Pergamon

Bioorganic & Medicinal Chemistry 6 (1998) 707–719

BIOORGANIC & MEDICINAL CHEMISTRY

8-(Sulfostyryl)xanthines: Water-soluble A_{2A}-Selective Adenosine Receptor Antagonists†

Christa E. Müller,[a,*] Jesús Sandoval-Ramírez,[a,‡] Ulrike Schobert,[a] Uli Geis,[a] Wolfram Frobenius[a] and Karl-Norbert Klotz[b]

[a]*Institut für Pharmazie und Lebensmittelchemie, Julius-Maximilians-Universität Würzburg, Pharmazeutische Chemie, Am Hubland, D-97074 Würzburg, Germany*

[b]*Institut für Pharmakologie und Toxikologie, Julius-Maximilians-Universität Würzburg, Versbacher Str. 9, D-97078 Würzburg, Germany*

Received 22 September 1997; accepted 24 November 1997

Abstract—8-(Sulfostyryl)xanthine derivatives were synthesized as water-soluble A_{2A}-selective adenosine receptor (AR) antagonists. *meta*- and *para*-sulfostyryl-DMPX (3,7-dimethyl-1-propargylxanthine) derivatives **11a** and **11b** exhibited high affinity to rat A_{2A}-AR in submicromolar concentrations, and were 20- to 30-fold selective versus rat A_1-AR. Styryl-DMPX derivatives were inactive at human A_{2B}- and A_3-AR. 1,3-Dipropyl-8-*p*-sulfostyrylxanthine (**13**) and its 7-methyl derivative (**14**) showed similar (**13**) or higher (**14**) A_{2A} affinity than **11a** and **11b** but showed no (**13**) or only a low degree (**14**) of selectivity versus A_1-, A_{2B}-, and A_3-AR. The A_{2A}-selective sulfostyryl-DMPX derivatives exhibit high water-solubility and may be useful research tools for in vivo studies.

Introduction

Cell membrane receptors for the physiological nucleoside adenosine are found in many organs and tissues.[1] Four different types of adenosine receptors (AR) have been identified and cloned, namely the high-affinity subtypes A_1 and A_{2A} and the low affinity subtypes A_{2B} and A_3.[2] All AR agonists known so far are derivatives of adenosine, the ribose moiety of the nucleoside being essential for agonism of the compounds.[3] The main classes of potent AR antagonists at least for the high affinity receptor subtypes A_1 and A_{2A} are (i) adenine derivatives and adenine analog, and (ii) xanthine derivatives.[4] The development of AR-antagonists with selectivity for the A_1-AR is most advanced as compared to other subtypes, several compounds being currently evaluated in clinical studies for the treatment of senile dementias, such as Alzheimer's disease, and for the prevention of acute renal failure.[5] A_{2A}-selective AR antagonists have potential as novel therapeutic agents for the treatment of Parkinson's disease. Further proposed indications include, for example, hypotension and acute ischemias.[6] The first AR antagonist described in the literature as A_{2A}-selective was 3,7-dimethyl-1-propargylxanthine (DMPX, **1**), a compound of low AR-affinity and low A_{2A}-selectivity versus A_1, however no selectivity versus A_{2B}-AR.[4,7] Nevertheless DMPX is still widely used as A_{2A}-antagonist in in vivo studies due to its good water-solubility and high bioavailability. During the past years potent and selective A_{2A}-AR antagonists have been developed, including 8-styrylxanthine derivatives (**2**–**5**);[8–10] among the most potent and most selective compounds are the 8-styryl-DMPX derivatives, such as **4** and **5**.[10] Further potent A_{2A}-selective AR antagonists include compounds that can be envisaged as adenine analogs, namely the pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines,[11] (e.g. SCH58261, and the triazolo-[1,5-*a*][1,3,5]triazine ZM241385.[12] A major

Key words: Xanthines; sulfonic acids; A_1-, A_{2A}-, A_{2B}-, A_3-adenosine receptors; A_{2A}-antagonists; water-solubility.

*Corresponding author. Tel: +49-(0)931-888-5440; Fax: +49-(0)931-888-5462; E-mail: mueller@pharmazie.uni-wuerzburg.de

†Preliminary results were presented at the International Symposium "Purines '96" in Milan, Italy; abstract published in *Drug Dev. Res.* **1996**, *37*, 112.

‡J. S.-R. was on leave from the Facultad de Ciencias Químicas, Benemérita Universidad Autónoma de Puebla (BUAP), Puebla, México, with support from Deutscher Akademischer Austauschdienst (DAAD).

0968-0896/98/$19.00
PII: S0968-0896(98)00025-X

problem associated with potent selective A_{2A}-antagonists is their typically low water-solubility, which limits their in vivo applicability. So far, only few efforts have been undertaken to improve water-solubility of A_{2A}-antagonists. Jacobson et al. synthesized a congener of 8-(*m*-aminostyryl)caffeine, a succinic acid amide, bearing a carboxylic function.[9] Solubility of that compound in basic solution (0.1 M K_2HPO_4) was high (19 mM/L), but may be lower at physiological pH values. AR antagonists with extraordinarily high water-solubility over a wide range of pH values are the 8-sulfophenylxanthines SPT (**15**) and DPSPX (**18**), compounds derived from the A_1-selective antagonists 8-phenyltheophylline and 1,3-dipropyl-8-phenylxanthine.[13,14] Despite lacking receptor subtype selectivity, these compounds have become important pharmacological tools due to their excellent water-solubility. Since sulfonic acids are deprotonated under physiological conditions, such compounds do not penetrate into the brain and are only peripherally active.[13,15]

In the present study, we prepared sulfo-derivatives of A_{2A}-selective styrylxanthines and evaluated their AR affinity and selectivity profile. Our goal was to obtain A_{2A}-selective antagonists with high water-solubility at physiological pH values useful as pharmacological tools and potential drugs without central stimulatory effects.

Results and Discussion

Chemistry

8-Sulfostyrylxanthines were synthesized from 5,6-diaminouracil derivatives and sulfocinnamic acids (Schemes 1 and 2). *p*-Sulfocinnamic acid **7a** was prepared by sulfonation of cinnamic acid with fuming sulfuric acid according to a procedure published in 1927.[16] Sulfonation yielded the *p*-substituted isomer **7a** as the main product. *m*-Sulfocinnamic acid was obtained as a by-product. Both isomers were separated by fractionating crystallization. The *meta*-sulfonated cinnamic acid derivative **7b** could alternatively be obtained by nucleophilic substitution of *m*-bromocinnamic acid with sodium sulfite as described (Method B).[16] The latter method is preferable for the preparation of **7b** resulting in higher yields. Isomers **7a** and **7b** could easily be distinguished by their ^1H and ^{13}C NMR spectra (see Experimental).

1-Propargylxanthine derivatives were prepared starting from 5,6-diamino-3-propargyluracil **6**[17,18] by condensation with *para*- or *meta*-sulfocinnamic acid **7a**, or **7b**, respectively. Amides **8a** and **8b** were methylated in the 3-position in analogy to described procedures.[18] Direct ring closure of **8a** and **8b** is not successful, since deprotonation in alkaline solution reduces the nucleophilicity of the 6-amino function. Ring closure in acidic media, however, leads to side reactions of the propargyl group.[19] Treatment of the methylated compounds **9a** and **9b** with a solution of sodium hydroxide in a mixture of methanol and water yielded xanthines **10a** and **10b**, which were methylated in the 7-position to yield the target compounds **11a** and **11b** (Scheme 1).

For the preparation of 1,3-dipropylxanthine derivatives the standard procedure was applied.[13,14] Thus, 5,6-diamino-1,3-dipropylxanthine (**12**) was condensed with sulfocinnamic acid **7a**, followed by intramolecular condensation in alkaline solution to xanthine **13**, which was methylated to **14** (Scheme 2).

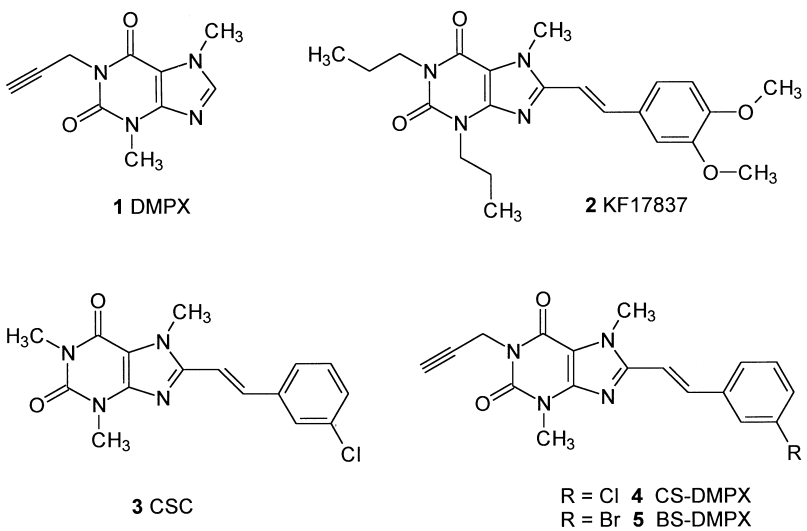
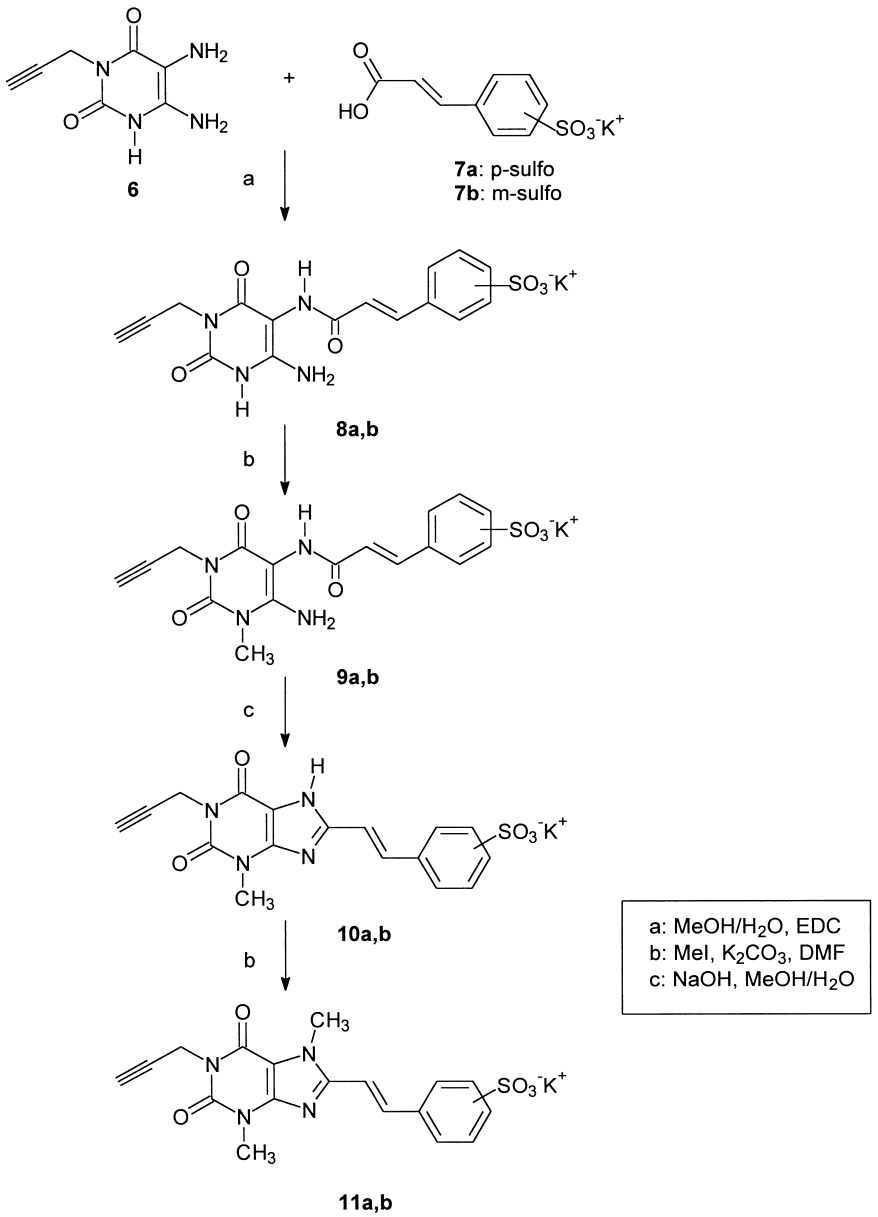


Chart 1. A_{2A}-Adenosine receptor antagonists with xanthine structure.

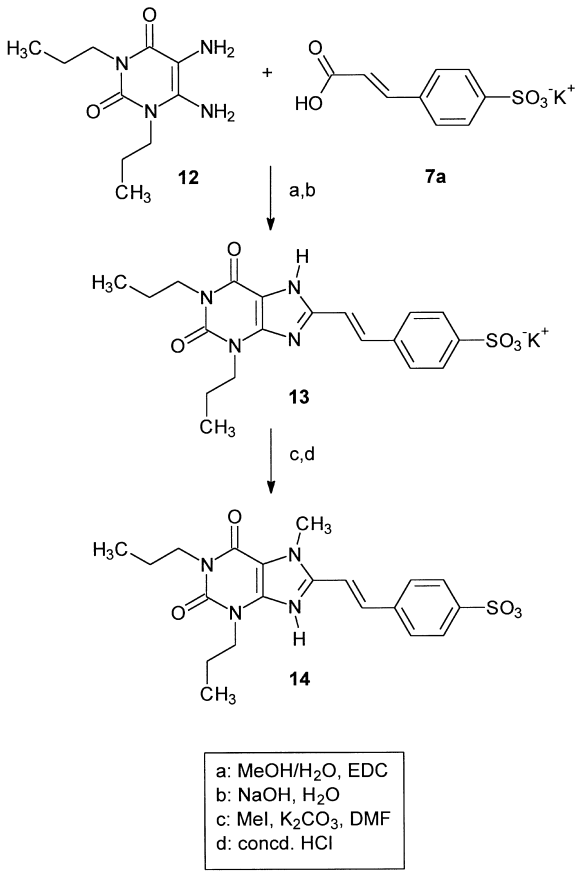


Scheme 1. Synthesis of 8-sulfostyryl-3,7-dimethyl-1-propargylxanthine (8-sulfostyryl-DMPX derivatives).

^{1}H and ^{13}C NMR spectral data for intermediate and final products confirmed the proposed structures (Tables 1–3). (*E*)-configuration of the double bond is retained as shown by the coupling constant of about 16 Hz for the vinylic protons of the sulfostyryl derivatives (Tables 1 and 2). For (*Z*)-configurated isomers a coupling constant of ca. 10–13 Hz would have to be expected.[20,21]

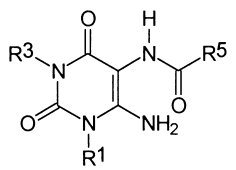
It had been difficult to obtain ^{13}C NMR spectra of 8-styrylxanthine derivatives investigated so far due to their low solubility in polar as well as nonpolar solvents, including water, dimethylsulfoxide (DMSO) and chloroform. Sulfostyrylxanthines, however, are well soluble in water and DMSO, and ^{13}C NMR spectra can be routinely recorded (Table 3).

Styrylxanthine derivatives had been observed to exhibit photo-induced isomerization in dilute solution.[10,20] We investigated *E*/*Z*-isomerization for one representative example of sulfostyrylxanthines, *p*-sulfostyryl-DMPX (**11a**), by measurement of the decrease in UV absorption at 356 nm, induced by exposure to normal daylight. Our



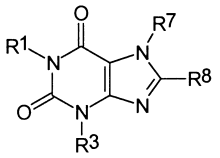
Scheme 2. Synthesis of 1,3-dipropyl-8-sulfostyrylxanthine derivatives.

Table 1. ^{1}H NMR data of intermediate carboxamidouracil derivatives



Compd	δ (ppm) in DMSO-d_6, J (Hz)			
	R^1	R^3	R^5	6-NH_2
8a	10.67 (br s, 1H)	3.05 (t, J = 2.3, 2H), 4.45 (d J = 2.3, 2H)	6.83 (d, J = 15.8, 1H, $H_{vinylic}$), 7.45 (d, J = 15.8, 1H, $H_{vinylic}$), 7.56 (d, J = 8.3, 2H, $H_{aromatic}$), 7.67 (d, J = 8.3, 2H, $H_{aromatic}$), 8.74 (s, 1H, NH)	6.26 (br s, 2H)
8b	10.62 (br s, 1 H)	3.05 (t, J = 2.1, 1H), 4.42 (d, J = 2.2, 2H)	6.85 (d, J = 16.0, 1H, $H_{vinylic}$), 7.36–7.86 (m, 5H, $H_{vinylic}$, $H_{aromatic}$), 8.75 (s, 1H, NH)	6.23 (br s, 2H)
9a	3.35 (s, 3H)	3.04 (t, J = 2.1, 1H), 4.50 (d, J = 2.1, 2H)	6.82 (d, J = 15.8, 1H, $H_{vinylic}$), 7.45 (d, J = 15.8, 1H, $H_{vinylic}$), 7.55 (d, J = 8.2, 2H, $H_{aromatic}$), 7.65 (d, J = 6.5, 2H, $H_{aromatic}$), 8.72 (s, 1H, NH)	6.84 (br s, 2H)
9b	3.32 (s, 3H)	3.04 (t, J = 2.0, 1H), 4.48 (d, J = 2.1, 2H)	6.82 (d, J = 15.7, 1H, $H_{vinylic}$), 7.40–7.66 (m, 5H, $H_{vinylic}$, $H_{aromatic}$)	6.86 (br s, 2H)

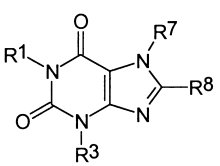
Table 2. [1]H NMR data of sulfostyrylxanthine derivatives



Compd	δ (ppm) in DMSO-d_6, J (Hz) R[1]	R[3]	R[7]	R[8]
10a	2.94 (t, J = 2.3, 1H), 4.58 (d, J = 3.43 (s, 3H) 2.3, 2H)		[a]	7.00 (d, J = 16.2, 1H, $H_{vinylic}$), 7.19 (d, J = 16.2, 1H, $H_{vinylic}$), 7.45 (d, J = 8.3, 2H, $H_{aromatic}$), 7.45 (d, J = 8.3, 2H, $H_{aromatic}$)
10b	2.95 (t, J = 1.8, 1H), 4.58 (d, J = 3.42 (s, 3H) 1.9, 2H)		[a]	7.01 (d, J = 16.1, 1H, $H_{vinylic}$), 7.18–7.75 (m, 5H, $H_{aromatic}$, $H_{vinylic}$)
11a	3.05 (t, J = 2.3, 1H), 4.57 (d, J = 3.46 (s, 3H) 2.3, 2H)		4.01 (s, 3H)	7.31 (d, J = 15.8, 1H, $H_{vinylic}$), 7.67 (d, J = 15.8, 1H, $H_{vinylic}$), 7.62 (d, J = 8.0, 2H, $H_{aromatic}$), 7.73 (d, J = 8.0, 2H, $H_{aromatic}$)
11b	3.08 (t, 1H), 4.57 (d, 2H)	3.45 (s, 3H)	4.03 (s, 3H)	7.06 (d, J = 15.9, 1H, $H_{vinylic}$), 7.36–7.79 (m, 5H, $H_{vinylic}$, $H_{aromatic}$)
13	0.82 (q, J = 12.8, J = 5.5, 6H, CH_3), 1.54 (sext., J = 15.0, J = 7.5, 2H, CH_2-CH_3), 3.82(t, J = 7.1, 2H, N-CH_2)	0.82 (q, J = 12.8, J = 5.5, 6H, CH_3, 1.69 (sext., J = 14.6, J = 7.5, 2H, CH_2-CH_3), 3.95 (t, J = 6.7, 2H, N-CH_2)	[a]	7.04 (d, J = 16.4, 1H, CH = CH), 7.58 (d, J = 8.4, 2H, $H_{aromatic}$), 7.62 (d, J = 16.3, 1H, CH = CH), 7.66 (d, J = 8.4, 2H, $H_{aromatic}$)
14	0.86, (q, J = 15.0, J = 7.4, 3H, CH_3), 1.54 (sext., J = 15.0, J = 7.5, 2H, CH_2-CH_3), 3.80 (t, J = 7.5, 2H, N-CH_2)	0.86 (q, J = 15.0, J = 7.4, 3H, CH_3), 1.71 (sext., J = 14.6, J = 7.5, 2H, CH_2-CH_3), 3. (t, J = 7.6, 2H, N-CH_2)	4.01 (s, 3H)	7.36 (d, J = 17.7, 1H, CH = CH), 7.61 (d, J = 8.6, 2H, $H_{aromatic}$) 7.63 (d, J = 15.2, 1H, CH = CH), 7.74 (d, J = 8.5, 2H, $H_{aromatic}$)

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

Table 3. [13]C NMR data of selected sulfostyrylxanthine derivatives



Compd	δ (ppm)in DMSO-d_6 C-2	C-4	C-5	C-6	C-8	R[1]	R[3]	R[7]	R[8]
11a	150.11	148.85*	107.09	153.16	149.97	29.47, 72.70, 79.65	29.92	31.47	112.96 (CH = CH), 126.00, 127.04, 135.50, 136.33, 138.27, 148.27 ($C_{aromatic}$, CH = CH)
13	150.86	148.58	107.45	154.07	149.61	11.23, 21.08, 42.34	11.41, 21.08, 44.61	—	116.50 (CH = CH), 126.45, 126.84, 134.70, 135.85, 148.58 ($C_{aromatic}$, CH = CH)
14	150.86	148.80	107.50	154.38	149.77	11.20, 20.96, 42.07	11.35, 21.02, 44.22	31.57	113.45 (CH = CH), 126.18, 127.27, 135.85, 136.15, 147.92 ($C_{aromatic}$, CH = CH)

*arbitrary assignment

results for **11a** were in accordance with results for other 8-styrylxanthine derivatives.[10,20] Thus, isomerization was much faster in dilute solutions (≤ 0.1 mM) as compared to more concentrated solutions. Isomerization was faster in methanol, or in aqueous buffer solution, respectively, than in DMSO. After ca. 30 min, a 0.01 mM solution in aqueous buffer reached its equilibrium (data not shown). It is concluded that under test conditions, stable mixtures of *E*- and *Z*-isomers are present in the dilute test solutions.

Pharmacology

The new compounds were tested in radioligand binding assays for affinity to A_1- and A_{2A}-adenosine receptors in rat cortical membrane, and rat striatal membrane preparations, respectively. The A_1-selective agonist [^{3}H]N^6-cyclohexyladenosine ([^{3}H]CHA) was used as A_1-ligand, and the A_{2A}-selective agonist [^{3}H]2-[4-[carboxyethyl)-phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine ([^{3}H] CGS21680) as A_{2A} ligand. Since a radioligand for A_{2B}-AR was not available, the inhibition of *N*-ethylcarboxamidoadenosine- (NECA-) stimulated adenylate cyclase by test compounds was measured using human recombinant A_{2B}-AR expressed in chinese hamster ovary (CHO) cells.[22] A_3-AR affinity was determined using human recombinant A_3-AR, expressed in CHO cells with [^{3}H]NECA as radioligand.

Structure–activity relationships

8-*p*-Sulfophenylxanthines had been introduced as non-selective AR-antagonists, which are freely soluble in water at physiological pH values.[13,14] The sulfonic acid group exhibits a pK_a value of < 1 (e.g. benzenesulfonic acid: p$K_a = -6.5$)[23] and is deprotonated under physiologic conditions. The introduction of a *p*-sulfo substituent in the A_1-selective 8-phenyltheophylline or its 1,3-dipropyl homolog had resulted in a large decrease in A_1-affinity, however it reduced A_{2A}-affinity to a lower degree.[13] Therefore, we investigated the introduction of a *p*-sulfo substituent into A_{2A}-selective xanthine derivatives.

8-*p*-Sulfophenyltheophylline (SPT, **15**) exhibits low affinity for A_1-, A_{2A}-, A_{2B}-, and A_3-AR in the micromolar concentration range (see Table 4). The compound is somewhat more potent at A_1- and A_{2B}- as compared to A_{2A}- and A_3-AR. The 1,3-dipropyl analog of **15**, 1,3-dipropyl-8-*p*-sulfophenylxanthine (DPSPX, **18**) is more potent at all AR subtypes, ca. 20-fold at A_1, 10-fold at A_{2A}, fivefold at A_{2B}- and ca. 60-fold at A_3-AR (see Table 4). Thus, DPSPX (**18**) is equipotent at A_1-, A_{2B}- and A_3-AR and about five to eightfold less potent at A_{2A}-AR. The corresponding 7-methylated xanthine derivatives **16** and **19** are considerably less potent than the 7-unsubstituted compounds **15** and **18** at A_1- and A_{2A}-AR. No data were available of their A_{2B}- and A_3-AR activity.

The 3-unsubstituted 1-propyl-8-*p*-sulfophenylxanthine (**17**) exhibits similar affinity as SPT (**15**) for A_1-, A_{2A}-, and A_{2B}-AR. Only at the A_3-AR affinity of **17** is several-fold higher compared to **15** demonstrating the importance of a 1-propyl substituent for high A_3-affinity of xanthine derivatives.

One of the most potent and selective A_{2A}-AR antagonists is 8-*m*-bromostyryl-DMPX (**5**).[10] Compound **5** has now been investigated at human A_{2B}- and A_3-AR. It was found that **5** exhibits no affinity for these 'low affinity AR' and is highly selective for A_{2A} versus all other AR subtypes.

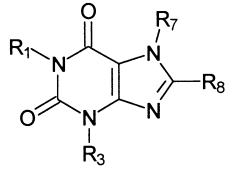
Introduction of a *para*- or *meta*-sulfo substituent into 8-styryl-DMPX (K_i $A_1 = 1.1\,\mu M$, $A_{2A} = 0.027\,\mu M$)[10] decreases AR-affinity of the compound five to eightfold at A_1-AR, and 9- to 11-fold at A_{2A}-AR. The resulting sulfostyryl-DMPX derivatives **11a** and **11b** retain A_{2A}-selectivity, which is 20-fold for **11a**, and 30-fold for **11b** versus A_1-AR. The compounds are virtually inactive at A_{2B}- and A_3-AR, like **5**, another 8-styryl-DMPX derivative.

In order to investigate which structural parameters are responsible for the high selectivity of compounds **11a**, **11b**, and **5** versus A_{2B}- and A_3-AR, 1,3-dipropyl-8-*p*-sulfostyrylxanthines were synthesized and compared with sulfostyryl-DMPX derivatives.

The 7-unsubstituted 1,3-dipropyl derivative **13** showed similar potency at all AR subtypes (150–670 nM) and therefore was virtually nonselective. As expected, 7-methylation resulted in a decrease in A_1-affinity (25-fold) and an increase in A_{2A}-affinity (fivefold). Compound **14** is the most potent, highly water-soluble A_{2A}-AR antagonist of the present series. Selectivity, however, is moderate (sevenfold versus A_1). 7-Methylation of **13** to **14** resulted in a slight decrease in A_{2B}- and A_3-affinity (twofold).

Since acidic functions on the 8-substituent of xanthine derivatives can enhance A_3-AR affinity,[24] and because acidic groups are also well tolerated by A_{2B}-AR (e.g. **13**,**14**) we investigated a carboxy-substituted 8-(2-naphthyl)xanthine derivative (**20**) at A_{2B}- and A_3-AR. The compound had been investigated as a sterically fixed analog of 8-styryl-DMPX.[26] Indeed, **20** was relatively potent at A_{2B}- and A_3-AR. This result is particularly surprising, since **20** is an 8-substituted DMPX derivative, which usually show very low activity at A_{2B}- and A_3-AR (e.g. **5**, **11a**, and **11b**). Compound **20** is about threefold selective for A_{2B}-AR versus A_1 and A_{2A} and ca. fivefold selective versus A_3 and thus could be a new lead for the development of A_{2B}-AR antagonists.

Table 4. Affinities of sulfostyrylxanthines and other xanthine derivatives for adenosine receptor subtypes



Compd	R^1	R^3	R^7	R^8	$K_i \pm$SEM[μM] A_1-Affinity Rat brain cortical membranes [^{3}H]CHA	$K_i \pm$SEM[μM] A_{2A}-Affinity Rat brain striatal membranes [^{3}H] CGS21680	K_i [μM] (95% confidence limits) A_{2B} Human recombinant receptor in CHO cell membranes Inhibition of NECA-stimulated AC	K_i [μM] (95% confidence limits) A_3-Affinity Human recombinant receptor in CHO cell membranes [^{3}H] NECA
8-(Sulfostyryl)xanthines								
10a *p*-sulfostyryl-MPX	propargyl	methyl	H	*p*-sulfostyryl	3.3 ± 0.3	0.73 ± 0.11	n.d.[a]	n.d.
11a *p*-sulfostyryl-DMPX	propargyl	methyl	methyl	*p*-sulfostyryl	4.9 ± 0.4	0.24 ± 0.05	> 30	> 100
11b *m*-sulfostyryl-DMPX	propargyl	methyl	methyl	*m*-sulfostyryl	8.9 ± 1.5	0.30 ± 0.09	> 30	> 100
13 1,3-dipropyl-8-*p*-sulfostyrylX	propyl	propyl	H	*p*-sulfostyryl	0.15 ± 0.03	0.24 ± 0.08	0.34 (0.14–0.83)	0.67 (0.404–1.113)
14 1,3-dipropyl-7-methyl-8-pSSX	propyl	propyl	methyl	*p*-sulfostyryl	0.38 ± 0.06	0.051 ± 0.004	0.77 (0.10–5.74)	1.27 (0.45–3.55)
8-(Sulfophenyl)xanthines								
15 SPT	methyl	methyl	H	*p*-sulfophenyl	14[38,b]	14[38,b]	1.2[39,c]	11[40]
16 8-*p*-sulfophenylcaffieine	methyl	methyl	methyl	*p*-sulfophenyl	> 250[38,b]	> 250[38,b]	n.d.	n.d.
17 1-propyl-8-sulfophenylX	propyl	H	H	*p*-sulfophenyl	2.2[33,b]	24[33,b]	0.82 (0.14–4.74)	1.73 (0.49–6.17)
18 DPSPX	propyl	propyl	H	*p*-sulfophenyl	0.21[38,b]	1.4[38,b]	0.25[41,d]	0.183[24,e]
19	propyl	propyl	methyl	*p*-sulfophenyl	15[38,f]	5.6[38,f]	n.d.	n.d.
Further xanthine derivatives								
5 BS-DMPX	Propargyl	methyl	methyl	*p*-bromostyryl	1.2[10]	0.0082[10]	> 10	> 10
20	Propargyl	methyl	H	6-carboxy-2-naphthyl	0.76[26]	0.75[26]	0.27 (0.15–0.49)	1.45 (0.34–6.26)

[a] n.d. = not determined.
[b][^{3}H]PIA was used as A_1-radioligand and [^{3}H]NECA as A_{2A}-radioligand.
[c]Inhibition of adenosine-induced stimulation of adenylate cyclase in human fibroblast cells.
[d]Inhibition of NECA-induced stimulation of adenylate cyclase in human erythroleukemia cells.
[e]Sheep A_3 receptor.
[f]Inhibition of NECA-induced stimulation of adenylate cyclase in PC12 cell membranes (K_b value).

In conclusion, 8-styryl-DMPX derivatives, including the water-soluble 8-sulfostyryl-DMPX derivatives **11a** and **11b**, exhibit selectivity for A_{2A}-AR versus A_1, and extraordinarily high selectivity versus A_{2B}- and A_3-AR, at which these compounds are virtually inactive. In contrast, 1,3-dipropyl-(7-methyl)-8-styrylxanthine derivatives (**13** and **14**) retain activity at A_{2B}- and A_3-AR. Propyl substitution in the 1- and 3-position is particularly important for high A_3-AR affinity.

Water solubility

A certain degree of water-solubility is a prerequisite for in vivo activity of a drug. Bruns and Fergus had postulated that the solubility over AR-affinity ratio of a compound (the so-called Bruns–Fergus or BF index) has to be greater than 100 in order to exhibit good in vivo activity.[27] For experimental drugs it is often desirable that they are highly water-soluble (e.g. in order to allow for parenteral application).

A major problem associated with AR-antagonists developed by in vitro screening methods is their generally low water-solubility, which limits their usefulness, especially for in vivo studies. Purine—including xanthine—derivatives, in particular, are often poorly soluble in water due to base-stacking and the formation of intermolecular hydrogen bonds.

Efforts have been undertaken to improve the water-solubility of potent A_1-selective AR-antagonists by introducing hydrophilic substituents into the molecules at positions where they are tolerated by the receptor.[5] Some of these 'second generation A_1-AR antagonists' are now under clinical development as drugs.[5] Solubility of the standard A_{2A}-antagonist KF17837 (**2**) has been reported to be as low as 0.06 g/mL (0.146 μM/L).[20] DMSO has to be used for in vivo studies to dissolve the compound, and solubility was reported to be a major limitation for the in vivo use of **2**.[28,29] Dionisotti et al. found the compound to be inactive in vivo in their experimental setting.[30]

Water solubilities of other A_{2A}-selective xanthine derivatives have not been reported. For chlorostyrylcaffeine (CSC, **3**), another standard A_{2A}-antagonist, dissolution in 45% aqueous 2-hydroxypropyl-β-cyclodextrin solution is recommended, and solubility in that mixture is given to be less than 0.3 g/L (< 900 μM/L).[31]

Potent non-xanthine A_{2A}-antagonists also exhibit low water-solubility. ZM241385, for example, had to be intravenously administered in a solution of 50% polyethylenglycol (PEG 400) and 50% aqueous NaOH solution (0.1 M) prepared by sonication.[32]

We investigated the solubilities and determined solubility over A_{2A}-AR affinity ratios for the potent, A_{2A}-selective xanthine derivatives **3** (CSC) and **4** (CS-DMPX), and for the new water-soluble sulfostyrylxanthine derivative **11a** (Table 5). These data were compared with values for the standard A_{2A} antagonist KF17837 (**2**), the therapeutically used xanthine derivatives theophylline (**21**) and caffeine (**22**), and the low-affinity A_2-AR antagonist DMPX (**1**). DMPX shows reasonable water-solubility (5 mM/L), the main reason,

Table 5. Water solubilities of A_{2A}-selective xanthine derivatives and reference compounds

Compd	K_i A_{2A} [μM] rat brain [^{3}H]CGS21680	A_{2A}-selectivity versus A_1 rat brain [^{3}H]CHA	Solubility [μM] ± SEM at rt in buffer pH 7.4 containing 1% DMSO	Solubility over A_{2A} affinity ratio (Bruns–Fergus index)
11a p-SS-DMPX	2.4	20	6,400 ± 1,750 (7,800)[a]	26,830
1 DMPX	8.6[10]	1.4[10,b]	5000[c]	580
4 CS-DMPX	0.013	100	0.296 ± 0.070 (0.476 ± 0.085)[d]	23
2 KF-17837	0.0085[10]	31[10]	0.146[20]	17
3 CSC	0.036[10] (0.054)[9]	> 28[10] (540)[9]	0.179 ± 0.014 (0.211 ± 0.030)[d]	5
21 Theophylline	9.4[10]	1.8[10]	44,400[e]	4720
22 Caffeine	22[10]	1[10]	108,000[e]	4910

[a]Solubility in water at room temperature determined by UV photometry (single determination).
[b]Exhibits somewhat higher A_{2A}-selectivity in a comparison of data from other test systems, (e.g fivefold selectivity versus [^{3}H]PIA binding to rat A_1-AR).[42]
[c]Solubility in water.[31]
[d]Solubility [μM] at room temperature in buffer pH 7.4 containing 2.5% DMSO.
[e]Solubility in water at 25 °C.[43]

why it is still widely used for in vivo studies despite its low potency and low selectivity towards A_1- and A_{2B}-AR. Theophylline and caffeine are both well soluble in water in millimolar concentrations. Solubility over A_{2A}-affinity ratio for both compounds is almost 5000. DMPX is somewhat less soluble, but still exhibits a Bruns-Fergus index of 580. The high affinity A_{2A}-antagonists, styrylxanthine derivatives **2**, **3**, and **4**, however, show very low water-solubility in submicromolar concentrations. As a consequence, their solubility over affinity ratio is only between 5 for CSC (**3**) and 23 for CS-DMPX (**4**), which is far below the required 100. In contrast, *p*-sulfostyryl-DMPX (**11a**) exhibits very high solubility of ca. 6 mM/L and a BF-index of more than 26,000. Solubility for **11a** was determined by the same radioreceptor assay that was used for the other compounds; an additional determination was made using UV-photometry. The values obtained with the two different methods were similar (Table 5).

In conclusion, we have developed A_{2A}-selective AR antagonists, which exhibit high water-solubility and may be useful research tools for in vivo studies.

Experimental

Chemistry

NMR spectra were performed on a Bruker WP-80 (^{1}H: 80 MHz, ^{13}C: 20 MHz), or a Bruker AC-250 spectrometer (^{1}H: 250 MHz, ^{13}C: 60 MHz), respectively. DMSO-d_6 or D_2O, respectively, was used as solvent. The chemical shifts of the remaining protons of the deuterated solvent served as internal standard. IR spectra were measured on a Perkin-Elmer 1750 spectrometer. All compounds were checked for purity by TLC on 0.2 mm aluminum sheets with silica gel 60 F_{254} (Merck); as eluent dichloromethane:methanol (9:1, or 99:1, respectively) was used. Melting points were taken on a Büchi 510 melting point apparatus and are uncorrected. Elemental analyses were performed by the Institute of Chemistry, University of Tübingen, or the Institute of Inorganic Chemistry, University of Würzburg, respectively. Satisfactory microanalyses were obtained for final products: C ± 0.4, H ± 0.32, N ± 0.35, unless otherwise noted.

The syntheses of compounds **5**, **17**, and **20** have been described.[10,26,33]

***p*-Sulfocinnamic acid and its potassium salt (7a).**[16] Finely ground cinnamic acid (50.0 g, 337 mmol) was dissolved in small portions in 75 mL of fuming sulfuric acid (containing 30% of SO_3) under vigorous stirring and cooling with an ice-bath, maintaining the temperature of the exothermic reaction below 35 °C. After all of the cinnamic acid had been added the reaction was stirred at rt for 30 min. The thick light-brown solution was carefully poured on 200 mL of ice water and left standing overnight at rt. The crystalline product that separated was collected by filtration using a sintered filter and washed with 100 mL of diluted sulfuric acid (25%) and subsequently with 100 mL of diethyl ether. After drying 42 g of *p*-sulfocinnamic acid were obtained as slightly orange crystals. A second crop (2 g) could be filtered off after cooling the filtrate.

***p*-Sulfocinnamic acid.** Yield: 57%; mp 82 °C. ^{1}H NMR (D_2O): δ 6.25 (d, J = 16.1 Hz, 1H, 2-H), 7.40 (d, J = 16.1 Hz, 1H, 3-H), 7.43 (d, J = 8.3 Hz, 2H, 2′-H, 6′-H), 7.61 (d, J = 8.3 Hz, 2H, 3′-H, 5′-H). ^{13}C NMR (D_2O): δ 121.90 (C-2), 128.57 (C-3′, C-5′), 131.24 (C-2′, C-6′), 139.25 (C-1′), 146.48 (C-4′), 147.29 (C-3), 172.79 (C-1).

***p*-Sulfocinnamic acid potassium salt (7a).** The *p*-sulfocinnamic acid was converted to its potassium salt by treatment with KOH, or K_2CO_3, respectively. Mp > 260 °C. ^{1}H NMR (D_2O): δ 6.54 (d, J = 16.1 Hz, 1H, 2-H), 7.68 (d, J = 16.1 Hz, 1H, 3-H), 7.69 (d, J = 8.3 Hz, 2H, 2′-H, 6′-H), 7.81 (s, J = 8.3 Hz, 2H, 3′-H, 5′-H). ^{13}C NMR (D_2O): δ 122.12 (C-2), 128.58 (C-2′, C-6′), 131.28 (C-3′, C-5′), 139.41 (C-1′), 146.41 (C-4′), 147.27 (C-3), 173.04 (C-1)

***m*-Sulfocinnamic acid and its potassium salt (7b).** Method A:[16] Sulfonation of cinnamic acid yields a mixture of *p*- and *m*-sulfocinnamic acid. After the main product, *p*-sulfocinnamic acid, had been filtered off (see above, preparation of **7a**) the aq filtrate was diluted to 1 L and 400 g of $BaCO_3$ was added with vigorous stirring. The formed precipitate of $BaSO_4$ was filtered off and washed with 200 mL of H_2O. The combined solutions were evaporated to dryness and the residue was dissolved in MeOH. K_2CO_3 was added in small portions until effervescence ceased. The precipitate was filtered off and the filtrate was concentrated to precipitate **7b**, which was collected by filtration. Purification was achieved by several recrystallizations from MeOH. Yield: 10.3 g (11%); mp > 260 °C. ^{13}C NMR (D_2O): δ 121.65 (C-2), 127.57 (C-2′), 129.83 (C-4′), 132.30 (C-5′), 133.53 (C-6′), 137.38 (C-1′), 145.82 (C-3′), 147.49 (C-3), 173.16 (C-1)

***m*-Sulfocinnamic acid.** Free *m*-sulfocinnamic acid was obtained by treatment of **7b** with sulfuric acid furnishing orange crystals. ^{1}H NMR (DMSO-d_6): δ 6.52 (d, J = 16.1 Hz, 1H, 2-H), 7.36 7.85 (m, 5H, 3-H, $H_{aromat.}$). Method B:[16] To a boiling solution of 3.7 g (44.0 mmol) of sodium bicarbonate in 25 mL of water, 10 g (44.0 mmol) of *m*-bromocinnamic acid and 5.8 g of an aq sodium bisulfite solution (92%, 51.0 mmol) was

added and the mixture was refluxed until a sample gave no precipitate when acidified. Then, 7.0 g of an aq sodium bisulfite solution (92%, 52.7 mmol) and 0.5 g (3.13 mmol) of $CuSO_4$ were added and the mixture was heated in a sealed tube at 175 °C for 45 h. After removing the formed precipitate, the colorless filtrate was treated with 5 g (29.2 mmol) of $Ba(OH)_2$ to precipitate sulfate and excess sulfite, which were removed by filtration. To the filtrate another 15 g of $Ba(OH)_2$ (87.5 mmol) was added, the solution was concentrated to 20 mL and then heated to reflux until no more barium sulfite precipitated. The precipitate formed was removed from time to time by filtration. The filtrate was then cooled in a mixture of ice and NaCl = 3:1 (w/w) and filtered again. The filtrate was saturated with CO_2 and filtered one more time. The filtrate was then acidified with 6 N HCl to a pH value of 0. Some of the unreacted *m*-bromocinnamic acid precipitated, further bromocinnamic acid was extracted with diethyl ether. The aq layer was separated, heated to boiling and treated with 6 g (28.8 mmol) of $BaCl_2$ in 25 mL of water. On cooling, a precipitate of the barium salt of *m*-sulfocinnamic acid formed which was filtered off and recrystallized from water; yield: 73%. The free acid was obtained by treatment with sulfuric acid; yield: 97%; mp 74 °C. The potassium salt **7b** can be prepared from the acid as described above (Method A)

6-Amino-3-propargyl-5-(*p*-sulfocinnamoyl)aminouracil potassium salt (8a). A suspension of 2.0 g (11.1 mmol) of 5,6-diamino-3-propargyluracil (**6**),[17,18] 2.13 g (11.1 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and 2.96 g (11.1 mmol) of the monopotassium salt of *p*-sulfocinnamic acid (**7a**) in 60 mL of water and 1 mL of MeOH was stirred vigorously at rt overnight. After that period of time no more diaminouracil could be detected by TLC. The reaction mixture was concentrated in vacuo and the precipitate was collected by filtration. The solid was washed with MeOH and dried at 90 °C affording 3.69 g (77.6%) of a white solid. $C_{16}H_{13}KN_4O_6S*H_2O$: C, calcd 43.04; found, 42.53; H, N. Mp > 300 °C. IR, cm^{-1}: 3470, 3271, 3214, 2125, 1737, 1676, 1651, 1626.

6-Amino-1-methyl-3-propargyl-5-(*p*-sulfocinnamoyl)aminouracil potassium salt (9a). To a suspension of 2.14 g (5 mmol) of **8a** and 1.38 g (10 mmol) of K_2CO_3 in 20 mL of DMF 0.4 mL (6 mmol) of methyl iodide was added. The reaction mixture was stirred for 8 h at rt. Then water (30 mL) was added. The precipitate formed was filtered off and washed with water, then with MeOH, and finally with diethyl ether. After drying, 2.04 g (92%) of a white solid was obtained. $C_{18}H_{13}KN_4O_6S*3H_2O$: C, calcd 41.12; found, 39.40; H, N. Mp 301–302 °C (decomp.). IR, cm^{-1}: 3464, 3253, 3011, 2125, 1703, 1671, 1632, 1591.

3-Methyl-1-propargyl-8-(*p*-sulfostyryl)xanthine potassium salt (10a). Compound **9a** (3.0 g, 6.78 mmol) was suspended in 100 mL of MeOH, and then 2 mL of a 20% aq solution of NaOH was added. The reaction mixture was heated to reflux for 3 h. After cooling to rt 80 mL of diethyl ether were poured into the flask producing a precipitate, which was filtered off, washed with diethyl ether, and dried in an oven at 90 °C. Yield: 2.14 g (74%); mp > 300 °C. $C_{17}H_{13}KN_4O_5S*2\ H_2O$: C, H, N. IR, cm^{-1}: 3425, 3271, 2125, 1674, 1628, 1593.

3,7-Dimethyl-1-propargyl-8-(*p*-sulfostyryl)xanthine potassium salt (11a). Compound **10a** (0.93 g, 2.19 mmol) and K_2CO_3 (1.5 g, 11 mmol) were suspended in 20 mL of DMF and 0.58 mL (9.3 mmol) of methyl iodide was added. The reaction mixture was stirred at rt overnight. The precipitate formed was collected by filtration and washed with DMF. Filtrate and washings were combined and concentrated nearly to dryness in vacuo. Then 20 mL of diethyl ether was added generating a white precipitate, which was filtered off and washed with diethyl ether. Purification was achieved by dissolution in hot methanol, filtration, and subsequent concentration of the methanol solution. The slightly yellowish solid was dried in the oven. Yield: 83%; mp > 300 °C. $C_{18}H_{15}KN_4O_5S*2\ H_2O$: C, H, N. IR, cm^{-1}: 2125, 1706, 1661, 1598, 1545, 1483.

6-Amino-3-propargyl-5-(3-sulfostyrylcarboxamido)uracil (8b). To a suspension of 0.477 g (2.09 mmol) of 3-sulfocinnamic acid (**7b**) and 0.370 g (2.05 mmol) of 5,6-diamino-3-propargyluracil[17,18] in 17 mL of MeOH 0.402 g (2.09 mmol) of EDC was added and the mixture was stirred at rt for 24 h. The mixture was concentrated in vacuo and the precipitate collected by filtration and washed with MeOH. Yield: 0.50 g (63%); mp > 250 °C.

6-Amino-1-methyl-3-propargyl-5-(3-sulfostyrylcarboxamido)uracil (9b). Compound **8b** (0.400 g, 1.03 mmol) was dissolved in 10 mL of DMF. Methyl iodide (1.49 g, 10.3 mmol, 642 μL) and K_2CO_3 (0.313 g, 2.27 mmol) were added and the mixture was stirred at rt for 48 h. The solvent was removed in vacuo and the residue was dissolved in 25% aq HCl solution. Solid impurities were removed by filtration. The filtrate was evaporated to dryness and the residue suspended in MeOH. The light-yellow product was collected by filtration. Yield: 0.190 g (46%); mp > 250 °C.

3-Methyl-1-propargyl-8-*p*-sulfostyrylxanthine monosodium salt (10b). Compound **9b** (0.240 g, 0.595 mmol) was dissolved in a mixture of 40.0 mL of MeOH and 6 mL of water. After the addition of 17.0 mL 20% aq NaOH solution the mixture was heated to 65 °C for 3 h. After cooling, the volume was reduced in vacuo, and the

formed precipitate was collected by filtration. Yield: 0.180 g (74%); mp >250 °C.

3,7-Dimethyl-1-propargyl-8-(3-sulfostyryl)xanthine potassium salt (11b). Compound **10b** (0.100 g, 0.242 mmol), K_2CO_3 (0.185 g, 1.35 mmol) and methyl iodide (0.148 g, 1.14 mmol, 71.3 μL) were suspended in 2.5 mL of DMF and stirred at rt for 48 h. The formed precipitate was filtered off and washed with DMF. The filtrate was evaporated to dryness, and the residue was taken up in 5 mL of MeOH. After the addition of 30 mL of diethyl ether a light-yellow precipitate was obtained which was collected by filtration. Yield: 0.070 g (66%); mp 255 °C. $C_{18}H_{15}KN_4O_5S$*3 H_2O: C, H, N.

1,3-Dipropyl-8-*p*-sulfostyrylxanthine monopotassium salt (13). 5,6-Diamino-1,3-dipropyluracil (**12**, 0.919 g, 4.06 mmol) was dissolved in a mixture of 10 mL of water and 10 mL of MeOH. 4-Sulfocinnamic acid monopotassium salt (**7a**, 1.13 g, 4.26 mmol) and EDC (0.814 g, 4.26 mmol) were added. The mixture was stirred at rt for 24 h. Then the solvent was removed in vacuo and the residue was suspended in a small amount of MeOH. Diethyl ether was added to precipitate 6-amino-1,3-dipropyl-5-(*p*-sulfostyrylcarboxamido)uracil, which was collected by filtration.

6-Amino-1,3-dipropyl-5-(*p*-sulfostyrylcarboxamido)uracil monopotassiun salt. ^{1}H NMR (DMSO-d_6) δ 0.88 (m, 6H, 2×CH_3), 1.60 (m, 4H, 2×CH_2-CH_3), 3.71 (t, 2H, N-CH_2), 3.81 (t, 2H, N-CH_2), 6.71 (s, 2H, NH_2), 6.82 (d, J = 16.1, 1H, CH = CH), 7.42 (d, J = 15.9, 1H, CH = CH), 7.53 (d, J = 7.9, 2H, $H_{aromatic}$), 7.63 (d, J = 8.0, 2H, $H_{aromatic}$), 8.68 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 10.93 (CH_3), 11.38 (CH_3), 15.84 (2×$C$$H_2$-$CH_3$), 42.55 (N-$CH_2$), 44.72 (N-$CH_2$), 74.90 (C-5), 111.83 (CH = CH), 126.50 (2×$C_{aromatic}$), 127.12 (2×$C_{aromatic}$), 135.21, 149.28 ($C_{aromatic}$, CH = CH), 150.46 (C-2), 151.49 (C-6), 159.20 (C-4), 165.44 (C = O).

Ring closure to the xanthine **13** was achieved by dissolution of the uracil derivative in 20 mL of 10% aq NaOH solution, heating for 20 min at 60 °C, filtration of the hot solution, and precipitation of the product by acidification of the filtrate with concd HCl solution to a pH value of 4. Yield: 0.870 g (49%); mp >300 °C. $C_{19}H_{21}KN_4O_5S$* H_2O: C, H, N.

1,3-Dipropyl-7-methyl-8-*p*-sulfostyrylxanthine (14). Compound **13** (0.200 g, 0.438 mmol) and K_2CO_3 (0.303 g, 2.19 mmol) were suspended in DMF. Methyl iodide (120 μL, 1.93 mmol) was added and the mixture was stirred for 2 days at rt. The solvent was removed in vacuo and the residue was taken up in water, precipitated by the addition of concd HCl, and collected by filtration. Yield: 0.120 g (63%); mp >250 °C. $C_{20}H_{24}N_4O_5S$* H_2O: C, H, N.

Pharmacological Methods

Materials

Radiolabelled compounds were from NEN Life Sciences, Dreieich, Germany. All other materials were from sources as described earlier.[22,34]

A_1- and A_{2A}-Adenosine receptor radioligand binding assays. Inhibition of binding of [^{3}H]N^6-cyclohexyladenosine ([^{3}H]CHA) to A_1-adenosine receptors of rat cerebral cortical membranes and inhibition of [^{3}H]2-[4-(carboxyethyl)phenylethylamino]-5′-*N*-ethylcarboxamido-adenosine ([^{3}H]CGS21680) to A_{2A}-adenosine receptors of rat striatal membranes were assayed as described.[34] Inhibition of the receptor-radioligand binding was determined by a range of 5 to 6 concentrations of the compounds in triplicate in at least two to three separate experiments. The Cheng Prusoff equation[35] and K_D values of 1 nM for [^{3}H]CHA and 14 nM for [^{3}H]CGS21680 were used to calculate the K_i values from the IC_{50} values, determined by the nonlinear curve fitting program Prism (GraphPad, San Diego, California, USA).

Transfected cells and membranes containing human A_{2B}- and A_3-AR. CHO cells were stably transfected with the human A_{2B}- and A_3-adenosine receptors as described.[22] Cells were grown on petri dishes (Ø 140 mm) to confluency. Then cells were washed and used immediately for membrane preparation for measurement of AC activity (A_{2B}), or frozen in the dishes and kept at −25 °C until membranes were prepared for binding studies (A_3).

For the preparation of crude membranes for A_3-receptor binding frozen cells were thawed and then scraped off the petridishes in hypotonic buffer (5 mM Tris/HCl, 2 mM EDTA, pH 7.4). The cell suspension was homogenized (Ultra-Turrax, 2×15 s at full speed) and the homogenate was spun for 10 min at 1,000 *g*. The supernatant was then centrifuged for 40 min at 50,000 *g*. The membrane pellet was resuspended in 50 mM Tris/HCl, pH 8.25 containing 1 mM EDTA and 10 mM $MgCl_2$, frozen in liquid nitrogen at a protein concentration of 1–3 mg/mL and stored at −80 °C.

For the measurement of adenylate cyclase activity (A_{2B}) a slightly modified protocol with only one centrifugation step was used. Fresh cells were homogenized and the homogenate was sedimented for 30 min at 54,000 *g*. The resulting pellet was resuspended in 50 mM Tris/HCl pH 7.4 and used for the adenylate cyclase assay immediately.

Adenylate cyclase activity

The procedure was carried out as described previously[36] with minor modifications. Membranes were incubated with about 150,000 cpm of [α-^{32}P]ATP for 20 min in the

incubation mixture as described[36] without EGTA and NaCl. IC_{50} values for concentration-dependent inhibition of NECA-stimulated adenylate cyclase (5 μM NECA) caused by the antagonists under investigation were calculated with the Hill equation. Hill coefficients in all experiments were near unity. Dissociation constants (K_i) for antagonist at A_{2B}-adenosine receptors were then calculated with the Cheng–Prusoff equation.[35]

A_3-AR radioligand binding assay

Dissociation constants (K_i values) for the antagonists at A_3-adenosine receptors were determined in radioligand competition experiments. The nonselective agonist [^{3}H]NECA was used as the radioligand at a concentration of 10 nM. Binding was carried out in a total volume of 200 μL in 96-well filter-bottom microplates (Millipore MultiScreen MAFC) with 20–25 μg of membrane protein in 50 mM Tris/HCl with 1 mM EDTA and 10 mM $MgCl_2$, pH 8.25. Samples were incubated for 3 h at 25 °C, filtered and washed as described.[22] Data were analyzed by nonlinear curve-fitting with the the program SCTFIT.[37]

Solubility determination

Solubilities of compounds were determined according to the method of Bruns and Fergus.[27] A 10 mM solution of compounds in DMSO was prepared, diluted 1:100, or 1:40, respectively, in Tris–HCl buffer, 50 mM, pH 7.4, and allowed to reach equilibrium with shaking for 24 h at rt in the dark. After centrifugation, the supernatant was filtered through cotton. Several dilutions of these saturated stock solutions were made in the buffer and an A_{2A}-AR binding assay was performed (see above). The solubility of each compound was then calculated by dividing the IC_{50} value of a compound, determined separately, by the fold dilution of the saturated solution required to give 50% inhibition of radioligand binding.

Solubility of the highly water-soluble sulfostyryl xanthine **11a** was additionally determined by UV-spectroscopy.

Acknowledgements

The expert technical assistance of Ms. Sonja Kachler is gratefully acknowledged. J.S.-R. was supported by a grant from the Deutscher Akademischer Austauschdienst (DAAD). C.E.M. is grateful for support by the Fonds der Chemischen Industrie.

References

1. Fredholm, B; Abbracchio, M. P.; Burnstock, G.; Daly, J. W.; Harden, T. K.; Jacobson, K. A.; Leff, P.; Williams, M. *Pharm. Rev*. **1994**, *46*, 143.

2. Collis, M. G.; Hourani, S. M. O. *Trends Pharmacol. Sci*. **1993**, *14*, 360.

3. Müller, C. E.; Scior, T. *Pharm. Acta Helv*. **1993**, *68*, 77.

4. Müller, C. E.; Stein, B. *Current Pharm. Design* **1996**, *2*, 501.

5. Müller, C. E. *Exp. Opin. Ther. Patents* **1997**, *7*, 419.

6. Baraldi, P. G.; Cacciari, B.; Spalluto, G.; Borioni, A.; Viziano, M.; Dionisotti, S.; Ongini, E. *Curr. Med. Chem*. **1995**, *2*, 707.

7. Seale, T. W.; Abla, K. A.; Shamim, M. T.; Carney, J. M.; Daly, J.W. *Life Sci*. **1988**, *43*, 1671.

8. Shimada, J.; Suzuki, F.; Nonaka, H.; Ishii, A.; Ichikawa, S. *J. Med. Chem*. **1992**, *35*, 2342.

9. Jacobson, K. A.; Gallo-Rodriguez, C.; Melman, N.; Fischer, B.; Maillard, M.; van Bergen, A.; van Galen, P. J. M.; Karton, Y. *J. Med. Chem*. **1993**, *36*, 1333.

10. Müller, C. E.; Geis, U.; Hipp, J.; Schobert, U.; Frobenius, W; Pawlowski, M.; Suzuki, F.; Sandoval-Ramírez, J. *J. Med. Chem*. **1997**, *40*, 4396.

11. Baraldi, P. G.; Cacciari, B.; Spalluto, G.; Pineda de las Infantas y Villatoro, M. J.; Zocchi, C.; Dionisotti, S.; Ongini, E. *J. Med. Chem*. **1996**, *39*, 1164.

12. Poucher, S. M.; Keddie, J. R.; Singh, P.; Stoggall, S. M.; Caulkett, P. W. R.; Jones, G.; Collis, M. G. *Br. J. Pharmacol*. **1995**, *115*, 1096.

13. Daly, J. W.; Padgett, W.; Shamim, M. T.; Butts-Lamb, P.; Waters, J. *J. Med. Chem*. **1985**, *28*, 487.

14. Hamilton, H. W.; Ortwine, D. F.; Worth, D. F.; Badger, E.W.; Bristol, J. A.; Bruns, R. F.; Haleen, S.J.; Steffen, R. P. *J. Med. Chem*. **1985**, *28*, 1071.

15. Baumgold, J.; Nikodijevic, O.; Jacobson, K. A. *Biochem. Pharmacol*. **1992**, *43*, 889.

16. Moore, F. J.; Tucker, G. R. *J. Am. Chem. Soc*. **1927**, *49*, 258.

17. Müller, C. E. *Synthesis* **1993**, 125–128.

18. Müller, C. E.; Sandoval-Ramírez, J.A. *Synthesis* **1995**, 1295.

19. Müller, C. E. *J. Org. Chem*. **1994**, *59*, 1928.

20. Nonaka, Y.; Shimada, J.; Nonaka, H.; Koike, N.; Aoki, N.; Kobayashi, H.; Kase, H.; Yamaguchi, K.; Suzuki, F. J. *J. Med. Chem*. **1993**, *36*, 3731.

21. Friebolin, H. Ein- *und zweidimensionale NMR-Spektroskopie (One- and two-dimensional NMR spectroscopy)*, 2nd ed. 1992, VCH Weinheim, 1992; p. 99 ff.

22. Klotz, K.-N.; Hessling, J.; Hegler, J.; Owman, C.; Kull, B.; Fredholm, B. B.; Lohse, M. J. *Naunyn-Schmiedeberg's Arch. Pharmacol*. **1998**, *357*, 1.

23. Cerfontain, H.; Koeberg-Felder, A.; Kruk, C. *Tetrahedron Lett*. **1975**, 3639.

24. Linden, J.; Taylor, H. E.; Robeva, A. S.; Tucker, A. L.; Stehle, J. H.; Rivkees, S. A.; Fink, J. S.; Reppert, S. M. *Mol. Pharmacol*. **1993**, *44*, 524.

25. Salvatore, C. A.; Jacobson, M. A.; Taylor, H. E.; Linden, J.; Johnson, R. G. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 10365.

26. Müller, C. E.; Schobert, U.; Hipp, J.; Geis, U.; Frobenius, W.; Pawlowski, M. *Eur. J. Med. Chem*. **1997**, *32*, 709.

27. Bruns, R. F.; Fergus, J. H. *J. Pharm. Pharmacol*. **1989**, *41*, 590.

28. Jackson, E. K.; Herzer, W. A.; Suzuki, F. *J. Pharmacol. Exp. Ther*. **1993**, *267*, 1304.

29. Nagashima, K.; Karasawa, A. *Life Sci*. **1996**, *59*, 761.

30. Dionisotti, S.; Conti, A.; Sandoli, D.; Zocchi, C.; Gatta, F.; Ongini, E. *Br. J. Pharmacol*. **1994**, *112*, 659.

31. Research Biochemicals International, One Strathmore Road, Natick, MA 01760-2447, USA, Catalog 1997/1998.

32. Keddie, J. R.; Poucher, S. M.; Shaw, G. R.; Brooks R. Collis M. G. *Eur. J. Pharmacol*. **1996**, *301*, 107.

33. Müller, C. E.; Shi, D.; Manning Jr., M.; Daly, J. W. *J. Med. Chem*. **1993**, *36*, 3341.

34. Müller, C. E.; Sauer, R.; Geis, U.; Frobenius, W.; Talik, P.; Pawlowski, M. *Arch. Pharm.—Pharm. Med. Chem*. **1997**, *330*, 181–189.

35. Cheng, Y.-C.; Prusoff, W. H. *Biochem. Pharmacol*. **1973**, *22*, 3099.

36. Klotz, K.-N.; Cristalli, G; Grifantini, M; Vittori, S.; Lohse, M. J. *J. Biol. Chem*. **1985**, *260*, 14659.

37. De Lean, A.; Hancock, A. A.; Lefkowitz, R. J. *Mol. Pharmacol*. **1982**, *21*, 5.

38. Daly, J. W. in *Role of adenosine and adenine nucleotides in the biological system*; Imai, S.; Nakazawa, M. Eds.; Elsevier: Amsterdam, 1991; p. 119.

39. Bruns, R. F. *Biochem. Pharmacol*. **1981**, *30*, 325.

40. Martin, P. L.; Wysocki, R. J. Jr.; Barrett, R. J.; May, J. M.; Linden, J. *J. Pharmacol. Exp. Ther*. **1996**, *276*, 490.

41. Feoktistov, I.; Biaggioni, I. *Mol. Pharmacol*. **1993**, *43*, 909.

42. Daly, J. W.; Hide, I.; Müller, C. E.; Shamim, M. *Pharmacology* **1991**, *42*, 309.

43. Paruta, A. N.; Sheth, B. B. *J. Pharm. Sci*. **1966**, *55*, 896.