Pyrazole-Related Nucleosides. Synthesis and Antiviral/Antitumor Activity of Some Substituted Pyrazole and Pyrazolo[4,3-*d*]-1,2,3-triazin-4-one Nucleosides[1]

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Several pyrazole and pyrazolo[4,3-*d*]-1,2,3-triazin-4-one ribonucleosides were prepared and tested for antiviral/antitumor activities. Appropriate heterocyclic bases were prepared by standard methodologies. Glycosylation of pyrazoles **6a–e,g,i** and of pyrazolo[4,3-*d*]-1,2,3-triazin-4-ones **12f–l** mediated by silylation with hexamethyldisilazane, with 1-β-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose, gave in good yields the corresponding glycosides **7a–e,g, 8g,i, 13f,h,k,** and **14f**, but could not be applied to compounds **12g,i,j,l**. To overcome this occurrence, a different strategy involving the preparation, diazotization, and in situ cyclization of opportune pyrazole glycosides **9** and **10** was required. Moreover derivatives having the general formula **5** were considered not only as synthetic intermediates in the synthesis of **3** but also as carbon bioisosteres of ribavirin **4**. All compounds were evaluated in vitro for cytostatic and antiviral activity. The pyrazolo[4,3-*d*]-1,2,3-triazin-4-one nucleosides that resulted were substantially devoid of any activity; only **15h,k** showed a moderate cytostatic activity against T-cells. However, pyrazole nucleosides **9b,c,e** were potent and selective cytotoxic agents against T-lymphocytes, whereas **9e** showed a selective, although not very potent, activity against coxsackie B1.

Introduction

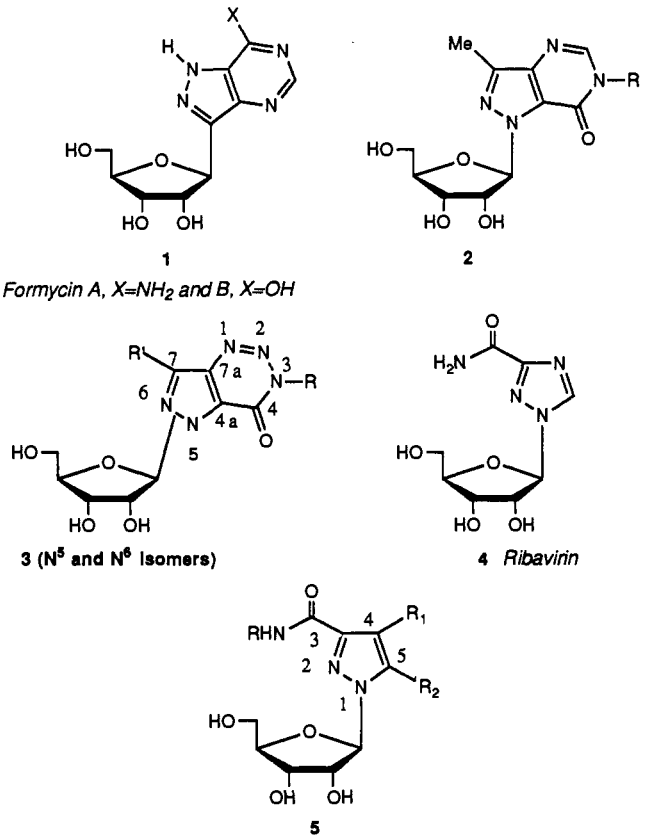
Formycin A and B (**1**), two pyrazolo[4,3-*d*]pyrimidine nucleoside antibiotics, have attracted considerable interest due to their broad spectrum of biological properties.[2,3] Synthetic efforts in this area have led to both sugar- and base-modified analogues[4,5] of **1**. Among the latter is a series of N^1-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-7-one derivatives (**2**), whose synthesis has been previously reported from this laboratory.[6] The significant antiherpes activity shown by some of these compounds and the interesting biological activities of 2-azapurine analogues of natural nucleosides,[7] have led us to design and synthesize new 5-aza analogues[8] of **2**, namely N^5- and N^6-β-D-ribofuranosylpyrazolo[4,3-*d*]-1,2,3-triazin-4-ones (**3**). We also briefly explored pyrazole derivatives having general formula **5**, which were intermediates in the synthesis of **3** and could be considered as carbon bioisosteres of ribavirin[9] (**4**). In fact, a variety of pyrazole analogues[10,11] of ribavirin have been synthesized and the importance of the amido group in conferring to the molecules the property of inducing cellular differentiation has been pointed out.[12] As matter of fact, the synthesis of compounds **9a,b** and **18b** has been already reported,[13,14] and **9a** has been claimed to possess antitumor properties. However, to the best of our knowledge, no biological data have been published yet.

In order to synthesize compounds **3**, a suitable methodology securing the regio- and stereocontrol of the glycosylation reaction was needed. To this end, two different strategies were examined which allowed the preparation of both N^5 and N^6 isomers and represent a useful approach to the synthesis of otherwise unavailable N^6 derivatives in this series.

The title compounds were evaluated in vitro for cytostatic activity and inhibitory effects against DNA and RNA viruses, HIV-1 included.

Chemistry

Pyrazole derivatives **6a–e** were prepared according to reported procedures.[15,16] Compounds **6f–k** were obtained according to a reactions sequence previously developed in our laboratories,[15,17] starting from a 1,6-dioxopiperazine, in turn prepared by reaction of 3-carboxy-4-nitro-5-methylpyrazole with thionyl chloride, with opportune amines. Compound **6l** was prepared by reaction of **6b** with a 30% aqueous ammonia solution (100 °C).[18]



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(1) Presented in part at the Third International Conference on Antiviral Research, Brussels, Belgium, April 1990. Manfredini, S.; Baraldi, P. G.; Guarneri, M.; Marongiu, M. E.; Pani, A.; La Colla, P. Synthesis and in vitro antiviral activity of some pyrazole-related nucleosides. *Antiviral Research.* Supplement 1, **1990**, Abs 55.

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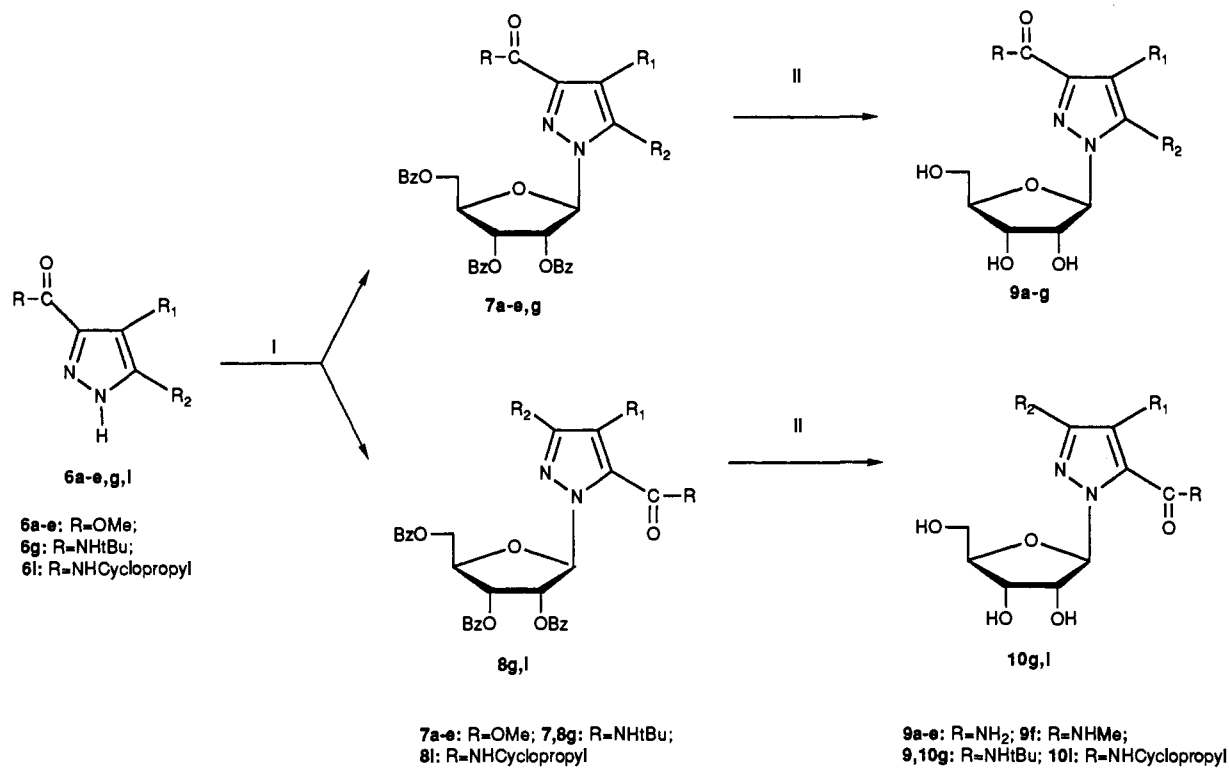
Scheme I[a]



[a] (i) CF_3SO_3H, MeCN, Me_3SiCl, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose; (ii) NH_3/MeOH or $MeNH_2$/MeOH (**9f**). **a**: R_1 = H, R_2 = H; **b**: R_1 = NO_2, R_2 = H; **c**: R_1 = Br, R_2 = H; **d**: R_1 = NO_2, R_2 = Me; **e**: R_1 = I, R_2 = H; **f**: R_1 = NO_2, R_2 = Me; **g**: R_1 = NO_2, R_2 = Me; **i**: R_1 = NO_2, R_2 = Me.

Pyrazole-3-carboxylic acid methyl esters **6a–e** and 4-nitropyrazole-3-(N-substituted)carboxamides **6g,i** (Scheme I) were glycosylated by following and adapting the procedure developed by Vorbruggen and co-workers.[19] N^1-β-D-Ribofuranosyl-3-carbomethoxypyrazoles **7a–e** were obtained in good yields; traces of the α-anomer could be detected only in the case of **7c**, compound **6g** gave both the 3-carboxamido (**7g**) and 5-carboxamido (**8g**) isomers,

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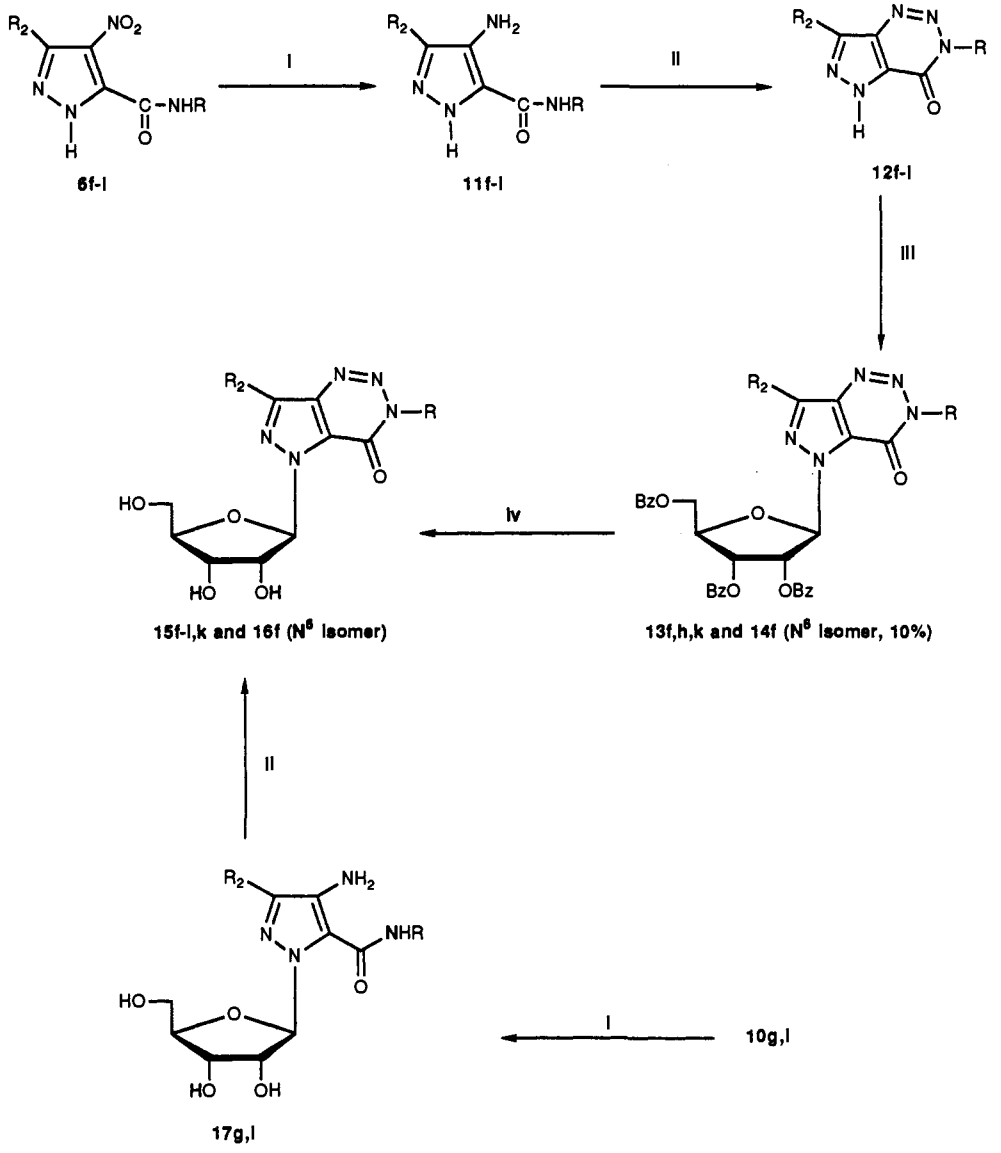
Scheme II[a]



[a] (i) $NaBH_4$, Pd/C 10% or H_2, Pd/C 10%, 20 psi; (ii) $NaNO_2$, AcOH; (iii) CF_3SO_3H, MeCN, Me_3SiCl, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose; (iv) NH_3/MeOH. **f**: R = Me, R_2 = Me; **g**: R = tBu, R_2 = Me; **h**: R = CH_2Ph, R_2 = Me; **l**: R = cyclopropyl, R_2 = Me; **j**: R = cyclopentyl, R_2 = Me; **k**: R = n-C_5H_{11}, R_2 = Me; l: R = H, R_2 = H.

whereas compound **6i** gave only the 5-carboxamido isomer **8i**. Deprotection of these compounds with methanolic ammonia or methanolic methylamine (for compound **7d** to give **9f**) furnished the amides **9a–g** and **10g,i** in appreciable yields.

3-Substituted pyrazolo[4,3-*d*]-1,2,3-triazin-4-ones **12f–l** were prepared by reduction of the 4-nitropyrazole-5-carboxamides **6f–l**[17,18,20] to produce the amines **11f–l**, which were in situ diazotizated[21] and cyclized according to our previously described procedure[17] (Scheme II). Initial attempts[8] using tin(IV) chloride as glycosylation catalyst resulted in very low yields of N^5-nucleoside derivatives as α/β mixtures, likely as a consequence of the basic nature of the heterocyclic base; no traces of N^6 derivatives could be detected. Direct glycosylation of the heterocyclic bases **12f–l**, as described above,[19] usually gave N^5-products as β-anomers (Scheme II); minor amounts of N^6 product, as β-anomer, were obtained in the case of **14f** (10%). Surprisingly, in the case of compounds **12g,i,j,l** glycosylation did not occur or gave very low yields and/or complex mixtures. This was likely due to the steric hindrance of the branched alkyl chain in the silylation step and to side reactions in the case of **12l**. Finally, deprotection of **13f,h,k** and **14f** by treatment with methanolic ammonia at 4 °C (instead of room temperature) proceeded with improved yields, avoiding degradation of the pyrazolo[4,3-*d*]-1,2,3-triazin-4-one moiety. In order to explore a different synthetic approach to the unavailable **13g,i,j,l** and N^6 regioisomers, a different strategy involving intermediates **5** was employed. Thus, compounds **10g,i** (5-carboxamido isomers) were in turn reduced at the nitro group to give **17g,i** (5-carboxamido isomers) (Scheme II); diazotization and in situ cyclization afforded the N^5-β-D-ribofuranosylpyrazolo[4,3-*d*]-1,2,3-triazin-4-ones **15g,i**. Following the same procedure (Scheme III), compounds **9b,d,f,g** (3-carboxamido isomers) were taken to the expected N^6-β-D-ribofuranosylpyrazolo[4,3-*d*]-1,2,3-triazin-

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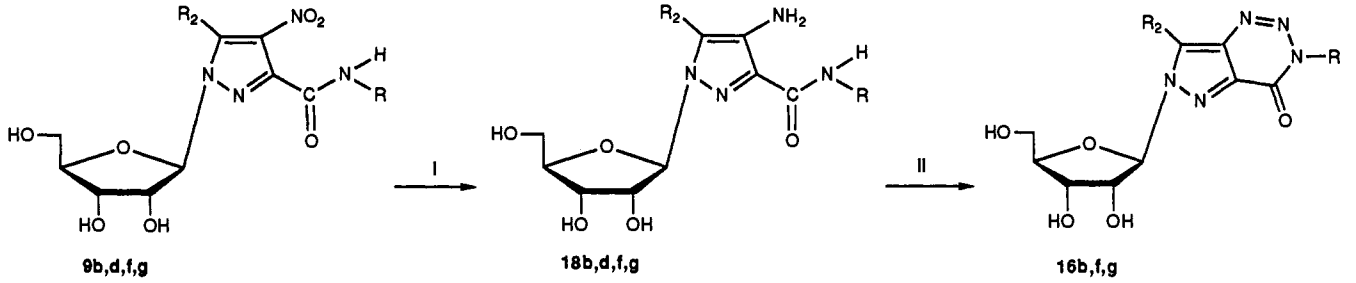
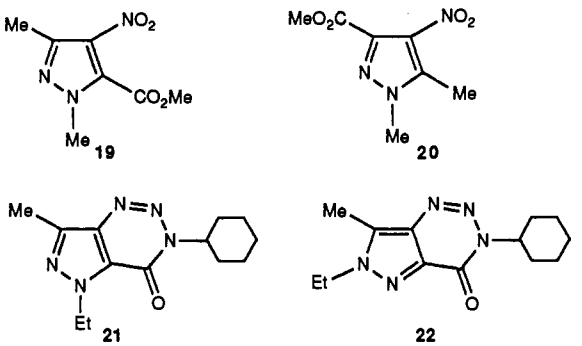
Scheme III[a]



[a] (i) H_2, Pd/C 10%, 20 psi; (ii) $NaNO_2$, AcOH. **b**: R = H, R_2 = H; **d**: R = H, R_2 = Me; **f**: R = Me, R_2 = Me; **g**: R = tBu, R_2 = Me.

4-ones **16b,f,g**. In the case of compound **18d**, complex mixtures of products were observed.

Regiochemistry of glycosylation reactions was determined either by UV spectroscopy or by ^1H and ^{13}C NMR analyses. It has been demonstrated that alkylation of the pyrazolo[3,4-*d*]pyrimidine ring at the N^2-position induces a bathochromic shift in the wavelength of maximum absorption with the respect to the unsubstituted heterocycle or the N^1 isomer.[5] Similarly N^6 isomers in the pyrazolo-[4,3-*d*]-1,2,3-triazin-4-one series showed an indicative bathochromic shift (λ_{max} typical 296 nm in methanol) with respect to the unsubstituted base and the corresponding N^5 isomer (λ_{max} typical 280 nm in methanol); these data were also confirmed by comparison with the UV data of the *N*-alkylpyrazolo[4,3-*d*]-1,2,3-triazin-4-ones **22** and **21** (λ_{max} 295 and 281 nm in methanol, respectively), prepared as model compounds. Similar bathochromic shift was not observed in the pyrazolo nucleosides series. Either the unsubstituted or the 3-carboxamido and 5-carboxamido isomers did not show any significative shift in the UV maximum absorption (λ_{max} typical 275–285 nm in methanol): that has been confirmed by the UV data of the two reference compounds *N*-alkylpyrazoles **19** and **20**, obtained in our previous studies[22] (λ_{max} 273 and 275 nm in methanol, respectively). In this case ^1H and ^{13}C NMR were used to accomplish the structural attributions. However all attributions were further corroborated by ^1H and ^{13}C NMR data, which were comparable to literature values for *N*-alkylpyrazoles.[23-26] Assignment of the anomeric configurations rested on ^1H NMR data: the H-1′ signal of α-anomers was observed at lower fields than that of β ones (**9c**: α = δ 5.97, J = 5.6 Hz; β = δ 5.63, J = 4.2 Hz) and the coupling constant H1′–H2′ usually ranged about 5–7 Hz for α-anomers and 2–4 Hz for β ones.[13,14,27] ^1H NMR can also give additional informations on the regiochemistry: downfield shift in anomeric protons (typical δ 5.7) can be observed in the case of glycosylation of the nitrogen close to the carbonyl group.[13] In the case of compounds **10g,i** (5-carboxamido isomers), the shift of anomeric protons was similar to that of the other regioisomers (3-carboxamido isomers), likely as a consequence of the steric hindrance at the carbonyl level which does not allow the typical deshielding effect on H-1′. However, hydrogenation of the nitro group restored the typical shift-range of glycosylated compounds (**17g,i**; 5-carboxamido isomers). Furthermore, ^{13}C NMR data allowed us to confirm the structures assigned. In agreement with the Pugmuire and Grant principle[26] and the pyrazole literature,[23-25] the C-5, as well as the methyl signal on C-3, of a N^1-glycosylated 5-carboxamidopyrazole nucleus are generally shifted to fields lower than the C-3 and methyl signal on C-5 of the 3-carboxamido isomer.[28]



Biological Results and Discussion

Cytostatic Activity. Compounds **9a–g**, **10g,i**, **15f–i,k**, **16f**, **17g,i**, and **18b,d,g** were tested in vitro for antiproliferative activity against fibroblast-like cells (Vero), murine lymphocytic leukemia (L1210), and human B (Raji) and T (C8166, MT4, and H9) lymphoblastoid cell lines. Some pyrazole derivatives were also tested against H9 cells chronically infected with the HIV-1 (H9/IIIB). Metotrexate (MTX) and adriamycin (ADM), which are of proved efficacy against numerous neoplasias, haematologic malignancies included, were used as reference compounds.

None of the pyrazole and pyrazolo[4,3-*d*]-1,2,3-triazin-4-one nucleosides inhibited the growth of Vero, L1210, and Raji cells at concentrations higher than 1000 μM. By contrast, various pyrazoles selectively inhibited the growth of T-lymphoblastoid cells (Table I). Compounds bearing

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(27) Naik, S. R.; Witkowski, J. T.; Robins, R. K. Synthesis of nucleosides of 5-substituted-1,2,4-triazole-3-carboxamides. *J. Heterocycl. Chem.* **1974**, *11*, 57–61 and references cited therein.

(28) Further informations were drawn from ^{13}C NMR data of some *N*-alkylpyrazoles and *N*-alkylpyrazolo[4,3-*d*]-1,2,3-triazin-4-one derivatives obtained in our previous studies (see refs 19, 22) or prepared as reference compounds (**21**, **22**).

Table I. Cytostatic Activity of Pyrazole and Pyrazolo[4,3-*d*]-1,2,3-triazin-4-one Nucleosides **9a–e**, **15h,k**, and **18b,d**

	ID_{50}[a] (μM)						
compd	Vero	L1210	Raji	C8166	MT4	H9	H9/IIIB
9a	>1030	>1030	1030	900	>1030	1030	>1030
9b	>1730	>1730	>1730	12	80	>1730	>1730
9c	>1550	>1550	>1550	4	20	50	50
9d	>1650	>1650	>1650	>1650	1490	>1650	>1650
9e	>1350	>1350	>1350	8	27	40	20
15h	>1340	>1340	>1340	250	370	800	ND
15k	>1400	>1400	1400	280	540	710	ND
18b	>1830	>1830	1280	320	470	580	550
18d	>1840	>1840	>1840	>1840	>1840	>1840	>1840
MTX	0.02	0.04	0.02	0.03	0.03	0.03	0.03
ADM	0.1	0.02	0.01	0.01	0.01	0.01	0.01

[a] Inhibitory dose 50: compound concentrations required to reduce by 50% the number of cells after three cell cycles. Values represent the mean of three separate experiments. Variation was less than 15%.

Table II. Effect of Pyrazole and Pyrazolo[4,3-*d*]-1,2,3-triazin-4-one Nucleosides **9a–e**, **15h,k**, and **18b,d** on Virus Multiplication

	ED_{50}[a] (μM)					ED_{90}[b] (μM):
compd	HSV-1	ASFV	polio	coxsackie	VSV	HIV-1
9a	>2050	>2050	>2050	>2050	>2050	>900
9b	>1730	>1730	>1730	>1730	>1730	25.4
9c	>1550	>1550	>1550	>1550	>1550	12.2
9d	>1650	>1650	>1650	>1650	>1650	>1650
9e	>1350	>1350	>1350	120	>1350	20
15h	>1340	>1340	>1340	>1340	>1340	>250
15k	>1400	>1400	>1400	>1400	>1400	>200
18b	>1830	>1830	>1830	>1830	>1830	>320
18d	>1840	>1840	>1840	>1840	>1840	>1840
ACQ	0.04	>1300	–	–	–	–
GUAN	–	–	125	–	–	–
AZT	–	–	–	–	–	0.003

[a] Effective dose 50: compound concentrations required to reduce by 50% the number of plaques in Vero cells. Plaque numbers in untreated cultures were 130 (HSV-1), 140 (ASFV), 140 (polio), 120 (coxsackie), 150 (VSV). [b] Effective dose 90: compound concentrations required to reduce by 90% the HIV-1 yield in C8166 cells. Virus titre in untreated controls was 2.3×10^5 $CCID_{50}$/mL.

a bromine (**9c**) or iodine (**9e**) atom at position 4 of the pyrazole ring showed a potent cytostatic activity against all the T-cell lines tested (ID_{50} range = 4–50 μM). The substitution of the halogen atom for a nitro group (**9b**) resulted in a potent and selective cytostatic activity against C8166 and MT4, but not against H9 and H9/IIIB cells. The presence of an amino group at the same position (**18b**) significantly diminished the cytostatic activity, whereas the absence of substituents at position 4 (**9a**), or the addition of a methyl group at position 5 (**9d** and **18d**), abolished the activity. Among pyrazolo[4,3-*d*]-1,2,3-triazin-4-one nucleosides, **15h,k** showed a selective, although not potent cytostatic activity against T-cells (ID_{50} range = 250–800 μM).

When compared to MTX and ADM, **9c** and **9e** were 100–1000-fold less potent as cytostatic agents. However, these pyrazole derivatives showed a 30–400-fold greater selectivity against the panel of T-lymphoblastoid cells tested. Therefore they may be of value for the selective treatment of T-cell malignancies.

At the present time, the mode of action of these compounds is unknown. However, it is perhaps worthy to note that C8166, MT4, and H9/IIIB cells carry and express an integrated retrovirus genome (either HTLV I or HIV), whereas H9 cells are devoid of any DNA provirus. Thus, these results suggest that **9b,c,e** are not targeted at a viral gene product.

Antiviral Activity. Pyrazole and pyrazolo[4,3-*d*]-1,2,3-triazin-4-one derivatives were also tested in vitro against herpes simplex type 1 (HSV-1), african swine fever (ASFV), polio, coxsackie, vescicular stomatitis virus (VSV), and HIV-1. Acycloguanosine (ACG), guanidine (GUAN), and azidothymidine (AZT) were used as reference compounds for HSV-1, polio, and HIV-1, respectively (Table II).

All pyrazolo[4,3-*d*]-1,2,3-triazin-4-one derivatives were devoid of any antiviral activity. Among pyrazole nucleosides, compound **9e** showed a selective, although not potent, activity against the coxsackie virus. Moreover, compounds **9b,c,e** inhibited the HIV-1 multiplication in acutely infected C8166 cells. These antichronically infected cells (data not shown) were obtained at doses affecting cell proliferation. Thus, it can hardly be considered selective.

Experimental Section

Chemistry. Melting points were obtained in open capillary tubes and are uncorrected. Reaction courses and product mixtures were routinely monitored by thin-layer chromatography (TLC) on silica gel precoated F254 Merck plates with detection under 254-nm UV lamp, and/or by spraying the plates with 10% H_2SO_4/MeOH and heating. Nuclear magnetic resonance (^1H NMR, ^{13}C NMR) spectra were determined in d_6-DMSO or $CDCl_3$ solutions with a Bruker AC-200 spectrometer. Peak positions were given in parts per million (δ) downfield from tetramethylsilane as internal standard. Ultraviolet spectra were recorded in methanol on a JASCO 510 spectrometer. Analytical HPLC was performed on a Bruker LC21-C instrument on Rainin (Dynamax 12m) C_{18} columns. Column chromatography was performed with Merck 60–200-mesh silica gel. Room temperature varied between 22 and 25 °C. All drying operations were performed over anhydrous magnesium sulfate. Microanalysis were in agreement with calculated values within ±0.4%.

Starting Materials. Starting compounds **6a–l** were prepared by standard methods according to reported procedures.[6,16,17,18] Pyrazolo[4,3-*d*]-1,2,3-triazin-4-one derivatives **12f–l** were prepared following our previously reported procedure.[17] Reference compounds **19–22**, used for UV and ^1H and ^{13}C NMR studies, have been prepared by standard procedures in our laboratory.[17,22]

General Procedure for Glycosylation of Pyrazole and Pyrazolo[4,3-*d*]-1,2,3-triazin-4-one Derivatives 6a–e,g,i and 12f–l. 1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (3.8 g, 7.5 mmol), hexamethyldisilazane (1.15 mL, 5.5 mmol), and a catalytic

amount of ammonium sulfate were added, under positive nitrogen pressure, to a suspension of each of the heterocycles **6a–e,g,i** and **12f–l** (5 mmol) in 50 mL of freshly dry-distilled acetonitrile. The mixture was heated at reflux conditions, with the exclusion of moisture, until complete dissolution was obtained. Then, trimethylsilyl chloride (0.75 mL, 6 mmol) and trifluoromethanesulfonic acid (1.05 mL, 12 mmol) were added. Reaction courses were monitored with TLC (EtOAc/hexane). After 3 h the reaction mixture was cooled to room temperature, diluted with dichloromethane (50 mL), and extracted with a saturated $NaHCO_3/H_2O$ solution. The aqueous phase was then extracted with dichloromethane (2 × 50 mL), and the combined organic phases were washed with saturated $NaCl/H_2O$ solution, dried, and evaporated. The residue was quickly purified from the lower moving unreacted heterocycles by elution on a silica gel short column (7 cm × 9 cm, 150 g of silica gel, eluent EtOAc/hexane) and then evaporated. Yields on crude residues were the following: **7a**, syrup (98%); **7b**, mp 41 °C (70%); **7c**, syrup (95%); **7d**, syrup (72%); **7e**, syrup (85%); **7g**, syrup (65%); **8g**, syrup (30%); **8i**, syrup (75%); **13f**, syrup (85%); **13h**, mp 70 °C (85%); **13k**, syrup (82%); **14f**, syrup (10%).

A sample of pure material gave analytical data consistent with the assigned structures.

General Procedure for Deprotection of Derivatives 7a–e,g, 8g,i, 13f,h,k, and 14f. The protected compounds (2.5 mmol) were dissolved at 4 °C in 50 mL of methanolic ammonia (**7a–e,g, 8g,i, 13f,h,k,** and **14f**) or methanolic methylamine (**7d**) (both saturated at −15 °C) and let to stir, in a firmly capped flask, at 4 °C for 24 h. After this time TLC (CH_2Cl_2/MeOH, 8/2) indicated complete reaction, and the mixture was evaporated to dryness. The residues were triturated with ether (3 × 50 mL), and the insoluble solids were purified by column chromatography (CH_2Cl_2/MeOH, 8/2). After evaporation of appropriate fractions, the residues were usually crystallized by methanol with diffusion of Et_2O.[29] Analytical, UV ($\epsilon \times 10^{-3}$), and ^1H NMR (δ) data are as follows.

Compound 9a:[13] yield 80%; mp 133–135 °C.

Compound 9b:[14] yield 90%; mp 152–153 °C.

Compound 9c: yield 80%; mp 148–150 °C; UV (MeOH) λ_{max} 207 (11.40); λ shoulder 240 (4.71); ^1H NMR (d_6-DMSO) 3.40–3.70 (m, 2 H, H-5′,5″), 3.91 (m, 1 H, H-4′), 4.12 (m, 1 H, H-3′), 4.3 (m, 1 H, H-2′), 4.98 (t, 1 H, J = 5.5 Hz, OH), 5.17 (d, 1 H, J = 5.4 Hz, OH), 5.52 (d, 1 H, J = 5.8 Hz, OH), 5.63 (d, 1 H, J = 4.2 Hz, H-1′), 7.39 (br s, 1 H, $CONH_2$), 7.57 (br s, 1 H, $CONH_2$), 8.32 (s, 1 H, H-5). Anal. ($C_9H_{12}BrN_3O_5$) C, H, N.

Compound 9d: yield 82%; mp 191–194 °C; UV (MeOH) λ_{max} 204 (11.67), 270 (6.45); λ_{min} 244 (4.12); ^1H NMR (d_6-DMSO) 2.62 (s, 3 H, Me), 3.30–3.60 (m, 2 H, H-5′,5″), 3.92 (m, 1 H, H-4′), 4.16 (m, 1 H, H-3′), 5.56 (m, 1 H, H-2′), 4.75 (t, 1 H, J = 5.7 Hz, OH), 5.20 (d, 1 H, J = 5.8 Hz, OH), 5.50 (d, 1 H, J = 5.8 Hz, OH), 5.78 (d, 1 H, J = 4.0 Hz, H-1′), 7.77 (br s, 1 H, $CONH_2$), 8.01 (br s, 1 H, $CONH_2$). Anal. ($C_{10}H_{14}N_4O_7$) C, H, N.

Compound 9e: yield 74%; mp 181–184 °C; UV (MeOH) λ_{max} 205 (14.28), 257 (2.83); λ_{min} 246 (2.71); ^1H NMR (d_6-DMSO) 3.40–3.70 (m, 2 H, H-5′,5″), 3.92 (m, 1 H, H-4′), 4.11 (m, 1 H, H-3′), 4.31 (m, 1 H, H-2′), 4.95 (t, 1 H, J = 5.5 Hz, OH), 5.14 (d, 1 H, J = 5.4 Hz, OH), 5.49 (d, 1 H, J = 5.8 Hz, OH), 5.65 (d, 1 H, J = 4.3 Hz, H-1′), 7.32 (br s, 1 H, $CONH_2$), 7.52 (br s, 1 H, $CONH_2$), 8.26 (s, 1 H, H-5). Anal. ($C_9H_{12}IN_3O_5$) C, H, N.

Compound 9f: yield 88%; mp 203–205 °C; UV (MeOH) λ_{max} 208 (7.14), 273 (5.63); λ_{min} 238 (2.81); ^1H NMR (d_6-DMSO) 2.62 (s, 3 H, Me), 3.03 (d, 3 H, J = 4.4 Hz, NHMe), 3.30–3.60 (m, 2 H, H-5′,5″), 3.92 (m, 1 H, H-4′), 4.16 (m, 1 H, H-3′), 4.56 (m, 1 H, H-2′), 4.75 (t, 1 H, J = 5.7 Hz, OH), 5.20 (d, 1 H, J = 5.8 Hz, OH), 5.50 (d, 1 H, J = 5.8 Hz, OH), 5.78 (d, 1 H, J = 4.0 Hz, H-1′), 8.01 (br, 1 H, CONH). Anal. ($C_{11}H_{16}N_4O_7$) C, H, N.

Compound 9g: yield 87%; mp 68 °C; UV (MeOH) λ_{max} 205 (15.57), 273 (7.68); λ_{min} 242 (6.75); ^1H NMR (d_6-DMSO) 1.33 (s, 9 H, tBu), 2.64 (s, 3 H, Me), 3.40–3.60 (m, 2 H, H-5′,5″), 3.92 (m, 1 H, H-4′), 4.11 (m, 1 H, H-3′), 4.56 (m, 1 H, H-2′), 4.76 (t, 1 H, J = 5.6 Hz, OH), 5.27 (d, 1 H, J = 5.4 Hz, OH), 5.47 (d, 1 H, J = 5.6 Hz, OH), 5.77 (d, 1 H, J = 4.4 Hz, H-1′), 8.16 (br s, 1 H, CONH). Anal. ($C_{14}H_{22}N_4O_7$) C, H, N.

Compound 10g: yield 93%; syrup; UV (MeOH) λ_{max} 203 (17.46), 274 (8.06); λ shoulder 225 (8.27); λ_{min} 245 (4.36); ^1H NMR (d_6-DMSO) 1.35 (s, 9 H, tBu), 2.43 (s, 3 H, Me), 3.40–3.60 (m, 2 H, H-5′,5″), 3.90 (m, 1 H, H-4′), 4.17 (m, 1 H, H-3′), 4.41 (m, 1 H, H-2′), 4.74 (br, 1 H, OH), 5.40 (br, 1 H, OH), 5.49 (br, 1 H, OH), 5.52 (d, 1 H, J = 3.4 Hz, H-1′), 8.71 (br s, 1 H, CONH). Anal. ($C_{14}H_{22}N_4O_7$) C, H, N.

Compounds 10i: yield 76%; mp 145–146 °C; UV (MeOH) λ_{max} 205 (19.52), 274 (9.59); λ_{min} 242 (4.69); ^1H NMR (d_6-DMSO) 0.55 (m, 2 H, cyclopropyl); 0.76 (m, 2 H, cyclopropyl), 2.49 (s, 3 H, Me), 2.87 (m, 1 H, cyclopropyl), 3.40–3.62 (m, 2 H, H-5′,5″), 3.92 (m, 1 H, H-4′), 4.16 (m, 1 H, H-3′), 4.48 (m, 1 H, H-2′), 4.76 (t, 1 H, J = 5.6 Hz, OH), 5.26 (d, 1 H, J = 5.6 Hz, OH), 5.54 (d, 1 H, J = 5.6 Hz, OH), 5.56 (d, 1 H, J = 3.6 Hz, H-1′), 9.12 (d, 1 H, J = 4.4 Hz, CONH). Anal. ($C_{13}H_{18}N_4O_7$) C, H, N.

Compound 15f: yield 85%; mp 170–173 °C; UV (MeOH) λ_{max} 217 (4.83), 282 (3.77); λ_{min} 245 (1.68); ^1H NMR (d_6-DMSO) 2.75 (s, 3 H, Me), 3.30–3.56 (m, 2 H, H-5′,5″), 3.86 (s, 3 H, NMe), 3.98 (m, 1 H, H-4′), 4.29 (m, 1 H, H-3′), 4.64 (m, 1 H, H-2′), 4.77 (t, 1 H, J = 6.0 Hz, OH), 5.31 (d, 1 H, J = 6.0 Hz, OH), 5.56 (d, 1 H, J = 6.0 Hz, OH), 6.02 (d, 1 H, J = 3.8 Hz, H-1′). Anal. ($C_{11}H_{15}N_5O_5$) C, H, N.

Compound 15h: yield 80%; mp 137 °C; UV (MeOH) λ_{max} 210 (21.76), 281 (9.81); λ_{min} 248 (3.31); ^1H NMR (d_6-DMSO) 2.65 (s, 3 H, Me), 3.40–3.70 (m, 2 H, H-5′,5″), 4.00 (m, 1 H, H-4′), 4.10 (m, 1 H, H-3′), 4.60 (m, 1 H, H-2′), 4.80 (t, 1 H, J = 5.7 Hz, OH), 5.28 (d, 1 H, J = 5.5 Hz, OH), 5.54 (d, 1 H, J = 5.7 Hz, OH), 5.66 (s, 2 H, CH_2), 6.45 (d, 1 H, J = 4.2 Hz, H-1′), 7.40 (m, 5 H, Ar). Anal. ($C_{17}H_{19}N_5O_5$) C, H, N.

Compound 15k: yield 80%; mp 107–109 °C; UV (MeOH) λ_{max} 204 (11.43), 281 (9.70); λ_{min} 250 (4.23); ^1H NMR (d_6-DMSO) 0.87 (m, 3 H, Me), 1.30 (m, 4 H, CH_2), 1.79 (m, 2 H, CH_2), 2.60 (s, 3 H, Me), 3.40–3.70 (m, 2 H, H-5′,5″), 3.95 (m, 1 H, H-4′), 4.27 (m, 1 H, H-3′), 4.40 (m, 2 H, CH_2-N), 4.55 (m, 1 H, H-2′), 4.79 (t, 1 H, J = 5.7 Hz, OH), 5.27 (d, 1 H, J = 5.5 Hz, OH), 5.53 (d, 1 H, J = 5.7 Hz, OH), 6.44 (d, 1 H, J = 4.1 Hz, H-1′). Anal. ($C_{15}H_{23}N_5O_5$) C, H, N.

Compound 16f: yield 90%; mp 158–160 °C; UV (MeOH) λ_{max} 213 (22.89), 296 (8.41); λ_{min} 252 (2.84); ^1H NMR (d_6-DMSO) 2.75 (s, 3 H, Me), 3.30–3.56 (m, 2 H, H-5′,5″), 3.87 (s, 3 H, NMe), 3.99 (m, 1 H, H-4′), 4.26 (m, 1 H, H-3′), 4.65 (m, 1 H, H-2′), 4.78 (t, 1 H, J = 6.0 Hz, OH), 5.32 (d, 1 H, J = 6.0 Hz, OH), 5.58 (d, 1 H, J = 6.0 Hz, OH), 6.02 (d, 1 H, J = 3.8 Hz, H-1′). Anal. ($C_{11}H_{15}N_5O_5$) C, H, N.

Preparation of Substituted 4-Amino-N^1-β-D-Ribofuranosylpyrazole-3-carboxamides and -5-carboxamides 18b,d,f,g and 17g,i. A solution of **9b,d,f,g** and **10g,i** (3.3 mmol) in methanol (50 mL) was bubbled for 10 min with dry nitrogen, and then 10% palladium on charcoal (0.1 g) was added. The suspension was shaken at 20 psi in a Parr apparatus until the absorption of hydrogen had ceased (approximately 4 h). The catalyst was removed by filtration on a Celite pad, and the cake was washed with methanol (4 × 50 mL). The filtrate was evaporated in vacuo to give crude compounds which were crystallized from MeOH with diffusion of Et_2O.[29] Analytical, UV ($\epsilon \times 10^{-3}$), and ^1H NMR (δ) data follow.

Compound 17g: yield 68%; mp 160–162 °C; UV (MeOH) λ_{max} 203 (8.33), 235 (8.48), 278 (5.28); λ_{min} 218 (6.97), 256 (4.90); ^1H NMR (d_6-DMSO) 1.38 (s, 9 H, tBu), 2.09 (s, 3 H, Me), 3.25–3.65 (m, 2 H, H-5′,5″), 3.83 (m, 1 H, H-4′), 4.16 (m, 3 H, H-3′), 4.49 (m, 1 H, H-2′), 4.79 (t, 1 H, J = 5.6 Hz, OH), 5.02 (d, 1 H, J = 5.6 Hz, OH), 5.22 (d, 1 H, J = 5.3 Hz, OH), 6.43 (d, 1 H, J = 3.6 Hz, H-1′), 8.29 (s, 1 H, CONH). Anal. ($C_{14}H_{24}N_4O_5$) C, H, N.

Compound 17i: yield 77%; mp 50–51 °C; UV (MeOH) λ_{max} 202 (8.05), 238 (8.30), 286 (4.39); λ_{min} 218 (7.09), 268 (3.98); ^1H NMR (d_6-DMSO) 0.53 (m, 2 H, cyclopropyl), 0.69 (m, 2 H, cyclopropyl), 2.06 (s, 3 H, Me), 2.78 (m, 1 H, cyclopropyl), 3.15–3.56 (m, 2 H, H-5′,5″), 3.78 (m, 1 H, H-4′), 4.11 (m, 1 H, H-3′), 4.18 (br, 2 H, NH_2), 4.40 (m, 1 H, H-2′), 4.74 (t, 1 H, J = 5.8 Hz, OH), 4.98 (d, 1 H, J = 5.9 Hz, OH), 5.19 (d, 1 H, J = 5.5 Hz, OH), 6.25 (d, 1 H, J = 3.4 Hz, H-1′), 8.21 (d, 1 H, J = 4.2 Hz, CONH). Anal. ($C_{13}H_{20}N_4O_5$) C, H, N.

Compound 18b:[14] yield 70%; mp 153–155 °C.

(29) Robins, M. J.; Mengel, R.; Jones, R. A.; Fouron, Y. Nucleic acid related compounds. 22. Transformation of ribonucleoside 2′,3′-O-ortho esters into halo, deoxy, and epoxy sugar nucleosides using acyl halides. Mechanism and structure of products. *J. Am. Chem. Soc.* **1976**, *98*, 8204–8213.

Compound 18d: yield 74%; mp 156–160 °C; UV (MeOH) λ_{max} 205 (13.86), 287 (3.34); λ_{min} 255 (1.24); ^{1}H NMR (d_6-DMSO) 2.13 (s, 3 H, Me), 3.20–3.70 (br, 2 H, NH_2), 3.30–3.62 (m, 2 H, H-5′,5″), 3.88 (m, 1 H, H-4′), 4.20 (m, 1 H, H-3′), 4.58 (m, 1 H, H-2′), 4.75 (br, 1 H, OH), 5.07 (br, 1 H, OH), 5.38 (br, 1 H, OH), 5.56 (d, 1 H, J = 4.2 Hz, H-1′), 7.11 (br s, 1 H, $CONH_2$), 7.20 (br s, 1 H, $CONH_2$). Anal. ($C_{10}H_{16}N_4O_5$) C, H, N.

Compound 18f: yield 80%; mp 160–163 °C; UV (MeOH) λ_{max} 204 (14.41), 287 (3.88); λ_{min} 258 (2.04); ^{1}H NMR (d_6-DMSO) 2.13 (s, 3 H, Me), 2.72 (d, 3 H, J = 4.6 Hz, CONHMe), 3.30–3.60 (m, 2 H, H-5′,5″), 3.85 (m, 1 H, H-4′), 4.18 (m, 1 H, H-3′), 4.45 (s br, 2 H, NH_2); 4.58 (m, 1 H, H-2′), 4.73 (t, 1 H, J = 5.3 Hz, OH), 5.03 (d, 1 H, J = 5.5 Hz, OH), 5.33 (d, 1 H, J = 5.4 Hz, OH), 5.54 (d, 1 H, J = 4.1 Hz, H-1′), 7.73 (q, 1 H, J = 4.6 Hz, CONH). Anal. ($C_{11}H_{18}N_4O_5$) C, H, N.

Compound 18g: yield 81%; mp 142–143 °C; UV (MeOH) λ_{max} 205 (18.49), 288 (4.99), 230 (7.12); λ_{min} 260 (3.01); ^{1}H NMR (d_6-DMSO) 1.38 (s, 9 H, tBu), 2.15 (s, 3 H, Me), 3.35–3.60 (m, 2 H, H-5′,5″), 3.87 (m, 1 H, H-4′), 4.13 (m, 1 H, H-3′), 4.47 (br s, 2 H, NH_2), 4.54 (m, 1 H, H-2′), 4.83 (t, 1 H, J = 5.7 Hz, OH), 5.13 (d, 1 H, J = 5.6 Hz, OH), 5.33 (d, 1 H, J = 6.0 Hz, OH), 5.56 (d, 1 H, J = 4.6 Hz, H-1′), 6.8 (br s, 1 H, CONH). Anal. ($C_{14}H_{24}N_4O_5$) C, H, N.

Preparation of Substituted N^5- and N^6-β-D-Ribofuranosylpyrazolo[4,3-*d*]-1,2,3-triazin-4-ones 15g,i and 16b,f,g. A solution of sodium nitrite (0.164 g, 2.4 mmol) in H_2O (10 mL) was added dropwise to a cooled (0 °C) solution of **17g,i** and **18b,d,f,g** (2.2 mmol) in an acetic acid/water mixture (3:1, 40 mL). After 30 min TLC (CH_2Cl_2/MeOH, 8/2) indicated complete reaction. The solution was concentrated to small volume (2 mL) and made alkaline with concentrated ammonium hydroxide, and the pH was adjusted to 6.5 using Dowex 50W (H^+). The mixture was filtered and evaporated to dryness to give a syrup which was finally purified by column chromatography (CH_2Cl_2/MeOH, 9/1). Evaporation of appropriate fractions gave the expected compounds **15g,i** and **16b,f,g**. Crystallizations were conduced in MeOH with diffusion of Et_2O.[29] Analytical, UV ($\epsilon \times 10^{-3}$), and ^{1}H NMR (δ) data follow.

Compound 15g: yield 85%; syrup; UV (MeOH) λ_{max} 206 (22.46), 277 (10.06); λ_{min} 247 (4.02); ^{1}H NMR (d_6-DMSO) 1.74 (s, 9 H, tBu), 2.59 (s, 3 H, Me), 3.38–3.62 (m, 2 H, H-5′,5″), 3.96 (m, 1 H, H-4′), 4.23 (m, 1 H, H-3′), 4.56 (m, 1 H, H-2′), 4.79 (br, 1 H, OH), 5.32 (br, 1 H, OH), 5.50 (br, 1 H, OH), 6.48 (d, 1 H, J = 4.1 Hz, H-1′). Anal. ($C_{14}H_{21}N_5O_5$) C, H, N.

Compound 15i: yield 70%; syrup; UV (MeOH) λ_{max} 212 (19.90), 282 (9.01); λ_{min} 249 (3.40); ^{1}H NMR (d_6-DMSO) 1.14 (m, 4 H, cyclopropyl), 2.57 (s, 3 H, Me), 3.52–3.96 (m, 4 H, H-5′,5″, H-4′, H-cyclopropyl), 4.22 (m, 1 H, H-3′), 4.53 (m, 1 H, H-2′), 4.76 (br, 1 H, OH), 5.28 (br, 1 H, OH), 5.52 (br, 1 H, OH), 6.42 (d, 1 H, J = 4.1 Hz, H-1′). Anal. ($C_{13}H_{17}N_5O_5$) C, H, N.

Compound 16b: yield 60%; mp 240 °C dec; UV (MeOH) λ_{max} 205 (4.73), 275 (2.03); λ_{min} 247 (0.13); ^{1}H NMR (d_6-DMSO) 3.47–3.78 (m, 2 H, H-5′,5″), 3.98 (m, 1 H, H-4′), 4.23 (m, 1 H, H-3′), 4.64 (m, 1 H, H-2′), 4.77 (t, 1 H, J = 6.0 Hz, OH), 5.30 (d, 1 H, J = 5.8 Hz, OH), 5.56 (d, 1 H, J = 5.8 Hz, OH), 5.97 (d, 1 H, J = 3.3 Hz, H-1′), 9.30 (s, 1 H, H-7), 15.00 (s, 1 H, CONH). Anal. ($C_9H_{11}N_5O_5$) C, H, N.

Compound 16f: yield 65%; mp 156–160 °C; ^{1}H NMR and UV data of this compound have been already reported in this paper. Anal. ($C_{11}H_{15}N_5O_5$) C, H, N.

Compound 16g: yield 62%; mp 72–73 °C; UV (MeOH) λ_{max} 204 (24.35), 287 (7.86), 297 (7.89); λ_{min} 256 (4.01), 292 (7.79); ^{1}H NMR (d_6-DMSO) 1.69 (s, 9 H, tBu), 2.73 (s, 3 H), 3.40–3.60 (m, 2 H), 3.99 (m, 1 H), 4.24 (m, 1 H), 4.63 (m, 1 H), 4.77 (t, 1 H, J = 5.6 Hz), 5.29 (d, 1 H, J = 5.5 Hz), 5.55 (d, 1 H, J = 5.4 Hz), 6.00 (d, 1 H, J = 3.6 Hz). Anal. ($C_{14}H_{21}N_5O_5$) C, H, N.

Biochemistry. Cells. The following cells were used: H9, CD4$^+$ T-cells which are permissive to HIV replication but are partially resistant to its cytopathyc effect; H9/IIIB, a H9 subline which is persistently infected with the HTLV-IIIB (HIV-1) virus; C8166 and MT4, CD4$^+$ T-cells containing a HTLV-I genome of which only the tat gene is expressed. In C8166 cells the HIV-1 induces an easy detectable, syncytium-forming cytopathic effect (CPE); L1210, lymphocytic mouse leukemia cells; Raji, human lymphoblast-like cells from a Burkitt lymphoma. All these cell lines were grown at 37 °C in a CO_2 incubator in RPMI-1640 medium supplemented with 10% foetal calf serum (FCS), 100 units/mL penicillin and 100 mg/mL streptomycin. In addition, Vero (African green monkey kidney) monolayers were used. These cells were grown in Dulbecco's modified MEM supplemented with 10% newborn calf serum (NCS) and antibiotics. The absence of mycoplasma contamination was checked periodically.

Viruses. The HIV-1 used in the assays was obtained from culture supernatants of H9/IIIB cells collected at the end of an exponential growth phase. Aliquots were made and stored at −70 °C. The titre of virus stock solutions varied between 2 and 4 × 10^5 cell culture infectious doses fifty ($CCID_{50}$) per mL. Virus stocks of Herpes simplex type 1 (HSV-1, ATCC VR 733), African Swine Fever (ASFV, Lisbona 60 strain), Vescicular Stomatitis (VSV, ATCC VR 158), Polio type 1 (Sabin strain), and Coxsackie B1 (Coxs., ATCC VR 28) were obtained in Vero cells and had a titre of 5 × 10^7 plaque forming units (PFU) per mL, 2 × 10^7 PFU per mL, 3 × 10^7 PFU per mL, 5 × 10^7 PFU per mL, 1 × 10^8 PFU mL, respectively.

Cytostatic Evaluation. L1210, Raji, C8166, MT4, H9, and H9/IIIB cells were suspended at a density of 1 × 10^5 cells per mL in growth medium and cultured with various concentrations of the compounds. Cell numbers were determined with a Coulter counter after incubation at 37 °C for 72 h (36 h in the case of L1210). Vero cells were seeded at a density of 1 × 10^5 cells per mL and allowed to adhere overnight. Growth medium containing various concentrations of the compounds was then added. After a 3-day incubation at 37 °C, the number of cells was determined with a Coulter counter after trypsinization of the monolayers. Cells growth at each dose level was expressed as a percentage of growth in untreated controls and dose resulting in 50% inhibition of growth was determined by linear regression technique.

Anti-HIV Assays. Exponentially growing C81166 cells were suspended at a density of 1 × 10^8 cells per mL and then infected with 1 × 10^5 $CCID_{50}$ of HIV-1. After a 2-h incubation at 37 °C, the inoculum was removed, the cells were washed three times and then were resuspended at 1 × 10^5 per mL in RPMI-1640 containing 10% FCS, in the absence or in the presence of the test compounds. After a 3-day incubation at 37 °C, the number of syncytia was evaluated at the inverted microscope and the amount of infectious virus produced was determined by end-point titration.

HIV Titration. Titration of HIV was performed in C8166 cells by the standard limiting dilution method (dilution 1:2, four replica wells per dilution) in 96-well plates. After 4 days at 37 °C syncytia were scored at the light microscope and virus titres were expressed as $CCID_{50}$ per mL according to the Reed and Muench method.

Antiviral Assays. Plaque-reduction tests were performed according to Collins and Bauer in Vero cell monolayers. The plaque counts obtained in the presence of the compounds were expressed as percentage of those obtained in untreated controls and plotted against the logarithm of drug concentrations. Dose–response lines were drawn by linear regression technique, and 50% effective doses (ED_{50}) were calculated.

Acknowledgment. This work was supported in part by grant no. 4206 37 from Istituto Superiore di Sanità (Progetto AIDS 1989) and Ministero della Ricerca Scientifica e Tecnologica (research grant 40%). The authors wish to thank the NMR staff: Prof. A. C. Veronese for discussions, Dr. A. Casolari and Mr. P. Orlandini for taking the NMR spectra, and Mr. V. Periotto for skillful technical assistance.

Registry No. **6a**, 15366-34-4; **6b**, 138786-86-4; **6c**, 81190-89-8; **6d**, 27116-86-5; **6e**, 136944-79-1; **6f**, 68375-43-9; **6g**, 138786-87-5; **6h**, 80030-79-1; **6i**, 138786-88-6; **6j**, 138787-22-1; **6k**, 138787-23-2; **6l**, 65190-36-5; **7a**, 138786-89-7; **7b**, 138786-90-0; **7c**, 138786-91-1; **7d**, 138786-92-2; **7e**, 138786-93-3; **7g**, 138786-94-4; **8g**, 138786-95-5; **8i**, 138786-96-6; **9a**, 138786-97-7; **9b**, 138786-98-8; **9c**, 138786-99-9; **9d**, 138787-00-5; **9e**, 138787-01-6; **9f**, 138787-02-7; **9g**, 138787-03-8; **10g**, 138787-04-9; **10i**, 138787-05-0; **11f**, 68375-44-0; **11g**, 138787-24-3; **11h**, 80030-85-9; **11i**, 138787-25-4; **11j**, 138787-26-5; **11k**, 138787-27-6; **11l**, 67221-50-5; **12f**, 138787-28-7; **12g**, 138787-29-8; **12h**, 115869-70-0; **12i**, 138787-30-1; **12j**, 138787-31-2; **12k**, 138787-32-3; **12l**, 138787-33-4; **13f**, 138787-06-1; **13h**, 138787-07-2; **13k**, 138787-08-3; **14f** N^6-isomer, 138787-09-4; **15f**, 138787-10-7; **15h**, 138787-11-8; **15i**, 138787-12-9; **15k**, 138787-13-0;

16b, 138787-20-9; 16f N^6-isomer, 138787-14-1; 16g, 138787-21-0; 17g, 138787-15-2; 17i, 138787-16-3; 18b, 61241-10-9; 18d, 138787-17-4; 18f, 138787-18-5; 18g, 138787-19-6; 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose, 6974-32-9.

Supplementary Material Available: Table I, analytical and spectral data (^{1}H NMR) of compounds **7a–e,g**, **8g,i**, **13f,h,k**, and **14f**; Table II, spectral data, ^{13}C NMR and ^{1}H NMR (anomeric protons), of compounds **9a–g**, **10g,i**, **17g,i**, **18b,d,f,g**, **19**, and **20**; Table III, spectral data, ^{13}C NMR and ^{1}H NMR (anomeric protons), of compounds **15f–i,k**, **16b,f,g**, **21**, and **22**; Table IV, cytostatic activity for all tested compounds; and Table V, antiviral activity for all tested compounds (9 pages). Ordering information is given on any current masthead page.

8-Polycycloalkyl-1,3-dipropylxanthines as Potent and Selective Antagonists for A_1-Adenosine Receptors

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With the aim of characterizing the hydrophobic interactions between xanthines and the A_1 receptor site, 1,3-dipropyl-8-substituted xanthines were synthesized. Introduction of a quaternary carbon and the conformationally restricted cyclopentyl moiety into the 8-position of xanthines enhanced the adenosine A_1 antagonism. 1,3-Dipropyl-8-(3-noradamantyl)xanthine (**42**) was identified to be a selective and the most potent A_1 receptor antagonist reported to date. Under our structure–activity relationship, the 8-substituent of xanthine antagonists and the N^6-substituent of adenosine agonists appears to bind to the same region of the A_1 receptor.

Introduction

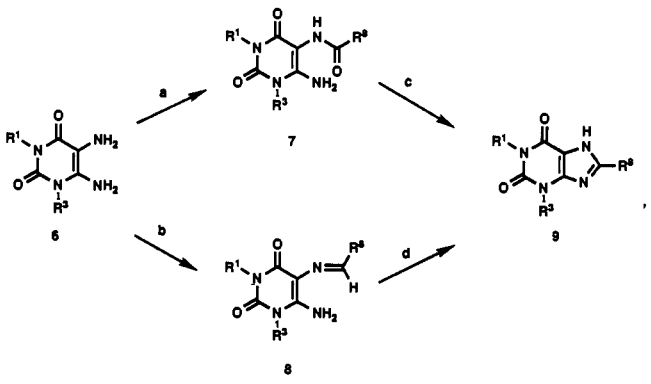
Adenosine elicits a wide variety of physiological responses[1] via interactions with two major subtypes of extracellular receptors, designated as A_1 and A_2. The two receptor subtypes were originally defined in terms of different effects on adenylate cyclase.[2,3] The A_1 receptor inhibits adenylate cyclase, whereas the A_2 receptor is stimulatory to this enzyme.

Considerable efforts to search for selective antagonists have been invested in order to elucidate the physiological role of adenosine and develop therapeutic agents.[4,5] Theophylline (**1**) and caffeine (**2**) (Figure 1) exert pharmacological effects primarily through blockade of adenosine receptors.[6] However, they are virtually nonselective antagonists and have weak affinity for A_1 and A_2 receptors. Studies of structure–activity relationships of xanthines[7-13] revealed that alkyl substitution such as propyl group at the 1- and 3-positions markedly increased affinity to A_1 and A_2 receptors. On the other hand, introduction of a hydrophobic substituent into the 8-position resulted in potent and selective A_1 antagonists such as 8-cyclopentyl-1,3-dipropylxanthine (**4**)[10,13,14] and 8-(dicyclopropylmethyl)-1,3-dipropylxanthine (**5**).[15] Although cycloalkyl or phenyl substitution at the 8-position of 1,3-dialkylxanthines was discovered to increase affinity to adenosine receptors, it is still uncertain what kinds of hydrophobic space at the 8-position are needed for the activity and selectivity.[16] As part of a program to develop adenosine A_1 antagonists as therapeutic agents, we synthesized a series of xanthines bearing a 5-membered heterocyclic, bulky alkyl, or cycloalkyl group at the 8-position and examined effects of substituents on A_1 and A_2 adenosine receptor binding.

Chemistry

Synthetic methods are outlined in Scheme I. Acylation of the appropriate 5,6-diaminouracil[17] (**6**) with a carboxylic acid or its acid chloride, followed by treatment with aqueous sodium hydroxide or phosphorous oxychloride

Scheme I[a]



[a] (a) R^8COCl, Py or R^8CO_2H, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride, dioxane–H_2O. (b) R^8CHO, AcOH, EtOH. (c) NaOH (aq), dioxane, reflux or $POCl_3$, reflux. (d) $FeCl_3$, EtOH, reflux.

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