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Discovery of highly potent dual EP_2 and EP_3 agonists with subtype selectivity

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ABSTRACT

The cyclic carbamate derivatives, 2-{[2-((4*S*)-4-{(1E,3*R*)-8-fluoro-3-hydroxy-4,4-dimethyl-1-octenyl}-2-oxo-1,3-oxazolidin-3-yl)ethyl]sulfanyl}-1,3-thiazole-4-carboxylic acid (**5**) and 2-{[2-((4*S*)-4-{(1E,3*R*)-3-[1-(4-fluorobutyl)cyclobutyl]-3-hydroxy-1-propenyl}-2-oxo-1,3-oxazolidin-3-yl)ethyl]sulfanyl}-1,3-thiazole-4-carboxylic acid (**7**) were identified as the first potent dual EP_2 and EP_3 agonists with selectivity against the EP_1 and EP_4 subtypes. Compounds **5** and **7** demonstrated highly potent dual EP_2 and EP_3 agonist activity with EC_{50} values of 10 nM or less. In addition, these compounds possess structural features distinct from natural prostaglandins, such as a cyclic carbamate moiety, a dimethyl or cyclobutyl group and a terminal fluorine atom.

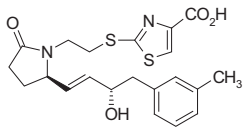
Prostanoid receptors are members of the G-protein coupled receptor superfamily. Receptors for prostaglandin E_2 (PGE_2) can be classified into four subtypes, EP_1, EP_2, EP_3, EP_4.[1] The diverse biological activities of PGE_2 are considered to be expressed as a hybrid of the activities mediated by 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 the 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. The primary drugs currently used for UAB are a cholinesterase inhibitor, distigmine bromide and a muscarinic receptor agonist, bethanechol chloride. The systemic cholinergic side effects of these two drugs negatively impact this therapy.

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] In addition our pharmacological tests revealed that an EP_3 agonist contracts the bladder and an EP_2 agonist relaxes the urethra (American Urology Association, 2015).

Our purpose was to develop PGE_2 analogs possessing highly potent dual EP_2 and EP_3 agonist activity with selectivity against the other two subtypes because a dual EP_2 and EP_3 agonist has the potential as an effective therapeutic addressing unmet medical needs for UAB.

So far, a potent dual EP_2 and EP_3 agonist with selectivity against the EP_1 and EP_4 receptor subtypes has not been identified. On the other hand, a dual EP_2 and EP_4 agonist with selectivity against



1

	EP_1	EP_2	EP_3	EP_4
Mouse Binding Assay *Ki* (nM)	$>10^4$	9.3	540	0.41
Rat Functional Assay EC_{50} (nM)	-	90	-	0.79

Figure 1. EP_2 and EP_4 dual agonist **1**.

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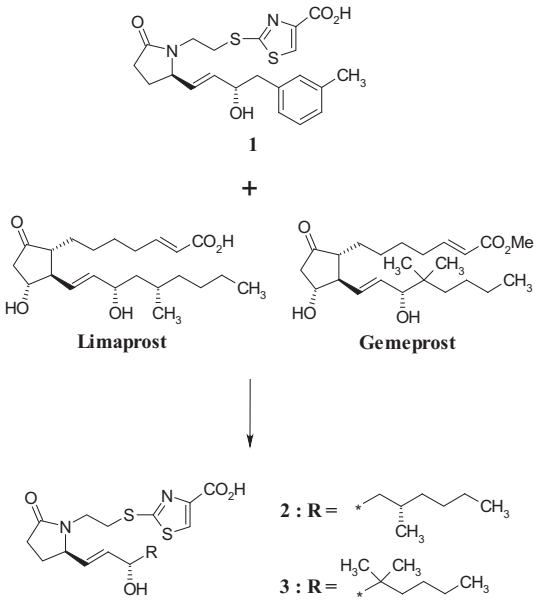
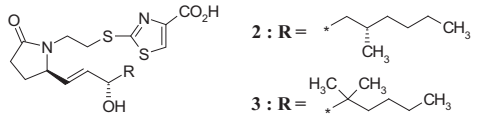


Figure 2. Molecular design of γ-lactam PGE analogs.

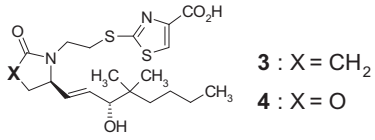
Table 1
Activity profiles of γ-lactam derivatives



Compd	Human functional assay, EC_{50}[a] (nM)		
	EP_2	EP_3	EP_4
2	0.39	310	3.0
3	0.91	8.4	4.2

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

Table 2
Effect of the incorporation of oxygen atom into 5-membered ring



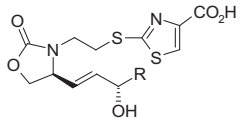
Compd	Human functional assay, EC_{50}[a] (nM)		
	EP_2	EP_3	EP_4
3	0.91	8.4	4.2
4	7.4	50	320

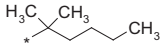
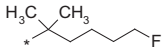
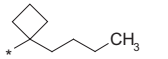
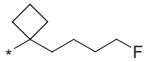
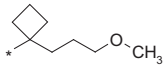
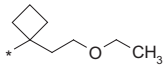
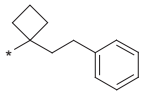
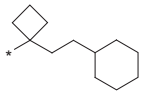
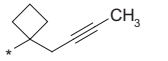
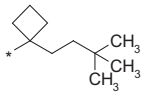
[a] EC_{50} values represent the mean of at least two experiments.

the EP_1 and EP_3 receptor subtypes was reported (compound **1** in Fig. 1).[7]

Our first molecular design for a dual EP_2 and EP_3 agonist is described in Figure 2. At first, an increase in affinity for the EP_3 receptor was required for compound **1**. The ω side chain of limaprost or gemeprost, which are prostaglandin E_1 (PGE_1) analogs in clinical use with high affinity for the EP_3 receptor, was introduced into **1**. The activity profiles of the resulting γ-lactam derivatives **2** and **3** are shown in Table 1. Of the two resulting compounds, *gem*-dimethyl **3** demonstrated potent EP_3 agonist activity comparable

Table 3
Activity profiles of cyclic carbamate derivatives



Compd	R	Human functional assay, EC_{50}[a] (nM)			Human binding assay K_i[a] (nm)
		EP_2	EP_3	EP_4	EP_1
4		7.4	50	320	120
5		5.7	4.7	1220	431
6		2.9	3.9	73	220
7		2.9	10	195	1080
8		18	25	705	>10,000
9		29	2606	745	8418
10		160	27	465	802
11		60	7.4	8390	1332
12		21	18	240	89
13		3700	6.7	4710	149

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

Table 4
Pharmacokinetics profile of **7** in rats

Iv dosing (0.01 mg/kg)		Oral dosing (1 mg/kg)	
CL (mL/min/kg)	$T_{1/2}$ (h)	AUC (μg·h/mL)	*F* (%)
9.5	4.4	0.041	2.5

to its EP_2 and EP_4 agonist activity. Therefore, the second step was to optimize the 5-membered ring and the ω side chain of **3** toward reduction of EP_4 agonist activity.

According to the published data,[8] lipophilicity at 5-membered ring seems to relate to EP_4 agonist activity. Therefore, the reduction of EP_4 agonist activity can be expected by the incorporation of oxygen atom into 5-membered ring. As shown in Table 2, the effect of modification of the 5-membered ring was investigated. The functional assay revealed that cyclic carbamate **4** showed a distinct decrease in EP_4 agonist activity versus its lactam counterpart **3** as expected.

Structure–activity relationships (SAR) of the cyclic carbamate derivatives are shown in Table 3. First, the effect of ω side chain was investigated. The functional assays for human EP_2–EP_4 receptor subtypes and the binding affinity for human EP_1 receptor subtype were performed to determine subtype selectivity. Surprisingly, the incorporation of a terminal fluorine atom into **4** enhanced EP_3 agonist activity while reducing EP_4 agonist activity.