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Synthesis and evaluation of a γ-lactam as a highly selective EP_2 and EP_4 receptor agonist

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Abstract—γ-Lactam analogs (**2**) of EP_4 receptor agonists were identified by substitution of the pyrazolidinone ring (**1**) with a pyrrolidinone ring. Several compounds (such as **2a**, **2h**) with high potency, selectivity and acceptable PK profiles were discovered. These were assessed in animal models of ovulation induction and bronchoconstriction.

Prostaglandins are known to have a broad range of biological actions in diverse tissues through binding to specific receptors on the plasma membrane.[1] Four subtypes of the PGE_2 receptor have been identified: EP_1, EP_2, EP_3, and EP_4, which mediate a wide variety of biological activities.[1] Of these four receptors, three are involved in the modulation of cAMP levels.[2] Activation of the EP_3 receptor results in a reduction of the intracellular cAMP level. In contrast, activation of EP_2 receptor and the EP_4 receptor increases the intracellular cAMP level, which is linked to the treatment of infertility. The EP_1 receptor is involved in regulating intracellular calcium levels. The EP_2 and the EP_4 receptors are interesting pharmacological targets because of their important regulatory roles in numerous physiological processes, suggesting that agonists may be useful in preventing and/or treating preterm labor, ovulation induction, asthma, fertility disorders, undesired blood clotting, sexual dysfunction, bone resorption, and inflammatory disorders, and other diseases. Additional roles for EP receptors have been reported, including smooth muscle relaxation in cat trachea for EP_2, vasodilation and anti-inflammatory activity for EP_4.[3] EP_2 and EP_4 receptor agonists have been proven to be beneficial for the treatment of preterm labor by suppressing uterine contraction and inducing oophorus maturation required for fertilization.[1b]

Several research groups have been investigating the improvement of pharmacological properties of PGE_2, which shows non-selective binding to the EP receptors (in-house binding data K_i = 9.1, 4.9, 0.33, and 0.79 nM for h-EP_1, h-EP_2, h-EP_3, and h-EP_4, respectively) and chemical and metabolic instability.[4] Until now, efforts to improve the selectivity and chemical stability of PGE_2 have been focused on only two general chemical modifications;[5] replacement of the α-alkenyl side chain with the more chemically stable phenylethyl group and substitution of heterocyclic rings for the 11-hydroxy cyclopentanone moiety.[5]

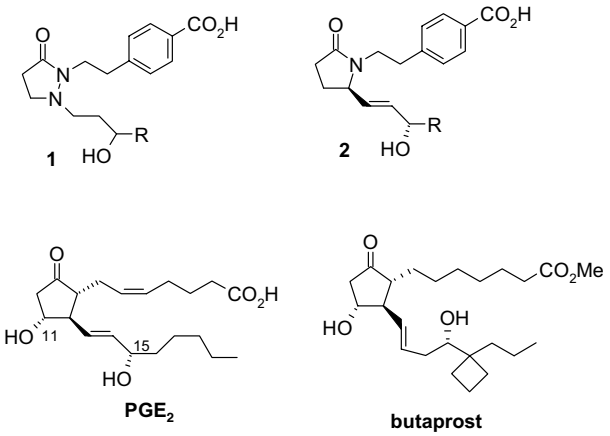

Figure 1. PGE_2 and prostaglandin derivative.

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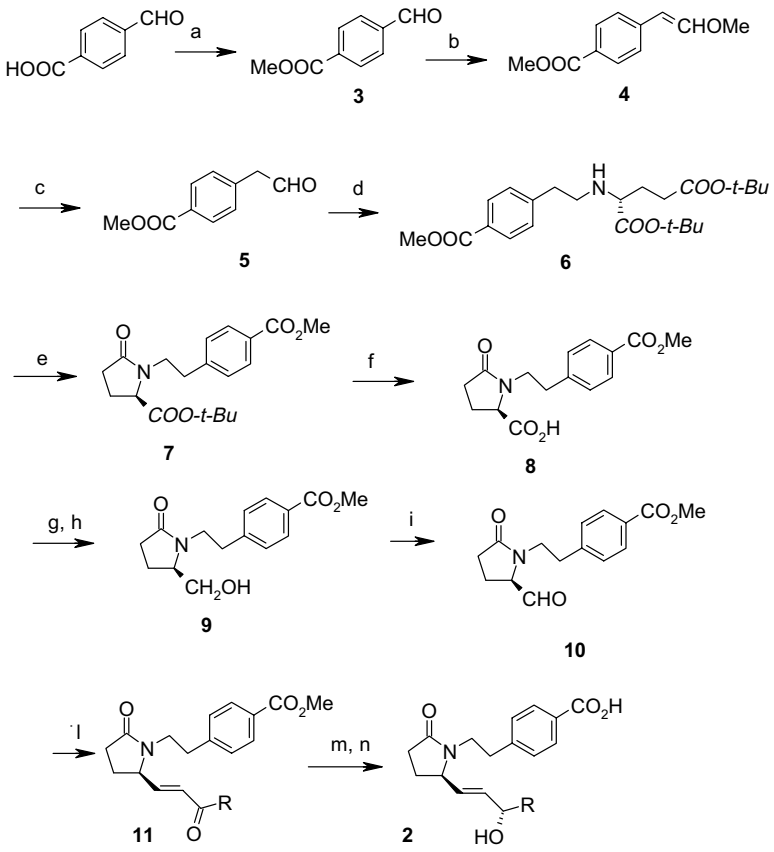
In our previous communication, we described pyrazolidinone analogs (**1** in Fig. 1) that were EP_4 receptor agonists.[6] Poor oral bioavailability limited assessment of these compounds in animal models. In order to overcome this problem, we investigated pyrrolidinone (γ-lactam) derivatives (**2** in Fig. 1). Derivatives (**2** in Fig. 1) showed good PK properties with high potency for the EP_4 receptor. In this report, we describe the synthesis, the structure-activity relationship of this series of compounds, as well as the results from in vivo animal model studies.

The approach for the synthesis of γ-lactams is outlined in Scheme 1. Alcohol **9** was the key intermediate for construction of the final γ-lactam derivatives **2**.The (*R*)-stereochemistry of compound **9** (corresponding to the natural PGE_2 stereochemistry) was inherited from the starting material H-D-Glu(O-*t*-Bu)-O-*t*-Bu·HCl (ee > 95%). Reductive amination of aldehyde **5** with H-D-Glu(O-*t*-Bu)-O-*t*-Bu·HCl smoothly provided triesters **6**, which underwent intramolecular cyclization to afford lactam **7**. The three-step conversion of **7** by hydrolysis, anhydride formation, and then reduction with $NaBH_4$ gave the key intermediate **9**.[7] Conversion of alcohol **9** into enone **11** was accomplished via Swern oxidation followed by Wittig olefination. The enone **11** can be reduced with $NaBH_4$ and $CeCl_3$ (for example R groups in compounds **2f**, **2g**) or by chiral reduction using Corey's procedure.[8] Saponification of the esters with NaOH in THF/MeOH/H_2O, followed by preparative reverse phase HPLC, furnished the desired target compound **2**.

Individual compounds were tested in vitro in the human EP_2/EP_4 receptor binding assays and also in the human EP_2/EP_4 functional assays.[5d] ^{3}H-PGE2/4 binding is evaluated by counting the plates on the top count using the ^{3}H SPA dpm2 program. % Binding and K_i values for inhibitors are calculated based on the one site competition parameter using the graphpad.? prism program. EP2/4 EC_{50} was evaluated by measuring total cAMP (intra- and extra- cellular) by using a cAMP-screen ELISA System (Tropix, #CS1000). The binding and functional data are summarized in Table 1. Compound **2a** with the $PGE_2\omega$-side chain showed high affinity binding against both EP_2 and EP_4 receptors (h-EP_2 K_i = 120 nM, h-EP_4 K_i = 2 nM). Interestingly, this compound also showed very good in vitro potency against the EP_4 receptor with an EC_{50} of 0.2 nM, about 15-fold more potent than PGE_2 itself (EC_{50} = 3.0 nM). In vitro activity of this compound displayed 60- or 750-fold selectivity for the EP_4 receptor versus the EP_2 receptor. Compounds with shorter alkyl groups (such as **2c**, **2d**,



Scheme 1. Reagents and conditions: (a) $SOCl_2$, MeOH, 91%; (b) $ClPh_3PCH_2OMe$, NaOMe, MeOH then benzene 73%; (c) aqueous H_2SO_4, THF, 94%; (d) i—H-D-Glu (O-*t*-Bu)-O-*t*-Bu.HCl, Et_3N; ii—HOAc, MeOH, $NaBH_3CN$; (e) xylene, reflux, 59%, 2 steps; (f) TFA, 0 °C to rt; (g) *N*-methylmorpholine, *i*-BuOC(O)Cl, THF; (h) $NaBH_4$, THF/H_2O, 54%, 3 steps; (i) $(COCl)_2$, DMSO, Et_3N, DCM, 92%; (l) i—NaH, THF, 0 °C; ii—$(MeO)_2P(O)CH_2C(O)R$, 0 °C to rt, 90%; (m) $NaBH_4$ and $CeCl_3$, MeOH, H_2O or (*R*)-2-methyl-CBS-oxazaborolidine, BH_3·THF, rt, 80%; (n) NaOH, H_2O/MeOH/THF, 100%.

Table 1. 15-Hydroxy pyrrolidin-2-one derivatives

Compound	R	h-EP_2 K_i (nM)	h-EP_2 EC_{50} (nM)	h-EP_4 K_i (nM)	h-EP_4 EC_{50}
2a	*n*-Pentyl	120	15	2	0.02
2b	*n*-Hexyl	54		1	
2c	*n*-Butyl	425	80	1	0.04
2d	*n*-Propyl	25,000		27	
2e	Et	>10,000		490	
2f		141	19	9	0.3
2g		21	3	2	0.3
2h	Bn	2540	434	0.05	0.03
2i	*m*-ClBn	1000	250	0.02	0.03
PGE_2		4.9		0.79	
Butaprost		110		>10,000	

2e) exhibited a dramatic decrease in EP_2 and EP_4 potency (**2a** vs **2e**). Butaprost was reported to have high selectivity for the EP_2 receptor over other EP subtypes.[9] Compound **2g**, with butaprost ω-side chain, increased remarkably the EP_2 potency while still maintained greater affinity for the EP_4 receptor.

Introduction of an aromatic moiety in the ω-chain increased by approximately 40-fold the EP_4 affinity and dramatically increased selectivity (from 60-fold for **2a** to 50,000-fold for **2h**) over the EP_2 receptor. Introduction of appropriate substituents at the *meta* position of the phenyl ring (for example **2i**) had similar selectivity and potency for EP_4 receptor. Similar results have been reported by the ONo's researchers.[10]

Rat pharmacokinetics data showed that compound **2a** exhibited high intravenous distribution rate with 42.44 L/kg, high area under the curve (AUC_{48}, 18095.3 h*ng/mL for iv, 35175.6 h*ng/mL for *sc*, 14657.8 h*ng/mL for per os), a long half-life time (14.30 h for iv, 31.61 for sc, 20.77 for per os), time to maximum (T_{max}: 0.083 h for iv, 0.25 h for sc, 4.0 h for per os), low clearance rate of 0.55 L/kg/h, and good bioavailability (33% per os, 82% for subcutaneous) when dosing with 10 mg/kg. This compares favorably against the relatively low oral bioavailability of 6.7% for the pyrazolidinone **1**.[6]

The in vivo ovulation induction activity of several compounds in CD-1 adult female mice (10-week-old) was evaluated.[11] The general protocol for ovulation induction is described as follows. PMSG (pregnant mare serum gonadotropin) (Calbiochem, cat #367222) and hCG (Serono) are diluted in PBS. PGE2 (Cayman, Ann Arbor MI) is dissolved in ethanol and diluted with 0.154 M $NaHCO_3$ Buffer (pH 8.0) to final concentration of ethanol of less than 3%. A test compound (based on solubility) is pre-dissolved in ethanol, DMSO or other reagent. Test compound is then diluted with saline or other diluents such as PBS or NP3S (5% *N*-methyl-pyrrolidinone/30% PEG-400/25% PEG-200/20% Propylene Glycol in saline). PMSG serves to stimulate follicle growth and maturation. Mature follicles will ovulate when an ovulation triggering dose of hCG or an hCG replacement (PGE2 or the test compounds) is administered. 5 IU PMSG in 200 UL PBS (i.p.) is injected and then after 48 h hCG or hCG replacement (PGE2 or test compounds, sc, iv or ps routes) is injected. After 18 h, animals are sacrificed by CO2 asphyxiation and abdominal cavities are opened using fine scissors and forceps. Uterus, oviducts, and ovaries are collected and placed in pre-labeled dishes containing phosphate-buffered saline (PBS). The collected tissues are transferred to the laboratory and intact oviduct carefully dissected out from uterus and ovary under the dissection microscope. The dissected oviducts are placed on the glass microscopic slide and covered with another slide. Two slides are taped on two edges. The numbers of ovulated ova in the oviducts are counted using upright microscope with 4× objective and recorded.

For an evaluation of the oral activity of compound **2a**, two experiments were conducted. The first experiment was conducted with non-fasted animals and the second one was conducted in 24 h fasted animals (water provided). The results demonstrate that the compound effectively induced ovulation when administered *po* (ED_{50} = 21.97 mg/kg in non-fasted animals and 21.1 mg/kg in the fasted animals) and the fasting did not affect in vivo activity.

Several compounds (Table 2) were tested in the same model by different routes of administration (po and sc). hCG and PGE2 were used to be reference samples. The results in Table 2 demonstrate that EP_4 agonists were able to stimulate ovulation induction in mature mice by both routes of administration.[12] Compound **2 h** was the most potent with an EC_{50} = 0.32 mg/kg (sc route) and 1 mg/

Table 2. The ovulation induction (ED_{50}, mg/kg)

Compound	Ovulation induction (ED_{50}, mg/Kg)	
	sc	po
2a	3.8	22
2f	3	18
2i	n.a.	1
2h	0.32	1
2g	n.a.	1
Butaprost	>30	n.a.

kg (po route), respectively, which is consistent with its high binding affinity ($K_i = 0.05$ nM) and potency ($EC_{50} = 0.03$ nM) for EP_4. In contrast, butaprost did not show any activity when administered at up to 30 mg/kg (sc route) suggesting that EP_4 might be more important than EP_2 in mediating this biological response.

The guinea pig pulmonary-cholinergic in vivo model is generally used to test potential therapeutics for the treatment of asthma in humans.[13] In our studies, groups of 3 Duncan Hartley male or female guinea pigs, weighing 250 ± 50 g, were anesthetized with sodium pentobarbital (50 mg/kg ip) and succinylcholine chloride. The trachea was cannulated and tracheal pressure was recorded through a sidearm of the cannula connected to a P23ID Statham transducer. Mean arterial pressure was monitored from a cannulated carotid artery, and heart rate was obtained from a chest-affixed electrode. The jugular vein was cannulated for iv administration of the test compounds in a volume of 1 mL/kg. Cholinergic-induced bronchoconstrictor responses, reflected as increases in tracheal pressure (cm H_2O), were elicited by administration of methacholine hydrochloride (10 μg/kg base weight iv). Compound **2a** was injected iv at doses 30 ng/kg to 0.3 mg/kg. Notably, significant methacholine-induced bronchoconstriction (>50%) inhibition was observed at doses >3 μg/kg. The calculated effective dose (ED_{50}) for Compound **2a** was approximately 1.7 μg/kg, while not altering the blood pressure or heart rate. This compound showed activity in dilation of bronchiolar muscles, which resulted in inhibition of methacholine-induced bronchomuscle constriction.

Conclusions. In summary, we have found that analogs of PGE_2 wherein the hydroxyl cyclopentanone ring has been replaced by a pyrrolidin-2-one ring are potentially useful EP_4 receptor agonists. Compound **2a** displayed good PK parameters and showed activity in two in vivo animal models. These compounds also exhibited an anti-inflammatory activity and were a potent anabolic agent for bone.[11] More in vivo animal data will be reported elsewhere.

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