N^6-Substituted Adenosine Receptor Agonists. Synthesis and Pharmacological Activity as Potent Antinociceptive Agents[1]

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Novel N^6-(indol-3-yl)alkyl derivatives of adenosine were synthesized. The adenosine receptor affinity and the antinociceptive activity of these compounds were assessed in binding studies and the phenylbenzoquinone-induced writhing test. Most of these analogues exhibited a potent analgesic activity without side effects. Among them, compound **3c** (UP 202-32) bound to A_1 (K_i = 110 nM) and A_2 (K_i = 350 nM) adenosine receptors in a specific manner since it did not interact with many other receptors, especially opioid binding sites. The antinociceptive activity in the phenylbenzoquinone assay (ED_{50} = 3.3 mg/kg po) was antagonized by 8-cyclopentyltheophylline, suggesting that an adenosinergic mechanism underlies the analgesic activity observed with this compound. The data obtained with these new N^6-substituted adenosine receptor agonists emphasize the interest of such compounds in the treatment of pain.

Since the recognition of the hypotensive, sedative, antispasmodic, and vasodilatory actions of adenosine,[2] a number of adenosine analogues have been synthesized and tested. From these studies, compounds such as N^6-cyclohexyladenosine (CHA), N^6-(R)-(1-phenyl-2-propyl)-adenosine (R-PIA), and N-ethyladenosine-5′-uronamide (NECA) were issued (Figure 1). More recently, on the basis of observations that morphine enhances adenosine release from the rat cerebral cortex,[3–5] a potential role for adenosine in analgesia has been postulated.[6] The antinociceptive potency of various adenosine analogues administered intrathecally in rats and mice has been found to correlate with the affinity for A_1 receptors,[7,8] although a delayed phase associated with A_2 receptors has also been reported.[9] However a number of side effects were observed with the tested analogues (i.e., CHA, R-PIA) at the analgesic doses.[9,10]

The possibility of inducing analgesia without major side effects with such compounds might be of interest in the development of future drugs for pain treatment in humans. Our strategy to prepare new adenosine-related compounds was then based on the following observations: The structure–activity relationships already published for N^6-substituted adenosines[11] showed that the interesting area for modulation of the A_1 binding properties is the N^6 region while the activity at the A_2 receptor is largely due to the N^6-(2-phenylethyl)amino moiety. A heteroarylethyl group such as 1-substituted-3-ethylindole known for its CNS penetration properties[12] would be an interesting pharmacophore to study. We consequently prepared new N^6-[(indol-3-yl)ethyl]adenosine compounds[13] of formula **3a–at** (Figure 1) whose binding results to A_1 and A_2 receptors and antinociceptive activities are described in this paper.

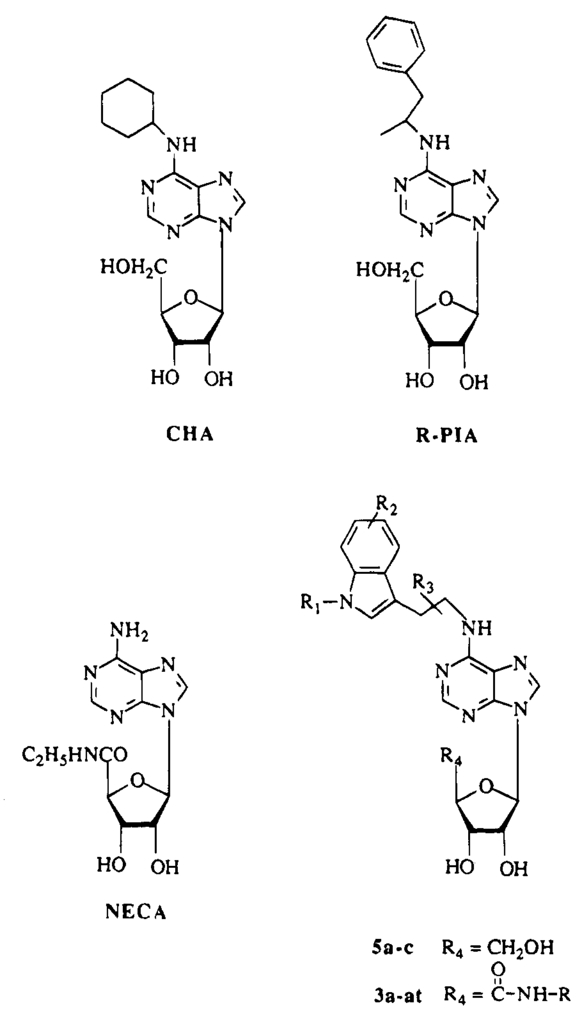

Figure 1.

Chemistry

The commercially available inosine was transformed to 6-chloroadenosine according to the known literature methods[14,15] involving acetylation of the free alcohols, treatment with $POCl_3$, and deacetylation in the presence of ammonia at low temperature. The known 2′,3′-isopropylideneadenosine-5-carboxylic acid[16–20] was converted to the corresponding acid chloride by reacting it with thionyl chloride. Reaction of the latter with

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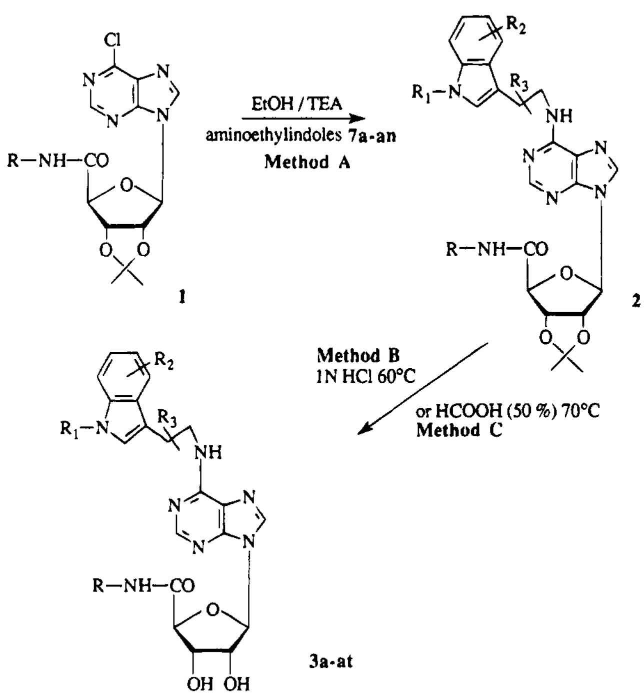
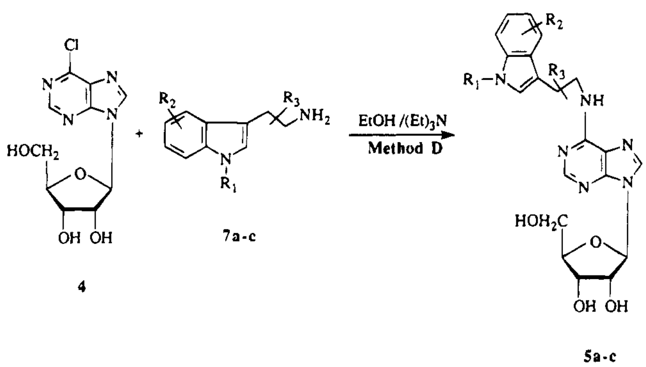
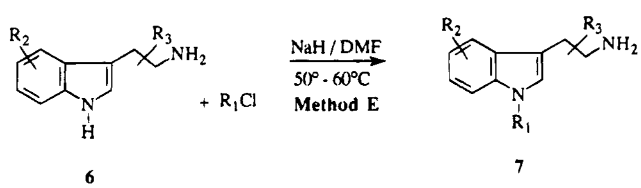
Scheme 1



Scheme 2



Scheme 3



different amines gave the desired amides **1**.[13] The title compounds **3a–at** were obtained by the reaction of 6-chloro ribofuranuronamide **1** with different substituted (aminoethyl)indoles (method A) and then by the removal of the isopropylidene group in acidic medium, 1 N HCl (method B) or HCOOH (50%) (method C), as outlined in Scheme 1. To obtain compounds **5a–c**, 1-substituted-3-(2-aminoethyl)indoles **7a–c** were reacted directly on the 6-chloroadenosine **4** according to Scheme 2 (method D). Most of the 1-substituted-3-(2-aminoethyl)indoles involved in structures **3a–at** and **5a–c** were prepared in one step by a new method[21] (method E) starting from the corresponding tryptamines **6** as shown in Scheme 3. The classical reaction scheme involving the intermediate formylindoles was also used to obtain 1-substituted-3-(2-aminoethyl)indoles as outlined in Scheme 4 (methods F–H), especially for compound **7e** which failed to give the attempted product by method E. The physical–chemical properties of the unknown compounds **7a–r** are presented in Table 4. The physical–chemical data of intermediates **9a–k** and **10a,b** obtained by methods F and G are presented in Tables 1 and 2. Compounds **6a–e** were prepared according to the literature methods[22,23] as shown in Scheme 5 (method I). The physical–chemical data of compounds **6a–e** as well as of the intermediates of method H, **13a,b**, **14a–e**, and **15a–e**, are presented in Table 3. All physical–chemical data and preparation methods for the final products **3a–at** and **5a–c** are summarized in Table 5. The intermediary compounds **2** were amorphous solids and only identified by their ^{1}H NMR spectra.

Structure–Activity Relationships

Pharmacological data of the final products **3a–at** are given in Table 6. Each of the compounds was evaluated for A_1 and A_2 adenosine receptor binding affinity and then in the antinociceptive assay.

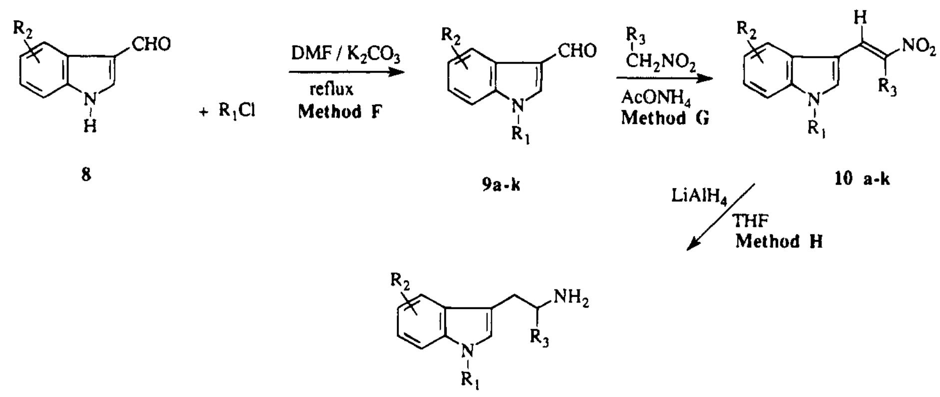
Analysis of the A_1/A_2 binding results concerning the sugar moiety showed no difference between alcohols and amides (**5a,b** vs **3a,ao**). Among the different amide functions prepared keeping the N^6-substituent constant (compounds **3a,l,t** in the one hand, **3g,aj,am,as,at** in the other hand), the highest activity was observed for R = ethyl or cyclopropyl group. Further lengthening of the R group with the introduction of a heteroatom into the side chain (compounds **3t,am**) was unpromising and not further exemplified. Concerning the N^6-substituent region, its importance in the receptor binding studies was also mentioned before.[10,11,32] We studied three levels of modulation on the indole moiety including the substitution on the indole cycle, the modulation on the ethylamino side chain, and the substitution on the nitrogen of the indole.

When one considers compounds **3g,s,w,x,al**, which are 5-substituted indoles, with the same N-substituent (5-substituents respectively H, Cl, CH_3, OCH_3, SCH_3), no significant difference was observed. However we noticed that the 2-position substituent's size may influence the activity on both A_1 and A_2 receptors (compounds **3g,y,ah**). In this region, hydrogen was the most effective substituent for A_1/A_2 binding affinities. For the 2-methyl group a slight decrease was observed, while for the 2-phenyl substituent the binding affinity decreased dramatically. One can assume that the steric hindrance caused by a phenyl group placed the indolyl moiety in an unfavorable position for binding to the receptor.

A methyl group in both α and β positions of the ethylamino side chain was tolerated. However, while the A_1 binding affinity was kept at the same level, the A_2 affinity was reduced, thereby improving the A_1 selectivity. For compound **3g**, the A_1/A_2 ratio was 4.8, while for compounds **3ae,ag**, the same ratio was 12 and 12.5, respectively.

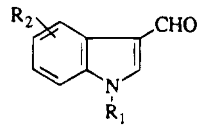
An important variation was the substitution of the indole's nitrogen. This substitution seems to be required for high affinity, and among various alkyl groups, the most efficient was the $CH_2CH_2N(CH_3)_2$ group (compounds **3h–j,ak**). Selectivity was always in favor of A_1 with a K_i value of 8.3 nM for compound **3j**. When the N-substituent contains an aromatic ring (compounds **3o–q,ai,ao**), the magnitude was phenyl ~ 2-pyridyl ~

Scheme 4



7a-l

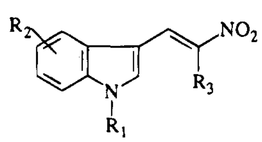
Table 1. Physical Data of Intermediates **9a–k** Obtained by Method F



compd	R_1	R_2	% yield[a]	mp (°C)[b]	recryst solvent
9a	CH_2-(4-chlorophenyl)	H	78	122	EtOH
9b	CH_2-phenyl	H	81	111	EtOH
9c	CH_2-(2,6-dichlorophenyl)	H	85	160	methoxy-ethanol
9d	$CH_2CH_2OCH_3$	H	98	oil	*c*
9e	cyclopentyl	H	60	oil	*c*
9f	isopropyl	H	90	oil[d]	*c*
9g	CH_2-(4-methylphenyl)	H	88	118	O-(iPr)$_2$[e]
9h	CH_2-(3,4-dimethylphenyl)	H	98	oil	*c*
9i	CH_2-(2,5-dimethylphenyl)	2-CH_3	57	155	*c*
9j	cyclopentyl	2-CH_3	25	oil	*f*
9k	H	2-phenyl	99	253	H_2O[e]

[a] Yields are not optimized. [b] Melting points are uncorrected. [c] Purified by column chromatography (SiO_2, 10% methanol/chloroform). [d] Crystallized on standing, mp ≤50 °C. [e] Crystallization solvent. [f] Purified by column chromatography (SiO_2, 5% methanol/chloroform).

Table 2. Physical Data of Intermediates **10a–k** Obtained by Method G



compd	R_1	R_2	R_3	% yield[a]	mp (°C)[b]	recryst solvent
10a	CH_2-(4-chlorophenyl)	H	H	86	178	CH_3NO_2[c]
10b	CH_2-phenyl	H	H	71	130	*c*
10c	CH_2-(2,6-dichlorophenyl)	H	H	86	170	*c*
10d	$CH_2CH_2OCH_3$	H	H	81	132	*c*
10e	cyclopentyl	H	H	80	oil	*d*
10f	isopropyl	H	H	98	oil	*d*
10g	CH_2-(4-methylphenyl)	H	H	91	172	*c*
10h	CH_2-(3,4-dimethylphenyl)	H	H	64	135	*c*
10i	CH_2-(2,5-dimethylphenyl)	2-CH_3	CH_3	85	160	iPrOH[e]
10j	cyclopentyl	2-CH_3	CH_3	69	oil	*d*
10k	H	2-phenyl	H	75	220	iPrOH[e]

[a,b] See corresponding footnotes in Table 1. [c] Crystallization in the reaction medium (CH_3NO_2). [d] Purified by column chromatography (SiO_2, CH_2Cl_2). [e] Crystallization solvent.

3-pyridyl > naphthyl. Different substituents on the aromatic ring were tested, especially in the case of a phenyl ring. No obvious structure–activity relationship was observed suggesting that this substitution was not critical for the binding affinity (compounds **3a,e–g,n,z,ac,ad,an,ao,aq,ar**).

Analysis of the antinociceptive activity concerning the sugar moiety showed that the amide function was better than the alcohols (**5a,b** vs **3a,ao**). The analgesic activity dropped with a ramified or bulky amide function (see **3a,l,t** on the one hand and **3g,aj,am,as,at** on the other hand). The best function for the antinociceptive effect was an ethyl- or cyclopropyl-substituted amide.

Concerning the N^6-substituent region, among the substituents on position 5 of the indole (keeping the same N-substitution), the best results were obtained with CH_3 and H (compounds **3w,g**). Compounds with 5-Cl and 5-OCH_3 (compounds **3s,x**) were less active. The potency order of the analgesic activity of compounds substituted on the 2-position of the indole was H > CH_3 > phenyl as observed in the binding studies.

The methyl group in both α and β positions of the ethylamino side chain has no considerable influence on the analgesic activity which remains very efficient (compounds **3g,ae,ag**). The slight modification of the A_1/A_2 ratio, noticed below, seemed not to interfere with the antinociceptive effect.

The substituent nature of the indole's nitrogen plays an important role in the antinociception. The ED_{50} value of the unsubstituted compound **3m** was 35 mg/kg, while the methylated compound **3ap** was 10-fold more active with an ED_{50} value of 3 mg/kg. Compounds substituted with different alkyl groups showed potent analgesic activity (ED_{50} < 2 mg/kg), except the cyclopropylmethyl-substituted compound **3ak**. Products substituted with unsaturated bond-containing groups, such as allyl or propargyl (**3u,v**), were slightly less active, while the aromatic ring-containing compounds showed high activities in the major cases. It was however noticed that some compounds (i.e., **3p**) had high analgesic activity despite their low binding activity. Concerning the benzyl group, substitutions on the 2-position were most favorable for the analgesic activity (**3ao,ar,an,ac**) irrespective of the nature of the 5′-substituent. From these data, compounds such as **3c,d,g,j,aa,ac** emerged as the most active products. The compound **3c** (UP 202-32), as an example among them, was further investigated in pharmacological studies, and the first results are given hereunder.

Scheme 5. Method I

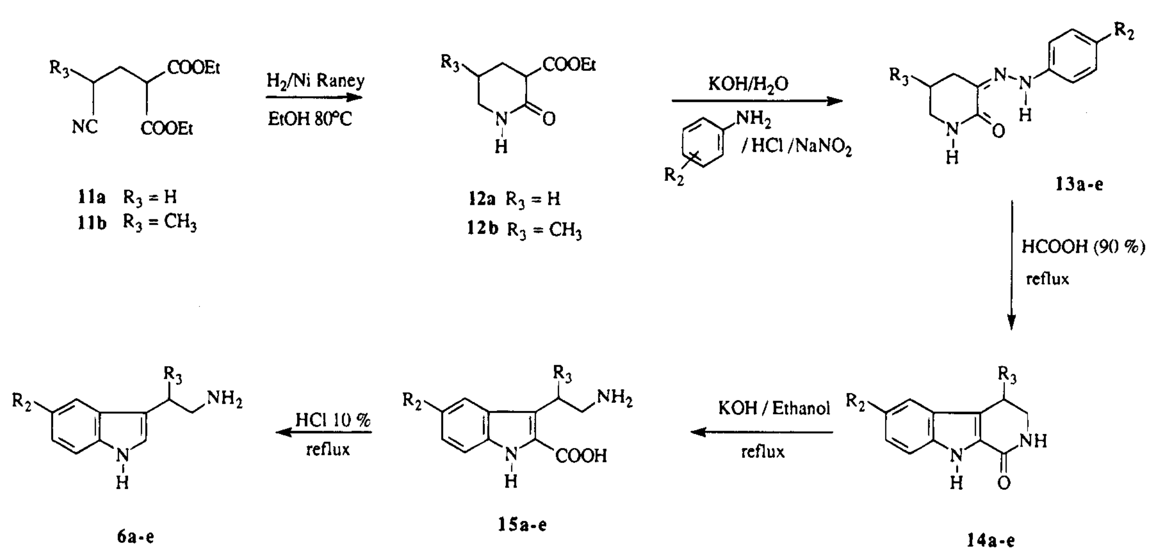


Table 3. Physical Data of Intermediates **13a–e**, **14a–e**, **15a–e**, and **6a–e** Involved in Method H

compd	R_2	R_3	% yield[a]	mp (°C)[b]	recryst solvent
13a	H	CH_3	57	231	H_2O[c]
13b	Cl	H	62	248	H_2O[c]
13c	CH_3	H	52	256	H_2O[c]
13d	OCH_3	H	29	199	H_2O[d]
13e	SCH_3	H	62	228	EtOH
14a	H	CH_3	52	175	EtOH
14b	Cl	H	87	218	H_2O[c]
14c	CH_3	H	96	175	H_2O[c]
14d	OCH_3	H	75	265	H_2O[c]
14e	SCH_3	H	59	208	CH_3CN[d]
15a	H	CH_3	≤100	255	H_2O[c]
15b	Cl	H	≤100	278[e]	H_2O[c,d]
15c	CH_3	H	≤100	254	H_2O[c]
15d	OCH_3	H	≤100	259–264	H_2O[c]
15e	SCH_3	H	≤100	257	EtOH
6a	H	CH_3	78	189[f]	iPrOH[g]
6b	Cl	H	72	288[f]	Et_2O[g]
6c	CH_3	H	64	286[f]	Et_2O[g]
6d	OCH_3	H	72	120	Et_2O[g]
6e	SCH_3	H	67	250[f]	iPrOH[g]

[a,b] See corresponding footnotes in Table 1. [c] Crystallized in the reaction medium (H_2O). [d] Purified first by column chromatography (SiO_2, 10% methanol/chloroform) and then recrystallized. [e] Lit.[22] mp 257–258 °C. [f] Melting point of the hydrochloride. [g] Crystallization solvent.

As is shown in Figure 2, adenosinergic mechanism of action of UP 202-32 was evaluated. The compound bound to A_1 and A_2 receptors and was a selective compound since it did not interact (K_i > 10 μM) with adrenergic (α_1, α_2, β), muscarinic, dopamine (D_1, D_2), serotonin (5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C}), opioid (μ, δ, κ), histamine (H_1) benzodiazepine, GABA-A, and NK_1 receptors. Furthermore, the antinociceptive effect of UP 202-32 (**3c**) observed in the phenylbenzoquinone assay (ED_{50} = 3.3 mg/kg po) appeared to be mediated by an adenosinergic mechanism since it was inhibited by 8-cyclopentyltheophylline (see Figure 2). It will be interesting to see if this compound displays effects at the newly discovered A_{2b} or A_3 adenosine receptors.

In conclusion, we synthesized new N^6-(indol-3-yl)alkyl derivatives of adenosine whose adenosine receptor affinity and marked antinociceptive properties without side effects were evidenced. These data suggest a neuromodulator role of adenosine in analgesia and emphasize the interest of such compounds in the treatment of pain.

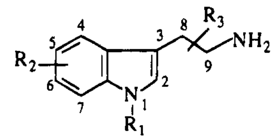
Experimental Section

Melting points were determined on an Electrothermal capillary melting point apparatus and are not corrected. The identity of all compounds was confirmed by ^1H NMR (200 MHz, Bruker AC 200 spectrometer, solvent Me_2SO-d_6 otherwise noted, TMS = 0 ppm), infrared (Perkin-Elmer 298 spectrometer), and microanalytical data (Carlo Erba, elemental analyzer, Model 1106). All reactions were followed by TLC on Merck Silica gel plates (60F-254). Merck silica gel (0.063–0.200 mm) was used for column chromatography. Unless literature references are given, the starting materials were commercially available or prepared according to the literature.[13,21] The following methods A–I are described for specific products. However, identical procedures may be applied to analogous compounds.

Method A. *β*-D-Ribofuranuronamide, 1-[6-[[2-(1-cyclopentylindol-3-yl)ethyl]amino]-9*H*-purin-9-yl]-*N*-cyclopropyl-1-deoxy-2,3-*O*-(1-methylethylidene)- (2) (R = cyclopropyl, R_1 = cyclopentyl, R_2 = R_3 = H). A mixture of 20 g (90 mmol) of 1-cyclopentyl-3-(2-aminoethyl)indole (**7e**) obtained by methods F–H, 21.8 g (58 mmol) of 2′,3′-*O*-isopropylidene-*N*-cyclopropyl-6-chloropurine-5′uronamide (**1**) (R = cyclopropyl),[13] 27 mL (190 mmol) of triethylamine, and 300 mL of ethanol was refluxed for 6 h under a nitrogen atmosphere. The ethanol was evaporated and 400 mL of dichloromethane added. This solution was washed with brine. Drying ($MgSO_4$) the organic layer and evaporation gave a brown oil purified by column chromatography (SiO_2, 5% methanol/chloroform) to yield 31.9 g (96%) of *β*-D-ribofuranuronamide, 1-[6-[[2-(1-cyclopentylindol-3-yl)ethyl]amino]-9*H*-purin-9-yl]-*N*-cyclopropyl-1-deoxy-2,3-*O*-(1-methylethylidene)- (*R* = cyclopropyl; R_1 = cyclopentyl, R_2 = R_3 = H) (**2**) as an amorphous solid. ^1H NMR ($CDCl_3$): δ 0.26 (m, 2H, CH_2 cyclopropyl), 0.67 (m, 2H, CH_2 cyclopropyl), 1.38 (s, 3H, CH_3 methylidene), 1.63 (s, 3H, CH_3 methylidene), 1.87 (m, 6H, cyclopentyl), 2.2 (m, 2H, cyclopentyl), 2.61 (m, 1H, cyclopropyl), 3.15 (t, 2H, CH_2 ethylamine, J = 7 Hz), 3.99 (m, 2H, CH_2 ethylamine), 4.69 (d, 1H, ribofuranose, J = 1.4 Hz), 4.29 (s, 1H, ribofuranose), 4.57 (s, 1H, ribofuranose), 4.77 (m, 1H, CH cyclopentyl), 5.31 (m, 2H, ribofuranose), 5.97 (d, 1H, ribofuranose, J = 3.1 Hz), 7.15 (m, 4H, 3H indole and 1H purine), 7.39 (d, 1H, indole, J = 8 Hz), 7.56 (m, 1H, NH ethylamine), 7.64 (d, 1H, indole, J = 7.8 Hz), 7.73 (s, 1H, purine), 8.37 (s, 1H, NH amide).

Method B. *β*-D-Ribofuranuronamide, 1-[6-[[2-(1-cyclopentylindol-3-yl)ethyl]amino]-9*H*-purin-9-yl]-*N*-cyclopropyl-1-deoxy- (3c). A mixture of 5.1 g (8.9 mmol) of *β*-D-ribofuranuronamide, 1-[6-[[2-(1-cyclopentylindol-3-yl)ethyl]-

Table 4. 1-Substituted-3-(2-aminoethyl)indoles **7a–r**



compd	R_1	R_2	R_3	prep methods	% yield[a]	mp (°C)[b]	recryst[c] solvent	formula
7a	CH_2-(4-chlorophenyl)	H	H	FGH	74	212	EtOH	$C_{17}H_{17}ClN_2{\cdot}HCl$
7b	CH_2-phenyl	H	H	FGH	58	178	iPrOH	$C_{17}H_{18}ClN_2{\cdot}HCl$
7c	CH_2-(2,6-dichlorophenyl)	H	H	FGH	86[e]	68	c	$C_{17}H_{16}Cl_2N_2$
7d	$CH_2CH_2OCH_3$	H	H	FGH	60	oil	c	$C_{13}H_{17}N_2O$
7e	cyclopentyl	H	H	FGH	58	oil	c	$C_{15}H_{20}N_2$
7f	isopropyl	H	H	FGH	53	oil	c	$C_{13}H_{18}N_2$
7g	CH_2-(4-methylphenyl)	H	H	FGH	85	oil	c	$C_{18}H_{20}N_2$
7h	CH_2-(3,4-dimethylphenyl)	H	H	FGH	89	oil	c	$C_{19}H_{22}N_2$
7i	CH_2-(2,5-dimethylphenyl)	2-CH_3	H	FGH	61	250	Et_2O[d]	$C_{20}H_{24}N_2{\cdot}HCl$
7j	cyclopentyl	2-CH_3	H	FGH	90	oil	c	$C_{16}H_{21}N_2$
7k	CH_2-(2,5-dimethylphenyl)	H	9-CH_3	FGH	47	87	iPr_2O[d]	$C_{16}H_{22}N_2$
7l	cyclopentyl	H	9-CH_3	FGH	85	oil	c	$C_{16}H_{22}N_2$
7m	CH_2-(2,5-dimethylphenyl)	H	8-CH_3	IE	61	178	iPrOH[d]	$C_{20}H_{24}N_2{\cdot}HCl$
7n	CH_2-(2-methoxyphenyl)	H	H	E	46	oil	c	$C_{18}H_{20}N_2O$
7o	H	2-phenyl	H	GH	73	266	iPrOH[d]	$C_{16}H_{16}N_2{\cdot}HCl$
7p	CH_3	H	H	f	53	201	iPrOH[d]	$C_{11}H_{14}N_2{\cdot}HCl$
7q	CH_2-(2-fluoro-4-bromophenyl)	H	H	E	35	oil	c	$C_{17}H_{16}BrFN_2$
7r	CH_2-(2-methylphenyl)	H	H	E	47	175	iPrOH[d]	$C_{18}H_{20}N_2{\cdot}HCl$

[a] Yields are not optimized and correspond to the recrystallized product in the final step (method H or E). [b] Melting points are uncorrected and correspond to the product for which the formula is given. [c] All compounds were purified by column chromatography on silica gel [elution with 5% isopropylamine/chloroform except for **7d** (elution with 10% isopropropylamine/chloroform) and **7n** (elution with 10% methanol/dichloromethane)] and used as such if the purity was acceptable. Further purification was achieved on salts (see formula) by recrystallization. [d] Crystallization solvent. [e] Reduction according to method H was performed in an ethyl ether (2)/THF (1) mixture instead of THF to prevent dechlorination. [f] Synthesis according to the literature method (see ref 24).

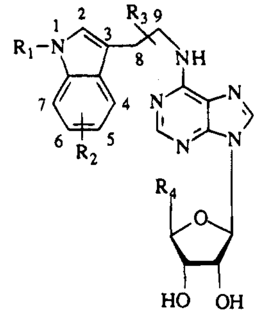
amino]-9*H*-purin-9-yl]-*N*-cyclopropyl-1-deoxy-2,3-*O*-(1-methylethylidene)- (**2**) obtained by method A and 110 mL of HCl (1 N) was heated for 3 h at 60 °C. Upon cooling, the mixture was neutralized with a saturated solution of $NaHCO_3$ and then extracted twice with ethyl acetate. The organic layers were washed with water, dried on magnesium sulfate, and concentrated to yield 4.5 g of a brown solid. Purification by chromatography (SiO_2, 5% methanol/chloroform) and crystallization in acetonitrile yielded 2.1 g (44%) of β-D-ribofuranuronamide, 1-[6-[[2-(1-cyclopentylindol-3-yl)ethyl]amino]-9*H*-purin-9-yl]-*N*-cyclopropyl-1-deoxy- (**3c**), mp 141–142 °C. ^1H NMR: δ 0.49 (m, 2H, CH_2 cyclopropyl), 0.72 (m, 2H, CH_2 cyclopropyl), 1.75 (m, 6H, CH_2 cyclopentyl), 2.1 (m, 2H, CH_2 cyclopentyl), 2.72 (m, 1H, cyclopropyl), 3.04 (m, 2H, CH_2 ethylamine), 3.79 (m, 2H, CH_2 ethylamine), 4.14 (m, 1H, ribofuranose), 4.29 (s, 1H, ribofuranose), 4.57 (s, 1H, ribofuranose), 4.82 (m, 1H, CH cyclopentyl), 5.58 (m, 1H, OH ribofuranose), 5.78 (m, 1H, OH ribofuranose), 5.95 (d, 1H, ribofuranose, J = 7.5 Hz), 7.07 (m, 2H, indole), 7.3 (s, 1H, H_2 indole), 7.45 (d, 1H, indole, J = 8 Hz), 7.63 (d, 1H, indole, J = 7.2 Hz), 8.15 (m, 1H, NH ethylamine), 8.28 (s, 1H, purine), 8.41 (s, 1H, purine), 8.96 (d, 1H, NH amide, J = 3.7 Hz).

Method C. β-D-Ribofuranuronamide, *N*-cyclopropyl-1-deoxy-1-[6-[[2-[1-(2,5-dimethylbenzyl)indol-3-yl]ethyl]-amino]-9*H*-purin-9-yl]- (3g). A mixture of 19.6 g (31 mmol) of β-D-ribofuranuronamide, *N*-cyclopropyl-1-deoxy-1-[6-[[2-[1-(2,5-dimethylbenzyl)indol-3-yl]ethyl]amino]-9*H*-purin-9-yl]-2,3-*O*-(1-methylethylidene)- obtained by method A and 590 mL of formic acid (50%) was heated for 1.25 h at 70 °C. The excess of formic acid was removed, water was added, and the mixture was concentrated in vacuo. Methanol was then added and the mixture concentrated. The white solid obtained upon triturating in water was filtered and purified by column chromatography (SiO_2, 10% methanol/chloroform). Crystallization in a mixture of methanol/ethyl ether yielded 12.8 g (69%) of the expected β-D-ribofuranuronamide, *N*-cyclopropyl-1-deoxy-1-[6-[[2-[1-(2,5-dimethylbenzyl)indol-3-yl]ethyl]amino]-9*H*-purin-9-yl]- (**3g**) as a monohydrate, mp 130–132 °C. ^1H NMR: δ 0.51 (m, 2H, CH_2 cyclopropyl), 0.72 (m, 2H, CH_2 cyclopropyl), 2.11 (s, 3H, CH_3), 2.17 (s, 3H, CH_3), 2.72 (m, 1H, CH cyclopropyl), 3.05 (t, 2H, CH_2 ethylamine, J = 6.5 Hz), 3.79 (m, 2H, CH_2 ethylamine), 4.15 (m, 1H, ribofuranose), 4.30 (s, 1H, ribofuranose), 4.60 (m, 1H, ribofuranose), 5.28 (s, 2H, CH_2 benzyl), 5.58 (d, 1H, OH ribofuranose, J = 5.9 Hz), 5.79 (d, 1H, OH ribofuranose, J = 3.7 Hz), 5.97 (d, 1H, ribofuranose, J = 7.5 Hz), 6.58 (s, 1H, benzyl), 7.1 (m, 5H, indole and benzyl), 7.36 (d, 1H, indole, J = 7.6 Hz), 7.68 (d, 1H, indole, J = 7 Hz), 8.09 (m, 1H, NH ethylamine), 8.27 (s, 1H, purine), 8.41 (s, 1H, purine), 8.97 (d, 1H, NH amide, J = 3.6 Hz).

Method D. N^6-[2-(1-Benzylindol-3-yl)ethyl]adenosine (5b). A mixture of 8 g (28 mmol) of 1-benzyl-3-(2-aminoethyl)-indole prepared according to methods F–H, 200 mL of ethanol, 4.2 g (42 mmol) of triethylamine, and 4 g (14 mmol) of 6-chloropurine riboside was refluxed for 6 h. After standing for one night at room temperature, the crystalline product was filtered off and washed with ethanol. Purification by column chromatography (SiO_2, 10% ethanol/dichloromethane) followed by recrystallization in ethanol gave 4.4 g (63%) of the expected N^6-[2-(1-benzylindol-3-yl)ethyl]adenosine (**5b**), mp 158 °C. ^1H NMR: δ 3.05 (t, 2H, CH_2, J = 8.1 Hz), 3.7 (m, 2H, CH_2), 4 (m, 1H, ribofuranose), 4.16 (m, 1H, ribofuranose), 4.6 (q, 1H, ribofuranose, J = 6.2 and 11.4 Hz), 5.22 (d, 1H, OH, J = 5.6 Hz), 5.35 (s, 2H, CH_2 benzyl), 5.5 (m, 2H, 2OH), 5.9 (d, 1H, ribofuranose, J = 6.2 Hz), 7.2 (m, 9H), 7.6 (d, 1H), 8.02 (m, 1H, NH), 8.26 (s, 1H, purine), 8.37 (s, 1H, purine).

Method E. 1-(2,5-Dimethylbenzyl)-3-(2-aminoethyl)-indole[13,21] (7) (R_1 = dimethylbenzyl, R_2 = R_3 = H). To a solution of tryptamine (32.6 g, 200 mmol) in 300 mL of dry DMF was added 9.2 g (230 mmol) of NaH (60%) under a nitrogen atmosphere. The mixture was stirred at room temperature for 30 min, and 34 mL (230 mmol) of 2,5-dimethylbenzyl chloride was added dropwise. The reaction was slightly exothermic. The temperature was then maintained at 50–55 °C for 3 h. Upon cooling, the solution was filtered off and the solid washed with DMF. The organic layer was concentrated; the residue was taken up with 300 mL of chloroform, washed twice with water, dried, and concentrated. A brown oil was obtained. When purified by column chromatography (SiO_2, 5% isopropylamine/chloroform) followed by treatment with a 2-propanol/HCl (6 N) solution, it gave 30.4 g (48%) of the expected 1-(2,5-dimethylbenzyl)-3-(2-aminoethyl)indole as a hydrochloride, mp 184 °C. ^1H NMR: δ 2.13 (s, 3H, CH_3), 2.23 (s, 3H, CH_3), 3.04 (s, 4H, CH_2CH_2), 5.3 (s,

Table 5. Physical Data and Preparation Methods of Compounds **3a–at** and **5a–c**



compd	R_1	R_2	R_3	R_4	prep methods[a]	% yield[b]	mp (°C)[c]	recryst[d] solvent	formula[e]
5a	CH_2-(4-chlorophenyl)	H	H	CH_2OH	FGH D	67	181	EtOH	$C_{27}H_{27}ClN_6O_4$
5b	CH_2-phenyl	H	H	CH_2OH	FGH D	63	158	EtOH[f]	$C_{27}H_{28}N_6O_4$
5c	CH_2-(2,6-dichlorophenyl)	H	H	CH_2OH	FGH D	58	192	EtOH[f]	$C_{27}H_{26}Cl_2N_6O_4$
3a	CH_2-(4-chlorophenyl)	H	H	CONH cyclopropyl	FGH AB	57	225	*g*	$C_{30}H_{30}ClN_7O_4$
3b	$CH_2CH_2OCH_3$	H	H	CONH cyclopropyl	FGH AB	53	132	CH_3CN/iPr_2O[g,h]	$C_{26}H_{31}N_7O_5$
3c	cyclopentyl	H	H	CONH cyclopropyl	FGH AB	44	141−142	CH_3CN[g,h]	$C_{28}H_{33}N_7O_4$
3d	isopropyl	H	H	CONH cyclopropyl	FGH AB	55	135	CH_3CN[h]	$C_{26}H_{31}N_7O_4$
3e	CH_2-(4-methylphenyl)	H	H	CONH cyclopropyl	FGH AB	45	144	Et_2O	$C_{31}H_{33}N_7O_4{\cdot}H_2O$
3f	CH_2-(3,4-dimethylphenyl)	H	H	CONH cyclopropyl	FGH AB	56	134	Et_2O[g,h]	$C_{32}H_{35}N_7O_4{\cdot}0.9H_2O$
3g	CH_2-(2,5-dimethylphenyl)	H	H	CONH cyclopropyl	EAC	69	130−132	$MeOH/Et_2O$[h]	$C_{32}H_{35}N_7O_4{\cdot}H_2O$
3h	CH_2CH_2-morpholino	H	H	CONH cyclopropyl	EAC	79 [52]	142	iPrOH	$C_{29}H_{36}N_8O_5{\cdot}C_6H_8O_7$
3i	$CH_2CH_2N(CH_3)_2$	H	H	CONH cyclopropyl	EAC	64 [66]	130−131	iPrOH	$C_{27}H_{34}N_8O_4{\cdot}C_6H_8O_7$
3j	CH_2CH_2-piperidino	H	H	CONH cyclopropyl	EAC	83 [73]	138	EtOH[h,i]	$C_{30}H_{38}N_8O_4{\cdot}C_6H_8O_7$
3k	CH_2CH_2-pyrrolidino	H	H	CONH cyclopropyl	EAC	74 [67]	125	EtOH[h,i]	$C_{29}H_{36}N_8O_4{\cdot}C_6H_8O_7$
3l	CH_2-(4-chlorophenyl)	H	H	$CONHC(CH_3)_2CH_2OH$	FGH AC	31	189	*j*	$C_{31}H_{34}ClN_7O_5$
3m	H	H	H	CONH cyclopropyl	AC	45	130−131	CH_3CN[g,h]	$C_{23}H_{25}N_7O_4{\cdot}0.2H_2O$
3n	CH_2-(3,4-dichlorophenyl)	H	H	CONH cyclopropyl	EAB	38	141	$MeOH/Et_2O$[g,h]	$C_{30}H_{29}Cl_2N_7O_4{\cdot}0.8H_2O$
3o	CH_2-(3-pyridyl)	H	H	CONH cyclopropyl	EAC	61	239	$MeO(CH_2)_2OH$	$C_{29}H_{30}N_8O_4{\cdot}0.5MeO(CH_2)_2OH$
3p	CH_2-(1-naphthyl)	H	H	CONH cyclopropyl	EAC	35	146	iPrOH[g]	$C_{34}H_{33}N_7O_4$
3q	CH_2-(2-pyridyl)	H	H	CONH cyclopropyl	EAC	30	122	Et_2O[h,k]	$C_{29}H_{30}N_8O_4$
3r	CH_2-(4-chlorophenyl)	5-Cl	H	CONH cyclopropyl	IE AC	77	154	H_2O[h]	$C_{30}H_{29}Cl_2N_7O_4{\cdot}1.1H_2O$
3s	CH_2-(2,5-dimethylphenyl)	5-Cl	H	CONH cyclopropyl	IE AC	49	139	EtOH	$C_{32}H_{34}ClN_7O_4{\cdot}1.1H_2O$
3t	CH_2-(4-chlorophenyl)	H	H	$CONHCH_2CH_2OCH_3$	IE AC	57	193	EtOH[h]	$C_{30}H_{32}ClN_7O_5$
3u	$CH_2CH{=}CH_2$	H	H	CONH cyclopropyl	EAC	44	117	*m*	$C_{26}H_{29}N_7O_4{\cdot}0.9H_2O$
3v	$CH_2C{\equiv}CH_2$	H	H	CONH cyclopropyl	EAC	58	123	*m*	$C_{26}H_{27}N_7O_4{\cdot}H_2O$
3w	CH_2-(2,5-dimethylphenyl)	5-CH_3	H	CONH cyclopropyl	IE AC	52	129	*m*	$C_{33}H_{37}N_7O_4{\cdot}0.8H_2O$
3x	CH_2-(2,5-dimethylphenyl)	5-OCH_3	H	CONH cyclopropyl	IE AC	53	182	*m*	$C_{33}H_{35}N_7O_5{\cdot}0.1H_2O$
3y	CH_2-(2,5-dimethylphenyl)	2-CH_3	H	CONH cyclopropyl	FGH AC	66	144	pentane[h]	$C_{33}H_{37}N_7O_4{\cdot}0.7H_2O$
3z	CH_2-(4-methoxyphenyl)	H	H	CONH cyclopropyl	EAC	72	134	MeOH[h]	$C_{31}H_{33}N_7O_5{\cdot}0.8H_2O$
3aa	cyclopentyl	2-CH_3	H	CONH cyclopropyl	FGH AC	54	140	Et_2O[h]	$C_{29}H_{35}N_7O_4$
3ab	H	2-phenyl	H	CONH cyclopropyl	G[n]H AC	42	180	iPrOH	$C_{29}H_{29}N_7O_4{\cdot}0.4H_2O$
3ac	CH_2-[2-(*N*,*N*-dimethylamino)phenyl]	H	H	CONH cyclopropyl	EAC	81	128−129	Et_2O[h]	$C_{32}H_{36}N_8O_4$
3ad	CH_2-(3-nitrophenyl)	H	H	CONH cyclopropyl	EAC	36	129	Et_2O[h,l]	$C_{30}H_{30}N_8O_6{\cdot}0.3H_2O$
3ae	CH_2-(2,5-dimethylphenyl)	H	9-CH_3	CONH cyclopropyl	FGH AC	46	135	*m*	$C_{33}H_{37}N_7O_4$
3af	cyclopentyl	H	9-CH_3	CONH cyclopropyl	FGHJ AC	49	130	Et_2O[h]	$C_{29}H_{35}N_7O_4$
3ag	CH_2-(2,5-dimethylphenyl)	H	8-CH_3	CONH cyclopropyl	I[o]E AC	56	137	Et_2O[g,h]	$C_{33}H_{37}N_7O_4$
3ah	CH_2-(2,5-dimethylphenyl)	2-phenyl	H	CONH cyclopropyl	GHE AC[n]	62	136	pentane[h]	$C_{38}H_{39}N_7O_4{\cdot}0.75H_2O$
3ai	CH_2-(5-chloro-2-thienyl)	H	H	CONH cyclopropyl	EAC	70	137	Et_2O[h]	$C_{28}H_{28}ClN_7O_4S{\cdot}H_2O$
3aj	CH_2-(2,5-dimethylphenyl)	H	H	$CONHCH_2CH_3$	EAC	46	125	CH_3CN	$C_{31}H_{35}N_7O_4{\cdot}0.45H_2O$
3ak	CH_2-cyclopropyl	H	H	CONH cyclopropyl	EAC	38	134	MeOH[h]	$C_{27}H_{31}N_7O_4{\cdot}0.5H_2O$
3al	CH_2-(2,5-dimethylphenyl)	5-SCH_3	H	CONH cyclopropyl	IE AC	38	137	MeOH[h]	$C_{33}H_{37}N_7O_4S{\cdot}0.55H_2O$
3am	CH_2-(2,5-dimethylphenyl)	H	H	$CONHCH_2CH_2$-morpholino	EAC	69	114	Et_2O[h]	$C_{35}H_{42}N_8O_5$
3an	CH_2-(2-methoxyphenyl)	H	H	CONH cyclopropyl	EAC	61	117	Et_2O[h]	$C_{31}H_{33}N_7O_5$
3ao	CH_2-phenyl	H	H	CONH cyclopropyl	EAC	47	138	MeOH[h]	$C_{30}H_{31}N_7O_4{\cdot}H_2O$
3ap	CH_3	H	H	CONH cyclopropyl	p AC	44	168	CH_3CN	$C_{24}H_{27}N_7O_4$
3aq	CH_2-(2-fluoro-4-bromophenyl)	H	H	CONH cyclopropyl	EAC	57	136	MeOH[h]	$C_{30}H_{29}BrFN_7O_4{\cdot}H_2O$
3ar	CH_2-(2-methylphenyl)	H	H	CONH cyclopropyl	EAC	68	177	CH_3CN[h]	$C_{31}H_{33}N_7O_4$
3as	CH_2-(2,5-dimethylphenyl)	H	H	$CONH_2$	EAC	48	187	Et_2O[h]	$C_{29}H_{31}N_7O_4$
3at	CH_2-(2,5-dimethylphenyl)	H	H	$CONHCH_3$	EAC	48	192	CH_3CN	$C_{30}H_{33}N_7O_4$

[a] Preparation methods involve, in the first part, the methods for the preparation of the 1-substituted-3-(2-aminoethyl) indoles **7** used and, in the second part, the preparation method of the compounds **1a,b**. [b] Yields are not optimized and correspond to the recrystallized product in the final step. Values in brackets [*X*] indicate the percent yield of the salt formation after recrystallization. [c] Melting points are uncorrected and correspond to the product whose formula is given. [d] All compounds were purified first by column chromatography (elution with 10% methanol/chloroform, otherwise noted) and then recrystallized if needed. [e] Analyses for C, H, and N were ±0.4% of the expected values for the formula shown. [f] Column chromatography elution with 10% ethanol/dichloromethane. [g] Column chromatography elution with 5% methanol/chloroform. [h] Crystallization solvent. [i] Column chromatography elution with 20% methanol/chloroform. [j] Two successive column elutions with 10% methanol/chloroform. [k] Three successive column elutions with 10% methanol/chloroform, 20% isopropylamine/chloroform, and 10% methanol/dichloromethane, respectively. [l] Two successive column elutions with 10% methanol/chloroform and then 10% methanol/dichloromethane. [m] Elution with 10% methanol/chloroform. [n] Synthesis of (2-phenyl)indole was achieved according to the literature method (see ref 25). [o] Synthesis of 2-[1-(2,5-dimethylbenzyl)indol-3-yl]propylamine was done according to the literature (see ref 26). [p] Synthesis according to the literature method (see ref 24).

Table 6. Pharmacological Activity of Compounds **3a–at** and **5a–c**

	affinity for adenosine receptors						
	A1			A2			
	% displacement[a]			% displacement[a]			analgesic activity,[b]
compd	10^{-5} M	10^{-7} M	K_i (nM)	10^{-5} M	10^{-7} M	K_i (nM)	ED_{50} (mg/kg po)
5a	100 ± 1	71 ± 1	11	97 ± 1	59 ± 1	140	40.8 (27.7–60.2)
5b	100 ± 1	67 ± 1	50	95 ± 1	30 ± 2	250	>30 NDD[d]
5c	92 ± 1	15 ± 3	NC[c]	91 ± 1	35 ± 2	NC	inactive
3a	98 ± 1	46 ± 2	50	91 ± 1	30 ± 2	320	0.9 (0.6–1.5)
3b	98 ± 1	61 ± 2	23	90 ± 1	19 ± 1	370	1.2 (0.7–2.2)
3c	96 ± 1	21*	110	90*	28 ± 2	350	1.5 (1.1–2.1)
3d	87 ± 2	4 ± 8	120	91 ± 2	14 ± 3	390	1.6 (0.8–3.4)
3e	97 ± 1	37 ± 10	130	89 ± 4	21 ± 6	340	<3 NDD
3f	96 ± 1	29 ± 1	160	83 ± 1	13 ± 5	820	>30 NDD
3g	93 ± 1	26 ± 3	140	85 ± 3	9 ± 4	670	2.4 (1.8–3.3)
3h[e]	100 ± 5	52 ± 4	NC	88 ± 3	20 ± 2	NC	>30[f]
3i[e]	100 ± 1	83 ± 4	NC	95 ± 3	51 ± 6	NC	>60 NDD
3j[e]	99 ± 1	90 ± 4	8.3	96 ± 1	44 ± 1	120	7.3 (3.1–17.1[g])
3k[e]	100 ± 3	96 ± 2	7.5	95 ± 1	53 ± 5	58	44.2 (28.8–67.9)
3l	79 ± 3	5 ± 2	NC	50 ± 8	0*	NC	inactive
3m	100 ± 1	91 ± 1	7.9	68 ± 2	29 ± 1	560	34.9 (20.2–60.3)
3n	100 ± 1	52 ± 1	140	81 ± 1	24 ± 5	1400	>30 NDD
3o	97*	78 ± 5	26	98 ± 1	30 ± 9	183	>30 NDD
3p	93 ± 2	20 ± 5	370	76 ± 6	8 ± 1	1400	2.4 (1.6–3.6)
3q	96 ± 3	73 ± 4	39	94 ± 1	28 ± 3	390	<3 NDD
3r	100 ± 6	49 ± 4	NC	79 ± 2	23 ± 2	NC	inactive
3s	99 ± 3	47 ± 9	150	77 ± 1	10 ± 4	660	6.3 (4.7–8.5)
3t	90 ± 4	13 ± 8	890	36 ± 4	0 ± 2	>10000	inactive
3u	100*	56 ± 5	39	80 ± 3	17 ± 7	280	5.3 (2.7–10.7)
3v	95 ± 1	64 ± 5	NC	83 ± 0	25 ± 7	NC	<10 NDD
3w	100 ± 2	50 ± 2	62	70 ± 1	3 ± 7	890	0.9 (0.6–1.4)
3x	100 ± 2	16 ± 2	NC	91 ± 3	17 ± 1	NC	46.3 (30.2–71.1)
3y	56 ± 8	5 ± 4	NC	60 ± 3	7 ± 2	NC	1.2 (0.4–4.3)
3z	98 ± 3	42 ± 2	NC	100 ± 1	59 ± 3	NC	71.6 (15.2–338)
3aa	77 ± 6	15 ± 5	895	92 ± 1	6 ± 1	2180	0.5 (0.2–1.2)
3ab	84 ± 1	20 ± 9	NC	71 ± 14	10 ± 5	NC	27.1 (17.2–42.8)
3ac	99 ± 1	33 ± 6	170	92 ± 7	5 ± 7	760	1.9 (0.8–4.4)
3ad	100 ± 2	63 ± 1	29	99 ± 1	46 ± 4	182	<10 NC
3ae	96 ± 2	21 ± 2	180	84 ± 1	9 ± 2	2200	3.6 (2.1–6.3)
3af	96 ± 2	45 ± 6	NC	85 ± 1	21 ± 7	NC	2.2 (1.2–4.0)
3ag	97 ± 4	28 ± 2	200	61 ± 5	0 ± 7	2500	0.9 (0.4–2.0)
3ah	31 ± 4	6 ± 3	NC	23 ± 6	0 ± 4	NC	>30 NDD
3ai	100 ± 2	47 ± 4	NC	82 ± 1	6 ± 2	NC	26.2 (9.8–69.9)
3aj	92 ± 4	38 ± 6	160	86 ± 2	12 ± 5	660	<2 NDD
3ak	97 ± 1	45 ± 3	67	87 ± 2	25 ± 3	190	>10 NDD
3al	82 ± 6	13 ± 3	NC	76 ± 6	8 ± 6	NC	>10 NDD
3am	5 ± 6	5 ± 5	NC	19 ± 2	0 ± 7	NC	>60
3an	100 ± 2	42 ± 4	170	89 ± 1	23 ± 2	810	2.3 (1.4–3.9)
3ao	99 ± 2	35 ± 1	40	94 ± 1	41 ± 3	270	18.1 (10.3–31.7)
3ap	99 ± 1	76 ± 3	14	91 ± 2	29 ± 3	270	3 (0.7–7.1)
3aq	96 ± 2	40 ± 3	NC	92 ± 3	23 ± 4	NC	inactive
3ar	98 ± 1	40 ± 1	NC	93 ± 1	27 ± 3	NC	<3
3as	87 ± 2	17 ± 3	510	61 ± 1	0 ± 1	2600	>30
3at	80 ± 2	12 ± 5	1400	68 ± 2	0 ± 5	3000	10.2 (5.4–15.2)

[a] Values are the mean ± SEM of three determinations except for values marked with an asterisk where only one determination was performed (see the Experimental Section). [b] ED_{50} in phenylbenzoquinone-induced writhing test with 95% fiducial limits (see the Experimental Section). [c] NC: not calculated. [d] NDD: not dose dependent. The ED_{50} value was not calculable by the linear regression method, although 50% inhibition was observed at the indicated dose. [e] Affinity values for compounds **3h–k** were given on salts. The affinity of the corresponding base is as follows. Compound **3h**: A1 (10^{-5} M) 98 ± 1 (10^{-7} M) 63 ± 3; A2 (10^{-5} M) 90 ± 1 (10^{-7} M) 12 ± 4. Compound **3i**: A1 (10^{-5} M) 100 ± 2 (10^{-7} M) 91 ± 1; A2 (10^{-5} M) 94 ± 1 (10^{-7} M) 44 ± 1. Compound **3j**: A1 (10^{-5} M) 99 ± 2 (10^{-7} M) 94 ± 2; A2 (10^{-5} M) 92 ± 1 (10^{-7} M) 46 ± 3. Compound **3k**: A1 (10^{-5} M) 100 ± 1 (10^{-7} M) 82 ± 10; A2 (10^{-5} M) 92 ± 1 (10^{-7} M) 69 ± 1. [f] Value for the base. [g] Activity for the salt as citrate. The result for an oxalate was ED_{50} = 10.2 (7.1–14.6).

2H, CH_2N indole), 6.59 (s, 1H), 7.1 (m, 5H), 7.4 (d, 1H, J = 7.8 Hz), 7.6 (d, 1H, J = 7.5 Hz), 8.1 (s, 3H, NH_2 and HCl).

Method F. 1-Cyclopentyl-3-formylindole (9e) (R_1 = cyclopentyl, R_2 = H). A mixture of 20 g (140 mmol) of 3-formylindole, 15.6 mL (150 mmol) of chlorocyclopentane, 100 mL of DMF, and 20.7 g (150 mmol) of K_2CO_3 was refluxed for 2 h. Upon cooling, the solid was filtered off and washed with DMF and the DMF phase concentrated. The residue was taken up with chloroform, washed twice with water, dried, and concentrated to give a brown oil which was purified by column chromatography (SiO_2, 10% methanol/chloroform) to yield 17.8 g (60%) of the expected 1-cyclopentyl-3-formylindole as an oil. ^{1}H NMR ($CDCl_3$): δ 1.8 (m, 6H, CH_2 cyclopentyl), 2.2 (m, 2H, CH_2 cyclopentyl), 4.8 (m, 1H, CH cyclopentyl), 7.4 (m, 2H, indole), 7.81 (s, 1H, H_2 indole), 8.3 (m, 1H, indole), 9.99 (s, 1H, CHO).

Method G. 1-Cyclopentyl-3-(2-nitrovinyl)indole (10e) (R_1 = cyclopentyl, R_2 = R_3 = H). 1-Cyclopentyl-3-formylindole (**9e**) (17.8 g, 83 mmol) prepared according to method F was mixed with 85 mL (1.6 mol) of nitromethane and 5 g (65 mmol) of ammonium acetate. The mixture was refluxed for 30 min and excess of nitromethane evaporated. The residue was taken up with dichloromethane, washed with water, dried, concentrated, and then purified by column chromatography (SiO_2, CH_2Cl_2) to yield 16.8 g (80%) of 1-cyclopentyl-3-(2-nitrovinyl)indole (**10e**) as an oil. ^{1}H NMR ($CDCl_3$): δ 1.9 (m, 6H, CH_2 cyclopentyl), 2.21 (m, 2H, CH_2 cyclopentyl), 4.7 (m,

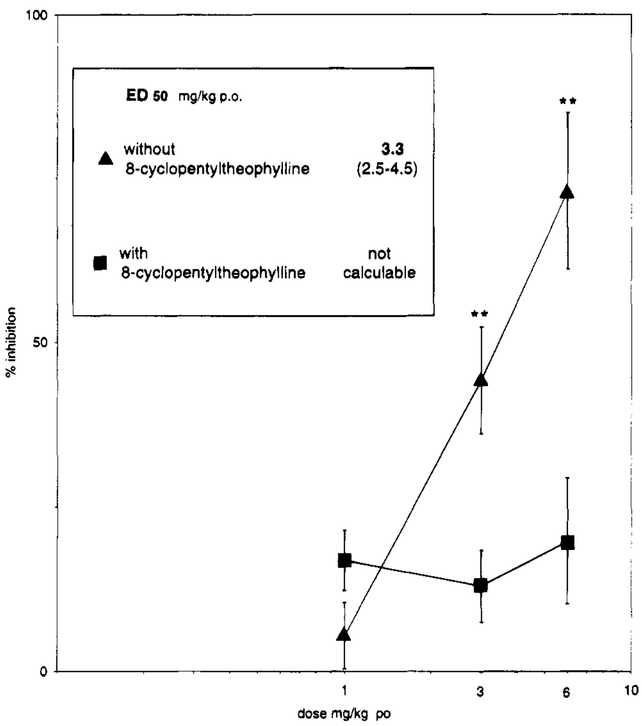


Figure 2. Effect of 8-cyclopentyltheophylline on UP202-32 (**3c**)-induced antinociception in the phenylbenzoquinone-induced writhing assay. $n = 6$ (see the Experimental Section). $^{**}p < 0.01$ as compared to the 8-cyclopentyltheophylline-treated group.

1H, CH cyclopentyl), 7.35 (m, 3H, indole), 7.58 (s, 1H, H_2 indole), 7.67 (m, 2H, vinyl and indole), 8.2 (d, 1H, vinyl, J = 13.3 Hz).

Method H. 1-Cyclopentyl-3-(2-aminoethyl)indole (7e). 1-Cyclopentyl-3-(2-nitrovinyl)indole (**10e**) (16.8 g, 66 mmol) prepared according to method G and 100 mL of THF were added dropwise to a mixture of 13.7 g (360 mmol) of $LiAlH_4$ and 100 mL of THF. The reaction was exothermic. Upon complete addition, the mixture was refluxed for 1.5 h. A saturated solution of H_2O/Na_2SO_4 was added dropwise at 5 °C to destroy the excess of $LiAlH_4$. The resulting mixture was filtered on Celite and extracted with ethyl acetate. The organic layers were concentrated, and the residue was chromatographed (SiO_2, 5% isopropylamine/chloroform) to give 8.8 g (58%) of 1-cyclopentyl-3-(2-aminoethyl)indole (**7e**) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 1.56 (s, 2H, NH_2), 1.85 (m, 6H, CH_2 cyclopentyl), 2.17 (m, 2H, CH_2 cyclopentyl), 2.95 (m, 4H, CH_2-CH_2N), 4.75 (m, 1H, CH cyclopentyl), 7.16 (m, 3H, indole), 7.37 (d, 1H, indole, J = 8.3 Hz), 7.59 (d, 1H, indole, J = 7.7 Hz).

Method I. Ethyl 2-Oxo-5-methylpiperidine-3-carboxylate (12b) ($R_3 = CH_3$). A mixture of 26.7 g (117 mmol) of ethyl 2-carbethoxy-4-cyanopentanoate (**11b**),[26] 500 mL of ethanol, and 1 g of Raney nickel was heated for 6 h under H_2 pressure (80 kg) at 80 °C. The catalyst was filtered on Celite and washed with methanol, and the organic layers were concentrated. Crystallization in light petroleum gave 17.7 g (82%) of ethyl 2-oxo-5-methylpiperidine-3-carboxylate (**12b**) as a white solid, mp 108 °C (lit.[26] mp 100–101 °C). ^{1}H NMR: δ 0.92 (d, 3H, CH_3, J = 5.8 Hz), 1.18 (t, 3H, CH_3 ester, J = 7.05 Hz), 1.6 (m, 1H), 1.9 (m, 2H), 2.78 (m, 1H), 3.13 (m, 1H), 3.3 (m, 1H), 4.09 (q, 2H, CH_2 ester, J = 7.05 Hz), 7.73 (s, 1H, NH).

2,3-Dioxo-5-methylpiperidine 3-Phenylhydrazone (13a) (R_2 = H, $R_3 = CH_3$). Ethyl 2-oxo-5-methylpiperidine-3-carboxylate (**12b**) 36.5 g (200 mmol) prepared as above was dissolved in 500 mL of water containing 14 g (250 mmol) of potassium hydroxide. The solution was stirred at room temperature overnight. This solution cooled in an ice bath was treated with a solution of benzene diazonium chloride (prepared as follows: 24 g (0.26 mol) of aniline was mixed with 400 mL of water and 60 mL of concentrated HCl. The mixture was cooled in an ice bath, and a solution of 20 g (0.29 mol) of $NaNO_2$ and 500 mL of water was introduced dropwise and the mixture stirred for 30 min. The pH of the solution was adjusted to 4.5 with a 10% solution of Na_2CO_3 (250 mL) before mixing with the benzene diazonium chloride solution). The pH of the resulting solution was adjusted to pH 5 by the addition of acetic acid. Stirring was continued for 4 h at 0 °C. The orange solid obtained was filtered and dried to yield 24.9 g (57%) of the expected phenylhydrazone **13a**, mp 231 °C (lit.[26] mp 238–239 °C).

4-Methyl-1-oxo-1,2,3,4-tetrahydro-β-carboline (14a) (R_2 = H, $R_3 = CH_3$). The phenylhydrazone **13a** prepared as above (24.9 g, 115 mmol) in 500 mL of formic acid (90%) was boiled under reflux for 1 h. The mixture was diluted with 200 mL of water, and hot ethanol was added until the brown oil separated in the solution. Upon cooling, the β-carboline obtained was filtered off and recrystallized from ethanol to yield 11 g (48%) of 4-methyl-1-oxo-1,2,3,4-tetrahydro-β-carboline (**14a**), mp 175 °C (lit.[26] mp 204–206 °C). ^{1}H NMR: δ 1.31 (d, 3H, CH_3, J = 6.5 Hz), 3.24 (m, 2H, CH_2), 3.58 (m, 1H, CH), 7.05 (t, 1H, J = 7 and 7.7 Hz), 7.21 (t, 1H, J = 7 and 7.7 Hz), 7.4 (d, 1H, J = 8 Hz); 7.58 (s, 1H, NH), 8 (d, 1H, J = 8 Hz), 11.6 (s, 1H, NH indole).

3-(2-Amino-1-methylethyl)indole-2-carboxylic Acid (15a) (R_2 = H, $R_3 = CH_3$). 4-Methyl-1-oxo-1,2,3,4-tetrahydro-β-carboline (**14a**) (11.9 g, 60 mmol) prepared as above was added portionwise to a mixture of 26.6 mL of ethanol (50%) and 29.3 g (520 mmol) of KOH. The resulting mixture was refluxed for 6 h and kept at room temperature for a night. The solvent was then removed in vacuo and 100 mL of water added. The solution was filtered and acidified with acetic acid. The solid obtained was washed with water, ethanol, and ethyl ether and then dried to yield 13.1 g (100%) of the expected acid, mp 255 °C (lit.[26] mp 242–243 °C). ^{1}H NMR: δ 1.51 (d, 3H, CH_3, J = 7.3 Hz), 3.1 (m, 2H, CH_2), 4.03 (m, 1H, CH), 6.93 (t, 1H, J = 7.1 and 7.7 Hz), 7.07 (t, 1H, J = 7.7 and 7.1 Hz), 7.36 (d, 1H, J = 8 Hz), 7.64 (d, 1H, J = 8 Hz), 11.04 (s, 1H, NH indole).

3-(2-Amino-1-methylethyl)indole (6a) (R_2 = H, R_3 = CH_3). 3-(2-Amino-1-methylethyl)indole-2-carboxylic acid (**15a**) (13.4 g, 0.06 mol) prepared as above was refluxed with 10% hydrochloric acid (440 mL) for 2 h. Upon cooling, the solution was made alkaline with 30% sodium hydroxide, extracted twice with ether, dried, and concentrated. The brown oil was treated with 9 mL of a 6 N 2-propanol/hydrochloric acid solution to yield 9.9 g (78%) of the expected 3-(2-amino-1-methylethyl)indole (**6a**) hydrochloride as a white solid, mp 189 °C (lit.[26] mp 224–226 °C for the picrate). ^{1}H NMR: δ 1.38 (d, 3H, CH_3, J = 6.8 Hz), 2.98 (m, 1H, CH), 3.37 (m, 1H, CH), 7.09 (m, 2H), 7.24 (s, 2H, H_2 indole), 7.38 (d, 1H, J = 7.9 Hz), 7.62 (d, 1H, J = 7.6 Hz), 8.14 (s, 3H, NH_2 and HCl), 11.09 (s, 1H, NH indole).

Pharmacology. The compounds were dissolved in water if available as soluble salts or suspended in an aqueous 1% gum arabic, 0.1% sodium chloride, 0.001% Tween 80 solution when available as free bases. They were administered orally or intraperitoneally under a volume of 0.5 mL/20 g and 0.2 mL/20 g, respectively.

Phenylbenzoquinone-Induced Writhing in Mice. The nociceptive reaction was induced following the method of Siegmund et al.[31] One hour after oral administration of the test compound, the mice received intraperitoneally 0.20–0.24 mL of a hydroalcoholic 0.02% phenylbenzoquinone solution. The number of nociceptive reactions (writhings and stretches) were counted from the 5th to the 10th minute. In the protocol of analgesic activity inhibition with 8-cyclopentyltheophylline, used as an A_1 selective adenosine receptor antagonist, an ineffective analgesic dose of 8-cyclopentyltheophylline (10 mg/kg ip) was administered 30 min after the oral administration of UP 202-32 (**3c**). Groups of six animals were used for treated and control groups. For each treated group, the inhibition of painful reactions was calculated from unpaired values in comparison with the mean control value. The ED_{50} value (dose for which the nociceptive reaction was decreased by 50%) was determined by linear regression on quantitative values.

Binding Studies. A_1 Binding Assays. Preparation of Whole Rat Brain Membranes. Rat brain membranes were obtained according to a previous method.[27] Rats were decapitated and whole brains rapidly removed at 4 °C. They were rapidly washed in ice-cold saline solution, dried, and weighed. They were homogenized in 25 volumes of ice-cold incubation buffer (Tris-HCl, 50 mM, pH 7.4) using an Ultra-Turrax homogenizer and centrifuged at 1090*g* for 10 min at 4 °C. The supernatant was centrifuged a second time at 48000*g* for 20 min at 4 °C. The pellet was resuspended in 4 volumes of ice-cold incubation buffer and homogenized with the Ultra-Turrax homogenizer. The suspension was incubated with adenosine deaminase (ADA) (1 U/mL of suspension) for 30 min at room temperature with gentle stirring and centrifuged as before. The pellet was resuspended in 10 volumes of ice-cold incubation buffer and homogenized with the Ultra-Turrax ice-cold homogenizer. The suspension was stored at 4 °C until required or stored at −20 °C. Receptor binding studies were carried out as previously described by Schwabe and Trost,[27] with slight modifications. Incubations (2 mL) were performed at 20 °C for 30 min in poly(styrene) tubes containing incubation buffer, 1 mL of the tissue preparation, 2.5 nM [^{3}H]PIA, and various concentrations of the competing drugs. Nonspecific binding was defined as that remaining in the presence of 10 μM PIA. The reaction was terminated by rapid filtration of the solution through Whatman GF/B glass fiber filters, and the latter were washed three times with incubation buffer. The radioactivity was counted in a liquid scintillation β counter with 47% efficiency. Each assay was performed in triplicate.

A_2 Binding Assays. Preparation of Rat Striatal Membranes. Rat striatal membranes were obtained according to a previously described method.[28] Briefly, the striata were disrupted in 10 volumes of ice-cold homogenization buffer (Tris-HCl, 50 mM, $MgCl_2$, 10 mM, pH 7.7) using an Ultra-Turrax homogenizer and centrifuged at 48340*g* for 10 min at 4 °C. These operations were repeated on the resulting pellet. Before the centrifugation, the suspension was incubated with ADA (1 U/mL of suspension) for 30 min at room temperature, with gentle stirring. The pellet was resuspended in 5 volumes of ice-cold homogenization buffer and homogenized with the Ultra-Turrax homogenizer. This suspension was stored frozen at −80 °C. On the day of use, it was thawed to room temperature and resuspended in 15 more volumes of ice-cold homogenization buffer. It was further homogenized with the Ultra-Turrax homogenizer and then with the glass-Teflon potter. Receptor binding studies were carried out as previously described by Bruns et al.[28] with slight modifications. Incubations (2 mL) were performed at 25 °C for 60 min in poly(styrene) tubes containing incubation buffer (Tris-HCl, 50 mM, $MgCl_2$, 10 mM, cyclopentyladenosine, 0.11 μM, pH 7.7), 1 mL of the tissue preparation, 50 μL of 4 nM [^{3}H]NECA, and various concentrations of the test compounds. Nonspecific binding was defined as that remaining in the presence of 5 μM NECA. The reaction was terminated by rapid filtration of the solution through Whatman GF/B glass fiber filters, and the latter were washed three times with washing buffer (Tris-HCl, 50 mM, $MgCl_2$, 10 mM, pH 7.7). The radioactivity was counted in a liquid scintillation β counter with 47% efficiency. Each assay was performed in triplicate.

Data Analysis. Competition data were analyzed using the nonlinear regression program LIGAND[29] adapted for an IBM-PC[30] and obtained from Elsevier-Biosoft (Cambridge, England). The concentration of unlabeled drug causing 50% displacement of the radioligand from its binding site (IC_{50} value) was calculated by log−logit linear regression analysis of data (EBDA). Then, the latter were analyzed by a nonlinear regression program (LIGAND) assuming a model of one binding site. The inhibition constant (K_i) value was calculated according to the Cheng−Prusoff equation.[33] Each K_i value was determined from one experiment, each assay being performed in triplicate.

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