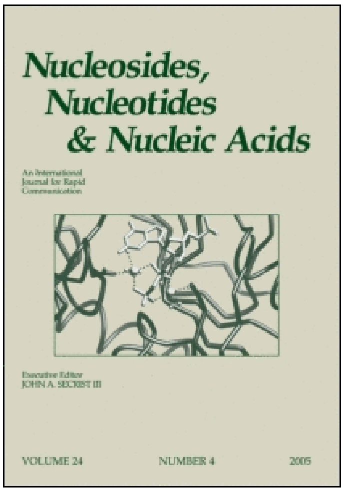

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Adenosine and 2-Chloroadenosine Deaza-Analogues as Adenosine Receptor Agonists[1]

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ADENOSINE AND 2-CHLOROADENOSINE DEAZA-ANALOGUES AS ADENOSINE RECEPTOR AGONISTS [1]

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Abstract. Several deaza-analogues of adenosine and 2-chloroadenosine have been examined for their adenosine receptor affinity. It was found that the relative contribution of the nitrogen atoms of the purine moiety to binding at A_1 rat brain adenosine receptor, follows the order $N^7 > N^3 > N^1$. The affinity of the adenosine analogues for the adenosine rat brain receptor was besides compared with their activity as inhibitors of platelet aggregation. A synthesis of 2-chloro-1-deazaadenosine by two alternative routes starting from 7-nitroimidazo[4,5-b]pyridine-4-oxide is also reported.

In the past decade, the role of adenosine as a 'local hormone' has been firmly established and many investigators have proposed that adenosine is a neurotrasmitter or neuromodulator which acts on several physiological systems.[2] Although adenosine can affect a variety of physiological functions, particular attention has been directed towards the identification of those actions which might have clinical utility.[3] In recent years extracellular adenosine and adenine nucleotide receptors have been identified. It appears that in many cells, adenosine receptors (P_1) are coupled

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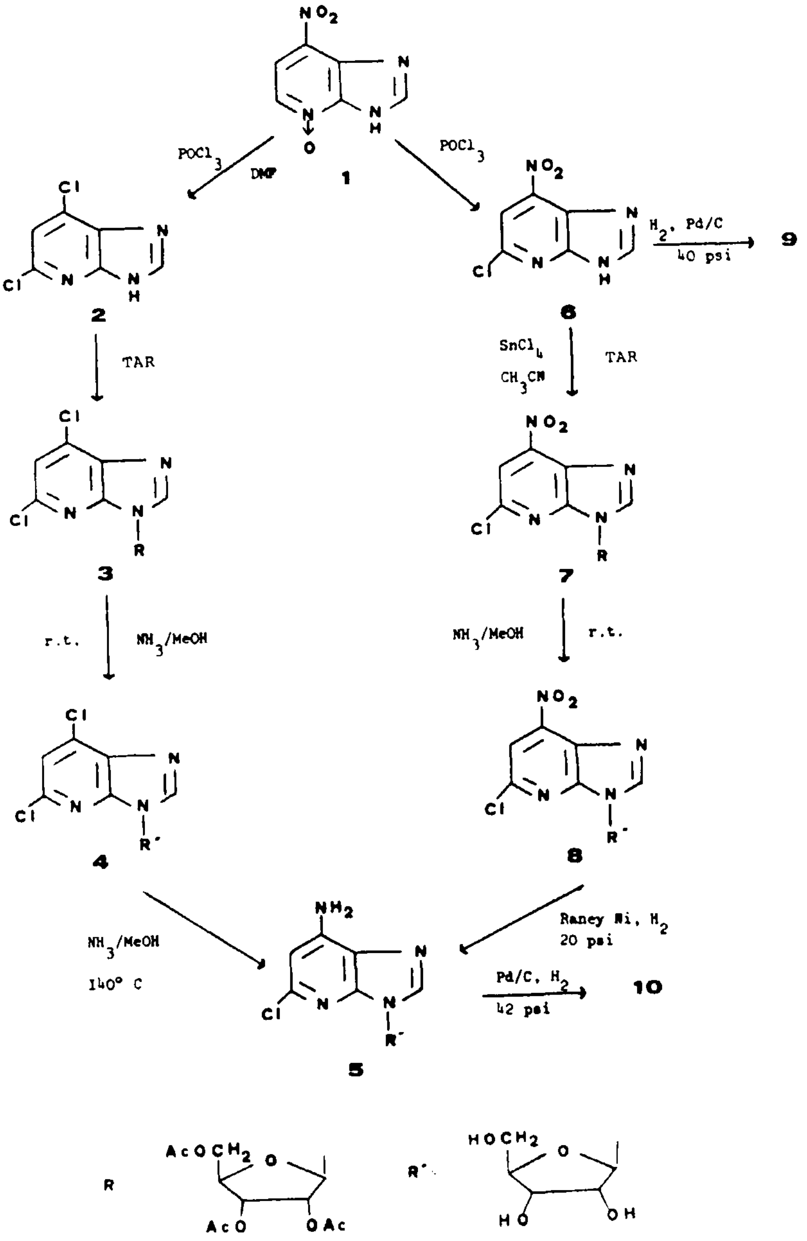
to adenylate cyclase while nucleotide receptors (P_2) probably regulate ionic channels.[3] A variety of adenosine analogues have been used to study the adenosine receptors and as a result two receptor subtypes, A_1 and A_2 have been identified.[3] The A_1 is a high-affinity receptor which inhibits adenylate cyclase, while the low affinity A_2 receptor stymulates adenylate cyclase.[4] Radioligand binding studies have been used to identify A_1 receptors in isolated membrane preparations. Using the tritiated adenosine analogues $[^3H]N^6$-L-phenylisopropyladenosine ($[^3H]$PIA), $[^3H]N^6$-cyclohexyladenosine ($[^3H]$CHA) and $[^3H]$2-chloroadenosine which are selective to this receptor subtype, A_1 receptors have been identified in both brain and fat adipose tissue.[3]

There have been several attempts to characterize the A_2 receptor using radioligand binding studies. However, such studies have been hampered by the lack of a selective A_2 receptor ligand. Recent studies have suggested that $[^3H]5'$-N-ethylcarboxamidoadenosine ($[^3H]$NECA) is a selective ligand for this receptor in human platelets,[5] whereas Yeung and Green have reported that $[^3H]$NECA binds to both A_1 and A_2 receptors in rat striatum.[6] Therefore at present an adequate ligand for the study of A_2 receptor is still lacking. One potentially useful method for the study of this receptor is the use of human platelets instead of brain tissue since platelets have been shown to possess receptors for adenosine that have been classified as A_2 which mediate inhibition of platelet aggregation via an activation of adenylate cyclase.[7,8]

In order to get further informations about the structure-activity relationships and to investigate the role of the purine nitrogens on stimulatory and inhibitory adenosine receptors we have examined the effects of several deaza-analogues of adenosine and 2-chloroadenosine on two different cell types. The displacement of [^{3}H]CHA binding in the rat brain was used as parameter of the activity on A_1 receptor and inhibition of platelet aggregation induced by ADP as an indication of A_2 receptor activity.

Chemistry. 7-Deazaadenosine (**12**) and 2-chloroadenosine (**14**) were purchased from Sigma Chemical Co. The syntheses of 1-deazaadenosine (**10**) and 1,3-dideazaadenosine (**13**) were accomplished by modification of previous methods and have been described elsewhere.[9] 3-Deazaadenosine (**11**) and 2-chloro-3-deazaadenosine (**15**) were synthesized using known procedure.[10] The synthesis of 7-amino-5-chloro-3-ß-D-ribofuranosyl-3H-imidazo[4,5-b]pyridine (2-chloro-1-deazaadenosine) (**5**) was carried out by the two sequences shown in the Scheme 1. 7-Nitroimidazo[4,5-b]pyridine-4-oxide (**1**) is the starting material in both methods; treatment of **1** with phosphoryl chloride in dimethylformamide gave the dichloro derivative **2** while reaction of the same compound **1** with only phosphoryl chloride gave the chloro-nitro derivative **6** as major product and a small amount of **2**, which can be separated from **6** by means of silica gel column chromatography. The structure proof of **6** was obtained by catalytic hydrogenolysis of the chlorine and simultaneous reduction of the nitro group to give 7-amino-3H-imidazo[4,5-b]pyridine (**9**).[11]

Fusion of **2** with tetra-O-acetyl-ß-D-ribofuranose in the presence of a catalytic amount of p-toluenesulfonic acid followed by treatment with methanolic ammonia at 140°C gave the desired 2-chloro-1-deazaadenosine **5**.[12] The proof of structure of **5** was obtained by catalytic hydrogenolysis of the chlorine which gave 1-deazaadenosine (**10**).[11] Fusion of **6** with tetra-O-acetyl-ß-D-ribofuranose did not afford the desired compound


NO2
N
N
H
O
1
POCl3
DMF
POCl3
Cl
Cl
2
TAR
Cl
Cl
R
3
r.t.
NH3/MeOH
Cl
Cl
R'
4
NH3/MeOH
140° C
NO2
Cl
6
H2, Pd/C
40 psi
9
SnCl4
CH3CN
TAR
NO2
Cl
R
7
NH3/MeOH
r.t.
NO2
Cl
R'
8
Raney Ni, H2
20 psi
NH2
Cl
R'
5
Pd/C, H2
42 psi
10
R
AcOCH2
O
AcO
OAc
R'
HOCH2
O
HO
OH

SCHEME 1

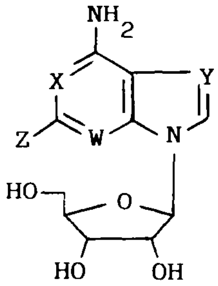
7. Alternatively, ribosylation of 6 was carried out in dry acetonitrile in the presence of freshly distilled stannic chloride at room temperature for 12 h to give the blocked nucleoside 7 in 46% yield. Deacetylation of 7 in methanolic ammonia afforded 5-chloro-7-nitro-ß-D-ribofuranosyl-3H-imidazo-[4,5-b]pyridine (8), which was converted into 2-chloro-1-deazaadenosine (5) by catalytic reduction of nitro group with Raney nickel at 30 psi.

BIOLOGICAL ACTIVITY

Adenosine analogues were evaluated for their ability to displace [^{3}H]CHA binding in rat brain preparations. This activity is expressed as the IC_{50} values and as percent of residual binding at 10^{-4}M final concentrations. 7-Deaza (12) and 1,3-dideazaadenosine (13) appear to be rather ineffective (IC_{50} > 100 M). On the other hand, 1-deazaadenosine (10) is able to displace [^{3}H]CHA binding and shows an IC_{50} of 1.4 µM. Although 3-deazaadenosine (11) retains some activity its IC_{50} is approximately ten fold higher than that of compound 10. Introduction of a chlorine atom into 2-position of 3-deazaadenosine (11) produces a further reduction in [^{3}H]CHA binding affinity. In contrast substitution of the chlorine atom at the 2-position of 1-deazaadenosine (compound 5, Scheme 1) produces significant increase in the activity. 2-Chloro-1-deazaadenosine (5) is in fact the most potent among the deaza-adenosine derivatives and it is approximately 2.6 fold more potent than compound 10 but about 47 fold less potent than 2-chloroadenosine (14).

These adenosine analogues were further examined for their biological activity at A_2 adenosine receptor by measuring ADP-induced aggregation of human platelets. As shown in Table 1, 3-deaza- (11), 7-deaza- (12), 1,3-dideaza- (13) and 2-chlo-

TABLE 1. Effect of adenosine analogues on the inhibition of [^{3}H]CHA binding to rat brain membranes and on the inhibition of human platelet aggregation induced by ADP. The values are means of three triplicate determinations.



no.		X	W	Y	Z	IC_{50} µM	Residual binding at 10^{-4}M % of control	Inhibition of aggregation at 10^{-4}M %	RAD
	adenosine	N	N	N	H			93[a]	1[a]
10	1-deaza	CH	N	N	H	1.4 ± 2.51	1.0 ± 3.2	82[a]	0.88[a]
11	3-deaza	N	CH	N	H	21.5 ± 3.45	24.0 ± 8	< 10[a]	< 0.1[a]
12	7-deaza	N	N	CH	H	> 100	63.0 ± 13	< 10[a]	< 0.1[a]
13	1,3-dideaza	CH	CH	N	H	> 100	58.0 ± 6.5	< 10[a]	< 0.1[a]
14	2-chloroadenosine	N	N	N	Cl	0.012 ± 0.72	0.5 ± 0.8	100	1.07
5	2-chloro-1-deaza	CH	N	N	Cl	0.56 ± 1.27	2.1 ± 2.7	82	0.88
15	2-chloro-3-deaza	N	CH	N	Cl	> 100	59.0 ± 7.1	< 10	< 0.1

[a] Reference 9.

ro-3-deazaadenosine (15) are ineffective in inhibiting platelet aggregation at 10^{-4}M concentration. In contrast however, compounds 10 and 5 are active and seem to be equipotent as platelet aggregation inhibitors. Nevertheless also in this biological test as well as in radioligand binding studies described above all the compounds are less potent than 2-chloroadenosine (14). Previous data obtained by Bruns, by measuring the cyclic AMP level in intact VA13 cells, showed that 3-deazaadenosine is inactive and 7-deazaadenosine is a very weak agonist in increasing cyclic AMP in human fibroblasts.[13] It is evident that substitution of CH for any one of nitrogen atoms of purine moiety results in a loss of affinity for the adenosine receptors both in the displacement of [^{3}H]CHA binding and in the inibition of platelet aggregation.

1-Deazaadenosine and 2-chloro-1-deazaadenosine are the most effective deaza-compounds evaluated while 3-deazaadenosine shows the highest degree of discrimination between the two classes of adenosine receptors. In fact the last compound is a weak displacer of [^{3}H]CHA binding but is substantially ineffective as inhibitor of ADP-induced platelet aggregation. These data indicate that the relative contribution of the nitrogen atoms of the purine moiety to binding at A_1 rat brain adenosine receptor is the following: $N^7 > N^3 > N^1$. On the other hand the presence of a nitrogen atom at positions 3 and 7 of adenosine appears to be critical for the A_2 receptor activity. These results suggest that introduction of substituent on purine ring rather than modification of purine moiety could increase the activity and selectivity on these receptors.

EXPERIMENTAL SECTION

The melting points were determined with a Büchi apparatus and are uncorrected. The ^{1}H NMR spectra were obtained with a Varian EM-390 90-MHz spectrometer, using Me_4Si (tetramethylsilane) as internal standard. TLC were carried out on silica gel 60 F-254 (Merck) precoated TLC plates. For column chromatography, silica gel 60 (Merck) was used.

5,7-Dichloro-3H-imidazo[4,5-b]pyridine (2). To an ice-cooled solution of 60 ml of $POCL_3$ and 30 ml of dry dimethylformamide was added portionwise 3 g (15.9 mmol) of 7-nitroimidazo-[4,5-b]pyridine-4-oxide (1).[9] The mixture was refluxed for 10 min and the excess phosphoryl chloride was removed in vacuo. The residue was stirred with crushed ice and neutralized with saturated Na_2CO_3 solution and then extracted several times with EtOAc. The combined extracts were dried (Na_2SO_4) and evaporated in vacuo to a residue, which was chromatographed on a silica gel column. Elution with $CHCl_3$-nC_6H_{14}-MeOH (7:2:1) gave 1.56 g (50%) of 2 as a chromatografically pure solid: mp 274-275°C (lit.[14] mp 275-276°C).

5,7-Dichloro-3-(2',3',5'-tri-O-acetyl-ß-D-ribofuranosyl)-3H-imidazo[4,5-b]pyridine (3). An intimate mixture of 2 (1 g, 5.31 mmol) and 1,2,3,5-tetra-O-acetyl-ß-D-ribofuranose (3.38 g, 10.62 mmol) was heated to 170°C with stirring. To the molten mixture p-toluenesulfonic acid (50 mg) was added and the melt was heated at 170°C under reduced pressure (12-15 mm) for 20 min. The resulting solid was neutralized with saturated K_2CO_3 solution and extracted several times with $CHCl_3$. The chloroform extracts were dried with anhydrous Na_2SO_4 and evaporated in vacuo to a residue, which was chromatographed on a silica gel column. Elution with $CHCl_3$-nC_6H_{14}-MeOH (80:15:5) yielded 1.1 g (47%) of 3 as a vetrous pure solid.[11]

5,7-Dichloro-3-ß-D-ribofuranosyl-3H-imidazo[4,5-b]pyridine (4). A solution of 3 (5 g, 11.2 mmol) in 90 ml of methanol saturated at 0°C with ammonia was set aside at room temperature for 24 h. The solvent was removed in vacuo and the residue was chromatographed on a silica gel column eluting with $CHCl_3$-MeOH (87:13). The resulting solid was crystallized from water to give 1.6 g (43%) of **4**: mp 155-157°C (lit.[11] mp 155-156°C).

7-Amino-5-chloro-3-ß-D-ribofuranosyl-3H-imidazo[4,5-b]pyridine (5) (2-chloro-1-deazaadenosine). Method A. A solution of 0.8 g (2.5 mmol) of **4** in 15 ml of methanolic ammonia (saturated at 0°C) was heated in a sealed tube at 140°C for 24 h. The solvent was removed in vacuo and the residue was chromatographed on a silica gel column eluting with $CHCl_3$-MeOH-NH_3 (80-18-2). Crude compound **5** was recrystallized from absolute ethanol (450 mg, 64%): mp 117-120°C; ^1H NMR (Me_2SO-d_6) δ 1.11 (t, 3, CH_3 ethanol), 3.50 (q, 2, CH_2 ethanol), 3.66 (m, 2, CH_2-5'), 4.00 (m, 1, H-4'), 4.20 (m, 1, H-3'), 4.60 (m, 1, H-2'), 5.92 (d, 1, J=6Hz, H-1'), 6.47 (s, 1, H-2), 6.86 (s, 2, NH_2), 8.38 (s, 1, H-6).

Anal. Calcd for $C_{11}H_{13}ClN_4O_4 \cdot C_2H_5OH$: C, 45.02; H, 5.52; N, 16.50. Found: C, 44.91; H, 5.40; N, 16.33.

Method B. To a solution of 200 mg (0.6 mmol) of **8** in 30 ml of methanol was added 500 mg of Raney nickel catalyst, and the mixture was shaken with hydrogen at 30 psi for 2 h. The catalyst was removed by filtration, the filtrate was evaporated and the resulting solid was recrystallized from absolute ethanol to give 150 mg (70%) of **5**.

5-Chloro-7-nitro-3H-imidazo[4,5-b]pyridine (6). 3 g (15.9 mmol) of 7-nitroimidazo[4,5-b]pyridine-4-oxide (**1**)[9] was added portionwise to 30 ml of cooled (-20°C) phosphorylchloride and the suspension was heated at 110°C for 1 h. The phosphorylchloride was removed in vacuo to give a residue which was neutralized with saturated Na_2CO_3 solution and then extracted several times with $CHCl_3$. The chloroform extracts were dried with anhydrous Na_2SO_4 and evaporated in vacuo to a residue, which was chromatographed on a silica gel column. Elution with $CHCl_3$-nC_6H_{14}-MeOH (5:4:1) yielded 1.6 g (49%) of **6** as a yellow-orange solid and 0.3 g (10%) of **2**. Analytical sample of **6** was obtained by recrystallization from ethanol-water (1:1):mp >300°C; ^1H NMR (Me_2SO-d_6) δ 8.06 (s, 1, H-6), 8.86 (s, 1, H-2).

Anal. Calcd for $C_6H_3ClN_4O_2$: C, 36.29; H, 1.52; N, 28.21. Found: C, 36.53; H, 1.36; N, 27.98.

5-Chloro-7-nitro-3-(2',3',5',-tri-O-acetyl-ß-D-ribofuranosyl)-3H-imidazo[4,5-b]pyridine (7). To a mixture of 0.64 g (2.01 mmol) of 1,2,3,5-tetra-O-acetyl-ß-D-ribofuranose in 50 ml of dry acetonitrile and 0.4 g (2.01 mmol) of **6** was added 0.23 ml of stannic chloride dissolved in 20 ml of dry acetonitrile, and the solution was stirred at room temperature for 12 h. The reaction mixture was concentrated to small volume, and then 1.2 g of $NaHCO_3$ and 4 ml of H_2O were added. The solution was evaporated to dryness in vacuo and the residue was extracted with boiling chloroform (100 ml x 3). The extracts were dried (Na_2SO_4) and evaporated, and the residue was chromatographed on a silica gel column eluting with $CHCl_3$-MeOH (96.5:3.5) to give 0.42 g (46%) of **7** as a viscous pure solid: ^1H NMR (Me_2SO-d_6) δ 6.41 (d, 1, J=5Hz,H-1'), 8.17 (s, 1, H-6), 9.05 (s, 1, H-2).

Anal. Calcd for $C_{17}H_{17}ClN_4O_9$: C, 44.69; H, 3.75; N, 12.20. Found: C, 44.92; H, 3.61; N, 12.45.

5-Chloro-7-nitro-3-ß-D-ribofuranosyl-3H-imidazo[4,5-b]pyridine (8). A solution of **7** (0.5 g, 1.09 mmol) in 15 ml of methanol saturated at 0°C with ammonia was set aside at room temperature for 3 days. The solvent was removed in vacuo and the residue was chromatographed on a silica gel column eluting with $CHCl_3$-MeOH (9:1) to give 0.25 g (69.4%) of 8 as a chromatografically pure solid: mp > 300°C, dec; ^1H NMR (Me_2SO-d_6) δ 5.94 (d, 1, J=5.5 Hz, H-1'), 6.90 (s, 1, H-6), 8.55 (s, 1, H-2).

Anal. Calcd for $C_{11}H_{11}ClN_4O_6$: C, 39.95; H, 3.35; N, 16.94. Found: C, 39.68; H, 3.49; N, 16.67.

7-Amino-3H-imidazo[4,5-b]pyridine (9). To a solution of 0.2 g (2 mmol) of **6** in 50 ml of ethanol-water (2:1) was added 0.15 g of 10% Pd/C, and the mixture was shaken with hydrogen at 40 psi for 6 h. The catalyst was removed by filtration, the filtrate was evaporated, and the residue was chromatographed on a silica gel column. Elution with EtOAc-MeOH-NH_3 (90:9:1) gave 0.14 g (70%) of **9** as a pure solid: mp 257-259°C. The base was converted to the hydrochloride which was identical with an autentic sample.[15]

7-Amino-3-ß-D-ribofuranosyl-3H-imidazo[4,5-b]pyridine (1-deazaadenosine) (10). To a solution of 0.2 g (0.66 mmol) of **5** in 20 ml of H_2O was added 0.1 g of 10% Pd/C, and the mixture was shaken with hydrogen at 42 psi for 2 h. The catalyst was removed and the filtrate was concentrated to dryness. Recrystallization from H_2O afforded 0.13 g (75%) of **10** as colourless needles, mp 262-263°C,dec. (Lit,[15] mp 263-264°C).

Biological Methods. Male Sprague Dawley rats (Charles River, Calco, Italy) weighing 200-300 g were killed by decapitation, their brains removed and disrupted in 20 vol. of Tris-HCl buffer at pH 7.7 with a Ultra Turrax homogenizer (30", set 5). The homogenate was centrifuged at 50,000 x g for 10 min, the pellet resuspended in buffer, centrifuged again, and finally resuspended in buffer containing 2 U.I./ml of adenosine deaminase (Sigma, Type III). After 30 min, incubation at 37°C, the membranes were recentrifuged and the pellet was stored at -70°C. The measurement of [^{3}H]CHA binding was performed using filtration technology.[16,17]

400 µl of membranes suspension, containing 400-600 µg protein were incubated at 25°C with 5 nM [^{3}H]CHA in a final volume of 1 ml of incubation buffer. After a 2 h incubation, samples were collected on Whatman GF/B glass-fiber filters under reduced pressure, washed three times with 5 ml buffer and assayed for radioactivity in a liquid scintillation counter. Protein determination was performed according to the method of Lowry et al.[18]

Dose-inhibition curves were generated with five to seven concentrations of drugs in triplicate samples. Nonspecific binding was determined in the presence of 10 µM of L-PIA. 2-Chloro-1-deazaadenosine, 3-deazaadenosine and 7-deazaadenosine were dissolved in 10 µl HCl 37% and brought to the final volume with buffer (pH 7.4). IC_{50} values were determined by computer analysis using an Apple IIe as described by Benfenati and Guardabasso.[19]

All the compounds listed in Table 1 were tested also as inhibitors of ADP human platelet aggregation <u>in vitro</u> according to the method of Born and Cross,[20] using a 1060 S ELVI 840 platelet aggregation meter. The aggregative agent ADP was purchased from Mascia Brunelli (Centropa AGP test); blood was obtained by venipuncture in the forearms of apparently healty humans and collected in polyethylene tubes containing a 1:9 volume of 3.8% sodium citrate. Platelet-rich plasma was obtained by centrifugation at 1200 rpm for 10 min, while platelet-poor plasma was obtained by centrifugation at 4500 rpm for 20 min. The platelet-rich plasma was adjusted to 250,000 platelets/ml by adding platelet-poor plasma. A 50 µl aliquot of the test sample dissolved in Michaelis buffer was added to the test control. The cuvette was placed in the aggregation meter and allowed to incubate at 37°C for 5 min, after which 50 µl of ADP was added to the platelet-rich plasma. The final concentration of each sample was 1×10^{-4} M. The per cent inhibition of aggregation by a test compound was calculated by dividing the maximal deflection in the optical density curve in the presence of the compound by that observed in the control, then moltiplying by 100. As inhibition percentage differed from preparation to preparation of platelet-rich plasma, relative potency (RAD) to a reference standard (adenosine) in the same concentration was a direct measure of the potency of inhibition.

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