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3-Acrylamide-4-aryloxyindoles: Synthesis, biological evaluation and metabolic stability of potent and selective EP_3 receptor antagonists

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ABSTRACT

A series of potent and selective EP_3 receptor antagonists are described. Utilizing a pharmacophore model developed for the EP_3 receptor, a series of 3,4-disubstituted indoles were found to be efficient ligands for this target. These compounds showed high selectivity over IP, FP and other EP receptors. An optimized molecule **7c** featured a sound profile and potency in the functional rat and human platelet aggregation assays.

The prostanoids constitute a family of endogenous signaling ligands arising from phospholipase A_2-mediated release of arachadonic acid followed by its further transformation through a series of synthases. The prostanoid family includes prostaglandins D_2, E_2, $F_{2\alpha}$, I_2 and thromboxane A_2. These ligands preferentially binds the series of G-coupled protein receptors DP_1 and CRTH(DP_2), EP_1 through EP_4, FP, IP and TP, respectively.[1,2] Activation of these receptors controls biological homeostasis.[3] Of these, the EP_3 receptor has been shown to play key roles in sodium and water reabsorption in kidney tubules,[4] smooth muscle contraction of the GI tract,[3] acid secretion,[5] uterine contraction during fertilization and implantation,[6] fever generation[7] and hyperalgesia.[8] Eight isoforms of the human EP_3 receptor have been detailed (EP_{3I} through EP_{3VI}, EP_{3e} and EP_{3f}) which are distinguished through unique cytoplasmic C-terminus ends ranging from 6 to 66 amino acids in length.[9]

Recently, we have identified DNA variants of the gene encoding for the EP_3 receptor that confer a significantly increased risk for development of peripheral arterial disease (PAD).[10] Utilizing EP_3 KO mice, it has been shown that the stimulatory effects of PGE_2 on a platelet aggregation are exerted specifically through the EP_3 receptor.[11a] Based on this evidence we reasoned that the development of potent and selective EP_3 antagonists may provide a novel therapeutic entry into the treatment of PAD through modulation of platelet aggregation. A subsequent report from the Fabre lab demonstrated that inflammation/rupturing of the existing plaque releases PGE_2 locally and promotes vicinal platelet aggregation through the platelet EP_3 receptor.[11b]

A series of reports from the Merck–Frosst labs[12] have documented the synthesis, biological activity and selectivity of three different series of *ortho*-substituted cinnamyl and dihydrocinnamyl derivatives as highly potent and selective human EP_3 receptor antagonists.

In a preceding paper in this series we described a series of 3-thioaryl ether (**A**) and 3-sulfonylaryl indoles (**B**) which were potent and selective antagonists of the EP_3 receptor (Fig. 1).[13] In order to further expand the diversity of the EP_3 receptor antagonists and to improve their physico-chemical and pharmacokinetic properties we investigated other chemotypes.[14] Several fused bicyclo[4.3.0]

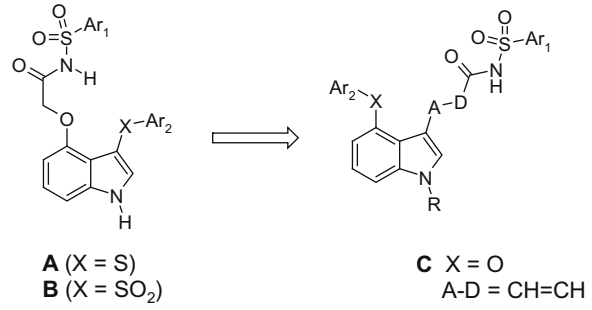

Figure 1. 3,4-Disubstituted indole analogs.

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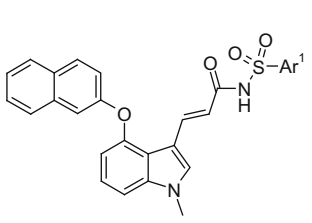
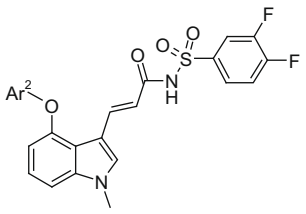
aromatic scaffolds afforded the proper spatial arrangement for the pharmacophoric entities in the targeted molecules. Of these scaffolds we inquired whether the acidic and lipophilic appendages of the previously disclosed 3,4-disubstituted indoles could be interchanged off of the indole ring and retain the EP_3 binding affinity experience. A priori, this exchange seem reasonable expecting the acidic and lipophilic appendages would project from indole core at a similar dihedral angle and thus the EP_3 receptor could be occupied with a compound of similar topology. In the current disclosure, we outline the synthesis and the biological evaluation of 3,4-disubstituted indole analogs **C**.

The target 3-acrylsulfonamide-4-aryloxy indole analogs, reported in Table 1, were synthesized from 4-bromo-1-methyl indole **2** (Scheme 1). The 4-aryloxy group was introduced by Ullmann-type diaryl ether formation using CuI/*N*,*N*-dimethylglycine as a catalyst.[15] Formylation[16] of **3** with DMF/$POCl_3$ afforded the corresponding 4-formyl-3-aryloxy indoles **4** in 33–95% yield. Wittig–Horner reaction of **4** provided the 4-acryl ester-3-aryloxy indoles **5**. Esters **5** were saponified to afford free acids **6** in 40–90% yield after extraction under acidic conditions. EDCI-mediated coupling of compounds **6** with the appropriate sulfonamide at room temperature resulted in the corresponding acylsulfonamides **7–9** (20–60% yield).

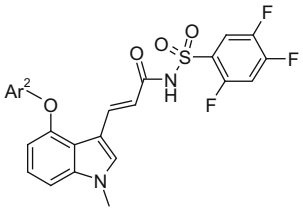
Table 1
IC_{50} for hEP_3 receptor binding affinity for selected compounds



7a-r



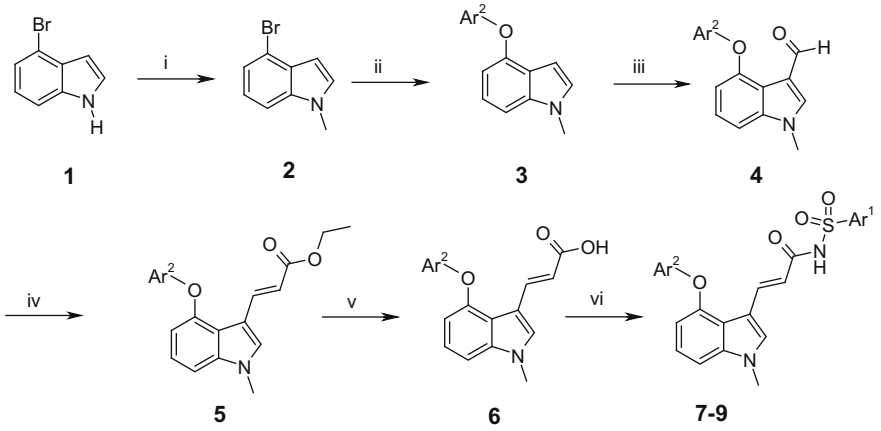
8a-j



9a-l

Compound	Ar^1	Ar^2	EP_3 receptor binding (nM)		
			Normal buffer	10% human serum[a]	Fold-shift in 10% human serum[b]
7a	4,5-Dichlorothiophene	2-Naphthyl	4.6	89.6	19.4
7b	3,4-Difluorophenyl	2-Naphthyl	7.4	15.9	2.1
7c	2,4,5-Trifluorophenyl	2-Naphthyl	9.2	28..0	3.1
7d	4-Fluorophenyl	2-Naphthyl	9.4	182.3	19.4
7e	2-Chlorophenyl	2-Naphthyl	18.4	1682	91.3
7f	3-Chlorophenyl	2-Naphthyl	15.7	35.0	2.2
7g	3,4-Dichlorophenyl	2-Naphthyl	11.8	92.9	7.8
7h	2,4-Dichlorophenyl	2-Naphthyl	54.1	1542	28.5
7i	3,5-Dichlorophenyl	2-Naphthyl	8.7	321.3	37.0
7j	2,4-Difluorophenyl	2-Naphthyl	3.5	115.3	32.7
7k	2,5-Difluorophenyl	2-Naphthyl	6.5	156.7	24.3
7l	2,6-Difluorophenyl	2-Naphthyl	5.9	709.6	120.6
7m	3,5-Difluorophenyl	2-Naphthyl	44.9	156.7	3.5
7n	3-Fluorophenyl	2-Naphthyl	17.5	115.5	6.6
7o	2-Fluorophenyl	2-Naphthyl	11.9	646.4	54.3
7p	4-Chlorophenyl	2-Naphthyl	21.9	1100	50.2
7q	4-Methoxyphenyl	2-Naphthyl	100.8	PD	—
7r	Pentafluorophenyl	2-Naphthyl	5.1	PD	—
8a	3,4-Difluorophenyl	3,4-Dichlorophenyl	12.4	50.8	4.1
8b	3,4-Difluorophenyl	2,4-Dichlorophenyl	11.8	23.7	2.0
8c	3,4-Difluorophenyl	4-Chlorophenyl	27.3	776.2	28.4
8d	3,4-Difluorophenyl	3,4-Difluorophenyl	4.7	195.9	41.9
8e	3,4-Difluorophenyl	2,4-Difluorophenyl	18.5	PD	—
8f	3,4-Difluorophenyl	3-Chloro-4-fluorophenyl	26.9	81.5	3.0
8g	3,4-Difluorophenyl	4-Chloro-3-fluorophenyl	23.4	612.4	26.3
8h	3,4-Difluorophenyl	4-Chloro-2-fluorophenyl	7.2	124.0	17.2
8i	3,4-Difluorophenyl	2-Chloro-4-fluorophenyl	13.8	145.8	10.5
8j	3,4-Difluorophenyl	3-Methoxyphenyl	4.4	PD	—
8k	3,4-Difluorophenyl	6-Quinolinyl	1.9	129.8	68.7
8l	3,4-Difluorophenyl	2-Quinoxalinyl	6.1	216.5	35.3
9a	2,4,5-Trifluorophenyl	3,4-Dichlorophenyl	2.6	45.2	17.1
9b	2,4,5-Trifluorophenyl	2,4-Dichlorophenyl	3.2	16.7	5.2
9c	2,4,5-Trifluorophenyl	4-Chlorophenyl	3.0	392.9	131.7
9d	2,4,5-Trifluorophenyl	3,4-Difluorophenyl	3.8	209.5	55.0
9e	2,4,5-Trifluorophenyl	2,4-Difluorophenyl	84.8	PD	PD
9f	2,4,5-Trifluorophenyl	3-Chloro-4-fluorophenyl	9.7	61.1	6.3
9g	2,4,5-Trifluorophenyl	4-Chloro-3-fluorophenyl	6.7	53.9	8.0
9h	2,4,5-Trifluorophenyl	4-Chloro-2-fluorophenyl	4.4	155.7	35.6
9i	2,4,5-Trifluorophenyl	2-Chloro-4-fluorophenyl	18.1	316.3	17.5
9j	2,4,5-Trifluorophenyl	3-Methoxyphenyl	5.1	PD	PD
9k	2,4,5-Trifluorophenyl	6-Quinolinyl	8.5	167.8	19.8
9l	2,4,5-Trifluorophenyl	2-Quinoxalinyl	7.2	128.2	17.7

[a] PD = partial displacement of [^{3}H]-PGE_2 in the radioligand assay at 20 μM (highest concentration).
[b] Fold-shift value is ratio of IC_{50} in the presence and absence of human serum.



Scheme 1. Reagents and conditions: (i) NaH, CH_3I, DMF, rt, 3 h, 100%; (ii) Ar^2–OH, CuI, *N*,*N*-dimethylglycine HCl salt, Cs_2CO_3, dioxane, 105 °C, 2–3 d; (iii) DMF, $POCl_3$, 50 °C, 1 h, then aq NaOH, 33–95% from **2**; (iv) NaH, triethyl phosphonoacetate, THF, rt, 10 min then compound **4**, THF, 60 °C, 16 h; (v) aq, NaOH, THF, MeOH, 50 °C, 3 h, 40–90% from **4**; (vi) Ar^1–SO_2–NH_2, DMAP, EDCI, CH_2Cl_2, rt, 16 h, 20–60%.

The SAR data and diversity of Ar^1/Ar^2 groups from the previously reported 1,7- and 3,4-peri-substituted indole series[13,17] served as starting point for evaluating SAR for the current series. An analog with the best Ar^1/Ar^2 group from series **A** (Ar^1 = 4,5-dichloro-2-thiophene, Ar^2 = naphthyl)[13] was prepared providing analog **7a**. Compound **7a** exhibited essentially identical activity in the hEP_3 radioligand binding assay in normal buffer (IC_{50} = 4.6 nM) versus the corresponding compound from series **A** (IC_{50} = 9.7 nM). This data strongly supported our hypothesis that the acidic and lipophilic appendages could be interchanged between the 3,4-peri-disubstitued indole series **A** and **C** with retention of hEP_3 binding affinity.

To optimize the Ar^1 (acylsulfonamide) group for the current series we elected to retain the 2-naphthyl substituent (Ar^2). Our previous studies have consistently shown that the derivatives endowed with this moiety were potent and isoform selective EP_3 antagonists.[13,17] Results from the human EP_3 receptor binding[13] in both normal buffer and in the presence of 10% human serum[18] are given in Table 1 (**7a–r**). Gratifyingly, except for the Ar^1 = 4-MeO–C_6H_4, all derivatives displayed EP_3 receptor activity in the low nM range. Except for **7c**, *ortho*-substituted Ar^1 groups showed significant plasma protein binding in the presence of human serum. SAR studies suggested that in this series binding affinity in the absence or presence of plasma proteins (IC_{50}) followed the increase of the electron deficiency[19] for the Ar^1. For example, comparison of data for the pair of molecules **7d**/**7p**, **7b**/**7g** and **7j**/**7h**, suggested that fluorine substituent(s) were generally favored over chlorine group(s). Based on this initial set of SAR data, we prioritized compounds **7b** and **7c** that demonstrated superior EP_3 binding affinity and low plasma protein binding as prototypes for further investigation. In addition, we expected the fluorine substituted phenyl derivatives to provide overall better physico-chemical properties. Thus, the next round of SAR was conducted with the Ar^1 = 3,4-difluorophenyl (**8a–l**) and 2,4,5-trifluorophenyl (**9a–l**).

In order to enhance both solubility and formulability of the targeted compounds, we introduced heterocyclic substituents with basic nitrogen(s) (e.g., quinolinyls **8k** and **9k** and quinoxalinyls **8l** and **9l**). Although these molecules afforded single digit nM potency in the normal buffer binding assay, they suffered from the significant plasma protein binding.

A number of potent molecules from the SAR studies exhibited sound selectivity against a panel of prostanoid receptors (generally >100- to 5000-fold) (Table 2).

Table 3 provides a summary of metabolic stability for the prioritized analogs in liver microsomes. Good metabolic stability was observed for the pair **7b**/**7c** and compound **8b** in all species examined with the exception of rat.

Table 2
Selectivity of the selected compounds for hEP_3 versus other prostanoid receptors

Compound	Prostanoid panel binding (μM)					
	hEP_3	EP_1	EP_2	EP_4	IP	FP
7b	0.0074	1.42	12.5	12.5	1.88	3.59
8a	0.0124	>10	4.2	20.4	ND	2.4
8b	0.0096	3.86	9.6	14.6	ND	1.97
8f	0.0269	ND	>10	>10	4.1	1.82
8h	0.0072	>10	>10	>10	>10	3.19
9a	0.0026	5.32	7.68	13.0	ND	4.64
9b	0.0032	ND	9.3	>10	ND	3.57
9f	0.0097	>10	>10	>10	ND	3.16
9g	0.0061	1.37	10	10	ND	2.23

IC_{50} (μM) values reported are from receptor binding assays with displacement of the respective radioligand.
ND = value not determined.

Table 3
In vitro metabolic stability of selected 3-acrylamide-4-aryloxy indoles in the microsomal assays

Compound	Microsome metabolic stability[a]				
	Human	Rat	Mouse	Dog	Monkey
7b	63.6	24.6	62	69.7	59
7c	91.2	32.0	ND	90.4	ND
7f	79.0	33.3	ND	80.6	ND
8a	58.8	14.9	ND	ND	ND
8b	74.7	30.6	69	104	53.8
8f	91.5	32	ND	ND	ND
8h	113	40	ND	ND	ND
9b	74.7	39.1	ND	ND	ND

[a] Each compound at 5 μM was incubated with liver microsomes representing 0.8 mg/mL protein concentration and the percent parent remaining at 30 min, as determined by LC–MS/MS, is reported.

Both compounds **7b** and **7c** (Table 3), were subsequently evaluated in a functional rat platelet aggregation assay. The molecules exhibited an EC_{50} value of 12.8 and 11.6 nM, respectively, which compares favorably, with potency of previously reported compounds.[13,20] Compound **7c** also exhibited an EC_{50} value of 8.1 nM in a human platelet aggregation inhibition assay.

Selected compounds were evaluated in a functional cell-based assays with CHO-KI cells transfected with the EP_{3II} receptor to con-

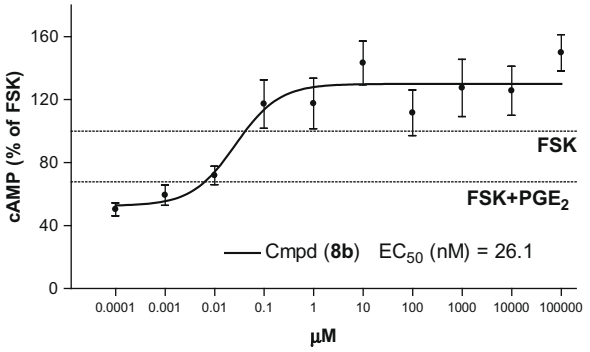


Figure 2. Dose–response of compound (**8b**) on CHO-K1 cells transfected with hEP_{3D} receptor in normal buffer.

Table 4
Mouse iv exposure screen

Compound	Average plasma concentration (ng/mL)	Average brain concentration (ng/g)	Ratio plasma/brain
7b	3420	55.7	61.4
7c	1518	29.1	52.2

Compounds were dosed in NMRI mice, $n = 3$ per compound, at 2 mg/kg in 10% solutol/PBS formulation. Samples were analyzed at 15 min.

firm EP_3 receptor antagonism.[21,22] A representative dose–response curve is shown in Figure 2 for compound **8b** (EC_{50} = 26.1 nM).

SAR studies and initial follow-up evaluation of the synthesized 3,4-disubstituted indoles prioritized the compounds **7b** and **7c** for further studies. Both of these compounds gave relatively low brain exposures while still showing relatively high plasma levels at 15 min post dose, plasma concentration of compounds (**7b**, 6.6 μM and **7c**, 2.8 μM). These data favored our intended therapeutic use as anti-thrombotic agents (Table 4).

The data presented here supported our hypothesis that indole derived analogs representing peri-substitution pattern as represented by series **A**, **B** and **C** provides a sound approach to potent and isoform selective hEP_3 antagonists. For the 3-acrylamide-4-aryloxyindole series, an optimized compound (**7c**) was selected as both potent and selective hEP_3 receptor antagonist with sound functional activity in platelet aggregation studies.

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18. Ref. 12c lists data which includes determinations of binding affinity to the EP_3 receptor in the presence of 0.05% HSA. This data indicates a propensity of these compounds to strongly bind to serum proteins. Hence, we found it imperative to routinely screen each strongly potent compound from the normal buffer assay in the presence of 10% human serum to provide useful information on the free fraction of the compound available for future in vivo examinations.
19. Previous studies have shown that strongly electron rich heteroaromatic Ar^1 substituents of these acylsulfonamides suffer from poorer binding affinity to the EP_3 receptor in normal buffer in addition to exhibiting a high propensity to suffer plasma protein binding. For an example, refer to structures **7g** and **8g** in Ref. 13.
20. Two of our lead compounds, labeled **7aa** and **8aa** in Ref. 13, exhibited EC_{50} in the rat platelet aggregation studies of 358 nM and 136 nM, respectively.
21. The hEP_{3II} (also known as hEP_{3D}) receptor isoform exhibited slightly higher affinity for PGE_2 and higher inhibitory efficiency compared to the other human EP_3 isoforms tested (data not shown). Therefore, hEP_{3II} was selected for both the primary binding screening assay and the functional cell-based assay.
22. Stably transfected CHO-K1 cells expressing the hEP_{3II} receptor were plated into 96-well plates at a cell density of 10^5 cells/well and cultured overnight at 37 °C, 5% CO_2 in culture media supplemented with 10% FIBS, 2% PS and 1 mg/mL Geneticin. Cells were washed once with PBS and pre-incubated in fresh, serum- and antibiotic-free medium containing 1 mM IBMX (3-isobutyl-1-methylxanthine, Sigma) for 30 min at 37 °C. After pre-incubation, cells were incubated with PGE_2 (5×10^{-9} M) and FSK (5×10^{-6} M, Sigma), in absence or presence of the testing compound at the appropriate concentrations (dose–response 10^{-4}–10^{-13} M). Cells were then incubated for an additional 10 min at 37 °C. Reactions were terminated by aspiration of medium and addition of 200 μl of lysis buffer 1B (cAMP EIA System kit, Amersham). cAMP levels were determined using a commercially available cAMP EIA System kit (Amersham). Raw OD values, were transformed into amount of cAMP (fmol/well) using GraphPad Prism 3.02 for Windows (GraphPad Software). For EC_{50} calculations in dose–response experiments, sigmoid non-linear regressions were performed.