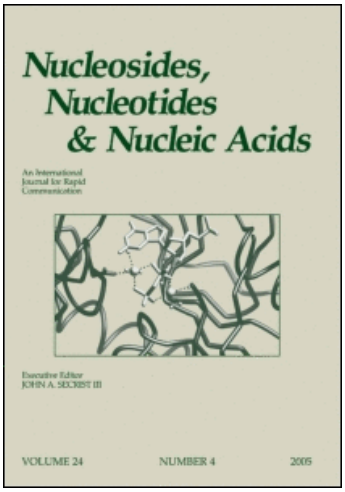

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Synthesis and Biological Activity of 4-Amino-1-(β-D-ribofuranosyl)imidazo[4,5-d]pyridazin-7-one

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 4-AMINO-1-(ß-D-RIBOFURANOSYL)IMIDAZO[4,5-*d*]PYRIDAZIN-7-ONE

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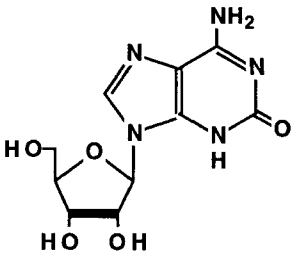
Abstract. The Lewis acid catalyzed ribosylation of 5(4)-cyano-4(5)-(5-methyl-1,2,4-oxadiazol-3-yl)-1H-imidazole (**2**) with 1-O-acetyl-2,3,5-tri-O-benzoyl-ß-D-ribose gave only 4-(5-methyl-1,2,4-oxadiazol-3-yl)-1-(2,3,5-tri-O-benzoyl-ß-D-ribofuranosyl)imidazole-5-carbonitrile (**3**). Treatment of **3** with methanolic ammonia gave 4-(5-methyl-1,2,4-oxadiazol-3-yl)-1-(ß-D-ribofuranosyl)imidazole-5-carbonitrile (**4**). Treatment of **4** with hydrogen peroxide in ammonia gave 4-(5-methyl-1,2,4-oxadiazol-3-yl)-1-(ß-D-ribofuranosyl)imidazole-5-carboxamide (**5**). When **5** was treated with sodium hydride in dimethyl-sulfoxide a rearrangement (mononuclear heterocyclic rearrangement, m.h.r.) occurred to give a modest 17% yield of 4-acetamido-1-(ß-D ribofuranosyl)imidazo[4,5-*d*]pyridazin-7-one (**6**). Treatment of **6** with aqueous ammonia gave 4-amino-1-(ß-D-ribofuranosyl)imidazo[4,5-*d*]pyridazin-7-one (**1**). The synthesis of compound **1** using the m.h.r. for the preparation of a single regioisomer of the imidazo[4,5-*d*]pyridazin-7-one ring system, has demonstrated the potential of this methodology. Neither compound **5** nor **6** affected the growth or replication of human foreskin fibroblasts (HFF cells) or human cytomegalovirus (HCMV). In contrast, compound **1** inhibited the replication of HCMV (IC_{50}=29 µM) but produced visual cytotoxicity in uninfected HFF cells (IC_{50}=70µM). Compound **1** also inhibited the proliferation of L1210 murine leukemic cells (IC_{50}=25µM), whereas the precursors **4** and **6** did not.

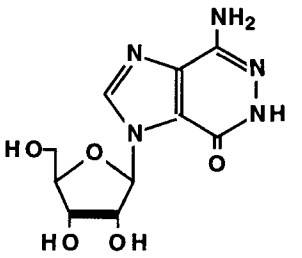
INTRODUCTION

The biological activity associated with the naturally occurring nucleosides isoguanosine (crotonoside) and 1-methylisoguanosine has been well documented. Isoguanosine inhibits the inducible binding sites for cytidine uptake in *E. coli* [1] and elicits a significant accumulation of cellular cAMP levels in slices of guinea pig cerebral cortex.[2] The methyl analog, 1-methylisoguanosine, has displayed muscle relaxant and antiinflammatory properties.[3] We have been recently involved in studies to afford

azapentalene analogs of adenosine utilizing the mononuclear heterocyclic rearrangement[4] (m.h.r.) of an appropriately substituted heterocycle. We now report on our use of this methodology for the preparation of the isoguanosine analog in the



Isoguanosine



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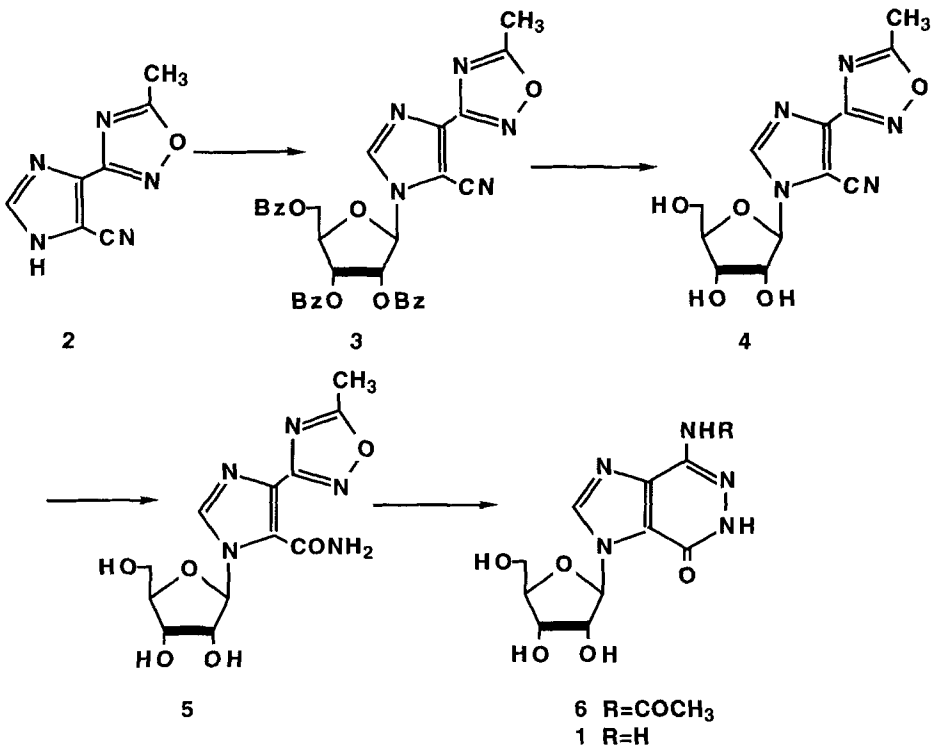
imidazo[4,5-*d*]pyridazine ring system, 4-amino-1-(ß-D-ribofuranosyl)imidazo[4,5-*d*]-pyridazin-7-one (**1**).

As part of our ongoing program to discover potential anticancer and antiviral drugs, pyrrolo[2,3-*d*]pyrimidine nucleoside analogs related to adenosine have been prepared which have significant antiproliferative activity or activity against herpesviruses such as herpes simplex virus type 1 (HSV-1) and human cytomegalovirus (HCMV).[5,6,7,8] We also have been interested in the antiproliferative effects and antiviral activity of 4-amino-pyrrolo[2,3-*d*]pyridazin-7-one nucleosides[9,10] which are closely related to the imidazo[4,5-*d*]pyridazin-7-ones described herein. Consequently, we have evaluated the target compound **1** and selected precursors for antiproliferative and antiviral activity.

RESULTS AND DISCUSSION

Chemistry. The synthesis (Scheme I) of the title compound **1** begins with the Lewis acid catalyzed ribosylation[11,12] of persilylated 4(5)-(5-methyl-1,2,4-oxadiazol-3-yl)-1H-imidazole-5(4)-carbonitrile (**2**)[13] with 1-O-acetyl-2,3,5-tri-O-benzoyl-ß-D-ribose to give 4-(5-methyl-1,2,4-oxadiazol-3-yl)-1-(2,3,5-tri-O-benzoyl-ß-D-



Scheme I

ribofuranosyl)imidazole-5-carbonitrile (**3**). The site of ribosylation was assumed to be the imidazole nitrogen adjacent to the cyano group.

This assignment was based initially on steric considerations. The actual position of ribosylation was subsequently determined at a later stage in the synthesis via a comparison of the chemical shift of the anomeric proton of **1** with the anomeric protons of structurally similar imidazo[4,5-*d*]pyridazine nucleosides.[14] Treatment of **3** with methanolic ammonia gave 4-(5-methyl-1,2,4-oxadiazol-3-yl)-1-(ß-D-ribofuranosyl)imidazole-5-carbonitrile (**4**) in 80% yield. A conversion of the 5-nitrile group in **3** to a carboxamide group was accomplished with hydrogen peroxide in aqueous ammonia to give 4-(5-methyl-1,2,4-

oxadiazol-3-yl)-1-(ß-D-ribofuranosyl)imidazole-5-carboxamide (**5**). When **5** was heated at 100 °C in dimethyl sulfoxide in the presence of sodium hydride, a mononuclear heterocyclic rearrangement (m.h.r.) occurred to give a modest 17% yield of 4-acetamido-1-(ß-D-ribofuranosyl)imidazo[4,5-*d*]pyridazin-7-one (**6**).

The ^{1}H NMR spectrum of **5** revealed a typical pattern for the carboxamide group, two individual peaks for the non-equivalent protons at δ 7.8 and 8.2. The methyl group at the 5-position of the oxadiazole ring is a singlet located at δ 2.57. The product of the rearrangement **6** has a very different ^{1}H NMR spectrum. The methyl group singlet is now found much further upfield (δ 2.05) than the chemical shift of δ 2.57 observed in the spectrum of **5**. This upfield chemical shift is indicative of an acetyl group attached to an exocyclic amine. There are two singlets found downfield at δ 12.62 and 10.12, respectively, corresponding to the newly formed 4-acetamido group and the N_6H of the pyridazine ring. The anomeric proton is a doublet ($J_{1'2'}$ = 4.86 Hz) centered at δ 6.38. The chemical shift of δ 6.38 for the anomeric proton is consistent solely with a structure where the keto functionality is adjacent, ie., in the 7-position of the imidazo[4,5-*d*]pyridazine ring system. Otter and coworkers synthesized and reported[14] the ^{1}H NMR data for the 7-one, 4-one, 7-amino and 4-amino derivatives of 1-(ß-D-ribofuranosyl)imidazo[4,5-*d*]pyridazine. Only the anomeric proton for the 7-one was found at δ 6.38 providing further proof for our initial structural assignment for **3**. The chemical shift for the anomeric proton for the other three compounds were in the range of δ 5.9 to 5.99.

The acetyl group was removed from the 4-amino group by treating **6** with concentrated aqueous ammonia at 75 °C for 24 h. The product (**1**) was isolated from the reaction mixture and recrystallized from water.

The ^{1}H NMR spectrum for **1** displayed sharp singlets located at δ 11.6 and 8.63, which were attributed to the N_6H and C_2H protons, respectively. The amino group was assigned to a singlet located at δ 5.81. The chemical shift (δ 6.37) for the anomeric proton remains essentially unchanged from that observed for **6**. The mass spectrum (FAB+)

displayed as a base peak a m/z of 284. This mass would be consistent with a protonated form of **1**. A prominent peak was also observed at a m/z of 152 corresponding to the molecular weight of the protonated heterocycle *via* the loss of the sugar moiety.

Biological Activity. The target adenosine analog **1** inhibited proliferation of L1210 murine leukemic cells with an IC_{50} of 25 μM. Neither the imidazole precursor **4** nor the 4-N-acetylated precursor **6** inhibited cellular proliferation in this system. Compounds **5** and **6**, along with **1**, were also evaluated against HCMV and for cytotoxicity in human foreskin fibroblasts (HFF cells). Compounds **5** and **6** were neither cytotoxic to HFF cells nor significant inhibition of the growth (plaque reduction assay) of HCMV at the highest concentration tested, 100 μM. However, the 50% inhibitory concentration (IC_{50}) for **1** against HCMV was 29 μM. This compound also exhibited some cytotoxicity against uninfected HFF cells (IC_{50}=70μM) thereby establishing that activity against HCMV was only partially separated from cell toxicity. In contrast, the activity of ganciclovir against HCMV (IC_{50}=8μM) was well separated from visual cytotoxicity (IC_{50}>100μM).

EXPERIMENTAL SECTION

Proton magnetic resonance (^{1}H NMR) spectra were obtained with a Bruker WP270SY spectrometer (solutions in dimethyl-d_6 sulfoxide or deuteriochloroform with tetramethylsilane as internal standard), with chemical shift values reported in δ, parts per million, relative to the internal standard. Infrared spectra were recorded on a Nicolet 5DXB Ft-IR spectrophotometer. Melting points were determined with a Thomas-Hoover capillary apparatus and are uncorrected. Thin layer chromatography was run on glass plates coated (0.25 mm) with silica gel. Compounds of interest were detected by either ultraviolet lamp (254 nm), iodine vapors, or treatment with sulfuric acid followed by heating. Evaporations were performed at 40 °C under reduced pressure with a rotary evaporator. Elemental analysis were performed by M-H-W Laboratories, Phoenix, AZ.

4-(5-Methyl-1,2,4-oxadiazol-3-yl)-1-(2,3,5-tri-O-benzoyl-ß-D-ribofuranosyl)imidazole-5-carbonitrile (3). A mixture of 4(5)-(5-methyl-1,2,4-oxadiazol-3-yl)imidazole-5(4)-carbonitrile[13] (**2**, 10.0 g, 57 mmol) and bis(trimethylsilyl)trifluoroacetamide (BSTFA) (16 mL, 60 mmol) in acetonitrile (350 mL) was heated at reflux under an atmosphere of argon. After 2 h, the solution was cooled to room temperature with an ice bath and 1-O-acetyl-2,3,5,-tri-O-benzoyl-ß-D-ribofuranose (29 g, 57.5 mmol) was added in one portion. Immediately following dissolution of the added sugar, stannic chloride (10 mL, 85.5 mmol, 1.5 equivalents) was added and the slightly warm solution was stirred. After 3 h, the clear yellow solution was concentrated *in vacuo* (14 torr, 40 °C) to give a viscous residue. Ethyl acetate (300 mL) was added to the residue followed by the addition of saturated aqueous sodium bicarbonate (100 mL). This mixture was stirred vigorously for 15 minutes and then filtered through a bed of Celite. The filter bed was washed with ethyl acetate (100 mL), and the washing was added to the original filtrate. The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo* to afford **3** as a hard foam (38.8 g, 100%): IR (KBr) 2235, 1729, 1602, 1581, 1490, 1455, 1363 and 1265 cm-1; ^1H NMR (Me_2SO-d_6) δ 8.63 (s,1H, C_2H), 7.97-7.89 (m, 6H, Ar's), 7.67-7.60 (m, 3H, Ar's), 7.49-7.41 (m, 6H, Ar's), 6.57 (d, 1H, 1'-H, $J_{1,2}$= 5.18 Hz), 6.12-6.08 (t, 1H, 2'-H), 5.99-5.95 (t, 1H, 3' or 4'-H), 4.95-4.93 (dd, 1H, 3' or 4'-H), 4.76-4.71 (m, 2H, 5'-CH_2), 2.66 (s, 3H, CH_3). *Anal.* Calcd for $C_{33}H_{25}N_5O_8 \cdot 0.5\ H_2O$: C, 63.05; H, 4.17; N, 11.14. Found: C, 63.21; H, 4.21; N, 10.90.

4-(5-Methyl-1,2,4-oxadiazol-3-yl)-1-(ß-D-ribofuranosyl)imidazole-5-carbonitrile (4). Ammonia (g) was bubbled into a room temperature solution of **3** (38.8 g, 57 mmol) in methanol (500 mL). After 1 h the flow of ammonia was ceased and the solution allowed to stir overnight. After 13 h, the solution was concentrated *in vacuo* (14 torr, 40 °C) to 125 mL at which time crystallization of the product occurred. The mixture containing product was cooled in an ice bath and the white solid collected by filtration. The product was washed successively with acetone and ethyl ether and then

dried (100 °C, 0.5 torr) for 8 h to afford **4** (14.2 g, 81%, mp 201-203.5 °C): IR (KBr) 3466, 3241, 3114, 2592-2917, 2235, 1574, 1490, 1272, 1096, 1061, 906, 814, 758 and 639 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.67 (s, 3H, CH_3), 3.52-3.68 (m, 2H, 5'-H's), 3.95-4.0 (dd, 1H, 3' or 4'-H), 4.05-4.12 (dd, 1H, 3' or 4'-H), 4.35-4.4 (dd, 1H, 2'-H), 5.06 (t, 1H, 5'-OH), 5.33 (d, 1H, 2' or 3'-OH), 5.68-5.73 (m, 2H, 1'-H and 2' or 3'-OH), 8.53 (s, 1H, C_2H). *Anal.* Calcd for $C_{12}H_{13}N_5O_5$: C, 46.90; H, 4.26; N, 22.79. Found: C, 47.17; H, 4.31; N, 23.00.

4-(5-Methyl-1,2,4-oxadiazol-3-yl)-1-(ß-D-ribofuranosyl)imidazole-5-carboxamide (5). Compound **4** (12 g, 39 mmol) was added to a mixture of 6 *N* aqueous ammonia (250 mL) and 30% aqueous hydrogen peroxide (250 mL) and the mixture stirred for 1.5 h. The thick white mixture was filtered to give a white solid paste which was washed successively with cold water, acetone and ethyl ether. The product was dried (100 °C, 0.5 torr) for 48 h to give **5** (10.09 g, 79%, mp 178-181 °C); IR (KBr) 3493-3072, 1669, 1622, 1568, 1457, 1229, 984, 912, 896 and 832 cm^{-1}; ^1H NMR (Me_2SO-*d* 6) δ 2.61 (s, 3H, CH3), 3.56-3.59 (m, 1H, 5'-H), 3.65-3.68 (m, 1H, 5'-H), 3.87-3.89 (m, 1H, 4'-H), 4.04-4.08 (m, 1H, 2' or 3'-H), 4.13-4.18 (m, 1H, 2' or 3'-H), 5.06-5.10 (m, 2H, OH's), 5.41 (d, 1H, OH), 5.98 (d, 1H, 1'-H, $J_{1,2}$= 3.9 Hz), 7.82 (s, 1H, NH), 8.24 (s, 1H, NH), 8.35 (s, 1H, C_2H). *Anal.* Calcd for $C_{12}H_{15}N_5O_6$: C, 44.30; H, 4.65; N, 21.51. Found: C, 44.10; H, 4.55; N, 21.33.

4-Acetamido-1-(ß-D-ribofuranosyl)imidazo[4,5-*d*]pyridazin-7-one (6). A mixture of sodium hydride (2.7 g of 50% sodium hydride as a mineral oil suspension, 56 mmol) was warmed in dimethyl sulfoxide (400 mL) at 75 °C for 30 minutes. Compound **5** (9.0 g, 27.7 mmol) was added, and the mixture heated at 100 °C for 45 minutes. The reaction mixture was then cooled to room temperature and acetic acid (3.1 mL) added. Concentration (0.5 torr, 50 °C) of the reaction mixture afforded a rose colored residue which was dissolved in methanol (50 mL) and this solution was placed on the top of a short silica gel column (10 x 10 cm, 200 g 70-230 mesh, chloroform/methanol 1:1) and the product fractions combined and concentrated to give a semi-solid residue. The

solid was dissolved in warm methanol (75 mL) and upon cooling this solution a light pink solid precipitated. The product was dried (0.5 torr, 100 °C) for 18 h to give **6** (1.6 g, 17%, mp 225-229 °C): IR (KBr)3552-2800, 3416, 16666, 1568, 1504, 1418, 1253 and 1136 cm^{-1}; ^1H NMR (Me_2SO-*d* 6) δ 2.05 (s, 3H, $COCH_3$), 3.57-3.58 (m, 1H, 5'-H), 3.65-3.67 (m, 1H, 5'-H), 3.94 (m, 1H, 3' or 4'-H), 4.11 (m, 1H, 3' or 4'-H), 4.38 (dd, 2'-H), 5.11 (t, 1H, 5'-$CH_2O\underline{H}$), 5.19 (d, 1H, 3'-OH), 5.53 (d, 1H, 2'-OH), 6.38 (d,1H, 1'-H), 8.73 (s, 1H, C2H), 10.12 ($N\underline{H}COCH_3$), 12.62 (s, 1H, N_6H); mass spectrum FAB^+ (EI) m/z 326 (M+1, 55% of base peak), FAB^- (EI) m/z 324 (M-1, 41% of base peak). *Anal.* Calcd for $C_{12}H_{15}N_5O_6$: C, 44.30; H, 4.65; N, 21.51. Found: C, 43.96; H, 4.81; N, 21.15.

4-Amino-1-(ß-D-ribofuranosyl)imidazo[4,5-*d*]pyridazin-7-one (1). A solution of **6** (500 mg, 1.54 mmol) in concentrated aqueous ammonium hydroxide (50 mL) was heated at 75 °C for 24 h. The solution was cooled (ice bath) and the precipitate collected by filtration. The moist solid was dissolved in hot water (5 mL) and the solution allowed to cool. The product crystallized and was collected by filtration and then successively washed with acetone and ethyl ether. The product was dried (0.5 torr, 100 °C) for 24 h to give **1** (317 mg, 70%, mp): IR (KBr) 3600-2800, 1673, 1630 and 1569 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.56-3.59 (m, 1H, 5'-H), 3.64-3.67 (m,1H, 5'-H), 3.93 (dd, 1H, 3' or 4'-H), 4.10 (dd, 1H, 3' or 4'-H), 4.37 (dd, 1H, 2'-H), 5.10-5.15 (m, 2H, 5'-OH and 2' or 3'-OH), 5.48 (d, 1H, 2' or 3'-OH), 5.81 (s, 2H, NH_2), 6.37 (d, 1H, 1'-H, $J_{1,2}$ 5.4 Hz), 8.63 (s, 1H, C_2H), 11.61 (s, 1H, N_6H). Mass spectrum FAB^+ (EI) m/z 284 (M+1, base pk), 152 ((M+1)-sugar, 92% of base pk). *Anal.* Calcd for $C_{10}H_{13}N_5O_5 \cdot 0.5\ H_20$: C, 41.09; H, 4.83; N, 23.97. Found: C, 41.27; H, 5.03; N, 24.12.

In Vitro Antiproliferative Studies. The *in vitro* cytotoxicity against L1210 was evaluated as described previously.[15] L1210 cells were grown in static suspension culture using Fischer's medium for leukemic cells of mice with 10% heat-inactivated (56 °C, 30 min) horse serum. The growth rate was calculated from determinations of cell number

twice daily for 96 h, in the presence of various concentrations of the test compound. Growth rate was defined as the slope of the semilogarithmic plot of cell number against time for the treated culture as a percent of the slope for the control culture. This parameter was determined experimentally by calculating the ratio of the population doubling time (T_d) of control cells to the T_d of treated cells. When the growth rate decreased during the experiment, the rate used was the final rate attained. The IC_{50} was defined as the concentration required to decrease the growth rate of 50% of the control.

***In Vitro* Antiviral Evaluation.** (a) **Cells and Viruses.** Diploid human foreskin fibroblasts (HFF cells) were grown in minimal essential medium (MEM) with Earle's salts [MEM(E)] supplemented with 10% fetal bovine serum. Cells were passaged according to conventional procedures as detailed previously.[5] A plaque-purified isolate, P_O, of the Towne strain of HCMV was used and was a gift of Dr. M. F. Stinski, University of Iowa.

(b) **Assays for Antiviral Activity.** HCMV plaque reduction experiments were performed using monolayer cultures of HFF cells by a procedure similar to that described previously[5], with the exceptions that the virus inoculum (0.2 mL) contained approximately 50-100 plaque forming units (pfu) of HCMV and the compounds to be assayed were dissolved in the overlay medium.

(c) **Cytotoxicity Assays.** Cytotoxicity produced in HFF cells was estimated by visual scoring of cells not affected by virus infection in the plaque reduction assays described above. Drug-induced cytopathology was estimated at 30-fold magnification and scored on a zero to four plus basis on the day of staining for plaque enumeration.[5]

(d) **Data Analysis.** Dose-response relationships were constructed by linearly regressing the percent inhibition of parameters derived in the preceding sections against $\log_{10}$ of drug concentration. Fifty-percent inhibitory (IC_{50}) concentrations were calculated from the regression lines. Samples containing positive controls (ganciclovir for HCMV)

were used in all assays. Results from sets of assays were rejected if inhibition by the positive control deviated from its mean response by more than 1.5 standard deviations.

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