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Replacing the cyclohexene-linker of FR181157 leading to novel IP receptor agonists: Orally active prostacyclin mimetics. Part 6

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Abstract—The synthesis and biological activity of novel derivatives of our previously reported IP receptor agonist FR181157 is described. SAR studies to replace the cyclohexene-linker of FR181157 led to the discovery of compound **1i** (FR207845) as a potent non-prostanoid PGI_2 mimetic with good oral bioavailability.

Prostacyclin (PGI_2) primarily derived from vascular endothelium is one of the metabolites of arachidonic acid and has an important role as an inhibitor of platelet aggregation and as a potent vasodilator.[1] Although these pharmacological properties are considered to be clinically useful, the therapeutic application of PGI_2 is limited due to its inherent instability.[1,2] Since the report that octimibate, known as an inhibitor of acyl-CoA:cholesterol *O*-acyltransferase (ACAT), acts as a non-prostanoid IP receptor agonist appeared in 1990,[3] intensive studies to identify new non-prostanoid PGI_2 mimetics with chemical and metabolic stability have been performed.[4–7] These investigations led us to a research program directed at the development of a new class of prostacyclin analogues, and we have investigated and reported several novel series of non-prostanoid PGI_2 mimetic.[8] From these series, FR181157 which has a cyclohexene core structure with a chiral center was identified as a potent orally active non-prostanoid IP receptor agonist (Fig. 1).[8b] However, a metabolism study revealed the existence of epoxides as active metabolites exhibiting 5- to 10-fold more potent human platelet aggregation inhibitory activity than the parent compound, and in a rat hepatic injury model FR181157 showed unpredictable potent activity derived from its complicated metabolism.[8e] Although the stability and toxicity of epoxides of FR181157 have not been fully investigated, in general, some kinds of epoxides are reactive with biogenic substances and sometimes induce unpredictable toxicological effects such as hepatotoxicity and genotoxicity.[9] Therefore, we aimed to investigate new linker structures without a double bond to prevent production of epoxides as active metabolites with likely reactivity. In this letter, we wish to disclose the synthesis and biological activities of FR181157-related derivatives prepared by replacing the cyclohexene-linker as novel PGI_2 mimetics.

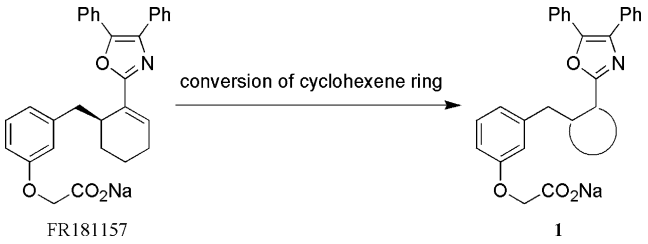

Figure 1. Conversion of the cyclic linker of FR181157.

The synthetic route to compounds **1a** and **1c–i** newly prepared in this letter is illustrated in Schemes 1–4.

Keywords: Prostacyclin; PGI_2; IP receptor; Agonist.
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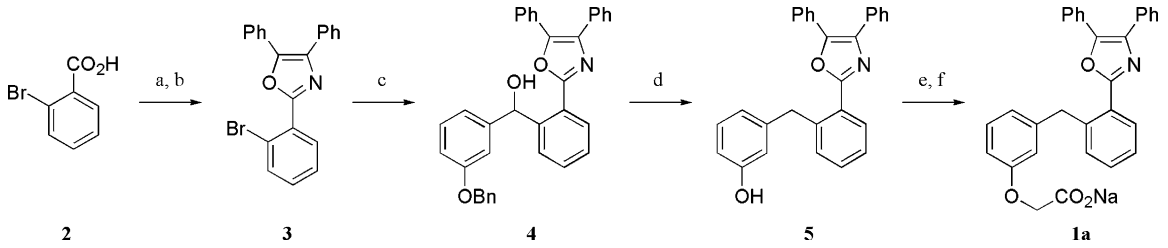
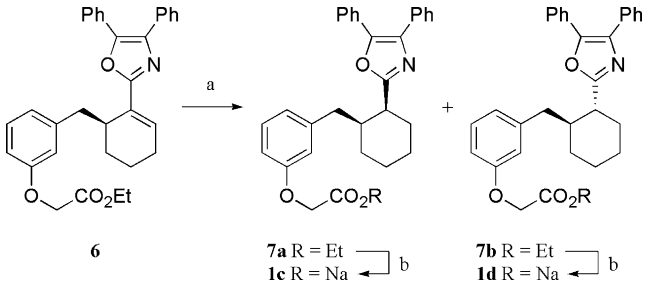
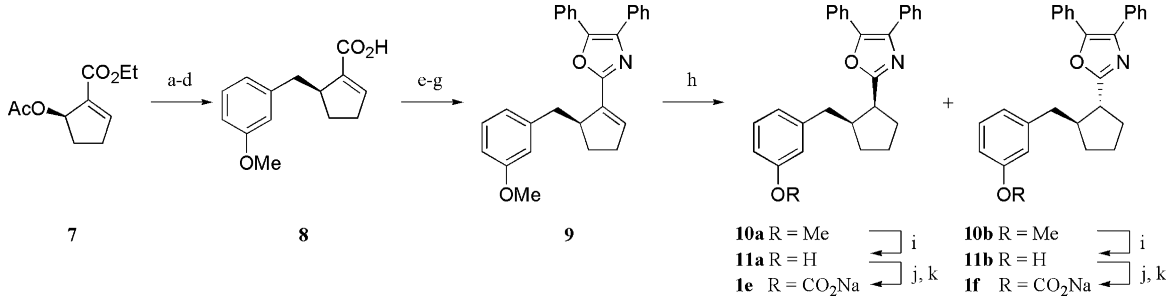


Scheme 1. Reagents: (a) benzoin, DMAP, EDC, CH_2Cl_2 (61%); (b) $AcONH_4$, AcOH (78%); (c) i—Mg, THF; ii—3-benzyloxybenzaldehyde (two steps 54%); (d) H_2, 10% Pd/C, EtOAc, MeOH, HCl (28%); (e) methyl bromoacetate, K_2CO_3, DMF (98%); (f) 1 N NaOH, DME (90%).

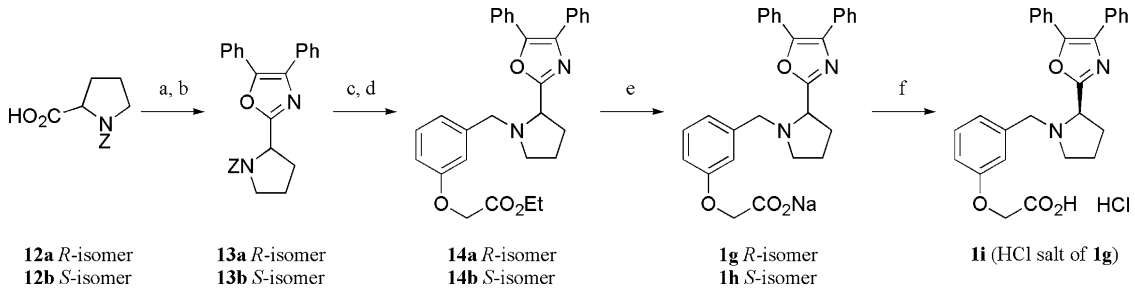


Scheme 2. Reagents: (a) i—H_2, 10% Pd/C, EtOAc; ii—SiO_2 column separation (43% for **7a**; 38% for **7b**); (b) 1 N NaOH, THF, EtOH (87% for **1c**; 72% for **1d**).

Compound **1b** was already reported in our previous paper.[8b] Compound **1a** having a benzene-linker was prepared from 2-bromobenzoic acid by construction of the diphenyloxazole ring, metalation, treatment with a benzaldehyde derivative, deprotection–dehydroxylation, and introduction of an ethyl acetate moiety, followed by hydrolysis (Scheme 1). Synthesis of **1c–d** with a cyclohexane-linker was accomplished by hydrogenation of optically active ester **6**,[8b] an intermediate for the synthesis of FR181157, followed by separation of the isomers and hydrolysis (Scheme 2). Optically active cyclopentane compounds **1e–f** were prepared from optically pure acetate **7**[10] as shown in Scheme 3. Treatment of **7** with 3-methoxybenzyl Grignard reagent in the presence of CuI was accompanied by a reduction in optical purity, and was followed by hydrolysis, co-recrystallization with (+)-α-phenylethylamine from Et_2O to enhance the optical purity, and treatment with 1 N HCl gave carboxylic acid **8** whose optical purity was determined to be >99% ee after transformation to a diphenyloxazole derivative **9**. Subsequent hydrogenation, separation of the isomers, demethylation, and introduction of an ester moiety,



Scheme 3. Reagents: (a) 3-methoxybenzylchloride, Mg, CuI, THF (quant., 77% ee); (b) 1 N NaOH, EtOH, dioxane (71%); (c) i—(+)-α-phenylethylamine, Et_2O; ii—recrystallization from EtOAc–hexane (2×) (67%); (d) 1 N HCl, EtOAc (quant.); (e) $SOCl_2$, CH_2Cl_2; (f) benzoin, pyridine, CH_2Cl_2; (g) $AcONH_4$, AcOH (three steps 80%, >99% ee); (h) i—H_2, 10% Pd/C, EtOH; ii—SiO_2 column separation (50% for **10a**; 42% for **10b**); (i) BBr_3, CH_2Cl_2; (j) ethyl bromoacetate, K_2CO_3, DMF (two steps 85% from **10a**; 85% for **10b**); (k) 1 N NaOH, EtOH (86% for **1e**; 86% for **1f**).



Scheme 4. Reagents: (a) benzoin, DMAP, EDC, CH_2Cl_2 (quant. from **12a**; 96% from **12b**); (b) $AcONH_4$, AcOH (80% for **13a**; 94% for **13b**); (c) H_2, 10% Pd/C, MeOH (98% from **13a**; 98% from **13b**); (d) ethyl 3-(bromomethyl)phenoxyacetate, K_2CO_3, DMF (95% for **14a**; 44% for **14b**); (e) 1 N NaOH, EtOH (96% for **1g**; 87% for **1h**); (f) i—neutralized with 1 N HCl, then 4 N HCl in EtOAc, Et_2O; ii—recrystallization from MeOH–Et_2O (69% from **1g**).

followed by hydrolysis, provided the target molecules. Optically active **1g** and **1h** were prepared from *Z*-protected D- or L-proline (**12**), respectively. Construction of the diphenyloxazole ring, deprotection, alkylation with benzylbromide derivative, followed by hydrolysis, afforded the object compounds.

The compounds prepared were evaluated for their ability to inhibit aggregation of ADP-induced human and rat platelets in platelet-rich plasma as PGI_2 receptor agonistic activity, and is expressed in Table 1 as the nanomolar concentration of a compound required to inhibit 50% of the aggregation (IC_{50}).

Replacement of the cyclohexene-linker of FR181157 by benzene to delete an isolated double bond and simultaneously a chiral center resulted in compound **1a** with reduced activity. In our previous paper,[8b] compound **1b** was reported to show a similar tendency. Therefore, it was considered that the sp^2 carbon atom, to which the left part benzyl moiety is attached, may cause an unfavorable effect on the interaction of agonists with the receptor. To investigate the importance of the double bond in cyclohexene of FR181157, we synthesized and evaluated cyclohexane analogues **1c–d**. These were only twofold less active than the parent compound, and no effect of cis/trans stereochemistry on the human platelet aggregation inhibitory activity was observed. The retained activity of these two compounds led us to investigation of ring size, and accordingly, cyclopentane analogues **1e** and **1f** were prepared and exhibited slightly more potent activity than FR181157. Looking at the lipophilicity, the Clog*P* value of natural prostacyclin is 2.33.[11] On the other hand, that of FR181157 calculated as a free form is quite high (6.88), and such a high lipophilic compound does not meet Lipinski's 'rule of five,' a well-known method to predict drug-likeness.[12] From this point of view, cyclopentane analogues **1e** and **1f** are still lipophilic, and besides have two chiral centers requiring a more complicated synthetic route than FR181157. Therefore, we planned to introduce a nitrogen atom as a functionality to reduce the lipophilicity and simultaneously delete one of two chiral centers. In general, proline is a well-known useful chiral synthon, because it is relatively inexpensive and both D- and L-enantiomers with high optical purity are easily available. Thus, both enantiomers **1g** and **1h** were readily synthesized from D- and L-proline and were assessed. As a result, **1h** showed potent activity comparable to FR181157, and **1g** was slightly more potent. The stereochemistry of these two compounds gave a great effect on rat platelet aggregation. Compound **1g** retained the activity, however, its enantiomer **1h** resulted in complete loss of activity for rat platelets. The species difference of **1g** (15-fold) was smaller than FR181157 (20-fold), and also much smaller than previously reported our another type of diphenylcarbamate compound FK-788 (80-fold).[8d] It is well known that this class of PGI_2 mimetics has a species difference.[3b,8c–e] Based on the results discussed above, compound **1g**, easily synthesized from D-proline, with a relatively small species difference and lower Clog*P* (4.85, calculated as a free form), was selected for further evaluation.

Table 1. Biological activity of prepared compounds

Compound	Cyclic linker	ADP-induced platelets aggregation inhibitory activity[a] IC_{50} (nM)	
		Human[b]	Rat[c]
1a		930	ND
1b		533	ND
1c		115	ND
1d		114	ND
1e		53	ND
1f		39	ND
1g		48	700
1h		58	>10000
FR181157		60	1200

ND denotes not determined.
[a] Values are the average of two experiments.
[b] Evaluated at a concentration of 2.5 μM ADP.
[c] Evaluated at a concentration of 2.0 μM ADP.

PGI_2 receptor binding was examined by the conventional ligand binding assay based on the displacement of [^{3}H]-iloprost from the cloned human PGI_2 receptor (IP).[13] **1g** exhibited high binding affinity for the IP receptor with a K_i value of 76 nM, and was comparable to that of 60 nM shown for FR181157. A pharmacokinetic (PK) study with **1i** (a crystalline hydrochloride salt of **1g**)[14] in fasted rats ($n = 3$) revealed its good oral bioavailability ($F = 41\%$) which is comparable to that of FR181157 ($F = 50\%$), although C_{max} and AUC were relatively lower (**1i**: $C_{max} = 15.5 \pm 1.3$ ng/mL, AUC = 81.7 ± 1.8 ng h/mL at 1.0 mg/kg po; FR181157: $C_{max} = 16.4 \pm 0.88$ ng/mL, AUC = 147.0 ± 14.3 ng h/mL at 0.32 mg/kg po).

In summary, we have prepared novel analogues of FR181157 replacing the cyclohexene-linker and assessed them as IP receptor agonists. Amongst them, the unique zwitter compound **1g** with the large benefit of synthetic

accessibility, relatively small species difference, and lower C log *P* was identified as a potent non-prostanoid PGI_2 mimetic designed not to produce reactive metabolites such as epoxide. A PK study of **1i** (FR207845), a hydrochloride salt of **1g**, revealed its good oral bioavailability in rats.

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14. Physical data for FR207845 (**1i**): mp 119–121 °C; $[\alpha]_D^{22}$ +9.2° (*c* 0.93, MeOH); ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.00–2.30 (2H, m), 2.30–2.70 (2H, m), 3.30–3.70 (2H, m), 4.40–4.70 (4H, m), 4.85–5.10 (1H, m), 6.80–7.70 (14H, m), 11.34 (1H, br), 13.01 (1H, br); IR (KBr) 2952, 2536, 1720, 1597, 1498, 1448, 1387 cm^{-1}; APCI-MS *m/z* 455 $(M+H-HCl)^+$. Anal. Calcd for $C_{28}H_{27}ClN_2O_4$: C, 68.50; H, 5.54; N, 5.71. Found: C, 68.14; H, 5.43; N, 5.86.