1,8-Disubstituted Xanthine Derivatives: Synthesis of Potent A_{2B}-Selective Adenosine Receptor Antagonists

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3-Unsubstituted xanthine derivatives bearing a cyclopentyl or a phenyl residue in the 8-position were synthesized and developed as A_{2B} adenosine receptor antagonists. Compounds bearing polar substituents were prepared to obtain water-soluble derivatives. 1-Alkyl-8-phenylxanthine derivatives were found to exhibit high affinity for A_{2B} adenosine receptors (ARs). 1,8-Disubstituted xanthine derivatives were equipotent to or more potent than 1,3,8-trisubstituted xanthines at A_{2B} ARs, but generally less potent at A_1 and A_{2A}, and much less potent at A_3 ARs. Thus, the new compounds exhibited increased A_{2B} selectivity versus all other AR subtypes. 9-Deazaxanthines (pyrrolo[2,3-*d*]pyrimidindiones) appeared to be less potent at A_{2B} ARs than the corresponding xanthine derivatives. 1-Propyl-8-p-sulfophenylxanthine (**17**) was the most selective compound of the present series, exhibiting a K_i value of 53 nM at human A_{2B} ARs and showing greater than 180-fold selectivity versus human A_1 ARs. Compound **17** was also highly selective versus rat A_1 ARs (41-fold) and versus the other human AR subtypes (A_{2A} > 400-fold and A_3 > 180-fold). The compound is highly water-soluble due to its sulfonate function. 1-Butyl-8-*p*-carboxyphenylxanthine (**10**), another polar analogue bearing a carboxylate function, exhibited a K_i value of 24 nM for A_{2B} ARs, 49-fold selectivity versus human and 20-fold selectivity versus rat A_1 ARs, and greater than 150-fold selectivity versus human A_{2A} and A_3 ARs. 8-[4-(2-Hydroxyethylamino)-2-oxoethoxy)phenyl]-1-propylxanthine (**29**) and 1-butyl-8-[4-(4-benzyl)piperazino-2-oxoethoxy)phenyl]xanthine (**35**) were among the most potent A_{2B} antagonists showing K_i values at A_{2B} ARs of 1 nM, 57-fold (**29**) and 94-fold (**35**) selectivity versus human A_1, ca. 30-fold selectivity versus rat A_1, and greater than 400-fold selectivity versus human A_{2A} and A_3 ARs. The new potent, selective, water-soluble A_{2B} antagonists may be useful research tools for investigating A_{2B} receptor function.

Introduction

The nucleoside adenosine plays an important physiological role acting via four different subtypes of G-protein-coupled receptors (GPCR), A_1, A_{2A}, A_{2B}, and A_3.[1] A_1 and A_{2A} adenosine receptors (ARs) are stimulated by low (submicromolar to low nanomolar) adenosine concentrations, while higher adenosine levels (micromolar concentrations) are required for the activation of A_{2B} and A_3 ARs in the body.[2] In artificial systems with high adenosine receptor expression, A_1, A_{2A}, and A_3 ARs have been shown to be stimulated by low adenosine concentrations, but the A_{2B} AR still requires micromolar adenosine concentrations.[3,4] Increased levels of adenosine, which might be sufficient to stimulate low affinity ARs, are found under pathophysiological conditions, e.g., as a result of hypoxic or ischemic conditions, after massive cell death, or as a consequence of inflammatory processes.[5,6]

The existence in brain of high and low affinity A_2 ARs was demonstrated by Daly and co-workers in 1983.[7] Such receptors were later named A_{2A} and A_{2B} ARs, respectively. The low affinity A_{2B} AR has now been cloned from various species including rat, mouse, and human.[1,8] The homology of the amino acid sequences of rodent and human A_{2B} receptors is 86−87%, while it is much higher if rat and mouse sequences are being compared (96%).[8] The A_{2B} ARs show a ubiquitous distribution, with highest levels being found in the large intestine, mast cells, and hematopoietic cells, while lower levels are detected in other organs, such as brain and liver.[8]

A_{2B} ARs, like A_{2A} ARs, are positively coupled to adenylate cyclase via G_s; however, coupling to phospholipase C via G_q resulting in mobilization of intracellular calcium and direct coupling to calcium channels (stimulation of calcium influx) have also been described.[1,8]

Adenosine may cause mast cell degranulation,[9] vasodilation,[10] chloride secretion in epithelial cells,[11,12] growth inhibition of smooth muscle cells,[13] enhanced synthesis of cytokines in astrocytes,[14] and stimulation of glucose production in rat hepatocytes[15] via A_{2B} adenosine receptors. Potential therapeutic applications

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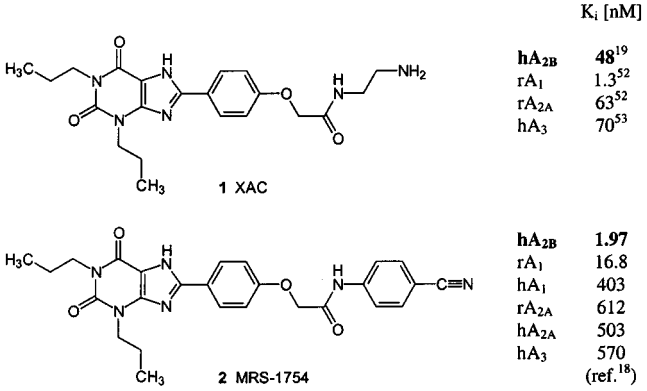
Chart 1. Nonselective Standard Xanthine Derivative XAC (**1**) and A_{2B}-Selective Analogue **2**[a]



[a] h = human, r = rat.

postulated for A_{2B} AR antagonists include asthma and chronic obstructive pulmonary disease,[6,16,17] type II diabetes,[15] cystic fibrosis,[11] secretory diarrhoea associated with inflammation,[12] and Morbus Alzheimer.[14]

A thorough investigation of the (patho)physiological roles of A_{2B} ARs, however, has so far been hampered by a lack of selective tools. Only a few structure−activity relationship studies of A_{2B} receptor ligands have been published.[2,4,15,18−22] Neither potent nor selective A_{2B} agonists are available.[4,23] Only recently, the first selective A_{2B} antagonists have been described.[18] A series of 1,3-disubstituted 8-phenylxanthines derived from the xanthine amine congener XAC (**1**) proved to be potent A_{2B} antagonists (see Chart 1). One of the best compounds was MRS-1754 (**2**), exhibiting a K_i value of 1.97 nM at human A_{2B} ARs and selectivity versus the other human AR subtypes. However, the compound was only moderately selective versus rat A_1 ARs (8.5-fold).[18] In addition, it is highly lipophilic, possessing very low water solubility. Compound **1** has been prepared in tritiated form for radioligand binding studies at recombinant A_{2B} receptors.[24] Furthermore, 2-alkynyl-9-methyladenine derivatives have been described as potent, but nonselective A_{2B} antagonists that were orally active in a mouse model of diabetes.[15]

The present study was aimed at identifying and developing potent A_{2B} antagonists with high selectivity versus the other AR subtypes combined with good water solubility. Starting point was a study published by Bruns in 1980,[25] in which a large series of compounds, including many xanthine derivatives, were investigated in functional studies in a human fibroblast cell line expressing A_{2B} ARs. Analysis of the stucture−activity relationships (SAR) revealed that the 1-substituent but not the 3-substituent of xanthine derivatives might be important for high A_{2B} affinity.[2] An 8-phenyl substituent largely increased A_{2B} affinity.[2,25] We have now synthesized and evaluated a series of 3-unsubstituted xanthine derivatives, most of which are bearing polar substituents in the 8-position to increase water solubility. In a few cases the corresponding 1,3-disubstituted xanthines and 9-deazaxanthines (pyrrolo[2,3-*d*]pyrimidindiones) were investigated for comparison.

Results and Discussion

Chemistry. The synthesis of some of the investigated 3-unsubstituted xanthine derivatives has previously been described.[26] 3-Substituted-5,6-diaminouracils **3a**−**3c** and 1,3-dipropyl-5,6-diaminouracil **3d** were used as starting compounds (Schemes 1 and 2). For the preparation of benzylidene derivatives **4aa**, **4ba**, **4ab**, **4bb**, **4cb**, **4bc**, **4ad**, **4be**, and **4af**, diaminouracils **3a**−**3c** were reacted with different (unsubstituted or *p*-substituted) aldehydes in ethanol at reflux temperature.[26] Subsequent ring closure of these derivatives was performed either by stirring the imines in thionyl chloride overnight, followed by refluxing for 1 h,[27] or by reflux in ethanol in the presence of anhydrous ferric chloride for 3 h affording 1,8-disubstituted xanthine derivatives **5**−**13**. Compound **14** was prepared by heating of **4ad** in thionyl chloride for 30 min to afford the acid chloride derivative of **11**, which was subsequently converted to the methyl ester **14** by refluxing it with methanol for 30 min.[28]

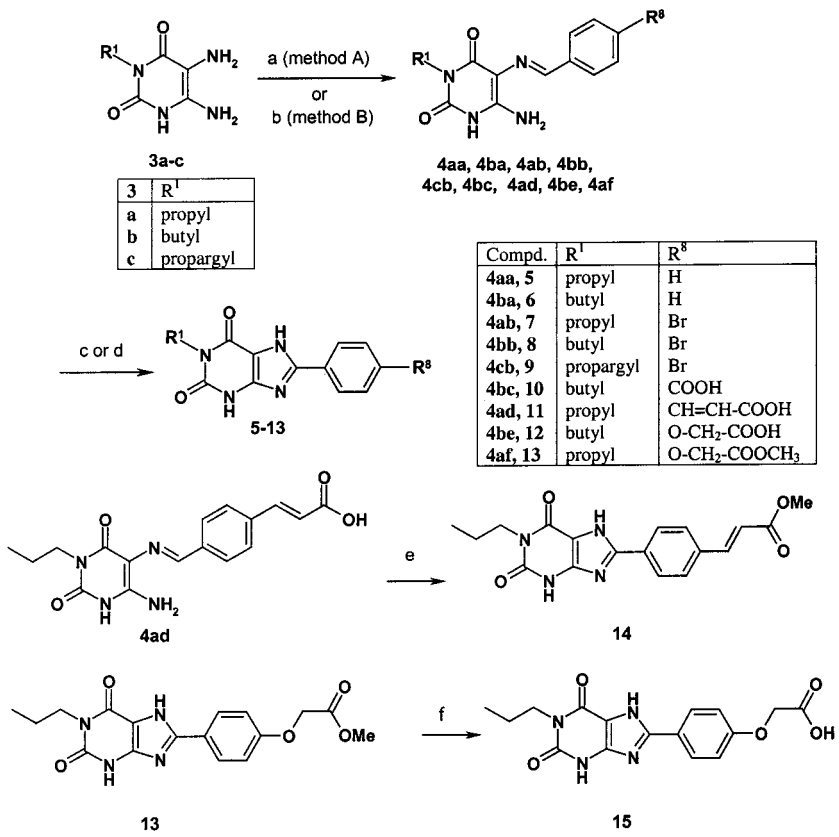
Xanthine **15** was prepared by hydrolysis of methyl ester **13** in dimethylformamide in the presence of 0.1 N aqueous sodium carbonate solution upon heating.[29] Carboxamide derivatives **16ag**, **16bg**, **16db**, and **16dh** were prepared by the reaction of 5,6-diaminouracils **3a**, **3b**, or **3d** with *p*-substituted benzoic acid derivatives, using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) as condensing agent (Scheme 2).[30] Ring closure to xanthines **17**−**18** was achieved either by heating of **16ag** or **16bg**, respectively, in hexamethyldisilazane in the presence of a catalytic amount of ammonium sulfate for 50 h at 140 °C[31] or by refluxing in trimethylsilylpolyphosphate at 160−180 °C for 1 h.[26] Xanthine derivatives **19** and **20** were obtained by heating **16db** or **16dh** in methanol in the presence of 10% sodium hydroxide at 70 °C for 30 min.[32]

Compound **21** was prepared by condensation of 5,6-diamino-1-propyluracil **3a** with 2,6-naphthalene dicarboxylic acid in dimethylformamide in the presense of *N*-methylmorpholine and isobutylformate.[33] Ring closure of **21** was achieved by refluxing it in hexamethyldisilazane in the presence of trimethylchlorosilane (TMSCl) and *p*-toluenesulfonic acid for 36 h affording compound **22** (Scheme 2).

An alternative procedure was used for the preparation of compound **27**, since neither *p*-carboxymethylbenzoic acid nor *p*-carboxymethylbenzaldehyde were commercially available (Scheme 3). Thus, *p*-chloromethylbenzoic acid **23** was treated with sodium cyanide in the presence of sodium carbonate in tetrahydrofurane to afford *p*-cyanomethylbenzoic acid **24**.[34,35] Compound **24** was condensed with 5,6-diamino-1-butyluracil **3b** as above affording the carboxamide derivative **25**, which subsequently underwent ring closure in hexamethyldisilazane to give compound **26**. Compound **26** was hydrolyzed by aqueous sulfuric acid under reflux for 6 h affording phenylacetic acid derivative **27**.[34]

Xanthine amide derivatives **28**−**37** were prepared by three different methods (Scheme 4). Amide derivatives **31** and **37** were prepared by direct condensation of **12** with the appropriate piperazine derivatives at room temperature in anhydrous dimethylformamide/dichloromethane (1:1) in the presence of *N*-(3-dimethylaminopropyl)-*N*′-ethyl-carbodiimide hydrochloride and 4-(dimethylamino)pyridine. Amide derivatives **30** and **32**−**36** were prepared by conversion of **12** to its acid chloride derivative by refluxing it in thionyl chloride for 4 h at

Scheme 1. Synthesis of 1,8-Disubstituted Xanthine Derivatives[a]

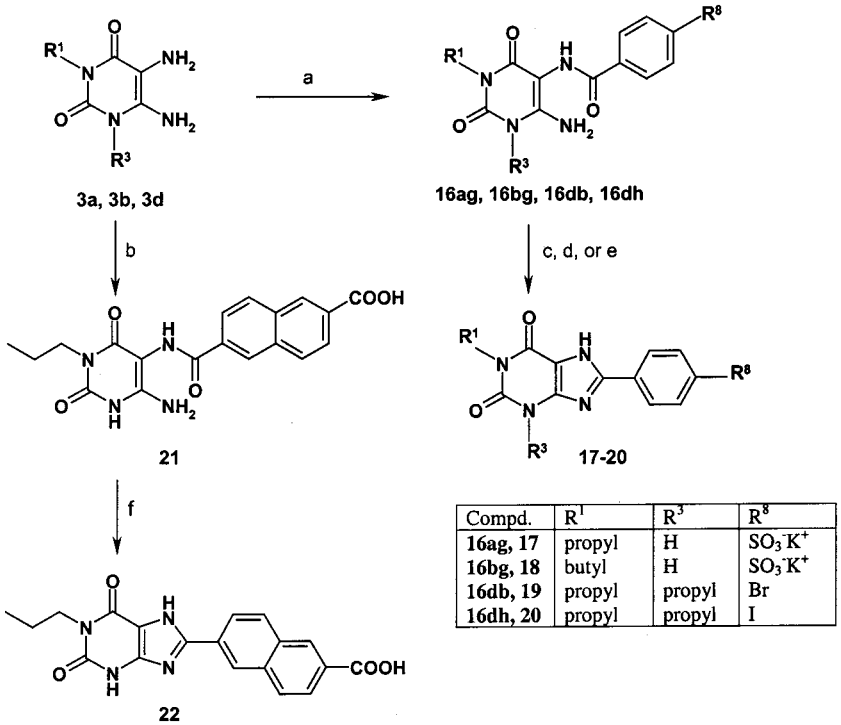


3	R^1
a	propyl
b	butyl
c	propargyl

Compd.	R^1	R^8
4aa, 5	propyl	H
4ba, 6	butyl	H
4ab, 7	propyl	Br
4bb, 8	butyl	Br
4cb, 9	propargyl	Br
4bc, 10	butyl	COOH
4ad, 11	propyl	CH=CH-COOH
4be, 12	butyl	O-CH_2-COOH
4af, 13	propyl	O-CH_2-$COOCH_3$

[a] (a) 4-(Un)substituted benzaldehyde, ethanol, reflux; (b) 4-(un)substituted benzaldehyde, ethanol, acetic acid, rt or reflux; (c) 1. $SOCl_2$ at 0 °C, 2. reflux, 3. stirring overnight at room temperature; (d) $FeCl_3$, ethanol, reflux; (e) 1. $SOCl_2$, reflux, 2. methanol, reflux; (f) DMF, aq Na_2CO_3, steam bath.

Scheme 2. Synthesis of 1,8-Di- and 1,3,8-Trisubstituted Xanthine Derivatives[a]

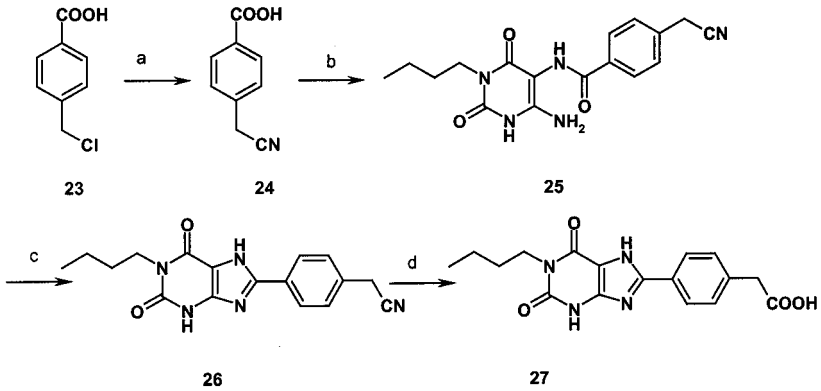


Compd.	R^1	R^3	R^8
16ag, 17	propyl	H	$SO_3^-K^+$
16bg, 18	butyl	H	$SO_3^-K^+$
16db, 19	propyl	propyl	Br
16dh, 20	propyl	propyl	I

[a] (a) 4-Substituted benzoic acid, MeOH or MeOH/H_2O (1:1), EDC, 24 h, rt; (b) *N*-methylmorpholine, isobutylformate, 2,6-naphthalene dicarboxylic acid, DMF; (c) 1. HMDS, $(NH_4)_2SO_4$, reflux, 2. MeOH; (d) 1. PPSE, 160−180 °C, 2. MeOH; (e) 1. MeOH, aq NaOH, 70 °C, 2. aq HCl; (f) 1. HMDS, TMSCl, *p*-toluene sulfonic acid, reflux 36 h, 2. H_2O, ΔT.

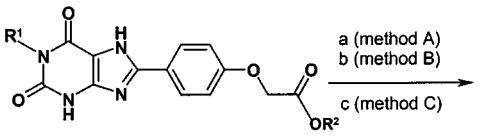
70 °C. After distillation of excess thionyl chloride, the residue was dissolved in a mixture of anhydrous pyridine and dichloromethane, then the appropriate amine derivative was added.[18] In some cases, the yields

Scheme 3. Synthesis of 1-Butyl-8-(4-carboxymethyl)phenylxanthine[a]

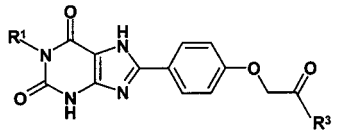


[a] (a) NaCN, aq $NaHCO_3$, THF, 20−25 °C, 48 h; (b) 3-butyl-5,6-diaminouracil, MeOH/H_2O (1:1), EDC, rt, overnight; (c) 1. HMDS, $(NH_4)_2SO_4$, 140 °C, 50 h, 2. MeOH/H_2O; (d) 1. concd H_2SO_4, H_2O, reflux, 6 h, 2. H_2O, 3. NaOH/HCl.

Scheme 4. Synthesis of Amide Derivatives of 3-Unsubstituted 8-Phenylxanthines[a]



12, R^1= butyl, R^2= H
13, R^1= propyl, R^2= CH_3



28-37

Compd.	R^1	R^3
28	propyl	2-aminoethylamino
29	propyl	2-hydroxyethylamino
30	butyl	4-carboxymethylphenylamino
31	butyl	4-ethylpiperazin-1-yl
32	butyl	4-acetylpiperazin-1-yl
33	butyl	4-ethoxycarbonylpiperazin-1-yl
34	butyl	4-phenylpiperazin-1-yl
35	butyl	4-benzylpiperazin-1-yl
36	butyl	4-(2-methoxyphenyl)piperazin-1-yl
37	butyl	4-benzyloxycarbonylpiperazin-1-yl

[a] (a) Amine derivative, EDC, DMAP, DMF/CH_2Cl_2, rt, 24−48 h; (b) amine derivative, pyridine/CH_2Cl_2, rt, 24−48 h; (c) amine derivative, DMF, ΔT, 24−48 h.

obtained by this method were better than that obtained by the carbodiimide method, and also the side products were less, resulting in an easier purification procedure. Amide derivatives **28** and **29** were prepared by direct reaction of xanthine methyl ester **13** with amine derivatives in hot dimethylformamide.[28]

In Table 1, yields, melting points, and analytical data for intermediate and final products are collected. ^1H and ^{13}C NMR data of all final compounds and most intermediate products were recorded and were in accordance with the proposed structures. NMR data are available as Supporting Information.

Biological Evaluation. All compounds were investigated in radioligand binding studies at rat brain A_1 and A_{2A} ARs using [^{3}H]2-chloro-N^6-cyclopentyladenosine (CCPA) and [^{3}H]3-(3-hydroxypropyl)-7-methyl-8-(*m*-methoxystyryl)-1-propargylxanthine (MSX-2), respectively, as radioligands. Selected compounds were also evaluated in binding assays at human recombinant A_1 and A_{2A} ARs stably expressed in Chinese hamster ovary (CHO) cells in order to assess selectivity in humans. The compounds were evaluated for A_{2B} affinity in radioligand binding assays at human recombinant receptors using [^{3}H]4-[2-[[7-amino-2-(furyl)1,2,4-triazolo[2,3-*a*]1,3,5-triazin-5-yl]amino]ethyl]phenol (ZM-241385) as radioligand. Selected compounds were additionally investigated in binding assays at human recombinant A_3 ARs with the A_3-selective antagonist radioligand [^{3}H]2-phenyl-8-ethyl-4-methyl-(8*R*)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*i*]purin-5-one (PSB-11).

Structure−Activity Relationships. Determined affinities of 3-unsubstituted 1,8-disubstituted xanthine derivatives are collected in Tables 2 and 3. Data of a few corresponding 1,3,8-trisubstituted xanthines, including the standard AR antagonists 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, **39**) and 1,3-dipropyl-8-*p*-sulfophenylxanthine (DPSPX, **42**), are given for comparison.

8-Cyclopentyl-1,3-dipropylxanthine (DPCPX or CPX, **39**) is a standard antagonist for A_1 ARs. It also exhibits considerable affinity for A_{2B} receptors (K_i = 51 nM) and has been used in tritiated form as a radioligand for A_{2B} AR binding assays.[19] 8-Cyclopentyl-1-propylxanthine (**38**) retained A_{2B} affinity but was much less potent at A_1 ARs than its 3-propyl derivative **39**.

8-Cyclopentyl substitution is known to be favorable for high A_1 affinity only. 8-Phenyl substitution, however, appears to generally increase affinity of xanthine derivatives for all AR subtypes.[2,25] Therefore most of the new compounds were 8-phenylxanthine derivatives. 8-Phenylxanthine (**40**) itself was more potent at A_{2B} (K_i = 810 nM) than at A_1 and A_{2A} ARs and exhibited 3-fold selectivity versus A_1 ARs. The introduction of a 1-alkyl substituent led to a large increase in AR affinity, which was most pronounced at A_{2B} receptors. The order of potency was 1-propyl ≥ 1-butyl > 1-ethyl for A_1, A_{2A}, and A_{2B} ARs. The A_{2B} selectivity of 1-alkyl-8-phenylxanthines **41**, **5,** and **6** was 3- to 8-fold (versus rat A_1 ARs) and much higher versus the other AR subtypes. Introduction of a bromine atom into the para-position of the phenyl ring (compounds **7**, **8**) further increased AR affinity. The increase was similar for A_1, A_{2A}, and A_{2B} receptors. Therefore, A_{2B} selectivity was not improved. 1-Propargyl-8-*p*-bromophenylxanthine (**9**) was somewhat less potent than the corresponding 1-propyl

Table 1. Yields, Melting Points, and Analytical Data of the Intermediate and Final Products

comp	formula	MW	anal.[a]	yield [%]	mp [°C]
4aa	$C_{14}H_{16}N_4O_2$	272.31	nd[b]	80	204−205
4ba	$C_{15}H_{18}N_4O_2$	286.34	C,[c] H, N	86	165−166
4ab	$C_{14}H_{15}N_4O_2Br$	351.20	C, H, N	82	250
4bb	$C_{15}H_{17}N_4O_2Br$	365.23	C, H, N	69	234
4cb	$C_{14}H_{11}N_4O_2Br \cdot H_2O$	365.19	C, H,[d] N	79	237−238
4bc	$C_{16}H_{18}N_4O_4$	330.35	nd	88	269−270
4ad	$C_{17}H_{18}N_4O_4 \cdot 0.5C_2H_5OH \cdot H_2O$	383.41	C, H, N	80	230 (dec)
4be	$C_{17}H_{20}N_4O_5$	360.37	nd	82	172−173
4af	$C_{17}H_{20}N_4O_5 \cdot 0.5H_2O$	369.38	C, H, N	95	230
5	$C_{14}H_{14}N_4O_2$	270.29	nd	80	>300 (lit. mp >300)[54]
6	$C_{15}H_{16}N_4O_2$	284.32	C,[e] H, N	82	351−352
7	$C_{14}H_{13}N_4O_2Br$	349.19	C, H, N	37	>250
8	$C_{15}H_{15}N_4O_2Br$	363.22	C, H, N	71	>270
9	$C_{14}H_9N_4O_2Br \cdot H_2O$	363.18	C, H, N	79	322−323
10	$C_{16}H_{16}N_4O_4 \cdot 0.25H_2O$	332.84	C, H, N	80	310−311
11	$C_{17}H_{16}N_4O_4 \cdot 0.5H_2O$	349.35	C, H, N	91	>250
12	$C_{17}H_{18}N_4O_5 \cdot 0.5H_2O$	369.17	C, H, N	89	302−303
13	$C_{17}H_{18}N_4O_5$	358.35	C, H, N	90	>250
14	$C_{18}H_{18}N_4O_4$	354.36	HRMS[f]	70	>250
15	$C_{16}H_{16}N_4O_5 \cdot 1.5H_2O$	371.39	C, H,[g] N	83	>250
16ag	$C_{14}H_{15}N_4O_6SK$	406.46	nd	75	>300 (lit. mp >300)[26]
16bg	$C_{15}H_{17}N_4O_6SK$	420.49	nd	72	>300
16db	$C_{17}H_{21}N_4O_3Br \cdot H_2O$	427.30	C, H, N	79	193
16dh	$C_{17}H_{21}N_4O_3I \cdot H_2O$	474.30	C, H, N	87	211
17	$C_{14}H_{13}N_4O_5SK \cdot 1.5H_2O$	415.52	C, H, N	53	>300 (lit. mp >300)[26]
18	$C_{15}H_{15}N_4O_5SK \cdot 1.5H_2O$	429.50	C, H,[h] N	50	>300
19	$C_{17}H_{19}N_4O_2Br$	391.27	C, H, N	84	>270
20	$C_{17}H_{19}N_4O_2I$	438.27	C, H, N	68	>270
21	$C_{19}H_{18}N_4O_5$	382.38	nd	70	>250
22	$C_{19}H_{16}N_4O_4 \cdot 0.5H_2O$	373.37	C, H, N	90	>250
25	$C_{17}H_{19}N_5O_3$	341.37	nd	78	283−285 (dec)
26	$C_{17}H_{17}N_5O_2$	336.88	C, H, N	69	>300
27	$C_{17}H_{18}N_4O_4$	349.57	C, H, N	76	>300
28	$C_{18}H_{22}N_6O_4 \cdot 0.5H_2O$	395.42	C, H, N	77	>250
29	$C_{18}H_{21}N_5O_5$	387.39	C, H, N	83	>250
30	$C_{25}H_{25}N_5O_6 \cdot 0.5CH_2Cl_2$	533.97	C, H, N	42	288−289
31	$C_{23}H_{30}N_6O_4 \cdot 0.4CH_2Cl_2$	488.50	C, H,[i] N	38	276−277
32	$C_{23}H_{28}N_6O_5 \cdot 0.6CH_2Cl_2$	519.48	C, H, N	38	294−295
33	$C_{24}H_{30}N_6O_6 \cdot 0.6CH_2Cl_2$	549.50	C, H, N[j]	41	281−283
34	$C_{27}H_{30}N_6O_4 \cdot 0.4CH_2Cl_2$	536.55	C, H, N[k]	55	280−281
35	$C_{28}H_{32}N_6O_4 \cdot 0.3CH_2Cl_2$	542.09	C, H, N	45	262−263
36	$C_{28}H_{32}N_6O_5 \cdot 0.5CH_2Cl_2$	575.07	C, H, N	40	269−270
37	$C_{29}H_{32}N_6O_6 \cdot 0.4CH_2Cl_2$	594.58	C, H, N	42	266−267

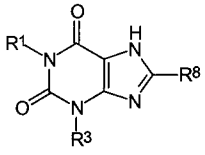
[a] Analyses were within ±0.4% of calculated values unless otherwise noted. [b] nd= not determined (intermediate products). [c] Calcd, 62.92; found, 62.30. [d] Calcd, 3.60; found, 3.12. [e] Calcd, 63.37; found, 62.83. [f] High-resolution mass in EI mode (*m*/*z*) determined to be within acceptable limits: calcd, 354.1328; found, 354.1327. [g] Calcd, 5.17; found, 4.50. [h] Calcd, 4.23; found, 4.76. [i] Calcd, 6.37; found, 6.82. [j] Calcd, 15.30; found, 14.83. [k] Calcd, 15.67; found, 16.18.

derivative **7**, but it showed improved selectivity versus A_1 ARs. The 1-alkyl-8-*p*-bromophenylxanthines **7** and **8** were investigated at human A_3 ARs. They were considerably less potent at A_3 receptors than at A_{2B} ARs. 1-Propyl substitution appeared to be optimal for high A_3 affinity. The order of potency was 1-propyl > 1-propargyl > 1-butyl at A_3 ARs. 1,3-Dipropyl-8-phenylxanthine derivatives with halogen substitution in the *p*-phenyl position (**19**, **20**) were prepared for comparison. 1,3-Dipropyl-8-*p*-bromophenylxanthine (**19**) exhibited about the same A_{2B} affinity as the corresponding 3-undisubstituted xanthine **7** (**19**, K_i = 3.8 nM; **7**, K_i = 2.7 nM). However, the additional 3-propyl residue in **19** led to an increase in A_{2A} and A_1 affinity, resulting in decreased selectivity for A_{2B} ARs. Replacement of bromine (in **19**) for iodine (in **20**) resulted in an increase in affinity for A_1, A_{2A}, and A_{2B} ARs, which was more pronounced at A_1 and A_{2A} (3-fold) than at A_{2B} receptors (2-fold).

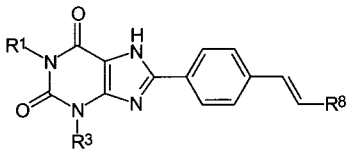
9-Deazaxanthine derivatives (pyrrolo[2,3-*d*]pyrimidindiones) had earlier been found to be potent AR antagonists exhibiting increased selectivity for A_1 ARs versus A_{2A} receptors as compared to xanthine derivatives.[36] We have now investigated 9-deaza analogues **44** and **45** of 3-unsubstituted 1-methyl- and 1-propyl-8-phenylxanthine. Both compounds exhibited lower A_{2B} affinity than the corresponding xanthine derivatives (compare 1-methyl-8-phenylxanthine[25] and **44**: 3.5-fold difference; **5**/**45**: 9-fold difference). Thus, 9-deazaxanthines exhibited increased A_1 selectivity not only versus A_{2A}[36] but also versus A_{2B} ARs. Interestingly, *N*-propyl-substituted deazaxanthine **45** was relatively potent at human A_3 ARs (K_i = 380 nM).

To increase water solubility of the highly lipophilic, insoluble 8-phenylxanthine derivatives, polar groups, e.g., acidic or basic functions, were introduced into the para-position of the phenyl ring. A carboxylate group (compound **10**) in 1-butyl-8-phenylxanthine (**6**) was well tolerated by A_{2B} ARs but not by the other AR subtypes. Thus, benzoic acid derivative **10** was a potent (K_i = 24 nM) and selective A_{2B} antagonist (49-fold versus human A_1, 20-fold versus rat A_1, 158-fold versus rat A_{2A}, 193-fold versus human A_3). The corresponding phenyl acetic acid derivative **27** was slightly more potent at A_1, A_{2B},

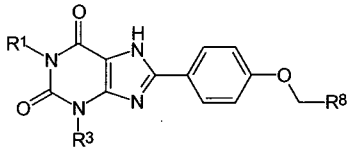
Table 2. Adenosine Receptor Affinities and A_{2B} Selectivities of Xanthine and 9-Deazaxanthine Derivatives



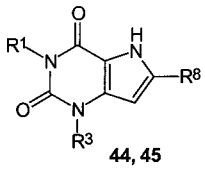
5-10, 17, 18, 20, 22, 27, 38-42



11, 14, 43



12, 13, 15



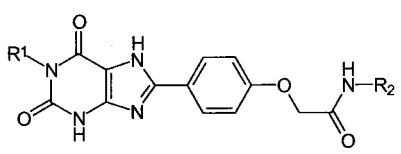
44, 45

				$K_i \pm$ SEM [nM] or % inhibition of radioligand binding at 10 μM				A_{2B} selectivity		
compd	R^1	R^3	R^8	rat (or human) A_1 vs [^{3}H]CCPA ($n = 3$)	rat (or human) A_{2A} vs [^{3}H]MSX-2 ($n = 3$)	human A_{2B} vs [^{3}H]ZM-241385 ($n = 2$)	human A_3 vs [^{3}H]PSB-11 ($n = 2$)	A_1/A_{2B}	A_{2A}/A_{2B}	A_3/A_{2B}
8-Mono-, 1,8-Di-, and 1,3,8-Trisubstituted Xanthine Derivatives										
38	propyl	H	cyclopentyl	14[26]	580[26]	34.4 ± 10.3	nd	0.4	169	nd
39 (DPCPX)	propyl	propyl	cyclopentyl	0.9[52]	470[52]	51[50]	795 ± 139	0.02	9	16
40	H	H	phenyl	2500[26]	21000[26]	810 ± 150	nd	3	26	nd
41	ethyl	H	phenyl	150[26]	1800[26]	19 ± 1.5	950 ± 32	8	95	50
5	propyl	H	phenyl	31 ± 2.7 67[26]	458 ± 71 1900[26]	4.7 ± 3.3	nd	7	97	nd
6	butyl	H	phenyl	40 ± 22	642 ± 151	11.8 ± 1.2	nd	3	54	nd
7	propyl	H	*p*-bromophenyl	18 ± 5	336 ± 80	4.4 ± 0.38	173 ± 9	5	84	43
8	butyl	H	*p*-bromophenyl	13 ± 9	57 ± 38	2.7 ± 1.6	45% ± 9	5	21	>1000
9	propargyl	H	*p*-bromophenyl	60 ± 8.8	199 ± 4.6	6.8 ± 0.2	477 ± 1	9	29	70
19	propyl	propyl	*p*-bromophenyl	5.7 ± 1.6	39 ± 7	3.8 ± 0.57	nd	2	10	nd
20	propyl	propyl	*p*-iodophenyl	2.1 ± 1.1	14 ± 6	2.5 ± 0.55	nd	1	6	nd
10	butyl	H	*p*-carboxyphenyl	481 ± 83.1 (1181 ± 88)[a]	3800 ± 458 (55% ± 11)[a]	24 ± 6	4622 ± 323	20 (49)[a]	158	193
27	butyl	H	*p*-(carboxymethyl)-phenyl	207 ± 105	5905 ± 1258	15.1 ± 4.3	2857 ± 16	14	391	189
17 (PSB-1115)	propyl	H	*p*-sulfophenyl	2200[26] (35% ± 6)[a]	24000[26]	53.4 ± 18.2	14% ± 20	41 (>180)[a]	453	>180
18	butyl	H	*p*-sulfophenyl	475 ± 34	8070 ± 1850	70 ± 12	39% ± 8	7	115	>140
42 (DPSPX)	propyl	propyl	*p*-sulfophenyl	210[52]	1400[52]	250[55]	183 (s)[b,56]	1	6	1
22	propyl	H	6-carboxy-2-naphthyl	110 ± 9	43% ± 10 (55% ± 4)[a]	13 ± 1.8	1184 ± 169	8	>700	91
8-(Phenylacrylic acid)xanthine Derivatives										
11	propyl	H	COOH	68 ± 11	13% ± 15	14.5 ± 8.2	1217 ± 231	5	>500	84
43[18]	propyl	propyl	COOH	15[18]	800[18]	60[18]	30[18]	0.25	13	0.5
14	propyl	H	$COOCH_3$	91 ± 13	30% ± 10	26 ± 21	1119 ± 147	3.5	>500	43
8-(4-Carboxymethyloxyphenyl)xanthine Derivatives										
15	propyl	H	COOH	145 ± 3	4% ± 13	372 ± 51	nd	0.4	>1000	nd
12	butyl	H	COOH	81 ± 43	1877 ± 908	10.8 ± 8.3	1192 ± 147	7	171	108
13	propyl	H	$COOCH_3$	15 ± 6	709 ± 71	3.7 ± 0.3	80.6 ± 10.9	4	177	22
9-Deazaxanthine Derivatives										
44	methyl	H	phenyl	97[36]	2000[36]	520 ± 78	2098 ± 299	0.2	4	4
45	propyl	H	phenyl	39[36] (45 ± 2)[a]	1200[36] (58% ± 11)[a]	42 ± 0.8	380 ± 177	1 (1)[a]	29	9

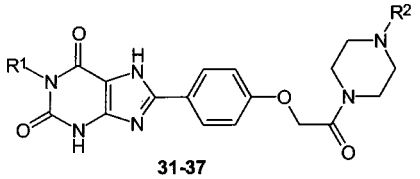
[a] Human recombinant receptors ($n = 2$). [b] Sheep A_3 receptor.

and A_3 ARs and somewhat less potent at A_{2A} ARs, retaining high affinity and some selectivity for A_{2B} ARs. A para-sulfonate group in 1-propyl- and 1-butyl-8-phenylxanthine (compounds **17** and **18**) was less well tolerated than a carboxylate group. However, it was again better tolerated by the A_{2B} than by the other AR subtypes yielding A_{2B} antagonists with improved selectivity. 1-Propyl-8-*p*-sulfophenylxanthine (**17**) was the compound with the highest selectivity of the present series. It showed a K_i value at human A_{2B} receptors of 53 nM and was greater than 180-fold selective versus human A_1, 41-fold selective versus rat A_1, and more than 180-fold selective versus rat A_{2A} and human A_3 ARs. The corresponding 1-butyl-8-p-sulfophenylxanthine (**18**) was less potent at A_{2B} ARs ($K_i = 70$ nM) but more potent at A_1 and A_{2A} ARs, thus exhibiting reduced selectivity. Data for 1,3-dipropyl-8-*p*-sulfophenylxanthine (DPSPX, **42**), a nonselective, water-soluble standard antagonist, were included (compare **42** and **17**). The additional propyl group in **42** results in largely increased A_1 (11-fold), A_{2A} (17-fold), and particularly A_3 affinity (>50-fold). In contrast, A_{2B} affinity is reduced in **42** (5-fold) compared to the 1,8-disubstituted xanthine **17**. It can be concluded from these and other data (see below) that the 3-substituent in xanthines is important for A_1, A_{2A}, and particularly for A_3 affinity but not for affinity to A_{2B} ARs. Sulfophenylxanthine derivatives, such as **42** and 8-*p*-sulfophenyltheophylline, do not penetrate into the central nervous system (CNS) and are only peripherally active.[37,38] Thus, 1-propyl-8-p-sulfophenylxanthine (**17**) is expected to be a peripheral A_{2B} antagonist without effects on the CNS. Perorally applied **17** is probably not being absorbed due to its highly polar character and may exert its actions only locally in the intestine, where a high density of A_{2B} ARs is known to exist. 1-Propyl-8-*p*-sulfophenylxanthine (**17**) has been investigated in a functional assay at human recombinant A_{2B} ARs.[39] It was shown to inhibit NECA-induced stimulation of adenylate cyclase with a K_i value of 820 nM.[39] An X-ray structure of **17** revealed a nearly coplanar conformation of the xanthine ring system on the 8-phenyl substituent.[40]

Table 3. Adenosine Receptor Affinities and A_{2B} Selectivities of Amide Derivatives of 3-Unsubstituted 8-Phenylxanthines



28-30



31-37

			$K_i \pm$ SEM [nM] or % inhibition of radioligand binding at 10 μM						
			rat (or human) A_1 vs [^{3}H]CCPA	rat (or human) A_{2A} vs [^{3}H]MSX-2	human A_{2B} vs [^{3}H]ZM-241385	human A_3 vs [^{3}H]PSB-11	A_{2B} selectivity		
compd	R^1	R^2					A_1/A_{2B}	A_{2A}/A_{2B}	A_3/A_{2B}
Amides of 8-(4-Carboxymethyloxyphenyl)xanthines									
28	propyl	2-aminoethyl	24 ± 4	365 ± 30	10 ± 0.8	nd	2	37	nd
29	propyl	2-hydroxyethyl	35 ± 6 (68 ± 35)[a]	2139 ± 1278	1.2 ± 0.5	422 ± 35	29 (57)[a]	2139	422
30	butyl	*p*-carboxymethylphenyl	41 ± 4.1	479 ± 97	5.3 ± 0.2	676 ± 224	8	90	128
Piperazinyl Amides of 8-(4-Carboxymethyloxyphenyl)xanthines									
31	butyl	ethyl	18 ± 8.5	290 ± 170	5.5 ± 0.4	nd	3	53	nd
32	butyl	acetyl	30 ± 16	450 ± 292	6.5 ± 0.3	nd	5	69	nd
33	butyl	ethyloxycarbonyl	20 ± 9.6	900 ± 485	6.1 ± 0.3	nd	3	148	nd
34	butyl	phenyl	17 ± 8.7	43% ± 11	14.7 ± 0.4	nd	1	>500	nd
35	butyl	benzyl	37 ± 11 (122 ± 54)[a]	550 ± 65 (55% ± 10)[a]	1.3 ± 0.2	475 ± 114	28 (94)[a]	423	365
36	butyl	2-methoxyphenyl	24 ± 6	320 ± 224	1.2 ± 0.4	102 ± 2	20	267	102
37	butyl	benzyloxycarbonyl	15 ± 1.7	130 ± 133	2.9 ± 1.4	nd	5	45	nd

[a] Human recombinant receptors ($n = 2$).

In a previous study,[39] 1-propargyl-3-methyl-8-(6-carboxy-2-naphthyl)xanthine, originally developed as an A_{2A} antagonist,[33] had been found to be somewhat selective for A_{2B} ARs. We have now synthesized the corresponding 1-propyl-8-(6-carboxy-2-naphthyl)xanthine (**22**). As expected, the compound was very potent at A_{2B} ARs ($K_i = 13$ nM). Selectivity versus A_{2A} and A_3 ARs was high, but it was much lower versus rat A_1 ARs (8-fold). A structurally related compound, the phenylacrylic acid derivative **11**, showed nearly the same K_i values as the naphthyl carboxylic acid **22**. Data for the 1,3-dipropyl analogue (**43**) of **11** have been published.[18] A comparison of **11** and **43** again showed that the 3-propyl residue leads to an increase in A_1 (4.5-fold) and A_{2A} (>12-fold) affinity and a dramatic increase in A_3 affinity (41-fold), but reduces A_{2B} affinity (4-fold). While the dipropyl derivative **43** was slightly A_1-selective, the 3-unsubstituted **11** was A_{2B}-selective. Methylation of the carboxylate of **11** leading to **14** had no major effects on affinity at all AR subtypes—as seen with compounds **10** and **27**—confirming that the acidic group is not required for high AR affinity.

Carboxymethyloxyphenyl derivatives (**12**, **13**, **15**) can be envisaged as bioisosteric analogues of carboxynaphthyl (**22**) and phenyl acrylic acid derivatives (**11**, **14**). 1-Propyl-(8-(4-carboxymethyloxy)phenyl)xanthine (**15**), however, exhibited considerably (>20-fold) lower A_{2B} affinity than the bioisosteric compounds **22** and **11**. At A_1 ARs, **15** was only slightly weaker than **11** and **22** and was therefore not selective for A_{2B} ARs. Replacement of the 1-propyl substituent (in **15**) by 1-butyl (compound **12**) resulted in a 34-fold increase in A_{2B} affinity, while A_1 affinity was increased only by 2-fold. The corresponding 1,3-dibutyl derivative described by Jacobson et al.[18] was more potent at A_1, A_{2A}, and particularly A_3 ARs. However, it was less potent at A_{2B} ARs than its 3-unsubstituted analogue **12**. Methylation of the carboxylate **15** increased affinity, leading to a potent A_{2B} antagonist (**13,** $K_i = 3.7$ nM) with moderate selectivity versus A_1 and A_3 and high selectivity versus A_{2A} ARs.

A second series of xanthine derivatives was prepared in which the carboxylic acid derivatives **11**, **12**, and **15** were coupled with amines bearing polar groups in order to increase water solubility (Table 3). All 3-unsubstituted xanthine amides exhibited high A_{2B} affinity with K_i values at low nanomolar concentrations. The hydroxyethylamide **29** of 1-propyl-8-(4-carboxymethyloxyphenyl)xanthine (**15**) exhibited a similarly high A_{2B} affinity ($K_i = 1.2$ nM) but was more selective versus A_1 ARs (59-fold versus human, 29-fold versus rat A_1 ARs). In comparison, the corresponding aminoethylamide **28**, in which the terminal hydroxy group in **29** was formally replaced by an amino group, was 10-fold less potent at A_{2B} receptors but showed increased A_1 and A_{2A} affinity. Compound **28** is a 3-unsubstituted analogue of the 1,3-dipropylxanthine XAC (**1**). In comparison with **1**, **28** exhibited decreased A_1 (20-fold) and A_{2A} (6-fold) affinity and increased A_{2B} affinity. This result confirms that 3-unsubstituted xanthines exhibit increased A_{2B} selectivity. Amide **30** bearing a terminal carboxylate was well tolerated by A_{2B} ARs and was quite selective versus the other AR subtypes.

A series of seven piperazinyl amides was prepared (compounds **31**−**37**). The *N*-alkyl- and *N*-aryl-piperazinylamides contained a basic nitrogen atom, which can be protonated leading to increased water solubility. All piperazinylamides exhibited high affinity for A_{2B} ARs. Their K_i values ranged from 1 to 15 nM (Table 3). Large substituents, e.g., benzyl, 2-methoxyphenyl, and benzyloxycarbonyl, were well tolerated. There was not much difference in affinities of ethyl (**31**) and acetyl (**32**) or benzyl (**35**) and benzyloxycarbonyl (**37**) derivatives. The most potent compound of the series was the *N*-benzylpiperazine derivative **35** of 1-butyl-8-(4-carboxymethyloxyphenyl)xanthine, exhibiting a K_i value of 1.3 nM

at human A_{2B} ARs and high selectivity versus the other AR subtypes.

In conclusion, 3-unsubstituted xanthine derivatives bearing a cyclopentyl or (substituted) phenyl residue in the 8-position were found to exhibit high potency at A_{2B} ARs and increased selectivity in comparison with xanthines bearing a 3-substituent. Some of the new A_{2B} antagonists were not only highly selective versus human but also versus rat A_1 ARs. A_{2B}-selective AR antagonists with high water solubility were obtained, which may be useful research tools to investigate the (patho)-physiology and pharmacology of A_{2B} receptors.

Experimental Section

Chemical Synthesis. ^{1}H and ^{13}C NMR spectra were performed on a Bruker Avance 500 MHz spectrometer. DMSO-d_6 was used as solvent. The chemical shifts of the deuterated solvent served as internal standard: δ ^{1}H: 2.50; ^{13}C: 39.1. The mass spectra were performed on an MS-50 A.E.I. mass spectrometer at the Institute of Organic Chemistry, University of Bonn. Purity of the prepared compounds was checked by TLC on silica gel 60 F_{254} (Merck) aluminum plates, using dichloromethane:methanol (9:1) or dichloromethane:methanol (3:1) as the mobile phase. Melting points were determined on a Büchi 530 melting point apparatus and are uncorrected. Elemental microanalyses were performed at the Pharmaceutical Institute, University of Bonn.

8-Cyclopentyl-1-propylxanthine (**38**),[26] 8-phenylxanthine (**40**),[41] 1-ethyl-8-phenylxanthine (**41**),[26] 3-methyl-6-phenyl-1,5-dihydropyrrolo[3,2-*d*]pyrimidin-2,4-dione (**43**),[36] and 6-phenyl-3-propyl-1,5-dihydropyrrolo[3,2-*d*]pyrimidin-2,4-dione (**44**)[36] were prepared as described. 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX **39**) was obtained from commercial sources. 3-Substituted 5,6-diaminouracils (**3a**−**3c**) were prepared from 6-aminouracil via regioselective alkylation followed by nitrosation and reduction as described.[42,43] 1,3-Disubstituted 6-aminouracils were prepared from urea derivatives as described followed by nitrosation and reduction to obtain 1,3-disubstituted 5,6-diaminouracils.[44]

6-Amino-5-benzylidenamino-3-propyluracil (4aa), 6-Amino-5-benzylidenamino-3-butyluracil (4ba), 6-Amino-5-(4-bromobenzyliden)amino-3-propargyluracil (4cb), 6-Amino-3-butyl-5-(4-carboxybenzyliden)aminouracil (4bc), 6-Amino-5-cinnamylidenamino-3-propyluracil (4ad), and 6-Amino-3-butyl-5-(4-carboxymethyloxybenzyliden)aminouracil (4be). General Procedure (Method A). To a solution of 3-substituted 5,6-diaminouracil (2.8 mmol) **3a**, **3b**, or **3c**, in ethanol, was added an equimolar amount of the appropriate aldehyde. The reaction mixture was refluxed for 4−5 h with monitoring by TLC. The reaction mixture was allowed to cool, and the reaction product was collected by filtration, dried, and recrystallized from ethanol.

6-Amino-5-(4-bromobenzylidenamino)-3-propyluracil (4ab), 6-Amino-5-(4-bromobenzylidenamino-3-butyluracil (4bb), and 6-Amino-5-(4-methoxycarbonylmethoxybenzylidenamino)-3-propyluracil (4af). General Procedure (Method B). To a solution of 3-substituted 5,6-diaminouracil (2.8 mmol) **3a**, **3b**, or **3c**, in ethanol, was added an equimolar amount of the appropriate aldehyde followed by a few drops of acetic acid. The reaction mixture was stirred at room temperature for 1 h and then precipitated by the addition of water to yield **4ab** and **4bb**. In case of **4af**, the starting compounds were refluxed for 0.5 h and then cooled, and the product was collected by filtration.

6-Amino-3-propyl-5-(4-sulfophenyl)carboxamidouracil (16ag), 6-Amino-3-butyl-5-(4-sulfophenyl)carboxamidouracil (16bg), 6-Amino-5-(4-bromophenyl)carboxamido-1,3-dipropyluracil (16db), and 6-Amino-1,3-dipropyl-5-(4-iodophenyl)carboxamidouracil (16dh). General Procedure. To a solution of 5,6-diaminouracil (2.8 mmol) **3a**, **3b**, or **3d**, in methanol or methanol:water (1:1) was added an equimolar amount of the appropriate acid derivative, and then an equimolar or slightly excessive amount of *N*-(3-(dimethylamino)-propyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) was added. The reaction mixture was stirred overnight at room temperature. The product was separated either by evaporation of the solvent in vacuo followed by suspension of the residue in a small amount of methanol and subsequent filtration, or by precipitation of the product by the addition of water followed by filtration.

6-Amino-5-(6-carboxy)naphth-2-oylamino-3-propyluracil (21). 2,6-Naphthalenedicarboxylic acid (0.22 g, 1 mmol) was dissolved in 15 mL of DMF, then 0.1 g (1 mmol) of *N*-methylmorpholine was added, and the reaction mixture was cooled in an ice bath. Isobutylchloroformate (0.14 g, 1 mmol) was added, and 25 min later 5,6-diamino-3-propyluracil (**3a**, 0.19 g, 1 mmol) was added. A precipitate was formed within 10−20 min, which was filtered off and washed with water.

8-Phenyl-1-propylxanthine (5), 1-Butyl-8-phenylxanthine (6), 8-(4-Bromophenyl)-1-propargylxanthine (9), 1-Butyl-8-(4-carboxy)phenylxanthine (10), 8-Cinnamyl-1-propylxanthine (11), 1-Butyl-8-(4-carboxymethyloxy)-phenylxanthine (12), and 8-(4-Methoxycarbonylmethoxy)-phenyl-1-propylxanthine (13). General Procedure. 3-Substituted 6-amino-5-benzylidenaminouracil **4aa**, **4ba**, **4cb**, **4bc**, **4ad**, **4be**, or **4af** (4.4 mmol) was dissolved at 0 °C in 120 mL of thionyl chloride. The reaction mixture was refluxed for 1 h, and then the mixture was stirred at room temperature overnight. In the case of **4ad**, the solution was refluxed for 30 min and subsequently stirred for 2 h. Compound **4af** was stirred for 2 h at 70 °C only. Thionyl chloride was distilled off, the residue was suspended in iced water and then filtered, and the residue was washed with water affording the expected products, which were recrystallized by dissolving them in DMF followed by dropwise addition of water.

8-(4-Bromophenyl)-1-propylxanthine (7), 8-(4-Bromophenyl)-1-butylxanthine (8). Benzylidene derivatives **4ab** or **4bb** (1.71 mmol) and 2.07 mmol of anhydrous ferric chloride were refluxed for 3 h in 15 mL of methanol. The reaction mixture was cooled, 30 mL of water was added, and the formed precipitate was collected by filtration. The products were recrystallized by dissolving them in DMF followed by dropwise addition of water.

1-Propyl-8-(4-sulfophenyl)xanthine (17) and 1-Butyl-8-(4-sulfophenyl)xanthine (18). HMDS Method. Carboxamide derivative **16ag** or **16bg** (2.71 mmol) was dissolved in 50 mL of hexamethyldisilazane (HMDS) in the presence of a catalytic amount of $(NH_4)_2SO_4$. The reaction mixture was refluxed at 140 °C for 50 h. HMDS was distilled off in vacuo, and the residue was treated with 10 mL of methanol and 10 mL of water. The formed precipitate was filtered off and recrystallized from water.

PPSE Method. Carboxamide derivative **16ag** or **16bg** (2.71 mmol) was refluxed in 8 mL of polyphosphoric acid trimethylsilyl ester (PPSE) at 160−180 °C for 1 h. After cooling, the reaction mixture was treated with 20 mL of methanol and the formed precipitate was filtered off and recrystallized from water.

8-(4-Bromophenyl)-1,3-dipropylxanthine (19) and 8-(4-Iodophenyl)-1,3-dipropylxanthine (20). Carboxamide derivative **16db** or **16dh** (0.977 mmol) was refluxed in a mixture of 10 mL of methanol and 10 mL of 10% aqueous NaOH solution for 30 min at 70 °C. The reaction mixture was filtered while hot. The methanol was distilled off, and the residue was taken up in H_2O and acidified with HCl to pH 4. The precipitate was filtered off and washed with 20 mL of water.

8-(6-Carboxynaphth-2-yl)-1-propylxanthine (22). Compound **21** (0.1 g, 0.26 mmol) was dissolved in 20 mL of HMDS, and 0.1 mL of trimethylchlorosilane (TMSCl) and 20 mg of *p*-toluenesulfonic acid were added. The reaction mixture was refluxed for 36 h. HMDS was removed in vacuo, then water was added, and the mixture was boiled for 10 min. The obtained suspension was filtered after cooling.

1-Butyl-8-[4-(carboxymethyl)phenyl]xanthine (27). (a) Preparation of 4-Cyanomethylbenzoic Acid (24). A solution of 4-chloromethylbenzoic acid (**23**, 3 g, 17.6 mmol) in THF

was carefully added to a saturated solution of $NaHCO_3$ (15 mL). NaCN (5.08 g, 103.6 mmol) was added followed by H_2O (16.5 mL). The reaction mixture was kept at 20−25 °C for 48 h. Then it was cooled in an ice bath and acidified with concentrated HCl to pH 4 (with caution). THF was removed by evaporation under reduced pressure (caution!), and then the solution was acidified with HCl to pH 2. The brown solid which had formed was collected, washed with H_2O, and then dissolved in 1 N NaOH (30 mL). Acidification of the charcoal-treated and filtered solution afforded the expected product **24** which was collected by filtration.

(b) Preparation of 6-Amino-3-butyl-5-(4-cyanomethylbenzoyl)amidouracil (25). Compound **3b** (0.93 g, 4.7 mmol), an equimolar amount of **24** (0.76 g, 4.7 mmol), and EDC (0.9 g, 4.7 mmol) were dissolved in 40 mL of CH_3OH:H_2O (1:1, v/v). The reaction mixture was stirred at room temperature overnight, the solvent was evaporated in vacuo, and the residue was suspended in a small amount of methanol and then filtered to afford the expected product **25** which was recrystallized from methanol.

(c) Preparation of 1-Butyl-8-[4-(cyanomethyl)phenyl]-xanthine (26). Compound **25** (0.5 g, 1.46 mmol) was dissolved in 50 mL of HMDS in the presence of a catalytic amount of $(NH_4)_2SO_4$. The reaction mixture was refluxed for 50 h. The reaction was monitored by TLC until the starting compound had completely disappeared. HMDS was distilled off in vacuo, and to the residue were added 10 mL of CH_3OH and 10 mL of H_2O. Then the precipitate was filtered off and recrystallized by dissolving it in DMF and subsequent dropwise addition of H_2O.

(d) Preparation of 1-Butyl-8-[4-(carboxymethyl)phenyl]xanthine (27). Compound **26** (0.25 g, 0.77 mmol) was dissolved in 3.4 mL of H_2O and 3 mL of H_2SO_4 and refluxed for 6 h. Then the reaction mixture was cooled to room temperature, 10 mL of H_2O was added, and the mixture was left in the refrigerator overnight. After filtration, the solid product was dissolved in aqueous NaOH solution, reprecipitated by addition of aqueous HCl (to pH 4), then filtered off, and recrystallized by dissolving it in DMF followed by the dropwise addition of H_2O.

Synthesis of Amide Derivatives 28−37. 1-Butyl-8-[4-((4-ethylpiperazin-1-yl)-2-oxo-ethoxy)phenyl]xanthine (31) and 1-Butyl-8-[4-((4-benzyloxycarbonylpiperazin-1-yl)-2-oxoethoxy)phenyl]xanthine (37). Method A (Carbodiimide Method). A solution of **12** (0.558 mmol), the desired amine derivatives (1.116 mmol), EDC (1.116 mmol), and 4-(dimethylamino)pyridine (DMAP, 0.346 mmol) in 22 mL of anhydrous DMF:CH_2Cl_2 (1:1, v/v) was stirred at room temperature for 48 h (**31**) or for 3 days (**37**) with follow up by TLC. The mixture was evaporated to dryness under reduced pressure, and the residue was dissolved in 40 mL of CH_2Cl_2 and a few drops of MeOH and left for precipitation. The precipitate was filtered off and recrystallized from CH_2Cl_2:CH_3OH (8:2) affording the desired products **31** and **37**.

1-Butyl-8-[4-((4-carboxymethyl)phenylamino-2-oxo-ethoxy)phenyl]xanthine(30), 1-Butyl-8-[4-((4-acetyl-piperazin-1-yl)-2-oxoethoxy)phenyl]xanthine (32), 1-Butyl-8-[4-((4-ethoxycarbonyl-piperazin-1-yl)-2-oxo-ethoxy)phenyl]xanthine (33), 1-Butyl-8-[4-((4-phenylpiperazin-1-yl)-2-oxoethoxy)phenyl]xanthine (34), 1-Butyl-8-[4-((4-benzyl-piperazin-1-yl)-2-oxoethoxy)phenyl]xanthine (35), and 1-Butyl-8-[4-((4-(2-metoxyphenyl-piperazin-1-yl)-2-oxo-ethoxy)phenyl]xanthine (36). Method B (Acyl Chloride Method). A solution of **12** (0.558 mmol) in 11 mL of thionyl chloride was stirred at 70°C for 4 h. Then the excess of thionyl chloride was removed by evaporation. To the residue was added a solution of the desired amine derivative (1.116 mmol) in 22 mL of anhydrous pyridine:CH_2Cl_2 (1:1, v/v), and the mixture was stirred at room temperature for 24−48 h and subsequently evaporated to dryness under reduced pressure. Isolation and purification of compounds **30** and **32**−**36** was achieved as described above for compounds **31** and **37**.

8-[4-((2-Aminoethylamino)-2-oxoethoxy)phenyl]-1-propylxanthine (28). Method C. Compound **13 (**200 mg, 0.558 mmol) was dissolved at 150 °C in DMF, then the solution was cooled to 60 °C, and 67 mg (1.116 mmol) of ethylenediamine was added. After stirring for 2 days at room temperature, the reaction mixture was concentrated producing a precipitate which was filtered off and washed with water and methanol affording the expected product **28**.

8-[4-((2-Hydroxyethylamino)-2-oxoethoxy)phenyl]-1-propylxanthine (29). Compound **13** (100 mg, 0.279 mmol) was dissolved in hot DMF, 17 mg (0.279 mmol) of ethanolamine was added at 40 °C, and the mixture was stirred overnight at room temperature. The formed precipitate was filtered off and washed with methanol affording the expected product **29**.

8-(4-Methylcarboxyethylidene)phenyl-3-propylxanthine (14). Compound **4g** (0.2 g, 0.58 mmol) was suspended in 10 mL of $SOCl_2$, and the mixture was refluxed for 30 min. Then the excess of $SOCl_2$ was distilled off. The solid residue was cooled in an ice bath, and 15 mL of methanol was added. The suspension was refluxed for 30 min. The cream-colored solid was filtered off and washed with methanol.

8-(4-Carboxymethyloxyphenyl)-1-propylxanthine (15). Compound **13** (0.36 g 1 mmol) was dissolved in 5 mL of DMF, and 5 mL of 0.1 N aqueous Na_2CO_3 solution was added. The reaction mixture was heated in a steam bath for 30 min. Then the solvent was concentrated, and the mixture was filtered. The filtrate was acidified (to pH 3) with concentrated HCl, and the formed precipitate was filtered off.

Biological Assays. Materials. Radioligands were obtained from the following sources: [^{3}H]CCPA from NEN Life Science (54.9 Ci/mmol), [^{3}H]MSX-2 from Amersham (85 Ci/mmol), [^{3}H]-ZM241385 from Tocris (17 Ci/mmol), and [^{3}H]PSB-11 from Amersham (53 Ci/mmol). The nonradioactive precursors of [^{3}H]-MSX-2 and [^{3}H]PSB-11 were synthesized in our laboratory.

Membrane Preparations. Membranes from Chinese hamster ovary (CHO) cells stably transfected with the human A_1, the human A_{2A}, or the human A_3 AR were prepared as described.[45] For A_{2B} adenosine receptor assays, commercially available membrane preparations containing the human A_{2B} AR were obtained from Biotrend (Cologne, Germany).

Frozen rat brains obtained from Pel Freez, Rogers, AR were dissected to obtain cortical membrane preparations for A_1 assays, and striatal membrane preparations for A_{2A} assays as described.[46,47]

Radioligand Binding Assays. Stock solutions of the compounds were prepared in dimethyl sulfoxide (DMSO); the final concentration of DMSO in A_{2B} assays was 1%, and in the other assays not more than 2.5%. The radioligand concentrations were as follows: [^{3}H]CCPA:[48] 0.5 nM (rat or human A_1); [^{3}H]MSX-2:[49] 1.0 nM (rat or human A_{2A}); [^{3}H]ZM241385:[50] 5 nM (human A_{2B}); [^{3}H]PSB-11:[51] 0.5 nM (human A_3). Binding assays were performed essentially as described.[45,48−50] The A_3 binding assay is described below. About 40−70 μg/mL of protein were used in the assays. Membranes were preincubated for 30 min with 0.12 IU/mL of adenosine deaminase in order to remove endogenous adenosine. Curves were determined using 6−7 different concentrations of test compounds spanning 3 orders of magnitude. At least two to three separate experiments were performed, each in duplicate (human receptors) or triplicate (rat receptors).

A_3 Binding Assays. Binding assays were performed using [^{3}H]PSB-11[51] in 50 mM TRIS−HCl buffer at pH 7.4. Assays were incubated on a shaking water bath at 23 °C for 30 min. Nonspecific binding was determined in the presence of 100 μM of *R*-PIA and amounted to less than 5% of total binding. Total binding was determined in the presence of 2% DMSO, and ca. 50 μg of protein per tube (containing a final volume of 0.5 mL) was added to start the reaction. Termination of the incubation was performed by rapid filtration through GF/B glass fiber filters, presoaked in rinse buffer, using a Brandel 48 channel harvester. Filters were washed three times with 2 mL of ice-cold rinse buffer each. Radioactivity of the punched-out wet filters was counted after 9 h of preincubation with 3 mL of Ultima Gold scintillation cocktail (Canberra Packard, Dreieich, Germany).

Data Analysis. Data were analyzed using Graph Pad PRISM Version 3.0 (San Diego, CA). For non-linear regression analysis, the Cheng−Prusoff equation and K_D values of 0.5 nM (rat A_1) and 0.61 nM (human A_1) for [^{3}H]CCPA, 8.0 nM (rat A_{2A}) and 7.3 nM (human A_{2A}) for [^{3}H]MSX-2, 33 nM for [^{3}H]ZM241385, and 4.9 nM for [^{3}H]PSB-11 were used to calculate K_i values from IC_{50} values.

Supporting Information Available: ^{1}H and ^{13}C NMR data of synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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