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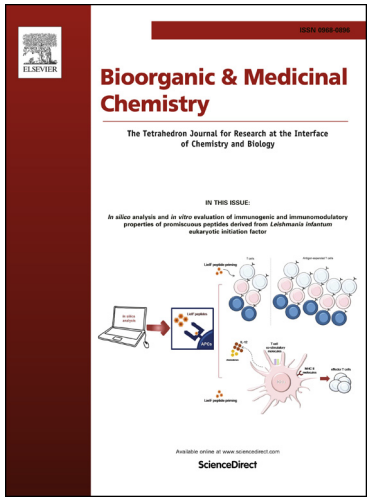

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Synthesis and Evaluation of a Potent, Well-balanced EP_2/EP_3 Dual Agonist

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ABSTRACT

A highly potent and well-balanced dual agonist for the EP_2 and EP_3 receptors is described. Optimization of the lead compound was accomplished in consideration of the relative agonist activity against each EP subtype receptor and the pharmacokinetic profile. As the result, 2-[(2-{(1R,2R)-2-[(1E,4S)-5-cyclopentyl-4-hydroxy-4-methyl-1-penten-1-yl]-5-oxocyclopentyl}ethyl)thio]-1,3-thiazole-4-carboxylic acid (**10**) showed excellent potency (human EC_{50} EP_2 = 1.1 nM, EP_3 = 1.0 nM) with acceptable selectivity over the EP_1 and EP_4 subtypes (>2000-fold). Further fine-tuning of compound **10** led to identification of **ONO-8055** as a clinical candidate. **ONO-8055** was effective at an extremely low dose (0.01 mg/kg, po, bid) in rats, and dose-dependently improved voiding dysfunction in a monkey model of underactive bladder (UAB). **ONO-8055** is expected to be a novel and highly promising drug for UAB.

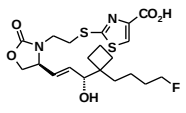
1. Introduction

Prostanoid receptors are members of the G-protein coupled receptor superfamily. Receptors for prostaglandin E_2 (PGE_2) can be classified into four subtypes: EP_1, EP_2, EP_3, and EP_4.[1] The diverse biological activities of PGE_2 are considered to be expressed as a hybrid of the activities mediated by these four EP receptor subtypes. Among them, the EP_2 receptor subtype[2,3] induces smooth muscle relaxation,[4] while the EP_3 receptor subtype inhibits smooth muscle relaxation.[5] Underactive bladder (UAB) represents dysfunctional conditions of the bladder where patients are unable to produce an effective voiding contraction. The most common clinical signs are an elevation of post-void residual urine volume and the lowering of urine flow rate. These symptoms have a profoundly negative impact on quality of life. PGE_2 is considered to act on both bladder and urethral smooth muscle. It has been reported that PGE_2 prompts contraction of the isolated bladder and relaxation of the isolated urethra.[6] Furthermore, our pharmacological tests revealed that an EP_3 selective agonist contracts the bladder and an EP_2 selective agonist relaxes the urethra (American Urology Association 2015).

We have already reported compound **1** as a highly potent EP_2 and EP_3 dual agonist.[7] Compound **1** showed low clearance (CL = 9.5 mL/min/kg) as shown in Table 1. In spite of low clearance, however, the bioavailability of **1** in rats was quite low (F_{rat} = 2.5%) due to its low permeability. Compound **1** also showed inadequate selectivity against the EP_4 receptor (human EC_{50} EP_2 = 2.9 nM, EP_3 = 10 nM, EP_4 = 195 nM). The EP_4 receptor subtype was also suggested to be involved in smooth muscle relaxation.[8] In terms of a manageable drug, EP_4 agonist activity must be removed because of a dramatic change in systemic blood pressure by acting on vascular smooth muscle.[9]

In this article, well-balanced, a highly selective and potent EP_2/EP_3 dual agonist is described. After extensive structural optimization from previously reported compound **1**, **ONO-8055** was identified as a clinical candidate for treatment of UAB. In European Phase I trials, **ONO-8055** was well-tolerated at multiple doses up to 700 μg bid oral dosing.[10]

Table 1. Permeability and PK Profiles of Compound **1**



1

cmpd	Caco-2 × 10^{-6}	Rat PK profile[a]

	(cm/s)	AUC (μg • h/mL)	CL (mL/min/kg)	F (%)
1	0.56	0.041	9.5	2.5

[a]Compound **1** was administered at 0.01 mg/kg, i.v. and 1 mg/kg, p.o.

2. Results and discussion

2.1. Structure-activity and physical property relationships

At first, we focused on the improvement of EP_4 subtype selectivity. According to previous reports,[11] the position of the hydroxyl group at C-15 is likely to affect EP_4 agonist activity directly. C-15 is derived from the numbering for prostaglandin E_2, as shown in Figure 1. Displacement of hydroxyl group at C-15 in lead compound **1** to next carbon (C-16) sharply reduced EP_4 agonist activity (Table 2). The resulting homoallylic tertiary alcohol **2** retained high EP_2 and EP_3 agonist activity, and showed slightly improved permeability. Permeability is one of the most important factors for an orally administered drug and it correlates closely with the lipophilicity of the compound. In addition to Caco-2 cell permeability studies, parallel artificial membrane permeability assay (PAMPA) was used for a simple evaluation of permeability.

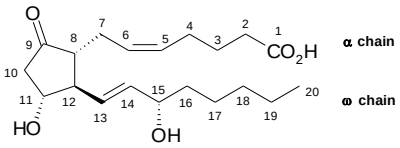
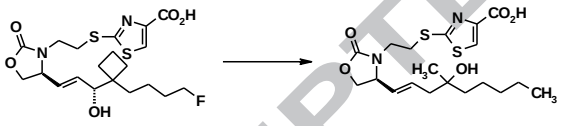


Figure 1. Structure of Prostaglandin E_2

Table 2. Structure Activity and Physical Property Relationship for Cyclic carbamate Compounds



1 **2**

cmpd	Caco-2 × 10^{-6} (cm/s)	Human Functional Assay, EC_{50} (nM)[a]		
		hEP_2	hEP_3	hEP_4
1	0.56	2.0	10	195
2	1.0	2.1	3.3	3380

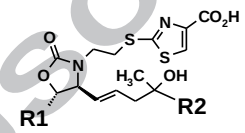
[a]EC_{50} values respectively represent the mean of at least two experiments.

In order to further improve permeability, we next tried to increase lipophilicity of compound **2**. As shown in Table 3, the incorporation of cyclohexyl group into ω-side chain and a methyl moiety onto the cyclic carbamate portion increased clog P, which is an indicator for lipophilicity, and also increased PAMPA value, as expected. However, EP_2 agonist activity of both compounds **3** and **4** was significantly reduced, suggesting that EP_2 agonist activity might be incompatible with improvements to permeability via introduction of hydrophobic groups.

Meanwhile, as the number of lone pair electrons increases, permeability generally tends to decrease because of a corresponding increase in the polar surface area and degree of hydration. Therefore, we also tried to cut down on the number of heteroatoms present in the compound. Removal of oxygen and nitrogen atom from cyclic carbamate in **2** led to cyclopentanone **5**, which is closer structurally to natural prostaglandins. As shown at Table 4, compound **5** showed a decreased topological polar surface area (TPSA)[12] in comparison to compound **2**, with concomitant balanced EP_2 agonist activity and high permeability. Unfortunately, cyclopentanone **5** exhibited inferior EP_4 subtype selectivity in comparison to cyclic carbamate **2**.

Furthermore, we individually prepared and evaluated two diastereomers at C-16 of compound **5**. As shown in Table 5, the *S* epimer (**5S**) turned out to be a highly active EP_2 and EP_3 dual agonist. The absolute configuration was determined as described below.

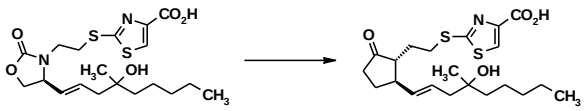
Table 3. Structure-Activity and Physical Property Relationship for Cyclic Carbamate Compounds



cmpd	R1	R2	Functional assay EC_{50} (nM)[a] hEP_2	hEP_3	hEP_4	PAMPA × 10^{-6} (cm/s) at pH=6.2	Caco-2 × 10^{-6} (cm/s)	cLogP
2	**H**		**2.1**	**3.3**	**3380**	**2.9**	**1.0**	**4.1**
3	**H**		**92**	**8.8**	**>10000**	**9.6**	**N.T.**[b]	**4.7**
4	**Me**		**3400**	**36**	**>10000**	**19**	**N.T.**[b]	**5.2**

[a]Assay protocols are provided in Experiments section. EC_{50} values represent the mean of more than two experiments.
[b]Not tested

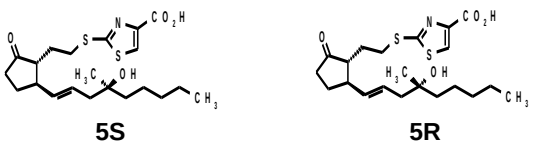
Table 4. Structure Activity and Physical Property Relationship for Cyclic Carbamate Compound and Cyclopentanone Compound



2 **5**

cmpd	Functional assay EC_{50} (nM)[a] hEP_2	hEP_3	hEP_4	PAMPA × 10^{-6} (cm/s) at pH=6.2	Caco-2 × 10^{-6} (cm/s)	cLogP	TPSA
2	**2.1**	**3.3**	**3380**	**2.9**	**1.0**	**4.1**	**100**
5	**0.48**	**2.7**	**91**	**40**	**4.1**	**4.6**	**87**

Table 5. Structure Activity Relationship for Cyclopentanone Compound **5S** and **5R** Isomer



5S **5R**

cmpd	Human Functional Assay, EC_{50}

	(nM)[a]		
	hEP_2	hEP_3	hEP_4
5S	0.086	0.91	146
5R	6.8	16	2540

[a]EC_{50} values represent the mean of at least two experiments.

Table 6. Pharmacokinetic Profiles in Rats and Physical Properties of compound **1** and **5S**

cmpd	Oral dose[a]					Intravenous dose[a]					TPSA	Clog *P*
	Dose	C_{max}	$T_{1/2}$	AUC	F	Dose	CL	$T_{1/2}$	AUCinf	Vss		
	(mg/kg)	(ng/mL)	(hr)	(ng h/mL)	(%)	(mg/kg)	(mL/min/kg)	(hr)	(ng h/mL)	(mL/kg)		
1	1	3.7	-	41	2.5	0.01	9.5	4.4	17	728	100	3.7
5S	0.1	0.96	4.1	8.9	17	0.1	34	11	51	107000	87	4.6

[a] Each value is the mean of three animals in rat study.

2.2. Further modification toward a clinical candidate

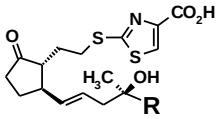
The pharmacokinetic (PK) profile of compound **5S** was evaluated and compared to the lead compound **1** (Table 6). Compound **5S** exhibited 17% bioavailability in rats due to its higher permeability versus **1**. According to this result, compound **5S** could be considered to be a key compound toward a clinical candidate. The concept behind an EP_2/EP_3 dual agonist is to synergistically maximize drug potency while minimizing adverse effects to the circulatory system. As mentioned earlier, our goal is to identify well-balanced EP_2 and EP_3 agonist activity with high selectivity over the EP_4 subtype. Specifically, EP_4 agonist activity that drastically lowers blood pressure must be eliminated.

Finally, we focused on improving the EP_4 subtype selectivity of compound **5S** while maintaining the other desired properties. The structure-activity relationship of the resulting derivatives is described in Table 7. The data for compounds **6**-**9** indicated that the bulkiness of ω side chain reduced EP_4 agonist activity, but also tended to weaken EP_2 agonist activity. No major change was seen in EP_3 agonist activity. This trend was especially noticeable for compound **9**, where the bulky *tert*-butyl group on the ω side chain adequately improved EP_4 subtype selectivity but also resulted in a drastic loss of EP_2 agonist activity even in comparison with the trifluoromethylated **8**. In terms of the bulkiness of ω side chain, homology modeling based on publicly disclosed data (GPCRdb, http://gpcrdb.org/alignment) supports the difficulty in balancing EP_2 and EP_3 agonist activity. According to the homology model, the binding pocket in the EP_2 receptor seems to be narrower and less tolerant of bulkiness than in the EP_3 receptor, as shown in Figure 2. EP_2 and EP_3 receptors respectively have Phe280 + Leu304 and Leu298 + Ala335 toward their binding pocket. This means there is larger bulge protruding into the binding region of the EP_2 receptor. Therefore, we replaced the *tert*-butyl group of compound **9** with a flatter cyclopentyl group. As expected, EP_2 agonist activity of cyclopentylated **10** was once again equal to EP_3 agonist activity, whereas selectivity against the EP_4 subtype remained high. In this case, the size of the cyclopentyl group turned out to be suitable to bind to both EP_2 and EP_3 receptor and not to bind to the EP_4 receptor. As an even more favorable characteristic, compound **10** also showed high selectivity against the EP_1 subtype (EC_{50} = 2,000 nM). Regarding the configuration at C-16, we synthesized the corresponding C-16(*R*) epimer **11** and reconfirmed that the *S*-isomer **10** is more active (Table 8).

At this point, we tried to confirm whether an EP_2/EP_3 dual agonist indicates the desired biological activity. When compound **10** was preliminarily evaluated by the use of a rat lumber spinal canal stenosis derived UAB model, it reduced the residual volume of urine with oral dosing of 0.03 mg/kg.[13] This result meant an EP_2/EP_3 dual agonist can be a novel drug for treatment of UAB, and motivated us to optimize compound **10** toward a clinical candidate.

Further modification of the ω-side chain terminus in compound **10** finally resulted in identification of our clinical candidate, **ONO-8055**. As mentioned above, the bulkiness of the ω-side chain terminus is a key factor for an EP_2/EP_3 dual agonist. **ONO-8055** was designed in consideration of the size of terminus and the physical property as well. **ONO-8055**, which possessed *S* stereochemistry at C-16, exhibits both well-balanced and highly potent EP_2 and EP_3 agonist activity, in addition to a good pharmacokinetic profile in both rats and cynomolgus monkeys (for example, *iv* clearance of **ONO-8055**: <5 mL/min/kg). In the rat UAB model, **ONO-8055** demonstrated the efficacy with oral dosing of 0.01 mg/kg.[13]

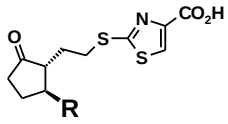
Table 7. Structure Activity Relationship for Cyclopentanone Compounds

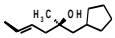
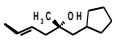


cmpd	R	Functional assay EC_{50} (nM)[a]			EP4 selectivity vs EP_2 / vs EP_3	Balance of EP_2 / EP_3
		hEP_2	hEP_3	hEP_4		
5S		**0.086**	**0.91**	**146**	**1698 / 160**	**0.095**
6		**0.068**	**0.80**	**26**	**382 / 33**	**0.085**
7		**0.11**	**3.0**	**578**	**5255 / 193**	**0.037**
8		**0.37**	**3.5**	**127**	**343 / 36**	**0.11**
9		**31**	**3.4**	**>10000**	**>323 / >2941**	**9.1**
10		**1.1**	**1.0**	**2636**	**2396 / 2636**	**1.1**

[a]EC_{50} values represent the mean of at least two experiments.

Table 8. Structure Activity Relationship for Diastereomeric Isomers



cmpd	R	Functional assay EC_{50} (nM) [a] hEP2	hEP3	hEP4	EP_4 selectivity vs EP_2 / vs EP_3	Balance of EP_2 / EP_3
10		1.1	1.0	2636	2396 / 2636	1.1
11		2.0	32	>10000	>5000 / >313	0.063
ONO-8055		0.67	0.7	339	506 / 484	1.0

[a]EC_{50} values represent the mean of at least two experiments.

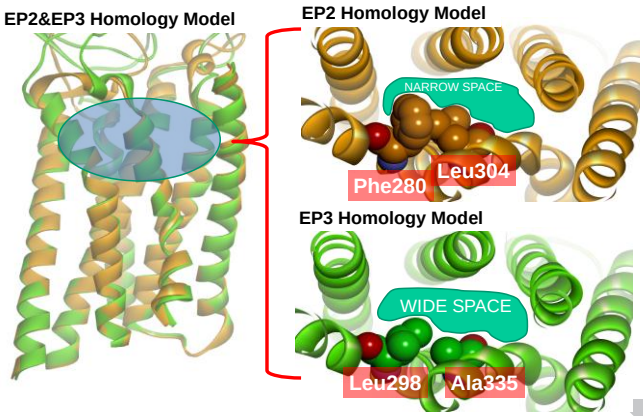


Figure 2. Homology models of EP_2 and EP_3 receptors

Regarding an expanded *in vivo* evaluation, **ONO-8055** improved the lower urinary tract dysfunctions of neurogenic UAB in a rat lumber spinal canal stenosis model with twice-daily (bid) oral dosing of 0.01 mg/kg.[14] It is worth noting that **ONO-8055** had little effect on blood pressure and heart rate at up to 0.3 mg/kg oral dosing. This strongly suggests that an appropriate balance between EP_2 and EP_3 agonist activity alleviates cardiovascular risks, as was predicted. Furthermore, **ONO-8055** was compared with a cholinesterase inhibitor, distigmine, in a monkey UAB model.[15] Distigmine is one of the primary drugs currently used for UAB. As shown in Figure 3, the voided volume was dose-dependently increased by a 2-hour iv infusion of 0.003, 0.03 and 0.3 ng/kg/min of **ONO-8055**. The voided volume at 0.3 ng/kg/min was the same as that at 30 ng/kg/min of distigmine. **ONO-8055** demonstrated much higher *in vivo* efficacy than the currently used drug for UAB.

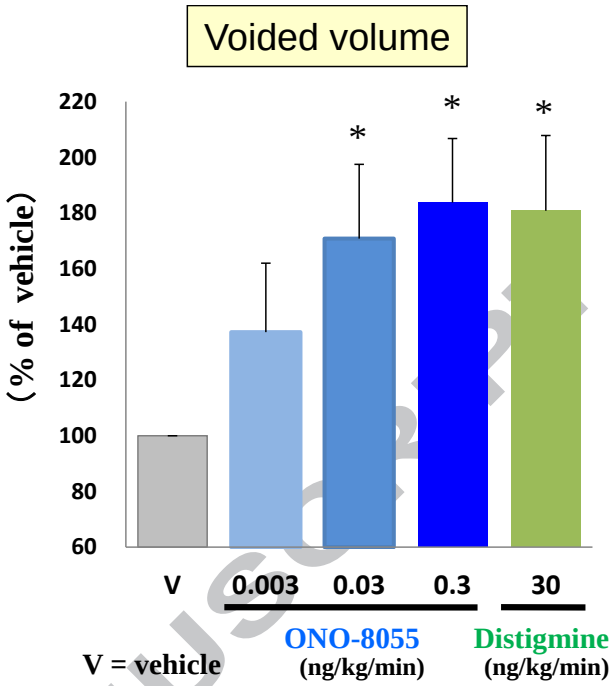


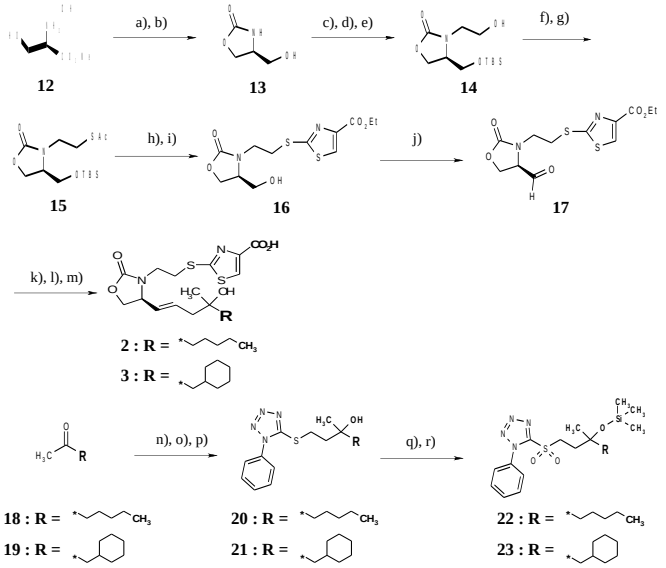
Figure 3. The effect of **ONO-8055** and distigmine in monkey UAB model

3. Chemistry

The syntheses of **2** and **3** are described in Scheme 1. Commercially available *D*-serine methyl ester hydrochloride **12** was treated with triphosgene to form the corresponding cyclic carbamate. Subsequent reduction of the methyl ester afforded alcohol **13**. Protection of the hydroxyl group of **13** was followed by *N*-alkylation of cyclic carbamate, reduction of the introduced ethyl ester moiety, mesylation of the resulting alcohol **14** and substitution by thioacetate to extend the α side chain. Treatment of thioacetate **15** with ethyl 2-bromo-1,3-thiazole-4-carboxylate and removal of TBS group provided alcohol **16**. Oxidation of alcohol **16**, Julia olefination with sulfone **22** or **23**, and removal of the TMS protection by aqueous HCl were followed by hydrolysis to provide **2** or **3**, respectively. Regarding preparation of sulfone **22** or **23**, at first Reformatsky reaction between ketones **18** or **19** and ethyl bromoacetate was performed, followed by reduction of the ethyl ester moiety. The primary alcohol of the resulting diol was tosylated and displaced with 1-phenyl-1H-tetrazole-5-thiol to provide sulfide **20** or **21**. Each sulfide was oxidized with *m*-CPBA and protected at the tertiary alcohol with TMS to furnish sulfone **22** or **23**, respectively.

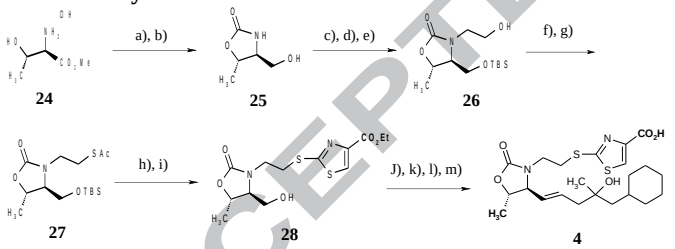
The synthesis of **4** is described in Scheme 2. Compound **4** was prepared by the use of *D*-threonine methyl ester hydrochloride **24** instead of *D*-serine methyl ester hydrochloride **12** as the starting material and followed an otherwise identical route as shown in Scheme 1.

Scheme 1. Syntheses of 2 and 3[a]



[a]Reagents and conditions: (a) K_2CO_3, water, triphosgene, toluene, 0 °C; (b) $NaBH_4$, EtOH, rt, 53% (for 2 steps); (c) TBSCl, imidazole, DMF, rt; (d) ethyl bromoacetate, KO*t*Bu, THF, rt; (e) $NaBH_4$, THF-EtOH, rt, 86% (for 3 steps); (f) MsCl, Et_3N, CH_2Cl_2, 0 °C; (g) KSAc, DMF, rt, 100% (for 2 steps); (h) ethyl 2-bromo-1,3-thiazole-4-carboxylate, tributylphosphine, K_2CO_3, EtOH, 50 °C; (i) TBAF, THF, rt, 78% (for 2 steps); (j) SO_3-Py, Et_3N, DMSO, EtOAc, 10 °C; (k) KHMDS, **22** or **23**, DME, -78 °C, 16-26% (for 2 steps); (l) 4N HCl(aq), EtOAc, 0 °C; (m) 2N NaOH(aq), EtOH, 0 °C, 33-65% (for 2 steps); (n) ethyl bromoacetate, zinc, iodine, dioxane, 0 °C; (o) $LiAlH_4$, THF, 0 °C, 50-70% (for 2 steps); (p) 5N NaOH(aq), *n*-Bu_4NBr, TsCl, toluene, 0 °C, then 1-phenyl-1H-tetrazole-5-thiol, 60 °C, 80-86%; (q) *m*-CPBA, CH_2Cl_2, (r) TMSCl, imidazole, CH_2Cl_2, 75-82% (for 2 steps).

Scheme 2. Synthesis of 4[a]



[a]Reagents and conditions: (a) K_2CO_3, water, triphosgene, toluene, 0 °C; (b) $NaBH_4$, EtOH, rt, 88% (for 2 steps); (c) TBSCl, imidazole, DMF, rt; (d) ethyl bromoacetate, KO*t*Bu, THF, rt; (e) $NaBH_4$, THF-EtOH, rt, 94% (for 3 steps); (f) MsCl, Et_3N, CH_2Cl_2, 0 °C; (g) KSAc, DMF, rt, 100% (for 2 steps); (h) ethyl 2-bromo-1,3-thiazole-4-carboxylate, tributylphosphine, K_2CO_3, EtOH, 50 °C; (i) TBAF, THF, rt, 83% (for 2 steps); (j) SO_3-Py, Et_3N, DMSO, EtOAc, 10 °C; (k) KHMDS, **23**, DME, -78 °C, 78% (for 2 steps); (l) 4N HCl(aq), EtOAc, 0 °C; (m) 2N NaOH(aq), EtOH, 0 °C, 88% (for 2 steps);

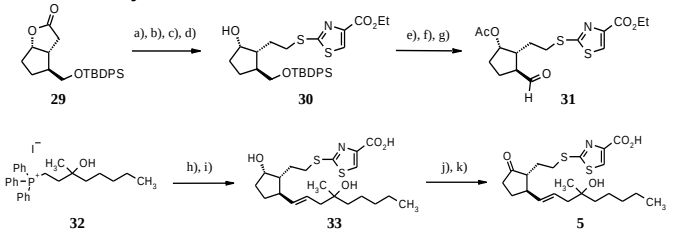
The synthesis of **5** is described in Scheme 3. Corey lactone **29** was first reduced with $LiAlH_4$ to make the core structure. The resulting diol was mesylated at the primary alcohol, protected at the secondary alcohol with TMS, substituted by thioacetate and treated with ethyl 2-bromo-1,3-thiazole-4-carboxylate to extend the α side chain. Removal of TMS group furnished alcohol **30**. Subsequent acetylation of the hydroxyl group of **30,** deprotection of TBDPS group and oxidation of the resulting alcohol provided aldehyde **31**. *E*-selective Wittig olefination[16] with phosphonium salt **32** and aldehyde **31** followed by hydrolysis provided carboxylic acid **33**. After temporary protection of the carboxylic acid with TBS and oxydation of the secondary hydroxyl group, we obtained the desired compound **5**.

As mentioned earlier, the *S*-configuration at C-16 is the more active form. The isomers were separated and the more active configuration was defined as follows: the syntheses of **5S** and **5R** are described in Scheme 4. Compound **33** was at first protected at the secondary alcohol with acetyl group, and then macrolactonization employing the Yamaguchi conditions afforded a mixture of two isomers (**34S** and **34R**). Fortunately, these two isomers had a distinct difference in polarity and were separable by silica gel column chromatography. In addition, when the cyclohexyl analog of **5** was prepared in parallel, the more polar macrolactone **40** was obtained as crystals. Therefore, the absolute configuration of **40** was determined by X-ray crystallography as shown in Figure 4. According to this result, the more polar macrolactone and the less active target compound derived from it was determined to be the C-16(*R*) isomer. In other words, the more active epimer is derived from the less polar macrolactone and has the *S*-configuration at C-16. In terms of the absolute configuration, it is also consistent with our knowledge in a variety of prostaglandin analogs which we synthesized so far.

After chromatographic separation of the two epimers, each macrolactone (**34S** or **34R**) was hydrolyzed to give the corresponding ring-opened compound, followed by ethyl esterification of carboxylic acid and oxidation of secondary alcohol. The obtained ester (*S* or *R*-isomer) was hydrolyzed to furnish the desired compound **5S** or **5R**, respectively.

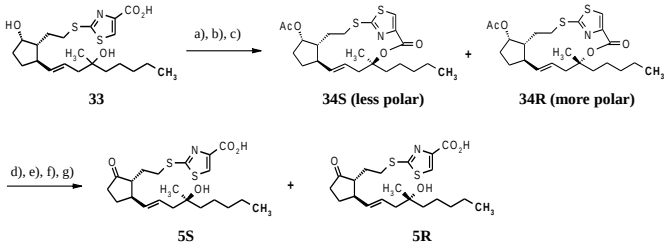
The syntheses of **6-10** are described in Scheme 5. Compounds **6-10** all possess the more active *S*-configuration at C-16. The requisite sulfones **37a-e** were prepared in a similar way to that shown in Scheme 1. Julia olefination between aldehyde **31** and sulfones **37a-e** gave the corresponding *E*-olefins, followed by hydrolysis of the ethyl ester and deprotection of TMS group. Subsequent macrolactonization of the resulting unmasked carboxylic acids **38a-e** and purification by silica gel column chromatography were performed in a similar fashion. The desired compounds **6-10** were derived from the corresponding less polar macrolactones **39a-e**. The *R*-isomer **11** in Table 8 was derived from the more polar maclolactone produced in the process of preparation of **10** as well.

Scheme 3. Synthesis of 5[a]



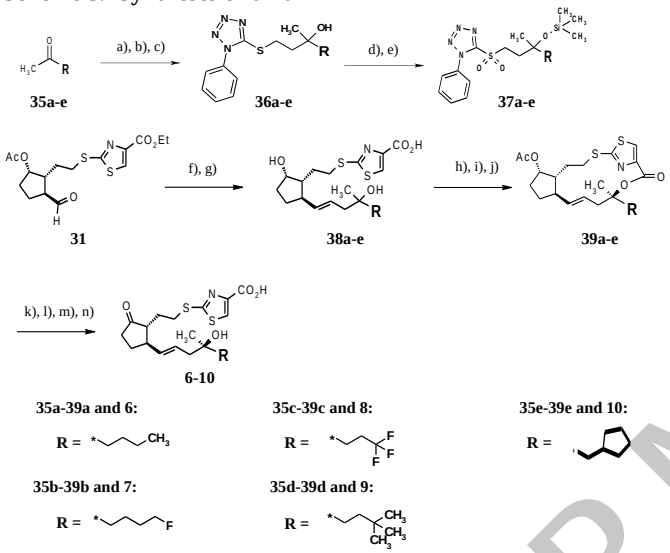
[a]Reagents and conditions: (a) $LiAlH_4$, THF, 0 °C; (b) MsCl, DIPEA, THF, -5 °C, then TMSCl, AcSK, K_2CO_3, DMF, 50 °C; (c) ethyl 2-bromo-1,3-thiazole-4-carboxylate, tributylphosphine, K_2CO_3, EtOH, 50 °C; (d) 1N HCl(aq), THF, 0 °C, 38% (for 4 steps); (e) Ac_2O, Py, DMAP, rt; (f) TBAF, THF, rt; (g) SO_3-Py, Et_3N, DMSO, EtOAc, 10 °C, 97% (for 3 steps); (h) *n*-BuLi, **31**, THF, -78 °C; (i) 2N NaOH(aq), MeOH, rt, 37% (for 2 steps); (j) TBSCl, TEA, Toluene; (k) SO_3-Py, DIPEA, DMSO, EtOAc, 0 °C, 62% (for 2 steps).

Scheme 4. Syntheses of 5S,5R[a]



[a]Reagents and conditions: (a) Ac_2O, pyridine, rt; (b) 2,4,6-trichlorobenzoyl chloride, DMAP, toluene, 100 °C ; (c) separation by silica gel column chromatography, 35% (for **34S**), 25% (for **34R**) (for 3 steps); (d) 2N NaOH(aq), THF/MeOH, rt; (e) ethyl iodide, K_2CO_3, DMF ; (f) SO_3-Py, DIPEA, DMSO, EtOAc, 0 °C; (g) LiOH, DME/H_2O, 80% (for **5S**), 72% (for **5R**) (for 4 steps).

Scheme 5. Syntheses of 6-10[a]



[a]Reagents and conditions: (a) ethyl bromoacetate, zinc, iodine, dioxane, 0 °C; (b) $LiAlH_4$, THF, 0 °C, 50-70% (for 2 steps); (c) 5N NaOH(aq), *n*-Bu_4NBr, TsCl, toluene, 0 °C, then, 1-phenyl-1H-tetrazole-5-thiol, 60 °C, 80-86%; (d) *m*-CPBA, CH_2Cl_2; (e) TMSCl, imidazole, CH_2Cl_2, 75-82% (for 2 steps) ; (f) KHMDS, **37a-e**, DME, -78 °C; (g) 2N NaOH(aq), EtOH, rt, then 1N HCl(aq), 64-77% (for 2 steps); (h) Ac_2O, Pyridine, rt; (i) 2,4,6-trichlorobenzoyl chloride, DMAP, toluene, 100 °C, (j) separation by silica gel column chromatography, 20-35% (for less polar lactone for 3 steps) (k) 2N NaOH(aq), THF/MeOH, rt; (l) ethyl iodide, K_2CO_3, DMF; (m) SO_3-Py, DIPEA, DMSO, EtOAc, 0 °C; (n) LiOH, DME/H_2O, 68-79% (for 4 steps).

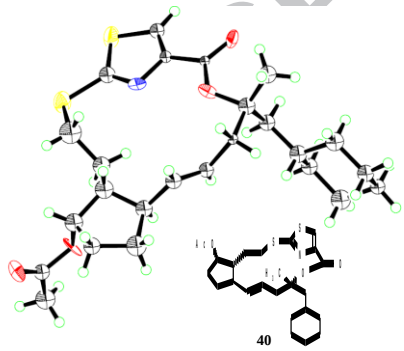


Figure 4. X-ray crystallography of macrolactone **40**

4. Conclusion

In summary, the optimization of compound **1** was carried out to improve the PK profile and EP_4 subtype selectivity while maintaining the acceptable balance between EP_2 and EP_3 agonist activity. As a result, 2-[(2-{(1R,2R)-2-[(1E,4S)-5-cyclopentyl-4-hydroxy-4-methyl-1-penten-1-yl]-5-oxocyclopentyl}ethyl)thio]-1,3-thiazole-4-carboxylic acid (**10**) showed excellent potency and good balance (human EC_{50} EP_2 = 1.1 nM, EP_3 = 1.0 nM) with excellent EP_1 and EP_4 subtype selectivity (>2000-fold). Moreover, **ONO-8055**, which is the derivative of compound **10**, dose-dependently increased voided volume in monkey UAB model at a dose of 0.03 ng/kg, *iv*. In a European Phase I trial, **ONO-8055** proved to be well-tolerated at multiple doses up to 700 μg bid oral dosing. **ONO-8055** is under development as a clinical candidate and it is expected to be novel and highly promising drug for the treatment of UAB.

5. Experiments

5.1. Chemistry

Analytical samples were homogeneous as confirmed by TLC, and afforded spectroscopic results consistent with the assigned structures. NMR spectra were recorded as designated on either a Varian Mercury 300 spectrometer or INOVA-500 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) were obtained on a JEOL JMS-DX303HF spectrometer. Atmospheric pressure chemical ionization (APCI) mass spectra were determined by Hitachi M-1200H spectrometer. IR spectra were measured on a JASCO FTIR-430 spectrometer. Elemental analyzes were performed with a Perkin–Elmer PE2400 series II CHNS/O Analyzer and were only indicated as the elements within ±0.4% of the theoretical values unless otherwise noted. Purity analysis was carried out by the following HPLC or LC/MS system. HPLC: Agilent Technologies 1200 Series with Unison UK-C18 (4.6 x 150mm). LC/MS: Waters ACQUITY UPLC system fitted by with Waters Micromass ZQ-2000 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 F254). 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). *tert*-butyl methyl ether (^{t}BuOMe), diisopropyl ether (^{i}Pr$_2$O), (acetonitrile (CH_3CN), triethylamine (Et_3N), trifluoroacetic acid (TFA).

5.1.1. (4S)-4-(hydroxymethyl)-1,3-oxazolidin-2-one (13)

Commercial available *D*-serine methyl ester hydrochloride (5.76 g) was dissolved in water (52.0 mL), and stirred with potassium bicarbonate (4.08 g) for 10 minutes. To the resulting solution were added potassium bicarbonate (5.63 g) and a solution of triphosgene (14.3 g) in toluene (26.0 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 2 hours, and then diluted with ethyl acetate. The aqueous layer was removed and concentrated in vacuo. The resulting residue was dispersed into methylene chloride, dried over anhydrous sodium sulfate and concentrated in vacuo. The ethanolic solution (73.0 mL) of the obtained residue was treated with sodium borohydride (1.34 g) and stirred for 3 hours at 0 °C. The reaction mixture was quenched with saturated ammonium chloride at 0 °C and stirred at room temperature for 30 minutes. The resulting mixture was filtered and concentrated. The obtained residue was purified by column chromatography on silica gel (ethyl acetate : methanol = 4 : 1) to give compound **13** (2.28 g, 53%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.62 (dd, *J* = 11.4, 5.7 Hz, 1H), 3.73 (m, 1H), 4.01 (m, 1H), 4.23 (m, 1H), 4.49 (t, *J* = 8.7 Hz, 1H), 5.99 (s, 1H).

5.1.2. (4R)-4-({[tert-butyl(dimethyl)silyl]oxy}methyl)-3-(2-hydroxyethyl)-1,3-oxazolidin-2-one (14)

To a solution of alcohol **13** (2.28 g) in dimethylformamide (20.0 mL) was added imidazole (1.60 g) and *tert*-butyldimethylsilyl chloride (3.09 g). The resulting solution was stirred at room temperature overnight, then poured into ice and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous sodium sulfate and concentrated. The obtained residue was dissolved in anhydrous tetrahydrofuran (45.0 mL), treated with potassium *tert*-butoxide (2.41 g), when cooled in ice. After stirring for 10 minutes, the reaction mixture was treated with 2-bromoethyl acetate (2.40 mL), and stirred at room temperature for 3 hours. The reaction was quenched with saturated ammonium chloride, and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated. To a solution of the obtained residue in tetrahydrofuran/ethanol (7:1) (46.0 mL) was added sodium borohydride (2.22 g). The reaction mixture was stirred at room temperature for 2 hours, then poured into ice/saturated ammonium chloride and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous sodium sulfate and concentrated to give compound **14** (4.62 g, 86%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.09 (s, 6H), 0.90 (s, 9H), 2.76 (s, 1H), 3.45 (m, 2H), 3.83 (m, 5H), 4.13 (dd, *J* = 8.6, 5.6 Hz, 1H), 4.38 (dd, *J* = 8.7, 8.6 Hz, 1H).

5.1.3. S-{2-[(4R)-4-({[tert-butyl(dimethyl)silyl]oxy}methyl)-2-oxo-1,3-oxazolidin-3-yl]ethyl} ethanethioate (15)

Methanesulfonyl chloride (1.43 mL) was added dropwise to a solution of the compound **14** (4.58 g) and triethylamine (3.50 mL) in methylene chloride (32.0 mL) under cooling with ice. The reaction mixture was stirred at 0 °C for 1 hour, then quenched with water and extracted with ethyl acetate. The organic layer was washed with 1N hydrochloric acid, water and brine, dried over anhydrous magnesium sulfate and concentrated. To a solution of the obtained residue in dimethylformamide (17.0 mL) was added potassium thioacetate (1.91 g). The reaction mixture was stirred at room temperature for 2 hours, then quenched with water, and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous sodium sulfate and concentrated to give compound **15** (5.56 g, 100%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.08 (s, 6H), 0.89 (s, 9H), 2.35 (s, 3H), 3.07 (m, 2H), 3.31 (m, 1H), 3.57 (m, 1H), 3.68 (dd, J=10.8, 4.0 Hz, 1H), 3.79 (m, 1H), 3.93 (m, 1H), 4.12 (m, 1H), 4.33 (t, J=8.7 Hz, 1H).

5.1.4. ethyl 2-({2-[(4S)-4-(hydroxymethyl)-2-oxo-1,3-oxazolidin-3-yl]ethyl}thio)-1,3-thiazol-4-carboxylate (16)

To a solution of the compound **15** (5.56 g), ethyl 2-bromo-1,3-thiazole-4-carboxylate (4.34 g) and tributylphosphine (0.46 mL) in ethanol (36.0 mL) was added potassium carbonate (3.70 g) under cooling with ice. The reaction mixture was stirred at room temperature for 1 hour and subsequently at 50 °C overnight. The reaction was quenched with water, and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous magnesium sulfate and concentrated. To a solution of the obtained residue in tetrahydrofuran (18.0 mL) was added a solution of 1M tetrabutylammonium iodide in tetrahydrofuran (18.4 mL) at 0 °C. The reaction mixture was stirred at room temperature for 1 hour, then treated with water, and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous magnesium sulfate and concentrated. The crude product was purified by column chromatography on silica gel (*n*-hexane : ethyl acetate = 2 : 3), to give compound **16** (4.29 g, 78%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (m, 3H), 3.66 (m, 6H), 4.14 (m, 2H), 4.39 (m, 3H), 8.02 (s, 1H).

5.1.5. ethyl 2-({2-[(4R)-4-formyl-2-oxo-1,3-oxazolidin-3-yl]ethyl}thio)-1,3-thiazole-4-carboxylate (17)

To a solution of the compound **16** (338 mg) in dimethylsulfoxide (3.40 mL)/ethyl acetate (6.80 mL) were added triethylamine (1.00 mL) and sulfur trioxide-pyridine complex (650 mg) at 10 °C. The reaction mixture was stirred at room temperature for 1 hour, then treated with 1N hydrochloric acid, and extracted with ethyl acetate. The organic layer was washed water and brine, dried over anhydrous sodium sulfate and concentrated to give compound **17** (336 mg, 100%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (t, *J* = 7.0 Hz, 3H), 3.84 (m, 9H), 8.02 (m, 1H), 9.81 (s, 1H).

5.1.6. 3-methyl-1-[(1-phenyl-1H-tetrazol-5-yl)thio]-3-octanol (20)

To a solution of commercial available 2-heptanone (10.1 mL) in 1,4-dioxane (150 mL) were added ethyl bromoacetate (9.6 mL), zinc (8.5 g) and iodine (3.7 g) at room temperature. The reaction mixture was sonicated for 2 hours, then carefully treated with 1N hydrochloric acid, and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous magnesium sulfate and concentrated. The residue was purified by flush column chromatography (*n*-hexane : ethyl acetate = 15 : 1). A solution of the obtained compound (13.86 g) in tetrahydrofuran (100 mL) was added dropwise to a suspension of lithium aluminum hydride (3.9 g) in tetrahydrofuran (100 mL) at 0 °C. After 35 minutes, the reaction was diluted with ethyl acetate at 0°C and quenched with 5N hydrochloric acid (20 mL) until the evolution of gas ceased. The solution temperature was raised to room temperature and the solution was stirred overnight. The reaction solution was dried over anhydrous magnesium sulfate, filtrated through Celite and concentrated. The obtained residue was purified by column chromatography (*n*-hexane : ethyl acetate = 1:1) to give diol compound (10.31 g). To a solution of the diol compound (8.05 g) in toluene (98 mL) were added tetrabutylammonium bromide (1.62 g) and 2N sodium hydroxide (98 mL) at 0°C. The resulting mixture was treated with a suspension of tosyl chloride (10.5 g) in toluene (40 mL). The reaction mixture was warmed to room temperature and stirred for 1 hour, followed by treatment with a solution of 1-phenyl-1H-tetrazole-5-thiol (10.74 g) in toluene. The reaction mixture was stirred at 60 °C for 3.5 hours, and extracted with *tert*-butoxymethyl ether. The organic layer was washed with brine, dried over anhydrous magnesium sulfate and concentrated. The obtained residue was purified by silica gel column chromatography (*n*-hexane : ethyl acetate = 81 : 19) to give compound **20** (14.15 g, 86%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, *J* = 7.5 Hz, 3H), 1.25 (s, 3H), 1.42 (m, 6H), 1.57 (m, 2H), 1.65 (s, 1H), 2.04 (m, 2H), 3.57 (m, 2H), 7.73 (m, 5H).

5.1.7. 5-({3-methyl-3-[(trimethylsilyl)oxy]octyl}sulfonyl)-1-phenyl-1H-tetrazole (22)

To a solution of the compound **20** (14.0 g) in methylene chloride (200 mL) was added *meta*-chloroperbenzoic acid (27.3 g) at 0 °C. The reaction mixture was warmed to room temperature and stirred overnight. The reaction was quenched

with an aqueous saturated sodium bicarbonate, evaporated to remove methylene chloride and extracted with ethyl acetate. The organic layer was washed with an aqueous saturated sodium bicarbonate solution and brine, dried over anhydrous magnesium sulfate and concentrated to give sulfone compound (15.4 g).

To a solution of the sulfone compound (15.4 g) in methylene chloride (87 mL) were added imidazole (8.92 g) and trimethylsilyl chloride (11.1 mL) at 0 °C. The reaction mixture was stirred for 1 hour, and then treated with water, and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous magnesium sulfate and concentrated. The obtained residue was purified by column chromatography (*n*-hexane : ethyl acetate = 25 : 1), to give compound **22** (16.95 g, 82%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.12 (s, 9 H), 0.90 (t, *J* = 6.0 Hz, 3H), 1.39 (m, 9H), 1.58 (m, 2H), 2.20 (m, 2H), 3.94 (m, 2H), 7.86 (m, 5H).

5.1.8. (4S,5S)-4-(hydroxymethyl)-5-methyl-1,3-oxazolidin-2-one (25)

Starting from *D*-threonine methyl ester hydrochloride following the same procedure as compound **13** provided compound **25**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 (d, *J* = 6.0 Hz, 3H), 3.62 (m, 3H), 4.51 (m, 1H), 6.20 (s, 1H).

5.1.9. (4S,5S)-4-({[dimethyl(2-methyl-2-propanyl)silyl]oxy}methyl)-3-(2-hydroxyethyl)-5-methyl-1,3-oxazolidin-2-one (26)

Starting from compound **25** following the same procedure as compound **15** provided compound **26**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.09 (s, 6H), 0.90 (s, 9H), 1.44 (d, *J* = 6.0 Hz, 3H), 3.43 (m, 3H), 3.78 (m, 4H), 4.38 (m, 1H).

5.1.10. S-{2-[(4S,5S)-4-({[dimethyl(2-methyl-2-propanyl)silyl]oxy}methyl)-5-methyl-2-oxo-1,3-oxazolidin-3-yl]ethyl}ethanethioate (27)

Starting from compound **26** following the same procedure as compound **15** provided compound **27**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.08 (s, 6H), 0.89 (s, 9H), 1.42 (d, *J* = 6.0 Hz, 3H), 2.35 (s, 3H), 3.07 (m, 1H), 3.30 (m, 1H), 3.43 (m, 1H), 3.55 (m, 1H), 3.72 (m, 2H), 4.11 (m, 1H), 4.37 (m, 1H) .

5.1.11. ethyl 2-({2-[(4S,5S)-4-(hydroxymethyl)-5-methyl-2-oxo-1,3-oxazolidin-3-yl]ethyl}thio)-1,3-thiazole-4-carboxylate (28)

Starting from compound **27** following the same procedure as compound **17** provided compound **28**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (m, 6H), 3.60 (m, 6H), 3.84 (m, 2H), 4.40 (m, 2H), 8.02 (s, 1H).

5.1.12. 2-[(2-{(4S,5S)-4-[(1E)-5-cyclohexyl-4-hydroxy-4-methyl-1-penten-1-yl]-5-methyl-2-oxo-1,3-oxazolidin-3-yl}ethyl)thio]-1,3-thiazole-4-carboxylic acid (4)

To a solution of the compound **23** (10.1 g) in dimethoxyethane (79 mL) was slowly added dropwise potassium hexamethyldisilazide (0.5M in toluene, 47.5 mL) at –78 °C. The reaction mixture was stirred at –78 °C for 54 minutes, and treated with a solution of the compound **28** (9.84 g) in dimethoxyethane (79 mL). After stirring at –78 °C for 25 minutes, the reaction mixture was warmed to 0 °C and stirred for 50 minutes. The reaction was quenched with an aqueous saturated sodium bicarbonate solution, and extracted with ethyl acetate. The organic layer was washed with an aqueous saturated sodium bicarbonate solution and brine, dried over anhydrous magnesium sulfate, filtrated through Celite and concentrated under reduced pressure. The obtained residue was purified by column chromatography (*n*-hexane : ethyl acetate = 84 : 16) to give olefin compound (6.62 g). To a solution of the olefin compound (6.53 g) in ethyl acetate (114 mL) was slowly added dropwise 4N hydrogen chloride/ethyl acetate solution (14 mL) at 0 °C and the resulting solution was stirred for 10 minutes. An aqueous saturated sodium bicarbonate solution was slowly added dropwise to the reaction solution, followed by extraction with ethyl acetate. The organic layer was washed with an aqueous saturated sodium bicarbonate solution and brine, dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The obtained residue was purified by column chromatography (*n*-hexane : ethyl acetate = 40 : 60) to give alcohol compound (3.81 g). To a solution of the alcohol compound (3.7 g) in methanol (37 mL) was added dropwise 2N aqueous sodium hydroxide solution (11 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 45 minutes. After cooling to 0 °C, 2N hydrochloric acid was added thereto and the solution was extracted with ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous magnesium sulfate and concentrated. The obtained residue was purified by column chromatography (*n*-hexane : ethyl acetate = 1 : 1) to give compound **4** as colorless viscous oil (2.95 g, 69%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.86 (m, 19H), 2.76 (m, 4H), 3.73 (m, 4H), 3.93 (m, 1H), 4.28 (m, 1H), 5.36 (dd, *J* = 15.2, 9.0 Hz, 1H), 6.08 (m, 1H), 8.10 (s, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 18.66, 26.15, 26.41, 26.94, 27.33, 30.99, 31.04, 33.63, 35.21, 35.28, 35.34, 35.37, 41.41, 41.45, 45.35, 45.37, 49.37, 49.81, 67.06, 73.30, 73.35, 76.48, 127.44, 128.09, 129.02, 129.09, 129.45, 135.05, 135.14, 146.53, 157.69, 161.45, 161.49, 165.12, 165.14.

HRMS (ESI, pos.) $C_{22}H_{33}O_5N_2S_2$ (M + H) +, Obs. 469.1825, Calc. 469.6378.

5.1.13. 2-[(2-{(4S)-4-[(1E)-4-hydroxy-4-methyl-1-nonen-1-yl]-2-oxo-1,3-oxazolidin-3-yl}ethyl)thio]-1,3-thiazole-4-carboxylic acid (2)

Starting from compound **17** and compound **22** following the same procedure as compound **4** provided compound **2** as pale yellow amorphous.

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.02 (m, 3H), 1.64 (m, 11H), 2.47 (m, 2H), 5.07 (m, 9H), 5.57(m, 1H), 6.24 (m, 1H), 8.33 (m, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 14.05, 18.70, 22.62, 22.63, 23.55, 26.68, 26.90, 30.93, 30.99, 32.28, 32.30, 41.42, 41.50, 41.84, 42.29, 44.40, 44.44, 67.05, 67.07, 72.84, 72.97, 76.55, 76.49, 127.51, 127.54, 128.99, 134.96, 134.98, 146.37, 157.63, 161.30, 161.44, 165.16, 165.20.

HRMS (ESI, pos.) $C_{19}H_{29}O_5N_2S_2$ (M + H) +, Obs. 429.1512, Calc. 429.5740.

5.1.14. 1-cyclohexyl-2-methyl-4-[(1-phenyl-1H-tetrazol-5-yl)thio]-2-butanol (21)

Starting from commercial available 1-cyclohexylacetone following the same procedure as compound **20** provided compound **21**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.87 (m, 16H), 2.11 (m, 2H), 3.56 (m, 2H), 7.67 (m, 5H).

5.1.15. 5-({4-cyclohexyl-3-methyl-3-[(trimethylsilyl)oxy]butyl}sulfonyl)-1-phenyl-1H-tetrazole (23)

Starting from compound **21** following the same procedure as compound **22** provided compound **23**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.13 (s, 9H), 1.81 (m, 16H), 2.15 (m, 2H), 3.88 (m, 2H), 7.76 (m, 5H).

5.1.16. 2-[(2-{(4S)-4-[(1E)-5-cyclohexyl-4-hydroxy-4-methyl-1-penten-1-yl]-2-oxo-1,3-oxazolidin-3-yl}ethyl)thio]-1,3-thiazole-4-carboxylic acid (3)

Starting from compound **17** and compound **22** following the same procedure as compound **4** provided compound **3** as colorless viscous oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.90 (m, 16H), 2.44 (m, 2H), 2.83 (s, 2H), 3.79 (m, 4H), 4.04 (m, 1H), 4.50 (m, 2H), 5.38 (dd, *J* = 14.9, 8.6 Hz, 1H), 6.11 (m, 1H), 8.09 (s, 1H).

HRMS (ESI, pos.) $C_{21}H_{31}O_5N_2S_2$ (M + H) +, Obs. 455.1669, Calc. 455.6112.

5.1.17. Ethyl 2-({2-[(1R,2S,5S)-2-({[tert-butyl(diphenyl)silyl]oxy}methyl)-5-hydroxycyclopentyl]ethyl}thio)-1,3-thiazole-4-carboxylate (30)

To a solution of (3aR,4S,6aS)-4-({[*tert*-butyl(diphenyl)silyl]oxy}methyl)hexahydro-2H-cyclopenta[b]furan-2-one (1.00 g) in anhydrous tetrahydrofuran (9.00 mL) was added lithium aluminum hydride (97.0 mg) at 0 ºC. The reaction mixture was stirred at 0 ºC for 20 minutes, and treated with water, and extracted with ethyl acetate. The organic layer was washed with an aqueous saturated solution of sodium tartrate and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. A mixed solution of the obtained residue and diisopropylethylamine (1.29 mL) in anhydrous tetrahydrofuran (9.00 mL) was added at −5 ºC to a solution of methanesulfonyl chloride (0.23 mL) in anhydrous tetrahydrofuran (5.00 mL). The reaction mixture was stirred for 20 minutes, and then quenched with anhydrous methanol (43.0 μL) at −5 ºC for 15 minutes. The resulting mixture was treated with trimethylsilyl chloride (0.49 mL) at −5 ºC, stirred at room temperature for 10 minutes, subsequently treated with potassium carbonate (1.10 g), potassium thioacetate (578 mg) and anhydrous dimethylformamide (20.0 mL), and further stirred at 50 ºC for 5 hours. The reaction mixture was poured into iced water, extracted with *tert*-butyl methyl ether, washed with water and brine, dried over anhydrous sodium sulfate and concentrated. To a solution of the obtained residue in ethanol (13.0 mL) were added tri-*n*-butylphosphine (0.07 mL), ethyl 2-bromo-1,3-thiazole-4-carboxylate (657 mg) and potassium carbonate (770 mg). The mixture was stirred at room temperature for 1 hour, and subsequently stirred at 50 ºC overnight. The reaction mixture was diluted in ethyl acetate, and washed with an aqueous saturated solution of ammonium chloride, water and brine, dried over anhydrous sodium sulfate and concentrated. The obtained residue was dissolved in tetrahydrofuran (8.60 mL), cooled to 0 ºC, and treated with 1N hydrochloric acid (1.86 mL). The resulting mixture was stirred for 30 minutes at room temperature, then diluted in ethyl acetate, washed with water and brine, dried over anhydrous sodium sulfate and concentrated. The crude product was purified by column chromatography on silica gel (*n*-hexane : ethyl acetate = 4 : 1) to give compound **30** (624 mg, 38%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05 (s, 9H), 1.38 (t, *J* = 7.1 Hz, 3H), 1.77 (m, 8H), 2.82 (m, 1H), 3.20 (s, 1H), 3.58 (m, 3H), 4.41 (m, 3H), 7.41 (m, 6H), 7.65 (m, 4H), 7.96 (s, 1H).

5.1.18. Ethyl 2-({2-[(1R,2S,5S)-2-(acetyloxy)-5-formylcyclopentyl]ethyl}thio)-1,3-thiazole-4-carboxylate (31)

To a solution of compound **30** (42.2 g) in pyridine (75 mL) were continuously added at 0 ºC acetic anhydride (13 mL) and 4-*N*,*N*-dimethylaminopyridine (453 mg). The reaction mixture was stirred at room temperature for 2 hours, and then diluted in ethyl acetate. The organic layer was removed, washed with 1N hydrochloric acid and brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The obtained residue was dissolved in tetrahydrofuran (140 mL) and treated with 1M tetrabutylammonium fluoride in tetrahydrofuran (110 mL) at 0 ºC. After stirring at room temperature for 1 hour, the reaction was quenched with water and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The obtained residue was purified by column chromatography on silica gel (*n*-hexane : ethyl acetate = 7 : 3) to give alcohol (27.0 g). The obtained alcohol (2.95 g) was dissolved in dimethyl sulfoxide (20 mL) and ethyl acetate (30 mL), treated with triethylamine (7.8 mL) and sulfur trioxide-pyridine complex (4.5 g) at 10 ºC, and stirred at room temperature for 1 hour. To the reaction solution was added 1N hydrochloric acid and the resulting mixture was extracted with ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous sodium sulfate, and concentrated to give compound **31** (2.93 g, 97%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.49 (m, 3H), 2.15 (m, 9H), 2.51 (m, 1H), 2.84 (m, 1H), 3.31 (m, 2H), 4.48 (m, 2H), 5.37 (m, 1H), 8.02 (s, 1H), 9.67 (d, *J* = 2.7 Hz, 1H).

5.1.19. 2-[(2-{(1R,2S,5R)-2-hydroxy-5-[(1E)-4-hydroxy-4-methyl-1-nonen-1-yl]cyclopentyl}ethyl)thio]-1,3-thiazole-4-carboxylic acid (33)

To a solution of (3-hydroxy-3-methyloctyl)(triphenyl)phosphonium iodide **32** (800 mg) in anhydrous tetrahydrofuran (9.00 mL) was added *n*-butyllithium (1.60M in hexane, 1.90 mL) at room temperature. After stirring for 1 hour, the resulting solution was treated with a solution of the compound **31** (349 mg) in anhydrous tetrahydrofuran (6.00 mL) at –78 °C and then stirred for 2 hours. The reaction mixture was warmed to room temperature, and poured into ice water, followed by extraction with ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The obtained residue was purified by column chromatography on silica gel (*n*-hexane : ethyl acetate = 4 : 1). The obtained product was dissolved in methanol (1.00 mL) and treated with 2N aqueous sodium hydroxide solution (0.19 mL) at 0 °C. The resulting solution was stirred at room temperature for 1 hour and acidified with an aqueous solution of 2N hydrochloric acid (0.30 mL), followed by extraction with ethyl acetate. The extract liquid was washed with water and brine, dried over anhydrous sodium sulfate and concentrated to give compound **33** (148 mg, 37%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (t, *J* = 6.0 Hz, 3H), 1.13 (s, 3H), 1.30 (m, 12H), 1.79 (m, 2H), 2.02 (m, 3H), 2.40 (m, 1H),

2.88 (m, 1H), 3.56 (m, 1H), 4.50 (m, 1H), 5.32 (m, 1H), 5.45 (m, 1H), 8.02 (s, 1H).

5.1.20. 2-[(2-{(1R,2R)-2-[(1E)-4-hydroxy-4-methyl-1-nonenyl]-5-oxocyclopentyl}ethyl)sulfanyl]-1,3-thiazole-4-carboxylic acid (5)

To a solution of compound **33** (325 mg) in toluene (3.80 mL) were added triethylamine (0.12 mL), *t*-butyl dimethyl silyl chloride (130 mg) at 0 °C and the solution was stirred for 1 hour. Ethyl acetate was added to the reaction solution, which was filtered and concentrated under reduced pressure. The obtained residue was dissolved in dimethylsulfoxide (1.20 mL)/ethyl acetate (2.40 mL) and then treated with diisopropylamine (0.80 mL) and sulfur trioxide-pyridine complex (363 mg) at 0 °C, followed by stirring for 30 min. The reaction mixture was diluted with water, poured into 1N hydrochloric acid, and extracted with ethyl acetate. The organic layer was washed with an aqueous saturated ammonium chloride solution, water and brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (*n*-hexane : ethyl acetate = 1 : 1) to give compound **5** as yellow oil (199 mg, 62%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.6 Hz, 3H), 2.59 (m, 21H), 3.75 (m, 4H), 5.51 (dd, *J* = 15.0, 9.0 Hz, 1H), 5.77 (m, 1H), 8.10 (s, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 14.06, 22.66, 23.56, 26.54, 27.62, 28.25, 31.98, 32.34, 37.54, 41.95, 44.57, 46.34, 53.77, 72.95, 127.39, 127.44, 136.08, 146.41, 161.03, 166.10, 219.11.

HRMS (ESI, pos.) $C_{21}H_{32}O_4NS_2$ (M + H) +, Obs. 426.1767, Calc. 426.6131.

5.1.21. (5R,6S,9R,10E,13S)-13-methyl-15-oxo-13-pentyl-14-oxa-2,18-dithia-19-azatricyclo[14.2.1.0~5,9~]nonadeca-1(19),10,16-trien-6-yl acetate (34S)

Starting from compound **33** following the same procedure as compound **39e** provided compound **34S** (less polar lactone).

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, *J* = 7.0 Hz, 3H), 1.46 (m, 13H), 2.16 (m, 8H), 2.39 (m, 1H), 2.68 (m, 1H), 2.88 (m, 2H), 3.32 (m, 1H), 5.31 (m, 1H), 5.43 (m, 1H), 5.62 (m, 1H), 7.94 (s, 1H).

5.1.22. (5R,6S,9R,10E,13R)-13-methyl-15-oxo-13-pentyl-14-oxa-2,18-dithia-19-azatricyclo[14.2.1.0~5,9~]nonadeca-1(19),10,16-trien-6-yl acetate (34R)

Starting from compound **33** following the same procedure as compound **39e** provided compound **34R** (more polar lactone).

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, *J* = 7.0 Hz, 3H), 1.66 (m, 20H), 2.38 (m, 3H), 2.92 (m, 2H), 3.24 (m, 1H), 5.31 (m, 1H), 5.43 (m, 1H), 5.58 (m, 1H), 7.94 (s, 1H).

5.1.23. 2-[(2-{(1R,2R)-2-[(1E,4S)-4-hydroxy-4-methyl-1-nonen-1-yl]-5-oxocyclopentyl}ethyl)thio]-1,3-thiazole-4-carboxylic acid (5S)

Starting from compound **34S** following the same procedure as compound **10** provided compound **5S**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (m, 3H), 2.59 (m, 21H), 3.75 (m, 4H), 5.52 (dd, *J* = 15.0, 7.8 Hz, 1H), 5.77 (m, 1H), 8.11 (s, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 14.06, 22.65, 23.55, 26.54, 27.62, 28.25, 31.98, 32.33, 37.54, 41.95, 44.57, 46.35, 53.77, 72.95, 127.38, 127.44, 136.09, 146.41, 161.03, 166.10, 219.11.

HRMS (ESI, pos.) $C_{21}H_{32}O_4NS_2$ (M + H) +, Obs. 426.1767, Calc. 426.6131.

5.1.24. 2-[(2-{(1R,2R)-2-[(1E,4R)-4-hydroxy-4-methyl-1-nonen-1-yl]-5-oxocyclopentyl}ethyl)thio]-1,3-thiazole-4-carboxylic acid (5R)

Starting from compound **34R** following the same procedure as compound **10** provided compound **5R**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (m, 3H), 2.58 (m, 21H), 3.67 (m, 4H), 5.53 (dd, *J* = 15.0, 8.2 Hz, 1H), 5.77 (m, 1H), 8.11 (s, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 14.07, 22.63, 23.55, 26.53, 27.62, 28.22, 31.98, 32.31, 37.55, 41.92, 44.57, 46.34, 53.77, 72.94, 127.38, 127.42, 136.09, 146.41, 161.01, 166.11, 219.11.

HRMS (ESI, pos.) $C_{21}H_{32}O_4NS_2$ (M + H) +, Obs. 426.1767, Calc. 426.6131.

5.1.25. 3-methyl-1-[(1-phenyl-1H-tetrazol-5-yl)thio]-3-heptanol (36a)

Starting from commercial available 2-hexanone following the same procedure as compound **20** provided compound **36a**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92 (t, *J* = 7.5 Hz, 3H), 1.25 (s, 3H), 1.34 (m, 4H), 1.52 (m, 2H), 2.03 (m, 2H), 3.47 (m, 2H), 7.57 (m, 5H).

5.1.26. 5-({3-methyl-3-[(trimethylsilyl)oxy]heptyl}sulfonyl)-1-phenyl-1H-tetrazole (37a)

Starting from compound **36a** following the same procedure as compound **22** provided compound **37a**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.12 (s, 9H), 0.92 (t, *J* = 6.0 Hz, 3H), 1.30 (m, 7H), 1.56 (m, 2H), 2.06 (m, 2H), 3.81 (m, 2H), 7.76 (m, 5H).

5.1.27. 7-fluoro-3-methyl-1-[(1-phenyl-1H-tetrazol-5-yl)thio]-3-heptanol (36b)

Starting from commercial available 5-hexene-2-one following the same procedure as compound **20** provided compound **36b**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (s, 3H), 1.67 (m, 6H), 2.03 (m, 2H), 3.47 (m, 2H), 4.39 (dt, *J* = 57.0, 6.0 Hz, 2H), 7.57 (m, 5H).

5.1.28. 5-({7-fluoro-3-methyl-3-[(trimethylsilyl)oxy]heptyl}sulfonyl)-1-phenyl-1H-tetrazole (37b)

Starting from compound **36b** following the same procedure as compound **22** provided compound **37b**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.12 (s, 9H), 1.54 (m, 7H), 1.70 (m, 2H), 2.06 (m, 2H), 3.81 (m, 2H), 4.39 (dt, *J* = 57.0, 6.0 Hz, 2H), 7.74 (m, 5H).

5.1.29. 6,6,6-trifluoro-3-methyl-1-[(1-phenyl-1H-tetrazol-5-yl)thio]-3-hexanol (36c)

Starting from commercial available 5,5,5-trifluoropentane-2-one following the same procedure as compound **20** provided compound **36c**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24 (s, 3H), 1.56 (m, 2H), 2.03 (m, 2H), 2.31 (m, 2H), 3.45 (m, 2H), 7.57 (m, 5H).

5.1.30. 1-phenyl-5-({6,6,6-trifluoro-3-methyl-3-[(trimethylsilyl)oxy]hexyl}sulfonyl)-1H-tetrazole (37c)

Starting from compound **36c** following the same procedure as compound **22** provided compound **37c**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.12 (s, 9H), 1.34 (m, 3H), 1.58 (m, 2H), 2.09 (m, 2H), 2.31(m, 2H), 3.80 (m, 2H), 7.75 (m, 5H)..

5.1.31. 3,6,6-trimethyl-1-[(1-phenyl-1H-tetrazol-5-yl)thio]-3-heptanol (36d)

Starting from commercial available 5,5-dimethylhexane-2-one following the same procedure as compound **20** provided compound **36d**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (s, 9H), 1.23 (s, 3H), 1.33 (m, 2H), 1.54 (m, 2H), 2.04 (m, 2H), 3.46 (m, 2H), 7.57 (m, 5H).

5.1.32. 1-phenyl-5-({3,6,6-trimethyl-3-[(trimethylsilyl)oxy]heptyl}sulfonyl)-1H-tetrazole (37d)

Starting from compound **36d** following the same procedure as compound **22** provided compound **37d**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.12 (s, 9H), 0.89 (s, 9H), 1.30 (m, 5H), 1.55 (m, 2H), 2.07 (m, 2H), 3.80 (m, 2H), 7.76 (m, 5H).

5.1.33. 1-cyclopentyl-2-methyl-4-[(1-phenyl-1H-tetrazol-5-yl)thio]-2-butanol (36e)

Starting from commercial available 4-cyclopentyl-2-butanone following the same procedure as compound **20** provided compound **36e**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.16 (m, 4H), 1.27 (s, 3H), 1.60 (m, 5H), 1.88 (m, 2H), 2.03 (m, 2H), 3.48 (m, 2H), 7.56 (m, 5H).

5.1.34. 5-({4-cyclopentyl-3-methyl-3-[(trimethylsilyl)oxy]butyl}sulfonyl)-1-phenyl-1H-tetrazole (37e)

Starting from compound **36e** following the same procedure as compound **22** provided compound **37e**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.12 (s, 9H), 1.35 (m, 8H), 1.66 (m, 6H), 2.06 (m, 2H), 3.81 (m, 2H), 7.76 (m, 5H).

5.1.35. 2-[(2-{(1R,2R,5S)-2-[(1E)-5-cyclopentyl-4-hydroxy-4-methyl-1-penten-1-yl]-5-hydroxycyclopentyl}ethyl)thio]-1,3-thiazole-4-carboxylic acid (38e)

Potassium hexamethyldisilazide in toluene (0.5 M, 3.24 mL) was slowly added dropwise to a solution of compound **37e** (705 mg) in 1,2-dimethoxyethane (4.0 mL) at –78 ºC, followed by stirring at the same temperature for 30 min. To the reaction mixture, a solution of compound **31** (300 mg) in 1,2-dimethoxyethane (3.0 mL) was slowly added dropwise at –78 ºC. After string at the same temperature for 30 min, the reaction temperature was raised to 0 ºC. A saturated aqueous solution of sodium hydrogen carbonate was added, followed by extraction with ethyl acetate. The organic layer was washed with water and saturated brine, dried over anhydrous sodium sulfate, and concentrated. The resulting residue was purified by silica gel column chromatography (*n*-hexane : ethyl acetate = 8 :1) to obtain olefin compound (313 mg).

To an ethanol solution (3.0 mL) of olefin compound (313 mg), 2N aqueous sodium hydroxide solution (1.2 mL) was added under ice cooling, followed by stirring at room temperature overnight. The reaction mixture was acidified with 1N hydrochloric acid. After stirring for 30 min, the reaction mixture was diluted with water and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated to obtain compound **38e** (227 mg, 64%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.15 (s, 3H), 1.49 (m, 8H), 1.88 (m, 5H), 2.08 (m, 2H), 2.20 (m, 2H), 2.48 (m, 4H), 2.93 (m, 1H), 3.55 (m, 1H), 4.52 (m, 1H), 5.37 (m, 1H), 5.48 (m, 1H), 8.07 (s, 1H).

5.1.36. (5R,6S,9R,10E,13S)-13-(cyclopentylmethyl)-13-methyl-15-oxo-14-oxa-2,18-dithia-19-azatricyclo[14.2.1.0~5,9~]nonadeca-1(19),10,16-trien-6-yl acetate (39e)

Acetic anhydride (0.06 mL) was added to a pyridine solution (1.5 mL) of compound **38e** (134 mg) under ice cooling, followed by stirring at room temperature overnight. The reaction mixture was poured into 1N hydrochloric acid and extracted with ethyl acetate. The organic layer was washed with saturated brine, dried over anhydrous sodium sulfate, and concentrated to furnish acetate compound (131 mg).

A solution of acetate compound (131 mg) in toluene (53 mL) was treated with 4,4-dimethylaminopyridine (323 mg) at room temperature and then heated to 100 ºC. 2,4,6-trichlorobenzoyl chloride (0.25 mL) was added thereto. After stirring for 15 min at 100 ºC, the reaction mixture was cooled to room temperature, then poured into a saturated aqueous solution of sodium hydrogen carbonate, and extracted with ethyl acetate. The organic layer was washed with water and brine, dried with anhydrous sodium sulfate, and concentrated under reduced pressure. The resulting crude product was purified by silica gel column chromatography (*n*-hexane : ethyl acetate = 15 : 1) to obtain compound **39e** (less polar lactone, 49 mg, 35%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20 (m, 4H), 1.66 (m, 8H), 1.89 (m, 5H), 2.22 (m, 6H), 2.37 (m, 2H), 2.82 (m, 2H), 3.34 (m, 2H), 5.31 (m, 1H), 5.45 (m, 1H), 5.62 (m, 1H), 7.95 (s, 1H).

5.1.37. 2-[(2-{(1R,2R)-2-[(1E,4S)-5-cyclopentyl-4-hydroxy-4-methyl-1-penten-1-yl]-5-oxocyclopentyl}ethyl)thio]-1,3-thiazole-4-carboxylic acid (10)

Compound **39e** (183 mg) was dissolved in a mixed solvent of methanol (1.3 mL) and tetrahydrofuran (2.6 mL), and treated with a 2N aqueous solution of sodium hydroxide (0.59 mL). The reaction mixture was stirred at room temperature overnight, subsequently poured into 1N hydrochloric acid and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous sodium sulfate, and concentrated to give diol carboxylic acid compound (187 mg).

Potassium carbonate (218 mg) and ethyl iodide (0.064 mL) were added to a dimethylformamide solution (2.0 mL) of diol carboxylic acid compound (174 mg), followed by stirring at room temperature overnight. The reaction solution was poured into water and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous sodium sulfate, and concentrated to provide ethyl ester compound (173 mg).

Diisopropylethylamine (0.51 mL) and sulfur trioxide·pyridine complex (286 mg) were added to a dimethyl sulfoxide (1.2 mL)/ethyl acetate (2.4 mL) solution of ethyl ester compound (173 mg) under ice cooling, followed by stirring for 15 min. The reaction was quenched with water, and extracted with ethyl acetate. The organic layer was washed with 1N hydrochloric

acid, water and brine, dried with anhydrous sodium sulfate, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (*n*-hexane : ethyl acetate = 3 : 2) to furnish keto ethyl ester (110 mg).

The keto ethyl ester (110 mg) was dissolved in 1,2-dimethoxyethane (2.0 mL)/water (1.0 mL), and treated with lithium hydroxide (16.0 mg) under ice cooling, followed by stirring at room temperature for 2 hours. The reaction mixture was poured into a 5% aqueous solution of potassium hydrogen sulfate and extracted with ethyl acetate. The organic layer was washed with water and brine, dried with anhydrous sodium sulfate, and concentrated in vacuo. The resulting residue was purified by silica gel column chromatography (*n*-hexane : ethyl acetate = 1 : 1 → methanol : ethyl acetate = 1 : 10) to provide compound **10** as pale yellow viscous oil (90.5 mg, 72%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 2.32(m, 22H), 2.59(m, 2H), 3.09(m, 2H), 3.36(t, *J* = 7.5 Hz, 2H), 5.51(dd, *J* = 15.0, 6.0 Hz, 1H), 5.70(dt, *J* = 15.0, 7.4 Hz, 1H), 8.10(s, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 25.00, 25.06, 27.09, 27.61, 28.24, 31.99, 34.50, 34.56, 36.00, 37.54, 45.37, 46.34, 48.04, 53.75, 73.33, 127.43, 127.50, 136.09, 146.40, 161.06, 166.11, 219.14.

HRMS (ESI, pos.) $C_{22}H_{32}O_4NS_2$ (M + H) +, Obs. 438.1767, Calc. 438.6238.

Rotation $[\alpha]_D$: -34.56 (c = 1.05, $CHCl_3$)

5.1.38. 2-[(2-{(1R,2S,5R)-2-hydroxy-5-[(1E)-4-hydroxy-4-methyl-1-octen-1-yl]cyclopentyl}ethyl)thio]-1,3-thiazole-4-carboxylic acid (38a)

Starting from compound **37a** instead of compound **37e** following the same procedure as compound **38e** provided compound **38a**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91 (t, *J* = 7.0 Hz, 3H), 1.15 (s, 3H), 1.42 (m, 12H), 2.02 (m, 3H), 2.45 (m, 1H), 3.13 (m, 1H), 3.57 (m, 1H), 4.52 (m, 1H), 5.36 (m, 1H), 5.48 (m, 1H), 8.08 (s, 1H).

5.1.39. (5R,6S,9R,10E,13S)-13-butyl-13-methyl-15-oxo-14-oxa-2,18-dithia-19-azatricyclo[14.2.1.0~5,9~]nonadeca-1(19),10,16-trien-6-yl acetate (39a)

Starting from compound **38a** instead of compound **38e** following the same procedure as compound **39e** provided compound **39a**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91 (t, *J* = 7.0 Hz, 3H), 1.36 (m, 11H), 2.06 (m, 7H), 2.38 (m, 1H), 2.66 (m, 1 H), 2.88 (m, 2 H), 3.32 (m, 2 H), 5.31 (m, 1 H), 5.43 (m, 1 H), 5.60 (m, 1 H), 7.94 (s, 1 H).

5.1.40. 2-[(2-{(1R,2R)-2-[(1E,4S)-4-hydroxy-4-methyl-1-octen-1-yl]-5-oxocyclopentyl}ethyl)thio]-1,3-thiazole-4-carboxylic acid (6)

Starting from compound **39a** instead of compound **39e** following the same procedure as compound **10** provided compound **6** as colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92 (t, *J* = 7.5 Hz, 3H), 1.16 (s, 3H), 1.37 (m, 4H), 1.53 (m, 2H), 1.77 (m, 1H), 2.27 (m, 7H), 2.59 (m, 2H), 3.51 (m, 2H), 5.50 (dd, *J* = 15.0, 8.0 Hz, 1H), 5.77 (m, 1H), 8.09 (s, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 14.12, 23.22, 26.08, 26.55, 27.60, 28.24, 32.00, 37.54, 41.71, 44.57, 46.34, 53.75, 72.92, 127.37, 127.43, 136.10, 146.44, 161.04, 166.08, 219.10.

HRMS (ESI, pos.) $C_{20}H_{30}O_4NS_2$ (M + H) +, Obs. 412.1611, Calc. 412.5865.

5.1.41. 2-[(2-{(1R,2R,5S)-2-[(1E)-8-fluoro-4-hydroxy-4-methyl-1-octen-1-yl]-5-hydroxycyclopentyl}ethyl)thio]-1,3-thiazole-4-carboxylic acid (38b)

Starting from compound **37b** instead of compound **37e** following the same procedure as compound **38e** provided compound **38b**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.15 (s, 3H), 1.42 (m, 12H), 2.03 (m, 3H), 2.42 (m, 1H), 3.11 (m, 1H), 3.55 (m, 1H), 4.36 (dt, *J* = 47.4, 6.0 Hz, 2H), 4.50 (m, 1H), 5.32 (m, 1H), 5.44 (m, 1H) 8.08 (s, 1H).

5.1.42. (5R,6S,9R,10E,13S)-13-(4-fluorobutyl)-13-methyl-15-oxo-14-oxa-2,18-dithia-19-azatricyclo[14.2.1.0~5,9~]nonadeca-1(19),10,16-trien-6-yl acetate (39b)

Starting from compound **38b** instead of compound **38e** following the same procedure as compound **39e** provided compound **39b**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.34 (m, 11H), 2.04 (m, 7H), 2.39 (m, 1H), 2.64 (m, 1H), 2.85 (m, 2H), 3.30 (m, 2H), 4.34 (dt, *J* = 47.4, 6.0 Hz, 2H), 5.30 (m, 1H), 5.46 (m, 1H), 5.61 (m, 1H), 7.94 (s, 1H).

5.1.43. 2-[(2-{(1R,2R)-2-[(1E,4S)-8-fluoro-4-hydroxy-4-methyl-1-octen-1-yl]-5-oxocyclopentyl}ethyl)thio]-1,3-thiazole-4-carboxylic acid (7)

Starting from compound **39b** instead of compound **39e** following the same procedure as compound **10** provided compound **7** as yellow viscous oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ. 1.19(s, 3H), 2.60 (m, 16H), 3.44 (m, 2H), 4.46 (dt, *J* = 47.4, 6.0Hz, 2H), 5.53 (dd, *J* = 15.3, 8.1 Hz, 1H), 5.70 (dt, *J* = 15.3, 7.5 Hz, 1H), 8.11 (s, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 19.72, 26.52, 27.63, 28.22, 30.81, 31.99, 37.51, 41.45, 44.58, 46.38, 53.73, 72.75, 84.00, 127.10, 127.50, 136.38, 146.36, 161.03, 166.08, 219.03.

HRMS (ESI, pos.) $C_{20}H_{29}O_4NFS_2$ (M + H) +, Obs. 430.1517, Calc. 430.5770.

5.1.44. 2-[(2-{(1R,2S,5R)-2-hydroxy-5-[(1E)-7,7,7-trifluoro-4-hydroxy-4-methyl-1-hepten-1-yl]cyclopentyl}ethyl)thio]-1,3-thiazole-4-carboxylic acid (38c)

Starting from compound **37c** instead of compound **37e** following the same procedure as compound **38e** provided compound **38c**

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.15 (s, 3H), 1.48 (m, 8H), 2.05 (m, 3H), 2.33 (m, 2H), 2.46 (m, 1H), 3.16 (m, 1H), 3.59 (m, 1H), 4.50 (m, 1H), 5.35 (m, 1H), 5.46 (m, 1H), 8.09 (s, 1H).

5.1.45. (5R,6S,9R,10E,13S)-13-methyl-15-oxo-13-(3,3,3-trifluoropropyl)-14-oxa-2,18-dithia-19-azatricyclo[14.2.1.0~5,9~]nonadeca-1(19),10,16-trien-6-yl acetate (39c)

Starting from compound **38c** instead of compound **38e** following the same procedure as compound **39e** provided compound **39c**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36 (m, 7H), 2.06 (m, 7H), 2.38 (m, 3H), 2.68 (m, 1H), 2.85 (m, 2H), 3.31 (m, 2H), 5.34 (m, 1H), 5.45 (m, 1H), 5.63 (m, 1H), 7.95 (s, 1H).

5.1.46. 2-[(2-{(1R,5R)-2-oxo-5-[(1E,4S)-7,7,7-trifluoro-4-hydroxy-4-methyl-1-hepten-1-yl]cyclopentyl}ethyl)thio]-1,3-thiazole-4-carboxylic acid (8)

Starting from compound **39c** instead of compound **39e** following the same procedure as compound **10** provided compound **8** as colorless viscous oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.19 (s, 3H), 1.81 (m, 3H), 2.31 (m, 9H), 2.57 (m, 2H), 3.37 (t, *J* = 6.7 Hz, 2H), 5.53 (dd, *J* = 15.1, 7.6 Hz, 1H), 5.75 (m, 1H), 8.10 (s, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 26.39, 27.55, 28.12, 28.46, 31.83, 33.43, 33.45, 37.50, 44.97, 46.18, 53.62, 71.25, 126.36, 127.50, 136.89, 146.12, 161.04, 166.25, 218.98.

HRMS (ESI, pos.) $C_{19}H_{25}O_4NF_3S_2$ (M + H) +, Obs. 452.1172, Calc. 452.5313.

5.1.47. 2-[(2-{(1R,2S,5R)-2-hydroxy-5-[(1E)-4-hydroxy-4,7,7-trimethyl-1-octen-1-yl]cyclopentyl}ethyl)thio]-1,3-thiazole-4-carboxylic acid (39c)

Starting from compound **37d** instead of compound **37e** following the same procedure as compound **38e** provided compound **38d**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (s, 9H), 1.15 (s, 3H), 1.24 (m, 2H), 1.39 (m, 6H), 1.88 (m, 2H), 2.10 (m, 3H), 2.44 (m, 1H), 3.13 (m, 1H), 3.57 (m, 1H), 4.52 (m, 1H), 5.37 (m, 1H), 5.46 (m, 1H), 8.06 (s, 1H).

5.1.48. (5R,6S,9R,10E,13S)-13-(3,3-dimethylbutyl)-13-methyl-15-oxo-14-oxa-2,18-dithia-19-azatricyclo[14.2.1.0~5,9~]nonadeca-1(19),10,16-trien-6-yl acetate (39d)

Starting from compound **38d** instead of compound **38e** following the same procedure as compound **39d** provided compound **39d**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (s, 9H), 1.30 (m, 7H), 2.06 (m, 9H), 2.39 (m, 1H), 2.65 (m, 1H), 2.88 (m, 2H), 3.32 (m, 2H), 5.31 (m, 1H), 5.44 (m, 1H), 5.62 (m, 1H), 7.95 (s, 1H).

5.1.49. 2-[(2-{(1R,2R)-2-[(1E,4S)-4-hydroxy-4,7,7-trimethyl-1-octen-1-yl]-5-oxocyclopentyl}ethyl)thio]-1,3-thiazole-4-carboxylic acid (9)

Starting from compound **39d** instead of compound **39e** following the same procedure as compound **10** provided compound **9** as yellow viscous oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89(s, 9H), 1.33(m, 5H), 1.53(m, 2H), 1.77(m, 1H), 2.30(m, 7H), 2.58(m, 2H), 3.35(t, *J* = 7.3 Hz, 2H), 5.54(dd, *J* = 15.7, 8.2 Hz, 1H), 5.76(m, 1H), 8.11(s, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 26.66, 27.52, 28.24, 29.35, 30.02, 31.97, 36.55, 37.56, 37.62, 44.38, 46.21, 53.72, 72.90, 127.32, 127.37, 136.06, 146.38, 160.87, 166.10, 219.15.

HRMS (ESI, pos.) $C_{22}H_{34}O_4NS_2$ (M + H) +, Obs. 440.1924, Calc. 440.6397.

5.1.50. 2-[(2-{(1R,2R)-2-[(1E,4R)-5-cyclopentyl-4-hydroxy-4-methyl-1-penten-1-yl]-5-oxocyclopentyl}ethyl)thio]-1,3-thiazole-4-carboxylic acid (11)

Starting from more polar lactone derived from compound **38e** following the same procedure as compound **10** provided compound **11** as colorless viscous oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 2.34 (m, 22H), 2.63 (m, 2H), 3.23 (m, 2H), 3.37 (t, *J* = 7.3 Hz, 2H), 5.51 (dd, *J* = 15.1, 6.0 Hz, 1H), 5.69 (dt, *J* = 15.1, 7.2 Hz, 1H), 8.10 (s, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 24.96, 25.07, 27.14, 27.53, 28.24, 31.96, 34.47, 34.58, 36.07, 37.52, 45.46, 46.39, 47.78, 53.78, 73.36, 127.38, 127.48, 136.18, 146.41, 161.08, 166.12, 219.08.

HRMS (ESI, pos.) $C_{22}H_{32}O_4NS_2$ (M + H) +, Obs. 438.1767, Calc. 438.6238.

Rotation $[\alpha]_D$: -36.15 (c = 1.15, $CHCl_3$)

5.1.51. (5R,6S,9R,10E,13R)-13-(cyclohexylmethyl)-13-methyl-15-oxo-14-oxa-2,18-dithia-19-azatricyclo[14.2.1.0~5,9~]nonadeca-1(19),10,16-trien-6-yl acetate (40)

Starting from compound **23** and compound **31** following the same procedure as compound **39e** provided more polar maclolactone compound **40**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (m, 6H), 1.56 (m, 12H), 2.02 (m, 7H), 2.45 (m, 3H), 2.92 (m, 2H), 3.25 (m, 1H), 5.31 (m, 1H), 5.45 (m, 1H), 5.59 (m, 1H), 7.92 (s, 1H).

5.2. Biology

To measure each EP agonistic activity, CHO cells expressing human EP_1, EP_2, EP_3, and EP_4 receptors were used.

To measure EP_1 and EP_3 agonistic activities, 100 μL loading medium (medium containing 5 μmol/L fura-2-AM solution, 2.5 mmol/L probenecid, 20 μmol/L indomethacin, and 10 mmol/L HEPES) was added, and the cells were incubated for about 60 minutes at 37 °C in a CO_2 (5% CO_2, 95% air) incubator to incorporate the fura-2. An assay buffer [120 μL HBSS containing 1 w/v% albumin from bovine serum (Sigma-Aldrich Corp.), 2.5 mmol/L probenecid, 2 μmol/L indomethacin, and 20 mmol/L HEPES] was added. The intracellular calcium level was determined using a Fluorescence Drug Screening System (FDSS-3000, Hamamatsu Photonics KK). Then, 2.45 min after the start of measurement at 37°C, compound (0.03 to 300 nmol/L for EP_3, 300 to 10,000 nmol/L for EP_1) and PGE_2 (0.03 to 300 nmol/L for EP_3, 0.1 to 1,000 nmol/L for EP_1) were added. Measurements were taken at approximately 3-sec intervals, and the indicator of the change in intracellular calcium level was the change in the fluorescence intensity ratio (f340/f380) at 500 nm during irradiation with two alternating wavelengths of excitation light (340 nm, 380 nm).

To measure EP_2 and EP_4 agonistic activities, 500 μL of assay medium A (MEM Alpha, Invitrogen Corp.) containing 2 μmol/L diclofenac (Sigma-Aldrich Corp.) was added. Then, 450 μL of assay medium B [MEM Alpha containing 2 μmol/L diclofenac, 1 mmol/L 3-isobutyl-1-methylxanthine (IBMX), and 1 w/v% albumin from bovine serum (Sigma-Aldrich Corp.)] was added. Next, compound (0.003 to 10 nmol/L for EP_2, 0.3 to 10,000 nmol/L for EP_4) and PGE_2 (0.03 to 300 nmol/L for EP_2, 0.01 to 3,000 nmol/L for EP_1) were added. Finally, 500 μL of 10 w/v% trichloroacetic acid solution (Wako Pure Chemical Industries) was added to stop the reaction. Then, 300 μL of supernatant from the centrifuged cell suspension was transferred to a polyethylene tube and mixed with 300 μL of cAMP extraction solvent [chloroform (Kishida Chemical Co., Ltd.) containing 0.45 mol/L tri-n-octylamine (Tokyo Chemical Industry Co., Ltd.)].

Approximately 80 μL of the aqueous layer from the centrifuged mixture was transferred to a polyethylene tube, and the level of cAMP was assayed using a cAMP EIA kit [Amersham cAMP Biotrak enzyme immunoassay (EIA) System (dual range), GE Healthcare UK Ltd.].

The compound or control response rate (%) was used as an evaluation indicator, where 100% represents the maximum increase in intracellular calcium level of PGE_2 in hEP_3-CHO and hEP_1-CHO and 100% represents maximum cAMP production of PGE_2 in hEP_2-CHO and hEP_4-CHO.

5.3. Pharmacokinetics studies

The pharmacokinetics of compounds were studied following single intravenous and oral administration to male rats. The IV and PO solution formulations contained wellsolve/H_2O = 1/9 (Celeste) and sodium hydroxide (two equivalents). All IV and PO formulations were given as solution. Animals were fasted prior to oral dosing in single-dose studies. Oral bioavailability was estimated using non-cross-over study designs for the rat (n = 3). Plasma samples were assayed for compounds using protein precipitation by acetonitrile/ethanol (70/30, v/v) followed by HPLC/MS/MS analysis employing negative-ion Turbo IonSpray ionization. Plasma concentration-time data were analyzed by-compartmental methods.

5.4. Parallel artificial membrane permeability assay (PAMPA)

The PAMPA System (Pion) should be used for assay. Dilute the System Solution Concentrate (Pion) with water, and add DMSO to prepare a system solution (in 5% DMSO). Add 0.5 mol/L NaOH to the system solution using an automated buffer preparation system to make three different buffers of pH 5.0, 6.2 and 7.4. Add 150-μL of the buffers per well in a UV plate to serve as blanks for determination of UV absorption (190-498 nm). Add 5 μL of DMSO solution containing 10 mM the test compound to 1000 μL of the system solution, and filter by suction through a 96-well filter plate (0.2 μm PVDF hydrophilic membrane, Corning) (final compound concentration of 50 μM). Add 150-μL of the filtrate per well in the UV plate to serve as the reference for determination of UV absorption (190-498 nm). Add 200 μL of the remaining filtrate per well in the donor plate, and use as the donor solution. Make a membrane by dropping 4 μL of oil (GIT-0 Liquid, Pion) onto the filter of the acceptor plate. Add a 200-μL aliquot of the acceptor solution (Acceptor Sink Buffer, pH 7.4, Pion) per well in the acceptor plate. Place the acceptor plate on the donor plate. After a 4-h incubation, transfer 150-μL aliquots from the donor and acceptor solutions to the UV plate for determination of UV absorption (190-498 nm). The membrane permeability coefficient should be calculated from the observed values obtained as above using the PAMPA Evolution Command Software.

5.5. Caco-2 membrane permeability assay

Grow Caco-2 cells on a 12-well Costar Transwell plate (with a collagen-coated fine porous polycarbonate membrane) until a confluent monolayer is formed. Prepare buffer for permeability assay using 10 mM HEPES and Hank's balanced salt solution containing 15 mM glucose and0.2 mM Lucifer yellow with the pH being adjusted at 7.4. Add the test substance solution to the apical side (for the A-to-B permeability) or the basolateral side (for the B-to-A permeability) of the Caco-2 cell monolayer at a final concentration of 1 μM, and then incubate the plate in a humidified incubator (5% CO_2, 37 °C). Two h later, take aliquots from the receiver chambers. Each determination was performed in duplicate. The flux of co-dosed lucifer yellow was also measured for each monolayer to ensure no damage was inflicted to the cell monolayers during the flux period. Determine test substance concentrations in the samples by LC/MS/MS.

The apparent permeability coefficient (Papp) is calculated according to the following equation:

$Papp = (dCr/dt) \times Vr/(A \times C0)$

dCr/dt: slope of compound accumulation in the receiver compartment over time (μM/s)

Vr: volume of the receiver compartment (cm^3)

A: area of the cell monolayer (1.13 cm^2 for 12-well Transwell plate)

C0: initial normality of the buffer (μM)

The involvement of efflux transporter(s) such as P-gp is suspected if the ratio of the B-to-A Papp to the A-to-B Papp is 2 or more.

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Graphical Abstract

Synthesis and Evaluation of a Potent, Well-balanced EP_2/EP_3 Dual Agonist

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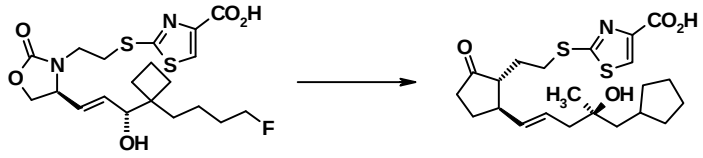

human EC_{50} EP_2 = 1.1 nM, EP_3 = 1.0 nM,
EP_1 = 2000 nM, EP_4 = 2636 nM