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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.



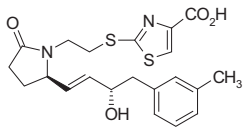
Prostanoid receptors are members of the G-protein coupled receptor superfamily. Receptors for prostaglandin $E_2$ ($PGE_2$) can be classified into four subtypes, $EP_1$, $EP_2$, $EP_3$, $EP_4$.[1] The diverse biological activities of $PGE_2$ are considered to be expressed as a hybrid of the activities 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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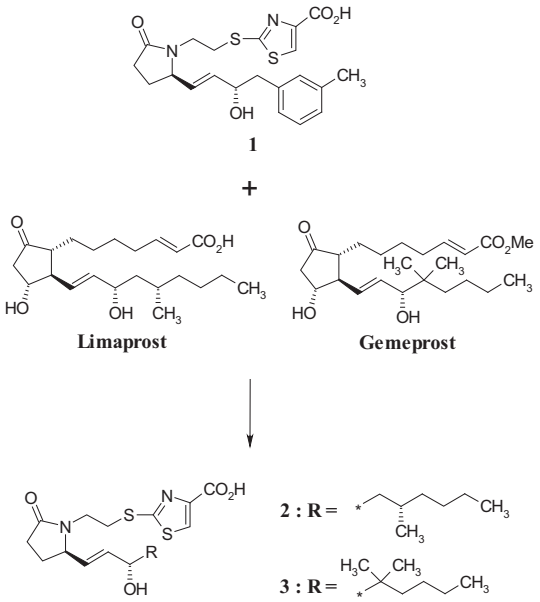
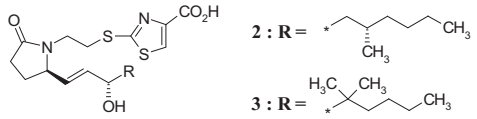



**Figure 2.** Molecular design of γ-lactam PGE analogs.

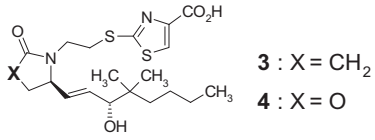
**Table 1**
Activity profiles of γ-lactam derivatives




| 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



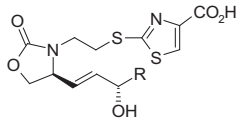

| Compd | Human functional assay, $EC_{50}$[a] (nM) | | |
|---|---|---|---|
| | $EP_2$ | $EP_3$ | $EP_4$ |
| **3** | 0.91 | 8.4 | 4.2 |
| **4** | 7.4 | 50 | 320 |

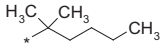
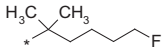
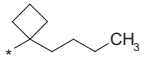
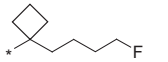
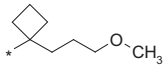
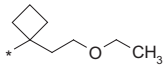
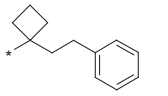
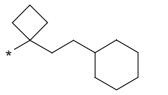
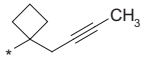
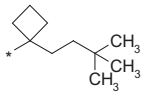
[a] $EC_{50}$ values represent the mean of at least two experiments.

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.

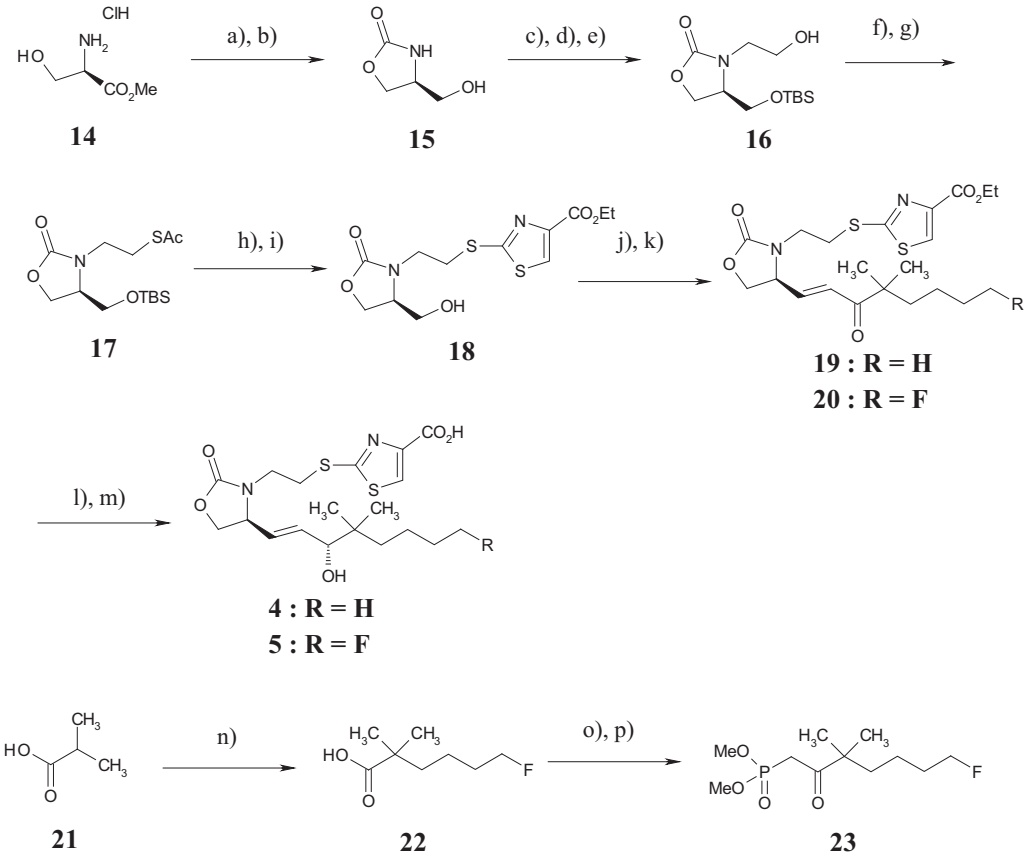
**Table 4**
Pharmacokinetics profile of **7** in rats

| 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.




**Scheme 1.** Syntheses of **4** and **5**. Reagents and conditions: (a) $K_2CO_3$, water, triphosgene, toluene, 0 °C; (b) $NaBH_4$, EtOH, rt, 51% (for 2 steps); (c) TBSCl, imidazole, DMF, rt; (d) bromo ethyl acetate, KO*t*Bu, THF, rt; (e) $NaBH_4$, THF–EtOH, rt, 89% (for 3 steps); (f) MsCl, $Et_3N$, $CH_2Cl_2$, 0 °C; (g) KSAc, DMF, rt, 99% (for 2 steps); (h) ethyl 2-bromo-1,3-thiazole-4-carboxylate, tributylphosphine, $K_2CO_3$, EtOH, 50 °C; (i) TBAF, THF, rt, 76% (for 2 steps); (j) $SO_3$-Py, $Et_3N$, DMSO, EtOAc, 10 °C; (k) NaH, dimethyl (3,3-dimethyl-2-oxoheptyl) phosphonate or **23**, THF, 0 °C, 46–59% (for 2 steps); (l) $NaBH_4$, MeOH, AcOH, −78 °C, then separation by silica gel column chromatography; (m) NaOH (aq), MeOH, 0 °C, 26–44% (for 2 steps); (n) 1-Bromo-4-fluorobutane, *n*-BuLi, *i*-$Pr_2NH$, THF, 0 °C; (o) $SOCl_2$, reflux; (p) dimethyl methylphosphonate, *n*-BuLi, THF, −78 °C, 57% (for 3 steps).

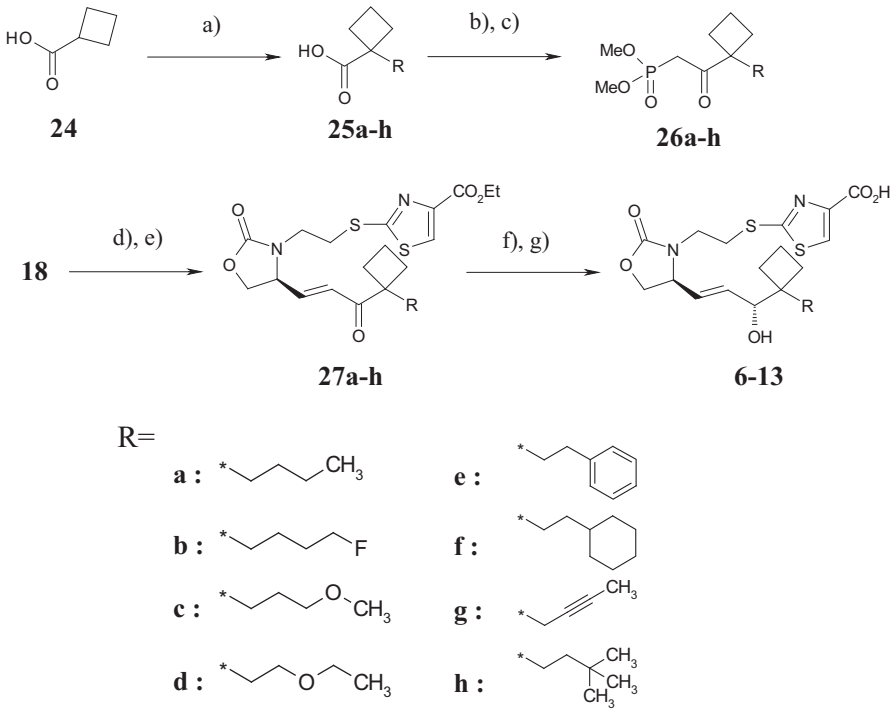
The resulting compound **5** indicated the possibility of potent and selective dual $EP_2$ and $EP_3$ agonist. Furthermore, compound **7** which possesses a cyclobutyl group in place of the *gem*-dimethyl in **5** preserved the feature of a potent dual $EP_2$ and $EP_3$ agonist. Meanwhile, the activities of other cyclobutyl derivatives **8** and **9** possessing terminal alkoxy group dropped overall. Compound **9**, in particular, suffered a significant loss in $EP_3$ agonist activity. In contrast, the incorporation of a bulky terminal group like phenyl, cyclohexyl or *tert*-butyl led to a selective $EP_3$ agonist. In addition, the linear ω side chain of internal alkyne **12** restored the $EP_1$ binding affinity. Regarding the cyclic carbamate derivatives, the combination of sterically-bulky dimethyl or cyclobutyl group and the terminal fluorine atom turned to be advantageous potent dual $EP_2$ and $EP_3$ agonist activity with selectivity against the $EP_1$ and $EP_4$ subtypes. Compounds **5** and **7** were expected to be good candidates for a UAB drug.

Unfortunately, the acceptable in vitro dual $EP_2$ and $EP_3$ agonist activity of compounds **5** and **7** was offset by pharmacokinetic problems. Table 4 shows the bioavailability of **7** is only 2.5% in rats. So, optimization of these compounds toward improvement in pharmacokinetic properties must be done to elaborate a clinical candidate.

The syntheses of **4** and **5** are described in Scheme 1. Commercially available D-serine methyl ester hydrochloride **14** was treated with triphosgene to form cyclic carbamate. Subsequent reduction of the methyl ester afforded alcohol **15**. Protection of the hydroxyl group of **15** was followed by N-alkylation of cyclic carbamate, reduction of the ethyl ester, mesylation of the resulting alcohol **16** and substitution by thioacetate to extend the α side chain. Treatment of thioacetate **17** with ethyl 2-bromo-1,3-thiazole-4-carboxylate and removal of TBS group provided alcohol **18**. Horner–Emmons olefination between the aldehyde derived from **18** and dimethyl (3,3-dimethyl-2-oxoheptyl)phosphonate or **23** provided **19** or **20**, respectively. Reduction of ketone **19** or **20** resulted in a diastereomeric mixture of each corresponding alcohol. The desired alcohols were separated by silica gel column chromatography, followed by hydrolysis to provide **4** and **5**, respectively. The configuration of the hydroxyl group was determined using the Mosher ester method.[9] Regarding preparation of phosphonate **23**, at first carboxylic acid **21** was α-alkylated with 1-bromo-4-fluorobutane to prepare **22**. Carboxylic acid **22** was converted into the corresponding acid chloride, and then treated with dimethyl methylphosphonate to give phosphonate **23**.

The syntheses of **6**–**13** are described in Scheme 2. Alkylation of cyclobutyl carboxylic acid **24** with alkylbromide (R-Br) afforded carboxylic acids **25a**–**h**. Acid chlorides derived from carboxylic acids **25a**–**h** were coupled with dimethyl methylphosphonate to prepare the corresponding phosphonates **26a**–**h**. Oxidation of **18** and Horner–Emmons reaction with **26a**–**h** were performed to provide alkenes **27a**–**h**. Ketones **27a**–**h** were reduced with $NaBH_4$ and then purified by silica gel column chromatography. The desired alcohols were hydrolyzed with aqueous NaOH to give **6**–**13**.

In summary, we have discovered compounds **5** and **7** as the first highly potent dual $EP_2$ and $EP_3$ agonists with selectivity against the $EP_1$ and $EP_4$ subtypes. The cyclic carbamate, dimethyl or cyclobutyl group and terminal fluorine atom were considered key moieties for dual $EP_2$ and $EP_3$ agonist activity. Further optimization of **5** and **7** to improve their pharmacokinetic profiles and subtype selectivity will be reported in due course.




**Scheme 2.** Syntheses of **6**–**13**. Reagents and conditions: (a) R-Br, *n*-BuLi, *i*-$Pr_2NH$, THF, 0 °C; (b) $SOCl_2$, reflux; (c) dimethyl methylphosphonate, *n*-BuLi, THF, −78 °C, 54–87% (for 3 steps); (d) $SO_3$-Py, $Et_3N$, DMSO, EtOAc, 10 °C; (e) NaH, **26a**–**h**, THF, 0 °C, 28–77% (for 2 steps); (f) $NaBH_4$, MeOH, AcOH, −78 °C, then separation by a silica gel column chromatography; (g) NaOH(aq), MeOH, 0 °C, 22–40% (for 2 steps).

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