

Imidazo[2,1-*i*]purin-5-ones and Related Tricyclic Water-Soluble Purine Derivatives: Potent A_{2A}- and A₃-Adenosine Receptor Antagonists[†]

Christa E. Müller,^{*,‡} Mark Thorand,[‡] Ramatullah Qurishi,[‡] Martina Diekmann,[‡] Kenneth A. Jacobson,[§] William L. Padgett,[§] and John W. Daly[§]

Pharmaceutical Institute Poppelsdorf, University of Bonn, Bonn, Germany, and Laboratory of Bioorganic Chemistry, NIDDK, National Institutes of Health, Bethesda, Maryland

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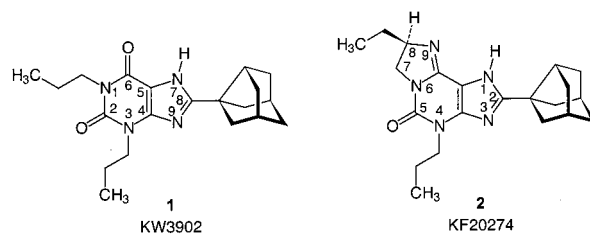
A series of tricyclic imidazo[2,1-*i*]purinones and ring-enlarged analogues derived from xanthine derivatives have been prepared as adenosine receptor (AR) antagonists. In comparison with xanthines, the tricyclic compounds exhibit increased water solubility due to a basic nitrogen atom, which can be protonated under physiological conditions. Substituents were introduced that confer high affinity for A_{2A} or A₃ ARs, respectively. A new capillary electrophoresis method was developed for the determination of the enantiomeric purity of selected chiral products using native and modified β -cyclodextrins as chiral discriminators. The compounds were investigated in radioligand binding assays at rat brain A₁ and A_{2A} ARs. Selected compounds were additionally investigated in radioligand binding assays at human recombinant A₃ ARs and in functional studies (adenylate cyclase assays) at A₁ ARs of rat fat cell membranes, A_{2A} ARs of rat PC 12 cell membranes, and mouse A_{2B} ARs of NIH 3T3 cell membranes. Structure–activity relationships were similar to those of corresponding xanthine derivatives. The 2-styrylimidazopurinones were less potent at A_{2A} ARs as compared to 8-styrylxanthine derivatives. The most potent compound at A_{2A} ARs was (*S*)-1,4-dimethyl-8-ethyl-2-styrylimidazo[2,1-*i*]purinone (*S*-**25**) exhibiting a *K*_i value of 424 nM at rat A_{2A} ARs. The compound was highly selective for A_{2A} receptors vs A₁ and A₃ ARs. Selectivity vs A_{2B} ARs, however, was low. Among the 1-unsubstituted 2-phenyl-imidazo[2,1-*i*]purin-5-one derivatives, very potent and highly selective antagonists for human A₃ ARs were identified. The most potent A₃ antagonist of the present series was (*R*)-4-methyl-8-ethyl-2-phenyl-imidazo[2,1-*i*]purin-5-one (*R*-**24**) exhibiting a *K*_i value of 2.3 nM and high selectivity for A₃ receptors vs all other AR subtypes.

Introduction

Adenosine receptors (ARs) are a class of G-protein-coupled receptors (GPCRs) that comprise four subtypes, designated A₁, A_{2A}, A_{2B}, and A₃.² All ARs may be coupled to adenylyl cyclase (AC) as a second messenger system. A₁ and A₃ AR stimulation leads to an inhibition of the enzyme, while A_{2A} and A_{2B} AR activation causes a stimulation of AC.²

Selective antagonists at AR subtypes are of considerable interest as novel therapeutics;^{3,4} for example, A₁ AR antagonists are being developed as kidney protective diuretics, for the prevention of renal failure, and as cognition enhancers;⁵ A_{2A} selective AR antagonists are promising new drugs for the treatment of Parkinson's disease.⁶ A₃ AR antagonists have been postulated as novel antiinflammatory and antiallergic agents,⁷ while A_{2B} AR antagonists may possess antiasthmatic properties.⁸ All AR subtypes have been cloned from various species, including rat and humans. While differences between the two species are moderate for A₁ (amino acid sequence identity: 95%), A_{2A} (84%), and A_{2B} (86%) ARs, there are considerable differences between the rat and

Chart 1. A₁ Selective AR Antagonists: Xanthine and Derived Imidazo[2,1-*i*]purin-5-one Derivative



the human A₃ ARs (only 74% sequence identity).^{2–4} The A₃ AR is also unique with regard to tissue distribution in different species: High densities of A₃ ARs are found in the testis of rats and only low levels in most other rat tissues. In humans, the highest A₃ receptor densities are found in lung and liver and only moderate levels in most other tissues.⁹

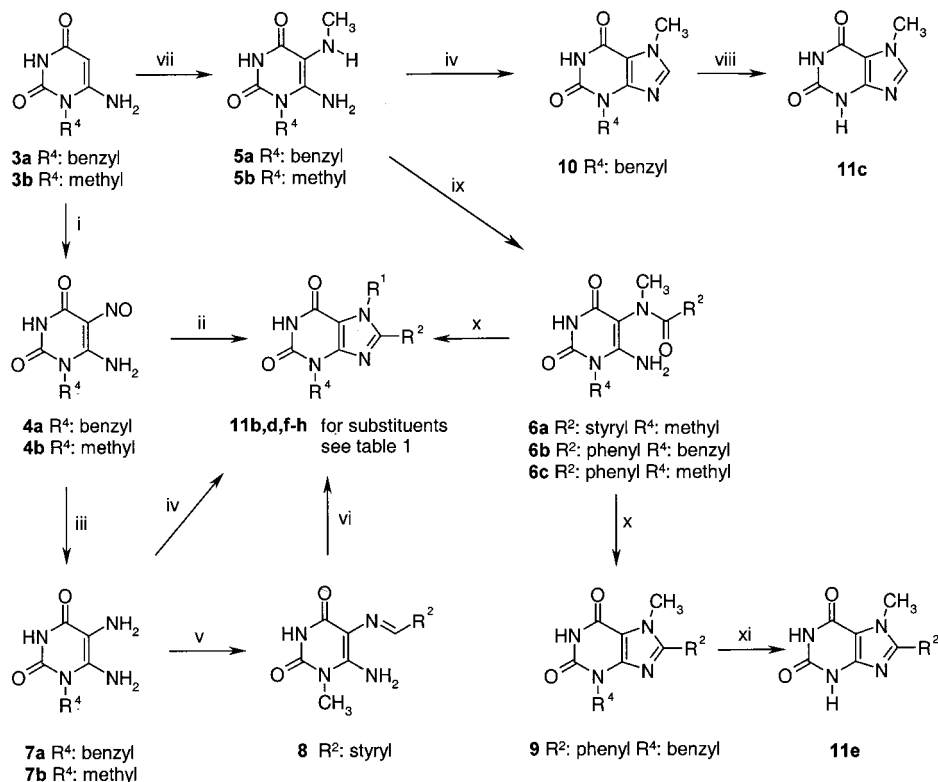
A number of potent and subtype selective AR antagonists have been developed during the past decades.^{3–7,9,10} However, a major problem is that most of the potent AR antagonists are highly lipophilic and therefore exhibit low water solubility, which may limit their in vivo applicability. A₁ AR antagonists with increased water solubility have been developed⁵ including tricyclic imidazo[2,1-*i*]purinone derivatives, such as KF 20274 (**2**) (Chart 1).¹¹ Compound **2** is structurally derived from an A₁ selective xanthine derivative, 1,3-dipropyl-8-(3-

* To whom correspondence should be addressed. Tel: +49-228-73-2301. Fax: +49-228-73-2567. E-Mail: christa.mueller@uni-bonn.de.

[†] Significant parts of the present results are based on data from the Ph.D. thesis of J. Hipp¹ and are so cited.

[‡] University of Bonn.

[§] National Institutes of Health.

Scheme 1. Preparation of Intermediate Xanthines Used as Starting Compounds for the Synthesis of Tricyclic Purinone Derivatives^a

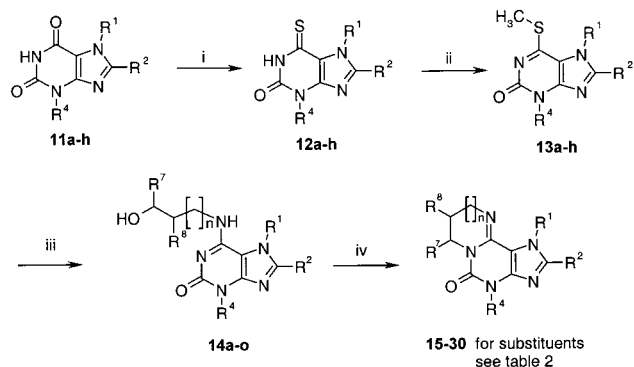
^a Reagents: (i) NaNO₂, H₂O/CH₃COOH. (ii) C₆H₅CH₂NH₂·HCl, C₆H₅CH₂NH₂, 3 h, 170 °C. (iii) Na₂S₂O₄, H₂O/NH₃. (iv) (C₂H₅O)₃CH, 12 h Δ. (v) Cinnamic aldehyde, MeOH/CH₃COOH, 1 h, room temperature. (vi) SOCl₂, 8 h, room temperature. (vii) 1, Br₂, 0 °C, 30 min, room temperature; 2, CH₃NH₂, 70 °C, 4 h. (viii) AlCl₃, toluene, 1 h, 70 °C. (ix) **6a**: cinnamic acid, EDC, 10 h, room temperature; **6b,c**: PhCOCl, pyridine, 12 h, room temperature. (x) NaOH/EtOH, 1 h, Δ. (xi) BBr₃, toluene, 2 h, 80 °C.

noradamantyl)xanthine (KW 3902, **1**, Chart 1). Increased water solubility was obtained by introduction of a third ring, an imidazoline ring, which contains a basic nitrogen atom subject to protonation at physiological pH. This compound shows a high degree of stereoselectivity at guinea pig A₁ ARs, the *R* enantiomer (*K*_i = 2.7 nM), being about 40-fold more potent than the *S* enantiomer (*K*_i = 120 nM). At rat A_{2A} ARs, both enantiomers are nearly equipotent (*R*, *K*_i = 290 nM; *S*, *K*_i = 250 nM).¹¹

In the present study, we tried to combine substituents that are known to enhance affinity in xanthine derivatives for A_{2A} ARs (e.g., 8-styryl, 7-methyl) or A₃ ARs (e.g., 8-phenyl) with the imidazo[2,1-*i*]purin-5-one nucleus. Furthermore, we investigated the influence of variations of the imidazoline ring, including substitutions, chiral derivatives, and ring enlargement. Our goal was to develop novel AR antagonists with increased water solubility and high selectivity for either A_{2A} or A₃ ARs.

Results and Discussion

Chemistry. The syntheses of the tricyclic purinone derivatives can be subdivided into two parts: First, appropriate xanthine derivatives were synthesized (Scheme 1 and Table 1), which were subsequently converted to the tricyclic purinone derivatives (Scheme 2).¹ The 1-substituted uracil derivatives **3a,b** required as starting compounds were synthesized according to Papesch and Schröder.¹² Starting from these compounds (**3a,b**), the xanthine derivatives could be obtained by

Scheme 2. Synthesis of Imidazo[2,1-*i*]purinone Derivatives and Ring-Enlarged Analogues^a

^a Reagents: (i) P₂S₅, pyridine, 8 h, Δ. (ii) MeI, NaOH/EtOH, 1 h, room temperature. (iii) Amino alcohol, DMSO, 1 h, 150 °C. (iv) SOCl₂, 1 h Δ.

different pathways (Scheme 1).¹ One method was their conversion to the nitroso uracils (**4a,b**). The nitroso compound **4b** was reacted with a mixture of benzylamine and benzylamine hydrochloride at high temperatures to yield xanthine derivative **11d**. The diamino uracils **7a,b** were obtained by reduction of the nitroso uracils **4a,b** using sodium dithionite. Because these compounds are unstable due to oxidative dimerization in the presence of oxygen,¹³ they were used for the next reaction step without further purification. The diamino uracil derivative **7a** was reacted with triethyl orthoformate to yield the xanthine derivative **11b**. Alternatively, the diamino uracil derivative **7b** was condensed

with cinnamic aldehyde to yield the imine **8**, which was oxidatively cyclized with thionyl chloride¹⁴ to afford xanthine **11g**. For the synthesis of 3,7,8-tri- or 7,8-disubstituted xanthine derivatives, the 1-substituted 5-*N*-methylamino-6-aminouracil derivatives **5a,b** were prepared by bromination of **3a,b** and subsequent substitution with methylamine.^{15–17} The obtained compound **5a** was converted to xanthine **10** using triethyl orthoformate. Debenzylation to obtain xanthine derivative **11c**, unsubstituted in the 3-position, was achieved by the Lewis acid aluminum trichloride in toluene. Compound **5b** was condensed with cinnamic acid to **6a**. Condensation of **5a,b** with benzoyl chloride afforded the amides **6b,c**. The acylated compounds **6a–c** were cyclized in sodium hydroxide solution in a mixture of water and ethanol to yield the xanthine derivatives **9** and **11f,g**. To obtain xanthine derivative **11e** without substitution in position 3 (R^4), the benzyl group of **9** was removed by reaction with boron tribromide yielding the xanthine derivative **11e**.

In the second part of the synthesis¹ (Scheme 2 and Table 2), the xanthine derivatives **11a–h** were converted to the tricyclic purine derivatives according to the route described by Shimada et al.¹⁸ The xanthine derivatives **11a–h** were thionated with phosphorus pentasulfide in dry pyridine under reflux to yield 6-thioxanthines **12a–h**. After methylation with methyl iodide, the compounds **13a–h** were treated with amino alcohols in dimethyl sulfoxide (DMSO) under reflux conditions to obtain the amino-substituted purines **14a–o**. The final ring closure to the tricyclic purine derivatives **15–30** was performed by cyclization of **14a–o** with thionyl chloride at high temperature under reflux conditions.¹ As expected, yields for cyclization of unbranched hydroxyalkyl derivatives (e.g., **17**, **27**, **29**, and **30**) depended on the ring size of the formed product: formation of five and six ring derivatives proceeded in high yields, while reduced yields were observed in preparation of the diazepine and the diazocine derivatives **29** and **30**.

Cyclization of chiral compounds with a methyl or ethyl substituent in the α -position with regard to the amino group (**18**, **19**, and **23–26**) yielded products with retained stereochemistry in good yields, as described for similar cyclization reactions.¹¹ On the other hand, cyclization of chiral compounds with a methyl substituent β to the amino group (**20**) resulted in inversion of the configuration. As shown for analogous cyclization reactions,¹⁹ the initial chlorination of the alcohol with thionyl chloride without the addition of pyridine follows an S_N1 mechanism with retention of configuration, while the subsequent step, the actual ring closure, resulted in inversion of the stereocenter.¹⁹

An alternative method for the amination of purinone derivatives, such as hypoxanthine, inosine, and xanthosine, via silylation and subsequent nucleophilic substitution of the trimethylsilyloxy group has been described in the literature.²⁰ This would allow for the preparation of hydroxyalkylaminopurinones, such as **14a–o**, in a one pot procedure starting from the xanthine derivatives. We treated 3,7-dimethylxanthine (theobromine), 3-methylxanthine, and the corresponding 8-phenyl derivatives with hexamethyldisilazane and

ethanolamine in the presence of catalyst (*p*-toluene-sulfonic acid hydrate, ammonium sulfate, or trimethylsilyltriflate, respectively) without or in the presence of solvent (toluene or pyridine) at 120–160 °C for up to 5 days.¹ However, no amination was observed. Yields and analytical data for intermediate and final products are collected in Tables 1 and 2.

Determination of Enantiomeric Purity. Enantiomeric purity has been investigated exemplarily for compounds *R*-**19** and *S*-**19** with ¹H nuclear magnetic resonance (NMR) spectroscopy using tris[3-(heptafluoropropylhydroxymethyl)-*d*-camphorato]europium(III) as a shift reagent.^{1,21,22} In contrast to the solution of racemate *RS*-**19**, only a single enantiomer could be detected in solutions of *R*-**19** and *S*-**19**, respectively.¹

Because NMR methods may not be sensitive enough for the quantitative determination of enantiomeric purity, we developed a novel capillary electrophoresis (CE) method using cyclodextrins (CDs) as chiral selectors added to the running buffer. The compounds were detected and identified by using a diode array detector scanning their UV spectra from 190 to 400 nm. Racemates *RS*-**19** and *RS*-**24** were selected for the development of a method for chiral separation. For all separations, a constant current of 90 μ A was applied. Two untreated fused silica capillaries, 60 (50 cm effective length) and 50 cm (40 cm effective length), were used for the investigations. Crucial to the new method was the use of two different buffer solutions simultaneously, the chiral selector added to the inlet buffer differing from that in the outlet buffer. While the inlet buffer contained native β -CD, the outlet buffer contained sulfated β -CD (*s*- β -CD). If native β -CD was used only, a baseline chiral separation could not be achieved. The separations were performed at pH 4.5 (phosphate buffer). The negatively charged *s*- β -CD molecules are believed to strongly interact with the positively charged chiral compounds. Low concentrations of *s*- β -CD ranging from 1.5 to 10 mg/mL added only to the outlet buffer were sufficient to obtain excellent baseline separations as shown in Figure 1. This can be explained by the large countercurrent mobility of *s*- β -CDs.^{23,24} The optimal concentration for β -CD in the inlet buffer was found to be 6 mM. Higher concentrations of β -CD led to a decrease in resolution. The use of carboxymethyl- β -cyclodextrin (CMCD) instead of *s*- β -CD resulted in low chiral resolution. A combination of hydroxypropyl- β -CD (HPCD) with *s*- β -CD in the inlet buffer and *s*- β -CD in the outlet buffer also gave good results as shown for compound *R*-**24** in Table 3. The investigated enantiomers generally exhibited high purity (98–100%) (Table 3). The chiral separations were powerful enough for injecting very high amounts of concentrated samples. We were able to detect contaminations by the other enantiomer of below 1%, e.g., *R*-**24** contained only 0.32 and 0.29% of the corresponding *S* enantiomer (Table 3, Figure 1). However, the racemate *RS*-**20** could not be resolved by the new method, possibly due to a methyl group at the chiral center (vs the larger ethyl group in **19** and **24**), resulting in smaller steric differences. Another explanation could be the larger distance of the chiral center (C7 in **20** vs C8 in **19** and **24**) from the basic nitrogen atom (N9), which is believed to interact electrostatically with the negatively charged *s*- β -CD.

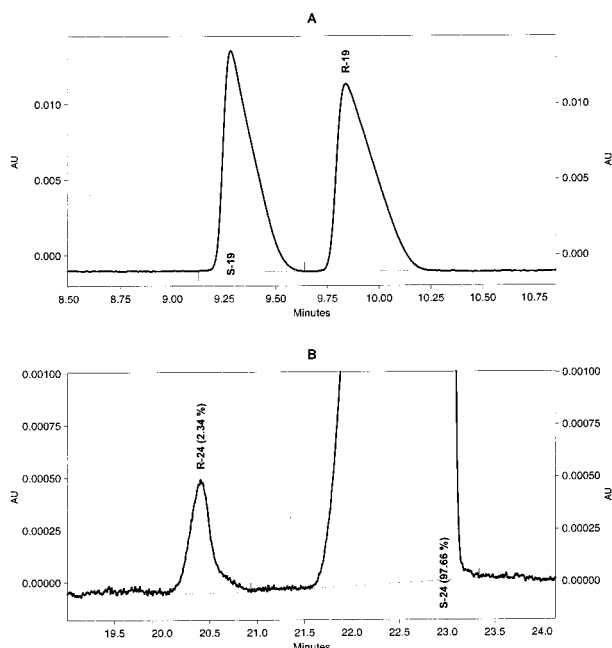


Figure 1. Electropherograms of chiral separation of two chiral compounds. Fused silica capillary 40 cm effective length \times 75 μ m ID \times 375 OD: constant current, 90 μ A; injection time, 5 s with 0.5 psi. (A) Racemate *RS-19*. Inlet buffer: phosphate 50 mM, β -CD 6 mM, pH 4.5; outlet buffer: phosphate 50 mM, sulfated β -CD 10 mg/mL, pH 4.5. (B) Compound *S-24*. Inlet buffer: phosphate 50 mM, β -CD 6 mM, pH 4.5; outlet buffer: phosphate 50 mM, sulfated β -CD 1.5 mg/mL, pH 4.5.

Table 1. Intermediate Xanthines Used for the Preparation of Tricyclic Purinone Derivatives

compd	R ¹	R ²	R ⁴	mp ¹ (°C) (lit. mp)	yield ¹ (%)
11a	CH ₃	H	CH ₃	290–295	<i>a</i>
11b	H	H	benzyl	>250 (>300) ³⁴	90
11c	CH ₃	H	H	>270 (>330) ¹⁶	75
11d	H	phenyl	CH ₃	>250 (>360) ³⁵	46
11e	CH ₃	phenyl	H	>250	80
11f	CH ₃	phenyl	CH ₃	>250 (300) ³⁶	85
11g	H	styryl	CH ₃	>270	80
11h	CH ₃	styryl	CH ₃	>270	88

^a Theobromine (**11a**) was commercially available.

Water Solubility and Estimation of *pK_a* Values.

The solubility of two representative imidazo[2,1-*i*]purin-5-ones was determined by UV spectroscopy.¹ In Table 4, solubilities of compounds **17** and **22** are listed. For comparison, solubilities of corresponding xanthine derivatives are included. The introduction of an 8-phenyl group into theophylline results in a drastic reduction of solubility. The effect of an 8-styryl residue is even more dramatic. On the other hand, 7-methyl-substituted xanthines generally display higher water solubility than the corresponding 7-unsubstituted compounds (compare theophylline and caffeine derivatives). The two imidazopurinone derivatives **17** and **22** possess significantly higher water solubility than caffeine and 8-phenylcaffeine, respectively.¹ It is expected that the other imidazopurinones also will possess higher water solubility than the corresponding substituted xanthines.

To assess whether imidazo[2,1-*i*]purinone derivatives are protonated at physiologic pH value, UV spectra of

1,4-dimethylimidazo[2,1-*i*]purin-5-one (**17**) were recorded at various pH values in steps of one pH unit from pH 2 to pH 13.¹ At pH equal to or greater to 8, the compound is present as the free base ($UV_{max} = 287$ nm), while at pH values of 7 or lower a bathochromic shift of 8 nm to a UV_{max} of 295 nm along with a hyperchromic effect is observed (data not shown).¹ Therefore, the *pK_a* value can be expected to be around physiologic pH of 7.4, and the compound should be at least partially protonated under physiological conditions.

Biological Evaluation. All imidazopurinones were investigated in radioligand binding studies at rat brain A₁ and A_{2A} ARs using [³H]N⁶-cyclohexyladenosine (CHA), and [³H]-2-[[[4-(carboxyethyl)phenyl]ethyl]amino]-5'-*N*-(ethylcarboxamido)adenosine (CGS21680), respectively, as radioligands.¹ Selected compounds were evaluated in AC assays at rat A₁ and A_{2A} ARs: Antagonism of *R*-PIA-elicited inhibition of AC via A₁ ARs in rat fat cell membranes and antagonism of NECA-induced stimulation of AC via A_{2A} ARs in rat pheochromocytoma (PC12) cell membranes was measured. A₃ AR affinity was determined at recombinant human A₃ ARs stably expressed in HEK-293 cells using [¹²⁵I]N²-methyl-3,4-dihydroxy-5-[6-[3-iodobenzyl]amino]-9*H*-9-purinyl]-3-tetrahydro-2-furancarboxamide ([¹²⁵I]AB-MECA) as a radioligand. Activity at A_{2B} ARs was determined by AC assays in NIH 3T3 fibroblast cells (derived from Swiss mouse embryo cells): the inhibition of the NECA-induced stimulation of adenosine cyclic 3',5'-phosphate (cAMP) accumulation by the imidazopurinones was determined.

Because data from different species (rat, mouse, and human) were compared, species differences have to be taken into account. However, for A₁, A_{2A}, and A_{2B} ARs, differences between human and rodent receptors have been shown to be moderate due to high amino acid sequence homologies.² In contrast, large species differences have been reported between human and rodent A₃ ARs, most known A₃ antagonists being inactive or only weakly active at rat A₃ while exhibiting high affinity for human A₃ receptors.^{2,7}

Structure–Activity Relationships (SAR). 2-Unsubstituted Tricyclic Purinone Derivatives. 2-Unsubstituted 1,4-dimethyl-imidazo[2,1-*i*]purin-5-one (**17**), corresponding to caffeine (**31**), was selected as a lead structure.¹ Ring enlargement of the dihydroimidazole ring in **17** to the corresponding pyrimido- (**27**), diazepino- (**29**), and diazocino- (**30**) purinone derivatives led to an increase in A₁ AR affinity; the optimum was reached with the seven ring compound **29**. At A_{2A} ARs, the five, six, and seven ring derivatives (**17**, **27**, and **29**) exhibited similar affinities, while the eight ring derivative **30** was considerably weaker. Thus, the imidazopurinone **17** showed the highest degree of A_{2A} selectivity (ca. 4-fold) as compared with the higher homologues and was selected for further modifications.¹ If the methyl group in the 4-position was lacking (corresponding to the 3-substituent in xanthines), the A_{2A} selectivity of **17** was lost shown in the marked reduction in A_{2A} affinity in **15**. A larger substituent in the 4-position, a benzyl group (compound **16**), was well-tolerated by the A_{2A} but less by the A₁ ARs. The imidazoline ring positions seven and eight of the lead structure **17** were substituted with methyl or ethyl groups (compounds

Table 2. Synthesized Tricyclic Purinone Derivatives: Substitution Pattern, Method of Preparation, Yields, and Analytical Data¹

compd ^a	stereo-chemistry	<i>n</i>	R ¹	R ²	R ⁴	R ⁷	R ⁸	method	yield (%)	formula	analyses	mp (°C)	[α] _D ²⁰ ^b
15		0	CH ₃	H	H	H	H	A	49	C ₈ H ₉ N ₅ O·HCl·0.7H ₂ O	C, H, N	>250	
16		0	CH ₃	H	benzyl	H	H	A	41	C ₁₅ H ₁₅ N ₅ O·2HCl	C, H, N ^c	235	
17		0	CH ₃	H	CH ₃	H	H	A	75	C ₉ H ₁₁ N ₅ O·2HCl·0.2H ₂ O	C, H, N	>250	
<i>R</i> - 18	R	0	CH ₃	H	CH ₃	H	CH ₃	C	35	C ₁₀ H ₁₃ N ₅ O·2HCl·0.3H ₂ O	C, H, N	>250	2.3
<i>S</i> - 18	S	0	CH ₃	H	CH ₃	H	CH ₃	C	39	C ₁₀ H ₁₃ N ₅ O·2HCl·0.5H ₂ O	C, H, N	>250	-2.8
<i>RS</i> - 19	rac	0	CH ₃	H	CH ₃	H	C ₂ H ₅	B	45	C ₁₁ H ₁₅ N ₅ O·2HCl·0.25H ₂ O	C, H, N	>250	0
<i>R</i> - 19	R	0	CH ₃	H	CH ₃	H	C ₂ H ₅	B	42	C ₁₁ H ₁₅ N ₅ O·2HCl·0.1H ₂ O	C, H, N	>250	4.2
<i>S</i> - 19	S	0	CH ₃	H	CH ₃	H	C ₂ H ₅	B	32	C ₁₁ H ₁₅ N ₅ O·2HCl·0.1H ₂ O	C, H, N	>250	-3.9
<i>RS</i> - 20	rac	0	CH ₃	H	CH ₃	CH ₃	H	B	48	C ₁₀ H ₁₃ N ₅ O·2HCl·0.1H ₂ O	C, H, N	175	0
<i>R</i> - 20	R	0	CH ₃	H	CH ₃	CH ₃	H	B	52	C ₁₀ H ₁₃ N ₅ O·2HCl·0.5H ₂ O	C, H, N	200	-3.4
<i>S</i> - 20	S	0	CH ₃	H	CH ₃	CH ₃	H	B	54	C ₁₀ H ₁₃ N ₅ O·2HCl·H ₂ O	C, H, N	200	3.3
21		0	CH ₃	phenyl	H	H	H	A	38	C ₁₄ H ₁₃ N ₅ O·HCl·0.6H ₂ O	C ^d , H, N	>250	
22		0	CH ₃	phenyl	CH ₃	H	H	A	45	C ₁₅ H ₁₅ N ₅ O·HCl·H ₂ O	C, H, N	>250	
<i>RS</i> - 23	rac	0	CH ₃	phenyl	CH ₃	H	C ₂ H ₅	D	29	C ₁₇ H ₁₉ N ₅ O·HCl·0.3H ₂ O	C, H, N	>250	0
<i>R</i> - 23	R	0	CH ₃	phenyl	CH ₃	H	C ₂ H ₅	D	27	C ₁₇ H ₁₉ N ₅ O·HCl·0.5H ₂ O	C, H, N	>250	2.4
<i>S</i> - 23	S	0	CH ₃	phenyl	CH ₃	H	C ₂ H ₅	D	28	C ₁₇ H ₁₉ N ₅ O·HCl·0.3H ₂ O	C, H, N	>250	-2.6
<i>RS</i> - 24	rac	0	H	phenyl	CH ₃	H	C ₂ H ₅	D	36	C ₁₆ H ₁₇ N ₅ O·HCl·3H ₂ O	C, H, N	>250	0
<i>R</i> - 24	R	0	H	phenyl	CH ₃	H	C ₂ H ₅	D	35	C ₁₆ H ₁₇ N ₅ O·HCl·2H ₂ O	C, H, N ^e	>250	4.5
<i>S</i> - 24	S	0	H	phenyl	CH ₃	H	C ₂ H ₅	D	35	C ₁₆ H ₁₇ N ₅ O·HCl·2H ₂ O	C, H, N	>250	-4.8
<i>R</i> - 25	R	0	CH ₃	styryl	CH ₃	H	C ₂ H ₅	D	16	C ₁₉ H ₂₁ N ₅ O	335.174 ^f	>250	3.3
<i>S</i> - 25	S	0	CH ₃	styryl	CH ₃	H	C ₂ H ₅	D	14	C ₁₉ H ₂₁ N ₅ O	335.175 ^f	>250	-3.2
<i>R</i> - 26	R	0	H	styryl	CH ₃	H	C ₂ H ₅	D	32	C ₁₈ H ₁₉ N ₅ O·HCl·0.6H ₂ O	C, H, N	>250	3.2
<i>S</i> - 26	S	0	H	styryl	CH ₃	H	C ₂ H ₅	D	28	C ₁₈ H ₁₉ N ₅ O·HCl·0.7H ₂ O	C, H, N	>250	-3.1
27		1	CH ₃	H	CH ₃	-	-	A	52	C ₁₀ H ₁₃ N ₅ O·2HCl·0.2H ₂ O	C, H, N	>250	
28		1	CH ₃	Cl	CH ₃	-	-	A	30	C ₁₀ H ₁₂ N ₅ OCl·HCl·0.1H ₂ O	C, H, N	261	
29		2	CH ₃	H	CH ₃	-	-	A	27	C ₁₁ H ₁₅ N ₅ O·2HCl·0.1H ₂ O	C, H, N ^g	198	
30		3	CH ₃	H	CH ₃	-	-	A	14	C ₁₂ H ₁₇ N ₅ O·2HCl	C, H, N	210	

^a Hydrochlorides of imidazopurinones. ^b Solvent, methanol; concentration, 1.00 g/100 mL. ^c N: calcd, 18.81; found, 18.35. ^d C: calcd, 53.46; found, 53.04. ^e N: calcd, 19.04; found, 18.52. ^f Determined by high-resolution mass spectroscopy. ^g N: calcd, 22.74; found, 22.24.

Table 3. Determination of Enantiomeric Purity of Selected Compounds by CE Using CDs as Chiral Selectors

compd	CD ^a added to inlet buffer ^b	CD ^a added to outlet buffer ^b	capillary effective length (cm)	determined purity ± RSD ^c (%)	determined impurity ^d ± RSD (%)
<i>R</i> - 19	β-CD (6 mM)	s-β-CD (10 mg/mL)	40	99.23 ± 0.03 (<i>n</i> = 3)	0.77 ± 0.03
<i>R</i> - 24 ^e	β-CD (6 mM)	s-β-CD (1.5 mg/mL)	40	99.68 ± 0.01 (<i>n</i> = 2)	0.32 ± 0.01
<i>R</i> - 24 ^e	HPCD (6 mM) + s-β-CD (1.5 mg/mL)	s-β-CD (1.5 mg/mL)	50	99.71 ± 0.04 (<i>n</i> = 5)	0.29 ± 0.04
<i>S</i> - 24	β-CD (6 mM)	s-β-CD (1.5 mg/mL)	40	97.69 ± 0.04 (<i>n</i> = 3)	2.31 ± 0.04

^a β-CD, β-cyclodextrin; s-β-CD, sulfated β-cyclodextrin; HPCD, 2-hydroxypropyl-cyclodextrin. ^b Phosphate buffer, 50 mM, pH 4.5. ^c RSD = relative standard deviation. ^d By the other enantiomer. ^e Two different batches.

Table 4. Solubilities of Imidazo[2,1-*f*]purin-5-one Derivatives in Comparison with Xanthines¹

compd	R ¹	R ²	water solubility (μM) ^a
Xanthines			
theophylline	H	H	44 400 ^{b,37}
8-phenyltheophylline	H	phenyl	10 ³⁸
8-styryltheophylline	H	styryl	0.22
caffeine	methyl	H	108 000 ^{b,37}
8-phenylcaffeine	methyl	phenyl	487
8-styrylcaffeine	methyl	styryl	2.8
Imidazopurinones ^c			
17	methyl	H	1 210 000
22	methyl	phenyl	1450

^a At 20 °C unless otherwise noted. ^b At 25 °C. ^c Hydrochlorides.

18–20) yielding chiral molecules. An *R*-methyl group in the 7-position (*R*-**20**) increased A₁ affinity (4-fold) and decreased A_{2A} affinity (2-fold), while the *S* enantiomer (*S*-**20**) showed increased A_{2A} affinity (2-fold) with no change in A₁ affinity in comparison with the unsubstituted compound **17**. Thus, the *R* enantiomer (*R*-**20**) was somewhat A₁ selective and the *S* enantiomer (*S*-**20**) was

slightly A_{2A} selective; however, the degree of stereoselectivity was low. A methyl group in the 8-position did not increase but rather decreased AR affinity (compound **18**), while an (*S*)-configured ethyl group (*S*-**19**) increased A_{2A} affinity and thus was favorable for high A_{2A} selectivity.

At human A₃ ARs, the lead compound **17** exhibited 40% displacement of radioligand binding at a concentration of 10 μM. Modifications as in compounds **15** (4-unsubstituted), *RS*-**20** (7-methyl), **27** (six ring analogue), **29** (seven ring analogue), and **30** (eight ring analogue) reduced A₃ affinity yielding compounds without significant A₃ receptor binding at a concentration of 10 μM. Introduction of an ethyl group in the 8-position (*RS*-**19**) yielded a compound with low A₃ affinity (28% displacement at 100 μM).

2-Substituted Tricyclic Purinone Derivatives.

An increase in AR affinity could be obtained by the introduction of substituents in the 2-position of the tricyclic purinone derivatives (corresponding to the 8-position in xanthines).¹ Chlorination of the imidazopurinone **27** to its 2-chloro derivative **28** slightly increased both A₁ (4-fold) and A_{2A} affinity (2-fold) (Table 5). A phenyl group in the 2-position (compounds **21–23**) resulted in a large increase in A₁ AR affinity with no major effect on A_{2A} affinity (compare **21** to **15** and

Table 5. AR Affinities and Selectivities of Tricyclic Purinone Derivatives

						K_i (μM) \pm SEM or % displacement at indicated concentration (in brackets)		
						A ₁ affinity rat brain cortical membranes ¹ [³ H]CHA	A _{2A} affinity rat brain striatal membranes ¹ [³ H]CGS21680	A ₃ affinity human recombinant receptors [¹²⁵ I]AB-MECA
compd	R ¹	R ²	R ⁴	R ⁷	R ⁸			
2-Unsubstituted Imidazopurinones								
15	methyl	H	H	H	H	65.5 \pm 7.6	40.9 \pm 6.3	> 10 ^a
16	methyl	H	benzyl	H	H	24% (30 μM)	16.1 \pm 3.8	nd ^b
17	methyl	H	methyl	H	H	79.6 \pm 0.6	20.7 \pm 3.0	40% (10 μM)
<i>R</i> - 18	methyl	H	methyl	H	(<i>R</i>)-methyl	14% (3 μM)	12% (30 μM)	nd
<i>S</i> - 18	methyl	H	methyl	H	(<i>S</i>)-methyl	10% (10 μM)	46% (30 μM)	nd
<i>RS</i> - 19	methyl	H	methyl	H	(<i>R,S</i>)-ethyl	46% (250 μM)	16.2 \pm 0.6	28% (100 μM)
<i>R</i> - 19	methyl	H	methyl	H	(<i>R</i>)-ethyl	75.5 \pm 4.5	28.3 \pm 5.4	nd
<i>S</i> - 19	methyl	H	methyl	H	(<i>S</i>)-ethyl	50% (250 μM)	10.9 \pm 1.2	nd
<i>RS</i> - 20	methyl	H	methyl	(<i>R/S</i>)-methyl	H	59 \pm 8	64.6 \pm 9.8	> 10 ^a
<i>R</i> - 20	methyl	H	methyl	(<i>R</i>)-methyl	H	23 \pm 4	50.4 \pm 13.4	nd
<i>S</i> - 20	methyl	H	methyl	(<i>S</i>)-methyl	H	70 \pm 6	12.0 \pm 1.9	nd
2-Substituted Imidazopurinones								
21	methyl	phenyl	H	H	H	1.83 \pm 0.9	11.9 \pm 1.6	0.047 \pm 0.0048
22	methyl	phenyl	methyl	H	H	9.7 \pm 2.6	20.3 \pm 2.6	3.33 \pm 0.72
<i>RS</i> - 23	methyl	phenyl	methyl	H	(<i>R,S</i>)-ethyl	6.1 \pm 0.8	19.2 \pm 3.9	17% (10 μM)
<i>R</i> - 23	methyl	phenyl	methyl	H	(<i>R</i>)-ethyl	17.5 \pm 5.0	36.3 \pm 1.2	nd
<i>S</i> - 23	methyl	phenyl	methyl	H	(<i>S</i>)-ethyl	4.7 \pm 0.3	16.2 \pm 4.6	nd
<i>RS</i> - 24	H	phenyl	methyl	H	(<i>R,S</i>)-ethyl	0.265 \pm 0.1	3.1 \pm 0.5	nd
<i>R</i> - 24 (PSB-11)	H	phenyl	methyl	H	(<i>R</i>)-ethyl	0.44 \pm 0.1	2.1 \pm 0.14	0.0023 \pm 0.0011
<i>S</i> - 24	H	phenyl	methyl	H	(<i>S</i>)-ethyl	0.115 \pm 0.001	3.33 \pm 0.93	0.0098 \pm 0.0038
<i>R</i> - 25	methyl	styryl	methyl	H	(<i>R</i>)-ethyl	21.7 \pm 3.8	0.547 \pm 0.035	1.70 \pm 0.48
<i>S</i> - 25	methyl	styryl	methyl	H	(<i>S</i>)-ethyl	14.9 \pm 2.6	0.424 \pm 0.007	30.6 \pm 8.3
<i>R</i> - 26	H	styryl	methyl	H	(<i>R</i>)-ethyl	0.95 \pm 0.15	0.89 \pm 0.01	0.64 \pm 0.199
<i>S</i> - 26	H	styryl	methyl	H	(<i>S</i>)-ethyl	0.73 \pm 0.13	0.67 \pm 1.73	> 10 ^a
Ring-Enlarged Analogues of Imidazopurinones								
27	methyl	H	methyl			43.2 \pm 13.4	28.5 \pm 5.2	> 10 ^a
28	methyl	Cl	methyl			10.5 \pm 6.1	11.9 \pm 0.87	nd
29	methyl	H	methyl			19.8 \pm 9.5	18.5 \pm 1.3	> 10 ^a
30	methyl	H	methyl			60.3 \pm 8.1	51 \pm 4	> 10 ^a
Xanthine Derivatives (for Comparison)								
	R ⁷	R ⁸	R ³	R ¹	name			
31	methyl	H	methyl	methyl	caffeine	41 ³⁹	43 ³⁹	13.3 ⁷
32	methyl	phenyl	methyl	methyl	8-phenylcaffeine	15 ⁴⁰	25 ⁴⁰	na ^c
33	H	phenyl	methyl	methyl	8-phenyltheophylline	0.089 ³⁹	0.83 ³⁹	na
34	methyl	styryl	methyl	methyl	8-styrylcaffeine	3.9 ⁴¹	0.094 ⁴¹	na
35	H	styryl	methyl	methyl	8-styryltheophylline	0.65 ⁴¹	0.29 ⁴¹	na

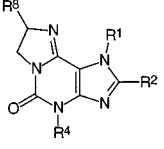
^a nd = not determined. ^b Less than 10% displacement at 10 μM . ^c Data not available.

22,23 to **19**). Compound **24** lacking a 1-methyl group was particularly potent. None of the compounds were highly selective for A₁ receptors. A 2-styryl had about the same effect as a phenyl group at A₁ ARs; however, in the case of compound **25**, it led to a dramatic increase in affinity for A_{2A} ARs yielding potent A_{2A} antagonists *R*-**25** and *S*-**25** with up to 40-fold selectivity for A_{2A} vs A₁ ARs. 2-Styryl-substituted imidazopurinones that were lacking a methyl group in the 1-position (compounds *R*-**26** and *S*-**26**) were 20-fold more potent at A₁ ARs but exhibited somewhat reduced affinity for A_{2A} ARs as compared to the 1-methyl analogues *R*-**25** and *S*-**25**. A similar effect had been observed with 8-styryl-xanthine derivatives with regard to methylation in the 7-position.²⁵ It was postulated that a neighboring methyl group might force the styryl residue into the putatively bioactive cisoid conformation.²⁶ For binding to the A₁ AR, however, the unsubstituted NH is an important

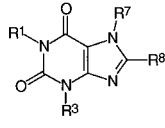
hydrogen bond donor.^{5,27} For the 2-phenyl imidazopurinones, 1-methyl substitution was highly unfavorable. 2-Phenyl derivatives lacking the 1-methyl group were ca. 40-fold more potent at A₁ ARs than their methylated analogues (compare *R*-**23**/*R*-**24**, *S*-**23**/*S*-**24**). At the A_{2A} ARs, this effect was less pronounced.

The introduction of a 2-phenyl substituent had an even more dramatic effect on A₃ affinity than it had on A₁ and A_{2A} affinity of imidazopurinone derivatives. The A₃ affinity of 1-methylimidazopurinone **15** was increased in the 2-phenyl derivative **21** by greater than 200-fold (K_i A₃ = 47 nM, >38-fold selective vs A₁, A_{2A}, and A_{2B} ARs). An additional methyl group in the 4-position of **21** (compound **22**) led to a 70-fold reduction in A₃ affinity, while A₁ affinity was only reduced by 5-fold, and A_{2A} affinity was reduced by 2-fold. An additional ethyl group in the 8-position of the imidazole ring (compound *RS*-**23**) resulted in a further

Table 6. Functional Antagonist Activity of Selected Imidazopurinones at A₁, A_{2A}, and A_{2B} ARs in Comparison with Standard Xanthine Derivatives



Imidazopurinones



Xanthines

compd	R ¹	R ²	R ⁴	R ⁸	K _B ± SEM (μM)		
					A ₁	A _{2A}	A _{2B}
Imidazopurinones							
21	methyl	phenyl	H	H	0.50 ± 0.11	17 ± 2	7.0 ± 0.3
R-24	H	phenyl	methyl	(R)-ethyl	0.43 ± 0.10	2.5 ± 0.2	2.1 ± 0.3
S-24	H	phenyl	methyl	(S)-ethyl	0.063 ± 0.021	4.3 ± 0.1	7.6 ± 7.3
S-25	methyl	styryl	methyl	(S)-ethyl	33 ± 10	1.0 ± 0.1	3.7 ± 0.3
Xanthines							
11f	R ⁷	R ⁸	R ³	R ¹			
caffeine	methyl	phenyl	H	methyl	0.32 ± 0.03	5.7 ± 0.7	5.2 ± 0.9
theophylline	methyl	H	methyl	methyl	60 ^a	36 ± 4 ^a	25 ± 2
enprofylline	H	H	methyl	methyl	8.7 ^a	13.7 ± 0.4 ^a	16 ± 8
	H	H	propyl	H	32 ± 2 ^b	120 ± 17 ^a	18 ± 4

^a Data from Choi; et al. *Life Sci.* **1988**, *43*, 387–398; Hide; et al. *Mol. Pharmacol.* **1992**, *41*, 352–359; Ukena; et al. *Life Sci.* **1986**, *39*, 743–750; and Brackett; Daly. *Biochem. Pharmacol.* **1994**, *47*, 801–804. ^b Binding data: Rat cerebral cortical membranes, [³H]R-PIA. Müller; et al. *J. Med. Chem.* **1993**, *36*, 3341–3349; also, 81 μM. Shamim; et al. *J. Med. Chem.* **1989**, *32*, 1231.

decrease in A₃ affinity without much influence on A₁ and A_{2A} affinity. However, the corresponding 1-unmethylated 2-phenyl-substituted imidazopurinones (**R-24**, **S-24**) were very potent ligands for the human A₃ AR exhibiting affinities in the low nanomolar concentration range (**R-24/S-24**). Compound **R-24** was highly selective for A₃ ARs vs all other AR subtypes and very potent showing a K_i value of 2.3 nM at human A₃ ARs. 2-Styryl substitution (compounds **25** and **26**) also increased A₃ affinity, however, to a much smaller extent than 2-phenyl substitution.

Stereoselectivity. Chiral 2-unsubstituted (**18–20**) and 2-phenyl-substituted imidazopurinones (**23** and **24**) bearing substituents at the imidazoline ring showed low degrees of enantioselectivity at ARs. High affinity compounds displayed the same low degree of enantioselectivity as compounds with lower AR affinity. 2-Styryl-substituted imidazopurinones (**25** and **26**) also showed low enantioselectivities at A₁ and A_{2A} ARs, but larger differences were observed at A₃ receptors (**R-25** > **S-25**, 18-fold; **R-26** > **S-26**, >>16-fold). Thus, the degree of enantioselectivity (i) depended on the receptor subtype and (ii) was affected by substitution in the 1-position (methyl or hydrogen) and the 2-position (styryl, phenyl, or hydrogen). While the A₃ AR preferred the *R*-configured enantiomers of 8-substituted derivatives, the A₁ AR showed a preference for the *S* enantiomers in the case of 2-phenyl and 2-styryl derivatives and for the *R* enantiomers in the case of 2-unsubstituted imidazopurinones. Enantioselectivity of 8-substituted imidazopurinones was lowest at the A_{2A} AR; in most cases, the *S* enantiomer was slightly more potent than the *R* enantiomer. One exception was seen with the 2-phenyl derivatives **R-24/S-24**; in this case, the *R* enantiomer was only slightly more potent than the *S* enantiomer. At the A₁ ARs, higher (4-fold) and opposite stereoselectivity was observed for the 2-phenyl-substituted imidazopurinone **R-24/S-24** (*S* > *R*) in comparison with the enantioselectivity at A_{2A} ARs. A_{2B} and A₃ ARs both exhibited ca. 4-fold stereoselectivity for the *R* enantiomer **R-24** over the *S* enantiomer **S-24**, which was

opposite to the results at the A₁ AR. Thus, despite only moderate degrees of stereoselectivity of the compounds vs one particular AR subtype, certain pure enantiomers may exhibit a much higher degree of receptor subtype selectivity than their counterparts as shown for the enantiomeric pair **R-24/S-24**. The *S* enantiomer was only 12-fold A₃ selective vs A₁, while the *R* enantiomer was 190-fold selective for the A₃ AR vs A₁.

Comparison with Xanthines. SARs for the various imidazopurinones roughly paralleled those of corresponding xanthine derivatives at A₁ and A_{2A} ARs. Published data of corresponding xanthine derivatives are included in Table 5 for comparison. The lead structure **17** can be compared with caffeine (**31**). Larger alkyl substituents (propyl, butyl) in the 1-position of xanthine derivatives may lead to an increase in A₁ and A_{2A} affinity,^{4,5} similarly as observed with increasing ring size in tricyclic compounds (**17**, **27**, and **29**). 8-Phenyl (**33**) and 8-styryl (**35**) substitution in xanthines and 7-methylation of those 8-substituted xanthine derivatives (**32** and **34**) result in similar effects at A₁ and A_{2A} ARs as analogous substitution in the corresponding positions of imidazopurinones. Therefore, it is very likely that the tricyclic purinone derivatives interact with the same binding site of the receptors and in a similar binding mode as the xanthines. A recently published molecular modeling study, in which the A₁ selective antagonists KW 3902 (**1**) and KF 20274 (**2**) were compared, came to the same conclusion.²⁸ In general, tricyclic imidazopurinone derivatives appeared to be somewhat less potent at A₁ and A_{2A} ARs than corresponding xanthine derivatives. Limited data on SARs of xanthine derivatives at A₃ ARs preclude a similar conclusion for the A₃ receptors at present.

Functional Assays. A selection of four compounds (**21**, **R-24**, **S-24**, and **S-25**) was investigated in functional studies at A₁, A_{2A}, and A_{2B} ARs. K_B values obtained from AC assays are listed in Table 6 in comparison with literature data for selected xanthine derivatives. The imidazopurinones were clearly shown to act as antagonists at ARs. K_B values obtained in AC assays correlated

well with K_i values from binding studies at A_1 and A_{2A} ARs. The investigated 2-phenyl- and 2-styrylimidazopyriminones showed moderate antagonist potency at A_{2B} ARs with K_B values in micromolar concentrations. Activities at A_{2A} and A_{2B} receptors were similar. Thus, the styryl derivative **S-25**, which was 35-fold selective for A_{2A} receptors vs A_1 and 72-fold selective vs A_3 ARs, exhibited only low selectivity vs A_{2B} ARs (4–9-fold). However, compounds **21**, **R-24**, and **S-24**, that were selective for A_3 ARs vs A_1 and A_{2A} , were also A_3 selective vs A_{2B} ARs.

Conclusion

The investigation of the SARs of imidazo[2,1-*i*]purinones and ring-enlarged analogues derived from xanthine derivatives resulted in the development of novel A_{2A} and A_3 AR antagonists exhibiting enhanced water solubility in comparison with corresponding xanthine derivatives. 2-Phenyl-substituted **R-24** exhibited the highest A_3 affinity of the present series with a K_i value of 2.3 nM and 190-fold selectivity vs A_1 and greater than 900-fold selectivity vs A_{2A} and A_{2B} ARs. Thus, **R-24** will be a useful research tool for future studies at human A_3 ARs. 1-Methyl-2-styryl-substituted **S-25** was the most potent A_{2A} antagonist with a K_i value of 424 nM, 35- and 72-fold selectivity vs A_1 and A_3 ARs, respectively, and 4–9-fold selectivity vs A_{2B} receptors. This study provides novel lead compounds for the development of A_{2A} and A_3 AR selective antagonists, respectively, exhibiting good water solubility, which is an important prerequisite for in vivo activity.

Experimental Section

Synthetic Procedures.¹ NMR spectra were performed on a Bruker AC-80 (^1H : 80 MHz, ^{13}C : 20 MHz), a Bruker AC200 (^1H : 200 MHz, ^{13}C : 50 MHz), a Bruker AC250 (^1H : 250, ^{13}C : 60 MHz), or a Bruker DMX 600 (^1H : 600 MHz, ^{13}C : 150 MHz) spectrometer. DMSO- d_6 was used as solvent. The chemical shifts are reported in parts per million (δ). Signals of the deuterated solvent served as internal standard: δ ^1H : 2.50 ppm; ^{13}C : 39.1 ppm. Coupling constants are in Hertz (Hz). UV spectra were recorded on an HP8452A spectrometer equipped with a diode array detector (Hewlett-Packard). All compounds were checked for purity by thin-layer chromatography (TLC) using aluminum sheets with silica gel 60 F_{254} (Merck). For column chromatography, silica gel (0.05–0.2 mm, Merck) was used. Dry column chromatography was performed using silica gel H according to Stahl (Merck). Enantiomeric purity of compounds **R-19** and **S-19** was exemplarily determined by ^1H NMR spectroscopy in the presence of tris[3-(heptafluoropropylhydroxymethyl)-*d*-camphorato]europium(III) as a shift reagent as described.^{21,22} Only one enantiomer could be detected by this method. Optical rotation was measured with a Perkin-Elmer 241 polarimeter using solutions in methanol (1.00%). The melting points were determined with a Büchi 510 or a Büchi 530 melting point apparatus and are uncorrected. Elemental analyses were performed by the Institute of Chemistry, University of Tübingen, the Institute of Inorganic Chemistry, University of Würzburg, or the Pharmaceutical Institute, Department of Pharmaceutical Chemistry, University of Bonn, respectively. The mass spectra were obtained on a 8200 Finnigan-MAT mass spectrometer.

Only selected ^1H NMR data for one representative member of each class of compounds are given. Complete ^1H NMR data and selected ^{13}C NMR data are available as Supporting Information. Theobromine (**11a**) was commercially available. Chiral amino alcohols were purchased from Merck, Aldrich, or Fluka in the highest available purity grade (typically at least 96% ee, usually ca. 99% ee).

6-Amino-1-benzyl-5-nitrosouracil (4a). 6-Amino-1-benzyluracil (**3a**, 10.85 g, 50 mmol) was suspended in 125 mL of

water. Acetic acid (125 mL) was added, and the suspension was heated to 70 °C. Slowly, 6.9 g (100 mmol) of NaNO_2 in 22 mL of H_2O was added. The suspension was stirred for 1 h at room temperature. After it was cooled to 4 °C, the precipitate was collected by filtration and purified by recrystallization from $\text{H}_2\text{O}:\text{EtOH}$ (50:50). Yield, 90%. ^1H NMR: δ 5.09 (s, 2H, CH_2), 7.20–7.40 (m, 5H, ar), 9.13 (s, 1H, N–O–H), 11.68 (s, 1H, N–H), 13.35 (s, 1H, =NH).

6-Amino-1-methyl-5-nitrosouracil (4b). 6-Amino-1-methyluracil (**3b**, 5.0 g, 36 mmol) was suspended in 150 mL of 50% aqueous acetic acid. The suspension was kept at a temperature of 60 °C until all solid had dissolved. After it was cooled to room temperature, 5 g of NaNO_2 (72 mmol) in 20 mL of H_2O was added over a period of 30 min. The solution was stirred for 1 h. After it was cooled to 4 °C, the precipitate was collected by filtration, subsequently suspended in H_2O , filtered off, and dried. An analytical sample was recrystallized from H_2O . Yield, 93%. ^1H NMR: δ 3.20 (s, 2H, CH_3), 8.94, 11.16 (2^*s , 1H, NH, NOH), 13.11 (s, 1H, N3–H).

6-Amino-1-benzyl-5-*N*-methylaminouracil (5a) and 6-Amino-5-*N*-methylamino-1-methyluracil (5b). To a mixture of 41 mmol of **3a** (8.9 g) or **3b** (5.78 g), respectively, and 3.44 g (41 mmol) of NaHCO_3 in 40 mL of MeOH, 6.55 g (41 mmol) of Br_2 was added at 0 °C over a period of 30 min. After it was stirred at room temperature for 30 min, the solution was cooled to 4 °C, and the white precipitate was collected by filtration. The solid was dissolved in 17 mL of aqueous methylamine solution (30%) and stirred at a temperature of 70 °C for a period of 4 h. The methylamine was subsequently removed by distillation. The residue was taken up in EtOH and brought to a pH value of 7 by adding concentrated HCl solution and kept at 4 °C for a period of 5 h. The product was then collected by filtration and purified by recrystallization from H_2O (**5a**) (yield, 75%) or EtOH (**5b**) (yield, 70%). ^1H NMR: **5a** δ 2.37 (s, 3H, CH_3), 2.82 (s, 1H, NH), 5.08 (s, 2H, CH_2), 6.46 (s, 2H, NH_2), 7.10–7.40 (m, 5H, ar), 10.47 (s, 1H, NH). **5b**: δ 2.36 (s, 3H, CH_3), 2.78 (s, 1H, NH), 3.21 (s, 3H, CH_3), 6.38 (s, 2H, NH_2), 10.48 (s, 1H, NH).

1*N*-(6-Amino-1-methyl-2,4-dioxo-1,2,3,4-tetrahydro-5-pyrimidinyl)-1*N*-methyl-3-phenyl-(*E*)-2-propenamide (6a). To a solution of **5a** in 40 mL of MeOH, (*E*)-cinnamic acid (0.72 g, 5.05 mmol) and *N*-(diethylaminopropyl)-*N*-ethylcarbodiimide-HCl (EDC) (0.98 g, 5.1 mmol) were added. The mixture was stirred for 10 h. Then, 20 mL of H_2O was added, and the mixture was stirred for 3 h. The precipitated product was collected by filtration and purified by dissolving it in 11 mL of dimethylformamide (DMF) and subsequent precipitation by the addition of 30 mL of H_2O . Yield, 1.04 g (69%). ^1H NMR: δ 2.95 (s, 3H, CH_3), 3.27 (s, 3H, CH_3), 6.74 (d, 1H, =CH), 7.06 (s, 2H, NH_2), 7.30–7.65 (m, 6H, ar + =CH), 10.66 (s, 1H, NH).

6-Amino-1-benzyl-5-methyl(phenyl)carboxamido-2,3-dioxo-1,2,3,4-tetrahydropyrimidine (6b) and 6-Amino-1-methyl-5-methyl(phenyl)carboxamido-2,3-dioxo-1,2,3,4-tetrahydro-pyrimidine (6c). A suspension of 14 mmol of **5a** (4.90 g) or **5b** (3.84 g) in 30 mL of dry pyridine was cooled to 0 °C. Benzoyl chloride (2.23 mL, 16 mmol) was added upon stirring. The mixture was stirred for 12 h, and then, the solvent was removed by distillation. After 30 mL of H_2O was added, the precipitate was filtered off and recrystallized from EtOH/ H_2O (50/50). The yields amounted to 73% for both compounds. ^1H NMR: **6b** δ 3.01 (s, 3H, CH_3), 4.94 (s, 2H, CH_2), 6.79 (s, 2H, NH_2), 7.15–7.45 (m, 10H, ar), 10.62 (s, 1H, NH). **6c**: δ 2.96 (s, 3H, CH_3), 3.33 (s, 3H, CH_3), 7.00–7.40 (m, 5H, ar), 10.46 (s, 1H, NH).

5,6-Diamino-1-benzyluracil (7a) and 5,6-Diamino-1-methyluracil (7b). 6-Amino-5-nitrosouracil **4a** (2.5 g, 10 mmol) or **4b** (1.7 g, 10 mmol) was dissolved in a mixture of 50 mL of 12.5% aqueous ammonia solution. $\text{Na}_2\text{S}_2\text{O}_4$ (3.5 g, 20 mmol) in 15 mL of H_2O was slowly added. The solution was reduced to half of its volume by rotary evaporation and then cooled to 4 °C. The precipitate was filtered off and used for the next reaction without any further purification due to instability.

6-Amino-1-methyl-5-[3-phenyl-(*E*,2*E*)-2-propenylidene-amino]-1,2,3,4-tetrahydro-2,4-pyrimidinone (8). 1-Methyl-5,6-diaminouracil **7b** (3.9 mmol) was dissolved in 30 mL of MeOH and 200 μ L (3.5 mmol) of acetic acid. The cinnamic aldehyde (0.48 mL, 3.8 mmol) was added, and the mixture was stirred for 1 h. The product precipitated after the addition of 30 mL of H₂O, was filtered off, and was dried over P₂O₅. Yield, 91%. ¹H NMR: δ 3.34 (s, 3H, CH₃), 6.97 (s, 2H, NH₂), 6.98 (m, 1H, C=CH), 7.20–7.55 (m, 6H, ar + C=CH), 9.47 (1H, N=CH), 10.71 (s, 1H, NH).

3-Benzyl-7-methyl-8-phenylxanthine (9). Pyrimidine derivative **6b** (5.26 g, 15 mmol) was suspended in a mixture of 26 mL of 2 N NaOH and 8 mL of EtOH. The suspension was refluxed for 1 h, then cooled to 4 °C, and diluted with 34 mL of H₂O. The solution was acidified to pH 5 by the addition of acetic acid. The precipitated product was collected by filtration and purified by recrystallization from H₂O:EtOH (50:50). Yield, 85%. ¹H NMR: δ 3.97 (s, 3H, CH₃), 7.20–7.40 (m, 5H, ar), 7.45–7.60 (m, 3H, ar), 7.70–7.9 (m, 2H, ar).

3-Benzyl-7-methylxanthine (10). 6-Amino-1-benzyl-5-methylaminouracil **5a** (1.0 g, 4 mmol) was suspended in triethylorthoformate (20 mL) and refluxed for 12 h. After the mixture was cooled to room temperature, the formed precipitate was filtered off and purified by recrystallization from H₂O:EtOH (50:50). Yield, 91%. ¹H NMR: δ 3.85 (s, 3H, CH₃), 5.09 (s, 2H, CH₂), 7.10–7.35 (m, 5H, ar), 7.97 (s, 1H, CH), 11.22 (s, 1H, NH).

3-Benzylxanthine (11b). 1-Benzyl-5,6-diaminouracil **7a** (6.5 g, 28 mmol) was suspended in 130 mL of triethylorthoformate and refluxed for 8 h. After the mixture was cooled to room temperature, the precipitate was collected by filtration and subsequently recrystallized from H₂O:MeOH (50:50). Yield, 90%. ¹H NMR: δ 5.14 (s, 2H, CH₂), 7.20–7.40 (m, 5H, ar), 8.03 (s, 1H, CH), 11.21 (s, 1H, NH), 13.45 (s, 1H, NH).

7-Methylxanthine (11c). 3-Benzyl-7-methylxanthine (**10**, 1.54 g, 6 mmol) and dry AlCl₃ (1.6 g, 12 mmol) were suspended in 6 mL of dry toluene. The mixture was stirred for 1 h at 70 °C. After it was cooled to room temperature, 30 mL of ice-cold H₂O was added over a period of 3 h. After it was stirred for 2 h, the precipitate was collected by filtration and dried at 80 °C. Yield, 75%. ¹H NMR: δ 3.83 (s, 3H, CH₃), 7.90 (s, 1H, CH), 10.86 (s, 1H, NH), 11.53 (s, 1H, NH).

3-Methyl-8-phenylxanthine (11d). A suspension of 6-amino-1-methyl-5-nitrosouracil (**4b**, 8.5 g, 35.1 mmol) and benzylamine hydrochloride (8 g, 55.7 mmol) in 20 mL of benzylamine was heated at 170 °C for 3 h. After it was cooled to room temperature, the suspension was diluted with 40 mL of EtOH. The precipitate was filtered off, suspended in 40 mL of H₂O, and stirred for 2 h. The white precipitate was collected by filtration and subsequently recrystallized from acetic acid. The collected product was washed with a large amount of H₂O (ca. 200 mL) and dried at 110 °C. Yield, 46%. ¹H NMR: δ 3.48 (s, 3H, CH₃), 7.40–8.20 (m, 5H, ar).

7-Methyl-8-phenylxanthine (11e). 3-Benzyl-7-methyl-8-phenylxanthine (**9**, 3.32 g, 10 mmol) was dissolved in 100 mL of dry toluene, BBr₃ (10 g, 40 mmol) was added, and the mixture was stirred at 80 °C for 2 h. After the mixture was cooled to room temperature, 30 mL of ice-cold H₂O was added over a period of 1 h. The mixture was stirred for 2 h. The product was collected by filtration and purified by recrystallization from H₂O. Yield, 80%. ¹H NMR: δ 3.94 (s, 3H, CH₃), 7.40–7.60 (m, 3H, ar), 7.70–7.90 (m, 2H, ar), 10.90 (s, 1H, NH), 11.60 (s, 1H, NH).

3,7-Dimethyl-8-phenylxanthine (11f). Compound **6c** (1.65 g, 6 mmol) was suspended in 13 mL of 2 N NaOH and 4 mL of EtOH and refluxed for 1 h. The mixture was cooled to 4 °C and diluted with 17 mL of H₂O. Acetic acid was added until the product precipitated. The solid was collected by filtration and washed with 20 mL of H₂O. The product could be recrystallized from acetic acid. Yield, 85%. ¹H NMR: δ 3.39 (s, 3H, CH₃), 3.97 (s, 3H, CH₃), 7.44–7.83 (m, 5H, ar), 11.16 (s, 1H, NH).

3-Methyl-8-styrylxanthine (11g). Compound **8** (9.46 g, 35 mmol) was dissolved in 50 mL of thionyl chloride. The solution

was stirred for 8 h at room temperature. Excess SOCl₂ was removed by vacuum distillation. To the residue, saturated aqueous NaHCO₃ solution was added. The product was collected by filtration. Yield, 80%. ¹H NMR: δ 3.41 (s, 3H, CH₃), 7.03 (d, 1H, C=CH), (7.30–7.70 (m, 6H, ar + C=CH), 11.12 (s, 1H, NH), 13.55 (s, 1H, NH).

3,7-Dimethyl-8-styrylxanthine (11h). Compound **6a** (0.54 g, 1.8 mmol) was dissolved in a mixture of 4 mL of EtOH and 2 mL of 2 N NaOH. The solution was refluxed for 2 h. After it was cooled to room temperature, concentrated HCl solution was added until a pH value of 4 was achieved. The precipitate was collected by filtration and subsequently purified by recrystallization from H₂O:EtOH (50:50). Yield, 88%. ¹H NMR: δ 3.35 (s, 3H, CH₃), 3.85 (s, 3H, CH₃), 6.74 (d, 1H, C=CH), 7.35–7.85 (m, 6H, ar + C=CH), 11.10 (s, 1H, NH).

6-Thioxanthines (12a–h). General Procedure. A mixture of xanthine **10a–h** (55.4 mmol) and phosphorus pentasulfide (20 g, 90 mmol) in 200 mL of dry pyridine was refluxed for 8 h. After the mixture was cooled to room temperature, 400 mL of H₂O was added over a period of 2 h. The solution was reduced in vacuo to one-third of its volume. The precipitate was collected by filtration and suspended in 100 mL of 2 N NaOH. The solution was filtered, and the product was precipitated by adding dilute HCl solution to achieve a pH value of 4 and collected by filtration. The product can be purified by recrystallization from EtOH in the presence of charcoal. ¹H NMR: **12d** δ 3.49 (s, 3H, CH₃), 7.40–7.60 (m, 3H, ar), 8.10–8.30 (m, 2H, ar).

6-Methylsulfanylxanthines (13a–h). General Procedure. 6-Thioxanthine **12a–h** (5 mmol) was suspended in 12.5 mL of 0.5 N NaOH. EtOH (ca. 5 mL) was added until a clear solution was obtained. At room temperature, 7.1–20 mmol (0.44–1.25 mL) of CH₃I was added slowly. After it was stirred for 1 h, the product was collected by filtration, washed with H₂O (10 mL), and recrystallized from H₂O or a mixture of H₂O:EtOH (50:50). ¹H NMR: **13d** δ 2.66 (s, 3H, CH₃), 3.55 (s, 3H, CH₃), 7.50–7.60 (m, 3H, ar), 8.10–8.30 (m, 2H, ar).

6-(Hydroxyalkylamino)xanthines (14a–o). 6-Methylsulfanylxanthine **13a–h** (1 mmol) and 5 mmol of the appropriate amino alcohol in 1 mL of DMSO was heated for 1 h at 150 °C. The solvent and the excess amount of alcohol were removed in vacuo. The workup was done using four different methods: The residue was recrystallized from EtOH (method A); prior to recrystallization, the residue was purified by dry column flash chromatography as previously described²⁹ (method B); or the residue was column chromatographed with CHCl₃:methanol (gradient from 9:1 to 3:1) (method D). A fourth method was dissolution of the residue in methanol and acidification to a pH value of ca. 3 by adding concentrated HCl solution. The precipitate was then collected by filtration and recrystallized from EtOH (method C). ¹H NMR: **R-14i** δ 0.95 (t, 3H, CH₃), 1.52, 1.71 (m, 2H, CH₂), 3.35–3.65 (m, 3H, CH₂–CH), 3.52 (s, 3H, CH₃), 4.2 (vbr, 1H, OH), 7.50–7.70 (m, 3H, ar) 8.05–8.25 (m, 2H, ar), 10.08 (d, 1H, NH).

Tricyclic Purinone Derivatives (15–30). 1-Methyl-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*i*]purin-9-ium-5-one chloride (**15**), 4-benzyl-1-methyl-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*i*]purin-9-ium-5-one chloride (**16**), 1,4-dimethyl-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*i*]purin-9-ium-5-on chloride (**17**), 1,4,8-trimethyl-(8*R*)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*i*]purin-9-ium-5-one chloride (**R-18**), 1,4,8-trimethyl-(8*S*)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*i*]purin-9-ium-5-one chloride (**S-18**), 8-ethyl-1,4-dimethyl-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*i*]purin-9-ium-5-one chloride (**RS-19**), 8-ethyl-1,4-dimethyl-(8-*R*)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*i*]purin-9-ium-5-one chloride (**R-19**), 8-ethyl-1,4-dimethyl-(8-*S*)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*i*]purin-9-ium-5-one chloride (**S-19**), 1,4,7-trimethyl-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*i*]purin-9-ium-5-one chloride (**RS-20**), 1,4,7-trimethyl-(7*R*)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*i*]purin-9-ium-5-one chloride (**R-20**), 1,4,7-trimethyl-(7*S*)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*i*]purin-9-ium-5-one chloride (**S-20**), 1-methyl-2-phenyl-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*i*]purin-9-ium-5-one chloride (**21**), 1,4-dimethyl-2-phenyl-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*i*]purin-9-ium-5-one chloride (**22**), 8-eth-

yl-1,4-dimethyl-2-phenyl-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*f*]purin-9-ium-5-on chloride (*RS-23*), 8-ethyl-1,4-dimethyl-2-phenyl-(*8R*)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*f*]purin-9-ium-5-one chloride (*R-23*), 8-ethyl-1,4-dimethyl-2-phenyl-(*8S*)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*f*]purin-9-ium-5-one chloride (*S-23*), 8-ethyl-4-methyl-2-phenyl-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*f*]purin-9-ium-5-one chloride (*RS-24*), 8-ethyl-4-methyl-2-phenyl-(*8R*)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*f*]purin-9-ium-5-one chloride (*R-24*), 8-ethyl-4-methyl-2-phenyl-(*8S*)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*f*]purin-9-ium-5-one chloride (*S-24*), 8-ethyl-1,4-dimethyl-2-[2-phenyl-(*E*)-1-ethenyl]-(*8R*)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*f*]purin-9-ium-5-one chloride (*R-25*), 8-ethyl-1,4-dimethyl-2-[2-phenyl-(*E*)-1-ethenyl]-(*8S*)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*f*]purin-9-ium-5-one chloride (*S-25*), 8-ethyl-4-methyl-2-[2-phenyl-(*E*)-1-ethenyl]-(*8R*)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*f*]purin-9-ium-5-one chloride (*R-26*), 8-ethyl-4-methyl-2-[2-phenyl-(*E*)-1-ethenyl]-(*8S*)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*f*]purin-9-ium-5-one chloride (*S-26*), 1,4-dimethyl-1,4,5,7,8,9-hexahydropyrimido[2,1-*f*]purin-10-ium-5-on chloride (*27*), 2-chloro-1,4-dimethyl-1,4,5,7,8,9-hexahydropyrimido[2,1-*f*]purin-10-ium-5-one chloride (*28*), 1,4-dimethyl-4,5,6,8,9,10-hexahydro-1*H*[1,3]diazepino[2,1-*f*]purin-11-ium-5-one chloride (*29*), 1,4-dimethyl-1,4,5,7,8,9,10,11-octahydro-[1,3]diazocino[2,1-*f*]purin-12-ium-5-one chloride (*30*).

To 1 mL of thionyl chloride (cooled to 0 °C), 0.5 mmol of 6-(hydroxylalkylamino)xanthine (**14a–o**) was added. The mixture was refluxed for 60 min. Excess thionyl chloride was removed by vacuum distillation. Workup was performed using five different methods.

Method A. The residue was purified by dry column flash chromatography as previously described²⁹ and subsequently recrystallized from EtOH.

Method B. To the residue, a saturated solution of NaHCO₃ was added and the product was extracted with CHCl₃, purified by dry column flash chromatography,²⁹ and subsequently recrystallized from EtOH.

Method C. The residue was purified by dry column flash chromatography.²⁹ The solvent was removed by rotary evaporation. The residue was suspended in ethyl acetate, and HCl gas was blown through the mixture. The precipitate was collected by filtration and recrystallized from EtOH.

Method D. The residue was purified by column chromatography using CHCl₃:methanol (gradient from 9:1 to 3:1), suspended in CHCl₃, filtered off, and recrystallized from EtOH.

Method E. The residue was purified by column chromatography using CHCl₃:methanol (gradient from 9:1 to 3:1) and subsequently recrystallized from EtOH.

The purification method, yield, analytical characterization, mp, and where applicable optical rotation of compounds **15–30** are provided in Table 2. ¹H NMR: *R-24* δ 0.91 (t, 3H, CH₃), 1.20–1.40 (m, 2H, CH₂), 3.73 (s, 3H, CH₃), 3.70–3.90 (m, 2H, CH₂), 4.20–4.40 (m, 1H, CH), 7.30–7.50 (m, 3H, ar), 8.10–8.20 (m, 2H, ar). Additional NMR data are provided in Supporting Information.

Determination of Water Solubility.¹ A saturated solution of the test compounds in water was prepared. After the undissolved residue was filtered off, the solution was diluted with water and the UV absorption was determined at λ_{max}. The concentration of the solution was calculated using a previously determined standard calibration curve for each compound.

Chiral Separation and Quantitative Determination of Enantiomeric Purity by CE. The experiments were performed on a P/ACE CE system MDQ glycoprotein (Beckman Coulter Instruments, Fullerton, CA) equipped with a diode array detection system. The electrophoretic separations were carried out using eCAP untreated fused silica capillaries (60 (50 cm effective length) or 50 cm (40 cm effective length) × 75 μm internal diameter (ID) × 375 μm outside diameter (OD) obtained from Beckman Coulter). The separation was performed using an applied constant current of 90 μA and a data acquisition rate of 8 Hz. Analytes were detected and identified using a diode array detector, which provided UV spectra of the compounds by scanning the absorption from 190 to 400

nm. The CE instrument was fully controlled through a PC, which operated with the analysis software 32 KARAT obtained from Beckman Coulter. The evaluation of the electropherograms was done using the same software. The capillary temperature was kept constant at 25 °C. The temperature of the sample-storing partition was adjusted to 25 °C.

β-CD (28707), sulfated β-CD sodium salt (28248), (2-hydroxypropyl)-β-CD (56332), and carboxymethyl-β-CD (21906) were purchased from Fluka (Buchs, Switzerland). Potassium dihydrogen phosphate for the running buffer was purchased from Kraemer and Martin (Bonn, Germany).

For the preparation of the samples, 10 mM stock solutions in DMSO were diluted 1:100 with distilled water containing 3 mM HCl. Sometimes, higher sample concentrations had to be prepared to make sure that even small amounts of impurities by the undesired enantiomer could be detected.

The capillary was conditioned every day by rinsing it with 0.1 M sodium hydroxide solution for 20 min and with water for 10 min before starting the measurements. Between the runs, the capillary was conditioned by rinsing with 0.1 N HCl solution for 1 min, then with distilled water for 1 min, and subsequently with buffer for 1 min. Sample injections were made hydrodynamically applying 0.5 psi pressure for 5–20 s at the inlet side of the capillary. After the work was completed, the capillary was rinsed with 0.1 M sodium hydroxide solution for 20 min and with water for 10 min followed by drying with air for 3 min before shutting down the instrument. For rinsing procedures, 30 or 40 psi of pressure was applied.

Radioligand Binding Assays. Inhibition of binding of CHA to A₁ ARs of rat cerebral cortical membranes and inhibition of CGS21680 to A_{2A} ARs of rat striatal membranes were assayed as previously described.^{1,30} 2-Chloroadenosine (10 μM for the A₁, 20 μM for the A_{2A} assay) was used to define nonspecific binding. Inhibition of the receptor radioligand binding was determined over a range of at least 5–6 concentrations of the compounds in triplicate in at least three separate experiments. The Cheng–Prusoff equation and K_D values of 1 nM for [³H]CHA and 14 nM for [³H]CGS21680 were used to calculate K_i values from IC₅₀ values, determined by the nonlinear curve fitting program Graph Pad Prism 2.01 (GraphPad, San Diego, CA). Binding of [¹²⁵I]AB-MECA to human A₃ ARs stably expressed in HEK-293 cells (Receptor Biology, Inc., Baltimore, MD) was determined as described.³¹

AC Assays. Antagonism of *R*-PIA-elicited inhibition of AC via A₁ ARs in rat fat cell membranes and antagonism of NECA-elicited stimulation of AC via A_{2A} ARs in rat PC12 cell membranes were assayed as described.³² Inhibition of NECA-induced stimulation of AC by test compounds was determined at A_{2B} receptors in NIH 3T3 fibroblast cell membranes as described.³³ K_B values were calculated using the Schild equation, and the ratio of EC₅₀ values for NECA activation or the ratio of IC₅₀ values for *R*-PIA inhibition were calculated in the presence and absence of antagonist.

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

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