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# Identification and optimization of novel 1,3,4-oxadiazole $EP_1$ receptor antagonists

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**Abstract**—A novel series of oxadiazole $EP_1$ receptor antagonists was identified by replacing the amide of a known glycine sulfonamide derivative with a 1,3,4-oxadiazole. Optimization of the substitution patterns on the three aromatic rings led to the identification of high affinity $EP_1$ receptor antagonists. The derivative with highest affinity displayed a binding $IC_{50}$ of 2.5 nM ($pIC_{50}$ 8.6).


The $EP_1$ receptor is one of four receptor subtypes that is activated by prostaglandin $E_2$ ($PGE_2$).[1] Preclinical evidence has shown that $EP_1$ receptor antagonism could provide a potential treatment for several conditions such as inflammatory pain,[2] neuropathic pain,[3] postoperative pain,[4] over active bladder[5] and ischaemic stroke.[6]

We have previously reported some of our efforts to identify novel $EP_1$ receptor antagonists.[2,7–11] To this end, we recently disclosed a new series of glycine sulfonamides,[12] exemplified by **1a** (Fig. 1), which was identified as part of a high throughput screen (HTS). Optimization of this series gave compounds such as **1b** and **1c** (Fig. 1).[12]

Compounds from the glycine sulfonamide series generally suffered from poor metabolic stability (when assessed in rat and human liver microsomes in vitro). Incubation of compounds from this series with human liver microsomes allowed qualitative metabolite identification by mass spectrometry. The major routes of metabolism were oxidation of the alkyl groups on the A- and B-rings and dealkylation of the amide. Thus, we wished to replace the amide of this series and establish whether the metabolically labile alkyl groups on the A- and B-rings could be replaced by halogens or trifluoromethyl groups.

Herein, we describe our work within this series, where we have replaced the amide in compounds of type **1a–c** with an oxadiazole. This change resulted in a novel series of $EP_1$ antagonists which we hoped would have better metabolic stability and better developability characteristics than the amides typified by **1a–c**.

The first step in this project was to understand the role of the amide in compounds such as **1b**. Thus, in order to investigate if the amide was an important pharmacophore we synthesized the ester **1d** (Table 1). Based on the modest decrease in affinity of **1d** relative to **1b** we hypothesized that the amide and ester were acting as hydrogen bond acceptors (HBA). The decreased affinity of **1d** could be explained by the weaker hydrogen bond acceptor ability of the ester moiety relative to the amide.[13]

Thus, we sought to replace the amide of **1b** (or the ester of **1d**) with a heterocycle with HBA potential such as the 1,3,4-oxadiazole. We felt that the vicinal arrangement of the lone pairs of electrons on the nitrogen atoms would

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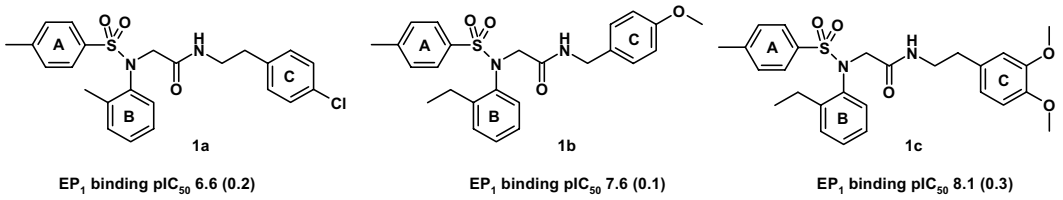
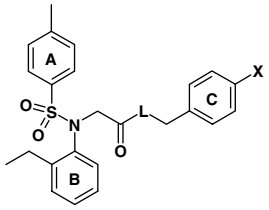



**Figure 1.** Profile of HTS hit **1a** and optimized derivatives **1b** and **1c**, with standard deviations in parentheses.

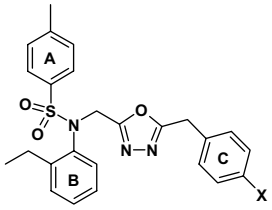
**Table 1.** Binding data for amide **1b** and corresponding ester **1d**



| Compound | L | X | Binding $pIC_{50}$[a] |
|---|---|---|---|
| **1b** | NH | OMe | 7.6 ± 0.1 |
| **1d** | O | OMe | 7.2 ± 0.2 |

[a] Values are means of three experiments.

**Table 2.** SAR for substitution of the C-ring



| Compound | X | Binding $pIC_{50}$[a] | FLIPR $pK_i$[a] |
|---|---|---|---|
| **2a** | H | 6.0 ± 0.1 | 6.4[b] |
| **2b** | Cl | 6.4 ± 0.1 | 6.5 ± 0.2[c] |
| **2c** | OMe | 6.9 ± 0.1 | 6.2 ± 0.4 |
| **2d** | OEt | 6.9 ± 0.1 | 7.4 ± 0.1 |

[a] Values are means of at least three experiments.
[b] Single experiment.
[c] Mean of two experiments.

mimic the lone pairs of electrons on the carbonyl oxygen atom[14] whilst improving metabolic stability.

Before undertaking the synthesis of the oxadiazole derivatives we carried out molecular overlays with the minimized structures of **1c** and an oxadiazole analogue (**2c**), using FLO,[15] Figure 2. This indicated a good overlap of the three phenyl rings (A, B, and C), including pendant functionality, and the sulfonamide moieties. Furthermore, the carbonyl group of the amide was very close to the nitrogen atoms of the oxadiazole, implying that a similar HBA interaction could possibly be formed by the oxadiazole.

Based on these observations we initiated the synthesis of oxadiazole analogues.

Compound affinities were determined using a [$^3$H]-$PGE_2$ binding assay at the human $EP_1$ receptor recombinantly expressed in CHO cell membranes.[7] Compounds were also profiled in a functional assay using the recombinant $EP_1$ receptor in CHO cells to measure their ability to block the intracellular $Ca^{2+}$ mobilization driven by $PGE_2$ ($Ca^{2+}$ mobilization measured by FLIPR).[7]

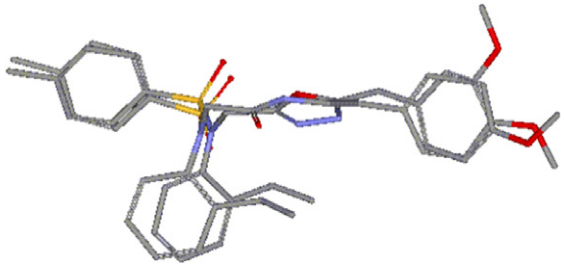


**Figure 2.** Molecular overlay of amide **1c** with oxadiazole **2c**.

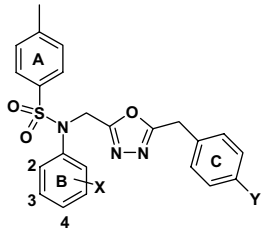
Thus, in the first round of synthesis we fixed the tosyl group (ring A) and the *ortho*-ethyl aniline (ring B), found in compounds **1b** and **1c**, and investigated substitution of the right hand side phenyl ring (ring C), (Table 2) utilising functionality that had proven beneficial for activity within the glycine sulfonamide series.[12] As expected, the 4-chloro, 4-methoxy and 4-ethoxy derivatives **2b–d** showed higher activity than the unsubstituted derivative **2a** (Table 2). Pleasingly, analogue **2c** (binding $pIC_{50}$ 6.9, $IC_{50}$ 126 nM) displayed a 5-fold decrease in affinity (binding assay) relative to the amide **1b** (binding $pIC_{50}$ 7.6, $IC_{50}$ 25 nM) and only a 2-fold decrease in affinity relative to the ester **1d** (binding $pIC_{50}$ 7.2, $IC_{50}$ 63 nM).

Replacement of the ester/amide by 1,2,4-oxadiazoles was also briefly investigated. Neither of the regioisomeric 1,2,4-oxadiazole analogues of **2b** gave an advantage over the parent (**2b**) in terms of binding affinity (results not shown), hence we focused on the 1,3,4-oxadiazoles.

Encouraged by the results in Table 2, we fixed a 4-alkoxy substituent on the C-ring and investigated optimization of the substituents on the aniline moiety (B-ring) (Table 3).

These results demonstrated a marked improvement in activity, as measured by binding affinity and functional antagonism, was possible by adding a second substituent in the 3-position of ring B. A chlorine atom in the 3-position proved optimal for affinity, whereas in the 2-position a methyl group or chlorine atom resulted in equal activity (Table 3).

**Table 3.** Initial exploration of substitution of the aniline moiety, B-ring



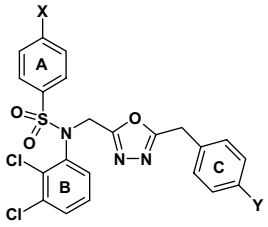
| Compound | X | Y | Binding $pIC_{50}$[a] | FLIPR $pK_i$[a] |
|---|---|---|---|---|
| **3a** | 2-Me, 3-$CF_3$ | OMe | 7.2 ± 0.2 | 6.4 ± 0.1 |
| **3b** | 2-Me, 3-Cl | OMe | 8.2 ± 0.2 | 7.4 ± 0.2 |
| **3c** | 2-Me, 3-Cl | OEt | 8.4 ± 0.3 | 7.3 ± 0.4 |
| **3d** | 2,3-diCl | OMe | 8.1 ± 0.2 | 7.4 ± 0.2 |
| **3e** | 2,3-diCl | OEt | 8.2 ± 0.1 | 7.5 ± 0.2 |

[a] Values are means of at least three experiments.

Fixing the 2-methyl-3-chloro aniline as ring B, we explored a wider range of substituents on various positions of the C-ring (Table 4). Initial results showed that the alkoxy groups present in compounds **3a–e** could be replaced by related electron donating groups such as the dimethylamino group present in analogue **4a**. We also found that the 4-trifluoromethyl group, as in **4b**, was an effective replacement for the 4-alkoxy group, thereby demonstrating that electron donating groups were not required for activity. Based on this result we investigated a range of halogenated C-ring analogues. Although the monofluorinated derivatives **4c–e** were less active, the 2-chloro analogue **4f** showed promising activity. Addition of a second chlorine atom retained activity, compounds **4h–i** (Table 4).

Returning to the 2,3-dichloro aniline (B-ring), we sought to investigate potential replacements for the methyl group on the sulfonamide A-ring (Table 5). Replacement of the methyl group in **3d** by a chlorine atom, as in **5a**, resulted in a moderate decrease in binding affinity but negligible change in activity in the functional assay. However, replacement of the methyl group in **3e** with a trifluoromethyl group, compound **5b**, led to a marked improvement in affinity in the binding assay and increased activity in the functional assay (Table 5).

**Table 5.** SAR for replacement of the 4-methyl group on the A-ring

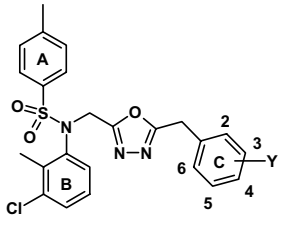


| Compound | X | Y | Binding $pIC_{50}$[a] | FLIPR $pK_i$[a] |
|---|---|---|---|---|
| **3d** | Me | OMe | 8.1 ± 0.2 | 7.4 ± 0.2 |
| **5a** | Cl | OMe | 7.7 ± 0.1 | 7.2 ± 0.1[b] |
| **3e** | Me | OEt | 8.2 ± 0.1 | 7.5 ± 0.2 |
| **5b** | $CF_3$ | OEt | 8.6 ± 0.1 | 8.2 ± 0.5 |

[a] Values are means of at least three experiments.
[b] Value is a mean of two experiments.

Finally, in order to ascertain whether the methylene linker was optimal we investigated various isosteric and homologated linkers (Table 6). Replacement of the methylene group by an oxygen atom was poorly tolerated (**6a**) in the binding assay although this compound still retained activity in the functional assay (FLIPR). However, the amino replacements **6b–d** retained activity in both assays. Analogue **6d** again highlighted the beneficial effect of the 4-trifluoromethyl group on the A-ring. Finally, the homologated analogues **6e–g** also retained activity. These results correlate well with the activity of the homologated analogues in the glycine sulfonamide series where it was found that both benzyl and phenethyl amides were active (Table 6).[12]

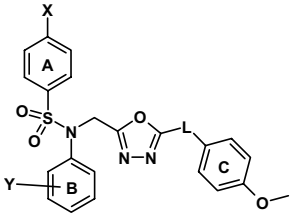
**Table 4.** SAR for substitution of the C-ring



| Compound | Y | Binding $pIC_{50}$[a] | FLIPR $pK_i$[a] |
|---|---|---|---|
| **4a** | 4-$NMe_2$ | 7.9 ± 0.1 | 7.7 ± 0.4 |
| **4b** | 4-$CF_3$ | 7.3 ± 0.1 | 6.8 ± 0.2 |
| **4c** | 4-F | 6.7 ± 0.1 | 6.3 ± 0.3 |
| **4d** | 2-F | 6.3 ± 0.3 | 6.2 ± 0.4 |
| **4e** | 3-F | 6.5 ± 0.2 | 6.3 ± 0.2 |
| **4f** | 2-Cl | 7.1 ± 0.2 | 6.4 ± 0.1 |
| **4g** | 3-Cl | 6.8 ± 0.1 | 5.7 ± 0.2 |
| **4h** | 2,6-diCl | 7.2 ± 0.2 | 6.3 ± 0.3 |
| **4i** | 2,4-diCl | 7.2 ± 0.1 | 6.0 ± 0.3 |

[a] Values are means of at least three experiments.

**Table 6.** SAR for derivatives with alternative linker groups



| Compound | X | Y | L | Binding $pIC_{50}$[a] | FLIPR $pK_i$[a] |
|---|---|---|---|---|---|
| **6a** | Me | 2-Et | O | <6 | 7.0 ± 0.4 |
| **6b** | Me | 2-Me, 3-Cl | NH | 7.5 ± 0.3 | 7.1 ± 0.2 |
| **6c** | Me | 2,3-diCl | NH | 7.4 ± 0.2 | 7.3 ± 0.1 |
| **6d** | $CF_3$ | 2,3-diCl | NH | 8.1 ± 0.0 | 7.2 ± 0.2 |
| **6e** | Me | 2-Et | $CH_2O$ | 7.6 ± 0.1 | 6.9 ± 0.1 |
| **6f** | Me | 2-Et | $CH_2NH$ | 6.3 ± 0.1 | 6.1 ± 0.2 |
| **6g** | Me | 2-Me, 3-Cl | $CH_2NH$ | 7.7 ± 0.1 | 7.1 ± 0.2 |

[a] Values are means of at least three experiments.

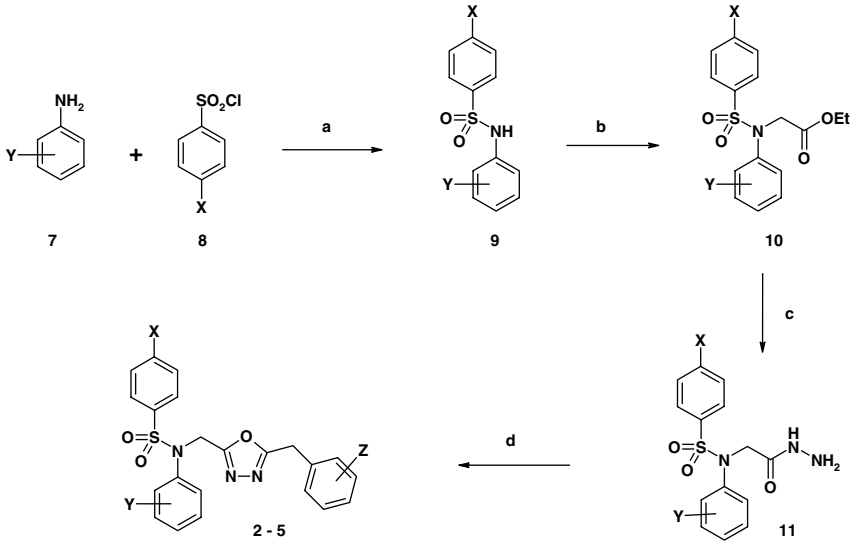
The synthesis of analogues was carried out as described in Schemes 1–4. All final compounds were characterized by LC/MS and $^1$H NMR spectroscopy.

Reaction of an appropriately substituted aniline, **7**, with a sulfonyl chloride, **8**, gave the corresponding sulfonamides **9**. Alkylation with ethyl bromoacetate gave esters **10**. Condensation with hydrazine gave the hydrazides **11** which were converted directly to the 1,3,4-oxadiazoles **2**–**5**[16] by reaction with substituted phenyl acetic acids in neat phosphorus oxychloride under microwave conditions at 100 °C (Scheme 1).

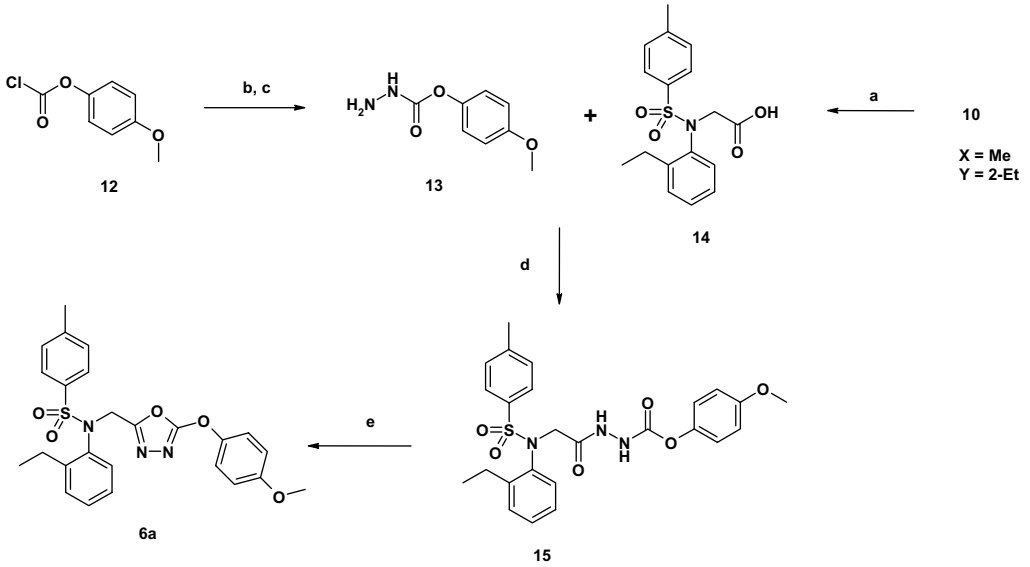
The oxygen-linked derivative **6a** was prepared as described in Scheme 2. Reaction of 4-methoxyphenyl chloroformate, **12**, with *tert*-butyl carbazate followed by deprotection gave **13**. Amide formation with glycine sulfonamide derivative **14** under standard conditions gave **15**, which was dehydrated with Burgess reagent[17] under microwave conditions to give **6a**.

The amino-linked derivatives **6b**–**d** were prepared as described in Scheme 3. Reaction of an appropriately substituted phenyl isocyanate derivative, **16**, with hydrazine gave the amino urea derivative **17**. Condensation of **17** with the glycine sulfonamide derivatives **14a** in phosphorus oxychloride under microwave irradiation at 100 °C furnished the oxadiazoles **6b**–**d**.

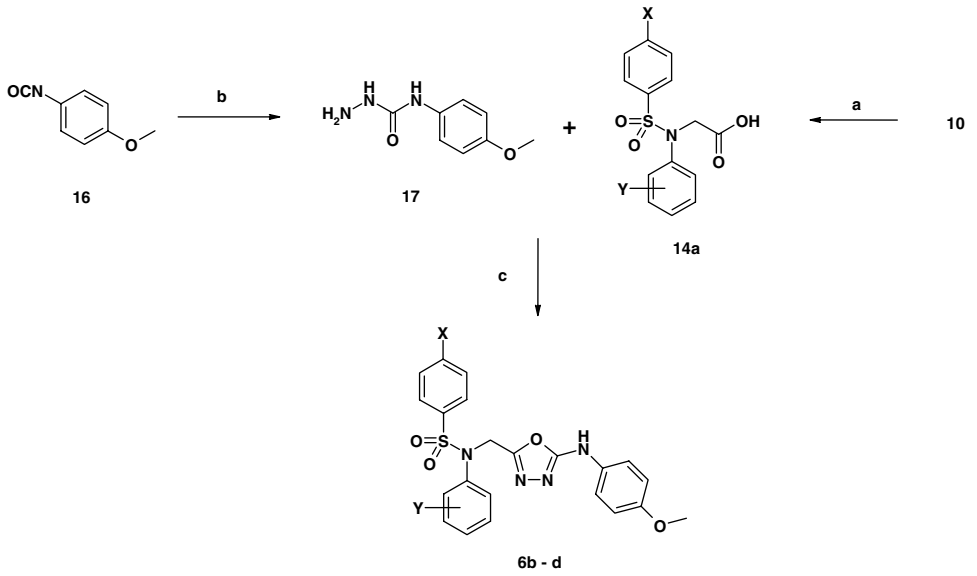
The homologated oxy and amino analogues **6e**–**g** were prepared as described in Scheme 4. Condensation of



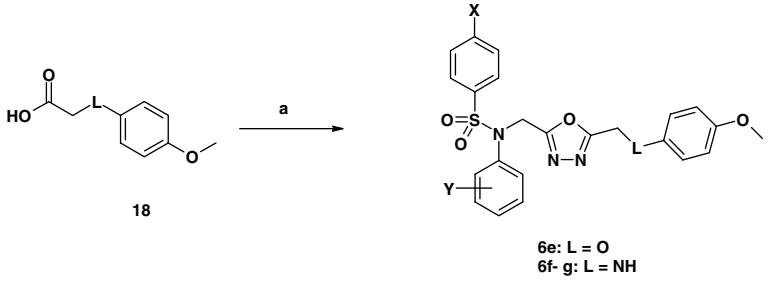

**Scheme 1.** Reagents and condition: (a) DCM, pyridine; (b) NaH, DMF, ethyl bromoacetate; (c) hydrazine hydrate, heat or microwave; (d) phenylacetic acid derivative, $POCl_3$, μ-wave, 100 °C, 30 min.




**Scheme 2.** Reagents and conditions: (a) EtOH, 2 M NaOH; (b) $BocNHNH_2$, EtOAc, TEA, 0 °C; (c) TFA then sat. $NaHCO_3$; (d) EDAC, HOBt, *N*-ethylmorpholine, DMF; (e) Burgess reagent, THF, μ-wave, 130 °C, 15 min.




**Scheme 3.** Reagents and conditions: (a) EtOH, 2 M NaOH; (b) DCM, hydrazine hydrate, −60 °C to rt; (c) $POCl_3$, μ-wave, 100 °C, 20 min.




**Scheme 4.** Reagents and conditions: (a) **11**, $POCl_3$, μ-wave, 100 °C, 20–40 min.

the required carboxylic acid derivative (**18**) with hydrazide **11** in neat phosphorus oxychloride under microwave irradiation at 100 °C yielded the desired oxadiazoles.

In conclusion, we have generated a novel series of 1,3,4-oxadiazole $EP_1$ receptor antagonists by replacing the amide group in a previously described series by a biosteric oxadiazole. Optimization of this series revealed that a 2,3-disubstituted aniline gave higher affinity than the starting 2-ethyl aniline. The tosyl group was best replaced by a 4-trifluoromethylbenzenesulfonyl group which led to a significant increase in binding affinity. The optimal compound in terms of in vitro activity was derivative **5b**, with a binding $IC_{50}$ of 2.5 nM ($pIC_{50}$ 8.6) and a functional $K_i$ of 6.3 nM (FLIPR $pK_i$ 8.2). Unfortunately the compounds described herein did not display sufficiently improved metabolic stability relative to the glycine sulfonamide series when assessed in rat and human liver microsomes in vitro.[18]

## Acknowledgment

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16. Analytical data for compound **3c**: $^1$H NMR $\delta_H$ (400 MHz, $CDCl_3$) 1.42 (3H, t), 2.13 (3H, s), 2.45 (3H, s), 3.92–4.15 (4H, m), 4.74–4.98 (2H, m), 6.43 (1H, d), 6.77–6.92 (3H, m), 7.02–7.17 (2H, m), 7.20–7.40 (3H, m), 7.48–7.60 (2H, m); *m/z* $[M+H]^+$ 512 and 514.
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