Orally Active GPIIb/IIIa Antagonists: Synthesis and Biological Activities of Masked Amidines as Prodrugs of 2-[(3*S*)-4-[(2*S*)-2-(4-Amidinobenzoylamino)-3-(4-methoxyphenyl)propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid

Shuji KITAMURA,*[,a] Hideto FUKUSHI,[b] Toshio MIYAWAKI,[a] Masaki KAWAMURA,[c] Zen-ichi TERASHITA,[c] and Takehiko NAKA[a]

Medicinal Chemistry Research Laboratories,[a] Discovery Research Laboratories V,[b] and Pharmacology Research Laboratories I,[c] Takeda Chemical Industries, Ltd., 2–17–85, Jusohonmachi, Yodogawa-ku, Osaka 532–8686, Japan.
Received August 24, 2000; accepted November 20, 2000

To improve the *in vivo* potency of the potent GPIIb/IIIa antagonist 2-[(3*S*)-4-[(2*S*)-2-(4-amidinobenzoylamino)-3-(4-methoxyphenyl)propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic acid (4), the amidino group was converted to an oxadiazole ring, thiadiazole ring or substituted amidoxime group. These groups were expected to be metabolized to an amidino group *in vivo*. The compounds synthesized were evaluated for their potency to inhibit the *ex vivo* adenosine 5′-diphosphate (ADP)-induced aggregation of guinea pig platelets. Among the compounds examined, the methoxycarbonyloxyamidine 8a exhibited the most potent *ex vivo* inhibitory activity with a fast onset and prolonged duration of action after oral administration.

Key words GPIIb/IIIa antagonist; antiplatelet effect, masked amidine, prodrugs for arylamidines

The activation, adhesion and aggregation of platelets are important processes in the initiation of thrombus formation at sites showing high-grade stenosis, ruptured atheromatous plaque and endothelial damage within arteries. Recent studies on the biochemical mechanism of platelet activation indicate that the final obligatory step in aggregation is the cross-linking of the plasma protein fibrinogen and platelet membrane glycoprotein IIb/IIIa (GPIIb/IIIa) exposed on activated platelets.[1—4] In the last decade, intensive effort has been devoted to the development of GPIIb/IIIa inhibitors to treat and prevent thrombotic disorders such as unstable angina and myocardial infarction.[5—12] The clinical efficacy of the GPIIb/IIIa inhibitors has been demonstrated by positive results obtained with ReoPro[13,14] (anti-GPIIb/IIIa c7E3 Fab antibody), Integrelin[15,16] (cyclic peptide) and Aggrastat[17,18] (non-peptide small molecule) used as intravenous treatments for acute thrombosis.

Orally active GPIIb/IIIa antagonists are required to expand the therapeutic utility of this class of agents and provide effective chronic treatment for patients with recurrent vascular events. Although a number of potent low molecular weight GPIIb/IIIa antagonists have been reported, their oral bioavailabilities are insufficient for practical use. The low bioavailability might be ascribed to the highly polar nature of these compounds due to the presence of a strong basic group and a carboxyl group. To improve the oral absorption of these compounds, a prodrug strategy has often been used. For example, Ro-48-3657 (**1**),[11] BIBU-104 (**2**)[19] and EMD-122347 (**3**)[20] exhibit sufficient oral bioavailabily, and **2** and **3** are currently being examined in clinical trials (Chart 1).

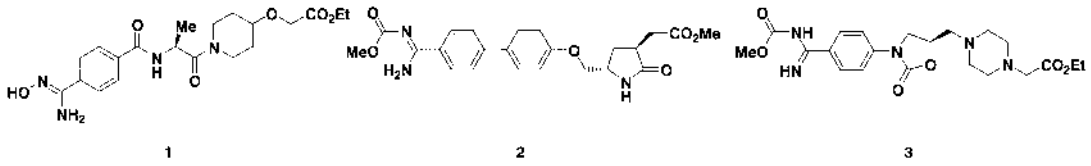
We recently reported the discovery of the potent GPIIb/IIIa antagonist 2-[(3*S*)-4-[(2*S*)-2-(4-amidinobenzoylamino)-3-(4-methoxyphenyl)propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic acid (**4**) (Chart 1).[21] Following oral administration to guinea pigs, **4** was not absorbed sufficiently, and absolute bioavailability of **4** was not high enough for practical use. Our initial effort to obtain orally active ester prodrugs of **4** resulted in failure. In the case of the ester prodrugs of **4**, due to unselective hydrolysis of the two ester groups, the prodrugs were not efficiently converted to the parent active form **4**.[22,23] We then turned our attention to masking the amidino group. While there are numerous reports on prodrugs of carboxylic acids, only a few prodrugs of aromatic amidines have been reported, for example, amidoximes,[11,19] *O*-methylamidoximes,[24] alkoxycarbonylamidines[25,26] and alkoxycarbonyloxyamidines.[26] In the clinical trials of the potent angiotensin II receptor antagonist TAK-536,[27,28] the amidine derivative (M-1) in which the oxadiazolone ring in TAK-536 has been converted to an amidino group was detected as one of the metabolites (Chart 1). This suggested that an oxadiazolone ring might be able to serve as a masked amidino group and prompted us to investigate new *in vivo* amidino group equivalents. Since there are many pharmacologically important amidines with poor oral bioavailability, the development of masked amidino groups is of great significance. In this paper, we describe the synthesis and pharmacological profiles of prodrugs of **4** based on the creation of an oxadiazolone ring.

Chemistry

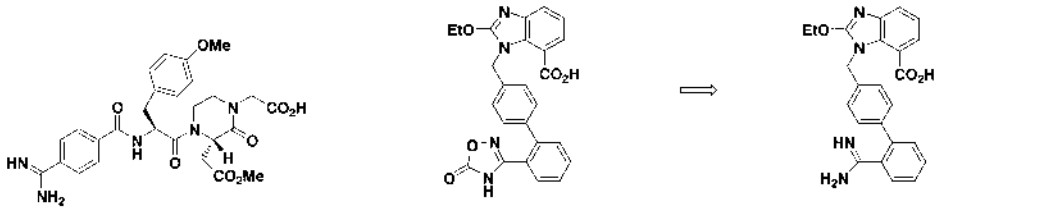
The target compounds **7a**—**l** and **8a**—**d** were synthesized as outlined in Chart 2 using **5**[21] as the starting material. Hydrogenolysis of **5** in the presence of 10% Pd–C to remove the benzyloxycarbonyl (Z) group and subsequent condensation with appropriate benzoic acids (**11**, **14**, **15**, **19**, **20**, **21**, **25**, **30**, **31**, **35**, **39** or **40**) gave the intermediates **6a**—**l**. Acid hydrolysis of the *tert*-butoxycarbonyl groups with trifluoroacetic acid (TFA) provided **7a**—**l**. Acylation of the amidoxime carboxylate **6c** followed by acid hydrolysis afforded **8a**—**d**.

The benzoic acids used in the above condensation reactions were prepared from the 4-cyanobenzoates **9** and **22** (Charts 3—5). Treatment of **9** with hydroxylamine hydrochloride, followed by hydrolysis with 2 N NaOH, provided the amidoxime **11**.[29] Cyclization of **10** with 1,1′-carbonyldiimidazole (CDI) and with 1,1′-thiocarbonyldiimidazole (TCDI) followed by hydrolysis afforded the oxadiazolone **14**

* To whom correspondence should be addressed. e-mail: Kitamura_Shuji@takeda.co.jp

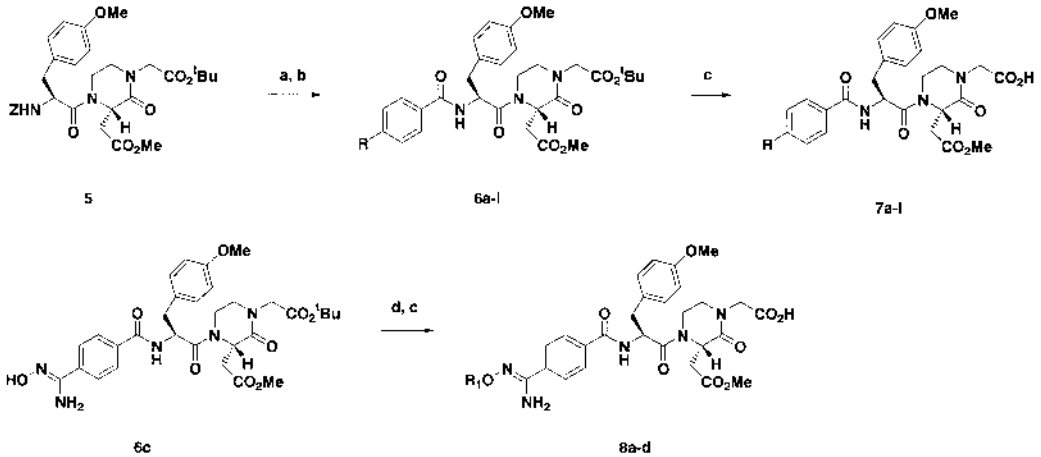


1 2 3



4 TAK-536 M-1

Chart 1

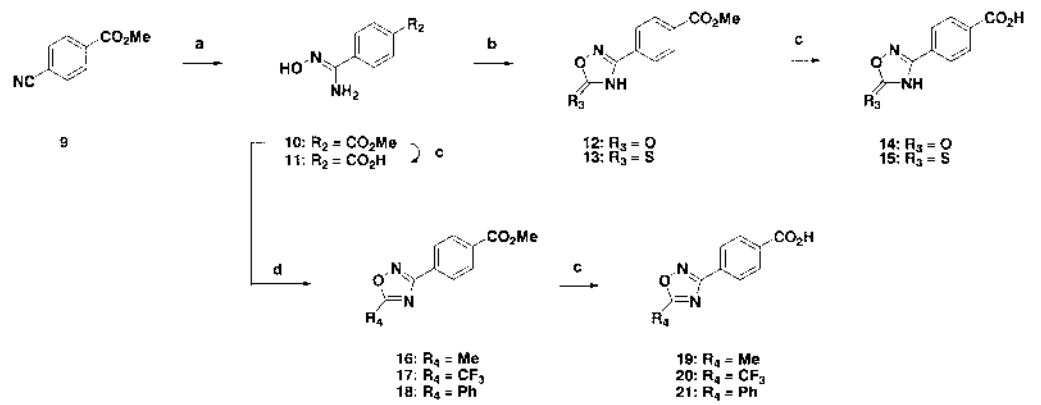


5 6a-l 7a-l

6c 8a-d

Reagents; (a) 10%Pd-C, H_2, MeOH; (b) 11, 14, 15, 19, 20, 21, 25, 30, 31, 35, 39 or 40, EDC, DMF; (c) CF_3CO_2H, CH_2Cl_2; (d) methyl chloroformate, methyl chlorothioformate or acetyl chloride, K_2CO_3, 1,4-dioxane; or ethyl isocyanate, CH_2Cl_2.

Chart 2



9

10: R_2 = CO_2Me
11: R_2 = CO_2H

12: R_3 = O
13: R_3 = S

14: R_3 = O
15: R_3 = S

16: R_4 = Me
17: R_4 = CF_3
18: R_4 = Ph

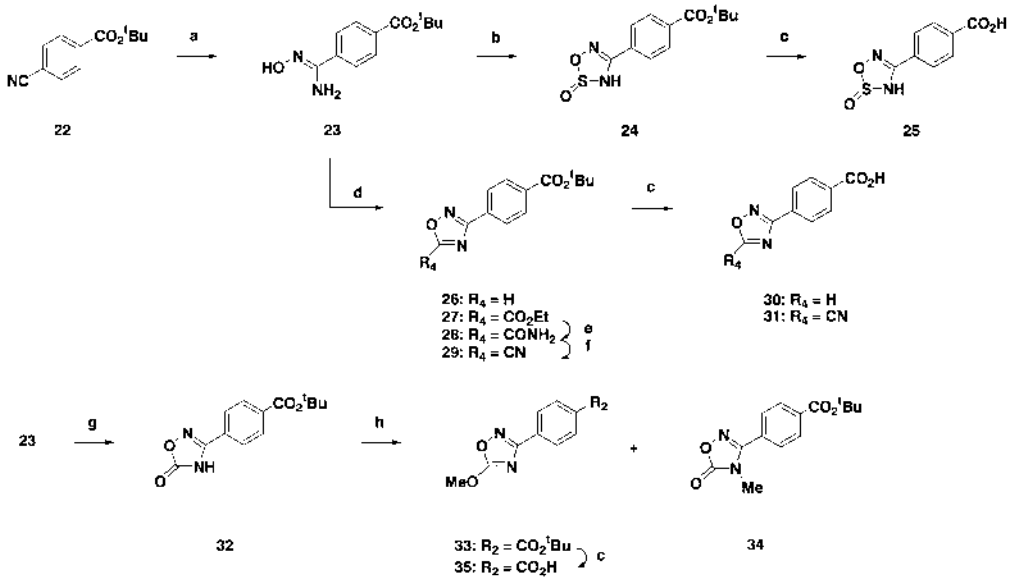
19: R_4 = Me
20: R_4 = CF_3
21: R_4 = Ph

Reagents; (a) NH_2OH•HCl, $NaHCO_3$, MeOH; (b) CDI, 1,4-dioxane; or TCDI, 1,8-diazabicyclo[5,4,0]undec-7-ene,1,4-dioxane; (c) 2N NaOH; (d) acetic anhydride, CH_2Cl_2; $(CF_3CO)_2O$, CH_2Cl_2; or benzoyl chloride, pyridine, xylene, 140°C.

Chart 3

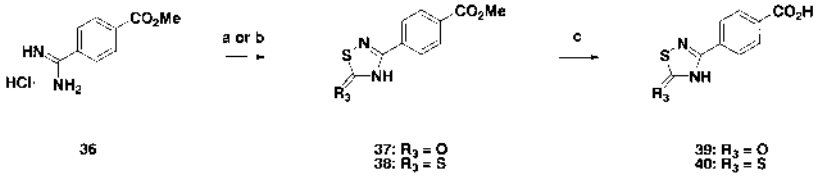
and the oxadiazolethione **15**, respectively. The 5-methyloxadiazole **19**, 5-trifluoromethyloxadiazole **20** and 5-phenyloxadiazole **21** were obtained by cyclization of **10** with the corresponding acid anhydride or benzoyl chloride followed by hydrolysis (Chart 3).

tert-Butyl 4-[amino(hydroxyimino)methyl]benzoate, **23**,



Reagents: (a) NH_2OH·HCl, $NaHCO_3$, *tert*-BuOH, H_2O; (b) $SOCl_2$, pyridine, CH_2Cl_2, -20°C to -5°C; (c) CF_3CO_2H, CH_2Cl_2; (d) trimethyl orthoformate, BF_3·Et_2O, 80 to 110°C; or ethyl chloroglyoxylate, pyridine, CH_2Cl_2; (e) conc. NH_4OH; (f) $(CF_3CO)_2O$, pyridine; (g) CDI, 1,4-dioxane; (h) CH_2N_2, EtOAc.

Chart 4



Reagents: (a) chlorocarbonylsulfenyl chloride, Na_2CO_3, CH_2Cl_2, H_2O; (b) CS_2, S, 28% NaOMe, MeOH; (c) 2N NaOH.

Chart 5

which was obtained from **22** with hydroxylamine hydrochloride, was cyclized with thionyl chloride followed by acid hydrolysis to provide the oxathiadiazolone **25**. Reaction of **23** with $CH(OCH_3)_3$ in the presence of boron trifluoride diethyl etherate ($BF_3 \cdot Et_2O$) gave the oxadiazole **26**. Treatment of **23** with ethyl chloroglyoxylate in the presence of pyridine provided the 5-ethoxycarbonyloxadiazole **27**, which was converted to the 5-cyanooxadiazole **29** by amidation and dehydration. Acid hydrolysis of **26** and **29** gave the benzoic acids **30** and **31**, respectively. The intermediate **23** was converted to the oxadiazolone **32**, which was methylated with diazomethane to give a mixture of the 5-methoxyoxadiazole **33** and the *N*-methyloxadiazole **34**. After the separation of **33** and **34** by silica gel column chromatography, **33** was converted to the corresponding benzoic acid **35** by acid hydrolysis (Chart 4).

Cyclization of the methyl 4-[amino(imino)methyl]benzoate hydrochloride **36** with chlorocarbonyl sulfenyl chloride and subsequent saponification provided the thiadiazolone **39**. The reaction of **36** with carbon disulfide and sulfur in the presence of sodium methoxide gave the thiadiazolethione **38**, which was hydrolyzed to the carboxylic acid **40** (Chart 5).

Table 1. Metabolic Conversion[a] of **7a** to **4**

	Generated **4** (%)	Remaining **7a** (%)
Liver homogenate	14.5	22.4
Small intestine homogenate	Not detected	73.4
Plasma	Not detected	57.9

a) Compond **7a** was incubated in guinea pig liver homogenate, small intestine homogenate and plasma at 37 °C for 1 h and then assayed by HPLC as described in the Experimental section.

Results and Discussion

First of all, to evaluate the potential usefulness of an oxadiazolone ring as a masked amidino group, metabolic conversion of the oxadiazolone **7a** to the amidine **4** using guinea pig liver homogenate, small intestine homogenate and plasma was examined (Table 1). After incubation of **7a** in liver homogenate at 37 °C for 1 h, the amount of **4** generated and that of **7a** remaining were determined to be 15 and 22% based on the initial amount of **7a**, respectively. After 1 h incubation in small intestine homogenate and plasma, no amount of **4** was detected, and the amount of **7a** recovered was 73 and 58%, respectively. This suggested that the oxadi-

azolone ring could be metabolized to the amidino group by the reductive liver enzymes,[30] but not by esterases in the plasma or small intestine. The metabolic pathway was considered to be the cleavage of the N–O bond in the oxadiazolone ring, followed by elimination of carbon dioxide from the carbamic acid intermediate to lead to **4**.

The results of the *in vitro* metabolic study encouraged us to evaluate **7a** *in vivo* using guinea pigs. The *ex vivo* inhibitory effect of **7a** on the adenosine 5′-diphosphate (ADP)-induced platelet aggregation in guinea pig platelet rich plasma (PRP) is shown in Fig. 1. Oral administration of **7a** at a dose of 0.3 mg/kg resulted in 91% inhibition at 8 h, and 38% inhibition was still observed at 24 h. On the contrary, **4** inhibited the platelet aggregation by 65 and 30% at 2 and 8 h, respectively. Since the *in vitro* antiplatelet effect of **7a** was very weak even at a high concentration (Table 2), **7a** is considered to be metabolized to the biological active form **4**, of which the plasma concentration could be estimated from the *ex vivo* potency of **7a** after oral administration, in the body. While improved *ex vivo* potency and prolonged duration of action were observed with **7a** as compared to **4**, the onset of its action was slower than that of **4**. This lag might be ascribed to sluggish conversion of **7a** to **4**, because the maximal concentration of **7a** in the plasma[31] was observed 2 h after oral administration (data not shown).

To achieve a quick onset of action, some thioanalogs were examined because it was thought they might be more easily converted than **7a** to **4**. In fact, incubation of the thiadiazolone **7d** in guinea pig liver homogenate at 37 °C for 1 h generated more **4** than in the case of **7a** (39% *vs.* 15%). The *ex vivo* inhibitory effects of the thioanalogs **7b** and **7d**—**f**, whose *in vitro* antiplatelet effects were remarkably decreased (IC_{50}: >4 μM, Table 2), are presented in Fig. 1. Among these thioanalogs, the oxathiadiazolone **7f** showed a potent in-

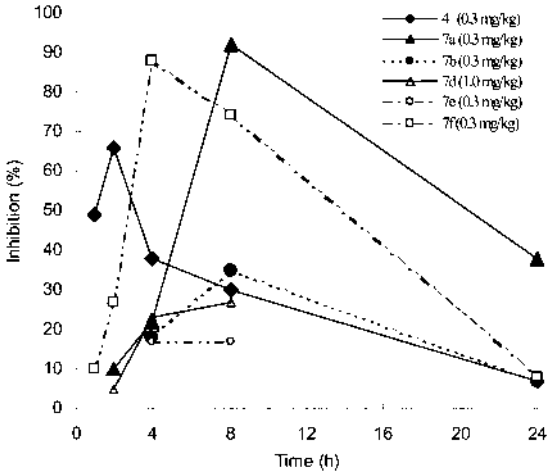
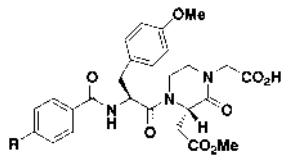
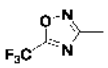
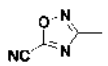
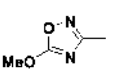
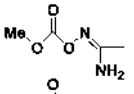
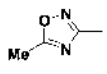
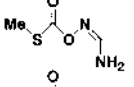
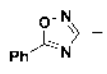
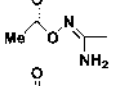
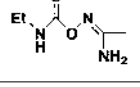


Fig. 1. Effects of Oxadiazolone, Oxadiazolethione, Thiadiazolone, Thiadiazolethione and Oxathiadiazolone Derivatives on *ex Vivo* (*p.o.*) ADP-Induced Platelet Aggregation in Guinea Pigs (n=4)

Table 2. *In Vitro* Inhibitory Effects of Compounds **4**, **7a—l** and **8a—d**



Compound	R=	Inhibitory effect[a] IC_{50} (nM)	Compound	R=	Inhibitory effect[a] IC_{50} (nM)
4		13	**7i**		17000
7a		>30000	**7j**		11000
7b		30000	**7k**		20000
7d		>30000	**7l**		9000
7e		>30000	**7c**		1900
7f		4400	**8a**		2000
7g		16000	**8b**		320
7h		>30000	**8c**		300
			8d		270

a) *In vitro* inhibition of ADP-induced platelet aggregation in guinea pig PRP. See the Experimental section for details.

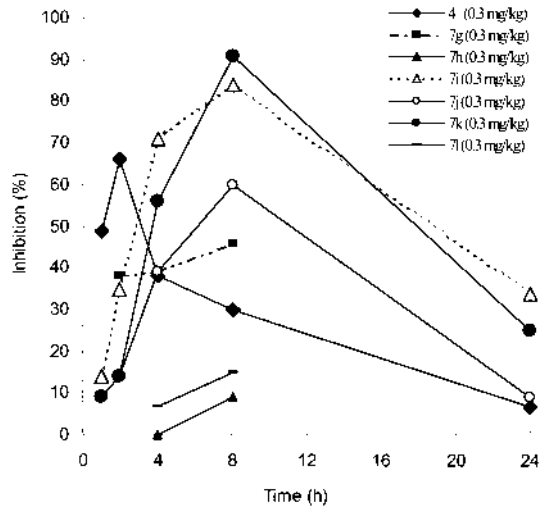


Fig. 2. Effects of Oxadiazole Derivatives on *ex Vivo* (*p.o.*) ADP-Induced Platelet Aggregation in Guinea Pigs ($n=4$)

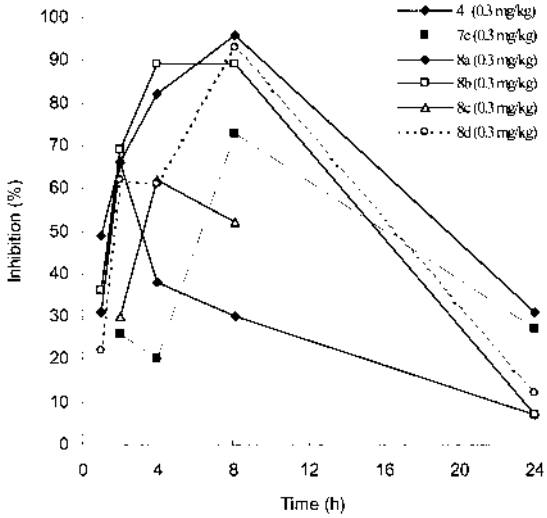


Fig. 3. Effects of Amidoxime Derivatives on *ex Vivo* (*p.o.*) ADP-Induced Platelet Aggregation in Guinea Pigs ($n=4$)

hibitory effect comparable to that of **7a** and a faster onset of action than **7a**. On the contrary, the other thioanalogs, **7b**, **7d** and **7e**, did not show enhanced *ex vivo* potency.

Although the *ex vivo* activity of **4** was improved by conversion to **7a** or **7f**, more rapid onset of action was required. We then investigated some oxadiazoles, which might be metabolized to the amidino group through the same pathway as **7a**. The *ex vivo* antiplatelet effect and the *in vitro* antiplatelet activities of the oxadiazoles **7g**—**7l** are summarized in Fig. 2 and Table 2. These compounds also had diminished *in vitro* antiplatelet activities (Table 2). Conversion of the amidino group to an oxadiazole ring (**7j**) did not enhance the *ex vivo* activity as compared to that of **4**. A dramatic decrease was observed with the 5-methyloxadiazole **7g** and the 5-methoxyoxadiazole **7l**, which possessed an electron donating group at the 5-position of the oxadiazole ring. On the contrary, introduction of an electron withdrawing group, such as a trifluoromethyl or a cyano group, increased the *ex vivo* antiplatelet effect. Oral administration of the 5-trifluoromethyloxadiazole **7i**, and the 5-cyanooxadiazole **7k** at a dose of 0.3 mg/kg, caused 71 and 56% inhibition at 4 h and 84 and 91% inhibition at 8 h, respectively. Introduction of the electron withdrawing group might facilitate the cleavage of the N–O bond to form the amidino group. The 5-phenyloxadiazole **7h** was much less potent than **7j**, providing only 9% of the maximal inhibition. While a phenyl group is a weak electron withdrawing group, the chemical stability of the phenyloxadiazole ring might prevent the reductive cleavage. The oxadiazoles **7i** and **7k**, bearing electron withdrawing groups at the 5-position, had *ex vivo* potency better than that of **4** but comparable to that of **7a** and **7f**.

We also evaluated the substituted amidoximes **7c** and **8a**—**d**, which are regarded as acyclic analogs of the corresponding oxadiazolone ring-bearing compounds (Fig. 3). The replacement of the amidino group with an amidoxime group (**7c**) did not increase the *ex vivo* activity as compared to that of **4**. We might ascribe the inefficacy of this modification to the presence of the hydroxyl group in **7c** which reduced its lipophilicity. To increase the lipophilicity of **7c**, the hydroxyl group was masked to lead to the amidoxime derivatives **8a**—**d**. The methoxycarbonyloxyamidine **8a** showed a pronounced inhibitory effect, fast onset and prolonged duration of action, which were superior to those of the other compounds prepared in this study. The inhibitory effect of **8a** at 0.3 mg/kg *p.o.* was found to be 66, 82, 96 and 31% at 2, 4, 8 and 24 h, respectively. The metabolic pathway of **8a** is considered to proceed *via* amidoxime **7c**,[32] which is thought to be converted to amidine **4** by reductive enzymes in the liver.[11,19] Rahmathullah and co-workers[26] reported the application of an alkoxycarbonyloxyamidino group as a masked amidino group to improve oral efficacy of an anti-*Pneumocystis carinii* agent, but that trial resulted in failure. Our study would be the first example showing the usefulness of an alkoxycarbonyloxyamidine group as a masked amidino group. The ethoxy- and the propoxycarbonyloxyamidines also displayed potent and long lasting *ex vivo* inhibitory activities, although these were inferior to that of **8a** (data not shown). A faster onset of action and similar inhibitory activity were observed after oral administration of the methylthiocarbonyloxyamidine **8b** as compared to **8a**; however, its duration of action was shorter than that of **8a**. The ethylcarbamyloxyamidine **8d** also showed as potent inhibitory activity as **8a**, but the onset of the action was slower than that of **8a**. While the amidoxime derivatives **8a,b** and **8d** demonstrated good oral activity, enhanced *ex vivo* activity was not observed with the acetoxyamidine **8c**.

The oral bioavailabilities of **4** and **8a**, in terms of **4**, in guinea pigs after a dose of 1 mg/kg *i.v.* (**4**) and 30 mg/kg *p.o.* (**4**) or 10 mg/kg *p.o.* (**8a**), were found to be 2.8 and 5.3%, respectively (Fig. 4). The oral bioavailability as **4** was increased by about 2-fold with the methoxycarbonyloxyamidine **8a**.

In conclusion, cyclic amidino groups, such as an oxadiazolone group, an oxathiadiazolone group, a 5-trifluoromethyloxadiazole group and a 5-cyanoloxadiazole group, as well as substituted amidoxime groups, such as a methoxycarbonyloxyamidino group and a methylthiocarbonyloxyamidino group, provided useful masked amidino groups to obtain orally active potent GPIIb/IIIa antagonists with a fast onset and prolonged duration of action. It is well known that there is no almighty prodrug promoiety for a carboxyl group.

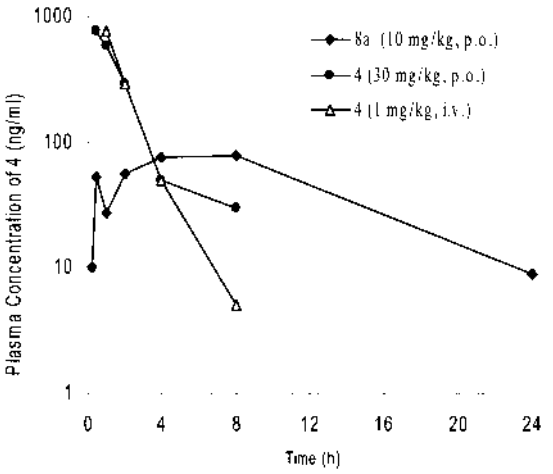


Fig. 4. Plasma Concentration of **4** after *i.v.* Administration of **4** and *p.o.* Administration of **4** and **8a** ($n=3$) in Guinea Pigs

Plasma concentration of **4** at 24 h after *p.o.* administration of **4** was under the limit of quantitation (<5 ng/ml).

The best promoieties are dependent on the nature of the carboxylic acid. This is also true in the case of arylamidines, and a variety of promoieties should therefore be provided as candidates. The masked amidino groups discussed in this study could be additional examples to be considered. Although dramatic improvement of the oral bioavailability was not accomplished with the compounds examined in this study because of a remaining carboxyl group, these new masked amidino groups are expected to be useful for other arylamidines.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were obtained on a Hitachi IR-215 spectrometer. ^{1}H-NMR spectra were recorded on a Varian Gemini 200 (200 MHz) spectrometer. Chemical shifts are given in δ value (ppm) with tetramethylsilane (TMS) as the internal standard. The following abbreviations are used: s=singlet, d=doublet, t=triplet, dd=double doublet, m=multiplet. Optical rotations were recorded on a JASCO DIP-370 digital polarimeter.

Methyl 2-[(2*S*)-4-[2-(*tert*-Butoxy)-2-oxoethyl]-1-[(2*S*)-3-(4-methoxyphenyl)-2-[[4-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)benzoyl]amino]-propanoyl-3-oxopiperazinyl]acetate (6a) A suspension of **5** (0.95 g, 1.59 mmol) and 10% Pd–C (0.25 g) in MeOH (50 ml) was hydrogenated under balloon pressure for 1 h at room temperature. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to give amine. To a solution of this amine in *N*,*N*-dimethylformamide (DMF) (12 ml) were added 4-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)benzoic acid **14** (0.36 g, 1.75 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (0.4 g, 2.09 mmol) at room temperature. After being stirred for 1 h, the mixture was diluted with ethyl acetate (EtOAc), washed with 5% aqueous $KHSO_4$, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc–MeOH, 4 : 1) to give **6a** (0.76 g, 73%) as a colorless amorphous powder. ^{1}H-NMR ($CDCl_3+D_2O$) δ: 1.46 (9H, s), 2.40—3.90 (8H, m), 3.59 (3H, s), 3.74 (3H, s), 3.94 (2H, s), 5.13 (1H, t, J=6.0 Hz), 5.23—5.42 (1H, m), 6.79 (2H, d, J=8.6 Hz), 7.12 (2H, d, J=8.6 Hz), 7.65—7.98 (4H, m).

Methyl 2-[(2*S*)-4-[2-(*tert*-Butoxy)-2-oxoethyl]-1-[(2*S*)-3-(4-methoxyphenyl)-2-[[4-(5-thioxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)benzoyl]amino]-propanoyl-3-oxopiperazinyl]acetate (6b) The title compound was prepared from **5** and 4-(5-thioxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)benzoic acid **15** according to the procedure described in the preparation of **6a** as a colorless amorphous powder. ^{1}H-NMR ($CDCl_3+D_2O$) δ: 1.43 (9H, s), 2.00—4.20 (10H, m), 3.52 (3H, s), 3.70 (3H, s), 5.00—5.45 (2H, m), 6.60—6.90 (2H, m), 7.00—7.80 (6H, m).

Methyl 2-[(2*S*)-1-[(2*S*)-2-[[4-[Amino(hydroxyimino)methyl]benzoyl]-amino]-3-(4-methoxyphenyl)propanoyl]-4-[2-(*tert*-butoxy)-2-oxoethyl]-3-oxopiperazinyl]acetate (6c) The title compound was prepared from **5** and 4-[amino(hydroxyimino)methyl]benzoic acid **11** according to the procedure described in the preparation of **6a** as a colorless amorphous powder. ^{1}H-NMR ($CDCl_3+D_2O$) δ: 1.45 (9H, s), 2.30—3.40 (6H, m), 3.60—4.00 (4H, m), 3.63 (3H, s), 3.78 (3H, s), 5.03—5.50 (2H, m), 6.83 (2H, d, J=8.0 Hz), 7.18 (2H, d, J=8.0 Hz), 7.31 (2H, d, J=8.0 Hz), 7.50 (2H, d, J=8.0 Hz).

Methyl 2-[(2*S*)-4-[2-(*tert*-Butoxy)-2-oxoethyl]-1-[(2*S*)-3-(4-methoxyphenyl)-2-[[4-(5-thioxo-4,5-dihydro-1,2,4-thiadiazol-3-yl)benzoyl]-amino]propanoyl-3-oxopiperazinyl]acetate (6e) The title compound was prepared from **5** and 4-(5-thioxo-4,5-dihydro-1,2,4-thiadiazol-3-yl)benzoic acid **40** according to the procedure described in the preparation of **6a** as a colorless amorphous powder. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (9H, s), 2.50—3.35 (6H, m), 3.62 (3H, s), 3.72—3.78 (2H, m), 3.79 (3H, s), 3.96 (3H, s), 5.05 (1H, t, J=5.6 Hz), 5.24—5.42 (1H, m), 6.84 (2H, d, J=8.6 Hz), 7.05 (1H, m), 7.16 (2H, d, J=8.6 Hz), 7.89 (2H, d, J=8.4 Hz), 8.32 (2H, d, J=8.4 Hz).

Methyl 2-[(2*S*)-4-[2-(*tert*-Butoxy)-2-oxoethyl]-1-[(2*S*)-3-(4-methoxyphenyl)-2-[[4-(2-oxo-2,3-dihydro-1,2λ^4,3,5-oxathiadiazol-4-yl)benzoyl]-amino]propanoyl-3-oxopiperazinyl]acetate (6f) The title compound was prepared from **5** and 4-(2-oxo-2,3-dihydro-1,2λ^4,3,5-oxathiadiazol-4-yl)benzoic acid **25** according to the procedure described in the preparation of **6a** as a colorless amorphous powder. ^{1}H-NMR ($CDCl_3+D_2O$) δ: 1.47 (9H, s), 2.60—4.20 (10H, m), 3.66 (3H, s), 3.73 (3H, s), 5.05—5.40 (2H, m), 6.68 (2H, d, J=8.4 Hz), 6.87 (2H, d, J=8.4 Hz), 7.86 (2H, d, J=8.6 Hz), 7.92 (2H, d, J=8.4 Hz).

Methyl 2-[(2*S*)-4-[2-(*tert*-Butoxy)-2-oxoethyl]-1-[(2*S*)-3-(4-methoxyphenyl)-2-[[4-(5-methyl-1,2,4-oxadiazol-3-yl)benzoyl]amino]propanoyl-3-oxopiperazinyl]acetate (6g) The title compound was prepared from **5** and 4-(5-methyl-1,2,4-oxadiazol-3-yl)benzoic acid **19** according to the procedure described in the preparation of **6a** as a colorless amorphous powder. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (9H, s), 2.55—2.80 (1H, m), 2.68 (3H, s), 2.95—3.30 (5H, m), 3.63 (3H, s), 3.73—3.86 (2H, m), 3.78 (3H, s), 3.95 (2H, s), 5.05 (1H, t, J=5.5 Hz), 5.22—5.40 (1H, m), 6.84 (2H, d, J=8.8 Hz), 7.09 (1H, d, J=8.0 Hz), 7.16 (2H, d, J=8.8 Hz), 7.90 (2H, d, J=8.6 Hz), 8.14 (2H, d, J=8.6 Hz).

Methyl 2-[(2*S*)-4-[2-(*tert*-Butoxy)-2-oxoethyl]-1-[(2*S*)-3-(4-methoxyphenyl)-2-[[4-(5-phenyl-1,2,4-oxadiazol-3-yl)benzoyl]amino]propanoyl-3-oxopiperazinyl]acetate (6h) The title compound was prepared from **5** and 4-(5-phenyl-1,2,4-oxadiazol-3-yl)benzoic acid **21** according to the procedure described in the preparation of **6a** as a colorless amorphous powder. ^{1}H-NMR ($CDCl_3$) δ: 1.47 (9H, s), 2.55—3.30 (4H, m), 3.00 (2H, d, J=5.4 Hz), 3.64 (3H, s), 3.75—3.90 (2H, m), 3.78 (3H, s), 3.96 (2H, s), 5.08 (1H, t, J=5.4 Hz), 5.25—5.45 (1H, m), 6.85 (2H, d, J=8.0 Hz), 7.18 (2H, d, J=8.0 Hz), 7.25—7.45 (1H, m), 7.50—7.70 (3H, m), 7.93 (2H, d, J=8.4 Hz), 8.18—8.30 (2H, m), 8.25 (2H, d, J=8.4 Hz).

Methyl 2-[(2*S*)-4-[2-(*tert*-Butoxy)-2-oxoethyl]-1-[(2*S*)-3-(4-methoxyphenyl)-2-[[4-(5-trifluoromethyl-1,2,4-oxadiazol-3-yl)benzoyl]amino]-propanoyl]-3-oxopiperazinyl]acetate (6i) The title compound was prepared from **5** and 4-(5-trifluoromethyl-1,2,4-oxadiazol-3-yl)benzoic acid **20** according to the procedure described in the preparation of **6a** as a colorless amorphous powder. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (9H, s), 2.50—3.30 (6H, m), 3.64 (3H, s), 3.70—3.90 (2H, m), 3.78 (3H, s), 3.96 (2H, s), 5.06 (1H, t, J=5.5 Hz), 5.24—5.40 (1H, m), 6.85 (2H, d, J=8.6 Hz), 7.10—7.30 (1H, m), 7.17 (2H, d, J=8.6 Hz), 7.94 (2H, d, J=8.4 Hz), 8.20 (2H, d, J=8.4 Hz).

Methyl 2-[(2*S*)-4-[2-(*tert*-Butoxy)-2-oxoethyl]-1-[(2*S*)-3-(4-methoxyphenyl)-2-[[4-(1,2,4-oxadiazol-3-yl)benzoyl]amino]propanoyl]-3-oxopiperazinyl]acetate (6j) The title compound was prepared from **5** and 4-(1,2,4-oxadiazol-3-yl)benzoic acid **30** according to the procedure described in the preparation of **6a** as a colorless amorphous powder. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (9H, s), 2.50—3.90 (8H, m), 3.63 (3H, s), 3.79 (3H, s), 3.96 (2H, s), 5.06 (1H, t, J=5.4 Hz), 5.20—5.40 (1H, m), 6.85 (2H, d, J=8.8 Hz), 7.17 (2H, d, J=8.8 Hz), 7.08—7.23 (1H, m), 7.93 (2H, d, J=8.6 Hz), 8.21 (2H, d, J=8.4 Hz), 8.81 (1H, s).

Methyl 2-[(2*S*)-4-[2-(*tert*-Butoxy)-2-oxoethyl]-1-[(2*S*)-2-[[4-(5-cyano-1,2,4-oxadiazol-3-yl)benzoyl]amino]-3-(4-methoxyphenyl)propanoyl]-3-oxopiperazinyl]acetate (6k) The title compound was prepared from **5** and 4-(5-cyano-1,2,4-oxadiazol-3-yl)benzoic acid **31** according to the procedure described in the preparation of **6a** as colorless crystals, mp 185—187 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (9H, s), 2.60—3.40 (4H, m), 2.99 (2H, d, J=5.6 Hz), 3.64 (3H, s), 3.72—3.88 (2H, m), 3.78 (3H, s), 3.96 (2H, s), 5.05 (1H, t, J=5.4 Hz), 5.23—5.39 (1H, m), 6.85 (2H, d, J=8.6 Hz), 7.10—7.25 (1H, m), 7.16 (2H, d, J=8.6 Hz), 7.94 (2H, d, J=8.8 Hz), 8.18 (2H, d, J=8.8 Hz). IR (KBr) cm^{-1}: 1740, 1660, 1650, 1610, 1510, 1445, 1290, 1250, 1230, 1150. *Anal.* Calcd for $C_{33}H_{36}N_6O_9$: C, 59.99; H, 5.49; N, 12.72. Found: C, 59.85; H, 5.45; N, 12.60.

Methyl 2-[(2*S*)-4-[2-(*tert*-Butoxy)-2-oxoethyl]-1-[(2*S*)-2-[4-(5-methoxy-1,2,4-oxadiazol-3-yl)benzoyl]amino]-3-(methoxyphenyl)propanoyl]-3-oxopiperazinyl]acetate (6l) The title compound was prepared from **5** and 4-(5-methoxy-1,2,4-oxadiazol-3-yl)benzoic acid **35** according to the procedure described in the preparation of **6a** as a colorless amorphous powder. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (9H, s), 2.55—3.30 (4H, m), 2.99 (2H, d, J=5.6 Hz), 3.63 (3H, s), 3.70—3.80 (2H, m), 3.78 (3H, s), 3.95 (2H, s), 4.29 (3H, s), 5.06 (1H, t, J=5.5 Hz), 5.25—5.40 (1H, m), 6.88 (2H, d, J=8.6 Hz), 7.05—7.20 (1H, m), 7.16 (2H, d, J=8.6 Hz), 7.88 (2H, J=8.6 Hz), 8.10 (2H, d, J=8.8 Hz).

2-[(3*S*)-3-(2-Methoxy-2-oxoethyl)-4-[(2*S*)-3-(4-methoxyphenyl)-2-[[4-(5-thioxo-4,5-dihydro-1,2,4-thiadiazol-3-yl)benzoyl]amino]propanoyl]-2-oxopiperazinyl]acetic Acid (7e) To a solution of **6e** (0.76 g, 11.1 mmol) in CH_2Cl_2 (5.0 ml) was added TFA (5.0 ml) at room temperature. After being stirred for 2 h at room temperature, the mixture was concentrated under reduced pressure. The residue was purified by column chromatography (CHP-20, gradient elution: H_2O to 30% aqueous CH_3CN) to give **7e** (0.44 g, 33%) as a colorless amorphous powder, $[\alpha]_D^{20}$ +4.4° (c=0.69, dimethyl sulfoxide (DMSO)). ^{1}H-NMR (DMSO-d_6) δ: 2.60—2.86 (2H, m), 2.86—4.25 (8H, m), 3.48 (3H, s), 3.69 (3H, s), 4.90 (1H, m), 6.81 (2H, d, J=8.8 Hz), 7.22 (2H, d, J=8.8 Hz), 7.96 (2H, d, J=8.4 Hz), 8.22 (2H, d, J=8.4 Hz), 8.89 (1H, d, J=8.2 Hz). IR (KBr) cm^{-1}: 1732, 1651, 1539, 1512, 1454, 1404, 1248, 1179. *Anal.* Calcd for $C_{28}H_{29}N_5O_8S_2\cdot 0.1Et_2O\cdot 0.5H_2O$: C, 52.96; H, 4.85; N, 10.87. Found: C, 52.93; H, 4.85; N, 10.94.

Following compound **7a**—**c** and **7f**—**l** were prepared according to the procedure described in the preparation of **7e**.

2-[(3*S*)-3-(2-Methoxy-2-oxoethyl)-4-[(2*S*)-3-(4-methoxyphenyl)-2-[[4-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)benzoyl]amino]propanoyl]-2-oxopiperazinyl]acetic Acid (**7a**): Colorless crystals (EtOH), mp 157—161 °C. $[\alpha]_D^{20}$ +56.4° (c=0.44, MeOH). ^{1}H-NMR (DMSO-d_6+D_2O) δ: 2.60—2.85 (2H, m), 2.85—4.20 (8H, m), 3.49 (3H, s), 3.70 (3H, s), 4.89 (1H, t, J=6.0 Hz), 4.92—5.20 (1H, m), 6.82 (2H, d, J=8.6 Hz), 7.22 (2H, d, J=8.6 Hz), 7.80—8.00 (4H, m). IR (KBr) cm^{-1}: 1780, 1730, 1640, 1510, 1440, 1300, 1250, 1180. *Anal.* Calcd for $C_{28}H_{29}N_5O_{10}\cdot 1.5H_2O$: C, 54.02; H, 5.18; N, 11.25. Found: C, 54.31; H, 5.05; N, 11.25.

2-[(3*S*)-3-(2-Methoxy-2-oxoethyl)-4-[(2*S*)-3-(4-methoxyphenyl)-2-[[4-(5-thioxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)benzoyl]amino]propanoyl]-2-oxopiperazinyl]acetic Acid (**7b**): A colorless amorphous powder, $[\alpha]_D^{20}$ +69.3° (c=0.63, H_2O). ^{1}H-NMR (D_2O) δ: 2.10—3.17 (6H, m), 3.35—4.05 (5H, m), 3.51 (3H, s), 3.77 (3H, s), 4.95 (1H, t, J=6.4 Hz), 6.87 (2H, d, J=8.4 Hz), 7.11 (2H, d, J=8.4 Hz), 7.75 (2H, d, J=8.4 Hz), 7.86 (2H, d, J=8.4 Hz). IR (KBr) cm^{-1}: 1728, 1634, 1543, 1512, 1441, 1400, 1345, 1304, 1248. *Anal.* Calcd for $C_{28}H_{29}N_5O_9S\cdot 7H_2O$: C, 45.59; H, 5.87; N, 9.49. Found: C, 45.30; H, 5.17; N, 9.58.

2-[(3*S*)-4-[(2*S*)-2-[[4-Amino(hydroxyimino)methyl]benzoyl]amino]-3-(4-methoxyphenyl)propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid Trifluoroacetate (**7c**): A colorless amorphous powder, $[\alpha]_D^{20}$ +55.4° (c=0.81, MeOH). ^{1}H-NMR (D_2O) δ: 2.15—3.20 (6H, m), 3.53 (3H, s), 3.56—4.10 (4H, m), 3.76 (3H, s), 4.98 (1H, t, J=6.5 Hz), 5.00—5.18 (1H, m), 6.90 (2H, d, J=8.6 Hz), 7.19 (2H, d, J=8.6 Hz), 7.74 (2H, d, J=8.4 Hz), 7.86 (2H, d, J=8.4 Hz). IR (KBr) cm^{-1}: 1730, 1640, 1510, 1440, 1250, 1200, 1180, 1140. *Anal.* Calcd for $C_{27}H_{31}N_5O_9\cdot CF_3COOH\cdot H_2O$: C, 49.65; H, 4.88; N, 9.98. Found: C, 49.72; H, 4.88; N, 10.14.

2-[(3*S*)-3-(2-Methoxy-2-oxoethyl)-4-[(2*S*)-3-(4-methoxyphenyl)-2-[[4-(2-oxo-2,3-dihydro-1,2λ^4,3,5-oxathiadiazol-4-yl)benzoyl]amino]propanoyl]-2-oxopiperazinyl]acetic Acid (**7f**): A colorless amorphous powder, $[\alpha]_D^{20}$ +58.6° (c=0.60, MeOH). ^{1}H-NMR (DMSO-d_6+D_2O) δ: 2.50—4.20 (10H, m), 3.49 (3H, s), 3.70 (3H, s), 4.90 (1H, t, J=5.4 Hz), 4.96—5.22 (1H, m), 6.81 (2H, d, J=8.4 Hz), 7.22 (2H, d, J=8.4 Hz), 7.80—8.10 (4H, m). *Anal.* Calcd for $C_{27}H_{29}N_5O_{10}S\cdot 1.5H_2O$: C, 50.46; H, 5.02; N, 10.90. Found: C, 50.76; H, 5.08; N, 11.07.

2-[(3*S*)-3-(2-Methoxy-2-oxoethyl)-4-[(2*S*)-3-(4-methoxyphenyl)-2-[[4-(5-methyl-1,2,4-oxadiazol-3-yl)benzoyl]amino]propanoyl]-2-oxopiperazinyl]acetic Acid (**7g**): Colorless crystals (MeOH–Et_2O), mp 165—166 °C. $[\alpha]_D^{20}$ +61.3° (c=0.73, MeOH). ^{1}H-NMR ($CDCl_3$+D_2O) δ: 2.67 (3H, s), 2.30—3.30 (6H, m), 3.62 (3H, s), 3.70—3.90 (2H, m), 3.79 (3H, s), 4.10 (2H, d, J=5.8 Hz), 5.05 (1H, t, J=5.4 Hz), 5.25—5.45 (1H, m), 6.83 (2H, d, J=8.6 Hz), 7.14 (2H, d, J=8.6 Hz), 7.89 (2H, d, J=8.4 Hz), 8.15 (2H, d, J=8.4 Hz). IR (KBr) cm^{-1}: 1740, 1660, 1540, 1510, 1450, 1250, 1180. *Anal.* Calcd for $C_{29}H_{31}N_5O_9$: C, 58.68; H, 5.29; N, 11.80. Found: C, 58.43; H, 5.02; N, 11.77.

2-[(3*S*)-3-(2-Methoxy-2-oxoethyl)-4-[(2*S*)-3-(4-methoxyphenyl)-2-[[4-(5-phenyl-1,2,4-oxadiazol-3-yl)benzoyl]amino]propanoyl]-2-oxopiperazinyl]acetic Acid (**7h**): Colorless crystals (MeOH–Et_2O), mp 179—181 °C. $[\alpha]_D^{20}$ +56.7° (c=0.97, MeOH). ^{1}H-NMR (DMSO-d_6) δ: 2.65—2.85 (2H, m), 2.90—4.25 (8H, m), 3.50 (3H, s), 3.70 (3H, s), 4.91 (1H, t, J=6.0 Hz), 5.00—5.20 (1H, m), 6.83 (2H, d, J=8.4 Hz), 7.24 (2H, d, J=8.4 Hz), 7.60—7.85 (3H, m), 8.03 (2H, d, J=8.4 Hz), 8.10—8.27 (2H, m), 8.18 (2H, d, J=8.4 Hz), 8.98 (1H, d, J=8.0 Hz). IR (KBr) cm^{-1}: 1730, 1660, 1610, 1540, 1510, 1490, 1450, 1410, 1350, 1300, 1270, 1250, 1190, 1030, 735. *Anal.* Calcd for $C_{34}H_{33}N_5O_9$: C, 62.28; H, 5.07; N, 10.68. Found: C, 62.13; H, 5.00; N, 10.64.

2-[(3*S*)-3-(2-Methoxy-2-oxoethyl)-4-[(2*S*)-3-(4-methoxyphenyl)-2-[[4-(5-trifluoromethyl-1,2,4-oxadiazol-3-yl)benzoyl]amino]propanoyl]-2-oxopiperazinyl]acetic Acid (**7i**): A colorless amorphous powder, $[\alpha]_D^{20}$ +54.2° (c=0.98, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 2.45—3.35 (6H, m), 3.62 (3H, s), 3.65—3.95 (2H, m), 3.78 (3H, s), 4.08 (1H, s), 4.10 (1H, s), 5.06 (1H, t, J=5.4 Hz), 5.20—5.45 (1H, m), 6.83 (2H, d, J=7.6 Hz), 7.15 (2H, d, J=7.6 Hz), 7.35—7.40 (1H, m), 7.94 (2H, d, J=7.2 Hz), 8.20 (2H, d, J=7.2 Hz). IR (KBr) cm^{-1}: 1740, 1640, 1510, 1250, 1220, 1180, 1160, 990. *Anal.* Calcd for $C_{29}H_{28}F_3N_5O_9\cdot 0.5H_2O$: C, 53.05; H, 4.45; N, 10.67. Found: C, 53.23; H, 4.32; N, 10.69.

2-[(3*S*)-3-(2-Methoxy-2-oxoethyl)-4-[(2*S*)-3-(4-methoxyphenyl)-2-[[4-(1,2,4-oxadiazol-3-yl)benzoyl]amino]propanoyl]-2-oxopiperazinyl]acetic Acid (**7j**): Colorless crystals (EtOAc–iso-Pr_2O), mp 175—177 °C. $[\alpha]_D^{20}$ +61.7° (c=1.01, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 2.45—3.95 (8H, m), 3.62 (3H, s), 3.77 (3H, s), 4.03 (1H, d, J=17.2 Hz), 4.17 (1H, d, J=17.2 Hz), 5.07 (1H, t, J=5.4 Hz), 5.15—5.45 (1H, m), 6.83 (2H, d, J=8.6 Hz), 7.15 (2H, d, J=8.6 Hz), 7.23—7.40 (1H, m), 7.92 (2H, d, J=8.8 Hz), 8.20 (2H, d, J=8.8 Hz), 8.81 (1H, s). IR (KBr) cm^{-1}: 3390, 1730, 1660. *Anal.* Calcd for $C_{28}H_{29}N_5O_9\cdot 0.3H_2O$: C, 57.49; H, 5.10; N, 11.97. Found: C, 57.57; H, 5.00; N, 11.86.

2-[(3*S*)-4-[(2*S*)-2-[[4-(5-Cyano-1,2,4-oxadiazol-3-yl)benzoyl]amino]-3-(4-methoxyphenyl)propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (**7k**): Colorless crystals, mp 177—179 °C. $[\alpha]_D^{20}$ +59.6° (c=0.66, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 2.40—3.35 (4H, m), 2.98 (2H, d, J=5.6 Hz), 3.61 (3H, s), 3.65—3.90 (2H, m), 3.76 (3H, s), 4.08 (1H, s), 4.12 (1H, s), 5.05 (1H, t, J=5.1 Hz), 5.20—5.42 (1H, m), 6.82 (2H, d, J=8.6 Hz), 7.13 (2H, d, J=8.6 Hz), 7.38 (1H, d, J=8.4 Hz), 7.94 (2H, d, J=8.4 Hz), 8.14 (2H, d, J=8.4 Hz). IR (KBr) cm^{-1}: 1730, 1650, 1610, 1510. *Anal.* Calcd for $C_{29}H_{28}N_6O_9$: C, 57.61; H, 4.67; N, 13.90. Found: C, 57.42; H, 4.69; N, 13.68.

2-[(3*S*)-4-[(2*S*)-2-[[4-(5-Methoxy-1,2,4-oxadiazol-3-yl)benzoyl]amino]-3-(4-methoxyphenyl)propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (**7l**): A colorless amorphous powder, $[\alpha]_D^{20}$ +60.8° (c=0.99, MeOH). ^{1}H-NMR (DMSO-d_6) δ: 2.50—4.20 (10H, m), 3.49 (3H, s), 3.70 (3H, s), 4.28 (3H, s), 4.90 (1H, t, J=5.8 Hz), 5.00—5.20 (1H, m), 6.87 (2H, d, J=8.6 Hz), 7.22 (2H, d, J=8.6 Hz), 7.85—8.10 (4H, m), 8.94 (1H, d, J=7.6 Hz). IR (KBr) cm^{-1}: 1780, 1730, 1660, 1610, 1510, 1380, 1250. *Anal.* Calcd for $C_{29}H_{31}N_5O_{10}\cdot 0.1H_2O$: C, 56.97; H, 5.14; N, 11.45. Found: C, 56.72; H, 5.13; N, 11.45.

2-[(3*S*)-3-(2-Methoxy-2-oxoethyl)-4-[(2*S*)-3-(4-methoxyphenyl)-2-[[4-(5-oxo-4,5-dihydro-1,2,4-thiadiazol-3-yl)benzoyl]amino]propanoyl]-2-oxopiperazinyl]acetic Acid (7d) A suspension of **5** (1.8 g, 3.0 mmol) and 10% Pd–C (0.32 g) in MeOH (80 ml) was hydrogenated under balloon pressure for 1 h at room temperature. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to give amine. Then, to a solution of this amine in DMF (18 ml) were added 4-(5-oxo-4,5-dihydro-1,2,4-thiadiazol-3-yl)benzoic acid **39** (0.74 g, 3.32 mmol) and EDC (0.76 g, 3.6 mmol) at room temperature. After being stirred for 1 h, the mixture was diluted with EtOAc, washed with 5% aqueous $KHSO_4$, dried over $MgSO_4$, and concentrated under reduced pressure to give crude **6d**.

Then, a mixture of **6d**, CH_2Cl_2 (5.0 ml) and TFA (5.0 ml) was stirred at room temperature for 2 h. The mixture was concentrated under reduced pressure and the residue was recrystallized from EtOH to give **7d** (0.87 g, 47%) as colorless crystals, mp 224—226 °C. $[\alpha]_D^{20}$ +8.0° (c=0.69, DMSO). ^{1}H-NMR (DMSO-d_6+D_2O) δ: 2.62—2.88 (2H, m), 2.88—4.20 (8H, m), 3.48 (3H, s), 3.69 (3H, s), 4.89 (1H, t, J=6.2 Hz), 4.98—5.20 (1H, m), 6.81 (2H, d, J=8.5 Hz), 7.21 (2H, d, J=8.5 Hz), 7.93 (2H, d, J=8.5 Hz), 8.01 (2H, d, J=8.5 Hz). IR (KBr) cm^{-1}: 1740, 1710, 1660, 1640, 1510, 1450, 1240. *Anal.* Calcd for $C_{28}H_{29}N_5O_9S\cdot 0.5H_2O$: C, 54.19; H, 4.87; N, 11.28. Found: C, 54.24; H, 4.73; N, 11.38.

2-[(3*S*)-4-[(2*S*)-2-[[4-[Amino[(methoxycarbonyl)oxy]imino]methyl]-benzoyl]amino]-3-(4-methoxyphenyl)propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (8a) To a mixture of **6c** (3.50 g, 5.59 mmol), potassium carbonate (0.39 g, 2.80 mmol) and 1,4-dioxane (11.2 ml) was added dropwise methyl chloroformate (0.45 ml, 5.87 mmol) at room

temperature. After being stirred for 1 h at room temperature, the mixture was poured into aqueous 5% $KHSO_4$ and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc) to give ester (3.23 g, 85%) as a yellow amorphous powder. Then, a mixture of the ester, TFA (5.0 ml) and CH_2Cl_2 (5.0 ml) was stirred for 1 h at room temperature. The mixture was concentrated under reduced pressure and the residue was purified by column chromatography (CHP-20, gradient elution: H_2O to 30% aqueous CH_3CN) to give **8a** (2.76 g, 91%) as a colorless amorphous powder, $[\alpha]_D^{20}$ +13.8° (c=0.83, DMSO). ^{1}H-NMR (DMSO-d_6) δ: 2.50—4.50 (10H, m), 3.48 (3H, s), 3.69 (3H, s), 3.78 (3H, s), 4.89 (1H, t, J=6.0 Hz), 4.95—5.20 (1H, m), 6.80 (2H, d, J=8.4 Hz), 6.82—7.04 (2H, m), 7.21 (2H, d, J=8.4 Hz), 7.75 (2H, d, J=8.6 Hz), 7.87 (2H, d, J=8.6 Hz), 8.87 (1H, d, J=7.8 Hz). *Anal.* Calcd for $C_{29}H_{33}N_5O_{11}\cdot H_2O$: C, 53.95; H, 5.46; N, 10.85. Found: C, 54.01; H, 5.48; N, 10.92.

2-[(3*S*)-4-[(2*S*)-2-[[4-[Amino[[(methylsulfanyl)carbonyl]oxy]imino]-methyl]benzoyl]amino]-3-(4-methoxyphenyl)propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (8b) The title compound was prepared from **6c** and methyl chlorothioformate according to the procedure described in the preparation of **8a** as a yellow amorphous powder, $[\alpha]_D^{20}$ +55.0° (c=0.79, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 2.37 (3H, s), 2.40—4.20 (10H, m), 3.57 (3H, s), 3.74 (3H, s), 5.04 (1H, t, J=5.6 Hz), 5.15—5.40 (1H, m), 5.45—5.90 (2H, m), 6.79 (2H, d, J=8.6 Hz), 7.34—7.54 (1H, m), 7.09 (2H, d, J=8.6 Hz), 7.72 (2H, d, J=8.6 Hz), 7.79 (2H, d, J=8.6 Hz). *Anal.* Calcd for $C_{29}H_{33}N_5O_{10}S\cdot 0.5H_2O$: C, 53.37; H, 5.25; N, 10.73. Found: C, 53.40; H, 5.49; N, 10.78.

2-[(3*S*)-4-[(2*S*)-2-[[4-[[(Acetyloxy)imino](amino)methyl]benzoyl]-amino]-3-(4-methoxyphenyl)propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxo-piperazinyl]acetic Acid (8c) The title compound was prepared from **6c** and acetyl chloride according to the procedure described in the preparation of **8a** as a colorless amorphous powder, $[\alpha]_D^{20}$ +13.1° (c=1.00, DMSO). ^{1}H-NMR (DMSO-d_6) δ: 2.14 (3H, s), 2.60—4.55 (10H, m), 3.47 (3H, s), 3.69 (3H, s), 4.89 (1H, t, J=6.0 Hz), 4.95—5.20 (1H, m), 6.80 (2H, d, J=8.4 Hz), 6.80—7.05 (2H, m), 7.21 (2H, d, J=8.4 Hz), 7.77 (2H, d, J=8.4 Hz), 7.87 (2H, d, J=8.4 Hz), 8.87 (1H, d, J=7.8 Hz). *Anal.* Calcd for $C_{29}H_{33}N_5O_{10}\cdot 0.5H_2O$: C, 56.12; H, 5.52; N, 11.28. Found: C, 56.19; H, 5.38; N, 11.19.

2-[(3*S*)-4-[(2*S*)-2-[[4-[Amino[[(ethylamino)carbonyl]oxy]imino]-methyl]benzoyl]amino]-3-(4-methoxyphenyl)propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (8d) The title compound was prepared from **6c** and ethyl isocyanate according to the procedure described in the preparation of **8a** as a colorless amorphous powder, $[\alpha]_D^{20}$ +45.6° (c=1.01, MeOH). ^{1}H-NMR (DMSO-d_6) δ: 1.08 (3H, t, J=7.0 Hz), 2.58—2.83 (2H, m), 2.88—4.25 (10H, m), 3.47 (3H, s), 3.69 (3H, s), 4.89 (1H, t, J=6.0 Hz), 4.95—5.25 (1H, m), 6.72—7.00 (4H, m), 7.23 (2H, d, J=8.8 Hz), 7.30—7.45 (1H, m), 7.80—8.00 (4H, m), 8.87 (1H, d, J=8.0 Hz). IR (KBr) cm^{-1}: 3400, 1730, 1640, 1510, 1440, 1300, 1250, 1180. *Anal.* Calcd for $C_{30}H_{36}N_6O_{10}\cdot 2H_2O$: C, 51.87; H, 5.80; N, 12.10. Found: C, 52.01; H, 5.74; N, 12.00.

Methyl 4-[Amino(hydroxyimino)methyl]benzoate (10) A mixture of methyl 4-cyanobenzoate (16.5 g, 102 mmol), hydroxylamine hydrochloride (7.2 g, 102 mmol), sodium hydrogencarbonate (8.82 g, 110 mmol) and MeOH (200 ml) was stirred for 30 min at room temperature and refluxed for 3 h. After being cooled, water (400 ml) was added to the mixture. The precipitated crystals were collected, washed with water and Et_2O and dried to provide **10** (16.1 g, 83%) as colorless needles, mp 171—173 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.86 (3H, s), 5.93 (2H, s), 7.82 (2H, d, J=8.4 Hz), 7.95 (2H, d, J=8.4 Hz), 9.91 (1H, s). IR (KBr) cm^{-1}: 1717, 1651, 1609, 1593, 1431, 1323, 1288, 1186, 1113, 951. *Anal.* Calcd for $C_9H_{10}N_2O_3$: C, 55.67; H, 5.19; N, 14.43. Found: C, 55.57; H, 5.22; N, 14.39.

Methyl 4-(5-Oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)benzoate (12) To a suspension of **10** (3.95 g, 20.3 mmol) in 1,4-dioxane (20 ml) was added CDI (4.0 g, 24.7 mmol) at room temperature and the mixture was stirred for 30 min at 110 °C. After being cooled, solvent was removed by evaporation and then water was added to the residue. The mixture was adjusted to pH 2 with 3 N HCl, and the precipitate was collected and washed with water. Recrystallization from DMF–EtOAc gave **12** (3.1 g, 69%) as colorless crystals, mp 278—280 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.88 (3H, s), 7.94 (2H, d, J=8.4 Hz), 8.06 (2H, d, J=8.4 Hz). IR (KBr) cm^{-1}: 1780, 1690, 1530, 1470, 1430, 1305, 1290. *Anal.* Calcd for $C_{10}H_8N_2O_4$: C, 54.55; H, 3.66; N, 12.72. Found: C, 54.42; H, 3.80; N, 12.95.

Methyl 4-(5-Thioxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)benzoate (13) To a suspension of **10** (4.00 g, 20.6 mmol) and TCDI (90%, 4.28 g, 21.6 mmol) in 1,4-dioxane (20 ml) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (98%, 3.35 g, 21.6 mmol) at room temperature, and the mixture was stirred for 30 min at 80 °C. After being cooled, the solvent was removed by evaporation and then water was added to the residue. The mixture was adjusted to pH 2 with 3 N HCl, and the precipitate was collected, washed with water, and dried to provide **13** (4.74 g, 97%) as colorless crystals, mp 202—203 °C (dec.). ^{1}H-NMR ($CDCl_3$+DMSO-d_6) δ: 3.96 (3H, s), 8.02 (2H, d, J=8.5 Hz), 8.17 (2H, J=8.5 Hz). IR (KBr) cm^{-1}: 1790, 1694, 1597, 1553, 1464, 1435, 1416, 1323, 1287, 1148, 1117. *Anal.* Calcd for $C_{10}H_8N_2O_3S$: C, 50.84; H, 3.41; N, 11.86. Found: C, 51.05; H, 3.32; N, 12.02.

4-(5-Thioxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)benzoic Acid (15) A suspension of **13** (4.74 g, 20 mmol), 2 N NaOH (30 ml) and MeOH (50 ml) was stirred for 16 h at room temperature, and the solvent was removed by evaporation. Water was added to the residue, and the mixturte was adjusted to pH 2 with 3 N HCl. The precipitate was collected, washed with water, and dried to give **15** (2.27 g, 51%) as colorless crystals, mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 8.01 (2H, d, J=8.0 Hz), 8.12 (2H, d, J=8.0 Hz). IR (KBr) cm^{-1}: 1690, 1590, 1550, 1480, 1430, 1320, 1290. *Anal.* Calcd for $C_9H_6N_2O_3S$: C, 48.64; H, 2.74; N, 12.61. Found: C, 48.80; H, 2.84; N, 12.84.

4-(5-Oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)benzoic Acid (14) The title compound was prepared from **12** according to the procedure described in the preparation of **15** as colorless crystals (DMF–EtOAc), mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 7.93 (2H, d, J=8.4 Hz), 8.11 (2H, d, J=8.4 Hz). IR (KBr) cm^{-1}: 1800, 1740, 1690, 1550, 1430, 1320, 1290, 950, 760. *Anal.* Calcd for $C_9H_6N_2O_4\cdot 0.1$DMF: C, 52.33; H, 3.16; N, 13.76. Found: C, 52.14; H, 3.29; N, 13.89.

Methyl 4-(5-Methyl-1,2,4-oxadiazol-3-yl)benzoate (16) To a solution of **10** (1.94 g, 10 mmol) in CH_2Cl_2 (20 ml) was added acetic anhydride (2.25 ml, mmol) at room temperature, and the mixture was stirred for 9 h at 80 °C. After being cooled, the mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc) to give **16** (1.90 g, 87%) as colorless crystals, mp 146—148 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.68 (3H, s), 3.96 (3H, s), 8.15 (4H, s). IR (KBr) cm^{-1}: 1720, 1600, 1440, 1410, 1280, 1265, 1120, 1110, 730. *Anal.* Calcd for $C_{11}H_{10}N_2O_3$: C, 60.55; H, 4.62; N, 12.84. Found: C, 60.37; H, 4.61; N, 12.90.

Methyl 4-(5-Trifluoromethyl-1,2,4-oxadiazol-3-yl)benzoate (17) To a suspension of **10** (1.94 g, 10 mmol) in CH_2Cl_2 (20 ml) was added trifluoroacetic anhydride (3.15 g, 15 mmol) at room temperature. After being stirred for 3 h at room temperature, the mixture was diluted with toluene and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc) to give **17** (2.0 g, 74%) as colorless crystals (hexane), mp 78—79 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.97 (3H, s), 8.20 (4H, s). IR (KBr) cm^{-1}: 1720, 1620, 1530, 1430, 1320, 1280, 1220, 1160, 1110, 1090, 995, 870, 760, 710. *Anal.* Calcd for $C_{11}H_7F_3N_2O_3$: C, 48.54; H, 2.59; N, 10.29. Found: C, 48.59; H, 2.60; N, 10.48.

Methyl 4-(5-Phenyl-1,2,4-oxadiazol-3-yl)benzoate (18) To a mixture of **10** (1.94 g, 10 mmol), pyridine (1.04 ml, 13 mmol) and xylene (50 ml) was added benzoyl chloride (1.39 ml, 12 mmol), and the mixture was stirred for 1 h at 140 °C. After being cooled, the mixture was diluted with EtOAc, washed with water, saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was recrystallized from hexane to provide **18** (2.54 g, 91%) as colorless crystals, mp 151—152 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.97 (3H, s), 7.50—7.70 (3H, m), 8.10—8.35 (6H, m). IR (KBr) cm^{-1}: 1705, 1688, 1613, 1534, 1433, 1325, 1290, 1207, 1192, 1161. *Anal.* Calcd for $C_{16}H_{12}N_2O_3$: C, 68.56; H, 4.32; N, 9.99. Found: C, 68.08; H, 4.35; N, 9.91.

4-(5-Methyl-1,2,4-oxadiazol-3-yl)benzoic Acid (19) The title compound was prepared from **16** according to the procedure described in the preparation of **15** as colorless crystals, mp 271—273 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.69 (3H, s), 8.11 (4H, s). IR (KBr) cm^{-1}: 1688, 1597, 1566, 1535, 1431, 1416, 1319, 1288, 1260. *Anal.* Calcd for $C_{10}H_8N_2O_3$: C, 58.82; H, 3.95; N, 13.72. Found: C, 58.83; H, 4.01; N, 13.93.

4-(5-Trifluoromethyl-1,2,4-oxadiazol-3-yl)benzoic Acid (20) The title compound was prepared from **17** according to the procedure described in the preparation of **15** as colorless crystals, mp 234—237 °C. ^{1}H-NMR (DMSO-d_6) δ: 8.16 (2H, d, J=8.0 Hz), 8.22 (2H, d, J=8.0 Hz). IR (KBr) cm^{-1}: 1705, 1688, 1534, 1433, 1325, 1291, 1207, 1192, 1161. *Anal.* Calcd for $C_{10}H_5F_3N_2O_3$: C, 46.53; H, 1.95; N, 10.85. Found: C, 46.52; H, 1.91; N, 11.03.

4-(5-Phenyl-1,2,4-oxadiazol-3-yl)benzoic Acid (21) The title compound was prepared from **18** according to the procedure described in the preparation of **15** as colorless crystals, mp 265—267 °C. ^{1}H-NMR (DMSO-d_6) δ: 7.60—7.81 (3H, m), 8.10—8.30 (2H, m), 8.15 (2H, d, J=8.8 Hz),

8.23 (2H, d, J=8.8 Hz). IR (KBr) cm^{-1}: 1700, 1410, 1290, 730. *Anal.* Calcd for $C_{15}H_{10}N_2O_3$: C, 67.67; H, 3.79; N, 10.52. Found: C, 67.35; H, 3.61; N, 10.56.

***tert*-Butyl 4-[Amino(hydroxyimino)methyl]benzoate (23)** A mixture of *tert*-butyl 4-cyanobenzoate (9.0 g, 44.3 mmol), hydroxylamine hydrochloride (3.08 g, 44.3 mmol), $NaHCO_3$ (4.0 g, 48.0 mmol), water (10 ml) and *tert*-BuOH (100 ml) was stirred for 1.5 h at 80 °C. After being cooled, the mixture was diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was recrystallized from hexane–EtOAc (4 : 1) to provide **23** (8.0 g, 76%) as colorless crystals, mp 153—155 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.60 (9H, s), 4.93 (2H, s), 7.68 (2H, d, J=8.8 Hz), 8.01 (2H, d, J=8.8 Hz), 9.30—9.80 (1H, m). IR (KBr) cm^{-1}: 1705, 1670, 1410, 1370, 1320, 1310, 1300, 1170, 1150, 1130, 1120, 950, 870, 850, 780, 710. *Anal.* Calcd for $C_{12}H_{16}N_2O_3$: C, 61.00; H, 6.83; N, 11.86. Found: C, 61.03; H, 6.70; N, 11.90.

***tert*-Butyl 4-(2-Oxo-2,3-dihydro-1,2λ^4,3,5-oxathiadiazol-4-yl)benzoate (24)** To a mixture of **23** (2.0 g, 8.46 mmol), pyridine (1.47 ml, 16.9 mmol) and CH_2Cl_2 (17 ml) was added dropwise a solution of thionyl chloride (0.68 ml, 9.31 mmol) in CH_2Cl_2 (17 ml) at −20 °C. After being stirred for 40 min at −5 °C, the mixture was washed with water and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was recrystallized from Et_2O–petroleum ether (1 : 1) to provide **24** (1.77 g, 74%) as colorless crystals, mp 150—152 °C (dec.). ^{1}H-NMR ($CDCl_3$) δ: 1.62 (9H, s), 7.78 (2H, d, J=8.8 Hz), 8.08 (2H, d, J=8.8 Hz). IR (KBr) cm^{-1}: 1705, 1291, 1169. *Anal.* Calcd for $C_{12}H_{14}N_2O_4S$: C, 51.05; H, 5.00; N, 9.92. Found: C, 51.25; H, 4.79; N, 9.92.

4-(2-Oxo-2,3-dihydro-1,2λ^4,3,5-oxathiadiazol-4-yl)benzoic Acid (25) To a solution of **24** (1.0 g, 3.54 mmol) in CH_2Cl_2 (3 ml) was added TFA (3 ml) at room temperature. After being stirred for 1 h at room temperature, the mixture was diluted with toluene and concentrated under reduced pressure to provide **25** (0.93 g, quant.) as a colorless powder. ^{1}H-NMR (DMSO-d_6) δ: 7.98 (2H, d, J=8.6 Hz), 8.12 (2H, d, J=8.6 Hz). IR (KBr) cm^{-1}: 1684, 1422, 1292, 1190. *Anal.* Calcd for $C_8H_6N_2O_4S \cdot 0.25H_2O$: C, 41.65; H, 2.84; N, 12.14. Found: C, 41.83; H, 2.74; N, 11.84.

***tert*-Butyl 4-(1,2,4-Oxadiazol-3-yl)benzoate (26)** A suspension of **23** (4.72 g, 20 mmol) in trimethyl orthoformate (20 ml, 183 mmol) was added $BF_3 \cdot Et_2O$ (2 drops) at room temperature, and the mixture was stirred for 30 min at 110 °C. After being cooled, the mixture was diluted with EtOAc, washed with water and saturated aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was recrystallized from hexane to provide **26** (3.7 g, 75%) as colorless crystals, mp 127—129 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.62 (9H, s), 8.11 (2H, d, J=8.8 Hz), 8.19 (2H, d, J=8.8 Hz), 8.80 (1H, s). IR (KBr) cm^{-1}: 3125, 2975, 1705, 1580, 1530, 1410, 1370, 1310, 1290, 1270, 1250, 1180, 1160, 1120, 1105, 890, 845, 730. *Anal.* Calcd for $C_{13}H_{14}N_2O_3$: C, 63.40; H, 5.73; N, 11.38. Found: C, 63.43; H, 5.71; N, 11.21.

Ethyl 3-[4-(*tert*-Butoxycarbonyl)phenyl]-1,2,4-oxadiazole-5-carboxylate (27) To a mixture of **23** (4.72 g, 20 mmol), pyridine (4.0 ml) and CH_2Cl_2 (40 ml) was added ethyl chloroglyoxylate (2.68 ml, 24 mmol) at room temperature. After being stirred for 24 h at room temperature, the mixture was washed with water and saturated aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was recrystallized from hexane to provide **27** (5.5 g, 87%) as colorless crystals, mp 136—137 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.50 (3H, t, J=7.0 Hz), 1.62 (9H, s), 4.59 (2H, q, J=7.0 Hz), 8.11 (2H, d, J=8.8 Hz), 8.22 (2H, d, J=8.8 Hz). IR (KBr) cm^{-1}: 1750, 1707, 1586, 1561, 1530, 1466, 1370, 1312, 1296, 1190, 1169, 1123, 1013, 849, 725. *Anal.* Calcd for $C_{16}H_{18}N_2O_5$: C, 60.37; H, 5.70; N, 8.80. Found: C, 60.35; H, 5.79; N, 8.84.

***tert*-Butyl 4-(5-Carbamoyl-1,2,4-oxadiazol-3-yl)benzoate (28)** A mixture of **27** (2.0 g, 6.3 mmol), 25% aqueous NH_3 (2 ml) and tetrahydrofuran (100 ml) was stirred for 44 h at room temperature. The mixture was concentrated under reduced pressure and the residue was recrystallized from EtOH to provide **28** (1.0 g, 55%) as colorless crystals, mp 202—205 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.63 (9H, s), 6.05—6.22 (1H, m), 6.95—7.12 (1H, m), 8.12 (2H, d, J=8.8 Hz), 8.18 (2H, d, J=8.8 Hz). IR (KBr) cm^{-1}: 1710, 1690, 1610, 1580, 1400, 1370, 1330, 1310, 1300, 1280, 1230, 1170, 1120, 1110. *Anal.* Calcd for $C_{14}H_{15}N_3O_4$: C, 58.13; H, 5.23; N, 14.53. Found: C, 57.98; H, 5.33; N, 14.45.

***tert*-Butyl 4-(5-Cyano-1,2,4-oxadiazol-3-yl)benzoate (29)** To a mixture of **28** (0.58 g, 2.0 mmol), pyridine (0.4 g, 5.0 mmol) and 1,4-dioxane (8 ml) was added trifluoroacetic anhydride (0.34 ml, 2.4 mmol) at 0 °C. After being stirred for 13 h at room temperature, the mixture was diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane–EtAOc, 2 : 1) to provide **29** (0.18 g, 33%) as colorless crystals (hexane), mp 102—104 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.62 (9H, s), 8.16 (4H, s). IR (KBr) cm^{-1}: 2250, 1705, 1550, 1310, 1300, 1250, 1160, 1130, 730. *Anal.* Calcd for $C_{14}H_{13}N_3O_3$: C, 61.99; H, 4.83; N, 15.49. Found: C, 61.86; H, 4.87; N, 15.44.

4-(1,2,4-Oxadiazol-3-yl)benzoic Acid (30) To a solution of **26** (0.49 g, 2.0 mmol) in CH_2Cl_2 (4 ml) was added TFA (4 ml) at room temperature. After being stirred for 1 h at room temperature, the mixture was diluted with toluene and concentrated under reduced pressure. The residue was recrystallized from EtOAc–MeOH to provide **30** (0.33 g, 90%) as colorless crystals, mp 216 °C. ^{1}H-NMR (DMSO-d_6) δ: 8.13 (2H, d, J=8.8 Hz), 8.19 (2H, d, J=8.8 Hz), 9.78 (1H, s). IR (KBr) cm^{-1}: 1680, 1580, 1535. *Anal.* Calcd for $C_9H_6N_2O_3$: C, 56.85; H, 3.18; N, 14.73. Found: C, 56.87; H, 3.14; N, 14.64.

4-(5-Cyano-1,2,4-oxadiazol-3-yl)benzoic Acid (31) The title compound was prepared from **29** according to the procedure described in the preparation of **30** as colorless crystals (Et_2O), mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 8.17 (4H, s). IR (KBr) cm^{-1}: 1680, 1580, 1550, 1530, 1430, 1410, 1340, 1320, 1290, 1250. *Anal.* Calcd for $C_{10}H_5N_3O_3 \cdot 0.1H_2O$: C, 55.36; H, 2.42; N, 19.37. Found: C, 55.67; H, 2.67; N, 18.78.

***tert*-Butyl 4-(5-Methoxy-1,2,4-oxadiazol-3-yl)benzoate (33)** To a solution of **23** (2.36 g, 10 mmol) in 1,4-dioxane (25 ml) was added CDI (2.1 g, 13 mmol) at room temperature and the mixture was stirred for 30 min at 90 °C. After being cooled, the mixture was concentrated under reduced pressure and the residue was partitioned between EtOAc (250 ml) and 5% aqueous $KHSO_4$. Then, an excess solution of diazomethane in diethyl ether (15—20 mmol) was added dropwise to the organic layer at room temperature. After being kept standing for 18 h, the mixture was washed with water and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane–EtAOc, 2 : 1) to provide **33** (1.40 g, 51%) as colorless crystals (hexane–EtAOc, 8 : 1), mp 133—134 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.62 (9H, s), 4.29 (3H, s), 8.07 (4H, s). IR (KBr) cm^{-1}: 1700, 1620, 1580, 1500, 1410, 1380, 1370, 1320, 1310, 1290, 1250, 1170, 1120, 1110, 1040, 1020, 980, 960, 880, 870, 850, 750, 710. *Anal.* Calcd for $C_{14}H_{16}N_2O_4$: C, 60.86; H, 5.84; N, 10.14. Found: C, 60.96; H, 5.76; N, 10.27.

4-(5-Methoxy-1,2,4-oxadiazol-3-yl)benzoic Acid (35) The title compound was prepared from **33** according to the procedure described in the preparation of **30** as colorless crystals (hexane), mp 240—241 °C. ^{1}H-NMR ($CDCl_3$) δ: 4.28 (3H, s), 8.05 (2H, d, J=8.8 Hz), 8.11 (2H, d, J=8.8 Hz). IR (KBr) cm^{-1}: 1690, 1620, 1580, 1530, 1490, 1410, 1380, 1320, 1290, 750. *Anal.* Calcd for $C_{10}H_8N_2O_4$: C, 54.55; H, 3.66; N, 12.72. Found: C, 54.53; H, 3.51; N, 12.76.

Methyl 4-(5-Oxo-4,5-dihydro-1,2,4-thiadiazol-3-yl)benzoate (37) To a mixture of methyl 4-[amino(imino)methyl]benzoate hydrochloride **36** (7.82 g, 36.4 mmol), sodium carbonate (12.2 g, 115 mmol), H_2O (50 ml) and CH_2Cl_2 (50 ml) was added dropwise chlorocarbonylsulfenyl chloride (98%, 5.0 g, 37.4 mmol) at 0 °C with vigorous stirring. After being stirred for 1 h at room temperature, the mixture was adjusted to pH 2 with conc. HCl and concentrated under reduced pressure. Water was added to the residue, and the precipitate was collected, washed with water, and dried to give **37** (3.8 g, 44%) as a yellow powder. Recrystallization from DMF–EtOAc gave **37** as colorless crystals, mp 263—265 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.89 (3H, s), 8.09 (4H, s). IR (KBr) cm^{-1}: 1720, 1715, 1665, 1655, 1280, 1110. *Anal.* Calcd for $C_{10}H_8N_2O_3S$: C, 50.84; H, 3.41; N, 11.86. Found: C, 50.73; H, 3.35; N, 11.84.

Methyl 4-(5-Thioxo-4,5-dihydro-1,2,4-thiadiazol-3-yl)benzoate (38) A mixture of **36** (6.14 g, 28.6 mmol), CS_2 (5.7 g, 75.0 mmol), sulfur (1.2 g, 37.4 mmol), 28% sodium methoxide (17.4 g, 90.2 mmol) and MeOH (30 ml) was refluxed for 6 h. After being cooled, the mixture was concentrated under reduced pressure. Water was added to the residue and the mixture was adjusted to pH 2 with 3 N HCl. The precipitate was collected and recrystallized from MeOH to give **38** (3.3 g, 46%) as colorless needles, mp 201—203 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.90 (3H, s), 8.10 (2H, d, J=8.6 Hz), 8.18 (2H, d, J=8.6 Hz). IR (KBr) cm^{-1}: 3200, 1710, 1540, 1450, 1435, 1320, 1290, 1220, 1200, 1120, 1070. *Anal.* Calcd for $C_{10}H_8N_2O_2S_2 \cdot 0.5H_2O$: C, 45.96; H, 3.47; N, 10.72. Found: C, 46.04; H, 3.07; N, 10.78.

4-(5-Oxo-4,5-dihydro-1,2,4-thiadiazol-3-yl)benzoic Acid (39) The title compound was prepared from **37** according to the procedure described in the preparation of **15** as colorless crystals (DMF–EtOAc), mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 8.06 (4H, s). IR (KBr) cm^{-1}: 1694, 1667, 1454, 1424, 1306, 1288. *Anal.* Calcd for $C_9H_6N_2O_3S \cdot 0.1DMF$: C, 48.66; H, 2.94; N, 12.81. Found: C, 48.66; H, 3.15; N, 13.05.

4-(5-Thioxo-4,5-dihydro-1,2,4-thiadiazol-3-yl)benzoic Acid (40) The title compound was prepared from **38** according to the procedure described

in the preparation of **15** as colorless crystals (DMF–MeOH), mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 8.06 (2H, d, J=8.4 Hz), 8.16 (2H, d, J=8.4 Hz). IR (KBr) cm^{-1}: 1703, 1543, 1447, 1427, 1325, 1304, 1289, 1233. *Anal.* Calcd for $C_9H_6N_2O_2S_2 \cdot 0.8S$: C, 40.96; H,2.29; N, 10.62. Found: C, 40.71; H, 2.39; N, 10.61.

In Vitro* Platelet Aggregation Studies** Blood was collected by aortic puncture from anesthetized guinea pigs with sodium pentobarbital. Blood was withdrawn into a plastic syringe containing 3.15% sodium citrate (1 : 10 citrate/blood, v/v). PRP and platelet-poor plasma (PPP) were obtained by centrifugation at 1000×g*** for 3—5 s and 1000×***g*** for 20 min at room temperature respectively. The platelet count in PRP was adjusted to $4\times10^5/\mu$l using an automatic blood cell counter (Sysmex E2500, Toaiyoudenshi Co., Tokyo, Japan). Platelet aggregation was measured using an 8 channel aggregometer (Hematracer VI, Niko Bioscience, Tokyo, Japan). PRP (250 μl), in a cuvette stirred at 1000 rpm, was prewarmed for 2 min at 37 °C with various concentrations of test compounds (25 μl). The change in light transmittance was measured after the addition of aggregating agents (25 μl) to the cuvette. Submaximal concentration of aggregating agents were used in each experiment.

***Ex Vivo* Platelet Aggregation Studies** Male Hartley guinea pigs (300—400 g) were used. At various times after *p.o.* administration of test compounds, blood was collected, and PRP and PPP were prepared as described for the *in vitro* study. ADP (20 μl) was added to the cuvette containing the prewarmed PRP (220 μl). In order to eliminate the possible influence of the sensitivity differences of guinea pig platelets to ADP, which is dependent on the animal lot and experimental conditions including drug administration protocol, two or three vehicle-treated animals were used as a control at each measuring point. The percentage inhibition of platelet aggregation in drug-treated animals was determined by comparison with the aggregation in the controls at each point.

Metabolic Conversion Test The liver (28 g) and small intestine (9 g) from Std Hartley guinea pigs were homogenized with ice-cooled saline (5 and 2.5 ml, respectively) and then centrifugated. The supernatant were collected for analysis. The plasma was prepared by centrifugation of heparinized whole blood. The compounds **7a** and **7d** (prepared as a 100 μg/ml solution in saline, 100 μl) were incubated with each homogenate (900 μl) and plasma (900 μl) at 37 °C for 1 h. The incubation was terminated by addition of 3 ml of acetonitrile. Then, the samples were centrifugated at 10000 rpm for 15 min, and the supernatants obtained were analyzed by HPLC. HPLC analysis was performed using a Develosil ODS HG-5 reverse phase column (4.6×250 mm) with a mixture of acetonitrile and 20 mM phosphate buffer adjusted pH 3 with phosphoric acid containing 5 mM 1-heptanesulfonate (25 : 75, v/v) as a mobile phase at a flow rate of 1 ml/min, and UV detection at 230 nm. The retention times for **7a**, **7d** and their parent compound **4** were 31.0, 45.8 and 9.0, respectively.

References and Notes

1) Plow E. F., Pierschbacher M. D., Ruoslahti E., Marguerie G. A., Ginsberg M. H., *Proc. Natl. Acad. Sci. U.S.A.*, **82**, 8057—8061 (1985).
2) Gartner T. K., Bennett J. S., *J. Biol. Chem.*, **260**, 11891—11894 (1985).
3) Plow E. F., Pierschbacher M. D, Ruoslahti E., Marguerie G., Ginsberg M. H., *Blood*, **70**, 110—115 (1987).
4) Phillips D. R., Charo I. F., Parise L. V., Fitzgerald L. A., *Blood*, **71**, 831—843 (1988).
5) Gan Z.-R., Gould R. J., Jacobs J. W., Friedman P. A., Polokoff M. A., *J. Biol. Chem.*, **263**, 19827—19832 (1988).
6) Scarborough R. M., Naughton M. A., Teng W., Rose J. W., Phillips D. R., Nannizzi L., Arfsten A., Campbell A. M., Charo I. F., *J. Biol. Chem.*, **268**, 1066—1073 (1993).
7) Samanen J., Ali F., Romoff T., Calvo R., Sorenson E., Vasko J., Storer B., Berry D., Bennett D., Strohsacker M., Powers D., Stadel J., Nichols A., *J. Med. Chem.*, **34**, 3114—3125 (1991).
8) McDowell R. S., Blackburn B. K., Gadek T. R., McGee L. R., Rawson T., Reynolds M. E., Robarge K. D., Somers T. C., Thorsett E. D., Tischler M., Webb R. R. II, Venuti M. C., *J. Am. Chem. Soc.*, **116**, 5077—5083 (1994).
9) Egbertson M. S., Chang C. T.-C., Duggan M. E., Gould R. J., Halczenko W., Hartman G. D., Laswell W. L., Lynch J. J., Jr., Lynch R. J., Manno P. D., Naylor A. M., Prugh J. D., Ramjit D. R., Sitko G. R., Smith R. S., Turchi L. M., Zhang G., *J. Med. Chem.*, **37**, 2537—2551 (1994).
10) Zablocki J. A., Rico J. G., Garland R. B., Rogers T. E., Williams K., Schretzman L. A., Rao S. A., Bovy P. R., Tjoeng F. S., Lindmark R. J., Toth M. V., Zupec M. E., McMackins D. E., Adams S. P., Miyano M., Markos C. S., Milton M. N., Paulson S., Herin M., Jacqmin P., Nicholson N. S., Panzer-Knodle S. G., Haas N. F., Page J. D., Szalony J. A., Taite B. B., Salyers A. K., King L. W., Campion J. G., Feigen L. P., *J. Med. Chem.*, **38**, 2378—2394 (1995).
11) Weller T., Alig L., Beresini M., Blackburn B., Bunting S., Hadváry P., Müller M. H., Knopp D., Levet-Trafit B., Lipari M. T., Modi N. B., Müller M., Refino C. J., Schmitt M., Schönholzer P., Weiss S., Steiner B., *J. Med. Chem.*, **39**, 3139—3147 (1996).
12) Eldred C. D., Evans B., Hindley S., Judkins B. D., Kelly H. A., Kitchin J., Lumley P., Porter B., Ross B. C., Smith K. J., Taylor N. R., Wheatcroft J. R., *J. Med. Chem.*, **37**, 3882—3885 (1994).
13) EPIC Investigators, *N. Engl. J. Med.*, **330**, 956—961 (1994).
14) Topol E. J., Califf R. M., Weisman H. F., Ellis S. G., Tcheng J. E., Worley S., Ivanhoe R., George B. S., Fintel D., Weston M., Sigmon K., Anderson K. M., Lee K. L., Willerson J. T., EPIC Investigators, *Lancet*, **343**, 881—886 (1994).
15) Harrington R. A., Schulman S. P., Kleiman N. S., Lincoff A. M., Goldschmidt-Clermont P. J., Joseph D., Sigmon K. N., Parker J., Marchant K., Kitt M. M., *Circulation*, **90** (Suppl. I), I-232. Abstract (1994).
16) Tcheng J. E., Harrington R. A, Kottke-Marchant K., Kleiman N. S., Ellis S. G., Kereiakes D. J., Mick M. J., Navetta F. I., Smith J. E., Worley S. J., Miller J. A., Joseph D. M., Sigmon K. N., Kitt M. M., du Mee C. P., Califf R. M., Topol E. J., *Circulation*, **91**, 2151—2157 (1995).
17) Kereiakes D., Kleiman N., Ambrose J., Cohen M., Rodriguez S., Palabrica T., Herrmann H. C., Sutton J., Weaer W. D., McKee D., Sax F. L., *Circulation*, **90** (Suppl. I), I-21. Abstract (1994).
18) Théroux P., White H., David D., Van de Werf F., Nienaber C. A., Charbonnier B., Erhardt L., Gill J., Hillis W. S., Jennings G., Tan L.-B., Deschenes N., Fitzpatrick V., Sax F. L., *Circulation*, **90** (Suppl. I), I-231. Abstract (1994).
19) Müller, T. H., Schurer H., Waldmann L., Bauer E., Himmelsbach F., Binder K., *Thromb. Haemost.*, **69**, 975, Abstract 1557 (1993).
20) Gante J., Juraszyk H., Raddatz P., Wuriger H., Bernotat-Danielowski S., Melzer G., Rippmann F., *Bioorg. Med. Chem. Lett.*, **6**, 2425—2430 (1996).
21) Kitamura S., Fukushi H., Miyawaki T., Kawamura M., Terashita Z., Sugihara H., Naka T., *Chem. Pharm. Bull.*, **49**, 258—267 (2001).
22) In a pharmacokinetics study of our previous GPIIb/IIIa antagonist TAK-029[23] in rats, the dicarboxyl derivative was not observed in plasma after oral administration of TAK-029. The ester group at the 3-position of the 2-oxopiperazine scaffold was assumed to be stable in plasma, and the ester form (TAK-029) was regarded as the biological active form by itself (unpublished data). Therefore, in the case of **4**, we considered that the ester form (**4**) is the biological active form by itself as well as TAK-029.
23) Sugihara H., Fukushi H., Miyawaki T., Imai Y., Terashita Z., Kawamura M., Fujisawa Y., Kita S., *J. Med. Chem.*, **41**, 489—502 (1998).
24) Boykin D. W., Kumar A., Hall J. E., Bender B. C., Tidwell R. R., *Bioorg. Med. Chem. Lett.*, **6**, 3017—3020 (1996).
25) Shahrokh Z., Lee E., Olivero A. G., Matamoros R. A., Robarge K. D., Lee A., Weise K. J., Blackburn B. K., Powell M. F., *Pharm.Res.*, **15**, 434—441 (1998).
26) Rahmathullah S. M., Hall J. E., Bender B. C., McCurdy D. R., Tidwell R. R., Boykin D. W., *J. Med. Chem.*, **42**, 3994—4000 (1999).
27) Kohara Y., Imamiya E., Kubo K., Wada T., Inada Y., Naka T., *Bioorg Med. Chem. Lett.*, **5**, 1903—1908 (1995).
28) Kohara Y., Kubo K., Imamiya E., Wada T., Inada Y., Naka T., *J. Med. Chem.*, **39**, 5228—5235 (1996).
29) Müller G., *Chem. Ber.*, **18**, 2485—2486 (1885).
30) Clement B., Jung F., *Drug Metab. Dispos.*, **22**, 486—497 (1994).
31) Compound **7a** was not detected in plasma at 8 h after oral administration of **7a**.
32) After addition of **8a** to plasma, about 70% of **8a** was converted to **7c** within 30 min.