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Peri-substituted hexahydro-indolones as novel, potent and selective human EP_3 receptor antagonists

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ABSTRACT

A series of peri-substituted [4.3.0] bicyclic non-aromatic heterocycles have been identified as potent and selective hEP_3 receptor antagonists. These molecules adopt a hair-pin conformation that overlaps with the endogenous ligand PGE_2 and fits into an internally generated EP_3 pharmacophore model. Optimized compounds show good metabolic stability and improved solubility over their corresponding bicyclic aromatic analogs.

Prostanoids play an essential role in vascular homeostasis, including regulation of platelet function. These molecules act through the specific membrane receptors belonging to the superfamily of G-protein coupled receptors (GPCRs). Among these prostanoids, thromboxane A_2 (TxA_2) is a potent stimulator of platelet aggregation, whereas prostaglandin I_2 (PGI_2) inhibits its activation. Another signaling molecule, prostaglandin E_2 (PGE_2) binds preferentially to the EP family of receptors, namely EP_{1-4}.[1] It has been shown that EP_3 receptor on the platelet is required for PGE_2 potentiation of coagonist signaling. Studies utilizing EP_3 KO mice showed that the stimulatory effects of PGE_2 on platelet aggregation are exerted specifically through EP_3 receptor.[2] PGE_2 has been reported to have biphasic effect on the platelet response. Specifically, it potentiates platelet aggregation at low concentrations and inhibits this effect at higher concentrations.[3] In addition to its involvement in platelet function, PGE_2 also plays a key role in the regulation of ion transport,[4] smooth muscle contraction of the GI tract, acid secretion, uterine contraction during fertilization and implantation,[5] fever generation, and hyperalgesia.[6] Development of specific antagonists of EP_3 receptor are of interest as anti-thrombotic agents.

Recently, we reported two series of peri-substituted bicyclic heteroaromatic molecules[7,8] which featured sound potency towards the hEP_3 receptor and high prostanoid receptor specificity. Derivatives of indoles **1** and dihydroindolones **2** also showed good antagonist activity in both cellular and secondary rat platelet aggregation assays.[7,8] However, these molecules displayed elevated clog*P*, clog*D* and possessed low aqueous solubility. Our preliminary structure–activity relationship (SAR) demonstrated that attempts to modify the solubility of compounds **1** and **2** through incorporation of either polar or basic functionalities in the bicyclic template or as Ar^1/Ar^2 substituents resulted in a significant reduction in affinity for EP_3 receptor (data not shown).[9] Another approach undertaken to improve the solubility of the [4.3.0] bicyclic framework involved altering the planarity of compounds **1** and **2**, and thus reducing the potential of these compounds for intermolecular π-stacking. We proposed that the [4.3.0] bicyclic system of the hexahydro-indolone analog **3** would possess more favorable physico-chemical parameters than series **1** and **2**, and thus display reduced lipophilicity and improved solubility/formulation properties. Furthermore, this [4.3.0] bicyclic core should provide for the proper arrangement of the requisite pharmacophoric features, in a manner analogous to our previously reported bicyclic heteroaromatic series,[7,8] to provide active EP_3 analogs (Fig. 1). Comparison of the energy-minimized[10] structure **3** with the previously reported indole **1** and 2-oxoindole [dihydroindolone] **2** derivatives, showed good spatial and electronic overlap (Fig. 1). The selection of Ar^1 and Ar^2 substituent groups was also geared towards improvement of solubility. This report describes the design, synthesis and SAR for the non-aromatic [4.3.0] bicyclic series **3**.

The hexahydro-indolone analogs **3a** were readily accessible from (1-methyl-2-oxo-cyclohexyl)-acetic acid (**4a**),[11] as shown in

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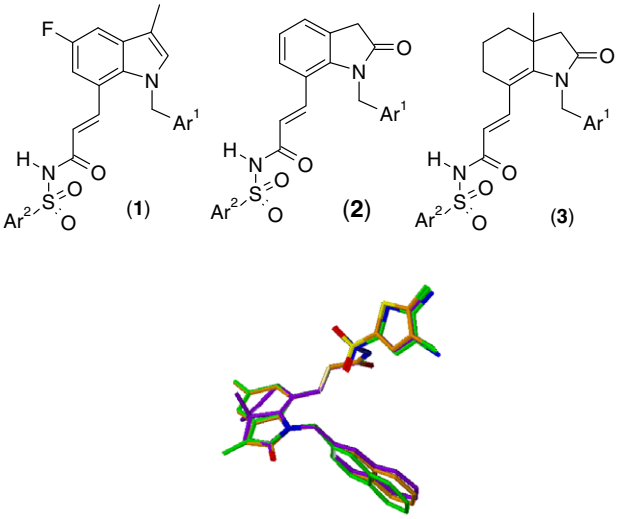
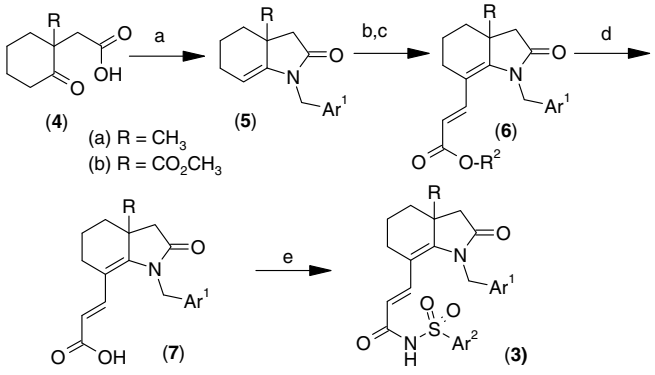


Figure 1. Overlay of AM1 energy minimized[10] indole analog (**1a**, green), indolone (**2a**, orange) and hexahydro-indolone (**3aa** purple), where Ar^1 = 2-naphthyl and Ar^2 = 4,5-dichloro-2-thiophene.

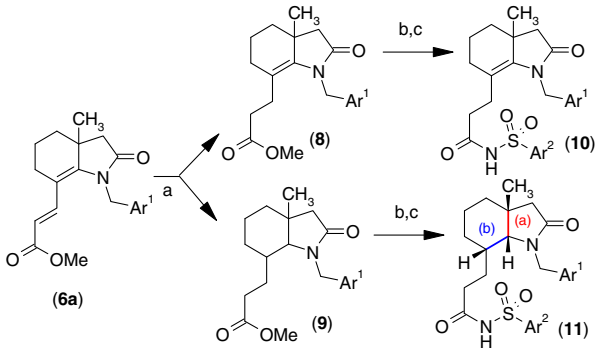
Scheme 1. Introduction of the first point of diversity in these analogs involved cyclization of **4a** with various benzyl amines using a modification of the protocol reported by Padwa et al. and afforded compounds **5a**.[12] Bromination of hexahydro-indolones **5a** with NBS provided the respective vinyl bromide intermediates. These were converted to methyl acrylates **6a** ($R^2 = CH_3$) via standard Heck coupling protocols. Saponification of **6a** provided the key intermediate acid **7a** which was reacted with arylsulfonamide using EDCI in the presence of DMAP to provide the acylsulfonamides **3a**.[13]

Analogs **3b**, featuring an ester functionality at the quaternary bridgehead carbon ($R = CO_2CH_3$), were accessed via the same synthetic route (Scheme 1) using **4b** for condensation with a benzylic amine followed by Heck coupling with *tert*-butyl acrylate. The orthogonally protected intermediate **6b** (R^2 = *t*-Bu), was deprotected with excess TFA in CH_2Cl_2 to yield the key mono-acid intermediate **7b**.

Hydrogenation of the methyl ester intermediate **6a** ($R^2 = CH_3$) in the presence of palladium on charcoal provided a mixture of tetra-substituted olefin and saturated analogs **8** and **9**, respectively. These esters were separated by chromatography, saponified and reacted with substituted arylsulfonamides to afford compounds **10** and **11**, respectively (Scheme 2).



Scheme 1. Reagents and conditions: (a) $Ar_1CH_2NH_2$, xylenes, 140 °C; (b) NBS, CH_2Cl_2; (c) acrylate ester, $Pd(OAc)_2$, (*o*-tolyl)$_3$P, TEA, DMF; (d) NaOH, H_2O, THF, MeOH or TFA, CH_2Cl_2; (e) $ArSO_2NH_2$, EDCI, DMAP, CH_2Cl_2.



Scheme 2. Reagents and conditions: (a) i—H_2, 10% Pd/C, EtOH; ii—silica-gel column chromatography; (b) NaOH, H_2O, MeOH, THF; (c) $Ar^2SO_2NH_2$, EDCI, DMAP, CH_2Cl_2.

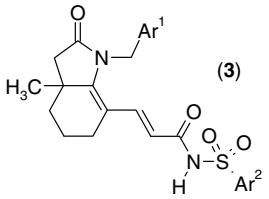
Similar to the optimized indole **1a** and dihydroindolone **2a** derivatives (Ar^1 = 2-naphthyl, Ar^2 = 4,5-dichloro-thiophenyl), molecule **3aa** showed good activity in the hEP_3 receptor binding assay [hEP_3 IC_{50} = 7.0, 1.5 and 6.8 nM for compounds **1a**, **2a** and **3aa**, respectively] and low IC_{50} shift in the presence of human serum [fold-shift = 7.0, 7.6 and 7.4 for compounds **1a**, **2a** and **3aa**, respectively] indicating favorable plasma protein binding properties.[14] Encouraged by these results, we undertook a detailed SAR study for Ar^1 and Ar^2 groups in this [4.3.0] bicyclic series (Table 1). A number of substituted phenyl derivatives at Ar^1 (**3aa**–**ah**) were evaluated. With the exception of **3ac**, all analogs afforded both good affinity for hEP_3 and low-to-modest plasma protein binding. 4,5-Dichloro substituents on the thiophene sulfonamide portion (Ar^2 substituents) consistently improved activity of the resulting molecules [see Table 1, **3ac** vs **3aj** (25-fold)]. The Ar^2 group as a phenyl substituted with two halogens (chloro or fluoro substituents, **3ay** and **3ar**) provided good hEP_3 affinity. However, these molecules also displayed large fold shift for hEP_3 binding in the presence of human serum (**3al**, **3am**, **3ao**, **3ay**, **3az**). Detailed data analysis did not show a direct correlation of their lipophilicity ($\mathrm{clog}D_{7.4}$) and fold-shift in IC_{50} values. For example, analogs **3an** versus **3as** and **3at** versus **3ap** showed essentially similar $\mathrm{clog}D_{7.4}$ values while these gave very different IC_{50} in the presence of plasma protein components.[15] This effect of substitution pattern rather than simple $\mathrm{clog}D_{7.4}$ (or $\mathrm{clog}P$) was further highlighted by comparison of Ar^2 isomers which display essentially identical activity in the binding assay while showing very different PPB effect. For example 3,4- versus 3,5-difluoro phenyl groups Ar^2 displayed similar affinities (within 2-fold) for hEP_3 receptor but different plasma protein fold-shifts (7.8/33.7 for **3aq/3am** and 4.2/62.4 for **3ap/3ao** pairs). Interestingly, derivative **3az** with higher $\mathrm{clog}D_{7.4}$ value versus **3al** afforded 8-fold lower protein binding. Similar to the indole series,[7,8] incorporation of meta-methoxy substituents in Ar^1 also provided very potent analogs, but with varying plasma protein binding effect (Table 1, **3ah**, **3ap**, **3ao** and **3ax**). Fluoro substituted Ar^1 and Ar^2 groups (**3as** and **3aw**) exhibited sound hEP_3 affinity, low fold-shift in the presence of plasma proteins and good metabolic stability in a number of species.[16]

An additional approach to improve aqueous solubility of these bicyclic systems was to replace the angular methyl group with a hydrophylic substituent such as $-CH_2OH$. The neopentyl nature of this hydroxyl group should have low potential for phase-II metabolism. Analogs containing a carbomethoxy group as an intermediate to the $-CH_2OH$ derivatives were prepared. However, attempted reduction of the angular ester group to corresponding hydroxyl methyl, under a variety of conditions, was not successful. The analogs containing the quaternary carbomethoxy group exhibited reduced hEP3 activity in the presence and absence of 10% human serum in the receptor binding assay relative to the angular

methyl derivatives; compare pairs **3af** versus **3ba** and **3av** versus **3bc** (Tables 1 and 2).

Reduction of the exocyclic double bond in **3aw** yielded **10w.** The molecule retained affinity for the hEP_3 receptor (Table 2, compare **10w** versus **3aw**) but displayed poor metabolic stability in rat liver microsomal preparations with 4% parent remaining after 30 min. Further reduction of the endocyclic olefin in **10w** provided octahydro-indolone analog **11w** that was 300-fold less active in hEP_3 binding assay compared with the hexahydro analog **10w**. ^1H NMR analyses showed the com-

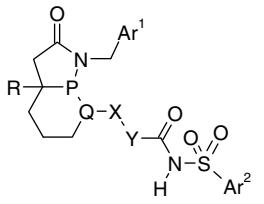
Table 1
Biological activity and metabolic stability of hexahydro-indolones



Compound	Ar^1	Ar^2	hEP_3 IC_{50}[a] (nM)	hEP_3 fold-shift[b]	clog$D_{7.4}$[c]	Metabolic stability[d]	
						Rat	Human
3aa	2-Naphthyl	4,5-Dichloro thiophen-2-yl	6.8	7.4	4.9	54	64
3ab	2,3-Dichloro phenyl	4,5-Dichloro thiophen-2-yl	4.4	6.6	4.8	69	80
3ac	2,4-Dichloro phenyl	4,5-Dichloro thiophen-2-yl	2.3	38.4	4.9	60	58
3ad	3-Chloro phenyl	4,5-Dichloro thiophen-2-yl	2.2	16.3	4.3	ND	ND
3ae	3-Fluoro phenyl	4,5-Dichloro thiophen-2-yl	9.5	8.2	3.8	18	67
3af	3,4-Difluoro phenyl	4,5-Dichloro thiophen-2-yl	3.5	17.7	3.7	22	80
3ag	4-Fluoro phenyl	4,5-Dichloro thiophen-2-yl	3.8	3.0	3.8	50	74
3ah	3-Methoxy phenyl	4,5-Dichloro thiophen-2-yl	6.4	4.8	3.6	64	62
3ai	2,4-Dichloro phenyl	5-Chloro thiophen-2-yl	13.4	6.9	4.0	68	75
3aj	2,4-Dichloro phenyl	2-Thiophenyl	57.2	25.2	3.0	79	78
3ak	2,4-Dichloro phenyl	4-Fluoro phenyl	15.2	21.2	3.8	64	70
3al	3,4-Difluoro phenyl	3,5-Difluoro phenyl	9.2	217.2	4.3	ND	ND
3am	2,3-Dichloro phenyl	3,5-Difluoro phenyl	3.8	33.7	4.2	ND	ND
3an	4-Fluoro phenyl	3,5-Difluoro phenyl	84.1	5.4	3.1	ND	ND
3ao	3-Methoxy phenyl	3,5-Difluoro phenyl	5.6	62.4	3.0	ND	ND
3ap	3-Methoxy phenyl	3,4-Difluoro phenyl	12.9	4.2	2.8	42	76
3aq	2,3-Dichloro phenyl	3,4-Difluoro phenyl	3.4	7.8	3.9	ND	ND
3ar	2,4-Dichloro phenyl	3,4-Difluoro phenyl	3.4	18.0	4.1	ND	ND
3as	3,4-Difluoro phenyl	3,4-Difluoro phenyl	9.4	3.2	2.9	82	79
3at	4-Fluoro phenyl	3,4-Difluoro phenyl	2.5	15.9	2.9	ND	ND
3au	2,3-Dichloro phenyl	2,4,5-Trifluoro phenyl	4.3	5.8	3.8	50	49
3av	2,4-Dichloro phenyl	2,4,5-Trifluoro phenyl	3.7	7.0	4.0	37	62
3aw	3,4-Difluroro phenyl	2,4,5-Trifluoro phenyl	7.7	9.3	2.9	89	75
3ax	3-Methoxy phenyl	2,4,5-Trifluoro phenyl	5.6	6.2	2.7	84	76
3ay	2,4-Dichloro phenyl	3,5-Dichloro phenyl	9.8	61.2	5.3	62	70
3az	3,4-Difluoro phenyl	3,5-Dichloro phenyl	5.6	26.8	4.2	45	68

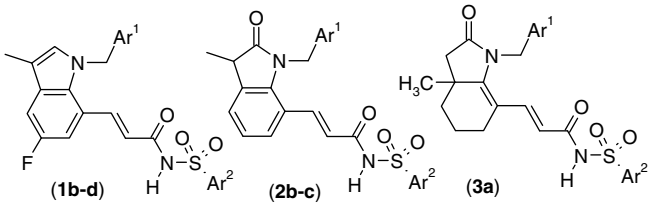
[a] Experimental IC_{50} values from displacement binding analysis with a minimum of three experiments per value. Displacement binding was assessed with [^3H]-PGE_2 for human EP_3 receptor membrane preparations in buffer.
[b] Fold-shift = (IC_{50} in the presence of 10% human serum)/(IC_{50} in buffer (a)).
[c] The values shown are from ACDLabs, version 9.0.
[d] Percent parent compound remaining after 30-min incubation with respective liver microsomal preparations, as determined by LC/MS/MS. ND, not determined.

Table 2
Bioactivity of hexa- and octahydro-indolones



Compound	Ar^1	Ar^2	R	P–Q	X–Y	hEP_3 IC_{50} (nM)	PPB fold-shift
10w	3,4-Difluoro phenyl	2,4,5-Trifluoro phenyl	Me	C=C	CH_2CH_2	1.8	33.2
11w	3,4-Difluoro phenyl	2,4,5-Trifluoro phenyl	Me	CHCH	CH_2CH_2	556.9	25.7
3aw	3,4-Difluoro phenyl	2,4,5-Trifluoro phenyl	Me	C=C	CH=CH	7.7	9.3
3ba	3,4-Difluoro phenyl	4,5-Dichloro-thiophen-2-yl	CO_2Me	C=C	CH=CH	29.9	308.5
3bb	2,4-Dichloro phenyl	3,4-Difluoro phenyl	CO_2Me	C=C	CH=CH	20.6	22.0
3bc	2,4-Dichloro phenyl	2,4,5-Trifluoro phenyl	CO_2Me	C=C	CH=CH	11.2	47.1

Table 3
Aqueous solubility, clog*D* and HPLC retention times for selected compounds



Compound	Ar^1	Ar^2	Aqueous solubility (mg/mL)[b]	clog$D_{7.4}$	HPLC RT min[17]
1b	3,4-Difluoro phenyl	2,4,5-Trifluoro phenyl	<0.04	4.4	27.79
2b	3,4-Difluoro phenyl	2,4,5-Trifluoro phenyl	1.58	3.1[a]	26.32
3aw	3,4-Difluoro phenyl	2,4,5-Trifluoro phenyl	>3.0[c]	2.9	23.54
1c	3,4-Difluoro phenyl	3,4-Difluoro phenyl	ND	4.5	27.83
2c	3,4-Difluoro phenyl	3,4-Difluoro phenyl	ND	3.2[a]	22.09
3as	3,4-Difluorophenyl	3,4-Difluoro phenyl	ND	2.9	23.58
1d	3,4-Difluoro phenyl	3,5-Difluoro phenyl	ND	4.7	27.93
3al	3,4-Difluoro phenyl	3,5-Difluoro phenyl	ND	2.6	23.73
1e	3-Methoxy phenyl	2,4,5-Trifluoro phenyl	ND	4.3	27.56
3ax	3-Methoxy phenyl	2,4,5-Trifluoro phenyl	ND	2.7	22.89
1f	4-Fluoro phenyl	3,5-Difluoro phenyl	ND	4.8	27.67
3an	4-Fluoro phenyl	3,5-Difluoro phenyl	ND	3.1	23.23
1g	4-Fluoro phenyl	4,5-Dichloro thiophen-2-yl	ND	5.4	30.25
3ag	4-Fluoro phenyl	4,5-Dichloro thiophen-2-yl	ND	3.8	26.10

[a] clog$D_{7.4}$ for the keto form.
[b] The data is for solubility in 50 mM PSB buffer (pH 7.4) and should represent thermodynamic solubility, as the samples were stirred overnight, filtered and analyzed by HPLC and concd determined using a 5-point standard curve.
[c] The sample was homogenous, so the actual solubility is higher than the value shown.

pound **11w** to have *syn*(*a*):*syn*(*b*) orientations, as shown in Scheme 2.[18]

A selected series of the more potent analogs were examined against a panel of prostanoid receptors to assess their EP_3 selectivity. All compounds tested displayed >1000× selectivity (Table 4) in radioligand displacement binding assays against other PGE_2 (EP_1, EP_2 and EP_4) and against the FP receptors.

The hexahydro-indolone analogs with good activity in the hEP_3 radioligand displacement binding assay and exhibiting low potential for PPB were shown to exhibit potent and full antagonist activity in the functional assay.[19] This data for selected analogs is shown in Table 5.

Table 4
Prostanoid receptor activity for potent hexahydro-indolone derivatives

Compound	hEP_1	hEP_2	hEP_4	hFP
3aa	6.9	IA	IA	10.2
3ag	IA	IA	IA	27.4
3ah	IA	IA	IA	6.1
3an	IA	IA	IA	4.2
3ap	1.4	IA	7.5	6.4
3as	IA	2.3	7.7	16.4
3au	IA	IA	IA	7.4
3av	11.6	IA	20.8	7.6
3aw	IA	13.9	6.2	3.8

IC_{50} (μM) values reported are from receptor binding assays.
IA, inactive; these analogs gave essentially no significant inhibition at 20 μM, maximum assay concentration, in this assay.

Table 5
Antagonist activity on CHO-cells expressing human EP_3 receptors

Compound	**3ab**	**3af**	**3ah**	**3aq**	**3au**	**3aw**	**3az**
IC_{50} (nM)	1.7	5.2	5.8	3.5	4.5	3.5	3.2

Derivatives containing the acryl acylsulfonamide functionality featured the best activity and sound metabolic stability (Table 1). An α,β unsaturated amide is not commonly found in registered therapeutics[20] as a structural feature due to its perceived potential to undergo Michael addition. However, the nature of the double bond in these compounds (e.g., **3aw)** suggests that this should not be a liability as the acylsulfonamide is a carboxylic acid mimetic.[7] Moreover, the aryl acrylic acylsulfonamide anion present at physiological pH[21] is likely to feature neither chemical nor in vivo reactivity. Relevant synthetic papers[22] point out that the α,β unsaturated amide functionality is a sub-optimal Michael acceptor.

The hexahydro-indolone series showed reduced retention times on C_{18} reverse phase HPLC, compared with their aromatic counterparts **1** and **2**, suggesting improved hydrophilicity and solubility. This is corroborated by (a) aqueous solubility data for a series of indole, dihyroindolone and hexahydro-indolone analogs (set-1: **1a** → **2b** → **3aw**;) as shown in Table 3 and (b) a Scatter plot of the clog$D_{7.4}$ versus HLPC retention time provided a correlation coefficient of $r^2 = 0.85$ for the entire data shown in Table 3. For example, compound **3aw** displayed >3 mg/mL solubility in PBS buffer at pH 7.4. This molecule showed good functional activity as it blocked inhibition of forskolin-stimulated production of cAMP by PGE_2 in CHO-K1 cells expressing human EP_3 with IC_{50} = 3.5 nM. Several representative molecules from this class are being further evaluated in the panel of functional assays in order to select an optimized lead candidate.

In conclusion, we described both synthesis and SAR of peri-substituted hexahydro-indolones featuring good overlap with the previously reported bicyclic series. These series led to the identification of analogs exhibiting high affinity for the human EP_3 receptor, favorable selectivity across the panel of prostanoid receptors, full antagonism at the human EP_3 receptor in cellular assay and good metabolic stability across multiple species. In addition, hexahydro-indolones displayed improved aqueous solubility suitable for further preclinical development.

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9. 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 Ar^1 substituents also resulted in analogs with very poor activity in the hEP_3 receptor binding assay.
10. For compounds **1a**, **2a** and **3aa**, 2D–3D structure conversion was performed using CONCORD 6.0 followed by energy minimization using MMFF94 force field with conjugate gradient method using SYBYL7.0. Gasteiger–Huckel charges were assigned and then each minimized structure was subjected to full conformational search using systematic search (SS) varying all rotatable bonds by 10° torsion increment and only two conformation of the olefin (*cis* and *trans*) were allowed. The lowest energy conformer for each molecule thus obtained was subsequently subjected to AM1 semi-empirical SCF MO energy minimization using 'MMOK' and 'Precise' for augmenting convergence criteria. Each geometry optimized structure with AM1 charges was finally used for alignment based on the electrostatic charge similarity index principle as reported by Burt, C.; Richards, W. G.; Huxley, P. The application of molecular similarity calculations. *J. Comp. Chem.* **2004**, *11*, 1139. The resulting overlap of **1a**, **2a** and **3aa** is shown in Figure 1.
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13. *Synthesis of analog* ***3as****, as a representative example:* A solution of **4a**[11] (1 equiv) and the 3,4-difluorobenzyl amine (1 equiv) in *m*-xylene was refluxed for 3 h. The reaction mixture was concentrated in vacuo, and residue was purified via silica gel chromatography using 10–20% CH_2Cl_2/hexanes as eluent to obtain the desired hexahydro-indol-2-ones product (**5a**) in 85% yield. Compound **5a** was then dissolved in CH_2Cl_2, cooled to 0 °C and Br_2 (1 equiv) was added drop-wise. The reaction mixture was stirred until bromine color disappeared. Et_3N (3 equiv) was added in one portion, and the reaction mixture was stirred at room temperature for 10 min. The reaction mixture was washed with water (3×), and dried over anhydrous $MgSO_4$. The CH_2Cl_2 solution was concentrated in vacuo to provide the desired vinyl bromide in 99% yield. To solution of the vinyl bromide (1 equiv) and Et_3N (10 equiv) in DMF were added methyl acrylate (1.1 equiv), $Pd(OAc)_2$ (0.1 equiv), and tri-*o*-tolyl phosphine (0.3 equiv). The reaction mixture was heated at 100 °C for 16 h, and then allowed to cool to room temperature. The reaction mixture was filtered through Celite, the Celite was washed with CH_2Cl_2, organic layer was washed with water, brine and dried over anhydrous $MgSO_4$. Organic layer was concentrated in vacuo. The residue was purified via silica gel chromatography, using 15% hexanes/CH_2Cl_2 to obtain the acrylate ester (**6a**, $R^2 = CH_3$). Hydrolysis of methyl esters with aqueous NaOH (3 equiv) in THF/MeOH (2:1) provided, after acidification (with 1 N HCl to pH 3) and extractive work-up with EtOAc, the desired acrylic acid (**6a**, R^2 **=** H) in 39% yield for the two steps. Subsequent coupling with 3,4-diflurorbenzenesulfonamide (1.2 equiv) and DMAP (2.4 equiv) in CH_2Cl_2 using EDCI (2 equiv) was carried out at room temperature. The reaction mixture was washed with 1 N HCl, water, brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was triturated with CH_2Cl_2/hexanes to obtain the desired product **3as** in 28% yield. ^{1}H NMR ($CDCl_3$) 1.19 (s, 3H), 1.57 (m, 1H), 1.87 (m, 3H), 2.19 (d, J = 6.8 Hz, 2H), 2.44 (d, J = 1.2 Hz, 2H), 4.75 (d, J = 16.4 Hz, 1H), 5.17 (d, J = 16.0 Hz, 1H), 5.53 (d, J = 14.8 Hz, 1H), 7.04 (m, 3H), 7.35 (ddd, J = 16.8, 9.2, 7.6 Hz, 1H), 7.72 (d, J = 14.8 Hz, 1H), 7.88 (m, 2H), 7.93 (ddd, J = 9.2, 7.2, 2.4 Hz, 1H). LC/MS (95%) ESI-Calcd 522.5 *m*/*z*. Found: 522 *m*/*z*.
14. Based on the data previously reported for the cinnamyl acylsulfonamide series Juteau, H.; Gareau, Y.; Labelle, M.; Sturino, C. F.; Sawyer, N.; Tremblay, N.; Lamontagne, S.; Carriere, M. C.; Denis, D.; Metters, K. M. *Bioorg. Med. Chem.* **2001**, *9*, 1977. we conducted our in vitro assays in the absence and presence of human serum to evaluate potential for plasma protein binding (PPB).
15. The shift in plasma protein was measured in the presence of mouse and human serum and human-serum albumin, and none show a simple correlation with clog*P* or clog*D*.
16. Compound **3aw** also showed good metabolic stability with dog and monkey liver microsomal preparations providing 87% and 54% parent remaining at 30 min, respectively.
17. Waters symmetry C_{18} column, 4.6 mm × 250 mm, 5 μm; flow rate: 1.0 mL/min; mobile phase A: water (0.05% TFA), linear gradient from 95% A to 95% B over 35 min.
18. All four possible isomers, *syn*(*a*)–*syn*(*b*), *syn*(*a*)–*anti*(*b*), *anti*(*a*)–*anti*(*b*) and *anti*(*a*)–*syn*(*b*) were energy minimized using semi-empirical, AM1 Hamiltonian. This data indicated that the *syn*(*a*)–*syn*(*b*) and *syn*(*a*)–*anti*(*b*) to have relatively close energy (<1 kcal/mol apart); while the two *anti*(*a*)–*syn*(*b*) and *anti*(*a*)–*anti*(*b*) isomers have much higher energy (~7–7.5 kcal/mol). The 1D and 2D ^{1}H NMR analyses of **11w** supports the *syn*(*a*)–*syn*(*b*) assignments. These data support that the isomer isolated to be low energy isomer. Also, analysis of the two *syn*(*a*) isomers with the EP_3 antagonist pharmacophore model is consistent with the observed loss in EP_3 binding.
19. CHO-K1 cells stably expressing the hEP_3 receptor were treated with increasing concentrations of a test compound for 10 min at 37 °C in the presence of 5 μM forskolin and 5 nM PGE_2. Control cells treated with a combination of forskolin and PGE_2 showed a 40% inhibition over forskolin-induced cAMP increase. This inhibition was reversed in a dose-dependent manner by the test compound.
20. Tranilast containing vinylogous amide functionality has been reported to be launched in Japan and Korea for the treatment of allergic rhinitis, asthma and atopic dermatitis by Kessie Pharmaceuticals, Ltd, Data source Iddb3.
21. For analogs listed in Table 1, pK_a range from 3.5 to 4.2. Compound **3aw**, pK_a = 3.4 (ACDLabs version 9.0).
22. (a) Carroll, F. A. *Perspectives on Structure and Mechanism in Organic Chemistry*: Brooks/Cole Publishing Co., 1998, pp 628, report that substituents that are particularly effective in stabilizing nucleophilic addition to α,β unsaturated carbonyl compounds include aldehydes, ketone, esters and other carboxylic acid derivatives except amide.; (b) Perlmutter, P. *Conjugate Addition Reaction in Organic Synthesis*; Pergamon Press: Oxford, England, 1992.