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Discovery of CP-533536: An EP_2 receptor selective prostaglandin E_2 (PGE_2) agonist that induces local bone formation

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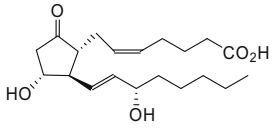
ABSTRACT

Sulfonamides, exemplified by **3a**, were identified as highly selective EP_2 agonists. Lead optimization led to the identification of CP-533536, **7f**, a potent and selective EP_2 agonist. CP-533536 demonstrated the ability to heal fractures when administered locally as a single dose in rat models of fracture healing.

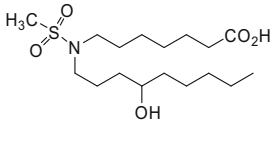
Bone has the unique ability to heal, however factors such as aging, metabolic diseases, and smoking can lead to delayed healing or non-union of fractured bones. The ability to induce and accelerate bone healing in conditions where bone repair has been impaired remains an unmet medical need and would significantly reduce the cost and morbidity associated with osteoporotic fractures. Fracture healing involves a cascade of events in which various growth and differentiation factors have been shown to play a role. Prostaglandin E_2 (PGE_2), is known to increase bone formation in humans and animals when dosed systemically and enhance bone formation and healing in animals when dosed locally.[1] The pharmacological activity of PGE_2 results from its action on four g-protein coupled cell surface receptor subtypes, EP_1, EP_2, EP_3 and EP_4. Of the four PGE_2 receptor subtypes, EP_2 and EP_4 have been envisaged as the two receptors most likely to be responsible for PGE_2's actions on bone.[2] We sought a non-prostanoid, subtype selective PGE_2 agonist that would induce local bone formation, and would be better tolerated than previously studied non-selective prostanoids. We recently reported our discovery of EP_2 and EP_4 selective agonists and their ability to restore bone locally as well as systemically in an osteopenic rat model.[3] Herein, we report the SAR which led to the discovery of potent and selective EP_2 agonists and their ability to induce local bone healing.[4]

Acyclic prostanoid **2** (Fig. 1), reported previously,[5] was characterized in our labs as a weak, but selective EP_2 agonist. Preparation of the benzylic alcohol derivative, **3a** (Table 1), provided improved EP_2 potency and maintained selectivity. Sulfonamide **3a** was locally injected into the bone marrow of the proximal tibial metaphysis of 6-week-old male rats.[6] A single injection dose-dependently increased new bone formation in the injected site of the marrow cavity, thus supporting the hypothesis that EP_2 agonists can promote bone formation locally. Medicinal chemistry efforts were therefore directed towards improving potency while maintaining selectivity. Results of our SAR efforts which led to the identification of our clinical candidate are reported herein.

A range of aliphatic bottom chains (3-octanol replacements), acid linkers (heptanoic acid replacements), and alkyl and aryl sulfonamide caps were explored. A variety of approaches were em-



Prostaglandin E2, **1**
rEP_2 IC_{50} = 5.2 nM, EC_{50} = 0.2 nM
rEP_4 IC_{50} = 2.1 nM, EC_{50} = 0.7 nM



2
rEP_2 IC_{50} = 225 nM, EC_{50} = 134 nM
rEP_4 IC_{50} = 2533 nM

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

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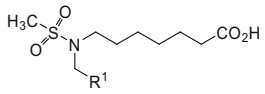
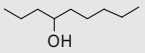
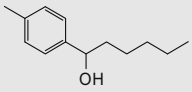
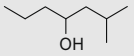
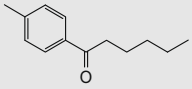
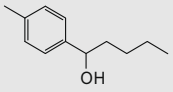
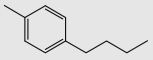
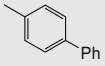
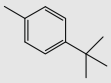
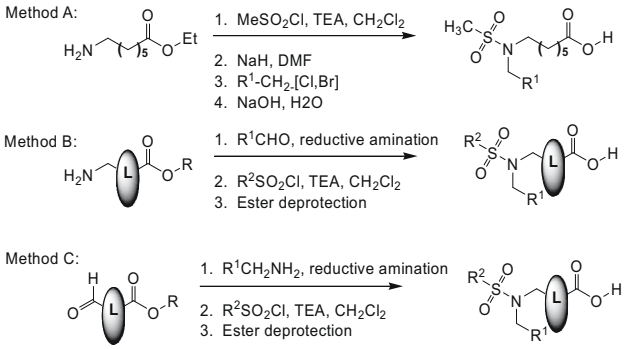
Table 1
In vitro results for methyl sulfonamide heptanoic acid analogs.



Compound	R^1	IC_{50} rEP_2 (nm)	IC_{50} rEP_4 (nm)	EC_{50} (cAMP) rEP_2 (nm)
2		225	2533	134
3a		93	>3200	44
3b		2503	>3200	NT
3c		636	>3200	887
3d		502	>3200	423
3e		160	>3200	94
3f		289	>3200	457
3g		590	2467	NT



Scheme 1. General methods for the preparation of sulfonamides.

ployed to access these analogs[4a] and general methods are provided in Scheme 1. For the preparation of heptanoic acids, Method A was followed. Formation of the methyl sulfonamide by treatment of ethyl 7-aminoheptanoate with methane sulfonyl chloride, alkylation with the appropriate alkyl halide and subsequent saponification provided the desired sulfonamide acids (Table 1). A similar approach was employed for the preparation of compounds in Fig. 2. For compounds where a linker was incorporated into the alkyl chain, either Method B or C was followed depending on availability of the amine or aldehyde starting intermediates. In either case, reductive amination, sulfonamide formation, and ester deprotection provided analogs depicted in Tables 2 and 3.

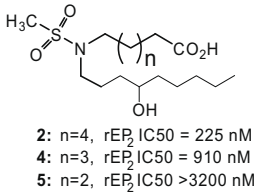


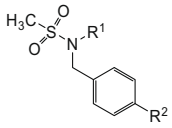
Figure 2. In vitro potency of truncated acids.

Compounds prepared were tested at the rat EP_2 and EP_4 receptor subtypes for their ability to bind and to stimulate release of cAMP and data is presented in Tables 1–3.[7] The in vitro numbers reflect the average of at least *n* of three determinations. All compounds with functional activity were full agonists as compared to PGE_2 in the cAMP assay. In addition to EP_2 potency and selectivity, our objectives for a fracture healing agent included single dose delivery to the fracture site. We also desired a compound that would be highly cleared from the systemic circulation if leakage from the site occurred.

SAR studies on the bottom chain (Table 1) demonstrated that the benzylic alcohol was not required for binding or functional activity. A variety of alkyl and aryl groups could be incorporated without substantial loss in binding potency or selectivity versus EP_4.

Changes to the heptanoic acid side-chain were explored for potency improvements. Truncation of the 6-carbon linker provided significant loss in activity (Fig. 2). Incorporation of a heteroatom into the 6-carbon linker also led to significant loss in binding activity (**6a**, Table 2). Based on these data, a series of analogs was pre-

Table 2
In vitro results for methyl sulfonamide analogs



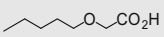
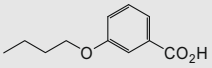
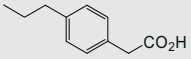
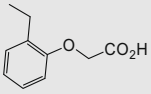
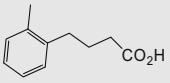
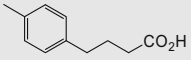
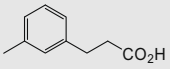
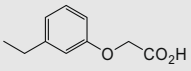
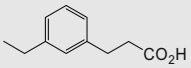
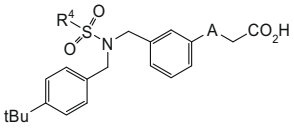
Compound	R^1	R^2	IC_{50} rEP_2 (nM)	IC_{50} rEP_4 (nM)	EC_{50} (cAMP) rEP_2 (nM)
6a		*n*-Butyl	701	>3200	407
6b		*n*-Butyl	3127	>3200	NT
6c		*n*-Butyl	833	>3200	413
6d		*n*-Butyl	>3200	>3200	NT
6e		*n*-Butyl	494	>3200	102.7
6f		*n*-Butyl	654	>3200	587
6g		*n*-Butyl	>3200	>3200	NT
6h		*t*-Butyl	792	>3200	203
6i		*n*-Butyl	270	>3200	34.4
6j		*t*-Butyl	379	>3200	48

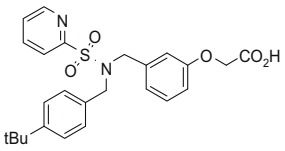
Table 3
In vitro results for aryl sulfonamide analogs



Compound	R^4	A	IC_{50} rEP_2 (nM)	IC_{50} rEP_4 (nM)	EC_{50} (cAMP) rEP_2 (nM)
7a	Phenyl	CH_2	278	>3200	0.2
7b	2-Pyridyl	CH_2	52	>3200	2.3
7c	1-Methylimidazolyl	0	326	>3200	58
7d	4-Chlorophenyl	CH_2	>3200	>3200	NT
7e	2-Thiazolyl	CH_2	63	>3200	1.1
7f CP-533536	3-Pyridyl	0	50	>3200	0.3

pared where a phenyl linker was incorporated into the chain with the goal of exploring various vectors while trying to maintain the appropriate chain length (Table 2). More promising linkers from Table 2 (e.g., **6h** and **6j**) which provided an attractive balance of potency and selectivity were further optimized by modification of the sulfonamide cap.

Because our objective was a compound that provided a short systemic half-life, we chose the metabolically labile *tert*-butyl group for our sulfonamide optimization studies. Replacement of the methyl sulfonamide with aryl provided significant improvements in functional potency (Table 3). The 3-pyridyl sulfonamide **7f**, CP-533536, demonstrated excellent in vitro potency against



7f, CP-533536
Rat $t_{1/2}$ = 0.33 h, Cl = 56 mL/min/kg, Vss = 0.49 L/kg

Figure 3. Pharmacokinetic parameters of CP-533536 after i.v. administration of 1 mg/kg in the male Sprague–Dawley rat.

Table 4
PQCT parameters from rat tibia (*n* of 10) after a single injection with CP-533536, Example **7f**. Values are given as percent changes as compared to vehicle

Measurement	0.3 mg/kg	1.0 mg/kg	3.0 mg/kg
Total bone area	−4.7	4.4	20.6*
Bone mineral content	2.5	26.3*	53.5*
Bone mineral density	7.5*	20.7*	27.5*

* $p < 0.001$ versus vehicle.

EP_2 and selectivity against a broad panel of other targets. This compound was therefore selected for in vivo evaluation.

To determine if **7f** met our half-life objectives pharmacokinetic parameters were determined in the rat. This compound indeed demonstrated high i.v. clearance as well as low volume of distribution leading to a very short half-life (Fig. 3).

In order to meet the desired single dose requirement for a fracture healing agent, it was necessary to identify a formulation that would maintain drug locally at the fracture site for an extended time period. To achieve this objective we found that administration of the EP_2 agonist as a matrix with poly(D,L-lactide-co-glycolide) (PLGH) provided an attractive profile.[8] The CP-533536 PLGH matrix was directly injected into the marrow cavity of the tibia in a rat to assess the potential for local bone growth. Seven days after the single injection, the bone was analyzed cross-sectionally using peripheral quantitative computerized tomography (PQCT) for bone formation (Table 4).[9] Dose dependent increases in bone was observed after a single dose of compound. These data support the hypothesis that local administration of an EP_2 agonist will promote bone formation.[3b,3c]

In summary, optimization of the weak, but selective non-prostanoid EP_2 agonist **3** led to the discovery of CP-533536, a non-prostanoid, highly potent and selective EP_2 agonist that promotes bone formation and improves fracture healing in rat models. Further evaluation is underway to assess the potential of CP-533536 to improve bone healing in humans.

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6. All procedures were reviewed and approved by the Institutional Animal Care & Use Committee.
7. Receptor binding assay: membranes were prepared from stably transfected HEK-293 cells expressing the rat EP_2 or rat EP_4 receptor subtypes. Determination of cAMP: cellular cAMP levels were determined in the HEK-293 rat EP_2 or rat EP_4 cell line. Rat in vitro data was collected because we used rat as our pre-clinical efficacy model.
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