Lactams as EP_4 Prostanoid Receptor Agonists. 3. Discovery of *N*-Ethylbenzoic Acid 2-Pyrrolidinones as Subtype Selective Agents

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Received August 30, 2004

Abstract: Two distinct synthetic schemes were applied to access heteroatom-containing α-chain lactams or lactams terminated as aryl acids. The latter lactams were devised using a pharmacophore for EP_4 receptor activity. γ-Lactams were characterized for their prostanoid EP receptor affinities and EP_4 activity and found to be selective for the EP_2 and EP_4 receptors or selective for the EP_4 subtype. Benzoic acid **17** displayed enhanced in vivo exposure relative to **3**.

Osteoporosis is the loss of bone mass due to an imbalance in the resorption and the remodeling processes. The onset of this imbalance can lead to an increased risk of fracture which may be anticipated for both women and men after age 50. Postmenopausal women in particular can present bone loss and thus have been of primary interest for effective drug therapy. The inhibition of bone resorption has been the established therapy with agents such as bisphosphonates or estrogen and estrogen-related compounds.[1] While stabilizing existing bone mass at best, the antiresorptives do not give rise to a net increase in bone.

Intermittently dosed parathyroid hormone[2] and related peptides[3] have been reported to stimulate bone formation.

Body and colleagues have recently reported a head-to-head comparison of the effectiveness of either approach. The bisphosphonate alendronate (Fosamax, 10 mg/day, oral) or recombinant human parathyroid hormone (Forteo, 40 μg/day, s.c.) was dosed for 14 months to postmenopausal women with established osteoporosis. The Forteo treated group responded more favorably in increased bone mineral density and more importantly in reduction of fracture rate.[4] While the bone anabolic approach is clinically advantageous for individuals at risk of fracture, the peptidic nature of Forteo requires parenteral administration.

The potential to discover an orally active bone-forming agent was instilled by the reports that prostaglandin (PG) E_2 **1** and misoprostol **2** (Figure 1) promote bone growth in mammals.[5,6] The interest in selectively activating a single prostanoid receptor subtype is borne out of the observations that **1** and **2** (i.e., nonselective agonists) exert many physiological effects in human.[6a,7] It was hoped that by selective stimulation of only the EP_4 receptor,[8] most of the undesirable effects might be avoided.

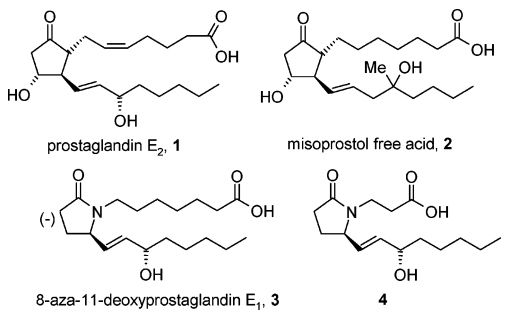

Figure 1. Structures of **1**−**4**.

We have previously described a functional assay for the EP_4 receptor and the optimization of the ω-chain of **3** toward high EP_4 subtype selectivity.[9a] We next sought to improve the in vivo half-life of active and selective EP_4 agonists.[9b] The focus of this report is to outline some design criteria and syntheses and to describe selected in vitro pharmacologic and pharmacokinetic data of lactams related to **3**.

Cyclopentane prostaglandins having an aliphatic α-side chain are known to be metabolized by β-oxidation.[7] An abbreviated pharmacokinetic analysis of lactam **3** was conducted in rat (Table 2, vide infra). The products of β-oxidation and 13,14-reduction of **3** were identified in circulation. In addition, the β-oxidation product **4** was found upon sampling plasma from the portal vein following oral dosing of **3** at 3 mpk. This latter finding pointed to the high lability of heptanoic acids to extrahepatic based metabolism, thus severely eroding the presence of active parent in circulation.

One approach to stabilize ligands toward β-oxidation is to prepare lactams containing a heteroatom in the α-chain. Following some experimentation, lactam **5**[10] was employed to produce thioether ligands via TIPS ether **6** as shown in Scheme 1. Diol **7** was converted to desired α-chain materials without resorting to 2° hydroxyl protection. The structures of the esters leading to acids **8** and **9** were supported by 2D NMR. The regiochemistry of mesylation of **7** was confirmed because alkylation of **5** with methyl 7-chloro-5-thiaheptanoate furnished the same thioether ligand. To produce 3-oxa derivatives (see Table 2), lactam **5** was alkylated with ethyl 7-bromo-3-oxaheptanoate[11] to produce **10** and **11** according to precedence.[9a]

Another approach to obviate the β-oxidation process is to prepare lactams bearing an aromatic acid. Interphenylene analogues of prostaglandins are known in which the double bond (e.g., PGE_2) is replaced in the α-chain.[12] However, *m*-phenylenealkyl acids were found to be inactive at the EP_4.[9b] To our knowledge, Tani and colleagues were the first to report a benzoic acid as a potent prostaglandin agonist (as a butaprost derivative selective for the mouse EP_2 subtype).[13] The benzoic acid prototype **12** was prepared according to *N*-alkylation

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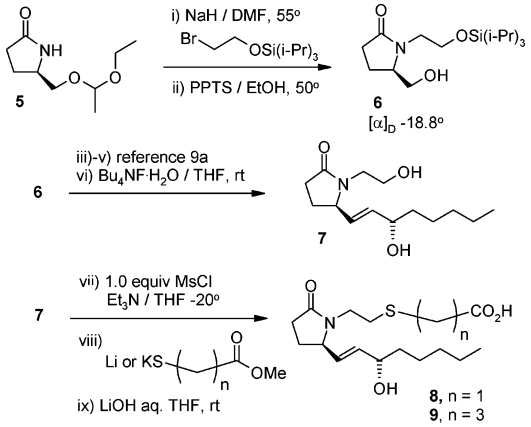
10.1021/jm049290a CCC: $27.50
Published on Web 11/10/2004

Scheme 1. Preparation 8-Aza-5-thia-11-deoxyprostaglandins



chemistry shown in Scheme 1 using ethyl 3-(3-bromopropyl)benzoate. Acid **12** displayed measurable affinity at the target receptor (Table 1), and thus we elected to pursue related aryl acids using pharmacophore searching.

The α-chain conformers of **1**, **3**, and **9** were sampled via a Monte Carlo simulation using the Schrödinger software to identify a predictive model of activity for the EP_4 receptor. The lower side-chain features were simplified to a small hydrophobe ($-CH{=}CH_2$) and thus approximated not to perturb the upper chain conformations.

Figure 2 displays an α-chain pharmacophore that was generated using Catalyst software based on the conformers of **1**, **3**, and **9**. The carbonyl oxygen of the lactam and the carboxylate are highlighted with a distance of 8.6 Å. A number of candidate structures varying linker and benzoic acid isomers were analyzed with the model. *A minimum chain length of two methylenes was predicted by this model to place the carboxylate of the p-benzoic acid to populate the conformers readily accessible to **1**, **3**, and **9**.* A pharmacophore overlay of one predicted active α-chain conformation of **9** and the proposed benzoic acid **16** is shown in Figure 2.

Not surprisingly, the treatment of the salt of **5** with an electrophile to generate the desired phenethyl ester was problematic. *N*-Ethyl-linked benzoic acids were prepared in a highly optically enriched form as shown in Scheme 2. The hydroxyl of (*S*)-5-hydroxymethylbutyrolactone was protected as the benzyl ether, and the lactone was condensed with 4-(2-aminoethyl)benzoic acid. The amidoalcohol **14** was sequentially treated with a single equivalent of MsCl and excess *tert*-butoxide to generate the core lactam.[14] The crystalline alcohol **15** resulted upon hydrogenolysis.

Hydroxymethyl lactam **15** served as the common intermediate for all the ethyl-linked benzoic acid lactams in this report. The synthesis of benzoic acid **22** is typical for this class and is exemplified in Scheme 2. The completion of **16**, **18** [from methyl 5-(2-bromoethyl)-thiophene 2-carboxylate],[15] **19**, and **21** is analogous to results from our earlier report.[9a] Saturated ω-chain ligands **17** and **20** were produced from the esters, leading to **16** and **19**, respectively, by reduction (1 atm of H_2, 10% Pd−C, alcohol, 2−3 h) prior to saponification.

Pharmacologic data are presented in Table 1 for compounds containing an aryl feature in the α-chain.

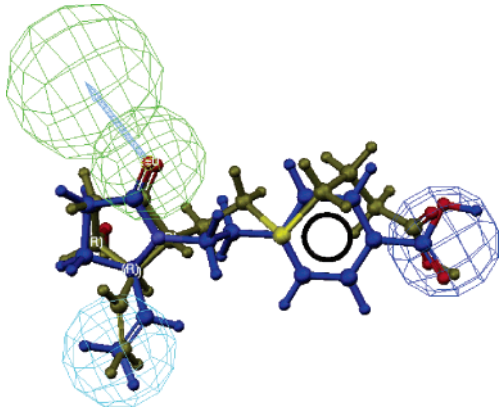
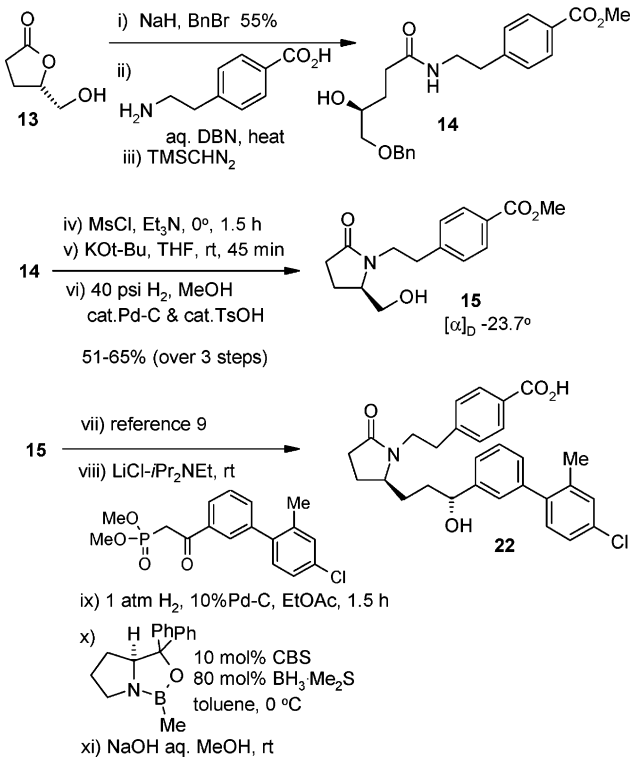


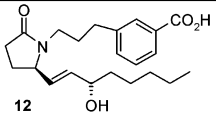
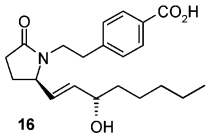
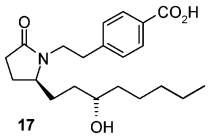
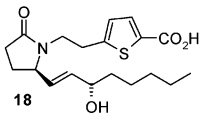
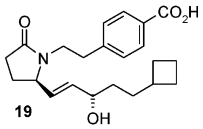
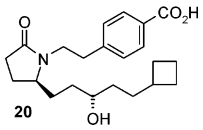
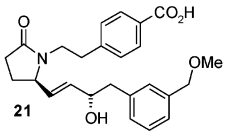
Figure 2. Predicted active conformation of **9** (brown-yellow) and **16** (blue) using a model of EP_4 activity. Features are negative ionizable (blue), hydrogen bond acceptor (green), hydrophobe (cyan).

Scheme 2. Syntheses of Alcohol **15** and Benzoic Acid **22**



Ligands were first assessed for their functional activity at the human EP_4 receptor and then profiled for their affinity to the hEP subtypes[9a] as warranted. The data suggest that the extended carboxylate conformers (to the lactam ring) presented by propyl-linked benzoate **12** do not stimulate the EP_4 receptor. The α-chain presented by the ethyl-linked benzoate **16** and thiophene 2-carboxylate **18** confers high potency at the EP_4 subtype, with the latter showing good subtype selectivity. However, **16** and the ω-cyclobutyl **19** possess modest subtype selectivity because these compounds show affinity for the EP_2 subtype. This selectivity is further eroded by the increased flexibility presented in the ω-chain of ligands **17** and **20**. The affinity data of **17** and **20** reveal that these ligands are coselective for the EP_2 and EP_4 subtypes. High subtype selectivity for the α-chain benzoates is restored when the ω-chain contains

Table 1. Prostanoid E-Type Receptor Profile of Lactams Bearing an Arene α-Chain

Structure	Binding Affinity, K_i (nM) EP_1	EP_2	EP_3	EP_4	Activity, EC_{50} (nM) EP_4
 **12**	nd[a]	nd	nd	900[b]	>10,000
 **16**	>100,000 (2)	130	11,000	2.9 (6)	11 (9)
 **17**	nd	35 (9)	90,300	17 (12)	130 (6)
 **18**	nd	3,700	>100,000	6.9	12 (9)
 **19**	nd	75 (6)	79,000	1.0 (6)	0.2
 **20**	nd	220 (6)	77,000	18 (6)	150 (6)
 **21**	nd	2,500	>100,000	0.7	5.9 (6)
22	18,000 (2)	5,100	54,000	0.94 (6)	0.04

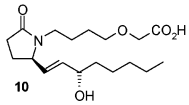
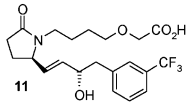
[a] nd= not determined. [b] The value is the average of three determinations except where noted in parentheses.

an appropriately substituted benzyl (e.g., **21**) or biphenyl (e.g., **22**) as observed earlier.[9a] The substituted benzyl feature of **21** was reported by Maruyama and colleagues (as a PGE_1 derivative) to impart high murine EP_4 subtype selectivity.[16]

The lactam prostanoids appear to undergo in vivo metabolism analogous to their cyclopentanone counterparts. The pharmacokinetics data of Table 2 show that lactam **3** is subject to rapid degradation. Of note, the i.v. clearance rate of **3** was measured to be faster (~150%) than hepatic blood flow (HBF). The products of both β-oxidation and 13,14-reduction of **3** were identified in circulation. The 3-oxa acid **10** displayed a marked decrease in clearance rate (~40% of HBF) compared to acid **3** but with no improvement in $t_{1/2}$. This can be clarified by noting their difference in volume of distribution (i.e., V_β for **3** was 6.9 L/kg and **10** was 1.5 L/kg).

Further insight was gained upon assessing the extent of protein binding of the two lactams. Ligand **3** displayed much higher plasma protein binding than **10**. The potency of 3-oxa ligands could be improved but at the expense of in vivo exposure (e.g., **11** presents CL at 60% of HBF). The benzoic acids **16** and **17** are moderate to high-clearance compounds with i.v. rates estimated at 65−80% HBF. Finally, the saturated ω-chain ligand **17** displayed a prolonged $t_{1/2}$ mainly due to its high V_β of 15.3 L/kg.

Table 2. Select Data of Modified Lactam Prostanoids

Structure	hEP4 receptor EC_{50} (K_i)[a]	Rat Pharmacokinetics[b] CL (L/kg/h)	$t_{1/2}$ (h)
3	56 (4.5)	5.5	0.87
8	10,700 (530)	nd[c]	nd
9	17 (3.8)[d]	0.63	0.76
 **10**	2,100 (nd)	1.4	0.75
 **11**	180 (7)	2.2	0.56[e]
16	11 (2.9)	2.5	0.78[e]
17	130 (17)	3	3.5

[a] The value is the average of at least three determinations expressed in nanomolar. [b] The average value following i.v. dosing with 1.0 mg/kg of lactam and the parent compound quantified with LC-MS/MS and detected to 0.1 ng/mL. [c] nd = not determined. [d] Additional binding data (K_i) for **9**: EP_1 nd; EP_2 4700 nM; EP_3 1900 nM. [e] Estimate due to nonlinear terminal phase.

The introduction of an ether or a thioether feature in the α-chain of 8-aza-11-deoxyPGE$_1$ leads to compounds of slower clearance from the circulation of rat. The thioether **9** presents favorable EP$_4$ receptor activity. However, it poses a short $t_{1/2}$ and it primarily degrades to the poorly active dinor acid **8** in vivo (rat).[9b] Ethers **10** and **11** displayed disappointing in vivo $t_{1/2}$ compared to the $t_{1/2}$ of **3**. The extent of protein binding for **10** was estimated to be much less than **3**, therefore decreasing V_β and having a deleterious effect toward the in vivo $t_{1/2}$ of **10**.

In summary, aryl acids can be prepared with two-carbon linkage to the nitrogen of the 2-pyrrolidinone template to produce ligands of mixed subtype selectivity for the EP$_2$ and EP$_4$ prostanoid receptors or greater than 500-fold selectivity for the EP$_4$ receptor as judged by affinity measurements. Significantly, benzoic acid **16** was predicted to be active at the EP$_4$ subtype based on a pharmacophore model. This differs from the classical modified PG upper side chains prior to identification of the EP$_4$ receptor. Acid **16** displays an improved i.v. clearance rate compared to **3**, and the "13,14-dihydro ligand" **17** illustrates the effect of the ω-chain alkene on in vivo PG metabolism.

11-Deoxylactam prostanoids undergo prostaglandin-like metabolism in vivo. The processes of β-oxidation (in mitochondria), 15-dehydrogenation (likely in the lung), and 13,14-reduction have all been observed for lactam **3**.

Acknowledgment. We are grateful to Dr. J. M. Muchowski, Dr. E. B. Sjogren, and Prof. E. J. Corey for their insights and to Dr. N. Illangasekare for his NMR expertise. We are also grateful to the staff of Analytical Chemistry and Laboratory Animal Technology departments at Roche Palo Alto for their assistance in characterizing the compounds described herein.

Supporting Information Available: Detailed experimental information for the compounds prepared according Schemes 1 and 2 and for the protocol for rat pharmacokinetic analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM049290A