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3,4-Disubstituted indole acylsulfonamides: A novel series of potent and selective human 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 shown to be high affinity ligands for this target. These compounds showed high selectivity over IP, FP and other EP receptors and are potent antagonists in functional assays.

Prostanoids, acting through specific membrane receptors belonging to the superfamily of G-protein-coupled receptors (GPCRs) have an essential role in vascular homeostasis, including platelet function regulation. Among various bioactive prostanoids, prostaglandin E_2 (PGE_2) binds preferentially to the EP_1, EP_2, EP_3, and EP_4 receptors; prostaglandin I_2 (PGI_2) to the IP receptor and thromboxane A_2 (TXA_2) to the TP receptor.[1] TxA_2 is a potent stimulator of platelet aggregation, whereas PGI_2 inhibits their activation. PGE_2 has been reported to have a biphasic effect on platelet response, potentiating their aggregation at low concentrations and inhibiting it at higher concentrations.[2] PGE_2 binding to the EP_3 receptor has also been found to play a key role in the contraction of the uterus,[3] inhibition of gastric excretion,[4] modulation of neurotransmitter release,[5] sodium and water reabsorption in kidney tubules,[6] and fever generation and hyperalgesia.[7] Population genetic studies conducted in Iceland have identified DNA variants of the gene encoding the EP_3 receptor that confers a significantly increased risk for peripheral arterial disease (PAD).[8] EP_3 knockout mice data showed that the stimulatory effects of PGE_2 on platelet aggregation were exerted specifically through the EP_3 receptor.[9] A subsequent report from the Fabre lab demonstrated that inflammation/rupturing of existing plaque released PGE_2 locally and promoted vicinal platelet aggregation through the platelet EP_3 receptor.[10] During the course of our studies on the novel antithrombotic agents, we became interested in pursuing potent and selective EP_3 antagonists as novel anti-platelet agents that do not cause excessive bleeding.[11]

Ortho-substituted cinnamyl and dihydrocinnamyl acylsulfonamides have previously been reported as highly potent and selective human EP_3 receptor antagonists. However, these reported analogs suffered from the high plasma protein binding.[12] As a result, molecules featuring favorable in vitro potency and selectivity properties failed to show potency in assays containing high concentration of human plasma proteins.

As reported previously, our drug discovery effort at deCODE allowed for the identification of peri-substituted bicyclic heterocycles as prototypical EP_3 receptor antagonists.[13] Based on this pharmacophore, we have arrived at the series of 3,4-disubstituted indole analogs **1** displaying high affinity for the hEP_3 receptor and low plasma protein binding. Unfortunately, this series of analogs showed high metabolic activity, in particular, with human liver microsomes.[13]

LC/MS/MS analysis of the human liver microsome incubate revealed rapid metabolism at the benzylic [$CH2^*$] position of the molecule **1** to provide analog **2** (X = CO) that displayed poor EP_3 activity (Table 1). In the regimented approach to overcome this undesirable species dependent metabolism, we undertook a detailed investigation of the analogs featuring heteroatom replacement of a benzylic methylene specifically focusing on the respective sulfur analogues. This paper describes generation and biological evaluation of a series of 3-substituted thioethers and respective sulfone analogs (X = S, SO_2, Fig. 1). Analysis of the

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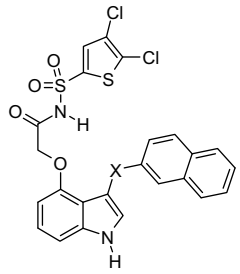
Table 1
Activity of C3-carbon-linked 3,4-disubstituted indole analogs.



Compound	X	hEP_3 IC_{50} (nM)
1	CH_2	3.0
2	CO	545

literature suggested that this functionality was present in investigational small molecules.[14]

The synthetic pathway towards sulfides **7** was designed to allow for the late-stage introductions of the two aryl/heteroaryl groups Ar^1/Ar^2 providing a versatile approach to a diverse matrix of relevant derivatives. The synthesis of the 3-arylsulfonyl-4-oxyacetyl indoles commenced with the selective *O*-alkylation of 4-hydroxyindole (**3**, Scheme 1). Hydrolysis of the ester **4**, followed by the EDCI-mediated coupling of acid **5** with the appropriate sulfonamide furnished the corresponding acyl sulfonamides **6** in 50–85% yield. Reaction of **6** with the appropriate thiol (1.5 eq.) and a mixture of iodine–potassium iodide (1:1, 2 eq.) in a mixture of ethanol and water (5:2) for 2–3 days at room temperature resulted in the targeted sulfides **7** in 40–71% yield.[15] sulfides (**7**) were subsequently oxidized with OXONE (3 eq.) to afford the corresponding sulfones **8** in good-to-excellent yield (82–97%).[16]

For the structure–activity relationship (SAR) evaluation at Ar^1 (acylsulfonamide) position, the Ar^2 group present in the active carba-analog **1** was kept as a 2-naphthyl substituent. Radio-ligand binding assay data for the human EP_3 receptor using [^{3}H-PGE_2][13] in both buffer and in the presence of 10% human serum are given in Table 2 (column [A] and [B]). The unsubstituted (**7l**/**8l**) and *ortho*-substituted [2-chloro (**7c**/**8c**) or 2,4-dimethoxy (**7b**/**8b**)] thioethers afforded 15- to 60-fold greater hEP_3 activity when compared to the corresponding sulfones. However, these molecules were consistently highly plasma bound. The bis-*ortho* substituted isoxazole derivatives **7g**/**8g** were among the least active analogs. Halogen substituted *meta*- or *meta*/*para*-phenyl sulfonamides provided sulfide and sulfone analogs with relatively similar hEP_3 binding affinity. Electron donating methoxy substituents showed high protein binding, with the 4-methoxyphenyl analog **8j** featuring the largest discrimination (>750-fold) between the sulfide and sulfone derivatives.

Bioisosteric replacement of phenyl and halogenated phenyls with the corresponding thiophene and chlorothiophene functionalities allowed for both enhanced hEP_3 binding affinity and reduction in plasma protein binding. This effect was observed for both sulfide (**7d** to **7k** to **7i**) and sulfone (**8d** to **8k** to **8i**) derivatives. The 4,5-dichlorothiophenesulfonamide moiety for Ar^1 yielded potent sulfide/sulfone pair **7i**/**8i**. Specifically, the sulfone **8i** gave 5-fold better EP3 activity compared to the corresponding sulfide. Therefore, 4,5-dichlorothiophene acylsulfonamide was retained as Ar^1 substituent to explore SAR for Ar^2 (Table 2, see columns labeled [C] and [D]).

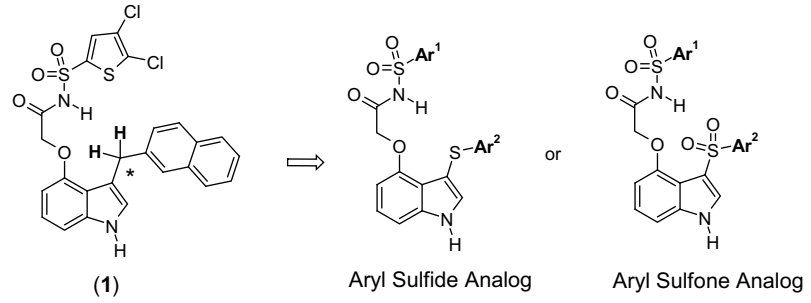
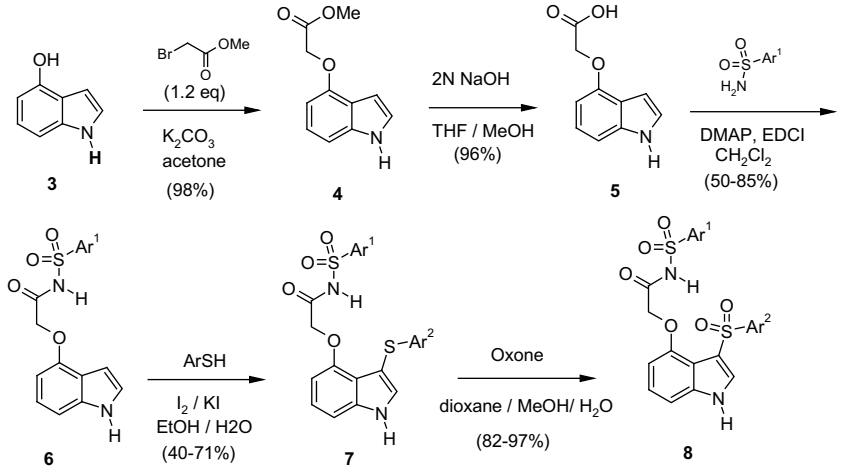
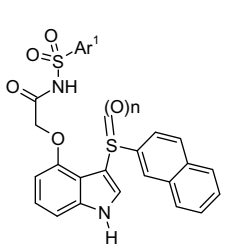


Figure 1.

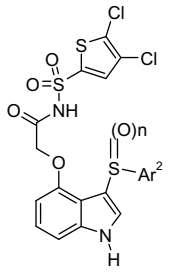


Scheme 1. Synthetic route to 4-alkoxy-3-thioether indoles.

Table 2
IC_{50} of hEP_3 receptor binding affinity for selected compounds.



7a-l, n = 0
8a-l, n = 2



7o-ak n = 0
8o-am n =2

Compound	Ar^1	*n*	hEP_3 Binding nM [A]		Compound	*n*	hEP_3 Binding [B]	
			IC_{50} (nM) NB	FS			IC_{50} (nM) NB	FS
7a	3,4-Difluorophenyl	0	2.0	0.7	**8a**	2	3.6	16.3
7b	2,5-Dimethoxyphenyl	0	1.2	134	**8b**	2	18.8	PD
7c	2-Chlorophenyl	0	1.4	48.7	**8c**	2	79.6	PD
7d	2-Thiophene	0	3.0	27.5	**8d**	2	40.2	PD
7e	3,5-Dichlorophenyl	0	3.6	2.3	**8e**	2	8.8	14.5
7f	3,5-Difluorophenyl	0	5.7	4.2	**8f**	2	3.9	27
7g	3,5-Dimethyl-4-isoxazole	0	122.1	PD	**8g**	2	41.0	PD
7h	3-Chlorophenyl	0	2.0	2.2	**8h**	2	5.9	PD
7i	4,5-Dichlorothiophene	0	9.7	0.3	**8i**	2	1.7	12.9
7j	4-Methoxyphenyl	0	0.7	651	**8j**	2	544.4	PD
7k	5-Chlorothiophene	0	4.5	3.6	**8k**	2	9.2	40.3
7l	Phenyl	0	2.2	10.1	**8l**	2	52.0	13.9
Compound	Ar^2	*n*	hEP_3 Binding [C]		Compound	*n*	hEP_3 Binding [D]	
			IC_{50} (nM) NB	FS			IC_{50} (nM) NB	FS
7o	2-(5-Methyl)thiadiazolyl	0	36.7	PD	**8o**	2	198	PD
7p	2,4-Dichlorophenyl	0	27.6	0.2	**8p**	2	44.8	5.6
7q	2,4-Difluorophenyl	0	18.3	1.9	**8q**	2	13.8	PD
7r	2,4-Dimethylphenyl	0	1.3	5.2	**8r**	2	5.7	33.2
7s	2,5-Dimethoxyphenyl	0	24.1	24.6	**8s**	2	6.1	7.9
7t	2-Benzothiazolyl	0	7.6	9.8	**8t**	2	44	7.6
7u	2-Chloro-4-fluorophenyl	0	1.8	2.6	**8u**	2	20.2	10.7
7v	2-Chlorophenyl	0	13.7	5.8	**8v**	2	43	PD
7w	2-Methoxyphenyl	0	24	13.2	**8w**	2	11.9	19.7
7x	2-Pyridinyl	0	2.8	PD	**8x**	2	95	PD
7y	2-Quinolinyl	0	4.7	3.9	**8y**	2	18.8	11.6
7z	3-(1,2,4-triazolyl)	0	37.9	PD	**8z**	2	236.2	PD
7aa	3,4-Dichlorophenyl	0	1.3	1.1	**8aa**	2	4.7	3.6
7ab	3,4-Dimethoxyphenyl	0	9.3	1.6	**8ab**	2	11.4	PD
7ac	4-Acetamidophenyl	0	3.6	43.2	**8ac**	2	27.8	5.0
7ad	4-Chlorophenyl	0	3.4	4.1	**8ad**	2	14.3	31.3
7ae	4-Methoxyphenyl	0	0.5	22.6	**8ae**	2	30.0	PD
7af	5-(1-Methyl)tetrazolyl	0	8.2	58.3	**8af**	2	88.4	PD
7ag	Phenyl	0	5.2	34.0	**8ag**	2	16.7	PD
7ah	2-(1 Methyl)benzimidazolyl	0	2.5	10.5	**7ai**	0	4.3	PD
7aj	2-Imidazolyl	0	66.1	PD	**7ak**	0	266	PD
8am	3-Methoxyphenyl	2	9.4	33.7				

NB, binding assay in normal buffer; FS, fold-shift in presence of 10% human serum.
PD, partial displacement of 3[H]-PGE2 at 20 μM, highest concentration.

With the exception of 2-pyridyl (**7x/8x**) and 4-methoxyphenyl (**7ae/8ae**) analogs, which gave 35- and 60-fold lower hEP_3 activity for sulfone versus sulfide, respectively; a number of other substituents gave a relatively narrow separation between sulfide–sulfone pairs. For the Ar^2 substituents, *ortho* substituted phenyls, bicyclics and 5-membered heterocytes led to the derivatives displaying considerable plasma protein binding. In fact, for several of these examples (**8o**,**8v**,**8z**,**8af**) full displacement of the radioligand was not achieved even at 20μM, the highest assay concentration.

In our hands, Ar^1 substituents endowed with strong electron donating groups or that were heteroaromatics consistently furnished ligands with poor hEP_3 receptor activity and these analogs were highly plasma bound. These data were in accord with our pharmacophore model[12] placing the Ar^1 substituent in a hydrophobic environment. Based on the SAR data from Table 2, sulfides **7i, 7p**, **7u**, and **7aa** and the corresponding sulfones displayed good affinity for the hEP_3 receptor along with the low fold-shift in the presence of human serum.

A selected set of compounds from Table 2 exhibiting good hEP_3 activity and low PPB fold-shift were evaluated against a panel of prostanoid receptors, exhibiting sound selectivity (>300–30,000, Table 3). Metabolic stability for the optimized sulfide/sulfone pairs in the rat and human liver microsome assay is summarized in Table 4. Sulfones consistently showed greater metabolic stability than their sulfide counterparts. Notably, a sulfide/sulfone pair **7aa**/**8aa** featured good stability when incubated in the presence of both rat and human liver microsomes.

Table 3
Fold selectivity[a] of selected compounds for the hEP_3 receptor versus other prostanoid receptors.

Compound	hIP versus hEP_3	hFP versus hEP_3	hEP_4 versus hEP_3	hEP_2 versus hEP_3	hEP_1 versus hEP_3
7y	838	671	3290	1682	ND
7r	31555	401	1861	1293	13090
7ab	227	1053	1492	1474	563
7u	2243	521	5282	5741	3726
7ad	2816	445	2034	1401	ND
7aa	4481	596	1768	950	10769
7t	1058	300	2391	1656	ND
7h	1298	1031	1360	1094	12261
8s	7298	2120	1363	ND	ND
8aa	2709	384	4218	1452	5189
7p	3350	2029	8875	6230	3520
8am	2171	1871	21391	1074	5421
7al	5138	728	13421	7625	950
7f	1518	614	1496	2110	478
7a	2151	570	1233	3604	713
7n	ND	5540	9672	442	2867
7m	4176	1088	2900	3730	1099

ND, not determined.
[a] Ratio (IC_{50} receptor shown/IC_{50} hEP_3 receptor).

Table 4
Metabolic stability versus rat and human liver microsomal preparations (in vitro).[a]

Compound	**7p**	**8p**	**7ad**	**8ad**	**7y**	**8y**	**7aa**	**8aa**
Human	37	79	52	75	23	60	46	69.7
Rat	0	72	14	98	35	68	74	68.8

[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. For comparison, compound **1** under these assay conditions provided 68% and 2% parent remaining for rat and human liver microsomes, respectively.

Table 5
Primary and secondary assay results for the sulfide and sulfone pair, **7aa** and **8aa**.

Compound	hEP_3 (nM)[a]	mEP_3 (nM)[a]	Rat platelet aggregation IC_{50}[b] (nM)
7aa	1.7	15.5	358
8aa	4.7	2.0	136

[a] IC_{50} from displacement binding assays using ^3H-PGE_2.
[b] The assay was performed using PGE_2 and collagen as coaggregants in the presence of 20% rat serum.

Sulfide/sulfone derivatives **7aa** and **8aa** showed (i) high affinity for the hEP_3 and mEP_3 receptor (Table 5), (ii) consistent stability in both rat and human liver microsomes assay and (iii) good selectivity against the family of prostanoid receptors, were further evaluated ex vivo using a platelet aggregation assay (rat-platelet rich plasma, PRP).[17] Both analogs afforded full antagonism of the hEP_3 receptor in the cAMP functional assay. The sulfone **8aa** gave 2.5-fold better activity in the rat platelet aggregation assay compared to the corresponding sulfide **7aa,** and the sulfone **8aa** showed better activity versus mouse receptor in the binding assay as well. (Table 5). Compound **8aa** also showed low fold-shift in the presence of human serum. The hEP_3 binding curve in the normal buffer and in the presence of 10% human serum for is shown graphically in Figure 2.

The molecule **8aa** was further selected for the in vivo efficacy studies.

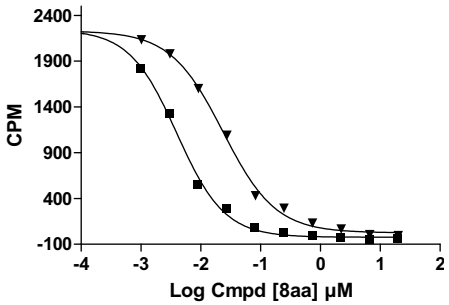


Figure 2. Dose–response curve showing IC_{50} for hEP_3 binding assay in normal buffer (–■–) and in the presence of 10% human serum (–▼–).

In conclusion, the 3,4-disubstituted indole template yielded a series of potent and selective hEP_3 antagonists. In our SAR studies, we have successfully addressed both plasma protein binding and metabolic liability of the lead candidates from this series. Compounds **7aa** and **8aa** displayed full antagonistic behavior against the target hEP_3 receptor (IC_{50} = 0.3 and 1 nM for **7aa** and **8aa**, respectively) in the cell-based assay. These molecules also displayed good potency in the platelet aggregation studies and were selected for in vivo efficacy studies.

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