Studies on Anti-platelet Agents. V.[1] Synthesis and Structure–Activity Relationship of 3-Substituted 5,6-Bis(4-methoxyphenyl)-1,2,4-triazines

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The syntheses and structure–activity relationships of a series of 3-substituted 5,6-bis(4-methoxyphenyl)-1,2,4-triazines as anti-platelet agents based on cyclooxygenase (CO) inhibition are described. Of these compounds, 1-[5,6-bis(4-methoxyphenyl)-1,2,4-triazin-3-yl]carbonyl-4-methylpiperazine (10) exhibited potent CO inhibition *in vitro* ($IC_{50} = 2.8 \times 10^{-7}$ M) with vasodilatory activity ($ED_{50} = 4.5 \times 10^{-5}$ M). Compound 10 also showed potent *ex vivo* activities, completely preventing platelet aggregation induced by arachidonic acid and collagen at 6 h after oral administration of 3.2 and 1.0 mg/kg. The *ex vivo* potency of 10 is more than three times that of aspirin. Moreover, 10 demonstrated no gastrointestinal side effect in rats even at 100 mg/kg in spite of its potent CO inhibition activity, while aspirin, the most widely-used anti-platelet drug, showed gastrointestinal side effects in a dose-dependent manner (32, 100, and 320 mg/kg) in our study. These results suggested that 10 is a very attractive candidate for development as an anti-platelet drug since an aspirin-like anti-platelet agent, based on CO inhibition and being free from gastrointestinal side effects, is a major goal of thromboembolic research.

Keywords platelet aggregation; vasodilation; cyclooxygenase inhibition; aspirin dilemma; 3-substituted 5,6-bis(4-methoxyphenyl)-1,2,4-triazine

In previous papers,[1,2] we synthesized 2-substituted 4,5-bis(4-methoxyphenyl)thiazoles and 2-substituted 4,5-bis(4-methoxyphenyl)pyrimidines and found that, for example, compounds (**1a**: FR122047[2] and **1b**[1]) possess potent and long-lasting anti-platelet activity based on cyclooxygenase (CO) inhibition, with vasodilatory activity. These novel anti-platelet agents were designed on the basis of structural analyses of other CO inhibitors, that is, it is prerequisite for CO inhibitors to have a bis(4-methoxyphenyl) moiety and a hetero ring including a nitrogen atom between the substituents and the 4-methoxyphenyl ring (Fig. 1).

In our continuing studies, we synthesized 3-substituted 5,6-bis(4-methoxyphenyl)-1,2,4-triazines and tested them for their anti-platelet and vasodilatory effects. Although 3-substituted 5,6-bis(4-methoxyphenyl)-1,2,4-triazines have previously been reported as anti-platelet agents,[3] only a few derivatives with simple substituents such as methyl at the 3 position of triazine ring were disclosed.

We describe here the syntheses and structure–activity relationships of 3-substituted 5,6-bis(4-methoxyphenyl)-1,2,4-triazines, and an evaluation of their pharmacological effects in terms of CO inhibition *in vitro*, platelet aggregation *ex vivo*, and vasodilatory activity *in vitro*. The reason for measurement of vasodilatory activity is that this activity is considered beneficial in the treatment of thrombotic disease by anti-platelet drugs. Ulcerogenic activity of the most potent derivative *ex vivo* in this study is also described since the use of aspirin, the most widely-used anti-platelet drug based on CO inhibition, is restricted by the gastrointestinal side effects (aspirin dilemma).[4]

Chemistry

The synthetic routes to the desired 3-substituted 5,6-bis(4-methoxyphenyl)-1,2,4-triazines are shown in Chart 1. Reaction of several thioamides (**2**) with hydrazine hydrate and subsequent condensation with anisil (**3**) in refluxing ethanol (EtOH) gave 3-substituted 5,6-bis(4-methoxyphenyl)-1,2,4-triazines (**4**—**8**). Aminolysis of ethyl 5,6-bis(4-methoxyphenyl)-1,2,4-triazine-3-carboxylate (**4**) with the corresponding amines at 80—90 °C afforded several 3-carbamoyl derivatives (**9**—**14**). Deprotection of the *N*-protected amino derivatives (**5**—**7**) with acids (37% HCl for **5**, 4 N HCl/dioxane for **6**—**7**) gave 3-aminoalkyl derivatives (**15**—**17**). Coupling of **15** and **16** with ethyl or/and isopropyl isocyanate gave *N'*-substituted-ureidoalkyl derivatives (**18**—**19**, **21**). Condensation of **15** and **17** with 3-oxo-2,3,4,5-tetrahydro-6-pyridazinic acid by 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) hydrochloride in the presence of 1-hydroxybenzotriazole at room temperature gave **20** and **22**. *N*-Methylation of 5,6-bis(4-methoxyphenyl)-3-(4-pyridyl)-1,2,4-triazines (**4**) by methyl iodide and subsequent reduction by sodium borohydride gave 5,6-bis(4-methoxyphenyl)-3-(1-methyl-1,2,5,6-tetrahydropyridin-4-yl)-1,2,4-triazine (**23**).

Biological Results and Discussion

Inhibitory activity on malondialdehyde (MDA) synthesis was measured to evaluate the CO inhibition of the novel compounds in this study. MDA is formed by the CO-catalyzed oxygenation of arachidonic acid (A.A.) in the synthesis of prostanoids so that inhibitory activity on MDA synthesis is considered to be consistent with CO-inhibitory activity. Vasodilatory activity of several derivatives was also tested since we consider that this activity may be beneficial in the treatment of thrombotic disease, while aspirin has no vasodilatory activity ($ED_{50} > 6.0 \times 10^{-4}$ M, Table I). The results on anti-MDA production and vasodilatory activities of the novel triazines are shown in Table I.

We first evaluated 5,6-bis(4-methoxyphenyl)-1,2,4-triazine-3-(*N*-substituted)carboxamides (**9**—**14**), since we

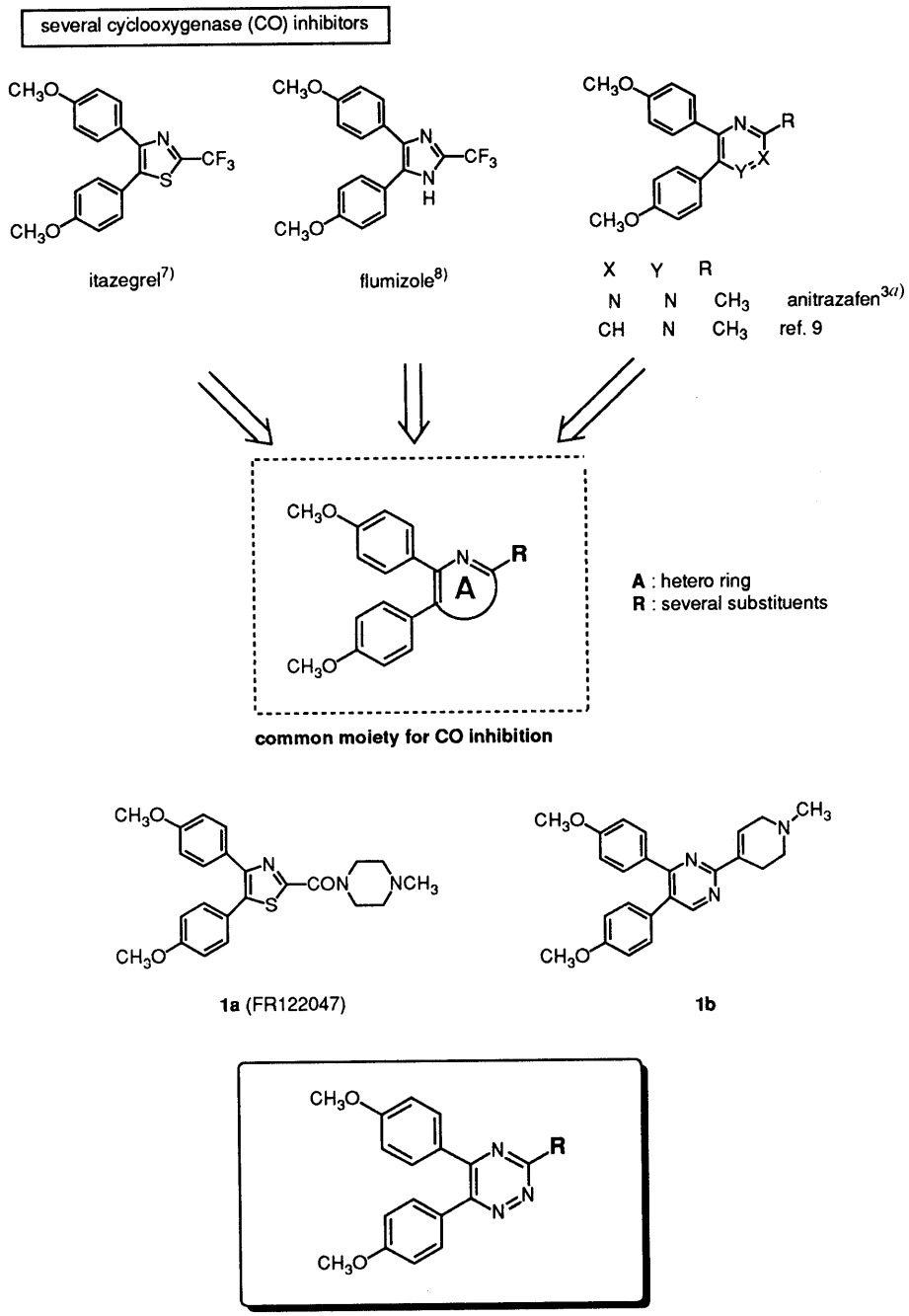
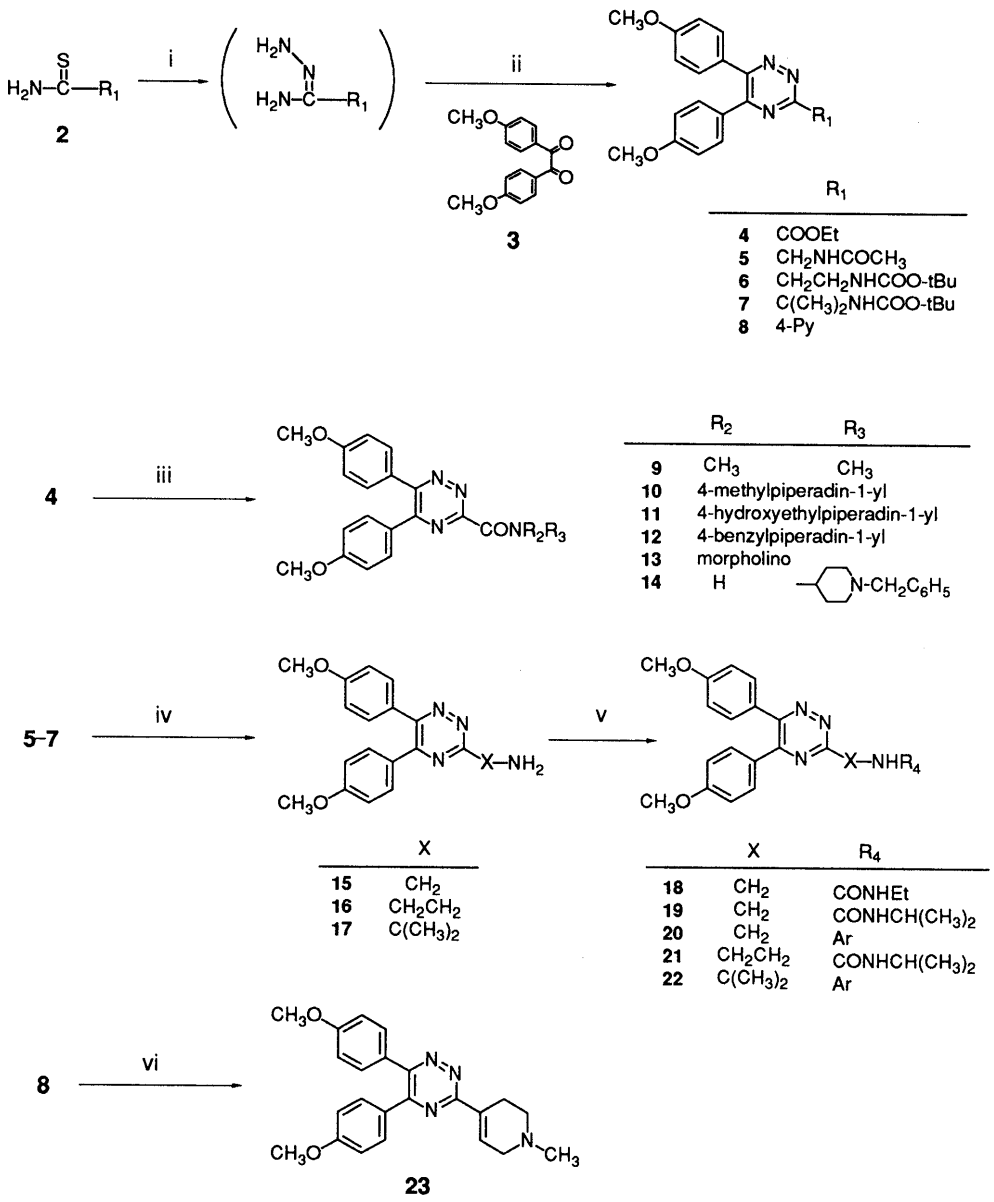


Fig. 1. Evolution of 3-Substituted 5,6-Bis(4-methoxyphenyl)-1,2,4-triazines as Anti-platelet Agents

had observed that 4,5-bis(4-methoxyphenyl)thiazole-2-(*N*-substituted)carboxamides exhibited potent and long-lasting anti-platelet activity in the previous study.[2] 3-Dimethylaminocarbonyl-5,6-bis(4-methoxyphenyl)triazine (**9**) showed potent anti-MDA production activity ($IC_{50}=5.5\times10^{-8}$ M), which was *ca.* 290 times more potent than that of aspirin ($IC_{50}=1.6\times10^{-5}$ M). Compound **9**, however, showed a weak vasodilating effect (47.3 % effect at 10^{-4} M). Substitution of the dimethylamino group with 1-methylpiperazine (**10**) was carried out since (4-methylpiperazin-1-yl)carbonyl was shown to be a potent anti-platelet agent with vasodilatory action in the previous paper (**1a**: FR122047).[2] Compound **10** exhibited potent anti-MDA ($IC_{50}=2.8\times10^{-7}$ M) and vasodilatory activities ($ED_{50}=4.5\times10^{-5}$ M). In order to increase the anti-MDA activity while retaining vasodilatory activity, several derivatives of **10** were synthesized (**11**—**14**). Replacement of the methyl group of **10** with a hydroxyethyl group (**11**) was accompanied with a loss of anti-MDA activity (−9.0% inhibition at 10^{-7} M) while a compound with a benzyl group showed a large increase in the anti-MDA production effect ($IC_{50}=9.0\times10^{-8}$ M), being three times more potent than **10**. Replacement of the *N*-methylpiperazine ring of **10** with morpholine gave **13** with potent anti-MDA activity ($IC_{50}=7.4\times10^{-8}$ M) and a decrease of the vasodilatory effect (17.4% effect at 10^{-7} M).

Next, 5,6-bis(4-methoxyphenyl)-3-(*N*-substituted aminoalkyl)-1,2,4-triazine derivatives (**18**—**22**) were evaluated since 4,5-bis(4-methoxyphenyl)-2-(*N*-substituted



reagents: (i) NH_2NH_2/EtOH, r.t., 1.5 h; (ii) **3**, reflux, overnight; (iii) HNR_2R_3, 80—90 °C, 2.5 h; (iv) 37% HCl, reflux, 2 h (for **15**); 4 N HCl/dioxane, r.t. overnight (for **16**, **17**); (v) R_4NCO/THF and MeOH, r.t., 4.5 h (for **18**, **19**, **21**); R_4–COOH, EDC·HCl HOBt/DMF, r.t., 3 h (for **20**, **22**); (vi) a) MeI/MeOH and $CHCl_3$, r.t. overnight, b) $NaBH_4$/MeOH and H_2O, r.t., 2.5 h. tBu = *tert*-butyl. Ar = (2,3,4,5-tetrahydro-3-pyridazinon-6-yl)carbonyl. HOBt = 1-hydroxybenzotriazole.

Chart 1

aminoalkyl)thiazoles have shown potent anti-MDA and vasodilatory effects previously.[2] The *N*-ethylureido derivative (**18**) showed potent anti-MDA activity ($IC_{50} = 4.0 \times 10^{-8}$ M), which was 400 times that of aspirin, with weak vasodilatory activity (40.5% effect at 10^{-4} M). Replacement of the ethyl group of **18** with an isopropyl group provided **19** with a potent anti-MDA effect ($IC_{50} = 6.4 \times 10^{-8}$ M) and vasodilatory activity ($ED_{50} = 7.3 \times 10^{-5}$ M). Replacement of the methylene moiety of **19** with ethyl gave **21** with a small increase in anti-MDA activity ($IC_{50} = 4.0 \times 10^{-8}$ M). Introduction of a 2,3,4,5-tetrahydro-3-pyridazinone ring onto the amino group (**20**) was accompanied by a loss of anti-MDA activity (48% inhibition at 10^{-7} M). Addition of dimethyl to the methylene moiety of **20** gave **22** with moderate anti-MDA activity ($IC_{50} = 6.1 \times 10^{-7}$ M).

5,6-Bis(4-methoxyphenyl)-3-(1-methyl-1,2,5,6-tetrahydropyridin-4-yl)-1,2,4-triazine (**23**) was synthesized and tested because 4,5-bis(4-methoxyphenyl)-2-(1-methyl-1,2,5,6-tetrahydropyridin-4-yl)pyrimidine (**1b**, Fig. 1) exhibited potent and long-lasting anti-platelet activities in the previous study.[1] Compound **23**, however, showed weak anti-MDA activity (30.1% inhibition at 10^{-6} M).

Next, we performed an *ex vivo* study in guinea pigs on two compounds, **10** and **12**, since **10** exhibited potent CO inhibition activity with the strongest vasodilatory activity and **12** showed *ca.* three times more potent anti-MDA activity than **10** (Table II). Compound **10** showed potent *ex vivo* activity, that is, **10** completely prevented platelet aggregation induced by A.A. and collagen at 6 h after oral

TABLE I. Yields, Analytical Data, and Pharmacological Data for 3-Substituted 3,6-Bis(4-methoxyphenyl)-1,2,4-triazines

	Yield[a] (%)	mp (°C)	Formula	Analysis (%) Calcd C	Calcd H	Calcd N	Found C	Found H	Found N	MDA Inhibition[b,c] *in vitro* IC_{50} (M)	Vasodilatory[b] rat aorta, *in vitro* ED_{50} (M)
9	16.0	50—60	$C_{24}H_{20}N_4O_3 \cdot 1/10H_2O$	65.60	5.56	15.30	65.52	5.66	14.91	5.5×10^{-8}	$>10^{-4}$ (47.3%)
10	60.8	252—254	$C_{23}H_{25}N_5O_3$	60.59	5.75	15.36	60.20	5.80	15.21	2.8×10^{-7}	4.5×10^{-5}
11	48.8	141—147	$C_{24}H_{27}N_5O_4 \cdot HCl \cdot 9/5H_2O$	55.61	6.14	13.51	55.76	6.19	13.30	$>10^{-7}$ (−9.0%)	5.5×10^{-5}
12	61.3	126—129	$C_{29}H_{29}N_5O_3$	70.29	5.90	14.13	70.35	5.89	14.29	9.0×10^{-8}	—
13	68.2	65—70	$C_{22}H_{22}N_4O_3 \cdot 3/10H_2O$	64.16	5.53	13.60	64.13	5.47	13.46	7.4×10^{-8}	$>10^{-4}$ (17.4%)
14	60.4	195 (dec.)	$C_{31}H_{33}N_5O_3 \cdot 3/5H_2O$	69.24	6.24	13.46	69.31	6.16	13.51	$>10^{-6}$ (14.0%)	—
18	79.8	69—74	$C_{21}H_{23}N_5O_3 \cdot 2/5H_2O$	62.96	5.99	17.48	62.97	6.00	17.15	4.0×10^{-8}	$>10^{-4}$ (40.5%)
19	82.5	80—83	$C_{22}H_{25}N_5O_3 \cdot 2/5H_2O$	63.72	6.27	16.89	63.99	6.28	16.56	6.4×10^{-8}	7.3×10^{-5}
20	5.6	183—187	$C_{23}H_{22}N_6O_4$	61.88	4.97	18.82	61.58	5.12	18.55	$>10^{-7}$ (48.0%)	—
21	33.3	157—160	$C_{23}H_{27}N_5O_3 \cdot 3/4H_2O$	63.51	6.60	16.10	63.47	6.36	15.72	4.0×10^{-8}	—
22	83.4	221—222	$C_{25}H_{26}N_6O_4 \cdot 2/5H_2O$	62.33	5.61	17.45	62.53	5.77	17.17	6.1×10^{-7}	—
23	61.0	163—165	$C_{23}H_{24}N_4O_2 \cdot 2/5H_2O$	69.82	6.32	14.16	69.88	6.02	14.11	$>10^{-6}$ (30.1%)	—
Aspirin										1.6×10^{-5}	$>6.0 \times 10^{-4}$

a) Yields from the corresponding precursors. *b*) The evaluation methods are described in Experimental. When the IC_{50} or ED_{50} values could not be defined even at the corresponding maximum concentration, the activity percentage values are shown in parentheses. *c*) MDA production induced by A.A. MDA is formed from the CO catalyzed oxygenation of A.A. in the synthesis of prostanoids so that inhibitory activity on MDA synthesis is considered to be consistent with CO-inhibitory activity. —, not tested.

TABLE II. *Ex Vivo* Activities in Guinea Pigs of Novel Triazines[a]

	Dose[b] (mg/kg)	A.A. 1 h	A.A. 6 h	Collagen 1 h	Collagen 6 h
10	3.2	—	100	—	100
	1.0	81	100	—	93
12	3.2	—	11	—	56
	1.0	—	18	—	−9
Aspirin	3.2	64	—	9	—

a) The evaluation methods are described in Experimental. *b*) Inhibitory activities on guinea pig platelet aggregation induced by A.A. and collagen were measured 1 or/and 6 h after oral administration of each compound. —, not tested.

TABLE III. Induction of Acute Stomach Lesion and Inhibition of Collagen-Induced Platelet Aggregation in Rats by **10** and Aspirin[a]

Compound	Dose (mg/kg)	Ulcer index[b]	Number of rats with ulceration
10	100	0.0±0.0	0/5
Aspirin	10	0.6±0.6	1/5
	32	2.0±0.8	3/5
	100	2.2±0.6	4/5
	320	3.8±0.2	5/5

a) Experimental methods are described in the experimental section. *b*) Values are means±S.E. for five animals.

administration of 3.2 and 1.0 mg/kg while **12** showed disappointing *ex vivo* results. It was very interesting that replacement of the methyl group with a benzyl group resulted in a large reduction of *ex vivo* potency; this indicated that modification of the substituent at the basic nitrogen of the piperazine ring can strongly influence the pharmacokinetic properties of triazine derivatives, such as absorption, distribution, metabolism, and/or excretion. The *ex vivo* potency of **10** is more than three times that of aspirin.

Finally, an ulcerogenesis study in rats was conducted on **10**, having excellent anti-platelet activity *ex vivo*, since it is known that the use of CO inhibitors such as aspirin tends to cause gastrointestinal ulceration, restricting clinical use. The reason for gastrointestinal ulceration by aspirin is considered to be that aspirin prevents not only the synthesis of thromboxane A_2 (TXA_2) in platelets but also that of prostaglandin I_2 in vascular endothelial cells, which induces stomach ulcers (aspirin dilemma).[4] Interestingly **10** had no gastrointestinal side effect even at 100 mg/kg (Table III) in spite of its potent CO-inhibitory activity, while aspirin showed gastrointestinal side effects in a dose-dependent manner (32, 100, and 320 mg/kg) in this study. This result is very interesting, since development of an aspirin-like anti-platelet drug, based on CO inhibition and being free from gastrointestinal side effects, is a major goal of thromboembolic research.

Experimental

Melting point determinations were performed in a capillary melting point apparatus (Thomas Hoover). All melting points are uncorrected. Thin layer chromatography (TLC) was performed on Merck Silica gel 60 F-254 plate. For normal chromatography, Merck Silica gel type 60 (size 70—230 mesh) was used. All evaporations were performed with a rotary evaporator under water aspiration. The structures of all compounds were confirmed by their infrared (IR, Hitachi 260-10), mass (MASS, Finigann MAT TSQ70 mass spectrometer), and 200 MHz proton nuclear magnetic resonance (^{1}H-NMR, Bruker AC200P) spectra. The chemical shift values are reported in parts per million on the δ scale from internal standard tetramethylsilane. All compounds evaluated in bio-assays were analyzed for C, H, N and the results were within 0.4% of the calculated theoretical values. No attempt was made to maximize the yields. All *in vitro* values are means for three experiments. *Ex vivo* values are means for five animals.

Ethyl 5,6-Bis(4-methoxyphenyl)-1,2,4-triazine-3-carboxylate (4) A mixture of ethoxycarbonylthioamide (**2**, 5.00 g, 37.5 mmol) and hydrazine hydrate (1.82 ml, 37.5 mmol) in ethanol (EtOH) was stirred at room temperature for 1.5 h. Anisil (**3**, 10.5 g, 37.5 mmol) was added thereto, and then the mixture was stirred under reflux overnight. After removal of the solvent, the resulting yellow precipitate was washed with isopropylether (IPE) to give **4** (12.03 g, 87.8%).

Compounds **5**—**8** were prepared similarly. Their analytical data are shown in Table IV.

1-[5,6-Bis(4-methoxyphenyl)-1,2,4-triazin-3-yl]carbonyl-4-methylpiperazine (10) A mixture of **4** (1.00 g, 2.74 mmol) and 1-methylpiperazine (1.82 ml, 16.4 mmol) was stirred at 80—90°C for 4.5 h. The reaction

TABLE IV. Physical Data for 5,6-Bis(4-methoxyphenyl)-3-substituted-1,2,4-triazines

	Mass m/z	IR (Nujol) (cm^{-1})	^{1}H-NMR (δ in DMSO-d_6, J=Hz)
4	365 (M^+)	1745, 1640, 1600, 1580, 1510, 1490	1.35 (3H, t, J=7), 3.78 (3H, s), 3.80 (3H, s), 4.38 (2H, q, J=7), 6.98 (2H, d, J=9), 7.03 (2H, d, J=9), 7.53 (2H, d, J=9), 7.55 (2H, d, J=9)
5	364 (M^+)	1650, 1600, 1590	2.13 (3H, s), 3.80 (3H, s), 3.83 (3H, s), 4.89 (2H, d, J=6), 6.85 (2H, d, J=9), 6.88 (2H, d, J=9), 7.56 (2H, d, J=9), 7.61 (2H, d, J=9)
6	436 (M^+)	3320, 1680, 1605, 1580, 1510	1.33 (9H, s), 3.18 (2H, t, J=6), 3.48 (2H, t, J=6), 3.78 (3H, s), 3.80 (3H, s), 6.95 (2H, d, J=9), 6.99 (2H, d, J=9), 7.43 (2H, d, J=9), 7.52 (2H, d, J=9)
7	750 (M^+)	3250, 3110, 1690, 1600, 1570, 1510	1.0—1.4 (9H, br s), 1.68 (6H, s), 3.79 (3H, s), 3.80 (3H, s), 6.97 (2H, d, J=9), 7.01 (2H, d, J=9), 7.46 (2H, d, J=9), 7.51 (2H, d, J=9)
8	370 (M^+)	1600, 1580, 1510, 1490	3.81 (3H, s), 3.82 (3H, s), 7.00 (2H, d, J=9), 7.04 (2H, d, J=9), 7.56 (2H, d, J=9), 7.67 (2H, d, J=9), 8.41 (2H, d, J=5), 8.86 (2H, d, J=5)
9	364 (M^+)	1650, 1600, 1510	2.98 (3H, s), 3.10 (3H, s), 3.79 (3H, s), 3.80 (3H, s), 6.96 (2H, d, J=9), 7.01 (2H, d, J=9), 7.53 (2H, d, J=9), 7.54 (2H, d, J=9)
10	418 (M^+)	3400 (br s), 2400, 1645, 1600, 1580, 1490	2.81 (3H, s), 3.1—4.1 (8H, m), 3.80 (3H, s), 3.81 (3H, s), 4.62 (1H, br s), 6.98 (2H, d, J=9), 7.03 (2H, d, J=9), 7.53 (2H, d, J=9), 7.54 (2H, d, J=9)
11	449 (M^+)	3350 (br s), 1660, 1610	3.0—4.1 (12H, m), 3.80 (3H, s), 3.81 (3H, s), 6.98 (2H, d, J=9), 7.03 (2H, d, J=9), 7.53 (2H, d, J=9), 7.54 (2H, d, J=9)
12[a]	495 (M^+)	1630, 1600, 1480	2.53 (2H, t, J=4.8), 2.62 (2H, t, J=4.8), 3.57 (4H, m, piperidine and NCH_2), 3.83 (3H, s), 3.85 (3H, s), 3.91 (2H, t, J=4.8), 6.88 (2H, d, J=9), 6.93 (2H, d, J=9), 7.58 (2H, d, J=9), 7.64 (2H, d, J=9)
13	406 (M^+)	1650, 1605, 1580	5.45 (4H, m), 5.58 (4H, m), 3.78 (3H, s), 3.81 (3H, s), 6.96 (2H, d, J=9), 7.01 (2H, d, J=9), 7.52 (2H, d, J=9), 7.53 (2H, d, J=9)
14	509 (M^+)	1690, 1600, 1520, 1490	1.5—2.4 (7H, m), 2.88 (2H, m), 3.54 (2H, s), 3.84 (3H, s), 3.86 (3H, s), 6.86 (2H, d, J=9), 6.92 (2H, d, J=9), 7.59 (2H, d, J=9), 7.68 (2H, d, J=9), 8.01 (1H, d, J=8.2)
15	322 (M^+)	1900, 1605, 1580, 1520	3.80 (3H, s), 3.81 (3H, s), 4.52 (2H, br s), 6.9—7.1 (4H, m), 7.49 (2H, d, J=9), 7.64 (2H, d, J=9), 9.00 (3H, br s)
16	336 (M^+)	3350, 1600, 1510	3.1—3.6 (4H, m), 3.79 (3H, s), 3.80 (3H, s), 6.97 (2H, d, J=9), 7.01 (2H, d, J=9), 7.46 (2H, d, J=9), 7.53 (2H, d, J=9), 8.11 (3H, br s)
17	350 (M^+)	3370, 1600, 1570, 1490	1.79 (6H, s), 3.80 (3H, s), 3.81 (3H, s), 6.99 (2H, d, J=9), 7.03 (2H, d, J=9), 7.50 (2H, d, J=9), 7.65 (2H, d, J=9), 8.93 (3H, br s)
18	393 (M^+)	3300, 1635, 1605, 1560, 1490	1.01 (3H, t, J=5), 3.03 (2H, t, J=5), 3.78 (3H, s), 3.80 (3H, s), 4.64 (2H, d, J=6), 6.23 (1H, d, J=6), 6.56 (1H, d, J=6), 6.95 (2H, d, J=9), 7.00 (2H, d, J=9), 7.45 (2H, d, J=9), 7.52 (2H, d, J=9)
19	407 (M^+)	3300, 1640, 1605, 1560, 1490	1.06 (6H, d, J=6), 3.65 (2H, m), 3.78 (3H, s), 3.80 (3H, s), 4.63 (2H, d, J=6), 6.14 (1H, d, J=8), 6.45 (1H, t, J=6), 6.94 (2H, d, J=9), 6.99 (2H, d, J=9), 7.45 (2H, d, J=9), 7.52 (2H, d, J=9)
20	446 (M^+)	3400, 3200, 3105, 1695, 1670, 1660, 1610, 1520	2.44 (2H, t, J=8), 2.78 (2H, t, J=8), 3.78 (3H, s), 3.80 (3H, s), 4.77 (2H, d, J=6), 6.95 (2H, d, J=9), 7.00 (2H, d, J=9), 7.45 (2H, d, J=9), 7.49 (2H, d, J=9), 8.75 (1H, d, J=6), 11.20 (1H, s)
21	421 (M^+)	3300, 1630, 1600, 1570, 1490	0.98 (6H, d, J=7), 3.18 (2H, t, J=7), 3.5—3.7 (3H, m), 3.79 (3H, s), 3.80 (3H, s), 5.73 (1H, d, J=8), 5.85 (1H, t, J=6), 6.96 (2H, d, J=9), 6.99 (2H, d, J=9), 7.45 (2H, d, J=9), 7.52 (2H, d, J=9)
22	474 (M^+)	1660, 1600, 1510	1.83 (6H, s), 2.42 (2H, t, J=8), 2.72 (2H, t, J=8), 3.79 (3H, s), 3.80 (3H, s), 6.97 (2H, d, J=9), 7.02 (2H, d, J=9), 7.48 (2H, d, J=9), 7.53 (2H, d, J=9)
23	388 (M^+)	1660, 1570, 1510	3.33 (3H, s), 2.64 (2H, d, m), 2.76 (2H, br s), 3.17 (2H, br s), 3.78 (3H, s), 3.79 (3H, s), 6.95 (2H, d, J=9), 7.00 (2H, d, J=9), 7.38 (1H, m), 7.48 (2H, d, J=9), 7.53 (2H, d, J=9)

a) ^{1}H-NMR spectrum was measured in $CDCl_3$.

mixture was poured onto a mixture of water and ethyl acetate (AcOEt), and the organic layer was washed with water and brine, dried over magnesium sulfate ($MgSO_4$), and evaporated. The resulting oil was dissolved in a mixture of EtOH and diethyl ether (Et_2O), and an excess of ethanol solution of hydrogen chloride was added thereto. The resulting precipitate was recrystallized from EtOH and Et_2O to give **10** (0.76 g, 60.8%), mp 252—254 °C.

Other 3-(substituted carbamoyl)triazines **9**, **11**—**14** were obtained similarly. Their analytical data are shown in Tables I and IV.

3-Aminomethyl-5,6-bis(4-methoxyphenyl)-1,2,4-triazine (15) A mixture of **5** (0.79 g, 2.17 mmol) and 37% hydrochloric acid (5 ml) was stirred under reflux for 2 h. The reaction mixture was poured onto water and AcOEt, and the mixture was adjusted to pH 10 with 4 N NaOH. The AcOEt layer was washed with water, dried over $MgSO_4$, and evaporated. The resulting oil was dissolved in EtOH and Et_2O, and an excess of EtOH solution of hydrogen chloride was added thereto. After evaporation, the resulting precipitate was recrystallized from EtOH and Et_2O to give **15** as the hydrochloride (0.18 g, 23.1%). Analytical data are shown in Table IV.

3-(1-Amino-1,1-dimethylmethyl)-5,6-bis(4-methoxyphenyl)-1,2,4-triazine (17) A 4 N dioxane solution of hydrogen chloride (30 ml) was added with ice-cooling to a solution of **7** (2.50 g, 5.55 mmol) in methylene chloride (CH_2Cl_2, 20 ml), and the mixture was stirred at room temperature overnight. After evaporation, the resulting precipitate was washed with IPE to give **17** as the hydrochloride (2.15 g, 100%), mp 245—247 °C.

Another 3-aminoalkyl derivative **16** was obtained similarly. The analytical data are shown in Table IV.

3-[(3-Ethylureid-1-yl)methyl]-5,6-bis(4-methoxyphenyl)-1,2,4-triazine (18) Compound **15** (0.80 g, 2.23 mmol) was dissolved in chloroform ($CHCl_3$) and a saturated aqueous solution of $NaHCO_3$. The organic phase was washed with brine, dried over $MgSO_4$, and evaporated. The resulting residue was dissolved in tetrahydrofuran (THF, 20 ml) and methanol (MeOH, 7 ml), and methyl isocyanate (0.21 ml, 2.68 mmol) was added thereto. The reaction mixture was stirred at room temperature for 4.5 h. After evaporation, the resulting precipitate was recrystallized from IPE and EtOH to give **18** (0.70 g, 79.8%), mp 69—74 °C.

Other ureido derivatives **19** and **21** were prepared similarly. The analytical data are shown in Tables I and IV.

6-{*N*-[(5,6-Bis(4-methoxyphenyl)-1,2,4-triazin-3-yl]methyl}carbamoyl-2,3,4,5-tetrahydro-3-pyridazinone (20) Compound **15** (1.00 g, 2.79 mmol) was dissolved in chloroform ($CHCl_3$) and a saturated

aqueous solution of $NaHCO_3$. The organic phase was washed with brine, dried over $MgSO_4$, and evaporated. The residue was dissolved in dimethylformamide (DMF, 10 ml), then 3-oxo-2,3,4,5-tetrahydro-6-pyridazinic acid (0.40 g, 2.79 mmol), 1-hydroxybenzotriazole hydrate (0.43 g, 2.79 mmol), and EDC hydrochloride (0.53 g, 2.79 mmol) were added thereto. The reaction mixture was stirred at room temperature for 3 h, and poured into a mixture of water and AcOEt. The AcOEt phase was washed with 1 N HCl, saturated aqueous solution of $NaHCO_3$, water, and brine, dried over $MgSO_4$, and evaporated. The resulting residue was purified by silica gel column chromatography [$CHCl_3$–MeOH (95 : 5)]. After fractions containing product were combined and evaporated, and the resulting oil was dissolved in EtOH and Et_2O. An excess of EtOH solution of hydrogen chloride was added thereto, and the resulting precipitate was recrystallized from EtOH and Et_2O to give **20** (0.07 g, 5.6%), mp 183—187 °C.

Compound **22** was obtained similarly. Their analytical data are shown in Tables I and IV.

5,6-Bis(4-methoxyphenyl)-3-(1-methyl-1,2,5,6-tetrahydropyridin-4-yl)triazine (23) A mixture of **8** (0.50 g, 1.35 mmol) and methyl iodide (0.75 ml, 1.62 mmol) in a mixture of MeOH (2 ml) and $CHCl_3$ (18 ml) was stirred at room temperature overnight. MeOH (30 ml) and water (10 ml) were added to the reaction mixture, and then sodium borohydride (0.10 g, 2.70 mmol) was added. The reaction mixture was stirred at room temperature for 2.5 h. The resulting precipitate was collected by filtration, washed with water, and recrystallized from $CHCl_3$ and Et_2O to give **23** (0.32 g, 61.0%), 163—165 °C. Analytical data are shown in Tables I and IV.

Pharmacological Tests Blood from male Hartley guinea-pigs was collected into plastic tubes containing 3.8% sodium citrate (1/10 volume of blood), and blood from male Sprague-Dawley rats was collected into tubes containing 2.2% sodium citrate. Platelet-rich plasma (PRP) was obtained by centrifugation of blood at $120 \times g$ for 10 min and platelet-poor plasma (PPP) was obtained by centrifugation of the remaining blood at $1500 \times g$ for 15 min. Platelet aggregation was studied by the turbidimetric method of Born and Cross[5] with an NKK Hematracer 1.

MDA Formation: PRP from rabbits was centrifuged at $150 \times g$ for 15 min. The pellets were suspended in 0.002% saponin–1% ammonium oxalate solution (Technicon). After further centrifugation of the tubes for 10 min, the platelets were resuspended in phosphate-buffered saline (PBS, pH 7.4) at a concentration of 10^9 platelets/ml. MDA was measured by the modified method of Placer *et al.*[6] Platelet suspension (0.9 ml) was preincubated with 0.1 ml of a solution of drug for 5 min at 37 °C and the reaction was started by the addition of 20 μl of 2.5 mM A.A.; the incubation lasted 3 min. The reaction was terminated by addition of 1 ml of thiobarbituric acid reagent, followed by boiling for 10 min. After centrifugation of the test tubes at $1500 \times g$ for 10 min, the absorption of the supernatant solution was measured at 532 nm.

Vasodilatory Activity: Helical strips of rat thoracic aorta were suspended in an organ bath containing Tyrode solution gassed with 95% O_2–5% CO_2 at 37 °C under 0.5 g load. Contraction was induced by addition of KCl solution (final concentration 30 mM). After the tonus reached a plateau, drug solution (dissolved in dimethyl sulfoxide (DMSO)) was added cumulatively and, finally, 10^{-4} M papaverine was added to obtain maximum relaxation. Activities of the test compound were expressed as ED_{50} values, *i.e.*, dose required to relax the isolated rat aorta by 50%.

Ex Vivo Studies on Platelet Aggregation: Male Hartley guinea-pigs weighing 200—300 g were used after a 24 h fast and male Sprague-Dawley rats weighing about 200 g were used after an overnight fast. Blood was obtained from the abdominal aorta under ether anesthesia at scheduled times after oral administration of drugs. The final concentration of collagen was 0.5 μg/ml for guinea-pigs and 2.0 μg/ml for rats. A.A. was used at 50 μM in guinea-pigs. The percent inhibition was calculated from the total aggregation.

Gastro-Ulcerogenic Activity: Male Sprague-Dawley rats were used after a 24 h fast. Drugs were orally administered to groups of five rats, which were killed for autopsy 5 h thereafter. The stomachs were macroscopically inspected and scored as follows: 0, no evidence of gastric lesions; 1, spotty submucosal hemorrhage; 2, some areas of submucosal hemorrhage or appearance of erosion; 3, widespread adherence of blood and large areas of submucosal hemorrhage or one to four small ulcers; 4, more than four small ulcers or one large ulcer (diameter: >3 mm); 5, numerous large ulcers.

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References

1) Part IV: A. Tanaka, Y. Motoyama, and H. Takasugi, *Chem. Pharm. Bull.*, **42**, 1828 (1994).
2) A. Tanaka, H. Sakai, Y. Motoyama, T. Ishikawa, H. Takasugi, *J. Med. Chem.*, **37**, 1189 (1994).
3) *a*) P. P. K. Ho, P. N. Benslay, W. B. Lacefield, W. Preifer, Abstract Papers, American Chemical Society/Chemical Society of Japan Chemical Congress, Honolulu, April 1979, I, Medi 059; H. R. Sullivan, W. M. Miller, D. G. Stark, P. G. Wood, *Xenobiotica*, **11**, 9 (1981); *b*) S. Konno, H. Yamanaka, JUC Pharma. SCI'87, Honolulu, December 1987, Poster Abstracts, p. S158.
4) K. D. Rainsford, *Agents Actions*, **5**, 326 (1975).
5) G. C. R. Born, M. J. Cross, *J. Physiol.*, **168**, 178 (1963).
6) Z. A. Placer L. L. Cushman, B. C. Johnson, *Anal. Biochem.*, **16**, 359 (1966).
7) R. H. Rynbrandt, E. E. Nishiawa, D. P. Balogoyen, A. R. Mendoza, K. A. Annis, *J. Med. Chem.*, **24**, 1507 (1981).
8) J. G. Lombardino, E. H. Weisman, *J. Med. Chem.*, **17**, 1182 (1974).
9) A. Ohta, Y. Akita, T. Watanabe, H. Hasegawa, S. Yaguchi, T. Wakabayashi, Abstracts of Papers, 8th Medicinal Chemistry Symposium, Osaka, November 1986, p. 5.