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Discovery of a Potent and Selective Agonist of the Prostaglandin EP_4 Receptor

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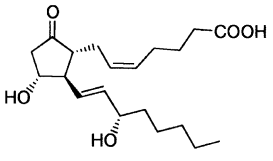
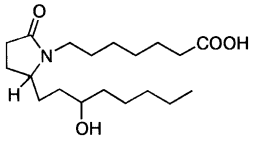
Abstract—Analogues of PGE_2 wherein the hydroxycyclopentanone ring has been replaced by a lactam have been prepared and evaluated as ligands for the EP_4 receptor. An optimized compound (**19a**) shows high potency and agonist efficacy at the EP_4 receptor and is highly selective over the other seven known prostaglandin receptors.

Prostaglandin E_2 (PGE_2) is a potent pro-inflammatory mediator which manifests a variety of activities in the body through interaction with four distinct subtypes of receptor (termed EP receptors) namely EP_1, EP_2, EP_3 and EP_4.[1,2] PGE_2 has multiple effects on bone including stimulation of resorption and formation[3] as well as other systemic effects including induction of diarrhea and hypotension. PGE_2 has been shown to stimulate bone formation in rats and humans in vivo.[4] However, the multiple side effects of PGE_2 have made it unsuitable for use as a bone-forming therapy. The major prostaglandin E receptor on bone cells is EP_4 and EP_4 receptor knockout mice have shown significant defects in bone metabolism.[5] A recent study utilizing an EP_4 receptor antagonist has shown that the in vivo bone forming effects of PGE_2 in rats are effectively blocked by co-administration of the antagonist.[6] Thus the hypothesis has been formed that a selective EP_4 receptor agonist could be a useful agent to promote bone growth.[7]

Receptor binding assays are available in our laboratory for all eight of the prostaglandin receptors[8] and utilizing these assays we have screened for selective ligands at the EP_4 receptor. Such screening resulted in the identification of compound **1**[9] which exhibited nanomolar binding at the EP_4 receptor and essentially no activity at any other receptor in the prostaglandin receptor panel (entry 1, Table 1). The compound also displayed agonism in a functionally coupled cellular assay ($EC_{50} = 770$ nM). Compound **1** was a mixture of four isomers and considering general homology with PGE_2, it was anticipated that activity should reside in one isomer only. Resynthesis of **1** as a pair of C-12 isomers (entries 2 and 3) revealed that the 12-(*R*)-enantiomer (corresponding to the natural PGE_2 stereochemistry) displayed the large majority of the activity. Analogues of **1** incorporating a 13,14 *trans*-double bond (entries 4 and 5) were prepared and again activity resided essentially in the 12-(*R*)-enantiomer (these compounds were still unresolved at the C-15 center).



PGE_2



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Our goal was to develop an EP_4 receptor agonist which would serve as an in vivo probe in a variety of assays of bone formation and therefore we sought to identify an analogue with a degree of metabolic stability which would be manifested in an improved in vivo half-life. PGE_2 has an extremely short half-life in vivo. As pros-

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Table 1. Binding data for compounds at the prostaglandin receptors

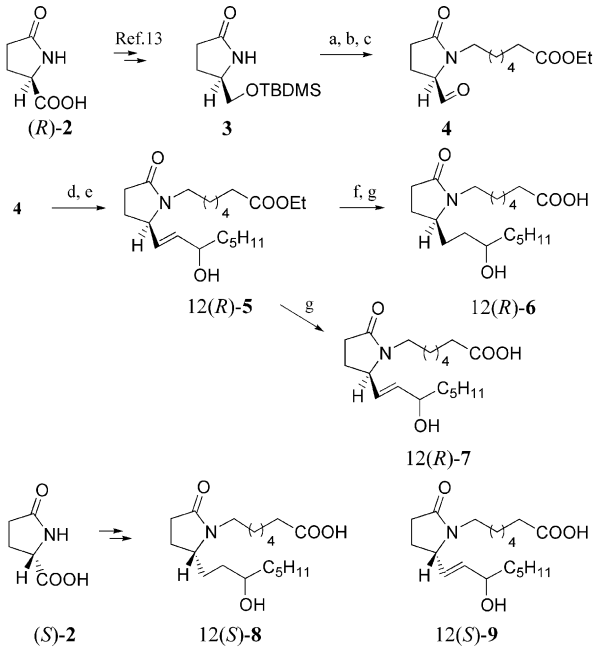
Entry	Compd	Binding K_i (nM)			
		EP_4	EP_2	EP_3	EP_1, DP, FP, IP, TP
1	**1**	35 ($n=1$)	3000	2000	>3000
2	**6**	28 ($n=2$)	4050	700	>13,000
3	**8**	1110 ($n=1$)	3610	2315	>13,000
4	**7**	6.0±0.5 ($n=5$)	6680	419	>13,000
5	**9**	1410 ($n=1$)	>13,000	1220	>13,000
6	**10a**	3500 ($n=1$)	>13,000	>13,000	>13,000
7	**10b**	2.6±0.1 ($n=6$)	>13,000	>13,000	>13,000
8	**10c**	3.6±0.2 ($n=3$)	>13,000	>13,000	>13,000
9	**10d**	102 ($n=2$)	>13,000	2014	>13,000
10	**12**	2100 ($n=1$)	>13,000	>13,000	>13,000
11	**10e**	62 ($n=1$)	>13,000	10,560	>13,000
12	**10f**	12 ($n=1$)	>13,000	3340	>13,000
13	**15a**	443±48 ($n=4$)	>13,000	>13,000	>13,000
14	**16a**	13±1 ($n=5$)	>13,000	>13,000	>13,000
15	**16b**	140 ($n=1$)	>13,000	>13,000	>13,000
16	**16d**	243 ($n=1$)	>13,000	>13,000	>13,000
17	**16c**	66 ($n=1$)	>13,000	>13,000	>13,000
18	**19a** + **19b**	2.5±0.2 ($n=5$)	>13,000	>13,000	>13,000
19	**19a**	1.2±0.2 ($n=4$)	>13,000	>13,000	>13,000
20	**19b**	230±40 ($n=3$)	>13,000	>13,000	>13,000

taglandin metabolism is known to involve both β-oxidation of the acid chain and terminal oxidation of the lipophilic chain, it was decided to explore whether these chains could be modified to inhibit or block the expected metabolism while maintaining potency at the EP_4 receptor and full functional efficacy. Initially, a variety of compounds were prepared wherein the terminal lipophilic group had been replaced by an aryl ring or a cyclohexyl ring. Introduction of the cyclohexyl ring adjacent to the hydroxy group at C-15 was accompanied by a dramatic drop loss in binding activity (entry 6). Replacement of the C-17 to 20 chain with a phenyl ring yielded a potent binding compound which retained its in vitro efficacy in the cell assay ($EC_{50}=28$ nM) (see entry 7). Substitution of the aryl ring by a methoxy methyl group (entry 8) (a substitution shown to be optimal in other studies on EP_4-selective PGE_2 analogues)[10] yielded a potent compound (**10c**) but with no advantage over **10b**. Homologation of the chain (entry 9) led to an important drop in activity. A major route of in vivo metabolism of the prostaglandins involves 15-dehydrogenation.[11] Although such oxidation was not observed in hepatocyte incubations (with **10b**), this type of oxidation is known to generally take place through extra-hepatic metabolism in mammals.[12] We therefore prepared the corresponding 15-methyl substituted analogue (**10c**). The compound, however, was dramatically less potent (entry 10). In order to block the 16-position to potential benzylic oxidation, 16,16-distubstituted analogues (entry 11 and 12) were prepared and were found to largely retain the binding activity compared to the **10b**. However, these substitutions did not lead to a significant increase in in vitro metabolic stability and so it was decided to maintain the terminal methylene aryl group as in compound **10b** and investigate potential substitution and analogues on the acid-bearing chain.

Preliminary in vivo evaluation of compound **10b** dosed intravenously to a rat revealed a relatively short half-life ($t_{1/2}=<20$ min) and in vitro metabolism studies in rat hepatocytes indicated significant β-oxidation of the acid chain. Compounds bearing a β-sulfur atom in an acidic chain are blocked to β-oxidation and, therefore, the corresponding β-thio compound (**16a**, entry 14) was prepared and was found to be approximately 4-fold less potent than the corresponding methylene compound (**10b**). Homologation of the chain led to a 10-fold loss in binding activity (entry 15). Examination of the metabolic fate of compound **16a** in incubation with rat hepatocytes indicated the compound was significantly more metabolically stable than **10b** (data not shown). A major metabolite, however, was observed and was identified as the corresponding sulfoxide. An alternative method to block β-oxidation of the acid chain could be to replace the acid group with an acid equivalent such as a tetrazole that may not be recognized by the β-oxidation enzymes. Thus, a variety of tetrazolyl analogues of **10b** were prepared and evaluated for their potency, binding and metabolism. The S-tetrazole compound (entry 16) was less potent than the corresponding straight chain acid. However, the homologue (entry 17) retained considerable activity at the EP_4 receptor. Replacement of the acid group in compound **10b** with a tetrazole, however, led to a compound approximately 2-fold *more* potent than the parent (entry 18). This compound was resolved into its two diastereoisomers (isomeric at C-15) (entries 19 and 20) through chromatographic separation and virtually all the binding activity was found to reside with one isomer (**19a**) ($IC_{50}=1.4$ nM; IC_{50} for PGE2 is 0.7 nM) presumably having the natural PGE_2 stereochemistry at C-15. This compound was evaluated in rat hepatocytes for metabolic stability and found to be essentially unchanged after incubation for 2 h. Dosed intravenously in rats, the compound had a much improved half-life ($t_{1/2}=$ca 2 h). Examination of bile from rats dosed with **19a** indicated that the compound was excreted largely unchanged. Compound **19a** was shown to be highly selective for the EP4 receptor with no significant binding observed at greater than 14 μM on any of the other receptors. Compound 19a was a full agonist in the cell efficacy assay with an EC_{50} of 2.5±1.0 nM ($n=5$). This is comparable to the EC_{50} of PGE_2 itself [$EC_{50}=3.0\pm0.4$ nM ($n=8$)].

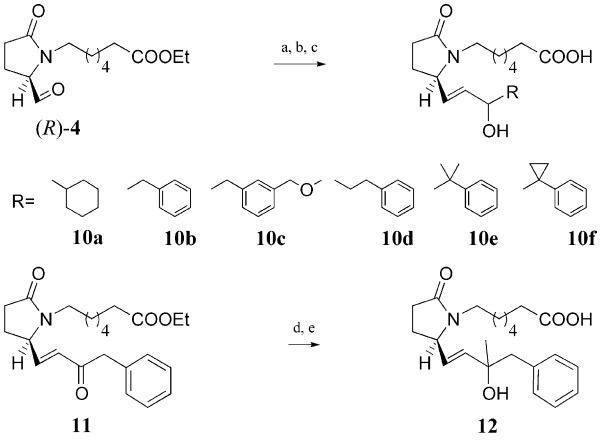
Compound **3** was synthesized from (*R*)-pyroglutamic acid (**2**) through a series of reactions described in ref 13 (Scheme 1).

The sodium salt of **3** was alkylated with ethyl 7-bromoheptanoate under phase-transfer catalysis (88% yield). After deprotection with HF–Pyridine complex, Dess–Martin oxidation of the alcohol led to the aldehyde **4** (84% yield). Wittig reaction with the sodium salt of dimethyl (2-oxoheptyl)phosphonate afforded the intermediate enone (55% yield) which was reduced to the allylic alcohol **5**. Saponification of **5** produced the acid **7** in 94% yield. Catalytic hydrogenation of **5** with PtO_2 in AcOEt followed by saponification with 1 N LiOH gave the acid **6**. Using the methodology decribed above, compounds **8** and **9** were obtained similar yields starting from (*S*)-pyroglutamic acid [(*S*)-**2**].



Scheme 1. Reagents and conditions: (a) (i) NaH, DMF, 50 °C; (ii) $BrCH_2(CH_2)_5COOEt$, nBu_4NI, 50 °C, 88%; (b) HF-Py, CH_2Cl_2, 68%; (c) Dess–Martin periodinane, CH_2Cl_2, 84%; (d) (i) NaH, DMA, 0 °C; (ii) $(MeO)_2P(O)CH_2COC_5H_{11}$, rt, 55%; (e) $NaBH_4$, EtOH, −20 °C, 92%; (f) PtO_2; AcOEt, H_2, 87%; (g) LiOH, H_2O, THF, MeOH, 94%.

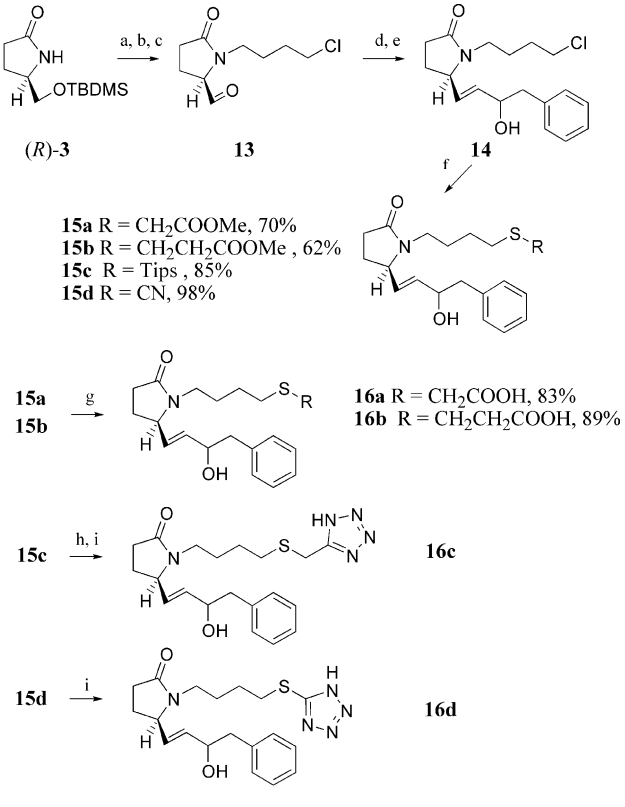
The optimization of the β-chain started with aldehyde (*R*)-**4** (Scheme 2) which was reacted the appropriate β-ketophosphonate using NaH as base in THF (yields from 16 to 72%). The resultant enones were then reduced to intermediate allylic alcohols using $NaBH_4$ in EtOH at −20 °C (yields from 65 to 85%). Ester saponification afforded compounds **10a–f** (yields from 38 to 96%). In the case of compounds **10e** and **10f**, the reduction was run using the Luche reaction ($NaBH_4$ and $CeCl_3 \cdot 7H_2O$ in EtOH–H_2O at 0 °C) in order to prevent the over-reduction of the double bond. Tertiary alcohol **12** was synthesized by reaction of enone **11** with one equivalent of freshly prepared methylcerium reagent in THF (33%), followed by saponification of the intermediate ester (66%).



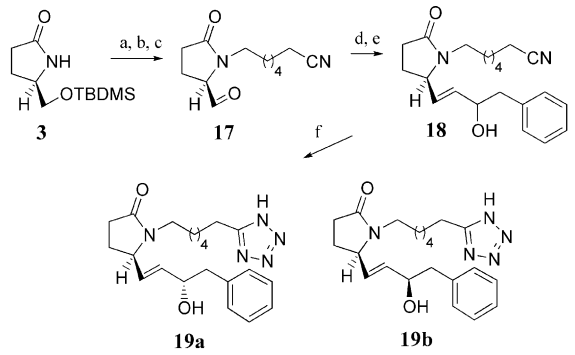
Scheme 2. Reagents and conditions: (a) (i) NaH DME 0 °C; (ii) $(MeO)_2$-$P(O)CH_2COR$, rt; (b) $NaBH_4$, EtOH, −20 °C; (c) LiOH, H_2O, THF, MeOH; (d) 1 equiv MeLi, 2 equiv $CeCl_3$, THF, −78 °C, 33%; (e) LiOH, H_2O, THF, MeOH, 66%.

The synthesis of analogues with optimized acid-bearing chains started with the previously synthesized amide (*R*)-**3** (Scheme 3). *N*-Alkylation of the amide (*R*)-**3** followed by alcohol deprotection and Dess–Martin oxidation afforded aldehyde **13** in good yield. Wittig reaction with sodium dimethyl (2-oxo-3-phenylpropyl)phosphonate gave the intermediate enone that was immediately reduced to the allylic alcohol **14** using $NaBH_4$ in EtOH at −20 °C. Chlorine displacement using various alkylsulfides produced compounds **15a–d** in good yields. Compounds **15a** and its homologue **15b** were saponified using 1 N LiOH to afford **16a** and **16b** in good yields (respectively, 83 and 89%). Sulfide **15c** was reacted with bromoacetonitrile in the presence of nBu_4NF to afford the intermediate nitrile (94% yield), which was then heated at 120 °C for 3 h with nBu_3SnN_3 to give the tetrazole **16c** (88% yield). Similarly **15d** was transformed into the tetrazole **16d** in good yield (Scheme 4).

The introduction of a terminal tetrazole started with the *N*-alkylation of amide 12(*R*)-**3** with NaH and 5-bromoheptanenitrile under phase-transfer catalysis followed by deprotection of the alcohol (84% yield) and Dess–Martin oxidation (84%) affording the aldehyde **17**. A Wittig reaction of **17** with the sodium salt of dimethyl (2-oxo-3-phenylpropyl)phosphonate produced the intermediate enone (84%yield), which was reduced with $NaBH_4$ in EtOH at −20 °C to provide the nitrile **18**.



Scheme 3. Reagents and conditions: (a) (i) NaH, DMF, 50 °C; (ii) $Br(CH_2)_4Cl$, Bu_4NI, 50 °C, 87%; (b) HF-Py, CH_2Cl_2, 90%; (c) Dess–Martin periodinane, CH_2Cl_2, 95%; (d) (i) NaH, DME, 0 °C; (ii) $(MeOH)_2$-$P(LO)CH_2COCH_2Ph$, rt, 62%; (e) $NaBH_4$, EtOH, −20 °C, 90%; (f) RSH, MeONa, DMF, Bu_4NI, 50 °C; (g); LiOH, H_2O–THF–MeOH, rt; (h) $BrCH_2CN$, nBu_4NF, THF, rt 94%; (i) nBu_3SnN_3, neat, 120 °C, 3 h.



Scheme 4. Reagents and conditions (a) (i) NaH, DMF, 50 °C; (ii) $BrCH_2(CH_2)_5CN$, nBu_4NI, 50 °C, 84% (b) HF-Py, CH_2Cl_2, 87%; (c) Dess–Martin periodinane, CH_2Cl_2, 88% (d) (i) NaH, DME, 0 °C; (ii) $(MeO)_2$-P(O)CH_2COCH_2Ph, rt, 75%; (e) $NaBH_4$, EtOH, −20 °C; (f) Bu_3SnN_3 neat 120 °C, 3 h, 80%.

Finally the tetrazole moiety was introduced by reaction of **18** with 3 equiv of nBu_3SnN_3 neat at 120 °C for 3 h (80% from enone). Reverse-phase HPLC allowed the separation of both diasterioisomers eluting sequentially in a ratio (**19a**/**19b**) (1:2) in the favor of the putative 15(*R*)-alcohol **19b**.

Biological Results and Discussion

Receptor binding assays were performed using cell membranes from HEK293ebna cells recombinantly expressing the corresponding human prostanoid cDNA's.[8] EP4 agonist potency and efficacy were evaluated utilizing a stable clone of pSV40-EP4 transfected into HEK293 cells that expresses approximately 50 fmol/mg EP4 receptor. Whole cell cAMP assays were performed essentially as described in Slipetz et al.[14] with the following modifications. Assays were performed with cells in suspension in a total of 0.2 mL HBSS containing 2 mM IBMX (phosphodiesterase type IV inhibitor). IBMX and PGE_2 or the test compound were added to the incubation mixture in DMSO to a final vehicle concentration of 1.8% (v/v) (kept constant in all samples). The reaction was initiated by the addition of 1×10^5 cells per incubation, samples were incubated at 37 °C for 10 min, and the reaction was terminated by immersing the samples in boiling water for 3 min. Measurement of cAMP was performed by a [^{125}I]cAMP scintillation proximity assay. For in vivo and in vitro metabolism methods, see methods described by Nicoll-Griffith et al.[15]

Analogues of PGE_2 wherein the hydroxy cyclopentanone ring has been replaced by a lactam were found to exhibit potent and selective agonism at the EP_4 receptor.[16] Thus one can conclude that the C-11 hydroxy group present in PGE_2 is not necessary for either binding or agonism at this receptor. Indeed, one could conclude that lack of a corresponding hydroxy group in these lactam analogues might be responsible for a measure of the selectivity observed for the EP_4 receptor over the other prostaglandin receptors. The natural stereochemistry at C-12 and putatively also at C-15 was found to be important for activity at the receptor. The carboxyl group can be replaced by an acid equivalent such as a tetrazole with comparable or enhanced activity. The optimized compound (**19a**) displays a high degree of selectivity and potency and in vivo half-life much superior to PGE_2. This compound and related analogues are now being studied in a number of biological assays to determine whether an EP_4 selective agonist can serve as a useful therapeutic for treatment of osteoporosis.

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