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3-(2-Aminocarbonylphenyl)propanoic acid analogs as potent and selective EP3 receptor antagonists. Part 3: Synthesis, metabolic stability, and biological evaluation of optically active analogs

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ABSTRACT

A series of 3-(2-aminocarbonylphenyl)propanoic acid analogs possessing the (1*R*)-1-(3,5-dimethylphenyl)-3-methylbutylamine moiety on the carboxyamide side chain were synthesized and evaluated for their binding affinity for the EP1-4 receptors and their antagonist activity for the EP3 receptor. Rational drug design based on the structure of the metabolites in human liver microsomes led us to the discovery of another series of analogs. Several compounds were further evaluated for their in vivo efficacy in rats after oral administration and also for their pharmacokinetic profiles including in vitro stability in the liver microsomes.

1. Introduction

It is well known that prostaglandins (PGs) and thromboxane (TX), both of which are the oxidative metabolites of arachidonic acid, play important roles in maintaining homeostasis. The chemical structures of PGs consist of the modified cyclopentane ring and two side chains (α-chain and ω-chain) attached to the ring. PGs are classified into nine series (PGA, PGB, PGC, PGD, PGE, PGF, PGG, PGH, and PGI) by their structural features of the cyclopentane ring.[1] Among them, PGE_2, which is biologically synthesized from PGH_2 by the action of PGE_2 synthase, is known to be the most abundantly produced prostaglandin in humans and exhibits multiple pharmacological actions through four specific receptor subtypes EP1-4. EP3 receptor subtype is distributed in various tissues including brain, kidney, uterus, and gastrointestinal tract. Various actions of PGE_2 such as hyperalgesia,[2] pyrexia,[3] uterine contraction,[4] gastric acid secretion,[5] platelet aggregation,[6] and thrombosis[7] are also known to be mediated by the EP3 receptor. As such, the EP3 receptor subtype could be one of the most promising therapeutic targets in this field.

In our previous reports,[8,9] a series of 3-(2-aminocarbonyl-4-phenoxymethylphenyl)propanoic acid analogs possessing a 1-(3,5-dimethylphenyl)-3-methylbutylamine moiety on the carboxyamide side chain which were synthesized as a racemate, were found to be excellent inhibitors of the PGE_2-induced uterine contraction in pregnant rats after intraduodenal administration. Based on the information described above, further investigation of the optically active analogs was carried out. Herein, we report the synthesis of a series of 3-(2-aminocarbonylphenyl)propanoic acid analogs possessing a (1*R*)-1-(3,5-dimethylphenyl)-3-methylbutylamine moiety on the carboxamide side chain, evaluation of their in vitro activity for the EP3 receptor subtype, and their stability in liver microsomes including the identification of two major metabolites. Several compounds were also evaluated for their in vivo efficacy after oral administration.

2. Chemistry

Synthesis of test compounds is outlined in Schemes 1–4. The optically active amines (*S*)-**27** and (*R*)-**27** were synthesized as shown in Schemes 1 and 2, respectively. Asymmetric induction was accomplished by the Ellman's chiral *t*-butyl sulfinamide method.[10] As shown in Scheme 1, optically active *N*-sulfinyl imine **25** was prepared by the dehydrative condensation reaction of (*S*)-*t*-butyl sulfinamide (**24**) with isovaleraldehyde in the presence of pyridinium *p*-toluenesulfonate and anhydrous magnesium sulfate. While nucleophilic addition of 3,5-dimethylphenylmagnesium

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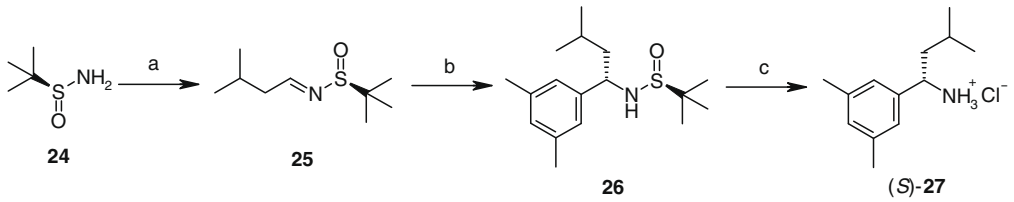
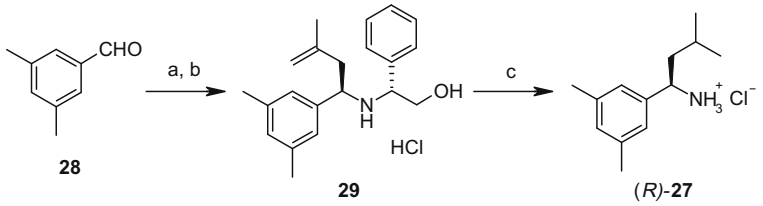
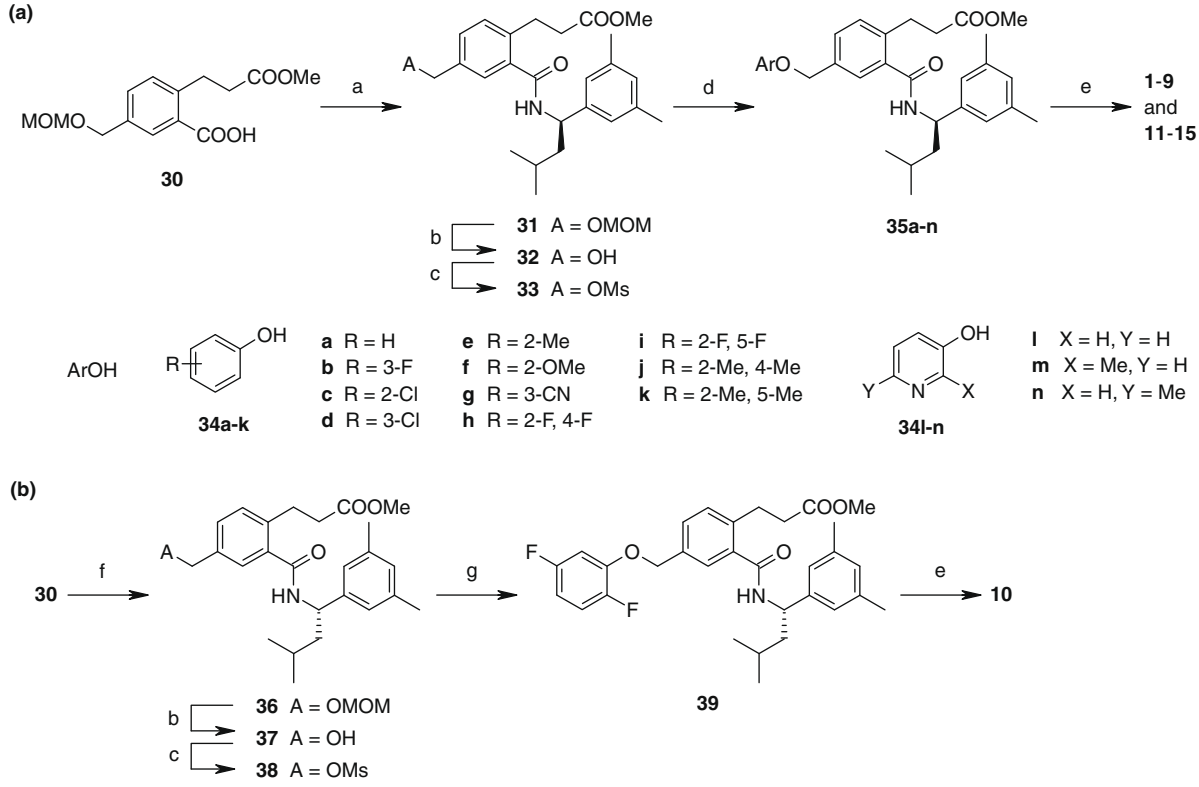


Scheme 1. Synthesis of (*S*)-**27**. Reagents: (a) isovaleraldehyde, PPTS, $MgSO_4$, CH_2Cl_2; (b) 3,5-dimethylphenylmagnesium bromide, THF, CH_2Cl_2; (c) HCl–dioxane, MeOH.



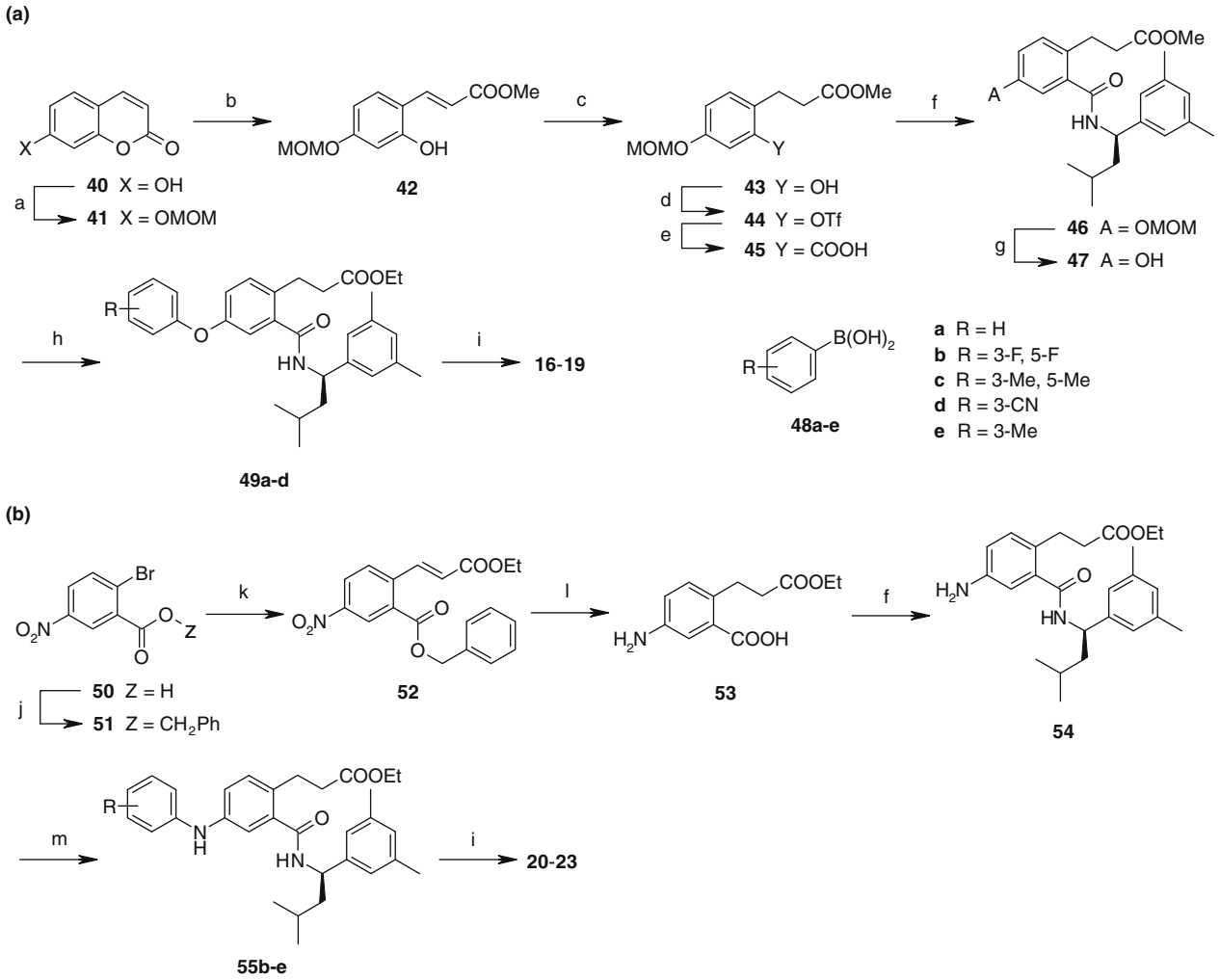
Scheme 2. Practical synthesis of (*R*)-**27**. Reagents: (a) (*R*)-phenylglycinol, toluene and then 2-methylallylmagnesium chloride; (b) HCl–EtOAc and then recrystallization from *i*-PrOH/hexane; (c) H_2, PtO_2, EtOH.



Scheme 3. Synthesis of **1**–**15**. Reagents: (a) (*R*)-**27**, EDC·HCl, HOBt, *N*-methylmorpholine, DMF; (b) HCl-dioxane, MeOH; (c) MsCl, Et_3N, THF; (d) **34a**–**n**, NaH, DMF; (e) NaOHaq, THF, MeOH; (f) (*S*)-**27**, EDC·HCl, HOBt, *N*-methylmorpholine, DMF; (g) 2,5-difluorophenol (**34i**), NaH, DMF.

bromide to the *N*-sulfinyl imine **25** afforded **26**, which was obtained with good diastereomeric selectivity of 93:7, purification by column chromatography on silica gel was needed to remove the minor diastereomer. The diastereomeric ratio was determined by using ^{1}H NMR spectroscopy. Removal of the *t*-butylsulfinyl group under acidic conditions afforded (*S*)-**27**. Since the acidic deprotection of the *t*-butylsulfinyl group proceeded smoothly in mild acidic condition, the stereochemistry was thought to be retained without affecting the benzylic C–N bond.[10] Using essentially the same procedure as described above, the corresponding enantiomer (*R*)-**27** could be prepared from (*R*)-*t*-butyl sulfinamide.

Since (*R*)-enantiomers were found to show significantly more potent in vitro activity than the corresponding (*S*)-enantiomers for the purposes of our SAR study, we needed a more efficient synthetic method than the one described above to obtain optically pure (*R*)-**27**. An alternative synthetic method is presented in Scheme 2.[11] Optically active (*R*)-**27** was prepared from 3,5-dimethylbenzaldehyde (**28**) by the following sequential procedures: (1) Formation of Schiff base from 3,5-dimethylbenzaldehyde (**28**) and (*R*)-phenylglycinol; (2) the nucleophilic addition of 2-methylallylmagnesium chloride resulting in a free form of **29** with good diastereomeric selectivity of 92:8 which was determined by using



Scheme 4. Synthesis of **16**–**23**. Reagents: (a) MOMCl, *i*-Pr_2NEt, DMF; (b) NaH, MeOH, THF; (c) H_2, Pd–C, MeOH; (d) Tf_2O, pyridine, CH_2Cl_2; (e) CO, $Pd(OAc)_2$, DPPF, KOAc, DMF; (f) (*R*)-**27**, EDC·HCl, HOBt, *N*-methylmorpholine, DMF; (g) HCl-dioxane, MeOH; (h) phenylboronic acids **48a**–**d**, $Cu(OAc)_2$, Et_3N, molecular sieves, CH_2Cl_2; (i) NaOH aq, THF, MeOH; (j) benzyl bromide, K_2CO_3, DMF; (k) ethyl acrylate, $Pd(OAc)_2$, DPPF, Et_3N, DMSO; (l) H_2, Pd–C, EtOH; (m) phenylboronic acids **48b**–**e**, $Cu(OAc)_2$, TEMPO, Et_3N, molecular sieves, CH_2Cl_2.

^{1}H NMR spectroscopy; (3) treatment of the free form of **29** with hydrogen chloride in ethyl acetate; (4) fractional crystallization from *i*-PrOH/hexane to afford **29** as a single diastereomer; (5) catalytic hydrogenolysis of **29** to afford (*R*)-**27**. Exclusive cleavage of the C–N bond attached to 2-phenylethanol moiety to afford (*R*)-**27** was accomplished by using platinum oxide as a catalyst with retention of the stereochemistry of another benzylic carbon. Purification by tedious column chromatography on silica gel was needed to remove the minor diastereomer in the synthetic route shown in Scheme 1, while purification by fractional crystallization afforded much more effective alternative method. Thus, the synthetic route shown in Scheme 2 was considered to be more useful for the scale-up synthesis of (*R*)-**27**. Absolute configuration of (*R*)-**27** was determined based on the X-ray crystallographic structure analysis of **29**, as shown in Figure 1.[12]

4-Aryloxymethyl analogs **1–15** were synthesized as shown in Schemes 3. All analogs listed in Scheme 3 were synthesized from the benzoic acid **30**, which was prepared from 7-methylcoumarin as described in our previous paper.[9] As shown in Schemes 3a, **31** was synthesized from **30** and (*R*)-**27** using an EDC/HOBt coupling method. Acidic deprotection of **31** followed by methanesulfonylation provided **33** which is a common intermediate for the syntheses of **1–9** and **11–15**. Nucleophilic substitution reaction of **33** with the anion prepared from **34a**–**n** in the presence of sodium hydride resulted in **35a**–**n**, respectively, alkaline hydrolysis of which afforded **1–9** and **11–15**, respectively. Synthesis of **10** which is an enan-

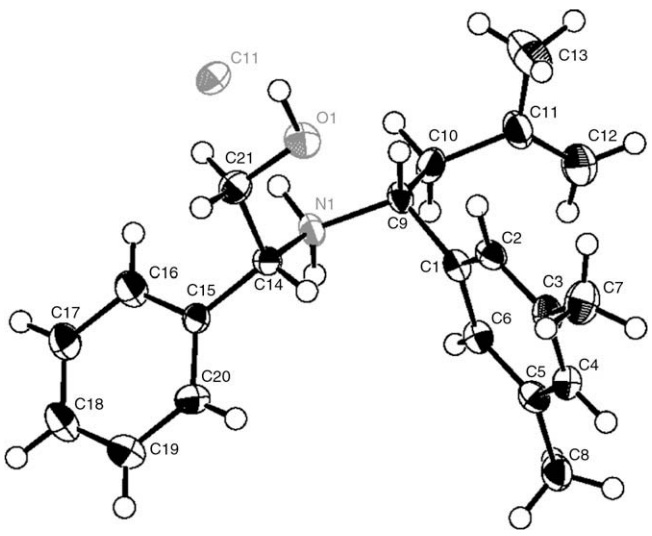
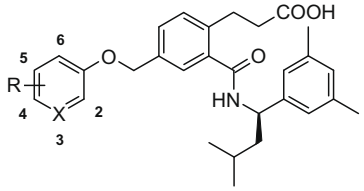


Figure 1. Perspective drawing of the X-ray crystal structure of **29**.

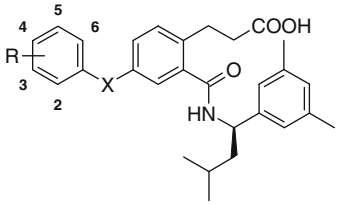
Table 1
Effect of the aryloxymethyl side chain on activity profiles and metabolic stability in liver microsomes



Compound	R	X	Absolute configuration	Binding affinity K_i (nM)				Function IC_{50}[a] (nM)	Metabolic stability (% remaining)	
				EP1	EP2	EP3	EP4	EP3	HLM[b]	RLM[c]
1	H	CH	*R*	>10,000	>10,000	0.41	75	5.5	51	27
2	3-F	CH	*R*	>10,000	>10,000	0.37	170	6.6	46	31
3	2-Cl	CH	*R*	4400	>10,000	0.29	130	2.9	62	40
4	3-Cl	CH	*R*	4400	>10,000	0.16	790	1.8	50	43
5	2-Me	CH	*R*	>10,000	>10,000	0.19	100	1.9	73	58
6	2-OMe	CH	*R*	4100	>10,000	0.67	55	7.3	70	90
7	3-CN	CH	*R*	>10,000	>10,000	0.28	72	2.7	57	70
8	2-F, 4-F	CH	*R*	>10,000	>10,000	0.29	110	3.3	65	38
9	2-F, 5-F	CH	*R*	>10,000	>10,000	0.068	230	1.6	37	21
10	2-F, 5-F	CH	*S*	>10,000	>10,000	1.4	3000	19	56	19
11	2-Me, 4-Me	CH	*R*	>10,000	>10,000	1.4	85	4.3	93	69
12	2-Me, 5-Me	CH	*R*	>10,000	>10,000	0.33	92	1.7	61	83
13	H	N	*R*	>10,000	>10,000	0.50	460	2.0	80	100
14	2-Me	N	*R*	>10,000	>10,000	0.55	460	4.4	68	57
15	4-Me	N	*R*	>10,000	>10,000	0.48	600	5.4	60	70

[a] EP3 antagonist activity was evaluated in the presence of 1% BSA.
[b] HLM: human liver microsomes.
[c] RLM: rat liver microsomes.

Table 2
Activity profiles and metabolic stability in liver microsomes of phenoxy analogs **16**–**19** and anilino analogs **20**–**23**



Compound	R	X	Binding affinity K_i (nM)				Function IC_{50} (nM)[a]	Metabolic stability (% remaining)	
			EP1	EP2	EP3	EP4	EP3	HLM[b]	RLM[c]
16	H	O	>10,000	>10,000	7.6	250	39	96	76
17	3-F, 5-F	O	>10,000	>10,000	0.85	410	11	100	89
18	3-Me, 5-Me	O	>10,000	>10,000	0.28	120	2.9	85	74
19	3-CN	O	>10,000	>10,000	0.28	43	3.8	73	63
20	3-F, 5-F	NH	>10,000	>10,000	0.77	430	6.9	82	72
21	3-Me, 5-Me	NH	>10,000	3000	0.23	300	2.0	97	93
22	3-CN	NH	8700	>10,000	0.36	220	4.9	95	71
23	3-Me	NH	>10,000	5200	0.39	180	5.5	93	91

[a] EP3 antagonist activity was evaluated in the presence of 1% BSA.
[b] HLM: human liver microsomes.
[c] RLM: rat liver microsomes.

tiomer of **9** is shown in Scheme 3b. Dehydrative condensation reaction of **30** with (*S*)-**27** afforded **36**, acidic deprotection of which provided **37**. Methanesulfonylation of **37** followed by substitution of **38** with the phenoxide prepared from 2,5-difluorophenol (**34i**) in the presence of sodium hydride provided **39**, alkaline hydrolysis of which resulted in the corresponding carboxylic acid **10**.

Synthesis of 4-phenoxy analogs **16–19** is outlined in Scheme 4a. Protection of the hydroxy group of 7-hydroxycoumarin (**40**) as the corresponding methoxymethyl ether afforded **41**, ring-opening reaction of which resulted in unsaturated ester **42**.[13] Catalytic hydrogenation of **42** afforded methyl propanoate **43**. Trifluoromethanesulfonylation of **43** afforded **44**, which was converted to the carboxylic acid **45** by the palladium-catalyzed insertion reaction of carbon monoxide. Dehydrative condensation of **45** with (*R*)-**27** followed by acidic deprotection gave phenol **47**. Cross-coupling reaction of phenol **47** and the phenylboronic acid (**48a**) in the presence of a stoichiometric amount of copper(II) acetate resulted in diphenyl ether **49a**.[14] Using optionally substituted phenylboronic acids **48b–d** instead of **48a**, **49b–d** were prepared from **47**, respectively. Alkaline hydrolysis of **49a–d** resulted in the carboxylic acids **16–19**, respectively.

4-Anilino analogs **20–23** were synthesized from 2-bromo-5-nitrobenzoic acid (**50**) as described in Scheme 4b. Heck reaction of **51**, which was prepared from **50**, with ethyl acrylate afforded

unsaturated ester **52**. Catalytic hydrogenation of **52** provided **53**, condensation reaction of which with (*R*)-**27** afforded **54**. The coupling reaction of aniline **54** with optimally substituted phenylboronic acids **48b–e** in the presence of copper (II) acetate and TEMPO afforded **55b–e**,[15] alkaline hydrolysis of which provided the corresponding carboxylic acids **20–23**, respectively.

3. Results and discussion

Test compounds listed in Tables 1 and 2 were biologically evaluated for their inhibition of the specific binding of a radiolabeled ligand, [^{3}H]PGE$_2$ to membrane fractions prepared from cells stably expressing each mouse EP1, EP2, EP3α, and EP4 receptors. The EP3 receptor antagonist activity was determined by a Ca^{2+} assay using mouse EP3α receptors expressed on CHO cells in the presence of 1% bovine serum albumin (BSA).

We previously reported a series of 3-(2-aminocarbonyl-4-phenoxymethylphenyl)propanoic acid analogs possessing the 1-(3,5-dimethylphenyl)-3-methylbutylamine moiety on the carboxyamide side chain, which were synthesized as racemates for the SAR study.[9] Since (*R*)-enantiomers possessing carboxyamide side chains derived from the (*R*)-α-substituted benzylamine was thought to show stronger activity relative to the corresponding (*S*)-enantiomers derived from the (*S*)-α-substituted benzylamine from our previous results,[8] we focused on the SAR study of the (*R*)-enantiomers. Results are summarized in Table 1. Unsubstituted phenoxymethyl analog **1** exhibited potent in vitro activities (K_i = 0.41 nM and IC_{50} = 5.5 nM for the EP3 receptor) and relatively less potent binding affinity for EP4 receptor (K_i = 75 nM), while it exhibited no affinity for the EP1 and EP2 receptors at concentrations tested up to 10,000 nM. Replacement of the phenoxy moiety of **1** with 2-methoxyphenoxy and 3-cyanophenoxy moieties afforded **6** and **7**, respectively, with a similar retention of the binding affinity and subtype selectivity for other EP receptors. Replacement of the phenoxy moiety of **1** with 3-fluorophenoxy, 2-chlorophenoxy, 3-chlorophenoxy, and 2-methylphenoxy moieties afforded **2–5**, respectively, with a tendency of retained affinity for the EP3 receptor and reduced affinity for the EP4 receptor. Thus, analogs **2–5** exhibited more subtype selectivity for EP3 relative to **1** (**1**: EP4/EP3 = 180; **2–5**: EP4/EP3 = 460, 450, 4900, 530). Replacement of the phenoxy moiety of **1** with disubstituted phenoxy moieties such as 2,4-difluorophenoxy, 2,5-difluorophenoxy, 2,4-dimethylphenoxy, and 2,5-dimethylphenoxy moieties afforded **8**, **9**, **11**, and **12**, respectively. Analogs **8** and **9** tended to show an increased affinity for the EP3 receptor and decreased affinity for EP4 receptor relative to **1**. Analog **11** tended to show reduced affinity for the EP3 receptor relative to **1** while it showed retained affinity for the EP4 receptor. Analog **12** again showed nearly equipotent binding affinity for EP3 and EP4 receptors relative to **1**. The corresponding (*S*)-enantiomer **10** was reconfirmed to be less active than the (*R*)-enantiomer **9**. Replacement of the phenoxy moiety of **1** with pyridin-3-yloxy, 2-methylpyridine-3-yloxy, and 6-methylpyridine-3-yloxy moieties afforded **13–15**, respectively, with nearly equipotent affinity for the EP3 receptor and reduced affinity for the EP4 receptor. As a result, these analogs **13–15** exhibited improved selectivity for the EP3 receptor with retained antagonist activity for the EP3 receptor.

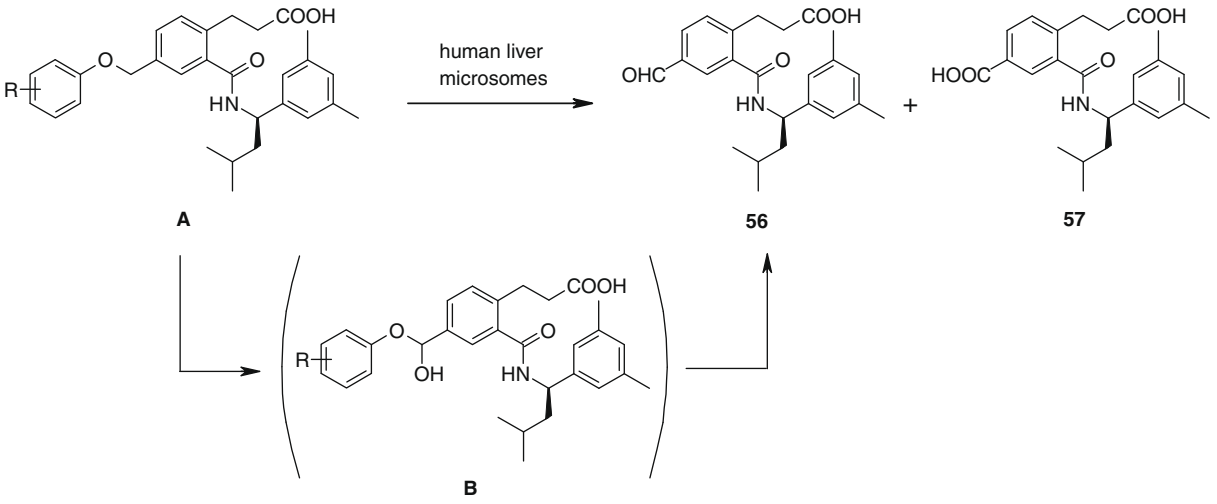
Table 3
In vivo efficacies of **5**, **9**, **12**, **18**, and **21** after oral administration

Compound	In vivo efficacy[a] (%inh., po)		
	0.1 mg/kg	0.3 mg/kg	1 mg/kg
5	29 ± 13	53 ± 10	98 ± 6.4
9	47 ± 11	85 ± 8.0	NT[b]
12	NT[b]	60 ± 6.3	84 ± 4.4
18	NT[b]	42 ± 9.1	79 ± 5.4
21	52 ± 15[c]	67 ± 5.9	77 ± 6.0

[a] Evaluation at 4 h after oral administration.
[b] NT: Not tested.
[c] Evaluation at 2 h after oral administration.

Since good oral activity should be derived from good pharmacokinetic profiles, we focused on the improvement of the pharmacokinetic profiles of these analogs. To propose a synthetic strategy to improve their pharmacokinetic profiles, in vitro stability of **1–15** in human liver microsomes (HLM) and rat liver microsomes (RLM) was evaluated. Results are summarized in Table 1. Most of the tested phenoxymethyl analogs, in which one or two substituents were introduced into the phenoxy moieties, tended to show moderate stability in both HLM and RLM. Replacement of the phenoxy moiety with a pyridine-3-yloxy moiety was also effective for providing in vitro stabilization.

Qualitative analysis of the in vitro metabolites of several phenoxymethyl analogs incubated in the presence of HLM was also carried out. As shown in Scheme 5, treatment of test compound **A** with HLM afforded an aldehyde **56** and the corresponding carboxylic acid **57** as the main metabolites, both of which were estimated to be produced via presumed hemiacetal **B**. Treatment of test com-



Scheme 5. Identification of the metabolites of phenoxymethyl analogs in human liver microsomes.

Table 4
Pharmacokinetic profiles of **5**, **9**, **12**, **18**, and **21** in rats

Compound	Route	Dose (mg/kg)	AUC (μg h/mL)	$t_{1/2}$ (h)	CL_{tot} (mL/min/kg)	V_{ss} (L/kg)	C_{max} (μg/mL)	F (%)
5	iv	2.7	0.89	0.4	51.5	1.24		
	po	10	1.01	1.6			0.33	31
9	iv	1.2	0.681	0.3	29.8	0.39		
	po	10	1.02	5.0			0.19	18
12	iv	1.8	1.37	0.4	22.0	0.67		
	po	10	4.10	2.2			1.35	54
18	iv	2	1.37	0.4	24.1	0.56		
	po	10	1.43	4.6			0.95	21
21	iv	2	2.67	2.0	11.5	1.03		
	po	10	3.27	2.7			1.37	25

pound **A** with RLM afforded mainly the same metabolites as described above. Structure determination of metabolites **56** and **57** was carried out by LC-NMR.

Based on the results described above, we directed our efforts towards synthesizing a series of 4-phenoxy analogs **16–19** and 4-anilino analogs **20–23** because these analogs do not have a benzylic moiety which may be susceptible to metabolism. Introduction of one or two substituents into the terminal phenyl moiety was also carried out as illustrated by **17–23**. All of the analogs listed in Table 2 exhibited good stability in the presence of liver microsomes. These analogs were also evaluated for their binding affinity for EP1-4 receptor and antagonist activity for the EP3 receptor. Compounds **17–23**, which showed excellent subtype selectivity, exhibited potent binding affinity and antagonist activity for the EP3 receptor, while **16** showed weaker antagonist activity relative to the other tested compounds.

Further biological evaluation of above-mentioned analogs was carried out. The inhibitory effect of PGE_2-induced uterine contraction in pregnant rats was investigated as a beneficial effect. The efficacy of **5**, **9**, **12**, **18**, and **21** was expressed as % inhibition at 4 h after oral administration, as shown in Table 3. All analogs exhibited efficacy after their oral administration in dose-dependent manner. The inhibitory effect was more than 50% at less than 1 mg/kg of oral administration. Among the tested compounds, 2,5-difluorophenoxymethyl analog **9** and 3,5-dimethylanilino analog **21** showed nearly 50% inhibition at 0.1 mg/kg of oral administration, while they showed 85% inhibition and 67% inhibition, respectively, at 0.3 mg/kg of oral administration.

The pharmacokinetic profiles of **5**, **9**, **12**, **18**, and **21** were also investigated. Results are summarized in Table 4. Compound **12**, which showed improved stability in the presence of HLM and RLM, exhibited a good maximum concentration (C_{max} = 1.35 μg/mL at 10 mg/kg po) and good bioavailability (F = 54%). Compound **21**, which also showed improved stability in the presence of HLM and RLM, had the lowest total clearance (CL_{tot} = 11.5 mL/min/kg) among the tested compounds. **21** also showed a good maximum concentration (C_{max} = 1.37 μg/mL at 10 mg/kg po) and moderate bioavailability (F = 25%). Compounds **5**, **9**, and **18** exhibited moderate bioavailability with F% of 31%, 18%, and 21%, respectively, and maximum concentration (C_{max}) of 0.33, 0.19, and 0.95 μg/mL at 10 mg/kg po, respectively. Compound **9**, which showed the most potent in vivo efficacy among the tested compounds, showed the lowest C_{max} with the longest half time ($t_{1/2}$) after oral administration.

The in vivo efficacies of **9** and **21** seemed to be close, while pharmacokinetic profiles of **9** and **21** did not always reflect the pharmacological efficacies. To explain the relationship between the in vivo efficacy and the pharmacokinetic profiles of these two compounds, their inhibitory effects on the PGE_2-induced uterine contraction in pregnant rats after their intravenous administration were evaluated. The inhibitory effect of **9** was 96 ± 5.2% after 5 min of intravenous administration at 0.03 mg/kg, while the inhibitory effect of **21** was 57 ± 3.9%. As such, compound **9** was found to be more potent than **21**. Based on the results described above, compound **9** was estimated to show nearly same efficacy as that of **21**, which possesses better pharmacokinetic profiles after their oral administration.

4. Conclusion

Further optimization of 3-(2-aminocarbonylphenyl)propanoic acid analogs possessing a (1*R*)-1-(3,5-dimethylphenyl)-3-methylbutylamine moiety on the carboxyamide side chain was carried out. A series of analogs possessing the 4-aryloxymethyl side chain were found to exhibit potent antagonist activity for the EP3 receptor with high subtype selectivity. Identification of the metabolites of 4-phenoxymethyl analogs in the presence of HLM resulted in the discovery that these analogs are metabolically susceptible at their benzylic position. Thus, rational design and synthesis of 4-phenoxy and 4-anilino analogs both of which do not have the benzylic carbon atom, led us to the discovery of another series of selective EP3 receptor antagonists. Several compounds were evaluated for their inhibitory effect against the PGE_2-induced uterine contraction in pregnant rats after their oral administration. Both series of analogs showed potent efficacy in this animal model. Among the tested analogs, compounds **9** and **21** exhibited relatively potent oral activity. Based on the results described above, these potent and selective EP3 receptor antagonists are expected to have potential for the treatment of diseases mediated by the EP3 receptor subtype.

5. Experimental

5.1. Chemistry

5.1.1. General procedures

Analytical samples were homogeneous as confirmed by TLC, and afforded spectroscopic results consistent with the assigned structures. Proton nuclear magnetic resonance spectra (^{1}H NMR) were taken on a Varian Mercury 300 spectrometer, Varian GEMINI-200 or VXR-200s spectrometer using deuterated chloroform ($CDCl_3$), deuterated dimethylsulfoxide (DMSO-d_6) or deuterated methanol (CD_3OD) as the solvent. Fast atom bombardment (FAB-MS, HRMS) and electron ionization (EI) mass spectra were obtained on a JEOL JMS-DX303HF spectrometer. Atmospheric pressure chemical ionization (APCI) mass spectra were determined on a HITACHI MI200H spectrometer. Infrared spectra (IR) were measured in a Perkin-Elmer FT-IR 1760X spectrometer. Melting points and results of elemental analyses were uncorrected. Optical rotations were measured in a JASCO DIP-1000 digital polarimeter. The enantiomer excess (% ee) was determined by the following HPLC system: Agilent Technologies 1200 Series with a CHIRALCEL

OD-RH (4.6 × 150 mm), eluting with 0.15 M aqueous KPF_6/acetonitrile = 60/40, flow rate of 1.0 mL/min, column temperature of 40 °C, detection with UV (210 nm). Column chromatography was carried out on silica gel [Merck Silica Gel 60 (0.063–0.200 mm), Wako gel C-200, or Fuji Silysia FL60D]. Thin layer chromatography was performed on silica gel (Merck TLC or HPTLC plates, Silica Gel 60 F254). The following abbreviations for solvents and reagents are used; *t*-butylmethylether (TBME), *N*,*N*-dimethylformamide (DMF), dimethylsulfoxide (DMSO), ethanol (EtOH), ethyl acetate (EtOAc), methanol (MeOH), tetrahydrofuran (THF), 1,1′-bis(diphenylphosphino)ferrocene (DPPF), *N*,*N*-diisopropylethylamime (DIPEA), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl), 1-hydroxybenzotriazole (HOBt), isopropanol (*i*-PrOH), methanesulfonyl chloride (MsCl), methanol (MeOH), methoxymethyl chloride (MOMCl), pyridinium *p*-toluenesulfonate (PPTS), 2,2,6,6-tetramethylmorpholine oxide (TEMPO), triethylamine (TEA), and trifluoromethanesulfonic anhydride (Tf_2O).

5.1.2. (S_S)-2-Methyl-*N*-[(1*E*)-3-methylbutylidene]propane-2-sulfinamide (25)

A solution of **24** (1.75 g, 14.4 mmol), isovaleraldehyde (3.10 mL, 28.9 mmol), PPTS (181 mg, 0.720 mmol) and $MgSO_4$ (8.67 g, 72.0 mmol) in CH_2Cl_2 (30 mL) was stirred for 5 h at room temperature under an argon atmosphere. The reaction mixture was concentrated in vacuo and the resultant residue was purified by column chromatography on silica gel (EtOAc/hexane, 100/0 to 50/50) to yield **25** (2.61 g, 96%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (t, J = 5.3 Hz, 1H), 2.47–2.38 (m, 2H), 2.16–1.98 (m, 1H), 1.20 (s, 9H), 0.99 (d, J = 6.6 Hz, 6H).

5.1.3. (S_S)-*N*-[(1*S*)-1-(3,5-Dimethylphenyl)-3-methylbutyl]-2-methylpropane-2-sulfinamide (26)

To a stirred solution of **25** (250 mg, 1.32 mmol) in CH_2Cl_2 (5 mL) was added dropwise 3,5-dimethylphenylmagnesium bromide (2 M in THF, 1.32 mL, 2.64 mmol) at −78 °C under an argon atmosphere. After being stirred for −78 °C at 12 h, the reaction was quenched with aqueous NH_4Cl and then the resultant mixture was extracted with EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The resultant residue was purified by column chromatography on silica gel (EtOAc/hexane, 1/9 to 1/1) to yield **26** (320 mg, 82%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.91 (s, 3H), 4.35–4.26 (m, 1H), 3.25 (d, J = 3.9 Hz, 1H), 2.31 (s, 6H), 1.87–1.76 (m, 1H), 1.68–1.55 (m, 1H), 1.52–1.38 (m, 1H), 1.21 (s, 9H), 0.92 (d, J = 6.6 Hz, 3H), 0.86 (d, J = 6.6 Hz, 3H).

5.1.4. (1*S*)-1-(3,5-Dimethylphenyl)-3-methylbutylamine hydrochloride ((*S*)-27)

To a stirred solution of **26** (200 mg, 0.677 mmol) in MeOH (1 mL) was added 4 M HCl in dioxane (1 mL) at room temperature. After being stirred for 1 h, the reaction mixture was concentrated in vacuo. The resultant residue was washed with EtOAc/hexane to yield (*S*)-**27** (136 mg, 88%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.41 (br s, 3H), 7.11 (s, 2H), 7.00 (s, 1H), 4.17–4.04 (m, 1H), 2.27 (s, 6H), 1.82–1.64 (m, 2H), 1.38–1.22 (m, 1H), 0.86 (d, J = 6.6 Hz, 3H), 0.82 (d, J = 6.6 Hz, 3H); Optical rotation $[\alpha]_D^{23}$ +20.7 (*c* 1.94, MeOH); HPLC retention time 5.50 min, 94% ee.

5.1.5. (2*R*)-2-{[(1*R*)-1-(3,5-Dimethylphenyl)-3-methyl-3-buten-1-yl]amino}-2-phenylethanol hydrochloride (29)

A solution of **28** (49.4 g, 0.368 mol) and (*R*)-phenylglycinol (50.5 g, 0.368 mol) in toluene (350 mL) was refluxed for 15 h to obtain the imine by azeotropic distillation to remove water. To the stirred solution of the imine was added dropwise 2-allylmethylmagnesium chloride which was prepared from 3-chloro-2-methylpropene (76.7 g, 0.847 mol) and magnesium (51.5 g, 2.12 mol) in THF (1700 mL) over 3 h at 0 °C under an argon atmosphere. After being stirred for 30 min at 0 °C, the reaction was quenched with aqueous NH_4Cl and water. The organic layer was separated and then the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The resultant residue was dissolved in EtOAc (500 mL) and then 4 M HCl in dioxane (100 mL) was added. After concentration in vacuo, the resultant residue was recrystallized from *i*-PrOH/hexane to yield **29** (77.1 g, 61% in three steps) as an off-white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.52 (br s, 2H), 7.39–7.20 (m, 5H), 6.94 (s, 2H), 6.81 (s, 1H), 5.44 (br s, 1H), 4.70 (s, 1H), 4.63 (s, 1H), 4.40–4.20 (m, 2H), 4.19–4.09 (m, 1H), 3.88–3.78 (m, 1H), 3.11 (dd, J = 14, 4.4 Hz, 1H), 2.94 (dd, J = 14, 11 Hz, 1H), 2.17 (s, 6H), 1.49 (s, 3H).

5.1.6. (1*R*)-1-(3,5-Dimethylphenyl)-3-methylbutylamine hydrochloride ((*R*)-27)

A suspension of **29** (33.0 g, 95.4 mmol) and PtO_2 (4.60 g) in EtOH (330 mL) was stirred for 40 h at 60 °C under an atmosphere of hydrogen. The reaction mixture was filtered through a pad of Celite and the filtrate was concentrated in vacuo. The resultant residue was recrystallized from EtOH/EtOAc to yield (*R*)-**27** (12.8 g, 58%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.41 (br s, 3H), 7.11 (s, 2H), 7.01 (s, 1H), 4.15–4.05 (m, 1H), 2.27 (s, 6H), 1.82–1.66 (m, 2H), 1.37–1.21 (m, 1H), 0.86 (d, J = 6.6 Hz, 3H), 0.82 (d, J = 6.6 Hz, 3H); Optical rotation $[\alpha]_D^{22}$ -21.1 (*c* 2.08, MeOH); HPLC retention time 3.99 min, 99% ee.

5.1.7. Methyl 3-{2-({[(1*R*)-1-(3,5-dimethylphenyl)-3-methylbutyl]amino}carbonyl)-4-(hydroxymethyl)phenyl} propanoate (32)

A solution of **30** (4.00 g, 14.2 mmol), (*R*)-**27** (3.23 g, 14.2 mmol), *N*-methylmorpholine (1.72 mL, 15.6 mmol), EDC·HCl (2.99 g, 15.6 mmol) and HOBt (3.84 g, 28.4 mmol) in DMF (15 mL) was stirred for 12 h at room temperature under an argon atmosphere. The reaction was quenched with 1 M aqueous solution of HCl and then the resultant mixture was extracted with EtOAc/hexane = 1/1 solution. The organic layer was washed with aqueous $NaHCO_3$, water and brine, dried over Na_2SO_4, and concentrated in vacuo to yield **31**, which was used for the next step without purification. To a stirred solution of **31** in MeOH (10 mL) was added 4 M HCl in dioxane (10 mL) at room temperature and the reaction mixture was stirred for 30 min. After concentration in vacuo, the resultant residue was recrystallized from EtOAc/hexane to yield **32** (5.21 g, 89% in two steps) as an off-white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35–7.27 (m, 2H), 7.20 (d, J = 7.8 Hz, 1H), 6.97 (s, 2H), 6.90 (s, 1H), 6.59 (d, J = 8.4 Hz, 1H), 5.22–5.10 (m, 1H), 4.64 (s, 2H), 3.62 (s, 3H), 3.07–2.92 (m, 2H), 2.76–2.56 (m, 2H), 2.31 (s, 6H), 1.86–1.52 (m, 3H), 0.99 (d, J = 6.0 Hz, 3H), 0.98 (d, J = 6.0 Hz, 3H).

5.1.8. 3-[2-({[(1*R*)-1-(3,5-Dimethylphenyl)-3-methylbutyl]amino}-carbonyl)-4-(phenoxymethyl)phenyl]propanoic acid (1)

To a stirred solution of **32** (333 mg, 0.810 mmol) and TEA (169 μL, 1.22 mmol) in THF (3 mL) was added MsCl (75 μL, 0.973 mmol) at 0 °C under an argon atmosphere. After being stirred for 30 min at 0 °C, the reaction mixture was diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo to yield **33**, which was used for the next step without purification. To a stirred solution of **34a** (91 mg, 0.972 mmol) in DMF (1 mL) was added NaH (60% in oil, 34 mg, 0.891 mmol) at 0 °C under an argon atmosphere and the reaction mixture was stirred for 20 min at room temperature. To the reaction mixture was added **33** in DMF (1 mL) at 0 °C. After being stirred for 1 h at room temperature, the reaction was quenched with aqueous NH_4Cl and then the resultant mixture was extracted with TBME. The organic layer was washed with 0.5 M aqueous solution of NaOH, water

and brine, and dried over $MgSO_4$. Concentration in vacuo afforded **35a** which was used for the next step without purification. To a stirred solution of **35a** in THF (2 mL) and MeOH (2 mL) was added 2 M aqueous solution of NaOH (2 mL) at room temperature. After being stirred for 12 h at room temperature, the reaction mixture was acidified with 1 M aqueous solution of HCl and then extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The resultant residue was recrystallized from EtOAc/hexane to yield **1** (201 mg, 52% in three steps) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46–7.38 (m, 2H), 7.34–7.25 (m, 3H), 7.02–6.93 (m, 5H), 6.91 (s, 1H), 6.28 (d, J = 8.2 Hz, 1H) 5.22–5.11 (m, 1H), 5.02 (s, 2H), 3.09–2.97 (m, 2H), 2.72 (t, J = 6.6 Hz, 2H), 2.21 (s, 6H), 1.83–1.54 (m, 3H), 0.98 (d, J = 6.3 Hz, 6H); MS (FAB, Pos.) m/e 474 $(M+H)^+$; HRMS (Pos.) calcd for $C_{30}H_{36}NO_4$: 474.2644; found: 474.2645; Optical rotation $[\alpha]_D^{24}$ +18.3 (c 1.00, MeOH).

5.1.9. 3-{2-({[(1*R*)-1-(3,5-Dimethylphenyl)-3-methylbutyl]amino}carbonyl)-4-[(3-fluorophenoxy)methyl]phenyl}propanoic acid (2)

The titled compound was synthesized in the same manner as described for **1** using **34b** instead of **34a** as a white powder. Yield 80% in three steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44–7.38 (m, 2H), 7.33–7.18 (m, 2H), 6.95 (s, 2H), 6.91 (s, 1H), 6.76–6.63 (m, 3H), 6.31 (d, J = 8.2 Hz, 1H), 5.23–5.14 (m, 1H), 5.00 (s, 2H), 3.08–2.95 (m, 2H), 2.72 (t, J = 7.5 Hz, 2H), 2.31 (s, 6H), 1.85–1.55 (m, 3H), 0.98 (d, J = 6.3 Hz, 6H); MS (FAB, Pos.) m/e 492 $(M+H)^+$; HRMS (Pos.) calcd for $C_{30}H_{35}FNO_4$: 492.2550; found: 492.2553; Optical rotation $[\alpha]_D^{24}$ +17.2 (c 1.00, MeOH).

5.1.10. 3-[4-[(2-Chlorophenoxy)methyl]-2-({[(1*R*)-1-(3,5-dimethylphenyl)-3-methylbutyl]amino}carbonyl)phenyl]propanoic acid (3)

The titled compound was synthesized in the same manner as described for **1** using **34c** instead of **34a** as a white powder. Yield 74% in three steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (d, J = 1.7 Hz, 1H), 7.45–7.37 (m, 2H), 7.28 (d, J = 8.1 Hz, 1H), 7.24–7.17 (m, 1H), 6.99–6.90 (m, 5H), 6.32 (d, J = 8.7 Hz, 1H), 5.21–5.13 (m, 1H), 5.12 (s, 2H), 3.10–2.98 (m, 2H), 2.72 (t, J = 7.1 Hz, 2H), 2.31 (s, 6H), 1.85–1.57 (m, 3H), 0.99 (d, J = 6.2 Hz, 3H), 0.98 (d, J = 6.2 Hz, 3H); MS (FAB, Pos.) m/e 508 $(M+H)^+$; HRMS (Pos.) calcd for $C_{30}H_{35}ClNO_4$: 508.2255; found: 508.2255; Optical rotation $[\alpha]_D^{24}$ +20.3 (c 1.14, MeOH).

5.1.11. 3-[4-[(3-Chlorophenoxy)methyl]-2-({[(1*R*)-1-(3,5-dimethylphenyl)-3-methylbutyl]amino}carbonyl)phenyl]propanoic acid (4)

The titled compound was synthesized in the same manner as described for **1** using **34d** instead of **34a** as a white powder. Yield 55% in three steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43–7.37 (m, 2H), 7.31–7.17 (m, 2H), 6.98–6.94 (m, 2H), 6.91 (s, 1H), 6.87–6.81 (m, 1H), 6.30 (d, J = 8.9 Hz, 1H), 5.22–5.12 (m, 1H), 5.00 (s, 2H), 3.09–2.96 (m, 2H), 2.73 (t, J = 7.2 Hz, 2H), 2.31 (s, 6H), 1.85–1.55 (m, 3H), 0.99 (d, J = 6.6 Hz, 6H); MS (FAB, Pos.) m/e 508 $(M+H)^+$; HRMS (Pos.) calcd for $C_{30}H_{35}ClNO_4$: 508.2255; found: 508.2256; Optical rotation $[\alpha]_D^{24}$ +15.9 (c 1.00, MeOH).

5.1.12. 3-{2-({[(1*R*)-1-(3,5-Dimethylphenyl)-3-methylbutyl]amino}carbonyl)-4-[(2-methylphenoxy)methyl]phenyl}propanoic acid (5)

The titled compound was synthesized in the same manner as described for **1** using **34e** instead of **34a** as an off-white powder. Yield 30% in three steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44–7.38 (m, 2H), 7.27 (d, J = 8.1 Hz, 1H), 7.19–7.11 (m, 2H), 6.95 (s, 2H), 6.92–6.83 (m, 3H), 6.28 (d, J = 8.4 Hz, 1H), 5.22–5.12 (m, 1H), 5.03 (s, 2H), 3.09–2.97 (m, 2H), 2.71 (t, J = 7.2 Hz, 2H), 2.30 (s, 6H), 2.26 (s, 3H), 1.83–1.57 (m, 3H), 0.99 (d, J = 6.6 Hz, 3H), 0.98 (d, J = 6.6 Hz, 3H); MS (FAB, Pos.) m/e 488 $(M+H)^+$; HRMS (Pos.) calcd for $C_{31}H_{38}NO_4$: 488.2801; found: 488.2802; Optical rotation $[\alpha]_D^{24}$ +17.9 (c 0.90, MeOH).

5.1.13. 3-{2-({[(1*R*)-1-(3,5-Dimethylphenyl)-3-methylbutyl]amino}carbonyl)-4-[(2-methoxyphenoxy)methyl]phenyl}propanoic acid (6)

The titled compound was synthesized in the same manner as described for **1** using **34f** instead of **34a** as a white powder. Yield 47% in three steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44–7.39 (m, 2H), 7.28–7.23 (m, 1H), 7.00–6.80 (m, 7H), 6.32 (d, J = 8.7 Hz, 1H), 5.21–5.11 (m, 1H), 5.09 (s, 2H), 3.86 (s, 3H), 3.10–2.95 (m, 2H), 2.71 (t, J = 7.5 Hz, 2H), 2.30 (s, 6H), 1.80–1.55 (m, 3H), 0.97 (d, J = 6.3 Hz, 6H); MS (FAB, Pos.) m/e 504 $(M+H)^+$; HRMS (Pos.) calcd for $C_{31}H_{38}NO_5$: 504.2750; found: 504.2750; Optical rotation $[\alpha]_D^{24}$ +16.3 (c 0.99, MeOH).

5.1.14. 3-[4-[(3-Cyanophenoxy)methyl]-2-({[(1*R*)-1-(3,5-dimethylphenyl)-3-methylbutyl]amino}carbonyl)phenyl]propanoic acid (7)

The titled compound was synthesized in the same manner as described for **1** using **34g** instead of **34a** as a white powder. Yield 59% in three steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42–7.35 (m, 3H), 7.32–7.25 (m, 2H), 7.21–7.15 (m, 2H), 6.96 (s, 2H), 6.91 (s, 1H), 6.37 (d, J = 8.6 Hz, 1H), 5.22–5.12 (m, 1H), 5.03 (s, 2H), 3.07–2.99 (m, 2H), 2.76–2.68 (m, 2H), 2.31 (s, 6H), 1.85–1.55 (m, 3H), 0.99 (d, J = 6.6 Hz, 6H); MS (FAB, Pos.) m/e 499 $(M+H)^+$; HRMS (Pos.) calcd for $C_{31}H_{35}N_2O_4$: 499.2597; found: 499.2595; Optical rotation $[\alpha]_D^{24}$ +15.4 (c 1.06, MeOH).

5.1.15. 3-[4-[(2,4-Difluorophenoxy)methyl]-2-({[(1*R*)-1-(3,5-dimethylphenyl)-3-methylbutyl]amino}carbonyl)phenyl]-propanoic acid (8)

The titled compound was synthesized in the same manner as described for **1** using **34h** instead of **34a** as a white powder. Yield 28% in three steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43–7.37 (m, 2H), 7.27 (d, J = 7.8 Hz, 1H), 6.98–6.83 (m, 5H), 6.81–6.72 (m, 1H), 6.34 (d, J = 8.2 Hz, 1H), 5.21–5.12 (m, 1H), 5.04 (s, 2H), 3.08–2.95 (m, 2H), 2.74–2.67 (m, 2H), 2.31 (s, 6H), 1.84–1.55 (m, 3H), 0.99 (d, J = 6.3 Hz, 6H); MS (FAB, Pos.) m/e 510 $(M+H)^+$; HRMS (Pos.) calcd for $C_{30}H_{34}F_2NO_4$: 510.2456; found: 510.2458; Optical rotation $[\alpha]_D^{24}$ +17.4 (c 1.11, MeOH).

5.1.16. 3-[4-[(2,5-Difluorophenoxy)methyl]-2-({[(1*R*)-1-(3,5-dimethylphenyl)-3-methylbutyl]amino}carbonyl)phenyl]-propanoic acid (9)

The titled compound was synthesized in the same manner as described for **1** using **34i** instead of **34a** as a white powder. Yield 58% in three steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.38 (m, 2H), 7.29 (d, J = 7.8 Hz, 1H), 7.07–6.97 (m, 1H), 6.96 (s, 2H), 6.91 (s, 1H), 6.76–6.68 (m, 1H), 6.65–6.56 (m, 1H), 6.31 (d, J = 8.4 Hz, 1H), 5.21–5.11 (m, 1H), 5.06 (s, 2H), 3.11–2.93 (m, 2H), 2.72 (t, J = 6.0 Hz, 2H), 2.31 (s, 6H), 1.85–1.56 (m, 3H), 0.99 (d, J = 6.3 Hz, 6H); MS (FAB, Pos.) m/e 510 $(M+H)^+$; HRMS (Pos.) calcd for $C_{30}H_{34}F_2NO_4$: 510.2456; found: 510.2456; Optical rotation $[\alpha]_D^{24}$ +14.9 (c 2.04, MeOH).

5.1.17. 3-[4-[(2,4-Dimethylphenoxy)methyl]-2-({[(1*R*)-1-(3,5-dimethylphenyl)-3-methylbutyl]amino}carbonyl)phenyl]-propanoic acid (11)

The titled compound was synthesized in the same manner as described for **1** using **34j** instead of **34a** as an off-white powder. Yield 48% in three steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.37 (m, 2H), 7.30–7.25 (m, 1H), 7.00–6.87 (m, 5H), 6.75 (d, J = 8.2 Hz, 1 H) 6.24 (d, J = 8.8 Hz, 1H), 5.22–5.13 (m, 1H), 5.01 (s, 2H), 3.09–2.99 (m, 2H), 2.73 (t, J = 6.9 Hz, 2H), 2.31 (s, 6H), 2.26 (s, 3H),

2.23 (s, 3H), 1.81–1.57 (m, 3H), 0.99 (d, J = 6.3 Hz, 3H), 0.98 (d, J = 6.3 Hz, 3H); MS (FAB, Pos.) m/e 502 $(M+H)^+$; HRMS (Pos.) calcd for $C_{32}H_{40}NO_4$: 502.2957; found: 502.2951; Optical rotation $[\alpha]_D^{24}$ +16.8 (c 1.01, MeOH).

5.1.18. 3-[4-[(2,5-Dimethylphenoxy)methyl]-2-({[(1*R*)-1-(3,5-dimethylphenyl)-3-methylbutyl]amino}carbonyl)phenyl]-propanoic acid (12)

The titled compound was synthesized in the same manner as described for **1** using **34k** instead of **34a** as an off-white powder. Yield 33% in three steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.37 (m, 2H), 7.28 (d, J = 7.7 Hz, 1H), 7.04 (d, J = 7.2 Hz, 1H), 6.95 (s, 2H), 6.90 (s, 1H), 6.73–6.68 (m, 2H), 6.27 (d, J = 8.8 Hz, 1H), 5.22–5.12 (m, 1H), 5.02 (s, 2H), 3.10–2.96 (m, 2H), 2.72 (t, J = 7.2 Hz, 2H), 2.31 (s, 3H), 2.30 (s, 6H), 2.22 (s, 3H), 1.84–1.54 (m, 3H), 0.99 (d, J = 6.2 Hz, 3H), 0.98 (d, J = 6.2 Hz, 3H); MS (FAB, Pos.) m/e 502 $(M+H)^+$; HRMS (Pos.) calcd for $C_{32}H_{40}NO_4$: 502.2957; found: 502.2947; Optical rotation $[\alpha]_D^{24}$ +18.9 (c 1.02, MeOH).

5.1.19. 3-{2-({[(1*R*)-1-(3,5-Dimethylphenyl)-3-methylbutyl]-amino}carbonyl)-4-[(pyridin-3-yloxy)methyl]phenyl}propanoic acid (13)

The titled compound was synthesized in the same manner as described for **1** using **34l** instead of **34a** as a white powder. Yield 36% in three steps; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.08 (s, 1H), 8.76 (d, J = 8.4 Hz, 1H), 8.35 (s, 1H), 8.17 (d, J = 4.0 Hz, 1H), 7.46–7.40 (m, 2H), 7.37–7.28 (m, 3H), 6.95 (s, 2H), 6.84 (s, 1H), 5.17 (s, 2H), 5.02–4.92 (m, 1H), 2.84 (t, J = 7.5 Hz, 2H), 2.45 (t, J = 7.5 Hz, 2H), 2.24 (s, 6H), 1.78–1.56 (m, 2H), 1.46–1.35 (m, 1H), 0.92 (d, J = 6.6 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H); MS (FAB, Pos.) m/e 475 $(M+H)^+$; HRMS (Pos.) calcd for $C_{29}H_{35}N_2O_4$: 475.2597; found: 475.2602; Optical rotation $[\alpha]_D^{22}$ +17.4 (c 0.45, MeOH).

5.1.20. 3-(2-({[(1*R*)-1-(3,5-Dimethylphenyl)-3-methylbutyl]-amino}carbonyl)-4-{[(2-methylpyridin-3-yl)oxy]methyl}-phenyl)propanoic acid (14)

The titled compound was synthesized in the same manner as described for **1** using **34m** instead of **34a** as a white powder. Yield 29% in three steps; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.08 (s, 1H), 8.79 (d, J = 8.8 Hz, 1H), 8.02–7.98 (m, 1H), 7.45–7.28 (m, 4H), 7.17 (dd, J = 8.1, 4.8 Hz, 1H), 6.95 (s, 2H), 6.84 (s, 1H), 5.15 (s, 2H), 5.02–4.91 (m, 1H), 2.84 (t, J = 7.5 Hz, 2H), 2.44 (t, J = 7.5 Hz, 2H), 2.39 (s, 3H), 2.25 (s, 6H), 1.80–1.60 (m, 2H), 1.45–1.32 (m, 1H), 0.93 (d, J = 6.2 Hz, 3H), 0.89 (d, J = 6.2 Hz, 3H); MS (FAB, Pos.) m/e 489 $(M+H)^+$; HRMS (Pos.) calcd for $C_{30}H_{37}N_2O_4$: 489.2753; found: 489.2755; Optical rotation $[\alpha]_D^{22}$ +20.6 (c 0.48, MeOH).

5.1.21. 3-(2-({[(1*R*)-1-(3,5-Dimethylphenyl)-3-methylbutyl]-amino}carbonyl)-4-{[(6-methylpyridin-3-yl)oxy]methyl}-phenyl)propanoic acid (15)

The titled compound was synthesized in the same manner as described for **1** using **35n** instead of **35a** as a white powder. Yield 26% in three steps; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.07 (s, 1H), 8.80 (d, J = 8.1 Hz, 1H), 8.00 (d, J = 4.8 Hz, 1H), 7.45–7.33 (m, 3H), 7.31 (d, J = 8.1 Hz, 1H), 7.17 (dd, J = 8.4, 4.8 Hz, 1H), 6.95 (s, 2H), 6.84 (s, 1H), 5.15 (s, 2H), 5.02–4.91 (m, 1H), 2.84 (t, J = 7.8 Hz, 2H), 2.48–2.41 (m, 2H), 2.39 (s, 3H), 2.24 (s, 6H), 1.80–1.60 (m, 2H), 1.45–1.31 (m, 1H), 0.93 (d, J = 6.4 Hz, 3H), 0.89 (d, J = 6.4 Hz, 3H); MS (FAB, Pos.) m/e 489 $(M+H)^+$; HRMS (Pos.) calcd for $C_{30}H_{37}N_2O_4$: 489.2753; found: 489.2750; Optical rotation $[\alpha]_D^{23}$ +20.1 (c 0.56, MeOH).

5.1.22. Methyl 3-{2-({[(1*S*)-1-(3,5-dimethylphenyl)-3-methylbutyl]amino}carbonyl)-4-(hydroxymethyl)phenyl} propanoate (37)

The titled compound was synthesized in the same manner as described for **32** using (*S*)-**27** instead of (*R*)-**27** as a white powder. Yield 75% in two steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38–7.30 (m, 2H), 7.23 (d, J = 7.8 Hz, 1H), 6.97 (s, 2H), 6.90 (s, 1H), 6.46 (d, J = 9.3 Hz, 1H), 5.22–5.12 (m, 1H), 4.67 (d, J = 5.7 Hz, 2H), 3.62 (s, 3H), 3.08–2.97 (m, 2H), 2.71–2.60 (m, 2H), 2.31 (s, 6H), 1.86–1.59 (m, 3H), 0.99 (d, J = 6.3 Hz, 3H), 0.98 (d, J = 6.6 Hz, 3H).

5.1.23. 3-[4-[(2,5-Difluorophenoxy)methyl]-2-({[(1*S*)-1-(3,5-dimethylphenyl)-3-methylbutyl]amino}carbonyl)phenyl]-propanoic acid (10)

The titled compound was synthesized in the same manner as described for **1** using **37** and **34i** instead of **32** and **34a**, respectively, as a white powder. Yield 89% in three steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.38 (m, 2H), 7.29 (d, J = 7.8 Hz, 1H), 7.09–6.98 (m, 1H), 6.96 (s, 2H), 6.91 (s, 1H), 6.78–6.69 (m, 1H), 6.66–6.56 (m, 1H), 6.31 (d, J = 8.4 Hz, 1H), 5.22–5.12 (m, 1H), 5.06 (s, 2H), 3.11–2.93 (m, 2H), 2.72 (t, J = 6.0 Hz, 2H), 2.31 (s, 6H), 1.84–1.52 (m, 3H), 0.99 (d, J = 6.3 Hz, 6H); MS (FAB, Pos.) m/e 510 $(M+H)^+$; HRMS (Pos.) calcd for $C_{30}H_{34}F_2NO_4$: 510.2456; found: 510.2457; Optical rotation $[\alpha]_D^{24}$ −15.8 (c 1.02, MeOH).

5.1.24. 7-(Methoxymethoxy)-2*H*-chromen-2-one (41)

To a stirred solution of **40** (100 g, 0.617 mol) and DIPEA (161 mL, 0.925 mol) in DMF (500 mL) was added dropwise methoxymethyl chloride (70.3 mL, 0.925 mol) at 0 °C under an argon atmosphere. After being stirred for 4 h at room temperature, the reaction mixture was poured into cold aqueous $NaHCO_3$ and EtOAc/hexane = 1/1 solution. The organic layer was separated and then the aqueous layer was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo to yield **41** as a pale yellow solid, which was used for the next step without purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64 (d, J = 9.6 Hz, 1H), 7.39 (d, J = 8.7 Hz, 1H), 7.01 (d, J = 2.4 Hz, 1H), 6.96 (dd, J = 8.7, 2.4 Hz, 1H), 6.28 (d, J = 9.6 Hz, 1H), 5.24 (s, 2H), 3.49 (s, 3H).

5.1.25. Methyl (2*E*)-3-[2-hydroxy-4-(methoxymethoxy)phenyl]-acrylate (42)

To a stirred suspension of NaH (63% in oil, 46.9 g, 1.23 mol) in THF (300 mL) was added dropwise MeOH (60.0 mL, 1.46 mol) at 0 °C under an argon atmosphere and the reaction mixture was stirred for 20 min at room temperature. To the resultant suspension was added dropwise **41** in THF (1000 mL) and MeOH (100 mL) at 0 °C. After being stirred for 40 min at 60 °C, the reaction was quenched with aqueous NH_4Cl and successively 2 M aqueous solution of HCl at 0 °C, and then the resultant mixture was extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The resultant residue was washed with EtOAc (500 mL) and hexane (1000 mL) to yield **42** (100 g, 68% in two steps) as a yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (d, J = 16 Hz, 1H), 7.39 (d, J = 8.5 Hz, 1H), 6.62 (dd, J = 8.5, 2.2 Hz, 1H), 6.54 (d, J = 2.2 Hz, 1H), 6.51 (d, J = 16 Hz, 1H), 6.01 (s, 1H), 5.17 (s, 2H), 3.81 (s, 3H), 3.47 (s, 3H).

5.1.26. Methyl 3-[2-hydroxy-4-(methoxymethoxy)phenyl]-propanoate (43)

A suspension of **42** (90.0 g, 0.378 mol) and Pd–C (10% wet type, 8.40 g) in MeOH (1000 mL) was stirred for 7 h at room temperature under an atmosphere of hydrogen. The reaction mixture was filtered through a pad of Celite and the filtrate was concentrated in vacuo to yield **43** (92.1 g, quant.) as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.24 (s, 1H), 6.97 (d, J = 8.2 Hz, 1H), 6.61 (d,

J = 2.5 Hz, 1H), 6.57 (dd, J = 8.2, 2.5 Hz, 1H), 5.13 (s, 2H), 3.69 (s, 3H), 3.46 (s, 3H), 2.84 (t, J = 6.1 Hz, 2H), 2.69 (t, J = 6.1 Hz, 2H).

5.1.27. Methyl 3-(4-(methoxymethoxy)-2-{[(trifluoromethyl)sulfonyl]oxy}phenyl)propanoate (44)

To a stirred solution of **43** (82.8 g, 0.345 mol) and pyridine (33.5 mL, 0.414 mol) in CH_2Cl_2 (300 mL) was added dropwise Tf_2O (63.8 mL, 0.379 mol) at 0 °C under an argon atmosphere. After being stirred for 10 min at 0 °C, the reaction was quenched with water and then the resultant mixture was extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo to yield **44**, which was used for the next step without purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.24 (d, J = 8.4 Hz, 1H), 7.04–6.96 (m, 2H), 5.16 (s, 2H), 3.68 (s, 3H), 3.47 (s, 3H), 2.98 (t, J = 7.5 Hz, 2H), 2.63 (t, J = 7.5 Hz, 2H).

5.1.28. 5-(Methoxymethoxy)-2-(3-methoxy-3-oxopropyl)-benzoic acid (45)

A suspension of **44**, potassium acetate (169 g, 1.73 mol), DPPF (7.65 g, 13.9 mmol) and $Pd(OAc)_2$ (1.55 g, 6.90 mmol) in DMF (400 mL) was stirred for 24 h at 90 °C under an atmosphere of carbon monoxide. The reaction mixture was filtered through a pad of Celite. Water was added to the filtrate and then the resultant solution was extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The resultant residue was passed through the column chromatography on silica gel (EtOAc/hexane, 1/1) and then above obtained residue was recrystallized from EtOAc/hexane to yield **45** (51.4 g, 56% in two steps) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (d, J = 2.7 Hz, 1H), 7.24 (d, J = 8.7 Hz, 1H), 7.17 (dd, J = 8.7, 2.7 Hz, 1H), 5.20 (s, 2H), 3.67 (s, 3H), 3.49 (s, 3H), 3.27 (t, J = 7.6 Hz, 2H), 2.68 (t, J = 7.6 Hz, 2H).

5.1.29. Methyl 3-[4-hydroxy-2-({[(1*R*)-3-methyl-1-phenylbutyl]-amino}carbonyl)phenyl]propanoate (47)

The titled compound was synthesized in the same manner as described for **32** using **45** instead of **30** as a white powder. Yield 95% in two steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.01 (d, J = 8.2 Hz, 1H), 6.96 (s, 2H), 6.88 (s, 1H), 6.85 (d, J = 9.6 Hz, 1H), 6.81 (d, J = 2.7 Hz, 1H), 6.74 (dd, J = 8.2, 2.7 Hz, 1H), 6.68 (br s, 1H), 5.18–5.08 (m, 1H), 3.62 (s, 3H), 2.97–2.84 (m, 2H), 2.72–2.54 (m, 2H), 2.29 (s, 6H), 1.84–1.52 (m, 3H), 0.98 (d, J = 6.3 Hz, 3H), 0.97 (d, J = 6.3 Hz, 3H).

5.1.30. 3-[2-({[(1*R*)-1-(3,5-Dimethylphenyl)-3-methylbutyl]-amino}carbonyl)-4-phenoxyphenyl]propanoic acid (16)

A suspension of **47** (367 mg, 0.922 mmol), **48a** (338 mg, 2.77 mmol), TEA (643 μL, 4.61 mmol) $Cu(OAc)_2$ (168 mg, 0.922 mmol) and molecular sieves (60 mg) in CH_2Cl_2 (2 mL) was stirred for 12 h at room temperature. The reaction mixture was diluted with EtOAc and then filtered through a pad of Celite. The filtrate was separated and the organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The resultant residue was purified by column chromatography on silica gel (EtOAc/hexane, 1/3) to yield **49a** which was dissolved in THF (2 mL) and MeOH (2 mL) and then 2 M aqueous solution of NaOH (2 mL) was added at room temperature. After being stirred for 12 h at room temperature, the reaction mixture was acidified with 1 M aqueous solution of HCl and then extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The resultant residue was recrystallized with EtOAc/hexane to yield **16** (293 mg, 71% in two steps) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40–7.30 (m, 2H), 7.17 (d, J = 8.1 Hz, 1H), 7.16–7.09 (m, 1H), 7.04–6.82 (m, 7H), 6.19 (d, J = 8.4 Hz, 1H), 5.18–5.08 (m, 1H), 3.08–2.92 (m, 2H), 2.71 (t, J = 6.9 Hz, 2H), 2.29 (s, 6H), 1.80–1.50 (m, 3H), 0.97 (d, J = 6.3 Hz, 3H), 0.96 (d, J = 6.3 Hz, 3H); MS (FAB, Pos.) m/e 460 $(M+H)^+$; HRMS (Pos.) calcd for $C_{29}H_{34}NO_4$: 460.2488; found: 460.2497; Optical rotation $[\alpha]_D^{24}$ +19.6 (c 0.94, MeOH).

5.1.31. 3-[4-(3,5-Difluorophenoxy)-2-({[(1*R*)-1-(3,5-dimethylphenyl)-3-methylbutyl]amino}carbonyl)phenyl]propanoic acid (17)

The titled compound was synthesized in the same manner as described for **16** using **48b** instead of **48a** as a beige powder. Yield 59% in two steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30–7.25 (m, 1H), 7.06–6.98 (m, 2H), 6.94 (s, 2H), 6.90 (s, 1H), 6.59–6.43 (m, 3H), 6.28 (d, J = 9.0 Hz, 1H), 5.20–5.09 (m, 1H), 3.07–2.96 (m, 2H), 2.72 (t, J = 7.5 Hz, 2H), 2.30 (s, 6H), 1.83–1.52 (m, 3H), 0.97 (d, J = 6.4 Hz, 6H); MS (FAB, Pos.) m/e 496 $(M+H)^+$; HRMS (Pos.) calcd for $C_{29}H_{32}F_2NO_4$: 496.2299; found: 496.2301; Optical rotation $[\alpha]_D^{24}$ +16.7 (c 1.02, MeOH).

5.1.32. 3-[4-(3,5-Dimethylphenoxy)-2-({[(1*R*)-1-(3,5-dimethylphenyl)-3-methylbutyl]amino}carbonyl)phenyl]propanoic acid (18)

The titled compound was synthesized in the same manner as described for **16** using **48c** instead of **48a** as a white powder. Yield 89% in two steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20 (d, J = 8.4 Hz, 1H), 7.00 (d, J = 2.6 Hz, 1H), 6.97–6.88 (m, 4H), 6.77 (s, 1H), 6.61 (s, 2H), 6.19 (d, J = 8.6 Hz, 1H), 5.19–5.08 (m, 1H), 3.04–2.96 (m, 2H), 2.72 (t, J = 7.2 Hz, 2H), 2.29 (s, 6H), 2.28 (s, 6H), 1.82–1.52 (m, 3H), 0.96 (d, J = 6.3 Hz, 3H), 0.95 (d, J = 6.3 Hz, 3H); MS (FAB, Pos.) m/e 488 $(M+H)^+$; HRMS (Pos.) calcd for $C_{31}H_{38}NO_4$: 488.2801; found: 488.2801; Optical rotation $[\alpha]_D^{22}$ +16.3 (c 0.49, MeOH).

5.1.33. 3-[4-(3-Cyanophenoxy)-2-({[(1*R*)-1-(3,5-dimethylphenyl)-3-methylbutyl]amino}carbonyl)phenyl]propanoic acid (19)

The titled compound was synthesized in the same manner as described for **16** using **48d** instead of **48a** as a white powder. Yield 37% in two steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47–7.36 (m, 2H), 7.30–7.25 (m, 1H), 7.24–7.16 (m, 2H), 7.04–6.95 (m, 2H), 6.94 (s, 2H), 6.90 (s, 1H), 6.32 (d, J = 8.8 Hz, 1H), 5.19–5.09 (m, 1H), 3.07–2.96 (m, 2H), 2.73 (t, J = 7.5 Hz, 2H), 2.29 (s, 6H), 1.85–1.51 (m, 3H), 0.97 (d, J = 6.6 Hz, 6H); MS (FAB, Pos.) m/e 485 $(M+H)^+$; HRMS (Pos.) calcd for $C_{30}H_{33}N_2O_4$: 485.2440; found: 485.2440; Optical rotation $[\alpha]_D^{24}$ +10.8 (c 0.57, MeOH).

5.1.34. Benzyl 2-bromo-5-nitrobenzoate (51)

To a stirred suspension of 2-bromo-5-benzoic acid (**50**) (25.0 g, 0.102 mol), K_2CO_3 (21.2 g, 0.153 mol) in DMF (150 mL) was added benzyl bromide (12.7 mL, 0.107 mol) at 0 °C under an argon atmosphere. After being stirred for 30 min at 60 °C, the reaction mixture was poured into water and then extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The resultant residue was washed with EtOAc/hexane to yield **51** (30.9 g, 90%) as a yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.64 (d, J = 2.7 Hz, 1H), 8.16 (dd, J = 8.7, 2.7 Hz, 1H), 7.86 (d, J = 8.7 Hz, 1H), 7.49–7.39 (m, 5H), 5.42 (s, 2H).

5.1.35. Benzyl 2-[(1*E*)-3-ethoxy-3-oxoprop-1-en-1-yl]-5-nitrobenzoate (52)

A solution of **51** (30.9 g, 91.8 mmol), ethyl acrylate (19.9 mL, 0.184 mol), $Pd(OAc)_2$ (2.06 g, 9.18 mmol), DPPF (5.09 g, 9.18 mmol) and TEA (64.0 mL, 0.459 mol) in DMSO (185 mL) was stirred for 1.5 h at 80 °C under an argon atmosphere. The reaction mixture was diluted with EtOAc/hexane = 1/1 and 1 M aqueous solution of HCl, and then filtered through a pad of Celite. The filtrate was separated and the organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The resultant residue was purified by column chromatography on silica gel (EtOAc/hex-

ane, 1/7 to 1/5) to yield **52** (11.8 g, 36%) as a brown solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.82 (d, J = 2.1 Hz, 1H), 8.45 (d, J = 16 Hz, 1H), 8.36 (dd, J = 8.7, 2.1 Hz, 1H), 7.74 (d, J = 8.7 Hz, 1H), 7.49–7.39 (m, 5H), 6.38 (d, J = 16 Hz, 1H), 5.42 (s, 2H), 4.29 (q, J = 7.2 Hz, 2H), 1.35 (t, J = 7.2 Hz, 3H).

5.1.36. 5-Amino-2-(3-ethoxy-3-oxopropyl)benzoic acid (53)

A suspension of **52** (11.8 g, 33.2 mmol) and Pd–C (10% wet type, 2.36 g) in EtOH (300 mL) was stirred for 5 h at room temperature under an atmosphere of hydrogen. The reaction mixture was filtered through a pad of Celite and the filtrate was concentrated in vacuo to yield **53** (7.72 g, 98%) as a pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (d, J = 2.7 Hz, 1H), 7.10 (d, J = 8.1 Hz, 1H), 6.81 (dd, J = 8.1, 2.7 Hz, 1H), 4.11 (q, J = 7.2 Hz, 2H), 3.20 (t, J = 7.5 Hz, 2H), 2.64 (t, J = 7.5 Hz, 2H), 1.23 (t, J = 7.2 Hz, 3H).

5.1.37. Ethyl 3-[4-amino-2-({[(1*R*)-3-methyl-1-phenylbutyl]amino}carbonyl)phenyl]propanoate (54)

A solution of **53** (600 mg, 2.52 mmol), (*R*)-**27** (633 mg, 2.78 mmol), *N*-methylmorpholine (305 μL, 2.78 mmol), EDC·HCl (531 mg, 2.77 mmol), and HOBt (681 mg, 5.04 mmol) in DMF (7 mL) was stirred for 12 h at room temperature under argon atmosphere. The reaction mixture was diluted with water and then extracted with TBME. The organic layer was washed with aqueous $NaHCO_3$, water and brine, dried over $MgSO_4$, and concentrated in vacuo. The resultant residue was purified by column chromatography on silica gel (EtOAc/hexane, 1/1) to yield **54** (659 mg, 64%) as a pale brown powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.00 (d, J = 8.7 Hz, 1H), 6.96 (s, 2H), 6.89 (s, 1H), 6.69–6.61 (m, 2H), 6.48 (d, J = 9.0 Hz, 1H), 5.20–5.07 (m, 1H), 4.07 (q, J = 7.2 Hz, 2H), 3.63 (s, 2H), 2.90 (t, J = 7.4 Hz, 2H), 2.69–2.50 (m, 2H), 2.31 (s, 6H), 1.86–1.53 (m, 3H), 1.20 (t, J = 7.2 Hz, 3H), 0.99 (d, J = 6.0 Hz, 3H), 0.98 (d, J = 6.0 Hz, 3H).

5.1.38. 3-[4-[(3,5-Difluorophenyl)amino]-2-({[(1*R*)-1-(3,5-dimethylphenyl)-3-methylbutyl]amino}carbonyl)phenyl]-propanoic acid (20)

A suspension of **54** (80 mg, 0.195 mmol), **48b** (61 mg, 0.389 mmol), TEA (54 μL, 0.389 mmol), $Cu(OAc)_2$ (7.1 mg, 38.9 μmol), and molecular sieves (20 mg) in CH_2Cl_2 (2 mL) was stirred for 24 h at room temperature. The reaction mixture was diluted with EtOAc and then filtered through a pad of Celite. The filtrate was separated and the organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The resultant residue was purified by column chromatography on silica gel (EtOAc/hexane, 1/4 to 1/3) to yield **55b** which was dissolved in THF (1 mL) and MeOH (1 mL) and then 2 M aqueous solution of NaOH (1 mL) was added at room temperature. After being stirred for 12 h at room temperature, the reaction mixture was neutralized with 1 M aqueous solution of HCl and then extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The resultant residue was recrystallized from EtOAc/hexane to yield **20** (60 mg, 62% in two steps) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.05 (s, 1H), 8.79 (d, J = 8.1 Hz, 1H), 8.72 (s, 1H), 7.20 (d, J = 8.4 Hz, 1H), 7.09 (dd, J = 8.4, 2.3 Hz, 1H), 7.02 (d, J = 2.3 Hz, 1H), 6.94 (s, 2H), 6.83 (s, 1H), 6.72–6.63 (m, 2H), 6.57–6.48 (m, 1H), 5.01–4.89 (m, 1H), 2.78 (t, J = 7.9 Hz, 2H), 2.47–2.40 (m, 2H), 2.23 (s, 6H), 1.78–1.61 (m, 2H), 1.44–1.32 (m, 1H), 0.92 (d, J = 6.4 Hz, 3H), 0.90 (d, J = 6.4 Hz, 3H); MS (FAB, Pos.) m/e 495 $(M+H)^+$; HRMS (Pos.) calcd for $C_{29}H_{33}F_2N_2O_3$: 495.2459; found: 495.2455; Optical rotation $[\alpha]_D^{22}$ −14.8 (c 1.22, DMF).

5.1.39. 3-(4-[(3,5-Dimethylphenyl)amino]-2-{[(1*R*)-1-(3,5-dimethylphenyl)-3-methylbutyl]carbamoyl}phenyl)propanoic acid (21)

The titled compound was synthesized in the same manner as described for **20** using **48c** instead of **48b** as an off-white powder. Yield 78% in two steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.12 (d, J = 8.4 Hz, 1H), 7.07–6.98 (m, 2H), 6.93 (s, 2H), 6.89 (s, 1H), 6.67 (s, 2H), 6.61 (s, 1H), 6.25 (d, J = 8.6 Hz, 1H), 5.27–5.04 (m, 1H), 3.08–2.84 (m, 2H), 2.69 (t, J = 7.6 Hz, 2H), 2.29 (s, 6H), 2.26 (s, 6H), 2.00–1.37 (m, 3H), 0.97 (d, J = 6.2 Hz, 3H), 0.96 (d, J = 6.2 Hz, 3H); MS (FAB, Pos.) m/e 487 $(M+H)^+$; HRMS (Pos.) calcd for $C_{31}H_{39}N_2O_3$: 487.2961; found: 487.2958; Optical rotation $[\alpha]_D^{20}$ −8.91 (c 0.66, $CHCl_3$).

5.1.40. 3-[4-[(3-Cyanophenyl)amino]-2-({[(1*R*)-1-(3,5-dimethylphenyl)-3-methylbutyl]amino}carbonyl)phenyl]propanoic acid (22)

The titled compound was synthesized in the same manner as described for **20** using **48d** instead of **48b** as a pale yellow powder. Yield 57% in two steps; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.77 (d, J = 8.8 Hz, 1H), 8.62 (s, 1H), 7.43–7.32 (m, 3H), 7.23–7.17 (m, 2H), 7.08 (dd, J = 8.3, 5.3 Hz, 1H), 7.00 (d, J = 2.4 Hz, 1H), 6.93 (s, 2H), 6.83 (s, 1H), 5.00–4.90 (m, 1H), 2.78 (t, J = 7.9 Hz, 2H), 2.42 (t, J = 7.9 Hz, 2H), 2.23 (s, 6H), 1.78–1.59 (m, 2H), 1.44–1.32 (m, 1H), 0.92 (d, J = 6.3 Hz, 3H), 0.90 (d, J = 6.3 Hz, 3H); MS (FAB, Pos.) m/e 484 $(M+H)^+$; HRMS (Pos.) calcd for $C_{30}H_{34}N_3O_3$: 484.2600; found: 484.2600; Optical rotation $[\alpha]_D^{23}$ −33.2 (c 0.80, CH_3CN).

5.1.41. 3-{2-({[(1*R*)-1-(3,5-Dimethylphenyl)-3-methylbutyl]-amino}carbonyl)-4-[(3-methylphenyl)amino]phenyl}propanoic acid (23)

The titled compound was synthesized in the same manner as described for **20** using **48e** instead of **48b** as a white powder. Yield 71% in two steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19–7.10 (m, 2H), 7.06–6.99 (m, 2H), 6.93 (s, 2H), 6.89 (s, 1H), 6.88–6.82 (m, 2H), 6.78 (d, J = 7.5 Hz, 1H), 6.24 (d, J = 8.4 Hz, 1H), 5.18–5.09 (m, 1H), 2.99–2.91 (m, 2H), 2.70 (t J = 7.7 Hz, 2H), 2.30 (s, 3H), 2.29 (s, 6H), 1.81–1.53 (m, 3H), 0.97 (d, J = 6.4 Hz, 3H), 0.96 (d, J = 6.4 Hz, 3H); MS (FAB, Pos.) m/e 473 $(M+H)^+$; HRMS (Pos.) calcd for $C_{30}H_{37}N_2O_3$: 473.2804; found: 473.2804; Optical rotation $[\alpha]_D^{22}$ −8.08 (c 0.71, $CHCl_3$).

5.2. Pharmacology

5.2.1. mEP1-4 receptor binding assay

Competitive binding studies were conducted using radiolabeled ligands and membrane fractions prepared from Chinese hamster ovary (CHO) cells stably expressing the prostanoid receptors mEP1-4. Membranes from CHO cells expressing prostanoid receptors were incubated with radiolabeled ligand (2.5 nM [^{3}H]PGE_2) and test compounds at various concentrations in assay buffer (10 mM KH_2PO_4–KOH buffer containing 1 mM EDTA, 10 mM $MgCl_2$ and 0.1 mM NaCl, pH 6.0). Incubation was carried out at 25 °C for 60 min except for mEP1 which was incubated for 20 min. Incubation was terminated by filtration through a Whatman GF/B filter. The filter was subsequently washed with ice-cold buffer (10 mM KH_2PO_4–KOH buffer containing 0.1 mM NaCl, pH 6.0), and the radioactivity on the filter was measured in 6 mL of liquid scintillation (ACSII) mixture with a liquid scintillation counter. Nonspecific binding was achieved by adding excess amounts of unlabeled PGE_2 in assay buffer. The half maximal inhibitory concentration of specific binding (IC_{50} value) was estimated from the regression curve. The K_i value (M) was calculated according to the following equation:

$K_i = IC_{50}/(1 + [L]/K_d)$

where $[L]$ is the concentration of radiolabeled ligand, and K_d is the dissociation constant of radiolabeled ligand for the prostanoid receptor of interest.

5.2.2. mEP3 receptor antagonist activity

To confirm that test compounds antagonized the mEP3 receptor and to estimate the extent of antagonism for the mEP3 receptor, a functional assay was performed by measuring PGE_2-stimulated increases in intracellular Ca^{2+}. The cells expressing mEP3α receptor were seeded at 1×10^4 cells/well in 96 well plates and cultured for two days with 10% FBS (fetal bovine serum)/minimum essential medium Eagle alpha modification (αMEM) in an incubator (37 °C, 5% CO_2). The cells in each well were rinsed with phosphate buffer (PBS(−)), and load buffer (10% FBS/αMEM containing 5 μM of Fura 2/AM, 20 μM of indomethacin, and 2.5 mM of probenecid) was added. After incubation for 1 h, the cells in each well were rinsed with assay buffer (Hank's balanced salt solution (HBSS) containing 1% (w/v) BSA, 2 μM of indomethacin, 2.5 mM of probenecid, and 10 mM of HEPES-NaOH) twice. Then 90 μL of assay buffer was added to each well and the cells were incubated in the dark at room temperature for 1 h. After the addition of a solution containing test compound (30 μL) and PGE_2 (30 μL), which were prepared with assay buffer, the intracellular calcium concentration was measured with a fluorescence drug screening system (FDSS-3000, Hamamatsu Photonics). The fluorescence intensities emitted at 500 nm using excitation wavelengths of 340 nm and 380 nm were measured. The percent inhibition from the increase in the intracellular Ca^{2+} concentration induced by PGE_2 (10 nM) was calculated relative to the maximum Ca^{2+} concentration that occurred in the absence of the test compound (100%). This was then used to estimate the IC_{50} value.

5.2.3. Inhibitory effect of test compounds on the PGE_2-induced uterine contraction in pregnant rats

Fed pregnant rats were anesthetized with pentobarbital (20 mg/20 mL/kg ip) and urethane (1.5 g/5 mL/kg sc) then fixed in the dorsal position. A midline incision was made in the lower abdomen and a small incision was made near the cervical area of the right or left uterine horn, and a balloon catheter (Okamoto Medical Industry, for rats) was inserted between the uterine wall and the amnion. After suturing the abdominal incision, the intraballoon pressure was loaded to approximately 10 mmHg. Uterine motility was recorded on a recticoder (WR3320 or WR3701, GRAPHTEC) via a pressure transducer (Life Kit DX-360, Nihon Kohden Corp.) and a strain pressure amplifier (AP-601G, Nihon Kohden Corp.). After spontaneous uterine motility was kept at a stable level, an increased uterine contraction was elicited by an intravenous administration of PGE_2 (30 μg/kg). Test compound (5 mL/kg in 0.5% methylcellulose (MC)) or vehicle (0.5% MC) was orally administrated 4 h prior to PGE_2 administration. Subtraction of the area under the uterus pressure-time curve (AUC) for 10 min before PGE_2 administration from the AUC for 10 min after PGE_2 administration was determined to be the increased uterine contraction (=ΔS). The inhibitory effect of a test compound (% inhibition) was calculated according to the following equation:

$$\text{Inhibitory effect (\% inhibition)} = \{[\Delta S(\text{vehicle}) - \Delta S(\text{test compound})]/\Delta S(\text{vehicle})\} * 100$$

where ΔS (test compound) is the increased uterine contraction of administration of a test compound, and ΔS (vehicle) is the increased uterine contraction of administration of vehicle.

5.2.4. The stability in human and rat microsomes

The test compound (5 μL, 10 mM in DMSO) was diluted with 195 μL of 50% acetonitrile in water to make a 250 μM solution of the test compound. Phosphate buffer (0.1 M, 245 μL) containing 0.5 mg/mL rat or human liver microsomes and NADPH-Co-factor was added to a reaction container, pre-warmed to 37 °C in a water bath, and incubated for 5 min. The reaction was initiated by the addition of 5 μL of the solution of the test compound (in 0.975% acetonitrile with 0.05% DMSO, final concentration of 5 μM). After the initiation of the reaction, a 20 μL aliquot was taken from the solution immediately and transferred to 180 μL of acetonitrile containing the internal standard (warfarin) to terminate the reaction. A 20 μL aliquot of the mixture was stirred with 180 μL of 50% acetonitrile on a plate with a filter for deproteinization and filtered by suction. The filtrate was used as the standard sample. After incubation for 15 min, a 20 μL aliquot was taken from the solution and then treated with the same procedure described above to obtain the reaction sample. The obtained samples were measured by an LC-MS/MS system. The percent remaining (%) was calculated by dividing the peak area ratio (i.e., test compound/I.S.) for the reaction sample by the peak area ratio for the standard sample and multiplying by 100.

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References and notes

1. Narumiya, S.; Sugimoto, Y.; Ushikubi, F. *Physiol. Rev.* **1999**, *79*, 1193.
2. Minami, T.; Nakano, H.; Kobayashi, T.; Sugimoto, Y.; Ushikubi, F.; Ichikawa, A.; Narumiya, S.; Ito, S. *Br. J. Pharmacol.* **2001**, *133*, 438.
3. Ushikubi, F.; Segi, E.; Sugimoto, Y.; Murata, T.; Matsuoka, T.; Kobayashi, T.; Hizaki, H.; Tuboi, K.; Katsuyama, M.; Ichikawa, A.; Tanaka, T.; Yoshida, N.; Narumiya, S. *Nature* **1998**, *395*, 281.
4. (a) Senior, J.; Marshall, K.; Sangha, R.; Baxter, G. S.; Clayton, J. K. *Br. J. Pharmacol.* **1991**, *102*, 747; (b) Goureau, O.; Tanfin, Z.; Marc, S.; Harbon, S. *Am. J. Physiol.* **1992**, *263*, C257.
5. Chen, M. C. Y.; Amirian, D. A.; Toomey, M.; Sanders, M. J.; Soll, A. H. *Gastroenterology* **1988**, *94*, 1121.
6. Ma, H.; Hara, A.; Xiao, C. Y.; Okada, Y.; Takahata, O.; Nakaya, K.; Sugimoto, Y.; Ichikawa, A.; Narumiya, S.; Ushikubi, F. *Circulation* **2001**, *104*, 1176.
7. Gross, S.; Tilly, P.; Hentsch, D.; Vonesch, J. L.; Fabre, J. E. *J. Exp. Med.* **2007**, *204*, 311.
8. Asada, M.; Obitsu, T.; Nagase, T.; Tanaka, M.; Yamaura, Y.; Takizawa, H.; Yoshikawa, K.; Sato, K.; Narita, M.; Ohuchida, S.; Nakai, H.; Toda, M. *Bioorg. Med. Chem.* **2010**, *18*, 80.
9. Asada, M.; Iwahashi, M.; Obitsu, T.; Kinoshita, A.; Nakai, Y.; Onoda, T.; Nagase, T.; Tanaka, M.; Yamaura, Y.; Takizawa, H.; Yoshikawa, K.; Sato, K.; Narita, M.; Ohuchida, S.; Nakai, H.; Toda, M. *Bioorg. Med. Chem.* **2010**, *18*, 1641.
10. Corgan, D. E.; Liu, G.; Ellman, J. *Tetrahedron* **1999**, *55*, 8883.
11. Takahashi, H.; Chida, Y.; Yoshii, T.; Suzuki, T.; Yanaura, S. *Chem. Pharm. Bull.* **1986**, *34*, 2071.
12. CCDC 768381 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by e-mailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.
13. Ganguly, N.; Sukai, A. K.; De, S. *Synth. Commun.* **2001**, *31*, 301.
14. Evans, D. A.; Katz, J. L.; West, T. R. *Tetrahedron Lett.* **1998**, *39*, 2937.
15. Lam, P. Y. S.; Vincent, G.; Clark, C. G.; Deudon, S.; Jadhav, P. K. *Tetrahedron Lett.* **2001**, *42*, 3415.