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Bioorganic & Medicinal Chemistry Letters 14 (2004) 1655–1659

Lactams as EP_4 prostanoid receptor subtype selective agonists. Part 1: 2-Pyrrolidinones-stereochemical and lower side-chain optimization

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Received 18 September 2003; revised 22 January 2004; accepted 22 January 2004

Respectfully and warmly dedicated to the memory of Brian H. Vickery, deceased June 2002.

Abstract—A series of 7-[(5*R*)-substituted 2-oxo-1-pyrrolidinyl]-heptanoic acids were prepared, their isomeric purity determined, and pharmacologically evaluated. Lactams with affinity for the EP_4 receptor displayed agonist behavior. The lower side-chain of the lactam template could be substituted to afford ligands (e.g., **17**, **24**, **30**, **31**, and **33**) of high potency and greater than 1000-fold affinity for EP_4 versus the other EP prostanoid receptors.
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Biosynthetic products of arachidonic acid, namely prostaglandin E_2 (**1**, PGE_2), are released and exert an effect(s) at the site where they are produced. PGE_2 is a potent paracrine (tissue) and autocrine (cell) mediator by acting on at least four pharmacologically distinct receptors: the prostanoid EP_1, EP_2, EP_3, and EP_4 receptors[1] which are widely distributed throughout the body.

Suda et al. reported that the EP_4 receptor plays a critical role in bone biology in vitro.[2] A current hypothesis in this arena is that selective stimulation of the EP_4 receptor will lead to preferential effects in bone tissue and spare effects on the cardiovascular, gastrointestinal and reproductive systems and other tissues that are observed by systemic administration of PGE_2 and other non-selective agonists. We sought to identify a chemically simplified class that would possess structure–activity relationship(s) towards EP_4 subtype selectivity. A program was initiated based on our preliminary finding that racemic 8-aza-11-deoxyPGE_1 **2**[3] behaved as an agonist at the EP_4 receptor.[4] The focus of this letter is to describe the preparation and in vitro pharmacological evaluation of some ω-side-chain derivatives of lactam **6** (Fig. 1).

The functional activity of compounds was assessed by a gene reporter assay. Recombinant EP_4 receptor[5] in conjunction with cAMP response element and luciferase[6] were stably transfected in CHO cells. Table 1 displays the activity for all four stereoisomers of 8-aza-11-deoxyPGE_1 skeleton that were prepared discretely as previously described.[7] Lactam **6** was the most potent agonist of the four isomers and it was not surprising to find that it bears the natural configurations (12*R*, 15*S*) of **1**.

To access new compounds, alcohol **7**[7] served as a key intermediate as outlined in Scheme 1. It was oxidized to the corresponding aldehyde under Swern conditions and directly subjected to olefination conditions.[8] The enantiomeric purity of **8** (R′ = *n*-pentyl) was determined by chiral stationary phase HPLC to be 97% ee [t_R 43.8 min with Chiracel® OJ column, eluant: 5% *i*-PrOH in *n*-hexane; $[\alpha]_D^{22}$ −1.1° (*c* 2.0, EtOH), lit. as the methyl

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doi:10.1016/j.bmcl.2004.01.063

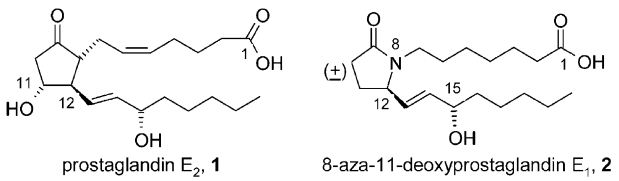
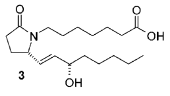
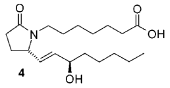
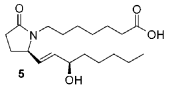
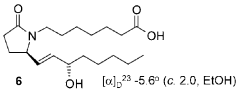


Figure 1. Structures of **1** and **2**.

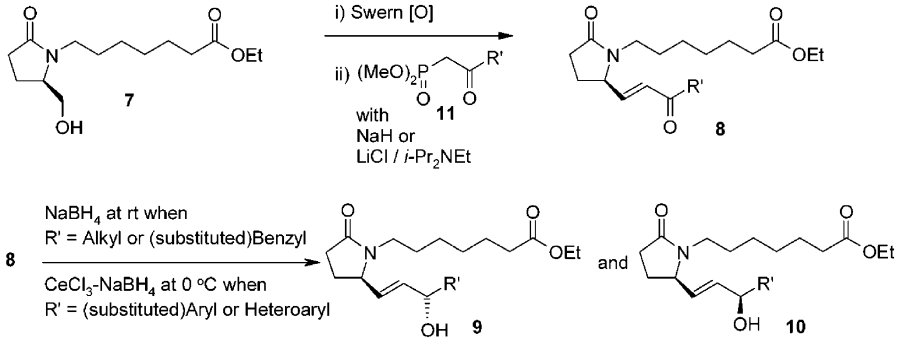
Table 1. EP_4 Prostanoid receptor activity: stereochemical relationships of 8-aza-11-deoxyprostanoids

Absolute configurations	Structure	EC_{50} (nM)[a]
(12*R*,15*S*)	**1**	6.1[b]
(12*S*,15*S*)	 **3**	>100,000
(12*S*,15*R*)	 **4**	200,000
(12*S*,15*R*)[c]	 **5**	6100
(12*S*,15*R*)[d]	 **6** $[\alpha]_D^{23}$ -5.6° (*c* 2.0, EtOH)	56 (12)

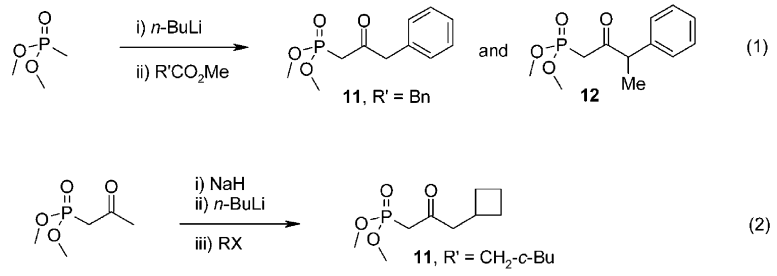
[a] The value is the average of a minimum of three determinations except where noted in parenthesis.
[b] Slipetz, D.; Buchanan, S.; Mackereth, C.; Brewer, N.; Pellow, V.; Hao, C.-m.; Adam, M.; Abramovitz, M.; Metters, K. M. *Biochem. Pharmacol.* **2001**, *62*, 997 reported EC_{50} 0.5 nM for **1** (or 3.1 nM pretreated with 1.0 μM of **1** 30 min prior to assay).
[c] The C-15 dr of **5**:**6** was determined to be 20:1 by HPLC: t_R 5.6 min (Zorbax SB-Phenyl column, eluant: 20% CH_3CN, 0.5% TFA in water for 1.0 min then linear gradient to 45% CH_3CN over 5.0 min).
[d] The C-15 dr of **5**:**6** was determined to be 1:120 t_R 5.3 min.

ester $[\alpha]_D^{29}$ −0.9° (*c* 2.0, EtOH)[7]]. Alcohols **9** and **10** were produced by hydride-based methods depicted in Scheme 1. The C-15 diastereomers were resolved (>96% dr) by silica gel chromatography as their ethyl esters when R′≠aryl (eluant: 1:1 ethyl acetate:toluene or 10% *i*-PrOH in 1:3 ethyl acetate:hexane). Allylic alcohol **9** (R′≠aryl) was more efficiently prepared by treatment of **8** with 10–20 mol% (*R*)-2-methyloxazaborolidine and 70 mol% BH_3–SMe_2 according to the method of Corey et al.[9] When **8** (R′ = aryl) was treated to these asymmetric conditions, an inseparable mixture of **9** and **10** as well as the corresponding saturated alcohols was obtained. The 13,14-dihydro-15-aryl compounds were synthesized to >95% dr to circumvent this problem and others (vide infra). Hydrogenation of **8** (R′ = aryl) at 1 atm H_2, catalytic Pd-C, EtOAc, 1.5 h, followed by reduction [10 mol% (*S*)-2-methyl-CBS, 70 mol% BH_3-SMe_2, toluene, 0 °C][9] produced the (15*R*) configured benzylic alcohol. The final acids were generated under standard conditions (5 equiv of LiOH or NaOH in aq MeOH, 0 °C to rt).

Scheme 2 displays methods of preparing the β-ketophosphonates **11** that were employed in Scheme 1. The most routine method of preparation is shown in eq 1.[10] We identified an additional product **12**, when R′ = Bn, if this reaction were allowed to warm above −78 °C. Phosphonates **11** and **12** were separated by silica chromatography. The undesired methylated product likely formed by subsequent deprotonation of **11** and then neutral dimethyl methylphosphonate behaved as an electrophilic source of methyl to result in **12**. In some instances, it was more expedient to use an alternative method to prepare **11** when R′ = alkyl, or cycloalkylalkyl eq 2.[11]

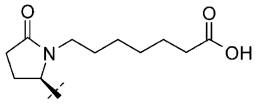


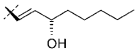
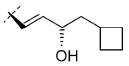
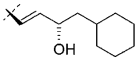
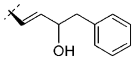
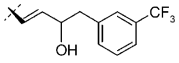
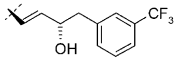
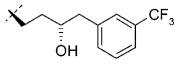
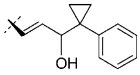
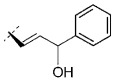
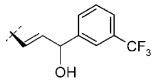
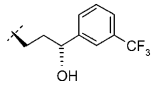
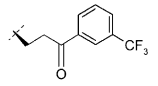
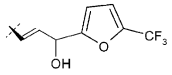
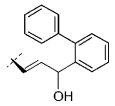
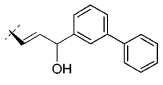
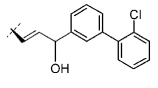
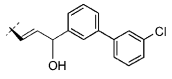
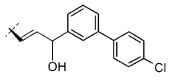
Scheme 1. General scheme for elaboration of lactams.



Scheme 2. Preparation of β-ketophosphonates.

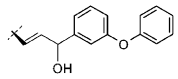
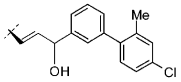
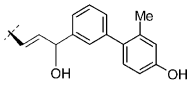
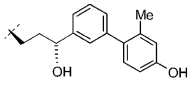
Table 2. E-Type prostanoid receptor profile of selected lower side chain (12*R*)-8-Aza-11-deoxy-prostaglandins



Compound	Structure	Binding affinity, K_i (nM)[a]				Activity, EC_{50} (nM)[a] hEP_4
		EP_1	EP_2	EP_3	EP_4	
1	**1**	4.5 (2)	6.4 (6)	8.0	0.8 (27)	6.1
6		> 100,000 (2)	4500	3000 (18)	4.5 (6)	56 (12)
13		nd[b]	> 100,000	56,000	4.2	200 (6)
14		nd	71,000	16,000 (6)	7.5	120
15		nd	31,000	100,000 (6)	6.1	140 (6)
16		> 100,000 (2)	nd	12,000 (6)	nd	17 (9)
17		> 100,000 (2)	29,000	13,000	1.6 (6)	12 (15)
18		nd	22,000 (6)	38,000 (6)	16	460 (9)
19		28,000 (2)	164,000	20,000 (6)	12 (6)	180 (6)
20		nd	41,000	nd	2,300	> 10,000
21		nd	68,000	131,000	140	500 (9)
22		nd	nd	12,000	7.4	130 (6)
23		nd	nd	nd	nd	> 10,000
24		nd	58,000	4,900	10	40
25		nd	nd	nd	nd	42,000 (9)
26		nd	32,000	> 100,000	360	360 (6)
27		nd	42,000	> 100,000	6.2	40 (6)
28		nd	85,000	> 100,000	420 (6)	170 (6)
29		nd	4200	> 100,000	390 (9)	50 (6)

(*continued on next page*)

Table 2 (*continued*)

Compound	Structure	Binding affinity, K_i (nM)[a]				Activity, EC_{50} (nM)[a] hEP_4
		EP_1	EP_2	EP_3	EP_4	
30		50,000 (2)	nd	>100,000	32	85 (9)
31		nd	>100,000	38,000	3.1	0.8 (6)
32		nd	>100,000	>100,000	4.9	13 (6)
33		nd	26,000	>100,000	8.2	15 (6)

The straight line (at C-15) denotes a dr of 1:1 for the compound.
[a] The value is the average of a minimum of three determinations except where noted in parentheses.
[b] nd = Not determined.

Selected lactams bearing the native PGE_1 α-chain are shown in Table 2. Compounds were studied for their affinity at the EP_1,[12] EP_2,[4b] EP_3,[13] and EP_4[5] receptors following a determination of their activity at the EP_4 (vide supra). The affinity estimates generated for **1** are in agreement with published reports[4] and the lactam **6** was found to confer subtype selectivity for EP_4. Data from compounds **6**, and **13**–**19** illuminated a SAR aligned with a published report.[14a] Data for **16** (1:1 dr at C-15) and **17** [>96% dr and assigned (15*S*) based on precedence[9]] demonstrated that the greater potency at EP_4 resides with the naturally configured isomer. This finding was also recently reported by Billot et al.[15] The 13,14-dihydro- **18** and the 15-keto- **24** compounds are less potent at EP_4 and are reflections of historical findings for PGs.

In order to increase activity at EP_4, the 15-phenyl compounds were elaborated and activity was found to be more reliant on *meta*- substitution. A comparison of the pairs **15** and **16** versus **20** and **21** demonstrate this finding (Table 2). Compound **24** displayed a favorable profile but was found to be acid sensitive. It degraded approximately 75% at pH 2 at 40 °C over 3 days as compared to **21** and **22** which suffered no measurable loss under the same conditions. Investigation of substituents of **20** produced the following: *meta*-phenyl- (e.g., **26**) or-phenoxy-substitution (e.g., **30**) restored EP_4 activity. Furthermore, a small 2′-substituent of the biphenyl system (e.g., **27**) was optimum for EP_4 affinity and activity. It was also found that the presentation of a 13,14-single bond and the (15*R*)-configured hydroxyl restored high potency (e.g., **22** and **33**) versus their more rigid counterparts (**21** and **32**, respectively). The 2′,4′-disubstituted biphenyls **31** and **32** could be substituted with a distal (4′-) hydrophobic or polar and potentially ionizable moiety and retain high subtype selectivity and excellent activity at the EP_4 receptor.

In summary, 8-aza-11-deoxy-(12*R*)-PGE_1 derivatives can be prepared in high diastereomeric purity. The 1-heptanoic-2-pyrrolidinone template was utilized to generate agonists of nanomolar potency at the EP_4 receptor. This class presents structure–activity relationships in both the 15- and 16-phenyl series. Ligands with ω-chain *meta*-biphenyl or *meta*-phenoxyphenyl substitution can display greater than 1000-fold subtype selectivity for EP_4, as determined by affinity measurements. The 15-biphenyl subclass allows for the removal of the metabolically labile 13,14-unsaturation and flexibility of the nature of the distal substituent.

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8. Most enones of this letter were prepared according to: Blanchette, M. A.; Choy, W.; Davis, J. T.; Essenfeld, A. P.; Masamune, S.; Roush, W. R.; Sakai, T. *Tetrahedron Lett.* **1984**, *25*, 2183 Preparation of enone 8, R′ = *n*-pentyl is representative: A-78° CH_2Cl_2 (90 mL) solution of dimethyl sulfoxide (1.27 mL, 16.5 mmol) was treated with oxalyl chloride (2 M CH_2Cl_2, 7.2 mL, 14.4 mmol),

drop wise, and stirred for 15 min. Alcohol 7 (2.8 g, 10.4 mmol) was dissolved in CH_2Cl_2 (5 mL) and added to the -78° solution of activated DMSO and stirred for an additional 15 min. At which time, Et_3N was added rapidly and the resulting suspension was allowed to warm to rt before being poured into a separatory funnel containing aqueous $NaHCO_3$ and CH_2Cl_2. The combined organic extract was dried over Na_2SO_4, filtered, and the volatiles were removed. ^1H NMR analysis of the crude aldehyde (3.1 g) suggested the purity was 90% which was immediately treated with CH_3CN (15 mL), LiCl (385 mg, 9.1 mmol), and dimethyl 2-oxoheptylphosphonate (1.0 equiv, 1.72 mL, 8.3 mmol) at rt. The suspension was treated with iPr_2NEt (1.5 mL, 8.7 mmol) and stirred at rt for 17 h. The enone 8 (1.66 g, 4.55 mmol) was isolated by extraction (H_2O and EtOAc 4 × 50 mL), dried over Na_2SO_4, and subjected to SGC (eluant: 1:1 hexane:EtOAc): $[\alpha]_D^{22}$ -1.1° (c 2.0, EtOH), lit. as the methyl ester $[a]_D^{29}$ -0.9° (c 2.0, EtOH).[7]

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