dissolved in 1 N NaOH (1.5 L), and filtered. The filtrate was neutralized with 6 N HCl, and the light yellow solid was collected, washed with water, and dried. Recrystallization from ethanol/DMF gave 17 (12 g).

4-(1,6-Dihydro-2-methyl-6-oxo-3-pyridinyl)benzenecarbothioamide (18). H_2S was bubbled into a solution of 17 (6 g, 28.6 mmol) in DMF (160 mL) for 40 min. 1,2-Ethanediamine (2.7 g, 45 mmol) was then added followed by stirring the mixture overnight at room temperature. The mixture was poured into ice–water (800 mL) and the precipitate was filtered, washed thoroughly with water, dried, and recrystallized from CH_3OH to give 5 g of 18.

5-[4-(4,5-Dihydro-1*H*-imidazol-2-yl)phenyl]-6-methyl-2-(1*H*)-pyridinone (19). A mixture of 18 (5 g, 0.02 mol) and 1,2-ethanediamine (30 mL) was heated with stirring in an oil bath at 100 °C for 0.5 h. The mixture was cooled, poured into water (300 mL), and acidified to pH 2. The suspension was filtered, and the filtrate was adjusted to pH 10. The solid was collected, washed with water, dried, and crystallized from DMF to give 19 (2 g).

Pharmacological Methods. 1. Anesthetized Dog Model. Adult mongrel dogs of either sex were anesthetized with pentobarbital, 35 mg/kg, IV, and were subsequently maintained under anesthesia with a continuous infusion of pentobarbital, 5 mg/kg per h. The trachea was intubated, but the animals were permitted to breathe spontaneously. A cannula was inserted into the femoral vein for administering test agents. A Miller catheter tip pressure transducer (Model PC-350) was inserted into the ascending aorta via the femoral artery for measuring left ventricular blood pressure. Needle electrodes were placed subcutaneously for recording a lead II electrocardiogram (ECG).

Left ventricular and aortic blood pressures were recorded on a strip chart recorder. Heart rate, using a biotachometer triggered from the R wave of the ECG, and the first derivative of left ventricular blood pressure (dP/dt), obtained with a differentiator amplifier coupled to the corresponding pressure amplified, were also recorded. Data analyses were performed with a digital computer. A period of 30 min was utilized to obtain control data prior to administration of test agent. Depending on solubility of the agent, compounds were dissolved in 0.9% saline solution or in dilute HCl or NaOH (0.1 or 1.0 N) and were diluted to volume with normal saline. Each dose of the test agent was administered in a volume of 0.1 mL/kg over a period of 1 min unless otherwise designated. Limited solubility may require adjustments in the volume of the solution that was administered. The test agents were administered in an ascending dose manner. Usually, half-log intervals were maintained between doses, with typical dosing consisting of four to six doses (for example, 0.01, 0.03, 0.3, 1.0 mg/kg) in order to establish any dose–response relationships. A 10–30-min interval was used between doses. Only one compound was adminstered to any one animal. The inotropic activity of a compound was determined by measuring changes in dP/dt_{max} of left ventricular pressure.

2. Conscious Dog Model. Adult mongrel dogs were prepared by surgically implanting devices for measuring ECG, aortic blood pressure, aortic blood flow, and left ventricular blood pressure. These animals were allowed to recover from surgery for at least 2 weeks prior to undergoing testing. On the day of the test, the dogs were caged and connected to appropriate interfacing for recording the indicated cardiovascular parameters on a strip chart recorder. Heart rate, aortic blood pressure, left ventricular blood pressure, and aortic blood flow were measured directly; myocardial contractility was determined by obtaining dP/dt_{max} of left ventricular blood pressure and dQ/dt_{max} of aortic blood flow. Cardiac output and total peripheral resistance were derived from heart rate, aortic flow, and aortic blood pressure. Data analyses were performed with a digital computer. The test agent was then administered by gavage to the fasted dog either as a solution or as a suspension in a single dose or multiple-dose fashion.

Data are expressed as means ± SEM. Statistical analysis of the data was performed by using a Student's t test for paired or unpaired data. The probability value, $p < 0.05$, was accepted as level of significance.

Acknowledgment. We thank Dr. F. A. Mackellar and his associates for spectral determinations and microanalyses. We also thank Dr. S. E. Burke, R. E. Potoczak, R. Mcnish, and D. M. Boucher for biological testing and D. Kobylarz-Singer for performing phosphodiesterase studies. We greatly appreciate the technical assistance of E. Badger.

Selective Thromboxane Synthetase Inhibitors and Antihypertensive Agents. New Derivatives of 4-Hydrazino-5*H*-pyridazino[4,5-*b*]indole, 4-Hydrazinopyridazino[4,5-*a*]indole, and Related Compounds

A. Monge,*† P. Parrado,† M. Font,‡ and E. Fernández-Alvarez§

Departamento de Quimica Orgánica y Farmacéutica, Universidad de Navarra, Pamplona, Spain, Antibióticos, S. A., Madrid, Spain, and Instituto de Quimica Orgánica General del C.S.I.C., Juan de la Cierva, 3, 28006 Madrid, Spain.
Received May 27, 1986

A series of new derivatives of 4-hydrazino-5*H*-pyridazino[4,5-*b*]indole (**5**) and 4-hydrazinopyridazino[4,5-*a*]indole (**12**) have been synthesized to investigate their activities as selective thromboxane synthetase inhibitors as well as antihypertensive agents. Several of the prepared compounds were found to be selective thromboxane synthetase inhibitors, in concordance with the Gorman model.[1] The most potent were 8-(benzyloxy)-3,4-dihydro-4-oxo-5*H*-pyridazino[4,5-*b*]indole (**3c**) and 8-methoxy-4-hydrazino-5*H*-pyridazino[4,5-*b*]indole (**5a**). This last compound did not inhibit prostacyclin formation and showed an antihypertensive activity similar to that of hydralazine. The acute toxicity in mice for **5a**·HCl is about 2.2 times less than that for hydralazine.

The action of antithrombotic drugs that act on platelet aggregation has been largely due to inhibition of platelet cyclooxygenase, the foremost example being acetylsalicylic acid (ASA).[2] A more effective approach may be the selective inhibition of thromboxane (TXA_2) synthetase.[3] TXA_2, which rapidly hydrolyzes under physiological conditions to TXB_2, is a potent vasoconstrictor and platelet aggregating agent.[4] An additional advantage would be

† Universidad de Navarra.
‡ Antibióticos, S. A.
§ Instituto de Quimica Orgánica General del C.S.I.C.

(1) Gorman, R. *Advances in Prostaglandin and Thromboxane Research*; Samuelsson, B., Kamwell, P. W., Paleotti, R., Eds.; Raven: New York, 1980; p 417.
(2) Moncada, S.; Vane, J. R. *J. Med. Chem.* **1980**, *23*, 591.
(3) (a) Verstraete, M. *Arzneim.-Forsch./Drug Res.* **1983**, *33*, 1405. (b) Smith, J. B. *Arzneim.-Forsch./Drug Res.* **1983**, *33*, 1357.

0022-2623/87/1830-1029$01.50/0

that some precursor of TXA_2, like prostaglandin H_2 (PGH_2), may increase the production of the vasodilator prostacyclin (PGI_2) in the vessel wall.[5] The control of the PGI_2/TXA_2 system may have a great biological significance in the treatment or prophylaxis of several cardiovascular diseases.[6] That control has a particular interest in elderly people.[7] The goal of the antihypertensive therapy in those cases is no longer just the control of elevated blood pressure as to protect the people against other cardiovascular complications.

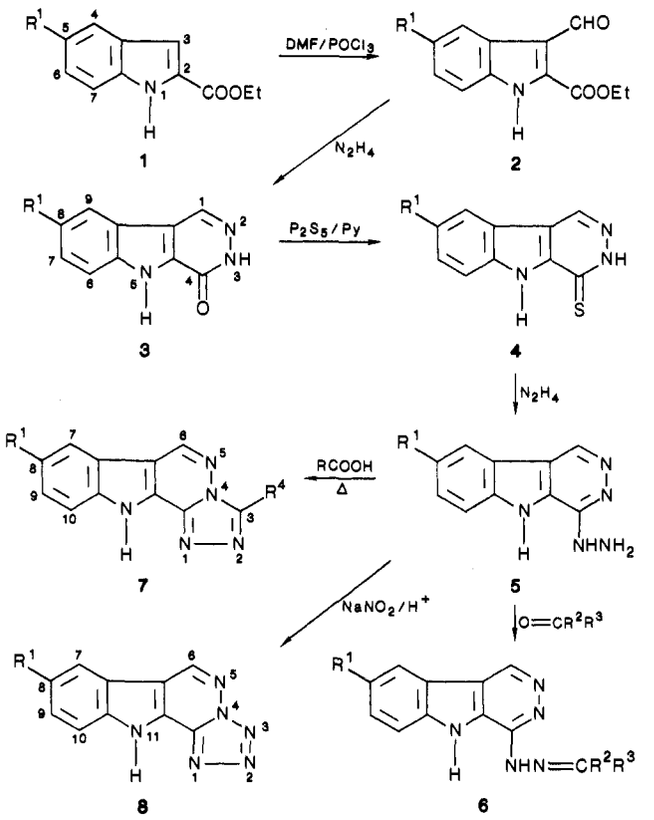
Based on the last considerations, our target is the regulation of the PGI_2/TXA_2 system by compounds with antihypertensive effects and that are structurally related to hydralazine[8] (1-hydrazinophthalazine) and dihydralazine (1,4-dihydrazinophthalazine), peripheral vasodilators used in the treatment of hypertension. In this paper we present our most recent results on the antihypertensive and thromboxane synthetase inhibition activities of a new series of pyridazino[4,5-*b*]indole and pyridazino[4,5-*a*]indole derivatives.

The antiaggregation properties of several indolic derivatives is well documented,[9a] and there are also many examples of selective TXA_2 synthetase inhibitors.[9b] The interest in that therapeutic approximation is not new,[10] but to our knowledge, this is the first effort to obtain indole compounds with both biological profiles.

This contribution is a continuation of our previous work on the synthesis and study of potential antihypertensive[11] agents as well as inhibitors of platelet aggregation[12] of hydrazino derivatives of pyridazine.

The demonstration of selective inhibitory actions of the

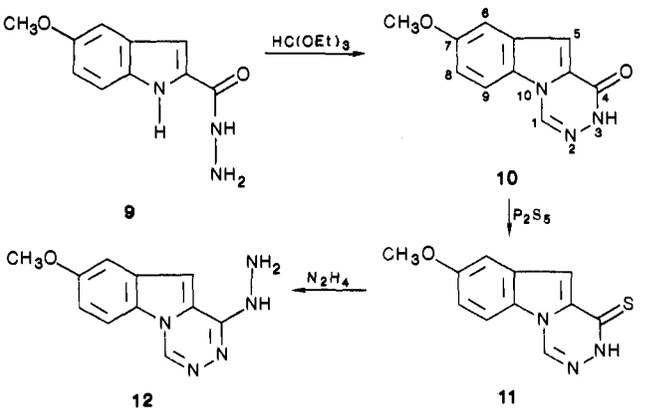
Scheme I



1 2 3 4 5 6 7 8

compound	R^1	R^2	R^3	R^4
1a, 2a, 3a, 4a, 5a	CH_3O			
1b, 2b, 3b, 4b, 5b	C_2H_5O			
1c, 2c, 3c, 4c, 5c	$C_6H_5CH_2O$			
1d, 2d, 3d, 5d	HO			
6a	CH_3O	CH_3	CH_3	
6b	CH_3O	C_6H_5	H	
6c	$C_6H_5CH_2O$	C_6H_5	H	
6d	$C_6H_5CH_2O$	$CH_3(CH_2)_2$	H	
7a	CH_3O			H
7b	CH_3O			CH_3
7c	$C_6H_5CH_2O$			H
7d	$C_6H_5CH_2O$			CH_3
8a	CH_3O			
8b	$C_6H_5CH_2O$			

Scheme II



9 10 11 12

compounds was determined in concordance with the Gorman model,[1] namely, the inhibition of the second wave of platelet aggregation induced by adenosine 5′-diphosphate (ADP), inhibition of aggregation induced by arachidonic acid (AA), and prostaglandin H_2 (PGH_2) and

(4) Moncada, S.; Gryglewski, R.; Bunting, S.; Vane, J. R. *Nature (London)* **1976**, *263*, 232.
(5) Nijkamp, F. P.; Moncada, S.; White, H. L.; Vane, J. R. *Eur. J. Pharmacol.* **1979**, *44*, 179.
(6) Szczeklik, A.; Gryglewsky, R.J.; Musial, J.; Grodzinska, L.; Serwonska, M.; Marcinkiewicz, E. *Thromb. Diath. Hemorrh.* **1978**, *40*, 66. (b) Nicolau, K. C. *Ann. Rep. Med. Chem.* **1979**, *14*, 178.
(7) Lamy, P. P. *Pharm. Int.* **1986**, *7*, 46.
(8) Reece, P. A. *Med. Res. Rev.* **1981**, *1*, 73.
(9) (a) Cross, P. E.; Dickinson, R. P. *Second SCI-RSC Medicinal Chemistry Symposium*; Emmett, J. C., Ed.; Smith Kline & French Research: Cambridge, England, 1984, pp 268–285. (b) Cross, P. E.; Dickinson, R. P.; Parry, M. P.; Randall, M. J. *J. Med. Chem.* **1986**, *29*, 342.
(10) Wright, W. B.; Press, J. B.; Chan, P. S.; Marsico, J. W.; Haug, M. F.; Lucas, J.; Tuber, J.; Tomcufcik, A. S. *J. Med. Chem.* **1986**, *29*, 523.
(11) (a) Monge, A.; Gil, M. J.; Fernández-Alvarez, E. *J. Heterocycl. Chem.* **1984**, *21*, 1271. (b) Monge, A.; Palop, J. A.; Tabar, P.; Fernández-Alvarez, E. *J. Heterocycl. Chem.* **1984**, *21*, 397. (c) Monge, A.; Aldana, I.; Parrado, P.; Daries, P.; Fernández-Alvarez, E. *An. Quim. Ser. C* **1983**, *79*, 462. (d) Monge, A.; Aldana, I.; Font, M.; Parrado, P.; Fernández-Alvarez, E.; Fuentes, J. A. *An. Quim. Ser. C.* **1983**, *79*, 242. (e) Monge, A.; Palop, J. A.; Gracia, I.; Fernández-Alvarez, E. *An. R. Acad. Farm.* **1982**, *48*, 213. (f) Monge, A.; Aldana, I.; Parrado, P.; Font, M.; Fernández-Alvarez, E. *J. Pharm. Sci.* **1982**, *71*, 1406. (g) Monge, A.; Aldana, I.; Fernández-Alvarez, E. *J. Heterocycl. Chem.* **1981**, *18*, 1533. (h) Monge, A.; Palop, J. A.; Antón, M. J.; Busto, C.; Sanfeliú, E.; Fernández-Alvarez, E. *An. R. Acad. Farm.* **1983**, *49*, 659. (i) Monge, A.; Palop, J. A.; Sanfeliú, E.; Antón, M. J.; Busto, C.; Parrado, P.; Fernández-Alvarez, E. *An. Quim. Ser. C* **1983**, *79*, 401.
(12) (a) Monge, A.; Aldana, I.; Erro, A.; Parrado, P.; Font, M.; Rocha, E.; Prieto, J.; Fremont-Smith, M.; Fernández-Alvarez, E. *An. R. Acad. Farm* **1984**, *50*, 365. (a) Monge, A.; Aldana, I.; Erro, A.; Parrado, P.; Font, M.; Rocha, E.; Prieto, J.; Quiroga, J.; Fernández-Alvarez, E. *Rev. Farmacol. Clin. Exp.* **1984**, *1*, 131. (b) Monge, A.; Aldana, I.; Erro, A.; Parrado, P.; Font, M.; Rocha, E.; Prieto, J.; Fremont-Smith, M.; Fernández-Alvarez, E. *Acta Farm. Bonaerense* **1984**, *3*, 21.

Table I. Physical Properties of **2–5**

no.	R^1	X	yield–method[a]	crystn solvent[b]	mp, °C	formula[c]
2a	CH_3O		80% from **1a**	D	246–248	$C_{13}H_{13}NO_4$
2b	C_2H_5O		85% from **1b**	D	225–227	$C_{14}H_{15}NO_4$
2c	$C_6H_5CH_2O$		95% from **1c**	EtOH/D	244–245	$C_{19}H_{17}NO_4$
2d	HO		80% from **1d**	D	>250	$C_{12}H_{11}NO_4$
3a	CH_3O	O	75% from **2a**	D	>250	$C_{11}H_9N_3O_2$
3b	C_2H_5O	O	98% from **2b**	D	>250 dec	$C_{12}H_{11}N_3O_2$
3c[e]	$C_6H_5CH_2O$	O	90% from **2c**	D	>250	$C_{17}H_{13}N_3O_2$
3d	HO	O	98% from **2d**	DMF/EtOH	>250	$C_{10}H_7N_3O_2$
			15% from **3a** or **3c**			
4a	CH_3O	S	80% from **3a**	D	>250 dec	$C_{11}H_9N_3OS$
4b	C_2H_5O	S	70% from **3b**	D	246–248	$C_{12}H_{11}N_3OS$
4c	$C_6H_5CH_2O$	S	98% from **3c**	D	>250	$C_{17}H_{13}N_3OS$
5a	CH_3O		75% from **4a**	DMF/H_2O	>250	$C_{11}H_{11}N_5O$
5b	C_2H_5O		95% from **4b**	DMF/H_2O	223–225	$C_{12}H_{13}N_5O$
5c[f]	$C_6H_5CH_2O$		90% from **4c**	DMF/H_2O	>250	$C_{17}H_{15}N_5O$
5d	HO		95% from **5c**	EtOH	>250 dec	$C_{10}H_9N_5O{\cdot}2BrH$
			15% from **5a**			

[a] See Experimental Section. Yield as analytically pure sample [b] D = dioxane. [c] All compounds showed satisfactory elemental analysis. [d] Previously reported.[18] [e] This compound was previously reported[20] with mp 282 °C and erroneously[18,21] with mp 273–274 °C. [f] Previously reported.[11d,f]

Table II. Physical Properties of **6–8**

no.	R^1	R^2	R^3	R^4	yield–method[a]	crystn solvent[b]	mp, °C	formula[c]
6a	CH_3O	CH_3	CH_3		90% from **5a**	D	>250	$C_{14}H_{15}N_5O$
6b	CH_3O	C_6H_5	H		90% from **5a**	D/EtOH	>250	$C_{18}H_{15}N_5O$
6c	$C_6H_5CH_2O$	C_6H_5	H		90% from **5c**	DMF	>250	$C_{24}H_{19}N_5O$
6d	$C_6H_5CH_2O$	$CH_3(CH_2)_2$	H		90% from **5c**	DMF	>250	$C_{21}H_{21}N_5O$
7a	CH_3O			H	90% from **5a**	DMF	>250	$C_{12}H_9N_5O$
7b	CH_3O			CH_3	90% from **5a**	DMF	>250	$C_{13}H_{11}N_5O$
7c	$C_6H_5CH_2O$			H	90% from **5c**	DMF	>250	$C_{18}H_{13}N_5O$
7d[d]	$C_6H_5CH_2O$			CH_3	90% from **5c**	DMF	>250	$C_{19}H_{15}N_5O$
8a	CH_3O				98% from **5a**	DMF/EtOH	>250	$C_{11}H_8N_6O$
8b	$C_6H_5CH_2O$				98% from **5c**	DMF	>250	$C_{17}H_{12}N_6O$

[a] See Experimental Section. Yield as analytically pure samp.e [b] D = dioxane. [c] All compounds showed satisfactory elemental analysis. [d] Previously reported.[11f]

prostaglandin E_2 production with AA as aggregating agent with concomitant inhibition of TXA_2 production.

Chemistry

5*H*-Pyridazino[4,5-*b*]indole derivatives were prepared according to Scheme I. The starting compounds **1** were obtained by well-known methods (see the Experimental Section) and formylated by the Vilsmeier–Haack reaction to give **2** with good yields. Boiling **2** with 90% hydrazine hydrate gave **3**. Compound **3d** was also obtained in smaller yields by treating **3a** or **3c** with hydrobromic acid. Treating **3a–c** with phosphorus pentasulfide in boiling pyridine gave **4a–c**. Compound **4d** could not be obtained from **3d** in this way. By treatment of **4a–c** with boiling 90% hydrazine hydrate, we obtained **5a–c** in good yield, and some salts (hydrochlorides, sulfates) were prepared by standard methods. Compound **5d** was obtained by treating **5c** with boiling hydrobromic acid and in smaller yield from **5a** in a similar way. The attempted catalytic (Pd/C) hydrogenolysis of **5c** to **5d** was not useful under several conditions, and **5c** was generally recovered. Some hydrazones of **5** were obtained by standard methods. Reaction of **5** with boiling acetic or formic acid gave **7**, and nitrosation ($NaNO_2/H^+$) of **5a,c** gave **8a,b**. Theoretically, compounds **8** can adopt a tetrazole structure, as shown in Scheme I, or an azide structure. The chemistry of the tetrazole–azide equilibrium for a series of condensed systems derived from tetrazole is well documented.[13] The IR spectra of **8** (KBr tablets) did not show any signal at about 2000 cm^{-1}, characteristic of an azido group. This suggests that in the crystalline state compounds **8** have the tetrazole structure as represented in Scheme I.

Pyridazino[4,5-*a*]indole derivatives were prepared according to Scheme II. Formylation of **9**, as previously reported for similar compounds,[14] gave **10**, which when treated with phosphorus pentasulfide yielded **11**. Reaction of **11** with boiling 90% hydrazine hydrate gave **12**.

Biology: Results and Discussion

A. Platelet Aggregation and Thromboxane Synthetase Inhibition Activities. Compounds **3**, **5**, and **10–12** reported in this paper were studied also as potential inhibitors of the blood platelet aggregation induced by ADP and/or AA, PGH_2, and adrenaline. Table IIIA summarizes the results for compounds with higher significant activity at 10 and 5 μM. The other related compounds reported in this paper had no significant activity. Compounds **3c** and **5a**·HCl were the most potent inhibitors in all of these experiments. These compounds were inhibitors of the in vitro platelet aggregation induced by ADP, AA, and PGH_2, and compound **5a**·HCl also inhibited the aggregation induced by adrenaline.

Compounds **3c** and **5a**·HCl were subsequently studied with ASA as reference as potential inhibitors of thromboxane synthetase in the in vitro blood platelet aggregation induced by AA and in the ex vivo blood platelet aggregation induced by ADP and AA. Table IIIB,C summarizes the results. In both types of experiments, **3c** and **5a**·HCl showed an inhibitory effect on the synthesis of TXB_2 and a stimulation of the synthesis of prostaglandin E_2 (PGE_2). Both effects are more significant with **5a**·HCl. To a 100 μM concentration, this last compound as compared with

(13) Monge, A.; Aldana, I.; Rabbani, M. M.; Fernández-Alvarez, E. *J. Heterocycl. Chem.* **1980**, *17*, 77 and references cited therein.

(14) Monge, A.; Aldana, I.; Lezamiz, I.; Fernández-Alvarez, E. *Synthesis* **1984**, 159.

Table III.[a] Blood Platelet Interactions

A. Effect on Platelet Aggregation (in Vitro Assay; $n = 5$)

compd	final concn, M	inhibition of platelet aggregation (%) induced by ADP-II[b]	ADP-II[c]	DD[b]	AA[c]	PGH_2[c]	adrenaline
3a	10^{-5}	*[d]	100	*	*		
3b	10^{-5}	*	100	*	*		
3c	10^{-5}	60	100	100	100	100	
	5×10^{-6}	40	80	*	*		
3d	10^{-5}	87	100	*	*		
	5×10^{-6}	*	100	*	*		
5a·HCl	10^{-5}	100	100	100	100	100	100
5b·HCl	10^{-5}	100	100	*	*		
5c·HCl	10^{-5}	*	*	*	*		
10	10^{-5}	*	100	*	*		
11	10^{-5}	*	100	*	*		
12	10^{-5}	100	100	*	*		
hydralazine hydrochloride	10^{-5}	*	*	*	*		

B. Effects on Thromboxane Synthetase Activity on in Vitro Platelet Aggregation Induced by AA

compd	final concn, M	n	PGE_2 % increase	TXB_2 % inhibn
3c	10^{-5}	5	270	50
5a·HCl	10^{-5}	5	47	40
ASA	10^{-5}	5	0	100

C. Effect on Platelet Aggregation and Thromboxane Synthetase Activity (Modified ex Vivo Assay)

compd	dose, mg/kg	inhibition of platelet aggregation (%) induced by ADP-II	AA	increase % PGE_2	inhibn % TXB_2
3c	100	100	100	30	50
5a·HCl	30	*	*		
5a·HCl	50	*	*		
5a·HCl	100	100	100	35	95
hydralazine hydrochloride	30	*	*		
hydralazine hydrochloride	50	*	*		
hydralazine hydrochloride	100	*	75		
ASA	30	*	*		
ASA	50	100	100	0	100
ASA	100	100	100	0	100

D. Effect on PGI_2 Release from Rat Aortic Tissue ($n = 4$)[a]

compd	time of incubation, min	final concn, (M)	inhibn (% of control)	compd	time of incubation, min	final concn, (M)	inhibn (% of control)
5a·HCl	10	10^{-4}	50	ASA	10	10^{-4}	80
	25		45		25		80
	30		35		30		80
	35		25		35		80

[a] See Experimental Section for details. [b] PRP incubated for 5 min at 37 °C. [c] PRP incubated for 60 min at 37 °C. [d] (*) no significant values ($p \leq 5\%$).

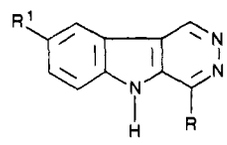
ASA showed (Table IIID) no significant inhibitory effect on the PGI_2 release from rat aortic tissue. This is about 50% that for ASA at the same concentration and conditions.

B. Antihypertensive Activity. The antihypertensive effect of the compounds was measured in spontaneously hypertensive rats (SHR). Table IV summarizes our preliminary results for antihypertensive activity after intraperitoneal, oral, and intravenous administration to SHR for compounds **3**–**8**, **12**, and hydralazine. Compounds **3**, **4**, **6**–**8**, and **12** showed only a weak activity, and so our previous results[11d,f] for other structural analogues seem to be confirmed. The introduction of the hydrazino group in the position 4 of the 5*H*-pyridazino[4,5-*b*]indole system considerably increased the antihypertensive activity and toxicity. However, the low activity of compounds **6a** and **6b** as compared with that of compounds **5** is surprising. Considering together the results of Table IV and our previous results,[11d,f] the following decreasing order of activity may be stated for compounds **5**: hydralazine $\geq$ **5a** $\approx$ **5d** $\geq$ **5** (R′ = H) $\geq$ **5b** $\gg$ **5c**. The other compounds were less active, as shown in Table IV.

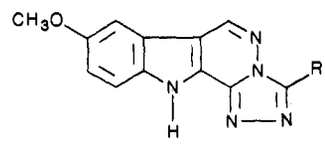
Table V summarizes the results for **5a**, **5b**, **5d**, and hydralazine for a short time after iv administration. In these experiments the decreasing order of activity seems to be **5d** > hydralazine $\approx$ **5a** > **5b**. In the experiments summarized in Table I, some results were also obtained on the effects of the compounds on the cardiac rhythm (CR). In comparison to a dose of 1 mg/kg ip, the following increasing order of stimulation was observed: hydralazine $\approx$ **5a** $\approx$ **5b** $\ll$ **5d** and at a higher dose **5b** $\approx$ **5a** $\ll$ **5d**. On the other hand, the LD_{50} values for **5a**, **5b**, **5d**, and **5** (R′ = H) are about 2.2–2.5 times less toxic than for hydralazine, and the less active compound **5c** is about 10 times less toxic than hydralazine. The acute toxicity in mice for **5a**·HCl is 225 ± 12 mg/kg (for hydralazine hydrochloride as reference in our laboratory[11d] it is 100 mg/kg).

In conclusion, the pyridazino[4,5-*b*]indoles derivatives reported herein represent a novel class of selective TXA_2 synthetase inhibitors. This series of compounds also show interesting antihypertensive activities. A new series of compounds related to **5a**·HCl with potential antihypertensive and antiaggregatory activities are in course in our laboratory. All the above-reported results suggest that

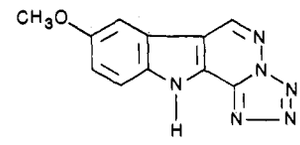
Table IV. Antihypertensive Activity in Spontaneously Hypertensive Rats[a]



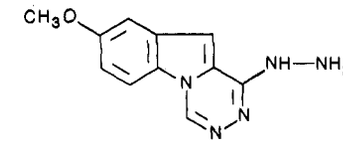
3-6



7



8

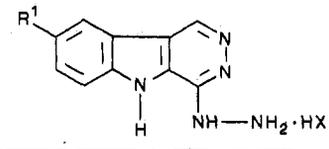


12

compd	R	R^1	dose, mg/kg	control AP	control CR	percentage falls in AP and CR: 1 h AP	1 h CR	3 h AP	3 h CR	5 h AP	5 h CR	24 h AP	24 h CR
3a	OH	CH_3O	25[b]	197	608	10	7	10	5	5	*		
3b	OH	C_2H_5O	25[b]	226	614	*[e]	6	*	*	*	*	*	*
3c	OH	$C_6H_5CH_2O$	50[b]	199	387			12	10	13	15	7	9
3d	OH	OH	25[b]	191	444			*	−11	*	−7	*	−8
4b	SH	CH_3O	25[b]	228	615	10	*	5	*	*	*	*	*
5a	$NHNH_2$	CH_3O	25[b]	197	610	17	−8	32	−8	30	−10	5	*
5a·HCl	$NHNH_2$	CH_3O	25[c]	226	450	58	22	57	19	50	24	10	38
5a·HCl	$NHNH_2$	CH_3O	5[c]	223	585	50	−9	35	−8	26	−6	9	*
5a·HCl	$NHNH_2$	CH_3O	1[c]	209	553	32	9	5	8	*	*	*	*
5a·HCl	$NHNH_2$	CH_3O	25[d]	205	561	50	*	40	−14	34	−14	15	*
5b	$NHNH_2$	C_2H_5O	25[b]	228	638	29	−20	28	−11	24	−9	5	*
5b·HCl	$NHNH_2$	C_2H_5O	25[c]	248	597	57	*	57	−17	51	−20	34	−11
5b·HCl	$NHNH_2$	C_2H_5O	5[c]	224	512	59	*	37	*	32	−7	12	*
5b·HCl	$NHNH_2$	C_2H_5O	1[c]	214	528	29	6	10	−5	*	−10	*	10
5b·HCl	$NHNH_2$	C_2H_5O	25[d]	223	511	16	11	5	*	*	*	*	*
5b·HCl	$NHNH_2$	C_2H_5O	5[d]	226	512	15	−11	10	*	*	*	*	*
5c	$NHNH_2$	$C_6H_5CH_2O$	25[b]	200	385			12	15	13	20	7	7
5c	$NHNH_2$	$C_6H_5CH_2O$	5[b]	203	467			5	16	13	*	7	*
5c	$NHNH_2$	$C_6H_5CH_2O$	1[b]	196	404			*	8	*	6	*	9
5c	$NHNH_2$	$C_6H_5CH_2O$	25[d]	207	455			6	21	10	*	5	*
5c·H_2SO_4	$NHNH_2$	$C_6H_5CH_2O$	25[c]	218	500	21	9	32	7	26	8	*	*
5c·HCl	$NHNH_2$	$C_6H_5CH_2O$	25[c]	235	590	18	−7	33	−13	28	−15	9	−16
5d·2HBr	$NHNH_2$	OH	25[c]	228	444	70	19	67	16	63	44	28	18
5d·2HBr	$NHNH_2$	OH	5[c]	231	430	27	64	16	30	10	24	6	28
5d·2HBr	$NHNH_2$	OH	1[c]	223	477	*	22	−6	13	*	19		
5d·2HBr	$NHNH_2$	OH	25[d]	230	507	29	17	27	24	8	8	*	*
6a	$NHN{=}C(CH_3)_2$	CH_3O	25[b]	197	521			13	−11	14	−8	7	−10
6b	$NHN{=}CHC_6H_5$	CH_3O	25[b]	207	485			6	−20	8	*	*	−13
7a	H		25[b]	226	450			*	10	11	*	*	*
7b	CH_3		25[b]	209	552			10	−5	8	7	*	*
8			25[b]	195	412			12	*	8	*	6	*
12·HCl			25[b]	200	628	9	*	*	*	*	*	*	*
12·HCl			25[b]	210	635	*	*	*	*	*	*		
hydralazine hydrochloride			1[c]	205	385	20	−7	20	−5	10	−2	*	*

[a] See Experimental Section for details. [b] Ip. [c] Ip (normal saline). [d] Po. [e] (*) no significant values ($p \leq 5\%$).

Table V. Antihypertensive Activities in Spontaneously Hypertensive Rats



compd	R^1	X	dose, mg/kg	percentage falls in systolic blood pressure, directly measured on carotid[a]: control	5 min	10 min	15 min	30 min	45 min	60 min	75 min	90 min	120 min	180 min	240 min
5a·HCl	CH_3O	Cl	1	250	30		36	40		60	54		40	36	30
5b·HCl	C_2H_5O	Cl	1	220	16	18	20	32	36	41	32	25	*[b]	*	*
5d·HBr	HO	Br	1	225	33	44	60	56	56	67	56	56	*	*	*
hydralazine hydrochloride			1	250	40		48	48		52	60		20	*	*

compd	R^1	X	dose, mg/kg	percentage increase in the cardiac rhythm, directly measured on the carotid: control	5 min	15 min	30 min	60 min	75 min	120 min	180 min	240 min
5a-HCl	CH_3O	Cl	1	365	*	11	12	10	*	*	*	*
hydralazine hydrochloride			1	375	*	13	20	*	*			

[a] Iv (vehicle normal saline) on the jugular vein. [b] (*) no significant values ($p \leq 5\%$).

5a·HCl is a potentially promising antihypertensive agent, which inhibits the blood platelet aggregation through the inhibition of the synthesis of TXB_2 and stimulation of the synthesis of PGE_2 from arachidonic acid.

Experimental Section

All the new compounds were characterized by elemental analysis, IR spectra, and, in most of the cases, ^1H NMR spectra. The IR spectra were recorded for all compounds on a Perkin-

Elmer 681 instrument, using potassium bromide tablets. The ^1H NMR spectra were obtained on Perkin-Elmer R-32 (90 MHz) and/or Varian EM-390 (90 MHz) instruments, with Me_4Si as internal standard at a concentration of about 0.1 g/mL. The IR and ^1H NMR spectra were consistent with assigned structures. Melting points were determined on a hot plate of a microscope (Reisert's apparatus) and are uncorrected. Elemental analysis obtained from vacuum-dried samples (over phosphorus pentoxide at 1–2 mm Hg, 2–3 h at about 50–60 °C) were within 0.4% of theoretical values.

Ethyl 5-Alkoxyindole-2-carboxylates 1a–c. These compounds were obtained from the respective *p*-alkoxyanilines and ethyl α-methylacetoacetate by the Japp–Klingemann reaction[15] as previously reported. **1a**:[16] mp 157–159 °C. **1b**:[16] mp 173–175 °C. **1c**:[17] mp 162–164 °C.

Ethyl 5-Hydroxyindole-2-carboxylate (1d). From **1c** by catalytic (Pd/C) hydrogenolysis,[17] mp 151 °C.

Ethyl 5-Alkoxy(or hydroxy)-3-formylindole-2-carboxylates 2. These compounds were obtained by formylation ($DMF/POCl_3$) of compounds 1 through the Vilsmeier–Haack reaction[15] as previously reported for **2c**.[18] Compounds **2a**, **2b**, and **2d** were also prepared by the procedure described above. Compound **2a**: from **1a**, as yellow needles; IR 3400 (NH), 1725 (C=O, ester), 1660 (C=O, aldehyde), 805, 860 (aromatic 1,2,4-trisubstitution) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.36 (3 H, t, CH_3), 3.80 (3 H, s, CH_3O), 4.43 (2 H, c, CH_2), 7.02 (1 H, dd, H-6), 7.45 (1 H, d, H-7), 7.58 (1 H, d, H-4) ($J_{4,6} \approx 2$ Hz, $J_{6,7} \approx 7$ Hz), 10.75 (1 H, s, CHO), 13.3 (1 H, brs, NH). Anal. ($C_{13}H_{13}NO_4$) C, H, N.

8-Alkoxy(or hydroxy)-3,4-dihydro-4-oxo-5*H*-pyridazino[4,5-*b*]indoles 3. These compounds were obtained by boiling a mixture of the respective compound **2** and 90% hydrazine hydrate for 2–3 h, as previously reported[19] for similar compounds. When the reaction mixture was cooled, the product crystallized. Compound **3a**: from **2a**, as white-yellow needles; IR 3280 (NH), 1650 (br s, C=O, C=N), 810, 900 (aromatic 1,2,4-trisubstitution) cm^{-1}; ^1H NMR (Me_2SO-d_6, 35 °C) δ 3.88 (3 H, s, CH_3O), 7.18 (1 H, dd, H-7), 7.57 (1 H, d, H-6), 7.70 (1 H, d, H-9, $J_{7,9} \approx 2.5$ Hz, $J_{6,7} \approx 8$ Hz), 8.75 (1 H, s, H-1), 12.7 (1 H, br s), 13.0 (1 H, br s) (NH and CONH). Anal. ($C_{11}H_9N_3O_2$) C, H, N. The preparation of compounds **3b–d** involved the procedure described above. Compound **3d** was also obtained in a smaller yield by boiling (1 h) a mixture of **3a** or **3c** (1 g) and 47% hydrobromic acid (10 mL). The cold reaction mixture was neutralized and the collected solid washed with water and recrystallized.

8-Alkoxy-3,4-dihydro-4-thioxo-5*H*-pyridazino[4,5-*b*]indoles 4. A mixture of the respective **3** (10 mmol, **3a–c**), dried pyridine (25 mL), and phosphorus pentasulfide (2.25 g, 10 mmol), protected with a tube of anhydrous calcium chloride, was boiled for 4 h. The color of the mixture changed from white-yellow to red. Solvent was removed in vacuo and the residual material (a mixture of a solid and an oil) was treated as indicated in each case. Compound **4a**: from **3a**. The residual material was stirred (0.5 h) with 5% ammonium hydroxide (50 mL) and the solid collected, washed with water, and recrystallized, giving pale yellow crystals: IR 3000–3200 (four bands, NH), 1620 (C=N), 1500, 1250 (N—C=S), 810, 890 (aromatic 1,2,4-trisubstitution) cm^{-1}; ^1H NMR (Me_2SO-d_6, 35 °C) δ 4.05 (3 H, s, CH_3O), 7.38 (1 H, dd, H-7), 7.80 (1 H, d, H-6), 7.92 (1 H, d, H-9) ($J_{6,7} \approx 8$ Hz, $J_{7,9} = 3$ Hz), 9.25 (1 H, s, H-1), 12.8 (1 H, s, S=CNH), 14.8 (1 H, s, NH-indole). Anal. ($C_{11}H_9N_3OS$) C, H, N. Compounds **4b** and **4c**: The residual material was extracted with hot dioxane. Solvent was removed in vacuo and the solid residue washed several times with 5% ammonium hydroxide and recrystallized.

8-Alkoxy-4-hydrazino-5*H*-pyridazino[4,5-*b*]indoles 5. A suspension of the respective **4** (60 mmol, **4a–c**) and 90% hydrazine hydrate (20 mL) was boiled for 5 h with stirring. The product that crystallized on cooling was collected, washed with water, and recrystallized. Compound **5a**: from **4a**, as pale yellow crystals; IR 3280 (NH), 1630 (C=N), 810, 890 (aromatic 1,2,4-trisubstitution) cm^{-1}; ^1H NMR (F_3CCOOH) δ 4.10 (3 H, s, CH_3O), 7.10–8.0 (3 H, m, H-6, H-7, H-9), 9.60 (1 H, s, H-1). Anal. ($C_{11}H_{11}N_5O$) C, H, N. The monohydrochloride of **5a** was obtained by recrystallization of **5a** from HCl/ethanol: mp >250 °C; IR 2500–3400 (br s, NH), 1620 (C=N), 820, 879 (aromatic 1,2,4-trisubstitution) cm^{-1}. Anal. ($C_{11}H_{11}N_5O$·HCl) C, H, N. Compounds **5b** and **5c** were also preparing by using the procedure described above. The monohydrochloride of **5b** was prepared by recrystallization of **5b** from HCl/ethanol. The monohydrochloride of **5c** was prepared by recrystallization of **5c** from HCl/ethanol; the acid sulfate was obtained by addition of concentrated sulfuric acid to a solution of **5c** in ethanol.

4-Hydrazino-8-hydroxy-5*H*-pyridazino[4,5-*b*]indole (5d). This compound could not be obtained by catalytic (Pd/C) hydrogenolysis of the benzyloxy derivative **5c** under different conditions, and generally the starting material and recovered. The compound was obtained by boiling (1.5 h) a solution of **5c** (10 mmol) with 47% hydrobromic acid (15 mL). Solvent was removed in vacuo and the residue dried by repeated addition and evaporation in vacuo with benzene. The dihydrobromide of **5d** was obtained. In a similar way, **5d** was also obtained in smaller yield from **5a**.

Hydrazones of 8-Alkoxy-4-hydrazino-5*H*-pyridazino[4,5-*b*]indole 6. These compounds were obtained by boiling a mixture of the respective **5** (30 mmol), the corresponding aldehyde or ketone (2 mL), and ethanol (10 mL) for 1–4 h (TLC). When the reaction mixture was cooled, the compound crystallized. The yields were about 90%. The following compounds were obtained. Compound **6a**: from **5a** and propanone, as pale yellow crytals; IR 3110 (NH), 1650 (C=N) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.05 (6 H, s, 2 CH_3), 3.80 (3 H, s, CH_3O), 7.00 (1 H, dd, H-7), 7.50 (2 H, m, H-6, H-9), 8.50 (1 H, s, H-1) ($J_{6,7} \approx 3$ Hz). Anal. ($C_{14}H_{15}N_5O$) C, H, N. Compound **6b**: from **5a** and benzaldehyde. Compound **6c**: from **5c** and benzaldehyde. Compound **6d**: from **5c** and butyraldehyde.

8-Alkoxy-11*H*-1,2,4-triazolo[4,3-*b*]pyridazino[4,5-*b*]indoles 7. A mixture of the respective **5** (30 mmol) and formic or acetic acid (40 mL) was boiled for 4–5 h (TLC). When the reaction mixtures, was cooled, the respective **7** crystallized. The following compounds were obtained in yields about 90%. Compound **7a**: from **5a** and formic acid. Compound **7b**: from **5a** and acetic acid. Compound **7c**: from **5c** and formic acid. Compound **7d**: from **5c** and acetic acid (previously reported[11f]).

8-Alkoxy-11*H*-tetrazolo[1,5-*b*]pyridazino[4,5-*b*]indoles 8. To a stirred mixture of the respective **5** (30 mmol) and powdered sodium nitrite (1.6 g, 20 mmol), cooled in an ice bath, was added hydrochloric acid (1 N, 40 mL) and the mixture was stirred for 24 h. The precipitate was collected, washed with water, and recrystallized. Compound **8a**: from **5a**, as pale pink crystals; IR 3050–3250 (br s, NH), 1650, 1630 (C=N) cm^{-1}; ^1H NMR (F_3CCOOH) δ 3.85 (3 H, s, CH_3O), 7.15 (1 H, dd, H-9), 7.55 (1 H, d, H-10), 7.80 (1 H, br s, H-7), 9.50 (1 H, s, H-6). Anal. ($C_{11}H_8N_6O$) C, H, N. Compound **8b** was also prepared by the procedure described above, from **5c**.

3,4-Dihydro-7-methoxy-4-oxo-1,2,4-triazino[4,5-*a*]indole (10). A mixture of **9**[16] (2.0 g, 10 mmol), *N,N*-dimethylformamide (20 mL), and ethyl orthoformate (2.0 g, 14 mmol) was boiled for 4 h. Solvent was removed in vacuo and the residue recrystallized: mp >250 °C (dioxane); yield about 80% of spongy white crystals; IR 3100, 3200 (NH), 1680 (C=O), 1630 (C=N) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.80 (3 H, s, CH_3O), 7.10 (1 H, dd, H-8), 7.22 (1 H, s, H-5), 7.28 (1 H, d, H-6), 8.05 (1 H, d, H-9) ($J_{6,8} \approx 3$ Hz, $J_{8,9} \approx 9$ Hz), 8.94 (1 H, s, H-1), 12.4 (1 H, s, CONH). Anal. ($C_{11}H_9N_3O_2$) C, H, N.

3,4-Dihydro-7-methoxy-4-thioxo-1,2,4-triazino[4,5-*a*]indole (11). This compound was prepared from **10** in a similar way as above described for **4c**: mp 221–223 °C (dioxane); yield about 98% of yellow colored crystals; IR 3200 (NH), 1540, 1200 (S=C=N) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.70 (3 H, s, CH_3O), 6.90 (1

(15) Remers, W. A. In *Indoles*; Houlihan, W. J., Ed.; Wiley: New York, 1979; Part 3, p 357.
(16) Monge, A.; Fernández-Alvarez, E. *An. Quim.* **1972**, *68*, 1153.
(17) Fernández-Alvarez, E.; Lora-Tamayo, M.; Monge, A. *Bull. Soc. Chim. Fr.* **1969**, 1932.
(18) Monge, A.; Huarte, V.; Palop, J. A.; Martinez, M. T.; Fernández-Alvarez, E. *An. Quim.* **1976**, *72*, 267.
(19) Monge, A.; Aldana, I.; Fernandez-Alvarez, E. *Eur. J. Med. Chem.* **1978**, *13*, 573.
(20) Monge, A.; Palop, J. A.; Martinez, M. T.; Fernández-Alvarez, E. *An. Quim., Ser. C* **1983**, *79*, 242.
(21) Monge, A.; Palop, J. A.; Martinez, M. T.; Fernández-Alvarez, E. *An. Quim.* **1979**, *75*, 889.

H, dd, H-8), 7.15 (2 H, d for H-6 and s for H-5), 7.40 (1 H, d, H-9) ($J_{8,9} \approx 9$ Hz, $J_{6,8} \approx 3$ Hz), 9.60 (1 H, s, H-1). Anal. ($C_{11}H_9N_3OS$) C, H, N.

4-Hydrazino-7-methoxy-1,2,4-triazino[4,5-*a*]indole (12). This compound was prepared from 11 in a similar way as described above for **5a–c**: yield about 70% of a pale gray solid; mp 248 °C dec (dioxane); IR 3200, 3300 (NH) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.80 (3 H, s, CH_3O), 6.45 (2 H, s, NH_2), 6.85 (1 H, dd, H-8), 7.15 (1 H, d, H-6), 7.25 (1 H, s, H-5), 7.45 (1 H, d, H-9) ($J_{8,9} \approx 9$ Hz, $J_{6,8} \approx 3$ Hz, 8.50 (1 H, s, H-1), 11.50 (1 H, s, NH). The monohydrochloride was obtained when **12** was recrystallized for HCl/EtOH. Anal. ($C_{11}H_{11}N_5O$) C, H, N.

Effect on Platelet Aggregation. I. In Vitro Assay. Human blood was obtained from the antecubital vein and was treated with 3.8% sodium citrate (1 part of citrate to 9 parts of blood). Platelet-rich plasma (PRP) was prepared by centrifuging the citrated blood at 800 rpm for 10 min at room temperature and then separated. The remaining blood was centrifuged at 2000 rpm for 10 min at room temperature to obtain the Platelet-poor plasma (PPP). The platelet count was adjusted with PPP to 200 000 platelets/mL. Platelet aggregation was measured by the absorbance method of Born and Cross,[22] using a platelet aggregation meter (Dual Channel Aggro Meter). In the test, 0.1 mL of the test sample dissolved in normal saline and 10% ethylene glycol was added to a polyethylene cube containing 0.4 mL of PRP. The platelets with compound were incubated 5–60 min at 37 °C at which time 0.05 mL of aggregating agent was added (ADP, 2×10^{-5} M; arachidonic acid (AA), 0.6 mM; prostaglandin H_2 (PGH_2), 2.8 μM; adrenaline, 0.6 μM. The final concentration of each tested compound was 10^{-5} M, and the most active compounds were also tested at a final concentration of 5×10^{-6} M. All the experiments were done with acetylsalicylic acid (ASA) as the control for 100% inhibition. Table III summarizes the results.

II. Effects on Thromboxane Synthetase Activity on in Vitro Platelet Aggregation. The demonstration of selective inhibition actions of the compounds was determined according to the Gorman model.[1] The following data were obtained: inhibition of the second wave of platelet aggregation induced by ADP, inhibition of aggregation induced by AA and PGH_2, and PGE_2 production with AA as aggregating agent with concomitant inhibition of TXB_2 production. PGE_2 and TXB_2 levels in the test samples following aggregation were determined by radioimmunoassay according to methods previously described.[23] Radioimmunoassay studies were performed on the PRP samples that experienced inhibited platelet aggregation following compound incubation and addition of AA and inhibited second wave formation with ADP. Table IIIB summarizes the results.

III. Modified ex Vivo Assay. Ex vivo platelet inhibitory aggregation was determined following oral administration of the most active compounds to five male or female guinea pigs weighing 250–300 g, which were fasted overnight. The compounds were administered at a dose of 100 mg/kg, either dissolved or suspended in normal saline or normal saline containing 0.2% (carboxymethyl)cellulose and 1.0% Tween 80. Control groups of guinea pigs were dosed with vehicle. At a specified time after dosing, the animals were anesthetized with ether. Blood was collected from the heart and was treated with 3.8% sodium citrate (1 part of citrate to 9 parts of blood). PRP and PPP were prepared by centrifuging the citrated blood as indicated above. The platelet count was adjusted to 250 000 platelets/mL. Platelet aggregation was measured by the absorbance method[22] as indicated above. PGE_2 and TXB_2 levels in the test samples following aggregation were determined by radioimmunoassay according to the method described above. Table IIIC summarizes the results.

Effects on PGI_2 Release. Female Wistar rats (200–220 g) were treated with heparin (200 IU/kg ip) and killed 10 min later by a blow on the head. The animals were exsanguinated. The dorsal aorta was rapidly removed and rinsed with ice-cold Krebs–Henseleit buffer. The vessel was opened longitudinally, and pieces of about 25 mm^2 were prepared by using a punching device. These tissue pieces were stored in ice-cold oxygenated Krebs–Henseleit buffer for approximately 1 h, and the bath fluid was changed every 10 min to remove PGI_2 synthesized by the vessel wall. A piece of rat aortic tissue was transferred into 1 mL of Krebs–Henseleit buffer at 37 °C, and the basal release of PGI_2 was studied by measuring the antiaggregatory effect of the incubate on ADP (2×10^{-5} M) induced primary aggregation of human PRP in 10–40-min incubation periods.[24] Each cuvette contained 0.4 mL of PRP + 0.020 mL of Krebs–Henseleit buffer or vessel incubate + 0.050 mL of ADP. The incubation with **5a**·HCl (10^{-4} M final concentration) was done for 10 min prior to transfer of the vessel probes. ASA was used as standard. $^3H_6$-keto-$PGF_{1\alpha}$ levels in the test samples following aggregation were determined by radioimmunoassay as a parameter of PGI_2 formation. Table IIID summarizes the results.

Antihypertensive Activity. Compounds to be tested were first evaluated for antihypertensive activity in unanesthetized spontaneously hypertensive, 8-week-old, male and female rats of the Okamoto strain, weighing 300–350 g and with systolic blood pressure levels $\geq$ 190 mmHg and cardiac rhythm $\geq$ 400 and $\leq$ 650 beats/min. Changes in the arterial pressure (AP) and cardiac rhythm (CR) 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 five control animals, and AP and CR were measured at the indicated times. The tested compounds were administered (ip, po, 2.5 mL/kg) either dissolved or suspended in normal saline or normal saline containing 0.2% (carboxymethyl)cellulose and 1.0% Tween 80, at an initial dose of 25 mg/kg, and the most active compounds were then tested at lower concentrations. The results of these experiments are shown in Table IV. The most active compounds, dissolved in normal saline, were also tested after iv administration to the jugular vein of anesthetized animals (sodium pentobarbital, 70 mg/kg), and changes of the AP and CR were registered by direct measure (two canals Letigraf 2000 polygraph) on the carotid. A summary of the results of these experiments is given in Table V.

Acute Toxicity. Swiss male mice weighing 20 ± 2 g were used. The animals were treated ip at least at four different doses. The LD_{50} values were calculated 7 days after drug administration by means of probit analysis according to Miller and Tainter.[25]

Acknowledgment. This work was kindly supported by Antibióticos S.A. (España). We are especially grateful to the Fondo de Investigaciones Sanitarias (F.I.S.) for a grant to Dr. M. Font (1982–1985).

(22) Born, G. U. R.; Cross, M. J. *J. Physiol.* **1963**, *168*, 178.
(23) Salmon, J. A. *Prostaglandins* **1978**, *15*, 383.
(24) Darius, H.; Schrör, K. *Arzneim.-Forsch./Drug Res.* **1985**, *35*, 55.
(25) Batson, M. C. *An. Introduction to Statistics in the Medical Sciences*; Burger's Publishing Co.: Minneapolis, 1963; p 64.