New Indole and Triazino[5,4-b]indol-4-one Derivatives: Synthesis and Studies as Inotropics and Inhibitors of Blood Platelet Aggregation

ARTICLE *in* ARCHIV DER PHARMAZIE · JANUARY 1992

Impact Factor: 1.53 · DOI: 10.1002/ardp.19923250712 · Source: PubMed

9 AUTHORS, INCLUDING:

Ignacio Aldana
Universidad de Navarra
119 PUBLICATIONS **1,637** CITATIONS
SEE PROFILE

Maria Font
Universidad de Navarra
75 PUBLICATIONS **760** CITATIONS
SEE PROFILE

Juan Jose Martinez Irujo
Universidad de Navarra
39 PUBLICATIONS **525** CITATIONS
SEE PROFILE

Elena Alberdi
Universidad del País Vasco / Euskal Herriko…
57 PUBLICATIONS **2,180** CITATIONS
SEE PROFILE

New Indole and Triazino[5,4-*b*]indol-4-one Derivatives: Synthesis and Studies as Inotropics and Inhibitors of Blood Platelet Aggregation

Antonio Monge[a)*], Ignacio Aldana[a)], Maria Font[a)], José Angel Arraras[a)], Esteban Santiago[b)], Maria José Lopez-Unzu[b)], Juan José Martínez de Irujo[b)], and Elena Alberdi[b)]

Centre of Investigation in Applied Pharmacobiology, University of Navarre, 31080 Pamplona, Spain
[a)] Medicinal Chemistry, [b)] Biochemistry

Eldiberto Fernández-Alvárez

National Centre of Organic Chemistry. Juan de la Cierva 3, 28006 Madrid, Spain

Received May 15, 1991

New triazino[5,4-*b*]indol-4-one derivatives carrying amino groups in position 3 were synthetized and tested as inotropic agents and inhibitors of platelet aggregation. **2h**, **2p**, **5p**, and **6g** are the most active as inotropic agents.
Compounds were tested as inhibitors of platelet aggregation induced by adenosine 5'-diphosphate (ADP) and arachidonic acid (AA) (guinea pig whole blood). **2k**, **2p**, **5o**, **6d**, **6m**, and **6o** are the most active as inhibitors of the platelet aggregation induced by AA. **6d**, **6h**, and **6o** are most active compounds also in the aggregation induced by ADP.
Radioinmunoassay studies, following AA induced aggregation, measuring thromboxane B_2 (TXB_2) and prostaglandin E_2 (PGE_2) were carried out on compounds **2b**, **2d**, **2f**, **2g**, **2h**, **2i**, **2k**, **2m**, **2o**, **2p**, **2r**, **5i**, **5j**, **5k**, **5r**, and **5f**, which inhibit platelet aggregation induced by AA. None of the compounds tested turned out to be selective inhibitors.
Compounds **2h** and **2p** showed both inotropic and platelet aggregation inhibiting activity.

Neue Indol- und Triazin[5,4-*b*]indol-4-on-Derivate: Synthese und Prüfung auf inotrope und plättchenaggregationshemmende Wirkung

Es wurden neue Triazin[5,4-*b*]indol-4-on-Derivate mit Aminogruppen in Stellung 3 synthetisiert und auf inotrope und plättchenaggregationshemmende Wirkung geprüft. **2h**, **2p**, **5p** und **6g** sind die stärksten inotropen Wirkstoffe in den entspr. Reihen. Die synthetisierten Verbindungen wurden als Hemmer der Plättchenaggregation, die mittels Adenosin-5'-diphosphat (ADP) und Arachidonsäure (AA) induziert wurde, geprüft (Meerschweinchen Gesamtblut). **2k**, **2p**, **5o**, **6d**, **6m** und **6o** sind die stärksten Hemmer der mittels (AA) induzierten Plättchenaggregation. **6d**, **6h** und **6o** sind die am stärksten wirksamen Verbindungen bei der mittels ADP induzierten Aggregation. Radioimmunologische Studien mit Bestimmung von Thromboxan B_2 (TXB_2) und Prostaglandin E_2 (PGE_2) nach AA-induzierter Aggregation wurden mit den Verbindungen **2b**, **2d**, **2f**, **2g**, **2h**, **2i**, **2k**, **2m**, **2o**, **2p**, **2r**, **5i**, **5j**, **5k**, **5r** und **5f** durchgeführt, die sich als Hemmer der durch AA-induzierten Aggregation erwiesen. Keine von den geprüften Verbindungen erwies sich als selektiver Hemmer. Verbindungen **2h** und **2p** haben inotrope und Plättchenaggregation-hemmende Eigenschaften.

Nowadays, congestive heart failure is treated with different inotropic agents[1)], some of which [carbazeram[2)], amrinone[3)], milrinone[4)], piroxinone[5)], imazodan[6)], and sulmazole[7)]] are selective inhibitors of the adenosine 3',5'-cyclic phosphate phosphodiesterase (cAMP-PDE) of the cardiac muscle (Chart 1). This inhibition of PDE seems to be the principle action mechanism, responsible for the positive inotropic activity of these cardiotonic compounds.

A new generation of cardiotonic compounds is emerging now, having a good balance between the inotropic and vasodilator activities. They also can cause a delay and even a reversion in the evolution of an illness which may prolongate the life of a patient with congestive heart failure. Therefore, another interesting approximation can be the use of a cardiotonic agent with platelet aggregation inhibitory activity in patients with a myocardial infarction history and a risk of coronary or pulmonary thrombosis[8,9)].

Preferably used antithrombotic drugs with activity on platelet aggregation act at the level of platelet cyclooxygenase. The most representative example is acetylsalicyclic acid (ASA)[10)]. A more effective therapeutic approach might be selective inhibition of thromboxane A_2 synthetase[11)], because thromboxane A_2 (TXA)l which is a potent vasoconstrictor[12)] and platelet aggregating agent, under physiological conditions rapidly hydrolyzes to TXB_2. An additional advantage in using these drugs could be that as a consequence of the inhibition of the enzyme, the accumulation of some precursors of TXA_2, such as protaglandin H_2 (PGH_2), could be deviated to the synthesis of the vasodilator prostacyclin (PGI_2) in the vessel wall[13)]. In this way, by controlling the PGI_2/TXA_2 system, a greater effectiveness can be obtained in the treatment or prophylaxis of several cardiovascular diseases[14)], and in a special way for elderly patients[15)].

Our objective is the synthesis of positive inotropic agents, vasodilators with antiaggregatory activity, by modification of the PGI_2/TXA_2 system.

The synthesis of a new series of pyridazino[4,5-*b*]indole derivatives and the study of their activity as antihypertensive agents and inhibitors of blood platelet aggregation have been reported[16)]; new pyrimido[5,4-*b*]indoles have been reported as inhibitors of the platelet aggregation[17)]. Some 1,2,3-triazino[5,6-*b*]indoles are also active[18)]. Finally some precursors of pyrimido[5,4-*b*]indole such as 3-aminoindole-2-carbohydrazine, inhibit platelet aggregation[19)]. Some examples of heterocyclic benzopyridazines, pyridines, pyrimidines, and pyridopyrimidine systems ... etc. with basic character and inotropic activity are shown in Chart 1. A wide range of systems could also be added to these examples.

Basing on the antecedents found in our laboratory, we have started the search for positive inotropic agents, with complementary activity in the cardiovascular system, for indoles condensed with heterocyclic systems. The synthesis and preliminary biological study of new triazino[5,4-*b*]indole-4-one derivatives are presented in this paper.

  0365-6233/92/0707-0439 $ 3.50 + .25/0

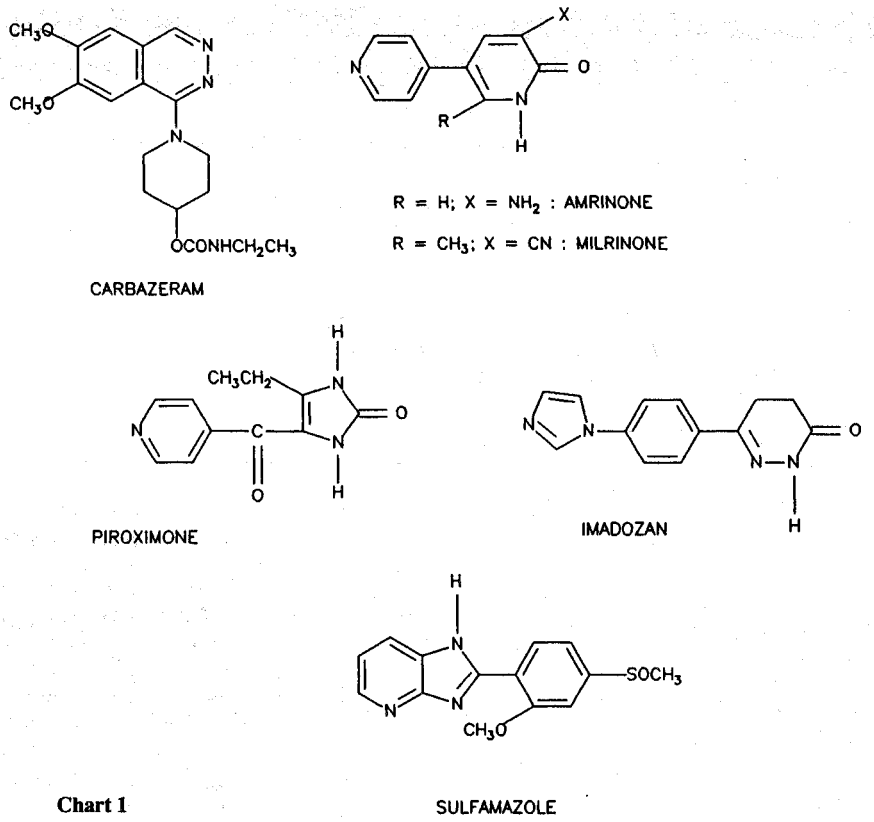


Chart 1

Chemistry

Compounds were synthetized as shown in the Chart 2. Ethyl 3-aminoindole-2-carboxylate (**1**) was prepared as described from 4-aminobenzonitrile[20].

Treatment of **1** with aromatic aldehydes/H^+ leads to the corresponding *Schiff* bases **2**. Reaction of **1** with hydrazine hydrate leads to the corresponding hydrazide **3**. When compounds **2** were treated with hydrazine hydrate, **3** was obtained by hydrolysis of the imine group and substitution by hydrazine in the ethyl carboxylate rest.

3-aminoindole-2-carbohydrazine (**3**) reacts with aromatic aldehydes to **4** or **5**, depending upon utilization of the aldehyde in excess or equimolar, respectively.

3-amino-1,2,3-triazino[5,4-*b*]indole-4-ones (**6** are obtained by reacting **5** with NO_2^-. By reaction with hydrazine hydrate the triazines **6** hydrolyze to 3-amino-1,2,3-triazino[5,4-*b*]indole-4-one **8** (68%). When the reaction was carried out with an excess of hydrazine hydrate in the absence of solvent, the azine of 3-amino-2-formyl indole **9** was obtained.

3-amino-5*H*-pyrimido[5,4-*b*]indole-4-one **7** is obtained by reaction of 3-aminoindole-2-carbohydrazine (**3**) or compound **5** with DMF.

Tables 1-4 summarize the physical and chemical properties of compounds **2**, **4**, **5**, and **6**, respectively.

Biology: Results and Discussion

A. *Inhibition of PDE*

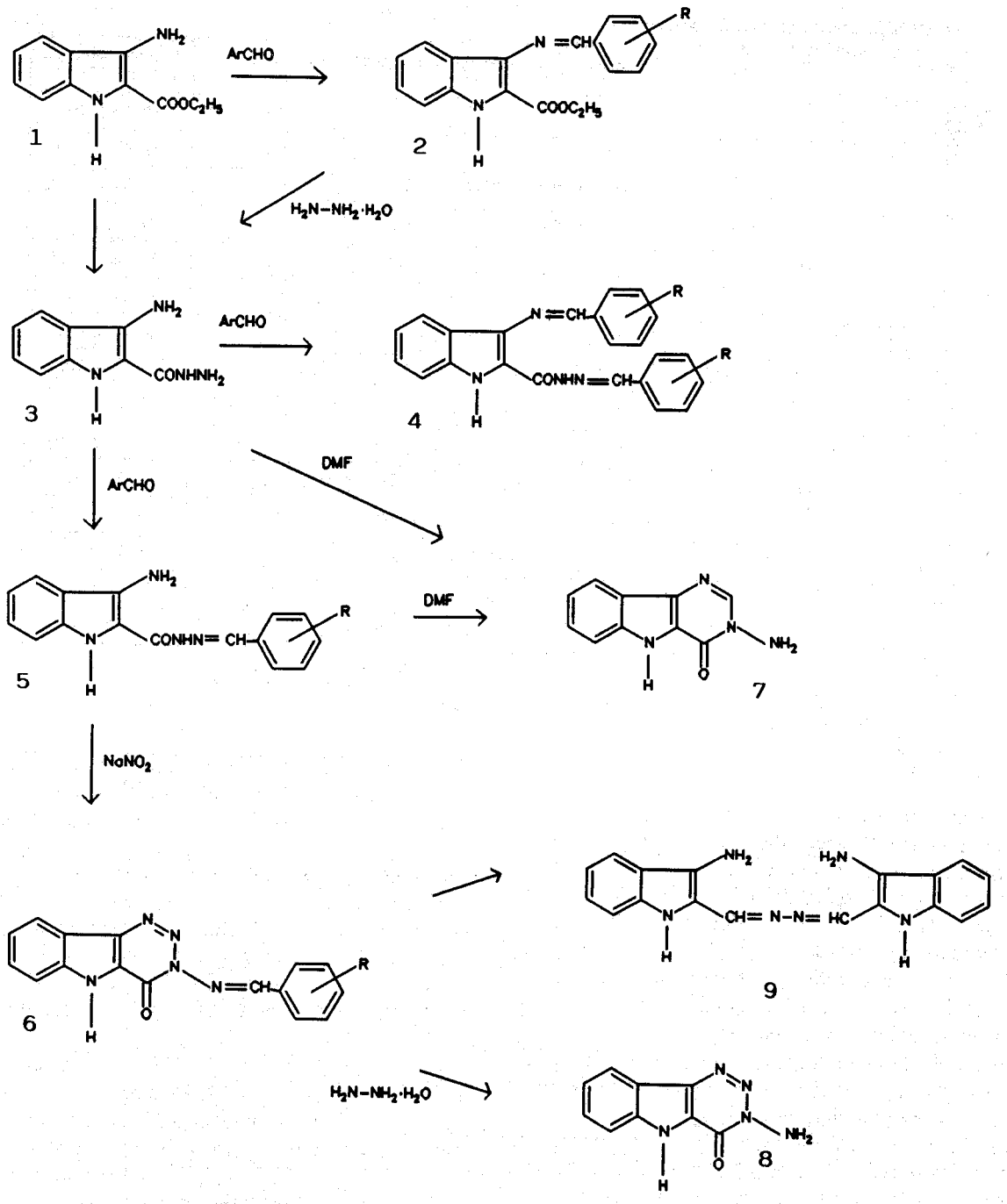
Determination of the inotopic activity of the synthetized compounds was initiated with the study of their behavior on the enzymatic activity of isolated phosphodiesterases (PDE) using the technique of *Reeves* et al.[21]. Starting from cardiac dog tissue, isoenzyme PDE-IV (high affinity c-AMP, inhibitable by c-GMP and cardiotonic inhibitors such as Amrinone, Milrinone, ... etc.) was isolated by ionic exchange chromatography with a DEAE-sepharose column (according to *Karya* and *Dage*[22] this tissue has a greater proportion of PDE-IV). In this preparation compounds **2** show an unequal activity, especially **2e** (51%), **2h** (42%), **2p** (44%), and **2r** (40%). Products **2s**, **2t**, and **4** could not be tested due to solubility problems (Table 2).

In general, the activity of compounds **5** is superior to that of series **2**. **5p**, IC_{50} = 113 µM, stands out for its activity. **5g**, **5k**, or **5h** also show significant activity (Table 3). Compounds **6** are less active than compounds **2** and **5** (Table 4). In this series 3-(4'-carboxybenzylidenamino)-1,2,3-triazino[5,4-*b*]indole-4-one, **6g**, shows an IC_{50} = 171 µM. In this test, Amrinone (Wincoram^r) has IC_{50} = 126 µM.

The results obtained in this test indicate a superior activity for noncyclic products in relation to the cyclic ones (Tables 1-4).

B. *Inhibition of platelet aggregation in whole blood*

Compounds were tested as inhibitors of platelet aggregation, induced by ADP and AA in guinea pig whole blood, as described and the relation of structure activity was analyzed. As antiaggregants, compounds **2** are generally the most active. In the aggregation induced by AA for series **2** (Table 5), a positive influence can be deduced, exerted by



Compound	R	Compound	R
a	4'-C_6H_5	i	4'-H
b	4'-$N(CH_3)_2$	j	4'-Cl
c	4'-$COOCH_3$	k	4'-OCH_3
d	4'-$OCH_2C_6H_5$	o	4'-$NHCH_3$
e	4'-NO_2	p	4'-OH
f	3',4'-methylendioxo	r	4'-CH_3
g	4'-COOH	s	2'-OH
h	3',4',5'-trimethoxy	t	4'-CF_3

Chart 2

the substituent in position 4', in both the donor character of electrons and in the ability to form hydrogen bonds. In series **5** (Table 6) influence in the donor character of hydrogen bonds, exerted by the substituent in position 4' is

Table 1: Structure and biological properties of **2**

Compd. no.	R	m.p. °C	recrystall. solvent	yield %	mol. form.	% Inhibition at 100 μM PDE-IV
2a	4'-C_6H_5	206-7°C	EtOH	66%	$C_{24}H_{20}N_2O_2$	18.10 ± 4.28
2b	4'-$N(CH_3)_2$	217°C	EtOH/H_2O	71%	$C_{20}H_{21}N_3O_2$	17.92 ± 9.49
2c	4'-$COOCH_3$	193°C	ISprOH	76%	$C_{20}H_{18}N_2O_4$	20.00 ± 13.31
2d	4'-$OCH_2C_6H_5$	150-1°C	EtOH/H_2O	78%	$C_{25}H_{22}N_2O_3$	26.51 ± 11.36
2e	4'-NO_2	226°C	AcOEt	70%	$C_{18}H_{13}N_3O_4$	51.22 ± 13.44
2f	3',4'-methylendioxy	165°C	MeOH/H_2O	80%	$C_{19}H_{16}N_2O_4$	20.21 ± 13.25
2g	4'-COOH	>250°C	Dioxane/EtOH	64%	$C_{19}H_{16}N_2O_4$	28.84 ± 6.04
2h	3',4',5'-trimethoxy	126.5°C	EtOH/H_2O	74%	$C_{21}H_{22}N_2O_5$	42.13 ± 12.02
2i	4' -H	132°C	EtOH/H_2O	65%	$C_{18}H_{16}N_2O_2$	22.91 ± 7.37
2j	4' -Cl	202-3°C	ISprOH/Dioxane	65%	$C_{18}H_{15}ClN_2O_2$	28.41 ± 8.68
2k	4' -OCH_3	156-7°C	EtOH	67%	$C_{19}H_{18}N_2O_3$	22.98 ± 13.79
2m	4' -OC_6H_5	157-8°C	Dioxane/EtOH	72%	$C_{24}H_{20}N_2O_3$	29.90 ± 11.57
2.o	4' -$NHCOCH_3$	228°C	MeOH/H_2O	80%	$C_{20}H_{19}N_3O_3$	39.17 ± 12.24
2p	4' -OH	177°C(d)	EtOH/H_2O	62%	$C_{18}H_{16}N_2O_3$	44.19 ± 13.46
2r	4' -CH_3	171.5-172.5	MeOH/H_2O	74%	$C_{19}H_{18}N_2O_2$	40.00 ± 15.68
2s	2' -OH	222°C	EtOH/H_2O	72%	$C_{18}H_{16}N_2O_3$	n.d.
2t	4' -CF_3	173-4°C	EtOH/H_2O	59%	$C_{19}H_{15}N_2O_2F_3$	n.d.
Amrinone						52

Table 2: Physical properties of **4**[a)]

Compd. no	R	m.p. °C	recrystall. solvent	yield %	mol. form.
4a	4'-C_6H_5	>250°C	IsprOH/Dioxane	78%	$C_{35}H_{26}N_4O$
4b	4'-$N(CH_3)_2$	>250°C	DMF/Dioxane	82%	$C_{27}H_{28}N_6O$
4c	4'-$COOCH_3$	>250°C	EtOH/DMF	82%	$C_{27}H_{22}N_4O_5$
4e	4'-NO_2	>250°C	DMF	85%	$C_{23}H_{16}N_6O_5$
4f	3',4'-methylendioxy	>250°C	Dioxane/EtOH	79%	$C_{25}H_{18}N_4O_5$
4g	4'-COOH	>250°C	DMF	78%	$C_{25}H_{18}N_4O_5 \cdot \frac{1}{2}H_2O$
4h	3',4',5'-trimethoxy	>250°C	DMF	74%	$C_{29}H_{30}N_4O_7$
4i	4'-H	>250°C	AcOEt	85%	$C_{23}H_{18}N_4O$
4j	4'-Cl	>250°C	Dioxane	86%	$C_{23}H_{16}Cl_2N_4O$
4k	4'-OCH_3	>250°C	MeOH/DMF	84%	$C_{25}H_{22}N_4O_3$
4m	4'-O-C_6H_5	>246°C	AcOEt	85%	$C_{35}H_{26}N_4O_3$
4.o	4'-$NHCOCH_3$	>250°C	DMF/Dioxane	84%	$C_{27}H_{24}N_6O_3$
4p	4'-OH	>250°C	EtOH/DMF	80%	$C_{23}H_{18}N_4O_3$
4r	4'-CH_3	>250°C	AcOEt/DMF	80%	$C_{25}H_{22}N_4O$

a) no biological data; insoluble compounds

conceivable. From the data obtained for series **6** (Table 7) in the inhibition of the aggregation induced by AA, an influence of steric factors is evident.

Of the more active compounds in the test with PDE-IV, **5h** and **5k** present activity as antiaggregant agents also (Table 4).

Table 3: Structure and biological properties of **5**

Compd. no	R	m.p. °C	recrystall. solvent	yield %	mol. form.	% inhibition at 100 μM PDE-IV	IC_{50}
5a	4'-C_6H_5	222-3°C	Dioxane	84%	$C_{22}H_{18}N_4O_2$	13.23 ± 5.52	
5b	4'-$N(CH_3)_2$	184°C	EtOH	75%	$C_{18}H_{19}N_5O$	14.81 ± 5.22	
5c	4'-$COOCH_3$	220-1°C	Dioxane/EtOH	81%	$C_{18}H_{16}N_4O_3$	9.25 ± 3.37	
5d	4'-$OCH_2C_6H_5$	165-166°C	Dioxane	82%	$C_{23}H_{20}N_4O_2$	13.67 ± 3.82	
5e	4'-NO_2	249°C	Dioxane	85%	$C_{16}H_{13}N_5O_3$	10.69 ± 5.75	
5f	3',4'-methylendioxy	205-6°C	EtOH	71%	$C_{17}H_{14}N_4O_3$	32.17 ± 5.71	
5g	4'-COOH	>250°C	EtOH/Dioxane	80%	$C_{17}H_{14}N_4O_3$	42.56 ± 5.83	139
5h	3',4',5'-trimethoxy	191-2°C	Dioxane	82%	$C_{19}H_{20}N_4O_4$	39.52 ± 5.27	159
5i	4'-H	190-1°C	EtOH	80%	$C_{16}H_{14}N_4O$	30.94 ± 4.56	
5j	4'-Cl	225°C	AcOEt/EtOH	79%	$C_{16}H_{13}ClN_4O$	20.72 ± 5.21	
5k	4'-OCH_3	185°C(d)	AcOEt	80%	$C_{17}H_{16}N_4O_3$	45.16 ± 3.77	126
5m	4'-OC_6H_5	196-7°C(d)	EtOH	77%	$C_{22}H_{17}N_4O_2$	4.87 ± 5.24	
5.o	4'-$NHCOCH_3$	238°C(d)	Dioxane	78%	$C_{18}H_{17}N_5O_2$	5.23 ± 3.47	
5p	4'-OH	270-1°C(d)	EtOH/Dioxane	78%	$C_{16}H_{14}N_4O_2$	51.07 ± 5.32	113
5r	4'-CH_3	199-200(d)	EtOH	79%	$C_{17}H_{16}N_4O$	26.22 ± 3.37	
5t	4'-CF_3	206-207°(d)	Dioxane/EtOH	83%	$C_{17}H_{13}N_4OF_3$	n.d.	
Amrinone						52	126

Table 4: Structure and biological properties of **6**

Compd. no	R_1	m.p. °C	recrystall. solvent	yield %	mol. form.	% inhibition at 100 μM PDE-IV	IC_{50}
6a	4'-C_6H_5	246-7°(d)	Dioxane	80%	$C_{22}H_{15}N_5O$	2.33 ± 4.04	
6c	4'-OCH_3	240-1°(d)	DMF/EtOH	79%	$C_{18}H_{13}N_5O_3$	0.36 ± 1.00	
6d	4'-$OCH_2C_6H_5$	234-5°(d)	DMF	89%	$C_{23}H_{17}N_5O_2$	3.82 ± 6.64	
6e	4'-NO_2	243-4°(d)	DMF	85%	$C_{16}H_{10}N_6O_3$	1.56 ± 2.46	
6f	-3',4'-methylendioxy	232-4°(d)	Dioxane	76%	$C_{17}H_{11}N_5O_3$	5.02 ± 6.01	
6g	4'-COOH	>250°C	DMF	82%	$C_{17}H_{11}N_5O_3$	42.93 ± 4.36	171
6h	3',4',5'-trimethoxy	232-3°(d)	DMF/EtOH	84%	$C_{19}H_{17}N_5O_4$	18.26 ± 2.67	
6i	4'-H	>250°C	Dioxane	80%	$C_{16}H_{11}N_5O$	14.76 ± 1,85	
6j	4'-Cl	247-8°C	DMF/Dioxane	86%	$C_{16}H_{10}ClN_5O$	2.52 ± 3.27	
6k	4'-OCH_3	190-1°(d)	EtOH/DMF	83%	$C_{17}H_{13}N_5O_2$	18.37 ± 8.50	
6m	4'-O-C_6H_5	230-1°(d)	DMF/EtOH	85%	$C_{22}H_{15}N_5O_2$	5.04 ± 1.12	
6.o	4'-$NHCOCH_3$	>250°C	DMF/H_2O	84%	$C_{18}H_{14}N_6O_2.H_2O$	8.03 ± 5.60	
6p	4'-OH	>250°C	DMF/EtOH	76%	$C_{16}H_{11}N_5O_2$	3.71 ± 1.86	
6r	4'-CH_3	232°(d)	Dioxane/DMF	74%	$C_{17}H_{13}N_5O$	0	
6t	4'-CF_3	240-1°C	DMF	78%	$C_{17}H_{10}N_3OF_3$	n.d.	
Amrinone						52	126

C. *Activity on Thromboxane A_2 Synthetase*

TXB_2 and PGE_2 levels of those samples, in which an inhibition 80% of the aggregation induced by AA was detected, were determined by radioinmunoassay. The data show that these compounds have a behavior which coincides with that anticipated for an inhibitor of cyclooxygenase, such as acetylsalicylic acid (Table 8).

Table 5: Effect of **2** and reference drug on platelet aggregation[a)]

Compound[b]	Final concent. (M)	% inhibition of whole blood platelet aggreg. induced by[c] ADP[d]	AA[e]
2a	5.10^{-4}	48.25 ± 10.23	48.86 ± 10.71
	$2.5\ 10^{-4}$	27.11 ± 6.48	23.38 ± 12.26
	10^{-4}	0	n.s.
2b	5.10^{-4}	43.13 ± 12.86	94.71 ± 8.47
	$2.5\ 10^{-4}$	17.00 ± 9.89	51.29 ± 39.02
	10^{-4}	0	n.s.
2c	5.10^{-4}	n.s.	n.s.
2d	5.10^{-4}	46.75 ± 20.22	99.14 ± 2.13
	$2.5\ 10^{-4}$	n.s.	43.50 ± 22.39
	10^{-4}	0	n.s.
2e	5.10^{-4}	44.50 ± 8.74	32.33 ± 8.64
	$2.5\ 10^{-4}$	n.s.	0
2f	5.10^{-4}	21.80 ± 6.89	99.00 ± 2.57
	$2.5\ 10^{-4}$	n.s.	n.s.
2g	5.10^{-4}	29.14 ± 14.59	99.00 ± 2.57
	$2.5\ 10^{-4}$	0	89.57 ± 19.76
	10^{-4}	--	n.s.
2h	5.10^{-4}	n.s	100
	$2.5\ 10^{-4}$	0	91.67 ± 12.76
	10^{-4}	--	n.s.
2i	5.10^{-4}	36.60 ± 16.30	97.50 ± 6.43
	$2.5\ 10^{-4}$	0	96.00 ± 6.11
	10^{-4}	----	n.s.
2j	5.10^{-4}	n.s.	n.s.
2k	5.10^{-4}	21.56 ± 9.73	98.50 ± 3.86
	$2.5\ 10^{-4}$	0	47.00 ± 20.12
	10^{-4}	----	23.67 ± 9.88
2m	5.10^{-4}	80.00 ± 16.06	99.50 ± 1.29
	$2.5\ 10^{-4}$	20.17 ± 13.82	40.25 ± 23.65
	10^{-4}	0	n.s.
2.o	5.10^{-4}	23.13 ± 10.98	97.33 ± 6.85
	$2.5\ 10^{-4}$	0	79.00 ± 8.07
	10^{-4}	----	n.s.
2p	5.10^{-4}	0	99.67 ± 0.86
	$2.5\ 10^{-4}$	----	100
	10^{-4}	---	97.40 ± 4.93
	5.10^{-5}	---	n.s.
2r	5.10^{-4}	0	78.33 ± 25.57
	$2.5\ 10^{-4}$	---	n.s.
2s	5.10^{-4}	65.75 ± 12,38	68.50 ± 9.83
ASA	5.10^{-3}	100	100
	5.10^{-4}	15.00 ± 12.75	30.00 ± 10.50

a) see Experiment. Part for details; b) incubated for 60 min. at 37°C; c) $\bar{X}$ ± S.E.M., $p \leq 0.05$ (n = 5-8); d) $2.3 \cdot 10^{-5}$M; $5.10 \cdot 10^{-4}$M.

This research work has been supported by The Upjohn Company within the National Plan of Scientific and Technological Investigation of Spain.

Experimental Part

IR spectra: Perkin-Elmer 681, KBr tablets.- ^{1}H-NMR spectra: Brucker AC-200E, Perkin-Elmer R-32 (90 MHz), or Perkin-Elmer R-24 (60 MHz), Me_4Si as int. standard, concentration about 0.1 g/ml.- ^{13}C-NMR spectra: Brucker AC-200E (50 MHz) with Me_4Si as int. standard.- Mass spectra: HP-5890 (GC/HPLC/DIP). All spectra were consistent with assigned structures.- Melting points: hot plate of a microscope (Reisert's apparatus), uncorrected.- Elemental analysis (Carlo Erba Elemental Analyzer) were

Table 6: Effect on platelet aggregation (whole blood)[a)]

Compound[b]	Final concentration (M)	% inhibition of platelet aggregation induced by[c] ADP[d]	AA[e]
5a	5.10^{-4}	81.83 ± 15.70	45.00 ± 10.61
	$2.5\ 10^{-4}$	34.13 ± 11.25	23.51 ± 10.26
	10^{-4}	n.s.	0
5b	5.10^{-4}	34.83 ± 5.73	n.s.
	$2.5\ 10^{-4}$	n.s.	0
5c	5.10^{-4}	38.00 ± 16.89	58.14 ± 13.47
	$2.5\ 10^{-4}$	23.00 ± 9.38	9.71 ± 9.84
	10^{-4}	n.s.	0
5d	5.10^{-4}	43.67 ± 12.55	28.67 ± 6.25
	$2.5\ 10^{-4}$	26.50 ± 8.08	0
	10^{-4}	0	---
5e	5.10^{-4}	69.50 ± 8.92	72.67 ± 6.82
	$2.5\ 10^{-4}$	n.s.	n.s.
5f	5.10^{-4}	n.s.	100
	$2.5\ 10^{-4}$	0	93.43 ± 5.03
	10^{-4}	---	n.s.
5g	5.10^{-4}	n.s.	0
5h	5.10^{-4}	n.s.	93.14 ± 6.89
	$2.5\ 10^{-4}$	0	n.s.
5i	5.10^{-4}	41.67 ± 17.87	100
	$2.5\ 10^{-4}$	n.s.	64.14 ± 25.18
	10^{-4}	0	0
5j	5.10^{-4}	49.00 ± 20.63	82.75 ± 31.38
	$2.5\ 10^{-4}$	n.s.	n.s.
5k	5.10^{-4}	47.87 ± 18.97	100
	$2.5\ 10^{-4}$	n.s.	67.43 ± 32.23
	10^{-4}	0	0
5m	5.10^{-4}	40.17 ± 8.17	23.25 ± 9.84
	$2.5\ 10^{-4}$	38.71 ± 10.74	0
	10^{-4}	0	---
5.o	5.10^{-4}	70.33 ± 15.70	69.63 ± 21.75
	$2.5\ 10^{-4}$	43.57 ± 12.78	46.67 ± 11.76
	10^{-4}	27.50 ± 6.19	30.17 ± 17.15
5p	5.10^{-4}	0	0
5r	5.10^{-4}	38.50 ± 24.51	84.29 ± 25.63
	$2.5\ 10^{-4}$	35.00 ± 18.94	60.20 ± 37.50
	10^{-4}	n.s.	n.s.
ASA	5.10^{-3}	100	100
	$5\ 10^{-4}$	15.00 ± 12.75	30.00 ± 10.50

a) see Experiment. Part for details; b) incubated for 60 min. at 37°C; c) $\bar{X}$ ± S.E.M., $p \leq 0.05$ (n = 5-8); d) $2.3 \cdot 10^{-5}$ M; $5.10 \cdot 10^{-4}$ M.

obtained from vacuum-dried samples (over P_2O_5 at 1-2 mm Hg, 24 h at about 60-80°C).

Ethyl 3-aminoindole-2-carboxylate (**1**)

This compound was obtained from 4-aminobenzonitrile as reported[20)] as a yellow microcrystalline solid. Yield 50%, m.p. 150-152°C (MeOH/H_2O).

Ethyl 3-(4'-R-benzylidenamino)indole-2-carboxylates, **2**, *General Method*

A mixture of **1** (2.5 mmol) and the respective aldehyde with 25% HCl (10 drops) and EtOH (25 ml) was refluxed for 8 h and then left to cool at room temp. The residue that precipitated was separated and washed with

Table 7: Effect on platelet aggregation (whole blood)[a]

Compound[b]	Final concentration (M)	% inhibition of platelet aggregation induced by[c] ADP[d]	AA[e]
6a	5.10^{-4}	55.64 ± 20.50	55.00 ± 26.61
	$2.5\ 10^{-4}$	24.86 ± 16.99	20.75 ± 5.47
	10^{-4}	n.s.	n.s.
6c	5.10^{-4}	75.80 ± 6.35	81.00 ± 18.81
	$2.5\ 10^{-4}$	41.17 ± 12.19	53.43 ± 6.04
	10^{-4}	n.s.	0
6d	5.10^{-4}	---	---
	$2.5\ 10^{-4}$	78.33 ± 6.82	84.63 ± 8.86
	10^{-4}	68.88 ± 7.13	50.33 ± 15.13
	5.10^{-5}	23.14 ± 7.73	35.38 ± 7.48
	$2.5\ 10^{-5}$	n.s.	0
6e	5.10^{-4}[f]	---	---
	$2.5\ 10^{-4}$	75.00 ± 7.74	74.88 ± 5.87
	10^{-4}	20.63 ± 10.51	n.s.
	5.10^{-5}	n.s.	0
6f	5.10^{-4}	51.17 ± 12.83	48.63 ± 6.11
	$2.5\ 10^{-4}$	n.s.	n.s.
6g	5.10^{-4}[f]	---	---
	$2.5\ 10^{-4}$	n.s.	0
6h	5.10^{-4}	98.83 ± 3.00	95.00 ± 3.03
	$2.5\ 10^{-4}$	77.00 ± 6.03	57.17 ± 17.63
	10^{-4}	54.50 ± 17.15	n.s.
	5.10^{-5}	n.s.	0
6i	5.10^{-4}	n.s.	n.s.
6j	5.10^{-4}	53.20 ± 16.78	50.20 ± 8.16
	$2.5\ 10^{-4}$	31.20 ± 16.31	n.s.
	10^{-4}	n.s.	0
6k	5.10^{-4}	n.s.	n.s.
6m	5.10^{-4}[f]	---	---
	$2.5\ 10^{-4}$	71.33 ± 10.13	62.60 ± 7.60
	10^{-4}	44.38 ± 9.93	47.17 ± 23.68
	5.10^{-5}	n.s.	n.s.
6.o	5.10^{-4}	100	98.60 ± 2.42
	$2.5\ 10^{-4}$	94.83 ± 7.04	97.75 ± 3.06
	10^{-4}	70.71 ± 10.62	77.63 ± 7.84
	5.10^{-5}	n.s.	33.00 ± 15.54
6p	5.10^{-4}	69.83 ± 9.67	80.75 ± 9.20
	$2.5\ 10^{-4}$	56.57 ± 7.79	46.57 ± 11.93
	10^{-4}	n.s.	n.s.
6r	5.10^{-4}[f]	---	---
	$2.5\ 10^{-4}$	53.67 ± 12.81	46.00 ± 9.71
	10^{-4}	n.s.	n.s.
ASA	5.10^{-3}	100	100

a) see Experiment. Part for details; b) incubated for 60 min. at 37°C; c) $\bar{X}$ ± S.E.M., $p \leq 0.05$ (n = 5-8); d) $2.3 \cdot 10^{-5}$ M; $5.10 \cdot 10^{-4}$ M.

EtOH/H_2O, thereby obtaining the desired compound in good yield (Table 1).

3-Aminoindole-2-carbohydrazide (**3**)

Ethyl 3-aminoindole-2-carboxylate (**1**) (2.05 g, 10 mmol) was suspended in hydrazine hydrate. The mixture was refluxed for 4 h and poured onto H_2O/ice (100 ml). The precipitated solid was washed with H_2O. Pale brown needles, yield 79%, m.p. 165°C (EtOH/H_2O)[19]. ^{1}H-NMR (DMSO-d_6, 90 MHz): δ (ppm) = 4.35 (2H, bs, NH_2), 5.45 (2H, bs, NH_2), 6.75-7.25 (3H, m, aromat.), 7.60 (1H, d, J = 8.00 Hz, H-4), 8.60 (1H, bs, NHCO), 10.25 (1H, s, NH indole).- IR: 3450 (m, NH); 3320; 3260 (s, NH); 1640 (s, C=O); 730 (1,2 disubst) cm^{-1}.

Table 8: Effect on Thromboxane Synthetase Activity on *in vitro* platelet aggregation induced by AA (whole blood)[a)]

Compound[b]	Final concentration (M)	% from basal values: PGE_2	TXB_2
2b	5.10^{-4}	-68.80 ± 12.52	-92.60 ± 7.78
	$2.5\ 10^{-4}$	-99.50 ± 1.59	-96.00 ± 0.12
2d	5.10^{-4}	-70.17 ± 13.98	-94.33 ± 5.34
2f	5.10^{-4}	-77.20 ± 9.67	-96.25 ± 4.38
2g	5.10^{-4}	-73.00 ± 19.65	-93.75 ± 4.18
	$2.5\ 10^{-4}$	-94.00 ± 8.23	-97.80 ± 2.39
2h	5.10^{-4}	-78.00 ± 9.60	-96.50 ± 6.43
	$2.5\ 10^{-4}$	-87.00 ± 10.35	-98.25 ± 3.76
2i	5.10^{-4}	-74.60 ± 9.19	-95.50 ± 4.95
	$2.5\ 10^{-4}$	-61.00 ± 20.50	-85.75 ± 15.50
2k	5.10^{-4}	-81.00 ± 7.12	-97.50 ± 4.77
2m	5.10^{-4}	-67.40 ± 20.62	-96.50 ± 7.17
2.o	5.10^{-4}	-83.00 ± 6.99	-97.75 ± 4.18
	$2.5\ 10^{-4}$	-86.20 ± 19.66	-96.80 ± 6.88
2p	5.10^{-4}	-81.83 ± 7.72	-97.50 ± 3.79
	$2.5\ 10^{-4}$	-88.86 ± 9.51	-98.75 ± 3.01
	10^{-4}	-61.50 ± 11.80	-92.75 ± 4.18
2r	5.10^{-4}	-78.00 ± 15.51	-91.00 ± 25.41
5i	5.10^{-4}	-82.75 ± 6.14	-96.75 ± 4.57
5j	5.10^{-4}	- 47[b]	- 43[b]
5k	5.10^{-4}	-90.10 ± 8.40	-75.80 ± 12.45
5r	5.10^{-4}	- 63[b]	- 74[b]
5f	5.10^{-4}	- 100	- 100
ASA	5.10^{-3}	- 100	- 100

a) see Experim. Part for details;
b) n = 3.

N'-(4'-R-Benzylidene)-3-(4'-R-benzylideneamino)indole-2-carbohydrazides, **4**, *General Method*

The respective aldehyde (3.3 mmol) and 25% HCl (2 drops) were added to a solution of **3** (1.5 mmol) in ethanol (30 ml). The mixture was refluxed for 1 h, left to cool at room temp., and filtered. The solid residue was washed with ethanol (2 x 10 ml) and recrystallized (Table 2).

N'-(4'-R-Benzylidene)-3-aminoindole-2-carbohydrazides, **5**, *General method*

3 (2.6 mmol) was dissolved in EtOH (20 ml). The respective aldehyde (2.6 mmol) was added, the reaction mixture refluxed for 10 min and left to cool at room temp. The precipitated solid was washed with hot H_2O and recrystallized (Table 3).

3-(4'-R-Benzylidenamino)-1,2,3-triazino[5,4-b]indol-4-one, **6**, *General method*

Conc. HCl (0.5 ml) was added to a suspension of the respective **5** (1.71 mmol) in H_2O (25 ml). Subsequently 20% aqueous $NaNO_2$ (5 ml) was added. The mixture was stirred for 5 h at room temp. and left to set over 10 h. It was filtered and the solid obtained was washed with hot H_2O and recrystallized (Table 4).

Table of 1H-NMR data

Compound	δ (ppm)
2a	1.26 (3H, t, CH_3) 4.30 (2H, q, CH_2) 7.12 (1H,t, H-5) 7.33 (1H, t, H-6) 7.48 (4H, m, Ar) 7.76 (4H, m, Ar) 7.86 (2H, d, H-3′ and H-5′) 8.10 (2H, d, H-2′ and H-6′) 8.87 (1H, s, CH=N) 11.71 (1H, s, NH indole).
2b	1.22 (3H, t, CH_3) 3.00 (6H, s, $(CH_3)_2$-N) 4.26 (2H, q, CH_2) 6.78 (2H, d, H-3′ and H-5′) 7.10 (1H, t, H-5) 7.25 (1H, t, H-6) 7.43 (1H, d, H-7) 7.66 (1H, d, H-4) 7.78 (2H, d, H-2′ and H-6′) 8.55 (1H, s, CH) 11.48 (1H, s, NH indole).
2c	1.25 (3H, t, CH_3) 3.89 (3H, s, CH_3-O) 4.27 (2H, q, CH_2) 7.12 (1H, t, H-5) 7.33 (1H, t, H-6) 7.48 (1H, d, H-7) 7.78 (1H, d, H-4) 8.12 (4H, s, H-2′, H-3′, H-5′ and H-6′) 8.94 (1H, s, CH=N) 11.81 (1H, s, NH indole).
2d	1.22 (3H, t, CH_3) 4.25 (2H, t, CH_2) 5.20 (3H, s, CH_2-O) 7.11 (1H, t, H-5) 7.17 (2H, d, H-3′ and H-5′) 7.30-7.50 (7H, m, Ar) 7.67 (1H, d, H-4) 7.94 (2H, d, H-2′ and H-6′) 8.69 (1H, s, CH=N) 11.58 (1H, s, NH indole).
2e	1.29 (3H, t, CH_3) 4.32 (2H, q, CH_2) 7.15 (1H, t, H-5) 7.35 (1H, t, H-6) 7.48 (1H, d, H-7) 7.86 (1H, d, H-4) 8.26 (2H, d, H-2′ and H-6′) 9.39 (2H, d, H-3′ and H-5′) 9.06 (1H, s, CH=N) 11.91 (1H, s, NH indole)
2f	1.30 (3H, t, CH_3) 4.35 (2H, q, CH_2) 6.18 (2H, s, -CH_2-) 7-7.78 (7H, m, H-4, H-5, H-6, H-7, H-2′, H-5′and H-6′) 8.68 (1H, s, CH=N) 11.83 (1H, bs, NH indole).
2g	1.27 (3H, t, CH_3) 4.30 (2H, q, CH_2) 7.13 (1H, t, H-5) 7.34 (1H, t, H-6) 7.48 (1H, d, H-7) 7.78 (1H, d, H-4) 8.12 (4H, s, H-2′, H-3′, H-5′ and H-6′) 8.94 (1H, s, CH) 11.80 (1H, s, NH indole) 13.00 (1H, bs, COOH).
2h	1.27 (3H, t, CH_3) 3.75 (3H, s, CH_3) 3.87 (6H, s, CH_3-O) 4.28 (2H, q, CH_2) 7.10 (1H, t, H-5) 7.35 (3H, m, H-6, H-2′ and H-6′) 7.43 (1H, d, H-7) 7.72 (1H, d, H-4) 8.74 (1H, s, CH) 11.67 (1H, s, NH indole).
2i	1.24 (3H, t, CH_3) 4.31 (2H, q, CH_2) 7.13 (1H, t, H-5) 7.31 (1H, t, H-6) 7.46 (1H, d, H-7) 7.54 (3H, s, H-3′, H-4′ and H-5′) 7.71 (1H, d, H-4) 8.00 (2H, d, H-2′ and H-6′) 8.83 (1H, s, CH=N) 11.78 (1H, s, NH indole).
2j	1.23 (3H, t, CH_3) 4.29 (2H, q, CH_2) 7.10 (1H, t, H-5) 7.32 (1H, t, H-6) 7.45 (1H, d, H-7) 7.60 (2H, d, H-3′ and H-5′) 7.74 (1H, d, H-4) 8.02 (2H, d, H-2′ and H-6′) 8.83 (1H, s, CH) 11.74 (1H, s, NH indole).
2k	1.23 (3H, t, CH_3) 3.85 (3H, s, CH_3-O-) 4.26 (2H, q, CH_2) 7.09 (3H, m, H-5, H-3′ and H-5′) 7.30 (1H, t, H-5) 7.43 (1H, d, H-7) 7.66 (1H, d, H-4) 7.95 (2H, d, H-2′ and H-6′) 8.70 (1H, s, CH=N) 11.59 (1H, s, NH).
2m	1.23 (3H, t, CH_3) 4.27 (2H, q, CH_2) 7.05-7.50 (10H, m, Ar) 7.69 (1H, d, H-4) 8.01 (2H, d, H-2′ and H-6′) 8.76 (1H, s, CH=N) 11.63 (1H, s, NH indole).
2.o	1.22 (3H, t, CH_3) 2.09 (3H, s, CH_3) 4.27 (2H, q, CH_2) 7.07 (1H, t, H-5) 7.30 (1H, t, H-6) 7.43 (1H, d, H-7) 7.67 (1H, d, H-4) 7.75 (2H, d, H-3′ and H-5′) 7.92 (2H, d, H-2′ and H-6′) 8.68 (1H, s, CH) 11.59 (1H, s, NH indole).
2p	1.23 (3H, t, CH_3) 4.27 (2H, q, CH_2) 6.93 (2H, d, H-3′ and H-5′) 7.08 (1H, t, H-5) 7.31 (1H, t, H-6) 7.45 (1H, d, H-7) 7.67 (1H, d, H-4) 7.85 (2H, d, H-2′ and H-6′) 8.63 (1H, s, CH=N) 10.15 (1H, s, OH) 11.56 (1H, s, NH indole).
2r	1.22 (3H, t, CH_3) 2.39 (3H, s, CH_3-Ph) 4.27 (2H, q, CH_2) 7.05 (1H, t, H-5) 7.30-7.40 (3H, m, H-6 + H-3′ and H-5′) 7.45 (1H, d, H-7) 7.67 (1H, d, H-4) 7.89 (2H, d, H-2′ and H-6′) 8.74 (1H, s, CH) 11.65 (1H, s, NH indole).
2s	1.36 (3H, t, CH_3) 4.40 (2H, q, CH_2) 6.92-7.00 (2H, m, Ar) 7.19 (1H, t, Ar) 7.32-7.40 (2H, m, Ar) 7.51 (1H, d, Ar) 7.73 (1H, d, Ar) 8.14 (1H, d, Ar) 9.34 (1H, s, CH=N) 12.06 (1H, s, NH indole) 13.54 (1H, s, OH).
2t	1.27 (3H, t, CH_3) 4.31 (2H, q, CH_2) 7.14 (1H, t, H-5) 7.35 (1H, t, H-6) 7.47 (1H, d, H-7) 7.81 (1H, d, H-4) 7.92 (2H, d, H-2′ and H-6′) 8.24 (2H, d, H-3′ and H-5′) 8.98 (1H, s, CH=N) 11.84 (1H, s, NH indole).
4a	7.20-7.60 (9H, m, Ar) 7.75-7.85 (6H, m, Ar) 7.98 (4H, t, Ar) 8.26 (3H, d, Ar) 8.46 (1H, s, Ar) 9.40 (1H, s, CH) 12.25 (1 H, s, NH) 12.44 (1H, s, NH).
4b	3.00 (6H, s, $(CH_3)_2$ N-) 3.08 (6H, s, $(CH_3)_2$-N) 6.80 (2H, d, Ar) 6.93 (2H, d, Ar) 7.15 (1H, t, H-5) 7.31 (1H, t, H-6) 7.50 (1H, d, H-7) 7.64 (2H, d, Ar) 7.94 (2H, d, Ar) 8.12 (1H, d, H-4) 8.20 (1H, s, CH=N) 9.12 (1H, s, CH=N) 11.94 (1H, s, NH) 12.37 (1H, s, NH).
4c	3.89 (3H, s, CH_3) 3.92 (3H, s, CH_3) 7.25-7.37 (2H, m, Ar) 7.56 (1H, d, Ar) 7.97 (2H, d, Ar) 8.08 (2H, d, Ar) 8.21-8.30 (5H, m, Ar) 8.44 (1H, s, CH=N) 9.40 (1H, s, CH=N) 12.30 (1H, s, NH) 12.40 (1 H, s, NH).
4d	5.20 (2H, s, -CH_2-) 5.28 (2H, s, -CH_2-) 6.90-7.15 (4H, m, Ar) 7.20-7.65 (14H, m, Ar) 7.70-7.90 (3H, m, Ar) 8.10 (1H, d, Ar) 8.33 (1H, s, CH=N) 9.35 (1H, s, CH=N) 12.11 (1H, s, NH) 12.34 (1H, s, NH)
4e	no data, insoluble compound.
4f	6.11 (2H, s, OCH_2O) 6.18 (2H, s, O-CH_2-O) 6.95-7.65 (8H, m, Ar) 8.10-8.25 (2H, m, Ar) 8.33 (1H, s, CH=N) 9.27 (1H, s, CH=N) 12.15 (1H, s, NH) 12.30 (1H, NH).

Table of 1H-NMR data **Cont.**

Compound	δ (ppm)
4g	7.30 (2H, m, Ar) 7.56 (1H, d, Ar) 7.95 (3H, m, Ar) 8.08 (2H, d, Ar) 8.23 (4H, s, Ar) 8.42 (1H, s, CH=N) 9.38 (1H, s, CH=N) 12.33 (1H, s, NH) 13.15 (1H, bs, NH).
4h	3.73 (3H, s, CH_3) 3.77 (3H, s, CH_3) 3.86 (6H, s, CH_3) 3.96 (6H, s, CH_3) 7.00 (2H, s, Ar) 7.23-7.35 (2H, m, Ar) 7.50-7.57 (3H, m, Ar) 8.22 (1H, d, Ar) 8.39 (1H, s, CH=N) 9.27 (1H, s, CH=N) 12.13 (1H, s, NH) 12.24 (1H, s, NH).
4i	7.00-7.80 (12H, m, Ar) 7.85-8.15 (2H, m, Ar) 8.28 (1H, s, CH) 9.20 (1H, s, CH) 12.0 (1H, s, NH) 12.25 (1H, s, NH).
4j	7.21-7.33 (3H, m, Ar) 7.56 (2H, d, Ar) 7.70 (2H, d, Ar) 7.84 (2H, d, Ar) 8.11-8.22 (3H, m, Ar) 8.30 (1H, s, CH) 9.29 (1H, s, CH) 12.24 (2H, bs, NH).
4k	3.80 (3H, s, CH_3) 3.88 (3H, s, CH_3) 7.03 (2H, d, Ar) 7.18 (3H, d, Ar) 7.30 (1H, t, H-5) 7.51 (1H, d, H-7) 7.74 (2H, d, Ar) 8.06 (2H, d, Ar) 8.14 (1H, d, H-4) 8.24 (1H, s, CH=N) 9.21 (1H, s, CH=N) 12.05 (1H, s, NH) 12.27 (1H, s, NH).
4m	7.06-7.51 (17H, m, Ar) 7.80 (2H, d, Ar) 8.15 (3H, d, Ar) 8.32 (1H, s, CH=N) 9.28 (1H, s, CH=N) 12.14 (1H, s, NH) 12.28 (1 H, s, NH).
4o	2.08 (3H, s, CH_3) 2.11 (3H, s, CH_3) 7.19 (1H, t, H-5) 7.32 (1H, t, H-6) 7.52 (1H, d, H-7) 7.71 (4H, s, Ar) 7.83 (2H, d, Ar) 8.06 (2H, d, Ar) 8.16 (1H, d, H-4) 8.28 (1H, s, CH=N) 9.21 (1H, s, CH=N) 10.17 (1H, s, NH) 10.27 (1H, s, NH) 12.10 (1H, s, NH) 12.30 (1H, s, NH).
4p	6.90 (2H, d, Ar) 7.03 (2H, d, Ar) 7.19 (1H, t, H-5) 7.32 (1H, t, H-6) 7.53 (1H, d, H-7) 7.66 (2H, d, Ar) 7.99 (2H, d, Ar) 8.15 (1H, d, H-4) 8.24 (1H, s, CH) 9.19 (1H, s, CH) 10.16 (2H, bs, OH) 12.05 (1H, s, NH) 12.31 (1H, s, NH).
4r	2.38 (3H, s, CH_3) 2.45 (3H, s, CH_3) 7.33 (3H, m, Ar) 7.49 (3H, m, Ar) 7.72 (2H, d, Ar) 7.85 (1H, d, Ar) 8.04 (2H, d, Ar) 8.25 (1H, d, Ar) 8.32 (1H, s, CH=N) 9.29 (1H, s, CH=N) 12.16 (1H, s, NH) 12.35 (1H, s, NH).
5a	5.93 (2H, bs, NH_2) 6.92 (1H, t, H-5) 7.22 (1H, t, H-6) 7.32-7.49 (3H, m, Ar) 7.70-7.85 (8H, m, Ar) 8.24 (1H, s, CH) 10.10 (1H, s, NH) 11.00-11.40 (1H, bs, NH).
5b	2.96 (6H, s, CH_3) 5.7-6.1 (2H, bs, NH_2) 6.76 (2H, d, H-3′ and H-5′) 6.94 (1H, t, H-5) 7.22 (1H, t, H-6) 7.35 (1H, d, H-7) 7.57 (2H, d, H-2′ and H-6′) 7.75 (1H, d, H-4) 8.08 (1H, s, CH=N) 10.14 (1H, s, NH indole) 10.8-11.2 (1H, bs, NHCO).
5c	3.97 (3H, s, CH_3) 6.02 (2H, bs, NH_2) 6.95 (1H, t, H-5) 7.25 (1H, t, H-6) 7.36 (1H, d, H-7) 7.77 (1H, d, H-4) 8.04 (2H, d, H-3′and H-5′) 8.17 (2H, d, H-2′ and H-6′) 8.28 (1H, s, CH) 10.11 (1H, s, NH) 11.00-11.50 (1H, bs, NH).
5d	5.18 (2H, s, CH_2) 5.90 (2H, bs, NH_2) 6.95 (1H, t, H-5) 7.12 (2H, d, H-3′ and H-5′) 7.24 (1H, t, H-6) 7.30-7.46 (6H, m, Ar) 7.73 (3H, m, Ar) 8.17 (1H, s, CH) 10.11 (1H, s, NH) 11.09 (1H, bs, NH).
5e	5.80-6.20 (2H, bs, NH_2) 6.95 (1H, t, H-5) 7.25 (1H, t, H-6) 7.34 (1H, d, H-7) 7.78 (1H, d, H-4) 8.02 (2H, d, H-2′ and H-6′) 8.30 (2H, d, H-3′ and H-5′) 8.29 (1H, s, CH=N) 10.12 (1H, s, NH indole) 11.00-11.50 (1H, bs, NH).
5k	3.66 (3H, s, CH_3) 5.76 (2H, bs, NH_2) 6.79 (1H, t, H-5) 6.87 (2H, d, H-3′ and H-5′) 7.08 (1H, t, H-6) 7.20 (1H, d, H-7) 7.62-7.53 (3H, m, H-4, H-2′ and H-6′) 8.02 (1H, s, CH) 9.96 (1H, s, NH) 10.7-11.1 (1H, bs, NH)
5f	5.7-6.02 (2H, bs, NH_2) 6.10 (2H, s, CH_2) 6.9-7.0 (2H, m, Ar) 7.17-7.30 (2H, m, Ar) 7.32-7.37 (2H, m, Ar) 7.75 (1H, d, H-4) 8.15 (1H, s, CH) 10.12 (1H, s, NH) 10.9-11.3 (1H, bs, NH).
5g	6.97 (1H, t, H-5) 7.25 (1H, t, H-6) 7.36 (1H, d, H-7) 7.80 (1H, d, H-4) 7.89 (2H, d, H-2′ and H-6′) 8.04 (2H, d, H-3′ and H-5′) 8.30 (1H, s, CH) 10.15 (1H, s, NH indole) 11-11.5 (1H, bs, NHCO).
5h	3.72 (3H, s, CH_3) (1H, t, H-5) 7.07 (2H, s, H-2′and H-6′) 7.24 (1H, t, H-6) 7.31 (1H, d, H-7) 7.77 (1H, d, H-4) 8.15 (1H, s, CH=N) 10.14 (1H, s, NH indole).
5i	5.6-6.0 (2H, bs, NH_2) 6.77 (1H, t, H-5) 7.07 (1H, t, H-6) 7.18 (1H, d, H-7) 7.31 (3H, d, Ar) 7.59 (3H, d, Ar) 8.05 (1H, s, CH) 9.95 (1H, NH indole) 10.9-11.4 (1H, bs, NHCO)
5j	5.8-6.2 (2H, bs, NH_2) 6.94 (1H, t, H-5) 7.24 (1H, t, H-6) 7.35 (1H, d, H-7) 7.53 (2H, d, H-3′ and H-5′) 7.77 (3H, d, H-4, H-2′ and H-6′) 8.22 (1H, s, CH) 10.92 (1H, s, NH) 11.00-11.50 (1H, s, NH).
5m	5.93 (2H, bs, NH_2) 6.94 (1H, t, H-5) 7.08 (3H, m) 7.23 (3H, m) 7.42 (3H, m) 7.77 (3H, d) 8.21 (1H, CH) 10.92 (1H, s, NH) 11.0-11.5 (1H, bs, NH).
5.o	2.07 (3H, s, CH_3) 6.94 (1H, t, H-5) 7.23 (1H, t, H-6) 7.34 (1H, d, H-7) 7.69 (4H, s, H-2′, H-3′, H-5′ and H-6′) 7.76 (1H, d, H-4) 8.16 (1H, s, CH) 10.11 (1H, s, NH) 10.14 (1H, s, NH) 10.9-11.4 (1H, bs, NHCO).
5p	5.72 (2H, bs, NH_2) 6.70 (2H, d, H-2′ and H-6′) 6.80 (1H, t, H-5) 7.07 (1H, t, H-6) 7.20 (1H, d, H-7) 7.43 (2H, d, H-3′ and H-5′) 7.60 (1H, d, H-4) 7.97 (1H, s, CH=N) 9.74 (1H, s,-OH) 9.94 (1H, s, NHCO) 10.8-11.2 (1H, bs, NH indol).

Table of 1H-NMR data **Cont.**

compound	δ (ppm)
5r	2.35 (3H, s, CH_3) 5.8-6.2 (2H, bs, NH_2) 6.96 (1H, t, H-5) 7.30 (4H, m, H-6 + H-7 + H-3′ and H-5′) 7.66 (2H, d, H-2′ and H-6′) 7.80 (1H, d, H-4) 8.23 (1H, s, CH) 10.17 (1H, s, NH indole) 10.8-11.4 (1H, bs, NHCO).
5t	5.4-6.6 (2H, bs, NH_2) 6.94 (1H, t, H-5) 7.20 (1H, t, H-6) 7.33 (1H, d, H-7) 7.75 (3H, m, H-4 + H-2′ and H-6′) 8.04 (2H, d, H-3′ and H-5′) 8.30 (1H, s, CH=N) 10.10 (1H, s, NH indole) 10.6-12 (1H, b.s, CO-NH).
6a	7.48 (4H, m, Ar) 7.66 (2H, m, Ar) 7.81 (2H, d, Ar) 7.92 (2H, d, Ar) 8.16 (2H, d, Ar) 8.26 (1H, d, Ar) 9.36 (1H, s, CH) 13.45 (1H, s, NH indole).
6c	3.80 (3H, s, CH_3) 7.42 (2H, d, H-3′ and H-5′) 7.65 (2H, s, H-5 + H-6) 7.90 (2H, d, H-2′ + H-5′) 8.19 (2H, m, H-7 + H-4) 9.11 (1H, s, CH) 13.35 (1H, s, NH indole).
6d	5.23 (2H, s, CH_2) 7.22 (2H, d, Ar) 7.38-7.65 (8H, m, Ar) 8.00 (2H, d, Ar) 8.25 (1H, d, Ar) 9.17 (1H, s, CH) 13.30 (1H, bs, NH indole).
6e	7.48 (1H, t, H-5) 7.67 (2H, d, H-2′ and H-6′) 8.26-8.45 (5H, m, H-6 + H-7 + H-4 + H-3′ and H-5′) 9.54 (1H, s, CH=N) 13.48 (1H, s, NH indole).
6f	6.18 (2H, s, CH_2) 7.12 (1H, d, Ar) 7.42-7.65 (5H, m, Ar) 8.25 (1H, d, Ar) 9.15 (1H, s, CH) 13.33 (1H, bs, NH indole).
6g	7.48 (1H, t, H-5) 7.67 (2H, d, H-6 + H-7) 8.17 (4H, m, H-2′ + H-3′ and H-5′ + H-6′) 8.27 (1H, d, H-4) 9.43 (1H, s, CH) 13.30 (1H, bs, OH) 13.45 (1H, s, NH indole).
6h	3.81 (3H, s, CH_3) 3.92 (6H, s, $2CH_3$) 7.43-7.53 (3H, m, H-5 + H-2′ and H-6′) 7.62-7.74 (2H, m, H-6 + H-7) 8.30 (1H, d, H-4) 9.23 (1H, s, CH=N) 13.38 (1H, bs, NH indole).
6i	7.45 (1H, t, H-5) 7.59-7.70 (5H, m, Ar) 8.05 (2H, m, Ar) 8.24 (1H, d, H-4) 9.30 (1H, s, CH) 13.36 (1H, bs, NH indole).
6j	7.44 (1H, t, H-5) 7.64 (4H, m, H-5 + H-6 + H-3′ and H-5′) 8.02 (2H, d, H-2′ and H-6′) 8.23 (1H, t, H-4) 9.32 (1H, s, CH) 13.37 (1H, s, NH indole).
6k	3.85 (3H, s, CH_3) 7.12 (2H, d, H-3′ and H-5′) 7.44 (1H, t, H-5) 7.62 (2H, m, H-6 + H-7) 7.98 (2H, d, H-2′ and H-6′) 8.22 (1H, d, H-4) 9.15 (1H, s, CH) 13.29 (1H, s, NH indole).
6m	6.87-7.65 (11H, m, Ar) 8.06 (2H, d, Ar) 8.25 (1H, d, Ar) 9.24 (1H, s, CH) 13.28 (1H, bs, NH indole).
6.o	2.12 (3H, s, CH_3) 7.49 (1H, t, H-5) 7.66 (2H, m, H-6 + H-7) 7.81 (2H, d, H-3′ and H-5′) 8.00 (2H, d, H-2′ and H-6′) 8.27 (1H, d, H-4) 9.28 (1H, s, CH) 10.33 (1H, s, NHCO) 13.35 (1H, s, NH indole).
6p	6.77 (2H, d, H-4′ + H-5′) 7.24 (1H, t, H-5) 7.45 (2H, d, H-6 + H-7) 7.71 (2H, d, H-2′ and H-6′) 8.02 (1H, d, H-4) 8.89 (1H, s, CH) 10.22 (1H, s, OH) 13.09 (1H, s, NH indole)
6r	2.44 (3H, s, CH_3) 7.43 (3H, m, H-5, H-3′ and H-5′) 7.67 (2H, m, H-6 and H-7) 7.97 (2H, d, H-2′ and H-6′) 8.26 (1H, d, H-4) 9.25 (1H, s, CH) 13.38 (1H, s, NH indole).
6t	7.44 (1H, t, H-6) 7.65 (2H, m, H-7 and H-8) 7.93 (2H, d, H-2′ and H-6′) 8.25 (3H, H-9′, H-3′ and H-5′) 9.44 (1H, s, CH=N) 13.38 (1H, s, NH indole).

(J $\underline{CH_3}$-CH_2-O= 6.95-7.12; J CH_3-$\underline{CH_2}$-O= 7.00-7.10; $J_{4,5}$= 7.80-8.14; $J_{4,5,6}$= 7.30-7.90; $J_{5,6,7}$= 7.00-7.45; $J_{7,6}$= 8.00-8.31 $J_{2',3'}$= 7.96-8.64; $J_{6',5'}$= 7.96-8.64)

3-Amino-5H-pyrimido[5,4-b]indol-4-one (**7**)

3 (2.63 mmol) dissolved in DMF (10 ml), was refluxed for 10 h. Then, the solvent was removed *in vacuo*. The solid obtained was stirred with EtOH (2 ml), filtered *in vacuo*, and then washed with cold EtOH and hot H_2O, thereby obtaining **7**[19] as white crystals. Yield 52%, m.p. 250°C (DMF/EtOH).- ^{1}H-NMR (DMSO-d_6, 60 MHz): δ (ppm) = 3.95 (s, 2H, NH_2), 7.10-7.60 (m, 3H, H-6, H-7, H-9), 8.03 (dd, J = 8.10/8.00 Hz, 1H, H-8), 9.00 (s, 1H, H-2), 12.15 (s, 1H, NH).- IR: 3160-3080 (s, N-H); 1680 (s, C=O); 730 (s, 1,2-disubst.) cm^{-1}.- ($C_{10}H_8N_4O_2$) C,H,N.

3-Amino-4-oxo-1,2,3-triazino[5,4-b]indole (**8**)

6k (0.5 g, 1.56 mmol) was suspended in EtOH (25 ml). 100% hydrazine hydrate (3 ml) was added and the reaction mixture was refluxed for 5 h. When it cooled to room temp., an earth-coloured solid precipitated, which was washed with H_2O and EtOH: brown needles. Yield 60%, m.p. > 225°C (EtOH/DMF).- ^{1}H-NMR (DMSO-d_6, 200 MHz): δ (ppm) = 6.98 (2H, s, NH_2), 7.43 (1H, t, J = 8.18 Hz, H-7), 7.57-7.68 (2H, m, H-6 and H-8), 8.24 (1H, d, J = 7.74 Hz, H-9), 12.98 (1H, bs, NH indole).- IR: 3360 (NH); 3190 (NH); 1700 (C=O); 1630 (C=C); 750 (1,2-disubst.) cm^{-1}.

Azine of 3-amino-2-formylindole (**9**)

A suspension of **6k** in hydrazine hydrate (10 ml) was refluxed for 5 h. After the solution had cooled to room temp., excess hydrazine hydrate was removed *in vacuo*. The residue was stirred with EtOH/H_2O (1:1), filtered and a very bright orange solid was obtained. Yield 44%, m.p. 238-240°C (DMF/EtOH).- ^{1}H-NMR (DMSO-d_6, 200 MHz): δ (ppm) = 5.71 (2H, bs, NH_2), 6.88 (1H, t, J = 7.40 Hz, H-5), 7.10-7.25 (2H, m, H-6 + H-7), 7.66 (1H, d, J = 7.85 Hz, H-4), 8.75 (1H, s, CH=N), 10.29 (1H, s, NH indole).- ^{13}C-NMR (DMSO-d_6, 50 MHz): δ (ppm) = 110.3 (C-3), 113.6 (C-7), 116.2

Table of C, H, N

2a	$C_{24}H_{20}N_2O_2$	C: Calcd. 78.3 Found 78.6	H: Calcd. 5.43. Found 5.62	N: Calcd. 7.6. Found 7.6.
2b	$C_{20}H_{21}N_3O_2$	C: Calcd. 71.7; Found 71.4.	H: Calcd. 6.27; Found 6.43.	N: Calcd. 12.5; Found 12.4.
2c	$C_{20}H_{18}N_2O_4$	C: Calcd. 68.6; Found 68.9.	H: Calcd. 5.14; Found 5.19.	N: Calcd. 8.0; Found 8.0.
2d	$C_{25}H_{22}N_2O_3$	C: Calcd. 75.4; Found 75.5.	H: Calcd. 5.52; Found 5.66.	N: Calcd. 7.0; Found 7.0.
2e	$C_{18}H_{13}N_3O_4$	C: Calcd. 64.1; Found 64.1.	H: Calcd. 4.45; Found 4.59.	N: Calcd. 12.5; Found 12.3.
2f	$C_{19}H_{16}N_2O_4$	C: Calcd. 67.9; Found 67.6.	H: Calcd. 4.76; Found 4.92.	N: Calcd. 8.3; Found 8.2.
2g	$C_{19}H_{16}N_2O_4$	C: Calcd. 67.9; Found 68.1.	H: Calcd. 4.76; Found 4.90.	N: Calcd. 8.3; Found 8.2.
2h	$C_{21}H_{22}N_2O_5$	C: Calcd. 65.9; Found 66.2.	H: Calcd. 5.76; Found 5.88.	N: Calcd. 7.3; Found 7.3.
2i	$C_{18}H_{16}N_2O_2$	C: Calcd. 74.0; Found 74.4.	H: Calcd. 5.48; Found 5.70.	N: Calcd. 9.6; Found 9.7.
2j	$C_{18}H_{15}N_2O_2Cl$	C: Calcd. 66.2; Found 66.2.	H: Calcd. 4.59; Found 4.76.	N: Calcd. 8.6; Found 8.6.
2k	$C_{19}H_{18}N_2O_3$	C: Calcd. 70.8; Found 71.2.	H: Calcd. 5.59; Found 5.64.	N: Calcd. 8.7; Found 8.4.
2m	$C_{24}H_{20}N_2O_3$	C: Calcd. 75.0; Found 75.2.	H: Calcd. 5.20; Found 5.39.	N: Calcd. 7.3; Found 6.9.
2.o	$C_{20}H_{19}N_3O_3$	C: Calcd. 68.8; Found 68.8.	H: Calcd. 5.44; Found 5.61.	N: Calcd. 12.0; Found 12.0.
2p	$C_{18}H_{16}N_2O_3$	C: Calcd. 70.1; Found 69.7.	H: Calcd. 5.20; Found 5.39.	N: Calcd. 9.1; Found 8.8.
2r	$C_{19}H_{18}N_2O_2$	C: Calcd. 74.5; Found 74.3.	H: Calcd. 5.88; Found 6.04.	N: Calcd. 9.2; Found 9.1.
2s	$C_{18}H_{16}N_2O_3$	C: Calcd. 70.1; Found 70.2.	H: Calcd. 5.19; Found 5.25.	N: Calcd. 9.1; Found 8.9.
2t	$C_{19}H_{15}N_2O_2F_3$	C: Calcd. 63.3; Found 63.0.	H: Calcd. 4.16; Found 4.22.	N: Calcd. 7.8; Found 7.9.
4a	$C_{35}H_{26}N_4O$	C: Calcd. 81.1; Found 81.3.	H: Calcd. 5.02; Found 5.20.	N: Calcd. 10.8; Found 10.8.
4b	$C_{27}H_{28}N_6O$	C: Calcd. 71.7; Found 71.9.	H: Calcd. 6.19; Found 6.34.	N: Calcd. 18.6; Found 18.7.
4c	$C_{27}H_{22}N_2O_5$	C: Calcd. 67.2; Found 67.1.	H: Calcd. 4.56; Found 4.57.	N: Calcd. 11.6; Found 11.6.
4e	$C_{23}H_{16}N_6O_5$	C: Calcd. 60.5; Found 60.5.	H: Calcd. 3.51; Found 3.59.	N: Calcd. 18.4; Found 18.2.
4f	$C_{25}H_{18}N_4O_5$	C: Calcd. 66.1; Found 66.2.	H: Calcd. 3.96; Found 4.00.	N: Calcd. 12.3; Found 12.2.
4g	$C_{25}H_{18}N_4O_5$	C: Calcd. 64.8; Found 64.9.	H: Calcd. 4.10; Found 4.06.	N: Calcd. 12.1; Found 12.1.
4h	$C_{29}H_{30}N_4O_7$	C: Calcd. 63.7; Found 63.6.	H: Calcd. 5.79; Found 5.60.	N: Calcd. 10.3; Found 10.1.
4i	$C_{23}H_{18}N_4O$	C: Calcd. 75.8; Found 75.8.	H: Calcd. 4.96; Found 5.09.	N: Calcd. 14.8; Found 15.2.
4j	$C_{23}H_{16}N_4OCl_2$	C: Calcd. 63.5; Found 63.2.	H: Calcd. 3.68; Found 3.75.	N: Calcd. 12.9; Found 12.8.
4k	$C_{25}H_{22}N_4O_3$	C: Calcd. 70.4; Found 70.7.	H: Calcd. 5.16; Found 5.24.	N: Calcd. 13.1; Found 13.1.
4m	$C_{33}H_{26}N_4O_3$	C: Calcd. 76.4; Found 76.7.	H: Calcd. 4.73; Found 4.80.	N: Calcd. 10.2; Found 10.3.

Table of C, H, N Cont.

4o	$C_{27}H_{24}N_6O_3 \cdot H_2O$	C: Calcd. 65.1; Found 65.1.	H: Calcd. 5.02; Found 5.24.	N: Calcd. 16.9; Found 16.9.
4p	$C_{23}H_{18}N_4O_3$	C: Calcd. 69.3; Found 69.4.	H: Calcd. 4.52; Found 4.72.	N: Calcd. 14.1; Found 13.9.
4r	$C_{25}H_{22}N_4O$	C: Calcd. 76.1; Found 76.3.	H: Calcd. 5.58; Found 5.81.	N: Calcd. 14.2; Found 14.3.
5a	$C_{22}H_{18}N_4O_2$	C: Calcd. 74.6; Found 74.4.	H: Calcd. 5.08; Found 5.14.	N: Calcd. 15.8; Found 15.8.
5b	$C_{18}H_{19}N_5O$	C: Calcd. 67.3; Found 67.2.	H: Calcd. 5.92; Found 6.08.	N: Calcd. 21.8; Found 21.6.
5c	$C_{18}H_{16}N_4O_3$	C: Calcd. 64.3; Found 64.3.	H: Calcd. 4.76; Found 4.87.	N: Calcd. 16.7; Found 16.7.
5d	$C_{23}H_{20}N_4O_2$	C: Calcd. 71.9; Found 71.9.	H: Calcd. 5.20; Found 5.33.	N: Calcd. 14.6; Found 14.4.
5e	$C_{16}H_{15}N_5O_3$	C: Calcd. 59.4; Found 59.5.	H: Calcd. 4.02; Found 4.00.	N: Calcd. 21.7; Found 21.6.
5f	$C_{17}H_{14}N_4O_3$	C: Calcd. 63.4; Found 63.3.	H: Calcd. 4.35; Found 4.37.	N: Calcd. 17.4; Found 17.3.
5g	$C_{17}H_{14}N_4O_3$	C: Calcd. 63.3; Found 63.0.	H: Calcd. 4.34; Found 4.54.	N: Calcd. 17.4; Found 17.5.
5h	$C_{19}H_{20}N_4O_4$	C: Calcd. 61.9; Found 62.1.	H: Calcd. 5.43; Found 5.62.	N: Calcd. 15.2; Found 14.9.
5i	$C_{16}H_{14}N_4O$	C: Calcd. 69.1; Found 69.2.	H: Calcd. 5.03; Found 5.22.	N: Calcd. 20.1; Found 20.4.
5k	$C_{17}H_{16}N_4O_3$	C: Calcd. 66.2; Found 66.5.	H: Calcd. 5.19; Found 5.35.	N: Calcd. 18.2; Found 18.1.
5j	$C_{16}H_{13}N_4Cl$	C: Calcd. 61.4; Found 61.2.	H: Calcd. 4.16; Found 4.26.	N: Calcd. 17.9; Found 17.8.
5m	$C_{22}H_{17}N_4O_2$	C: Calcd. 71.5; Found 71.2.	H: Calcd. 4.60; Found 4.73.	N: Calcd. 15.2; Found 15.1.
5.o.	$C_{18}H_{17}N_5O_2$	C: Calcd. 64.5; Found 64.6.	H: Calcd. 5.07; Found 5.31.	N: Calcd. 20.9; Found 20.8.
5p	$C_{16}H_{14}N_4O_2$	C: Calcd. 65.3; Found 65.4.	H: Calcd. 4.76; Found 4.96.	N: Calcd. 19.1; Found 19.1.
5r	$C_{17}H_{16}N_4O$	C: Calcd. 69.9; Found 69.7.	H: Calcd. 5.48; Found 5.86.	N: Calcd. 19.2; Found 18.8.
5t	$C_{17}H_{13}N_4O$	C: Calcd. 58.9; Found 59.2.	H: Calcd. 3.76; Found 3.57.	N: Calcd. 16.2; Found 15.9.
6a	$C_{22}H_{15}N_5O$	C: Calcd. 72.3; Found 72.4.	H: Calcd. 4.11; Found 4.30.	N: Calcd. 19.2; Found 19.3.
6c	$C_{18}H_{13}N_5O_3$	C: Calcd. 62.2; Found 62.4.	H: Calcd. 3.75; Found 3.91.	N: Calcd. 20.2; Found 19.9.
6d	$C_{23}H_{17}N_5O_2$	C: Calcd. 69.9; Found 69.5.	H: Calcd. 4.11; Found 4.35.	N: Calcd. 17.7; Found 15.6.
6e	$C_{16}H_{10}N_6O_3$	C: Calcd. 57.5; Found 57.3.	H: Calcd. 2.99; Found 2.95.	N: Calcd. 25.1; Found 25.0.
6f	$C_{17}H_{11}N_5O_3$	C: Calcd. 61.3; Found 61.2.	H: Calcd. 3.30; Found 3.42.	N: Calcd. 21.0; Found 21.1.
6g	$C_{17}H_{11}N_5O_3$	C: Calcd. 61.3; Found 60.9.	H: Calcd. 3.30; Found 3.46.	N: Calcd. 21.0; Found 20.8.
6h	$C_{19}H_{17}N_5O_4$	C: Calcd. 60.2; Found 60.4.	H: Calcd. 4.48; Found 4.63.	N: Calcd. 18.5; Found 18.6.
6i	$C_{16}H_{11}N_5O$	C: Calcd. 66.4; Found 66.6.	H: Calcd. 3.80; Found 4.02.	N: Calcd. 24.2; Found 24.3.
6j	$C_{16}H_{10}N_5OCl$	C: Calcd. 59.3; Found 59.5.	H: Calcd. 3.09; Found 3.14.	N: Calcd. 21.6; Found 21.8.

Table of C, H, N **Cont.**

6k	$C_{17}H_{13}N_5O_2$	C: Calcd. 63.9; Found 64.0.	H: Calcd. 4.07; Found 4.13.	N: Calcd. 21.9; Found 22.0.
6m	$C_{22}H_{15}N_5O_2$	C: Calcd. 69.3; Found 69.2.	H: Calcd. 3.9; Found 4.1.	N: Calcd. 18.4; Found 18.0.
6.o	$C_{18}H_{14}N_6O.H_2O$	C: Calcd. 59.3; Found 59.6.	H: Calcd. 4.39; Found 4.51.	N: Calcd. 23.1; Found 23.1.
6p	$C_{16}H_{11}N_5O_2$	C: Calcd. 62.9; Found 63.0.	H: Calcd. 3.61; Found 3.84.	N: Calcd. 22.9; Found 22.9.
6r	$C_{17}H_{13}N_5O$	C: Calcd. 67.3; Found 67.2.	H: Calcd. 4.29; Found 4.45.	N: Calcd. 23.1; Found 23.2.
6t	$C_{17}H_{10}N_5OF_3$	C: Calcd. 57.1; Found 57.5.	H: Calcd. 2.80; Found 2.84.	N: Calcd. 19.6; Found 19.3.

(C-6), 118.4 (C-4 or C-5), 118.8 (C-4 or C-5), 123.4 (C-2), 131.3 (C-3a), 136.2 (C-7a), 148.2 (CH=N).- MS (70 eV) m/z: 316 (28, M^+), 285 (13), 159 (100), 131 (43), 104 (16), 57 (20).- IR: 3410 (s, NH); 3200 (s, NH); 1600 (s, C=N); 745 (s, o-disubst.) cm^{-1}.

Isolation of Phosphodiesterases (PDE) and Assay of Activity

Four peaks of cyclic nucleotide phosphodiesterase activity (PDE I, PDE II, PDE IV, and PDE V)[22,23] from dog heart were separated by chromatography on a DEAE-sepharose-CL-6B (Pharmacia Fine Chemicals) column using a procedure essentially similar to that of *Reeves* et al.[21] for the separation of PDE activity from human and guinea pig cardiac ventricle. 25 g of dog heart tissue were homogenized in 250 ml of 20 mM Bis-Tris, 5 mM 2-mercaptoethanol, 2 mM benzamidine, 2 mM EDTA and 50 mM sodium acetate, pH 6.5, within a Polytron isntrument (3 times at setting 16.5 during 10 seconds). Phenylmethanesulphonyl fluoride was dissolved in propan-2-ol and added to the buffer immediately before homogenization, giving a final concentration of 50 µM. The homogenate was ultracentrifuged for 30 min at 105.000 x g and the supernatant was applied to a DEAE-Sepharose CL-6B column (11 x 2.5 cm), pre-equilibrated with a homogenization buffer. The column was washed with 100 ml of homogenization buffer and the PDE activities were eluted with a linear gradient of 0.05 - 1.0 M sodium acetate in the homogenizing buffer. A flow rate of 80 ml/h was used throughout the ion-exchange chromatography; 10 ml fractions were collected and assayed for PDE activity. Four activities could be distinguished on the basis of elution profiles measured at 0.5 µM cyclic-AMP, 25 µM cyclic-AMP, 0.5 µM cyclic-AMP + 10^{-4} M amrinone and 0.5 µM cyclic-AMP + 2 µM cyclic-GMP. Peaks three and four (PDE-IV and V) were separated once more by chromatography in order to improve their isolation. The peaks were collected, diluted with an equal volume of homogenization buffer without sodium acetate, applied to a column (17 cm x 1.5 cm) pre-equilibrated with homogenization buffer with 350 mM sodium acetate (instead of 50 mM), and eluted with a linear gradient of 350 mM - 1 M sodium acetate in homogenization buffer.

Peak I (PDE-I) is a high Vmax PDE activity which corresponds to the Ca^{2+}/calmodulin-stimulated PDE; peak II (PDE-II) is a cGMP-stimulated cAMP-PDE; peak III (PDE-IV) and peak IV (PDE-V) are both high affinity cAMP PDE. The former (sub-type IV) is a cGMP-inhibitable form which is also sensitive to the cardiotonic PDE-inhibitors, whereas the later (sub-type V) is a cGMP non-inhibitable form which is quite insensitive to the cardiotonic agents but strongly inhibited by rolipram[22].

PDE activity was assayed by the batch method of *Thompson* et al.[24]. All the chemicals were dissolved in DMSO. The final concentration of DMSO was 2.5%.IC_{50} values were obtained for peak three (PDE-IV) by incubation of the enzyme at 1 µM c-AMP and by a range of inhibiting concentrations from 10^{-6} to 10^{-4} M.

Platelet Aggregation Using Guinea Pig Whole Blood

Guinea pig whole blood was obtained by direct cardiac puncture of anaesthetized (Et_2O) female guinea pigs weighing ≥ 450 g. Blood was collected in 4.5 ml portions in Vacutainer 64625 silicone-coated tubes containing 0.5 ml of 3.8% sodium citrate. Normally, blood from 5-8 guinea pigs was collected.

Aggregation tests were carried out using the chronolog whole Blood Aggregometer and following *Cardinal* and *Flowers* method[25]. The operating principle of the whole blood aggregometer involves measurement of the electrical impedance between two electrodes immersed in the whole blood sample. When platelets aggregate in the whole blood aggregometer, they coat the electrodes to a greater or lesser degree, thereby impeding the current between the electrodes to an extent proportional to the amount of aggregation that has occurred 5 min after stirring in the aggregating agent (ADP, $2 \cdot 7\ 10^{-5}$ M; Arachidonic Acid, AA, $5 \cdot 10^{-4}$ M). Test drugs were dissolved in DMSO (6.6 µl final volume DMSO/ml in cuvette). Citrated whole blood was distributed in 500 µl aliquots into aggregometer cuvettes containing 500 µl of normal saline. Test drug solutions or DMSO were added (6.6 µl) and the cuvettes were incubated for 60 min at 37°C. Incubation was followed by the addition of aggregating agents (50 µl). The extent of aggregation of test samples was compared to the extent of aggregation of control samples and is expressed as "percent of control".

Effects on Thromboxane Synthetase Activity on in vitro Platelet Aggregation

The demonstration of selective inhibition effects of the compounds was determined according to the modified *Gorman* model[26]. PGE_2 and TXB_2 levels in the test samples, following aggregation, were determined by radioimmunoassay (RIA), according to[27].

RIA studies were carried out on the whole blood samples that experienced inhibited platelet aggregation following compound inhibition and addition of AA.

References

1 (a) W. Vn der Saal, J.P. Hölck, W. Damp, A. Mertens, and B. Müller-Beckmann, J. Med. Chem. *32*, 1481 (1989).
(b) A.S. Bell, S.F. Campbell, D.S. Morris, D.A. Roberts, and M.H. Stefaniak, J. Med. Chem. *32*, 1552 (1989).
(c) I. Sircar, R.P. Steffen, G. Bobowski, S.f. Burke, R.S. Newton, R.E. Weishaar, J.A. Bristol, and D.B. Evans, J. Med. Chem. *32* 342 (1989).
(d) J.A. Bristol, Ed. "Cardiovascular Drugs", pp. 257-304, John Wiley and Sons, New York, 1986.

2 S.F. Campbell, J.C. Donilewicz, A.G. Evans, and A.L. Ham, Pat. 4.289.772 (1981); C.A. *96*, 919P (1982).

3 A. Ward, R.N. Brodgen, R.C. Heel, I.M. Speight, and G.S. Avery, Drugs *24*, 468 (1983).

4 A.A. Alonsi, J.M. Canter, M.J. Montenaro, D.J. Fort, and R.A. Ferrari, J. Cardiovasc. Pharmacol. *5*, 792 (1983).
5 T. Dasiy, L.J. Wille, and R.C. Dage, J. Cardiovasc. Pharmacol. *6*, 50 (1984).
6 J.A. Bristol, J. Sircar, W.H. Moos, D.B. Evans, and R.E. Weishaar, J. Med. Chem. *27*, 1099 (1984).
7 W. Diederen and R. Kadatz, Arzneim.-Forsch. *31*, 146 (1981).
8 E.H. Sonnenblick and S. Factor, Prog. Cardiovasc. Dis. *27*, 395 (1985).
9 C. Nordin, Heart Failure **1**, 256 (1986).
10 S. Moncada and J.R. Vane, J. Med. Chem. *23*, 591 (1980).
11 M. Verstraete, Arzneim.-Forsch. *33* 1405 (1983).
12 S. Moncada, K. Gryglewski, S. Bunting, and J.R. Vane, Nature (London) *263*, 232 (1976).
13 F.P. Nijkamp, S. Moncada, H.L. White, and J.R. Vane, Eur. J. Pharmacol. *44*, 179 (1979).
14 K.C. Nicolaou, G.P. Gasie, and W.F. Barnette, Angew. Chem. *90*, 360 (1978); Angew. Chem. Int. Ed. Engl. *90*, 293 (1978).
15 P.P. Lamy, Pharm. Int. *7*, 46 (1986).
16 A. Monge, P. Parrado, M. Font, and E. Fernández-Alvarez, J. Med. Chem. *30*, 1029 (1987).
17 A. Monge, J.A. Palop. T. Goñi, and E. Fernández-Alvarez, An. Real Acad. Farm. *54*, 5 (1988); C.A. *111*, 23471d (1989).
18 A. Monge, J.A. Palop, C. Ramirez, and E. Fernández-Alvarez, Acta Farmaceutica Bonaerense *6*, 157 (1987); C.A. *109*, 121991b (1988).
19 A. Monge, J.A. Palop, P. Parrado, C. Pérez-Ilzarbe, and E. Fernández-Alvarez, J. Heterocycl. Chem. *24*, 437 (1987).
20 P. Unangst, J. Heterocycl. Chem. *20*, 495 (1983).
21 M.L. Reeves, B.K. Leigh, and P.J. England, Biochem. J. *241*, 535 (1987).
22 T. Kariya and R.C. Dage, Biochem. Pharmacol. *37*, 3267 (1988).
23 Nomenclature recommendations, Adv. Cyclic Nucleotide Protein Phosphorylation Res. *16*, IV (1984).
24 W.J. Thompson, W.L. Terasaki, P.M. Epstein, and S.J. Strada, Advances in Cyclic Nucleotide Research *10*, 69 (1979).
25 D.C. Cardinal and R.J. Flowers, J. Pharm. Meth. *3*, 135 (1980).
26 R. Gorman, "Advances in Prostaglandin and Thromboxane Research", p. 147, B. Samuelsson, P.W. Kamwell, and R. Paleotti, Eds., Raven Press, New York, 1980.
27 J.A. Salmon, Prostaglandins *15*, 383 (1978).

[Ph951]