2-[N'-(3-Arylallylidene)hydrazino]adenosines Showing A_{2a} Adenosine Agonist Properties and Vasodilation Activity

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A series of 2-[N'-(3-arylallylidene)hydrazino]adenosines were prepared and studied in binding and functional assays to assess their potency for the A_{2a} compared with the A_1 adenosine receptor. These analogs possess A_{2a} receptor affinity in the low nanomolar range associated with weak interaction with the A_1 receptor. Among the compounds, in rat tissues, 2-[N'-[3-(4-nitrophenyl)allylidene]hydrazinoadenosine (**5g**) had the most potent affinity for the A_{2a} receptor, the K_i value being 23 nM. The type and position of substituents on the phenyl ring show a moderate influence on biological activity, allowing the conclusion that the latter is mostly due to the allylidenehydrazino side chain. From functional experiments 2-[N'-(2-furylmethylidene)hydrazino]adenosine (**4b**), 2-[N'-(3-phenylallylidene)hydrazino]adenosine (**5a**) and 2-[N'-[3-(2-furyl)allylidene]hydrazino]adenosine (**5b**) appeared to be potent in inducing vasorelaxation (an A_{2a}-mediated response) without appreciable effects on heart rate (an A_1-mediated action). While the lack of effects on heart rate is clearly explained by the poor affinity for A_1 receptors, more difficult appears the interpretation of vasorelaxant properties displayed by some compounds. Affinity for A_{2a} has a major role, but other types of interactions, yet to be identified, may play a part.

Introduction

Adenosine exerts its physiological actions through activation of four distinct subtypes of cell surface receptors which have been recently classified as A_1, A_{2a}, A_{2b}, and A_3.[1] Structure–activity relationship (SAR) efforts made over several years have made it clear that the structure of adenosine is critical to retain agonist activity at adenosine receptors.[2] Structural modification at N^6, C-2, or 5' yields a variety of adenosine analogs with different potency and selectivity for each individual receptor subtype. Thus, N^6 substitutions such as N^6-cyclohexyladenosine and N^6-cyclopentyladenosine lead to selective A_1 agonists.[2,3] Modifications of the C-2 position, especially when combined with the 5'-(*N*-ethylcarboxamido) substitution, confer high potency and selectivity at A_{2a} receptors. Typical examples are 2-[2-(4-methoxyphenyl)ethoxy]adenosine,[4] 2-[(2-cyclohexylethyl)amino]adenosine,[5] and 2-[[4-(2-carboxyethyl)phenethyl]amino]adenosine-5'-(*N*-ethyluronamide) (CGS 21680).[6] Recently, it has been reported that introduction of appropriate substitutions in both N^6 and 5' positions lead to compounds which preferentially interact with the A_3 receptor.[7]

Given the variety of physiological effects specifically modulated by each adenosine receptor subtype,[7b] there is continous interest toward the synthesis of more potent and selective adenosine agonists. With regard to A_{2a} agonists, it is known that such compounds produce vasodilation,[8] inhibit platelet aggregation[9] and neutrophil adhesion to vascular endothelium, and reduce generation of oxygen free radicals by activated neutrophils.[10] Therefore, selective A_{2a} receptor agonists have the potential for the treatment of cardiovascular pathologies such as hypertension and ischemic heart diseases.[11] It has been demonstrated that in the C-2 position a polar linkage group such as –NH–, –O–, –C≡C–, or –NH–N=, followed by a lipophilic group such as cyclohexyl or phenyl, is required in order to achieve high affinity for the A_{2a} receptor. Olsson et al.[12] reported the synthesis of a series of 2-(N'-alkylidenehydrazino)- and 2-(N'-aralkylidenehydrazino)adenosines as potent and selective A_{2a} agonists in which the alkyl or aryl group is directly bonded to the hydrazone carbon. Studies of several C-2 adenosine derivatives have shown that a spacer is needed between the polar atom or group and the lipophilic moiety at the C-2 position to enhance affinity at A_{2a} receptors. In the case of 2-(N'-aralkylidenehydrazino)adenosines, it has been reported that a $-CH_2-$ or $-CH_2CH_2-$ group inserted between the hydrazone carbon and the aryl substituents does not affect the A_{2a} potency significantly.

Our compounds were designed with the aim to find new A_{2a} agonists and improve knowledge on the effects of the C-2 side chain structure by inserting a –CH=CH– spacer between the hydrazone carbon and the aryl substituent. With this background it was of interest to assess whether the presence of a rigid and conjugated group could affect the potency and selectivity of new adenosine derivatives for A_{2a} receptors.

Results and Discussion

Chemistry. Reacting 2-hydrazinoadenosine (**1**) with an aryl aldehyde (**2a,b**) or a substituted arylpropenal (**3a–i**) produced the corresponding hydrazones **4a,b** and **5a–i**, respectively (Scheme 1). The reactions were performed with equimolar amounts of aldehyde and **1** in water with a catalytic amount of acetic acid. Alternatively, reaction in refluxing methanol was used for compounds **5d,h,i**. The structures of all products were confirmed by ^1H NMR data which show that in the case

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Scheme 1

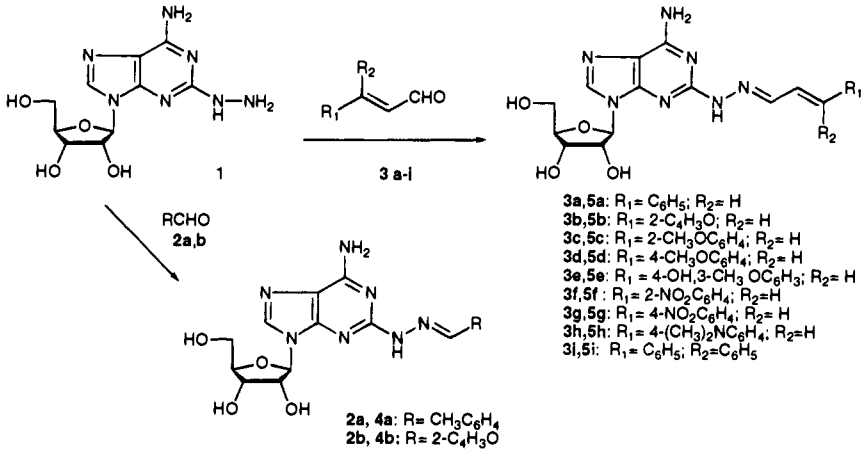
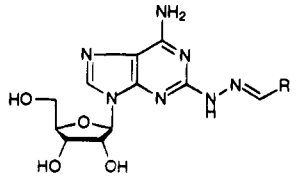


Table 1. *In Vitro* Pharmacological Activity of Compounds **4a,b**



4a,b

		binding assay[a] K_i (nM)			functional activity[b] EC_{50} (nM)	
compd	R	rat brain (A_1)	rat striatum (A_{2a})	selectivity (A_1/A_{2a})	rat atria (A_1)	rat aorta (A_{2a})
CGS 21680		569 (511–634)	11 (9.4–13)	52	>10 μM	115 (53–251)
NECA		10 (9.4–12)	7.8 (6.6–9.1)	1.3	55 (35–78)	394 (209–792)
CCPA		1.3 (1.1–1.4)	650 (555–762)	0.002	8.2 (4.4–15)	>10 μM
4a	4-$CH_3C_6H_4$	1770 (1270–2460)	29 (26–32)	62	>10 μM	747 (425–1240)
4b	2-C_4H_3O	1850 (1750–1950)	42 (33–53)	44	>10 μM	195 (105–308)

[a] Receptor binding affinity at A_1 and A_{2a} receptors was determined using [^{3}H]CHA and [^{3}H]CGS 21680 as radioligands, respectively. Data are geometrical means from at least three separate experiments; 95% confidence limits are in parentheses. [b] Data are means from at least three separate experiments; 95% confidence limits are in parentheses.

of products **5** the *E*-configuration of the C=C double bond of the starting aldehydes was retained as expected. This is demonstrated by a coupling constant of 10 Hz.

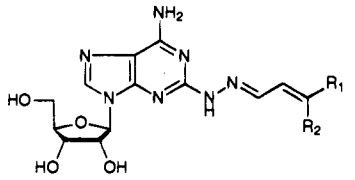
Receptor Binding Affinity. Affinity of the 2-(*N'*-aralkylidenehydrazino)adenosines **4** and 2-[*N'*-(arylallylidene)hydrazino]adenosines **5** with A_1 and A_{2a} receptors was evaluated using radioligand binding assays. A_{2a} receptors were studied in competition assays in rat striatum using [^{3}H]CGS 21680 as radioligand.[13] Affinity for A_1 receptors was determined in rat whole brain using N^6-cyclohexyladenosine.[14] Adenosine-5'-(*N*-ethyluronamide) (NECA), CGS 21680, and 2-chloro-N^6-cyclopentyladenosine (CCPA) were included as reference compounds. The results are reported in Tables 1 and 2.

Data for compounds **4** and **5** show that lengthening of the alkylidene side chain with the conjugated double bond retained affinity at the A_{2a} receptor. In fact, both **4a,b** showed A_{2a} affinity and selectivity similar to that of compounds **5a,b**. Compound **5g** is the most potent of the series showing an A_{2a} affinity of 23 nM. Changing the position of the nitro group from para to ortho (**5f**) led to a decrease in the affinity for both receptors. The introduction of an *o*-methoxy group (**5c**) produced a decrease in A_{2a} affinity and an increase in A_1 potency. The methoxy group in the para position (**5d**) instead retained an affinity similar to that of compound **5g**. The *m*-methoxy *p*-hydroxy analog **5e** also retained good A_{2a} affinity. Surprisingly, *N,N*-dimethylamino substitution (**5h**) greatly enhanced A_1 affinity and decreased A_{2a} potency leading to a nonselective compound. The present data allow the conclusion that biological activity is associated mostly with the allylidenehydrazino side chain itself, and only minor effects are due to the type and ring position of substituents. The presence of a bulky chain as in the diphenyl derivative **5i** is detrimental for A_{2a} affinity (IC_{50} >1000 nM), meanwhile A_1 potency is increased clearly showing that the A_{2a} receptors do not tolerate bulky substituents in this position.

Effects in Isolated Tissues. Negative chronotropic activity (A_1) was tested in spontaneously beating rat atria and A_{2a}-mediated vasodilation in rat aorta precontracted with prostaglandin F2α (3 μM) according to a method described elsewhere.[8] Results are summarized in Tables 1 and 2.

Both the compounds of series **4** have shown A_{2a} selectivity and vasodilating properties. In rat aorta preparation compound **4a** was weakly active being

Table 2. *In Vitro* Pharmacological Activity of Compounds **5a**–i



5a-i

compd	R_1	R_2	binding assay[a] K_i (nM) rat brain (A_1)	rat striatum (A_{2a})	selectivity (A_1/A_{2a})	functional activity[b] EC_{50} (nM) rat atria (A_1)	rat aorta (A_{2a})
CGS 21680			569 (511–634)	11 (9.4–13)	52	>10 μM	115 (53–251)
NECA			10.4 (9.4–12)	7.8 (6.6–9.1)	1.3	55 (35–78)	394 (209–792)
CCPA			1.3 (1.1–1.4)	650 (555–762)	0.002	8.2 (4.4–15)	>10 μM
5a	C_6H_5	H	1050 (962–1140)	38 (37–40)	27	>10 μM	269 (162–464)
5b	2-C_4H_3O	H	1670 (1150–2400)	83 (62–113)	20	4340 (1500–12600)	800 (401–1600)
5c	2-$CH_3OC_6H_4$	H	264 (193–359)	384 (257–574)	0.7	1970 (229–787)	529 (277–4920)
5d	4-$CH_3OC_6H_4$	H	425 (344–426)	59 (38–91)	7	>10 μM	677 (173–1260)
5e	4-OH-3-$CH_3OC_6H_3$	H	545 (473–629)	27 (19–39)	20	4890 (3160–7550)	281 (141–560)
5f	2-$NO_2C_6H_4$	H	935 (737–1190)	54 (38–76)	17	>10 μM	638 (296–2370)
5g	4-$NO_2C_6H_4$	H	524 (509–539)	23 (16–32)	23	>10 μM	482 (322–721)
5h	4-$(CH_3)_2NC_6H_4$	H	101 (67–152)	170 (102–284)	0.6	866 (410–1830)	728 (250–1740)
5i	C_6H_5	C_6H_5	64 (52–78)	>1000		>1860	704 (185–1550)

[a] Receptor binding affinity at A_1 and A_{2a} receptors was determined using [^{3}H]CHA and [^{3}H]CGS 21680 as radioligands, respectively. Data are geometrical means from at least three separate experiments; 95% confidence limits are in parentheses. [b] Data are means from at least three separate experiments; 95% confidence limits are in parentheses.

effective in the high nanomolar range. This weak response appears to be in contrast with the good affinity found in binding studies (K_i 29 nM). Unlike the other agonists examined, compound **4a** showed a different shape of dose–response curve, as indicated by the slope value which was 2.53 ± 0.23 compared with the range of 1.14 ± 0.08 and 1.72 ± 0.18 ($p < 0.05$) displayed by the other adenosine analogs. This different response may well depend upon interaction of **4a** with other receptor systems. Conversely, compound **4b** induced vasorelaxation similar to that observed for the reference A_{2a} agonist CGS 21680.

As regards compounds **5a**–**i**, they selectively induced relaxation of rat aorta with maximal effect ranging between 70% and 100% of precontraction values. As shown in Figure 1 compounds **5a,e** were the most potent vasodilating agents with EC_{50} values similar to that obtained for CGS 21680. The *p*-nitro derivative **5g** appeared to be somewhat less potent in inducing vasorelaxation than **5e**, even though their binding affinity was similar. Moreover, changing the position of the nitro group from para to ortho led to an even less vasorelaxant effect, as evidenced in binding studies. Compounds **5b**–**d,f,h,i** displayed a weak vasorelaxant effect. Except for **5h**, all compounds did not inhibit chronotropic activity in rat atria, a finding which was expected on the basis of binding studies. Compound **5h**, having an affinity of 100 nM (K_i value) for A_1 receptors, showed a measurable decrease of A_1-mediated beating rate at 866 nM (EC_{50} value). The agonist **5i** relaxed rat aorta without inducing significant negative chronotropic effect. The latter finding appears to be in contrast with binding data, where the compound showed an A_1 affinity of 64 nM.

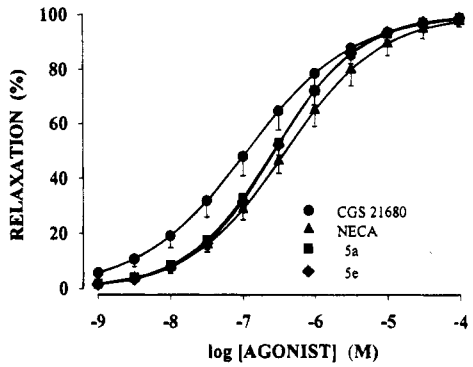


Figure 1. Mean dose–response curve for vasorelaxant activity induced by CGS 21680 (●), NECA (▲), and selected new adenosine agonists **5a** (■) and **5e** (◆) in isolated rat aorta. Each response is expressed as the percentage of the maximal relaxation in vessels precontracted by PGF2α (3 μM). Each data point is the mean with vertical bars showing 95% confidence limits of at least four experiments.

Conclusions

Compounds **4a,b** and **5a**–**g** show an appreciable A_{2a} receptor affinity and good A_{2a} versus A_1 selectivity. The activity found for the 2-[*N'*-(3-arylallylidene)hydrazino]-adenosines indicates that the rigidity of the C-2 side chain, due to the insertion of a CH=CH spacer between the hydrazone carbon and the aryl substituent, leads

to compounds with the same order of activity as the analog hydrazones of aryl aldehydes. Compounds 2-[*N'*-[3-(4-nitrophenyl)allylidene]hydrazino]adenosine (**5g**) and 2-[*N'*-[3-(4-hydroxy-3-methoxyphenyl)allylidene]hydrazino]adenosine (**5e**) had the most potent affinity for the A_{2a} receptors with K_i values around 20 nM, whereas the best separation between A_{2a} and A_1 receptors was found for **4a** with a ratio of 62. However these results indicate that the type and ring position of substituents weakly affect binding affinity and selectivity. From functional experiments compounds **4b** and **5a,b** appear to be potent in inducing vasorelaxation without appreciable effects on heart rate. The lack of effects on heart rate is clearly explained by their poor affinity for A_1 receptors; more difficult to explain is the interpretation of vasorelaxant properties displayed by some compounds. Affinity for A_{2a} receptors has a major role, but other types of interactions yet to be identified may play a part. For example, it has been recently shown that some A_{2a} agonists may produce vasorelaxation through mechanisms such as those involving K^+ channels, not strictly related to stimulation of A_{2a} receptors.[15] Further studies will be necessary to elucidate this aspect. It is important however to outline that the best information on the profile of new adenosine analogs can be obtained using binding assays combined with functional studies.

Experimental Section

Chemistry. Melting points were determined using a Büchi 510 (capillary) apparatus or an Electrothermal 9100 apparatus and are uncorrected. ^{1}H NMR spectra (ppm, DMSO-d_6 as solvent) were obtained with either a Brucker AC 200 or an EM-390 Varian instrument. Chemical shifts (δ) are given in ppm, and the coupling constants (J) are given in Hz. TLC utilized ready to use silica gel plates. Column chromatography was accomplished on silica gel (Kieselgel 60, 230–400 mesh, 230 ASTM; Merck) with the eluant indicated. 2-Hydrazinoadenosine is a known compound.[12a] Starting aldehydes were commercial products. Compound **4a** was prepared according to literature.[12b] Analyses indicated by the symbols of the elements were within ±0.4% of theoretical values.

General Procedure A for the Preparation of Compounds 4b and 5a–c,e–g. To a solution of 1 (1 mM) in H_2O (5 mL) containing 2 drops of CH_3COOH was added the aldehyde **2** or **3** (1 mM). The reaction mixture was stirred at room temperature until completion of the reaction (TLC). The residue resulting from solvent evaporation was purified to obtain the final product.

General Procedure B for the Preparation of Compounds 5d,h,i. A mixture of 1 (1 mM) and aldehyde (1.5 mM) **3d,h,i** in methanol (10 mL) was heated at reflux until completion of the reaction (TLC). The residue resulting from solvent evaporation was purified to obtain the final product.

2-[*N'*-(2-Furylmethylidene)hydrazino]adenosine (4b). The title compound was prepared according to the general procedure A. Reaction time 8 h. Purification by washing with diethyl ether. Yield 63%. Mp 198 °C dec. ^{1}H NMR: δ 3.10–5.90 (br, 3H, OH), 3.60 (m, 2H, C*H*$_2$OH), 3.96 (m, 1H, 4'-H), 4.16 (m, 1H, 3'-H), 4.48 (m, 1H, 2'-H), 5.80 (d, 1H, 1'-H, J = 5.19 Hz), 6.69 (s, 1H, furyl proton), 7.23 (s, 1H, furyl proton), 7.89, 8.11, 8.42 (s, 3H, CH=N, H-8, 1H, furyl proton), 9.00 (br, 2H, NH_2),12.2 (br, 1H, NH). Anal. ($C_{15}H_{17}N_7O_5$) C,H,N.

2-[*N'*-(3-Phenylallylidene)hydrazino]adenosine (5a). The title compound was prepared according to the general procedure A. Reaction time 1 h. Purification by washing with ethanol. Yield 85%. Mp 168 °C dec. ^{1}H NMR: δ 3.60 (m, 2H, C*H*$_2$OH), 3.95 (m, 1H, 4'-H), 4.16 (m, 1H, 3'-H), 4.40 (d, 1H, OH) 4.60 (m, 1H, 2'-H), 5.00–5.80 (3H, OH), 5.82 (d, 1H, 1'-H, J = 6.31), 6.83–7.71 (m, 9H, 5H aromatic protons, NH_2, Ph–CH–, CH=N) 7.94 (d, 1H, CH=N, J = 6.31 Hz), 8.15 (s, 1H, H-8), 11.00 (br, 1H, NH). Anal. ($C_{19}H_{20}N_7O_4$) C,H,N.

2-[*N'*-[3-(2-Furyl)allylidene]hydrazino]adenosine (5b). The title compound was prepared according to the general procedure A. Reaction time 15 min. Purification by washing with 2-propanol. Yield 62%. Mp 145 °C dec. ^{1}H NMR: δ 3.65 (m, 2H, C*H*$_2$OH), 3.95 (m, 1H, 4'-H), 4.16 (m, 1H, 3'-H), 4.40 (d, 1H, OH) 4.60 (m, 1H, 2'-H), 5.12 (d, 1H, OH), 5.42 (d, 1H, OH), 5.80 (d, 1H, 1'-H, J = 6.80 Hz), 6.55–6.75 (m, 4H, 2H furyl protons, C*H*=C*H*–CH=N), 7.09 (br, 2H, NH_2), 7.73 (s, 1H furyl proton), 7.87 (d, 1H, –CH=N, J = 8.06 Hz), 8.06 (s, 1H, H-8), 10.66 (br, 1H, NH). Anal. ($C_{17}H_{19}N_7O_5$) C,H,N.

2-[*N'*-[3-(2-Methoxyphenyl)allylidene]hydrazino]adenosine (5c). The title compound was prepared according to the general procedure A. Reaction time 15 min. Purification by column chromatography by eluting with CH_2Cl_2/MeOH (10:1, v/v). Yield 12%. Mp 189 °C dec. ^{1}H NMR: δ 3.50 (m, 2H, C*H*$_2$OH), 3.89 (s, 3H, CH_3O), 3.94 (m, 1H, 4'-H), 4.16 (m, 1H, 3'-H), 4.45 (m, 1H, 2'-H), 5.80 (d, 1H, 1'-H, J = 6.27 Hz), 6.97–7.40 (m, 6H, 4H aromatic protons, Ph–CH–, CH–C*H*=N), 8.05 (d, 1H, CH=N, J = 8.83 Hz), 8.43 (s, 1H, H-8), 12.20 (br, NH). Anal. ($C_{20}H_{23}N_7O_5$) C,H,N.

2-[*N'*-[3-(4-Methoxyphenyl)allylidene]hydrazino]adenosine (5d). The title compound was prepared according to the general procedure B. Reaction time 15 min. Purification by column chromatography by eluting with CH_2Cl_2/MeOH (10:1, v/v). Yield 23%. Mp 170 °C dec. ^{1}H NMR: δ 3.50 (m, 2H, C*H*$_2$OH), 3.80 (s, 3H, CH_3O), 3.94 (m, 1H, 4'-H), 4.16 (m, 1H, 3'-H), 4.60 (m, 1H, 2'-H), 5.80 (d, 1H, 1'-H, J = 5.62 Hz), 6.70–7.11 (m, 5H, 2H aromatic protons, Ph–CH=C*H*, NH_2), 7.51 (2H, aromatic protons), 7.88 (1H, Ph–CH), 8.02–8.08 (2H, H-8, CH=N), 12.20 (br, NH). Anal. ($C_{20}H_{23}N_7O_5$) C,H,N.

2-[*N'*-[3-(4-Hydroxy-3-methoxyphenyl)allylidene]hydrazino]adenosine (5e). The title compound was prepared according to the general procedure A. Reaction time 15 min. Purification by washing with ethanol. Yield 39%. Mp 150 °C dec. ^{1}H NMR: δ 3.60 (m, 2H, C*H*$_2$OH), 3.83 (s, 3H, CH_3O), 3.93 (m, 1H, 4'-H), 4.14 (m, 1H, 3'-H), 4.57 (m, 1H, 2'-H), 5.11 (br, 1H, OH) 5.20 (d, 1H, OH), 5.82 (d, 1H, 1'-H, J = 6.51), 6.66–7.24 (m, 7H, 3H aromatic protons, NH_2, Ph–CH–, C*H*–CH=N), 7.87 (d, 1H, CH=N, J = 8.83 Hz), 8.12 (s, 1H, H-8), 9.27 (s, 1H, *m*-OH–Ph), 10.62 (br, 1H, NH). Anal. ($C_{20}H_{23}N_7O_6$) C,H,N.

2-[*N'*-[3-(2-Nitrophenyl)allylidene]hydrazino]adenosine (5f). The title compound was prepared according to the general procedure A. Reaction time 15 min. Purification by washing with ethanol. Yield 39%. Mp 147 °C dec. ^{1}H NMR: δ 3.60 (m, 2H, C*H*$_2$OH), 3.93 (m, 1H, 4'-H), 4.14 (m, 1H, 3'-H), 4.60 (m, 1H, 2'-H), 5.08–5.46 (3H, OH) 5.83 (d, 1H, 1'-H, J = 6.03 Hz), 6.99–7.23 (m, 4H, NH_2, Ph–CH–, C*H*–CH=N), 7.49–7.57 (m, 1H, aromatic proton), 7.49–7.57 (m, 1H, aromatic proton), 7.91–8.00 (m, 3H, CH=N, 2H aromatic proton), 8.11 (s, 1H, H-8), 10.85 (br, 1H, NH). Anal. ($C_{19}H_{20}N_8O_6$) C,H,N.

2-[*N'*-[3-(4-Nitrophenyl)allylidene]hydrazino]adenosine (5g). The title compound was prepared according to the general procedure A. Reaction time 15 min. Purification by column chromatography by eluting with CH_2Cl_2/MeOH (8.5:1, v/v). Yield 25%. Mp 160 °C dec. ^{1}H NMR: δ 3.60 (m, 2H, C*H*$_2$OH), 3.94 (m, 1H, 4'-H), 4.17 (m, 1H, 3'-H), 4.60 (m, 1H, 2'-H), 5.10–5.46 (3H, OH), 5.84 (d, 1H, 1'-H, J = 6.68), 6.95 (d, 1H, Ph–C*H*–, J = 15.80 Hz), 7.11 (br, NH_2), 7.29 (dd, 1H, CH–C*H*–CH=N, J = 15.80, 9.97 Hz), 7.84 (d, 2H, aromatic protons, J = 8.79 Hz), 7.95 (d, 1H, CH=N, J = 9.97 Hz), 8.11 (s, 1H, H-8), 9.21 (d, 2H, aromatic protons), 10.90 (br, 1H, NH). Anal. ($C_{19}H_{20}N_8O_6$) C,H,N.

2-[*N'*-[3-[4-(Dimethylamino)phenyl]allylidene]hydrazino]adenosine (5h). The title compound was prepared according to the general procedure B. Reaction time 1 h 20 min. Purification by column chromatography by eluting with CH_2Cl_2/MeOH (10:1, v/v). Yield 32%. Mp 190 °C dec. ^{1}H NMR: δ 3.60 (m, 2H, C*H*$_2$OH), 3.94 (m, 1H, 4'-H), 4.17 (m, 1H, 3'-H), 4.47 (m, 1H, 2'-H), 4.90–5.70 (3H, OH), 5.80 (d, 1H, 1'-H, J = 5.86), 6.69–6.82 (m, 4H, 2H aromatic protons, Ph–CH–, CH–C*H*=N), 8.05 (d, 1H, CH=N, J = 8.83 Hz), 8.43 (s, 1H, H-8), 12.20 (br, NH). Anal. ($C_{21}H_{25}N_7O_6$) C,H,N.

2-[N'-(3,3-Diphenylallylidene)hydrazino]adenosine (5i). The title compound was prepared according to the general procedure B. Reaction time 1 h 20 min at room temperature. Purification by column chromatography by eluting with CH_2Cl_2/MeOH (10:1, v/v). Yield 85%. Mp 168 °C dec. ^{1}H NMR: δ 3.60 (m, 2H, CH_2OH), 3.95 (m, 1H, 4'-H), 4.17 (m, 1H, 3'-H), 4.60–4.68 (m, 2H, 2'-H, OH), 5.12 (d, 1H, OH), 5.39 (d, 1H, OH), 5.79 (d, 1H, 1'-H, J = 6.30), 6.94 (d, 1H, CH–CH=N, J = 9.89 Hz), 7.02 (br, 2H, NH_2), 7.23–7.50 (m, 10H, aromatic protons), 7.75 (d, 1H, CH=N, J = 9.89 Hz), 8.04 (s, 1H, H-8), 10.59 (br, 1H, NH). Anal. ($C_{25}H_{25}N_7O_4$) C,H,N.

Biological Assays. A_1 and A_{2a} Receptor Binding Assays. Cerebral membranes were obtained from male Sprague–Dawley rats (Charles River, Calco, Italy) weighing 150–200 g. Adenosine A_1 and A_{2a} receptor binding assays were performed according to Bruns et al.[14] and Jarvis et al.[13] using [^{3}H]-*N*-cyclohexyladenosine ([^{3}H]CHA) and [^{3}H]-2-[[4-(2-carboxyethyl)phenethyl]amino]adenosine-5'-(*N*-ethylcarboxamide) ([^{3}H]CGS 21680), respectively. The IC_{50}'s were estimated by probit analysis.[16] K_i values were calculated from the Cheng–Prusoff equation[17] using 1 nM as the K_d for [^{3}H]CHA and 18.5 nM for [^{3}H]CGS 21680 in A_1 and A_{2a} binding studies, respectively.

Isolated Tissues. Rats were sacrificed by decapitation; both heart and thoracic aorta were removed and placed in Krebs–Henseleit's solution according to a method described elsewhere.[8] Spontaneously beating rat atria were used to measure drug interaction with A_1 receptors. The decrease in heart rate evoked by cumulative addition of agonist was measured. Vascular tissue was specifically used to measure the interaction of adenosine analogs with A_{2a} receptors. Specimens of rat aorta were cleaned of connective tissue, cut into rings, and allowed to equilibrate in organ baths. Submaximal contractions of vascular rings were obtained by PGF2α (3 μM). The compounds were then added cumulatively, and vascular relaxation was measured isometrically. The relationship between the contractile response (y) and the log dose was fitted by a four-constant logistic function as described by De Lean et al.[18] Both EC_{50} and E_{max} were estimated as constants of the logistic function by nonlinear least squares methods.[19] Comparison between both E_{max} and EC_{50} values obtained in different conditions was performed using a three-way ANOVA model.[20] The average dose–response function was computed as mean constant curve (i.e., a curve whose constants are the mean of those estimated from each preparation). The effective dose of each compound was expressed as mean EC_{50} with 95% confidence limits. The analysis was carried out by SAS PROC GLM.[21]

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