Design, Synthesis, and Antiviral Evaluations of 1-(Substituted benzyl)-2-substituted-5,6-dichlorobenzimidazoles as Nonnucleoside Analogues of 2,5,6-Trichloro-1-(β-D-ribofuranosyl)benzimidazole

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Received August 20, 1997

We have recently reported that certain ribosylated polyhalogenated benzimidazoles are potent and selective inhibitors of HCMV replication at noncytotoxic concentrations. To extend the structure−activity relationship beyond these first-generation compounds, we alkylated 5,6-dichloro-2-substituted-benzimidazoles with either a series of substituted benzyl halides or (2-bromoethyl)benzene to obtain five series of nonnucleoside analogues. Evaluation of these compounds for activity against herpes viruses revealed that the new compounds were less active than the benzimidazole ribonucleosides against human cytomegalovirus (HCMV) and inactive against herpes simplex virus type 1 (HSV-1). However, as part of our broader antiviral testing, we found that some of these compounds were active against HIV. Comparisons of the biological data revealed that a chloro or bromo group was required at the 2-position for the best separation of activity against HIV and cytotoxicity. Evaluation of the most active compounds against drug-resistant HIV suggested that they act by a mechanism other than inhibition of reverse transcriptase.

Introduction

Human cytomegalovirus (HCMV) has become a formidable opponent in the battle against infection in humans. Although relatively nonpathogenic in an intact immune system, HCMV is a leading cause of birth defects in the United States and causes morbidity and mortality in immunocompromised individuals, transplant recipients, and AIDS patients.[1−4] The two most widely used agents for the treatment of HCMV disease are ganciclovir and foscarnet.[5−7] Cidofovir,[8] a new agent against HCMV, has recently been approved for use in the United States. Unfortunately, toxicity associated with these agents,[5−8] poor oral bioavailability, high relapse rates, and drug resistance[9] have made their use less than optimal.

As a part of our search for new antiviral agents, we have been exploring benzimidazole nucleosides as potential agents for the treatment of HCMV infections. In 1954 Tamm, Folkers and co-workers first reported the synthesis and antiviral activity of halogenated benzimidazole nucleosides.[10,11] They found that 5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole (DRB) has multiple biological activities including activity against RNA[11] and DNA[12] viruses. DRB inhibits viral[13] and cellular[14] RNA synthesis most likely as a consequence of inhibiting cellular RNA polymerase II.[15] Thus, DRB affects multiple cellular processes, and its antiviral activity is poorly separated from cytotoxicity. Consequently, DRB has little potential as an antiviral drug.[11,16] The early studies by Tamm and co-workers prompted us to synthesize a series of 2-substituted benzimidazole ribonucleosides as potential anticancer agents[17] and more recently to examine the 2-substituted analogues of DRB for activity against HCMV. We found that 2,5,6-trichloro-1-(β-D-ribofuranosyl)benzimidazole (TCRB) and its 2-bromo analogue (BDCRB) are potent and selective inhibitors of HCMV replication at noncytotoxic concentrations.[18] Both compounds act by a unique mechanism which does not involve inhibition of DNA synthesis[19] but does involve inhibition of DNA processing.[20] As part of a structure−activity relationship study,[21,22] acyclic analogues of TCRB, BDCRB, and related nucleosides have been synthesized.[22] None of the compounds, including the acyclovir and ganciclovir analogues of TCRB were active.

To better understand the requirement of a sugar moiety for the potent and selective activity of TCRB and the lack of activity of the acyclic analogues, we decided to initiate the preparation of other nonnucleoside analogues in which the β-D-ribofuranosyl moiety has been replaced with a benzyl or phenylethyl group. A benzyl group at the N-1 nitrogen position also makes it convenient to employ the rational drug design method developed by Topliss.[23] We concentrated our efforts on 2-amino-, 2-chloro-, and 2-bromo-5,6-dichlorobenzimidazoles based upon our prior observations that the ribosides of these latter two heterocycles were potent and selective inhibitors of HCMV and that the 2-amino analogue was somewhat active, albeit more cytotoxic.[18] In addition, several other derivatives were synthesized using the same rationale. These compounds were

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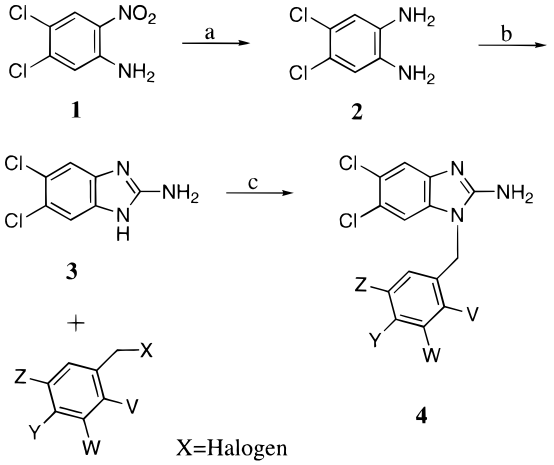
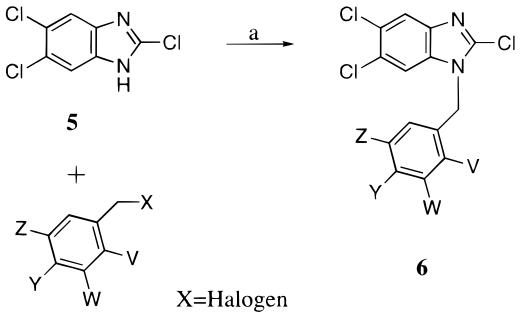
S0022-2623(97)00559-1 CCC: $15.00
Published on Web 03/15/1998

Scheme 1.[a] Synthesis of the 2-Amino-5,6-dichloro-1-(substituted benzyl)benzimidazole Series



[a] Reagents: (a) hydrogen gas at 40 psi, Raney nickel, ethanol; (b) cyanogen bromide, water, methanol; (c) sodium hydroxide, acetonitrile.

Scheme 2.[a] Synthesis of the 1-(Substituted benzyl)-2,5,6-trichlorobenzimidazole Series



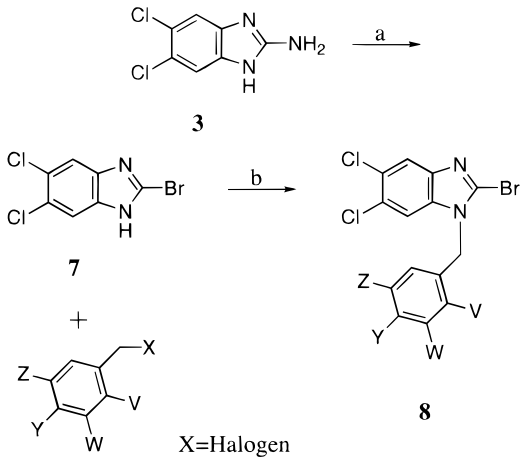
[a] Reagents: (a) sodium hydroxide, acetonitrile.

evaluated for antiviral activity against two herpesviruses [HCMV and herpes simplex virus type 1 (HSV-1)] and human immunodeficiency virus (HIV) in an effort to establish structure−activity relationships (SAR's). We report herein that the compounds were essentially inactive against the herpesviruses, but the testing provided an interesting SAR against HIV.

Results and Discussion

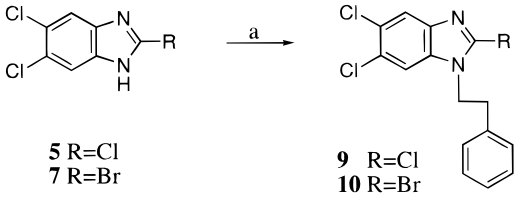
Chemistry. For the synthesis of the requisite target compounds in the first series (Scheme 1), we used commercially available 4,5-dichloro-2-nitroaniline (**1**) as our starting material instead of the considerably more expensive 4,5-dichloro-1,2-phenylenediamine. A reduction of the nitro group without concomitant removal of the chloro groups restricted the choice of reducing agents and conditions. We found that treatment of **1** with Raney nickel in the presence of hydrogen gas effected a clean reduction of the nitro group without any affect on the chloro group to provide a good yield of 4,5-dichloro-1,2-phenylenediamine (**2**). The ring annulation of **2** was accomplished with cyanogen bromide in acetonitrile to afford 2-amino-5,6-dichlorobenzimidazole (**3**) in good yield. A series (**4a**−**o**) of 2-amino-5,6-dichloro-1-(substituted benzyl)benzimidazoles (**4**) were prepared by alkylating compound **3** with the appropriately substituted benzyl halide in the presence of sodium hydroxide.

Scheme 3.[a] Synthesis of the 2-Bromo-5,6-dichloro-1-(substituted benzyl)benzimidazole Series



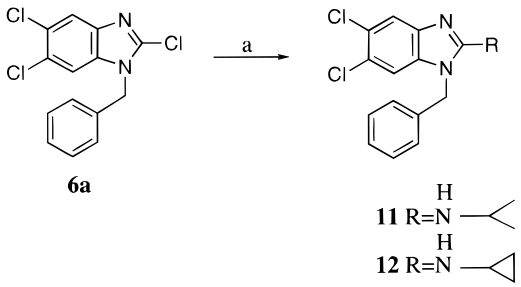
[a] Reagents: (a) $CuBr_2$, *tert*-butyl nitrite, acetone; (b) sodium hydroxide, acetonitrile.

Scheme 4.[a] Synthesis of 1-(Phenylethyl)-2,5,6-trichlorobenzimidazole and 2-Bromo-5,6-dichloro-1-(phenylethyl)benzimidazole



[a] Reagents: (a) (2-bromoethyl)benzene, sodium hydroxide, acetonitrile.

Scheme 5.[a] Synthesis of 1-Benzyl-5,6-dichloro-2-(isopropylamino)benzimidazole and 1-Benzyl-2-(cyclopropylamino)-5,6-dichlorobenzimidazole

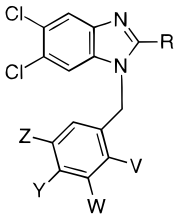


[a] Reagents: (a) isopropylamine or cyclopropylamine at 60 °C in a sealed vessel.

For the synthesis of the target compounds in the second series (Scheme 2), we prepared compound **5** by published methods.[18] A series (**6a**−**o**) of 1-(substituted benzyl)-2,5,6-trichlorobenzimidazoles (**6**) were prepared by alkylating compound **5** with the appropriately substituted benzyl halide in the presence of sodium hydroxide.

For the synthesis of the target compounds in the third series (Scheme 3), we elected to use compound **3** as our starting material. A diazotization of compound **3** with *tert*-butyl nitrite was followed by a displacement of the diazo group by a bromo group to furnish 2-bromo-5,6-dichlorobenzimidazole (**7**). A series of 2-bromo-5,6-

Table 1. Activity against Herpesviruses and Cytotoxicity of 1-(Substituted benzyl)-2-substituted-5,6-dichlorobenzimidazoles



						50% inhibitory concentration (μM)			
						antiviral activity			
						HCMV[a]	HSV-1[b]	cytotoxicity[c]	
no.	R	V	W	Y	Z	plaque	ELISA	visual	growth
4a	NH_2	H	H	H	H	37[d]	152	58[d]	170[d]
4b	NH_2	H	H	Cl	H	>100[e]	>100	>100	45
4c	NH_2	H	H	OCH_3	H	>100	>100	>100	45
4d	NH_2	H	H	CH_3	H	30	80	32	50
4e	NH_2	H	Cl	Cl	H	40	50	32	35
4f	NH_2	H	Cl	H	H	>100	70	>100	65
4g	NH_2	H	H	$C(CH_3)_3$	H	22[d]	60[d]	32[d]	50[d]
4h	NH_2	H	H	CF_3	H	32		32	
4i	NH_2	H	CH_3	H	H	>100	80	>100	65
4j	NH_2	H	CF_3	H	H	32	60	32	45
4k	NH_2	Cl	H	H	H	32	>100	32	
4l	NH_2	H	CF_3	H	CF_3	32	60	32	
4m	NH_2	H	H	NO_2	H	>100	100	>100	35
4n	NH_2	H	NO_2	H	H	>100	>100	>100	65
4o	NH_2	H	H	F	H	12[d]	70	37[d]	70
6a	Cl	H	H	H	H	>100[d]	>100[d]	>100[d]	>100[d]
6b	Cl	H	H	Cl	H	>100	>100	>100	>100
6c	Cl	H	H	OCH_3	H	>100	>100	>100	>100
6d	Cl	H	H	CH_3	H	>100	>100	32	>100
6e	Cl	H	Cl	Cl	H	>100	>100	100	>100
6f	Cl	H	Cl	H	H	>100	>100	100[d]	
6g	Cl	H	H	$C(CH_3)_3$	H	>100	>100	>100	>100[d]
6h	Cl	H	H	CF_3	H	>100	>100	>100	>100
6i	Cl	H	CH_3	H	H	>100	>100	>100	>100
6j	Cl	H	CF_3	H	H	>100	>100	>100	>100
6k	Cl	Cl	H	H	H	30	>100	>100	156[d]
6l	Cl	H	CF_3	H	CF_3	>100	>100	>100	>100[d]
6m	Cl	H	H	NO_2	H	>100	>100	>100	183[d]
6n	Cl	H	NO_2	H	H	>100	>100	>100	239[d]
6o	Cl	H	H	F	H	>100	>100	>100	>100[d]
8a	Br	H	H	H	H	>100[d]	>100[d]	>100[d]	>100[d]
8b	Br	H	H	Cl	H	>100	>100	>100	>100
8c	Br	H	H	OCH_3	H	>100	>100	>100	55
8d	Br	H	H	CH_3	H	>100	>100	>100	>100
8e	Br	H	Cl	Cl	H	>100	>100	>100	>100
8f	Br	H	Cl	H	H	>100	70	>100	>100
8g	Br	H	H	$C(CH_3)_3$	H	>100		>100	
8h	Br	H	H	CF_3	H	>100	>100	>100	>100
8i	Br	H	CH_3	H	H	>100[d]		>100[d]	
8j	Br	H	CF_3	H	H	>100[d]		>100[d]	
8k	Br	Cl	H	H	H	>10	>100[d]	>10	78[d]
8l	Br	H	CF_3	H	CF_3	>100	65	>100	70
8m	Br	H	H	NO_2	H	>100		>100	40
8n	Br	H	NO_2	H	H	>100		>100	150
8o	Br	H	H	F	H	>100[d]		>100[d]	

[a] Plaque reduction assays were performed in duplicate as described in the text. [b] Compounds were assayed by ELISA in quadruplicate wells. [c] Visual cytotoxicity was scored on HFF cells at time of HCMV plaque enumeration. Inhibition of KB cell growth was determined as described in the text in quadruplicate assays. [d] Average of IC_{50}'s from two to five separate experiments. [e] A greater than sign (>) indicates IC_{50} not reached at the noted (highest) concentration tested.

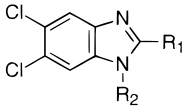
dichloro-1-(substitutedbenzyl)benzimidazoles (**8a**−**o**) were synthesized by the alkylation of compound **7** with the appropriately substituted benzyl halide in the presence of sodium hydroxide.

Compounds **9** and **10** were prepared by alkylating compounds **5** and **7**, respectively, with (2-bromoethyl)-benzene in the presence of sodium hydroxide in acetonitrile (Scheme 4).

Compounds **11** and **12** were prepared by displacing the 2-chloro group of **6a** with either isopropylamine or cyclopropylamine, respectively, at 60 °C in a sealed vessel (Scheme 5).

All new compounds reported in this research investigation were characterized using ^{1}H-nuclear magnetic resonance spectroscopy, ^{13}C-nuclear magnetic resonance spectroscopy, elemental analysis, and thin-layer chromatography. More detailed proton and carbon assignments of **3** representative compounds (**4a**, **6b**, and **8i**) were obtained using distortionless enhancement by polarization transfer spectroscopy (DEPT), hetero-

Table 2. Activity against Herpesviruses and Cytotoxicity of 1-Phenylethyl- and 1-Benzyl-2-substituted-5,6-dichlorobenzimidazoles



			50% inhibitory concentration (μM)			
			antiviral activity			
			HCMV[a]	HSV-1[b]	cytotoxicity[c]	
no.	R_1	R_2	plaque	ELISA	visual	growth
9	Cl	phenylethyl	>100[d]		>100	
10	Br	phenylethyl	>100	>100	>100	>100
11	isopropylamino	benzyl	22	50	22	45
12	cyclopropylamino	benzyl	18	>100	33	60
TCRB[e]	Cl	β-D-ribose	2.9	102	238	210
BDCRB[e]	Br	β-D-ribose	0.7	130	118	>100

[a,b,c] See footnotes to Table 1. [d] A greater than sign (>) indicates IC_{50} not reached at the noted (highest) concentration tested. [e] Data also reported in ref 18; average derived from three to six experiments.

nuclear shift correlation spectroscopy (HetCor), and correlation spectroscopy via long-range coupling (COLOC) on a 500 MHz Bruker Avance DRX 500.

Biology. Evaluation of 2-amino-1-benzyl-5,6-dichlorobenzamidazole (**4a**) and its 2-chloro (**6a**) and 2-bromo (**8a**) analogues established that the unsubstituted benzyl analogues were either inactive (**6a** and **8a**) or weakly active and cytotoxic (**4a**) against HCMV and HSV-1 (Table 1). By incorporating substituents on the benzyl moiety, suggested by the Topliss tree approach, we attempted to increase antiviral activity and reduce cytotoxicity in the three series of compounds (Table 1). In the 2-amino-5,6-dichloro-1-(substituted benzyl)benzimidazole series (**4**), compounds **4d**, **4e**, **4g**, **4h**, **4j**, **4k**, **4l**, and **4o** showed some activity against HCMV but with similar cytotoxicity. Consequently, this antiviral activity most likely was a result of cytotoxicity. Compound **4o** was the most active with a 50% inhibitory concentration (IC_{50}) of 12 μM vs HCMV. However, a 50% cytotoxic concentration (CC_{50}) of 37 μM (in vitro therapeutic index = 3) excludes it from being a potential antiviral agent.

The parent compound (**6a**) of the 2-chloro series (**6**) had no activity. Likewise, all of the compounds in this series were inactive with the exception of the *o*-chloro-substituted compound (**6k**) which had an IC_{50} of 30 μM against HCMV. In the 2-bromo series (**8**), the parent compound (**8a**) and all its derivatives were inactive against HCMV.

Two analogues closely related to compounds **6a** and **8a** also were made and tested. Like compounds **6a** and **8a**, 1-(phenylethyl)-2,5,6-trichlorobenzimidazole (**9**) and the 2-bromo homologue (**10**) were inactive against HCMV (Table 2). In addition, two analogues similar to those in the 2-amino series (**4**) were synthesized based upon the activity of 5,6-dichloro-2-(isopropylamino)-β-L-ribofuranosylbenzimidazole (1263W94) against HCMV.[24] Both 1-benzyl-5,6-dichloro-2-(isopropylamino)benzimidazole (**11**) and the 2-cyclopropylamino analogue (**12**) were active in the HCMV assay, but with only a small separation from cytotoxic concentrations (Table 2).

To better understand the activity of the three amine-substituted compounds (**4a**, **11**, **12**), their activity against HCMV was compared in wild-type (wt) and drug-resistant HCMV. Dose−response curves for each compound (not shown) were identical in wt and TCRB-resistant strains[25] and in wt and in 1263W94-resistant strains[24] of HCMV. Together, these data suggest that the compounds act by a mechanism different from either TCRB or 1263W94 or that the observed effects were due to cytotoxicity.

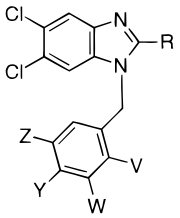
When screened for activity in the HSV-1 assay, no compounds in any of the five series were active at noncytotoxic concentrations (Tables 1 and 2).

All the compounds in the five series also were evaluated for activity against human immunodeficiency virus type 1 (HIV-1) using a syncytial plaque assay. Although many of the compounds in the 2-amino series (**4**) inhibited plaque formation, the activity was not well separated from cytotoxicity, and therefore specific antiviral activity against HIV-1 is questionable (Table 3). Cytotoxicity in CEM-SS cells measured by [^{3}H]dThd incorporation into total acid precipitable material (Table 3) was similar to cytotoxicity in HFF and KB cells reported in Table 1. The most promising was compound **4h**, with an IC_{50} of 1.8 μM and a CC_{50} of 20 μM (in vitro therapeutic index = 11). In the 2-chloro series (**6**), **6b**, **6c**, **6d**, **6i**, **6k**, **6m**, **6n**, and **6o** had modest activity against HIV-1 in the syncytial plaque assay (Table 3). Of these, compound **6b** was the most promising, with an IC_{50} of 9.1 μM and a CC_{50} of greater than 100 μM. In the 2-bromo series (**8**), compounds **8a**, **8c**, **8i**, **8j**, **8l**, **8m**, **8n**, and **8o** also were active in the HIV-1 syncytial plaque assay (Table 3). Of these, compound **8i** was the most promising, with an IC_{50} of 3.5 μM and a CC_{50} of greater than 100 μM.

When comparing trends among the series of compounds (Table 3), we found that the 2-chloro series (**6**) and the 2-bromo series (**8**) are less toxic than the 2-amino series (**4**). Overall, in the HIV-1 assay the 2-chloro series (**6**) was less active than or equally active to the 2-amino series (except **4m**) and the 2-bromo series (except **8b**, **8d**). A comparison between the 2-amino series (**4**) and the 2-bromo series (**8**) showed no trend. Compounds **4b**, **4d**, **4e**, **4f**, and **4g** showed greater activity in the HIV-1 assay in the 2-amino series than the 2-bromo series where compounds **8c**, **8i**, **8j**, **8m**, **8n**, and **8o** showed greater or equal activity.

Selected compounds in the 2-chloro and 2-bromo series were examined in greater detail by assaying them against virus which was resistant to AZT or to the

Table 3. Activity of 1-(Substituted benzyl)-2-substituted-5,6-dichlorobenzimidazoles against Human Immunodeficiency Virus



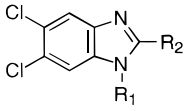
						50% inhibitory concentration (μM)	
no.	R	V	W	Y	Z	HIV syncytial plaque[a]	cytotoxicity [^{3}H]dThd[b]
4b	NH_2	H	H	Cl	H	10	32
4c	NH_2	H	H	OCH_3	H	12	27
4d	NH_2	H	H	CH_3	H	20	40
4e	NH_2	H	Cl	Cl	H	>6[c,d]	30
4f	NH_2	H	Cl	H	H	>30	37
4g	NH_2	H	H	$C(CH_3)_3$	H	10[d]	27
4h	NH_2	H	H	CF_3	H	1.8[d]	20
4i	NH_2	H	CH_3	H	H	12	48
4j	NH_2	H	CF_3	H	H	>6	34
4k	NH_2	Cl	H	H	H	6	36
4l	NH_2	H	CF_3	H	CF_3	>10	17
4m	NH_2	H	H	NO_2	H	21	32
4n	NH_2	H	NO_2	H	H	21[d]	47
4o	NH_2	H	H	F	H	32	30
6b	Cl	H	H	Cl	H	9.1[d]	>100
6c	Cl	H	H	OCH_3	H	26[d]	>100
6d	Cl	H	H	CH_3	H	33[d]	>100
6e	Cl	H	Cl	Cl	H	>100[d]	>100
6f	Cl	H	Cl	H	H	100[d]	>100
6g	Cl	H	H	$C(CH_3)_3$	H	>100	>100
6h	Cl	H	H	CF_3	H	>100	>100
6i	Cl	H	CH_3	H	H	14[d]	>100
6j	Cl	H	CF_3	H	H	>100[d]	>100
6k	Cl	Cl	H	H	H	15[d]	70
6l	Cl	H	CF_3	H	CF_3	>100	>100
6m	Cl	H	H	NO_2	H	7.8[d]	51
6n	Cl	H	NO_2	H	H	20[d]	>100
6o	Cl	H	H	F	H	37[d]	>100
8a	Br	H	H	H	H	27	>100
8b	Br	H	H	Cl	H	>100	>100
8c	Br	H	H	OCH_3	H	6[d]	60
8d	Br	H	H	CH_3	H	>100	>100
8e	Br	H	Cl	Cl	H	>100	>100
8f	Br	H	Cl	H	H	>100	>100
8g	Br	H	H	$C(CH_3)_3$	H	100[d]	>100
8h	Br	H	H	CF_3	H	>100	>100
8i	Br	H	CH_3	H	H	3.5[d]	>100
8j	Br	H	CF_3	H	H	12[d]	>100
8k	Br	Cl	H	H	H	15[d]	74
8l	Br	H	CF_3	H	CF_3	5.9[d]	>100
8m	Br	H	H	NO_2	H	14[d]	>100
8n	Br	H	NO_2	H	H	5.3[d]	>100
8o	Br	H	H	F	H	30	>100
zidovudine (AZT)						0.0026[e]	1.9

[a] Syncytial plaque assay was performed in duplicate using CEM-SS cells. [b] Incorporation of [^{3}H]dThd was used as a measure of cytotoxicity. Average results from duplicate experiments are presented. [c] A greater than sign (>) indicates IC_{50} not reached at the noted (highest) concentration tested. [d] Average of duplicate or triplicate experiments. [e] Mean from nine separate experiments ± standard deviation of 0.0024.

nonnucleoside reverse transcriptase (RT) inhibitor, TIBO. Data in Table 4 establish that other than compound **6b**, all of the compounds were active against HIV-1 that was resistant to either AZT or TIBO, thereby suggesting that the compounds are not RT inhibitors. In contrast, none of the compounds with the possible exception of **8n** were active against HIV-2.

To better understand the activity of the benzylbenzimidazoles against HIV-1, selected compounds were assayed in a different manner by determining their effect on the production of reverse transcriptase (RT) in HIV-1-infected cultures. (This assay measures RT activity in culture supernatants as a marker for infectious virus multiplication, it does not measure the direct effect of compounds on RT itself.) In contrast to the activity observed in the syncytial plaque assay, little activity was observed using the RT marker assay (Table 5). The 2-amino compounds **4b** and **4h** were active but at concentrations near those which were cytotoxic to CEM-SS cells (Table 3). Compounds in the 2-chloro and 2-bromo series were found to be inactive except for compound **8i**—the most active compound in the series—and it was nearly 20-fold less active in the RT assay. The identical activity of AZT in both assays

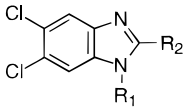
Table 4. Activity of 5,6-Dichloro-1-(substituted benzyl)-2-substituted-benzimidazoles against Selected Strains of HIV-1 and HIV-2



			50% inhibitory concentration (μM)[a]				
			HIV-1				
no.	R_1	R_2	III_B[b] (wt)	C-691[c] or H-112[c] (wt)	G-762[c] or G-910[c] (AZT^r)	A-17 ($TIBO^r$)	HIV-2
6b	*p*-ClBn	Cl	9.1	30	255	19[d]	>30[e]
6i	*m*-CH_3Bn	Cl	14	37	13	16[d]	>30
6k	*o*-ClBn	Cl	15			13[d]	>30
6o	*p*-FBn	Cl	37			19	>30
8i	*m*-CH_3Bn	Br	3.5	8.7	9.0	17	143
8m	*p*-NO_2Bn	Br	14	11	6.9	11	66
8n	*m*-NO_2Bn	Br	5.0	8.7	1.1	1.0	28
zidovudine (AZT)			0.0026	0.002	>10		

[a] Syncytial plaque assay was performed in duplicate sets using CEM-SS cells. [b] Data from Table 3. [c] Matched pairs of HIV sensitive (wt) and resistant to AZT (AZT^r). C-691 and G762 were used with **6** series compounds and zidovudine; H-112 and G910 were used with **8** series compounds. [d] Average of duplicate experiments. [e] A greater than sign (>) indicates IC_{50} not reached at the noted (highest) concentration tested.

Table 5. Comparison of the Activity of 5,6-Dichloro-1-(substituted benzyl)-2-substituted-benzimidazoles against Human Immunodeficiency Virus in Different Assays



			50% inhibitory concentration (μM) HIV assay	
no.	R_1	R_2	syncytial plaque[a]	RT[b]
4b	*p*-ClBn	NH_2	10	10
4h	*p*-CF_3Bn	NH_2	1.8	20
6d	*p*-CH_3Bn	Cl	33	>100
6m	*p*-NO_2Bn	Cl	7.8	>100
8c	*p*-OCH_3Bn	Br	6	>100
8e	*m*,*p*-Cl_2Bn	Br	>100	>100
8i	*m*-CH_3Bn	Br	3.5	66
8l	*m*-CF_3Bn	Br	5.9	>100
8n	*m*-NO_2Bn	Br	5.3	>32
zidovudine (AZT)			0.0026 ± 0.0024[c]	0.0031 ± 0.0039[c]

[a] Syncytial plaque assay was performed in duplicate sets using CEM-SS cells; data from Table 3. [b] Reverse transcriptase (RT) assay was performed in triplicate, also in CEM-SS cells. [c] Means ± standard deviations from 9 and 31 separate experiments.

validates the assays and virtually proves that the benzylbenzimidazoles are acting by a mechanism different than AZT. Furthermore, the lack of activity of these compounds in the RT marker assay suggests that the compounds are inhibiting a late step in virus multiplication with the production of noninfectious virus particles.

In summary, N-1 benzyl and phenylethyl analogues of TCRB were synthesized to expand our understanding of the structure−activity relationship among nonnucleoside benzimidazole analogues. However, the compounds which we prepared were inferior in activity to the benzimidazole ribonucleosides as agents against HCMV. Furthermore, the antiviral activity was poorly separated from the cytotoxicity. As part of our broader antiviral testing, we found these compounds were inactive against HSV-1 but active against HIV-1. We found that the Topliss tree provided some useful suggestions for substitutions on the 1-benzyl group; for example, if one assumes that **6b** is equally as active as **6a** in the HIV assay (Table 3), then the Topliss tree will lead to **6m** via **6d**, **6f**, and **6k**. In some cases, activity against HIV-1 was well separated from cytotoxicity (i.e., **6b** and **8i**). Using these as lead compounds, it is plausible that other modifications could uncover agents which are more active against HIV-1.

Experimental Section

Evaporations were carried out under reduced pressure (water aspirator) with the bath below 50 °C. Silica gel 60 230−400 mesh (E. Merck, Darmstadt, West Germany) was used for chromatography. Thin-layer chromatography was performed on silica gel GHLF-254 plates (Merck Reagents) using a 9:1 chloroform:methanol solvent system. Melting points were determined with a Thomas-Hoover capillary apparatus and are uncorrected. Proton magnetic resonance (^{1}H NMR) spectra were obtained with a Bruker WP270SY spectrometer (solutions in dimethyl-d_6 sulfoxide), with chemical shift values reported in δ, parts per million, relative to the internal standard. Carbon magnetic resonance (^{13}C NMR) spectra were obtained at 90 MHz in DMSO-d_6 with an IBM WM-360 spectrometer. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ.

4,5-Dichloro-1,2-phenylenediamine (2). 4,5-Dichloro-2-nitroaniline (**1**) (51.7 g, 250 mmol) and Raney nickel (8 g) were

mixed in ethanol (500 mL) and hydrogenated under an average hydrogen pressure of 40 psi. Hydrogen consumption ceased once the reduction was complete. The reaction mixture was filtered and evaporated to dryness to obtain 4,5-dichloro-1,2-phenylenediamine (**2**): yield 44.55 g (98%); mp 163−165 °C (lit.[25] mp 162−163 °C).

2-Amino-5,6-dichlorobenzimidazole (3). 4,5-Dichloro-1,2-phenylenediamine (**2**) (10.0 g, 57 mmol) was added to a mixture of water (120 mL), methanol (120 mL), and cyanogen bromide (11.3 mL of a 5.0 M solution, 57 mmol) and left to stir overnight. This mixture was decolorized with charcoal and adjusted to pH >9, with concentrated NH_4OH, to precipitate orangish-brown crystals of 2-amino-5,6-dichlorobenzimidazole (**3**): yield 10.38 g (91%); mp 259−261 °C (lit.[18] mp 250−251 °C).

2-Amino-5,6-dichloro-1-(substituted benzyl)benzimidazole (4). 2-Amino-5,6-dichlorobenzimidazole (**3**) (0.152 g, 0.75 mmol) and sodium hydroxide (0.03 g, 0.75 mmol) were dissolved in acetonitrile (40 mL). After approximately 1 h of stirring, the appropriately substituted benzyl halide (0.75 mmol) was added and the mixture was heated at reflux. When only one spot was present on TLC, the reaction mixture was cooled to room temperature, evaporated to dryness, dissolved in methanol, decolorized with charcoal, filtered, and evaporated to dryness. The product was dissolved in hot methanol and recrystallized from a mixture of methanol and water to obtain pure **4a**−**o**.

2-Amino-1-benzyl-5,6-dichlorobenzimidazole (4a). **3** was reacted with benzyl chloride (0.127 g, 1.0 mmol): yield 0.14 g (64%); R_f 0.52; yellow solid; mp 255−257 °C; ^{1}H NMR (DMSO-d_6) δ 5.29 (2H, s, CH_2), 6.94 (2H, s, NH_2) 7.17 (2H, d, C2′-H and C6′-H), 7.27 (1H, t, C4′-H), 7.31 (1H, s, C4-H), 7.34 (2H, dd, C3′-H and C5′-H), 7.36 (1H, s, C7-H); ^{13}C NMR (DMSO-d_6) δ 45.62 (CH_2), 110.07 (C4), 116.27 (C7), 120.50 (C3a or C5), 123.55 (C6), 127.76 (C2′, C6′), 128.32 (C4′), 129.50 (C3′, C5′), 135.03 (C7a), 137.42 (C1′), 144.06 (C3a), 157.83 (C2); Anal. ($C_{14}H_{11}N_3Cl_2$) C, H, N.

2-Amino-1-(*p*-chlorobenzyl)-5,6-dichlorobenzimidazole (4b). **3** was reacted with *p*-chlorobenzyl chloride (0.12 g, 0.75 mmol): yield 0.11 g (45%); R_f 0.46; light brown crystals; mp 247−249 °C; ^{1}H NMR (DMSO-d_6) δ 5.26 (2H, s), 6.92 (2H, s), 7.15−7.18 (2H, d), 7.30 (1H, s), 7.37−7.39 (2H, s), 7.40 (1H, s); ^{13}C NMR (DMSO-d_6) δ 44.95, 110.04, 116.26, 121.20, 123.97, 129.33, 129.61, 133.02, 134.36, 135.69, 142.89, 157.20. Anal. ($C_{14}H_{10}N_3Cl_3$) C, H, N.

2-Amino-1-(*p*-methoxybenzyl)-5,6-dichlorobenzimidazole (4c). **3** was reacted with *p*-methoxybenzyl chloride (0.12 g, 0.75 mmol): yield 0.11 g (45%); R_f 0.52; light yellow powder; mp 199−200 °C; ^{1}H NMR (DMSO-d_6) δ 3.70 (3H, s), 5.17 (2H, s), 6.86−6.90 (4H, m), 7.13−7.15 (2H, d), 7.27 (1H, s), 7.35 (1H, s); ^{13}C NMR (DMSO-d_6) δ 45.00, 55.66, 110.04, 114.67, 116.03, 120.85, 123.61, 128.65, 128.98, 129.16, 142.93, 157.15, 159.29; Anal. ($C_{15}H_{13}N_3Cl_2O$) C, H, N.

2-Amino-1-(*p*-methylbenzyl)-5,6-dichlorobenzimidazole (4d). **3** was reacted with *p*-methylbenzyl chloride (0.11 g, 0.75 mmol): yield 0.16 g (70%); R_f 0.52; light brown needles; mp 231−233 °C; ^{1}H NMR (DMSO-d_6) δ 2.23 (3H, s), 5.22 (2H, s), 6.93 (2H, s), 7.05 (2H, d), 7.19 (2H, d), 7.29 (1H, s), 7.32 (1H, s); ^{13}C NMR (DMSO-d_6) δ 20.58, 44.63, 109.25, 115.39, 119.68, 122.69, 126.94, 129.15, 133.47, 136.69, 143.12, 156.93. Anal. ($C_{15}H_{13}N_4Cl_2$) C, H, N.

2-Amino-1-(*m*,*p*-dichlorobenzyl)-5,6-dichlorobenzimidazole (4e). **3** was reacted with *m*,*p*-dichlorobenzyl chloride (0.15 g, 0.75 mmol): yield 0.17 g (62%); R_f 0.52; light yellow powder; mp 247−249 °C; ^{1}H NMR (DMSO-d_6) δ 5.81 (2H, s), 7.49 (2H, s,), 7.59−7.61 (1H, d), 7.85 (1H, s), 7.98−7.99 (2H, s), 8.11−8.14 (1H, d); ^{13}C NMR (DMSO-d_6) δ 43.96, 109.45, 115.87, 120.38, 123.36, 127.34, 129.16, 130.46, 131.14, 131.45, 134.09, 143.11, 156.83, 137.8. Anal. ($C_{14}H_9N_3Cl_4$) C, H, N.

2-Amino-1-(*m*-chlorobenzyl)-5,6-dichlorobenzimidazole (4f). **3** was reacted with *m*-chlorobenzyl chloride (0.12 g, 0.75 mmol): yield 0.15 g (61%); R_f 0.52; light yellow crystals; mp 229−230 °C; ^{1}H NMR (DMSO-d_6) δ 5.28 (2H, s), 6.94 (2H, s,), 7.06−7.08 (1H, d), 7.23 (1H, s), 7.30−7.36 (3H, m), 7.42 (1H, s); ^{13}C NMR (DMSO-d_6) δ 44.44, 109.46, 115.83, 120.00, 123.26, 125.71, 126.92, 127.75, 130.86, 133.00, 134.18, 139.18, 143.14, 157.00. Anal. ($C_{14}H_{10}N_3Cl_3$) C, H, N.

2-Amino-1-(*p*-*tert*-butylbenzyl)-5,6-dichlorobenzimidazole (4g). **3** was reacted with *p*-*tert*-butylbenzyl bromide (0.17 g, 0.75 mmol): yield 0.19 g (72%); R_f 0.61; white crystals; mp 263−265 °C; ^{1}H NMR (DMSO-d_6) δ 1.21 (9H, s), 5.23 (2H, s), 6.91 (2H, s), 7.07−7.10 (2H, 2 s), 7.29−7.37 (4H, 2d); ^{13}C NMR (DMSO-d_6) δ 31.03, 34.00, 44.45, 109.22, 115.41, 119.67, 122.50, 125.36, 126.63, 133.54, 134.22, 143.18, 149.92, 156.96. Anal. ($C_{18}H_{20}N_3Cl_2$) C, H, N.

2-Amino-1-(*p*-(trifluoromethyl)benzyl)-5,6-dichlorobenzimidazole (4h). **3** was reacted with p-trifluoromethylbenzyl bromide (0.18 g, 0.75 mmol): yield 0.13 g (48%); R_f 0.45; light brown needles; mp 248−250 °C; ^{1}H NMR (DMSO-d_6) 5.45 (2H, s), 6.95 (2H, s), 7.29−7.35 (4H, m), 7.71 (1H, s), 7.84 (1H, s). Anal. ($C_{15}H_{10}N_3Cl_2F_3$) C, H, N.

2-Amino-1-(*m*-methylbenzyl)-5,6-dichlorobenzimidazole (4i). **3** was reacted with *m*-methylbenzyl chloride (0.11 g, 0.75 mmol): yield 0.13 g (58%); R_f 0.52; light brownish crystals; mp 189−191 °C; ^{1}H NMR (DMSO-d_6) δ 2.24 (3H, s), 5.22 (2H, s), 6.89−6.93 (3H, m), 6.99 (1H, s), 7.05−7.07 (1H, d), 7.17−7.21 (1H, t), 7.29−7.31 (2H, d); ^{13}C NMR (DMSO-d_6) δ 21.23, 45.03, 109.43, 115.66, 120.00, 122.92, 124.17, 127.64, 128.35, 128.77, 134.44, 136.71, 138.01, 143.43, 157.22. Anal. ($C_{15}H_{13}N_3Cl_2$) C, H, N.

2-Amino-1-(*m*-(trifluoromethyl)benzyl)-5,6-dichlorobenzimidazole (4j). **3** was reacted with *m*-trifluoromethylbenzyl chloride (0.18 g, 0.75 mmol): yield 0.19 g (70%); R_f: 0.39; light brownish crystals; mp 220−223 °C; ^{1}H NMR (DMSO-d_6) δ 5.34 (2H, s), 6.97 (2H, s), 7.31 (1H, s), 7.39−7.40 (1H, d), 7.45 (1H, s), 7.50−7.65 (3H, m); ^{13}C NMR (DMSO-d_6) δ 44.47, 109.31, 115.74, 120.20, 123.66, 124.37, 125.65, 127.56, 129.94, 130.90, 134.06, 138.03, 143.04, 156.80, 129.26. Anal. ($C_{15}H_{10}N_3Cl_2F_3$) C, H, N.

2-Amino-1-(*o*-chlorobenzyl)-5,6-dichlorobenzimidazole (4k). **3** was reacted with *o*-chlorobenzyl chloride (0.12 g, 0.75 mmol): yield 0.17 g (69%); R_f 0.61; light brownish crystals; mp 231−233 °C; ^{1}H NMR (DMSO-d_6) δ 5.34 (2H, s), 6.42−6.45 (1H, d), 6.91 (2H, s), 7.22−7.23 (2H, m), 7.28−7.34 (3H, m), 7.50−7.53 (1H, d); ^{13}C NMR (DMSO-d_6) δ 43.52, 109.43, 115.80, 120.06, 123.22, 126.92, 127.70, 129.25, 129.83, 132.12, 133.81, 134.32, 143.49, 157.37. Anal. ($C_{14}H_{10}N_3Cl_3$) C, H, N.

2-Amino-1-(*m*,*m*-bis(trifluoromethyl)benzyl)-5,6-dichlorobenzimidazole (4l). **3** was reacted with *m*,*m*-bis-(trifluoromethyl)benzyl bromide (0.23 g, 0.75 mmol): yield 0.11 g (35%); R_f 0.52; white crystals; mp 233−235 °C; ^{1}H NMR (DMSO-d_6) δ 5.47 (2H, s), 7.00 (2H, s), 7.32 (1H, s), 7.54 (1H, s), 7.83 (2H, s), 8.02 (1H, s); ^{13}C NMR (DMSO-d_6) δ 44.02, 109.07, 115.65, 120.09, 121.29, 123.15, 127.64, 130.27, 130.64, 133.83, 140.08, 143.03, 156.65. Anal. ($C_{16}H_9N_3Cl_2F_6$) C, H, N.

2-Amino-1-(*p*-nitrobenzyl)-5,6-dichlorobenzimidazole (4m). **3** was reacted with *p*-nitrobenzyl chloride (0.13 g, 0.75 mmol): yield 0.11 g (44%); R_f 0.39; yellow powder; mp 276−279 °C; ^{1}H NMR (DMSO-d_6) δ 5.44 (2H, s), 6.96 (2H, s), 7.32−7.40 (4H, m), 8.19−8.20 (2H, d); ^{13}C NMR (DMSO-d_6) δ 44.58, 109.40, 115.87, 120.18, 123.27, 124.06, 128.16, 134.30, 143.44, 144.55, 147.16, 157.09. Anal. ($C_{14}H_{10}N_4Cl_2O_2$) C, H, N.

2-Amino-1-(*m*-nitrobenzyl)-5,6-dichlorobenzimidazole (4n). **3** was reacted with *m*-nitrobenzyl chloride (0.13 g, 0.75 mmol): yield 0.19 g (74%); R_f 0.36; yellow powder; mp 296−298 °C; ^{1}H NMR (DMSO-d_6) δ 5.43 (2H, s), 7.02 (2H, s), 7.32 (1H, s), 7.48 (1H, s), 7.55−7.65 (2H, m), 8.07−8.13 (2H, m); ^{13}C NMR (DMSO-d_6) δ 44.06, 109.11, 115.57, 119.96, 121.59, 122.39, 123.00, 130.18, 133.40, 133.94, 138.81, 143.08, 153.00, 156.74. Anal. ($C_{14}H_{10}N_4Cl_2O_2$) C, H, N.

2-Amino-1-(*p*-fluorobenzyl)-5,6-dichlorobenzimidazole (4o). **3** was reacted with *p*-fluorobenzyl chloride (0.11 g, 0.75 mmol): yield 0.16 g (70%); R_f 0.52; white fluffy needles; mp 225−227 °C; ^{1}H NMR (DMSO-d_6) δ 5.25 (2H, s), 6.92 (2H,

s), 7.15−7.29 (5H, m), 7.39 (1H, s); ^{13}C NMR (DMSO-d_6) δ 44.37, 109.53, 115.59, 115.77, 115.83, 120.24, 123.17, 129.23, 129.32, 132.82, 139.19, 143.13. Anal. ($C_{14}H_{10}N_3Cl_2F$) C, H, N.

1-(Substituted benzyl)-2,5,6-trichlorobenzimidazole (6). 2,5,6-Trichlorobenzimidazole[18] (**5**) (0.1661 g, 0.75 mmol) and sodium hydroxide (0.03 g, 0.75 mmol) were dissolved in acetonitrile (40 mL). After approximately 1 h of stirring, the appropriately substituted benzyl halide (0.75 mmol) was added and the mixture was heated at reflux. Once thin-layer chromatography showed only one spot, the reaction mixture was cooled to room temperature, evaporated to dryness, dissolved in methanol, decolorized with charcoal, filtered, and evaporated to dryness. The product was dissolved in hot methanol and recrystallized from a mixture of methanol and water to obtain pure **6a**−**o**.

1-Benzyl-2,5,6-trichlorobenzimidazole (6a). **5** was reacted with benzyl bromide (0.17 g, 1.0 mmol): yield 0.21 g (90%); R_f 0.90; yellow solid; mp 180−182 °C. Anal. ($C_{14}H_9N_2Cl_3$) C, H, N.

1-(*p*-Chlorobenzyl)-2,5,6-trichlorobenzimidazole (6b). **5** was reacted with *p*-chlorobenzyl chloride (0.12 g, 0.75 mmol): yield 0.17 g (66%); R_f 0.82; white crystals; mp 194−196 °C; ^{1}H NMR (DMSO-d_6) δ 5.56 (2H, s, CH_2), 7.24 (2H, d, C2′-H and C6-H), 7.42 (2H, d, C3′-H and C5′-H), 7.97 (1H, s, C4-H), 8.12 (1H, d, C7-H); ^{13}C NMR (DMSO-d_6) δ 47.51 (CH_2), 113.47 (C4), 121.07 (C7), 126.41 (C3a or C5), 126.99 (C6), 129.76 (C2′, C3′, C5′, C6′), 133.50 (C4′), 135.39 (C7a), 135.47 (C1′), 141.38 (C3a or C5), 143.40 (C2). Anal. ($C_{14}H_8N_2Cl_4$) C, H, N.

1-(*p*-Methoxybenzyl)-2,5,6-trichlorobenzimidazole (6c). **5** was reacted with *p*-methoxybenzyl chloride (0.12 g, 0.75 mmol): yield 0.17 g (65%); R_f 0.81; yellow crystals; mp 159−160 °C. Anal. ($C_{15}H_{11}N_2Cl_3O$) C, H, N.

1-(*p*-Methylbenzyl)-2,5,6-trichlorobenzimidazole (6d). **5** was reacted with *p*-methylbenzyl chloride (0.11 g, 0.75 mmol): yield 0.13 g (54%); R_f 0.81; white crystals; mp 174−176 °C. Anal. ($C_{15}H_{11}N_2Cl_3$) C, H, N.

1-(*m*,*p*-Dichlorobenzyl)-2,5,6-trichlorobenzimidazole (6e). **5** was reacted with *m*,*p*-dichlorobenzyl chloride (0.15 g, 0.75 mmol): yield 0.19 g (67%); R_f 0.80; white crystals; mp 224−226 °C. Anal. ($C_{14}H_7N_2Cl_5$) C, H, N.

1-(*m*-Chlorobenzyl)-2,5,6-trichlorobenzimidazole (6f). **5** was reacted with *m*-chlorobenzyl chloride (0.12 g, 0.75 mmol): yield 0.16 g (63%); R_f 0.81; white crystals; 170−172 °C. Anal. ($C_{14}H_8N_2Cl_4$) C, H, N.

1-(*p*-*tert*-Butylbenzyl)-2,5,6-trichlorobenzimidazole (6g). **5** was reacted with *p*-*tert*-butylbenzyl bromide (0.17 g, 0.75 mmol): yield 0.21 g (77%); R_f 0.81; white crystals; mp 222−226 °C. Anal. ($C_{18}H_{17}N_2Cl_3$) C, H, N.

1-(*p*-(Trifluoromethyl)benzyl)-2,5,6-trichlorobenzimidazole (6h). **5** was reacted with *p*-(trifluoromethyl)benzyl bromide (0.18 g, 0.75 mmol): yield 0.19 g (67%); R_f 0.81; white crystals; mp 198−200 °C. Anal. ($C_{15}H_8N_2Cl_3F_3$) C, H, N.

1-(*m*-Methylbenzyl)-2,5,6-trichlorobenzimidazole (6i). **5** was reacted with *m*-methylbenzyl chloride (0.11 g, 0.75 mmol): yield 0.16 g (67%); R_f 0.81; yellow crystals; mp 138−141 °C. Anal. ($C_{15}H_{11}N_2Cl_3$) C, H, N.

1-(*m*-(Trifluoromethyl)benzyl)-2,5,6-trichlorobenzimidazole (6j). **5** was reacted with *m*-(trifluoromethyl)benzyl chloride (0.18 g, 0.75 mmol): yield 0.17 g (60%); R_f 0.81; white crystals; mp 175−177 °C. Anal. ($C_{15}H_8N_2Cl_3F_3$) C, H, N.

1-(*o*-Chlorobenzyl)-2,5,6-trichlorobenzimidazole (6k). **5** was reacted with *o*-chlorobenzyl chloride (0.12 g, 0.75 mmol): yield 0.15 g (57%); R_f 0.80; white crystals; mp 120−122 °C. Anal. ($C_{14}H_8N_2Cl_4$) C, H, N.

1-(*m*,*m*-Bis(trifluoromethyl)benzyl)-2,5,6-trichlorobenzimidazole (6l). **5** was reacted with *m*,*m*-bis(trifluoromethyl)benzyl bromide (0.23 g, 0.75 mmol): yield 0.25 g (75%); R_f 0.81; white crystals; mp 173−175 °C. Anal. ($C_{16}H_7N_2Cl_3F_6$) C, H, N.

1-(*p*-Nitrobenzyl)-2,5,6-trichlorobenzimidazole (6m). **5** was reacted with *p*-nitrobenzyl chloride (0.13 g, 0.75 mmol): yield 0.18 g (67%); R_f 0.80; yellow crystals; mp 200−204 °C. Anal. ($C_{14}H_8N_3Cl_3O_2$) C, H, N.

1-(*m*-Nitrobenzyl)-2,5,6-trichlorobenzimidazole (6n). **5** was reacted with *m*-nitrobenzyl chloride (0.13 g, 0.75 mmol): yield 0.19 g (71%); R_f 0.80; white crystals; mp 198−200 °C. Anal. ($C_{14}H_8N_3Cl_3O_2$) C, H, N.

1-(*p*-Fluorobenzyl)-2,5,6-trichlorobenzimidazole (6o). **5** was reacted with *p*-fluorobenzyl chloride (0.11 g, 0.75 mmol): yield 0.19 g (75%); R_f 0.80; white crystals; mp 215−217 °C. Anal. ($C_{14}H_8N_2Cl_3F$) C, H, N.

2-Bromo-5,6-dichlorobenzimidazole (7). To a mixture of $CuBr_2$ (17.87 g, 80 mmol) in dry acetone (160 mL) was added 90% *tert*-butyl nitrite (8 mL). The reaction mixture was stirred at room temperature for 10 min before 2-amino-5,6-dichlorobenzimidazole (**3**) (8.08 g, 40 mmol) was added portionwise over a period of 10 min at 60 °C. After 30 min of stirring, *tert*-butyl nitrite (8 mL, 67 mmol) was added and the mixture stirred for 1.5 h. This reaction mixture was concentrated and partitioned between 2 N HBr and ethyl acetate. The ethyl acetate layer was washed with saturated NaCl solution, dried over Na_2SO_4, decolorized with charcoal, and evaporated. The residue was recrystallized in methanol to yield 2-bromo-5,6-dichlorobenzimidazole (**7**) crystals: R_f 0.17; orange crystals; mp 233−234 °C (lit.[18] mp 233−234 °C); ^{1}H NMR (DMSO-d_6) 13.62 (1H, s), 7.81 (s, 2H).

2-Bromo-5,6-dichloro-1-(substituted benzyl)benzimidazole (8). 2-Bromo-5,6-dichlorobenzimidazole (**7**) (0.200 g, 0.75 mmol) and sodium hydroxide (0.03 g, 0.75 mmol) were dissolved in acetonitrile (40 mL). After approximately 1 h of stirring, the appropriately substituted benzyl halide (0.75 mmol) was added and the mixture was set to reflux. Once thin-layer chromatography showed only one spot, the reaction mixture was cooled to room temperature, evaporated to dryness, dissolved in a mixture of methanol and tetrahydrofuran, decolorized with charcoal, filtered, and evaporated to dryness. The product was dissolved in a hot mixture of methanol and tetrahydrofuran and recrystallized from a mixture of methanol, tetrahydrofuran, and water to obtain pure **8a**−**o**.

1-Benzyl-2-bromo-5,6-dichlorobenzimidazole (8a). **7** was reacted with benzyl bromide (0.128 g, 0.75 mmol): yield 0.13 g (64%); R_f 0.73; white crystals; mp 194−196 °C. Anal. ($C_{14}H_9N_2Cl_2Br$) C, H, N.

2-Bromo-1-(*p*-chlorobenzyl)-5,6-dichlorobenzimidazole (8b). **7** was reacted with *p*-chlorobenzyl chloride (0.12 g, 0.75 mmol): yield 0.20 g (67%); R_f 0.58; white crystals; mp 206−208 °C. Anal. ($C_{14}H_8N_2Cl_3Br$) C, H, N.

2-Bromo-1-(*p*-methoxybenzyl)-5,6-dichlorobenzimidazole (8c). **7** was reacted with *p*-methoxybenzyl chloride (0.12 g, 0.75 mmol): yield 0.14 g (48%); R_f 0.65; light brown crystals; mp 156−158 °C. Anal. ($C_{15}H_{11}N_2Cl_2BrO$) C, H, N.

2-Bromo-1-(*p*-methylbenzyl)-5,6-dichlorobenzimidazole (8d). **7** was reacted with *p*-methylbenzyl chloride (0.11 g, 0.75 mmol): yield 0.20 g (72%); R_f 0.65; white powder; mp 194−195 °C. Anal. ($C_{15}H_{11}N_2Cl_2Br$) C, H, N.

2-Bromo-1-(*m*,*p*-dichlorobenzyl)-5,6-dichlorobenzimidazole (8e). **7** was reacted with *m*,*p*-dichlorobenzyl chloride (0.15 g, 0.75 mmol): yield 0.20 g (63%); R_f 0.58; white crystals; mp 222−224 °C. Anal. ($C_{14}H_7N_2Cl_4Br$) C, H, N.

2-Bromo-1-(*m*-chlorobenzyl)-5,6-dichlorobenzimidazole (8f). **7** was reacted with *m*-chlorobenzyl chloride (0.12 g, 0.75 mmol): yield 0.22 g (76%); R_f 0.52; white powder; mp 166−168 °C. Anal. ($C_{14}H_8N_2Cl_3Br$) C, H, N.

2-Bromo-1-(*p*-*tert*-butylbenzyl)-5,6-dichlorobenzimidazole (8g). **7** was reacted with *p*-*tert*-butylbenzyl bromide (0.17 g, 0.75 mmol): yield 0.21 g (67%); R_f 0.52; white crystals; mp 230−231 °C. Anal. ($C_{18}H_{17}N_2Cl_2Br$) C, H, N.

2-Bromo-1-(*p*-(trifluoromethyl)benzyl)-5,6-dichlorobenzimidazole (8h). **7** was reacted with *p*-(trifluoromethyl)-benzyl bromide (0.18 g, 0.75 mmol): yield 0.25 g (79%); R_f 0.58; white crystals; mp 195−197 °C. Anal. ($C_{15}H_8N_2Cl_2BrF_3$) C, H, N.

2-Bromo-1-(*m*-methylbenzyl)-5,6-dichlorobenzimidazole (8i). **7** was reacted with *m*-methylbenzyl chloride (0.11 g, 0.75 mmol): yield 0.20 g (72%); R_f 0.58; light brown powder; mp 160−162 °C; ^{1}H NMR (DMSO-d_6) δ 2.25 (3H, s, CH_3) 5.51 (2H, s, CH_2), 6.91 (1H, d, C6′-H), 7.01 (1H, s, C2′-H), 7.10 (1H, d, C4′-H), 7.23 (1H, t, C5′-H), 7.96 (1H, s, C4-H), 8.06 (1H, s, C7-H); ^{13}C NMR (DMSO-d_6) δ 21.84 (CH_3), 49.07 (CH_2), 113.4 (C4), 120.84 (C7), 124.63 (C6′), 126.15 (C3a or C5), 126.76 (C6), 128.1 (C2′), 129.42 (C4′), 129.64 (C5′), 134.15 (C2), 135.94 (C7a), 136.4 (C1′), 138.98 (C3′), 142.84 (C3a or C5). Anal. ($C_{15}H_{11}N_2Cl_2Br$) C, H, N.

2-Bromo-1-(*m*-(trifluoromethyl)benzyl)-5,6-dichlorobenzimidazole (8j). **7** was reacted with *m*-(trifluoromethyl)-benzyl chloride (0.18 g, 0.75 mmol): yield 0.21 g (65%); R_f 0.58; yellow crystals; mp 172−174 °C. Anal. ($C_{15}H_8N_2Cl_2BrF_3$) C, H, N.

2-Bromo-1-(*o*-chlorobenzyl)-5,6-dichlorobenzimidazole (8k). **7** was reacted with *o*-chlorobenzyl chloride (0.12 g, 0.75 mmol): yield 0.23 g (40%); R_f 0.80; white crystals; mp 120−122 °C. Anal. ($C_{14}H_8N_2Cl_3Br$) C, H, N.

2-Bromo-1-(*m*,*m*-bis(trifluoromethyl)benzyl)-5,6-dichlorobenzimidazole (8l). **7** was reacted with *m*,*m*-bis-(trifluoromethyl)benzyl bromide (0.23 g, 0.75 mmol): yield 0.25 g (69%); R_f 0.58; white needles; mp 168−170 °C. Anal. ($C_{16}H_7N_2Cl_2BrF_6$) C, H, N.

2-Bromo-1-(*p*-nitrobenzyl)-5,6-dichlorobenzimidazole (8m). **7** was reacted with *p*-nitrobenzyl chloride (0.13 g, 0.75 mmol): yield 0.23 g (77%); R_f 0.84; yellow powder; mp 203−205 °C. Anal. ($C_{14}H_8N_2Cl_2BrO_2$) C, H, N.

2-Bromo-1-(*m*-nitrobenzyl)-5,6-dichlorobenzimidazole (8n). **7** was reacted with *m*-nitrobenzyl chloride (0.13 g, 0.75 mmol): yield 0.25 g (82%); R_f 0.58; light brown crystals; mp 202−204 °C. Anal. ($C_{14}H_8N_2Cl_2BrO_2$) C, H, N.

2-Bromo-1-(*p*-fluorobenzyl)-5,6-dichlorobenzimidazole (8o). **7** was reacted with *p*-fluorobenzyl chloride (0.11 g, 0.75 mmol): yield 0.19 g (67%); R_f 0.58; white crystals; mp 215−217 °C. Anal. ($C_{14}H_8N_2Cl_2BrF$) C, H, N.

1-(Phenylethyl)-2,5,6-trichlorobenzimidazole (9). 2,5,6-Trichlorobenzimidazole (**5**) (0.1661 g, 0.75 mmol) and sodium hydroxide (0.03 g, 0.75 mmol) were dissolved in acetonitrile (40 mL). After approximately 1 h of stirring, (2-bromoethyl)-benzene (0.14 g, 0.75 mmol) was added and the mixture was heated at reflux. Once thin-layer chromatography showed only one spot, the reaction mixture was cooled to room temperature, evaporated to dryness, dissolved in methanol, decolorized with charcoal, filtered, and evaporated to dryness. The product was dissolved in hot methanol and recrystallized from a mixture of methanol and water to obtain pure **9**: yield 0.15 g (62%); R_f 0.82; light brown crystals; mp 179−181 °C; ^{1}H NMR (DMSO-d_6) δ 3.00 (2H, t), 4.45 (2H, t), 7.07−7.22 (5H, m), 7.85 (1H, s), 7.90 (1H, s); ^{13}C NMR (DMSO-d_6) δ 34.57, 45.82, 112.65, 119.80, 125.05, 125.62, 126.70, 128.35, 128.96, 134.28, 137.37, 140.27, 142.40. Anal. ($C_{15}H_{11}N_2Cl_3$) C, H, N.

2-Bromo-1-(phenylethyl)-5,6-dichlorobenzimidazole (10). 2-Bromo-5,6-dichlorobenzimidazole (**7**) (0.20 g, 0.75 mmol) and sodium hydroxide (0.03 g, 0.75 mmol) were dissolved in acetonitrile (40 mL). After approximately 1 h of stirring, (2-bromoethyl)benzene (0.14 g, 0.75 mmol) was added and the mixture was heated at reflux. Once thin-layer chromatography showed only one spot, the reaction mixture was cooled to room temperature, evaporated to dryness, dissolved in a mixture of methanol and tetrahydrofuran, decolorized with charcoal, filtered, and evaporated to dryness. The product was dissolved in a mixture of hot methanol and tetrahydrofuran and recrystallized from a mixture of methanol, tetrahydrofuran, and water to obtain pure **10**: yield 0.18 g (63%); R_f 0.78; white crystals; mp 179−181 °C; ^{1}H NMR (DMSO-d_6) δ 3.00 (2H, t), 4.44 (2H, t), 7.08−7.24 (5H, m), 7.86 (1H, s), 7.90 (1H, s); ^{13}C NMR (DMSO-d_6) δ 34.72, 46.69, 112.58, 119.63, 124.86, 125.52, 126.71, 128.36, 128.98, 132.86, 134.67, 137.34, 141.72. Anal. ($C_{15}H_{11}N_2Cl_2Br$) C, H, N.

1-Benzyl-2-(isopropylamino)-5,6-dichlorobenzimidazole (11). 1-Benzyl-2,5,6-trichlorobenzimidazole (**6a**) (200 mg, 0.64 mmol) was dissolved in isopropylamine (20 mL), sealed in a pressure vessel, and heated to 60 °C with constant stirring. After 5 days, the solvent was removed and the residue was dissolved in chloroform and eluted off a silica gel column (3.5 × 8 cm) using chloroform as the solvent. The fractions showing the desired compound were combined, evaporated to dryness, and recrystallized from methanol and water to obtain pure **11**: yield 0.08 g (37%); R_f 0.30 (ethyl acetate:hexane, 1:9); white solid; mp 175−177 °C; ^{1}H NMR (DMSO-d_6) δ 1.20 (6H, d), 4.07 (1H, m), 5.30 (2H, s), 6.90−9.63 (1H, d), 7.10 (1H, s), 7.14 (1H, s), 7.23−7.36 (5H, m). Anal. ($C_{17}H_{17}N_3Cl_2$) C, H, N.

1-Benzyl-2-(cyclopropylamino)-5,6-dichlorobenzimidazole (12). 1-Benzyl-2,5,6-trichlorobenzimidazole (**6a**) (700 mg, 2.25 mmol) was dissolved in cyclopropylamine (30 mL), sealed in a pressure vessel, and heated to 60 °C with constant stirring. After 5 days, the solvent was removed and the residue was washed with chloroform to obtain a white precipitate. The precipitate was recrystallized from methanol and water to obtain pure **12**: yield 0.20 g (27%); R_f 0.10 (chloroform); white crystals; mp 194−196 °C; ^{1}H NMR (DMSO-d_6) δ 0.53 (2H, m), 0.72 (2H, m), 2.80 (1H, m), 3.35 (1H, s), 5.27 (2H, s), 7.09 (1H, s), 7.11 (1H, s), 7.25−7.45 (5H, m). Anal. ($C_{17}H_{15}N_3Cl_2$) C, H, N.

Cell Culture Procedures. The routine growth and passage of KB and human foreskin fibroblasts (HFF cells) were performed in monolayer cultures using minimal essential medium (MEM) with either Hanks salts [MEM(H)] or Earle salts [MEM(E)] supplemented with 10% calf serum (KB cells) or 10% fetal bovine serum (HFF cells). The sodium bicarbonate concentration was varied to meet the buffering capacity required. Cells were passaged at 1:2 to 1:10 dilutions according to conventional procedures by using 0.05% trypsin plus 0.02% EDTA in a HEPES-buffered salt solution. Similar suspension culture conditions were employed for CEM-SS cells.

Virological Procedures. The Towne strain, plaque-purified isolate P_o, of HCMV was kindly provided by Dr. Mark Stinski, University of Iowa. The KOS strain of HSV-1 was used in most experiments and was provided by Dr. Sandra K. Weller, University of Connecticut. Stock HCMV was prepared by infecting HFF cells at a multiplicity of infection (moi) of <0.01 plaque-forming units (pfu) per cell as detailed previously.[27] High-titer HSV-1 stocks were prepared by infecting KB cells at an moi of <0.1 also as detailed previously.[27] Virus titers were obtained using monolayer cultures of HFF cells for HCMV and monolayer cultures of BSC-1 cells for HSV-1 as described earlier.[28] The HIV-1 strain III_B producer cell line $H9III_B$ was obtained through the courtesy of Dr. R. C. Gallo. Matched pairs of pre-AZT and post-AZT human treatment isolates of HIV-1 which were sensitive and resistant to AZT were obtained from Dr. D. D. Richman[29] through the AIDS Research and Reference Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD. The A17 variant of HIV-1, resistant to nonnucleoside RT inhibitors including TIBO,[30] was obtained from Dr. A. M. Emini through the AIDS Research and Reference Program; HIV-2 also was obtained from the latter program. HIV-1 strain III_B was assayed in CEM-SS cells as described previously by Kucera et al.[31,32]

Antiviral Assays, HCMV. A plaque assay was used for HCMV. HFF cells in 24-well cluster dishes were infected with approximately 100 pfu of HCMV per cm^2 cell sheet using the procedures detailed above. Following virus adsorption, compounds dissolved in growth medium were added to duplicate wells in four to eight selected concentrations. After incubation at 37 °C for 7 days, cell sheets were fixed and stained with crystal violet and microscopic plaques enumerated as described above. Drug effects were calculated as a percentage of reduction in number of plaques in the presence of each drug concentration compared to the number observed in the absence of drug. For yield reduction assay, the procedure devised by us[28] was used. HFF cells were planted as described above in 96-well cluster dishes, incubated overnight, and infected with HCMV at a moi of 0.5−1 pfu per cell. After virus adsorption, innoculum was replaced with 0.2 mL of fresh medium contain-

ing test compounds in a manner so that six compounds could be tested in duplicate on a single plate with concentrations from 100 to 0.14 μM. Plates were incubated at 37 °C for 7 days and subjected to one cycle of freezing and thawing, and aliquots from each of the wells were transferred to a fresh 96-well monolayer culture of HFF cells. Each column of the original drug-containing plate was diluted across a new separate plate in this manner. Cultures were incubated, plaques were enumerated, and titers were calculated as described.[28]

HSV-1. An ELISA developed by us[33] was employed to detect HSV-1. Ninety-six-well cluster dishes were planted with 10 000 BSC-1 cells per well in 200 μL per well of MEM(E) plus 10% calf serum. After overnight incubation at 37 °C, selected drug concentrations in quadruplicate and HSV-1 at a concentration of 100 pfu/well were added. Following a 3-day incubation at 37 °C, medium was removed, plates were blocked and rinsed, and horseradish peroxidase conjugated rabbit anti-HSV-1 antibody was added. Following removal of the antibody containing solution, plates were rinsed and then developed by adding a solution of tetramethylbenzidine as substrate. The reaction was stopped with H_2SO_4, and absorbance was read at 450 and 570 nm. Drug effects were calculated as a percentage of the reduction in absorbance in the presence of each drug concentration compared to absorbance obtained with virus in the absence of drug.

HIV-1. Two separate assays were employed to evaluate the activities of compounds in cells acutely infected with HIV-1. The syncytial plaque assay performed as previously described by us[32] was used to measure the effect of compounds on HIV-1. Briefly, CEM-SS cells rendered adherent to substrate by means of poly-L-lysine were infected with HIV-1 strain III_B in the presence and absence of selected concentrations of compounds. Syncytial plaques were enumerated at 30-fold magnification 7 days postinfection. In the second assay, reverse transcriptase (RT) was employed as a marker for HIV-1. CEM-SS cells were infected at a moi of approximately 0.001 pfu per cell with strain III_B of HIV-1 in a minimal volume of stock virus in growth medium. Cultures were incubated at 37 °C for 2 h to permit virus adsorption, washed and then diluted to 5×10^5 cells/mL with RPMI 1640 containing 10% fetal bovine serum. One-tenth milliliter was then added to each well of a 96-well cluster dish. Fresh medium (0.1 mL with 10% fetal bovine serum) containing test compounds in twice the desired final concentration was added to triplicate wells at seven concentrations ranging from 100 to 0.14 μM. After 6 days of incubation, supernatant samples were taken and the amount of RT activity was measured by the incorporation of [^{3}H]dTTP into acid insoluble material using the assay described by White et al.[34] Drug effects were calculated as a percentage of the reduction in counts per minute (cpm) in the presence of each drug concentration compared to the cpm obtained in the absence of drug.

Cytotoxicity Assays. Several different assays were used to explore cytotoxicity of selected compounds using methods we have detailed previously. (i) Cytotoxicity produced in stationary HFF cells was determined by microscopic inspection of cells not affected by the virus used in plaque assays.[27] (ii) The effect of compounds during two population doublings of KB cells was determined by crystal violet staining and spectrophotometric quantitation of dye eluted from stained cells as described earlier.[35] Briefly, 96-well cluster dishes were planted with KB cells at 3000−5000 cells per well. After overnight incubation at 37 °C, test compound was added in quadruplicate at six to eight concentrations. Plates were incubated at 37 °C for 48 h in a CO_2 incubator, rinsed, fixed with 95% ethanol, and stained with 0.1% crystal violet. Acidified ethanol was added and plates read at 570 nm in a spectrophotometer designed to read 96-well ELISA assay plates. (iii) Inhibition of DNA synthesis in CEM-SS cells was measured by determining the effect of compounds on the uptake of [^{3}H]dThd into total acid precipitable material as detailed earlier.[32]

Data Analysis. Dose−response relationships were constructed by linearly regressing the percent inhibition of parameters derived in the preceding sections against log drug concentrations. Fifty-percent inhibitory (IC_{50}) concentrations were calculated from the regression lines. Samples containing positive controls (acyclovir for HSV-1, ganciclovir for HCMV, zidovudine for HIV-1, and 2-acetylpyridine thiosemicarbazone for cytotoxicity) were used in all assays.

Acknowledgment. The authors thank Chirag Patel, Julie M. Breitenbach, Nathan P. Iyer, Dr. Mary S. Ludwig, Carolyn Oh, and Roger G. Ptak for expert technical performance of biological assays, Jack Hinkley for his large-scale preparation of starting materials, and Marina Savic for assistance with manuscript preparation. This study was supported by research grants U01-25739, U01-AI31718, R01-AI33332, and R01-AI-36872 from the National Institute of Allergy and Infectious Diseases, Research Agreement DRDA-942921, and by training grant T32-GM07767 from the National Institutes of Health.

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JM970559I