Synthesis and Structure−Activity Relationships of 3,7-Dimethyl-1-propargylxanthine Derivatives, A_{2A}-Selective Adenosine Receptor Antagonists†

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A series of 8-substituted derivatives of 3,7-dimethyl-1-propargylxanthine (DMPX) was synthesized and investigated as A_{2A} adenosine receptor antagonists. Different synthetic strategies for the preparation of DMPX derivatives and analogues were explored. A recently developed synthetic procedure starting from 3-propargyl-5,6-diaminouracil proved to be the method of choice for the preparation of this type of xanthine derivatives. The novel compounds were investigated in radioligand binding studies at the high-affinity adenosine receptor subtypes A_1 and A_{2A} and compared with standard A_{2A} adenosine receptor antagonists. Structure−activity relationships were analyzed in detail. 8-Styryl-substituted DMPX derivatives were identified that exhibit high affinity and selectivity for A_{2A} adenosine receptors, including 8-(*m*-chlorostyryl)-DMPX (CS-DMPX, K_i A_{2A} = 13 nM, 100-fold selective), 8-(*m*-bromostyryl)-DMPX (BS-DMPX, K_i A_{2A} = 8 nM, 146-fold selective), and 8-(3,4-dimethoxystyryl)-DMPX (K_i A_{2A} = 15 nM, 167-fold selective). These and other novel compounds are superior to the standard A_{2A} adenosine receptor antagonists KF17837 (**4**) and CSC (**5**) with respect to A_{2A} affinity and/or selectivity.

Introduction

Adenosine receptors (AR) are currently subdivided into four subtypes, all of which have been cloned from different species including humans.[1−4] The most thoroughly investigated subtypes are the high-affinity receptors A_1 and A_{2A}, both of which are activated by adenosine in low, nanomolar, or at least submicromolar concentrations, depending on the cell system investigated.[1] A variety of potent and selective agonists for both subtypes has been developed, all structurally derived from the physiological agonist adenosine.[1,2] Antagonists for the A_1 AR have also been identified. Compounds derived from the alkaloid theophylline bearing lipophilic substituents in the 8-position, such as 8-phenyltheophylline and 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), are potent A_1-selective AR antagonists.[5] Further structural classes, including adenines,[6] 7-deazaadenines (pyrrolo[2,3-*d*]pyrimidines and pyrimido[4,5-*b*]indoles),[7] 7-deaza-8-azapurines (pyrazolo[3,4-*d*]pyrimidines),[8] and triazoloquinoxalines, as well as related compounds,[9] were identified and developed as A_1 AR antagonists.

In addition to their A_1 AR affinity, many xanthines are also active at A_2 AR.[1,2,5] It has taken, however, quite long until the first potent A_{2A}-selective AR xanthines were identified. In contrast to the wealth of A_1 antagonists that are known, much fewer A_{2A} AR antagonists have been described in the literature so far, and structure−activity relationship (SAR) studies of A_{2A} antagonists are limited (for a recent review see ref 10). Potent and selective A_{2A} AR antagonists, however, are needed as pharmacological tools and have been proposed as drugs, especially as a novel therapy for the treatment of Morbus Parkinson.[11]

One of the first A_2-selective AR antagonists described in the literature was 3,7-dimethyl-1-propargylxanthine (DMPX, **2**, Chart 1).[12] The compound was shown to exhibit A_2 selectivity in vitro as well as in vivo.[13] Activity and selectivity of DMPX, however, is low. Later on it was found that 7-methylation of 8-substituted theophylline derivatives increased A_{2A} selectivity.[14] The resulting caffeine derivatives, such as 8-cyclohexylcaffeine (**3**), showed A_{2A} selectivity only in certain test systems and relatively low affinity for the receptors.[5,14] Nevertheless, that discovery eventually led to the recent development of further xanthine derivatives with increased A_{2A} affinity and selectivity, such as 8-(3,4-dimethoxystyryl)-1,3-dipropyl-7-methylxanthine (KF17837, **4**)[15] and 8-(*m*-chlorostyryl)caffeine (CSC, **5**).[16] Some non-xanthine A_{2A} AR antagonists have also been described, such as the triazoloquinazoline CGS15943 and various new, improved analogues,[17] and the thiazoloquinazoline HTQZ and structurally related tricyclic compounds.[18]

Despite its low affinity and A_{2A} selectivity in vitro, DMPX is still widely used as a standard A_{2A} antagonist in pharmacological studies, particularly in in vivo experiments, due to its relatively high water solubility

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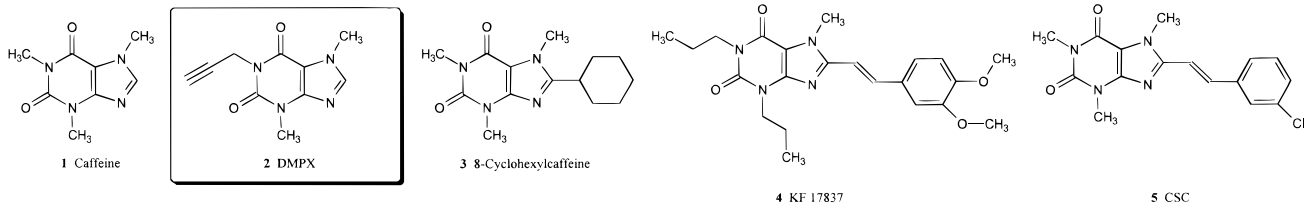
Chart 1. Selection of A_2 Adenosine Receptor Antagonists with Xanthine Structure



and bioavailability. The development of A_{2A} antagonists with enhanced potency, selectivity, and water solubility is desirable.

In the present study we have attempted to develop new A_{2A} AR antagonists with improved properties. DMPX was selected as a lead structure. Our approach was to introduce various substituents in the 8-position of DMPX and/or to replace the substituents in the 1-, 3-, or 7-position of 8-substituted DMPX derivatives by (ar)alkyl residues. Structure−activity relationships were analyzed in detail. Various synthetic strategies were developed and applied to obtain 8-substituted DMPX derivatives and related xanthines with different substituents in the 1-, 3-, 7-, and 8-positions.

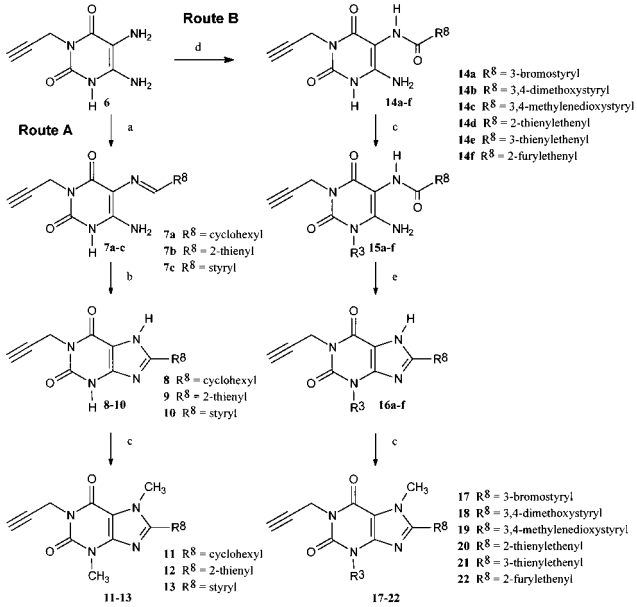
Results and Discussion

Chemistry. DMPX itself had been obtained by Daly and co-workers by alkylation of theobromine (3,7-dimethylxanthine) with propargyl bromide in ethanol/water in the presence of sodium hydroxide as a base.[12]

For the preparation of 8-substituted DMPX derivatives, the standard procedure for the synthesis of xanthines bearing different substituents in the 1- and 3-position,[19] namely alkylation of 6-amino-1-methyluracil with propargyl bromide in the 3-position under basic conditions, according to Papesch and Schroeder,[20] had not been successful in our hands,[21] probably due to the high reactivity of the triple bond.[22] An alternative alkylation procedure after silylation of 6-amino-1-methyluracil had provided the desired 6-amino-1-methyl-3-propargyluracil only in relatively low yield.[21] Therefore, three alternative strategies, A, B, and C, were investigated and applied for the synthesis of 8-substituted DMPX derivatives as depicted in Schemes 1 and 2.

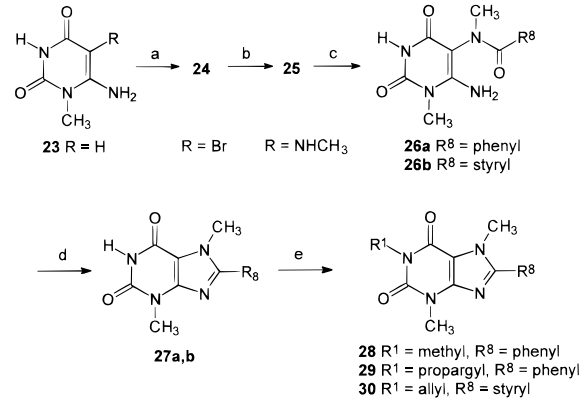
(A) Following the procedure developed for the preparation of 1,8-disubstituted xanthines,[21] 3-propargyl-5,6-diaminouracil (**6**)[23,24] was condensed with the appropriate aldehyde to yield the imines **7a**−**c**. Compounds **7a** and **7b** could subsequently be subjected to oxidative ring closure using ferric chloride[25] for the preparation of **8** and **9**. The same reaction conditions lead to a degradation of styryl-substituted imine **7c**. In this case ring closure could be performed with thionyl chloride[26] at room temperature, which afforded **10** in short reaction time and very pure form. 1,8-Disubstituted xanthines **8**−**10** were bismethylated using methyl iodide/potassium carbonate in dimethylformamide (DMF) under mild conditions. 8-Cyclohexyl as well as aryl (2-thienyl, styryl) derivatives of DMPX were obtained in high yields by this method (Scheme 1). The limited availability of different aldehydes, however, prompted us to pursue yet a different approach to 8-substituted DMPX derivatives, in which the easily available carboxylic acids could be employed. Another drawback of this method, with regard to the preparation of analogues, is that substituents in the 3- and 7-position have to be identical, since selective alkylation is difficult to perform.

Scheme 1. Synthesis of 8-Substituted DMPX Derivatives: Methods A and B[a]



[a] (a) R^8CHO, acetic acid, MeOH or EtOH; (b) $FeCl_3$ in EtOH, reflux, or $SOCl_2$ at rt; (c) MeI, K_2CO_3, DMF; (d) R^8COOH, EDC, MeOH; (e) NaOH, H_2O, MeOH, 60 °C.

Scheme 2. Synthesis of 8-Substituted DMPX Derivatives: Method C[a]



[a] (a) Br_2; (b) CH_3NH_2; (c) R_8COOH, EDC or R_8COCl, pyridine; (d) NaOH; (e) R_1X; X = Br, I.

(B) Most DMPX derivatives investigated in the present study were obtained by this alternative route which had recently been developed in our group (Scheme 1).[24] Thus, 5,6-diamino-3-propargyluracil (**6**) was condensed with a carboxylic acid by means of a carbodiimide. The 5-amino group of 3-substituted 5,6-diaminouracils bearing no substituent at the 1-position is particularly reactive. Therefore, satisfactory to excellent yields of various amides could be obtained. Uracil derivatives unsubstituted in the 1-position, such as **14**, can only be

cyclized under harsh basic or acidic conditions under which the propargyl function is attacked.[21,22] Therefore, methylation in the 1-position of diaminouracil derivatives **14** is required as the following step in order to enhance the nucleophilicity of the 6-amino group for subsequent alkaline ring closure of compounds **15** under mild conditions.[21] Final methylation of compounds **16** in the 7-position yielded the desired DMPX derivatives **17**−**22**. 8-Styryl-DMPX derivatives bearing substituents on the phenyl ring and heterocyclic analogues of 8-styryl-DMPX were prepared. The synthesis of further 8-substituted DMPX derivatives and analogues (**38**−**45**) has already been described elsewhere.[24]

(C) A third method for the synthesis of 8-substituted DMPX derivatives and analogues was investigated (Scheme 2). Thus, 6-amino-1-methyl-5-(methylamino)-uracil **25** was prepared essentially as described[27] starting from 1-methyl-6-aminouracil (**23**)[20] via bromination in the 5-position to **24**, followed by nucleophilic displacement of bromine by methylamine. Condensation of the amino uracil **25** with a carboxylic acid chloride, or a carboxylic acid by means of a carbodiimide, respectively, and subsequent ring closure of compounds **26** under strong basic conditions afforded 8-substituted 3,7-dimethylxanthines **27**. It was observed that ring closure of such uracil derivatives lacking a substituent in the 3-position required harder conditions compared to corresponding 1,3-disubstituted uracil derivatives. Thus, a derivative of **26b** bearing methoxy substituents in the 3- and 4-position of the styryl residue gave no ring-closed product under a variety of ring closure conditions, due to cleavage of the amide bond under the drastic reaction conditions required. Such methoxy-substituted 6-amino-5-(cinnamoylamino)uracil derivatives appear to hydrolyze particularly easily under basic conditions. If a 3-substituent is lacking, the uracil derivative is deprotonated under basic ring closure conditions, which reduces its reactivity, and the labile amide bond of those methoxy derivatives is hydrolyzed already under conditions that are not yet sufficient for ring closure.

Alkylation of **27** in the 1-position under mild conditions led to 8-phenyl-DMPX (**29**), which had been synthesized before by method (A) starting from 8-phenyl-1-propargylxanthine.[21] 8-Phenylcaffeine (**28**), which was required as a standard compound for comparison, and 1-allyl-3-methyl-8-styrylxanthine (**30**) were obtained analogously.

The (3,4-dimethoxystyryl)-DMPX (**18**), which could not be obtained by this method, was eventually obtained by method B, which appears to be the method of choice for the preparation of 8-substituted DMPX derivatives and analogues.

Further 1,3,7,8-tetrasubstituted xanthines, including 8-(3,4-dimethoxystyryl)caffeine (**34**) and 7-methyl- and 7-propargyl-3-isobutyl-1-methyl-8-phenylxanthine (**35** and **36**), were prepared by standard procedures.[19]

Compound **34** had been synthesized earlier by Jacobson et al. in 4% overall yield in three steps starting from 1,3-dimethyl-5,6-diaminouracil.[16] No yields for the single steps were published. We found that the critical step in that reaction sequence is the ring closure reaction of 6-amino-1,3-dimethyl-5-((3,4-dimethoxycinnamoyl)amino)uracil under basic conditions. Using the published method,[16] hydrolysis of the amide bond rather than ring closure was observed as the main reaction in our hands. We have now developed optimized ring closure conditions for the preparation of **34** leading to an increase in overall yield from 4%[16] to 22%. Hydrolysis of the amide bond may also be the reason for the low yields of the thienyl- and furylethenyl-substituted xanthines **16d**−**f**.

Yields, melting points, and elemental analyses data for the majority of intermediate and all final products are listed in Table 1. The compounds investigated were further characterized by their ^{1}H NMR spectra. For selected compounds ^{13}C NMR spectra were recorded (Supporting Information).

(*E*)-Configurated cinnamic acid derivatives were used as starting compounds for the preparation of 8-styryl-substituted xanthines. The (*E*)-configuration was retained during the course of the synthesis as documented by the coupling constant of the vinylic protons (^3J ca. 16 Hz).

The propargyl group in DMPX derivatives, which is rather reactive and can be hydrated in strong acidic or basic medium, remained intact under the applied reaction conditions, as shown by the typical signals in the ^{1}H NMR spectrum with a long-range coupling constant of 2.3−2.4 Hz for the propargyl protons and the typical signal for the propargyl carbon atoms in the ^{13}C NMR spectra (Supporting Information).

The 7-methyl group in 8-aryl- (compound **12**) and 8-styryl-substituted compounds (**13**, **17**−**22**) is shifted downfield as compared to 8-alkyl- (**11**) or 8-unsubstituted xanthine derivatives[21] from 3.8−3.9 to 4.0−4.1 ppm.

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 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)phenyl]ethyl]amino]-5′-*N*-(ethylcarbonyl)amino]adenosine (CGS21680) as A_{2A} ligand. In addition, some compounds were also tested versus another frequently used A_1 agonist radioligand, [^{3}H]-N^6-(*R*)-(phenylisopropyl)adenosine (*R*-PIA). Before [^{3}H]CGS21680 had become available as a radioligand, the nonselective agonist [^{3}H]-5′-[(*N*-(ethylcarbonyl)amino]adenosine (NECA) was generally used in A_{2A} AR binding assays. An A_1-selective AR agonist, N^6-cyclopentyladenosine (CPA), was added in order to block A_1 AR present in striatal tissue. We tested some compounds versus both radioligands, [^{3}H]NECA and [^{3}H]CGS21680 in order to compare data.

Some standard compounds (**1, 2, 4, 5, 28, 29, 31, 34**) were tested in our assay systems for comparison with literature data. Our results were generally consonant with data obtained by other groups, with few exceptions. For CSC (**5**) a K_i value at A_1 AR of 28 μM had been determined using [^{3}H]*R*-PIA as radioligand.[16] In our assay using [^{3}H]CHA, we were not able to determine an IC_{50} and a K_i value due to limited solubility of the compound. We measured 17% inhibition of [^{3}H]CHA binding at a concentration of compound **5** of 1 μM. Higher concentrations gave results indicating lacking solubility of the compound. No sigmoidal curve could be obtained. For compound **34**, which was resynthe-

Table 1. Yield, Melting Point, and Analytical Data of Intermediate and Final Products

compd.	yield (%)	mp (°C)	formula	elemental analyses
7b	78	242	$C_{12}H_{10}N_{4O}2$	C,H,N,S
7c	96	266	$C_{16}H_{14}N_4O_2$	C,H,N
9	64	299	$C_{12}H_8N_4O_2S$	C,H,N,S
10	87	>300	$C_{16}H_{12}N_4O_2$	C,H,N
11	63	207	$C_{16}H_{14}N_4O_2$	C,H,N
12	72	>270	$C_{14}H_{12}N_4O_2S$	C,H,N
13	93	232	$C_{18}H_{16}N_4O_2$	C[a],H,N
14a	96	268	$C_{16}H_{13}N_4O_3Br$	C,H,N
14b	50	>275	$C_{18}H_{18}N_4O_5$	*c*
14c	71	292	$C_{17}H_{14}N_4O_5$	C,H,N
14d	82	281	$C_{14}H_{12}N_4N_3S$	C,H,N,S
14e	29	285	$C_{14}H_{12}N_4O_3S$	C,H,N,S
14f	45	278	$C_{14}H_{12}N_4O_4$	C,H,N
15a	71	253	$C_{17}H_{15}N_4O_3Br\cdot H_2O$	C,H,N
15b	45	273	$C_{19}H_{20}N_4O_5$	C[a],H,N
15c	85	277	$C_{18}H_{16}N_4O_5$	C[a],H,N
15d	24	271	$C_{15}H_{14}N_4O_3S\cdot H_2O$	C,H,N,S
15e	53	272	$C_{15}H_{14}N_4O_3S$	C,H,N,S
15f	68	270	$C_{15}H_{14}N_4O_4$	C,H,N
16a	59	270	$C_{17}H_{13}N_4O_2Br$	C,H,N
16b	56	>270	$C_{19}H_{18}N_4O_4\cdot 1.25H_2O$	C,H,N
16c	78	>300	$C_{18}H_{14}N_4O_4$	C,H,N
16d	24	290	$C_{15}H_{12}N_4O_2S$	C,H,N,S
16e	24	273	$C_{15}H_{12}N_4O_2S$	C,H,N,S
16f	37	271	$C_{15}H_{12}N_4O_3$	C,H,N
17	34	200	$C_{18}H_{15}N_4O_2Br\cdot H_2O$	C[a],H,N
18	60	267	$C_{20}H_20N_4O_4\cdot 0.7H_2O$	C,H,N
19	74	260	$C_{19}H_{16}N_4O_4$	C,H,N
20	67	234	$C_{16}H_{14}N_4O_2S$	C,H,N,S
21	72	245	$C_{16}H_{14}N_4O_2S$	C,H,N,S
22	60	242	$C_{16}H_{14}N_4O_3$	C,H,N[b]
24	66	>270 (lit.[27] mp 286−288)	$C_5H_6N_3O_2Br$	C,H,N
25	70	243−244 (lit.[27] mp 238−240)	$C_6H_{10}N_4O_2$	C,H,N
26a	73	>270 (lit.[44] mp > 300)	$C_{13}H_{14}N_4O_3$	C,H,N
26b	69	>270	$C_{15}H_{16}N_4O_3$	C,H,N
27a	85	>270 (lit.[44] mp > 300)	$C_{13}H_{12}N_4O_2$	C,H,N
27b	88	>270	$C_{15}H_{14}N_4O_2$	C,H,N
28	80	178 (lit.[45] mp 186−188)	$C_{14}H_{14}N_4O_2$	C,H,N
29	78	239 (lit.[21] mp 237)	$C_{16}H_{14}N_4O_2$	C,H,N
30	71	206	$C_{18}H_{18}N_4O_2$	C,H,N
34	73	236−238 (lit.[16] mp 230−232)	$C_{18}H_{20}N_4O_4\cdot 0.5H_2O$	C,H,N
35	68	130	$C_{17}H_{20}N_4O_2$	C,H,N
36	83	152	$C_{19}H_{20}N_4O_2$	C,H,N

[a] C found (calcd): **9**, 52.9 (51.8); **13**, 66.6 (65.6); **15b**, 56.5 (57.0); **15c**, 56.5 (56.0); **17**, 52.9 (51.8). [b] N found (calcd): **22**, 17.4 (18.0). [c] Not determined (intermediate product).

sized in our laboratory, we obtained an about 10-fold higher A_{2A} affinity than that described in the literature.[16]

[^{3}H]*R*-PIA and [^{3}H]CHA data were very similar (see **9, 10, 13, 38**). A comparison of [^{3}H]NECA and [^{3}H]-CGS21680 data, however, showed significant differences. K_i values determined with [^{3}H]CGS21680 were generally lower (up to 9-fold, see compound **9**) compared to data obtained with [^{3}H]NECA for xanthines investigated (see compounds **9, 10, 13, 38**; also see standard xanthines **1, 2, 28**, and **31**). Therefore, A_{2A} selectivity appears greater if CGS21680 is used as radioligand. Jarvis et al., who introduced CGS21680 as an A_{2A}-specific radioligand, had observed similar differences for some but not all xanthines that were compared.[28] They observed up to 7-fold higher K_i values for xanthines when determined with NECA as compared to CGS21680 as a radioligand.

Structure−Activity Relationships

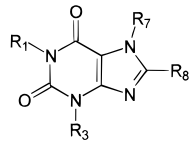
Comparison: Caffeine−DMPX. Caffeine (**1**) is a weak, nonselective AR antagonist. Theophylline (**31**) is more potent but also shows low selectivity for either receptor subtype (Table 2). The big breakthrough in the development of A_1-selective AR antagonists was, first, the introduction of lipophilic substituents in the 8-position of theophylline (**31**), such as phenyl and cycloalkyl residues, and second, the exchange of the 1,3-methyl groups for 1,3-dipropyl residues, as in 8-cyclopentyl-1,3-dipropylxanthine (DPCPX).[1,2,5]

The caffeine analogue DMPX (**2**, 3,7-dimethyl-1-propargylxanthine) is somewhat more potent than caffeine, particularly at A_{2A} AR. DMPX exhibits A_{2A} selectivity up to 10-fold, depending on the test systems that are being compared,[12,13,29] while caffeine is nonselective.

The introduction of certain 8-substituents into caffeine and 1,3-dipropyl-7-methylxanthine led to potent A_{2A}-selective antagonists, the most potent compounds bearing a styryl residue as in 8-styrylxanthine derivatives **4** and **5**.[15,16] In the present study we investigated the effects of 8-substitution of the A_2-selective AR antagonist DMPX and compared 8-substituted DMPX derivatives with corresponding caffeine derivatives.

8-Substitution of DMPX with cyclohexyl (**11**), phenyl (**29**), 2-thienyl (**12**), and styryl residues (**13, 17−22, 38**)

Table 2. A_1 and A_{2A} Adenosine Receptor Affinities of Novel DMPX Derivatives and Related Xanthines



compound		R_1	R_3	R_7	R_8	K_i (μM) ± SEM: A_1 rat brain cortical membranes vs [^{3}H]*R*−PIA	A_1 rat brain cortical membranes vs [^{3}H]CHA	A_{2A} rat brain striatal membranes vs [^{3}H]NECA	A_{2A} rat brain striatal membranes vs [^{3}H]CGS 21680	A_{2A} selectivity (A_1/A_{2A})[a]
Caffeine Derivatives and Standard Xanthines										
caffeine	**1**	methyl	methyl	methyl	H	41[b]	26 ± 9	43[b]	22 ± 11	1
DMPX	**2**	propargyl	methyl	methyl	H	45[c]	12 ± 1	16[c]	8.6 ± 1.6	1.4
theophylline	**31**	methyl	methyl	H	H	14[b]	17 ± 4	19[b]	9.4 ± 6.0	1.8
1-propargyl-3-methylxanthine	**32**	propargyl	methyl	H	H	0.82[d]	nd[e]	4.8[d]	nd	0.2
8-cyclohexylcaffeine	**3**	methyl	methyl	methyl	cyclohexyl	28[f]	nd	9.3[f]	nd	3
8-phenylcaffeine	**28**	methyl	methyl	methyl	phenyl	15[f]	33 ± 3	25[f]	19 ± 5	1.7
8-styrylcaffeine	**33**	methyl	methyl	methyl	styryl	3.89[g]	nd	nd	0.094[g]	41
8-(3,4-dimethoxystyryl)caffeine	**34**	methyl	methyl	methyl	3,4-dimethoxystyryl	13.8[g]	9.6 ± 3.1	nd	0.018 ± 0.0051 (0.197)[g]	533
KF17837	**4**	propyl	propyl	methyl	3,4-dimethoxystyryl	nd	0.26 ± 0.004 (0.390)[i]	nd	0.0085 ± 0.0012 (0.0079)[i]	31
8-(*m*-chlorostyryl)caffeine (CSC)	**5**	methyl	methyl	methyl	3-chlorostyryl	28[g]	> 1 (17%)[h]	nd	0.036 ± 0.006 (0.054)[g]	>28
DMPX Derivatives and Analogues										
8-cyclohexyl-DMPX	**11**	propargyl	methyl	methyl	cyclohexyl	nd	2.8 ± 0.3	nd	4.5 ± 1.5	0.6
8-phenyl-DMPX	**29**	propargyl	methyl	methyl	phenyl	6.1[d]	3.2 ± 0.8	6.4[d]	3.3 ± 0.7	1
	35	methyl	Isobutyl	methyl	phenyl	nd	4.4 ± 0.5	nd	14 ± 2	0.3
	36	methyl	Isobutyl	propargyl	phenyl	nd	2.8 ± 0.3	nd	3.5 ± 0.4	0.8
	9	propargyl	H	H	2-thienyl	0.12 ± 0.01	0.18 ± 0.09	5.1 ± 0.3	0.56 ± 0.08	0.3
8-(2-thienyl)-DMPX	**12**	propargyl	methyl	methyl	2-thienyl	nd	0.95 ± 0.09	nd	1.8 ± 0.3	0.5
	10	propargyl	H	H	styryl	2.1 ± 0.5	1.3 ± 0.3	1.9 ± 0.6	0.31 ± 0.02	4.2
8-styryl-DMPX	**13**	propargyl	methyl	methyl	styryl	2.7 ± 0.8	1.1 ± 0.2	0.14 ± 0.05	0.027 ± 0.005	41
	30	allyl	methyl	methyl	styryl	nd	9.2 ± 0.9	nd	0.063 ± 0.014	146
	37	propargyl	methyl	H	3-chlorostyryl	0.25 ± 0.06	nd	0.41 ± 0.17	nd	0.6
8(-*m*-chlorostyryl)-DMPX (CS-DMPX)	**38**	propargyl	methyl	methyl	3-chlorostyryl	2.5 ± 0.1	1.3 ± 0.1	0.047 ± 0.007	0.013 ± 0.001	100
	39	propargyl	propyl	methyl	3-chlorostyryl	nd	0.102 ± 0.014	nd	0.0051 ± 0.0007	20
	40	propargyl	Isobutyl	H	3-chlorostyryl	nd	0.083 ± 0.008	nd	0.046 ± 0.003	1.8
	41	propargyl	Isobutyl	methyl	3-chlorostyryl	nd	0.240 ± 0.038	nd	0.015 ± 0.009	16
	42	propargyl	benzyl	H	H	nd	0.86 ± 0.07	nd	0.88 ± 0.14	1
	43	propargyl	benzyl	methyl	3-chlorostyryl	nd	2.3 ± 0.5	nd	0.068 ± 0.011	34
	44	propargyl	methyl	propargyl	3-chlorostyryl	nd	0.20 ± 0.03	nd	0.061 ± 0.003	3
	45	propyl	methyl	methyl	3-chlorostyryl	nd	0.54 ± 0.16	nd	0.031 ± 0.003	17
8-(*m*-bromostyryl)-DMPX (BS-DMPX)	**17**	propargyl	methyl	methyl	3-bromostyryl	nd	1.2 ± 0.1	nd	0.0082 ± 0.0003	146
	18	propargyl	methyl	methyl	3,4-dimethoxystyryl	nd	2.5 ± 0.3	nd	0.015 ± 0.0054	167
	16c	propargyl	methyl	H	3,4-(methylenedioxy)styryl	nd	0.84 ± 0.09	nd	0.17 ± 0.02	4.9
	19	propargyl	methyl	methyl	3,4-(methylenedioxy)styryl	nd	1.5 ± 0.3	nd	0.035 ± 0.002	43
	20	propargyl	methyl	methyl	2-thienylethenyl	nd	4.1 ± 0.3	nd	0.34 ± 0.12	1.4
	21	propargyl	methyl	methyl	3-thienylethenyl	nd	0.75 ± 0.02	nd	0.024 ± 0.06	31
	22	propargyl	methyl	methyl	2-furylethenyl	nd	1.9 ± 0.01	nd	0.25 ± 0.16	8

[a] [^{3}H]CHA data/[^{3}H]CGS 21680 data as far as available. [b] Grahner et al.[30] [c] Daly et al.[29] [d] Müller et al.[21] [e] nd = not determined. [f] Daly[5] [g] Jacobson et al.[16] [h] Percent inhibition at indicated concentration; full curve could not be determined due to limited solubility. [i] Nonaka et al.[33]

generally increased A_1 and A_2 AR affinity of DMPX (Table 2). The same effect had been observed for caffeine derivatives, with the exception of the 8-phenyl derivative (**28**), which was equally active to caffeine at both receptor subtypes.

A comparison of four caffeine derivatives with corresponding DMPX derivatives (**3/11**, **28/29, 33/13, 5/38**) shows that 8-substituted DMPX derivatives are at least about 2−3-fold more potent AR antagonists than corresponding caffeine derivatives at both receptor subtypes. One exception is, however, the 8-(3,4-dimethoxystyryl)-DMPX **18**, which is virtually equipotent to the corresponding caffeine derivative **34** at A_{2A} AR but slightly more potent at A_1 AR, thus leading to a reduction in selectivity. This result demonstrates that subtle changes in the substitution pattern of the 8-styryl substitutent can influence the structure−activity relationships of substituents in the xanthine 1- and 3-positions.

The 1,3-dipropyl-7-methyl analogue **4** (KF 17837) exhibits increased affinity compared to the corresponding DMPX derivative **18** and the caffeine derivative **34** at both receptor subtypes, about 2-fold at A_{2A} AR, and 10-fold (compared to **18**), or 37-fold (compared to **34**), at A_1 AR, thus yielding a compound with high affinity but low selectivity (31-fold for **5** versus more than 100-fold for **18** and **34**).

The 7-Position. 7-Methylation of theophylline (**31**) led to a decrease in A_1 and A_{2A} AR affinity, caffeine (**1**) being about 2-fold less potent at A_1 and A_{2A} AR than **31**. The same effect is observed in 1-propargyl-3-methylxanthine (**32**).[21] Methylation causes a 3-fold decrease in A_{2A} AR affinity, but the decrease is much more pronounced at A_1 AR (55-fold). Thus, the A_1-selective 1-propargyl-3-methylxanthine (**32**) is turned into an A_{2A}-selective compound (DMPX, **2**) by 7-methylation. The N7-hydrogen of xanthines has been shown to be an important hydrogen bond donor in A_1 and A_{2A} AR binding,[30] but may be of greater importance for binding to A_1 AR for many xanthine derivatives.

Similarly, in 1-propargyl-8-(2-thienyl)xanthine (**9**) introduction of methyl groups in the 3- and 7-positions (compound **12**) led to a decrease in A_1 (5-fold) and A_{2A} (3-fold) AR affinity. In an 8-styryl derivative (**10**), however, the same modification led to no change in A_1 affinity, but an increase in A_{2A} affinity (>10-fold). The effect of methylation of 1-propargyl-3-(ar)alkyl-8-styrylxanthines could be observed in three compounds (**37/38, 40/41**, **16c/19**). The general finding was a decrease in A_1 AR affinity (2−10-fold), and at the same time an increase in A_{2A} affinity (3−9-fold). This effect appears to be specific for styryl substituents.

7-Methylation of 8-phenyl- and related 8-aryl-substituted xanthines will lead to a steric interaction between the methyl hydrogen atoms and the phenyl ortho-hydrogen atoms. This interaction may force the aryl ring into an unfavorable (noncoplanar?) conformation. For styryl and analogous 8-substituents in which the aryl moiety is linked to the xanthine via an ethenyl bridge, the situation is different. Coplanar conformation, for example, should be possible provided the (*E*)-configurated double bond is in a cisoid conformation, in which methyl hydrogen atoms and ethenyl hydrogen atoms do not interact.

The steric hindrance of a coplanar or nearly coplanar conformation should be reduced if a substituent other than methyl was located in the 7-position. As an example we introduced a propargyl residue in the 7-position, which indeed has quite different effects than the methyl substituent. At A_1 AR 7-propargyl was superior to a 7-methyl substituent (cf. **35/36**, **38/44**). The same effect had been observed with the 8-unsubstituted caffeine analogue 7-propargyl-1,3-diethylxanthine.[5] At A_{2A} AR the effect depended on the 8-substituent. For the 8-phenyl derivative, 7-propargyl was superior to 7-methyl (**35/36**), as had been seen with the 8-unsubstituted derivative.[5] This effect, however, was reversed in the 8-styryl derivatives (**38/44**): The propargyl derivative was 5-fold less potent compared to the methyl derivative. Steric hindrance may be needed to force the styryl residue in a favorable conformation.

In analogy to the current standard A_{2A} antagonist 8-chlorostyrylcaffeine (CSC, **5**), we synthesized the 8-(*m*-chlorostyryl)-DMPX (CS-DMPX, **38**) and investigated SAR of analogues with variations in the 1-, 3-, and 8-positions. So far, only two 8-styrylxanthine derivatives with different substituents in the 1- and 3-positions (1-methyl-3-propylxanthines) have been described.[31] Most derivatives investigated so far were 1,3-dimethyl- or 1,3-dipropyl-substituted xanthines. Few 1,3-diethyl- and 1,3-diallyl-8-styrylxanthines have also been described.[16]

The 1-Position. CS-DMPX (**38**) bearing a propargyl substituent in the 1-position was compared to the 1-methyl analogue CSC (**5**) and to an analogue in which the propargyl residue was replaced by *n*-propyl (**45**). Propargyl was the best substituent in this series (compound **38**) for high A_{2A} affinity, the methyl-substituted derivative **5** was about 3-fold less potent and equally potent to the 1-propyl derivative **45**. At A_1 AR, however, the 1-propyl derivative was the most potent compound, and therefore, **45** exhibited the least selectivity of the three derivatives.

In a series of 8-(3,4-dimethoxystyryl)-3,7-dimethylxanthines, three different substituents in the 1-position were compared: methyl (in caffeine derivative **34**), propargyl (in DMPX derivative **18**), and allyl (in **30**). Again, propargyl was the best substituent with regard to A_{2A} affinity, but methyl was nearly equipotent. Allyl substitution decreased A_{2A} AR affinity about 4-fold compared to propargyl.

The 3-Position. Four different substituents in the 3-position were investigated. The 3-methyl group in CS-DMPX (**38**) was exchanged for *n*-propyl (in **39**), isobutyl (in **41**), or benzyl (in **43**). The isobutyl derivative showed similar A_{2A} affinity as the methyl derivative, but A_1 affinity was increased 5-fold, leading to a compound with lower A_{2A} selectivity compared to the DMPX derivative **38**. A similar effect of a 3-isobutyl substituent could be observed in a comparison of 8-phenylcaffeine (**28**) and its 3-isobutyl analogue **35**. The latter was severalfold more potent than the methyl derivative at A_1 AR but not at A_{2A} AR, where both showed similar potency. A 3-benzyl residue decreased A_1 (2-fold) as well as A_{2A} affinity (5-fold), and **43** was therefore less potent and less selective than **38**. The most potent A_{2A} antagonist investigated in the present study was the 3-*n*-propyl analogue **39** of CS-DMPX (**38**) with a K_i value of 5.1 nM. Exchange of methyl for

propyl in the 3-position of **38** increased A_{2A} affinity 2.5-fold, but at the same time, A_1 affinity was increased nearly 13-fold, leading to a decrease in selectivity. Large substituents in the 3-position of xanthines increase A_1 selectivity in 8-phenyl- as well as in 8-styrylxanthines.

The 8-Position. The largest set of compounds investigated in the present study were DMPX derivatives bearing different substituents in the 8-position. 8-Cyclohexyl- (**11**), 8-phenyl- (**29**), and 8-(2-thienyl)-DMPX (**12**) were moderately potent at both receptor subtypes in the low micromolar range and nonselective. DMPX derivatives bearing a 2-(hetero)arylethenyl substituent in the 8-position (**13, 17**−**22, 38**) all exhibited similar potency at A_1 AR. Thus, the structure of the 8-substituent in DMPX derivatives had very little influence on A_1 AR affinity of the compounds, ranging from ca. 1−4 μM. At A_{2A} AR, however, 8-(2-arylethenyl) substitution of DMPX led to a large increase in A_{2A} AR affinity (compounds **13**, **17**−**22**, **38**) and subtle structural changes in the substituent pattern led to big changes in receptor affinity.

We investigated different substituents on the phenyl ring of 8-styryl-DMPX, namely the 3-chloro (**38**), the 3-bromo (**17**), the 3,4-dimethoxy (**18**), and the 3,4-methylenedioxy (**19**) derivatives. Compound **19** was similarly potent and A_{2A}-selective as the parent compound 8-styrylxanthine **13**, but may be somewhat more water-soluble due to polar substituents. 3-Halo substitution increased A_{2A} affinity, 2-fold for chlorine (in **38**) and 3-fold for bromine (in **17**). Since A_1 affinity remained virtually unaltered, A_{2A} selectivity was also significantly increased. 8-(*m*-Bromostyryl)-DMPX (BS-DMPX, **17**) is about as potent at A_{2A} AR as the standard A_{2A} antagonist KF17837 (**4**), but its selectivity is much greater (146-fold versus 31-fold). Compared with the commercially available high-affinity A_{2A} antagonist CSC (**5**), **17** is several times more potent and at least as selective as **5**. 8-(3,4-Dimethoxystyryl)-DMPX (**18**) showed about the same A_{2A} affinity and selectivity as 8-(*m*-chlorostyryl)-DMPX (**38**)

In a further series of compounds, the phenylethenyl (styryl) substituent was replaced by heteroarylethenyl residues with furyl and thienyl as bioisosteric heterarylic replacements for the phenyl ring. 2-Furyl (in **22**) and 2-thienyl replacement (in **20**) of phenyl (in 8-styryl-DMPX, **13**) led to compounds that were about 10-fold less potent at A_{2A} AR than **13**, but still about 10-fold more potent compared to 8-phenyl- (**29**) or 8-(2-thienyl)-DMPX (**12**). The 3-thienylethenyl analogue (**21**) of 8-styryl-DMPX (**13**), however, was equally potent to **13**. It appears that the heteroatom in **21** is in vicinity to the *m*-halogen atom in compounds **17** and **38**.

***(E/Z)*-Isomerization.** Styryl derivatives are known to undergo photoisomerization.[32,33] 8-Styrylxanthine derivative **4**, which had been investigated in this respect, isomerized when irradiated by a fluorescent lamp in dilute solution to yield a stable mixture of ca. 82% (*Z*) and ca. 18% (*E*)-configurated isomer.[33] The (*E*)-isomer was shown to be a much more potent AR antagonist compared to the (*Z*)-isomer.[33] The commercially available styrylxanthine CSC (**5**) had also been described to photoisomerize, but isomer ratios had not been determined.[34] We selected one of the most potent and selective compounds of the present series, 8-(*m*-chlorostyryl)-DMPX (**38**) for closer investigation of the photoisomerization process. In fact, fast isomerization was observed at normal daylight or when irradiated with UV light (366 nm) in methanolic solution containing 0.1 mM **38**, as measured by a decrease in UV absorption at 356 nm, the absorption maximum of the (*E*)-isomer. After 45 min the (*E*/*Z*)-equilibrium of the UV-irradiated solution was reached. HPLC analysis of the products revealed that the stable mixture contained ca. 57% (*E*)- and ca. 43% (*Z*)-isomer. The photoisomerization process appeared to be somewhat slower in DMSO solution as compared to methanol.

We conclude that all data for styrylxanthines in Table 2 represent results from stable mixtures of (*E*/*Z*)-isomers. Since the photoisomerization process is very fast in dilute solutions most published data for styrylxanthine derivatives are probably results from (*E*/*Z*)-mixtures.[15,16,31]

Low-Affinity Adenosine Receptor Subtypes. The 8-styrylxanthine derivatives **4** and **5** had been investigated at the low-affinity A_{2B} and A_3 AR subtypes. Both styrylxanthines showed low activity at A_{2B} AR[35,36] in the micromolar concentration range. CSC (**5**) was inactive at rat A_3 AR in concentrations up to 10 μM.[37] Preliminary results with BS-DMPX (**17**) at human recombinant A_{2B} and A_3 AR indicate that **17** is inactive at both receptor subtypes in high concentrations (10 μM; Klotz, K.-N.; Müller, C. E., unpublished). Therefore it appears that A_{2A}-selective styryl-DMPX derivatives are not only selective versus the high-affinity A_1 AR, but also exhibit a high degree of selectivity versus low-affinity subtypes A_{2B} and A_3.

Experimental Section

Synthetic Procedures. 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. 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) 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.

Standard compounds caffeine (**1**), DMPX (**2**), theophylline (**31**), and 8-(*m*-chlorostyryl)caffeine (CSC, **5**) were commercially available. KF17837 (**4**) was synthesized as described.[15] The synthesis of xanthines **37**−**45** has been described elsewhere.[24]

6-Amino-3-propargyl-5-(2-thenylideneamino)uracil (7b). 5,6-Diaminopropargyluracil **6**[24] (0.53 g, 2.9 mmol) and thiophene-2-carbaldehyde (0.4 g, 3.6 mmol) are dissolved in EtOH (10 mL). Acetic acid (1.5 mL) is added, and the mixture is heated under reflux for 3 h. After cooling, H_2O (20 mL) is added to the solution, and the resulting precipitate is collected by filtration: ^{1}H NMR δ (ppm) 2.49 (t, 1H, propargyl CH), 4.46 (d, 2H, propargyl CH_2), 6.44 (s, 2H, C6−NH_2), 7.14 (dd, 1H, C4′-H), 7.38, 7.53 (2 x dd, 2 × 1H, C3′-H, C5′-H), 9.73 (s, 1H, N=CH), 10.90 (br s, 1H, N1−H); ^{13}C NMR δ (ppm) 28.5 (propargyl CH_2), 72.4 (propargyl CH), 79.9 (propargyl C2′), 97.9 (C5), 127.9, 128.7, 144.0, 145.5 (arom), 148.4, 151.7 (N=CH, C2, C6), 157.4 (C4).

***(E)*-6-Amino-3-propargyl-5-styrylideneaminouracil (7c).** Compound **6**[24] (0.7 g, 3.9 mmol) is dissolved in MeOH (30 mL). (*E*)-Cinnamic aldehyde (0.5 mL, 3.8 mmol) and 3 drops of acetic acid are added, and the solution is stirred at room temperature for 30 min. After the addition of H_2O (30 mL), the precipitated yellow product is collected by filtration and washed with

H_2O: ^1H NMR δ (ppm) 3.16 (t, 1H, propargyl CH), 4.56 (d, 2H, propargyl CH_2), 6.70 (br s, 2H, NH_2), 6.70−7.62 (m, 7H, $H_{vinylic}$ and H_{arom}), 9.52 (br s, 1H, N=CH), 11.15 (br s, 1H, N1−H); ^{13}C NMR δ (ppm) 26.6 (propargyl CH_2), 72.6 (propargyl CH), 80.1 (propargyl C2′), 99.0 (C5), 126.8, 128.4, 128.9, 131.4, 136.6, 137.1 (arom), 148.6, 152.1 (N=CH, C2, C6), 157.2 (C4).

1-Propargyl-8-(2-thienyl)xanthine (9). Compound **7b** (0.57 g, 2.1 mmol) and anhydrous $FeCl_3$ (0.40 g, 2.5 mmol) are dissolved in anhydrous EtOH (10 mL). The mixture is refluxed for 7 h. After cooling, H_2O (20 mL) is added, and the product is collected by filtration. The compound is dissolved in 1 N NaOH (15 mL), insoluble material is removed by filtration, and the product is precipitated by acidification of the solution with concentrated HCl.

(*E*)-1-Propargyl-8-styrylxanthine (10). Compound **7c** (0.13 g, 0.44 mmol) is dissolved in $SOCl_2$ (10 mL) and stirred at room temperature for 1 h. $SOCl_2$ is removed by distillation in vacuo. The residue is treated with ice water, and the precipitate is collected by filtration. The product is purified by dissolution in DMF (5 mL) and subsequent precipitation by the addition of H_2O (20 mL).

Bismethylation of 1,8-Disubstituted Xanthines: Preparation of Compounds 11−13. 1,8-Disubstituted xanthine (**8**,[21] **9**, or **10**, respectively; 1.5 mmol), K_2CO_3 (0.43 g, 3.1 mmol), and MeI (1.9 mL, 60 mmol) are dissolved in dimethylformamide (DMF, 10 mL) and stirred at room temperature for 12 h. The product is precipitated by the addition of H_2O (20 mL), collected by filtration, and washed with H_2O. Purification is achieved by dissolution in DMF and subsequent precipitation by the addition of H_2O. Starting products and monomethylated derivatives can be extracted with 10% NaOH solution.

6-Amino-3-propargyl-5-(carbonylamino)uracils (14a−f). General Procedure. 3-Propargyl-5,6-diaminouracil[24] (**1**, 0.7 g, 3.9 mmol) is dissolved in MeOH (30 mL), and the appropriate carboxylic acid (1.1 equiv) and 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC, 0.822 g, 4.3 mmol) is added. The mixture is stirred at room temperature for 24 h. Then, H_2O (30 mL) is added to complete the precipitation of the product which is collected by filtration and washed with H_2O.

6-Amino-1-methyl-3-propargyl-5-(carbonylamino)-uracils (15a−f). General Procedure. Compound **14a−e** (3.0 mmol) is dissolved in DMF (30 mL). After the addition of K_2CO_3 (0.50 g, 3.6 mmol) and MeI (4.0 mL, 64 mmol), the suspension is stirred at room temperature for 24 h. The product is precipitated by the addition of H_2O (45 mL), collected by filtration, and washed with H_2O.

1,3,8-Trisubstituted Xanthines (16a,c−f). General Procedure. Compound **15a**, or **15c−f**, respectively (2.0 mmol), is suspended in a mixture of MeOH (155 mL) and H_2O (22 mL). After the addition of an aqueous 10% NaOH solution (66 mL), the yellow solution is heated to 60 °C for 3 h. Then MeOH is distilled off. The aqueous solution is brought to pH 4 by the addition of concentrated HCl. The product precipitates as a white solid which is collected by filtration and washed with H_2O. Purification is achieved by dissolution in 10% aqueous NaOH solution and subsequent precipitation with HCl or, alternatively, by dissolution in DMF and precipitation by the addition of H_2O.

1-Propargyl-3-methyl-8-(3,4-dimethoxystyryl)xanthine (16b). Compound **15b** (0.38 g, 1 mmol) is suspended in 120 mL of 0.5 N NaOH and 100 mL of EtOH and stirred at 60 °C for 18 h. After being cooled to room temperature, the solution is acidified with 2 N HCl to pH 4, EtOH is evaporated, and the remaining aqueous solution is cooled to 4 °C. The precipitated product is collected by filtration and dried at 80 °C. Purification was achieved by column chromatography (eluent MeOH:CH_2Cl_2 = 1:9) followed by recrystallization from 95 mL of EtOH:H_2O = 50:50 (v/v): ^1H NMR δ (ppm) 2.92 (t, ^4J = 2.4 Hz, 1 H, C−H), 3.47 (s, 3 H, N3−CH_3), 3.75 (s, 3 H, O−CH_3), 3.89 (s, 3 H, O−CH_3), 4.57 (d, ^4J = 2.4 Hz, 2H, CH_2), 6.86 (d, J = 16 Hz, 1H, $H_{vinylic}$), 6.9−7.2 (m, 6 H, $H_{vinylic+arom}$).

1,3,7,8-Tetrasubstituted Xanthines (17−22 and 35, 36). General Procedure. Xanthine derivative **16a−f** (0.30 mmol, for the preparation of **17−22**), or 3-isobutyl-1-methyl-8-phenylxanthine (8-phenyl-IBMX,[38] 0.30 mmol, for the preparation of **35** and **36**), respectively, is dissolved in DMF (7.0 mL). After the addition of K_2CO_3 (0.070 g, 0.51 mmol) and MeI (0.2 mL, 3.29 mmol, for **17−22** and **35**), or propargyl bromide (0.12 g, 1 mmol, for **36**), the mixture is stirred for 24 h. The product is precipitated by the addition of H_2O (30 mL), cooled, and collected by filtration. Purification of the product is achieved by dissolution in DMF and precipitation by the addition of H_2O. Unreacted 7-unsubstituted xanthine can be extracted with 10% aqueous NaOH solution.

6-Amino-5-bromo-1-methyluracil (24). A mixture of 6-amino-1-methyluracil (**23**)[20] (3.44 g, 41 mmol), MeOH (40 mL), and $NaHCO_3$ (3.41 g, 41 mmol) is cooled to 4 °C, and Br_2 (6.55 g, 41 mmol) is added during a period of 30 min. The reaction mixture is kept stirring for another 30 min at room temperature and then cooled to 4 °C. The white precipitate is filtered off and recrystallized from H_2O: ^1H NMR δ (ppm) 3.30 (s, 3H, CH_3−N), 7.02 (br s, 2H, NH_2), 10.90 (v br s, 1H, H−N); ^{13}C NMR δ (ppm) 29.9 (N7−CH_3), 70.5 (C5), 149.9 (C2), 152.8 (C4), 158.2 (C6).

6-Amino-1-methyl-5-(methylamino)uracil (25). Compound **24** (3.52 g, 16 mmol) and 17 mL (160 mmol) of an aqueous solution of methylamine (30%) are heated at 70 °C for 4 h. Excess methylamine is evaporated, the residue suspended in 20 mL of ethanol, and the pH of the solution adjusted to 7 by the gradual addition of 2 N HCl solution. The flask is allowed to stand for 5 h at 0 °C, during which time the methylammonium bromide which had formed dissolves. The product is filtered off and recrystallized from ethanol: ^1H NMR δ (ppm) 2.36 (s, 3H, N−CH_3), 2.78 (br s, 1H, N−CH_3), 3.21 (s, 3H, N−CH_3), 6.38 (s, 2H, NH_2), 10.48 (br s, 1H, N−H); ^{13}C NMR δ (ppm) 34.1 (N1−CH_3), 40.1 (N7−CH_3), 104.0 (C5), 155.1 (C2), 157.5 (C4), 165.8 (C6).

6-Amino-5-(*N*-benzoyl-*N*-methylamino)-1-methyluracil (26a). A suspension of 2.39 g (14 mmol) of **25** in 30 mL of dry pyridine is cooled to 0 °C, and 2.23 mL (16 mmol) of benzoyl chloride is added dropwise with stirring. The mixture is stirred overnight and then evaporated to dryness. The residue is treated with 30 mL of H_2O, collected by filtration, and recrystallized from EtOH:H_2O = 50:50 (v/v) to give white crystals: ^1H NMR δ (ppm) 2.96 (s, 3H, N(CH_3)CO), 3.33 (s, 3H, N1−CH_3), 7.0−7.4 (m, 5H, H_{arom}), 10.46 (v br s, 1H, N−H); ^{13}C NMR δ (ppm) 28.9 (N1−CH_3), 34.3 (N7−CH_3), 96.0 (C5), 125.4, 127.1, 128.8, 137.4 (arom), 149.7, 152.6 (C2, C6), 159.0 (C4), 172.9 (C=O exocyclic).

(*E*)-6-Amino-5-(*N*-cinnamoyl-*N*-methylamino)-1-methyluracil (26b). Compound **25** (0.85 g, 5 mmol), (*E*)-cinnamic acid (0.72 g, 5.05 mmol), and EDC (0.98 g, 5.1 mmol) are suspended in 40 mL of MeOH and stirred overnight. After the addition of 60 mL of H_2O the mixture is stirred for 3 h and the precipitate filtered off, air-dried, dissolved in DMF and precipitated by the addition of 40 mL of H_2O to give 1.04 g of white crystals: ^1H NMR δ (ppm) 2.95 (s, 3H, CH_3), 3.27 (s, 3H, CH_3), 6.74 (d, J = 16 Hz, $H_{vinylic}$), 7.06 (s, 2H, NH_2), 7.30−7.65 (m, 6 H, $H_{arom+vinylic}$), 10.66 (s, 1 H, N−H); ^{13}C NMR δ (ppm) 29.1 (N−CH_3), 34.3 (N1−CH_3), 93.7 (C5), 108.9, 118.7 (vinylic), 127.8, 128.7, 129.3, 140.4 (arom), 150.2 (C6), 153.3 (C4), 159.5 (C2), 167.8 (C=O, exocyclic).

3,7-Dimethyl-8-phenylxanthine (27a). Compound **26a** (1.65 g, 6 mmol) is refluxed in 13 mL of 2 N NaOH and 4 mL of ethanol for 1 h. The hot solution is allowed to cool to 4 °C, diluted with 17 mL of water, and acidified with acetic acid to form a white precipitate, which is collected by filtration: ^1H NMR δ (ppm) 3.39 (s, 3H, N3−CH_3), 3.97 (N7−CH_3), 7.44−7.83 (m, 5H, H_{arom}), 11.16 (v br s, 1H, N−H); ^{13}C NMR δ (ppm) 28.4 (N3−CH_3), 33.5 (N7−CH_3), 128.2, 128.7, 129.0, 130.1 (arom), 149.1 (C4), 150.8 (C8), 151.0 (C2), 155.0 (C6).

(*E*)-3,7-Dimethyl-8-styrylxanthine (27b). Compound **26b** (0.54 g, 1.8 mmol) is refluxed in 2 mL of 2 N NaOH and 4 mL of EtOH for 1 h. After being cooled to 4 °C, the solution is acidified with 2 N HCl, and the white precipitate is collected by filtration and recrystallized from H_2O:EtOH = 50:50 (v/v): ^1H NMR δ (ppm) 3.35 (s, 3H, N3−CH_3), 3.85 (s, 3H, N7−CH_3), 6.74 (d, ^3J = 16 Hz, 1 H), 7.35−7.85 (m, 6H, $H_{vinylic+arom}$), 11.1 (s, 1H, N−H).

8-Phenylcaffeine (28). To a mixture of **27a** (0.154 g, 0.6 mmol) and K_2CO_3 (0.1 g, 0.73 mmol) in DMF (30 mL) is added

dropwise MeI (60 μL, 9.5 mmol). The mixture is stirred at room temperature for 48 h. The product is precipitated by the addition of H_2O (60 mL). After the mixture is cooled to 4 °C, the precipitate is collected by filtration and washed with H_2O.

3,7-Dimethyl-8-phenyl-1-propargylxanthine (29). To a solution of **27a** (0.154 g, 0.6 mmol) and K_2CO_3 (0.1 g, 0.73 mmol) in DMF (30 mL) is added dropwise MeI (68 μL, 9 mmol). The mixture is stirred at room temperature for 48 h. The product is precipitated by the addition of H_2O (70 mL). After the mixture is cooled to 4 °C, the precipitate is collected by filtration and washed with H_2O.

***(E)*-1-Allyl-3,7-dimethyl-8-styrylxanthine (30).** To a solution of **27b** (0.27 g, 1 mmol) and K_2CO_3 (0.2 g, 1.5 mmol) in DMF (10 mL) is added allyl bromide (0.18 g, 1.5 mmol). The mixture is stirred at 50 °C for 3 h. The product is precipitated by the addition of H_2O (30 mL). After the mixture is cooled to 4 °C the precipitate is collected by filtration and washed with H_2O.

8-(3,4-Dimethoxystyryl)caffeine (34). 6-Amino-5-[(3,4-dimethoxycinnamoyl)amino]-1,3-dimethyluracil[16] (0.36 g, 1 mmol) is suspended in 120 mL of 0.5 N NaOH and 100 mL of EtOH and stirred at 60 °C for 18 h. After being cooled to room temperature, the solution is acidified to pH 4 by the addition of 2 N HCl. After evaporation of the EtOH, the aqueous solution is cooled to 4 °C. The precipitate is collected by filtration and dried at 80 °C. Purification is achieved by column chromatography (eluent MeOH:CH_2Cl_2 = 1:9) followed by recrystallization from 80 mL of EtOH:H_2O = 50:50 (v/v) to yield 0.18 g (53%) of 8-(3,4-dimethoxystyryl)theophylline (mp >250 °C): ^{1}H NMR δ (ppm) 3.19 (s, 3 H, N−CH_3), 3.41 (s, 3 H, N−CH_3), 3.75 (s, 3 H, O−CH_3), 3.81 (s, 3 H, O−CH_3), 6.86 (d, ^3J = 16 Hz, 1 H, $H_{vinylic}$), 6.9−7.3 (m, 4 H, $H_{vinylic+arom}$).

8-(3,4-Dimethoxystyryl)theophylline (0.1 g, 0.3 mmol) is dissolved in 5 mL of DMF. After the addition of 0.1 g (7.3 mmol) of K_2CO_3 followed by 60 mL (0.9 mmol) of MeI, the mixture is stirred at room temperature for 12 h. The precipitation of the product is completed by the addition of 10 mL of H_2O. The product is collected by filtration and dried in an oven at 80 °C overnight.

Photoisomerization of 38. A solution of **38** (0.1 mM) in MeOH, or DMSO, is irradiated by UV light (366 nm). Samples are taken at time intervals of 5−10 min, and UV spectra are immediately recorded. A methanolic solution of **38**, treated as described above, which had reached its equilibrium, is subjected to HPLC separation with UV detection at 280 nm, using an RP18 column and acetonitrile: H_2O = 60:40 as eluent, essentially as described.[33,39] Retention times were 6.18 min for the (*E*)- and 5.37 min for the (*Z*)-isomer.

Adenosine Receptor Assays. 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)phenyl]ethyl]amino]-5′−[*N*-(ethylcarbonyl)-amino]adenosine (CGS21680) to A_2 adenosine receptors of rat striatal membranes were assayed as described.[28,40] 2-Chloroadenosine (10 μM) was used to define nonspecific binding. Inhibition of the receptor−radioligand binding was determined by a range of five or six concentrations of the compounds in triplicate in at least three (A_1) or two (A_{2A}) separate experiments. The Cheng−Prusoff equation[41] 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, CA). For selected compounds, additional A_1AR binding studies with [^{3}H]-N^6-(*R*)-phenylisopropyladenosine ([^{3}H]*R*-PIA) as A1 radioligand[42] and [^{3}H]NECA as A_{2A} radioligand in the presence of 50 nM N^6-cyclopentyladenosine[43] were performed as described. A K_D value of 1 nM for [^{3}H]PIA and 8.5 nM for [^{3}H]NECA was used for the calculation of K_i values.

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Supporting Information Available: ^{1}H and selected ^{13}C NMR data of final products (4 pages). Ordering information is given on any current masthead page.

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