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3-(2-Aminocarbonylphenyl)propanoic acid analogs as potent and selective EP3 receptor antagonists. Part 1: Discovery and exploration of the carboxyamide side chain

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ABSTRACT

A series of 3-(2-aminocarbonyl-4-phenoxymethylphenyl)propanoic acid analogs were synthesized and evaluated for their EP3 antagonist activity in the presence of additive serum albumin. Several compounds were biologically evaluated for their in vivo efficacy with respect to the PGE_2-induced uterine contraction in pregnant rats as well as their pharmacokinetics. The discovery process of these potent and selective EP3 antagonists and their structure activity relationship are also presented.

1. Introduction

Prostanoids, which include prostaglandins and thromboxanes play important roles in a multitude of significant physiological processes. They are believed to mediate the prostanoid receptors, which are known to be a member of the G-protein coupled receptor superfamily. Prostanoid receptors consist of EP, FP, IP, TP and DP receptors.[1] Recently, the CRTH2 receptor has been identified as another prostanoid receptor due to its affinity with PGD_2.[2] The EP receptor which responds to PGE_2 is divided into four subtypes EP1, EP2, EP3 and EP4. The EP3 receptor, whose cDNA was already cloned, is distributed in the brain, kidney, uterus and other organs. Recent studies suggest that PGE_2 acts through the EP3 receptor to function in areas of hyperalgesia,[3] pyrexia,[4] uterine contraction,[5] gastric acid secretion,[6] platelet aggregation[7] and thrombosis.[8] Therefore, a subtype selective EP3 antagonist would be a useful drug for the treatment of pain, pyrexia, threatened abortion and peripheral occupied arterial disease (PAOD). Recently, several selective EP3 antagonists have been disclosed by Merck,[9] de CODE[10] and GlaxoSmithKline.[11]

In our previous paper,[12] compound **1** was identified as a new chemical lead for further optimization. In the process of our further investigations, carboxyamide analogs in which the naphthalen-2-ylethoxy moiety of **1** was replaced by an aminocarbonyl moiety, were found to show a smaller shift of the antagonist activity relative to **1** when the concentration of bovine serum albumin (BSA) in the assay system was increased. This structure–activity relationship (SAR) led us to synthesize a series of carboxyamide analogs which proved to be subtype selective antagonists with excellent in vivo efficacy. We herein report the discovery of a series of 3-(2-aminocarbonyl-4-phenoxymethylphenyl)propanoic acid analogs possessing an *N*-(α-substituted arylmethyl)carboxyamide side chain as potent and selective EP3 antagonists (Scheme 1). Their in vitro antagonist activity in the presence of 1% additive BSA and in vivo antagonist activity with respect to the PGE_2-induced uterine contraction in pregnant rats are discussed.

2. Chemistry

Synthesis of test compounds listed in Tables 1–3 is outlined in Schemes 2–4.

Compound **2** was synthesized as shown in Scheme 2. Addition of *t*-butoxide to an acyl chloride, which was prepared from 2-iodo-5-methylbenzoic acid (**17**), afforded *t*-butyl ester **18**. Bromination of **18** followed by a substitution reaction with pyrazole provided **19**. Heck reaction of the iodide **19** with ethyl acrylate afforded **20**. Acidic deprotection of **20** followed by catalytic hydrogenation provided carboxylic acid **22**. Amidation of an acyl chloride which was prepared from **22**, with 1-naphthalenemethylamine afforded **23**. Alkaline hydrolysis of **23** resulted in **2**.

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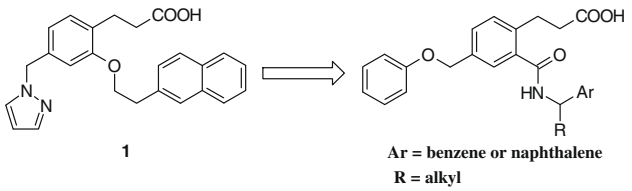


Scheme 1. New scaffold possessing *N*-(α-substituted arylmethyl)carboxyamide side chain as potent and selective EP3 antagonists.

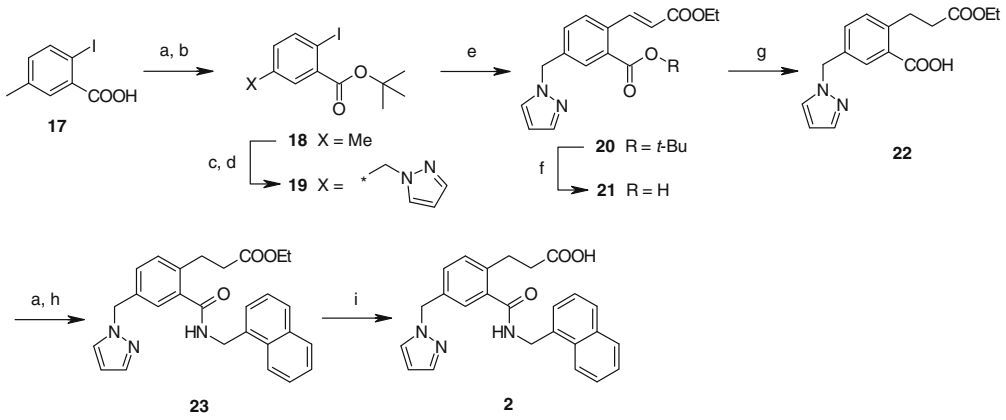
Synthesis of **3**–**16** is shown in Scheme 3. The common intermediate **27** was prepared from **18** as described in Scheme 3a. Bromination of **18** followed by a substitution reaction with a phenoxide afforded **24**, which was then subjected to a Heck reaction with ethyl acrylate to afford **25**. Sodium borohydride reduction of **25** in the presence of nickel chloride afforded the saturated ester **26**, acidic deprotection of which gave **27**. *N*-(Naphthylmethyl)carboxyamide analogs **3** and **14**–**16** were synthesized as shown in Scheme 3b. Dehydrative condensation of **27** with naphthylmethylamines **28**–**31** afforded **32**–**35**, respectively, alkaline hydrolysis of which provided the corresponding carboxylic acids **3** and **14**–**16**, respectively. *N*-(Benzyl)carboxyamide analogs **4**–**11** were synthesized as described in Scheme 3c. Condensation reaction of **27** with benzylamines **36**–**43** afforded **44**–**51**, respectively, alkaline hydrolysis of which resulted in the corresponding carboxylic acids **4**–**11**, respectively. Synthesis of *N*-(2-phenylethyl)carboxyamide analogs **12** and **13** is outlined in Scheme 3d. Condensation reaction of **27** with 2-phenylethylamines **52** and **53** afforded **54** and **55**, respectively, alkaline hydrolysis of which gave **12** and **13**, respectively.

Several amines such as **29**, **39**–**42** and **53** were synthesized according to Scheme 4. Synthesis of **29** is shown in Scheme 4a. Deprotonation of **56** with lithium diisopropylamide followed by alkylation with *i*-butyl iodide afforded **57**, reaction of which with diphenylphosphoryl azide in *t*-butyl alcohol in the presence of triethylamine afforded **58**. Removal of the *t*-butoxycarbonyl group of **58** under acidic conditions afforded **29**. Synthesis of **39** and **41** are described in Scheme 4b. Alkylation of **59** with alkyl iodides **60** and **61** followed by alkaline hydrolysis afforded **62** and **63**, respectively, Curtius rearrangement of which followed by treatment with *t*-BuOH provided **64** and **65**, respectively. Acidic deprotection of **64** and **65** provided **39** and **41**, respectively. Synthesis of **40** is shown in Scheme 4c. Reaction of **67**, which was prepared from **66** and *N*,*O*-dimethylhydroxylamine hydrochloride, with *i*-butylmagnesium bromide provided **68**. Reductive amination of **68** with ammonium acetate in the presence of sodium cyanoborohydride followed by treatment with hydrogen chloride afforded **40**. Compound **42** was prepared from benzaldehyde (**69**) as shown in Scheme 4d. Reaction of **69** with 2-methyl-1-propenylmagnesium bromide afforded **70**, the hydroxyl group of which was replaced by azide to provide **71**. Lithium aluminum hydride reduction of **71** afforded **72**, protection of whose amino group with a benzyloxycarbonyl group provided **73**. Oxidation of the double bond of **73** with molecular oxygen in the presence of $Co(acac)_2$ followed by treatment with triphenylsilylhydride afforded **74**, deprotection of which by catalytic hydrogenation resulted in amino alcohol **42**. Synthesis of **53** was described in Scheme 4e. Substitution reaction of 1-iodo-3-methylbutane (**75**) with silver nitrite afforded **76**, nitro Aldol condensation of which with benzaldehyde in the presence of *n*-butylamine and acetic acid provided **77**. Sodium borohydride reduction of **77** in the presence of boron trifluoride etherate afforded **53**.

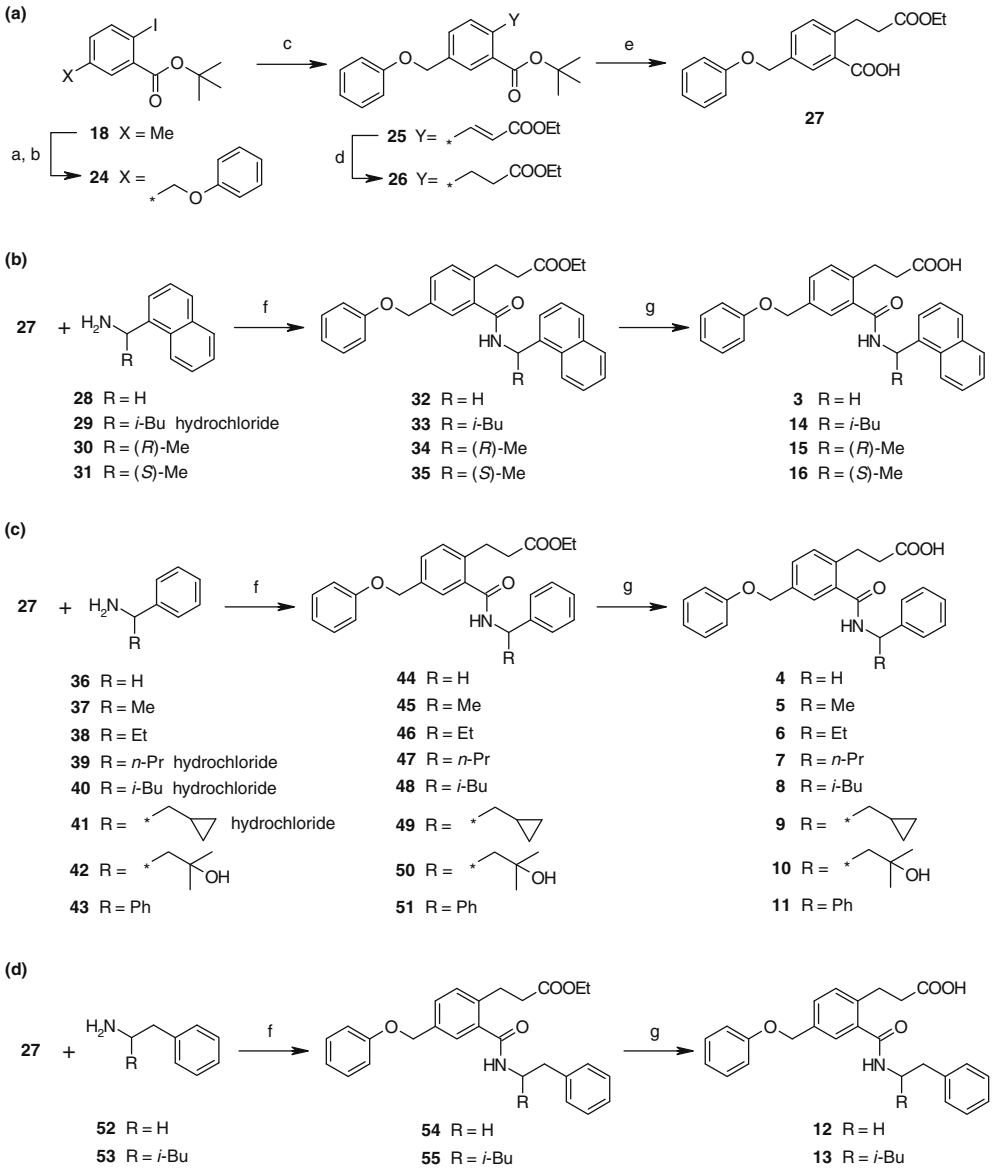
3. Results and discussion

Test compounds listed in Tables 1–3 were biologically evaluated for their inhibition of the specific binding of a radiolabeled ligand, $[^3H]PGE_2$, to membrane fractions prepared from cells stably expressing mouse EP1, EP2, EP3α or EP4 receptors. EP3 receptor antagonist activity was determined by a Ca^{2+} assay using mouse EP3α receptor expressed on CHO cells in the presence of bovine serum albumin (BSA).

In our previous paper,[12] we reported the discovery of **1** as a structurally novel and subtype selective EP3 receptor antagonist. Through further investigation, we identified *N*-(naphthylmethyl)carboxyamide analogs **2** and **3**, and carried out the evaluation of their EP3 antagonist activity in the presence of 0.1%, 1% and 2% additive BSA.[13] These results are summarized in Table 1. Compound **1** showed more potent antagonist activity in the presence of 0.1% BSA relative to **2** and **3**, while they showed nearly equipotent antagonist activity in the presence of 1% and 2% BSA, respectively. The most remarkable shift of the IC_{50} value was observed for the ether analog **1**, with 160-fold shift. As a result, the antagonist activities of carboxyamide analogs **2** and **3** were less influenced by additive BSA relative to **1**. Additionally, the shift of the IC_{50} value was not always parallel with an increase of a clog *P* value for **1**–**3**. The potency of the antagonist activity of the carboxyamide analog **3** was less influenced by BSA for its higher clog *P* value (5.11) relative to the ether analog **1**. Based on the SAR described above, further optimization of the carboxyamide analog **3** was considered to be more favored than that of the ether analog **1** to improve in vivo potency.



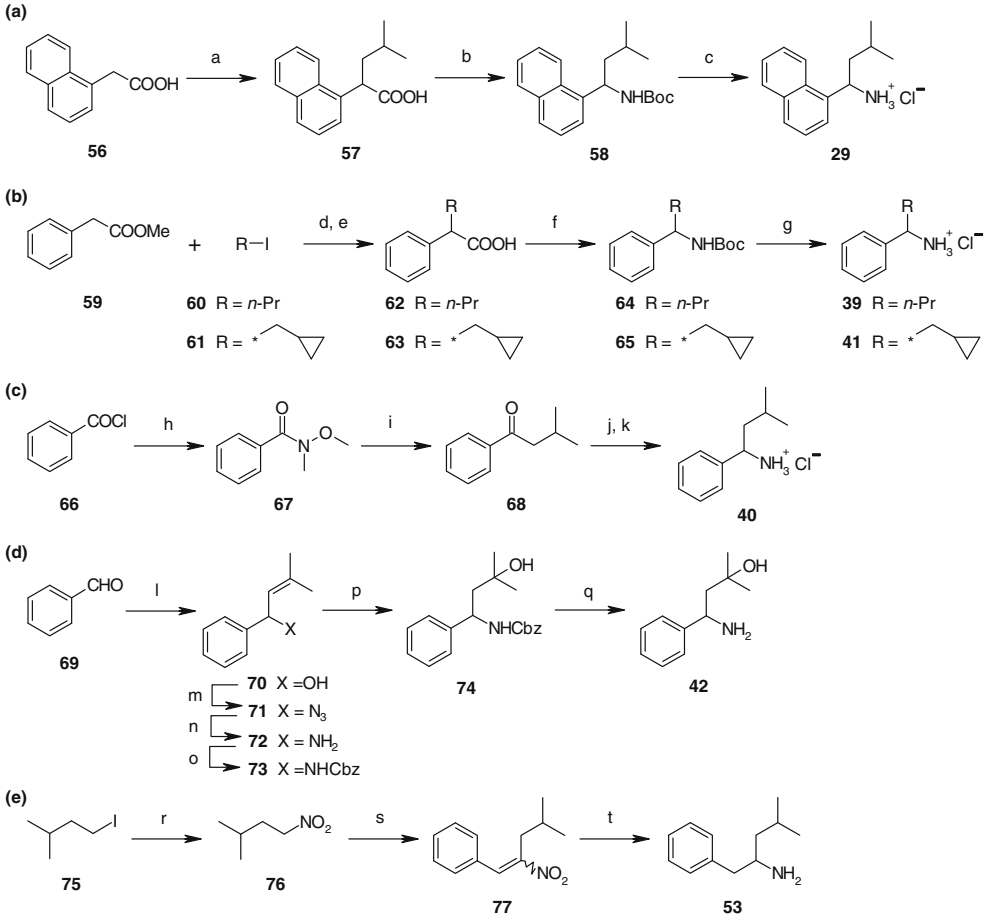
Scheme 2. Synthesis of **2**. Reagents: (a) $(COCl)_2$, DMF, toluene; (b) *t*-BuOK, THF, 75% from **17**; (c) NBS, benzoyl peroxide, CCl_4; (d) pyrazole, NaH, DMF, 55% from **18**; (e) ethyl acrylate, $Pd(OAc)_2$, DPPF, TEA, DMSO, 79%; (f) TFA, anisole, 76%; (g) H_2, Pd–C, EtOH, dioxane, 73%; (h) 1-naphthalenemethylamine, pyridine, CH_2Cl_2, 78% from **22**; (i) NaOHaq, THF, MeOH, 62%.



Scheme 3. Synthesis of **3**–**16**. Reagents: (a) NBS, AIBN, CCl_4; (b) phenol, NaH, DMF, 60% from **18**; (c) ethyl acrylate, $Pd(OAc)_2$, DPPF, TEA, DMSO, 69%; (d) $NaBH_4$, $NiCl_2 \cdot 6H_2O$, THF, EtOH, 100%; (e) TFA, anisole, 88%; (f) EDC·HCl, HOBt, NMM, DMF; (g) NaOHaq, THF, MeOH, 38–96% from **27**.

A large number of carboxyamide analogs were synthesized and evaluated. Among them, the effect of the benzylic substituent from the *N*-benzylamine moiety on the activity profiles was investigated and the results are summarized in Table 2. The *N*-(α-substituted benzyl)carboxyamide analogs were found to exhibit significantly increased binding affinity to the EP3 receptor relative to *N*-benzylcarboxyamide analog **4**. *N*-(α-Methylbenzyl)carboxyamide analog **5** showed 8.4-fold more potent binding affinity and improved antagonist activity relative to **4**. Replacement of the benzylic methyl group of **5** with other alkyl groups such as ethyl, *n*-propyl, *i*-butyl and cyclopropylmethyl afforded **6**–**9**, respectively. Compounds **6** and **7** showed more potent binding affinity (2.5-fold and 3.8-fold, respectively) and antagonist activity (4.2-fold and 9.2-fold, respectively) relative to **5**. *N*-(α-Isobutylbenzyl)carboxyamide analog **8** had the maximum potency in both the binding affinity and the antagonist activity assays. Compound **8** showed 19-fold more potent binding affinity and 32-fold more potent antagonist activity relative to **5**. *N*-(α-Cyclopropylmethylbenzyl)carboxyamide analog **9** showed 6.1-fold less potent binding affinity and 3.8-fold less potent antagonist activity relative to **8**. *N*-[α-(2-Hydroxy-2-methylpropyl)benzyl]carboxyamide analog **10** displayed a remarkably reduced potency with 79-fold less potent binding affinity and 40-fold less potent antagonist activity relative to **8**. Replacement of the benzylic methyl group of **5** with a phenyl group afforded **11** with slightly increased activity. As a result, it was concluded that the EP3 receptor recognized the lipophilicity and the bulkiness of the benzylic substituents of **5**–**11**. Among them, the benzylic isobutyl group of **8** was identified as the most optimized substituent.

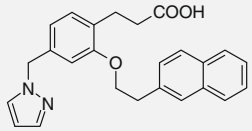
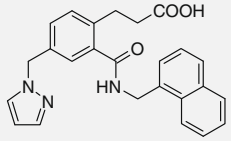
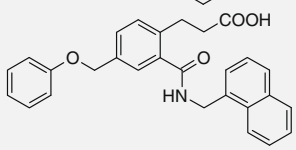
To obtain more detailed SAR, *N*-(2-phenylethyl)carboxyamide analogs **12**–**13** and *N*-(naphtylmethyl)carboxyamide analogs **14**–**16** were synthesized and evaluated. Results are summarized in Table 3. Replacement of the *N*-benzyl moiety of **4** with an *N*-(2-phenylethyl) moiety afforded **12** with a remarkable reduction in the binding affinity. Introduction of an isobutyl group into **12** afforded **13** with significant increases in the binding affinity and antagonist activity, while the potency of **13** was less than **8**. Based on the SAR described above, the *N*-benzyl moiety was determined to be more favored than the *N*-(2-phenylethyl) moiety. Introduction of an isobutyl group into the benzylic position of the *N*-(naphtylm-



Scheme 4. Synthesis of **29**, **39**–**42** and **53**. Reagents: (a) *i*-BuI, LDA, THF, 49%; (b) DPPA, TEA, *t*-BuOH, 50%; (c) HCl–dioxane, MeOH, 100%; (d) LDA, THF; (e) NaOHaq, THF, MeOH, 86–88% from **59**; (f) DPPA, TEA, *t*-BuOH; (g) HCl–dioxane, MeOH, 62% from **62** for **39**, 25% from **63** for **41**; (h) *N*,*O*-dimethylhydroxylamine hydrochloride, TEA, CH_2Cl_2, 89%; (i) *i*-BuMgBr, THF, 61%; (j) $NaBH_3CN$, $AcONH_4$, molecular sieves, MeOH; (k) HCl–dioxane, MeOH, 60% from **68**; (l) 2-methyl-1-propenylmagnesium bromide, THF, 34%; (m) DPPA, DBU, toluene, 42%; (n) $LiAlH_4$, THF, 72%; (o) CbzCl, *i*-Pr_2NEt, THF, 94%; (p) O_2, $Co(acac)_2$, Ph_3SiH, THF, 72%; (q) H_2, Pd–C, THF, MeOH, 100%; (r) $AgNO_2$, Et_2O, 80%; (s) benzaldehyde, *n*-$BuNH_2$, AcOH, MeOH; (t) $NaBH_4$, $BF_3{\cdot}OEt_2$, THF, 40% from **76**.

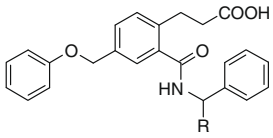
ethyl) moiety of **3** afforded **14** with slightly more potent binding affinity (3.2-fold) and remarkably increased antagonist activity (390-fold), with an IC_{50} value of 2.0 nM. The effect of the stereochemistry of the benzylic position on the activity profiles was also investigated using commercially available amines. Introduction of a (*R*)-methyl group into the benzylic position of **3** afforded **15** which exhibited improved potency with a K_i value of 0.22 nM and an IC_{50} value of 39 nM. Compound **15** showed 95-fold more

Table 1
Effect of the additive BSA on IC_{50} values of **1**–**3**

Compound	Structure	Function IC_{50} (nM)			IC_{50} shift[b]	clog *P*
		0.1%[a]	1%[a]	2%[a]		
1		22	580	3600	160	4.98
2		120	520	3000	19	3.31
3		68	770	3200	47	5.11

[a] Concentration of additive BSA.
[b] IC_{50} shift = IC_{50}(BSA 2%)/IC_{50}(BSA 0.1%).

Table 2
Activity profiles of *N*-(benzyl)carboxyamide analogs



Compound	R	Binding K_i (nM) EP3	Function IC_{50} (nM)[a] EP3
4		110	>10000
5		13	2200
6		5.2	520
7		3.4	240
8		0.70	68
9		4.3	260
10		55	2700
11		7.7	1400

[a] Antagonist activity was evaluated in the presence of 1% BSA.

potent binding affinity and 240-fold more potent antagonist activity relative to **16** which had a (*S*)-methyl group in the benzylic position of **3**. Among the tested compounds listed in Table 3, compounds **8**, **14** and **15** showed subnanomolar binding affinities for the EP3 receptor. All of these compounds showed nearly equipotent binding affinities in the absence of BSA. Compound **14** exhibited 5.9-fold decrease of the antagonist activity in the presence of 1% BSA relative to the binding affinity, while **8** and **15** showed much more decrease. The results were speculated to be due to the influence of the additive BSA on the presumed protein binding. The increase in the binding affinity of these analogs listed in Table 3 for the EP3 receptor was accompanied by an increase in the binding affinity for the EP4 receptor, however sufficient subtype selectivity (K_i for EP4/K_i for EP3) was still maintained for **3** (310-fold), **8** (2300-fold), **13** (110-fold), **14** (790-fold) and **15** (450-fold).

Some of the more potent compounds such as **8**, **14** and **15** were evaluated for their in vivo EP3 antagonist activity which was measured by the inhibitory effect on PGE_2-induced uterine contraction in pregnant rats. Results are summarized in Table 4. The inhibitory effect of **8**, **14** and **15** at 3 mg/kg of intraduodenal administration was 71%, 83% and 54%, respectively, relative to the control (vehicle administration). Although it displayed a significant increase in the in vitro antagonist activity relative to **8**, compound **14** did not show a reasonable increase in the in vivo antagonist activity. The inconsistency between the in vitro and in vivo results was ascribed to the presumed poor pharmacokinetic profiles of **14**.

Table 4
In vivo EP3 antagonist activity of **8**, **14** and **15**

Compound	%inhib. (3 mg/kg id)
8	71%
14	83%
15	54%

Table 5
Pharmacokinetic profiles of **8** and **14** in rats (10 mg/kg po and 10 mg/kg iv)[a]

Compound	CL_{tot} (mL/min/kg)	$t_{1/2}$ (h)	C_{max} (μg/mL)	V_{ss} (L/kg)	F (%)
8	15.3	12	2.66	2.2	31
14	16.0	2.1	0.28	0.8	3.9

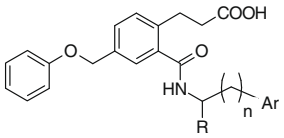
[a] Compounds were administered as Na salt.

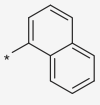
Compounds **8** and **14** were investigated for their pharmacokinetic profiles, as shown in Table 5. Their oral dosing to rats (10 mg/kg) resulted in remarkable difference in C_{max} values (2.66 μg/mL and 0.28 μg/mL) despite close clearance (CL) values (15.3 mL/min/kg and 16.0 mL/min/kg) after intravenous dosing (10 mg/kg). An apparent difference in $t_{1/2}$ values (12 h and 2.1 h) was also noted. As a result, the oral bioavailability of **14** (F = 3.9%) was found to be much lower than that of **8** (F = 31%). The unexpectedly weak in vivo potency of **14** compared with its potent in vitro activity was likely derived from the above-described poor pharmacokinetic data.

4. Conclusion

Starting with further chemical modifications of the previously reported EP3 antagonist **1**, 3-(2-aminocarbonylphenyl)propanoic

Table 3
Activity profiles of *N*-(arylmethyl) and *N*-(arylethyl)carboxyamide analogs



Compound	Ar	R	*n*	Binding K_i (nM) EP1	EP2	EP3	EP4	Function IC_{50} (nM)[a] EP3
8			0	>10000	>10000	0.70	1600	68
12			1	>10000	>10000	210	>10000	>10000
13			1	>10000	>10000	18	1900	1100
3			0	5000	1500	1.1	340	770
14			0	>10000	>10000	0.34	270	2.0
15			0	>10000	1100	0.22	100	39
16			0	>10000	9200	21	1100	9500

[a] Antagonist activity was evaluated in the presence of 1% BSA.

acid analogs **2** and **3**, whose potency was less influenced by BSA relative to **1**, were identified. Further optimization provided **8**, **14** and **15** possessing a *N*-(α-substituted arylmethyl)carboxyamide moiety as the carboxyamide side chain. They were found to show better potency and good subtype selectivity from in vitro evaluation relative to **2**–**3** and moderate in vivo EP3 antagonist activity. Among them, compound **14** showed remarkable improved antagonist activity, with an IC_{50} value of 2.0 nM. Although it exhibited improvement in antagonist activity, **14** did not exhibit suitable in vivo efficacy relative to **8**. This discrepancy between in vitro and in vivo results was ascribed to differences in their PK profiles. Further optimization of **8** and **14** to improve their in vivo efficacy will be reported in due course.

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 mass spectra (FAB-MS, HRMS) and electron ionization (EI) were obtained on a JEOL JMS-DX303HF spectrometer. Atmospheric pressure chemical ionization (APCI) was 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. 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*-butylalcohol (*t*-BuOH), *t*-butylmethylether (TBME), diethylether (Et_2O), *N*,*N*-dimethylformamide (DMF), dimethylsulfoxide (DMSO), ethanol (EtOH), ethyl acetate (EtOAc), methanol (MeOH), tetrahydrofuran (THF), acetic acid (AcOH), NBS (*N*-bromosuccinimide), DBU (1,8-diazabicyclo[5.4.0]undec-7-ene), 1,1′-bis(diphenylphosphino)-ferrocene (DPPF) and triethylamine (TEA).

5.1.2. *tert*-Butyl 2-iodo-5-methylbenzoate (18)

To a stirred solution of **17** (27.5 g, 0.105 mol) in toluene (350 mL) were added successively oxalyl chloride (9.90 mL, 0.120 mol) and a drop of DMF. After being stirred for 1 h at 50 °C, the reaction mixture was evaporated to give an acyl chloride. To a stirred suspension of potassium *t*-butoxide (14.1 g, 0.130 mol) in THF (330 mL) was added dropwise the acyl chloride in THF (200 mL) at 0 °C under argon atmosphere. After being stirred for 15 min, the reaction mixture was treated with cold water and aqueous NH_4Cl, and then extracted with Et_2O. 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, 1/10) to yield **18** (25.1 g, 75% in two steps) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (d, J = 8.1 Hz, 1H), 7.48 (d, J = 2.1 Hz, 1H), 6.91 (dd, J = 8.1, 2.1 Hz, 1H), 2.32 (s, 3H), 1.62 (s, 9H).

5.1.3. *tert*-Butyl 2-iodo-5-(1*H*-pyrazol-1-ylmethyl)benzoate (19)

To a stirred solution of **18** (15.0 g, 47.1 mmol) in CCl_4 (150 mL) were added NBS (9.70 g, 54.2 mmol) and benzoyl peroxide (1.70 g, 7.10 mmol), and the reaction mixture was refluxed for 16 h under argon atmosphere. After cooling to room temperature, the resultant mixture was filtered through a pad of Celite. The filtrate was poured into water and then extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo to yield a bromide, which was used for the next step without purification. To the stirred solution of pyrazole (5.10 g, 75.4 mmol) in DMF (150 mL) was added NaH (62.7% in oil, 2.70 g, 70.7 mmol) at 0 °C under argon atmosphere. After being stirred for 40 min at room temperature, the bromide in DMF (150 mL) was added dropwise at 0 °C. After being stirred for 15 min, the reaction mixture was quenched with aqueous NH_4Cl and extracted with Et_2O. 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, 2/5) to yield **19** (9.90 g, 55% in two steps) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (d, J = 8.1 Hz, 1H), 7.62–7.50 (m, 2H), 7.46–7.37 (m, 1H), 6.91 (dd, J = 8.1, 2.7 Hz, 1H), 6.30 (t, J = 2.3 Hz, 1H), 5.29 (s, 2H), 1.60 (s, 9H).

5.1.4. *tert*-Butyl 2-[(1*E*)-3-ethoxy-3-oxoprop-1-en-1-yl]-5-(1*H*-pyrazol-1-ylmethyl)benzoate (20)

A solution of **19** (7.30 g, 19.0 mmol), ethyl acrylate (4.10 mL, 38.0 mmol), TEA (13.3 mL, 95.0 mmol), DPPF (1.05 g, 1.90 mmol) and $Pd(OAc)_2$ (426 mg, 1.90 mmol) in DMSO (100 mL) was refluxed for 5 h under argon atmosphere. To the reaction mixture was added water and the resulting mixture was extracted with MTBE. 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, 2/5) to yield **20** (5.40 g, 79%) as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (d, J = 16 Hz, 1H), 7.73 (d, J = 1.5 Hz, 1H), 7.57 (d, J = 1.5 Hz, 1H), 7.52 (d, J = 8.1 Hz, 1H), 7.43 (d, J = 2.1 Hz, 1H), 7.28 (dd, J = 8.1, 2.1 Hz, 1H), 6.31 (t, J = 1.5 Hz, 1H), 6.24 (d, J = 16 Hz, 1H), 5.36 (s, 2H), 4.27 (q, J = 7.2 Hz, 2H), 1.60 (s, 9H), 1.33 (t, J = 7.2 Hz, 3H).

5.1.5. 2-[(1*E*)-3-Ethoxy-3-oxoprop-1-en-1-yl]-5-(1*H*-pyrazol-1-ylmethyl)benzoic acid (21)

To a stirred solution of **20** (1.00 g, 2.80 mmol) in anisole (4 mL) was added trifluoroacetic acid (4 mL) at room temperature. After being stirred for 1.5 h at 50 °C, the reaction mixture was concentrated in vacuo. The resultant residue was triturated with Et_2O to yield **21** (639 mg, 76%) as a white powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.32 (d, J = 16 Hz, 1H), 7.90–7.86 (m, 1H), 7.82 (d, J = 8.0 Hz, 1H), 7.70 (d, J = 1.8 Hz, 1H), 7.48 (d, J = 1.8 Hz, 1H), 7.38 (dd, J = 8.0, 1.8 Hz, 1H), 6.48 (d, J = 16 Hz, 1H), 6.28 (t, J = 1.8 Hz, 1H), 5.41 (s, 2H), 4.18 (q, J = 7.2 Hz, 2H), 1.24 (t, J = 7.2 Hz, 3H).

5.1.6. 2-(3-Ethoxy-3-oxopropyl)-5-(1*H*-pyrazol-1-ylmethyl)benzoic acid (22)

A suspension of **21** (530 mg, 1.80 mmol) and 5% Pd/C (53 mg) in EtOH (5 mL) and dioxane (5 mL) was stirred for 2 h at room temperature under hydrogen atmosphere. The reaction mixture was filtered through a pad of Celite and the filtrate was concentrated in vacuo. The resultant residue was triturated with Et_2O to yield **22** (392 mg, 73%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.09 (s, 1H), 7.63 (d, J = 2.1 Hz, 1H), 7.49 (d, J = 2.1 Hz, 1H), 7.32–7.24 (m, 2H), 6.31 (t, J = 2.1 Hz, 1H), 5.34 (s, 2H), 4.10 (q, J = 7.1 Hz, 2H), 3.30 (t, J = 7.6 Hz, 2H), 2.67 (t, J = 7.6 Hz, 2H), 1.21 (t, J = 7.1 Hz, 3H).

5.1.7. Ethyl 3-[2-{[(1-naphthylmethyl)amino]carbonyl}-4-(1*H*-pyrazol-1-ylmethyl)phenyl]propanoate (23)

To a stirred solution of **22** (311 mg, 1.03 mmol) in toluene (3 mL) were added successively oxalyl chloride (0.135 mL,

1.54 mmol) and a drop of DMF. After being stirred for 1 h at room temperature, the reaction mixture was evaporated to give an acyl chloride which was used for the next step without purification. To a stirred solution of 1-naphthalenemethylamine (210 mg, 1.34 mmol) and pyridine (0.125 mL, 1.55 mmol) in CH_2Cl_2 (2 mL) was added the acyl chloride in CH_2Cl_2 (1 mL) at 0 °C under argon atmosphere. After being stirred for 15 min, the reaction mixture was diluted with EtOAc and then successively washed with water, 0.5 M HCl, aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated in vacuo. The resultant residue was purified by column chromatography on silica gel (EtOAc/hexane, 2/1) to yield **23** (356 mg, 78% in two steps) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (d, J = 8.7 Hz, 1H), 7.92–7.80 (m, 2H), 7.61–7.40 (m, 5H), 7.34 (d, J = 2.1 Hz, 1H), 7.22–7.10 (m, 3H), 6.59 (br s, 1H), 6.24 (t, J = 2.1 Hz, 1H), 5.23 (s, 2H), 5.07 (d, J = 5.7 Hz, 2H), 4.03 (q, J = 7.2 Hz, 2H), 3.04 (t, J = 7.4 Hz, 2H), 2.65 (t, J = 7.4 Hz, 2H), 1.18 (t, J = 7.2 Hz, 3H).

5.1.8. 3-[2-{[(1-Naphthylmethyl)amino]carbonyl}-4-(1*H*-pyrazol-1-ylmethyl)phenyl]propanoic acid (2)

To a stirred solution of **23** (328 mg, 0.743 mmol) in THF (2 mL) and MeOH (1 mL) was added 2 M NaOH (1 mL) at room temperature. After being stirred for 12 h, the reaction mixture was acidified with 1 M HCl and then 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 triturated with MeOH/Et_2O/hexane to yield **2** (189 mg, 62%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.11 (s, 1H), 8.93 (t, J = 5.7 Hz, 1H), 8.16 (m, 1H), 7.95 (m, 1H), 7.85 (dd, J = 7.1, 2.2 Hz, 1H), 7.79 (d, J = 2.4 Hz, 1H), 7.61–7.42 (m, 5H), 7.28–7.13 (m, 3H), 6.25 (t, J = 2.0 Hz, 1H), 5.29 (s, 2H), 4.89 (d, J = 5.7 Hz, 2H), 2.88 (t, J = 7.8 Hz, 2H), 2.48 (t, J = 7.8 Hz, 2H); IR (KBr) 3310, 3128, 2913, 2523, 1728, 1638, 1528, 1435, 1402, 1288, 1209, 1193, 1164, 1068, 989, 963, 836, 792, 763, 620 cm^{-1}; MS (APCI, Neg.) *m/e* 412 $(M-H)^-$; HRMS (Pos.) calcd for $C_{25}H_{24}N_3O_3$: 414.1818; found: 414.1818.

5.1.9. *tert*-Butyl 2-iodo-5-(phenoxymethyl)benzoate (24)

To a stirred solution of **18** (9.17 g, 28.6 mmol) in CCl_4 (60 mL) were added NBS (10.2 g, 57.1 mmol) and AIBN (940 mg, 5.86 mmol), and the reaction mixture was refluxed for 18 h under argon atmosphere. After cooling to room temperature, the resultant mixture was filtered through a pad of Celite and the filtrate was concentrated in vacuo to yield a bromide, which was used for the next step without purification. To a stirred suspension of NaH (62.7% in oil, 1.20 g, 31.5 mmol) in DMF (50 mL) was added phenol (2.69 g, 28.6 mmol) in DMF (30 mL) at 0 °C under argon atmosphere and the mixture was stirred for 30 min at room temperature. To the reaction mixture was added dropwise the bromide in DMF (30 mL) at 0 °C. After being stirred for 1 h at room temperature, the resultant mixture was quenched with aqueous NH_4Cl and then extracted with Et_2O. 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, 1/100–1/40) to yield **24** (7.10 g, 60% in two steps) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.94 (d, J = 8.1 Hz, 1H), 7.73 (d, J = 2.1 Hz, 1H), 7.32–7.18 (m, 3H), 7.01–6.92 (m, 3H), 5.03 (s, 2H), 1.62 (s, 9H).

5.1.10. *tert*-Butyl 2-[(1*E*)-3-ethoxy-3-oxoprop-1-en-1-yl]-5-(phenoxymethyl)benzoate (25)

The titled compound was synthesized in the same manner as described for **20** using **24** instead of **19** as a white solid. Yield 69%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (d, J = 16 Hz, 1H), 7.58 (d, J = 1.5 Hz, 1H), 7.52 (d, J = 8.1 Hz, 1H), 7.40–7.30 (m, 3H), 7.10–6.90 (m, 3H), 6.28 (d, J = 16 Hz, 1H), 5.10 (s, 2H), 4.28 (q, J = 7.2 Hz, 2H), 1.62 (s, 9H), 1.34 (t, J = 7.2 Hz, 3H).

5.1.11. *tert*-Butyl 2-(3-ethoxy-3-oxopropyl)-5-(phenoxymethyl)benzoate (26)

To a stirred solution **25** (4.54 g, 11.9 mmol) in THF (48 mL) and MeOH (12 mL) were added successively $NiCl_2{\cdot}6H_2O$ (3.11 g, 13.1 mmol) and $NaBH_4$ (1.80 g, 47.5 mmol) at 0 °C under argon atmosphere. After being stirred for 1 h, the resultant mixture was quenched with acetone and water, diluted with Et_2O and filtered through a pad of Celite. The filtrate was separated and the organic layer was washed with water and brine, and dried over $MgSO_4$. Concentration in vacuo yielded **26** (4.51 g, 100%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86 (d, J = 1.5 Hz, 1H), 7.47 (dd, J = 8.1, 2.1 Hz, 1H), 7.32–7.25 (m, 3H), 7.01–6.94 (m, 3H), 5.04 (s, 2H), 4.13 (q, J = 7.2 Hz, 2H), 3.24 (t, J = 7.5 Hz, 2H), 2.64 (t, J = 7.5 Hz, 2H), 1.60 (s, 9H), 1.24 (t, J = 7.2 Hz, 3H).

5.1.12. 2-(3-Ethoxy-3-oxopropyl)-5-(phenoxymethyl)benzoic acid (27)

The titled compound was synthesized in the same manner as described for **21** using **26** instead of **20** as a white solid. Yield 88%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (d, J = 1.8 Hz, 1H), 7.57 (d, J = 8.1 Hz, 1H), 7.38–7.24 (m, 3H), 7.00–6.95 (m, 3H), 5.07 (s, 2H), 4.13 (q, J = 7.2 Hz, 2H), 3.25 (t, J = 8.1 Hz, 2H), 2.70 (t, J = 8.1 Hz, 2H), 1.23 (t, J = 7.2 Hz, 3H).

5.1.13. 3-[2-{[(1-Naphthylmethyl)amino]carbonyl}-4-(phenoxymethyl)phenyl]propanoic acid (3)

A solution of **27** (197 mg, 0.600 mmol), **28** (104 mg, 0.660 mmol), *N*-methylmorpholine (73 μL, 0.660 mmol), EDC·HCl (138 mg, 0.720 mmol) and HOBt (162 mg, 1.20 mmol) in DMF (2 mL) was stirred for 3 h at room temperature under argon atmosphere. The reaction mixture was acidified with 1 M HCl and then extracted with EtOAc. The organic layer was washed with aqueous $NaHCO_3$, water and brine, and dried over Na_2SO_4. Concentration in vacuo afforded **32**, which was used for the next step without purification. To a stirred solution of **32** in THF (1.5 mL) and MeOH (1.5 mL) was added 2 M NaOH (1.5 mL) at room temperature. After being stirred for 12 h at room temperature, the reaction mixture was acidified with 1 M HCl and then 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 triturated with EtOAc/hexane to yield **3** (187 mg, 50% in two steps) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (d, J = 8.4 Hz, 1H), 7.92–7.79 (m, 2H), 7.62–7.35 (m, 6H), 7.31–7.20 (m, 3H), 6.98–6.84 (m, 3H), 6.37 (t, J = 5.1 Hz, 1H), 5.08 (d, J = 5.1 Hz, 2H), 4.95 (s, 2H), 3.10 (t, J = 7.5 Hz, 2H), 2.76 (t, J = 7.5 Hz, 2H); IR (KBr) 1703, 1636, 1599, 1531, 1496, 1302, 1241, 776, 754, 690 cm^{-1}; MS (APCI, Neg.) *m/e* 438 $(M-H)^-$; HRMS (Pos.) calcd for $C_{28}H_{26}NO_4$: 440.1862; found: 440.1858.

5.1.14. 3-[2-({[3-Methyl-1-(1-naphthyl)butyl]amino}carbonyl)-4-(phenoxymethyl)phenyl]propanoic acid (14)

The titled compound was synthesized in the same manner as described for **3** using **29** instead of **28** as a white powder. Yield 74% in two steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.32 (d, J = 8.7 Hz, 1H), 7.88 (d, J = 7.8 Hz, 1H), 7.80 (d, J = 8.4 Hz, 1H), 7.61–7.25 (m, 9H), 7.00–6.90 (m, 3H), 6.38 (d, J = 8.7 Hz, 1H), 6.14 (dt, J = 8.7, 7.2 Hz, 1H), 4.99 (s, 2H), 3.04 (t, J = 7.2 Hz, 2H), 2.74 (t, J = 7.2 Hz, 2H), 2.01–1.90 (m, 2H), 1.80 (m, 1H), 1.13 (d, J = 6.6 Hz, 3H), 1.01 (d, J = 6.6 Hz, 3H); IR (KBr) 3296, 2956, 1700, 1637, 1600, 1531, 1301, 1239, 1173, 1035, 796, 776, 758, 692 cm^{-1}; MS (FAB, Pos.) *m/e* 496 $(M+H)^+$; HRMS (Pos.) calcd for $C_{32}H_{34}NO_4$: 496.2488; found: 496.2495.

5.1.15. 3-[2-({[(1*R*)-1-(1-Naphthyl)ethyl]amino}carbonyl)-4-(phenoxymethyl)phenyl]propanoic acid (15)

The titled compound was synthesized in the same manner as described for **3** using **30** instead of **28** as a white powder. Yield 95% in two steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.23 (d, J = 7.8 Hz, 1H), 7.89 (d, J = 7.5 Hz, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.62–7.25 (m, 9H), 6.98–6.88 (m, 3H), 6.36 (d, J = 8.4 Hz, 1H), 6.14 (m, 1H), 4.97 (s, 2H), 3.10 (t, J = 7.2 Hz, 2H), 2.78 (t, J = 7.2 Hz, 2H), 1.80 (d, J = 6.6 Hz, 3H); IR (KBr) 3290, 2923, 1705, 1636, 1541, 1496, 1304, 1244, 775, 752, 474 cm^{-1}; MS (FAB, Pos.) *m/e* 454 $(M+H)^+$; HRMS (Pos.) calcd for $C_{29}H_{28}NO_4$: 454.2018; found: 454.2017.

5.1.16. 3-[2-({[(1*S*)-1-(1-Naphthyl)ethyl]amino}carbonyl)-4-(phenoxymethyl)phenyl]propanoic acid (16)

The titled compound was synthesized in the same manner as described for **3** using **31** instead of **28** as a white powder. Yield 96% in two steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22 (d, J = 7.8 Hz, 1H), 7.89 (d, J = 7.8 Hz, 1H), 7.83 (d, J = 8.1 Hz, 1H), 7.62–7.25 (m, 9H), 6.98–6.88 (m, 3H), 6.36 (d, J = 7.8 Hz, 1H), 6.14 (m, 1H), 4.97 (s, 2H), 3.09 (t, J = 7.5 Hz, 2H), 2.78 (t, J = 7.5 Hz, 2H), 1.80 (d, J = 6.9 Hz, 3H); IR (KBr) 3290, 2977, 1704, 1636, 1541, 1496, 1302, 1244, 775, 753 cm^{-1}; MS (FAB, Pos.) *m/e* 454 $(M+H)^+$; HRMS (Pos.) calcd for $C_{29}H_{28}NO_4$: 454.2018; found: 454.2018.

5.1.17. 3-[2-[(Benzylamino)carbonyl]-4-(phenoxymethyl)phenyl]propanoic acid (4)

The titled compound was synthesized in the same manner as described for **3** using **36** instead of **28** as a white powder. Yield 38% in two steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46–7.25 (m, 10H), 6.99–6.92 (m, 3H), 6.48 (m, 1H), 5.00 (s, 2H), 4.61 (d, J = 5.7 Hz, 2H), 3.10 (t, J = 7.5 Hz, 2H), 2.77 (t, J = 7.5 Hz, 2H); IR (KBr) 3292, 3031, 2924, 1704, 1640, 1599, 1540, 1496, 1455, 1304, 1239 cm^{-1}; MS (APCI, Neg.) *m/e* 388 $(M-H)^-$; HRMS (Pos.) calcd for $C_{24}H_{24}NO_4$: 390.1705; found: 390.1704.

5.1.18. 3-(4-(Phenoxymethyl)-2-{[(1-phenylethyl)amino]carbonyl}phenyl)propanoic acid (5)

The titled compound was synthesized in the same manner as described for **3** using **37** instead of **28** as a white powder. Yield 58% in two steps; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.92 (d, J = 7.8 Hz, 1H), 7.48–7.20 (m, 10H), 7.07–6.91 (m, 3H), 5.13 (m, 1H), 5.09 (s, 2H), 2.87 (t, J = 7.9 Hz, 2H), 2.60–2.40 (m, 2H), 1.44 (d, J = 7.2 Hz, 3H); IR (KBr) 3296, 1716, 1637, 1524, 1496, 1249, 754, 698 cm^{-1}; MS (APCI, Neg.) *m/e* 402 $(M-H)^-$; HRMS (Pos.) calcd for $C_{25}H_{26}NO_4$: 404.1862; found: 404.1873.

5.1.19. 3-(4-(Phenoxymethyl)-2-{[(1-phenylpropyl)amino]carbonyl}phenyl)propanoic acid (6)

The titled compound was synthesized in the same manner as described for **3** using **38** instead of **28** as a white powder. Yield 82% in two steps; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.83 (d, J = 8.4 Hz, 1H), 7.44–7.18 (m, 10H), 7.03–6.90 (m, 3H), 5.08 (s, 2H), 4.86 (m, 1H), 2.84 (t, J = 7.8 Hz, 2H), 2.46 (t, J = 7.8 Hz, 2H), 1.82–1.64 (m, 2H), 0.90 (t, J = 7.5 Hz, 3H); IR (KBr) 3294, 2961, 1703, 1637, 1533, 1496, 1456, 1303, 1241, 1033, 820 752, 700 cm^{-1}; MS (APCI, Neg.) *m/e* 416 $(M-H)^-$; HRMS (Pos.) calcd for $C_{26}H_{28}NO_4$: 418.2018; found: 418.2017.

5.1.20. 3-(4-(Phenoxymethyl)-2-{[(1-phenylbutyl)amino]carbonyl}phenyl)propanoic acid (7)

The titled compound was synthesized in the same manner as described for **3** using **39** instead of **28** as a white powder. Yield 76% in two steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47–7.24 (m, 10H), 7.02–6.91 (m, 3H), 6.41 (d, J = 8.1 Hz, 1H), 5.15 (m, 1H), 5.03 (s, 2H), 3.08–2.97 (m, 2H), 2.78–2.69 (m, 2H), 1.98–1.74 (m, 2H), 1.52–1.23 (m, 2H), 0.96 (t, J = 7.2 Hz, 3H); IR (KBr) 3290, 2956, 1719, 1637, 1601, 1531, 1496, 1300, 1244, 1034, 941, 843, 753, 697 cm^{-1}; MS (APCI, Neg.) *m/e* 430 $(M-H)^-$; HRMS (Pos.) calcd for $C_{27}H_{30}NO_4$: 432.2175; found: 432.2174.

5.1.21. 3-[2-{[(3-Methyl-1-phenylbutyl)amino]carbonyl}-4-(phenoxymethyl)phenyl]propanoic acid (8)

The titled compound was synthesized in the same manner as described for **3** using **40** instead of **28** as a white powder. Yield 82% in two steps; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.82 (d, J = 8.4 Hz, 1H), 7.44–7.16 (m, 10H), 7.04–6.88 (m, 3H), 5.08 (s, 2H), 5.03 (m, 1H), 2.90–2.75 (m, 2H), 2.45 (t, J = 8.4 Hz, 2H), 1.80–1.56 (m, 2H), 1.43 (m, 1H), 0.92 (d, J = 6.9 Hz, 3H), 0.90 (d, J = 6.9 Hz, 3H); IR (KBr) 3292, 2955, 1703, 1636, 1601, 1531, 1496, 1451, 1301, 1244, 1173, 1080, 1033, 944 cm^{-1}; MS (APCI, Neg.) *m/e* 444 $(M-H)^-$; HRMS (Pos.) calcd for $C_{28}H_{32}NO_4$: 446.2331; found: 446.2339.

5.1.22. 3-[2-{[(2-Cyclopropyl-1-phenylethyl)amino]carbonyl}-4-(phenoxymethyl)phenyl]propanoic acid (9)

The titled compound was synthesized in the same manner as described for **3** using **41** instead of **28** as a white powder. Yield 98% in two steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (m, 1H), 7.48–7.22 (m, 9H), 7.00–6.93 (m, 3H), 6.62 (d, J = 7.2 Hz, 1H), 5.25 (m, 1H), 5.02 (s, 2H), 3.03 (t, J = 7.2 Hz, 2H), 2.74 (t, J = 7.2 Hz, 2H), 1.88–1.72 (m, 2H), 0.66 (m, 1H), 0.55–0.40 (m, 2H), 0.20–0.01 (m, 2H); IR (KBr) 3296, 2915, 1717, 1634, 1602, 1530, 1497, 1449, 1292, 1245, 941, 846, 750, 698 cm^{-1}; MS (APCI, Neg.) *m/e* 442 $(M-H)^-$; HRMS (Pos.) calcd for $C_{28}H_{30}NO_4$: 444.2175; found: 444.2164.

5.1.23. 3-[2-{[(3-Hydroxy-3-methyl-1-phenylbutyl)amino]carbonyl}-4-(phenoxymethyl)phenyl]propanoic acid (10)

The titled compound was synthesized in the same manner as described for **3** using **42** instead of **28** as a white powder. Yield 55% in two steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (s, 1H), 7.47–7.17 (m, 11H), 7.00–6.86 (m, 3H), 5.04 (s, 2H), 4.98 (d, J = 10 Hz, 1H), 3.10 (t, J = 7.2 Hz, 2H), 2.78 (t, J = 7.2 Hz, 2H), 2.15 (dd, J = 15, 10 Hz, 1H), 1.73 (d, J = 15 Hz, 1H), 1.62 (s, 3H), 1.57 (s, 3H); IR (KBr) 3296, 2915, 1717, 1634, 1602, 1530, 1497, 1449, 1292, 1245, 941, 846, 750, 698 cm^{-1}; MS (APCI, Neg.) *m/e* 460 $(M-H)^-$; HRMS (Pos.) calcd for $C_{28}H_{32}NO_5$: 462.2280; found: 462.2285.

5.1.24. 3-[2-[(Benzhydrylamino)carbonyl]-4-(phenoxymethyl)phenyl]propanoic acid (11)

The titled compound was synthesized in the same manner as described for **3** using **43** instead of **28** as a white powder. Yield 87% in two steps; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.39 (d, J = 8.7 Hz, 1H), 7.43–7.20 (m, 15H), 7.03–6.90 (m, 3H), 6.36 (d, J = 8.7 Hz, 1H), 5.07 (s, 2H), 2.85 (t, J = 7.8 Hz, 2H), 2.44 (m, 2H); IR (KBr) 3288, 3030, 1716, 1639, 1600, 1524, 1495, 1245, 1031, 753, 693 cm^{-1}; MS (APCI, Neg.) *m/e* 464 $(M-H)^-$; HRMS (Pos.) calcd for $C_{30}H_{28}NO_4$: 466.2018; found: 466.2015.

5.1.25. 3-(4-(Phenoxymethyl)-2-{[(2-phenylethyl)amino]carbonyl}phenyl)propanoic acid (12)

The titled compound was synthesized in the same manner as described for **3** using **52** instead of **28** as a white powder. Yield 75% in two steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42–7.20 (m, 10H), 7.00–6.90 (m, 3H), 6.25 (m, 1H), 4.98 (s, 2H), 3.72 (dt, J = 6.0, 6.9 Hz, 2H), 3.01 (t, J = 7.2 Hz, 2H), 2.94 (t, J = 6.9 Hz, 2H), 2.74 (t, J = 7.2 Hz, 2H); IR (KBr) 3328, 2921, 2521, 1704, 1598, 1557, 1492, 1454, 1383, 1287, 1219, 1196 cm^{-1}; MS (APCI, Neg.) *m/e* 402 $(M-H)^-$; HRMS (Pos.) calcd for $C_{25}H_{26}NO_4$: 404.1862; found: 404.1861.

5.1.26. 3-[2-{[(1-Benzyl-3-methylbutyl)amino]carbonyl}-4-(phenoxymethyl)phenyl]propanoic acid (13)

The titled compound was synthesized in the same manner as described for **3** using **53** instead of **28** as a white powder. Yield 62% in two steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43–7.16 (m, 10H), 7.04–6.93 (m, 3H), 5.76 (d, J = 9.0 Hz, 1H), 4.99 (s, 2H), 4.53 (m, 1H), 3.02–2.66 (m, 6H), 1.72 (m, 1H), 1.52–1.35 (m, 2H), 0.97 (d, J = 6.0 Hz, 3H), 0.95 (d, J = 6.0 Hz, 3H); IR (KBr) 3337, 1719, 1640, 1529, 1496, 1243, 816, 751, 702, 689 cm^{-1}; MS (APCI, Neg.) m/e 458 $(M-H)^-$; HRMS (Pos.) calcd for $C_{29}H_{34}NO_4$: 460.2488; found: 460.2498.

5.1.27. 4-Methyl-2-(1-naphthyl)pentanoic acid (57)

To a stirred solution of **56** (2.50 g, 13.4 mmol) in THF (20 mL) was added dropwise lithium diisopropylamide (1.8 M in heptane/THF/ethylbenzene, 15.3 mL, 27.5 mmol) at −78 °C under argon atmosphere and the reaction mixture was stirred for 1 h at −78 °C. To the resultant mixture was added dropwise isobutyl iodode (3.70 g, 20.1 mmol) in THF (5 mL) and the reaction mixture was allowed to warm to room temperature. The resultant mixture was quenched with water and then extracted with hexane. The aqueous layer was acidified with 2 M HCl and then extracted with EtOAc. The organic layer was washed with water and brine, and dried over $MgSO_4$. Concentration in vacuo yielded **57** (1.58 g, 49%) as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 (d, J = 8.3 Hz, 1H), 7.86 (dd, J = 7.8, 1.8 Hz, 1H), 7.78 (d, J = 8.3 Hz, 1H), 7.58–7.42 (m, 4H), 4.52 (dd, J = 8.3, 6.9 Hz, 1H), 2.22–2.12 (m, 1H), 1.87–1.76 (m, 1H), 1.69–1.55 (m, 1H), 0.97 (d, J = 6.6 Hz, 3H), 0.93 (d, J = 6.6 Hz, 3H).

5.1.28. *tert*-Butyl [3-methyl-1-(1-naphthyl)butyl]carbamate (58)

To a stirred solution of **57** (1.00 g, 4.10 mmol) in *t*-BuOH (12 mL) were successively added TEA (0.570 mL, 4.10 mmol) and diphenylphosphoryl azide (0.930 mL, 4.10 mmol) and the mixture was refluxed for 24 h under argon atmosphere. After concentration in vacuo, the resultant residue was purified by column chromatography on silica gel (EtOAc/hexane, 1/20) to yield **58** (650 mg, 50%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19 (m, 1H), 7.85 (d, J = 8.1 Hz, 1H), 7.75 (t, J = 8.4 Hz, 1H), 7.56–7.43 (m, 4H), 5.58 (s, 1H), 4.84 (m, 1H), 1.88–1.65 (m, 3H), 1.42 (s, 9H), 1.07 (d, J = 6.0 Hz, 3H), 0.95 (d, J = 6.0 Hz, 3H).

5.1.29. 3-Methyl-1-(1-naphthyl)butan-1-amine hydrochloride (29)

To a stirred solution of **58** (431 mg, 1.37 mmol) in MeOH (5 mL) was added 4 M HCl in dioxane (10 mL) and the mixture was stirred for 2 h at room temperature. After concentration in vacuo, the resultant residue was washed with EtOAc to yield **29** (345 mg, 100%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.50 (br s, 3H), 8.22 (d, J = 8.4 Hz, 1H), 8.01 (d, J = 8.1 Hz, 1H), 7.98 (d, J = 8.7 Hz, 1H), 7.79 (d, J = 7.2 Hz, 1H), 7.68–7.55 (m, 3H), 5.19 (t, J = 7.2 Hz, 1H), 1.46–0.90 (m, 3H), 0.89 (d, J = 6.6 Hz, 3H), 0.85 (d, J = 6.6 Hz, 3H).

5.1.30. 2-Phenylpentanoic acid (62)

To a stirred solution of **59** (4.00 g, 26.6 mmol) in THF (90 mL) was added dropwise lithium diisopropylamide (1.8 M in heptane/THF/ethylbenzene, 14.6 mL, 29.2 mmol) at −78 °C under argon atmosphere and the reaction mixture was stirred for 1 h at −78 °C. To the reaction mixture was added dropwise **60** (7.75 mL, 79.8 mmol) and the reaction mixture was allowed to warm up to room temperature and then stirred for 3 h. The resultant mixture was quenched with aqueous NH_4Cl 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 hydrolyzed with 1 M NaOH (20 mL) in THF (20 mL) and MeOH (20 mL). After being stirred for 3 h at 50 °C, the reaction mixture was extracted with hexane. The aqueous layer was acidified with 2 M HCl and then extracted with EtOAc. The organic layer was washed with water and brine, and dried over $MgSO_4$. Concentration in vacuo afforded **62** (4.10 g, 86%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34–7.22 (m, 5H), 3.57 (m, 1H), 2.05 (m, 1H), 1.76 (m, 1H), 1.38–1.20 (m, 2H), 0.91 (t, J = 6.6 Hz, 3H).

5.1.31. 1-Phenylbutan-1-amine hydrochloride (39)

To a stirred solution of **62** (2.50 g, 14.0 mmol) in *t*-BuOH (18 mL) were added TEA (5.85 mL, 42.0 mmol) and diphenylphosphoryl azide (3.17 mL, 14.7 mmol), and the mixture was refluxed for 12 h under argon atmosphere. The resultant mixture was diluted with EtOAc, 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, 1/9) to yield **64** which was treated with 4 M HCl in dioxane (12 mL) and MeOH (8 mL). After being stirred for 4 h at room temperature, the mixture was concentrated in vacuo. The resultant residue was recrystallized with EtOAc/hexane to yield **39** (1.60 g, 62% in two steps) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.60 (br s, 3H), 7.54–7.33 (m, 5H), 4.16 (m, 1H), 1.99–1.59 (m, 2H), 1.28–0.96 (m, 2H), 0.82 (t, J = 7.5 Hz, 3H).

5.1.32. 3-Cyclopropyl-2-phenylpropanoic acid (63)

The titled compound was synthesized in the same manner as described for **62** using **61** instead of **60** as a yellow oil. Yield 88% in two steps; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50–7.22 (m, 5H), 3.69 (t, J = 7.2 Hz, 1H), 1.92 (m, 1H), 1.76 (m, 1H), 0.64 (m, 1H), 0.45–0.35 (m, 2H), 0.01–0.00 (m, 2H).

5.1.33. 2-Cyclopropyl-1-phenylethanamine hydrochloride (41)

The titled compound was synthesized in the same manner as described for **39** using **63** instead of **62** as a white powder. Yield 25% in two steps; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.60 (br s, 3H), 7.56–7.50 (m, 2H), 7.45–7.35 (m, 3H), 4.22 (t, J = 6.9 Hz, 1H), 1.81–1.73 (m, 2H), 0.56–0.25 (m, 3H), 0.11 (m, 1H), −0.07 (m, 1H).

5.1.34. *N*-Methoxy-*N*-methylbenzamide (67)

To a stirred solution of *N*,*O*-dimethylhydoxylamine hydrochloride (1.66 g, 17.0 mmol) and TEA (9.90 mL, 71.0 mmol) in CH_2Cl_2 (20 mL) was added **66** (4.65 mL, 14.2 mmol) at 0 °C under argon atmosphere. After being stirred for 2 h, the reaction mixture was quenched with water 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 purified by column chromatography on silica gel (EtOAc/hexane, 1/6–1/3) to yield **67** (2.09 g, 89%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70–7.60 (m, 2H), 7.50–7.34 (m, 3H), 3.56 (s, 3H), 3.36 (s, 3H).

5.1.35. 3-Methyl-1-phenylbutan-1-one (68)

To a stirred solution of **67** (1.00 g, 6.05 mmol) in THF (20 mL) was added isobutylmagnesium chloride (1 M in THF, 12.7 mL, 12.7 mmol) at 0 °C under argon atmosphere. After being stirred for 16 h at 40 °C, the reaction mixture was quenched with aqueous NH_4Cl at 0 °C 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 purified by column chromatography on silica gel (EtOAc/hexane, 1/8) to yield **68** (598 mg, 61%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00–7.90 (m, 2H), 7.56 (m, 1H), 7.51–7.40 (m, 2H), 2.84 (d, J = 6.9 Hz, 2H), 2.30 (m, 1H), 1.00 (d, J = 6.6 Hz, 6H).

5.1.36. 3-Methyl-1-phenylbutan-1-amine (40)

To a stirred suspension of **68** (300 mg, 1.85 mmol), ammonium acetate (1.43 g, 18.5 mmol) and molecular sieves (2.00 g) in MeOH (5 mL) was added $NaBH_3CN$ (81.4 mg, 13.0 mmol) at room temperature under argon atmosphere. After being stirred for 72 h, the reaction mixture was acidified with 5 M HCl and then filtered through a pad of Celite. The filtrate was neutralized with 5 M NaOH and then extracted with Et_2O. 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, 1/8) to yield a free amine, which was treated with 4 M HCl in dioxane (1 mL) and MeOH (1 mL) to yield **40** (180 mg, 60% in two steps) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.47 (br s, 3H), 7.56–7.48 (m, 2H), 7.48–7.34 (m, 3H), 4.22 (t, J = 7.8 Hz, 1H), 1.81–1.70 (m, 2H), 1.36–1.20 (m, 1H), 0.86 (d, J = 6.6 Hz, 3H), 0.81 (d, J = 6.6 Hz, 3H).

5.1.37. 3-Methyl-1-phenylbut-2-en-1-ol (70)

To a stirred solution of **69** (5.00 g, 49.2 mmol) in THF (10 mL) was added dropwise 2-methyl-1-propenylmagnesium bromide (0.5 M in THF, 100 mL, 50.0 mmol) at −78 °C under argon atmosphere. After being stirred for 15 min at 0 °C, the reaction mixture was quenched with 1 M 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 purified by column chromatography on silica gel (EtOAc/hexane, 1/5) to yield **70** (2.71 g, 34%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42–7.19 (m, 5H), 6.60 (d, J = 16 Hz, 1H), 6.36 (d, J = 16 Hz, 1H), 1.43 (s, 6H).

5.1.38. (1-Azido-3-methylbut-2-en-1-yl)benzene (71)

To a stirred solution of **70** (2.70 g, 16.6 mmol) in toluene (35 mL) were added successively diphenylphosphoryl azide (4.30 mL, 20.0 mmol) and DBU (3.00 mL, 20.0 mmol) at 0 °C under argon atmosphere. After being stirred for 12 h at room temperature, the reaction mixture was quenched with cold water and then extracted with Et_2O. 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, 1/9) to yield **71** (1.30 g, 42%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47–7.21 (m, 5H), 6.57 (d, J = 16 Hz, 1H), 6.24 (d, J = 16 Hz, 1H), 1.46 (s, 6H).

5.1.39. 3-Methyl-1-phenylbut-2-en-1-amine (72)

To a stirred suspension of $LiAlH_4$ (180 mg, 4.74 mmol) in THF (15 mL) was added dropwise **71** (900 mg, 4.80 mmol) in THF (6 mL) at room temperature under argon atmosphere. The reaction mixture was stirred for 15 min and then quenched with aqueous Na_2SO_4. The resulting white precipitate was removed by filtration through a pad of Celite and the filtrate was concentrated in vacuo. The resultant residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH, 9/1) to yield **72** (558 mg, 72%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46–7.14 (m, 5H), 6.47 (d, J = 16 Hz, 1H), 6.33 (d, J = 16 Hz, 1H), 1.31 (s, 6H).

5.1.40. Benzyl (3-methyl-1-phenylbut-2-en-1-yl)carbamate (73)

To a stirred solution of **72** (400 mg, 2.49 mmol) and diisopropylethylamine (0.870 mL, 4.99 mmol) in THF (8 mL) was added benzyloxycarbonyl chloride (0.300 mL, 3.00 mmol) at 0 °C under argon atmosphere. After being stirred for 12 h at room temperature, the reaction mixture was poured into water 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 purified by column chromatography on silica gel (EtOAc/hexane, 1/5–1/3) to yield **73** (691 mg, 94%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42–7.10 (m, 10H), 6.46 (d, J = 8.1 Hz, 1H), 6.37 (d, J = 8.1 Hz, 1H), 5.07 (s, 2H), 4.89 (br s, 1H), 1.51 (s, 6H).

5.1.41. Benzyl (3-hydroxy-3-methyl-1-phenylbutyl)carbamate (74)

To a stirred solution of **73** (93 mg, 0.310 mmol) in THF (1.6 mL) were added triphenylsilane (80.0 μL, 0.630 mmol) and $Co(acac)_2$ (4.0 mg, 16.0 μmol) at room temperature. The reaction mixture was stirred for 12 h at room temperature under oxygen atmosphere and then concentrated in vacuo. The resultant residue was purified by column chromatography on silica gel (EtOAc/hexane, 1/2) to yield **74** (70 mg, 72%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39–7.24 (m, 10H), 5.91 (s, 1H), 5.07 (d, J = 12 Hz, 1H), 5.04 (d, J = 12 Hz, 1H), 4.94 (m, 1H), 2.25 (s, 1H), 2.07 (dd, J = 15, 9.9 Hz, 1H), 1.81 (d, J = 15 Hz, 1H), 1.55 (s, 3H), 1.43 (s, 3H).

5.1.42. 4-Amino-2-methyl-4-phenylbutan-2-ol (42)

A mixture of **74** (70 mg, 0.220 mmol), 10% Pd/C (4 mg) in EtOH (2.5 mL) was vigorously stirred for 1 h under an atmosphere of hydrogen. The reaction mixture was filtered through a pad of Celite and the filtrate was concentrated in vacuo to yield **42** (39 mg, quant.) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42–7.18 (m, 5H), 5.03 (dd, J = 9.3, 3.6 Hz, 1H), 2.65 (br s, 3H), 1.69–1.54 (m, 2H), 1.35 (s, 3H), 1.21 (s, 3H).

5.1.43. 3-Methyl-1-nitrobutane (76)

To a stirred suspension of $AgNO_3$ (8.80 g, 57.2 mmol) in Et_2O (40 mL) was added **75** (7.57 g, 38.2 mmol) in Et_2O (10 mL) at 0 °C under argon atmosphere. After being stirred for 20 h at room temperature in a dark place, the reaction mixture was filtered through a pad of Celite and the filtrate was carefully concentrated in vacuo to yield **76** (3.60 g, 80%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.41 (t, J = 7.2 Hz, 2H), 1.92 (dt, J = 6.9, 7.1 Hz, 2H), 1.68 (m, 1H), 0.96 (d, J = 6.6 Hz, 6H).

5.1.44. 4-Methyl-1-phenylpentan-2-amine (53)

To a stirred solution of **76** (3.30 g, 28.2 mmol) in MeOH (20 mL) were added AcOH (1.08 mL, 18.8 mmol) and n-$BuNH_2$ (1.86 mL, 18.8 mmol) at 0 °C under argon atmosphere. After being stirred for 36 h at room temperature, the reaction mixture was poured into water 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 purified by column chromatography on silica gel (EtOAc/hexane, 1/20) to yield **77**. To a stirred suspension of $NaBH_4$ (740 mg, 19.5 mmol) in THF (30 mL) were added successively $BF_3{\cdot}OEt_2$ (2.90 mL, 23.4 mmol) and **77** in THF (10 mL) at 0 °C under argon atmosphere. After being stirred for 15 h at 60 °C, the reaction mixture was successively quenched with water and 1 M HCl at 0 °C, and then extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo to yield **53** (260 mg, 40%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40–7.10 (m, 5H), 3.06 (m, 1H), 2.79 (m, 1H), 2.42 (m, 1H), 1.88–1.10 (m, 5H), 1.04–0.60 (m, 6H).

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:

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

Where $[L]$: Concentration of radiolabeled ligand, and K_d: 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 estimate potencies 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 2 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, 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 0.1% (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 urethane (1.5 g/5 mL/kg sc) and 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, a test compound (5 mL/kg in 0.5% methylcellulose (MC)) or vehicle (0.5% MC) was intraduodenally administrated. After 30 min, an increased uterine contraction was elicited by an intravenous administration of PGE_2 (30 μg/kg). 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 measured as 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): an increased uterine contraction of administration of a test compound, and ΔS (vehicle): an increased uterine contraction of administration of vehicle.

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13. We considered that the antagonist activity was more closely related to the in vivo efficacy than the binding affinity. For such a reason, we investigated the influence of additive BSA on the EP3 antagonist activity using our assay system. The binding affinity for EP1–4 receptor subtypes was evaluated in the absence of BSA.