Bioorganic & Medicinal Chemistry 17 (2009) 5989–6002

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Synthesis, biological evaluation, and metabolic stability of acrylamide derivatives as novel CCR3 antagonists

Ippei Sato [a,*], Koichiro Morihira [a], Hiroshi Inami [a], Hirokazu Kubota [a], Tatsuaki Morokata [a], Keiko Suzuki [a], Kazuki Ohno [a], Yosuke Iura [b], Aiko Nitta [b], Takayuki Imaoka [b], Toshiya Takahashi [b], Makoto Takeuchi [a], Mitsuaki Ohta [a], Shin-ichi Tsukamoto [a]

[a] Drug Discovery Research, Astellas Pharma Inc., 21 Miyukigaoka, Tsukuba-shi, Ibaraki 305-8585, Japan
[b] Pharmaceutical Research Laboratories, Toray Industries, Inc., 6-10-1 Tebiro, Kamakura, Kanagawa 248-0036, Japan

ARTICLE INFO

Article history:
Received 3 June 2009
Revised 25 June 2009
Accepted 26 June 2009
Available online 3 July 2009

Keywords:
Allergic diseases
CCR3 antagonists
Acrylamide derivatives
CL_{int}

ABSTRACT

Our laboratory has identified several acrylamide derivatives with potent CCR3 inhibitory activity. In the present study, we evaluated the in vitro metabolic stability (CL_{int}; mL/min/kg) of these compounds in human liver microsomes (HLMs), and assessed the relationship between their structures and CL_{int} values. Among the compounds identified, *N*-{(3*R*)-1-[(6-fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}-2-[1-(2-hydroxybenzoyl)piperidin-4-ylidene]acetamide (**30j**) was found to be a potent inhibitor (IC_{50} = 8.4 nM) with a high metabolic stability against HLMs.

1. Introduction

The chemokines are a family of small (8–12 kDa), heparin-binding, basic proteins that chemoattract and activate leukocytes such as monocytes, lymphocytes, eosinophils, and basophils. To date, over 50 distinct chemokines have been characterized and subsequently classified into four sub-families, CC chemokines, CXC chemokines, C chemokines, and CX_3C chemokines. This classification is based on the positions of their first two conserved N-terminal cysteine residues. The chemokine receptors are members of the seven-transmembrane G-protein coupled receptor (GPCR) family, which are expressed on various immune and inflammatory cells. Chemokines bind to and activate these receptors in order to exert their biological effects.[1a–e]

CC chemokine receptor 3 (CCR3), which is predominantly expressed on eosinophils, plays an important role in mediating the eosinophil chemotaxis induced by eosinophilic chemokines such as eotaxin-1, -2, -3, MCP-3, -4, and RANTES. The recruitment of eosinophils to sites of inflammation is thought to be a feature of various inflammatory diseases, such as allergic asthma and atopic dermatitis. Thus, using small molecule CCR3 antagonists to inhibit this recruitment process may lead to significant advances in the treatment of eosinophil-related allergic diseases.[2a,b]

In previous papers, we reported a series of 6-fluoro-2-naphthyl derivatives, **1**[3a] and **2**,[3b] as novel types of CCR3 antagonists (Fig. 1). However, their ability to inhibit CCR3 (IC_{50} = 20 and 23 nM, respectively) was slightly weaker than that of other CCR3 inhibitors reported by other groups.[4a–c] Additionally, compound **1** was found to have a potent CYP2D6 inhibition, as previously disclosed, and further examination revealed that compound **2**, which had improved CYP2D6 inhibition, exhibited poor permeability in a PAMPA (pH 6.5: 4.7×10^{-6} cm/s) likely due to the low *C* log $D_{7.4}$ value caused by the N-oxide moiety. Therefore, to improve the CCR3 inhibitory activity of compound **1**, we attempted the further structural optimization of **1** in the early stages of this study by adding a novel backbone. We planned a synthetic strategy based on previously reported key pharmacophores for CCR3 inhibitory activity. One pharmacophore is a basic nitrogen atom in the center of the molecule, and the other pharmacophores are the two aryl rings, a 6-fluoronaphthalene moiety and another aryl ring, at the terminus (Fig. 2).[3a] We first modified the linkage between the phenylene ring and the basic nitrogen atom of **1** and found that (**3*R***)-**17**, which has a pyrrolidine ring instead of the tropane ring, showed a moderate activity. We chose this pyrrolidine derivative as the next lead compound, and its optimization led to the discovery of several acrylamide derivatives that were potent CCR3 inhibitors.

In this paper, we detail the results of research on the structure–activity relationships (SARs) of these acrylamide derivatives as well as their in vitro metabolic stability against human liver microsomes (HLMs).

* Corresponding author. Tel.: +81 29 863 6732; fax: +81 29 852 5387.
E-mail address: ippei.sato@jp.astellas.com (I. Sato).

0968-0896/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2009.06.066

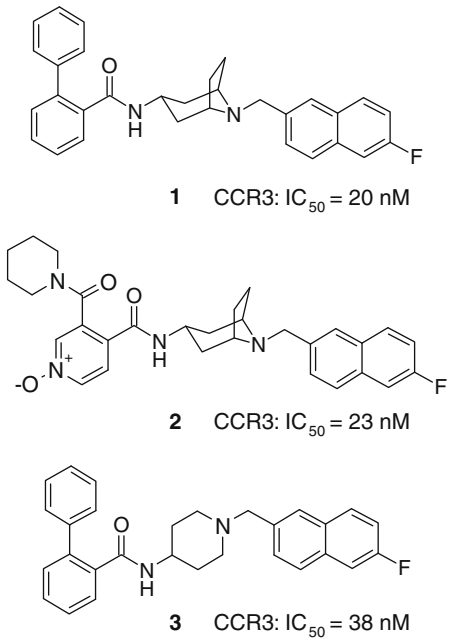


Figure 1. The structure of our previously reported compounds.[3a,b]

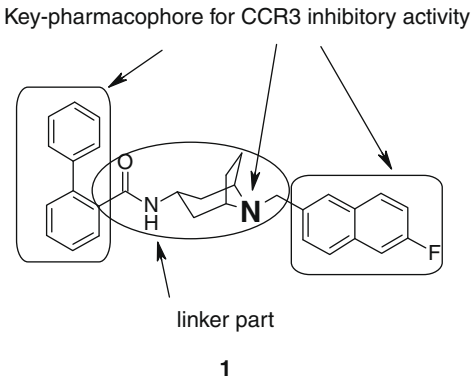


Figure 2. The key pharmacophore for CCR3 inhibitory activity.[3a]

2. Chemistry

Scheme 1 summarizes the synthesis of compounds **7**, **8**, **11**, (***rac***)-**14**, (***rac***)-**17**, (**3*S***)-**17**, and (**3*R***)-**17**. The alkylation of homopiperazine (**4**) with 2-(bromomethyl)-6-fluoronaphthalene (**5**)[5] yielded amine **6**. The condensation of **6** with biphenyl-2-carboxylic acid and biphenyl-2-ylacetic acid using WSC·HCl as the coupling reagent afforded **7** and **8**, respectively. The alkylation of *tert*-butyl piperazine-1-carboxylate (**9**) with **5**, followed by removal of the Boc group under acidic condition yielded the amine **10**, which was converted into **11** in the same manner as for **8**. Compound (***rac***)-**13** was synthesized by condensation of the amine (***rac***)-**12** with biphenyl-2-carboxylic acid, followed by deprotection of the Boc group. The alkylation of (***rac***)-**13** with **5**, and subsequent conversion to its hydrochloride salt via treatment with 4 M HCl (g)/EtOAc yielded (***rac***)-**14**. Compounds (***rac***)-**17**, (**3*S***)-**17**, and (**3*R***)-**17** were synthesized in the same manner as for **11**, respectively.

The synthetic routes of compounds **19a, 20a,b, 21a, 22a,b,** and **24** are shown in Scheme 2. Compounds **19a** and **20a** were prepared from (**3*R***)-**16** and appropriate acid chlorides, and for compound **20b**, compound **18**[3a] was used as a starting material instead of (**3*R***)-**16**. Compounds **21a** and **23** were prepared from (**3*R***)-**16** and appropriate carboxylic acids via condensation with WSC·HCl. The Boc group of **23** was removed under acidic conditions, and subsequent benzoylation yielded **24**. Benzenesulfonyl isocyanate was reacted with the free amines of (**3*R***)-**16** and **18**[3a] to afford **22a** and **22b**, respectively.

Scheme 3 illustrates the synthesis of compounds **26**, **29**, and **30a –30s**. The condensation of (**3*R***)-**16** with (diethoxyphosphoryl)acetic acid quantitatively yielded Horner–Wadsworth–Emmons (HWE) reagent **25**. Compounds **26** and **27** were obtained via the HWE reaction between **25** and appropriate ketones. By using compound **25** slightly more than sodium hydride in this step, a good yield of the *exo*-olefin compound was selectively obtained. The Boc group of **27** was removed under acidic conditions, and subsequent condensation with the corresponding commercially available carboxylic acids or acid chlorides yielded **29** and **30a–30s**. The intermediate ketone (**34**), which corresponded to compound **26**, was prepared via the Mitsunobu reaction between **33** and phenol, followed by acidic hydrolysis of the ketal group (Scheme 4).

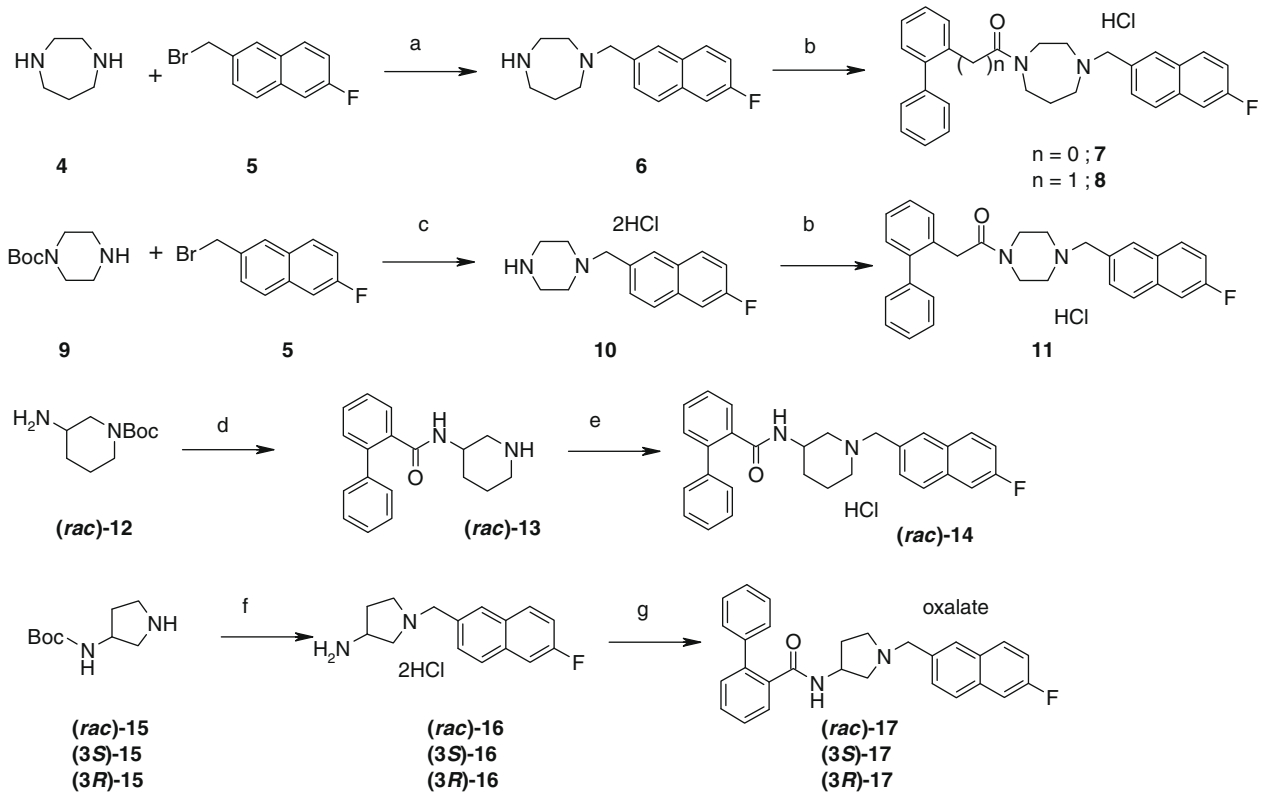
3. Results and discussion

Each synthesized compound's ability to inhibit (IC_{50}) eotaxin-induced Ca^{2+} influx was evaluated using CCR3-expressing pre-B cells. The lead compound **1** demonstrated potent CCR3 inhibitory activity with an IC_{50} value of 20 nM.

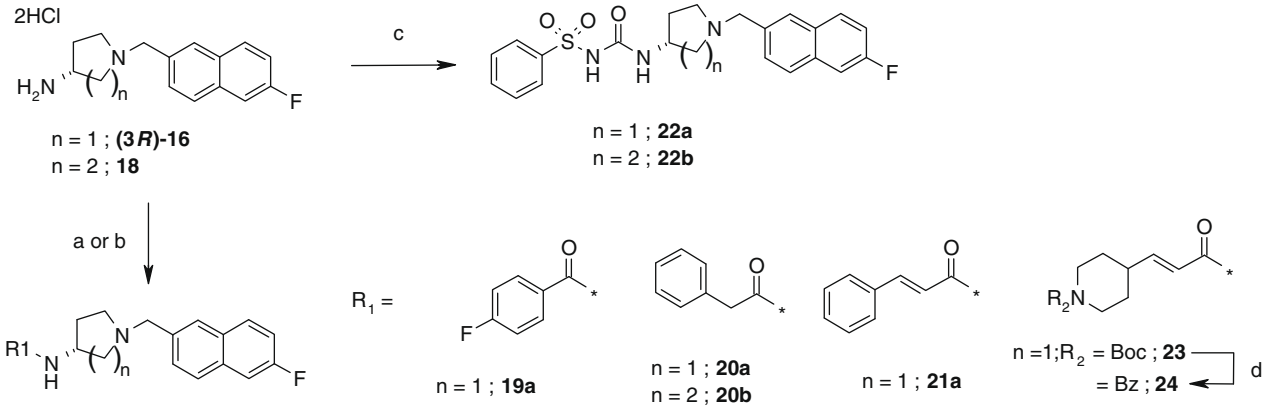
We previously reported that for optimal CCR3 inhibitory activity, the distance between the phenylene ring and the basic nitrogen of **1** was a more important factor than the nature of the linkage elements and that the piperidine derivative **3** was approximately two-fold less potent than **1**.[3a] In order to find alternative linkage structures, we initially reinvestigated the effect of replacing the tropane ring of **1** with another nitrogen-containing ring system (Table 1). Homopiperazines (**7** and **8**) and piperazine (**11**) derivatives had no inhibitory effect at 10 μM, irrespective of their linkage length.

However, the activity of 3-aminopiperidine [(***rac***)-**14**] and the 3-aminopyrrolidine [(***rac***)-**17**] derivatives was moderate. Comparison of the two enantiomers of (***rac***)-**17** revealed that the (3*R*)-isomer was approximately 20-fold more potent than the (3*S*)-isomer. Therefore, the structure–activity relationships (SARs) of the (3*R*)-3-aminopyrrolidine derivative [(**3*R***)-**17**] were further studied.

To simplify the SAR results, the terminal phenyl ring of the biphenyl moiety was removed, and the amide linkage between the phenyl ring and pyrrolidine was converted. The results were compared with those of piperidine derivatives that were already reported (**19b** and **21b**) (Table 2).[3a] The results obtained for pyrrolidine derivatives were markedly different from those obtained for piperidine derivatives. That is, while the length of linkage was an important factor for determining the inhibitory potency of piperidine derivatives, the potency of the pyrrolidine derivatives did not change after elongation of the linker. Among these compounds, the acrylamide derivative (**21a**) showed the most potent activity (Table 2). Based on these data, we decided to further elongate the linker part of **21a** by incorporating a rigid structure between the phenyl ring and the acrylamide double bond. We expected tolerance for various structural conversions in the linker part since all compounds listed out in Table 2 exhibited a moderate activity. The results are summarized in Table 3. The piperidinecarboxamide derivatives (**30a**), in which the acrylamide double bond was *exo*-substituted at the 4-position on the piperidine ring, showed extremely potent inhibitory activity (IC_{50} = 4.4 nM). Compared with **30a**, compound **24**, which has a linkage one atom longer than that of **30a**, showed a weak activity. Compound **26**, the cyclohexyl analogue of **30a**, also proved to be less active than **30a**, whose potency could be attributed to a newly introduced amide oxygen atom. We



Scheme 1. Synthesis of compounds **7**, **8**, **11**, **14**, and **17**. Reagents and conditions: (a) K_2CO_3, MeCN, rt, 20 h, 77%; (b) ArCOOH, WSC·HCl, HOBt, DMF, rt, then 4 M HCl/EtOAc; (c) K_2CO_3, MeCN, rt, 4.5 h, then 4 M HCl/EtOAc, EtOAc–MeOH, rt, overnight, 86% for two steps; (d) biphenyl-2-carboxylic acid, WSC·HCl, HOBt, THF, rt, 18 h, then 2 M HCl/EtOAc, rt, 4 h, 21% for two steps; (e) **5**, K_2CO_3, MeCN, rt, 25 h, then 4 M HCl/EtOAc, 20% for two steps; and (f) **5**, K_2CO_3, MeCN, rt, then, 4 M HCl/EtOAc, EtOAc, rt; (g) biphenyl-2-carboxylic acid, WSC·HCl, HOBt, Et_3N, DMF, rt, then oxalic acid.

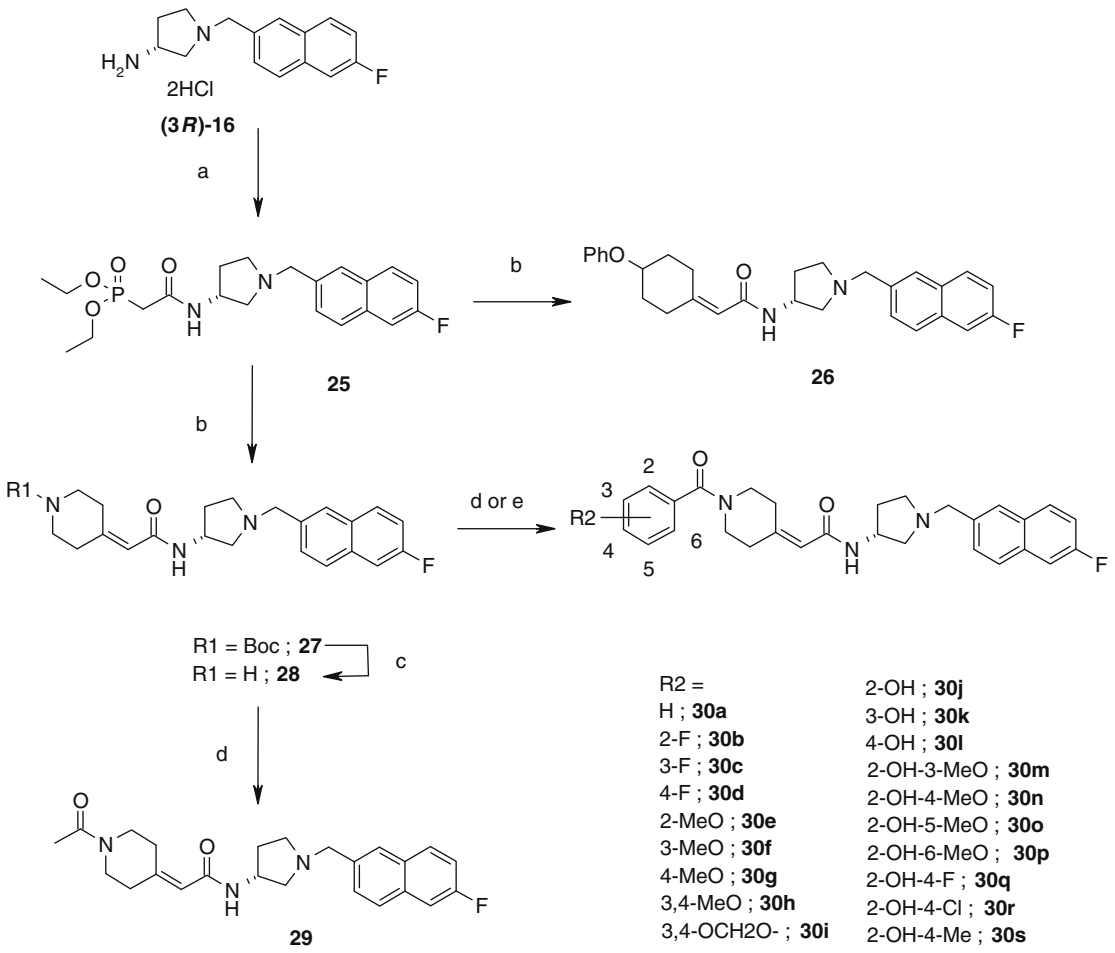


Scheme 2. Synthesis of compounds **19**–**22,** and **24**. Reagents and conditions: (a) ArCOCl, Et_3N, CH_2Cl_2, rt; (b) ArCOOH, WSC·HCl, HOBt, Et_3N, DMF, rt; (c) benzenesulfonyl isocyanate, MeCN, 0 °C; and (d) 4 M HCl/EtOAc, EtOAc, rt, overnight, then BzCl, Et_3N, CH_2Cl_2, rt, 3 h, 41% for two steps.

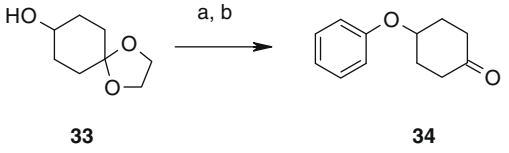
believed that the rigid structure of the piperidinecarboxamide moiety enhanced inhibitory activity by locking the terminal benzoyl moiety into an active conformation. In order to confirm the importance of the terminal phenyl ring, the phenyl moiety of **30a** was replaced with a methyl group. The activity of the acetamide derivative (**29**) significantly decreased relative to that of **30a**. These results also suggested that the newly added carbonyl moiety and terminal phenyl ring would work synergistically to increase the CCR3 inhibitory activity. Thus, we created a potent inhibitor with high novelty by modifying the acrylamide derivatives.

However, although compound **30a** showed better membrane permeability than **2**, as measured in a PAMPA (19.9×10^{-6} cm/s vs 4.7×10^{-6} cm/s), its metabolic stability against human liver microsomes (HLMs) in vitro was poor. Its clearance value (CL_{int} value) was 338 mL/min/kg, which is greater than those of **2** (89 mL/min/kg) and several commercial drugs.[6]

With the aim of improving both activity and metabolic stability, we next investigated the effects of various substituents around the benzoyl moiety of **30a** (Table 4). The introduction of an electron-withdrawing fluorine atom at the 2-position (**30b**) on the phenyl ring of **30a** yielded a similar activity, but the same introduction at the 3- or 4-position led to activity decreases of about threefold and fivefold, respectively. Introduction of an electron-donating methoxy moiety, at the 2- or 3-position retained the activity of



Scheme 3. Synthesis of compounds **26**, **29**, and **30a**–**30s**. Reagents and conditions: (a) (diethoxyphosphoryl)acetic acid, WSC·HCl, HOBt, Et_3N, DMF, rt, 3.5 h, quant.; (b) NaH (60% oil dispersion), DME, rt, 0.5 h, then ketone, DME, rt; (c) TFA, CH_2Cl_2, rt, 0.5 h, 97%; and (d) ArCOCl, CH_2Cl_2; (e) ArCOOH, WSC·HCl, HOBt, THF, rt.

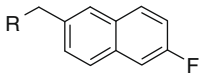


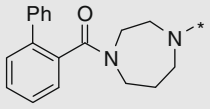
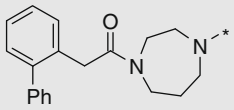
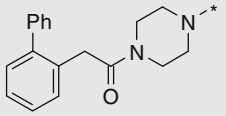
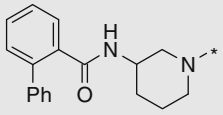
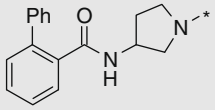
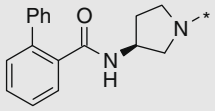
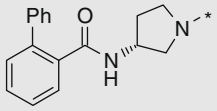
Scheme 4. Synthesis of compound **34**. Reagents and conditions: (a) phenol, PPh_3, DEAD, THF, rt, 12 h, 88% and (b) 3 M HCl aq, THF, rt, 8 h, 72%.

30a, while introduction at the 4-position (**30g**) slightly enhanced the activity (IC_{50} = 1.8 nM). The activity of the 3,4-dimethoxy derivative (**30h**) was similar to that of **30a**, and the 3,4-methylenedioxy derivative (**30i**) exhibited a slightly improved activity (IC_{50} = 1.6 nM). Incorporation of the hydroxyl moiety at the 2-position (**30j**) slightly reduced the inhibitory activity, while that at the 3- or 4-position resulted in activities equal to that of **30a**. These results indicated that the CCR3 inhibitory activity was more susceptible to an electron factor than to a steric factor placed near the terminal benzoyl part, and that the incorporation of electron-donating groups at the 4-position would improve the activity.

This series of compounds was also evaluated for its in vitro human liver microsomal (HLM) metabolic stability (CL_{int}; mL/min/kg). For both the fluorine- and methoxy-substituted derivatives, the rank order for metabolic stability (CL_{int}) is: 2-substituted > 3-substituted > 4-substituted. The 4-fluorine (**30d**) and 4-methoxy (**30g**) derivatives generated much smaller CL_{int} values than **30a**. On the other hand, both the 2- and 3-substituted derivatives resulted in larger CL_{int} values than that for **30a**. Furthermore, both 3,4-dimethoxy (**30h**) and the 3,4-methylenedioxy (**30i**) derivatives had CL_{int} values smaller than that for **30a**. These results suggested that the 4-position of benzoyl moiety would be the most susceptible to metabolic reactions induced by HLMs, and that the CL_{int} values could be improved by blocking there. The phenol derivatives (**30j**, **30k**, and **30l**) yielded interesting results; the CL_{int} value of the 4-substituted derivative (**30l**) was smaller than that of 3-substituted derivative (**30k**), which is consistent with the results described above. However, that of the 2-substituted derivative (**30j**) was much smaller than that of **30l**, which had the smallest CL_{int} value in this series of compounds. One reason why this occurred could be the intramolecular hydrogen bond (confirmed to exist in the NMR spectrum of **30j**) between the phenolic hydroxyl moiety and carbonyl oxygen of the amide moiety. In addition, the geometrically optimized structure of the model compound (*N*-2-hydroxybenzoylpiperidine), which was obtained by calculation on the HF/6-31* level using the GAUSSIAN 98 program[9], also suggested this (Fig. 3). We speculated that this carbonyl moiety plays an effective role in the interaction between CYPs of hepatic microsomes and ligands, and that the intramolecular hydrogen bond in **30j** decreased their interaction. To confirm this speculation, we evaluated the ability of the carbonyl moiety to accept the hydrogen bond by calculating the interaction energies between ligands (using substituted *N*-benzoylpiperidine as a model compound) and water molecule (as a model of hydrogen bond donor in a receptor protein). The detailed method is described in Section 5.

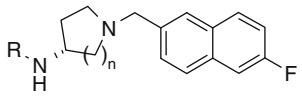
Table 1
CCR3 inhibitory activity of 6-fluoronaphthalene derivatives

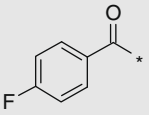
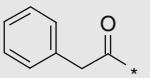
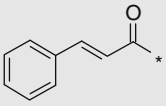
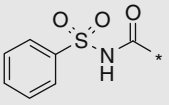


Compound	R[a]	IC_{50}[b] (nM)
1[c]		20 ± 3.9
2[d]		23 ± 4.7
3[c]		38 ± 5.4
7		NE@10 μM[e]
8		NE@10 μM[e]
11		NE@10 μM[e]
(***rac***)-**14**		550 ± 180
(***rac***)-**17**		320 ± 89
(**3*S***)-**17**		2500 ± 1100
(**3*R***)-**17**		140 ± 16

[a] Structures of **1**, **2**, and **3** are shown in Figure 1.
[b] The IC_{50} values are shown as the means ± SEM for at least three determinations.
[c] See Ref. 3a.
[d] See Ref. 3b.
[e] No effect at 10 μM.

Table 2
CCR3 inhibitory activity of 6-fluoronaphthalene derivatives



Compound	R[a]	*n*	IC_{50}[b] (nM)
1[c]			20 ± 3.9
3[c]			38 ± 5.4
19a		1	230 ± 72
19b[b]		2	640 ± 270
20a		1	170 ± 9.1
20b		2	300 ± 150
21a		1	100 ± 21
21b[b]		2	9700 ± 3700
22a		1	250 ± 60
22b		2	2000 ± 470

[a] Structures of **1** and **3** are shown in Figure 1.
[b] The IC_{50} values are shown as the means ± SEM for at least three determinations.
[c] See Ref. 3a.

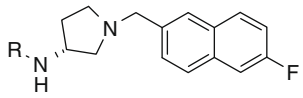
The results showed that the interaction energy between the *N*-2-hydroxybenzoylpiperidene, the model compound of **30j**, and a water molecule was much lower than that of the other model ligands (Table 5). This result led us to predict that the degree of interaction energy between **30j** and CYPs would be lower than that of the other compounds because of the intramolecular hydrogen bond, and that this weaker interaction meant metabolic stability.

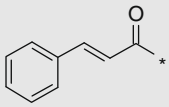
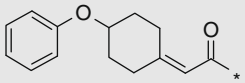
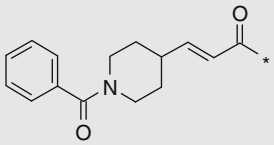
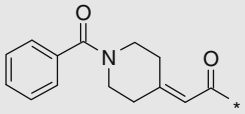
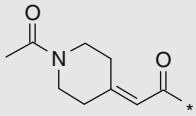
These favorable results prompted us to attempt to further improve the CCR3 inhibitory activity and retain the metabolic stability of **30j**, and we expected to do this by incorporating an additional substituent at the benzoyl part (Table 6). First, the electron-donating methoxy moiety that increased the inhibitory activity in the study of the mono-substituted derivative (described above) was substituted at the most appropriate position. The results showed that the CCR3 inhibitory activity of the 4-substituted derivative (**30n**) was slightly more potent than that of **30j** (IC_{50} = 3.3 nM). A substitution at the 5-position (**30o**) resulted in less activity (IC_{50} = 14 nM) and those at the 3- (**30m**) and 6-positions (**30p**) resulted in much less activity than that of **30j** (IC_{50} = 38 and 45 nM, respectively). The effects of introducing other substituents at the 4-position were then investigated (Table 6). While both the electron-withdrawing fluorine and chlorine derivatives had an activity equal to that of **30j**, the electron-donating methyl derivative exhibited the most potent activity in this series of compounds (IC_{50} = 0.98 nM).

As we expected, all phenolic derivatives listed out in Table 6 had CL_{int} values smaller than those of the corresponding non-phenolic derivatives (**30p** vs **30e**, **30f** vs **30m** and **30o**, **30n** vs **30g**, and **30d** vs **30q**). However, all their CL_{int} values were larger than that of **30j**, which indicates that the increase in molecular lipophilicity caused by each additional substituent was responsible for the microsomal metabolic lability and that additional methoxy and methyl moieties would be sensitive to be metabolized by HLMs. For our series of acrylamide derivatives, the structural modification of the benzoyl moiety produced substantial changes in metabolic stability against HLMs.

Compounds **30j** and **30s** were identified as having potent CCR3 inhibitory activity, with CL_{int} values smaller than that of **30a**, and also showed potent dissociation constant (K_b) values (1.0 and 0.44 nM, respectively), which were determined using the method reported by Lazareno and Bridsall (Table 7).[7] Further, compounds **30j** and **30s** proved to be CCR3-selective inhibitors since they showed no inhibitory effects on Ca^{2+} influx signals induced by other chemokines at 10 μM (using RANTES for CCR1 and CCR5, and MCP-1 for CCR2, respectively). With regard to species cross-reactivity, compounds **1** and **2** showed markedly weak inhibition against mouse and monkey CCR3 as shown in Table 7, while **30j** showed more potent mouse and monkey CCR3 inhibitory activity

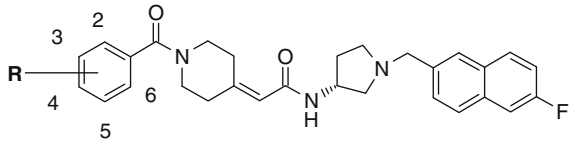
Table 3
CCR3 inhibitory activity of 6-fluoronaphthalene derivatives



Compound	R	IC_{50}[a] (nM)
1		20 ± 3.9
21a		100 ± 21
26		210 ± 15
24		900 ± 2.3
30a		4.4 ± 1.2
29		160 ± 59

[a] The IC_{50} values are shown as the means ± SEM for at least three determinations.

Table 4
CCR3 inhibitory activity and CL_{int} value of acrylamide derivatives



Compound	R	IC_{50}[a] (nM)	CL_{int} (mL/min/kg)
30a	H	4.4 ± 1.2	338
30b	2-F	4.8 ± 1.1	590
30c	3-F	12 ± 3.4	459
30d	4-F	22 ± 7.1	237
30e	2-OMe	5.1 ± 2.3	1147
30f	3-OMe	5.8 ± 3.3	500
30g	4-OMe	1.8 ± 0.5	252
30h	3,4-OMe	3.6 ± 0.3	246
30i	3,4-OCH_2O-	1.6 ± 0.1	238
30j	2-OH	8.4 ± 2.8	<64
30k	3-OH	5.0[b]	335
30l	4-OH	5.1[b]	120

[a] The IC_{50} values are shown as the means ± SEM for at least three determinations.
[b] Mean of two experiments.

(IC_{50} = 200 and 7.2 nM, respectively) than **1** or **2**. In addition, compound **30j** exhibited weaker CYP2D6 inhibition than **1** (IC_{50} = 29 μM vs 0.02 μM) and no significant CYP inhibition against other major subtypes such as CYP1A2, 2C9, 2C19, and 3A4 (Table 7).

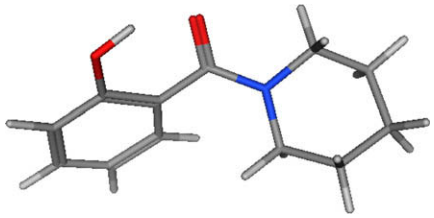
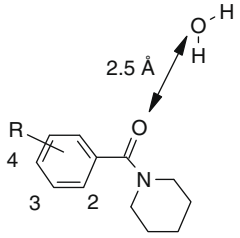


Figure 3. The geometrically optimized structure of model compound of **30j**.

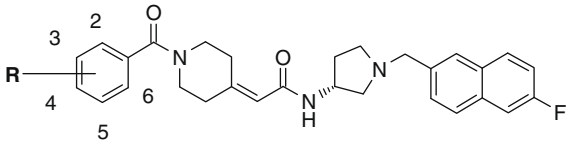
Table 5
Interaction energy between the ligand (model compound) and the water molecule



R	Interaction energy[a] (kcal mol^{-1})
H	−2.02
2-F	−1.82
3-F	−1.14
4-F	−2.24
2-MeO	−2.01
3-MeO	−1.79
4-MeO	−2.03
2-OH	0.89
3-OH	−2.38
4-OH	−1.97

[a] The detailed method is described in Section 5.

Table 6
CCR3 inhibitory activity of acrylamide derivatives



Compound	R	IC_{50}[a] (nM)	CL_{int} (mL/min/kg)
30a	H	4.4 ± 1.2	338
30j	2-OH	8.4 ± 2.8	<64
30m	2-OH-3-OMe	38 ± 10	162
30n	2-OH-4-OMe	3.3 ± 1.4	232
30o	2-OH-5-OMe	14 ± 3.2	119
30p	2-OH-6-OMe	45 ± 8.3	192
30q	2-OH-4-F	6.0 ± 0.8	116
30r	2-OH-4-Cl	7.5 ± 2.6	87
30s	2-OH-4-Me	0.98 ± 0.32	156

[a] The IC_{50} values are shown as the means ± SEM for at least three determinations.

With regard to metabolic stability, we selected compound **30j** for evaluating the pharmacokinetic properties by dosing 1 mg/kg IV and 3 mg/kg PO (Table 8). Compound **30j** exhibited an acceptable half-life (2.9 h), short maximum drug concentration time (0.8 h), and moderate oral bioavailability (35%). Membrane permeability of **30j** was 29.8×10^{-6} cm/s (pH 6.5) as measured in a PAMPA, and was 4.0×10^{-6} cm/s as measured in a Caco-2 cell assay (P_{app}) (Table 7). These properties are highly desirable and strongly support that **30j** would exhibit good absorption on oral administra-

Table 7
Comparison of pharmacological profiles between **1**, **2**, **30j**, and **30s**

	1	2	30j	30s
Human CCR3, IC_{50} (nM)	20	23	8.4	0.98
Human K_b, (nM)[a]	3.7	1.4	1.0	0.44
Human CCR1, IC_{50} (nM)	NE[c]	NE[c]	NE[c]	NE[c]
Human CCR2, IC_{50} (nM)	NE[c]	NE[c]	NE[c]	NE[c]
Human CCR5, IC_{50} (nM)	NE[c]	NE[c]	NE[c]	NE[c]
Mouse CCR3, IC_{50} (nM)[b]	NE[c]	1100	230	NT[d]
Monkey CCR3, IC_{50} (nM)[b]	200	613	7.2	NT[d]
CL_{int} (mL/min/kg)	NT[d]	87	<64	156
PAMPA ($\times 10^{-6}$ cm/s)	>30	4.7	29.8	27.3
Caco-2, P_{app} ($\times 10^{-6}$ cm/s)	NT[d]	NT[d]	4.0	NT[d]
CYP2D6 inhibition, IC_{50} (μM)	0.02	29	29	NT[d]

[a] See Ref. 7.
[b] See Refs. 8a–c.
[c] No effect at 10 μM.
[d] Not tested.

tion to humans. Further biological and pharmacokinetic evaluations of **30j** are now carried out, and further structural optimization studies are also planned to determine the SAR of our acrylamide derivatives, and the results of these compounds' biological and pharmacokinetic evaluations will be reported in due course.

4. Conclusions

In summary, to improve the CCR3 inhibitory activity of the lead compound **1**, we designed novel compounds based on previously reported key pharmacophores, which led to the successful establishment of a novel series of acrylamide derivatives with potent activity. The SAR studies showed that the CCR3 inhibitory activity would be susceptible to electrons around the terminal benzoyl part, and that the incorporation of electron-donating groups at the 4-position would be critical for potent activity. In addition, the study of the relationship between structure and metabolic stability against HLMs (CL_{int}; mL/min/kg) revealed that the structural modification of the benzoyl moiety gave rise to considerable changes in the CL_{int} values. The 2-hydroxy derivatives (**30j**) had the lowest CL_{int} values in this series of compounds. The intramolecular hydrogen bond between the 2-hydroxy moiety and the carbonyl oxygen of the amide moiety effectively improved microsomal metabolic stability by decreasing the amount of energy used to interact with CYPs of hepatic microsomes. Finally, the introduction of an electron-donating methyl moiety at the 4-position of the benzoyl moiety of **30j** led to the identification of **30s**, which had the most potent CCR3 inhibitory activity in this series of compounds.

Based on the desirable properties of **30j**, including its high metabolic stability against HLMs, moderate bioavailability in mice, and high membrane permeability, we expected **30j** to show good pharmacokinetic properties in humans.

Table 8
Pharmacokinetic properties of **30j** in mice

	30j	
	IV[a] (1 mg/kg)	PO[a] (3 mg/kg)
CL_{tot} (mL/min/kg)	88 ± 7.9	—
$t_{1/2}$ (h)	2.1 ± 0.5	2.9 ± 0.3
T_{max} (h)	—	0.8 ± 0.3
C_{max} (h)	—	41 ± 3
V_{dss} (mL/kg)	9877 ± 692	—
$AUC_{0-\infty}$ (ng h/mL)	190 ± 17	200 ± 47
F (%)	35 ± 8	

[a] The values are shown as the means ± SEM for at least three determinations.

5. Experimental

5.1. Chemistry

In general, reagents and solvents were used as purchased without further purification. Melting points were determined with a Yanaco MP-500D melting point apparatus and left uncorrected. ^{1}H NMR spectra were recorded on a JEOL JNM-LA300 or a JEOL JNM-EX400 spectrometer. Chemical shifts were expressed in δ (ppm) values with tetramethylsilane as an internal standard (NMR descriptions; s = singlet, d = doublet, t = triplet, dt = double triplet, m = multiplet, and br = broad peak). Mass spectra were recorded on a JEOL JMS-LX2000 spectrometer. High resolution (HR)-mass spectra were recorded using a Waters QTOF Premier spectrometer. The elemental analyses were performed with a Yanaco MT-5 microanalyzer (C, H, and N) and Yokogawa IC-7000S ion chromatographic analyzer (halogens) and were within ±0.4% of the theoretical values.

5.1.1. 1-[(6-Fluoro-2-naphthyl)methyl]-1,4-diazepane (6)

To a solution of homopiperazine (**4**) (2.09 g, 20.9 mmol) in MeCN (50 mL) were added **5** (1.0 g, 4.18 mmol) and K_2CO_3 (1.73 g, 12.54 mmol), and the mixture was stirred at room temperature for 20 h. This mixture was concentrated in vacuo. The residue was then partitioned between $CHCl_3$ and H_2O, and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 95:5–85:15) to yield **6** (832 mg, 77%) as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) 1.72–1.82 (m, 2H), 2.67–2.76 (m, 4H), 2.88–2.94 (m, 2H), 2.95–3.00 (m, 2H), 3.80 (s, 2H), 7.20–7.28 (m, 1H), 7.42 (dd, J = 9.9, 2.6 Hz, 1H), 7.55 (d, J = 8.6 Hz, 1H), 7.71–7.82 (m, 3H); MS (FAB) m/z = 259 $[M+H]^+$.

5.1.2. 1-(Biphenyl-2-ylcarbonyl)-4-[(6-fluoro-2-naphthyl)-methyl]-1,4-diazepane hydrochloride (7)

To a solution of **6** (460 mg, 1.78 mmol) in DMF (10 mL) were added biphenyl-2-carboxylic acid (378 mg, 1.78 mmol), HOBt (289 mg, 2.14 mmol), and WSC·HCl (412 mg, 2.14 mmol), and this mixture was stirred at room temperature overnight. The mixture was then partitioned between EtOAc and satd $NaHCO_3$ aq, and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude oil was converted to its hydrochloride salt by treating it with 4 M HCl (g)/EtOAc. The crude solid was recrystallized from MeOH–EtOAc to yield **7** (224 mg, 26%) as a colorless solid. Mp: 230–232 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.24–1.28 (m, 1H), 1.62–1.80 (m, 1H), 2.00–2.40 (m, 1H), 2.63–2.89 (m, 1H), 2.95–3.55 (m, 5H), 4.08–4.35 (m, 2H), 4.36–4.60 (m, 1H), 7.24–7.60 (m, 10H), 7.73–7.94 (m, 2H), 7.94–8.20 (m, 3H), 11.35–11.83 (m, 1H); MS (FAB) m/z = 439 $[M+H]^+$. Anal. Calcd for $C_{29}H_{27}FN_2O{\cdot}HCl{\cdot}0.2H_2O$: C, 72.78; H, 5.98; N, 5.85; Cl, 7.41; F, 3.97. Found: C, 72.85; H, 5.99; N, 5.77; Cl, 7.39; F, 3.96.

5.1.3. 1-(Biphenyl-2-ylacetyl)-4-[(6-fluoro-2-naphthyl)methyl]-1,4-diazepane hydrochloride (8)

Compound **8** was prepared from **6** and biphenyl-2-ylacetic acid in a manner similar to that described for compound **7**, in a yield of 33% as a colorless solid. Mp: 212 °C (dec) (MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.84–2.07 (m, 1H), 2.24–2.37 (m, 1H), 2.62–3.00 (m, 2H), 3.17–3.71 (m, 7H), 4.05–4.15 (m, 1H), 4.36–4.52 (m, 2H), 7.17–7.41 (m, 9H), 7.48–7.56 (m, 1H), 7.77–7.88 (m, 2H), 7.98–8.08 (m, 2H), 8.12–8.19 (m, 1H), 11.35 (s, 1H); MS (FAB) m/z = 453 $[M+H]^+$. Anal. Calcd for $C_{30}H_{29}FN_2O{\cdot}HCl$: C, 73.68; H, 6.18; N, 5.73; Cl, 7.25; F, 3.88. Found: C, 73.79; H, 6.05; N, 5.69; Cl, 7.25; F, 3.89.

5.1.4. 1-[(6-Fluoro-2-naphthyl)methyl]piperazine dihydrochloride (10)

To a solution of *tert*-butyl piperazine-1-carboxylate (**9**) (770 mg, 4.14 mmol) in MeCN (15 mL) were added **5** (990 mg, 3.76 mmol) and K_2CO_3 (1.56 g, 11.3 mmol), and the mixture was stirred at room temperature for 4.5 h. The residue was then partitioned between EtOAc and satd $NaHCO_3$ aq, and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. To a solution of this crude compound in EtOAc (20 mL) and MeOH (10 mL) was added 4 M HCl (g)/EtOAc (30 mL), and the mixture was stirred at room temperature overnight. The precipitate was collected by filtration, washed with EtOAc and Et_2O, and dried in vacuo to yield **10** (1.02 g, 86% for two steps) as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) 3.08–3.82 (m, 8H), 4.45–4.65 (m, 2H), 7.51 (ddd, J = 8.8, 8.8, 2.5 Hz, 1H), 7.79 (dd, J = 10.3, 2.0 Hz, 1H), 7.80–7.90 (m, 1H), 7.98–8.08 (m, 2H), 8.17–8.20 (m, 1H), 9.76 (s, 1H), 11.00–12.55 (m, 2H); MS (FAB) m/z = 245 $[M+H]^+$.

5.1.5. 1-(Biphenyl-2-ylacetyl)-4-[(6-fluoro-2-naphthyl)methyl]-piperazine hydrochloride (11)

Compound **11** was prepared from **10** and biphenyl-2-ylacetic acid in a manner similar to that described for compound **7**, in a yield of 33% as a colorless solid. Mp: 218–220 °C (MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.70–2.91 (m, 2H), 3.05–3.15 (m, 1H), 3.23–3.48 (m, 3H), 3.55–3.68 (m, 2H), 3.81–3.92 (m, 1H), 4.34–4.54 (m, 3H), 7.17–7.40 (m, 9H), 7.52 (ddd, J = 8.8, 8.8, 2.4 Hz, 1H), 7.86–7.85 (m, 2H), 7.98–8.08 (m, 2H), 8.12–8.18 (m, 1H), 11.60 (s, 1H); MS (FAB) m/z = 439 $[M+H]^+$. Anal. Calcd for $C_{29}H_{27}FN_2O{\cdot}HCl$: C, 73.33; H, 5.94; N, 5.90; Cl, 7.46; F, 4.00. Found: C, 73.37; H, 5.94; N, 5.95; Cl, 7.50; F, 4.11.

5.1.6. (*rac*)-*N*-Piperidin-3-ylbiphenyl-2-carboxamide [(*rac*)-13]

To a solution of (***rac***)-**12** (720 mg, 3.59 mmol) in THF (10 mL) were added biphenyl-2-carboxylic acid (712 mg, 3.59 mmol), HOBt (583 mg, 4.31 mmol), and WSC·HCl (830 mg, 4.31 mmol), and this mixture was stirred at room temperature for 18 h. The mixture was then partitioned between EtOAc and satd $NaHCO_3$ aq, and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. To a solution of this crude compound in EtOAc (30 mL) was added 4 M HCl (g)/EtOAc (30 mL), and the mixture was stirred at room temperature for 4 h. The mixture was concentrated in vacuo. The residue was dissolved in H_2O, and washed with Et_2O. The aqueous layer was alkalified with satd $NaHCO_3$ aq and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to yield (***rac***)-**13** (210 mg, 21% for two steps) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) 1.12–1.33 (m, 2H), 1.38–1.49 (m, 1H), 1.56–1.65 (m, 1H), 2.13 (dd, J = 11.8, 9.3 Hz, 1H), 2.28–2.38 (m, 1H), 2.64–2.78 (m, 2H), 3.53–3.64 (m, 1H), 7.29–7.41 (m, 8H), 7.45–7.51 (m, 1H), 7.85 (d, J = 8.4 Hz, 1H); MS (FAB) m/z = 281 $[M+H]^+$.

5.1.7. (*rac*)-*N*-{1-[(6-Fluoro-2-naphthyl)methyl]piperidin-3-yl}-biphenyl-2-carboxamide hydrochloride [(*rac*)-14]

To a solution of (***rac***)-**13** (196 mg, 0.70 mmol) in MeCN (5 mL) were added **5** (167 mg, 0.70 mmol) and K_2CO_3 (193 mg, 1.40 mmol), and the mixture was stirred at room temperature for 25 h. The residue was then partitioned between EtOAc and satd $NaHCO_3$ aq, and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 98:2–95:5). The crude oil was converted to its hydrochloride salt by treating it with 4 M HCl (g)/EtOAc. The crude solid was recrystallized from MeOH–EtOAc to yield (***rac***)-**14** (70 mg, 20%) as a colorless solid. Mp: 138–142 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.15–1.32 (m, 1H), 1.63–1.75 (m, 1H), 1.77–1.88 (m, 2H), 2.76–2.95 (m, 1H), 2.95–3.25 (m, 2H), 3.30–3.42 (m, 1H), 4.01–4.15 (m, 1H), 4.23–4.57 (m, 3H), 7.15–7.33 (m, 5H), 7.34–7.44 (m, 3H), 7.46–7.58 (m, 2H), 7.74–7.85 (m, 2H), 7.98–8.18 (m, 3H), 8.28 (d, J = 7.8 Hz, 1H), 10.68–10.95 (m, 1H); MS (FAB) m/z = 439 $[M+H]^+$. Anal. Calcd for $C_{29}H_{27}FN_2O{\cdot}HCl{\cdot}0.8H_2O$: C, 71.17; H, 6.10; N, 5.72; Cl, 7.24; F, 3.88. Found: C, 71.36; H, 5.96; N, 5.72; Cl, 7.34; F, 3.74.

5.1.8. (3*R*)-1-[(6-Fluoro-2-naphthyl)methyl]pyrrolidin-3-amine dihydrochloride [(3*R*)-16]

To a solution of *tert*-butyl (3*R*)-pyrrolidin-3-ylcarbamate [(3*R*)-**15**] (20.2 g, 108 mmol) in MeCN (300 mL) were added **5** (31.12 g, 130 mmol) and K_2CO_3 (17.99 g, 130 mmol), and the mixture was stirred at room temperature for 7 h. The residue was then partitioned between EtOAc and H_2O, and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 98:2) to yield *tert*-butyl {(3*R*)-1-[(6-fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}carbamate (28.35 g, 76%). To a solution of this compound in EtOAc (500 mL) was added 4 M HCl (g)/EtOAc (200 mL), and the mixture was stirred at room temperature for 3 h. The mixture was concentrated in vacuo to yield (**3*R*)-16** (26.32 g, quant.) as a pale pink amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.00–2.60 (m, 2H), 3.18–3.46 (m, 1H), 3.51–3.79 (m, 3H), 3.81–4.15 (m, 1H), 4.54–4.75 (m, 2H), 7.52 (ddd, J = 9.2, 8.8, 2.5 Hz, 1H), 7.75–7.90 (m, 2H), 7.98–8.03 (m, 2H), 8.15–8.23 (m, 1H), 8.50–8.90 (m, 3H), 11.45–12.08 (m, 1H); MS (FAB) m/z = 245 $[M+H]^+$.

5.1.9. (3*S*)-1-[(6-Fluoro-2-naphthyl)methyl]pyrrolidin-3-amine dihydrochloride [(3*S*)-16]

Compound (**3*S*)-16** was prepared from (3*S*)-pyrrolidin-3-ylcarbamate and **5** in a manner similar to that described for compound (**3*R*)-16**, in a yield of 53% as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.00–2.60 (m, 2H), 3.18–3.46 (m, 1H), 3.51–3.79 (m, 3H), 3.81–4.15 (m, 1H), 4.54–4.75 (m, 2H), 7.52 (ddd, J = 9.2, 8.8, 2.5 Hz, 1H), 7.75–7.90 (m, 2H), 7.98–8.03 (m, 2H), 8.15–8.23 (m, 1H), 8.50–8.90 (m, 3H), 11.45–12.08 (m, 1H); MS (ESI) m/z = 245 $[M+H]^+$.

5.1.10. (*rac*)-1-[(6-Fluoro-2-naphthyl)methyl]pyrrolidin-3-amine dihydrochloride [(*rac*)-16]

Compound (***rac***)-**16** was prepared from (*rac*)-pyrrolidin-3-ylcarbamate and **5** in a manner similar to that described for compound (**3*R*)-16**, in a yield of 77% as a pale brown amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.00–2.60 (m, 2H), 3.18–3.46 (m, 1H), 3.51–3.79 (m, 3H), 3.81–4.15 (m, 1H), 4.54–4.75 (m, 2H), 7.52 (ddd, J = 9.2, 8.8, 2.5 Hz, 1H), 7.75–7.90 (m, 2H), 7.98–8.03 (m, 2H), 8.15–8.23 (m, 1H), 8.50–8.90 (m, 3H), 11.45–12.08 (m, 1H); MS (FAB) m/z = 245 $(M+H)^+$.

5.1.11. *N*-{(3*R*)-1-[(6-Fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}biphenyl-2-carboxamide oxalate [(3*R*)-17]

To a solution of (**3*R*)-16** (250 mg, 0.79 mmol) in DMF (5 mL) were added biphenyl-2-carboxylic acid (171 mg, 0.87 mmol), Et_3N (0.36 mL, 2.60 mmol), HOBt (117 mg, 0.87 mmol), and WSC·HCl (181 mg, 0.95 mmol), and this mixture was stirred at room temperature overnight. The mixture was then partitioned between EtOAc and satd $NaHCO_3$ aq, and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 94:6–90:10). The crude oil was converted to its oxalate by treating it with oxalic acid. This solid was recrystallized from EtOH–EtOAc to yield (**3*R*)-17** (224 mg, 55%) as a colorless powder. Mp: 183–185 °C. $[\alpha]_D^{25}$ +1.99 (c 0.20, EtOH). ^{1}H NMR

(400 MHz, DMSO-d_6) δ: 1.66–1.75 (m, 1H), 2.05–2.20 (m, 1H), 2.58–2.70 (m, 1H), 2.95–3.10 (m, 2H), 3.16–3.28 (m, 1H), 4.14 (d, J = 12.7 Hz, 1H), 4.23 (d, J = 12.7 Hz, 1H), 4.30–4.39 (m, 1H), 7.18 (t, J = 7.2 Hz, 1H), 7.27 (dd, J = 7.7, 7.3 Hz, 2H), 7.31–7.33 (m, 2H), 7.35–7.44 (m, 3H), 7.46–7.54 (m, 2H), 7.59 (d, J = 9.0 Hz, 1H), 7.76 (dd, J = 10.2, 2.5 Hz, 1H), 7.95–7.98 (m, 2H), 8.04 (dd, J = 9.0, 5.8 Hz, 1H), 8.42 (d, J = 6.8 Hz, 1H); MS (ESI) m/z = 425 $[M+H]^+$. Anal. Calcd for $C_{28}H_{25}FN_2O{\cdot}C_2H_2O_4$: C, 70.03; H, 5.29; N, 5.44; F, 3.69. Found: C, 70.02; H, 5.28; N, 5.49; F, 3.65.

5.1.12. *N*-{(3*S*)-1-[(6-Fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}biphenyl-2-carboxamide oxalate [(3*S*)-17]

Compound (**3*S***)-**17** was prepared from (**3*S***)-**16** and biphenyl-2-carboxylic acid in a manner similar to that described for compound (**3*R***)-**17**, in a yield of 58% as a colorless powder. Mp: 183–185 °C (EtOH–EtOAc). $[\alpha]_D^{25}$ −1.99 (c 0.20, EtOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.66–1.75 (m, 1H), 2.05–2.20 (m, 1H), 2.58–2.70 (m, 1H), 2.95–3.10 (m, 2H), 3.16–3.28 (m, 1H), 4.14 (d, J = 12.7 Hz, 1H), 4.23 (d, J = 12.7 Hz, 1H), 4.30–4.39 (m, 1H), 7.18 (t, J = 7.2 Hz, 1H), 7.27 (dd, J = 7.7, 7.3 Hz, 2H), 7.31–7.33 (m, 2H), 7.35–7.44 (m, 3H), 7.46–7.54 (m, 2H), 7.59 (d, J = 9.0 Hz, 1H), 7.76 (dd, J = 10.2, 2.5 Hz, 1H), 7.95–7.98 (m, 2H), 8.04 (dd, J = 9.0, 5.8 Hz, 1H), 8.42 (d, J = 6.8 Hz, 1H); MS (ESI) m/z = 425 $[M+H]^+$. Anal. Calcd for $C_{28}H_{25}FN_2O{\cdot}C_2H_2O_4$: C, 70.03; H, 5.29; N, 5.44; F, 3.69. Found: C, 70.06; H, 5.30; N, 5.47; F, 3.67.

5.1.13. *N*-{(*rac*)-1-[(6-Fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}biphenyl-2-carboxamide fumarate [(*rac*)-17]

Compound (***rac***)-**17** was prepared from (***rac***)-**16** and biphenyl-2-carboxylic acid in a manner similar to that described for compound (**3*R***)-**17**, in a yield of 28% as a pale brown powder. Mp: 165–168 °C (EtOH–EtOAc). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.49–1.62 (m, 1H), 1.95–2.08 (m, 1H), 2.27 (d, J = 9.8, 4.8 Hz, 1H), 2.50–2.65 (m, 2H), 2.74–2.81 (m, 1H), 3.74 (d, J = 12.7 Hz, 1H), 3.81 (d, J = 12.7 Hz, 1H), 4.16–4.28 (m, 1H), 6.61 (s, 2H), 7.18–7.28 (m, 3H), 7.32–7.56 (m, 8H), 7.70 (dd, J = 10.2, 2.5 Hz, 1H), 7.84–7.92 (m, 2H), 8.04 (dd, J = 9.6, 5.7 Hz, 1H), 8.25 (d, J = 6.8 Hz, 1H); MS (FAB) m/z = 425 $(M+H)^+$. Anal. Calcd for $C_{28}H_{25}FN_2O{\cdot}C_4H_4O_4$: C, 71.10; H, 5.41; N, 5.18; F, 3.51. Found: C, 70.01; H, 5.40; N, 5.18; F, 3.51.

5.1.14. 4-Fluoro-*N*-{(3*R*)-1-[(6-fluoro-2-naphthyl)methyl]-pyrrolidin-3-yl}benzamide (19a)

To a solution of (**3*R***)-**16** (284 mg, 0.90 mmol) in CH_2Cl_2 (10 mL) were added Et_3N (0.37 mL, 2.69 mmol) and 4-fluorobenzoyl chloride (149 mg, 0.94 mmol), and the mixture was stirred at room temperature for 0.5 h. The mixture was then partitioned between EtOAc and H_2O, and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 98:2–90:10). The residue was recrystallized from MeCN to yield **19a** (131 mg, 40%) as a colorless crystal. Mp: 157–159 °C. $[\alpha]_D^{25}$ −4.99 (c 0.10, $CHCl_3$). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.77–1.86 (m, 1H), 2.11–2.22 (m, 1H), 2.42–2.48 (m, 1H), 2.52–2.59 (m, 1H), 2.62–2.70 (m, 1H), 2.82–2.89 (m, 2H), 3.75 (s, 2H), 4.33–4.44 (m, 1H), 7.24–7.31 (m, 2H), 7.40 (ddd, J = 8.8, 8.8, 2.5 Hz, 1H), 7.55 (d, J = 8.8 Hz, 1H), 7.68 (dd, J = 10.3, 2.5 Hz, 1H), 7.84–8.00 (m, 5H), 8.49 (d, J = 6.9 Hz, 1H); MS (FAB) m/z = 367 $[M+H]^+$. Anal. Calcd for $C_{22}H_{20}F_2N_2O$: C, 72.12; H, 5.50; N, 7.65; F, 10.37. Found: C, 72.05; H, 5.42; N, 7.67; F, 10.39.

5.1.15. *N*-{(3*R*)-1-[(6-Fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}-2-phenylacetamide (20a)

Compound **20a** was prepared from (**3*R***)-**16** and phenylacetyl chloride in a manner similar to that described for compound **19a**, in a yield of 15% as a colorless crystal. Mp: 114–116 °C (MeCN). $[\alpha]_D^{25}$ +41.12 (c 0.107, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.53–1.63 (m, 1H), 2.06–2.16 (m, 1H), 2.31–2.37 (m, 1H), 2.39–2.49 (m, 1H), 2.63–2.70 (m, 2H), 3.37 (s, 2H), 3.68 (d, J = 13.2 Hz, 1H), 3.74 (d, J = 13.2 Hz, 1H), 4.09–4.19 (m, 1H), 7.16–7.29 (m, 5H), 7.41 (ddd, J = 8.8, 8.8, 2.7 Hz, 1H), 7.53 (d, J = 8.3 Hz, 1H), 7.68 (dd, J = 10.5, 2.7 Hz, 1H), 7.82–7.88 (m, 2H), 7.96 (dd, J = 9.2, 5.8 Hz, 1H), 8.23 (d, J = 5.9 Hz, 1H); MS (FAB) m/z = 363 $[M+H]^+$. Anal. Calcd for $C_{23}H_{23}FN_2O$: C, 76.22; H, 6.40; N, 7.73; F, 5.24. Found: C, 76.16; H, 6.43; N, 7.71; F, 5.18.

5.1.16. *N*-{1-[(6-Fluoro-2-naphthyl)methyl]piperidin-4-yl}-2-phenylacetamide (20b)

Compound **20b** was prepared from **18**[3a] and phenylacetyl chloride in a manner similar to that described for compound **19a**, in a yield of 46% as a colorless crystal. Mp: 168–171 °C (MeCN). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.27–1.39 (m, 2H), 1.80–1.89 (m, 2H), 2.08–2.27 (m, 2H), 2.69–2.79 (m, 2H), 3.55 (s, 2H), 3.58 (s, 2H), 3.76–3.87 (m, 1H), 5.22 (d, J = 7.8 Hz, 1H), 3.74 (d, J = 13.2 Hz, 1H), 7.20–7.38 (m, 6H), 7.41 (dd, J = 10.0, 2.7 Hz, 1H), 7.45 (d, J = 8.3 Hz, 1H), 7.66–7.74 (m, 2H), 7.77 (dd, J = 9.1, 5.7 Hz, 1H); MS (FAB) m/z = 377 $[M+H]^+$. Anal. Calcd for $C_{24}H_{25}FN_2O$: C, 76.57; H, 6.69; N, 7.44; F, 5.05. Found: C, 76.82; H, 6.80; N, 7.44; F, 5.18.

5.1.17. (2*E*)-*N*-{(3*R*)-1-[(6-fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}-3-phenylacrylamide (21a)

Compound **21a** was prepared from (**3*R***)-**16** and (2*E*)-3-phenylacrylic acid in a manner similar to that described for compound (**3*R***)-**17**, in a yield of 37% as a colorless powder. Mp: 172–174 °C (EtOAc). $[\alpha]_D^{25}$ −15.00 (c 0.10, $CHCl_3$). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.57–1.70 (m, 1H), 2.11–2.23 (m, 1H), 2.38–2.50 (m, 2H), 2.65–2.80 (m, 2H), 3.68–3.79 (m, 2H), 4.25–4.36 (m, 1H), 6.64 (d, J = 15.8 Hz, 1H), 7.33–7.47 (m, 5H), 7.51–7.57 (m, 3H), 7.67 (dd, J = 10.3, 2.4 Hz, 1H), 7.84–7.89 (m, 2H), 7.97 (dd, J = 9.0, 5.9 Hz, 1H), 8.25 (d, J = 7.0 Hz, 1H); MS (ESI) m/z = 375 $[M+H]^+$. Anal. Calcd for $C_{24}H_{23}FN_2O$: C, 76.98; H, 6.19; N, 7.49; F, 5.07. Found: C, 77.02; H, 6.26; N, 7.49; F, 5.15.

5.1.18. *tert*-Butyl 4-[(1*E*)-3-({(3*R*)-1-[(6-fluoro-2-naphthyl)-methyl]pyrrolidin-3-yl}amino)-3-oxoprop-1-en-1-yl]piperidine-1-carboxylate (23)

Compound **23** was prepared from (**3*R***)-**16** and (2*E*)-3-[1-(*tert*-butoxycarbonyl)piperidin-4-yl]acrylic acid[10] in a manner similar to that described for compound (**3*R***)-**17**, in a yield of 96% as a colorless amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.24–1.39 (m, 1H), 1.60–1.74 (m, 2H), 2.18–2.39 (m, 3H), 2.60–2.84 (m, 4H), 2.89–2.96 (m, 1H), 3.75 (s, 2H), 4.02–4.20 (m, 2H), 4.50–4.60 (m, 1H), 5.69 (d, J = 15.3 Hz, 1H), 5.85 (d, J = 7.8 Hz, 1H), 6.76 (dd, J = 15.3, 6.6 Hz, 1H), 7.21–7.30 (m, 1H), 7.43 (dd, J = 9.8, 2.2 Hz, 1H), 7.48 (d, J = 8.4 Hz, 1H), 7.70–7.82 (m, 3H); MS (ESI) m/z = 482 $[M+H]^+$.

5.1.19. (2*E*)-3-(1-Benzoylpiperidin-4-yl)-*N*-{(3*R*)-1-[(6-fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}acrylamide (24)

To a solution of **23** (430 mg, 0.89 mmol) in EtOAc (3 mL) was added 4 M HCl (g)/EtOAc (3 mL), and the mixture was stirred at room temperature overnight. The mixture was concentrated in vacuo. To a solution of this crude compound (405 mg, 0.89 mmol) in CH_2Cl_2 (5 mL) were added Et_3N (0.42 mL, 3.04 mmol) and BzCl (147 mg, 1.04 mmol), and the mixture was stirred at room temperature for 3 h. The mixture was then partitioned between EtOAc and H_2O, and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 99:1–92:8). The residue was recrystallized from hexane–EtOAc to yield **24** (172 mg, 41% for two steps) as a colorless powder. Mp: 104–106 °C (hexane–EtOAc). $[\alpha]_D^{25}$ +13.00 (c 0.10, $CHCl_3$). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.20–1.40 (m, 2H), 1.53–1.86 (m, 3H), 2.08–2.18 (m, 1H), 2.32–2.50 (m, 3H), 2.61–2.75 (m, 2H), 2.78–

3.18 (m, 2H), 3.45–3.66 (m, 1H), 3.67–3.76 (m, 2H), 4.17–4.28 (m, 1H), 4.34–4.58 (m, 1H), 5.90 (d, *J* = 15.5 Hz, 1H), 6.56 (d, *J* = 15.5, 5.4 Hz, 1H), 7.32–7.48 (m, 6H), 7.53 (d, *J* = 8.4 Hz, 1H), 7.67 (dd, *J* = 10.3, 2.4 Hz, 1H), 7.82–7.88 (m, 2H), 7.96 (dd, *J* = 9.0, 5.8 Hz, 1H), 8.06 (d, *J* = 7.0 Hz, 1H); MS (ESI) *m/z* = 486 $[M+H]^+$. Anal. Calcd for $C_{30}H_{32}FN_3O_2{\cdot}0.2H_2O$: C, 73.66; H, 6.68; N, 8.59; F, 3.88. Found: C, 73.59; H, 6.66; N, 8.53; F, 3.91.

5.1.20. *N*-({(3*R*)-1-[(6-Fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}carbamoyl)benzenesulfonamide (22a)

A solution of (**3*R***)-**16** (390 mg, 1.23 mmol) in EtOAc (50 mL) was washed with 1 M NaOH aq and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. To an ice-cooled solution of this product in MeCN (50 mL) was added benzenesulfonyl isocyanate (283 mg, 1.55 mmol) in MeCN (3 mL) dropwise over 10 min, and this mixture was stirred at room temperature for 0.5 h. To this mixture was then added PS-trisamine (800 mg), and this mixture was stirred slowly at room temperature for 4 h. The residue was then diluted with MeOH (300 mL), filtered, and concentrated in vacuo. The crude solid was recrystallized from MeOH to yield **22a** (154 mg, 30%) as a colorless crystal. Mp: 150–152 °C. $[\alpha]_D^{25}$ +27.27 (*c* 0.11, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.42–1.58 (m, 1H), 2.04–2.16 (m, 1H), 2.36–2.50 (m, 2H), 2.59–2.70 (m, 1H), 2.72–2.82 (m, 1H), 3.70–3.88 (m, 2H), 3.94–4.02 (m, 1H), 6.54–6.72 (m, 1H), 7.43 (ddd, *J* = 8.8, 8.8, 2.5 Hz, 1H), 7.50–7.65 (m, 4H), 7.70 (dd, *J* = 10.2, 2.5 Hz, 1H), 7.83–7.90 (m, 5H), 7.98 (dd, *J* = 9.2, 5.8 Hz, 1H); MS (FAB) *m/z* = 428 $[M+H]^+$. Anal. Calcd for $C_{22}H_{22}FN_3O_3S{\cdot}0.5H_2O$: C, 60.53; H, 5.31; N, 9.63; F, 3.51; S, 7.35. Found: C, 60.57; H, 5.16; N, 9.82; F, 4.49; S, 7.48.

5.1.21. *N*-({1-[(6-Fluoro-2-naphthyl)methyl]piperidin-4-yl}carbamoyl)benzenesulfonamide hydrochloride (22b)

Compound **22b** was prepared from **18** and benzenesulfonyl isocyanate in a manner similar to that described for compound **22a**, in a yield of 13% as a colorless amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.62–2.01 (m, 4H), 2.91–3.12 (m, 2H), 3.17–3.40 (m, 2H), 3.45–3.58 (m, 1H), 4.39 (s, 2H), 7.08 (d, *J* = 5.9 Hz, 1H), 7.41 (ddd, *J* = 8.6, 8.5, 2.4 Hz, 1H), 7.60 (dd, *J* = 7.5, 7.5 Hz, 2H), 7.65–7.71 (m, 1H), 7.73–7.83 (m, 2H), 7.90 (d, *J* = 7.5 Hz, 2H), 7.95–8.08 (m, 2H), 8.12 (s, 1H), 10.49–10.80 (m, 2H); MS (FAB) *m/z* = 442 $[M+H]^+$. Anal. Calcd for $C_{23}H_{24}FN_3O_3S{\cdot}HCl$: C, 57.79; H, 5.27; N, 8.79; Cl, 7.42; F, 3.97; S, 6.71. Found: C, 57.86; H, 5.21; N, 8.79; Cl, 7.45; F, 3.85; S, 6.71.

5.1.22. Diethyl [2-({(3*E*)-1-[(6-fluoro-2-naphthyl)methyl]-pyrrolidin-3-yl}amino)-2-oxoethyl]phosphonate (25)

To a solution of (**3*R***)-**16** (10.3 g, 32.5 mmol) in DMF (100 mL) were added (diethoxyphosphoryl)acetic acid (6.52 g, 34.1 mmol), HOBt (2.19 g, 16.2 mmol), WSC·HCl (6.85 g, 35.7 mmol), and Et_3N (9.05 mL, 64.9 mmol), and this mixture was stirred at room temperature for 3.5 h. The mixture was then partitioned between $CHCl_3$ and H_2O, and the organic layer was washed with satd $NaHCO_3$ aq, dried over Na_2SO_4, filtered, and concentrated in vacuo to yield compound **25** (15.3 g, quant.) as a brown oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.15–1.21 (m, 6H), 1.52–1.62 (m, 1H), 2.08–2.18 (m, 1H), 2.34–2.44 (m, 2H), 2.63–2.72 (m, 2H), 3.57 (s, 2H), 3.68 (d, *J* = 13.2 Hz, 1H), 3.75 (d, *J* = 13.2 Hz, 1H), 3.93–4.04 (m, 4H), 4.10–4.20 (m, 1H), 7.42 (ddd, *J* = 9.3, 8.8, 2.9 Hz, 1H), 7.54 (d, *J* = 8.7 Hz, 1H), 7.68 (dd, *J* = 10.2, 2.5 Hz, 1H), 7.84–7.88 (m, 2H), 7.98 (dd, *J* = 8.8, 5.9 Hz, 1H), 8.15 (d, *J* = 7.3 Hz, 1H); MS (FAB) *m/z* = 423 $[M+H]^+$.

5.1.23. *N*-{(3*R*)-1-[(6-Fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}-2-piperidin-4-ylideneacetamide (28)

To a solution of NaH (60% oil dispersion, 1.19 g, 29.8 mmol) in DME (30 mL) was added **25** (13.7 g, 32.5 mmol) in DME (100 mL) dropwise over 0.5 h, and this mixture was stirred at room temperature for 0.5 h. To this mixture was added *tert*-butyl 4-oxopiperidine-1-carboxylate (5.40 g, 27.1 mmol) in DME (50 mL) dropwise, and this mixture was stirred at room temperature for 3 h. The mixture was then partitioned between $CHCl_3$ and H_2O, and the organic layer was washed with satd $NaHCO_3$ aq, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH/NH_4OH = 99:1:0.1–96:4:0.1) to yield compound **27** (13.0 g, quant.) as a colorless amorphous solid. To a solution of the above-obtained compound (9.23 g, 19.7 mmol) in CH_2Cl_2 (50 mL) was added TFA (50 mL), and the mixture was stirred at room temperature for 0.5 h. The mixture was then concentrated in vacuo. The residue was dissolved in $CHCl_3$, and this organic layer was washed with satd $NaHCO_3$ aq, dried over Na_2SO_4, filtered, and concentrated in vacuo to yield compound **28** (7.05 g, 97%) as a pale red amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.54–1.63 (m, 1H), 2.04–2.15 (m, 3H), 2.32 (dd, *J* = 9.5, 5.7 Hz, 1H), 2.42–2.49 (m, 1H), 2.58–2.83 (m, 8H), 3.69 (d, *J* = 13.1 Hz, 1H), 3.73 (d, *J* = 13.1 Hz, 1H), 4.14–4.25 (m, 1H), 5.59 (s, 1H), 7.41 (ddd, *J* = 8.8, 8.8, 2.4 Hz, 1H), 7.53 (d, *J* = 8.3 Hz, 1H), 7.68 (dd, *J* = 10.5, 2.7 Hz, 1H), 7.82–7.88 (m, 2H), 7.92–8.00 (m, 2H); MS (FAB) *m/z* = 368 $[M+H]^+$.

5.1.24. 4-Phenoxycyclohexanone (34)

To a solution of **33** (4.82 g, 30.5 mmol) in THF (100 mL) were added phenol (2.73 g, 29.0 mmol), PPh_3 (7.60 g, 29.0 mmol), and DEAD (5.31 g, 30.5 mmol), and this mixture was stirred at room temperature for 12 h. The mixture was then concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc = 5:1) to yield 8-phenoxy-1,4-dioxaspiro[4.5]decane (5.94 g, 88%) as a colorless oil. To an ice-cooled solution of the above-obtained compound (5.94 g, 25.4 mmol) in THF (200 mL) was added 3 M HCl aq (60 mL), and the mixture was stirred at room temperature for 8 h. The mixture was neutralized with $NaHCO_3$ (pH 8), and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc = 7:1–5:1) to yield **34** (3.78 g, 72%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.02–2.12 (m, 2H), 2.25–2.37 (m, 4H), 2.65–2.75 (m, 2H), 4.67–4.73 (m, 1H), 6.94–7.01 (m, 3H), 7.29–7.35 (m, 2H); MS (FAB) *m/z* = 191 $[M+H]^+$.

5.1.25. *N*-{(3*R*)-1-[(6-Fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}-2-(4-phenoxycyclohexylidene)acetamide (26)

Compound **26** was prepared from **25** and **34** in a manner similar to that described for compound **27**, in a yield of 79% as a pale yellow amorphous solid. $[\alpha]_D^{25}$ +27.99 (*c* 0.10, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.51–1.69 (m, 3H), 1.85–2.02 (m, 2H), 2.05–2.22 (m, 2H), 2.24–2.36 (m, 2H), 2.42–2.50 (m, 1H), 2.56–2.68 (m, 2H), 2.68–2.76 (m, 1H), 3.22–3.29 (m, 1H), 3.66–3.76 (m, 2H), 4.14–4.25 (m, 1H), 4.53–4.59 (m, 1H), 5.66 (s, 1H), 6.88–6.98 (m, 3H), 7.24–7.31 (m, 2H), 7.40 (ddd, *J* = 8.8, 8.8, 2.0 Hz, 2H), 7.53 (d, *J* = 8.3 Hz, 1H), 7.68 (dd, *J* = 10.3, 2.5 Hz, 1H), 7.83–7.88 (m, 2H), 7.94–8.00 (m, 2H); MS (FAB) *m/z* = 459 $[M+H]^+$. Anal. Calcd for $C_{29}H_{31}FN_2O_2$: C, 75.96; H, 6.81; N, 6.11; F, 4.14. Found: C, 75.60; H, 6.88; N, 6.08; F, 4.10.

5.1.26. 2-(1-Benzoylpiperidin-4-ylidene)-*N*-{(3*R*)-1-[(6-fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}acetamide (30a)

To an ice-cooled solution of **28** (275 mg, 0.75 mmol) in CH_2Cl_2 (5 mL) was added BzCl (110 mg, 0.79 mmol), and the mixture was stirred at 0 °C for 5.5 h. The mixture was then partitioned between EtOAc and H_2O, and the organic layer was washed with satd $NaHCO_3$ aq and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 100:0–97:3) to yield **30a** (210 mg, 60%) as a

colorless amorphous solid. $[\alpha]_D^{25}$ +36.36 (*c* 0.11, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.53–1.64 (m, 1H), 2.03–2.36 (m, 4H), 2.40–2.50 (m, 1H), 2.59–2.77 (m, 2H), 2.80–3.05 (m, 2H), 3.28–3.45 (m, 2H), 3.49–3.80 (m, 4H), 4.13–4.26 (m, 1H), 5.73 (s, 1H), 7.37–7.48 (m, 6H), 7.53 (d, *J* = 8.3 Hz, 1H), 7.67 (dd, *J* = 10.2, 2.5 Hz, 1H), 7.82–7.88 (m, 2H), 7.97 (dd, *J* = 8.8, 5.8 Hz, 1H), 8.05 (d, *J* = 6.8 Hz, 1H); MS (FAB) *m/z* = 472 $[M+H]^+$. Anal. Calcd for $C_{29}H_{30}FN_3O_2{\cdot}0.5H_2O$: C, 72.48; H, 6.50; N, 8.74; F, 3.95. Found: C, 72.80; H, 6.42; N, 8.76; F, 3.88.

5.1.27. 2-(1-Acetylpiperidin-4-ylidene)-*N*-{(3*R*)-1-[(6-fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}acetamide (29)

Compound **29** was prepared from **28** and acetyl chloride in a manner similar to that described for compound **30a**, in a yield of 17% as a pale yellow amorphous solid. $[\alpha]_D^{25}$ −11.05 (*c* 0.057, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.50–1.66 (m, 1H), 2.01 (s, 3H), 2.04–2.16 (m, 2H), 2.18–2.23 (m, 1H), 2.28–2.35 (m, 1H), 2.43–2.50 (m, 1H), 2.59–2.74 (m, 2H), 2.80–2.85 (m, 1H), 2.89–2.95 (m, 1H), 3.35–3.50 (m, 4H), 3.66–3.76 (m, 2H), 4.15–4.24 (m, 1H), 5.71 (s, 1H), 7.40 (ddd, *J* = 9.2, 8.8, 2.5 Hz, 1H), 7.53 (d, *J* = 8.3 Hz, 1H), 7.68 (dd, *J* = 10.3, 2.5 Hz, 1H), 7.82–7.88 (m, 2H), 7.97 (dd, *J* = 8.3, 5.9 Hz, 1H), 8.01–8.06 (m, 1H); HR-MS calcd for $C_{24}H_{29}FN_3O_2$ *m/z* = 410.2244 $[M+H]^+$. Found: 410.2231.

5.1.28. 2-[1-(2-Fluorobenzoyl)piperidin-4-ylidene]-*N*-{(3*R*)-1-[(6-fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}acetamide (30b)

Compound **30b** was prepared from **28** and 2-fluorobenzoyl chloride in a manner similar to that described for compound **30a**, in a yield of 86% as a colorless amorphous solid. $[\alpha]_D^{25}$ +35.99 (*c* 0.10, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.54–1.66 (m, 1H), 2.04–2.19 (m, 2H), 2.24–2.36 (m, 2H), 2.40–2.48 (m, 1H), 2.58–2.75 (m, 2H), 2.80–3.03 (m, 2H), 3.18–3.23 (m, 1H), 3.24–3.30 (m, 1H), 3.58–3.76 (m, 4H), 4.13–4.25 (m, 1H), 5.71, 5.76 (each s, 1H), 7.25–7.33 (m, 2H), 7.36–7.45 (m, 2H), 7.46–7.55 (m, 2H), 7.64–7.70 (m, 1H), 7.84–7.88 (m, 2H), 7.93–7.99 (m, 1H), 8.04–8.09 (m, 1H); MS (FAB) *m/z* = 490 $[M+H]^+$. Anal. Calcd for $C_{29}H_{29}F_2N_3O_2{\cdot}0.4H_2O$: C, 70.12; H, 6.05; N, 8.46; F, 7.65. Found: C, 70.12; H, 5.97; N, 8.36; F, 7.82.

5.1.29. *N*-{(3*R*)-1-[(6-Fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}-2-[1-(3-methoxybenzoyl)piperidin-4-ylidene]acetamide (30c)

Compound **30c** was prepared from **28** and 3-fluorobenzoyl chloride in a manner similar to that described for compound **30a**, in a yield of 54% as a pale yellow amorphous solid. $[\alpha]_D^{25}$ +30.90 (*c* 0.11, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.52–1.63 (m, 1H), 2.05–2.31 (m, 4H), 2.41–2.50 (m, 1H), 2.59–2.76 (m, 2H), 2.86–3.05 (m, 2H), 3.50–3.76 (m, 6H), 3.78 (s, 3H), 4.14–4.26 (m, 1H), 5.73 (s, 1H), 7.23–7.33 (m, 3H), 7.40 (ddd, *J* = 9.2, 8.8, 2.6 Hz, 1H), 7.46–7.55 (m, 2H), 7.68 (dd, *J* = 10.3, 2.5 Hz, 1H), 7.82–7.88 (m, 2H), 7.97 (dd, *J* = 8.8, 5.9 Hz, 1H), 8.06 (d, *J* = 6.8 Hz, 1H); MS (FAB) *m/z* = 490 $[M+H]^+$. Anal. Calcd for $C_{29}H_{29}F_2N_3O_2{\cdot}0.4H_2O$: C, 70.12; H, 6.05; N, 8.46; F, 7.65. Found: C, 70.01; H, 6.02; N, 8.22; F, 7.73.

5.1.30. 2-[1-(4-Fluorobenzoyl)piperidin-4-ylidene]-*N*-{(3*R*)-1-[(6-fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}acetamide (30d)

Compound **30d** was prepared from **28** and 4-fluorobenzoyl chloride in a manner similar to that described for compound **30a**, in a yield of 83% as a colorless amorphous solid. $[\alpha]_D^{25}$ +29.16 (*c* 0.12, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.52–1.64 (m, 1H), 2.05–2.34 (m, 4H), 2.40–2.50 (m, 1H), 2.59–2.75 (m, 2H), 2.88–3.04 (m, 2H), 3.50–3.77 (m, 6H), 4.13–4.25 (m, 1H), 5.71 (s, 1H), 7.23–7.30 (m, 2H), 7.40 (ddd, *J* = 8.8, 8.8, 2.5 Hz, 1H), 7.45–7.55 (m, 3H), 7.67 (dd, *J* = 10.8, 2.7 Hz, 1H), 7.82–7.87 (m, 2H), 7.96 (dd, *J* = 9.0, 5.6 Hz, 1H), 8.06 (d, *J* = 6.8 Hz, 1H); MS (FAB) *m/z* = 490 $[M+H]^+$. Anal. Calcd for $C_{29}H_{29}F_2N_3O_2{\cdot}0.25H_2O$: C, 70.50; H, 6.02; N, 8.51; F, 7.69. Found: C, 70.34; H, 6.00; N, 8.39; F, 7.97.

5.1.31. *N*-{(3*R*)-1-[(6-Fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}-2-[1-(2-methoxybenzoyl)piperidin-4-ylidene]acetamide (30e)

Compound **30e** was prepared from **28** and 2-methoxybenzoyl chloride in a manner similar to that described for compound **30a**, in a yield of 75% as a colorless amorphous solid. $[\alpha]_D^{25}$ +34.99 (*c* 0.10, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.51–1.64 (m, 1H), 2.03–2.36 (m, 4H), 2.38–2.48 (m, 1H), 2.58–2.75 (m, 2H), 2.80–2.98 (m, 2H), 3.05–3.21 (m, 2H), 3.56–3.73 (m, 4H), 3.78 (s, 3H), 4.12–4.25 (m, 1H), 5.69, 5.74 (each s, 1H), 6.96–7.02 (m, 1H), 7.05–7.10 (m, 1H), 7.16–7.21 (m, 1H), 7.35–7.44 (m, 2H), 7.50–7.56 (m, 1H), 7.64–7.70 (m, 1H), 7.81–7.88 (m, 2H), 7.93–8.00 (m, 1H), 8.02–8.08 (m, 1H); MS (FAB) *m/z* = 502 $[M+H]^+$. Anal. Calcd for $C_{30}H_{32}FN_3O_3{\cdot}0.25H_2O$: C, 71.20; H, 6.47; N, 8.30; F, 3.75. Found: C, 71.11; H, 6.36; N, 8.22; F, 3.72.

5.1.32. *N*-{(3*R*)-1-[(6-Fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}-2-[1-(3-methoxybenzoyl)piperidin-4-ylidene]acetamide (30f)

Compound **30f** was prepared from **28** and 3-methoxybenzoyl chloride in a manner similar to that described for compound **30a**, in a yield of 78% as a colorless amorphous solid. $[\alpha]_D^{25}$ +30.83 (*c* 0.12, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.53–1.63 (m, 1H), 2.03–2.38 (m, 4H), 2.39–2.50 (m, 1H), 2.58–2.75 (m, 2H), 2.84–3.04 (m, 2H), 3.50–3.75 (m, 6H), 3.78 (s, 3H), 4.13–4.25 (m, 1H), 5.72 (s, 1H), 6.92–6.98 (m, 2H), 6.99–7.04 (m, 1H), 7.35 (dd, *J* = 7.8, 7.8 Hz, 1H), 7.37–7.43 (m, 1H), 7.53 (d, *J* = 8.3 Hz, 1H), 7.67 (dd, *J* = 10.3, 2.5 Hz, 1H), 7.82–7.88 (m, 2H), 7.96 (dd, *J* = 9.3, 5.9 Hz, 1H), 8.05 (d, *J* = 7.3 Hz, 1H); MS (FAB) *m/z* = 502 $[M+H]^+$. Anal. Calcd for $C_{30}H_{32}FN_3O_3{\cdot}0.5H_2O$: C, 70.57; H, 6.51; N, 8.23; F, 3.72. Found: C, 70.70; H, 6.41; N, 8.19; F, 3.80.

5.1.33. *N*-{(3*R*)-1-[(6-Fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}-2-[1-(4-methoxybenzoyl)piperidin-4-ylidene]acetamide (30g)

To a solution of **28** (300 mg, 0.82 mmol) in THF (6 mL) were added 4-methoxybenzoic acid (131 mg, 0.86 mmol), HOBt (55 mg, 0.41 mmol), and WSC·HCl (172 mg, 0.90 mmol), and this mixture was stirred at room temperature overnight. The mixture was then partitioned between $CHCl_3$ and H_2O, and the organic layer was washed with satd $NaHCO_3$ aq, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH/NH_4OH = 99:1:0.1–98:2:0.1) to yield **30g** (150 mg, 36%) as a colorless amorphous solid. $[\alpha]_D^{25}$ +30.99 (*c* 0.10, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.53–1.64 (m, 1H), 2.05–2.16 (m, 1H), 2.18–2.27 (m, 2H), 2.29–2.35 (m, 1H), 2.40–2.50 (m, 1H), 2.59–2.75 (m, 2H), 2.89–2.97 (m, 2H), 3.35–3.61 (m, 4H), 3.68 (d, *J* = 13.2 Hz, 1H), 3.73 (d, *J* = 13.2 Hz, 1H), 3.80 (s, 3H), 4.14–4.25 (m, 1H), 5.73 (s, 1H), 6.98 (d, *J* = 8.8 Hz, 2H), 7.35–7.43 (m, 3H), 7.53 (d, *J* = 8.3 Hz, 1H), 7.68 (dd, *J* = 10.2, 2.4 Hz, 1H), 7.82–7.88 (m, 2H), 7.97 (dd, *J* = 8.8, 5.9 Hz, 1H), 8.05 (d, *J* = 6.9 Hz, 1H); MS (FAB) *m/z* = 502 $[M+H]^+$. Anal. Calcd for $C_{30}H_{32}FN_3O_3{\cdot}0.3H_2O$: C, 71.04; H, 6.48; N, 8.29; F, 3.75. Found: C, 71.01; H, 6.46; N, 8.22; F, 3.63.

5.1.34. 2-[1-(3,4-Dimethoxybenzoyl)piperidin-4-ylidene]-*N*-{(3*R*)-1-[(6-fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}acetamide (30h)

Compound **30h** was prepared from **28** and 3,4-dimethoxybenzoic acid in a manner similar to that described for compound **30g**, in a yield of 68% as a slightly brown amorphous solid. $[\alpha]_D^{25}$ +9.06 (*c* 0.11, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.52–1.63 (m, 1H), 2.05–2.17 (m, 1H), 2.18–2.27 (m, 2H), 2.29–2.35 (m, 1H), 2.40–2.50 (m, 1H), 2.59–2.74 (m, 2H), 2.89–2.99 (m, 1H), 3.35–3.62 (m, 4H), 3.66–3.76 (m, 2H), 3.77 (s, 3H), 3.79 (s, 3H), 4.14–4.24 (s, 1H), 5.75 (s, 1H), 6.94–7.01 (m, 3H), 7.41 (ddd,

J = 8.8, 8.8, 2.5 Hz, 1H), 7.53 (d, *J* = 8.3 Hz, 1H), 7.67 (dd, *J* = 10.2, 2.9 Hz, 1H), 7.85–7.88 (m, 3H), 7.97 (dd, *J* = 9.3, 5.8 Hz, 1H), 8.05 (d, *J* = 6.9 Hz, 1H); MS (FAB) *m/z* = 532 $[M+H]^+$. Anal. Calcd for $C_{31}H_{34}FN_3O_4{\cdot}0.5H_2O$: C, 68.87; H, 6.53; N, 7.77; F, 3.51. Found: C, 68.70; H, 6.61; N, 7.71; F, 3.48.

5.1.35. 2-[1-(1,3-Benzodioxol-5-ylcarbonyl)piperidin-4-ylidene]-*N*-{(3*R*)-1-[(6-fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}acetamide (30i)

Compound **30i** was prepared from **28** and 3,4-methylenedioxybenzoic acid in a manner similar to that described for compound **30g**, in a yield of 56% as a slightly brown amorphous solid. $[\alpha]_D^{25}$ +26.14 (*c* 0.13, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.52–1.64 (m, 1H), 2.05–2.17 (m, 1H), 2.18–2.25 (m, 2H), 2.29–2.36 (m, 1H), 2.40–2.50 (m, 1H), 2.59–2.75 (m, 2H), 2.90–2.98 (m, 1H), 3.35–3.60 (m, 4H), 3.66–3.78 (m, 2H), 4.14–4.26 (m, 1H), 5.72 (s, 1H), 6.07 (s, 2H), 6.90–6.99 (m, 3H), 7.41 (ddd, *J* = 8.8, 8.8, 2.5 Hz, 1H), 7.53 (d, *J* = 8.8 Hz, 1H), 7.67 (dd, *J* = 10.2, 2.9 Hz, 1H), 7.82–7.88 (m, 3H), 7.97 (dd, *J* = 9.1, 5.7 Hz, 1H), 8.05 (d, *J* = 6.8 Hz, 1H); MS (FAB) *m/z* = 516 $[M+H]^+$. Anal. Calcd for $C_{30}H_{30}FN_3O_4{\cdot}0.75H_2O$: C, 68.10; H, 6.00; N, 7.94; F, 3.59. Found: C, 68.32; H, 5.87; N, 7.90; F, 3.74.

5.1.36. *N*-{(3*R*)-1-[(6-Fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}-2-[1-(2-hydroxybenzoyl)piperidin-4-ylidene]acetamide (30j)

Compound **30j** was prepared from **28** and 2-hydroxybenzoic acid in a manner similar to that described for compound **30g**, in a yield of 59% as a colorless amorphous solid. $[\alpha]_D^{25}$ +22.30 (*c* 0.25, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.51–1.63 (m, 1H), 2.05–2.27 (m, 3H), 2.28–2.35 (m, 1H), 2.40–2.50 (m, 1H), 2.58–2.74 (m, 2H), 2.83–2.99 (m, 2H), 3.10–3.40 (m, 2H), 3.50–3.77 (m, 4H), 4.13–4.24 (m, 1H), 5.71 (s, 1H), 6.80–6.89 (m, 2H), 7.12 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.19–7.24 (m, 1H), 7.41 (ddd, *J* = 8.8, 8.8, 2.9 Hz, 1H), 7.53 (d, *J* = 8.8 Hz, 1H), 7.67 (dd, *J* = 10.2, 2.4 Hz, 1H), 7.82–7.88 (m, 2H), 7.96 (dd, *J* = 8.8, 5.8 Hz, 1H), 8.04 (d, *J* = 7.4 Hz, 1H), 9.76 (s, 1H); MS (FAB) *m/z* = 488 $[M+H]^+$. Anal. Calcd for $C_{29}H_{30}FN_3O_3{\cdot}0.25H_2O$: C, 70.78; H, 6.25; N, 8.54; F, 3.86. Found: C, 70.99; H, 6.10; N, 8.50; F, 3.90.

5.1.37. *N*-{(3*R*)-1-[(6-Fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}-2-[1-(3-hydroxybenzoyl)piperidin-4-ylidene]acetamide (30k)

Compound **30k** was prepared from **28** and 3-hydroxybenzoic acid in a manner similar to that described for compound **30g**, in a yield of 22% as a colorless amorphous solid. $[\alpha]_D^{25}$ +54.00 (*c* 0.10, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.52–1.63 (m, 1H), 2.04–2.36 (m, 4H), 2.40–2.50 (m, 1H), 2.59–2.78 (m, 2H), 2.82–3.02 (m, 2H), 3.35–3.78 (m, 6H), 4.15–4.23 (m, 1H), 5.72 (s, 1H), 6.65–6.84 (m, 3H), 7.22 (dd, *J* = 7.9, 7.8 Hz, 1H), 7.40 (ddd, *J* = 8.9, 8.9, 2.6 Hz, 1H), 7.53 (d, *J* = 8.4 Hz, 1H), 7.67 (dd, *J* = 10.3, 2.4 Hz, 1H), 7.81–7.89 (m, 3H), 7.96 (dd, *J* = 9.0, 5.9 Hz, 1H), 8.03 (d, *J* = 7.0 Hz, 1H), 9.64 (br s, 1H); MS (ESI) *m/z* = 488 $[M+H]^+$. HR-MS calcd for $C_{29}H_{30}FN_3O_3$ *m/z* = 488.2344 $[M+H]^+$. Found: 488.2349.

5.1.38. *N*-{(3*R*)-1-[(6-Fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}-2-[1-(4-hydroxybenzoyl)piperidin-4-ylidene]acetamide (30l)

Compound **30l** was prepared from **28** and 4-hydroxybenzoic acid in a manner similar to that described for compound **30g**, in a yield of 74% as a colorless amorphous solid. $[\alpha]_D^{25}$ +35.99 (*c* 0.10, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.52–1.63 (m, 1H), 2.04–2.16 (m, 1H), 2.17–2.25 (m, 2H), 2.32 (dd, *J* = 9.3, 4.8 Hz, 1H), 2.42–2.50 (m, 1H), 2.59–2.67 (m, 1H), 2.69–2.75 (m, 1H), 2.86–2.96 (m, 2H), 3.39–3.59 (m, 4H), 3.69 (d, *J* = 7.2 Hz, 1H), 3.73 (d, *J* = 7.2 Hz, 1H), 4.17–4.23 (m, 1H), 5.72 (s, 1H), 6.79 (d, *J* = 8.5 Hz, 2H), 7.27 (d, *J* = 8.5 Hz, 2H), 7.40 (ddd, *J* = 8.9, 8.9, 2.5 Hz, 1H), 7.53 (d, *J* = 8.6 Hz, 1H), 7.67 (dd, *J* = 10.3, 2.3 Hz, 1H), 7.82–7.88 (m, 3H), 7.96 (dd, *J* = 9.0, 5.8 Hz, 1H), 8.03 (d, *J* = 7.0 Hz, 1H), 9.80 (br s, 1H); MS (ESI) *m/z* = 488 $[M+H]^+$. Anal. Calcd for $C_{29}H_{30}FN_3O_3{\cdot}0.75H_2O{\cdot}0.25CHCl_3$: C, 66.71; H, 6.03; N, 7.91; Cl, 5.01; F, 3.58. Found: C, 66.31; H, 6.06; N, 7.86; Cl, 4.95; F, 3.55.

5.1.39. *N*-{(3*R*)-1-[(6-Fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}-2-[1-(2-hydroxy-3-methoxybenzoyl)piperidin-4-ylidene]-acetamide (30m)

Compound **30m** was prepared from **28** and 2-hydroxy-3-methoxybenzoic acid in a manner similar to that described for compound **30g**, in a yield of 67% as a pale brown amorphous solid. $[\alpha]_D^{25}$ +31.99 (*c* 0.10, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.53–1.64 (m, 1H), 2.05–2.26 (m, 3H), 2.29–2.35 (m, 1H), 2.40–2.50 (m, 1H), 2.59–2.65 (m, 2H), 2.83–3.00 (m, 2H), 3.09–3.30 (m, 2H), 3.50–3.75 (m, 4H), 3.81 (s, 3H), 4.13–4.26 (m, 1H), 5.70 (s, 1H), 6.70 (dd, *J* = 7.8, 1.5 Hz, 1H), 6.81 (dd, *J* = 8.8, 8.8 Hz, 1H), 6.98 (dd, *J* = 8.8, 1.5 Hz, 1H), 7.40 (ddd, *J* = 8.8, 8.8, 2.5 Hz, 1H), 7.53 (d, *J* = 8.3 Hz, 1H), 7.68 (dd, *J* = 10.5, 2.7 Hz, 1H), 7.82–7.88 (m, 3H), 7.97 (dd, *J* = 8.8, 5.8 Hz, 1H), 8.05 (d, *J* = 6.8 Hz, 1H), 9.00 (s, 1H); MS (FAB) *m/z* = 518 $[M+H]^+$. Anal. Calcd for $C_{30}H_{32}FN_3O_4{\cdot}0.5H_2O$: C, 68.42; H, 6.32; N, 7.98; F, 3.61. Found: C, 68.53; H, 6.21; N, 7.91; F, 3.51.

5.1.40. *N*-{(3*R*)-1-[(6-Fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}-2-[1-(2-hydroxy-4-methoxybenzoyl)piperidin-4-ylidene]-acetamide (30n)

Compound **30n** was prepared from **28** and 2-hydroxy-4-methoxybenzoic acid in a manner similar to that described for compound **30g**, in a yield of 16% as a colorless amorphous solid. $[\alpha]_D^{25}$ +30.99 (*c* 0.10, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.52–1.64 (m, 1H), 2.05–2.23 (m, 3H), 2.29–2.34 (m, 1H), 2.42–2.50 (m, 1H), 2.59–2.66 (m, 1H), 2.68–2.74 (m, 1H), 2.88–2.93 (m, 2H), 3.35–3.50 (m, 5H), 3.66–3.76 (m, 2H), 3.72 (s, 3H), 4.14–4.23 (m, 1H), 5.71 (s, 1H), 6.40–6.45 (m, 2H), 7.40 (ddd, *J* = 8.8, 7.2, 2.4 Hz, 1H), 7.52 (d, *J* = 8.0 Hz, 1H), 7.67 (dd, *J* = 10.4, 2.4 Hz, 1H), 7.82–7.87 (m, 2H), 7.97 (dd, *J* = 9.2, 5.6 Hz, 1H), 8.04 (d, *J* = 7.2 Hz, 1H), 9.88 (s, 1H); MS (FAB) *m/z* = 518 $[M+H]^+$. Anal. Calcd for $C_{30}H_{32}FN_3O_4{\cdot}0.5H_2O$: C, 68.42; H, 6.32; N, 7.98; F, 3.61. Found: C, 68.78; H, 6.43; N, 7.96; F, 3.56.

5.1.41. *N*-{(3*R*)-1-[(6-Fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}-2-[1-(2-hydroxy-5-methoxybenzoyl)piperidin-4-ylidene]-acetamide (30o)

Compound **30o** was prepared from **28** and 2-hydroxy-5-methoxybenzoic acid in a manner similar to that described for compound **30g**, in a yield of 32% as a colorless amorphous solid. $[\alpha]_D^{25}$ +36.36 (*c* 0.11, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.52–1.64 (m, 1H), 2.05–2.25 (m, 3H), 2.29–2.36 (m, 1H), 2.39–2.50 (m, 1H), 2.58–2.75 (m, 2H), 2.83–3.00 (m, 2H), 3.50–3.67 (m, 4H), 3.68 (s, 3H), 3.69–3.76 (m, 2H), 4.14–4.25 (m, 1H), 5.72 (s, 1H), 6.69 (d, *J* = 2.9 Hz, 1H), 6.76–6.84 (m, 2H), 7.40 (ddd, *J* = 8.8, 8.8, 3.0 Hz, 1H), 7.57 (d, *J* = 8.3 Hz, 1H), 7.67 (dd, *J* = 10.2, 2.5 Hz, 1H), 7.82–7.86 (m, 2H), 7.97 (dd, *J* = 9.2, 5.8 Hz, 1H), 8.04 (d, *J* = 6.9 Hz, 1H), 9.27 (s, 1H); MS (FAB) *m/z* = 518 $[M+H]^+$. Anal. Calcd for $C_{30}H_{32}FN_3O_4{\cdot}0.25H_2O$: C, 69.01; H, 6.27; N, 8.05; F, 3.64. Found: C, 68.80; H, 6.35; N, 7.82; F, 3.43.

5.1.42. *N*-{(3*R*)-1-[(6-Fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}-2-[1-(2-hydroxy-6-methoxybenzoyl)piperidin-4-ylidene]-acetamide (30p)

Compound **30p** was prepared from **28** and 2-hydroxy-6-methoxybenzoic acid in a manner similar to that described for compound **30g**, in a yield of 49% as a pale pink amorphous solid. $[\alpha]_D^{25}$ +27.18 (*c* 0.103, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.52–1.65 (m, 1H), 2.02–2.25 (m, 4H), 2.29–2.36 (m, 1H), 2.59–2.75 (m, 2H), 2.85–2.95 (m, 2H), 3.09–3.15 (m, 1H), 3.16–3.22 (m, 1H), 3.55–3.78 (m, 7H), 4.13–4.27 (m, 1H), 5.67, 5.73 (each s,

1H), 6.46–6.54 (m, 2H), 7.09–7.16 (m, 1H), 7.37–7.45 (m, 1H), 7.50–7.56 (m, 1H), 7.64–7.71 (m, 1H), 7.82–7.90 (m, 2H), 7.94–8.00 (m, 1H), 8.01–8.09 (m, 1H), 9.60, 9.61 (each s, 1H); MS (FAB) m/z = 518 $[M+H]^+$. Anal. Calcd for $C_{30}H_{32}FN_3O_4$: C, 68.66; H, 6.30; N, 8.01; F, 3.62. Found: C, 68.42; H, 6.15; N, 7.87; F, 3.61.

5.1.43. 2-[1-(4-Fluoro-2-hydroxybenzoyl)piperidin-4-ylidene]-*N*-{(3*R*)-1-[(6-fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}acetamide (30q)

Compound **30q** was prepared from **28** and 4-fluoro-2-hydroxybenzoic acid in a manner similar to that described for compound **30g**, in a yield of 31% as a colorless amorphous solid. $[\alpha]_D^{25}$ +24.99 (c 0.10, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.53–1.64 (m, 1H), 2.05–2.23 (m, 3H), 2.29–2.34 (m, 1H), 2.40–2.50 (m, 1H), 2.59–2.66 (m, 1H), 2.67–2.74 (m, 1H), 2.85–2.99 (m, 2H), 3.35–3.76 (m, 4H), 4.12–4.25 (m, 1H), 5.71 (s, 1H), 6.60–6.70 (m, 2H), 7.18 (dd, J = 8.3, 6.9 Hz, 1H), 7.40 (ddd, J = 8.8, 8.8, 2.5 Hz, 1H), 7.53 (d, J = 8.3 Hz, 1H), 7.68 (dd, J = 10.2, 2.4 Hz, 1H), 7.82–7.88 (m, 2H), 7.97 (dd, J = 8.8, 5.8 Hz, 1H), 8.05 (d, J = 7.8 Hz, 1H), 10.33 (s, 1H); MS (FAB) m/z = 506 $[M+H]^+$. Anal. Calcd for $C_{29}H_{29}F_2N_3O_3\cdot 0.2H_2O$: C, 68.41; H, 5.82; N, 8.25; F, 7.46. Found: C, 68.31; H, 5.83; N, 8.18; F, 7.47.

5.1.44. 2-[1-(4-Chloro-2-hydroxybenzoyl)piperidin-4-ylidene]-*N*-{(3*R*)-1-[(6-fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}acetamide (30r)

Compound **30r** was prepared from **28** and 4-chloro-2-hydroxybenzoic acid in a manner similar to that described for compound **30g**, in a yield of 63% as a colorless amorphous solid. $[\alpha]_D^{25}$ +32.06 (c 0.103, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.53–1.64 (m, 1H), 2.03–2.27 (m, 3H), 2.29–2.35 (m, 1H), 2.39–2.50 (m, 1H), 2.57–2.75 (m, 2H), 2.82–3.01 (m, 2H), 3.10–3.28 (m, 2H), 3.49–3.75 (m, 4H), 4.12–4.25 (m, 1H), 5.71 (s, 1H), 6.88–6.93 (m, 2H), 7.14–7.18 (m, 1H), 7.40 (ddd, J = 8.8, 8.8, 3.0 Hz, 2H), 7.53 (d, J = 8.8 Hz, 1H), 7.68 (dd, J = 10.3, 2.5 Hz, 1H), 7.82–7.88 (m, 2H), 7.97 (dd, J = 9.3, 5.9 Hz, 1H), 8.05 (d, J = 7.3 Hz, 1H), 10.33 (s, 1H); MS (FAB) m/z = 522 $[M+H]^+$. Anal. Calcd for $C_{29}H_{29}ClFN_3O_3\cdot 0.5H_2O$: C, 65.59; H, 5.69; N, 7.91;Cl, 6.68; F, 3.58. Found: C, 65.74; H, 5.67; N, 8.05; Cl, 6.45; F, 3.49.

5.1.45. *N*-{(3*R*)-1-[(6-Fluoro-2-naphthyl)methyl]pyrrolidin-3-yl}-2-[1-(2-hydroxy-4-methylbenzoyl)piperidin-4-ylidene]-acetamide (30s)

Compound **30s** was prepared from **28** and 2-hydroxy-4-methylbenzoic acid in a manner similar to that described for compound **30g**, in a yield of 27% as a pale yellow amorphous solid. $[\alpha]_D^{25}$ +31.99 (c 0.10, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.52–1.63 (m, 1H), 2.05–2.22 (m, 3H), 2.24 (s, 3H), 2.29–2.35 (m, 1H), 2.40–2.50 (m, 1H), 2.59–2.73 (m, 2H), 2.87–2.97 (m, 2H), 3.10–3.65 (m, 4H), 3.68 (d, J = 12.7 Hz, 1H), 3.73 (d, J = 12.7 Hz, 1H), 4.13–4.25 (m, 1H), 5.71 (s, 1H), 6.63–6.68 (m, 2H), 7.05 (d, J = 7.8 Hz, 1H), 7.40 (ddd, J = 9.2, 8.8, 2.6 Hz, 2H), 7.53 (d, J = 8.3 Hz, 1H), 7.68 (dd, J = 10.3, 2.5 Hz, 1H), 7.82–7.88 (m, 2H), 7.97 (dd, J = 9.3, 5.9 Hz, 1H), 8.04 (d, J = 7.3 Hz, 1H), 9.65 (s, 1H); MS (FAB) m/z = 502 $[M+H]^+$. Anal. Calcd for $C_{30}H_{32}FN_3O_3\cdot 0.5H_2O$: C, 70.57; H, 6.51; N, 8.23; F, 3.72. Found: C, 70.72; H, 6.53; N, 8.15; F, 3.60.

5.2. Biology

5.2.1. Measurement of intracellular Ca^{2+} concentrations

CCR3-transfected B300-19 cells[11] were loaded with 5 μM Fura-2 acetoxymethyl ester in RPMI 1640 medium containing 1% fetal bovine serum for 30 min at 37 °C. After two washes, the cells were resuspended at a concentration of 2×10^6 cells/mL in 20 mM HEPES buffer containing 0.1% BSA, 130 mM NaCl, 5.4 mM KCl, 1 mM $MgCl_2$, 2.5 mM $CaCl_2$, and 5.5 mM glucose. The cell suspension (490 μL) was transferred into cuvettes and placed under constant agitation. Changes in fluorescence were monitored at 25 °C using a spectrophotometer at excitation wavelengths of 340 nm and 380 nm and an emission wavelength of 510 nm. Calculation of Ca^{2+} concentration was performed using the Kd for the Ca^{2+} binding at 224 nm. The antagonist was dissolved in 100% DMSO solution (1 μL) and added to the cuvette 1 min prior to the addition of eotaxin (final concentration of 50 ng/mL). Linear regression analysis using EXSAS-STAT was used to calculate the IC_{50} values. Values are reported as means ± SEM of triplicate experiments.

5.2.2. In vitro human liver microsomal (HLM) metabolic stability

Pooled human liver microsomes (Cat No. H161, lot 14, Gentest Corporation) were diluted in 0.1 M KH_2PO_4/K_2HPO_4 buffer, pH 7.4, containing 8 mM $MgCl_2$, 10 mM G6P, and 2 units/mL G6PDH. The incubation mixtures (1.0 mL total volume), which contained 0.1 mg/mL of microsomal proteins and 0.5 μmol/mL of substrates, were pre-incubated for 5 min at 37 °C. The reactions were initiated by the addition of NADPH (50 mM, 10 μL) and stopped at the appropriate time points (0, 10, 20, and 30 min) by the addition of 100 μL of 20% TCA aq. The incubation mixtures were then added to an internal standard (5 μmol/L in 10% DMSO, 100 μL) and centrifuged for 5 min at 3000 rpm. One milliliter of the supernatant was purified by solid phase extraction column (OASIS HLB, 30 mg/1 cc, Waters) and was then analyzed using LC–MS/MS.

5.3. Interaction energy calculation method

To evaluate the ability to accept hydrogen bonds, the interaction energies between ligands and water molecule (as a model hydrogen bond donor in a protein) were obtained using ab initio quantum mechanical calculations. In this study, the interaction energy is defined as

$$E\ (\text{interaction}) = E\ (\text{complex}) - E\ (\text{ligand}) - E\ (\text{water})$$

where E (interaction) is the interaction energy. E (complex), E (ligand), and E (water) are the energy of the ligand/water complex, that of the ligand, and that of the water molecule, respectively, which were calculated as described below. Initial coordinates were built and optimized at the molecular mechanics level using Molecular Operating Environment software (MOE, Chemical Computing Group, Inc.). The distance between the carboxyl group of the ligand and the oxygen atom of the water molecule was set at 2.5 Å. After geometrical optimizations were performed on the HF/6-31* level using the GAUSSIAN 98 program,[9] the single point energy for the complex, ligand, and water molecule was calculated on the B3LYP/6-311+G** level.

Acknowledgments

The authors wish to thank the staff of the Division of Analysis & Pharmacokinetics Research Laboratories for their help with the evaluation of in vitro human liver microsomal (HLM) metabolic stability (CL_{int}) as well as with the elemental analysis and spectral measurements.

References and notes

1. (a) Heath, H.; Qin, S.; Rao, P.; Wu, L.; LaRosa, G. J.; Kassam, N.; Ponath, P. D.; Mackay, C. R. *J. Clin. Invest.* **1997**, *99*, 178; (b) Uguccioni, M.; Mackay, C. R.; Ochensberger, B.; Loetscher, P.; Rhis, S.; LaRosa, G. J.; Rao, P.; Ponath, P. D.; Marco, B.; Dahinden, C. A. *J. Clin. Invest.* **1997**, *100*, 1137; (c) Ochi, H.; Hirani, W. M.; Yuan, Q.; Friend, D. S.; Austen, K. F.; Boyce, J. A. *J. Exp. Med.* **1999**, *190*, 267; (d) Sallusto, F.; Mackay, C. R.; Lanzavecchia, A. *Science* **1997**, *277*, 2005; (e)

Murphy, P. M.; Baggiolini, M.; Chero, I. F.; Herbert, C. A.; Horuk, R.; Matsushima, K.; Miller, L. H.; Oppenheim, J. J.; Power, C. A. *Pharmacol. Rev.* **2000**, *52*, 145.
2. (a) Barnes, P. J.; Chung, K. F.; Page, C. P. *Pharmacol. Rev.* **1998**, *50*, 515; (b) Busse, W. W.; Banks-schlegel, S.; Wenzel, S. E. *J. Allergy Clin. Immunol.* **2000**, *106*, 1033.
3. (a) Sato, I.; Morihira, K.; Inami, H.; Kubota, H.; Morokata, T.; Suzuki, K.; Hamada, N.; Iura, Y.; Nitta, A.; Imaoka, T.; Takahashi, T.; Takeuchi, M.; Ohta, M.; Tsukamoto, S. *Bioorg. Med. Chem.* **2008**, *16*, 144; (corrigendum): Sato, I.; Morihira, K.; Inami, H.; Kubota, H.; Morokata, T.; Suzuki, K.; Hamada, N.; Iura, Y.; Nitta, A.; Imaoka, T.; Takahashi, T.; Takeuchi, M.; Ohta, M.; Tsukamoto, S. *Bioorg. Med. Chem.* **2008**, *16*, 7078; (b) Sato, I.; Morihira, K.; Inami, H.; Kubota, H.; Morokata, T.; Suzuki, K.; Iura, Y.; Nitta, A.; Imaoka, T.; Takahashi, T.; Takeuchi, M.; Ohta, M.; Tsukamoto, S. *Bioorg. Med. Chem.* **2008**, *16*, 8607.
4. For example: (a) Naya, A.; Kobayashi, K.; Ishikawa, M.; Ohwaki, K.; Saeki, T.; Noguchi, K.; Ohtake, N. *Chem. Pharm. Bull.* **2003**, *51*, 697; (b) Ting, P. C.; Lee, J. F.; Wu, J.; Umland, S. P.; Aslanian, R.; Cao, J.; Dong, Y.; Garlisi, C. G.; Gilbert, E. J.; Huang, Y.; Jakway, J.; Kelly, J.; Liu, Z.; McCombie, S.; Shah, H.; Tian, F.; Wan, Y.; Shih, N. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1375; (c) Santella, J. B., III; Gardner, D. S.; Yao, W.; Shi, C.; Reddy, P.; Tebben, A. J.; Delucca, G. V.; Wacker, D. S.; Watson, P. S.; Welch, P. K.; Wadman, E. S.; Davies, P.; Solomon, K. A.; Graden, D. M.; Yeleswaram, S.; Mandlekar, S.; Kariv, I.; Decicco, C. P.; Ko, S. S.; Carter, P.; Duncia, J. V. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 576.
5. Bird, T. G. C.; Edwards, P. N.; Crawley, G. C.; Girodeau, J. M. M.; Edwards, M. P.; Kingston, J. F. ICI Pharma., EP Patent 0351194, 1990.
6. Obach, R. S. *Drug Metab. Dispos.* **1999**, *27*, 1350.
7. Lazareno, S.; Birdsall, N. J. M. *Trends Pharmacol. Sci.* **1993**, *14*, 237. The K_b values are shown as the means of three experiments.
8. The mouse and monkey CCR3 inibitory activities were evaluated using the same method of our previous reports ($n = 3$): (a) Morokata, T.; Suzuki, K.; Masunaga, Y.; Taguchi, K.; Morihira, K.; Sato, I.; Fujii, M.; Takizawa, S.; Torii, Y.; Yamamoto, N.; Kaneko, M.; Yamada, T.; Takahashi, K.; Simizu, Y. *J. Pharmacol. Exp. Ther.* **2006**, *317*, 244; (b) Suzuki, K.; Morokata, T.; Morihira, K.; Sato, I.; Takizawa, S.; Kaneko, M.; Takahashi, K.; Simizu, Y. *Biochem. Biophys. Res. Commun.* **2006**, *339*, 1217; (c) Suzuki, K.; Morokata, T.; Morihira, K.; Sato, I.; Takizawa, S.; Kaneko, M.; Takahashi, K.; Simizu, Y. *Eur. J. Pharmacol.* **2007**, *563*, 224.
9. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A.; Stratmann, R. E.; Burant, L. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowsko, J.; Ortiz, J. V.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. GAUSSIAN *98 (Version A.1)*; Gaussian: Pittsburgh, PA, 1998.
10. Yamanaka, T.; Ohkubo, M.; Takahashi, F.; Kato, M. *Tetrahedron Lett.* **2004**, *45*, 2843.
11. Sato, K.; Kawasaki, H.; Nagayama, H.; Serizawa, R.; Ikeda, J.; Morimoto, C.; Yasunaga, K.; Yamaji, N.; Tadokoro, K.; Juji, T.; Takahashi, T. A. *Blood* **1999**, *93*, 34.