Anti-Platelet Aggregation Activity of Some Pyrazines

Akihiro OHTA,*^a Hiromitsu TAKAHASHI,^a Naoomi MIYATA,^a Hiroyuki HIRONO,^a Toyotaka NISHIO,^a Etsuo UCHINO,^a Kenji YAMADA,^a Yutaka AOYAGI,^a Yasushi SUWABE,^b Masayuki FUJITAKE,^b Takahiro SUZUKI,^b and Kazuo OKAMOTO^b

Tokyo University of Pharmacy and Life Science,^a 1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan and Terumo Corporation,^b 2-44-1 Hatagaya, Shibuya-ku, Tokyo 151, Japan.

Received April 14, 1997; accepted June 26, 1997

This report describes the anti-platelet aggregation activity of 48 pyrazines. Among alkyl- and arylpyrazines tested, 2,3-diphenylpyrazines showed the strongest anti-platelet aggregation activity. Then, various substituents were introduced into the phenyl groups, and the 2,3-bis(*p*-methoxyphenyl)pyrazine derivatives were consequently found to possess considerably strong inhibitory activity.

Key words alkylpyrazine; arylpyrazine; anti-platelet aggregation; rabbit PRP; 2,3-bis(*p*-methoxyphenyl)pyrazine

Several anti-platelet aggregants, such as ticlopidine, cilostazol and prostaglandin E₁ derivatives, have been proposed in the clinical field. Tetramethylpyrazine, isolated from *Ligusticum wallichii* FRANCH, also has anti-platelet aggregation activity and is used for clinical application in China.¹⁾ This substance is also a component of *Jatropha podagrica* HOOK, an African higher plant,²⁾ and is a metabolite of *Bacillus natto*.³⁾

While studying the chemical reactions of pyrazines and syntheses of naturally occurring pyrazines, our attention was directed to the anti-platelet aggregation activity of pyrazines. Certain phenylpyrazines were consequently found to possess high activity and patents have been taken out for these inventions.⁴⁾ This report will arrange the results obtained so far.

The anti-platelet aggregation activity of some alkylpyrazines has been reported also by Igarashi *et al.*,⁵⁾ but positive confirmation of this property has not yet been made. After our applications for platelet aggregation, 5-alkoxy-2,3-bis(*p*-methoxyphenyl)pyrazines were reported to have potent anti-platelet aggregation activity in a series which studied the chemical reactions of *N*-heterocycles.⁶⁾

MATERIALS AND METHODS

Materials Tetramethylpyrazine (**1**) was purchased from Aldrich Chemical Company, Inc. (Wisconsin, U.S.A.) and purified by sublimation. Compounds **2–9** and **11** were prepared by the methods previously reported.^{7–11)} Compound **10**¹²⁾ was obtained by the cross-coupling reaction of 5,6-dichloro-2,3-diphenylpyrazine⁷⁾ with 2 molar equivalents of trimethylaluminum in the presence of tetrakis(triphenylphosphine)palladium, while compounds **12–26** were prepared through the condensation of 5,6-dihydro-2,3-diphenylpyrazine⁷⁾ derived from benzil and ethylenediamine, using carbonyl compounds such as acetone, benzaldehyde derivatives, and 2-naphthalene carbaldehyde in the presence of potassium hydroxide, as shown in Chart 1.

Compounds **27–31** were prepared by the condensation between benzil derivatives and 1,2-diaminopropane and subsequent dehydrogenation (Chart 2).

Preparation of compounds **32–42** was achieved by the coupling reaction of *p*-anisil and ethylenediamine, and by the subsequent appropriate reactions.

Compounds **43–48** were prepared by the condensation reaction between *p*-anisil and the corresponding diamines. In the case of the preparation of **44**, the condensation between *p*-anisil and 1,2-diaminocyclohexane was followed by dehydrogenation using sulfur.

Experimental No correction is made for melting and boiling points. ¹H-NMR spectra were taken on a Varian EM-390 instrument in CDCl₃, except the spectrum of **41**, which was taken on a Varian GME-300 instrument in *d*₆-DMSO, using tetramethylsilane as the internal standard. Mass spectra (MS) were obtained using a Hitachi M-80B spectrometer. Products were purified by open column chromatography on silica gel or by medium-pressure column chromatography. Medium-pressure column chromatography was carried out using a UVILOG ALPL-100 pump, UVILOG 5111A ultraviolet (UV) detector (Oyo-bunko kiki Co., Ltd., Tokyo), and Kieselgel

Chart 1. Reactions of 2,3-Diphenyl-5,6-dihydropyrazine with Carbonyl Compounds

Chart 2. Synthesis of 2,3-Bis(*p*-substituted phenyl)-5-methylpyrazines

* To whom correspondence should be addressed.

Fig. 1. Some 2,3-Bis(*p*-methoxyphenyl)pyrazines

60 (Merck AG, Darmstadt) as the packing material.

Platelet aggregation was measured with an NBS Hema Trace IV (Niko Bioscience Co., Ltd., Tokyo). Platelet aggregation was assessed by a method using rabbit PRP. The IC_{50} value was the concentration at which there was maximum (50%) inhibition of platelet aggregation induced by arachidonic acid and collagen.

The Reaction of 2,3-Bis(*p*-substituted phenyl)-5,6-dihydropyrazines with Carbonyl Compounds A solution of 2,3-bis(*p*-substituted phenyl)-5,6-dihydropyrazine (10 mmol), a carbonyl compound (10 mmol) and KOH (12 mmol) in MeOH (20 ml) was heated under reflux for 1 h. After MeOH was evaporated *in vacuo*, water (50 ml) was added to the residue. The resulting mixture was extracted with AcOEt 3 times. After being dried with Na_2SO_4 , the solvent was evaporated *in vacuo*, and the residue was purified by column chromatography on silica gel.

2,3-Diphenyl-5-isopropylpyrazine (12) Colorless prisms (MeOH–H₂O); mp 69–70 °C; Yield 60%; MS: m/z 274 (M^+); 1H -NMR: 1.42 (6H, d, $J=7$ Hz), 3.20 (1H, m, $J=7$ Hz), 7.20–7.53 (10H, m), 8.47 (1H, s) ppm; *Anal.* Calcd for $C_{19}H_{18}N_2$: C, 83.17; H, 6.61; N, 10.21. Found: C, 83.39; H, 6.58; N, 10.19.

2,3-Diphenyl-5-benzylpyrazine (13) Colorless prisms (hexane); mp 118–119 °C; Yield 99%; MS: m/z 274 (M^+); 1H -NMR: 4.27 (2H, s), 7.20–7.60 (15H, m), 8.47 (1H, s) ppm; *Anal.* Calcd for $C_{23}H_{18}N_2$: C, 85.68; H, 5.63; N, 8.69. Found: C, 85.68; H, 5.53; N, 8.67.

2,3-Diphenyl-5-(1-naphthylmethyl)pyrazine (14) Colorless prisms (MeOH–H₂O); mp 91–92 °C; Yield 89%; MS: m/z 372 (M^+); 1H -NMR: 4.70 (2H, s), 7.10–7.60 (14H, m), 7.67–8.00 (2H, m), 8.07–8.33 (1H, m), 8.30 (1H, s) ppm; *Anal.* Calcd for $C_{27}H_{20}N_2$: C, 87.06; H, 5.41; N, 7.52. Found: C, 86.96; H, 5.43; N, 7.50.

5-(*p*-Bromobenzyl)-2,3-diphenylpyrazine (15) Colorless needles (MeOH); mp 100–101 °C; Yield 97%; MS: m/z 401 (M^+); 1H -NMR: 4.23 (2H, s), 7.20–7.57 (14H, m), 8.48 (1H, s) ppm; *Anal.* Calcd for $C_{23}H_{17}BrN_2$: C, 68.83; H, 4.27; N, 6.98. Found: C, 68.84; H, 4.24; N, 6.97.

5-(*o*-Chlorobenzyl)-2,3-diphenylpyrazine (16) Colorless prisms (MeOH–H₂O); mp 112–114 °C; Yield 74%; MS: m/z 356 (M^+); 1H -NMR: 4.40 (2H, s), 7.17–7.60

(14H, m), 8.47 (1H, s) ppm; *Anal.* Calcd for $C_{23}H_{17}ClN_2$: C, 77.41; H, 4.80; N, 7.85. Found: C, 77.13; H, 4.81; N, 7.83.

5-(*m*-Chlorobenzyl)-2,3-diphenylpyrazine (17) Colorless prisms (MeOH–H₂O); mp 58–59 °C; Yield 94%; MS: m/z 356 (M^+); 1H -NMR: 4.18 (2H, s), 7.13–7.50 (14H, m), 8.40 (1H, s); *Anal.* Calcd for $C_{23}H_{17}ClN_2$: C, 77.41; H, 4.80; N, 7.85. Found: C, 77.35; H, 4.75; N, 7.80.

5-(*p*-Chlorobenzyl)-2,3-diphenylpyrazine (18) Colorless prisms (iso-PrOH); mp 106 °C; Yield 94%; MS: m/z 356 (M^+); 1H -NMR: 4.20 (2H, s), 7.13–7.50 (14H, m), 8.40 (1H, s) ppm; *Anal.* Calcd for $C_{23}H_{17}ClN_2$: C, 77.41; H, 4.80; N, 7.85. Found: C, 77.54; H, 4.80; N, 7.86.

5-(*o*-Methylbenzyl)-2,3-diphenylpyrazine (19) Colorless prisms (MeOH–H₂O); mp 106–107 °C; Yield 93%; MS: m/z 336 (M^+); 1H -NMR: 2.37 (3H, s), 4.27 (2H, s), 7.10–7.53 (14H, m), 8.28 (1H, s) ppm; *Anal.* Calcd for $C_{24}H_{20}N_2$: C, 85.68; H, 5.99; N, 8.33. Found: C, 85.70; H, 6.02; N, 8.35.

5-(*p*-Methylbenzyl)-2,3-diphenylpyrazine (20) Colorless prisms (MeOH–H₂O); mp 70–71 °C; Yield 94%; MS: m/z 336 (M^+); 1H -NMR: 2.33 (3H, s), 4.23 (2H, s), 7.00–7.57 (14H, m), 8.47 (1H, s) ppm; *Anal.* Calcd for $C_{24}H_{20}N_2$: C, 85.68; H, 5.99; N, 8.21. Found: C, 85.76; H, 5.90; N, 8.33.

5-(*o*-Methoxybenzyl)-2,3-diphenylpyrazine (21) Colorless prisms (MeOH–H₂O); mp 93–94 °C; Yield 93%; MS: m/z 352 (M^+); 1H -NMR: 3.82 (3H, s), 4.25 (2H, s), 6.87 (1H, d, $J=7$ Hz), 6.97 (1H, d, $J=7$ Hz), 7.13–7.50 (12H, m), 8.40 (1H, s) ppm; *Anal.* Calcd for $C_{24}H_{20}N_2O$: C, 81.79; H, 5.72; N, 7.95. Found: C, 81.60; H, 5.64; N, 7.97.

5-(*m*-Methoxybenzyl)-2,3-diphenylpyrazine (22) Colorless prisms (MeOH–H₂O); mp 65–66 °C; Yield 97%; MS: m/z 352 (M^+); 1H -NMR: 3.82 (3H, s), 4.25 (2H, s), 6.87 (1H, d, $J=7$ Hz), 6.97 (1H, d, $J=7$ Hz), 7.13–7.50 (12H, m), 8.40 (1H, s) ppm; *Anal.* Calcd for $C_{24}H_{20}N_2O$: C, 81.79; H, 5.72; N, 7.95. Found: C, 82.02; H, 5.74; N, 7.97.

5-(*p*-Methoxybenzyl)-2,3-diphenylpyrazine (23) Colorless prisms (MeOH–H₂O); mp 102–103 °C; Yield 94%; MS: m/z 352 (M^+); 1H -NMR: 3.73 (3H, s), 4.17 (2H, s), 6.83 (2H, d, $J=9$ Hz), 7.13 (12H, m), 8.37 (1H, s) ppm; *Anal.* Calcd for $C_{24}H_{20}N_2O$: C, 81.79; H, 5.72; N, 7.95.

Found: C, 81.89; H, 5.70; N, 8.01.

2,3-Diphenyl-5-(3,4-dimethoxybenzyl)pyrazine (24) Colorless prisms (MeOH-H₂O); mp 95–97 °C; Yield 92%; MS: m/z 382 (M⁺); ¹H-NMR: 3.85 (6H, s), 4.18 (2H, s), 6.83–7.00 (3H, m), 7.17–7.57 (10H, m), 8.45 (1H, s) ppm; *Anal.* Calcd for C₂₅H₂₂N₂O₂: C, 78.51; H, 5.80; N, 7.33. Found: C, 78.79; H, 5.80; N, 7.39.

2,3-Diphenyl-5-(3,4-methylenedioxybenzyl)pyrazine (25) Colorless needles (MeOH-H₂O); mp 103–104 °C; Yield 95%; MS: m/z 366 (M⁺); ¹H-NMR: 4.10 (2H, s), 5.83 (2H, s), 6.70 (1H, s), 6.75 (2H, d, $J=7$ Hz), 7.10–7.47 (10H, m), 8.33 (1H, s) ppm; *Anal.* Calcd for C₂₄H₁₈N₂O₂: C, 78.67; H, 4.95; N, 7.65. Found: C, 78.41; H, 4.91; N, 7.60.

5-(*p*-Dimethylaminobenzyl)-2,3-diphenylpyrazine (26) Colorless prisms (MeOH-H₂O); mp 80–82 °C; Yield 92%; MS: m/z 365 (M⁺); ¹H-NMR: 2.88 (6H, s), 4.13 (2H, s), 6.70 (2H, d, $J=9$ Hz), 7.17–7.53 (12H, m), 8.40 (1H, s) ppm; *Anal.* Calcd for C₂₅H₂₃N₃: C, 82.16; H, 6.34; N, 11.50. Found: C, 82.14; H, 6.30; N, 11.50.

2,3-Bis(*p*-methoxyphenyl)-5-benzylpyrazine (37) Colorless prisms (MeOH-H₂O); mp 107–109 °C; Yield 92%; MS: m/z 382 (M⁺); ¹H-NMR: 3.77 (6H, s), 4.22 (2H, s), 6.80 (2H, d, $J=9$ Hz), 6.82 (2H, d, $J=9$ Hz), 7.17–7.50 (9H, m), 8.33 (1H, s) ppm; *Anal.* Calcd for C₂₅H₂₂N₂O₂: C, 78.51; H, 5.80; N, 7.33. Found: C, 78.42; H, 5.77; N, 7.29.

2,3-Bis(*p*-methoxyphenyl)-5-(2-thienyl)methylpyrazine (38) Colorless prisms (MeCN); mp 189–190 °C; Yield 88%; MS: m/z 388 (M⁺); ¹H-NMR: 3.77 (6H, s), 4.40 (2H, s), 6.80 (2H, d, $J=9$ Hz), 6.92 (2H, d, $J=9$ Hz), 6.67–7.00 (2H, m), 7.18 (1H, m), 7.37 (2H, d, $J=9$ Hz), 7.43 (2H, d, $J=9$ Hz), 8.40 (1H, s) ppm; *Anal.* Calcd for C₂₃H₂₀N₂O₂S: C, 71.11; H, 5.19; N, 7.21. Found: C, 71.19; H, 5.25; N, 7.22.

Syntheses of 2,3-Bis(*p*-substituted phenyl)-5-methylpyrazines 1,2-Diaminopropane (9 mmol) was added dropwise to a solution of *p,p'*-disubstituted benzil (7 mmol) in EtOH (35 ml) at room temperature. The mixture was heated under reflux for 1 h and condensed to a half volume *in vacuo*. After cooling, the crystals which appeared were collected with suction and purified by column chromatography on silica gel or by recrystallization. The dihydro compound (2.8 mmol) thus obtained was heated with powdered sulfur (6 mmol) at 140 °C on an oil bath and the product was purified by column chromatography on silica gel, followed by recrystallization.

2,3-Bis(*p*-bromophenyl)-5-methylpyrazine (27) Colorless prisms (MeOH); mp 164–165 °C; Yield 40%; MS: m/z 404 (M⁺); ¹H-NMR: 2.63 (3H, s), 7.34 (8H, m), 8.43 (1H, s) ppm; *Anal.* Calcd for C₁₇H₁₂Br₂N₂: C, 50.52; H, 2.99; N, 6.93. Found: C, 50.75; H, 2.95; N, 7.00.

2,3-Bis(*p*-chlorophenyl)-5-methylpyrazine (28) Colorless prisms (hexane); mp 168–169 °C; Yield 43%; MS: m/z 314 (M⁺); ¹H-NMR: 2.60 (3H, s), 7.28 (8H, m), 8.40 (1H, s) ppm; *Anal.* Calcd for C₁₇H₁₂Cl₂N₂: C, 64.78; H, 3.84; N, 8.89. Found: C, 64.42; H, 3.82; N, 8.78.

2,3-Bis(*p*-fluorophenyl)-5-methylpyrazine (29) Colorless prisms (MeOH); mp 96–97 °C; Yield 44%; MS: m/z 282 (M⁺); ¹H-NMR: 2.62 (3H, s), 6.97 (4H, t, $J=9$ Hz), 7.33 (4H, m), 8.40 (1H, s) ppm; *Anal.* Calcd for

C₁₇H₁₂F₂N₂: C, 72.33; H, 4.29; N, 9.92. Found: C, 72.53; H, 4.28; N, 9.97.

2,3-Bis(*p*-methylphenyl)-5-methylpyrazine (30) Colorless prisms (MeOH-H₂O); mp 111–112 °C; Yield 96%; MS: m/z 274 (M⁺); ¹H-NMR: 2.30 (6H, s), 2.59 (3H, s), 7.05 (4H, d, $J=8.4$ Hz), 7.29 (2H, d, $J=8.4$ Hz), 7.31 (2H, d, $J=8.4$ Hz), 8.38 (1H, s) ppm; *Anal.* Calcd for C₁₉H₁₈N₂: C, 83.17; H, 6.61; N, 10.21. Found: C, 83.30; H, 6.56; N, 10.19.

2,3-Bis(*p*-methoxyphenyl)-5-methylpyrazine (31) Colorless prisms (MeOH); mp 120–121 °C; Yield 44%; MS: m/z 306 (M⁺); ¹H-NMR: 2.55 (3H, s), 3.73 (6H, s), 6.76 (4H, d, $J=9$ Hz), 7.32 (2H, d, $J=9$ Hz), 7.34 (2H, d, $J=9$ Hz), 8.32 (1H, s) ppm; *Anal.* Calcd for C₁₉H₁₈N₂O₂: C, 74.49; H, 5.92; N, 9.12. Found: C, 74.63; H, 6.20; N, 9.12.

Synthesis of 2,3-Bis(*p*-methoxyphenyl)-5-chloropyrazine and 2,3-Bis(*p*-methoxyphenyl)-5,6-dichloropyrazine 2,3-Bis(*p*-methoxyphenyl)pyrazine 1-oxide (12.7 mmol) was heated in POCl₃ (20 ml) under reflux for 1 h, and the mixture was then poured into ice water. The solution was made alkaline with powdered K₂CO₃, and a yellowish orange solid was collected by suction and purified by column chromatography on silica gel.

2,3-Bis(*p*-methoxyphenyl)pyrazine 1,4-dioxide was treated with POCl₃ as stated above. The product was purified also by column chromatography on silica gel, followed by recrystallization.

2,3-Bis(*p*-methoxyphenyl)-5-chloropyrazine (39) Pale yellow prisms (EtOH); mp 127–128 °C; Yield 76%; MS: m/z 326 (M⁺); ¹H-NMR: 3.77 (6H, s), 6.77 (4H, d, $J=7$ Hz), 7.33 (4H, d, $J=7$ Hz), 8.42 (1H, s) ppm; *Anal.* Calcd for C₁₈H₁₅ClN₂O₂: C, 66.16; H, 4.63; N, 8.57. Found: C, 65.91; H, 4.63; N, 8.60.

2,3-Bis(*p*-methoxyphenyl)-5,6-dichloropyrazine Colorless needles (hexane); mp 120–121 °C; Yield 78%; MS: m/z 360 (M⁺); ¹H-NMR: 3.77 (6H, s), 6.83 (4H, d, $J=7.5$ Hz), 7.13 (4H, d, $J=7.5$ Hz) ppm; *Anal.* Calcd for C₁₈H₁₄Cl₂N₂O₂: C, 59.85; H, 3.91; N, 7.76. Found: C, 60.02; H, 3.88; N, 7.79.

Synthesis of 2,3-Bis(*p*-methoxyphenyl)-5-substituted and 5,6-Disubstituted Pyrazines by the Coupling Reaction These substances were prepared by the coupling reaction of 2,3-bis(*p*-methoxyphenyl)-5-chloro- or 5,6-dichloropyrazine with alkyl metals in the reported manner.^{8,11)}

2,3-Bis(*p*-methoxyphenyl)-5,6-dimethylpyrazine (32) Colorless needles (EtOH); mp 106.5–108 °C; Yield 87%; MS: m/z 320 (M⁺); ¹H-NMR: 2.57 (6H, s), 3.77 (6H, s), 6.78 (4H, d, $J=7.5$ Hz), 7.37 (4H, d, $J=7.5$ Hz) ppm; *Anal.* Calcd for C₂₀H₂₀N₂O₂: C, 74.97; H, 6.29; N, 8.74. Found: C, 74.89; H, 6.25; N, 8.96.

2,3-Bis(*p*-methoxyphenyl)-5-ethylpyrazine (33) Colorless needles (EtOH); mp 76.5–78 °C; Yield 87%; MS: m/z 320 (M⁺); ¹H-NMR: 1.37 (3H, t, $J=7$ Hz), 2.87 (2H, q, $J=7$ Hz), 3.77 (6H, s), 6.77 (4H, d, $J=7.5$ Hz), 7.33 (2H, d, $J=7.5$ Hz), 7.37 (2H, d, $J=7.5$ Hz), 8.33 (1H, s) ppm; *Anal.* Calcd for C₂₀H₂₀N₂O₂: C, 74.97; H, 6.29; N, 8.74. Found: C, 75.02; H, 6.41; N, 8.73.

2,3-Bis(*p*-methoxyphenyl)-5,6-diethylpyrazine (34) Colorless viscous oil; bp 188–194 °C/0.075 torr; Yield 70%; MS: m/z 348 (M⁺); ¹H-NMR: 1.25 (6H, t, $J=$

7.5 Hz), 2.80 (4H, q, $J=7.5$ Hz), 3.65 (6H, s), 6.70 (4H, d, $J=9$ Hz), 7.32 (4H, d, $J=9$ Hz) ppm.

2,3-Bis(*p*-methoxyphenyl)-5-butylpyrazine (35) Pale yellow viscous oil; bp 175–180 °C/0.06 torr; Yield 53%; MS: m/z 348 (M^+); $^1\text{H-NMR}$: 0.93 (3H, t, $J=7.5$ Hz), 1.38 (2H, m), 1.78 (2H, m), 2.85 (2H, t, $J=7.5$ Hz), 3.75 (6H, s), 6.82 (4H, d, $J=9$ Hz), 7.42 (4H, dd, $J=2$ Hz), 8.38 (1H, s) ppm.

2,3-Bis(*p*-methoxyphenyl)-5,6-dibutylpyrazine (36) Pale yellow viscous oil; bp 195–200 °C/0.06 torr; Yield 30%; MS: m/z 404 (M^+); $^1\text{H-NMR}$: 0.93 (6H, t, $J=7.5$ Hz), 1.43 (4H, m), 1.77 (4H, m), 2.83 (4H, t, $J=7.5$ Hz), 3.70 (6H, s), 6.73 (4H, d, $J=9$ Hz), 7.35 (4H, d, $J=9$ Hz) ppm.

2,3-Bis(*p*-methoxyphenyl)-5-cyanopyrazine (40) Pale yellow needles (MeOH); mp 110–112 °C; Yield 84%; MS: m/z 317 (M^+); $^1\text{H-NMR}$: 3.77 (6H, s), 6.80 (4H, d, $J=9$ Hz), 7.43 (2H, d, $J=9$ Hz), 7.45 (2H, d, $J=9$ Hz), 8.70 (1H, s) ppm; IR (KBr) cm^{-1} : 2260; *Anal.* Calcd for $\text{C}_{19}\text{H}_{15}\text{N}_3\text{O}_2$: C, 71.91; H, 4.76; N, 13.24. Found: C, 71.89; H, 4.63; N, 13.27.

Synthesis of 2,3-Bis(*p*-methoxyphenyl)-5-pyrazine-carboxylic Acid (41) 2,3-Bis(*p*-methoxyphenyl)-5-cyanopyrazine (6.3 mmol) was dissolved in a mixture of MeOH (40 ml), dioxan (30 ml) and 20% KOH (20 ml), and heated under reflux for 4 h. The solvent was evaporated *in vacuo*, and the residual solution was acidified with 5% HCl and extracted with CH_2Cl_2 . After the extract was dried with Na_2SO_4 , the solvent was evaporated *in vacuo*. The resulting solid was recrystallized from EtOH.

Colorless needles; mp 234–235 °C; Yield 95%; MS: m/z 336 (M^+); IR (KBr): 1690 cm^{-1} ; $^1\text{H-NMR}$: 3.761 (3H, s), 3.764 (3H, s), 6.91 (2H, d, $J=8.9$ Hz), 6.93 (2H, d, $J=8.8$ Hz), 7.40 (2H, d, $J=8.6$ Hz), 7.42 (2H, d, $J=8.7$ Hz), 9.09 (1H, s) ppm; *Anal.* Calcd for $\text{C}_{19}\text{H}_{16}\text{N}_2\text{O}_4$: C, 67.85; H, 4.80; N, 8.33. Found: C, 67.67; H, 4.85; N, 8.34.

Synthesis of 2,3-Bis(*p*-methoxyphenyl)-5-methoxycarbonylpyrazine (42) The carboxylic acid (41) (1 mmol) described above was heated in a mixture of MeOH (5 ml) and conc. H_2SO_4 (0.1 ml) under reflux for 1 h. The solvent was evaporated *in vacuo* and water (15 ml) was added to the residue. The resulting solution was made alkaline with powdered K_2CO_3 and extracted with CH_2Cl_2 . The extract was treated as usual to give crystals, which were recrystallized from MeOH to give colorless needles; mp 140–141 °C; Yield 91%; MS: m/z 350 (M^+); $^1\text{H-NMR}$: 3.77 (6H, s), 3.97 (3H, s), 6.78 (4H, d, $J=9$ Hz), 7.43 (4H, d, $J=9$ Hz), 9.12 (1H, s) ppm; *Anal.* Calcd for $\text{C}_{20}\text{H}_{18}\text{N}_2\text{O}_4$: C, 68.56; H, 5.18; N, 8.00. Found: C, 68.40; H, 5.11; N, 7.93.

Synthesis of 2,3-Bis(*p*-methoxyphenyl)quinoxalines and 2,3-Bis(*p*-methoxyphenyl)pyridopyrazines *p*-Anisil (1 mmol) and the corresponding diaminoaromatics (1 mmol) were dissolved in an appropriate solvent such as MeOH (20 ml) and heated under reflux for 1 h. The products were purified by column chromatography on silica gel, followed by recrystallization.

Compound 44 was prepared by the condensation of *p*-anisil and 1,2-diaminocyclohexane. The product was then dehydrogenated by heating with sulfur, as described

before.

2,3-Bis(*p*-methoxyphenyl)quinoxaline (43) Colorless needles (MeOH); mp 151–153 °C; Yield 80%; MS: m/z 342 (M^+); $^1\text{H-NMR}$: 3.78 (6H, s), 6.83 (4H, d, $J=9$ Hz), 7.46 (4H, d, $J=9$ Hz), 7.67 (2H, dd, $J=6$, 3 Hz), 8.08 (2H, dd, $J=6$, 3 Hz) ppm; *Anal.* Calcd for $\text{C}_{22}\text{H}_{18}\text{N}_2\text{O}_2$: C, 77.17; H, 5.30; N, 8.18. Found: C, 77.03; H, 5.31; N, 8.16.

2,3-Bis(*p*-methoxyphenyl)-5,6,7,8-tetrahydroquinoxaline (44) Yellow prisms (MeCN); mp 189–190 °C; Yield 36%; MS: m/z 346 (M^+); $^1\text{H-NMR}$: 1.95 (4H, m), 3.00 (4H, m), 3.77 (6H, s), 6.78 (4H, d, $J=9$ Hz), 7.35 (4H, d, $J=9$ Hz) ppm; *Anal.* Calcd for $\text{C}_{22}\text{H}_{22}\text{N}_2\text{O}_2$: C, 76.27; H, 6.40; N, 8.09. Found: C, 75.99; H, 6.38; N, 8.13.

2,3-Bis(*p*-methoxyphenyl)benzo[*g*]quinoxaline (45) Pale yellow needles (MeCN); mp 185–187 °C; Yield 71%; MS: m/z 392 (M^+); $^1\text{H-NMR}$: 3.80 (6H, s), 6.87 (4H, d, $J=9$ Hz), 7.50 (2H, m), 7.53 (4H, d, $J=9$ Hz), 8.05 (2H, dd, $J=6$, 3 Hz), 8.67 (2H, s) ppm; *Anal.* Calcd for $\text{C}_{26}\text{H}_{20}\text{N}_2\text{O}_2$: C, 79.57; H, 5.14; N, 7.14. Found: C, 79.63; H, 5.14; N, 7.29.

2,3-Bis(*p*-methoxyphenyl)benzo[*f*]quinoxaline (46) Colorless needles (MeOH); mp 165–166 °C; Yield 27%; MS: m/z 392 (M^+); $^1\text{H-NMR}$: 3.77 (6H, s), 6.80 (4H, d, $J=9$ Hz), 7.53 (4H, d, $J=9$ Hz), 7.63 (2H, d, $J=9$ Hz), 7.67 (3H, m), 7.97 (2H, s), 9.28 (1H, m) ppm; *Anal.* Calcd for $\text{C}_{26}\text{H}_{20}\text{N}_2\text{O}_2$: C, 79.57; H, 5.14; N, 7.14. Found: C, 79.72; H, 5.04; N, 7.27.

2,3-Bis(*p*-methoxyphenyl)pyrido[2,3-*b*]pyrazine (47) Yellow prisms (MeOH– H_2O); mp 137–139 °C; Yield 22%; MS: m/z 343 (M^+); $^1\text{H-NMR}$: 3.77 (6H, s), 6.80 (2H, d, $J=9$ Hz), 6.82 (2H, d, $J=9$ Hz), 7.47 (2H, d, $J=9$ Hz), 7.53 (1H, m), 7.58 (2H, d, $J=9$ Hz), 8.38 (1H, dd, $J=9$, 1.5 Hz), 9.98 (1H, dd, $J=4.5$, 1.5 Hz) ppm; *Anal.* Calcd for $\text{C}_{21}\text{H}_{17}\text{N}_3\text{O}_2$: C, 73.45; H, 4.99; N, 12.24. Found: C, 73.37; H, 4.93; N, 12.37.

2,3-Bis(*p*-methoxyphenyl)pyrido[3,4-*b*]pyrazine (48) Yellow needles (iso-PrOH); mp 153–154 °C; Yield 25%; MS: m/z 343 (M^+); $^1\text{H-NMR}$: 3.77 (6H, s), 6.83 (4H, d, $J=9$ Hz), 7.48 (4H, d, $J=9$ Hz), 7.87 (1H, d, $J=6$ Hz), 8.72 (1H, d, $J=6$ Hz), 9.50 (1H, s) ppm; *Anal.* Calcd for $\text{C}_{21}\text{H}_{17}\text{N}_3\text{O}_2$: C, 73.45; H, 4.99; N, 12.24. Found: C, 73.34; H, 4.92; N, 12.14.

Biological Test Platelet aggregation was assessed by the method¹³⁾ using rabbit PRP. The IC_{50} value was the concentration at which there was maximum (50%) inhibition of platelet aggregation induced by arachidonic acid or collagen.

RESULTS AND DISCUSSION

Inhibitory Activity on Arachidonic Acid- and Collagen-Induced Platelet Aggregation Various types of pyrazines prepared were submitted to *in vitro* tests for the inhibition of platelet aggregation induced by arachidonic acid and collagen in platelet-rich plasma (PRP) from rabbit. The activities of the pyrazines are summarized in Table 1. As a standard substance, aspirin was used.

Alkylpyrazines (1–3) indicated only weak inhibitory activity, as already reported.⁵⁾ Monophenylpyrazines (4–6) showed somewhat stronger activity than alkylpyrazines (1–3). The activity of methylphenylpyrazines

Table 1. Anti-Platelet Aggregation Activity of Some Alkyl- and Arylpyrazines

Compound	R ₁	R ₂	R ₃	R ₄	IC ₅₀	
					AA ^{a)}	Collagen
1	Me	Me	Me	Me	> 10 ⁻³	9.7 × 10 ⁻⁴
2 ⁸⁾	Me	Et	H	Et	6.7 × 10 ⁻⁴	6.2 × 10 ⁻⁴
3 ⁸⁾	Me	iso-Pr	Me	iso-Pr	7.4 × 10 ⁻⁵	> 10 ⁻³
4 ⁹⁾	Ph	H	H	H	1.4 × 10 ⁻⁴	7.2 × 10 ⁻⁵
5 ⁷⁾	Ph	Me	H	H	2.4 × 10 ⁻⁵	> 10 ⁻⁴
6 ¹⁰⁾	Ph	H	H	Me	5.5 × 10 ⁻⁵	> 10 ⁻⁴
7 ⁷⁾	Ph	Ph	H	H	1.4 × 10 ⁻⁴	1.1 × 10 ⁻⁴
8 ⁸⁾	Ph	Ph	Me	H	7.4 × 10 ⁻⁷	4.3 × 10 ⁻⁶
9 ⁸⁾	Ph	Me	H	Ph	1.4 × 10 ⁻⁶	2.2 × 10 ⁻⁷
10 ¹²⁾	Ph	Ph	Me	Me	4.1 × 10 ⁻⁷	1.7 × 10 ⁻⁵
11 ¹¹⁾	Ph	Ph	Et	H	4.1 × 10 ⁻⁷	3.1 × 10 ⁻⁵
12	Ph	Ph	iso-Pr	H	8.6 × 10 ⁻⁷	8.1 × 10 ⁻⁶
13	Ph	Ph	CH ₂ Ph	H	4.5 × 10 ⁻⁶	1.3 × 10 ⁻⁵
14	Ph	Ph	^{b)}	H	4.3 × 10 ⁻⁵	3.1 × 10 ⁻⁵
		Aspirin			2.4 × 10 ⁻⁵	8.8 × 10 ⁻⁵

a) arachidonic acid; b) naphthylmethyl.

Table 2. Anti-Platelet Aggregation Activity of 2,3-Diphenyl-5-benzylpyrazine Derivatives

Compound	X	IC ₅₀ (M)	
		AA	Collagen
15	4-Br	8.4 × 10 ⁻⁵	7.4 × 10 ⁻⁵
16	2-Cl	4.5 × 10 ⁻⁶	4.4 × 10 ⁻⁵
17	3-Cl	8.2 × 10 ⁻⁵	3.4 × 10 ⁻⁵
18	4-Cl	1.6 × 10 ⁻⁵	> 10 ⁻⁴
19	2-Me	8.0 × 10 ⁻⁶	4.6 × 10 ⁻⁵
20	4-Me	4.4 × 10 ⁻⁶	4.2 × 10 ⁻⁵
21	2-MeO	8.2 × 10 ⁻⁶	4.2 × 10 ⁻⁶
22	3-MeO	4.5 × 10 ⁻⁶	1.4 × 10 ⁻⁵
23	4-MeO	1.7 × 10 ⁻⁴	3.5 × 10 ⁻⁵
24	3,4-Di-MeO	4.4 × 10 ⁻⁵	1.4 × 10 ⁻⁵
25	3,4-Methylenedioxy	8.0 × 10 ⁻⁶	8.4 × 10 ⁻⁵
26	4-Me ₂ N	4.4 × 10 ⁻⁶	3.6 × 10 ⁻⁵

(5, 6) toward arachidonic acid-induced platelet aggregation was essentially the same as that of aspirin.

The activity of 2,3-diphenylpyrazine (7) was almost the same as that of the monophenylpyrazine derivatives (4–6). However, the monomethyl derivative (8) of 2,3-diphenylpyrazine (7) showed stronger activity than the one of compound 7. To examine the effects of substituents, an additional methyl group was introduced into compound 8, and, moreover, the methyl group of compound 8 was replaced by ethyl, isopropyl, benzyl, and naphthylmethyl groups. Among these substances, compounds 10, 11 and 12 showed basically the same activity as com-

Table 3. Anti-Platelet Activity of 2,3-Bis(*p*-substituted phenyl)-5-methylpyrazines

Compound	X	IC ₅₀ (M)	
		AA	Collagen
8	H	7.4 × 10 ⁻⁷	4.3 × 10 ⁻⁶
27	Br	> 10 ⁻⁵	9.4 × 10 ⁻⁶
28	Cl	4.5 × 10 ⁻⁶	2.6 × 10 ⁻⁵
29	F	5.1 × 10 ⁻⁶	2.6 × 10 ⁻⁶
30	Me	2.5 × 10 ⁻⁶	3.5 × 10 ⁻⁶
31	MeO	1.0 × 10 ⁻⁸	5.0 × 10 ⁻⁷

Table 4. Anti-Platelet Aggregation Activity of 2,3-Bis(*p*-methoxyphenyl)-5- and 5,6-Substituted Pyrazines

Compound	R ₁	R ₂	IC ₅₀ (M)	
			AA	Collagen
31	Me	H	1.0 × 10 ⁻⁸	5.0 × 10 ⁻⁷
32	Me	Me	5.2 × 10 ⁻⁸	9.2 × 10 ⁻⁷
33	Et	H	2.5 × 10 ⁻⁸	3.2 × 10 ⁻⁷
34	Et	Et	3.9 × 10 ⁻⁸	1.2 × 10 ⁻⁷
35	Bu	H	1.3 × 10 ⁻⁸	1.6 × 10 ⁻⁸
36	Bu	Bu	2.1 × 10 ⁻⁸	1.6 × 10 ⁻⁸
37	CH ₂ Ph	H	2.0 × 10 ⁻⁷	5.0 × 10 ⁻⁶
38	*	H	4.4 × 10 ⁻⁷	7.9 × 10 ⁻⁷
39	Cl	H	4.2 × 10 ⁻⁹	6.2 × 10 ⁻⁸
40	CN	H	2.4 × 10 ⁻⁸	1.5 × 10 ⁻⁸
41	COOH	H	2.6 × 10 ⁻⁷	5.0 × 10 ⁻⁶
42	COOMe	H	3.0 × 10 ⁻⁹	1.3 × 10 ⁻⁷

*: thienylmethyl.

pound 8.

On the other hand, 3-methyl-2,6-diphenylpyrazine (9) showed somewhat stronger activity than monophenylpyrazines (4–6). However, investigation of the preparation and platelet aggregation activity of 2,6-diphenylpyrazine was not undertaken, since the preparative procedure of 2,6-diphenylpyrazines is tedious.

To find compounds of greater activity, certain groups were introduced into the benzene ring of the benzyl group of compound 13. Although this compound showed about 5 times stronger inhibitory than that of aspirin, the activities of products 15–26 are the same as that of aspirin, as shown in Table 2.

Because compound 8 showed fairly strong activity, some substituents were introduced into the phenyl group to aim at even stronger activity. As Table 3 shows, the introduction of a methoxy group increased the activity.

Table 5. Anti-Platelet Aggregation Activity of 2,3-Bis(*p*-methoxyphenyl)pyrazine Derivatives

No.	IC ₅₀ (M)	
	AA	Collagen
43	3.8×10^{-8}	9.8×10^{-9}
44	1.5×10^{-8}	2.4×10^{-8}
45	$< 10^{-10}$	1.5×10^{-9}
46	1.5×10^{-10}	$< 10^{-10}$
47	1.7×10^{-8}	1.1×10^{-7}
48	1.6×10^{-8}	4.0×10^{-7}

On the basis of these data (Table 3), some substituents were introduced into the pyrazine ring of 2,3-bis(*p*-methoxyphenyl)pyrazine. Table 4 shows that all compounds prepared have strong activity. Among these, compounds **39** and **42** showed the strongest activity, especially against arachidonic acid aggregation.

The activity of some 2,3-bis(*p*-methoxyphenyl)pyrazines, condensed with benzene, cyclohexane, naphthalene and pyridine rings, was also tested. Table 5 shows that these compounds also have strong activity. Especially, the activity of **45** and **46** was quite strong.

To determine the potent substances in this area, research on the synthesis of new pyrazines and on biological tests continues.

REFERENCES

- 1) Dai X., Bache R. J., *J. Cardiovas. Pharmacol.*, **7**, 841—849 (1985).
- 2) Kosuge T., Kamiya H., Adachi T., *Yakugaku Zasshi*, **84**, 448—451 (1964).
- 3) a) Ojewole J. A. O., Odebiyi O. O., *Planta Medica*, **38**, 332—338 (1980); b) Ojewole J. A. O., *ibid.*, **42**, 227—228 (1981).
- 4) a) Wakabayashi T., Hasegawa H., Ohta A., Jpn. Kokai Tokkyo Koho, JP, 61212522 (1986) [*Chem. Abstr.*, **106**, 78766f (1987)]; b) Wakabayashi T., Hasegawa H., Ohta A., Eur. Pat. Appl., Ep 194,686 (1986) [*Chem. Abstr.*, **106**, 102321e (1987)]; c) Suwabe Y., Ushijima H., Hijikura K., Sakuragi S., Suzuki T., Akita Y., Ohta A., PCT Int. Appl., WO, 8,904,308 (1989) [*Chem. Abstr.*, **111**, 153841k (1989)].
- 5) Igarashi M., Kanno H., Tanaka K., Asada T., Yamaguchi R., Shirai T., Yamanaka M., Namiki K., Namiki M., *J. Med. Soc. Toho. Japan*, **33**, 261—264 (1986).
- 6) Konno S., Matsuya Y., Kumazawa M., Amano M., Kokubo T., Sagi M., Yamanaka H., *Yakugaku Zasshi*, **113**, 40—52 (1993).
- 7) Ohta A., Masano S., Iwakura S., Tamura Y., Watahiki H., Tsutsui M., Akita Y., Watanabe T., *J. Heterocycl. Chem.*, **19**, 465—473 (1982).
- 8) Ohta A., Inoue A., Watanabe T., *Heterocycles*, **22**, 2317—2321 (1984).
- 9) Ohta A., Watanabe T., Akita Y., Yoshida M., Toda S., Akamatsu T., Ohno H., Suzuki A., *J. Heterocycl. Chem.*, **19**, 1061—1067 (1982).
- 10) Ohta A., Imazeki A., Itoigawa Y., Yamada H., Takagai C., Sano H., Watanabe T., *J. Heterocycl. Chem.*, **20**, 311—320 (1983).
- 11) Ohta A., Ohta M., Igarashi Y., Sacki K., Yuasa K., Mori T., *Heterocycles*, **26**, 2449—2454 (1987).
- 12) Boyer J. H., Mikol G. J., *J. Heterocycl. Chem.*, **9**, 1325—1330 (1972).
- 13) Born G. V. R., *Nature (London)*, **194**, 927—929 (1962).