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Bioorganic & Medicinal Chemistry Letters 17 (2007) 6572–6575
Bioorganic & Medicinal Chemistry Letters

Synthesis and evaluation of novel pyrazolidinone analogs of PGE_2 as EP_2 and EP_4 receptors agonists

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Received 23 August 2007; revised 18 September 2007; accepted 21 September 2007
Available online 26 September 2007

Abstract—Replacement of the hydroxy cyclopentanone ring in PGE_2 with chemically more stable heterocyclic rings and substitution of the unsaturated α-alkenyl chain with a metabolically more stable phenethyl chain led to the development of potent and selective analogs of PGE_2. Compound **10f** showed the highest potency and selectivity for EP_4 the receptor.

Prostaglandins are derived from arachidonic acid in a two-step enzymatic reaction and act as autocrine and paracrine lipid mediators.[1] Prostaglandin E_2 (PGE_2) is the most well known prostanoid derivative and exhibits a broad range of biological actions in diverse tissues through binding to specific receptors on the plasma membrane. Four subtypes of the PGE receptor are known (EP_1, EP_2, EP_3 and EP_4) and have been demonstrated to belong to the G protein-coupled rhodopsin-type receptor superfamily. Recent developments in the molecular biology of the prostanoid receptors have enabled the investigation of roles specific to each receptor by disruption of the respective gene.[1] The EP_2 and the EP_4 receptors are interesting pharmacological targets because of their important regulatory roles in numerous physiological processes, including bronchodilation, fertility, bone resorption, and inflammation. Activation of the EP_2 and EP_4 receptor increase the intracellular cAMP level, which is linked to the treatment of preterm labor by suppressing uterine contraction. Because EP_2 receptor is induced in the cumulus in response to gonadotropins and EP_2 receptor system works as a positive-feedback loop to induce of oophorus maturation required for fertilization.[1b]

PGE_2, the natural ligand for these receptors, despite its high potency, is not selective toward the individual EP receptors. Also it is degraded quickly into inactive products under physiological conditions, with a half-life of 30 s to a few minutes.[2] Natural prostaglandins are susceptible to three major modes of metabolic inactivation: ω-chain hydroxy oxidation, β-oxidation of the.α-chain, and oxidation of the ω-chain terminus.[2] Furthermore, PGE_2 is chemically unstable since the hydroxyl group on C-11 can easily undergo elimination, leading to enone derivatives like PGA_2.[2c] In the last two decades, efforts to improve the selectivity and chemical and metabolic stability have been described[3–6] (Fig. 1).

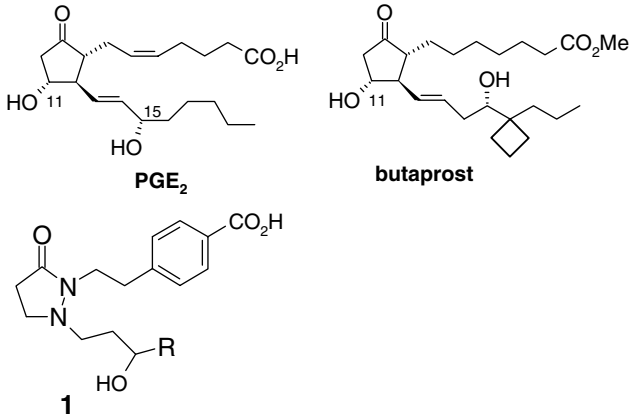

Figure 1. PGE_2 and pyrazolidinone derivative.

Keywords: Prostaglandin; EP2; EP4; Agonist.
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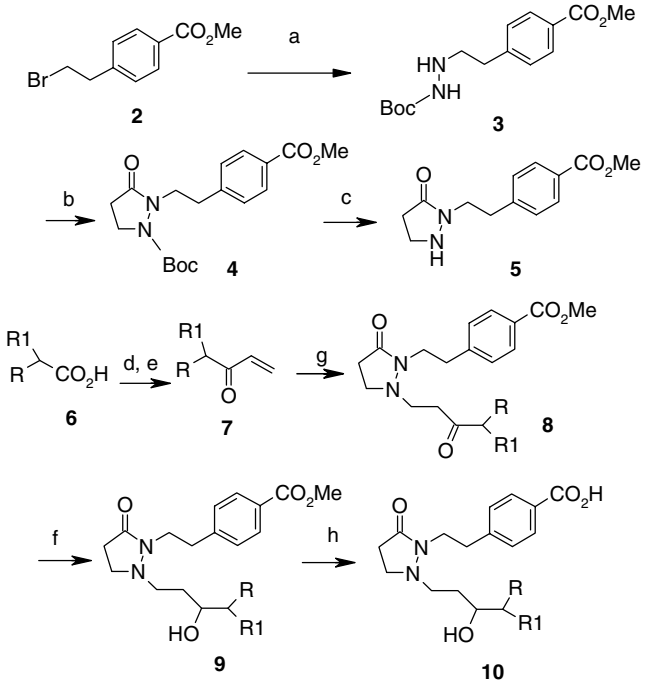
doi:10.1016/j.bmcl.2007.09.074

In order to improve the pharmacological properties of PGE_2, we have examined the replacement of the α-alkenyl side chain with the more stable phenethyl chain and the substitution of the hydroxy cyclopentanone moiety with different heterocyclic rings.[3,4,6] This report describes the synthesis and structure-activity relationship of a series of pyrazolidin-3-one derivatives of general structure **1** bearing different R groups in the ω-chain.

Synthesis of the pyrazolidin-3-one derivatives is outlined in Scheme 1. Introduction of the side chain in the α-position was achieved by alkylation of commercially available *tert*-butyl hydrazinecarboxylate with the phenethyl bromide derivative **2** in the presence of a weak base. The pyrazolidinone ring was then formed in one step by reaction of chloropropanoyl chloride with the hydrazide intermediate **3** under basic conditions.[7] Deprotection of the Boc group afforded the versatile intermediate **5** which was extensively used in our research effort for the introduction of the ω-chain.

Preparation of the pyrazolidinone derivatives bearing the hydroxy group on C-15 of the ω-chain was achieved by Michael addition of the pyrazolidinone intermediate **5** to enone **7**.[8] As described in Scheme 1, the carboxylic acid **6** was converted to the corresponding Weinreb amide using EDCI and HOBt in DMF.[9] Reaction of the Weinreb amide with vinyl magnesium bromide provided enone derivative **7** that was used directly without further purification.[10] Michael addition of **5** to enone **7** was carried out in EtOH at reflux for 2 h to furnish **8** in good to quantitative yields. The ketone intermediate **8** was transformed in 2 steps with excellent yield into the final desired product **10** via reduction of **8** with $NaBH_4$ in the presence of $CeCl_3$ and saponification of the ester group of **9**.



Scheme 1. Reagents and conditions: (a) *tert*-butyl hydrazinecarboxylate, $NaHCO_3$, ACN, reflux, 15 h; (b) 3-chloropropanoyl chloride, K_2CO_3, DMF, rt, 18 h; (c) TFA, DCM, RT, 1 h; (d) *N*,*O*-dimethylhydroxylamine, EDCI, HOBt, Et_3N, DMF, rt, 18 h; (e) vinylmagnesium bromide, THF, 0 °C, 1 h; (g) **5**, EtOH, reflux, 2 h; (f) $NaBH_4$, $CeCl_3$, EtOH, H_2O, RT, 1 h; (h) NaOH, H_2O, MeOH, THF, rt, 18 h.

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.[4] The data in Table 1 indicate that the best results are obtained when a straight PGE_2-like chain is introduced at the ω-position (compound **10a**). Introduction of branching in the chain led to a remarkable decrease in activity, especially for the EP_4 receptor. Furthermore, it is interesting to note that compound **10a** showed 10-fold selectivity for the EP_4 receptor over the EP_2 receptor.

The ONO's researchers reported that the introduction of a *meta*-position in phenyl group in the ω-chain increased selectivity and potency for EP_4 receptor.[6b] Several 15-hydroxy-16-aryl pyrazolidinone derivatives have been synthesized and their in vitro data are summarized in Table 2. As can be seen, the introduction of a simple aromatic group as **10d** led to a remarkable improvement in EP_4 affinity (100-fold over EP_2) compared to the corresponding alkyl derivative **10a** (10-fold over EP_2). Development of SAR for substituents at the *meta* position of the aromatic ring led to the conclusion that the best results are obtained with the introduction of halogen atoms like iodo, bromo and chloro. Interesting results are also obtained with the introduction of bulky groups (**10m** and **10n**). There is still little known about the structural determinants of the EP_2 and EP_4 receptors that are required for the binding of PGE_2, or the residues that dictate the selectivity of EP_2 or EP_4 receptors. It is not at all clear that *meta*-position contributes significantly to binding or function at EP_4 receptor.

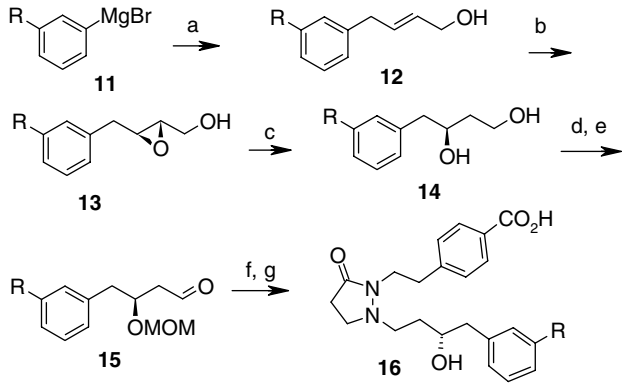
Due to the promising results in this series, we decided to develop an enantioselective synthesis for these EP_4 agonists. As described in Scheme 2, the commercially available butadiene monooxide was converted to allylic alcohol **12** by reaction with aryl magnesium bromide **11** in the presence of a catalytic amount of CuCN. The allylic alcohol was then subjected to Sharpless epoxidation condition with (+)-diethyl *L*-tartrate to furnish the epoxide **13**.[11] Regioselective ring opening of epoxide **13** with Red-Al[12] reduction afforded the diol derivative **14** (ee% >97% determined with chiral HPLC). Selective protection of the secondary alcohol was achieved in a two-step procedure by first using trimethyl orthoformate and then reducing the cyclic ether intermediate with

Table 1. 15-Hydroxy 16-alkyl pyrazolidinone derivatives

Compound	CH_2RR1	h-EP_2 K_i (μM)	h-EP_2 EC_{50} (μM)	h-EP_4 K_i (μM)	h-EP_4 EC_{50} (μM)
10a	$(CH_2)_4CH_3$	2.2	0.595	0.25	0.005
10b	$CH_2CH(Me)_2$	4.41	15	2	0.277
10c	$CH(Me)C_3H_7$	2.76	5	6	nd

Table 2. 15-Hydroxy-16-aryl pyrazolidinone derivatives (R1=H)

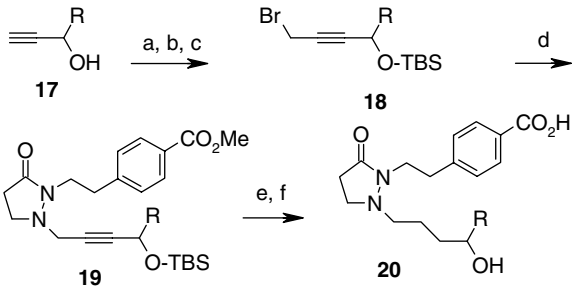
Compound	R	h-EP$_2$ K_i (μM)	h-EP$_2$ EC$_{50}$ (μM)	h-EP$_4$ K_i (μM)	h-EP$_4$ EC$_{50}$ (μM)
10d	Ph	6.2		0.068	
10e	*m*-I-Ph	2.36		0.027	
10f	*m*-Br-Ph	5.45		0.013	0.0002
10g	*m*-OCF$_3$-Ph	2.83		0.059	
10h	*m*-F-Ph	6.29		0.036	
10i	*m*-CF3-Ph	6		0.057	
10l	*m*-Cl-Ph	5.69		0.018	0.0002
10m	*m*-(ethynylcyclopropyl) phenyl	8.34		0.33	
10n	*m*-(ethynylphenyl) phenyl	0.56		0.044	
PGE$_2$		0.0049		0.00079	
Butaprost		0.11		>10	



Scheme 2. Reagents and conditions: (a) 2-vinyloxirane, CuCN, THF, −78 °C, 2 h; (b) *L*-DET, Ti(O-*i*-Pr)$_4$, *t*-BuO$_3$H, molecular sieves, DCM, −16 °C, 4 h; (c) Red-Al, THF, 0 °C, 15 h; (d) (i) trimethyl orthoformate, CSA, DCM, RT, 2 h (ii) Dibal-H, −70 °C, 2 h; (e) Dess-Martin reagent, DCM, rt, 1 h; (f) Na(OAc)$_3$BH, AcOH, DCE, rt, 2 h; (g) (i) HCl (conc), MeOH, rt, 1 h; (ii) NaOH, H$_2$O, MeOH, THF, rt, 18 h.

DIBAL-H. Oxidation of the resulting alcohol intermediate using Dess-Martin periodine reagent afforded the aldehyde intermediate **15** in quantitative yield, which was used to alkylate the pyrazolidinone ring under standard procedure followed by saponification of the ester groupto yield the desired chiral pyrazolidinone derivatives **16**. The in vitro activity of the compounds is summarized in Table 3. The **16** with chiral center at C-15 did not improve the binding affinity and potency (**16a** vs **10d**, **16b** vs **10f**, **16c** vs **10e**). It indicates that the chiral center at C-15 was not necessary to contribute the potency and selectivity.

In order to improve the selectivity for the EP$_2$ receptor, we have investigated the synthesis of pyrazolidinone



Scheme 3. Reagents and conditions: (a) TBSCl, Imidazole, DMF, rt, 18 h; (b) (i) *n*-BuLi, THF, −78 °C, 10 min, (ii) paraformaldehyde, rt, 2 h; (c) PPh$_3$, CBr$_4$, DCM, rt, 18 h; (d) **5**, K$_2$CO$_3$, NaI, DMF, 50 °C, 1 h; (e) H$_2$ (1 atm), Pd/C 10%, MeOH, rt, 2 h; (f) (i) HCl 4 M, dioxane, rt, 1 h; (ii) NaOH, H$_2$O, MeOH, THF, rt, 18 h.

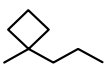
analogs bearing the hydroxyl group at C-16, similar to the known EP$_2$ selective agonist butaprost, which exhibited a high selectivity (25000-fold) for EP$_2$ receptor.[13] Synthesis of the 16-hydroxyl pyrazolidinone derivatives is outlined in Scheme 3. Protection of the propargyl alcohol derivative **17** followed by formylation gave the new propargyl alcohol in good yield. Conversion of the alcohol into the bromide using triphenylphosphine and carbon tetrabromide in DCM gave the corresponding propargyl bromide **18** in quantitative yield, which was used to alkylate pyrazolidinone **5**. Hydrogenation and deprotection of the TBS group and hydrolysis of the methyl ester furnished the desired compounds of general formula **20** in good yield.

The in vitro activities are listed in Table 4. Moving the hydroxy group from C-15 to C-16 decreased dramatically in activity toward the EP$_4$ receptor while maintain-

Table 3. 15-Hydroxy-16-aryl pyrazolidinone derivatives

Compound	R	h-EP$_2$ K_i (μM)	h-EP$_2$ EC$_{50}$ (μM)	h-EP$_4$ K_i (μM)	h-EP$_4$ EC$_{50}$ (μM)
16a	H	9.2	nd	0.134	nd
16b	*m*-Br	3.07	>10	0.013	0.0001
16c	*m*-I	2.03	nd	0.026	nd

Table 4. 16-Hydroxy pyrazolidinone derivatives

Compound	R	h-EP$_2$ K_i (μM)	h-EP$_2$ EC$_{50}$ (μM)	h-EP$_4$ K_i (μM)
20a	(CH$_2$)$_4$CH$_3$	2.44	1.3	2
20b		0.75	0.393	>50

ing the affinity for EP_2 (**10a** vs **20a**). An analog with the butaprost-like cyclobutyl group at C-17 (**20b**) increased the EP_2 activity to a certain degree, but showed a sharp decrease in EP_4 activity. However, **20b** still showed less selectivity and potency for EP_2 receptor.

In summary, for this series of pyrazolidinone derivatives, compound **10f** was found to be the most potent and selective for the EP_4 receptor than the EP_2 receptor. The rat pharmacokinetics data showed that compound **10f** exhibited a high intravenous distribute rate (199.66 L/Kg), a moderate clearance rate (1.44 L/Kg/h), a half-life time (4.25 h), and a low oral bioavailability (6.7%). The low oral bioavailability might possibly be caused by its low permeability.[14] The low bioavailability limited the assessment of these compounds in animal model with per os route. The in vivo ovulation induction of compound **10f** in CD-1 adult female mice (10-week-old) was evaluated. Preliminary results showed that a 3 mg/kg dose of **10f** stimulated rupture of the follicle and the induction and release of four oocytes into the fallopian tube.11 oocytes were released when dosing 30 mg/kg at subcutaneous administration.[15] **10f** also exhibited good efficacy in a dose-dependent manner (from 3 to 30 mg/kg).

Conclusions. In summary, we have found that analogs of PGE_2 wherein the hydroxyl cyclopentanone ring has been replaced by a pyrazolidinone ring are potent EP_4 receptor agonists. In particular, the pyrazolidin-2-one derivatives having a phenethyl α-chain showed the best in vitro profile among the different series explored. Modulation of the ω-chain allowed us to find a potent and selective EP_4 agonist (for example **10f**). Introduction of a 16-hydroxy group on the ω-chain (**20a**–**b**) did not obviously improve the potency for the EP_2, but dramatically decreased EP_4 receptor activity.

Acknowledgment

We are grateful to Dr. Lesley Liu-Bujalski for proofreading the manuscript.

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14. Compound **10f** has low permeability with 9% passage, 10 papp $\times 10^{-6}$ cm/s. Rat plasma protein binding of compound **10f** (C = 10 mM, 2 h) was 61% unbound. After 1 h, live microsomes degradation was 7% in rat and 0% in human, respectively. The solubility of compound **10f** was measured to be 0.89 mg/mL in 2% DMSO in HBSS or 0.84 mg/mL in 2%DMSO in saline. The result at intravenous administration is not disclosed here.
15. We tested compounds in ovulation induction in two routes: intravenous, subcutaneous. 2% DMSO + saline was used to be vehicle control and FSH was a positive control.