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Discovery of highly selective EP4 receptor agonists that stimulate new bone formation and restore bone mass in ovariectomized rats

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Abstract—Heptanoic acid lactams, exemplified by **2**, were identified as highly selective EP4 agonists via high throughput screening. Lead optimization led to the identification of lactams with a 30-fold increase in EP4 potency in vitro. Compounds demonstrated robust bone anabolic effects when administered in vivo in rat models of osteoporosis.

Prostaglandin E_2 (PGE_2, **1**, Fig. 1) is an endogenous ligand with high affinity to four receptor subtypes designated EP1–EP4. PGE_2 is a local mediator of a number of physiological responses including analgesia, smooth muscle relaxation, and vasodilation.[1] In addition, administration of PGE_2 in animals stimulates new bone formation and increases bone mass and strength.[2] There is significant interest in the discovery of a therapeutic agent that can form new bone and therefore treat patients with established osteoporosis; however, side effects (diarrhea, hypotension) preclude the use of PGE_2 for this purpose. Previous reports suggest that agonism of the EP4 receptor is important for mediating PGE_2's bone anabolic effects.[3] We describe our efforts to identify potent and EP4-selective compounds in a lactam series.[4,5]

Directed high throughput screening led to the identification of our lead heptanoic acid lactam **2a** (Fig. 1), a selective EP4 agonist with good in vitro potency (see Table 1). The pharmacokinetic properties of **2a** were benchmarked in the Sprague–Dawley rat. Lactam **2** exhibits low bioavailability (approximately 20%), high iv clearance (101 mL/min/kg), and a short half-life (0.1 h). SAR goals were to improve EP4 potency and oral bioavailability. Homolog **3** and truncated phenyl derivative **4** (Fig. 2) had reduced EP4 potency (see Table 1). We therefore focused chemistry efforts on the synthesis of benzyl analogs and explored phenyl substituent effects on EP4 potency. In addition, we hypothesized that β-oxidation of the heptanoic acid side chain, a significant clearance pathway for PGE_2,[6] may contribute to the high clearance observed with **2a**. A survey of a variety of heptanoic acid replacements led to the identification of propyl thiophene and propyl benzene carboxylic acid analogs (**9**–**23**) as potent, EP4-selective compounds. In vitro data,

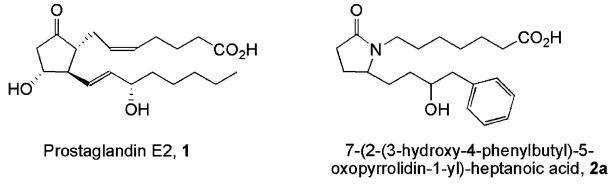

Figure 1. Structures of **1** and **2a**.

Keywords: Bone anabolic agent; EP4; EP2; Prostaglandin; Lactam; Osteoporosis.
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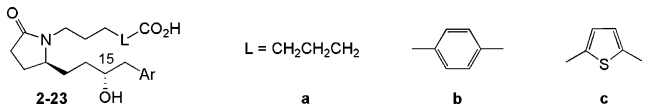
Table 1. In vitro results for selected lactam analogs



Compound	L	Ar	IC_{50} hEP1 (nM)	IC_{50} rEP2 (nM)	IC_{50} hEP3 (nM)	IC_{50} rEP4 (nM)	EC50 rEP4 nM (% vs PGE_2)
2a	a	Ph[b]	>3200	>3200	>3200	54 ± 31	32.5 ± 34 (114)
2b	b	Ph[a]	>3200	>3200	>3200	250 ± 184	
2c	c	Ph[b]		690 ± 156		21 ± 4	1.7 ± 0.2 (88)
3	a			>3200		>3200	
4	a	CH_2Ph[a]		>3200		2705 ± 700	
9c	c	2-Naphthyl[b]		>3200		65 ± 38	500 ± 28 (73)
10b	b	4-Fluorophenyl[a]	>3200	>3200	>3200	2767 ± 379	
10c	c	4-Fluorophenyl		1205 ± 64		70 ± 36	22 ± 14 (70)
11b	b	3-Fluorophenyl[a]		>3200		126 ± 48	
11c	c	3-Fluorophenyl[b]		640 ± 71		17 ± 7	2.4 ± 2 (97)
12c	c	4-Chlorophenyl[b]	>3200	86 ± 44	>3200	80 ± 64	40 ± 24 (90)
13a	a	3-Chlorophenyl[a]		>3200		22 ± 3	8.8 ± 6.7 (89)
13b	b	3-Chlorophenyl[a]	>3200	>3200	>3200	42 ± 35	425 ± 35 (106)
13c	c	3-Chlorophenyl		188 ± 109		1.1 ± 1.1	0.6 ± 0.7 (70)
14c	c	2-Biphenyl[b]		125 ± 35		1600 ± 566	
15a	a	3-Biphenyl		>3200		337 ± 154	195 ± 106 (101)
15b	b	3-Biphenyl[a]		>3200		47 ± 43	440 ± 260 (97)
15c	c	3-Biphenyl[b]	18,550	135 ± 49	20,000	50 ± 11	203 ± 138 (95)
16a	a	3-Trifluoromethylphenyl		>3200		21 ± 11	13 ± 13 (75)
16b	b	3-Trifluoromethylphenyl		>3200		70 ± 27	105 ± 62 (105)
16c	c	3-Trifluoromethylphenyl		483 ± 237		6 ± 1	0.5 ± 0.7 (102)
17a	a	3-Cyanophenyl		>3200		388 ± 134	96 ± 19 (104)
18a	a	3-Hydroxyphenyl		>3200		1202 ± 436	170 ± 28 (100)
19a	a	3-Methoxymethylphenyl	>3200	>3200	>2000	62 ± 50	18 ± 25 (89)
20a	a	3-Methoxyethylphenyl		>3200		210 ± 134	92 ± 96 (82)
21a	a	3-Trifluoromethoxy-phenyl		>3200		59 ± 37	50 ± 3 (78)
22a	a	3-Phenoxyphenyl[b]	>3200	>3200	>3200	536 ± 52	
22b	b	3-Phenoxyphenyl[a]		>3200		220 ± 127	
23c	c	4-Ethylphenyl[b]		1200 ± 283		140 ± 42	275 ± 49 (82)

Data are presented ±SD and are the means of at least two independent measurements.
[a] Mixture of isomers at C-12 and C-15.
[b] Mixture of isomers at C-15 alcohol.

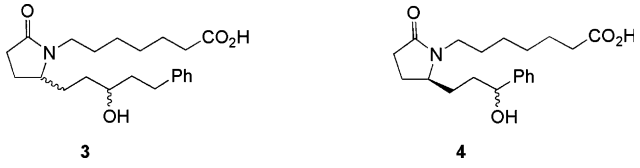
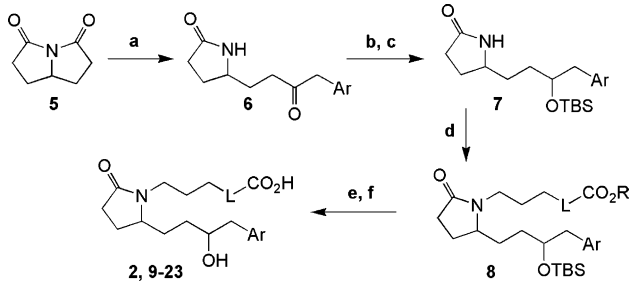


Figure 2. Structures of **3** and **4**.

pharmacokinetic properties as well as our in vivo results for selected analogs are presented.

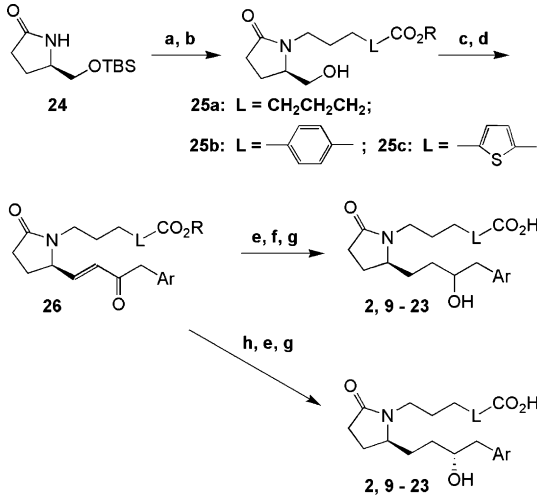
The synthesis of the requisite lactams as racemates is described in Scheme 1. Addition of the appropriate Grignard reagent into tetrahydro-6*H*-pyrrolizine-3,5-dione (**5**) provided benzylic ketone **6**.[7] Reduction of the ketone, followed by TBS protection of the resulting alcohol, generated lactam **7** as an approximate 1:1 mixture of diastereomers. N-Alkylation, followed by saponification, provided the desired lactam acids (**2**, **9**–**23**).

The chiral alcohols **25a**, **b**, and **c** served as key intermediates in the enantioselective synthesis of lactam analogs

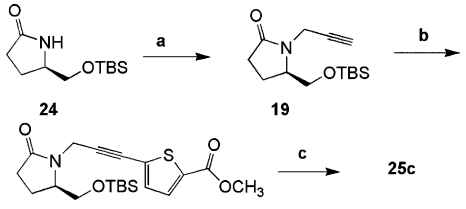


Scheme 1. Reagents and conditions: (a) $ArCH_2MgX$, CH_2Cl_2, 0 °C; (b) $NaBH_4$, EtOH, 0 °C; (c) TBSCl, imidazole, DMAP, DMF; (d) NaHMDS, DMF, RX; (e) TBAF, THF; (f) NaOH, MeOH.

(Scheme 2).[8] Alkylation of intermediate **24**[9] with either methyl 7-bromoheptanoate or methyl 4-(3-bromopropyl)benzoate[10] followed by TBS deprotection provided **25a** or **25b**, respectively. Intermediate **25c** was prepared as described in Scheme 3. Conversion to the enone **26** was accomplished using modified Horner–Emmons conditions. In some cases, **26** was hydrogenated, reduced with sodium borohydride, and deprotected to generate



Scheme 2. Reagents and condition: (a) NaHMDS, DMF, RBr; (b) 1 N HCl, MeOH; (c) EDC, DMSO, pyridinium trifluoroacetate, benzene; (d) NaH, $ArCH_2PO(OR)_2$; (e) H_2, Pd/C; (f) $NaBH_4$, EtOH; (g) NaOH, MeOH; (h) (*R*)-2-methyl-CBS-oxazaborolidine, catecholborane, CH_2Cl_2, −45 °C.



Scheme 3. Reagents and condition: (a) DMF, NaHMDS, propargyl bromide, 0 °C; (b) Methyl 5-bromothiophene-2-carboxylate, Et_3N, $Pd(PPh_3)_4$, CuI, CH_3CN; (c) H_2, Pd/C.

a diastereomeric mixture of alcohols for in vitro testing. Diastereo-enriched allylic alcohol was prepared via reduction with (*R*)-2-methyl-CBS-oxazaborolidine and catecholborane at −45 °C.[11] Subsequent hydrogenation and saponification provided the desired lactams (**2**, **9**–**23**).

Compounds were tested for binding at the EP receptors in HEK-293 cells, which were stably expressed. Analogs with good EP4 binding potency were assessed for their functional activity by measuring cAMP levels in HEK-293 cells expressing the rEP4 receptor. Unless otherwise stated, the lactam analogs were full agonists as compared to PGE_2 in the cAMP assay. In vitro data are shown in Table 1.

The lactam analogs displayed excellent selectivity for the rEP4 receptor versus EP1 and EP3. Typically, addition of a small substituent on the meta position of the phenyl ring maintained or enhanced EP4 potency, whereas para substitution had a slight detrimental effect on potency. The thiophene acid side chains ('**c**' compounds in Table 1) generally had reduced rEP4 to rEP2 binding selectivity as compared to the heptanoic acid and benzoic acid side chains. However, enhanced EP4 functional potency was achieved with the thiophene acid top chain.

A summary of pharmacokinetic parameters of selected lactam derivatives in the Sprague–Dawley rat is shown in Table 2. Compounds are generally characterized as having high clearance, low volume of distribution, and low oral bioavailability. Replacement of the heptanoic acid with a propyl thiophene did little to reduce clearance. The reduced oral exposure of **16c** relative to **2a** and **16a** is presumably due to decreased absorption. Due to poor oral PK, analogs were tested in our in vivo models using a subcutaneous (sc) route of administration.

A sub-set of compounds was tested for bone anabolic activity in an established osteopenic rat model.[12] Female rats (3.5–4 months) were ovariectomized (OVX) and, 5 weeks post-ovariectomy they were dosed daily (sc), with compound for 28 days.[13] No remarkable side effects, such as GI effects, were observed with these subtype selective agents at any dose. Peripheral distal femur metaphysis showed that EP4 receptor selective agonists can restore volumetric bone mineral content and density in osteopenic rats. Selected bone efficacy parameters are provided in Table 3. Administration of EP4 receptor agonists increased serum osteocalcin (a bone formation marker) and bone formation rate (by bone histomorphometric analysis). Compound **16c** showed

Table 2. Pharmacokinetics in Sprague–Dawley rat for selected lactam analogs

Compound	Cl (mL/min kg)	% *F*	Oral $t_{1/2}$ (h)	iv $t_{1/2}$ (h)	sc $t_{1/2}$ (h)	V_d (L/kg)
2a	101	21	0.45	0.1	0.35	0.40
16a	71	17	0.42	0.2	0.43	0.46
16c	70	1	0.87	0.2	0.3	0.49

Table 3. Selected PQCT parameters from distal femoral metaphysis showing EP4 receptor selective agonists increased bone mineral content and density

Compound	Dose (mg/kg)	Total content	Total density	Cortical area	Cortical thickness	Cortical content
8a	30	16.43	23.65	26.03	43.05	30.40
16a	10	15.34	18.73	21.93	34.12	29.78
16a	30	32.53	23.70	66.92	124.19	57.70
16c	1	20.59	22.88	34.31	52.69	35.76
16c	3	23.72	26.36	41.77	62.57	43.27

Values are given as percent increase ($p < 0.01$ for all values) compared with OVX rats treated with vehicle.

comparable efficacy to our initial lead **2a** at 1/30th the dose. These data correlate well with the improved in vitro functional potency of **16c**. In addition, excellent efficacy was achieved with QD dosing of short acting compounds (sc $t_{1/2} \sim 0.3$ h). These data demonstrate the importance of the EP4 receptor in bone formation and in restoring bone lost due to estrogen deficiency.

In conclusion, we have shown that a selective EP4 agonist **2a** provides robust bone anabolism in an osteopenic rat model. Chemical modification led to compound **16c**, which was about 30× more potent in vivo. Selective EP4 receptor agonists have potential as an anabolic therapy for established osteoporotic patients.

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13. A full report on the in vivo data has been accepted for publication (Ke, H. et al. *J. Bone Mineral Res.*).