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Bioorganic & Medicinal Chemistry Letters 15 (2005) 3284–3287
Bioorganic & Medicinal Chemistry Letters

Metabolism investigation leading to novel drug design: Orally active prostacyclin mimetics. Part 4

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Received 18 February 2005; revised 11 April 2005; accepted 22 April 2005

Abstract—A metabolism study of FR181157 (**1**) led to the discovery of new oxazole derivatives as active metabolites. The metabolite **6** with an epoxy ring exhibited high anti-aggregative potency with an IC_{50} of 5.8 nM and potent binding affinity for the human recombinant IP receptor with a K_i value of 6.1 nM and selectivity for human IP receptor over all other members of the human prostanoid receptor family.

1. Introduction

In the previous article, we described the design, synthesis, and pharmacology of a new diphenyloxazole derivative with a cyclohexene ring FR181175 (**1**) as a PGI_2 agonist.[1] Tables 1 and 2 show the profiles of **1** and the related compound **2**, which has the cyclopentene ring instead of the cyclohexene ring. **1** and **2** had similar potency and affinity for IP receptors in vitro; however, **1** had 30-fold more potent in vivo activity, such as in the D-GalN/LPS-induced rat hepatic injury model.[2] Compound **2** exhibited 3-fold improved PK properties over **1**, particularly bioavailability and duration time in rats. These results suggested that active metabolites of **1** may exist with resultant improvement of in vivo potency. After exploring the metabolites of **1** using rat liver microsomes, we found the active metabolite **M-4**, which exhibited 10-fold improved potency for the IP receptor. Herein, we describe a stereoselective synthesis and the biological activity of the active metabolite **M-4**.

2. Metabolism study

The time course of metabolite formation for **1** in rat liver microsomes is shown in Figure 1.[3] When **1** (10 μM) was incubated with rat liver microsomes (1 mg protein/ml), four metabolites were formed and named, **M-1**, **M-2**, **M-3**, and **M-4**, according to the elution order on HPLC. **M-1**, **M-2**, and **M-4** were isolated by HPLC, PGI_2 agonist activities of these metabolites were determined. **M-1** and **M-2** were slightly less potent than the parent compound; however, **M-4** exhibited dramatically improved activity toward inhibition of ADP-induced aggregation in human platelets. The chemical structures of these metabolites were determined by LC/MS/MS analysis and are shown in Scheme 1. Based on the LC/MS analysis, **M-1**, **M-2**, and **M-4** had an $[M+H]^+$ ion at *m/z* 482, 16 Da larger than that of **1**, and the fragmentation patterns of these metabolites suggested that the addition of an oxygen atom had occurred on the cyclohexene moiety. The identification of **M-1**, **M-2**, and **M-4** was confirmed by comparing their MS/MS spectra and HPLC retention times with those of the corresponding synthetic authentic compounds. We also explored the metabolite of **2** and identified the metabolites with similar structures. The re-synthesized epoxide derivatives did not exhibit

Keywords: Metabolism; Prostacyclin mimetic; IP receptor.
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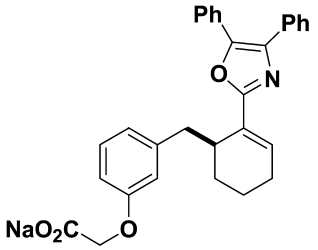
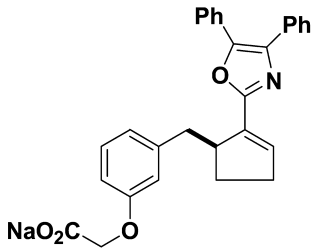
doi:10.1016/j.bmcl.2005.04.076

Table 1. Pharmacological profiles of **1** and **2**



(*S*)-1: FR181157

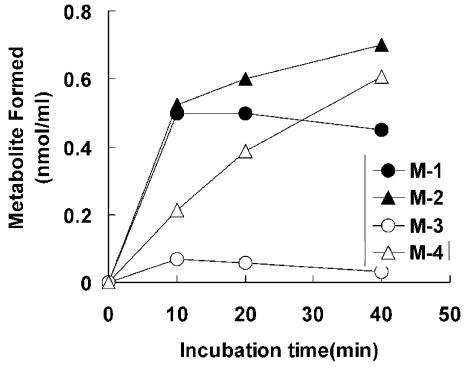


(S)-2: FR181560

	In vitro			In vivo
	Function assay: IC_{50} (nM)		Binding assay: K_i (nM)	Effective dose in rat hepatic injury model (mg/kg, po)
	Human	Rat	Human	
(*S*)-**1**	60 ± 5.2	1200 ± 170	54 ± 0.36	0.032
(*S*)-**2**	56 ± 1.9	2800 ± 40	41 ± 0.24	1.0

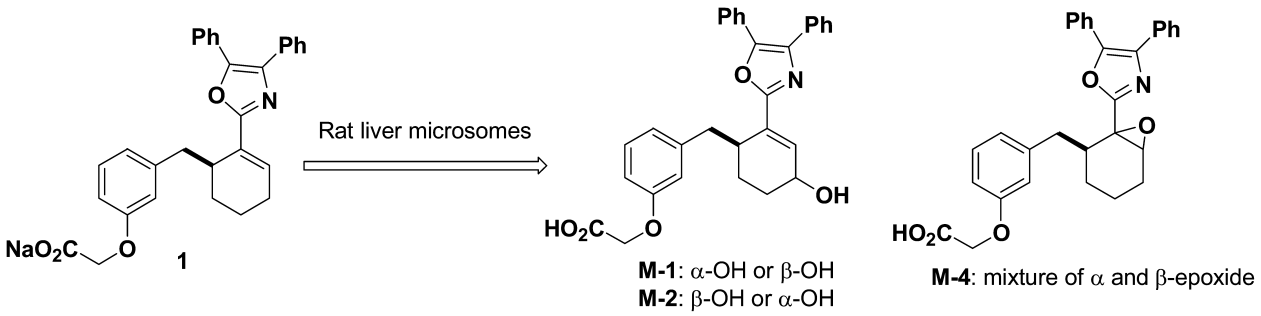
Table 2. Pharmacokinetic profiles of **1** and **2** in rats

	po (fasted), $n = 3$			iv, $n = 3$		Ba (%)
	Dose (mg/kg)	C_{max} (ng/ml)	AUC (ng h/ml)	$t_{1/2}\beta$ (h)	CL_{tot} (ml/min/kg)	
(*S*)-**1**	0.32	16.4 ± 0.88	147 ± 14.3	6.6 ± 0.33	17.7 ± 0.33	50
(*S*)-**2**	0.32	45.2 ± 29.6	425 ± 45.7	17 ± 3.3	9.19 ± 0.16	78

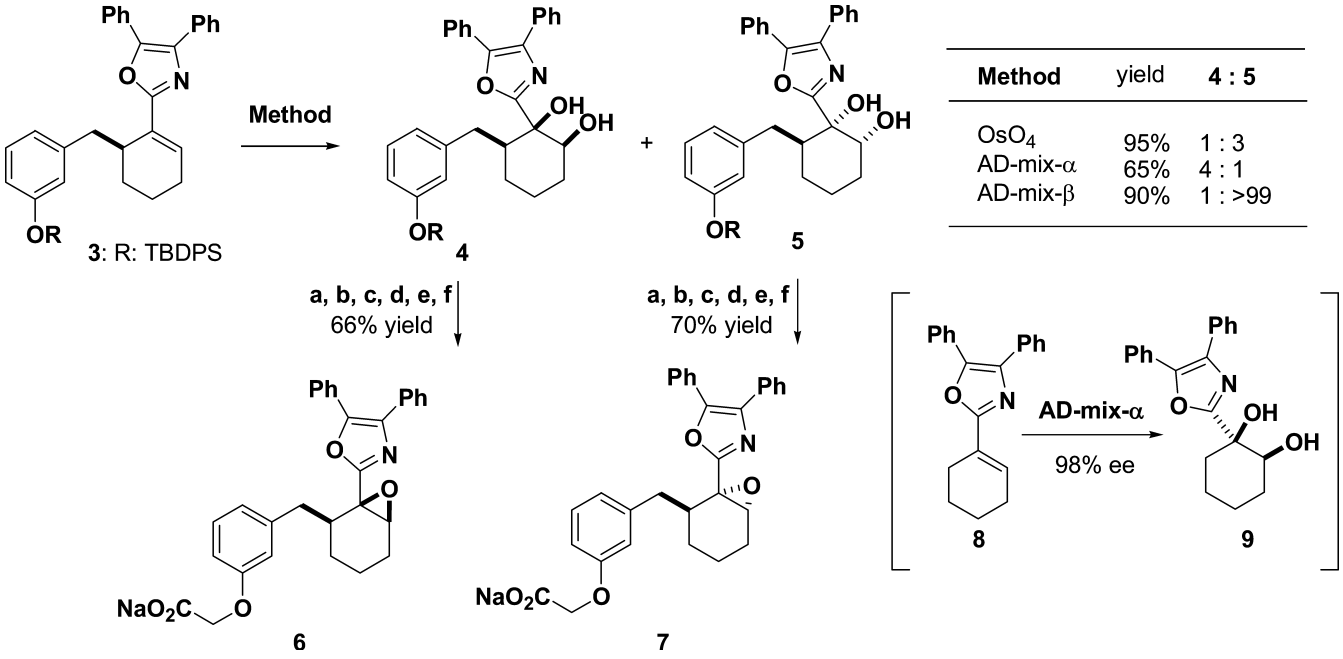


Inhibition of ADP-induced aggregation of human platelets:	IC_{50} (nM)
1	60
M-1	98
M-2	116
M-4	14

Figure 1. Rat microsome-induced metabolite formation from **1**.



Scheme 1.



Scheme 2. Reagents: (a) $HC(OMe)_3$, cat. *p*-TsOH, CH_2Cl_2; (b) AcBr, CH_2Cl_2; (c) K_2CO_3, MeOH; (d) TBAF, THF, (e) ethyl bromoacetate, K_2CO_3, DMF; (f) NaOH, EtOH.

improved PGI_2 potency of inhibition of ADP-induced aggregation using human and rat platelets.

3. Chemistry

The synthetic strategy for the optically active epoxide is illustrated in Scheme 2. We planned to prepare these compounds by controlling the stereochemistry at the epoxy part on the cyclohexene ring. The required optically active epoxides were obtained via dihydroxylation of the olefin **3** with the Sharpless AD reagent and OsO_4.[4] The optical active intermediate **3** was produced by the previously reported asymmetric synthesis.[1] Asymmetric dihydroxylation of **3** by OsO_4 provided the mixture of the diol **4** and **5** with a 1:3 ratio in 95% yield. Under the standard conditions with 1 mol% of AD-mix-β, with a better matching pair for the substrate, the enantiomerically pure diol **5** was obtained in 90% yield. On the other hand, under the conditions with AD-mix-α with mismatching pair, the diols **4** and **5** with 4:1 a ratio were obtained in 65% yield. The previous work indicated that AD-mix-α is effectively covering the α-face of the cyclohexene **8** and selective approach from the β-face of **8** should result.[1] After separation of **4** and **5** by column chromatography, the diols were smoothly converted to the epoxide using the method involving a cyclic acetoxonium intermediate,[5] followed by the standard method to give the corresponding products **6** and **7**, in 66% and 70% yield, respectively.

4. Biological activity

The metabolite **6** possesses potent PGI_2 agonist activity and especially excellent selectivity over all other human PG receptors. Table 3 shows the results of the functional assay by measuring the inhibition of ADP-induced platelet aggregation using human, rat, dog, and monkey platelet-rich plasma. The epoxide **6** was 10-fold more potent than mother compound **1** in human and 4-fold more potent in rat. The diastereomer **7** was 5-fold more

Table 3. Function assay of the epoxide derivatives[a]

Compound	Function assay: IC_{50} (nM)			
	Human	Rat	Dog	Monkey
6	5.8 ± 1.2	300 ± 28	1470 ± 376	12 ± 1.3
7	12	NT	NT	NT
1	60 ± 5.2	1200 ± 170	1300 ± 180	188 ± 11

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

Table 4. Affinity of **6** and **1** to eight prostanoid receptors

Compound	Human K_i (nM)[a]							
	IP	DP	FP	TP	EP_1	EP_2	EP_3	EP_4
6	6.1 ± 0.11	2900	>10,000	930	>10,000	1400	3200	7200
1	54 ± 0.36	>1000	>1,000	>1000	>1,000	>1000	6800	1020

[a] K_i determination are averages of at 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.

Table 5. Pharmacokinetic profile of **6**[a]

	po (fasted), $n = 3$			iv, $n = 3$		Ba (%)
	Dose (mg/kg)	C_{max} (ng/ml)	AUC (ng h/ml)	$t_{1/2}\beta$ (h)	CL_{tot} (ml/min/kg)	
Rat	1	67.9 ± 10.9	168.2 ± 36.3	26.9 ± 3.2	18.6 ± 0.63	20

[a] Results are shown as means ± SE.

potent. The newly designed **6** showed excellent affinity for the IP receptor with $K_i = 6.1$ nM and excellent selectivity over other human prostanoid receptors (receptor selectivity >100) in Table 4.[6] **6** exhibited powerful potency in vivo in the D-GalN/LPS-induced rat hepatic injury model, comparable to **1**. Table 5 shows the pharmacokinetic profiles of **6**, which displayed moderate oral bioavailability in rat.

5. Conclusion

We have explored the metabolites of **1** using rat liver microsomes and identified the active metabolites **M-1**, **M-2**, and **M-4**. The potent metabolite **M-4** was a mixture of **6** and **7**, which were re-synthesized and evaluated. The isomer **6** exhibited the strongest potency for the IP receptor of all oxazole derivatives we had produced and also excellent selectivity over the other eight PG receptors. These results indicate that the active metabolite of **1** may contribute to enhancement of the potency in vivo. Furthermore, **6** emerged as a new candidate as an orally active prostacyclin mimetic with high IP receptor selectivity.

Acknowledgment

We express our thanks to Dr. David Barrett for his critical reading of the manuscript.

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3. In vitro metabolism: incubation mixtures (0.5 ml) contained rat liver microsomes (1 mg/ml), 10 μM **1**, 2 mM NADP, 10 mM glucose-6-phosphate, 5 mM $MgCl_2$, 0.5 U glucose-6-phosphate dehydrogenase, and 100 mM phosphate buffer (pH 7.4). After preincubation at 37 °C for 5 min, the reaction was started by addition of **1**. Incubations were carried out at 37 °C for 10–40 min and the reactions were stopped by adding 0.1 ml of 1 N HCl and diethyl ether. The mixtures were shaken for 10 min and centrifuged at 1700*g* for 5 min. The organic phase (3 ml) was removed and evaporated to dryness. The residue was dissolved in 100 μl methanol and analyzed by HPLC. For the purpose of isolation and purification of metabolites, large-scale incubation of **1** (200 μM; 110 ml incubation) was carried out with rat liver microsomes (2 mg/ml) for 40 min. The diethyl ether extracts were evaporated to dryness and dissolved in methanol for isolation by HPLC.
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