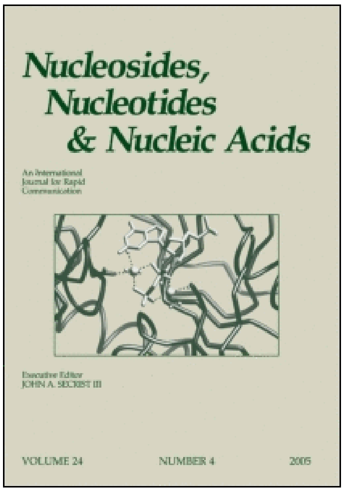

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# Design, Synthesis and Antiviral Activity of α-L-Arabinofuranosyl Derivatives of 2-Substituted-5,6-dichlorobenzimidazoles

Jean-Luc Girardet[a]; John C. Drach[a]; Stanley D. Chamberlain[b]; George W. KosLzalka[b]; Leroy B. Townsend[a]
[a] Department of Medicinal Chemistry, College of Pharmacy, Department of Chemistry, College of Literature, Sciences and Art, and Department of Biologic and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, Michigan [b] Glaxo Division of Chemistry, Glaxo Wellcome Co., Research Triangle Park, NC

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NUCLEOSIDES & NUCLEOTIDES, 17(12), 2389-2401 (1998)

# DESIGN, SYNTHESIS AND ANTIVIRAL ACTIVITY OF α-L-ARABINOFURANOSYL DERIVATIVES OF 2-SUBSTITUTED-5,6-DICHLOROBENZIMIDAZOLES

Jean-Luc Girardet, John C. Drach, Stanley D. Chamberlain,§ George W. Koszalka§ and Leroy B. Townsend.*

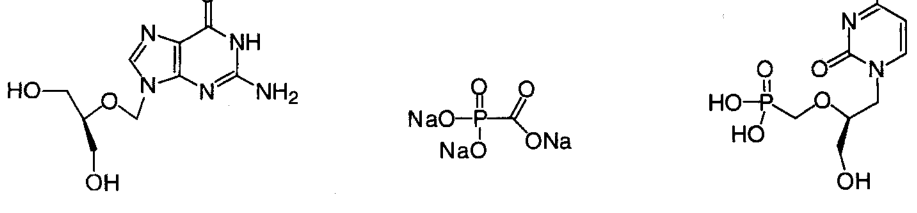
Department of Medicinal Chemistry, College of Pharmacy, Department of Chemistry, College of Literature, Sciences and Art, and Department of Biologic and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, Michigan 48109-1065. §Glaxo Division of Chemistry, Glaxo Wellcome Co., Research Triangle Park, NC 27709.

**Abstract**: A number of 2-substituted-5,6-dichloro-1-(α-L-arabinofuranosyl)benzimidazoles have been prepared by condensation of 2-bromo-5,6-dichlorobenzimidazole or 2,5,6-trichlorobenzimidazole with tetra-*O*-acetyl-L-arabinofuranose. 2-Alkylamino derivatives were prepared by a substitution of the 2-chloro group with the appropriate amines. All target compounds were evaluated for activity against HCMV and HSV-1. The 2-chloro and 2-bromo derivatives showed moderate activity against HCMV at non-cytotoxic concentrations.

## Introduction

Human cytomegalovirus (HCMV) is one of eight viruses of the Herpes viridae family. It has been estimated that more than half of all Americans are infected with this virus.[1] HCMV infections in immunocompetent individuals are usually asymptomatic; however, in immunocompromised patients, HCMV infections are often life threatening. Transplant recipients[2] and individuals with acquired immunodeficiency syndrome (AIDS)[3] often have clinical manifestations of the disease. HCMV is also a leading cause of birth defects as a consequence of *in utero* fetal infection.[4]

Currently, there are three FDA-approved drugs available for the treatment of HCMV infections: ganciclovir,[5] foscarnet[6] and cidofovir[7] (FIG. 1).



ganciclovir foscarnet cidofovir

FIG. 1

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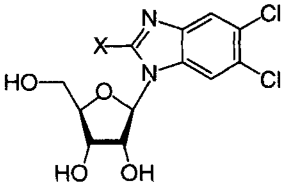
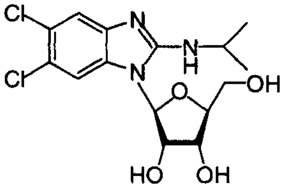
Unfortunately, ganciclovir, foscarnet and cidofovir are associated with significant side effects and have limited oral bioavailability. Moreover, virus strains resistant to each of these drugs have emerged.[8] Consequently, there is need for more potent and selective antiviral agents to treat HCMV infections.

As part of our search for new antiviral drugs, a number of benzimidazole derivatives have been synthesized.[9-14] Certain of these compounds, including 2,5,6-trichloro-1-(β-D-ribofuranosyl)benzimidazole (TCRB) and its 2-bromo analog (BDCRB), showed potent activity against HCMV with low cellular toxicity at concentrations inhibiting viral growth.[9] In addition to these D-carbohydrate derivatives, several L-carbohydrate derivatives have been synthesized and evaluated for their antiviral properties.[15,16] From among these, 5,6-dichloro-2-isopropylamino-1-(β-L-ribofuranosyl)benzimidazole (1263W94, FIG. 2) was chosen for clinical trials due to its potent and selective activity *in vitro* and its superior pharmacokinetic properties.



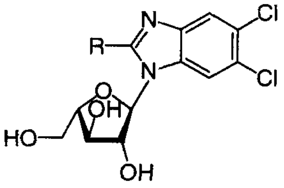
X = Cl: TCRB
X = Br: BDCRB



1263W94

FIG. 2

Biological evaluations of TCRB and BDCRB have established that both compounds share a unique mechanism of action, which involves the inhibition of viral DNA processing and virus assembly.[17] In contrast, the mechanism of action of 1263W94 appears to be different from that of TCRB and BDCRB, and involves inhibition of viral DNA synthesis.[15] TCRB, BDCRB and 1263W94 do not require phosphorylation to exhibit antiviral activity, and a removal of the 5'-hydroxyl group increases the antiviral activity of TCRB and BDCRB[14,18] while it slightly decreases but does not eliminate the antiviral potency of 1263W94.[16]



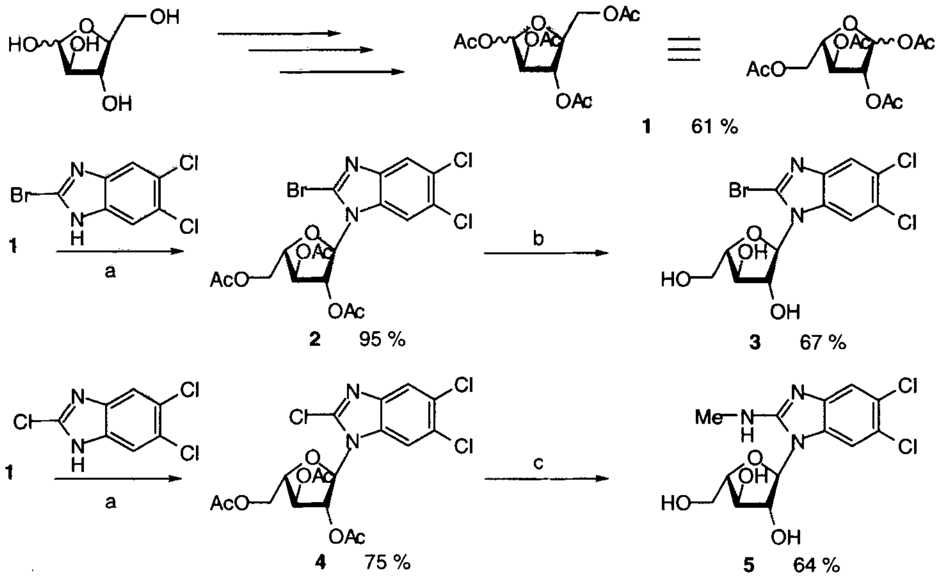
2-substituted-α-L-arabinosyl series

FIG. 3

As part of the studies designed to better understand the structure activity relationship in regard to the carbohydrate moiety, we elected to synthesize a variety of 5,6-dichloro-2-substituted-1-(α-L-arabinofuranosyl)benzimidazoles (FIG. 3) which are in fact 3', 4' epimers of the β-D-nucleosides TCRB and BDCRB.

## Results and Discussion

Starting with commercially available L-arabinose, tetra-*O*-acetyl-L-arabinofuranose (**1**) was prepared, in three steps in 61% yield after silica gel chromatography, from a procedure developed by Guthrie and Smith[19] and generalized by Kam *et al.*[20] (SCHEME 1).

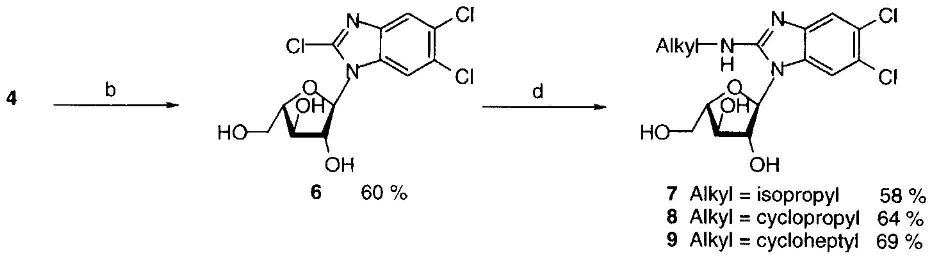


a) BSA, $CH_3CN$, then TMSTf. b) $Na_2CO_3$, EtOH, $H_2O$. c) $CH_3NH_2$, MeOH.

SCHEME 1

In a fashion analogous to the preparation of TCRB and BDCRB,[9] 2,5,6-trichlorobenzimidazole[21] (TCB) and 2-bromo-5,6-dichlorobenzimidazole[9] (BDCB) were silylated, individually, with *N,O*-bis(trimethylsilyl)acetamide (BSA) in dry acetonitrile and then glycosylated with **1** in the presence of trimethylsilyl triflate (TMSTf) to give **2** and **4** respectively. Deprotection of **2** and **4** under basic conditions furnished 2-bromo-5,6-dichloro-1-(α-L-arabinofuranosyl)benzimidazole (**3**) in 67% yield and 2,5,6-trichloro-1-(α-L-arabinofuranosyl)benzimidazole (**6**) in 60% yield, respectively (SCHEMES 1 and 2).

As reported previously in the literature,[22] the 2-chloro substituent of 1-substituted benzimidazoles can be conveniently displaced by a variety of nucleophiles. Following this



b) $Na_2CO_3$, EtOH, $H_2O$. d) $AlkylNH_2$, EtOH, 60 °C.

SCHEME 2

approach, 5,6-dichloro-2-methylamino-1-(α-L-arabinofuranosyl)benzimidazole (**5**) could be prepared in one step by the direct treatment of **4** with a 2 M solution of methylamine in methanol at room temperature. The preparation of 5,6-dichloro-2-isopropylamino-1-(α-L-arabinofuranosyl)benzimidazole (**7**), 2-cyclopropylamino-5,6-dichloro-1-(α-L-arabinofuranosyl)benzimidazole (**8**) and 2-cycloheptylamino-5,6-dichloro-1-(α-L-arabinofuranosyl)benzimidazole (**9**) was more conveniently accomplished by treating the deprotected nucleoside **6** with the appropriate primary amine in ethanol at 60 °C (SCHEME 2). Compounds **3**, **5**, **6**, **8** and **9** were obtained as crystalline solids while **7** was obtained as an amorphous solid.

Target compounds **3**, **5-9** were evaluated for activity against HCMV and HSV-1 (TABLE I). Among these, compounds **3** and **6** were found to have moderate activity against

TABLE I: Antiviral Activity and Cytotoxicity

| R = substituent of the 2-position | | 50% Inhibitory Concentration (μM) | | | |
|---|---|---|---|---|---|
| | | Antiviral Activity | | Cytotoxicity[c] | |
| | | HCMV[a] | HSV-1[b] | | |
| Compound | R | Plaque | ELISA | Visual | Growth |
| **3** | bromo | 21[d] | > 100 | > 100[d] | > 100 |
| **5** | methylamino | > 100 | > 100 | > 100 | > 100 |
| **6** | chloro | 51[d] | > 100 | > 100[d] | > 100 |
| **7** | isopropylamino | > 100 | > 100 | > 100 | > 100 |
| **8** | cyclopropylamino | > 100 | > 100 | > 100 | > 100 |
| **9** | cycloheptylamino | 32 | 60 | 21 | 100 |
| foscarnet[e] | | 39 ± 26 | - | > 100 | - |
| ganciclovir[f] | | 7.4 ± 6.5 | 3.5 ± 2.1 | > 100 | > 100 |
| TCRB[g] | | 2.8 | > 100 | > 100 | > 100 |

[a] Plaque reduction assays were performed in duplicate as described in the text. [b] All compounds were assayed by ELISA in quadruplicate wells. [c] Visual cytotoxicity was scored on HFF cells at the time of plaque enumeration. Inhibition of KB cell growth was determined in quadruplicate assays. [d] Average of duplicate or triplicate experiments. [e] Average ± standard deviation from 15 experiments. [f] Average ± standard deviation from 108 and 3 experiments, respectively. [g] Average ± standard deviation from 5 experiments.

HCMV at non-cytotoxic concentrations. However, these compounds were less potent against HCMV than TCRB. They were also less potent than 2-bromo-5,6-dichloro-1-(α-L-lyxofuranosyl)benzimidazole[23] and 2,5,6-trichloro-1-(α-L-lyxofuranosyl)benzimidazole[23] which are respectively 4'-epimers of BDCRB and TCRB. This shows the importance of the 3'-hydroxyl orientation in this series. No activity was detected for **3** and **6** against HSV-1. Compounds **5**, **7** and **8** were inactive against HCMV and HSV-1, and not cytotoxic, as was found for their α-L-lyxofuranosyl analogs.[23] Compound **9** was moderately active against both viruses, but its antiviral activity was not differentiated from its cytotoxicity and therefore probably was a consequence of it.

## Experimental

General Chemical Procedures: Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Silica gel, SilicAR 40-63 microns 230-400 mesh (Mallinckrodt) was used for chromatography. Thin layer chromatography (TLC) was performed on prescored SilicAR 7GF plates (Analtech, Newark, DE). Compounds were visualized by illuminating with UV light (254 nm) and/or by treatment with 10 % methanolic sulfuric acid followed by charring on a hot plate. Evaporations were carried out under reduced pressure (water aspirator) with the bath temperature not exceeding 50 °C, unless specified otherwise. $^1$H NMR spectra were recorded on either a Brüker 300, 360 or 500 MHz instrument. Chemical shift values are expressed in δ values (ppm) relative to the standard shift of the residual DMSO-$d_5$ (fixed at 2.50 ppm) contained in the solvent DMSO-$d_6$. All $^1$H NMR assignments reported were made by homonuclear decoupling experiments. Microanalytical results were performed by the University of Michigan, Department of Chemistry. Unless otherwise noted, all materials were obtained from commercial suppliers.

2-Bromo-5,6-dichloro-1-(2,3,5-tri-*O*-acetyl-α-L-arabinofuranosyl)benzimidazole (**2**): 2-Bromo-5,6-dichlorobenzimidazole[9] (265 mg, 1.0 mmol) was suspended in acetonitrile (30 mL) and the mixture was stirred at 60 °C. BSA (365 μL, 1.5 mmol) was added, and the reaction mixture stirred for 5 min. Compound **1**[24] (320 mg, 1.0 mmol) in acetonitrile (10 mL) and TMSOTf (290 μL, 1.5 mmol) were added to the clear solution, and the mixture was allowed to reach room temperature and stirred for an additional 20 h. The mixture was concentrated under reduced pressure, and the residue from the evaporation dissolved with chloroform (30 mL). The solution was washed with an aqueous saturated hydrogencarbonate solution (5 mL) and then water (3 x 5 mL), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was subjected to silica gel chromatography [2 x 15 cm, eluent: gradient of methanol (0-1%) in chloroform] to give **2** as a foam (496 mg, 95%). Compound **2** was used without further purification.

$^1$**H NMR** (DMSO-$d_6$): δ 8.07 and 8.02 (2 s, 2 H, H-4 and H-7), 6.37 (d, 1 H, $J$ = 5.4 Hz, H-1'), 5.6 (m, 1 H, H-2'), 5.41 (t, 1 H, $J$ = 3.4 Hz, H-3'), 4.9-4.8 (m, 1 H, H-4'), 4.35 (dd, 1 H, $J$ = 6.0 and 12.2 Hz, H-5'), 4.28 (dd, 1 H, $J$ = 3.5 and 12.1 Hz, H-5''), 2.17, 2.09 and 2.05 (3 s, 9 H, 3 $CH_3CO$).

2-Bromo-5,6-dichloro-1-(α-L-arabinofuranosyl)benzimidazole (**3**):

To a solution of **2** (393 mg, 0.75 mmol) in a 9:1 solution of ethanol and water (15 mL) was added sodium carbonate (517 mg, 4.9 mmol), and the reaction mixture was stirred 16 h at room temperature. Acetic acid (1 mL) was added and the mixture was evaporated to dryness. The residue from the evaporation was dissolved in a mixture of chloroform (50 mL), ethyl acetate (25 mL) and then washed with water (50 mL). The aqueous layer was washed with chloroform (25 mL) and then with ethyl acetate (25 mL). The combined organic extracts were dried over anhydrous sodium sulfate, filtered and the filtrate evaporated under reduced pressure. The residue was subjected to silica gel chromatography [2 x 15 cm, eluent: gradient of methanol (5-10%) in chloroform] to give **3** which crystallized from a mixture of acetonitrile and methanol (200 mg, 67%).

**mp**: 184-186 °C; $^1$**H NMR** (DMSO-$d_6$): δ 7.99 and 7.95 (2 s, 2 H, H-4 and H-7), 5.91 (d, 1 H, $J$ = 6.9 Hz, H-1'), 5.80 (d, 1 H, $J$ = 5.2 Hz, OH-2'), 5.57 (d, 1 H, $J$ = 6.3 Hz, OH-3'), 4.95 (t, 1 H, $J$ = 4.8 Hz, OH-5'), 4.5-4.4 (m, 1 H, H-2'), 4.2-4.1 (m, 2 H, H-3' and H-4'), 3.7-3.5 (m, 2 H, H-5' and H-5''); **Anal.**: Calc. for $C_{12}H_{11}BrCl_2N_2O_4$: C, 36.21; H, 2.79; N, 7.04. Found: C, 36.54; H, 2.79; N, 7.06.

2,5,6-Trichloro-1-(2,3,5-tri-*O*-acetyl-α-L-arabinofuranosyl)benzimidazole (**4**):

2,5,6-Trichlorobenzimidazole[21] (221 mg, 1.0 mmol) was suspended in acetonitrile (30 mL) and the mixture was stirred at 60 °C. BSA (365 µL, 1.5 mmol) was added, and the reaction mixture stirred for 30 min. Compound **1**[24] (320 mg, 1.0 mmol) in acetonitrile (10 mL) and TMSOTf (290 µL, 1.5 mmol) were added to the clear solution, and the mixture was allowed to reach room temperature and stirred for an additional 16 h. The mixture was concentrated under reduced pressure, and the residue from the evaporation dissolved with chloroform (30 mL). The solution was washed with an aqueous saturated hydrogencarbonate solution (5 mL), then with water (3 x 5 mL), dried over anhydrous sodium sulfate, filtered and the filtrate concentrated under reduced pressure. The residue was subjected to silica gel chromatography [2 x 15 cm, eluent: gradient of methanol (0-1%) in chloroform] to give **4** as a glass (360 mg, 75%). Compound **4** was used without further purification.

$^1$**H NMR** (DMSO-$d_6$): δ 8.07 and 8.02 (2 s, 2 H, H-4 and H-7), 6.37 (d, 1 H, $J$ = 5.4 Hz, H-1'), 5.6 (m, 1 H, H-2'), 5.41 (t, 1 H, $J$ = 3.4 Hz, H-3'), 4.9-4.8 (m, 1 H, H-4'), 4.35 (dd, 1 H, $J$ = 6.0 and 12.2 Hz, H-5'), 4.28 (dd, 1 H, $J$ = 3.5 and 12.1 Hz, H-5''), 2.17, 2.09 and 2.05 (3 s, 9 H, 3 $CH_3CO$).

5,6-Dichloro-2-methylamino-1-(α-L-arabinofuranosyl) benzimidazole (**5**)

Compound **4** (300 mg, 0.63 mmol) was dissolved in a 2 M solution of methylamine in methanol (12.6 mL), the flask was sealed and the reaction mixture stirred at room temperature for 18 h. The mixture was decanted and evaporated to dryness. The residue from the evaporation was dissolved in a 4:1 mixture of ethyl acetate and dichloromethane (50 mL), and washed with water (3 x 5 mL). The organic layer was dried over sodium sulfate, filtered and evaporated under reduced pressure. The residue was subjected to silica gel chromatography [2 x 15 cm, eluent: gradient of methanol (5-7.5%) in dichloromethane] to give **5** which crystallized from acetonitrile with a small amount of methanol (140 mg, 64%).

**mp**: 164-166 °C; **$^1$H NMR** (DMSO-$d_6$): δ 7.48 and 7.40 (2 s, 2 H, H-4 and H-7), 6.93 (d, 1 H, NH, $J$ = 4.4 Hz), 5.79 (d, 1 H, OH-2', $J$ = 4.9 Hz), 5.69 (d, 1 H, H-1', $J$ = 6.5 Hz), 5.62 (d, 1 H, OH-2', $J$ = 5.7 Hz), 4.88 (t, 1 H, OH-5', $J$ = 5.3 Hz), 4.21 (m, 1 H, H-2'), 4.1-4.0 (m, 1 H, H-3' and H-4'), 3.7-3.5 (m, 2 H, H-5',5''), 2.89 (d, 3 H, $CH_3$, $J$ = 4.2 Hz); **Anal.** Calcd for $C_{13}H_{15}Cl_2N_3O_4$: C, 44.84; H, 4.35; N, 12.07. Found: C, 44.56; H, 4.37; N, 11.79.

2,5,6-Trichloro-1-(α-L-arabinofuranosyl)benzimidazole (**6**):

Compound **4** (300 mg, 0.63 mmol) was dissolved in a 9:1 solution of ethanol and water (12.4 mL). To this stirred solution was added sodium carbonate (431 mg, 4.1 mmol), and the reaction mixture was stirred at room temperature for 16 h. Acetic acid (1 mL) was added and the mixture was evaporated to dryness. The residue was dissolved in a mixture of chloroform (50 mL) and ethyl acetate (25 mL) and then washed with water (50 mL). The aqueous layer was washed with chloroform (25 mL) and then ethyl acetate (25 mL). The combined organic extracts were dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure. The residue was subjected to silica gel chromatography [2 x 15 cm, eluent: gradient of methanol (5-7.5%) in chloroform] to give **6** which crystallized from a mixture of acetonitrile and methanol (130 mg, 60%).

**mp**: 173-175 °C; **$^1$H NMR** (DMSO-$d_6$): δ 8.00 and 7.96 (2 s, 2 H, H-4 and H-7), 5.91 (d, 1 H, $J$ = 6.9 Hz, H-1'), 5.85 (d, 1 H, $J$ = 5.5 Hz, OH-2'), 5.59 (d, 1 H, $J$ = 6.5 Hz, OH-3'), 4.98 (t, 1 H, $J$ = 5.3 Hz, OH-5'), 4.5-4.4 (m, 1 H, H-2'), 4.2-4.1 (m, 2 H, H-3' and H-4'), 3.7-3.5 (m, 2 H, H-5' and H-5''); **Anal.**: Calc. for $C_{12}H_{11}Cl_3N_2O_4$: C, 40.76; H, 3.14; N, 7.92. Found: C, 40.90; H, 3.16; N, 7.87.

5,6-Dichloro-2-isopropylamino-1-(α-L-arabinofuranosyl)benzimidazole (**7**)

Compound 6̲ (200 mg, 0.57 mmol) was dissolved in ethanol (5.7 mL) and isopropylamine (4 mL, 47 mmol) was added. The flask was sealed and the reaction mixture stirred at 60 °C for 2 days. The mixture was transfered to a round bottom flask and evaporated to dryness. The residue from the evaporation was dissolved in a 4:1 mixture of ethyl acetate and dichloromethane (50 mL), and washed with water (3 x 5 mL). The organic extract was dried over sodium sulfate, filtered and evaporated under reduced pressure. The residue was subjected to silica gel chromatography [2 x 15 cm, eluent: gradient of methanol (5-10 %) in dichloromethane] to give **7** as a glass (125 mg, 58%).

**mp**: 125-127 °C; **$^1$H NMR** (DMSO-$d_6$): δ 7.47 and 7.39 (2 s, 2 H, H-4 and H-7), 6.79 (d, 1 H, NH, *J* = 7.4 Hz), 5.8-5.6 (m, 3 H, H-1', OH-2' and OH-3'), 4.91 (t, 1 H, OH-5', *J* = 5.5 Hz), 4.4 (m, 1 H, H-2'), 4.1-3.9 (m, 3 H, H-3', H-4' and $C\underline{H}(CH_3)_3$), 3.7-3.5 (m, 2 H, H-5',5''), 1.19 (d, 6 H, $CH(C\underline{H}_3)_3$, *J* = 6.3 Hz); **Anal.** Calcd for $C_{15}H_{19}Cl_2N_3O_4$.1/10 $CH_2Cl_2$: C, 47.14; H, 5.03; N, 10.92. Found: C, 47.32; H, 4.99; N, 10.71.

### 2-Cyclopropylamino-5,6-dichloro-1-(α-L-arabinofuranosyl)benzimidazole (**8**)

Compound **6** (175 mg, 0.50 mmol) was dissolved in ethanol (3.0 mL) and isopropylamine (3.0 mL, 43 mmol) was added. The flask was sealed and the reaction mixture stirred at 60 °C for 2 days. The mixture was decanted and evaporated to dryness. The residue was dissolved in a 4:1 mixture of ethyl acetate and dichloromethane (50 mL), and washed with water (2 x 10 mL). The organic extract was dried over sodium sulfate, filtered and evaporated under reduced pressure. The residue was subjected to silica gel chromatography [2 x 15 cm, eluent: gradient of methanol (5-12%) in dichloromethane] to give **8** which crystallized from dichloromethane with a small amount of methanol (120 mg, 64%).

**mp**: 136-138 °C; **$^1$H NMR** (DMSO-$d_6$): δ 7.50 and 7.47 (2 s, 2 H, H-4 and H-7), 7.23 (d, 1 H, NH, *J* = 7.4 Hz), 5.77 (d, 1 H, H-1', *J* = 5.2 Hz), 5.71 (d, 1 H, OH-2', *J* = 6.4 Hz), 5.67 (d, 1 H, OH-3', *J* = 6.0 Hz), 4.89 (t, 1 H, OH-5', *J* = 5.6 Hz), 4.4 (m, 1 H, H-2'), 4.0 (m, 2 H, H-3', H-4'), 3.6 (m, 1 H, H-5'), 3.5 (m, 1 H, H-5''), 2.75 (m, 1 H, CH cyclopropyl), 0.7 and 0.55 (2 m, 4 H, 2 $CH_2$ cyclopropyl); **Anal.** Calcd for $C_{15}H_{17}Cl_2N_3O_4$.1/10 $CH_2Cl_2$: C, 47.39; H, 4.53; N, 10.98. Found: C, 47.19; H, 4.46; N, 10.80.

### 2-Cycloheptylamino-5,6-dichloro-1-(α-L-arabinofuranosyl)benzimidazole (**9**)

Compound **6** (150 mg, 0.42 mmol) was dissolved in ethanol (6.3 mL) and cycloheptylamine (4.9 mL) was added. The flask was sealed and the reaction mixture stirred at 60 °C for 2 days. The mixture was decanted and evaporated to dryness at 65 °C under high vacuum. The residue was dissolved in dichloromethane (30 mL), and washed with water (2 x 10 mL). The organic extract was dried over sodium sulfate, filtered and evaporated under reduced pressure. The residue was subjected to silica gel chromatography [2 x 15 cm, eluent: gradient of methanol (0-6%) in dichloromethane] to give **9** which crystallized from toluene with a few drops of methanol (125 mg, 69%).

**mp**: 139-141 °C; **$^1$H NMR** (DMSO-$d_6$): δ 7.47 and 7.39 (2 s, 2 H, H-4 and H-7), 6.77 (d, 1 H, NH, *J* = 7.6 Hz), 5.8-5.7 (m, 3 H, H-1', OH-2' and OH-3'), 4.90 (t, 1 H, OH-5', *J* = 5.5

Hz), 4.4 (m, 1 H, H-2'), 4.1-3.9 (m, 3 H, H-3', H-4' and $\underline{C\underline{H}}(CH_2)_n$), 3.7-3.5 (m, 2 H, H-5',5''), 2.0-1,9 (bs, 2 H, cycloheptyl), 1.7-1.4 (m, 10 H, cycloheptyl); **Anal.** Calcd for $C_{19}H_{25}Cl_2N_3O_4$.1/3 MeOH: C, 52.65; H, 6.02; N, 9.53. Found: C, 52.68; H, 5.95; N, 9.35.

## Methods

### Biological evaluation.

**Cell culture procedures**. The routine growth and passage of KB, BSC-1 and HFF cells was 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 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.

**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 (m.o.i.) of <0.01 plaque-forming units (p.f.u.) per cell as detailed previously.[25] High titer HSV-1 stocks were prepared by infecting KB cells at an m.o.i. of <0.1 also as detailed previously.[25] Virus titers were determined using monolayer cultures of HFF cells for HCMV and monolayer cultures of BSC-1 cells for HSV-1 as described earlier.[26]

**HCMV plaque reduction assay.** HFF cells in 24-well cluster dishes were infected with approximately 100 p.f.u. 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, 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.

**HSV-1 ELISA.** An ELISA developed in our laboratories was employed[27] 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 p.f.u./well were added. Following a 3-day incubation at 37 °C, medium was removed, plates were blocked, rinsed, and horse radish peroxidase conjugated rabbit anti-HSV-1 antibody was added. Following removal of the antibody containing solution, plates were rinsed, and then developed by adding 150 µl per well of 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.

**Cytotoxicity assays**. Two different assays were used: (*i*) Cytotoxicity produced in stationary HFF cells was determined by microscopic inspection of cells not affected by the virus used in plaque assays.[25] (*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.[28] 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 hours 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.

**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 concentrations ($IC_{50}$'s) were calculated from the regression lines. Samples containing positive controls (acyclovir for HSV-1, ganciclovir for HCMV, and 2-acetylpyridine thiosemicarbazone for cytotoxicity) were used in all assays.

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