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Original paper

New derivatives of 5*H*-pyridazino (4,5-*b*) indole and 1,2,4-triazino (4,5-*a*) indole and related compounds as inhibitors of blood platelet aggregation, anti-hypertensive agents and thromboxane synthetase inhibitors

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Summary — This paper reports the synthesis of a series of hydrazone **2**, tetrazole **3**, triazole **4**, pyrrole **5**, **8**, pyrazole **6**, **9**, **12** and 1,2,4-triazino[4,5-*a*]indole **10** derivatives, obtained from 4-hydrazino-8-methoxy-5*H*-pyridazino[4,5-*b*]indole **1a**, 4-hydrazino-8,9-dimethoxy-5*H*-pyridazino[4,5-*b*]indole **1b**, 5-methoxy- and 5,6-dimethoxyindole-2-carbohydrazides **7** and 4-hydrazino-7-methoxy-1,2,4-triazino[4,5-*a*]indole **11**. All these new products and some other related compounds, previously reported by the authors, were studied as inhibitors of platelet aggregation in whole guinea pig blood, induced by arachidonic acid (AA), adenosine-5′-diphosphate (ADP) or collagen. Some of the most active anti-aggregating compounds were also studied as anti-hypertensive agents in spontaneously hypertensive rats (SHR) and as inhibitors of thromboxane synthetase. Acute toxicities in mice were also determined for the most interesting compounds.

Résumé — Nouveaux dérivés du 5*H*-pyridazino[4,5-*b*]indole et du triazino-1,2,4[4,5-*a*]indole et composés apparentés inhibiteurs de l'agrégation plaquettaire, agents anti-hypertenseurs et inhibiteurs de la thromboxane synthétase. *Cet article relate la synthèse de différents types d'hydrazone **2**, tétrazole **3**, triazole **4**, pyrrole **5**, **8**, pyrazole **6**, **9**, **12** et de dérivés du triazino-1,2,4[4,5-a]indole, obtenus à partir de l'hydrazino-4 méthoxy-8 5H-pyridazino[4,5-b]indole **1a**, l'hydrazino-4 diméthoxy-8,9 5H-pyridazino[4,5-b]indole **1b**, des méthoxy-5 et diméthoxy-5,6 indole carbohydrazides-2 7 et de l'hydrazino-4 méthoxy-7 triazino-1,2,4[4,5-a]indole **11**. Tous ces nouveaux produits chimiques et quelques autres composés proches, préalablement divulgués par les auteurs, furent étudiés comme inhibiteurs de l'agrégation plaquettaire dans le sang complet de cochon d'Inde, laquelle fut provoquée au moyen d'acide arachidonique (AA), d'adénosine-5′-diphosphate (ADP) ou de collagène. Quelques composés anti-agrégants plaquettaires des plus actifs furent aussi étudiés en tant qu'inhibiteurs de la thromboxane synthétase, chez des rats spontanément hypertendus. La toxicité aiguë chez la Souris fut aussi déterminée pour les composés les plus intéressants.*

pyridazino[4,5-*b*]indoles / triazino[4,5-*b*]indoles / platelet aggregation inhibitors / thromboxane synthetase inhibitors / anti-hypertensive agents

Introduction

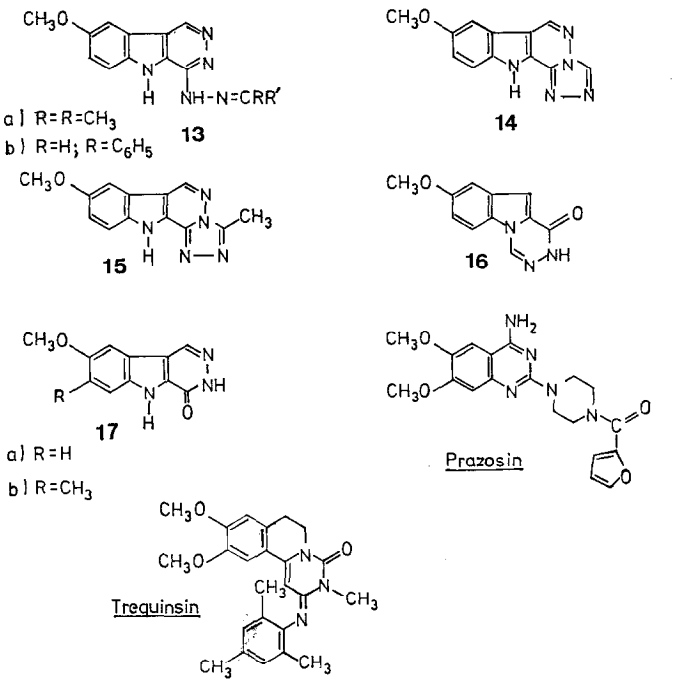
For some time, anti-thrombotic drugs that prevent platelet aggregation by inhibition of platelet cyclooxygenase have been the most widely studied, the foremost example being acetylsalicylic acid [1]. However, a more efficient approach may be the selective inhibition of thromboxane (TAX_2) synthetase. TXA_2, which under physiological conditions rapidly hydrolyzes to TXB_2, is a potent vasoconstrictor and platelet aggregating agent [2—4]. In addition, the inhibition of the production of TXA_2 may increase the production of the vasodilator, prostacyclin (PC). In this way, the control of the ratio *PC/TX* may have a great biological significance in the treatment of cardiovascular disorders [5], particularly in elderly people [6].

As a continuation of our previous work [7, 8] on the synthesis and biological properties of 5*H*-pyridazino[4,5-*b*]-indole derivatives and related compounds, we report in this paper the synthesis and biological study of a new series of 5*H*-pyridazino[4,5-*b*]indole and 1,2,4-triazino-[4,5-*a*]indole derivatives and some related compounds.

In a previous paper [7], we have reported the synthesis and biological study of a long series of 8-substituted (methoxy hydroxy, benzyloxy) derivatives of 4-hydrazino-5*H*-pyridazino[4,5-*b*]indole and the most promising properties were displayed by the 4-hydrazino-8-methoxy-5*H*-pyridazino-[4,5-*b*]indole **1a** and some of its derivatives.

On the other hand, the presence of an *O*-dimethoxyphenyl moiety in several compounds with well-demonstrated anti-hypertensive and anti-aggregating properties [9],

prazosin [10, 11] and trequinsin [12] (see Scheme 1) being perhaps the most significant examples, induced us to synthesize [2—4] the 4-hydrazino-8,9-dimethoxy-5*H*-pyridazino-[4,5-*b*]indole **1b**.



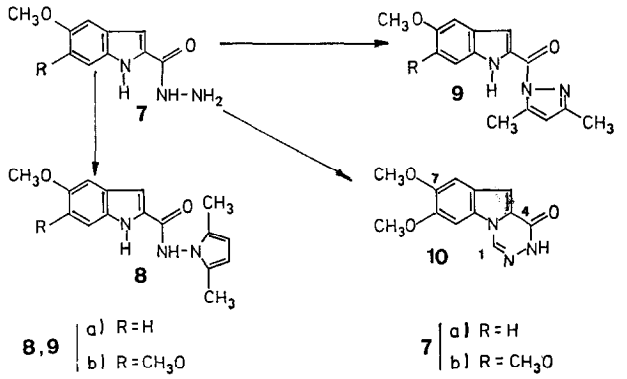
Scheme 1.

We report in this paper the synthesis and the preliminary results of the study of the anti-hypertensive, anti-aggregating and thromboxane synthetase inhibition properties of some hydrazone, tetrazole, triazole, pyrrole and pyrazole derivatives of 4-hydrazino-8,9-dimethoxy-5*H*-pyridazino[4,5-*b*]-indole **1b**. Some related compounds from 4-hydrazino-8-methoxy-5*H*-pyridazino[4,5-*b*]indole **1a**, 5-methoxy- and 5,6-dimethoxyindole-2-carbohydrazides **7** and 4-hydrazino-7-methoxy-1,2,4-triazino[4,5-*a*]indole **11**, were also studied.

Chemistry

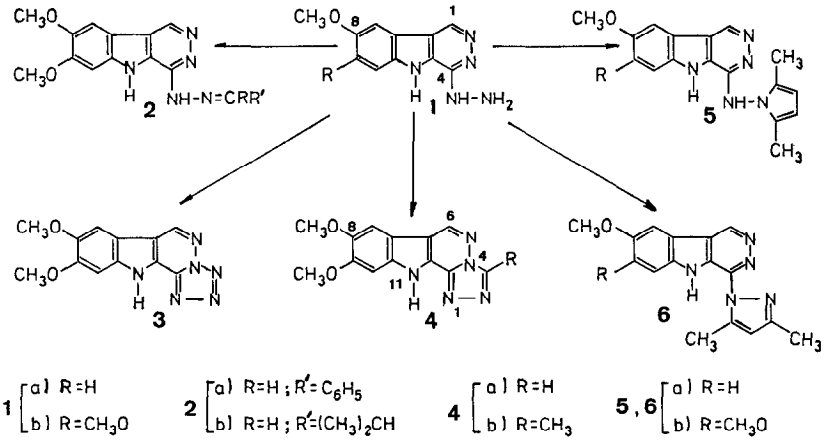
These compounds were obtained as outlined in Schemes 2—4 and all of them were characterized by elemental analysis and IR and ^{1}H NMR spectra which are detailed under Experimental protocols.

The hydrazones **2** were obtained by standard methods. Treating **1b** with nitrous acid as previously reported [7] for similar compounds, gave the tetrazole derivative **3**. The IR spectra of **3** did not show any of the characteristic bands at about 2250—2080 and 1380—1180 cm^{-1} for the azido group (—N_3) and so its structure, at least in the solid state, is tetrazolic. Boiling **1b** with formic and acetic acids gave, respectively, **4a** and **4b**. Compound **4a** was also obtained by treating **1b** with ethyl *ortho*formate. Pyrrole derivatives **5** and **8** were obtained by treating the hydrazines **1** and the hydrazides **7**, respectively, with 2,5-hexanedione.



Scheme 3.

Scheme 4.



Scheme 2.

In a similar way, the reaction of **1**, **7** and **11** with acetylacetone gave the pyrazole derivatives **5**, **9** and **12**, respectively. Finally, by treating **7** with ethyl *ortho*formate, as previously reported [7] for similar compounds, we obtained **10**.

Results and Discussion

All the new compounds described in this paper, as well as the previously reported compounds **1a**, **11**, **13**—**16**, **17a** [7], **1b**, **7b**, **17b** [8] and **7a** [13], were assayed *in vitro* as potential inhibitors of platelet aggregation induced by adenosine-5′-diphosphate (ADP), arachidonic acid (AA) or collagen in whole guinea pig blood. Standard assays were carried out at a final concentration of 500 μM and only the most active compounds were also tested at lower concentrations. Table I summarizes the results. Compound **1a**, the most active compound previously reported by us [1] on the aggregation of platelet-rich plasma from human blood, induced by ADP, AA, PGH_2 or adrenaline, was now active on aggregation induced by ADP, or AA, but not on that induced by collagen. On the other hand, **1b** was active only on the aggregation induced by collagen.

The most active compounds in the AA induced platelet aggregation assay were further studied as inhibitors of thromboxane synthetase. Changes in prostaglandin E_2 (PGE_2) and TXB_2 levels in the platelet aggregation of

Table I. *In vitro* inhibition of platelet aggregation.

Compound	Final conc. (M)	% Inhibition of the aggregation induced by:		
		AA	ADP	collagen
Hydralazine·HCl[a]	5×10^{-4}	20	0	35
1a·HCl[a,b]	5×10^{-4}	100	100	0
1b[b]	5×10^{-4}	0	0	100
	10^{-4}	—	—	30
2a	5×10^{-4}	80	55	70
2b	5×10^{-4}	50	60	70
13a	5×10^{-4}	100	65	40
	10^{-4}	55	0	0
13b	5×10^{-4}	20	0	55
3	5×10^{-4}	100	100	75
	10^{-4}	50	30	0
14	5×10^{-4}	20	20	25
4a	5×10^{-4}	100	45	10
	10^{-4}	100	45	10
	5×10^{-5}	0	—	—
4b	5×10^{-5}	100	90	75
	10^{-5}	0	50	—
15	5×10^{-4}	35	65	55
5a[c]	5×10^{-4}	100	100	100
	10^{-4}	0	0	0
5b	10^{-4}	40	15	75
7a	10^{-4}	0	25	10
7b	5×10^{-4}	30	20	100
	10^{-4}	—	—	95
	5×10^{-5}	—	—	30
8a	10^{-4}	10	0	50
8b	10^{-4}	40	0	50
6a	5×10^{-4}	60	60	75
6b	5×10^{-4}	100	70	100
	10^{-4}	100	30	85
	5×10^{-5}	30	0	35
9a	10^{-4}	25	15	55
9b	5×10^{-4}	20	100	65
	10^{-4}	—	0	—
12c	5×10^{-4}	35	25	70
10	5×10^{-4}	100	46	100
	10^{-4}	100	—	100
	5×10^{-5}	0	—	30
16	10^{-4}	66	35	5
17a	5×10^{-4}	24	40	0
17b	5×10^{-4}	100	30	100
	10^{-4}	100	—	100
	5×10^{-5}	0	—	5
11	5×10^{-4}	90	15	100
	10^{-4}	20	—	50

[a]In normal saline solution.
[b]Determined with platelet-rich plasma.
[c]In DMSO-d_6 solution.

whole guinea pig blood were measured by radioimmunoassay and the results are summarized in Table II. Some of the assayed compounds, at a concentration of 500 μM, showed very significant decreases of TXB_2 and increases of PGE_2 levels. At that concentration, **5a** showed 63% inhibition for TXB_2 and 54% stimulation for PGE_2, while **13a** showed 70% and 25% inhibition for TXB_2 and PGE_2, respectively.

Table II. Inhibition of thromboxane synthetase and stimulation of the prostaglandin E_2 production on the *in vitro* inhibition of the platelet aggregation induced by arachidonic acid.

Compd.	Final conc. (M)	PGE_2 % increase	TXB_2 % inhibition
17b	5×10^{-4}	20	25
	10^{-4}	[a]	[a]
10	5×10^{-4}	16	38
	10^{-4}	−10	[a]
11	5×10^{-4}	36	42
13a	5×10^{-4}	−25	70
3	5×10^{-4}	20	+20
4a	5×10^{-4}	20	10
4b	5×10^{-4}	30	13
6b	5×10^{-4}	50	26
	10^{-4}	30	20
5a	5×10^{-4}	54	63
ASA[b]	5×10^{-4}	0	100

[a]No significant values (p <5%).
[b]Acetylsalicylic acid.

On the other hand, a selection of the most active compounds in the platelet aggregation assay were also studied as potential anti-hypertensive agents in spontaneously hypertensive rats (SHR), at a standard dose (i.p.) of 30 mg/kg. Table III summarizes the results in percentage of falls in the arterial pressure (AP) at 1, 3, 5 and 24 h after dosing. Compounds **1a** and **1b** were the most active and both of them also showed a short-time significant activity at a dose of 1 mg/kg.

Table III. Anti-hypertensive activity in spontaneously hypertensive rats.

Compd.	Dose (mg/kg)	Control AP	Percentage falls in AP 1 h	3 h	5 h	24 h
10	30	235	10	[a]	[a]	[a]
16	30	200	10	10	5	[a]
1a	30	226	60	58	50	12
	1	210	34	5	[a]	[a]
1b	30	233	50	45	44	6
	1	236	30	25	30	[a]
2b	30	210	7	[a]	[a]	[a]
13a	30	200	15	15	12	10
4b	30	217	[a]	[a]	[a]	[a]
15	30	226	5	10	12	[a]
6b	30	262	10	5	[a]	[a]
6a	30	229	5	[a]	[a]	[a]
5b	30	224	12	10	7	[a]
5a	30	201	10	[a]	[a]	[a]
11·HCl	30	200	[a]	[a]	[a]	[a]

[a]No significant values ($p \leq 5\%$).
AP: arterial pressure.

Table IV shows the acute toxicities in male Swiss mice for the most interesting compounds.

Table IV. Acute toxicities.

Compound	DL_{50} (mg/kg)
17a	>1000
17b	>1000
1a	215±12
1b	225±25
2b	575±18
13a	380±30
4b	>1000
15	>1000
6b	980±35
6a	>1000
5b	960±50
5a	800±45
Hydralazine·HCl	100

Experimental protocols

Chemistry

Melting points were determined on a Reichert microscope and they are uncorrected. Elemental microanalyses were obtained from vacuum-dried samples (over phosphorus pentoxide at 1—2 mm Hg, 12 h, at about 50-60°C). Analyses indicated by elemental symbols were within ±0.4% of the theoretical values. IR spectra were recorded on a Perkin—Elmer 681 apparatus, using potassium bromide tablets; the frequences are expressed in cm^{-1}. ^1H NMR spectra were recorded on a Perkin—Elmer R-32 (90 MHz) instrument, with tetramethylsilane (TMS) as the internal reference, at a concentration of about 0.1 g/ml, with DMSO-d_6 (dimethylsulfoxide) or the indicated solvents; the chemical shifts are reported in δ (ppm) from TMS. The abbreviations (s, m, t, *etc.*) are the usual.

The course of most of the reactions was observed by thin—layer chromatography (TLC), carried out on silica gel (DSF-5, Camaga) plates of about 0.3 mm thickness, with benzene:dioxane:acetic acid (90:25:4, v/v) as the solvent. The plates were scanned under ultraviolet light, λ = 254 and 366 nm.

The following compounds were obtained according to previously reported methods: **1a, 11, 13, 14, 15, 16, 17a** [7]; **1b, 7b, 17b** [8]; **7a** [13].

Hydrazones **2**

4-(N²-Benzylidenehydrazino)-7,8-dimethoxy-5H-pyridazino[4,5-b]indole **2a**

A mixture of **1b** (1.0 g, 3.8 mmol) and benzaldehyde (5 ml) was boiled for 4 h. On cooling the reaction mixture, the product crystallized. The orange colored crystals were collected and washed with warm 50% (v/v) ethanol/dioxane and then with ether; mp: >250°C, yield: 1.2 g (95%).

Anal. $C_{19}H_{17}N_5O_2$ (C, H, N). IR: 1630 (CN); 770, 750, 690 (aromatic substitutions). ^1H NMR (TFA): 4.05 (s, 3H) and 4.10 (s, 3H) for 2 CH_3O; 7.40 (s, 1H-6); 7.60—7.70 (m, 6H, H-9 and C_6H_5); 8.40 (bs, 1H, CH=N); 9.45 (s, 1H-1).

4-(N²-Isobutylidenehydrazino)-7,8-dimethoxy-5H-pyridazino[4,5-b]-indole **2b**

A mixture of **1b** (1.0 g, 3.8 mmol) and isobutyraldehyde (3 ml) was boiled for 1 h. The product, which crystallized on cooling the mixture, was collected and washed with dioxane; spongy white crystals, mp: >250°C (from dimethylformamide (DMF)), yield: 1.1 g (95%).

Anal. $C_{16}H_{19}N_5O_2$ (C, H, N). IR: 3160 (NH); 1620 (CN). ^1H NMR

(TFA): 1.75 (d, 6H, 2 CH_3); 2.10 (m, 1H, CH); 4.15 (s, 3H); and 4.20 (s, 3H) for 2 CH_3O; 7.45 (s, 1H-6); 7.82 (s, 1H-9); 955 (s, 1H-1).

8,9-Dimethoxy-11H-tetrazolo[1,5-b]pyridazino[4,5-b]indole **3**
To a stirred mixture of powdered **1b** (1.0 g 3.8 mmol) and sodium nitrite (0.26 g, 3.8 mmol), cooled in an ice bath, concentrated HCl (3 ml) was added dropwise. The mixture was stirred for 24 h at room temperature, the precipitate was collected, washed with water and recrystallized to give grayish crystals, mp: >250°C (from DMF), yield: 1.0 g (96%).

Anal. $C_{12}H_{10}N_6O_2$ (C, H, N). IR: 1660 (CN), 1630 (N=N). ^1H NMR: 3.95 (s, 6H, 2 CH_3O); 7.13 (s, 1H-10); 7.85 (s, 1H-7); 9.50 (s, 1H-6).

Triazole derivatives **4**

8,9-Dimethoxy-11H-1,2,4-triazolo[3,4-b]pyridazino[4,5-b]indole **4a**
Method A. A mixture of **1b** (1.0 g, 3.8 mmol) and formic acid (25 ml) was boiled for 4 h. The cooled mixture was poured onto crushed ice (30 g) and the precipitate collected, washed with water and recrystallized to give white crystals, mp: >250°C (from DMF), yield: 1.0 g (98%).
Method B. A mixture of **1b** (1.0 g, 3.8 mmol) and ethyl *ortho*formate (10 ml) was boiled for 8 h. Solvents were removed by rotatory evaporation under vacuum and the residue treated with ethanol to give white crystals of **4a**, yield 0.9 g (95%).

Anal. $C_{13}H_{11}N_5O_2$ (C, H, N). IR: 3100 (NH), 1640 (CN). ^1H NMR (TFA): 4.15 (s, 6H, 2 CH_3O); 7.43 (s, 1H-10); 7.83 (s, 1H-7); 9.48 (s, 1H-6); 9.60 (s, 1H-3).

8,9-Dimethoxy-3-methyl-11H-1,2,4-triazolo[3,4-b]pyridazino[4,5-b]indole **4b**
A mixture of **1b** (1.0 g, 3.8 mmol) and acetic acid (20 ml) was boiled for 8 h. Solvent was removed under vacuum by rotatory evaporation and the solid residue collected, washed with warm water and recrystallized to give white crystals, mp: >250°C (from dioxane/DMF), yield: 0.8 g (75%).

Anal. $C_{14}H_{13}N_5O_2$ (C, H, N). IR: 1640 (CN). ^1H NMR (TFA): 2.90 (s, 3H, CH_3); 4.00 (s, 6H, 2 CH_3O); 7.25 (s, 1H-10); 7.65 (s, 1H-7); 9.32 (s, 1H-6).

Pyrrole derivatives **5** *and* **8**

4-[(2,5-Dimethylpyrrol-1-yl)amino]-8-methoxy-11H-pyridazino[4,5-b]-indole **5a**
To a stirred mixture of **1a** (2.29 g, 10 mmol) and acetic acid (15 ml), 2,5-hexanedione (1.6 g, 14 mmol) was added dropwise. The mixture was warmed for 8 h, at about 60°C and the solid material which precipitated upon cooling was collected and stirred for 30 min with a saturated aqueous solution of sodium bicarbonate. The solid was collected by filtration, washed several times with water and recrystallized to give **5a**, mp: >250°C (from ethanol), yield: 1.4 g (45%).

Anal. $C_{17}H_{17}N_5O$ (C, H, N). IR: 3270, 3160, 3100 (NH), 1610 (CN). ^1H NMR: 2.05 (s, 6H, 2 CH_3); 3.90 (s, 3H, CH_3O); 5.75 (s, 2H, pyrrole); 7.10—7.80 (m, 3H, indole); 9.40 (s, 1H-1).

4-[(2,5-Dimethylpyrrol-1-yl)amino]-8,9-dimethoxy-11H-pyridazino[4,5-b]indole **5b**
This compound was obtained in a similar way to that reported above for **5a**, but starting with **1b** (2.59 g, 10 mmol), acetic acid (15 ml) and 2,5-hexanedione (1.4 g, 12 mmol); mp: 243—245°C (d) (from 2-propanol), orange-colored crystals, yield: 2.5 g (75%).

Anal. $C_{18}H_{19}N_5O_2$ (C, H, N). IR: 3200—3100 (NH); 1630—1610 (CN). ^1H NMR: 2.05 (s, 6H, 2 CH_3); 3.90 (s, 6H, 2 CH_3O); 5.77 (s, 2H pyrrole); 7.20 (s, 1H-6); 7.75 (s, 1H-9); 9.05 (s, 1H-1).

2-[N-(2,5-Dimethylpyrrol-1-yl)carbamoyl]-5-methoxyindole **8a**
A mixture of **7a** (1.0 g, 4.9 mmol), 2,5-hexanedione (3 ml) and ethanol (10 ml) was boiled for 8 h. Solvents were removed under vacuum by rotatory evaporation and the residual material treated with ether. The precipitate was collected and recrystallized, mp: >250°C (from ethanol), yield: 1.1 g (80%).

Anal. $C_{16}H_{17}N_3O_2$ (C, H, N). IR: 3400, 3290 (NH), 1660 (CO). ^1H NMR: 2.10 (s, 6H, 2 CH_3); 3.80 (s, 3H, CH_3O); 5.75 (s, 2H pyrrole); 6.80—7.50 (m, 4H, H-3, H-4, H-6, H-7 indole); 11.35 (bs, iH) and 11.80 (bs, 1H) for 2 NH.

2-[N-(2,5-Dimethylpyrrol-1-yl)carbamoyl]-5,6-dimethoxyindole **8b**
A mixture of **7b** (1.0 g, 4.25 mmol), 2,5-hexanedione (2 ml) and ethanol (10 ml) was boiled for 6 h. On cooling the mixture, the product crystallized, mp: >250°C (from dioxane), yield: 1.0 g (75%).

Anal. $C_{17}H_{19}N_3O_3$ (C, H, N). IR: 3350, 3290 (NH), 1650 (CO). ^1H NMR: 2.10 (s, 6H, 2 CH_3); 3.80 (s, 6H, 2 CH_3O); 5.75 (s, 2H pyrrole); 6.95 (s, 1H), 7.15 (s, 1H), 7.28 (s, 1H) for H-3, H-4, H-7; 11.40 (bs, 1H) and 11.85 (bs, 1H) for 2 NH.

Pyrazole derivatives **6**, **9** *and* **12**

4-(3,5-Dimethylpyrazol-1-yl)-8-methoxy-11H-pyridazino[4,5-b]indole **6a**
A mixture of **1a** (1.5 g, 6.5 mmol) and acetylacetone (5 ml) was boiled for 6 h. The solid which precipitated upon cooling was collected, washed with ether and recrystallized to give **6a**, mp: 184—186°C (dioxane), yield: 2.9 g (70%).

Anal. $C_{16}H_{15}N_5O$ (C, H, N). IR: 3420 (NH), 1590 (CN). ^1H NMR: 2.45 (s, 3H) and 2.67 (s, 3H) for 2 CH_3; 3.95 (s, 3H, CH_3O); 6.29 (s, 1H pyrazole); 7.20—7.40 (m, 2H) and 7.80—8.00 (m, 1H) for H-7, H-9, H-10; 9.82 (s, 1H-1); 11.70 (bs, 1H, NH).

4-(3,5-Dimethylpyrazol-1-yl)-8,9-dimethoxy-11H-pyridazino[4,5-b]indole **6b**
This compound was obtained in similar way to that described above for **6a**, but starting with **1b**; mp: 225—227°C (from dioxane), yield: 1.5 g (70%).

Anal. $C_{17}H_{17}N_5O_2$ (C, H, N). IR: 3300 (NH), 1630, 1600 (CN). ^1H NMR: 2.48 (s, 3H) and 2.80 (s, 3H) for 2 CH_3; 4.00 (s, 6H, 2 CH_3O); 6.30 (s, 1H pyrazole); 7.50 (s, 1H-6); 7.95 (s, 1H-9); 9.80 (s, 1H-1); 11.80 (bs, 1H, NH).

2-(3,5-Dimethylpyrazolyl-1-carbonyl)-5-methoxyindole **9a**
A mixture of **7a** (1.0 g, 4.9 mmol) and acetylacetone (10 ml) was boiled for 6 h. Solvents were removed under vacuum by rotatory evaporation and the residue treated with ether (5 ml). The pink-colored solid was collected and recrystallized; mp: 108—112°C (from 2-propanol), yield: 0.85 g (65%).

Anal. $C_{15}H_{15}N_3O_2$ (C, H, N). IR: 3400 (NH), 1650 (CO). ^1H NMR ($CDCl_3$): 2.30 (s, 3H) and 2.60 (s, 3H) for 2 CH_3; 3.80 (s, 3H, CH_3O); 5.95 (s, 1H pyrazole); 6.80—7.60 (m, 4H, H-3, H-4, H-6, H-7); 11.00 (bs, 1H, NH).

2-(3,5-Dimethylpyrazolyl-1-carbonyl)-5,6-dimethoxyindole **9b**
This compound was obtained in similar way to that described above for **9a**, but starting with **7b**; mp: 159—162°C (from 2-propanol) as orange-colored needles, yield: 1.1 g (85%).

Anal. $C_{16}H_{17}N_3O_3$ (C, H, N). IR: 3350 (NH), 1650—1670 (CO). ^1H NMR ($CDCl_3$): 2.35 (s, 3H), 2.68 (s, 3H) for 2 CH_3; 4.00 (s, 6H, 2 CH_3O); 6.10 (s, 1H pyrazole); 6.90 (s, 1H), 7.10 (s, 1H) and 7.65 (s, 1H) for H-3, H-4, H-7; 11.20 (bs, 1H, NH).

4-(3,5-Dimethylpyrazol-1-yl)-7-methoxy-1,2,4-triazino[4,5-a]indole **12**
This compound was prepared in similar way to that described above for **9a**, but starting with **11** and boiling the reaction mixture for 8 h; mp: >250°C (from dioxane), yield: 1.2 g (85%).

Anal. $C_{16}H_{15}N_5O$ (C, H, N). IR: 1640 (CN). ^1H NMR (TFA): 2.80 (s, 3H), 2.90 (s, 3H) for 2 CH_3; 4.10 (s, 3H, CH_3O); 7.10—7.60 (m, 4H, H-5, H-6, H-8, H-9); 7.85 (s, 1H-1).

3,4-Dihydro-7,8-dimethoxy-4-oxo-1,2,4-triazino[4,5-a]indole **10**
A mixture of **7b** (1.0 g, 4.25 mmol) *N,N*-dimethylformamide (10 ml) and ethyl *ortho*formiate (2.5 ml) was boiled for 4 h. Solvents were removed under vacuum by rotatory evaporation and the residue recrystallized to give yellow-colored needles, mp: >250°C (from dioxane), yield: 0.80 g (85%).

Anal. $C_{12}H_{11}N_3O_3$ (C, H, N). IR: 3190 (NH), 1660 (CO). ^1H NMR: 3.80 (s, 6H, 2 CH_3O); 7.20—7.30 (m, 3H, H-5, H-6, H-9); 8.65 (s, 1H, H-1); 11.75 (bs, 1H, NH).

Biological assays

In vitro *assay of platelet aggregation*
Guinea pig blood was obtained from anesthetized (ethyl ether) animals (300—350 g) by direct cardiac puncture. The blood was collected

in polyethylene tubes (Venoject) containing a 3.8% sodium citrate solution (1 part citrate solution to 9 parts blood). Platelet aggregation was measured using a total blood aggregation meter (Chrono-Long) according to a previously reported method [14] and using siliconized glass tubes. In the test, 0.5 ml of whole blood, 0.5 ml of normal saline and 0.05 ml of a solution (only the solvent for control) of the compound to be tested were preincubated for 5—60 min at 37°C, at which time, 0.05 ml of a solution of the aggregating agents was added (final concentration, ADP: 50 μM; AA: × μM; collagen: 0.2 mg/ml). The changes in the electric impedance were registered for 5 min at 37°C and, in each case, the activity has been expressed (Table I) as the % inhibition of the aggregation, measured at 5 min for the control.

In general, compounds were tested initially at a final concentration of 500 μM and the most active were also tested at 100 and/or 50 μM. The compounds were dissolved in a suitable solvent: normal saline, 10% ethylenglycol in normal saline or dimethyl sulfoxide [15] (in this case, the final concentration of the solvent in the mixture for preincubation was not greater than 6.6 μl/ml).

Effects on thromboxane synthetase and prostaglandin production on the in vitro *platelet aggregation*

The demonstration of selective inhibition of thromboxane synthetase was determined according to the Gorman model [16], in whole guinea pig blood, on aggregation induced by arachidonic acid (AA). Changes in PGE_2 and TXB_2 levels in the test samples following aggregation were determined by radioimmunoassay according to methods previously reported [17]. Acetylsalicylic acid (100 μM) was tested as the reference. Table II summarizes the results for the test compounds.

Anti-hypertensive activity

Compounds to be tested were evaluated for anti-hypertensive activity in unanesthetized spontaneously hypertensive, 20 week old, male and female Okamoto strain rats, weighing 300—350 g and with systolic blood pressure levels ⩾200 mm Hg. Changes in the arterial pressure (AP) were measured on the tails of the animals by mechanical transduction (W+W, BP recorder 8005) and registered on paper. A dose was given to each of the 5 control animals and AP were measured at the indicated times. The tested compounds were administered (i.p., 2.5 ml/kg) either dissolved or suspended in normal saline or normal saline containing 0.2% carboxymethylcellulose and 1.0% Tween 80. The initial dose was 30 mg/kg and the most active compounds were then tested at a dose of 1 mg/kg. The results of these experiments are summarized in Table III.

Acute toxicity

Male Swiss mice in lots of 6 animals were used. The animals were treated i.p. with least at 4 different doses with mortalities between 0 and 100%. LD_{50} values were calculated 7 days after drug administration by means of probit analysis according to Miller and Taimter [18].

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