Eur J Med Chem (1991) 26, 729–733

Short communication

Synthesis and *in vitro* study of platelet antiaggregant activity of 2(4)-imidazol-1-yl-4(2)-cycloalkylamino pyrimidines

L Del Corona, G Signorelli, S Manzardo, A Pinzetta, G Coppi*

Research Centre, Poli Industria Chimica, Via Volturno 48, 20089 Rozzano (MI), Italy

(Received 15 June 1990; accepted 25 March 1991)

Summary — The synthesis of some 2(4)-imidazol-1-yl-4(2)-cycloalkylaminopyrimidines are reported. The compounds demonstrated a marked antiaggregating activity superior to dipyridamole, ASA, ticlopidine and similar to that of indobufen.

Résumé — Synthèse et étude *in vitro* de l'activité anti-agrégante de 2(4)-imidazol-1-yl-4(2)-cycloalkylaminopyrimidines. *Quelques 2(4)-imidazol-1-yl-4(2)-cycloalkylaminopyrimidines ont été synthétisées. Ces composés possèdent une activité prononcée anti-agrégante supérieure à celle du dipyridamole, de l'ASA, et de la ticlopidine et proche de celle de l'indobufen.*

substituted pyrimidines / antiaggregant activity

Introduction

Dipyridamole is employed satisfactorily in the treatment of several diseases of the cardiocirculatory system characterized by thrombotic accidents [1]. Starting from the principle that the compound's specific activity could be correlated with the notable polarity of the structure owing to nitrogen-rich rings of pyrimidine, piperidine and diethanolamine residues, our aim was to verify to what extent these properties were connected to the structural complexity of the molecule, since neither pyrimidine, piperidine nor diethanolamine exhibit them. The most immediate approach in this respect seemed be 'halving' the molecule into 2 fragments similar to each other by keeping the same ratio between polar substituents and central nucleus and consequently the requisite possibly responsible for the activity. As substituents besides piperidine we introduced pyrrolidine, morpholine, imidazole and also *N*-methyl-*N*-cyclopropylamine.

*Correspondence and reprints

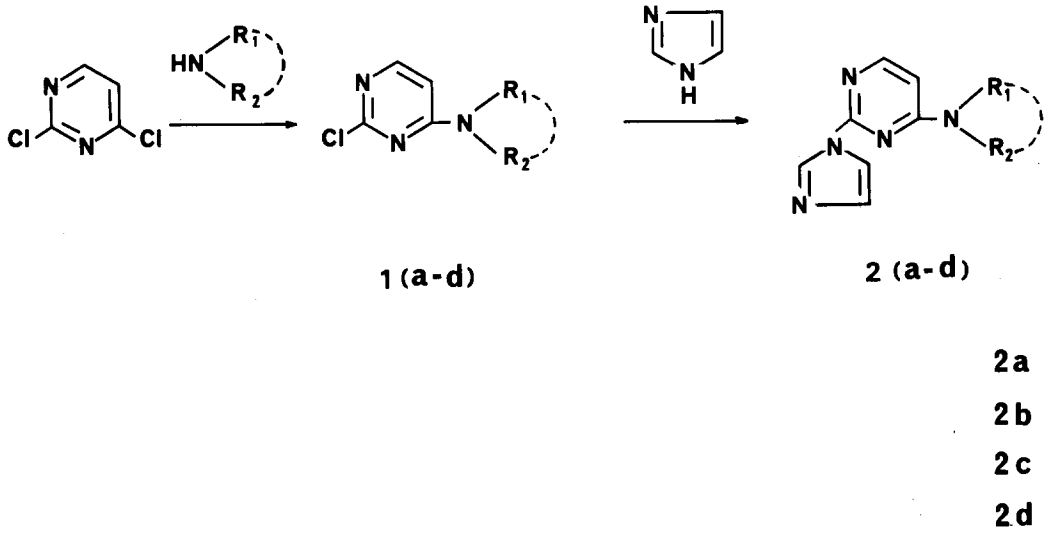
Abbreviations: ASA: acetylsalicilic acid; DMF: dimethyl formamide; ADP: adenosine diphosphoric acid; CNS: central nervous system; PRP: platelet-rich plasma; PPP: platelet-poor plasma

Chemistry

We used 2 routes of synthesis, according to the amine to be introduced and to the position (2 or 4) of the pyrimidine. Scheme 1 shows the sequence used to prepare the compounds in which the 2 substituent is imidazole and the 4 substituent is a cycloamine. As the chlorine atom in position 4 is more reactive [2–11] than the one in the position 2, the substitution with cycloamine proceeds selectively in ethanolic solution to give the 2-chloro-4-cycloamine **1**, which is heated in DMF with imidazole to produce the disubstituted compound **2** in very good yield. Inversion of the reagent sequence in order to prepare 2-piperidinyl-4-imidazolyl-pyrimidine did not give good results owing to the difficulty in obtaining pure 2-chloro-4-imidazolyl-pyrimidine by reacting 2,4-dichloropyrimidine and imidazole, and to the consequent reaction with piperidine that leads to mixtures of products from which 2,4-dipiperidinylpyrimidine was separated and unambiguously recognized.

A satisfactory route to 2-cycloamino-4-imidazolyl-pyrimidines **4** is shown in scheme 2.

2-Carbethoxymethylthio-4-chloro-pyrimidine [12] is heated in DMF with imidazole, and the produced 4-imidazolyl derivative **3** reacted with piperidine to give the corresponding compound **4** in good yield.



1 (a-d)

2 (a-d)

	R_1	R_2
2a	$-(CH_2)_5-$	
2b	$-(CH_2)_4-$	
2c	$-(CH_2)_2O(CH_2)_2-$	
2d	$-CH_3$	

Scheme 1.

All compounds in tables I and II were obtained by these procedures. They were characterized by elemental analysis and ^{1}H-NMR spectrum.

Pharmacological results and discussion

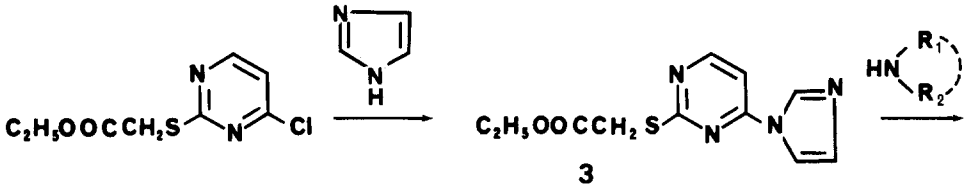
The activity of the compounds on *in vitro* platelet aggregation was determined by Born's turbidimetric method [13] utilizing as inductors arachidonic acid, collagen and ADP. The approximate acute toxicity, CNS activity and interactions with some drugs in mice were determined by a standardized method [14].

Table III reports all the results in comparison to 4 references standards: indobufen, ticlopidine, dipyridamole and acetylsalicylic acid (ASA).

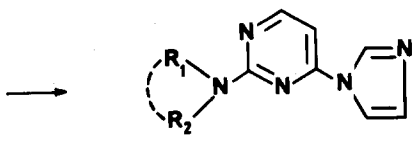
All the new compounds show a strong dose-dependent inhibition of *in vitro* guinea-pig platelet aggregation by arachidonic acid, collagen and ADP.

The most active compounds on aggregation induced by collagen in decreasing order were: **2c** = indobufen > **2d** ≥ **2a** > **4a** > **4c** ≥ **4b** ≥ **2b** > ASA ≥ dipyridamole > ticlopidine.

The most active compounds on aggregation induced by arachidonic acid in decreasing order were: **2c** = **2d** > **2a** > **4b** ≥ **2b** > **4c** > indobufen > **4a** > dipyridamole ≥ ASA > ticlopidine.



3

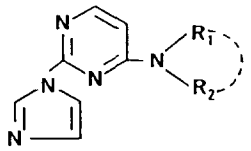


4 (a-c)

	R_1	R_2
4a	$-(CH_2)_5-$	
4b	$-(CH_2)_2O(CH_2)_2-$	
4c	$-(CH_2)_4-$	

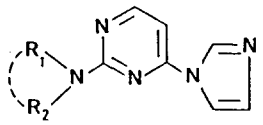
Scheme 2.

Table I. 2-(Imidazol-1-yl)-4-cycloalkylamino-pyrimidines.



Compd	*R_1* *R_2*	*Yield %*	*mp (°C)*	*Recryst solvent*	*Formula*	*MW*	*Analysis (C, H, N)*
2a	$-(CH_2)_5-$	75	198–200	EtOH	$C_{12}H_{15}N_5 \cdot HCl$	265.75	See Experimental
2b	$-(CH_2)_4-$	86.6	235–237	MeOH	$C_{11}H_{15}N_5 \cdot HCl$	251.72	^{1}H-NMR ($CDCl_3$): δ 1.90–2.20 (m, 4H, CH_2-CH_2); 3.40–3.64 (dt, 4H, J = 6 Hz, CH_2-N-CH_2, 6.35 (d, 1H, J = 6 Hz, H_5 pyrim); 7.45 (t, 1H, J = 2 Hz, H_4 imid); 8.08 (t, 1H, $J \approx 2$ Hz, H_5 imid); 8.10 (d, 1H, J = 6 Hz, H_6 pyrim); 9.30 (t, 1H, J = 2 Hz, H_2 imid)
2c	$-(CH_2)_2-O-(CH_2)_2-$	77	213–215	MeOH	$C_{11}H_{13}N_5O \cdot HCl$	267.72	^{1}H-NMR ($CDCl_3$): δ 3.60 (t, 4H, J = 5 Hz, CH_2N); 3.90 (t, 4H, $J \approx 5$ Hz, CH_2O); 6.60 (d, 1H, J = 6 Hz, H_5 pyrim); 7.50 (t, 1H, J = 2 Hz, H_4 imid); 8.08 (t, 1H, $J \approx 2$ Hz, H_5 imid); 8.22 (d, 1H, J = 6 Hz, H_6 pyrim); 9.42 (t, 1H, $J \approx$ 2 Hz, H_2 imid)
2d	$-CH_3$ 	73	171–173	EtOH/ Et_2O	$C_{11}H_{13}N_5 \cdot HCl$	251.72	^{1}H-NMR ($CDCl_3$): δ 0.70–0.85 (m, 2H, CH_2); 1.0–1.10 (m, 2H, CH_2); 2.73 (symm m, 1H, CH); 3.20 (s, 3H, CH_3); 6.90 (d, 1H, J = 6 Hz, H_5 pyrim); 7.46 (t, 1H, J = 2 Hz, H_4 imid); 8.09 (t, 1H, J = 2 Hz, H_5 imid); 8.19 (d, 1H, J = 6 Hz, H_6 pyrim); 9.30 (t, 1H, J = 2 Hz, H_2 imid)

Table II. 2-Cycloalkylamino-4-(imidazol-1-yl)-pyrimidines.



Compd	*R_1* *R_2*	*Yield %*	*mp (°C)*	*Recryst solvent*	*Formula*	*MW*	*Analysis (C, H, N)*
4a	$-(CH_2)_5-$	53.5	238–240	MeOH	$C_{12}H_{15}N_5 \cdot HCl$	265.75	See Experimental
4b	$-(CH_2)_2-O-(CH_2)_2-$	65.5	212–215	EtOH	$C_{11}H_{13}N_5O \cdot 2HCl$	304.18	^{1}H-NMR (DMSO–d_6): δ 3.65 (t, 4H, J = 5 Hz, CH_2N); 3.82 (t, 4H, J = 5 Hz, CH_2O); 7.30 (d, 1H, J = 6 Hz, H_5 pyrim); 7.92 (t, 1H, $J \approx 2$ Hz, H_4 imid); 8.60 (t, 1H, J = 2 Hz, H_5 imid); 8.70 (d, 1H, J = 6 Hz, H_6 pyrim); 10.2 (t, 1H, $J \approx$ 2 Hz, H_2 imid)
4c	$-(CH_2)_4-$	77	170–172	EtOH	$C_{11}H_{13}N_5 \cdot HCl$	251.72	^{1}H-NMR ($CDCl_3$ + DMSO–d_6): δ 1.90–2.05 (m, 4H, CH_2-CH_2); 3.40–3.65 (m, 4H, CH_2-N-CH_2); 7.00 (d, 1H, J = 6 Hz, H_5 pyrim); 7.43 (t, 1H, J = 2 Hz, H_4 imid); 7.98 (t, 1H, J = 2 Hz, H_5 imid); 8.45 (d, 1H, J = 6 Hz, H_6 pyrim); 9.90 (t, 1H, J = 2 Hz, H_2 imid)

Table III. Approximate acute toxicity in mice and activity on platelet aggregation *in vitro*.

Compounds	*Approx LD_{50} (mg/kg os*	*IC_{50} (μg/ml) Collagen (20 μg/ml final conc)*	*Arachidonic acid (24–40 μg/ml final conc)*	*ADP (1.7 μg/ml final conc)*
2a	400	3.2	0.5	19.4
2b	400	7.3	1.4	36.7
2c	300	2.3	0.2	15.0
2d	200	3.0	0.2	35.4
4a	600	4.5	8.5	42.0
4b	700	7.1	1.1	37.6
4c	300	6.5	2.3	40.2
Indobufen	1200	2.3	3.0	16.3
Ticlopidine	1200	125.4	129.9	147.5
Dipyridamole	> 1600	15.6	24.4	55.8
ASA	1600	12.1	29.5	29.6

The most active compounds on aggregation induced by ADP in decreasing order were: **2c** ≥ indobufen > **2a** > ASA > **2d** ≥ **2b** ≥ **4b** > **4c** ≥ **4a** > dipyridamole > ticlopidine.

The new compounds show oral acute toxicities in mice ranging from 200–700 mg/kg *per os*; they show CNS depression and generally interactions of varying degree with tremorine and amphetamine.

Regarding the structure–activity relationship it seems important that the imidazol-1-yl group be in the 2-position to obtain very active antiaggregating compounds. The most interesting groups in the 4-position, in decreasing order, are: morpholino, *N*-methyl-*N*-cyclopropylamino, piperidino and pirrolidino.

In conclusion, all the new compounds show a marked *in vitro* platelet antiaggregating activity superior to dipyridamole, ASA, ticlopidine and similar to that of indobufen.

The most interesting compound (**2c**) has the imidazol-1-yl group in the 2-position and the morpholino group in 4-position.

Other studies are necessary in order to determine the mechanism of action of these compounds taking into account cyclo-oxygenase inhibition for ASA and indobufen, cAMP for dipyridamole and fibrinogen receptors for ticlopidine.

Experimental protocols

Chemistry

Melting points were taken from a Büchi 535 apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a Varian Geminy (200 MHz) spectrometer using tetramethylsilane as internal standard. Microanalysis values are within ± 0.4% of theoretical values.

2-Chloro-4-(piperidin-1-yl)pyrimidine ***1a***
To a solution of 7.5 g (0.05 mol) of 2.4-dichloropyrimidine and 7 ml (0.05 mol) of triethylamine in 50 ml ethanol, a solution of 4.25 g (0.05 mol) of piperidine in 10 ml ethanol was added with temperature maintained between 20–25°C. After 1 h the solvent was distilled under vacuum and the solid residue treated with water. The wet solid was dissolved in methylene chloride, the organic phase separated, the solvent distilled and the solid triturated with petrol ether obtaining 7.7 g of 2-chloro-4-(piperidin-1-yl)pyrimidine, mp = 83–85°C. Anal calcd for $C_9H_{12}ClN_3$: C, 54.67; H, 6.11; N, 21.24; Cl, 17.95. Found: C, 54.64; H, 6.20; N, 21.18; Cl, 17.77. ^{1}H NMR ($CDCl_3$): δ 1.55–1.80 (m, 6H, CH_2-(CH_2)$_3$-CH_2); 3.5–3.82 (m, 4H, CH_2-N-CH_2); 6.38 (d, 1H, J = 6 Hz, H_6 pyrim); 7.98 (d, 1H, J = 6 Hz, H_5 pyrim).

2-(Imidazol-1-yl)-4-(piperidin-1-yl)pyrimidine hydrochloride ***2a***
Product **1a** (9.87 g; 0.05 mol) was dissolved in DMF (20 ml), 6.8 g (0.1 mol) of imidazole was added and the mixture was heated to 100°C for 60 min. The solvent was evaporated uv, the residue shaken with water/toluene, the organic phase separated, evaporated to dryness, the solid dissolved in ethanol and the solution treated with hydrogen chloride. The product was precipitated by adding ethyl ether, filtered and dried to give 9.95 g (75%), mp = 198–200°C (recrystallized from ethanol). Anal calcd for $C_{12}H_{15}N_5$·HCl: C, 54.23; H, 6.07; N, 26.35. Found: C, 54.28; H, 5.98; N, 26.26. ^{1}H NMR ($CDCl_3$): δ 1.60–1.85 (m, 6H, CH_2-(CH_2)$_3$-CH_2); 3.70 (broad s, 4H, CH_2-N-CH_2); 6.55 (d, 1H, J = 6 Hz, H_5 pyrim); 7.45 (t, 1H, $J \approx$ 2 Hz, H_4 imid); 8.08 (t, 1H, $J \approx$ 2 Hz, H_5 imid); 8.12 (d, 1H, J = 6 Hz, H_6 pyrim); 9.30 (t, 1H, $J \approx$ 2 Hz, H_2 imid).
2-Chloro-4-(pyrrolidin-1-yl)pyrimidine **1b**. (64% from ethylacetate/petrol ether), mp = 120–122°C. 2-Chloro-4-(morpholin-1-yl)pyrimidine **1c**. (57% from ethylacetate) mp = 110–112°C. 2-Chloro-4-(*N*-methyl-*N*-cyclopropyl)amino **1d**. (69% from petrol ether) mp = 46–48°C.

2-Carbethoxymethylthio-4-(imidazol-1-yl)pyrimidine ***3***
To a mixture of 11.7 g (0.05 mol) of 2-carbethoxymethylthio-4-chloropyrimidine [12] and 7 ml (0.05 mol) of triethylamine in 15 ml of DMF was added a solution of 3.4 g (0.05 mol) of imidazole in 10 ml DMF and the whole was heated at 100°C for 5 h. The solvent was evaporated, water was added, the product was extracted with ethyl acetate and after evaporation of the solvent, the residue was dissolved in ethanol and treated with a little excess of nitric acid. The precipitated nitrate of 2-carbethoxymethylthio-4-(imidazol-1-yl)pyrimidine was filtered, alkalinized and the base crystallized from ethylacetate yielding 9.27 g, 70%; mp = 90–92°C. Anal calcd for $C_{11}H_{12}N_4O_2S$; C, 50.00; H, 4.57; N, 21.19; S, 12.11. Found: C, 49.93; H, 4.58; N, 21.12; S, 12.08. ^{1}H NMR ($CDCl_3$): δ 1.28 (t,

3H, J = 8 Hz, CH_3); 3.90 (s, 2H, SCH_2); 4.22 (q, 2H, J = 8 Hz, OCH_2CH_3); 7.0 (d, 1H, J = 6 Hz, H_5 pyrim); 7.22 (t, 1H, $J \approx$ 2 Hz, H_4 imid); 7.66 (t, 1H, $J \approx$ 2 Hz, H_5 imid); 8.40 (t, 1H, $J \approx$ 2 Hz, H_6 pyrim); 8.56 (d, 1H, J = 6 Hz, H_2 imid).

2-(Piperidin-1-yl)-4-(imidazol-1-yl)pyrimidine hydrochloride ***4a***
Ten g of **3** (0.037 mol) and 50 ml of piperidine were refluxed for a night, the excess piperidine was distilled under vacuum, the residue was shaken with water/toluene, the organic phase washed with water, dried, the solvent evaporated, the residue dissolved in ethanol and treated with HCl. The product was precipitated by adding ethyl ether, filtered and dried at 110°C under vacuum to give 6.1 g (53.5%), mp = 238–240°C (recrystallized from methanol). Anal calcd for $C_{12}H_5N_5$·HCl: C, 54.23; H, 6.07; N, 26.35. Found: C, 54.18; H, 6.10; N, 26.31. ^{1}H NMR (DMSO-d_6): δ 1.50–1.80 (m, 6H, CH_2-(CH_2)$_3$-CH_2); 3.85 (t, 4H, J = 6 Hz, CH_2-N-CH_2); 7.22 (d, 1H, J = 6 Hz, H_5 pyrim); 7.90 (s, 1H, H_4 imid); 8.50 (s, 1H, H_5 imid); 8.63 (d, 1H, J = 6 Hz, H_6 pyrim); 10.03 (s, 1H, H_2 imid).

Attempt to prepare 2-(piperidin-1-yl)-4-(imidazol-1-yl)pyrimidine via 2-chloro-4-(imidazol-1-yl)pyrimidine
To a suspension of 1.5 g (0.05 mol) of 80% sodium hydride in 15 ml of DMF, 3.4 g (0.05 mol) of imidazole in 10 ml of DMF were added. The temperature was raised to 70°C, and then the mixture was warmed with stirring at 100°C for 40 min. After cooling to 10°C, the suspension was quickly transferred in a separatory funnel stopped with a calcium chloride tube and it was dropped in a solution of 7.45 g (0.05 mol) of 2,4-dichloropyrimidine in 15 ml of DMF stirred at 5–10°C. After 30 min at 10°C, DMF was distilled off under vacuum, the residue was taken up in a small volume of water, the solution was saturated with potassium carbonate and extracted with toluene. The extract contained 4 products having in TLC (*i*-propyl ether/toluene/methanol, 60/30/10) R_f < starting product. After evaporation of the solvent, the oily residue was taken up in a small volume of ethanol and the solution was acidified with nitric acid. The precipitate (a mixture of 3 products) was dissolved in water, basified with sodium hydroxide and extracted with toluene, the organic solution was dried and evaporated to give an oily residue that was taken up in 30 ml of DMF. To the solution 3.4 g (0.04 mol) of piperidine and 5.6 ml of triethylamine (0.04 mol) were added and the whole was warmed at 140°C for 4–5 h. DMF was evaporated, to the residue water was added and the oil was extracted with ethyl acetate, dried over sodium sulphate and evaporated yielding an oily residue. This was taken up in ethanol and acidified with 65% nitric acid. By adding ethyl ether a solid product precipitated; it was collected and recrystallized from ethanol. It melted at 153–153.5°C. The free base was obtained in aqueous medium by adding sodium hydroxide and collecting the precipitate. After recrystallization from aqueous ethanol, 1.1 g, mp = 58–59°C, of 2,4-di(piperidin-1-yl)pyrimidine were obtained. Anal calcd for $C_{14}H_{22}N_4$: C, 68.26; H, 9.00; N, 22.73. Found: C, 68.17; H, 9.07; N, 22.68. ^{1}H NMR ($CDCl_3$): δ 1.5–1.75 (m, 12H, [CH_2(CH_2)$_3$$CH_2$]$_2$); 3.5 (t, 4H, CH_2-N-CH_2 (2)); 3.65 (t, 4H, CH_2-N-CH_2 (4)); 5.82 (d, 1H, J = 6 Hz, H_5 pyrim); 7.9 (d, 1H, J = 6 Hz, H_6 pyrim).

Pharmacology

Activity on platelet aggregation in vitro
The activity on *in vitro* platelet aggregation was determined according Born's turbidimetric method [13] with the following modifications.

Platelet-rich plasma (PRP) was prepared from guinea pig blood drawn into syringes containing 3.8% sodium citrate (1 ml of citrate/9 ml of blood). PRP was obtained from the blood of guinea pig by centrifugation at low speed (75 *g* for 20 min) to obtain the supernatant, or PRP.

Platelet-poor plasma (PPP) was obtained from the precipitated fraction of PRP by centrifugation at 1350 *g* for 20 min. The platelet count was measured by a Coulter counter (Kontron) and PRP was diluted with homogeneous PPP to adjust the platelet count in 3–5 x 10^5 cells/ml for use in the study. Silicon-treated glassware or plastic–ware was used for the preparation of PRP.

The antiplatelet aggregation activity of the compounds and the reference drugs was determined by Born's turbidimetric [1] method using an aggregometer (Elvi mod 840 with Logos (Omniscribe 176) register). A 240-µl volume of PRP was placed in the microcuvettes of the aggregometer, and a fixed amount (10 µl) of the test drug solutions or vehicle solution was added, and this was incubated at 37°C for 3 min. After incubation, platelet aggregation was induced by the addition of 10 µl of a normal saline solution of adenosine diphosphate (Mascia Brunelli), 5 µl of a collagen suspension diluted with normal saline (Mascia Brunelli) and 3–5 µl of a saline solution of arachidonic acid (Sigma Chemical). The aggregometer was calibrated with PRP (transmittance = 0%) and PPP (transmittance = 100%). The inhibition of aggregation was calculated as percent in comparison with the aggregation curve obtained with vehicle. The reference drugs utilized were: acetylsalicylic acid (ASA), dipyridamole, indobufen, ticlopidine. IC_{50} (the drug concentration that inhibited induced platelet aggregation by 50%) was calculated by the regression line method.

Determination of approximate acute toxicity, CNS activity and interactions with some drugs in mice
Male COBS CD-1 mice (C River), weighing 20 g, were used. The compounds were given orally in a 10% arabic gum suspension (0.1 ml/10 g) to mice fasted for 3 h. The range of doses were 25, 50, 100, 200, 400, 800 and 1600 mg/kg; control mice only received 10% arabic gum suspension.

The determination of approximate acute toxicity, CNS activity and interactions with strychnine, pentylenetetrazol, amphetamine, tremorine and reserpine were determined according Morpurgo [14].

References

1 Goodman and Gilman's (1985) *The Pharmacological Basis of Therapeutics*. MacMillan Publ Co, 7th edn, 1352
2 Roy-Burman P, Roy D, Sen D (1960) *Naturwissenschaften* 47, 515
3 Roy-Burman P, Roy D, Sen D (1961) *Naturwissenschaften* 48, 737
4 Roy-Burman P, Roy D, Sen D (1962) *Naturwissenschaften* 49, 494
5 Koppel H, Spring RH, Robins RK, Cleng CC (1961) *J Org Chem* 27, 181
6 Brown DJ, Jacobens NW (1962) *J Chem Soc* 3172
7 Brown DJ, Harper JG (1963) *J Chem Soc* 1276
8 Ballweg H (1964) *Ann Chemie* 673, 153
9 Nitta Y, Okui K, Ita K (1965) *Chem Pharm Bull* 13, 557
10 Nagpal KL, Dhar MM (1967) *Tetrahedron* 23, 1297
11 Zagulyalva OA, Bukhatkina NV, Hamaev VP (1978) *Zh Org Khim* 14 (2), 409–413
12 Santilli AA, Scotese AC, Tomarelli RM (1973) *Ger Offen* 2.314.160
13 Born GVR (1962) *Nature (Lond)* 194, 927
14 Morpurgo C (1971) *Arzneim Forsch* 21, 1727