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Discovery of AAT-008, a novel, potent, and selective prostaglandin EP4 receptor antagonist

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ABSTRACT

Starting from acylsufonamide HTS hit **2**, a novel series of *para*-*N*-acylaminomethylbenzoic acids was identified and developed as selective prostaglandin EP4 receptor antagonists. Structural modifications on lead compound **4a** were explored with the aim of improving potency, physicochemical properties, and animal PK predictive of QD (once a day) dosing regimen in human. These efforts led to the discovery of the clinical candidate AAT-008 (**4j**), which exhibited significantly improved pharmacological profiles over grapiprant (**1**).

Prostaglandin E_2 (PGE_2) is a pro-inflammatory mediator generated from arachidonic acid by the action of cyclooxygenase (COX) isoenzymes under inflammatory conditions. Four PGE_2 receptor subtypes (EP1, EP2, EP3, and EP4) responsible for different pharmacological properties have been cloned and classified.[1,2] The EP4 subtype, a G-protein-coupled receptor (GPCR), stimulates cyclic adenosine monophosphate (cAMP) production[3] and is distributed in a wide variety of tissues suggesting an important role of EP4 receptor in PGE_2-mediated biological events such as inflammation,[4] pain,[5] and cancer.[6–10] Selective blockade of the PGE_2 signaling through the EP4 receptor pathway represents an attractive approach to discover novel analgesic, immunomodulating, and antineoplastic agents. Analgesic potentials are clearly supported by the reduction of pain and inflammation in EP4 receptor knock out/knock down animals and the similar results using EP4 receptor selective antagonists in animal studies.[11,12] Since the PGE_2-EP4 signal blockade does not affect actions of the other subtype PGE_2 receptors as well as other prostanoids, selective EP4 antagonists might provide analgesic effects without adverse events observed with nonsteroidal anti-inflammatory drugs (NSAIDs) or cyclooxygenase-2 (COX-2) inhibitors,[13–15] and are expected to be a new therapy for the treatment of both acute and chronic inflammatory pain. In addition, selective EP4 receptor antagonists are also expected to offer an attractive therapeutic approach for autoimmune diseases such as inflammatory bowel disease (IBD), rheumatoid arthritis (RA), and multiple sclerosis (MS)[16–18] through the inhibition of interleukin-23 (IL-23) production and suppression of T helper 1 (Th1) and T helper 17 (Th17). Moreover, the recent reports suggest that the EP4 receptor which is expressed in certain types of cancer promotes tumor cell proliferation and metastasis.[19] Therefore, selective antagonism of the EP4 receptor might have significant clinical potential for the treatment of colorectal, breast, prostate, lung, gastric, bladder, head and neck, hepatocellular, pancreatic, and ovarian cancers. Herein we report novel and selective EP4 receptor antagonists of benzoic acid with the nicotinamide or benzamide scaffolds.

Grapiprant (Fig. 1) is a selective antagonist for prostaglandin E_2 (PGE_2) receptor subtype 4 (EP4) identified as a clinical candidate for the treatment of inflammatory pain associated with osteoarthritis (OA). It is currently under development for use in humans[20] and dogs.[21] The projected dosing regimen of grapiprant

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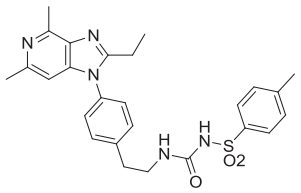

Fig. 1. Selective EP4 antagonist, grapiprant under development.

for humans is 50–100 mg, PO, BID (twice a day) based on the pharmacology and pharmacokinetic (PK) data in clinical studies.

In order to identify another clinical candidate with improved efficacy, safety, and pharmacokinetic profiles with potential for QD (once a day) dosing, we embarked on a back-up discovery project to discover the second generation of grapiprant (**1**).[22] The target profiles of this candidate are summarized as follows. The back-up candidate should demonstrate improved potency over grapiprant, i.e.; (1) antagonize PGE_2-mediated cAMP elevation in transfectants expressing human EP4 receptor-with a $pA_2 > 8.6$; (2) exhibit selectivity of >100 over other PG receptor subtypes; (3) clearly demonstrate improved oral activity over grapiprant in key acute and chronic inflammatory pain models; i.e., carrageenan induced mechanical hyperalgesia and complete Freund's adjuvant (CFA) induced weight bearing deficit in the rat; (4) demonstrate a PK profile in animals predictive of QD dosing in human. From a medicinal chemistry perspective, the back-up compound should have improved physicochemical characteristics; lower molecular weight and lower lipophilicity while improving efficacy and PK profile. In order to meet the above criteria, we pursued an alternative core structure different from the sulfonylurea since the structure–activity relationships (SAR) studies around grapiprant revealed compounds consisting of the sulfonylurea core had extremely low volume of distribution and high to moderate clearance in rats and other experimental animals.

High throughput screening (HTS) of the Pfizer compound library using a human EP4 functional assay measuring PGE_2-induced cAMP formation in HEK-293 cells expressing human EP4 receptor and the subsequent verification in a membrane binding assay using [^{3}H]PGE_2 resulted in the identification of *N*-acyl sulfonamide **2** as a moderately potent EP4 antagonist (IC_{50}: 302 nM) without EP4 selectivity over other subtypes (Fig. 2). HTS hit **2** has high molecular weight (MW: 610.08) and is quite lipophilic (ALogP: 5.70, cLogD: 5.80).[23] Further screening of HTS hit **2** were initiated by using the in-house compound libraries. First, a bioisosteric transformation[24,25] of the *N*-acyl phenylsulfonamide moiety to the corresponding carboxy group was well tolerated and resulted in the identification of compound **3** with reduced molecular weight. Compound **3** displayed modest EP4 selective functional antagonism (IC_{50}: 370 nM) and good stability in human liver microsomes (HLM) ($T_{1/2} > 120$ min), however it did not have acceptable physicochemical properties (typically for lead compounds, solubility in phosphate-buffered saline (PBS) > 10 μM; molecular weight <400; ALogP < 4) (Fig. 2). Next, with further reduction of MW and lipophilicity (ALogP and cLogD) in mind, a structural similarity search by using a simplified pharmacophore query as shown in Fig. 2 led to identify ca. 1000 compounds. The subsequent filtering of these compounds by applying Lipinski's rule of 5[26,27] and eliminating compounds with toxicophore[28–30] narrowed down to 50 hit compounds. These 50 compounds were evaluated in a series of assays of functional EP4 receptor antagonism, binding selectivity against EP receptors, HLM stability, human cytochrome P450 inhibition, aqueous solubility, and membrane permeability. As a result, compound **4a** was shown to be superior to the others, demonstrating EP4 selective functional antagonism (IC_{50}: 575 nM), binding affinity for EP4 receptor (*Ki*: 73 nM), stability in HLM ($T_{1/2} > 120$ min), no notable CYP 450 inhibition, high Caco-2 cell permeability (P_{app}: 25×10^{-6} cm/s),[31] good solubility in PBS (>10 μM), lower MW (MW: 384.33), and acceptable lipophilicity (ALogP: 3.61, cLogD: 2.14). The hit-to-lead efforts resulted in the identification of compound **4a** that has a core structure of *para*-*N*-acylaminomethylbenzoic acid (Fig. 2). The molecules containing carboxylic acid often have undesirable metabolic instability, limited permeability, and potential toxicities. Despite the drawbacks of the carboxylic acid functional group, **4a** exhibited selective EP4 functional antagonism with a suitable metabolic profile *in vitro* and good physicochemical properties. Thus, we envisaged that the optimization efforts around **4a** would provide the backup candidate that meets the target profiles.

The optimization of the lead compound (**4a**) was initiated and the initial key SAR and the results of the structural modifications of lead compound **4a** are summarized in Fig. 3. Replacement of the carboxylic acid moiety with other functional groups led to loss of functional activity against EP4 receptor. Although the corresponding tetrazole exhibited slightly increased functional activity, the tetrazole analog was a substrate for efflux pumps and a strong inhibitor of CYP3A4. Shifting the carboxylic acid moiety from *para*- to *meta*-position showed loss of intrinsic activity. The benzoic acid moiety was not replaceable by nicotinic acid, cyclohexanecarboxylic acid, or 4-thiazolecarboxylic acid. Moreover, modifications of the amide moiety by exchanging the nitrogen with methylene, reduction of the amide carbonyl, or *N*-methylation of the nitrogen

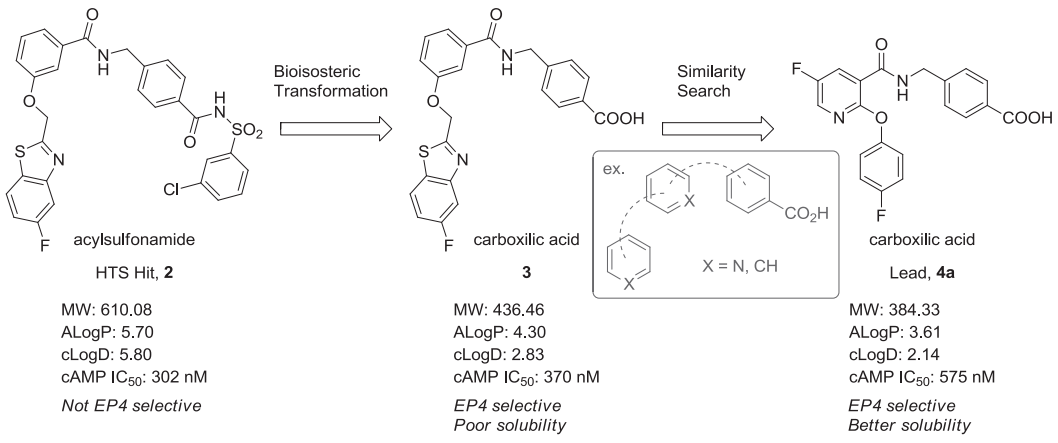

Fig. 2. Hit to lead: genesis of benzoic acid EP4 antagonists.