7-Deazaadenines Bearing Polar Substituents: Structure−Activity Relationships of New A_1 and A_3 Adenosine Receptor Antagonists†

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A series of 28 new pyrrolo[2,3-*d*]pyrimidine-4-amines, pyrimido[4,5-*b*]indole-4-amines, and tetrahydropyrimido[4,5-*b*]indole-4-amines was synthesized and their adenosine receptor affinity determined in radioligand binding assays at rat A_1 and A_{2A} adenosine receptors (ARs). Selected compounds were additionally investigated in binding assays at recombinant A_3 ARs. The 2-phenyl residue in (*R*)-7-(1-methylbenzyl)-2-phenylpyrrolo[2,3-*d*]pyrimidine-4-amine (ADPEP, **1**) and in the corresponding pyrimido[4,5-*b*]indole (APEPI, **3**) could be bioisosterically replaced by heterocyclic rings, such as 2-thienyl and 4-pyridyl. The resulting compounds retained high affinity and selectivity for A_1 ARs. Judging from the investigation of selected compounds, it appears that they are also potent at human A_1 ARs and selective not only versus A_{2A} ARs but also highly selective versus A_{2B} and A_3 ARs. The *p*-pyridyl-substituted derivatives **11** and **27** (APPPI) may be interesting pharmacological tools due to their fluorescent properties. Pyrrolo-[2,3-*d*]pyrimidine-4-amine derivatives which were simultaneously substituted at N7 and N^4, combining the substitution pattern of ADPEP (**1**) and DPEAP (**2**), showed very low affinity for A_1 ARs. This finding supports our previously published hypothesis of different binding modes for pyrrolopyrimidines, such as ADPEP (**1**) and DPEAP (**2**). DPEAP (**2**), a pyrrolo[2,3-*d*]-pyrimidine-4-amine substituted at the amino group (N^4), was found to exhibit high affinity for human A_3 ARs (K_i = 28 nM), whereas N^4-unsubstituted analogues were inactive. DPEAP (**2**) and related compounds provide new leads for the development of antagonists for the human A_3 AR.

Introduction

Adenosine receptors (ARs) belong to the superfamily of G-protein-coupled receptors and are currently subdivided into the following subtypes: A_1, A_{2A}, A_{2B}, and A_3.[1,2] A_1 and A_3 AR activation can lead to an inhibition of adenylate cyclase activity, while A_{2A} and A_{2B} AR activation causes a stimulation of adenylate cyclase. In addition, coupling to other second-messenger systems has been described, including calcium or potassium ion channels (A_1) or phospholipase C (A_1, A_{2B}, A_3).[3]

Agonists for ARs are all derived from the physiological agonist adenosine. The ribose moiety appears to be essential for agonistic activity.[4] Adenine derivatives and analogues lacking the ribose moiety have been shown to act as antagonists at ARs.[2,4] A variety of different classes of heterocyclic compounds has been described to possess antagonistic activity at ARs, including xanthines, adenines, 7-deazaadenines, 7-deaza-8-azapurines, triazolo[1,5-*a*]quinoxalines, and pyrazolo-[1,5-*a*]pyridines.[5−11] For the well-known A_1 and A_{2A} ARs many agonists and antagonists have been developed during the past decades.[5−11]

However, compounds which are believed to be highly selective for A_1 and A_{2A} ARs, respectively, have to be reevaluated, since (i) they may not be selective versus A_{2B} and/or A_3 ARs and (ii) they may only be selective in some species, such as rat but not humans. In fact, several generally used "selective" ligands have recently been found to be not very selective when tested in the now available cell culture systems expressing the human AR subtypes, e.g., the A_{2A} agonist CGS21680 is only 2-fold selective for human A_{2A} versus human A_3 ARs.[12−14] The A_3 AR is the latest member of the AR family. Recently, A_3 AR agonists (adenosine derivatives) and antagonists (including flavonoids, dihydropyridines, triazolonaphthyridine, isoquinoline, quinazoline derivatives) have been developed.[13−19]

Truly selective AR antagonists for the various subtypes are needed as pharmacological tools and are also of considerable interest as potential drugs. Possible therapeutic applications for A_1 antagonists include cognitive deficits, renal failure, and cardiac arrhythmias,[5] while A_{2A} antagonists may be beneficial for patients suffering from Morbus Parkinson.[20] A_3 Antagonists are associated with cerebroprotective properties.[14,21]

During our search for novel AR antagonists, it was found that 7-deazaadenines with the pyrrolo[2,3-*d*]-

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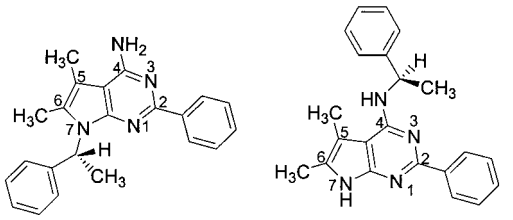
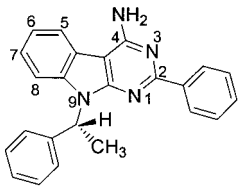
Chart 1. Potent A_1 AR Antagonists with 7-Deazaadenine Structure: Pyrrolo[2,3-*d*]pyrimidines **1** and **2** and Pyrimido[4,5-*b*]indole **3**



ADPEP (**1**)

DPEAP(**2**)



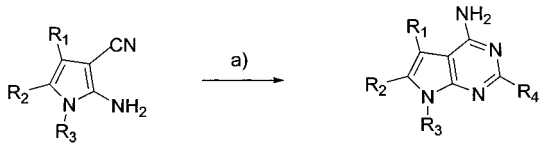
APEPI (**3**)

pyrimidine and pyrimido[4,5-*b*]indole structure (see Chart 1) were potent and selective AR antagonists.[11,22,23] Pyrrolo[2,3-*d*]pyrimidine-4-amines bearing a 2-phenyl substituent turned out to be particularly active at A_1 ARs and highly selective for that receptor subtype.[11,23] Thus, ADPEP (**1**) exhibited a K_i value at A_1 ARs of rat brain of 4.7 nM and ca. 800-fold selectivity as compared to the high-affinity A_{2A} ARs of rat striatum. APEPI (**3**) showed a K_i value at A_1 ARs of rat brain of 2.6 nM and greater than 2000-fold selectivity toward A_{2A} ARs.[11,23] The compounds exhibited a high degree of stereoselectivity, the (*R*)-configurated stereoisomers being 20−100-fold more potent than the (*S*)-enantiomers.

In the present study we have prepared and investigated a series of new 7-deazaadenine derivatives, mainly with variation of the substituent in the 2-position. Thus, pyrrolo[2,3-*d*]pyrimidines, tetrahydropyrimido[4,5-*b*]indoles, and pyrimido[4,5-*b*]indoles were prepared bearing a substituted 2-phenyl group, or in which the 2-phenyl substituent was bioisosterically replaced by heteroaromatic rings, or a 1-methylbenzyl residue. Our goal was to investigate the electronic and steric requirements of the ARs with respect to the 2-substituent in these classes of compounds and to gain further insight into their structure−activity relationships (SARs). Furthermore, we tried to increase the water solubility of the highly lipophilic parent compounds by the introduction of basic heterocycles (e.g., pyridyl residues) which would allow for the formation of more polar hydrochlorides.

An isomer of pyrrolo[2,3-*d*]pyrimidine **1** (ADPEP), in which the (*R*)-1-methylbenzyl substituent was moved from the pyrrole nitrogen atom to the exocyclic amino group (DPEAP, **2**), had proven to be a similarly potent A_1 AR antagonist as compared to ADPEP (**1**). On the basis of these results, we had postulated different binding modes for the two classes of compounds: 7-substituted pyrrolopyrimidines, such as ADPEP (**1**), on the one hand and N^4-substituted derivatives, such as DPEAP (**2**), on the other hand.[11] In our computer model, the two compounds have been aligned in such a way that the (*R*)-1-methylbenzyl substituents are overlapping and a hydrogen bond donor (the exocyclic amino group in **1** and the pyrrole N7-H in **3**) and a nearby hydrogen bond acceptor (N3 in **1** and N1 in **3**) are superimposed.[11]

Scheme 1. Syntheses of Pyrrolo[2,3-*d*]pyrimidines, Tetrahydropyrimido[4,5-*b*]indoles, and Pyrimido[4,5-*b*]indoles (Method A)[a]



4-7 **9-28**

[a] For R_1−R_4, see Chart 2 and Table 1. Reaction conditions: (a) appropriate carbonitrile, sodium methylate, 2-propanol.

Recently, Campbell et al. synthesized a series of analogues of DPEAP (**2**), in which the substituent at the exocyclic amino group was replaced by hydrophilic residues.[24] The new derivatives were investigated at recombinant human A_1 and A_{2A} ARs. Some of them, including the *N*-(*trans*-4-hydroxycyclohexyl) derivative proved to be potent and selective antagonists at human A_1 ARs.[24] We have now prepared further derivatives substituted at the exocyclic amino group (analogues of DPEAP, **2**), including derivatives which are disubstituted at N^4 and N7. These compounds were synthesized and investigated at ARs in order to test our previously designed pharmacophore model of different binding modes.

Chemistry

Most of the products prepared were chiral, and in most cases only the (*R*)-enantiomers, which had previously been shown to be the eutomers, were synthesized.

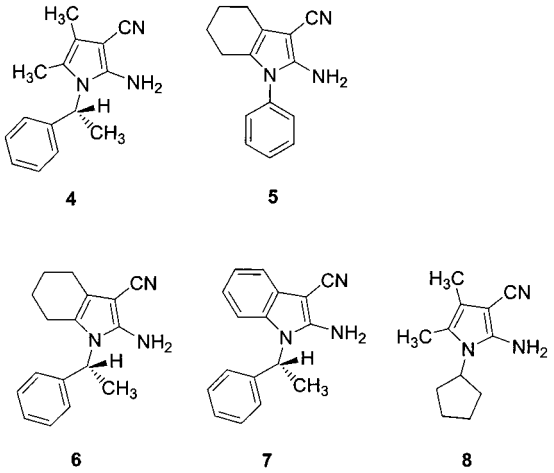
Ring-closure reactions (educts **4**−**8**, see Chart 1) to pyrrolo[2,3-*d*]pyrimidines and pyrimido[4,5-*b*]indoles were performed either in a one-pot reaction (method A, Scheme 1) or in two steps via acylamido derivatives and subsequent ring closure (method B, Scheme 3).

Method A: One-Pot Synthesis. 5,6-Dimethylpyrrolo[2,3-*d*]pyrimidine-4-amines **9**−**17** were prepared starting from the pyrrole derivative **4** (Chart 2 and Scheme 1). Compound **4** was obtained in a one-pot reaction developed by Eger et al. condensing 3-hydroxy-2-butanone and (*R*)-1-methylbenzylamine in the presence of *p*-toluenesulfonic acid, followed by base-catalyzed cyclization with malonodinitrile as described.[25] Tetrahydroindoles **5** and **6** (Chart 2) were prepared analogously.[25e] Ring closure was carried out using the appropriate carbonitriles in the presence of sodium methylate in 2-propanol (Scheme 1).

Reaction of pyrrolo[2,3-*d*]pyrimidine **11** with 2-propiolic acid methyl ester at room temperature yielded **29**. The 7-unsubstituted pyrrolo[2,3-*d*]pyrimidine **30** was prepared from racemic compound **17**. Dealkylation in the 7-position by heating with polyphosphoric acid yielded racemic **30**.[25d] Due to its low affinity at ARs, pure enantiomers of **30** were not prepared. The hydroxyphenyl-substituted pyrrolo[2,3-*d*]pyrimidine **31** was prepared from methoxyphenyl derivative **16** by cleavage of the methyl ether using the Lewis acid boron tribromide (Scheme 2).

Indole **7** was prepared from tetrahydroindole **6** by oxidation using dichlorodicyanoquinone (DDQ) in tet-

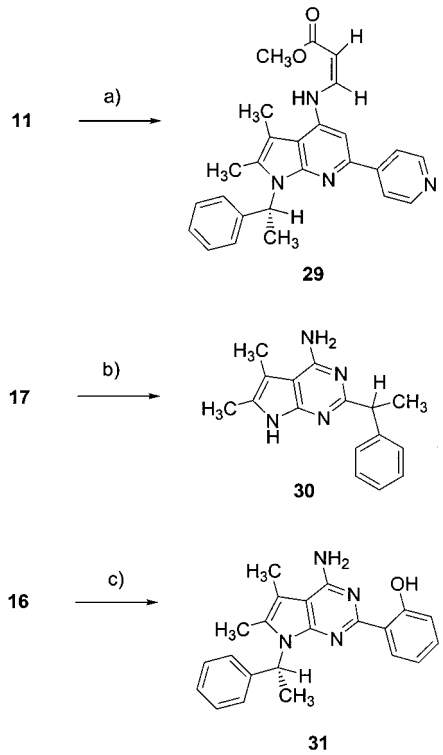
Chart 2. Educts Synthesized



rahydrofuran (Chart 2). Reaction of tetrahydroindole **5** with the appropriate pyridinecarbonitriles in the presence of sodium methylate in 2-propanol yielded the 2-phenyltetrahydropyrimido[4,5-*b*]indoleamine derivatives **18**−**20** following described procedures (Scheme 1).[11,25] Analogously, compounds **21**−**24** were prepared using the tetrahydroindole **6**, and **25**−**28** were obtained starting from indole **7** (Scheme 1).

Method B: Two-Step Synthesis. Pyrroles **4** and **8** (Chart 2) were used as starting material to prepare N^4-substituted 5,6-dimethylpyrrolo[2,3-*d*]pyrimidines (Scheme 3). Thus, the pyrroles were reacted with the appropriate benzoyl chloride derivative to yield the acylated pyrroles **32**− **34**, respectively. Pyrrolo[2,3-*d*]-pyrimidine derivatives **35**−**37** were prepared by cyclization of **32**−**34** using phosphorus pentoxide in a mixture of dimethylcyclohexylamine and water at 190 °C (Scheme 3). Reaction of **35**−**37** with phosphorus oxychloride yielded chloro-substituted 5,6-dimethylpyrrolo[2,3-*d*]-pyrimidines **38**−**40**. The desired N^4-substituted 5,6-dimethylpyrrolo[2,3-*d*]pyrimidines **41**−**45** were prepared from **38**−**40** by reaction with the appropriate amines (Scheme 3).

Scheme 2. Syntheses of Amino-Substituted Pyrrolo[2,3-*d*]pyrimidines **29**−**31**[a]



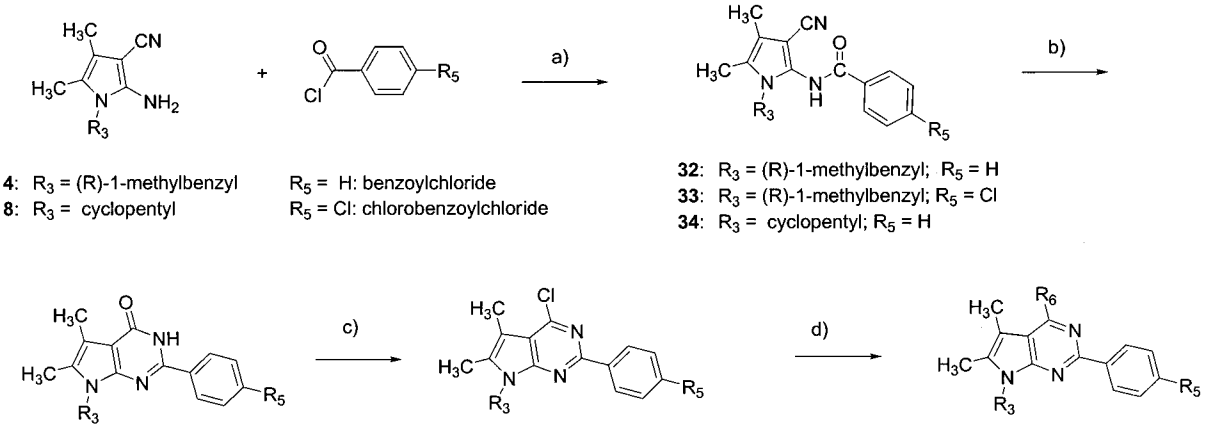
[a] Reaction conditions: (a) propiolic acid methylate, glacial acetic acid; (b) phosphorus pentoxide; (c) boron tribromide, CH_2Cl_2.

Reagents, yields, and analytical data are given in Table 1. ^1H and ^{13}C NMR spectral data were in accordance with the proposed structures. Selected NMR data are given in the Experimental Section. Further NMR data are available as Supporting Information.

Biological Evaluation

The compounds were tested in radioligand binding assays for affinity at A_1 and A_{2A} ARs in rat brain cortical

Scheme 3. Syntheses of N^4-Substituted Pyrrolo[2,3-*d*]pyrimidine Derivatives (Method B)[a]



4: R_3 = (R)-1-methylbenzyl
8: R_3 = cyclopentyl

R_5 = H: benzoylchloride
R_5 = Cl: chlorobenzoylchloride

32: R_3 = (R)-1-methylbenzyl; R_5 = H
33: R_3 = (R)-1-methylbenzyl; R_5 = Cl
34: R_3 = cyclopentyl; R_5 = H

35: R_3 = (R)-1-methylbenzyl; R_5 = H
36: R_3 = (R)-1-methylbenzyl; R_5 = Cl
37: R_3 = cyclopentyl; R_5 = H

38: R_3 = (R)-1-methylbenzyl; R_5 = H
39: R_3 = (R)-1-methylbenzyl; R_5 = Cl
40: R_3 = cyclopentyl; R_5 = H

41: R_3 = (R)-1-methylbenzyl; R_5 = H; R_6 = (R)-1-methylbenzylamino
42: R_3 = (R)-1-methylbenzyl; R_5 = H; R_6 = cyclopentylamino
43: R_3 = (R)-1-methylbenzyl; R_5 = H; R_6 = ethylendiamino
44: R_3 = (R)-1-methylbenzyl; R_5 = Cl; R_6 = 2-amino-butan-1-ol
45: R_3 = cyclopentyl; R_5 = H; R_6 = 2-amino-butan-1-ol

[a] Reaction conditions: (a) pyridine, CH_2Cl_2, 0 °C; (b) phosphorus pentoxide, *N*,*N*-dimethylcyclohexylamine, H_2O, 190 °C; (c) $POCl_3$; (d) appropriate amine derivative.

Table 1. Reagents, Yields, and Analytical Data of the New Compounds Synthesized

compd	reagent, g (mmol)	yield [g (%)]	formula	anal.[a]	TS-MS or EI-MS (70 eV) *m/z*	mp (°C)
	Type **I**: Pyrrolo[2,3-*d*]pyrimidine-4-amine Derivatives					
9	2-pyridinecarbonitrile, 1.9 (18)	1.3 (21%)	$C_{21}H_{21}N_5$	C, H, N	TS-POS: 344 [M + H$^+$]	233
10	3-pyridinecarbonitrile, 1.9 (18)	2.8 (45%)	$C_{21}H_{21}N_5$	C, H, N	TS-POS: 344 [M + H$^+$]	249
11	4-pyridinecarbonitrile, 1.9 (18)	3.4 (55%)	$C_{21}H_{21}N_5$	C, H, N	TS-POS: 344 [M + H$^+$]	171
12	4-pyridinecarbonitrile *N*-oxide, 2.2 (18)	4.1 (64%)	$C_{21}H_{21}N_5O$	C, H, N	EI-MS: 359 [M$^+$]	264
13	2-pyrazinecarbonitrile, 1.9 (18)	1.2 (20%)	$C_{20}H_{20}N_6$	C, H, N[b]	TS-POS: 345 [M + H$^+$]	210
14	2-thiophenecarbonitrile, 2.0 (18)	3.6 (58%)	$C_{20}H_{20}N_4S$	C, H, N	TS-NEG: 347 [M − H$^+$]	168
15	2-furonitrile, 1.7 (18)	2.5 (42%)	$C_{20}H_{20}N_4O$	C, H, N	TS-POS: 333 [M + H$^+$]	193
16	2-methoxybenzonitrile, 2.4 (18)	1.8 (27%)	$C_{23}H_{24}N_4O$	C, H, N	EI-MS: 372 [M$^+$]	125
17	(*R*,*S*)-1-methylphenylacetonitrile, 2.4 (18)	1.4 (38%)	$C_{24}H_{26}N_4$	C, H, N	EI-MS: 370 [M$^+$]	144
29	**11**, 0.70 (2)	0.60 (70%)	$C_{25}H_{25}N_5O_2$	C, H, N	TS-POS: 428	175
30	**17**, 2.6 (7)	1.4 (76%)	$C_{16}H_{18}N_4$	C, H, N	NCI: 265 [M − H$^+$]	165
31	**16**, 0.37 (1)	0.08 (21%)	$C_{22}H_{22}N_4O$	C, H, N	TS-POS: 359 [M + H$^+$]	154
34	**8**, 2.5 (12.5)	4.4 (35%)	$C_{19}H_{21}N_3O$	C, H, N	TS-POS: 308 [M + H$^+$]	180
37	**34**, 2.5 (8)	0.847 (69%)	$C_{19}H_{21}N_3O$	C, H, N	TS-POS: 308 [M + H$^+$]	266
39	**36**, 3.0 (8)	1.6 (52%)	$C_{22}H_{19}N_3Cl_2$	C, H, N	TS-POS: 397 [M$^+$+ 2]	133
40	**37**, 0.615 (2)	0.352 (54%)	$C_{19}H_{20}N_3Cl$	C, H, N	TS-POS: 326 [M$^+$]	158
41	**38**, 1.085 (3)	1.003 (77%)	$C_{30}H_{30}N_4$	C, H, N	TS-POS: 447 [M + H$^+$]	145
42	**38**, 0.543 (1.5)	0.511 (83%)	$C_{27}H_{30}N_4$	C, H, N	TS-POS: 411 [M + H$^+$]	176
43	**38**, 0.732 (1.5)	0.704 (91%)	$C_{24}H_{28}N_5$	C, H, N	TS-POS: 387 [M + H$^+$]	122
44	**39**, 1.189 (3.0)	0.687 (51%)	$C_{26}H_{29}N_4OCl$	C, H, N	TS-POS: 448 [M$^+$]	232
45	**40**, 0.326 (1.0)	0.351 (93%)	$C_{23}H_{30}N_4O$	C, H, N	TS-POS: 379 [M$^+$]	70
	Type **II**: Tetrahydropyrimido[4,5-*b*]indole-4-amine Derivatives					
18	2-pyridinecarbonitrile, 1.0 (10)	2.1 (62%)	$C_{21}H_{19}N_5$	C, H, N	EI-MS: 341 [M$^+$]	266
19	3-pyridinecarbonitrile, 1.0 (10)	1.2 (35%)	$C_{21}H_{19}N_5$	C, H, N	TS-POS: 342 [M + H$^+$]	252
20	4-pyridinecarbonitrile, 1.0 (10)	0.7 (21%)	$C_{21}H_{19}N_5$	C, H, N[c]	EI-MS: 342 [M + H$^+$]	233
21	2-pyridinecarbonitrile, 2.6 (25)	1.2 (13%)	$C_{23}H_{23}N_5$	C, H, N	EI-MS: 369 [M$^+$]	249
22	3-pyridinecarbonitrile, 2.6 (25)	1.0 (11%)	$C_{23}H_{23}N_5$	C, H, N	EI-MS: 369 [M$^+$]	199
23	4-pyridinecarbonitrile, 2.6 (25)	2.0 (22%)	$C_{23}H_{23}N_5$	C, H, N	EI-MS: 369 [M$^+$]	156
24	4-pyridinecarbonitrile *N*-oxide, 3.0 (25)	6.0 (62%)	$C_{23}H_{23}N_5O$	C, H, N	EI-MS: 385 [M$^+$]	242
	Type **III**: (*R*)-(1-Methylbenzyl)pyrimido[4,5-*b*]indole-4-amine Derivatives					
25	2-pyridinecarbonitrile, 1.0 (10)	1.2 (13%)	$C_{23}H_{19}N_5$	C, H, N	TS-NEG: 365 [M$^+$]	111
26	3-pyridinecarbonitrile, 1.0 (10)	1.4 (38%)	$C_{23}H_{19}N_5$	C, H, N	EI-MS: 364 [M$^+$]	197
27	4-pyridinecarbonitrile, 1.0 (10)	1.2 (33%)	$C_{23}H_{19}N_5$	C, H, N	EI-MS: 365 [M$^+$]	170
28	2-thiophenecarbonitrile, 1.1 (10)	2.7 (73%)	$C_{22}H_{18}N_4S$	C, H, N	EI-MS: 370 [M$^+$]	110

[a] Elemental analyses were within 0.4% of calculated values, unless otherwise noted. [b] Calcd: 69.75; 5.85; 24.40. Found: 69.22; 5.67; 24.99. [c] Calcd: 73.88; 5.61; 20.51. Found: 73.77; 6.03; 19.93.

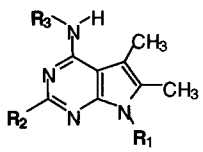
membrane and rat striatal membrane preparations, respectively. [^{3}H]N^6-Cyclohexyladenosine ([^{3}H]CHA, 1 nM) or [^{3}H]-2-chloro-N^6-cyclopentyladenosine ([^{3}H]CCPA, 0.5 nM), respectively, was used as the A_1 ligand and CGS21680 ([^{3}H]-2-[[[[4-(carboxyethyl)phenyl]ethyl]-amino]-5′-*N*-(ethylcarbonyl)amino]adenosine, 5 nM) as the A_{2A} ligand. All compounds were dissolved in DMSO and diluted into aqueous buffer solution. Compound **11** was additionally tested at recombinant human A_1 ARs expressed in Chinese hamster ovary (CHO) cells using [^{3}H]CCPA (0.5 nM). The A_3 AR affinity was determined at human recombinant A_3 ARs expressed in CHO cells using [^{3}H]-5′-[(*N*-(ethylcarbonyl)amino]adenosine (NECA, 10 nM) as radioligand. The inhibition of NECA-stimulated adenylate cyclase by test compounds was measured using human recombinant A_{2B} ARs expressed in CHO cells.[12]

Results and Discussion

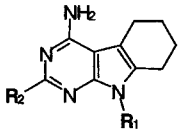
7-Deazaadenines have been investigated as antagonists at A_1 and A_{2A} ARs. In earlier studies we showed that 7-deazaadenines are antagonists at A_1 and A_{2A} ARs in adenylate cyclase assays.[11,22,23] In contrast to the 7-deazaadenine derivatives presented, all AR agonists and partial agonists described so far contain a (substituted) ribose or an analogous structure.[14] The most potent compound of this series, **27** (APPPI), has additionally been investigated in [^{35}S]GTPγS binding studies at A_1 ARs and clearly shown to be an A_1 AR antagonist (data not shown). On the basis of these results, it is assumed that all 7-deaazaadenines described herein are antagonists at ARs.

The 2-Substituent. 2-Substituted pyrrolo[2,3-*d*]pyrimidine-4-amines (**I**), tetrahydropyrimido[4,5-*b*]indole-4-amines (**II**), and pyrimido[4,5-*b*]indole-4-amines (**III**), in which the 2-phenyl group was bioisosterically replaced by heterocyclic rings, were investigated (Table 2). Most of these compounds showed only low or negligible affinity for the A_{2A} ARs and were A_1-selective. However, these modifications led to a decrease in A_1 AR affinity compared to the parent, 2-phenyl-substituted compounds ADPEP (**1**) and APEPI (**3**). While 2-furyl (**15**), 2-thienyl (**14**), and 4-pyridyl (**11**) rings were well-tolerated at the 2-position of pyrrolo[2,3-*d*]pyrimidine-4-amines, 2- or 3-pyridyl (compounds **9** and **10**), 4-pyridyl-*N*-oxide (**12**), or 2-pyrazinyl (**13**) substituents in the same position led to a large reduction in A_1 AR affinity. Similarly, in the pyrimidoindole series (**III**), a 2-thienyl (**28**) or 4-pyridyl (**27**) substituent in the 2-position was much better tolerated than 2- or 3-pyridyl residues (**25**, **26**). In general, the pyrimidoindoles (**III**) were somewhat more potent as compared to the corresponding pyrrolopyrimidines (**I**), and similar SARs were observed, with one striking exception: The 2-(3-pyridyl) derivative in the pyrimidoindole series (**III**) retained A_1 affinity (**26**, K_i = 92 nM), while the analogous pyrrolopyrimidine **10** was inactive at high concentrations (Table 2). The rank order of potency for bioisosteric analogues of the pyrrolopyrimidine ADPEP (**1**) was 2-furyl (**15**) ≥ 2-thienyl (**14**) ≥ 4-pyridyl (**11**) > 2-pyrazinyl (**13**) > 2-pyridyl (**9**) > 3-pyridyl (**10**). The rank order of potency for analogues of the pyrimidoindole APEPI (**3**) was 4-pyridyl (**27**) ≥ 2-thienyl (**28**) > 3-pyridyl (**26**) > 2-pyridyl (**25**). The different SAR for the 3-pyridyl-substituted

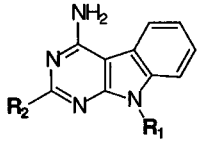
Table 2. AR Affinities of Pyrrolo[2,3-*d*]pyrimidine-4-amines, Tetrahydropyrimido[4,5-*b*]indole-4-amines, and Pyrimido[4,5-*b*]indole-4-amines



I
Pyrrolo[2,3-*d*]pyrimidine-4-amines



II
Tetrahydropyrimido[4,5-*b*]-indole-4-amines



III
Pyrimido[4,5-*b*]-indole-4-amines

				$K_i \pm$ SEM (μM)		
compd	R_1	R_2	R_3	A_1 AR vs [^{3}H]CHA or [^{3}H]CCPA rat brain cortex	A_{2A} AR vs [^{3}H]CGS21680 rat striatum	A_3 AR vs [^{3}H]NECA human recomb receptors
			Pyrrolo[2,3-*d*]pyrimidine-4-amines (**I**)			
1 (ADPEP)	(*R*)-1-methylbenzyl	phenyl	H	0.0047[11]	3.71[11]	
2 (DPEAP)[d]	H	phenyl	(*R*)-1-methylbenzyl	0.0067[11]	23% (30 μM)[11]	0.028 (10.4−77.8)[c]
9	(*R*)-1-methylbenzyl	2-pyridyl	H	2.1 ± 0.3	43% (30 μM)[b]	
10	(*R*)-1-methylbenzyl	3-pyridyl	H	35% (100 μM)	3% (30 μM)[b]	
11	(*R*)-1-methylbenzyl	4-pyridyl	H	0.046 ± 0.017 (0.0088 ± 0.0013[e])	1.3 ± 0.1	
12	(*R*)-1-methylbenzyl	4-pyridyl *N*-oxide	H	0.49 ± 0.025[a]	5.9 ± 2.7	
13	(*R*)-1-methylbenzyl	2-pyrazinyl	H	0.56 ± 0.12	6.6 ± 0.5	
14	(*R*)-1-methylbenzyl	2-thienyl	H	0.019 ± 0.002[a]	5.4 ± 0.75	
15	(*R*)-1-methylbenzyl	2-furyl	H	0.016 ± 0.005[a]	0.49 ± 0.08	
16	(*R*)-1-methylbenzyl	2-methoxyphenyl	H	38% (30 μM)[a,b]	14% (30 μM)[b]	
17	(*R*)-1-methylbenzyl	(*R,S*)-1-methylbenzyl	H	33% (30 μM)[a,b]	38% (30 μM)[b]	
29	(*R*)-1-methylbenzyl	4-pyridyl	acrylic acid methylate	3.0 ± 0.25	2.3 ± 0.19	2.64 ± 0.32
30	H	(*R,S*)-1-methylbenzyl	H	6.84 ± 0.001[a]	10% (10 μM)[b]	
31	(*R*)-1-methylbenzyl	2-hydroxyphenyl	H	0.24 ± 0.04[a]	46% (30 μM)[b]	
41	(*R*)-1-methylbenzyl	phenyl	(*R*)-1-methylbenzyl	18.3 ± 1.0	26% (30 μM)[b]	11.4 ± 2.6
42	(*R*)-1-methylbenzyl	phenyl	cyclopentyl	41% (30 μM)[b]	19% (30 μM)[b]	8.65 ± 2.97
43	(*R*)-1-methylbenzyl	phenyl	aminoethyl	3.58 ± 1.64[a]	2% (30 μM)[b]	5.58 ± 0.53
44	(*R*)-1-methylbenzyl	4-chlorphenyl	(*R,S*)-2-butan-1-ol	2.6 ± 0.75[a]	36% (30 μM)[b]	0.57 ± 0.26
45	cyclopentyl	phenyl	(*R,S*)-2-butan-1-ol	1.24 ± 0.41[a]	22% (30 μM)[b]	1.87 ± 0.23
			Tetrahydropyrimido[4,5-*b*]indole-4-amines (**II**)			
18	phenyl	2-pyridyl		8.9 ± 2.0	42% (30 μM)[b]	
19	phenyl	3-pyridyl		7.9 ± 2.3	44% (30 μM)[b]	
20	phenyl	4-pyridyl		0.6 ± 0.3	28% (10 μM)[b]	
21	(*R*)-1-methylbenzyl	2-pyridyl		12.5 ± 1.8	38% (30 μM)[b]	
22	(*R*)-1-methylbenzyl	3-pyridyl		1.3 ± 0.03	46% (30 μM)[b]	
23	(*R*)-1-methylbenzyl	4-pyridyl		8% (100 μM)[a]	2% (30 μM)[b]	>10
24	(*R*)-1-methylbenzyl	4-pyridyl *N*-oxide		1.85 ± 0.29[a]	50% (30 μM)[b]	
			Pyrimido[4,5-*b*]indole-4-amines (**III**)			
3 (APEPI)[d]	(*R*)-1-methylbenzyl	phenyl		0.0026[11]	6.2[11]	>1
25	(*R*)-1-methylbenzyl	2-pyridyl		0.54 ± 0.15[a]	6% (1 μM)[b]	
26	(*R*)-1-methylbenzyl	3-pyridyl		0.092 ± 0.017[a]	47% (10 μM)[b]	
27 (APPPI)[d]	(*R*)-1-methylbenzyl	4-pyridyl		0.021 ± 0.002	1.85 ± 0.2	>10
28	(*R*)-1-methylbenzyl	2-thienyl		0.036 ± 0.006[a]	32% (3 μM)[b]	

[a] [^{3}H]CCPA was used as radioligand. [b] Percent inhibition at the indicated concentration. [c] 95% confidence limits. [d] IC_{50} at human A_{2B} ARs > 10 μM. [e] K_i value at human recombinant A_1 ARs expressed in CHO cells, determined with [^{3}H]CCPA as radioligand.

compounds **10** and **26** could indicate different binding modes for some members of the two series.

Tetrahydropyrimido[4,5-*b*]indole derivatives (**II**) exhibited only weak A_1 AR affinity as compared to the corresponding pyrrolopyrimidines (**I**) and pyrimidoindoles (**III**); K_i values at the A_1 AR were in the micromolar concentration range (compounds **18**−**24**, Table 2). It appears that a flat aromatic ring system, as in the pyrimidoindoles, is well-accommodated by the A_1 AR. The tetrahydroyprimidoindole ring system (**II**) is unfavorable, probably due to steric interference with the receptor protein. The less spacious dimethyl substituents in the pyrrolopyrimidines (**I**) are better tolerated by the receptor than the additional saturated ring in the compound series **II**.

In a recent publication, it has been shown that methoxy substitution in the meta- or para-position of the phenyl ring in ADPEP (**1**) led to ca. 20-fold reduction in the A_1 AR affinity.[11] In the present study, it was found that the same substituent in the ortho-position led to an even more drastic reduction in A_1 AR affinity. Thus, compound **16** showed an IC_{50} value of greater than 30 μM (Table 2). The smaller hydroxyl substituent (compound **31**) was better tolerated (K_i A_1 = 0.24 μM) but still led to a 50-fold reduction as compared to the unsubstituted parent compound **1**. The receptor does not appear to accept large substituents in the ortho-position of the 2-phenyl group, as it does not tolerate nitrogen atoms (2-pyridyl substitution) at the same position (compounds **9** and **21**). A chlorine atom in the ortho-position, however, only led to a 2-fold decrease in A_1 AR affinity.[11]

One compound bearing a 1-methylbenzyl substituent in the 2-position instead of phenyl but lacking the (*R*)-1-methylbenzyl residue at the pyrrole nitrogen in the pyrrolopyrimidine series (**I**) was prepared (compound

30). It exhibited moderate A_1 AR affinity ($K_i = 6.8\ \mu M$) and thus was ca. 5-fold weaker as compared to the analogous 2-phenyl derivative.[23] Since the chiral compound **30** was not superior to the 2-phenyl derivative, the single enantiomers were not prepared.

One of the more potent compounds, the *p*-pyridyl-substituted pyrrolopyrimidine **11** was additionally investigated at human recombinant A_1 ARs. It was found that compound **11** showed high affinity for the human A_1 AR exhibiting a K_i value of 8.8 nM.

It was observed that the pyridyl-substituted pyrrolopyrimidines (**9**−**11**), tetrahydropyrimidoindoles (**21**−**23**), and pyrimidoindoles (**25**−**27**) showed intensive fluorescent properties. Acidification leading to a protonation of the pyridyl nitrogen atom increased water solubility of the compounds and also strongly enhanced fluorescence of the compounds. A more detailed description of this effect is currently under investigation. The *p*-pyridyl-substituted compounds **11** and **27**, potent and selective A_1 AR ligands with fluorescent properties, might be useful pharmacological tools.

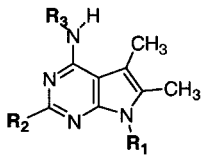
Substitution of the Exocyclic Amino Group. Since 2-phenylpyrrolopyrimidines bearing a 1-methylbenzyl substituent, either on the pyrrole nitrogen (N7, e.g., **1**, ADPEP) or on the exocyclic amino group (N^4, e.g., **2**, DPEAP), were potent A_1 AR antagonists, we investigated whether a combination of N7- and N^4-substitution would be additive leading to an increase in A_1 AR affinity. However, the bis(1-methylbenzyl)-substituted derivative **41** exhibited only low A_1 AR affinity ($K_i = 18.3\ \mu M$). All other bis-substituted compounds (**29**, **41**−**45**), including the N^4-cyclopentyl derivative **42**, exhibited only low A_1 AR affinity. These results support our previously postulated pharmacophore model for the binding of AR antagonists.[5,11] 7-Substituted pyrrolopyrimidine derivatives and N^4-substituted derivatives appear to exhibit different binding modes at A_1 ARs. It is very likely that the 7-substituent in ADPEP (**1**) and the N^4-substituent in DPEAP (**2**) occupy the same receptor region.[5,11]

A_{2B} and A_3 ARs. Initially, three representative compounds, the N^4-substituted pyrrolopyrimidine **2** (DPEAP), the 2-*p*-pyridyl-substituted pyrimidoindole **27** (APPPI), and the 2-phenyl-substituted pyrimidoindole **3** (APEPI) were investigated in radioligand binding assays at human A_3 ARs and in adenylate cyclase assays at human A_{2B} ARs. These experiments were performed in order to assess A_1 selectivity of the compounds versus A_{2B} and A_3 ARs. All these compounds did not show any antagonistic effects at A_{2B} AR up to concentrations of 10 μM. APPPI (**27**) and APEPI (**3**), pyrimidoindole derivatives which are **un**substituted at the exocyclic amino group, did not show binding to A_3 ARs at the investigated concentrations either (Table 2). However, DPEAP (**2**), a pyrrolopyrimidine derivative substituted at the amino group, exhibited surprisingly high affinity for human A_3 ARs ($K_i = 28$ nM). Thus, DPEAP (**2**) is only 4-fold selective for A_1 versus A_3 ARs. Due to its high affinity for A_3 ARs, DPEAP (**2**) has been selected as a new lead compound for the development of A_3 AR antagonists. Further N^4-substituted pyrrolopyrimidine derivatives (**29**, **41**−**45**) of the present series were investigated at A_3 ARs. In contrast to DPEAP (**2**), all these derivatives bore an additional substituent at the pyrrole nitrogen N7, either (*R*)-1-methylbenzyl (**29, 41**−**44**) or cyclopentyl (**45**). Substitution at N^4 comprised lipophilic residues, including (*R*)-1-methylbenzyl (**41**) and cyclopentyl (**42**), as well as more polar, hydrophilic residues, such as amino- and hydroxyalkyl (**43**−**45**) and acrylic acid methyl ester (**29**). Substituents in the 2-positions were those known to be well-tolerated by the ARs (phenyl, *p*-chlorophenyl, *p*-pyridyl). All of the compounds exhibited affinity to A_3 ARs in the micromolar concentration range. The additional (*R*)-1-methylbenzyl substituent at the pyrrole nitrogen in compound **41** as compared to **2** (DPEAP) caused a 400-fold decrease in A_3 affinity. Thus, bis-substitution at N^4 and N7 appears to be unfavorable for A_3 affinity as it is for A_1 AR affinity (see above). A variety of different − polar and nonpolar − N^4-substituents was tolerated by the A_3 AR. The most potent compound, besides DPEAP (**2**), was the hydroxybutyl-substituted derivative **44** showing a K_i value of 570 nM at human A_3 ARs; it was about 5-fold selective for A_3 versus A_1 ARs. Interestingly, the introduction of a *p*-chloro substituent in the 2-phenyl group led to ca. 3-fold increase in A_3 affinity, while it caused ca. 2-fold reduction of the A_1 affinity (Table 2).

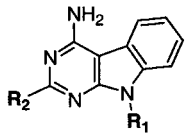
Water Solubility. Many high-affinity AR antagonists exhibit low water solubility, which limits their usefulness as pharmacological tools.[5,6d,26] Potent A_1 AR antagonists with the pyrrolo[2,3-*d*]pyrimidine or pyrimido-[4,5-*b*]indole structure have been shown to be highly lipophilic and soluble in water only in micromolar concentrations.[11] The present study was aimed at improving the water solubility of the compounds by introducing polar substituents in the 2-position (e.g., exchange of phenyl for pyridyl) and at the N^4-amino group (e.g., aminoethyl, hydroxybutyl substitution). Water solubility of selected compounds was determined by an HPLC/UV method (Table 3). Stock solutions of the compounds were prepared in DMSO and diluted into buffer to obtain a saturated solution containing 1% DMSO. As expected, the replacement of the 2-phenyl group in APEPI (**3**) by pyridyl residues resulted in a ca. 6−10-fold increase in water solubility (compounds **25**, **27**, Table 3). Due to the lower affinities of compounds **25** and **27**, their solubility-over-affinity ratio was not improved as compared to APEPI (**3**). An even higher increase in water solubility was achieved by the introduction of polar substituents at the exocyclic amino group (compounds **43**−**45**). These compounds were 20−50-fold better soluble than APEPI (**3**). The higher solubility, however, was associated with lower AR affinity. The best of the new compounds was the *p*-pyridyl analogue of APEPI, compound **27**, which showed increased water solubility and a solubility-over-affinity ratio of greater than 100. This has been postulated as a prerequisite for compounds to be active in vivo.[26]

In conclusion, new potent, selective A_1 AR antagonists have been prepared, including 2-*p*-pyridyl-substituted analogues of ADPEP (**1**) and APEPI (**3**), which may prove to be useful pharmacological tools due to their fluorescent properties. N^4-Substituted pyrrolo[2,3-*d*]-pyrimidine-4-amines provide new lead compounds for the development of A_3-selective AR antagonists.

Table 3. Solubility and Solubility-over-Affinity Ratios of Selected Pyrrolo[2,3-*d*]pyrimidines and Pyrimido[4,5-*b*]indoles



I
Pyrrolo[2,3-*d*]pyrimidines



III
Pyrimido[4,5-*b*]indoles

compd	R_1	R_2	R_3	solubility[a] (μM)	A_1 affinity	ratio solubility/A_1 affinity
			Pyrimido[4,5-*b*]indoles (**III**)			
3 (APEPI)	(*R*)-1-methylbenzyl	phenyl		0.48[11b]	0.0026[11b]	185
25	(*R*)-1-methylbenzyl	2-pyridyl		5.0	0.54	9
27 (APPPI)	(*R*)-1-methylbenzyl	4-pyridyl		3.0[b]	0.021	142
			Pyrrolo[2,3-*d*]pyrimidines (**I**)			
43	(*R*)-1-methylbenzyl	phenyl	aminoethyl	24.2	3.58	7
44	(*R*)-1-methylbenzyl	4-chlorphenyl	(*R,S*)-2-butan-1-ol	11.2	2.6	4
45	cyclopentyl	phenyl	(*R,S*)-2-butan-1-ol	13.2	1.24	11

[a] Determined in 50 mM TRIS-HCl buffer, pH 7.4, containing the indicated amount of DMSO. [b] Estimated from the validation data. The solubility is smaller than the limit of quantification (3.6 μM) but higher than the limit of detection (2.4 μM).

Experimental Section

Chemistry. NMR spectra were measured on a Varian Gemini 300 spectrometer (^{1}H: 300 MHz; ^{13}C: 75 MHz). The chemical shifts of the remaining protons of the deuterated solvents served as internal standard: δ (^{1}H: DMSO-d_6 = 2.50, $CDCl_3$ = 7.24, MeOD = 3.35 and 4.78; ^{13}C: DMSO-d_6 = 39.7, $CDCl_3$ = 77.0, MeOD = 49.3). All compounds were checked for purity by TLC on 0.2-mm aluminum sheets with silica gel 60 F_{254} (Merck): hexane:ethyl acetate (2:1), toluene:acetone: formic acid (60:39:1) or ethyl acetate, respectively, were used as eluents.

Melting points were taken on a Büchi 535 melting point apparatus or a Gallenkamp variable heater and are uncorrected. Thermospray (negative and positive ionization) and electron ionization mass spectra were recorded on an MS Engine HP 5989A mass spectrometer (Hewlett-Packard). Elemental analyses, NMR spectra, and mass spectra were performed by the Institute of Organic Chemistry, University of Leipzig. Additional NMR data are available as Supporting Information.

Compounds **4**−**6** and **8** were obtained as described.[11,25]

2-Amino-1-(1-methylbenzyl)indole-3-carbonitrile (7). A solution of 6.6 g (25 mmol) of **6** and 11.4 g (50 mmol) of 2,3-dichloro-5,6-dicyanoquinone (DDQ) in 50 mL of tetrahydrofuran was refluxed for 3 h. After removing excess solvent in vacuo the residue was dissolved in 1000 mL of ethyl acetate and diethyl ether (1:1) and washed three times with 1000 mL of 0.1 M aqueous NaOH solution and finally with water. The organic phase was dried with anhydrous Na_2SO_4 and evaporated in vacuo: ^{1}H NMR (DMSO-d_6) δ 1.84 (d, 3H, CHCH_3, J = 7 Hz), 5.88 (q, 1H, CHCH$_3$, J = 7 Hz), 6.68−6.76 (m, 2H, H-7, H-6), 6.90−6.97 (m, 1H, H-5), 7.08 (s, 2H, NH_2), 7.17 (d, 1H, H-4, J = 7.7 Hz), 7.22−7.39 (m, 5H, aromatic).

2-Substituted Derivatives of 5,6-Dimethyl-7*H*-7-(1-methylbenzyl)pyrrolo[2,3-*d*]pyrimidines 9−17. General Procedure: A suspension of 4.3 g (18 mmol) of **4**, 1.9 g (36 mmol) of sodium methylate, and 18 mmol of the appropriate carbonitrile derivative (see Table 1) in 50 mL of 2-propanol was refluxed for 8 h. After cooling to about 30 °C, the mixture was diluted with 20 mL of EtOH to keep sodium methylate in solution. After cooling, the precipitated crystals were filtered off and recrystallized from 50 mL of EtOH:H_2O (1:1). Reagents, yields and selected analytical data are given in Table 1.

(*R*)-5,6-Dimethyl-7-(1-methylbenzyl)-2-(2′-pyridyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-4-amine (9): ^{1}H NMR (DMSO-d_6) δ 1.99 (d, 3H, $CHCH_3$, ^3J = 7.14 Hz), 2.08 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 6.24 (q, 1H, $CHCH_3$, ^3J = 7.14 Hz), 6.66 (s, br, NH_2), 7.17−7.39 (m, 6H, phenyl, 5′-pyridyl), 7.85 (t, 1H, 4′-pyridyl, J = 7.6 Hz), 8.31−8.34 (d, 1H, 3′-pyridyl, J = 7.7 Hz), 8.62−8.63 (d, 1H, 6′-pyridyl, J = 4.0 Hz); ^{13}C NMR (DMSO-d_6) δ 10.53 (CH_3), 10.65 (CH_3), 19.25 (CHCH_3), 51.00 ($CHCH_3$), 101.57 (C-4a), 105.71 (C-5), 122.69, 123.48 (C-3′ and C-5′), 126.17, 126.89, 128.39 (aromatic CH), 129.79 (C-6), 136.44 (C-4′), 142.04 (aromatic ipso-C), 148.89 (C-6′), 150.83, 155.09, 156.32, 157.28 (C-2′, C-7a, C-4, C-2).

(*R*)-5,6-Dimethyl-7-(1-methylbenzyl)-2-(3′-pyridyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-4-amine (10): ^{1}H NMR ($CDCl_3$) δ 2.03 (d, 3H, $CHCH_3$, ^3J = 7.14 Hz), 2.09 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 5.16 (s, 2H, NH_2), 6.34 (q, 1H, $CHCH_3$, ^3J = 7.14 Hz), 7.22−7.34 (m, 6H, aromatic and pyridyl-H), 8.59 (dd, 1H, pyridyl-H, J = 1.6 and 3.3 Hz), 8.64 (dt, 1H, pyridyl-H, J = 8.0 Hz), 9.60 (d, 1H, pyridyl-H, J = 1.4 Hz).

(*R*)-5,6-Dimethyl-7-(1-methylbenzyl)-2-(4′-pyridyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-4-amine (11): ^{1}H NMR ($CDCl_3$) δ 2.05 (d, 3H, $CHCH_3$, $^3J_{H,H}$ = 7.2 Hz), 2.06 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 5.22 (s, br, 2H, NH_2), 6.37 (q, 1H, $CHCH_3$, ^3J = 7.2 Hz), 7.22−7.31 (m, 5H, phenyl), 8.27 (d, 2H, pyridyl, J = 6.0 Hz), 8.66 (d, 2H, pyridyl, J = 5.8 Hz).

(*R*)-5,6-Dimethyl-7-(1-methylbenzyl)-2-(4-pyridyl-*N*-oxide)-7*H*-pyrrolo[2,3-*d*]pyrimidine-4-amine (12): ^{1}H NMR ($CDCl_3$) δ 2.00 (d, 3H, $CHCH_3$, ^3J = 7.14 Hz), 2.09 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 5.13 (s, 2H, NH_2), 6.33 (q, 1H, CHCH$_3$, ^3J = 7.14 Hz), 7.18−7.31 (m, 5H, aromatic), 8.21 (d, 2H, pyridyl-H, J = 7.2 Hz), 8.30 (d, 2H, pyridyl-H, J = 7.2 Hz).

(*R*)-5,6-Dimethyl-7-(1-methylbenzyl)-2-(2′-pyrazinyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-4-amine (13): ^{1}H NMR ($CDCl_3$) δ 2.03 (d, 3H, $CHCH_3$, ^3J = 7.41 Hz), 2.10 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 5.38 (s, 2H, NH_2), 6.34 (q, 1H, CHCH$_3$, ^3J = 7.41 Hz), 7.19−7.30 (m, 5H, aromatic), 8.52−8.70 (m, 2H, pyrazinyl-H), 9.66−9.68 (q, 1H, pyrazinyl-H).

(*R*)-5,6-Dimethyl-7-(1-methylbenzyl)-2-(2′-thienyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-4-amine (14): ^{1}H NMR (DMSO-d_6) δ 1.99 (d, 3H, $CHCH_3$, ^3J = 7.35 Hz), 2.09 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 6.04 (q, 1H, CHCH$_3$, ^3J = 7.35 Hz), 6.47 (s, 2H, NH_2), 7.08 (dd, 1H, 4′-thienyl, ^3J = 4.95 and 3.51 Hz), 7.24−7.29 (m, 5H, phenyl), 7.49 (d, 1H, thienyl, ^3J = 4.95 Hz), 7.73 (d, 1H, thienyl, ^3J = 3.51 Hz).

(*R*)-5,6-Dimethyl-2-(2′-furyl)-7-(1-methylbenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-4-amine (15): ^{1}H NMR (DMSO-d_6) δ 1.95 (d, 3H, $CHCH_3$, ^3J = 7.1 Hz), 2.03 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 6.16 (q, 1H, CHCH$_3$), 6.51 (s, br, NH_2), 6.57 (s, 1H, furyl, ^3J = 7.1 Hz), 7.00 (d, 1H, furyl, ^3J = 2.9 Hz), 7.16−7.32 (m, 5H, phenyl), 7.72 (s, 1H, furyl).

(*R*)-5,6-Dimethyl-2-(2-methoxyphenyl)-7-(1-methylbenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-4-amine (16): ^{1}H NMR (DMSO-d_6) δ 1.94 (d, 3H, $CHCH_3$, ^3J = 7.14 Hz), 2.08 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 3.71 (s, 3H, OCH_3), 6.07 (q, 1H, $CHCH_3$, ^3J = 7.14 Hz), 6.39 (s, 2H, NH_2), 6.96 (t, 1H, J = 7.4 Hz), 7.04 (d, 1H, J = 8.3 Hz), 7.21−7.30 (m, 5H, aromatic), 7.40 (dd, 2H, aromatic, J = 1.6 and 5.9 Hz).

5,6-Dimethyl-2(*R,S*)-(1-methylbenzyl)-7(*R*)-(1-methylbenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-4-amine (17): ^{1}H NMR ($CDCl_3$) δ 1.70 (d, 2 × 3H, CHC*H*$_3$, J = 7.14 Hz), 2.04 (d, 3H, CHC*H*$_3$, J = 7.14 Hz), 2.07 (d, 3H, CHC*H*$_3$, J = 7.14 Hz), 2.10 (s, 3H, CH_3), 2.14 (s, 3H, CH_3), 2.26 (s, 2 × 3H, CH_3), 4.2 (m, 2 × 1H, C*H*CH_3, J = 7.14 Hz), 5.32 (s, 2 × 2H, NH_2), 6.11 (q, 1H, $CHCH_3$, J = 7.14 Hz), 6.20 (q, 1H, $CHCH_3$, J = 7.14 Hz), 7.24−7.30 (m, 20 H, aromatic).

2-Substituted Derivatives of 5,6,7,8-Tetrahydro-9-phenyl-9*H*-pyrimido[4,5-*b*]indoles 18−20. General Procedure: A suspension of 2.4 g (10 mmol) of **5**, 1.0 g (20 mmol) of sodium methylate, and 10 mmol of the appropriate carbonitrile derivative (see Table 1) in 20 mL of 2-propanol was refluxed for 6 h. After cooling to about 30 °C, the mixture was diluted with 10 mL of EtOH to keep sodium methylate in solution. After cooling, the precipitated crystals were filtered off and recrystallized from 100 mL of EtOH. Reagents, yields and selected analytical data are given in Table 1.

(*R*)-5,6,7,8-Tetrahydro-9-phenyl-2-(2′-pyridyl)-9*H*-pyrimido[4,5-*b*]indole-4-amine (18): ^{1}H NMR (MeOD) δ 1.81−1.91 (m, 4H, CH_2CH_2), 2.49−2.50 (d, 2H, CH_2, J = 5.2 Hz), 2.87−2.89 (d, 2H, CH_2, J = 5.6 Hz), 7.40−7.42 (m, 1H, 5′-pyridyl), 7.50−7.57 (m, 5H, phenyl), 7.87 (dt, 1H, 4′-pyridyl, J = 1.2 and 7.1 Hz), 8.05 (d, 1H, 3′-pyridyl, J = 7.95 Hz), 8.66 (d, 1H, 6′-pyridyl, J = 4.32 Hz).

(*R*)-5,6,7,8-Tetrahydro-9-phenyl-2-(3′-pyridyl)-9*H*-pyrimido[4,5-*b*]indole-4-amine (19): ^{1}H NMR (MeOD) δ 0.50 (m, 4H, CH_2−CH_2), 1.16 (t, 2H, CH_2, ^3J = 5.3 Hz), 1.52 (t, 2H, CH_2, ^3J = 5.6 Hz), 6.01−6.16 (m, 5H, aromatic), 6.27 (m, 1H, 5′-pyridyl), 7.22 (d, 1H, 6′-pyridyl, $^3J_{5',6'}$ = 4.2 Hz), 7.39 (d, 1H, 4′-pyridyl, $^3J_{4',5'}$ = 8.0 Hz), 7.91 (s, 1H, 2′-pyridyl).

(*R*)-5,6,7,8-Tetrahydro-9-phenyl-2-(4′-pyridyl)-9*H*-pyrimido[4,5-*b*]indole-4-amine (20): ^{1}H NMR ($CDCl_3$) δ 1.88−1.95 (m, 4H, CH_2), 2.63 (t, 2H, CH_2, ^3J = 6.0 Hz), 2.95 (t, 2H, CH_2, ^3J = 6.0 Hz), 5.13 (s, 2H, NH_2), 7.45−7.55 (m, 5H, aromatic), 8.18 (dd, 2H, pyridyl-H, J = 1.4 and 3.2 Hz), 8.62 (dd, 2H, pyridyl-H, J = 1.1 and 4.7 Hz).

2-Substituted Derivatives of 5,6,7,8-Tetrahydro-9-(1-methylbenzyl)-9*H*-pyrimido[4,5-*b*]indoles 21−24. General Procedure: A suspension of 6.6 g (25 mmol) of **6**, 2.7 g (50 mmol) of sodium methylate, and 25 mmol of the appropriate carbonitrile derivative (see Table 1) in 50 mL of 2-propanol was refluxed for 6 h. After cooling to about 30 °C, the mixture was diluted with 20 mL of EtOH to keep sodium methylate in solution. After cooling, the precipitated crystals were filtered off and recrystallized from 50 mL of EtOH. Reagents, yields and selected analytical data are given in Table 1.

(*R*)-5,6,7,8-Tetrahydro-9-(1-methylbenzyl)-2-(2′-pyridyl)-9*H*-pyrimido[4,5-*b*]indole-4-amine (21): ^{1}H NMR (MeOD) δ 1.68−1.74 (m, 4H, CH_2−CH_2), 1.97 (d, 3H, CHC*H*$_3$, ^3J = 7.14 Hz), 2.19−2.26 (m, 1H, CH_2), 2.55−2.61 (m, 1H, CH_2), 2.87 (br, s, 2H, CH_2), 6.35 (q, 1H, C*H*CH_3, ^3J = 7.14 Hz), 7.18−7.28 (m, 5H, aromatic), 7.39−7.41 (m, 1H, 5′-pyridyl), 7.87 (m, 1H, 4′-pyridyl), 8.40 (d, 1H, 3′-pyridyl, ^3J = 8.0 Hz), 8.63 (d, 1H, 6′-pyridyl, ^3J = 4.2 Hz).

(*R*)-5,6,7,8-Tetrahydro-9-(1-methylbenzyl)-2-(3′-pyridyl)-9*H*-pyrimido[4,5-*b*]indole-4-amine (22): ^{1}H NMR (DMSO-d_6) δ 1.67−1.71 (m, 4H, CH_2−CH_2), 1.98 (d, 3H, CHC*H*$_3$, ^3J = 7.14 Hz), 2.49 (m, 2H, CH_2), 2.82 (m, 2H, CH_2), 6.05 (q, 1H, C*H*CH_3, ^3J = 7.14 Hz), 6.51 (s, 2H, NH_2), 7.20−7.29 (m, 5H, 9-phenyl), 7.44 (m, 1H, 5′-pyridyl, $^3J_{5',6'}$ = 4.65 Hz), 8.55−8.60 (m, 2H, 4′-pyridyl, 6′-pyridyl, $^3J_{5',6'}$ = 4.65 Hz), 9.4 (s, 1H, 2′-pyridyl).

(*R*)-5,6,7,8-Tetrahydro-9-(1-methylbenzyl)-2-(4′-pyridyl)-9*H*-pyrimido[4,5-*b*]indole-4-amine (23): ^{1}H NMR (MeOD) δ 1.66−1.71 (4H, CH_2−CH_2), 1.98 (d, ^3J = 7.08 Hz, 3H, CHC*H*$_3$), 2.49−2.51 (m, 2H, CH_2), 2.83 (br, s, 2H, CH_2), 6.07 (q, 1H, ^3J = 7.14 Hz, C*H*CH_3), 6.56 (s, 2H, NH_2), 7.20−7.29 (m, 5H, 9-phenyl), 8.22 (dd, 2H, pyridyl), 8.70 (t, 2H, pyridyl).

(*R*)-5,6,7,8-Tetrahydro-9-(1-methylbenzyl)-2-(4′-pyridyl-*N*-oxide)-9*H*-pyrimido[4,5-*b*]indole-4-amine (24): ^{1}H NMR (DMSO-d_6) δ 1.63−1.72 (m, 4H, CH_2−CH_2), 1.97 (d, 3H, $CHCH_3$, ^3J = 7.14 Hz), 2.37 (m, 1H, CH_2), 2.55 (m, 1H, CH_2), 2.81 (m, 2H, CH_2), 6.06 (q, 1H, $CHCH_3$, ^3J = 7.14 Hz), 6.52 (s, 2H, NH_2), 7.24−7.33 (m, 5H, aromatic), 8.19−8.30 (m, 4H, pyridyl-H).

2-Substituted Derivatives of (*R*)-9-(1-Methylbenzyl)-9*H*-pyrimido[4,5-*b*]indole-4-amines 25−28. General Procedure: A suspension of 2.4 g (10 mmol) of **7**, 1.1 g (20 mmol) of sodium methylate, and 10 mmol of the appropriate carbonitrile derivative (see Table 1) in 20 mL of 2-propanol was refluxed for 8 h. After cooling to about 30 °C, the mixture was diluted with 10 mL of EtOH to keep sodium methylate in solution. After cooling, the precipitated crystals were filtered off and recrystallized from 50 mL of EtOH:H_2O (1:1). Reagents, yields and selected analytical data are given in Table 1.

(*R*)-9-(1-Methylbenzyl)-2-(2′-pyridyl)-9*H*-pyrimido[4,5-*b*]indole-4-amine (25): ^{1}H NMR (DMSO-d_6) δ 2.09 (d, 3H, $CHCH_3$, ^3J = 7.14 Hz), 6.74 (q, 1H, $CHCH_3$, ^3J = 7.14 Hz), 7.26−7.37 (m, 9H, aromatic), 7.81−7.84 (m, 2H, pyridyl-H), 8.63 (d, 1H, pyridyl-H, J = 7.95 Hz), 8.83 (d, 1H, pyridyl-H, J = 3.84 Hz).

(*R*)-9-(1-Methylbenzyl)-2-(3′-pyridyl)-9*H*-pyrimido[4,5-*b*]indole-4-amine (26): ^{1}H NMR (DMSO-d_6) δ 2.11 (d, 3H, $CHCH_3$, J = 7.2 Hz), 6.68 (q, 1H, $CHCH_3$, J = 7.14 Hz), 7.24−7.40 (m, 9H, aromatic CH), 7.79−7.82 (m, 1H, pyridyl-H), 8.68 (br, s, 1H, pyridyl-H), 8.79 (d, 1H, pyridyl-H, J = 7.98 Hz), 9.75 (br, s, 1H, pyridyl-H).

(*R*)-9-(1-Methylbenzyl)-2-(4′-pyridyl)-9*H*-pyrimido[4,5-*b*]indole-4-amine (27): ^{1}H NMR (MeOD) δ 2.10 (d, 3H, CHC*H*$_3$, ^3J = 7.14 Hz), 6.57 (q, 1H, C*H*CH_3, ^3J = 7.14 Hz), 7.22−7.44 (m, 9H, aromatic, indole-H), 8.32 (d, 2H, pyridyl-H, ^3J = 6.0 Hz), 8.40 (d, 1H, NH, J = 7.4 Hz), 8.72 (d, 2H, pyridyl-H, ^3J = 6.0 Hz).

(*R*)-9-(1-Methylbenzyl)-2-(thienyl)-9*H*-pyrimido[4,5-*b*]-indole-4-amine (28): ^{1}H NMR ($CDCl_3$) δ 2.10 (d, 3H, $CHCH_3$, ^3J = 7.14 Hz), 5.43 (br, s, 2H, NH_2), 6.62 (q, 1H, $CHCH_3$, ^3J = 7.14 Hz), 7.14 (dd, 1H, 4′-thienyl-H), 7.18−7.44 (m, 9H, aromatic, indole-H), 7.74 (m, 1H, thienyl-H), 8.04 (dd, 1H, thienyl-H).

(*R*)-3-[5,6-Dimethyl-7-(1-methylbenzyl)-2-pyridin-4-yl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-ylamino]acrylic Acid Methylate (29). A solution of **11** (700 mg, 2 mmol) and propiolic acid methylate (600 mg, 7 mmol) in 5 mL of glacial acetic acid was stirred at room temperature for 12 h. After evaporation in vacuo the residual oil was treated with 5 mL of acetone to yield crystalline **29**: ^{1}H NMR ($CDCl_3$) δ 2.06 (d, 3H, CH−CH_3, ^3J = 7.14 Hz), 2.20 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 3.70 (s, 3H, OCH_3), 5.19 (d, 1H, ^3J = 8.73 Hz), 6.18 (q, 1H, CH−CH_3, ^3J = 7.14 Hz), 7.25−7.32 (m, 5H, aromatic), 8.26 (d, 2H, pyridyl-H, ^3J = 5.5 Hz), 8.46 (dd, 1H, ^3J = 8.73 and 11.5 Hz), 8.67 (d, 2H, pyridyl-H, ^3J = 4.7 Hz), 10.84 (d, 1H, NH, $^3J_{cis}$ = 11.5 Hz); ^{13}C NMR ($CDCl_3$) δ 10.14, 10.67 (2 x CH_3), 19.21 (*C*H*C*H$_3$), 51.00 (*C*HCH$_3$), 52.20 (OCH_3), 91.89 (*C*HC═O), 103.68 (C-4a), 104.72 (C-5), 121.27 (C-3′ and C-5′), 126.32, 127.15, 128.46 (aromatic CH), 133.96 (C-6), 140.19, 141.42 (C-4′, aromatic ipso-C), 145.21 (*C*H−NH), 149.28, 151.66, 153.39 (C-4, C-7a, C-2), 150.04 (C-2′ and C-6′), 169.66 (C═O).

(*R,S*)-5,6-Dimethyl-2-(1-methylbenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-4-amine (30). A mixture of **17** (2.6 g, 7 mmol) in 35 g of polyphosphoric acid was stirred for 3 h at 70−80 °C. The mixture was poured into ice-cold water and sufficient, concentrated ammonia solution was subsequently added to obtain a pH value of 10. The precipitated crystals were collected by filtration, washed with water, and recrystallized from ethanol: ^{1}H NMR (DMSO-d_6) δ 1.56 (d, 3H, CHC*H*$_3$ ^3J = 7.14 Hz), 2.16 (s. 3H, CH_3), 2.21 (s, 3H, CH_3), 4.20 (q, 1H, C*H*CH_3, ^3J = 7.14 Hz), 6.95 (s, 2H, NH_2), 7.11−7.35 (m, 5H, aromatic), 11.44 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 10.12, 10.48 (2 × CH_3), 20.38 (*C*H*C*H$_3$), 45.93 (*C*HCH$_3$), 100.39 (C-4a), 104.61 (C-5), 125.97, 127.16, 127.22, 127.40, 128.04, 128.22 (aromatic C and C-6), 144.76 (aromatic ipso-C), 149.75, 155.10, 161.53 (C-2, C-4, C-7a).

(*R*)-5,6-Dimethyl-2-(2-hydroxyphenyl)-7-(1-methylbenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-4-amine (31). A solution of **16** (373 mg, 1 mmol) in CH_2Cl_2 was cooled to 0 °C. Boron tribromide (1.0 mL, 10 mmol) was added and the mixture was

stirred at 0 °C for 1 h and subsequently at room temperature for 24 h. After the mixture had cooled to −10 °C, 10 mL of water was slowly added. The mixture was then extracted three times with 20 mL of CH_2Cl_2 each, and the organic phase was dried over anhydrous Na_2SO_4 and evaporated in vacuo. The precipitated crystals were recrystallized from 10 mL of ethanol: ^{1}H NMR ($CDCl_3$) δ 2.02 (d, 3H, $CHCH_3$, ^3J = 7.3 Hz), 2.11 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 5.14 (s, 2H, NH_2), 6.21 (q, 1H, C*H*CH_3, ^3J = 7.3 Hz), 6.89−6.97 (m, 2H, aromatic), 7.22−7.32 (m, 7H, aromatic), 8.44 (dd, 1H, aromatic, J = 1.6 and 6.3 Hz), 13.81 (br, s, 1H, OH); ^{13}C NMR ($CDCl_3$) δ 10.98 (2 × CH_3), 19.15 (CH*C*H_3), 51.75 (*C*HCH_3), 101.45 (C-4a), 105.87 (C-5), 117.34, 118.56 (aromatic CH), 120.34 (C-2′), 126.37, 127.24, 128.60, 128.76 (aromatic CH), 130.59 (C-6), 131.21 (aromatic CH), 141.38 (aromatic ipso-C), 155.02, 156.89, 159.75 (C-7a, C-4, C-2).

Derivatives of (*R*)-2-Benzamido-4,5-dimethyl-1-(1-methylbenzyl)-1*H*-pyrrole-3-carbonitrile (32 and 33). General Procedure: To an ice-cold solution of 30 mmol of **4** in 20 mL of CH_2Cl_2 was added 5 mL of pyridine followed by the addition of 32 mmol of the appropriate benzoyl chloride derivative. After 1 h of stirring the mixture in an ice bath, 10 mL of petroleum ether (bp 40−60 °C) was added to complete the precipitation of the product. The precipitate was collected by filtration and recrystallized from EtOH/H_2O.

2-Benzamido-1-cyclopentyl-4,5-dimethyl-1*H*-pyrrole-3-carbonitrile (34). To an ice-cold solution of 12.5 mmol of **8** in 10 mL of CH_2Cl_2 was added 2.5 mL of pyridine followed by the addition of 1.6 mL (12.5 mmol) of benzoyl chloride. After 1 h of stirring the mixture in an ice bath, 5 mL of petroleum ether (bp 40−60 °C) was added. The resulting hygroscopic residue was purified by flash chromatography. The product was eluted with petroleum ether:ethyl acetate (3:1). After evaporation of the solvent, the precipitate was recrystallized from ethyl acetate. Reagents, yields and some analytical data are given in Table 1: IR (KBr) ν 3216, 2214, 1644, 1510, 1486, 1456, 1394, 1302, 1288, 1246; ^{1}H NMR (DMSO-d_6) δ 1.51−1.55 (m, 2H, CH_2), 1.68−1.70 (m, 2H, CH_2), 1.91−2.00 (m, 4H, CH_2), 2.03 (s, 3H, CH_3), 2.19 (s, 3H, CH_3), 4.55 (quint, 1H, CH), 7.54−7.64 (m, 3H, phenyl-H), 8.00 (d, 2H, phenyl-H, J = 7.0 Hz), 10.18 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 9.77, 10.53 (CH_3), 24.90, 30.85 (CH_2), 55.69 (CH), 90.41 (C-3), 113.24, 115.77 (C-4, CN), 124.26 (C-5), 127.72, 128.60, 132.26 (aromatic CH), 130.63 (C-2), 132.85 (aromatic ipso-C), 167.01 (C=O).

Derivatives of 7*H*-Pyrrolo[2,3-*d*]pyrimdin-4(3*H*)-one (35−37). General Procedure: H_2O (4.5 g, 250 mmol) was added dropwise to a mixture of 22.7 g (160 mmol) of phosphorus pentoxide and 20.2 g (160 mmol) of *N*,*N*-dimethylcyclohexylamine with stirring and ice cooling. The viscous mixture was heated (to ca. 220 °C) until a homogeneous solution was obtained. After cooling to 190 °C, 8 mmol of the appropriate 2-benzamido-4,5-dimethyl-1-1*H*-pyrrole-3-carbonitrile (**32**−**34**) was added with stirring. The temperature was kept at 190−200 °C for 4 h. After allowing to cool to 90 °C, a pH value of 12 was adjusted by the addition of 2 N NaOH (ca. 150 mL), to obtain a separated amine phase. The precipitate was collected by filtration, washed with H_2O and subsequently with acetone, and recrystallized from 800 mL of EtOH. Reagents, yields and some analytical data are given in Table 1.

7-Cyclopentyl-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3-*d*]-pyrimidin-4(3*H*)-one (37): IR (KBr) ν 3434, 3144, 3060, 2954, 2868, 1660, 1550, 1522, 1394, 692, 528; ^{1}H NMR (DMSO-d_6) δ 1.67 (m, 2H, CH_2), 2.02 (m, 4H, CH_2−CH_2), 2.25 (s, br, 5H, CH_3 + CH_2), 2.26 (s, 3H, CH_3), 4.81 (quint, 1H, CH), 7.50 (m, 3H, phenyl-H), 8.10 (m, 2H, phenyl-H), 11.84 (s, br, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 9.80, 10.00 (CH_3), 24.94, 31.18 (CH_2), 55.17 (*C*H), 105.61 (C-4a), 108.93 (C-5), 127.05, 130.38 (C-6 + aromatic ipso-C), 128.49, 128.56 (aromatic CH), 133.18 (aromatic CH), 146.34, 148.40 (C-7a, C-2), 159.65 (C=O).

4-Chloro-Substituted 5,6-Dimethyl-2-phenyl-7-(1-methylbenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidines 38 and 39. General Procedure: A mixture of 8 mmol of the appropriate 7*H*-pyrrolo[2,3-*d*]pyrimidin-4(*3H*)-one (**35** or **36**) and 20 mL of $POCl_3$ was refluxed for 1 h. Excess reagent was removed in vacuo. The residue was poured on water, collected by filtration, washed until neutral reaction, and recrystallized from 500 mL of acetone or 750 mL of ethanol, respectively.

(*R*)-4-Chloro-2-phenyl-5,6-dimethyl-7-(1-methylbenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (38): IR (KBr) ν 3440, 2940, 2344, 1654, 1600, 1560, 1530, 1452, 1424, 1170, 1042; ^{1}H NMR ($CDCl_3$) δ 2.08 (d, 3H, $CHCH_3$, $^3J_{H,H}$ = 7.2 Hz), 2.16 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 6.40 (q, 1H, C*H*CH_3, $^3J_{H,H}$ = 7.2 Hz), 7.23−7.47 (m, 8H, phenyl-H), 8.48−8.50 (d, 2H, phenyl-H); ^{13}C NMR ($CDCl_3$) δ 10.14 (CH_3), 11.44 (CH_3), 19.29 (CH*C*H_3), 52.14 (*C*HCH_3), 107.19 (C-4a), 114.71 (C-5), 126.47, 127.42, 128.02, 128.38, 128.66 (aromatic CH), 129.77 (C-6), 135.40, 141.20 (aromatic ipso-C), 150.63, 152.60, 156.16 (C-7a, C-4, C-2).

(*R*)-4-Chloro-2-(4-chlorophenyl)-5,6-dimethyl-7-(1-methylbenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (39): IR (KBr) ν 2924, 1664, 1526, 1452, 1428, 1392, 1382, 1090, 842, 786, 702; ^{1}H NMR ($CDCl_3$) δ 2.06 (d, 3H, $CHCH_3$, $^3J_{H,H}$ = 7.35 Hz), 2.16 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 6.37 (q, 1H, $CHCH_3$, $^3J_{H,H}$ = 7.35 Hz), 7.21−7.33 (m, 5H, phenyl-H), 7.40−7.46 (m, 2H, phenyl-H), 8.42 (d, 2H,phenyl-H, J = 8.7 Hz); ^{13}C NMR ($CDCl_3$) δ 10.12 (CH_3), 11.45 (CH_3), 19.26 ($CHCH_3$), 52.14 ($CHCH_3$), 107.32 (C-4a), 114.83 (C-5), 126.43, 127.46, 128.57, 128.68, 129.31 (aromatic CH), 135.70, 135.81, 136.38 (C-6, ipso- und para-C), 141.07 (aromatic ipso-C), 150.62, 152.44, 155.08 (C-7a, C-4, C-2).

4-Chloro-7-cyclopentyl-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (40). A mixture of 2 mmol (615 mg) of **37** and 3 mL of $POCl_3$ was refluxed for 1 h. Excess reagent was removed in vacuo. The residue was poured on water, collected by filtration, washed until neutral reaction, and recrystallized from 500 mL of acetone: IR (KBr) ν 2956, 2870, 1602, 1530, 1472, 1452, 1440, 1424, 1400, 1198, 702; ^{1}H NMR (DMSO-d_6) δ 1.74 (m, 2H, CH_2), 2.10 (m, 4H, CH_2−CH_2), 2.38 (s, 3H, CH_3), 2.42 (s + m, 3H, CH_3, 2H, CH_2), 4.93 (quint, 1H, CH), 7.50 (m, 3H, phenyl-H), 8.34 (d, 2H, phenyl-H, J = 6.57 Hz); ^{13}C NMR (DMSO-d_6) δ 9.91, 10.54 (CH_3), 24.98, 30.56 (CH_2), 55.74 (CH), 104.75 (C-4a), 114.70 (C-5), 127.28, 128.60, 129.92 (aromatic CH), 137.26, 137.31 (C-6, aromatic ipso-C), 149.36, 151.08, 154.29 (C-7a, C-4, C-2).

(*R*,*R*)-5,6-Dimethyl-7-(1-methylbenzyl)-2-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-4-(1-methylbenzyl)amine (41). A solution of 1.1 g (3 mmol) of **38** and 3.6 g (30 mmol) of *(R)*-1-methylbenzylamine in 20 mL of ethanol was refluxed for 70 h. After cooling, the precipitated crystals were collected by filtration and recrystallized from ethanol: IR (KBr) ν 3448, 1592, 1572, 1458, 1444, 1426, 1408, 1388, 1374, 766, 700; ^{1}H NMR (DMSO-d_6) δ 1.63 (d, 3H, NH−CH−CH_3, $^3J_{H,H}$ = 6.9 Hz), 2.00 (d, 3H, $CHCH_3$, $^3J_{H,H}$ = 7.1 Hz), 2.11 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 5.63 (m, 1H, NH−CH−CH_3, J = 6.9 and 7.4 Hz), 6.16 (q, 1H, CH−CH_3, $^3J_{H,H}$ = 7.1 Hz), 6.35 (d, 1H, NH, $^3J_{H,H}$ = 7.4 Hz), 7.21−7.39 (m, 11H, phenyl-H), 7.55 (d, 2H, phenyl-H, J = 7.38 Hz), 8.25 (dd, 2H, phenyl-H, J = 1.6 and 6.4 Hz); ^{13}C NMR (DMSO-d_6) δ 10.53 (CH_3), 10.87 (CH_3), 19.33 (CH*C*H_3), 22.69 (CH*C*H_3), 49.58 (*C*HCH_3), 51.30 (*C*HCH_3), 101.40 (C-4a), 105.14 (C-5), 126.12, 126.23, 126.31, 126.87, 127.16, 127.99, 128.11, 128.35, 128.84 (aromatic CH), 129.18 (C-6), 139.19, 142.17, 145.93 (aromatic ipso-C), 150.58, 154.64, 155.08 (C-7a, C-4, C-2).

(*R*)-5,6-Dimethyl-7-(1-methylbenzyl)-2-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-4-cyclopentylamine (42). A solution of 543 mg (1.5 mmol) of **38** in 1.48 mL (15 mmol) of cyclopentylamine was refluxed for 5 h. After cooling, the precipitated crystals were collected by filtration and recrystallized from ethanol: IR (KBr) ν 3456, 2942, 1592, 1574, 1544, 1496, 1458, 1442, 1428, 774, 704; ^{1}H NMR (DMSO-d_6) δ 1.63−1.64 (m, 6H, cyclopentyl-H), 2.00−2.03 (d, 3H, $CHCH_3$, $^3J_{H,H}$ = 7.2 Hz), 2.10 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 2.50 (m, 2H, CH_2), 4.63 (q, 1H, CH-cyclopentyl), 5.86 (d, 1H, NH, J = 6.6 Hz), 6.17 (q, 1H, CH−CH_3, $^3J_{H,H}$ = 7.2 Hz), 7.20−7.43 (m, 8H, phenyl-H), 8.35−8.38 (d, 2H, phenyl-H); ^{13}C NMR (DMSO-d_6) δ 10.51 (CH_3), 10.71 (CH_3), 19.35 (CH*C*H_3), 23.67 (*C*H_2CH_2), 32.44 (*C*H_2-CH*C*H_2), 51.26 (*C*HCH_3), 52.19 (CH_2*C*HCH_2), 101.46 (C-4a), 105.09 (C-5), 126.22, 126.84, 127.13, 128.05, 128.34, 128.83

(aromatic CH), 128.89 (C-6), 139.37, 142.24 (aromatic ipso-C), 150.42, 154.81, 155.88 (C-7a, C-4, C-2).

(*R*)-*N*¹-[5,6-Dimethyl-7-(1-methylbenzyl)-2-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]ethanediamine (43). A solution of 732 mg (1.5 mmol) of **38** in 5.0 mL of ethylenediamine was refluxed for 6 h. After cooling, 20 mL of an ice-cold mixture of water and ethanol was added. The precipitated crystals were collected by filtration and recrystallized from 200 mL of ethanol: IR ν 3396, 1592, 1576, 1542, 1496, 1444, 1426, 1382, 1324, 700; ^{1}H NMR (DMSO-d_6) δ 2.01 (d, 3H, $CHCH_3$, $^3J_{H,H}$ = 7.14 Hz), 2.09 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 2.89 (t, 2H, CH_2, J = 5.7 Hz), 3.63 (q, 2H, CH_2, J = 5.7 Hz), 6.16 (q, 1H, $CHCH_3$, $^3J_{H,H}$ = 7.14 Hz), 6.49 (s, 1H, NH), 7.21−7.42 (m, 9H, Phenyl-H), 8.37 (d, 2H, NH_2, J = 7.4 Hz); ^{13}C NMR (DMSO-d_6) δ 10.50 (CH_3), 10.73 (CH_3), 19.35 ($CHCH_3$), 40.92 (CH_2), 43.08 (CH_2), 51.25 ($CHCH_3$), 101.36 (C-4a), 105.11 (C-5), 126.22, 126.84, 127.18 (aromatic CH), 127.99 (C-6), 128.03, 128.34, 128.83 (aromatic CH), 139.33, 142.25 (aromatic ipso-C), 150.39, 154.84, 156.09 (C-7a, C-4, C-2).

(*R,S*)-2-[4-Chlorophenyl-5,6-dimethyl-7(*R*)-(1-methylbenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-ylamino]butan-1-ol (44). A solution of 1.2 g (3.0 mmol) of **39** in 10 mL of freshly distilled 2-aminobutanol was refluxed for 7 h. The resulting oil was mixed with silica gel and the mixture was put on a silica gel column. After purification by column chromatography with petroleum ether:ethyl acetate (2:1), the precipitate was recrystallized from 200 mL of methanol: IR (KBr) ν 3428, 2938, 1660, 1600, 1486, 1458, 1090, 1064, 728, 696, 540; ^{1}H NMR ($CDCl_3$) δ 0.96 (t, 3H, CH_2CH_3, $^3J_{H,H}$ = 7.4 Hz), 1.31 (m, 1H, CH_2, J = 6.9 Hz), 1.47 (m, 1H, CH_2, J = 7.0 Hz), 2.03 (d, 3H, $CHCH_3$, J = 7.14 Hz), 2.06 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 2.77 (m, br, 1H, CH, J = 4.11 Hz), 3.30 (dq, 1H, CH_2OH, J = 8.0 Hz), 3.61 (dd, 1H, CH_2OH, J = 3.7 + 6.8 Hz), 6.28 (q, 1H, $CHCH_3$, 7.14 Hz), 7.20−7.32 (m, 5H, phenyl-H), 7.44 (d, 2H, pCl-phenyl-H, J = 8.5 Hz), 8.31 (d, 2H, pCl-phenyl-H, J = 8.5 Hz); ^{13}C NMR ($CDCl_3$) δ 9.86 (CH_2CH_3), 10.49 (CH_3), 10.96 (CH_3), 19.42 ($CHCH_3$), 27.33 (CH_2CH_3), 51.83 ($CHCH_3$), 54.53 (CH), 66.43 (CH_2OH), 105.56 (C-4a), 111.60 (C-5), 126.41, 127.20, 128.55, 128.75, 128.81 (aromatic CH), 129.27, 131.79 (C-6, para-Cl−C), 136.65, 141.57 (aromatic ipso C), 148.07, 148.22, 161.74 (C-7a, C-4, C-2).

(*R,S*)-2-[7-Cyclopentyl-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-ylamino]butan-1-ol (45). A solution of 326 mg (1.0 mmol) of **40** in ca. 5 mL of freshly distilled 2-aminobutanol was refluxed for 1 h. The resulting oil was mixed with silica gel and the mixture was put on a silica gel column. After purification by column chromatography with petroleum ether:ethyl acetate (2:1), the resulting oil was lyophilized: IR (KBr) ν 3452, 2960, 2866, 1594, 1574, 1542, 1460, 1446, 1428, 1412, 1378, 1324, 774, 702; ^{1}H NMR ($CDCl_3$) δ 1.09 (t, 3H, CH_3, J = 7.5 Hz), 1.74 (m, 4H, CH_2CH_2), 2.02−2.17 (m, 2H, CH_2), 2.33 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 2.48−2.52 (m, 2H, CH_2), 3.72 (dd, 1H, CH_2−O, J = 2.5 + 8.0 Hz), 3.94 (dd, 1H, CH_2−O, J = 2.3und 8.2 Hz), 4.29 (m, 1H, CH_2CH*CH_2), 4.83 (quint, 1H, CH, J = 8.7 Hz), 5.16 (d, 1H, NH, J = 5.8 Hz), 7.38−7.48 (m, 3H, phenyl-H), 8.36 (d, 2H, phenyl-H, J = 7.14 Hz); ^{13}C NMR ($CDCl_3$) δ 10.56 (CH_2CH_3), 11.01 (CH_3), 11.37 (CH_3), 25.13 (CH_2CH_3), 25.49 (2 × CH_2), 31.14 (CH_2), 31.25 (CH_2), 55.83 (CH), 56.10 (C*H), 68.68 (CH_2-OH), 102.19 (C-4a), 104.01 (C-5), 127.67, 128.28, 129.02 (aromatic CH), 130.01 (C-6), 139.44 (aromatic ipso-C), 150.47, 155.46, 157.13 (C-7a, C-4, C-2).

Solubility Determination. The solubility of selected compounds was determined using HPLC/UV. A 10 mM solution of the compounds in DMSO was prepared, diluted 1:100 in TRIS-HCl buffer, 50 mM, pH 7.4, and allowed to reach equilibrium by shaking overnight at room temperature. After centrifugation (1000*g*), the supernatant was filtered through cotton. Several dilutions of these saturated stock solutions were made in the buffer, on the basis of the estimated solubility. These dilutions were injected into the HPLC system and analyzed by UV detection. The solubility of the compounds was then calculated using calibration curves, previously carried out. The method was validated according to the following parameters: selectivity, precision, accuracy, linearity, range, limit of detection (LOD), and limit of quantification (LOQ). Detailed data are available as Supporting Information.

Radioligand Binding Assays. The compounds were tested in radioligand binding assays for affinity to A_1 and A_{2A} ARs in rat cortical membrane and rat striatal membrane preparations, respectively. An A_1-selective agonist radioligand, either [^{3}H]-N^6-cyclohexyladenosine (CHA, 1 nM) or [^{3}H]-2-chloro-N^6-cyclohexyladenosine (CCPA, 0.5 nM), respectively, was used as the A_1 ligand and the A_{2A}-selective agonist [^{3}H]-2-[[[[4-(carboxyethyl)phenyl]ethyl]amino]-5′-*N*-(ethylcarbonyl)amino]-adenosine (CGS21680, 5 nM) as the A_{2A} ligand, as previously described.[9b,27,28] Inhibition of receptor−radioligand binding was determined by a range of 5−7 concentrations of the compounds in triplicate in at least three separate experiments.

A_3 AR affinities to human recombinant receptors expressed in CHO cell membranes were determined as described using [^{3}H]-5′-[(*N*-(ethylcarbonyl)amino]adenosine (NECA) as the A_3 radioligand.[12]

The Cheng−Prusoff equation and K_D values of 1 nM for [^{3}H]-CHA (A_1), 0.2 nM for [^{3}H]CCPA (A_1), 14 nM for [^{3}H]CGS21680 (A_{2A}), and 6 nM for [^{3}H]NECA (A_3) were used to calculate the K_i values from IC_{50} values, determined by the nonlinear curve-fitting program Prism, version 2.0 (GraphPad, San Diego, CA).[29]

Radioligand Binding at Human Recombinant A_1 ARs. Membrane preparation: After growing CHO cells expressing human A_1 ARs, they were washed and frozen in the dishes at −80 °C.[12] For the preparation of membranes, the cells were thawed followed by scraping them off the Petri dishes using TRIS buffer (pH 7.4, 50 mM). The cell suspension was homogenized on ice (polytron, 10 s with high speed) and then centrifuged for 20 min at 4 °C and 40000*g*. After resuspending the pellet in TRIS puffer, it was washed twice and stored in aliquots at −80 °C with a protein concentration of 2.3 mg/mL.

Binding assay: Radioligand binding assays were performed essentially as described.[12,30] [^{3}H]CCPA was used as the A_1 ligand in a concentration of 0.5 nM. The protein concentration in the assays was 70−100 μg/mL. A K_d value of 0.7 nM was used to calculate K_i values.[30] The nonspecific binding amounted to 10% of the total binding.

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Supporting Information Available: IR and ^{1}H and ^{13}C NMR data of intermediate and final products synthesized; validation data of solubility determination. This material is available free of charge via the Internet at http://pubs.acs.org.

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