Bioorganic & Medicinal Chemistry 20 (2012) 702–713

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

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

Synthesis and evaluation of novel modified γ-lactam prostanoids as EP4 subtype-selective agonists

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ARTICLE INFO

Article history:
Received 7 November 2011
Revised 3 December 2011
Accepted 5 December 2011
Available online 13 December 2011

Keywords:
Prostaglandin
EP4 receptor
Agonist
TNF-alpha

ABSTRACT

To identify chemically and metabolically stable subtype-selective EP4 agonists, design and synthesis of a series of modified γ-lactam prostanoids has been continued. Prostanoids bearing 2-oxo-1,3-oxazolidine, 2-oxo-1,3-thiazolidine and 5-thioxopyrrolidine as a surrogate for the γ-hydroxycyclopentanone without a troublesome 11-hydroxy group were identified as highly subtype-selective EP4 agonists. Among the tested, several representative compounds demonstrated in vivo efficacy after oral dosing in rats. Their pharmacokinetic and structure–activity relationship studies are presented.

1. Introduction

Prostaglandin E_2 (PGE_2) derived from arachidonic acid is one of the most well-known prostanoids, and it exhibits a broad range of biological actions in diverse tissues. These biological actions have been known to mediate four subtypes of PGE_2 receptor (EP1, EP2, EP3 and EP4).[1] Among them, the EP4 receptor subtype is of great interest as a drug discovery target because of its important regulatory roles in numerous physiological processes, including bronchodilation, bone resorption, and inflammation.[2] Activation of the EP4 receptor subtype increases the intracellular cAMP level, which is linked to the treatment of diseases based on the above-described mechanism of actions. Despite its high potency, PGE_2, a natural ligand for all of these receptor subtypes, shows strong nonselective affinity to all the EP receptor subtypes.

Natural PGs are susceptible to three major modes of metabolic inactivation: oxidation of the 15-hydroxy group, β-oxidation of the α-chain, and oxidation of the ω-chain terminus. Furthermore, PGE_2 is chemically unstable since the 11-hydroxyl group can easily undergo an elimination reaction, leading to PGA_2.

Efforts to improve the chemical and/or metabolic instability of PGE_2 while maintaining the potency and the subtype selectivity have been attempted. Previously, we reported 3,7-dithiaPGE analogs as highly subtype-selective EP4 agonists, although they were still susceptible to metabolism in addition to general chemical instability.[3] Recently, many γ-lactam prostanoids and their related structures have been reported as agonists for the EP2 and/or EP4 receptors with properties of increased chemical and metabolic stability.[4–7] We also reported γ-lactam prostanoids bearing the 16-(*meta*-substituted)phenyl ω-chain as a subtype-selective EP4 agonist and an EP2&EP4 dually selective agonist based on the molecular design as schematically described in Figure 1.[8–10] The tertiary amide of the γ-lactam moiety of **II** was assumed to have a double bond character, which can retain the four atoms of the 7-, 8-, 9- and 10-positions (prostanoic acid numbering in Fig. 3) in an identical plane, and was designed based on a hybrid structure derived from an equilibrium mixture consisting of **Ia** and **Ib**. Based

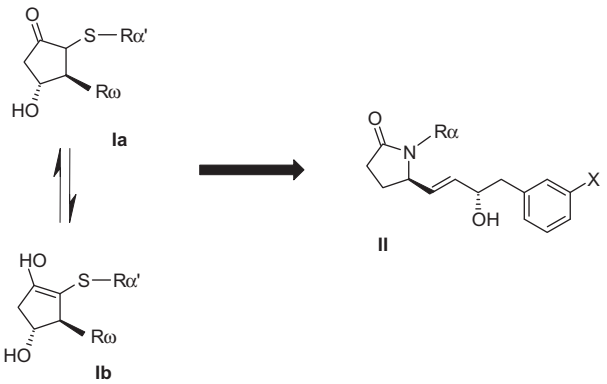

Figure 1. Reported template for EP4 receptor agonist.

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doi:10.1016/j.bmc.2011.12.009

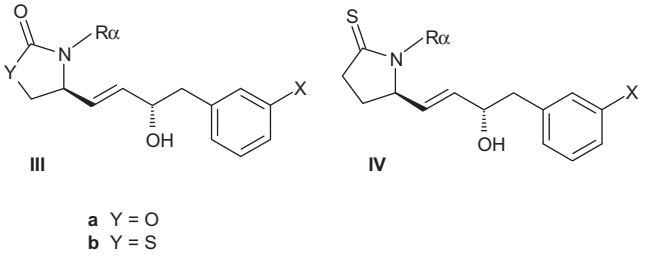


Figure 2. New templates for EP4 receptor agonists.

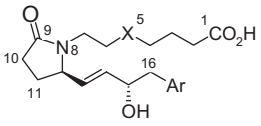


Figure 3. Numbering of the γ-lactam prostanoid.

on the same concept as described above, 2-oxo-1,3-oxazolidine, 2-oxo-1,3-thiazolidine and 5-thioxopyrrolidine (Fig. 2) were also considered to be surrogates for the γ-lactam moiety of **II**. Thus, structures **IIIa–b** and **IV** were proposed as new templates for a subtype-selective EP4 agonist with increased chemical and/or metabolic stability in addition to the γ-lactam prostanoids.

Herein, we report the synthesis and structure–activity relationship (SAR) study of modified γ-lactam prostanoids **IIIa–b** and **IV** as prostanoids bearing 2-oxo-1,3-oxazolidine, 2-oxo-1,3-thiazolidine and 5-thioxopyrrolidine as surrogates for the γ-hydroxycyclopentanone without a troublesome 11-hydroxy group as highly subtype-selective EP4 agonists. All of them were designed based on the same synthetic concept as previously described for the γ-lactam prostanoids since these modified γ-lactam prostanoids could be synthesized as their optically active forms from commercially available starting materials.

2. Chemistry

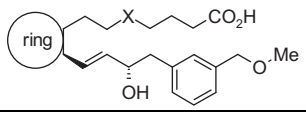
Synthesis of test compounds listed in Tables 2–4 is described in Schemes 1–4. 2-Oxo-1,3-oxazolidine prostanoids **3–12** were synthesized as outlined in Scheme 1a.

Sodium borohydride reduction of an acid anhydride prepared by the reaction of the commercially available **28** with isobutyl chloroformate in the presence of *N*-methylmorpholine afforded an alcohol **29** in good yield. Acidic deprotection of **29** with 4 N hydrogen chloride in ethyl acetate followed by cyclization with 1,1′-carbonyldiimidazole in the presence of triethylamine resulted in a cyclic urethane **30**. N-Alkylation of **30** with ethyl bromoacetate in the presence of potassium *tert*-butoxide afforded **31**. Sodium borohydride reduction of **31** afforded an alcohol **32**. *O*-Methanesulfonylation of **32** followed by the substitution reaction with potassium thioacetate provided a thioacetate **33**. Deacylation and then *S*-alkylation of **33** with butyl 4-iodobutanoate followed by acidic debenzylation with trifluoroacetic acid in the presence of thioanisole produced **34**, which was converted to prostanoids **3–12** by the following sequential reactions: (k) oxidation with dimethyl sulfoxide in the presence of sulfur trioxide and *N*,*N*-diisopropylethylamine; (l) Horner–Emmons reaction with phosphonates **35a–j** in the presence of sodium hydride[9]; (m) stereoselective reduction catalyzed with (*R*)-CBS[11]; and (n) alkaline hydrolysis.

Phosphonates **35h–i** were prepared as described in Scheme 1b. Cross-coupling reaction of the commercially available (3-bromophenyl)acetic acid with phenylboronic acid in the presence of palladium catalyst afforded 3-(phenyl)phenylacetic acid **36**. Preparation of the Weinreb amide from **36** followed by its reaction with the anion prepared from dimethyl methylphosphonate in the presence of *n*-butyl lithium afforded a phosphonate **35h**. Another phosphonate **35i** was prepared from (3-trifluoromethoxyphenyl)acetic acid according to the same procedures as described above.

Synthesis of 2-oxo-1,3-thiazolidine prostanoids **13–18** were synthesized as outlined in Scheme 2. Reaction of D-cysteine with triphosgene in aqueous alkaline conditions followed by treatment

Table 1
Activity profiles of prostanoids bearing 16-(3-methoxymethyl)phenyl as a ω-chain moiety



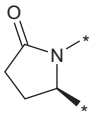
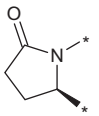
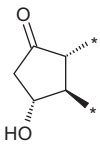
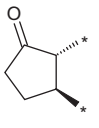
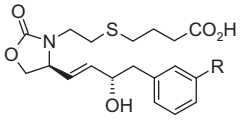
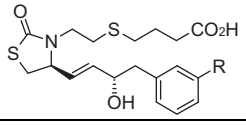
Compound	ring	X	Binding assay (K_i, nM) mEP1	mEP2	mEP3	mEP4	Functional assay EC_{50} (nM) rEP4
1		CH_2	>10^4	>10^4	>10^4	10	15
2		S	>10^4	8500	>10^4	8.0	1.3
26		S	>10^4	620	56	0.7	1.4
27		S	>10^4	470	190	2.4	1.1

Table 2
Activity profiles of 2-oxo-1,3-oxazolidine prostanoids



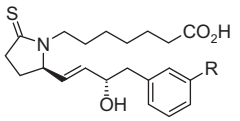
Compound	R	Binding assay (K_i, nM) mEP1	mEP2	mEP3	mEP4	Functional assay EC_{50} (nM) rEP4
3	H	>10^4	>10^4	6400	4.2	36
4	Me	4400	>10^4	2800	2.5	33
5	Et	2400	>10^4	2700	7.9	18
6	*n*Pr	>10^4	>10^4	1900	2.4	93
7	CF_3	>10^4	>10^4	1600	2.0	12
8	F	1200	>10^4	4000	20	35
9	Cl	>10^4	>10^4	630	1.5	23
10	Ph	>10^4	>10^4	>10^4	28	450
11	OCF_3	>10^4	>10^4	1700	29	160
12	CH_2OMe	>10^4	>10^4	>10^4	7.2	24

Table 3
Activity profiles of 2-oxo-1,3-thiazolidine prostanoids



Compound	R	Binding assay (K_i, nM) mEP1	mEP2	mEP3	mEP4	Functional assay EC_{50} (nM) rEP4
13	H	>10^4	>10^4	39	0.65	4.8
14	Et	4600	>10^4	65	0.88	1.9
15	*n*Pr	>10^4	>10^4	1200	0.98	6.1
16	CF_3	>10^4	>10^4	280	0.97	4.3
17	F	>10^4	>10^4	1800	1.5	5.7
18	CH_2OMe	>10^4	>10^4	>10^4	1.2	2.4

Table 4
Activity profiles of 5-thioxopyrrolidine prostanoids



Compound	R	Binding assay (K_i, nM) mEP1	mEP2	mEP3	mEP4	Functional assay EC_{50} (nM) rEP4
19	H	>10^4	>10^4	2300	1.5	29
20	Et	>10^4	1100	2300	0.83	9.4
21	*n*Pr	>10^4	6800	2000	0.40	27
22	CF_3	>10^4	>10^4	450	0.38	10
23	F	>10^4	>10^4	1700	0.69	26
24	CH_2OMe	>10^4	1400	6300	2.0	7.3
25	Me (5-thia)	2800	1700	980	0.50	7.7

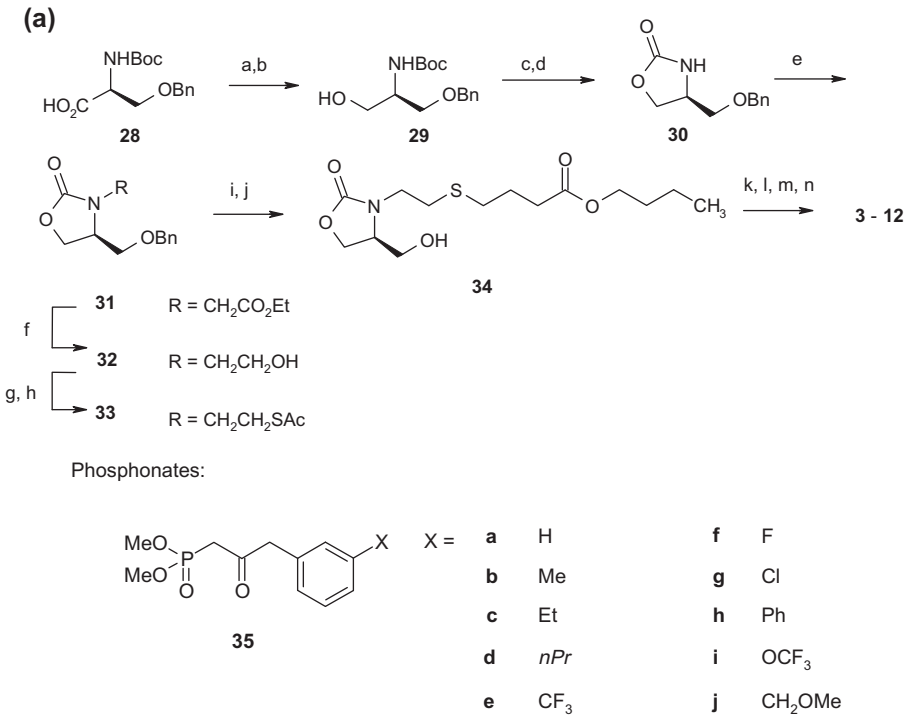
with diazomethane afforded 2-oxo-1,3-thiazolidine **37**. Sodium borohydride reduction of **37** provided an alcohol **38**, which was converted to **39** by the following sequential reactions: (d) *O*-protection with *tert*-butyldimethylsilyl chloride (TBSCl); (e) N-alkylation with ethyl bromoacetate in the presence of *tert*-butoxide; (f) sodium borohydride reduction; (g) *O*-methanesulfonylation; and (h) substitution reaction with potassium thioacetate. Deacetylation and then *S*-alkylation of **39** with ethyl 4-bromobutanoate in the presence of *tert*-butoxide followed by deprotection with tetrabutylammonium fluoride (TBAF) afforded **40**, which was converted to the prostanoids **13**–**18** according to the same procedure as described for the synthesis of **3**–**12** from **34**.

Synthesis of 5-thioxopyrrolidine prostanoids **19**–**24** is outlined in Scheme 3. *O*-Protection of **41**[8] with TBSCl followed by thiation of the amide oxygen with Lawesson's reagent afforded **42**, deprotection of which with TBAF provided **43**. Conversion of **43** to their prostanoid analogs was carried out according to the usual PG synthesis resulting in **19**–**24**.

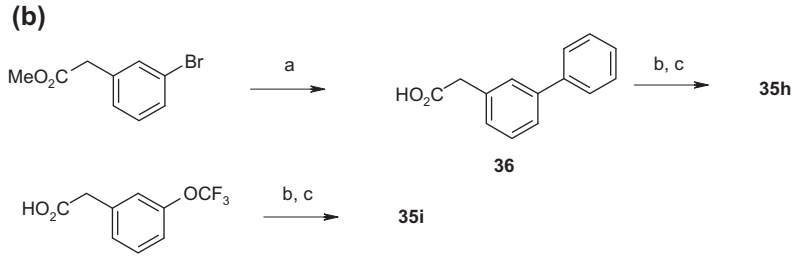
The corresponding 5-thiaprostanoid analog **25** was synthesized as shown in Scheme 4. Thiation of the amide oxygen of the prostanoids **44**[9] was carried out by using Lawesson's reagent to afford the corresponding thioamide **45**, deprotection of which with TBAF followed by alkaline hydrolysis resulted in **25**.

3. Results and discussion

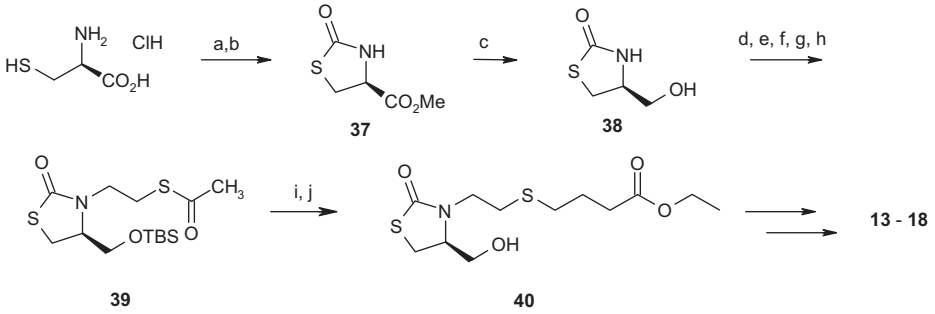
The binding assay was conducted according to the reported method with minor modifications.[12] The binding constants (K_i values) for the mouse EP receptors (mEP1, mEP2, mEP3 and mEP4) were determined by a competitive binding assay of the test



Scheme 1a. Synthesis of **3**–**12** reagents: (a) isobutyl chloroformate, *N*-methyl morpholine, DME; (b) $NaBH_4$, H_2O, 96% in two steps; (c) 4 N HCl, AcOEt, 79%; (d) 1,1′-carbonyldiimidazole, Et_3N, THF, 66%; (e) ethyl bromoacetate, potassium *tert*-butoxide, DMF, 75%; (f) $NaBH_4$, THF, MeOH, 94%; (g) MsCl, Et_3N, THF; (h) potassium thioacetate, K_2CO_3, DMF, 88%; (i) butyl 4-iodobutanoate, *n*-butanol, potassium *tert*-butoxide, DMF, 74%; (j) TFA, thioanisole, 52%; (k) SO_3–Py, *i*Pr_2NEt, DMSO, AcOEt; (l) phosphonates **35a**–**j**, NaH, THF; (m) (*R*)-Me–CBS, BH_3–THF, THF; (n) aq NaOH, MeOH, DME.



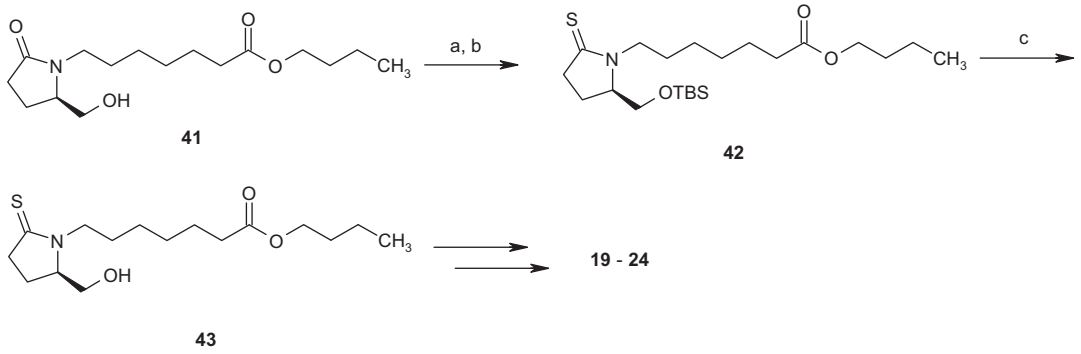
Scheme 1b. Preparation of phosphonates **35h**–**i**. Reagents: (a) Phenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME; (b) *N*,*O*-dimethylhydroxylamine hydrochloride, EDC–HCl, Et_3N, CH_3CN; (c) dimethyl methylphosphonate, *n*-BuLi, toluene.



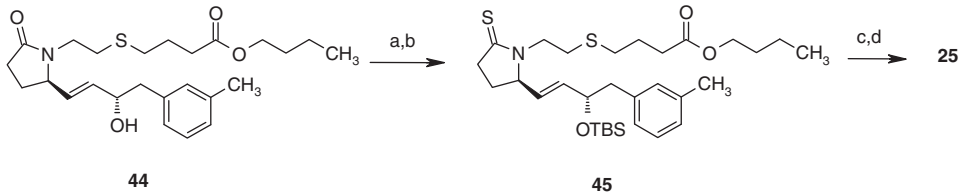
Scheme 2. Synthesis of **13**–**18**. Reagents: (a) Triphosgene, aq NaOH, dioxane; (b) CH_2N_2, AcOEt, 93% in two steps; (c) $NaBH_4$, EtOH, 36%; (d) TBSCl, imidazole, DMF; (e) ethyl bromoacetate, potassium *tert*-butoxide, THF; (f) $NaBH_4$, THF, EtOH; (g) MsCl, Et_3N, THF; (h) potassium thioacetate, K_2CO_3, DMF, 96% in five steps; (i) ethyl 4-bromobutanoate, potassium *tert*-butoxide, THF; (j) TBAF, THF, 84% in two steps.

compounds using radiolabeled ligands such as $[^3H]PGE_2$. Prior to the in vivo evaluation, the rat functional activity (EC_{50} values) was conducted by a cell-based assay (EC_{50} for rEP4).

Results of the in vitro evaluation of the test compounds are shown in Tables 1–4. As the standard compounds for SAR discussion, activity profiles of γ-lactam prostanoids **1**, **2** and the cyclo-



Scheme 3. Synthesis of **19**–**24**. Reagents: (a) TBSCl, imidazole, DMF; (b) Lawesson's reagent, toluene, 74%; (c) TBAF, THF, 80%.



Scheme 4. Synthesis of **25**. Reagents: (a) TBSCl, imidazole, DMF, 79%; (b) Lawesson's reagent, toluene; (c) TBAF, THF, 86% in two steps; (d) aq NaOH, MeOH, THF, 85%.

pentanone prostanoids **26**–**27** are described in Table 1. The 5-methylene γ-lactam analog **1** exhibited nearly equipotent activity and EP4 subtype selectivity with the corresponding 5-thia analog **2** in the mouse binding assay, while **2** showed nearly 10-fold more potency in the rat cell-based functional assay. As reported previously,[3] the 16-(3-methoxymethyl)phenyl moiety was the most optimized 16-substituent (prostanoic acid numbering) in a series of γ-hydroxycyclopentanone prostanoids including **26**. The corresponding 11-deoxy analog **27** was also found to show almost the same subtype selectivity and potency as **26** (in-house data).[8] However, these two cyclopentanone analogs showed less subtype selectivity relative to the γ-lactam analogs owing to their increased receptor affinity for EP2 and EP3, while they showed nearly the same potency of the rat functional activity (EC_{50} values) as that of **2**. Thus, both the γ-lactam prostanoids **1** and **2** tended to show better EP4 subtype selectivity than the cyclopentanone prostanoids **26**–**27**, while the corresponding 5-thia analog **2** as well as the cyclopentanone prostanoids **26**–**27** tended to show more potency than the 5-methylene γ-lactam analog **1** in terms of rat EP4 functional activity.

Keeping these results in mind, a series of 2-oxo-1,3-oxazolidine prostanoids were synthesized as their 5-thia analogs and evaluated. Results are shown in Table 2. The corresponding 16-substitutent 2-oxo-1,3-oxazolidine prostanoids was again optimized with regard to this new template. As shown in Table 2, all the tested analogs **3**–**12** exhibited good EP4 subtype selectivity and potent EP4 receptor affinity. Among them, 16-(3-fluoro)phenyl analog **8**, 16-(3-phenyl)phenyl analog **10** and 16-(3-trifluoromethoxy)phenyl analog **11** demonstrated significantly less potent receptor affinity for the EP4 receptor subtype than **3**–**7**, **9**, and **12** because of unknown reasons. Functional activities of these compounds in the rat cell-based assay are also described in Table 2. Most of the compounds showed 1.8–16-fold reduction in their potency of EC_{50} values in terms of the rat functional activity relative to the K_i values of the mouse EP4 receptor affinity, while the 16-(3-propyl)phenyl analog **6** showed remarkable reduction (38-fold) of the rat EC_{50} value relative to the K_i value of the mouse EP4 receptor affinity. One of the plausible reasons for the remarkable reduction in the rat functional activity of **6** relative to **3**–**5** and its relatively stronger mouse receptor affinity was estimated to be due to the increased lipophilicity of the 16-(3-propyl)phenyl moiety relative to those of the 16-phenyl, 16-(3-methyl)phenyl and 16-(3-ethyl)phenyl moieties since the higher lipophilicity causes more protein binding in the cell-based assay. The 16-(3-fluoro)phenyl analog **8** did not show a significant difference between the potency of the mouse EP4 binding affinity and the rat functional activity. The significantly less potent rat functional activities of **10** and **11** were considered to be due to their weaker EP4 receptor affinities relative to those of the other compounds.

A series of 2-oxo-1,3-thiazolidine prostanoids were synthesized also as their 5-thia analogs **13**–**18** and biologically evaluated. Results are summarized in Table 3. It is of great interest to compare the activity profiles of these 2-oxo-1,3-thiazolidine prostanoids with their corresponding 2-oxo-1,3-oxazolidine prostanoids because of their similarities in structures. The 16-phenyl analog **13** and 16-(3-ethyl)phenyl analog **14** showed very strong affinity for the mouse EP4 receptor subtype while they showed increased affinity for the mouse EP3 receptor subtype. The increased affinity of **13** and **14** seems to be one of the clear differences from the corresponding 2-oxo-1,3-oxazolidine series **3** and **5**, respectively. The 16-(3-propyl)phenyl analog **15** also exhibited very strong affinity for the mouse EP4 receptor subtype with good subtype selectivity, while its rat functional activity did not show such as remarkable reduction as that of **6**. The 16-(3-trifluoromethyl)phenyl analog **16** showed very strong affinity for the mouse EP4 receptor subtype with slightly increased affinity for the mouse EP3 receptor subtype relative to the corresponding 2-oxo-1,3-oxazolidine analog **7**. The 16-(3-fluoro)phenyl analog **17** exhibited potent affinity for the mouse EP4 receptor subtype with good subtype selectivity, while it showed more increased mouse EP4 receptor affinity and rat functional activity than **8**. The 16-(3-methoxymethyl)phenyl analog **18** showed excellent activity profiles regarding both the mouse EP4 receptor affinity and the subtype selectivity, which are more potent than the corresponding 2-oxo-1,3-oxazolidine analog **12**. Activity profiles of the most optimized structure in the mouse receptor subtype selectivity were found to be those of **18**. All the analogs **13**–**18**

Table 5
Biological evaluation and pharmacokinetic parameters of representative compounds

Compound	Rat TNF-α inhibition ED_{50} (mg/kg)	rEC_{50} (nM)	Pharmacokinetic parameters[a]				
			C_{max} (ng/mL)	AUC (ng h/mL)	CL_{tot} (mL/min/kg)	V_{ss} (mL/kg)	B.A. (%)
1	1	15	1.6[b]	6.4[b]	35	386	1.2
4	>1	33	5.4	16.1	47	291	4.5
7	3	12	3.8	16.7	67	631	6.7
13	1	4.8	2.7	4.0	56	792	3.5
14	1	1.9	10.4	19.5	75	538	8.6
15	1	6.1	11.4	17.7	52	1280	6.3
16	1	4.3	5.3	14.6	51	512	5.5
18	1	1.2	2.6	6.1	47	325	2.0
24	3	7.3	6.5	20.6	102	530	12.3
25	1	7.7	3.4	29.9	61	1760	9.8

[a] 1 mg/kg, po and 0.1 mg/kg, iv.
[b] C_{max} and AUC were normalized to a dose of 1 mg/kg.

showed more potent rat functional activities relative to the corresponding 2-oxo-1,3-oxazolidine analogs **3**, **5**–**8** and **12**, respectively.

Replacement of the γ-lactam carbonyl of **1** and **2** with a thiocarbonyl moiety afforded 5-thioxopyrrolidine analogs as illustrated by **19**–**25** also with excellent affinity for the mouse EP4 receptor subtype, good to excellent subtype selectivity and excellent rat functional activity as shown in Table 4. This series of prostanoids were first synthesized and evaluated as the 5-methylene analogs since we wanted to avoid introduction of more than two sulfur atoms into a compound because of the predicted oxidative metabolism. Also it was fortunate that all the synthesized 5-methylene analogs **19**–**24** exhibited nearly equipotent mouse EP4 receptor affinity and excellent subtype selectivity relative to the only synthesized 5-thia analog **25** bearing a 16-(methyl)phenyl ω-chain. All the tested compounds **19**–**25** tended to have less potent rat functional activity relative to the corresponding 2-oxo-1,3-thiazolidine analogs.

As shown in Table 5, oral efficacy of the representative compounds **1**, **4**, **7**, **13**–**16**, **18** and **24**–**25** selected based on their in vitro activity profiles were evaluated for their inhibitory activity of LPS-induced TNF-α production and pharmacokinetic (PK) parameters in rats. Rat functional assay results (rEC_{50} values) are again listed for purposes of comparison. Their PK parameters are also listed. Compounds **1**, **13**–**18** and **25** had ED_{50} values of 1 mg/kg and **4**, **7** and **24** demonstrated less effective ED_{50} values after oral dosing. We could not find any special SAR between their ED_{50} values and their rat EC_{50} values. The parameters from the PK study (1 mg/kg, po and 0.1 mg/kg, iv) are listed in Table 5. According to the PK data described above, compounds **14** and **15** showed higher C_{max} values relative to the others. Compounds **4**, **7**, **14**–**16** and **24**–**25** showed higher oral exposure (AUC values). Relatively higher tissue permeability (V_{ss} values) and AUC values of **15** and **25** may suggest that once compounds are distributed into the tissues, they circulate systemically resulting in prolongation of their blood concentration. Compound **24** exhibited the highest clearance (CL_{tot}) while others showed relatively lower CL_{tot} values. Compounds **24** and **25** demonstrated the best bioavailability (BA). However, we could not find any clear SAR among the values of ED_{50}, rEC_{50}, C_{max}, AUC, CL_{tot}, V_{ss} and BA, as systemic effects were estimated to result in a hybrid of EC_{50} values and PK parameters.

4. Conclusion

In conclusion, the 2-oxo-1,3-oxazoline, 2-oxo-1,3-thiazolidine and 5-thioxopyrrolidine were presented as candidates of the surrogate for the cyclopentanone and the γ-lactam frameworks.

Among the tested compounds, all the optimized 2-oxo-1,3-thiazolidine prostanoids **13**–**18** were efficacious at 1 mg/kg (oral dosing) while 2-oxo-1,3-oxazolidine prostanoids **4**, **7** and 5-thioxopyrrolidine prostanoids **24**, **25** tended to show less in vivo potency. However, we could not find any clear SAR in their biological and PK data. Further efforts to identify more optimized orally active EP4 selective agonists are being continued in our lab.

5. Experimental

5.1. Chemistry

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 methanol (CD_3OD) and deuterated dimethylsulfoxide (DMSO-d_6) 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. Column chromatography was carried out on silica gel [Merck Silica Gel 60 (0.063–0.200 μm), Wako gel C-200, or Fuji Silysia FL60D]. Thin layer chromatography was performed on silica gel (Merck TLC or HPTLC plates, Silica Gel 60 F_{254}). The following abbreviations for solvents and reagents are used; diethyl ether (Et_2O), *N*,*N*-dimethylformamide (DMF), dimethylsulfoxide (DMSO), ethanol (EtOH), ethyl acetate (EtOAc), methanol (MeOH), tetrahydrofuran (THF), methanol (MeOH), dichloromethane (CH_2Cl_2), chloroform ($CHCl_3$), dimethoxyethane (DME), acetonitrile (CH_3CN), sulfur trioxide/pyridine complex (SO_3–Py), 4-(dimethylamino)pyridine (DMAP), tetrabutylammonium fluoride (TBAF), *tert*-butyldimethylsilyl (TBS) chloride.

5.1.1. (*R*)-2-(*tert*-Butoxycarbonylamino)-3-benzyloxypropanol (29)

To a stirred solution of **28** (9.28 g, 31.7 mmol) and *N*-methyl morpholine (3.7 mL, 33.7 mmol) in DME (70 mL) was added isobutyl chloroformate (4.3 mL, 33.3 mmol) at −20 °C under argon atmosphere. After being stirred for 30 min at 0 °C, the reaction mixture was filtered to remove the resulting precipitates. To a cooled suspension of sodium borohydride (2.5 g, 66.5 mmol) in water (30 mL) was added the filtrate at 0 °C. Stirring was continued for additional 30 min at the same temperature. The reaction mixture was diluted with EtOAc, washed with hydrochloric acid, water, brine, and dried over Na_2SO_4. The organic layer was removed by evaporation to give an alcohol **29** as a colorless oil (8.47 g, 96%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (s, 9H), 3.60–3.83 (m, 6H), 4.54 (s, 2H), 5.17 (br s, 1H), 7.40–7.28 (m, 5H).

5.1.2. (4*S*)-4-[(Benzyloxy)methyl]-1,3-oxazolidin-2-one (30)

To a stirred solution of **29** (8.56 g, 30.3 mmol) in EtOAc (10 ml) was added 4 N hydrogen chloride/EtOAc (30 mL) at 0 °C under argon atmosphere, and the stirring was continued for 3 h. The reaction mixture was evaporated. The remaining organic solvent was further removed by repeated evaporation with toluene. To the resulting residue was added EtOAc/hexane (1:5, 10 mL) and the resulting mixture was stirred for 1 h. The resulting precipitates were removed by filtration to afford an amino alcohol hydrochloride as a white powder (5.23 g, 79%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.15 (m, 3H), 7.38–7.25 (m, 5H), 5.32 (m, 1H), 4.55 (s, 2H), 3.65–3.52 (m, 4H).

To a stirred solution of the above-described amino alcohol hydrochloride (5.13 g, 23.6 mmol) and triethylamine (6.9 mL, 49.4 mmol) in THF (100 mL) was added 1,1′-carbonyldiimidazole (4.6 g, 28.3 mmol) at room temperature under argon atmosphere. After being stirred for 2.5 h, the reaction mixture was diluted with EtOAc, washed with water, brine, and dried over Na_2SO_4. The organic layer was evaporated, and the resulting residue was purified by column chromatography on silica gel (EtOAc) to give **30** as a colorless oil (3.23 g, 66%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.44–3.48 (m, 2H), 4.04 (m, 1H), 4.14 (m, 1H), 4.55 (m, 1H), 4.56 (s, 2H), 5.88 (br s, 1H), 7.28–7.40 (m, 5H).

5.1.3. Ethyl {(4*S*)-4-[(benzyloxy)methyl]-2-oxo-1,3-oxazolidin-3-yl}acetate (31)

To a stirred solution of **30** (3.22 g, 15.5 mmol) and ethyl bromoacetate (2.1 mL, 18.6 mmol) in DMF (35 mL) was added potassium *tert*-butoxide (2.1 g, 18.6 mmol) at 0 °C under argon atmosphere. After being stirred for 3 h at room temperature, the reaction was quenched with saturated aq NH_4Cl. The reaction mixture was extracted with EtOAc (×3). The combined organic layers were washed with H_2O (×2), brine, dried over $MgSO_4$ and evaporated. The resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 1:2) to give an ester **31** as a colorless oil (3.43 g, 75%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.23 (t, J = 7.2 Hz, 3H). 3.52 (m, 1H), 3.60 (m, 1H), 4.05 (m, 1H), 4.14 (m, 1H), 4.15 (q, J = 7.2 Hz, 2H), 4.23 (m, 1H), 4.44 (m, 1H), 4.47 (m, 1H), 4.57 (s, 2H), 7.25–7.40 (m, 5H).

5.1.4. (4*S*)-4-[(Benzyloxy)methyl]-3-(2-hydroxyethyl)-1,3-oxazolidin-2-one (32)

To a stirred solution of **31** (3.43 g, 11.7 mmol) in MeOH (10 mL) and THF (50 mL) was added sodium borohydride (1.1 g, 29.2 mmol) at 0 °C under argon atmosphere. After being stirred at room temperature for 3 h, the reaction was quenched with H_2O. The reaction mixture was extracted with EtOAc (×3). The combined organic layers were washed with brine, and dried over $MgSO_4$. Removal of the solvent by evaporation gave an alcohol **32** as a colorless oil (2.75 mg, 94%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.36–3.63 (m, 4H), 3.72–3.88 (m, 2H), 4.06 (m, 1H), 4.12 (m, 1H), 4.38 (m, 1H), 4.57 (s, 2H), 7.28–7.40 (m, 5H).

5.1.5. *S*-(2-{(4*S*)-4-[(Benzyloxy)methyl]-2-oxo-1,3-oxazolidin-3-yl}ethyl) ethanethioate (33)

To a stirred solution of **32** (2.72 g, 10.8 mmol) in THF (20 mL) and triethylamine (2.1 mL, 15.2 mmol) was added methanesulfonyl chloride (1.0 mL, 13.0 mL) at 0 °C under argon atmosphere. Stirring was continued at room temperature for 30 min. To the reaction mixture was added potassium carbonate (3.0 g, 21.6 mmol) and a solution of potassium thioacetate (2.5 g, 21.6 mmol) in DMF (50 mL). Stirring was continued at 50 °C for additional 90 min. After being cooled to room temperature, the reaction was quenched with 1 N hydrochloric acid. The reaction mixture was extracted with EtOAc twice. The combined organic layers were washed with H_2O twice, brine, dried over $MgSO_4$ and evaporated to afford a thioacetate **33** as a pale brown oil (2.93 g, 83% in two steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.36 (s, 3H), 3.34 (m, 1H), 3.51–3.63 (m, 3H), 4.04 (m, 1H), 4.12 (m, 1H), 4.35 (m, 1H), 4.57 (s, 2H), 7.27–7.40 (m, 5H).

5.1.6. Butyl 4-({2-[(4*S*)-4-(hydroxymethyl)-2-oxo-1,3-oxazolidin-3-yl]ethyl}thio)butanoate (34)

To a stirred solution of thioacetate **33** (2.92 g, 9.40 mmol) in butanol (6 mL) and DMF (6 mL) was added potassium *tert*-butoxide (1.27 g, 11.3 mmol) and *n*-butyl 4-iodobutanoate (3.57 g, 13.2 mmol) at 0 °C under argon atmosphere. After being stirred at room temperature for 2 h, the reaction was quenched with saturated aq NH_4Cl. The reaction mixture was extracted with EtOAc, washed with H_2O (×2), brine, dried over $MgSO_4$ and evaporated to give an ester as a brown oil (2.84 g). A solution of the above-described ester in thioanisole (5 mL) was treated with trifluoroacetic acid (5 mL) at 50 °C under argon atmosphere for 10 h. The reaction mixture was evaporated. The resulting residue was purified by column chromatography on silica gel (EtOAc) to give a desilylated ester **34** as a colorless oil (1.56 g, 52%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95 (t, J = 7.4 Hz, 3H), 1.43–1.47 (m, 2H), 1.59–1.68 (m, 2H), 1.89–2.00 (m, 2H), 2.43 (t, J = 7.1 Hz, 2H), 2.64 (t, J = 7.1 Hz, 2H), 2.74–2.95 (m, 2H), 3.40 (m, 1H), 3.55 (m, 1H), 3.64 (m, 1H), 3.83 (m, 1H), 3.93 (m, 1H), 4.08 (t, J = 6.9 Hz, 2H), 4.25 (m, 1H), 4.38 (m, 1H).

5.1.7. 4-[(2-{(4*S*)-4-[(1*E*,3*S*)-3-Hydroxy-4-phenylbut-1-enyl]-2-oxo-1,3-oxazolidin-3-yl}ethyl)sulfanyl]butanoic acid (3)

To a stirred solution of the alcohol **34** (500 mg, 1.57 mmol) in EtOAc (3 mL) and *N*,*N*-diisopropylethylamine (0.98 mL, 9.36 mmol) was added a solution of SO_3–Py (750 mg, 4.71 mmol) in DMSO (2.5 mL) at 0 °C under argon atmosphere. After being stirred at the same temperature for 30 min, the reaction was quenched with 1 N hydrochloric acid. The reaction mixture was diluted with EtOAc, washed with brine, dried over $MgSO_4$ and evaporated to yield the corresponding aldehyde as a yellow oil. To a stirred solution of a phosphonate **35a** (455 mg, 1.88 mmol) in THF (15 mL) was added sodium hydride (62.7% in mineral oil, 73.2 mg, 1.88 mmol) in several portions at 0 °C under argon atmosphere and stirring was further continued at ambient temperature for 90 min. To the reaction mixture was added a solution of the above-described aldehyde in THF (15 mL) at 0 °C and stirring was continued at room temperature for 20 min. The reaction was quenched with acetic acid. The reaction mixture was diluted with EtOAc, washed with aq $NaHCO_3$, water, brine, dried over $MgSO_4$ and evaporated to give an enone as a pale yellow oil.

To a stirred solution of the above-described enone (285 mg) in THF (5 mL) was added a solution of (*R*)-2-methyl-*CBS*-oxazaborolidine (1.0 M in toluene, 0.24 mL, 0.24 mmol) at room temperature under argon atmosphere. To the reaction mixture was added dropwise a solution of borane–THF complex (1.0 M in THF, 0.49 mL, 0.49 mmol) over 5 min. The resulting solution was stirred for 1 h, and then treated with MeOH (0.3 mL). Stirring was continued for additional 5 min. The reaction mixture was diluted with EtOAc, washed with 1 N hydrochloric acid, water, aq $NaHCO_3$, brine, and dried over Na_2SO_4. After evaporation, the resulting residue was purified by column chromatography on silica gel (EtOAc) to give an allyl alcohol as a colorless oil.

A solution of the above-described allyl alcohol in EtOH (3 mL), DME (1 mL) and 2 N sodium hydroxide (0.5 mL) was stirred at ambient temperature for 16 h. After neutralization with 2 N hydrochloric acid (0.5 mL) under cooling, the reaction mixture was extracted with EtOAc three times, and the combined organic layers were washed with brine, dried over Na_2SO_4 and evaporated. The resulting residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH, 50:1–20:1) to afford **3** as a white powder

(154 mg, 24% in four steps): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.88–1.92 (m, 2H), 2.60–2.64 (m, 8H), 3.06 (m, 1H), 3.45 (m, 1H), 3.89 (dd, J = 8.1, 6.8 Hz, 1H), 4.30–4.39 (m, 3H), 5.55 (m, 1H), 5.89 (dd, J = 15.6, 5.3 Hz, 1H), 7.24–7.30 (m, 5H); IR (film): 3454, 3026, 2911, 1729, 1704, 1474, 1437, 1217, 1141, 1095, 1021, 874, 764, 697, 484 cm^{-1}; FAB-MS (m/z): 380 (M+H)$^+$; HRMS-FAB (m/z): [M+H]$^+$ calcd for $C_{19}H_{26}NO_5S$, 380.1532; found, 380.1527.

5.1.8. 4-[(2-{(4*S*)-4-[(1*E*,3*S*)-3-Hydroxy-4-(3-methylphenyl)but-1-enyl]-2-oxo-1,3-oxazolidin-3-yl}ethyl)sulfanyl]butanoic acid (4)

Compound **4** was prepared from **34** according to the same procedure as described for the preparation of **3** from **34** as a pale yellow oil (43% in four steps): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.85–1.96 (m, 2H), 2.36 (s, 3H), 2.44–2.89 (m, 8H), 3.11 (m, 1H), 3.45 (m, 1H), 3.91 (dd, J = 8.2, 8.0 Hz, 1H), 4.28–4.51 (m, 3H), 5.57 (ddd, J = 15.4, 8.8, 1.4 Hz, 1H), 5.90 (dd, J = 15.4, 5.2 Hz, 1H), 6.97–7.11 (m, 3H), 7.22 (t, J = 7.4 Hz, 1H); IR (film): 3410, 1732, 1420, 1235, 1028, 761, 704 cm^{-1}; FAB-MS (m/z): 394 (M+H)$^+$; HRMS-FAB (m/z): [M+H]$^+$ calcd for $C_{20}H_{28}NO_5S$, 394.1688; found, 394.1681.

5.1.9. 4-[(2-{(4*S*)-4-[(1*E*,3*S*)-4-(3-Ethylphenyl)-3-hydroxybut-1-enyl]-2-oxo-1,3-oxazolidin-3-yl}ethyl)sulfanyl]butanoic acid (5)

Compound **5** was prepared from **34** according to the same procedure as described for the preparation of **3** from **34** as a pale yellow oil (50% in four steps): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.24 (t, J = 7.5 Hz, 3H), 1.87–1.90 (m, 2H), 2.60–2.70 (m, 9H), 3.10 (m, 1H), 3.40–3.45 (m, 2H), 3.91 (m, 1H), 4.38–4.42 (m, 3H), 5.58 (ddd, J = 15.4, 8.5, 1.2 Hz, 1H), 5.90 (dd, J = 15.4, 5.3 Hz, 1H), 6.98–7.01 (m, 2H), 7.11 (m, 1H), 7.23 (m, 1H); IR (film): 3412, 2963, 2928, 2360, 1732, 1606, 1540, 1444, 1419, 1312, 1232, 1159, 1087, 1026, 976, 912, 797, 762, 704, 667, 517, 472, 456 cm^{-1}; FAB-MS (m/z): 408 (M+H)$^+$; HRMS-FAB (m/z): [M+H]$^+$ calcd for $C_{21}H_{30}NO_5S$, 408.1845; found, 408.1847.

5.1.10. 4-[(2-{(4*S*)-4-[(1*E*,3*S*)-3-Hydroxy-4-(3-propylphenyl)but-1-enyl]-2-oxo-1,3-oxazolidin-3-yl}ethyl)sulfanyl]butanoic acid (6)

Compound **6** was prepared from **34** according to the same procedure as described for the preparation of **3** from **34** as a pale yellow oil (22% in four steps): ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.94 (t, J = 7.3 Hz, 3H) 1.63–1.67 (m, 2H), 1.89–1.92 (m, 2H), 2.55–2.62 (m, 10H), 3.10 (m, 1H), 3.45 (m, 1H), 3.90 (dd, J = 8.1, 6.8 Hz, 1H), 4.30–4.39 (m, 3H), 5.58 (m, 1H), 5.90 (dd, J = 15.5, 5.2 Hz, 1H), 7.00–7.04 (m, 3H), 7.22 (m, 1H); IR (film): 3423, 3022, 2928, 2871, 1731, 1444, 1234, 1027 cm^{-1}; FAB-MS (m/z): 422 (M+H)$^+$; HRMS-FAB (m/z): [M+H]$^+$ calcd for $C_{22}H_{32}NO_5S$, 422.2001; found, 422.2001.

5.1.11. 4-{[2-((4*S*)-4-{(1*E*,3*S*)-3-Hydroxy-4-[3-(trifluoromethyl) phenyl]but-1-enyl}-2-oxo-1,3-oxazolidin-3-yl)ethyl]sulfanyl} butanoic acid (7)

Compound **7** was prepared from **34** according to the same procedure as described for the preparation of **3** from **34** as a pale yellow oil (10% in four steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.88–1.90 (m, 2H), 2.50–2.57 (m, 6H), 2.89–2.92 (m, 2H), 3.10 (m, 1H), 3.46 (m, 1H), 3.88 (dd, J = 8.1, 6.4 Hz, 1H), 4.42 (m, 3H), 5.58 (m, 1H), 5.90 (dd, J = 15.4, 5.1 Hz, 1H), 7.45 (m, 4H); IR (film): 3407, 2924, 1732, 1469, 1447, 1435, 1330, 1190, 1121, 1074, 1026, 977, 801, 763, 705 cm^{-1}; FAB-MS (m/z): 448 (M+H)$^+$; HRMS-FAB (m/z): [M+H]$^+$ calcd for $C_{20}H_{25}F_3NO_5S$, 448.1408; found, 448.1407.

5.1.12. 4-[(2-{(4*S*)-4-[(1*E*,3*S*)-4-(3-Fluorophenyl)-3-hydroxybut-1-enyl]-2-oxo-1,3-oxazolidin-3-yl}ethyl)sulfanyl]butanoic acid (8)

Compound **8** was prepared from **34** according to the same procedure as described for the preparation of **3** from **34** as a pale yellow oil (10% in four steps): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.90–1.94 (m, 2 H), 2.55–2.68 (m, 6 H), 2.80–2.91 (m, 2H), 3.10 (m, 1H), 3.44 (m, 1H), 3.90 (dd, J = 8.1, 6.6 Hz, 1H), 4.34–4.42 (m, 3H), 5.60 (m, 1H), 5.88 (m, 1H), 6.97 (m, 3H), 7.30 (m, 1 H); IR (film): 3410, 2925, 1729, 1631, 1602, 1501, 1436, 1292, 1153, 1047, 953 cm^{-1}; FAB-MS (m/z): 398 (M+H)$^+$; HRMS-FAB (m/z): [M+H]$^+$ calcd for $C_{19}H_{25}FNO_5S$, 398.1437; found, 398.1436.

5.1.13. 4-[(2-{(4*S*)-4-[(1*E*,3*S*)-4-(3-Chlorophenyl)-3-hydroxybut-1-enyl]-2-oxo-1,3-oxazolidin-3-yl}ethyl)sulfanyl]butanoic acid (9)

Compound **9** was prepared from **34** according to the same procedure as described for the preparation of **3** from **34** as a pale yellow oil (11% in four steps): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.87–1.98 (m, 2H) 2.44–2.77 (m, 6H), 2.80–2.84 (m, 2H), 3.10 (m, 1H), 3.46 (m, 1H), 3.89 (dd, J = 8.5, 8.2 Hz, 1H), 4.43 (dd, J = 8.5, 8.2 Hz, 1H), 4.29–4.50 (m, 2H), 5.56 (ddd, J = 15.4, 8.5, 1.4 Hz, 1H), 5.88 (dd, J = 15.4, 5.2 Hz, 1H), 7.10 (m, 1H), 7.20–7.32 (m, 3H); IR (film): 2922, 1731, 1599, 1478, 1429, 1081, 784, 704 cm^{-1}; FAB-MS (m/z): 414 (M+H)$^+$; HRMS-FAB (m/z): [M+H]$^+$ calcd for $C_{19}H_{25}ClNO_5S$, 414.1142; found, 414.1158.

5.1.14. 3-Biphenylacetic acid (36)

To a stirred solution of (3-bromo)phenylacetic acid (4.00 g, 18.26 mmol), Na_2CO_3 (2.13 g, 20.1 mmol) and phenylborinic acid (2.45 g, 20.1 mmol) in DME (45 mL) was added *tetrakis*-triphenylphosphine palladium (200 mg) at room temperature under argon atmosphere. After being stirred at 100 °C for 16 h, the reaction mixture was cooled to room temperature and filtered through Celite to remove the precipitates. The filtrate was diluted with EtOAc, washed with 2 N hydrochloric acid, brine, dried over $MgSO_4$ and evaporated to afford **36** as a pale yellow powder (3.82 g, 99%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.72 (s, 2H), 7.25–7.62 (m, 9H).

5.1.15. 3-(3-Biphenyl)-2-oxopropanephosphonate (35h)

To a stirred solution of *N,O*-dimethyl hydroxylamine hydrochloride (2.64 g, 27.0 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (4.49 g, 27.0 mmol) and triethylamine (3.76 mL, 27.0 mmol) in CH_3CN (30 mL) was added a solution of **36** (3.82 g, 18.0 mmol) in CH_3CN (20 mL) at room temperature under argon atmosphere. After being stirred for 1 h, the reaction was quenched with water. The reaction mixture was diluted with EtOAc, washed with 2 N hydrochloric acid, water, then brine, dried over $MgSO_4$ and evaporated to give a Weinreb amide as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.43–7.27 (m, 9H), 3.84 (s, 2H), 3.63 (s, 3H), 3.21 (s, 3H).

To a stirred solution of dimethyl methylphosphonate (2.32 g, 18.7 mmol) in toluene (40 mL) was added dropwise a solution of *n*-BuLi (1.57 M in hexane, 11.9 mL, 18.7 mmol) at −78 °C under argon atmosphere, and stirring was continued for 1 h at the same temperature. To the reaction mixture was added a solution of the above-described Weinreb amide (18.0 mmol) in toluene (13 mL), and stirring was continued for additional 2 h at the same temperature. The reaction was quenched with acetic acid. The reaction mixture was allowed to warm up to room temperature with stirring, diluted with EtOAc, washed with water, then brine, dried over $MgSO_4$ and evaporated. The resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 2:1–0:1) to give a phosphonate **35h** as a colorless oil (3.12 g, 73% in two steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.14 (d, J = 22.8 Hz, 2H), 3.70 (s, 2H), 3.76 (s, 3H), 3.82 (s, 3H), 7.20–7.62 (m, 9H).

5.1.16. 4-[(2-{(4*S*)-4-[(1*E*,3*S*)-4-(1,1′-Biphenyl-3-yl)-3-hydroxy but-1-enyl]-2-oxo-1,3-oxazolidin-3-yl}ethyl)sulfanyl]butanoic acid (10)

Compound **10** was prepared from **34** according to the same procedure as described for the preparation of **3** from **34** as a pale yel-

low oil (40% in four steps): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.84–1.90 (m, 2H), 2.48–2.53 (m, 6H), 2.92–3.01 (m, 4H), 3.40 (m, 1H), 3.88 (m, 1H), 4.28–4.35 (m, 2H), 4.51 (m, 1H), 5.55 (dd, J = 15.4, 8.6 Hz, 1H), 5.90 (dd, J = 15.4, 5.5 Hz, 1H), 7.17 (m, 1H), 7.49 (m, 8H); IR (film): 3438, 3032, 2921, 1732, 1439, 1419, 1232, 1086, 1024, 976, 759, 729, 703 cm^{-1}; FAB-MS (m/z): 456 (M+H)$^+$.

5.1.17. 3-(3-Trifluoromethoxyphenyl)-2-oxopropanephospho nate (35i)

Compound **35i** was prepared from (3-trifluoromethoxyphenyl)acetic acid according to the same procedure as described for the preparation of **35h** from **36** as a colorless oil (58% in two steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.13 (d, J = 21.0 Hz, 2H), 3.78 (s, 3H), 3.82 (s, 3H), 3.94 (s, 2H), 7.04–7.21 (m, 3H), 7.37 (m, 1H).

5.1.18. 4-{[2-((4*S*)-4-{(1*E*,3*S*)-3-Hydroxy-4-[3-(trifluoromethoxy)phenyl]but-1-enyl}-2-oxo-1,3-oxazolidin-3-yl)ethyl]sulfanyl} butanoic acid (11)

Compound **11** was prepared from **34** according to the same procedure as described for the preparation of **3** from **34** as a pale yellow oil (27% in four steps): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.88–1.91 (m, 2H), 2.55–2.59 (m, 6H), 2.85–2.89 (m, 2H), 3.10 (m, 1H), 3.46 (m, 1H), 3.89 (m, 1H), 4.37–4.44 (m, 3H), 5.59 (ddd, J = 15.4, 8.51, 1.4 Hz, 1H), 5.89 (dd, J = 15.4, 5.3 Hz, 1H), 7.11 (m, 3H), 7.36 (m, 1H); IR (film): 3734, 2925, 2360, 2342, 1869, 1733, 1654, 1614, 1589, 1488, 1446, 1420, 1259, 1217, 1161, 1087, 1026, 1003, 976, 763 cm^{-1}; FAB-MS (m/z): 464 (M+H)$^+$.

5.1.19. 4-{[2-((4*S*)-4-{(1*E*,3*S*)-3-Hydroxy-4-[3-(methoxymethyl) phenyl]but-1-enyl}-2-oxo-1,3-oxazolidin-3-yl)ethyl]sulfanyl} butanoic acid (12)

Compound **12** was prepared from **34** according to the same procedure as described for the preparation of **3** from **34** as a pale yellow oil (18% in four steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.88–1.92 (m, 2H), 2.40–2.65 (m, 8H), 3.20 (m, 1H), 3.39 (m, 1H), 3.43 (s, 3H), 3.92 (m, 1H), 4.41 (m, 3H), 4.47 (s, 2H), 5.63 (m, 1H), 5.92 (dd, J = 15.4, 4.9 Hz, 1H), 7.24 (m, 4H); IR (film): 3842, 3411, 2924, 1732, 1421, 1384, 1311, 1233, 1158, 1087, 1026, 976, 892, 762, 704 cm^{-1}; FAB-MS (m/z): 424 (M+H)$^+$; HRMS-FAB (m/z): [M+H]$^+$ calcd for $C_{21}H_{30}NO_6S$, 424.1794; found, 424.1798.

5.1.20. Methyl (4*S*)-2-oxo-1,3-thiazolidine-4-carboxylate (37)

To a stirred solution of D-cysteine hydrochloride (5.26 g, 30.0 mmol) in 1 N sodium hydroxide (90 mL) was added a solution of triphosgene (8.88 g, 30.0 mmol) in 1,4-dioxane (60 mL) at 0 °C under argon atmosphere. After being stirred at the same temperature for 1 h, the reaction mixture was evaporated. To the resulting residue was added warm acetonitrile. The resulting precipitates were removed by filtration and the filtrate was evaporated. A solution of the resulting residue in EtOAc was treated with diazomethane to give **37** (4.49 g, 93%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.64 (dd, J = 11.4, 5.3 Hz, 1H), 3.68–3.81 (m, 1H), 3.83 (s, 3H), 4.45 (dd, J = 7.9, 5.3 Hz, 1H), 6.05 (m, 1H).

5.1.21. (4*S*)-4-(Hydroxymethyl)-1,3-thiazolidin-2-one (38)

To a stirred solution of **37** (5.30 g, 32.9 mmol) in EtOH (50 mL) was added sodium borohydride (1.38 g, 36.2 mmol) at 0 °C under argon atmosphere. After being stirred at room temperature for 3 h, the reaction was quenched with aq NH_4Cl. The reaction mixture was extracted with EtOAc three times. The combined organic layers were washed with brine, dried over $MgSO_4$ and evaporated. The resulting residue was purified by column chromatography on silica gel (EtOAc/MeOH, 1:0–10:1) to afford **38** as a white powder (1.55 g, 36%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.19 (dd, J = 11.2, 5.1 Hz, 1H), 3.34–3.48 (m, 3H), 3.72 (m, 1H), 4.99 (t, J = 5.7 Hz, 1H), 8.03 (m, 1H).

5.1.22. *S*-{2-[(4*S*)-4-(*tert*-Butylsilyloxymethyl)-2-oxo-1,3-thiaz olidin-3-yl]ethyl} ethanethioate (39)

To a stirred solution of **37** (1.49 g, 11.2 mmol) was added TBS chloride (1.85 g, 12.3 mmol) and imidazole (1.14 g, 16.8 mmol) in DMF (15 mL) at 0 °C under argon atmosphere. After being stirred at room temperature for 30 min, the reaction was quenched with water. The reaction mixture was diluted with EtOAc. The organic layer was washed with aq $NaHCO_3$, brine, dried over $MgSO_4$ and evaporated to give a TBS ether as a colorless oil.

To a stirred solution of the above-described TBS ether in THF (15 mL) was added potassium *tert*-butoxide (1.38 g, 12.3 mmol) at 0 °C under argon atmosphere, and then stirring was continued at 0 °C for 15 min. To the stirred suspension was added ethyl bromoacetate (2.05 g, 12.3 mmol) at 0 °C and stirring was continued at room temperature for additional 1 h. The reaction was quenched with aqueous NH_4Cl. The reaction mixture was extracted with EtOAc three times. The combined organic layers were washed with H_2O (×2), brine, dried over $MgSO_4$ and evaporated to give an ester as a colorless oil (3.97 g).

To a stirred solution of the above-described ester in MeOH (5 mL) and THF (50 mL) was added sodium borohydride (509 mg, 13.4 mmol) at 0 °C under argon atmosphere. After being stirred at room temperature for 3 h, the reaction was quenched with aq NH_4Cl. The reaction mixture was extracted with EtOAc (×3). The combined organic layers were washed with brine, dried over $MgSO_4$ and evaporated to give an alcohol as a colorless oil (3.28 g). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.09 (s, 6H), 0.91 (s, 9H), 2.50 (m, 1H), 3.18 (m, 1H), 3.38–3.48 (m, 2H), 3.64 (m, 1 H), 3.71–3.84 (m, 4H), 3.92–4.01 (m, 1H).

To a stirred solution of the above-described alcohol in THF (15 mL) and triethylamine (2.34 mL, 16.8 mmol) was added methanesulfonyl chloride (0.91 mL, 11.8 mL) at 0 °C under argon atmosphere. Stirring was continued at 0 °C for 1 h. To the reaction mixture was added potassium carbonate (341 mg, 2.47 mmol) and a solution of potassium thioacetate (1.35 g, 11.8 mmol) in DMF (20 mL) and the stirring was continued at 50 °C for additional 16 h. After being cooled to room temperature, the reaction was quenched with 1 N hydrochloric acid. The reaction mixture was extracted with EtOAc (×2). The combined organic layers were washed with H_2O (×2), brine, dried over $MgSO_4$ and evaporated to afford a thioacetate **39** as a pale yellow oil (3.78 g, 96% in five steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.09 (s, 6H), 0.90 (s, 9H), 2.36 (s, 3H), 3.02–3.09 (m, 2H), 3.14–3.32 (m, 2H), 3.38 (dd, J = 11.1, 7.8 Hz, 1H), 3.66–3.84 (m, 3H), 3.93 (m, 1H).

5.1.23. Ethyl 4-({2-[(4*S*)-4-(hydroxymethyl)-2-oxo-1,3-thiazoli din-3-yl]ethyl}thio) butanoate (40)

To a stirred solution of thioacetate **39** (3.78 g, 10.8 mmol) in EtOH (10 mL) and THF (10 mL) was added potassium *tert*-butoxide (1.46 g, 13.0 mmol) and ethyl 4-bromobutyrate (2.54 g, 13.0 mmol) at 0 °C under argon atmosphere. After being stirred at room temperature for 2 h, the reaction was quenched with aq NH_4Cl. The reaction mixture was extracted with EtOAc, washed with water (×2), brine, dried over $MgSO_4$ and evaporated to give a sulfide as a yellow oil.

A solution of the above-described sulfide in THF (10 mL) was treated with TBAF (16.2 mL, 1 M in THF, 16.2 mmol) at room temperature under argon atmosphere for 1 h. After addition of brine, the reaction mixture was extracted with EtOAc (×3). The combined organic layers were dried over $MgSO_4$ and evaporated. The resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 1:1–0:1) to give an alcohol **40** as a colorless oil (2.78 g, 84%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.27 (t, J = 7.1 Hz, 3H), 1.86–1.99 (m, 2H), 2.39–2.45 (m, 3H), 2.60 (t, J = 7.4 Hz, 2H), 2.79 (t, J = 6.6 Hz, 2H), 3.22–3.33 (m, 1H), 3.35–3.47 (m, 2H), 3.58–3.80 (m, 2H), 3.82–4.00 (m, 2H), 4.13 (q, J = 7.1 Hz, 2H).

5.1.24. 4-[(2-{(4*S*)-4-[(1*E*,3*S*)-3-Hydroxy-4-phenylbut-1-enyl]-2-oxo-1,3-thiazolidin-3-yl}ethyl)sulfanyl]butanoic acid (13)

Compound **13** was prepared from **40** according to the same procedure as described for the preparation of **3** from **34** as a yellow oil (30% in four steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.88–1.90 (m, 2H), 2.44–2.59 (m, 6H), 2.88–2.97 (m, 4H), 3.38 (m, 1H), 3.59 (m, 1H), 4.32 (m, 1H), 4.46 (m, 1H), 5.65 (dd, J = 15.4, 8.4 Hz, 1H), 5.86 (dd, J = 15.4, 5.3 Hz, 1H), 7.20–7.30 (m, 5H); IR (film): 3934, 3856, 3844, 3788, 3399, 3060, 3027, 2925, 1713, 1651, 1495, 1440, 1396, 1282, 1211, 1133, 1097, 1030, 977, 912, 841, 747, 702, 666, 607, 524, 470, 456 cm^{-1}; FAB-MS (m/z): 396 $(M+H)^+$; HRMS-FAB (m/z): $[M+H]^+$ calcd for $C_{19}H_{26}NO_4S_2$, 396.1303; found, 396.1303.

5.1.25. 4-[(2-{(4*S*)-4-[(1*E*,3*S*)-4-(3-Ethylphenyl)-3-hydroxybut-1-enyl]-2-oxo-1,3-thiazolidin-3-yl}ethyl)sulfanyl]butanoic acid (14)

Compound **14** was prepared from **40** according to the same procedure as described for the preparation of **3** from **34** as a yellow oil (29% in four steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.23 (t, J = 7.5 Hz, 3H), 1.88–1.92 (m, 2H), 2.48–2.59 (m, 8H), 2.90–2.94 (m, 4H), 3.39 (m, 1H), 3.59 (m, 1H), 4.33 (m, 1H), 4.46 (m, 1H), 5.67 (dd, J = 15.4, 8.4 Hz, 1H), 5.87 (dd, J = 15.4, 5.3 Hz, 1H), 7.00–7.06 (m, 3H), 7.25 (m, 1H); IR (film): 3410, 3105, 3022, 2963, 2929, 2871, 1711, 1650, 1560, 1488, 1440, 1397, 1282, 1211, 1134, 1102, 1031, 976, 911, 849, 797, 736, 704, 663, 584, 504 cm^{-1}; FAB-MS (m/z): 424 $(M+H)^+$; HRMS-FAB (m/z): $[M+H]^+$ calcd for $C_{21}H_{30}NO_4S_2$, 424.1616; found, 424.1612.

5.1.26. 4-[(2-{(4*S*)-4-[(1*E*,3*S*)-3-Hydroxy-4-(3-propylphenyl)but-1-enyl]-2-oxo-1,3-thiazolidin-3-yl}ethyl)sulfanyl]butanoic acid (15)

Compound **15** was prepared from **40** according to the same procedure as described for the preparation of **3** from **34** as a yellow oil (33% in four steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.94 (t, J = 7.3 Hz, 3H), 1.62–1.65 (m, 2H), 1.89–1.93 (m, 2H), 2.56–2.59 (m, 7H), 2.90–2.96 (m, 5H), 3.38 (m, 1H), 3.60 (m, 1H), 4.32 (m, 1H), 4.46 (m, 1H), 5.68 (dd, J = 15.4, 8.4 Hz, 1H), 5.87 (dd, J = 15.4, 5.1 Hz, 1H), 7.00–7.05 (m, 3H), 7.24 (m, 1H); IR (film): 3390, 2956, 2928, 2870, 1709, 1649, 1441, 1397, 1282, 1211, 1134, 1103, 1032, 974, 786, 704 cm^{-1}; FAB-MS (m/z): 438 $(M+H)^+$; HRMS-FAB (m/z): $[M+H]^+$ calcd for $C_{22}H_{32}NO_4S_2$, 438.1773; found, 438.1768.

5.1.27. 4-{[2-((4*S*)-4-{(1*E*,3*S*)-3-Hydroxy-4-[3-(trifluoromethyl)phenyl]but-1-enyl}-2-oxo-1,3-thiazolidin-3-yl)ethyl]sulfanyl}butanoic acid (16)

Compound **16** was prepared from **40** according to the same procedure as described for the preparation of **3** from **34** as a yellow viscous oil (15% in four steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.88–1.90 (m, 2H), 2.73–2.76 (m, 10H), 3.39 (dd, J = 11.2, 7.5 Hz, 1H), 3.61 (m, 1H), 4.34 (m, 1H), 4.49 (m, 1H), 5.68 (m, 1H), 5.85 (m, 1H) 7.40–7.47 (m, 4H); IR (film): 3410, 2926, 2864, 1712, 1649, 1442, 1400, 1330, 1283, 1202, 1162, 1122, 1074, 1034, 977, 801, 704, 663 cm^{-1}; FAB-MS (m/z): 464 $(M+H)^+$; HRMS-FAB (m/z): $[M+H]^+$ calcd for $C_{20}H_{25}F_3NO_4S_2$, 464.1177; found, 464.1174.

5.1.28. 4-[(2-{(4*S*)-4-[(1*E*,3*S*)-4-(3-Fluorophenyl)-3-hydroxybut-1-enyl]-2-oxo-1,3-thiazolidin-3-yl}ethyl)sulfanyl]butanoic acid (17)

Compound **17** was prepared from **40** according to the same procedure as described for the preparation of **3** from **34** as a yellow viscous oil (19% in four steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.90–1.92 (m, 2H), 2.70–2.76 (m, 10H), 3.40 (dd, J = 11.1, 7.6 Hz, 1H), 3.61 (m, 1H), 4.34 (m, 1H), 4.47 (m, 1H), 5.66 (m, 1H), 5.85 (m, 1H), 6.94–6.98 (m, 3H), 7.28 (m, 1H); IR (film): 3407, 2926, 2872, 1718, 1648, 1587, 1488, 1444, 1399, 1281, 1246, 1212, 1140, 1033, 976, 944, 886, 786, 753, 692 cm^{-1}; FAB-MS (m/z): 414 $(M+H)^+$; HRMS-FAB (m/z): $[M+H]^+$ calcd for $C_{19}H_{25}FNO_4S_2$, 414.1209; found, 414.1202.

5.1.29. 4-{[2-((4*S*)-4-{(1*E*,3*S*)-3-Hydroxy-4-[3-(methoxymethyl)phenyl]but-1-enyl}-2-oxo-1,3-thiazolidin-3-yl)ethyl]sulfanyl}butanoic acid (18)

Compound **18** was prepared from **40** according to the same procedure as described for the preparation of **3** from **34** as a yellow viscous oil (65% in four steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.88–1.90 (m, 2H), 2.54–2.57 (m, 6H), 2.80–2.86 (m, 2H), 2.97 (dd, J = 11.2, 6.6 Hz, 1H), 3.07 (m, 1H), 3.39 (m, 1H), 3.42 (s, 3H), 3.57 (m, 1H), 4.32 (m, 1H), 4.45 (s, 2H), 4.46 (m, 1H), 5.65 (m, 2H), 5.67 (ddd, J = 15.3, 8.4, 1.3 Hz, 1H), 5.86 (dd, J = 15.3, 5.1 Hz, 1H), 7.13 (m, 1H), 7.20–7.24 (m, 2H), 7.29 (m, 1H); IR (film): 3409, 2926, 1725, 1720, 1654, 1649, 1440, 1386, 1311, 1281, 1210, 1093 cm^{-1}; FAB-MS (m/z): 440 $(M+H)^+$; HRMS-FAB (m/z): $[M+H]^+$ calcd for $C_{21}H_{30}NO_5S_2$, 440.1565; found, 440.1564.

5.1.30. Butyl 7-[(2*R*)-2-(*tert*-butyldimethylsilyloxymethyl)-5-thioxo-1-pyrrolidinyl]heptanoate (42)

To a stirred solution of **41** (3.28 g, 11.0 mmol) was added TBS chloride (1.82 g, 12.1 mmol) and imidazole (1.12 g, 16.5 mmol) in DMF (20 mL) at 0 °C under argon atmosphere. After being stirred at room temperature for 30 min, the reaction was quenched with H_2O. The reaction mixture was diluted with EtOAc, washed with aqueous $NaHCO_3$, brine, dried over $MgSO_4$ and evaporated to give TBS ether as a colorless oil (5.25 g).

To a stirred solution of the above-described TBS ether in toluene (12 mL) was added Lawesson's reagent (2.67 g, 6.6 mmol) at room temperature under argon atmosphere. After being stirred at 50 °C for 2 h, the reaction was quenched with water. The reaction mixture was diluted with EtOAc, washed with aqueous $NaHCO_3$, brine, and dried over $MgSO_4$. The combined organic layers were dried over $MgSO_4$ and evaporated. The resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 9:1–4:1) to give **42** as a dark green oil (3.47 g, 74%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.04 (s, 6H), 0.06 (s, 3H), 0.86–0.91 (m, 9H), 0.95 (t, J = 7.2 Hz, 3H), 1.32–1.46 (m, 6H), 1.55–1.68 (m, 8H), 1.92 (m, 1H), 2.30 (t, J = 7.4 Hz, 2H), 2.95 (m, 1H), 3.30 (m, 1H), 3.62–3.83 (m, 2H), 3.96 (m, 1H), 4.09 (t, J = 7.2 Hz, 2H), 4.25 (m, 1H).

5.1.31. Butyl 7-[(2*R*)-2-(hydroxymethyl)-5-thioxo-1-pyrrolidinyl]heptanoate (43)

A solution of **42** (3.47 g, 8.09 mmol) in THF (8 mL) was treated with TBAF (8.9 mL, 1 M in THF, 8.9 mmol) at room temperature under argon atmosphere for 1 h. After addition of brine, the reaction mixture was extracted with EtOAc (×2). The combined organic layers were dried over $MgSO_4$ and evaporated. The resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 4:1–1:1) to give an alcohol **43** as a colorless oil (2.04 g, 80%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.94 (t, J = 7.2 Hz, 2H), 1.25–1.47 (m, 6H), 1.54–1.91 (m, 8H), 1.95 (m, 1H), 2.13 (m, 1H), 2.30 (t, J = 7.4 Hz, 2H), 2.86–3.17 (m, 2H), 3.37 (m, 1H), 3.71 (m, 1H), 3.85 (m, 1H), 3.96–4.13 (m, 3H), 4.24 (m, 1H).

5.1.32. 7-{(2*R*)-2-[(1*E*,3*S*)-3-Hydroxy-4-phenylbut-1-enyl]-5-thioxopyrrolidin-1-yl}heptanoic acid (19)

Compound **19** was prepared from **43** according to the same procedure as described for the preparation of **3** from **34** as a yellow viscous oil (18% in four steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28–1.34 (m, 4H), 1.64–1.70 (m, 5H), 2.24 (m, 1H), 2.35 (t, J = 7.3 Hz, 2H), 2.90–2.98 (m, 5H), 4.07 (m, 1H), 4.37–4.39 (m, 2H), 5.53 (m, 1H), 5.77 (m, 1H), 7.20–7.29 (m, 5H); IR (film): 3398, 2859, 1708, 1495, 1455, 1421, 1263, 1227, 1151, 1124, 1097, 1029, 975, 749, 684 cm^{-1}; FAB-MS (m/z): 376 $(M+H)^+$; HRMS-FAB (m/z): $[M+H]^+$ calcd for $C_{21}H_{30}NO_3S$, 376. 1946; found, 376. 1954.

5.1.33. 7-{(2*R*)-2-[(1*E*,3*S*)-4-(3-Ethylphenyl)-3-hydroxybut-1-enyl]-5-thioxopyrrolidin-1-yl}heptanoic acid (20)

Compound **20** was prepared from **43** according to the same procedure as described for the preparation of **3** from **34** as a yellow viscous oil (18% in four steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.24 (t, J = 7.7 Hz, 3H), 1.30–1.36 (m, 4H), 1.60–1.70 (m, 5H), 2.18–2.23 (m, 2H), 2.34 (t, J = 7.2 Hz, 2H), 2.59–2.66 (m, 2H), 2.90–2.95 (m, 4H), 4.06 (m, 1H), 4.34–4.38 (m, 2H), 5.55 (dd, J = 15.9, 9.2 Hz, 1H), 5.79 (m, 1H), 7.15–7.20 (m, 3H), 7.24 (m, 1H); IR (film): 3377, 3023, 2932, 2859, 1709, 1490, 1457, 1421, 1374, 1321, 1263, 1227, 1153, 1100, 1030, 975 cm^{-1}; FAB-MS (m/z): 404 (M+H)$^+$; HRMS-FAB (m/z): [M+H]$^+$ calcd for $C_{23}H_{34}NO_3S$, 404.2259; found, 404.2255.

5.1.34. 7-{(2*R*)-2-[(1*E*,3*S*)-3-Hydroxy-4-(3-propylphenyl)but-1-enyl]-5-thioxopyrrolidin-1-yl}heptanoic acid (21)

Compound **21** was prepared from **43** according to the same procedure as described for the preparation of **3** from **34** as a yellow viscous oil (14% in four steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.94 (t, J = 7.41 Hz, 3H), 1.32–1.35 (m, 4H), 1.60–1.70 (m, 7H), 2.21–2.24 (m, 2H), 2.34 (t, J = 7.2 Hz, 2H), 2.54–2.57 (m, 2H), 2.95–2.96 (m, 4H), 4.06 (m, 1H), 4.36–4.38 (m, 2H), 5.55 (dd, J = 15.4, 8.6 Hz, 1H), 5.79 (m, 1H), 7.00–7.04 (m, 3H), 7.23 (m, 1H); IR (film): 3374, 3022, 2931, 2860, 1709, 1489, 1421, 1263, 1208, 1100, 1030, 974, 787, 733, 704 cm^{-1}; FAB-MS (m/z): 418 (M+H)$^+$; HRMS-FAB (m/z): [M+H]$^+$ calcd for $C_{24}H_{36}NO_3S$, 418.2416; found, 418.2413.

5.1.35. 7-((2*R*)-2-{(1*E*,3*S*)-3-Hydroxy-4-[3-(trifluoromethyl) phenyl]but-1-enyl}-5-thioxopyrrolidin-1-yl)heptanoic acid (22)

Compound **22** was prepared from **43** according to the same procedure as described for the preparation of **3** from **34** as a yellow viscous oil (13% in four steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28–1.32 (m, 4H) 1.66–1.68 (m, 5H), 2.20–2.23 (m, 1H), 2.34 (t, J = 7.2 Hz, 2H), 2.98–3.01 (m, 5H), 4.05 (m, 1H), 4.33 (m, 1H), 4.48 (m, 1H), 5.56 (m, 1H), 5.78 (m, 1H), 7.40–7.45 (m, 4H); IR (film): 3046, 2935, 2861, 1710, 1494, 1451, 1422, 1329, 1264, 1227, 1200, 1163, 1122, 1074, 1033, 976, 800, 704 cm^{-1}; FAB-MS (m/z): 444 (M+H)$^+$; HRMS-FAB (m/z): [M+H]$^+$ calcd for $C_{22}H_{29}F_3NO_3S$, 444.1820; found, 444.1817.

5.1.36. 7-{(2*R*)-2-[(1*E*,3*S*)-4-(3-Fuorophenyl)-3-hydroxybut-1-enyl] -5-thioxopyrrolidin-1-yl}heptanoic acid (23)

Compound **23** was prepared from **43** according to the same procedure as described for the preparation of **3** from **34** as a yellow viscous oil (11% in four steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.33–1.36 (m, 4H), 1.68–1.71 (m, 5H), 2.26 (m, 1H), 2.35 (t, J = 7.3 Hz, 2H), 2.90–2.97 (m, 5H), 4.07 (m, 1H), 4.35 (m, 1H), 4.43 (m, 1H), 5.54 (m, 1H), 5.76 (m, 1H), 6.93–6.96 (m, 3H) 7.28 (m, 1H); IR (film): 3376, 3041, 2935, 2860, 1709, 1616, 1587, 1489, 1448, 1421, 1374, 1321, 1250, 1228, 1185, 1142, 1098, 1032, 976, 787, 753, 693 cm^{-1}; FAB-MS (m/z): 394 (M+H)$^+$; HRMS-FAB (m/z): [M+H]$^+$ calcd for $C_{21}H_{29}FNO_3S$, 394.1852; found, 394.1858.

5.1.37. 7-((2*R*)-2-{(1*E*,3*S*)-3-Hydroxy-4-[3-(methoxymethyl) phenyl]but-1-enyl}-5-thioxopyrrolidin-1-yl) heptanoic acid (24)

Compound **24** was prepared from **43** according to the same procedure as described for the preparation of **3** from **34** as a brown oil (64% in four steps). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.20–1.90 (m, 10H), 2.33 (t, J = 7.2 Hz, 2H), 2.15–2.40 (m, 2H), 2.75–3.10 (m, 4H), 3.10–3.38 (m, 1H), 3.43 (s, 3H), 3.85–4.02 (m, 1H), 4.47 (s, 2H), 4.25–4.50 (m, 2H), 5.59 (dd, J = 15.4, 8.4 Hz, 1H), 5.82 (dd, J = 15.4, 5.0 Hz, 1H), 7.10–7.40 (m, 4H); IR (film): 3377, 2931, 2858, 1709, 1490, 1421, 1380, 1263, 1227, 1191, 1156, 1096, 1033, 975, 915, 793, 757, 733, 703 cm^{-1}; FAB-MS (m/z): 420 (M+H)$^+$; HRMS-FAB (m/z): [M+H]$^+$ calcd for $C_{23}H_{34}NO_5S$, 420.2209; found, 420.2208.

5.1.38. Butyl 4-[(2-{(2*R*)-2-[(1*E*,3*S*)-3-(*tert*-butyldimethylsilyl oxy)-4-(3-methylphenyl)but-1-enyl]-5-thioxopyrrolidin-1-yl} ethyl)sulfanyl]butanoate (45)

To a stirred solution of **44** (11.2 g, 25.0 mmol) was added TBS chloride (4.13 g, 27.5 mmol) and imidazole (2.55 g, 37.5 mmol) in DMF (70 mL) at 0 °C under argon atmosphere. After being stirred at room temperature for 30 min, the reaction was quenched with H_2O. The reaction mixture was diluted with EtOAc, and washed with aq $NaHCO_3$, brine, and dried over $MgSO_4$. The organic solvent was removed by evaporation to give TBS ether as a colorless oil (13.5 g).

To a stirred solution of the above-described TBS ether in toluene (100 mL) was added Lawesson's reagent (5.30 g, 13.1 mmol) at room temperature under argon atmosphere. After being stirred at 50 °C for 1 h, the reaction was quenched with H_2O. The reaction mixture was diluted with EtOAc, washed with aq $NaHCO_3$, brine, dried over $MgSO_4$. The combined organic layers were dried over $MgSO_4$ and evaporated. The resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 7:1–3:1) to give **45** as a colorless oil (10.5 g, 76%). ^{1}H NMR (300 MHz, $CDCl_3$): δ −0.15 (s, 3H), −0.07 (s, 3H), 0.86 (s, 9H), 0.94 (t, J = 7.2 Hz, 3H), 1.32–1.46 (m, 2H), 1.56–1.66 (m, 2H), 1.76 (m, 1H), 1.84–2.00 (m, 2H), 2.24 (m, 1H), 2.33 (s, 3H), 2.43 (t, J = 7.2 Hz, 2H), 2.56–2.87 (m, 5H), 2.91–3.07 (m, 2H), 3.26 (m, 1H), 4.08 (s, 3H), 4.25–4.50 (m, 2H), 5.40 (dd, J = 15.2, 6.6 Hz, 1H), 5.73 (dd, J = 15.2, 5.7 Hz, 1H), 6.88–7.07 (m, 3H), 7.16 (m, 1H).

5.1.39. 4-[(2-{(2*R*)-2-[(1*E*,3*S*)-3-Hydroxy-4-(3-methylphenyl) but-1-enyl]-5-thioxopyrrolidin-1-yl}ethyl)sulfanyl]butanoic acid (25)

A solution of **45** (10.5 g, 18.2 mmol) in THF (50 mL) was treated with TBAF (50.0 mL, 1 M in THF, 50.0 mmol) at room temperature under argon atmosphere for 15 h. After addition of water, the reaction mixture was extracted with EtOAc (×3). The combined organic layers were dried over $MgSO_4$ and evaporated to give an alcohol as a colorless oil (7.2 g).

A solution of the above-described allyl alcohol in EtOH (28 mL), THF (28 mL) and 1 N sodium hydroxide (23 mL) was stirred at ambient temperature for 18 h. After neutralization with 1 N hydrochloric acid (23 mL) under cooling, the reaction mixture was extracted with EtOAc (×3). The combined organic layers were washed with brine, dried over Na_2SO_4 and evaporated. The resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 1:1–0:1) to afford **25** as a white powder (5.7 g, 90% in two steps): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.70–2.00 (m, 3H), 2.27 (m, 1H), 2.35 (s, 3H), 2.39–3.10 (m, 12H), 3.37 (m, 1H), 4.13 (m, 1H), 4.38–4.52 (m, 2H), 5.55 (ddd, J = 15.3, 8.7, 1.2 Hz, 1H), 5.82 (dd, J = 15.3, 5.1 Hz, 1H), 6.95–7.11 (m, 3H), 7.22 (dd, J = 7.5, 7.5 Hz, 1H); IR (film): 2922, 1713, 1608, 1487, 1322, 1229, 1161, 1032, 976, 752, 701, 665, 440 cm^{-1}; FAB-MS (m/z): 408 (M+H)$^+$; HRMS-FAB (m/z): [M+H]$^+$ calcd for $C_{21}H_{30}NO_3S_2$, 408.1667; found, 408.1667.

5.2. Biological assays

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, which stably express the prostanoid receptors mEP1–4. Membranes from CHO cells expressing prostanoid receptors were incubated with a radiolabeled ligand (i.e. 2.5 nM [^{3}H]PGE$_2$) and test compounds at various concentrations in an assay buffer (i.e. 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, with the exception of mEP1, which was incubated for 20 min. Incubation was terminated via 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 a 6 mL liquid scintillation (ACSII) mixture with a liquid scintillation counter. Non-specific binding was achieved by adding excess amounts of unlabeled PGE_2 in the assay buffer. The concentration that causes 50% of inhibition (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. Measurement of cAMP production

Chinese hamster ovary (CHO) cells expressing mouse or rat EP4 receptor were cultured in 24-well plates (1×10^5 cells/well). After 2 days, the media were removed and cells were washed with 500 μL of Minimum Essential Medium (MEM) and incubated for 10 min in 500 μL of buffer (MEM containing 2 μM of diclofenac) at 37 °C. After the removal of buffer via suction, cells were pre-incubated in 450 μL of assay medium (containing 1% of BSA) for 10 min at 37 °C. The reaction was started with the addition of each test compound in 50 μL of assay buffer. After incubation for 10 min at 37 °C, the reaction was terminated by adding 500 μL of ice-cold 10% trichloroacetic acid. cAMP production was determined via a cAMP radioimmunoassay kit (Amersham).

5.2.3. LPS-induced changes in plasma TNF-α levels in rats

LPS was dissolved in a sterile saline solution (1 mg/mL), and the test compound was dissolved in a sterile saline solution containing 20% HP-β-CD. Then, the test compound was orally administered to seven-week-old Sprague–Dawley rats (Charles River, Japan). After 30 min, an intravenous administration of LPS (10 μg/2 mL/kg) was given, and 90 min after the LPS injection, blood samples were withdrawn into heparinized syringes via aortic puncture. Following centrifugation at 12,000 rpm for 3 min at 4 °C, plasma was recovered and immediately frozen at 80 °C. Plasma TNF-α concentrations were determined via an ELISA kit (Biosource).

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