Bioorganic & Medicinal Chemistry Letters 20 (2010) 2658–2664

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

1,7-Disubstituted oxyindoles are potent and selective EP_3 receptor antagonists

Nian Zhou [a], Alexandre M. Polozov [a], Matthew O'Connell [a], James Burgeson [a], Peng Yu [a], Wayne Zeller [a], Jun Zhang [a], Emmanuel Onua [a], Jose Ramirez [b], Gudrun A. Palsdottir [b], Gudrun V. Halldorsdottir [b], Thorkell Andresson [b], Alex S. Kiselyov [a], Mark Gurney [a,b], Jasbir Singh [a,*]

[a] *deCODE Chemistry, 2501 Davey Road, Woodridge, IL 60517, USA*
[b] *deCODE genetics, Inc. Reykjavik, Iceland*

ARTICLE INFO

Article history:
Received 10 December 2009
Revised 5 February 2010
Accepted 8 February 2010
Available online 25 February 2010

ABSTRACT

A series of novel 1,7-disubstituted oxyindoles were shown to be potent and selective EP_3 receptor antagonists. Variation of substitution pattern at the C-3 position of indole enhanced in vitro metabolic stability of the resulting derivatives. Series **27a–c** showed >1000-fold selectivity over a panel of prostanoid receptors including IP, FP, EP_1, EP_2 and EP_4. These agents also featured low CYP inhibition and good activity in the functional rat platelet aggregation assay.

Prostanoids generated in vivo from arachidonic acid act through specific membrane-bound G protein-coupled receptors (GPCRs) and play an essential role in vascular homeostasis, including regulation of platelet function. Specifically, PGE_2 preferentially binds to a family of receptors referred to as EP_{1-4}.[1] Low levels of PGE_2 potentiate platelet aggregation while at higher concentrations this effect is inhibited.[2] Furthermore, studies utilizing knock-out mice showed that the stimulatory effects of PGE_2 on platelet aggregation are exerted specifically through the EP_3 receptor.[3] Notably, EP_3 receptors are not expressed in the arterial wall. Therefore, selective EP_3 receptor antagonists are expected to be anti-thrombotic agents that do not increase risk of bleeding.[4]

Previously, we have reported identification and SAR of potent, isoform selective hEP_3 receptor antagonists derived from 1,7-disubstituted indoles. In our hands, these molecules displayed strong platelet aggregation in both ex vivo and in vivo settings (Fig. 1, structure **A**).[5] During structure–activity relationship (SAR) studies we noticed that basic Ar^1 or Ar^2 functionalities lead to a significant reduction of hEP_3 affinity.[6] In this Letter, we followed the original pharmacophore hypothesis[4] and studied indolone derivatives with the specific focus to improve physicochemical properties of the resultant derivatives. We have previously reported that the nonaromatic hexahydro-indolone core did furnish potent hEP_3 antagonists.[7] However, in spite of good potency in the binding

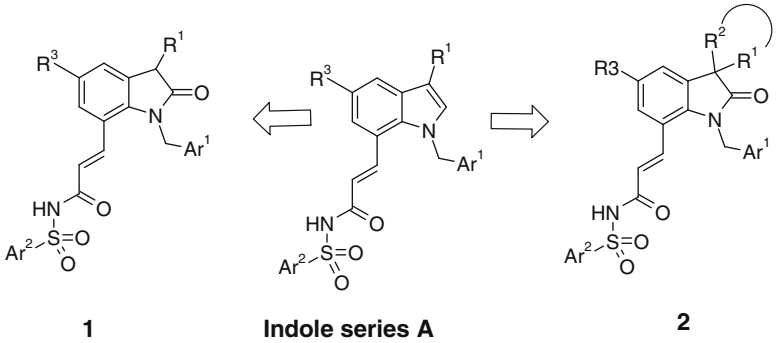


Figure 1. Proposed indolone series of derivatives **1** and **2**.

* Corresponding author. Tel.: +1 630 904 2218.
E-mail addresses: JSingh@deCODE.com, Jasbir@JasinDS.com (J. Singh).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.02.028

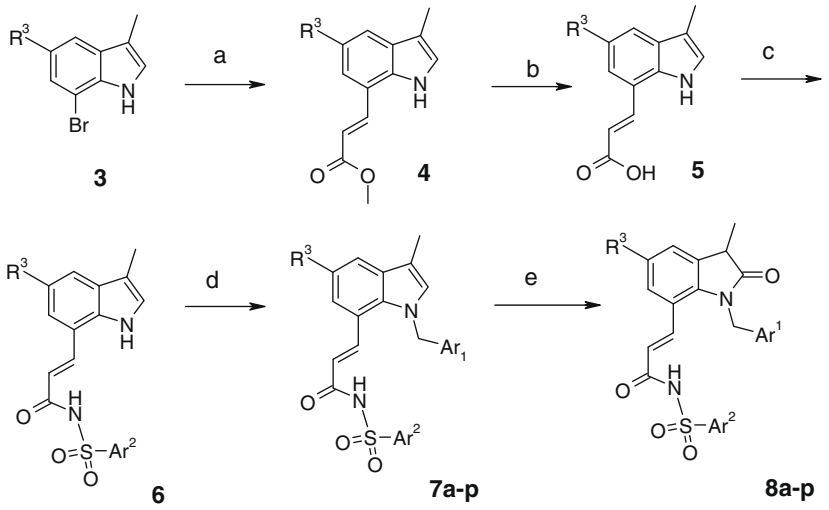
assay, this series showed somewhat lower activity in the functional rat platelet aggregation assay.[8] It was anticipated that the indolone templates, which retain the aromatic characteristics of the indole series A would provide improved activity in the functional assay. Moreover, the indolone analogs are expected to exhibit enhance GI permeability and PK characteristics due to keto–enol tautomerization of the carbonyl moiety. Chemical feasibility of this chemotype also allowed for the additional diversity at the C-3 position.

This Letter describes EP_3 receptor binding affinities, liver microsome stability, prostanoid selectivity and rat platelet aggregation studies of the novel 1,7-substituted oxyindole series represented by the generic structures **1** and **2** (Fig. 1).

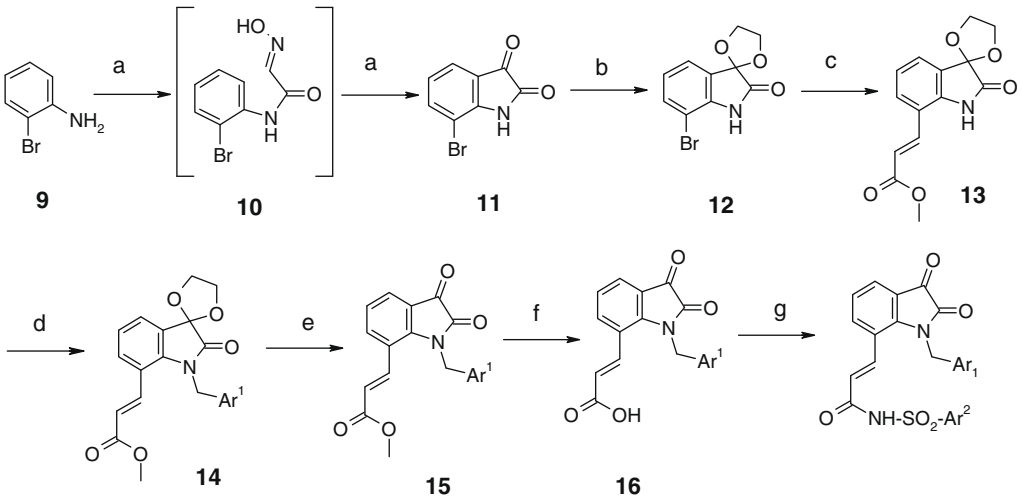
First, we prepared a series of 3-methyl oxyindoles. α-Methyl substituted oxyindoles ($R^1 = CH_3$, $R^2 = H$) were accessed via oxidation of the corresponding 3-methyl-indoles. Starting 7-bromo-3-methylindole (**3a**, $R^3 = H$) was subjected to Heck olefination conditions with methyl acrylate to afford **4** (Scheme 1). Compound **4** was saponified to yield acrylic acid **5** followed by its coupling with a diverse aryl sulfonamides to furnish the respective derivatives **6**. Alkylation of the indole nitrogen with benzylic halides provided 1,7-disubstituted indoles **7a–p** that were converted to the targeted oxyindoles **8a–p** via oxidation with DMSO/concentrated HCl/acetic acid system. These compounds, as shown, imply presence of a chiral center. However, due to keto–enol tautomerism this center will be expected to equilibrate under physiological conditions.

In a subsequent synthetic effort, we prepared a 3,3-disubstituted oxyindole series. Isatin core was selected as a convenient starting point as the C-3 position may be derivatized by reduction, reductive amination and addition reactions to rapidly produce the targets of interest (Schemes 2 and 3). The key oxyindole molecules from this series were produced from the isatin intermediates **15** and **16**.

The synthesis of compound **16** commenced with the reaction of 2-bromoaniline (**9**) with chloral hydrate and hydroxylamine to afford the intermediate hydroxylamine (**10**).[9] Heating of **10** in the concentrated H_2SO_4 afforded 7-bromoisatin (**11**).[9] Direct Heck

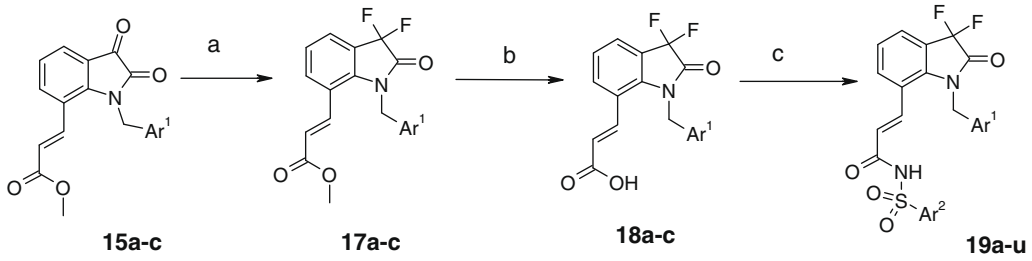


Scheme 1. Reagents and conditions: (a) methyl acrylate, Et_3N, $Pd(OAc)_2$, tri-*o*-tolylphosphine, 100 °C, 4 h, sealed tube, (82%); (b) aq NaOH, THF, CH_3OH, room temperature, 16 h (100%); (c) $Ar^2SO_2NH_2$, EDCI, DMAP, CH_2Cl_2, rt, 2–16 h, 80–94%; (d) NaH, DMF or THF, $ArCH_2X$, rt, 16 h, 20–85%; (e) DMSO, HCl–AcOH, rt, 2–5.5 h, 54–91%.



Ar¹ = 2,4-Dichlorophenyl, 2-Naphthyl, 3,4-Difluorophenyl

Scheme 2. Reagents and conditions: (a) $Cl_3CCHO{\cdot}H_2O$, $NH_2OH{\cdot}HCl$, reflux, 30 min, then H_2SO_4, 50–80 °C, 30 min, 44%, two steps; (b) ethylene glycol, *p*TsOH, benzene, reflux, 23 h, 90%; (c) methyl acrylate, $Pd(OAc)_2$, (*o*-tolyl)$_3$P, Et_3N, sealed tube, 100 °C, 6 h, 81%; (d) $ArCH_2X$, K_2CO_3, DMF, 50 °C, 3 h, 77–84%; (e) concentrated HCl, MeOH, 90 °C, 3 h, 77–83%; (f) NaOH, CH_3OH, H_2O, 50 °C, 4 h, 78–83%; (g) $Ar^2SO_2NH_2$, EDCI, DMAP, CH_3CN, rt, 8–70 h, 60–85%.



Scheme 3. Reagents and conditions: (a) DAST, CH_2Cl_2, rt, 18 h, 90–99%; (b) NaOH, CH_3OH, H_2O, 50 °C, 4 h, 45–91%; (c) $Ar^2SO_2NH_2$, EDCI, DMAP, CH_3CN, rt, 16–72 h, 62–80%.

coupling of bromoisatin **11** with methyl acrylate did not yield the anticipated 7-isatin acrylate presumably due to the significant side reactions involving active C-3 carbonyl group of isatin. Thus, compound **11**, was converted to the respective ethylene acetal **12**.[10] This protection facilitated both Heck coupling to furnish **13** and a subsequent N-alkylation to result in compound **14**. This four-step conversion of **11** to **14** followed by its deprotection afforded the acrylate **15** in a 52% yield. Saponification of methyl ester **15** provided acrylic acid **16**.

Conversion of **15** into our second set of desired target compounds involved treatment with DAST in CH_2Cl_2 to provide *gem*-difluoro derivatives **17a–c** (Scheme 3). Saponification of **17a–c** afforded the corresponding acrylic acids **18a–c**. Ar^1 = 2,4-dichlorophenyl, 2-naphthyl or 3,4-difluorophenyl substituents were selected as optimal from our earlier SAR studies of unsaturated analogs.[6,7] EDCI-mediated coupling of **18a–c** with aryl sulfonamides provided targeted molecules **19a–u** in a 62–80% overall yields.

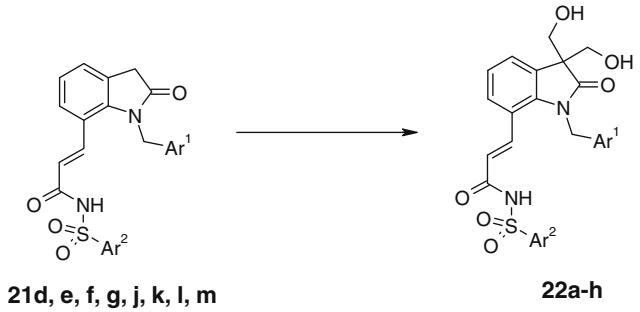
Methylene oxyindoles **21a–q** were accessed in a 23–94% overall yields by a two-step sequence involving reduction of **16a–c** with hydrazine[7] to oxyindoles **20a–c** followed by their coupling with the respective aryl sulfonamides (Scheme 4).

Several selected compounds **21** underwent α-bis-hydroxymethylation in basic formalin solution to provide molecules **22** (Scheme 5).

Saponification of compounds **14a** and **14b** afforded acrylic acids **23a** and **23b**, respectively (Scheme 6). A standard acylsulfonamide coupling protocol described above (Scheme 1) afforded target compounds **24a–c** in 65–89% yields.

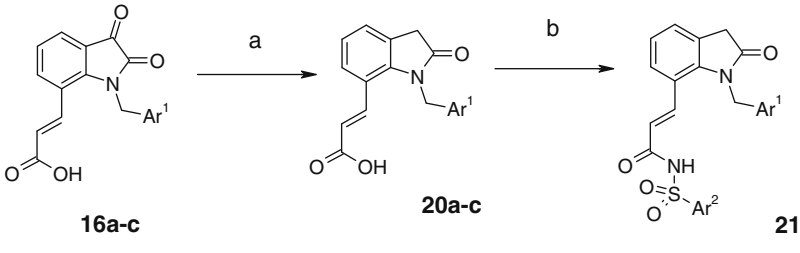
Acylsulfonamide formation using compound **16** afforded isatin derivatives **25a–f**. Compound **26** was made through the condensation of compound **25a** with 2-mercaptoethylamine. A series of α-hydroxy oxyindoles (**27a–c**) were produced by reduction of the corresponding isatin derivatives **25a–e** (Scheme 7).

In our previous Letter[4,7] we have reported that Ar^1 = 2,4-dichlorophenyl, 2-naphthyl and 3,4-difluoro-phenyl groups displayed good hEP_3 receptor affinity. As shown in Table 1, compounds **8a–p** showed high binding affinity for the EP_3 receptor in normal buffer providing low nanomolar and/or sub-nanomolar IC_{50}S. When the receptor binding assay was performed in the presence of 10% human serum (HS), series **8** analogs, showed low-to-moderate fold shift (<10×) in binding affinities with the exception of Ar^2 as 2-thiophenyl (**8a** and **8g**) or Ar^1 as 3,4-difluorophenyl (**8n–p**) indicating their low potential for protein plasma binding. Several compounds from this series were further evaluated for stability using liver microsome incubations (Table 1). Unfortunately, these displayed low metabolic stability in both human and rodent liver microsomes. Incorporation of the fluorine atom at the C-5 position of indole core improved metabolic stability albeit at the expense of the plasma binding properties, as these analogs only displayed partial activity in the receptor binding assay in the presence of human serum.

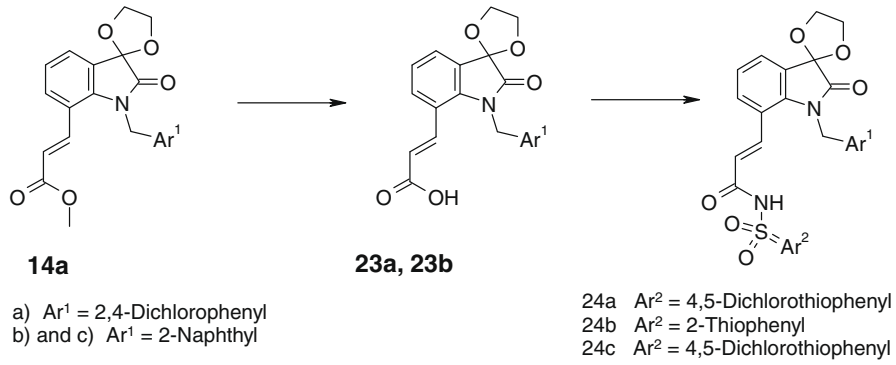


Scheme 5. Reagents and conditions: Na_2CO_3, 37% aq CH_2O, dioxane, rt, 18–60 h, 22–64%.

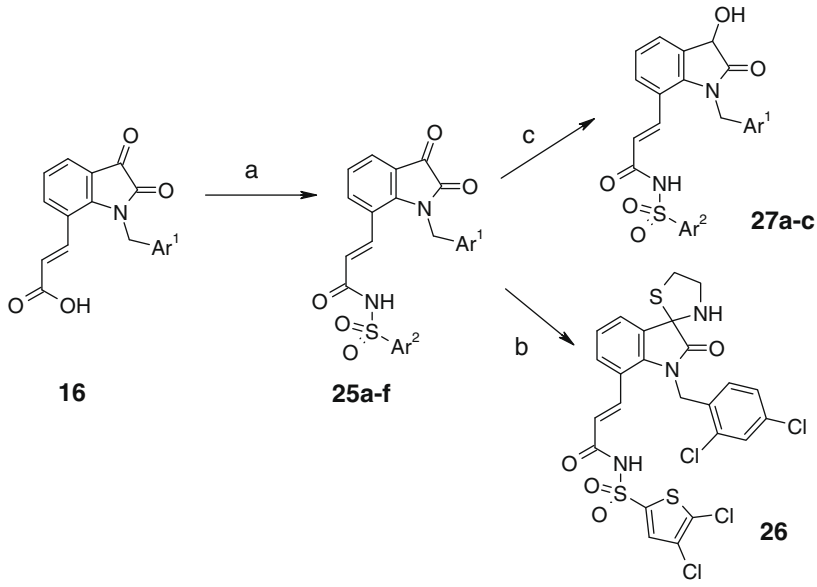
The most compelling initial biological data for the series **8** were obtained from the rat platelet aggregation studies, the respective IC_{50} values ranging from single digit to double digit nanomolar (Table 2). This further supported our notion that indolone core would provide improved functional activity. These results triggered additional structural modifications within this chemotype



Scheme 4. Reagents and conditions: (a) $NH_2NH_2{\cdot}H_2O$, 60 °C, 18 h, 73%; (b) $Ar^2SO_2NH_2$, EDCI, DMAP, CH_2Cl_2 (or CH_3CN or THF), rt, 16–72 h, 23–94%.



Scheme 6. Reagents and conditions: (a) LiOH, CH_3OH, THF, H_2O, 50 °C, 4 h, 67–95%; (b) $Ar^2SO_2NH_2$, EDCI, DMAP, CH_2Cl_2, rt, 16–72 h, 65–89%.



Scheme 7. Reagents and conditions: (a) $Ar^2SO_2NH_2$, EDCI, DMAP, CH_2Cl_2, rt, 16–72 h, 38–77%; (b) $HSCH_2CH_2NH_2$, $TsOH{\cdot}H_2O$, 40%; (c) $NaBH_4$, MeOH, H_2O, 0 °C, 1 min, 57–82%.

aimed at the enhanced liver microsome stability. We speculated that liver microsome instability most likely originated from the CYP-mediated oxidation of the C-3 benzylic position of oxyindole. We next focused on the α,α-difluoro derivatives **19**. As shown in Table 2, many of these compounds retained high binding affinity for the EP_3 receptor and showed relatively low fold shift in the receptor binding assays in the presence of 10% HS. Furthermore, series **19** molecules showed good metabolic stability for human, rat and mouse liver microsomes (Table 1), however they suffered a setback in the rat platelet aggregation assay showing reduced activity with the IC_{50} values exceeding 800 nM. Compound **19** also displayed tendency for a mild CYP inhibition, most notably for the CYP3A4 (BFC used as a substrate). Consequently, these series were not pursued further.

Next, we investigated α-methylene oxyindole series **21**. With the exception of **21b** and **21h** (Ar^2 = 2-thiophene), all compounds from this class exhibited IC_{50} <3 nM for the EP_3 receptor in normal buffer. In addition, except for the compounds featuring Ar^1 = 3,4-difluorophenyl, other derivatives from this chemotype displayed low fold shifts in the presence of 10% HS following the trend observed for the series **8** and **19**. Considering combined evidence, we focused on preparing oxyindole derivatives with Ar^1 = 2,4-dichlorophenyl or 2-naphthyl.[4,7] Analogs from series **21** gave the poorest results in the rat platelet aggregation assay (Table 2) and thus these were not pursued further.

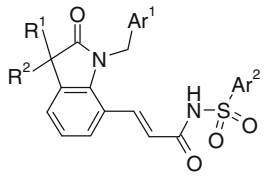
In order to both improve metabolic stability and physiochemical parameters of the oxyindole derivatives, we further pursued modification at the C-3 of oxyindole core specifically by varying R^1/R^2 moieties. *gem*-Disubstituted derivatives including **22** and **24** showed both reduced EP_3 receptor affinity in normal buffer and high fold shifts with a 10% HS. Compound **26** featuring a protonizable nitrogen displayed good EP_3 receptor affinity but it suffered from high protein binding as well.

Molecules **25a–f** exhibited single to double digit nanomolar binding affinities and moderate fold shifts in the presence of HS. Analogs **27a–c** afforded high affinity for the EP_3 receptor, a promising inhibition profile in the rat platelet aggregation assay (Table 2) and reduced potential for the plasma protein binding.

Importantly, compounds **27a–c** were considerably more stable towards human and rodent liver microsomes treatment. Three specific α-hydroxy oxyindole analogs (**27a–c**) furnished high selectivity for the EP_3 receptor in the panel of other prostanoid receptors (Table 3). These agents did not inhibit CYP isoforms including 1A2, 2D6 and subset of 3A4 isozymes. However, they did affect the 3A4 site that metabolizes DBF and showed relatively high inhibition of CYP2C9 (Table 4).

The intrinsic PK parameters for the α-hydroxy oxyindole **27a** following iv dosing to mouse and rat are shown in Table 5. Although the half-life was similar, the clearance and volume of distribution was lower in rat than that observed in mouse.

Table 1
Binding affinities (IC_{50}s) for compound series **8**, **19**, **21**, **22**, **24**, **25**, **26** and **27** in normal buffer and 10% human serum, and liver microsome stabilities (% parent remaining after 30 min incubation) for human (HLM), rat (RLM), mouse (MsLM), dog (DLM) and monkey (MkLM)



Cmpd	R^1	R^2	Ar^1	Ar^2	hEP_3 NB[a] (nM)	hEP_3 HS[b] (nM)	Metabolic stability[c]		
							HLM	RLM	MsLM
C3-monomethyl analogs									
8a	CH_3	H	2,4-Dichlorophenyl	2-Thiophenyl	2.4	660			
8b	CH_3	H	2,4-Dichlorophenyl	4,5-Dichlorothiophenyl	3.4	23.9			
8c	CH_3	H	2,4-Dichlorophenyl	3,4-Difluorophenyl	0.4	3.3	64.7	62.1	16
8d	CH_3	H	2,4-Dichlorophenyl	3,4-Dichlorophenyl	0.9	9.8			
8e	CH_3	H	2,4-Dichlorophenyl	3-Chlorophenyl	0.5	6.8	49.5	23.6	7.26
8f	CH_3	H	2,4-Dichlorophenyl	2,4,5-Trifluorophenyl	0.5	4.3	48.4	22.4	4.85
8g	CH_3	H	2-Naphthyl	2-Thiophenyl	3.5	96.5	43.5	48	32.6
8h	CH_3	H	2-Naphthyl	4,5-Dichlorothiophenyl	4.4	3.2	48.2	8.0	67.7
8i	CH_3	H	2-Naphthyl	3,4-Difluorophenyl	0.6	20			
8j	CH_3	H	2-Naphthyl	2,4,5-Trifluorophenyl	0.3	5.4	13.7	17.6	5.1
8k	CH_3	H	2-Naphthyl	3-Chlorophenyl	0.4	2.8	24.8	13.1	5.63
8l	CH_3	H	2-Naphthyl	3,4-Chlorophenyl	0.6	4.1	36.4	10.3	5.83
8n	CH_3	H	3,4-Difluorophenyl	3,4-Dichlorophenyl	0.9	95.8			
8o	CH_3	H	3,4-Difluorophenyl	2,4,5-Trifluorophenyl	1.4	10.7			
8p	CH_3	H	3,4-Difluorophenyl	3-Chlorophenyl	4.0	39.0			
19a	F	F	2,4-Dichlorophenyl	4,5-Dichlorothiophenyl	3.8	64.0	94.4	94.8	100
19b	F	F	2,4-Dichlorophenyl	2-Thiophenyl	237	PD[d]			
19c	F	F	2,4-Dichlorophenyl	2,4,5-Trifluorophenyl	0.2	5.6	74.6	77.4	60.6
19d	F	F	2,4-Dichlorophenyl	3,4-Difluorophenyl	0.2	6.5	74.4	80.1	71
19e	F	F	2,4-Dichlorophenyl	3,4-Chlorophenyl	0.2	5.5	73.1	70.4	82.8
19f	F	F	2,4-Dichlorophenyl	3-Chlorophenyl	0.2	7.7	66.6	62.2	31.2
19g	F	F	2,4-Dichlorophenyl	4-Chlorophenyl	1.1	251			
19h	F	F	2,4-Dichlorophenyl	4-Fluorophenyl	0.2	64.7			
19i	F	F	2-Naphthyl	4,5-Dichlorothiophenyl	11.6	20.8	79.3	71.8	80.7
19j	F	F	2-Naphthyl	2-Thiophenyl	201.8	PD			
19k	F	F	2-Naphthyl	2,4,5-Trifluorophenyl	0.3	9.3	68.8	54.2	47.9
19l	F	F	2-Naphthyl	3,4-Difluorophenyl	0.2	5.0	68.1	45	47.1
19m	F	F	2-Naphthyl	3,4-Chlorophenyl	0.3	4.5	79.4	62.1	41.3
19n	F	F	2-Naphthyl	3-Chlorophenyl	0.1	1.9	64.5	21	21.6
19o	F	F	2-Naphthyl	4-Fluorophenyl	2.7	27.2			
19p	F	F	2-Naphthyl	4-Chlorophenyl	5.5	125.1			
19q	F	F	3,4-Difluorophenyl	4,5-Dichlorothiophenyl	7.2	979.9			
19r	F	F	3,4-Difluorophenyl	2,4,5-Trifluorophenyl	16.5	720.9			
19s	F	F	3,4-Difluorophenyl	3,4-Difluorophenyl	1.3	251.1			
19t	F	F	3,4-Difluorophenyl	3,4-Chlorophenyl	1.3	PD			
19u	F	F	3,4-Difluorophenyl	3-Chlorophenyl	5.0	416.3			
C3-methylene (unsubstituted) series									
21a	H	H	2,4-Dichlorophenyl	4,5-Dichlorothiophenyl	2.9	92.8	76.8	46.3	56.8
21b	H	H	2,4-Dichlorophenyl	2-Thiophenyl	56.0	PD			
21c	H	H	2,4-Dichlorophenyl	3-Chlorophenyl	0.5	9.4	50.4	21	33.9
21d	H	H	2,4-Dichlorophenyl	2,4,5-Trifluorophenyl	0.2	4.4	61.6	50.6	70.1
21e	H	H	2,4-Dichlorophenyl	3,4-Difluorophenyl	0.7	3.3	54.3	23.5	39.1
21f	H	H	2,4-Dichlorophenyl	3,4-Chlorophenyl	1.2	3.3	53.3	26.8	56.1
21g	H	H	2-Naphthyl	4,5-Dichlorothiophenyl	1.3	5.4	105	32.9	63.8
21h	H	H	2-Naphthyl	2-Thiophenyl	23.6	PD			
21i	H	H	2-Naphthyl	2,4,5-Trifluorophenyl	0.3	10.2	73.9	62.5	
21j	H	H	2-Naphthyl	3,4-Difluorophenyl	0.4	6.9	65.9	43	13.2
21k	H	H	2-Naphthyl	3,4-Chlorophenyl	0.5	5.2	66.5	46	15.7
21l	H	H	2-Naphthyl	3-Chlorophenyl	0.6	8.1	56.8	29.8	7.8
21m	H	H	3,4-Difluorophenyl	3,4-Difluorophenyl	0.9	47.8			
21n	H	H	3,4-Difluorophenyl	3,4-Chlorophenyl	0.3	55.4			
21o	H	H	3,4-Difluorophenyl	3-Chlorophenyl	0.4	35.5			
21p	H	H	3,4-Difluorophenyl	2,4,5-Trifluorophenyl	0.7	42.0			
21q	H	H	3,4-Difluorophenyl	4,5-Dichlorothiophenyl	0.4	55.4			
C3-disubstituted analogs									
22a	CH_2OH	CH_2OH	2-Naphthyl	4,5-Dichlorothiophenyl	2.3	243.1			
22b	CH_2OH	CH_2OH	2-Naphthyl	3,4-Difluorophenyl	11.6	186.9			
22c	CH_2OH	CH_2OH	3,4-Difluorophenyl	3,4-Difluorophenyl	45.4	PD			

Table 1 (*continued*)

Cmpd	R^1	R^2	Ar^1	Ar^2	hEP_3 NB[a] (nM)	hEP_3 HS[b] (nM)	Metabolic stability[c]		
							HLM	RLM	MsLM
C3-spiro-analogs									
24a	$-OCH_2CH_2O-$		2,4-Dichlorophenyl	4,5-Dichlorothiophenyl	3.8	PD		28.4[e]	
24b	$-OCH_2CH_2O-$		2-Naphthyl	2-Thiophenyl	13.0	679.0		37.8[e]	
24c	$-OCH_2CH_2O-$		2-Naphthyl	4,5-Dichlorothiophenyl	7.9	82.4			
26	$-SCH_2CH_2NH-$		2,4-Dichlorophenyl	4,5-Dichlorothiophenyl	4.9	880			
C3-oxo (isatin) analogs									
25a	$R^1 = R^2 = O$		2,4-Dichlorophenyl	4,5-Dichlorothiophenyl	6.5	136.5			
25b	$R^1 = R^2 = O$		2-Naphthyl	2-Thiophenyl	13.7	260.0			
25c	$R^1 = R^2 = O$		2-Naphthyl	4,5-Dichlorothiophenyl	12.3	15.0			
25d	$R^1 = R^2 = O$		2-Naphthyl	4-Fluorophenyl	1.6	33.9			
25e	$R^1 = R^2 = O$		2-Naphthyl	3,4-Chlorophenyl	1.0	12.7			
25f	$R^1 = R^2 = O$		2-Naphthyl	2,4,5-Trifluorophenyl	0.6	17.4			
C3-hydroxy (α-hydroxy oxyindole) analogs									
27a	OH	H	2-Naphthyl	4-Fluorophenyl	0.9	19.4	97.7	103.3	83.4
27b	OH	H	2-Naphthyl	3,4-Difluorophenyl	1.6	7.6	89.3	87.9	72.7
27c	OH	H	2-Naphthyl	2,4,5-Trifluorophenyl	0.6	9.4	95.6	99.7	73.5

[a] NB: normal assay buffer.
[b] HS: hEP_3 IC_{50} in the presence of 10% human serum.
[c] (1) Data shown as percent parent remaining at 30 min following incubation with liver microsomes. (2) HLM: human liver microsomes, RLM: rat liver microsomes, MsLM: mouse liver microsomes, DLM: dog liver microsomes; MkLM: monkey liver microsomes. (3) DLM: dog liver microsomes data for selected compounds: **8g** 81.8%, **8**h 67.7%, **19a** 83.3%, **21g** 79.5%. (4) MkLM: monkey liver microsomes data for selected compounds: **8g** 35.3%, **19a** 67.3%, **21a** 76.9%.
[d] PD: Partial displacement of ^3H-PGE_2 in the in vitro assay.
[e] These analogs were stable in the assay buffer in the absence of microsomes.

Table 2
Rat platelet aggregation data for selected compounds

Compd no.	EC_{50} (nM)
8b	47
8h	2
8j	19
21c	290
21d	361
21e	137
21f	316
21g	27
21i	1089
21j	246
21l	405
27a	63
27b	87
27c	69

Table 3
IC_{50} (μM) activity for analogs from radioligand displacement assays across prostanoid isoforms panel

Compd	hEP2	hEP4	hFP	hIP
27a	>20	15.1	>20	>20
27b	>20	2.3	>20	>20
27c	>20	8.7	>20	>20

Table 4
hCYP inhibition with compounds **27b**–**e**

Compd	3A4[a]	3A4[b]	2C9	2C19	1A2	h2D6
27a	>10	3.06	1.04	4.89	>10	>10
27b	>10	2.12	1.02	6.18	>10	>10
27c	>10	2.33	0.8	5.38	>10	>10

[a] Utilized BFC, 7-benzyloxy-4-trifluoromethylcoumarin, as substrate.
[b] Utilized DBF, dibenzylfluorescein as substrate.

In conclusion, we have described SAR of 1,7-disubstituted oxyindoles where the C3 substituent modifications were guided by both plasma protein binding and metabolic stability. We identified an optimized series of 3-hydroxy oxyindoles (**27a–c**) that afforded high affinity for the human EP_3 receptor, over >1000× selectivity across a panel of prostanoid receptors, good stability in the liver microsomes assay, and low plasma binding potential. In addition, these compounds exhibited good activity in the rat platelet aggregation assay for EP3 antagonism. Detailed pharmacokinetic data and in vivo pharmacology for the prioritized molecules will be reported elsewhere.

Table 5
Pharmacokinetic parameters for compound **27a** following iv dosing (6 mg/kg)

Species	$t_{1/2}$ (h)	Cl_pred (ml/h/kg)	Vss, pred (ml/kg)
Mouse	2.10	4584	5663
Rat	1.75	2510	2405

References and notes

1. (a) Abramovitz, M.; Adam, A.; Boie, Y.; Godbout, C.; Lamontagne, S.; Rochette, C.; Sawyer, N.; Tremblay, N. M.; Belley, M.; Gallant, M.; Dufresne, C.; Gareau, Y.; Ruel, R.; Juteau, H.; Labelle, M. *Biochim. Biophys. Acta* **2000**, *1483*, 285; (b) Kiryiama, A.; Ushukubi, K.; Kobayashi, T.; Hirata, M.; Sugimoto, Y.; Narumiya, S. *Br. J. Pharmacol.* **1997**, *122*, 217.
2. Gross, S.; Tilly, P.; Hentsch, D.; Vonesch, J. L.; Fabre, J. E. *J. Exp. Med.* **2007**, *20*, 311.
3. Fabre, J.; Nguyen, M.; Athirakul, K.; Coggins, K.; McNeish, J. D.; Austin, S.; Parise, L. K.; FitzGerald, G. A.; Coffman, T. M.; Koller, B. H. *J. Clin. Invest.* **2001**, *107*, 603.
4. Singh, J.; Zeller, W.; Zhou, N.; Hategen, G.; Mishra, R.; Polozov, A.; Yu, P.; Onua, E.; Zhang, J.; Zembower, D.; Kiselyov, A.; Ramírez, J.; Sigthorsson, G.; Bjornsson, J.; Thorsteinnsdottir, M.; Andrésson, T.; Bjarnadottir, M.; Magnusson, O.; Stefansson, K.; Gurney, M. *ACS Chem. Biol.* **2009**, *4*, 115.
5. Singh, J.; Zeller, W.; Zhou, N.; Hategen, G.; Mishra, R.; Polozov, A.; Yu, P.; Onua, E.; Zhang, J.; Ramírez, J.; Sigthorsson, G.; Thorsteinnsdottir, M.; Kiselyov, A.; Zembower, D.; Andrésson, Þ.; Gurney, M. *J. Med. Chem.* **2010**, *53*, 18.
6. For example, replacement of indole with 5-azaindole core led to over 100-fold drop in activity versus the corresponding indole analog. Incorporation of (e.g., 2-pyridyl methyl as Ar1 substituents also resulted in analogs with very poor activity in the hEP3 receptor-binding assay.
7. O'Connell, M.; Zeller, W.; Burgeson, J.; Mishra, R. K.; Ramirez, J.; Kiselyov, A. S.; Andrésson, T.; Gurney, M. E.; Singh, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 778.
8. In spite of excellent potency in the receptor binding assays (IC_{50} <10 nM), the hexa-hydroindolone analogs, that were evaluated, displayed low μM potency in the functional rat platelet aggregation assay.

9. For reports of conversions of anilines to their respective isatins via this sequence, see: (a) Schmid, R.; Cereghetti, M.; Heiser, B.; Schonholzer, P.; Hansen, H.-J. *Helv. Chim. Acta* **1988**, *71*, 897; (b) Ma, D.; Wu, Q. *Tetrahedron Lett.* **2000**, *41*, 9089; (c) Tokunaga, T.; Hume, W. E.; Umezome, T.; Okazaki, K.; Ueki, Y.; Kumagai, K.; Hourai, S.; Nagamine, J.; Seki, H.; Taiji, M.; Noguchi, H.; Nagata, R. *J. Med. Chem.* **2001**, *44*, 4641; (d) Natarajan, A.; Fan, Y.-H.; Chen, H.; Guo, Y.; Iyasere, J.; Harbinski, F.; Christ, W. J.; Aktas, H.; Halperin, J. A. *J. Med. Chem.* **2004**, *47*, 1882; (e) Nicolaou, K. C.; Chen, D. Y.-K.; Huang, X.; Ling, T.; Bella, M.; Snyder, S. A. *J. Am. Chem. Soc.* **2004**, *126*, 12888; (f) Ferandin, Y.; Bettayeb, K.; Kritsanida, M.; Lozach, O.; Polychronopoulos, P.; Magiatis, P.; Skaltsounis, A.-L.; Meijer, L. *J. Med. Chem.* **2006**, *49*, 4638; (g) Kaila, N.; Janz, K.; DeBernardo, S.; Bedard, P. W.; Camphausen, R. T.; Tam, S.; Tsao, D. H. H.; Keith, J. C., Jr.; Nickerson-Nutter, C.; Shilling, A.; Young-Sciame, R.; Wang, O. *J. Med. Chem.* **2007**, *50*, 21; (h) Kaila, N.; Janz, K.; Huang, A.; Moretto, A.; DeBernardo, S.; Bedard, P. W.; Tam, S.; Clerin, V.; Keith, J. C., Jr.; Tsao, D. H. H.; Sushkova, N.; Shaw, G. D.; Camphausen, R. T.; Schaub, R. G.; Wang, Q. *J. Med. Chem.* **2007**, *50*, 40.

10. (a) Bass, R. J. *Tetrahedron Lett.* **1971**, *16*, 1087; (b) Rajopadhyet, M.; Popp, F. D. *J. Med. Chem.* **1988**, *31*, 1001.