Synthesis and Structure-Activity Relationships of Deazaxanthines: Analogs of Potent A_1- and A_2-Adenosine Receptor Antagonists†,‡

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Received November 3, 1993®

A set of 22 9-deazaxanthines (pyrrolo[3,2-*d*]pyrimidine-2,4-diones) and three 7-deazaxanthines (pyrrolo[2,3-*d*]pyrimidine-2,4-diones) with various substituents in the 1-, 3-, 7- or 9-, and 8-positions was synthesized and investigated in A1 and A2a adenosine receptor binding assays at rat brain cortical membranes and rat brain striatal membranes, respectively. 9-Deazaxanthines showed structure-activity relationships that were similar to those of xanthines. They were about equipotent to the corresponding xanthines at A2a adenosine receptors. 9-Deazaxanthines were generally at least 2-3-fold more potent than xanthines at A1 receptors and therefore exhibited higher A1 selectivities compared to the xanthines. 1,3-Dimethyl-8-(2-naphthyl)-9-deazaxanthine (**19e**) showed high affinty (K_i = 26 nM) and selectivity for A1 adenosine receptors. A hydroxyl function at N7 of 9-deazaxanthines was unfavorable for A1 and A2a receptor binding. 7-Deazaxanthines were considerably less potent compared to xanthines and to 9-deazaxanthines at both receptor subtypes.

Introduction

Extracellular adenosine receptors (ARs) are divided into subtypes with high affinity (A1, A2a) and those with low affinity (A2b, A3) for adenosine.[1] Recently, a further low-affinity subtype, designated A4, was postulated.[2] While A1-, A2-, and A3ARs were cloned, a gene encoding for the A4AR has not been identified yet.[1] The development of potent and subtype-selective agonists and antagonists for ARs has been an active area of research due to the therapeutic potential of those compounds.[3-5] All adenosine receptor agonists are derivatives of the endogenous nucleoside adenosine with an intact ribose moiety and substitution preferably at N^6 or C2. The main class of AR antagonists are the xanthines. A1-, A2a-, and A2bARs are blocked by xanthines. The recently cloned A3ARs of sheep[6] and humans[7] are also blocked by xanthines, while A3- and A4ARs of rats appear to be xanthine-insensitive.[1] Structure-activity relationships (SARs) of xanthines at the high-affinity A1- and A2aARs have been extensively investigated. Substitution at the 1-position with methyl, propyl, or other small substituents is required for high A1- and A2AR affinity. Substitution at the 3-position with methyl or propyl can improve A1- and A2AR activity but is not absolutely necessary for high receptor affinity.[8] Substitution at the 7-position generally reduces AR affinity, the A1 affinity to a greater extent than A2 affinity. 9-Substituted xanthines exhibit very low activity or are inactive at ARs. The introduction of 8-phenyl or 8-cyclopentyl residues leads to a large increase in receptor affinity and A1 selectivity of the compounds. Recently, potent A2-selective xanthines have been described, the most potent compounds bearing an 8-styryl residue and a 7-methyl function.[9-11]

It has been hypothesized that xanthines bind to the same site of ARs as adenosine derivatives.[12-15] Different models have been developed which are to explain the binding of xanthine antagonists with respect to adenosine agonists at ARs.[12-14] These models were based on the established structure-activity relationshis of adenosines and xanthines at ARs. In the present study, 7- and 9-deazaxanthines were synthesized and investigated in A1- and A2aAR binding assays at rat brain membranes. The goal was to get information about the role of the nitrogens in the binding of xanthines to ARs and to improve the affinity and/or selectivity of the compounds.

Chemistry

Pyrrolo[2,3-*d*]pyrimidine-2,4-diones (7-deazaxanthines) **21** and **22** were synthesized according to published procedures.[16,17] The 7-methyl derivative **23** was obtained by methylation of **22** with excess methyl iodide in DMF in the presence of K_2CO_3.

Pyrrolo[3,2-*d*]pyrimidine-2,4-diones (9-deazaxanthines) were synthesized as depicted in Schemes 1-3. Condensation of *N*-methylurea with ethyl acetoacetate afforded 3,6-dimethyluracil (**1a**) as described.[18] The preparation of 6-methyl-3-propyluracil (**1b**) was performed analogously but required a higher concentration of NaOH for the ring closure. N1-Alkylation of **1a** with excess methyl iodide in an aqueous solution of NaOH afforded **2a**. 6-Methyl-3-propyluracil (**1b**) was treated with excess propyl bromide in a solution of 15% NaOH in a mixture of ethanol-H_2O (1:1) to afford **2b**. Introduction of a nitro group in the 5-position was achieved by treating 1,3,6-trimethyluracil (**2a**) with a mixture of equal volumes of fuming HNO_3 and concentrated H_2SO_4.[19] For the nitration of the 3-monosubstituted 6-methyluracils **1a** and **1b** and the 1,3-dipropyl derivative **2b**, the ratio of fuming HNO_3 to concentrated H_2SO_4 had to be changed to 1:1.5 to obtain **3a** and **4b** and to 1:2 for the synthesis of the monopropyl derivative **3b**. 6-[(Dimethylamino)vinyl]uracils **5a,b** and **6a,b** were synthesized by treating the appropriate 6-methyl-5-nitrouracils with DMF-diethyl acetal in DMF.[20] Ring closure to 6-unsubstituted pyrrolo[3,2-*d*]pyrimidine-2,4-diones or to 6-dimethylamino derivatives, respectively, was achieved by reduction with Pd/C in a hydrogen atmosphere.[20] The reaction took place under normal air pressure at room

† Dedicated to Prof. Dr. Hermann J. Roth on the occasion of his 65th birthday.

‡ Preliminary results were presented in Milan Purines 92; abstract published in *Int. J. Purine Pyrimidine Res.* 1992, *3*, 92.

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® Abstract published in *Advance ACS Abstracts*, April 15, 1994.

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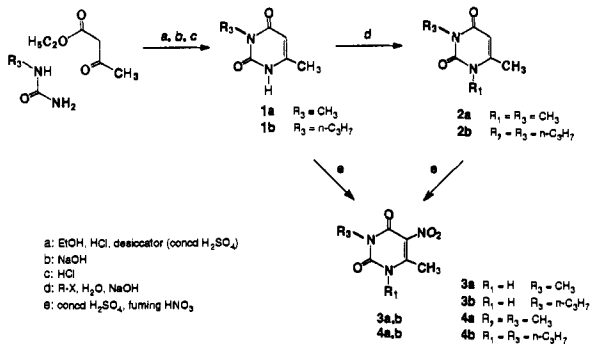
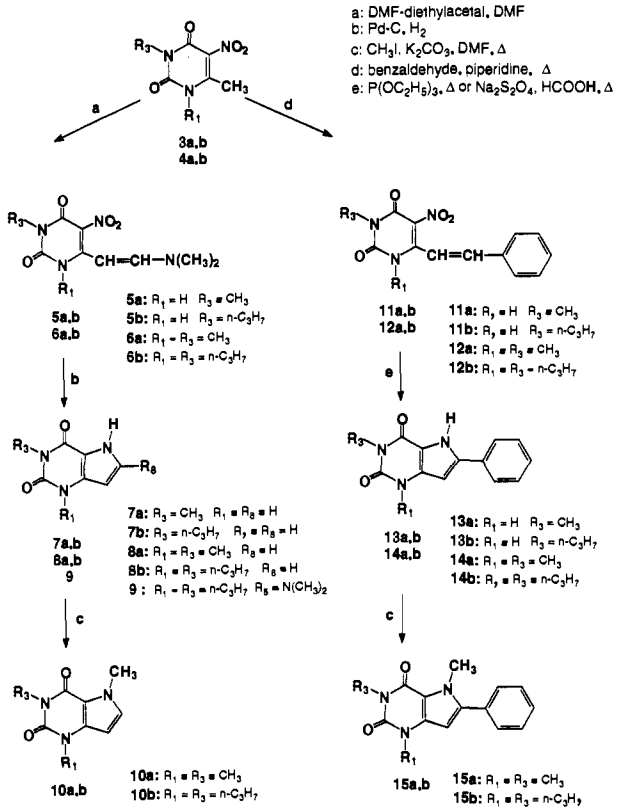
Scheme 1. Synthesis of 3-Monoalkyl- and 1,3-Dialkyl-5-nitrouracils



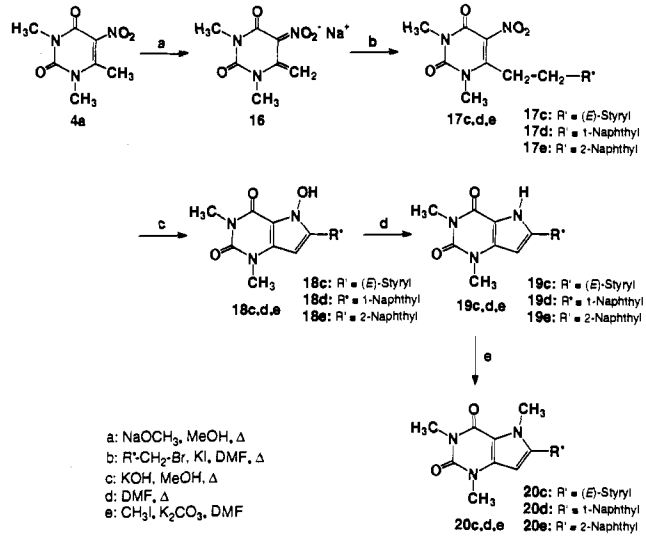
Scheme 2. Synthesis of 6-Unsubstituted- and 6-Phenylpyrrolo[3,2-*d*]pyrimidines



temperature, and the reduction of **5a,b** and **6a** afforded the corresponding 6-unsubstituted pyrrolo[3,2-*d*]pyrimidines as sole products. The reductive ring closure of the dipropyluracil **6b** yielded two products: the 6-unsubstituted (**8b**) and the 6-(dimethylamino)pyrrolo[3,2-*d*]pyrimidine **9**. Methylation of **8a,b** with excess methyl iodide in DMF in the presence of K_2CO_3 yielded the 1,3,5-trialkyl derivatives **10a** and **10b**.

5-Nitro-6-styryluracils (**11a,b** and **12a,b**) were prepared by reaction of the 6-methyl-5-nitro derivatives **3a,b** and **4a,b** with benzaldehyde in the presence of piperidine.[21,22] The 3-monoalkylated compounds were obtained as piperidinium salts due to their acidic hydrogen at N1. Neutralization with acetic acid afforded the free acids **11a** and **11b**. Reduction of the 5-nitro group gave the 6-phenyl-substituted pyrrolo[3,2-*d*]pyrimidines **13a,b** and **14a,b**. The monopropyl derivative **13b** was prepared by reductive ring closure of **11b** with sodium dithionite in formic acid.[23] Compound **13b** could be separated from the formed sulfur-containing precipitate by extraction with organic solvents. Compounds **13a** and **14a,b** showed rather low solubility in organic solvents. Therefore, triethyl phosphite was used

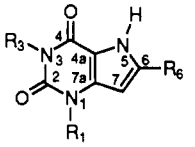
Scheme 3. Synthesis of 6-Styryl- and 6-Naphthylpyrrolo[3,2-*d*]pyrimidines



as a reducing agent.[21] The 5-methylated derivatives **15a** and **15b** were obtained by methylation of **14a** and **14b** with excess methyl iodide in DMF in the presence of K_2CO_3. 6-Styryl- and 6-naphthylpyrrolo[3,2-*d*]pyrimidine-2,4-diones were prepared as outlined in Scheme 3. 1,3,6-Trimethyl-5-nitrouracil **4a** was treated with a solution of 30% sodium methylate in methanol to yield 1,3,6-trimethyl-5-nitrouracil monosodium salt (**16**).[24] Compound **16** was reacted with (*E*)-3-bromo-1-phenyl-1-propene in the presence of potassium iodide to afford **17c**, or with 1- or 2-(bromomethyl)naphthalene to the derivatives **17d** and **17e**, respectively.[25] The 5-hydroxypyrrolo-[3,2-*d*]pyrimidine-2,4-diones **18c-e** were obtained by heating **17c-e** in a methanolic solution containing an equimolar amount of KOH. Refluxing of the 5-hydroxy derivatives in DMF for 1 h afforded the deoxygenated compounds **19c–e**. The 6-substituted pyrrolo[3,2-*d*]-pyrimidine derivatives **19c–e** were treated with excess methyl iodide in DMF in the presence of K_2CO_3 to afford the corresponding 5-methylated compounds **20c–e**.

The compounds were characterized by ^1H- and ^{13}C-NMR spectra. Selected ^{13}C-NMR spectral data are listed in Table 1. In analogy to xanthines,[8] the chemical shift of the C2 carbon of the pyrrolo[3,2-*d*]pyrimidine-2,4-diones is constantly at about 151 ppm. The C4 signal appears between 152 and 156 ppm. The C4 carbon of these compounds corresponds to the C4 of the uracils and the C6 of the xanthines (see Figure 1). The chemical shift of C4 depends on substitution in the 5-position. Methylation of the N5 nitrogen of 6-unsubstituted (**10a,b**) and 6-phenyl-substituted (**15a,b**) derivatives results in a downfield shift of about 1 ppm. The signal of the C4a carbon is constantly at 108–110 ppm, except for the 6-dimethylamino derivative **9**. Here, the signal is shifted to 103 ppm, due to the mesomeric effects of the nitrogen of the dimethylamino group in the 6-position. The chemical shift of C6 is influenced by two effects: firstly, the substitution at C6 itself, and secondly, the N5 substitution pattern. The C6 signal of C6-unsubstituted compounds (**7a,b, 8a,b**) appears at 127.0 ppm. C6-Phenyl-substituted compounds (**13a,b, 14a,b**) show a signal at 139 ppm. In both cases the replacement of the N5 hydrogen by a methyl group results in an upfield shift of 3.5 ppm (**10a,b, 15a,b**). The signals at 90–95 ppm (76.6 ppm for compound **9**) can be assigned to the C7 carbon by DEPT (distortionless enhancement by polarization transfer) experiments. The carbon C7a

Table 1. ^{13}C-NMR Data of Selected Pyrrolo[3,2-*d*]pyrimidines



compd	chemical shifts (δ) in DMSO-d_6 (ppm)											
	N1 R	N3 R	N5 R	C2	C4	C4a	C6	C7	C7a	C6 R		
	C6-H and 6-(Dimethylamino)-Substituted Pyrrolo[3,2-*d*]pyrimidine-2,4-diones											
7a		26.7		151.3	155.4	110.1	127.0	94.8	132.7			
7b		11.2, 21.0, 41.0		151.1	155.3	110.1	127.0	94.7	132.7			
8a	31.8	27.5		151.0	154.8	109.9	127.0	95.0	135.1			
8b	11.2, 20.9, 46.3	11.0, 20.7, 41.8		150.7	154.6	110.1	127.1	95.1	134.6			
9	11.2, 21.1, 46.1	11.0, 20.6, 41.5		150.9[a]	152.4	103.5	151.0[a]	76.6	137.5	39.7		
10a	31.7	27.7	35.8	152.6	156.1	111.0	130.4	93.3	135.5			
10b[b]	11.4, 21.4, 46.9	11.2, 21.0, 42.6	35.8	151.3	156.0	110.8	130.3	93.3	135.3			
	6-Phenylpyrrolo[3,2-*d*]pyrimidine-2,4-diones									C1′	C4′	CH arom
13a		26.8		151.3	155.3	110.0	139.6	92.4	133.6	130.8	128.1	125.5, 128.8
13b		11.2 21.0 41.1		151.1	155.1	111.1	139.4	92.4	133.7	130.8	128.2	125.5, 128.8
14a	31.8	27.6		151.1	154.6	110.8	139.2	93.0	136.0	130.8	128.2	125.5, 128.8
14b	11.2 21.0 46.3	11.0 20.7 41.8		150.6	154.2	110.8	139.4	93.0	136.0	131.5	128.2	125.5, 128.8
15a	31.6	27.5	33.3	150.8	155.2	110.5	142.4	94.9	135.3	130.5		128.8–129.0
15b	11.4 21.5 46.9	11.3 21.1 42.7	33.6	151.2	156.1	111.8	143.1	94.3	135.2	130.8	128.9	128.7, 129.2

compd	N1 CH_3	N3 CH_3	N5 CH_3	C2	C4	C4a	C6	C7	C7a	C6 R
	6-Styryl- and 6-Naphthylpyrrolo[3,2-*d*]pyrimidine-2,4-diones									
18c	31.0	27.4		150.8	153.0	107.9	131.3	86.2	136.0[a]	CH=CH 113.8, 131.6 C1′ 136.3,[a] CH arom 126.4–128.7
18e	31.2	27.6		151.0	153.5	108.9	132.9[a]	89.6	137.2	C quart. 132.6,[a] 131.4[a] CH arom 125.4–128.3
20c[b]	31.5	27.4	31.5	150.8	154.9	110.0	140.1	91.2	136.5[a]	CH=CH 115.4, 132.4 C1′ 135.4,[a] CH arom 126.8–128.8
20e[b]	31.7	27.9	33.8	151.7	156.1	111.5	143.1	94.5	135.7	C quart. 133.1, 128.0 CH arom 126.4–128.7

[a] Assignments are arbitrary. [b] $CDCl_3$ was used as solvent.

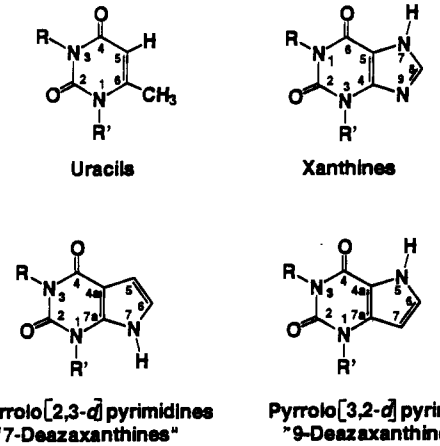


Figure 1. Nomenclature and numbering system of uracils, xanthines, and deazaxanthines.

signal of N1-unsubstituted compounds appears at 132.7 ppm (**7a,b**) or 133.6 ppm (**13a,b**). Replacement of the N1 hydrogen by alkyl substituents (**8a, 8b, 14a, 14b**) results in an upfield shift of about 2–3 ppm for carbon C7a.

6-Styryl- and 6-naphthyl derivatives exhibit poor solubility in various solvents. The N5-unsubstituted (**19c–e**) derivatives could only be dissolved in DMSO-d_6 if a few drops of NaOD were added. Therefore the NMR data are not directly comparable with the data of other compounds, which are dissolved in DMSO-d_6 only. In case of compounds **18c** and **18e**, the N5-nitrogen is substituted by a hydroxyl group. While the chemical shifts of C2, C4, and C7a are comparable to those of other pyrrolo[3,2-*d*]-pyrimidines, the signals of C4a, C6, and C7 are shifted downfield due to the influence of the N5-hydroxyl group. The signals of the N5-methylated 6-styryl (**20c**) and 6-(2-naphthyl) (**20e**) derivatives correlate with those of other N5-methylated compounds (**10a,b, 15a,b**).

Biological Evaluation

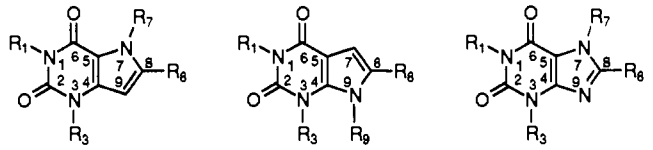
Deazaxanthines and xanthines were tested in radioligand binding assays for affinity at A1 and A2a adenosine receptors in rat cortical membrane, and rat striatal membrane preparations, respectively. [^{3}H]-N^6-(*R*)-(Phenylisopropyl)adenosine (*R*-PIA) was used as A1 ligand, and [^{3}H]-5′-(*N*-ethylcarboxamido)adenosine (NECA) as A2a ligand in the presence of 50 nM N^6-cyclopentyladenosine, the latter to block A1 receptors present in the striatal tissue.

Results and Discussion

A set of 22 9-deazaxanthines (pyrrolo[3,2-*d*]pyrimidine-2,4-diones) and three 7-deazaxanthines (pyrrolo[2,3-*d*]-pyrimidine-2,4-diones) was synthesized and investigated in A1 and A2 adenosine receptor binding assays at rat brain membranes. The AR affinities of the deazaxanthines were compared with those of corresponding xanthines where possible. In order to facilitate this comparison, the xanthine numbering system is used for all compounds (see Figure 1).

All 9-deaza- and 7-deazaxanthines investigated exhibited specific binding to ARs. 9-Deazaxanthines showed high affinity to A1- and A2ARs (see Table 2). 1-Methyl-9-deazaxanthine (**7a**) was a potent A1AR ligand (K_i = 2.9 μM) with 11-fold selectivity for that receptor subtype. 1-Propyl-9-deazaxanthine was about 2-fold more potent at both receptors. The introduction of a methyl or a propyl

Table 2. Adenosine Receptor Affinities of Deazaxanthine and Corresponding Xanthine Derivatives



9-deazaxanthines 7-deazaxanthines xanthines

compd	R_1	R_3	R_7/R_9	R_8	$K_i \pm$ SEM (μM) A1 receptors vs [^{3}H]-*R*-PIA rat brain cortex	$K_i \pm$ SEM (μM) A2 receptors vs [^{3}H]NECA rat striatum	A1 selectivity A2/A1
				9-Deazaxanthine			
7a	methyl	H	H	H	2.9 ± 0.5	34 ± 13	12
8a	methyl	methyl	H	H	5.4 ± 0.4	12 ± 4	2
10a	methyl	methyl	methyl	H	32 ± 5	72 ± 6	2
7b	propyl	H	H	H	1.7 ± 0.12	18 ± 0.1	11
8b	propyl	propyl	H	H	0.44 ± 0.06	5.1 ± 0.4	12
9	propyl	propyl	H	$N(CH_3)_2$	0.13 ± 0.02	16 ± 0.36	123
10b	propyl	propyl	methyl	H	2.3 ± 0.2	13 ± 2	6
13a	methyl	H	H	phenyl	0.097 ± 0.001	2.0 ± 1.4	21
14a	methyl	methyl	H	phenyl	0.047 ± 0.03	0.51 ± 0.04	11
15a	methyl	methyl	methyl	phenyl	3.6 ± 0.1	14 ± 3	4
13b	propyl	H	H	phenyl	0.039 ± 0.020	1.2 ± 0.6	31
14b	propyl	propyl	H	phenyl	0.013 ± 0.003	0.45 ± 0.06	35
15b	propyl	propyl	methyl	phenyl	0.11 ± 0.01	5.2 ± 0.5	47
18c	methyl	methyl	OH	(*E*)-styryl	0.56 ± 0.05	1.8 ± 0.3	3
18d	methyl	methyl	OH	1-naphthyl	>30 (47%)[a]	>100 (38%)[a]	
18e	methyl	methyl	OH	2-naphthyl	0.22 ± 0.07	1.0 ± 0.1	5
19c	methyl	methyl	H	(*E*)-styryl	0.165 ± 0.010	0.64 ± 0.12	4
19d	methyl	methyl	H	1-naphthyl	>30 (33%)[a]	>30 (1.2%)[a]	
19e	methyl	methyl	H	2-naphthyl	0.026 ± 0.009	>0.5 (34%)[a]	>19
20c	methyl	methyl	methyl	(*E*)-styryl	2.6 ± 0.3	0.26 ± 0.08	0.1
20d	methyl	methyl	methyl	1-naphthyl	9.2 ± 7.9	>30 (22%)[a]	>3
20e	methyl	methyl	methyl	2-naphthyl	0.27 ± 0.09	1.1 ± 0	4
				7-Deazaxanthine			
21	methyl	methyl	H	H	43 ± 1	>250 (39%)[a]	6
22	methyl	methyl	H	phenyl	3.1 ± 0.4	12 ± 2	4
23	methyl	methyl	methyl	phenyl	11 ± 5	40 ± 0	4

		R_1	R_3	R_7	R_8	A1	A2	A2/A1
					Xanthine (X)			
24	1-methylX	methyl	H	H	H	36[b]	47[b]	1
25	theophylline	methyl	methyl	H	H	14 ± 1 (14[c])	19 ± 2 (25[c])	1
26	caffeine	methyl	methyl	methyl	H	41 ± 6 (44[c])	43 ± 9 (45[c])	1
27	1-propylX	propyl	H	H	H	13[b]	33[b]	3
28	1,3-dipropylX	propyl	propyl	H	H	0.7[c]	6.6[c]	9
29	7-methyl-1,3-dipropylX	propyl	propyl	methyl	H	7.0[d]	10[d]	1
30	1-methyl-8-phenylX	methyl	H	H	phenyl	0.30[b]	2.2[b]	7
31	8-phenyltheophylline	methyl	methyl	H	phenyl	0.089 ± 0.004 (0.086[c])	0.83 ± 0.12 (0.85[c])	9
32	8-phenylcaffeine	methyl	methyl	methyl	phenyl	15[c]	25[c]	2
33	8-phenyl-1-propylX	propyl	H	H	phenyl	0.067[b]	1.9[b]	28
34	8-phenyl-1,3-dipropylX	propyl	propyl	H	phenyl	0.010[c]	0.18[c]	18
35	7-methyl-8-phenyl-1,3-dipropylX	propyl	propyl	methyl	phenyl	2.3[c]	15[c]	7
36	1,3-dimethyl-8-styrylX	methyl	methyl	H	(E)-styryl	0.65[e]	0.29[e]	0.4
37	1,3,7-trimethyl-8-styrylX	methyl	methyl	methyl	(E)-styryl	3.9[e]	0.094[e]	0.02

[a] Percent inhibition of radioligand binding at the indicated concentration; compounds are insoluble in the assay buffer at higher concentrations. [b] Müller et al., 1993.[10] [c] Daly, 1991.[26] [d] Daly et al., 1991.[27] [e] Jacobson et al., 1993.[10] [^{3}H]CGS 21680 was used as A2 radioligand in that study. [^{3}H]NECA binding data have been reported to be consonant with [^{3}H]CGS 21680 binding data.[28]

group in the 3-position of 9-deazaxanthines resulted in an increase in A1- and A2AR affinity, with the exception of 9-deazatheophylline **8a**, which was less potent compared to **7a** at the A1AR. Methylation in the 7-position resulted in a decrease in A1- and A2AR affinity of 9-deazaxanthines, except for 8-styryl and 8-(2-naphthyl) compounds, which exhibited a decrease in A1 affinity, but an increase in A2AR affinity. Thus, compound **20c** is A2-selective (10-fold). Replacement of the N7 hydrogen by a hydroxyl group, which could also function as a hydrogen donor, resulted in decreased A1 and A2AR affinity.

Substitution of 9-deazaxanthines with a phenyl group in the 8-position drastically increases AR affinity, particularly at the A1AR. An (*E*)-styryl substituent in the 8-position leads to an increase in A1- and A2AR affinity. Naphthyl substituents were introduced in the 8-position of 9-deazaxanthines, since the 2-naphthyl derivatives can be envisaged as ring-constrained styryl analogs. 8-(1-Naphthyl)-9-deazaxanthines (**19d, 20d**) exhibited only very low affinity for A1- and A2ARs. In contrast, 8-(2-naphthyl)-9-deazaxanthines (**19e, 20e**) were potent AR ligands. The theophylline analog **19e** was a very potent ligand at A1ARs (K_i = 26 nM) with selectivity for that receptor subtype. The 8-(2-naphthyl)-9-deazacaffeine analog **20e** was synthesized because of its similarity to the 8-styrylcaffeines, which are A2-selective. The 2-naphthyl group in the 8-position, however, led to an increase in A1 affinity and a decrease in A2 affinity, compared to the

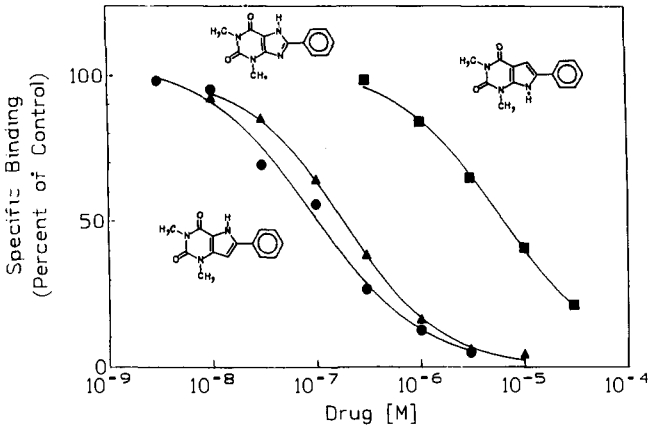


Figure 2. A1 adenosine receptor binding curves of (▲)8-phenyltheophylline (31), (●) 8-phenyl-9-deazatheophylline (14a), and (■) 8-phenyl-7-deazatheophylline (22). Data points are means of at least three different experiments.

8-styryl residue, resulting in A1-selective compounds. Substitution of 1,3-dipropyl-9-deazaxanthine (8b) with a dimethylamino group in the 8-position resulted in a 3-fold increase in A1AR affinity but a 3-fold decrease in A2AR affinity producing a potent highly A1-selective (123-fold) compound (9).

The structure–activity relationships of 9-deazaxanthines in most cases paralleled those of xanthine derivatives. Thus, we conclude that 9-deazaxanthines bind to adenosine receptors in the same binding mode as the corresponding xanthines.

The affinities of 9-deazaxanthines to A1- and A2ARs were directly compared with those of analogous xanthines in some cases. As a comparison, binding data of xanthines obtained in our laboratory (compounds 25, 26, and 31), or published data from the laboratory of Dr. John W. Daly, NIDDK, NIH, were used (see Table 2). Our assay procedures were essentially the same as those used by Daly *et al.*,[8,26,27] and we obtained virtually identical results for three standard xanthines (25, 26, and 31).

At the A2AR, 9-deazaxanthines were about equipotent to their 9-nitrogen-containing counterparts. At A1ARs, however, 9-deazaxanthines were in most cases significantly more potent compared to xanthines. Generally, the potency of 9-deazaxanthines was about 2–3-fold higher than that of xanthines. Remarkable exceptions to this rule were the 1-monosubstituted xanthines, 1-methylxanthine (24) and 1-propylxanthine (27). The corresponding 9-deaza analogs 7a and 8a were 12-fold or 8-fold, respectively, more potent at the A1AR. Other compounds which did not follow the above rule were the 1,3-dipropyl-8-phenylxanthine analogs. While the 9-deaza analog of 1,3-dipropyl-8-phenylxanthine (14b) was about equipotent to the xanthine 34, the respective 7-methyl derivatives differed greatly in A1AR affinity. The 9-deaza compound 15b exhibited 21-fold higher A1AR affinity compared to the xanthine 35.

The higher potency of 9-deazaxanthines compared to xanthines at A1ARs results in an increased A1 selectivity of these compounds.

Three 7-deazaxanthines were synthesized, and their AR affinities were compared with those of xanthines and 9-deazaxanthines. All 7-deazaxanthines were several-fold less potent compared to the 9-deaza isomers at both receptor subtypes. The 7-deazatheophylline derivatives (21 and 22) were also considerably less potent than the analogous xanthines at both receptors. The 7-deaza analog

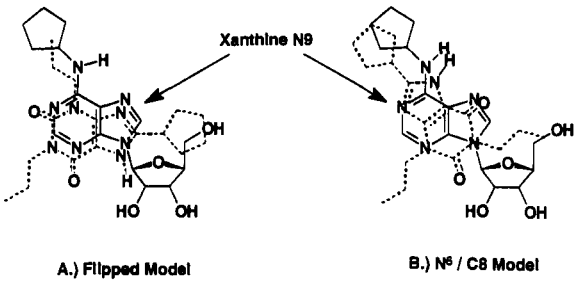


Figure 3. Binding models for the adenosine receptor antagonist 1,3-dipropyl-8-cyclopentylxanthine (dashed line) with respect to the agonist N^6-cyclopentyladenosine (solid line): (A) flipped model, (B) N^6/C8 model.

of the very potent A1-selective AR antagonist 8-phenyltheophylline was 35-fold less potent than the parent compound and 66-fold less potent than the 9-deaza isomer at the A1AR (compare 22, 31, and 14a; see Figure 2). Due to the lower affinity of the compounds at the A2AR, the differences were somewhat lower in a comparison of A2AR affinities of xanthines and deazaxanthines. 7-Deaza-8-phenylcaffeine (23) exhibited similar A1- and A2AR affinity as the corresponding xanthine (32). Both compounds are lacking the N7 hydrogen for hydrogen bonding to the receptors and thus show relatively low receptor affinity.

In earlier studies, a range of 7-deazaxanthines and few 9-deazaxanthines had been investigated *in vivo* for their diuretic, cardiac stimulatory, and CNS-stimulatory activities and compared to the activities of corresponding xanthines.[20,29] These xanthine effects are thought to be mainly due to adenosine receptor antagonism, but other mechanisms of action could also play a role.[30] *In vivo*, 7-deaza- and 9-deazatheophylline and -caffeine were less potent than the analogous xanthines.[20,29] The *in vivo* results obtained by Senda and Hirota[20,29] do not correlate with the AR affinities of the 7- and 9-deazaxanthines and cannot be explained at present.

Cristalli *et al.*[31] had synthesized adenosine analogs which were lacking a nitrogen atom in the 1-, 3-, or 7-position. These deazaadenosines were investigated in binding and functional assays at A1- and A2ARs. The result was that substitution of any one of the nitrogen atoms for CH in the 1-, 3-, or 7-deazaadenosines caused a decrease or loss of affinity for both receptor subtypes. The most potent deazaadenosines, however, were the 1-deazaadenosines which were subsequently investigated in more detail.[32] All 1-deazaadenosines exhibited somewhat lower affinity to A1- and A2ARs compared to the corresponding adenosines. The majority of 1-deazaadenosines, however, showed higher A1-selectivity compared to adenosines.[32]

In a computer model published by Peet *et al.*,[13] the so-called N^6-C8 model, the authors describe a binding mode for xanthines with respect to adenosine, in which the N^6-substituent of adenosine and the 8-substituent of xanthines are overlapping (see Figure 3). As a consequence, the N1 nitrogen of adenosines and the N9 nitrogen of xanthines coincide. The findings that the N1 of adenosines and the N9 of xanthines are both not critical for high affinity binding of the compounds to ARs appears to support this binding model. The fact that both 1-deazaadenosines and 9-deazaxanthines are more A1-selective than the parent N-containing compounds is another result in favor of the N^6-C8 model. The improved potency of 9-deazaxanthines compared to xanthines and the decreased activity of 1-deazaadenosines compared to adenosines, however,

indicates differences in the significance of the concerning nitrogen atoms for binding to the ARs.

In a different model, it was postulated that adenosines and xanthines bind to ARs in a so-called flipped orientation with respect to each other (see Figure 3).[12] This would mean that the N7 nitrogen of adenosines coincides with the N9 nitrogen of xanthines. The N7 of adenosines, however, is critical for binding of adenosine to the receptors. 7-Deazaadenosine showed an IC_{50} in binding to the A1AR of rat cortical membranes of greater than 100 μM.[31] The compound was nearly inactive in functional assays at human platelet A2a[31] and human fibroblast A2bARs.[33] The N7 nitrogen of adenosine thus appears to be an important hydrogen-bond acceptor in AR binding. According to our experimental results, the lacking of the N9 of xanthines does not decrease AR affinity of the compounds. Therefore, it appears unlikely that nitrogen atoms N7 of adenosines and N9 of xanthines are binding to the same receptor sites.

Another class of deaza compounds that was investigated at ARs are the 7-deazaadenines.[34] In contrast to the 7-deazaadenosines that are lacking AR affinity, 7-deazaadenines have been shown to exhibit high affinity and selectivity for A1ARs. They belong to the most potent not-xanthine AR antagonists. It is assumed that their binding mode to the A1AR differs from the binding mode of adenosines.[34]

Xanthines that are unsubstituted in the 7-position can exist in the N7-H or the N9-H tautomeric form. For molecular modelling studies of ARs and their ligands it is important to known which tautomer is the active one that binds to the receptors. Most AR modeling studies conducted so far used the N7-H tautomers of xanthines without discussing the matter of tautomerization. One study considered the N9-H tautomer of xanthines.[14]

In crystals as well as in aqueous solution, the N7-H tautomeric form of xanthines predominates or is found exclusively.[35,36] Molecular calculations of the two tautomeric forms of theophylline using the semiempirical AM1 method resulted in an energetic difference of about 15 kJ/mol, the N7-H tautomer being more stable. In a thermodynamical equilibrium the low-energy tautomer should be predominant, as was already demonstrated.[35,36] However, the difference in energy is small. It is possible that the receptor induces a change of the tautomeric form normally present, if the association between receptor and ligand were better for the high-energy tautomer. The ligands with the highest affinities for ARs are those with a substituent, such as a phenyl group, in the 8-position. Therefore, we also did molecular calculations on 8-phenylxanthines in order to investigate if 8-substitution changes the tautomeric proportion. Again, the difference in energy between the two forms proved to be approximately 15 kJ/mol with the N7-H tautomeric form being the energetically favored one. From these data, it cannot be decided which tautomer is the one that binds to the ARs.

The investigation of 7-deaza- and 9-deazaxanthines at ARs contributes to the question of which tautomeric form actually binds to the receptors. In 7- and 9-deazaxanthines tautomerization is no longer possible. 7-Deazaxanthines can be envisaged as fixed N9-H tautomers, while 9-deazaxanthines can be seen as fixed N7-H tautomers. The affinities of a range of xanthines and 9-deazaxanthines are similar and 9-deazaxanthines exhibit much higher affinity for ARs compared to 7-deazaxanthines. We therefore conclude that xanthines bind to ARs in the N7-H tautomeric form.

Experimental Section

Synthesis. Melting points were measured with a Büchi 510 apparatus and are uncorrected. NMR spectra were run on a Bruker WP 80 spectrometer or a Bruker AC-250 spectrometer; DMSO-d_6 was used as solvent, unless otherwise noted. In some cases it was necessary to add a few drops of an aqueous solution of NaOD (40%) in order to get the compounds into solution in DMSO-d_6. ^{13}C-NMR data are given in Table 1 or are available as supplementary material. All compounds were checked for purity by TLC on 0.2-mm aluminum sheets with silica gel 60 F_{254} (Merck); as eluent CH_2Cl_2–MeOH, 9:1 (E I) or 99:1 (E II) or ethyl acetate (E III) was used. Elemental analyses were performed by the Institute of Chemistry, University of Tübingen. Intermediates were used without further purification. The xanthine derivatives **25**, **26**, and 31 were obtained from commercial sources.

1,3-Dimethylpyrrolo[2,3-*d*]pyrimidine-2,4-dione (21) was synthesized as described;[16] ^{1}H-NMR δ (ppm) 3.20 (s, 3H, N3-CH_3), 3.42 (s, 3H, N1-CH_3), 6.35, 6.77 (2 m, 2 × 1H, C5-H, C6-H), 11.70 (br s, 1H, N7-H). Anal. ($C_8H_9N_3O_2$) C, H, N.

1,3-Dimethyl-6-phenylpyrrolo[2,3-*d*]pyrimidine (22) was synthesized as described;[17] ^{1}H-NMR δ (ppm) 3.23 (s, 3H, N3-CH_3), 3.54 (s, 3H, N1-CH_3), 6.84 (s, 1H, C7-H), 7.21–7.77 (m, 5H, arom), 11.57 (br s, 1H, N7-H). Anal. ($C_{14}H_{13}N_3O_2$) C: calcd, 65.9; found, 65.3, H, N.

3,6-Dimethyluracil (1a). **1a** was synthesized as described:[18] yield 50%; mp 259 °C (lit.[18] mp 262 °C); ^{1}H-NMR δ 2.02 (s, 3H, C6-CH_3), 3.08 (s, 3H, N3-CH_3), 5.44 (s, 1H, C5-H), 11.10 (br s, 1H, N1-H).

6-Methyl-3-propyluracil (1b). **1b** was synthesized in analogy to **1a**. The reaction mixture consisted of 26 g (0.23 mol) of ethyl acetoacetate, 20.4 g (0.2 mol) of *N*-propylurea, 10 mL of EtOH, and 8 drops of concentrated HCl. The residue was dissolved in an aqueous solution of 15 g of NaOH in 70 mL of H_2O and stirred until it became white and slimy. Acidifing the solution with concd HCl and cooling to 8 °C afforded a white precipitate: yield 45%; mp 184 °C (lit.[37] mp 184 °C); ^{1}H-NMR δ 0.82 (t, 3H, N3-$CH_2CH_2CH_3$), 1.48 (sext, 2H, N3-$CH_2CH_2CH_3$), 2.01 (s, 3H, C6-CH_3), 3.67 (t, 2H, N3-CH_2), 5.43 (s, 1H, C5-H), 11.07 (br s, 1H, N1-H).

1,3,6-Trimethyluracil (2a). To a suspension of 10 g (0.07 mol) of **1a** in 120 mL of a 15% aqueous NaOH was added 43.7 mL (0.7 mol) of methyl iodide dropwise. The mixture was refluxed for 40 h, concentrated *in vacuo*, and extracted with CH_2Cl_2. The organic phase was dried and evaporated *in vacuo*: yield 65%; mp 112 °C (lit.[38] mp 112 °C); ^{1}H-NMR δ 2.22 (s, 3H, C6-CH_3), 3.13 (s, 3H, N3-CH_3), 3.30 (s, 3H, N1-CH_3), 5.61 (s, 1H, C5-H).

6-Methyl-1,3-dipropyluracil (2b). To a suspension of 10 g (0.06 mol) of **1b** in a mixture of 15% aqueous NaOH (150 mL) and ethanol (150 mL) was added dropwise 127.5 mL (1.4 mol) of propyl bromide. The mixture was refluxed for 13 h, and the product was isolated as described for **2a**: yield 74%; mp 62 °C; ^{1}H-NMR δ 0.85 (m, 6H, 2 $NCH_2CH_2CH_3$), 1.52 (m, 4H, 2 $NCH_2CH_2CH_3$), 2.25 (s, 3H, C6-CH_3), 3.72 (m, 4H, 2 N-CH_2), 5.90 (s, 1H, C5-H).

3,6-Dimethyl-5-nitrouracil (3a), 6-Methyl-5-nitro-3-propyluracil (3b), 1,3,6-Trimethyl-5-nitrouracil (4a), and 6-Methyl-5-nitro-1,3-dipropyluracil (4b). General procedure: a mixture of a given volume of concentrated H_2SO_4 and 20 mL of fuming HNO_3 was cooled to 0–5 °C, and then 0.065 mol of the appropriate 5-unsubstituted uracil (**1a,b**, **2a,b**) was gradually added and, if necessary, the reaction mixture was stirred for a certain time (see Table 3). Then it was carefully poured on ice. The precipitate was collected by filtration and washed with H_2O. The dipropyl derivative **4b** separated as an oil and was purified over a silica gel column, using CH_2Cl_2–MeOH, 9:1, as an eluent. Reaction conditions, yields, and analytical data are given in Table 3.

3-Methyl-6-[2-(dimethylamino)vinyl]-5-nitrouracil (5a), 3-Propyl-6-[2-(dimethylamino)vinyl]-5-nitrouracil (5b), 1,3-Dimethyl-6-[2-(dimethylamino)vinyl]-5-nitrouracil (6a), and 1,3-Dipropyl-6-[2-(dimethylamino)vinyl]-5-nitrouracil (6b). General procedure: To a suspension or solution of 0.01 mol of

Table 3. Reaction Conditions, Yields, and Analytical Data of 5-Nitrouracils

compd	concd H_2SO_4 (mL)	reaction time[a]	yield (%)	mp (°C)	^{1}H-NMR spectral data: chemical shifts (δ) in DMSO-d_6 (ppm) N1 R	N3 R	C6-CH_3
3a	30	20 min	68	230	12.10	3.13	2.29
3b	40	30 min	78	185	12.09	0.85, 1.50, 3.72	2.29
4a	20	-	79	153[b]	3.40	3.21	2.38
4b	30	1 h	49	61	0.88, 1.59, 3.80	0.85, 1.59, 3.78	2.39

[a] After addition of uracil to reaction mixture is completed. [b] Literature mp 150 °C.

Table 4. Yields and Analytical Data of 6-Substituted Uracils

compd	mp (°C)	yield (%)	^{1}H-NMR spectral data: chemical shifts (δ) in DMSO-d_6 (ppm) N1 R	N3 R	C6 C*H*=C*H* 2 d (J, Hz)	C6 CH=CH*R*
5a	260 dec	90	10.94	3.14[a]	5.27 (13), 8.08 (12.9)	2.87, 3.10[a] (6H) ($N(CH_3)_2$)
5b	237	84	10.99	0.85, 1.52, 3.69	5.26 (12.9), 8.08 (12.9)	3.35 (6H) ($N(CH_3)_2$)
6a	182	66	3.40	3.16	4.79 (12.8), 7.06 (12.8)	2.89 (6H) ($N(CH_3)_2$)
6b	92	55	0.95, 1.64, 3.88	0.91, 1.69, 3.85	4.45 (12.9), 6.88 (12.9)	2.94 (6H) ($N(CH_3)_2$)
11a	>300	68	11.97	3.18	6.99 (16), 7.78 (16)	7.44–7.67 ($5H_{arom}$)
11b	278	74	11.96	0.87, 1.57, 3.77	6.98 (16), 7.78 (16)	7.45–7.67 ($5H_{arom}$)
12a	185[b]	66	3.38	3.24	6.98 (16.5), 7.19 (16.5)	7.45–7.64 ($5H_{arom}$)
12b	125	74	0.88, 1.57, 3.85	0.85, 1.57, 3.81	7.02 (16.4), 7.21 (16.4)	7.42–7.65 ($5H_{arom}$)

			N1 CH_3	N3 CH_3	$C_6CH_2CH_2$		$C_6CH_2CH_2R$
17c	126	55	3.56	3.39	2.63	2.82	6.16 (dt, 1H, J = 15.7 Hz, J' = 6.9 Hz, CH), 6.50 (d, 1H, J = 15.7 Hz, CH), 7.28 ($5H_{arom}$)
17d	210[c]	80	3.51	3.24	3.07	3.44	7.46–7.62 ($4H_{arom}$), 7.83–8.04 ($3H_{arom}$)
17e	174	83	3.51	3.39	2.93	3.12	7.29–7.89 ($6H_{arom}$), 7.64 (C1″-H_{arom})

[a] Assignments are arbitrary. [b] Literature mp 190–192 °C.[19] [c] Literature mp 235 °C.[25]

Table 5. Synthesis and Analytical Data of 6-Unsubstituted Pyrrolo[3,2-*d*]pyrimidine-2,4-diones

compd	starting compound (mmol)	eluent for purification	mp (°C)	yield (%)	formula	analyses	^{1}H-NMR spectral data: chemical shifts (δ) in DMSO-d_6 (ppm) N1 R	N3 R	N5 H	C7 H d (J, Hz)	C6 R d (J, Hz)
7a	5a (4.2)	E I	>300	21	$C_7H_7N_3O_2$	C, H, N	11.11	3.19	11.88	5.86 (2.9)	7.15 (2.9)
7b	5b (3.7)	E III	>300	14	$C_9H_{11}N_3O_2$	C, H, N	11.04	0.85, 1.50, 4.79	11.87	5.83 (2.7)	7.14 (2.7)
8a	6a (6.5)	E I	215[a]	36	$C_8H_9N_3O_2$	C, H; N[b]	3.38	3.24	12.09	6.17 (2.8)	7.25 (2.8)
8b[c]	6b (3.2)	E III	107	16	$C_{12}H_{17}N_3O_2$	C, H, N	0.88, 1.64, 3.84	0.85, 1.58, 3.83	12.04	6.18 (2.8)	7.24 (2.8)
9[d]	6b (3.2)	E III	220	22	$C_{14}H_{22}N_4O_2$	C, H, N	0.86, 1.60, 3.82	0.84, 1.53, 3.75	10.81	5.29 (2.2)	2.87 ($N(CH_3)_2$)

[a] Literature[20] mp 212 °C. [b] N: calcd, 23.5; found, 22.1. [c] R_f = 0.83. [d] R_f = 0.70.

the appropriate 5-nitrouracil (**3a,b**, **4a,b**) in 5 mL of dry DMF was added 2.6 mL (0.015 mol) of DMF–diethyl acetal, and the mixture was stirred at room temperature for 2 h. Then, diethyl ether was added to the reaction mixture and the precipitate was collected by filtration and washed with diethyl ether. The dipropyl derivative **6b** separated as an oil and was purified over a silica gel column with CH_2Cl_2–MeOH, 99:1, as an eluent. Yields and analytical data of **3a,b** and **4a,b** are given in Table 4.

3-Methylpyrrolo[3,2-*d*]pyrimidine-2,4-dione (7a), 3-Propylpyrrolo[3,2-*d*]pyrimidine-2,4-dione (7b), 1,3-Dimethylpyrrolo[3,2-*d*]pyrimidine-2,4-dione (8a), 1,3-Dipropylpyrrolo[3,2-*d*]pyrimidine-2,4-dione (8b), and 1,3-Dipropyl-6-(dimethylamino)pyrrolo[3,2-*d*]pyrimidine-2,4-dione (9). General procedure: To a suspension of 1.0 g of the appropriate 6-[2-(dimethylamino)vinyl]-5-nitrouracil in 20 mL of MeOH was added 0.5 g of Pd–C (10%) under an argon atmosphere. The mixture was stirred under a hydrogen atmosphere and normal air pressure at room temperature for 2 h. It was filtered over kieselgur, the kieselgur layer was thoroughly washed with MeOH, the filtrate was evaporated, and the product was purified by column chromatography. For filtration of the slightly soluble products **7a,b** and **8a**, those were transformed to their sodium salts by treating with 15% aqueous NaOH. After filtration the solution was acidified with concentrated HCl to obtain the free acids. Molar equivalents, eluents for column chromatography, yields, and analytical data are given in Table 5.

3-Methyl-5-nitro-6-styryluracil (11a), 5-Nitro-3-propyl-6-styryluracil (11b), 1,3-Dimethyl-5-nitro-6-styryluracil (12a), and 5-Nitro-1,3-dipropyl-6-styryluracil (12b). General procedure: A mixture of 0.01 mol of the appropriate 5-nitrouracil (**3a,b** and **4a,b**), 1.0 g (0.01 mol) of benzaldehyde, or 2.0 g (0.02 mol) of benzaldehdye for the preparation of **11b**, respectively, and 1 mL of piperidine in 45 mL of ethanol was refluxed for 3–5 h. The reaction mixture was concentrated *in vacuo*, and the precipitate was collected by filtration and washed with ethanol. Compounds **11a** and **11b** were obtained as piperidinium salts. Neutralization with HOAc afforded the free acids which were washed with water and subsequently with ethanol. Yields and analytical data are given in Table 4.

3-Methyl-6-phenylpyrrolo[3,2-*d*]pyrimidine-2,4-dione (13a), 1,3-Dimethyl-6-phenylpyrrolo[3,2-*d*]pyrimidine-2,4-dione (14a), and 6-Phenyl-1,3-dipropylpyrrolo[3,2-*d*]pyrimidine-2,4-dione (14b). General procedure: A suspension of 7.0 mmol of the appropriate 5-nitro-6-styryluracil (**11a, 12a,b**) in triethyl phosphite (7 mL) was refluxed for 5 h under an argon atmosphere. The mixture was allowed to stand at room temperature overnight, and the precipitate was collected by filtration, washed with EtOH, and recrystallized from MeOH–H_2O. The precipitate of **14b** was soluble in EtOH and was therefore washed with diethyl ether. Yields and analytical data are given in Table 6.

6-Phenyl-3-propylpyrrolo[3,2-*d*]pyrimidine-2,4-dione (13b). A mixture of 1.37 g (5.0 mmol) of **11b** and 4.35 g (25 mmol) of sodium dithionite in formic acid (50 mL) was refluxed for 10 h. The reaction mixture was evaporated, and the residue was suspended in boiling H_2O. The H_2O-insoluble product was filtered off, washed with water, and recrystallized from MeOH. Yield and analytical data are given in Table 6.

1,3,6-Trimethyl-5-nitrouracil Monosodium Salt (16). To a suspension of 6.0 g (0.025 mol) of **3a** in dry MeOH was dropwise added 4.7 mL (0.025 mol) of $NaOCH_3$ (30%) in MeOH, and the mixture was refluxed for 2–3 h. After cooling the precipitate was filtered off and washed with dry methanol: yield 76%; mp 250 °C; ^{1}H-NMR δ 3.05 (s, 3H, N3-CH_3), 3.13 (s, 3H, N1-CH_3), 4.35, 5.65 (2 s, 2H, C6=CH_2).

1,3-Dimethyl-6-(4-phenyl-3-butenyl)-5-nitrouracil (17c), 1,3-Dimethyl-6-[2-(1-naphthyl)ethyl]-5-nitrouracil (17d), and 1,3-Dimethyl-6-[2-(2-naphthyl)ethyl]-5-nitrouracil (17e). General procedure: A suspension of **16** (1.1 g, 5.0 mmol), KI (0.3

Table 6. Yields and Analytical Data of 6-Substituted Pyrrolo[3,2-*d*]pyrimidine-2,4-diones

					^{1}H-NMR spectral data: chemical shifts (δ) in DMSO-d_6 (ppm)				
compd	mp (°C)	yield (%)	formula	analyses	N1 R	N3 R	N5 H/OH	C7 H	C6 R
13a	>300	6	$C_{13}H_{11}N_3O_2$	C, H, N	11.18	3.23	12.22	6.31	7.37, 7.87 (5H$_{arom}$)
13b	>300	21	$C_{15}H_{15}N_3O_2$	C, H, N	11.16	0.87, 1.56, 3.82	12.22	6.30	7.29–7.44, 7.88 (5H$_{arom}$)
14a	>300[a]	16	$C_{14}H_{13}N_3O_2$	C, H, N	3.47	3.26	12.41	6.73	7.34–7.47, 7.91 (5H$_{arom}$)
14b	170	11	$C_{18}H_{21}N_3O_2$	C, H, N	0.92, 1.68, 3.88	0.87, 1.63, 3.88	12.35	6.76	7.31–7.54 (5H$_{arom}$)
18c	266	67	$C_{16}H_{15}N_3O_3$	C, H, N	3.36	3.22	11.88	6.44	7.15 (CH, J = 16 Hz), 7.28–7.60 (6H)
18d[b]	250[c]	49	$C_{18}H_{15}N_3O_3$	C, H; N[d]	3.50	3.24		5.65	7.45–8.02 (6H$_{arom}$), 8.41 (C1′-H)
18e	279	63	$C_{18}H_{15}N_3O_3$	C, H, N	3.40	3.26	12.01	6.53	7.54–8.02 (6H$_{arom}$), 8.41 (C1′-H)
19c[b]	>290	63	$C_{16}H_{15}N_3O_2$	C, H, N	3.32	3.22	12.13[e]	6.05	6.88 (CH, J = 16.4 Hz), 7.08–7.44 (6H)
19d[b]	>300[f]	61	$C_{18}H_{15}N_3O_2$	C, H; N[g]	3.42	3.27		6.35	7.50–7.97 (7H$_{arom}$)
19e[b]	>280	31	$C_{18}H_{15}N_3O_2$	C, H, N	3.41	3.25		6.60	7.41–8.11 (6H$_{arom}$), 8.36 (C1′-H)

[a] Literature[21] mp >300 °C. [b] In DMSO-d_6/NaOD. [c] Literature[25] mp 230–235 °C. [d] N: calcd, 13.1; found, 12.0. [e] In DMSO-d_6. [f] Literature[25] mp >300 °C. [g] N: calcd, 13.8; found, 13.2.

Table 7. Synthesis and Analytical Data of N5/N7-Methylated Pyrrolo[3,2-*d*]- and -[2,3-*d*]pyrimidine-2,4-diones

								^{1}H-NMR spectral data: chemical shifts (δ) in DMSO-d_6 (ppm)				
compd	reaction time (h)	reaction temp (°C)	purification method	mp (°C)	yield (%)	formula	analyses	N1 R	N3 R	N5 CH_3	C7 H	C6 R
10a	4	50	PI	165[a]	40	$C_8H_{11}N_3O_2$	C, H, N	3.44	3.39	3.98	5.86	6.85 (d, J = 2.8 Hz)
10b	3	rt	PI	84	81	$C_{13}H_{19}N_3O_2$	C, H, N	0.92, 1.75, 3.98	0.92, 1.75, 3.83	3.98	5.84	6.83 (d, J = 2.9 Hz)
15a	4	50	PI	190	86	$C_{15}H_{15}N_3O_2$	C, H, N	3.38	3.23	3.89	6.34	7.50 (5H$_{arom}$)
15b	3	rt	PI	116	96	$C_{19}H_{23}N_3O_2$	C, H, N	0.97, 1.74, 3.99	0.97, 1.74, 3.88	3.96	5.94	7.45 (5H$_{arom}$)
20c[b]	3	70	P II	138	60	$C_{17}H_{17}N_3O_2$	C, H, N	3.47	3.40	4.17	6.17	6.98 (d, J = 16.2 Hz) 7.13 (d, J = 16.2 Hz) 7.33 (2H), 7.51 (3H)
20d	3	70	P II	159	74	$C_{19}H_{17}N_3O_2$	H; C,[c] N[d]	3.42	3.28	3.60	6.39	7.56–8.12 (7H)
20e[b]	3	70	P II	185	80	$C_{19}H_{17}N_3O_2$	C, H, N	3.50	3.44	4.02	6.06	7.50–7.88 (6H) 7.95 (C1′-H)
23	4	50	P I	170	56	$C_{15}H_{15}N_3O_2$	C, H, N	3.73[e]	3.24	3.75[e,f]	6.05[g]	7.44 (5H$_{arom}$)

[a] Literature[20] mp 170 °C. [b] In $CDCl_3$. [c] C: calcd, 71.5; found, 69.3. [d] N: calcd, 13.2; found, 12.0. [e] Assignments are arbitrary. [f] N7 CH_3. [g] C5 H.

g, 1.8 mmol), and the appropriate aryl halogenide (6.0 mmol; (*E*)-3-bromo-1-phenyl-1-propene for the synthesis of **17c**, 1-(bromomethyl)naphthalene for the synthesis of **17d**, and 2-(bromomethyl)naphthalene for the synthesis of **17e**) in dry DMF (10 mL) was heated at 80 °C under an argon atmosphere for 2 h. To the reaction mixture was added MeOH (30 mL), and the yellow precipitate was collected by filtration and washed with MeOH. Yields and analytical data are given in Table 4.

5-Hydroxy-1,3-dimethyl-6-styrylpyrrolo[3,2-*d*]pyrimidine-2,4-dione (18c), 5-Hydroxy-1,3-dimethyl-6-(1-naphthyl)pyrrolo[3,2-*d*]pyrimidine-2,4-dione (18d), and 5-Hydroxy-1,3-dimethyl-6-(2-naphthyl)pyrrolo[3,2-*d*]pyrimidine-2,4-dione (18e). General procedure: To a solution of potassium hydroxide (0.123 g, 2.2 mmol) in ethanol (30 mL) was added 2.0 mmol of the appropriate 6-aralkyluracil, and the mixture was refluxed for 15 min. The precipitate was collected by filtration and washed with MeOH. The residue was neutralized with HOAc, and the gelatinous precipitate was filtered off and washed with water and subsequently with MeOH. Yields and analytical data are given in Table 6.

1,3-Dimethyl-6-styrylpyrrolo[3,2-*d*]pyrimidine-2,4-dione (19c), 1,3-Dimethyl-6-(1-naphthyl)pyrrolo[3,2-*d*]pyrimidine-2,4-dione (19d), and 1,3-Dimethyl-6-(2-naphthyl)-pyrrolo[3,2-*d*]pyrimidine-2,4-dione (19e). General procedure: a solution or suspension of 2.5 mmol of the appropriate 5-hydroxypyrrolo[3,2-*d*]pyrimidine in DMF (12 mL) was prepared and heated under reflux for 1 h. After cooling, the precipitate was collected by filtration and washed with MeOH. Purification was achieved by dissolution in DMSO/NaOH 15% and precipitation by HCl. The product was thoroughly washed with MeOH. Yields and analytical data are given in Table 6.

1,3,5-Trimethylpyrrolo[3,2-*d*]pyrimidine-2,4-dione (10a), 5-Methyl-1,3-dipropylpyrrolo[3,2-*d*]pyrimidine-2,4-dione (10b), 1,3,5-Trimethyl-6-phenylpyrrolo[3,2-*d*]pyrimidine-2,4-dione (15a), 5-Methyl-6-phenyl-1,3-dipropylpyrrolo[3,2-*d*]pyrimidine-2,4-dione (15b), 1,3,5-Trimethyl-6-styrylpyrrolo-[3,2-*d*]pyrimidine-2,4-dione (20c), 1,3,5-Trimethyl-6-(1-naphthyl)pyrrolo[3,2-*d*]pyrimidine-2,4-dione (20d), 1,3,5-Trimethyl-6-(2-naphthyl)pyrrolo[3,2-*d*]pyrimidine-2,4-dione (20e), and 1,3,7-Trimethyl-6-phenylpyrrolo[2,3-*d*]pyrimidine-2,4-dione (23). General procedure: Methyl iodide (0.19 mL, 0.03 mol) was added to a suspension of the appropriate pyrrolopyrimidine (3.0 mmol) and 4.6 g (3.3 mmol) of K_2CO_3 in 10 mL of absolute DMF. The mixture was stirred at room temperature or 50–70 °C, respectively, for 3–4 h. After cooling, H_2O was added, the precipitate was collected by filtration and washed with water. Purification was achieved by recrystallization from MeOH/H_2O (P I) or by dissolution in DMSO and precipitation by the addition of H_2O (P II). Reaction conditions, purification, yields, and analytical data are given in Table 7.

Receptor Binding Assays. Inhibition of binding of [^{3}H]-(*R*)-N^6-(phenylisopropyl)adenosine (R-PIA) to A1 adenosine receptors of rat cortical membranes and inhibition of binding of [^{3}H]-5′-(*N*-ethylcarboxamido)adenosine (NECA) to A2 adenosine receptors of rat striatal membranes were assayed as described.[39-41] 2-Chloroadenosine (10 μM) was used in the A1 binding assay and theophylline (5 mM) in the A2 biding assay to determine nonspecific binding. The A1-selective adenosine receptor agonist N^6-cyclopentyladenosine (N^6-CPA) was present in the A2 binding assay to block A1 adenosine receptors present in the striatal membranes. Inhibition of binding by a range of concentrations of xanthines and deazaxanthines was determined in triplicate in three separate experiments. K_i values were calculated using the Cheng–Prusoff equation[42] with K_d values of 1 nM for *R*-PIA and 8.5 nM for NECA.

Semiempirical Calculations. The calculations were based on the crystal structure of theophylline.[43] The atomic coordinates were read into the molecular modeling software program Sybyl (Sybl molecular modeling software, program version 6.0, Tripos Assoc. Inc., 1993) with the *Crysin* command and transformed into one molecule of theophylline. For the 8-phenyl derivatives, a phenyl ring was added in the 8-position with standard geometry parameters. The N9-H tautomers were built by moving the hydrogen from the N7 nitrogen to N9 using Sybl. The resulting structures were calculated with the semiempirical method AM1[44] as implemented in MOPAC 6.0 (MOPAC 6.0, QCPE No. 455; United States Air Force Academy, 1990). All geometry parameters were optimized, and the PRECISE option was used. No rotation of the phenyl ring was performed. However, as full geometry optimization was done for all molecules, it appears not

very likely that significant changes in the energy differences of the respective tautomers are to be expected due to a rotation of the phenyl ring. Sybyl was installed on an E&S ESV3/32 graphics workstation, MOPAC on the Convex C3850 of the University of Tübingen.

Supplementary Material Available: ^{13}C-NMR spectral data of 6-methyluracils, 6-substituted uracils, and selected pyrrolo[3,2-*d*]- and [2,3-*d*]pyrimidine-2,4-diones (3 pages). Ordering information is given on any current masthead page.

Acknowledgment. S. Winiwarter gratefully acknowledges support by the Austrian Science Foundation (FWF).

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