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Metabolism investigation leading to novel drug design 2: Orally active prostacyclin mimetics. Part 5

Fujiko Takamura,[a] Akira Tanaka,[b] Hisashi Takasugi,[b] Kiyoshi Taniguchi,[b] Mie Nishio,[c] Jiro Seki[c] and Kouji Hattori[b,*]

[a]*Biopharmaceutical and Pharmacokinetic Research Laboratories, Fujisawa Pharmaceutical Co., Ltd, 2-1-6 Kashima, Yodogawa-ku, Osaka 532-8514, Japan*
[b]*Medicinal Chemistry Research Laboratories, Fujisawa Pharmaceutical Co., Ltd, 2-1-6 Kashima, Yodogawa-ku, Osaka 532-8514, Japan*
[c]*Medicinal Biology Research Laboratories, Fujisawa Pharmaceutical Co., Ltd, 2-1-6 Kashima, Yodogawa-ku, Osaka 532-8514, Japan*

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Abstract—A metabolism study of FK788 (**2**) led to the discovery of new diphenylcarbamoyl derivatives as prostacyclin mimetics without the PG skeleton. We designed and evaluated PGI_2 mimetics based on blocking the main metabolic pathway of FK788. The new compound **7c** was found to be equipotent to FK788 towards PGI_2 agonist activity and metabolically more stable than FK788.

In the previous paper, we have described the design, synthesis and pharmacology of a new diphenylcarbamoyl derivative with a tetrahydronaphthalene skeleton as a PGI_2 agonist.[1] FK788: **2** has been selected as a promising therapeutic candidate, and exhibited the same potency as prostacyclin analogues and also excellent selectivity over other human prostanoid receptors. After a single oral dosing of 12.5 μg/body **2** in healthy volunteers, the maximum plasma concentration (C_{max}), the area under the plasma concentration–time curve (AUC) and the elimination half-life ($t_{1/2}$) were 156.9 pg/mL, 327 pg h/mL and 1.5 h, respectively. Despite the observation that **2** was well absorbed from intestine (calculated bioavailability: 55%),[2] its elimination half-life was relatively short and insufficient for once daily dosing. Metabolic studies indicated there were three primary metabolic sites for **2**.[3] Accordingly, we set out to block these sites of metabolism in order to obtain more efficacious compounds. We disclose herein a series of tetrahydronaphthalene-based PGI_2 agonists with suitable pharmacokinetics properties for further development (Fig. 1).

The time courses of metabolite formation from **2** in rat and human liver microsomes are shown in Figure 2.[4] When **2** was incubated with rat and human liver microsomes, five metabolites were formed and named, M-1, M-2, M-3, M-4 and M-5, according to the elution order on HPLC. M-2 was the main metabolite in both rat and human liver microsomes, as shown in Figure 2. The chemical structures of M-2, M-4 and M-5 were confirmed by comparing their MS/MS spectra and HPLC retention times with those of the corresponding authentic

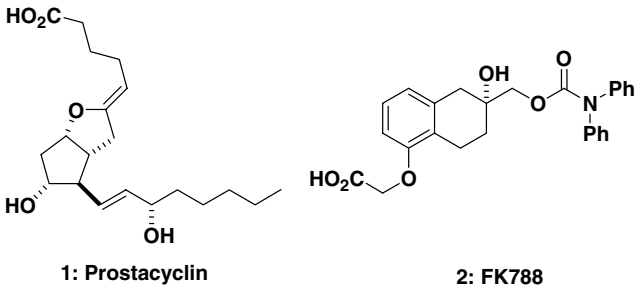

Figure 1.

Keywords: Prostacyclin; FK788; PG; PGI2; Metabolism.
* Corresponding author at present address: Chemistry Research Laboratories, Astellas Pharmaceutical Co. Ltd, 21, Miyukigaoka, Tsukuba-shi, Ibaraki 305-8585, Japan. Tel.: +81 29 863 7179; fax: +81 29 852 2971; e-mail: kouji.hattori@jp.astellas.com

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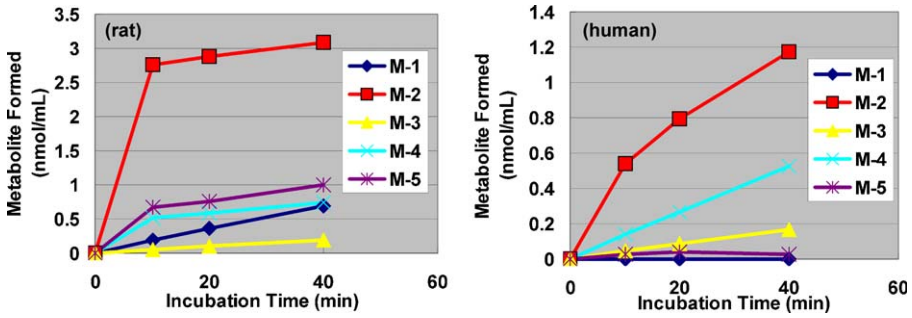
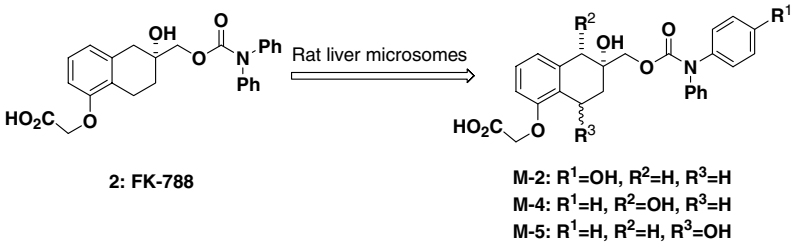
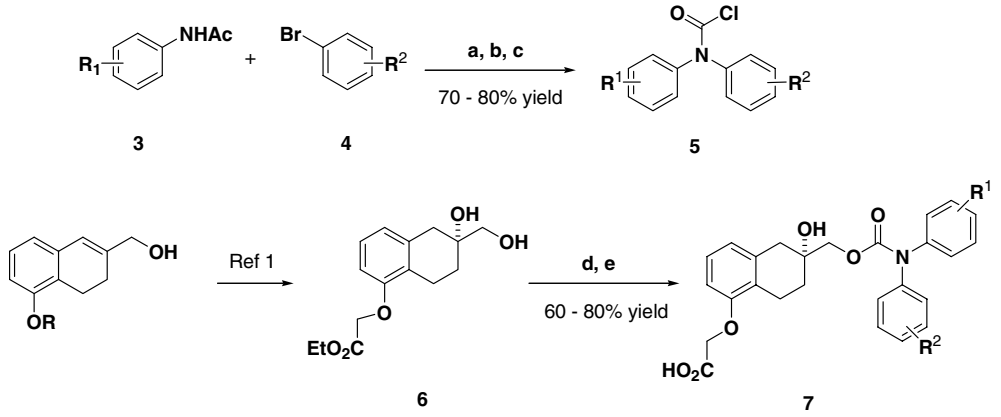


Figure 2. Rat and human microsome-induced metabolite formation from FK788: **2**.



Inhibition of ADP-induced aggregation of human and rat platelets	IC_{50} (nM)	
	Human	Rat
2	18	1400
M-2	35	2490
M-4	1290	>10000
M-5	198	>10000

Scheme 1. Metabolite of FK788 by rat liver microsomes.

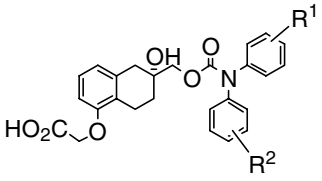


Scheme 2. Reagents: (a) CuCl, K_2CO_3, xylene; (b) 1 N-NaOH, EtOH; (c) triphosgen, pyridine, CH_2Cl_2; (d) **5**, pyridine (e) 1 N-NaOH, EtOH.

compounds in Scheme 1.[5] PGI_2 activity of M-2 towards inhibition of ADP-induced aggregation in human or rat platelets was of similar potency compared to the parent compound. On the other hand, M-4 and M-5 showed 70-fold and 10-fold less activity. After intravenous administration of FK788 in rats, M-2 and glucuronide of M-2 were found in the bile at 12% and 33% of the administered dose, respectively. Since M-2 and glucuronide of M-2 were rapidly excreted in the bile, plasma concentration of M-2 after oral administration of FK788 would be low.[3] From the results of this metabolism study, it could be considered that blockage of the main metabolic pathway *p*-oxidation of the benzene moiety would be effective to improve the metabolic stability of **2** without affecting the potency.

Diphenylcarbamoyl derivatives in which a proton was replaced by halogen atoms for this study were prepared as shown in Scheme 2. Copper-catalyzed amination reactions[6] of aryl halides with *N*-acyl aryl amines produced di-aryl amines. Hydrolysis of the products

Table 1. SAR of the diphenylcarbamoyl derivatives



Compound	R^1	R^2	Functional assay: IC_{50}[a] (nM)		CL_{int} (mL/min/kg)[b]
			Human	Rat	Rat
2(FK788)	H	H	18	1400	31.9
7a	*p*-F	H	13	4580	10.3
7b	*p*-Br	H	49	6490	12.3
7c	*p*-F	*p*-F	59	>10000	4.6
7d	*m*-F	*m*-F	14	>10000	13.5
7e	*o*-F	*o*-F	63	NT	44.8
7f	*p*-F	*o*-F	22	5110	11.8
7g	*p*-F	*m*-F	25	>10000	11.7

[a] Results are average of two or three experiments.
[b] Compounds were incubated at 37 °C for 60 min with rat liver microsomes in the presence of the NADPH-generating system.

with 4 N NaOH solution, followed by treatment with triphosgen in pyridine, gave the corresponding diphenylcarbamoyl chlorides **5**. Treatment of optically active diol **6**[1] with **5** in pyridine led to smooth conversion to the desired (*2R*)-hydroxy carbamoyl derivative, followed by hydrolysis of ester with 1 N NaOH solution to afford the desired compounds **7** in 60–80% overall yield from **6**.

Table 1 shows the results of biological evaluation of the prepared derivatives. Functional assay by measuring inhibition of ADP-induced platelet aggregation using human and rat platelet rich plasma, and the metabolic rate using rat liver microsomes was examined for the novel diphenylcarbamoyl derivatives. *p*-Fluoro **7a** and *m*-difluoro **7d** maintained similar PGI_2 activity, while *p*-bromo **7b**, *p*-difluoro **7c**, and *o*-difluoro **7e** exhibited approximately 3-fold less activity than the parent compound **2**. The metabolic rate of substitution at the *para*-position of the phenyl ring was slower than that of the parent compound, and particularly, *p*-difluoro **7c** was metabolically the most advantageous among all compounds.

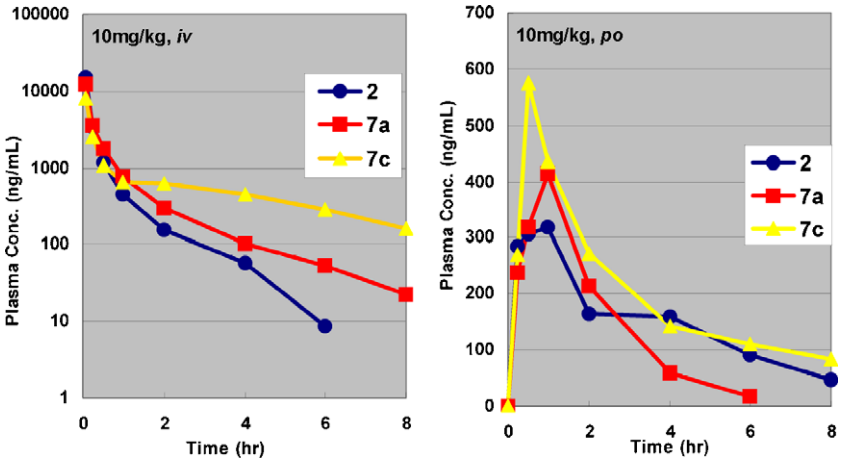
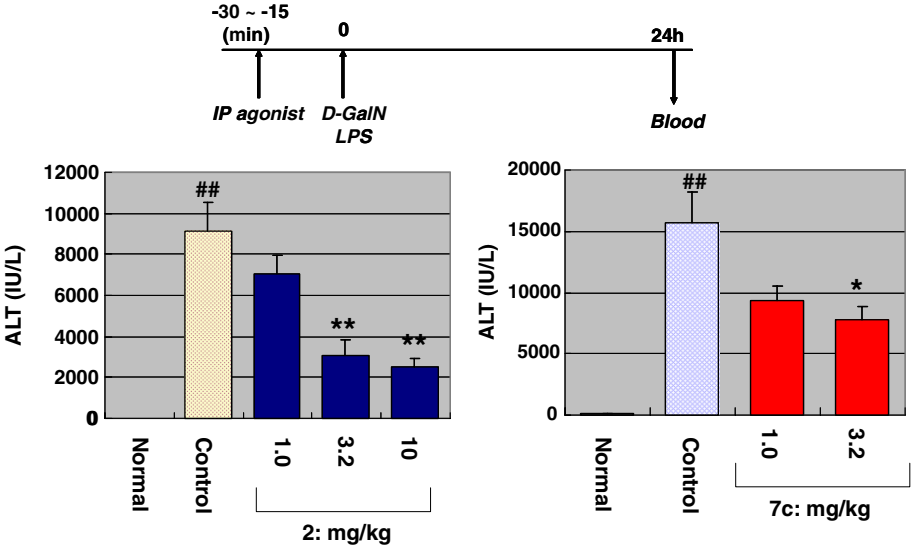


Figure 3. PK profiles of **2**, **7a**, and **7c** by rat cassette-dosing.

Table 2. Pharmacokinetic profiles in rats

Compound	po (fasted)			iv		
	Dose (mg/kg)	C_{max} (ng/mL)	AUC (ng/mL h)	$t_{1/2}$ β (h)	CL_{tot} (mL/min/kg)	*F*(%)
2	10	282	601	1.8	38.0	9.6
7a	10	434	961	1.6	32.9	19.2
7c	10	575	1607	3.0	30.3	29.5



##: p<0.01 *vs* Normal, *: p,0.05 *vs* control, ** p,0.01 *vs* control

Figure 4. Effect of **2** and **7c** in rat hepatic injury model by D-GalN/LPS, $N = 15$.

Figure 3 and Table 2 show the pharmacokinetic profiles after administration of a mixture of **7a**, **7c** and **2** at a dose of 10 mg/kg each to rats by cassette-dosing.[7] The rank order of elimination half-lives after intravenous administration was **7c** > **7a** > **2** and also **7c** displayed a higher plasma concentration level after oral administration compared to **2**, which is explained by the smaller in vitro clearance of **7c**. Enhanced metabolic stability by blockage of specific metabolic sites of **2** resulted in improved bioavailability.

Next, we examined a rat hepatic injury model with D-galactosamine hydrochloride (D-GalN) and lipopolysaccharide (LPS), as shown in Figure 4.[8] The compounds **7c** and **2** exhibited a similar protection effect at a dose of 3.2 mg/kg, even though **7c** had more than 7-fold less potent in vitro activity of rat.[9] This result suggested that the improved bioavailability of **7c** leads to the resultant improvement of in vivo potency.

In this communication, we have explored the metabolites of **2** using rat and human liver microsomes and identified the major metabolite, M-2. Blockage of oxidation at the *para*-position of the benzene moiety greatly improved the metabolic stability of **2**. Among the new derivatives, compound **7c** was found to have improved bioavailability and duration time than **2**. These results indicate that **7c** was a potential new candidate with potent PGI_2 activity and preferable pharmacokinetic profile.

Acknowledgment

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References and notes

1. Hattori, K.; Tanaka, A.; Okitsu, O.; Tabuchi, S.; Taniguchi, K.; Nishio, M.; Koyama, S.; Higaki, M.; Seki, J.; Sakane, K. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3091.
2. The bioavailability (*F*) was calculated from the following equation: $F = Qh/Qh + (Dose/AUC^{*}BP)$, where Qh and BP, respectively, are liver blood flow in humans (1500 mL/min/70 kg) and blood-to-plasma ratio of FK788 (0.57).
3. Glucuronidation of FK788 was not observed in rat excrement after intravenous administration of FK788, which is explained by being stable towards glucuronidation in vitro.
4. ^{14}C-FK788 (10 μmol/L) was incubated at 37 °C with rat and human liver microsomes (1 mg protein/mL) in the presence of a NADPH-generating system. Incubation mixtures were analyzed by radio-HPLC and the concentrations of formed metabolites were determined.
5. FK788 was incubated at 37 °C with rat liver microsomes in the presence of a NADPH-generating system. M-2, M-4 and M-5 in incubation mixtures were isolated by HPLC and their chemical structures were determined by LC/MS/MS and NMR analysis.
6. Weston, P. E.; Adkins, H. *J. Am. Chem. Soc.* **1928**, *50*, 859.
7. A dosing solution containing **7a**, **7c** and **2** was prepared in PEG400 and the dose of each compound was 10 mg/kg. After administration of the dosing solution to male rats, the blood was collected from cannulated femoral artery. The blood samples were centrifuged to separate plasma. The plasma samples were analyzed by LC/MS/MS for determination of plasma concentrations of **7a**, **7c** and **2**.
8. (a) Rahman, T. M.; Hodgson, H. J. F. *Int. J. Exp. Path.* **2000**, *81*, 145; (b) Jonker, A. M.; Dijkhuis, F. W. J.; Kroese, F. G. M.; Hardonk, M. J.; Grond, J. *Hepatology* **1990**, *11*, 622; (c) Galanos, C.; Freudenberg, M. A.; Reutter, W. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 5939.
9. The compounds **2** and **7c** were orally administered to rats 15–30 min prior to treatment with D-GalN (300 mg/kg)/LPS (0.32 μg/kg), and after 24 h, the blood was collected with a syringe from the abdominal artery. The separated plasma samples were examined for ALT levels by auto-analyzer.