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A novel series of potent and selective EP_4 receptor ligands: Facile modulation of agonism and antagonism

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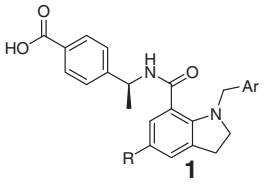
ABSTRACT

A novel series of EP_4 ligands, based on a benzyl indoline scaffold, has been discovered. It was found that agonism and antagonism in this series can be easily modulated by minor modifications on the benzyl group. The pharmacokinetic, metabolic and pharmacological profiles of these compounds was explored. It was found that these compounds show good pharmacokinetics in rat and are efficacious in pre-clinical models of pain and inflammation.

Arthritis is a chronic inflammatory condition leading to bone and cartilage destruction. Accumulated evidence over past decades suggests that PGE_2 plays an important role in the pathogenesis of this disease.[1] Inhibition of prostaglandin E2 (PGE_2) production by NSAIDs and, more recently, Cox-2 inhibitors relieves arthritis symptoms.

PGE_2 is the ligand of four subtype of EP receptors: $EP_{1\text{-}4}$. In a mouse model of collagen-antibody induced arthritis (CAIA), $EP_4-/-$ mice, unlike $EP_{1-3}-/-$ mice, showed remarkable symptom resistance (paw swelling/redness, ankylosis). This suggests that the effect of PGE_2 in inflammation is mediated predominantly by the EP_4 receptor.[2] To further support EP_4 antagonism as a valid strategy for treating inflammatory pain, we recently demonstrated, using highly selective EP_1, EP_3 and EP_4 antagonists, that EP_4, not EP_1 or EP_3, is the primary receptor involved in joint inflammation and pain in rodent models of rheumatoid and osteoarthritis.[3a] Moreover, since EP_4 antagonism does not interfere with the biosynthesis of important prostanoids, such as prostacyclin (PGI_2) and thromboxane A_2 (TxA_2), it is plausible that a highly selective EP_4 antagonist would be a safer alternative for relieving arthritis symptoms and thus lack the potential cardiovascular side effects observed with NSAIDs and Cox-2 inhibitors.[4]



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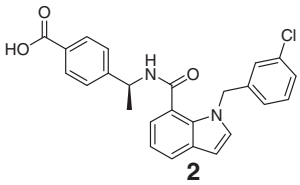

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We previously disclosed the discovery of several series of selective EP_4 antagonists.[3] We demonstrated that these compounds exhibited excellent efficacy and GI tolerability. In this Letter, we describe the SAR, pharmacokinetics, metabolism and in vivo efficacy of a new series of EP_4 ligands containing the indoline template shown in general structure **1**.

In a previous communication,[3d] we described indole **2** as a potent antagonist of EP_4 with a K_i of 2.7 nM. It was highly selective against the EP_1, EP_2, EP_3, FP, IP, TP (>1000-fold) receptors but was only sixfold selective against the DP receptor. Subsequently, it was found that replacement of the indole core with an indoline moiety (**1a,** Table 1) resulted in a sixfold increase of potency on the EP_4 receptor, as well as a substantial improvement in selectivity against DP.

The general synthetic route to the indolines is described in Scheme 1. Commercially available *N*-Boc indoline (**3**) was treated with *s*-BuLi, then quenched with CO_2 to provide acid **4**.[5] Standard HATU amide coupling conditions were used to couple **4** with

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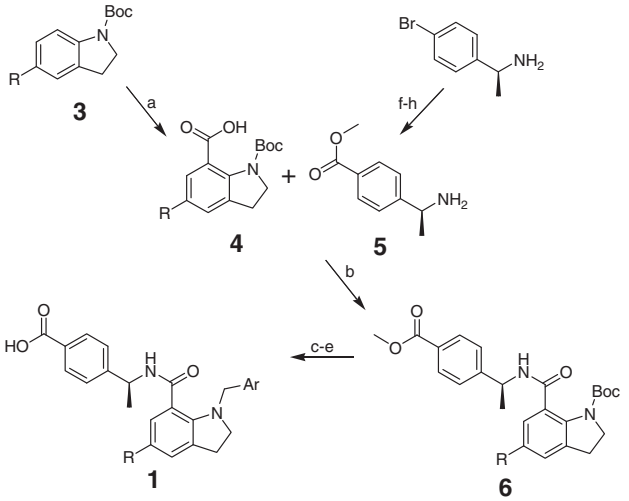
Table 1
SAR of compounds of general structure **1** (R = H)

	Ar	EP_4 K_i, nM	Func. assay[a] $IC_{50}(EC_{50})$ nM	EP_2[b]/EP_4	DP[b]/EP_4
2		2.7	6.0	>1000	16
1a	3-Cl	0.44	>10,000 (0.82)	595	>1000
1b	3,4-Cl	0.34	1.1	338	241
1c	4-Cl	0.44	0.92	>1000	>1000
1d	4-F	1.8	6.2	488	907
1e	4-CF_3	0.37	2.1	>1000	>1000
1f	3-CF_3	0.40	>10,000 (0.20)	>1000	>1000
1g	4-OCF_3	1.5	2.8	687	880
1h	4-CN	3.1	7.3	603	916
1i	4-Ph	0.28	1.3	139	57
1j	3,5-Br	0.33	>10,000 (0.36)	151	209
1k	4-COOH	54	—	>179	>73
1l	2-Br,4-Cl	2.3	8.3	>1000	114
1m	4-$CONH_2$	30	73	>1000	>1000
1n	3-Pyridyl	76	258	>127	>50
1o	4-*t*-Bu	14	114	>692	>281
1p	4-Me	0.78	5.8	>1000	>1000
1q	2-Br,4-CF_3	6.7	4.7	795	88
1r	3-$CONH_2$	30	72	>1000	461
1s	3-CN	7.5	212	582	>1000

[a] Functional antagonist assay: inhibition of PGE_2 evoked intracellular cAMP elevation in HEK293 cells expressing EP_4 receptors; agonist assay: potentiation of cAMP elevation in the same cell line in the absence of PGE_2

[b] All compounds described in this Letter are greater than 1000-fold selective versus EP_1, EP_3, FP, IP and TP.



Scheme 1. Synthesis of compounds of general structure **2**. Reagents: (a) *s*-BuLi, TMEDA, CO_2, THF; (b) HATU, DIPEA, ACN; (c) TFA, DCM; (d) NaH, $ArCH_2X$, THF; (e) NaOH, MeOH, THF; (f) Boc_2O, TEA, MeOH; (g) MeLi, *n*-BuLi, CO_2, THF; (h) TMSCl/MeOH.

commercially available amine **5** to give **6**. Afterwards, the amine was deprotected and the indoline NH was benzylated with the appropriate benzyl halide. Finally, hydrolysis of the methyl ester provided compounds of general structure **1**.

Interestingly, it was found that indoline **1a** behaved as a full agonist of EP_4 with an EC_{50} of 0.82 nM (Table 1), and was inactive (IC_{50} >10000 nM) in inhibiting PGE_2 evoked cAMP elevation in HEK293 cells over-expressing human EP_4. Furthermore, we observed that it was possible to switch from agonist to antagonist by varying the aryl substitution pattern on the benzyl indoline. For example, addition of a *para*-substituent such as Cl on the benzyl group provided compound **1b** as an antagonist (IC_{50} = 1.1 nM). Omission of the 3-Cl (**1c**) still provided a full antagonist and this modification also resulted in an increase in selectivity on DP and EP_2 (from 241 and 338-fold, respectively, to >1000-fold). It appeared that a variety of 4-substituents on the benzyl group (Cl, F, CF_3, OCF_3, CN, Ph, $COONH_2$, *t*-Bu and Me) were tolerated for antagonist activity. An alternate way of producing antagonists was to add a polar group at the 3 position of the benzyl group. For example, 3-pyridyl (**1n**), 3-$CONH_2$ (**1r**) and 3-CN (**1s**) behaved as antagonists albeit with loss in potency. Incorporation of less polar groups at the 3 position resulted in agonists (**1a**, **1f**, **1j**). Also noteworthy, the 2- and 5-substitutions examined (**1j**, **1l**, **1q**) resulted in a loss of selectivity. Interestingly, this agonist/antagonist switch was not observed with the indole series[3d] (all the indole derivatives prepared are antagonists).

With regards to potency, it appears that small non-polar *para*-substituents (Cl, F, CF_3 and CH_3) are superior. With respect to selectivity, 4-Cl, 4-CF_3 and 4-CH_3 are the most selective (>1000-fold selectivity vs all other prostanoid receptors). Overall, compound **1c** (4-Cl) and **1e** (4-CF_3) have the best potency and selectivity profile. Both are highly potent in both the binding (K_i = 0.4 nM) and functional assays (IC_{50} = 1.0–2.0 nM), and are >1000-fold selective versus all other prostanoid receptors.

Other SAR of interest is shown in Table 2. The tetrazole carboxylic acid isostere **10** was prepared as shown in Scheme 2, and binding data indicates that the tetrazole group is a suitable replacement for the carboxylic acid. Also, substitution at the 5-position of the indoline is tolerated, but loss of selectivity is observed against EP_2 and DP with groups larger than fluorine (**1t**–**1v**).

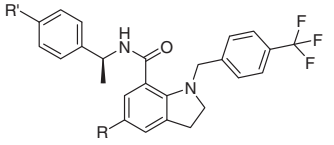
The functional potency of **1c** and **1e** was measured in whole blood. This functional cellular assay measures the blockade of inhibition of TNFα induced IP-10 release by a specific EP_4 agonist.[3] Both compounds are very potent in this assay with IC_{50}s of 63 and 34 nM, respectively.

Pharmacokinetic experiments of select compounds were performed in rats. Animals were dosed with the potassium salts at 20 mg/kg PO in 0.5% methocel and 5 mg/kg IV in 60% PEG 200. Half-life, bioavailability and clearance data are shown in Table 3. All compounds showed good to excellent bioavailability and low to moderate clearance.

In order to investigate the metabolism of the series, rat hepatocyte incubation experiments were performed with compound **1e**, and the analysis of the incubation mixture was done by LC/MS. After a 2 h incubation, 36% of parent compound was remaining. Two major metabolites were observed and were identified as the glucuronide and taurine conjugates of **1e**. Indoles and metabolites resulting from oxidation on the indoline core were among the other minor metabolites observed.

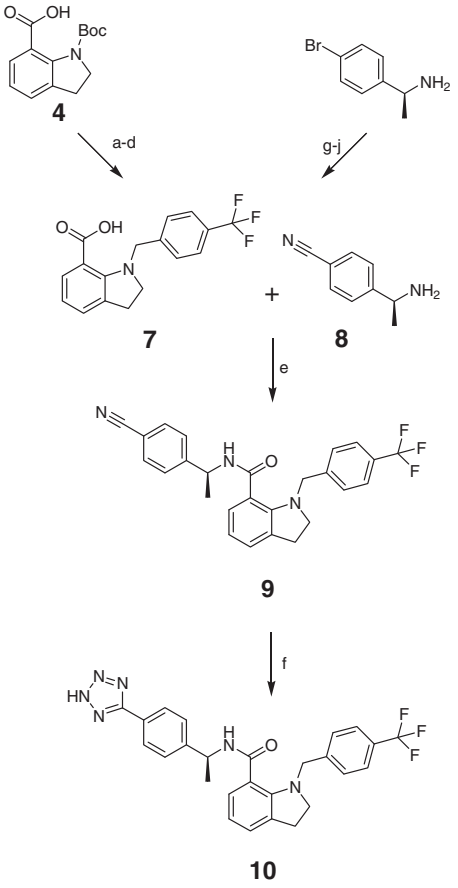
Because of the high amount of metabolism on the acid moiety, the tetrazole replacement of the carboxylic acid (**10**) could have

Table 2



	R	R'	EP4 *Ki*, nM	Func. assay[a] IC_{50}, nM	EP2/EP4	DP/EP4
1e	H	COOH	0.28	1.6	>1000	>1000
10	H	Tetrazole	1.0	3.5	>1000	>1000
1t	Cl	COOH	0.40	2.2	647	670
1u	CF_3	COOH	0.66	3.0	153	504
1v	F	COOH	0.37	2.1	>1000	>1000

[a] Functional assay: inhibition of PGE_2 evoked intracellular cAMP elevation in HEK293 cells expressing EP_4 receptors.



Scheme 2. Synthesis of tetrazole **10**. Reagents: (a) CH_2N_2, MeOH; (b) TFA, DCM; (c) NaH, 4-CF_3ArCH_2Br, THF; (d) KOH, MeOH, THF, Δ; (e) HATU, DIPEA, ACN; (f) azidotributyltin, toluene, Δ, AcOH; (g) Boc_2O, TEA, MeOH; (h) CuI, NaI, *rac*-trans-*N*,*N*′-dimethylcyclohexane-1,2-diamine, dioxane, Δ; (i) CuI, NaCN, *N*,*N*′-dimethylethylenediamine, toluene, Δ; (j) HCOOH, DCM.

Table 3
Pharmacokinetics

	$t_{1/2}$ (h)	*F* (%)	Cl (mL/min/kg)
1a	4.4	65	26
1b	4.8	73	12
1c	4.7	102	9
1e	4.1	48	11
10	2.6	54	28

potentially altered the pharmacokinetics; however, this modification did not improve half-life or lower clearance in rats (Table 3).

In order to better understand the clearance pathway of these compounds, a radiolabelled version of **1e** was prepared from **1q** by palladium catalyzed tritiation. A rat bile cannulation study was performed with ^{3}**H-1e**, and LC/MS analysis of the bile indicated that the compound is mostly excreted as glucuronide and taurine conjugates, with very small amounts of parent. In total, 86% of the total radioactivity was recovered in the first 24 hours from bile.

In order to predict the potential of toxicity resulting from covalent protein labelling from acyl glucoronide migration or oxidative metabolism,[6] in vitro and in vivo covalent labelling studies were performed with ^{3}**H-1e**. It was found that the extent of covalent protein labelling was relatively low in both rat and human hepatocytes (56 and 28 pmole-eq/mg @ 2 h, respectively). And an in vivo covalent protein labelling study in rat also showed a relatively small amount of labelling (5 pmol/mg protein).

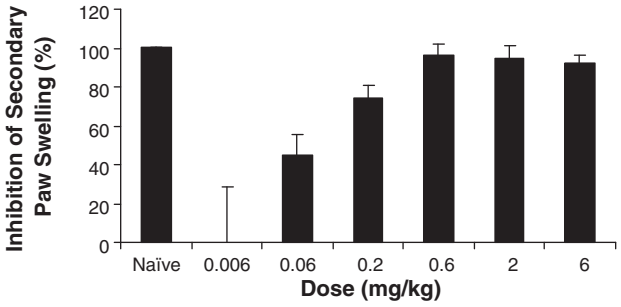


Figure 1. Inhibition by **1e** of secondary paw swelling in rats with AIA. Complete Freund's adjuvant was injected into left hind paw on day 0. Compound **1e** was administered starting on day 9, once daily for 9 days. Secondary paw volume was measured at day 18. The percentage of inhibition was calculated from the difference between a treated group (n = 7/group) and a vehicle control. Vertical bars show SEM.

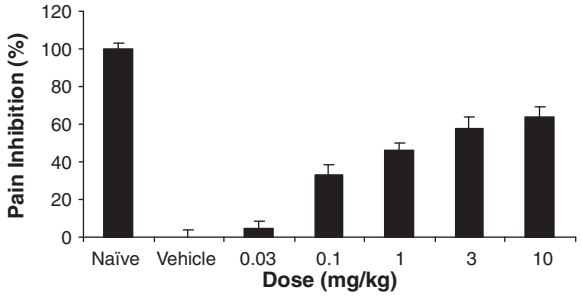


Figure 2. Inhibition by **1e** of carrageenan-induced mechanical hyperalgesia. Carrageenan is injected into the rat's paw at t = 0 h and **1e** was administered at t = 2 h. The hyperalgesic response is measured at t = 3 h by applying mechanical pressure to the paw and measuring the latency in paw withdrawal. **, p <0.01 versus vehicle control by one way ANOVA followed by Dunnett's test. Vertical bars show SEM (n = 5/group).

The efficacy of compound **1e** was evaluated in both the rat adjuvant-induced arthritis model (AIA), and in the rat carrageenan-induced mechanical hyperalgesia model. In the AIA, **1e** dose dependently reduced secondary paw swelling with NSAID/Coxib like efficacy (Fig. 1) and completely inhibited secondary paw swelling at a dose of 0.6 mg/kg/day. In the carrageenan-induced mechanical hyperalgesia model, **1e** showed dose dependant inhibition of pain with a maximum of 64% inhibition relative to vehicle at 10 mg/kg (Fig. 2).

In conclusion, a novel benzyl indoline series of EP_4 ligands was discovered. Agonism and antagonism can be modulated by simple modifications on the benzyl moiety. The compounds in this series have good pharmacokinetic properties and are efficacious in pre-clinical models of pain and inflammation.

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