Bioorganic & Medicinal Chemistry Letters xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of substituted-2,4-dimethyl-(naphthalene-4-carbonyl) amino-benzoic acid as potent and selective EP4 antagonists

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ARTICLE INFO

Article history:
Received 19 August 2015
Revised 5 November 2015
Accepted 8 November 2015
Available online xxxx

Keywords:
Arthritis pain
EP4 receptor antagonist
Prostaglandin E2
Human whole blood assay

ABSTRACT

A novel series of EP4 antagonists, based on a quinoline scaffold, has been discovered. Medicinal chemistry efforts to optimize the potency of the initial hit are described. A highly potent compound in a clinically relevant human whole blood assay was identified. Selectivity and pharmacokinetic profiles of this compound are discussed.

Arthritis pain affects millions of patients in the United States alone and is a leading cause of disability.[1,2] Treatments often include NSAIDs (nonsteroidal anti-inflammatory drugs) or cyclooxygenase 2 (COX-2) inhibitors, which provide broad analgesic efficacy but also carry substantial cardiovascular (CV) and gastrointestinal (GI) risk.[3] Arthritis patients with a poor cardiovascular profile, such as hypertension, or an intolerance to NSAIDS may be precluded from using these types of analgesics. Thus, there is a need for safer alternative therapies for osteoarthritis and rheumatoid arthritis pain.

Multiple literature reports in the past decade demonstrate that prostaglandin E2 (PGE2) plays an important role in the pathogenesis of arthritis pain and that inhibition of PGE2 production either by using NSAIDs or COX-2 inhibitors provides predictable and marketable efficacy. PGE2 is known to exert its effects through four G-protein coupled receptors (GPCR) receptors, EP1-4. Studies with selective prostaglandin receptor knockout animals have shown that EP4 is the primary receptor involved in joint and inflammatory pain.[4] Hence, a selective EP4 antagonist may be useful in treating arthritis pain. In addition, it has been suggested that since EP4 antagonism does not interfere directly with the biosynthesis of prostanoids, such as PGE2, prostacyclin (PGI2), and thromboxane A2 (TxA2),[5–7] a selective EP4 antagonist may not exhibit the CV and GI side effects seen with NSAIDs and COX-2 inhibitors.[8]

This therapeutic potential has triggered significant efforts in the pharmaceutical industry that have led to the discovery of a variety of EP4 antagonists.[9] Several examples of compounds advancing to clinical studies have been reported, in particular CJ-023423[10,11] (**1**) and PGN-1531[12] (**2**) have been studied extensively (Fig. 1).

From a drug discovery perspective, in vitro assays have been developed to understand both potency, and prostanoid selectivity, as well as function of EP4 in a native tissue assay. For example, Murase et al reported[13] that CJ-042794 (**3**, Fig. 1) competitively inhibited PGE2-evoked elevations of intracellular cAMP levels in HEK293 cells overexpressing human EP4 receptor. Similar assays for the other EP receptor subtypes have been reported as well. It is also known that EP4 antagonism modulates PGE2 mediated production of TNFα in lipopolysaccharide (LPS) stimulated human whole blood (hWB).[13] In this assay, CJ-042794 reliably reversed the inhibitory effects of PGE2 on LPS-induced TNFα production in a concentration-dependent manner. The whole blood assay provides a measure of EP4 antagonist function in a clinically relevant tissue and provides a means to assess the potential for in vivo activity.[14,15] This type of assay provides both a measure of preclinical potency and an effective target engagement biomarker to facilitate clinical translation.[16]

In this manuscript we will disclose our efforts towards the identification and optimization of substituted 2,4-dimethyl-(quinoline-4-carbonyl)amino-benzoic acid derivatives as EP4 antagonists. During the course of our research, commercially available quinoline **5** (Table 1) was identified as a hit from screening. It had reasonable in vitro antagonist activity (128 nM) at hEP4, with

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http://dx.doi.org/10.1016/j.bmcl.2015.11.023
0960-894X/

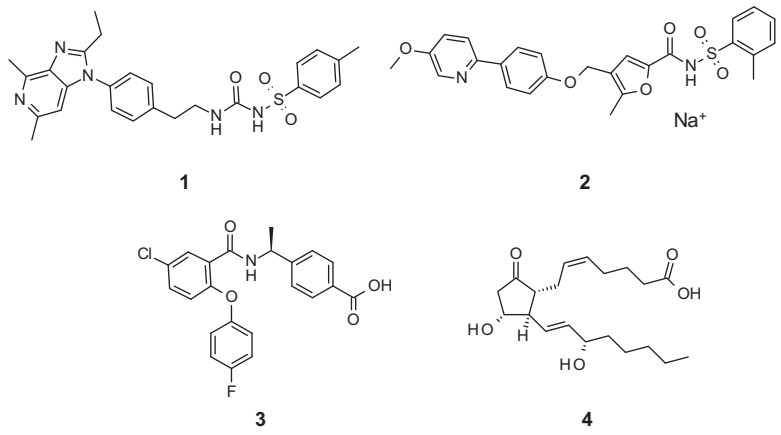
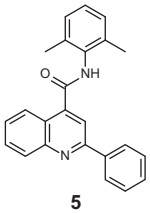
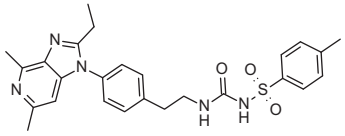


Figure 1. Examples of EP4 antagonists and PGE2 (**4**).

Table 1
Compound profile for initial hit from screening

Property	 5	 1
MW	352.44	491.61
hEP4 binding K_i (nM)[a]	512 + 254 (5)	448 ± 276 (8)
hEP4 functional cAMP IC_{50} (nM)[a,b]	128 ± 42.8 (7)	11.7 ± 8 (6)
LEAN[c]	0.26	0.23
hWB IC_{50} (nM)[d]	>30000 (3)	1520 ± 1030 (82)
Rat hepatic microsome metabolism (%)	64	5
Human hepatic microsome metabolism (%)	71	2
Measured aqueous solubility, pH = 6, (mg/mL)	<0.001	0.977
*c*Log*P*[e]	6.28	3.55

[a] Data is shown as K_i or IC_{50} values + SEM and number of experiments (n) in parenthesis.
[b] The EP4 functional assay measured the inhibition of PGE2-induced cAMP accumulation in HEK 293 cells.
[c] LEAN values based on functional activity. LEAN is an internal metric for ligand efficiency and is defined as $-\log(IC_{50})$/number of non-hydrogen atoms.
[d] Results are expressed as the geometric mean ± standard deviation; n = number of independent determinations. The standard deviation is calculated by the delta method, being $SD\log IC_{50} \times$ geometric mean $\times \ln(10)$.
[e] *c*Log*P*, calculated using Marvin and calculator plugin freeware (www.chemaxon.com, ChemAxon Kft, Budapest, Hungary).

a LEAN ratio[17,18] of 0.26 [LEAN is an internal metric for ligand efficiency and is defined as −log(IC50)/number of non-hydrogen atoms]. Unfortunately, compound **5** was inactive in the hWB assay. Furthermore, high lipophilicity (*c*Log*P* > 6) and significant in vitro metabolic liability in rodent microsomes (>60%, Table 1), suggested poor PK properties for this compound. Understanding these property issues, we decided to launch a medicinal chemistry effort to explore optimization of this scaffold.

Our overall effort was influenced by the expectation that the relationship between the human whole blood activity and the plasma exposure profile would ultimately drive clinical efficacy. This approach relied on the ability of a compound to maintain projected human plasma concentrations in excess of the hWB IC_{50} value. The focus of our SAR effort was to understand the potential of this series to achieve potent hWB activity, while incorporating modifications to obtain a suitable PK profile.

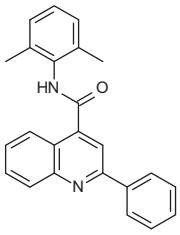
PGE2 (**4**, Fig. 1), the endogenous ligand of EP4, contains both hydroxyl and carboxyl functional groups indicating that significant polarity is tolerated by the receptor. We hypothesized that introduction of polar groups in compound **5**, might have positive effect in modulating the overall lipophilicity addressing metabolism and solubility issues while potentially affording more activity.

We also explored changes to the central core of the molecule to include naphthalene analogs. Structures of these compounds are shown in Table 2. Known EP4 antagonist **1** and initial hit **5** are also highlighted for comparison in Table 2. Compounds were characterized initially with their in vitro binding activity and functional potencies and then evaluated in the more demanding hWB assay as warranted.

As shown in Table 2, introduction of a benzoic acid was not tolerated as an R^2 substituent (**6a**). However, introduction of the same moiety as an R^1 substituent led to potent compound (**6b**) with a functional activity of 11 nM. Compound **6b** showed activity in the hWB assay comparable to clinical compound **1**. Further modification to a benzylic alcohol as R^2 substituent (**6c**, **6d**) provided slight improvements in the functional as well as in hWB assay. The incorporation of a benzoic acid in R^1 with either *para*- or *meta*-substitution seemed to be beneficial for the binding and functional activity, giving >10-fold improvement over **5**. Remarkably, *meta*-benzoic acid analog **6d** was >5-fold more potent in hWB activity than standard **1**.

In the naphthalene scaffold, we observed similar trends for the phenyl R^2 analogs. Compounds **7a** and **7b** demonstrated robust binding and functional activity that translated well to hWB activity

Table 2
SAR efforts to optimize initial hit



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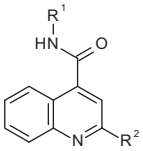

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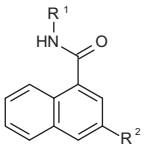

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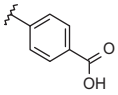
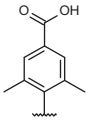
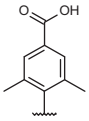
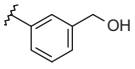
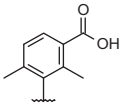
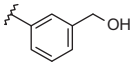
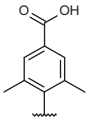
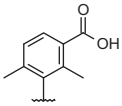
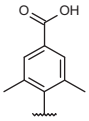
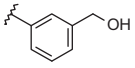
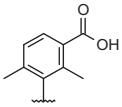
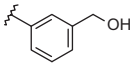
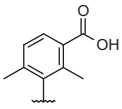
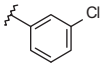
Compound	R^1	R^2	hEP4 K_i[a] (nM)	hEP4 cAMP IC_{50}[a,b] (nM)	LEAN value[c]	hWB IC_{50}[d] (nM)	*c*Log *D*[e]
1	—	—	448 ± 276 (8)	11.7 ± 8 (6)	0.23	1520 ± 1030 (82)	*c*Log *P* = 3.08
5	—	—	512 ± 254 (5)	128 ± 42.8 (7)	0.26	>30,000 (3)	*c*Log *P* = 6.28
6a			>19,200	>25,000	—	>30,000 (3)	2.75
6b			39.2 ± 36.4 (2)	11.2 ± 2.32 (4)	0.26	1980 ± 1650 (5)	2.88
6c			52.3 (1)	3.08 ± 1.76 (7)	0.26	501 ± 495 (6)	2.11
6d			17 (1)	5.20 ± 2.94 (3)	0.26	312 ± 295 (5)	2.03
7a			8.04 ± 4.63 (2)	3.48 ± 2.11 (5)	0.28	623 ± 244 (3)	3.33
7b			6.72 ± 6.32 (3)	5.65 ± 1.91 (3)	0.27	577 ± 175 (6)	3.24
7c			3.54 ± 0.48 (3)	1.11 ± 0.73 (4)	0.28	136 ± 86 (2)	2.56
7d			1.98 ± 1.24 (3)	2.42 ± 1.1 (5)	0.28	6.2 ± 4.5 (3)	2.48
7e			3.49 ± 2.7 (10)	3.62 ± 0.74 (3)	0.27	243 ± 116 (6)	3.85

[a] Data is shown as K_i or IC_{50} values + SEM and number of experiments (*n*) in parenthesis.
[b] The EP4 functional assay measured the inhibition of PGE2-induced cAMP accumulation in HEK 293 cells.
[c] LEAN values based on functional activity. LEAN is an internal metric for ligand efficiency and is defined as $-\log(IC_{50})$/number of non-hydrogen atoms.
[d] Results are expressed as the geometric mean ± standard deviation; *n* = number of independent determinations. The standard deviation is calculated by the delta method, being $SD\log IC_{50} \times$ geometric mean $\times \ln(10)$.
[e] *c*Log *D* at pH = 7.4, calculated using Marvin and calculator plugin freeware (www.chemaxon.com, ChemAxon Kft, Budapest, Hungary).

(IC_{50} ~0.6 μM). The benzylic alcohol R^2 substituent (**7c** and **7d**) led again to improve binding and functional potency. Compound **7d** showed outstanding hWB potency (IC_{50} ~6 nM), >250 fold more potent than clinical compound **1**. Chlorophenyl substitution also provided a potent in vitro compound (**7e**), although the effect on hWB activity was not as pronounced as with benzylic alcohol **7c**. Noteworthy, a linear correlation between binding or functional activity and hWB activity was not apparent. Since the hWB assay uses a native tissue matrix, additional parameters such as protein binding and cell type influence the response.

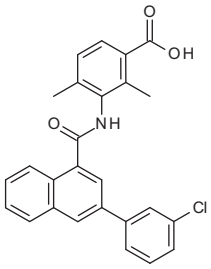
In general, the incorporation of a carboxylic acid group into the phenyl amide portion of our initial hit compound **5** led to significant improvement of the in vitro activity with meaningful translation to our whole blood assay. Lean values for these compounds

Table 3
Rat in vitro and in vivo ADME profiling

Compound	hWB (nM)	Rat hepatic microsome metabolism[a] (%)	Clearance, iv[b] (mL/min/kg)	po, C_{max}[c] (ng/mL)	po, T_{max}[c] (h)	% F[b,c]	C_{max}/IC_{50}
6c	501	<1	59.6	42.3	0.25	3	0.20
7d	6.2	<1	23.8	85.7	0.25	6	33
7e	243	7	1.9	10,900	1	100	104

[a] Microsomal incubation was a 30-min incubation in the presence of rat liver microsomes and NADPH.
[b] Dosed 1 mg/kg iv.
[c] Dosed 5 mg/kg po.

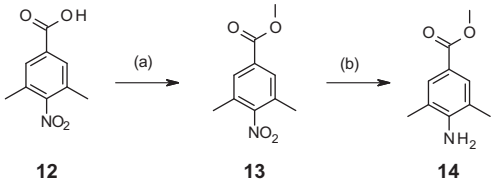
Table 4
In vitro data for **7e**



7e

Assay	Results[a]
hEP_1 binding	K_i > 19.5 μM; IC_{50} > 25 μM (2)
hEP_2 binding	K_i = 2.8 μM; IC_{50} = 3.7 μM (2)
hEP_3 binding	K_i > 17.5 μM; IC_{50} > 25 μM (2)
hEP_4 binding	K_i = 3.49 nM (10) IC_{50} = 5.62 nM (10)
CYP1A2, CYP2B6, CYP2C19, CYP2C8, CYP2C9, CYP2D6 CYP3A4 inhibition	IC_{50} > 10 μM for all

[a] Value represents mean of two or more experiments.



12 **13** **14**

Scheme 2. Synthesis of *para*-substituted amino benzoic ester. Reagents and conditions: (a) $SOCl_2$, MeOH, 80 °C, 16 h, (98%); (b) Fe powder, MeOH, concd HCl, 0 °C–80 °C, 16 h, (99%).

were >0.26 which is better than known EP4 antagonist **1** with a LEAN = 0.23. The naphthalene yielded more potent in vitro compounds as reflected in the greater LEAN values.

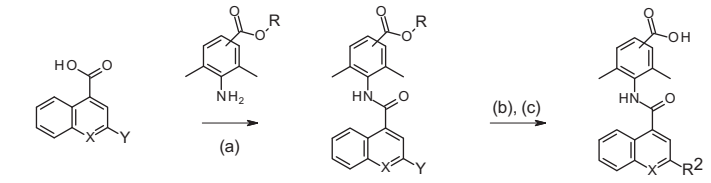
After positively assessing the scaffold for hWB activity, the next step was the evaluation of ADME parameters for this new scaffold. To be thorough, the assessment was conducted for a few compounds, including **7d**, and others with some structural differences. The pharmacokinetic profiles for representative compounds are given in Table 3.

An initial evaluation of rat hepatic microsome stability showed that all compounds (**6c**, **7d** and **7e**) were more stable than initial hit **5** (<10% vs 64% respectively). However, the in vivo rat PK profile was not directly connected to the microsomal stability. The iv clearance ranged from minimal clearance (**7e**) to greater than hepatic flow (**6c**, Table 3). Upon oral administration, compound **7e** showed much greater C_{max} than the other two compounds and was 100% bioavailable. Compound **7d** delivered a C_{max} with 33-fold over the hWB IC_{50} whereas the C_{max} for compound **7e** was >100-fold the hWB IC_{50} value.

Based on these data, compound **7e** was selected for additional evaluation. It was submitted to assess selectivity against the other known EP receptors (Table 4). No detectable binding was observed with either EP1 or EP3. Binding to EP2 was >800-fold weaker than the EP4 binding. Compound **7e** was found to have no inhibitory activity against several CYP enzymes in vitro up to 10 μM. The risk of drug-drug interactions with **7e** was deemed low.

The compounds described in Table 2 were synthesized[15] by the representative method shown below in Scheme 1. Amino ester intermediates **14** and **18** were prepared as shown in Schemes 2 and 3. T_3P mediated coupling[19] of 3-chloro or 3-bromo substituted quinoline **8** or naphthalene **9** carboxylic acids with *para*- or *meta*-substituted amino-benzoic esters led to key intermediates **10** or **11**. Suzuki coupling of quinoline **10** or naphthalene **11** derivatives with relevant substituted phenyl boronic acids allowed for the versatile synthesis of diverse precursors. Finally, hydrolysis of these penultimate intermediates under basic conditions led to corresponding quinolone (**6a–d**) and naphthalene (**7a–e**) analogs in good yields.

In conclusion, we have discovered a new scaffold with potent EP4 binding and antagonist activity (<15 nM). More importantly,



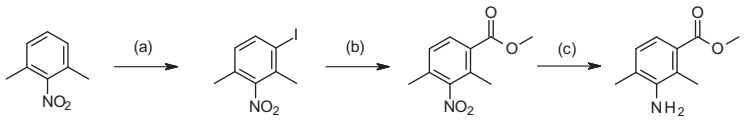
8, X = N; Y = Cl, Br
9, X = CH; Y = Cl, Br

10, X = N; Y = Cl, Br
11, X = CH; Y = Cl, Br

6a-d, X = N
7a-e, X = CH

R = CH_3, CH_2CH_3

Scheme 1. General synthetic route to quinoline and naphthalene derivatives. Reagents and conditions: (a) T_3P, CH_2Cl_2, DIEA, room temperature, 24 h, (11–48%); (b) 1,4-dioxane, water, substituted phenyl boronic acid, K_2CO_3, $PdCl_2$(dppf), CH_2Cl_2, 110 °C, 3 h, (57–90%); (c) 1 N NaOH, 1:1 THF/MeOH, (47–85%).



15 16 17 18

Scheme 3. Synthesis of *meta*-substituted amino benzoic esters. Reagents and conditions: (a) AcOH, H_2SO_4, I_2, and HIO_4, 90 °C, 7 days, (95%); (b) $Pd(OAc)_2$, dppb, CH_3CN, Et_3N, MeOH, CO (80 psig), 100 °C, 2 h, (98%); (c) MeOH, 10% Pd/C, H_2 (atm), 6 days, (65%).

efforts incorporating a carboxylic acid moiety rendered compounds with clinically relevant hWB activity. Additional optimization introducing hydroxy benzylic substituents led to the discovery of compound **7d** (hWB = 6 nM), one of the most potent EP4 antagonist reported to date. ADME assessment of a set of representative compounds, showed favorable PK profile for compound **7e** with human whole blood potency of 250 nM, yielding the best combination of activity and exposure for this series. Compound **7e** was selective against other EP receptor subtypes and CYP enzymes. Further exploration of this series of compounds is in progress and will be disclosed in due course.

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