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

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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).

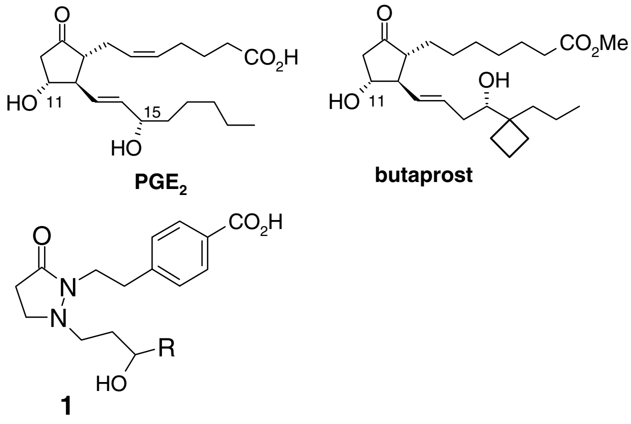

Figure 1. PGE_2 and pyrazolidinone derivative.

Keywords: Prostaglandin; EP2; EP4; Agonist.

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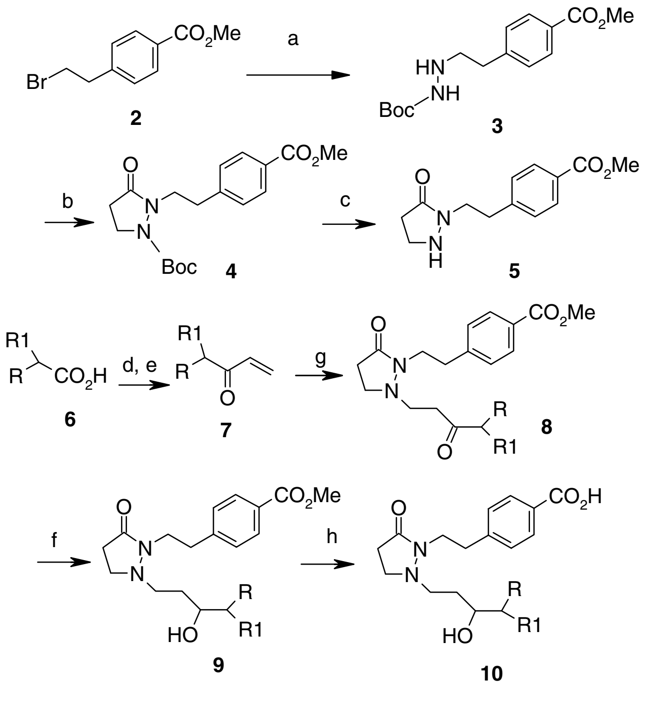
In order to improve the pharmacological properties of PGE$_2$, we have examined the replacement of the α-alkenyl side chain with the more stable phenethyl chain and the substitution of the hydroxy cyclopentanone moiety with different heterocyclic rings.[3,4,6] This report describes the synthesis and 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$_2$CO$_3$, DMF, rt, 18 h; (c) TFA, DCM, RT, 1 h; (d) *N,O*-dimethylhydroxylamine, EDCI, HOBt, Et$_3$N, 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$_2$O, RT, 1 h; (h) NaOH, H$_2$O, 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$_2$RR1	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$_2$)$_4$CH$_3$	2.2	0.595	0.25	0.005
10b	CH$_2$CH(Me)$_2$	4.41	15	2	0.277
10c	CH(Me)C$_3$H$_7$	2.76	5	6	nd