



Published in final edited form as:

Antivir Chem Chemother. ; 20(1): 37–46. doi:10.3851/IMP782.

L-Valine Ester of Cyclopropavir - a New Antiviral Prodrug

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Abstract

The L-Valine ester of antiviral agent cyclopropavir, valcyclopropavir (**6**), was synthesized and evaluated for antiviral properties. Prodrug (**6**) inhibited replication of HCMV virus (Towne and AD169 strain) in HFF cells to approximately the same extent as the parent drug cyclopropavir (**5**). Stability of **6** toward hydrolysis at pH 7.0 roughly corresponds to that of valganciclovir (**2**). Pharmacokinetic studies in mice established that the oral bioavailability of valcyclopropavir (**6**) was 95%.

Introduction

Efficacy of antiviral nucleoside analogues is often hampered by their low solubility and bioavailability. To address this deficiency, several types of prodrugs have been synthesized [1]. Amino acid esters were found especially advantageous. Thus, valine ester prodrugs of acyclovir (**1**, valacyclovir, Valtrex, Chart 1) [2–4] and ganciclovir (**2**, valganciclovir, Valcyte) [5,6] are already part of our armament for fighting infections caused by herpes virus 1 and 2 (HSV-1 and HSV-2), varicella zoster virus (VZV) and human cytomegalovirus (HCMV). Additional advantage of these prodrugs resides in their ability to facilitate the membrane transport by using peptide transporters [6,7] PEPT1 and PEPT2. L-Valine ester prodrugs are readily hydrolyzed by chemical or esterase-catalyzed hydrolysis and/or a biphenyl hydrolase-like protein (BPHL, valaciclovirase) [8]. More recently, this approach has been extended to nucleoside analogue 2'-deoxy-L-cytidine (L-dC) [9] effective against hepatitis B (HBV) and 2'-C-methylcytidine [10] which is an anti-hepatitis C (HCV) agent. Both L-valine esters, Val-L-dC (**3**, valtorcitabine) [11] and valopicitabine (**4**) [12] have improved pharmacokinetic profiles over the parent analogues.

In our laboratory, we have developed a new class of antiviral agents -methylenecyclopropane analogues of nucleosides [13]. Among these analogues, cyclopropavir (**5**) exhibits antiviral parameters superior to ganciclovir [14–16] and it is a subject of current preclinical studies. In

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Disclosure statement

The authors declare no competing interest.

order to improve therapeutic efficacy of cyclopropavir (**5**) we have turned our attention to L-valine ester - valcyclopropavir (**6**). The synthesis, antiviral activity and pharmacokinetic parameters of analogue **6** is the subject of this communication.

Methods

Synthesis

The mass spectra (MS) were determined in an electrospray ionization (ESI) mode. Valcyclopropavir (**6**) was synthesized as described below. Cyclopropavir (**5**) was synthesized by GLSynthesis, Inc., Worcester, Massachusetts by previously published method [14]. All other chemicals were analytical reagent grade and all solvents were HPLC grade.

(Z)-9-[[2-(N-tert-Butoxycarbonyl-L-valyloxymethyl)-2-(tetrahydropyranyloxymethyl)-cyclopropylidene]methyl]guanine (8**)**—A mixture of N-(tert-butoxycarbonyl)-L-valine (BOCValOH, 955 mg, 4.4 mmol) and dicyclohexylcarbodiimide (DCC, 537 mg, 2.6 mmol) in dichloromethane (36 mL) was stirred at room temperature under nitrogen for 3 h. The insoluble portion was filtered off and solvent from the filtrate was evaporated in vacuo. The resultant N-(t-butoxycarbonyl)-L-valine anhydride/(BOCVal)₂O was dissolved in N,N-dimethylformamide (DMF, 50 mL) and compound **7** (0.7 g, 2.02 mmol) was added followed by 4-dimethylaminopyridine (DMAP, 25 mg, 0.3 mmol). The mixture was stirred at room temperature for 40 h, solvent was removed in vacuo and the crude product was chromatographed on a silica gel column using CH₂Cl₂ - MeOH (30: 1 to 8: 1) to give compound **8** (0.81 g, 73%) as a solid, mp 228–230 °C. UV λ_{max} (ethanol) 274 nm (ε 11,200), 231 nm (ε 27,100). ESI-MS (MeOH + NaCl) 547 (M + H, 10.9), 569 (M + Na, 100.0). The NMR spectra could not be obtained because of poor solubility.

(Z)-9-[[2-Hydroxymethyl-2-(L-valyloxymethyl)cyclopropylidene]methyl]guanine Hydrochloride (6**)**—A solution of compound **8** (0.5 g, 0.92 mmol) in 10% HCl/ethyl acetate (50 mL) was stirred at room temperature for 1.5 h. The volatile components were removed in vacuo, the crude product was chromatographed on a silica gel column using CH₂Cl₂ - MeOH (10: 1 to 5: 1). Evaporation of solvents afforded product **6** as a white solid after trituration of the residue with methanol and ethyl acetate (280 mg, 76.4%), mp >300 °C, [α]_D²⁰ 21.0° (c 1.0, DMSO). UV (ethanol) λ_{max} 273 nm (ε 12,600), 230 nm (ε 29,100). ¹H NMR (DMSO-d₆, 400 MHz) δ 10.92 (s, 1H, NH), 8.51 (bs, 3H, NH₃⁽⁺⁾ of Val), 8.22, 8.18 (2s, 1H, H₈), 7.16 (s, 1H, H_{1'}), 6.78 (s, 2H, NH₂), 5.36 (poorly resolved t, 1H, OH), 4.38–4.25 (m, 2H, H_{5''}), 3.82–3.73 (m, 2H, H_{5'}), 3.46–3.42 (m, 1H, CHα of Val, partly overlapped with H₂O), 2.15–2.12, 2.03–1.96/2m, 1H, CH(CH₃)₂/, 1.51–1.46 (m, 2H, H_{3'}), 0.94, 0.89 (2d, J = 6.4, 6.8 Hz), 0.84–0.83 (poorly resolved apparent t, 6H, CH₃); ¹³C NMR 169.5, 169.4 (C=O), 157.1 (C₆), 155.0 (C₂), 150.4, 150.3 (C₄), 134.9, 134.8 (C₈), 116.9, 116.5 (C_{2'}), 116.4 (C_{2'}, C₅), 112.2 (C_{1'}), 67.5, 66.9 (C_{5'}), 63.2, 63.1 (C_{5''}), 58.0 (Cα of Val), 29.93, 29.88 (C_{4'}), 27.8, 27.7 (CH(CH₃)₂), 18.9, 18.7, 18.3, 18.2 (CH₃), 12.4, 12.1 (C_{3'}). ESI-MS (MeOH - KOAc) 363 (100.0, M + H), 401 (36.9, M + K). For analysis it was dried at 100 °C in vacuo. Anal. Calcd. for C₁₆H₂₂N₆O₄ x 1.2 HCl x 1.4 H₂O: C, 44.55; H, 6.08; Cl, 9.86; N, 19.48. Found: C, 44.93; H, 5.87; Cl, 9.63; N, 19.13.

Kinetics of Hydrolysis of Valcyclopropavir (6**) at pH 7.0**—A solution of compound **6** (1 mg, 2.25 μmol) in 0.02 M Na₂HPO₄ (pH 7.0, 1 mL) was stirred at room temperature for 36 h. At selected time intervals, aliquots of the mixture was analyzed by HPLC on DuPont Zorbax NH column 250 × 4.6 mm, mobile phase 0.05 M KH₂PO₄ - acetonitrile (3: 1), flow rate 1 mL/min., UV detection at 273 nm, see Figure 1.

Antiviral Assays—Plaque reduction assays in human foreskin fibroblasts (HFF's) were performed with HCMV strains Towne and AD169 as described [14,15].

Stability of the Prodrug Valcyclopropavir (6) in Mouse Plasma and Whole Blood

—Male Swiss-Webster mice (Taconic Farms, Germantown, Pennsylvania) were anesthetized and blood collected (ca. 1 ml per animal) by cardiac puncture into Monoject[®] No. 8881-311149 collection tubes (Tyco Healthcare, Mansfield, Massachusetts), containing an anticoagulant [0.04 ml of 7.5% EDTA(K₃)]. The blood was cooled in ice and used immediately for plasma preparation and stability studies. For plasma preparation, half of the collected blood was centrifuged for 10 min. at 1100 × G in a refrigerated centrifuge (4°C). For stability at 0°C, ice cooled blood and plasma samples were spiked with a 10 mM solution of the prodrug (6) in DMSO to a final concentration of 50 μM. The samples were kept in ice water. For stability at 37°C, the plasma and blood samples were brought to 37°C by placing in warm water, and then in a thermostatic water bath. Aliquots of 200 μL plasma, and 400 μL blood were taken at the indicated times. The plasma samples were frozen immediately in a dry ice/acetone mixture. The blood samples were centrifuged in a refrigerated centrifuge (4°C) for 5 min. at 2200 × G. Plasma (150 μL) was removed, frozen in a dry ice/acetone mixture and stored at -20°C until analyzed. The samples were analyzed for valcyclopropavir (6), and cyclopropavir (5) by the HPLC method describe below.

Bioavailability of Cyclopropavir (5) after IV and PO Dosing of the Prodrug Valcyclopropavir (6) to Mice

1. Dosing and blood sampling of mice: Male Swiss-Webster mice were obtained from Taconic Farms, Germantown, Pennsylvania. Prodrug (6) was dissolved in physiological saline at 4.92 mg/mL. Mice (n=3/group/time point) were injected intravenously (IV) via the tail vein at a dose of 24.6 mg/kg (5 mL/kg, ca. 140 uL/animal), or given orally, by gavage (IO), at a dose of 49.2 mg/kg (10 mL/kg, ca. 300 μL/animal). Mice given the IV dose had a mean body weight of ca. 28 g. Mice given the IO dose were fasted overnight before dosing and had a mean body weight on the day of dosing of ca. 30 g. At the indicated sampling times, (10, 20, 40, 80, 160, and 240 min. for IV; and 20, 40, 60, 120, 240, and 320 min. for IO), animals were anesthetized, sacrificed, and blood collected by cardiac puncture. Blood samples were cooled on ice, and immediately subjected to centrifugation at 4°C for plasma preparation. The plasma samples were frozen on dry ice and stored at -20°C until analyzed.

2. Analytical methods

A. Preparation of standard solutions and spiked plasma calibration samples: Six standard solutions, containing a mixture of prodrug (6) and cyclopropavir (5), at concentrations of 0.6, 2.0, 6.0, 20.0 and 60.0 μg/mL, were prepared by diluting DMSO stock solutions (10 mg/mL) with acetonitrile. For spiked plasma calibration samples, aliquots of 250 μL of the standard solutions were mixed with equal volume of mouse plasma in polypropylene microfuge tubes. For extraction efficiency determination, another set of standard solutions, (250 μL), were mixed with an equal volume of water. All tubes were vortexed for 30 sec, maintained at room temperature for 30 min., vortexed again for 30 sec, and then centrifuged for 15 min. at 13,000 rpm. The supernatants (375 μL each) were pipetted from each tube into a new microfuge tube, and evaporated to dryness in a Speed-Vac (4–5 hours). DMSO (250 μL) was added to each residue, and the samples were vortexed for 60 min. The solutions were centrifuged for 15 min. at 13,000 rpm, and transferred to HPLC vials. Each sample was analyzed by HPLC by the method described below.

B. Preparation and extraction of plasma samples from dosed mice: The plasma samples were thawed on ice, and aliquots (250 μL) were immediately transferred to microfuge tubes, and

deproteinated by addition of acetonitrile, (250 μ L). The samples were further processed as described above for the spiked plasma standards.

C. HPLC method: The HPLC analysis was performed on a Varian ProStar® HPLC System consisting of two pumps, a variable wavelength detector, an autosampler, and a desktop computer for HPLC control, data acquisition and data analysis. The data analysis software was the Varian® Star Chromatography Workstation (version 6.0). Compound elution was performed on a 4.6 \times 150 mm Ace 5 C18 HPLC column (Advanced Chromatography Technologies). The mobile phase consisted of reservoir A, containing 100% water with 0.1% TFA; and reservoir B, containing 100% methanol. Each solvent was filtered through a 0.45 μ m nylon filter and degassed before use. Elution from the column was at a flow rate of 1 mL/min. using the following gradient program: a linear gradient of 0% B to 30% B from zero to 18 min., then a linear gradient from 30% to 100% B for 1 min., hold at 100% B for 2 min., followed by a linear gradient from 100% to 0% B for 1 min., and then equilibration by a hold at 0% B for 5 min.. Compound elution was monitored by UV absorbance at 274 nm. Total analysis time was 27 min. per sample. Retention times were 9.8 min. for cyclopropavir (**5**) and 14.6 min. for the prodrug (**6**).

D. Data analysis: Peak areas from HPLC analysis of plasma samples were measured, and concentrations of cyclopropavir (**5**) and prodrug (**6**) were calculated based on the standard curves generated with the plasma spiked samples. The extraction efficiency for each compound was determined by comparing the peak areas for spiked plasma samples with those for standard samples processed with water instead of plasma. The average extraction efficiency was 67% for cyclopropavir (**5**), and 88% for the prodrug (**6**). The limit of detection and the limit of quantification, determined as 3x and 5x the intensity of the matrix peaks in the regions of detection of the analytes were 0.24 μ g/mL and 0.40 μ g/ml for cyclopropavir (**5**), and 0.048 μ g/mL and 0.080 μ g/mL for the prodrug (**6**). The PK modeling and analysis was done using the PK modeling program WinNonLin® (Pharsight, Mountain View, CA).

Results

A. Antiviral activity

Prodrug **6** inhibited replication of HCMV in two strains of virus, Towne and AD169 (Table 1). These studies confirmed that its potency was comparable to ganciclovir, which was used as a control, and was also similar to cyclopropavir (**5**) reported previously [14]. These results confirmed that valcyclopropavir (**6**) was readily hydrolyzed to cyclopropavir (**5**) inside the virus-infected cells or in the culture medium that contains bovine plasma. However, its efficacy relative to cyclopropavir (**5**) cannot be discerned from these data.

B. Conversion of valcyclopropavir (**6**) to cyclopropavir (**5**) *in vitro* in mouse plasma and whole blood

The prodrug valcyclopropavir (**6**) was converted into the parent drug, cyclopropavir (**5**) when incubated at 37°C in mouse blood or plasma with a half-life of 1 h 20 min., and 40.4 min., respectively (Figure 2, Table 2). At 0°C the conversion was more than an order of magnitude slower, with estimated half-lives of 22 h (blood) and 12 h (plasma). In a control experiment, blood and plasma samples were processed immediately after spiking with the prodrug (no incubation). The level of the cyclopropavir peak detected in those samples corresponded to a 3.7% (plasma) and 9.0% (blood) conversion of valcyclopropavir (**6**) to cyclopropavir (**5**). This is assumed to take place during the plasma preparation and sample processing, and should not affect the overall kinetic parameters observed for the *in vitro* or *in vivo* metabolism of the prodrug (**6**), since it is the same for all incubation/sampling times. In our opinion, this is small

conversion does not warrant correction of the dose-dependent PK parameters for the prodrug (6).

C. IV dosing

The average plasma concentration of the prodrug (6), and cyclopropavir (5) are plotted in Figure 3.

Prodrug (6)—The plasma concentration data for prodrug (6) were fitted into a single compartment bolus IV first order elimination model:



$$C(T)=D/V*EXP(-K_{10}*T),$$

where

C is the plasma concentration, mg/L

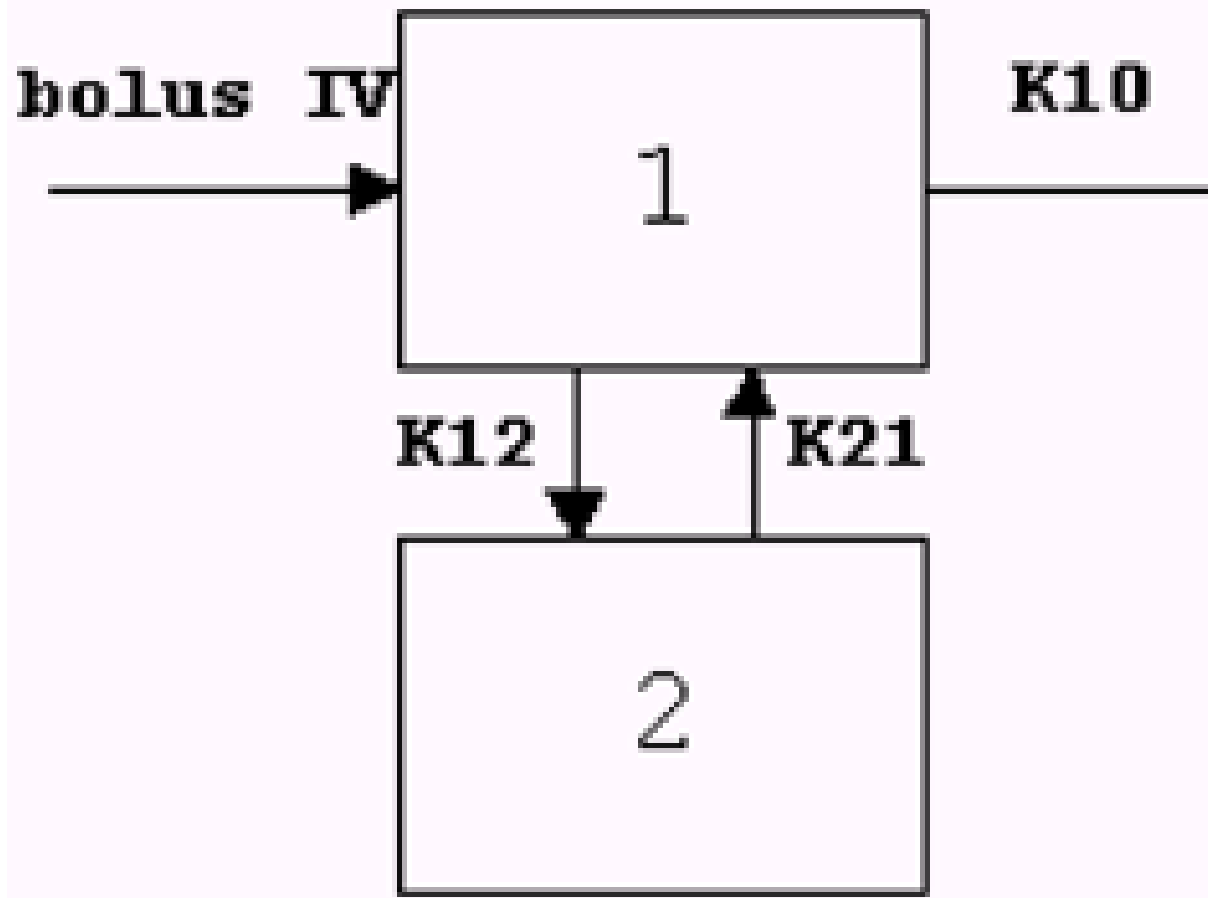
D is the dose, mg/kg

V is the apparent volume of distribution, L

K_{10} is the elimination rate constant, L/min.

T is the time, min.

The best fit curve is presented in Figure 4A, and the best fit PK parameters are presented in Table 3. Analysis of the plasma concentration profile suggested that the prodrug (6) is converted by a fast process to cyclopropavir (5). At the same time, some amount of the prodrug (6) remains in a depot compartment, and is released by a slow process. This results in a large half-life and volume of distribution parameter in the single compartment model (Table 3). The data for the prodrug (6), therefore, was fitted to a two compartment bolus IV 1st order elimination model as described below:



$$C(T) = A \cdot \text{EXP}(-\text{ALPHA} \cdot T) + B \cdot \text{EXP}(-\text{BETA} \cdot T)$$

where

C is the plasma concentration, mg/L

$$A = D/V \cdot (\text{ALPHA} - K_{21}) / (\text{ALPHA} - \text{BETA})$$

$$B = -D/V \cdot (\text{BETA} - K_{21}) / (\text{ALPHA} - \text{BETA})$$

ALPHA and BETA are the roots of the quadratic equation $r^2 + (K_{12} + K_{21} + K_{10}) \cdot r + K_{21} \cdot K_{10} = 0$

D is the dose, mg/kg

V is the apparent volume of distribution, L

K_{12} is the rate constant for transfer from compartment 1 to compartment 2, L/min.

K_{21} is the rate constant for transfer from compartment 2 to compartment 1, L/min..

K_{10} is the elimination rate constant, L/min.

T is the time, min.

The best fit graph and corresponding parameters are presented in Figure 4B and Table 4. The parameters are more consistent with the properties expected of the prodrug (6). The lack of very early time points resulted in high coefficients of variability, especially of the parameters highly influenced by K_{12} .

Cyclopropavir (5)—The plasma concentration of cyclopropavir (5) was first modeled using a single compartmental first order elimination model, in which the last three data points were used to estimate the terminal elimination rate constant, β (Figure 5A). The PK parameters based on this analysis are presented in Table 4.

The time dependence of the cyclopropavir (5) plasma concentration was also assessed by using a two compartmental model as described below:



$$C(T) = \frac{D \cdot K_{01}}{V \cdot (K_{01} - K_{10})} * (\exp(-K_{10} \cdot T) - \exp(-K_{01} \cdot T))$$

where:

0 is the valcyclopropavir (6) occupied compartment,

1 is the main, sampling compartment

C is the plasma concentration of 5 in the sampling compartment, mg/L;

D is the dose, mg/kg (for simplification of the calculation a dose of 16.2 mg/kg of cyclopropavir was used, which is equivalent to a valcyclopropavir dose of 24.6 mg/kg)

V is the apparent volume of distribution, L;

K_{01} is the first order input rate constant, i.e. the rate constant for conversion of prodrug (6) to cyclopropavir (5), L/min.;

K_{10} is the rate constant for elimination of cyclopropavir (5) from the plasma compartment, L/min.;

T is the time from the prodrug (6) dosing, min.

The best fit graph and corresponding parameters are presented in Figure 5B and Table 6. The PK parameters obtained by this analysis are in good overall agreement with the corresponding PK parameters, obtained by the single compartment method (Table 5). However, since the elimination of the prodrug (6) does not follow first order kinetics there is significant uncertainty in the estimation of the input rate constant K_{01} .

D. Oral dosing

The results of the plasma sample analysis after a single oral dose of 49.2 mg of prodrug (6) in mice is presented in Figure 6. No prodrug (6) was detected in the oral samples (limit of detection = 0.048 $\mu\text{g/mL}$). The absorption phase took less than 10 min. and was not observed. The \log_{10} of the observed experimental cyclopropavir (5) concentrations demonstrated an overall linear decline (Figure 6B, red line). The data was fitted into a first order elimination non-compartmental model. The model-predicted and experimental cyclopropavir concentrations are shown in Table 7, and shown plotted in semilog form in Figure 6B. The PK parameters

derived from the above model are presented in Table 8. The prodrug (**6**) oral bioavailability, calculated according to the formula: $OB\% = (IV_{AUC}/Oral_{AUC}) \cdot (IV \text{ dose}/Oral \text{ dose}) \cdot 100$, was found to be very high at 95.3%.

Discussion

Chemistry

The previously described [17] tetrahydropyranyl (THP) cyclopropavir (**7**) which is available from cyclopropavir in two steps (73% yield) served as a convenient starting material for synthesis of valcyclopropavir (**6**, Scheme 1). The method described [2] for synthesis of valacyclovir (**1**) was followed. Reaction of **7** with N-(t-butoxycarbonyl)-L-valine anhydride (BOCVal)₂O in DMF under catalysis with 4-dimethylaminopyridine (DMAP) gave intermediate **8** in 73% yield. The BOC and THP group were removed in acid medium (10% HCl in ethyl acetate) to give valcyclopropavir (**6**) as a hydrochloride in 76% yield. Similar to valganciclovir (**2**) [3], prodrug (**6**) is a mixture of two stereoisomers. The stereoisomeric ratio of **2** determined from the ¹H NMR spectrum is 2: 1.

As expected, prodrug (**6**) has a limited stability at pH 7.0 (Figure 1) with the half-life of 22.5 h at room temperature. For comparison, the half-life of valganciclovir (**2**) [18] at pH 7.08 and 37 °C is 11 h.

Pharmacokinetic (PK) studies

Prodrug (**6**) is converted to cyclopropavir (**5**) *in vitro* in mouse whole blood and blood plasma, and *in vivo* after both intravenous (IV) and oral (PO) administration. Prodrug (**6**) is quickly and efficiently absorbed by the gastrointestinal tract, with PO bioavailability of 95%. The high apparent volume of distribution of **5** after both IV and PO dosing with prodrug (**6**), is suggestive of possible sequestering of either **6** or **5** in non-sampling compartments. The PK profile of prodrug (**6**) after IV dosing also supports the possibility of non-uniform distribution of **6** with sequestering outside of the plasma compartment.

Acknowledgments

The work described herein was supported by SBIR grant 5 R44 AI054135, contract NO1-AI30049 and research grant RO1-CA32779 from the National Institutes of Health, Bethesda, Maryland. We thank L. M. Hrihorczuk from the Central Instrumentation Facility, Department of Chemistry, Wayne State University for mass spectra, Kathy Borysko at University of Michigan for plaque assays, Sofya Dvoskin and George Wright from GLSynthesis for valuable assistance in bioavailability studies.

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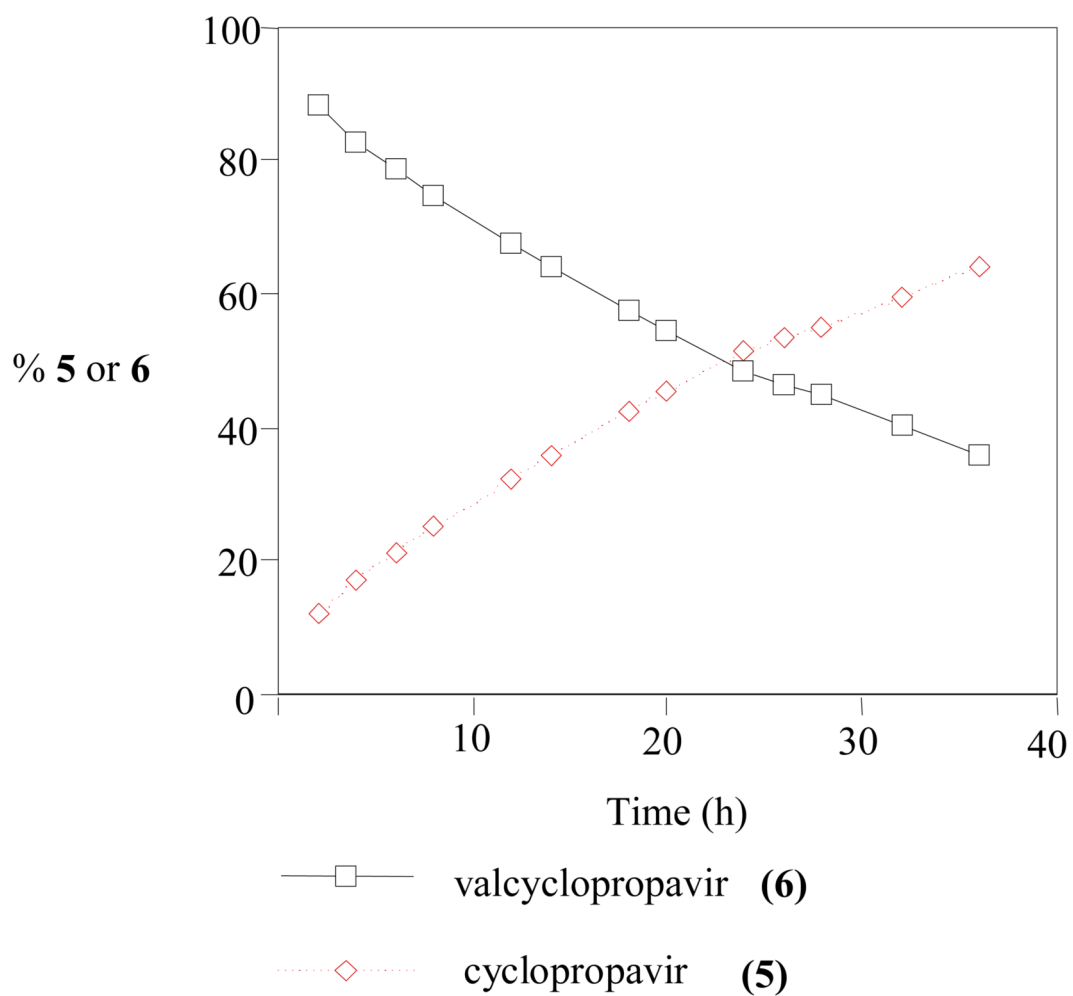


Figure 1.
Kinetics of hydrolysis of prodrug 6 at pH 7.0 and room temperature.

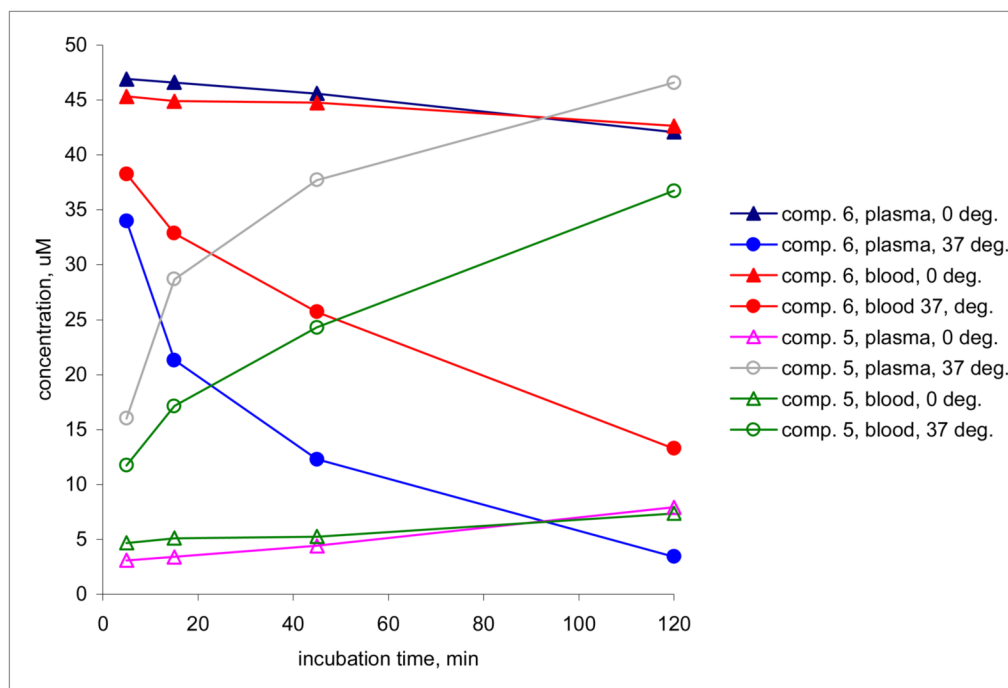


Figure 2. Conversion of prodrug 6 into cyclopropavir (5) after incubation at 0 and 37 °C in mouse whole blood and plasma.

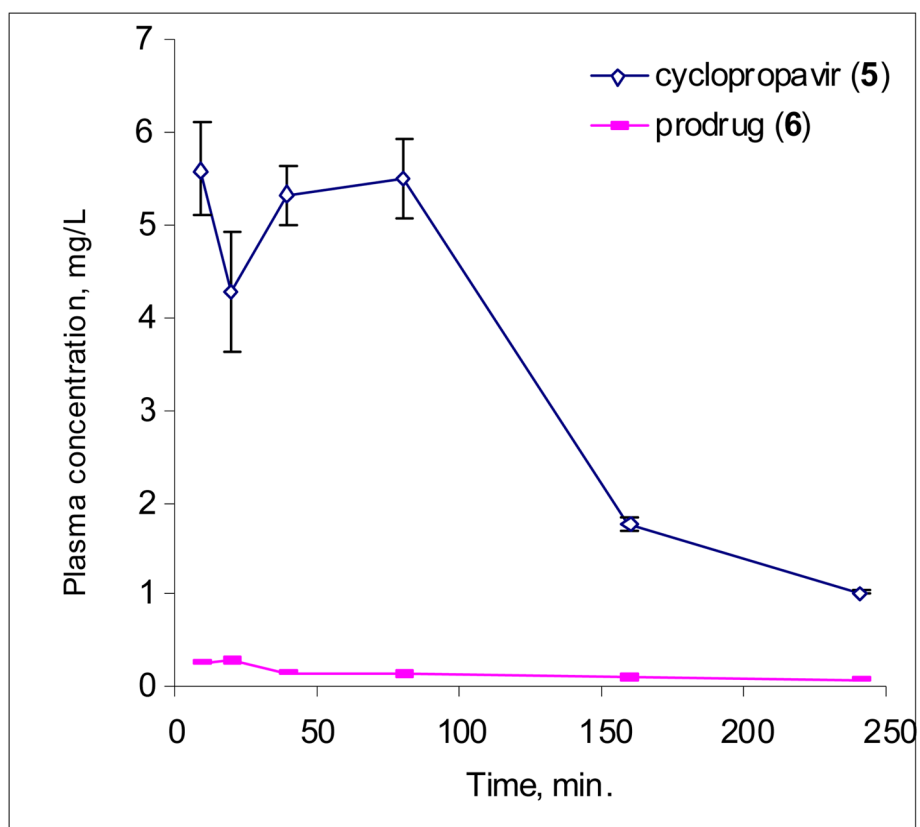


Figure 3. Plasma concentrations of cyclopropavir (5), and prodrug 6 vs. time after single IV bolus injection of 24.6 mg/kg prodrug in mice.

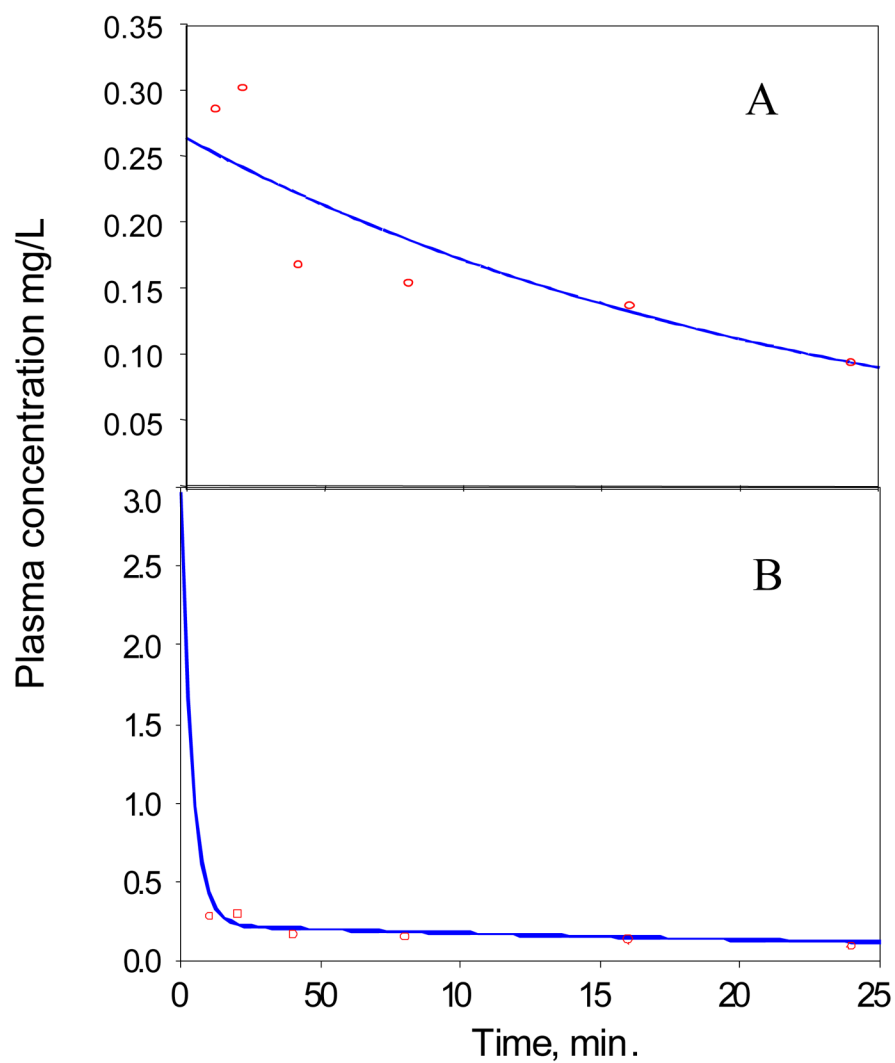


Figure 4. PK modeling of the experimental data for prodrug (**6**) after IV bolus injection in mice. **A**, Single compartment first order elimination model. **B**, Two compartments first order distribution and elimination model.

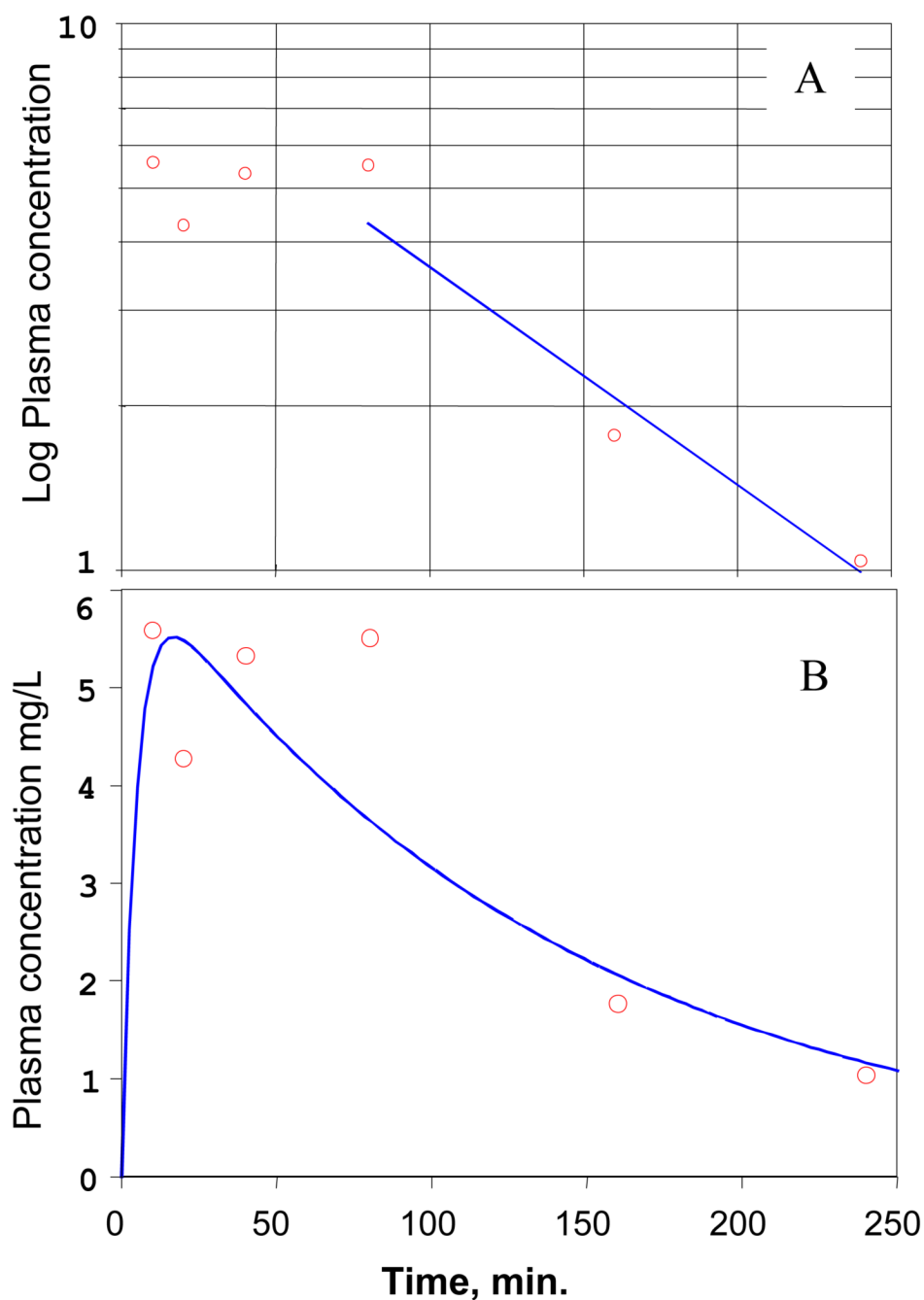


Figure 5. PK modeling of the experimental data for cyclopropavir (**5**) after IV bolus injection of prodrug **6** in mice (○ observed, — predicted). **A**, Single compartment first order elimination modeling: Logarithmic plot of the average plasma concentration of cyclopropavir (**5**) vs. time. The straight line is the linear regression fit to the last three data points (1/Y weighting), which was used to estimate the terminal elimination rate constant β . **B**, Two compartmental analysis. See text for model description.

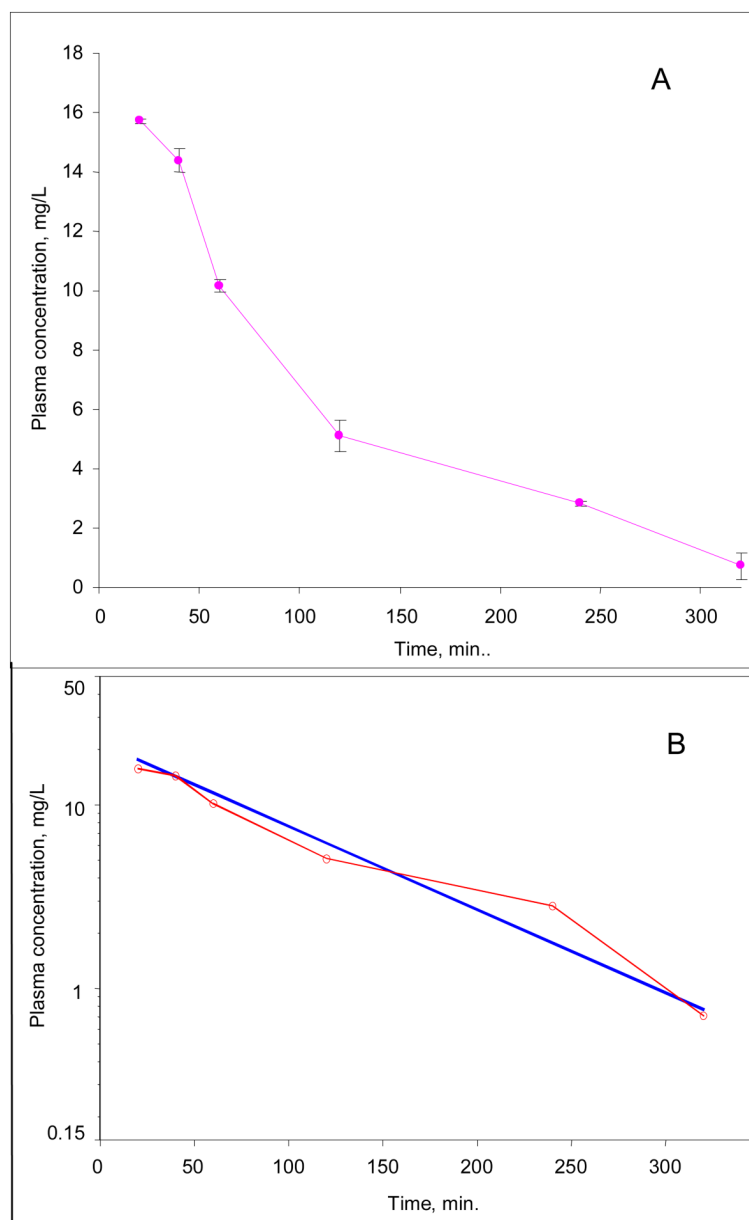
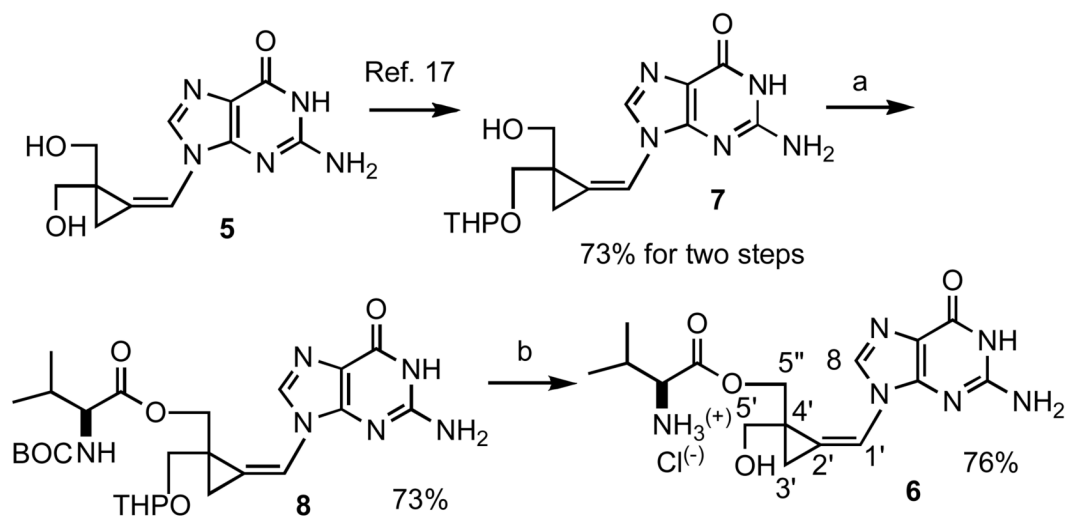


Figure 6. Plot of plasma concentrations of cyclopropavir **5** vs. time in mice after a single oral dose of 49.2 mg/kg of prodrug **6**. **A**, normal plot; **B**, log plots of the experimental (o—o—o) and modeled (—) plasma concentrations.



a. (BOCV_{al})₂O, DMAP, DMF. b. 10% HCl in AcOEt.

Scheme 1.

Synthesis of valcyclopropavir (**6**)

a. (BOCV_{al})₂O, DMAP, DMF. b. 10% HCl in AcOEt.

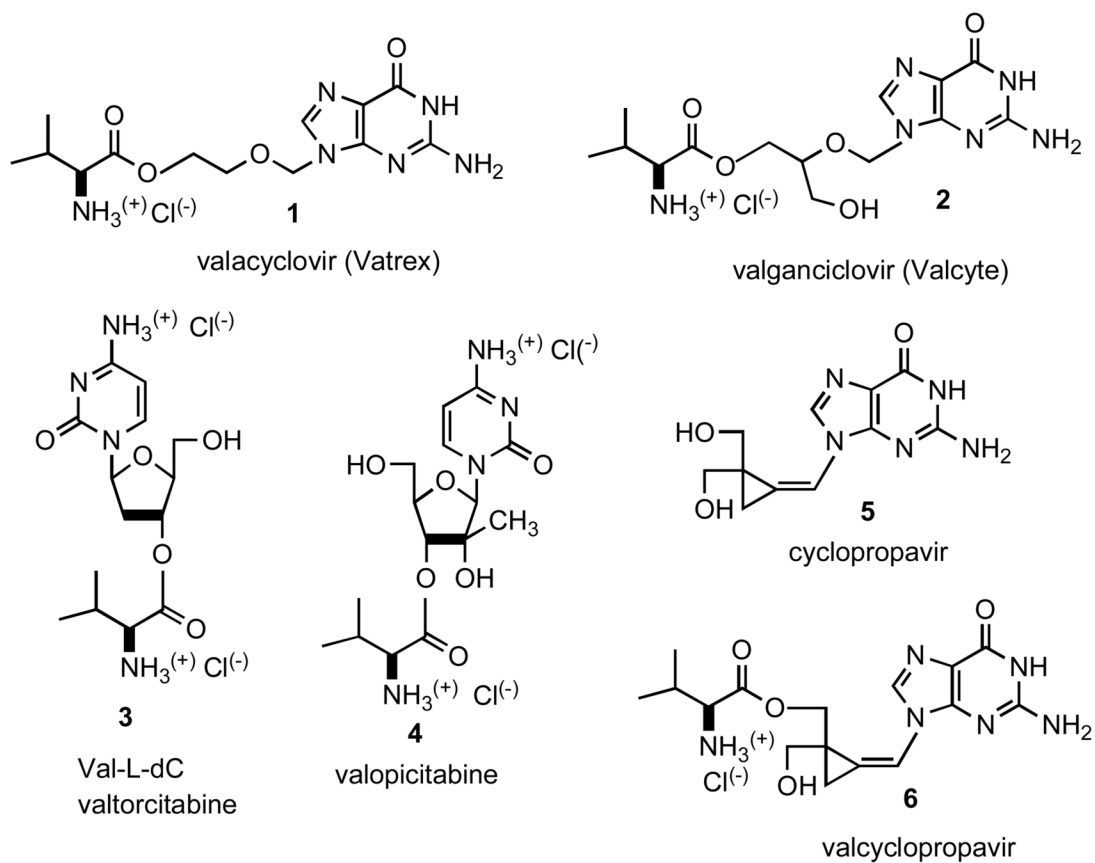


Chart 1.

Table 1Anti-HCMV activity of valcyclopropavir (**6**).

Compound	HCMV/HFF ^a , EC ₅₀ /CC ₅₀ (μM)	
	Towne ^b	AD169 ^c
Valcyclopropavir (6) ^d	0.21/>100	2.9/>300
Ganciclovir	1.58±1.53/>100	3.6 ± 1.4/>100
Cyclopropavir (5) ^e	0.42±0.23/>100	1.2 ± 0.8/>380

^aEfficacy and cytotoxicity in human foreskin fibroblasts (HFF's) as determined by plaque reduction assay.

^bVisual cytotoxicity in uninfected HFF's used in plaque reduction assays.

^cCytotoxicity by neutral red assay.

^dResults from single assays.

^eEC₅₀ values for cyclopropavir (**5**) were reported previously for both strains of the virus [14].

Table 2Conversion of valcyclopropavir (**6**) to cyclopropavir (**5**) in mouse whole blood and plasma at 0 and 37°C.

Incubation conditions	First order rate constant, min. ⁻¹	half live min.	R ²
Plasma 0°C	0.0010	727	0.9925
Plasma 37°C	0.0172	40.4	0.9998
Blood 0°C	0.0005	1310	0.9645
Blood 37°C	0.0087	80	0.9997

Table 3Single compartment first order elimination PK parameters for prodrug (**6**) after IV dosing in mice.

Parameter	Best fit	CV%
Apparent volume of distribution, V_D , L/kg	93.5	11.9
Area under the curve, AUC, mg.min/L	61.2	7.33
Rate constant elimination, K , min^{-1}	0.0043	14.8
Half-life, $T_{1/2}$, min.	161	23.9
Intercept (time zero concentration), C_{max} , mg/L	0.263	11.9
Clearance (Cl), L/min.	0.402	7.33

Table 4

Two compartments first order distribution and elimination PK parameters for prodrug (**6**) after bolus IV dosing in mice.

Parameter	Best Fit	CV%
Volume of main compartment, V, L/kg	0.0753	14.9
Area under the curve, AUC, mg.min/L	97.1	85.6
Rate constant of distribution phase, α , min. ⁻¹	0.25	536
Half-life of distribution phase, T _{1/2} (α), min.	2.73	535
Rate constant of elimination phase, β , min. ⁻¹	0.00263	114
Half-life of elimination phase, T _{1/2} (β), min.	301	114
Intercept (time zero concentration) of distribution phase, C ₀ (A), mg/L	36.5	1234
Intercept (time zero concentration) of elimination phase, C ₀ (B), mg/L	1.037	12.3
Clearance (Cl), L/min.	0.217	222

Table 5

Single compartment first order elimination PK parameters for cyclopropavir (**5**) after IV dosing of prodrug (**6**) in mice. The last three data points were used to model the elimination rate constant.^a

Parameter	Best Fit
Rate constant of elimination phase, β , min. ⁻¹	0.0092
Half-life based on terminal elimination phase, $T_{1/2}(\beta)$, min.	75.5
Time of maximum plasma concentration, T_{max} , min.	10
Maximum plasma concentration, C_{max} , mg/L	5.59
Volume of distribution based on the terminal phase, V_z , L	1.94
Area under the curve at infinity, AUC_{∞} , mg.min/L	902
Total plasma clearance (Cl), L/min.	0.018

^aIn this analysis a complete conversion of the prodrug (**6**) to cyclopropavir (**5**) was assumed, which would result in a equivalent dose of cyclopropavir (**5**) of 16.2 mg/kg.

Table 6

Two compartmental analysis of cyclopropavir (**5**) plasma levels after IV bolus dosing of prodrug (**6**). See text for model description.

Parameter	Best Fit	CV%
Volume of distribution, V, L/kg	2.61	21.0
Area under the curve, AUC, mg.min/L	874	22.3
Input rate constant, K ₀₁ , min. ⁻¹	0.21	121
Elimination rate constant, K ₁₀ , min. ⁻¹	0.0071	32.7
Elimination Half-life of T _{1/2} K ₁₀ , min.	97	32.7
Time of maximum plasma concentration, T _{max} , min.	16.7	85.8
Maximum plasma concentration, C _{max} , mg/L	5.52	15.0
Clearance (Cl), L/min.	0.0185	22.3

^a A complete conversion of the prodrug (**6**) to cyclopropavir (**5**) is assumed, which would result in a equivalent dose of cyclopropavir (**5**) of 16.2 mg/kg.

Table 7

Experimental and model-predicted concentrations of cyclopropavir (**5**) and “area under the curve” for oral dosing of prodrug (**6**).

Time, min.	Exp. conc. mg/L	Predicted conc. mg/L	Area under the curve, AUC mg.min/L
20	15.71		157.1
40*	14.39	14.75	458.1
60*	10.17	11.94	703.7
120*	5.10	6.330	1161.8
240*	2.82	1.780	1637.0
320*	0.71	0.764	1778.2

* Time point included in modeling of the elimination phase.

Table 8PK parameters of cyclopropavir (**5**) after oral dosing of prodrug (**6**).

Parameter	Best Fit
Rate constant of oral elimination phase, λ_z , 1/min.	0.0106
Half-life of oral elimination phase, $T_{1/2\lambda_z}$, min.	65.6
Time of maximum plasma concentration, T_{max} , min.	≤ 20
Maximum plasma concentration, C_{max} , mg/L	≥ 15.7
Volume of distribution based on the terminal phase, V_z , L	2.52
Area under the curve at infinity, AUC_{∞} , mg.min/L	1845
Total body clearance (Cl), L/min.	0.0267
Oral bioavailability, %	95.3

^a In this analysis, a complete conversion of the bioavailable prodrug (**6**) to cyclopropavir (**5**) was assumed, which would result in a dose equivalent to 32.4 mg/kg of cyclopropavir (**5**).