of concentrated H_2SO_4 at 0–10 °C. The mixture was stirred at 0–15 °C for 4 h, poured over ice, made alkaline with an excess of NH_4OH, and extracted with CH_2Cl_2. The CH_2Cl_2 solution was dried and concentrated at reduced pressure to dryness. Purification of the residue (13 g) by column chromatography (SiO_2, 55 g; eluent, CH_2Cl_2 and then ether) gave, on concentration of the ether fraction, 4.6 g (29%) of **22** as a crystalline solid: mp 116–117 °C; IR ($CHCl_3$) 1685 (C=O) cm^{-1}; NMR ($CDCl_3$) δ 3.06 (m, 2) and 3.91 (m, 2) (A_2B_2 system, CH_2CH_2), 3.82 (s, 3, CH_3), 6.8–7.1 (m, 2, arom H), 7.4–7.6 (m, 2, arom H), 7.82 (d, J = 8 Hz, 1, C_{11} H); mass spectrum, m/e 333 (M^+). Anal. ($C_{17}H_{13}Cl_2NO_2$) C, H, N.

8-Chloro-1-(2-chloro-4-methoxyphenyl)-3,4-dihydro-4-[(dimethylamino)methylene]-5*H*-2-benzazepinone (23). A mixture of 6.4 g (19 mmol) of **22**, 12 mL of DMF, and 6 mL of dimethylformamide dimethyl acetal was heated on a steam bath for 1 h. The reaction mixture was concentrated at reduced pressure to dryness, and the residue was crystallized from a mixture of CH_2Cl_2 and ether to give 5.8 g (77%, mp 144–145 °C) of **23** as a yellow solid. Recrystallization from a mixture of ether and CH_2Cl_2 gave **23** as yellow needles: mp 145–147 °C; IR ($CHCl_3$) 1648 (C=O) cm^{-1}; NMR ($CDCl_3$) δ 3.26 (s, 6, CH_3), 3.60 (d, J = 13 Hz, 1) and 4.85 (d, J = 13 Hz, 1) (AB system, C_3 H), 6.8–7.0 (m, 2, arom H), 7.4–7.6 (m, 2, arom H), 7.76 (s, 1, =CH), 8.00 (d, J = 8 Hz, 1, C_{11} H); mass spectrum, m/e 388 (M^+). Anal. ($C_{20}H_{18}Cl_2N_2O_2$) C, H, N.

9-Chloro-7-(2-chloro-4-methoxyphenyl)-5*H*-pyrimido[5,4-*d*][2]benzazepine (24). A mixture of 8 g (20 mmol) of **23**, 9 g (86 mmol) of formamidine acetate, and 90 mL of formamide was heated on a steam bath for 16 h. The mixture was poured into ice–water and extracted with CH_2Cl_2. The CH_2Cl_2 solution was dried and concentrated at reduced pressure to dryness. The residue crystallized from ether to give 5.7 g (77%; mp 104–108 °C) of **24** as a yellow solid. Recrystallization from ether gave **24** as yellow prisms: mp 108–112 °C; NMR ($CDCl_3$) δ 3.81 (s, 3, CH_3), 4.0–5.0 (br s, 2, C_5 H), 6.8–7.0 (m, 2, arom H), 7.2–7.7 (m, 3, arom H), 8.27 (d, J = 8 Hz, 1, C_{11} H), 8.81 (s, 1, C_4 H), 9.29 (s, 1, C_2 H); mass spectrum, m/e 369 (M^+). Anal. ($C_{19}H_{13}Cl_2N_3O$) C, H, N.

3-Chloro-4-(9-chloro-5*H*-pyrimido[5,4-*d*][2]benzazepin-7-yl)phenol Hydrochloride Hydrate (4). A solution of 0.9 g (2.4 mmol) of **24** in 50 mL of CH_2Cl_2 was added slowly to a stirred solution of 3 g (12 mmol) of boron tribromide in 25 mL of CH_2Cl_2. The mixture was refluxed with stirring for 23 h, poured into a small amount of ice, and concentrated at reduced pressure to a small volume. The residue was dissolved in THF and diluted with an excess of dilute aqueous NaOH. The solution was stirred at room temperature for 10 min, diluted with H_2O, and extracted with ether. The ether layer which contained starting material was separated and discarded. The aqueous solution was neutralized with acetic acid and extracted with CH_2Cl_2. The CH_2Cl_2 solution was dried and concentrated at reduced pressure to dryness. The residue (0.5 g) was dissolved in an excess of methanolic HCl and diluted with ether, and the resulting precipitate was collected by filtration to give 0.3 g (29%; mp 247–250 °C dec) of **4** as a yellow solid. Recrystallization from a mixture of MeOH and ether gave **4** as yellow needles: mp 249–251 °C dec; IR (KBr) 3400 (OH) and 2660, 2020, 1900 (C=NH) cm^{-1}; NMR (Me_2SO-d_6) δ 4.80 (br s, 2, C_5 H), 7.0–7.2 (m, 2, arom H), 7.4–8.2 (m, 5, arom H), 8.39 (d, J = 8 Hz, 1, C_{11} H), 9.12 (s, 1, C_4 H), 9.40 (s, 1, C_2 H). Anal. ($C_{18}H_{11}ClN_3O{\cdot}HCl$) C, H, N.

The free base of **4** was prepared by dissolving the HCl salt in dilute aqueous NaOH, neutralizing the solution with acetic acid, and extracting the base into CH_2Cl_2. The CH_2Cl_2 solution was dried and concentrated at reduced pressure to dryness. The residue crystallized from a mixture of CH_2Cl_2 and ether and gave the free base of **4** as cream-colored prisms: mp 208–210 °C; IR (KBr) 2760, 2660 (OH), 1595, 1570 (arom) cm^{-1}; NMR (Me_2SO-d_6) δ 4.0–5.5 (vbr s, 2, C_5 H), 6.7–6.9 (m, 2, arom H), 7.15 (d, J = 2 Hz, 1, arom H), 7.38 (d, J = 9 Hz, 1, arom H), 7.73 (dd, J = 2 and 8 Hz, 1, C_{10} H), 8.20 (d, J = 8 Hz, C_{11} H), 8.93 (s, 1, C_4 H), 9.22 (s, 1, C_2 H), 10.10 (br s, 1, OH); mass spectrum, m/e 355 (M^+). Anal. ($C_{18}H_{11}Cl_2N_3O$) C, H, N.

Metabolite A (compound **2**): NMR (CD_3OD) δ 5.47 (s, 1, C_5 H), 7.18 (d, J = 2 Hz, 1, arom H), 7.2–7.8 (m, 5, arom H), 8.27 (d, J = 8 Hz, 1, C_{10} H), 9.14 (s, 1, arom H), 9.22 (s, 1, arom H); mass spectrum, m/e 355 (M^+).

Metabolite B (compound **3**): NMR (CD_3OD) δ 4.51 (br s, 2, C_5 H), 7.18 (d, J = 2 Hz, 1, arom H), 7.2–7.8 (m, 5, arom H), 8.26 (d, J = 8 Hz, 1, C_{10} H), 8.85 (d, J = 2 Hz, 1, arom H), 9.16 (d, J = 2 Hz, 1, arom H).

Metabolite C (compound **4**): NMR ($CDCl_3/CD_3OD$, 5:1) δ 4.08 (br s, 1) and 4.77 (br s, 1) (AB system, C_5 H), 6.78 (s, 1, arom H), 6.82 (d, J = 2 Hz, 1, arom H), 7.25 (d, J = 2 Hz, 1, arom H), 7.3–7.5 (m, 1, arom H), 7.66 (dd, J = 2 and 8 Hz, C_9 H), 8.22 (d, J = 8 Hz, 1, C_{10} H), 8.83 (s, 1, C_4 H), 9.25 (s, 1, C_2 H); mass spectrum, m/e 355 (M^+).

Acknowledgment. We thank our Physical Chemistry Department and, in particular, Dr. W. Benz for mass spectra, Dr. F. Scheidl for elemental analysis, S. Traiman for IR spectra, and Dr. T. Williams for NMR spectra. We thank Drs. W. Dairman, W. D. Horst, and R. O'Brien and their staffs for detemining and providing the pharmacological data.

Registry No. 1, 76988-39-1; 2, 76988-67-5; 3, 76988-77-7; 4, 86709-89-9; 4 (free base), 86709-90-2; 5, 76988-65-3; 6, 76988-66-4; 7, 76988-73-3; 8, 86709-91-3; 9, 76988-75-5; 10, 76988-76-6; 11, 76988-78-8; 12, 86709-92-4; 13, 86709-93-5; 14, 76988-79-9; 15, 86709-94-6; 16, 86709-95-7; 18, 86709-96-8; 19, 86709-97-9; 20, 86709-98-0; 21, 86709-99-1; 21·HCl, 86710-00-1; 22, 86710-01-2; 23, 86710-02-3; 24, 86710-03-4; *N*-propargylphthalimide, 7223-50-9; formamidine acetate, 3473-63-0; dimethylfurmamide dimethyl acetal, 4637-24-5.

Supplementary Material Available: Tables IV and V, the bond lengths and angles in compound **3**; Tables VI and VII, the final atomic and anisotropic thermal parameters for compound **3**; Tables VIII and IX, the bond lengths and angles in compound **12**; and Tables X and XI, the final atomic and anisotropic thermal parameters for **12** (9 pages). Ordering information is given on any current masthead page.

C_4-Substituted 1-β-D-Ribofuranosylpyrazolo[3,4-*d*]pyrimidines as Adenosine Agonist Analogues

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Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, Michigan 48105. *Received March 7, 1983*

The synthesis of four novel C_4-substituted 1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidines is reported, and the compounds were examined as adenosine receptor agonist analogues. Neither receptor affinity nor biological activity was as potent as the purine counterparts. Adenosine agonists appear to be sensitive to modification of the purine base, with a nitrogen atom in the 7 position necessary for efficacy.

In recent years, several investigators have proposed adenosine to be a neurotransmitter or neuromodulator acting on a variety of physiological systems. Receptor classifications have been defined by several investigators

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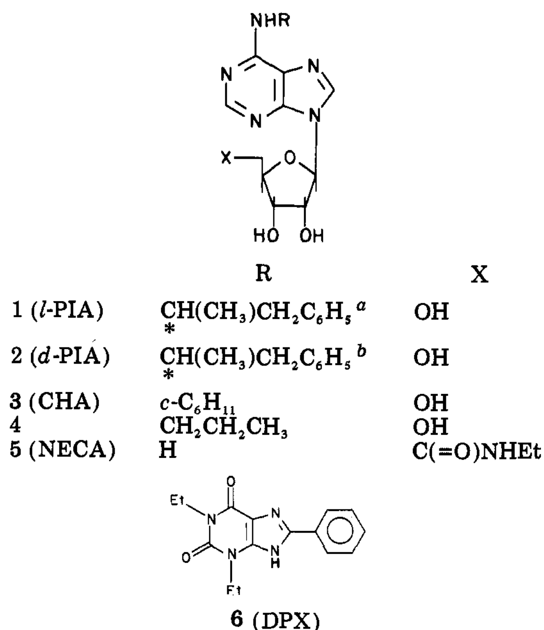
Chart I



[a] * = R. [b] * = S.

for adenosine in this regard,[1] although a standard "nomenclature" has not yet been established. Thus, in addition to the less well-defined intracellular "P site", there appear to be extracellular "R-site" receptors. The R-site (also identified as P_1) receptors are further divided into the A_1 (or R_I) receptor, in which agonist binding inhibits accumulation of cyclic AMP, and the A_2 (or R_A) receptor, which increases cyclic AMP upon activation. Both the A_1 and A_2 receptors are competitively inhibited by methylxanthines. A selective A_1 or A_2 antagonist is not known at the present time. An extracellular P_2 receptor has also been proposed that is sensitive to ATP and mediates prostaglandin release. There is no known specific antagonist for this receptor.

Modified adenosine analogues have been identified as potent A_1 and A_2 receptor agonists.[2] (−)-*N*-(1-Methyl-2-phenylethyl)adenosine (*l*-phenylisopropyladenosine, *l*-PIA, **1**), (+)-*N*-(1-methyl-2-phenylethyl)adenosine (*d*-phenylisopropyladenosine, *d*-PIA, **2**), N^6-cyclohexyladenosine (CHA, **3**), and N^6-propyladenosine (**4**) (see Chart I) have been found to displace radiolabeled CHA in rat brain preparations with IC_{50}'s in the nanomolar range. These compounds are modified at the N-6 position of the adenine nucleus, while the ribose ring is intact. Stereochemical requirements for the A_1 receptor are illustrated by *d*- and *l*-PIA, which differ only in the configuration of the amphetamine side chain. *l*-PIA shows greater affinity than *d*-PIA for the A_1 receptor, while both compounds have approximately the same affinity for the A_2 receptor.[3] *N*-Ethyladenosine-5′-carboxamide (NECA, **5**) is an agonist that also possesses nanomolar affinity. It is modified in the 5′-position of the ribose ring, while the adenosine base is unchanged. NECA is reported to have a greater A_2 affinity than either *d*- or *l*-PIA, although it does bind to both the A_1 and A_2 receptors.

Potent xanthine antogonists, such as 1,3-diethyl-8-phenylxanthine (DPX, **6**[4] have been developed that have a hundred times greater affinity for adenosine receptors than the classical antagonist theophylline. None of these xanthines is selective for A_1 or A_2 receptors.[5]

Adenosine, acting through its receptor system, is hypothesized to be involved in the regulation of wide range of physiological responses. Among these are the contraction/relaxation of various smooth muscle tissues, including trachea and lung,[6] ileum,[7] and taeni coli.[8] Adenosine has been implicated by several laboratories in the regulation of coronary blood flow.[9] In the peripheral vasculature it causes vasodilation and hypotension.[10] Conversely, it is a vasoconstrictor in the kidney.[11] Adenosine has been proposed as a factor in the contraction of cardiac smooth muscle[12] and may influence platelet aggregation (ADP may be more significant in regards to aggregation).[13] Receptors for adenosine in adipose cells possibly help regulate fat metabolism.[14] Adenosine has been suggested as a neurotransmitter, cotransmitter, or neuromodulator in the brain and at various nerve terminals.[15] Excess adenosine is implicated in some immunological disorders.[16]

The in vivo effects of adenosine antagonists, the methylxanthines, are well documented. Several authors have proposed that an adenosine receptors mechanism may be involved in some of these responses, such as bronchodilatation or tachycardia, rather than phosphodiesterase inhibition. Both of these mechanisms remain controversial.[17]

Adenosine analogues, particularly *l*-PIA, have also been shown to have profound physiological effects. In addition to hypotensive and negative chronotropic responses, sedative activity at very low doses has been observed with these compounds.[18]

Separation of the CNS, cardiac, and other effects of these type of compounds, either by physiochemical or receptor differentiation means, would allow selective development of therapeutically useful drugs. With this in

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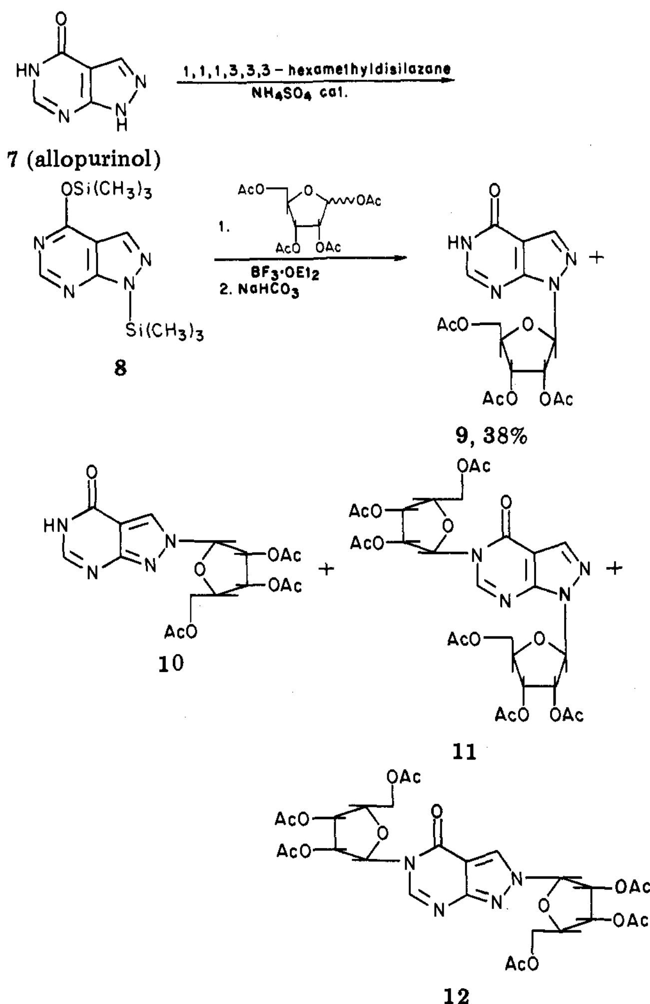
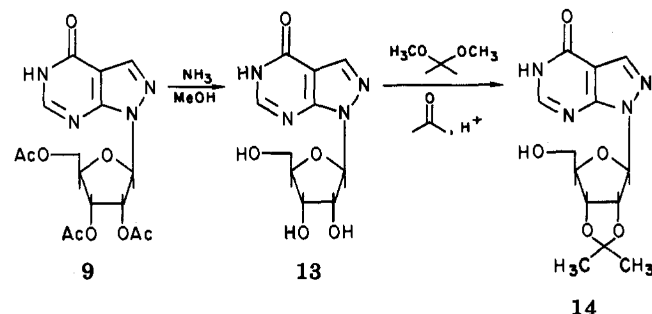
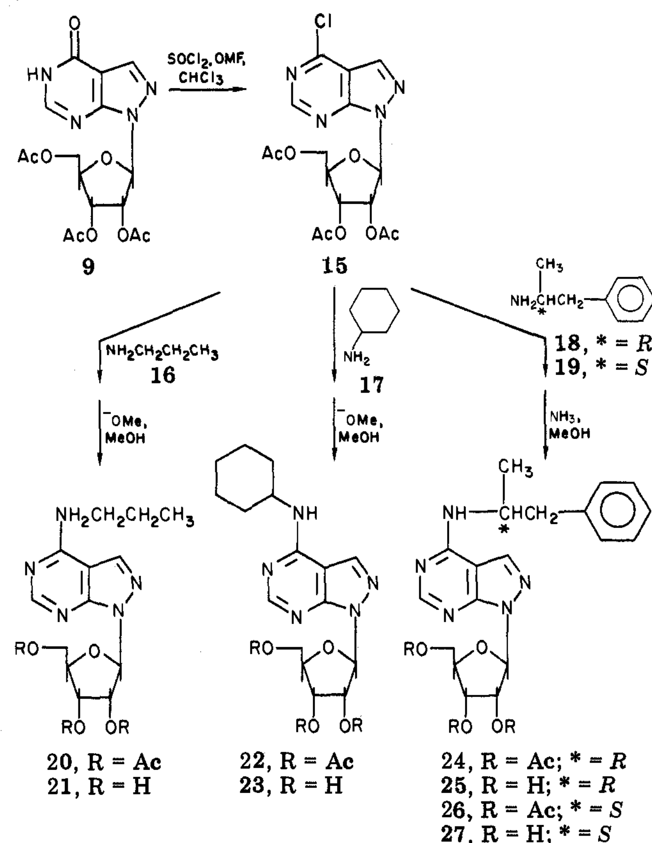
Scheme I



Scheme II



Scheme III



mind we decided to synthesize the ribosylated pyrazolo[3,4-*d*]pyrimidine analogues of *d*- and *l*-PIA, CHA, and N[6]-propyladenosine. These novel compounds were then examined for their adenosine receptor affinity and biological activity.

Chemistry. The synthesis of the pyrazolo[3,4-*d*]pyrimidine analogues of *d*- and *l*-PIA, CHA, and *N*[6]-propyladenosine is shown Scheme I. Ribosylation of allopurinol (**7**) was carried out according to standard procedures developed for pyrimidines and purines utilizing an acylated sugar and Lewis acid catalysis with the silyated derivative of allopurinol.[19] Following bicarbonate workup, a pale yellow syrup was obtained, which showed four spots on thin layer chromatography. There were isolated by preparative chromatography on silica gel, eluting with a chloroform–acetone gradient.

The structural assignments of these four compounds were based on ^{1}H NMR, ^{13}C NMR, IR, elemental analysis, optical rotation, and their UV spectra (See Experimental Section). The UV data are in accordance with literature values for synthetic and naturally occuring ribosylallopurinols, and parallel the UV spectra of the corresponding ribosylated C-4 amino-substituted allopurinols and the methylated and dimethylated allopurinols.[20]

The desired N-1 isomer **9** represented 30% of the reaction mixture. A small amount of material was deacylated (NH_3/MeOH) and compared on analytical HPLC with a sample of the N-1 allopurinol ribose **13** obtained from biological sources.[21] In addition, the 2′,3′-isopropylidene derivative **14** was synthesized in order to determine the proportion of α and β anomers resulting from the fusion of the sugar and base (see Scheme II). Applying Imbach's methodology to the isopropylidene compound[22] and by close examination of the 300-mHz ^{1}H NMR,[23] we found the N-1 isomer **9** to have 90% of the ribose moiety in the β-configuration.

The triacetate **9** was chlorinated by refluxing in thionyl chloride/chloroform with a catalytic amount of *N*,*N*-di-

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Table I

IC_{50}, nM	R	IC_{50}, nM
3.7	$CH(CH_3)CH_2C_6H_5$ [b]	47% inhibn at 10^{-4} M [a]
95.7	$CH(CH_3)CH_2C_6H_5$ [c]	31% inhibn at 10^{-4} M [a]
2.4	$c\text{-}C_6H_{11}$	20 200
4	$CH_2CH_2CH_3$	76 500

[a] IC_{50}'s were not determined for these compounds. [b] * = *R*. [c] * = *S*.

ethylaniline. The chloride **15** is somewhat more labile than the corresponding 6-chloropurine riboside. It was subjected to nucleophilic displacement utilizing 2 equiv of amines **16–19** (See Scheme III). The resulting 4-substituted derivatives **20**, **22**, **24**, and **26** were deprotected most successfully by using methoxide ion, followed by treatment with an acidic ion exchange resin, to afford, after purification, the desired purine analogues **21**, **23**, **25**, and **27**.

Biological Results

Compounds **21**, **23**, **25**, and **27** were evaluated for their ability to displace tritiated CHA in rat brain preparations (see Experimental Section). Table I compares these pyrazolo[3,4-*d*]pyrimidines with their purine counterparts. It is apparent that transposition of a nitrogen atom and a carbon atom within the five-membered ring of the nucleoside structure has a profound effect on affinity for adenosine receptors in this assay.

Compound **23**, the analogue of CHA, was evaluated for cardiovascular[24] and CNS[25] activity. It was found to have no significant effect on blood pressure or heart rate in spontaneously hypertensive rats at 30 mg/kg over 24 h. This is in contrast to the hypotensive and bradycardic responses to CHA seen at doses as low as 0.3 mg/kg in the same test system. Activity of **23** in tests surveying CNS effects was detected only as depression and ptosis at 300 mg/kg. This compares with marked depression, sedation, and passiveness noted with CHA at doses as low as 0.03 mg/kg in the same test system.

Compound **21**, the *N*-propyl analogue, was found to have no significant effect on blood pressure or heart rate in spontaneously hypertensive rats at 30 mg/kg. It produced no change in rate or force of contraction in guinea pig isolated atria at concentrations of up to 10^{-4} M rather than the decrease in rate and force noted with the other purine analogues.[26] As with **23**, slight depression and ptosis were the only central effects seen, again only at 300 mg/kg.

Compound **24**, the triacetate analogue of *l*-PIA, was examined for effects on guinea pig isolated atria. No change from control was seen up to 3×10^{-5} M.

It is obvious the pyrazolo[3,4-*d*]pyrimidine analogues synthesized in this study for comparison with standard adenosine reference agents were found to be significantly less active than the purines, not only in the receptor binding assay but also in biological screens for cardiovascular and CNS activity. This supports the hypothesis of an adenosine-mediated mechanism for vasodilation and bradycardia. The pyrazolo[3,4-*d*]pyrimidine isomers did not show great affinity in the adenosine receptor system, while the purine ribosides have nanomolar affinity in this system. Neither did the analogues exhibit activity in vitro or in vivo, while the purines were cardiac depressants and hypotensive agents at low doses. The parallel between receptor binding and biological activity is illustrated further in the CNS, where sedation and depression were seen only with the compounds that also showed good affinity for the receptor.

This study suggests that adenosine and adenosine agonists act through a receptor system that is sensitive to modification of the purine base. The only modification in this case is the transposition of a carbon and nitrogen in the imidazole/pyrazole ring. It is tempting to postulate that a hydrogen-bonding interaction at N-7 in the purine ring system is necessary for affinity and efficacy. Lack of a heteroatom in this position, as with the pyrazolo[3,4-*d*]pyrimidines, has a dramatic effect on both of these parameters. This is supported further by the lack of activity seen with tubercidin (7-deazaadenosine) and formycin (7-deaza-8-azaadenosine) in binding to adenosine receptors in human fibroblasts.[2a] Physiological selectivity of adenosine analogues would seem to be better controlled by modification of substituents, perhaps at N-6 or the 5′-position of the ribose ring, rather than alteration of the purine base.

Experimental Section

Chemistry. All melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were obtained on the Digilab FTS-14 IR spectrometer; ^1H NMR spectra were obtained on either a Varian EM-390 or Varian XL-300 instrument; all values are reported in parts per million (δ) from $(CH_3)_4Si$. ^{13}C NMR spectra were obtained on a Bruker WH90 FT NMR of Varian XL-200 instrument. Ultraviolet spectra were obtained on a Cary 118 UV/visible spectrometer. Elemental analyses were performed by the Warner-Lambert/Parke-Davis Analytical Chemistry Section. Rotations were obtained on a Perkin-Elmer 141 polarimeter. Thin-layer chromatograhy was performed on Silica Gel 60 F_{254} aluminum-backed plates. Preparative chromatography was conducted on the Waters Prep 500 utilizing silica cartridges.

Starting Materials. Allopurinol was purchased from Aldrich Chemical Co. and purified as noted. (+)-Amphetamine and (−)-amphetamine were synthesized and resolved in these laboratories. Cyclohexylamine was purchased from Eastman Kodak and distilled under vacuum. 1-propylamine was purchased from Aldrich Chemical Co. Reagents and solvents were purified and dried as noted.

Ribosylation of Allopurinol (9–12). Allopurinol (58.0 g, 0.426 mol) (dried over P_2O_5 under vacuum at 110 °C for 18 h), 250 mL of 1,1,1,3,3,3-hexamethyldisilazane (dried over 4Å molecular sieves), and 50 mg of analytical reagent grade ammonium sulfate were combined and refluxed with exclusion of moisture for 14 h. While the solution cooled, white crystals formed, which were filtered and vacuum dried to afford 93 g of **8** (0.332 mol, 78%), mp 94–96 °C (lit.[20] mp 86 °C). Compound **8**, without further purification, was dissolved in 1200 mL of anhydrous dioxane under a nitrogen atmosphere. β-D-Ribofuranose tetracetate (105.0 g, 0.332 mol), dried under vacuum, was added in a single portion.

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(25) CNS symptom etiology was examined in male Swiss-Webster mice (×4) at five dosages. Drugs were administered intraperitoneally in a mixed solvent system (60% Emulfor, 6% glacial acetic acid, 30% Me_2SO, and 4% water), and the mice were observed over 30 min.

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This solution was heated to reflux while 49 mL of freshly distilled boron trifluoride etherate was added dropwise over 2 h. After this addition was complete, the solution was refluxed for another 30 min. Upon cooling, it was poured carefully into 1.6 L of saturated aqueous sodium bicarbonate solution and stirred for 30 min. After filtering, the filtrate was extracted with chloroform (4 × 500 mL), and the combined organic layers were washed with ice–water (2 × 250 mL) and dried over sodium sulfate. Removal of solvent under reduced pressure and further drying in vacuo yielded 137.5 g of a yellow syrup. This syrup was chromatographed on silica gel eluting with 85:15 chloroform/acetone. Six fractions were collected and compared by thin-layer chromatography (eluting with 1:1 chloroform/acetone).

(a) **1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidin-4-one (9, Allopurinol Riboside Triacetate).** The fourth fraction collected (R_f 0.38) yielded 34.0 g (28%) of a hygroscopic white foam, mp 71–73 °C. This was identified as the desired N-1 allopurinol riboside 9 containing >90% β anomer and <10% α anomer, as determined by high-resolution 300-MHz ^{1}H NMR:[23] ^{1}H NMR ($CDCl_3$) δ 11.8 (br s, 1 H), 8.13 (s, 1 H), 7.98 (s, 1 H), 6.45 (d, *J* = 2 Hz, 1 H), 5.86 (d of d, *J* = 2 and 4 Hz, 1 H), 5.76 (d of d, *J* = 4 Hz, 1 H), 4.41 (m, 2 H), 4.17 (d of d, *J* = 4 and 7 Hz, 1 H), 2.1–2.0 (m, 9 H). By 300-MHz expansion: δ 6.45 (d, *J* = 2 Hz), 6.19 (d, *J* = 2.5 Hz, integrated for 9.4% of 6.45 doublet), 5.86 (d of d, *J* = 2 and 4 Hz), 5.76 (d of d, *J* = 4 Hz), 5.48 (d of d, *J* = 2.5 and 3.5 Hz, integrates for 9.1% of 5.86 peaks), 5.41 (d of d, *J* = 3.5 Hz, integrates for 7.9% of 5.76 peaks); UV (methanol) λ_{max} 251 (ε 6657), 270 (pH 11), 251 nm (pH 1); $[\alpha]^{20}$ −18.7° (*c* 1.11, methanol); IR 1750, 1700 cm^{-1}; ^{13}C NMR ($CDCl_3$) 169.9, 168.9, 168.8, 158.2, 152.4, 146.6, 135.6, 105.8, 85.8, 79.0, 73.0, 70.3, 62.5, 19.9, 19.7 ppm. Anal. ($C_{16}H_{18}N_4O_8\cdot{}^3/_4H_2O$) C, H, N.

(b) **1,5-Bis(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidin-4-one (11).** In order of elution, fraction 1 (R_f 0.62) yielded 29.54 g of a syrup, which partially solidified upon standing. The ^{1}H NMR spectrum showed the presence of unreacted β-D-ribofuranose tetraacetate in addition to the heterocyclic compound. This was confirmed by crystallization from methanol, yielding a white powder that was identical with the starting sugar by elemental analysis, IR, and NMR. The methanolic solution was concentrated, subjected to flash chromatography through 100 g of silica gel, and concentrated to yield 11 as a white foam, mp 53–57 °C, which still contained 16% unreacted sugar: ^{1}H NMR ($CDCl_3$) δ 8.15 (s, 1 H), 8.07 (s, 1 H), 6.37 (d, *J* = 3 Hz, 1 H), 6.2 (d, *J* = 4.5 Hz, 1 H), 5.8 (m, 2 H), 5.3 (m, 2 H), 4.0–4.5 (m, 7 H), 2.0–2.2 (m, 22 H); UV (methanol) λ_{max} 252, 251 (pH 1), 253 (pH 11) nm; IR (KBr) 1750, 1712 cm^{-1}; $[\alpha]^{20}$ −6.6° (*c* 1.05, methanol) [lit.[20] $[\alpha]^{20}$ −5.4° (*c* 1.0, methanol)].

(c) **2,5-Bis(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidin-4-one (12).** The second fraction eluted (R_f 0.54) yielded 16.5 g (7.6%) of a hygroscopic white foam, mp 71–73 °C, which was assigned structure 12: ^{1}H NMR ($CDCl_3$) δ 8.28 (s, 1 H), 8.13 (s, 1 H), 6.18 (d, *J* = 2.5 Hz, 1 H) 5.95 (d, *J* = 1.5 Hz, 1 H), 5.75 (d of d, *J* = 1.5 and 2.5 Hz, 1 H), 5.59 (d of d, *J* = 3 Hz, 1 H), 5.45 (m, 2 H), 4.18–4.46 (m, 6 H), 2.02 and 2.12 (m, 18 H); UV (methanol) λ_{max} 261, 260 (pH 1), 260 nm (pH 11); IR 1750, 1710 cm^{-1}; $[\alpha]^{20}$ −30.4° (*c* 1.10, methanol) [lit.[20] $[\alpha]^{20}$ −30.5° (*c* 1.00, methanol)]; ^{13}C NMR ($CDCl_3$) 169.5, 169.4, 168.7, 168.6, 168.5, 168.3, 157.6, 156.7, 145.7, 128.3, 106.0, 91.8, 86.4, 79.8, 79.0, 73.8, 72.7, 69.7, 69.3, 62.2, 62.1, 19.8, 19.5 ppm.

(d) Fraction 3 yielded 9.9 g of a yellow syrup, which showed two compounds by thin-layer chromatography (R_f 0.62 + 0.54). This was not purified further.

(e) Fraction 5 (R_f 0.29) afforded 5.6 g (4.3%) of a pale yellow foam, mp 55–60 °C. This was assigned structure 10: ^{1}H NMR ($CDCl_3$) δ 10.5 (br s, 1 H), 8.34 (s, 1 H), 7.98 (s, 1 H), 5.97 (d, *J* = 3 Hz, 1 H), 5.8 (d of d, *J* = 3.5 Hz, 1 H), 5.64 (d of d, *J* = 5 Hz, 1 H), 4.2–4.6 (m, 3 H), 2.1–2.2 (m, 9 H); UV (methanol) λ_{max} 260, 258 (pH 1), 281 nm (pH 11); IR (KBr) 1750, 1705, 1612 cm^{-1}; $[\alpha]^{20}$ −36.0° (*c* 1.10, methanol); ^{13}C NMR ($CDCl_3$) 169.8, 168.7, 168.6, 158.9, 158.6, 146.5, 127.9, 106.9, 91.8, 79.7, 73.8, 69.7, 62.2, 19.9, 19.6 ppm.

(f) The final fraction, 20.8 g, was a syrup showing two components by thin-layer chromatography (R_f 0.38 + 0.29). No further purification was done on this mixture. However, HPLC analysis (C-18 column) showed the mixture to contain 80% allopurinol riboside triacetate, for a total of 50.62 g (30% based on allopurinol).

1-β-D-Ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4-one (13, Allopurinol Riboside). Compound 9 (0.83 %, 2.1 mmol) was dissolved in 600 mL of methanol saturated with ammonia at 0 °C and stirred at 0 °C while continuing to bubble ammonia through the solution for 16 h. Removal of solvent afforded 0.60 g of a yellow syrup. Precipitation was induced by the addition of 2 mL each of ethyl acetate and ethanol. After filtration, 0.54 g (2.0 mmol, 96%) of a hygroscopic white powder was isolated: mp 161–162 °C; ^{1}H NMR (Me_2SO-d_6) δ 8.08 (s, 1 H), 8.05 (s, 1 H), 6.03 (d, *J* = 4.5 Hz, 1 H), 4.47 (d of d, *J* = 4.5 Hz, 1 H), 4.17 (d of d, *J* = 4.5 Hz, 1 H), 3.85 (d of d, *J* = 4.5 and 11.5 Hz, 1 H), 3.1–3.7 (m, 5 H); UV (methanol) λ_{max} 250 (ε 6.38 × 10^4), 250 (pH 1), 270 (pH 11) nm; IR (KBr) 3400, 1680, 1600 cm^{-1}; $[\alpha]^{20}$ −58.4° (*c* 1.10, DMF). Anal. ($C_{13}H_{16}N_4O_5\cdot{}^1/_2H_2O$) C, H, N.

1-[2,3-*O*-(1-Methylethylidene)-β-D-ribofuranosyl]pyrazolo[3,4-*d*]pyrimidin-4-one (14). Compound 13 (0.13 g, 0.49 mmol) was dissolved in 10 mL of acetone (dried over 4Å molecular sieves) containing 1.5 mL of 2,2-dimethoxypropane. Bis(*p*-nitrophenyl) hydrogen phosphate (0.21 g, 0.61 mmol, 1.25 equiv) was added, and the mixture was stirred at ambient temperature with exclusion of moisture. After 45 min, 15 mL of aqueous 0.1 N $NaHCO_3$ was added, and the reaction was stirred for 2 h. Removal of the acetone under reduced pressure afforded an aqueous solution, which was extracted with chloroform (3 × 100 mL). The combined organic layers were dried ($MgSO_4$) and filtered, and the filtrate was concentrated to a yellow oil. This was taken up in 2 mL of acetone, and precipitation was induced by 20 mL of isopropyl ether. Filtering and drying yielded 0.046 g (0.15 mmol, 30%) of a white amorphous solid: mp 79–82 °C; ^{1}H NMR (Me_2SO-d_6) δ 8.20 (s, 1 H), 8.12 (s, 1 H), 6.27 (d, *J* = 1.5 Hz, 1 H), 5.28 (d of d, *J* = 1.5 and 4.0 Hz, 1 H), 4.92 (d of d, *J* = 1.5 and 4.0 Hz, 1 H), 4.14 (d of t, *J* = 1.5 and 4.0 Hz, 1 H), 3.6 (br s, 1 H, exchanges with D_2O), 3.35 (d of d, *J* = 3.0 and 4.0 Hz, 2 H), 1.51 (s, 3 H), 1.32 (s, 3 H), 1.20 and 1.13 (2 s), these last two singlets integrate for 10% of the 1.51 + 1.32 singlets; IR (KBr) 3420, 1695, 1595 cm^{-1}. Anal. ($C_{13}H_{16}N_4O_5\cdot{}^1/_2H_2O$) C, H, N.

4-Chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (15). Allopurinol riboside triacetate (9; 10.0 g, 25.4 mmol) was dissolved in a solution of 275 mL of chloroform (dried over 4Å molecular sieves), 15 mL of dry DMF, and 60 mL of freshly distilled thionyl chloride. This was refluxed under a nitrogen atmosphere for 4 h and then evaporated to dryness in vacuo. Toluene (75 mL) was added to the residue and distilled to remove traces of thionyl chloride. The resulting solid was added carefully to 200 mL of ice–water, while maintaining the pH between 5 and 8 with Na_2CO_3. The aqueous solution was extracted with chloroform (3 × 250 mL), and the organic layers were combined, dried over sodium sulfate, and evaporated in vacuo to afford a yellow syrup. Addition of petroleum ether induced precipitation. After decanting, the resulting solid was dried with a stream of nitrogen, yielding 5.33 g (12.9 mmol, 51%) of 15: mp 107–110 °C (lit. mp 113–115 °C); ^{1}H NMR ($CDCl_3$) δ 8.77 (s, 1 H), 8.19 (s, 1 H), 6.60 (d, *J* = 3 Hz, 1 H), 5.96 (m, 1 H), 5.76 (t, *J* = 5 Hz, 1 H), 4.1–4.5 (m, 3 H), 2.1–2.2 (m, 9 H).

***N*-Cyclohexyl-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidin-4-amine (22).** Freshly distilled cyclohexylamine (0.96 g, 9.7 mmol) was dissolved in 25 mL of dichloromethane under nitrogen. To this was added dropwise 2.00 g of 15 (4.85 mmol) in 5 mL of dichloromethane. The solution was stirred at ambient temperature for 24 h, after which it was filtered and the solvent was removed under reduced pressure to afford 2.30 g of 22 as a tan foam (4.8 mmol, 99%): mp 58–60 °C; ^{1}H NMR ($CDCl_3$) δ 8.30 (s, 1 H), 7.92 (s, 1 H), 6.53 (d, *J* = 3 Hz, 1 H), 5.9 (d of d, *J* = 3 and 5 Hz, 1 H), 5.75 (t, *J* = 5 Hz, 1 H), 5. 45 (br s, 1 H), 4.0–4.5 (m, 4 H), 2.05–2.15 (m, 9 H), 1.2–2.0 (m, 10 H); UV (methanol) λ_{max} 284 (ε 1.32 × 10^5), 263 (ε 8.74 × 10^4), 226 (sh) nm; IR (KBr) 2935, 1750, 1610 cm^{-1}.

***N*-Cyclohexyl-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (23).** Compound 22 (2.15 g, 4.5 mmol) was dissolved in 15 mL of methanol (dried over 4Å molecular sieves) under a nitrogen atmosphere. Sodium methoxide (0.24 g, 1 equiv) was added at ambient temperature, and the mixture was stirred for 0.5 h. Dowex 50W-X8 H$^+$ resin was added in portions until pH 5. After the solution was filtered, the resin was washed with

methanol. The combined methanolic portions were evaporated in vacuo, and the resulting off-white foam was vacuum dried to afford 1.58 g of **23** (99%): mp 67–73 °C; ^{1}H NMR (Me_2SO-d_6) δ 8.20 (s, 2 H), 8.15 (br d, 1 H, exchanges with D_2O), 6.10 (d, J = 4.5 Hz, 1 H), 5.2–5.8 (m, 3 H, exchanges with D_2O), 4.57 (d of d, J = 4.5 Hz, 1 H), 3.89 (d of d, J = 4.5 and 6 Hz, 1 H), 3.50 (m, 2 H), 1.1–2.1 (m, 10 H); UV (methanol) λ_{max} 284 (ϵ 1.17 × 10^5), 265 nm (ϵ 7.96 × 10^4); IR (KBr) 2950, 1620, 1575 cm^{-1}. Anal. ($C_{16}H_{23}N_5O_4 \cdot {}^1/_2H_2O$) C, H, N.

***N*-Propyl-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4-amine (21).** Substitution of 2.00 g (4.85 mmol) of **15** by 1-propanamine was carried out in a manner analogous to the synthesis of **22**. The resulting 2.28 g of amber oil was subjected to flash chromatography (150 g of silica gel eluting with acetone) and then dissolved in anhydrous methanol under a nitrogen atmosphere. Sodium methoxide (0.26 g) was added in a single portion, and the suspension was stirred for 15 min at ambient temperature. Dowex 50Wx8 acidic resin was added until pH 7, the reaction mixture was filtered, and the resin was washed with methanol. The combined methanolic solutions were concentrated and vacuum dried to yield 1.15 g (77%) of tan foam: mp 69–71 °C; ^{1}H NMR δ 8.35 (t, 1 H, exchanges with D_2O), 8.26 (s, 1 H), 8.20 (s, 1 H), 6.10 (d, J = 5 Hz, 1 H), 4.9–5.4 (br s, 3 H, exchanges with D_2O), 4.60 (d of d, J = 5 Hz, 1 H), 4.22 (d of d, J = 5 Hz, 1 H), 3.92 (d of d, J = 5 and 10 Hz, 1 H), 3.5 (m, 4 H), 1.55 (m, J = 7.5 Hz, 2 H), 0.92 (t, J = 7.5 Hz, 3 H); UV (methanol) λ_{max} 283 (ϵ 1.27 × 10^4), 265 (sh), (pH 11) 282, 265 (sh) nm; IR (KBr) 3400, 3000, 2980, 1625, 1580 cm^{-1}. Anal. ($C_{13}H_{19}N_5O_4 \cdot {}^1/_2CH_3OH$) C, H, N.

(*R*)-*N*-(1-Methyl-2-phenylethyl)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidin-4-amine (24). Compound **15** (5.80 g, 14 mmol) was dissolved in 1:1 1-propanol/dichloromethane (dried over 4Å molecular sieves) at ambient temperature under a nitrogen atmosphere, and then (*R*)-α-methylbenzeneethanamine [(–)-amphetamine, **18**; 9.99 g, 73.9 mmol, 5 M excess] in 10 mL of 1-propanol was added dropwise with stirring. After refluxing for 3 h, a complex mixture was seen on thin-layer chromatography (silica, eluting with chloroform/acetone, 1:1). The reaction mixture was concentrated and chromatographed under medium pressure (200 g of silica gel eluting with chloroform/acetone, 4:1), the fractions with R_f 0.63 (original TLC system) were combined and dried, and the solvent was removed under reduced pressure. Compound **24** (0.94 g, 13%), mp 59–61 °C, was collected as an off-white foam: ^{1}H NMR ($CDCl_3$) δ 8.32 (s, 1 H), 7.86 (s, 1 H), 7.1–7.3 (m, 5 H), 6.54 (d, J = 3 Hz, 1 H), 5.95 (d of d, J = 3 and 5 Hz, 1 H), 5.75 (d of d, J = 5 Hz, 1 H), 5.5 (br d, 1 H), 4.1–4.6 (m, 4 H), 2.8–3.0 (m, 2 H), 2.05–2.2 (m, 9 H), 1.3 (d, J = 6 Hz, 3 H); UV (methanol) λ_{max} 283 (ϵ 14.8 × 10^5), 263 (ϵ 9.57 × 10^4) nm; IR (KBr) 3180, 1750, 1610, 1576 cm^{-1}; $[\alpha]^{20}$ –103° (*c* 0.60, DMF). Anal. ($C_{25}H_{29}N_5O_7 \cdot {}^1/_2H_2O$) C, H, N.

(*R*)-*N*-(1-Methyl-2-phenylethyl)-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (25). Compound **24** (0.20 g, 3.9 × 10^{-4} mol) was dissolved in 100 mL of methanol saturated with ammonia at 0–5 °C. The mixture was stirred at 0 °C for 2 h while bubbling ammonia through it and then stored at 0 °C for 18 h. After the solvents were removed, chromatography (50 g silica gel eluting with acetone/chloroform 1:3) afforded 0.15 g (99%) of white foam, mp 62–64 °C, which liquified upon exposure to air: ^{1}H NMR (Me_2SO-d_6) δ 8.5 (br s, 1 H), 8.21 (s, 1 H), 8.13 (s, 1 H), 7.1–7.25 (m, 5 H), 6.05 (d, J = 5 Hz, 1 H), 5.23 (d of d, J = 5 Hz, 1 H), 5.05 (d of d, J = 4.5 Hz, 1 H), 4.50 (m, 2 H), 4.16 (m, 1 H), 3.84 (d of d, J = 4.5 and 9 Hz, 1 H), 3.48 (m, 2 H), 2.7–3.1 (m, 3 H), 1.18 (d, J = 7 Hz, 3 H); UV (methanol) λ_{max} 284 (ϵ 1.35 × 10^5), 265 (ϵ 8.75 × 10^4) nm; IR (KBr) 3300, 1612, 1575 cm^{-1}; $[\alpha]^{20}$ –94.0° (*c* 1.0, DMF). Anal. ($C_{19}H_{23}N_5O_4 \cdot {}^3/_4H_2O$) C, H, N.

(*S*)-*N*-(1-Methyl-2-phenylethyl)-1-β-D-ribofuranosyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (27). Compound **15** (2.60 g, 6.3 mmol) was added in single portion to 3.90 g of (*S*)-α-methylbenzeneethanamine [(+)-amphetamine; 28.8 mmol, 4.5 M excess) in 25 mL of 1-propanol at ambient temperature under a nitrogen atmosphere. After stirring for 3 h, the reaction mixture was concentrated under reduced pressure. Without further purification, this was added to 500 mL of saturated methanolic ammonia at 0 °C, stirred for 1 h, and then refrigerated for 16 h. Upon removal of solvent, the resulting gum was chromatographed (200 g of silica gel eluting with acetone), the fractions with R_f 0.13 (thin-layer chromatography on silica eluting with 1:1 chloroform/acetone) were combined, and the acetone was removed in vacuo, affording 1.50 g of yellow oil. This was dissolved in a minimum amount of acetone and added dropwise to rapidly stirred isopropyl ether. After repeated precipitation and filtration, 0.16 g of impure **27** was collected as a white hygroscopic amorphous solid: mp 63–67 °C; ^{1}H NMR ($CDCl_3$) δ 8.27 (s, 1 H), 7.77 (s, 1 H), 7.0–7.15 (m, 5 H), 6.20 (d, J = 5 Hz, 1 H), 5.96 (br s, 1 H), 5.58 (m, 1 H), 4.82 (d of d, J = 5 Hz, 1 H), 4.45 (m, 2 H), 4.13 (m, 1 H), 3.7–3.8 (m, 3 H), 2.7–3.05 (m, 2 H), 1.19 (d, J = 6 Hz, 3 H); $[\alpha]^{20}$ +42.4° (*c* 1.033, DMF). Anal. ($C_{19}H_{23}N_5O_4 \cdot H_2O$) H, N; C: calcd, 56.56; found, 57.10.

Pharmacology. *N*^{6}-Cyclohexyl[^{3}H]adenosine Binding in Rat Brain Membranes. Rat brain (minus cerebellum and brain stem) membranes were prepared as described[27] in the presence of adenosine deaminase and incubated in triplicate with 1 nM [^{3}H]CHA at 25 °C for 1 h alone or with test agents. The unbound [^{3}H]CHA was separated by rapid filtration under reduced pressure, and the radiolabeled ligand retained by the filter was counted by liquid scintillation spectrophotometry. Specific binding for [^{3}H]CHA was determined as the total binding minus the binding obtained in the presence of 1 mM theophylline. IC_{50}'s were determined by the analysis of data from four doses (1, 2, 5, and 10 nM) by the following equation:

$$y = b + \frac{(m - b)c}{c + k}$$

where b = total binding, m = nonspecific binding, c = concentration of the drug, y = counts per minute, and k = IC_{50}.

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Registry No. **7**, 315-30-0; **8**, 51642-61-6; **9**, 64372-69-6; **10**, 67221-54-9; **11**, 59957-65-2; **12**, 59957-66-3; **13**, 16220-07-8; **14**, 53762-15-5; **15**, 78586-33-1; **18**, 156-34-3; **19**, 51-64-9; **20**, 86954-44-1; **21**, 86954-43-0; **22**, 86954-41-8; **23**, 86954-42-9; **24**, 86954-45-2; **25**, 86954-46-3; **26**, 86954-48-5; **27**, 86954-47-4; adenosine, 58-61-7; tetraacetyl-β-D-ribofuranose, 13035-61-5; cyclohexylamine, 108-91-8; 1-propanamine, 107-10-8.

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