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# Structure–activity relationships and pharmacokinetic parameters of quinoline acylsulfonamides as potent and selective antagonists of the $EP_4$ receptor

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**Abstract**—A new series of $EP_4$ antagonists based on a quinoline acylsulfonamide scaffold have been identified as part of our ongoing efforts to develop treatments for chronic inflammation. These compounds show subnanomolar intrinsic binding potency towards the $EP_4$ receptor, and excellent selectivity towards other prostanoid receptors. Acceptable pharmacokinetic profiles have also been demonstrated across a series of preclinical species.


Prostanoids (prostaglandins and thromboxanes) are important lipid hormones formed from arachidonic acid metabolism. Prostaglandin $E_2$ ($PGE_2$), in particular, is the principal proinflammatory prostanoid and is implicated in the pathogenesis of a number of diseases such as pain, fever, arthritis and cancer. Inhibition of $PGE_2$ production by NSAIDs and COX-2 inhibitors (Coxibs) relieves arthritis symptoms, and thus is the basis of widespread uses of these drugs as analgesics.[1] Unfortunately, the therapeutic utilities of these drugs are limited by their potential to cause either gastro-intestinal toxicity (by NSAIDs)[2] or cardiovascular (CV) side effects (by both NSAIDs and Coxibs).[3] The CV adverse events associated with these drugs are not clearly understood although it is speculated that inhibition of prostacyclin biosynthesis may cause the prothrombotic and hypertensive effects.[4] Therefore, there is a vast unmet medical need to discover alternatives for treating chronic ailments such as arthritis.

$PGE_2$ exerts its biological effects through four subtype EP receptors, $EP_{1–4}$. In a mouse model of collagen-antibody induced arthritis (CAIA), the $EP_4{}^{-/-}$ mice showed a remarkable resistance to both the incidences and symptom scores (paw swelling/redness, ankylosis) of arthritis compared to the wild type controls while the $EP_{1–3}{}^{-/-}$ mice showed no effect, suggesting that the effect of $PGE_2$ in chronic inflammation was mediated predominantly by the $EP_4$ receptor.[5] Lin et al. demonstrated that $EP_4$, not $EP_{1–3}$, contributed to inflammatory pain hypersensitivity in rats, providing further evidence that $EP_4$ antagonism is a valid strategy for treating inflammatory pain.[6] It is plausible that a selective $EP_4$ antagonist may ameliorate symptoms of chronic inflammation without the potential CV side effects observed with NSAIDs and COX-2 inhibitors since it does not interfere with biosynthesis of any prostanoids including prostacyclin and thromboxanes. In addition to its role in inflammation, the $EP_4$ receptor has also been implicated in destabilizing atherosclerotic plaques in human[7] and in developing tumour metastasis.[8] Therefore, $EP_4$ antagonists represent potential promising new therapeutic agents for treating inflammatory pain, atherosclerosis and cancer. In this paper, we describe the discovery of a highly potent $EP_4$ antagonist with excellent pharmacokinetic properties and in vivo potency.

At the outset of our investigations, acylsulfonamide **1** was a known $EP_4$ antagonist from the literature

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(Fig. 1).[9] While this compound showed low nanomolar intrinsic potency for binding with the $EP_4$ receptor,[10] it suffered from extensive shift in the presence of human serum. Further, in vivo hydrolysis of the acylsulfonamide moiety resulted in the formation of a circulating carboxylic acid which was shown to be a partial agonist for $EP_4$.[11] We sought to alleviate this issue by reversal of the orientation of the acylsulfonamide, with an eye to increasing potency through a combination of increasing rigidity through steric constraints, and by changing the polarity by the introduction of heterocyclic frameworks (Fig. 1).

The acylsulfonamide targets were prepared as shown in Scheme 1. Claisen condensation[12] of dimethyl succinate with dimethyl phthalate or dimethyl pyridine dicarboxylate **A**, then bis-alkylation of the resulting diphenol provided the alkylated dihydroquinones **B**. Conversion to the anhydrides **C** was accomplished by hydrolysis of the diesters under basic conditions followed by treatment of the resulting dicarboxylates with refluxing acetic anhydride. Bromides **D** were converted to protected sulfonamides **E** via oxidation of the intermediate thioimidate with chlorine gas.[13] Reduction to anilines **F**, then treatment with anhydride **C** under acidic conditions provided the phthalimides **G**. Sequential reductions provided lactams **H**, which in the case of the quinoline series were a readily separable mixture of regioisomers. Finally, formation of the acylsulfonamides **I** was accomplished by either treatment with the desired acid chloride, or from the acid using peptide coupling conditions.[14]

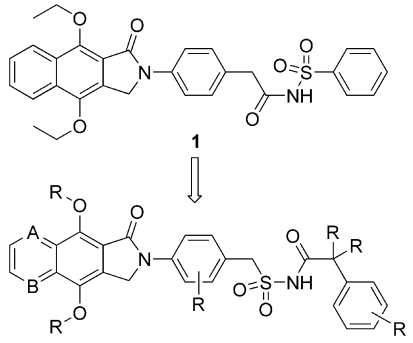
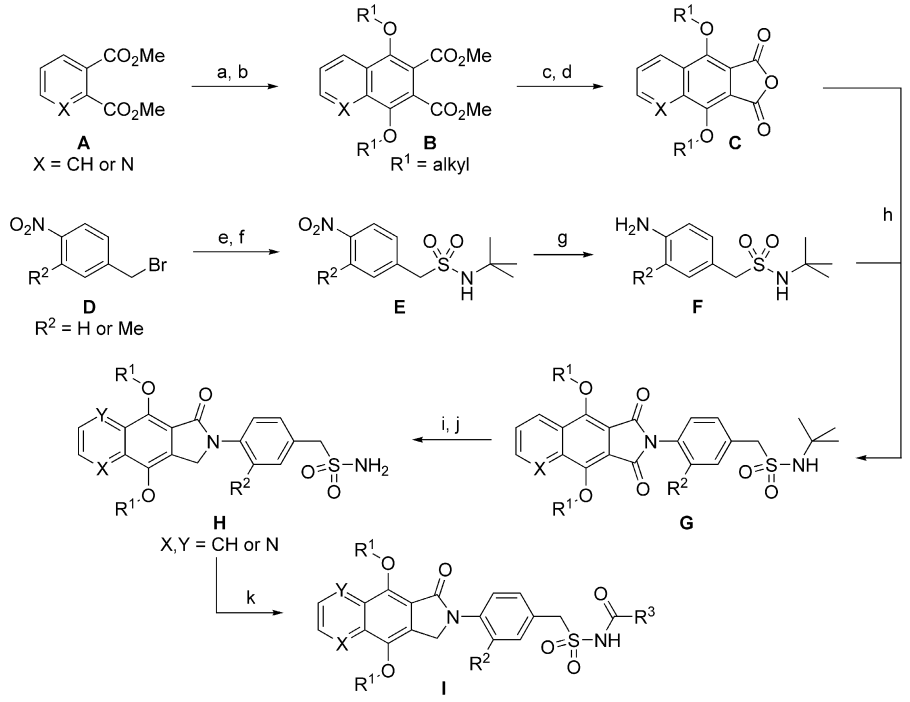



**Figure 1.** Planned SAR evolution.

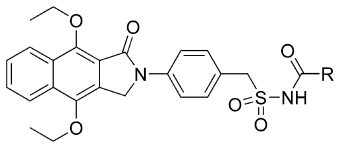
The first phase of our SAR investigation involved parallel synthesis of a ~50-member library of acylsulfonamides based on a naphthalene scaffold, differing in the acyl substituent. Selected results from this library are summarized in Table 1 below.

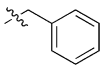
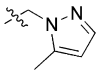
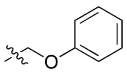
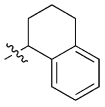
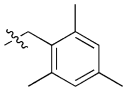
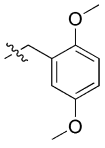
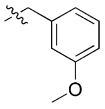
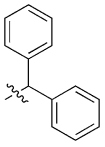
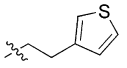
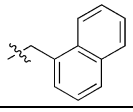
In terms of intrinsic binding affinity, good potency ($K_i$ < 10 nM) is maintained for a wide range of acyl substituents: methylene aryl (entries 1, 4–8 and 10), methylene heteroaryl (entry 2), methylene phenoxy (entry 3) and bis-methylene heteroaryl (entry 9). The extent of protein shift, however, is drastically affected by the constitution of this position. While a simple benzyl substituent leads to extensive protein shift (entry 1), substitution of the *ortho*-position of the aryl or heteroaryl ring significantly improves the potency in the presence of 10% human serum (entries 1, 5, 6 and 10). In particular, 2,5-dimethoxyphenyl (entry 6, 100-fold shifted) and naphthalene (entry 10, 10-fold shifted) provide the best results. For simplicity, moving forward, *ortho*-methoxy-




**Scheme 1.** Reagents: (a) $(MeO_2CCH_2)_2$, NaH, MeOH; (b) $R^1I$ or $R^1OTf$ $K_2CO_3$; (c) NaOH; (d) $Ac_2O$; (e) $H_2NCSNH_2$; (f) $Cl_2$, $^tBuNH_2$; (g) $H_2$, Pd/C; (h) AcOH; (i) $NaBH_4$; (j) TFA, $Et_3SiH$; (k) $R^3COCl$, $Et_3N$ or $R^3COOH$, EDC, DMAP.

**Table 1.** Library of naphthalene acylsulfonamides[a]



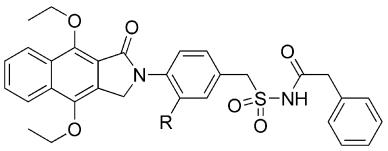
| Entry | R | Compound | $EP_4$ $K_i$ (nM) | |
|---|---|---|---|---|
| | | | 0% HS | 10% HS |
| 1 |  | (**2**) | 0.31 ± 0.01 | >4000 |
| 2 |  | (**3**) | 2.0 ± 0.4 | 600 ± 130 |
| 3 |  | (**4**) | 0.38 ± 0.08 | 1200 ± 500 |
| 4 |  | (**5**) | 1.0 ± 0.1 | 940 ± 470 |
| 5 |  | (**6**) | 0.43 ± 0.07 | 380 ± 60 |
| 6 |  | (**7**) | 0.37 ± 0.07 | 38 ± 12 |
| 7 |  | (**8**) | 2.4 ± 0.5 | 1600 ± 400 |
| 8 |  | (**9**) | 7.9 ± 6.0 | >4000 |
| 9 |  | (**10**) | 4.2 ± 0.6 | >4000 |
| 10 |  | (**11**) | 0.43 ± 0.20 | 4.8 ± 2.2 |

[a] Values are means from at least three experiments; HS, human serum; for details of the $EP_4$ binding assay see Ref. 10.

phenylacetate was selected since we postulated that the 5-methoxy substituent did little to diminish protein shift (entry 7), and compounds possessing naphthalene substitution exhibited poor bioavailability.

Concurrent with these explorations, optimization studies on the central and western ring were undertaken. In the case of the central ring, we were interested in the effect of introduction of *ortho*-substitution. Originally the aim of this substitution was to investigate the effect of changing the orientation of this ring with regards to crystal packing and solubility. As can be seen in Table 2, this modification has a much more beneficial effect: introduction of an *ortho*-methyl substituent diminished the protein shift from >10,000-fold to ~100-fold (entry 2 vs entry 1). We postulate that this is due to a disruption in albumin binding by deviation from planarity.

**Table 2.** Effect of *ortho*-substitution on the central ring[a]



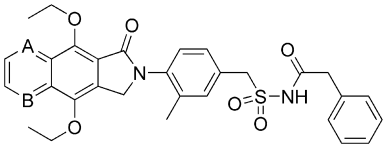
| Entry | R | Compound | $EP_4$ $K_i$ (nM) | |
|---|---|---|---|---|
| | | | 0% HS | 10% HS |
| 1 | H | (**2**) | 0.31 ± 0.01 | >4000 |
| 2 | Me | (**12**) | 0.25 ± 0.10 | 29 ± 11 |

[a] Values are means from at least three experiments; HS, human serum; for details of the $EP_4$ binding assay see Ref. 10.

For the western ring, we sought to replace the naphthalene ring with a quinoline. The benefits of this replacement were twofold: (a) synthetic access to the quinoline precursors was more favourable; and (b) introduction of a basic nitrogen would allow for the possibility of formation of a wider variety of salt forms for formulation. Further, we had hoped that altering the polarity of this region might have a positive effect on the extent of protein shift. As can be seen in Table 3, binding potency and shift are greatly affected by the regiochemistry of the quinoline ring: while the 6-carboxyquinoline (entry 2) exhibits similar potency and shift to the naphthalene (entry 1), the 7-carboxyquinoline (entry 3) is ~10-fold less potent and significantly more shifted. Although no benefit was observed in terms of potency and shift for the 6-carboxyquinoline, this scaffold was selected for further explorations due to the aforementioned practical benefits.

With an optimal scaffold selected, further SAR studies were performed on the phenylacetate (eastern) fragment through the synthesis of a second targeted library

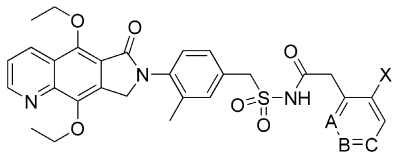
**Table 3.** Western fragment SAR[a]



| Entry | A | B | Compound | $EP_4$ $K_i$ (nM) | |
|---|---|---|---|---|---|
| | | | | 0% HS | 10% HS |
| 1 | CH | CH | (**12**) | 0.25 ± 0.10 | 29 ± 11 |
| 2 | CH | N | (**13**) | 0.76 ± 0.16 | 31 ± 28 |
| 3 | N | CH | (**14**) | 3.3 ± 1.3 | 660 ± 430 |

[a] Values are means from at least three experiments; HS, human serum; for details of the $EP_4$ binding assay see Ref. 10.

**Table 4.** Eastern SAR in the 6-carboxyquinoline series[a]



| Entry | A | B | C | X | Compound | $EP_4$ $K_i$ (nM) | |
|---|---|---|---|---|---|---|---|
| | | | | | | 0% HS | 10% HS |
| 1 | CH | CH | CH | H | (**13**) | 0.76 ± 0.16 | 31 ± 28 |
| 2 | CH | CH | CH | OMe | (**15**) | 0.74 ± 0.46 | 3.6 ± 1.0 |
| 3 | CH | CH | CH | $CF_3$ | (**16**) | 0.43 ± 0.11 | 350 ± 60 |
| 4 | CH | CH | CH | F | (**17**) | 0.48 ± 0.14 | 34 ± 16 |
| 5 | CH | CH | CH | Cl | (**18**) | 0.27 ± 0.07 | 37 ± 7 |
| 6 | CH | CH | CH | $OCF_3$ | (**19**) | 0.21 ± 0.06 | 56 ± 44 |
| 7 | CH | CH | CH | OEt | (**20**) | 0.27 ± 0.03 | 8.9 ± 5.4 |
| 8 | CH | CH | CH | $OCHF_2$ | (**21**) | 0.32 ± 0.08 | 13 ± 11 |
| 9 | N | CH | CH | H | (**22**) | 2.4 ± 1.0 | 13 ± 7 |
| 10 | CH | N | CH | H | (**23**) | 2.8 ± 0.9 | 48 ± 11 |
| 11 | CH | CH | N | H | (**24**) | 9.8 ± 4.1 | 160 ± 30 |
| 12 | CCl | CH | CH | Cl | (**25**) | 0.28 ± 0.05 | 4.8 ± 2.8 |
| 13 | COMe | CH | CH | OMe | (**26**) | 0.12 ± 0.09 | 2.3 ± 0.6 |

[a] Values are means from at least three experiments; HS, human serum; for details of the $EP_4$ binding assay see Ref. 10.

(Table 4). As expected from our initial library screen, introduction of an *ortho*-methoxy substituent resulted in a dramatic reduction in protein shift (entry 2 vs entry 1). While electron withdrawing substituents were tolerated at the *ortho*-position in terms of intrinsic potency (entries 2–5), this substitution did not provide any benefit in terms of protein shift. Replacement of the hydrogen atoms of the methoxy substituent with two or three fluorine atoms (entries 6 and 8) or an additional methyl group (entry 7) also resulted in an increase in protein shift. We investigated the replacement of the phenylacetate with the various isomers of pyridylacetates (entries 9–11). In all cases this resulted in a significant reduction in intrinsic binding potency, but interestingly the pyridin-2-yl isomer (entry 9) was only moderately shifted. Finally, we were interested in exploring the effect of substituting both *ortho* positions (entries 12 and 13). In the case of chloro and methoxy substitution, this addition further increased the intrinsic binding potency and, in the case of chlorine (entry 12), dramatically reduces the extent of protein shift.

Compound **15**, alternatively referred to as MF498 in other publications,[15] was selected for further profiling due to its excellent intrinsic potency and moderate protein shift. This compound was found to be potent ($IC_{50}$ = 1.1 ± 0.2 nM) and moderately shifted (~10-fold in the presence of 10% human serum) in an $EP_4$ functional assay[16] and was shown to be a full antagonist. A full prostanoid receptor screen was performed (Table 9, vide supra) and **15** was found to be nearly 1000-fold selective against all other receptors.

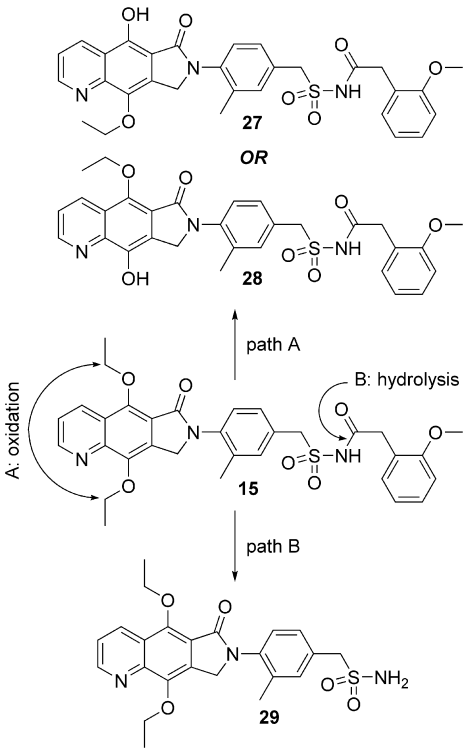
Analysis of the pharmacokinetic parameters of **15** showed that it exhibited acceptable absorption, half-life and clearance in a variety of species (Table 5). Two main circulating metabolites were observed (Scheme 2): phenol **27** or **28** and deacylated sulfonamide (**29**). Unlike the acid metabolite of **1**, sulfonamide **29** showed no functional activity towards the $EP_4$ receptor. The deethylation product was troubling, due to the possibility of further metabolic degradation to a quinone (not observed) which could act as a reactive intermediate leading to covalent protein labelling. Further, in vitro microsomal incubations across a variety of species indicated that acylsulfonamide hydrolysis leading to **29** was likely to be exacerbated in higher species, especially humans. For these reasons, further SAR explorations were undertaken with the goal of limiting these metabolic liabilities.

**Table 5.** Pharmacokinetic parameters for compound **15**[a]

| Species | Dose (mg/kg) | | $F$ (%) | $T_{1/2}$ (h) | Cl (mL/min/kg) | $V_{dss}$ (L/kg) |
|---|---|---|---|---|---|---|
| | po | iv | | | | |
| Rat | 20 | 5 | >99 | 2.7 | 7.8 | 1.6 |
| Guinea pig | 20 | 5 | 18 | 8.6 | 3.6 | 0.6 |
| Dog | 10 | 2 | 49 | 4.1 | 0.4 | 0.1 |

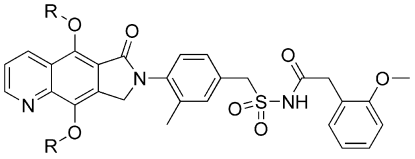
[a] The corresponding sodium salt was used for pharmacokinetic studies. $F$ denotes bioavailability; $T_{1/2}$ is the half-life in plasma, and is determined from the intravenous (iv) data; Cl is plasma clearance; $V_{dss}$ is the volume of distribution; po vehicle is 0.5% methocel; iv vehicle is 60% PEG 200.

In order to limit in vivo oxidative deethylation, the ethyl groups were replaced by the oxidatively more stable trifluoroethyl and isopropyl substituents (Table 6). While intrinsic binding potency was maintained, both compounds were more shifted in the presence of human serum. Nonetheless, it was hoped that by further tuning of the acylsulfonamide (eastern) terminus, acceptable levels of protein shift could be obtained with the trifluoroethyl replacement.




**Scheme 2.** Observed circulating metabolites of compound **15**.

**Table 6.** Diphenol diether SAR[a]



| Entry | R | Compound | $EP_4$ $K_i$ (nM) | |
|---|---|---|---|---|
| | | | 0% HS | 10% HS |
| 1 | $CH_2CH_3$ | (**15**) | 0.74 ± 0.46 | 3.6 ± 1.0 |
| 2 | $CH_2CF_3$ | (**30**) | 0.38 ± 0.10 | 17 ± 10 |
| 3 | $CH(CH_3)_2$ | (**31**) | 0.36 ± 0.15 | 26 ± 10 |

[a] Values are means from at least three experiments; HS, human serum; for details of the $EP_4$ binding assay see Ref. 10.

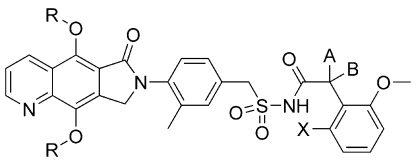
In an effort to reduce the extent of in vivo acylsulfonamide hydrolysis, the steric hindrance near the carbonyl group was increased (Table 7). Replacement of the methylene bridge with a cyclopropyl substituent resulted in the retention of binding potency and shift (entry 2 vs entry 1). Unfortunately, replacement of the ethyl moieties with trifluoroethyl groups resulted in the same increase in protein shift observed above (entry 3). Fortuitously, while an additional *ortho*-methoxy substituent had minimal effect in the diethyl case (entry 4 vs entry 1), the same replacement in the bis-trifluoroethyl series had a marked effect (entry 5 vs entry 3). Acylsulfonamide **35** exhibits similar intrinsic potency to our previous lead (**15**), but has improved protein shift, and possesses the oxidatively more stable trifluoroethyl substituents on the dihydroquinone moiety, and also has increased steric hindrance about the acylsulfonamide carbonyl. Compound **35** was thus selected for further in vitro and in vivo profiling.

Analysis of the pharmacokinetic parameters of compound **35** indicated a good overall profile across several species (Table 8). Further, in vitro metabolism in hepatocytes showed good correlation with the in vivo results, and demonstrated that human pharmacokinetics would be expected to be intermediate between dog and rhesus monkey. Acylsulfonamide hydrolysis was observed in vitro and in vivo (see Scheme 3), but to a lesser extent than was observed for compound **15**. No dealkylation of the western dihydroquinone was observed in any species.

Compound **35** was found to be potent ($IC_{50}$ = 3.2 ± 1.6 nM) and was only moderately shifted in the presence of 10% human serum (~4-fold) in an assay for functional $EP_4$ antagonism,[15] and was shown to be a full antagonist. A full prostanoid receptor screen was performed (Table 9), and **35** was found to be significantly less selective than **15**, but still showed a >50-fold selectivity against the other receptors.

Compound **35** was then submitted to an in vivo preclinical model for chronic inflammation: the rat adjuvent-induced arthritis (AIA) model.[17] This compound was found to be extremely potent in this model, with an

**Table 7.** Final SAR optimization[a]



| Entry | R | A | B | X | Compound | $EP_4$ $K_i$ (nM) | |
|---|---|---|---|---|---|---|---|
| | | | | | | 0% HS | 10% HS |
| 1 | $CH_2CH_3$ | H | H | H | (**15**) | 0.74 ± 0.46 | 3.6 ± 1.0 |
| 2 | $CH_2CH_3$ | –$CH_2CH_2$– | | H | (**32**) | 0.55 ± 0.29 | 4.2 ± 1.2 |
| 3 | $CH_2CF_3$ | –$CH_2CH_2$– | | H | (**33**) | 0.95 ± 0.58 | 12 ± 7 |
| 4 | $CH_2CH_3$ | –$CH_2CH_2$– | | OMe | (**34**) | 0.54 ± 0.24 | 2.3 ± 1.0 |
| 5 | $CH_2CF_3$ | –$CH_2CH_2$– | | OMe | (**35**) | 0.79 ± 0.48 | 1.6 ± 0.7 |

[a] Values are means from at least three experiments; HS, human serum; for details of the $EP_4$ binding assay see Ref. 10.

**Table 8.** Pharmacokinetic parameters for compound **35**[a]

| Species | Dose (mg/kg) | | $F$ (%) | $T_{1/2}$ (h) | Cl (mL/min/kg) | $V_{dss}$ (L/kg) | Metab.[b] (%) |
|---|---|---|---|---|---|---|---|
| | po | iv | | | | | |
| Mouse | 100 | 10 | 44 | 3.5 | 2.6 | 0.7 | — |
| Rat | 20 | 10 | 73 | 14.8 | 0.6 | 0.7 | 6 |
| Dog | 4 | 5 | 69 | 13.2 | 0.2 | 0.2 | 20 |
| Rhesus | 4 | 2 | 40 | 3.8 | 2.0 | 0.3 | 39 |
| Human | — | — | — | — | — | — | 36 |

[a] The corresponding sodium salt was used for pharmacokinetic studies. $F$ denotes bioavailability; $T_{1/2}$ is the half-life in plasma, and is determined from the intravenous (iv) data; Cl is plasma clearance; $V_{dss}$ is the volume of distribution; po vehicle is 0.5% methocel; iv vehicle is 60% PEG 200.
[b] In vitro metabolism in standard hepatocytes (20 μM **35**, $1 \times 10^6$ cells/0.5 mL, 2 h).

**Table 9.** Prostanoid receptor binding affinities ($K_i$, nM) for lead compounds **15** and **35**[a]

| Compound | $EP_1$ | $EP_2$ | $EP_3$ | $EP_4$ | DP | TP | FP | IP |
|---|---|---|---|---|---|---|---|---|
| **15** | >7000 | 1900 ± 800 | 4000 ± 1000 | 0.74 ± 0.46 | 1200 ± 300 | 630 ± 120 | 2000 ± 1000 | >7000 |
| **35** | >9000 | 300 ± 200 | 2700 ± 1500 | 0.79 ± 0.48 | 53 ± 16 | 39 ± 26 | 340 ± 240 | 2000 ± 1000 |

[a] Values are means from at least three experiments; for details of the prostanoid binding assays see Ref. 10.

$ED_{50}$ of approximately 0.005 mg/kg ($[\mathbf{35}]_{trough}$ = 30 nM) with once-daily dosing, making compound **35** one of the most potent compounds reported in the rat-AIA model. For comparison, the COX-2 inhibitor rofecoxib exhibited an $ED_{50}$ of 0.74 mg/kg/day in this model.[16]

In summary, we have presented a potent and selective $EP_4$ receptor antagonist (compound **35**). This compound exhibits subnanomolar binding potency for the $EP_4$ receptor and >50-fold selectivity against other prostanoid receptors. Compound **35** is also potent in a functional assay for $EP_4$ antagonism, and exhibits good pharmacokinetic properties across several preclinical species. Potency was optimized and protein shift was minimized by the introduction of *ortho*-substituents on the central and eastern aromatic rings, and metabolic liabilities were minimized by reducing the oxidative instability of the dihydroquinone ether groups, and by increasing the steric hindrance around the acylsulfonamide moiety. This compound has shown excellent efficacy in an in vivo preclinical model of inflammation, and further details of these discoveries will be reported in due course.

## References and notes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