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Synthesis and In Vitro Evaluation of Human FP-receptor Selective Prostaglandin Analogues

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Abstract—The in vitro evaluation of a series of saturated prostaglandins revealed that compounds with *omega* chain aromatic rings retain nanomolar potency for the human prostaglandin F receptor (hFP receptor), exemplified by compound **8**. In contrast, the double bonds are required for activity in the series with an acyclic *omega* chain as in $PGF_{2\alpha}$. © 2000 Published by Elsevier Science Ltd.

Introduction

The naturally-occurring prostaglandin $PGF_{2\alpha}$ has a wide range of pharmacological properties.[1] The primary mediator of its activity is thought to be the prostanoid receptor F, the FP receptor, following the nomenclature of Coleman.[2] FP-mediated events are thought to include the reduction of intraocular pressure,[3] the induction of estrus in some species and the induction of labor. However, while naturally-occurring prostaglandins are characterized by their activity against a particular prostaglandin receptor, they are not specific for any given receptor. Further, conflicting data exist in the literature on both the tissue and receptor selectivity of $PGF_{2\alpha}$ analogues.[4] The confusion may now be dispelled with the cloning and expression of the cell-surface receptors which are thought to mediate the effects of the prostaglandins.[5]

As part of a pharmacophore minimization effort in our bone anabolics group,[6] we examined the contribution that the double bonds had to the potency of $PGF_{2\alpha}$ and its analogues. Known hFP receptor ligands were thus converted to their corresponding saturated counterparts and tested in hFP receptor functional[7] (EC_{50}) and radioligand-displacement binding[8] (IC_{50}) assays. Data are averages of triplicate measurements.

Chemistry

All aromatic compounds were synthesized using a modification of the original Corey prostaglandin route as outlined in the Scheme for compounds **7** and **8**. Thus *meta*-trifluoromethylcinnamic acid (**9**) was saturated, then esterified with TMS-diazomethane to give the ester **10** in good yield. Treatment of methyl dimethylphosphonate with *n*-butyllithium followed by **10** provided ketophosphonate **11** in yields ranging from 50–80%. Ketophosphonate **11** was then treated with sodium hexamethyl-disilazane followed by condensation with Corey Lactone (**12**) to give the α,β-unsaturated ketone **13** in approximately 50% yield. Stereoselective reduction of C_{15} was realized by treatment of **13** with a premixed solution of BINAL and lithium aluminum hydride to give the C_{15} alcohol **14** as the only isomer isolated. Deprotection of the benzoate ester was accomplished with potassium carbonate in methanol to give diol **15**. The C_{11} and C_{15} alcohols were subsequently protected as their tetrahydropyranyl (THP) ethers to give **16**. Reduction of **16** with diisobutylaluminum hydride gave the corresponding lactol which was, without purification, condensed with the sodium salt of the stabilized phosphonium ylide of 4-carboxybutyl triphenylphosphonium bromide to install the prostaglandin α-chain with high *Z*-stereoselectivity (9:1 *Z:E*) providing **17**. Removal of the THP ethers with acetic acid and water gave **7**. Saturation of the 5,6 and 13,14 double bonds gave the tetrahydro analogue **8**.

The aliphatic compounds $PGF_{2\alpha}$ (**1**) and 16,16, dimethyl $PGF_{2\alpha}$ (**3**) were purchased (Cayman Chemical, Ann Arbor, MI) and tested without further purification. The

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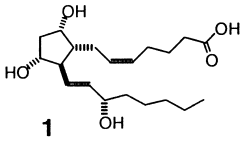
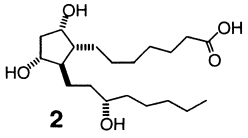
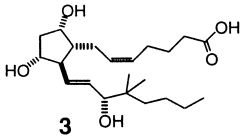
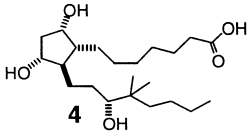
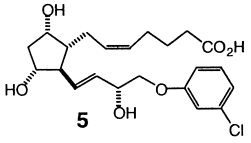
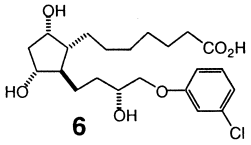
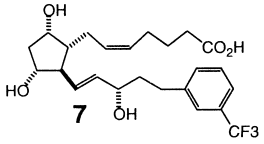
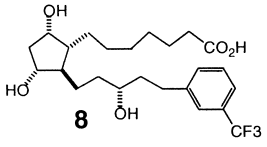
saturated aliphatic compounds **2** and **4** were synthesized by hydrogenation of the appropriate precursor with H_2 over 5% Pd/C in ethanol.

Results

As shown in Table 1, both $PGF_{2\alpha}$(**1**), and 16,16-dimethyl $PGF_{2\alpha}$(**3**) have nanomolar activity at the hFP receptor, in agreement with literature values on tissues. Also consistent, the saturated analogue of $PGF_{2\alpha}$(**2**) has an IC_{50} of 522 nM and an EC_{50} of 5000 nM, a 200-fold decrease in activity at the receptor. A similar decrease is observed upon hydrogenation of 16,16-dimethyl $PGF_{2\alpha}$(**4**). Thus, in the straight chain series the two double bonds may be necessary for high affinity binding.

Cloprostenol (**5**),[9] a 16-phenoxy-substituted ligand of the hFP receptor, upon hydrogenation, provides tetrahydrocloprostenol, (**6**) which *maintains* its potency at the human FP receptor with an EC_{50} of 9 nM and has an even better selectivity profile for the hFP receptor versus the hTP, hEP_1 and hEP_3 receptors (Table 1). Similarly, the 17-phenyl analogue, (**7**), and its tetrahydro version (**8**) gave hFP EC_{50}'s between 64 and 85 nM; again the saturated analogue has the better receptor selectivity profile. The syntheses of these analogues was accomplished as outlined in the Scheme.

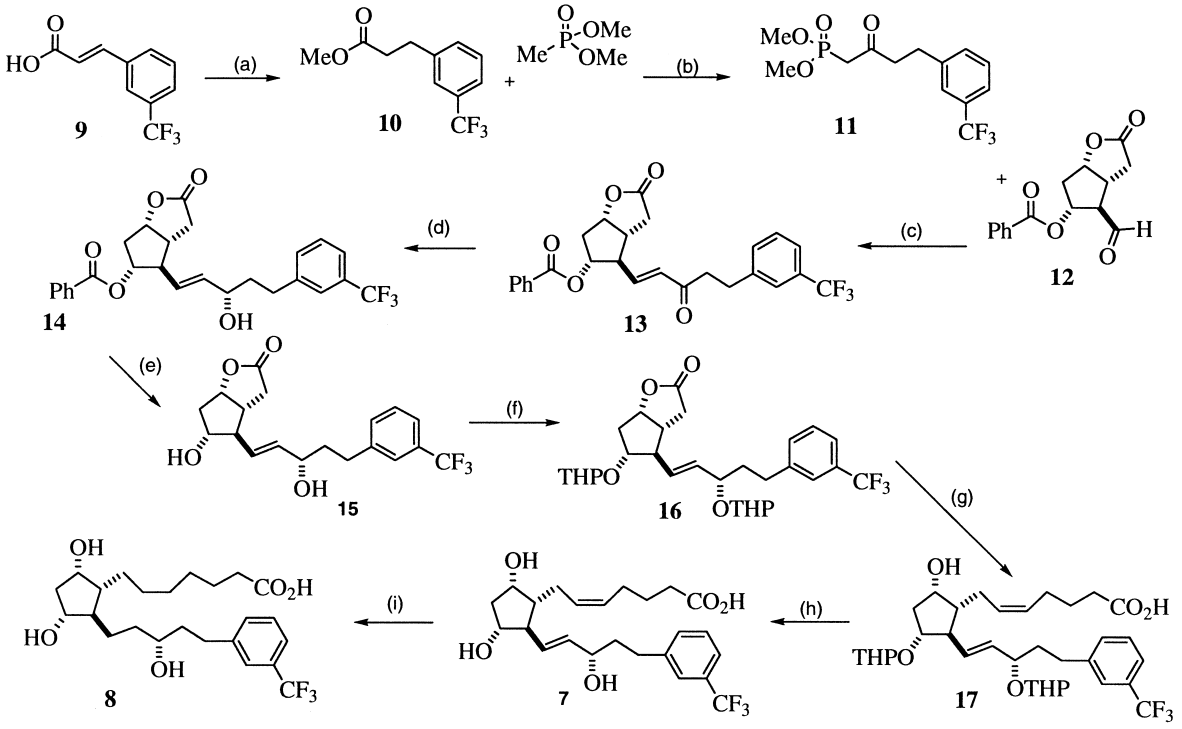
Table 1. hFP Binding and excitatory receptors' functional data for $PGF_{2\alpha}$ and derivatives

Compound	IC_{50} hFP	EC_{50} hFP	EC_{50} hTP	EC_{50} hEP_1	EC_{50} hEP_3
 **1**	2.5 nM	79 nM (75%[a])	1400 nM (95%[b])	600 nM (80%[c])	2300 nM (50%[d])
 **2**	522 nM	5000 nM (50%[a])	30,000 nM, (100%[b])	>10,000 nM, (< 50%[c])	>20,000 nM, (< 50%[d])
 **3**	16 nM	90 nM (150%[a])	24 nM, (95%[b])	300 nM, (80%[c])	2000 nM, (130%[d])
 **4**	>1000 nM	>20,000 nM (< 20%[a])	>20,000 nM (< 20%[b])	>20,000 nM (< 20%[c])	>20,000 nM (< 20%[d])
 **5**	1.0 nM	10 nM (100%[a])	2400 nM (100%[b])	760 nM (80%[c])	5000 nM (75%[d])
 **6**	1.0 nM	9 nM (100%)	8000 nM (90%[b])	19,000 nM (100%[c])	>20,000 nM (< 50%[d])
 **7**	10 nM	64 nM (87%[a])	2100 nM (50%[b])	>20,000 nM (< 50%[c])	>20,000 nM (< 50%[d])
 **8**	8.6 nM	85 nM (90%[a])	>20,000 nM (< 50%[b])	30,000 nM (100%[c])	>20,000 nM (< 50%[d])

[a]% of control ligand, fluprostenol.
[b]% of control ligand, U46619.
[c]% of control ligand, 17-ϕ-PGE_2.
[d]% of control ligand, sulprostone.

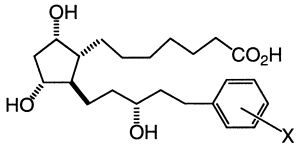
Structure–Activity Relationship

To investigate the breadth of this finding, we initiated a limited exploration of the structure–activity relationship (SAR) of the saturated prostaglandin analogues. Using the synthetic route outlined in Scheme 1, a number of aromatic prostaglandin analogues were synthesized and tested. Compounds with initial binding IC_{50}s >2000 nM (hFP) were considered inactive and not further tested. Data are shown in Table 2. Removal of the unsaturation in all cases resulted in molecules that did not appreciably bind to the hTP receptor, and only one example had measurable activity at the hEP_3 receptor, in contrast to the unsaturated series,[10] where they were found to activate both of these receptors in tissue preparations. Further, activity at the hEP_1 receptor was greatly reduced for all of the molecules in this series. Thus the initial selectivity trend observed was continued in these analogues. The potency at the hFP receptor, however, varied widely from compound to compound. The ability



Scheme 1. (a) H_2, 5% Pd/C, EtOAc; then $TMSCHN_2$, MeOH (b) *n*-BuLi, THF, 0 °C (c) NaHMDS, DME, (d)(*S*)-BINAL-H, $LiAlH_4$, THF, (e) K_2CO_3, MeOH overnight (f) 2.2 equiv DHP, cat. *p*-TSA, CH_2Cl_2 (g) DIBAL-H, −78 °C, then 2.1 equiv NaHMDS and $Ph_3P^+(CH_2)_4COOH\ Br^-$ (h) 1:1:1 THF: H_2O: HOAc; (i) H_2, 5% Pd/C, EtOAc.

Table 2. Human-FP receptor data for 17-phenyl analogues



Entry	X	IC_{50} hFP (Binding)[a]	IC_{50} hEP_1 (Binding)[b]	IC_{50} hEP_3 (Binding)[b]	IC_{50} TP (Binding)[c]
18	H	82 nM	1100 nM	>10,000	>10,000
19	*o*-F	76 nM	3100 nM	>10,000	>10,000
20	*m*-F	42 nM	10,000	3700 nM	>10,000
21	*p*-F	210 nM	3450	>10,000	>10,000
22	*o*-CF_3	2140 nM	Not Tested	Not Tested	Not Tested
23	*m*-CF_3	8.6 nM	>10,000	>10,000	>10,000
24	*p*-CF_3	3630 nM	Not Tested	Not Tested	Not Tested
25	*o*-CH_3	630 nM	10,000	>10,000	>10,000
26	*p*-CH_3	4400 nM	Not Tested	Not Tested	Not Tested
27	*o*-OCH_3	5890 nM	Not Tested	Not Tested	Not Tested
28	*m*-OCH_3	1620 nM	>10,000	>10,000	>10,000
29	*p*-OCH_3	290 nM	>10,000	>10,000	>10,000
30	*m*-CH_2CH_3	540 nM	>10,000	>10,000	>10,000
31	*m*-CN	210 nM	>10,000	>10,000	>10,000

of the molecule to displace $PGF_{2\alpha}$ from its receptor diminishes rapidly with the increasing aryl substituent size. The compound class containing a single fluoro substituent, (**19**, **20** and **21**) while active at all three positions on the aromatic ring, was slightly less potent at the *para* position where it also shows some undesirable hEP_1 activity. The other substituents show a divergent SAR; e.g. the *ortho* methyl is more potent than the *para*, while the *para*-methoxy is more potent than its *ortho* analogue. Finally, electronics may play a role, as the *m*-F (42 nM) is more potent than H (82 nM), but the *m*-CN at 210 nM is not. Thus the characteristics of the terminal ''pocket'' where the aromatic ring of the ligands fit plays a major role in determining their affinity.

Conclusions

In conclusion, it has been found that the double bonds present in the naturally-occurring prostaglandin $PGF_{2\alpha}$ are required for its nanomolar binding at the hFP receptor. Surprisingly, neither of these double bonds are necessary for similar potency levels in analogues wherein an aromatic ring has replaced the last four atoms of the prostaglandin skeleton. While compounds containing substituted aromatic rings were also found to be active at the hFP receptor, the SAR shows a relatively narrow scope for reasonable potency. The data further suggest an improvement in the compounds' selectivity is simultaneously achieved by removal of the unsaturation, especially toward the thromboxane (hTP) receptor. The SAR is not unambiguous, however, as both size and electronic interactions appear to be contributing to the final binding values. Finally, receptor modeling studies of the seven transmembrane domain region of the hFP receptor may help to rationalize this SAR. These data suggest that new FP-receptor selective analogues with a simplified skeleton can be found in a saturated series which might also show improved selectivity toward the hFP receptor over the other excitatory receptors.

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7. Compounds evaluated for functional activity in RAT-1 cells, transiently-transfected with a human prostanoid receptor (hFP, hEP_3, hEP_1, or hTP) having a constitutively-expressed β-galactosidase reporter gene stably-transfected therein. EC_{50} shown is after timed hydrolysis of galactosidase pseudsubstrate. For details: Messier, T.; Dorman, C.M.; Brauner, H.; Eubanks, D.; Brann, M. R. *Pharmacol. Toxicol.* **1995**, *76*, 308.

8. **Binding assays**: Compounds evaluated for their ability to displace [^{3}H]$PGF_{2\alpha}$ (hFP receptor assay) [^{3}H]PGE_2 (hEP_1; EP_3) [^{3}H]U46619 (hTP) in membrane preparations isolated from CHO-KI cells stably transfected with the receptor. The radiolabel (5 nM) membrane and test compound were incubated together 1h; plates harvested and DPM's counted.

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