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Synthesis and evaluation of γ-lactam analogs of PGE_2 as EP4 and EP2/EP4 agonists

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ABSTRACT

To identify topically effective EP4 agonists and EP2/EP4 dual agonists with excellent subtype selectivity, further optimization of the 16-phenyl ω-chain moiety of the γ-lactam 5-thia prostaglandin E analog and the 2-mercaptothiazole-4-carboxylic acid analog were undertaken. Rat in vivo evaluation of these newly identified compounds as their poly (lactide-co-glycolide) microsphere formulation, from which sustained release of the test compound is possible, led us to discover compounds that showed efficacy in a rat bone fracture healing model after its topical administration without serious influence on blood pressure and heart rate. A structure–activity relationship study is also presented.

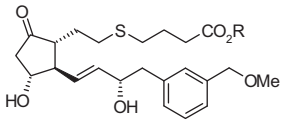
1. Introduction

Prostaglandins (PGs) are a group of lipid mediators that are produced from arachidonic acid in a variety of tissues under various physiological and pathophysiological conditions and serve to maintain local homeostasis.[1] Among them, PGE_2, which is the most well-known prostanoid derivative and exhibits a broad range of biological actions in diverse tissues through binding to specific receptors (EP1, EP2, EP3 and EP4), potently induces bone resorption in bone organ cultures. Repeated injection of PGE_2 also induces bone formation in a variety of animals including humans.[2] Despite its high potency, the use of PGE_2 as a therapeutic agent in the treatment of bone loss has been hindered by unwanted actions because of its nonspecific affinity toward the individual EP receptors. According to recent molecular biology developments, both the EP2 and EP4 receptors, activation of which increases the intracellular cAMP level, are interesting pharmacological targets because of their important regulatory roles in numerous physiological processes including bone metabolism.[2,3]

Natural PGE_2 is susceptible to metabolic inactivation: 15-OH oxidation, β-oxidation of the α-chain, and oxidation of the ω-chain terminus. Furthermore, PGE_2 is chemically unstable because of the easy elimination of the 11-OH. Several research groups have investigated strategies for improving the pharmacological properties of PGE_2.[4–7]

In a previous paper, we reported PGE analogs **1** and **2** (Fig. 1) as the highly potent EP4 selective agonists.[8] In the early stage of this project, we tried to prepare the poly (lactide-co-glycolide) (PLGA) microsphere formulation of the methyl ester **1** for its sustained release during topical evaluation. This attempt was not successful because of the low lipophilicity and instability of **1** as described above for PGE_2. In order to evaluate the in vivo efficacy in a bone fracture healing model, the sustained release of a chemically and metabolically more stable EP4 selective agonist was absolutely needed. Previously, we reported the discovery of chemically and metabolically stable γ-lactam analogs of PGE as EP4 subtype-selective agonists and the EP2/EP4 dual agonists.[9–11]

In this report, we describe the synthesis and evaluation of two series of γ-lactam PGE analogs **3** and **4** (Fig. 2) as an EP4 subtype-selective agonist and EP2/EP4 dual agonist, respectively, and optimization of their sustained release from the PLGA formulation. Some of the representative compounds were evaluated for their in vivo efficacy towards bone fracture healing.



1 R = Me
2 R = H active metabolite

Figure 1. Structure of reported EP4 agonist.

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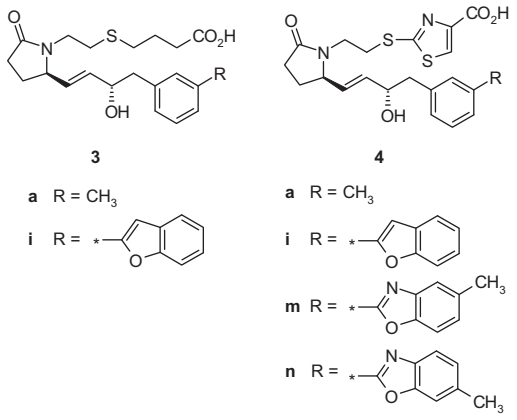


Figure 2. Molecular design of EP4 subtype-selective agonist **3** and EP2/EP4 dual agonist **4** optimized for their PLGA microsphere formulation.

Since evaluation of a subtype-selective agonist in the bone fracture healing model in rats requires 17 days, its sustained release from the carrier after the topical injection was considered to be an important factor necessary for good efficacy. For this reason, evaluation of the in vitro release profile of representative test compounds from PLGA microspheres was also carried out to estimate their systemic sustained release after topical injection.

2. Chemistry

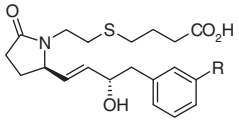
Test compounds listed in Tables 1 and 2 were synthesized as outlined in Schemes 1–5. Most of these compounds were synthesized by the conventional synthetic method for PGs: Horner–Emmons olefination followed by stereoselective reduction of the prochiral enones and then alkaline hydrolysis. Thus, this section starts with a description of the preparation of phosphonates (Fig. 3) for Horner–Emmons olefination.

Phosphonates **5a**, **5c**,**d** and **5g** were prepared as shown in Scheme 1a. Dehydrative condensation of 3-bromophenyl acetic acid with *N*,*O*-dimethylhydroxylamine hydrochloride in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl) and triethylamine afforded **6**, which was converted to **5a** by the reaction with lithium anion prepared from dimethyl methylphosphonate and *n*-butyl lithium. The palladium-catalyzed cross-coupling reaction of **6** with 4-methylphenylboronic acid, 4-methoxyphenylboronic acid or indole-6-boronic acid afforded **5c**, **5d** and **5g**, respectively.

Phosphonates **5j** and **5q**,**r** were prepared as described in Scheme 1b. Compound **8a** was prepared by the same method as described above. The palladium-catalyzed cross-coupling reaction of the corresponding trifluoromethanesulfonate with benzofuran-2-boronic acid afforded **8b**, which was converted to the phosphonate **5j** according to the same procedure as described above. Compound **8a** was converted to the phosphonate **5q** by the following sequential reactions: (1) trifluoromethanesulfonylation with trifluoroacetic acid anhydride and pyridine; (2) palladium-catalyzed condensation with pinacolborane; (3) palladium-catalyzed cross-coupling with 2-bromopyridine; (4) reaction with diethyl methylphosphonate in the presence of *n*-butyl lithium. O-silylation of **8a** with *tert*-butyldimethylsilyl chloride in the presence of imidazole provided **8e**, which was converted to the phosphonate **5r** by the same reaction as described above.

Preparation of phosphonates **5h**, **5l**–**p**, **5i** and **5k** is described in Scheme 1c. Dehydrative condensation of commercially available aminophenols and 3-iodobenzoic acid in the presence of

Table 1
Effect of the ω-chain structure on the activity profiles of 8-aza-5-thia-16aryl PGE_1 analogs



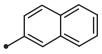
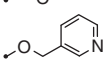
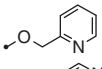
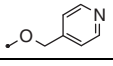
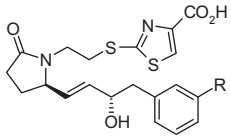
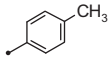
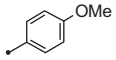
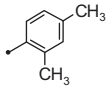
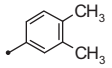
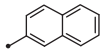
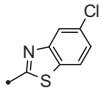
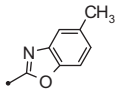
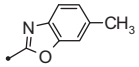
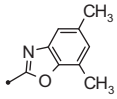
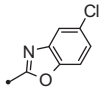
Compound	R	Binding assay (K_i, nM)				Functional assay (EC_{50}, nM)	*c*log*P*
		mEP1	mEP2	mEP3	mEP4	rEP4	
3a	CH_3	$>10^4$	$>10^4$	5800	1.8	5.7	2.2
3b	Ph	$>10^4$	$>10^4$	$>10^4$	1.1	52	3.6
3c		$>10^4$	2100	$>10^4$	0.6	96	4.8
3d		$>10^4$	6100	$>10^4$	4.8	86	2.3
3e		7100	$>10^4$	$>10^4$	4.4	28	3.5
3f		$>10^4$	3500	$>10^4$	0.28	85	3.6
3g		5000	1700	$>10^4$	0.2	13	3.1
3h		$>10^4$	720	$>10^4$	0.36	3.2	3.8
3i		$>10^4$	2000	$>10^4$	0.1	7.0	4.4
3j		$>10^4$	$>10^4$	$>10^4$	4.7	19	1.9
3k		$>10^4$	$>10^4$	$>10^4$	0.89	0.61	1.9
3l		$>10^4$	$>10^4$	$>10^4$	28	46	1.9

Table 2
Effect of the ω-chain structure on the activity profiles of ethyl-linked 2-mercaptothiazole-4-carboxylic acid analogs

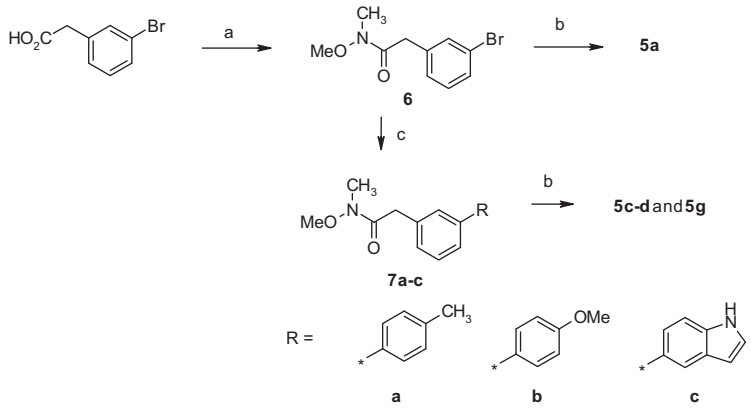


Compound	R	Binding assay (K_i, nM)				Functional assay (EC_{50}, nM)		*c*log*P*
		mEP1	mEP2	mEP3	mEP4	rEP2	rEP4	
4a	CH_3	>10^4	9.3	540	0.41	90	0.79	3.0
4b	Ph	4200	3.0	4300	0.94	1800	8.0	4.4
4c		6100	2.0	>10^4	0.43	380	15	4.9
4d		6300	1.7	>10^4	0.81	260	27	5.1
4e		5400	9.2	>10^4	1.7	4600	15	4.3
4f		2400	23	>10^4	3.5	>10^4	160	5.1
4g		2500	1.5	>10^4	0.35	1400	21	5.3
4h		>10^4	22	>10^4	0.012	>10^4	480	5.6
4i		2700	2.1	1700	0.12	1500	5.3	5.1
4j		>10^4	28	>10^4	0.12	1600	2.2	3.9
4k		8000	15	>10^4	0.19	2700	13	4.5
4l		680	22	>10^4	0.25	>10^4	9.7	5.3
4m		1300	21	>10^4	0.21	>10^4	8.8	4.4
4n		200	56	>10^4	0.16	3200	1.6	4.4
4o		7700	22	>10^4	0.21	8500	28	4.3
4p		3200	24	>10^4	0.15	>10^4	7.7	4.9
4q		650	67	>10^4	0.20	>10^4	4.9	4.6

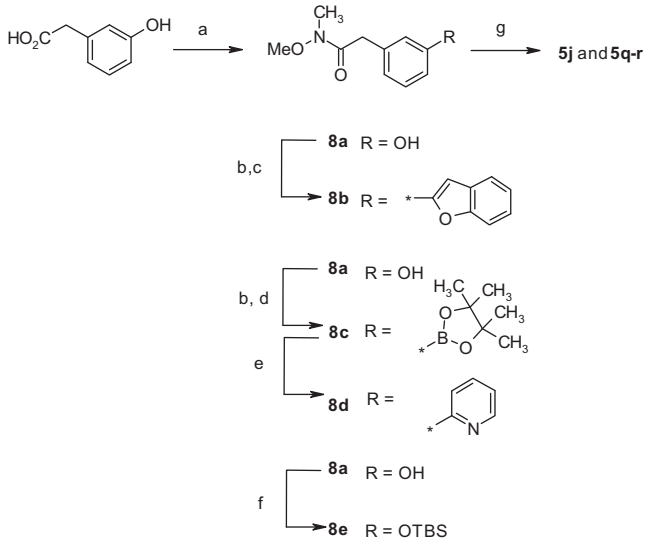
polyphosphoric acid (PPA) afforded oxazoles **9a–f**, respectively, which were converted to **10a–f**, respectively, by the following sequential reactions: (1) reaction with diethyl malonate in the presence of sodium hydride and cuprous iodide; (2) alkaline hydrolysis followed by decarboxylation; (3) condensation reaction with *N*,*O*-dimethylhydroxylamine in the presence of EDC-HCl and triethylamine. Compounds **10a–f** were converted to **5h** and **5l–p**, respectively, according to the same procedure as described above. Dehydrative condensation of the commercially available 2-aminothiophenols with 3-iodobenzoic acid in PPA afforded **9g–h**, respectively, which were converted to the phosphonates **5i** and **5k**, respectively, according to the same procedure as described above for the preparation of the corresponding benzoxazoles.

Phosphonate **5f** was prepared as shown in Scheme 1d. Esterification of 3-aminophenylacetic acid with methanol in the presence of thionyl chloride followed by N,N-dialkylation with 1,2-bis (bromomethyl)benzene afforded **11**, which was converted to the phosphonate **5f** according to the same procedure as described above. Preparation of phosphonates **5b** and **5e** was described in our preceding report.[13]

Synthesis of **3b–h** is outlined in Scheme 2a. Horner–Emmons olefination of the aldehyde **12** with the appropriate phosphonates in the presence of sodium hydride followed by stereoselective (*R*)-



Scheme 1a. Preparation of phosphonates **5a**, **5c**,**d** and **5g**: (a) *N*,*O*-dimethylhydroxylamine hydrochloride, EDC-HCl, Et_3N, CH_3CN; (b) dimethyl methylphosphonate, *n*-BuLi, toluene; (c) 4-methylphenylboronic acid or 4-methoxyphenylboronic acid, $Pd(PPh_3)_4$, or indole-6-boronic acid, $Pd(OAc)_2$, (*S*)-BINAP, aq Na_2CO_3, DME.



Scheme 1b. Preparation of phosphonates **5j** and **5q**,**r**: (a) *N*,*O*-dimethylhydroxylamine hydrochloride, EDC-HCl, Et_3N, CH_3CN; (b) trifluoromethanesulfonic anhydride, pyridine, CH_2Cl_2; (c) benzofuran-2-boronic acid, $PdCl_2(PPh_3)_2$, Et_3N, DMF; (d) pinacolborane, $PdCl_2$(dppf), Et_3N, CH_3CN; (e) 2-bromopyridine, $PdCl_2$(dppf), K_3PO_4, DME; (f) TBSCl, imidazole, DMF; (g) dimethyl methylphosphonate or diethyl methylphosphonate, *n*-BuLi, toluene.

CBS-reduction of the formed enones afforded **13a**–**g**, respectively, alkaline hydrolysis of which afforded **3b**–**h**, respectively.[10,14] Compounds **4c**, **4e** and **4j**,**k** were synthesized as shown in Scheme 2b. Horner–Emmons olefination of the aldehyde **14** with the appropriate phosphonates followed by stereoselective CBS-reduction provided **15a**–**e**, respectively, alkaline hydrolysis of which produced **4c**, **4e** and **4j**,**k**, respectively.[11,12]

Synthesis of **4i** and **4l**–**q** is described in Scheme 3a. Horner–Emmons olefination of the aldehyde **16** with the appropriate phosphonates followed by stereoselective CBS-reduction afforded **17a**–**g**.[11,12] Acidic deprotection of **17a**–**g** followed by reductive amination with the aldehyde **23**, which was prepared as shown in Scheme 3c, in the presence of sodium triacetoxyborohydride resulted in the γ-lactam framework **18a**–**g** accompanied by intramolecular cyclization, alkaline hydrolysis of which yielded **4i** and **4l**–**q**, respectively.

5-Thia-γ-lactam analog **3i** was synthesized as shown in Scheme 3b. Acidic deprotection of **17a** was carried out as described above followed by reductive amination with an aldehyde **19** in the presence of sodium triacetoxyborohydride to afford **20**, alkaline hydrolysis of which produced **3i**.[4]

Preparation of the aldehyde **23** is described in Scheme 3c. Nucleophilic substitution of 2-bromoacetoaldehyde diethylacetal with potassium thioacetate provided **20**, reaction of which with ethyl 2-bromothiazole-4-carboxylate in the presence of potassium carbonate in ethanol afforded **23**.

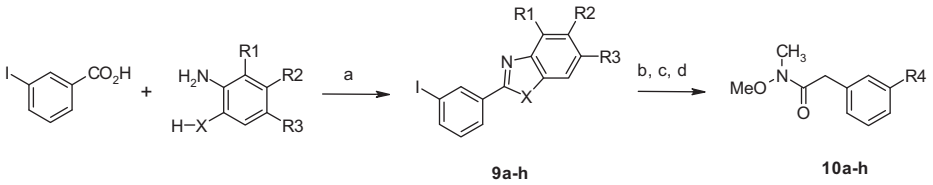
Synthesis of **4f**,**g** is described in Scheme 4. Horner–Emmons olefination of the aldehyde **16** with the phosphonate **5a** in the presence of sodium hydride followed by stereoselective CBS-reduction afforded **24**. Protection of the 15-hydroxy function of **24** as a TBS–ether provided **25**. The palladium-catalyzed cross-coupling reaction of **25** with 2,4-dimethylphenylboronic acid and 3,4-dimethylphenylboronic acid afforded **26a** and **26b**, respectively. Acidic deprotection of **26a**,**b** followed by reductive amination with the aldehyde **23** (Scheme 3c) afforded **27a**,**b** accompanied by intramolecular cyclization. Alkaline hydrolysis of **27a**,**b** yielded **4f**,**g**, respectively.

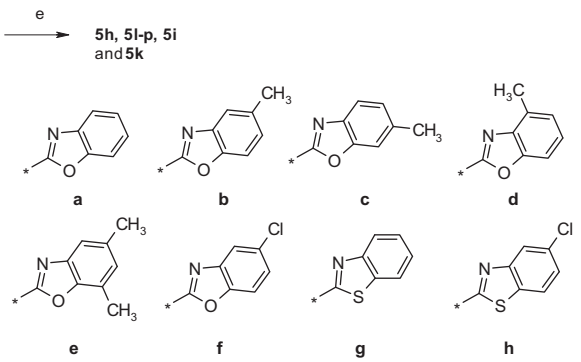
Synthesis of the additional 5-thia analogs **3j**–**l** is outlined in Scheme 5. Horner–Emmons olefination of the aldehyde **12** with the phosphonate **5q** followed by stereoselective CBS-reduction afforded **28**. Protection of the 15-hydroxy group of **28** as a tetrahydropyranyl ether followed by deprotection of the TBS–ether with tetrabutylammonium fluoride afforded **29**. O-alkylation of **29** with 2-hydroxymethylpyridine, 3-hydroxymethylpyridine and 4-hydroxymethylpyridine under Mitsunobu conditions afforded **30a**–**c**, respectively. Acidic deprotection of **30a**–**c** followed by alkaline hydrolysis resulted in **3j**–**l**, respectively.

3. Results and discussion

Compounds listed in Tables 1 and 2 were evaluated for their binding affinity using membrane fractions of CHO cells expressing the mouse EP-receptor. K_i values were determined by a competitive binding assay that was performed according to the method of Kiriyama et al. with minor modifications.[15]

Prior to rat in vivo evaluation, the compounds listed in Tables 1 and 2 were tested for their agonist activity towards rat EP2 and EP4 receptors. Chemical modifications were conducted to concurrently optimize the lipophilicity (*c*log*P*) and activity profiles so that sustained release of the most active compounds from PLGA microspheres could be achieved. Maintaining the optimal α-chains, we continued further chemical modifications of the ω-chain of **3** and **4** to investigate their SAR in more detail. Results are summarized in Tables 1 and 2. As an index of lipophilicity, *c*log*P* values of test compounds were calculated and are also summarized in the tables.





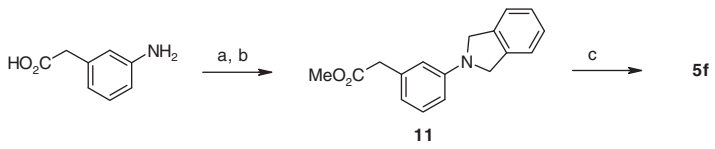
X = O

R1	R2	R3
H	H	H
H	CH_3	H
H	H	CH_3
CH_3	H	H
CH_3	H	CH_3
H	Cl	H

X = S

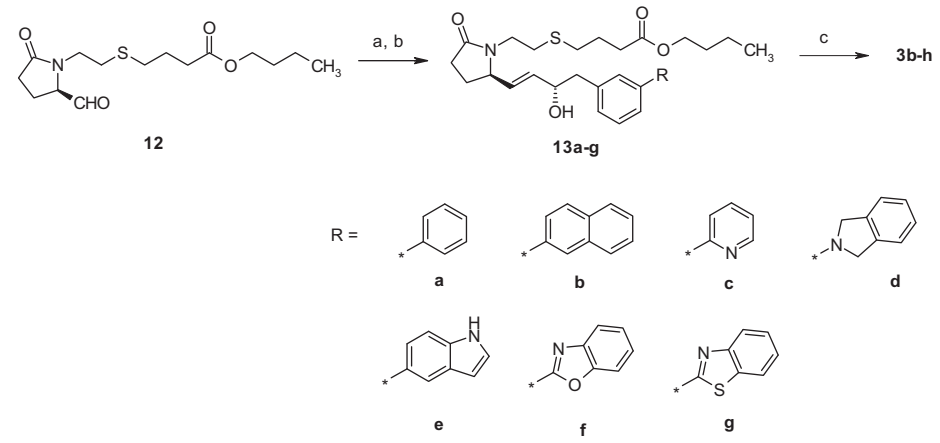
R1	R2	R3
H	H	H
H	Cl	H

Scheme 1c. Preparation of phosphonates **5h, 5l–p**, **5i** and **5k**: (a) 3-iodobenzoic acid, PPA; (b) diethyl malonate, NaH, CuI, DMI; (c) aq NaOH, DME then concd HCl; (d) *N*,*O*-dimethylhydroxylamine hydrochloride, EDC-HCl, Et_3N, CH_3CN; (e) dimethyl methylphosphonate, *n*-BuLi, toluene.

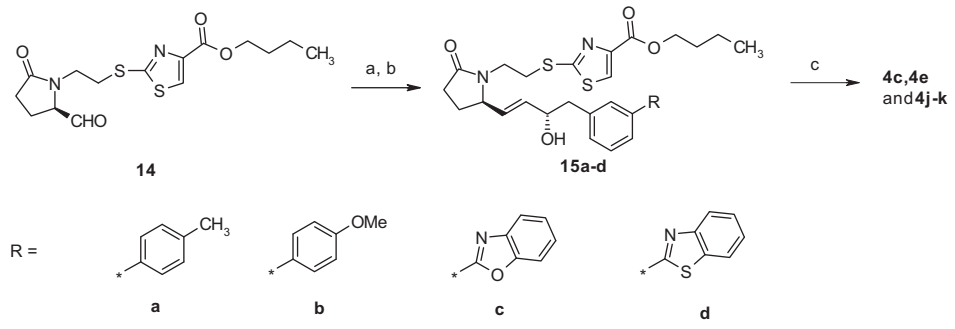


Scheme 1d. Preparation of phosphonate **5f**: (a) thionyl chloride, MeOH; (b) 1,2-dibenzyl bromide, K_2CO_3, DMPU; (c) dimethyl methylphosphonate, *n*-BuLi, toluene.

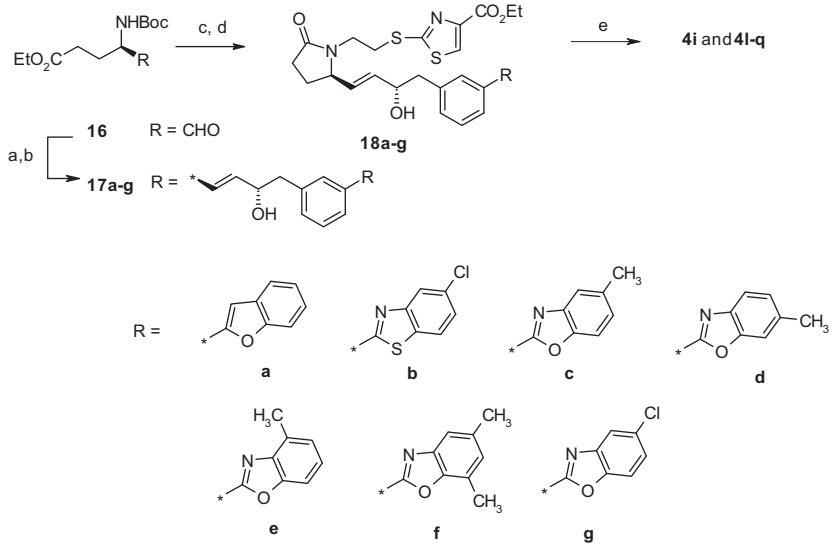
8-Aza-5-thia-16-(3-methyl)phenyl PGE_1 analog **3a**, which is one of the optimized EP4 subtype-selective agonists, was subjected to the above-described chemical modifications. Thus, the purpose of chemically modifying this compound was to identify a structure that shows a biological profile with higher mouse EP4 selectivity and more potent rat EP4 agonist activity with higher lipophilicity. Results are shown in Table 1. Replacement of the 3-methyl residue of **3a** with a phenyl or naphth-2-yl residue afforded **3b** and **3c**, respectively, with reduced rat agonist activities despite increased *c*log*P* values and the retention of the mouse EP4 subtype-selectivity. Replacement of the 3-methyl residue of **3a** with a pyridine-2-yl residue provided **3d** without an increase in the *c*log*P* value. Replacement of the 3-methyl residue of **3a** with a dihydroisoindol-2-yl moiety afforded **3e** with reduced rat agonist activity and an increased *c*log*P* value while it retained good mouse EP4 subtype-selectivity. Replacement of the 3-methyl residue of **3a** with an indol-5-yl residue afforded **3f** with reduced rat agonist activity. As described above, chemical optimization targeting profiles of strong rat EP4 agonist activity with increased lipophilicity and good EP4 subtype-selectivity was difficult. However, the desired profiles were achieved with a series of fused 5–6 heterobiaryl analogs, **3g**, **3h** and **3i**. Replacement of the 3-methyl residue of **3a** with a benzoxazol-2-yl, benzthiazol-2-yl or benzofuran-2-yl residue afforded **3g**, **3h** and **3i**, respectively. All of these three analogs demonstrated excellent mouse EP4 subtype-selectivity and rat agonist activity with increased *c*log*P* values. While the pyridylmethyl analogs **3j** and **3l** showed reductions in mouse EP4 affinity and rat agonist activity, 2-pyridylmethyl analog **3k** showed potent



Scheme 2a. Synthesis of **3b–h**. (a) the corresponding phosphonate, NaH, THF, (b) (*R*)-Me-CBS, BH_3–THF, THF; (c) aq NaOH, MeOH, THF.



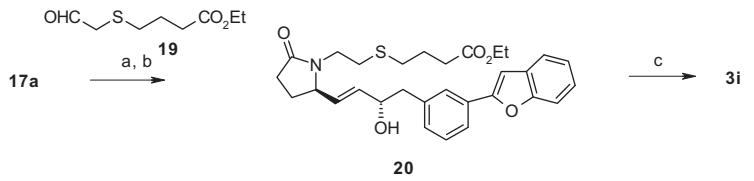
Scheme 2b. Synthesis of **4c, 4e** and **4j**,**k**. (a) The corresponding phosphonate, NaH, THF, (b) (*R*)-Me-CBS, BH_3–THF, THF; (c) aq NaOH, MeOH, THF.



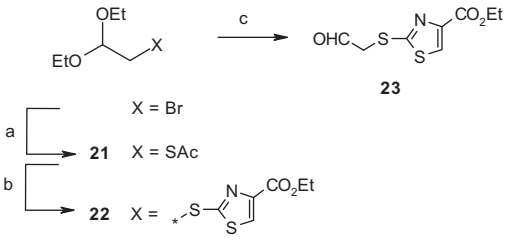
Scheme 3a. Synthesis of **4i** and **4l–q**. (a) the corresponding phosphonate, NaH, THF; (b) (*R*)-Me-CBS, BH_3–THF, THF; (c) HCl in dioxane, EtOH; (d) aldehyde **23**, $NaBH(OAc)_3$, THF, DMF; (e) aq NaOH, MeOH, THF.

mouse EP4 subtype-selectivity and rat agonist activity. Despite low *c*log*P* value of compound **3k**, high entrapping efficiency and/or extended release was expected due to the interaction between the basic residue of **3k** and acidic residue of PLGA micosphere. As a result, **3g**, **3h,** and **3i,** which bear the 5,6-fused heteroaromatic moiety as a ω-chain terminus, and **3k**, which bear the basic residue, were identified as the optimized compounds.

Secondly, 2-mercaptothiazole-4-carboxylic acid analog **4a**, which was identified as one of the optimized EP2/EP4 dual agonists, was also subjected to the above-described chemical modifications. Replacement of the 3-methyl residue of **4a** with a phenyl residue afforded **4b** with retention of the excellent mouse EP2/EP4 dual affinity. Based on the results described above, compounds **4c**–**4g** were also synthesized and evaluated for their mouse EP2/

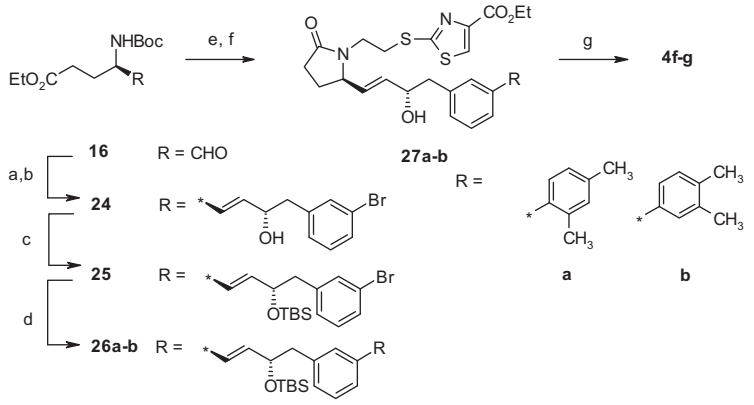


Scheme 3b. Synthesis of **3i**. (a) HCl in dioxane, EtOH; (b) aldehyde **19**, $NaBH(OAc)_3$, THF; (c) aq NaOH, MeOH, THF.

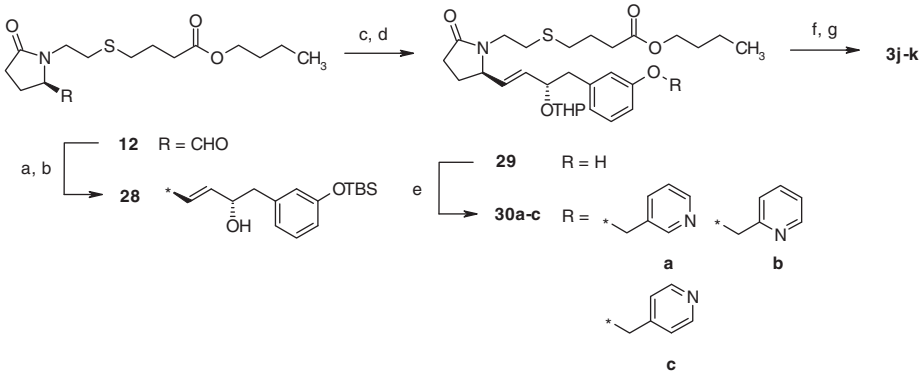


Scheme 3c. Preparation of aldehyde **23**: (a) potassium thioacetate, DMF, (b) ethyl 2-bromothiazole-4-carboxylate, K_2CO_3, EtOH; (c) 2 N HCl, CH_3CN.

EP4 dual selectivity.[12] All of these compounds showed excellent to good mouse EP2/EP4 dual selectivity and increased *c*log*P* values, while 2,4-dimethylphenyl analog **4f** showed reduced affinity for the mouse EP2/EP4 receptors and reduction of rat EP2/EP4 dual agonist activity. All of the listed compounds tended to show remarkable reduction of rat EP2 agonist activity. Compound **4h** exhibited significantly reduced rat EP2/EP4 agonist activity along with its particularly increased mouse EP4 receptor affinity.[12] Replacement of the methyl residue of **4a** with benzofuran-2-yl, benzoxazol-2-yl or benzthiazol-2-yl residues afforded **4i**–**4k**, respectively, with good EP2/EP4 dual selectivity, while they showed good to excellent rat EP4 agonist activity with remarkably reduced rat EP2 agonist activity. Introduction of a chloro or methyl residue into the thiazol-2-yl and oxazol-2-yl residues afforded **4l** and **4m**–**q**, respectively, with retention of mouse EP2/EP4 dual affinity and good rat EP4 agonist activity with remarkably reduced rat EP2 agonist activity. The *c*log*P* values of all the compounds **4b**–**q** were relatively higher than **4a**. Thus, this series of compounds exhibited EP2/EP4 dual subtype-selectivity in a mouse binding assay while they tended to show rat EP4 selective agonist activity because of the remarkably reduced rat EP2 agonist activity. These results indicate that the rat EP2 agonist activity is much more susceptible to the species differences than the rat EP4 agonist activity while the desired mouse EP2/EP4 dual selectivity may be reproduced in the human receptor assay and the functional assay.



Scheme 4. Synthesis of **4f** and **g**. (a) phosphonate **5a**, NaH, THF; (b) (*R*)-Me-CBS, BH_3–THF, THF; (c) *tert*-butyldimethylsilyl chloride, imidazole, DMF; (d) 2,4-dimethylphenylboronic acid or 3,4-dimethylphenylboronic acid, $Pd(OAc)_2$, (*S*)-BINAP, aq Na_2CO_3, DME; (e) HCl in dioxane, EtOH; (f) aldehyde **23**, $NaBH(OAc)_3$, THF; (g) aq NaOH, MeOH, THF.



Scheme 5. Synthesis of **3j–l**. (a) phosphonate **5q**, NaH, THF; (b) (*R*)-Me-CBS, BH_3–THF, THF; (c) DHP, *p*-TsOH, CH_2Cl_2; (d) tetrabutylammonium fluoride, THF; (e) 2-hydroxymethylpyridine or 3-hydroxymethylpyridine or 4-hydroxymethylpyridine, DEAD, PPh_3, THF; (f) 2 N HCl, MeOH; (g) aq NaOH, MeOH, THF.

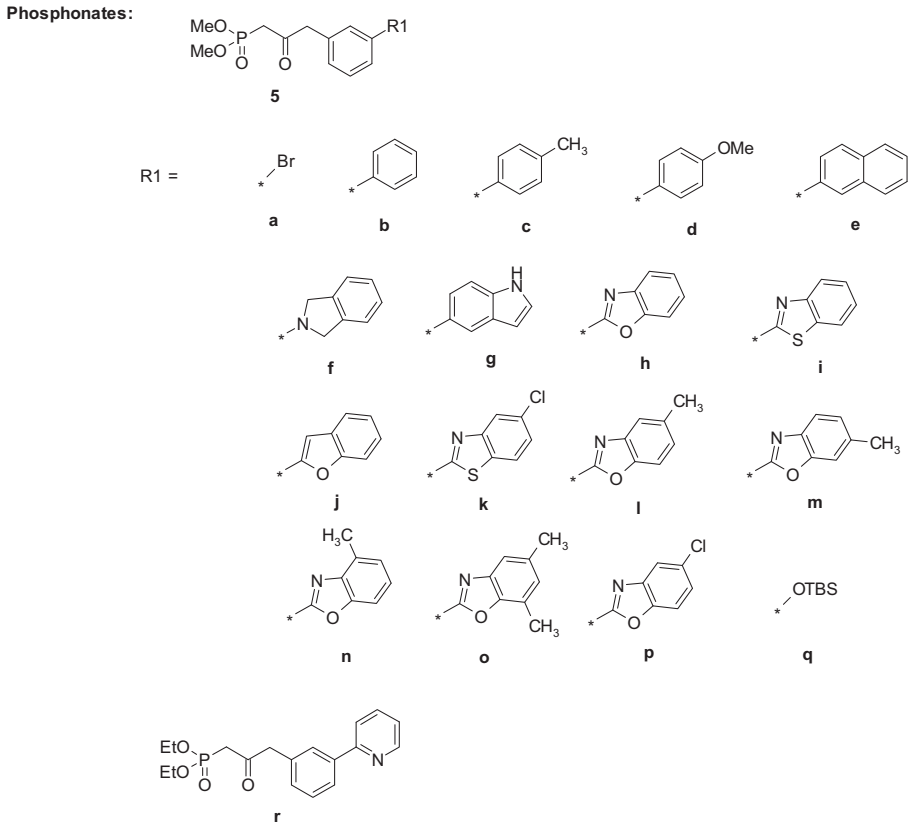


Figure 3. Phosphonates for the synthesis of analogs listed in Tables.

Table 3
PLGA microsphere entrapping efficiency of representative compounds

Compound	Entrapping efficiency (%)
1	22.0
3i	77.3
3k	17.2
4i	59.7
4m	70.2
4n	60.6

Table 4
In vitro release profile of representative compounds from PLGA microspheres

Compound	Remaining (%)				
	Days				
	0	7	14	21	28
3i	100	94.8	91.1	83.5	46.3
4i	100	95.7	88.6	80.1	60.8
4m[a]	100	99.6	93.1	85.2	79.3
4n	100	100	95	86.6	87.6

[a] Containing 5% of methanol in the buffer system because of low solubility of **5m**.

Prior to the rat in vivo evaluation, the compounds were also investigated for their entrapping efficiency from PLGA microspheres. As described in Table 3, compounds **3i**, **4i**, **4m** and **4n**, which showed relatively higher *c*log*P* values, displayed better entrapping efficacy relative to the others while **3k**, which showed a lower *c*log*P* value, displayed a lower entrapping effect. Expected interaction between basic residue of **3k** and acidic residue of PLGA microsphere was not demonstrated.

Compounds **3i**, **4i**, **4m** and **4n** were then evaluated for their in vitro release profiles from PLGA microspheres. As shown in Table 4, the in vitro sustained release of **3i**, **4i**, **4m** and **4n** from PLGA microspheres over 28 days was observed by measuring their remaining percentage in the PLGA microspheres. The remaining percentage of **3i**, **4i**, **4m** and **4n** in PLGA microspheres was 46.3%, 60.8%, 79.3% and 87.6% after 28 days, respectively. These results prompted us to investigate the rat in vivo study of these compounds using their PLGA formulation. According to their remaining percentages (%), a sufficient amount of test compounds remained in the PLGA microspheres even after 28 days of incubation. Thus, successful sustained release of these compounds from PLGA microspheres was observed.

They were also biologically evaluated for their effective dosage for the rat bone fracture healing model. Results are summarized in Table 5. Using the PLGA microsphere formulation described above, the efficacy of **3i**, **4i**, **4m** and **4n** in the rat bone fracture healing model was investigated. Table 5 shows the changes in mechanical properties of the healing fibulae. Seventeen days after the topical injection of the test compounds, the mechanical breaking strength of the fractured fibulae increased in a dose-dependent manner. In the fibulae treated with topical injection of 30, 100, 300 μg/kg of **3i**/PLGA, the breaking strength reached 95.4%, 136%, 154% of nonfractured fibulae, respectively, while it was 64.1% in the vehicle-treated fibulae. Among the 2-mercaptothiazole-4-carboxylic acid analogs, **4i** started to show efficacy at 10 μg/kg of the PLGA formulation. In the fibulae treated with topical injection of 1, 3, 10, 30 μg/kg of **4i**/PLGA, the breaking strength reached 76.1%, 83.3%, 104%, 116% of nonfractured fibulae, respectively, while it was 56.9% in the vehicle-treated fibulae. Based on the results described above, these subtype-selective agonists are expected to be promising drug candidates for treating bone fracture healing.

To evaluate the safety of these subtype-selective agonists, the effect of **3i**/PLGA (300, 1000, 3000 μg/kg), one of the representative

Table 5
Biological evaluation of representative compounds in a rat bone fracture healing model

Compound	Functional assay (EC_{50}, nM)		Breaking strength (% intact) compound/PLGA (μg/kg)						
	rEP2	rEP4	Vehicle	1	3	10	30	100	300
3i/PLGA	NT	7.0	64.1 ± 4.7	NT	NT	NT	95.4 ± 8.8	136.3 ± 9.5*	154 ± 18*
4i/PLGA	1500	5.3	56.9 ± 4.0	76.1 ± 8.2	83.3 ± 6.3	104.2 ± 7.3*	116.6 ± 11.7*	NT	NT
4m/PLGA	$>10^4$	8.8	69.9 ± 16.9	NT	77.7 ± 12.0	87.7 ± 7.7	98.1 ± 5.4	136.0 ± 38.3	144.3 ± 27.6
4n/PLGA	3200	1.6	69.9 ± 16.9	NT	84.4 ± 14.5	87.1 ± 6.4	71.6 ± 8.1	124.6 ± 9.9	167.4 ± 26.7

NT = not tested.
* p <0.05 Dunnett's test, n = 10.

Table 6
Effect of compound **3i**/PLGA on mean blood pressure and heart rate

Biological evaluation	% Change **3i**/PLGA (μg/kg)		
	300	1000	3000
Mean blood pressure	98.4	98.2	97.3
Heart rate	106.3	104.6	111.4

n = 4.

compounds of this series, on blood pressure and heart rate was investigated. Results are summarized in Table 6. At the higher dosages, **3i**/PLGA did not influence blood pressure and heart rate while it slightly increased heart rate at a dose of 3000 μg/kg.

4. Conclusion

In conclusion, two series of compounds were optimized in terms of their PLGA microsphere formulation while retaining EP4 subtype-selectivity and EP2/EP4 dual selectivity with good potency. Sustained release of the test compounds seemed to be successful based on their in vitro release profile from PLGA microspheres. Single topical injection of several representative compounds as their PLGA microsphere formulation was found to be effective in a rat bone fracture healing model. A series of γ-lactam 5-thia PGE analogs and a series of 2-mercaptothiazole-4-carboxylic acid PGE analogs showed EP4 subtype-selectivity and EP2/EP4 dual selectivity in a mouse receptor assay while the series showed EP4 selectivity because of the reduced rat EP2 functional activity of the 2-mercaptothiazole-4-carboxylic acid PGE analogs. More details will be reported in due course.

5. Experimental

5.1. Chemistry

5.1.1. General procedure

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 using deuterated chloroform ($CDCl_3$), deuterated methanol (CD_3OD) and deuterated dimethylsulfoxide (DMSO-d_6) as the solvent. Fast atom bombardment (FABMS, 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. 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; *N*,*N*-dimethylformamide (DMF), dimethylsulfoxide (DMSO), ethanol (EtOH), ethyl acetate (EtOAc), methanol (MeOH), tetrahydrofuran (THF), dichloromethane (CH_2Cl_2), chloroform ($CHCl_3$), dimethoxyethane (DME), acetonitrile (CH_3CN), *tert*-butyl methyl ether (MTBE), 1,3-dimethyl-2-imidazolidinone (DMI), *N*,*N*′-dimethylpropyleneurea (DMPU), tetrabutylammonium fluoride (TBAF).

5.1.2. *N*-Methoxy-*N*-methyl-2-(3-bromophenyl)acetamide (6)

To a stirred solution of *N*,*O*-dimethylhydroxylamine hydrochloride (6.81 g, 69.8 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (11.6 g, 60.5 mmol) and triethylamine (9.71 mL, 60.5 mmol) in CH_3CN (150 mL) was added a solution of (2-bromophenyl)acetic acid (10.0 g, 46.5 mmol) in CH_3CN (50 mL) at room temperature under argon atmosphere. After being stirred for 2 h, the reaction was quenched with water. The reaction mixture was diluted with EtOAc. The organic layer was washed with 2 N HCl, water, brine, and dried over $MgSO_4$. The organic solvent was removed by evaporation to give a Weinreb amide **6** as a colorless oil (8.16 g, 68%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.47–7.43 (m, 1H), 7.41–7.35 (m, 1H), 7.25–7.17 (m, 2H), 3.74 (s, 2H), 3.64 (s, 3H), 3.20 (s, 3H).

5.1.3. Dimethyl 3-(3-bromophenyl)-2-oxopropanephosphonate (5a)

To a stirred solution of dimethyl methylphosphonate (4.94 mL, 45.6 mmol) in toluene (100 mL) was added dropwise a solution of *n*-BuLi (1.57 M in hexane, 47.3 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 **6** (32.6 mmol) in toluene (20 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. The reaction mixture was diluted with EtOAc. The organic layer was washed with water, brine, and dried over $MgSO_4$. The organic solvent was removed by evaporation and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 2:1–0:1) to give a phosphonate **5a** as a yellow oil (7.76 g, 74%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.43–7.38 (m, 2H), 7.25–7.13 (m, 2H), 3.83 (s, 2H), 3.82 (s, 3H), 3.78 (s, 3H), 3.12 (d, J = 22.5 Hz, 2H).

5.1.4. *N*-Methoxy-*N*-methyl-2-(4′-methyl-3-biphenylyl) acetamide (7a)

To a stirred mixture consisting of a solution of **6** (1.20 g, 4.66 mmol) in DME (15 mL) and aqueous Na_2CO_3 (742 mg, 7.00 mmol) in H_2O (12 mL) were added 4-methylphenylboronic acid (951 mg, 7.00 mmol) and tetrakis(triphenylphosphine)palladium (60 mg, 0.052 mmol) under argon atmosphere. After being stirred at 90 °C for 2 h, the reaction mixture was cooled to room temperature. The resulting mixture was filtered through a pad of Celite. The filtrate was evaporated and the resulting residue purified by column chromatography on silica gel (hexane/EtOAc, 3:1–1:1) to afford **7a** as a pale brown solid (1.50 g, 100%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.53–7.43 (m, 3H), 7.41–7.34 (m, 1H), 7.33–7.19 (m, 4H), 3.83 (s, 2H), 3.62 (s, 3H), 3.21 (s, 3H), 2.38 (s, 3H).

5.1.5. Dimethyl [3-(4′-methyl-3-biphenylyl)-2-oxopropyl] phosphonate (5c)

Compound **5c** was prepared from **7a** according to the same procedure as described for the preparation of **5a** from **6** as a colorless oil (1.17 g, 76%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.53–7.37 (m, 4H), 7.29–7.16 (m, 4H), 3.96 (s, 2H), 3.81 (s, 3H), 3.77 (s, 3H), 3.10 (d, J = 22.5 Hz, 2H), 2.36 (s, 3H).

5.1.6. *N*-Methoxy-*N*-methyl-2-(4′-methoxy-3-biphenylyl) acetamide (7b)

Compound **7b** was prepared from **6** according to the same procedure as described for the preparation of **7a** from **6** as a pale yellow solid (1.58 g, 100%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.57–7.32 (m, 4H), 7.2–7.26 (m, 2H), 6.90–7.01 (m, 2H), 3.85 (s, 3H), 3.83 (s, 2H), 3.63 (s, 3H), 3.21 (s, 3H).

5.1.7. Dimethyl [3-(4′-methoxy-3-biphenylyl)-2-oxopropyl] phosphonate (5d)

Compound **5d** was prepared from **7b** according to the same procedure as described for the preparation of **5a** from **6** as a white solid (1.02 g, 63%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.56–7.35 (m, 5H), 7.20–7.13 (m, 1H), 7.02–6.94 (m, 2H), 3.95 (s, 2H), 3.85 (s, 3H), 3.81 (s, 3H), 3.77 (s, 3H), 3.14 (d, J = 22.0 Hz, 2H).

5.1.8. *N*-Methoxy-*N*-methyl-2-(3-(1*H*-indol-5-yl)phenyl) acetamide (7c)

To a stirred solution of the above-described Weinreb amide **6** (500 mg, 1.94 mmol) in DME (11 mL) and 2 M aqueous sodium carbonate (2.90 mL, 5.80 mmol) were added indole-2-boronic acid (623 mg, 3.87 mmol), (*S*)-2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (BINAP) (240 mg, 0.386 mmol) and palladium acetate (44 mg, 0.194 mmol) at under argon atmosphere. After being stirred at 80 °C for 15 h, the reaction mixture was cooled to room temperature. The resulting mixture was filtered through a pad of Celite, and the filtrate was evaporated and purified by column chromatography on silica gel (hexane/EtOAc, 4:1–1:1) to afford **7c** as a brown oil (411 mg, 72%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.23 (br s, 1H), 7.86 (s, 1H), 7.61–7.49 (m, 2H), 7.46–7.44 (m, 2H), 7.38 (t, J = 7.7 Hz, 1H), 7.29–7.21 (m, 2H), 6.64–6.57 (m, 1H), 3.85 (s, 2H), 3.62 (s, 3H),3.21 (s, 3H).

5.1.9. Dimethyl {3-[3-(1*H*-indol-5-yl)phenyl]-2-oxopropyl} phosphonate (5g)

Compound **5g** was prepared from **7c** according to the same procedure as described for the preparation of **5a** from **6** as a white solid (137 mg, 28%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.28 (br s, 1H), 7.81–7.87 (m, 1H), 7.57 (d, J = 7.69 Hz, 1H), 7.37–7.52 (m, 4H), 7.21–7.28 (m, 1H), 7.17 (d, J = 7.42 Hz, 1H), 6.57–6.63 (m, 1H), 3.96 (s, 2H), 3.81 (s, 3H), 3.77 (s, 3H), 3.15 (d, J = 3.1 Hz, 2H).

5.1.10. 2-(3-Hydroxyphenyl)-*N*-methoxy-*N*-methylacetamide (8a)

Compound **8a** was prepared from 3-hydroxyphenylacetic acid according to the same procedure as described for the preparation of **6** from 3-bromophenylacetic acid as a white solid (12.9 g, 100%).

5.1.11. 2-[3-(1-Benzofuran-2-yl)phenyl]-*N*-methoxy-*N*-methylacetamide (8b)

To a stirred solution of **8a** (12.9 g, 66.0 mmol) in CH_2Cl_2 (130 mL) and pyridine (6.4 mL, 79 mmol) was added trifluoromethanesulfonic anhydride (10.3 mL, 73 mmol) at 0 °C under argon atmosphere. After being stirred for 15 min, the reaction mixture was poured into cold 1 N HCl, and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$. The organic solvent was removed by evaporation to give a trifluoromethanesulfonate as a pale brown oil.

To a stirred solution of the above-described trifluoromethanesulfonate (66 mmol) in DMF (70 mL) were added triethylamine (36 mL, 260 mmol), benzofuran-2-boronic acid (21.0 g, 130 mmol), bis(triphenylphosphine)palladium dichloride (4.2 g, 5.98 mmol) under argon atmosphere. After being stirred at 60 °C for 15 h, the reaction mixture was cooled to room temperature, and was filtered through a pad of Celite. The filtrate was evaporated and purified by column chromatography on silica gel (hexane/EtOAc, 2:1) to afford **8b** as a pale brown oil (15.1 g, 77% in three steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.84–7.71 (m, 3H), 7.69–7.61 (m, 1H), 7.61–7.50 (m, 1H), 7.46–7.35 (m, 1H), 7.34–7.19 (m, 2H), 7.05–7.00 (m, 1H), 3.84 (s, 2H), 3.65 (s, 3H), 3.23 (s, 3H).

5.1.12. Dimethyl {3-[3-(1-benzofuran-2-yl)phenyl]-2-oxopropyl} phosphonate (5j)

Compound **5j** was prepared from **8b** according to the same procedure as described for the preparation of **5a** from **6** (Scheme 1a) as a yellow oil (13.9 g, 76%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.82–7.72 (m, 2H), 7.62–7.49 (m, 2H), 7.43 (t, J =7.69 Hz, 1H), 7.34–7.18 (m, 3H), 7.06–7.02 (m, 1H), 3.98 (s, 2H), 3.83 (s, 3H), 3.79 (s, 3H), 3.16 (d, J = 22.3 Hz, 2H).

5.1.13. *N*-Methoxy-*N*-methyl-2-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]acetamide (8c)

To a stirred solution of **8a** (4.87 mmol) in CH_3CN (20 mL) and triethylamine (2.04 mL, 14.6 mmol) were added pinacolborane (1.06 mL, 7.31 mmol) and dichloro[1,1′-bis(diphenylphosphino)ferrocene]palladium CH_2Cl_2 complex (200 mg, 0.244 mmol) at under argon atmosphere. After being stirred at 80 °C for 18 h, the reaction mixture was cooled to room temperature. The resulting mixture was filtered through a pad of Celite, and the filtrate was diluted with EtOAc. The organic layer was washed with 1 N HCl, brine and dried over Na_2SO_4. The organic solvent was removed by evaporation and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 3:1–1:1) to give **8c** as a pale brown oil (491 mg, 33%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.73 (dd, J = 15.9, 6.0 Hz, 1H), 5.52 (dd, J = 15.9, 8.4 Hz, 1H), 4.21–4.02 (m, 4H), 3.65 (m, 1H), 3.10 (m, 1H), 2.78–2.50 (m, 4H), 2.45–2.20 (m, 5H), 1.98–1.20 (m, 6H), 0.93 (t, J = 6.9 Hz, 3H), 0.65 (m, 1H), 0.40 (m, 2H), −0.02 (m, 2H).

5.1.14. *N*-Methoxy-*N*-methyl-2-[3-(2-pyridinyl)phenyl] acetamide (8d)

To a stirred solution of the above-described Weinreb amide **8c** (491 mg, 1.61 mmol) in DME (5 mL) and potassium phosphate (1.71 g, 8.05 mmol) were successively added 2-bromopyridine (381 mg, 2.41 mmol) and dichloro[1,1′-bis(diphenylphosphino) ferrocene]palladium CH_2Cl_2 complex (132 mg, 0.161 mmol) under argon atmosphere. After being stirred at 80 °C for 16 h, the reaction mixture was cooled to room temperature. The resulting mixture was filtered through a pad of Celite, and the filtrate was diluted with EtOAc. The organic layer was washed with H_2O, brine and dried over Na_2SO_4. The solvent was removed by evaporation and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 2:1–1:1) to give **8d** as a pale brown oil (289 mg, 70%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.68 (m, 1H), 7.93 (m, 1H), 7.86 (d, J = 7.8 Hz, 1H), 7.74 (m, 2H), 7.44 (t, J = 7.8 Hz, 1H), 7.34 (m, 1H), 7.22 (m, 1H), 3.87 (s, 2H), 3.63 (s, 3H), 3.21 (s, 3H).

5.1.15. Diethyl {2-oxo-3-[3-(2-pyridinyl)phenyl]propyl} phosphonate (5r)

Compound **5r** was prepared from **8d** using diethylmethyl phosphonate instead of dimethylmethyl phosphonate according to the same procedure as described for the preparation of **5a** from **6** (Scheme 1a) as a colorless oil (276 mg, 70%). ^{1}H NMR (300 MHz,

$CDCl_3$): δ 8.70 (m, 1H), 7.90 (m, 2H), 7.76–7.70 (m, 2H), 7.46 (t, J = 7.8 Hz, 1H), 7.32–7.10 (m, 2H), 4.20–4.03 (m, 4H), 4.00 (s, 2H), 3.13 (d, J = 22.8 Hz, 2H), 1.40–1.25 (m, 6H).

5.1.16. *N*-Methoxy-*N*-methyl-2-[3-(*tert*-butyldimethylsilyloxy) phenyl]acetamide (8e)

To a stirred solution of **8a** (12.8 g, 65.7 mmol) in DMF (60 mL) and imidazole (4.47 g, 65.7 mmol) was added *t*-butyldimethylsilyl chloride (9.90 g, 65.7 mmol) at room temperature under argon atmosphere. After being stirred for 1 h, the reaction mixture was diluted with EtOAc. The organic layer was washed with H_2O twice, brine and dried over $MgSO_4$. The solvent was removed by evaporation to give **8e** as a pale brown oil (19.3 g, 95%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.24–7.13 (m, 1H), 6.87–6.65 (m, 3H), 3.82 (s, 2H), 3.81 (s, 3H), 3.77 (s, 3H), 3.09 (d, J = 22.5 Hz, 2H), 0.98 (s, 9H), 0.18 (s, 6H).

5.1.17. Dimethyl {3-[3-(*tert*-butyldimethylsilyloxy)phenyl]-2-oxopropyl}phosphonate (5q)

Compound **5q** was prepared from **8e** according to the same procedure as described for the preparation of **5a** from **6** (Scheme 1a) as a colorless oil (16.3 g, 70%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.24–7.13 (m, 1H), 6.87–6.65 (m, 3H), 3.87–3.70 (m, 8H), 3.09 (d, J = 22.5 Hz, 2H), 0.98 (s, 9H), 0.24–0.12 (m, 6H).

5.1.18. 2-(3-Iodophenyl)-1,3-benzoxazole (9a)

To a stirred mixture of 3-iodobenzoic acid (5.00 g, 20.0 mmol) and polyphosphoric acid (25.0 g) was added 2-aminophenol (2.20 g, 20.2 mmol) under argon atmosphere. After being stirred at 200 °C for 5 h, the reaction mixture was cooled to room temperature. The resulting mixture was diluted with EtOAc and THF and washed with H_2O, saturated aqueous $NaHCO_3$, brine and dried over Na_2SO_4. The organic solvent was removed by evaporation to give **9a** as a pale brown powder (6.16 g, 96%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.66–8.59 (m, 1H), 8.27–8.18 (m, 1H), 7.91–7.83 (m, 1H), 7.82–7.74 (m, 1H), 7.64–7.54 (m, 1H), 7.43–7.34 (m, 2H), 7.31–7.21 (m, 1H).

5.1.19. *N*-Methoxy-*N*-methyl-2-[3-(1,3-benzoxazol-2-yl)phenyl] acetamide (10a)

To a stirred suspension of sodium hydride (63% dispersion in mineral oil, 1.37 g, 35.7 mmol) in DMI (18 mL) was added dropwise a solution of diethyl malonate (5.42 g, 35.7 mmol) at 0 °C under argon atmosphere, and stirring was continued for 20 min at room temperature. To the stirred mixture was successively added **9a** (6.16 g, 19.2 mmol) and copper iodide (6.80 g, 35.7 mmol), and stirring was continued for additional 4 h at 120 °C. The reaction mixture was cooled to room temperature and poured into a mixture of cold saturated aqueous NH_4Cl and EtOAc. The resulting mixture was filtered through a pad of Celite and the filtrate was washed with H_2O, brine, and dried over Na_2SO_4. The organic solvent was removed by evaporation and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 30:1–5:1) to give a diester as a yellow oil (6.27 g, 11.9 mmol).

A solution of the above-described diester (6.27 g, 11.9 mmol) in DME (15 mL) was treated with 2 N NaOH (24 mL, 120 mmol) at 60 °C for 2 h. The resulting reaction mixture was treated with conc. HCl (12 mL) and stirred at 60 °C for additional 2 h. To the reaction mixture was added 5 N NaOH and diisopropyl ether at 0 °C with vigorous stirring. The resulting precipitates were collected by filtration, dissolved in AcOEt and treated with 2 N HCl. The aqueous layer of the filtrate was further extracted with AcOEt after treating again with 2 N HCl. The combined organic layers were washed with H_2O, brine, and dried over Na_2SO_4. The organic solvent was removed by evaporation to give a phenylacetic acid as a beige powder (1.30 g, 43% in two steps).

To a stirred solution of *N,O*-dimethylhydroxylamine hydrochloride (0.53 g, 5.45 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.04 g, 5.45 mmol) and triethylamine (1.52 mL, 10.9 mmol) in CH_3CN (10 mL) was added a solution of the above-described phenylacetic acid (1.15 g, 4.54 mmol) in CH_3CN (3 mL) at room temperature under argon atmosphere. After being stirred for 6 h, the reaction was quenched with water. The reaction mixture was diluted with EtOAc. The organic layer was washed with 2 N HCl, water, brine, and dried over $MgSO_4$. The organic solvent was removed by evaporation to give a Weinreb amide **10a** as a pale brown solid (670 mg, 50%).

5.1.20. Dimethyl {3-[3-(1,3-benzoxazol-2-yl)phenyl]-2-oxopropyl} phosphonate (5h)

Compound **5h** was prepared from **10a** according to the same procedure as described for the preparation of **5a** from **6** as a yellow oil (650 mg, 80%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.19 (d, J = 7.7 Hz, 1H), 8.15–8.10 (m, 1H), 7.83–7.72 (m, 1H), 7.64–7.56 (m, 1H), 7.55–7.48 (m, 1H), 7.44–7.32 (m, 3H), 4.03 (s, 2H), 3.84 (s, 3H), 3.81 (s, 3H), 3.18 (d, J = 22.5 Hz, 2H).

5.1.21. 2-(3-Iodophenyl)-5-methyl-1,3-benzoxazole (9b)

To a stirred mixture of 3-iodobenzoic acid (5.00 g, 20.0 mmol) and polyphosphoric acid (25.0 g) was added 2-amino-4-methylphenol (2.49 g, 20.2 mmol) under argon atmosphere. After being stirred at 200 °C for 5 h, the reaction mixture was cooled to room temperature. The resulting mixture was diluted with EtOAc and THF, and washed with H_2O, saturated aqueous $NaHCO_3$, brine and dried over Na_2SO_4. The organic solvent was removed by evaporation and to give **9b** as a beige powder (5.99 g, 88%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.60 (m, 1H), 8.20 (d, J = 8.0 Hz, 1H), 7.85 (d, J = 8.0 Hz, 1H,) 7.55 (s, 1H), 7.46 (d, J = 8.2 Hz, 1H), 7.33–7.22 (m, 1H), 7.22–7.15 (m, 1H), 2.49 (s, 3H).

5.1.22. *N*-Methoxy-*N*-methyl-2-[3-(5-methyl-1,3-benzoxazol-2-yl)phenyl]acetamide (10b)

To a stirred suspension of sodium hydride (63% dispersion in mineral oil, 1.37 g, 35.7 mmol) in DMI (18 mL) was added dropwise a solution of diethyl malonate (5.42 gm 35.7 mmol) at 0 °C under argon atmosphere, and stirring was continued for 20 min at room temperature. To the reaction mixture were added **9b** (5.98 g, 17.8 mmol) and copper iodide (6.80 g, 35.7 mmol), and stirring was continued for additional 4 h at 120 °C. After being cooled to room temperature, the resulting mixture was poured into a mixture of saturated aqueous NH_4Cl and EtOAc under cooling. The resulting mixture was filtered through a pad of Celite, and the filtrate was washed with H_2O, brine, and dried over Na_2SO_4. The organic solvent was removed by evaporation and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 30:1–5:1) to give a diester as a yellow oil (6.27 g, 96%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.30–8.18 (m, 1H), 7.67–7.60 (m, 1H), 7.57–7.50 (m, 2H), 7.46 (d, J = 8.2 Hz, 1H), 7.36 (d, J = 1.1 Hz, 1H), 7.21–7.13 (m, 1H), 4.73 (s, 1H), 4.31–4.13 (m, 4H), 2.49 (s, 3H), 1.37–1.20 (m, 6H).

A solution of the above-described diester (6.27 g, 17.1 mmol) in DME (30 mL) was treated with 2 N NaOH (36 mL, 72 mmol) at 60 °C for 4 h. The resulting reaction mixture was treated with conc. HCl (8 mL), and stirred at 90 °C for additional 1 h. To the resulting reaction mixture was added 5 N NaOH and diisopropyl ether at 0 °C with vigorous stirring. The resulting precipitates were collected by filtration, dissolved in AcOEt and treated with 2 N HCl. The aqueous layer of the filtrate was further extracted with AcOEt after treating again with 2 N HCl. The combined organic layers were washed with H_2O, brine, and dried over Na_2SO_4. The organic solvent was removed by evaporation and to give a phenylacetic acid as a beige powder (2.53 g, 55%). ^{1}H NMR (300 MHz, $CDCl_3$):

δ 8.25–8.21 (m, 1H), 8.18–8.09 (m, 1H), 7.61–7.57 (m, 1H), 7.54–7.40 (m, 3H), 7.21–7.13 (m, 1H), 3.78 (s, 2H), 2.48 (s, 3H).

To a stirred solution of *N,O*-dimethylhydroxylamine hydrochloride (1.31 g, 13.4 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (2.22 g, 11.6 mmol) and triethylamine (1.87 mL, 13.4 mmol) in CH_3CN (20 mL) was added a solution of the above-described phenylacetic acid (2.53 g, 9.48 mmol) in CH_3CN (3 mL) at room temperature under argon atmosphere. After being stirred for 1 h, the reaction was quenched with water. The resulting reaction mixture was diluted with EtOAc, washed with 2 N HCl, water, then brine, and dried over $MgSO_4$. The organic solvent was removed by evaporation to give a Weinreb amide **10b** as a light yellow powder(2.19 g, 75%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.20–8.16 (m, 1H), 8.16–8.10 (m, 1H), 7.55 (m, 1H), 7.52–7.41 (m, 3H), 7.16 (dd, *J* = 8.2, 1.1 Hz, 1H), 3.88 (s, 2H),3.67 (s, 3H),3.22 (s, 3H), 2.49 (s, 3H).

5.1.23. Dimethyl {3-[3-(5-methyl-1,3-benzoxazol-2-yl)phenyl]-2-oxopropyl}phosphonate (5l)

Compound **5l** was prepared from **10b** according to the same procedure as described for the preparation of **5a** from **6** (Scheme 1a) as a colorless oil (2.10 g, 81%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.20–8.14 (m, 1H), 8.13–8.08 (m, 1H), 7.59–7.37 (m, 4H), 7.21–7.14 (m, 1H), 4.02 (s, 2H), 3.84 (s, 3H), 3.80 (s, 3H), 3.18 (d, *J* = 22.5 Hz, 2H), 2.49 (s, 3H).

5.1.24. 2-(3-Iodophenyl)-6-methyl-1,3-benzoxazole (9c)

Compound **9c** was prepared from 3-iodobenzoic acid and 2-amino-5-methylphenol according to the same procedure as described for the preparation of **9a** from 3-iodobenzoic acid and 2-aminophenol as a gray powder. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.49–8.41 (m, 1H), 8.20–8.14 (m, 1H), 8.02–7.93 (m, 1H), 7.68 (d, *J* = 8.0 Hz, 1H), 7.62–7.5 (m, 1H), 7.40 (t, *J* = 7.8 Hz, 1H), 7.28–7.19 (m, 1H), 2.47 (s, 3H).

5.1.25. *N*-Methoxy-*N*-methyl-2-[3-(6-methyl-1,3-benzoxazol-2-yl)phenyl]acetamide (10c)

Compound **10c** was prepared from **9c** according to the same procedure as described for the preparation of **10a** from **9a** as a brown oil (2.74 g, 60% in two steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.20–8.07 (m, 2H), 7.63 (d, *J* = 8.2 Hz, 1H), 7.51–7.44 (m, 2H), 7.40–7.35 (m, 1H), 7.20–7.13 (m, 1H), 3.88 (s, 2H), 3.67 (s, 3H), 3.22 (s, 3H), 2.51 (s, 3H).

5.1.26. Dimethyl {3-[3-(6-methyl-1,3-benzoxazol-2-yl)phenyl]-2-oxopropyl}phosphonate (5m)

Compound **5m** was prepared from **10c** according to the same procedure as described for the preparation of **5a** from **6** (Scheme 1a) as a brown oil (2.74 g, 60%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.19–8.13 (m, 1H), 8.12–8.07 (m, 1H), 7.63 (d, *J* = 8.2 Hz, 1H), 7.55–7.46 (m, 1H), 7.42–7.35 (m, 2H), 7.21–7.13 (m, 1H), 4.02 (s, 2H), 3.84 (s, 3H), 3.80 (s, 3H), 3.18 (d, *J* = 23.1 Hz, 2H), 2.51 (s, 3H).

5.1.27. 2-(3-Iodophenyl)-4-methyl-1,3-benzoxazole (9d)

Compound **9d** was prepared from 3-iodobenzoic acid and 2-amino-3-methylphenol according to the same procedure as described for the preparation of **9a** from 3-iodobenzoic acid and 2-aminophenol as a gray powder(5.0 g, 74%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.51–8.47 (m, 1H), 8.23–8.17 (m, 1H), 8.03–7.96 (m, 1H), 7.64–7.55 (m, 1H), 7.47–7.29 (m, 2H), 7.26–7.21 (m, 1H), 2.59 (s, 3H).

5.1.28. *N*-Methoxy-*N*-methyl-2-[3-(4-methyl-1,3-benzoxazol-2-yl)phenyl]acetamide (10d)

Compound **10d** was prepared from **9d** according to the same procedure as described for the preparation of **10a** from **9a** as a brown oil (3.86 g, 84% in two steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.25–8.10 (m, 2H), 7.52–7.43 (m, 2H), 7.40 (d, *J* = 8.0 Hz, 1H), 7.25–7.19 (m, 1H), 7.17–7.10 (m, 1H), 3.89 (s, 2H), 3.67 (s, 3H), 3.23 (s, 3H), 2.67 (s, 3H).

5.1.29. Dimethyl {3-[3-(4-methyl-1,3-benzoxazol-2-yl)phenyl]-2-oxopropyl}phosphonate (5n)

Compound **5n** was prepared from **10d** according to the same procedure as described for the preparation of **5a** from **6** (Scheme 1a) as a pale pink powder (2.9 g, 92%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.23–8.10 (m, 2H), 7.56–7.46 (m, 1H), 7.44–7.35 (m, 2H), 7.30–7.20 (m, 1H), 7.18–7.11 (m, 1H), 4.03 (s, 2H), 3.84 (s, 3H), 3.78 (s, 3H), 3.18 (d, *J* = 23.1 Hz, 2H), 2.67 (s, 3H).

5.1.30. 2-(3-Iodophenyl)-5,7-dimethyl-1,3-benzoxazole (9e)

Compound **9e** was prepared from 3-iodobenzoic acid and 2-amino-4,6-dimethylphenol according to the same procedure as described for the preparation of **9a** from 3-iodobenzoic acid and 2-aminophenol as a reddish purple powder (5.67 g, 80%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.49–8.44 (m, 1H), 8.22–8.14 (m, 1H), 8.02–7.94 (m, 1H), 7.44–7.35 (m, 2H), 7.07 (s, 1H), 2.51 (s, 3H), 2.39 (s, 3H).

5.1.31. *N*-Methoxy-*N*-methyl-2-[3-(5,7-dimethyl-1,3-benzoxazol-2-yl)phenyl]acetamide (10e)

Compound **10e** was prepared from **9e** according to the same procedure as described for the preparation of **10a** from **9a** as a red oil (1.30 g, 49%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.22–8.10 (m, 2H), 7.52–7.44 (m, 2H), 7.37 (s, 1H), 6.97 (s, 1H), 3.89 (s, 2H), 3.67 (s, 3H), 3.22 (s, 3H), 2.55 (s, 3H), 2.44 (s, 3H).

5.1.32. Dimethyl {3-[3-(5,7-dimethyl-1,3-benzoxazol-2-yl)phenyl]-2-oxopropyl}phosphonate (5o)

Compound **5o** was prepared from **10e** according to the same procedure as described for the preparation of **5a** from **6** (Scheme 1a) as an orange oil (1.14 g, 74%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.21–8.15 (m, 1H), 8.13–8.08 (m, 1H), 7.56–7.46 (m, 1H), 7.42–7.35 (m, 2H), 6.98 (s, 1H), 4.02 (s, 2H), 3.84 (s, 3H), 3.80 (s, 3H), 3.18 (d, *J* = 22.8 Hz, 2H), 2.55 (s, 3H), 2.45 (s, 3H).

5.1.33. 2-(3-Iodophenyl)-5-chloro-1,3-benzoxazole (9f)

Compound **9f** was prepared from 3-iodobenzoic acid and 2-amino-4-chlorophenol according to the same procedure as described for the preparation of **9a** from 3-iodobenzoic acid and 2-aminophenol as brown powder (6.4 g).

5.1.34. *N*-Methoxy-*N*-methyl-2-[3-(5-chloro-1,3-benzoxazol-2-yl) phenyl]acetamide (10f)

Compound **10f** was prepared from **9f** according to the same procedure as described for the preparation of **10a** from **9a** as a yellow oil (354 mg, 6%).

5.1.35. Dimethyl {3-[3-(5-chloro-1,3-benzoxazol-2-yl)phenyl]-2-oxopropyl}phosphonate (5p)

Compound **5p** was prepared from **10f** according to the same procedure as described for the preparation of **5a** from **6** (in Scheme 1a) as a yellow oil (130 mg, 31%).

5.1.36. 2-(3-Iodophenyl)-1,3-benzothiazole (9g)

Compound **9g** was prepared from 3-iodobenzoic acid and 2-aminothiophenol according to the same procedure as described for the preparation of **9a** from 3-iodobenzoic acid and 2-aminophenol as a reddish purple powder (3.15 g, 93%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.51–8.43 (m, 1H), 8.12–7.98 (m, 2H), 7.96–7.87 (m, 1H), 7.86–7.77 (m, 1H), 7.57–7.46 (m, 1H), 7.45–7.35 (m, 1H), 7.28–7.17 (m, 1H).

5.1.37. *N*-Methoxy-*N*-methyl-2-[3-(1,3-benzothiazol-2-yl) phenyl]acetamide (10g)

Compound **10g** was prepared from **9g** according to the same procedure as described for the preparation of **10a** from **9a** as an orange oil (791 mg, 43% in three steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.10–8.02 (m, 1H), 8.00–7.94 (m, 1H), 7.93–7.87 (m, 1H), 7.53–7.48 (m, 1H), 7.48–7.43 (m, 2H), 7.42–7.34 (m, 1H), 7.26 (s, 1H), 3.88 (s, 2H), 3.67 (s, 3H), 3.22 (s, 3H).

5.1.38. Dimethyl {3-[3-(1,3-benzothiaxazol-2-yl)phenyl]-2-oxopropyl}phosphonate (5i)

Compound **5i** was prepared from **10g** according to the same procedure as described for the preparation of **5a** from **6** (Scheme 1a) as a yellowish-green oil (809 mg, 85%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.11–8.04 (m, 1H), 8.02–7.97 (m, 2H), 7.95–7.88 (m, 1H), 7.56–7.45 (m, 2H), 7.43–7.33 (m, 2H), 4.02 (s, 2H), 3.84 (s, 3H), 3.80 (s, 3H), 3.18 (d, J = 22.8 Hz, 2H).

5.1.39. 2-(3-Iodophenyl)-5-chloro-1,3-benzothiazole (9h)

Compound **9e** was prepared from 3-iodobenzoic acid and 2-amino-4-chlorothiophenol according to the same procedure as described for the preparation of **9a** from 3-iodobenzoic acid and 2-aminophenol as a dark brown powder (8.60 g, 88%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.43–8.38 (m, 1H), 8.24–8.14 (m, 2H), 8.10–8.03 (m, 1H), 7.99–7.92 (m, 1H), 7.57–7.49 (m, 1H), 7.38 (t, J = 7.8 Hz, 1H).

5.1.40. *N*-Methoxy-*N*-methyl-2-[3-(5-chloro-1,3-benzothiazol-2-yl)phenyl]acetamide (10h)

Compound **10h** was prepared from **9h** according to the same procedure as described for the preparation of **10a** from **9a** as dark brown oil (2.24 g, 34% in three steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.05 (m, 1H), 8.01 (m, 1H), 7.97 (m, 1H), 7.81 (d, J = 8.5 Hz, 1H), 7.45 (m, 2H), 7.36 (dd, J = 8.5, 2.2 Hz, 1H), 3.88 (s, 2H), 3.69 (s, 3H), 3.23 (s, 3H).

5.1.41. Dimethyl {3-[3-(5-chloro-1,3-benzothiazol-2-yl)phenyl]-2-oxopropyl}phosphonate (5k)

Compound **5k** was prepared from **10h** according to the same procedure as described for the preparation of **5a** from **6** (Scheme 1a) as an orange oil (2.02 g, 76%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.05 (m, 1H), 7.97 (m, 2H), 7.82 (dd, J = 8.5, 1.0 Hz, 1H), 7.50 (m, 1H), 7.37 (m, 2H), 4.03 (s, 2H), 3.84 (s, 3H), 3.80 (s, 3H), 3.18 (d, J = 23.4 Hz, 2H).

5.1.42. Methyl [3-(1,3-dihydro-2*H*-isoindol-2-yl)phenyl]acetate (11)

To stirred MeOH (20 mL) was added dropwise thionyl chloride (1.46 mL, 20 mmol) at 0 °C under argon atmosphere and stirring was continued for 15 min. To the resulting mixture was added 3-aminophenylacetic acid (3.02 g, 20 mmol). After being stirred at room temperature for 3 h, the reaction mixture was evaporated to give an ester as a beige powder. To a stirred mixture consisting of a solution of the above-described ester (20 mmol) in *N*,*N*′-dimethylpropyleneurea (DMPU) (40 mL) and potassium carbonate (9.11 g, 66 mmol) was added 1,2-bis(bromomethyl)benzene (5.81 g, 22 mmol) at room temperature under argon atmosphere. After being stirred at 60 °C for 4 h, the reaction mixture was cooled to room temperature. The resulting reaction mixture was diluted with EtOAc, washed with H_2O, brine, and dried over Na_2SO_4. The organic layer was evaporated and the resulting residue was recrystallized from MTBE to give **11** as a purple powder (3.14 g, 59% in two steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.43–7.19 (m, 4H), 6.76–6.53 (m, 4H), 4.68 (s, 4H), 3.71 (s, 3H), 3.60 (s, 2H).

5.1.43. Dimethyl {3-[3-(1,3-dihydro-2*H*-isoindol-2-yl)phenyl]-2-oxopropyl}phosphonate (5f)

Compound **5f** was prepared from **11** according to the same procedure as described for the preparation of **5a** from **6** (Scheme 1a) as pale yellow oil (381 mg, 28%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40–7.24 (m, 5H), 6.68–6.47 (m, 3H), 4.65 (s, 4H), 3.87 (s, 2H), 3.82 (s, 3H), 3.78 (s, 3H), 3.13 (d, J = 22.5 Hz, 2H).

5.1.44. Butyl 4-({2-[(2*R*)-2-{(1*E*,3*S*)-4-[1,1′-biphenyl-3-yl]-3-hydroxy-1-buten-1-yl}-5-oxo-1-pyrrolidinyl] ethyl} thio) butanoate (13a)

To a stirred solution of phosphonate **5b** (223 mg, 0.70 mmol) in THF (3 mL) was added sodium hydride (62.3% in mineral oil, 23.1 mg, 0.60 mmol) at 0 °C under argon atmosphere and stirring was continued at ambient temperature for 90 min. To the resulting suspension was added a solution of aldehyde **12** (158 mg, 0.50 mmol) in THF (1 mL) at 0 °C and stirring was continued for 2 h. The reaction was quenched with acetic acid, diluted with EtOAc, washed with water, brine, and dried over $MgSO_4$. The organic solvent was removed by evaporation to give an enone as a pale yellow oil. To a stirred solution of the above-described enone (0.50 mmol) in THF (3.0 mL) was added a solution of (*R*)-2-methyl-CBS-oxazaborolidine (1.0 M in toluene, 0.15 mL, 0.15 mmol) at room temperature under argon atmosphere. To the resulting reaction mixture was added dropwise a solution of borane–THF complex (1.0 M in THF, 0.40 mL, 0.40 mmol) in 5 min. The resulting solution was stirred for 1 h, then treated with MeOH (0.1 mL) and stirring was continued for 5 min. The reaction mixture was diluted with EtOAc, washed with 1 N HCl, water, saturated $NaHCO_3$, brine, and dried over Na_2SO_4. The organic solvent was removed by evaporation, and the resulting residue was purified by column chromatography on silica gel (EtOAc/hexane, 2:1–1:10) to give an allyl alcohol **13a** as a yellow viscous oil (180 mg, 72% in two steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.58 (d, J = 7.0 Hz, 2H), 7.54–7.30 (m, 6H), 7.20 (d, J = 7.3 Hz, 1H), 5.84–5.73 (m, 1H), 5.51 (dd, J = 15.4, 8.8 Hz, 1H), 4.39–4.53 (m, 1H), 4.16–4.01 (m, 3H), 3.67–3.51 (m, 1H), 2.98–2.84 (m, 3H), 2.69–2.10 (m, 10H), 1.97 (m, 1H), 1.93–1.79 (m, 2H), 1.77–1.50 (m, 2H), 1.45–1.29 (m, 2H), 0.92 (t, J = 7.5 Hz, 3H).

5.1.45. 4-[(2-{(2*R*)-2-[(1*E*,3*S*)-4-(1,1′-Biphenyl-3-yl)-3-hydroxybut-1-enyl]-5-oxopyrrolidin-1-yl}ethyl)sulfanyl]butanoic acid (3b)

A solution of **13a** (180 mg, 0.353 mmol) in MeOH (1.5 mL), THF (1.5 mL) and 2 N NaOH (0.27 mL) was stirred at ambient temperature for 5 h. After neutralization with 2 N HCl (0.27 mL) under cooling, the reaction mixture was extracted with EtOAc three times, and the combined organic layers were washed with brine and dried over Na_2SO_4. The organic solvent was removed by evaporation. The resulting residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH, 100:1–20:1) to afford **3b** as a colorless oil (91 mg, 57%). IR (film): 3735, 3386, 3031, 2923, 1725, 1658, 1479, 1454, 1419, 1362, 1217, 1159, 1101, 1026, 976, 903, 798, 759, 729, 702, 667, 616 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.59–7.55 (m, 2H), 7.49–7.33 (m, 6H), 7.17 (d, J = 7.8 Hz, 1H), 5.76 (dd, J = 15.3, 6.0 Hz, 1H), 5.46 (dd, J = 15.3, 8.4 Hz, 1H), 4.45 (m, 1H), 4.09 (m, 1H), 3.57 (m, 1H), 2.98–2.82 (m, 3H), 2.61–2.26 (m, 8H), 2.18 (m, 1H), 1.92–1.78 (m, 2H), 1.63 (m, 1H); MS (FAB) *m/z*: 454 (M+H)$^+$; HRMS-FAB (*m/z*): [M+H]$^+$ calcd for $C_{26}H_{32}NO_4S$, 454.2052; found, 454.2050.

5.1.46. Butyl 4-({2-[(2*R*)-2-{(1*E*,3*S*)-4-[3-(2-naphthyl)phenyl]-3-hydroxy-1-buten-1-yl}-5-oxo-1-pyrrolidinyl]ethyl}thio) butanoate (13b)

Compound **13b** was prepared from **12** using the phosphonate **5e** instead of **5b** according to the same procedure as described for the

preparation of **13a** from **12** as a pale yellow solid (74 mg, 42% in two steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.03 (m, 1H), 7.96–7.82 (m, 3H), 7.73 (m, J = 8.5, 1.6 Hz, 1H), 7.67–7.37 (m, 5H), 7.30–7.17 (m, 1H), 5.81 (dd, J = 15.5, 5.9 Hz, 1H), 5.61–5.43 (m, 1H), 4.54–4.43 (m, 1H), 4.19–3.99 (m, 4H), 3.68–3.42 (m, 4H), 3.00–2.84 (m, 3H), 2.63–2.43 (m, 4H), 2.40–2.11 (m, 5H), 2.00 (m, 1H), 1.92–1.76 (m, 1H), 1.44–1.27 (m, 2H), 0.91 (t, J = 7.40 Hz, 3H).

5.1.47. 4-{[2-((2*R*)-2-{(1*E*,3*S*)-3-Hydroxy-4-[3-(2-naphthyl) phenyl]but-1-enyl}-5-oxopyrrolidin-1-yl)ethyl]sulfanyl} butanoic acid (3c)

Compound **3c** was prepared from **13b** according to the same procedure as described for the preparation of **3b** from **13a** as colorless amorphous (45 mg, 65%). IR (film): 3422, 3054, 2922, 1728, 1658, 1488, 1444, 1418, 1370, 1268, 1238, 1158, 1099, 1030, 973, 891, 859, 822, 791, 749, 706, 661, 625, 607, 570 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.03 (s, 1H), 7.94–7.82 (m, 3H), 7.73 (dd, J = 8.7, 2.1 Hz, 1H), 7.64–7.57 (m, 1H), 7.57–7.40 (m, 4H), 7.21 (d, J = 7.5 Hz, 1H), 5.79 (dd, J = 15.3, 6.0 Hz, 1H), 5.49 (ddd, J = 15.3, 8.7, 1.2 Hz, 1H), 4.54–4.44 (m, 1H), 4.14–4.04 (m, 1H), 3.66–3.52 (m, 1H), 3.00–2.85 (m, 3H), 2.60–2.10 (m, 9H), 1.90–1.60 (m, 3H); MS (FAB) *m/z*: 504 (M+H)$^+$; HRMS-FAB (*m/z*): [M+H]$^+$ calcd for $C_{30}H_{34}NO_4S$, 504.2209; found, 504.2199.

5.1.48. Butyl 4-({2-[(2*R*)-2-{(1*E*,3*S*)-4-(3-pyridin-2-ylphenyl)-3-hydroxy-1-buten-1-yl}-5-oxo-1-pyrrolidinyl]ethyl}thio)butanoate (13c)

Compound **13c** was prepared from **12** using the phosphonate **5r** instead of **5b** according to the same procedure as described for the preparation of **13a** from **12** as a pale yellow solid (105 mg, 48% in two steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.69 (d, J = 4.8 Hz, 1H), 7.92–7.70 (m, 4H), 7.41 (t, J = 7.5 Hz, 1H), 7.38–7.20 (m, 2H), 5.79 (dd, J = 15.0, 5.7 Hz, 1H), 5.51 (dd, J = 15.0, 8.4 Hz, 1H), 4.48 (m, 1H), 4.18–4.00 (m, 3H), 3.60 (m, 1H), 3.00–2.83 (m, 3H), 2.63–2.10 (m, 10H), 2.03 (br s, 1H), 1.87 (m, 2H), 1.78–1.50 (m, 3H), 1.38 (m, 2H), 0.92 (t, J = 7.5 Hz, 3H).

5.1.49. 4-[(2-{(2*R*)-2-[(1*E*,3*S*)-3-Hydroxy-4-(3-pyridin-2-ylphenyl)but-1-enyl]-5-oxopyrrolidin-1-yl}ethyl)sulfanyl] butanoic acid (3d)

Compound **3d** was prepared from **13c** according to the same procedure as described for the preparation of **3b** from **13a** as colorless amorphous (60 mg, 65%). IR (film): 3366, 2924, 1715, 1666, 1590, 1566, 1462, 1436, 1418, 1217, 1155, 1101,1033, 975, 914, 754, 666 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.74 (m, 1H), 7.93 (s, 1H), 7.84 (dt, J = 1.8, 7.8 Hz, 1H), 7.72 (d, J = 7.8 Hz, 1H), 7.65 (d, J = 8.1 Hz, 1H), 7.42 (t, J = 7.5 Hz, 1H), 7.37–7.23 (m, 2H), 5.88 (dd, J = 15.0, 4.5 Hz, 1H), 5.64 (ddd, J = 15.0, 9.0, 1.5 Hz, 1H), 5.45 (br s, 2H), 4.58 (m, 1H), 4.10 (m, 1H), 3.40 (m, 1H), 3.21 (m, 1H), 3.02–2.80 (m, 2H), 2.78–2.10 (m, 9H), 1.99–1.82 (m, 2H), 1.73 (m, 1H); MS (FAB) *m/z*: 455 (M+H)$^+$; HRMS-FAB (*m/z*): [M+H]$^+$ calcd for $C_{25}H_{31}N_2O_4S$, 455.1992; found, 455.2005.

5.1.50. Butyl 4-({2-[(2*R*)-2-{(1*E*,3*S*)-4-[3-(1,3-Dihydro-2*H*-isoindol-2-yl)phenyl]-3-hydroxy-1-buten-1-yl}-5-oxo-1-pyrrolidinyl]ethyl}thio)butanoate (13d)

Compound **13d** was prepared from **12** using the phosphonate **5f** instead of **5b** according to the same procedure as described for the preparation of **13a** from **12** as a colorless oil (86 mg, 53% in two steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.43–7.17 (m, 6H), 6.65–6.46 (m, 2H), 5.81 (dd, J = 15.4, 5.8 Hz, 1H), 5.55 (ddd, J = 15.4, 8.7, 1.1 Hz, 1H), 4.66 (s, 4H), 4.50–4.36 (m, 1H), 4.19–4.01 (m, 3H), 3.73–3.52 (m, 1H), 3.07–2.73 (m, 3H), 2.71–2.13 (m, 10H), 2.01–1.21 (m, 8H), 0.93 (t, J = 7.3 Hz, 3H).

5.1.51. 4-{[2-((2*R*)-2-{(1*E*,3*S*)-4-[3-(1,3-Dihydro-2*H*-isoindol-2-yl)phenyl]-3-hydroxybut-1-enyl}-5-oxopyrrolidin-1-yl)ethyl] sulfanyl}butanoic acid (3e)

Compound **3e** was prepared from **13d** according to the same procedure as described for the preparation of **3b** from **13a** as colorless amorphous (39 mg, 65%). IR (film): 3389, 3043, 2925, 2861, 1913, 1723, 1714, 1660, 1605, 1580, 1497, 1469, 1455, 1418, 1374, 1305, 1260, 1225, 1163, 1096, 1036, 976, 910, 850, 775, 733, 699, 664, 647, 571, 462 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.33 (m, 6H), 6.57 (m, 2H), 5.81 (dd, J = 15.7, 5.5 Hz, 1H), 5.55 (dd, J = 15.7, 8.8 Hz, 1H), 4.78 (m, 4H), 4.49 (m, 1H), 4.12 (m, 1H), 3.59 (m, 1H), 2.72 (m, 13H), 1.79 (m, 3H); MS (FAB) *m/z*: 495 (M+H)$^+$; HRMS-FAB (*m/z*): [M+H]$^+$ calcd for $C_{28}H_{35}N_2O_4S$, 495.2318; found, 495.2330.

5.1.52. Butyl 4-({2-[(2*R*)-2-{(1*E*,3*S*)-4-[3-(1*H*-indol-5-yl)phenyl]-3-hydroxy-1-buten-1-yl}-5-oxo-1-pyrrolidinyl]ethyl}thio) butanoate (13e)

Compound **13e** was prepared from **12** using the phosphonate **5g** instead of **5b** according to the same procedure as described for the preparation of **13a** from **12** as a colorless oil (74 mg, 46% in two steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.42–8.21 (m, 1H), 7.84 (s, 1H), 7.61–7.21 (m, 6H), 7.19–7.10 (m, 1H), 6.67–6.52 (m, 1H), 5.78 (dd, J = 15.5, 5.9 Hz, 1H), 5.48 (dd, J = 15.4, 8.8 Hz, 1H), 4.53–4.37 (m, 1H), 4.20–3.99 (m, 3H), 3.65–3.47 (m, 1H), 3.01–2.77 (m, 3H), 2.66–2.07 (m, 9H), 2.02–1.19 (m, 8H), 0.92 (t, J = 7.3 Hz, 3H).

5.1.53. 4-{[2-((2*R*)-2-{(1*E*,3*S*)-3-Hydroxy-4-[3-(1*H*-indol-5-yl)phenyl]but-1-enyl}-5-oxopyrrolidin-1-yl)ethyl]sulfanyl} butanoic acid (3f)

Compound **3f** was prepared from **13e** according to the same procedure as described for the preparation of **3b** from **13a** as colorless amorphous (40 mg, 60%). IR (film): 3410, 3032, 2925, 1714, 1647, 1604, 1458, 1419, 1348, 1310, 1247, 1161, 1097, 1066, 1042, 1026, 975, 894, 882, 792, 768, 732, 704, 671, 665, 643, 610, 600, 571, 564, 540 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.35 (br s, 1H), 7.84 (m, 1H), 7.46–7.40 (m, 4H), 7.25 (m, 2H), 7.14 (m, 1H), 6.60 (m, 1H), 5.78 (dd, J = 15.4, 5.8 Hz, 1H), 5.46 (ddd, J = 15.4, 8.8, 1.1 Hz, 1H), 4.48 (m, 1H), 4.07 (m, 1H), 3.54 (m, 1H), 2.90 (m, 3H), 2.09–1.80 (m, 13H); MS (FAB) *m/z*: 493 (M+H)$^+$; HRMS-FAB (*m/z*): [M+H]$^+$ calcd for $C_{28}H_{33}N_2O_4S$, 493.2161; found, 493.2170.

5.1.54. Butyl 4-({2-[(2*R*)-2-{(1*E*,3*S*)-4-[3-(1,3-benzoxazol-2-yl)phenyl]-3-hydroxy-1-buten-1-yl}-5-oxo-1-pyrrolidinyl] ethyl}thio)butanoate (13f)

Compound **13f** was prepared from **12** using the phosphonate **5h** instead of **5b** according to the same procedure as described for the preparation of **13a** from **12** as a colorless oil (80 mg, 45% in two steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.20–8.08 (m, 2H), 7.77 (m, 1H), 7.60 (m, 1H), 7.54–7.32 (m, 4H), 5.81 (dd, J = 15.1, 6.6 Hz, 1H), 5.60–5.48 (m, 1H), 4.49 (br s, 1H), 4.17–4.01 (m, 3H), 3.68–3.53 (m, 1H), 3.49 (s, 1H), 3.01–2.89 (m, 3H), 2.69–2.47 (m, 5H), 2.45–2.30 (m, 5H), 2.10–2.05 (m, 1H), 1.93–1.78 (m, 3H), 1.12–1.05 (m, 1H), 0.91 (t, J = 7.2 Hz, 3H).

5.1.55. 4-{[2-((2*R*)-2-{(1*E*,3*S*)-4-[3-(1,3-Benzoxazol-2-yl)phenyl] -3-hydroxybut-1-enyl}-5-oxopyrrolidin-1-yl)ethyl]sulfanyl} butanoic acid (3g)

Compound **3g** was prepared from **13f** according to the same procedure as described for the preparation of **3b** from **13a** as colorless amorphous (45 mg, 60%). IR (film): 3423, 3060, 2923, 2854, 1945, 1899, 1727, 1660, 1552, 1491, 1473, 1454, 1421, 1359, 1244, 1175, 1109, 1032, 1002, 977, 930, 879, 803, 793,

762, 747, 726, 698, 684 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.31 (s, 1H), 8.08 (d, J = 7.8 Hz, 1H), 7.82–7.74 (m, 1H), 7.64–7.56 (m, 1H), 7.48 (t, J = 7.8 Hz, 1H), 7.44–7.34 (m, 3H), 5.89 (dd, J = 15.6, 4.5 Hz, 1H), 5.63 (dd, J = 15.6, 7.5 Hz, 1H), 4.65–4.55 (m, 1H), 4.20–4.05 (m, 1H), 3.55–3.40 (m, 1H), 3.30–3.10 (m, 1H), 3.30 (m, 1H), 2.89 (m, 1H), 2.75–2.15 (m, 9H), 1.95–1.85 (m, 2H), 1.80–1.60 (m, 1H); MS (FAB) *m/z*: 495 $(M+H)^+$; HRMS-FAB (*m/z*): $[M+H]^+$ calcd for $C_{27}H_{31}N_2O_5S$, 495.1954; found, 495.1954.

5.1.56. Butyl 4-({2-[(2*R*)-2-{(1*E*,3*S*)-4-[3-(1,3-benzothiazol-2-yl) phenyl]-3-hydroxy-1-buten-1-yl}-5-oxo-1-pyrrolidinyl]ethyl} thio)butanoate (13g)

Compound **13g** was prepared from **12** using the phosphonate **5i** instead of **5b** according to the same procedure as described for the preparation of **13a** from **12** as a pale yellow oil (84 mg, 47% in two steps).

5.1.57. 4-{[2-((2*R*)-2-{(1*E*,3*S*)-4-[3-(1,3-Benzothiazol-2-yl)phen yl]-3-hydroxybut-1-enyl}-5-oxopyrrolidin-1-yl)ethyl]sulfanyl} butanoic acid (3h)

Compound **3h** was prepared from **13g** according to the same procedure as described for the preparation of **3b** from **13a** as a pale yellow amorphous powder (39 mg, 54%). IR (film): 3425, 3083, 3060, 3026, 2923, 2852, 1727, 1658, 1508, 1493, 1484, 1453, 1437, 1419, 1364, 1314, 1238, 1158, 1030, 976, 906, 760, 730, 697, 668, 615, 569, 560, 549, 522, 513 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.14 (s, 1H), 8.09 (d, J = 8.1 Hz, 1H), 7.91 (d, J = 7.8 Hz, 1H), 7.84 (d, J = 7.8 Hz, 1H), 7.56–7.32 (m, 4H), 5.88 (dd, J = 15.0, 5.1 Hz, 1H), 5.61 (ddd, J = 15.0, 8.7, 1.5 Hz, 1H), 4.60–4.45 (m, 1H), 4.20–4.05 (m, 1H), 3.55–3.40 (m, 1H), 3.25–3.05 (m, 1H), 3.01 (dd, J = 13.8, 4.8 Hz, 1H), 2.88 (dd, J = 13.8, 8.7 Hz, 1H), 2.70–2.10 (m, 9H), 1.96–1.82 (m, 2H), 1.80–1.60 (m, 1H); MS (FAB) *m/z*: 511 $(M+H)^+$; HRMS-FAB (*m/z*): $[M+H]^+$ calcd for $C_{27}H_{31}N_2O_4S_2$, 511.1725; found, 511.1738.

5.1.58. Butyl 2-({2-[(2*R*)-2-{(1*E*,3*S*)-4-(4′-methyl-1,1′-biphenyl-3-yl)-3-hydroxy-1-buten-1-yl}-5-oxo-1-pyrrolidinyl] ethyl}thio)-1,3-thiazole-4-carboxylate (15a)

Compound **15a** was prepared from aldehyde **14** using the phosphonate **5c** according to the same procedure as described for the preparation of **13a** from **12** as a colorless oil (42 mg, 18% in two steps). ^1H NMR (300 MHz, $CDCl_3$): δ 8.02–7.95 (m, 1H), 7.54–7.18 (m, 7H), 7.16–7.05 (m, 1H), 5.84 (dd, J = 15.4, 5.8 Hz, 1H), 5.51 (dd, J = 15.4, 8.8 Hz, 1H), 4.51–4.05 (m, 4H), 3.84–3.56 (m, 2H), 3.33 (t, J = 6.6 Hz, 2H), 3.25–3.13 (m, 1H), 2.94–2.78 (m, 2H), 2.44–2.06 (m, 6H), 1.78–1.60 (m, 3H), 1.49–1.31 (m, 2H), 0.94 (t, J = 7.4 Hz, 3H).

5.1.59. 2-[(2-{(2*R*)-2-[(1*E*,3*S*)-3-Hydroxy-4-(4′-methyl-1,1′-biphenyl-3-yl)but-1-enyl]-5-oxopyrrolidin-1-yl}ethyl)sulfanyl]-1,3-thiazole-4-carboxylic acid (4c)

Compound **4c** was prepared from **15a** according to the same procedure as described for the preparation of **3b** from **13a** as a white amorphous powder (29 mg, 80%). IR (film): 3415, 3114, 3022, 2921, 1716, 1662, 1483, 1422, 1324, 1214, 1101, 1029, 976, 922, 824, 787, 718, 665, 561 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.01 (s, 1H), 7.39–7.29 (m, 8H), 5.82 (dd, J = 15.4, 5.8 Hz, 1H) 5.51 (dd, J = 15.4, 8.5 Hz, 1H), 4.47 (m, 1H), 4.10 (m, 1H), 3.69 (m, 1H), 3.23 (m, 3H), 2.92 (m, 2H), 2.38 (m, 8H), 1.71 (m, 1H); MS (FAB) *m/z*: 509 $(M+H)^+$; HRMS-FAB (*m/z*): $[M+H]^+$ calcd for $C_{27}H_{29}N_2O_4S_2$, 509.1569; found, 509.1562.

5.1.60. Butyl 2-({2-[(2*R*)-2-{(1E,3*S*)-4-(4′-methoxy-1,1′-biphenyl-3-yl)-3-hydroxy-1-buten-1-yl}-5-oxo-1-pyrrolidinyl] ethyl}thio)-1,3-thiazole-4-carboxylate (15b)

Compound **15b** was prepared from the aldehyde **14** using the phosphonate **5d** instead of **5b** according to the same procedure as described for the preparation of **13a** from **12** as a colorless oil (29 mg, 29% in two steps). ^1H NMR (300 MHz, $CDCl_3$): δ 7.98 (s, 1 H), 7.58–7.28 (m, 5H), 7.15–7.05 (m, 1H), 7.02–6.89 (m, 2H), 5.84 (dd, J = 15.4, 5.8 Hz, 1H), 5.51 (dd, J = 15.4, 8.5 Hz, 1H), 4.48–4.08 (m, 4H), 3.85 (s, 3H), 3.74–3.59 (m, 1H), 3.33 (t, J = 6.6 Hz, 2H), 3.25–3.12 (m, 1H), 2.94–2.76 (m, 2H), 2.46–2.08 (m, 4H), 1.80–1.62 (m, 3H), 1.49–1.34 (m, 2H), 0.94 (t, J = 7.4 Hz, 3H).

5.1.61. 2-[(2-{(2*R*)-2-[(1*E*,3*S*)-3-Hydroxy-4-(4′-methoxy-1,1′-biphenyl-3-yl)but-1-enyl]-5-oxopyrrolidin-1-yl}ethyl)sulfanyl]-1,3-thiazole-4-carboxylic acid (4e)

Compound **4e** was prepared from **15b** according to the same procedure as described for the preparation of **3b** from **13a** as a white amorphous powder (48 mg, 76%). IR (film): 3422, 3114, 2933, 2837, 1718, 1662, 1609, 1516, 1483, 1461, 1421, 1295, 1272, 1247, 1181, 1100, 1027, 975, 922, 835, 798, 788, 749, 710, 664, 608, 572 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.07 (s, 1H), 7.22 (m, 8H), 5.82 (dd, J = 15.4, 6.0 Hz, 1H), 5.51 (dd, J = 15.4, 8.8 Hz, 1H), 4.46 (m, 1H), 4.09 (m, 1H), 3.85 (s, 3H), 3.69 (m, 1H), 3.21 (m, 3H), 2.53 (m, 7H), 1.70 (m, 1H); MS (FAB) *m/z*: 525 $(M+H)^+$; HRMS-FAB (*m/z*): $[M+H]^+$ calcd for $C_{27}H_{29}N_2O_5S_2$, 525.1518; found, 525.1520.

5.1.62. Butyl 2-({2-[(2*R*)-2-{(1E,3*S*)-4-[3-(1,3-benzoxazol-2-yl) phenyl]-3-hydroxy-1-buten-1-yl}-5-oxo-1-pyrrolidinyl]ethyl} thio)-1,3-thiazole-4-carboxylate (15c)

Compound **15c** was prepared from the aldehyde **14** using the phosphonate **5h** instead of **5b** according to the same procedure as described for the preparation of **13a** from **12** as a pale yellow amorphous powder (69 mg, 28% in two steps). ^1H NMR (300 MHz, $CDCl_3$): δ 8.14–8.07 (m, 2H), 7.97 (s, 1H), 7.78 (m, 1H), 7.60 (m, 1H), 7.45 (m, 1H), 7.40–7.31 (m, 3H), 5.88 (dd, J = 15.5, 5.6 Hz, 1H), 5.59 (m, 1H), 4.49 (m, 1H), 4.33–4.16 (m, 3H), 3.66 (m, 1H), 3.42–3.23 (m, 3H), 2.97–2.87 (m, 2H), 2.45–2.11 (m, 4H), 1.77–1.62 (m, 3H), 1.48–1.33 (m, 2H), 0.94 (t, J = 7.7 Hz, 3H).

5.1.63. 2-{[2-((2*R*)-2-{(1*E*,3*S*)-4-[3-(1,3-Benzoxazol-2-yl)phenyl] -3-hydroxybut-1-enyl}-5-oxopyrrolidin-1-yl)ethyl]sulfanyl}-1,3-thiazole-4-carboxylic acid (4j)

Compound **4j** was prepared from **15c** according to the same procedure as described for the preparation of **3b** from **13a** as a white powder (43 mg, 67%). IR (film): 3412, 3063, 2925, 1715, 1665, 1490, 1473, 1422, 1244, 1101, 1029, 1003, 977, 803, 793, 762 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.32 (s, 1H), 8.12 (s, 1H), 8.06 (d, J = 7.5 Hz, 1H), 7.90–7.82 (m, 1H), 7.64–7.58 (m, 1H), 7.50–7.36 (m, 4H), 5.94 (dd, J = 15.6, 4.5 Hz, 1H), 5.78 (dd, J = 15.6, 6.3 Hz, 1H), 4.70–4.50 (m, 1H), 4.15 (q, J = 7.2 Hz, 1H), 3.60–3.20 (m, 4H), 3.00 (dd, J = 14.4, 4.2 Hz, 1H), 2.85 (dd, J = 14.4, 9.0 Hz, 1H), 2.50–2.15 (m, 3H), 1.85–1.70 (m, 1H); MS (FAB) *m/z*: 536 $(M+H)^+$; HRMS-FAB (*m/z*): $[M+H]^+$ calcd for $C_{27}H_{26}N_3O_5S_2$, 536.1314; found, 536.1303.

5.1.64. Butyl 2-({2-[(2*R*)-2-{(1E,3*S*)-4-[3-(1,3-benzothiazol-2-yl)phenyl]-3-hydroxy-1-buten-1-yl}-5-oxo-1-pyrrolidinyl] ethyl}thio)-1,3-thiazole-4-carboxylate (15d)

Compound **15d** was prepared from aldehyde **14** using the phosphonate **5i** instead of **5b** according to the same procedure as described for the preparation of **13a** from **12** as a pale yellow amorphous powder (61 mg, 24% in two steps). ^1H NMR (300 MHz, $CDCl_3$): δ 8.05 (s, 1H), 8.00–7.86 (m, 4H), 7.56–7.28 (m, 4H), 5.90 (m, 1H), 5.60 (m, 1H), 4.48 (m, 1H), 4.35–4.15 (m, 3H), 3.75–3.59 (m, 2H), 3.44–3.32 (m, 2H), 3.26 (m, 1H), 2.97–2.84 (m, 2H), 2.42–2.12 (m, 3H), 1.77–1.63 (m, 2H), 1.47–1.34 (m, 2H), 0.99–0.87 (m, 3H).

5.1.65. 2-{[2-((2*R*)-2-{(1*E*,3*S*)-4-[3-(1,3-benzothiazol-2-yl) phenyl]-3-hydroxybut-1-enyl}-5-oxopyrrolidin-1-yl)ethyl] sulfanyl}-1,3-thiazole-4-carboxylic acid (4k)

Compound **4k** was prepared from **15d** according to the same procedure as described for the preparation of **3b** from **13a** as a white powder (36 mg, 65%). IR (film): 3059, 2921, 1946, 1716, 1664, 1484, 1315, 1236, 1027, 976, 919, 760, 728 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.16 (s, 1H) 7.95 (m, 4H), 7.44 (m, 4H), 5.79 (dd, J = 15.2, 6.8 Hz, 1H,) 5.40 (m, 1H), 4.40 (m, 1H), 4.24 (m, 1H), 3.58 (m, 1H), 3.25 (m, 2H), 2.91 (m, 3H), 2.22 (m, 3H), 1.67 (m, 1H); MS (FAB) *m/z*: 552 $(M+H)^+$; HRMS-FAB (*m/z*): $[M+H]^+$ calcd for $C_{27}H_{26}N_3O_4S_3$, 552.1085; found, 552.1073.

5.1.66. Ethyl (4*R*,5*E*,7*S*)-8-(3-(1-benzofuran-2-yl)phenyl)-7-hydroxy-4-({*tert*-butyoxycarbonyl}amino)-5-octenoate (17a)

Compound **17a** was prepared from the aldehyde **16** using the phosphonate **5j** according to the same procedure as described for the preparation of **13a** from **12** as a colorless amorphous powder (4.27 g, 76% in two steps). ^1H NMR (300 MHz, $CDCl_3$): δ 7.77–7.69 (m, 2H), 7.59 (m, 1H), 7.55–7.49 (m, 1H), 7.40 (m, 1H), 7.33–7.16 (m, 3H), 7.04 (s, 1H), 5.76 (m, 1H), 5.60 (m, 1H), 4.55 (m, 1H), 4.42 (m, 1H) 4.18–4.06 (m, 3H), 2.99–2.80 (m, 2H), 2.31 (t, J = 6.9 Hz, 2H), 1.91–1.71 (m, 2H), 1.41 (s, 9H), 1.25 (t, J = 7.7 Hz, 3H).

5.1.67. Ethyl 2-({2-[(2*R*)-2-{(1*E*,3*S*)-4-[3-(1-benzofuran-2-yl)phen yl]-3-hydroxy-1-buten-1-yl}-5-oxo-1-pyrrolidinyl] ethyl} thio)-1, 3-thiazole-4-carboxylate (18a)

A solution of **17a** (4.20 g, 8.50 mmol) in EtOH (10 mL) was treated with 4 N HCl in dioxane (3.2 mL, 25.5 mmol) at room temperature under argon atmosphere. After being stirred for 6 h, the reaction mixture was evaporated. The resulting residue was recrystallized from MTBE and AcOEt to give an amine hydrochloride as a white powder (2.81 g, 77%). To a stirred solution of aldehyde **23** (1.86 g, 8.07 mmol) in THF (25 mL) and DMF (3 mL) was added a solution of the above-described amine hydrochloride (2.31 g, 5.38 mmol) in THF (3 mL) at room temperature under argon atmosphere and stirring was continued for 1 h at 50 °C. To the stirred reaction mixture was added sodium acetoxyborohydride (1.71 g, 8.07 mmol) at 0 °C under argon atmosphere. After being stirred at room temperature for additional 15 h, the reaction mixture was diluted with EtOAc, washed with water, saturated aqueous $NaHCO_3$, brine, and dried over Na_2SO_4. The organic layer was evaporated and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 1:4–0:1) to give **18a** as a white amorphous (1.82 g, 60%). ^1H NMR (300 MHz, $CDCl_3$): δ 7.99 (s, 1H), 7.72 (s, 2H), 7.58 (d, J = 7.1 Hz, 1H), 7.51 (d, J = 7.1 Hz, 1H), 7.36 (t, J = 8.0 Hz, 1H), 7.31–7.12 (m, 3H), 7.03 (s, 1H), 5.83 (m, 1H), 5.60 (m, 1H), 4.45 (m, 1H), 4.39–4.29 (m, 2H), 4.18 (m, 1H), 3.66 (m, 1H), 3.40–3.30 (m, 2H), 3.23 (m, 1H), 2.96–2.80 (m, 2H), 2.45–2.10 (m, 3H), 1.80–1.60 (m, 2H), 1.36 (t, J = 7.0 Hz, 3H).

5.1.68. 2-{[2-((2*R*)-2-{(1*E*,3*S*)-4-[3-(1-Benzofuran-2-yl)phenyl]-3-hydroxybut-1-enyl}-5-oxopyrrolidin-1-yl)ethyl]sulfanyl}-1,3-thiazole-4-carboxylic acid (4i)

Compound **4i** was prepared from **18a** according to the same procedure as described for the preparation of **3b** from **13a** as colorless amorphous (1.62 g, 98%). IR (KBr): 3412, 3114, 2933, 1718, 1663, 1490, 1452, 1421, 1324, 1257, 1214, 1106, 1029, 976, 937, 795, 751, 720, 700 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.05 (s, 1H), 7.80–7.65 (m, 2H), 7.58 (d, J = 7.5 Hz, 1H), 7.51 (d, J = 7.5 Hz, 1H), 7.39 (t, J = 7.8 Hz, 1H), 7.35–7.15 (m, 3H), 7.03 (s, 1H), 5.82 (dd, J = 15.0, 5.7 Hz, 1H), 5.52 (d, J = 15.0, 8.7 Hz, 1H), 4.50 (m, 1H), 4.19–4.02 (m, 1H), 3.70 (m, 1H), 3.36–3.08 (m, 3H), 3.00–2.82 (m, 2H), 2.50–2.10 (m, 3H), 1.72 (m, 1H); MS (FAB) *m/z*: 535 $(M+H)^+$; HRMS-FAB (*m/z*): $[M+H]^+$ calcd for $C_{28}H_{27}N_2O_5S_2$, 535.1361; found, 535.1357.

5.1.69. Ethyl (4*R*,5*E*,7*S*)-8-(3-(5-methyl-1,3-benzoxazol-2-yl) phenyl)-7-hydroxy-4-({*tert*-butyoxycarbonyl}amino)-5-octenoate (17b)

Compound **17b** was prepared from the aldehyde **16** using the phosphonate **5l** according to the same procedure as described for the preparation of **13a** from **12** as a colorless amorphous powder (878 mg, 88% in two steps). ^1H NMR (300 MHz, $CDCl_3$): δ 8.10 (m, 2H), 7.55 (s, 1H), 7.50–7.34 (m, 3H), 7.16 (dd, J = 8.2, 1.7 Hz, 1H), 5.73 (m, 1H), 5.58 (m, 1H), 4.62 (m, 1H), 4.44 (m, 1H), 4.09 (q, J = 7.1 Hz, 2H), 3.04–2.82 (m, 2H), 2.49 (s, 3H), 2.30 (t, J = 7.4 Hz, 2H), 1.94–1.69 (m, 3H), 1.42 (s, 9H), 1.23 (t, J = 7.1 Hz, 3H).

5.1.70. Ethyl 2-({2-[(2*R*)-2-{(1*E*,3*S*)-4-[3-(5-methyl-1,3-benzoxazol-2-yl)phenyl]-3-hydroxy-1-buten-1-yl}-5-oxo-1-pyrrolidinyl] ethyl}thio)-1,3-thiazole-4-carboxylate (18b)

Compound **18b** was prepared from **17b** according to the same procedure as described for the preparation of **18a** from **17a** as a pale yellow viscous oil (282 mg, 58%). ^1H NMR (300 MHz, $CDCl_3$): δ 8.15–8.04 (m, 2H), 7.99 (s, 1H), 7.54 (s, 1H), 7.48–7.24 (m, 3H), 7.16 (dd, J = 8.2, 1.5 Hz, 1H), 5.87 (dd, J = 15.4, 5.5 Hz, 1H), 5.56 (m, 1H), 4.47 (m, 1H), 4.42–4.28 (m, 2H), 4.22 (m, 1H), 3.67 (m, 1H), 3.38 (t, J = 6.6 Hz, 2H), 3.27 (m, 1H), 2.99–2.86 (m, 3H), 2.49 (s, 3H), 2.42–2.12 (m, 3H), 1.68 (m, 1H), 1.36 (t, J = 7.1 Hz, 3H).

5.1.71. 2-{[2-((2*R*)-2-{(1*E*,3*S*)-3-Hydroxy-4-[3-(5-methyl-1,3-benzox azol-2-yl)phenyl]but-1-enyl}-5-oxopyrrolidin-1-yl) ethyl] sulfanyl}-1, 3-thiazole-4-carboxylic acid (4m)

Compound **4m** was prepared from **18b** according to the same procedure as described for the preparation of **3b** from **13a** as colorless amorphous (158 mg, 60%). IR (KBr): 3422, 2924, 1716, 1663, 1551, 1424, 1264, 1206, 1029, 797, 724, 567 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.47 (s, 1H), 8.14 (s, 1H), 8.04 (d, J = 7.8 Hz, 1H), 7.65 (s, 1H), 7.52–7.36 (m, 3H), 7.21 (dd, J = 8.4, 0.6 Hz, 1H), 5.94 (dd, J = 15.0, 4.5 Hz, 1H), 5.81 (m, 1H), 4.63 (m, 1H), 4.17 (m, 1H), 3.55–3.24 (m, 4H), 3.00 (dd, J = 14.7, 3.9 Hz, 1H), 2.84 (dd, J = 14.7, 9.0 Hz, 1H), 2.51 (s, 3H), 2.46–2.18 (m, 3H), 1.81 (m, 1H); MS (FAB) *m/z*: 550 $(M+H)^+$; HRMS-FAB (*m/z*): $[M+H]^+$ calcd for $C_{28}H_{28}N_3O_5S_2$, 550.1470; found, 550.1472.

5.1.72. Ethyl (4*R*,5*E*,7*S*)-8-(3-(6-methyl-1,3-benzoxazol-2-yl) phenyl)-7-hydroxy-4-({*tert*-butyoxycarbonyl}amino)-5-octeno ate (17c)

Compound **17c** was prepared from the aldehyde **16** using the phosphonate **5m** instead of **5b** according to the same procedure as described for the preparation of **13a** from **12** as a colorless amorphous powder (1.75 g, 89% in two steps).

5.1.73. Ethyl 2-({2-[(2*R*)-2-{(1*E*,3*S*)-4-[3-(6-methyl-1,3-benzoxazol-2-yl)phenyl]-3-hydroxy-1-buten-1-yl}-5-oxo-1-pyrrolidinyl]ethyl}thio)-1,3-thiazole-4-carboxylate (18c)

Compound **18c** was prepared from **17c** according to the same procedure as described for the preparation of **18a** from **17a** as a pale yellow viscous oil (313 mg, 47% in two steps). ^1H NMR (300 MHz, $CDCl_3$): δ 8.15–8.02 (m, 2H), 7.99 (s, 1H), 7.63 (d, J = 8.2 Hz, 1H), 7.49–7.30 (m, 3H), 7.17 (d, J = 7.7 Hz, 1H), 5.87 (dd, J = 15.4, 5.8 Hz, 1H), 5.56 (ddd, J = 15.4, 8.5, 1.1 Hz, 1H), 4.50 (m, 1H), 4.35 (q, J = 7.1 Hz, 2H), 4.25–4.14 (m, 1 H) 3.60 (m, 1H), 3.38 (m, 1H), 3.30 (m, 1H), 2.95–2.85 (m, 2H), 2.51 (s, 3H), 2.43–2.09 (m, 3H), 1.69 (m, 1H), 1.35 (t, J = 7.1 Hz, 3H).

5.1.74. 2-{[2-((2*R*)-2-{(1*E*,3*S*)-3-Hydroxy-4-[3-(6-methyl-1,3-benzoxazol-2-yl)phenyl]but-1-enyl}-5-oxopyrrolidin-1-yl) ethyl]sulfanyl}-1,3-thiazole-4-carboxylic acid (4n)

Compound **4n** was prepared from **18c** according to the same procedure as described for the preparation of **3b** from **13a** as colorless amorphous (245 mg, 82%). IR (film): 3060, 2941, 1723, 1683, 1551, 1474, 1418, 1381, 1277, 1149, 1032, 949, 892, 795, 724, 696, 569 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.20 (s, 1H), 8.05 (m, 2H), 7.66 (d, J = 8.2 Hz, 1H), 7.42 (m, 3H), 7.20 (d, J = 8.0 Hz, 1H), 5.92 (dd, J = 15.4, 4.9 Hz, 1H), 5.62 (ddd, J = 15.4, 8.8, 1.1 Hz, 1H), 4.49 (m, 1H), 4.19 (m, 1H), 3.60 (m, 1H), 3.34 (m, 3H), 2.90 (m, 2H), 2.33 (m, 7H), 1.75 (m, 1H); MS (FAB) *m/z*: 550 $(M+H)^+$; HRMS-FAB (*m/z*): $[M+H]^+$ calcd for $C_{28}H_{28}N_3O_5S_2$, 550.1470; found, 550.1472.

5.1.75. Ethyl (4*R*,5*E*,7*S*)-8-(3-(4-methyl-1,3-benzoxazol-2-yl) phenyl)-7-hydroxy-4-({*tert*-butyoxycarbonyl}amino)-5-octeno ate (17d)

Compound **17d** was prepared from the aldehyde **16** using the phosphonate **5n** according to the same procedure as described for the preparation of **13a** from **12** as a colorless amorphous powder (1.75 g, 89% in two steps).

5.1.76. Ethyl 2-({2-[(2*R*)-2-{(1*E*,3*S*)-4-[3-(4-methyl-1,3-benzoxazol-2-yl)phenyl]-3-hydroxy-1-buten-1-yl}-5-oxo-1-pyrrolidinyl] ethyl} thio)-1,3-thiazole-4-carboxylate (18d)

Compound **18d** was prepared from **17d** according to the same procedure as described for the preparation of **18a** from **17a** as a pale yellow viscous oil (313 mg, 47% in two steps).

5.1.77. 2-{[2-((2*R*)-2-{(1*E*,3*S*)-3-Hydroxy-4-[3-(4-methyl-1, 3-benzoxazol-2-yl)phenyl]but-1-enyl}-5-oxopyrrolidin-1-yl) ethyl]sulfanyl}-1,3-thiazole-4-carboxylic acid (4o)

Compound **4o** was prepared from **18d** according to the same procedure as described for the preparation of **3b** from **13a** as colorless amorphous (245 mg, 82%). IR (film): 3077, 2921, 1737, 1679, 1608, 1551, 1496, 1435, 1324, 1300, 1285, 1193, 1092, 983, 886, 784, 713 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.16 (s, 1H), 8.08 (m, 2H), 7.44 (m, 2H), 7.35 (d, J = 7.7 Hz, 1H), 7.27 (t, J = 7.8 Hz, 1H), 7.17 (d, J = 7.4 Hz, 1H), 5.88 (dd, J = 15.3, 5.4 Hz, 1H), 5.53 (dd, J = 15.3, 9.0 Hz, 1H), 4.43 (m, 1H), 4.20 (m, 1H), 3.63 (m, 1H), 3.40 (m, 1H), 3.29 (m, 2H), 2.91 (d, J = 6.6 Hz, 2H), 2.68 (s, 3H), 2.37 (m, 2H), 2.24 (m, 1H), 1.74 (m, 1H); MS (FAB) *m/z*: 550 $(M+H)^+$; HRMS-FAB (*m/z*): $[M+H]^+$ calcd for $C_{28}H_{28}N_3O_5S_2$, 550.1470; found, 550.1465.

5.1.78. Ethyl (4*R*,5*E*,7*S*)-8-(3-(5,7-dimethyl-1,3-benzoxazol-2-yl) phenyl)-7-hydroxy-4-({*tert*-butyoxycarbonyl}amino)-5-octenoate (17e)

Compound **17e** was prepared from the aldehyde **16** using the phosphonate **5o** instead of **5b** according to the same procedure as described for the preparation of **13a** from **12** as a colorless amorphous powder (347 mg, 27% in two steps). ^1H NMR (300 MHz, $CDCl_3$): δ 8.17–8.07 (m, 2H), 7.47 (m, 1H), 7.45–7.38 (m, 2H), 6.97 (s, 1H), 5.74 (m, 1H), 5.61 (m, 1H), 4.70 (m, 1H), 4.44 (m, 1H), 4.19–4.03 (m, 3H), 3.01–2.85 (m, 2H), 2.55 (s, 3H), 2.45 (s, 3H), 2.30 (t, J = 7.6 Hz, 2H), 1.91–1.69 (m, 2H), 1.43 (s, 9H), 1.25 (t, J = 7.4 Hz, 3H).

5.1.79. Ethyl 2-({2-[(2*R*)-2-{(1*E*,3*S*)-4-[3-(5,7-dimethyl-1,3-benzoxazol-2-yl)phenyl]-3-hydroxy-1-buten-1-yl}-5-oxo-1-pyrrolidinyl]ethyl}thio)-1,3-thiazole-4-carboxylate (18e)

Compound **18e** was prepared from **17e** according to the same procedure as described for the preparation of **18a** from **17a** as a pale amorphous powder (258 mg, 64% in two steps).

5.1.80. 2-{[2-((2*R*)-2-{(1*E*,3*S*)-4-[3-(5,7-Dimethyl-1,3-benzoxazol-2-yl)phenyl]-3-hydroxybut-1-enyl}-5-oxopyrrolidin-1-yl)ethyl] sulfanyl}-1,3-thiazole-4-carboxylic acid (4p)

Compound **4p** was prepared from **18e** according to the same procedure as described for the preparation of **3b** from **13a** as colorless amorphous (152 mg, 40%). IR (film): 3061, 2940, 2875, 1720, 1666, 1551, 1493, 1312, 1260, 1151, 1028, 942, 844, 718, 615 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.48 (s, 1H), 8.14 (s, 1H), 8.06 (m, 1H), 7.42 (m, 3H), 7.01 (s, 1H), 5.81 (m, 1H), 4.63 (m, 1H), 4.15 (m, 1H), 3.39 (m, 4H), 3.01 (m, 1H), 2.83 (m, 1H), 2.39 (m, 11H), 1.81 (m, 1H); MS (FAB) *m/z*: 564 $(M+H)^+$; HRMS-FAB (*m/z*): $[M+H]^+$ calcd for $C_{29}H_{30}N_3O_5S_2$, 564.1627; found, 564.1631.

5.1.81. Ethyl (4*R*,5*E*,7*S*)-8-(3-(5-chloro-1,3-benzothiazol-2-yl) phenyl)-7-hydroxy-4-({*tert*-butyoxycarbonyl}amino)-5-octenoate (17g)

Compound **17g** was prepared from aldehyde **16** using phosphonate **5k** instead of **5b** according to the same procedure as described for the preparation of **13a** from **12** as a white powder (1.12 g, 51% in two steps). ^1H NMR (300 MHz, $CDCl_3$): δ 8.07 (m, 1H), 7.96 (s, 1H), 7.91 (m, 1H), 7.82 (m, 1H) 7.50–7.33 (m, 3H), 5.77 (m, 1H), 5.60 (m, 1H), 4.70 (m, 1H), 4.45 (m, 1H), 4.18–4.05 (m, 3H), 2.97–2.88 (m, 2H), 2.30 (t, J = 7.4 Hz, 2H), 1.91–1.72 (m, 2H), 1.43 (s, 9H), 1.24 (t, J = 7.1 Hz, 3H).

5.1.82. Ethyl 2-({2-[(2*R*)-2-{(1*E*,3*S*)-4-[3-(5-chloro-1,3-benzothiazol-2-yl) phenyl]-3-hydroxy-1-buten-1-yl}-5-oxo-1-pyrrolidinyl]ethyl}thio)-1,3-thiazole-4-carboxylate (18g)

Compound **18g** was prepared from **17g** according to the same procedure as described for the preparation of **18a** from **17a** as a pale brown oil (254 mg).

5.1.83. 2-{[2-((2*R*)-2-{(1*E*,3*S*)-4-[3-(5-Chloro-1,3-benzothiazol-2-yl) phenyl]-3-hydroxybut-1-enyl}-5-oxopyrrolidin-1-yl)ethyl] sulfanyl}-1,3-thiazole-4-carboxylic acid (4l)

Compound **4l** was prepared from **18g** according to the same procedure as described for the preparation of **3b** from **13a** as a pale yellow powder (140 mg, 29% in two steps). IR (film): 3076, 2987, 2871, 1749, 1675, 1548, 1465, 1415, 1324, 1295, 1180, 1099, 1071, 870, 845, 795, 735, 710, 695 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.31 (s, 1H), 8.15 (m, 2H) 7.90 (m, 2H) 7.45 (m, 3H), 5.72 (dd, J = 15.4, 6.2 Hz, 1H), 5.33 (dd, J = 15.4, 8.8 Hz, 1H), 5.05 (m, 1H), 4.25 (m, 1H), 4.10 (m, 1H), 3.55 (m, 1H), 3.20 (m, 2H), 2.83 (m, 3H), 2.11 (m, 3H), 1.55 (m, 1H); MS (FAB) *m/z*: 586 $(M+H)^+$; HRMS-FAB (*m/z*): $[M+H]^+$ calcd for $C_{27}H_{25}ClN_3O_4S_3$, 586.0696; found, 586.0692.

5.1.84. Ethyl (4*R*,5*E*,7*S*)-8-(3-(5-chloro-1,3-benzoxazol-2-yl) phenyl)-7-hydroxy-4-({*tert*-butyoxycarbonyl}amino)-5-octenoate (17f)

Compound **17f** was prepared from aldehyde **16** using the phosphonate **5p** instead of **5b** according to the same procedure as described for the preparation of **13a** from **12** as a white powder (185 mg, 84% in two steps).

5.1.85. Ethyl 2-({2-[(2*R*)-2-{(1*E*,3*S*)-4-[3-(5-chloro-1,3-benzoxazol-2-yl)phenyl]-3-hydroxy-1-buten-1-yl}-5-oxo-1-pyrrolidinyl]ethyl}thio)-1,3-thiazole-4-carboxylate (18f)

Compound **18f** was prepared from **17f** according to the same procedure as described for the preparation of **18a** from **17a** as a pale amorphous powder (75 mg, 35% in two steps).

5.1.86. 2-{[2-((2*R*)-2-{(1*E*,3*S*)-4-[3-(5-Chloro-1,3-benzoxazol-2-yl)phenyl]-3-hydroxybut-1-enyl}-5-oxopyrrolidin-1-yl)ethyl] sulfanyl}-1,3-thiazole-4-carboxylic acid (4q)

Compound **4q** was prepared from **18f** according to the same procedure as described for the preparation of **3b** from **13a** as colorless amorphous (46 mg, 65%). IR (film): 3093, 2922, 2686, 1717, 1666, 1587, 1490, 1330, 1257, 1208, 1179, 1028, 975, 860, 725, 685 cm^{-1}; ^1H NMR1H NMR (300 MHz, $CDCl_3$): δ 8.17 (s, 1H), 8.05 (m, 2H), 7.71 (m, 1H), 7.64 (m, 1H), 7.44 (m, 3H), 5.80 (dd, *J* = 15.4, 7.0 Hz, 1H), 5.42 (dd, *J* = 15.4, 8.8 Hz, 1H), 4.40 (m, 1H), 4.25 (m, 1H), 3.59 (m, 1H), 3.23 (m, 1H), 2.92 (m, 3H), 2.24 (m, 3H), 1.67 (m, 1H); MS (FAB) *m/z*: 570 $(M+H)^+$; HRMS-FAB (*m/z*): $[M+H]^+$ calcd for $C_{27}H_{25}ClN_3O_5S_2$, 570.0924; found,570.0925.

5.1.87. Ethyl 4-({2-[(2*R*)-2-{(1*E*,3*S*)-4-[3-(1-benzofuran-2-yl)phenyl]-3-hydroxy-1-buten-1-yl}-5-oxo-1-pyrrolidinyl] ethyl}thio)butanoate (20)

Compound **20** was prepared from **17a** using aldehyde **19** instead of **23** according to the same procedure as described for the preparation of **18a** from **17a** as a beige amorphous powder (290 mg, 56% in two steps). ^1H NMR (300 MHz, $CDCl_3$): δ 7.79–7.68 (m, 2H), 7.59 (d, *J* = 6.6 Hz, 1H), 7.50 (m, 1H), 7.40 (m, 1H), 7.33–7.16 (m, 3H), 7.04 (s, 1H), 5.80 (m, 1H), 5.53 (m, 1H), 4.49 (d, *J* = 6.0 Hz, 1H), 4.18–4.03 (m, 3H), 3.62 (m, 1H), 3.01–2.85 (m, 2H), 2.65–2.45 (m, 3H), 2.43–2.13 (m, 6H), 2.01 (br s, 1H), 1.91–1.79 (m, 2H), 1.70 (m, 1H), 1.25 (t, *J* = 6.9 Hz, 3H).

5.1.88. 4-{[2-((2*R*)-2-{(1*E*,3*S*)-4-[3-(1-Benzofuran-2-yl)phenyl]-3-hydroxybut-1-enyl}-5-oxopyrrolidin-1-yl)ethyl]sulfanyl} butanoic acid (3i)

Compound **3i** was prepared from **20** according to the same procedure as described for the preparation of **3b** from **13a** as colorless amorphous (246 mg, 91%). IR (film): 3389, 2925, 1717, 1655, 1452, 1420, 1257, 751 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.76–7.70 (m, 2H), 7.59 (d, *J* = 8.4 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.40 (dd, *J* = 8.4, 8.4 Hz, 1H), 7.33–7.17 (m, 3H), 7.04 (s, 1H), 5.79 (dd, *J* = 15.3, 5.7 Hz, 1H), 5.51 (dd, *J* = 15.3, 8.4 Hz, 1H), 4.55–4.44 (m, 1H), 4.16–4.07 (m, 1H), 3.68–3.54 (m, 1H), 3.02–2.90 (m, 3H), 2.70–2.10 (m, 9H), 1.92–1.78 (m, 2H), 1.78–1.62 (m, 1H); MS (APCI) *m/z*: 492 $(M-H)^-$.

5.1.89. *S*-(2,2-Diethoxyethyl) ethanethioate (21)

To a stirred solution of bromoacetaldehyde diethylacetal (29.6 g, 150 mmol) in DMF (70 mL) was added potassium thioacetate (17.9 g, 157 mmol) at room temperature under argon atmosphere. After being stirred at 50 °C for 4 h, the reaction mixture was allowed to be cooled to room temperature. The reaction was quenched with 1 N HCl. The resulting reaction mixture was extracted with EtOAc (twice). The combined organic layers were washed with H_2O (twice), brine, and dried over $MgSO_4$. The organic solvent was removed by evaporation to afford a thioacetate **21** as a brown oil (28.2 g). ^1H NMR (300 MHz, $CDCl_3$): δ 4.50 (t, *J* = 5.5 Hz, 1H), 3.76–3.47 (m, 4H), 3.12 (d, *J* = 5.5 Hz, 2H), 2.35 (s, 3H), 1.22 (t, *J* = 7.1 Hz, 6H).

5.1.90. Ethyl 2-[(2,2-diethoxyethyl)thio]-1,3-thiazole-4-carboxylate (22)

To a stirred solution of thioacetate **21** (10.1 g, 52.5 mmol) in EtOH (60 mL) and DMF (60 mL) was added potassium carbonate (10.4 g, 75.0 mmol) and ethyl 2-bromothiazole-4-carboxylate (11.8 g, 50.0 mmol) at room temperature under argon atmosphere. After being stirred at room temperature for 15 h, the reaction was quenched with saturated aqueous NH_4Cl. The reaction mixture was diluted with EtOAc, washed with H_2O twice, brine, and dried over $MgSO_4$. The organic solvent was removed by evaporation to give a sulfide **22** as a yellow oil (16.8 g). ^1H NMR (300 MHz, $CDCl_3$): δ 8.02 (s, 1H), 4.77 (t, *J* = 5.4 Hz, 1H) 4.39 (q, *J* = 7.08 Hz, 2H), 3.81–3.52 (m, 4H), 3.47 (d, *J* = 5.5 Hz, 2H), 1.39 (t, *J* = 7.1 Hz, 3H), 1.22 (t, *J* = 7.1 Hz, 6H).

5.1.91. Ethyl 2-[(2-oxoethyl)thio]-1,3-thiazole-4-carboxylate (23)

A stirred solution of **22** (16.8 g, 50 mmol) in CH_3CN (150 mL) was treated with 2 N HCl (15 mL) at room temperature under argon atmosphere. After being stirred for 16 h, the reaction mixture was evaporated up to about half of its volume, then poured into water and extracted with AcOEt (five times). The organic solvent was removed by evaporation, the resulting residue was recrystallized from hexane to give **23** as pale brown powder (10.1 g, 87%). ^1H NMR (300 MHz, $CDCl_3$): δ 9.72 (t, *J* = 2.0 Hz, 1H), 8.05 (s, 1H), 4.39 (q, *J* = 7.1 Hz, 2H), 4.09 (d, *J* = 2.0 Hz, 2H), 1.39 (t, *J* = 7.1 Hz, 3H).

5.1.92. Ethyl (4*R*,5*E*,7*S*)-8-(3-bromophenyl)-7-hydroxy-4-({*tert*-butyoxycarbonyl}amino)-5-octenoate (24)

Compound **24** was prepared from the aldehyde **16** using the phosphonate **5a** instead of **5b** according to the same procedure as described for the preparation of **13a** from **12** as a colorless oil (840 mg, 70% in two steps). ^1H NMR (300 MHz, $CDCl_3$): δ 7.40–7.33 (m, 2H), 7.21–7.09 (m, 2H), 5.68 (m, 1H), 5.55 (m, 1H), 4.50 (br s, 1H), 4.35 (m, 1H), 4.19–4.07 (m, 3H), 2.86–2.71 (m, 2H), 2.32 (t, *J* = 7.7 Hz, 2H), 1.91–1.66 (m, 3H), 1.45 (s, 9H), 1.26 (t, *J* = 7.1 Hz, 3H).

5.1.93. Ethyl (4*R*,5*E*,7*S*)-8-(3-bromophenyl)-7-(*tert*-butyldimethylsilyloxy)-4-({*tert*-butyoxycarbonyl}amino)-5-octenoate (25)

To a stirred solution of **24** (590 mg, 1.29 mmol) in DMF (4.5 mL) and imidazole (176 mg, 2.59 mmol) was added *t*-butyldiphenylsilyl chloride (291 mg, 1.94 mmol) at room temperature under argon atmosphere. After being stirred for 1 h, the reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with water (twice), brine, and dried over $MgSO_4$. The organic layer was evaporated to give a silyl ether **25** as a colorless oil (742 mg, 100%). ^1H NMR (300 MHz, $CDCl_3$): δ 7.36–7.27 (m, 2H), 7.17–7.03 (m, 2H), 5.68–5.57 (m, 1H), 5.45 (m, 1H), 4.42 (br s, 1H), 4.23 (m, 1H), 4.17–4.05 (m, 3H), 2.75–2.60 (m, 2H), 2.30 (t, *J* = 7.6 Hz, 2H), 1.87–1.67 (m, 2H), 1.42 (s, 9H), 1.25 (t, *J* = 6.6 Hz, 3H), 0.81 (s, 9H), −0.14 (s, 3H), −0.25 (s, 3H).

5.1.94. Ethyl (4*R*,5*E*,7*S*)-8-(2′,4′-dimethyl-1,1′-biphenyl-3-yl)-7-(*tert*-butyldimethylsilyloxy)-4-({*tert*-butyoxycarbonyl}amino)-5-octenoate (26a)

To a stirred solution of **25** (330 mg, 0.579 mmol) in DME (4 mL) and 2 M aqueous sodium carbonate (0.87 mL, 1.74 mmol) were added 2,4-dimethylphenylboronic acid (173 mg, 1.15 mmol), (*S*)-2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (BINAP) (72 mg, 0.116 mmol) and palladium acetate (13 mg, 0.058 mmol) under argon atmosphere. After being stirred at 80 °C for 15 h, the reaction mixture was cooled to room temperature. The resulting mixture was filtered through a pad of Celite, and the filtrate was evaporated. The resulting residue was purified by column chromatography on silica gel (hexane/EtOAc, 4:1–1:1) to afford **26a** as a brown oil (321 mg, 93%). ^1H NMR (300 MHz, $CDCl_3$): δ 7.27 (s, 1H), 7.15–7.00 (m, 6H), 5.65 (m, 1H), 5.46 (m, 1H), 4.43 (m, 1H), 4.30 (m, 1H), 4.17–4.02 (m, 3H), 2.80–2.71 (m, 2H), 2.35 (s, 3H), 2.33–2.22 (m, 2H), 2.23 (s, 3H), 1.86–1.68 (m, 2H), 1.41 (s, 9H), 1.25 (t, *J* = 7.0 Hz, 3H), 0.79 (s, 9H), −0.13 (s, 3H), −0.24 (s, 3H).

5.1.95. Ethyl 2-({2-[(2*R*)-2-{(1E,3*S*)-4-(2′,4′-dimethyl-1,1′-biphenyl-3-yl)-3-hydroxy-1-buten-1-yl}-5-oxo-1-pyrrolidinyl] ethyl}thio)-1,3-thiazole-4-carboxylate (27a)

Compound **27a** was prepared from **26a** according to the same procedure as described for the preparation of **18a** from **17a** as a

pale amorphous powder (150 mg, 52%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.00 (s, 1H), 7.30 (m, 1H), 7.18 (m, 1H), 7.14–7.01 (m, 5H), 5.82 (m, 1H), 5.55 (m, 1H), 4.38 (m, 1H), 4.25–4.07 (m, 3H), 3.67 (m, 1H), 3.37 (t, J = 6.6 Hz, 2H), 3.20 (m, 1H), 2.87–2.80 (m, 2H), 2.36 (s, 3H), 2.31 (m, 1H), 2.23 (s, 3H), 2.21–2.10 (m, 2H), 1.73 (m, 1H), 1.27 (t, J = 7.0 Hz, 3H).

5.1.96. 2-[(2-{(2*R*)-2-[(1*E*,3*S*)-4-(2′,4′-Dimethyl-1,1′-biphenyl-3-yl)-3-hydroxybut-1-enyl]-5-oxopyrrolidin-1-yl}ethyl)sulfanyl]-1,3-thiazole-4-carboxylic acid (4f)

Compound **4f** was prepared from **27a** according to the same procedure as described for the preparation of **3b** from **13a** as colorless amorphous (142 mg, 100%). IR (film): 3407, 3112, 2924, 2858, 1717, 1663, 1649, 1639, 1561, 1506, 1479, 1458, 1421, 1326, 1214, 1098, 1028, 974, 921, 865, 822, 793, 714, 661, 594, 583, 568 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.07 (s, 1H), 7.35 (t, J = 8.0 Hz, 1H), 7.15 (m, 6H), 5.82 (m, 1H), 5.53 (m, 1H), 4.44 (m, 1H), 4.12 (m, 1H), 3.71 (m, 1H), 3.24 (m, 3H), 2.88 (d, J = 6.6 Hz, 2H), 2.36 (s, 3H), 2.33 (m, 3H), 2.23 (s, 3H), 1.74 (m, 1H); MS (FAB) *m/z*: 523 (M+H)$^+$; HRMS-FAB (*m/z*): [M+H]$^+$ calcd for $C_{28}H_{31}N_2O_4S_2$, 523.1725; found, 523.1720.

5.1.97. Ethyl (4*R*,5*E*,7*S*)-8-(3′,4′-Dimethyl-1,1′-biphenyl-3-yl)-7-(*tert*-butyldimethylsilyloxy)-4-({*tert*-butyoxycarbonyl}amino)-5-octenoate (26b)

Compound **26b** was prepared from **25** using 3,4-dimethylphenylboronic acid instead of 2,4-dimethylphenylboronic acid according to the same procedure as described for the preparation of **26a** from **25** as a pale amorphous powder (306 mg, 87%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.45–7.27 (m, 4H), 7.19 (d, J = 8.1 Hz, 1H), 7.10 (d, J = 7.3 Hz, 1H), 6.97 (m, 1H), 5.76 (m, 1H), 5.51 (m, 1H), 4.44 (m, 1H), 4.31 (s, 1H), 4.18–4.06 (m, 3H), 2.83–2.74 (m, 3H), 2.33 (s, 3H), 2.30 (s, 3H), 1.78 (s, 2H), 1.42 (s, 9H), 1.30–1.20 (m, 3H), 0.81 (s, 9H), −0.12 (s, 3H), −0.24 (s, 3H).

5.1.98. Ethyl 2-({2-[(2*R*)-2-{(1E,3*S*)-4-(3′,4′-Dimethyl-1,1′-biphenyl-3-yl)-3-hydroxy-1-buten-1-yl}-5-oxo-1-pyrrolidinyl]ethyl}thio)-1,3-thiazole-4-carboxylate (27b)

Compound **27b** was prepared from **26b** according to the same procedure as described for the preparation of **18a** from **17a** as a pale amorphous powder (150 mg, 55%).

5.1.99. 2-[(2-{(2*R*)-2-[(1*E*,3*S*)-4-(3′,4′-Dimethyl-1,1′-biphenyl-3-yl)-3-hydroxybut-1-enyl]-5-oxopyrrolidin-1-yl}ethyl)sulfanyl]-1,3-thiazole-4-carboxylic acid (4g)

Compound **4g** was prepared from **27b** according to the same procedure as described for the preparation of **3b** from **13a** as colorless amorphous (143 mg, 100%). IR (film): 3402, 3112, 2924, 2859, 1718, 1663, 1649, 1561, 1508, 1458, 1421, 1210, 1099, 1027, 881, 825, 790, 715, 610, 594 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.06 (s, 1H), 7.45–7.35 (m, 5H), 7.20–7.12 (m, 2H), 5.82 (dd, J = 15.3, 6.0 Hz, 1H), 5.50 (dd, J = 15.3, 9.0 Hz, 1H), 4.46 (m, 1H), 4.10 (m, 1H), 3.68 (m, 1H), 3.24 (m, 2H), 3.13 (m, 1H), 2.91 (m, 2H), 2.33 (m, 3H), 2.31 (s, 3H), 2.30 (m, 3H), 1.70 (m, 1H); MS (FAB) *m/z*: 523 (M+H)$^+$; HRMS-FAB (*m/z*): [M+H]$^+$ calcd for $C_{28}H_{31}N_2O_4S_2$, 523.1725; found, 523.1724.

5.1.100. Butyl 4-({2-[(2*R*)-2-{(1*E*,3*S*)-4-[3-(*tert*-butyldimethylsilyloxy)phenyl]-3-hydroxy-1-buten-1-yl}-5-oxo-1-pyrrolidinyl]ethyl}thio)butanoate (28)

Compound **28** was prepared from the aldehyde **12** using the phosphonate **5q** instead of **5b** according to the same procedure as described for the preparation of **13a** from **12** as a colorless oil (992 mg, 65% in two steps). ^{1}H NMR1H NMR (300 MHz, $CDCl_3$): δ 7.17 (t, J = 8.1 Hz, 1H), 6.89 (d, J = 8.1 Hz, 1H), 6.86–6.68 (m, 2H), 5.76 (dd, J = 15.3, 5.4 Hz, 1H), 5.52 (dd, J = 15.3, 8.4 Hz, 1H), 4.45–4.33 (m, 1H), 4.15–4.02 (m, 3H), 3.70–3.58 (m, 1H), 3.02–2.91 (m, 1H), 2.83–2.72 (m, 2H), 2.72–2.48 (m, 4H), 2.48–2.30 (m, 4H), 2.30–2.20 (m, 1H), 1.98–1.84 (m, 3H), 1.80–1.64 (m, 1H), 1.64–1.50 (m, 2H), 1.43–1.30 (m, 2H), 0.97 (s, 9H), 0.92 (t, J = 7.2 Hz, 1H), 0.20 (s, 6H).

5.1.101. Butyl 4-({2-[(2*R*)-2-{(1*E*,3*S*)-4-(3-hydroxyphenyl)-3-(tetrahydro-2*H*-pyran-2-yloxy)-1-buten-1-yl}-5-oxo-1-pyrrolidinyl]ethyl}thio)butanoate (29)

To a stirred solution of **28** (992 mg, 1.76 mmol) and 3,4-dihydropyran (0.32 mL, 3.52 mmol) in CH_2Cl_2 (10 mL) was added *p*-toluenesulfonic acid monohydrate (3.3 mg, 0.018 mmol) at room temperature under argon atmosphere After being stirred for 30 min, the reaction was quenched with several drops of triethylamine and the solvent was evaporated to give crude tetrahydropyranyl ether as a light yellow oil.

To a stirred solution of the above-described tetrahydropyranyl ether in THF (5 mL) was added TBAF (1.0 M in THF, 2.6 mL, 2.6 mmol) at room temperature under argon atmosphere. The solution was stirred for 1 h and then poured into saturated aqueous NH_4Cl. The reaction mixture was extracted with EtOAc. The organic layer was washed with water, brine and dried over Na_2SO_4. The organic solvent was evaporated and the residue was purified by column chromatography on silica gel (hexane/EtOAc, 2:1–1:2) to give **29** as a white solid. (873 mg, 93%) ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.12 (t, J = 7.7 Hz, 1H), 6.80–6.57 (m, 3H), 6.14 (m, 1H), 5.61 (m, 1H), 5.35 (m, 2H), 4.60 (m, 1H), 4.30 (m, 1H), 4.16–3.97 (m, 3H), 3.88–3.33 (m, 3H), 3.06 (m, 1H), 2.80–2.39 (m, 7H), 2.37–2.28 (m, 2H), 2.15 (m, 1H), 1.98–1.30 (m, 13H), 0.93 (t, J = 7.1 Hz, 3H).

5.1.102. Butyl 4-({2-[(2*R*)-2-{(1*E*,3*S*)-4-[3-(3-pyridinylmethoxy)phenyl]-3-(tetrahydro-2*H*-pyran-2-yloxy)-1-buten-1-yl}-5-oxo-1-pyrrolidinyl]ethyl}thio)butanoate (30a)

To a stirred solution of **29** (300 mg, 0.562 mmol), 3-hydroxymethylpyridine (73.5 mg, 0.674 mmol) and triphenylphosphine (177 mg, 0.674 mmol) in THF (7 mL) was added diethyl azodicarboxylate (40% in toluene, 2.98 mL, 0.674 mmol) at 0 °C under argon atmosphere. After stirring for 3 h at room temperature, the solution was diluted with EtOAc. The organic layer was washed with water, brine and dried over Na_2SO_4. The organic solvent was removed by evaporation and the resulting residue was purified by column chromatography on silica gel (hexane/AcOEt, 1:2–0:1) to give **30a** as a colorless oil (168 mg, 48%).

5.1.103. 4-{[2-((2*R*)-2-{(1*E*,3*S*)-3-Hydroxy-4-[3-(pyridin-3-ylmethoxy)phenyl]but-1-enyl}-5-oxopyrrolidin-1-yl)ethyl]sulfanyl}butanoic acid (3j)

To a stirred solution of **30a** (167 mg, 0.268 mmol) in MeOH (2 mL) was added 2 N HCl (1 mL) at room temperature under argon atmosphere. The heterogeneous reaction mixture was stirred for 30 min and the resulting clear solution was treated with 2 N NaOH (2 mL). After stirring for 20 min, the solution was diluted with water and extracted with ether. The aqueous layer was neutralized with 1 N HCl and then evaporated. The precipitating inorganic salt was removed by filtration through a pad of Celite and washed with EtOH and EtOAc repeatedly. The filtrate was evaporated and the resulting residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH, 1:0–100:1–50:1–30:1) to give **3j** as a colorless viscous oil (82 mg, 63%). IR (film): 3376, 2917, 1664, 1421, 1254, 1157, 1036, 752 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.72 (s, 1H), 8.45 (d, J = 3.3 Hz, 1H), 7.85 (d, J = 7.8 Hz, 1H), 7.39 (dd, J = 7.8, 3.3 Hz, 1H), 7.16 (dd, J = 8.1, 8.1 Hz, 1H), 7.04 (s, 1H), 6.80–6.75 (m, 2H), 5.85 (dd, J = 15.3, 4.8 Hz, 1H), 5.62 (dd, J = 15.3, 8.7 Hz, 1H), 5.24 (d, J = 13.2 Hz, 1H), 5.17 (d, J = 13.2 Hz, 1H), 4.35–4.30

(m, 1H), 4.20–4.10 (m, 1H), 3.53–3.40 (m, 1H), 3.30–3.16 (m, 1H), 2.81–2.38 (m, 10H), 2.35–2.12 (m, 1H), 1.95–1.80 (m, 2H), 1.81–1.62 (m, 1H); MS (FAB) *m/z*: 485 $(M+H)^+$; HRMS-FAB (*m/z*): [M+H]+ calcd for $C_{26}H_{33}N_2O_5S$, 485.2110; found, 485.2116.

5.1.104. Butyl 4-({2-[(2*R*)-2-{(1*E*,3*S*)-4-[3-(2-pyridinylmethoxy) phenyl]-3-(tetrahydro-2*H*-pyran-2-yloxy)-1-buten-1-yl}-5-oxo-1-pyrrolidinyl]ethyl}thio)butanoate (30b)

Compound **30b** was prepared from **29** according to the same procedure as described for the preparation of **30a** from **29** as a colorless oil (46 mg, 75%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.60 (d, J = 4.8 Hz, 1H), 7.72 (m, 1H), 7.52 (d, J = 8.1 Hz, 1H),7.27–7.10 (m, 2H), 6.90–6.78 (m, 3H), 5.78–5.30 (m, 2H), 5.19 (s, 2H), 4.62–4.30 (m, 2H), 4.07 (t, J = 7.5 Hz, 2H), 3.83–3.35 (m, 4H), 3.03–2.16 (m, 3H), 1.95–1.30 (m, 7H), 0.93 (t, J = 7.5 Hz, 3H).

5.1.105. 4-{[2-((2*R*)-2-{(1*E*,3*S*)-3-Hydroxy-4-[3-(pyridin-2-ylmethoxy)phenyl]but-1-enyl}-5-oxopyrrolidin-1-yl)ethyl] sulfanyl}butanoic acid (3k)

Compound **3k** was prepared from **30b** according to the same procedure as described for the preparation of **3j** from **30a** as colorless oil (25 mg, 62%). IR (film): 3383, 2924, 1715, 1666, 1598, 1488, 1437, 1419, 1383, 1266, 1157, 1100, 1048, 975, 758, 697, 666 cm $^{-1}$; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.57 (d, J = 5.4 Hz, 1H), 7.80 (dt, J = 1.5, 7.5 Hz, 1H), 7.60 (d, J = 7.5 Hz, 1H), 7.32 (m, 1H), 7.22 (t, J = 7.8 Hz, 1H), 6.99–6.85 (m, 2H), 6.80 (d, J = 7.8 Hz, 1H), 5.85 (dd, J = 15.0, 4.8 Hz, 1H), 5.59 (ddd, J = 15.0, 8.7, 1.2 Hz, 1H), 5.32 (s, 2H), 4.43 (m, 1H), 4.11 (m, 1H), 3.43 (m, 1H), 3.18 (m, 1H), 2.88–2.18 (m, 13H), 1.97–1.83 (m, 2H), 1.72 (m, 1H); MS (FAB) *m/z*: 485 $(M+H)^+$; HRMS-FAB (*m/z*): $[M+H]^+$ calcd for $C_{26}H_{33}N_2O_5S$, 485.2110; found, 485.2105.

5.1.106. Butyl 4-({2-[(2*R*)-2-{(1*E*,3*S*)-4-[3-(4-pyridinylmethoxy) phenyl]-3-(tetrahydro-2*H*-pyran-2-yloxy)-1-buten-1-yl}-5-oxo-1-pyrrolidinyl]ethyl}thio)butanoate (30c)

Compound **30c** was prepared from **29** according to the same procedure as described for the preparation of **30b** from **29** as a colorless oil (36 mg, 59%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.01 (d, J = 5.4 Hz, 2H), 7.35 (d, J = 5.4 Hz, 2H), 7.30–7.18 (m, 3H), 6.90–6.77 (m, 3H), 5.70 and 5.55–5.29 (m, 2H), 5.08 (s, 2H), 4.62, 4.50 and 4.40–4.28 (m, 2H), 4.07 (t, J = 6.6 Hz, 2H), 3.80 and 3.62–3.30 (m, 3H), 3.05–2.12 (m, 3H), 1.95–0.138 (m, 7H), 0.93 (t, J = 7.5 Hz, 3H).

5.1.107. 4-{[2-((2*R*)-2-{(1*E*,3*S*)-3-Hydroxy-4-[3-(pyridin-4-ylmethoxy)phenyl]but-1-enyl}-5-oxopyrrolidin-1-yl)ethyl] sulfanyl}butanoic acid (3l)

Compound **3l** was prepared from **30c** according to the same procedure as described for the preparation of **3j** from **30a** as colorless oil (19 mg, 60%). IR (film): 3382, 2924, 1715, 1666, 1611, 1584, 1488, 1446, 1418, 1384, 1256, 1158, 1047, 975, 803, 755 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.58 (d, J = 6.0 Hz, 2H), 7.39 (d, J = 6.0 Hz, 2H), 7.22 (t, J = 7.8 Hz, 1H), 6.88–6.70 (m, 3H), 5.72 (dd, J = 15.3, 5.7 Hz, 1H), 5.45 (dd, J = 15.3, 8.1 Hz, 1H), 5.12 (s, 2H), 4.32 (m, 1H), 4.11 (m, 1H), 3.59 (m, 1H), 3.30 (m, 1H), 2.99 (m, 1H), 2.78 (m, 2H), 2.69–2.12 (m, 10H), 1.98–1.80 (m, 2H), 1.63 (m, 1H); MS (FAB) *m/z*: 485 $(M+H)^+$; HRMS-FAB (*m/z*): $[M+H]^+$ calcd for $C_{26}H_{33}N_2O_5S$, 485.2110; found, 485.2102.

5.2. Pharmacological evaluation

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. Nonspecific 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 EP2 or 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. Biological evaluation

Animals: Sprague–Dawley male rats (CrJ:CD(SD)IGS, 8-weeks old, Charles River Laboratories (Atsugi, Japan), were maintained in a controlled environment of 12 h light/12 h dark at 24 ± 2 °C, humidified at 55 ± 15%. The animals were housed in polycarbonate cage. All animal experiments in this study were performed in accordance with ethical guidelines established by the Experimental Animal Care and Use Committee of ONO Pharmaceutical Co., Ltd.

Fracture models: Fibulae were fractured according to the method of Kawaguchi et al. with minor modification.[15] In brief, after anesthetizing the animals with sodium pentobarbital (30–50 mg/kg), the midshaft of each left fibula was exposed and cut sharply with bone cutter.

Determination of bone strength: Fibulae were removed from anesthetized rats to estimate mechanical strength 17 days after fracture. The three-point destructive bending test was performed with a bone-testing machine (Instron 5544 model, Instron Japan). Breaking strength was expressed as percentage of the mean value of nonfractured fibulae harvested from intact rats.

5.2.4. Pharmacological evaluation of the hypotensive effects of compound 3i/PLGA in rats

Sprague–Dawley male rats (Crj:CD(SD)IGS, 7–9 weeks old (Charles River Laboratories)) were anesthetized with urethane (1.5 g/kg, sc). Blood pressure (BP) was measured directly from the C3 arteria carotis tube catheter equipped with a pressure transducer. Heart rate was also calculated from the pulse wave of the BP. Test compounds were injected into the femoral muscle.

5.3. PLGA formulation

5.3.1. Preparation of PLGA microspheres containing compound 3i, 4i, 4m,n

Microspheres were prepared by the emulsion–solvent evaporation method.[16,17] Compound (10 mg) and PLGA 75–65 (90 mg) were dissolved in 1 mL of dichloromethane in the oil phase. The oil phase was gradually added into an aqueous 0.1% PVA (polyvinyl alcohol) solution stirred with a turbine-shaped mixer (Homomixer) at 6000 rpm to obtain o/w emulsion. The emulsion was continuously stirred gently with a magnetic stirrer for 3 h to remove CH_2Cl_2. After organic solvent evaporation, the PLGA microspheres were suspended in the PVA solution. The suspension was centrifuged at 3000 rpm for 10 min to precipitate the microspheres and remove any unreacted compound. The supernatant was then discarded and replaced with fresh water or aqueous medium containing 0.2% Tween 80. This washing procedure was performed repeatedly. The washed microsphere precipitation was lyophilized to remove residual organic solvent and water, and to recover compound/ microspheres in solid form.

5.3.2. Drug loading and encapsulation efficiency of compounds 3i, 4i, 4m,n PLGA microspheres

The appropriate quantity of compound compound-loaded PLGA microspheres was dissolved with *p*-hydroxy benzoic acid *n*-nonyl ester in acetonitrile. The concentration of compound in the solution was analyzed by high performance liquid chromatography (HPLC). The drug loading and the encapsulation efficiency were calculated as follows:

$$\text{Drug loading } (\%, \text{w/w}) = (M/W) \times 100$$

where,M = measured amount of compound in the compound-loaded-PLGA microspheres (μg),W = weight of compound-loaded-PLGA microspheres (μg),Encapsulation efficiency (%, w/w) = (Lm/Lt) × 100, where Lm = measured drug loading (%, w/w) and Lt = theoretical drug loading (%, w/w).

5.3.3. In vitro release profile of compound 3i, 4i, 4m,n PLGA microsphere

Compound-loaded PLGA microspheres were suspended in phosphate-buffered saline (PBS) containing 0.2% Tween 80 to adjust the concentration of compound to 100 μg/mL. The microspheres were then completely dispersed by vortex mixing and sonication. The solution was divided into 1 mL samples and incubated at 37 °C. At various time intervals, the aliquots were centrifuged for 5 min at 12,000 rpm (n = 3 for each). The supernatant was removed and the pellet was dissolved with *p*-hydroxy benzoic acid *n*-nonyl ester in acetonitrile. The concentration of compound in the solution was analyzed by high performance liquid chromatography (HPLC).

The remaining percentage of compound was calculated as follows:

$$R_x = P_x/(S_0 + P_0) \times 100$$

where, R_x = Remaining percentage of compound at x days (%),P_x = Amount of compound in the pellet of 1 mL sample at x days (μg),S_0 = Amount of compound in the supernatant of 1 mL sample at 0 day (μg),P_0 = Amount of compound in the pellet of 1 mL sample at 0 day (μg).

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