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Improving Potency, Selectivity, and Water Solubility of Adenosine A_1 Receptor Antagonists: Xanthines Modified at Position 3 and Related Pyrimido[1,2,3-*cd*]purinediones

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*The structure–activity relationships of xanthine derivatives related to the adenosine A_1 receptor antagonists 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) and 1,3-dipropyl-8-(3-noradamantyl)xanthine (KW3902) were investigated by focusing on variations of the 3-substituent. Aromatic residues were well tolerated by the A_1 receptor in that position. A moderate effect of stereochemistry was found for the 3-(1-phenylethyl)-substituted analogue of DPCPX (S > R) at A_1 and A_3 receptors, whereas the opposite stereoselectivity was observed at the A_2 receptor subtypes. A 3-hydroxypropyl substituent was found to be optimal for high A_1 affinity and selectivity. The most potent compound of the present series was 1-butyl-3-(3-hydroxypropyl)-8-(3-noradamantyl)xanthine (**10c**), which exhibits a K_i value of 0.124 nM at rat, and 0.7 nM at human adenosine A_1 receptors, combined with high selectivity (≥ 200-fold) versus the other receptor subtypes. The similarly potent 8-cyclopentyl-3-(3-hydroxypropyl)-1-propylxanthine was converted into a water-soluble phosphate prodrug, which may become a useful pharmacological tool for in vivo studies. 8-Alkyl-2-(3-noradamantyl)pyrimido[1,2,3-cd]purine-8,10-diones, which can be envisaged as xanthine analogues with a fixed 3-propyl substituent, were identified as a new class of potent, selective adenosine A_1 receptor antagonists. For example, compound **14** (8-butyl-substituted) exhibits a K_i value of 13.8 nM at human A_1 receptors. A selection of the most potent compounds was investigated in [^{35}S]GTPγS binding assays and showed inverse agonistic activity. Their efficacy was generally lower than that of the full inverse agonist DPCPX, and depended on subtle structural changes. Some of the new compounds belong to the most potent and selective A_1 antagonists described to date.*

Introduction

Adenosine receptors (ARs) belong to the superfamily of G-protein-coupled receptors (GPCRs).[1] Four distinct subtypes have been identified, A_1, A_{2A}, A_{2B}, and A_3 which are all of considerable interest as new drug targets.[2–6] Antagonists for A_{2A} ARs are currently under clinical development as novel therapeutics for Parkinson's disease and may also exhibit antidepressive and neuroprotective activity.[7,8] A_{2B} antagonists show anti-asthmatic, anti-inflammatory and analgesic activity,[9–11] while A_3 antagonists are of interest as potential anti-inflammatory and anti-asthmatic agents as well.[6,12] Antagonists for A_1 ARs are promising new drugs for the treatment of congestive heart failure, and are currently being evaluated as kidney-protective diuretics and positive inotropic agents.[13,14] The first generation of A_1 antagonists failed in preclinical or clinical studies. However, there is now a renewed interest in a second generation of A_1 antagonists that are not only potent and highly selective for the A_1 AR, but also exhibit favorable pharmacokinetic properties, including good water solubility. A major problem with the first generation of A_1 antagonists has been their high lipophilicity, which limits their applicability and activity in in vivo studies. Furthermore, it has become evident that certain species differences exist, and compounds that are receptor subtype-selective in rats may not be as selective in humans.[15] An example for this is the standard A_1 antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), which is highly potent and selective in rats, but somewhat less potent at human A_1 ARs, and only moderately selective versus human A_{2A} (20–40-fold) and A_{2B} receptors (10–20-fold). In a recent review article, IJzerman and

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colleagues came to the conclusion that "ligands truly selective for the human adenosine A_1 receptor are still scarce".[16] An ideal A_1 antagonist that could be further developed as a drug should exhibit the following profile: 1) a high affinity ($K_i \leq$ 10 nM) for the A_1 receptor, 2) a high selectivity versus other receptors (>100-fold), 3) moderate species differences (rodent versus human <10-fold), 4) good water solubility and bioavailability, 5) a suitable half-life in vivo (several hours), and 6) poor CNS penetration in the case of drugs intended for peripheral targets, for example, in the treatment of congestive heart failure. Two well-characterized, xanthine-based A_1 AR antagonists are depicted in Figure 1.

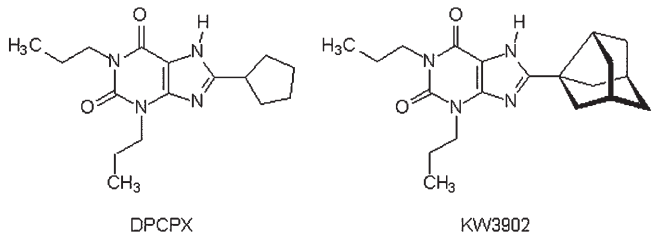


Figure 1. Standard xanthine adenosine A_1 receptor antagonists.

Over the past years it has also been reported that many antagonists for GPCRs exhibit full or partial inverse agonistic activity by stabilizing the inactive conformation of the receptors.[17] This can be of pharmacological relevance in tissues with a high receptor density, where the receptors show constitutive activity. In this case, an inverse agonist may lead to a functional response even in the absence of an agonist, by shifting the receptor conformation to an inactive state. DPCPX is the most potent inverse agonist for A_1 ARs described to date.[18]

Antagonists for A_1 ARs can be divided into two subgroups, xanthines and nonxanthines, three examples of which are depicted in Figure 2. Whereas DPCPX is the archetypical xanthine-based inhibitor, ADPEP and LUF 5751 are examples of nonxanthine-based inhibitors. The latter group is often structurally related to adenine, but can also include other mono-, bi-, and tricyclic nitrogen-containing heterocyclic compounds.[13] Recent pharmacophore models of A_1 AR antagonists based on diverse structures, including xanthine and nonxanthine antagonists, postulate the existence of a hydrogen bond donor–acceptor motif, and three lipophilic pockets, one of which can be filled best with a cycloalkyl moiety.[13,19]

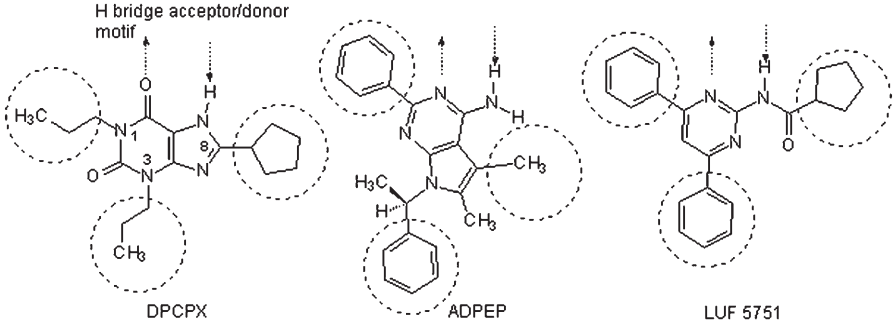


Figure 2. Pharmacophore model for A_1-selective adenosine receptor antagonists. Arrows indicate hydrogen bond acceptor–donor motifs; dashed circles indicate lipophilic pockets.[19]

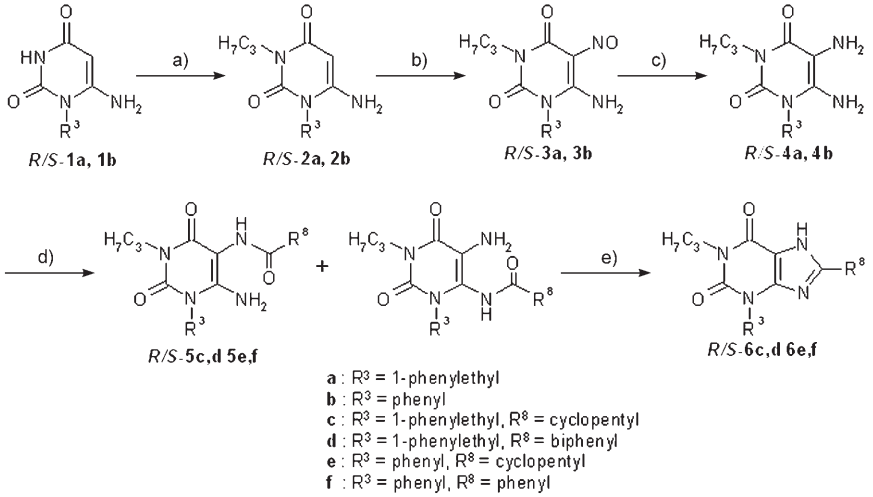
Whereas the structure–activity relationships (SARs) for the 1- and 8-substituents of xanthine derivatives have been extensively analyzed[13,20,21] the 3-substituent has been less well explored. The main reasons for this have been because of difficulties in synthesizing 1,3,8-trisubstituted xanthine derivatives with variations in the 3-position, which is not always straightforward. The majority of xanthine derivatives investigated so far bear identical substituents at N1 and N3, frequently dipropyl or dimethyl groups, because these residues are already introduced in the very first step of the classical Traube purine synthesis via suitably *N*,*N*-disubstituted urea derivatives. Over the past years we have developed synthetic methods which allow the straightforward preparation of xanthine derivatives with many different N3 substituents. This synthetic progress has now permitted us to explore the SARs of the 3-substituent of xanthine derivatives in more detail, to obtain more potent and selective A_1 AR antagonists. In addition, we discovered a new class of potent and selective A_1 AR antagonists, the 2,9-disubstituted pyrimido[1,2,3-*cd*]purine-8,10-diones, which can be envisaged as sterically fixed analogues of 1,8-substituted 3-propylxanthine derivatives. The new compounds were characterized at human and rat AR subtypes. Their antagonistic/inverse agonistic behavior was assessed by [^{35}S]GTPγS binding studies at human A_1 ARs. Furthermore, we developed a water-soluble prodrug of a potent, selective A_1 AR antagonists, which was discovered in the present study.

Results and Discussion

Chemistry

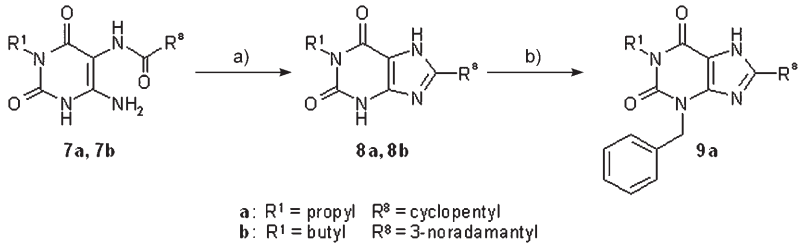
The synthesis of 1,3,8-trisubstituted xanthine derivatives containing a phenyl ring or a chiral 1-phenylethyl (1-methylbenzyl) residue in position N3 started from the corresponding 1-substituted 6-aminouracils via the classical Traube purine synthesis (see Scheme 1).[22] 6-Aminouracil derivatives (*R*)-**1a**, (*S*)-**1a**, and **1b** were obtained as previously described.[23] Alkylation of these compounds at N3 (corresponding to xanthine N1) was carried out in *N*,*N*-dimethylformamide in the presence of potassium carbonate by using propyl iodide at room temperature. Under these mild conditions, the reaction was regioselective and gave, in the case of the 1-phenyl-6-aminouracil, the crystalline product **2b**. The phenylethyl derivatives (*R*)-**2a** and (*S*)-**2a** were obtained as gluey oils.

Owing to the neighboring 6-amino group, products **2** are susceptible to electrophilic substitution in the 5-position by a nitrosyl cation, which is produced in situ from sodium ni-



Scheme 1. Synthesis of 1,3,8-trisubstituted xanthine derivatives: a) K_2CO_3, C_3H_7I, DMF, 20 h, RT; b) $NaNO_2$, CH_3COOH/H_2O, 24 h, RT; c) $Na_2S_2O_4$, NH_3/H_2O, 80 °C; d) R^8COOH, EDC, methanol, 24 h, RT; e) $NaOCH_3$, methanol, 3–12 h, reflux.

The ring closure reaction of 1-butyl-5-(3-noradamantyl)carboxamidouracil (**7b**) to **8b** was performed in hexamethyldisilazane at high temperature in analogy to a published procedure,[25] whereas 1-propyl-5-cyclopentanecarboxamidouracil (**7a**) was cyclized in 30% methanolic sodium methylate solution to yield **8a**.[24,26] Subsequent alkylation of xanthine derivative **8a** at N3 yielded 3-benzyl-8-cyclopentyl-1-propylxanthine (**9a**). Owing to the similar acidities of N3 and N7, a regioselective alkylation was not easy to perform. The benzylation of **8a** was carried out in



Scheme 2. Synthesis of 1,8-disubstituted xanthines and 3-benzyl-8-cyclopentyl-1-propylxanthine: a) $NaOCH_3$ (30%), 3 h, reflux (for **8a**) or HMDS, 18 h, reflux (for **8b**); b) benzyl bromide, KOH, EtOH, 1 h, reflux. HMDS = hexamethyldisilazane.

trite in aqueous acetic acid at room temperature. This functionality in position 5 allowed us to build up the purine structure by reducing the nitroso group with sodium dithionate in aqueous ammonia solution to yield an amino group. This reaction was performed in an inert argon atmosphere to decrease the formation of side products, and to reach nearly quantitative yields of the intermediates **4**. These were treated at room temperature with cyclopentylcarboxylic acid, benzoic acid, or biphenylcarboxylic acid in methanol in the presence of *N'*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC) to obtain the corresponding N^5-substituted amides **5c**, **d**, **e**, **f** as well as their N^6-substituted isomers. The reactivity of both amino groups seemed to be similar in the 1,3-disubstituted uracil derivatives **4**. The 6-carboxamidouracils were clearly more reactive, so that, even under mild reaction conditions, they were readily condensed to the desired 1,3,8-substituted xanthines **6**. The cyclization of the regioisomeric mixtures of 5- and 6-carboxamidouracil derivatives was performed under refluxing conditions in methanolic sodium methylate solution, both isomers yield the same products **6**. Therefore, the carboxamides were usually not isolated, but used without further purification in the last step. The resulting xanthines (*R*)-**6c**, (*S*)-**6c**, **6e**, and **6f** could be easily purified by recrystallization from methanol/water mixtures, whereas the biphenyl derivatives (*R*)-**6d**, (*S*)-**6d** had to be purified by column chromatography (Scheme 1).

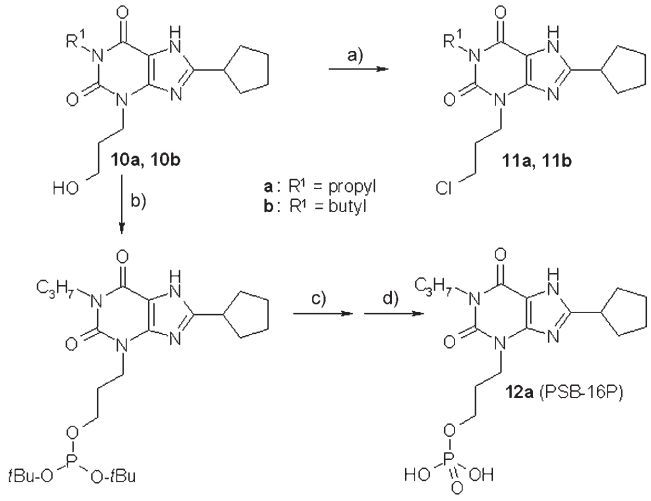
The 1,8-disubstituted xanthine derivative **8b** (1-butyl-8-(3-noradamantyl)xanthine) was obtained in analogy to the previously described procedure for the preparation of **8a** (8-cyclopentyl-1-propylxanthine, see Scheme 2).[24] the presence of potassium hydroxide in ethanol, and produced predominantly the 3-benzyl-8-cyclopentyl-1-propylxanthine (**9a**), which could be isolated in 52% yield after several extraction and recrystallization steps.

The syntheses of all other trisubstituted xanthines (**10a**, **10b**, and **10c**), and the preparation of the tricyclic 2,9-disubstituted 4,5-dihydro-6*H*,8*H*-pyrimido[1,2,3-*cd*]purine-8,10-diones **12**, **13**, **14** and **15** have been described previously.[26]

Preparation of a water-soluble phosphate prodrug

All synthesized xanthine derivatives possess moderate water solubility, as they contain large nonpolar aliphatic or aromatic residues. In radioligand binding studies it was shown that the 3-(3-hydroxypropyl)-substituted xanthine derivatives were the most potent and selective adenosine A_1 receptor ligands, exhibiting considerably improved properties compared with the standard A_1 antagonist DPCPX. The synthesized 3-(3-hydroxypropyl)xanthine derivatives would be suitable precursors to prepare more water-soluble derivatives such as phosphoric acid, sulfuric acid, or amino acid esters, which could act as prodrugs if they were cleaved in vivo by esterases. The 8-cyclopentyl derivatives (**10a** and **10b**) were chosen as starting com-

pounds to prepare phosphate prodrugs because they easily could be obtained in large quantities for further derivatization, while the somewhat more potent **10c** was more difficult to prepare and purify, and required an expensive precursor, 3-noradamantylcarboxylic acid. We had previously obtained a phosphate prodrug of the potent, A_{2A}-selective adenosine receptor antagonist MSX-2 by phosphorylation of the hydroxypropyl derivative using phosphorus oxychloride in trimethyl phosphate.[25,27] However, when we applied this method to the A_1-selective xanthine derivatives **10a** and **10b**, we did not observe phosphorylation of the hydroxy group, but substitution of the hydroxy group by chloride which yielded the chloropropyl derivatives **11a** and **11b**, as confirmed by mass spectrometry (Scheme 3). Therefore, the phosphate prodrug had to



Scheme 3. Synthesis of a water-soluble phosphate prodrug of an A_1-selective xanthine derivative: a) $POCl_3$, $OP(OMe)_3$, 2 h, RT; b) $(tBuO)_2P\text{-}N(Et)_2$, 1*H*-tetrazole, MeCN/THF, 2 h, RT; c) *t*BuOOH, 1 h, RT; d) CF_3COOH, CH_2Cl_2, 2 h, RT.

be prepared by a different approach by applying the multistep phosphoramidite method (Scheme 3).[27] After phosphitylation with di-*tert*-butyl-*N*,*N*-diethylphosphoramidite, the phosphorus was subsequently oxidized to yield the *tert*-butyl-phosphoric acid ester. Without prior isolation, the ester was cleaved by treatment with trifluoroacetic acid in dichloromethane. The resulting phosphoric acid ester derivative **12a** was detected as a single spot on thin-layer chromatography, and was visualized with a phosphate-specific spraying reagent. The phosphoric acid ester (free acid) was then converted into the more water-soluble disodium salt by treatment with an ion exchange resin.

Biological evaluation

The new compounds were initially investigated in radioligand binding studies at rat brain adenosine A_1 and A_{2A} receptors, and at human recombinant A_{2B} and A_3 receptors. Selected compounds were additionally investigated at human recombinant A_1 and A_{2A} receptors, and at recombinant rat A_3 receptors, to obtain information on the affinities and selectivities of the most interesting compounds in both species, rat and human. [^{3}H]2-Chloro-N^6-cyclopentyladenosine (CCPA) was used as an A_1-selective radioligand; [^{3}H]3-(3-hydroxypropyl)-7-methyl-8-(*m*-methoxystyryl)-1-propargylxanthine (MSX-2) was used for the A_{2A} radioligand binding assays, and [^{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) was used as an A_{2B} radioligand. For binding assays at human A_3 receptors, 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) was applied. As [^{3}H]PSB-11 is not suitable for labeling rat A_3 receptors, the agonist radioligand [^{125}I]AB-MECA had to be used for binding studies at rat A_3 receptors. The most potent compounds of the present series were investigated for their functional properties in ligand-mediated [^{35}S]GTPγS binding via recombinant human A_1 receptors.

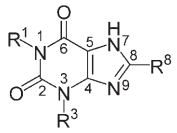
Structure–activity relationships

Ten 1,3,8-trisubstituted xanthines (*R*/*S*-**6c**, *R*/*S*-**6d**, **6e**, **6f**, **9a**, **10a**, **10b**, and **10c**), the 1,8-disubstituted xanthine derivative **8b**, and four 2,9-disubstituted 4,5-dihydro-6*H*,8*H*-pyrimido-[1,2,3-*cd*]purin-8,10-diones (compounds **13**–**16**) were investigated in radioligand binding assays at all four rat and/or human adenosine receptor subtypes. The respective affinities are summarized in Table 1. The potent A_1 antagonists DPCPX and KW3902, both 1,3-dipropylxanthine derivatives with a cycloalkyl residue in position 8, served as our lead structures. DPCPX is widely used as an experimental standard compound, whereas KW3902 has been developed for potential use as a drug.

The 8-cyclopentylxanthine derivative DPCPX is a very potent inhibitor at rat A_1 receptors (K_i = 0.5 nM), but is a 6-fold less potent inhibitor at human A_1 receptors (K_i = 3 nM). While DPCPX appears to be quite selective in rats, it shows only moderate selectivity in humans (20–40-fold versus A_{2A}, 10–20-fold versus A_{2B}, 100–1000-fold versus A_3). The analogous compound KW3902, in which the cyclopentyl group is replaced by a 3-noradamantyl residue, appears to be less potent at rat ARs but more potent and more selective at human ARs. However, the data for KW3902 at A_{2B} and A_3 receptors have not been available.

Our goal was to explore the structure–activity relationships (SARs) of the substituent in the 3-position of 1,3,8-trisubstituted xanthine derivatives. In our series of compounds, the substituent at N1 was always propyl or butyl, both of which have been shown to be favorable for the A_1 AR.[13,21,24,28] The 8-substituent was in most cases a cyclopentyl (as in DPCPX) or a 3-noradamantyl residue (as in KW3902). In addition, analogues bearing a phenyl or a biphenyl residue were investigated. The 3-substituents encompassed the aromatic residues phenyl, benzyl, and the chiral 1-methylbenzyl, the latter to study the stereoselectivity. Besides propyl, as in the lead structures, an unsubstituted analogue (compound **8b**) as well as 3-hydroxypropyl derivatives were also investigated. Furthermore, analogues in which the 3-propyl substituent was connected to the N7 nitrogen atom, resulting in 4,5-dihydro-6*H*,8*H*-pyrimido-[1,2,3-*cd*]purine-8,10-diones **13**–**16** were studied.

Table 1. Affinities of xanthine derivatives for adenosine receptor subtypes.



Compd	R^1	R^3	R^8	K_i [nM] Rat A_1[a]	Human A_1[a]	Rat A_{2A}[b]	Human A_{2A}[b]	Human A_{2B}[c]	Rat A_3[d]	Human A_3[e]
DPCPX	propyl	propyl	cyclopentyl	0.5 ± 0.2[39]	3.0 ± 0.8[39]	157 ± 6[39]	60 ± 45[f]	51[39]	>10000	243 ± 56[39]
KW3902	propyl	propyl	3-noradamantyl	12.6[40][g]	0.72[40][g]	510[40][f]	108[40][f]	n.d.	n.d.	n.d.
(*S*)-**6c**	propyl	(*S*)-1-phenylethyl	cyclopentyl	10.1 ± 2.1	n.d.	3500 ± 1400	n.d.	8000 ± 600	>10000 (22±7%)	85 ± 43
(*R*)-**6c**	propyl	(*R*)-1-phenylethyl	cyclopentyl	23.8 ± 3.5	n.d.	2400 ± 700	n.d.	2960 ± 30	n.d.	370 ± 76
(*S*)-**6d**	propyl	(*S*)-1-phenylethyl	biphenyl	>10000 (32±5%)	n.d.	>10000 (5±5%)	n.d.	>10000 (0%)	n.d.	455 ± 35
(*R*)-**6d**	propyl	(*R*)-1-phenylethyl	biphenyl	>10000 (9±5%)	>10000 (10±4%)	>10000 (10±5%)	>10000 (16±4%)	>10000 (0%)	n.d.	456 ± 45
6e	propyl	phenyl	cyclopentyl	1.01 ± 0.36	7.1 ± 1.1	492 ± 116	1200 ± 100	625 ± 135	n.d.	395 ± 135
6f	propyl	phenyl	phenyl	24.5 ± 7.34	n.d.	314 ± 57	600 ± 40	245 ± 115	n.d.	145 ± 35
9a	propyl	benzyl	cyclopentyl	8.70 ± 1.90	24.3 ± 8.7	511 ± 90	n.d.	n.d.	n.d.	54.6 ± 24.1
10a (PSB-16)	propyl	3-hydroxypropyl	cyclopentyl	0.57 ± 0.03	5.74 ± 0.37	664 ± 16	n.d.	194 ± 20	n.d.	3100 ± 200
10b	butyl	3-hydroxypropyl	cyclopentyl	0.45 ± 0.04	n.d.	582 ± 42	n.d.	n.d.	n.d.	1190 ± 178
10c (PSB-36)	butyl	3-hydroxypropyl	3-noradamantyl	0.124 ± 0.007	0.7 ± 0.2	552 ± 47	980 ± 20	187 ± 0.07	6500 ± 600	2300 ± 100
8b	butyl	H	3-noradamantyl	1.13 ± 0.24	n.d.	520 ± 110	n.d.	21.0 ± 1.0	n.d.	2400 ± 200

[a] Versus [^{3}H]CCPA. [b] Versus [^{3}H]MSX-2. [c] Versus [^{3}H]ZM-241385. [d] Versus [^{125}I]AB-MECA. [e] Versus [^{3}H]PSB-11. [f] Versus [^{3}H]CGS21680. [g] Versus [^{3}H]DPCPX.

As expected, in the 1-position both propyl and butyl were well tolerated by the A_1 AR; both substituents afforded similar A_1 affinities and selectivities (see **10a**/**10b** and **13**/**14**). In the 8-position of the xanthine derivatives **6**–**10**, cyclopentyl and 3-noradamantyl residues led to potent and selective A_1 AR antagonists ((*R*)-**6c**, (*S*)-**6c**, **6e**, **6f**, **8b**, **9a**, **10a**, **10b**, and **10c**). The 3-noradamantyl residue appeared to be somewhat superior to the cyclopentyl residue (compare: **10b**/**10c**). An 8-phenyl substituent, as in **6f**, had previously been shown to be favorable for A_1 ARs in xanthine derivatives, but also at the other receptor subtypes.[28] We have now investigated the effect of a biphenyl substituent in the 8-position ((*S*)-**6d** and (*R*)-**6d**). The biphenyl substituent was not well tolerated by the ARs with the exception of the human A_3 AR, at which the compounds showed K_i values of 455 nM (for (*S*)-**6d**) and 456 nM (for (*R*)-**6d**). The biphenyl derivatives (*R*)-**6d** and (*S*)-**6d** were A_3-selective.

The main focus of the present study was to investigate whether modification of the 3-substituent could lead to enhanced affinity and/or selectivity at A_1 ARs. Since the lipophilic pocket occupied by the 3-propyl group of DPCPX and KW3902 in A_1 receptor models is frequently filled with aromatic residues in nonxanthine AR antagonists (Figure 2), we investigated the replacement of the 3-propyl group by aromatic residues in xanthines as well. Compared with DPCPX, a 3-phenyl ring decreased the A_1 affinity of compound **6e** only slightly (rat A_1 $K_i = 1.01$ nM, human A_1 $K_i = 7.1$ nM). A_{2A}, A_{2B}, and A_3 affinity was also somewhat reduced, thus retaining good selectivity for the A_1 AR. An analogue of **6e**, compound **6f**, in which the cyclopentyl residue in position 8 was replaced by a phenyl ring, further reduced the A_1 affinity, but slightly increased affinity for the other receptor subtypes which resulted in an only moderately A_1-selective antagonist. The 3-benzyl analogue of DPCPX, **9a**, was somewhat less potent at A_1 ARs than the phenyl-substituted analogue **6e**, but showed increased A_3 affinity. Thus, the human A_3 AR appears to favor a benzyl substituent in the xanthine 3-position and a phenyl substituent at C8. The 3-substituent was further enlarged in compound **6c** featuring an (*S*)- or (*R*)-1-methylbenzyl residue. The A_1 affinity of these derivatives was similar to that of the benzyl derivative, but the A_{2A} affinity was reduced and the compound also showed low A_{2B} affinity. The human A_3 receptor tolerated the methylbenzyl residue, and the compounds showed moderate selectivity versus A_3, but good selectivity versus the A_2 receptor subtypes. For the first time the requirements of adenosine receptors with regard to a chiral substituent in the 3-position of xanthine derivatives have been explored. The *S*-enantiomer was 2-fold more potent at A_1 and 4-fold more potent at A_3 receptors, but the opposite was true for A_{2A} and A_{2B} receptors, where the *R*-enantiomer was more potent than the *S*-enantiomer. Although the degree of stereoselectivity was only moderate at the A_1 receptor, the *R*-enantiomer of **6c** was much more selective than the *S*-enantiomer with regard to the A_2 receptor subtypes ((*S*)-**6c**: 100-fold versus A_{2A}, 124-fold versus A_{2B}; (*R*)-**6c**: 346-fold versus A_{2A}, 792-fold versus A_{2B}). Thus, a chiral center at the 3-substituent could be used to enhance selectivity towards cer-

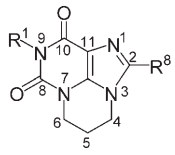
tain receptor subtypes. For the 8-biphenyl derivatives **6d**, no stereoselectivity was observed, but this is probably due to the low AR affinity of the compounds.

The introduction of a terminal hydroxy group at the 3-propyl residue of DPCPX retained affinity for the A_1 receptor but increased selectivity towards the A_{2A}, A_{2B} and A_3 ARs. Thus, compound **10a**, a hydroxylated DPCPX derivative (and a potential metabolite of DPCPX) exhibited a similar affinity for the rat and human A_1 AR relative to the parent DPCPX, but was somewhat more selective. The same modification at N3, in combination with a 1-butyl (instead of 1-propyl) and an 8-(3-noradamantyl) (instead of a 8-cyclopentyl) residue yielded the most potent compound of the present series: compound **10c** (PSB-36), which exhibited a subnanomolar K_i value of 0.124 nM at the rat A_1 AR, and thus belongs to the most potent A_1 AR ligands described to date. At the human A_1 adenosine receptor it was about 6-fold less potent, but still a highly potent ligand with a K_i value of 0.7 nM. Compound **10c** was highly A_1-selective at rat as well as human ARs. Compounds **8b**, which is a 3-unsubstituted analogue of **10c**, was 9-fold less potent at A_1 receptors, but demonstrated little change in the A_{2A} and A_3 receptor affinity. However, **8b** was 9-fold more potent at the A_{2B} receptor compared with the 3-substituted **10c** analogue, confirming that 3-unsubstituted xanthines are potent A_{2B} AR antagonists.[28] But, because **8b** is more potent at the A_1 than at the A_{2B} receptor, it is not A_{2B}-selective.

Finally, tricyclic 2,9-disubstituted 4,5-dihydro-6*H*,8*H*-pyrimido-[1,2,3-*cd*]purine-8,10-diones **13**–**16** were investigated. The compounds can also be envisaged as xanthine derivatives, in which the 3-propyl substituent is additionally attached to N9, and thus in a sterically fixed conformation. This results in the removal of the N–H function in position 1, which corresponds to the xanthine N7-position. In antagonist-binding models for the A_1 receptors, it has been suggested that this hydrogen atom is important for good affinity as it is required for hydrogen bonding between the receptor and the ligand.[13,19,29] Therefore, these tricyclic compounds are expected to show decreased affinity to the A_1 ARs. In fact, the direct analogue of DPCPX, compound **12**, showed dramatically reduced affinity for the A_1 receptor (2880-fold, compared with DPCPX). A_{2A} and A_{2B} affinities were also largely reduced. Compound **15**, An analogue of **12** which bears a butyl instead of a propyl group at N9 (corresponding to xanthine N1), and a phenyl instead of a cyclopentyl ring at C2 (corresponding to xanthine C8) was also a very weak A_1 ligand (K_i = 8630 nM), confirming that a hydrogen-bond donor was very important for high affinity to A_1, and also the other AR subtypes. Despite their moderate A_1 affinity, both compounds were still A_1-selective.

Surprisingly, the 2-(3-noradamantyl)-substituted tricyclic compounds **13** and **14**, which are analogues of KW3902, behaved differently. The direct analogue of KW3902, propyl derivative **13** (PSB-63), was a potent and selective A_1 AR ligand, and exhibited K_i values of 16.9 nM for rat, and 90.6 nM for human A_1 ARs (Table 2). It was highly selective for the rat A_1 versus the rat A_{2A} (1300-fold) AR, and for the human A_1 versus human A_{2A} (381-fold), human A_3 (110-fold), and human A_{2B} adenosine receptors (35-fold). The homologous butyl derivative **14** was similarly potent at A_1 (rat A_1 K_i = 40.6 nM, human A_1 K_i = 13.8 nM) and highly selective versus A_{2A} and A_{2B} receptors. However, the butyl derivative **14** also showed a relatively high affinity for the human A_3 AR (K_i = 188 nM). It appears that the butyl group optimally fits into a lipophilic pocket of the A_3 receptor protein and interacts with this pocket much better than the propyl residue. An alternative explanation could be that the longer butyl chain induces a slightly different, more favorable binding mode than the shorter propyl group. The divergent SARs of 1,3,8-trisubstituted xanthine derivatives on the one hand, and pyrimidopurinediones on the other hand may indicate different binding modes for the two classes of compounds. Since pyrimidopurinediones lack a hydrogen-bond donor function, they may bind in different ways depending on the 8-substituent (cyclopentyl/phenyl or 3-noradamantyl), which results in very different effects for cyclopentyl and phenyl, versus 3-noradamantyl substitution, in contrast to the situation for the 1,3,8-substituted xanthine derivatives featuring an N–H donor at the N7 position. Pyrimido[1,2,3-*cd*]purines represent a novel class

Table 2. Affinities of pyrimidopurinediones for adenosine receptor subtypes.



Compd	R^1	R^8	K_i [nM] Rat A_1[a]	Human A_1[a]	Rat A_{2A}[b]	Human A_{2A}[b]	Human A_{2B}[c]	Rat A_3[d]	Human A_3[e]
12	propyl	cyclopentyl	1440 ± 94	n.d.	12400 ± 3790	n.d.	42600[f]	n.d.	n.d.
13 (PSB-63)	propyl	3-noradamantyl	16.9 ± 2.8	90.6 ± 17.0	22000 ± 4200	34500 ± 1500	3190 ± 75	n.d.	> 10000 (24 ± 0%)
14	butyl	3-noradamantyl	40.6 ± 11.3	13.8 ± 3.7	23400 ± 2200	≈ 25000 (55 ± 9%)	22300 ± 5600	n.d.	188 ± 54
15	butyl	phenyl	8630 ± 500	n.d.	> 10000 (27 ± 4%)	n.d.	n.d.	≈ 10000 (50 ± 12%)	> 10000 (0%)

[a] Versus [^{3}H]CCPA. [b] Versus [^{3}H]MSX-2. [c] Versus [^{3}H]ZM-241385. [d] Versus [^{125}I]AB-MECA. [e] Versus [^{3}H]PSB-11. [f] Single value determined in duplicates.

of AR antagonists which exhibit SARs different from the classical xanthine derivatives.

As the affinity of the tricyclic compounds seems to rely on the 3-noradamantyl substituent, their bicyclic precursors (Figure 3) were also tested at three different concentrations

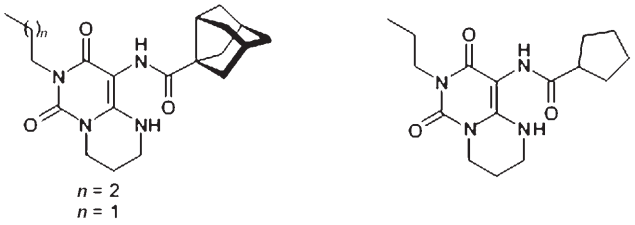


Figure 3. Bicyclic precursors of tricyclic pyrimido[1,2,3-*cd*]purinediones.

(2.5 μM, 25 μM and 250 μM) at the A_1 receptor. The roughly estimated K_i values derived from these inhibition tests were very high (5–20 μM) and did not differ much from the data for the corresponding cyclopentyl derivative (70 μM). Therefore we can conclude that the fixed tricyclic 4,5-dihydro-6*H*,8*H*-pyrimido[1,2,3-*cd*]purine-8,10-dione structure is an important determinant for the high A_1 affinity.

The most potent compounds of the present series, which bear a hydroxypropyl function in the 3-position offer the possibility to prepare prodrugs with improved pharmacokinetic properties, for example, enhanced water solubility. We have realized one example and synthesized a phosphate prodrug **12a** (PSB-16P) of the potent and selective A_1 antagonist **10a** (PSB-16), analogously to a previously developed prodrug (MSX-3) of an A_{2A}-selective antagonist (MSX-2).[25,27] Phosphorylation leads to a large increase in water solubility as reported in a number of previous publications.[27] MSX-3 has been shown to be an extremely useful pharmacological tool for in vivo studies of central A_{2A} ARs allowing systemic application by injections, for example, i.p. application, and even injection directly into certain brain areas.[30] It is rapidly hydrolyzed by ubiquitous phosphatases in vivo to release the potent, but highly lipophilic A_{2A} antagonist MSX-2. Similarly, the new A_1 prodrug **12a** (PSB-16P) or related prodrugs may become useful tools to study the role of adenosine A_1 receptors in animal models.

Species differences

In basic research as well as and in preclinical drug development, rodents (frequently rats or mice) are usually the primary species for in vivo studies. Therefore it is important to obtain pharmacological data for new compounds that are being developed as pharmacological tools not only for the human, but also for a rodent species. Species differences for human and rat A_1, A_{2A} and A_{2B} adenosine receptors have been described to be moderate for most ligands as a result of the high degree of sequence similarity for these three AR subtypes across the mammalian species (> 85%). In contrast, rat and human A_3 receptors show less than 75% similarity in the amino acid sequences,[2] and many A_3 antagonists that have been described exhibit high affinity only to the human but not to the rat A_3 receptor.[12] We initially screened our compounds at the "high-affinity" AR subtypes A_1 and A_{2A} using rat brain tissues (cortical membranes for A_1 and striatal membranes for A_{2A}) as a natural tissue source for these receptors with high receptor densities. Since A_{2B} and A_3 receptors are usually expressed in low density only, we used recombinantly expressed human receptors for testing the compounds' affinities. Selected compounds were additionally investigated at recombinantly expressed human A_1, human A_{2A}, and rat A_3 ARs, respectively.

As expected, species differences were only moderate for A_1 and A_{2A} receptors, but usually much larger at the A_3 AR. Affinities for the investigated compounds were in most cases somewhat weaker at human than with rat A_1 ARs (3–13-fold). The only exception was the pyrimidopurinedione **14**, which was 3-fold more potent at human than at rat A_1 receptors. Only very minor species differences were observed for the A_{2A} receptors; the determined K_i values at rat and human receptors were similar. In contrast, large species differences between human and rat A_3 ARs were determined for the xanthine derivatives DPCPX (K_i > 10000 nM at rat A_3 ARs; 243 nM at human A_3 ARs) and (*S*)-**6c** (K_i > 10000 nM at rat A_3 ARs; 85 nM at human A_3 ARs), but compound **10c**, which exhibited only relatively low affinity for the human A_3 AR (K_i = 2300 nM), was only 2.8-fold less potent for the rat A_3 AR (K_i = 6500 nM).

Functional properties

The most potent compounds of the present series were investigated in [^{35}S]GTPγS binding assays to study their intrinsic activity. The lead compounds DPCPX and KW3902 are antagonists at ARs. DPCPX has been shown to exhibit inverse agonistic activity at A_1 ARs, an effect which can best be demonstrated in cell lines overexpressing the A_1 AR. DPCPX is the compound with the highest intrinsic inverse agonistic activity described so far. In Figure 4A the effects of N^6-cyclopentyladenosine (CPA), DPCPX and 8-cyclopentyltheophylline (CPT) on [^{35}S]GTPγS binding to membranes of human embryonic kidney (HEK) cells overexpressing the human A_1 AR are shown: the full agonist CPA led to a dose-dependent enhancement of GTPγS binding from a basal level of 100% to 201% (see also Table 3). The determined EC_{50} value (2.9 nM) corresponds well with K_i value (2.3 nM) from radioligand binding studies.[4] In contrast, the full inverse agonist DPCPX led to a dose-dependent decrease of GTPγS binding from a basal level of 100% to 57%. Again, the determined IC_{50} value (2.9 nM) corresponds to the K_i value that was determined in radioligand binding studies (3.0 nM). CPT, an analogue of DPCPX in which the 1,3-dipropyl groups of DPCPX are replaced by methyl groups, also reduced GTPγS binding, but to a lesser extent; it can thus be characterized as a partial inverse agonist showing 66% of the intrinsic activity of DPCPX.

In Figure 4B and 4C and Table 3 the effects of selected newly synthesized xanthine and pyrimidopurinedione derivatives are shown. None of the investigated compounds led to an increase in GTPγS binding thus showing that they were all antagonists at A_1 ARs. In fact, like DPCPX, they led to a de-

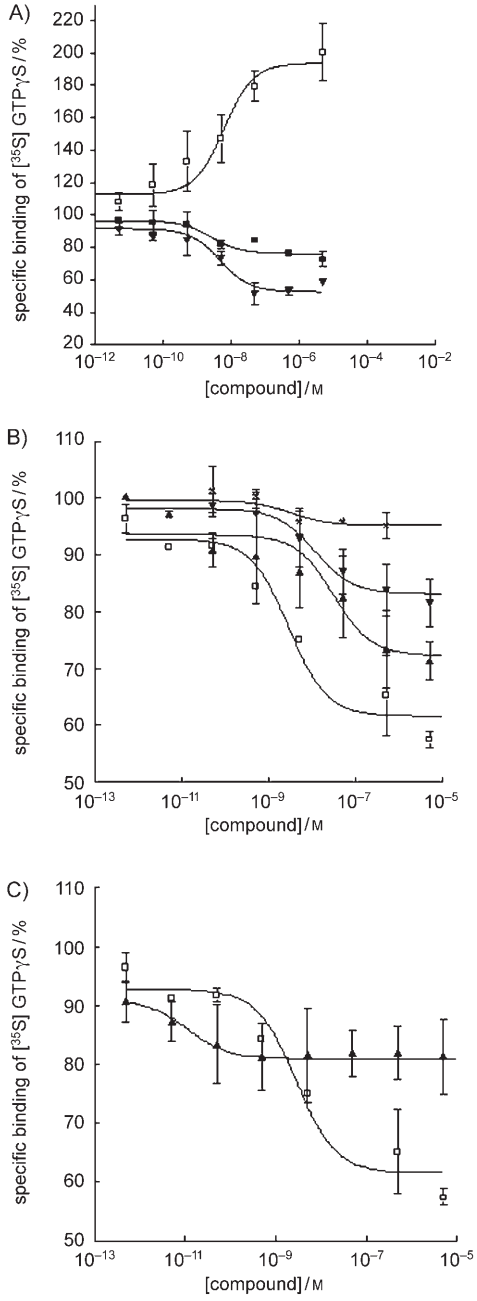


Figure 4. [^{35}S]GTPγS binding studies at human adenosine A_1 receptors, which are expressed in human embryonic kidney (HEK) cells. A) Curves for the standard compounds CPA (□, N^6-cyclopentyladenosine) as a full A_1 agonist, DPCPX (▼, 1,3-dipropyl-8-cyclopentylxanthine) as an A_1 antagonist with full inverse agonistic activity, and CPT (■, 8-cyclopentyltheophylline) as an A_1 antagonist with partial inverse agonistic activity. B) Effects of selected potent 1,3,8-substituted xanthine and 2,9-substituted pyrimidopurinedione derivatives in comparison with DPCPX (□): **9a** (*), 3-benzyl analogue of DPCPX; **6e** (▲), 3-phenyl analogue of DPCPX; **14** (▼), 2-(3-noradamantyl)-9-butyl-4,5-dihydro-6*H*,8*H*-pyrimido[1,2,3-*cd*]purine-8,10-dione. C) Effect of 1-butyl-3-(3-hydroxypropyl)-8-(3-noradamantyl)xanthine (▲, **10c**) in comparison with DPCPX (□).

crease in GTPγS binding, but to variable extents. None were as efficacious as the full inverse agonist DPCPX. Replacement of the 3-propyl residue of DPCPX by a phenyl ring (in **6e**) reduced the intrinsic activity to 47% of that of DPCPX. A benzyl group instead of a phenyl residue nearly abolished the intrinsic activity (compound **9a**). The tricyclic noradamantyl-substituted pyrimidopurinedione **14** and 1-butyl-3-(3-hydroxypropyl)-8-(3-noradamantyl)xanthine (**10c**), the most potent compound of the present series, showed low intrinsic activity relative to DPCPX: 44 and 42%, respectively. The determined IC_{50} values for the inhibition of GTPγS binding were in most cases consistent with K_i values from the radioligand binding studies (Table 3). It can be concluded that minor structural modifications may have a large influence on the inverse agonistic efficacy of xanthine derivatives. This may play a role in tissues with high A_1 AR expression where the receptors may be constitutively active.[17,18]

Table 3. GTPγS binding studies of selected compounds at human adenosine A_1 receptors in comparison with K_i values from adenosine A_1 receptor radioligand binding data.[a]

Compd	K_i [nM][b]		EC_{50} or IC_{50} [nM][c]	Intrinsic Activity [%][d]
	Rat A_1[e]	Human A_1	Human A_1	
CPA	0.32[4][f]	2.3[15]	2.9 ± 1.8	201 ± 17[g]
DPCPX	0.5[39]	3.0[39]	2.9 ± 1.0	100[h]
CPT	24[41][f]	n.d.	6.2 ± 3.1	66 ± 3
9a	8.7 ± 1.9	n.d.	9.3 ± 7.9	11 ± 1
6e	1.0 ± 0.4	7.1 ± 1.1	56 ± 47	47 ± 7
10c	0.12 ± 0.01	0.7 ± 0.2	0.012 ± 0.002	42 ± 5
14	40.6 ± 11.3	13.8 ± 3.7	75 ± 51	44 ± 2

[a] ARs expressed in human embryonic kidney (HEK) cells; values expressed as mean ± SEM. [b] Versus [^{3}H]CCPA. [c] Inhibition of [^{35}S]GTPγS binding. [d] Activity of inverse agonists normalized with respect to the full inverse agonist DPCPX set at 100%. [e] AR from rat brain cortical membranes. [f] Versus [^{3}H]N^6-(*R*)-phenylisopropyladenosine. [g] Value represents maximal stimulation by the full agonist CPA ($n = 3$) relative to basal (100%). [h] The full inverse agonist DPCPX reduced [^{35}S]GTPγS binding from basal (100%) to 57 ± 3% ($n = 4$).

Conclusions

In conclusion, we have explored the SAR of the substituent in the 3-position of xanthine derivatives related to the standard A_1 AR antagonists DPCPX and KW3902. This led to the finding that phenyl, benzyl and 1-phenylethyl residues are well tolerated by the A_1 ARs. The highest affinity and selectivity, however, was observed with the more hydrophilic and flexible 3-hydroxypropyl substituent, suggesting that the hydroxy group at that position may form a hydrogen bond to the A_1 receptor protein. The most potent compound of the present series was 1-butyl-3-(3-hydroxypropyl)-8-(3-noradamantyl)xanthine (**10c**) exhibiting a K_i value of 0.124 nM at rat and 0.7 nM at human adenosine A_1 receptors, combined with high selectivity (1400-fold versus human A_{2A}, 267-fold versus human A_{2B} and >3000-fold versus human A_3). It belongs to the most potent and selective A_1 antagonists described to date. We prepared the first water soluble phosphate prodrug of a highly potent and selective A_1 AR antagonist, which can be a useful pharmacological tool for in vivo studies. Finally, we discovered a new class of potent and selective A_1 AR antagonists, the 8-alkyl-2-(3-noradamantyl)pyrimido[1,2,3-*cd*]purine-8,10-diones.

Experimental Section

General remarks: NMR spectra were recorded on a Varian XL-300 (^{1}H: 300 MHz, ^{13}C: 75 MHz) or a Bruker DRX 500 (^{1}H: 500 MHz, ^{13}C: 125 MHz). Deuterated DMSO was used as a solvent unless otherwise noted. The chemical shifts of the remaining protons of the solvent were used as an internal standard: ^{1}H, 2.49 ppm; ^{13}C, 39.7 ppm. All chemical shifts (δ) are expressed in ppm. Coupling constants (J) are given in Hertz (Hz). The following abbreviations are used: pr = propyl, cp = cyclopentyl, ar = phenyl, pheth = phenylethyl, and hpr = hydroxypropyl. The reactions were monitored by TLC using aluminum sheets with silica gel 60 F_{254} (Merck). The melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Optical rotations were determined with a Perkin–Elmer 241 polarimeter. Elemental analyses were performed in the Pharmaceutical Institute, Pharmaceutical Chemistry Endenich, University of Bonn, using a VarioEL instrument (Elementar Analysensysteme GmbH, Hanau, Germany). Mass spectra were collected on an MS-50 A.E.I. (Manchester) mass spectrometer with an ionization energy of 70 eV. The 6-aminouracils with an α-branched substituent ((*R*)-**1a** and (*S*)-**1a**) or a phenyl substituent (in **1b**) in position 1 were synthesized as previously described,[23] and compound **8a** was prepared according to literature procedure.[24] Compounds **7b**, **10a**, **10b**, **10c**, **13**–**16** were synthesized by following a multistep procedure recently developed in our group.[26]

General procedure for the preparation of compounds (*R*)-2a, (*S*)-2a and 2b: A suspension of 1-substituted 6-aminouracil ((*R*)-**1a**, (*S*)-**1a**, or **1b**) (10 mmol) and potassium carbonate (3.5 g, 25 mmol) in *N*,*N*-dimethylformamide (40 mL) was stirred for 3 h at room temperature. Propyl iodide (2 mL, 3.4 g, 20 mmol) was then added, and the reaction mixture was stirred for about 20 h at room temperature. The progress of the reaction was controlled by TLC using dichloromethane/methanol (9:1) as the eluent. After all of the starting material had disappeared, water (50 mL) was added and the resulting mixture was washed three times with petrol ether (bp: 60 °C, 40 mL). Then the aqueous layer was extracted three times with dichloromethane (30 mL). The combined dichloromethane layers were dried with sodium sulfate, and after filtration, the solvent was removed by distillation under reduced pressure.

6-Amino-1-(1-phenylethyl)-3-propyluracil ((*R*)-2a and (*S*)-2a): The remaining brown oil was stirred in a mixture of diethyl ether and ethanol (1:1, *v*/*v*). The solvent was removed under vacuum, and the residue was used without further purification in the next step. Yield: *R* isomer: 1.18 g (51%), $[\alpha]_D^{20} = -184.82$ ($c = 0.94$, ethanol); *S* isomer: 1.04 g (45%); ^{1}H NMR (500 MHz): $\delta = 0.78$ (t, 3H, J = 7.5 Hz, CH_3-pr), 1.33–1.46 (m, 2H, CH_2-pr), 1.83 (d, 3H, $J = 7.6$ Hz, CH_3-pheth), 3.61–3.70 (m, 2H, N3CH_2), 4.81 (s, 1H, H5), 5.70–5.91 (m, 1H, N1CH), 6.65 (s, 2H, NH_2), 7.23–7.37 ppm (m, 5H, H-ar); ^{13}C NMR (125 MHz): $\delta = 11.4$ (CH_3-pr), 21.2 (CH_3-pheth), 31.1 (CH_2-pr), 36.1 (N1CH), 41.5 (N3CH_2), 76.5 (C5), 125.9, 127.1, 128.2, 128.6 (C-ar), 155.1 (C6), 161.8 (C2), 162.6 ppm (C4).

6-Amino-1-phenyl-3-propyluracil (2b):[31] The remaining yellowish product was stirred in petrol ether (10 mL), which was subsequently decanted. The residue was dissolved in a mixture of petrol ether and ethanol (1:4 *v*/*v*). After 24 h the precipitated product was collected by suction filtration to give 1.5 g (65%); mp: 178–181 °C (dec); ^{1}H NMR (500 MHz): $\delta = 0.83$ (t, 3H, $J = 7.6$ Hz, CH_3-pr), 1.51 (quint, 2H, $J = 7.6$ Hz, CH_2-pr), 3.68 (t, 2H, $J = 7.6$ Hz, N3CH_2), 4.81 (s, 1H, H5), 6.12 (s, 2H, NH_2), 7.33–7.56 ppm (m, 5H, H-ar); ^{13}C NMR (125 MHz): $\delta = 11.6$ (CH_3-pr), 21.3 (CH_2-pr), 41.6 (N3CH_2), 75.1 (C5), 129.6, 129.9, 130.3, 134.9 (C-ar), 151.5 (C6), 154.5 (C2), 162.0 ppm (C4); elemental analysis calcd (%) for $C_{13}H_{15}N_3O_2$: C 63.66, H 6.16, N 17.12; found: C 63.82, H 5.99, N 16.93.

6-Amino-5-nitroso-1-(1-phenylethyl)-3-propyluracil ((*R*)-3a and (*S*)-3a): 6-Amino-1-(1-phenylethyl)-3-propyluracil ((*R*)-**2a** or (*S*)-**2a**) (7.0 g, 25.6 mmol) was dissolved in a mixture of acetic acid and water (2:1, 75 mL) To improve the solubility, ethanol (6 mL) was added. Then sodium nitrite (4.5 g, 65 mmol) was added in small portions. After stirring the reaction mixture for 24 h at room temperature a solid precipitated. The solvent was decanted and extracted twice with dichloromethane (30 mL). Then the combined organic layers were washed with a saturated sodium bicarbonate solution (30 mL) and dried with sodium sulfate. The solvent was removed under reduced pressure, and the residue was used without further purification for the next step. To obtain a sample for analytical purposes, the residue was suspended in diethyl ether and stored at 4 °C for 4 h. The resulting dark violet crystals were collected by suction filtration. Yield: *R* isomer 4.1 g (53%); mp: 160 °C; *S* isomer: 3.5 g (45%); mp: 163 °C; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta =$ 0.83 (t, 3H, $J = 7.5$ Hz, CH_3-pr), 1.54–1.57 (m, 2H, CH_2-pr), 1.79 (d, 3H, $J = 7.2$ Hz, CH_3-pheth), 3.82–3.88 (m, 2H, N3CH_2), 6.10–6.21 (m, 1H, N1CH), 7.20–7.28 (m, 5H, H-ar), 13.47 ppm (s, 2H, NH_2); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 10.2$ (CH_3-pr), 14.5 (CH_3-pheth), 20.1 (CH_2-pr), 42.6 (N3CH_2), 51.5 (N1CH), 125.0, 127.3, 128.2, 136.3 (C-ar), 136.9 (C5), 144.9 (C2), 147.6 (C6), 159.5 ppm (C4); elemental analysis calcd (%) for $C_{15}H_{18}N_4O_3$: C 59.59, H 6.00, N 18.53; found (for (*R*)-**3a**): C 59.26, H 6.00, N 18.36; (for (*S*)-**3a**): C 59.15, H 6.34, N 18.51.

6-Amino-5-nitroso-1-phenyl-3-propyluracil (3b):[31] 6-Amino-1-phenyl-3-propyluracil (**2b**) (1.5 g, 6.12 mmol) was dissolved in a mixture of acetic acid and water (1:1, 80 mL) by heating the mixture to 80 °C. Then the solution was cooled to room temperature and sodium nitrite (1.2 g, 17.4 mmol) was added in small portions. After stirring for 1 h at room temperature the reaction mixture was cooled again to 4 °C and the violet precipitate was collected by suction filtration and washed with water (10 mL) to give 0.73 g (44%) of **3b**; mp: 215 °C (dec); ^{1}H NMR (300 MHz): $\delta = 0.92$ (t, 3H, $J = 7.5$ Hz, CH_3-pr), 1.66 (quint, 2H, $J = 7.6$ Hz, CH_2-pr), 3.87 (t, 2H, $J = 7.5$ Hz, N3CH_2), 7.44–7.62 (m, 5H, H-ar), 7.92 ppm (s, 2H, NH_2); ^{13}C NMR (75 MHz): $\delta = 11.2$ (CH_3-pr), 20.7 (CH_2-pr), 42.3 (N3CH_2), 128.6, 128.9, 129.9, 131.8 (C-ar), 139.1 (C5), 145.9 (C2), 148.8 (C6), 160.0 ppm (C4); elemental analysis calcd (%) for $C_{13}H_{14}N_4O_3$: C 56.91, H 5.14, N 20.42; found: C 56.64, H 5.12, N 19.99.

General procedure for the preparation of compounds (*R*)-4a, (*S*)-4a and 4b: 1-Substituted 6-amino-5-nitroso-3-propyluracil ((*R*)-**3a**, (*S*)-**3a**, or **3b**) (20 mmol) was dissolved in aqueous ammonia solution (12.5%, 200 mL) by heating at 80 °C. The insoluble residue was removed by filtration. Under an inert atmosphere of argon, sodium dithionite ($\approx$40 mmol) was added in small portions to the clear violet-colored solution until it became colorless. Then the mixture was cooled for 1 h to −5 °C and then brought to 4 °C. The resulting slightly yellow precipitate was separated by suction filtration, and was washed with small amounts of water.

5,6-Diamino-1-(1-phenylethyl)-3-propyluracil ((*R*)-4a, (*S*)-4a): *R* isomer: 2.5 g (42%); *S* isomer: 2.7 g (45%); ^{1}H NMR (300 MHz, 40 °C): $\delta = 0.82$ (t, 3H, $J = 7.5$ Hz, CH_3-pr), 1.55 (sext, 2H, $J = 7.6$ Hz, CH_2-pr), 1.85 (d, 3H, $J = 7.2$ Hz, CH_3-pheth); 3.21 (s, 2H, C6-NH_2), 3.76 (t, 2H, $J = 7.6$ Hz, N-CH_2), 5.63 (s, 2H, C5-NH_2), 7.21–7.54 ppm (m, 5H, H-ar); ^{13}C NMR (75 MHz): $\delta = 11.4$ (CH_3-pr), 13.3 (CH_3-pheth); 21.0 (CH_2-pr), 42.1 (N3CH_2), 51.2 (CH-pheth); 95.4 (C5), 128.4, 129.6, 129.9, 134.1 (C-ar), 144.3 (C2), 150.1 (C6), 160.3 ppm (C4).

5,6-Diamino-1-phenyl-3-propyluracil (4b):[31] 5.1 g (98%); mp: 174.2 °C; ^{1}H NMR (300 MHz): $\delta = 0.83$ (t, 3H, $J = 7.5$ Hz, CH_3-pr), 1.54 (sext, 2H, $J = 7.6$ Hz, CH_2-pr), 3.01 (s, 2H, C6-NH_2), 3.74 (t, 2H, $J = 7.6$ Hz, N3CH_2), 5.31 (s, 2H, C5-NH_2), 7.31–7.54 ppm (m, 5H, H-ar); ^{13}C NMR (75 MHz): $\delta = 11.4$ (CH_3-pr), 21.0 (CH_2-pr), 42.0 (N3CH_2), 96.4 (C5), 129.2, 129.6, 129.8, 135.1 (C-ar), 143.3 (C2), 149.5 (C6), 159.3 ppm (C4); elemental analysis calcd (%) for $C_{13}H_{16}N_4O_2$: C 59.98, H 6.20, N 21.52; found: C 59.83, H 6.33, N 21.45.

General procedure for the preparation of compounds (*R*)-5c, (*S*)-5c, (*R*)-5d, (*S*)-5d, 5e, 5f: 1-Substituted 5,6-diamino-3-propyluracil ((*R*)-**4a**, (*S*)-**4a**, **4b**) (4.2 mmol) was suspended in methanol (30 mL). Cyclopentanecarboxylic acid, benzoic acid, or 4-biphenylcarboxylic acid (4.5 mmol) was added under an inert atmosphere of argon. The resulting suspension was stirred for 30 min at room temperature. Then *N*-(3-dimethylaminoproyl)-*N'*-ethylcarbodiimide hydrochloride (0.9 g, 4.7 mmol) was added and the reaction mixture was stirred for 24 h at room temperature. The methanol was removed by distillation under reduced pressure and the yellow oily residue was used directly for the next step.

6-Amino-5-cyclopentylcarboxamido-1-(1-phenylethyl)-3-propyluracil ((*R*)-5c, (*S*)-5c): Yield: 0.34 g (21%).

5-(4-Biphenyl)carboxamido-1-(1-phenylethyl)-3-propyluracil ((*R*)-5d, (*S*)-5d): Yield: 0.51 g (26%).

6-Amino-5-cyclopentylcarboxamido-1-phenyl-3-propyluracil (5e): Yield: 0.33 g (22%).

6-Amino-5-phenylcarboxamido-1-phenyl-3-propyluracil (5f): Yield: 0.58 g (38%).

General procedure for the preparation of compounds (*R*)-6c, (*S*)-6c, 6e or 6f: A freshly prepared methanolic solution of sodium methylate (20%, 40 mL) was added to the oily 6-amino-5-carboxamidouracil derivatives ((*R*)-**5c**, (*S*)-**5c**, **5e**, or **5f**). The reaction mixture was kept at reflux for 3.5 h. It was then cooled to 40 °C, and water (5 mL) was added. The acidity was adjusted to pH 5 with concentrated hydrochloric acid. Then the reaction mixture was stored at 4 °C until a precipitate was formed. The solid product was collected by suction filtration, and was recrystallized from methanol/water, and was finally washed thoroughly with water.

8-Cyclopentyl-3-(1-phenylethyl)-1-propylxanthine ((*R*)-6c, (*S*)-6c): *R* isomer: 350 mg (52%); mp: 158 °C, $[\alpha]_D^{20} = +98.36$ ($c = 0.9$, ethanol); *S* isomer: 270 mg (40%); mp: 161 °C. $[\alpha]_D^{20} = -102.66$ ($c = 1.011$, ethanol); ^{1}H NMR (500 MHz): $\delta = 0.82$ (t, 3H, $J = 7.4$ Hz, CH_3-pr), 1.47–1.60 (m, 4H, 2CH_2-cp), 1.65–1.80 (m, 4H, CH_2-pr, CH_2-cp), 1.91–1.99 (m, 5H, CH_2-cp, CH_3-pheth), 3.09 (quint, 1H, $J = 7.9$ Hz, CH-cp), 3.81 (t, 2H, $J = 7.4$ Hz, N1CH_2), 6.09 (q, 1H, $J = 7.6$ Hz, CH-pheth), 7.19–7.37 (m, 5H, H-ar), 13.09 ppm (s, 1H, NH); ^{13}C NMR (125 MHz): $\delta = 11.2$ (CH_3-pr), 17.1 (CH_3-pheth), 21.0 (CH_2-pr), 25.1, 25.1, 32.0, 32.0 (CH_2-cp), 38.7 (CH-cp), 42.1 (N1CH_2), 53.0 (CH-pheth), 106.6 (C5), 126.6, 127.0, 127.1, 128.4, 140.8 (C-ar), 147.6 (C6), 150.5 (C8), 154.0 (C2), 157.6 ppm (C4); elemental analysis calcd (%) for $C_{21}H_{26}N_4O_2$: C 68.83, H 7.15, N 15.29; found (for (*R*)-**6c**): C 68.58, H 7.12, N 15.22; (for (*S*)-**6c**): C 68.44, H 7.12, N 15.00.

8-Cyclopentyl-3-phenyl-1-propylxanthine (6e): Yield: 0.3 g (63%); mp: 292.7 °C; ^{1}H NMR (500 MHz): $\delta = 0.87$ (t, 3H, $J = 7.8$ Hz, CH_3-pr), 1.53–1.70 (m, 8H, 3CH_2-cp, CH_2-pr), 1.91 (quart, 2H, $J = 8.2$ Hz, CH_2-cp), 3.04 (quint, 1H, $J = 7.9$ Hz, CH-cp), 3.85 (t, 2H, $J = 7.4$ Hz, N1CH_2), 7.38–7.51 (m, 5H, H-ar), 13.15 ppm (s, 1H, NH); ^{13}C NMR (125 MHz): $\delta = 11.3$ (CH_3-pr), 21.0 (CH_2-pr), 25.1, 31.8, 32.0 (CH_2-cp), 39.1 (CH-cp), 42.4 (N1CH_2), 106.6 (C5), 128.3, 128.8, 129.0, 135.9 (C-ar), 148.0 (C6), 150.7 (C8), 154.1 (C2), 157.9 ppm (C4); elemental analysis calcd (%) for $C_{19}H_{22}N_4O_2$: C 67.44, H 6.55, N 16.56; found: C 66.99, H 6.44, N 16.42.

3,8-Diphenyl-3-propylxanthine (6f): Yield: 185 mg (36%); mp: 348.2 °C (dec); ^{1}H NMR (500 MHz): $\delta = 0.88$ (t, 3H, $J = 7.5$ Hz, CH_3-pr), 1.64 (quint, 2H, $J = 7.6$ Hz, CH_2-pr), 3.92 (t, 2H, $J = 7.6$ Hz, N1CH_2), 7.44–7.79 (m, 10H, H-ar), 13.89 ppm (s, 1H, NH); ^{13}C NMR (125 MHz): $\delta = 11.6$ (CH_3-pr), 21.2 (CH_2-pr), 67.8 (N1CH_2), 108.4 (C5), 126.7, 128.0, 128.7, 128.9, 129.2, 129.3, 130.5, 136.9 (C-ar), 148.8 (C8), 150.0 (C6), 150.9 (C4), 154.6 ppm (C2); elemental analysis calcd (%) for $C_{20}H_{18}N_4O_2$: C 69.35, H 5.24, N 16.17; found: C 69.21, H 5.25, N 16.23.

General procedure for the preparation of compounds (*R*)-6d and (*S*)-6d: A freshly prepared methanolic solution of sodium methylate (20%, 35 mL) was added to the oily 6-amino-5-biphenylcarboxamido-1-(1-phenylethyl)-3-propyluracil ((*R*)-**5d** or (*S*)-**5d**). The reaction mixture was kept at reflux for 12 h. Water (30 mL) was then added, and the mixture was cooled to room temperature. The acidity was adjusted to pH 5 with concentrated hydrochloric acid. The precipitate was collected by suction filtration, and washed with water (20 mL) twice. Then the crude product was purified by column chromatography with dichloromethane/methanol (9:1) solvent. The resulting product was washed with diethyl ether (50 mL) and dried in vacuo to give either enantiomer of **6d**. *R* isomer: 2.0 g (45%); mp: 290 °C, $[\alpha]_D^{20} = +51.81$ ($c = 0.564$, ethanol); *S* isomer: 1.9 g (40%); mp: 288 °C, $[\alpha]_D^{20} = -51.02$ ($c = 0.568$, ethanol); ^{1}H NMR (500 MHz): $\delta = 0.86$ (t, 3H, $J = 7.5$ Hz, CH_3-pr), 1.57 (quint, 2H, $J = 7.7$ Hz, CH_2-pr), 2.00 (d, 3H, $J = 7.4$ Hz, CH_3-pheth), 3.86 (t, 2H, $J = 7.4$ Hz, N1CH_2), 6.22 (quart, 1H, $J = 7.3$ Hz, CH-pheth), 7.21–8.19 (m, 14H, H-ar), 13.86 ppm (s, 1H, NH);^{13}C NMR (125 MHz): $\delta = 11.3$ (CH_3-pr), 17.2 (CH_3-pheth), 20.0 (CH_2-pr), 42.4 (N1CH_2), 53.1 (N3CH), 108.5 (C5), 126.2, 127.0, 127.2, 128.1, 128.3, 129.1, 133.5, 139.2, 139.4, 140.7, 141.8, 142.8 (C-ar), 148.2 (C6), 150.5 (C8), 154.2 (C2), 165.3 ppm (C4); elemental analysis calcd (%) for $C_{28}H_{26}N_4O_2$: C 74.65, H 5.86, N 12.44; found (*R*)-**6d**: C 74.28, H 5.82, N 12.38; (*S*)-**6d**: C 74.49, H 5.72, N 12.44.

General procedure for the synthesis of compounds 11a and 11b: Under an inert atmosphere of argon, the 1-substituted 8-cyclopentyl-3-(3-hydroxypropyl)xanthine (**10a** or **10b**) (0.31 mmol) was suspended in freshly distilled trimethylphosphate. Phosphorus oxychloride (0.1 mL) was added to the resulting suspension, and the reaction mixture was stirred at room temperature for 2 h. Then water (10 mL) was added and the precipitate was isolated by suction filtration. The product was washed first with water (10 mL) and then with methanol (10 mL) to yield the pure white products **11a** and **11b**.

3-(3-Chloropropyl)-8-cyclopentyl-1-propylxanthine (11a): Yield: 36 mg (34%); mp: 182 °C; ^{1}H NMR (500 MHz): $\delta = 0.84$ (t, 3H, $J = 7.5$ Hz, CH_3-pr), 1.54 (sext, 2H, $J = 7.4$ Hz, CH_2-pr), 1.60–2.01 (m, 8H, 4CH_2-cp), 2.12 (quint, 2H, $J = 6.7$ Hz, CH_2hpr), 3.12 (quint, 1H, $J = 8.1$ Hz, CH-cp), 3.65 (t, 2H, $J = 6.5$ Hz, CH_2OH), 3.81 (t, 2H, $J = 7.4$ Hz, N1CH_2), 4.09 (t, 2H, $J = 6.7$ Hz, N3CH_2), 13.08 ppm (NH); ^{13}C NMR (125 MHz): $\delta = 11.3$ (CH_3-pr), 20.9 (CH_2-pr), 25.2 (2C, 2CH_2-cp), 31.0 (CH_2-hpr), 32.0 (2C, 2CH_2-cp), 38.9 (CH-cp), 40.8 (N3CH_2), 42.2 (N1CH_2), 42.9 (CH_2OH), 106.4 (C5), 147.7 (C8), 150.9 (C4), 154.0 ppm (C2), 158.0 (C6); MS: $m/z = 340.1$ (16) + 338.1 (48).

1-Butyl-3-(3-chloropropyl)-8-cyclopentylxanthine (11b): Yield: 27 mg (25%); ^{1}H NMR (300 MHz): $\delta = 0.90$ (t, 3H, $J = 7.3$ Hz, CH_3-bu), 1.30 (sext, 2H, $J = 7.4$ Hz, CH_2-bu), 1.53 (quint, 2H, $J = 7.2$ Hz, CH_2-bu), 1.63–2.07 (m, 8H, 4CH_2-cp), 2.14 (quint, 2H, $J = 6.7$ Hz, CH_2-hpr), 3.15 (quint, 1H, $J = 8.02$ Hz, CH-cp), 3.68 (t, 2H, $J = 6.7$ Hz, CH_2OH), 3.88 (t, 2H, $J = 7.3$ Hz, N1CH_2), 4.12 (t, 2H, $J = 6.9$ Hz,

$N3CH_2$), 13.08 ppm (NH); MS: $m/z = 352.2$ (16); 354.2 (50) + 316.1 (28).

Synthesis of 8-cyclopentyl-3-[3-O-(phosphatyl)propyl]-1-propylxanthine (12a): 8-Cyclopentyl-3-(3-hydroxypropyl)-1-propylxanthine (100 mg, 0.31 mmol) was dissolved in a mixture of THF (20 mL) and acetonitrile (10 mL) under an inert atmosphere of argon. Then 1-H-tetrazole (210 mg, 3.0 mmol) was added, followed by di-tert-butyl-*N*,*N*-diethyl phosphoramidite (0.3 mL). The reaction mixture was stirred for 2 h at room temperature. Then tert-butylhydroperoxide (70%, 5 mL, 35 mmol) was added and the mixture was stirred for another hour. Then it was cooled to 0 °C and a sodium bisulfite solution (5%, 15 mL) was added. The solution was stirred until the reaction temperature reached room temperature again. It was extracted five times with dichloromethane (10 mL), and the combined organic phases were washed twice with water (20 mL each) and dried over magnesium sulfate. The solvent was removed in vacuo and the resulting product was directly used for the subsequent step.

After the solid product was dissolved in dichloromethane (10 mL) trifluoroacetic acid (98%, 0.3 mL, 3.9 mmol) was added. The solution was kept for 2 h at room temperature and the solvent was subsequently removed in vacuo. The oily residue was co-evaporated with water until no white fumes appeared anymore. Then the white precipitate was isolated by suction filtration and washed with water (30 mL). The product was detected by TLC by using isopropanol/ammonia/water (6:3:1) as the eluent, and visualized by using a solution of 0.1% $FeCl_3 \cdot 6H_2O$ and 7% sulfosalicylic acid in 75% ethanol. Yield: 45 mg (36%); ^{1}H NMR (500 MHz): $\delta = 0.84$ (t, 3H, $J = 7.4$ Hz, CH_3-pr), 1.53–2.10 (m, 12H, 4 CH_2-cp, CH_2-pr, CH_2-hpr), 3.12 (quint, 1H, $J = 8.2$ Hz, CH-cp), 3.81 (t, 2H, $J = 7.4$ Hz, CH_2OP), 3.85 (t, 2H, $J = 6.7$ Hz, $N1CH_2$), 4.02 (t, 2H, $J = 7.4$ Hz, $N3CH_2$), 13.09 ppm (br, 1H, NH); ^{13}C NMR (125 MHz): $\delta = 11.3$ (CH_3-pr); 21.0 (CH_2-pr), 25.2 (2C, 2 CH_2-cp), 29.0 (CH_2-hpr), 32.1 (2C, CH_2-pr), 38.9 (CH-cp), 40.4 ($N3CH_2$), 42.1 ($N1CH_2$), 63.3 (d, $^1J = 4.7$ Hz, CH_2OP), 106.4 (C5), 147.6 (C8), 150.8 (C4), 154.0 (C2), 158.0 ppm (C6).

Synthesis of 1-butyl-8-noradamantylxanthine (8b): 6-Amino-3-butyl-5-(hexahydro-2,5-methanopentalene-3a)-carboxamido-2,4-dioxo-1,2,3,4-tetrahydropyrimidine (**7b**) (350 mg, 1mmol) was stirred in hexamethyldisilazane (30 mL) for 18 h under reflux. Then the solvent was removed by distillation under reduced pressure. The product was purified by column chromatography by using dichloromethane/methanol (100:2) as the eluent to afford **8b** (95 mg) in 29% yield. ^{1}H NMR (500 MHz): $\delta = 0.88$ (t, 3H, $J = 7.3$ Hz, CH_3-bu), 1.27 (sext, 2H, $J = 7.4$ Hz, CH_2-bu), 1.49 (quint, 2H, $J = 7.3$ Hz, CH_2-bu), 1.59 (m, 4H, C1*H*H-n, C6*H*H-n, $C7H_2$-n), 1.88 (m, 4H, C1*H*H-n, C3*H*H-n, C4*H*H-n, C6*H*H-n), 2.09 (m, 2H, C3*H*H-n, C4*H*H-n); 2.27 (m, 2H, C2H-n, C5H-n), 2.57 (t, 1H, $J = 6.7$ Hz, C6aH-n), 3.82 (t, 2H, $J = 7.3$ Hz, $N1CH_2$); 11.63 (br s, 1H, N3H), 12.80 ppm (br s, 1H, N7H); ^{13}C NMR (125 MHz): $\delta = 13.9$ (CH_3-bu), 19.7 (CH_3CH_2-bu), 29.9 (NCH_2CH_2-bu), 34.3 (C7-n), 37.1 (2C, C2-n, C5-n), 39.5 (NCH_2), 43.3(2C, C1-n, C6-n), 45.2 (C6a-n), 48.4 (2C, C3-n, C4-n), 49.0 (C3a-n), 106.6 (C5), 147.2 (C8), 151.1 (C4), 154.8 (C2), 160.2 ppm (C6).

Synthesis of 3-benzyl-8-cyclopentyl-1-propylxanthine (9a): 8-Cyclopentyl-1-propylxanthine **8a**[24] (0.2 g, 0.76 mmol), potassium hydroxide (63 mg, 1.1. mmol) and benzyl bromide (0.1 mL) were suspended in ethanol (10 mL). The reaction mixture was kept at reflux for 1 h, during which time it became clear. The suspension was cooled to room temperature, water (10 mL) was added, and the precipitate was collected by filtration. The starting material was removed by suspending the solid material in a solution of sodium hydroxide (1 N), in which only the starting material was soluble, and the purified product was collected by filtration. The product was washed with water, and after drying, it was purified by recrystallization from dichloromethane (5 mL) by the addition of diethyl ether (10 mL). Yield: 0.14 g (52%); mp: 217 °C; ^{1}H NMR ([D_6]DMSO/$CDCl_3$ = 4:1): $\delta = 0.33$ (t, 3H, CH_3-pr); 0.40–0.97 (m, 10H, cp-CH_2 and CH_2-pr); 2.20 (m, 1H, C1′H-cp); 3.02 (t, 2H, NCH_2-pr); 4.33 (s, 2H, CH_2-bn); 6.46 ppm (m, ar-5H); elemental analysis calcd (%) for $C_{20}H_{26}N_4O_2$: C 67.8, H 7.39, N 15.8; found: C 67.5, H 6.98, N 15.6.

Biological assays:

Materials: Radioligands were obtained from the following sources: [^{3}H]CCPA from NEN Life Science (54.9 Ci mmol^{-1}), [^{3}H]MSX-2 from Amersham (85 Ci mmol^{-1}), [^{3}H]ZM-241385 from Tocris (17 Ci mmol^{-1}), and [^{3}H]PSB-11 (53 Ci mmol^{-1}) and [^{125}I]AB-MECA (2000 Ci mmol^{-1}) from Amersham. 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.[15] For [^{35}S]GTPγS binding assays HEK cells expressing the human A_1 AR in high density were used. For assays at human A_{2B} and rat A_3 ARs, commercially available membrane preparations containing the human A_{2B}, or the rat A_3 AR, respectively, expressed in human embryonic kidney (HEK) cells, were obtained from Biotrend (Cologne, Germany). Frozen rat brains obtained from Pel Freez, Rogers, Arkansas, USA, were dissected to obtain cortical membrane preparations for A_1 assays, and striatal membrane preparations for A_{2A} assays as described.[32,33]

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: [^{3}H]CCPA:[34] 0.5 nM (rat and human A_1); [^{3}H]MSX-2:[35] 1.0 nM (rat and human A_{2A}); [^{3}H]ZM-241385:[36] 5 nM (human A_{2B}); [^{3}H]PSB-11:[37] 0.5 nM (human A_3). Binding assays were performed as described.[15,28,34–37] Approximately 50 μg mL^{-1} of protein were used in the assays. At least two to three separate experiments were performed, each in duplicate or triplicate.

[^{35}S]GTPγS binding assays: Membrane preparations of HEK293 cells expressing the human A_1 AR (5 μg per tube) were incubated with 0.1–0.5 nM [^{35}S]GTPγS (46.3 TBq mmol^{-1}, NEN) in a total volume of 200 μL in 50 mM Tris-HCl buffer pH 7.4 containing 1 mM EDTA, 5 mM $MgCl_2$, 1 mM dithiothreitol, 10 μM GDP, 100 mM NaCl, 2 IU mL^{-1} adenosine deaminase (ADA), 0.5% bovine serum albumin and test compound, according to Lorenzen et al.[38] Adenosine (10 nM) was added to prove that the amount of ADA was sufficient to remove endogenous adenosine as well as the added adenosine (data not shown). Nonspecific binding was determined by using 10 μM of unlabeled GTPγS. The incubations were terminated after 45 min at 25 °C by the addition of 1 mL of ice-cold buffer containing 50 mM Tris-HCl pH 7.4 and 5 mM $MgCl_2$, and rapid filtration through GF/B filters (Whatman) on a 48-channel Brandel cell harvester, followed by three washing steps with cold buffer (2 mL each). The wet filters were transferred into scintillation vials, 2 mL of scintillation cocktail (Ultima Gold, Canberra Packard, Dreieich, Germany) was added and the radioactivity was measured by liquid scintillation in a Tricarb 2100 TR liquid scintillation analyzer (Canberra, Packard, Dreieich, Germany).

Data analysis: Data were analyzed by using Graph Pad Prism Version 3.0 (San Diego, CA, USA). For the calculation of K_i values by nonlinear regression analysis, the Cheng–Prusoff equation and K_D

values of 0.5 nM (rat A_1), 0.61 nM (human A_1) for [^{3}H]CCPA, 8 nM for [^{3}H]MSX-2, 33 nM for [^{3}H]ZM-241385, 4.9 nM for [^{3}H]PSB-11, and 1.48 nM for [^{125}I]AB-MECA were used.

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