8-Azaxanthine Derivatives as Antagonists of Adenosine Receptors

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Received March 9, 1994®

A series of 1,3-dimethyl- and 1,3-dipropyl-8-azaxanthines, substituted at the N^8 or N^7 position with substituents which usually increase the affinity of the xanthines for the adenosine receptors, was synthesized and studied in radioligand binding experiments. The substitution of CH with N at the 8-position of both theophylline and caffeine dramatically reduced the affinity, as demonstrated by the fact that 8-azatheophylline and 8-azacaffeine were inert. The introduction of a methyl group at 8-position of 8-azatheophylline restored the antagonistic activity at A_2 receptors, while a 8-cycloalkyl substituent increased the affinity for both receptor subtypes. A more favorable effect on affinity was produced by the substitution of the 7-methyl group in 8-azacaffeine with cycloalkyl groups. 7-Cyclopentyl-1,3-dimethyl-8-azaxanthine was 3 times more potent than caffeine at A_1 receptors and 6 times less active at A_2 receptors. On the contrary, the 7-cyclohexyl-1,3-dimethyl-8-azaxanthine was more potent than caffeine at A_2 receptors. The substitution of 1- and 3-methyl groups with propyl in both 7- and 8-substituted 8-azatheophylline increased remarkably the affinity for A_1 receptors. The 7-cyclopentyl-1,3-dipropyl-8-azaxanthine appears to be one of the most potent and selective among 7-alkyl-substituted xanthines at A_1 receptors so far known. Because the 8-aza analogues of 8-substituted 1,3-dialkylxanthine were in any case less active than the corresponding xanthine derivatives, it was confirmed that the hydrogen atom at the 7-position of xanthines plays an important role in the binding to adenosine receptors.

Introduction

Adenosine is a neuromodulator which, acting through specific cell-surface receptors, elicits a range of physiological responses, including vasodilation, antilipolytic effect, inhibition of platelet aggregation, inhibition of lymphocyte functions, inhibition of insuline and potentiation of glucagon release, inhibition of neurotransmitter release from nerve endings, stimulation of steroidogenesis, potentiation of histamine release from mast cells, and erythropoietin production.[1] Potent and selective agonists have been developed for these receptors, of which at least two subclasses are known (A_1 and A_2).[1] All adenosine receptor agonists designed up to now are derivatives of adenosine. The most important class of adenosine antagonists are the xanthines.[2] The methylxanthines theophylline and caffeine exhibit a variety of pharmacological actions, including facilitation of the atrioventricular conductions, renal vasodilation, stimulation of the central nervous system and bronchodilation, primarily through blockade of adenosine receptors.[3] However, they are virtually nonselective antagonists and have weak affinity for A_1 and A_2 receptors. Affinity enhancement of xanthines can be obtained by modification of the 1-, 3-, and 8-position. Introduction of alkyl groups to positions 1 and 3 and substitution at the 8-position with aryl or cycloalkyl groups increases the affinity for both receptor subtypes.[4-8] Substitution at the 8-position with phenyl or cycloalkyl substituents enhances not only the affinity but also the selectivity for A_1 receptors.[6,8] Recently, it was found that the *p*-trifluoromethyl substitution of certain 8-phenylxanthines increases the potency at A_2 receptors.[9]

Some caffeine derivatives such as 3,7-dimethyl-1-propagyl-, 1,3-dimethyl-7-propyl-, and 1,3-dimethyl-7-allylxanthine and various 8-substituted caffeine derivatives, including 8-(trifluoromethyl)caffeine and 8-vinylcaffeine, are somewhat selective for A_2 receptors.[9-11] 8-Cycloalkyl-1,3,7-trialkylxanthines[7] and 8-styryl-1,3,7-trialkylxanthines[12,13] have high affinity and selectivity for A_2 receptors. The greater selectivity of many xanthine derivatives structurally related to caffeine for the A_2 receptors leads one to suppose that the xanthine N7-hydrogen is less involved in the binding to this subtype of adenosine receptors.

In order to obtain further information about the role played by this hydrogen in the interaction between the ligand and the receptor, we have synthesized a series of 1,3-dialkyl-8-azaxanthine substituted at the 8-position with substituents which usually increase the affinity for the adenosine receptors, such as the cycloalkyl- and 2-amino-4-chlorophenyl groups.[6,14] Such compounds may be considered analogues of 8-substituted theophylline derivatives in which the 7-hydrogen is lacking.

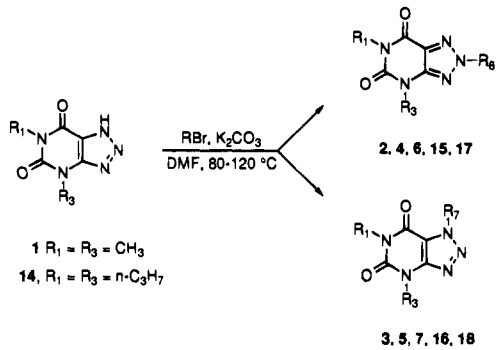
Chemistry

The 1,3-dimethyl-8-azaxanthines substituted at the 8-position with cyclopentyl, cyclohexyl, and *endo*-norbornyl groups were obtained as described in Scheme 1. Cycloalkylation of 1,3-dimethyl-8-azaxanthine[15] (**1**) with cyclopentyl, cyclohexyl, or *exo*-2-norbornyl bromide in DMF in the presence of K_2CO_3 at 80–120 °C gave a mixture of N^8 (**2**, **4**, and **6**) and N^7 (**3**, **5**, and **7**) regioisomers which were separated by chromatography on silica gel. The cycloalkylation position was determined by comparison of the ^{13}C NMR spectra. In Table 2 the chemical shift values are reported of C-4 and C-5 of compounds **2**–**17** and of 1,3,7-trimethyl-8-azaxanthine[15] (8-azacaffeine) and 1,3,8-trimethyl-8-azaxanthine[15] (8-methyl-8-azatheophylline) used as reference compounds.

® Abstract published in *Advance ACS Abstracts*, July 1, 1994.

0022-2623/94/1837-2970$04.50/0

Scheme 1



1 $R_1 = R_3 = CH_3$

14, $R_1 = R_3 = n\text{-}C_3H_7$

2: $R_1 = R_3 = CH_3$ R_8 = cyclopentyl

3: $R_1 = R_3 = CH_3$ R_7 = cyclopentyl

4: $R_1 = R_3 = CH_3$ R_8 = cyclohexyl

5: $R_1 = R_3 = CH_3$ R_7 = cyclohexyl

6: R1 = $R_3 = CH_3$ R_8 = endo-2-norbornyl

7: R1 = $R_3 = CH_3$ R_7 = endo-2-norbornyl

15: $R_1 = R_3 = n\text{-}C_3H_7$ R_8 = cyclopentyl

16: $R_1 = R_3 = n\text{-}C_3H_7$ R_7 = cyclopentyl

17: $R_1 = R_3 = n\text{-}C_3H_7$ R_8 = cyclohexyl

18: $R_1 = R_3 = n\text{-}C_3H_7$ R_7 = cyclohexyl

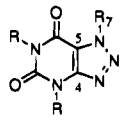
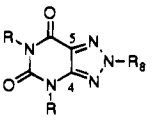
Table 1. Physicochemical Data of 8-Azaxanthine Derivatives

compd	% yield	mp, °C	PM[a]	R_f	cryst solvent	formula[b]
2	46.5	80–82	A	0.55	ethyl ether	$C_{11}H_{15}N_5O_2$
3	16.0	105–106	A	0.57	ethyl ether	$C_{11}H_{15}N_5O_2$
4	28.9	128–130	B	0.52	ethyl ether	$C_{12}H_{17}N_5O_2$
5	8.9	130–132	B	0.44	ethyl ether	$C_{12}H_{17}N_5O_2$
6	13.8	131–132	C	0.37	EtOH	$C_{13}H_{17}N_5O_2$
7	3.5	129–131	C	0.33	EtOH	$C_{13}H_{17}N_5O_2$
8	5.2	185–186	D	0.60	EtOH	$C_{12}H_9ClN_6O_4$
9	24.8	185–186	D	0.52	EtOH	$C_{12}H_9ClN_6O_4$
10	75.4	277–278	E	0.39	EtOH	$C_{12}H_{11}ClN_6O_2$
11	13.4	292–294	E	0.33	EtOH	$C_{12}H_{11}ClN_6O_2$
12	2.8	91–93	F	0.42	ethyl ether	$C_{12}H_{17}N_5O_3$
13	18.4	173–175	F	0.36	ethyl ether	$C_{12}H_{17}N_5O_3$
15	11.4	53–54	G	0.48	hexane	$C_{15}H_{23}N_5O_2$
16	7.3	oil	G	0.45		$C_{15}H_{23}N_5O_2$
17	46.2	70–72	H	0.66	hexane	$C_{16}H_{25}N_5O_2$
18	60.5	50–52	H	0.60	hexane	$C_{16}H_{25}N_5O_2$
19	10.2	148–150	I	0.39	EtOH	$C_{16}H_{17}ClN_6O_4$
20	3.4	91–93	I	0.33	EtOH	$C_{16}H_{17}ClN_6O_4$
21	60.6	198–200	J	0.26	EtOH	$C_{16}H_{19}ClN_6O_2$
22	25.5	oil	J	0.32		$C_{16}H_{19}ClN_6O_2$
23	1.5	oil	K	0.43		$C_{17}H_{27}N_5O_3$
24	15.6	oil	K	0.40		$C_{17}H_{27}N_5O_3$

[a] Purified by column chromatography on silica gel using the following eluents: A, cyclohexane/EtOAc (6:4); B, CH_2Cl_2/EtOAc (95.5); C, $CHCl_3$/cyclohexane (55:45); D, benzene/EtOAc (9:1); E, benzene/EtOAc (8:2); F, $CHCl_3$/MeOH/NH_4OH (98.5:1.25:0.25); G, CH_2Cl_2/EtOAc (99:1); H, CH_2Cl_2/cyclohexane/EtOAc (50:45:5); I, benzene/EtOAc (97:3); J, benzene/EtOAc (95:5); K, cyclohexane/EtOAc (65:35). [b] Analyses for C, H, and N were within ±0.4% of the calculated values.

As can be seen, the chemical shift of the C-4 of compounds **2**–**17** (149.2–150.9 ppm) is similar to that of 8-azatheophylline (148.6) and of 8-azacaffeine (150.3). This indicates that N^9 did not carry a substituent. The cycloalkylation posistion to N^7 isomers **3**, **5**, and **7** was assigned on the basis of the remarkable upfield shift of the C-5 signal compared to that observed for N^8 isomers **2**, **4**, and **6**[16] and of the similarity of the chemical shift of this signal to that of 8-azacaffeine. Therefore, compounds **2**, **4**, and **6** should be N^8-substituted isomers, as confirmed by the analogy of the chemical shift of their C-5 signal with that of 8-methyl-8-azatheophylline. The 8-(2-amino-4-chlorophenyl)-1,3-dimethyl-8-azaxanthine was prepared as reported in Scheme 2. 8-Azatheophylline was reacted with 2-nitro-1,4-dichlorobenzene to give a mixture of N^8- and N^7-arylated regioisomers (**8** and **9**), which were separated by silica gel column chromatography. Also in this case, the substitution position was determined on the basis of ^{13}C NMR as discussed above. The nitro derivative intermediates **8** and **9** were reduced with diborane–nickelous chloride system[16] to give the corresponding amino derivatives **10** and **11**.

Table 2. Selected ^{13}C NMR Data[a] of N^7- and N^8-Substituted-1,3-dialkyl-8-azaxanthines

Compd	R	R_7	R_8	C-4 (δ)	C-5 (δ)
8-Azatheophylline	CH_3	H		148.6	123.9
8-Azacaffeine	CH_3	CH_3		150.3	113.6
1,3,8-Trimethyl-8-azaxanthine	CH_3	CH_3		149.7	124.8
2	CH_3		cyclopentyl	149.2	124.2
3	CH_3	cyclopentyl		150.3	112.5
8	CH_3		2-nitro-4-chloro-phenyl	150.7	138.6
9	CH_3	2-nitro-4-chloro-phenyl		150.9	128.7
12	CH_3	*trans*-2-hydroxy-cyclohexyl		150.3	113.3
13	CH_3		*trans*-2-hydroxy-cyclohexyl	149.4	124.6
16	$n\text{-}C_3H_7$		cyclopentyl	149.0	124.7
17	$n\text{-}C_3H_7$		cyclohexyl	150.3	112.5

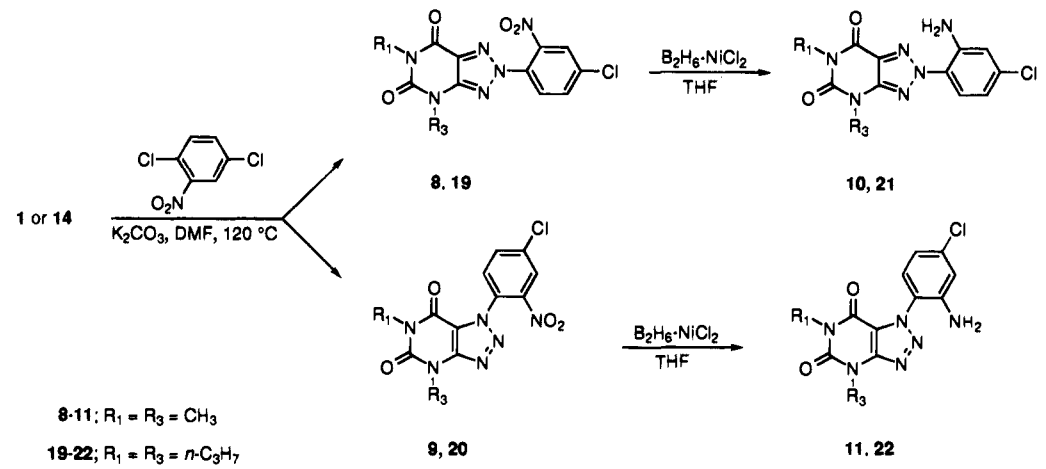
[a] DMSO-d_6.

In order to obtain further information about the interaction of this type of ligand with the adenosine receptor, we have also synthesized the 8-(*trans*-2-hydroxycyclohexyl)-1,3-dimethyl-8-azaxanthine (**13**). The presence of a hydroxy group close to N^7, able to form a hydrogen bond with the receptor (probably a histidyl residue),[1d] could compensate for the lack of the hydrogen atom on the N^7 and increase the water solubility of these very lipophilic products. Compound **13**, together with its N^7 isomer **12**, was prepared by reaction of **1** with cyclohexene oxide in DMF at 100 °C (Scheme 3). A mixture of two regioisomers (**12**, **13**) was obtained, which was purified by silica gel column chromatography. Also in this case, the cycloalkylation position was determined by comparison of the C-4 and C-5 signals in the ^{13}C NMR spectra (Table 2). In a similar way, starting from 1,3-dipropyl-8-azaxanthine (**14**), the corresponding 1,3-dipropyl analogues of **2**–**13** (**15**–**24**) were prepared. It was impossible to prepare the 1,3-dipropyl analogues of **6** and **7** because the 1,3-dipropyl-8-azaxanthine was resistent to the cycloalkylation by *exo*-2-bromonorborane. It was found that all the aza derivatives **2**–**13** and **15**–**24** were stable *in vitro*, in the conditions used for the biological evaluation, and insensitive to light.

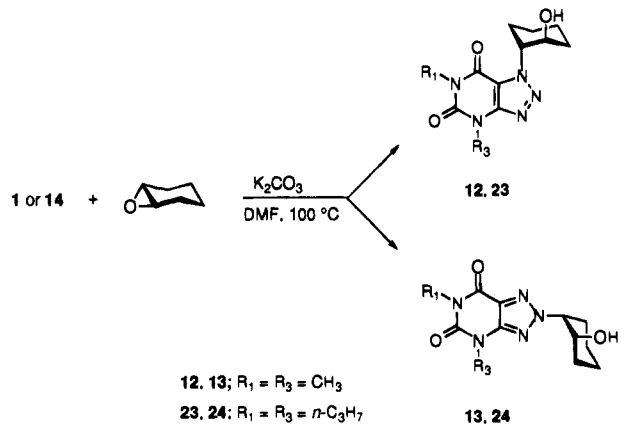
Biological Evaluation

The 8-substituted 8-azaxanthines and their 7-substituted isomers were tested in radioligand binding assays for affinity at A_1 and A_2 adenosine receptors in bovine brain cortical membranes, and bovine brain striatal membranes, respectively. [^3H]N^6-(*R*)-Phenylisopropy-

Scheme 2



Scheme 3



ladenosine (*R*-PIA) was used as A_1 ligand and [^{3}H]-2-[[*p*-(2-carboxyethyl)phenylethyl]amino]-5′-(*N*-ethylcarbamoyl)adenosine (CGS 21680) as A_2 ligand.

Results and Discussion

Compounds **2**–**7**, **10**–**13**, **15**–**18**, and **21**–**24** were evaluated in binding studies at A_1 and A_2 adenosine receptors; theophylline, caffeine, 8-azatheophylline, 8-azacaffeine, 8-methyl-8-azatheophylline, and 8-cyclopentyl-1,3-dipropylxanthine (CPX) were used as reference compounds. As can be seen from the data in Table 3, the substitution of the CH at the 8-position with N of both theophylline and caffeine had dramatic effects on affinity. The 8-carbon appears essential for activity since 8-azatheophylline and 8-azacaffeine were inert. This confirms the data found by Bruns in the case of inhibition induced by 8-azatheophylline of the increase in cyclic AMP caused by adenosine in human fibroblasts.[18] However, the introduction of a methyl group at the 8-position of 8-azatheophylline restored the antagonistic activity, at least at the A_2 receptors.

The substitution of the hydrogen at the 8-position of 8-azatheophylline with cycloalkyl groups increased the affinity, as found also for the xanthine derivatives, for adenosine receptors. The 8-(2-*endo*-norbornyl) derivative **6** is the most potent at both receptor subtypes, being only slightly less potent than theophylline. A still more favorable effect on affinity was produced by the substitution of the 7-methyl group in 8-azacaffeine with cycloalkyl groups. The cyclopentyl analogue **3** was about 3 times more potent than caffeine at A_1 receptors and 6 times less potent at A_2 receptors, being, therefore, selective for A_1 receptors. On the contrary, the 7-cyclohexyl and 7-(*endo*-2-norbornyl) derivatives **5** and **7** were more potent than caffeine at A_2 receptors and selective for this receptor subtype. The introduction of a hydroxy group in the 2′-trans-diequatorial position of cyclohexyl moiety of 7- and 8-cyclohexyl-8-azatheophylline is detrimental for the activity.

It was also found that insertion of the 2-amino-4-chlorophenyl group at the 8-position of 8-azatheophylline is able to restore the antagonistic activity, at least at A_1 receptors. A more favorable effect was observed when this group replaced the 7-methyl group of 8-azacaffeine. In fact, compound **11** was twice as potent as caffeine at A_1 receptors and had a comparable affinity for A_2 receptors.

The substitution of 1- and 3-methyl groups with propyl in both 7- and 8-substituted 8-azatheophylline derivatives increased the affinity remarkably, particularly for A_1 receptors. Thus 8-cyclopentyl- and 7-cyclopentyl-1,3-dipropyl-8-azaxanthine (**15** and **16**) were respectively 85- and 34-fold more potent than 8-cyclopentyl- and 7-cyclopentyl-1,3-dimethyl-8-azaxanthine (**2** and **3**). This effect is similar to that observed for xanthine derivatives, confirming that 8-azaxanthine binds the adenosine receptor in a similar way to the xanthines.[1d] Potency at both receptors also increased as 1- and 3-methyl groups were replaced by propyl ones in the 8-azacaffeine derivatives. A selectivity against A_1 receptors was observed in this case, too. The most active appeared to be the 7-cyclopentyl derivative **16** which is one of the most potent and selective caffeine analogues at A_1 receptors so far known. Owing to the potential of caffeine analogues as therapeutic agents (antiasthmatic, antithrombotics and cognitive enhancers), this compound deserves further investigation as a potential new drug.

It is interesting to note that a hydroxy group in the 2′-position of cyclohexyl moiety of 7-cyclohexyl-1,3-dipropyl-8-azaxanthine does not change the potency at both receptor subtypes, contrary to what was found by Daly and co-workers, who observed that the introduction of polar groups in the 7-substitutent of caffeine derivatives gave rise to compounds with low or no activity at adenosine receptors.[11] Since it was found that 7- and 8-alkyl-8-azapurine derivatives analogues of compounds reported in this paper have similar

Table 3. A_1 and A_2 Adenosine Receptors Binding of 7- and 8-Substituted-1,3-dialkyl-8-azaxanthines

compd	substituents	K_i (μM)[a] A_1[b]	A_2[c]	A_2/A_1
	Substituted 1,3-Dimethyl-8-azaxanthine			
2	8-cyclopentyl	110 ± 3	58 ± 4	0.5
3	7-cyclopentyl	11 ± 1.2	292 ± 20	26
4	8-cyclohexyl	36 ± 2.8	250 ± 18	6.9
5	7-cyclohexyl	23 ± 1.5	8.4 ± 1	0.36
6	8-(*endo*-2-norbornyl)	31 ± 1.5	49 ± 6	1.6
7	7-(*endo*-2-norbornyl)	18 ± 2.0	4.5 ± 1	0.25
10	8-(2-amino-4-chloro-phenyl)	24 ± 2.6	*d*	
11	7-(2-amino-4-chloro-phenyl)	14 ± 1.2	46 ± 4	3.3
12	7-(*trans*-2-hydroxy-cyclohexyl)	114 ± 11	*d*	
13	8-(*trans*-2-hydroxy-cyclohexyl)	*d*	*d*	
	Substituted 1,3-Dipropyl-8-azaxanthine			
15	8-cyclopentyl	1.3 ± 1.1	13 ± 0.9	10
16	7-cyclopentyl	0.34 ± 0.27	10 ± 0.7	29
17	8-cyclohexyl	1.6 ± 0.22	21.7 ± 1	13
18	7-cyclohexyl	1.1 ± 0.13	22.9 ± 2	21
21	8-(2-amino-4-chlorophenyl)	1.5 ± 0.18	28.9 ± 2	19
22	7-(2-amino-4-chlorophenyl)	44 ± 4	*d*	
23	7-(*trans*-2-hydroxy-cyclohexyl)	1.8 ± 0.2	13.5 ± 1.2	7.5
24	8-(*trans*-2-hydroxy-cyclohexyl)	1.4 ± 0.11	20% (20 mM)	
theophylline		8.5 ± 0.7	25 ± 2.6	3.0
caffeine		29 ± 1.5	48 ± 3.3	1.7
8-azatheophylline		*d*	*d*	
8-azacaffeine		*d*	*d*	
8-methyl-8-azatheophylline		*d*	52.8	
CPX[e]		(46 ± 3.2) × 10^{-5}	0.34 ± 0.02	740

[a] In one case the % inhibition at the highest concentration is given. [b] Displacement of [^{3}H]PIA in bovine brain cortical membranes. [c] Displacement of [^{3}H]CGS 21680 in bovine brain striatal membranes. [d] Inactive at the highest tested concentration (30 μM). [e] 8-Cyclopentyl-1,3-dipropylxanthine.

aromaticity,[19] the difference of affinity of 7- and 8-substituted isomers **2**–**7** and **15**–**18** could be due more to steric than electronic factors.

In conclusion, considering that 8-aza analogues of 8-substituted 1,3-dialkylxanthines were in any case much less active than the corresponding xanthine derivatives, it was confirmed that the hydrogen atom at the 7-position of 8-substituted 1,3-dialkylxanthines plays an important role in the binding to adenosine receptor, in particular to A_1 subtype. On the other hand, the substitution of the CH group with N in caffeine derivatives leads to compounds with equivalent or more potent affinity for A_1 receptors, especially when 1- and 3-methyl substituents are replaced with propyl and the 7-methyl group is substituted with a cycloalkyl substituent.

Experimental Section

Chemistry. Melting points were determined on a Buchi apparatus and are uncorrected. Elemental analyses were determined on a Carlo Erba Model 1106 analyzer. UV spectra were recorded with an HP 8452 A diode array spectrophotometer driven by an Olivetti M 24. Thin-layer chromatography (TLC) was performed on silica gel 60 F-254 plates, and silica gel 60 (Merck) for column chromatography was used. Nuclear magnetic resonance ^{1}H and ^{13}C spectra were determined, respectively, at 300 and 75 MHz with a Varian VXR-300 spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. All exchangeable protons were confirmed by addition of D_2O.

General Procedure for Compounds 2–7. This procedure is illustrated for the preparation of 1,3-dimethyl-8-cyclopentyl-8-azaxanthine (**2**) and 1,3-dimethyl-7-cyclopentyl-8-azaxanthine (**3**).

To 2 g (10.1 mmol) of 1,3-dimethyl-8-azaxanthine dissolved in 60 mL of DMF was added 1.84 g (13.3 mmol) of K_2CO_3. The suspension was stirred and heated at 80 °C for 15 min. Then 1.8 g (12.1 mmol) of cyclopentyl bromide was added to the reaction mixture, which was heated at 80 °C for 1 h. After cooling, the suspension was filtered and the solid was washed with ethyl acetate. The filtrate was evaporated to dryness and purified by chromatography on a silica gel column, eluting with CH_2Cl_2/ethyl acetate (95:5) to give 1.3 g of **2** as white solid. ^{1}H NMR (DMSO-d_6): δ 1.62–1.92 (m, 4 H, cyclopent C3 and C4), 2.20–2.25 (m, 4 H, cyclopent C2 and C5), 3.23 (s, 3 H, N1-CH_3), 3.48 (s, 3 H, N3-CH_3), 5.40 (m, 1 H, cyclopent C1). UV (MeOH): λ_{max} 278 nm.

Further elution of the chromatographic column gave 0.44 g of isomer **3** as white solid. ^{1}H NMR (DMSO-d_6): δ 1.62–1.87 (m, 4 H, cyclopent C3 and C4), 2.02–2.25 (m, 4 H, cyclopent C2 and C5), 5.12 (m, 1 H, cyclopent C1). UV (MeOH): λ_{max} 278 nm.

The ^{1}H NMR spectra of the other compounds were consistent with the assigned structure.

In the case of compounds **6** and **7** the reaction mixture was heated at 120 °C for 2 days.

General Procedure for Compounds 15–18. This procedure is illustrated for the preparation of 1,3-di-*n*-propyl-8-cyclohexyl-8-azaxanthine (**17**) and 1,3-di-*n*-propyl-7-cyclohexyl-8-azaxanthine (**18**).

To 2 g (8.4 mmol) of 1,3-di-*n*-propyl-8-azaxanthine (**14**) dissolved in 50 mL of DMF was added 1.4 g (10.1 mmol) of K_2CO_3. The suspension was stirred and heated at 80 °C for 15 min. Then 1.4 g (9.2 mmol) of cyclohexyl bromide was added to the reaction mixture which was stirred and heated at 80 °C for 1 h. After cooling, the inorganic salts were filtered off and washed with ethyl acetate. The filtrate was evaporated to dryness and purified by column chromatography on silica gel, eluting with CH_2Cl_2/cyclohexane/ethyl acetate (50:45:5) to give 1.24 g of **17** as white solid. ^{1}H NMR (DMSO-d_6): δ 0.91 (two overlapping t, 6 H, CH_3), 1.18–1.97 (m, 12 H, cyclohex C2a, C3, C4, C5, C6a and $CH_2CH_2CH_3$), 2.18 (m, 2 H, cyclohex C2e and C6e), 3.88 (m, 4 H, $CH_2CH_2CH_3$), 4.63 (m, 1 H, cyclohex C1). UV (MeOH): λ_{max} 278 nm.

Further elution of the chromatographic column gave the isomer **18** as white solid. ^{1}H NMR (DMSO-d_6): δ 0.90 (t, 3 H, CH_3), 0.94 (t, 3 H, CH_3), 1.22–2.20 (m, 14 H, cyclohex C2, C3, C4, C5, C6 and $CH_2CH_2CH_3$), 3.86 (pseudo t, 2 H, N1-CH_2-CH_2CH_3), 4.02 (pseudo t, 2 H, N3-$CH_2CH_2CH_3$), 4.92 (m, 1 H, cyclohex C1). UV (MeOH): λ_{max} 278 nm.

The ^{1}H NMR spectra of the other compounds were consistent with the assigned structure.

General Procedure for Compounds 8, 9, 19, and 20. This procedure is illustrated for the preparation of 1,3-dimethyl-8-(2-nitro-4-chlorophenyl)-8-azaxanthine (**8**) and 1,3-dimethyl-7-(2-nitro-4-chlorophenyl)-8-azaxanthine (**9**).

1,3-Dimethyl-8-azaxanthine (2 g, 11 mmol) was treated in 60 mL of DMF with 1.83 g (13.2 mmol) of K_2CO_3 and 2.33 g (12.1 mmol) of 2,5-dichloronitrobenzene at 120 °C for 15 h with stirring. After cooling, the inorganic salts were filtered off and washed with ethyl acetate, and the filtrate was evaporated to dryness. The residue was chromatographed on a silica gel column, eluting with benzene/ethyl acetate (9:1) to give 0.175 g of **8** as yellow solid. ^{1}H NMR (DMSO-d_6): δ 3.32 (s, 3 H, N1-CH_3), 3.48 (s, 3 H, N3-CH_3), 7.97 (dd, 1 H, J_1 = 3 Hz, J_2 = 8 Hz, phenyl C5), 8.20 (d, 1 H, J = 3 Hz, phenyl C3), 8.21 (d, 1 H, J = 8 Hz, phenyl C2). UV (Me OH): λ_{max} 316 nm.

Further elution of the chromatographic column gave the isomer **9** as yellow solid. ^{1}H NMR (DMSO-d_6): δ 3.31 (s, 3 H, N1-CH_3), 3.47 (s, 3 H, N3-CH_3), 8.07 (dd, 1 H, J_1 = 3 Hz, J_2 = 8 Hz, phenyl C5), 8.09 (d, 1 H, J = 3 Hz, phenyl C3), 8.36 (d, 1 H, J = 8 Hz, phenyl C6). UV (MeOH): λ_{max} 320 nm.

Compounds **19** and **20** were prepared in a similar way starting from **14**. The NMR spectra of these compounds were consistent with the assigned structures.

General Procedure for Compounds 10, 11, 21, and 22. This procedure is illustrated for the preparation of 1,3-dimethyl-7-(2-nitro-4-chlorophenyl)-8-azaxanthine (**11**).

Compound **9** (0.2 g, 0.6 mmol) and $NiCl_2$·$6H_2O$ (0.14 g, 0.6 mmol) were dissolved in tetrahydrofuran (2.2 mL) and MeOH (0.72 mL), and then borane–methyl sulfide complex (0.46 g, 3.56 mmol) was slowly added under nitrogen to the solution at 0 °C. The reaction mixture was stirred under nitrogen at room temperature for 1 h. After removal of the solvent, 10% hydrochloric acid (6.6 mL) was added to the residue, and the mixture was basified by the addition of concentrated ammonium hydroxide and extracted with ether. The extract was evaporated off, and the residue was crystallized. ^{1}H NMR (DMSO-d_6): δ 3.35 (s, 3 H, N1-CH_3), 3.52 (s, 3 H, N3-CH_3). 7.04 (dd, 1 H, J_1 = 3 Hz, J_2 = 8 Hz, phenyl C5), 7.38 (d, 1 H, J = 3 Hz, phenyl C3), 7.77 (d, 1 H, J = 8 Hz, phenyl C6), 8.90 and 9.10 (2 s, 2 H, NH_2). UV (MeOH): λ_{max} 300 and 348 nm.

In a similar way starting from nitro compounds **8**, **19**, and **20**, the amino derivatives **10**, **21**, and **22** were prepared. The ^{1}H NMR spectra of these compounds were consistent with the assigned structures.

General Procedure for Compounds 12, 13, 23, and 24. This procedure is illustrated for the preparation of 1,3-dimethyl-7-(*trans*-2-hydroxycyclohexyl)-8-azaxanthine (**12**) and its 8-substituted isomer **13**.

To 0.51 g (2.80 mmol) of 1,3-dimethyl-8-azaxanthine dissolved in 15 mL of DMF were added 0.47 g (3.42 mmol) of K_2CO_3 and 0.3 g (3.09 mmol) of cyclohexane oxide. The mixture was stirred and heated at 100 °C for 7 h. After cooling, the inorganic salts were filtered off and washed with ethyl acetate. Evaporation of the filtrate gave an oily residue which was purified by chromatography on a silica gel column, eluting with $CHCl_3$/MeOH/NH_4OH (98.5:1.25:0.25). Evaporation of the first eluate gave **12** as white solid. ^{1}H NMR (DMSO-d_6): δ 1.40 (br s, 3 H), 1.72–2.13 (m, 5 H), 3.27 (dd, 1 H, N1-CH_3), 3.54 (s, 3 H, N3-CH_3), 4.11 (ddd, 1 H, J_1 = 4.2, J_2 = 9.6 Hz, *CH*OH), 4.76 (dddd, 1 H, J_1 = 4.2, J_2 = 9.7, J_3 = 11.7 Hz, N7-CH), 5.0 (d, 1 H, J = 6 Hz, OH). UV (MeOH): λ_{max} 282 nm.

Further elution of the chromatographic column gave 0.244 g of the 8-substituted isomer **13**. ^{1}H NMR (DMSO-d_6): δ 1.40 (br s, 3 H), 1.72–2.13 (m, 5-H), 3.27 (dd, 1 H, N1-CH_3), 3.45 (s, 3 H, N3-CH_3), 3.87 (m, which was converted into a ddd after addition of D_2O, 1H, J_1 = 4.2, J_2 = 9.6 Hz, *CH*OH), 4.37 (dddd, 1 H, J_1 = 4.2, J_2 = 9.7, J_3 = 11.7 Hz, N7-CH), 4.99 (d, 1 H, J = 6 Hz, OH). UV (MeOH): λ_{max} 278 nm.

Biochemistry Assays: A_1 Receptors Binding. Bovine cerebral cortex was homogenized in ice-cold 0.32 M sucrose containing protease inhibitors, as previously described.[20] The homogenate was centrifuged at 1000*g* for 10 min at 4 °C and the supernatant again centrifuged at 48000*g* for 15 min at 4 °C. The final pellet was dispersed in 10 volumes of fresh buffer, incubated with adenosine deaminase (2 units/mL) to remove endogenous adenosine at 37 °C for 60 min, and then recentrifuged at 48000*g* for 15 min at 4 °C. The pellet was suspended in buffer and used in the binding assay.

The [^{3}H]CHA binding assay was performed in triplicate by incubating aliquots of the membrane fraction (0.2–0.3 mg of protein) at 25 °C for 45 min in 0.5 mL of Tris-HCl, pH 7.7, containing 2 nM $MgCl_2$, with approximately 1.2 nM [^{3}H]CHA. Nonspecific binding was defined in the presence of 50 μM *R*-PIA (*R*)-phenylisopropyladenosine). The assay was completed by filtration through Whatman GF/C glass microfibre filters under suction and washing twice with 5 mL of ice-cold buffer.

A_2 Receptors Binding. Bovine striatum was homogenized in 20 volumes of ice-cold 50 mM Tris-HCl, pH 7.5, containing 10 mM $MgCl_2$, and protease inhibitors. The membrane homogenate was centrifuged at 48000*g* for 10 min at 4 °C. The resulting pellet was resuspended in buffer containing 2 units/mL of adenosine deaminase and incubated at 37 °C for 30 min. The membrane homogenate was centrifuged, and the final pellet was frozen at −80 °C. Routine assays were performed in triplicate by incubating an aliquot of striatal membranes (0.2–0.3 mg of protein) in 50 mM Tris-HCl, pH 7.5, containing 10 mM $MgCl_2$ with approximately 5 nM [^{3}H]CGS 21680 in a final volume of 0.5 mL. Incubation was carried out at 25 °C for 90 min. Nonspecific binding was defined in the presence of 50 μM CGS 21680. Binding reactions were terminated by filtration through Whatman GF/C filters under reduced pressure. Filters were washed three times with 5 mL of ice-cold buffer and placed in scintillation vials. The radioactivity was counted in a 4-mL Beckman Ready-Protein scintillation cocktail in a scintillation counter. The compounds were dissolved in DMSO and added to the assay mixture to make a final volume of 0.5 mL. Blank experiments were carried out to determine the effect of the solvent (2%) on the binding. The concentrations of the tested compounds to produce 50% inhibition of specific [^{3}H]CHA or [^{3}H]CGS 21680 binding (IC_{50}) were determined from semilog plots of data from experiments of binding inhibition. The K_i values were calculated from the IC_{50} values using the equation $IC_{50}/(L/K_d)$.[21] For [^{3}H]CHA K_d = 10.5 nM and L = 1.2 nM; for [^{3}H]CGS 21680 K_d = 1 nM and L = 5 nM. Protein estimation was based on the reported method,[22] using bovine serum albumin as standard.

Acknowledgment. Financial support from CNR "Progetto Finalizzato Chimica Fine II" is gratefully acknowledged.

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