Aza-Analogs of 8-Styrylxanthines as A_{2A}-Adenosine Receptor Antagonists[1)]

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Summary

In the present study we synthesized aza-analogs of 8-styrylxanthines, in which the ethenyl bridge is replaced by an imine, amide, or azo function, in order to investigate structure-activity relationships of the 8-substituent of A_{2A}-selective xanthine derivatives. Thus, various 8-substituents were combined with theophylline or caffeine, respectively, and affinities of the novel compounds for adenosine A_1- and A_{2A}-receptors were determined and compared with those of analogous 8-styrylxanthine derivatives. 8-(Benzylideneamino)caffeine derivatives exhibited high affinity and selectivity for A_{2A}-adenosine receptors, but were unstable in aqueous buffer solution at physiological pH values. 8-(Phenylazo)caffeine derivatives were less potent than corresponding 8-styrylcaffeine derivatives at adenosine receptors. The most potent azo compound of the present series was 8-(*m*-chlorophenylazo)caffeine (**14b**) exhibiting a K_i value of 400 nM at A_{2A}-adenosine receptors and 20-fold selectivity versus A_1-receptors. Due to the facile synthetic access to 8-(phenylazo)xanthine derivatives, which are obtained by coupling of 8-unsubstituted xanthines with phenyldiazonium salts, **14b** may be an interesting new lead compound for the development of more potent and selective A_{2A}-antagonists with azo structure.

Introduction

Adenosine receptors (AR) belong to the superfamily of G-protein-coupled receptors. They can be subdivided into high-affinity subtypes, which are activated by adenosine in nanomolar concentrations (A_1 and A_{2A}), and low-affinity subtypes, for the activation of which high (micromolar) concentrations of adenosine are required (A_{2B}, A_3) [1]. All four AR subtypes have been cloned from different species, including humans [2]. Antagonists for the high-affinity AR subtypes are currently under development as novel drugs for the treatment of cognitive deficits, including Alzheimer's disease (A_1), as kidney-protective diuretics (A_1), antihypertensives (A_1), and for the treatment of Morbus Parkinson (A_{2A}) [3,4].

All AR *agonists* are nucleosides derived from the physiological agonist adenosine. The ribose moiety is essential for agonistic activity[1]. The most prominent class of AR *antagonists* are the xanthines, derivatives of the naturally occurring alkaloids theophylline and caffeine [1–3,5,6]. Several classes of non-xanthine AR antagonists have also been described, e.g. adenine[7], pyrrolo[2,3-*d*]pyrimidine[8,9], pyrido[2,3-*d*]-pyrimidine[10], pyrimido[4,5-*b*]indole[8,9], pyrazolo[3,4-*d*]-pyrimidine[11], and 1,8-naphthyridine[10] derivatives. During the past decade, a number of potent, selective A_1-AR antagonists could be developed, and it appears most likely, that A_1-antagonists with xanthine structure will be the first class of AR antagonists to reach the drug market in the near future.[3]

In contrast to the progress in the field of A_1-antagonists, only a limited number of A_{2A}-antagonists has been developed so far, and structure-activity relationship studies of A_{2A}-selective AR-antagonists are sparse. The first class of potent A_{2A}-selective antagonists described in the literature were the 8-styrylxanthine derivatives, such as 1,3-dipropyl-7-methyl-8-styrylxanthines, 8-styrylcaffeines, and 3,7-dimethyl-1-propargyl-8-styrylxanthines (8-styryl-DMPX derivatives) [12–14]. Detailed structure-activity relationships of 8-styrylxanthines with regard to the 1-, 3-, and 7-substituents have been described recently [14]. A 1-propargyl group combined with 3- and 7-methyl substitution was found to be optimal for high A_{2A}-affinity and -selectivity of 8-styrylxanthines. Bioisosteric exchange of the phenyl ring in the 8-styryl residue [14,15], or replacement of the isomerizable double bond by configurationally stable structures[16] resulted in a decrease in A_{2A}-affinity and/or selectivity.

In the present study, we synthesized analogs of 8-styrylxanthines, in which the styryl ethenyl bond was replaced by analogous nitrogen-containing structural elements, including imine, amide, or azo structures, respectively. Our goal was to investigate effects of such structural changes on AR affinity and selectivity of the compounds.

Chemistry

The compounds that were investigated can be generally described as aza-analogs of 8-styrylxanthines. We synthesized three different types of 8-substituted theophylline and caffeine derivatives. The first type of 8-substituents is characterized by an imine structure (-N=CH-), the second type by an amide structure (NHCO), and the third type by an azo-group (-N=N-) replacing the ethenyl function in the styryl residue. Some of the imine and amide compounds were also synthesized with an inverse structural element (-CH=N-; CONH).

[1)] Preliminary results were presented at the Spring Meeting ("Doktorandentagung") of the German Pharmaceutical Society in Kiel/Salzau 1996; abstract published in *Pharm. unserer Z.* **1996**, *25*, 208.

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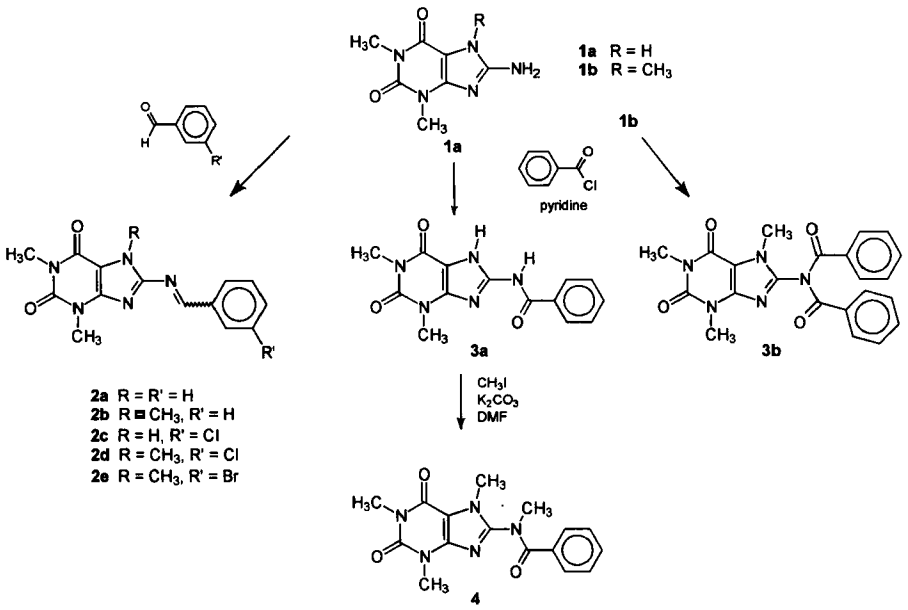
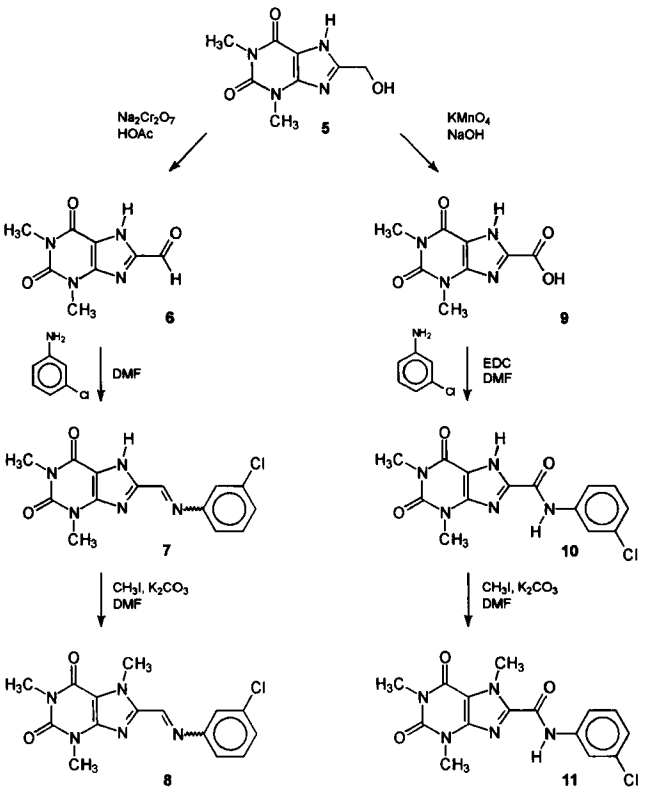


Scheme 1. Synthesis of imines and amides of 8-aminoxanthines.



Scheme 2. Synthesis of imines and amides starting from 8-(hydroxymethyl)-theophylline.

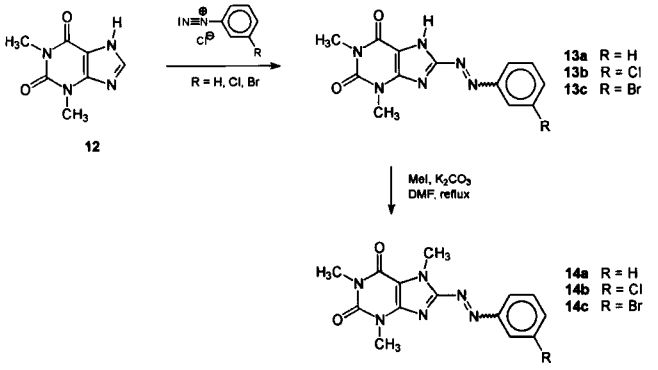
8-Benzylideneaminocaffeine derivatives **2b** and **2d** and 8-benzylideneaminotheophylline derivatives **2c** and **2e** were synthesized in analogy to the method described for unsubstituted 8-benzylideneaminotheophylline **2a** [17]. Thus, 8-aminotheophylline (**1a**)[17], or 8-aminocaffeine (**1b**)[17], respectively, were condensed with the appropriate benzaldehyde derivatives (Scheme 1). For the preparation of amides, 8-aminotheophylline (**1a**) was reacted with benzoyl chloride in pyridine to yield 8-(N-benzoylamino)theophylline (**3a**). Methylation of **3a** in dimethylformamide using excess methyl iodide in the presence of potassium carbonate yielded bis-methylated product **4** in high yield (see Scheme 1). Selective methylation in the 7-position was not possible due to the similarly high reactivities of the nitrogen atoms N-7 and N^8, which are part of a basic guanidine structure. Methylation of N-9 was not observed as shown by ^{1}H-NMR spectroscopy (see below). Benzoylation of 8-aminocaffeine (**1b**) did not yield the desired 8-(benzoylamino)caffeine, but resulted in the formation of bis-benzoylated product **3b**.

The "inverse imines" **7** and **8** and the "inverse amides" **10** and **11** were prepared as illustrated in Scheme 2. As starting material 8-hydroxymethyltheophylline (**5**) was used, which could easily be obtained by condensation and ring closure reaction of 1,3-dimethyl-5,6-diaminouracil with α-hydroxyacetic acid[18]. Oxidation of **5** with sodium dichromate in acetic acid selectively led to the aldehyde **6**[18], while oxidation with potassium permanganate in sodium hydroxide solution yielded carboxylic acid **9** [19]. 8-(3-Chlorophenyliminomethyl)theophylline **7** was prepared by the condensation of aldehyde **6** with 3-chloroaniline in hot dimethylformamide in high yield. Alkylation of **7** with methyl iodide under basic conditions yielded the N7-methylated 8-(3-chlorophenyliminomethyl)caffeine **8**. An alternative reaction sequence, which involved initial methylation of **6**, followed by condensation of the resulting caffeine-8-carbaldehyde with 3-chloroaniline was not successful, since methylation of aldehyde **6** resulted in a mixture of products.

Similarly, selective N7-methylation of carboxylic acid **9** could not be performed due to side reaction of the carboxylic group with methyl iodide. Compound **9**, however, could be condensed with 3-chloroaniline by means of a water-soluble carbodiimide to yield 8-N-(3-chlorophenyl)-8-carboxamidotheophylline (**10**). Xanthine **10** was selectively alkylated in the 7-position by the use of excess methyl iodide to yield 8-N-(3-chlorophenyl)-8-carboxamidocaffeine (**11**). The formation of a bis-methylated product, as observed in the methylation of xanthine **3a** to **4**, was obtained as a side product only after very long reaction times. This can easily be explained by the considerably lower basicity of the exocyclic amide nitrogen as compared to the N-7, which is part of a cyclic guanidine structure.

The azo-compounds **13a–c** were obtained by electrophilic coupling of *meta*-halogenated phenyldiazonium chlorides with equimolar quantities of theophylline (**12**) in the presence of potassium hydroxide. Subsequent methylation under basic conditions yielded 8-phenylazocaffeine derivatives **14a–c** (Scheme 3).

Yields and selected analytical data of final products are given in Table 1. Melting points of 8-substituted xanthines were generally above 300 °C, with few exceptions. The N7-



Scheme 3. Synthesis of 8-(phenylazo)xanthines

methylated compounds **4**, **8**, and **11** showed significantly decreased melting points compared to their 7-unsubstituted counterparts **3a**, **7**, and **10**. Obviously, the N7-hydrogen function is contributing to intermolecular stabilization by hydrogen bonding in the crystal lattice.

Elemental analyses, UV/VIS spectra, ^{1}H-NMR and ^{13}C-NMR data were consistent with the proposed structures. A selection of ^{1}H-NMR data is listed in Table 2. The chemical shifts for the methyl protons in xanthine derivatives allow for the assignment of the position of the methyl group [20]. Chemical shifts are as follows: N1-CH_3 (3.2–3.3 ppm in DMSO-d_6, 3.4-3.5 ppm in $CDCl_3$), N3-CH_3 (3.4–3.5 ppm in DMSO-d_6, 3.5–3.7 ppm in $CDCl_3$). The chemical shift of the

Table 1. Yields and analytical data of final products.

Compd	Yield [%]	Formula	Anal.	M_R [g/mol]	Mp [°C]	UV/VIS λ_{max} [nm] (solvent)
2b	89	$C_{15}H_{15}N_5O_2$	C,H,N	297.3	>300	369 ($CHCl_3$) 360 (Tris buffer[a])
2c	65	$C_{14}H_{12}N_5O_2Cl$	C,H,N[b]	317.7	>300	364 (Tris buffer)
2d	68	$C_{15}H_{14}N_5O_2Cl$	C,H,N	331.7	>300	363 (Tris buffer)
2e	60	$C_{15}H_{14}N_5O_2Br$	C,H,N	376.2	>300	374 ($CHCl_3$)
3a	82	$C_{14}H_{13}N_5O_3$	C,H,N	299.3	>300	309 (DMF)
3b	70	$C_{22}H_{19}N_4O_4$	C,H,N	417.4	241–242	282 (MeOH)
4	89	$C_{16}H_{17}N_5O_3$	C[c],H,N[d]	327.3	163–164	
7	86	$C_{14}H_{12}N_5O_2Cl$	C,H,N	317.7	>300	
8	88	$C_{15}H_{14}N_5O_2Cl$	C,H,N	331.7	216	352 (Tris buffer)
10	54	$C_{14}H_{12}N_5O_3Cl$	C,H,N	333.7	331–332	
11	86	$C_{15}H_{14}N_5O_3Cl$	C,H,N[e]	347.5	230–231	
13a	45	$C_{13}H_{12}N_6O_2$	C,H,N	284.3	>300	
13b	40	$C_{13}H_{11}N_6O_2Cl$	C,H,N	318.7	>300	
14a	64	$C_{14}H_{14}N_6O_2$	C[f],H,N	298.3	>300	401 (MeOH)
14b	63	$C_{14}H_{13}N_6O_2Cl$	C,H,N	332.8	>300	421 ($CHCl_3$)
14c	64	$C_{14}H_{13}N_6O_2Br$	C,H,N	377.3	>300	406 ($CHCl_3$)
20	78	$C_9H_{12}N_4O_3$	C,H,N	224.2	223–226 (224–226)[18]	

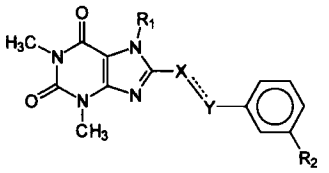
[a] Tris buffer: Tris(hydroxymethyl)aminomethane-HCl buffer, 50 mM, pH 7.4. [b] calcd, 22.04; found, 21.60; [c] calcd, 58.70; found, 58.23; [d] calcd, 21.39; found, 20.75; [e] calcd, 20.14; found, 21.10; [f] calcd, 56.37; found, 55.93.

Table 2. ^{1}H-NMR data of selected compounds[a].

Compd	N1-CH_3	N3-CH_3	N7-CH_3	8-Substituent
2c[b]	3.28	3.49	–	7.62–8.08 (m, 4H, arom.), 9.27 (s, 1H, CH)
2e[c]	3.43	3.59	4.09	7.35–8.19 (arom.), 9.20 (CH)
3a[c]	3.41	3.58		7.50–7.60 (m, 3H, arom.), 8.12 (d, 2H, o-arom.), 11.71 (s, 1H,NHCO), 12.04 (s, 1H, N7-H)
3b[c]	3.38	3.46	3.83	7.40–7.44 (m, 4H, arom.), 7.54–7.57 (m, 2H, arom.), 7.79–7.81 (m, 4H, arom.)
4[b]	3.19	3.37[d]	3.55	7.39–7.50 (m, 5H, arom.), 3.33[d] (s, 3H, N-CH_3)
7[b]	3.26	3.49	–	7.47–7.29 (m, 4H, arom.), 8.49 (s, 1H, CH).
8[b]	3.25	3.46	4.30	7.35–7.52 (m, 4H, arom.), 8.69 (s, 1H, CH).
10[b]	3.26	3.53	–	7.20 (d, J = 7.6 Hz, 1H, C6′-H), 7.40 (t, J = 8.0 Hz, 1H, C5′-H), 7.81 (d, J = 8.2 Hz, 1H, C4′-H), 8.02 (s, 1H, C2′-H), 10.72 (s, 1H, NH).
11[c]	3.42	3.61	4.45	7.15 (ddd, $J_{5'6'}$ = 8.0 Hz, $J_{2'6'}$ = 2.0 Hz, $J_{4'6'}$ = 1.2 Hz, 1H, C6′-H), 7.31 (t, J = 8.1 Hz, 1H, C5′-H), 7.52 (ddd, $J_{4'5'}$ = 8.1 Hz, $J_{2'4'}$ = 2.1 Hz, $J_{4'6'}$ = 1.2 Hz, 1H, C4′-H), 7.80 (t, J = 2.0 Hz, 1H, C2′-H), 9.16 (s, 1H, NH).
13a[b]	3.27	3.49	–	7.62–7.95 (m, 5H, arom.)
14b[c]	3.46	3.66	4.40	7.53–8.03 (m, 4H, arom.)
14c[c]	3.46	3.66	4.39	7.40–8.12 (m, 4H, arom.)
20[b]	3.20	3.40	3.95	4.57 (s, 2H, CH_2OH), 5.65 (s, 1H, OH)

[a] δ [ppm]; [b] in DMSO-d_6; [c] in $CDCl_3$; [d] arbitrary assignment.

Table 3. Apparent adenosine receptor affinities and selectivity of aza-analogs of 8-styrylxanthine derivatives.



Compound		X	Y	R^1	R^2	$K_i \pm SEM^a$ [μM] A_1-Affinity Rat brain cortical membranes [^{3}H]CHA	A_{2A}-Affinity Rat brain striatal membranes [^{3}H]CGS21680	A_{2A}-Selectivity (A_1/A_{2A})
8-Styrylxanthines								
15	8-Styryltheophylline	CH	CH	H	H	0.65[13]	0.29[13]	2.3
16	8-Styrylcaffeine	CH	CH	CH_3	H	3.9[13]	0.094[13]	42
17	8-*m*-Chlorostyrylcaffeine	CH	CH	CH_3	Cl	>1 (17 %) 28.2[13]	0.036 ± 0.006 0.054[13]	>28
Aza-analogs of 8-styrylxanthines **Imines Type I** (X = N, Y = CH)								
2a		N	CH	H	H	3.9± 1.6	9.4 ± 2.7	0.42
2b		N	CH	CH_3	H	>25 (43 %)	0.29 ± 0.08	>43
2c		N	CH	H	Cl	5.1 ± 0.8	6.3 ± 1.1	0.81
2d		N	CH	CH_3	Cl	25 (33 %)	0.93 ± 0.28	>13
2e		N	CH	CH_3	Br	>10 (27 ± 4 %)	2.91 ± 1.96	>3
Imines Type II (X = CH, Y = N)								
7		CH	N	H	Cl	>30 (11 ± 2 %)	> 30 (6 ± 4 %)	–
8		CH	N	CH_3	Cl	>30 (8 ± 5 %)	5.8 ± 1.3	>5
Azo Compounds (X = Y = N)								
13a		N	N	H	H	25.7 ± 2.3	11.3 ± 1.3	2.3
13b		N	N	H	Cl	>10 (32 ± 11 %)	10.8 ± 1.4	–
14a		N	N	CH_3	H	7.8 ± 0.6	0.61 ± 0.11	13
14b		N	N	CH_3	Cl	7.9 ± 1.5	0.40 ± 0.06	20
14c		N	N	CH_3	Br	7.0 ± 2.0	0.45 ± 0.04	16
Amides Type I (X = NR, Y = CO)								
3a		NH	CO	H	H	>25 (48 ± 6 %)	>25 (28 ± 3 %)	–
4		N-CH_3	CO	CH_3	H	>100 (18 ± 2 %)	>100 (19 ± 4 %)	–
Amides Type II (X = CO, Y = NR)								
10		CO	NH	H	Cl	42 ± 10	129 ± 41	0.3
11		CO	NH	CH_3	Cl	28 ± 7[b]	5.5 ± 2.7[b]	5

[a] In some cases K_i values could not be determined due to limited solubility of the compounds; therefore, percent inhibition of radioligand binding at the highest tested concentration is given.
[b] Results from two independent experiments.

N7-methyl group depends on substitution in the 8-position and is found at ca. 3.8 ppm (in DMSO-d_6) for 8-unsubstituted xanthines [20,21]. In 8-benzoylaminoxanthine **4**, the 7-methyl group is shifted upfield to 3.55 ppm. The other compounds exhibit shifts for the 7-methyl group between 4.0–4.3 ppm (in DMSO-d_6) or between 4.1–4.5 ppm (in $CDCl_3$) consistent with data for other 8-substituted xanthine derivatives bearing an aryl or styryl substituent [16,22]. Methylation in the 9-position of compound **4**, as well as of all other derivatives can be excluded, since this would cause a downfield shift of the methyl protons at N-3 from 3.3–3.4 ppm (in D_2O or DMSO-d_6) to about 3.7–3.8 ppm[23,24] due to mutual steric hindrance of the 3- and 9-methyl groups.

For one representative compound, imine **2a**, a ^{13}C-NMR spectrum was recorded (see Experimental). This could only be done at elevated temperatures (100 °C, in DMSO-d_6) due to the low solubility of the compound, which was even lower for other derivatives. ^{13}C-NMR data of **2a** correspond well with data for other 1,3,8-substituted xanthine derivatives [24]. Typically, C8 is shifted downfield in comparison with 8-unsubstituted analogs (by 13 ppm from 141 to 154 ppm) [20,21]. Only one set of signals was observed in this spectrum.

Biological Evaluation

The compounds were tested in radioligand binding assays for affinity to A_1 and A_{2A} adenosine receptors in rat cortical membrane, and rat striatal membrane preparations, respectively. The A1-selective agonist [^{3}H]N^6-cyclohexyladenosine (CHA) was used as A_1 ligand, and the A_{2A}-selective agonist [^{3}H]2-[4-(carboxyethyl)phenylethylamino]-5'-N-ethylcarboxamidoadenosine (CGS21680) as A_{2A} ligand.

Structure-Activity Relationships

The ethenyl group of the styryl substituent in 8-styryltheophylline (**15**), 8-styrylcaffeine (**16**), 8-*m*-chlorostyrylcaffeine (CSC, **17**) and its bromo-substituted analogon was replaced by an imine group. In one series (compounds **2a–e**) the nitrogen was adjacent to the xanthine carbon atom C-8 (type I imines), another series showed an inversed imine structure with the nitrogen adjacent to the phenyl ring (compounds **7** and **8**, type II imines). Furthermore, a series of azo compounds was prepared, in which both methine groups were replaced by nitrogen atoms (compounds **13a,b, 14a–c**). In addition, compounds were synthesized in which the ethenyl bridge of the styrylxanthines was replaced by an amide function (**3a,4**), or an inverse amide structure (**10,11**). In Table 3 the apparent K_i values obtained in radioligand binding assays for the new compounds are listed. Affinities of corresponding 8-styrylxanthine derivatives (**15–17**) are given for comparison.

In analogy to styrylxanthines, imines and azo derivatives can exist in two isomeric forms as (*E*)- and (*Z*)-isomers. Styrylxanthines have been shown to photoisomerize in dilute solution at normal daylight [14,25,26]. Therefore radioligand binding data in Table 3 represent test results from stable mixtures of both stereoisomers. Imines and azo compounds

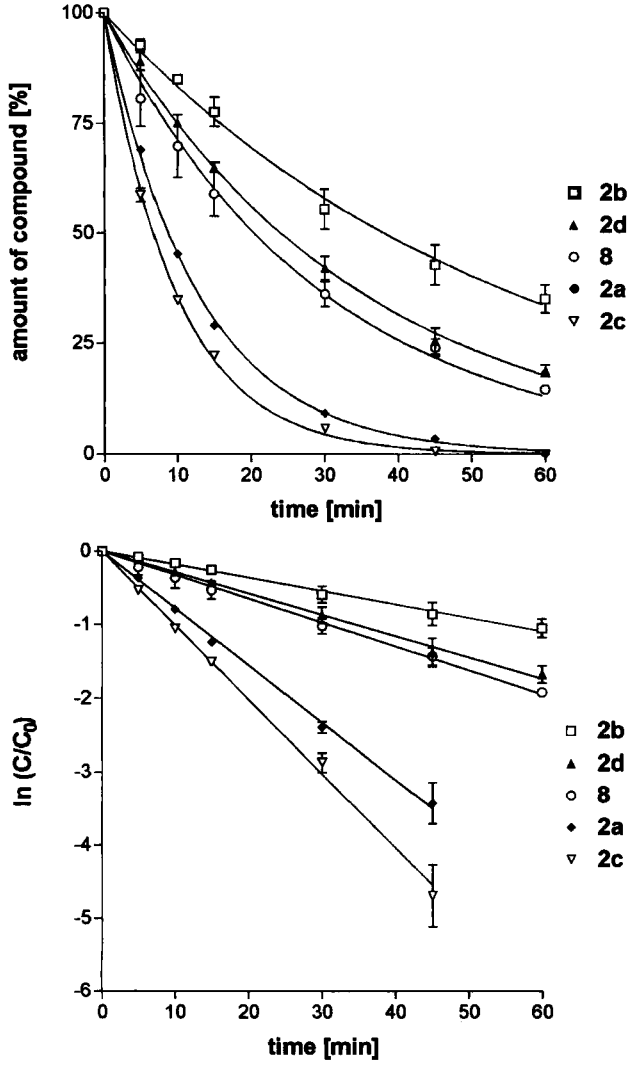


Figure 1. Hydrolysis rates in Tris-buffer (pH = 7.4) at 23 °C of compounds **2b** (slope of the curve, –0.0183; half life, 38 min; r^2 = 0.9791), **2d** (slope of the curve, –0.0290; half life, 24 min; r^2 = 0.9904), **8** (slope of the curve, –0.0314; half life, 20 min; r^2 = 0.9743), **2a** (slope of the curve, –0.0769; half life, 8.5 min; r^2 = 0.9987), **2c** (slope of the curve, –0.1020; half life, 6.5 min; r^2 = 0.9986).

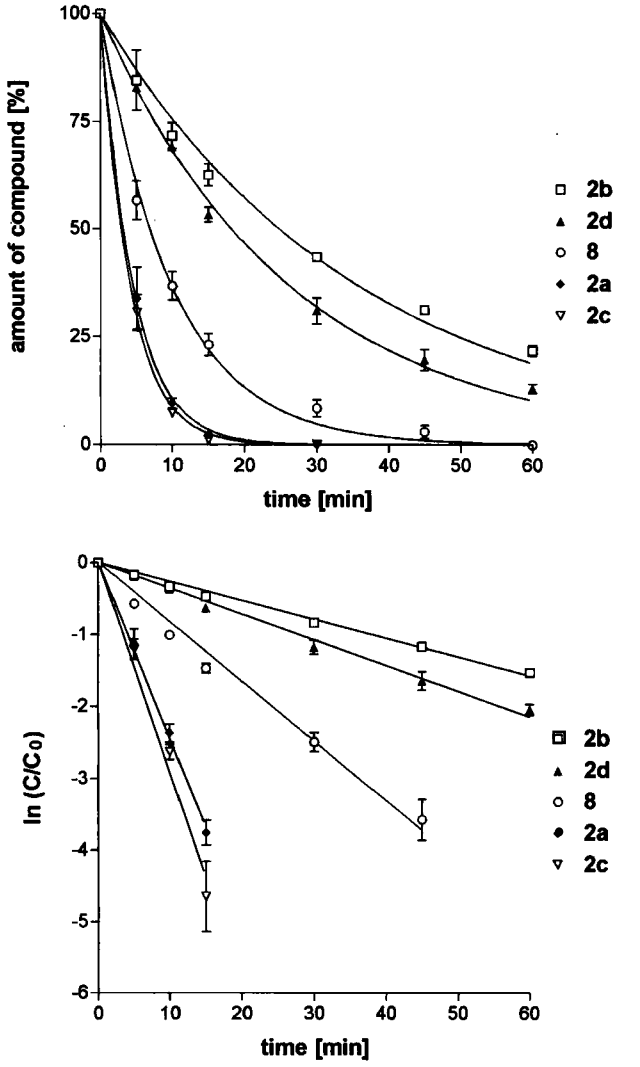


Figure 2. Hydrolysis rates in Tris-buffer (pH = 7.4) at 37 °C of compounds **2b** (slope of the curve, –0.0248; half life, 25 min; r^2 = 0.9812), **2d** (slope of the curve, –0.0346; half life, 18 min; r^2 = 0.9937), **8** (slope of the curve, –0.0773; half life, 7 min; r^2 = 0.9921), **2a** (slope of the curve, –0.2504; half life, 3.1 min; r^2 = 0.9843), **2c** (slope of the curve, –0.3074; half life, 2.8 min; r^2 = 0.9941).

investigated are also assumed to be mixtures of (*E*)- and (*Z*)-isomers.

All investigated aza-analogs of styrylxanthines appeared to be less potent compared to the corresponding styrylxanthines at both receptor subtypes. Imines of type I, derived from 8-aminoxanthines, were more potent than imines of type II, derived from xanthine-8-carbaldehydes (cp. **2c/7** and **2d/8**). Structure-activity relationships of imines roughly paralleled those of styrylxanthines [12–16]. Thus, caffeine derivatives were much more potent at A_{2A}-AR than theophylline derivatives, but less potent at the A1-AR (cp. **2a/2b, 2c/2d, 7/8**). A surprising result was, however, that the introduction of a *meta*-chloro-substituent into the phenyl ring of compound **2b** appeared to result in a 3-fold decrease in A_{2A} AR affinity (compound **2d**), while it generally causes a 2–3-fold increase in A_{2A} affinity in styrylxanthines (cp. **16/17**) [5,13,14]. A bromo instead of a chloro substituent in the *meta*-position had led to a further increase in A_{2A}-affinity in styrylxanthines [5,14], but appeared to have the opposite effect in the imines (see compound **2e**).

Since imines could be unstable under the test conditions, we investigated the possibility of hydrolysis of selected compounds (see Fig. 1 and 2). Five representative imines were incubated in aqueous buffer solution (tris(hydroxymethyl)-aminomethane hydrochloride buffer, 50 mM, pH 7.4) under the test conditions of the A_{2A}-AR assay (at 23 °C, Fig. 1) and the A_1-AR assay (at 37 °C, Fig. 2). Samples were taken in certain time intervals and investigated by UV photometry, in order to calculate the remaining amount of intact compound in solution. Indeed, all investigated imines were unstable in aqueous buffer solution. As expected, hydrolysis was significantly faster at 37 °C (A1 assay conditions) than at 23 °C (A_{2A} assay conditions). Measurement of the hydrolysis kinetics revealed an exponential decay of first order for all imines investigated. This is documented by the diagrams in Fig. 1 and 2 in which ln (C/C_0) (C = concentration of compound, C_0 = concentration at time zero) is plotted over the time (t); the diagrams show linear relationships for all compounds.

Methylation in the xanthine 7-position had a stabilizing effect on the imines, probably due to steric hindrance of the nucleophilic attack at the imine function. Thus caffeine derivatives **2b** and **2d** hydrolyzed much more slowly than theophylline derivatives **2a** and **2c**. Substitution on the phenyl ring with the electron-withdrawing chlorine atom caused the opposite effect, as expected; compound **2d** hydrolyzed considerably faster compared to **2b**. This may be the reason for the unexpectedly low observed A_{2A}-AR affinity of chloro-derivative **2d** in comparison with unsubstituted **2b** (see above).

Hydrolysis rate of type II imines was investigated (compounds **7** and **8**). Compound **8** showed a slightly faster hydrolysis at 23 °C (A_{2A}-AR assay conditions) than the corresponding type I imine **2d**. At higher temperatures (37 °C, A1-assay conditions), however, **8** hydrolyzed considerably faster than **2d** (Fig. 1 and 2).

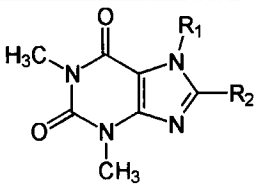
In accordance with the above mentioned results, compound **7** is the most unstable compound of the present series, as an imine type II compound, unsubstituted at N-7, and bearing a *meta*-chloro substituent on the phenyl ring. Its hydrolysis is so fast that after a few minutes no starting compound can be detected anymore by UV spectrophotometry. Therefore, no kinetic study was performed for **7**. On the other hand, compound **2b** is the most stable compound of this series, as a type I imine, methylated in the 7-position, containing an unsubstituted phenyl ring.

Due to the instability of the imines under test conditions, apparent AR-affinities as presented in Table 3 for imines **2a–e**, **7**, and **8** are underestimated. A_{2A}-receptor selectivity, on the other hand, is overestimated, since hydrolysis is faster under A1-assay conditions (37 °C) than under A_{2A}-assay conditions. Compound **2d** (imine type I) exhibited a similar rate of hydrolysis at 23 °C (half life: 24 min) as compound **8**, the corresponding imine type 2 (half life: 20 min at 23 °C). Apparent K_i values at A_{2A}-AR, however, differed by a factor of 6, **2d** (apparent K_i = 0.93 μM) being more potent than **8** (apparent K_i = 5.8 μM). Therefore it appears that a nitrogen atom adjacent to the xanthine 8-position is tolerated by the receptor, while a nitrogen atom adjacent to the phenyl ring, as in imines of type II, is less favorable and reduces A_{2A}-AR affinity.

Imine **2b** (type I) exhibits an apparent K_i value of 290 nM at A_{2A}-AR. The corresponding 8-styrylcaffeine **16** is about 3 times more potent (K_i = 94 nM). Taken into account the instability of **2b**, which is degraded after one hour of incubation in the A_{2A}-AR radioligand binding assay by 67%, it appears that imines of type I would have similarly high affinity for A_{2A}-AR compared to styrylxanthines. For **2b** a theoretical K_i value was calculated using corrected concentrations (33% remaining compound at the end of the assay procedure). A K_i value of 98 nM was obtained at A_{2A}-AR, which is indeed virtually identical with the A_{2A}-affinity of the corresponding styrylcaffeine **16** (K_i = 94 nM).

Hydrolysis of type I imines leads to 8-aminotheophylline (**1a**), or 8-aminocaffeine (**1b**), respectively, while hydrolysis of type II imines will produce theophylline-8-carbaldehyde (**6**), or caffeine-8-carbaldehyde (**19**), respectively. These xanthine derivatives could contribute to the measured AR-affinites presented for compounds **2a–e**, **7** and **8** in Table 3. Therefore we determined A_1- and A_{2A}-AR binding of these compounds. Data for **1a**, **1b**, **6** and **19** are presented together with AR-affinities of some xanthines which were starting compounds in the present study, including theophylline (**12**), caffeine (**18**), and 8-hydroxymethylcaffeine (**20**) in Table 4. Theophylline (**12**) and caffeine (**18**) are weak, non-selective AR-antagonists, theophylline being 2–3-fold more potent than caffeine. The introduction of an amino group in the 8-position of theophylline abolishes AR-affinity (compound **1a**). 8-Aminocaffeine (**1b**), however, also showing no affinity for A_1-AR, is somewhat more potent than theophylline and caffeine at A_{2A}-AR; the compound is A_{2A}-selective. Its A_{2A}-affinity, however, is much lower than that determined for the caffeine-imines **2b**, **2d**, and **2e** (Table 3). Theophylline-8-carbaldehyde (**6**), degradation product of imines **7** and **8**, exhibits only very low A_1- and A_{2A}-AR affinities, similar to those determined for 8-(hydroxymethyl)caffeine (**20**, see Table 4). We conclude that degradation products do not appear to contribute to the determined AR-affinities of the investigated imines **2a–e**.

Table 4. Adenosine receptor affinities of starting xanthines and degradation products for comparison.



		R^1	R^2	$K_i \pm$ SEM [μM] or percent inhibition of radioligand binding (in brackets) at concentration indicated [μM]	
				A_1-Affinity Rat brain cortical membranes [^{3}H]CHA	A_{2A}-Affinity Rat brain striatal membranes [^{3}H]CGS21680
12	Theophylline	H	H	14[20]a	22[20]b
18	Caffeine	CH_3	H	44[32]a	45[32]b
1a	8-Aminotheophylline	H	NH_2	>>25 (4%)[c]	>>25 (0%)[c]
1b	8-Aminocaffeine	CH_3	NH_2	>>25 (8%)[c]	13.9[c]
6	Theophylline-8-carbaldehyde	H	CHO	113[c]	>250 (42%)[c]
19	Caffeine-8-carbaldehyde	CH_3	CHO	101 ± 2[d]	46 ± 18
20	8-(Hydroxymethyl)caffeine	CH_3	CH_2OH	98[c]	61 ± 11[d]

[a] [^{3}H]PIA was used as radioligand in that study.

[b] [^{3}H]NECA was used as radioligand in that study.

[c] Result from single experiment.

[d] Result from two independent experiments.

In contrast to imines, azo compounds are stable in aqueous buffer solution. A series of five compounds was investigated, in which the ethenyl bond of styrylxanthines was formally exchanged for an azo function (compounds **13a, b, 14a–c**, Table 3). Phenylazoxanthines were less potent than corresponding styrylxanthine derivatives. In styrylxanthines, methylation in the 7-position increases A_{2A}-AR affinity, while decreasing A_1-affinity (see for example **15/16**) [5]. In the azo analogs, A_{2A}-affinity is also increased by 7-methylation of the xanthine structure (19-fold for **13a/14a**, 27-fold for **13b/14b**), but A_1-affinity is increased as well, although to a smaller extent (ca. 3-fold). As observed for styrylcaffeines (cp. **16/17**), a *meta*-chloro substituent on the phenyl ring increases A_{2A}-affinity about 2-fold, virtually without having any effect on A_1-affinity, thus increasing A_{2A}-selectivity (cp. **14a/14b**). Bromo- and chloro-substituted derivatives **14b** and **14c** were about equipotent at AR.

Azo compound **14a** showed lower A_{2A}-affinity than imine **2b** in our assay, despite partial degradation of **2b**. This result is another support for the finding that a nitrogen atom adjacent to the xanthine C-8 is tolerated, while a nitrogen atom adjacent to the phenyl ring, as in imines of type II and in azo compounds, leads to a reduction in AR-affinity. Nevertheless, 8-(phenylazo)xanthines are a novel class of A_{2A}-AR antagonists, which are easily accessible by coupling of 8-unsubstituted xanthine derivatives with phenyldiazonium salts. They may serve as new leads for the development of more potent and selective A_{2A}-AR antagonists.

In a further series of four compounds, the ethenyl bridge of styrylxanthines was replaced by an amide structure (compounds **3a,4,10,11**). Amide compounds proved to be better soluble in water than styrylxanthines, imines, or azo compounds, thus permitting testing of higher concentrations of these compounds (see Table 3). All amides were considerably less potent than corresponding styrylxanthines, and imine or azo analogs at A_{2A}-AR. 7-Methylation of xanthine **10** increased A_{2A}-AR affinity (5-fold) without much effect on A_1-affinity (compound **11**), yielding a weak, A_{2A}-selective compound (K_i A_{2A} = 5.5 μM). Bis-methylation in the 7-position and on the exocyclic amide nitrogen of compound **3** to compound **4** resulted in a decrease in AR-affinity at both receptor subtypes.

In conclusion, novel classes of A_{2A}-selective AR antagonists have been identified and investigated, which can be envisaged as aza analogs of 8-styrylcaffeine, containing an imine, amide or azo structure. The results of the present study will be useful for the development of more potent and selective A_{2A}-AR antagonists, which have a potential as novel drugs for the treatment of Parkinson's disease.

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Experimental

Melting points were determined with a Büchi 530 apparatus and are not corrected. Nuclear magnetic resonance spectra were determined using a Bruker AC-200 or a Bruker AMX-500 spectrometer (for ^{1}H-NMR spectra), or a Bruker AMX 400 (for ^{13}C-NMR spectra). Chemical shifts are given in ppm downfield from tetramethylsilane as an internal standard. UV spectra were recorded with a Perkin Elmer Lambda 12 spectrometer. UV spectra for kinetical investigations were determined with a Hewlett Packard 8452A diode array spectrophotometer. Microanalyses were performed by the chemistry department, University of Würzburg, using an Elemental Analyser, Carlo Erba Instruments and were within ± 0.4% of theoretical values for all elements listed, unless otherwise indicated.

Syntheses

8-Aminotheophylline (**1a**)[17] 8-aminocaffeine (**1b**)[17], 8-hydroxymethyltheophylline (**5**) [18], theophylline-8-carbaldehyde (**6**)[18], theophylline-8-carboxylic acid (**9**)[19] and caffeine-8-carbaldehyde (**19**)[18] were prepared according to literature methods.

*8-Benzylideneaminotheophylline (**2a**)[17], 8-benzylideneaminocaffeine (**2b**), 8-[1-(3-chlorophenyl)methylideneamino]theophylline (**2c**), 8-[1-(3-chlorophenyl)methylideneamino]caffeine (**2d**), 8-[1-(3-bromophenyl)-methylideneamino]caffeine (**2e**)*

General procedure:

A suspension of 1.0 g (5.1 mmol) of 8-aminotheophylline (**1a**), or 1.0 g (4.8 mmol) of 8-aminocaffeine (**1b**), respectively, in 30 mL of the appropriate 3-substituted benzaldehyde (50 equiv.) was refluxed for 2h. The product was allowed to crystallize over night. The precipitate was filtered, washed with methanol and recrystallized from DMF.

2a: ^{13}C-NMR (DMSO-d_6): δ = 27.8 (N1-CH_3), 29.9 (N3-CH_3), 106.9 (C5), 129.2, 129.7, 133.0, 135.3 (phenyl), 147.9 (C4), 151.5 (C2), 153.6, 154.3 (C6, C8), 165.7 (exocycl. -C=N-).

*8-N-Benzoylaminotheophylline (**3a**)*

A mixture of 1.95 g (0.01 mol) of 8-aminotheophylline (**1a**), 1.40 g (0.01 mol) of benzoyl chloride and 20 mL of pyridine was stirred at 70 °C for 2h. After cooling to room temp. 200 mL of water was added and the mixture was left standing overnight. The precipitate was separated by filtration, washed with water and recrystallized from DMF.

*8-(N,N-Dibenzoylamino)caffeine (**3b**)*

A mixture of 1.0 g (0.005 mol) of **1b**, 1.4 g (0.01 mol) of benzoyl chloride and 10 mL of anhydrous pyridine was heated at 70 ± 5°C with stirring. After 1 h the solid had dissolved and 15 min later product started to precipitate. After 2 h, water was added, the precipitate was filtered off, washed with water and recrystallized from ethanol.

*8-(N-Methyl-N-benzoylamino)caffeine (**4**)*

A mixture of 0.6 g (0.002 mol) of compound **3a**, 2.8 g (0.02 mol) of methyl iodide and 0.55 g (0.004 mol) of K_2CO_3 in 10 mL DMF were stirred at room temp. overnight. The mixture was diluted with water, the precipitate was filtered off, washed with water and recrystallized from ethanol (96%).

*8-(3-Chlorophenyliminomethyl)theophylline (**7**)*

Theophylline-8-carbaldehyde[18] (**6**, 0.64 g, 3.1 mmol) was dissolved in DMF (15 mL). 3-Chloroaniline (0.5 mL, 4.65 mmol) was added and the solution was stirred for 2 h. The formed precipitate was collected by filtration and washed with H_2O to yield yellow crystals.

*8-(3-Chlorophenyliminomethyl)caffeine (**8**)*

Compound **7** (1.2 g, 3.75 mmol) was dissolved in DMF (20 mL) by the addition of K_2CO_3 (1 g, 7.3 mmol). Methyl iodide (0.45 mL, 7.5 mmol) was added and the solution was stirred at room temp. for 4 h. The product was precipitated by the addition of 20 mL of H_2O. After filtration the yellow crystals were washed with cold water.

*8-N-(3-Chlorophenyl)-8-carboxamidotheophylline (**10**)*

Theophylline-8-carboxylic acid[19] (**9**, 0.5 g, 2.23 mmol) and 3-chloroaniline (0.26 mL, 2.5 mmol) were dissolved in DMF (15 mL) at 70 °C. After cooling, N-(3-dimethylaminopropyl)-N′-ethyl-carbodiimide hydrochloride (0.45 g, 2.25 mmol) was added and the solution was stirred for 72 h. The product was separated by the addition of 20 mL of H_2O. The precipitate was isolated by filtration and washed with water to yield a colorless product.

*8-N-(3-Chlorophenyl)-8-carboxamidocaffeine (**11**)*

Compound **10** (0.1 g, 0.30 mmol) was dissolved in DMF (10 mL) by the addition of K_2CO_3 (0.08 g, 0.58 mmol). Methyl iodide (0.5 mL, 8.1 mol) was added and the solution was stirred for 72 h at room temperature. The product separated after the addition of 15 mL of H_2O. The precipitate was collected by filtration and washed with water to yield a colorless product.

*8-(Phenylazo)theophylline (**13a**), 8-(3-chlorophenylazo)theophylline (**13b**), 8-(3-bromophenylazo)theophylline (**13c**)*

General procedure (in analogy to reference[27])

A cold solution of phenyldiazonium chloride, 3-chlorophenyldiazonium chloride, or 3-bromophenyldiazonium chloride, respectively, (10 mmol) was reacted with an equimolar quantity of theophylline dissolved in cold (0 °C) aq. KOH solution (5%,) with stirring. The precipitates were collected, washed with water and recrystallized from DMF (**13a,b**). Compound **13c** was used for the subsequent step without purification.

*8-(Phenylazo)caffeine (**14a**), 8-(3-chlorophenylazo)caffeine (**14b**), 8-(3-bromophenylazo)caffeine (**14c**)*

General procedure:

A mixture of **13a**, **13b**, or **13c**, respectively, (10 mmol) methyl iodide (20 mmol) and K_2CO_3 (10 mmol) was refluxed in DMF (50 mL) for 2 h. The product was allowed to crystallize overnight. The precipitate was filtered, washed with methanol and recrystallized from DMF.

*8-(Hydroxymethyl)caffeine (**20**)*

8-(Hydroxymethyl)theophylline[18] (**5**, 1.0 g, 4.75 mmol) was dissolved in 15 mL of DMF with the addition of K_2CO_3 (1.3 g, 9.5 mmol). Methyl iodide (0.6 mL, 9.6 mmol) was added and the solution was stirred at room temp. for 3 h. The product was precipitated by the addition of 15 mL of H_2O. After filtration, the colorless crystals were washed with cold water.

Radioligand Binding Assays

Inhibition of binding of [^{3}H]N^6-cyclohexyladenosine (CHA) to A_1-adenosine receptors of rat brain cortical membranes and inhibition of [^{3}H]2-[4-(carboxyethyl)phenylethylamino]-5′-N-ethylcarboxamidoadenosine (CGS21680) to A_{2A}-adenosine receptors of rat brain striatal membranes were assayed essentially as described.[28–30] As buffer tris(hydroxymethyl)aminomethane- (Tris-) HCl buffer, 50 mM, pH 7.4 (at room temp.) was used for all experiments. The incubation tubes for the A1-assay contained 50 µL of test compound dissolved in DMSO, or a control, respectively, 1.75 mL of buffer, 100 µL of radioligand solution in buffer to obtain a final concentration of 1 nM and 100 µL of membrane suspension treated with adenosine deaminase, to give a final volume of 2 mL. A_{2A}-assay tubes contained 25 µL of compound dissolved in DMSO, or a control, respectively, 0.725 mL of buffer, 50 µL of a $MgCl_2$ solution (200 mM) in buffer, 100 µL of radioligand solution in buffer to obtain a final concentration of 5 nM and 100 µL of membrane suspension treated with adenosine deaminase, to give a final volume of 1 mL. 2-Chloroadenosine (10 µM) was used to define nonspecific binding. DMSO concentration was 2.5 % (V/V) in all experiments. Incubation was performed at 37 °C for 1.5 h (A_1-assay), or at 23 °C for 1 h (A_{2A}-assay). Incubation was terminated by rapid filtration through glass fiber (GF/B) filters using a cell harvester. Filters were washed twice with ice-cold

buffer. Wet filter papers were incubated with scintillation cocktail for at least 6 h before radioactivity was counted. Inhibition of the receptor-radioligand binding was determined by a range of 5 to 6 concentrations of the compounds in triplicate in at least three separate experiments. The Cheng-Prusoff equation[31] and K_D values of 1 nM for [^{3}H]CHA and 14 nM for [^{3}H]CGS21680 were used to calculate the K_i values from the IC_{50} values, determined by the nonlinear curve fitting program PRISM™ (GraphPad, San Diego, California, USA).

Measurement of imine hydrolysis

Stock solutions of compounds in DMSO (0.4 mMol/L) were prepared and diluted in Tris-HCl buffer (50 mM, pH 7.40) to a final concentration of 10µM. The samples were incubated under assay conditions (A_1: 37°C, A_{2A}: 23°C). After appropriate time intervals, aliquots from the solutions were taken and UV spectra were recorded. The amount of each compound was determined by measuring the UV absorbance at its maximum (see Table 1). Hydrolysis products were shown to exhibit no significant absorbance at these maxima. Thus, imine concentrations could be determined by standard calibration.

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