2-(3-Pyridyl)thiazolidine-4-carboxamide Derivatives. III.[1,2] Synthesis of Metabolites and Metabolism of 2-(3-Pyridyl)thiazolidine-4-carboxamides YM461 and YM264 as Platelet-Activating Factor (PAF) Receptor Antagonists

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The metabolism of 2-(3-pyridyl)thiazolidine-4-carboxamides YM461[4] and YM264[5] was investigated, and their metabolites were compared with separately synthesized materials by measuring ^{1}H-NMR spectra, mass spectra, and HPLC retention times, and evaluated for platelet activating factor (PAF) antagonistic activity. YM461 was metabolized by two different metabolic pathways (cleavage of the thiazolidine ring and oxidation of the benzyl position), whereas YM264 was metabolized by three metabolic pathways. The minor metabolite M7 from YM264 possessed potent PAF antagonistic activity, as strong as YM264 and this existed as an active metabolite. From pharmacokinetics studies, YM264 was almost completely absorbed from the gastrointestinal tract, but readily metabolized in rats. In dogs, pharmacokinetic parameters of YM264 were significantly improved compared to those in rats, and YM264 tended to show better pharmacokinetics than YM461 due to an extension of the half-life period.

Key words PAF antagonist; metabolite; pharmacokinetics; YM264; YM461

In previous papers,[1,2] we reported the structure–activity relationships and active configuration of 2-(3-pyridyl)thiazolidine-4-carboxamides as platelet activating factor (PAF) antagonists. YM461[4] was synthesized by modification of 2,3-dihydrobenzothiazole derivative (**1**), which was found by random screening, and YM264[5] was designed and synthesized for prevention from oxidation, on the basis of results of metabolism of YM461. YM264 fortunately showed potent activities in various pharmacological tests, in contrast to YM461. In this paper, we report the metabolism and pharmacokinetics of YM461 and YM264, and the syntheses and PAF antagonistic activities of their metabolites.

Synthesis

The metabolites of YM461 and YM264 were isolated from rat urine by HPLC and TLC, and identified by comparison with separately synthesized ones by ^{1}H-NMR, mass spectra, and HPLC. Syntheses of the metabolites are shown in Chart 2. The *S*-methylcysteine derivatives were prepared from *N*-*tert*-butoxycarbonyl-*S*-methylcysteine (**2**). Metabolite **M1** was obtained from **2** by coupling with piperazine and subsequent deprotection of the *N*-*tert*-butoxycarbonyl substituent by trifluoroacetic acid in good yield. Metabolite **M3** was obtained from **4b** by treatment with nicotinoyl chloride. The *S*-oxide derivatives (**M2**, (+)-**M6**, (−)-**M6**) were prepared from the L-*S*-methylcysteine *S*-oxide (**5**), which was obtained from oxidation of **2** with *m*-chloroperbenzoic acid (*m*CPBA). Metabolite **M2** was obtained as a diastereoisomeric mixture, whereas (+)-**M6** and (−)-**M6** were obtained as single isomers, respectively, which were obtained from amides (**6c**) after separation by column chromatography. The pyridine *N*-oxide (**M7**) was synthesized as follows. The aldehyde (**8**), prepared according to Okano's method,[6] was treated with L-cysteine to afford acid (**10**) which was then coupled with piperazine to afford **M7**.

Results and Discussion

The composition of the metabolites of YM461 and YM264 were determined on the basis of the radioactivities in rat urine for 24 h after oral administration of [^{14}C]-YM461 and [^{14}C]-YM264, which were labeled at 2- and 3-position of the piperazine ring.

In the metabolic study of YM461, five metabolites (**M1**—**M5**) were identified. The *N*-dealkylated metabolite **M5** and the cysteine *S*-oxide (**M2**) were detected in approximately equal amounts as chief metabolites. Cysteine derivative **M1** and benzyl alcohol derivatives **M3** and **M4** were detected as minor metabolites. These results proved that YM461 was mainly metabolized by two metabolic pathways. The first is cleavage of the thiazolidine ring, and the other is oxidation of the benzyl position of the phenylpropyl substituent. YM461 exists as *cis* and *trans* diastereoisomers at the thiazolidine

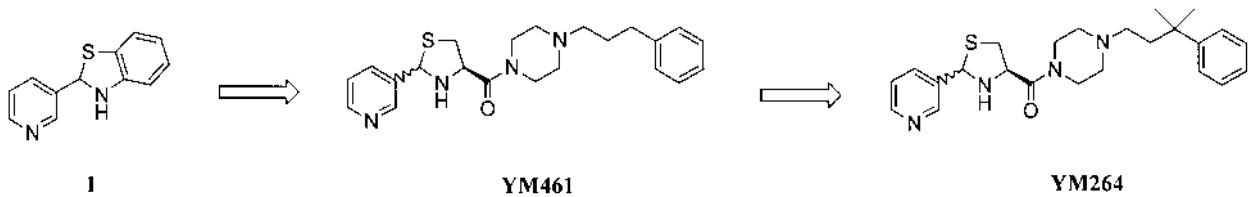

Chart 1

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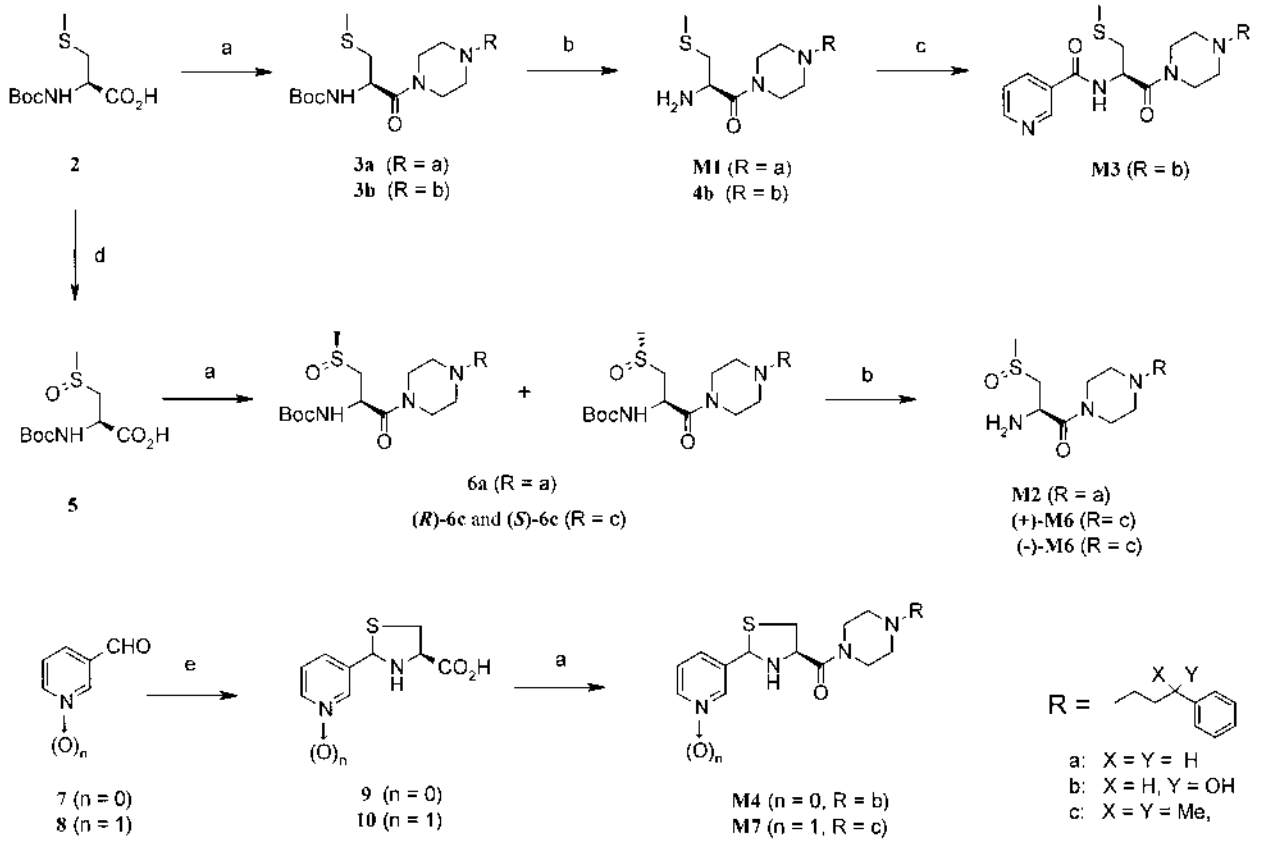

Chart 2

ring and is stabilized by the rapid equilibrium.[2] All cleaved metabolites were *S*-methylated L-cysteine derivatives. For these reasons, this cleavage of thiazolidine was presumably initiated by *S*-methyl conjugation and subsequent interruption of the equilibrium, to leading intermediate A, which was converted into metabolites **M1**, **M2**, and **M3** by hydrolysis and/or oxidation. *S*-(3-Oxo-3-phenylpropyl)mercapturic acid (*S*-(3-oxo-3-phenylpropyl)-*N*-acetylcysteine) was detected as one of the metabolites of the side chain. This metabolite is expected to be generated from a phenylvinylketone *via* through glutathione conjugation, which suggested that another chief metabolite **M5** was generated by retro-Michael type degradation of intermediate B after oxidation of benzylalcohol (**M4**). Nicotinic acid, nicotinealdehyde, and 3-phenylpropylpiperazine were also detected as metabolites.

In the previous paper,[2] it was reported that the 2-(3-pyridyl)thiazolidine skeleton was important for potency of PAF antagonistic activity, and that YM264 was synthesized to prevent the latter metabolic pathway in YM461, by introduction of dimethyl substitutents at the benzyl position. In the metabolic study of YM264, this compound showed a slightly different metabolic pathway and produced fewer metabolites due to the dimethyl substituent, and three metabolites were identified. Metabolite **M5** was also detected as the chief metabolite in YM264, and was more prevalent than in YM461, and 3-methyl-3-phenylbutanol was detected as its counter part. A mixture of (+)-**M6** and (−)-**M6** was detected as the second chief metabolite. These isomers were separated and identified, but there was no chiral selectivity in the oxidation of sulfide *in vivo*. Pyridine *N*-oxide (**M7**) was newly detected as a minor metabolite. These metabolites were generated by distinct metabolic pathways, and production of **M5** and 3-methyl-3-phenylbutanol indicated that direct *N*-dealkylation was the chief metabolic pathway in YM264.

Table 1. Composition of Metabolites in Rat Urine after Oral Administration of [^{14}C]-YM461 and [^{14}C]-YM264

Compd.	Metabolite	Composition (%)[a]
		Rat urine
YM461	**M1**	1.2
	M2	17.2
	M3+**M4**[b]	2.8
	M5	14.8
	YM461	0.8
YM264	**M5**	31.9
	M6	7.5
	M7	0.4
	YM264	0.1

a) Data was obtained from the pool urine (0—24 h) of rat and composition was calculated on the basis of their radioactivities to the dose. *b*) **M3**; minor metabolite, **M4**; major metabolite.

The synthesized metabolites were evaluated for PAF antagonistic activity by measuring the inhibition of PAF-induced platelet aggregation using rabbit platelet-rich plasma

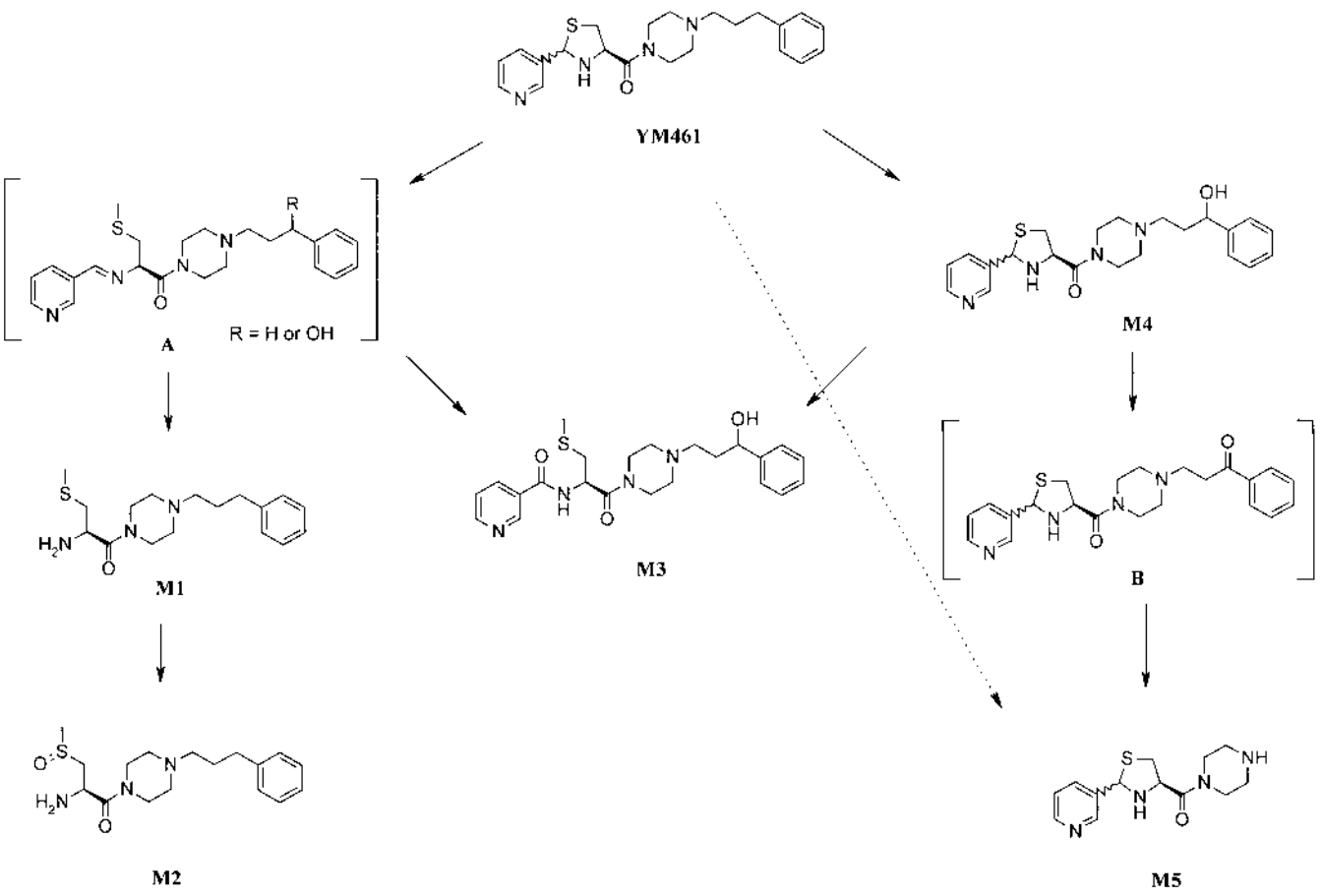

Chart 3

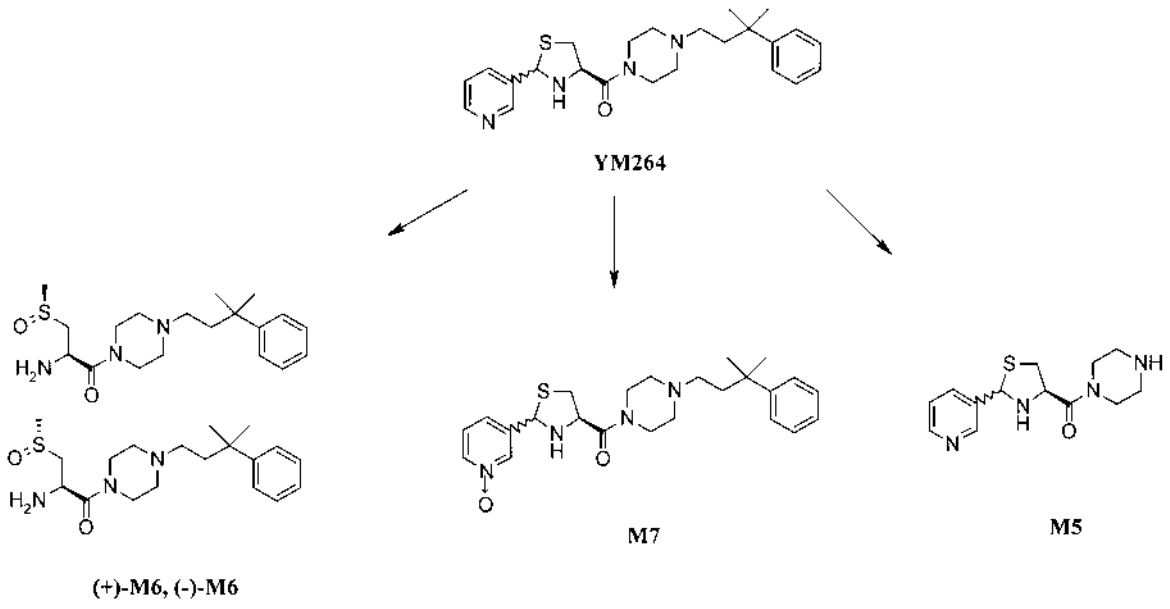

Chart 4

(PRP) and expressed as IC_{50} values (μM). All cleaved metabolites of thiazolidine (**M1**, **M2**, **M3**, (+)-**M6**, (−)-**M6**) lost PAF antagonistic activity. The metabolites containing a 2-(3-pyridyl)thiazolidine skeleton (**M4**) retained activity to some degree. The *N*-dealkylated metabolite **M5** had greatly reduced activity, whereas, pyridine *N*-oxide (**M7**) showed potent PAF antagonistic activity, as great as YM264 and existed as an active metabolite although it was only a minor metabolite.

Next, in order to investigate the effect of the dimethyl substituent, the pharmacokinetic parameters were compared for YM264 and YM461 in rats and dogs. YM264 and YM461

were given by intravenous or oral administration, and the plasma concentration of unchanged drug was determined by gas chromatography–mass spectrometry. After oral administration of YM264 at doses of 3, 10 and 30 mg/kg in rats, the plasma concentrations reached their maxima (C_{max}) within 0.5 h and decreased with half-lives of 0.25—0.86 h, and C_{max} and *AUC* increased non-linearly with the increase of dose. The absolute bioavailability calculated from the *AUC*'s after oral and intravenous administration at a dose of 3 mg/kg was 2.1%, but was recovered 31.8% at a dose of 30 mg/kg. The plasma concentration of YM264 at a dose of 3 mg/kg (*p.o.*) was about 1/4 to 1/6 in comparison with that of YM461, however, YM264 at this dose showed equal duration of the inhibitory effect on PAF-induced hemoconcentration in rats (*in vivo*) to YM461.[4,5]

Concerning the absorption of YM264, after oral administration of [^{14}C]-YM264 (3 mg/kg) to bile-duct cannulated rats, 33% of dosed radioactivity was excreted in the urine and 61% in the bile within 72 h. The sum of biliary and urinary excretion was 94%, which suggested that YM264 was almost completely absorbed from the gastrointestinal tract. However, the ratio of unchanged drug to total radioactivity in plasma was about 10% at 5 min after administration and 0.2% at 1 h and further there was only a very small amount of YM264 in urine. These results suggested that YM264 was readily metabolized in rats.

Whereas the C_{max} and *AUC* of YM264 in dogs were about 3.6 and 35 times higher than those in rats at a dose of 3 mg/kg (*p.o.*), and there were no significant differences in the pharmacokinetic parameters between YM264 and YM461. Parameters in monkeys closely resembled those in dogs. In the intravenous administration, the *AUC* of YM264 was about 1.4 times higher than that of YM461 due to an extension of the half-life period. YM461 and YM264 showed comparatively low absolute bioavailability (25% and 18% (32% at 10 mg/kg (*p.o.*)), however, there is the possibility to improve pharmacokinetics by an adequate administration method to avoid a first pass effect due to their high absorption ratios.

The introduction of the dimethyl substituent to give YM264 prevented oxidation of the benzyl position and extended the half-life period in plasma, however, it simultaneously increased the elimination rate of the *N*-alkyl substituent on the piperazine by metabolism, in contrast to YM461. Because direct *N*-dealkylation occurred as the chief metabolic pathway in YM264, it was not apparent how much retro-Michael degradation occurred in YM461 for **M5**, however, this modification resulted in improved pharmacokinetics in dogs.

In conclusion, the metabolism and pharmacokinetics of YM461 and YM264 were investigated. YM461 was metabolized by two metabolic pathways, and YM264 was metabolized by three metabolic pathways. The introduction of a dimethyl substituent promoted *N*-dealkylation in YM264, however, prolonged the half-life period in plasma in comparison with YM461. YM264 had 2—5 times higher potency in various pharmacological tests, compared to YM461, and was due to improvements in phamacokinetics. From these reasons, YM264 was selected as a promising candidate for treatment of pathogenesis of asthma, shock, glomerulonephritis and is currently under clinical study.

Table 2. PAF Antagonitic Activities of Metabolites of YM461 and YM264

Compd.	Platelet aggregation inhibition IC_{50} (μM)
M1	>100
M2	>100
M3	>100
M4	2.0
M5	50
(+)-**M6**	>100
(−)-**M6**	>100
M7	0.054
YM461	0.071
YM264	0.030

Experimental

Melting points were taken on a Yanaco MP-3 melting point apparatus and are uncorrected. ^{1}H-NMR were taken with a JEOL FX-90Q, FX-100 or GSX-400 spectrometer with tetramethylsilane as an internal standard. Mass spectra were determined on an MS-5970 or JEOL JMS-DX300 mass spectrometer. Column chromatography was carried out on silica gel (Wakogel C-200).

Unless otherwise noted, all reagents and solvents obtained from commercial suppliers were used without further purification. In general, organic extract was dried over Na_2SO_4 or $MgSO_4$ and the solvent was evaporated under

Table 3. Plasma Concentrations of Unchanged YM461 and YM264 by Oral Administration in Rats[a]

Compd.	Dose (mg/kg)	T_{max} (h)	C_{max} (μg/ml)	$t_{1/2}$ (h)	$AUC_{0\to\infty}$ (μg·kg/ml)	Bioav. (%)
YM461	3	0.17	0.256	0.19	0.133	—
YM264	3	0.17	0.058	0.25	0.011	2.1
	10	0.17	0.372	0.25	0.147	8.8
	30	0.50	1.425	0.45[b], 0.86[c]	1.603	31.8

a) Plasma concentration of unchanged drug was determined by gas chromatography-mass spectrometry (n=3). *b*) Data for 0 to 4 h. *c*) Data for 4 to 8 h.

Table 4. Plasma Concentrations of Unchanged YM461 and YM264 by Oral Administration in Dogs and Monkeys[a]

Compd.	Dose (mg/kg)	T_{max} (h)	C_{max} (μg/ml)	$t_{1/2}$ (h)	$AUC_{0\to\infty}$ (μg·kg/ml)	Bioav. (%)
YM461 (d)[b]	3	0.4	0.264	0.7	0.367	24.6
YM264 (d)[b]	3	0.3	0.211	1.8	0.386	18.3
YM264 (m)[b]	3	0.5	0.188	1.23	0.309	—

a) Plasma concentration of unchanged drug was determined by gas chromatography-mass spectrometry (dog; n=4, monkey; n=3). *b*) d; dog, m; monkey.

Table 5. Plasma Concentrations of Unchanged YM461 and YM264 by Intravenous Administration in Dogs[a)]

Compd.	Dose (mg/kg)	$t_{1/2}$ (h)	Vd_1 (l/kg)	$AUC_{0\to\infty}$ (μg·kg/ml)	Cl (l/h·kg)
YM461	1	1.1	1.54	0.498	2.0
YM264	1	2.0	1.41	0.701	1.4

a) Plasma concentration of unchanged drug was determined by gas chromatography-mass spectrometry (n=4).

reduced pressure. All nonaqueous reactions were performed in dry glassware under an atomosphere of dry Ar.

1-[2-(*tert*-Butoxycarbonylamino)-3-methylthiopropanoyl]-4-(3-phenylpropyl)piperazine (3a) A mixture of *N*-*tert*-butoxycarbonyl-*S*-methylcysteine (**2**, 4.70 g, 20 mmol), dicyclohexylcarbodiimide (4.33 g, 21 mmol), 1-hydroxybenzotriazole (2.84 g, 21 mmol), and 1-(3-phenylpropyl)piperazine (4.20 g, 20.6 mmol) in tetrahydrofuran (THF, 120 ml) was stirred overnight at room temperature. The reaction mixture was diluted with AcOEt, and the insoluble materials were removed by filtration. The filtrate was washed with aqueous $NaHCO_3$ and brine, dried and concentrated. The residue was chromatographed on silica gel eluting with $CHCl_3$–MeOH (10 : 1) to afford **3a** (5.10 g, 61%) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 1.45 (9H, s), 1.60—2.00 (2H, m), 2.16 (3H, s), 2.22—2.88 (10H, m), 3.48—3.75 (4H, m), 4.79 (1H, br q, J=8 Hz), 5.46 (1H, br d, J=12 Hz), 7.04—7.40 (5H, m). FAB-MS m/z: 422 (M^++1).

1-(2-Amino-3-methylthiopropanoyl)-4-(3-phenylpropyl)piperazine (M1) Trifluoroacetic acid (8 ml) was added to a solution of **3a** (3.01 g, 7.1 mmol) in CH_2Cl_2 (20 ml) and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated, and the residue was neutralized with saturated $NaHCO_3$. The mixture was concentrated and diluted with AcOEt, and the insoluble materials were removed by filtration. The filtrate was concentrated and chromatographed on silica gel eluting with $CHCl_3$–MeOH (10 : 1) to afford **M1** (890 mg, 37%) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 1.60—2.05 (2H, m), 2.10 (3H, s), 2.30—2.85 (10H, m), 3.44—3.80 (4H, m), 8.82 (1H, dd, J=5, 8 Hz), 7.05—7.42 (5H, m). FAB-MS m/z: 322 (M^++1). *Anal*. Calcd for $C_{17}H_{27}N_3OS$: C, 63.51; H, 8.46; N, 13.07; S, 9.97. Found: C, 63.27; H, 8.63; N, 12.88; S, 9.69.

1-(3-Hydroxy-3-phenylpropyl)-4-[3-methylthio-2-(3-pyridylcarbonylamino)propanoyl]piperazine (M3) Nicotinoyl chloride hydrochloride (229 mg, 1.3 mmol) was added to a solution of 1-(2-amino-3-methylthiopropanoyl)-4-(3-hydroxy-3-phenylpropyl)piperazine (**4b**, 376 mg, 1.2 mmol), prepared from 1-(3-hydroxy-3-phenylpropyl)piperazine[7)] by the above method, in $CHCl_3$ (6 ml) and stirred at room temperature for 30 min. The mixture was concentrated and diluted with AcOEt. The organic layer was washed with aqueous $NaHCO_3$ and brine, dried and concentrated. The residue was chromatographed on silica gel eluting with $CHCl_3$–MeOH (20 : 1) to afford **M3** (336 mg, 67%) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 1.60—2.05 (2H, m), 2.18 (3H, s), 2.25—2.80 (8H, m), 2.80—3.05 (2H, m), 3.65—3.85 (4H, m), 5.15—5.50 (1H, m), 7.10—7.50 (6H, m), 8.00—8.20 (1H, m), 8.65—8.80 (1H, m), 9.00—9.13 (1H, m). FAB-MS m/z: 442 (M^+). *Anal*. Calcd for $C_{23}H_{30}N_4O_3S\cdot0.8H_2O$: C, 60.45; H, 6.97; N, 12.26; S, 7.02. Found: C, 60.38; H, 6.89; N, 12.30; S, 7.00.

2-(*tert*-Butoxycarbonylamino)-3-methylsulfinylpropionic Acid (5) A solution of *m*CPBA (4.60 g, 29.4 mmol) in CH_2Cl_2 (20 ml) was added to a solution of **2** (6.27 g, 26.7 mmol) in CH_2Cl_2 (50 ml) at 0 °C, and the mixture was stirred overnight. The reaction mixture was concentrated and the residue was chromatographed on silica gel eluting with $CHCl_3$–MeOH (10 : 1) to afford **5** (4.39 g, 66%) as a solid. ^{1}H-NMR ($CDCl_3$) δ: 1.44 (9H, s), 2.74 and 2.79 (3H, s), 4.45—4.75 (2H, m), 5.80—6.05 (1H, m). FAB-MS m/z: 252 (M^++1).

1-[2-(*tert*-Butoxycarbonylamino)-3-methylsulfinylpropanoyl]-4-(3-phenylpropyl)piperazine (6a) Compound **6a** was prepared from **5** in 83% yield by a similar method to that described for **3a**. ^{1}H-NMR ($CDCl_3$) δ: 1.44 (9H, s), 1.60—1.95 (2H, m), 2.05—2.75 (6H, m), 2.63 and 2.68 (3H, s), 2.80—3.13 (2H, m), 3.52—3.75 (4H, m), 4.90—5.25 (1H, m), 5.62 (1H, br t, J=8 Hz), 7.05—7.40 (5H, m). FAB-MS m/z: 438 (M^++1).

1-[2-(*tert*-Butoxycarbonylamino)-3-methylsulfinylpropanoyl]-4-(3-methyl-3-phenylbutyl)piperazine (6c) A mixture of **6c** was prepared from **5** by a similar method to that described for **3a** and separated by column chromatography. Less polar **6c**; ^{1}H-NMR ($CDCl_3$) δ: 1.33 (6H, s), 1.43 (9H, s), 1.80—1.90 (2H, m), 2.10—2.20 (2H, m), 2.20—2.55 (6H, m), 2.67 (3H, s), 2.95—3.15 (2H, m), 3.50—3.75 (4H, m), 5.03 (1H, q, J=8 Hz), 5.47 (1H, d, J=8 Hz), 7.14—7.30 (5H, m). FAB-MS m/z: 466 (M^++1). More polar **6c**; ^{1}H-NMR ($CDCl_3$) δ: 1.33 (6H, s), 1.43 (9H, s), 1.80—1.90 (2H, m), 2.05—2.15 (2H, m), 2.25—2.45 (6H, m), 2.61 (3H, s), 2.80—2.90 (2H, m), 3.46—3.70 (4H, m), 5.13 (1H, br s), 5.55 (1H, br d, J=8 Hz), 7.15—7.30 (5H, m). FAB-MS m/z: 466 (M^++1).

1-[2-Amino-3-methylsulfinylpropanoyl]-4-(3-phenylpropyl)piperazine (M2) Compound **M2** was prepared from **6a** in 77% yield by a similar method to that described for **M1**. ^{1}H-NMR ($CDCl_3$) δ: 1.70—1.85 (2H, m), 2.25—2.75 (8H, m), 2.70 (3H, s), 2.80—2.90 (1H, m), 3.02—3.15 (1H, m), 3.20—3.30 (1H, m), 3.55—3.75 (4H, m), 4.40 (1H, t, J=5 Hz), 7.10—7.35 (5H, m), FAB-MS m/z: 337 (M^+). *Anal*. Calcd for $C_{17}H_{27}N_3O_2S\cdot0.5H_2O$: C, 58.93; H, 8.14; N, 12.13; S, 9.25. Found: C, 58.88; H, 8.15; N, 12.15; S, 8.95.

(+)-1-(2-Amino-3-methylsulfinylpropanoyl)-4-(3-methyl-3-phenylbutyl)piperazine ((+)-M6) Compound (+)-**M6** was prepared from less polar **6c** in 60% yield by a similar method to that described for **M1**. ^{1}H-NMR (CD_3OD) δ: 1.34 (6H, s), 1.85—1.95 (2H, m), 2.10—2.20 (2H, m), 2.30—2.55 (6H, m), 2.70 (3H, s), 2.91 (1H, dd, J=6, 13 Hz), 3.13 (1H, dd, J=6, 13 Hz), 3.45—3.70 (4H, m), 4.25 (1H, dd, J=6, 7 Hz), 7.10—7.40 (5H, m). FAB-MS m/z: 365 (M^+). $[\alpha]_D^{20}$ +53.5° (C=0.74, MeOH).

(−)-1-(2-Amino-3-methylsulfinylpropanoyl)-4-(3-methyl-3-phenylbutyl)piperazine ((−)-M6) Compound (−)-**M6** was prepared from more polar **6c** in 61% yield by a similar method to that described for **M1**. ^{1}H-NMR (CD_3OD) δ: 1.34 (6H, s), 1.85—1.95 (2H, m), 2.10—2.20 (2H, m), 2.35—2.50 (6H, m), 2.67 (3H, s), 2.80—2.90 (2H, m), 3.45—3.65 (4H, m), 4.22 (1H, dd, J=4, 9 Hz), 7.10—7.40 (5H, m). FAB-MS m/z: 365 (M^+). $[\alpha]_D^{20}$ −34.7° (C=0.76, MeOH).

1-(3-Hydroxy-3-phenylpropyl)-4-[2-(3-pyridyl)thiazolidine-4-carbonyl]piperazine (M4) Compound **M4** was prepared from 2-(3-pyridyl)-thiazolidine-4-carboxylic acid (**9**)[1)] and 1-(3-hydroxy-3-phenylpropyl)piperazine[8)] in 63% yield by a similar method to that described for **3a**. ^{1}H-NMR ($CDCl_3$) δ: 1.76—2.03 (2H, m), 2.30—2.80 (6H, m), 2.95—3.48 (2H, m), 3.48—3.94 (4H, m), 4.00—4.32 (1H, m), 4.95 (1H, t, J=1.5 Hz), 5.60 and 5.96 (1H, s), 7.16—7.50 (6H, m), 7.72—7.98 (1H, m), 8.44—8.64 (1H, m), 8.64—8.80 (1H, m). FAB-MS m/z: 412 (M^+). *Anal*. Calcd for $C_{22}H_{28}N_4O_2S\cdot0.3H_2O$: C, 63.22; H, 6.90; N, 13.41; S, 7.67. Found: C, 63.15; H, 6.88; N, 13.20; S, 7.51.

2-3′-Pyridylthiazolidine-4-carboxylic Acid 1′-Oxide (10) A solution of nicotinealdehyde *N*-oxide (**8**, 460 mg, 3.7 mmol) in EtOH (10 ml) was added to a solution of L-cysteine (453 mg, 3.7 mmol) in H_2O (10 ml) at room temperature and stirred overnight. The mixture was concentrated and the residue was diluted with EtOH. The mixture was concentrated and the residue was washed with CH_3CN to afford **10** (828 mg, 98%) as a solid. ^{1}H-NMR (DMSO-d_6) δ: 2.95—3.50 (2H, m), 3.84—4.22 (1H, m), 5.54 and 5.74 (1H, s), 7.29—7.58 (2H, m), 8.09—8.50 (2H, m). FAB-MS m/z: 227 (M^++1).

1-3′-Methyl-3′-phenylbutyl-4-2″-3‴-pyridylthiazolidine-4″-carbonylpiperazine 1‴-Oxide (M7) Compound **M7** was prepared from **10** in 42% yield by a similar method to that described for **3a**, as a solid, mp 177 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.27 (6H, s), 1.70—1.85 (2H, m), 2.00—2.10 (2H, m), 2.20—2.36 (4H, m), 2.80—3.06 (1H, m), 3.24—3.60 (4H, m), 4.12—4.33 (1H, m), 5.49 and 5.85 (1H, s), 6.62 (2H, s), 7.10—7.50 (7H, m), 8.00—8.40 (2H, m). FAB-MS m/z: 441 (M^++1). *Anal*. Calcd for $C_{24}H_{32}N_4O_2S\cdot C_4H_4O_4\cdot0.4H_2O$: C, 59.75; H, 6.41; N, 9.95; S, 5.70. Found: C, 59.82; H, 6.57; N, 9.83; S, 5.69.

Composition of Metabolites of YM461 and YM264 YM461: At 0—24 h after oral administration of 100 mg/kg of [^{14}C]-YM461 to male F344 rats, urine was collected, applied to a Sep-Pak C_{18} cartridge (Waters Corp., Milford, MA) and eluted with methanol. A portion of the eluate was spotted onto silica-gel plates to separate metabolites by two-dimensional thin-layer chromatography (TLC). Each band of silica-gel corresponding to the metabolite was detected by autoradiography and removed from the plate to measure its radioactivity with a liquid scintillation counter and calculate its fraction to the dose.

YM264: At 0—24 h after oral administration of 3 mg/kg of [^{14}C]-YM264 to male F344 rats, urine was collected, applied to a Sep-Pak C_{18} cartridge and eluted with methanol. A portion of the eluate was injected into a HPLC with a radiodetector to monitor chromatographic patterns of radioactivity. Each radioactive fraction corresponding to a metabolite was collected to measure its radioactivity and calculate its fraction to the dose.

PAF-Induced Platelet Aggregation Rabbit PRP was obtained by centrifugation at 270×***g*** for 10 min. The platelet concentrations were adjusted with platelet-poor plasma to 5×10^8 cells/ml. Platelet aggregation was measured by the method of Born[8)] using a Hema Tracer VI (Niko Bioscience,

Tokyo, Japan). Various concentrations of the test compounds were added to the PRP 2 min prior to the addition of PAF (10 nM). All experiments were carried out within 4 h following blood collection to avoid a decrease in the sensitivity of the platelets to the aggregation agent.

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