2 (Ar = Ph, Ar′ = 4-ClC_6H_4), 23187-09-9; **2** (Ar = Ph, Ar′ = 4-$(CH_3)_2NC_6H_4$), 73181-80-3; **2** (Ar = Ph, Ar′ = 4-$EtOC_6H_4$), 97060-26-9; **2** (Ar = 4-FC_6H_4, Ar′ = 4-$CH_3OC_6H_4$), 97071-44-8; **2** (Ar = 4-FC_6H_4, Ar′ = 4-$CH_3SC_6H_4$), 97060-27-0; **3a**, 3653-22-3; **3b**, 62894-49-9; **3c**, 34926-08-4; **3d**, 62894-54-6; **3e**, 49855-26-7; **3f**, 97059-92-2; **3g**, 97059-93-3; **3h**, 97059-94-4; **3i**, 62894-55-7; **3j**, 62939-41-7; **3k**, 62894-56-8; **3l**, 73918-90-8; **3m**, 73918-91-9; **3n**, 73918-89-5; **3o**, 60220-29-3; **3p**, 62894-32-0; **3q**, 62894-41-1; **3r**, 62894-48-8; **3s**, 62894-33-1; **3t**, 97059-95-5; **3u**, 94286-02-9; **3v**, 62894-25-1; **3w**, 62894-31-9; **3x**, 62894-28-4; **3y**, 97059-96-6; **3z**, 62894-37-5; **3aa**, 97059-97-7; **3bb**, 62894-34-2; **3cc**, 62894-39-7; **4a**, 97059-98-8; **4b**, 73919-13-8; **4c**, 62894-77-3; **4d**, 62894-74-0; **4e**, 73919-23-0; **4f**, 73919-16-1; **4g**, 73919-21-8; **4h**, 97059-99-9; **4i**, 62894-35-3; **4j**, 62894-79-5; **4k**, 97060-00-9; **4l**, 97060-01-0; **4m**, 62894-26-2; **4n**, 62894-76-2; **4o**, 62894-29-5; **4p**, 97060-02-1; **4q**, 97060-03-2; **4r**, 97060-04-3; **4s**, 62895-14-1; **4t**, 62894-38-6; **5a**, 62894-90-0; **5b**, 62894-85-3; **5c**, 73919-17-2; **5d**, 62895-01-6; **5e**, 62894-96-6; **5f**, 62894-75-1; **5g**, 97060-05-4; **5h**, 73919-15-0; **5i**, 97060-06-5; **5j**, 97060-07-6; **5k**, 62895-03-8; **5l**, 62895-04-9; **5m**, 97060-08-7; **5n**, 73919-22-9; **5o**, 73919-24-1; **5p**, 73919-20-7; **5q**, 97060-09-8; **5r**, 62894-36-4; **5s**, 97060-10-1; **5t**, 62894-80-8; **5u**, 62894-73-9; **5v**, 62894-81-9; **5w**, 97060-11-2; **5x**, 62894-27-3; **5y**, 62894-88-6; **5z**, 62939-40-6; **5aa**, 62894-30-8; **5bb**, 97060-12-3; **5cc**, 97060-13-4; **5dd**, 97060-14-5; **5ee**, 62895-16-3; **5ff**, 62895-15-2; **6a**, 62894-43-3; **6b**, 62894-40-0; **6c**, 62894-59-1; **6d**, 62894-58-0; **6e**, 62894-67-1; **6f**, 62894-63-7; **6g**, 73918-97-5; **6h**, 62894-60-4; **6i**, 62894-71-7; **6j**, 62894-57-9; **6k**, 62894-62-6; **6l**, 73918-96-4; **6m**, 73918-95-3; **6n**, 97060-15-6; **6o**, 62894-61-5; **6p**, 62894-65-9; **6q**, 62894-66-0; **6r**, 97060-16-7; **6s**, 62894-68-2; **6t**, 73918-99-7; **6u**, 73918-98-6; **6v**, 62894-70-6; **6w**, 62894-69-3; **6x**, 73918-83-9; **7a**, 62894-84-2; **7b**, 73919-11-6; **7c**, 62894-86-4; **7d**, 87483-32-7; **7e**, 62894-83-1; **8a**, 73919-03-6; **8b**, 71078-11-0; **8c**, 62894-78-4; **8d**, 62894-89-7; **8e**, 62894-87-5; **8f**, 62895-08-3; **8g**, 73919-14-9; **8h**, 73919-00-3; **8i**, 73919-12-7; **8j**, 97060-17-8; **8k**, 97060-18-9; **8l**, 62894-82-0; **8m**, 62894-98-8; **8n**, 71078-12-1; **8o**, 73919-10-5; **8p**, 62894-97-7; **8q**, 62895-12-9; **8r**, 62895-05-0; **8s**, 97060-19-0; **8t**, 62895-06-1; **8u**, 62895-07-2; **8v**, 97060-20-3; **8w**, 62895-09-4; **8x**, 73919-19-4; **8y**, 97060-21-4; **8z**, 73919-18-3; **8aa**, 62895-00-5; **8bb**, 62894-99-9; **8cc**, 73919-04-7; **8dd**, 62895-11-8; **8ee**, 73919-05-8; **8ff**, 62894-93-3; **8gg**, 97060-22-5; **9a**, 62894-51-3; **9b**, 62894-53-5; **9c**, 62894-52-4; **9d**, 62894-50-2; **10**, 62895-10-7; **11a**, 62894-92-2; **11b**, 62894-95-5; **11c**, 62894-94-4; **11d**, 62894-91-1; **12a**, 62894-46-6; **12b**, 73918-81-7; **12c**, 73918-88-4; **12d**, 73918-84-0; **12e**, 73918-86-2; **12f**, 73918-87-3; **13a**, 97060-23-6; **13b**, 85284-10-2; **14a**, 62894-47-7; **14b**, 73919-02-5; **14c**, 73919-09-2; **14d**, 71078-10-9; **14e**, 73919-07-0; 2-mercapto-4,5-bis[3,4-(methylenedioxy)phenyl]imidazole, 66660-00-2; 4,5-bis[3,4-(methylenedioxy)phenyl]imidazole, 5549-84-8; 4,4′-difluorobenzoin, 53458-16-5; thiourea, 62-56-6; 4,5-bis(4-fluorophenyl)imidazole, 68163-71-3; formamide, 75-12-7; 2-mercapto-4,5-bis(3-chlorophenyl)imidazole, 97060-24-7; 1-(tetrahydropyran-2-yl)-4,5-bis(4-fluorophenyl)imidazole, 74767-58-1.

8-Substituted Guanosine and 2′-Deoxyguanosine Derivatives as Potential Inducers of the Differentiation of Friend Erythroleukemia Cells[1]

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A variety of 8-substituted guanosine and 2′-deoxyguanosine derivatives were synthesized and tested as inducers of the differentiation of Friend murine erythroleukemia cells in culture. The most active agents in the guanosine series were 8-substituted $-N(CH_3)_2$, $-NHCH_3$, $-NH_2$, $-OH$, and $-SO_2CH_3$, which caused 68, 42, 34, 33, and 30% of erythroleukemia cells to attain benzidine positivity, a functional measure of maturation, at concentrations of 5, 1, 0.4, 5, and 5 mM, respectively. The 8-OH derivative of the 2′-deoxyguanosine series produced comparable activity, causing 62% benzidine-positive cells at a level of 0.2 mM. These findings indicate that 8-substituted analogues of guanosine and 2′-deoxyguanosine have the potential to terminate leukemia cell proliferation through conversion to end-stage differentiated cells.

Most of the chemotherapeutic agents employed for the treatment of the leukemias act by cytodestructive mechanisms. An alternative to this approach, which is based upon the concept that leukemia is a disease of blocked maturation, envisions the use of agents to convert neoplastic cells to end-stage cells with no proliferative capability through induction of differentiation. The Friend murine erythroleukemia has been shown to have the capacity to undergo both morphological and functional maturation after exposure to a large number of different chemicals including polar solvents,[3] hormones,[4] vitamins,[5,6] tumor promotors[7] and cancer chemotherapeutic agents.[8,9]

Among the antineoplastic agents that are capable of initiating maturation are the 6-thiopurines, 6-thioguanine, and 6-mercaptopurine. These antileukemic drugs are at best weak inducers of the differentiation of Friend erythroleukemia and HL-60 human promyelocytic leukemia cells[10-12] but are potent initiators of maturation in variants

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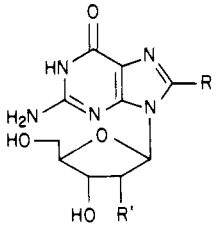
Chart I



Guanosine Series (R' = OH)

1, R = H
2, R = Br
3, R = $NHNH_2$
4, R = N_3
5, R = NH_2
6, R = $NHCH_3$
7, R = $NH(CH_3)_2$
8, R = NHOH
9, R = $OCH_2C_6H_5$
10, R = OH
11, R = OCH_3
12, R = SH
13, R = SCH_3
14, R = SO_2CH_3

2'-Deoxyguanosine Series (R' = H)

15, R = H
16, R = Br
17, R = OCH_3
18, R = $OCH_2C_6H_5$
19, R = OH

of these cell lines that lack hypoxanthine–guanine phosphoribosyltransferase (HGPRT) activity.[11,13-15] Analysis of metabolites of 6-thioguanine formed by parental and hypoxanthine–guanine phosphoribosyltransferase negative ($HGPRT^-$) HL-60 cells treated with this antimetabolite has demonstrated that the free base 6-thioguanine is responsible for inducing differentiation, while formation of the metabolite 6-thioguanosine 5'-phosphate is essential for cytotoxic activity.[15] The use of 8-aminoguanosine, an inhibitor of purine nucleoside phosphorylase (PNP),[6] in a mutant cell line of HL-60 lacking both HGPRT and deoxycytidine kinase activities, has provided evidence that 2'-deoxythioguanosine may also be an active form in the induction process.[15] On the basis of this biochemical information, we have hypothesized that modifications of guanosine and deoxyguanosine that prevent these nucleoside derivatives from serving as substrates for PNP and HGPRT could result in the attainment of purine nucleoside derivatives with differentiation-inducing activity in wild-type leukemia cells. To test this possibility, various 8-substituted derivatives of guanosine and 2'-deoxyguanosine were synthesized and their toxicities and capacities to induce erythroid differentiation were evaluated in Friend murine erythroleukemia cells.

Chemistry. A series of 8-substituted guanosine and 2'-deoxyguanosine analogues, shown in Chart I, has been synthesized from the corresponding 8-bromo derivatives **2** and **16**, which in turn were prepared from guanosine and 2'-deoxyguanosine by the methodology of Long et al.[17] The 8-hydrazine derivative **3** was obtained by refluxing **2** and ethanolic hydrazine.[18] Reaction of **3** with sodium nitrite in 5% hydrochloric acid at 0 °C gave the 8-azido analogue **4**. Catalytic hydrogenation (10% Pd/C) of **4** in EtOH–H_2 yielded the amino derivative **5**. Treatment of **2** and **16** with sodium methoxide and sodium benzyloxide in Me_2SO[19,20] afforded the respective 8-methoxy derivatives **11** and **17** and the 8-benzyloxy derivatives **9** and **18**. 8-Hydroxy analogues **10** and **19** were obtained by catalytic hydrogenation (10% Pd/C) of **9** and **18** in EtOH–H_2O (1:1, v/v). The 8-methylamino, 8-dimethylamino, and 8-hydroxylamino derivatives **6–8** were synthesized by heating the 8-bromo compound **2** with the corresponding amine in a Parr pressure reaction apparatus.[19,21] Refluxing **2** with thiourea in EtOH readily provided the 8-mercapto analogue **12**.[22] Methylation of **12** with dimethyl sulfate[22] gave 8-(methylthio)guanosine (**13**). Oxidation of **13** with hydrogen peroxide yielded the 8-methylsulfonyl derivative **14**.[19]

Because of the lability of the glycosidic bond, control of reaction conditions, especially temperature and reaction time, were critical in the synthesis of the 8-substituted 2'-deoxyguanosine derivatives, and cleavage of the glycosidic bond was observed during recrystallization of compounds **16** and **17** from boiling water.

Table I. Inhibition of Cellular Replication and Extent of Differentiation of Friend Erythroleukemia Cells Exposed to 8-Substituted Analogues of Guanosine[a]

compd	R	growth inhibn: ID_{50}, mM		differentiation: opt concn, mM	differentiation: % benzidine-pos cells
		parent	$HGPRT^-$		
1	-H	1.2	7.8		0
2	-Br	5.1	5.1	5	6
4	$-N_3$	3.6	3.6	2	12
5	$-NH_2$	1.7	1.7	0.4	34
6	$-NHCH_3$	2.9	4.9	1	42
7	$-N(CH_3)_2$	>5	>5	5	68
8	-NHOH	>5	ND[b]	6	7
9	$-OCH_2C_6H_5$	>8	>8	8	4
10	-OH	8	8	5	33
11	$-OCH_3$	>8	>8	8	7
12	-SH	3.5	3.5	2	4
13	$-SCH_3$	>8	>8	5	11
14	$-SO_2CH_3$	5.4	10	5	30
Me_2SO				200	70

[a] Inhibition of cellular replication and differentiation were measured as described in the Experimental Section. The percentage of benzidine-positive cells in nontreated cultures was less than 3%. The results for 8-hydrazinoguanosine (**3**) were omitted because of its instability in solution. 8-(Benzyloxy)guanosine (**9**) produced precipitation upon addition of the drug solution to the cell suspension. [b] ND = not determined.

Table II. Inhibition of Cellular Replication and Extent of Differentiation of Friend Erythroleukemia Cells Exposed to 8-Substituted Analogues of 2'-Deoxyguanosine[a]

compd	R	growth inhibn: ID_{50}, mM		differentiation: opt concn, mM	differentiation: % benzidine-pos cells
		parent	$HGPRT^-$		
15	-H	0.29	0.49		0
16	-Br	>5	>5	7	20
17	$-OCH_3$	>5	>5	5	22
18	$-OCH_2C_6H_5$	>5	>5	7	7
19	-OH	0.39	0.39	0.2	62

[a] Cells were evaluated as described in Table I. 8-(Benzyloxy)-deoxyguanosine produced precipitation upon addition of the drug solution to the cell suspension.

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Biological Results. The parent nucleosides guanosine and deoxyguanosine caused cytotoxicity to wild type Friend erythroleukemia cells, with ID_{50} values (concentration required for 50% inhibition of growth) of 1.2 and 0.29 mM, respectively. $HGPRT^-$ erythroleukemia cells were 6 times more resistant to guanosine than parental cells but were fully sensitive to the cytotoxic action of 2′-deoxyguanosine due to phosphorylation by deoxycytidine kinase.[23] In contrast, 8-substituted derivatives of guanosine and deoxyguanosine were for the most part less toxic to parental Friend cells than the physiological purine nucleosides (Tables I and II). Moreover, the growth of $HGPRT^-$ erythroleukemia cells was inhibited by these analogues at roughly the same concentrations occurring in parental cells, implying that the growth inhibitory activity exhibited by the modified nucleosides was independent of HGPRT activity. Because of the apparent lack of substrate activity for HGPRT exhibited by the nucleosides substituted in the 8-position of the purine nucleus, which significantly limited their capacity to function as conventional cytotoxic antimetabolites, it was anticipated that they might act as inducers of differentiation. The attainment of a differentiated state was assayed in parental wild type Friend cells by the measurement of the content of hemoglobin, a functional marker of erythroid maturation (Tables I and II). 8-Bromoguanosine, which has been reported to be a mitogen in lymphocytes and whose activity is not mediated through phosphorylated metabolites,[24] was a poor inducer of the maturation of Friend erythroleukemia cells. In contrast, 8-aminoguanosine, an inhibitor of purine nucleoside phosphorylase,[16] and its N-methylated derivatives possessed the capacity to induce maturation. ID_{50} values and optimal concentrations required for maximum induction increased with the introduction of methyl groups in the 8-NH_2, 8-$NHCH_3$, and 8-$NH(CH_3)_2$ series **5–7**; thus, the degree of induction at the optimal dose of each agent increased with increasing bulk and hydrophobicity. 8-Hydroxyguanosine (**10**) also showed some potency as an inducer of maturation. Evaluation of the O-substituted derivatives of this agent [i.e., 8-$OCH_2C_6H_5$ (**9**) and 8-OCH_3 (**11**)] was limited by solubility, and these agents could not be tested at concentrations greater than 8 mM.

The 8-substituted 2′-deoxyguanosines **16** and **17** were modestly active as inducers of maturation in parental Friend cells; whereas, the 8-hydroxy-2′-deoxyguanosine derivative **19** gave a relatively high percentage (62%) of benzidine-positive cells at a relatively low concentration (0.2 mM).

6-Thiopurines are effective initiators of differentiation of $HGPRT^-$ leukemia cells but are at best weak inducers in parental wild-type leukemic cells.[11-15] The present study demonstrates that some 8-substituted derivatives of guanosine and 2′-deoxyguanosine can function as inducers of differentiation in parental erythroleukemia cells, presumably because of their inability to be converted to the nucleotide level.

Experimental Section

Melting points were taken on a Thomas-Hoover Unimelt apparatus and are uncorrected. Thin-layer chromatography was performed on EM silica gel 60 F_{254} sheets (0.2 mm). IR spectra were recorded on a Perkin-Elmer 21 spectrophotometer. UV spectra were recorded on a Beckman 25 spectrophotometer, and NMR spectra were taken on a Varian T-60 or a WM 500 spectrometer at 60 or 500 MHz with Me_4Si as the internal reference. The elemental analyses were carried out by Baron Consulting Co., Analytical Services, Orange, CT. Where analyses are indicated only by symbols of the elements, the analytical results obtained for those elements were within ±0.4% of the theoretical values.

8-Bromoguanosine (2). This compound was prepared by the methodology of Long et al.:[17] yield 84%; mp 201–203 °C dec (lit.[17] mp 201–203 °C dec); UV (pH 1) λ_{max} 261 nm [lit.[17] UV (pH 1) λ_{max} 261 nm]; NMR (Me_2SO-d_6) δ 3.46–3.75 (m, 2 H, 5′-H), 3.75–4.01 (m, 1 H, 4′-H), 4.01–4.31 (m, 1 H, 3′-H), 4.82–5.38 (m, 4 H, 2′-H; 2′-, 3′-, and 5′-OH, D_2O exchangeable), 5.75 (d, 1 H, 1′-H), 6.48 (s, 2 H, 2-NH_2, D_2O exchangeable), 10.81 (br s, 1-NH, D_2O exchangeable).

8-Hydrazinoguanosine (3). Compound **3** was synthesized by a modification of the methodology of Saneyoshi.[18] 8-Bromoguanosine (**2**) (4 g, 11 mmol) was added with stirring to a solution of 4 mL of 95% hydrazine in 350 mL of EtOH. The reaction mixture (a clear solution) was refluxed, and a light yellow precipitate began to separate after 0.5 h. Refluxing was continued for an additional 20 h, at which time the reaction mixture was allowed to cool to room temperature. The resulting precipitate was filtered and recrystallized from boiling water to give white fine needles: 2 g (58%): mp >238 °C dec (lit.[18] mp 240 °C dec); UV (pH 1) λ_{max} 256 and 276 nm [lit.[18] UV (pH 1) λ_{max} 248 and 275 nm]; NMR (Me_2SO-d_6) δ 3.32–3.75 (m, 2 H, 5′-H), 3.76–4.01 (m, 1 H, 4′-H), 4.01–4.28 (m, 1 H, 3′-H), 4.86–5.18 (m, 4 H, 2′-H; 2′-, 3′-, and 5′-OH, D_2O exchangeable), 5.72 (d, 1 H, 1′-H), 6.00–6.62 (m, 5 H, 2-NH_2 and 8-$NHNH_2$, D_2O exchangeable).

8-Azidoguanosine (4). Compound **4** was synthesized according to the procedure of Saneyoshi:[18] yield 49%; mp >202 °C dec (lit.[18] mp 200 °C dec); UV (pH 1) λ_{max} 255 and 276 nm [lit.[18] UV (pH 1) λ_{max} 251 and 276 nm]; NMR (Me_2SO-d_6) δ 3.38–3.62 (m, 2 H, 5′-H), 3.62–4.01 (m, 1 H, 4′-H), 4.01–4.18 (m, 1 H, 3′-H), 4.78–5.51 (m, 4 H, 2′-H; 2′-, 3′-, and 5′-OH, D_2O exchangeable), 5.72 (d, 1 H, 1′-H), 6.42 (s, 2 H, 2-NH_2, D_2O exchangeable), 10.88 (br s, 1-NH, D_2O exchangeable); IR (KBr) 4.72 μm (azido).

8-Aminoguanine (5). The 8-azido derivative **4** was converted to the corresponding 8-amino analogue **5** by catalytic hydrogenation (10% Pd/C) in water: yield 65%; mp >241 °C dec (lit.[18] mp >240 °C dec); UV (pH 1) λ_{max} 248 and 287 nm [lit.[12] UV (pH 1) λ_{max} 249 and 288 nm].

8-(Methylamino)guanosine (6). Compound **6** was prepared by a modification of the procedure described by Long et al.[21] Dry methylamine (82 g) was absorbed into 100 mL of anhydrous MeOH, followed by the addition of 1 g (2.76 mmol) of 8-bromoguanosine (**2**). The reaction mixture was heated at 130 °C for 5 h in a Parr pressure reaction apparatus and then allowed to stand at room temperature overnight. The excess methylamine and solvent were evaporated in vacuo, and the residue was recrystallized from water to give 0.37 g (43%) of product: mp >196 °C dec (lit.[21] mp >200 °C dec); UV (pH 1) λ_{max} 253 and 289 nm [lit.[21] UV (pH 1) λ_{max} 252 and 289 nm); NMR (Me_2SO-d_6) δ 2.81 (d, 3 H, 8-NCH_3 collapsed to singlet after D_2O exchange), 3.50–3.76 (m, 2 H, 5′-H), 3.76–3.98 (m, 1 H, 4′-H), 3.98–4.12 (m, 1 H, 3′-H), 4.21–4.80 (m, 1 H, 2′-H), 4.82–5.40 (br s, 4 H, 8-NHC; 2′-, 3′-, and 5′-OH, D_2O exchangeable), 5.78 (d, 1 H, 1′-H), 6.21 (s, 2 H, 2-NH_2, D_2O exchangeable), 10.63 (br s, 1-NH, D_2O exchangeable).

8-(Dimethylamino)guanosine (7). 8-Bromoguanosine (1.00 g, 2.76 mmol) was added to 100 mL of methanolic dimethylamine solution (containing 66 g of dimethylamine). The reaction mixture was heated at 130 °C for 5 h in a Parr pressure reaction apparatus. The solvent was evaporated under reduced pressure to dryness, and the residue was recrystallized from water to afford 0.39 g (42%) of **7**: mp >211 °C dec; UV (pH 1) λ_{max} 264 and 286 (sh) nm [lit.[19] UV (pH 1) λ_{max} 261.5 and 282 (sh) nm]; NMR (Me_2SO-d_6) δ 2.73 [s, 6 H, 8-$N(CH_3)_2$], 3.42–3.72 (m, 2 H, 5′-H), 3.73–4.01 (m, 1 H, 4′-H), 4.01–4.26 (m, 1 H, 3′-H), 4.82–5.44 (m, 4 H, 2′-H; 2′-, 3′-, and 5′-OH, D_2O exchangeable), 5.64 (d, 1 H, 1′-H), 6.24 (s, 2 H, 2-NH_2, D_2O exchangeable), 10.96 (br s, 1-NH_2, D_2O exchangeable).

8-(Hydroxylamino)guanosine (8). 8-Bromoguanosine (**2**) (3.00 g, 8.28 mmol) was dissolved in anhydrous MeOH (250 mL) containing 6 g of hydroxylamine. The reaction mixture was heated at 100 °C for 20 h in a Parr pressure reaction apparatus. The reaction vessel was cooled, and a solid separated that was collected by filtration, washed with water and acetone, and air-dried: yield

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1.31 g (50%): mp 260 °C dec (lit.[21] mp >260 °C dec); UV (pH 1) λ_{max} 254 and 290 nm [lit.[21] UV (pH 1) λ_{max} 250 and 287.5 nm]; NMR (Me_2SO-d_6) δ 3.12–3.74 (m, 2 H, 5′-H), 3.74–4.01 (m, 1 H, 4′-H), 4.02–4.38 (m, 1 H, 3′-H), 4.52–5.28 (m, 5 H, 2′-H; 2′-, 3′-, and 5′-OH and 8-NOH, D_2O exchangeable), 5.64 (d, 1 H, 1′-H), 6.51 (s, 2 H, 2-NH_2, D_2O exchangeable).

8-(Benzyloxy)guanosine (9). 8-Bromoguanosine (2) in 8 mL of Me_2SO was added to a solution of 0.2 g of sodium dissolved in 7 mL of benzyl alcohol and 20 mL of Me_2SO. The reaction mixture was heated at 65 °C for 18 h and then cooled to room temperature. Glacial acetic acid (~1 mL) was added until the solution was neutral, and the resulting solution was poured slowly into 400 mL of ethyl ether. The ether layer was decanted and discarded. The remaining oily residue was then slowly poured into 100 mL of acetone. The precipitate that formed was filtered and stirred with 10 mL of water. The slurry was filtered, and the solid residue was recrystallized from H_2O–EtOH to yield 0.68 g (63%) of 9: mp 170–171 °C dec (lit.[20] mp 171–173 °C dec); UV (pH 1) λ_{max} 247 and 295 nm [lit.[20] UV (pH 1) λ_{max} 246 and 294 nm]; NMR (Me_2SO-d_6) δ 3.36–3.65 (m, 2 H, 5′-H), 3.66–3.88 (m, 1 H, 4′-H), 3.88–4.04 (m, 1 H, 3′-H), 4.60–4.94 (m, 4 H, 2′-H; 2′-, 3′-, and 5′-OH, D_2O exchangeable), 5.41 (s, 2 H, 8-OCH_2Ar), 5.64 (d, 1 H, 1′-H), 6.26 (s, 2 H, 2-NH_2, D_2O exchangeable), 7.21–7.53 (m, 5 H, 8-OCC_6H_5).

8-Hydroxyguanosine (10). A solution of 8-(benzyloxy)-guanosine (9; 0.50 g, 1.28 mmol) dissolved in 20 mL of hot EtOH–H_2O (1:1, v/v), was added to 0.3 g of 10% Pd/C suspended in 10 mL of water. The mixture was hydrogenated at 50 psi of hydrogen at room temperature for 16 h. The catalyst was removed by filtration and washed with 15 mL of hot water. The solvents were evaporated to dryness in vacuo to afford 0.31 g (79%) of product: mp 229–231 °C dec (lit.[20] mp 232–235 °C dec); UV (pH 1) λ_{max} 247 and 295 nm [lit.[20] UV (pH 1) λ_{max} 246 and 294 nm]; NMR (Me_2SO-d_6) δ 3.41–3.66 (m, 2 H, 5′-H), 3.67–3.98 (m, 1 H, 4′-H), 3.98–4.21 (m, 1 H, 3′-H), 4.71–5.22 (m, 4 H, 2′-H; 2′-, 3′-, and 5′-OH, D_2O exchangeable), 5.58 (d, 1 H, 1′-H), 6.46 (s, 2 H, 2-NH_2, D_2O exchangeable).

8-Methoxyguanosine (11). 8-Bromoguanosine (2; 0.72 g, 2.00 mmol) in 8 mL of Me_2SO was added to a solution of 0.20 g of sodium dissolved in 10 mL of anhydrous MeOH and 20 mL of Me_2SO. The reaction mixture was heated at 60–65 °C in an oil bath for 18 h. The solution was allowed to cool to room temperature, neutralized with glacial acetic acid, and then poured slowly into 400 mL of ethyl ether. The resulting precipitate was filtered and washed with acetone followed by water and then recrystallized from water to yield 0.51 g (84%) of product: mp >216 °C dec; UV (pH 1) λ_{max} 250 and 282 nm [lit.[19] UV (pH 1) λ_{max} 248 and 282 nm]; NMR (Me_2SO-d_6) δ 3.32–3.68 (m, 2 H, 5′-H), 3.69–4.01 (m, 1 H, 4′-H), 3.96 (s, 3 H, 8-OCH_3), 4.01–4.22 (m, 1 H, 3′-H), 4.56–4.89 (m, 4 H, 2′-H; 2′-, 3′-, and 5′-OH, D_2O exchangeable), 5.65 (d, 1 H, 1′-H), 6.41 (s, 2 H, 2-NH_2, D_2O exchangeable), 10.92 (br s, 1-NH, D_2O exchangeable).

8-Mercaptoguanosine (12). Thiourea (0.80 g, 10.26 mmol) was added to a suspension of 8-bromoguanosine (2) (2.00 g, 5.50 mmol) in 20 mL of absolute EtOH. The mixture was refluxed for 16 h and then cooled to room temperature. The resulting solid was collected by filtration and recrystallized from water to provide 1.40 g (58%) of 12: mp >218 °C dec (lit.[22] mp >220 °C dec); UV (pH 1) λ_{max} 303, 286, and 232 nm [lit.[22] UV (pH 1) λ_{max} 302, 285, and 230 nm]; NMR (Me_2SO-d_6) δ 3.54–3.72 (m, 2 H, 5′-H), 3.73–3.92 (m, 1 H, 4′-H), 4.12–4.32 (m, 1 H, 3′-H), 4.51–5.22 (m, 5 H, 2′-H; 2′-, 3′-, 5′-OH and 8-SH, D_2O exchangeable), 6.24 (d, 1 H, 1′-H), 6.42 (s, 2 H, 2-NH_2, D_2O exchangeable), 10.76 (br s, 1-NH, D_2O exchangeable).

8-(Methylthio)guanosine (13). Dimethyl sulfate (0.60 g, 6.38 mmol) was added to a mixture of 8-mercaptoguanosine (12; 1.20 g, 3.80 mmol) and 0.60 g of anhydrous K_2CO_3 in 18 mL of DMF. The reaction mixture was heated with stirring at 70–75 °C for 3 h, cooled to room temperature, and then poured into 250 mL of acetone. The solution was adjusted to pH 6 with glacial acetic acid, and the solid residue was filtered and recrystallized from water to afford 0.45 g (36%) of product: mp >201 °C dec (lit.[22] mp >200 °C dec); UV (pH 1) λ_{max} 289 (sh) and 273 nm [lit.[22] UV (pH 1) λ_{max} 289 (sh) and 272 nm]; NMR (Me_2SO-d_6) δ 2.54 (s, 3 H, 8-SCH_3), 3.42–3.70 (m, 2 H, 5′-H), 3.72–3.99 (m, 1 H, 4′-H), 3.99–4.18 (m, 1 H, 3′-H), 4.64–5.50 (m, 4 H, 2′-H; 2′-, 3′-, and 5′-OH, D_2O exchangeable), 5.64 (d, 1 H, 1′-H), 6.38 (s, 2 H, 2-NH_2, D_2O exchangeable), 10.74 (br s, 1-NH, D_2O exchangeable).

8-(Methylsulfonyl)guanosine (14). 8-(Methylthio)guanosine (0.26 g, 0.76 mmol) was dissolved in 3 mL of acetic acid, and 0.15 mL of 3% hydrogen peroxide was added to the acetic acid solution. The reaction mixture was stirred at room temperature for 48 h, the solvent was evaporated to dryness in vacuo, and the residue was crystallized from water to give 0.12 g (43%) of 14: mp >206 °C dec; UV (pH 1) λ_{max} 273 and 285 (sh) nm [lit.[19] UV (pH 1) λ_{max} 273 and 285 (sh) nm]; NMR (Me_2SO-d_6) δ 3.04 (s, 3 H, 8-SO_2CH_3), 3.36–3.72 (m, 2 H, 5′-H), 3.73–4.02 (m, 1 H, 4′-H), 4.04–4.36 (m, 1 H, 3′-H), 4.71–5.54 (m, 4 H, 2′-H; 2′-, 3′-, and 5′-OH, D_2O exchangeable), 6.12 (d, 1 H, 1′-H), 6.62 (s, 2 H, 2-NH_2, D_2O exchangeable), 10.67 (br s, 1-NH, D_2O exchangeable).

8-Bromo-2′-deoxyguanosine (16). Saturated bromine–water in aliquots of 15 mL was added to a suspension of 2′-deoxyguanosine (15; 5.00 g, 18.7 mmol) in 30 mL of water at a rate that permitted the yellow color of the reaction mixture to disappear between each addition. The colorless solid was then quickly collected by filtration and successively washed with 30 mL of cold water and 30 mL of acetone. The crude product was recrystallized from water, filtered, and dried to yield 3.70 g (57%) of 16: mp 208 °C dec (lit.[21] mp 210 °C dec); UV (pH 1) λ_{max} 262 nm [lit.[21] UV (pH 1) λ_{max} 261 nm]; NMR (Me_2SO-d_6) δ 2.10–2.39 (m, 2 H, 2′-H), 3.40–3.62 (m, 2 H, 5′-H), 3.63–3.84 (m, 1 H, 3′-H), 4.18–4.52 (m, 1 H, 4′-H), 4.76–5.32 (m, 2 H, 3′- and 5′-OH, D_2O exchangeable), 6.18 (t, 1 H, 1′-H), 6.42 (s, 2 H, 2-NH_2, D_2O exchangeable), 11.25 (br s, 1-NH, D_2O exchangeable).

8-Methoxy-2′-deoxyguanosine (17). Me_2SO (12 mL) was added to a solution of 0.2 g of sodium dissolved in 7 mL of anhydrous MeOH. To the resulting solution was added 8-bromo-2′-deoxyguanosine (16; 0.50 g, 1.44 mmol) in 8 mL of Me_2SO. The reaction mixture was heated at 60–65 °C with stirring for 18 h and cooled to room temperature, and glacial acetic acid was added to the solution until it was neutral. The solution was then slowly poured into 400 mL of ethyl ether. The resulting solid precipitate was collected by filtration, washed with water, and recrystallized from water to give 0.10 g (24%) of product: mp 204 °C dec; UV (pH 1) λ_{max} 249 nm (ε 13 400) and 281 (ε 8900); UV (pH 12) λ_{max} 252 nm (ε 11 780) and 269 (sh) (ε 10 366); NMR (Me_2SO-d_6) δ 2.20–2.32 (m, 2 H, 2′-H), 3.38–3.59 (m, 2 H, 5′-H), 3.60–3.82 (m, 1 H, 3′-H), 3.96 (s, 3 H, 8-OCH_3), 4.02–4.58 (m, 1 H, 4-H), 4.60–5.32 (m, 2 H, 3′- and 5′-OH, D_2O exchangeable), 6.12 (t, 1 H, 1′-H), 6.78 (s, 2 H, 2-NH_2, D_2O exchangeable). Anal. ($C_{11}H_{15}N_5O_5 \cdot 2H_2O$) C, H, N.

8-(Benzyloxy)-2′-deoxyguanosine (18). 8-Bromo-2′-deoxyguanosine (16; 0.68 g, 1.96 mmol) was added to a solution of 0.15 g of sodium dissolved in 5 mL of benzyl alcohol containing 15 mL of Me_2SO. The resulting solution was heated at ~65 °C for 18 h, cooled to room temperature, and neutralized with acetic acid. The solution was then poured slowly into 400 mL of ethyl ether. The precipitate that separated was collected by filtration and recrystallized from MeOH: yield 0.2 g (28%); mp >187 °C dec; UV (pH 1) λ_{max} 247 nm (ε 11 410) and 294 (ε 9820); UV (pH 12) λ_{max} 252 nm (ε 13 350) and 268 (sh) (ε 10 530); NMR (Me_2SO-d_6) δ 2.05–2.40 (m, 2 H, 2′-H), 3.61–3.82 (m, 1 H, 3′-H), 4.12–4.40 (m, 1 H, 4′-H), 4.56–5.21 (m, 2 H, 3′- and 5′-OH, D_2O exchangeable), 5.42 (s, 2 H, 8-OCH_2Ar), 6.14 (t, 1 H, 1′-H), 6.34 (s, 2 H, 2-NH_2, D_2O exchangeable), 7.38–7.59 (m, 5 H, 8-$OCH_2C_6H_5$), 10.98 (br s, 1-NH, D_2O exchangeable). Anal. ($C_{17}H_{19}N_5O_5 \cdot 2H_2O$) C, H, N.

8-Hydroxy-2′-deoxyguanosine (19). A solution of 8-(benzyloxy)-2′-deoxyguanosine (18; 0.20 g, 0.54 mmol) in 8 mL of H_2O and 8 mL of MeOH was hydrogenated at 50 psi of hydrogen at room temperature in the presence of 0.3 g of 10% Pd/C for 18 h. The catalyst was removed by filtration, and the solvent was evaporated to dryness in vacuo to yield 0.089 g (59%) of 19: mp 217–220 °C dec; UV (pH 1) λ_{max} 248 nm (ε 13 500) and 295 (ε 11 300); UV (pH 12) λ_{max} 248 nm (ε 13 400) and 282 (ε 11 140); NMR (Me_2SO-d_6) δ 1.94–2.32 (m, 2 H, 2′-H), 3.38–3.60 (m, 2 H, 5′-H), 3.61–3.82 (m, 1 H, 3′-H), 4.21–4.60 (m, 1 H, 4′-H), 4.61–5.32 (m, 3 H, 3′- and 4′-OH and 8-OH, D_2O exchangeable), 6.14 (t, 1 H, 1′-H), 6.41 (s, 2 H, 2-NH_2, D_2O exchangeable), 11.21 (s, 1 H, 1-NH, D_2O exchangeable). Anal. ($C_{10}H_{13}N_5O_5 \cdot H_2O$) C, H, N.

Biological Experimental Procedure. Friend erythroleukemia cells and HGPRT$^-$ variant erythroleukemia cells were passaged every 3 days at a level of 5×10^4 cells/mL in Dulbecco's

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).

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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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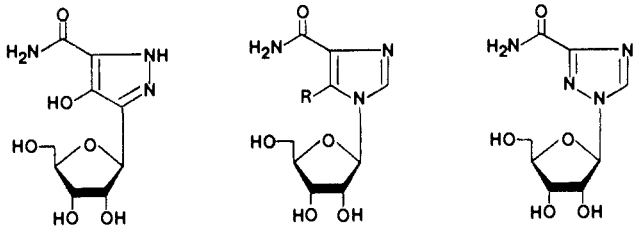
A number of 5-substituted imidazole-4-carboxamide ribonucleosides were prepared and tested for their biological activity. Treatment of 5-chloro-1-β-D-ribofuranosylimidazole-4-carboxamide (**2**) with methanethiol provided 5-(methylthio)-1-β-D-ribofuranosylimidazole-4-carboxamide (**3a**). Similar treatment 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]



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