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Discovery of a novel indole series of EP_1 receptor antagonists by scaffold hopping

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Abstract—We describe the medicinal chemistry approach that generated a novel indole series of EP_1 receptor antagonists. The SAR of this new template was evaluated and culminated in the identification of compound **12g** which demonstrated in vivo efficacy in a preclinical model of inflammatory pain.

Scaffold hopping involves transforming one pharmacophoric template into another.[1] The rationale behind this type of transformation is that the new template will carry some form of benefit or distinguishing factors over the starting template, for example, improved potency, different selectivity, or improved pharmacokinetics.[1] We have described several series of EP_1 receptor antagonists[2–4] as potential analgesics.[5] Compounds from these several series have shown efficacy in the Complete Freund's Adjuvant model of inflammatory pain.[2,4] As part of an ongoing medicinal chemistry programme we were interested in identifying further novel EP_1 receptor antagonists and profiling their potential for the treatment of inflammatory pain.

One series of EP_1 receptor antagonists we have reported is exemplified by the pyrazole derivative **1**.[4] We have also disclosed some related work from this series where we identified replacement heterocycles for the pyrazole, such as the thiazole derivatives **2** and **3** and the pyridine derivatives **5** and **6**. We found that the central methylene linker could be replaced by an amino linker when the heterocycle on the right-hand side was thiazole but not pyridine, compare compounds **4** and **7**, Figure 1.[6]

We were fascinated by this disparity in SAR and intrigued by the potential intramolecular bond that could be formed between the *ortho*-alkoxy group and the biaryl NH of generic structure **8**, Figure 2. We hypothesized that an intramolecular hydrogen bond could occur and that this could be mimicked by indoles such as **9** (Fig. 2).

However, we were uncertain if the indole would provide the bioactive conformation as the methylene-linked compounds were active and modeling suggested that in the lowest energy conformation thiazole derivative **3** resided in a non-planar conformation (Fig. 3).[7]

In order to test this hypothesis we synthesized compounds of general structure **9** (Fig. 2, R = Bn or *i*-Bu).

Compounds were tested in [^{3}H]-PGE_2 binding assay.[8] Selected compounds were also tested in a functional Ca^{2+} mobilization assay (FLIPR).[9]

The first compound prepared was **10a** and we were pleased to find that it was a potent EP_1 receptor antagonist with a binding pIC_{50} of 8.2 (IC_{50} 6.3 nM). Furthermore, **10a** showed potent activity as a functional EP_1 antagonist with a pK_i of 9.3 ± 0.3 (K_i 0.5 nM) in a functional assay (FLIPR) (Table 1).

Based on this result we went on to explore initial SAR in the 3-position of the indole and investigated some alkyl groups which would mimic preferred groups from the

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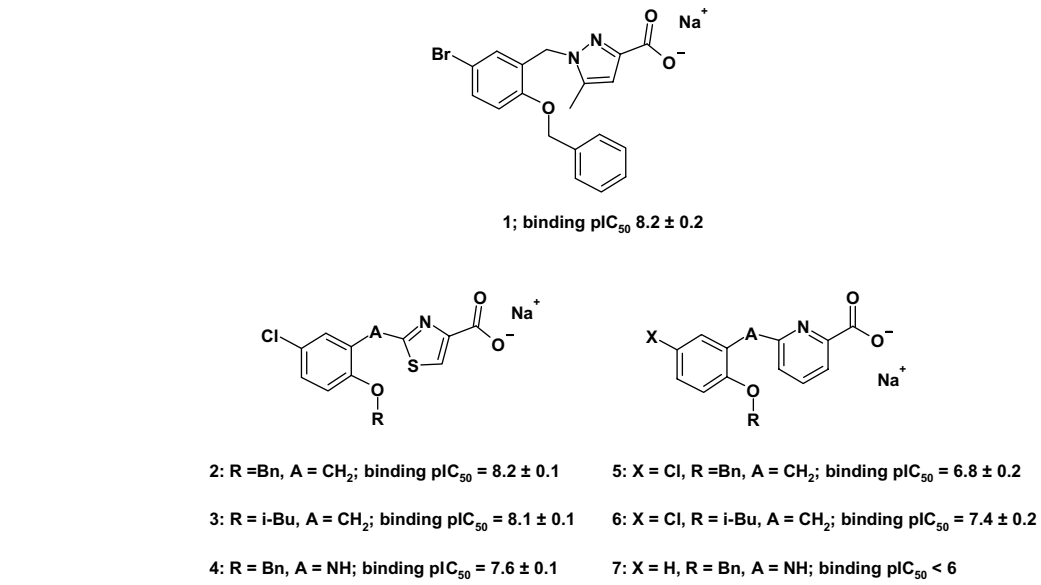


Figure 1. Selected GSK EP_1 receptor antagonists.

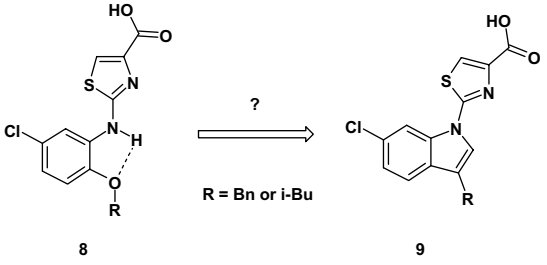


Figure 2. Capitalizing on the potential hydrogen bond between the NH and *ortho*-alkoxy group to generate a new indole series.

corresponding alkoxy series, Table 1. Thus, we prepared benzyl derivative **10b** which was surprisingly found to be 30-fold lower in affinity than **10a** in the binding assay, and 100-fold weaker in the FLIPR assay (pK_i 7.2 ± 0.4). Several alternative alkyl groups were investigated, **10c**–**10h**. The SAR shows that large groups such as CH_2CH_2t-Bu (**10e**) (FLIPR pK_i 8.4 ± 0.8) are best. Complete removal of the 3-substituent (**10i**) led to erosion of activity. A ketone was tolerated (**10j**) in terms of functionality, but the corresponding amide (**10k**) was considerably less active. Placement of basic functionality (**10l** and **10m**) severely diminished affinity. Ta-

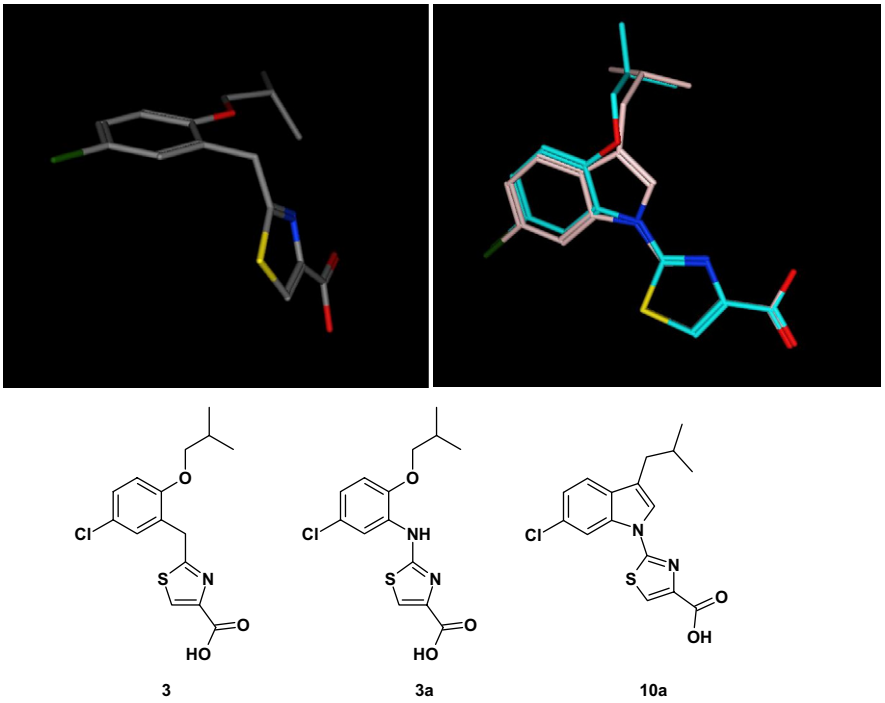
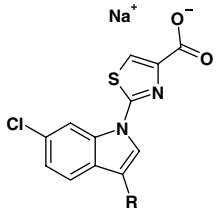


Figure 3. Energy minimized conformation of compound **3** (left-hand panel). Flexible alignment of compound **3a** (turquoise) with indole **10a** (pink) (right-hand panel).

Table 1. SAR for 3-substituents, compounds **10a–m**



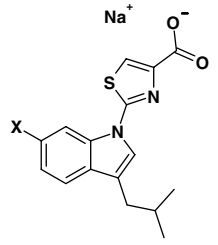
Compound	R	Binding pIC_{50}[a]
10a	*i*-Bu	8.2 ± 0.0
10b	CH_2Ph	6.8 ± 0.2
10c	*i*-Pr	7.4 ± 0.1
10d	CH_2t-Bu	7.5 ± 0.1
10e	CH_2CH_2t-Bu	8.2 ± 0.1
10f	Pr	7.5 ± 0.1
10g	Et	6.9 ± 0.1
10h	CH_2CF_3	6.6 ± 0.1
10i	H	<6
10j	CO*i*-Pr	7.0 ± 0.2
10k	$CONMe_2$	6.0 ± 0.1
10l	CH_2NMe_2	<6
10m	$CH_2CH_2NMe_2$	<6

[a] See note 8, values are means of at least three experiments.

ken together these results imply the group in the 3-position of the indole forms a lipophilic interaction with the receptor which is in accordance with our hypothesis of the receptor binding interaction for compounds such as **1**.

We next turned our attention to the 6-position of the indole (Table 2). It was found that the chlorine atom present in **10a** was extremely important. The des-chloro analogue **11a** showed nearly 30-fold less affinity for the EP_1 receptor in the binding assay and was nearly 30-fold lower in activity in the functional assay (FLIPR pK_i 7.9 ± 0.6). The fluoro analogue (**11b**) was approximately 10-fold less active than chloro derivative **10a**, however, the bromo (**11c**, FLIPR pK_i 9.7 ± 0.3), trifluoromethyl (**11d**, FLIPR pK_i 10.2 ± 0.3), and methyl (**11e**) analogues were of similar activity to the parent **10a**, in terms of binding affinity (Table 2).

Table 2. SAR for 6-substituents, compounds **11a–e**



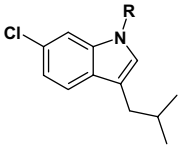
Compound	X	Binding pIC_{50}[a]
10a	Cl	8.2 ± 0.0
11a	H	6.8 ± 0.1
11b	F	7.4 ± 0.1
11c	Br	8.4 ± 0.1
11d	CF_3	7.9 ± 0.0
11e	Me	8.0 ± 0.3

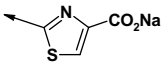
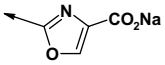
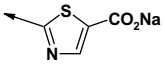
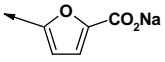
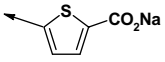
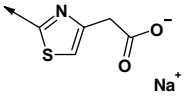
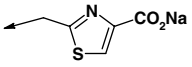
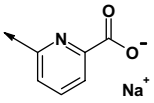
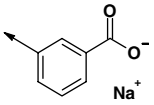
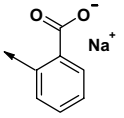
[a] See note 8, values are means of at least three experiments.

As the compounds in Figure 1 had shown they were amenable to alternative heterocyclic acids we next investigated this region of the molecule, namely the 1-position of the indole (Table 3).

The thiazole could be replaced by the analogous oxazole (**12a**, FLIPR pK_i 9.0 ± 0.5) with minimal change in activity. The regioisomeric thiazole (**12b**) was considerably weaker. Other 5-membered aromatic heterocycles with a heteroatom between the acid the indole, such as

Table 3. SAR for thiazole replacement, compounds **12a–j**



Compound	Ring	Binding pIC_{50}[a]
10a		8.2 ± 0.0
12a		8.1 ± 0.2
12b		7.0 ± 0.3
12c		7.6 ± 0.2
12d		7.3 ± 0.1
12e		6.8 ± 0.1
12f		8.2 ± 0.1
12g		7.2 ± 0.4
12h		6.2 ± 0.1
12i		<6
12j	CH_2CO_2Na	<6

[a] See note 8, values are means of at least three experiments.

furan **12c** and thiophene **12d** displayed activity intermediate between **12a** and **12b**. A methylene linker could be inserted between the acid and thiazole (**12e**), or, more favorably, between indole and thiazole (**12f**).

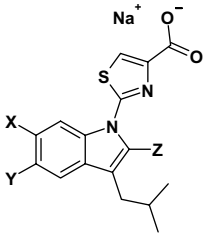
As we had found disparate SAR between compounds **4** and **7**, we were intrigued to see if the picolinic acid moiety would be tolerated in the 1-position of the indole template. Surprisingly, picolinate derivative **12g** was active, albeit with 10-fold lower activity than the parent thiazole **10a**. This result can be rationalized as an isobutyl group is preferred to benzyl in the 3-position of the indole (compare compound **10a** with **10b**). In the parent thiazole series, both isobutyl and benzyl groups provided equal affinity (compare **2** to **3** and **4**). However, in the parent picolinate series, the isobutyl group was preferred over benzyl (compare **5** to **6**), thus the fact that compound **7** was essentially inactive could be attributed to the benzyloxy group and thus the corresponding isobutoxy analogue may show activity.

Interestingly, the phenyl analogue **12h** showed extremely weak activity, highlighting the importance of a heteroatom *ortho* to the carboxylic acid. The corresponding *ortho*-benzoic acid derivative **12i** was essentially inactive, as was the acetic acid derivative **12j**.

Finally, we investigated further substitution around the indole ring. Moving the Cl atom from the 6-position (**10a**) to the 5-position (**13a**) led to a 100-fold decrease in affinity (Table 4). This result, in conjunction with the activity of the des-chloro analogue (**11a**) highlights the importance of a small lipophilic group in 6-position. Finally, addition of a methyl group to the 2-position of the indole also resulted in approximately 100-fold decrease in activity. The presence of the 2-methyl group may force the 3-isobutyl group into an unfavorable conformation for receptor binding. Taken together these results show low tolerance for the substitution of the indole ring (see Table 4).

Several compounds were profiled in vitro to assess their metabolic stability.[10] Data for key compounds is shown (Table 5) and is representative of the series.

Table 4. SAR for 5-, 6-, and 2-substituted indoles, compounds **13a** and **b**



Compound	X	Y	Z	Binding pIC_{50}[a]
10a	Cl	H	H	8.2 ± 0.0
11a	H	H	H	6.8 ± 0.1
13a	H	Cl	H	6.1 ± 0.2
13b	Cl	H	Me	6.0 ± 0.0

[a] See note 8, values are means of at least three experiments.

Table 5. In vitro metabolic stability data (microsomes) for selected compounds

Compound	CLi[a,b]	CLi[a,c]
10a	12.0	2.2
10d	1.9	2.7
10e	1.3	2.1
10f	11.0	1.3
12c	35.0	5.4
12g	4.3	3.6

[a] Intrinsic clearance values (mL/min/g liver), values are from one experiment.[10]
[b] Rat liver microsomes.
[c] Human liver microsomes.

Based on a combination of in vitro metabolic stability and structural similarity to compounds which had previously shown good in vivo efficacy,[2c] compound **12g** was profiled in the CFA model of hypersensitivity,[2b,c] data is depicted in Figure 4. Compound **12g** demonstrated a good dose–response relationship with a calculated ED_{50} of 2.17 mg/kg and efficacy equivalent to celecoxib at doses of 3 and 10 mg/kg (po). Bioanalysis of samples from this study revealed a dose-proportional increase in blood exposure, blood concentrations were 0.44, 1.23, and 6.04 μM at doses of 1, 3, and 10 mg/kg, respectively. Further analysis from this study (top dose only) showed low levels of the compound in the brain, Br:Bl 0.05.

This data highlights **12g** as a novel EP_1 receptor antagonist with excellent in vivo efficacy.

Compounds were synthesized according to the literature procedures or as outlined in Schemes 1–7. Full experimental details and characterizing data for key compounds has been described.[11]

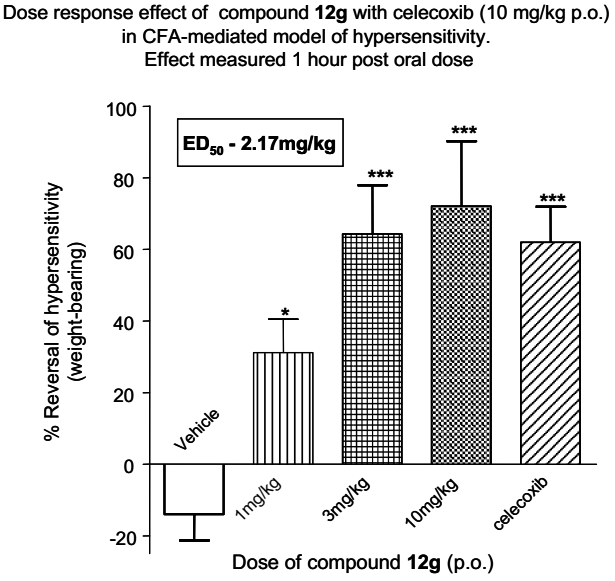
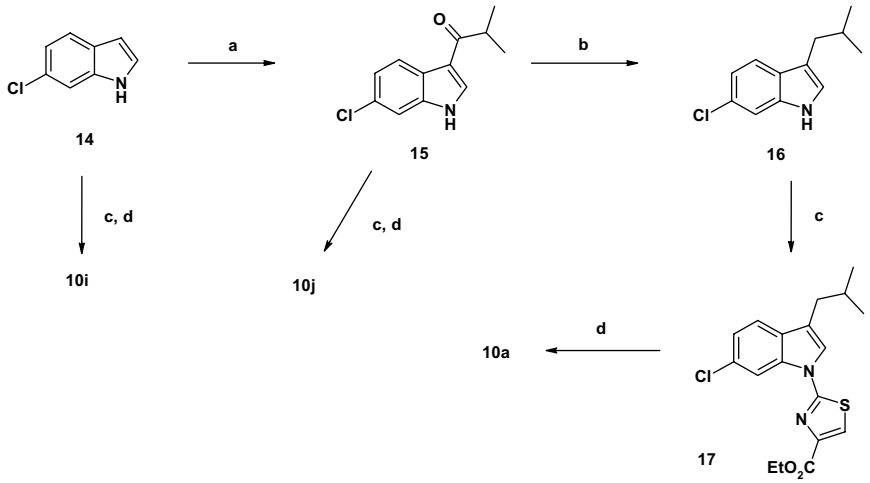
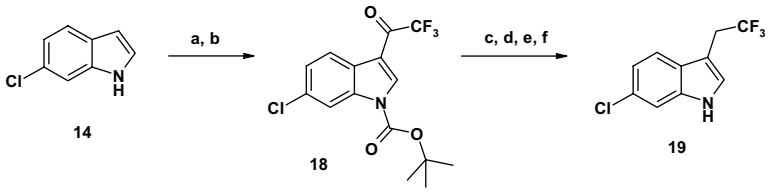


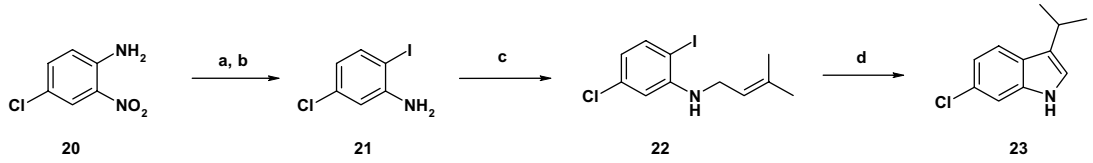
Figure 4. Dose–response data for compound **12g** in the CFA-induced model of hypersensitivity. Seven rats used per dose group. Efficacy readout taken 1 h post dose of compound. Compound dosed orally. Asterisks denote significance level, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.



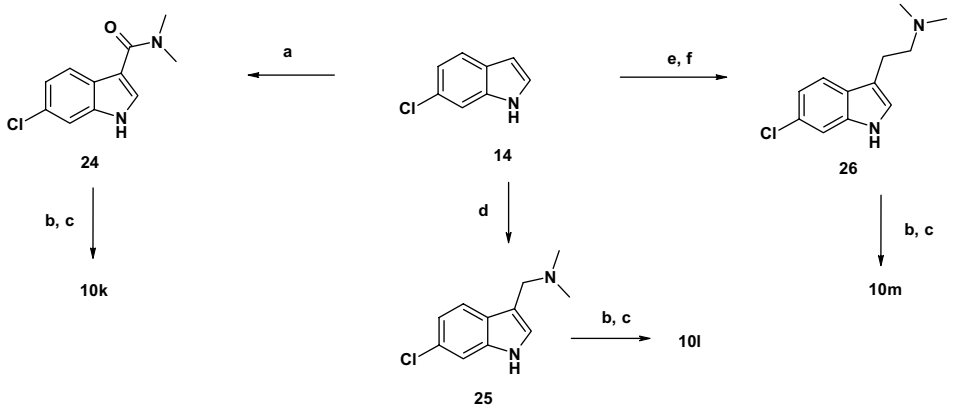
Scheme 1. Reagents and conditions: (a) 3 M MeMgBr in Et_2O 20 min, then 1 M $ZnCl_2$ in Et_2O 30 min, then isobutyryl chloride, Et_2O, 100%; (b) $LiAlH_4$, THF, rt then reflux 3 h; (c) CuI (5 mol%), K_3PO_4 (20 mol%), *rac-trans-N,N′*-dimethylcyclohexyane-1,2-diamine, ethyl-2-bromothiazole-4-carboxylate, PhMe, reflux; (d) aq NaOH, EtOH, 50–60 °C, 1 h.



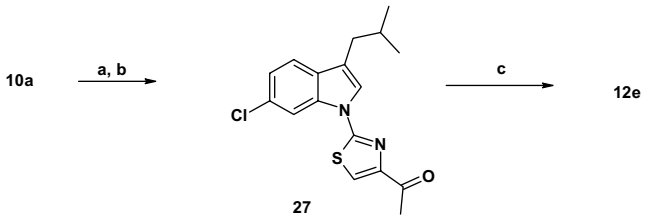
Scheme 2. Reagents and conditions: (a) TFAA, Et_2O, 0–4 °C; (b) Boc_2O, DMAP, DCM, rt, 1 h; (c) $NaBH_4$, EtOH, rt, 1 h; (d) phenyl chlorothionoformate, DCM, pyridine, DMAP, rt, 3 days; (e) Bu_3SnH, AIBN, PhMe, 80 °C, 3 h; (f) TFA.



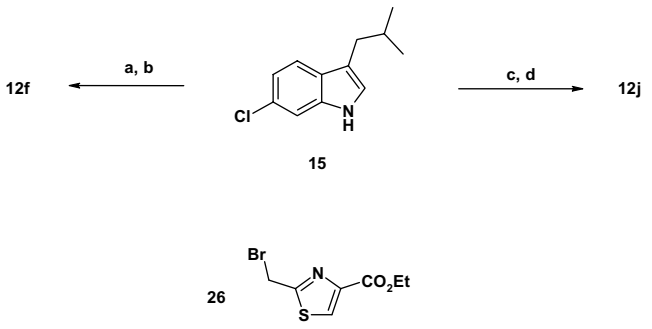
Scheme 3. Reagents and conditions: (a) H_2SO_4, $NaNO_2$, 0 °C to rt, then KI, H_2O; (b) Fe, $FeCl_3{\cdot}6H_2O$, EtOH, AcOH, reflux, 1 h; (c) LDA, THF, −78 °C to rt to −78 °C then 1-bromo-3-methylbut-2-ene, −78 °C to rt, 1 h; (d) $Pd(OAc)_2$, DMF, TEA, Bu_4NBr, 80 °C, 1 h.



Scheme 4. Reagents and conditions: (a) 3 M MeMgBr in Et_2O 20 min, then 1 M $ZnCl_2$ in Et_2O 30 min, then dimethylcarbamoyl chloride; (b) CuI (5 mol%), K_3PO_4 (20 mol%), *rac-trans-N,N′*-dimethylcyclohexyane-1,2-diamine, ethyl-2-bromothiazole-4-carboxylate, PhMe, reflux; (c) aq NaOH, EtOH, 50–60 °C, 1 h; (d) 40% aq $MeNH_2$, 40% aq HCHO, AcOH; (e) (i) $(COCl)_2$, Et_2O, rt, 4 h, 36%, (ii) 40% aq Me_2NH, THF, rt, 1 h, 52%; (f) $LiAlH_4$, THF, rt then reflux.



Scheme 5. Reagents and conditions: (a) EDC, HOBt, TEA, DCM, *N*-methyl-*O*-methyl hydroxylamine hydrochloride; (b) 3 M MeMgBr, Et_2O, THF; (c) (i) sulfur, morpholine, 130 °C overnight (ii) aq KOH, EtOH, reflux.



Scheme 6. Reagents and conditions: (a) 60% NaH, THF, **26**, rt; (b) EtOH, 2 M NaOH, rt; (c) 60% NaH, $BrCH_2CO_2Et$, THF, rt; (d) EtOH, 2 M NaOH, 70 °C.

Compound **10a** was prepared from 6-chloroindole (**14**) (Scheme 1) by acylation, to give **15**, and subsequent reduction to give **16**. The thiazole was then installed using conditions developed by Buchwald and co-workers[12] to give **17**. Hydrolysis of the ester **17** yielded the thiazole acid derivative (**10a**). Derivatives **10i** and **10j** were prepared directly from **14** and **15**, respectively, as outlined (Scheme 1). Derivatives **12a–d** and **12g–i** (Table 3) were prepared from intermediate **16** using similar conditions. The requisite bromides were commercially available, except for ethyl 2-bromo-1,3-oxazole-4-carboxylate which was prepared from ethyl 2-amino-1,3-oxazole-4-carboxylate (isoamyl nitrite, $CuBr_2$, MeCN, 60 °C).

Analogues **10b** and **10d–g** were prepared in analogous manner to **10a** (Scheme 1), reacting 6-chloroindole (**14**) with the appropriate acylating agent.

The 3-trifluoroethyl analogue (**10h**) was prepared by acylation of 6-chloroindole (**14**), followed by protection to give the *tert*-butyloxycarbonyl derivative **18** (Scheme 2). The ketone moiety was reduced to the corresponding alcohol which then underwent a modified Barton radical deoxygenation[13] and deprotection to give 6-chloro-3-trifluorethylindole (**19**) (Scheme 2). Buchwald chemistry [12] was again employed, as in Scheme 1, to convert **19** to **10h**.

Buchwald conditions[12] were also used to prepare derivative **10c** from 6-chloro-3-isopropylindole **23** (Scheme 3). The synthesis of **23** commenced from 4-chloro-2-nitroaniline (**20**) which was converted to the corresponding iodide by Sandmeyer reaction.[14] Reduction of the nitro moiety delivered 5-chloro-2-iodoaniline (**21**) which was alkylated to give **22**. Palladium-mediated cyclization[15] of **22** delivered the aforementioned intermediate **23** (Scheme 3).

Derivatives **10k–m** were prepared as outlined in Scheme 4. Reaction of 6-chloroindole (**14**) with dimethylcarbamoyl chloride gave **24** which was converted to **10k** using Buchwald conditions.[12] (Scheme 4). Derivative **25** was prepared from **14** using Mannich–Eschenmoser conditions[16] and was converted to **10l** via Buchwald coupling.[12] (Scheme 4). Homologated analogue **26** was prepared in three steps from **14** by acylation with oxalyl chloride, reaction with dimethylamine and reduction. Again, Buchwald chemistry was employed to convert **26** to **10m** (Scheme 4).

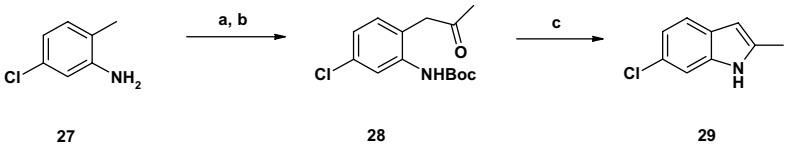
Compounds in Table 2 (**11a–e**) were prepared from commercially available 6-substituted indoles, as described in Scheme 1.

Derivative **12e** was prepared from **10a** (Scheme 5). Firstly, the carboxylic acid was converted to the corresponding methyl ketone (**27**) via the Weinreb amide.[17] Willgerodt–Kindler reaction[18] and subsequent hydrolysis furnished **12e** (Scheme 5).

Compounds **12f** and **12j** were prepared by alkylation of 6-chloro-3-isobutylindole (**15**) as outlined below (Scheme 6).

Derivative **13b** was prepared as described in Scheme 1 from 6-chloro-2-methylindole (**29**) which was prepared as described below (Scheme 7).

Compounds generally showed good selectivity (100- to 10,000-fold) over the EP_3 receptor subtype and similar or lower selectivity over the thromboxane (TP receptor), for example **10a** (EP_1 FLIPR pK_i 9.3, EP_3 FLIPR pK_i 6.5, TP FLIPR pK_i 7.5), **10e** (EP_1 FLIPR pK_i 8.4, EP_3 FLIPR pK_i 6.4, TP FLIPR pK_i 7.1), **11c** (EP_1 FLIPR pK_i 9.7, EP_3 FLIPR pK_i 6.4, TP FLIPR pK_i 7.3), **11d**



Scheme 7. Reagents and conditions: (a) Boc_2O, THF, rt to reflux; (b) sec-BuLi cyclohexane-THF, −40 to −20 °C, then −60 °C, *N*-methoxy-*N*-methylacetamide, then warmed to rt; (c) TFA, DCM, rt, 3 h.

(EP_1 FLIPR pK_i 10.2, EP_3 FLIPR pK_i 6.3), **12c** (EP_1 FLIPR pK_i 8.9, EP_3 FLIPR pK_i 6.3, TP FLIPR pK_i 6.4), **12g** (EP_1 FLIPR pK_i 7.7, EP_3 FLIPR pK_i 6.1).

In conclusion, we have identified a novel series of indole EP_1 receptor antagonists by scaffold hopping and explored the SAR in the 1-, 2-, 3-, 5-, and 6-positions. Although there are similarities between the SAR in this series and previous series there is some divergence, in particular the 3-position which forms a lipophilic interaction. Several compounds with high in vitro affinity were identified. From this, compound **12g** was found to combine the best balance of in vitro DMPK properties and in vitro EP_1 activity and was thus assessed in an in vivo model of inflammatory pain where it showed excellent efficacy, with an ED_{50} of 2.17 mg/kg, and equivalent efficacy to celecoxib.

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8. Compound potencies were determined using a radioligand binding assay where the ability of compounds to compete with tritiated prostaglandin E_2 ($[^3H]$-PGE_2) binding to the human EP_1 receptor was measured in Chinese hamster ovary-K1 (CHO-K1) cells into which a stable vector containing the EP_1 cDNA had previously been transfected. Cell membranes, competing compounds and $[^3]$-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 Packard TopCount scintillation counter.
9. The antagonist properties of compounds were assessed by their ability to inhibit the mobilization of intracellular calcium ($[Ca^{2+}]_i$) in response to activation of the EP_1 receptor by prostaglandin E_2 (PGE_2). The amount of calcium produced was assessed using a calcium-sensitive fluorescent dye such as Fluo-3, AM and a suitable instrument such as a Fluorimetric Imaging Plate Reader (FLIPR).
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