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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 4767–4771

# Identification of novel pyrazole acid antagonists for the $EP_1$ receptor

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Received 25 May 2006; revised 29 June 2006; accepted 29 June 2006
Available online 14 July 2006

**Abstract**—The discovery, synthesis and structure–activity relationship (SAR) of a novel series of $EP_1$ receptor antagonists is described. Pyrazole acid **4**, identified from a chemical array, had desirable physicochemical properties, an excellent in vitro microsomal inhibition and cytochrome P450 (CYP450) profile and good exposure levels in blood. This compound had an $ED_{50}$ of 1.3 mg/kg in a rat pain model. A range of more potent analogues in the in vitro assay was identified using efficient array chemistry. These $EP_1$ antagonists have potential as agents in the treatment of $PGE_2$ mediated pain.


Prostaglandin $E_2$ ($PGE_2$) is one of a number of prostaglandins generated through the metabolism of arachidonic acid and acts locally both in the periphery and CNS. $PGE_2$ mediates a multitude of pharmacological actions and acts predominantly through four identified GPCRs ($EP_{1-4}$).[1] $EP_1$ receptor-deficient mice show reduced pain sensitivity, similar to the effects of COX-2 inhibition with a NSAID (non-steroidal anti-inflammatory drug). $EP_1$ receptor antagonist ONO-8711 has shown efficacy in numerous preclinical models of pain in rats, including allodynia,[2] neuropathic pain,[3] and postoperative pain.[4] Furthermore, the $EP_1$ receptor antagonist ZD-6416 (**1**) has demonstrated human efficacy in visceral pain.[5] Hence, selective $EP_1$ receptor antagonists would be expected to be efficacious in the treatment of pain and, in addition, have the potential to circumvent the gastrointestinal (GI) side effects associated with NSAIDS,[6] and the cardiovascular side effects associated with COX-2 inhibitors.[7] Several small molecule $EP_1$ receptor antagonists have been identified by various companies, including ZD-6416 (**1**)[8,9] from AstraZeneca, ONO-8711 and ONO-8713 (**2**)[10] from Ono and thiophene **3**[11] from Merck Frosst, Figure 1.

In our efforts to discover new $EP_1$ antagonists we initiated a chemistry effort exploring azole acids as motifs. We chose azoles because of their prevalence in drug molecules that have good general physico-chemical properties and good bioavailability.[12] We report here the discovery, SAR and synthetic accessibility of pyrazole acids (e.g., compound **4**, Fig. 2) as a new series of $EP_1$ antagonists.

We chose to utilize the well-established lipophilic *ortho*-benzyloxyphenyl portion of known $EP_1$ antagonists (e.g., **3**) as a privileged fragment to attach to a variety of heterocyclic acids using simple alkylation chemistry, Scheme 1.

Alkylation of 54 azole esters with bromide **5** (detailed chemistry shown in Scheme 2) gave, after ester hydrolysis, the final products as azole acids. In some cases regioisomers were formed as a result of the azole alkylation chemistry, these were not separated but assayed as

*Keywords*: $EP_1$ antagonist; Pain; Pyrazole; Prostanoid.
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doi:10.1016/j.bmcl.2006.06.086

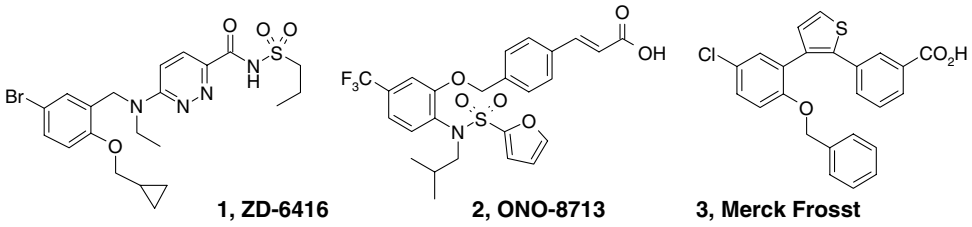



**Figure 1.** Selective 'non-prostanoid' $EP_1$ antagonists.

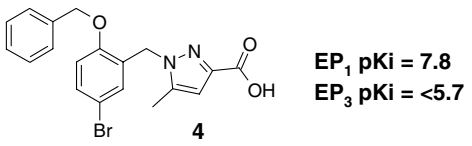



**Figure 2.**

mixtures. Our initial hit, **4**, was identified as a potent $EP_1$ antagonist having a $pK_i$ of 7.8 against $EP_1$ and $pK_i < 5.7$ against $EP_3$ in our FLIPR assays.[13] The $EP_1$ potency was confirmed in a $[^3H]PGE_2$ displacement binding assay ($pIC_{50}$ 8.2).[14] Pyrazole **4** was immediately attractive because of its relatively low molecular weight (401 Da), good solubility and selectivity against $EP_3$.

Compound **4** showed a low level of inhibition of CYP450 isoforms routinely tested and showed excellent stability in our microsomal clearance assay giving intrinsic clearances of <0.5 (rat) and 0.6 (human) mL/min/g liver (Table 1).

The in vivo pharmacokinetic (PK) profile of **4** was assessed in rat (Table 2) (dose = 3 mg/kg po). The data show a high level of **4** in blood after 0.5 h with a $C_{max}$ of 5.69 μM and an AUC/dose of 124 min kg/L suggesting good bioavailability (absolute bioavailability for this compound was not determined).

These data suggested that **4** and close analogues would have a suitable profile for oral administration. A chemistry programme was then initiated to explore the SAR around **4**.

**Table 1.** Physicochemical, in vitro metabolism and CYP450 profile of **4**

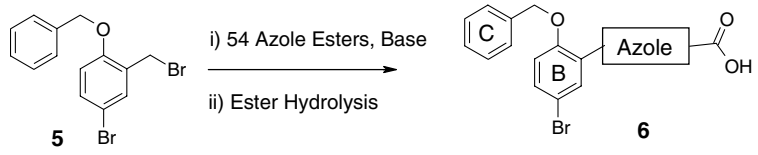
| Parameter | Value |
|---|---|
| Solubility @ pH 7.4 (mg/mL) | 0.85 |
| CYP450,[a] $IC_{50}$ (μM) | 21 @ 1A2, >100 @ 2C9,>100 @ 2C19, >100 @ 2D6, >100 @ 3A4 |
| Microsomal Cli (mL/min/g liver) | <0.5 rat; 0.6 human |
| Clog *P* | 5.1 |
| log *D* @ pH 7.4 | 1.15 |
| Molecular weight | 401 |

[a] In vitro CYP450 assay results using Gentest protocol.

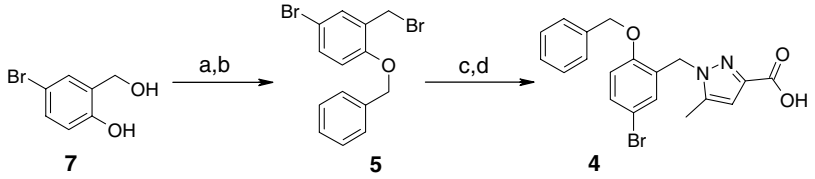
**Table 2.** In vivo PK data for compound **4**

| Parameter | Value |
|---|---|
| $C_{max}$ (μM) | 5.69 ± 1.41 |
| $T_{max}$ (h) | 0.5 |
| AUC (μM h) | 16.26 ± 6.49 |
| AUC/dose (min kg/L) | 124 ± 51 |

Initially we investigated the substitution on the pyrazole ring. Each of the compounds was prepared according to Scheme 2. Phenol **7** was selectively alkylated and the intermediate alcohol was then converted with $PBr_3$ to the bromide **5**. A selection of pyrazole esters (commercially available) were then alkylated with **5** under basic conditions ($KO^tBu$). Ester hydrolysis gave the final carboxylic acids. Any regioisomers formed from the alkylation step were separated by chromatography, characterized by NOE and HMBC NMR experiments and assayed individually.




**Scheme 1.** Array chemistry plan.



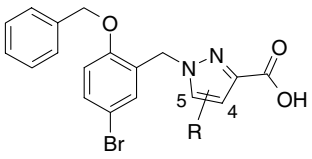

**Scheme 2.** Reagents and conditions: (a) EtOH, NaOH, BnBr; (b) $PBr_3$; (c) $KO^tBu$, EtOH, azole ester, 60 °C; (d) LiOH, 40 °C, EtOH.

No increase in potency was observed by varying either the substituent at the 4- or the 5-position of the pyrazole (Table 3). The des-methyl compound **8** showed similar potency to the hit molecule **4**. All analogues with substituents at the 4-position were less potent. Interestingly, increasing the size of the group at the 5-position from methyl to *n*-butyl (**11**) also led to a loss in potency. Introduction of a larger, more polar acetamide substituent also led to a decrease in potency (**10**).

We then investigated the effect on potency of the heterocycle and the importance of the regiochemistry of the carboxylic acid using the chemistry described in Scheme 2 and employing commercially available heterocyclic esters. Imidazole **18** and pyrazole **19** were inactive (Table 4) suggesting that the 1,3 relationship of the carboxylic acid and the methylene linker was required for optimal $EP_1$ potency. It also appeared that the heteroatom in the 2-position of the pyrazole was very important since the analogues **20**–**22** were far less potent. We believe that the 2-nitrogen between the carboxylic acid and methylene linker plays a significant role in varying the affinity to the receptor by influencing the acidity of the carboxylic acid (either by electron density effects or by means of an internal H-bond with the carboxylic acid) or by forming an additional H-bond with the receptor.

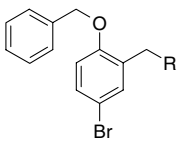
We next examined the substitution around phenyl ring C of the benzyl group. The synthesis of the analogues is described in Scheme 3. The hydrazine **24** was formed by reductive alkylation of *tert*-butyl carbazate with phenol aldehyde **23**. After TFA deprotection, the hydrazine **24** was treated with ethyl 2,4-dioxopentanoate (**25**) to yield predominantly the desired regioisomer **26** which was conveniently purified by preferential crystallization from the reaction mixture upon cooling. An efficient method[16] was then developed whereby, in the same pot, after alkylation of the phenol **26** (NaOH and alkylating agent), LiOH was added to affect ester hydrolysis. Final purification was achieved using chromatography.

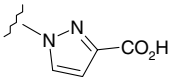
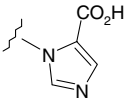
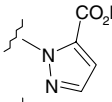
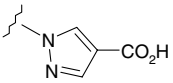
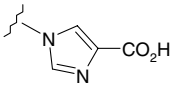
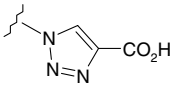
**Table 3.** $EP_1$ Potency of pyrazole substitutions




| Compound | R | Av $EP_1$ p$K_i$ (FLIPR)[13] | Av $EP_1$ p$IC_{50}$ (binding)[14] |
|---|---|---|---|
| **4** | 5-Me | 7.82 ± 0.38 | 8.19 ± 0.04 |
| **8** | 5-H | 7.26 ± 0.29 | 8.01 ± 0.10 |
| **9** | 5-(2-)Thiophene | 6.21 ± 0.14 | 6.71 ± 0.07 |
| **10** | 5-NHCOMe | <5.7 | |
| **11** | 5-″Bu | 6.01 ± 0.18 | |
| **12** | 4-Fluoro | 6.16 ± 0.32 | 6.91 ± 0.09 |
| **13** | 4-Me | 5.87 ± 0.16 | 6.37 ± 0.05 |
| **14** | 4-Br | <5.7 | 6.34 ± 0.06 |
| **15** | 4-$CH_2OH$ | <5.7 | 6.17 ± 0.11 |
| **16** | 4-$CF_3$ | <5.7 | 5.59 ± 0.14 |
| **17** | 4-Cl | <5.7 | |
| **3**[11] | n/a | 8.90 ± 0.60[15] | 8.45 ± 0. 04[15] |

**Table 4.** $EP_1$ Potency of heterocycle replacement



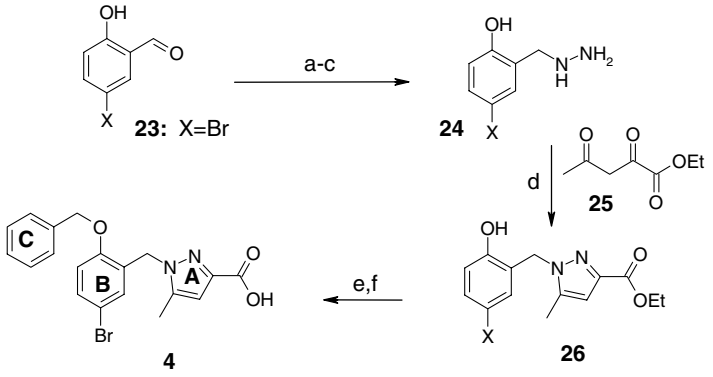

| Compound | R | Av $EP_1$ p$K_i$ (FLIPR)[13] |
|---|---|---|
| **8** |  | 7.26 ± 0.29 |
| **18** |  | <5.7 |
| **19** |  | <5.7 |
| **20** |  | <5.7 |
| **21** |  | <5.7 |
| **22** |  | <5.7 |

The results in Table 5 show that substitution at the 3-position of ring C was detrimental to potency (entries **35**–**41**). However, substitution at the 2- and/or 4-position led to analogues with up to 100-fold increase in potency for $EP_1$ with the 2,4-dichloro derivative (**27**) giving the highest functional potency (p$K_i$ 9.2). All compounds in Table 5 showed no inhibition in our $EP_3$ FLIPR assay (p$K_i$ < 5.7).

Finally, we undertook a combinatorial approach to investigate the effect of varying the bromine substituent in **4** with either Cl, F, OMe, or H, together with varying ring C with those substitutions that gave an increase in potency relative to **4** in Table 5. The syntheses of the four phenol intermediates were achieved by using the conditions described in Scheme 3.[16] Each of the four phenols was then alkylated with 7 chosen alkylating agents.

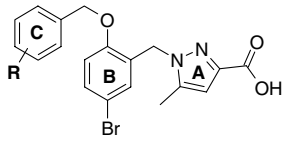
Table 6 shows the potency of selected results from the array with the bromo- and chloro-substituent, giving highest potency on central ring B. Replacement of the bromo with hydrogen (**46**) resulted in a 10-fold drop in potency. Figure 3 shows the results of the complete combinatorial array (and includes the original bromo compounds for comparative purposes) and illustrates that the SAR is consistent between the substituents on ring C and B, with 2,4-dichloro consistently giving the higher potency and the chloro substituent giving generally higher potency over bromo on ring B.

Each of the analogues **44**–**48** showed very low clearance in the microsomal clearance assay and low inhibitory



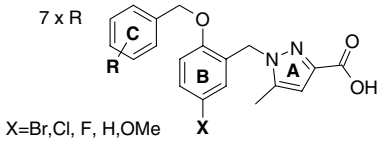

**Scheme 3.** Reagents and conditions: (a) AcOH, $BocNHNH_2$; (b) $Na(OAc_3)BH$; (c) TFA, $CH_2Cl_2$; (d) **25**, AcOH, reflux, 1 h; (e) EtOH, NaOH, RBr, 70 °C, 16 h; (f) EtOH, LiOH, 40 °C, 3 h.

**Table 5.** $EP_1$ Potency of benzyloxy analogues




| Compound | R | Av $EP_1$ p$K_i$ (FLIPR)[13] | Av $EP_1$ p$IC_{50}$ (binding)[14] |
|---|---|---|---|
| **27** | 2,4-DiCl | 9.31 ± 0.20 | 9.88 ± 0.32 |
| **28** | 2,4-DiF | 8.58 ± 0.41 | 9.28 ± 0.93 |
| **29** | 4-Cl | 8.06 ± 0.33 | 8.06 ± 0.51 |
| **30** | 4-F | 7.93 ± 0.34 | 8.13 ± 0.39 |
| **4** | H | 7.82 ± 0.38 | 8.19 ± 0.04 |
| **31** | 2-Cl | 7.54 ± 0.19 | 8.50 ± 1.40 |
| **32** | 2-MeO | 7.16 ± 0.19 | |
| **33** | 4-$CF_3$ | 7.01 ± 0.11 | 7.45 ± 0.50 |
| **34** | 2,6-DiF | 6.98 ± 0.15 | 7.83 ± 0.55 |
| **35** | 3-Br | 6.89 ± 0.23 | 7.75 ± 0.12 |
| **36** | 3-Cl | 6.37 ± 0.15 | 6.94 ± 0.66 |
| **37** | 3-Me | 6.28 ± 0.09 | 6.90 ± 0.53 |
| **38** | 3-$NO_2$ | 6.47 ± 0.54 | |
| **39** | 3-$CF_3$ | 5.96 ± 0.07 | 6.51 ± 0.61 |
| **40** | 3,5-DiCl | 5.95 ± 0.15 | |
| **41** | 3-$CF_3$,4-Cl | <5.7 | 6.24 ± 0.17 |
| **42** | 4-$^t$Bu | <5.7 | 5.91 ± 0.35 |
| **43** | 3,5-di$CF_3$ | <5.7 | 5.28 ± 0.23 |

**Table 6.** $EP_1$ Potency of examples from Scheme 3




| Compound | X | R | Av $EP_1$ p$K_i$ (FLIPR)[13] | Av $EP_3$ p$K_i$ (FLIPR)[2] |
|---|---|---|---|---|
| **27** | Br | 2,4-DiCl | 9.31 ± 0.20 | <5.7 |
| **44** | Cl | 2,4-DiCl | 9.26 ± 0.14 | <5.7 |
| **45** | Cl | 2,4-DiF | 8.95 ± 0.24 | <5.7 |
| **46** | H | 2,4-DiCl | 8.08 ± 0.01 | <5.7 |
| **47** | F | 2,4-DiCl | 7.69 ± 1.14 | 6.45 ± 0.14 |
| **48** | F | 2,4-DiF | 8.10 ± 0.16 | <5.7 |

potential of CYP450 isoforms (data not shown), similar to that described for **4**. A number of pyrazole acids have now been progressed through the established FCA (Freund's Complete Adjuvant) in vivo rat model of inflammatory pain.[17] Pyrazole **4** was effective in this model with an $ED_{50}$ of 1.3 mg/kg. This and related compounds are now the subject of further investigations.

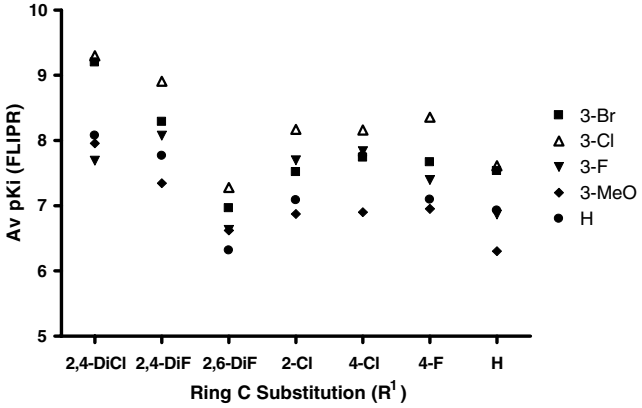



**Figure 3.** $EP_1$ SAR around rings B (shapes) and C (*x*-axis).

In summary, we have described the discovery, synthesis and structure–activity relationships (SARs) of a novel series of selective $EP_1$ antagonists. Pyrazole acid **4**, identified from a chemical array, had desirable physicochemical properties and was shown to attain high exposure levels in blood after oral administration. A range of potent analogues with potencies up to 9.3 (p$K_i$) were identified using efficient array chemistry. These $EP_1$ antagonists show potential as useful agents in the treatment of pain.

## Acknowledgments

We thank Pat McDonough and Klara Valko-Slegel for carrying out the physical chemical measurements.

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13. The p$K_i$ of compounds were measured using stable either $EP_1$ or $EP_3$ transfected CHO-K1 whole cells and by measuring inhibition of intracellular $Ca^{2+}$ mobilisation in response to $PGE_2$. Calcium sensitive dye (Fluo-3) was assessed using a Fluorimetric Image Plate Reader (FLIPR). Values are means of at least three ($EP_1$) or two ($EP_3$) experiments.
14. The binding assay was conducted using stable $EP_1$ transfected CHO-K1 membranes and [$^3$H]$PGE_2$ as the radio ligand. Cell membranes, compounds and [$^3$H]$PGE_2$ (3 nM final assay concentration) were incubated in a final volume of 100 μL for 30 min at 30 °C. The radioactivity retained was measured by liquid scintillation counting in a Packard TopCount scintillation counter. Values are means of at least four experiments.
15. These data agree well with data published by Merck Frosst[11] (binding p$K_i$ = 8.4, functional p$K_b$ = 8.15).
16. Typical experimental procedure (e.g., from compound **26** to **4**): The phenol (16.9 mg, 0.05 mmol) was dissolved in ethanol (0.5 mL) and 2 M sodium hydroxide (0.028 mL, 0.055 mmol) and stirred at room temperature for 5 min. The alkylating agent (10.4 mg, 0.05 mmol) in ethanol (0.5 mL) was added and the reaction mixture heated under nitrogen at 70 °C overnight. After cooling, the mixture was diluted with ethanol (0.5 mL) and a 0.5 M solution of lithium hydroxide in water (0.5 mL, 6.0 mg, 0.25 mmol) was added. The mixture was stirred at 40 °C for 3 h. After cooling, 2 M hydrochloric acid (0.15 mL, 0.3 mmol) was added and the mixture was diluted with water (2 mL). Dichloromethane (2 mL) was added and the mixture stirred vigorously. The organic layer was separated and the solvent removed in vacuo. The residue was purified by HPLC mass directed purification (fraction collection triggered by mass ion detection) to yield the title compound (isolated yields range from 26 to 80%). The purity of all compounds was greater than 85% by UV and NMR.
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