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Configurationally stable analogs of styrylxanthines as A_{2A} adenosine receptor antagonists*

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Summary — Configurationally stable analogs of the potent, A_{2A}-selective adenosine receptor (AR) antagonist 3,7-dimethyl-1-propargyl-8-styrylxanthine (8-styryl-DMPX, **3**) were synthesized and investigated in radioligand binding assays for affinity to the high-affinity A_1- and A_{2A}-AR subtypes of rat brain. All derivatives prepared, including compounds in which the styryl double bond was replaced by a cyclopropane ring or a triple bond, or in which it was integrated into a (hetero) cyclic ring system, were less potent and less selective compared to the parent compound **3**. The best compound of the present series was 8-(phenylethynyl)-DMPX (**21**), exhibiting a K_i value at A_{2A}-AR of 300 nM and a > 10-fold selectivity versus A_1-AR. In view of its configurational stability, **21** may be an interesting lead compound for the development of more potent A_{2A} antagonists by introducing appropriate substituents in the phenyl ring. Based on conformational analysis of 8-styrylxanthine and 8-(2-naphthyl)xanthine derivatives, it is hypothesized that the bioactive conformation of (*E*)-8-styryl substituents with regard to the imidazole ring of the xanthine nucleus at A_{2A}-AR may be nearly coplanar and *cis*oid, and may differ from the bioactive conformation of such xanthine derivatives at A_1-AR.

xanthine / styrylxanthine / synthesis / *E*-isomer / *Z*-isomer / adenosine receptor / A_{2A} adenosine receptor antagonist / bioactive conformation

Introduction

The physiological nucleoside adenosine plays a unique role in the mammalian organism since it is the only nucleoside for which specific cell membrane receptors are known to exist [1]. So far, four distinct adenosine receptor (AR) subtypes have been described on a pharmacological and molecular–biological basis [1, 2]. Antagonists for the 'high-affinity subtypes' [1], A_1 and A_{2A}, are currently under development as drugs, eg, diuretics with renal protective activity (A_1), cognitive enhancers (A_1), anti-Alzheimer (A_1) and anti-Parkinsonian agents (A_{2A}) [3–5].

The most prominent class of AR antagonists are the xanthine derivatives [1, 6]. Numerous A_1-selective AR antagonists have been developed [1]. The first A_{2A}-selective AR antagonist described in the literature was the caffeine analog, 3,7-dimethyl-1-propargylxanthine (DMPX), a compound of low AR affinity and selectivity [7, 8]. Nevertheless, DMPX is still widely used in in vivo studies due to its relatively good water solubility and high bioavailability. Progress in the development of A_{2A}-AR antagonists have been the investigation for 8-substituted caffeine derivatives such as 8-phenyl- and 8-cyclohexylcaffeine [9], the preparation of 8-styryltheophylline analogs [10] and finally the combination of both structures resulting in the first generation of potent and truly A_{2A}-selective AR antagonists, such as 8-(3,4-dimethoxystyryl)-1,3-dipropyl-7-methylxanthine (KF17837; **1**) [11] and 8-(3-chlorostyryl)caffeine (CSC; **2**) [12]. A further recent development has been the synthesis of 8-styryl-substituted DMPX derivatives, identifying 8-(3-bromostyryl)- (**5**) and 8-(3-chlorostyryl)-DMPX (**4**) which are superior to KF17837 (**1**) and CSC (**2**) with respect to A_{2A} receptor affinity and/or selectivity [13]. A few A_{2A}-selective non-xanthine AR antagonists have also been identified recently, including pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines, eg, SCH58261 [14] and triazolo[1,5-*a*][1,3,5]triazines such as ZM241385 [15].

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**Correspondence and reprints

Major drawbacks of the new A_{2A} antagonists with xanthine structure are: (i) their low water solubility, which limits their in vivo applicability [15, 16]; and (ii) their liability to isomerize in dilute solution when exposed to light [17]. Therefore if no special precautions are taken, test solutions usually contain a mixture of two isomers (*E*- and *Z*-configurations), with the *E*-isomer being considerably more potent, but present as the minor component in the stable mixtures of 8-styrylxanthines that have been investigated so far [13, 17, 18].

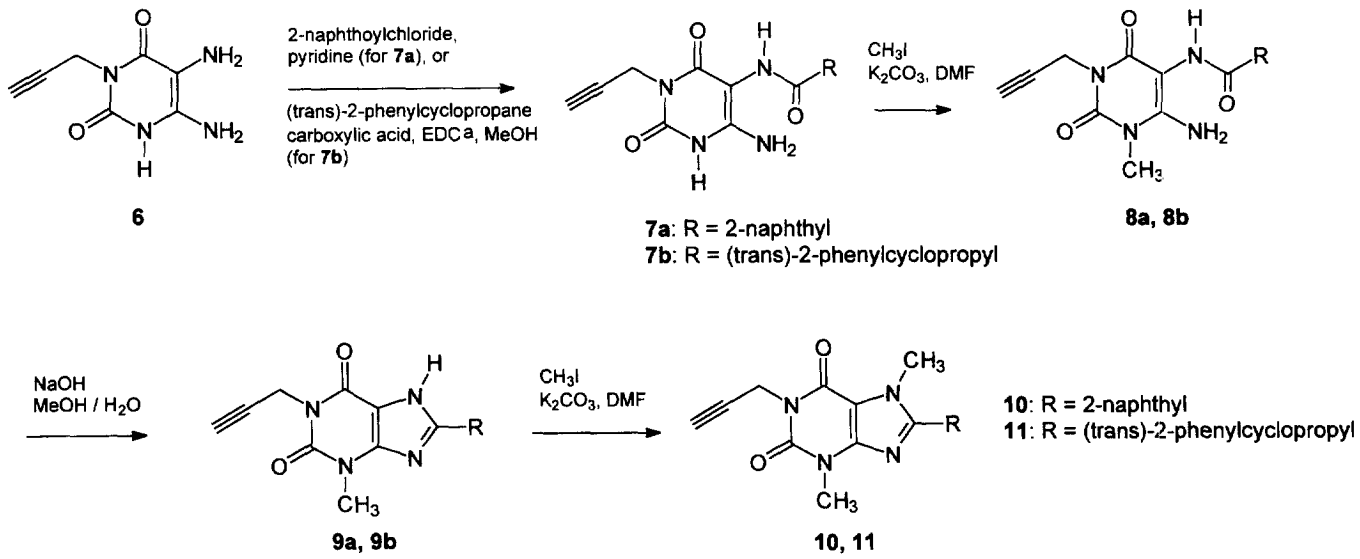
In the present study we synthesized and investigated styrylxanthine analogs, in which the ethenyl bridge is sterically fixed and *E*/*Z*-isomerization is no longer possible.

Chemistry

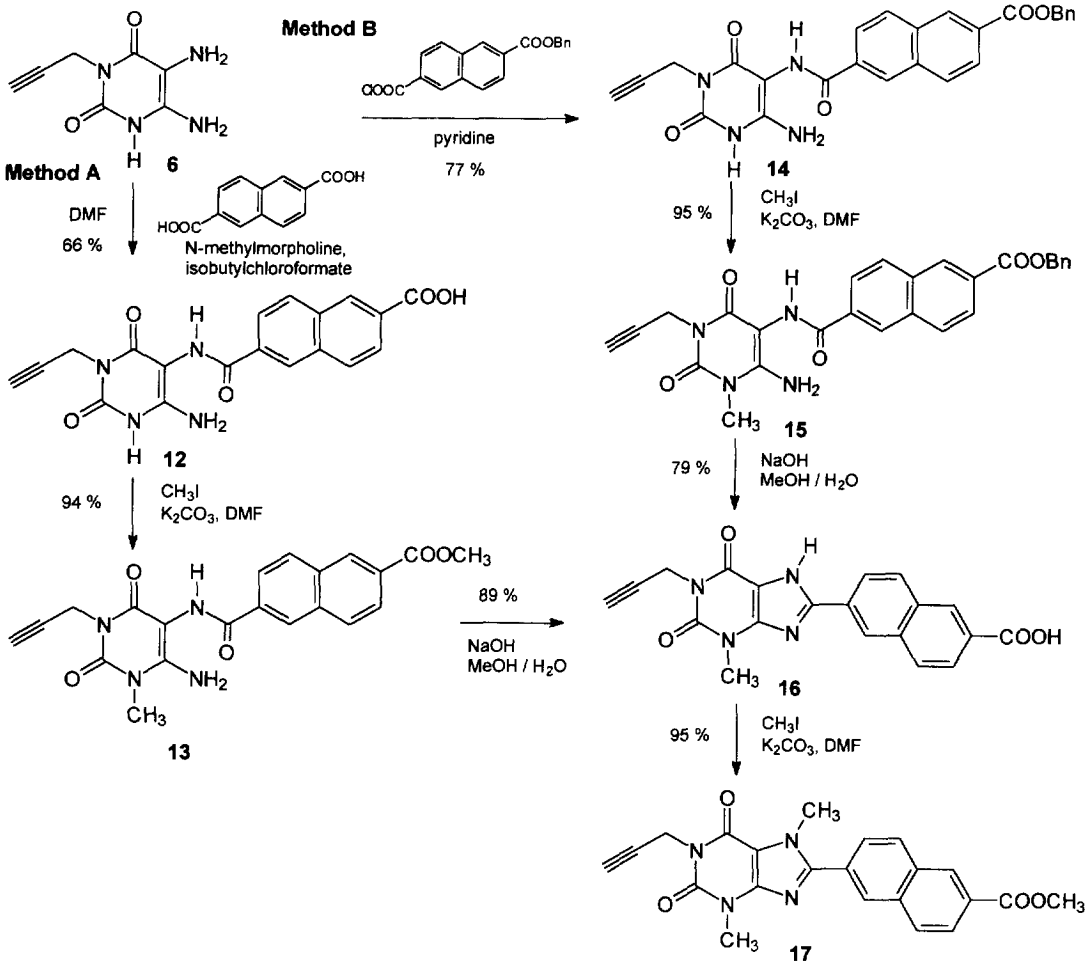
All products were prepared starting from 5,6-diamino-3-propargyluracil **6** [19, 20] (schemes 1–3). For the majority of compounds, a recently developed strategy for the preparation of xanthines with different substituents in the 1-, 3-, 7-, and 8-position was applied [20]. Thus, diaminouracil derivative **6** was reacted either with a carboxylic acid using a carbodiimide as condensing agent (for the preparation of **7b**), with a carboxylic acid chloride in pyridine (for the preparation of **7a** and **14**), or alternatively with a mixed carboxylic acid anhydride (for the synthesis of **12**). Subsequent methylation in the uracil 1-position with methyl iodide and potassium carbonate as a base in dimethylformamide (DMF) at room temperature yielded compounds **8a**, **8b**, **13** and **15** (schemes 1, 2). Ring closure to the xanthines **9a**, **9b** and **16** was achieved by treatment with dilute sodium hydroxide solution in a mixture of ethanol and water at reflux temperature. Methylation in the 7-position again with methyl iodide in the presence of potassium carbonate in DMF yielded the 8-substituted DMPX derivatives **10**, **11**, **17** and **21** in high yields.

For the coupling of 5,6-diamino-3-propargyluracil **6** with naphthaline-2,6-dicarboxylic acid derivatives, two alternative methods (*A* and *B*; scheme 2) were developed and compared. Diaminouracil **6** was condensed with a mixed anhydride prepared in situ from naphthaline-2,6-dicarboxylic acid and isobutylchloroformate *(Method A)*. Methylation of the resulting amide **12** yielded the bismethylated uracil derivative **13**, which was converted to the xanthine derivative **16**. Under alkaline ring closure conditions, the carboxylic acid methyl ester of **13** was hydrolyzed.

Alternatively, one carboxylic acid function of naphthalene-2,6-dicarboxylic acid was protected by benzylation, then the carboxylic acid chloride of the monobenzylester was prepared and subsequently reacted with diaminouracil **6** *(Method B)*. Ring closure in alkaline solution gave rise to hydrolysis of the benzyl



Scheme 1. Synthesis of 8-substituted xanthine derivatives.

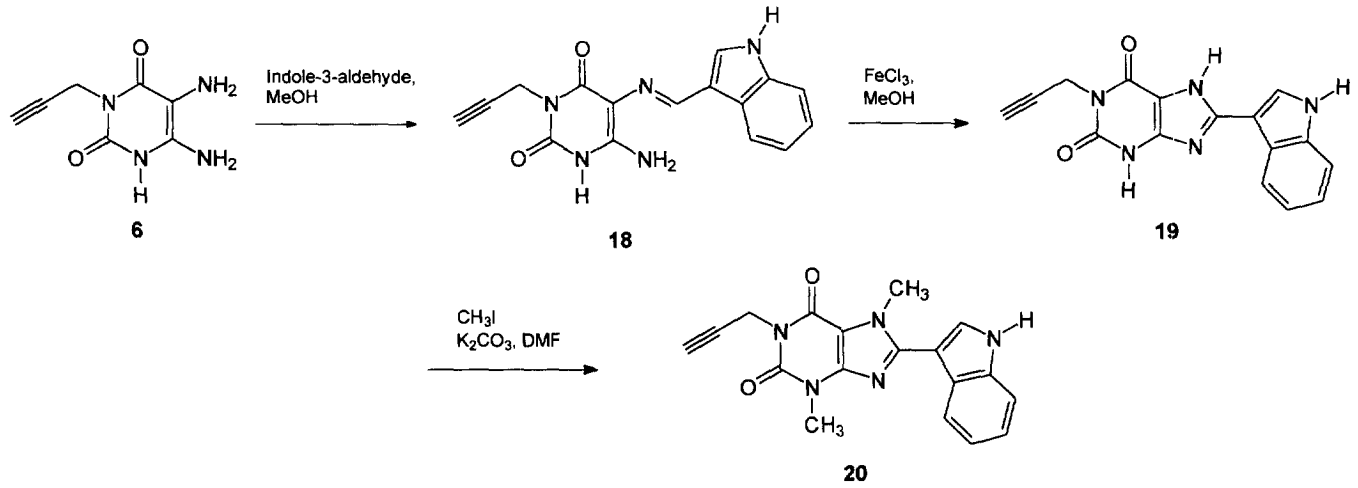


Scheme 2. Synthesis of 8-naphthylxanthines bearing polar substituents on the naphthyl ring.

ester, yielding **16**. Final methylation of **16** under mild conditions yielded bismethylated DMPX derivative **17**.

Both methods, ie, *A* and *B*, resulted in high yields of the desired xanthine derivatives. *Method A*, however, proved to be superior to *Method B* since it was faster (one step less) and particularly because the reaction from **6** to **12** was much cleaner than that from **6** to **14**, yielding a very pure product.

For the preparation of 8-indolyl-DMPX (**20**), an alternative route was used [21]. This was necessary since ring closure of a 5-(indolylcarboxamido)-substituted 6-amino-1-methyl-3-propargyluracil in alkaline solution in analogy to the ring closure reactions of compounds **8a**, **8b**, **13** and **15** was not successful but resulted in hydrolysis of the amide bond. In the alkaline ring closure reaction of such amides to xanthines nucleophilic attack at the carbonyl carbon atom by hydroxide ions competes with intramolecular nucleophilic attack by the uracil 6-amino group. The nature of the substituents at the carbonyl group influences its reactivity. The electron-rich heterocycle indole increases the electron density at the carbonyl group in the indole 3-position [32]. In addition, the indole nitrogen may be deprotonated in aqueous sodium hydroxide solution, which would further reduce electrophilicity of the carbonyl group by delocalization of the negative charge [32]. Thus the carbonyl group becomes less active towards nucleophiles. This may be a reason for the resulting hydroxide-catalyzed



Scheme 3. Synthesis of 8-(3-indolyl)-3,7-dimethyl-1-propargylxanthine.

hydrolysis rather than cyclization reaction. Thus, diaminouracil derivative **6** was condensed with indole-3-carbaldehyde and the Schiff base formed was oxidatively cyclized by ferric chloride. Methylation yielded the bismethylated xanthine **20**.

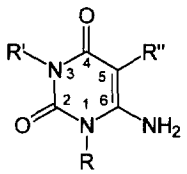
Yields, melting points and results from elemental analyses are given in table I. Intermediate and final products were characterized by their ^{1}H-NMR spectral data, which were in accordance with the proposed structures (see tables II, III).

Table I. Yields, melting points and analytical data of new compounds.

Compound	*Yield (%)*	*Formula*	*Molecular weight*	*Anal*[a]	*Mp (°C)*
7a	74	$C_{18}H_{14}N_4O_3$	334.34	C, H, N	216–217
7b	84	$C_{17}H_{16}N_4O_3$	324.34	C, H, N	245
8a	75	$C_{19}H_{16}N_4O_3$	348.49	C, H, N	277
8b	48	$C_{18}H_{18}N_4O_3$	338.37	C[b], H, N[c]	220
9a	97	$C_{19}H_{14}N_4O_2$	330.35	C, H, N[c]	299–300
9b	28	$C_{18}H_{16}N_4O_2 \cdot H_2O$	338.37	C[b], H, N	274
10	73	$C_{20}H_{16}N_4O_2$	344.37	C, H, N	279–280
11	96	$C_{19}H_{18}N_4O_2$	334.38	C, H, N	139
12	66	$C_{19}H_{14}N_4O_5$	378.35	C, H, N	>300
13	94	$C_{21}H_{18}N_4O_5$	406.39	C, H, N	>270
14	77	$C_{26}H_{20}N_4O_5$	468.47	C[b], H, N[c]	>300
15	95	$C_{27}H_{22}N_4O_5$	482.50	C, H, N	242–244
16	89[d]/79[e]	$C_{20}H_{14}N_4O_4$	374.36	C, H, N[c]	>300
17	95	$C_{22}H_{18}N_4O_4$	402.41	C, H, N	215–217
19	24[f]	$C_{16}H_{11}N_5O_2$	305.30	C, H, N	175
20	76	$C_{18}H_{15}N_5O_2$	333.35	C, H, N	305 (dec)
21	78	$C_{18}H_{14}N_4O_2$	318.11	[g]	233

[a]Elemental analysis within ± 0.4% for elements indicated unless otherwise noted; [b]C found (calc); **8a**: 64.39 (65.48); **9b**: 63.33 (63.79); **14**: 66.20 (66.66); [c]N found (calc); **8a**: 15.58 (16.08); **9a**: 16.50 (16.96); **14**: 11.50 (11.96); **16**: 14.54 (14.97); [d]starting from **13**; [e]starting from **15**; [f]overall yield in two steps; [g]high resolution mass spectrum: calc mass: 318.1114, found: 318.111; elemental analysis not performed.

Table II. ^{1}H-NMR data on selected uracil derivatives.



Compound	N1-R	N3-R'	C5-R''	C6-NH_2
		δ (ppm) in DMSO-d_6, J (Hz)		
7a	10.66 (br s, 1H, NH)	3.02 (t, ^4J = 2.1, 1H), 4.46 (d, ^4J = 2.1, 2H)	7.54–7.70 (m, 2H, Ar-H), 7.93–8.07 (m, 4H, Ar-H), 9.08 (s, 1H, NH), 8.58 (s, 1H, Ar-H)	6.25 (br s, 2H)
7b	10.58 (br s, 1H, NH)	3.02 (t, ^4J = 2.1, 1H), 4.42 (d, ^4J = 2.0, 2H)	1.24 (m, 1H, cyclopropyl), 1.38 (m, 1H, cyclopropyl), 2.08 (m, 1H, cyclopropyl), 2.29 (m, 1H, cyclopropyl), 7.09–7.32 (m, 5H, Ar-H), 8.71 (s, 1H, NH)	6.01 (br s, 2H)
8a	3.24 (s, 3H, CH_3)	3.02 (t, ^4J = 2.4, 1H), 4.51 (d, ^4J = 2.4, 2H)	7.53–7.65 (m, 2H, Ar-H), 7.93–8.01 (m, 4H, Ar-H), 8.60 (s, 1H, Ar-H), 9.09 (s, 1H, NH)	6.88 (br s, 2H)
8b	3.33 (s, 3H, CH_3)	3.03 (t, ^4J = 2.3, 1H), 4.48 (d, ^4J = 2.2, 2H)	1.23 (m, 1H, cyclopropyl), 1.38 (m, 1H, cyclopropyl), 2.09 (m, 1H, cyclopropyl), 2.29 (m, 1H, cyclopropyl), 7.08–7.32 (m, 5H, cyclopropyl), 8.70 (s, 1H, NH)	6.75 (br s, 2H)
12	10.70 (br s, 1H, NH)	3.02 (t, 1H), 4.45 (d, 2H)[a]	5.63–5.65 (m, 2H, Ar-H), 7.96–8.26 (m, 4H, Ar-H), 9.13 (s, 1H, NH)	6.30 (br s, 2H)
13	3.33 (s, 3H, CH_3)	3.02 (t, 1H), 4.50 (d, 2H)[a]	3.93 (s, 3H, CH_3), 7.98–8.30 (m, 4H, Ar-H), 8.65–8.66 (m, 2H, Ar-H), 9.17 (s, 1H, NH)	6.92 (br s, 2H)
14	10.73 (br s, 1H, NH)	3.02 (t, 1H), 4.47 (d, 2H)[a]	5.42 (s, 2H, benyl-CH_2), 7.33–7.53 (m, 5H, Ar-H), 7.98–8.32 (m, 4H, Ar-H), 8.65–8.70 (m, 2H, Ar-H), 9.15 (s, 1H, NH)	6.31 (br s, 2H)
15	3.37 (s, 3H, CH_3)	3.02 (t, 1H), 4.50 (d, 2H)[a]	5.42 (s, 2H, benyl-CH_2), 7.34–7.54 (m, 5H, Ar-H), 8.02–8.35 (m, 4H, Ar-H), 8.67–8.72 (m, 2H, Ar-H), 9.19 (s, 1H, NH)	6.90 (br s, 2H)

[a]Badly resolved signals; no reliable coupling constants could be determined.

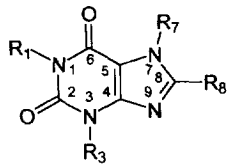
Pharmacology

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 A_1-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*-ethyl-carboxamidoadenosine (CGS21680) as A_{2A} ligand.

Results and discussion

8-Styrylxanthine derivatives including compounds **1**–**5** (see chart 1) belong to the most potent and selective A_{2A}-AR antagonists known so far (table IV). A major drawback of these compounds is their photo-induced *cis–trans*-isomerization in dilute solutions. Therefore we investigated compounds that could be envisaged as sterically fixed analogs of styrylxanthines. Since 1-propargyl-, 3-methyl- and 7-methyl-substitution is optimal for high A_{2A}-AR affinity and selectivity of xanthine derivatives [1, 13], we combined this substitution pattern with various 8-substituents that could undergo *cis–trans*-isomerization. Thus, 8-(2-naphthyl)- and 8-(3-indolyl)xanthines in which the ethenyl double bond of styryl is integrated into an aromatic ring system were prepared. It had been shown earlier that a 1-naphthyl substituent in the 8-position of 9-deazaxanthines is not well tolerated either by A_1- or

Table III. Selected ^{1}H-NMR data on new xanthine derivatives.



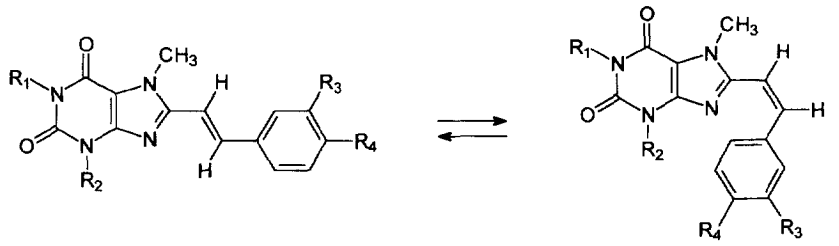
Compound	*δ (ppm) in DMSO-d_6, J (Hz)*							
	R^1		*R^3*		*R^7*		*R^8*
9a	3.07 4.63	(t, ^4J = 2.3, 1H), (d, ^4J = 2.4, 2H)	3.54	(s, 3H, CH_3)	[a]		7.51–7.67 (m, 2H, Ar-H), 7.89–8.29 (m, 4H, Ar-H), 8.69 (s, 1H, Ar-H)
9b	3.08 4.59	(t, ^4J = 2.3, 1H), (d, ^4J = 2.3, 2H)	3.42	(s, 3H, CH_3)	[a]		1.64 (m, 1H, cyclopropyl), 1.73 (m, 1H, cyclopropyl), 2.24 (m, 1H, cyclopropyl), 2.50 (m, cyclopropyl + DMSO-d_6), 7.18–7.33 (m, 5H, Ar-H)
10	2.89 4.69	(t, ^4J = 2.4, 1H), (d, ^4J = 2.4, 2H)	3.54	(s, 3H, CH_3)	4.09	(s, 3H, CH_3)	7.55–7.72 (m, 2H, Ar-H), 7.91–8.13 (m, 4H, Ar-H), 8.35 (s, 1H, Ar-H)
11	3.08 4.58	(t, ^4J = 2.4, 1H), (d, ^4J = 2.4, 2H)	3.48	(s, 3H, CH_3)	3.91	(s, 3H, CH_3)	1.57 (m, 1H, cyclopropyl), 1.69 (m, 1H, cyclopropyl), 2.46–2.58 (m, cyclopropyl + DMSO-d_6), 7.17–7.32 (m, 5H, Ar-H)
16	3.07	(t, 1H), 4.60 (d, 2H)	3.52	(s, 3H, CH_3)	[a]		8.04–8.22 (m, 4H, Ar-H), 8.63–8.71 (m, 2H, Ar-H)
17	3.08 4.61	(t, ^4J = 2.2, 1H), (d, ^4J = 2.2, 2H)	3.46	(s, 3H, CH_3)	4.07	(s, 3H, CH_3)	3.91 (s, 3H, CH_3), 7.91–8.32 (m, 4H, Ar-H), 8.64–8.66 (m, 2H, Ar-H)
19	3.41 4.66	(t, ^4J = 2.2, 1H), (d, ^4J = 2.1, 2H)	12.08	(s, 1H, NH)	13.29	(s, 1H, NH)	7.23 (m, 2H, indolyl), 7.54 (dd, ^3J = 6.6, 1H, indolyl), 8.01 (s, 1H, indolyl), 8.40 (dd, ^3J = 9.9, ^4J = 6.8, 1H, indolyl), 11.77 (s, 1H, indolyl NH)
20	3.11 4.63	(t, ^4J = 2.4, 1H), (d, ^4J = 2.3, 2H)	3.56 (s, 3H, CH_3)		4.11 (s, 3H, CH3)		7.22 (m, 2H, indolyl), 7.52 (dd, ^3J = 8.0, 1H, indolyl), 8.12 (s, 1H, indolyl), 8.35 (d, ^3J = 7.4, 1H, indolyl), 11.97 (s, 1H, indolyl NH)
21	3.13 4.62	(t, ^4J = 2.4,1H), (d, ^4J =2.4, 2H)	3.45 (s, 3H, CH_3)		4.02 (s, 3H, CH_3)		7.4–7.7 (m, 5H, Ar-H)

[a]N7-H could not be detected in some cases due to rapid exchange.

by A_{2A}-AR and that a 2-naphthyl substituent in the same position was favorable for AR affinity of that class of compounds [22].

8-(2-Naphthyl)-DMPX (**10**) is equally potent as its analog 8-styryl-DMPX (**3**) at A_1-AR where both compounds show low affinity, but is 14-fold less potent at A_{2A}-AR. In styrylxanthines, the introduction of a 7-methyl group generally leads to a decrease in A_1-AR and an increase in A_{2A}-AR (compare for example **22** and **4**). In the naphthyl analog (compare **9a** and **10**), a decrease in A_1 affinity (10-fold) can be observed by 7-methylation as expected; but A_{2A} affinity is also decreased by 7-methylation (3-fold) resulting in a compound (**10**) with a K_i value of 380 nM at A_{2A}-AR and low A_{2A} selectivity (3-fold). This effect parallels the effect of 7-methylation of 8-phenylxanthine and related 8-arylxanthine derivatives [9]. In these compounds, affinity is reduced at both receptor subtypes by 7-methylation. This effect may be caused: (a) by a loss of the N7-hydrogen as a hydrogen bond donor; and/or (b) by the interaction of the 8-aryl ring with the 7-methyl group, which may force the aryl ring into a conformation that is unfavorable for interaction with the receptors [13, 23]. The same type of interaction of methyl hydrogen atoms of naphthyl derivatives **10** and **17** with the α-hydrogen atoms of the naphthyl substituent is very likely.

A simple conformational analysis of 8-naphthyl- and 8-styryl-substituted 3-methyl-1-propargylxanthine (MPX) and DMPX derivatives was performed (see fig 1), with regard to the torsion angle between the purine heterocycle and the 8-substituent. The calcula-



E- (*trans*-) configuration

Z- (*cis*-) configuration

1: $R_1 = R_2 = n\text{-}C_3H_7$; R3 = R4 = OCH_3 (**KF17837**)
2: $R_1 = R_2 = CH_3$; R_3 = Cl; R_4 = H (**CSC**)
3: R_1 = propargyl; $R_2 = CH_3$; $R_3 = R_4$ = H (**8-Styryl-DMPX**)
4: R_1 = propargyl; $R_2 = CH_3$; R_3 = Cl; R_4 = H (**CS-DMPX**)
5: R_1 = propargyl; $R_2 = CH_3$; R_3 = Br; R_4 = H (**BS-DMPX**)

Chart 1. Potent selective A_{2A}-adenosine receptor antagonists with styrylxanthine structure: photo-induced *E/Z*-isomerization in dilute solution.

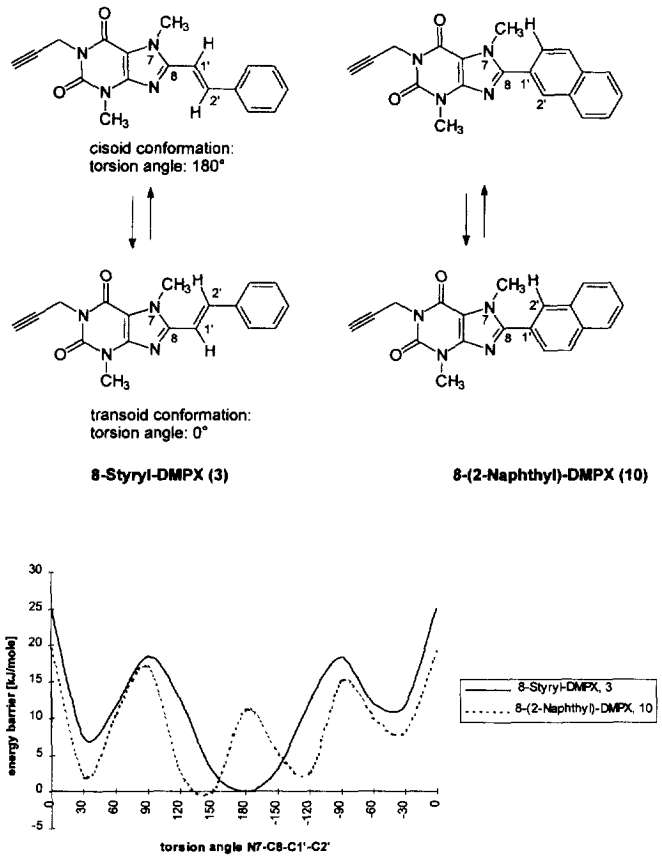
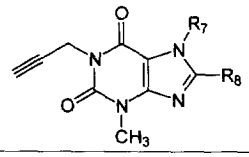
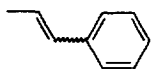
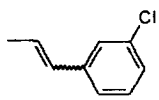
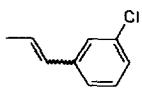
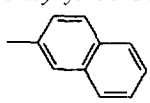
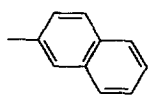
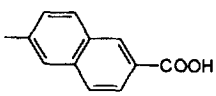
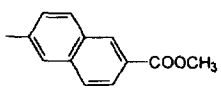
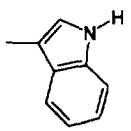
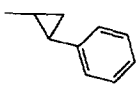
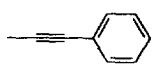


Fig 1. Conformational analysis of 7-methylated 8-styryl- and 8-naphthylxanthine derivatives.

Table IV. Adenosine receptor affinities of configurationally stable compounds in comparison with standard adenosine receptor antagonists.



Compound	R_7	R_8	$K_i \pm SEM$ (μM)		A_{2A}-*Selectivity* K_{iA1}/K_{iA2A}
			A_1 *Rat brain cortical membranes* [^{3}H]*CHA*	A_{2A} *Rat brain striatal membranes* [^{3}H]*CGS21680*	
8-Styrylxanthines (for comparison)					
3 (8-Styryl-DMPX)	CH_3		1.1 ± 0.2[a]	0.027 ± 0.005[a]	41
22	H		0.25 ± 0.06[a,b]	0.41 ± 0.17[a,c]	0.6
4 (CS-DMPX)	CH_3		1.3 ± 0.1[a]	0.013 ± 0.001[a]	100
Configurationally stable analogs of 8-styryl-DMPX (3)					
9a	H		0.092 ± 0.017	0.12 ± 0.04	0.8
10	CH_3		0.98 ± 0.14	0.38 ± 0.04	2.6
16	H		0.76 ± 0.15	0.75 ± 0.20	1.0
17	CH_3		2.2 ± 1.6	1.6 ± 0.3	1.4
20	CH_3		1.0 ± 0.1	0.30 ± 0.7	3
11	CH_3		4.6 ± 1.2	1.7 ± 0.3	3
21	CH_3		> 3 (32 ± 3 %)[d]	0.30 ± 0.07	> 10

[a]Müller et al [1, 13]; [b][^{3}H]R-PIA was used as radioligand; [c][^{3}H]NECA was used as radioligand; [d]IC_{50} value could not be determined due to limited solubility of the compound. Percent inhibition (in brackets) at the indicated concentration is given.

tion was based on Van der Waals interactions without taking into account other factors influencing conformation, such as electrostatic terms.

The 7-unsubstituted xanthine derivatives 8-styryl-MPX and 8-naphthyl-MPX (**9a**) show a very similar energy profile, exhibiting minima at torsion angles around 0° (–30° – +30°) and around 180° (+150° – –150°), and maxima around +90° and –90° (data not shown). Thus, a close-to-coplanar conformation (0 ± 30° or 180 ± 30°) appears to be favorable for 7-unsubstituted 8-naphthyl- and 8-styrylxanthine derivatives. This finding is supported by a recently determined X-ray structure of a 7-unsubstituted 8-phenylxanthine derivative, namely 1-propyl-8-*p*-sulfophenylxanthine, where a torsion angle of 155.3° (N7-C8-C1'-C2', see fig 1) was found in the crystal [24]. This value is close to our calculated global minimum for 8-naphthyl- and 8-styryl-MPX (see above). For A_1-selective 8-phenylxanthine derivatives a 40° or 50° deviation from coplanarity for the 8-phenyl ring with respect to the purine heterocycle had been determined as the most likely bioactive conformation at A_1-AR by two independent groups [23, 25]. However, the requirements of A_1-AR may differ from those for A_{2A}-AR in this respect. While A_1-AR prefers bulky cycloalkyl substitutents in the 8-position, A_{2A}-AR prefers flat, aromatic, conjugated residues, such as phenyl or styryl. For A_1-AR, the N7-hydrogen is very important as a hydrogen bond donor; therefore all 7-methylated xanthine derivatives, including 8-styrylxanthines, are less potent at A_1-AR compared to 7-unsubstituted analogs. For A_{2A}-AR, the N7-hydrogen appears to be of less importance since some methylated xanthine derivatives can be even more potent than their unsubstituted counterparts. This is especially true for 8-styrylxanthine derivatives.

7-Methylated 8-styryl-DMPX (**3**) showed a rather similar result in conformation analysis to the 7-unmethylated compounds with one exception (fig 1): an angle of around 0°, corresponding to the *trans*oid conformation, is unfavorable. 8-(2-Naphthyl)-DMPX (**10**), however, differed considerably from the 7-unmethylated xanthines and the 7-methyl-8-styrylxanthine **3**. As for 8-styryl-DMPX (**3**), an angle of around 0° is unfavorable, but in addition there is another energy maximum around 180°, corresponding to an energy minimum in 8-styryl-DMPX (**3**). Based on these results, it could be speculated that a nearly coplanar conformation of the 8-styryl substituent would be favorable for interaction of styrylxanthines with A_{2A}-AR, that a *cis*oid conformation (torsion angle ca 180°) is preferred, and that 7-methylation forces the 8-styryl residue in this favorable conformation and may for that reason produce high A_{2A}-AR affinity. For 8-naphthyl-, 8-phenyl- or similarly substituted xanthines this conformation is energetically unfavorable when the 7-position is methylated, due to a steric interaction between the 7-methyl group and the 8-substituent. It is concluded that the bioactive conformation of the 8-arylxanthine derivatives may differ at A_1- from that at A_{2A}-AR. A different explanation for the increase in A_{2A}-AR affinity by 7-methylation of 8-styrylxanthines would be that 7-methylated and 7-unmethylated 8-substituted xanthines have different binding modes at A_{2A}-AR, but there is no evidence as yet for the latter possibility.

Many potent AR antagonists are lacking reasonably good water solubility, which appears to be a prerequisite for in vivo activity of a pharmacological agent [26]. A number of potent, selective A_1-AR antagonists have been synthesized which bear functional groups that increase water solubility [1, 3]. Some of these compounds are now under development as drugs. Most potent, selective A_{2A} antagonists described so far are very lipophilic and exhibit only low water solubility, which limits their usefulness for in vivo studies [1].

We introduced polar functions into the naphthyl ring of 8-naphthyl-DMPX derivatives in order to obtain compounds with improved water solubility. In the 7-unsubstituted derivative **9a**, a 6-carboxy group in the naphthyl ring led to an 8-fold decrease in A_1- and a 6-fold decrease in A_{2A} affinity, resulting in a non-selective derivative **16**. A 6-methoxycarbonyl substitution in 7-methylated 8-(2-naphthyl)-DMPX (**10**) similarly reduced A_1- and A_{2A}-AR affinity (2-fold and 4-fold, respectively), resulting in a non-selective compound (**17**).

8-(3-Indolyl)-DMPX (**20**) exhibited very similar A_{2A}-AR affinity and selectivity to 8-(2-naphthyl)-DMPX (**10**).

In analog **11**, the ethenyl bridge of 8-styryl-DMPX (**3**) was replaced by a cyclopropyl ring structure. The substituents are attached in *trans*-configuration. The product is a mixture of two enantiomers exhibiting (*R*,*R*)- and (*S*,*S*)-configuration, respectively. Compared to the corresponding styryl derivative **3**, compound **11** is 3-fold less potent at A_1-AR, and 63-fold less potent at A_{2A}-AR, exhibiting a K_i value in the low micromolar range, and only 3-fold selectivity for A_{2A}-AR. The reasons for this low A_{2A} affinity may be the different steric arrangement of the substituents at the sp^3-hybridized carbon atoms of the cyclopropane ring as compared to the sp^2-hybridized carbon atoms of an ethenyl bond in styryl derivatives; conjugation of the phenyl residue to the xanthine heterocycle is no longer possible in **11**.

Replacement of the ethenyl bridge in **3** by an ethynyl group (in **21**) resulted in a 10-fold decrease in A_{2A}-AR affinity. Nevertheless, the resulting 8-(2-phenylethynyl)-DMPX **21** was the best A_{2A} antagonist of the present series with regard to A_{2A} affinity (K_i =

0.30 μM) and selectivity (> 10-fold versus A_1). The introduction of appropriate substituents in the phenyl ring of **21** could perhaps yield more potent and selective A_{2A}-AR antagonists with the advantage of being configurationally stable in contrast to the styrylxanthine derivatives.

Experimental protocols

Chemistry

NMR spectra were performed on a Bruker WP-80 (^{1}H: 80 MHz, ^{13}C: 20 MHz), or a Bruker AC-250 spectrometer (^{1}H: 250 MHz, ^{13}C: 60 MHz), respectively. DMSO-d_6 was used as solvent. The chemical shifts of the remaining protons of the deuterated solvent served as internal standard: δ ^{1}H: 2.50; ^{13}C: 39.7. Mass spectra were recorded on a 8200 Finnigan-MAT mass spectrometer, or a 90 Finnigan MAT mass spectrometer (for high resolution spectra), respectively. All compounds were checked for purity by TLC on 0.2 mm aluminum sheets with silica gel 60 F_{254} (Merck); as eluent dichloromethane/methanol (9:1, or 99:1, respectively) was used. Melting points were taken on a Büchi 510 melting point apparatus and are uncorrected. Elemental analyses were performed by the Institute of Chemistry, University of Tübingen, or the Institute of Inorganic Chemistry, University of Würzburg, respectively.

5,6-Diamino-3-propargyluracil **6** was prepared from 6-aminouracil via regioselective alkylation [27] followed by nitrosation and reduction as described [19, 20].

*6-Amino-5-(2-naphthalenecarboxamido)-3-propargyluracil **7a***
2-Naphthoyl chloride (0.76 g, 4 mmol) was added in portions to a suspension of compound **6** (0.9 g, 5.0 mmol) in pyridine (20 mL), and the clear solution obtained was stirred overnight at room temperature (rt). After H_2O (200 mL) had been added, the mixture was cooled in an ice bath and conc HCl was added to pH 3. The formed precipitate was filtered off, washed with H_2O and air-dried.

*6-Amino-5-(trans-2-phenylcyclopropanecarboxamido)-3-propargyluracil **7b***
Compound **6** (0.70 g, 3.87 mmol) was stirred with 0.64 g (3.95 mmol) of *trans*-2-phenylcyclopropane-carboxylic acid and 0.76 g (3.95 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride at rt overnight in methanol (30 mL). The product was precipitated by the addition of H_2O (30 mL), filtered off, washed with H_2O (10 mL) and air-dried.

*6-Amino-5-(carboxamido-substituted)-1-methyl-3-propargyluracils **8a, 8b, 13, 15***
Compound **7a**, **7b**, **12** or **14**, respectively, (5 mmol) was dissolved in DMF (20 mL). After the addition of K_2CO_3 (1.38 g, 10 mmol) MeI (0.85 g, 374 μL, 6 mmol) was added and the mixture was stirred at rt for 48 h. The product was precipitated by the addition of H_2O (30 mL), collected by filtration and washed with H_2O (10 mL). Further purification was achieved by dissolution in DMF (10 mL) and precipitation by the addition of 30 mL of H_2O (**8b**, **13**), or recrystallization from MeOH (**8a**), or EtOH (**15**).

*8-Substituted 3-methyl-1-propargylxanthines **9a, 9b, 16***
Compound **8a**, **8b**, or **15** respectively (3.0 mmol) was dissolved in a mixture of EtOH (100 mL) and 20% aq NaOH solution (20 mL) and refluxed for 2–3 h. After cooling, the solution was acidified to pH 4 by the addition of conc HCl, and the formed precipitate was collected by filtration and washed with H_2O (50 mL). Purification was achieved by dissolution in DMF (10 mL) and precipitation by the addition of H_2O (50 mL) for **9b**, by recrystallization from 90% acetic acid (**9a**), or by dissolution in 10% NaOH solution and precipitation by the addition of conc HCl solution (**16**).

*8-Substituted 3,7-dimethyl-1-propargylxanthines **10, 11, 17***
Compound **9a**, **9b** or **16** respectively (2.3 mmol) was dissolved in DMF (20 mL). K_2CO_3 (0.64 g, 4.6 mmol) and MeI (0.43 g, 187 μL, 3.0 mmol) was added. The mixture was stirred at rt overnight. Then H_2O (80 mL) was added to precipitate the product which was collected by filtration. The product was treated with 10% aq NaOH solution (15 mL) to dissolve impurities. Further purification was achieved by dissolution in DMF (10 mL) and precipitation by the addition of H_2O (50 mL).

*6-Amino-5-(2-(6-carboxy)naphthalenecarboxamido)-3-propargyluracil **12***
To a suspension of 2,6-naphthalene dicarboxylic acid (0.22g, 1 mmol) in DMF (10 mL) *N*-methylmorpholine (0.1 g, 1 mmol) and isobutylchloroformiate (0.14 g, 1 mmol) were added under cooling on an ice bath. To the obtained solution compound **6** (0.2 g, 1.2 mmol) in DMF (5 mL) was added dropwise and the mixture was stirred for 5 h. After dilution with H_2O the precipitate was filtered off, washed with H_2O and dried. Purification was achieved by dissolution in DMF and precipitation by the addition of H_2O.

*6-Amino-5-(2-(6-benzoyloxycarbonyl)naphthalenecarboxamido)-3-propargyl-uracil **14***
Napthalene dicarboxylic acid monobenzylester. A mixture of 0.66 g (3 mmol) of 2,6-naphthalene dicarboxylic acid, 0.36 g (3.6 mmol) of triethylamine, and 0.60 g (3.6 mmol) of benzyl bromide was heated at 60 ± 5 °C for 3 h. After cooling to rt, the mixture was diluted with 20 mL H_2O. The precipitated product was collected by filtration, washed with H_2O and dried at rt. Yield: 0.81 g (90%) of white powdered product; mp = > 300 °C. ^{1}H-NMR: δ (ppm) 5.42 (s, 2H, CH_2); 7.33–7.47 (m, 5H, Ar-H); 7.97–8.29 (m, 4H, Ar-H); 8.65–8.69 (m, 2H, Ar-H: H1' and H5').

*Amide **14***
2-Carboxy-6-benzoyloxynaphthalene (0.55 g, 1.8 mmol) was refluxed in $SOCl_2$ (20 mL) until completely dissolved (10–15 min). The excess of $SOCl_2$ was removed by evaporation to dryness, the residue was co-evaporated three times with dry toluene (10 mL) and dried in a desiccator over silica gel. Then a solution of compound **6** (0.36 g, 2 mmol) in pyridine (10 mL) was added under cooling in an ice bath and the temperature was slowly raised to rt. After 5 h of stirring, H_2O (100 mL) was added and the pH was adjusted to 2 by the addition of conc HCl. The precipitate was filtered off, washed with H_2O and dried. Purification was achieved by dissolution in DMF and subsequent precipitation by the addition of H_2O.

*8-(3-Indolyl)-1-propargylxanthine **19***
Compound **6** (0.68 g, 3.87 mmol) and indole-3-carbaldehyde (0.8 g, 5.51 mmol) were refluxed in a mixture of methanol (30 mL) and acetic acid (1.5 mL). After the solution was cooled to rt H_2O (70 mL) was added and the precipitated compound **18**, contaminated with educts, was filtered off and used without further purification in the subsequent step.

Compound **18** (900 mg) was refluxed with anhydrous $FeCl_3$ (0.62 g, 3.79 mmol) in ethanol (30 mL) for 3 h. After the addition of H_2O the precipitate was filtered off and dissolved in 1 N NaOH solution (120 mL). The product was precipitated by acidification with acetic acid. Purification was achieved by dissolution in DMF (20 mL) and precipitation by the addition of H_2O (30 mL).

8-(3-Indolyl)-3,7-dimethyl-1-propargylxanthine ***20***
Compound **19** (0.18 g, 0.59 mmol) was dissolved in DMF (15 mL). After the addition of K_2CO_3 (0.05 g, 1.83 mmol) and MeI (0.5 mL) the solution was stirred at rt for 24 h. The product was precipitated by the addition of H_2O (20 mL) and collected by filtration.

3,7-Dimethyl-8-(2-phenylethynyl)-1-propargylxanthine ***21***
3-Methyl-8-(2-phenylethynyl)-1-propargylxanthine[1] (0.010 g, 0.033 mmol) was dissolved in 500 µL DMF upon heating. After cooling the solution to rt, K_2CO_3 (0.010 mg, 0.072 mmol) was added and the mixture was stirred for 1 h. Then MeI (21 µL, 0.33 mmol) was added. After 12 h stirring at rt, H_2O (1.5 mL) was added to precipitate the product, which was recrystallized from H_2O/EtOH = 1:1. MS (EI, 70 eV): *m/z* = 67.1 (59%), 82.1 (28%), 122.1 (100%), 318.1 (76%). ^{13}C-NMR: δ (ppm) 29.7 (propargyl CH_2N); 33.2 (N7-CH_3); 69.7 (alkyne); 73.1 (propargyl C2'); 77.5 (alkyne); 79.5 (propargyl CH); 96.5 (C5); 129.2, 130.7, 132.1, 135.1 (phenyl); 147.8 (C4); 150.2 (C2); 153.2 (C6).

Conformational analysis

Molecular modelling studies were carried out on a Silicon Graphics Iris Indigo workstation using the software package Sybyl, program version 6.1. Structures were built using Sybyl. A torsion angle of 0° was assigned to the N7–C8–C1'–C2' torsion angle, assuming this conformation representing a possible energy minumum. Energy minimization was performed using the Sybyl force field with default values. A conformational analysis concerning this torsion angle was performed using the grid search routine of Sybyl with 30° increments, without using electrostatic terms or terms of periodic boundary conditions.

Pharmacological methods

Inhibition of binding of [^{3}H]*N*^{6}-cyclohexyladenosine (CHA) to A_1-adenosine receptors of rat cerebral cortical membranes and inhibition of [^{3}H]2-[4-(carboxyethyl)phenylethylamino]-5'-*N*-ethylcarboxamidoadenosine (CGS21680) to A_2-adenosine receptors of rat striatal membranes were assayed as described [28–30]. 2-Chloroadenosine (10 µM) was used to define non-specific binding. Inhibition of 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 InPlot®, version 4.03 (GraphPad, San Diego, California, USA).

[1]Preparation of this compound will be described elsewhere (Hipp J, Müller CE, manuscript in preparation).

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