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Development of an in vivo active, dual EP1 and EP3 selective antagonist based on a novel acyl sulfonamide bioisostere

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ABSTRACT

Recent preclinical studies demonstrate a role for the prostaglandin E_2 (PGE_2) subtype 1 (EP1) receptor in mediating, at least in part, the pathophysiology of hypertension and diabetes mellitus. A series of amide and *N*-acylsulfonamide analogs of a previously described picolinic acid-based human EP1 receptor antagonist (**7**) were prepared. Each analog had improved selectivity at the mouse EP1 receptor over the mouse thromboxane receptor (TP). A subset of analogs gained affinity for the mouse PGE_2 subtype 3 (EP3) receptor, another potential therapeutic target. One analog (**17**) possessed equal selectivity for EP1 and EP3, displayed a sufficient in vivo residence time in mice, and lacked the potential for acyl glucuronide formation common to compound **7**. Treatment of mice with **17** significantly attenuated the vasopressor activity resulting from an acute infusion of EP1 and EP3 receptor agonists. Compound **17** represents a potentially novel therapeutic in the treatment of hypertension and diabetes mellitus.

Prostanoids are a family of oxidative metabolites of arachidonic acid that act in a autocrine and paracrine fashion. The cyclooxygenase activity of COX-1 and COX-2 converts arachidonic acid to prostaglandin G_2 (PGG_2) and the peroxidase activity of the same enzymes reduces PGG_2 to prostaglandin H_2 (PGH_2). PGH_2 is then isomerized to the five principal prostanoids by their respective synthases. Prostanoids bind to and activate a family of cell surface G-protein coupled receptors.[1] Prostaglandin E_2 (PGE_2) is formed from PGH_2 by prostaglandin E synthases (cPGES, mPGES-1, mPGES-2) and is a major prostanoid produced by the kidney and the vasculature.[2] The bioactivity of PGE_2 is mediated through four subtypes of E-Prostanoid (EP) receptors, designated EP1-EP4.[3] EP2 and EP4 couple to stimulatory G-proteins, which increase intracellular cAMP when activated. EP3 canonically couples to inhibitory G-proteins, suppressing cAMP accumulation. Both EP1 and EP3 are known to induce calcium flux into the cell.[4,5] The tissue localization of each of these EP receptors produces diverse and sometimes opposing biological activities of PGE_2 in vivo.[3]

Hypertension and diabetes are the primary causes of 62% of patients with end-stage renal disease (ESRD) and 72% of patients that develop ESRD each year[6], which requires life-long dialysis or kidney transplantation for survival. Elimination of PGE_2 production with COX inhibitors,[7,8] like NSAIDs,[9] is not a viable option as highlighted in a number of clinical trials. Recent studies in rodents and humans have suggested a role for the EP1 receptor in mediating at least part of the pathophysiology of diabetes mellitus[10–12] and hypertension.[13–16] EP1 has been prosecuted as a potential therapeutic target for chronic pain.[17–21] As such, small molecule, drug-like antagonists of EP1 have been developed. Human prostanoid receptor-targeting molecules are often nonselective,[22] owing to the evolution of the EP family of GPCRs to recognize the same endogenous ligand, PGE_2. The molecular pharmacology of these compounds at mouse prostanoid receptors is less well known, often poorly selective, and not always comparable to human pharmacology.[23] In order to study these molecular targets more precisely, we developed EP1 antagonists selective for the mouse receptor to use in mouse models of hypertension and diabetes mellitus.

To develop antagonists selective for the mouse EP1 receptor, we started with compound **7** (Figure 1), synthesized as previously described (Scheme 1).[24] Diethyl dipicolinic acid (**1**) was reduced with $NaBH_4$ to **2**. Parikh–Doering oxidation of **2** with sulfur trioxide–pyridine complex and DMSO produced the unstable aldehyde **3**. 4-Chlorophenoxide was then reacted with **3**, followed by neutralization with HCl to form **4**. Reduction of the secondary alcohol

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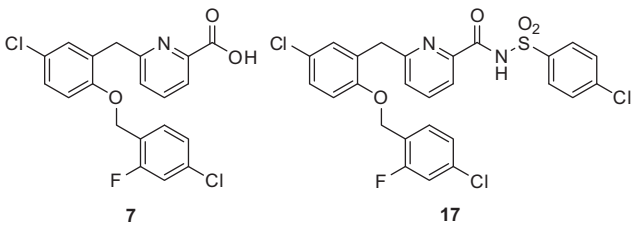


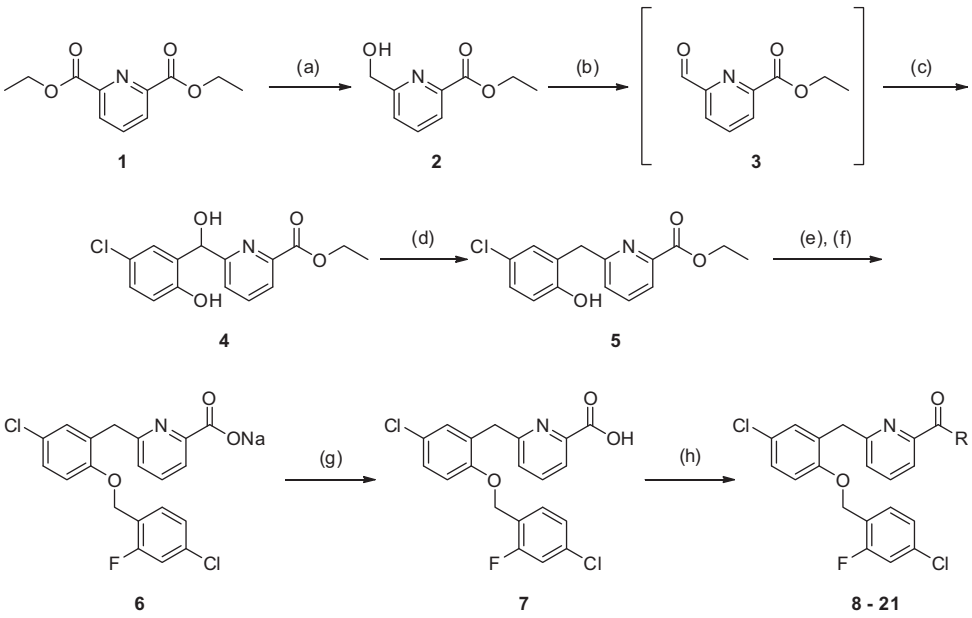
Figure 1. Lead picolinic acid-based human EP1 antagonist **7**, and 4-chloro-*N*-acylsulfonamide analog **17**.

of **4** under H_2 and Pd/C with the addition of H_2SO_4 and $ZnBr_2$ gave **5**. Alkylation of **5** with 2-fluoro-4-chlorobenzyl bromide and cleavage of the ester by refluxing with NaOH produced the sodium salt (**6**) of the lead (**7**) which was formed by protonation of **6**. The lead compound was shown to have good affinity for the human EP1 receptor and was stable in microsomes and S9 fractions of several species. However, **7** was previously reported to have a high-affinity interaction with the human thromboxane (TP) receptor.[24] We evaluated the molecular pharmacology of **7** at the mouse EP receptors as well as the mouse TP receptor.

Compound **7** was confirmed to be a functional antagonist of mEP1 in vitro and to have submicromolar affinity for the mouse EP1 receptor by Schild Analysis (Figure 2). **7** had no detectable affinity for mouse EP3 or EP4 receptors by radioligand binding assays. **7** had poor, but detectable affinity at mouse EP2, and suppressed signaling through mouse TP receptor at concentrations 100-fold higher than at the human receptor (Table 1), confirming the off-target activity of **7** at mouse TP.

Results from in vivo pharmacokinetics experiments (Table 2) revealed compound **7** to possess a moderate systemic plasma clearance (CL_p) and volume of distribution predicted at steady-state (V_{ss}), subsequently displaying a short half-life ($t_{1/2}$, >60 min) in mice receiving a parenteral administration of the EP1 receptor antagonist. We observed a bioavailability (%F) of approximately 14% following the oral administration (10 mg/kg) of **7** to mice.

Recently, Ostenfeld *et al.* have shown that in rats **7** is cleared primarily by glucuronidation and sequestration into the bile.[25] With the goal of inhibiting glucuronidation while improving molecular pharmacology of **7**, a series of carboxylic acid bioisosteres of **7** were pursued. *N*-acylsulfonamides are common carboxylic acid bioisosteres that have been successfully implemented in antagonists of angiotensin II AT1 receptors[26] as well as EP3 receptors.[27] A series of analogs (**8**–**21**) resulting from the amidation of **7** was prepared (Table 3). Tertiary amide analogues of **7** (**9** and **14**) were included to evaluate a structure–activity role of an acidic proton in ligand-receptor interactions. Each was synthesized by



Scheme 1. Reagents: (a) $NaBH_4$, EtOH (47%); (b) Pyr·SO_3, DMSO, DCM, (c) 4-chlorophenol, EtMgBr, DCM (54%); (d) H_2, Pd/C, H_2SO_4, $ZnBr_2$, EtOAc (69%); (e) 2-fluoro-4-chlorobenzyl bromide, K_2CO_3, EtOH; (f) NaOH, reflux; (g) HOAc, PhMe (68%); (h) RNH_2, R_2NH, or RSO_2NH_2, EDC·HCl, HOBt, DIPEA, DMF (10%–20%)

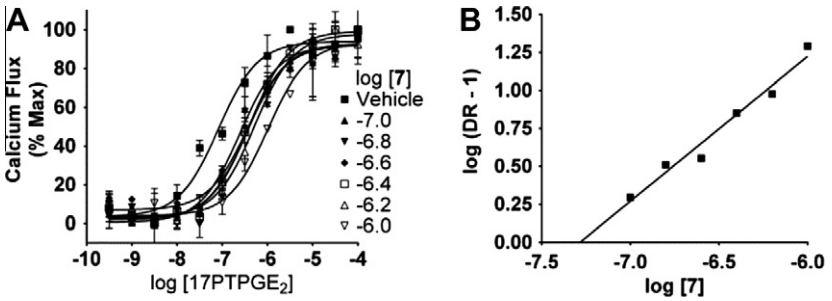


Figure 2. Concentration response curves (A) and transformed Schild regression; (B) for mEP1-expressing CHOk1 cells treated with six concentrations of **7** before being challenged with a range of concentrations of 17-phenyl-ω-trinor PGE_2 ($17PTPGE_2$), an EP1 agonist ($m = 0.953 \pm 0.085$, $pK_D = 7.283$, $r^2 = 0.9689$).

Table 1
Molecular pharmacology of **7** at mouse EP and TP receptors

mEP1 pK_D[a]	mEP1 K_D (nM)	mEP2 pK_I[b]	mEP3 pK_I[b]	mEP4 pK_I[b]	mTP pIC_{50}[b]
7.32 ± 0.08	47.9	5.76 ± 0.21	<6	<6	6.04 ± 0.18

[a] Value represents mean ± SEM of at least two independent experiments measured in duplicate.
[b] Values represent mean ± SEM of at least three independent experiments measured in triplicate.

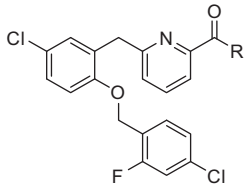
Table 2
Pharmacokinetic parameters for compound **7** following IV (1 mg/kg) or PO (10 mg/kg) dosing

$t_{1/2}$ (min)	CL_p (mL/min/kg)	V_{ss} (L/kg)	F (%)
65 ± 8.2	59.2 ± 6.4	0.90 ± 0.1	13.8 ± 2.1

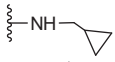
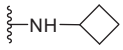
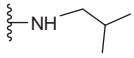
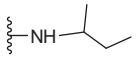
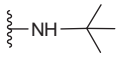
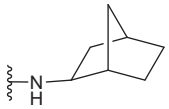
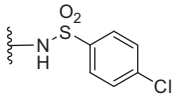
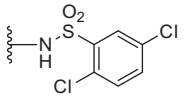
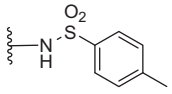
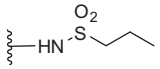
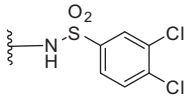
coupling **7** to a series of primary and secondary amines (**8**–**16**) and sulfonamides (**17**–**21**) employing common activators such as EDC·HCl, HOBt, and DIPEA in DMF (Scheme 1).

The molecular pharmacology observed for **8**–**21** was determined at mEP1–mEP4 and mTP (Table 3). Generally, *N*-acylsulf-

Table 3
Molecular pharmacology of amide and acylsulfonamide analogues of **7** at mouse EP and TP receptors



8 - 21

Compd.	R	mEP1 pK_D[a]	mEP1 K_D (nM)	mEP2 pK_I[b]	mEP3 pK_I[b]	mEP4 pK_I[b]	mTP pIC_{50}[b]
7	OH	7.32 ± 0.08	47.9	5.76 ± 0.21	<6	<6	6.04 ± 0.18
8		6.26 ± 0.02	549	<6	<6	<6	<6
9		4.80 ± 0.05	15800	<6	<6	<6	<6
10		6.04 ± 0.06	912	<6	<6	<6	<6
11		6.52 ± 0.36	302	<6	6.67 ± 0.03	<6	<6
12		5.86 ± 0.01	1380	<6	<6	<6	<6
13		5.36 ± 0.12	4360	<6	<6	<6	<6
14		6.35 ± 0.34	447	<6	<6	<6	<6
15		5.66 ± 0.13	2190	<6	<6	<6	<6
16	NH_2	5.58 ± 0.30	2630	<6	<6	<6	<6
17		7.39 ± 0.39	40.7	<6	6.97 ± 0.22	<6	<6
18		7.25 ± 0.32	53.7	<6	6.69 ± 0.13	<6	<6
19		6.67 ± 0.14	214	<6	<6	<6	<6
20		N.D.[c]	N.D.[c]	<6	<6	<6	<6
21		6.67 ± 0.16	214	<6	7.18 ± 0.05	<6	<6

[a] Values represent mean ± SEM of at least two independent experiments measured in duplicate.
[b] Values represent mean ± SEM of at least three independent experiments measured in triplicate.
[c] No functional antagonism was evident at concentrations exceeding 100 μM.

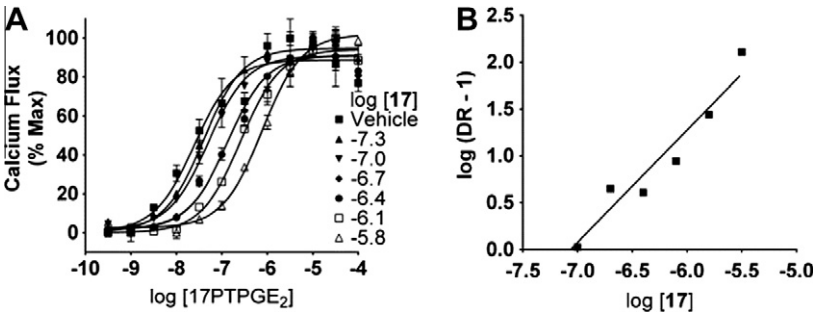


Figure 3. Concentration response curves (A) and transformed Schild regression; (B) for mEP1-expressing CHOk1 cells treated with six concentrations of **17** before being challenged with a range of concentrations of 17PTPGE$_2$ (m = 1.20 ± 0.12, pK_D = 7.063, r^2 = 0.9487).

Table 4
Intrinsic clearance of amide and *N*-acylsulfonamide analogs of **7** by mouse liver microsomes

Compd.	Cl_{int} (mL/min/kg)	CL_{HEP} (mL/min/kg)
7	84.7	43.6
8	11382	89.3
11	9806	89.2
14	7157	88.9
17	2260	86.6
19	5039	88.4
21	2994	87.4

onamides retained mEP1 affinity similar to **7** (representative data for **17**, Figure 3) while the amide series had reduced affinity for mEP1. Each analog displayed reduced affinity for mEP2 and mTP. Interestingly, four analogs (**11**, **17**, **18**, and **21**) displayed enhanced affinity for mEP3, a potential therapeutic target for hypertension- and diabetes mellitus-related ESRD. EP3 is of particular interest as it shares signaling pathways and endogenous ligands with EP1 and may represent a compensatory signaling pathway in the event of EP1 blockade.[3,5,16,28,29] These dual-selectivity compounds were confirmed to be functional antagonists of mEP3 by Schild analysis (data not shown).

We subsequently determined the intrinsic clearance (Cl_{int}) of several potent amide and *N*-acylsulfonamide analogs (Table 4). Results indicated an exceptional instability to metabolism in vitro, displaying estimated predicted hepatic clearance (CL_{HEP}) values that approached the hepatic blood flow in mice (Q_H, 90 mL/min/kg).

Results from metabolite identification studies in hepatic subcellular fractions indicated extensive biotransformation of the amide **11** and the *N*-acylsulfonamide **17**, including NADPH-independent hydrolysis (i.e., esterases) and NADPH-dependent oxidation (i.e., P450) of these analogs. Figure 4 depicts the metabolism of **17**, including the hydrolysis of the sulfonamide (**M1**), and P450-mediated oxidation of the methylene linker (**M2**) and benzylic oxidation (**M3**). The extent of plasma protein binding (fraction unbound, F_u) in mouse was determined to be extensive for three compounds assessed (F_u: **7** = 0.005, **11** = 0.010, **17** = 0.004).

Given the molecular pharmacology and in vitro metabolism data, we proceeded to evaluate the in vivo pharmacokinetics of **17**. Mice (n = 3) were subsequently administered a subcutaneous dose (5 mg/kg) with intermittent plasma collections to measure systemic levels of **17** (Figure 5). Compound **17** achieved a maximum plasma concentration (C_{max}) of 504 nM (±167) 2 h (t_{max}) following subcutaneous administration and displayed an area-under-the-curve (AUC) of 7508 nM*h.

To evaluate **17** as an antagonist of EP1 and EP3 in vivo, we measured blockade of mEP1 and mEP3 acute vasopressor activity in mice. Left common carotid arteries and right jugular veins of anesthetized mice were cannulated. Direct arterial pressure was measured via carotid catheter. Vasoactive substances were administered via jugular catheter. 17PTPGE$_2$ was used to acutely raise mean arterial pressure (MAP) via mEP1 and sulprostone was used for mEP3 (Figure 6). Agonists were administered IV through the

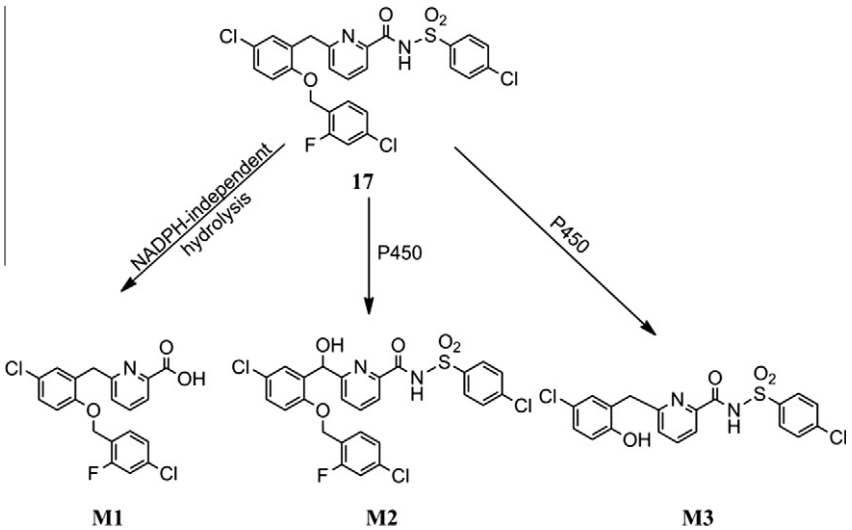


Figure 4. Metabolism of **17** in hepatic subcellular fractions.

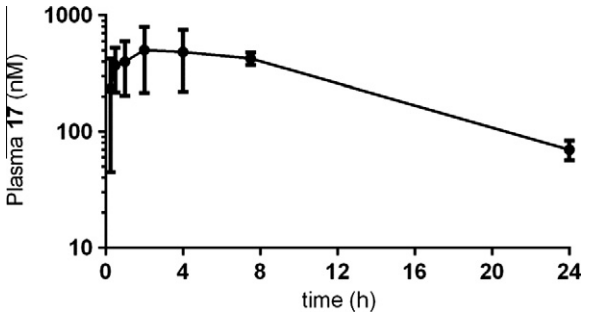


Figure 5. Plasma concentration-time profile of **17** following subcutaneous administration.

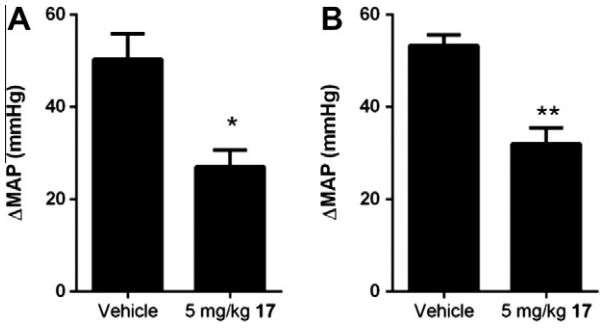


Figure 6. Change in mean arterial pressure (MAP) after intravenous infusion of (A) 17-phenyl-ω-trinor PGE_2 ($n = 3$ each, $^{*}P = 0.024$ by Student's two-tailed t test) or; (B) sulprostone ($n = 3$ each, $^{**}P = 0.007$ by Student's two-tailed t test) 2 h after subcutaneous injection of 5 mg/kg **17** or vehicle.

jugular catheter 2 h after subcutaneous administration of **17**. Pretreatment of mice with 5 mg/kg **17** administered subcutaneously significantly attenuated the pressor activity of an IV bolus of 20 μg/kg 17PTPGE$_2$ (ΔMAP 50.3 ± 5.5 mmHg vs 27.0 ± 3.6 mmHg). Pretreatment with **17** also significantly suppressed pressor activity of an IV bolus of 10 μg/kg sulprostone (ΔMAP 53.3 ± 2.3 mmHg vs 32.0 ± 3.5 mmHg). To ensure the observed effect was selective for EP-mediated vasoconstriction, phenylephrine (10 μg/kg) was shown to be unaffected by pretreatment with **17** (data not shown).

In conclusion, we have identified a novel, dual-selectivity antagonist (**17**) of the mouse EP1 and mouse EP3 receptors possessing an acylsulfonamide bioisostere for the prototypical carboxylic acid moiety of EP ligands. **17** was found to have indistinguishable affinity for mEP1 as for mEP3 (mEP1 pK_D vs mEP3 pK_I, $P = 0.40$, Student's two-tailed t test). **17** had improved selectively over mEP2 and mTP. **17** was less stable in mouse hepatic microsomes than **7**, due in part to hydrolysis of **17** to **7**, a problem effectively circumvented by subcutaneous administration of **17**. Finally, we confirmed **17** is a functional antagonist of mEP1 and mEP3 in vivo by blocking mEP1/mEP3-mediated acute vasopressor activity in anesthetized mice. While the attenuation of pressor activity appears to be incomplete, these results recapitulate experiments performed in mice with genetic disruptions of EP1.[13] Dual specificity EP1/EP3 antagonists represent a novel class of potential ESRD therapeutics we hypothesize will be more beneficial than blocking either receptor alone.

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