1). Anal. Calcd for $C_{29}H_{28}ClF_2N_3O_5\cdot0.5H_2O$: C, 59.95; H, 5.03; N, 7.23. Found: C, 60.01; H, 5.10; N, 6.81.

To a stirred solution of methyl 4-[1-[3-chloro-4-[*N*′-(5-fluoro-2-methylphenyl)ureido]phenylacetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]benzoate (368 mg, 0.64 mmol) in THF/MeOH (7 ml, 6:1, v/v) was added 0.25 N NaOH (5 ml, 1.25 mmol) and the reaction mixture was stirred at room temperature for 16 h. The mixture was acidified by the addition of 1 N HCl and extracted with $CHCl_3$/MeOH (5:1, v/v). The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel with $CHCl_3$/MeOH (10:1, v/v) as an eluent to give the title compound (272 mg, 76%) as a colorless solid. IR (KBr) 1710, 1685, 1604, 1529 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.23 (3H, s), 2.27–2.30 (2H, m), 3.61–3.71 (2H, m), 3.78–3.97 (3H, m), 4.04–4.66 (2H, m), 5.30–5.51 (1H, m), 6.73–6.77 (1H, m), 7.02–7.07 (2H, m), 7.13–7.20 (2H, m), 7.31–7.35 (1H, m), 7.79 (1H, d, J = 12.4 Hz), 7.85–7.90 (2H, m), 7.96–8.00 (1H, m), 8.66 (1H, s), 8.83 (1H, s), 12.63 (1H, broad s); MS (FAB), m/z 558 (M^++1). Anal. Calcd for $C_{28}H_{26}ClF_2N_3O_5\cdot1.5H_2O$: C, 57.49; H, 5.00; N, 7.18. Found: C, 57.77; H, 4.89; N, 6.86.

Compounds **20a**–**k** were prepared according to general procedure B

5.23. 4-[1-[3-Chloro-6-fluoro-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]benzoic acid (20a)

Yield 56% (two steps). Colorless powder. IR (KBr) 3353, 1604, 1531, 1253, 1168, 638 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.27 (3H, s), 2.30–2.33 (2H, m), 3.70–3.98 (5H, m), 4.08–4.70 (2H, m), 5.32–5.54 (1H, m), 6.99–7.07 (3H, m), 7.14–7.20 (2H, m), 7.37–7.42 (1H, m), 7.75 (1H, d, J = 7.8 Hz), 7.85–7.91 (2H, m), 7.99–8.04 (1H, m); MS (ESI), m/z 558 (M^++1). Anal. Calcd for $C_{28}H_{26}ClF_2N_3O_5\cdot1.25H_2O$: C, 57.93; H, 4.95; N, 7.24. Found: C, 58.12; H, 4.81; N, 6.91.

5.24. 4-[1-[2,5-Dichloro-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]benzoic acid (20b)

Yield 70% (two steps) Colorless powder. IR (KBr) 3340, 1604, 1511, 1251, 1166, 750 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.27 (3H, s), 2.30–2.35 (2H, m), 3.63–4.00 (5H, m), 4.12–4.70 (2H, m), 5.33–5.55 (1H, m), 6.94–7.20 (6H, m), 7.45–7.49 (1H, m), 7.76–7.81 (1H, m), 7.86–7.91 (2H, m), 8.26–8.31 (2H, m); MS (ESI), *m*/*z* 574 (M^++1). Anal. Calcd for $C_{28}H_{26}Cl_2FN_3O_5{\cdot}1.5H_2O$: C, 55.92; H, 4.86; N, 6.99. Found: C, 56.23; H, 4.78; N, 6.59.

5.25. 4-[1-[5-chloro-4-[*N*′-(2-methylphenyl)ureido]-2-methylphenylacetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]benzoic acid (20c)

Yield 70% (two steps). Colorless powder. IR (ATR) 3342, 1604, 1533, 1251, 1166, 755 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.17 (3H, s), 2.25 (3H, s), 2.29–2.32 (2H, m), 3.68–3.78 (2H, m), 3.80–3.99 (3H, m), 4.10-–4.43 (2H, m), 5.32–5.53 (1H, m), 6.98 (1H, t, *J* = 7.5 Hz), 7.03–7.07 (2H, m), 7.12–7.22 (3H, m), 7.78–7.80 (1H, m), 7.86–7.91 (3H, m); MS (FAB), *m*/*z* 554 (M^++1). Anal. Calcd for $C_{29}H_{29}ClFN_3O_5{\cdot}1.25H_2O$: C, 60.42; H, 5.51; N, 7.29. Found: C, 60.19, H, 5.38; N, 7.04.

5.26. 4-[1-[3-Chloro-4-[*N*′-(2-chlorophenyl)ureido]-6-fluorophenylacetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]benzoic acid (20d)

Yield 43% (two steps). Colorless powder. IR (KBr) 3322, 1529, 1249, 1168, 750, 644 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.26–2.33 (2H, m), 3.68–3.99 (5H, m), 4.10–4.69 (2H, m), 5.41–5.55 (1H, m), 7.04–7.10 (3H, m), 7.30–7.34 (1H, m), 7.39–7.49 (2H, m), 7.85–7.91 (2H, m), 7.96–7.99 (1H, m), 8.05–8.07 (1H, m); MS (ESI), *m*/*z* 578 (M^++1), 576 (M^+−1). Anal. Calcd for $C_{27}H_{23}Cl_2F_2N_3O_5{\cdot}1H_2O$: C, 54.37; H, 4.22; N, 7.05. Found: C, 54.41; H, 4.13; N, 6.80.

5.27. 4-[1-[4-[*N*′-(2-chlorophenyl)ureido]-2,5-dichlorophenylacetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]benzoic acid (20e)

Yield 46% (two steps). Colorless solid. IR (KBr) 3328, 1604, 1513, 1251, 1166, 750 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.27–2.34 (2H, m), 3.67–4.01 (5H, m), 4.12–4.72 (2H, m), 5.33–5.55 (1H, m), 7.05–7.10 (4H, m), 7.30 (1H, t, *J* = 7.8 Hz), 7.47–7.50 (2H, m), 7.86–7.91 (3H, m), 8.07 (1H, d, *J* = 8.6 Hz), 8.20–8.22 (1H, m); MS (ESI), *m*/*z* 594 (M^++1). Anal. Calcd for $C_{27}H_{23}Cl_3FN_3O_5{\cdot}1.25H_2O$: C, 52.53; H, 4.16; N, 6.81. Found: C, 52.78; H, 4.18; N, 6.31.

5.28. 4-[1-[5-Chloro-4-[*N*′-(2-chlorophenyl)ureido]-3-methylphenylacetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]benzoic acid (20f)

Yield 25% (two steps). Colorless powder. IR (ATR) 3338, 1583, 1531, 1438, 750 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.18 (3H, s), 2.26–2.33 (2H, m), 3.59–3.73 (2H, m), 3.84–3.99 (3H, m), 4.09–4.42 (2H, m), 5.32–5.54 (1H, m), 7.05–7.07 (3H, m), 7.20–7.24 (1H, m), 7.30 (1H, t, *J* = 8.0 Hz), 7.48 (1H, d, *J* = 7.8 Hz), 7.86–7.91 (3H, m), 8.10 (1H, d, *J* = 8.4 Hz); MS (FAB), *m*/*z* 574 (M^++1). Anal. Calcd for $C_{28}H_{26}Cl_2FN_3O_5{\cdot}1.25H_2O$: C, 56.34; H, 4.81; N, 7.04. Found: C, 56.28, H, 4.71; N, 6.72.

5.29. 4-[1-[4-[*N*′-(2-Bromophenyl)ureido]-3-chloro-6-fluorophenylacetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]benzoic acid (20g)

Yield 38% (two steps). Colorless powder. IR (KBr) 3320, 1525, 1434, 1249, 1168, 750 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.26–2.33 (2H, m), 3.58–3.98 (5H, m), 4.10–4.69 (2H, m), 5.32–5.55 (1H, m), 7.01–7.06 (3H, m), 7.34–7.44 (2H, m), 7.63–7.65 (1H, m), 7.85–7.98 (4H, m). Anal. Calcd for $C_{27}H_{23}BrClF_2N_3O_5{\cdot}0.5H_2O$: C, 51.32; H, 3.83; N, 6.65. Found: C, 51.29; H, 3.93; N, 6.36.

5.30. 4-[1-[4-[*N*′-(2-bromophenyl)ureido]-2,5-dichlorophenylacetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]benzoic acid (20h)

Yield 49% (two steps). Colorless solid. IR (KBr) 3328, 1604, 1513, 1434, 1251, 1166, 750 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.25–2.33 (2H, m), 3.63–4.00 (m, 5 H), 4.10–4.72 (2H, m), 5.42–5.55 (1H, m), 7.01–7.07 (4H, m), 7.35 (1H, t, *J* = 7.5 Hz), 7.47–7.50 (1H, m), 7.63 (1H, d, *J* = 7.3 Hz), 7.86–7.94 (4H, m), 8.19–8.21 (1H, m); MS (ESI), *m*/*z* 638 (M^++1). Anal. Calcd for $C_{27}H_{23}BrCl_2FN_3O_5{\cdot}0.25H_2O$: C, 50.37; H, 3.68; N, 6.53. Found: C, 50.57; H, 4.06; N, 5.99.

5.31. 4-[1-[4-[*N*′-(2-bromophenyl)ureido]-5-chloro-2-methylphenylacetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]benzoic acid (20i)

Yield 27% (two steps). Colorless powder. IR (ATR) 3324, 1581, 1529, 1434, 1025, 748 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.19 (3H, s), 2.25–2.32 (2H, m), 3.59–3.74 (2H, m), 3.85–4.00 (3H, m), 4.10–4.40 (2H, m), 5.32–5.54 (1H, m), 6.98–7.07 (3H, m), 7.20–7.24 (1H, m), 7.32–7.35 (1H, m), 7.62 (1H, d, *J* = 8.0 Hz), 7.84–7.91 (3H, m), 7.96 (1H, d, *J* = 6.8 Hz); MS (FAB), *m*/*z* 618 (M^++1). Anal. Calcd for $C_{28}H_{26}BrClFN_3O_5{\cdot}1.25H_2O$: C, 52.43; H, 4.48; N, 6.55. Found: C, 52.42, H, 4.34; N, 6.11.

5.32. 4-[1-[3-Chloro-4-[*N*′-(3-fluoro-2-methylphenyl)-ureido]phenylacetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]benzoic acid (20j)

Yield 53% (two steps). Light brown solid. IR (KBr) 1604, 1581, 1527, 1240, 1220, 1167, 773 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.17 (3H, s), 2.21–2.34 (2H, m), 3.65–3.68 (2H, m), 3.78–4.28 (4H, m), 4.38–4.71 (1H, m), 5.31–5.52 (1H, m), 6.87 (1H, t, *J* = 8.5 Hz), 7.04–7.07 (2H, m), 7.16–7.20 (2H, m), 7.32–7.36 (1H, m), 7.65 (1H, d, *J* = 8.1 Hz), 7.87–7.90 (2H, m), 7.99–8.01 (1H, m), 8.69 (1H, s), 8.73 (1H, s), 12.60 (1H, broad s); MS (ESI), *m*/*z* 558 (M^++1). Anal. Calcd for $C_{28}H_{26}ClF_2N_3O_5{\cdot}1H_2O$: C, 58.39; H, 4.90; N, 7.30. Found: C, 58.58; H, 4.78; N, 7.14.

5.33. 4-[1-[3-Chloro-4-[*N*′-(4-fluoro-2-methylphenyl)-ureido]phenylacetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]benzoic acid (20k)

Yield 76% (two steps). Colorless solid. IR (KBr) 1604, 1525, 1496, 1248, 1167, 773 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.18–2.32 (2H, m), 2.27 (3H, s), 3.47–3.97 (5H, m), 4.05–4.68 (2H, m), 5.31–5.52 (1H, m), 6.98 (1H, dt, *J* = 2,9, 8.6 Hz), 7.08–7.13 (3H, m), 7.14 (1H, t, *J* = 8.3 Hz), 7.31–7.35 (1H, m), 7.68–7.72 (1H, m), 7.86–7.91 (2H, m), 7.99–8.03 (1H, m), 8.55–8.59 (2H, m), 12.61 (1H, broad s); MS (ESI), *m*/*z* 558 (M^++1). Anal. Calcd for $C_{28}H_{26}ClF_2N_3O_5{\cdot}1H_2O$: C, 58.39; H, 4.90; N, 7.30. Found: C, 58.38; H, 4.73; N, 7.10.

5.34. VLA-4/VCAM-1 binding assay

A human VLA-4-expressing cell line, 4B4, was established at Pharmacopeia Inc., by transfecting both the α4 gene and β1 gene of VLA-4 into CHO-K1 cells. The 4B4 cells were maintained in Ham′s F-12 medium (Sigma Corporation) supplemented with 10% (v/v) fetal calf serum (REHATUIN Fetal Bovine Serum, Serologicals Corporation), 100 U/ml penicillin (Invitrogen Corporation), 100 μg/ml streptomycin (Invitrogen Corporation), and 2 mM L-glu-

tamine (Invitrogen Corp.) and 1 mg/ml G-418 (Geneticin, Invitrogen Corporation). An Eu-labeling Reagent (Perkin-Elmer Inc.) was used to labeled the human VCAM-1/Fc chimeria (R&D Systems Inc.). The Eu-labeled protein was purified by a PD-10 column (Amersham Biosciences KK.) and stored at −80 °C until use. All assays were performed in duplicate. In preparation for the assay, the 4B4 cells were suspended at 3×10^5 cells/ml in Ham's F-12 medium. One hundred microliters of the 4B4 cell suspension was placed into each well of a 96-well-culture plate (Costar Inc.). The plates were incubated at 37 °C in a 5% CO_2 atmosphere for 2 days. Prior to the assay, the medium was discarded and each well was washed twice with 300 μl of chilled Wash Buffer (25 mM Hepes, pH 7.5, 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 4 mM $MnCl_2$). Then, 50 μl of compound solution was added to a well, followed by 50 μl of 2 nM of Eu-labeled human VCAM-1/Fc chimera diluted with the Wash Buffer (final concentration: 1 nM). For assays conducted in the presence of human serum albumin, 50 μl of compound at various concentrations and an equal volume of 2 nM Eu-labeled human VCAM-1/Fc chimera in 6% (w/v) human serum albumin (Sigma Corporation) were distributed into each well (final concentration: 1 nM). The plates were incubated for 60 min at room temperature and the wells were washed 4 times with 300 μl of chilled Wash Buffer. Finally, 100 μl of the enhancement solution (Perkin-Elmer Inc.) was added to each well. The plates were placed on a shaker for 5 min. Eu fluorescence was then measured using a time-resolved fluorometer (DELFIA Research fluorometer, model 1234-001; Perkin-Elmer Inc.). The concentration of compound required for 50% inhibition in the assay was determined.

5.35. Bio-assay

Female BALB/c mice (8 weeks old, Charles River Japan, Inc.) were used. Each group consisted of four animals. The mice were orally administered the compound dissolved in 0.5% (w/v) methylcellulose (MC) at a dose of 10 mg/ml/kg. After 15 min, blood samples were collected via inferior vena cava from the animals under ether anesthesia. The blood samples were stood at room temperature and centrifuged at 2000 rpm for 10 min at 4 °C. The serum samples were subsequently stored in a −20 °C freezer prior to analysis. According to the VLA-4/VCAM-1 binding assay, instead of the compound solution, 50 μl of serum samples at various concentrations were added into each well (final concentration: 0.01–10%). As for the calibration curve, each diluted compound solution was also assayed in the presence of the same concentration of untreated mouse serum.

5.36. Distribution coefficient

The distribution coefficients (log *D*) at pH 7.4 were determined by the shake-flask method.[16] A 400 μM solution of each compound in a 2 ml *n*-octanol/2 ml PBS solution was placed on a shaker for 30 min. After centrifuging each solution separately at 3000 rpm for 10 min, an LC/MS method was used to assay each layer. The LC/MS system consisted of an 1100 Series LC/MSD (Agilent) and an X Terra® MSC18 3.5 μm, 3.0 × 30 mm column (Waters). The mobile phase was a 10 mM ammonium acetate buffer (pH 4.5)/0.05% (v/v) acetic acid mixture in acetonitrile; the gradient condition (95/5–10/90). An analyst software program (version 1.4, Applied Bio. Systems) was used to calculate the log *D*.

5.37. Madin–Darby canine kidney cell permeability

The cell permeability of the selected compounds was determined with Madin–Darby canine kidney (MDCK, American Type Culture Collection) cells. MDCK cells were maintained in Minimum Essential Medium (GIBCO) containing 10% (v/v) fetal bovine serum (GIBCO), a penicillin–streptomycin mixture (GIBCO), and L-glutamine. For the transport assay, cells were seeded into HTS 24-well transwells (Costar) at 1.65×10^5 cells/ml and grown for 6 days after seeding to allow the formation of a cell monolayer. Transport buffer was prepared using $NaHCO_3$ (final 0.35 g/l), D-glucose (final 3.5 g/l), Hepes (Sigma; final 10 mM), $CaCl_2$ (final 0.14 g/l), and $MgSO_4$ (final 0.098 g/l) in 10× Hanks' Balanced Salt Solution (GIBCO) and adjusted to pH 6.0 or 7.4 with 1 M HCl or 1 M NaOH. For each test compound, a dosing solution containing one of the compounds at a concentration of 10 μM in transport buffer (pH 6.0) (100 μl) was added to the apical (A) side of the monolayer. A blank solution containing 4% (w/v) BSA in transport buffer (pH 7.4) (600 μl), which was re-adjusted to pH 7.4, was added to the basolateral (B) side of the monolayer. Metoprolol was used as a positive control. After 1 h of incubation at 37 °C, aliquots of the basal solutions were analyzed on an LC/MS/MS system comprising an Alliance 2790 HPLC (Waters), Atlantis dC18, 2.1 mm ID × 20 mm *L*, 3 μm particle size column (Waters), and TSQ7000 mass spectrometer (ThermoQuest). The mobile phase consisted of a 10 mM HCO_2NH_4–acetonitrile step gradient 100/0–20/80–100/0. The concentrations of each compound in the basolateral solutions were determined from a peak area versus a concentration standard curve. For each compound, Eq. 1 was used to calculate an apparent permeability coefficient (P_{app}) from the LC/MS/MS-determined concentration in the basolateral compartment (C_b, μM) and the initial 10 μM concentration in the donor compartment. In the following equation, 3600 s is the total time for the measurement of compound flux, and 0.33 cm^2 is the area of the transwell filter.

$$P_{app}(10^{-6}\text{cm/s}) = (C_b \times 600\ \mu\text{l})/(10\ \mu\text{M} \times 3600\text{s} \times 0 : 33\ \text{cm}^2) \quad (1)$$

5.38. Murine asthma model

Female BALB/c AnNCrj mice (8 weeks old, Charles River Japan, Inc.) received an oral administration of cyclophosphamide dissolved in water at a dose of 150 mg/kg (day 0). On day 2 and 14,500 μg protein of *Ascaris suum* extract (LSL Co., Ltd) in 0.2 ml saline containing 4.5 mg aluminum hydroxide was injected intraperitoneally. On day 22, the mice were challenged intratracheally under anesthesia with 300 μg (30 μl) protein of *A. suum* extract. In the negative control group, sensitized mice were challenged with saline instead of the antigen.

5.38.1. Effect on eosinophil infiltration

Compound **20l**, which was dissolved in 0.5% MC containing 0.03% Tween 80, was orally administered 15 min before and 5, 13, 21, 29, and 37 h after the antigen challenge at a dose of 8, 20, or 50 mg/kg. Forty-eight hours after antigen challenge, the mice were sacrificed and BAL fluid was collected using tracheal polyethylene cannula with 2 × 0.5 ml Hanks' balanced salt solution. The cells in the BAL fluid were counted in a particle analyzer CDA-500 (Sysmex Corporation). Cytocentrifuged preparations (Cytospin 2; Shandon) were stained with Wright's stain solution (Muto Chemical Co., Ltd) for differential counts, based on standard morphologic criteria. The number of eosinophils was calculated by multiplying the total cell number by the percentage of eosinophils in a cytocentrifuged preparations.

5.38.2. Effect on hyper-responsiveness

Compound **20l**, which was dissolved in 0.5% MC containing 0.03% Tween 80, was orally administered 15 min before and 8, 24, 32 h after the antigen challenge at a dose of 15 or 75 mg/kg. The bronchial hyper-responsiveness in each mouse was estimated

from the increase in lung resistance by an acetylcholine chloride injection at 48 h after the antigen challenge. Ten minutes before the start of the measurement, the mice were anesthetized by an intraperitoneal injection of pentobarbital at a dose of 100 mg/kg. The trachea was cannulated and connected to a rodent ventilator (Model 683; Harvard Apparatus) with an in-line pressure transducer (DP45-28; Validyne Engineering Corporation) that was coupled to a pulmonary mechanics analyzer (Model 6; Buxco Electronics, Inc.). Flows were determined by measuring the differential pressure (DP45-14; Validyne Engineering Corporation) across six layers of 400-mesh wire cloth covering a 1.3-cm hole in a plethysmogragh box (Plyan-M; Buxco Electorics, Inc.). Mice were placed in the plethysmogragh box and then ventilated at 120 strokes/min with a stroke volume of 350 μL. After establishing a stable baseline of lung resistance, acetylcholine chloride (ACh; Sigma Corp.), dissolved in saline, was cumulatively administered (15.625–1000 μg/ml/kg) via the tail vein, and changes in lung resistance were monitored.

Increase of lung resistance (R_L) is calculated as follows.

Percent increase of R_L = [(Peak R_L induced by ACh injection) − (Baseline of R_L)] × 100/(Baseline of R_L)

5.39. Pharmacokinetic studies on rats

Male Sprague–Dawley rats [Crj: CD(SD) IGS, 7 weeks old, Charles River Laboratories] were used. The animals were fasted for 18 h prior to dosing. Each group consisted of four animals. The rats were orally administered the test compound at the doses of 2 mg/kg dissolved in 0.5% (w/v) MC with 3 equiv NaOH aqueous solution. The rats were intravenously administered the test compound at a dose of 1 mg/kg dissolved in saline with 3 equiv NaOH solution. Blood samples (0.4 ml) were collected at 0.08, (or 0.25 for po), 0.5, 1, 2, and 6 h after the administration. These analytical samples were stored at room temperature, followed by centrifugation at 15,000 rpm for 10 min at 4 °C. The plasma fractions were subsequently stored in a −20 °C freezer until being analyzed. The concentrations of the test compounds were determined by an LC/MS/MS method, comprised of an Alliance 2695 HPLC (Waters), Symmetry Shield RP8, 2.1 × 50 mm, 3.5 μm column (Waters), and TSQ–700 (Thermo Electron, Waltham, MA). The mobile phase consisted of 10 mM $HCOONH_4$ in water/methanol; the gradient condition was 90/10 to 10/90. The plasma concentrations versus the time data were analyzed by non-compartmental approaches using the WinNonlin software program (version 1.13.1 Pharsight, Mountain View, CA).

5.40. Pharmacokinetic studies on dogs

Male Beagle dogs (10–12 kg, LSG Corporation) were used. The animals were fasted for 18 h prior to dosing. Each group consisted of three animals. The test compound was dissolved in 0.5% (w/v) MC with 3 equiv NaOH for oral dosing or dissolved in saline with 3 equiv NaOH for intravenous dosing. The dose in each experiment was 1 mg/kg. Blood samples (1 ml) were collected after 0.08 (for iv), 0.25 (for po), 0.5, 1, 2, 4, 8, and 24 h. After the 4 h sampling, the animals were provided with food. These analytical samples were prepared and analyzed according to the pharmacokinetic studies on rats.

References and notes

1. Hynes, R. O. *Cell* **1987**, *48*, 549.
2. Elices, M. J.; Osborn, L.; Takeda, Y.; Crouse, C.; Luhowskyj, S.; Hemler, M. E.; Lobb, R. R. *Cell* **1990**, *60*, 577.
3. (a) Guan, J. L.; Hyens, R. O. *Cell* **1990**, *60*, 53; (b) Wayner, E. A.; Garcia-Pardo, A.; Humphries, M. J.; McDonald, J. A.; Carter, W. G. *J. Cell Biol.* **1989**, *109*, 1321.
4. (a) Vanderslice, P.; Bidiger, R. J.; Woodside, D. G.; Berens, K. L.; Holland, G. W.; Dixon, R. A. *Pulmon. Pharmacol. Ther.* **2004**, *17*, 1; (b) Yang, G. I.; Hagmann, W. H. *Med. Res. Rev.* **2003**, *23*, 369; (c) Tilley, J. W. *Expert Opin. Ther. Patents* **2002**, *12*, 991; (d) Jackson, D. Y. *Curr. Pharm. Des.* **2002**, *8*, 1229; (e) Yusuf-Makagiansar, H.; Anderson, M. E.; Yakovleva, T. V.; Murray, J. S.; Siahaan, T. J. *Med. Res. Rev.* **2002**, *22*, 146; (f) Holland, G. W.; Biediger, R. J.; Vandersilice, P.. *Annual Report in Medicinal Chemistry*. In *Vol. 37*; Academic press: New York, 2002. pp. 65; (g) Porter, J. R. *IDrugs* **2000**, *3*, 788.
5. Lin, K.; Ateenq, H. S.; Hsiunig, S. H.; Chong, L. T.; Zimmerman, C. N.; Castro, A.; Lee, W. C.; Hammond, C. E.; Kalkunte, S.; Chen, L. L.; Pepinsky, R. B.; Leone, D. R.; Sprague, A. G.; Abraham, W. M.; Gill, A.; Lobb, R. R.; Adams, S. P. *J. Med. Chem.* **1999**, *42*, 920.
6. (a) Yednock, T. A.; Cannon, C.; Fritz, L. C.; Sanchez-Madrid, F.; Steinman, L.; Karin, N. *Nature* **1992**, *356*, 63; (b) Piraino, P. S.; Yednock, T. A.; Freedman, S. B.; Messersmith, E. K.; Pleiss, M. A.; Vandevert, C.; Thorsett, E. D.; Karlik, S. J. *J. Neuroimmunol.* **2002**, *131*, 147.
7. Seiffge, D. *J. Rheumatol.* **1996**, *23*, 2086.
8. (a) Podolsky, D. K. *N. Engl. J. Med.* **1991**, *325*, 928; (b) Powrie, F.; Leach, M. W. *Ther. Immunol.* **1995**, *2*, 115.
9. (a) Miller, D. H.; Khan, O. A.; Sheremata, W. A.; Blumhardt, L. D.; Rice, G. P.; Libonati, M. A.; Willmer-Hulme, A. J.; Dalton, C. M.; Miszkiel, K. A.; O'Connor, P. W. *N. Engl. J. Med.* **2003**, *348*, 15; (b) Ghosh, S.; Goldin, E.; Gordon, F. H.; Malchow, H. A.; Madsen, J. R.; Rutgeerts, P.; Vynálek, P.; Zádorová, Z.; Palmer, T.; Donoghue, S. *N. Engl. J. Med.* **2003**, *348*, 24; (c) Steinman, L. *Nat. Rev. Drug Disc.* **2005**, *4*, 510; d Elan-Biogen official home page: http://www.tysabri.com/.
10. (a) Hijazi, Y.; Welker, H.; Dorr, A. E.; Tang, J.-P.; Blain, R.; Renzetti, L. M.; Abbas, R. *J. Clin. Pharmacol. Ther.* **2004**, *44*, 1368; (b) Chavez Lopez, F.; Shankar, A.; Thompson, M.; Shealy, B.; Locklear, D.; Rawalpally, T.; Cleary, T.; Gagliardi, C. *Org. Process Res. Dev.* **2005**, *9*, 1003; (c) Sircar, I.; Gudmundsson, K. S.; Martin, R. WO1999036393.; (d) Sagi, K.; Izawa, H.; Chiba, A.; Okuzumi, T.; Yoshimura, T.; Tanaka, Y.; Ono, M.; Murata, M. WO2003070709.; (e) Davenport, R. J.; Munday, J. R. *Drug Discovery Today* **2007**, *12*, 569.
11. Chiba, J.; Iimura, S.; Yoneda, Y.; Watanabe, T.; Muro, F.; Tsubokawa, M.; Iigou, Y.; Satoh, A.; Takayama, G.; Yokoyama, M.; Takashi, T.; Nakayama, A.; Machinaga, N. *Bioorg. Med. Chem.* **2007**, *15*, 1679.
12. Finger, G. C.; Gortatowski, M. J.; Shiley, R. H.; White, R. H. *J. Am. Chem. Soc.* **1959**, *81*, 94.
13. (a) RajanBabu, T. V.; Chenard, B. L.; Petti, M. A. *J. Org. Chem.* **1986**, *51*, 1704; (b) Zhu, J.; Beugelmans, R.; Bourdet, S.; Chastanet, J.; Roussi, G. *J. Org. Chem.* **1995**, *60*, 6389.
14. Chiba, J.; Iimura, S.; Yoneda, Y.; Sugimoto, Y.; Horiuchi, T.; Muro, F.; Ochiai, Y.; Ogasawara, T.; Tsubokawa, M.; Iigou, Y.; Takayama, G.; Taira, T.; Takata, Y.; Yokoyama, M.; Takashi, T.; Nakayama, A.; Machinaga, N. *Chem. Pharm. Bull.* **2006**, *54*, 1515.
15. Holzmann, B.; McIntyre, B. W.; Weissman, I. L. *Cell* **1989**, *56*, 37.
16. Manners, C. N.; Payling, D. W.; Smith, D. A. *Xenobiotica* **1989**, *19*, 1387.