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Key Structural Features of Prostaglandin E_2 and Prostanoid Analogs Involved in Binding and Activation of the Human EP_1 Prostanoid Receptor

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ABSTRACT

The structure-activity relationship (SAR) of prostaglandin (PG) E_2 at the human EP_1 prostanoid receptor (designated hEP_1) was examined via the binding and activation of this receptor by a series of 55 prostanoids and analogs. Using clonal human embryonic kidney 293 cell lines expressing recombinant hEP_1, affinity (K_i), potency (EC_{50}), and efficacy data were obtained using a radioligand competitive binding assay and an aequorin-based calcium functional assay. All compounds behaved as full agonists (90–100% of the response elicited by PGE_2) in this assay, and the correlation between the K_i and EC_{50} values was highly significant ($R^2 = 0.86$). The results from the SAR analysis can be summarized as follows: 1) the existence and configuration of hydroxyl groups at the 11 and 15 positions of PGE_2 and prostanoid analog structures play a critical role in agonist activity; 2) the carboxyl group is also important for activity and modification of the carboxylic acid to various esters results in greatly reduced affinity and potency; 3) the activity of structures with moderate or weak potency can be enhanced by modification of the ω-tail; and 4) modifications to the ketone at the 9-position are better tolerated, with 9-deoxy-9-methylene-PGE_2 being the most potent agonist tested in the functional assay. The impact of other modifications on agonist potency is also discussed. The results from this study have identified, for the first time, the key structural features of PGE_2 and related prostanoids and prostanoid analogs necessary for activation of hEP_1.

PGE_2 is a member of the 2-series of PGs that is derived de novo from arachidonic acid and constitutes the most abundant naturally occurring prostanoid (Campbell, 1990; Davies and MacIntyre, 1992). These autocrine and paracrine mediators are released in response to a variety of stimuli in many tissues, where they are involved in a broad spectrum of physiological and pathophysiological events (Coleman et al., 1989) and have been implicated in a number of therapeutic areas (Abramovitz and Metters, 1998).

PGE_2 elicits its effects primarily through interaction with four distinct prostanoid receptors: EP_1, EP_2, EP_3, and EP_4 (Coleman et al., 1994), members of the G-protein coupled receptor superfamily of integral serpentine plasma membrane proteins (Boie et al., 1995). The primary signaling pathways of the four EP receptor subtypes are as follows: EP_1 couples to elevation of $[Ca^{2+}]_i$ (Funk et al., 1993), EP_2 (Regan et al., 1994) and EP_4 (Bastien et al., 1994) couple to an increase in intracellular cAMP accumulation, and EP_3 couples to a decrease in intracellular cAMP levels (Boie et al., 1997; Jin et al., 1997).

When the four EP receptor subtypes are aligned by amino acid identity with the additional four members of the prostanoid receptor family (TP, Hirata et al, 1991; FP, Abramovitz et al., 1994; IP, Boie et al., 1994; DP, Boie et al., 1995), two subgroups are formed. Interestingly, the common element within each group is the signal transduction pathway to which the receptors couple rather than their preferred natural ligand: thus, $G_{\alpha q}/G_{\alpha i}$ for the EP_1, FP, TP, and EP_3 receptors and $G_{\alpha s}$ for the EP_2, EP_4, DP, and IP receptors (Boie et al., 1995; Toh et al., 1995). There are, in fact, no strictly conserved amino acid residues specific to the four EP subtypes that do not occur in one or more of the other four prostanoid receptors. This is reflected in preliminary molecular models of the EP receptors in which the few common residues found in the putative ligand binding pocket are also present in one or more of the non-EP prostanoid receptors (Yamamoto and Imai, 1996).

In view of this intriguing sequence information, it is relevant to identify the distinguishing structural features of PGE_2 and related prostanoid analogs that are important for the specific activation of each of the four EP receptor subtypes. This information would be particularly useful in the

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ABBREVIATIONS: PG, prostaglandin; SAR, structure-activity-relationship; HBSS, Hanks' balanced salt solution; MES, 2-(*N*-morpholino)ethanesulfonic acid; HEK, human embryonic kidney; hEP_1, human EP_1 prostanoid receptor. See Table 1 for compound abbreviations.

design of selective agonists. A number of SAR studies of prostanoids and prostanoid analogs had been conducted before the cloning of the receptors, using various bioassays from different mammalian species (Coleman et al., 1989 and references within). Unfortunately, the tissues chosen usually express mixed populations of two or more receptors, limiting interpretation of the results (Lawrence et al., 1992). Detailed SAR studies have yet to be published using recombinant systems expressing individual prostanoid receptors. The cloning of the prostanoid receptor family over the past several years (Coleman et al., 1994; Boie et al., 1995) has opened the door to evaluating compounds at all eight prostanoid receptors (Kiriyama et al., 1997; Abramovitz et al., 2000).

We describe in this report, for the first time, an SAR study of prostanoids and prostanoid analogs at the hEP_1 receptor using a well-defined recombinant hEP_1 heterologous expression system. A radioligand binding assay and a recently developed automated aequorin-based calcium functional assay (Ungrin et al., 1999) have been employed to obtain quantitative affinity and potency data for PGE_2 and related compounds at hEP_1. This data establishes that the 11α and 15(*S*) configuration of the hydroxyl groups, as well as the presence of the carboxylic acid moiety of the prostanoid structure, are crucial for hEP_1 receptor binding and activation.

Materials and Methods

Chemicals. Chemicals were purchased from the following vendors: Biomol (Plymouth Meeting, PA) and Cayman (Ann Arbor, MI), with the exception of prostaglandin E_1 methyl ester (**47**) and prostaglandin $F_{2\alpha}$ methyl ester (**50**), which were purchased from Sigma (Oakville, ON, Canada). Concentrations of the two compounds obtained from Sigma were verified by NMR spectroscopy. The compounds used are listed in Table 1. The numbers assigned to the compounds in Table 1 are used throughout the article.

Aequorin Luminescence Assay. The aequorin luminescence assay was performed using the hEP_1 expressing clonal cell line hEP_1-5/293-AEQ17, as described previously (Ungrin et al., 1999). Briefly, holo-aequorin was reconstituted in intact cells by charging 85%-confluent cultures for 1 h at 37°C in Ham's F12 medium (with 0.1% fetal bovine serum, 25 mM HEPES, at pH 7.3) (Life Technologies, Missisauga, ON, Canada) containing 30 μM reduced glutathione (Sigma, St. Louis, MO) and 8 μM coelenterazine cp (Molecular Probes, Eugene, OR). After charging, the cells were washed from the growth surface by pipetting up and down, rinsed once, and resuspended in Ham's F12 medium (modified as above) at 5×10^5 cells/ml. Experiments were performed using a Labsystems Luminoskan RS plate reading luminometer (Labsystems, Franklin, MA) with 3 integral peristaltic pumps and an internal orbital mixer. The luminometer was controlled by the Lskan Controller, custom software written in LabView (National Instruments, Austin, TX) and data was analyzed using a dedicated package of Excel Macros referred to as the Luminometer Data Analysis Macros (LDAM) developed at Merck Frosst.

Test compounds in 2 μL of dimethyl sulfoxide were diluted in 98 μl of modified Hanks' balanced salt solution (HBSS) (with 25 mM HEPES, at pH 7.3) (Life Technologies) in a Labsystems White Clinplate FB (Labsystems) 96-well plate and loaded into the luminometer. Wells were tested sequentially, starting at position A1, by rows. Cells (5×10^4 in 100 μL of Ham's F12 medium, modified as above) were injected into the well and light emission was recorded over 30 s ("Peak 1", recorded as a series of 60 0.5-s integrations). The cells were then lysed by injection of 25 μl of 0.9% Triton X-100 solution in modified HBSS, and light emission measured for an additional 10 s ("Peak 2", recorded as 20 0.5-s integrations). Succeeding drug dilutions were 3/10 and 1/3, alternatively, with 10 duplicated data points per series, covering 4.5 orders of magnitude in concentration.

Instability issues with prostacyclin (PGI_2) were addressed by loading this compound and the associated controls (PGE_2 and 6-keto-$PGF_{1\alpha}$) into the 96-well plate in 2 μl of ethanol. Modified HBSS (100 μL) was injected into the well using a third peristaltic pump immediately preceding the test. The samples were mixed thoroughly for 5 s using the orbital mixer built into the luminometer, the cells were added, and light emission was recorded immediately as described above. This procedure was repeated for all samples in the plate, including the PGE_2 control series.

Data Analysis. Peak integration values were obtained by summing the half-second integrations from the raw trace. Fractional luminescence for each well was determined by dividing the area under peak 1 by the total area under peaks 1 and 2. These calculations were performed using the Lskan Controller program, and a data file was generated containing both the raw traces, the calculated results for each well, drug concentrations, and the start time for each well. This data file was then subsequently analyzed using the LDAM software employing a modified version of the Levenberg-Marquardt four-parameter curve-fitting algorithm to calculate EC_{50} values.

Interexperimental variability was primarily related to minor day-to-day variations in cell/receptor sensitivity, resulting in parallel effects on all compounds ($R^2 = 0.79$, data not shown). Compensation was achieved via normalization to a PGE_2 concentration-response series repeated within each trial. The activity value from each trial was therefore reported as $\log_{10}(EC_{50}\ \text{cmpd}) - \log_{10}(EC_{50}\ PGE_2)$, where EC_{50} cmpd and EC_{50} PGE_2 were the molar concentrations of the test compound and PGE_2, respectively, required to elicit a response that was 50% of the maximum obtainable.

Radioligand Binding Assays. Radioligand binding assays were carried out on membranes prepared from the hEP_1-expressing clonal cell line hEP_1-HEK 293 (EBNA), as described previously (Abramovitz et al., 2000). Briefly, cells were harvested by incubation in enzyme-free cell dissociation buffer (Life Technologies, Inc.), washed in ice-cold phosphate-buffered saline, pH 7.4, and resuspended in 10 mM HEPES/KOH at pH 7.4 containing 1 mM EDTA. Membranes were prepared from harvested cells by lysis followed by differential centrifugation (1,000*g* for 10 min, then 160,000*g* for 30 min, all at 4°C). The 160,000*g* pellets were resuspended in 10 mM HEPES/KOH, pH 7.4, containing 1 mM EDTA at approximately 5 to 10 mg/ml by Dounce homogenization (Dounce A; 10 strokes), frozen in liquid nitrogen and stored at −80°C.

hEP_1 equilibrium competition binding assays were performed in a final incubation volume of 0.2 ml in 10 mM MES/KOH at pH 6.0 containing 1 mM EDTA, 10 mM $MgCl_2$, and 1 nM [^{3}H]PGE_2 (185 Ci/mmol; Amersham Pharmacia Biotech, Oakville, ON, Canada). The reaction was initiated by addition of 60 to 70 μg of membrane protein from the 160,000*g* pellet fraction. Ligands were added in dimethyl sulfoxide that was kept constant at 1% (v/v) in all incubations. Nonspecific binding was determined in the presence of 1 to 10 μM PGE_2. Incubations were conducted for 60 min at room temperature and terminated by rapid filtration through 96-well GF/C Unifilters (Canberra Packard, Mississauga, ON, Canada) prewetted in cold 10 mM MES/KOH at pH 6.0. The Unifilters were washed with 3 to 4 ml of the same buffer, dried for 90 min at 55°C, and the residual radioactivity bound to the individual filters determined by scintillation counting with addition of 50 μl of Ultima Gold F (Canberra Packard). Specific binding, which was calculated by subtracting nonspecific binding from total binding, accounted for 85 to 90% of the total binding and was linear with respect to the concentrations of radioligand and protein used. Total binding represented 5 to 10% of the radioligand added to the incubation media. Where necessary for comparison purposes, normalization was carried out as described above for the aequorin assay.

TABLE 1

Summary of all functional and affinity data, normalized relative to PGE_2 as described under *Materials and Methods*

The relative aequorin response data (Log_{10} relative aequorin) is presented as the average of triplicate experiments, ± 1 S.D., whereas for Log_{10} relative binding data (Log_{10} relative binding), both data points from duplicate experiments are presented. For ease of interpretation, the relative aequorin response is also presented as a simple nonlogarithmic ratio. Overall, in the activity assay, the EC_{50} value for PGE_2 = 2.90 nM (n = 69), and in the binding assay, the K_i value for PGE_2 = 9.9 nM (n = 18). Compound numbers are assigned based on rank order of potency in the aequorin assay. In the aequorin assay, all differences in activity of ≥0.5 log units (≥ 3-fold difference in EC_{50}) are statistically significant (2-tailed t test, α = 0.05 for each individual comparison) with the exception of those involving 19(*R*)-hydroxy PGE_2 (**32**). Statistical analysis was not possible for compounds **50** to **55** because the concentration-response curves did not reach a plateau at the maximum concentrations tested.

Compound	**Compound**	Trivial Name	Log_{10} relative aequorin ± SD (n = 3)	EC_{50} cmpd/EC_{50} PGE_2	Log_{10} relative binding (n = 2)		K_i (cmpd)/ K_i (PGE_2)
(5*Z*,11α,13*E*,15*S*)-11,15-Dihydroxy-9-methylene-prosta-5,13-dien-1-oic acid	**1**	9-Deoxy-9-methylene-prostaglandin E_2	−0.53 ± 0.11	0.30	−0.23	−0.03	0.75
[3α,*S*-[2*E*,3α,4α(1*E*,3*R**),5β,6α]]-5-[Hexahydro-5-hydroxy-4-(3-hydroxy-1-octenyl)-2(1H)-pentalenylidene]-pentanoic acid	**2**	Carbacyclin (carbaprostacyclin)	−0.20 ± 0.09	0.63	0.23	0.26	1.8
5-[Hexahydro-5-hydroxy-4-(3-hydroxy-4-methyl-1-octen-6-ynyl)-2(1H)-pentalenylidene]pentanoic acid	**3**	Iloprost	−0.18 ± 0.05	0.66	−0.05	0.03	0.98
(5*Z*,11α,13*E*,15*R*)-11,15-Dihydroxy-16,16-dimethyl-9-oxoprosta-5,13-dien-1-oic acid	**4**	16,16-Dimethyl-prostaglandin E_2	−0.16 ± 0.02	0.69	−0.82	−0.85	0.15
[1*R*-[1α(*Z*),2β(1*E*,3*S**),3α]]-7-[3-Hydroxy-2-(3-hydroxy-5-phenyl-1-pentenyl)-5-oxocyclopentyl]-5-heptenoic acid	**5**	17-Phenyl-ω-trinor-prostaglandin E_2	−0.15 ± 0.02	0.71	0.04	−0.77	0.43
[1α(*Z*),2β(1*E*,3*R**),3α,5α](±)-7-[3,5-Dihydroxy-2-(3-hydroxy-4-phenoxy-1-butenyl-)cyclopentyl]-5-heptenoic acid	**6**	16-Phenoxy-ω-tetranor-prostaglandin $F_{2\alpha}$	−0.05 ± 0.08	0.88	0.04	0.43	1.7
[1*R*-[1α(*Z*),2β(1*E*,3*S**),3α,5α]]-7-[3,5-Dihydroxy-2-(3-hydroxy-5-phenyl-1-pentenyl-)cyclopentyl]-5-heptenoic acid	**7**	17-Phenyl-ω-trinor-prostaglandin $F_{2\alpha}$	−0.04 ± 0.14	0.92	0.61	0.85	5.4
(5*Z*,11α,13*E*,15*S*)-11,15-Dihydroxy-9-oxoprosta-5,13-dien-1-oic acid	**8**	Prostaglandin E_2		1			1
(5*Z*,11α,13*E*,15*S*,17*Z*)-11,15-Dihydroxy-9-oxoprosta-5,13,17-trien-1-oic acid	**9**	Prostaglandin E_3	0.11 ± 0.10	1.3	0.86	0.95	8.0
(5*Z*,13*E*,15*R*)-15-Hydroxy-16,16-dimethyl-9-oxoprosta-5,13-dien-1-oic acid	**10**	11-Deoxy-16,16-dimethyl-prostaglandin E_2	0.26 ± 0.05	1.8	0.60	0.57	3.8
(11α,15*S*)-11,15-Dihydroxy-9-oxoprost-1-oic acid	**11**	13,14-Dihydro-prostaglandin E_1 (PGE_0)	0.36 ± 0.16	2.3	1.07	1.51	20
(5*Z*,11α,13*E*,15*S*)-11,15-Dihydroxy-15-methyl-9-oxoprosta-5,13-dien-1-oic acid	**12**	15(*S*)-15-Methyl-prostaglandin E_2	0.38 ± 0.15	2.4	0.77	0.85	6.5
[1*R*-[1α(*Z*),2β(1*E*,3*R**),3α]]-7-[3-Hydroxy-2-(3-hydroxy-4-phenoxy-1-butenyl)-5-oxocyclopentyl]-*N*-(methylsulfonyl)-5-heptenamide	**13**	Sulprostone	0.48 ± 0.13	3.0	1.16	1.24	16
(11α,13*E*,15*S*)-11,15-Dihydroxy-9-oxoprost-13-en-1-oic acid	**14**	Prostaglandin E_1	0.50 ± 0.14	3.2	1.12	0.97	11
(5*Z*,9α,11α,13*E*,15*S*)-9,11,15-Trihydroxyprosta-5,13-dien-1-oic acid	**15**	Prostaglandin $F_{2\alpha}$	1.00 ± 0.04	10	1.39	1.77	38
(5*Z*,9β,11α,13*E*,15*S*)-9,11,15-Trihydroxyprosta-5,13-dien-1-oic acid	**16**	9β-11α-Prostaglandin F_2	1.01 ± 0.11	10	2.00	1.64	66
(5*Z*,9β,11α,13*E*,15*S*)-11,15-Dihydroxy-9-chloro-15-cyclohexyl-ω-pentanor-prosta-5,13-dien-1-oic acid	**17**	ZK110841	1.10 ± 0.06	13	−0.45	−0.59	0.30
[1α,2β(1*E*,3*R**),3α]-7-[3-Hydroxy-2-(3-hydroxy-4-phenoxy-1-butenyl)-5-oxocyclopentyl]-4,5-heptadienoic acid methyl ester	**18**	Enprostil	1.10 ± 0.03	13	0.57	0.71	4.4
[1*R*-[1α(*Z*),2β(1*E*,3*S**),3α,5α]]-7-[2-(3-Cyclohexyl-3-hydroxy-1-propenyl)-3,5-dihydroxycyclopentyl]-5-heptenoic acid	**19**	15-Cyclohexyl-pentanor-prostaglandin $F_{2\alpha}$	1.19 ± 0.20	15	1.87	2.01	87
(13*E*,15*S*)-15-Hydroxy-9-oxo-16-phenoxy-ω-tetranorprost-13-en-1-oic acid	**20**	M&B 28767	1.21 ± 0.01	16	1.63	1.72	48
(5*Z*,9α,11α,13*E*,15*S*)-9,11,15-Trihydroxy-15-methyl-prosta-5,13-dien-1-oic acid	**21**	15(*S*)-15-Methyl-prostaglandin $F_{2\alpha}$ (Carboprost)	1.43 ± 0.07	27	2.15	2.25	160
[1*R*-[1α(*Z*),2β(1*E*,3*R**),3α,5α]]-7-[2-[4-(3-Chlorophenoxy)-3-hydroxy-1-butenyl]-3,5-dihydroxycyclopentyl]-5-heptenoic acid	**22**	Cloprostenol	1.51 ± 0.04	32	1.79	1.84	65
[1*R*-[1α(*Z*),2β(*R**),3α,5α]]-7-[3,5-Dihydroxy-2-(3-hydroxy-5-phenylpentyl)cyclopentyl]-5-heptenoic acid	**23**	Latanoprost free acid	1.61 ± 0.09	41	1.90	2.23	120
(5*Z*,13*E*,15*S*)-15-Hydroxy-9-oxoprosta-5,13-dien-1-oic acid	**24**	11-Deoxy-prostaglandin E_2	1.64 ± 0.04	43	2.00	2.15	120
(5*Z*,8β,11α,13*E*,15*S*)-11,15-Dihydroxy-9-oxoprosta-5,13-dien-1-oic acid	**25**	8-iso-Prostaglandin E_2	1.73 ± 0.05	53	2.14	1.99	120
(5*Z*,11β,13*E*,15*S*)-11,15-Dihydroxy-9-oxoprosta-5,13-dien-1-oic acid	**26**	11β-Prostaglandin E_2	1.78 ± 0.07	61	2.28	2.29	190

TABLE 1
Continued

Compound	**Compound**	Trivial Name	Log_{10} relative aequorin ± SD (n = 3)	EC_{50} cmpd/EC_{50} PGE_2	Log_{10} relative binding (n = 2)		K_i (cmpd)/ K_i (PGE_2)
(5*Z*,9α,11α,13*E*,15*S*)-9,11,15-Trihydroxy-6-oxo-prosta-5,13-dien-1-oic acid	**27**	6-keto-Prostaglandin $F_{1\alpha}$	1.97 ± 0.07	92	3.32	3.06	1500
(5*Z*,11α,13*E*)-11-Hydroxy-9,15-dioxoprosta-5,13-dien-1-oic acid	**28**	15-keto-Prostaglandin E_2	1.97 ± 0.12	93	2.60	2.15	240
(5*Z*,9α,11α,13*E*,15*S*)-6,9-Epoxy-11,15-dihydroxyprosta-5,13-dien-1-oic acid	**29**	Prostaglandin I_2	2.03 ± 0.12	110	N.D.	N.D.	N.D.
(9α,11α,13*E*,15*S*)-9,11,15-Trihydroxyprost-13-en-1-oic acid	**30**	Prostaglandin $F_{1\alpha}$	2.09 ± 0.08	120	2.89	2.55	530
(11β,13*E*,15*S*)-11,15-Dihydroxy-9-oxoprost-13-en-1-oic acid	**31**	11β-Prostaglandin E_1	2.15 ± 0.04	140	2.70	2.50	400
(5*Z*,11α,13*E*,15*S*,19*R*)-11,15,19-Trihydroxy-9-oxoprosta-5,13-dien-1-oic acid	**32**[a]	19(*R*)-Hydroxy prostaglandin E_2	2.19 ± 0.33	150	2.77	2.74	570
(5*Z*,11α)-11-Hydroxy-9,15-dioxo-prost-5-en-1-oic acid	**33**	13,14-Dihydro-15-keto-prostaglandin E_2	2.39 ± 0.05	250	2.98	2.67	670
(5*Z*,11α,13*E*,15*S*)-11,15-Dihydroxy-9-oxoprosta-5,13-dien-1-oic acid methyl ester	**34**	Prostaglandin E_2 methyl ester	2.40 ± 0.14	250	2.21	2.23	170
(13*E*,15*S*)-15-Hydroxy-9-oxoprost-13-en-1-oic acid	**35**	11-Deoxy-prostaglandin E_1	2.43 ± 0.05	270	2.85	2.33	390
(11α,13*E*)-11,16-Dihydroxy-16-methyl-9-oxoprost-13-en-1-oic acid	**36**	Misoprostol free acid	2.52 ± 0.08	330	3.42	2.92	1500
(5*Z*,9α,11α,15*S*)-9,11,15-Trihydroxyprost-5-en-1-oic acid	**37**	13,14-Dihydro-prostaglandin $F_{2\alpha}$	2.53 ± 0.10	340	3.22	2.79	1000
(11α,13*E*,15*R*)-11,15-Dihydroxy-9-oxoprost-13-en-1-oic acid	**38**	15(*R*)-Prostaglandin E_1	2.66 ± 0.06	460	3.32	3.15	1700
(5*Z*,8β,9α,11α,13*E*,15*S*)-9,11,15-Trihydroxyprosta-5,13-dien-1-oic acid	**39**	8-iso-Prostaglandin $F_{2\alpha}$	2.70 ± 0.04	510	2.94	2.97	900
(5*Z*,11α,13*E*,15*R*)-11,15-Dihydroxy-15-methyl-9-oxoprosta-5,13-dien-1-oic acid	**40**	15(*R*)-15-Methyl-prostaglandin E_2	2.73 ± 0.11	540	3.04	2.75	790
(5*Z*,13*E*,15*S*)-15-Hydroxy-9-oxoprosta-5,10,13-trien-1-oic acid	**41**	Prostaglandin A_2	2.87 ± 0.19	750	2.99	2.93	920
(5*Z*,11α,13*E*,15*R*)-11,15-Dihydroxy-9-oxoprosta-5,13-dien-1-oic acid	**42**	15(*R*)-Prostaglandin E_2	2.94 ± 0.10	870	3.50	3.29	2500
[1*R*-[1α,4α,5β(*Z*),6α(1*E*,3*S**)]]-7-[6-(3-Hydroxy-1-octenyl)-2-oxabicyclo[2.2.1]hept-5-yl]-5-heptenoic acid	**43**	U46619	3.00 ± 0.06	990	3.25	3.27	1800
(5*Z*,11α,13*E*,15*S*)-11,15-Dihydroxy-*N*-(2-hydroxyethyl)-9-oxoprosta-5,13-dien-1-amide	**44**	Prostaglandin E_2 ethanolamide	3.00 ± 0.05	1000	3.06	2.92	970
(5*Z*,9α,13*E*,15*S*)-9,15-dihydroxy-11-oxoprosta-5,13-dien-1-oic acid	**45**	Prostaglandin D_2	3.04 ± 0.05	1100	2.82	2.75	610
(13*E*,15*S*)-15-Hydroxy-9,11-dioxoprost-13-en-1-oic acid	**46**	Prostaglandin K_1	3.09 ± 0.11	1200	>3.50	3.40	2800
(11α,13*E*,15*S*)-11,15-Dihydroxy-9-oxoprost-13-en-1-oic acid methyl ester	**47**	Prostaglandin E_1 methyl ester	3.13 ± 0.20	1300	3.11	3.04	1200
(5*Z*,9α,11β,13*E*,15*S*)-9,11,15-Trihydroxyprosta-5,13-dien-1-oic acid	**48**	9α-11β-Prostaglandin F_2	3.16 ± 0.13	1500	3.36	3.27	2100
[1α(*Z*),2β(1*E*,3*R**),3α,5α]-(±)-7-[3,5-Dihydroxy-2-[3-hydroxy-4-[3-(trifluoromethyl)phenoxy]-1-butenyl]cyclopentyl]-5-heptenoic acid	**49**	Fluprostenol	3.30 ± 0.02	2000	3.79	3.74	5800
(5*Z*,9α,11α,13*E*,15*S*)-9,11,15-Trihydroxyprosta-5,13-dien-1-oic acid methyl ester	**50**	Prostaglandin $F_{2\alpha}$ methyl ester	>3.4[±] N.A.	>2400	3.57	3.18	2400
[1*R*-[1α(*Z*),2β(*R**),3α,5α]]-7-[3,5-Dihydroxy-2-(3-hydroxy-5-phenylpentyl)cyclopentyl]-5-heptenoic acid 1-methylethylester	**51**	Latanoprost isopropyl ester	>3.4 ± N.A.	>2600	2.52	2.83	470
(5*Z*,9α,11α,13*E*,15*R*)-9,11,15-Trihydroxyprosta-5,13-dien-1-oic acid	**52**	15(*R*)-Prostaglandin $F_{2\alpha}$	>3.6 ± N.A.	>4000	>3.6	>3.8	>5000
(5*Z*,13*E*,15*S*)-15-Hydroxy-9,11-dioxoprosta-5,13-dien-1-oic acid	**53**	Prostaglandin K_2	>3.9 ± N.A.	>8000	>3.6	>3.8	>5000
(5*Z*,9α,13*E*,15*S*)-9,11,15-Trihydroxythromboxa-5,13-dien-1-oic acid	**54**	Thromboxane B_2	>4.0 ± N.A.	>10000	>3.6	>3.8	>5000
(11α,13*E*)-11,16-Dihydroxy-16-methyl-9-oxoprost-13-en-1-oic acid methyl ester	**55**	Misoprostol methyl ester	≫4.0 ± N.A.	≫10000	3.64	3.70	4700

N.D., not determined; N.A., not applicable.
[a]Interexperimental variation was comparatively greater for this compound, the activity of which is significantly lower than that of M&B 28767 (**20**) and all more active compounds (**1–19**), and significantly higher than PGA_2 (**41**) and all less active compounds (**42–49**).

Results and Discussion

This SAR study was undertaken to elucidate the structural features of PGE_2 (Fig. 1) and 54 additional prostanoids and prostanoid analogs (structures shown in Fig. 2 and trivial names shown in Table 1) that significantly impact potency at hEP_1. Two previously described clonal cell lines (Ungrin et al., 1999, Abramovitz et al., 2000) that stably express recombinant hEP_1 (Funk et al., 1993) were used in this study (see *Materials and Methods*). To compare the two cell lines, saturation analysis was performed on hEP_1-5/AEQ17-HEK 293 cells to determine the affinity of PGE_2 for hEP_1 (K_D) as well as the level of receptor expression, as defined by the maximum number of detectable PGE_2 specific binding sites (B_{max}). The K_D value for PGE_2 was approximately 10 nM and the data conformed to a single-site binding model (data not shown). The level of hEP_1 receptor expression corresponded to a B_{max} value of 2.8 pmol/mg of protein. Membranes from parental AEQ17-293 cells were also tested for their ability to bind [^{3}H]PGE_2 under the same conditions used for hEP_1-5/AEQ17-293 and were negative (data not shown). The K_D and B_{max} values for the hEP_1-HEK 293 EBNA membranes used to obtain binding data were 25 nM and 7.3 pmol/mg, respectively, as reported previously (Abramovitz et al., 2000).

The rank orders of affinity and potency for all of the compounds tested at hEP_1 are shown in Table 1. In the functional assay, the EC_{50} value for PGE_2 was 2.90 nM ± 1.86 nM (n = 69). All of the compounds whose responses reached a plateau within the range of concentrations tested were as efficacious as PGE_2 with respect to the maximum response in the aequorin assay (90 to 100% effective relative to ≥ 3 μM PGE_2), behaving as full agonists at hEP_1 in this recombinant assay system. A representative example of the data generated in the aequorin assay for PGE_2 and three compounds, enprostil, cloprostenol, and fluprostenol, is shown in Fig. 3. It should be noted that partial agonism could potentially be masked in a cell line with a high receptor reserve such as the one used in this study. Of the 55 compounds tested at hEP_1, only four showed higher affinities than PGE_2 in the binding assay, whereas seven were more potent than PGE_2 in the functional assay.

Figure 4 depicts a direct comparison of receptor affinity and agonist potency for all the compounds for which K_i and EC_{50} values were obtained. Receptor binding results are presented on the x-axis, whereas results from the aequorin assay are presented on the y-axis. Theoretically, the points would be expected to lie along a straight line of slope 1 if agonist affinities and potencies were to correlate perfectly. This relationship is seen for the majority of the prostanoids tested, giving a best fit line of slope 0.87, and a correlation coefficient R^2 of 0.86 (Fig. 4). The hypothesized "barrier effect" (see below) affecting compounds with stronger binding than the natural ligand would be expected to interfere with linear correlation of the data. If we do not consider the compounds involved in this hypothesis (**1**, **3**, **4**, **5** and **17**), the slope becomes 1.01 and R^2 becomes 0.91 (data not shown). The effects of various ligand modifications on receptor activation are presented in Tables 2 through 9, organized by location on PGE_2. Effects on receptor binding are also discussed where they differ from the changes in activity.

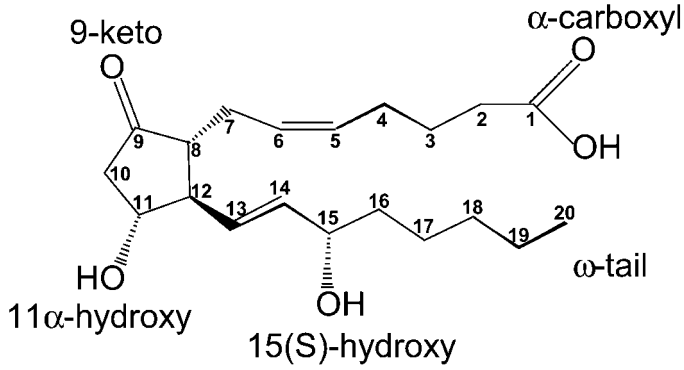


Fig. 1. Structure of PGE_2. Carbon atoms are numbered from 1 to 20 starting from the C-1 carboxyl group.

C-15 Position of PGE_2. The receptor is most sensitive to modifications at the C-15 position of prostanoid analogs (Tables 1 and 2). Methylation at the 15-position of PGE_2 (**8**) or $PGF_{2\alpha}$ (**15**), while maintaining the orientation of the hydroxyl group, yielded the 15(*S*)-15-methyl derivatives (**12**, **21**) and caused minor reductions in potency of 2.5- and 2.7-fold, respectively. However, inversion of the stereochemistry at this position to the 15(*R*) conformations of 15(*R*)-PGE_2 (**42**), 15(*R*)-PGE_1 (**38**), 15(*R*)-$PGF_{2\alpha}$ (**52**), and 15(*R*)-15-methyl-PGE_2 (**40**) resulted in substantial reductions in potency of 870, 140, >400, and 220-fold, respectively. Oxidation of the hydroxyl to a ketone, giving the primary metabolite of PGE_2, 15-keto-PGE_2 (**28**), resulted in 93-fold lower potency than the parent compound [the subsequent metabolite, 13,14-dihydro-15-keto-PGE_2 (**33**) is 250-fold less active]. In the case of misoprostol free acid (**36**), a 15-deoxy-16-hydroxy-16-methyl derivative of PGE_1 (**14**) racemic at the 16 position, potency was reduced 100-fold relative to PGE_1 (**14**) and 330-fold relative to PGE_2 (**8**), and binding was reduced 130- and 1500-fold relative to the same two compounds. Interestingly, in competition for [^{3}H]PGE_2 radioligand binding at the human EP_2, EP_3, and EP_4 receptors, misoprostol free acid, is 7, 24, and 29-fold less effective than PGE_2 (Abramovitz et al., 2000). This suggests that at these receptor subtypes, the 15-hydroxyl may not play as critical a role for agonist affinity as it does at hEP_1.

C-1 Position of PGE_2. Modifications to the carboxylic acid moiety at C-1 were also found to have extremely negative effects on the affinity and potency of the compounds tested, although the functional effects were generally more profound (Tables 1 and 3). In all cases in which a direct comparison could be made between compounds with and without such a modification, the modified compound was at least 60- and up to >400-fold less potent as an agonist. All such compounds also exhibited reduced affinity. Similarly, data with various methyl ester compounds supports the importance of the C-1 carboxylic acid for binding to the EP_2 (Stillman et al., 1998; Abramovitz et al., 2000), EP_3 (Audoly and Breyer, 1997; Abramovitz et al., 2000), and EP_4 (Stillman et al., 1998; Abramovitz et al., 2000) receptor subtypes.

Evidence from mutagenesis (Funk et al., 1993; Huang and Tai, 1995) and modeling (Yamamoto et al., 1993; Yamamoto and Imai, 1996) studies suggest that the C-1 carboxylic acid of prostanoids interacts with the conserved arginine residue in transmembrane domain VII that occurs in all eight prostanoid receptors (Boie et al., 1995). Although the carboxylic acid could interact with the arginine residue by forming both ionic and hydrogen bonds, it has recently been proposed that hydrogen bonding interactions are sufficient for the func-

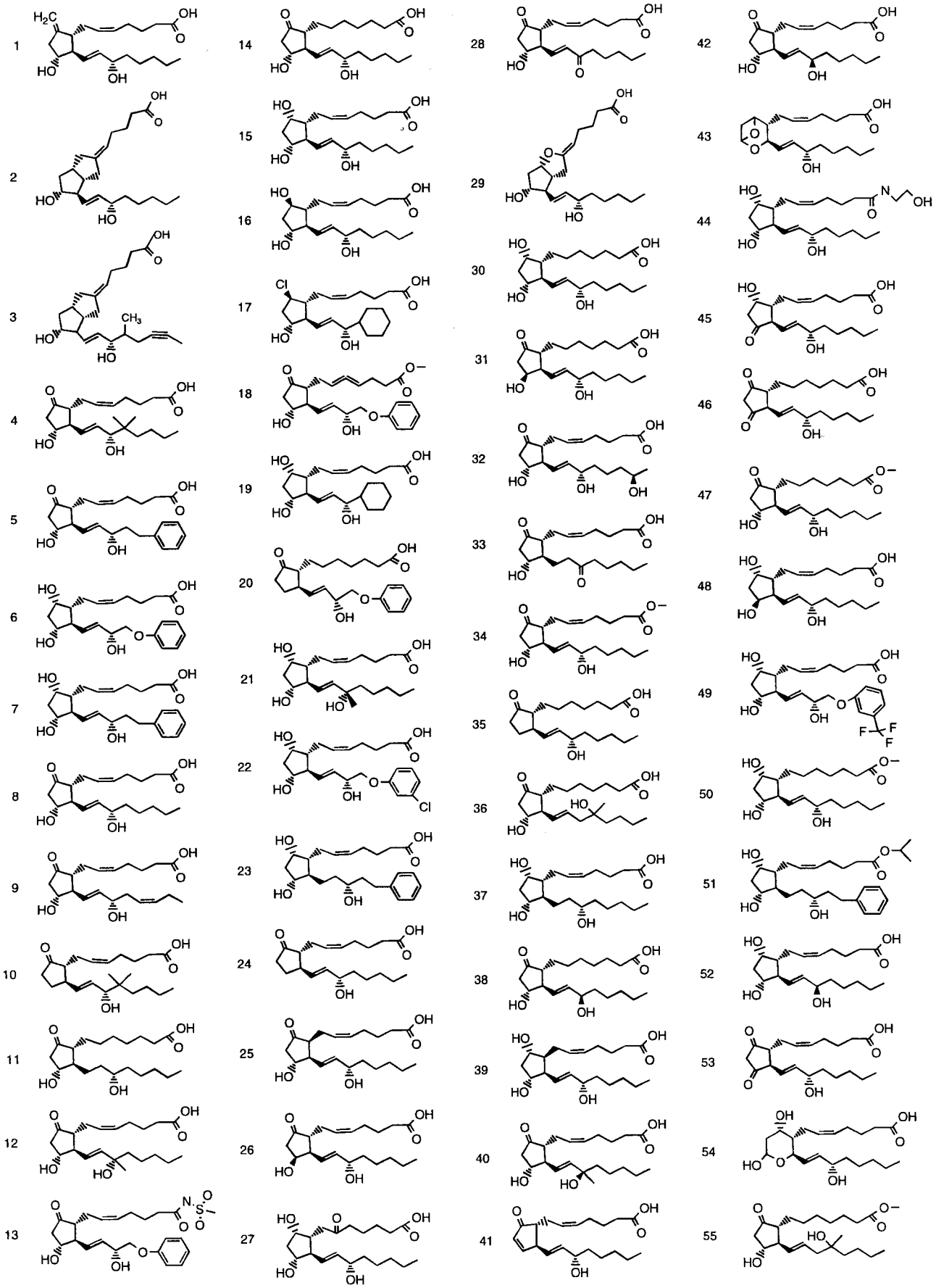


Fig. 2. Molecular structures of compounds used in the study, with compound numbers as in Table 1.

tional activation of all EP receptor subtypes (Chang et al., 1997). In the latter study, at mouse EP_1 for example, PGE_2-methyl ester (**34**) was only 2-fold less potent than PGE_2 (EC_{50} of 200 versus 400 nM) in a functional assay, although it displayed 15-fold less affinity in a binding assay. Our data contradicts this model in that modification of the C-1 carboxylic acid to various functional groups capable of hydrogen but not ionic bonding (for example compounds **34**, **44**, and **51**) greatly decreases the ability of these compounds to bind to and activate hEP_1. Conversion of the free acid to a methyl ester [PGE_2 methyl ester (**34**), PGE_1 methyl ester (**47**), $PGF_{2\alpha}$ methyl ester (**50**), and misoprostol (**55**)], isopropyl ester [latanoprost (**51**)] or ethanolamide group [PGE_2 ethanolamide (**44**)] caused a dramatic decrease in activity at the hEP_1 receptor.

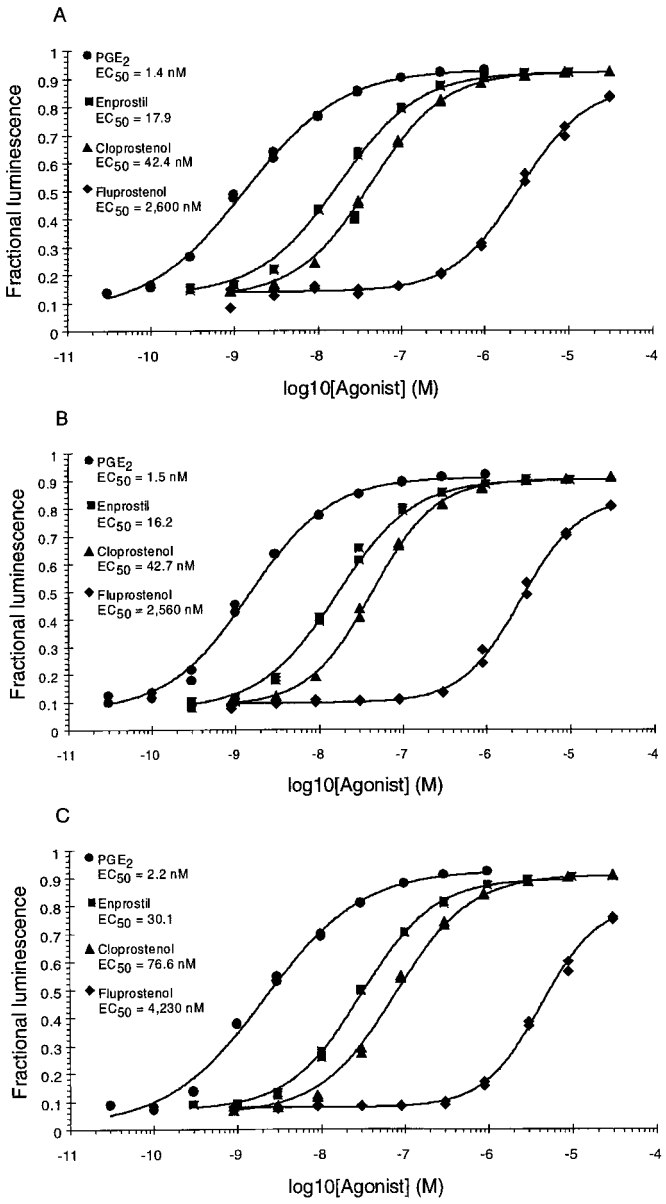


Fig. 3. Concentration response curves from hEP_1-5/293-AEQ17 cells challenged with PGE_2 (**8**), cloprostenol (**22**), enprostil (**18**), and fluprostenol (**49**) from three different experiments shown in A, B, and C. The fractional luminescence responses are plotted as a function of $\log_{10}$ agonist concentrations and the EC_{50} values shown on the graphs are calculated as described under *Materials and Methods*.

Exceptions of particular interest include sulprostone (**13**) and enprostil (**18**), which retained potency yet have modified α-carboxyl groups, a methanesulfonimide (acidic) and methyl ester, respectively. Because free acid forms were so much more potent than ester forms, except for the two compounds mentioned above, enprostil was checked by mass spectrometry, repurified by high-performance liquid chromatography, and retested in the aequorin assay to confirm that the methyl ester was still intact and was indeed the active compound in the assays, which was seen to be the case (data not shown).

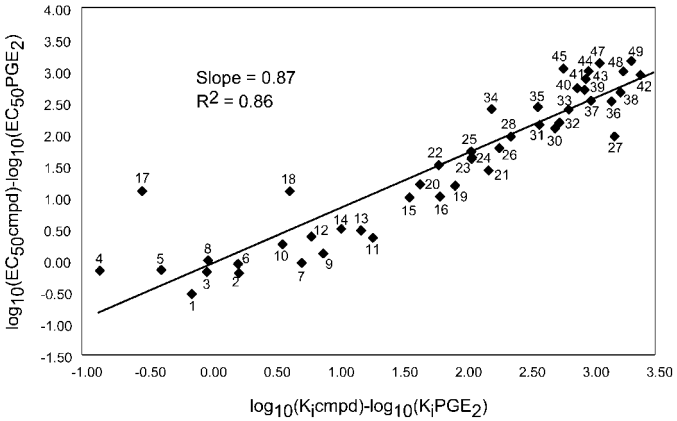
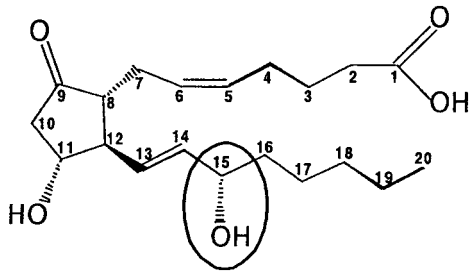


Fig. 4. Correlation of receptor binding and aequorin activity data. Both data sets are normalized to PGE_2 as described under *Materials and Methods*. Binding results for each compound are shown on the *x*-axis, whereas functional results from the aequorin assay are shown on the *y*-axis. The relevant compound reference number (see Table 1 and Fig. 2) is displayed adjacent to each data point. K_i and/or EC_{50} values for compounds 46 and 50 to 55 could not be ascertained because the concentration-response curves did not plateau at the maximum concentrations tested and hence are not included in the figure.

TABLE 2

The effects of modifications at the C-15 position on potency

Results are reported as fold reduction in potency relative to the root structure in each group with parenthetical values relative to PGE_2. For compounds in which no root structure was available (**bold**), results are reported relative to PGE_2. Compounds are numbered according to Table 1.

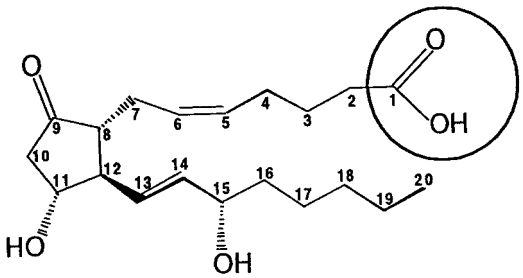


Compound	Trivial name	EC_{50} cmpd/ EC_{50} PGE_2
8	PGE_2	1
12	15(*S*)-15-Methyl-PGE_2	2.4
28	15-keto-PGE_2	93
40	15(*R*)-15-Methyl-PGE_2	540
42	15(*R*)-PGE_2	870
14	PGE_1	1 (3.2)
38	15(*R*)-PGE_1	140 (460)
15	$PGF_{2\alpha}$	1 (10)
21	15(*S*)-15-Methyl-$PGF_{2\alpha}$	2.7 (27)
52	15(*R*)-$PGF_{2\alpha}$	>400 (>4000)
36	**Misoprostol free acid**	**330**

TABLE 3

The effects of modifications to the carboxylic acid moiety on potency

Results are reported as fold reduction in potency relative to the root structure in each group with parenthetical values relative to PGE_2. For compounds where no root structure was available (**bold**), results are reported relative to PGE_2. Compounds are numbered according to Table 1.

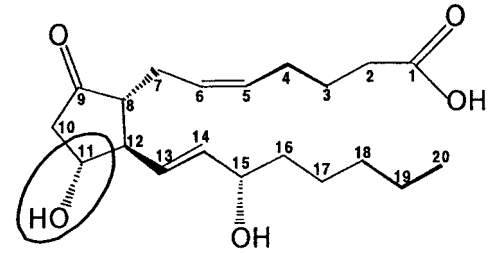


Compound	Trivial name	EC_{50} cmpd/EC_{50} PGE_2
8	PGE_2	1
34	PGE_2 methyl ester	250
44	PGE_2 ethanolamide	1000
14	PGE_1	1 (3.2)
47	PGE_1 methyl ester	420 (1300)
15	$PGF_{2\alpha}$	1 (10)
50	$PGE_{2\alpha}$ methyl ester	>240 (>2400)
23	Latanoprost free acid	1 (41)
51	Latanoprost isopropyl ester	>63 (>2600)
36	Misoprostol free acid	1 (330)
55	Misoprostol methyl ester	>300 (>10,000)
13	**Sulprostone**	**3.0**
18	**Enprostil**	**13**

TABLE 4

The effects of modifications at the C-11 position on potency

Results are reported as fold reduction in potency relative to the root structure in each group with parenthetical values relative to PGE_2. For compounds where no root structure was available (**bold**), results are reported relative to PGE_2. Compounds are numbered according to Table 1.



Compound	Trivial name	EC_{50} cmpd/ EC_{50} PGE_2
8	PGE_2	1
24	11-deoxy-PGE_2	43
26	11β-PGE_2	61
53	PGK_2 (11-keto-PGE_2)	>8,000
14	PGE_1	1 (3.2)
35	11-deoxy-PGE_1	84 (270)
31	11β-PGE_1	44 (140)
46	PGK_1 (11-keto-PGE_1)	390 (1200)
15	$PGF_{2\alpha}$	1 (10)
48	9α,11β-PGF_2	150 (1500)
45	PGD_2 (11-keto-$PGF_{2\alpha}$)	110 (1100)
4	16,16-dimethyl-PGE_2	1 (0.69)
10	11-deoxy-16,16-dimethyl-PGE_2	2.6 (1.8)
41	**PGA_2**	**750**
43	**U46619**	**990**
54	**TxB_2**	**>10,000**

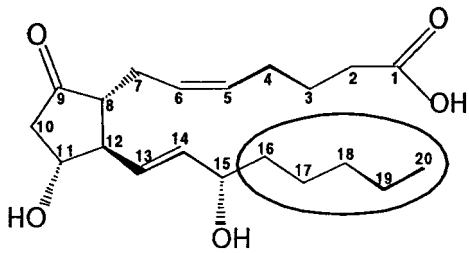
This ability to retain potency is, therefore, probably attributable to the presence of a phenoxy substitution at the C-16 position on both structures (see below and Table 5). It has also been suggested, in a prior study using a series of C-1 modified PGE_2 analogs tested in several bioassay systems, that at the C-1 position, acidity is more important than steric bulk for activity (Schaaf and Hess, 1979). This may also contribute to the potency of sulprostone (**13**) at hEP_1.

C-11 Position of PGE_2. Both potency and affinity are sensitive to alterations at the C-11 position (Tables 1 and 4). Removal of the hydroxyl group reduced potency by 43 and 84-fold for PGE_2 (**8**) and PGE_1 (**14**), respectively. A much smaller effect was observed in the case of 16,16-dimethyl-PGE_2 (**4**), a slightly more potent agonist than PGE_2, in which removal of the 11-hydroxy group reduced potency only 2.6-fold—another example of a ligand in which an ω-tail modification seems to protect against loss of potency (see below). PGA_2 (**41**) (11-deoxy-10,11-enyl-PGE_2) exhibited 748-fold less potency than PGE_2 (**8**). Inversion of the stereochemistry at the 11-position also significantly reduced the potencies of

TABLE 5

The effects of ω-tail modifications on potency

Results are reported as fold reduction in potency relative to the root structure in each group with parenthetical values relative to PGE_2. For compounds where no root structure was available (**bold**), results are reported relative to PGE_2. Compounds are numbered according to Table 1.



Compound	Trivial name	EC_{50} cmpd/ EC_{50} PGE_2
8	PGE_2	1
4	16,16-dimethyl-PGE_2	0.69
5	17-phenyl-ω-trinor-PGE_2	0.71
9	PGE_3	1.3
32	19(*R*)-hydroxy-PGE_2	153
24	11-deoxy-PGE_2	1 (43)
10	11-deoxy-16,16-dimethyl-PGE_2	0.042 (1.8)
35	11-deoxy-PGE_1	1 (270)
20	M&B28767 (16-phenoxy-ω-tetranor-)	0.059 (16)
15	$PGF_{2\alpha}$	1 (10)
6	16-phenoxy-ω-tetranor-$PGF_{2\alpha}$	0.088 (0.88)
7	17-phenyl-ω-trinor-$PGF_{2\alpha}$	0.094 (0.92)
19	15-cyclohexyl-ω-pentanor-$PGF_{2\alpha}$	1.5* (15)
22	Cloprostenol (16-*m*-chlorophenoxy-ω-tetranor-)	3.2 (32)
49	Fluprostenol (16-*m*-trifluoromethylphenoxy-ω-tetranor-)	200 (2000)
37	13,14-dihydro-$PGF_{2\alpha}$	1 (340)
23	Latanoprost free acid (17-phenyl-ω-trinor-)	0.12 (41)
2	Carbacyclin	1 (0.63)
3	Iloprost (16-methyl-18-yne-)	1.0 (0.66)
13	**Sulprostone**	**3.0**
17	**ZK110841**	**13**
18	**Enprostil**	**13**
36	**Misoprostol free acid**	**330**

*, not significantly different from the root structure.

compounds, as exemplified by analogs of PGE_2 (**8**) [11β-PGE_2 (**26**)], PGE_1 (**14**) [11β-PGE_1 (**31**)] and $PGF_{2\alpha}$ (**15**) [9α,11β-PGF_2 (**48**)], whose activities dropped 61, 44, and 150 -fold, respectively. Replacement of the 11-hydroxyl with a keto group again greatly reduced the potencies of analogs of PGE_2 (**8**) [PGK_2 (**53**)], PGE_1 (**14**) [PGK_1 (**46**)] and $PGF_{2\alpha}$ (**15**) [PGD_2 (**45**)] resulting in reductions of >8000, 387, and 110-fold, respectively, further highlighting the importance of the 11β-hydroxyl group of PGE_2 for potency at hEP_1.

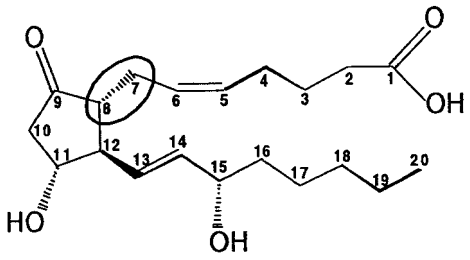
The ω-Tail of PGE_2. The ω-tail is the primary region of interest for modifications yielding increased potency (see Table 5). In particular, modifications that result in a phenyl ring commencing at the former C-18 position, such as the 16-phenoxy and 17-phenyl compounds, exhibit a significant positive influence, although it is not clear whether this is caused by steric or electronic effects. It is noteworthy that these phenylic modifications are capable of contributing significant improvements in potency (more than an order of magnitude) to structures that contain other deleterious substitutions, for example 11-deoxy-16,16-dimethyl-PGE_2 (**10**) is 24-fold more potent than 11-deoxy-PGE_2 (**24**). These effects, however, are not observed when the parent structure is a highly potent compound, such as PGE_2.

Taken together, these data suggest the existence of a virtual "barrier", beyond which modifications to the ω-tail are not able to improve potency. The mechanism through which this effect might operate is not obvious. Increased bulk beyond that of a phenyl group and/or modifications to the electronic structure of the group, as occur in the potent FP agonists cloprostenol (**22**) (16-*m*-chlorophenoxy-$PGF_{2\alpha}$) and fluprostenol (**49**) (16-*m*-trifluoromethylphenoxy-$PGF_{2\alpha}$), become counterproductive, reducing both potency and affinity at hEP_1. The presence of phenyl groups in this region also provides a potential explanation for the potencies of compounds such as sulprostone (**13**) and enprostil (**18**), which one would predict to be relatively inactive due to C-1 modifications. Another example of an activating modification is 16,16-dimethyl-PGE_2 (**4**) which displayed the greatest affinity (8-fold > PGE_2; Table 1) of all of the prostanoids tested in the hEP_1 binding assay and was fourth most potent in the aequorin assay.

TABLE 6

The effects of chiral inversion at the C8 position on potency

Results are reported as fold reduction in potency relative to the root structure in each group with parenthetical values relative to PGE_2. Compounds are numbered according to Table 1.



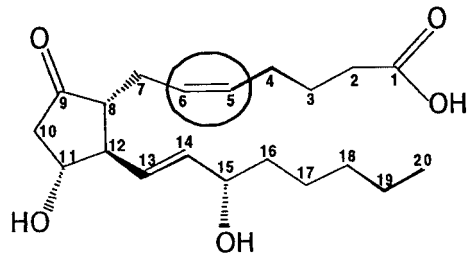
Compound	Trivial name	EC_{50} cmpd/ EC_{50} PGE_2
8	PGE_2	1
25	8-iso-PGE_2	53
15	$PGF_{2\alpha}$	1 (10)
39	8-iso-$PGF_{2\alpha}$	51 (510)

In contrast, replacement of the ω-tail with a cyclohexyl group starting at the 15 position [as in 15-cyclohexyl-ω-pentanor-$PGF_{2\alpha}$ (**19**)] resulted in minor reductions in affinity and potency. The addition of a double bond between the C-17 and C-18 position to give PGE_3 (**9**) results in a small reduc-

TABLE 7

The effects of saturation at the 5,6 double bond on potency

Results are reported as fold reduction in potency relative to the root structure in each group with parenthetical values relative to PGE_2. Compounds are numbered according to Table 1.

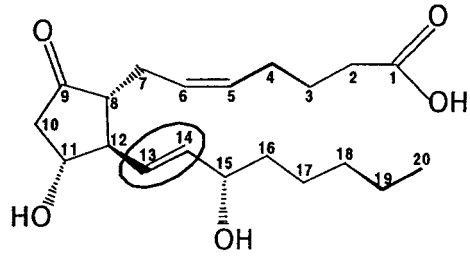


Compound	Trivial name	EC_{50} cmpd/ EC_{50} PGE_2
8	PGE_2	1
14	PGE_1	3.2
34	PGE_2 methyl ester	1 (250)
47	PGE_1 methyl ester	5.3 (1300)
42	15(*R*)-PGE_2	1 (870)
38	15(*R*)-PGE_1	0.53 (460)
26	11β-PGE_2	1 (61)
31	11β-PGE_1	2.3 (140)
24	11-deoxy-PGE_2	1 (43)
35	11-deoxy-PGE_1	6.3 (270)
53	PGK_2	1 (>8000)
46	PGK_1	<0.15 (1200)
15	$PGF_{2\alpha}$	1 (10)
30	$PGF_{1\alpha}$	12 (120)

TABLE 8

The effects of saturation at the 13,14 double bond on potency

Results are reported as fold reduction in potency relative to the root structure in each group with parenthetical values relative to PGE_2. Compounds are numbered according to Table 1.



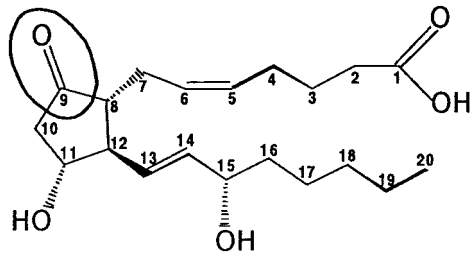
Compound	Trivial name	EC_{50} cmpd/ EC_{50} PGE_2
28	15-keto-PGE_2	1 (93)
33	13,14-dihydro-15-keto-PGE_2	2.7 (250)
14	PGE_1	1 (3.2)
11	13,14-dihydro-PGE_1 (PGE_0)	0.72* (2.3)
15	$PGF_{2\alpha}$	1 (10)
37	13,14-dihydro-$PGF_{2\alpha}$	34 (340)
7	17-phenyl-ω-trinor-$PGF_{2\alpha}$	1 (0.92)
23	Latanoprost free acid	45 (41)

*, not significantly different from the root structure.

TABLE 9

The effects of modifications at the C-9 position on potency

Results are as fold reduction in potency reported relative to the root structure in each group with parenthetical values relative to PGE_2. For compounds where no root structure was available (**bold**), results are reported relative to PGE_2. Compounds are numbered according to Table 1.



Compound	Trivial name	EC_{50} cmpd/ EC_{50} PGE_2
8	PGE_2	1
1	9-deoxy-9-methylene-PGE_2	0.30
15	$PGF_{2\alpha}$ (9α-hydroxy-)	10
16	9β,11α-PGF_2 (9β-hydroxy-)	10
14	PGE_1	1 (3.2)
30	$PGF_{1\alpha}$	39 (120)
12	15(*S*)-15-methyl-PGE_2	1 (2.4)
21	15(*S*)-15-methyl-$PGF_{2\alpha}$	11 (27)
42	15(*R*)-PGE_2	1 (870)
52	15(*R*)-$PGF_{2\alpha}$	>4.6 (>4000)
34	PGE_2 methyl ester	1 (250)
50	$PGF_{2\alpha}$ methyl ester	>9.6 (>2400)
25	8-iso-PGE_2	1 (53)
39	8-iso-$PGF_{2\alpha}$	9.5 (510)
5	17-phenyl-ω-trinor-PGE_2	1 (0.71)
7	17-phenyl-ω-trinor-$PGF_{2\alpha}$	1.3* (0.92)
17	ZK110841 (9β-chloro-)	1 (13)
19	15-cyclohexyl-ω-pentanor-$PGF_{2\alpha}$	1.2* (15)
29	PGI_2	1 (106)
2	Carbacyclin	0.006 (0.63)
43	**U46619**	**990**

*, not significantly different from the root structure

tion in potency accompanied by a larger reduction in affinity, whereas the presence of a chiral hydroxyl group with *R*-configuration at the C-19 position (**32**) reduces both by >2 orders of magnitude. The addition of a triple bond at the C-18 position and a methyl at the C-16 position has little overall effect when comparing carbacyclin (**2**) and iloprost (**3**); it is unclear, however, whether this is caused by a lack of effect of the modification itself or to the hypothetical "barrier effect" proposed above. Although iloprost is used in pharmacological studies of the IP receptor, as a substitute for the endogenous unstable prostanoid PGI_2, it is also a known potent agonist of EP_1 (Coleman et al., 1994, our data) and this activity at hEP_1 is shared by carbacyclin.

C-8 Position of PGE_2. Stereochemistry at the C-8 position is important for affinity and potency (Tables 1, 6), with chiral inversion at this position in PGE_2 (**8**) [to 8-iso-PGE_2 (**25**)] and $PGF_{2\alpha}$ (**15**) [to 8-iso-$PGF_{2\alpha}$ (**39**)] giving 53- and 51-fold reductions in potency, respectively. These compounds, known as isoprostanes, are thought to exert some, if not all, of their biological effects through the TP receptor. However, a recent study (Sametz et al., 2000) suggests that they may also act through an EP_1 subtype. Our data also suggests a potential role for EP_1, although the isoprostanes are weak agonists when compared with PGE_2. Specific receptors for isoprostanes have yet to be identified.

The 5,6 Double Bond of PGE_2. Saturation of the double bond present at the 5,6 position has relatively minor effects on affinity and potency (Tables 1 and 7). In general, this modification results in a small decrease in both parameters, exemplified by the reduction of the 5–6 double bond of PGE_2 (**8**) to form PGE_1 (**14**) which resulted in a 3-fold decrease in activity. In certain cases, however, the effect is slightly positive [e.g., PGK_2 (**53**) to PGK_1 (**46**) and 15(*R*)-PGE_2 (**42**) to 15(*R*)-PGE_1 (**38**)]. Interestingly, both these compounds are derived from a parent structure bearing a single modification that heavily impairs affinity and potency (see Fig. 2) and that might be expected to result in significant effects on the general conformation of the molecule. One possible explanation is that the increased flexibility resulting from the saturation of the double bond may allow these compounds to adopt a more active conformation.

13,14 Position of PGE_2. Saturation of the double bond at the 13,14 position also had variable consequences for activity (see Table 8), ranging from a slight increase for 13,14-dihydro-PGE_1 (**11**) to a 45-fold reduction for latanoprost free acid (**23**) compared with their parent compounds, PGE_1 (**14**) and 17-phenyl-ω-trinor-$PGF_{2\alpha}$ (**7**), respectively. In all cases, the effects on receptor affinities are detrimental, to a greater or lesser degree, as are the effects on potency with the exception of the conversion of PGE_1 (**14**), already one degree more saturated than the optimal natural ligand, PGE_2 (**8**), to 13,14-dihydro-PGE_1 (PGE_0) (**11**), where the difference is not statistically significant.

C-9 Position of PGE_2. Finally, modifications at the C-9 position have small effects on the potency of a given structure, although in certain cases larger effects are seen on the receptor binding results (see Tables 1 and 9). In general, conversion of the 9-keto group to a hydroxy group results in a loss of approximately 1 order of magnitude in potency. Comparison of results from $PGF_{2\alpha}$ (**15**) and 9β-PGF_2 (**16**) would suggest that the stereochemistry of the resulting chiral center is not of great significance. The effects of keto-to-hydroxy conversion are not statistically significant on the structure incorporating the phenyl substitution at the C-17 position [17-phenyl-ω-trinor-PGE_2 (**5**) versus 17-phenyl-ω-trinor-$PGF_{2\alpha}$ (**7**)]. Because 17-phenyl-ω-trinor-$PGF_{2\alpha}$ (**7**) is potent, this is thought to reflect the limit of improvement by ω-tail modifications (see discussion above) rather than an absence of effect from the conversion.

Replacement of the oxygen substituent at the C-9 position with a CH_2 group [9-deoxy-9-methylene-PGE_2 (**1**)] resulted in a 3-fold increase in potency, yielding the most effective agonist tested in this study. Replacement of a 9α-hydroxyl with a 9β-chlorine [ZK110841 (**17**) versus 15-cyclohexy-ω-pentanor-$PGF_{2\alpha}$ (**19**)] gave a dramatic improvement in receptor binding affinity with no significant increase in potency (see Table 1). This difference in affinity and potency was unusual among the compounds tested and may be of use in the design of novel competitive antagonists for this receptor. Within the context of the results discussed above, this effect is thought to be due to the chlorine substitution, rather than the stereochemical inversion. ZK110841 (**17**), commonly referred to as a potent DP receptor agonist (Coleman et al., 1994), also shows high affinity for not only EP_1 but also EP_2 and EP_4 receptor subtypes (Abramovitz et al., 2000).

In summary, this study has revealed the basic SAR of prostanoids and prostanoid analogs acting as agonists at the human EP_1 receptor. The configuration of the 15-hydroxyl group was the most critical for agonism, followed by the 1-carboxylic acid, the 11-hydroxyl group, and chirality at C-8, whereas the 9-keto and unsaturated carbon bonds were least important. Modifications to the ω-tail could also make substantial positive or negative contributions to agonist potency. This information suggests paths for further exploration in the area of receptor-ligand interactions at hEP_1, and the design of novel therapeutic agents.

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