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Discovery of diphenylcarbamate derivatives as highly potent and selective IP receptor agonists: Orally active prostacyclin mimetics. Part 3

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Abstract—The new classes of diphenylcarbamate derivatives with a tetrahydronaphthalene skeleton as highly potent and selective IP agonists have been discovered. The optimized diphenylcarbamate type compound FK-788: (*R*)-**4** exhibited potent antiaggregative potency with an IC_{50} of 18 nM and high binding affinity for the human recombinant IP receptor with K_i values of 20 nM and selectivity for human IP over all other members of the human prostanoid receptor family. Compound (*R*)-**4** was shown to exhibit good pharmacokinetic properties in rats and dogs, and also good bioavailability in healthy volunteers.
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1. Introduction

Prostanoid receptors are members of the G-protein coupled receptor superfamily. Recently, eight prostanoid receptors have been cloned and characterized.[1] PGE_2 will bind preferentially to the EP_1, EP_2, EP_3, and EP_4 receptors, PGD_2 to the DP receptor, $PGF_{2\alpha}$ to the FR receptor, PGI_2 to the IP receptor and TXA_2 to the TP receptor. The molecular characterization of these receptors has resulted in renewed interest in the field because the selectivity of compounds to human prostanoid receptors can now be determined.[2] However, success has been limited by the problems of identifying suitably selective ligands. Many of the problems encountered have been due to the existence of multiple prostanoid receptor subtypes and the lack of the selectivity of prostaglandin analogs.

Prostacyclin (PGI_2) is primarily derived from the vascular endothelium and plays an extremely important inhibitory role in platelet aggregation and as a vasodilator in maintaining homeostatic circulation.[3] Some groups have already disclosed novel PGI_2 analogs without a PG skeleton, which led us to create a novel PGI_2 mimetic with improved chemical and metabolic stability.[4] Hamanaka and co-workers disclosed AP-227 (**3**), which has a tetrahydronaphthalene scaffold with a benzhydrylimine group instead of the natural PG structure.[5] We have previously disclosed the new diphenyloxazole derivatives FR181157 (**1**) and FR193262 (**2**) as potent and orally active prostacyclin mimetics (Fig. 1). We explored a second structural series with improved potency and selectivity over all the other human PG receptors, and good pharmacokinetic profiles. Optimization of the benzhydrylimine moiety of **3** rapidly led to the identification of the potent diphenylcarbamate compound **11** in Figure 2. Despite high potency for the human IP receptor, compound **11** exhibited a lack of selectivity for the EP_3 receptor and poor PK properties, hindering further pharmacological evaluation. After extensive research, we selected FK-788: (*R*)-**4** as a potential candidate, since it exhibited highly potent and selective IP receptor activity and improved solubility and bioavailability. In this paper, we report the SAR of a series of diphenylcarbamate derivatives and the biological and pharmacokinetic profile of (*R*)-**4**.

Keywords: Diphenylcarbamate; Prostacyclin mimetic; IP receptor.
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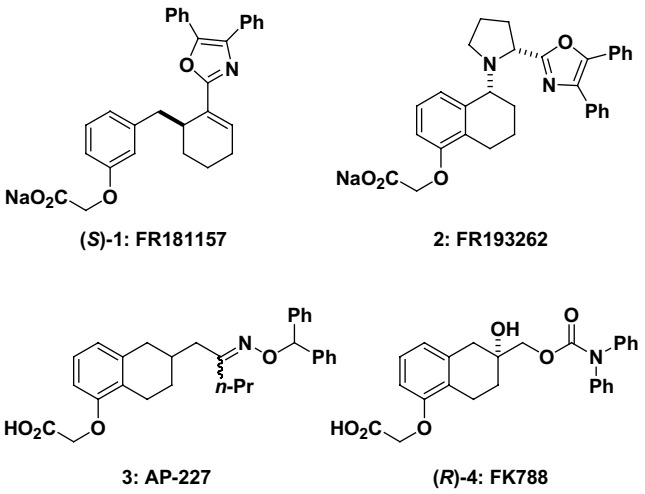


Figure 1.

2. Chemistry

The racemic diphenylcarbamate derivatives were synthesized by the routes shown in Scheme 1. Commercially available 5-hydroxytetralone **5** was converted in four steps to α,β-unsaturated ester **8**. Reduction of **8** with DIBAL in toluene, followed by acylation with diphenylcarbamoyl chloride in pyridine gave the desired carbamate **9**. Deprotection of phenol with tetrabutylammonium fluoride followed by *O*-alkylation with ethyl bromoacetate and hydrolysis afforded the phenoxyacetic acid **10**. Catalytic hydrogenation of **10** with Pd/C gave the corresponding unsaturated carbamate **11**. Similarly the α-alcohol **7** was transformed into α-alcohol carbamate derivatives, which were resolved into the two diastereoisomers (cis and trans at alcohol with a ratio 5:2) through chromatographic separation and followed by the same conditions to give *cis*-**14** and *trans*-**15** as racemates.

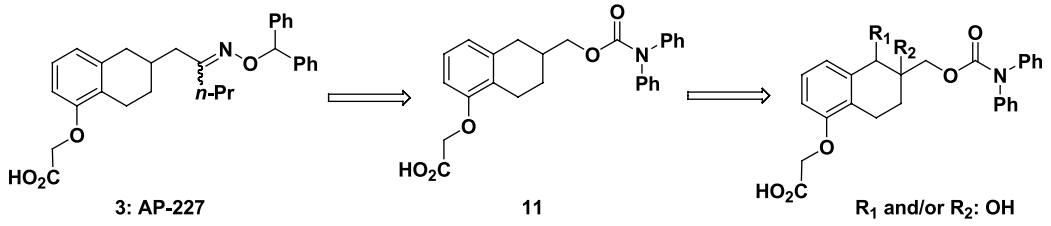
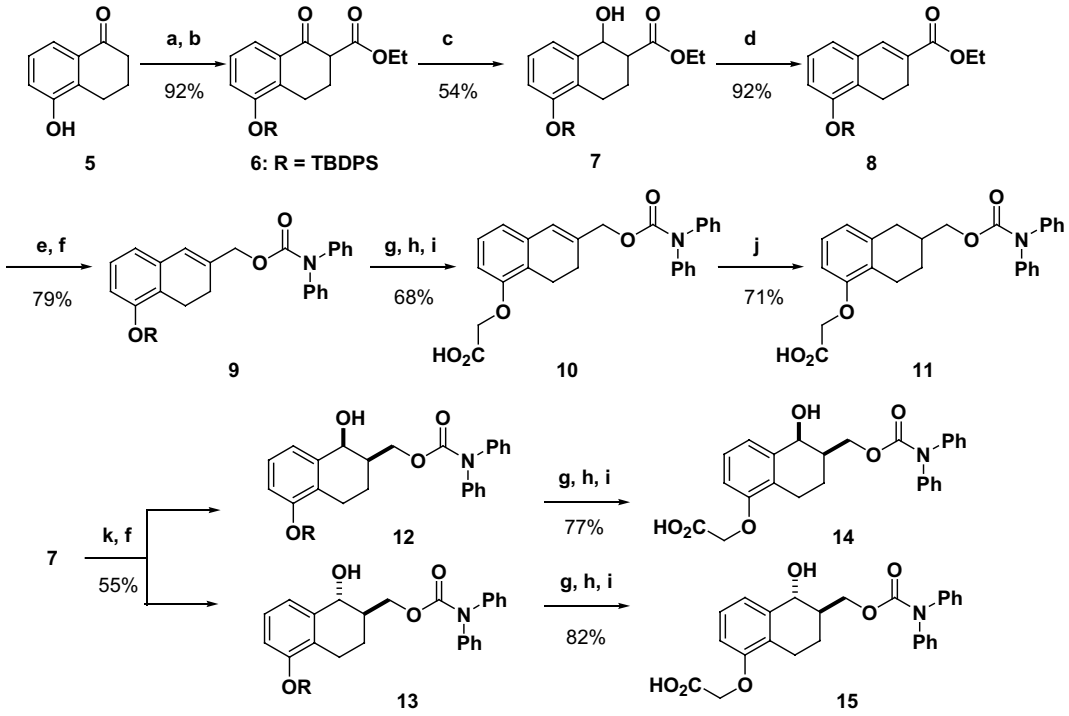
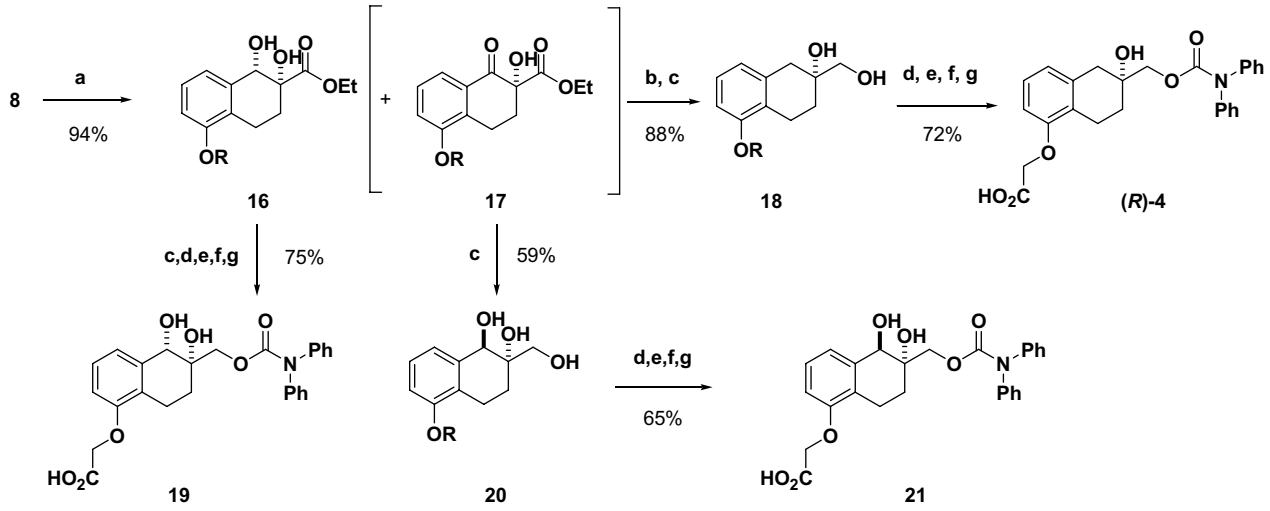


Figure 2.



Scheme 1. Reagents: (a) TBDPSiCl, imidazole, DMF; (b) $(EtO)_2CO$, NaH, Tol; (c) $NaBH_4$, EtOH–THF; (d) $KHSO_4$, Tol; (e) DIBAL-H, Tol; (f) $ClCON(Ph)_2$, Py; (g) *t*-BuNF, THF (h) ethyl bromoacetate, K_2CO_3, DMF; (i) NaOH, THF–EtOH; (j) Pd/C, AcOEt–EtOH; (k) $LiAlH_4$, THF.



Scheme 2. Reagents: (a) AD-mix-α, *t*-BuOH–H_2O; (b) PPh_3–CBr_4, CH_2Cl_2; (c) $LAlH_4$, THF; (d) $ClCON(Ph)_2$, Py; (e) *t*-BuNF, THF; (f) ethyl bromoacetate, K_2CO_3, DMF; (g) NaOH, THF–EtOH.

Scheme 2 shows asymmetric synthesis of diphenylcarbamate derivatives. The required optically active hydroxyl was obtained via the Sharpless AD reaction. Asymmetric dihydroxylation of **8** by the standard procedure[6] with 1 mol % of AD-mix-α provided the diol **16** in 82% yield and 92% ee with the ketone derivative **17** in 12% yield and 91% ee. The *cis*-diol **16** was converted to α-bromo intermediate by CBr_4 and PPh_3, followed by reduction with $LiAH_4$ to give the diol **18**. The diol **18** was smoothly converted to the desired β-hydroxy carbamate (*R*)-**4** using the same procedure. The optical pure product was obtained by crystallization of ethyl ester of **4** with ethanol–ether (>98% ee as determined by HPLC analysis with a chiral column: Chiralcel AD).[7,8] The (*S*)-enantiomer was prepared by the same method using AD-mix-β. The *cis*-diol derivative **19** was also prepared from the optically active *cis*-diol **16**. Reduction of the optically active ketone **17** by $LiAH_4$ stereoselectively produced the triol **20**, followed by the same condition to give the optically active *trans*-diol **21**.

3. Biological activity

Compound (*R*)-**4** displayed potent PGI_2 agonist activity and especially excellent selectivity over all other human PG receptors. Table 1 shows the in vitro SAR results for the diphenylcarbamate derivatives. PGI_2 receptor binding was examined by the conventional ligand binding assay based on the displacement of [^{3}H]-Iloprost from the cloned human IP receptor.[9] IC_{50} values in the functional assay were obtained by measuring inhibition of ADP-induced platelet aggregation using human, rat, dog, and monkey platelet rich plasma. Compound (*R*)-**4** exhibited high binding affinity for the IP receptor with a K_i of 20 nM and good antiaggregative potency with an IC_{50} of 18 nM. The enantiomer (*S*)-**4** was 15-fold less potent, and the *trans* α-hydroxy derivative **15** and the cis derivative **14** showed decreased potency, with IC_{50} values of 3200 and 170 nM, respectively, under the same conditions. Twofold improvement in activity also occurred by addition of *trans*-dihydroxy **21** at the α-and β-position of the tetrahydronaphthalene, while the addition of *cis*-dihydroxy **19** was 70-fold less active than (*R*)-**4**. The species difference suggested that inhibition of ADP-induced platelet aggregation using rat and dog platelet rich plasma was less potent than human (rat: IC_{50} = 1400 nM, dog: IC_{50} = 480 nM, monkey: IC_{50} = 13 nM). The newly designed (*R*)-**4** showed good affinity for the IP receptor and excellent selectivity over other human prostanoid receptors (receptor selectivity > 400) in Table 2.[1] Recent findings indicated that prostaglandin coupled with Gs may be responsible for the induction of HGF.[10] Figure 3 shows the effect of (*R*)-**4** on HGF production in cultured human dermal fibroblasts. Compound (*R*)-**4** increased HGF production in a concentration-dependent manner and apparent increases were observed at concentration of 1 nM or more. On the other hand, the launched Beraprost with PG skeleton increased HGF at the concentration of 3 nM (data not shown).[11,12]

Table 1. SAR of the diphenylcarbamate derivatives[a]

Compound	Function assay: IC_{50} (nM)			
	Human	Rat	Dog	Monkey
(*R*)-**4**: FK788	18 ± 0.6	1400 ± 600	480 ± 41	13 ± 0.3
(*S*)-**4**	270 ± 54	>10,000		
10	91 ± 29	>10,000		
11	53 ± 6.3	>10,000		
14	170	>10,000		
15	3200	>10,000		
19	1300	>10,000		
21	5.8	7400		
3: AP-227	150 ± 17	5600 ± 470		
Iloprost	2.5 ± 0.14			

[a] Results are the average of two or three experiments.

Table 3 shows the pharmacokinetic profiles in rats and dogs. Compound (*R*)-**4** without a PG skeleton displayed good oral bioavailability (rat: *F* = 38%, dog: *F* = 98%) in

Table 2. Affinity of (*R*)-**4** and derivatives to eight prostanoid receptors

Compound	Human K_i[a] (nM)							
	IP	DP	FP	TP	EP_1	EP_2	EP_3	EP_4
(*R*)-**4**	8.9 ± 0.53	2300	>10,000	>10,000	9600	>10,000	8200	>10,000
(*S*)-**1**	54 ± 0.36	>1000	>1000	>1000	>1000	>1000	6800	1020
2	12 ± 0.25	>1000	>1000	>1000	>1000	>1000	>10,000	>10,000
11	140	NT	NT	NT	>1000	>1000	1900	>10,000
21	110	NT	NT	NT	>1000	>1000	>10,000	>10,000

[a] K_i determination are averages of two or three experiments. Competitive binding assay based on the displacement of [^{3}H]-Iloprost for human IP receptor, [^{3}H]-PGD_2 for human DP receptors, [^{3}H]-PGF_2 for human FP receptors, [^{3}H]-SQ29548 for human TP receptor, and [^{3}H]-PGE_2 for human EP_{1-4} receptors.

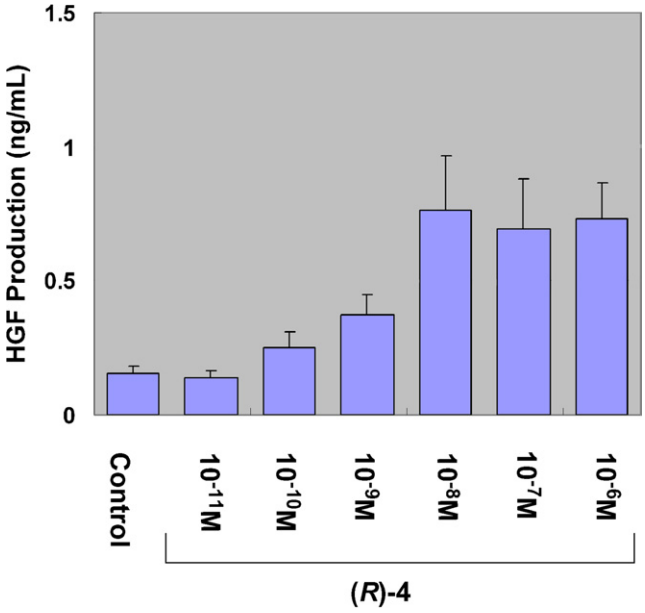


Figure 3. Effect of (*R*)-**4** on HGF production in human dermal fibroblasts.

both species. Furthermore, clinical studies of (*R*)-**4** (code number FK788) have shown excellent bioavailability in healthy volunteers with over 50% BA.

4. Conclusions

We designed and synthesized a series of diphenylcarbamate derivatives with a tetrahyronaphthalene skeleton as prostacyclin mimetics. The clinical candidate (*R*)-**4** with an optically active β-hydroxy group exhibited highly potent and selective IP receptor activity and good pharmacokinetic properties, especially, in healthy volunteers. Compound (*R*)-**4** displays good potency in vivo in several hepatitis models in rats and in mice infected with the replication-deficient recombinant adenovirus.[13] The detailed pharmacological work of (*R*)-**4** will be published in the near future.

Acknowledgments

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Table 3. Pharmacokinetic profiles of (*R*)-**4**[a]

	po (fasted) *n* = 3			iv *n* = 3		Ba (%)
	Dose (mg/kg)	C_{max} (ng/mL)	AUC (ng/mL h)	$t_{1/2}\beta$ (h)	CL_{tot} (mL/min/kg)	
Rat	10	342.4 ± 42.1	1594 ± 579	1.81 ± 0.43	37.95 ± 3.51	38
Dog	0.32	305.2 ± 28.5	2199 ± 300	7.94 ± 1.86	2.31 ± 0.33	98

[a] Results are shown as the mean ± SE.

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7. The assignment of the absolute configuration of **4** was determined by X-ray structural analysis of a complex with (*S*)-1-(1-naphthyl)ethylamine. This compound should be handled with extreme caution because of PGI_2 agonist activity and high skin permeability.
8. HPLC-conditions (Chiralcel AD, flow rate 1.0 mL/min, 1.5% EtOH/hexane) t_R = 14.8 min for ethyl ester of (*R*)-**4** and t_R = 13.2 min for ethyl ester of (*S*)-**4**.
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