modified Eagle's MEM supplemented with 50 units/mL of penicillin, 50 μg/mL of streptomycin, 2 mM L-glutamine, and 15% fetal bovine serum under 10% CO_2. To assess the capacity of compounds to induce erythroid differentiation, parental Friend cells ((7-8) × 10^4 cells/mL) in exponential growth were incubated with potential inducers, employing graded 2.5-fold increases in concentration. On day 3, the cell number was determined on a Coulter Model ZBI particle counter and the percent growth inhibition was calculated based on log cell number as described previously.[15] On day 6, the proportion of differentiated cells was determined cytologically by measuring the number of hemoglobin-containing cells that stained blue with an acid solution of 3,3',5,5'-tetramethylbenzidine peroxide[25] as described by Orkin et al.[26] The nucleosides were dissolved in either hot water or 0.02-0.2 N NaOH depending upon their solubility. Each compound was tested in two separate experiments where Me_2SO served as the positive control and caused 70% differentiation (Table I).

Acknowledgment. This research was supported in part by U.S. Public Health Service Grant CA-02817 from the National Cancer Institute.

Registry No. 1, 118-00-3; 2, 4016-63-1; 3, 22423-08-1; 4, 22423-10-5; 5, 28128-41-8; 6, 13389-05-4; 7, 7057-52-5; 8, 13389-07-6; 9, 3868-36-8; 10, 3868-31-3; 11, 7057-53-6; 12, 26001-38-7; 13, 2104-66-7; 14, 7057-50-3; 15, 961-07-9; 16, 13389-03-2; 17, 96964-89-5; 18, 96964-90-8; 19, 88847-89-6.

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Synthesis and Biological Activity of 5-Thiobredinin and Certain Related 5-Substituted Imidazole-4-carboxamide Ribonucleosides[1,2]

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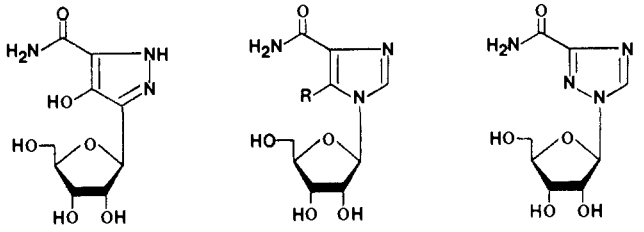
A number of 5-substituted imidazole-4-carboxamide ribonucleosides were prepared and tested for their biological activity. Treatment of 5-chloro-1-β-D-ribofuranosylimidazole-4-carboxamide (**2**) with methanethiol provided 5-(methylthio)-1-β-D-ribofuranosylimidazole-4-carboxamide (**3a**). Similar treatment of **2** with ethanethiol or benzenemethanethiol gave the corresponding 5-ethylthio and 5-benzylthio derivatives **3b** and **3c**. Oxidation of **3a** and **3b** with *m*-chloroperoxybenzoic acid furnished the corresponding sulfonyl derivatives **4a** and **4b**. Reductive cleavage of **3c** with sodium naphthalene or Na/NH_3 gave 5-mercapto-1-β-D-ribofuranosylimidazole-4-carboxamide (5-thiobredinin, **5**). Direct treatment of **2** with sodium hydrosulfide provided an alternate route to **5**, the structure of which was established by single-crystal X-ray analysis. 5-Thiobredinin has a zwitterionic structure similar to that of bredinin. Glycosylation of persilylated ethyl 5(4)-methylimidazole-4(5)-carboxylate (**6**) with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose in the presence of $SnCl_4$ provided a quantitative yield of the corresponding tri-*O*-benzoyl nucleoside **7**. Debenzoylation of **7** with $MeOH/NH_3$ at ambient temperature gave ethyl 5-methyl-1-β-D-ribofuranosylimidazole-4-carboxylate (**8**). Further ammonolysis of **8** or **7** at elevated temperature and pressure gave 5-methyl-1-β-D-ribofuranosylimidazole-4-carboxamide (**9**). All of these ribonucleosides were tested in Vero cell cultures and in mice against certain viruses. Compounds **3a** and **3c** exhibited significant activity against vaccinia virus in vitro, whereas **4a** was effective against Rift Valley fever virus in mice. 5-Thiobredinin failed to exhibit appreciable antiviral or cytostatic activity (against L1210 and P388) in cell culture.

The isolation and structural elucidation of naturally occurring nucleoside antibiotics pyrazofurin (pyrazomycin, 4-hydroxy-3-β-D-ribofuranosylpyrazole-5-carboxamide)[3] and bredinin (4-carbamoyl-5-hydroxy-1-β-D-ribofuranosylimidazole, **1a**)[4-6] has generated great interest in five-membered azolecarboxamide ribonucleosides. Pyrazofurin has shown broad-spectrum antiviral[3,7,8] and antitumor[7] activities in vitro. Canonico and co-workers[9] have recently tested the potency of pyrazofurin against several selected RNA viruses responsible for human hemorrhagic fever in vitro and observed an 80% reduction in plaque formation at 2-10 μg/mL of pyrazofurin. Efficacy of pyrazofurin against Rift Velley fever virus in mice resulted in a survival rate of 20% and generally prolonged life.[9]



† U.S. Army Medical Research Institute of Infectious Diseases.

(1) This work is taken, in part, from the Ph.D. Dissertation of S.G.W., Brigham Young University, Provo, UT, 1983.
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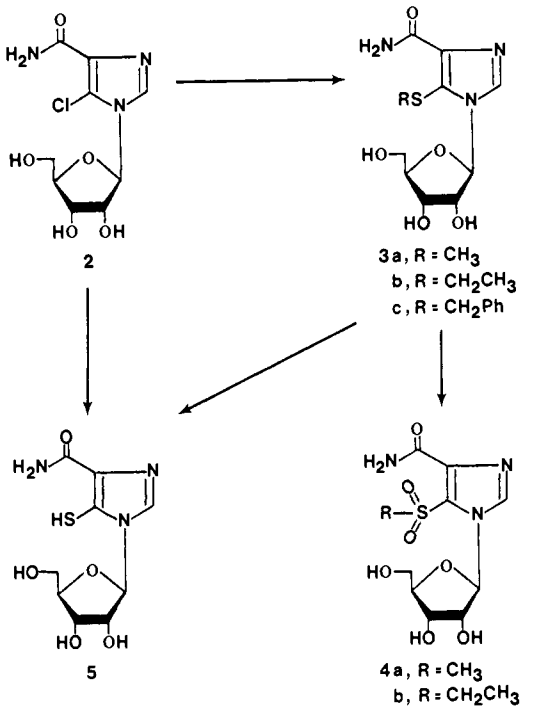
0022-2623/85/1828-1198$01.50/0

Although pyrazofurin exhibits a high degree of selectivity in its antiviral effects in vitro, the LD_{50} dose in mice is about 5 mg/kg per day, and it has a low chemotherapeutic index against MLV and vaccinia virus.[8,10,11] Like pyrazofurin, bredinin also exhibits broad-spectrum antiviral activity in vitro.[4] Bredinin is a potent immunosuppressive agent[4] and cytotoxic to L5178 Y cells.[12-15] However, it showed little effect on the life prolongation of mice inoculated with lymphatic leukemia L1210.[16] Recently in a separate in vitro and in vivo study it has been found that the aglycon of bredinin, 4-carbamoyl-5-hydroxyimidazole, is active against 6-mercaptopurine-resistant sublines of P388 and L1210 leukemia.[17,18] It has been suggested that the aglycon is converted to its active nucleotide form by APRTase and this nucleotide further blocks the de novo purine synthesis of GMP by inhibiting IMP dehydrogenase.[17] Bredinin exhibits beneficial effects on experimental rheumatoid arthritis[19,20] and has been used efficiently in preventing kidney transplant rejection in dogs[21] and in human.[22] The synthetic azole nucleoside ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide),[23] reported from our laboratories,[24] is a structural analogue of both pyrazofurin and bredinin. Ribavirin is a broad-spectrum antiviral agent[23,25] and is currently being investigated in human clinical trials against influenza,[26,27] hepatitis,[28,29] herpes,[30,31] Lassa fever,[32] and respiratory syncytial[33-35] viruses.

Scheme I



In addition to bredinin, several 5-substituted imidazole-4-carboxamide ribonucleosides have also shown significant antiviral activity. Among these derivatives 5-fluoro-1-β-D-ribofuranosylimidazole-4-carboxamide (**1b**)[36] had the greatest antiviral activity in vitro.[37] However, the antiviral potency was somewhat less than that of ribavirin in vitro.[38] 5-Chloro (**2**)[38] and 5-cyanomethyl (**1c**)[39,40] derivatives of 1-β-D-ribofuranosylimidazole-4-carboxamide, reported recently from our laboratories, also exhibited potent activity against several RNA viruses in vitro. Each of the above ribonucleosides contain a carboxamide group in the same relative position on the azole heterocycle. This suggests that certain azole nucleosides containing a carboxamide group may be of considerable importance in binding certain peptide functionalities of specific viral nucleic acid enzymes.[41] As a part of our ongoing synthetic program directed toward the preparation of new azolecarboxamide ribonucleosides, we have now prepared 5-

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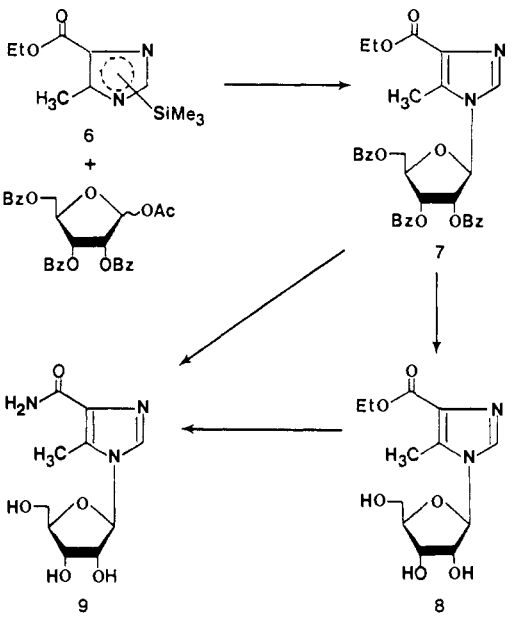
thiobredinin (5-mercapto-1-β-D-ribofuranosylimidazole-4-carboxamide, **5**) and several related 5-substituted imidazole-4-carboxamide ribonucleosides.

Chemistry. For the synthesis of 5-thiobredinin, 5-chloro-1-β-D-ribofuranosylimidazole-4-carboxamide (**2**), previously reported from our laboratories,[38] served as a viable starting material (Scheme I). Direct treatment of **2** with methanethiol in DMF provided a good yield of 5-(methylthio)-1-β-D-ribofuranosylimidazole-4-carboxamide (**3a**). A similar treatment of **2** with ethanethiol or benzenemethanethiol gave the corresponding 5-ethylthio and 5-benzylthio derivatives **3b** and **3c**, respectively. It was envisioned that oxidation of **3a** or **3b** to the corresponding sulfones might provide key precursor to 5-thiobredinin through a nucleophilic displacement reaction with an alkali-metal sulfide or hydrosulfide. The oxidation of **3a** or **3b** with *m*-chloroperoxybenzoic acid in EtOH readily gave the corresponding sulfonyl derivatives **4a** and **4b**, respectively. In the ^{1}H NMR (Me_2SO-d_6) of **4a**, all the protons except $C_{4'}$-H and $C_{5'}$-H_2, were shifted downfield as compared to those of **3a**. The SO_2CH_3 protons had a considerable downfield shift of 1.33 ppm, whereas the anomeric and C_2-H aromatic protons of **4a** were shifted by 0.37 and 0.27 ppm, respectively. A similar downfield shift for ethyl, anomeric, and aromatic protons were noted for **4b** as compared to those of **3b**. This downfield shift in **4a** and **4b** would be expected due to the sulfonyl group. To our surprise, attempts to displace the sulfonyl group in **4a** and **4b** with a sulfide nucleophile under a variety of experimental conditions resulted in the cleavage of the glycosidic bond. However, reductive cleavage of the thiobenzyl ether **3c** with either sodium in liquid ammonia or sodium naphthalene[42] in THF gave an intractable reaction mixture from which **5** was isolated in a 40% yield.

In an effort to improve the yield and isolation procedures of **5**, an alternate method was sought. Our earlier studies[38] suggested that the 5-chloro group in 5-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carbonitrile could be replaced directly by a thiol function by the nucleophilic displacement reaction with potassium hydrosulfide. Thus, the treatment of **2** with sodium hydrosulfide in MeOH at 135 °C for 72 h provided 5-mercapto-1-β-D-ribofuranosylimidazole-4-carboxamide (**5**) in more than 84% yield. In this method, the isolation of **5** is rather relatively simple. The structure of **5** was evident from its spectroscopic (ultraviolet and ^{1}H NMR) and elemental analysis and was finally established by single-crystal X-ray analysis.

The next target was the synthesis of 5-methyl-1-β-D-ribofuranosylimidazole-4-carboxamide (**9**) (Scheme II). We elected to use ethyl 5(4)-methylimidazole-4(5)-carboxylate[43] for our glycosylation studies, which on silylation by heating with hexamethyldisilazane (HMDS) in the presence of $(NH_4)_2SO_4$ gave syrupy trimethylsilyl derivative **6**. Distillation of the syrup under reduced pressure gave **6** as a pure, colorless liquid in more than 90% yield. Treatment of **6** with 1 equiv of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose in the presence of 1.4 molar equiv of anhydrous $SnCl_4$ in 1,2-dichloroethane at ambient temperature for 24 h gave a quantitative yield of crystalline ethyl 5-methyl-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-imidazole-4-carboxylate (**7**). Compound **7** was the only nucleoside product that could be isolated from the reaction mixture. No other isomers were detected. It has been well documented that the isomeric ratio of a Lewis acid mediated condensation of a silylated aglycon with a protected pentofuranose is influenced by a number of factors, such as temperature, solvent, blocking groups, etc.[44,45] The apparent exclusive formation of **7** in the present study seems to be influenced by the intermediacy of a stannic chloride–aglycon **6** complex,[39] which provides a regiospecific ribosylation of **6**. Although it is generally assumed that Lewis acids, such as $SnCl_4$, promote the formation of acyloxonium ions of fully acylated ribofuranoses,[46-48] no account of the interaction of silylated heterocycles with Lewis acids has been reported.[39]

Scheme II



While this work was in progress, a paper appeared[49] in which the glycosylation of unsilylated ethyl 5(4)-methylimidazole-4(5)-carboxylate with 2,3,5-tri-*O*-acetyl-D-ribofuranosyl chloride in the presence of mercuric cyanide in nitromethane has been described. However, that procedure provided a mixture of positional isomers, resulting in low yield of the desired per-O-acetylated N1-glycosyl derivative. Subsequent debenzoylation of **7** in our laboratory with $MeOH/NH_3$ at ambient temperature for 24 h provided ethyl 5-methyl-1-β-D-ribofuranosylimidazole-4-carboxylate (**8**). Further ammonolysis of **8** or **7** with liquid NH_3 at elevated temperature and pressure readily give the desired 5-methyl-1-β-D-ribofuranosylimidazole-4-carboxamide (**9**) in excellent yield. The glycosidic conformation of **9** has been determined by circular dichroism studies and reported recently from our laboratories.[51]

Single-Crystal X-ray Diffraction Analysis of 5-Thiobredinin (5)

Slow crystallization of 5-thiobredinin from water gave X-ray quality crystals. A suitable single crystal was

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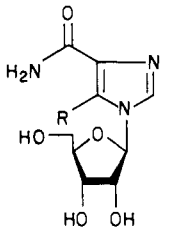
Table I. Comparative in Vitro Antiviral Activity of 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide (Ribavirin) and Certain 5-Substituted 1-β-D-Ribofuranosylimidazole-4-carboxamides in Vero Cells



compd	R	MTC,[a] M	ED_{50},[b] M				
			HSV-2	VV	Para 3	RVF	VEE
3a	SCH_3	1.6×10^{-3}	4.1×10^{-3}	2.8×10^{-4}	7.3×10^{-4}	1.8×10^{-3}	$>1.7 \times 10^{-3}$
3b	SC_2H_5	$>5.0 \times 10^{-3}$	$>5.0 \times 10^{-3}$	2.6×10^{-3}	$>5.0 \times 10^{-3}$	$>2.0 \times 10^{-3}$	$>2.0 \times 10^{-3}$
3c	SCH_2Ph	$>5.0 \times 10^{-3}$	$>5.0 \times 10^{-3}$	2.3×10^{-4}	4.4×10^{-3}	1.0×10^{-3}	1.0×10^{-3}
4a	SO_2CH_3	$>5.0 \times 10^{-3}$	5.0×10^{-3}	$>5.0 \times 10^{-3}$	$>5.0 \times 10^{-3}$	8.7×10^{-6}	6.5×10^{-4}
4b	$SO_2C_2H_5$	$>5.0 \times 10^{-3}$	$>5.0 \times 10^{-3}$	3.5×10^{-3}	$>5.0 \times 10^{-3}$	$>2.0 \times 10^{-3}$	$>2.0 \times 10^{-3}$
5	SH	$>5.0 \times 10^{-3}$	$>5.0 \times 10^{-3}$	3.8×10^{-3}	5.0×10^{-3}	$>2.0 \times 10^{-3}$	$>2.0 \times 10^{-3}$
9	CH_3	1.6×10^{-3}	4.0×10^{-3}	1.4×10^{-3}	5.0×10^{-4}	1.8×10^{-3}	$>1.7 \times 10^{-3}$
ribavirin[56]		$>5.0 \times 10^{-3}$	1.9×10^{-4}	9.0×10^{-5}	1.6×10^{-4}	1.1×10^{-3}	2.5×10^{-3}

[a] The maximum tolerated concentration (MTC) is the lowest concentration of compound that produced microscopically visible cytotoxicity assessed as described in the Experimental Section. [b] The ED_{50} is the concentration of compound that produced 50% inhibition of viral CPE as compared with nondrug controls.

mounted on a Nicolet P3 autodiffractometer, and the diffraction data were collected utilizing graphite monochromated Mo Kα radiation (λ = 0.71069 Å). The compound crystallizes in the monoclinic space group $P2_1$ with the cell dimensions a = 4.991 (1) Å, b = 12.857 (3) Å, c = 9.555 (1) Å; β = 93.73 (1) Å with Z = 2. The lattice parameters were obtained with a least-squares procedure involving 15 centered 2θ values ($22.5 < 2\theta < 30.0$). A total of 1707 reflections were measured to a $\sin\theta/\lambda$ limit of 0.65, using a variable-speed θ–2θ scan technique. Merging the data resulted in 1433 unique reflections with $I > 2\sigma(I)$, which were used in the structure solution and refinement.

The structure of 5-thiobredinin was solved by using a combination of heavy atom and direct method techniques. All crystallographic calculations were performed with use of the SHELX-76 program package.[52] All the non-hydrogen atoms were located in Fourier maps. Difference maps calculated after anisotropic refinement of the heavy atoms gave positions for all the hydrogen atoms of the molecular structure. With heavy atoms refined anisotropically and hydrogen atoms refined isotropically, the final residual values were R = 0.036 and R_w = 0.034. The weights were based on counting statistics.

A stereoscopic view of thiobredinin, drawn with the aid of a computer,[53] is shown in Figure 1 with conformation, atom labels, and bond lengths (angstroms). The result of this structure-determination study confirmed the β-anomeric configuration and the site of glycosyl attachment as N_1. Thiobredinin has a zwitterionic structure similar to that of bredinin.[5] The C_5–S interatomic distance of 1.708 (3) Å indicates that this bond has a double-bond character. The positive charge resides on N_3, which is protonated. The bond lengths in the imidazole ring and the glycon portion are similar to those found in bredinin.[5]

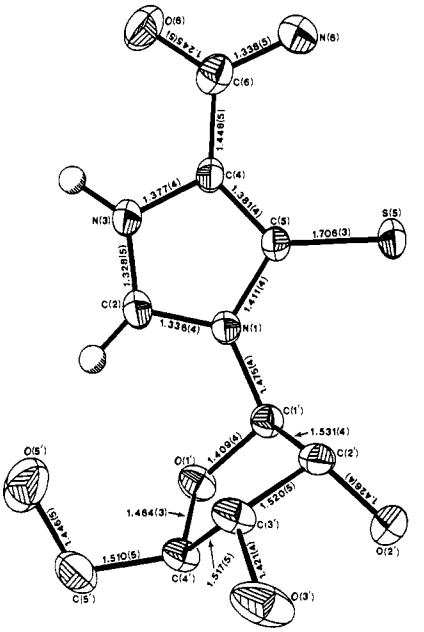


Figure 1. Stereoscopic view of 5-thiobredinin with the atom labels and bond lengths (angstroms).

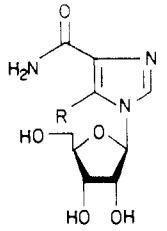
Biological Evaluations: Antiviral

The deblocked 5-substituted 1-β-D-ribofuranosylimidazole-4-carboxamides synthesized during this study were tested against herpes simplex type 2 (HSV2), vaccinia (VV), parainfluenza type 3 (Para 3), Rift Valley fever (RVF), and Venezuelan equine encephalomyelitis (VEE) viruses in vitro in parallel with 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (ribavirin) (Table I). It is evident that some of the (alkylthio)imidazole nucleosides possess good antiviral activity. Compounds **3a** and **3c** exhibited significant activity against VV in vitro. However, this activity is less than that of ribavirin, which in turn is not a very significant antiviral agent against VV. 5-Thiobredinin (**5**) failed to show any appreciable antiviral activity.

Of considerable interest is 5-(methylsulfonyl)-1-β-D-ribofuranosylimidazole-4-carboxamide (**4a**), which is active in vitro against certain other RNA viruses, like Rift Valley fever (RVF) and Venezuelan equine encephalitis (VEE). Although compounds **3c** and **4a** were effective in mice inoculated with RVF, compound **4a** was found to be more active against RVF (a therapeutic index of 2.80) in vivo (Table II). However, **4a** is less potent than ribavirin

(52) Sheldrick, G. M. SHELX-76, "A Program for X-ray Crystal Structure Determination", University of Cambridge, England, 1976.
(53) Sheldrick, G. M. SHELXTL, 4th revision, "An Integrated System for Solving, Refining and Displaying Crystal Structures from Diffraction Data", University of Gottingham, Federal Republic of Germany, 1983.

Table II. Comparative in Vivo Antiviral Activity of 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide (Ribavirin) and Certain 5-Substituted 1-β-D-Ribofuranosylimidazole-4-carboxamides



compd	R	MTC,[a] mg/kg	ther index[b] RVF	VEE
3a	SCH_3	>100	1.20	0.90
3b	SC_2H_5	>121	1.40	*c*
3c	SCH_2Ph	>146	1.90	c
4a	SO_2CH_3	>400	2.80	1.40
4b	$SO_2C_2H_5$	>134	1.21	*c*
5	SH	>110	1.10	c
ribavirin		>100	4.00	1.50

[a] The maximum tolerated dose in mice (milligrams/kilograms of body weight). [b] Therapeutic index, a measure of in vivo antiviral potential of a compound, is calculated as (geometric mean time to death of experimental group)/(geometric mean time to death of control group). For the purpose of this calculation, surviving animals are assigned a value of 28 days. A therapeutic index value of 1 indicated by beneficial effects of the drug. Values exceeding 2 are indicative of effective in vivo compounds. [c] Not determined.

against RVF in vivo.[54] In view of these findings, no further activiral testing of these compounds (**3a**, **3c**, and **4a**) is planned.

All the newly synthesized imidazole nucleosides that were tested for their inhibitory effects on the growth of L1210 and P388 leukemic cell lines in vitro failed to show significant activity at concentration levels less than 10^{-4} M, whereas the reference compound ribavirin exhibited inhibitory effects on these cell lines at 2.7×10^{-5} M.

Experimental Section

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H NMR) spectra were determined at 89.6 MHz with a JEOL FX 90Q spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. The presence of water as indicated by elemental analysis was verified by ^{1}H NMR. Infrared spectra (IR) were obtained on a Beckman Acculab 2 spectrophotometer and ultraviolet spectra (UV; sh = shoulder) were recorded on a Cary Model 15 spectrophotometer. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN, and Robertson Labs, Florham Park, NJ. Thin-layer chromatography (TLC) was run on silica gel 60 F-254 plates (EM Reagents). J. T. Baker silica gel (70–230 mesh) was used for column chromatography. Preparative liquid chromatography (LC) was run utilizing the Waters Prep 500 LC system. All solvents used were reagent grade. Detection of components on TLC was by UV light and with 10% H_2SO_4 in MeOH spray followed by heating. Evaporations were carried out under reduced pressure with the bath temperature below 30 °C. Reagent grade DMF was dried over molecular sieve (4A, 24 h) and freshly distilled before use. THF was distilled from $LiAlH_4$ before use.

5-(Methylthio)-1-β-D-ribofuranosylimidazole-4-carboxamide (3a). To a solution of 5-chloro-1-β-D-ribofuranosylimidazole-4-carboxamide[38] (**2**; 2.77 g, 10 mmol) in dry DMF (50 mL) was added a cold (−5 to −10 °C) ethanolic solution (50 mL) of methanethiol (CH_3SH bubbled in for 10 min) and *t*-BuOK (1.35 g, 12 mmol). The mixture was heated at 100 °C in an atmosphere of nitrogen for 3 h, before it was diluted with EtOH (10 mL), neutralized with Dowex-50 H^+ resin, and filtered. The filtrate was evaporated to dryness and the residue was dissolved in MeOH (25 mL), adsorbed on silica gel (10 g), and placed on a silica gel column (2.5 × 40 cm) prepacked in EtOAc. The column was eluted with EtOAc/H_2O/*n*-PrOH (4:2:1, upper phase). The homogeneous fractions were pooled, and the solvent was evaporated. The residue was crystallized from H_2O/EtOH to yield 2.30 g (79.6%): mp 156–158 °C; IR (KBr) ν 1325 (SCH_3), 1655 (C=O), 3200–3420 (OH) cm^{-1}; UV λ_{max} (nm) (ε × 10^{-3}) pH 1, 222 (9.4), 265 (sh) (1.3), pH 7, 230 (sh) (6.5), 271 (sh) (2.0), pH 11, 230 (sh) (7.8), 271 (sh) (2.3); ^{1}H NMR (Me_2SO-d_6) δ 2.40 (s, 3, SCH_3), 5.86 (d, 1, $J_{1',2'}$ = 4.0 Hz, C_1-H), 7.16 and 7.40 (br s, 2, $CONH_2$), 8.20 (s, 1, C_2-H). Anal. ($C_{10}H_{15}N_3O_5S$) C, H, N, S.

5-(Ethylthio)-1-β-D-ribofuranosylimidazole-4-carboxamide (3b). To a mixture of ethanethiol (1.0 g, 16.1 mmol) and NaH (50% in oil, 0.84 g, 17.5 mmol) in dry DMF (75 mL) was added **2** (4.15 g, 15 mmol) and the mixture heated at 100 °C for 1 h. The solvent was removed and the residue was worked up as described for **3a** to yield 2.0 g (44.0%): mp 80 °C (softens), 120 °C dec; IR KBr) ν 1480 (SEt), 1645 (C=O), 3400 (OH) cm^{-1}; UV λ_{max} (nm) (ε × 10^{-3}) pH 1, 217 (9.7), 260 (sh) (1.5), pH 7 and 11, 233 (sh) (7.0), 275 (sh) (2.4); ^{1}H NMR (Me_2SO-d_6) δ 1.06 (t, 3, CH_2CH_3), 2.90 (q, 2, CH_2CH_3), 5.87 (d, 1, $J_{1',2'}$ = 5.0 Hz, C_1-H), 7.16 and 7.38 (br s, 2, $CONH_2$), 8.22 (s, 1, C_2-H). Anal. ($C_{11}H_{17}N_3O_5S \cdot {}^1/_2H_2O$) C, H, N, S.

5-(Benzylthio)-1-β-D-ribofuranosylimidazole-4-carboxamide (3c). In the same manner as for **3a**, treatment of benzenemethanethiol (1.6 g, 13 mmol) in DMF containing *t*-BuOK (1.5 g, 13 mmol) with **2** (3.0 g, 11 mmol) gave the title compound as yellow needles: 2.8 g (69.6%); mp 90 °C (softens), 150 °C dec; IR (KBr) ν 1650 (C=O), 3340–3400 (OH) cm^{-1}; UV λ_{max} (nm) (ε × 10^{-3}) pH 1, 313 (sh) (4.6), pH 7 and 11, 310 (sh) (5.1); ^{1}H NMR (Me_2SO-d_6) δ 4.20 (s, 2, CH_2Ph), 5.52 (d, 1, $J_{1',2'}$ = 4.0 Hz, C_1-H), 7.18 and 7.32 (br s, 2, $CONH_2$), 7.22 (s, 5, C_6H_5), 8.15 (s, 1, C_2-H). Anal. ($C_{16}H_{19}N_3O_5S$) C, H, N, S.

5-(Methylsulfonyl)-1-β-D-ribofuranosylimidazole-4-carboxamide (4a). To a suspension of **3a** (2.9 g, 10 mmol) in EtOH (150 mL) was added *m*-chloroperoxybenzoic acid (80–90%, 5.5 g, 32 mmol) and the mixture was stirred at room temperature for 8 h with the exclusion of moisture. The solvent was evaporated to dryness and the residue was triturated with ether (5 × 50 mL). The ether-insoluble solid was crystallized from EtOH to yield 2.4 g (74.7%): mp 163–165 °C; IR (KBr) ν 1310 (SO_2CH_3), 1685 (C=O), 3120–3440 (OH) cm^{-1}; UV λ_{max} (nm) (ε × 10^{-3}) pH 1, 236 (9.0), pH 7, 219 (8.2), pH 11, 235 (sh) (7.1); ^{1}H NMR (Me_2SO-d_6) δ 3.53 (s, 3, SO_2CH_3), 6.23 (d, 1, $J_{1',2'}$ = 3.5 Hz, C_1-H), 7.50 and 7.72 (br s, 2, $CONH_2$), 8.47 (s, 1, C_2-H). Anal. ($C_{10}H_{15}N_3O_7S$) C, H, N, S.

5-(Ethylsulfonyl)-1-β-D-ribofuranosylimidazole-4-carboxamide (4b). In the same manner as for **4a**, the title compound was prepared with use of **3b** (1.0 g, 3.3 mmol) and *m*-chloroperoxybenzoic acid (1.5 g, 8.7 mmol) in EtOH (50 mL). The product was purified on a silica gel column (2 × 20 cm) with CH_2Cl_2/MeOH (3:1, v/v) as the solvent to yield 0.80 g (72.7%): mp 115 °C; IR (KBr) ν 1150, 1310 (SO_2CH_3), 1675 (C=O), 3300–3420 (OH) cm^{-1}; UV λ_{max} (nm) (ε × 10^{-3}) pH 1, 225 (sh) (8.4); ^{1}H NMR (Me_2SO-d_6) δ 1.18 (t, 3, CH_2CH_3), 3.72 (q, 2, CH_2CH_3), 6.20 (d, 1, $J_{1',2'}$ = 3.0 Hz, C_1-H), 7.22 and 7.52 (br s, 2, $CONH_2$), 8.52 (s, 1, C_2-H). Anal. ($C_{11}H_{17}N_3O_7S$) C, H, N, S.

5-Mercapto-1-β-D-ribofuranosylimidazole-4-carboxamide (5-Thiobredinin, 5). Method 1. To a solution of **2** (1.1 g, 4 mmol) in MeOH (15 mL) was added a solution of NaOMe/H_2S (1 N NaOMe in MeOH saturated with H_2S at 0 °C, 8 mL), and the mixture was heated in a Teflon liner steel bomb at 135 °C for 72 h. The solvent was evaporated to dryness. The residue was dissolved in water (25 mL) and the aqueous solution was acidified (pH 5) with acetic acid. The mixture was filtered to remove precipitated sulfur. The filtrate was concentrated to 15 mL and cooled and the separated solid was collected by filtration.

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Crystallization of the solid from water gave 0.71 g. Evaporation of the filtrate and purification of the residue on a silica gel column (2 × 30 cm) using $CHCl_3$/MeOH (10:4, v/v) gave an additional 0.21 g of **5**: total yield 0.92 g (84.4%); mp 208 °C dec; IR (KBr) ν 1085 (CSH), 1650 (C=O), 3360 (OH) cm^{-1}; UV λ_{max} (nm) ($\epsilon \times 10^{-3}$) pH 1, 290 (9.1), pH 7 and 11, 260 (sh) (6.0), 295 (10.3); ^1H NMR (Me_2SO-d_6) δ 6.0 (d, 1, $J_{1',2'}$ = 2.5 Hz, $C_{1'}$-H), 7.47 and 8.97 (br s, 2, $CONH_2$), 8.92 (s, 1, C_2-H). Anal. ($C_9H_{13}N_3O_5S \cdot {}^1/_2H_2O$) C, H, N, S.

Method 2. Sodium naphthalene solution[42] was added to a suspension of **3c** (2.0 g, 5.5 mmol) in dry THF (25 mL) until the dark green color of the reagent persisted. The mixture was stirred under nitrogen for 16 h and then opened to the atmosphere. Following the disappearance of the green color, the solvent was evaporated. The residue was washed with hexane (3 × 50 mL) to remove the naphthalene and then taken up in MeOH (50 mL). The methanolic solution was neutralized with Dowex 1-X8 H^+ resin and filtered and the filtrate taken to dryness. The residue was triturated with dry EtOH (10 mL) and the solid that separated was collected. Crystallization of the solid from water gave 0.60 g (40%) of the title compound, which was identical in all respects with **5** prepared by method 1.

Ethyl 5-Methyl-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazole-4-carboxylate (7). A mixture of dry ethyl 5(4)-methylimidazole-4(5)-carboxylate[43] (15.4 g, 100 mmol), HMDS (100 mL), and $(NH_4)_2SO_4$ (0.1 g) was heated under reflux for 15 h with the exclusion of moisture. Excess HMDS was removed by distillation and the residual syrup was distilled under reduced pressure to provide the trimethylsilyl derivative **6** as a colorless liquid: 20.3 g (90%); bp 98–100 °C (0.1 mm). To a solution of the trimethylsilyl derivative **6** (4.52 g, 20 mmol) in dry 1,2-dichloroethane (100 mL) was added 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (10.1 g, 20 mmol). The mixture was stirred for 10 min before anhydrous $SnCl_4$ (7.3 g, 28 mmol) was added to it. After stirring at ambient temperature for 24 h under anhydrous conditions, the mixture was evaporated to dryness. The residue was dissolved in EtOAc (200 mL) and poured over 5% aqueous $NaHCO_3$ solution (500 mL). The organic layer was separated, washed with 5% aqueous $NaHCO_3$ solution (2 × 150 mL), followed by water (3 × 150 mL), and then dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a syrupy residue, which crystallized on standing to yield 11.9 g (100%): mp 96–98 °C; IR (KBr) ν 1595, 1720 (C=O) cm^{-1}; UV λ_{max} (nm) ($\epsilon \times 10^{-3}$) (EtOH) 232 (46.6); ^1H NMR (Me_2SO-d_6) δ 1.26 (t, 3, CH_2CH_3), 2.60 (s, 3, C_5-CH_3), 4.20 (q, 2, CH_2CH_3), 6.36 (d, 1, $J_{1',2'}$ = 4.5 Hz, $C_{1'}$-H), 8.10 (s, 1, C_2-H). Anal. ($C_{33}H_{30}N_2O_9$) C, H, N.

Ethyl 5-Methyl-1-β-D-ribofuranosylimidazole-4-carboxylate (8). Compound **7** (5.98 g, 10 mmol) was combined with MeOH/NH_3 (150 mL, saturated at 0 °C) and stirred in a pressure bottle at room temperature for 24 h. The solvent was evaporated and the residue treated with boiling benzene (3 × 75 mL) to remove benzamide. The benzene insoluble solid was crystallized from H_2O/EtOH as needles to yield 2.4 g (84%): mp 144–145 °C (lit.[50] mp 144–145 °C); IR (KBr) ν 1715 (C=O of ester), 3200–3400 (OH) cm^{-1}; UV λ_{max} (nm) ($\epsilon \times 10^{-3}$) pH 1, 226 (12.0), pH 7, 240 (11.2), pH 11, 240 (10.0); ^1H NMR (Me_2SO-d_6) δ 1.30 (t, 3, CH_2CH_3), 2.53 (s, 3, C_5-CH_3), 3.60 (q, 2, CH_2CH_3), 5.60 (d, 1, $J_{1',2'}$ = 4.5 Hz, $C_{1'}$-H), 8.0 (s, 1, C_2-H). Anal. ($C_{12}H_{18}N_2O_6$) C, H, N.

5-Methyl-1-β-D-ribofuranosylimidazole-4-carboxamide (9). In the same manner as for **8**, treatment of either **7** (5.98 g, 10 mmol) or **8** (2.86 g, 10 mmol) in dry MeOH (100 mL) with liquid NH_3 (30 mL) in a steel bomb at 120 °C for 75 h gave, after crystallization from MeOH, 2.4 g (93.4%) of the title compound: mp 176–178 °C; IR (KBr) ν 1675 (C=O of amide), 3200–3400 (NH_2, OH) cm^{-1}; UV λ_{max} (nm) ($\epsilon \times 10^{-3}$) pH 1, 223 (10.5), pH 7, 237 (11.4), pH 11, 237 (10.2); ^1H NMR (Me_2SO-d_6) δ 2.53 (s, 3, C_5-CH_3), 5.53 (d, 1, $J_{1',2'}$ = 5.0 Hz, $C_{1'}$-H), 6.96 and 7.26 (2 br s, 2, $CONH_2$), 7.93 (s, 1, C_2-H). Anal. ($C_{10}H_{15}N_3O_5$) C, H, N.

Antiviral Evaluation. Test compounds were evaluated for their ability to inhibit virus-induced cytopathic effect (CPE) produced by herpes simplex virus type 2 (HSV-2, 333), vaccinia virus (VV), parainfluenza virus type 3 (para-3), Rift Valley fever virus (RVF, zagazig 501 strain), and Venezuelan equine encephalomyelitis virus (VEE, Trinidad donkey strain) in African green monkey kidney (Vero) cells (American Type Culture Collection, Rockville, MD). Vero cells were maintained in antibiotic-free Eagle minimum essential medium (EMEM) with Earle's salts supplemented with 10% heat inactivated newborn bovine serum (Grand Island Biological Co., Grand Island, NY). For antiviral experiments, cells were inoculated into 96-well tissue culture plates (Corning Glassworks, Corning, NY) at a concentration of 4 × 10^4 cells/0.2 mL per well and cultured for 24 h at 37 °C in 5% CO_2 to confluency.

Monolayers were inoculated with a predetermined number of $TCID_{50}$ (50% tissue culture infective dose) units of virus that will produce complete destruction of the cell monolayer in 72 h. The number of $TCID_{50}$ units in 0.1 mL/well were as follows: HSV-2, 100; VV, 200; para-3, 60. After 30-min adsorption at 37 °C, test compounds were added (0.1 mL/well) in seven 0.5 log concentrations ranging from 1 × 10^{-5} to 1 × 10^{-2} M, resulting in final well concentrations of 5 × 10^{-6} to 5 × 10^{-3} M. At each concentration, duplicate wells were used for evaluation of antiviral activity and single uninfected wells for cytotoxicity evaluation.

After 72-h incubation at 37 °C in 5% CO_2/air, the concentration of compound that produced 50% inhibition of virus plaque formation or viral CPE was determined (ED_{50}). A given concentration of compound was considered cytotoxic if it produced any microscopically visible changes in cellular morphology or in the density of the cell monolayer due to lysis, rounding up or detachment of cells. The lowest concentration where cytotoxicity to uninfected, confluent Vero cell monolayers was considered to be the maximum tolerated concentration (MTC).

The in vivo antiviral activity is reported in terms of therapeutic index, which is the measure of antiviral potential of a compound and is calculated as the geometric mean time to death of experimental group divided by geometric mean time to death of control group. For the purpose of this calculation, surviving animals are assigned a value of 28 days. A therapeutic index value of 1 indicated no beneficial effects of the drug. Values exceeding 2 are indicative of effective in vivo compounds.

Cytostatic Activity Evaluation. Compounds were evaluated for their ability to inhibit growth of murine leukemia L1210 and lymphoid neoplasm P388 (American Type Culture Collection, Rockville, MD) maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (Grand Island Biological Co., Grand Island, NY) and 20 mM Hepes buffer. For growth experiments, cells were adjusted to 1 × 10^5 cells/mL and distributed into 13 × 100 mm culture tubes (1 mL/tube). Test compounds were dissolved in growth medium, sterilized by passage through an 0.22-μm membrane filter, and added to tubes of cells (1 mL/tube). Compounds were tested in duplicate at log concentrations ranging from 1 × 10^{-7} to 1 × 10^{-4} M. Following 48-h incubation at 37 °C, cell counts were determined with a Coulter Model ZF cell counter. Cell growth in the presence of test compounds was expressed as a percentage of growth in untreated control wells, and the concentration of compound producing 50% inhibition of cell growth was determined (ID_{50}).

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Registry No. **2**, 59353-97-8; **3a**, 97151-16-1; **3b**, 97151-17-2; **3c**, 97151-18-3; **4a**, 97151-19-4; **4b**, 97151-20-7; **5**, 93194-37-7; **6**, 97151-21-8; **7**, 97151-22-9; **8**, 82877-39-2; **9**, 85665-04-9; methanethiol, 74-93-1; ethanethiol, 75-08-1; benzenemethanethiol, 100-53-8; ethyl 5(4)-methylimidazole-4(5)-carboxylate, 51605-32-4; 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose, 14215-97-5.