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Identification of novel glycine sulfonamide antagonists for the EP_1 receptor

Stephen C. McKeown,[a,*] Adrian Hall,[b,*] Richard Blunt,[a] Susan H. Brown,[b] Iain P. Chessell,[b] Anita Chowdhury,[b] Gerard M. P. Giblin,[b] Mark P. Healy,[b] Matthew R. Johnson,[b] Olivier Lorthioir,[a] Anton D. Michel,[b] Alan Naylor,[b] Xiao Lewell,[a] Shilina Roman,[b] Stephen P. Watson,[a] Wendy J. Winchester[b] and Richard J. Wilson[a]

[a]*Molecular Discovery Research, GlaxoSmithKline, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, UK*
[b]*Neurology and Gastrointestinal Centre of Excellence for Drug Discovery GlaxoSmithKline, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, UK*

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Abstract—A high-throughput screen targeting the EP_1 receptor identified non-acidic glycine sulfonamide derivative **2a** with a pK_i of 6.2. Analogue synthesis allowed a thorough investigation of the structure–activity relationship (SAR) and led to a 100-fold increase in recombinant potency.

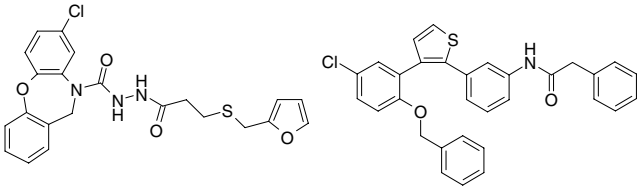
Prostaglandin E_2 (PGE_2) is one of a number of prostaglandins generated through the metabolism of arachidonic acid, and acts locally both in the periphery and CNS. PGE_2 mediates a multitude of pharmacological actions, acting predominantly through four identified G-protein coupled receptors (GPCRs, EP_{1-4}).[1] The EP_1 receptor has been of interest to the pharmaceutical industry due to the preclinical effects seen with small molecule antagonists in conditions such as inflammatory and neuropathic pain.[2–5]

In this paper, we describe the identification of a novel series of non-acidic glycine sulfonamides as potent EP_1 antagonists. We were particularly interested in these hit molecules because they do not contain the common acidic motif displayed in many EP_1 antagonists which, if present, would generally limit exposure to the CNS.[6] We wanted to explore molecules with CNS penetration since there is some evidence that centrally expressed EP_1 receptors are involved in certain types of pain.[7]

There have been several reports of carboxylic acid-containing EP_1 receptor antagonists.[8–11] However, there have been relatively few reports of non-acidic antagonists, such as those from Searle[12] (**1a**, SC-51322) and Merck Frosst[6] (**1b**), Figure 1.

A high-throughput screen of the GlaxoSmithKline compound collection, using a FLIPR based assay,[13] identified the glycine sulfonamide derivative **2a** with a functional pK_i of 6.2, and at least 10-fold selectivity over the EP_3 receptor, Figure 2.

Compound **2a** represented a novel EP_1 ligand that was chemically tractable, had low molecular weight and



1a[12], SC-51322; EP_1: pA_2 8.1 **1b[6], EP_1: pKi 8.4**

Figure 1. Known non-acidic EP_1 antagonists.

Keywords: EP_1 antagonist; Pain; Pyrazole; Prostanoid.
* Corresponding authors. Tel.: +44 1279 627558; fax: +44 1279 87 5414 (S.C.M.); tel.: +44 1279 623464 (A.H.); e-mail addresses: Steve.C.McKeown@gsk.com; Adrian.2.Hall@gsk.com

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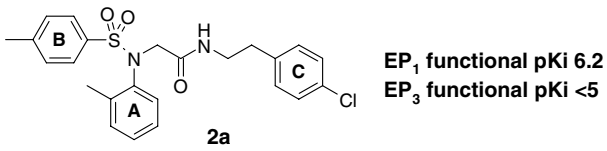
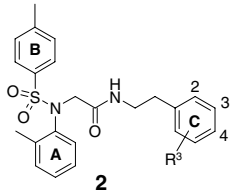


Figure 2. HTS hit compound **2a**.

possessed no carboxylic acid or similarly acidic group. We explored the structure–activity relationship (SAR) by varying four regions of the hit molecule **2a**.

Screening of phenethyl amide analogues (a selection is shown in Table 1) provided initial SAR around ring C. These were synthesized according to Scheme 1 (route A). It was found that the 3,4-dimethoxyphenethyl amide was one of the most preferred groups, compound **2b**, with a functional pK_i of 7.4. The most active analogue of the phenethyl amides was compound **2g** with a functional pK_i of 8.4. However, no further derivatives of this compound were prepared due to its higher molecular weight and polar surface area. It appears that substitution on this ring is required since potency of the unsubstituted ring, **2h**, is reduced to pK_i 5.8.

Table 1. SAR of the phenethylamide derivatives, **2a**–**2h**

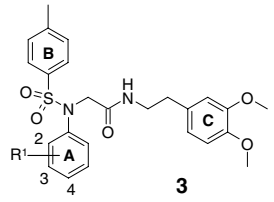


Compound	R^3	EP$_1$ functional pK_i[13]
2a	4-Cl	6.18 ± 0.30
2b	3,4-DiOMe	7.41 ± 0.23
2c	4-OMe	6.77 ± 0.18
2d	3-OH	6.69 ± 0.05
2e	4-NHSO$_2$Me	7.05 ± 0.07
2f	2-F–3,4-DiOMe	7.18 ± 0.01
2g	3-SO$_2$NHMe,4-OH	8.43 ± 0.06
2h	H	5.87 ± 0.22
1b		7.9 ± 0.7[14]

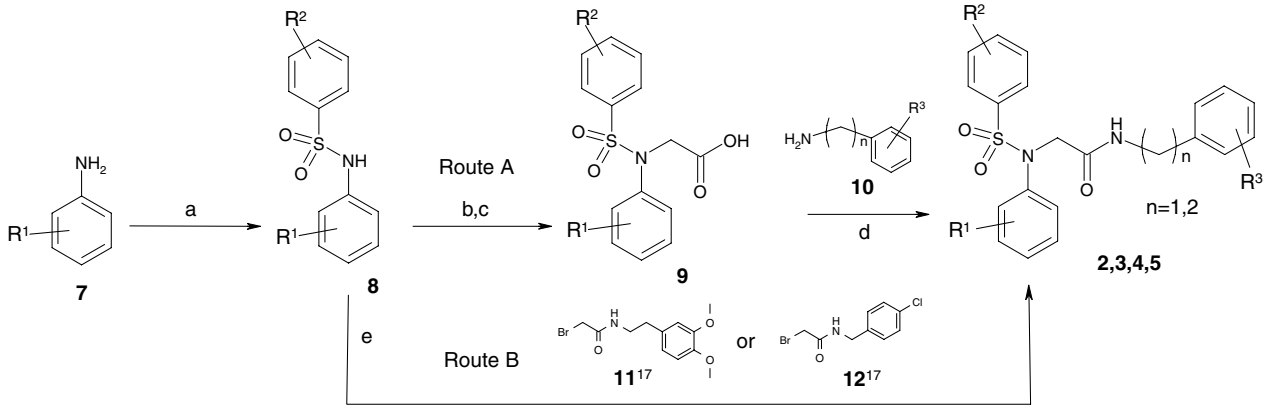
Fixing the newly discovered 3,4-dimethoxyphenethyl amide moiety, we next sought to explore the SAR around the aniline moiety (ring A), Table 2. These compounds were synthesized according to Scheme 1 (using route B).

Removal of the 2-methyl substituent present in **2b** reduced activity by approximately 16-fold (compound **3a** vs **2b**). Substitution of the aniline moiety at the 4-position generally led to a marked decrease in activity; electron-withdrawing groups such as F, Cl and Br were not well tolerated, compounds **3b**–**d**; and similarly, electron-donating groups such as MeO (**3f**) and Me (**3e**) showed no activity. Further SAR of the aniline moiety indicated that the 2- and 3-positions were amenable to substitution. For example, the 3-Me (**3g**) and 3-Cl (**3h**) derivatives displayed an almost 10-fold improvement in activity relative to the unsubstituted analogue **3a**. The 3-CF_3 (**3j**) and Br (**3i**) groups showed the highest activity of the 3-substituents, with functional pK_i values

Table 2. SAR for substitution (R^1) of the aniline moiety, **3a**–**3l**



Compound	R^1	EP$_1$ functional pK_i[13]
3a	H	6.23 ± 0.01
2b	2-Me	7.41 ± 0.23
3b	4-F	<5.8
3c	4-Cl	5.89 ± 0.23
3d	4-Br	<5.42
3e	4-Me	<5.95
3f	4-OMe	<5.8
3g	3-Me	7.21 ± 0.04
3h	3-Cl	7.21 ± 0.25
3i	3-Br	7.46 ± 0.32
3j	3-CF_3	7.51 ± 0.01
3k	2-Et	7.87 ± 0.28
3l	2,3-diMe	7.89 ± 0.19



Scheme 1. Reagents and conditions: (a) $ArSO_2Cl$, CH_2Cl_2, pyridine, 16 h; (b) 2 N NaOH, THF, $BrCH_2CO_2Et$, 16 h; (c) LiOH, THF, H_2O, 2 h; (d) **10**, EDCI, HOAT, DMF, CH_2Cl_2, 16 h; (e) **11** or **12**, NaOH, THF, reflux, 16 h.

of 7.5, indicating the preference of a bulky substituent at this 3-position.

Further exploration of the 2-position revealed that the methyl group could be replaced by an ethyl group (**3k**), leading to an increase in activity of approximately 2-fold. Combining the 2- and 3-substitution resulted in excellent activity as displayed by the 2,3-dimethyl derivative **3l** with a functional pK_i of 7.9, Table 2.

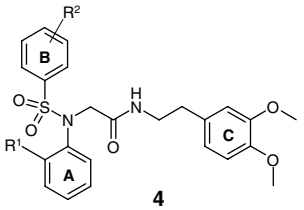
Next we investigated the SAR around the aryl sulfonamide, ring B. These compounds were synthesized using solid-phase methodologies described in Scheme 2. A selection of the results is shown in Table 3.

Substitution at the 4-position appears to be preferred with substitution at the 2- or 3-positions resulting in a loss of potency. Introduction of a methoxy group at the 4-position (**4g**) leads to a slight reduction in potency (pK_i 6.8). Compound **4a** with hydrogen in place of methyl is less potent with a pK_i of 6.1. Generally, no potency enhancement was observed over **3k** with any of these analogues.

Further profiling of compound **3k**, Figure 3, indicated excellent selectivity for the EP_1 receptor over EP_3[13] (functional pK_i < 5). When screened in a [^{3}H]PGE_2 binding assay in EP_1-transfected CHO cell membranes,[15] **3k** showed a pIC_{50} of 8.3. Schild analysis in the reporter gene assay[16] revealed **3k** to have a pA_2 of 8.3 with a slope of 0.9, indicating it to be a competitive antagonist.

Having completed the initial round of optimization, we next sought to expand the SAR by returning to further optimize the phenethyl amide moiety. Interestingly, we found that it was possible to truncate the phenethyl amide to the corresponding benzylamide with less than 10-fold decrease in activity, compound **5a**, Table 4. SAR was then obtained on a range of benzylamides. The compounds in Table 4 were synthesized according to Scheme 1 (route A).

Table 3. SAR for substitution (R^2) of the aryl sulfone moiety, **4a**–**4l**



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Compound	R^1	R^2	EP_1 functional pK_i[13]
3k	2-Et	4-Me	7.87 ± 0.28
2b	2-Me	4-Me	7.41 ± 0.23
4a	2-Me	H	6.13[a]
4b	2-Et	4-CF_3	7.72 ± 0.10
4c	2-Et	4-Br	7.54 ± 0.01
4d	2-Et	4-CN	7.50 ± 0.16
4e	2-Et	4-OCF_3	7.16 ± 0.19
4f	2-Et	2-Me	6.78 ± 0.14
4g	2-Et	4-OMe	6.77 ± 0.02
4h	2-Et	3-CN	6.62 ± 0.04
4i	2-Et	3-OCF_3	6.15 ± 0.27
4j	2-Et	3-Me	5.78 ± 0.06
4k	2-Et	2-Cl	5.70[a]
4l	2-Et	2-CN	<5.5

[a] Single experiment.

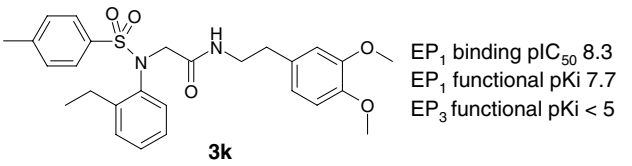
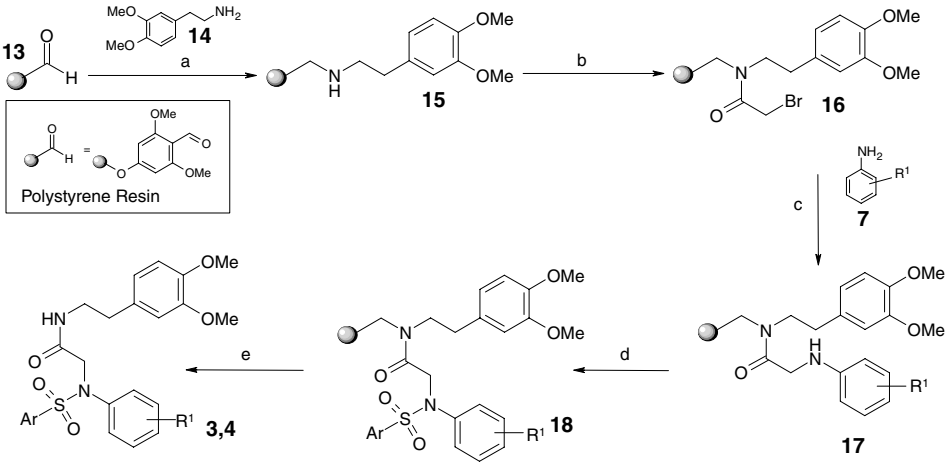


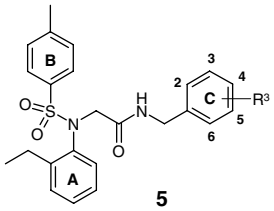
Figure 3. Profile of lead compound **3k**.

Single substitution at the 3-position led to a decrease in potency, for example, **5d** and **5e**. However, single methoxy substitution at the 4-position proved to be more successful. Compound **5f** showed good activity in both functional and binding assays. Replacement of the 4-methoxy group by Cl led to a further gain in activity, compound **5g**. The 4-Br analogue **5h** was found to be



Scheme 2. Reagents and conditions: (a) **13**, $NaBH(OAc)_3$, AcOH, CH_2Cl_2, NMP, 16 h; (b) bromoacetic acid, DIC, DMF, 16 h; (c) **7**, NMP, 120 °C, 20 min, microwave; (d) $ArSO_2Cl$, DIPEA, CH_2Cl_2,16 h; (e) TFA/H_2O, 2 h.

Table 4. SAR of substituted benzylamide compounds **5a–5k**



5

Compound	R^3	EP_1 functional pK_i[13]	EP_1 binding pIC_{50}[15]
5a	3,4-DiOMe	6.55 ± 0.05	7.6 ± 0.1
5b	H	6.65 ± 0.36	7.2 ± 0.2
5c	3-OMe	6.86 ± 0.47	7.1 ± 0.1
5d	3-OCF_3	6.37 ± 0.02	n/t
5e	3-Cl	5.85 ± 0.01	6.6 ± 0.1
5f	4-OMe	7.45 ± 0.24	7.6 ± 0.1
5g	4-Cl	7.94 ± 0.22	8.0 ± 0.3
5h	4-Br	7.64 ± 0.67	
5i	4-CF_3	6.68 ± 0.03	
5j	2-F	6.87[a]	
5k	2-MeO	5.97[a]	

[a] Single experiment.

slightly less active, and the 4-CF_3 analogue **5i** was significantly less active. Interestingly, the SAR around the phenethylamides (Table 1) and benzylamides (Table 4) appear to differ. For example, 4-Cl appears optimal and more potent than 3,4-dimethoxy substituent in the benzylamide series, whereas the reverse potency order is observed in the phenethylamide series.

A profile of **5g** is shown in Table 5 together with its selectivity against other prostanoid receptors. Compound **5g** is approximately 100-fold selective over EP_3 and is poorly active against the TP and FP receptors. It shows inhibition of CYP450 isoforms tested with the highest inhibition being against 3A4 (pIC_{50} 6.6). The high microsomal intrinsic clearance (CLi) observed for **5g** was typical of potent compounds described in Tables 1–4 (>50 mL/min/g liver in both rat and human microsomes).

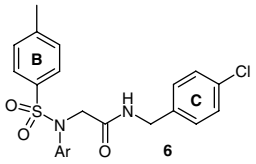
In an attempt to improve the poor metabolic stability of these molecules, we replaced the 2-ethylphenyl moiety with heterocycles in order to lower the log *P*, Table 6. These molecules were synthesized using similar conditions to those described in Scheme 1, route B. Replacement of the phenyl ring by an unsubstituted pyridine abolished activity, **6a** and **6b**. However, when a 2-methyl group was added to the 3-pyridyl isomer, moderate activity was observed, **6c**. None of the other pyridine analogues synthesized showed appreciable activity, **6d–g**, even when incorporating the preferred 2- or 3-methyl substituents observed in Table 2. The most active compound from this effort was the 1-ethyl-5-pyrazolyl analogue **6i** which displayed a functional pK_i of 7.1 and a binding pIC_{50} of 6.8. The corresponding Me analogue **6h** was slightly less active. However, the intrinsic clearance for **6i** remained high (30 and 42 mL/min/g liver in human and rat microsomes, respectively). Hence, it was generally observed that replacement of the A ring with heteroaryls led to less potent derivatives.

Table 5. Selectivity, in vitro metabolism and CYP450 profile of **5g**

Parameter	Value
EP_1 FLIPR[13] pK_i	7.94 ± 0.22
EP_1 binding[15] pK_i	8.0 ± 0.3
EP_3 FLIPR[13] pK_i	6.1[a]
TP pIC_{50}	<5.0
FP pIC_{50}	5.38 ± 0.05
CYP450[b], pIC50	4.5 at 1A2, 5.2 at 2C9, 5.7 at 2C19, 4.0 at 2D6, 6.6 at 3A4
Microsomal CLi (mL/min/g liver)	>50 rat; >50 human
Molecular weight	456

[a] Tested three times, inactive (<5.0) twice, active (6.1) once.
[b] In vitro CYP450 assay results using Gentest protocol.

Table 6. SAR of substituted benzylamide compounds **6a–6i**



6

Compound	Ar	EP_1 functional pK_i[13]	EP_1 binding pIC_{50}[15]
5g	2-Ethylphenyl	7.94 ± 0.22	8.0 ± 0.3
6a	3-Pyridyl	<5.84	<6
6b	2-Pyridyl	<5.84	<6
6c	2-Me-3-pyridyl	6.17[a]	6.7 ± 0.3
6d	3-Me-2-pyridyl	<5.84	6.1 ± 0.1
6e	6-Me-3-pyridyl	<5.84	<6
6f	6-Me-2-pyridyl	<5.84	<6
6g	5-Me-2-pyridyl	<5.84	<6
6h	1-Me-5-pyrazolyl	6.23 ± 0.06	6.4 ± 0.1
6i	1-Et-5-pyrazolyl	7.14 ± 0.07	6.8 ± 0.1

[a] Single experiment.

The molecules described in this paper were either synthesized using solution chemistry (Scheme 1) or solid-phase methodologies (Scheme 2) or alternatively were commercially available.

Scheme 1 shows the reaction of a substituted aniline **7** with the appropriate aryl sulfonyl chloride generating sulfonamide **8** which was subsequently alkylated with ethyl bromoacetate followed by basic hydrolysis to give the carboxylic acid **9**. Amide coupling with benzyl or phenethyl amine derivatives (**10**) under standard conditions furnished the desired amides (route A). Alternatively, some final products were prepared directly from sulfonamide **8** (via route B) by alkylation with bromide **11** or **12**.[17]

Scheme 2 shows the solid phase synthetic route to compounds **3** and **4**. Resin-bound amine **15** was formed via reductive alkylation of the phenethylamine **14** with aldehyde resin **13** using sodium triacetoxy borohydride. Bromoacetic acid was then directly coupled to the amines to give the alkylating agent **16**, which was reacted with the anilines (**7**) to furnish the resin-bound secondary anilines **17**. These were then reacted with the appropriate aryl sulfonyl chloride and subsequently cleaved from the resin to yield the final products.

Compounds synthesized by either Scheme 1 or Scheme 2 were purified using HPLC mass-directed purification (fraction collection triggered by mass ion detection).

In summary, we have described the synthesis and SAR of glycine sulfonamides as novel EP_1 receptor antagonists. Compound **3k** has been shown to be a competitive antagonist at EP_1 and chemical optimization around this template resulted in **5g** having an EP_1 functional pK_i of 7.8. Unfortunately, all of the compounds described here exhibited poor in vitro metabolic stability in rat and human microsomes. Further optimization of this template will be described in due course.

Acknowledgment

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13. The pK_i of compounds were calculated from IC_{50}'s measured using stable either EP_1 or EP_3 transfected CHO (Chinese hamster ovary)-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 two experiments unless specified.
14. These data agree well with data published by Merck Frosst.[6]
15. 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.
16. Schild analysis was conducted using Chinese hamster ovary (CHO) cells, stably transfected with the NFAT reporter gene and human EP_1 receptor (pCIN3 vector). Cells were quiesced 24 h prior to assay. Dose-related response curves were obtained to PGE_2 in the absence and presence of five concentrations of antagonist. Cells were incubated for 4 h to allow for transcription and translation of the reporter gene. The amount of reporter gene produced was assumed to be directly proportional to the level of receptor activation and this was measured by addition of Perkin Elmer Luclite reagent. The plates were then read on a Packard Topcount and the data analysed in Graphpad Prism. PGE_2 EC_{50} values in the absence and presence of antagonist were obtained. Dose ratios were calculated and then the log (dose ratio − 1) was plotted against Log (antagonist) concentration to produce the Schild graph from which the pA2 value and the slope were determined by linear regression.
17. Alkylating agents **11** and **12** can be prepared by coupling the appropriate amine in DMF with bromoacetic acid using DIC.