New Indole and Pyridazinoindole Analogs – Synthesis and Study as Inhibitors of Phosphodiesterases and as Inhibitors of Blood Platelet Aggregation

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***Key Words**: indole, pyridazinoindole, phosphodiesterases, platelet aggregation*

Summary

This paper presents the synthesis of new indole, pyridazino[4,5-*b*]-indole, and pyridazino[4,5-*a*]indole analogs as well as a study of their "*in vitro*" activity as inhibitors of different phosphodiesterases isolated from dog cardiac tissue, dog aorta, and bovine platelets; the study of their activity as inhibitors of platelet aggregation in guinea pig whole blood, with ADP and arachidonic acid (AA) as pro-aggregants, is also included. The selected compounds 8-benzyloxy-3,4-dihydro-1-(3,4,5-trimethoxy)benzylideneaminopyridazino[4,5-*b*]indole **14g**, and 8-benzyloxy-4-[(3,5-dimethyl)pyrazolyl]pyridazino[4,5-*b*]indole **20** present an interesting profile as potential inodilators, with a complementary, beneficial activity as inhibitors of the aggregation, activities which could possibly be related to the inhibition of the PDE's. Among the other compounds studied, 8-benzyloxy-3,4-dihydro-1-[4-(methyl)piperazino]acetamidopyridazino[4,5-*b*]indol-4-one **16c** and 8-benzyloxy-3,4-dihydro-1-[4-(2-methoxyphenyl)piperazino]acetamidopyridazino[4,5-*b*]indol-4-one **16f** stood out as inhibitors of platelet aggregation, with a mechanism that could possibly be related to the AA cascade.

Introduction

Congestive heart failure (CHF) is an illness which affects millions of persons throughout the world, and has a high death rate in spite of the efforts made in the therapeutic field over these past few years [1].

The traditional treatment of CHF has been based on the use of cardiac glycosides, diuretics, and vasodilators, either separately or in combination. However, the pronounced toxic effects and the narrow therapeutic index of the cardiac glycosides[2] have prompted an extensive search for alternatives to the conventional therapy of this disease, especially for those cases in which conventional long-term treatment is not advisable, having reached a high degree of deterioration or hemodynamic instability, such as in the case of patients with severe CHF requiring a more aggressive therapy, usually intravenous administration of therapeutic agents[3]. All of this led to the design of new cardiotonic agents such as amrinone [4], milrinone [5], enoximone [6], indolidan [7], and saterinone[8] (Figure 1), which in addition, combine the properties of positive inotropes with vasodilator activity in order to achieve maximum improvement in cardiac performance [9,10].

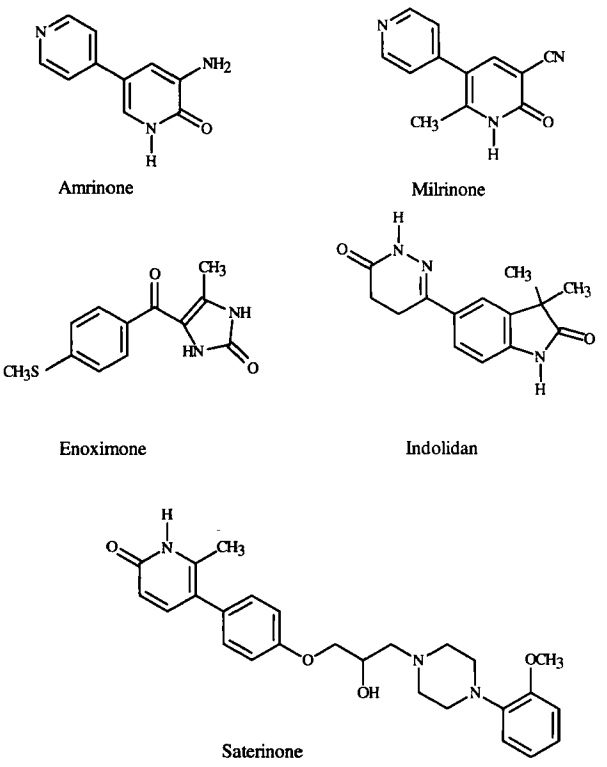

Figure 1

The mechanism of action of these compounds could be attributed, at least partly, to the inhibition of phosphodiestarase isoenzymes which are specific for cyclic adenosine-3′,5′-monophospate (cAMP) [11,12], and consequently to an increase in intracellular cAMP, varying the effectiveness of these compounds considerably for said increase [13].

These PDE inhibitors, which initially appeared to hold great therapeutic promise, have scarcely shown clinical benefits in controlled studies. This has interrupted the clinical development of some of them, as in the case of indolidan [14]; others, such as milrinone, are surrounded with controversy concerning their efficiency and even the safety in their use in patients

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with moderate-to-severe CHF [15]. All of this has nurtured the belief that inotropic therapy for heart failure is still an unfulfilled promise [16], and that this line of therapy should consequently be reexamined. This idea leads to the design of compounds which are selective in their inhibition of different PDE isoenzymes and present an additional biological profile that increases their efficiency in the pathologies associated with CHF, which, in turn, could retard or even reverse the progression of the disease, prolonging the life of CHF patients. This would be the case for compounds which also exhibit antiaggregatory activity.

With this objective in mind and as a continuation of our previous work [17–19], we now present the synthesis and preliminary "*in vitro*" evaluation of new derivatives of indole (**I**), pyridazino[4,5-*b*]indole (**II**), pyridazino[4,5-*b*]indol-4-one (**III**), and pyridazino[4,5-*a*]indole (**IV**) (Figure 2) as inhibitors of different PDE's and as inhibitors of platelet aggregation. The design of the new compounds presented in this report has been carried out using the biological data found for the pyridazino[4,5-*b*]indole derivatives, differently substituted in positions 1 and 4, previously described by our research team[17–19]. The structural modifications proposed may be briefly summarised as follows:

1. Introduction of substituents in position 5 of the indole ring. The benzyloxy group is chosen as substituent because it can be hydrolysed to yield the hydroxy group if the biological data indicate this to be appropriate.

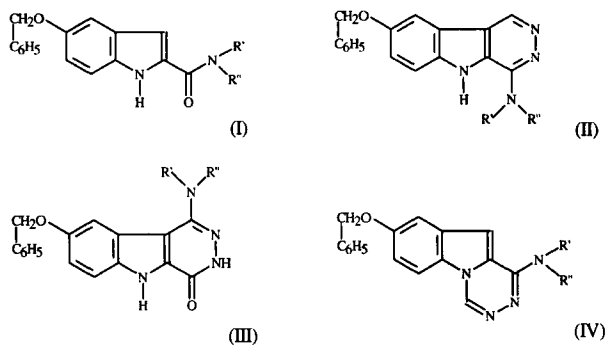
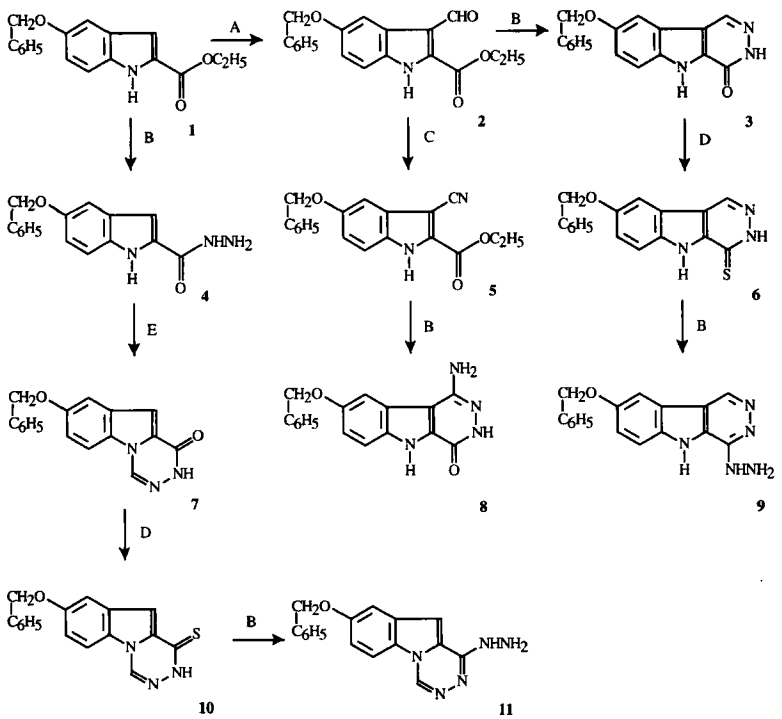


Figure 2

2. Relative disposition of the indole and pyridazine rings: [4,5-*a*] and [4,5-*b*] fusion are selected, and synthesis of parallel series of pyridazino[4,5-*a*]indole and pyridazino[4,5-*b*]indole derivatives are proposed.
3. Elimination of the pyridazine ring in those compounds of the preceding series that turned out to be more active, synthesizing a parallel series of indole derivatives.
4. Substituents in positions 1 and 4 of the pyridazinoindole ring were chosen on the basis of our previous work [18,19].



Scheme 1: A, $POCl_3$/DMF; B, $NH_2NH_2.H_2O$; C, $CH_3CH_2NO_2$/acetic acid/sodium acetate
D, S_5P_2/Pyridine; E, Ethyl orthoformate

The synthesis of two different series of compounds is proposed: one in which position 1 remains free and substituents such as pyrazole or aminopyrrole appear in position 4 and the other in which position 4 retains an oxy group, resulting in an amide residue which is frequently found in the products exhibiting the desired activity (Figure 1), while an amine group (subsequently modified to form imine and acetamide type bonds) is introduced in position 1. The biological activity data found in series of indole and triazino[5,4-*b*]indol-4-one derivatives described by our research team [20] were taken into account selecting this type of bonds, especially the imines. The presence of this type of substituents seems to increase the antiaggregatory activity.

Chemistry

The compounds were synthesized according to Schemes 1–3.

Ethyl 5-benzyloxyindole-2-carboxylate **1** [21] is treated with DMF and $POCl_3$, according to Vilsmeier's reaction, affording **2** [21] in high yield. Reaction of **2** with boiling 90 % hydrazine hydrate gives 8-benzyloxy-3,4-dihydropyridazino[4,5-*b*]indol-4-one **3** [21]. With pyridine as the solvent, **3** reacts with excess S_5P_2, under reflux, thereby yielding the corresponding thioxo derivative **6** [21]. On treatment of this compound (**6**) with 90 % hydrazine hydrate under reflux, 1-hydrazino-8-benzyloxypyridazino[4,5-*b*]indole **9** is obtained in good yield [21].

On starting with **1**, reaction with 90 % hydrazine hydrate as the solvent and reagent gives **4** in a good quantitative yield. **4** reacts with ethyl orthoformate to give 7-benzyloxy-1,2-dihydropyridazino[4,5-*a*]indol-1-one **7**. Parallel to the previous series, the oxygen in position 1 is exchanged for S, using S_5P_2 with pyridine as the solvent and under reflux. This affords **10**, which, when treated with 90 % hydrazine hydrate under reflux, leads to 1-hydrazino-7-benzyloxypyridazino[4,5-*a*]indole **11**.

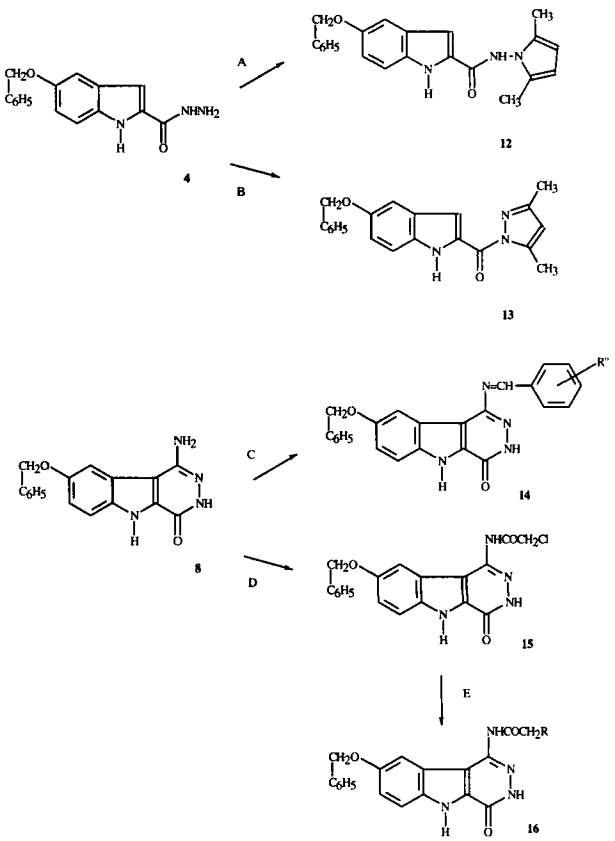
Treatment of **2** with nitroethane in an acetic acid/sodium acetate solution produces derivative **5**, which bears a cyano group in position 3. Reaction of this compound with 90% hydrazine hydrate leads to 1-amino-8-benzyloxy-3,4-dihydropyridazino[4,5-*b*]indol-4-one **8**.

Starting with the carbonylhydrazine **4**, and by reaction with acetonyl acetone, under reflux in an acid medium, aminopyrrole **12** is obtained; pyrazole **13** is also obtained from **4** (Scheme 2) but now by reaction with acetoacetone, under reflux.

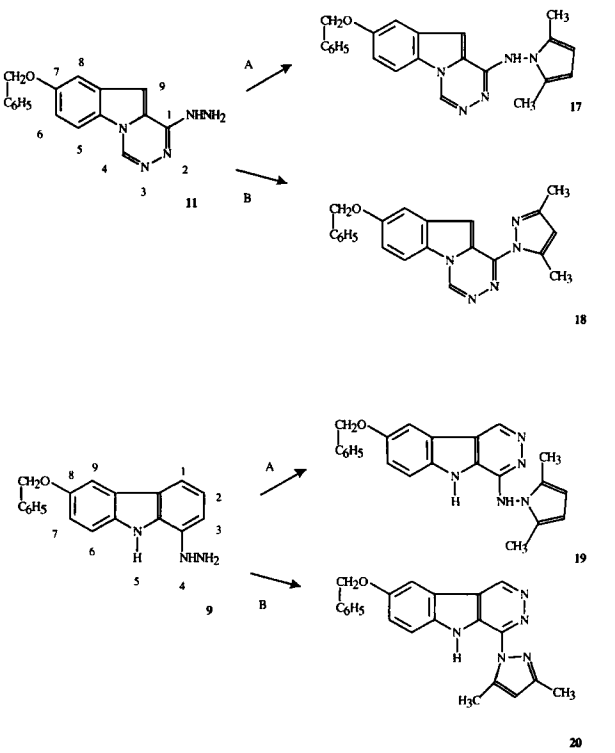
Reaction of compound **8** with different aldehydes and in the absence of a solvent furnishes the series of imines **14**, in low yields, except in the case of analog **14b** (R″ = 4′-OH).

Treating **8** with chloroacetyl chloride under reflux leads to amides **15**; the reaction of this compound with different amines leads to series **16**.

Reaction of 1-hydrazino-7-benzyloxypyridazino[4,5-*a*]indole, **11**, with acetonylacetone under reflux leads to the aminopyrrole **17** (Scheme 3). Reaction of **11** with acetylacetone leads to pyrazole **18**. Compounds **19** and **20** are obtained similarly, by reaction of **9** with acetonylacetone and acetoacetone respectively. These reactions give high yields except in the case of aminopyrrole **17**, where the appearance of a noncyclic secondary product is observed.



Scheme 2: A, $CH_3COCH_2CH_2COCH_3$; B, $CH_3COCH_2COCH_3$; C, Aldehydes D, $ClCH_2COCl$; E, Amines



Scheme 3 : A. $CH_3COCH_2CH_2COCH_3$; B. $CH_3COCH_2COCH_3$

Biology: Results and Discussion

The goal of our work is to obtain compounds which possess activity as inodilators, through the selective inhibition of cardiac and aorta phosphodiesterases. In parallel, it is of interest that these compounds possess a complementary effect that is beneficial from a hemodynamic point of view, as in the case of compounds with significant antiaggregatory activity that may or may not be related to the PDE inhibition of platelet origin.

With the aim of evaluating the biological activity of the new compounds, the following screening scheme has been carried out:

1. Inhibition of CGI-PDE isolated from dog heart, affording information concerning potential activity as inotropes.
2. Inhibition of other cardiac isoenzymes, with the object of evaluating the possible selectivity of the most active compounds.
3. Inhibition of PDE's isolated from dog aorta, as an indicator of their potential vasodilator character and of PDE's isolated from bovine platelets, as an indicator of their possible antiaggregatory activity related to PDE inhibition.
4. Inhibition of platelet aggregation induced by ADP and/or arachidonic acid (AA) in guinea pig whole blood, in order to obtain information about the potential antiaggregatory character related to AA cascade.

Table 1 shows the data obtained for the aminopyrrole or pyrazole derivatives, in the inhibition of cardiac CGI-PDE. On comparing the activity of the compounds derived from indole (**12** and **13**) with that of the pyridazinoindole derivatives, it could be deduced that the loss of the pyridazine ring fused with the indole ring provokes a decrease in the inhibitory activity on the CGI-PDE. Thus, whereas **13** proved to be inactive, its analog derived from pyridazino[4,5-*b*]indole **20** turned out to be the most active. Also, within this series, it can be observed that the relative disposition of the indole and pyridazine rings is important because while the pyridazino-[4,5-*a*]indole derivatives are found to be inactive (compounds **17** and **18**), the pyridazino[4,5-*b*]indole derivatives have significative activity. This is especially true of **20**, which in this assay turns out to be approximately ten times more active than the Amrinone used as reference: IC_{50} for **20** is 4.3 μM, whereas we found Amrinone to have an IC_{50} of 47.0 μM.

Therefore, an increase in the total surface of the molecule on going from an indole ring to a pyridazinoindole ring has beneficial results, especially when these rings fuse in a [4,5-*b*] manner.

In the series of pyridazino[4,5-*b*]indol-4-one derivatives **14** (Table 2), the introduction of an imine group in position 1 does not appear to increase the activity of the compounds as inhibitors of CGI-PDE ompared to that shown by derivative **8**, with a NH_2 group in position **1** and an IC_{50}= 4.3 μM. Within this series **14**, a definite substituent influence is observed; a greater activity is observed for the strong electron donor groups, as in the case of **14b** with an OH group in position 4′, with an IC_{50}= 29.2 μM, and especially in the case of **14g**, with three methoxy substituents and an IC_{50}= 5.5 μM. As in the previous case, all these compounds are significatively more active than Amrinone.

With regard to series **16** (Table 2), no improvement is observed in the activity when different amines are introduced on the acetamide radical of position 1 of the pyridazino [4,5-*b*]indol-4-one ring. Only **16c**, derived from methylpiperazine, has a significative activity with an IC_{50}= 63.7 μM, inferior to that of Amrinone.

On the basis of these results, the derivatives **14g** and **20** were selected for the subsequent PDE inhibition assays (Table 3). With regard to the cardiac isoenzymes, both compounds are inactive for PDE-I, especially **14g,** which, like Amrinone, slightly stimulates the enzymatic activity.

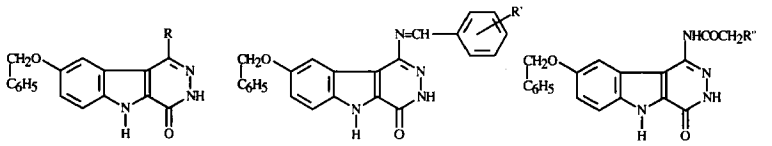
The results obtained for the isoenzymes isolated from dog aorta permit us to expect a vasodilator activity for these compounds; the superior inhibition shown for PDE-V with regard to PDE-I is striking. Just as in the case of cardiac isoenzymes, both compounds are significantly more active towards CGI-PDE. With regard to the isoenzymes of platelet

Table 1. Physical properties and dog heart CGI-PDE inhibitory activity for pyrazolyl and aminopyrrolyl derivatives[a].

No.	yield[b] (%)	mp, °C (recr.solv.)[c]	% Inh. PDE[d]	IC_{50} (μM)[e]	formula[f]
12	75	255–256 (A)	74.9 ± 1.1	21.5	$C_{22}H_{21}N_3O_2$
13	70	201–202 (B)	I[g]		$C_{21}H_{19}N_3O_2$
17	20	196–198 (A)	39.3 ± 2.4		$C_{23}H_{21}N_5O$
18	72	261–262 (C)	I		$C_{22}H_{19}N_5O$
19	71	132–233 (D)	60.4 ± 2.9	40.2	$C_{23}H_{21}N_5O$
20	65	196–198 (E)	72.8 ± 1.6	4.3	$C_{22}H_{19}N_5O$
Amrinone				47.0	

[a] See Scheme 2 for structures. [b] Value of the final transformation is expressed. [c] Recrystallization solvent: A, EtOH; B, 2-propanol; C, dioxane/DMF; D, EtOH/DMF; E, dioxane. [d] % inhibition at 100 μM. [e] Concentration-activity curves are carried out with four or more concentrations of test compounds; IC_{50} values are calculated from log curve. [f] All compounds are analyzed for C,H,N and results agreed to ± 0.4% of theoretical values. [g] I= inactive, % Inhibition ≤ 30 %.

Table 2. Physical properties and dog heart CGI-PDE inhibitory activity for 8-benzyloxy-3,4-dihydropyridazino[4,5-*b*]indol-4-one derivatives.



8 **14** **15, 16**

No.	R	R′	R″	yield[a] (%)	mp, °C (recr.solv.)[b]	% Inh. PDE[c]	IC_{50}[d] (μM)	formula[e]
8	NH_2	—	—	49	> 270 (A)	67.8 ± 6.4	4.3	$C_{17}H_{14}N_4O_2$
14a	—	4′-NO_2	—	27	> 300 (A)	I[f]		$C_{24}H_{17}N_5O_4$
14b	—	4′-OH	—	62	> 250 (A)	59.5 ± 0.4	29.2	$C_{24}H_{18}N_4O_3$
14c	—	4′-$COOCH_3$	—	36	> 250 (A)	I		$C_{26}H_{20}N_4O_4$
14d	—	4′-Cl	—	36	284–286 (B)	I		$C_{24}H_{17}ClN_4O_2$[g]
14e	—	4′-C_6H_5	—	22	> 300 (B)	I		$C_{30}H_{22}N_4O_2$[g]
14f	—	4′-CN	—	25	> 300 (B)	I		$C_{25}H_{17}N_5O_2$
14g	—	3′,4′,5′-OCH_3	—	25	> 250 (A)	62.8 ± 5.3	5.5	$C_{27}H_{24}N_4O_5$
15	—	—	Cl	30	> 250 (C)	I		$C_{19}H_{15}ClN_4O_3$
16a	—	—	morpholine	56	> 300 (B)	I		$C_{23}H_{23}N_5O_4$
16b	—	—	piperidine	83	> 300 (B)	I		$C_{24}H_{25}N_5O_3$
16c	—	—	methylpiperazine	73	> 300 (B)	60.3 ± 0.7	63.7	$C_{24}H_{26}N_6O_3$
16d	—	—	(4′-phenyl)piperidine	42	> 300 (B)	n.d.[h]		$C_{30}H_{28}N_5O_3$
16e	—	—	(4′-Cl)phenylpiperazine	36	> 300[i]	I		$C_{29}H_{27}ClN_6O_3$
16f	—	—	(2′-methoxy)phenylpiperazine	51	> 300 (B)	I		$C_{30}H_{30}N_6O_4$
16g	—	—	(3′-methoxy)phenylpiperazine	46	> 300 (B)	I		$C_{30}H_{30}N_6O_4$
16h	—	—	(2-pyridyl)piperazine	38	> 300 (B)	I		$C_{28}H_{27}N_7O_3$
Amrinone	—	—				47.0		

[a] Value of the final transformation is expressed. [b] Recrystallization solvent: A, DMF; B, EtOH/DMF; C, dioxane. [c] % inhibition at 100 μM. [d] Concentration-activity curves are carried out with four or more concentrations of test compounds; IC_{50} values are calculated from log curve. [e] All compounds are analyzed for C,H,N and results agreed to ± 0.4% of theoretical values. [f] I= inactive, % Inhibition ≤ 30. [g] Confirmed by MS-DIP [h] n.d.= no data, nonsoluble compounds. [i] Purified by flash column chromatography, CH_2Cl_2/MeOH 7.5:2.5, as mobile phase

Table 3. Inhibition rate of different PDE isoenzymes[a].

Ref. comp.	% Inhibition of PDE isolated from: Dog Heart			Dog Aorta			Bovine PRP[b]	
	PDE-I[c]	PDE-II[d]	PDE-IV[e]	PDE-I[f]	PDE-V[g]	CGI-PDE[e]	PDE-V[g]	CGI-PDE[e]
14g	—[h]	33.8 ± 2.6	44.8 ± 2.5	32.9 ± 4.2	50.2 ± 2.7	52.3 ± 3.1	15.8 ± 4.8	55.0 ± 1.2
20	26.2 ± 4.8	53.9 ± 1.2	79.7 ± 2.6	41.5 ± 4.7	62.9 ± 2.5	76.6 ± 1.2	100	69.9 ± 1.6
Amrinone	—[h]	3.1 ± 5.5	47.5 ± 3.4	16.0 ± 4.9	33.9 ± 4.2	66.7 ± 4.0	25.0 ± 4.1	59.0 ± 1.1

[a] Comparative data at 100 μM (See experimental section for details). [b] Platelet-Rich Plasma. [c] PDE-I: Ca^{2+}/CaM-PDE, cAMP 1 μM. [d] PDE-II: CGS-PDE, cAMP 25 μM. [e] PDE-IV: cAMP-PDE, cAMP 1 μM. [f] cAMP 1 μM. [g] cGMP 1 μM. [h] slightly stimulates the enzymatic activity.

Table 4. Effect on platelet aggregation (guinea pig whole blood) for pyrazolyl and aminopyrrolyl derivatives.

Ref. comp.	Final conc. (M)	%Inhb. of platelet aggregation induced by[a]: ADP[b]	AA[c]
12	10^{-4}[d]	52.00 ± 5.1	53.67 ± 8.1
	5×10^{-5}	I[e]	I
13	5×10^{-4}	I	I
17	5×10^{-4}	33.00 ± 14.8	35.14 ± 14.3
18	2.5×10^{-4}	51.50 ± 10.6	58.63 ± 11.4
	10^{-4}	I	I
19	5×10^{-4}	60.00 ± 9.54	93.33 ± 7.2
	2.5×10^{-4}	I	74.62 ± 17.6
	10^{-4}	–	58.45 ± 9.6
	5×10^{-5}	–	I
20	5×10^{-4}	41.75 ± 11.92	95.67 ± 6.9
	2.5×10^{-4}	I	80.45 ± 6.7
	10^{-4}	–	56.4 ± 10.1
	5×10^{-5}	–	I
ASA	5×10^{-3}	100	100
	5×10^{-4}	15.00 ± 12.7	30.0 ± 10.5

[a] $\bar{X}$ ± SEM; $p \leq 0.05$ (n = 5–8). [b] 2.3×10^{-5} M. [c] 5×10^{-4} M. [d] Not soluble at higher concentrations. [e] I = inactive, % Inhibition ≤ 30.

Table 5. Effect on platelet aggregation (Guinea Pig whole blood) for pyridazino[4,5-*b*]indol-4-one derivatives.

Ref. comp.	Final conc. (M)	% Inhb. of platelet aggregation induced by[a]: ADP[b]	AA[c]
8	5×10^{-4}	81.50 ± 5.96	76.75 ± 17.23
	2.5×10^{-4}	I[d]	32.86 ± 11.23
	10^{-4}	–	I
14a	5×10^{-4}	56.40 ± 7.80	46.67 ± 5.28
	2.5×10^{-4}	I	I
14b	5×10^{-4}	91.67 ± 1.97	98.00 ± 2.28
	2.5×10^{-4}	88.33 ± 4.68	83.71 ± 6.45
	10^{-4}	48.71 ± 8.98	51.20 ± 4.71
	5×10^{-5}	I	I
14c	5×10^{-4}	90.33 ± 2.34	98.83 ± 1.83
	2.5×10^{-4}	74.00 ± 7.80	74.14 ± 8.19
	10^{-4}	33.43 ± 5.94	40.43 ± 5.74
	5×10^{-5}	I	I
14d	5×10^{-4}	94.25 ± 3.28	96.33 ± 5.32
	2.5×10^{-4}	70.83 ± 5.98	73.67 ± 3.39
	10^{-4}	42.71 ± 13.19	40.75 ± 12.10
	5×10^{-5}	I	I

Table 5. *Continued.*

Ref. comp.	Final conc. (M)	% Inhb. of platelet aggregation induced by[a]: ADP[b]	AA[c]
14e	5×10^{-4}	64.25 ± 7.63	63.00 ± 5.76
	2.5×10^{-4}	I	I
14f	5×10^{-4}	89.50 ± 3.02	96.17 ± 5.95
	2.5×10^{-4}	75.20 10.62	79.50 ± 5.47
	10^{-4}	I	I
14g	5×10^{-4}	83.00 ± 6.48	94.50 ± 4.89
	2.5×10^{-4}	68.67 ± 11.15	59.50 ± 5.17
	10^{-4}	32.80 ± 7.40	36.80 ± 8.93
	5×10^{-5}	I	I
15	5×10^{-4}	65.33 ± 7.23	95.20 ± 6.72
	2.5×10^{-4}	48.67 ± 6.44	65.00 ± 5.52
	10^{-4}	I	I
16a	5×10^{-4}	55.50 ± 8.60	85.43 ± 7.21
	2.5×10^{-4}	34.83 ± 6.43	37.80 ± 11.24
	10^{-4}	I	I
16b	5×10^{-4}	77.57 ± 6.45	91.88 ± 6.73
	2.5×10^{-4}	65.33 ± 5.82	71.00 ± 10.71
	10^{-4}	I	I
16c	5×10^{-4}	91.00 ± 6.16	89.50 ± 4.93
	2.5×10^{-4}	87.50 ± 4.18	92.33 ± 5.43
	10^{-4}	86.25 ± 8.34	95.00 ± 6.16
	5×10^{-5}	I	89.80 ± 6.30
	2.5×10^{-5}	—	69.70 ± 7.10
	10^{-6}	—	I
16d	5×10^{-4}	I	I
16e	5×10^{-4}	86.17 ± 1.33	88.75 ± 12.82
	2.5×10^{-4}	50.50 ± 6.89	82.50 ± 20.22
	10^{-4}	I	63.83 ± 13.50
	5×10^{-5}	—	I
16f	5×10^{-4}	81.40 ± 6.69	94.40 ± 5.95
	2.5×10^{-4}	85.20 ± 2.59	98.00 ± 1.83
	10^{-4}	I	86.80 ± 2.28
	5×10^{-5}	—	74.40 ± 7.60
	2.5×10^{-5}	—	68.40 ± 6.70
	5×10^{-6}	—	36.15 ± 12.00
	2.5×10^{-6}	—	I
16g	5×10^{-4}	86.25 ± 6.69	90.50 ± 5.83
	2.5×10^{-4}	87.00 ± 1.26	89.00 ± 5.97
	10^{-4}	50.50 ± 18.50	82.67 ± 9.83
	5×10^{-5}	I	70.25 ± 11.02
	2.5×10^{-5}	—	I
16h	2.5×10^{-4}[e]	I	94.25 ± 5.56
	10^{-4}	—	96.63 ± 3.66
	5×10^{-5}	—	I
ASA	5×10^{-3}	100	100
	5×10^{-4}	15.00 ± 12.75	30.00 ± 10.50

[a] $\bar{X}$ ± SEM; $p \leq 0.05$ (n = 5–8). [b] 2.3×10^{-5} M. [c] 5×10^{-4} M. [d] I = inactive, % Inhibition ≤ 30. [e] Not soluble at higher concentrations.

origin, **14g** is practically inactive for PDE-V, whereas **20** is active for both isoenzymes.

With regard to the antiaggregatory activity in whole blood, all the compounds have been assayed against both ADP and AA (Tables 4, 5). Table 4 shows the activities obtained for the aminopyrrole and pyrazole derivatives. In this case, it can also be observed that the pyridazinoindole derivatives are significantly more active than the indole derivatives and that the most effective relative fusion between the indole and pyridazine is the [4,5-*b*] (compounds **19** and **20**). A greater activity is observed in both compounds when AA is used as the pro-aggregatory agent; in any case, under these assay conditions, these compounds show a greater activity than ASA, which was used as the control.

For the pyridazino[4,5-*b*]indol-4-one (Table 5) derivatives, a parallel behavior is observed against ADP and AA. In this case, the introduction of imine groups in position 1 of the ring provokes an improvement in the activity, which coincides with the preceding observations made by our research team [20], previously cited. The derivative **14g**, selected in the previous assay, shows a medium antiaggregatory character towards both ADP and AA.

Derivatives **16** (Table 5) turn out to be the most active in this assay, especially when the aggregation is induced with AA; noteworthy compounds are those which present groups derived from piperazine in position 1 (compounds **16c, 16e, 16f, 16g**), of which compounds **16c** and **16f**, at a concentration of 25 μM, still maintain an activity close to 70 %.

The data obtained in the different assays leads us to select derivatives **14g** and **20** for studies which would confirm the "*in vitro*" activities shown as inodilators and inhibitors of platelet aggregation. In the same way, compounds **16c** and **16f** were selected as antiaggregants for a subsequent, thorough study, in order to determine their ability to interact with the cascade of AA.

Experimental Part

Chemistry

Mps: Mettler FP82 hot stage apparatus with FP800/FP80 processor, Olympus 8091 microscope and video system; uncorrected.– IR spectra: Perkin-Elmer FT-681 spectrometer, KBr.– ^{1}H-NMR spectra: Bruker-AC 200E spectrometer, 200 MHz, $SiMe_4$ as an internal standard.– Mass spectra: Hewlett Packard HP-5988A, GC-HPLC-DIP instrument, at 70 eV.– Elemental Analyses: Carlo Erba Elemental Analyzer; vacuum dried samples (over P_2O_5 at 1–2 mm Hg, 24 h at 60 – 80 °C).

Compounds **1–4**, **6**, and **9** are prepared as described [21].

*Ethyl 5-benzyloxy-3-cyanoindol-2-carboxylate (**5**)*

A mixture of anhydrous sodium acetate (2.96 g, 36 mmol), acetic acid (8 mL), nitroethane (3.4 mL), and **2** (2.92 g, 9.2 mmol) is boiled for 15 h. The mixture is then cooled. The solid obtained is isolated by filtration, washed with abundant hot water, dried, and purified. Yield: 1.44 g, 49 %; as light brown needles; mp 200–201 °C (dioxane). Table 6.

*7-Benzyloxy-1,2-dihydropyridazino[4,5-a]indol-1-one (**7**)*

A mixture of **4** (1.0 g, 3.6 mmol), ethyl orthoformate (0.6 mL), and DMF (10 mL) is refluxed for 5 h. The solvents are removed under reduced pressure until approximately 1 mL of the mixture remains. The mixture is then set aside for 12 h. The solid that appears is filtered, dried, and recrystallized. Yield: 0.64 g, 62 %; as yellow solid. mp > 230 °C (dec.) (dioxane). Table 6.

*1-Amino-8-benzyloxy-3,4-dihydropyridazino[4,5-b]indol-4-one (**8**)*

A mixture of **5** (2.0 g, 6.25 mmol) and 90 % hydrazine hydrate (15 mL) is refluxed for 13 h. The solid obtained is isolated by filtration, washed first with abundant cold water and then with hot water, dried, and recrystallized. Tables 2 and 6.

*7-Benzyloxy-1,2-dihydropyridazino[4,5-a]indol-1-thione (**10**)*

A mixture of **7** (10 g, 3.40 mmol), a slight excess of S_5P_2, and anhydrous pyridine (15 mL) is boiled for 4h. The solvent is eliminated under reduced pressure and NH_4OH 25% (25 mL) is poured over the residue. The solid obtained is filtered and washed with abundant water, dried, and purified. Yield: 0.73 g, 70 %; as brown solid; mp > 230 °C (dec.) (dioxane) Table 6.

*7-Benzyloxy-1-hydrazinopyridazino[4,5-a]indole (**11**)*

A mixture of **10** (10 g, 32.5 mmol) and 90 % hydrazine hydrate (150 mL) is boiled for 9 h. Upon cooling, a solid appears. The solid is isolated by filtration and washed with abundant water, first with cold and later with hot. The solid is dried and recrystallized. Yield: 6.44 g, 65 %; as yellow solid; mp > 250 °C (dioxane). Table 6.

*5-Benzyloxy-2-[N-(2,5-dimethylpyrrol-1-yl)]carbamoylindole (**12**)*

A mixture of **4** (1.0 g, 3.6 mmol) and 2,5-hexanedione (5 mL) is refluxed for 6 h. This mixture is poured over crushed ice (50 g). The mixture is set aside for 12 h. The solid obtained is isolated, washed with abundant water, dried, and recrystallized. Black solid. Tables 1 and 6.

*5-Benzyloxy-2-(3,5-dimethylpyrazol-1-yl)carbamoylindole (**13**)*

A mixture of **4** (1.0 g, 3.6 mmol) and acetylacetone (10 mL) is refluxed for 25 h. The excess reagent is eliminated under reduced pressure. Ethyl ether (10 mL) is added to the residue. The mixture is boiled and then set aside at room temperature for 12 h. The solid obtained is isolated by filtration, dried, and recrystallized. White solid. Tables 1 and 6.

*8-Benzyloxy-1-iminopyridazino[4,5-b]indol-4-one derivatives **14**. General method*

A mixture of **8** (0.4 g, 1.3 mmol) and the corresponding aldehyde (3.0 mmol) is heated in a sand bath at the fusion temperature for 4 to 8 h. Upon cooling, the residue is treated with ethanol (25 mL) and the solid obtained is filtered, dried, and recrystallized. In this way, the following compounds are obtained (Tables 2 and 6):

*8-Benzyloxy-3,4-dihydro-1-(4-nitro)benzylidenaminopyridazino[4,5-b]indol-4-one (**14a**)*

From **8** and 4-nitrobenzaldehyde; as yellow solid.

*8-Benzyloxy-3,4-dihydro-1-(4-hydroxy)benzylidenaminopyridazino[4,5-b]indol-4-one (**14b**)*

From **8** and 4-hydroxybenzaldehyde; as yellow solid.

*8-Benzyloxy-3,4-dihydro-1-(4-methoxy)benzylidenaminopyridazino[4,5-b]indol-4-one (**14c**)*

From **8** and methyl 4-formylbenzoate; as yellow solid.

*8-Benzyloxy-1-(4-chloro)benzylidenamino-3,4-dihydropyridazino[4,5-b]indol-4-one (**14d**)*

From **8** and 4-chlorobenzaldehyde; as yellow solid.

*8-Benzyloxy-3,4-dihydro-1-(4-phenyl)benzylidenaminopyridazino[4,5-b]indol-4-one (**14e**)*

From **8** and methyl 4-biphenylaldehyde; as yellow solid.

Table 6. Spectroscopic data (IR, MS-DIP, and ^{1}H NMR) of the compounds.

No.	IR (KBr) cm^{-1} ν =	^{1}H NMR ([D_6]-DMSO)[a] δ =
5	3200 (NH), 2235 (CN), 1715 (C=O)	1.34 (t, 3H, CH_3), 4.40 (q, 2H, CH_2), 5.18 (s, 2H, CH_2O), 7.12 (d, *J* = 8.0, 1H, 7-H), 7.36–7.51 (m, 6H, aromatic H), 7.81 (s, 1H, 4-H), 13.00 (s, 1H, NH[b])
7[c]	3100, 3000 (NH), 1660 (C=O)	5.22 (s, 2H, CH_2O), 7.26–7.52 (m, 8H, aromatic H), 8.14 (s, 1H, 9-H), 9.10 (s, 1H, 4-H), 11.96 (s, 1H, NH[b])
8	3451 (NH), 1651 (C=O)	5.19 (s, 2H, CH_2O), 5.80 (s, 2H, NH_2[b]), 7.16 (d, *J* = 8.0, 1H, 7-H), 7.37–7.51 (m, 6H, aromatic H), 7.90 (s, 1H, 9-H), 11.65 (s, 1H, NH[b]), 11.92 (s, 1H, NH[b])
10	3150 (NH), 1540, 1200 (S=C–N)	5.16 (s, 2H, CH_2O), 7.10–7.50 (m, 8H, aromatic H), 8.12 (s, 1H, 9-H), 9.41 (s, 1H, H-4), 13.48 (s, 1H, NH[b])
11[d]	3270 (NH), 1519 (C–N)	5.11 (s, 2H, CH_2O), 6.40 (s, 2H, NH_2[b]), 6.90 (d, *J* = 8.1, 2H, aromatic H), 7.20 (s, 1H, aromatic H), 7.30–7.50 (m, 6H, aromatic H), 8.31 (s, 1H, 9-H), 8.49 (s, 1H, 4-H), 11.82 (s, 1H, NH[b])
12[e]	3390, 3250 (NH), 1655 (C=O)	2.06 (s, 6H, CH_3), 5.12 (s, 2H, CH_2O), 5.73 (s, 2H, CH pyrrole), 6.98 (d, *J* = 8.0, 2H, aromatic H), 7.24 (s, 1H, aromatic H), 7.33–7.51 (m, 6H, aromatic H), 11.26 (s, 1H, NH[b]), 11.76 (s, 1H, NH[b])
13[f]	3390 (NH), 1670 (C=O)	2.31 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 5.11 (s, 2H, CH_2O), 6.27 (s, 1H, CH pyrazol), 7.06 (d, *J* = 8.0, 2H, aromatic H), 7.32–7.51 (m, 5H, aromatic H), 7.73 (s, 1H, aromatic H), 8.33 (s, 1H, aromatic H), 11.83 (s, 1H, NH[b])
14a	3265 (NH), 1650 (C=O), 1519 (NO_2), 1341 (NO_2), 815 (1,4-disubst.)	5.27 (s, 2H, CH_2O), 7.29–7.66 (m, 8H, aromatic H), 8.42 (dd, 4H, 2′-H, 3′-H, 5′-H, 6′-H), 8.20 (s, 1H, CH=N), 12.71 (s, 1H, NH[b]), 12.79 (s, 1H, NH[b]).
14b	3290 (NH), 1640 (C=O), 1250 (C–O) 830 (1,4-disubst.)	5.21 (s, 2H, CH_2O), 7.00 (d, *J* = 8.0, 2H, 3′-H, 5′-H), 7.27–7.65 (m, 8H, aromatic H), 7.95 (d, *J* = 8.0, 2H, 2′-H, 6′-H), 8.87 (s, 1H, CH=N), 10.35 (s, 1H, OH[b]), 12.48 (s, 1H, NH[b]), 12.87 (s, 1H, NH[b])
14c	3280 (NH), 1640 (C=O), 1278 (C–O), 816 (1,4-disubst.)	3.88 (s, 3H, CH_3), 5.19 (s, 2H, CH_2O), 7.23–7.64 (m, 8H, aromatic H); 8.18 (dd, 4H, 2′-H, 3′-H, 5′-H, 6′-H), 9.08 (s, 1H, CH=N), 12.57 (s, 1H, NH[b]), 12.78 (s, 1H, NH[b])
14d[g]	3279–3065 (NH), 1648 (C=O), 816 (1,4-disubst.)	5.22 (s, 2H, CH_2O), 7.29–7.66 (m, 8H, aromatic H), 7.72 (d, *J* = 8.0, 2H, 2′-H, 6′-H), 8.17 (d, *J* = 8.0, 2H, 3′-H, 5′-H), 9.05 (s, 1H, CH=N), 12.63 (s, 1H, NH[b]), 12.78 (s, 1H, NH[b])
14e[h]	3296–3071 (NH), 1642 (C=O), 817 (1,4-disubst.)	5.23 (s, 2H, CH_2O), 7.28 (s, 5H, aromatic H), 7.44–7.58 (m, 5H, aromatic H), 7.71 (s, 1H, aromatic H), 7.82 (d, *J* = 8.0, 2H, aromatic H), 7.95 (d, *J* = 8.0, 2H, 3′-H, 5′-H), 8.22 (d, 2H, *J* = 8.0, 2′-H, 6′-H), 9.08 (s, 1H, CH=N), 12.61 (s, 1H, NH[b]), 12.74 (s, 1H, NH[b])
14f	3282–3070 (NH), 2226 (CN), 1657 (C=0), 817 (1,4-disubst.)	5.12 (s, 2H, CH_2O), 7.27–7.62 (m, 8H, aromatic H), 8.10 (d, *J* = 8.0, 2H, 3′-H, 5′-H), 8.29 (d, *J* = 8.0, 2H, 2′-H, 6′-H), 9.12 (s, 1H, CH=N), 12.68 (s, 1H, NH[b]), 12.77 (s, 1H, NH[b])
14g	3280 (NH), 1700 (C=0), 1130 (C–O), 700 (1,2,3,4-tetrasubst.)	3.76 (s, 3H, CH_3O in 4′), 3.88 (s, 6H, CH_3O in 3′ and 5′), 5.17 (s, 2H, CH_2O), 7.31–7.54 (m, 8H, aromatic H), 7.84 (s, 1H, aromatic H), 7.95 (s, 1H, aromatic H), 9.00 (s, 1H, CH=N), 12.59 (s, 1H, NH[b]), 12.70 (s, 1H, NH[b])
15	3406, 3245 (NH), 1665 (C=0)	4.46 (s, 2H, CH_2Cl), 5.13 (s, 2H, CH_2O), 7.22–7.57 (m, 8H, aromatic H), 10.80 (s, 1H, NH[b]), 12.70 (s, 1H, NH[b]), 12.81 (s, 1H, NH[b]).
16a	3195 (NH), 1638 (C=0)	2.57 (br.s, 4H, CH_2), 3.25 (s, 2H, CH_2), 3.60 (br.s, 4H, CH_2), 5.12 (s, 2H, CH_2O), 7.23–7.56 (m, 8H, aromatic H), 10.19 (s, 1H, NH[b]), 12.61 (br.s, 2H, NH[b])
16b	3197, 2940, 2815 (NH), 1636 (C=O)	1.60 (br.s, 4H, CH_2), 2.58 (br.s, 6H, CH_2), 3.25 (s, 2H, CH_2), 5.18 (s, 2H, CH_2O), 7.35–7.63 (m, 8H, aromatic H), 10.14 (s, 1H, NH[b]), 12.66 (br.s, 2H, NH[b])
16c	3206 (NH), 1648 (C=0)	2.50 (s, 3H, CH_3), 2.79 (br.s, 8H, CH_2), 3.65 (s, 2H, CH_2), 5.54 (s, 2H, CH_2O), 7.65–7.98 (m, 8H, aromatic H), 10.49 (s, 1H, NH[b]), 12.99 (s, 1H, NH[b]), 13.11 (s, 1H, NH[b])
16d	3194–2935 (NH), 1639 (C=0)	1.71 (s, 4H, CH_2), 2.35 (m, 1H, CH), 2.50 (s, 4H, CH_2), 5.13 (s, 2H, CH_2O), 7.19–7.57 (m, 13H, aromatic H), 10.21 (s, 1H, NH[b]), 12.64 (s, 1H, NH[b]), 12.77 (s, 1H, NH[b])
16e	3200 (NH), 1650 (C=0)	2.72 (s, 4H, CH_2), 3.16 (s, 4H, CH_2), 5.11 (s, 2H, CH_2O), 6.85 (d, *J* = 8.0, 2H, 3′-H, 5′-H), 7.35–7.43 (m, 10H, aromatic H), 7.53 (d, *J* = 8.0, 2H, 2′-H, 6′-H), 10.22 (s, 1H, NH[b]), 12.62 (s, 1H, NH[b]), 12.74 (s, 1H, NH[b]).
16f	3195 (NH), 1645 (C=0)	2.78 (s, 4H, CH_2), 3.01 (br.s, 6H, CH_2), 3.74 (s, 3H, CH_3O), 5.15 (s, 2H, CH_2O), 6.81–6.93 (m, 4H, aromatic H), 7.26–7.59 (m, 8H, aromatic H), 10.22 (s, 1H, NH[b]), 12.64 (s, 1H, NH[b]), 12.76 (s, 1H, NH[b])
16g	3183, 2928 (NH), 1635 (C=O)	2.50 (s, 4H, CH_2), 2.73 (br.s, 6H, CH_2), 3.96 (s, 3H, CH_3O), 5.13 (s, 2H, CH_2O), 6.33–6.47 (m, 4H, aromatic H), 7.04–7.57 (m, 8H, aromatic H), 10.21 (s, 1H, NH[b]), 12.63 (s, 1H, NH[b]), 12.75 (s, 1H, NH[b])
16h	3197 (NH), 1640 (C=O)	2.50 (s, 4H, CH_2), 2.69 (br.s, 6H, CH_2), 5.13 (s, 2H, CH_2O), 6.72 (t, *J* = 7.8, 2H, 4′-H, 5′-H pyridine), 6.75 (d, *J* = 8.0, 2H, 3′-H, 6′-H pyridine), 7.25–7.57 (m, 7H, aromatic H), 8.09 (d, *J* = 8.0, 1H, aromatic H), 10.22 (s, 1H, NH[b]), 12.62 (s, 1H, NH[b]), 12.73 (s, 1H, NH[b])

Table 6. *Continued*

No.	IR (KBr) cm^{-1} ν =	^1H NMR ([D_6]-DMSO)[a] δ =
17[i]	3219, 3412 (NH), 1600, 1573 (C=N)	1.91 (s, 6H, CH_3), 5.08 (s, 2H, CH_2O), 6.10 (s, 2H, CH pyrrole), 6.94–7.03 (m, 2H, aromatic H), 7.03 (s, 1H, NH[b]), 7.35–7.47 (m, 6H, aromatic H), 9.29 (s, 1H, 4-H), 12.09 (s, 1H, NH[b])
18[j]	3000–3300 (NH), 1630 (C=N)	2.50 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 5.12 (s, 2H, CH_2O), 6.92–6.98 (m, 1H, aromatic H), 7.19–7.51 (m, 9H, aromatic H), 11.97 (s, 1H, NH[b])
19[k]	3020, 3080,3150 (NH), 1650 (C=N)	2.10 (s, 6H, CH_3), 5.27 (s, 2H, CH_2O), 5.82 (s, 2H, CH pyrrole), 7.33–7.60 (m, 6H, aromatic H), 7.73–7.96 (m, 2H, aromatic H), 9.32 (s, 1H, 1-H), 11.78 (s, 1H, NH[b])
20[l]	3000–3300 (NH), 1650 (C=N)	2.40 (s, 3H, CH_3), 2.71 (s, 3H, CH_3), 5.24 (s, 2H, CH_2O), 6.29 (s, 1H, CH), 7.36–8.07 (m, 8H, aromatic H), 9.84 (s, 1H, 1-H), 11.00 (s, 1H, NH[b])

[a] δ ppm. *J* Hz [b] exchanges with D_2O. [c] M^+= 291. [d] M^+= 305. [e] M^+= 359. [f] M^+= 345. [g] M^+= 428. [h] M^+= 470. [i] M^+= 383. [j] M^+= 369. [k] M^+= 383 [l] M^+= 369.

8-Benzyloxy-1-(4-cyano)benzylidenamino-3,4-dihydropyridazino[4,5-b]indol-4-one (**14f**)

From **8** and 4-cyanobenzaldehyde; as yellow solid.

8-Benzyloxy-3,4-dihydro-1-(3,4,5-trimethoxy)benzylidenaminopyridazino[4,5-b]indol-4-one (**14g**)

From **8** and methyl 3,4,5-trimethoxybenzaldehyde; as cream solid.

8-Benzyloxy-1-(2-chloroacetamido)-3,4-dihydropyridazino[4,5-b]indol-4-one (**15**)

A mixture of **8** (1.0 g, 3.3 mmol) and chloroacetylchloride (4 mL) is refluxed for 1 h. Once the mixture is cooled, ethanol (100 mL) is added. The solid obtained is isolated by filtration, dried, and recrystallized. Violet solid. Tables 2 and 6.

1-(Amino)acetamido-8-benzyloxy-3,4-dihydropyridazino[4,5-b]indol-4-one derivatives **16**. *General method.*

A mixture of **15** (0.3 g, 0.8 mmol), the corresponding amine (3 mmol), and a few drops of triethylamine is refluxed for 8 to 50 h (in the case of the solid amines, DMF (15 mL) is also added). The solvents are eliminated under reduced pressure and the solid obtained is isolated and washed with abundant hot water. In this way, the following compounds are obtained (Tables 2 and 6):

8-Benzyloxy-3,4-dihydro-1-morpholinoacetamidopyridazino[4,5-b]indol-4-one (**16a**)

From **15** and morpholine; as white solid.

8-Benzyloxy-3,4-dihydro-1-piperidinoacetamidopyridazino[4,5-b]indol-4-one (**16b**)

From **15** and piperidine; as yellow solid.

8-Benzyloxy-3,4-dihydro-1[(4-methyl)piperazino]acetamidopyridazino[4,5-b]indol-4-one (**16c**)

From **15** and methylpiperazine; as pale yellow solid.

8-Benzyloxy-3,4-dihydro-1[(4-phenyl)piperidino]acetamidopyridazino[4,5-b]indol-4-one (**16d**)

From **15** and 4-phenylpiperidine; as brown solid.

8-Benzyloxy-1-[4-(4-chlorophenyl)piperazino]acetamido-3,4-dihydropyridazino[4,5-b]indol-4-one (**16e**)

From **15** and 1-(4-chloro)phenylpiperazine; as yellow solid.

8-Benzyloxy-3,4-dihydro-1-[4-(2-methoxyphenyl)piperazino]acetamidopyridazino[4,5-b]indol-4-one (**16f**)

From **15** and 1-(2-methoxy)phenylpiperazine; as white solid.

8-Benzyloxy-3,4-dihydro-1-[4-(3-methoxyphenyl)piperazino]acetamidopyridazino[4,5-b]indol-4-one (**16g**)

From **15** and 1-(3-methoxy)phenylpiperazine; as white solid.

8-Benzyloxy-3,4-dihydro-1-[4-(2-pyridyl)piperazino]acetamidopyridazino[4,5-b]indol-4-one (**16h**)

From **15** and 1-(2-pyridyl)piperazine; as brown solid.

7-Benzyloxy-1-[(2,5-dimethyl)pyrrol-1-ylamino]pyridazino[4,5-a]indole (**17**)

A mixture of **11** (2.0 g, 5.5 mmol) and 2,5-hexanedione (15 mL) is refluxed for 9 h. The excess reagent is eliminated and xilene (15 mL) is poured over the residue. The mixture is then refluxed for 15 h. The solvent is eliminated under reduced pressure and the solid obtained is isolated, washed with abundant water, dried, and recrystallized. Brown solid. Tables 1 and 6.

7-Benxyloxy-1-[(3,5-dimethyl)pyrazol-1-yl]pyridazino[4,5-a]indole (**18**)

Starting form **11**, and using the same procedure indicated for **13**. White solid. Tables 1 and 6.

8-Benzyloxy-4-[(2,5-dimethylpyrrol-1-ylamino]pyridazino[4,5-b]indole (**19**)

2,5-hexanedione (2 mL) is added dropwise to a mixture of **9** (1.0 g, 5.5 mmol) and acetic acid (15 mL). The mixture is then heated at 60°C for 8 h, maintaining constant and vigorous stirring. The mixture is then set aside to cool. The solid obtained is filtered, suspended in a solution of $NaHCO_3$ (2.0 g/20 mL H_2O), and heated to 60°C, with stirring, for 30 min. The mixture is allowed to cool, and the solid is isolated, washed with abundant water, dried, and recrystallized. Brown solid. Tables 1 and 6.

8-Benzyloxy-4-[(3,5-dimethyl)pyrazol-1-yl]pyridazino[4,5-b]indole (**20**)

A mixture of **9** (3.6 mmol) and acetylacetone (10 mL) is refluxed for 25 h. The excess reagent is eliminated under reduced pressure. Ethyl ether (10 mL) is poured over the residue. The mixture is boiled and then set aside at room temperature for 12 h. The solid obtained is isolated by filtration, dried, and recrystallized. White solid. Tables 1 and 6.

Biological Activity

Isolation of PDE and assays of activity

The different molecular forms of PDE were separated by DEAE-Sepharose anion exchange liquid chromatography as previously described[18,19], using the method of Reeves and associates[22], with minor modifications. PDE activity was determined by the batch method of Thompson and associates[23]. The nomenclature of PDE's follow that proposed by Beavo and Reifsnyder[24]. PDE-I corresponds to the Ca^{2+}/Calmodulin-stimulated PDE; PDE-II corresponds to the cGMP-stimulated PDE; PDE-III corresponds to the cGMP-inhibited PDE; PDE-IV corresponds to the cAMP-specific PDE and PDE-V is the specific cGMP-PDE. PDE-V isolated from aorta was not resolved from PDE-I and the cGMP activity measured with this isoenzyme probably includes some PDE-I contaminant activity.

Platelet aggregation: guinea pig whole blood

In guinea pig whole blood, the antiaggregatory activity against ADP and AA, has been determined by following previously reported methods[17,18,19], applying the Cardinal and Flowers method[25].

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