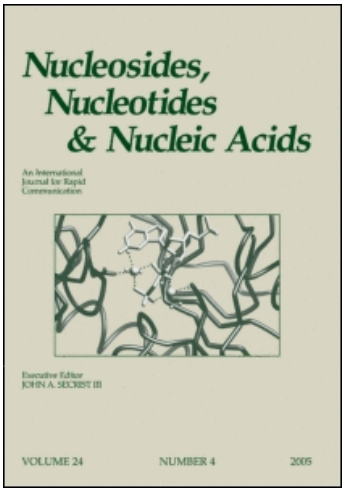


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis and Antiviral Activity of Some 2-Substituted 3-Formyl-and 3-Cyano-5,6-Dichloroindole Nucleosides

John D. Williams[a]; John C. Drach[ab]; Leroy B. Townsend[ac]
[a] Department of Medicinal Chemistry, College of Pharmacy, University of Michigan, Ann Arbor, Michigan, USA [b] Department of Biologic and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, Michigan, USA [c] Department of Chemistry, College of Literature, Sciences and the Arts, University of Michigan, Ann Arbor, Michigan, USA

To cite this Article Williams, John D. , Drach, John C. and Townsend, Leroy B.(2005) 'Synthesis and Antiviral Activity of Some 2-Substituted 3-Formyl-and 3-Cyano-5,6-Dichloroindole Nucleosides', Nucleosides, Nucleotides and Nucleic Acids, 24: 10, 1613 — 1626
To link to this Article: DOI: 10.1080/15257770500265836
URL: http://dx.doi.org/10.1080/15257770500265836

Nucleosides, Nucleotides, and Nucleic Acids, 24:1613–1626, 2005

ISSN: 1525-7770 print/1532-2335 online
DOI: 10.1080/15257770500265836

Taylor & Francis
Taylor & Francis Group

SYNTHESIS AND ANTIVIRAL ACTIVITY OF SOME 2-SUBSTITUTED 3-FORMYL- AND 3-CYANO-5,6-DICHLOROINDOLE NUCLEOSIDES

John D. Williams □ *Department of Medicinal Chemistry, College of Pharmacy, University of Michigan, Ann Arbor, Michigan, USA*

John C. Drach □ *Department of Medicinal Chemistry, College of Pharmacy, Department of Biologic and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, Michigan, USA*

Leroy B. Townsend □ *Department of Medicinal Chemistry, College of Pharmacy, Department of Chemistry, College of Literature, Sciences and the Arts, University of Michigan, Ann Arbor, Michigan, USA*

□ *A series of dichlorinated indole nucleosides has been synthesized and tested for activity against human cytomegalovirus (HCMV) and herpes simplex virus type-1 (HSV-1) and for cytotoxicity. The isopropylidene-protected analogs of the previously reported 3-formyl-2,5,6-trichloro-1-(β-D-ribofuranosyl)indole (FTCRI) and 3-cyano-2,5,6-trichloro-1-(β-D-ribofuranosyl)indole (CTCRI) were modified by nucleophilic displacement of the 2-chloro substituent using secondary amines. Deprotection of the intermediates provided 2-substituted analogs of FTCRI and CTCRI in good yield. There was a significant difference in reactivity between the isopropylidene-protected and the fully deprotected FTCRI and CTCRI with respect to nucleophilic displacement of the 2-chloro substituent using dialkylamines. This difference in reactivity was not observed with monoalkylamines or with alkoxides, and the corresponding 2-alkylamino- and 2-methoxy substituted analogs were synthesized from FTCRI and CTCRI directly. None of the synthesized analogs demonstrated potent antiviral activity without some corresponding cytotoxicity.*

Keywords Indole nucleoside; Nucleoside analog; TCRB; Antiviral; Human cytomegalovirus (HCMV)

This manuscript is dedicated to the memory of Dr. John A. Montgomery.

Received 12 October 2004; accepted 18 April 2005.

We thank Julie M. Breitenbach and Kathy Z. Borysko for expert performance of antiviral and cytotoxicity assays. These studies were supported by training grant T32-GM07767 and research grants U19-AI31718 and P01-AI46390 from the National Institutes of Health.

Address correspondence to Dr. Leroy B. Townsend, Department of Medicinal Chemistry, College of Pharmacy, University of Michigan, Ann Arbor, MI 48109-1065. Fax: 734-764-7547; E-mail: ltownsen@umich.edu

INTRODUCTION

Human cytomegalovirus (HCMV) is an opportunistic pathogen that is endemic in both industrialized and developing nations.[1] It is estimated that 50% of the American public is seropositive for HCMV.[2] Although HCMV poses little risk to healthy individuals, a variety of immunocompromised populations are susceptible to HCMV-related pathologies. AIDS patients, for example, are susceptible to retinitis and gastritis, transplant recipients are susceptible to organ rejection, and neonates are at risk for a host of birth defects and developmental disorders.[1,3]

Currently, there are five FDA-approved drugs available for the treatment of HCMV, namely ganciclovir,[4] valganciclovir,[5] cidofovir,[6] foscarnet,[7] and fomivirsen.[8] All of these compounds suffer limitations including poor bioavailability and toxicity. Furthermore, all of the licensed compounds (with the exception of fomivirsen) act upon the viral DNA polymerase, making the emergence of new drug-resistant viral strains more likely.

The search for new compounds with fewer or less severe limitations has led our laboratory to synthesize a wide range of nucleoside analogs, including 2,5,6-trichloro-1-(β-D-ribofuranosyl)benzimidazole (TCRB, **1**, Figure 1).[9] Although this compound demonstrated excellent antiviral activity and selectivity in vitro, it was degraded too rapidly in vivo to be of interest as a clinical candidate.[10] Further investigations led to the syntheses of numerous TCRB analogs with potentially stabilized glycosidic bonds including 3-formyl-2,5,6-trichloro-1-(β-D-ribofuranosyl)indole[11] (FTCRI, **2**, Figure 1) and 3-cyano-2,5,6-trichloro-1-(β-D-ribofuranosyl)indole[11] (CTCRI, **3**, Figure 1).

The antiviral activity and selectivity observed for FTCRI and CTCRI prompted us to initiate the synthesis and antiviral evaluation of additional FTCRI and CTCRI analogs in an effort to increase their antiviral activity or decrease their cytotoxicity. This study involved the reaction of FTCRI and CTCRI with a variety of nucleophiles in an effort to obtain a series of 2-substituted analogs. Evaluation of these resulting alkylamino- and ether-substituted indole nucleosides tested the structure-activity relationship at

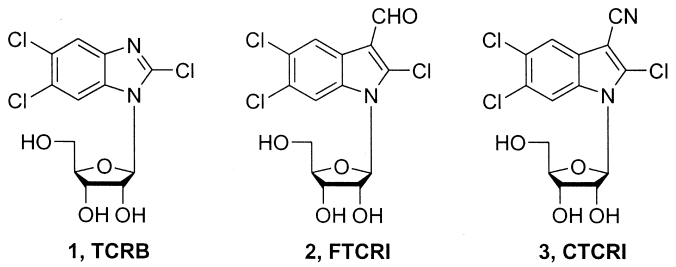


FIGURE 1 TCRB and analogous indole nucleosides.

the 2-position of the indole, and whether changes at this position would be tolerated.

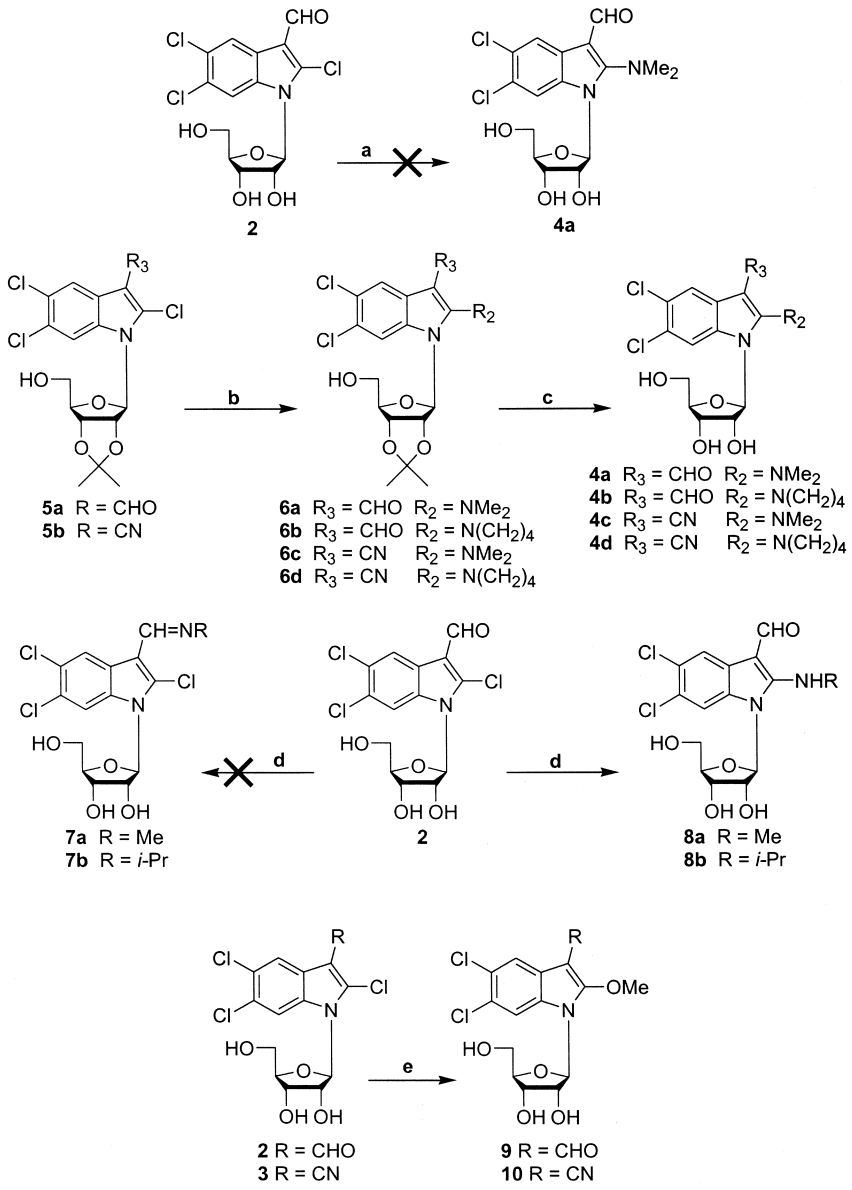
RESULTS AND DISCUSSION

Chemistry

It is well-documented that 2-chloroindoles with electron-withdrawing groups at the 3-position are susceptible to nucleophilic attack,[12,13] and this prompted us to investigate the nucleophilic displacement of the 2-chloro substituent of FTCRI with some simple dialkylamines. Our initial investigations centered on a very small-scale (<1 mg) reaction of dimethylamine with the isopropylidene-protected intermediate **5a** (prepared in a previous study[11]). This reaction was monitored by TLC, and a disappearance of starting material was observed. Having proved the feasibility of this nucleophilic displacement, we prepared a sufficient quantity of FTCRI for large-scale reactions in order to obtain additional analogs for antiviral testing.

Unexpectedly, when we reacted dimethylamine with the fully deprotected FTCRI, no reaction was observed by TLC. We increased the reaction temperature and prolonged the reaction time but still did not obtain any of the expected **4a**. However, a reaction of **5a** with dimethylamine on a 100 mg scale did provide the expected 3-formyl-2-dimethylamino-5,6-dichloro-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)indole (**6a**) in a rapid and facile reaction. The isopropylidene protecting group was removed with 90% aqueous trifluoroacetic acid to provide the corresponding fully deprotected nucleoside analog **4a** in good yield. An analogous procedure with pyrrolidine was used to synthesize 3-formyl-2-(*N*-pyrrolidino)-5,6-dichloro-1-(β-D-ribofuranosyl)indole (**4b**) via its isopropylidene-protected intermediate **6b**. The two corresponding 2-dialkylamino analogs of CTCRI were synthesized using the isopropylidene-protected analog **5b** to provide **6c** and **6d** in two steps.

This observed difference in reactivity between the deprotected nucleosides and their isopropylidene-protected congeners is rather difficult to explain. One would not expect a significant difference in the electronic properties of the heterocycle, given that the protecting group is so remote from the reaction center. Neighboring group assistance is also unlikely for essentially the same reason. The most likely explanation involves steric hindrance of the 2-chloro substituent of the indole. The rigid 5:5 ring system of the protected nucleoside must lock the indole system in a conformation which allows for effective nucleophilic displacement. The more flexible unprotected nucleoside, however, is less susceptible because the indole system is not held in the same favorable position found in the isopropylidene-protected analog.



SCHEME 1 Reagents and conditions: (**a**) $HNMe_2$, MeOH/DMF, 60°C, 24 h; (**b**) $HNMe_2$, DMF/H_2O, 20°C, 16 h *or* pyrrolidine, DMF, 20°C, 16 h; (**c**) 90% TFA, 20°C 2 min; (**d**) $MeNH_2$, 20°C, 30 min *or* *i*-$PrNH_2$, EtOH, 20°C, 16 h; (e) NaOMe, MeOH, 20°C, 30 min.

Given the difference we had previously observed in the reactivity between the isopropylidene-protected and fully deprotected nucleoside analogs, we assumed that the reaction of FTCRI (**2**, Scheme 1) with monoalkylamines would provide the imine derivatives **7a** and **7b**. However, in this instance, the 2-chloro substituent was displaced and the only isolated products were the now unexpected methylamino derivative **8a** and isopropylamino derivative **8b**. In a similar manner, the synthesis of the 2-methoxy analogs **9** and **10** was also easily achieved by the reaction of FTCRI (**2**) and CTCRI (**3**) with methanolic sodium methoxide. These results are quite surprising in light of the previous examples using dialkylamines. However, the difference in reactivity can again be explained by steric factors. Because the monoalkylamines and alkoxides are less bulky than the corresponding dialkylamines, they must be able to effectively attack even the hindered 2-chloro substituent of the fully deprotected nucleoside.

BIOLOGICAL EVALUATION

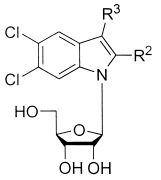
The compounds synthesized as described above were tested for antiviral activity against HCMV and HSV-1 and for cytotoxicity. Generally, all of the compounds synthesized in this study were less active against HCMV than the 2-chloro analogs **2** and **3** (see Table 1). None of the compounds demonstrated significant activity against HSV-1. The apparent activity of some compounds was probably a manifestation of cytotoxicity. For example, the 2-monoalkylamines **8a** and **8b** were particularly cytotoxic to human diploid fibroblasts. Because these modifications at the 2-position did not produce antiviral agents that were more potent or selective, further analogs were not pursued.

EXPERIMENTAL

General Procedures

All solvents were dried prior to use according to known procedures; all reagents were obtained from commercial sources or were synthesized from literature procedures, and were used without further purification unless otherwise noted. Air-sensitive reactions were performed under slight positive pressure of argon. Room temperature is assumed to be between 20–25°C. Evaporation of solvents was accomplished under reduced pressure (water aspirator, 12 mmHg), at less than 40°C, unless otherwise noted. Chromatography solvent systems are expressed in v:v ratios or as %v. Melting points were taken on a Mel-Temp apparatus, and are uncorrected. Thin layer chromatography was performed on silica gel GHLF plates from Analtech (Newark, DE). Chromatograms were visualized under UV light at 254 nm. ^{1}H NMR spectra were obtained at 500 MHz on a Bruker DRX500

TABLE 1 Antiviral Activity and Cytotoxicity of 2-Substituted Indole Nucleosides



			50% inhibitory concentration (μM)			
			Antiviral		Cytotoxicity	
No.	R^2	R^3	HCMV Plaque[a]	HSV-1 ELISA[b]	HFF Visual[c]	KB Growth[c]
4a	$-N(Me)_2$	$-CHO$	43	15	32	30
4b	$-N(CH_2)_4$	$-CHO$	7.0	15	10	10
4c	$-N(Me)_2$	$-CN$	17	>100[d]	100	90
4d	$-N(CH_2)_4$	$-CN$	15	>100	32	45
8a	$-NH(Me)$	$-CHO$	1.7	35	3.2	20
8b	$-NH(i-Pr)$	$-CHO$	3.6	35	3.2	25
9	$-OMe$	$-CHO$	4.0	20	32	55
10	$-OMe$	$-CN$	7.0	20	100	80
2[e]	$-Cl$	$-CHO$	0.23	40	45	45
3[e]	$-Cl$	$-CN$	0.55	15	32	65
TCRB[f]			2.9	102	238	210
GCV[g]			7.4	3.5	>100	>100

[a]Plaque reduction assays were performed in duplicate wells as described in the text.
[b]Compounds were assayed by ELISA in quadruplicate wells.
[c]Visual cytotoxicity was scored on HFF cells at the time of HCMV plaque enumeration in duplicate wells; inhibition of KB cell growth was determined in triplicate wells as described in the text.
[d]>100 indicates an IC_{50} greater than the highest concentration tested.
[e]Data for compounds **2** and **3** published previously as compounds **9a** and **9b**, respectively, in Williams et al.[11]
[f]Data for TCRB published previously as compound **9** in Townsend et al.[9]
[g]Averages from 108, 33, and 3 experiments, respectively, using ganciclovir (GCV).

spectrometer. ^{13}C NMR spectra were obtained at 125 MHz on a Bruker DRX500 spectrometer. Chemical shift values for ^1H determined relative to an internal tetramethylsilane standard (0.00 ppm); chemical shift values for ^{13}C were determined relative to the solvent used (39.52 ppm for DMSO-d_6 and 77.23 ppm for $CDCl_3$). Mass Spectrometry was performed at the University of Michigan Department of Chemistry Mass Spectrometry facility. Elemental Analysis was performed at the University of Michigan Chemistry Department Elemental Analysis facility.

3-Formyl-2-dimethylamino-5,6-dichloro-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)indole (6a). To a solution of 3-formyl-2,5,6-trichloro-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)indole[11] (**5a**, 100 mg, 0.24 mmol) dissolved in DMF (1 mL) was added 40% aqueous dimethylamine (1 mL). The resulting mixture was stirred at room temperature for 16 h, then the solvent was evaporated (0.5 mmHg, 40°C) to provide a pale yellow crystalline solid. The residue was suspended in water (10 mL) and brine (40 mL) and the aqueous suspension was extracted with EtOAc (2 × 25 mL). The com-

bined organic extracts were washed with brine (10 mL), dried over $MgSO_4$, filtered, and evaporated to yield a pale yellow crystalline solid. The solid was dissolved in $CHCl_3$ (1 mL) and subjected to column chromatography (40 × 350 mm) on silica gel with 1:2 hexane:EtOAc. Fractions containing product were pooled and evaporated to yield 81 mg (79%) of **6a** as a white crystalline solid: R_f 0.5 (1:2, hexane:EtOAc); mp 206–207°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.24 (s, 1H), 8.29 (s, 1H), 7.99 (s, 1H), 5.89 (d, 1H), 5.40 (t, 1H, D_2O exch.), 5.16 (m, 1H), 5.07 (m, 1H), 4.12 (m, 1H), 3.74 (s, 2H), 3.20 (s, 6H), 1.60 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 184.25, 158.96, 131.36, 126.13, 125.38, 124.85, 120.96, 114.99, 114.80, 106.38, 89.90, 83.62, 81.11, 79.53, 60.38, 45.02, 27.19, 25.35.

3-Formyl-2-(*N*-pyrrolidino)-5,6-dichloro-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)indole (6b). To a solution of 3-formyl-2,5,6-trichloro-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)indole[11] (**5a**, 100 mg, 0.24 mmol) dissolved in DMF (1 mL) was added pyrrolidine (1 mL). The resulting mixture was stirred at room temperature for 16 h, then the solvent was evaporated to provide a pale yellow oil. The residue was suspended in water (10 mL) and brine (40 mL) and extracted with EtOAc (2 × 25 mL). The combined organic extracts were washed with brine (10 mL), dried over $MgSO_4$, filtered, and evaporated to yield a pale yellow oil which solidified upon standing. The solid was dissolved in $CHCl_3$ (1 mL) and subjected to column chromatography (40 × 350 mm) on silica gel with 1:2 hexane:EtOAc. Fractions containing product were pooled and evaporated to yield 75 mg (69%) of **6b** as a white crystalline solid: R_f 0.5 (1:2, hexane:EtOAc); mp 119–120°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.04 (s, 1H), 8.30 (s, 1H), 7.86 (s, 1H), 5.88 (d, 1H), 5.33 (t, 1H, D_2O exch.), 4.99 (m, 2H), 4.05 (s, 1H), 3.62 (m, 6H), 1.99 (q, 4H), 1.51 (s, 3H), 1.28 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 183.29, 155.95, 131.88, 127.63, 125.21, 123.96, 120.48, 114.79, 114.69, 104.82, 90.66, 83.53, 80.98, 79.43, 60.28, 54.26, 27.19, 25.49, 25.33.

3-Cyano-2-dimethylamino-5,6-dichloro-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)indole (6c). 3-Cyano-2,5,6-trichloro-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)indole[11] (**5b**, 100 mg, 0.24 mmol) was dissolved in DMF (1 mL) to which was added 40% aqueous dimethylamine (1 mL). The resulting solution was stirred at room temperature for 16 h, then the solvent was removed under vacuum (0.5 mmHg, 40°C). The residue was dissolved in $CHCl_3$ (1 mL) and subjected to column chromatography (40 × 350 mm) on silica gel with 1:1 hex:EtOAc. Fractions containing product were pooled and evaporated to yield 89 mg (87%) of **6c** as a white crystalline solid: mp 172–174°C; R_f 0.6 (1:1, hex:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.66 (s, 1H), 7.58 (s, 1H), 5.91 (d, 1H), 5.09 (m, 2H), 4.14 (m, 1H), 4.07–3.91 (m, 2H), 3.10 (s, 6H), 2.10 (t, 1H, CD_3OD exch.), 1.62 (s, 3H), 1.40 (s, 3H).

^{13}C NMR (125 MHz,$CDCl_3$): δ 157.55, 130.46, 127.85, 127.02, 126.86, 119.62, 116.10, 115.65, 114.31, 90.15, 83.89, 82.10, 79.48, 75.08, 61.94, 44.35, 27.42, 25.52.

3-Cyano-2-(*N*-pyrrolidino)-5,6-dichloro-1-(2,3-di-*O*-isopropylidene-β-D-ribofuranosyl)indole (6d). 3-Cyano-2,5,6-trichloro-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)indole[11] (**5b**, 80 mg, 0.19 mmol) was dissolved in DMF (1 mL) to which was added pyrrolidine (1 mL). The resulting solution was stirred at room temperature for 16 h, then the solvent was removed under vacuum (0.5 mmHg, 40°C). The residue was dissolved in $CHCl_3$ (1 mL) and subjected to column chromatography (40 × 350 mm) on silica gel with 1:1 hex:EtOAc. Fractions containing product were pooled and evaporated to yield 73 mg (84%) of **6d** as a white crystalline solid: mp 129–130°C; R_f 0.6 (1:1, hex:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.57 (s, 1H), 7.53 (s, 1H), 5.93 (d, 1H), 5.07 (m, 2H), 4.11 (d, 1H), 4.05 (m, 1H), 3.95 (m, 1H), 3.71 (m, 2H), 3.64 (m, 2H), 2.03 (m, 4H), 1.87 (t, 1H, CD_3OD exch.), 1.60 (s, 3H), 1.36 (s, 3H). ^{13}C NMR (125 MHz,$CDCl_3$): δ 155.12, 130.59, 129.00, 127.01, 125.90, 119.03, 116.62, 116.08, 114.06, 90.74, 84.03, 82.27, 79.40, 71.82, 61.94, 53.04, 27.47, 25.84, 25.52.

3-Formyl-2-dimethylamino-5,6-dichloro-1-(β-D-ribofuranosyl)indole (4a). 3-Formyl-2-dimethylamino-5,6-dichloro-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)indole (**6a**, 74 mg, 0.17 mmol) was dissolved in 90% aqueous TFA (5 mL), and the resulting solution was stirred at room temperature for 2 min. The excess solvent was removed under vacuum, and the residual oil suspended in 5% aqueous Na_2CO_3 (20 mL) The aqueous suspension was extracted with EtOAc (2 × 25 mL) and the combined organic extracts were washed with brine (25 mL), dried over $MgSO_4$, filtered, and evaporated to yield a white solid. The crude material was dissolved in MeOH (1 mL) and subjected to column chromatography (40 × 350 mm) on C18-reverse phase silica gel with 75% MeOH/water. Fractions containing product were pooled and evaporated to yield 57 mg (85%) of **4a** as a white powder: mp 181–182°C; R_f 0.3 (10% MeOH/$CHCl_3$); ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.18 (s, 1H), 8.27 (s, 1H), 8.24 (s, 1H), 5.72 (d, 1H), 5.34 (m, 2H, D_2O exch.), 5.21 (d, 1H, D_2O exch.), 4.50 (q, 1H), 4.12 (t, 1H), 3.92 (d, 1H), 3.68 (s, 2H), 3.17 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 184.09, 159.90, 131.82, 126.27, 125.07, 124.48, 120.80, 115.46, 106.28, 88.41, 85.57, 70.33, 69.77, 61.15, 44.94. HRMS (EI) m/z calcd for $C_{16}H_{18}Cl_2N_2O_5$ 388.0593, found 388.0596. Anal calcd for $C_{16}H_{18}Cl_2N_2O_5 \bullet 1/4\ H_2O$: C, 48.81; H, 4.74; N, 7.11. Found: C, 48.69; H, 4.83; N, 6.94.

3-Formyl-2-pyrrolidino-5,6-dichloro-1-(β-D-ribofuranosyl)indole (4b). 3-Formyl-2-pyrrolidino-5,6-dichloro-1-(2,3-*O*-isopropylidene-β-D-ribofurano-

syl)indole (**6b**, 65 mg, 0.14 mmol) was dissolved in 90% aqueous TFA (5 mL), and the resulting solution was stirred at room temperature for 2 min. The excess solvent was removed under vacuum, and the residual oil suspended in 5% aqueous Na_2CO_3 (20 mL) The aqueous suspension was extracted with EtOAc (2 × 25 mL) and the combined organic extracts were washed with brine (25 mL), dried over $MgSO_4$, filtered, and evaporated to yield a white solid. The crude material was dissolved in MeOH (1 mL) and subjected to column chromatography (40 × 350 mm) on C18-reverse-phase silica gel with 75% MeOH/water. Fractions containing product were pooled and evaporated to yield 39 mg (66%) of **4b** as a white powder: mp 131–134°C; R_f 0.2 (10% MeOH/$CHCl_3$); ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.05 (s, 1H), 8.33 (s, 1H), 8.17 (s, 1H), 5.71 (d, 1H), 5.34 (m, 1H, D_2O exch.), 5.23 (d, 1H, D_2O exch.), 5.18 (d, 1H, D_2O exch.), 4.43 (q, 1H), 4.09 (m, 1H), 3.91 (m, 1H), 3.68 (d, 4H), 1.99 (m, 4H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 182.93, 156.97, 132.27, 127.85, 124.81, 123.41, 120.26, 115.04, 104.56, 89.19, 85.32, 69.77, 69.63, 61.00, 54.07, 25.52. HRMS (EI) m/z calcd for $C_{18}H_{20}Cl_2N_2O_5$ 414.0749, found 414.0733. Anal calcd for $C_{18}H_{20}Cl_2N_2O_5 \bullet 1/2\ H_2O$: C, 50.96; H, 4.99; N, 6.60. Found: C, 51.20; H, 5.21; N, 6.54.

3-Cyano-2-dimethylamino-5,6-dichloro-1-(β-D-ribofuranosyl)indole (4c). 3-Cyano-2-dimethylamino-5,6-dichloro-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)indole (**6c**, 100 mg, 0.23 mmol) was dissolved in 90% aqueous TFA (5 mL), and the resulting solution was stirred at room temperature for 2 min. The excess solvent was then removed under vacuum, and the residual oil suspended in 5% aqueous Na_2CO_3 (20 mL). The aqueous suspension was extracted with EtOAc (2 × 25 mL), and the combined organic extracts were washed with brine (25 mL), dried over $MgSO_4$, filtered, and evaporated to yield a white solid. The crude material was dissolved in MeOH (1 mL) and subjected to column chromatography (40 × 350 mm) on C18-reverse-phase silica gel with 75% MeOH/water. Fractions containing product were pooled and evaporated to yield 77 mg (85%) of **4c** as a white powder: mp 186–188°C; R_f 0.3 (10% MeOH/$CHCl_3$); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.34 (s, 1H), 7.59 (s, 1H), 5.69 (d, 1H), 5.37 (d, 1H, D_2O exch.), 5.35 (t, 1H, D_2O exch.), 5.20 (d, 1H, D_2O exch.), 4.51 (q, 1H), 4.13 (m, 1H), 3.92 (m, 1H), 3.67 (m, 2H), 3.08 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 158.43, 130.70, 127.62, 124.96, 124.62, 117.81, 115.95, 115.41, 88.13, 85.60, 72.63, 70.31, 69.73, 61.09, 43.53. HRMS (ES) m/z calcd for $C_{16}H_{17}Cl_2N_3O_4 \bullet Na \bullet MeOH$ 440.0756, found 440.0756. Anal calcd for $C_{16}H_{17}Cl_2N_3O_4 \bullet 1/2$ MeOH: C, 49.27; H, 4.76; N, 10.45. Found: C, 49.39; H, 4.37; N, 10.28.

3-Cyano-2-pyrrolidino-5,6-dichloro-1-(β-D-ribofuranosyl)indole (4d). 3-Cyano-2-pyrrolidino-5,6-dichloro-1-(2,3-*O*-isopropylidene-β-D-ribofura-

nosyl)indole (**6d**, 73 mg, 0.16 mmol) was dissolved in 90% aqueous TFA (5 mL), and the resulting solution was stirred at room temperature for 2 min. The excess solvent was then removed under vacuum, and the residual oil suspended in 5% aqueous Na_2CO_3 (20 mL). The aqueous suspension was extracted with EtOAc (2 × 25 mL), and the combined organic extracts were washed with brine (25 mL), dried over $MgSO_4$, filtered, and evaporated to yield a white solid. The crude material was dissolved in MeOH (1 mL) and subjected to column chromatography (40 × 350 mm) on C18-reverse-phase silica gel with 75% MeOH/water. Fractions containing product were pooled and evaporated to yield 53 mg (80%) of **4d** as a white powder: mp 186–188°C; R_f 0.4 (10% MeOH/$CHCl_3$); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.23 (s, 1H), 7.43 (s, 1H), 5.71 (d, 1H), 5.33 (d, 1H, D_2O exch.), 5.30 (d, 1H, D_2O exch.), 5.18 (d, 1H, D_2O exch.), 4.46 (q, 1H), 4.10 (m, 1H), 3.91 (m, 1H), 3.71–3.62 (m, 6H), 2.01–1.93 (m, 4H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 155.54, 130.83, 129.01, 124.82, 123.38, 116.72, 116.57, 115.72, 88.60, 85.45, 70.07, 69.59, 68.49, 60.99, 52.27, 25.26. HRMS (ES) m/z calcd for $C_{18}H_{19}Cl_2N_3O_4$ • Na • MeOH 466.0912, found 466.0915. Anal calcd for $C_{18}H_{19}Cl_2N_3O_4$: C, 52.44; H, 4.65; N, 10.19. Found: C, 52.13; H, 4.58; N, 9.71.

3-Formyl-2-methylamino-5,6-dichloro-1-(β-D-ribofuranosyl)indole (8a). 3-Formyl-2,5,6-trichloro-1-(β-D-ribofuranosyl)indole (**2**, 125 mg, 0.33 mmol) was dissolved in 33% ethylamine solution in ethanol (10 mL), and the resulting solution was stirred at room temperature for 30 min. The solvent was then evaporated to approx. 1 mL and diluted with EtOAc (50 mL). The suspension was washed with H_2O (20 mL) and brine (50 mL), then dried over $MgSO_4$, filtered and evaporated to yield a yellow residue. The residue was dissolved in MeOH (1 mL) and subjected to column chromatography (40 × 350 mm) on silica gel with 20% MeOH/$CHCl_3$. Fractions containing product were pooled and evaporated to yield a clear viscous residue which was dissolved in MeOH (1 mL) and subjected to column chromatography (40 × 350 mm) on C18-reverse-phase silica gel with 75% MeOH/H_2O. Fractions containing product were pooled and evaporated to yield a white solid which was recrystallized from MeOH/H_2O to yield 56 mg (43%) of **8a** as a white microcrystalline solid: mp 241–242°C; R_f 0.5 (20% MeOH/$CHCl_3$); ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.98 (s, 1H), 8.24 (s, 1H), 7.97 (q, 1H, D_2O exch.), 7.69 (s, 1H), 5.93 (t, 1H, D_2O exch.), 5.91 (d, 1H), 5.34 (d, 1H, D_2O exch.), 5.32 (d, 1H, D_2O exch.), 4.34 (q, 1H), 4.09 (t, 1H), 4.05 (s, 1H), 3.76–3.67 (m, 2H), 3.12 (d, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 181.72, 154.48, 133.66, 127.05, 123.71, 122.72, 119.70, 110.91, 100.20, 88.56, 86.10, 70.78, 70.30, 60.80, 33.45. HRMS (ES) m/z calcd for $C_{15}H_{16}Cl_2N_2O_5$ + H 375.0514, found 375.0511. Anal calcd for $C_{15}H_{16}Cl_2N_2O_5$ • 1/4 H_2O: C, 47.45; H, 4.38; N, 7.38. Found: C, 47.53; H, 4.59; N, 7.26.

3-Formyl-2-isopropylamino-5,6-dichloro-1-(β-D-ribofuranosyl)indole (8b). 3-Formyl-2,5,6-trichloro-1-(β-D-ribofuranosyl)indole (**2**, 171 mg, 0.45 mmol) was dissolved in isopropylamine (10 mL), and the resuluting solution was stirred at room temperature for 16 h. The solvent was then evaporated and the residue dissolved in EtOAc (50 mL). The suspension was washed with H_2O (20 mL) and brine (50 mL), then dried over $MgSO_4$, filtered, and evaporated to yield a yellow syrup. The residue was dissolved in MeOH (1 mL) and subjected to column chromatography (40 × 350 mm) on silica gel with 20% MeOH/$CHCl_3$. Fractions containing product were pooled and evaporated to yield a clear viscous residue which was dissolved in MeOH (1 mL) and subjected to column chromatography (40 × 350 mm) on C18-reverse phase silica gel with 75% MeOH/H_2O. Fractions containing product were pooled and evaporated to yield a white solid which was recrystallized from MeOH/H_2O to yield 76 mg (42%) of **8b** as a white microcrystalline solid: mp 143–145°C; R_f 0.6 (20% MeOH/$CHCl_3$); ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.90 (s, 1H), 8.19 (s, 1H), 7.73 (s, 1H), 7.26 (d, 1H, D_2O exch.), 5.87 (d, 1H, D_2O exch.), 5.68 (s, 1H, D_2O exch.), 5.37 (d, 1H, D_2O exch.), 5.29 (d, 1H), 4.35 (q, 1H), 4.10 (s, 1H), 4.07 (m, 1H), 4.00 (s, 1H), 3.70 (m, 2H), 1.28 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 181.55, 153.38, 133.43, 127.15, 123.96, 122.70, 119.37, 111.47, 99.04, 88.51, 85.88, 70.76, 69.80, 60.69, 48.99, 22.77, 22.55. HRMS (ES) m/z calcd for $C_{17}H_{20}Cl_2N_2O_5$ • Na • MeOH 457.0909, found 457.0912. Anal calcd for $C_{17}H_{20}Cl_2N_2O_5$ • 1/4 H_2O: C, 49.53; H, 5.13; N, 6.80. Found: C, 49.83; H, 5.04; N, 6.51.

3-Formyl-2-methoxy-5,6-dichloro-1-(β-D-ribofuranosyl)indole (9). 3-Formyl-2,5,6-trichloro-1-(β-D-ribofuranosyl)indole[11] (**2**, 100 mg, 0.26 mmol) was dissolved in dry MeOH (10 mL) to which was added sodium methoxide (20 mg, 0.37 mmol). The solution was stirred at room temperature for 30 min., then the solvent was removed under vacuum. The residue was suspended in 10% aqueous $NaHCO_3$, and the suspension extracted with EtOAc (2 × 50 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and evaporated to yield a white solid. The solid was dissolved in MeOH and subjected to column chromatography (40 × 350 mm) on C18-reverse-phase silica gel with 75% MeOH/H_2O. Fractions containing product were pooled and evaporated to yield 50 mg (51%) of **9** as a white crystalline solid: mp 198–199°C; R_f 0.2 (10% MeOH/$CHCl_3$); ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.21 (s, 1H), 8.34 (s, 1H), 8.31 (s, 1H), 5.84 (d, 1H), 5.41 (d, 1H, D_2O exch.), 5.30 (t, 1H, D_2O exch.), 5.23 (d, 1H, D_2O exch.), 4.41 (s, 4H), 4.13 (s, 1H), 3.95 (d, 1H), 3.69 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 183.28, 160.04, 129.51, 125.21, 124.83, 124.68, 121.12, 114.73, 100.30, 86.81, 85.93, 71.06, 69.75, 64.78, 61.18. HRMS (EI) m/z calcd for $C_{15}H_{15}Cl_2NO_6$ 375.0276, found 375.0278. Anal calcd for $C_{15}H_{15}Cl_2NO_6$: C, 47.89; H, 4.02; N, 3.72. Found: C, 47.73; H, 4.12; N, 3.72.

3-Cyano-2-methoxy-5,6-dichloro-1-(β-D-ribofuranosyl)indole (10). 3-Cyano-2,5,6-trichloro-1-(β-D-ribofuranosyl)indole (**3**, 100 mg, 0.27 mmol) was dissolved in dry MeOH (10 mL) to which was added sodium methoxide (100 mg, 1.9 mmol). The resulting solution was heated at reflux temperature for 2 h, then cooled to room temperature and the solvent evaporated. The residual solid was recrystallized from MeOH/H_2O to yield 68 mg (63%) of **10** as a white powder: mp 245–246°C; R_f 0.2 (10% MeOH/$CHCl_3$); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.36 (s, 1H), 7.65 (s, 1H), 5.80 (d, 1H), 5.37 (d, 1H, D_2O exch.), 5.28 (t, 1H, D_2O exch.), 5.20 (d, 1H, D_2O exch.), 4.39–4.37 (m, 4H), 4.10 (m, 1H), 3.93 (d, 1H), 3.66 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 158.44, 128.52, 126.05, 125.01, 124.81, 118.21, 115.02, 114.47, 86.89, 85.86, 71.06, 69.67, 65.16, 61.10, 60.81. HRMS (EI) m/z calcd for $C_{15}H_{14}Cl_2N_2O_5$ 372.0280, found 372.0265. Anal calcd for $C_{15}H_{14}Cl_2N_2O_5$: C, 48.28; H, 3.78; N, 7.51. Found: C, 47.97; H, 3.75; N, 7.34.

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.[14]

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.[15] High titer HSV-1 stocks were prepared by infecting KB cells at an m.o.i. of <0.1 also as detailed previously.[15] 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.[16] Briefly, HFF or BSC-1 cells were planted as described above in 96-well cluster dishes and incubated overnight at 37°C. The next day cultures were inoculated with HCMV or HSV-1 and serially diluted 1:3 across the remaining 11 columns of the 96-well plate. After virus adsorption the inoculum was replaced with fresh medium and cultures were incubated for seven days for HCMV, two or three days for HSV-1. Plaques were enumerated under 20-fold magnification in wells having the dilution which gave 5 to 20 plaques per well. Virus titers were calculated according

to the following formula: Titer (p.f.u./mL) = number of plaques $\times$ 5 $\times$ 3^n; where n represents the nth dilution of the virus used to infect the well in which plaques were enumerated.

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, the compounds, prepared as 10 mg/mL stock solutions in DMSO were diluted with growth medium and were added to duplicate wells in four to eight selected concentrations. After incubation at 37°C for 7–10 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 was employed[17] 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 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 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 for routine cytotoxicity testing. (a) Cytotoxicity produced in stationary HFF cells was determined by microscopic inspection of cells not affected by the virus used in plaque assays.[15] (b) 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.[18] 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.

Data Analysis. Dose response relationships were used to quantitate drug effects by linear regression of the percent inhibition of parameters derived

in the preceding assays against $\log_{10}$ drug concentrations. Fifty percent inhibitory concentrations (IC_{50}'s) were calculated from the linear portions of the regression lines. Samples containing positive controls (acyclovir for HSV-1, GCV for HCMV, and 2-acetylpyridine thiosemicarbazone for cytotoxicity) were used in all assays.

REFERENCES

1. Pass, R.F. Chapter 77: Cytomegalovirus. In *Fields Virology*, 4th Ed.; Knipe, D.M., Howley, P.M., Eds.; Lippincott, Williams & Wilkins: Philadephia, 2001; 2675–2705.
2. Alford, C.A.; Britt, W.J. Cytomegalovirus. In *The Human Herpesviruses*; Roizman, B., Whitley, R.J., Lopez, C., Eds.; Raven Press: New York, 1993; 227–256.
3. Britt, W.J. Infections associated with human cytomegalovirus. In *Herpesvirus Infections*; Glaser, R., Jones, J.F., Eds.; Marcel Dekker, Inc.: New York, 1994; 59–116.
4. Faulds, D.; Heel, R.C. Ganciclovir: A review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy in cytomegalovirus infections. Drugs **1990**, 39, 597–638.
5. Curran, M.; Noble, S. Valganciclovir. Drugs **2001**, 61, 1145–1150.
6. Lea, A.P.; Bryson, H.M. Cidofovir. Drugs **1996**, 52, 225–230.
7. Wagstaff, A.J.; Bryson, H.M. Foscarnet: A reappraisal of its antiviral activity, pharmacokinetic properties, and therapeutic use in immunocompromised patients with viral infections. Drugs **1994**, 48, 199–226.
8. Perry, C.M.; Barman-Balfour, J.A. Fomivirsen. Drugs **1999**, 57, 375–380.
9. Townsend, L.B.; Devivar, R.V.; Turk, S.R.; Nassiri, M.R.; Drach, J.C. Design, synthesis, and antiviral activity of certain 2,5,6-trihalo-1-(β-D-ribofuranosyl)benzimidazoles. J. Med. Chem. **1995**, 38, 4098–4105.
10. Good, S.S.; Owens, B.S.; Townsend, L.B.; Drach, J.C. The disposition in rats and monkeys of 2-bromo-5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole (BDCRB) and its 2,5,6-trichloro congener. Antiviral Res. **1994**, 23(S), 103.
11. Williams, J.D.; Chen, J.J.; Drach, J.C.; Townsend, L.B. Synthesis and antiviral activity of some 3-substituted 2,5,6-trichloroindole nucleosides. J. Med. Chem. **2004**, 47, 5753–5765.
12. Comber, M.F.; Moody, C.J. 2-Chloro-1-methoxymethylindole-3-carboxaldehyde: Introduction of nucleophiles into the indole 2-position and an approach to the unusual TrpHis fragment of moroidin. Synthesis **1992**, 731–733.
13. Monge, A.; Palop, J.; Ramirez, C.; Font, M.; Fernandez-Alvarez, E. New 5*H*-1,2,4-triazino[5,6-*b*]indole and aminoindole derivatives. Synthesis and studies as inhibitors of blood platelet aggregation, anti-hypertensive agents and thromboxane synthetase inhibitors. Eur. J. Med. Chem. **1991**, 26, 179–188.
14. Shipman, C., Jr.; Smith, S.H.; Carlson, R.H.; Drach, J.C. Antiviral activity of arabinofuranosyladenosine and arabinofuranosylhypoxanthine in herpes simplex virus-infected KB cells. Antimicrob. Agents Chemother. **1976**, 9, 120–127.
15. Turk, S.R.; Shipman, C., Jr.; Nassiri, M.R.; Genzlinger, G.; Krawczyk, S.H.; Townsend, L.B.; Drach, J.C. Pyrrolo[2,3-*d*]pyrimidine nucleosides as inhibitors of human cytomegalovirus. Antimicrob. Agents Chemother. **1987**, 31, 544–550.
16. Prichard, M.N.; Turk, S.R.; Coleman, L.A.; Engelhardt, S.L.; Shipman, C., Jr.; Drach, J.C. A microtiter virus yield reduction assay for the evaluation of antiviral compounds. J. Virol. Methods **1990**, 28, 101–106.
17. Prichard, M.N.; Shipman, C., Jr. A three dimensional model to analyze drug-drug interactions. Antiviral Res. **1990**, 14, 181–206.
18. Prichard, M.N.; Prichard, L.E.; Baguley, W.A.; Nassiri, M.R.; Shipman, C., Jr. Three-dimensional analysis of synergistic cytotoxicity of ganciclovir and zidovudine. Antiviral Res. **1991**, 35, 1060–1065.