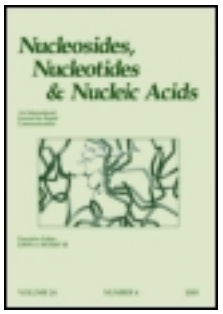

Nucleosides, Nucleotides and Nucleic Acids

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Synthesis and Evaluation of a Series of 2′-Deoxy Analogues of The Antiviral Agent 5,6-Dichloro-2-Isopropylamino-1-(β-L-Ribofuranosyl)-1H-Benzimidazole (1263W94)

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NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 19(1&2), 101–123 (2000)

SYNTHESIS AND EVALUATION OF A SERIES OF 2'-DEOXY ANALOGUES OF THE ANTIVIRAL AGENT 5,6-DICHLORO-2-ISOPROPYLAMINO-1-(β-L-RIBOFURANOSYL)-1H-BENZIMIDAZOLE (1263W94)

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This manuscript is dedicated to the memory of Dr. Gertrude B. Elion

ABSTRACT: A series of 2'-deoxy analogues of the antiviral agent 5,6-dichloro-2-isopropylamino-1-(β-L-ribofuranosyl)-1H-benzimidazole (1263W94) were synthesized and evaluated for activity against human cytomegalovirus (HCMV) and for cytotoxicity. The 2-substituents in the benzimidazole moiety correspond to those that were used in the 1263W94 series. In general, as was found in the 1263W94 series, cyclic and branched alkylamino groups were needed for potent activity against HCMV. Three analogues **3a, 3b** and **3d** were as potent as 1263W94. Further evaluation of two analogues, **3a** and **3b**, suggested that these 2'-deoxy analogues may act via a novel mechanism of action similar to that of 1263W94. These 2'-deoxy analogues generally lacked cytotoxicity in vitro. Pharmacokinetic parameters in mice and protein binding properties of **3a** were quite similar to 1263W94. However, the oral bioavailability of **3a** was only half of that observed for 1263W94.

INTRODUCTION

Human cytomegalovirus (HCMV) is a member of the herpes family of viruses. Primary HCMV infection is usually followed by an extended period of latency until an

event occurs to compromise the immune system. Then the virus can begin actively replicating, resulting in serious, debilitating, and even life threatening diseases. Among severely immunocompromised AIDS patients, for example, HCMV can cause retinitis, a condition that if left untreated, ultimately results in blindness.[1,2] Other equally serious and more systemic diseases include esophagitis, colitis and pneumonia.[2] The virus is shed in body fluids such as blood, saliva and semen resulting in transmission to uninfected individuals.

Four drugs currently are approved by the FDA for the treatment of HCMV infections. Three of these - ganciclovir,[1,2] foscarnet[3] and cidofovir[4] (Figure 1) - act via the inhibition of HCMV DNA polymerase, but ganciclovir and cidofovir require initial anabolism to their triphosphates. Although therapeutically useful, these three drugs are generally associated with dose-limiting side effects and poor oral bioavailability, thus reducing their clinical utility.[1] Resistant HCMV strains can develop in patients following prolonged therapy,[5] and since they share a common inhibition site, cross-resistance is a potential problem.[1] A more potent and efficacious agent to treat HCMV that acts via a novel mechanism of action, with limited toxicity and good oral bioavailability would thus fulfill a significant, unmet medical need.

Among potential HCMV agents with a novel mechanism of action, 2,5,6-trichloro-1-(β-D-ribofuranosyl)benzimidazole (TCRB) (**1**) and its 2-bromo analogue (BDCRB) (**2**) (Figure 2) were shown to be potent inhibitors of HCMV replication, and to have high therapeutic indices.[6] BDCRB does not inhibit either viral RNA synthesis, DNA synthesis, or protein synthesis in HCMV-infected cells. Its mechanism of action is a result of inhibition of viral DNA maturation by interacting with one or both of the components of the HCMV DNA cleavage apparatus: the UL89[7] and UL56.[8]

Unfortunately, BDCRB is rapidly cleared in both rats and monkeys,[9] which substantially limits its potential as an agent for the treatment of HCMV infections. An extensive search for a replacement for BDCRB with superior pharmacokinetic properties identified 1263W94 among a series of 1-(ß-L-ribofuranosyl)benzimidazoles evaluated at Glaxo Wellcome.[10, 11] The compound 1263W94 differs from BDCRB in that it is an L-

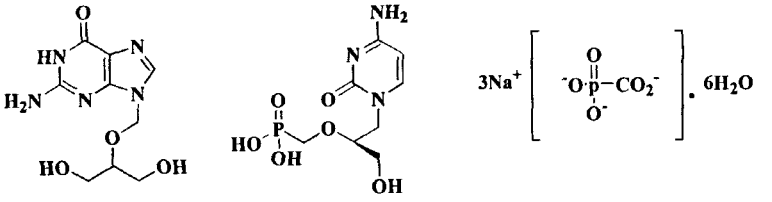


FIGURE 1

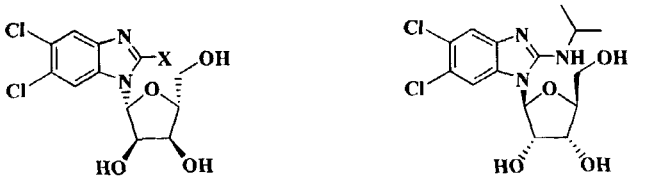
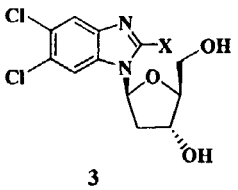


FIGURE 2

ribofuranose with an isopropylamino substituent in the 2-position of the benzimidazole heterocycle. This compound is as potent against HCMV in vitro as BDCRB and is metabolically more stable in rats, monkeys and humans. 1263W94 has recently been shown in a Phase I/II clinical trial to be well tolerated and efficacious in reducing viral load.[12] Additionally, 1263W94 inhibits HCMV DNA synthesis by a novel mechanism which does not involve a conversion to its triphosphate.[10, 11]

We report in this paper our efforts[13] to modify the L-ribofuranose moiety of the 1263W94 template to the 2'-deoxy-L-ribofuranose moiety. This work was part of a project to delineate the pharmacophore of the scaffold on which 1263W94 is based. This plan is depicted in structure **3** (Figure 3). Our focus was on the synthesis of structurally related analogues of the 1-(ß-L-ribofuranosyl)benzimidazoles, which differ from 1263W94 only in the absence of the 2'-hydroxy group in the L-ribofuranose moiety.



3

FIGURE 3

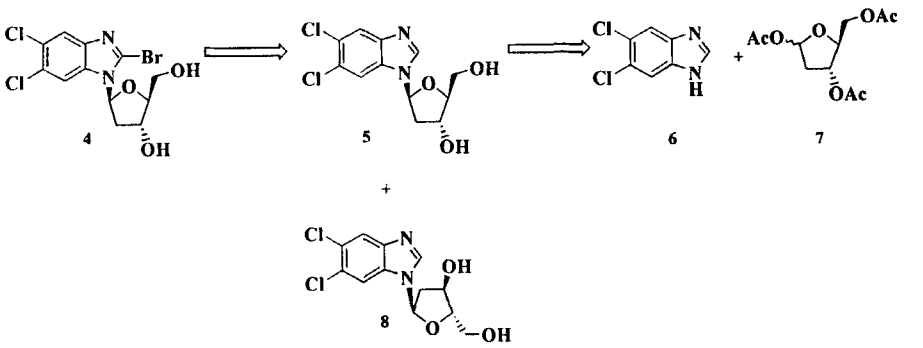
The 2-substituent in the benzimidazole heterocycle was found to play a significant role in the potency of the resultant analogues against HCMV in previous synthetic and SAR work on the 1-(ß-L-ribofuranosyl)benzimidazoles. In our work with the 2'-deoxy-L-ribofuranose analogues, we focused on varying the 2-substituents of the benzimidazole heterocycle. The substituents that we chose are similar to those in the (ß-L-ribofuranosyl)benzimidazole series and are those that have been shown to result in analogues with potent activity in vitro against HCMV in that series. The resultant compounds are shown in Table 1.

CHEMISTRY

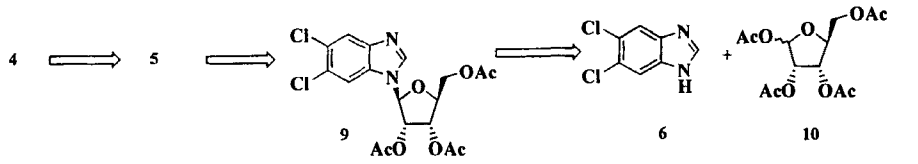
The synthetic strategy calls for the synthesis of common intermediate **4** from which the desired analogues **3** will be obtained by the displacement of the bromo group using the appropriate alkylamines. Retrosynthetic analysis, as shown in Schemes 1a and 1b, suggested two plausible routes for the synthesis of intermediate **4**.

Scheme 1a utilizes a convergent methodology starting from 2-deoxy-1,3,5-tri-*O*-acetyl-β-L-ribofuranose (**7**)[14] and 5,6-dichlorobenzimidazole (**6**).[15] Because of the absence of anchimeric assistance from a 2'-acetoxy group during the coupling of benzimidazole **6** to carbohydrate **7**, this reaction pathway is predicted to result in anomers **5** and **8**.

Scheme 1b, on the other hand, starts with 1,2,3,5-tetra-*O*-acetyl-β-L-ribofuranose (**10**) (L-TAR).[16] This is expected to lead to isomer **9** as the predominant product on



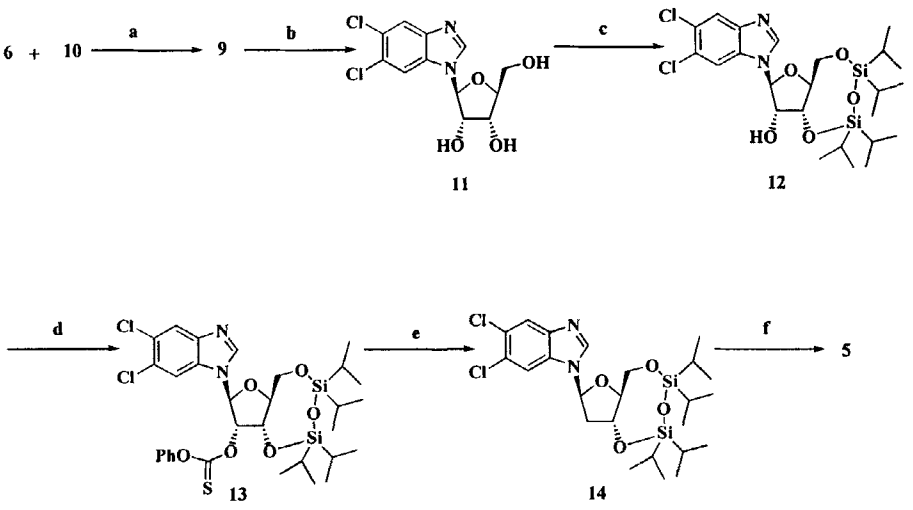
SCHEME 1a



SCHEME 1b

coupling with **6** because of the anchimeric assistance of the 2'-acetoxy group. Saponification of the acetyl groups, protection of the 3',5'-dihydroxy groups and deoxygenation of the 2'-hydroxy group is expected to lead to intermediate **5**. Bromination of **5** will then lead to **4**. Albeit divergent, however, because of the potentially more regiospecific outcome than that shown in Scheme 1a, the methodology depicted in Scheme 1b was adopted in our synthesis.

As shown in Scheme 2, the synthesis of **4** started with the coupling of benzimidazole **6** and L-TAR (**10**) under Vorbruggen's conditions using N,O-*bis*trimethylsilylacetamide and trimethylsilyl trifluoromethanesulfonate as the catalyst.[17] This resulted in the formation of the triacetate **9**. Saponification of **9** with sodium carbonate in a mixture of methanol, ethanol and water gave compound **11** (L-DRB). Prior to a removal of the 2'-hydroxy group, **11** was first converted to the tetraisopropyldisiloxy-protected **12** that was then transformed to the thiocarbonate **13**.[18]



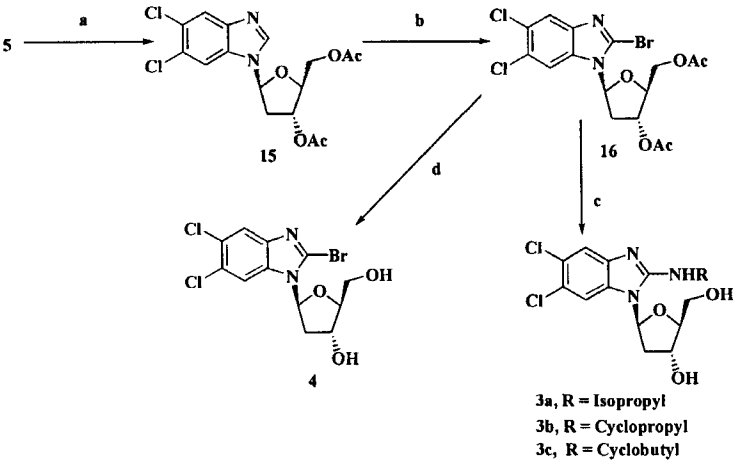
Conditions: (a) BSA, TMSO-triflate, CH_3CN; (b) Na_2CO_3, MeOH, EtOH, H_2O; (c) 1,3-dichloro-tetraisopropyldisiloxane, imidazole, DMF; (d) PhOC(=S)Cl, DMAP, CH_3CN; (e) Bu_3SnH, AIBN, toluene; (f) TEAF, THF.

SCHEME 2

Reaction of **13** with tributyltin hydride and AIBN as the free radical inducer in toluene gave the intermediate **14**.[18] The protecting group was removed with tetraethylammonium fluoride in tetrahydrofuran (THF) to give compound **5**.[19]

Our initial attempt at the synthesis of intermediate **4** by brominating **5** with N-bromosuccinimide (NBS) in THF resulted in a multitude of products with the aglycone 5,6-dichlorobenzimidazole (**6**) as the predominant one. We theorized that the glycosidic linkage was possibly weakened by the presence of the two "naked" hydroxy groups that could also react with NBS. We next attempted to brominate the tetraisopropyldisiloxy-protected **14** using the same conditions. This also resulted in a multitude of products, none of which could be isolated in sufficient quantities for structural analysis.

We solved this problem by first protecting the two hydroxy groups in **5** with acetyl groups (see Scheme 3) to obtain **15**. Compound **15** was then brominated with NBS in refluxing THF to obtain a moderate yield of the bromo intermediate **16**. Reacting **16** with an excess of the appropriate alkylamines resulted in the concomitant removal of the



Conditions: (a) Ac_2O, pyridine; (b) NBS, THF; (c) RNH_2, EtOH; (d) Na_2CO_3, MeOH, EtOH, H_2O.

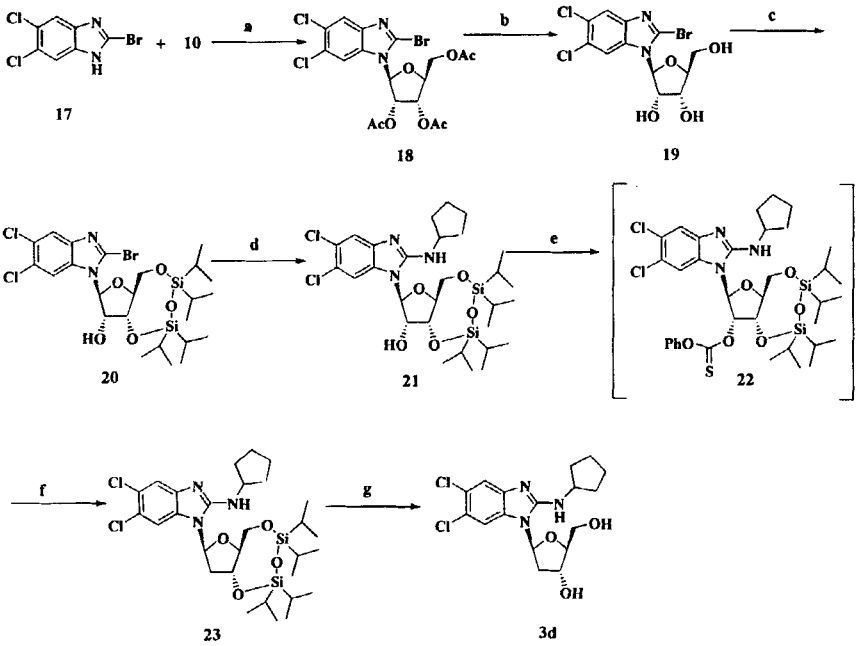
SCHEME 3

diacetyl groups and the formation of the desired analogues **3a, 3b** and **3c**. The bromo analogue **4** was obtained from **16** with sodium carbonate in a mixture of methanol, ethanol and water.

Unfortunately, for reasons not understood, the reaction of **16** with cyclopentylamine resulted in a poor yield of the desired **3d**. An alternative procedure, as depicted in Scheme 4, was devised. The key step in this procedure was the displacement of the bromo group in 2-bromo-5,6-dichloro-1-[3,5-*O*-(1,3-tetraisopropyldisiloxa-1,3-diyl)-β-L-ribofuranosyl]benzimidazole (**20**) by cyclopentylamine to give **21**. Thiocarbonate formation to **22** and the reaction of **22** with tributyltin hydride and AIBN gave the tetraisopropyldisiloxy-protected **23**. Removal of the protecting group with tetrabutylammonium fluoride gave the desired **3d** in a moderate yield. This method is also applicable to the synthesis of the other 2'-deoxy analogues.

RESULTS AND DISCUSSION

Much has been learned about the structure-activity relationship (SAR) for the (ß-L-ribofuranosyl)benzimidazole scaffold from the work that led to the identification of



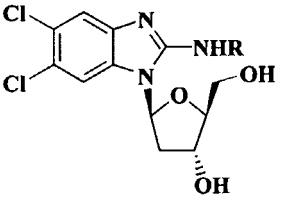
Conditions: (a) BSA, TMSO-triflate, CH_3CN; (b) Na_2CO_3, MeOH, EtOH, H_2O; (c) 1,3-dichloro-tetraisopropyldisiloxane, imidazole, DMF; (d) Cyclopentylamine, EtOH; (e) PhOC(=S)Cl, DMAP, CH_3CN; (f) Bu_3SnH, AIBN, toluene; (g) TBAF, THF.

SCHEME 4

1263W94. One important feature for antiviral activity and metabolic stability is the presence of 2-alkylamino substituents in the benzimidazole moiety. Relatively small, branched and cyclic alkylamino groups, in particular, were found to have a profound effect on potency against HCMV and on optimal pharmacokinetic profile. Thus, as mentioned earlier, in our ensuing work in the 2'-deoxy-(ß-L-ribofuranosyl)benzimidazole series, the choice of 2-substituents is based on those that had resulted in compounds with potent activity against HCMV in the 1-(ß-L-ribofuranosyl)benzimidazole series.

The compounds that were synthesized and evaluated are shown in Table 1. Their in vitro activity against HCMV was measured by inhibition of multicycle DNA synthesis using a hybridization assay in MRC-5 cells infected with HCMV strain AD169. Details of the assay are described in the experimental section. As shown in Table 1, the unsubstituted **5** was slightly active against HCMV in the DNA hybridization assay. This

TABLE 1. Activity Against HCMV of 5,6-Dichloro-1-(2-deoxy-β-L-ribofuranosyl)benzimidazoles.[a]



Compound	R	DNA Hybridization (IC_{50} in μM)
5	NHR = H	41 (1)[b]
4	NHR = Br	26 (1)
3a	$(CH_3)_2CH$	0.17 (6)
3b	Cyclopropyl	0.17 (2)
3c	Cyclobutyl	0.37 (2)
3d	Cyclopentyl	0.1 (2)
1263W94		0.12 (71)

[a]Details are described in the Experimental Section.
[b]The number in parenthesis represents the number of replicate assays. IC_{50} values are the average of these replicates.

was also the case for the corresponding 1-(ß-L-ribofuranosyl)benzimidazole analogue (IC_{50} value = 52 μM).

2-Bromo-(ß-L-2'-deoxyribofuranosyl)benzimidazole analogue **4** was about 26 times less active than the corresponding L-ribofuranose analogue, which had an IC_{50} value of 0.93 μM in the hybridization assay. Replacing this 2-bromo group by alkylamino groups gave compounds that were generally potent against HCMV. Three of these branched or cyclic analogues **3a**, **3b** and **3d** had IC_{50} values in the DNA hybridization assay in the range of the values for 1263W94. Further efforts to delineate the mechanism of action of these 2'-deoxy-(ß-L-ribofuranosyl)benzimidazole analogues using **3a** and **3b** suggested that these compounds act by inhibiting DNA synthesis. In a

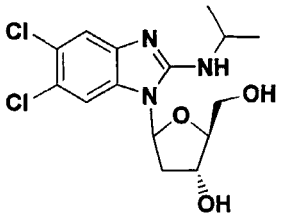
single-cycle DNA synthesis inhibition assay, **3a** and **3b** gave IC_{50} concentrations of <0.3 μM.[20] Exactly how these compounds inhibit this pathway is yet to be determined. Both compounds, however, did not inhibit DNA maturation. 1263W94 inhibits DNA synthesis by a novel mechanism which is yet to be elucidated, and 1263W94 does not inhibit either DNA maturation nor viral polymerase since its triphosphate analogue is inactive against the enzyme.[11] Given the similarity in the two series, they probably share the same mechanism of action.

To gauge their potential therapeutic utility, the cytotoxicity of all analogues was determined in the human leukemic cell lines IM-9 and Molt-4 and the lymphoma cell line U-937.[21, 22] As shown in Table 2, these 2'-deoxy-(ß-L-ribofuranosyl)benzimidazoles were generally poor inhibitors of the growth of these cell lines. **3a** and **3b** showed the highest therapeutic indices, as measured by the ratio of the average of the IC_{50} values of the three cell lines versus the IC_{50} value against HCMV (see Table 2). Further evaluation of the cytotoxic potential of **3a** and **3b** included an assay (Table 2) that gauged their likelihood to cause bone-marrow related toxicities.[23, 24] This assay measured the cytotoxicity of the compounds for early human bone marrow progenitors. The compounds **3a** and **3b** were not cytotoxic for these cells. The therapeutic indices (the average of the IC_{50} values of the two cell lines used versus the IC_{50} value against HCMV) for these two compounds were 230 and 500, respectively. For 1263W94 this index is around 740.

Finally, because of similarity in structure to 1263W94, compound **3a** was chosen for further studies of its pharmacokinetics and plasma protein binding. As presented in Table 3, both the pharmacokinetic parameters in mice and the plasma protein binding of **3a** were generally comparable to those of 1263W94. The oral bioavailability, however, was only about half that of 1263W94.

In conclusion, we have synthesized a series of 2'-deoxy-(ß-L-ribofuranosyl)benzimidazole analogues. The 2-substituents chosen for the benzimidazole heterocycle were designed to mimic those in the corresponding 1263W94 series. In general, 2-alkylamino substituted analogues had activity against HCMV that was in the range of that of the corresponding analogues in the L-ribofuranose series. Further studies

TABLE 3. Pharmacokinetic Parameters and Protein Binding of 3a in Mice.[a]



3a

Compound	AUC (hμg/mL)	Cl (L/Kg/hr)	t1/2 (h)	F (%)	Protein Binding (%)
3a	8.6	1.2	0.94	28	96.4
1263W94	8.2	1.2	1.2	69	96.4

[a]Details are described in the Experimental Section. Legend: AUC, area under the curve; Cl, clearance from plasma; t1/2, half-life; F, oral bioavailability.

TABLE 2. Cytotoxicity of 5,6-Dichloro-1-(2-deoxy-β-L-ribofuranosyl)benzimidazoles.

	Growth inhibition of cultured cells[a] (IC_{50} in μM)			Human bone marrow progenitor[b] (IC_{50} in μM)	
Compound	**IM-9**	**MOLT 4**	**U-937**	**CFU-GM**	**BFU-E**
5	120	140	48		
4	21	21	12		
3a	42	20	19	31	46
3b	110	73	120	99	64
3c	28	23	20		
3d	12	8	11		
1263W94	57	-	-	88	90

[a]See references 21 and 22 for experimental details.
[b]See references 23 and 24 for experimental details.

using analogues **3a** and **3b** suggested that these 2'-deoxy analogues inhibited viral DNA synthesis but the mechanism of action is currently unknown.

The 2'-deoxy L-ribofuranose analogues generally had low in vitro cytotoxicity against growth of leukemic and lymphomic cells, and against human bone marrow progenitor cell lines, resulting in favorable therapeutic indices. Finally, the pharmacokinetic parameters of **3a** in mice were similar to those of 1263W94, although oral bioavailability was only half of the latter.

EXPERIMENTAL SECTION

GENERAL: ^{1}H nmr spectra were recorded on Varian Unity Plus 400 MHz and 300 MHz NMR spectrometers. Mass spectral data were obtained using a Micromass Platform mass spectrometer utilizing atmospheric pressure chemical ionization. Melting points were obtained using a Melt-Temp II apparatus. Microanalyses were provided by Atlantic Microlab, Norcross, Georgia. All commercial reagents were used without further purification. All reactions were run under a nitrogen atmosphere. Flash column chromatography was performed according to reference 25. The reported experimental procedures are representative examples of many runs. Experimental details for the in vitro cytotoxicity studies (growth inhibition of cultured cells and human bone marrow progenitor) are described in references 21-24.

5,6-Dichloro-1-(2,3,5-tri-*O*-acetyl-β-L-ribofuranosyl)benzimidazole (9).

To a refluxed solution of 5,6-dichlorobenzimidazole (**6**)[15] (10 g, 52 mmol) and N,O-*bis*trimethylsilylacetamide (BSA) (13 mL, 52 mmol) in 200 mL of anhydrous acetonitrile was added trimethylsilyl trifluoromethanesulfonate (13 mL, 63 mmol). The resultant solution was stirred for 15 min, followed by the addition of 20 g of 1,2,3,5-tetra-*O*-acetyl-β-L-ribofuranose (**10**) (L-TAR).[16] The reaction mixture was refluxed for an additional 2 h, cooled and poured into 100 mL of cold saturated $NaHCO_3$. The mixture was then extracted with chloroform, and the chloroform extracts were washed with water. After drying ($MgSO_4$) and solvent removal, the crude product was purified by flash column chromatography on silica gel with EtOAc/hexane (7:3). This gave 18.3

g (79%) of **9** as an off-white foam. ^{1}H NMR (DMSO-d_6) δ 2.05 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 2.15 (s, 3H, CH_3), 4.3-5.0 (m, 3H, CH_2 & CH), 5.42 (unresolved m, 1H, CH), 5.62 (t, 1H, CH), 6.4 (d, 1H, CH), 8.0 (s, 1H, benzimidazole), 8.2 (s, 1H, benzimidazole), 8.63 (s, 1H, benzimidazole).

Anal. Calcd for $C_{18}H_{18}N_2O_7Cl_2 \cdot 0.5H_2O$: C, 47.59; H, 4.22; N, 6.17; Cl, 5.61. Found: C, 47.32; H, 4.05; N, 5.95; Cl, 15.33.

5,6-Dichloro-1-(β-L-ribofuranosyl)benzimidazole (L-DRB) (11).

A suspension of **9** (5.4 g, 12 mmol) and sodium carbonate (1.9 g, 18 mmol) in MeOH/EtOH/H_2O (25:25:6) was stirred at room temperature for 24 h. The reaction mixture was concentrated and then dissolved in EtOAc. After washing with saturated brine, drying ($MgSO_4$) and solvent removal, 2.5 g (65%) of **11** was obtained as a white solid: mp 210-212 °C; ^{1}H NMR (DMSO-d_6) δ 3.5-3.7 (m, 2H, CH_2), 3.9 (dd, 1H, CH), 4.0 (m, 1H, CH), 4.2 (q, 1H, CH), 5.2 (overlapping m, 2H, CH and OH), 5.5 (d, 1H, OH), 5.9 (d, 1H, OH), 7.9 (s, 1H, benzimidazole), 8.2 (s, 1H, benzimidazole), 8.5 (s, 1H, benzimidazole).

Anal. Calcd for $C_{12}H_{12}N_2O_4Cl_2$: C, 45.16; H, 3.79; N, 8.78; Cl, 22.22. Found: C, 45.02; H, 3.02; N, 8.81; Cl, 22.09.

5,6-Dichloro-1-[3,5-*O*-(1,1,3,3-tetraisopropyldisiloxa-1,3-diyl)-β-L-ribofuranosyl] benzimidazole (12).

A mixture of **11** (2.4 g, 7.5 mmol), 1,3-dichlorotetraisopropyldisiloxane (2.5 mL, 8.3 mmol) and imidazole (2 g, 30 mmol) in 30 mL of DMF was stirred for 12 h and then 100 mL of H_2O were added. The solid that had formed was collected by filtration and then dissolved in 50 mL of EtOAc/hexane (1:1). This organic phase was washed with saturated brine once. After drying ($MgSO_4$) and solvent removal, the crude product was purified by flash column chromatography on silica gel with MeOH/CH_2Cl_2 (1:100) as the eluent. This gave 2.62 g (62%) of **12** as a white solid: mp 148-150 °C; ^{1}H NMR (DMSO-d_6) δ 0.8-1.1 (m, 28H, $CH(CH_3)_2$), 3.9 (dd, 1H, CH_2), 4-4.1 (m, 1H, CHO), 4.12 (dd, 1H, CH_2O), 4.24 (dt, 2H, CHO), 5.74 (d, 1H, CHO(N)), 5.9 (d, 1H, OH), 7.9 (s, 2H, benzimidazole), 8.4 (s, 1H, benzimidazole).

Anal. Calcd for $C_{24}H_{38}N_2O_5Cl_2Si_2$: C, 51.28; H, 6.77; N, 4.99; Cl, 12.64. Found: C, 51.49 H, 6.85; N, 4.98; Cl, 12.63.

5,6-Dichloro-1-[2-phenoxythionoformyl-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxa-1,3-diyl)-β-L-ribofuranosyl]benzimidazole (13).

To an almost homogeneous solution of **12** (1 g, 1.8 mmol) in 40 mL of CH_3CN was added N, N-dimethylaminopyridine (0.66 g, 5.4 mmol). The resultant mixture was stirred for 5 min and was followed by the dropwise addition of phenyl chlorothionoformate (0.3 mL, 2.2 mmol). The mixture was stirred for 6 h and ~25 mL of EtOAc/hexane (1:1) was then added. This mixture was washed with saturated brine (2X25 mL). The aqueous solution was re-extracted once with 25 mL of EtOAc/hexane (1:1). The organic extracts were combined, dried ($MgSO_4$) and concentrated. The crude product was purified by flash column chromatography on silica with EtOAc/Hexane (1.5:6) as the eluent. This gave 0.6 g (48%) of **13** as a white foam. ^{1}H NMR (DMSO-d_6) δ 0.8-1.1 (m, 28H, $CH(CH_3)_2$), 3.9 (dd, 1H, CH_2), 4 (m, 1H, CH), 4.16 (dd, 1H, CH_2), 4.77 (dd, 1H, CH), 6.02 (dd, 1H, CH), 6.46 (d, 1H, CH), 7.14 (d, 2H, aromatic), 7.28 (t, 1H, aromatic), 7.42 (t, 2H, aromatic), 7.93 (s, 1H, benzimidazole), 8.02 (s, 1H, benzimidazole), 8.5 (s, 1H, benzimidazole).

Anal. Calcd for $C_{31}H_{42}N_2O_6Cl_2Si_2S$: C, 53.5; H, 6.03; N, 4.02; Cl, 10.06; S, 4.6. Found: C, 53.39; H, 5.97; N, 4.00; Cl, 10.19; S, 4.48.

5,6-Dichloro-1-[2-deoxy-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxa-1,3-diyl)-β-L-ribofuranosyl]benzimidazole (14).

A solution of **13** (0.39 g, 0.56 mmol), AIBN (0.06 g) and 0.6 mL (4.5 mmol) of tributyltin hydride in 10 mL of degassed toluene was refluxed under nitrogen for 2 h. The toluene was then removed in vacuo and the resultant concentrate was chromatographed on silica gel with methylene chloride/acetone (100:2) to give 0.13 g (43%) of **14** as an oil. ^{1}H NMR (DMSO-d_6) δ 0.8-1.1 (m, 28H, $4CH(CH_3)_2$), 2.3-2.5 (m, 1H, buried in the DMSO peak, CH_2), 2.7-2.8 (m, 1H, CH_2), 3.8-3.9 (m, 3H, CH and CH_2O), 4.63 (dd, 1H, CH), 6.38 (dd, 1H, CH), 7.93 (s, 1H, benzimidazole), 7.94 (s, 1H, benzimidazole), 8.5 (s, 1H, benzimidazole).

Anal. Calcd for $C_{24}H_{38}N_2O_4Cl_2Si_2$: C, 52.94; H, 6.98; N, 5.15; Cl, 12.87. Found: C, 53.12; H, 6.99; N, 4.96; Cl, 13.15.

5,6-Dichloro-1-(2-deoxy-β-L-ribofuranosyl)benzimidazole (5).

A mixture of **14** (0.18 g, 0.34 mmol), tetraethylammonium fluoride hydrate (1 mL of a 1M THF solution) in 5 mL of THF was stirred for 6 h. The THF was removed in

vacuo and the concentrate was purified on a preparative silica gel plate with 15% MeOH in CH_2Cl_2. This gave 0.02 g (19%) of **5** as a white solid: mp 155-157 °C; ^{1}H NMR (DMSO-d_6) δ 2.2-2.4 (m, 1H, CH_2), 2.5-2.7 (m, 1H, CH_2, partially buried by the DMSO peak), 3.6 (unresolved q, 2H, CH_2), 3.9 (d, 1H, CH), 4.4-4.5 (m, 1H, CH), 5.07 (t, 1H, CH), 5.4 (d, 1H, OH), 6.4 (t, 1H, OH), 8 (s, 1H, benzimidazole), 8.2 (s, 1H, benzimidazole), 8.6 (s, 1H, benzimidazole).

Anal. Calcd for $C_{12}H_{12}N_2O_3Cl_2$: C, 47.55; H, 3.99; N, 9.24, Cl, 23.39. Found: C, 47.44; H, 3.99; N, 9.14; Cl, 23.20.

2-Bromo-5,6-dichloro-1-(2-deoxy-β-L-ribofuranosyl)benzimidazole (4).

A mixture of **5** (3.6 g, 12 mmol), and acetic anhydride (2.7 mL, 29 mmol) in 70 mL of pyridine was stirred for 24 h. The pyridine was removed in vacuo and the resultant concentrate was dissolved in 100 mL of EtOAc, which was then washed with water. The aqueous solution was re-extracted once with 50 mL EtOAc. The organic layers were combined, dried over $MgSO_4$ and concentrated. Further drying at rt under high vacuum for 48 hours resulted in 4.56 g (98%) of 5,6-Dichloro-1-(2-deoxy-3,5-di-*O*-acetyl-β-L-ribofuranosyl)benzimidazole (**15**) as an oil. ^{1}H NMR (DMSO-d_6) δ 2 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.5-2.6 (m, 1H, CH_2), 2.8-2.9 (m, 1H, CH_2), 4.1-4.3 (m, 3H, CH_2 & CH), 5.2-5.3 (m, 1H, CH), 6.4 (dd, 1H, CH), 7.98 (s, 1H, benzimidazole), 8.02 (s, 1H, benzimidazole), 8. 6 (s, 1H, benzimidazole).

A mixture of **15** (0.21 g, 0.54 mmol) and NBS (0.2 g, 1.1 mmol) in 10 mL of THF was refluxed for 5 min. The resultant reaction mixture was poured into an ice/water-cooled mixture of chloroform/saturated $NaHCO_3$ (5:2). The organic layer was separated, washed with saturated $NaHCO_3$ (2X20 mL) and then water. After drying ($MgSO_4$), solvent removal and flash column chromatography on silica gel with MeOH/CH_2Cl_2 (1:50), 0.11 g (44%) of 2-bromo-5,6-dichloro-1-(2-deoxy-3,5-di-*O*-acetyl-β-L-ribofuranosyl)benzimidazole (**16**) was obtained as a white foam. ^{1}H NMR (DMSO-d_6) δ 2.13 (s, 3H, CH_3), 2.16 (s, 3H, CH_3), 2.4-2.6 (m, 1H, CH_2), 2.7-2.9 (m, 1H, CH_2), 4.3-4.4 (m, 2H, CH_2), 4.2-4.6 (m, 1H, CH), 5.3-5.4 (m, 1H, CH), 6.4 (dd, 1H, CH), 8.03 (s, 1H, benzimidazole), 8.05 (s, 1H, benzimidazole).

A suspension of **16** (0.1 g, 0.2 mmol) and Na_2CO_3 (0.03 g, 0.3 mmol) in MeOH/EtOH/H_2O (4:4:1) was stirred for 0.5 h. The suspension was concentrated and

then partitioned between EtOAc and saturated brine (2:1). The organic phase was separated, dried over $MgSO_4$ and evaporated to yield a white solid. The solid was suspended in EtOH, filtered, washed with hexane and dried, resulting in 0.05g (69%) of **4** as a white solid. An analytically pure sample was obtained by flash chromatographic purification on silica gel with MeOH/CH_2Cl_2 (1:20). Mp 175-176 °C; ^{1}H NMR (DMSO-d_6) δ 2.0-2.1 (m, 1H, CH_2), 2.4-2.5 (m, 1H, CH_2, partially buried by the DMSO peak), 3.6 (unresolved q, 2H, CH_2), 3.9 (d, 1H, CH), 4.4-4.5 (m, 1H, CH), 5.07 (t, 1H, CH), 5.2 (t, 1H, OH), 5.4 (d, 1H, OH), 6.3 (dd, 1H, CH), 7.9 (s, 1H, benzimidazole), 8.4 (s, 1H, benzimidazole).

Anal. Calcd for $C_{12}H_{11}N_2O_3BrCl_2 \cdot CH_3OH$: C, 37.71; H, 3.65; N, 6.76. Found: C, 37.49, H, 3.51; N, 6.58.

5,6-Dichloro-2-isopropylamino-1-(2-deoxy-β-L-ribofuranosyl)benzimidazole (3a).

A mixture of **16** (1.6 g, 3.4 mmol) and isopropylamine (10 mL) in 10 mL of EtOH was refluxed for 24 h. The reaction mixture was concentrated and the resultant concentrate was subjected to flash column chromatography on silica gel with 5% MeOH in CH_2Cl_2 as the eluent. This resulted in impure material that was rechromatographed on silica with EtOAc/hexane (4:1) to give 0.25 g (20%) of **3a** as a foam. ^{1}H NMR (DMSO-d_6) δ 1.2 (d, 6H, $CH(CH_3)_2$), 1.9-2.1 (m, 1H, CH_2), 2.3-2.4 (m, 1H, CH_2), 3.7 (unresolved s, 2H, CH_2), 3.8 (unresolved m, 1H, CH), 3.9-4.1 (m, 1H, CH), 4.4 (unresolved m, 1H, CH), 5.3 (d, 1H, OH), 5.5 (unresolved t, 1H, OH), 6.2 (dd, 1H, CH), 6.98 (d, 1H, NH), 7.4 (s, 1H, benzimidazole), 7.7 (s, 1H, benzimidazole).

Anal. Calcd for $C_{15}H_{19}N_3O_3Cl_2 \cdot 0.4EtOAc$: C, 50.41; H, 5.66; N, 10.62; Cl, 17.93. Found: C, 50.22; H, 5.54; N, 10.61; Cl, 17.90

2-Cyclopropylamino-5,6-dichloro-1-(2-deoxy-β-L-ribofuranosyl)benzimidazole (3b).

This reaction was run according to the procedure described for the synthesis of **3a**, using cyclopropylamine. A 29% yield of **3b** was obtained as an off-white solid: mp 140 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 0.3–0.6 (m, 2H, CH_2), 0.6-0.7 (m, 2H, CH_2), 1.9-2.1 (m, 1H, CH_2), 2.2-2.4 (m, 1H, CH_2), 2.6-2.8 (m, 1H, CH), 3.6 (unresolved m, 2H, CH_2), 3.8 (unresolved m, 1H, CH), 4.3 (unresolved m, 1H, CH), 5.3 (d, 1H, OH), 5.5 (unresolved t, 1H, OH), 6.2 (t, 1H, CH), 7.32 (s, 1H, NH), 7.4 (s, 1H, benzimidazole), 7.7 (s, 1H, benzimidazole).

Anal. Calcd for $C_{15}H_{17}N_3O_3Cl_2 \cdot 0.4CH_3OH \cdot 0.5CHCl_3$: C, 48.02; H, 4.86; N, 10.8; Cl, 22.33. Found: C, 47.93; H, 4.84; N, 10.83; Cl, 22.36.

2-Cyclobutylamino-5,6-dichloro-1-(2-deoxy-β-L-ribofuranosyl)benzimidazole (3c).

This reaction was run according to the procedure described for the synthesis of **3a**, using cyclobutylamine. A 64% yield of **3c** was obtained as an off-white solid: mp 180-182 °C; ^{1}H NMR (DMSO-d_6) δ 0.3–0.6 (m, 2H, CH_2), 0.6-0.7 (m, 2H, CH_2), 1.9-2.1 (m, 1H, CH_2), 2.2-2.4 (m, 1H, CH_2), 2.6-2.8 (m, 1H, CH), 3.6 (unresolved m, 2H, CH_2), 3.8 (unresolved m, 1H, CH), 4.3 (unresolved m, 1H, CH), 5.3 (d, 1H, OH), 5.5 (unresolved t, 1H, OH), 6.2 (t, 1H, CH), 7.32 (s, 1H, NH), 7.4 (s, 1H, benzimidazole), 7.7 (s, 1H, benzimidazole).

Anal. Calcd for $C_{16}H_{19}N_3O_3Cl_2 \cdot 0.2H_2O \cdot 0.4EtOAc$: C, 51.42; H, 5.54; N, 10.22; Cl, 17.25. Found: C, 51.43; H, 5.39; N, 10.08; Cl, 17.17.

2-Bromo-5,6-dichloro-1-[3,5-*O*-(1,1,3,3-tetraisopropyldisiloxa-1,3-diyl)-β-L-ribofuranosyl]benzimidazole (20).

2-Bromo-5,6-dichlorobenzimidazole (**17**)[6] (3 g, 7.6 mmol) and N,O-*bis*trimethylsilylacetamide (BSA) (2.9 mL, 11.4 mmol) in 100 mL of anhydrous acetonitrile was refluxed for 1 h. The reaction mixture was cooled to room temperature and this was followed by the addition of trimethylsilyl trifluoromethanesulfonate (4.5 mL, 23 mmol). After stirring for 15 min,1,2,3,5-tetra-*O*-acetyl-β-L-ribofuranose (**10**) (L-TAR) (3.6 g, 14.4 mmol) was added. The reaction mixture was stirred for 12 h at room temperature. This reaction was then partitioned between 200 mL of 10% $NaHCO_3$ and EtOAc. The organic layer was separated. After drying ($MgSO_4$) and solvent removal, a crude product was obtained which was re-crystallized from EtOH. This gave 3.95 g (100%) of 2-bromo-5,6-dichloro-1-(2,3,5-tri-*O*-acetyl-β-L-ribofuranosyl)benzimidazole (**18**) as yellow crystals: mp 140-142 °C; ^{1}H NMR (DMSO-d_6) δ 2 (s, 3H, CH_3), 2.2 (s, 6H, $2CH_3$), 4.4 (d, 1H, CH), 4.5-4.6 (m, 2H, CH_2), 5.4-5.5 (unresolved m, 1H, CH), 5.6 (t, 1H, CH), 6.2 (d, 1H, CH), 8 (s, 1H, benzimidazole), 8.1 (s, 1H, benzimidazole).

The synthesis of 2-bromo-5,6-dichloro-1-(β-L-ribofuranosyl)benzimidazole (**19**) followed the procedure described for compound **11**, using **18** (15.24 g, 9.6 mmol). After stirring for 4 h, the reaction was worked up as described for **11**. Following flash

chromatography on silica with 10% MeOH in CH_2Cl_2, 3.4 g (89%) of **19** was obtained as an off-white solid: mp 220 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 3.6-3.8 (m, 2H, CH_2), 4 (unresolved m, 1H, CH), 4.2 (unresolved m, 1H, CH), 4.4-4.5 (t, 1H, CH), 5.3 (br s, 1H, OH), 5.5 (br s, 2H, OH), 5.9 (d, 1H, CH), 8 (s, 1H, benzimidazole), 8.6 (s, 1H, benzimidazole).

The procedure for the synthesis of **20** followed that described for compound **12**, using **19** (3.4 g, 8.5 mmol). This gave 3.59 g (66%) of **20** as a colorless oil. ^{1}H NMR (DMSO-d_6) δ 0.8-1.2 (m, 28H, $CH(CH_3)_2$), 3.9-4.1 (m, 2H, CH_2), 4.1-4.3 (m, 1H, CH), 4.4-4.5 (m, 2H, CH), 5.6 (d, 1H, OH), 5.9 (d, 1H, CH), 7.9 (s, 1H, benzimidazole), 8.0 (s, 1H, benzimidazole).

Anal. Calcd for $C_{24}H_{37}N_2O_5BrCl_2Si_2$: C, 44.96; H, 5.78; N, 4.37. Found: C, 44.86; H, 5.8; N, 4.29.

2-Cyclopentylamino-5,6-dichloro-1-[3,5-*O*-(1,1,3,3-tetraisopropyldisiloxa-1,3-diyl)-β-L-ribofuranosyl]benzimidazole (21).

A mixture of **20** (1.39 g, 1.8 mmol), cyclopentylamine (3.5 mL, 36 mmol) and 30 mL of EtOH was refluxed for 48 h and then stirred for 12 h at room temperature. The reaction mixture was concentrated and the resultant crude product was purified by flash column chromatography on silica with 20% EtOAc in hexane to give 0.6 g (52%) of **21** as a white foam. ^{1}H NMR (DMSO-d_6) δ 0.8-1.2 (m, 28H, $CH(CH_3)_2$), 1.4-1.6 (m, 4H, cyclopentyl), 1.6-1.7 (m, 2H, cyclopentyl), 1.8-2 (m, 2H, cyclopentyl), 3.8 (br s, 1H, cyclopentyl), 3.9-4.2 (m, 3H, CH and CH_2), 4.3-4.4 (m, 2H, CH), 5.3 (unresolved d, 1H, OH), 5.7 (unresolved d, 1H, CH), 6.9 (d, 1H, NH), 7.2 (s, 1H, benzimidazole), 7.4 (s, 1H, benzimidazole).

Anal. Calcd for $C_{29}H_{47}N_3O_5Cl_2Si_2$: C, 53.97; H, 7.29; N, 6.51; Cl, 11.0. Found: C, 53.97; H, 7.29; N, 6.43; Cl, 10.9

5,6-Dichloro-2-cyclopentylamino-1-[2'-deoxy-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxa-1,3-diyl)-β-L-ribofuranosyl]benzimidazole (23).

To a mixture of **21** (0.56 g, 0.9 mmol) and 0.21 g (1.7 mmol) of N,N-dimethylaminopyridine in 20 mL of CH_3CN was added 0.14 mL (1 mmol) of phenyl chlorothionoformate. The resultant mixture was stirred for 12 h. The mixture was

partitioned between a solution of hexane/EtOAc (1:1) and saturated brine. The organic layer was collected, dried with $MgSO_4$ and concentrated. The resultant crude product was flash chromatographed on silica with 20% EtOAc in hexane as the eluent. This gave 0.4 g of **22** as white foam. Without further purification, a mixture of this foam, tributyltin hydride (0.13 mL, 0.5 mmol) and AIBN (8 mg, 0.5 mmol) in 25 mL of degassed toluene was refluxed for 1 h. Concentration of the reaction mixture and purification by flash column chromatography on silica with 20% EtOAc in hexane gave 0.29 g of a product that was still impure. Further chromatography on silica with CH_2Cl_2 as the eluent gave 0.13 g (24%) of **23** as a white foam. ^{1}H NMR (DMSO-d_6) δ 0.8-1.2 (m, 28H, $CH(CH_3)_2$), 1.4-1.6 (m, 4H, cyclopentyl), 1.6-1.7 (m, 2H, cyclopentyl), 1.8-2.0 (m, 2H, cyclopentyl), 2.2-2.3 (m, 1H, CH_2), 2.6-2.7 (m, 1H, CH_2), 3.6-3.7 (m, 1H, cyclopentyl), 3.98 (d, 2H, CH_2), 4-4.2 (m, 1H, CH), 4.6-4.8 (m, 1H, CH), 6.2 (1, 1H, CH), 6.83 (d, 1H, NH), 7.3 (s, 1H, benzimidazole), 7.4 (s, 1H, benzimidazole).

Anal. Calcd for $C_{29}H_{47}N_3O_4Cl_2Si_2$: C, 55.34; H, 7.47; N, 6.68; Cl, 11.29. Found: C, 55.3; H, 7.56; N, 6.64; Cl, 11.36.

5,6-Dichloro-2-cyclopentylamino-1-(2-deoxy-β-L-ribofuranosyl)benzimidazole (3d).

A mixture of **23** (0.5 g, 0.8 mmol) and tetrabutylammonium fluoride (1.9 mL of a 1M THF solution, 1.9 mmol) in 10 mL of THF was stirred for 1 h. The reaction mixture was concentrated to give a crude product that was first chromatographed on silica with 5% MeOH in CH_2Cl_2. The resultant product from this purification was re-chromatographed on silica with 10% MeOH in CH_2Cl_2 to give 0.21 g (67%) of **3d** as a colorless oil. ^{1}H NMR (DMSO-d_6) δ 1.4-1.6 (m, 4H, cyclopentyl), 1.6-1.7 (m, 2H, cyclopentyl), 1.8-1.9 (m, 2H, cyclopentyl), 2 (ddd, 1H, CH_2), 2.3 (ddd, 1H, CH_2), 3.6-3.7 (m, 2H, CH_2), 3.98 (d, 1H, cyclopentyl), 4-4.2 (m, 1H, CH), 4.3-4.4 (m, 1H, CH), 5.3 (d, 1H, OH), 5.4 (t, 1H, OH), 6.2 (dd, 1H, CH), 6.9 (d, 1H, NH), 7.3 (s, 1H, benzimidazole), 7.4 (s, 1H, benzimidazole).

Anal. Calcd for $C_{17}H_{21}N_3O_3Cl_2 \cdot 0.3H_2O$: C, 52.13; H, 5.56; N, 10.73; Cl, 18.10. Found: C, 52.19; H, 5.49; N, 10.71; Cl, 18.19.

Cells, viruses, and viral infection. Human diploid fibroblasts (MRC-5) were obtained from BioWhittaker, (Watersville, MD) and human cytomegalovirus (HCMV) strain

AD169 was obtained from American Type Culture Collection (Rockville, MD). Monolayer cultures were grown at 37 °C; in Eagle's minimal essential medium (MEM, GIBCO) supplemented with Earle's salts, L-glutamine, antibiotics, and 8% fetal calf serum (Hyclone Laboratories, Logan, UT) as described [MRU]. HCMV (strain AD169) was plaque purified and mycoplasma free (Gen Probe Mycoplasma Rapid Detection System, Fisher Scientific, Pittsburgh, PA).

For viral infection, monolayers were grown to confluence. The medium was removed; virus was added and suspended in the minimum volume of MEM with the serum concentration reduced to 2%. Plates were centrifuged at 1500 rpm for 10 min at 25 °C and incubated at 37 °C for 90 min to allow virus adsorption. MEM containing 2% fetal calf serum ± test compounds was added to the wells.

HCMV replication-assay. MRC-5 cells were seeded (1 x 10^4 cells/well), grown to confluence (~2 x 10^4 cells/well), and infected at a multiplicity of infection (MOI) of 0.01 infectious viral particles per cell. For drug studies, a range of eight concentrations of compound were added to define the dose-response curves for each inhibitor. After five days, 50 μL of lysis buffer (100 mM Tris-HCl, pH 8, 50 mM EDTA, 0.2% SDS, 0.1 mg/mL proteinase K) were added per well. Plates were incubated for 1 h at 55 °C after which the DNA was extracted by the addition and mixing of 65 μL of TE (10 mM Tris-HCl, 1 mM EDTA [pH 7.0])-saturated phenol-chloroform and 150 μL water. After centrifugation (2200 rpm for 15 min), a 50 μL sample of the aqueous phase was mixed with an equal volume of 0.6 M NaOH, denatured at 95 °C for 15 min and made to 1.5 M ammonium acetate, 1.5 M ammonium dihydrogen phosphate, 5 mM EDTA (pH 6.5). Samples were blotted onto GIBCO supported nitrocellulose filters (catalog no. 1465MH) on a 96-well BRL Convertible Filtration Manifold. Wells were rinsed with 200 μL of the above buffer. The DNA was cross-linked to the filter with a Stratlinker 1800 UV oven (Stratagene, La Jolla, CA) on the Autolink setting.

HCMV DNA on the blots was measured by quantitative DNA-DNA hybridization.[7] Values of IC50 were determined by fitting the data to the Hill equation using weighted linear regression.

Pharmacokinetic method and assay of compound 3a. Male CD-1 mice weighing 38 +/- 3 grams were dosed via the lateral tail vein with 10 ml/kg of a dose solution

comprised of 1 mg/ml **3a** in 20% propylene glycol-saline. Blood was removed by cardiac puncture under CO_2 anesthesia into EDTA-treated syringes. Plasmas were harvested by centrifugation. Plasma proteins were removed by precipitation with two volumes of acetonitrile, the precipitates removed by centrifugation, and an aliquot of the supernatant was diluted with an equal volume of water. Samples of 200 µl were injected onto a 4.6x250 mm Waters Symmetry ® C-18 reversed phase column equilibrated with 50% acetonitrile in a buffer of 0.05% triethylamine adjusted to pH 5.5 with acetic acid. The flow rate was 1-ml/min and the UV absorbance of the effluent was monitored at 305 nm. Five calibration standards spanning the range from 10 to 0.1 ug/ml were prepared in control mouse plasma and run with the unknowns. A calibration curve was constructed by linear regression of the ln-transformed peak areas and standard concentrations. The back-calculated concentrations of all calibration standards were within 5% of nominal. Pharmacokinetic parameters were calculated from a two-compartment exponential model of the plasma concentration-time data.

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