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SUMMARY

This project was designed to develop antiviral agents by adapting new enzymatic and pharmacokinetic principles to inhibition of the HIV-reverse transcriptase. Towards this end synthetic organic procedures were directed to synthesis of compounds in the following categories.

1. 3' Nucleosidee spiroxiranes and a series of related compounds having the potential to function as suicide inhibitor of the reverse transcriptase through the 3' functionality.
2. A series of sterol phosphonoformate analogs designed to improve delivery of the active phosphonoformate (PFA) moiety to sites of viral replication.
3. A series of 5' sterol ester derivatives of azido thymidine designed to improve the pharmacokinetics and blood half life of AZT.

Synthetic compounds were tested for antiviral activity against HIV and EIAV in tissue culture, against purified HIV-reverse transcriptase in enzyme kinetic studies, where appropriate and samples of synthetic analogs were supplied to the US Army Antiviral Program for screening against a battery of 10 viruses of interest as military disease hazards.

In addition to the targeted objectives, the serendipitous observation of a cell product that enhances the sensitivity of HIV-reverse transcriptase towards inhibtion by Foscarnet (PFA) by almost 1000 fold, was explored further resulting in preliminary identification of the sensitizing compound.

The major accomplishments in the categories listed above are summarized below and are described in more detail in the appropriate sections of the final report.

Organic synthetic procedures for the synthesis of a new class of compounds, the 3' nucleoside sproxiranes were developed. A series of these and related compounds and intermediates modified at the 2' and 3' functionalities were tested for activity against HIV and EIAV in tissue culture. Six compounds showing promising antiretroviral activity were identified. Several additional compounds were identified in the subsequent USAMRID screeing tests is being active against additional viruses including Punta, Toro, Vaccinia, and Yellow Fever.

A procedure for synthesis of sterol esters of phosphonoformate (FOSCARNET) was developed by

condensation of cholesterol chloroformate with the appropirate dialkylphosphite. Several of these compounds displayed greater antiviral activity than the parent compound PFA and side chains that enhanced (or detracted) from the activity were identified.

Three 5' cholesterol ester derivatives of AZT were synthesized and showed antiviral activity in tissue culture demonstrating that the cholesterol ester hydrolases of the cell could liberate the active moiety intracellularly. The ^{3}H radiolabelled AZT derivative of cholesterol sebacate was synthesized and used to evaluate blood and tissue half life of the ^{3}H-AZT moiety in mice. Administration of AZT as the sterol ester derivative inproved the blood half life of AZT by over 100 fold (< 1/2 hr to > 48 hr) and was accompanied by significant accumulation in tissues including brain.

The interaction of the effective antiviral compound 3' uridine sproxirane and the recombinant HIV reverse transcriptase enzyme was investigated in depth in order to characterize the suicide inhibition native of the interaction. This compound was designed as a potential suicide inhibitor of reverse transcriptase through the oxirane functionality at the 3' position. This compound showed good antiviral activity against HIV in cell culture and also against a second retrovirus (Equine Infectious Anemia).

In order to determine if the observed antiviral activity was due (as predicted) to inhibition of reverse transcriptase, the kinetic properties of 3' uridine spiroxirane were evaluated against the purified recombinant HIV-reverse transcriptase in vitro. In order to do this the triphosphate derivative of the nucleoside analog (the true putative inhibitor) was synthesized using a newly developed procedures for synthesizing nucleoside triphosphates.

It was found that the 3' uridine spiroxirane triphosphate derivative was an effective inhibitor of the HIV reverse transcriptase in the 0.1 to 1 micromolar range. The time course of the inhibition was progressive as expected for a suicide type inhibitor. Furthermore, the inhibition was not reversed by addition of excess template thus distinguishing it from a simple chain-terminating inhibitor and confirming that the inhibition resulted in the progressive and permanent inactivation of the enzyme characteristic of a suicide inhibitor. Thus it is concluded that the antiviral activity of the spiroxirane analogs we have synthesized is indeed related to their ability to function as suicide susbstrates for the HIV reverse transcriptase.

The compounds synthesized and supplied to USAMRID for antiviral testing are tabulated. The test data

indicating I_{50}'s, cytotoxicity and therapeutic index for all compounds showing antiviral activity are summarized on the Appendix to this final report.

The project was thus characterized by an unexpectedly high degree of success.

Of a total of 4a compounds synthesized and supplied to USAMRID for antiviral testing a total of 9 proved to have effective activity against one or more viruses of interest as military disease hazards including HIV, Punta, Toro, Vaccinia, and Yellow Fever. These compounds and the viruses against which they are effective are listed on the final page of this report. Futhermore, most of these showed low cytotoxicity in cell culture assays suggesting that they will be potentially useful therpeutic agents if future in vivo testing is ever carried out. Finally, the basic enzyme kinetic studies carried out in the extended (unfunded) 4th year of the contract, support the basic idea underlying these investigations, in that effective antiviral agents can be developed based upon the principle of suicide inhibition of key viral enzymes.

A. INTRODUCTION

Retroviruses are characterized by the anomalous storage of their genetic information in th form of RNA. They are a diverse group of organisms which have been shown to be causative agen in a number of mammalian and avian diseases states Lentiviruses are a subfamily o retroviruses which have been linked to the induction of arthritis, encephalitis, and slow neurologic diseases in certain species. Many of these viruses are characterized by their ability to develop nov antigenic variants that can escape temporarily from host immune surveillance Interest i retroviruses in human disease states has increased with the isolation of HTLV I and II as the causativ agents of adult human T cell leukemias and of HIV I and II as the cause of Acquired Immun Deficiency Syndrome (AIDS)

The most recent evaluation of the AIDS epidemic in the U.S. indicates that the curre total of approximately 70,000 AIDS cases is accompanied by a further 1 to 1.5 million infecte individuals, the majority of whom will progress to the clinically overt disease state in the next 5-years. In the near-term absence of an effective vaccine an increase in this infected/symptomle category is likely to continue for a number of years.

In addition to an antiviral therapy for acute cases of AIDS therefore, there is clearly pressin need for an effective antiviral drug regimen suitable for administration to large numbers of otherwi healthy individuals in order to arrest progression of the disease. By preventing viral replication, suc therapy may also reduce dissemination of the virus and thus indirectly fulfill some of the immedia functions of a vaccine. The current drug of choice, AZT can have undesirable side effects when use at maximum therapeutic dosages over prolonged periods. In addition HIV-variants with increase resistance to AZT have recently begun to appear. Combination therapy with drugs having a differe mechanism of action can reduce the likelyhood that resistant variants will appear. The drugs bei developed in this project are designed with the above potential for use in long-term combinatic therapy low of toxicity as a primary goal.

Replication of retroviruses is critically dependent upon a single enzyme called reve transcriptase (RT). This is an RNA-dependent DNA polymerase first found in the purified virio of Rous sarcoma virus by Temin and murine leukemia virus by Baltimore The enzy transcribes the viral RNA into DNA in the first step of viral replication. Since host cells do n contain reverse transcriptase, several molecules of the enzyme are packaged in each virion and ent the cell together with the viral RNA. It is at this stage that viral replication is most sensitive inhibitors of the reverse transcriptase, before amplification of the viral genome and pol gene directe synthesis of endogenous RT has taken place.

The principle that inhibitors of reverse transcriptase will inhibit replication of retoviruse is well established. For example, 3'-azido-3' deoxythymidine, the triphosphate of which inhibits th RT activity of HIV, is a potent inhibitor of virus replication in cultured H-9 cells in the rage of 1 10 micromolar and is being used successfully in patients with AIDS. Prolonged administratio may cause serious side effects. Another agent which has shown promise is phosphonoformate (PFA Foscarnet) which inhibits the RT activity of with an I_{50} of only 0.1 micromolar. However, muc higher concentrations of up to 340 micromolar are required for complete inhibition of HI replication in H-9 cells Other drugs including the dideoxycytidines which are also based upo inhibition of RT by chain termination of the viral template are in clinical trial. Since none of the drugs permanently inactivate the reverse transcriptase and since they do not accumulate intracellularly in significant amounts, virus replication will resume when blood levels of the drug decrease.

Since there is no complete animal model for HIV which reproduces the immunodeficiency, as an adjunct to the tissue culture studies thisproject will also utilize the Rauscher murine leukemia virus which has a reverse transcriptase with close homology to that of HIV, for in vivo testing of new antiviral drugs. This will be supplemented by testing drug toxicity in human T lymphocytes growing in tissue culture.

Suicide Inhibitors of Reverse Transcriptase

Suicide inhibitors, also known as Kcat inhibitors, are active site directed substrate analogs which contain a latent, reactive group. Following cleavage of the analog by the target enzyme the reactive group is released at the active site and inactivates the enzyme Inhibitors of this type have several potential therapeutic advantages int that they can be designed to be highly specific for

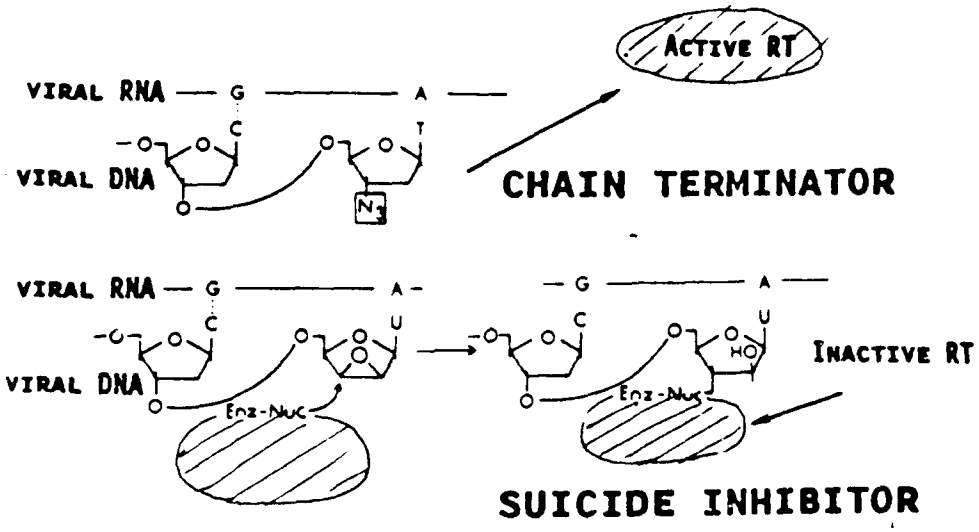
the target enzyme. Since most normal cells do not contain active or functional reverse transcriptases this reduces the possibility of side effects. Furthermore, by their nature, inhibitors of this type remain metabolically inert unless specifically cleaved by the target enzyme. Finally, the inhibition is permanent and irreversible, so that viral replication will not resume when the blood levels of the drug decrease. This feature could be particularly useful in long-term therapy of chronic human retrovirus infection.

Inhibition of retroviral replication by 3'-Nucleoside Spiroxirane Derivatives:

An apparent suicide inhibitor of E.Coli DNA polymerase was described by Abboud et al. It was shown that adenosine 2'3'-riboepoxide 5'-triphosphate irreversibly inactivated the enzyme by a covalent interaction. Because of the intrinsic reactivity of the epoxides however they are not suited for use under physiological conditions. We will therefore synthesize a series of nucleoside 3'-spiroxirane analogs. These are designed to inhibit the enzyme by an analogous suicide mechanism and are based upon the observation that the uridine 3'-ribospiroxirane derivative is an inhibitor of retroviral replication in cultured cells and selectively suppresses reverse transcriptase production by the VCF 21 (Moss) vaccinia HIV-RT recombinant. Furthermore at therapeutic levels it has no significant effects on ^{3}H-thymidine incorporation in cultured T-lymphocytes.

In the first step of replication of viral DNA by reverse transcriptase, activation of the primer 3'-OH by the enzyme results in incorporation of the suicide nucleotide by a normal 3',5'-phosphodiester linkage. At this point the inhibition resembles a simple chain termination similar to that produced by other 3'-blocked nucleoside analogs such as 3'-azidothymidine (AZT) and dideoxycytidine. However when the next step in the elongation reaction is attempted, the enzyme by activating the 3'-oxygen of the spiroxirane functionality becomes alkylated by the substrate and effectively commits suicide. A primary objective of these studies will be to characterize the kinetics of inhibition of the purified HIV-reverse transcriptase by the nucleoside spiroxirane triphosphates to determine if this second suicide step occurs and contributes to the observed antiviral activity of these compounds. Initial indications that the analogs can accomplish irreversible inactivation of intracellular RT support this possibility, and further efforts will be made to incorporate features into the molecule which favor the suicide pathway.

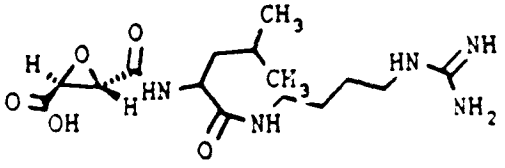
Because of the greater stability of the oxirane ring under physiological conditions these analogs offer several advantages over the simple epoxide types: A schematic representation of the consequences of an interaction with a suicide inhibitor versus a chain terminator is given below.



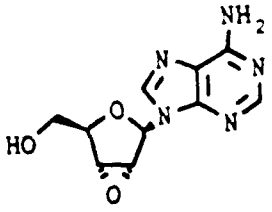
B. WORK ACCOMPLISHED

Epoxide Suicide Inhibitors:

Epoxide-containing irreversible (suicide) inhibitors have been reported for a number enzymes, the structures of two such compounds are shown below. One of the first, [N-(L-3-*tran* carboxyxiran-2-carbonyl)-L-leucyl]-amido (4-guanido) butane was isolated from *Aspergill japonicus* and was found to irreversibly inhibit cysteine proteases by alkylation of the sulfydr group at the active site.

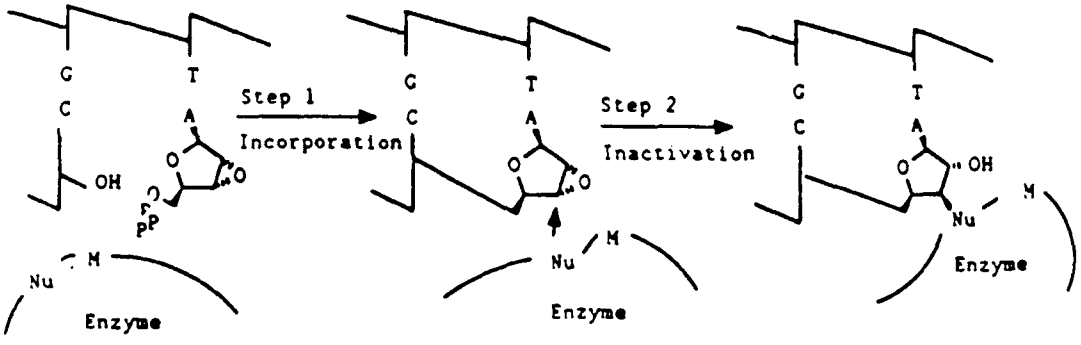


[N-(L-3-*trans*-carpoxioxiran-2-carbonyl) -L-leucyl]-amido(4-guanido)butane



9-(2,3-Anhydro-β-D-ribofuranosyl)adenine

A 2',3'-riboepoxynucleoside, 9-(2,3-anhydro-β-D-ribofuranosyl)adenine, was shown, as the 5' triphosphate, to irreversibly inhibit avian myeloblastosis virus DNA polymerase (a revers transcriptase) Its mechanism of inactivation, shown below, is believed to involve alkylation c the enzyme in the active site upon activation of the epoxide ring after incorporation of the nucleosid to the end of the growing DNA strand

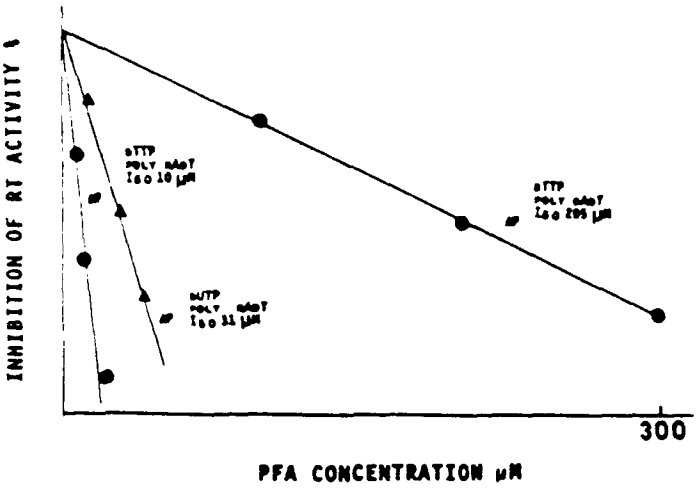


A proposed mechanism for RT inactivation by 2',3'-riboepoxyadenosine.

A similar compound, 1-(2,3-anhydro-β-D-lyxofuranosyl)cytidine, was recently synthesized by Broder et. al. and was found to inhibit HIV *in vitro* Although the mechanism of inactivation was not elucidated, irreversible inactivation was considered a possibility. We have also synthesized the corresponding 1-(2,3-anhydro-β-D-lyxofuranosyl) uridine and found it to have antiviral activity. However these epoxide derivatives unlike the spiroxiranes (see below) are also cytotoxic at higher concentrations probably owing to the greater reactivity of the epoxide ring

Preliminary Synthetic and Antiviral Screening Studies:

The reverse transcriptase of HIV is a highly error prone enzyme, a factor which probably contributes to the high mutation rate of the HIV genome. We have shown that reverse transcriptase readily incorporates dUTP in place of dTTP when transcribing from a poly rAdT template, and this incorporation, like that of dTTP, is particularly sensitive to inhibition by PFA (figure).



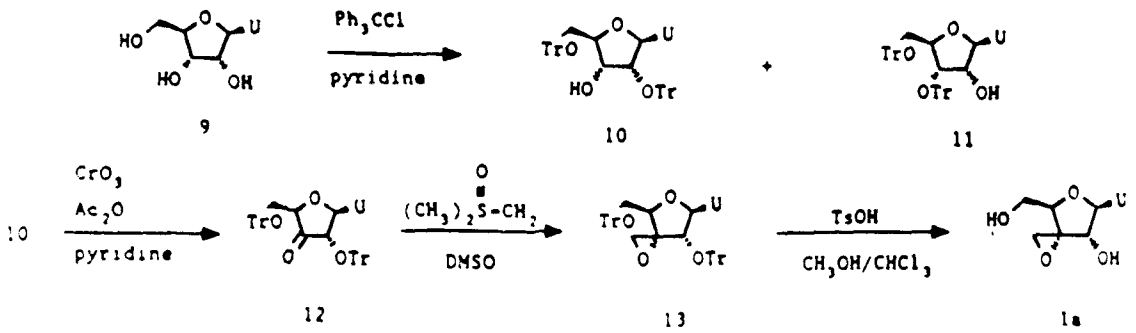
Reverse Transcriptase catalyzed Incorporation of d-UTP: Differential Inhibition of First-strand Synthesis by Foscarnet.

Since uridine is an unnatural base for the cellular DNA polymerases, initial studies were carried out using uridine nucleosides to enhance the antiviral selectivity.

Synthesis of Uridine 2' and 3'-Ribospiroxiranes:

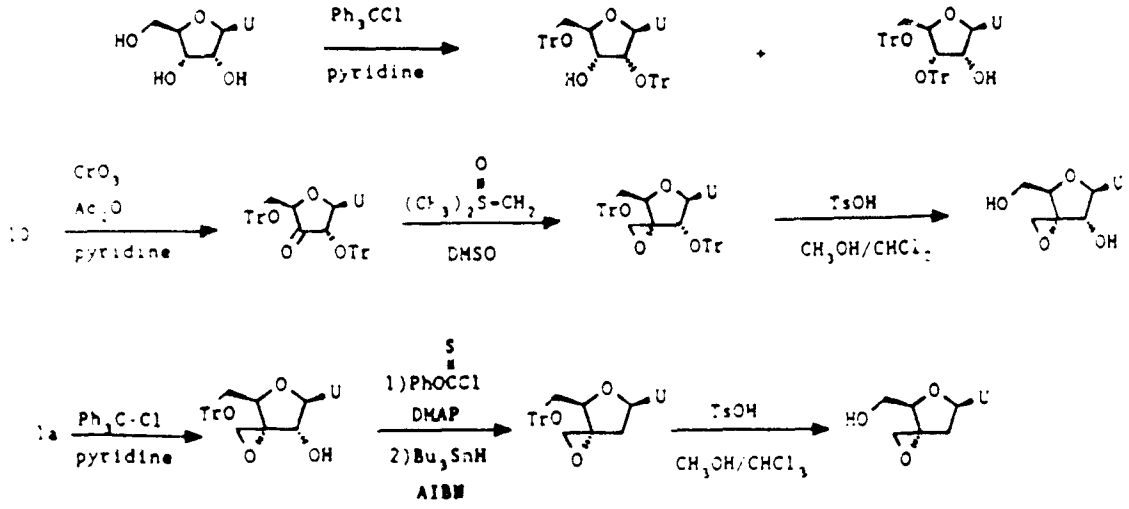
3'-uridine spiroxirane was synthesized from uridine by the sequence of reactions shown below. The procedure begins with the selective tritylation of uridine by the procedure of Cook and Moffat (38). Chromatographic separation of the isomers yielded the 2'5'and 3'5'-di-O-trityluridines in good yield. The 2',5'isomer was oxidized with chromium trioxide in pyridine/acetic anhydride to 3',5'-di-O-trityl 3'-ketouridine. Treatment of this compound with two equivalents of dimethyloxosulfonium methylide yielded the 2',5'-di-O-trityl spiroxirane derivative which upon mild acid catalyzed hydrolysis yielded the deprotected 3'-uridine spiroxirane.

The corresponding 2'-spiroxirane derivative was also prepared from the 2',3'-di-O-trityl isomer by analagous procedures and characterized by IR and NMR spectroscopy.



Synthesis of Spiroxirane Nucleosides:

This will be carried out by the general procedures used for synthesis of the uridine 3'-ibospro xirane described in section C above. The further extension of this to the synthesis of the 2'-piroxiranes from the 3'5'-di-O-trityl isomers and the 3'-dideoxy spiroxiranes from the 5'-mono-O-trityl derivative is summarized in the scheme below.



This route to the deoxynucleoside spiroxiranes is preferred since direct conversion of the orresponding deoxynucleosides by procedures analagous to steps 1-3 in the above scheme may lead base elimination following conversion to the 3'-keto derivative . Since trityl ethers of primary lcohols are more readily hydrolyzed than those of secondary alcohols it is not possible to obtain the '-mono-trityl uridine spiroxirane directly from the 3',5' derivative, which must first be completely eprotected and converted by the procedure of Michelson and Todd . Free radical deoxygenation f C-2 by the procedure of Acton et al via the 2'-O-phenylthiocarbonate followed by eprotection of the 5'-hydroxyl group yields the desired 2'-deoxy-3'-spiroxirane. The absolute onfigurations of the 3' and 2' uridine spiroxiranes obtained by this synthesis have not yet been ssigned. However it has been reported by Corey and Chaykousky , that dimethyloxo-sulfonium nethylide affords spiroepoxides with an equatorial methylene in reaction with 4 t-butyl yclohexanone, which would lead to spiroxiranes having the ribo-configurations illustrated. piroxiranes in the alternative lyxo configuration however may also be obtained by this general procedure by reaction of the 3'-ketoderivatives with dimethyl sulfonium methylide, as illustrated n the scheme below. In contrast to dimethyloxosulfonium methylide, this reagent yields piroexpoxides with an axially oriented methylene

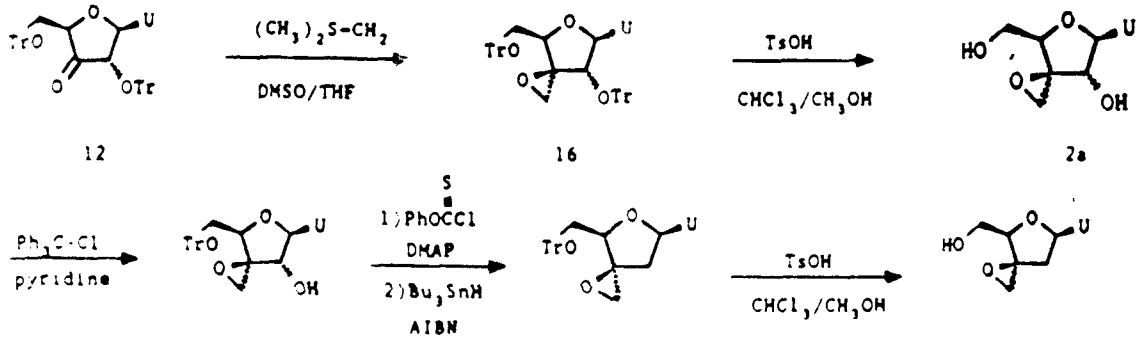
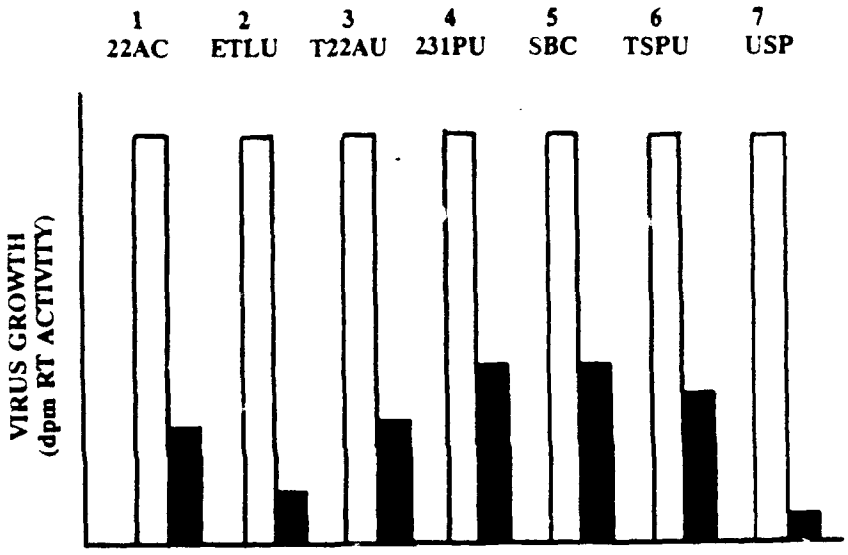


Fig.

Antiviral Activity of Uridine Spiroxiranes and Cytotoxicity Screening in Human T-Lymphocytes:

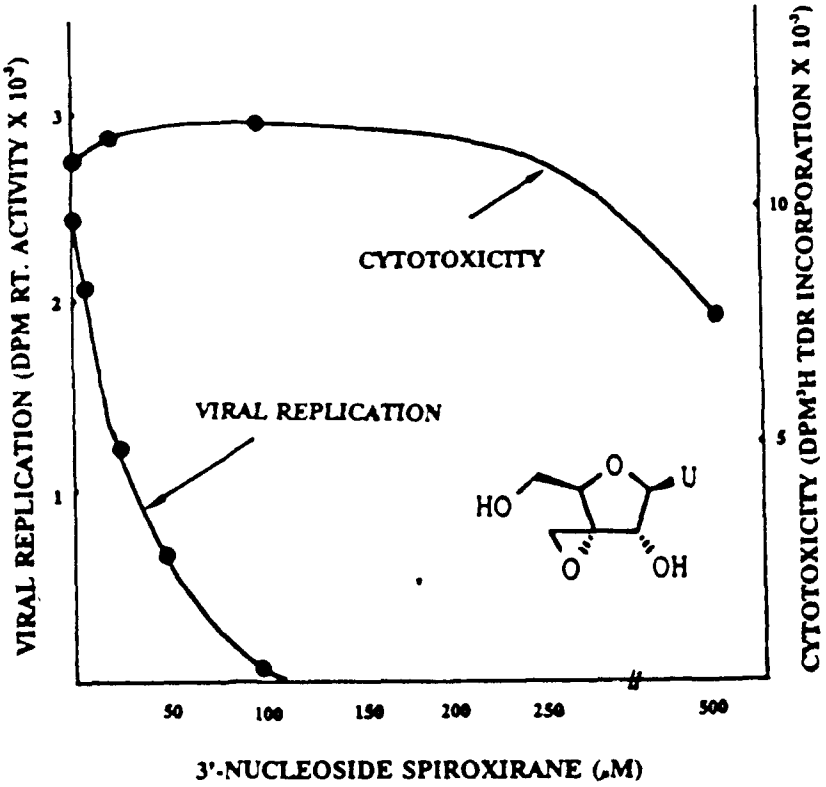
A series of approximately 30 nucleoside analogs, suggested by the considerations outlined in section B, were synthesized. These were screened for ability to inhibit replication of equine infectious anemia virus growing in equine derma. fibroblasts. This is a lentivirus having a close homology with HIV and is non pathogenic to humans. It is used as a preliminary screen for antiviral drugs before testing against HIV grown in A 3.01 human T-lymphocytes as described in section D. Cytotoxicity was evaluated at concentrations from 25-500μM in IL-2 supplemented CTLL T-Lymphocyte cultures pulsed with ^{3}H thymidine at 24 and 48 hours.



Antiviral Activity of Some Epoxy- and Oxirane Nucleosides:

Viral replication was monitored by RT assay on the pelletized virions from the culture medium. As illustrated in the figure above, 7 of these compounds inhibited viral replication by greater than 50% when present in the culture medium at a concentration of 100μM. The two most effective analogs were the uridine 2', 3'-lyxo epoxide (compound 2) and the uridine 3'-ribospiroxirane (compound 7). Both compounds were also tested for cytotoxicity by measuring their effects on ^{3}H-thymidine incorporation in the IL-2 dependent CTLL T-lymphocyte cell line in tissue culture.

The 3'-uridine spiroxirane derivative was added to control and EIAV infected cultures over a range of concentrations from 5 to 100μM and reverse transcriptase activity was assayed on the pelletized virions after 8 days. Virus replication was almost completely inhibited by 100μM concentrations of the 3' uridine spiroxirane the I_{50} being about 25μM. The corresponding 2'-spiroxirane used as a control had no effect at similar concentrations. The 3'-spiroxirane was non-toxic to T-lymphocytes,the I_{50} for inhibiting ^{3}H-thymidine incorporation being greater than 500μM. Since the epoxide derivative was somewhat inhibitory to T-cell proliferation at concentrations above 100μM, whereas the spiroxirane analog was not, the nucleoside spiroxirane family has been selected for further in depth characterization in this project.



Antiviral Activity of Uridine 3'-ribospiroxirane.

Cultures of equine dermal cells were infected with a standard inoculum of the EIAV retrovirus in the presence of increasing concentrations of uridine 3' spiroxirane. Virus replication was measured by following release of reverse transcriptase into the culture medium. In separate experiments growth inhibition of cultured T lymphocytes was evaluated by measuring incorporation of tritiated thymidine (TdR) into cellular DNA. Note the greater sensitivity of the viral versus the cellular polymerases presumably due to the ability of the cellular polymerases to "proofread" and excise the unusual nucleoside.

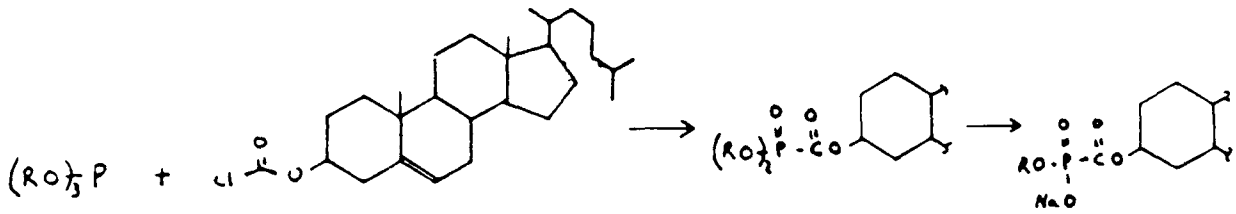
Antiviral Properties of the Sterol Phosphonoformates

Despite the sensitivity of the HIV-RT to inhibition by PFA, replication of the virus in tissue cultu is relatively insensitive, the I_{50} being of the order of 50 μM.

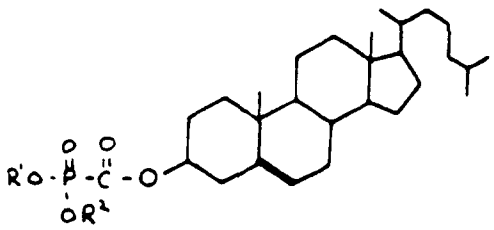
We have shown in previous studies, by use of sterol ester analogs such as the sterically hindered a hydrolysis resistant cholesterol $\alpha\alpha$-methyl ethyl caproate (CMEC), that intact cholesterol esters enter ce via a specific endocytotic transport process To enhance deliverability of the active PFA moiety to endosomal sites of viral replication, cholesteryl phosphonoformate ester analogs of PFA have be synthesized and characterized.

Cholesteryloxycarbonylphosphonoformates:

A series of mono- and di-alkyl cholesteryloxycarbonylphosphonoformates have been successfu prepared via an Arbozov reaction between trialkylphosphites and cholesterylchloroformate.



Triesters gave acceptable spectroscopic characteristics and were selectively hydrolyzed to the mon and di-sodium salts



Properties of Some Sterol Phosphonoformates.

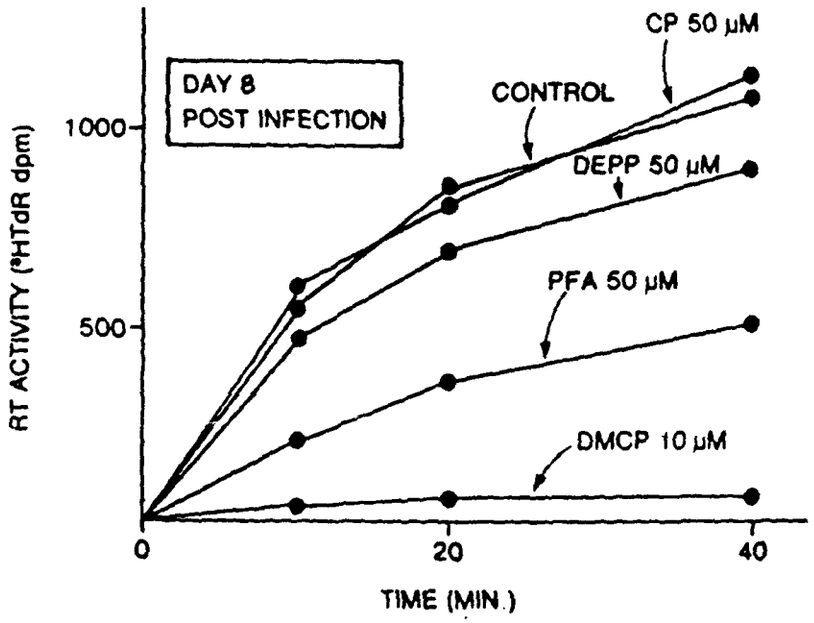
Cmpd,5	R^1	R^2	MP (C°)	R_f
a	C_2H_5	C_2H_5	110-111	0.60 (A)°
b	$CH_2\ CH_2\ CH\ (CH_3)_2$	$CH_2\ CH_2\ CH\ (CH_3)_2$	67-69	0.51 (B)
·	$CH_2\ CHO\ (CH_3)_2\ OCH_2$	$CH_2 CHO\ (CH_3)\ OCH_2$	106-108	0.30 (A)
d	$CH_2\ CH_2\ CH_2\ CO_2\ C_2H_5$	$CH_2\ CH_2\ CH_2\ CO_2\ C_2H_5$	oil	0.33 (A)
e	C_2H_5	Na	≥220	0.29 (C)
f	Na	Na	≥300	-

° Solvent systems: A, 50% ethyl acetate/hexanes, B, 20% ethyl acetate/hexanes, C, 52/14/1/1 $CHCl_3/CH_3OH$/con NH_4OH/H_2O.

A number of compounds of this type having both aromatic and aliphatic substituents have been test for antiviral activity in vitro, as detailed below. Incorporation of the sterol moiety in an appropriate triesi environment can result in compounds having 20-30 times the antiviral potency of the parent compound PF Table lists the effect of increasing the number and size of the R groups on the melting point a chromatographic mobilities of some of these derivatives.

Pending development of the low biohazard CPE assay for HIV infectivity described below, t antiviral potency of the drugs was evaluated against Equine Infectious Anemia Virus (EIAV) growing in fe equine fibroblasts. This is a lentivirus with a reverse transcriptase having the highest degree of homolo with HIV-1 RT. The assay consists in measurements of RT activity on pelletized supernatants of infect cultures 8 days following infection. These sterol esters of phosphonoformate show an interesting pattern antiviral activity in this system

ANTI-VIRAL ACTIVITY OF SOME STEROL PHOSPHONOFORMATES



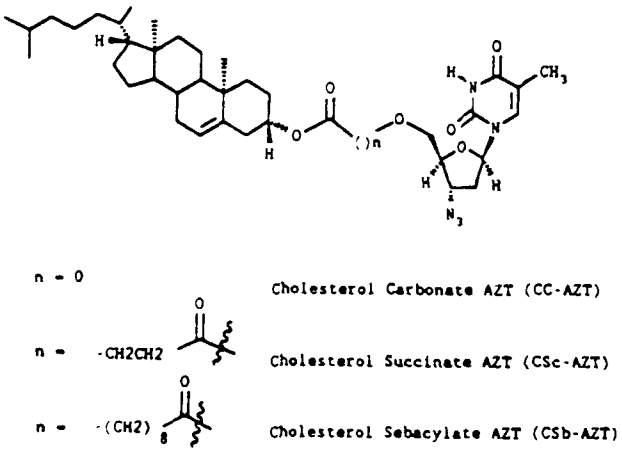
The approximate I_{50} for inhibition of EIAV replication by PFA is between 40 and 50 μM. The liethyl-phenyl-phosphonoformate ester (DEPP) is only about 20% as active as PFA and the doubly charged lisodium cholesterol phosphonoformate (CP) is essentially inactive. Substitution of the phenyl group with cholesterol group, however, results in a major enhancement of activity. For example, the closely related DMCP (lower curve, in which cholesterol is esterified to the formate moiety, has 10-20 times more nti-viral activity than the parent compound PFA. Some of the more active analogs display activities close o the theoretical maximum predicted by the enzyme kinetic studies.

The sterol phosphonoformates display another useful property since the antiviral effects persist fc a considerable time (up to 8 days) after removal of the drug from the culture medium. It seems likely th: this prolonged antiviral protection may be due to intracellular accumulation of the analog followed by slo hydrolysis and sustained release of the active PFA moiety. One of the objectives in this Project is to explo: this observation in more depth by measuring rates of intracellular accumulation and hydrolysis of ster phosphonoformates using ^{14}C-labelled compounds synthesized by analagous procedures. In this way ligan which enhance uptake can be distinguished from those which enhance (or reduce) hydrolysis. Th information will be used to enhance pharmacokinetic properties which may prove clinically useful.

SYNTHESIS AND ANTIVIRAL ACTIVITY OF AZT-STEROL DICARBOXYLATES:

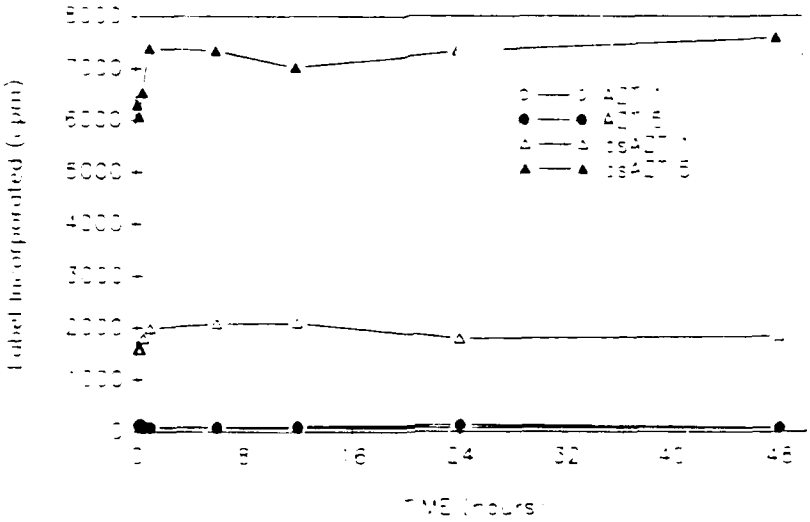
^3H-labelled AZT was prepared by NaB^3H_4 reduction of the 5'aldehyde derivative and coupled cholesterol by a C_{10} (Sebacate) linker as described in section D, to yield cholesteryl sebacyl ^3H-AZT (CSE AZT). Unlabelled cholesteryl succinyl AZT (CS-AZT) and cholesteryl carbonate AZT (CCAZT) having tl structures illustrated below, were also synthesized

STRUCTURES OF SOME SYNTHETIC AZT STEROL DIESTERS



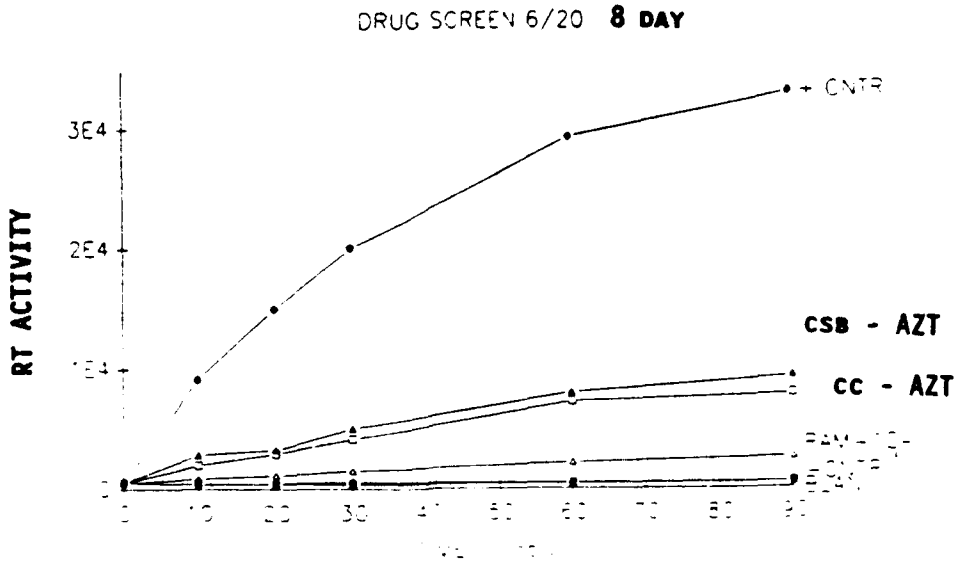
The uptake and accumulation of the ^3H-labelled AZT sterol sebacylate was compared with that c ^3H-AZT in the human T-lymphocyte cell line over a 48 hr period at two concentrations (1 μM and 5 μM in the medium. Cells were harvested at intervals and washed using a Mash harvester before countin Maximum accumulation and rentention (following the wash procedure) occured within 1 hour and w approximately 15 and 80 fold greater for the sterol derivatives than for free AZT at the two concentratior (1μM and 5μM) respectively

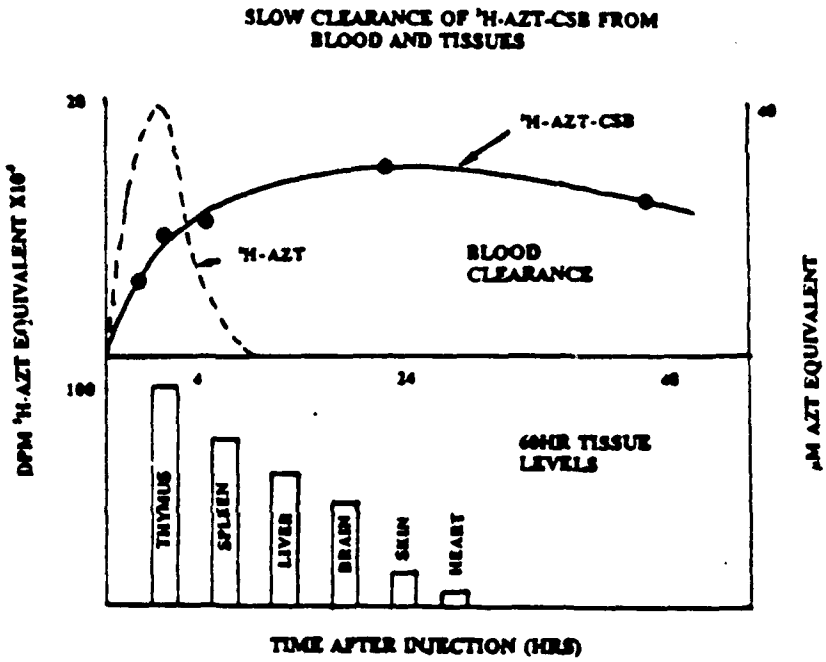
ENHANCED ACCUMULATION OF RADIOLABELLED CSB - AZT IN T LYMPHOCYTES



The unlabelled AZT-sterol dicarboxylates showed good antiviral activity at similar concentratio as measured by suppression of viral RT levels in culture supernatants Figure suggesting that the prodru were being hydrolyzed intracellularly to the free form. Use of the radiolabelled analogs to explore facto regulating this intracellular accumulation and release will be a major objective of the further studies propos in this project.

ANTIVIRAL ACTIVITY OF SOME STEROL AZT DIESTERS





Tissue Distribution of ³H-AZT-CSB 25 Hours and 60 Hours Following Injection of a Single 20mg/kg Equivalent Dose

Tissue	25 Hrs			60 Hrs		
	Sample dpm	dpm per mg	μM AZT equivalent	Sample dpm	dpm per mg	μM AZT equivalent
liver	42596	38.4	33	57594	62.6	54
spleen	10247	183	159	9873	80.3	70
thymus	1606	61.8	54	1661	139	121
ovary	--	--	--	2273	59.8	52
skin	1398	20.9	18	1589	8.4	7
stomach	11500	134	117	10779	38.2	33
lung	2311	25.1	22	11324	104	90
heart	21275	154	134	707	4.5	4
kidney	18073	38.8	34	7183	37.8	33
brain	3914	9.3	8	3922	42.5	37
muscle	2043	16.7	15	4516	36.7	32

SYNTHESIS OF NUCLEOSIDE TRIPHOSPHATE ANALOGS FOR ANTIVIRAL TESTING:

The classical procedures for synthesis of nucleotide triphosphates require relatively large quantities of the nucleoside precursor and frequently give poor yields.

Ludwig and Eckstein (J. Org. Chem. 1989, 54,631-635) have reported an approach involving condensation of an activated nucleoside derivative with inorganic pyrophosphate. In this synthesis 2-chloro-4H-1,3,20-benzodioxaphosphorin-4-one phosphitylates the 5'-hydroxy group of a nucleoside to form an intermediate 2, which on subsequent reaction with pyrophosphate produces a nucleosidylcyclotriphosphite 3. This intermediate is oxidized with iodine/water to furnish nucleoside 5'-triphosphate. The procedure is suitable for use with low milligram quantities.

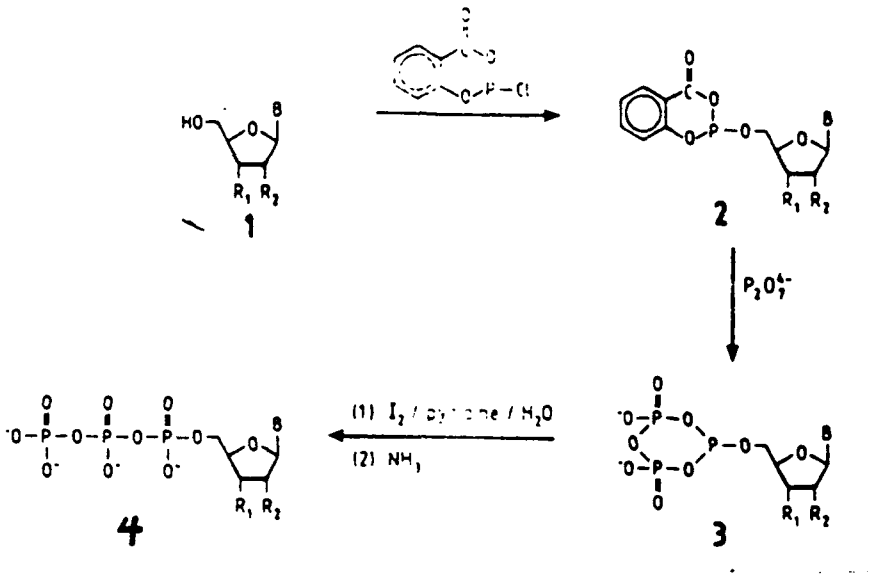
The series of reactions illustrated in Scheme I was successfully applied to microscale preparations of the 5'-triphosphates of both AZT and 3' uridine spiroxirane 1a which was previously synthesized and shown to have antiviral activity.

The formation of intermediate 3 was detected by ^{31}P NMR spectroscopy. The observed chemical shifts are comparable to those reported in the literature. The spectrum is of the ABX type where the AB part corresponds to the two phosphate groups with close but not identical shifts and the X part corresponds to the trivalent phosphorous atom. Oxidation of the intermediate 3 yields the normal triphosphate. In the ^{31}P NMR spectrum, the triplet of the trivalent phosphorous atom of the intermediate compound 3 disappears indicating complete oxidation.

We suspected some nucleotide by-products due to the contamination by a small amount of triphosphate. Also hydrolysis of intermediate 2 could result in phosphorous containing contamination detected by ^{31}P NMR. These by-products must copurify with the unreacted nucleoside on DEAE-cellulose chromatography.

The antiviral activity of the synthesized AZTTP was tested and proved comparable to, and in some cases even more effective than, a sample provided by the Burroughs Wellcome Co., thus validating the procedure. The nucleoside spiroxirane triphosphate may exist in two different isomeric forms which may or may not have identical antiviral activity. We have isolated one isomer that has proved antiviral activity. Isolation and characterization of the other isomer requires further investigation. The validated procedure was then applied to synthesize the 5' triphosphate of 3' uridine spiroxirane.

Scheme I



The phosphitylating agent 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one was purchased from Aldrich. Dimethylformamide (DMF) was dried over $MgSO_4$ and distilled under reduced pressure. Anhydrous dioxane was distilled from LAH. Anhydrous pyridine was prepared by fractional distillation of refluxing pyridine with potassium hydroxide.

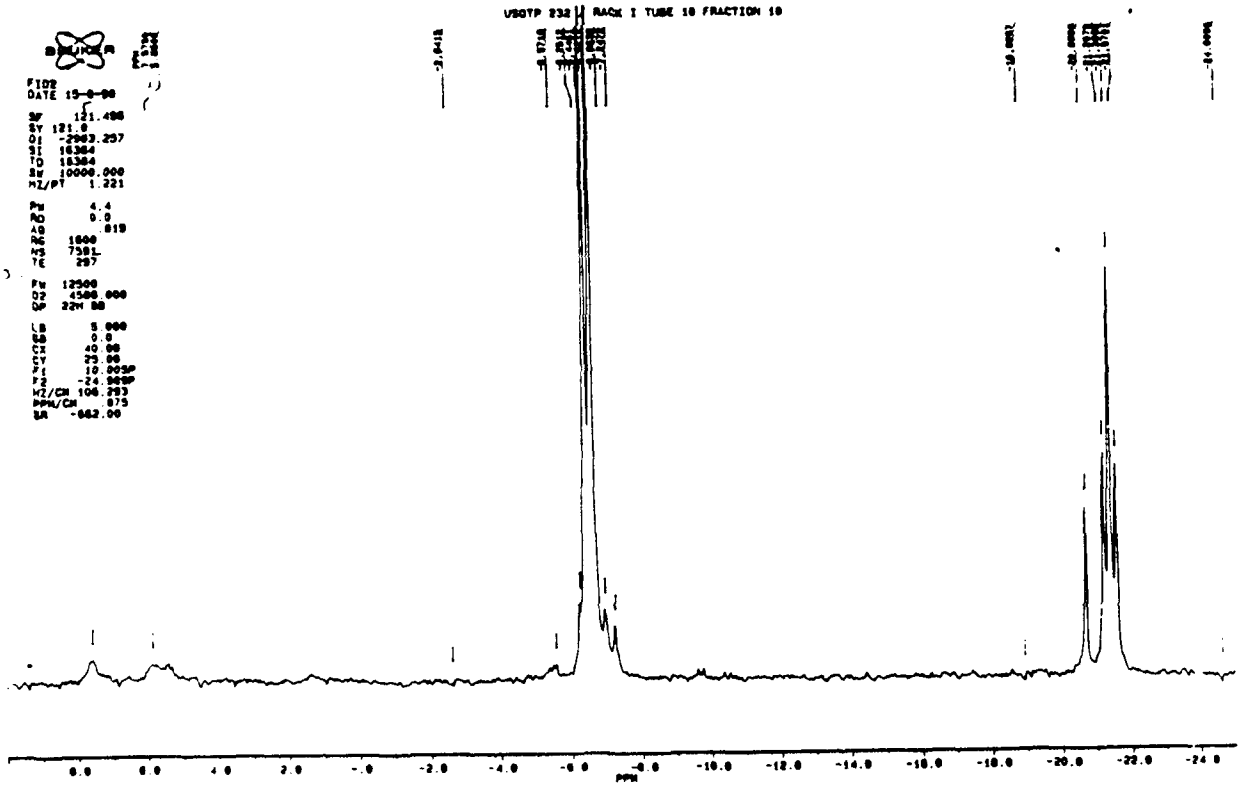
^{31}P NMR spectra were recorded on a Brucker 300 spectrometer with broad band decoupling. TLC was performed on either silica GHLF (Analtech) developed with isopropanol, ammonia, water (3:1:1) (system 1) or on DEAE-cellulose (Analtech) developed with 0.02 N hydrochloric acid (system 2).

Bis(tri-n-butylammonium) pyrophosphate. Tetrasodium diphosphate decahydrate (2.23 g, 5 mmol) was dissolves in water (50 ml), the solution was applied to a column of Dowex 50WXB in the H^+ form and the column was washed with water. The eluate was directly dropped into a cooled (ice water) and stirred solution of tri-n-butylamine (2.38 ml, 10 mmol) in ethanol (20 ml). The column was washed until the pH of the eluate increased to 5.0 (approximately 70 ml of water). The ethanol/water solution was evaporated to dryness and reevaporated twice with ethanol and finally with anhydrous DMF and diluted to 10 ml. This solution was stored over 4-Å molecular sieves.

Nucleoside spiroxirane 1a (100 μmol) was dissolved in anhydrous pyridine/DMF, 1/4, V/V and evaporated to dryness in vacuo. The residue was dried further over P_2O_5 under reduced pressure for 2 hours at room temperature. The reaction flask was filled with nitrogen, during all the following manipulations a small positive pressure of nitrogen was maintained in the reaction vessel. Anhydrous pyridine (200 μl) and DMF (800

μl) were injected through septum. A freshly prepared 1 M solution of 2-chloro-4H-1.3.2-benzodioxaphosphorin-4-one in anhydrous dioxane (110 μl, 110 μl) was then injected into the well-stirred solution of nucleoside. After 15 minutes a well-vortexed mixture of a 0.5 M solution of bis (tri-n-butylammonium) pyrophosphate in anhydrous DMF (300 μl) and tri-n-butylamine (100 μl) was quickly injected and the reaction mixture was stirred for 10 minutes. A solution of 1% iodine in pyridine/water (98/2, V/V) (2 ml, 157 μmol) was then added. After 15 minutes excess iodine was destroyed by adding a few drops of a 5% aqueous solution of $NaHSO_3$ and the reaction solution was evaporated to dryness. The residue was dissolved in water and applied to a DEAE-cellulose column which was eluted with a linear gradient of 800 ml of each 0.05 M and 1 M TEAB. The fractions were characterized by NMR spectroscopy. The presence of the characteristic triphosphate group was confirmed by NMR as indicated in the figure below.

NMR SPECTRUM OF 3' URIDINE SPIROXIRANE TRIPHOSPHATE DERIVATIVE



ENZYME INHIBITION STUDIES WITH 3' URIDINE SPIROXIRANE TRIPHOSPHATE

The recombinant HIV-reverse transcriptase was expressed in the vaccinia virus construct VCF21 as described in previous progress reports. The enzyme was purified from culture fluids by column chromatography on DEAE cellulose and carboxy-methyl cellulose columns. The purified enzyme was used to synthesize DNA using a poly rAdT template and following the enzyme activity by measuring incorporation of ^{3}H labelled dTTP. The synthesized ^{3}H DNA was collected on acid washed filter, and counted in a scintillation counter. It was found that 3' uridine spiroxirane was an excellent inhibitor of the enzyme in this system. The inhibition was time and concentration dependent consistent with the irreversible inhibition associated with a suicide inhibitor. More detailed kinetic studies on reversibility however will be required to establish this and to distinguish the kinetics observed from those of a chain terminating inhibitor.

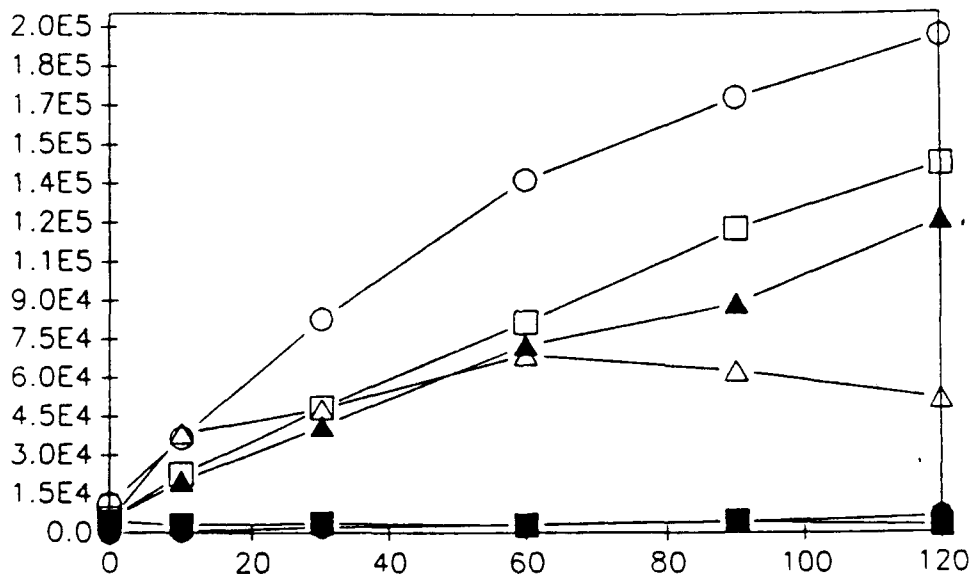


Figure: Activity of HIV-reverse transcriptase and inhibition by synthetic 3' uridine spiroxirane triphosphate

Key ○ control □ 0.05 μM △ .25 μM △ .25 μM ■ 2.5 μM ● Reagent blank

The triphosphate of 3' uridine spiroxirane was more potent as an inhibitor of the HIV reverse transcriptase than the triphosphate of AZT synthesized and tested in this system under the same conditions.

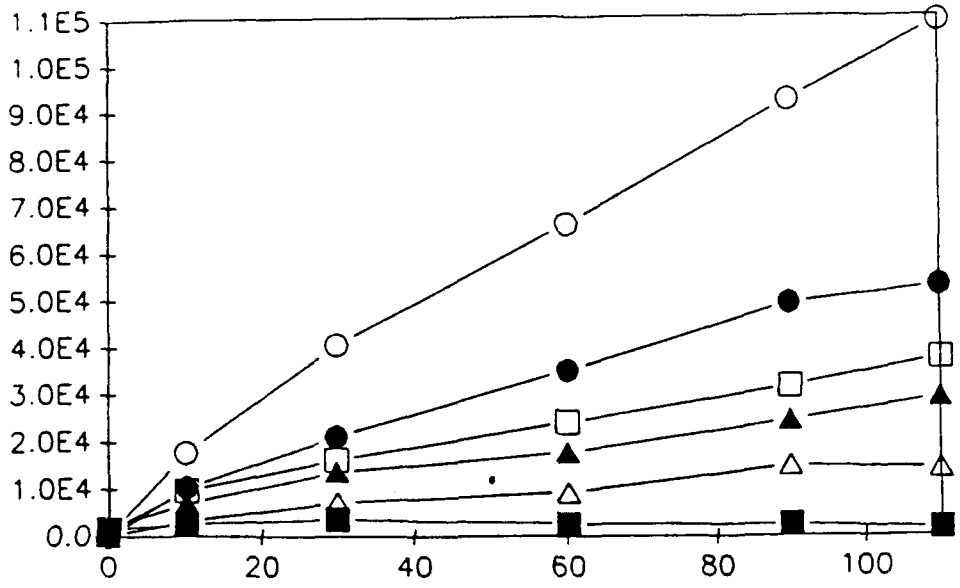


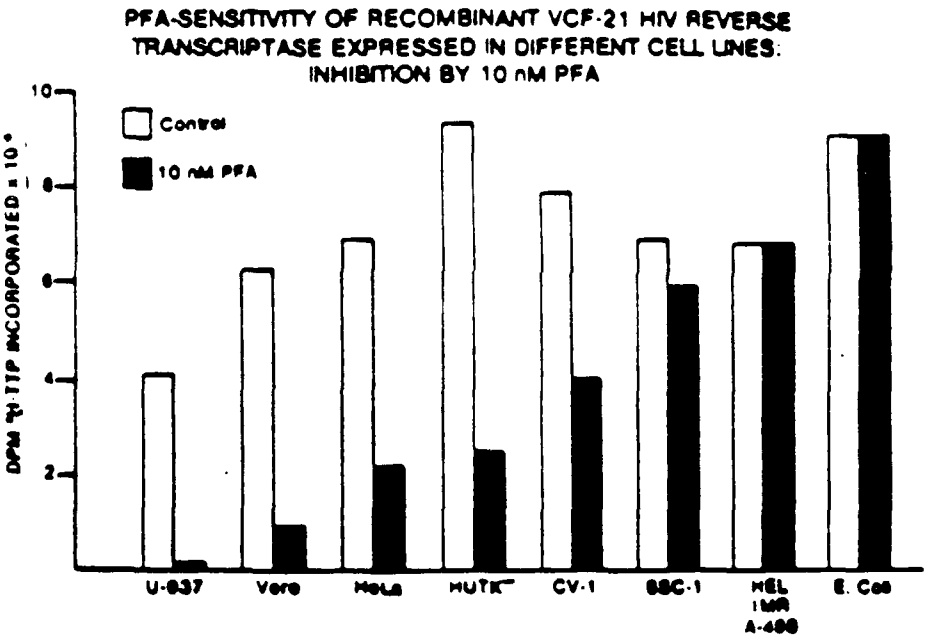
Figure: Inhibition of HIV-reverse transcriptase by synthetic AZT-triphosphate

Key ○ control ● .05 μM □ .25 μM △ .25 μM △ 2.5 μM ■ Reagent blank

Preliminary experiments have been conducted to characterize further the nature of the inhibition by 3' uridine spiroxirane triphosphate. The inhibition is further confirmed as irreversible suicide type by the fact that addition of excess template does not reverse it, thus distinguishing it from that of a chain terminating inhibitor where new template would be expected to overcome the inhibition.

Expression of HIV-Reverse Transcriptase In Different Cell Lines.

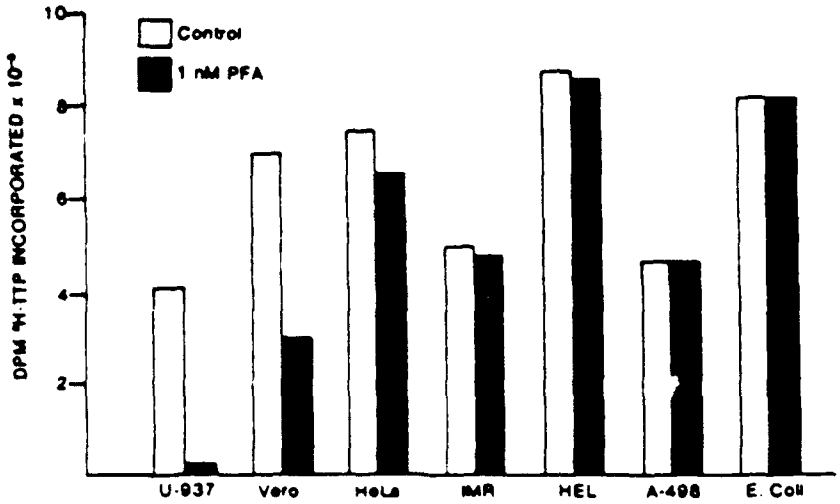
In order to determine if the recombinant HIV-reverse transcriptase was expressed in different forms depending upon the cell type, the vaccinia VCF-21 construct was grown in a number of different cell lines of both human, monkey and rodent origin. The expressed reverse transcriptase was tested for inhibition by Foscarnet at two different levels (1 and 10 nanomolar) and compared to the E. Coli recombinant HIV-RT (Kindly donated by Dr. Steven Hughes Fort Detrick M.D.) and the wild type HIV-RT. Both the wild type and E. Coli HIV-RT's were resistant to PFA showing essentially no inhibition at the 10nM level. Previous studies have shown that both enzymes have I_{50}'s for PFA in the 200-400 nM range. The recombinant HIV-RT expressed in eukaryotic cells however showed a range of phenotypes as indicated in figures 3 and 4 below. Both U-937 and Vero cells expressed enzyme sensitive to 1 nanomolar PFA, whereas human embryo lung and A-498 cells expressed RT-enzyme having wild-type sensitivity. Hela, HuTK- and CV-1 cells as observed previously expressed enzyme having intermediate PFA sensitivity



Sensitivity of Recombinant HIV-Reverse Transcriptases to 10 Nanomolar PFA.

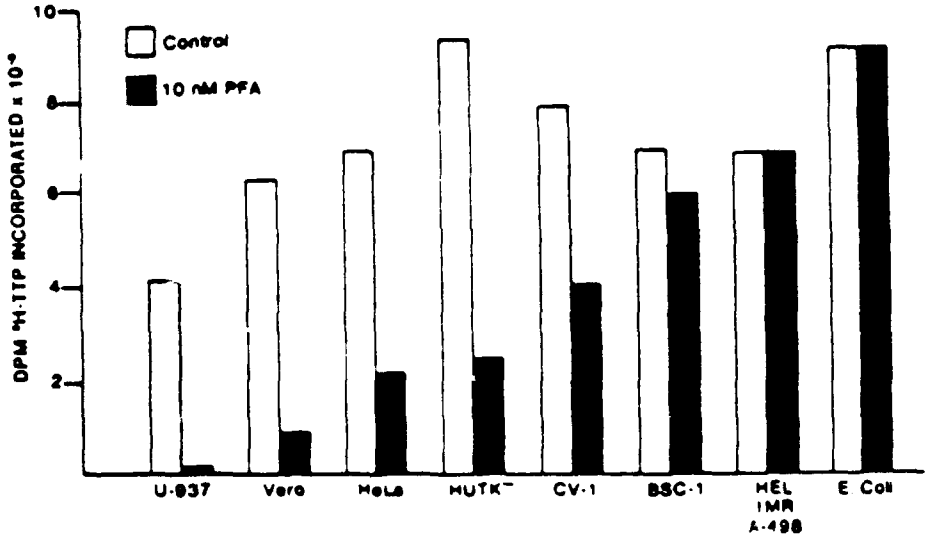
The VCF-21 vaccinia construct was grown in the indicated cell lines and the activity of the expressed reverse transcriptase was measured in the presence (dark blocks) or absence (open blocks) of 10 nanomolar PFA.

expressed RT-enzyme having wild-type sensitivity Hela, HuTK- and CV-1 cells as observed previously expressed enzyme having intermediate PFA sensitivity



Sensitivity of Recombinant HIV-Reverse Transcriptases to 1 Nanomolar PFA.

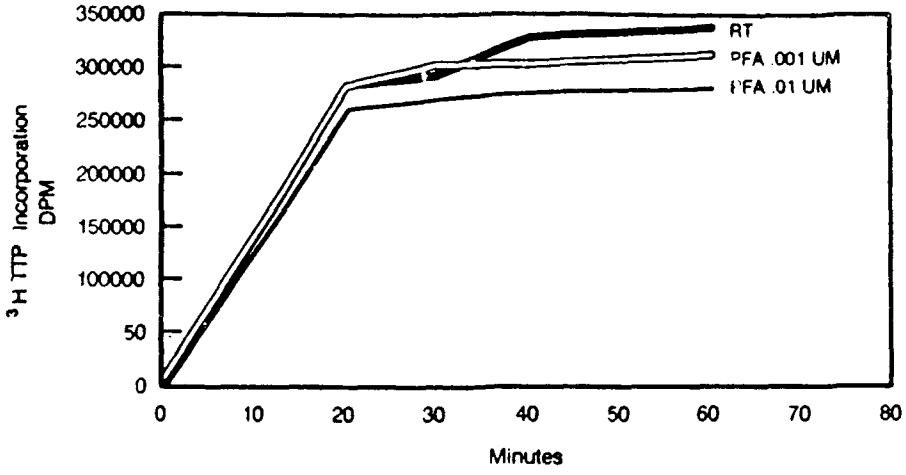
The VCF-21 vaccina construct was grown in the indicated cell lines and the activity of the expressed reverse transcriptase was measured in the absence (open blocks) or presence of 1 nanomolar PFA. The height of the open blocks is a measure of the yield of recombinant enzyme activity in the different cell lines. Note the extreme sensitivity of the HIV-RT to PFA when expressed in the U-937 macrophage and vero monkey kidney cell lines.



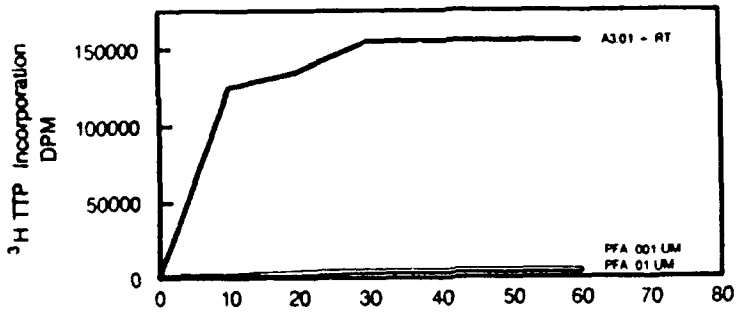
Sensitivity of Recombinant HIV-Reverse Transcriptases to 10 Nanomolar PFA.

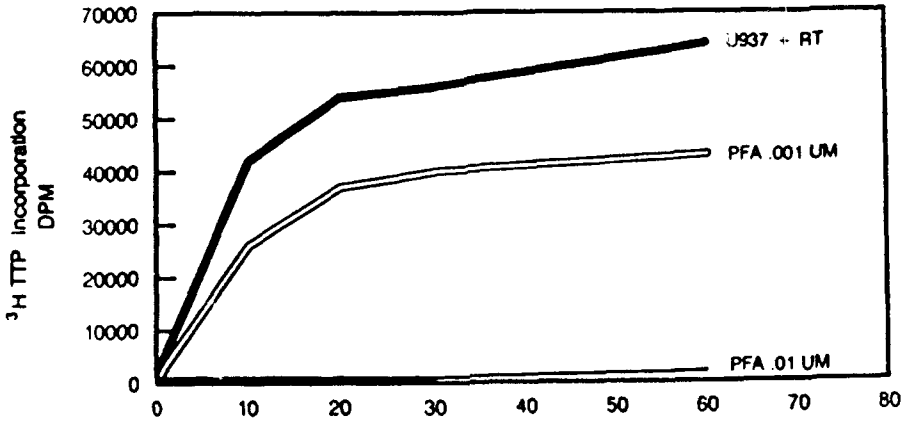
The VCF-21 vaccinia construct was grown in the indicated cell lines and the activity of the expressed reverse transcriptase was measured in the presence (dark blocks) or absence (open blocks) of 10 nanomolar PFA. Note that the enzyme produced in HEL I MR and A498 cultures resembles the E. Coli recombinant and wild type HIV-RT in being relatively resistant to PFA The enzyme expressed in HeLa, HVTK and CVI cells is of intermediate sensitivity and that expressed in U-937 and vero cells is maximally inhibited by PFA. Sensitivity of the vero enzyme remained following purification. Cross sensitizing experiments on mixtures of PFA sensitive and resistant preparations and measurements of RNAse H activity of all constructs are in progress

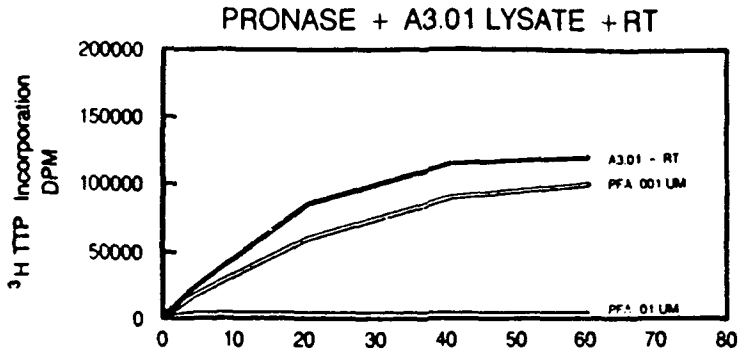
RECOMBINANT HIV RT (E. COLI) + FOSCARNET



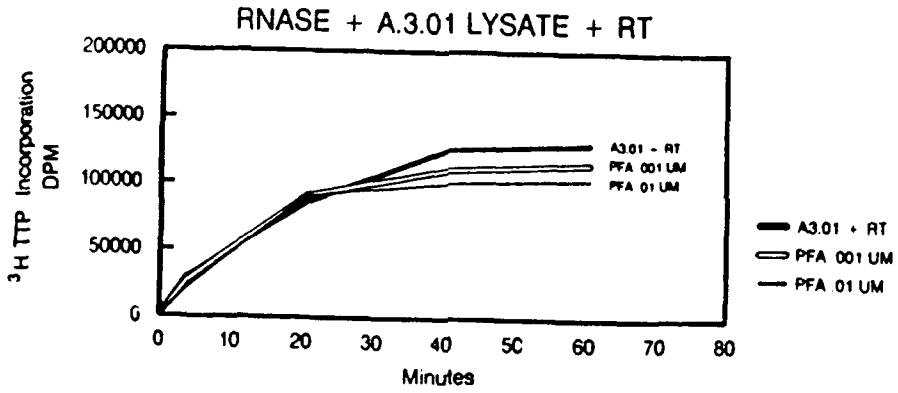
SENSITIVITY TO FOSCARNET INHIBITION OF HIV REVERSE TRANSCRIPTASE (PURIFIED) IN THE PRESENCE OF CELL LYSATES

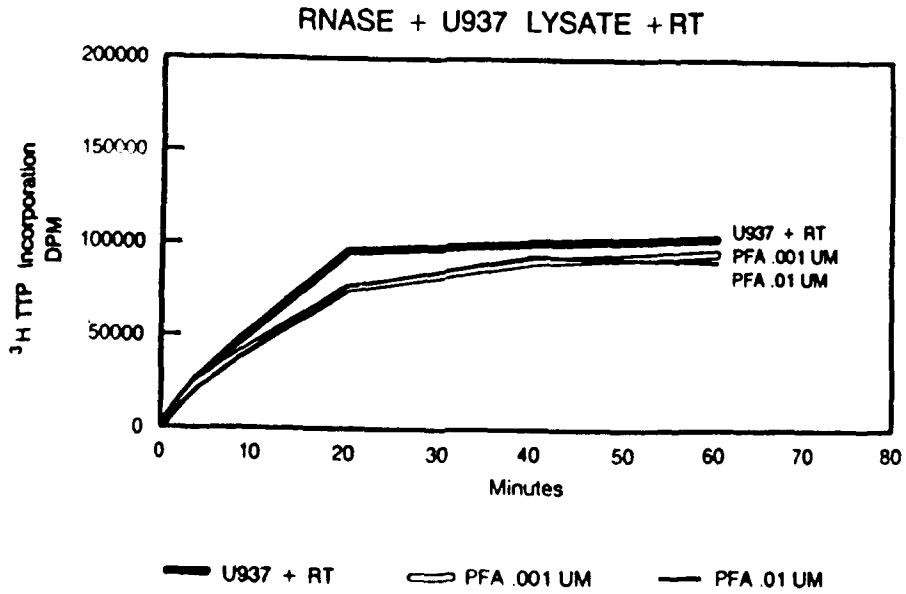






SENSITIVITY TO FOSCARNET INHIBITION OF HIV REVERSE TRANSCRIPTASE (PURIFIED) IN THE PRESENCE OF CELL LYSATES





Appendix I

PUBLICATIONS

PROJECT: DAMD 17-87-C-7171

TITLE: SUICIDE INHIBITOR OF REVERSE TRANSCRIPTASE IN THERAPY OF AIDS AND OTHER RETROVIRUSES.

PRINCIPAL INVESTIGATOR: Dr. J.M. Bailey, Ph.D., D.Sc.
Professor of Biochemistry

PRODUCTIVITY REPORT

Publications:

1. Enhanced sensitivity to Foscarnet of first-strand viral replication by recombinant HIV-reverse transcriptase. M.M. Lightfoote and J.M. Bailey. FASEB J. 4:1318 (1990).

2. Differential sensitivity of wild-type and recombinant HIV-Reverse transcriptase to inhibition by Foscarnet. M.M. Lightfoote and J.M. Bailey. Proc Vth Int. AIDS Conf. Montreal. 5:515 (1989).

3. M.M. Lightfoote and J.M. Bailey. Somatic Cell Modulation of HIV-Reverse Transcriptase Expression. Antiviral Chem. and Chemotherapy. (1989) in preparation.

4. Nucleotide and template selectivity for inhibition of reverse transcriptase by PFA: Implications for retroviral therapy. J.M. Bailey and M.M. Lightfoote. Proc. IVth Int. AIDS Conf. Montreal. 4:3223 (1988).

5. Differential sensitivity of wild-type and recombinant HIV-reverse transcriptase to inhibition by foscarnet. M.M. Lightfoote and J.M. Bailey. Proc. IVth Int. AIDS Conf. Montreal. (1989).

6. Antiviral activities of some sterol phosphonoformate diester. J.M. Bailey, K. Nelson, M. Lightfoote. J. Clinic. Exp. Ther. in preparation.

7. Nucleoside spiroxiranes: A new class of retroviral inhibitor. J.M. Bailey, K. Nelson, M. Lightfoote. J. Virol. in preparation.

8. Synthesis and antiviral activities of some sterol dicarboxylate esters of 3' Azido thymidine (AZT). J.M. Bailey, R.M. Mook, M. Lightfoote. J. Clin. Exp. Ther. in preparation.

9. Synthesis of mono and di-substituted cholesterol phosphonoformates by the Arbuzov reaction. J.M. Bailey and Keith Nelson. Tetrahedron Letters. in preparation.

10. Drug sensitizing RNA interactions with HIV reverse transcriptase. J.M. Bailey and M. Lightfoote. Biochem. Soc. Trans. (1991) in press.

Appendix II

COMPOUNDS SYNTHESIZED:

Compounds synthesized and prepared for shipment to USAMRIID for antiviral testing.

1. 2',0^2-Anhydrouridine
2. 2',0^2-Anhydrocytidine hydrochloride
3. 3',5'-Di-0-benzoyl-2'-0^2-anhydrouridine
4. 5'-0-t-Butyldimethylsilyl-3'-0-benzoyl-2',0'-anhydrouridine
5. 2',3'-Anhydro-5'-0-trityluridine
6. 3'-Deoxy-2'-thymidinene
7. N^3-Benzyl-2',5'-di-0-trityluridine
8. 5'-0-t-Butyldimethylsilylanhydrouridine
9. N^4-Benzoyleytidine
10. 2',3'-Di-0 -mesyl-5'-0-trityluridine
11. 5'-0 -t-Butyldimethylsilyl-2',3'-isopropylideneuridine
12. 2',3'-Isopropylideneuridine
13. 2',3'-0-Sulfinyluridine
14. 2',3'-Benzylideneuridine
15. N^4-Benzoyl-2',3'-0-Sulfinylcytidine
16. 2',3'-0-Sulfinylcytidine
17. 3',5'-Di-0-trityl-2'deoxy-2'-oxouridine
18. 3',5'-Di-0-t-butyldimethylsilyl-2'-deoxy-2'-oxouridine
19. 2',5'-Di-0-t-butyldimethylsilyl-3'-deoxy-3'-oxouridine
20. Diethyl (cholesteryloxycarbonyl) phosphonate
21. Disodium (cholesteryloxycarbonyl) phosphonate
22. Di-[1-(3-carboethoxypropyl)] cholesteryloxycarbonyl
23. Di-(2,3-isopropylideneglyceryl) cholesteryloxycarbonyl phosphonate
24. Di-[1-(3-methylbutyl)] cholesteryloxycarbonyl phosphonate
    Di-[1-(lithium 3-carboxypropyl)] cholesteryloxycarbonyl phosphonate
25. Sodium ethyl (cholesteryloxycarbonyl) phosphonate

26. Sodium 1-(3-carboxypropyl) 1-(30 carboethoxypropyl) [cholesteryloxycarbonyl] phosphonate

27. Adenosine 2',3'-Riboepoxide

28. Thymidine 5'-(1,3,2-dioxaphosphorin-2-oxide)

29. Thymidinene 5'-(1,3,2-dioxaphosphorin-2-oxide)

30. Thymidinene

31. 2-Ethoxy-5-chloro-6-methyl-1,3,2-dioxaphosphorin-5-ene-2-oxide

32. 2-Ethoxy-5-chloro-1,2-oxaphosphol-4-ene-2-oxide

33. 2,4-dichloro-5-methyl-1,3,2-dioxaphosphole-2-oxide

34. 2-methoxy-4,5-dimethyl-1,3,2-dioxaphole-2-oxide

35. Thymidine 3',5'-oxetane

Appendix III

TEST DATA ON ANTI HIV DRUGS

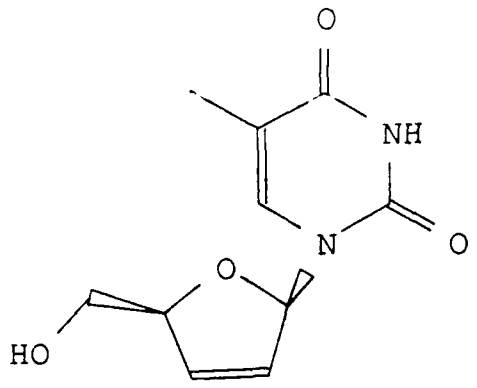
USAMRIID

Antiviral Drug Screening Program

05/18/90

STRUCTURE

CHIRAL



SUBMITTER	CTR NO	AVS NO
01141.01	KN-II-55	AVS-006466
DATE RECD	**AMT RECEIVED [mg]**	**MOL WT (au)**
12-28-89		224.218

HANDLING/STORAGE

SOLUBILITY

STABILITY

ALT NAME

2',3'-DIDEOXYTHYMIDINENE

COMPOUND NAME

2',3'-DIDEOXYTHYMIDINENE

SCREEN INSTRUCTION

PRIORITY=PT>VEE>YF>KHF>PIC>JE>SF>VV>AD2>VSV

IN VIVO TOXICITY [mg/kg]

HOST	VH	RTE	LD50	MTC	LAB	PR	DATE

IN VITRO SCREEN [ug/ml]

VIR	VR	VR+	ID50	CELL	MTC	TI	TI+	LAB	PRT	DATE
HIV			4.99	MT2	48.8	13.29		SO	MTT	
HIVC			.32	CEM	51.5	> 222.13		SO	MTT	
JE			NOT ACT	VERO	84.3	0		SO	MTT	90-03-06
PT			NOT ACT	VERO	111	0		SO	MTT	90-03-06
SF			NOT ACT	VERO	93.8	0		SO	MTT	90-03-06
VEE			NOT ACT	VERO	232	0		SO	MTT	90-03-09
YF			NOT ACT	VERO	100	0		SO	MTT	90-03-06

IN VIVO SCREEN [Dose = mg/kg]

VIR	HST	VR	VR+	DOSE	MTC	VEH	RTE	D	TOX	SP	L	PR	DATE

PLATE 1HK
DRUG 6466

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6466
TAI: 35.67 SI: 9.78

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.128	0.131	0.131	0.130	0.130	0.131	0.037	0.034	0.035	0.035	0.034	0.034
	tox	cc/vc	drug 6466 experimental			tox					cc/vc	
B	1.400	1.627	0.356	0.363	0.376	1.639					1.651	
C	1.362	1.625	0.418	0.386	0.384	1.622					1.588	
D	1.367	1.657	0.627	0.570	0.580	1.712					1.666	
E	1.430	0.369	1.821	1.729	1.850	1.785					0.358	
F	1.481	0.364	1.581	1.414	1.737	1.753					0.343	
G	0.160	0.368	0.127	0.130	0.142	0.156					0.338	
	drug 6466 colorimetric background											
H	0.133	0.129	0.131	0.135	0.131	0.132						

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

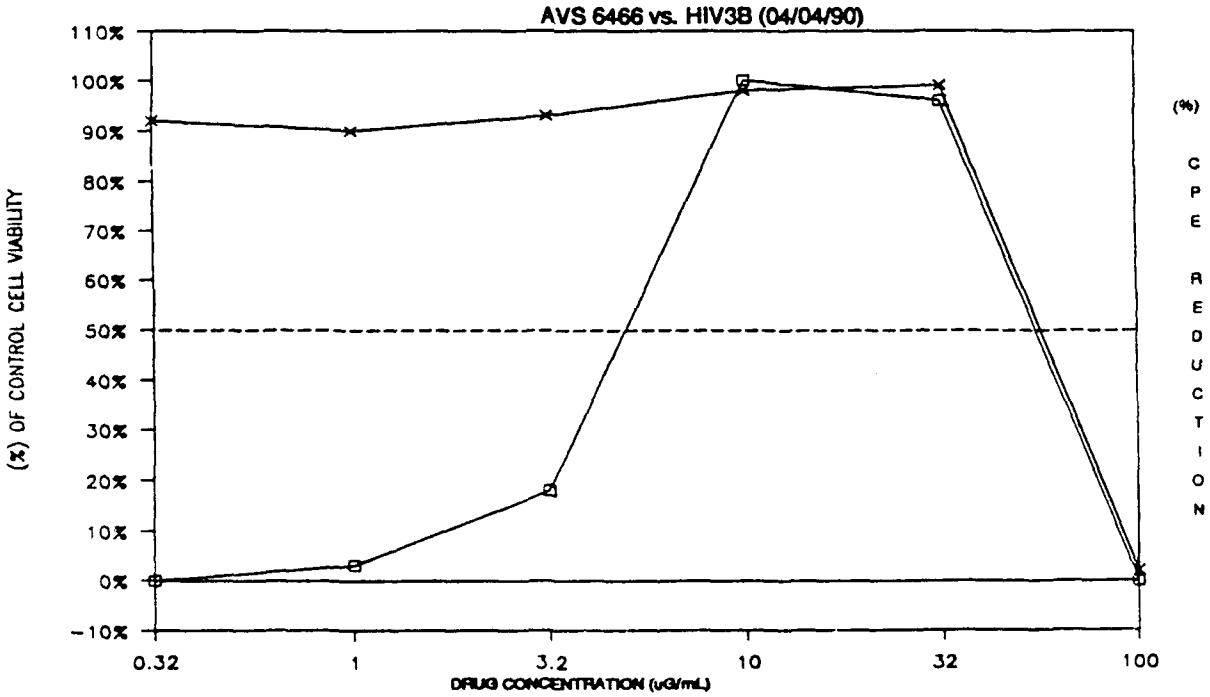
VIRUS	HIV3B		PROJECT #	6520-2
CELLS	MT2	Satisfactory; Active; Retest	SPONSOR	USAMRIID
SHIPMENT NUMBER	63		TEST DATE	04/04/90
STRN	2.5		DATE READ	04/12/90
REAGENT	0.130			
VIRUS CONTROL	0.227			
CELL CONTROL	1.506			
DIFFERENTIAL	1.279			

DRUG 6466	25%	50%	95%
TC (uG/mL)	48.80	66.40	97.90
IC (uG/mL)	3.53	4.99	9.33
ANTIVIRAL INDEX (AI)	13.84	13.29	10.49

DRUG 6466		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% RED. IN VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	0.32	0.006	0%	1.387	92%	0.002
C	1	0.038	3%	1.361	90%	0.001
D	3.2	0.231	18%	1.404	93%	0.005
E	10	1.442	100%	1.476	98%	0.001
F	32	1.222	96%	1.488	99%	-.001
high G *	100	-.227	0%	0.025	2%	0.003

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH



□ DRUG'S ANTIVIRAL EFFECT (% RED. IN VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

PRINTED 04/20/90

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PLATE 1KA
DRUG 6466

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6466
TAI: >85.47 SI: >161.06

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.118	0.109	0.116	0.115	0.112	0.114	0.034	0.034	0.034	0.034	0.034	0.033
		cc/vc					tox	drug 6466 experimental			cc/vc	tox
B		1.089					1.190	1.183	1.176	1.067	1.233	1.133
C		1.051					1.137	1.101	1.193	1.236	1.212	1.245
D		0.917					1.061	1.207	1.204	1.234	1.205	1.323
E		0.478					1.197	1.195	1.334	1.246	0.512	1.477
F		0.469					1.629	1.371	1.380	1.360	0.576	1.578
G		0.500					0.302	0.174	0.174	0.164	0.566	0.248
							drug 6466 colorimetric background					
H							0.144	0.137	0.135	0.126	0.121	0.132

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	HIVCRF		PROJECT #	6520-2
CELLS	CEM	Satisfactory; Active; Retest	SPONSOR	USAMRIID
SHIPMENT NUMBER	64	Low MOI	TEST DATE	04/26/90
STRN	RF??		DATE READ	05/03/90
REAGENT	0.114			
VIRUS CONTROL	0.404			
CELL CONTROL	1.004			
DIFFERENTIAL	0.600			

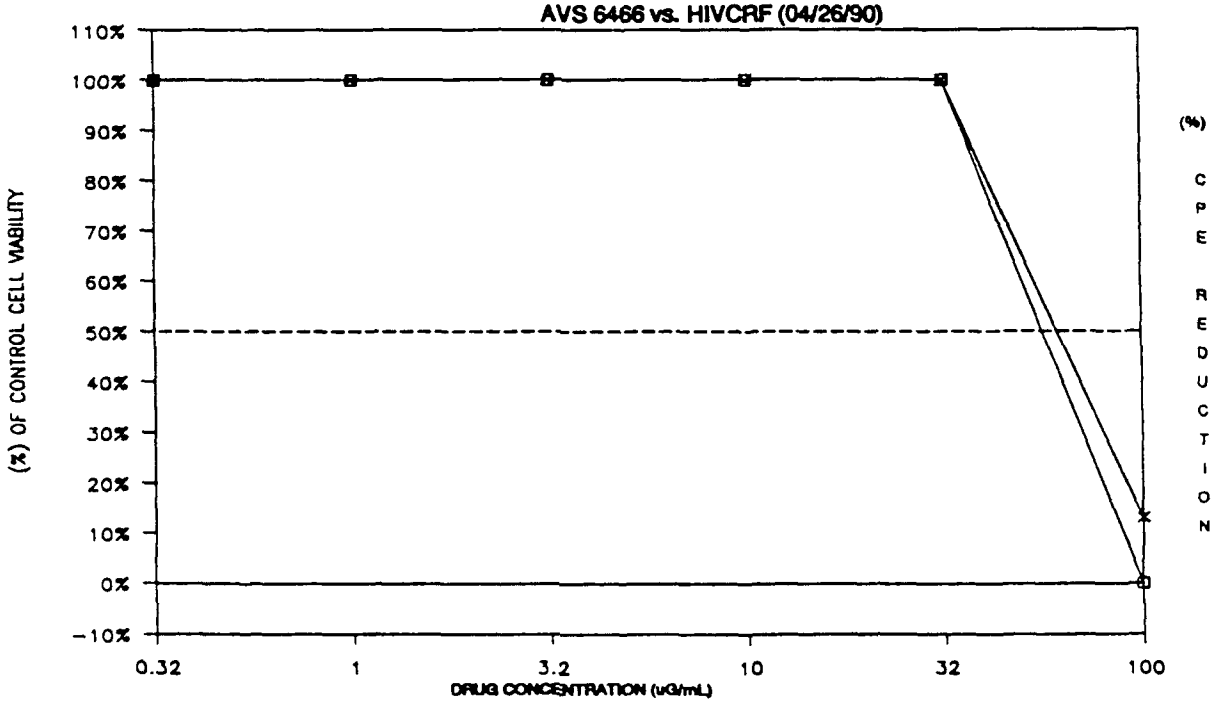
DRUG 6466	25%	50%	95%
TC (uG/mL)	51.50	71.10	> 100.00
IC (uG/mL)	< 0.32	< 0.32	< 0.32
ANTIVIRAL INDEX (AI)	> 161.06	> 222.13	> 312.50

DRUG 6466		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% RED. IN CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	0.32	0.606	100%	1.030	100%	0.018
C	1	0.652	100%	1.070	100%	0.007
D	3.2	0.685	100%	1.066	100%	0.012
E	10	0.720	100%	1.202	100%	0.021
F	32	0.830	100%	1.467	100%	0.023
high G *	100	-.377	0%	0.131	13%	0.030

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6466 vs. HIVCRF (04/26/90)



□ DRUG'S ANTIVIRAL EFFECT (% RED. IN VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

PRINTED 06/10/90

SOUTHERN RESEARCH INSTITUTE

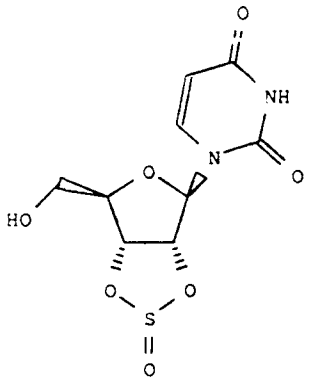
USAMRIID

Antiviral Drug Screening Program

03/26/90

STRUCTURE

CHIRAL



SUBMITTER	CTR NO	AVS NO
01141.01	KN-V-99	AVS-006442
DATE RECD	**AMT RECEIVED [mg]**	**MOL WT (au)**
12-28-89	74.00	290.253

HANDLING/STORAGE

SOLUBILITY

STABILITY

ALT NAME

2',3'-O-SULFINYL URIDINE

COMPOUND NAME

2',3'-O-SULFINYL URIDINE

SCREEN INSTRUCTION

PRIORITY=PT>VEE>YF>KHF>PIC>JE>SF>VV>ADE>VSV

IN VIVO TOXICITY [mg/kg]

HOST	VH	RTE	LD50	MTC	LAB PR	DATE

IN VITRO SCREEN [ug/ml]

VIR	VR	VR+	ID50	CELL	MTC	TI	TI+	LAB	PRT	DATE
JE			NOT ACT	VERO	183	0		SO	MTT	90-03-01
PT			100	VERO	170	2.39		SO	MTT	90-03-01
SF			NOT ACT	VERO	172	0		SO	MTT	90-03-01
VEE			NOT ACT	VERO	38.3	0		SO	MTT	90-03-02
YF			NOT ACT	VERO	171	0		SO	MTT	90-03-01

IN VIVO SCREEN [Dose = mg/kg]

VIR	HST	VR	VR+	DOSE	MTC	VEH	RTE	D TOX	SP	L PR	DATE

PLATE U9A
DRUG 6442

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6442
TAI: 15.38 SI: 1.70

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.042	0.041	0.040	0.042	0.039	0.042	0.001	0.001	0.001	0.001	0.001	0.001
	tox	cc/vc	drug 6442 experimental			tox					cc/vc	
B	0.898	0.951	0.392	0.299	0.337	0.956					0.746	
C	0.938	1.033	0.389	0.295	0.345	0.912					0.972	
D	1.011	1.139	0.419	0.423	0.434	1.020					0.800	
E	1.034	0.329	0.559	0.504	0.542	1.102					0.334	
F	1.115	0.389	0.663	0.626	0.656	1.181					0.342	
G	0.243	0.353	0.227	0.227	0.219	0.229					0.396	
	drug 6442 colorimetric background											
H	0.049	0.038	0.038	0.037	0.038	0.039						

tox=cell toxicity cc=cell control vc=virus control **BOLD** = highest drug conc values shown are optical densities

VIRUS	PT		PROJECT #	5975-1
CELLS	VERO	Satisfactory; Active; Retest	SPONSOR	USAMRIID
SHIPMENT NUMBER	63		TEST DATE	03/01/90
STRN	ADAMES		DATE READ	03/09/90
REAGENT	0.041			
VIRUS CONTROL	0.316			
CELL CONTROL	0.899			
DIFFERENTIAL	0.583			

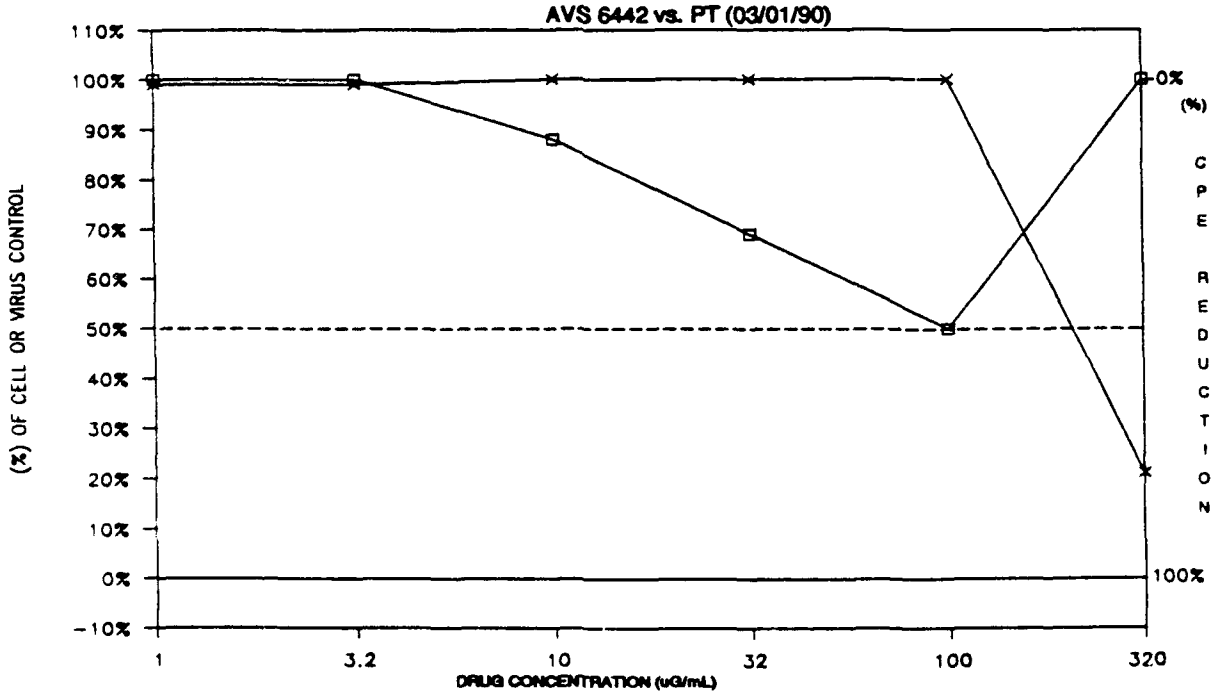
DRUG 6442	25%	50%	95%
TC (uG/mL)	170.00	239.00	> 320.00
IC (uG/mL)	22.20	100.00	------
ANTIVIRAL INDEX (AI)	7.65	2.39	------

DRUG 6442		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	-.013	100%	0.888	99%	-.002
C	3.2	-.011	100%	0.887	99%	-.003
D	10	0.072	88%	0.979	100%	-.004
E	32	0.181	69%	1.030	100%	-.003
F	100	0.294	50%	1.110	100%	-.003
high G *	320	-.141	100%	0.187	21%	0.008

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6442 vs. PT (03/01/90)



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

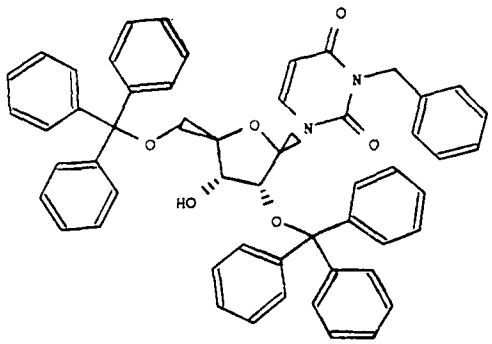
USAMRIID

Antiviral Drug Screening Program

03/26/90

STRUCTURE

CHIRAL



SUBMITTER	CTR NO	AVS NO
01141.01	KN-V-109	AVS-006443
DATE RECD	**AMT RECEIVED [mg]**	**MOL WT (au)**
12-28-89	86.00	818.979

HANDLING/STORAGE

SOLUBILITY

STABILITY

ALT NAME

N3-BENZYL-2',5'-DI-O-TRITYLURIDINE

COMPOUND NAME

N3-BENZYL-2',5'-DI-O-TRITYLURIDINE

SCREEN INSTRUCTION

PRIORITY=PT>VEE>YF>KHF>PIC>JE>SF>VV>AD2>VSV

IN VIVO TOXICITY [mg/kg]

HOST	VH	RTE	LD50	MTC	LAB PR	DATE

IN VITRO SCREEN [ug/ml]

VIR	VR	VR+	ID50	CELL	MTC	TI	TI+	LAB PRT	DATE
JE			NOT ACT	VERO	24.7	0		SO MTT	90-03-01
PT			77.1	VERO	210	> 4.15		SO MTT	90-03-01
SF			NOT ACT	VERO	> 320	0		SO MTT	90-03-01
VEE			NOT ACT	VERO	> 320	0		SO MTT	90-03-02
YF			NOT ACT	VERO	> 320	0		SO MTT	90-03-01

IN VIVO SCREEN [Dose = mg/kg]

VIR	HST	VR	VR+	DOSE	MTC	VEH	RTE	D TOX	SP L	PR	DATE

PLATE U9A
DRUG 6443

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6443
TAI: >10.57 SI: 2.72

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.042	0.041	0.040	0.042	0.039	0.042	0.001	0.001	0.001	0.001	0.001	0.001
		co/vo					tox	drug 6443 experimental			co/vo	tox
B		0.951					0.830	0.376	0.357	0.334	0.746	0.879
C		1.033					0.911	0.386	0.324	0.297	0.972	0.771
D		1.139					1.015	0.406	0.436	0.343	0.800	0.866
E		0.329					0.800	0.493	0.491	0.497	0.334	0.814
F		0.389					0.734	0.695	0.713	0.683	0.342	0.716
G		0.353					0.696	0.560	0.632	0.599	0.396	0.751
							drug 6443 colorimetric background					
H							0.059	0.044	0.040	0.039	0.040	0.040

tox=cell toxicity co=cell control vo=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	PT		PROJECT #	5975-1
CELLS	VERO	Satisfactory; Active; Retest	SPONSOR	USAMRIID
SHIPMENT NUMBER	63		TEST DATE	03/01/90
STRN	ADAMES		DATE READ	03/09/90
REAGENT	0.041			
VIRUS CONTROL	0.316			
CELL CONTROL	0.899			
DIFFERENTIAL	0.583			

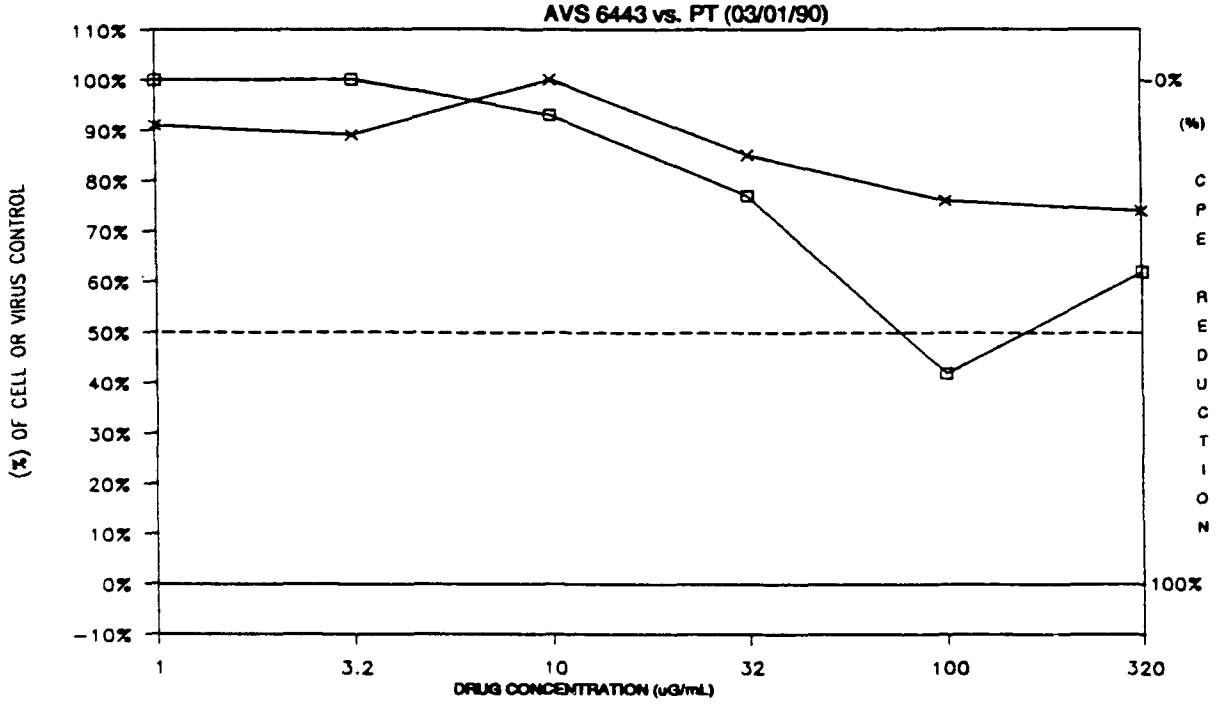
DRUG 6443	25%	50%	95%
TC (uG/mL)	210.00	> 320.00	> 320.00
IC (uG/mL)	34.20	77.10	-----
ANTIVIRAL INDEX (AI)	6.15	> 4.15	-----

DRUG 6443		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	-.001	100%	0.815	91%	-.001
C	3.2	-.021	100%	0.801	89%	-.001
D	10	0.040	93%	0.902	100%	-.002
E	32	0.137	77%	0.767	85%	-.001
F	100	0.337	42%	0.681	76%	0.003
high G *	320	0.222	62%	0.665	74%	0.018

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6443 vs. PT (03/01/90)



DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

PRINTED 03/15/90

SOUTHERN RESEARCH INSTITUTE

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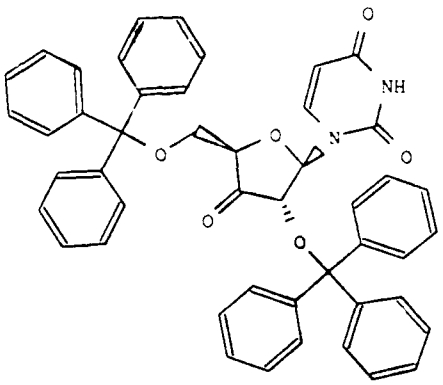
Antiviral Drug Screening Program

03/26/90

STRUCTURE

CHIRAL



SUBMITTER	CTR NO	AVS NO
01141.01	KN-VII-83	AVS-006444
DATE RECD	**AMT RECEIVED (mg)**	**MOL WT (au)**
12-28-89	79.00	726.837

HANDLING/STORAGE

SOLUBILITY

STABILITY

ALT NAME

3'-DEOXY-2',5'-DI-O-TRITYL-3'-OXOURIDINE

COMPOUND NAME

3'-DEOXY-2',5'-DI-O-TRITYL-3'-OXOURIDINE

SCREEN INSTRUCTION

PRIORITY=PT>VEE>YF>KHF>PIC>JE>SF>VV>AD2>VSV

IN VIVO TOXICITY [mg/kg]

HOST	VH	RTE	LD50	MTC	LAB PR	DATE

IN VITRO SCREEN [ug/ml]

VIR	VR	VR+	ID50	CELL	MTC	TI	TI+	LAB PRT	DATE
JE			NOT ACT	VERO	51	0		SO MTT	90-03-01
PT			79.2	VERO	> 320	> 4.04		SO MTT	90-03-01
SF			NOT ACT	VERO	30	0		SO MTT	90-03-01
VEE			NOT ACT	VERO	> 320	0		SO MTT	90-03-02
YF			NOT ACT	VERO	> 320	0		SO MTT	90-03-01

IN VIVO SCREEN [Dose = mg/kg]

VIR	HST	VR	VR+	DOSE	MTC	VEH	RTE	D TOX	SP L	PR	DATE

PLATE U9B

DRUG 6444

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6444

TAI: >24.65 SI: >4.04

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.043	0.042	0.045	0.041	0.040	0.042	0.001	0.001	0.001	0.001	0.001	0.001
	tox	cc/vc	drug 6444 experimental			tox					cc/vc	
B	1.033	0.918	0.365	0.330	0.321	0.864					0.837	
C	0.959	0.946	0.304	0.333	0.381	0.999					0.734	
D	0.956	1.054	0.433	0.451	0.414	0.883					0.631	
E	0.811	0.295	0.382	0.411	0.490	0.810					0.339	
F	0.753	0.355	0.577	0.706	0.655	0.748					0.369	
G	0.982	0.382	0.952	0.924	0.879	0.981					0.358	
	drug 6444 colorimetric background											
H	0.041	0.043	0.042	0.039	0.039	0.039						

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

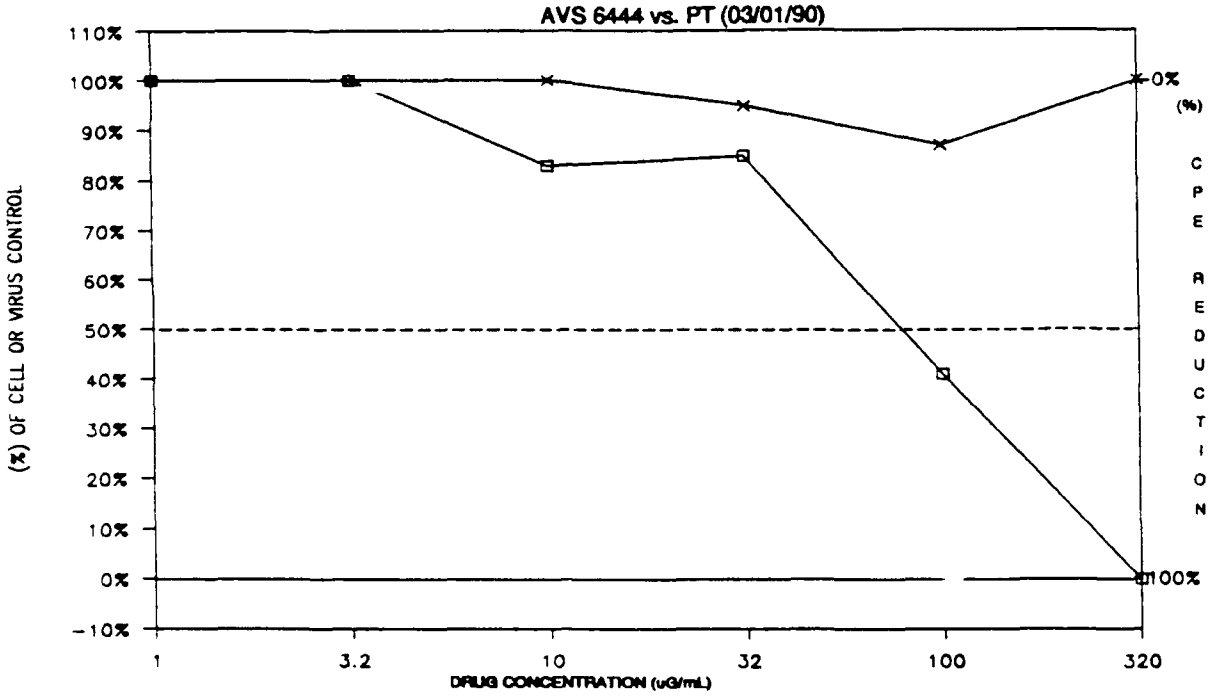
VIRUS	PT		PROJECT #	5975-1
CELLS	VERO	Satisfactory; Active; Retest	SPONSOR	USAMRIID
SHIPMENT NUMBER	63		TEST DATE	03/01/90
STRN	ADAMES		DATE READ	03/09/90
REAGENT	0.042			
VIRUS CONTROL	0.308			
CELL CONTROL	0.811			
DIFFERENTIAL	0.504			

DRUG 6444	25%	50%	95%
TC (uG/mL)	> 320.00	> 320.00	> 320.00
IC (uG/mL)	41.50	79.20	278.00
ANTIVIRAL INDEX (AI)	> 7.72	> 4.04	> 1.15

DRUG 6444		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	-.008	100%	0.909	100%	-.003
C	3.2	-.007	100%	0.940	100%	-.003
D	10	0.086	83%	0.870	100%	-.003
E	32	0.078	85%	0.768	95%	0.000
F	100	0.295	41%	0.707	87%	0.001
high G *	320	0.570	0%	0.940	100%	-.001

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

PRINTED 03/15/90

SOUTHERN RESEARCH INSTITUTE

PLATE UAR

DRUG 6444

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6444

TAI: >7.30 SI: ------

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.042	0.040	0.039	0.038	0.039	0.039	0.001	0.001	0.001	0.001	0.001	0.001
	tox	cc/vc	drug 6444 experimental			tox					cc/vc	
B	0.957	0.847	0.187	0.272	0.243	0.896					0.926	
C	0.960	1.048	0.275	0.297	0.280	1.005					0.782	
D	0.807	0.922	0.352	0.319	0.346	0.906					0.882	
E	0.817	0.213	0.421	0.441	0.493	0.717					0.218	
F	0.728	0.253	0.278	0.294	0.369	0.731					0.176	
G	0.972	0.297	0.071	0.062	0.069	0.798					0.190	
	drug 6444 colorimetric background											
H	0.042	0.042	0.043	0.041	0.041	0.039						

tox=cell toxicity cc=cell control vc=virus control **BOLD** = highest drug conc values shown are optical densities

VIRUS	YF		PROJECT #	5975-1
CELLS	VERO	Satisfactory; Active; Retest	SPONSOR	USAMRIID
SHIPMENT NUMBER	63		TEST DATE	03/01/90
STRN	ASIBI		DATE READ	03/09/90
REAGENT	0.040			
VIRUS CONTROL	0.185			
CELL CONTROL	0.862			
DIFFERENTIAL	0.677			

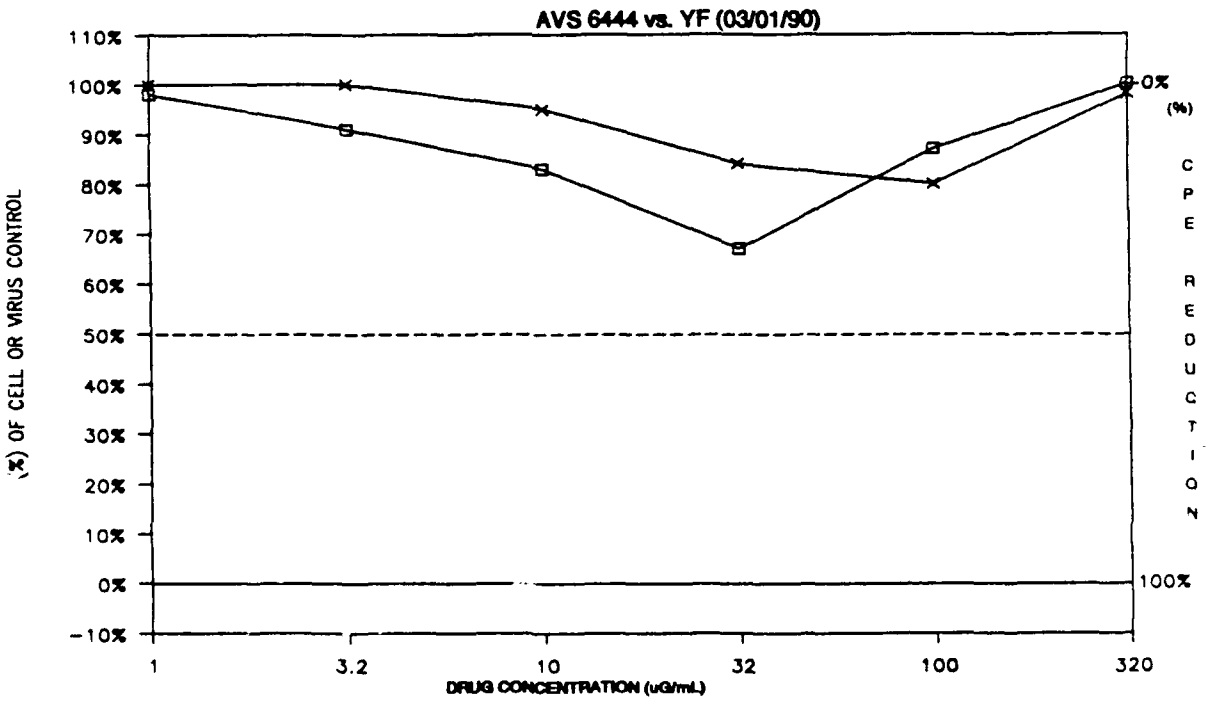
DRUG 6444	25%	50%	95%
TC (uG/mL)	> 320.00	> 320.00	> 320.00
IC (uG/mL)	17.90	-----	-----
ANTIVIRAL INDEX (AI)	> 17.89	-----	-----

DRUG 6444		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	0.011	98%	0.888	100%	-.001
C	3.2	0.058	91%	0.941	100%	0.002
D	10	0.113	83%	0.815	95%	0.002
E	32	0.224	67%	0.725	84%	0.003
F	100	0.086	87%	0.687	80%	0.003
high G *	320	-.160	100%	0.843	98%	0.003

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6444 vs. YF (03/01/90)



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

PRINTED 03/16/90

SOUTHERN RESEARCH INSTITUTE

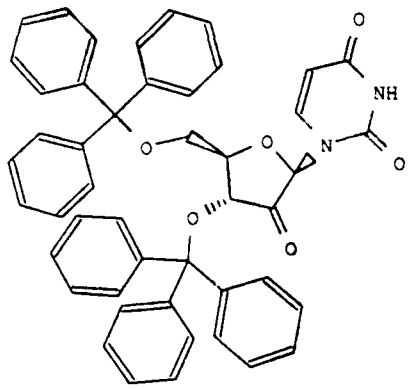
USAMRIID

Antiviral Drug Screening Program

03/26/90

STRUCTURE

CHIRAL



SUBMITTER	CTR NO	AVS NO
01141.01	KN-VII-21	AVS-006445
DATE RECD	**AMT RECEIVED [mg]**	**MOL WT (au)**
12-28-89	74.00	726.837

HANDLING/STORAGE

SOLUBILITY

STABILITY

ALT NAME

2'-DEOXY-3',5'-DI-O-TRITYL-2'-OXOURIDINE

COMPOUND NAME

2'-DEOXY-3',5'-DI-O-TRITYL-2'-OXOURIDINE

SCREEN INSTRUCTION

PRIORITY=PT>VEE>YF>KHF>PIC>JE>SF>VV>AD2>VSV

IN VIVO TOXICITY [mg/kg]

HOST	VH	RTE	LD50	MTC	LAB PR	DATE

IN VITRO SCREEN [ug/ml]

VIR	VR	VR+	ID50	CELL	MTC	TI	TI+	LAB	PRT	DATE
JE			NOT ACT	VERO	23.2	0		SO	MTT	90-03-01
PT			22.6	VERO	49	2.92		SO	MTT	90-03-01
SF			NOT ACT	VERO	43.3	0		SO	MTT	90-03-01
VEE			NOT ACT	VERO	32	0		SO	MTT	90-03-02
YF			18.9	VERO	48	3.45		SO	MTT	90-03-01

IN VIVO SCREEN [Dose = mg/kg]

VIR	HST	VR	VR+	DOSE	MTC	VEH	RTE	D TOX	SP	L PR	DATE

PLATE U9B
DRUG 6445

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6445
TAI: >17.14 SI: 2.17

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.043	0.042	0.045	0.041	0.040	0.042	0.001	0.001	0.001	0.001	0.001	0.001
		co/vo					tox	drug 6445 experimental			co/vo	tox
B		0.918					0.833	0.402	0.373	0.379	0.837	0.871
C		0.946					1.074	0.286	0.430	0.407	0.734	0.870
D		1.054					0.830	0.587	0.567	0.534	0.631	0.816
E		0.295					0.882	0.656	0.606	0.590	0.339	0.890
F		0.355					0.033	0.036	0.038	0.035	0.369	0.033
G		0.382					0.035	0.036	0.036	0.036	0.358	0.037
							drug 6445 colorimetric background					
H							0.038	0.044	0.043	0.040	0.039	0.039

tox=cell toxicity co=cell control vo=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	PT		PROJECT #	5975-1
CELLS	VERO	Satisfactory; Active; Retest	SPONSOR	USAMRIID
SHIPMENT NUMBER	63		TEST DATE	03/01/90
STRN	ADAMS		DATE READ	03/09/90
REAGENT	0.042			
VIRUS CONTROL	0.308			
CELL CONTROL	0.811			
DIFFERENTIAL	0.504			

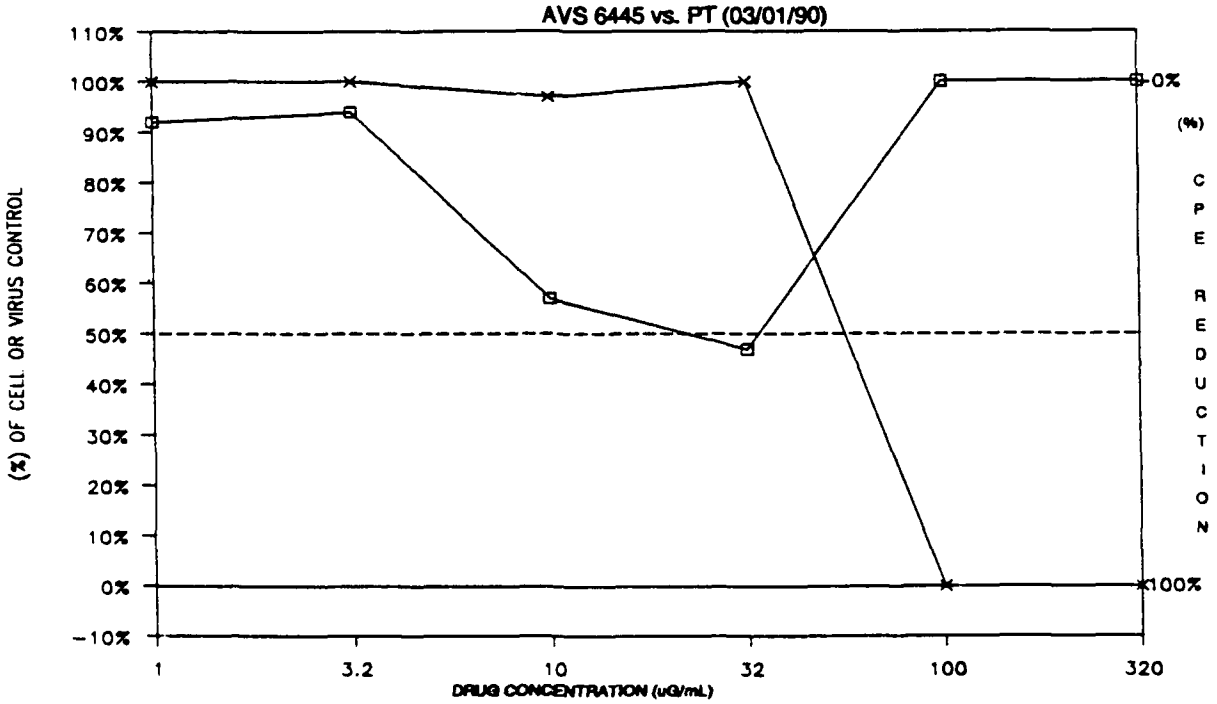
DRUG 6445	25%	50%	95%
TC (uG/mL)	49.00	66.00	96.60
IC (uG/mL)	5.74	22.60	-----
ANTIVIRAL INDEX (AI)	8.53	2.92	-----

DRUG 6445		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	0.038	92%	0.813	100%	-.003
C	3.2	0.028	94%	0.933	100%	-.003
D	10	0.215	57%	0.783	97%	-.002
E	32	0.267	47%	0.843	100%	0.001
F	100	-.315	100%	-.011	0%	0.002
high G *	320	-.310	100%	-.002	0%	-.004

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6445 vs. PT (03/01/90)



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

PRINTED 03/16/90

SOUTHERN RESEARCH INSTITUTE

PLATE UAR
DRUG 6445

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6445
TAI: >19.99 SI: 2.53

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.042	0.040	0.039	0.038	0.039	0.039	0.001	0.001	0.001	0.001	0.001	0.001
		cc/vc					tox	drug 6445 experimental			cc/vc	tox
B		0.847					0.912	0.290	0.356	0.317	0.926	0.943
C		1.048					0.867	0.433	0.430	0.426	0.782	0.925
D		0.922					0.767	0.426	0.441	0.474	0.882	0.834
E		0.213					0.857	0.621	0.700	0.670	0.218	0.916
F		0.253					0.034	0.033	0.034	0.034	0.176	0.042
G		0.297					0.035	0.036	0.035	0.035	0.190	0.038
							drug 6445 colorimetric background					
H							0.038	0.041	0.044	0.039	0.040	0.042

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	YF		PROJECT #	5975-1
CELLS	VERO	Satisfactory; Active; Retest	SPONSOR	USAMRIID
SHIPMENT NUMBER	63		TEST DATE	03/01/90
STRN	ASIBI		DATE READ	03/09/90
REAGENT	0.040			
VIRUS CONTROL	0.185			
CELL CONTROL	0.862			
DIFFERENTIAL	0.677			

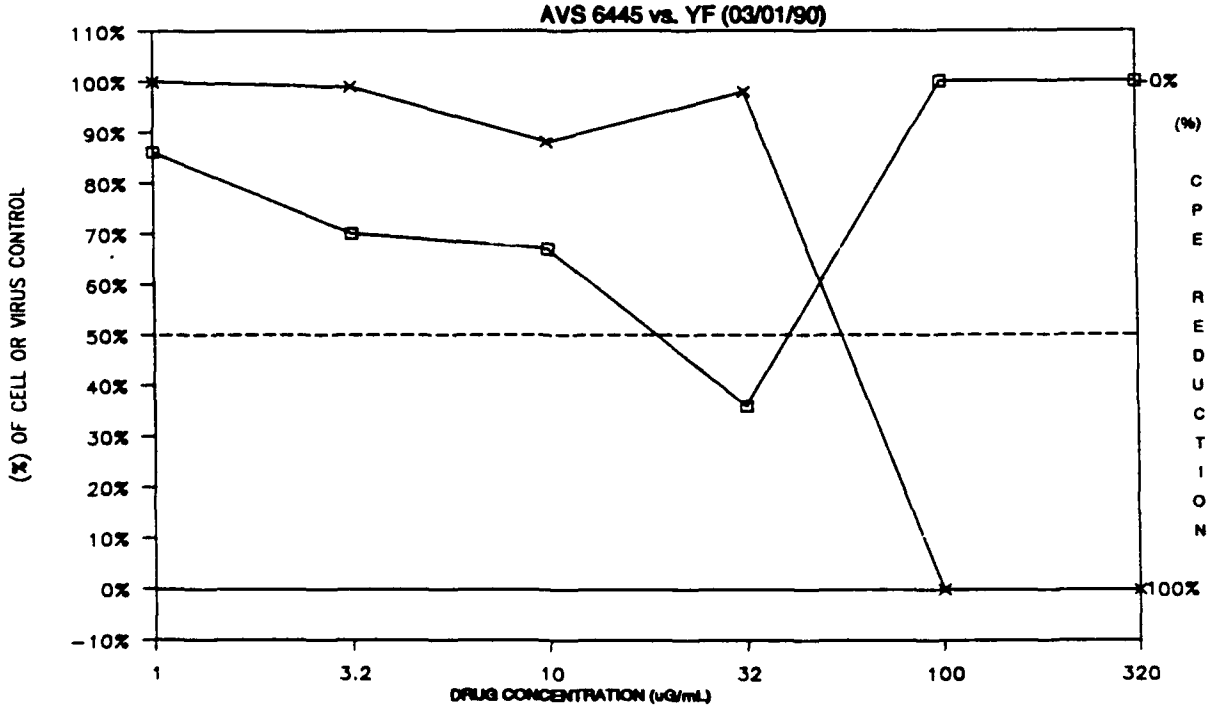
DRUG 6445	25%	50%	95%
TC (uG/mL)	48.00	65.30	96.50
IC (uG/mL)	2.22	18.90	-----
ANTIVIRAL INDEX (AI)	21.56	3.45	-----

DRUG 6445		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	0.094	86%	0.885	100%	0.003
C	3.2	0.204	70%	0.856	99%	0.001
D	10	0.224	67%	0.762	88%	-.001
E	32	0.435	36%	0.843	98%	0.004
F	100	-.193	100%	-.003	0%	0.002
high G *	320	-.187	100%	-.001	0%	-.002

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6445 vs. YF (03/01/90)



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

SOUTHERN RESEARCH INSTITUTE

Appendix IV

TEST DATA AGAINST OTHER VIRUSES

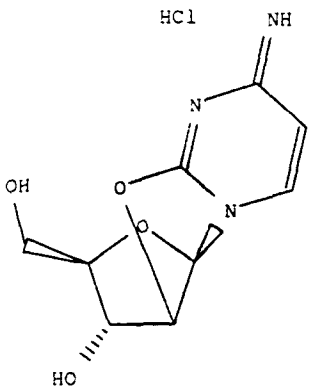
USAMRIID

Antiviral Drug Screening Program

09/21/90

STRUCTURE

CHIRAL



SUBMITTER	CTR NO	AVS NO
01141.01	KN-II-95	AVS-006467
DATE RECD	**AMT RECEIVED [mg]**	**MOL WT (au)**
12-28-89	72.60	261.667

HANDLING/STORAGE

SOLUBILITY

STABILITY

ALT NAME

2',O2-ANHYDROCYTIDINE HYDROCHLORIDE

COMPOUND NAME

2',O2-ANHYDROCYTIDINE HYDROCHLORIDE

SCREEN INSTRUCTION

PRIORITY=PT>VEE>YF>KHF>PIC>JE>SF>VV>AD2>VSV

IN VIVO TOXICITY [mg/kg]

HOST	VH	RTE	LD50	MTC	LAB	PR	DATE

IN VITRO SCREEN [ug/ml]

VIR	VR	VR+	ID50	CELL	MTC	TI	TI+	LAB	PRT	DATE
HIV			NOT ACT	MT2	< .32	0		SO	MTT	04-APR-90
HIV			NOT ACT	MT2	.09	0		SO	MTT	24-APR-90
JE			NOT ACT	VERO	3.05	0		SO	MTT	06-MAR-90
JE			NOT ACT	VERO	1.6	0		SO	MTT	22-MAR-90
PT			NOT ACT	VERO	> 320	0		SO	MTT	06-MAR-90
PT			NOT ACT	VERO	.81	0		SO	MTT	22-MAR-90
SF			NOT ACT	VERO	> 320	0		SO	MTT	06-MAR-90
SF			NOT ACT	VERO	.91	0		SO	MTT	22-MAR-90
VEE			NOT ACT	VERO	> 320	0		SO	MTT	09-MAR-90
VEE			NOT ACT	VERO	10	0		SO	MTT	23-MAR-90
VV			.18	VERO	1.86	10.53		SO	MTT	19-APR-90
VV			.25	VERO	2.01	8.03		SO	MTT	10-MAY-90
YF			NOT ACT	VERO	3.11	0		SO	MTT	06-MAR-90
YF			NOT ACT	VERO	.82	0		SO	MTT	22-MAR-90

IN VIVO SCREEN [Dose = mg/kg]

VIR	HST	VR	VR+	DOSE	MTC	VEH	RTE	D	TOX	SP	L	PR	DATE

PLATE 1HK
DRUG 6467

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6467
TAI: 0.00 SI: ——

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.128	0.131	0.131	0.130	0.130	0.131	0.037	0.034	0.035	0.035	0.034	0.034
		cc/vc					tox	drug 6467 experimental			cc/vc	tox
B		1.627					0.172	0.157	0.155	0.147	1.651	0.184
C		1.625					0.132	0.125	0.130	0.130	1.588	0.138
D		1.657					0.131	0.128	0.130	0.133	1.666	0.139
E		0.369					0.138	0.135	0.131	0.133	0.358	0.143
F		0.364					0.137	0.132	0.133	0.131	0.343	0.130
G		0.368					0.135	0.134	0.132	0.132	0.338	0.140
							drug 6467 colorimetric background					
H							0.125	0.129	0.131	0.129	0.130	0.132

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	HIV3B		PROJECT #	6520-2
CELLS	MT2	Satisfactory; Toxic; Retest	SPONSOR	USAMRIID
SHIPMENT NUMBER	63		TEST DATE	04/04/90
STRN	2.5		DATE READ	04/12/90
REAGENT	0.130			
VIRUS CONTROL	0.227			
CELL CONTROL	1.506			
DIFFERENTIAL	1.279			

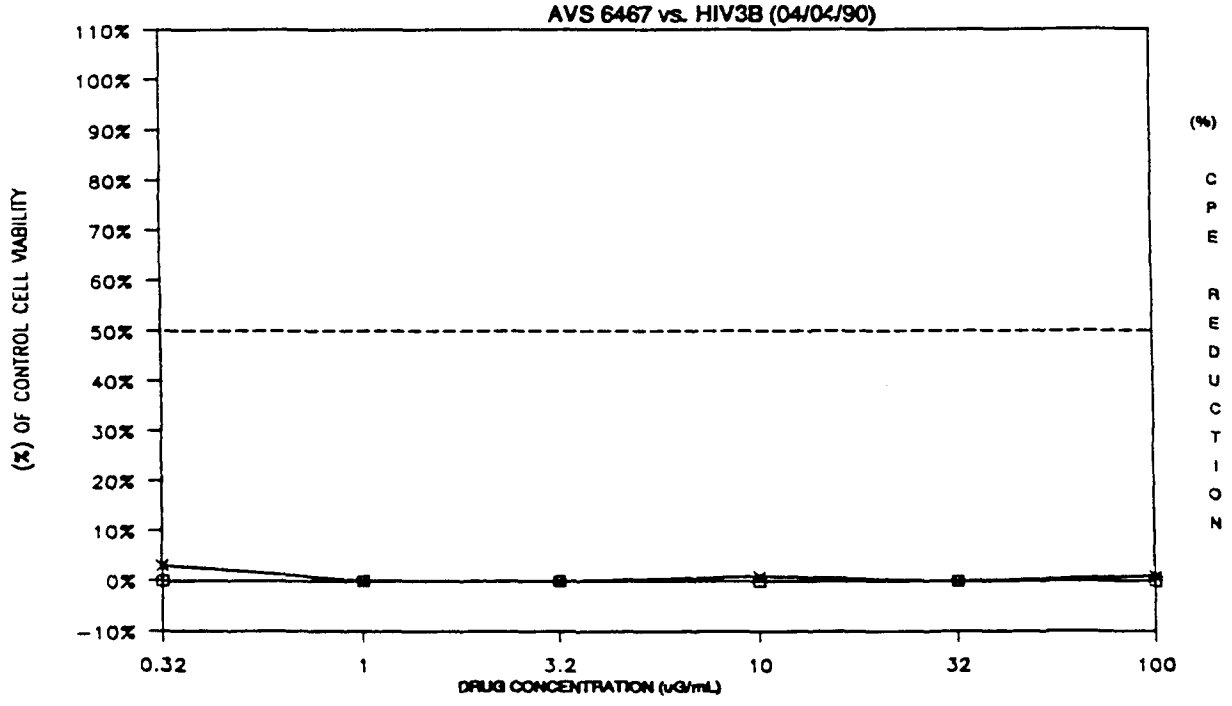
DRUG 6467	25%	50%	95%
TC (uG/mL)	< 0.32	< 0.32	< 0.32
IC (uG/mL)	-----	-----	-----
ANTIVIRAL INDEX (AI)	-----	-----	-----

DRUG 6467		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% RED. IN CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	0.32	-.206	0%	0.046	3%	0.002
C	1	-.228	0%	0.005	0%	0.000
D	3.2	-.225	0%	0.006	0%	-.001
E	10	-.225	0%	0.009	1%	0.001
F	32	-.224	0%	0.004	0%	-.001
high G *	100	-.219	0%	0.012	1%	-.005

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6467 vs. HIV3B (04/04/90)



□ DRUG'S ANTIVIRAL EFFECT (% RED. IN VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

PRINTED 04/20/90

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PLATE 1JP
DRUG 6467

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6467
TAI: >0.42 SI: ——

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.117	0.112	0.113	0.114	0.115	0.116	0.034	0.035	0.035	0.035	0.035	0.034
	tox	cc/vc	drug 6467 experimental			tox					cc/vc	
B	1.266	1.279	0.277	0.333	0.325	1.350					1.389	
C	1.184	1.290	0.307	0.305	0.307	1.295					1.346	
D	1.306	1.275	0.320	0.311	0.315	1.371					1.311	
E	1.114	0.289	0.257	0.245	0.268	1.244					0.280	
F	0.628	0.300	0.216	0.199	0.240	0.715					0.207	
G	0.124	0.301	0.119	0.124	0.119	0.124					0.294	
	drug 6467 colorimetric background											
H	0.116	0.113	0.113	0.113	0.112	0.115						

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	HIV3B		PROJECT #	6520-2
CELLS	MT2	Satisfactory	SPONSOR	USAMRIID
SHIPMENT NUMBER	63		TEST DATE	04/24/90
STRN	2.5		DATE READ	05/02/90
REAGENT	0.115			
VIRUS CONTROL	0.164			
CELL CONTROL	1.201			
DIFFERENTIAL	1.037			

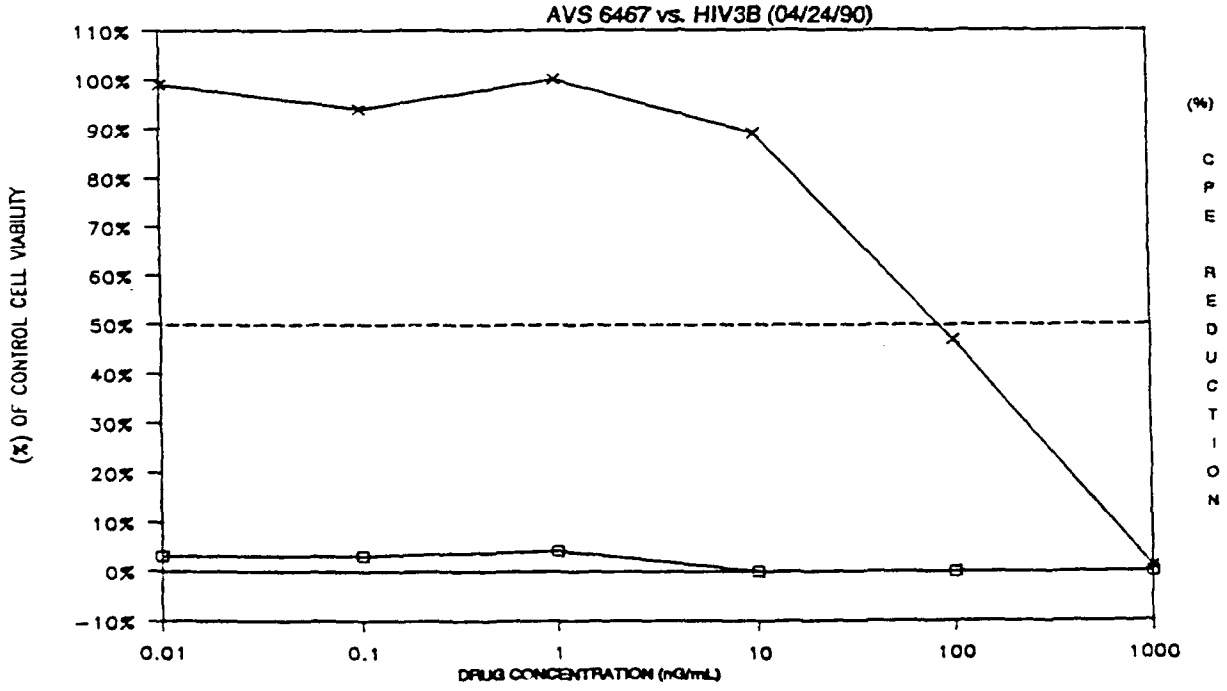
DRUG 6467	25%	50%	95%
TC (uG/mL)	0.040000	0.093600	0.922000
IC (uG/mL)	——	——	——
ANTIVIRAL INDEX (AI)	——	——	——

DRUG 6467		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (nG/mL)	MEAN O.D.	% RED. IN VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	0.01	0.032	3%	1.193	99%	0.001
C	0.1	0.031	3%	1.128	94%	-.003
D	1	0.039	4%	1.226	100%	-.002
E	10	-.020	0%	1.067	89%	-.002
F	100	-.058	0%	0.559	47%	-.002
high G *	1000	-.160	0%	0.007	1%	0.002

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6467 vs. HIV3B (04/24/90)



□ DRUG'S ANTIVIRAL EFFECT (% RED. IN VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

PRINTED 05/02/90

SOUTHERN RESEARCH INSTITUTE

PLATE 0U4
DRUG 6467

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6467
TAI: 23.24 SI: 4.65

	1	2	3	4	5	6	7	8	9	10	11	12
			reagent background						plastic background			
A	0.105	0.097	0.123	0.114	0.109	0.119	0.000	0.000	0.000	0.000	0.000	0.000
		cc/vc					tox	drug 6467 experimental			cc/vc	tox
B		1.314					1.488	0.153	0.166	0.134	1.321	1.330
C		1.339					1.510	0.179	0.148	0.205	1.470	1.424
D		1.312					1.553	0.258	0.206	0.325	1.410	1.427
E		0.182					1.415	1.153	1.398	1.302	0.160	1.541
F		0.180					1.058	0.847	0.883	0.733	0.173	0.825
G		0.174					0.450	0.426	0.446	0.427	0.142	0.400
									drug 6467 colorimetric background			
H							0.107	0.110	0.105	0.108	0.114	0.117

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	VV		PROJECT #	5975-4
CELLS	VERO	Satisfactory; Active; Retest	SPONSOR	USAMRIID
SHIPMENT NUMBER	63	RETEST AT 3.2 UG/ML	TEST DATE	04/19/90
STRN	LEDCA		DATE READ	04/25/90
REAGENT	0.111			
VIRUS CONTROL	0.057			
CELL CONTROL	1.250			
DIFFERENTIAL	1.193			

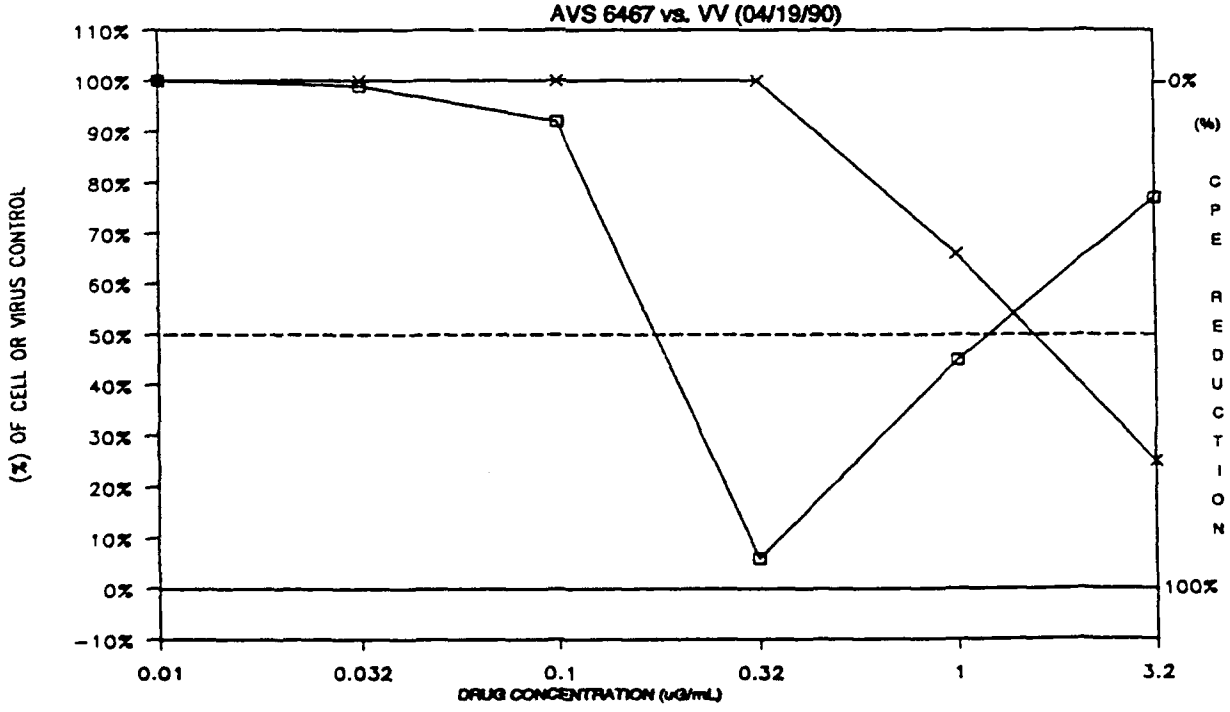
DRUG 6467	25%	50%	95%
TC (uG/mL)	0.82	1.86	> 3.20
IC (uG/mL)	0.13	0.18	----
ANTIVIRAL INDEX (AI)	6.52	10.53	----

DRUG 6467		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	0.01	-.023	100%	1.292	100%	0.006
C	0.032	0.006	99%	1.353	100%	0.003
D	0.1	0.098	92%	1.382	100%	-.003
E	0.32	1.122	6%	1.373	100%	-.006
F	1	0.654	45%	0.831	66%	-.001
high G *	3.2	0.269	77%	0.318	25%	-.004

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6467 vs. VV (04/19/90)



□ DRUG'S ANTIVIRAL EFFECT. (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

PRINTED 05/08/90

SOUTHERN RESEARCH INSTITUTE

PLATE 0VV
DRUG 6467

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6467
TAI: 17.08 SI: 3.66

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.108	0.113	0.110	0.114	0.111	0.121	0.000	0.000	0.000	0.000	0.000	0.000
		cc/vc					tox	drug 6467 experimental			cc/vc	tox
B		1.830					1.845	0.203	0.233	0.193	1.728	1.710
C		1.920					1.733	0.174	0.147	0.218	1.917	1.735
D		1.895					1.913	0.357	0.263	0.292	1.915	1.868
E		0.199					1.843	1.232	1.174	1.173	0.136	1.739
F		0.124					1.495	1.117	1.250	1.167	0.166	1.229
G		0.186					0.497	0.416	0.431	0.448	0.110	0.556
							drug 6467 colorimetric background					
H							0.104	0.106	0.109	0.104	0.116	0.109

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

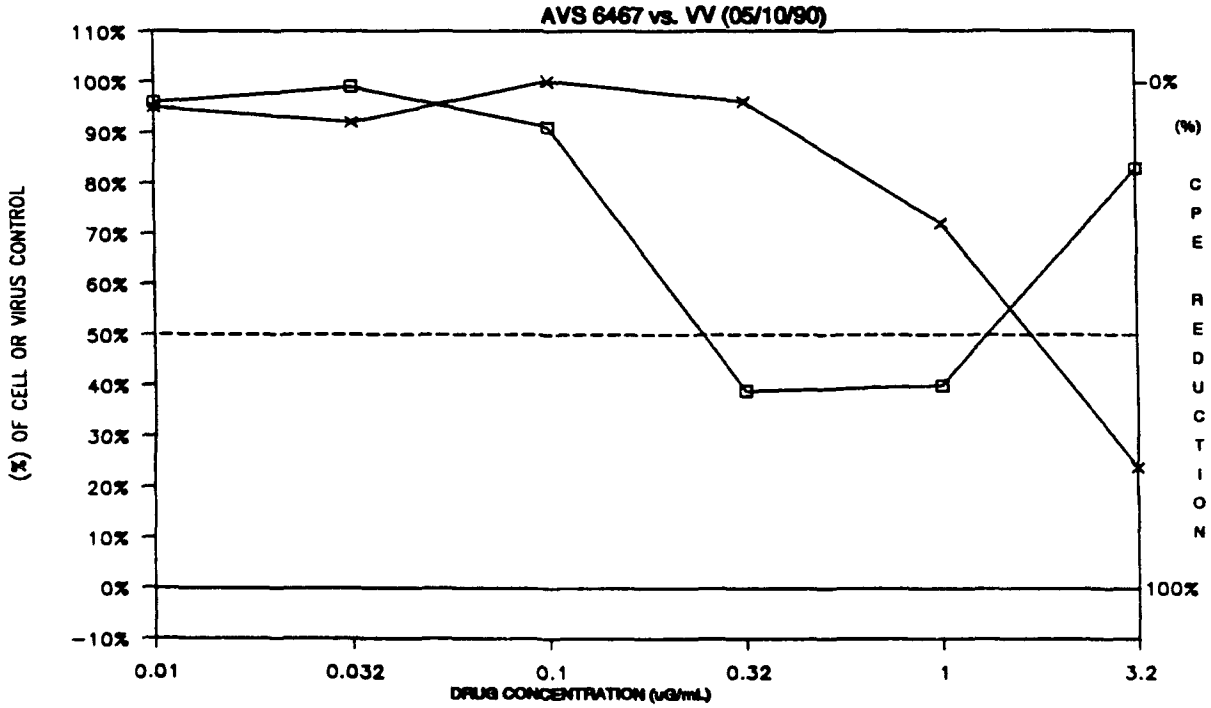
VIRUS	VV		PROJECT #	5975-4
CELLS	VERO	Satisfactory	SPONSOR	USAMRIID
SHIPMENT NUMBER	63	CONFIRMS ORIGINAL ACTIVITY	TEST DATE	05/10/90
STRN	LEDCA		DATE READ	05/16/90
REAGENT	0.113			
VIRUS CONTROL	0.041			
CELL CONTROL	1.755			
DIFFERENTIAL	1.714			

DRUG 6467	25%	50%	95%
TC (uG/mL)	0.92	2.01	> 3.20
IC (uG/mL)	0.14	0.25	-----
ANTIVIRAL INDEX (AI)	6.40	8.03	-----

DRUG 6467		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	0.01	0.060	96%	1.669	95%	-.004
C	0.032	0.023	99%	1.618	92%	0.003
D	0.1	0.160	91%	1.787	100%	-.009
E	0.32	1.044	39%	1.682	96%	-.004
F	1	1.032	40%	1.256	72%	-.007
high G *	3.2	0.287	83%	0.423	24%	-.009

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

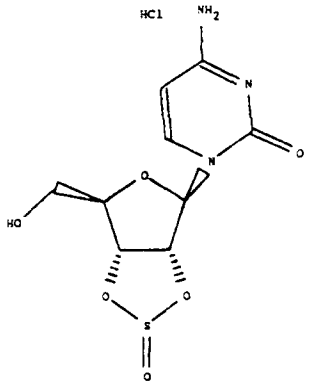
USAMRIID

Antiviral Drug Screening Program

09/21/90

STRUCTURE

CHIRAL



SUBMITTER	CTR NO	AVS NO
01141.01	KN-II-71	AVS-006462
DATE RECD	**AMT RECEIVED [mg]**	**MOL WT (au)**
12-28-89	72.40	325.729

HANDLING/STORAGE

SOLUBILITY

STABILITY

ALT NAME

2',3'-O-SULFINYLCYTIDINE HYDROCHLORIDE

COMPOUND NAME

2',3'-O-SULFINYLCYTIDINE HYDROCHLORIDE

SCREEN INSTRUCTION

PRIORITY=PT>VEE>YF>KHF>PIC>JE>SF>VV>AD2>VSV

IN VIVO TOXICITY [mg/kg]

HOST	VH	RTE	LD50	MTC	LAB PR	DATE

IN VITRO SCREEN [ug/ml]

VIR	VR	VR+	ID50	CELL	MTC	TI	TI+	LAB PRT DATE
HIV			NOT ACT	MT2	< .32	0		SO MTT04-APR-90
HIV			NOT ACT	MT2	.31	0		SO MTT24-APR-90
JE			NOT ACT	VERO	60.6	0		SO MTT06-MAR-90
PT			NOT ACT	VERO	93.4	0		SO MTT06-MAR-90
SF			NOT ACT	VERO	91.5	0		SO MTT06-MAR-90
VEE			NOT ACT	VERO	46.6	0		SO MTT09-MAR-90
VV			3.28	VERO	61.1	18.66		SO MTT29-MAR-90
VV			1.72	VERO	24.4	14.12		SO MTT19-APR-90
YF			NOT ACT	VERO	83	0		SO MTT06-MAR-90

IN VIVO SCREEN [Dose = mg/kg]

VIR	HST	VR	VR+	DOSE	MTC	VEH	RTE	D TOX	SP L	PR	DATE

PLATE 1HI

DRUG 6462

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6462

TAI: 0.00 SI: ——

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.137	0.135	0.136	0.136	0.137	0.138	0.035	0.033	0.034	0.034	0.035	0.042
	tox	cc/vc	drug 6462 experimental			tox					cc/vc	
B	0.327	1.655	0.203	0.202	0.208	0.323					1.743	
C	0.165	1.702	0.139	0.144	0.143	0.151					1.651	
D	0.139	1.663	0.130	0.133	0.130	0.137					1.644	
E	0.141	0.352	0.141	0.140	0.136	0.142					0.346	
F	0.138	0.358	0.132	0.132	0.131	0.133					0.368	
G	0.133	0.350	0.123	0.126	0.127	0.131					0.337	
	drug 6462 colorimetric background											
H	0.127	0.130	0.132	0.131	0.134	0.135						

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	HIV3B		PROJECT #	6520-2
CELLS	MT2	Satisfactory; Toxic; Retest	SPONSOR	USAMRIID
SHIPMENT NUMBER	63		TEST DATE	04/04/90
STRN	2.5		DATE READ	04/12/90
REAGENT	0.137			
VIRUS CONTROL	0.215			
CELL CONTROL	1.540			
DIFFERENTIAL	1.325			

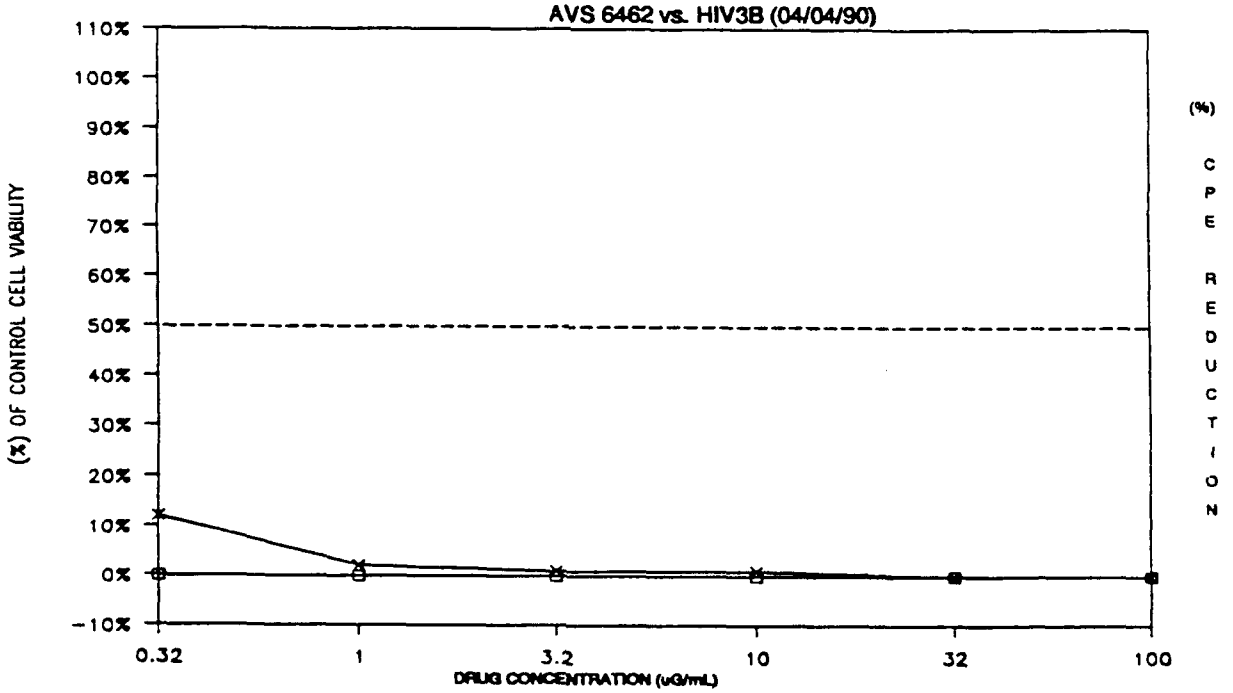
DRUG 6462	25%	50%	95%
TC (uG/mL)	< 0.32	< 0.32	0.80
IC (uG/mL)	-----	-----	-----
ANTIVIRAL INDEX (AI)	-----	-----	-----

DRUG 6462		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% RED. IN VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	0.32	-.146	0%	0.191	12%	-.002
C	1	-.207	0%	0.024	2%	-.003
D	3.2	-.215	0%	0.008	1%	-.006
E	10	-.208	0%	0.010	1%	-.005
F	32	-.213	0%	0.006	0%	-.007
high G *	100	-.217	0%	0.005	0%	-.010

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6462 vs. HIV3B (04/04/90)



□ DRUG'S ANTIVIRAL EFFECT (% RED. IN VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

PRINTED 04/20/90

SOUTHERN RESEARCH INSTITUTE

PLATE 1JO

DRUG 6462

IN VITRO ANTIVIRAL RESULTS

MTT ASSAY

DRUG: AVS 6462

TAI: 0.10 SI: ----

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.099	0.096	0.104	0.107	0.101	0.100	0.034	0.034	0.035	0.034	0.033	0.034
		cc/vc					tox	drug 6462 experimental			cc/vc	tox
B		1.277					1.202	0.325	0.302	0.296	1.184	1.192
C		1.183					1.204	0.317	0.328	0.336	1.249	1.164
D		1.477					1.277	0.318	0.322	0.327	1.201	1.297
E		0.309					1.193	0.320	0.302	0.316	0.271	1.098
F		0.288					0.906	0.218	0.264	0.275	0.303	0.842
G		0.266					0.125	0.121	0.118	0.122	0.292	0.119
							drug 6462 colorimetric background					
H							0.117	0.115	0.115	0.111	0.108	0.113

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	HIV3B		PROJECT #	6520-2
CELLS	MT2	Satisfactory	SPONSOR	USAMRIID
SHIPMENT NUMBER	63		TEST DATE	04/24/90
STRN	2.5		DATE READ	05/02/90
REAGENT	0.101			
VIRUS CONTROL	0.187			
CELL CONTROL	1.161			
DIFFERENTIAL	0.974			

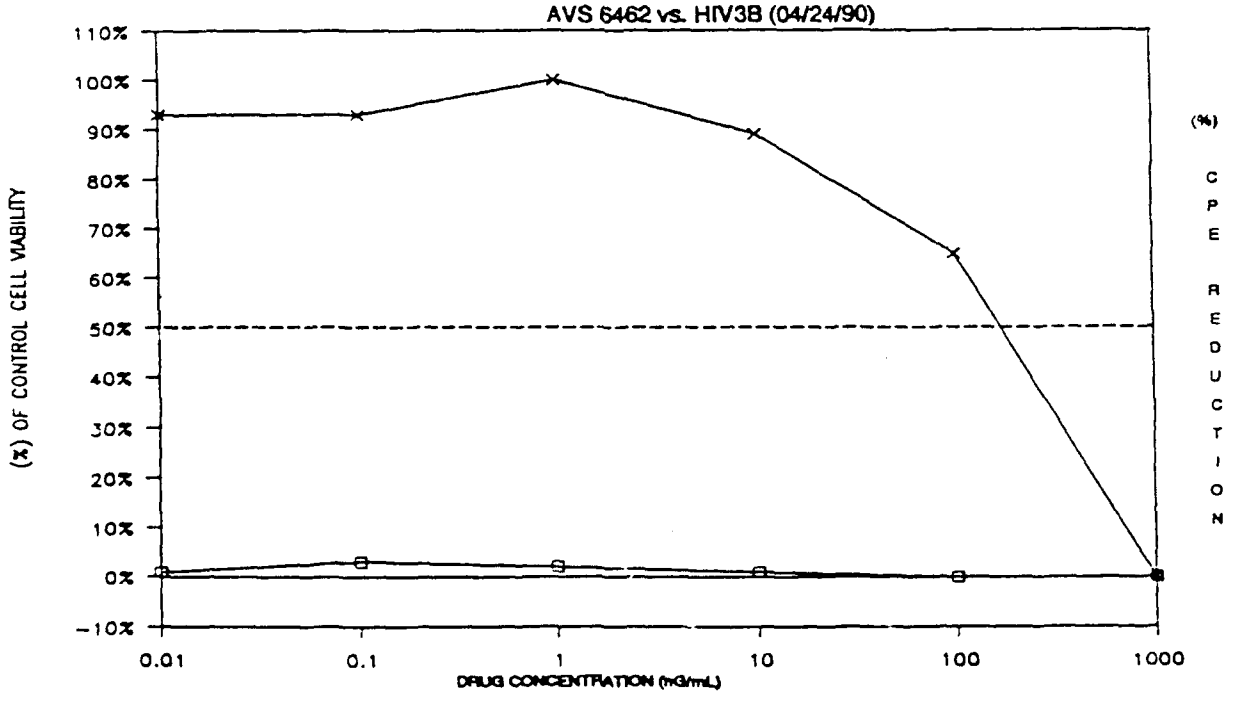
DRUG 6462	25%	50%	55%
TC (uG/mL)	0.062500	0.308000	0.931000
IC (uG/mL)	----	----	----
ANTIVIRAL INDEX (AI)	----	----	----

DRUG 6462		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (nG/mL)	MEAN O.D.	% RED. IN CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	0.01	0.008	1%	1.084	93%	0.012
C	0.1	0.032	3%	1.076	93%	0.007
D	1	0.024	2%	1.176	100%	0.010
E	10	0.011	1%	1.030	89%	0.014
F	100	-.050	0%	0.759	65%	0.014
high G *	1000	-.184	0%	0.005	0%	0.016

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6462 vs. HIV3B (04/24/90)



□ DRUG'S ANTIVIRAL EFFECT (% RED. IN VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

PRINTED 05/03/90

SOUTHERN RESEARCH INSTITUTE

PLATE 0U2
DRUG 6462

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6462
TAI: 30.00 SI: 9.69

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.125	0.117	0.119	0.120	0.124	0.130	0.000	0.000	0.000	0.000	0.000	0.000
	tox	cc/vc	drug 6462 experimental			tox					cc/vc	
B	1.284	1.387	0.125	0.155	0.151	1.443					1.322	
C	1.197	1.494	0.453	0.332	0.473	1.315					1.435	
D	1.183	1.466	1.131	1.258	1.282	1.466					1.513	
E	1.311	0.176	1.234	1.216	1.347	1.456					0.278	
F	0.415	0.176	0.359	0.399	0.430	0.446					0.187	
G	0.280	0.197	0.263	0.256	0.255	0.294					0.173	
	drug 6462 colorimetric background											
H	0.124	0.106	0.110	0.108	0.103	0.152						

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

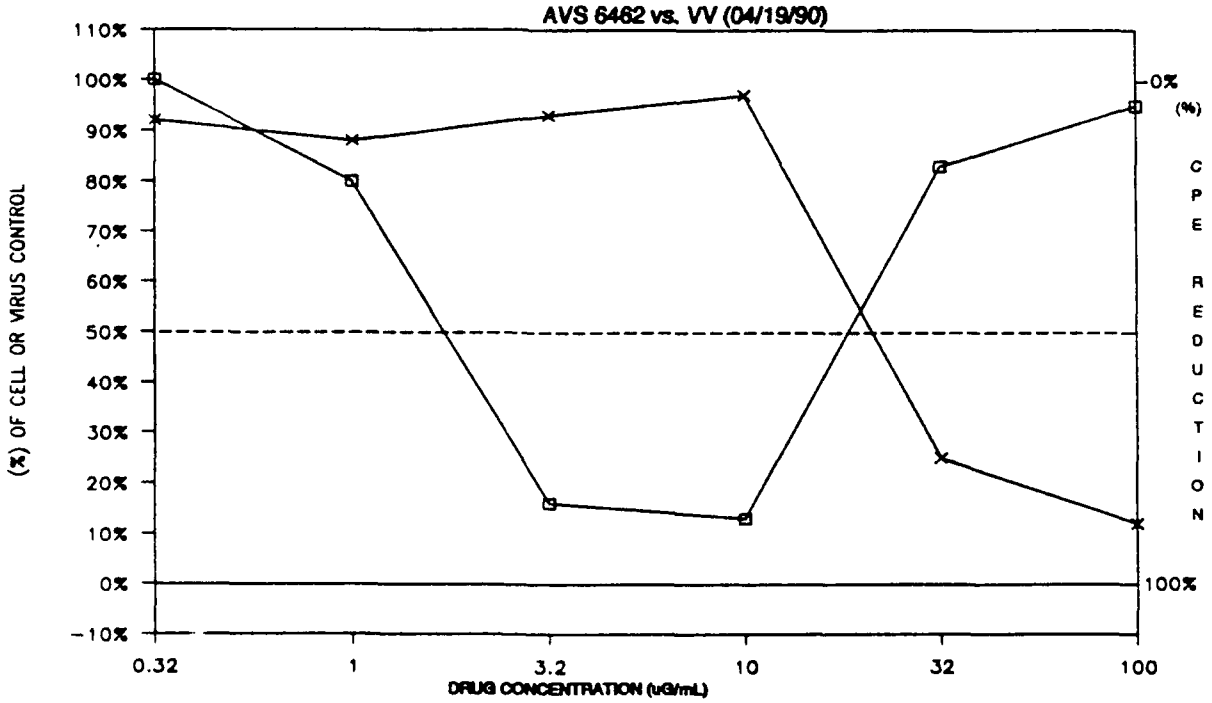
VIRUS	VV		PROJECT #	5975-4
CELLS	VERO	Satisfactory	SPONSOR	USAMRIID
SHIPMENT NUMBER	63	CONFIRMS ORIGINAL ACTIVITY	TEST DATE	04/19/90
STRN	LEDCA		DATE READ	04/25/90
REAGENT	0.123			
VIRUS CONTROL	0.075			
CELL CONTROL	1.314			
DIFFERENTIAL	1.238			

DRUG 6462	25%	50%	95%
TC (uG/mL)	16.70	24.46	> 100.00
IC (uG/mL)	1.10	1.72	-----
ANTIVIRAL INDEX (AI)	15.27	14.12	-----

DRUG 6462		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	0.32	-.084	100%	1.211	92%	0.029
C	1	0.242	80%	1.154	88%	-.020
D	3.2	1.041	16%	1.217	93%	-.015
E	10	1.081	13%	1.274	97%	-.013
F	32	0.215	83%	0.325	25%	-.017
high G *	100	0.059	95%	0.164	12%	0.001

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

PRINTED 05/08/90

SOUTHERN RESEARCH INSTITUTE

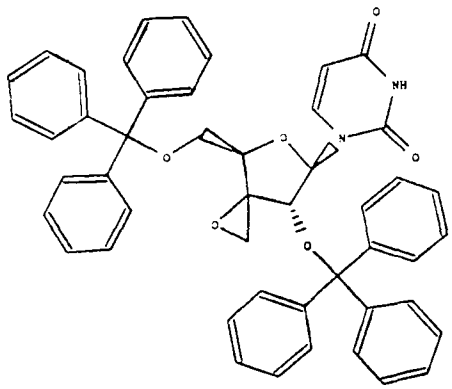
USAMRIID

Antiviral Drug Screening Program

09/21/90

STRUCTURE

CHIRAL



SUBMITTER	CTR NO	AVS NO
01141.01	KN-VII-87	AVS-006460
DATE RECD	**AMT RECEIVED [mg]**	**MOL WT (au)**
12-28-89	73.00	740.864

HANDLING/STORAGE

SOLUBILITY

STABILITY

ALT NAME

3'-DEOXY-2',5'-DI-O-TRITYL-3'-SPIROEPOXYURIDINE

COMPOUND NAME

3'-DEOXY-2',5'-DI-O-TRITYL-3'-SPIROEPOXYURIDINE

SCREEN INSTRUCTION

PRIORITY=PT>VEE>YF>KHF>PIC>JE>SF>VV>AD2>VSV

IN VIVO TOXICITY [mg/kg]

HOST	VH	RTE	LD50	MTC	LAB	PR	DATE

IN VITRO SCREEN [ug/ml]

VIR	VR	VR+	ID50	CELL	MTC	TI	TI+	LAB	PRT	DATE
HIV			NOT ACT	MT2	78.6	0		SO	MTT	28-MAR-90
HIV			22.4	CEM	92.7	4.14		SO	MTT	12-JUN-90
JE			NOT ACT	VERO	205	0		SO	MTT	06-MAR-90
JE			NOT ACT	VERO	514	0		SO	MTT	22-MAR-90
PT			NOT ACT	VERO	> 320	0		SO	MTT	06-MAR-90
PT			NOT ACT	VERO	346	0		SO	MTT	22-MAR-90
SF			NOT ACT	VERO	> 320	0		SO	MTT	06-MAR-90
SF			NOT ACT	VERO	605	0		SO	MTT	22-MAR-90
VEE			NOT ACT	VERO	> 320	0		SO	MTT	09-MAR-90
VEE			NOT ACT	VERO	556	0		SO	MTT	23-MAR-90
VV			NOT ACT	VERO	203	0		SO	MTT	29-MAR-90
YF			NOT ACT	VERO	266	0		SO	MTT	06-MAR-90
YF			49.5	VERO	251	5.07		SO	MTT	22-MAR-90

IN VIVO SCREEN [Dose = mg/kg]

VIR	HST	VR	VR+	DOSE	MTC	VEH	RTE	D	TOX	SP	L	PR	DATE

PLATE 1Q9
DRUG 6460

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6460
TAI: >30.24 SI: 2.79

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.302	0.273	0.281	0.414	0.402	0.296	0.079	0.081	0.295	0.252	0.130	0.071
		cc/vc					tox	drug 6460 experimental			cc/vc	tox
B		1.940					1.999	0.798	0.734	0.680	2.096	2.160
C		1.881					1.993	0.697	0.802	0.720	1.992	1.693
D		1.776					1.987	0.947	0.994	0.879	1.856	1.943
E		0.557					2.067	1.159	0.949	0.911	0.423	2.060
F		0.423					2.018	0.930	1.338	1.309	0.365	1.921
G		0.478					1.223	0.719	0.614	0.637	0.500	0.881
							drug 6460 colorimetric background					
H							0.354	0.270	0.282	0.268	0.273	0.313

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

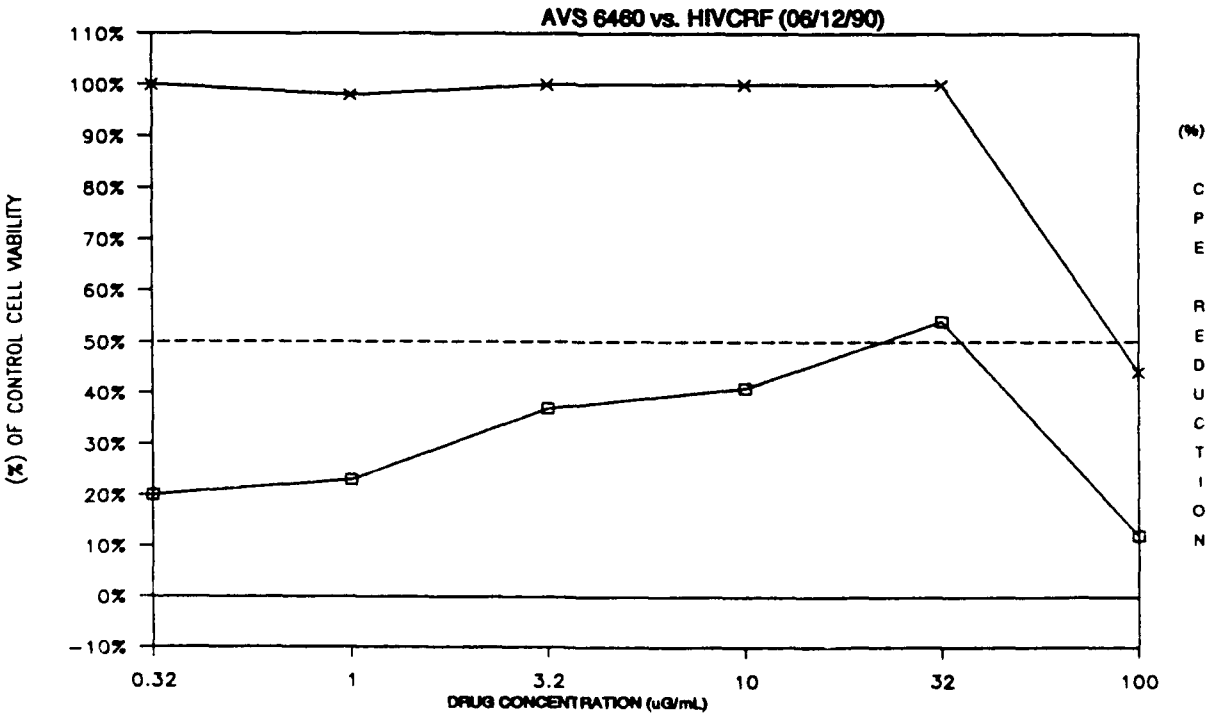
VIRUS	HIVCRF		PROJECT #	6520-2
CELLS	CEM	Satisfactory; Active; Retest	SPONSOR	USAMRIID
SHIPMENT NUMBER	63		TEST DATE	06/12/90
STRN	RF2		DATE READ	06/19/90
REAGENT	0.328			
VIRUS CONTROL	0.130			
CELL CONTROL	1.596			
DIFFERENTIAL	1.466			

DRUG 6460	25%	50%	95%
TC (uG/mL)	62.48	89.28	> 100.00
IC (uG/mL)	1.18	22.48	----
ANTIVIRAL INDEX (AI)	52.81	4.14	----

DRUG 6460		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% RED. IN CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	0.32	0.295	20%	1.767	100%	-.015
C	1	0.337	23%	1.570	98%	-.055
D	3.2	0.542	37%	1.697	100%	-.060
E	10	0.595	41%	1.782	100%	-.046
F	32	0.793	54%	1.700	100%	-.058
high G *	100	0.173	12%	0.698	44%	0.026

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH



□ DRUG'S ANTIVIRAL EFFECT (% RED. IN VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

PRINTED 06/21/90

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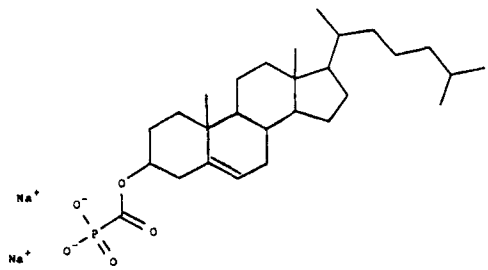
USAMRIID

Antiviral Drug Screening Program

09/21/90

STRUCTURE



SUBMITTER	CTR NO	AVS NO
01141.01	MS-I-48	AVS-006457
DATE RECD	**AMT RECEIVED [mg]**	**MOL WT (au)**
12-28-89	71.40	538.621

HANDLING/STORAGE

SOLUBILITY

STABILITY

ALT NAME

DISODIUM(CHOLESTERYLOXYCARBONYL)-PHOSPHONATE

COMPOUND NAME

DISODIUM(CHOLESTERYLOXYCARBONYL)-PHOSPHONATE

SCREEN INSTRUCTION

PRIORITY=PT>VEE>YF>KHF>PIC>JE>SF>VV>AD2>VSV

IN VIVO TOXICITY [mg/kg]

HOST	VH	RTE	LD50	MTC	LAB	PR	DATE

IN VITRO SCREEN [ug/ml]

VIR	VR	VR+	ID50	CELL	MTC	TI	TI+	LAB	PRT	DATE
HIV			NOT ACT	MT2	> 100	0		SO	MTT	28-MAR-90
HIV			77.6	CEM	> 100	> 1.29		SO	MTT	12-JUN-90
JE			NOT ACT	VERO	> 320	0		SO	MTT	06-MAR-90
PT			NOT ACT	VERO	> 320	0		SO	MTT	06-MAR-90
SF			NOT ACT	VERO	> 320	0		SO	MTT	06-MAR-90
VEE			NOT ACT	VERO	> 320	0		SO	MTT	09-MAR-90
VV			NOT ACT	VERO	165	0		SO	MTT	29-MAR-90
YF			NOT ACT	VERO	> 320	0		SO	MTT	06-MAR-90

IN VIVO SCREEN [Dose = mg/kg]

VIR	HST	VR	VR+	DOSE	MTC	VEH	RTE	D	TOX	SP	L	PR	DATE

PLATE 1Q8
DRUG 6457

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6457
TAI: >27.78 SI: >1.29

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.433	0.373	0.552	0.655	0.447	0.451	0.103	0.100	0.191	0.442	0.104	0.087
	tox	co/vo	drug 6457 experimental			tox					co/vo	
B	1.739	1.747	0.707	0.601	0.807	1.972					1.841	
C	1.606	1.765	0.674	0.715	1.069	1.794					1.750	
D	1.871	1.913	0.559	0.799	1.037	1.806					2.012	
E	1.782	0.522	0.965	0.990	0.742	1.916					0.811	
F	1.783	0.541	0.894	0.662	1.126	1.899					0.583	
G	1.691	0.602	1.069	1.161	1.241	1.811					0.652	
	drug 6457 colorimetric background											
H	0.345	0.405	0.387	0.396	0.393	0.382						

tox=cell toxicity co=cell control vo=virus control BOLD = highest drug conc values shown are optical densities

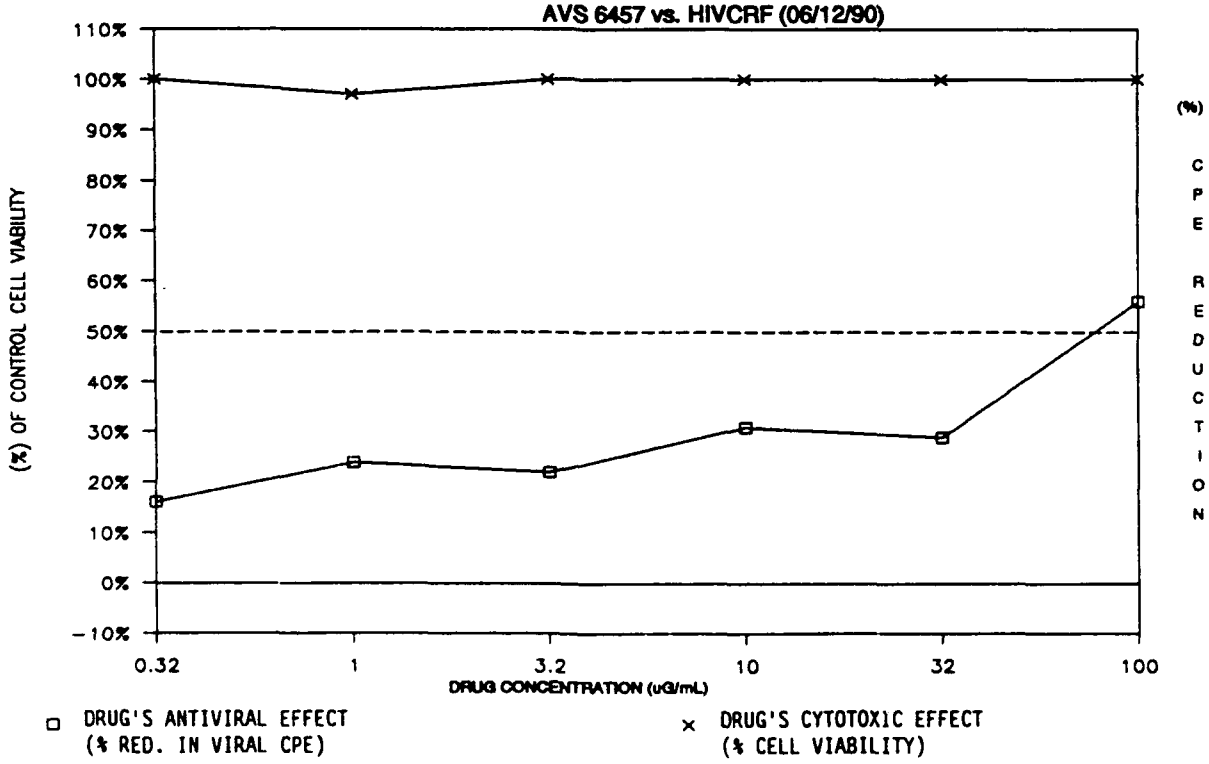
VIRUS	HIVCRF		PROJECT #	6520-2
CELLS	CEM	Satisfactory; Active; Retest	SPONSOR	USAMRIID
SHIPMENT NUMBER	63		TEST DATE	06/12/90
STRN	RF2		DATE READ	06/19/90
REAGENT	0.485			
VIRUS CONTROL	0.133			
CELL CONTROL	1.353			
DIFFERENTIAL	1.220			

DRUG 6457	25%	50%	95%
TC (uG/mL)	> 100.00	> 100.00	> 100.00
IC (uG/mL) *	4.68	77.00	------
ANTIVIRAL INDEX (AI)	> 21.37	> 1.29	------

DRUG 6457		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% RED. IN VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	0.32	0.190	16%	1.473	100%	-.103
C	1	0.293	24%	1.307	97%	-.092
D	3.2	0.269	22%	1.442	100%	-.089
E	10	0.379	31%	1.462	100%	-.098
F	32	0.356	29%	1.436	100%	-.080
high G *	100	0.679	56%	1.406	100%	-.140

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH



PRINTED 06/21/90

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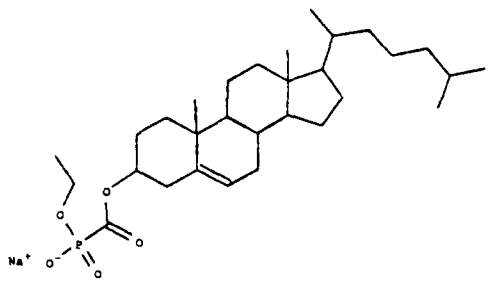
Antiviral Drug Screening Program

09/21/90

STRUCTURE



SUBMITTER	CTR NO	AVS NO
01141.01	MS-I-47	AVS-006456
DATE RECD	**AMT RECEIVED [mg]**	**MOL WT (au)**
12-28-89	79.20	544.694

HANDLING/STORAGE

SOLUBILITY

STABILITY

ALT NAME

SODIUM ETHYL(CHOLESTERYLOXYCARBONYL)-PHOSPHONATE

COMPOUND NAME

SODIUM ETHYL(CHOLESTERYLOXYCARBONYL)-PHOSPHONATE

SCREEN INSTRUCTION

PRIORITY=PT>VEE>YF>KHF>PIC>JE>SF>VV>AD2>VSV

IN VIVO TOXICITY [mg/kg]

HOST	VH	RTE	LD50	MTC	LAB PR	DATE

IN VITRO SCREEN [ug/ml]

VIR	VR	VR+	ID50	CELL	MTC	TI	TI+	LAB PRT DATE
HIV			NOT ACT	MT2	66	0		SO MTT28-MAR-90
HIV			NOT ACT	CEM	20.3	0		SO MTT12-JUN-90
JE			NOT ACT	VERO	70.1	0		SO MTT06-MAR-90
JE			NOT ACT	VERO	134	0		SO MTT18-APR-90
PT			NOT ACT	VERO	69.3	0		SO MTT06-MAR-90
PT			NOT ACT	VERO	120	0		SO MTT18-APR-90
SF			NOT ACT	VERO	69.4	0		SO MTT06-MAR-90
SF			NOT ACT	VERO	130	0		SO MTT18-APR-90
VEE			NOT ACT	VERO	95.1	0		SO MTT09-MAR-90
VEE			NOT ACT	VERO	214	0		SO MTT20-APR-90
VV			NOT ACT	VERO	53	0		SO MTT29-MAR-90
YF			NOT ACT	VERO	63.2	0		SO MTT06-MAR-90
YF			61	VERO	198	3.25		SO MTT18-APR-90

IN VIVO SCREEN [Dose = mg/kg]

VIR	HST	VR	VR+	DOSE	MTC	VEH	RTE	D TOX	SP L	PR	DATE

PLATE 1Q7
DRUG 6456

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6456
TAI: >10.33 SI: ——

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.463	0.349	0.539	0.566	0.386	0.388	0.253	0.256	0.399	0.448	0.239	0.199
		cc/vc					tox	drug 6456 experimental			cc/vc	tox
B		1.590					2.158	0.677	0.749	0.790	1.783	2.032
C		1.486					1.897	0.768	0.952	0.649	1.588	1.808
D		2.193					1.511	0.717	0.851	0.958	1.641	1.705
E		0.462					1.648	0.907	0.911	0.499	0.539	1.596
F		0.636					0.671	0.511	0.407	0.340	0.548	0.271
G		0.374					0.517	0.507	0.351	0.430	0.607	0.456
							drug 6456 colorimetric background					
H							0.437	0.442	0.455	0.454	0.455	0.522

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	HIVCRF		PROJECT #	6520-2
CELLS	CEM	Satisfactory	SPONSOR	USAMRIID
SHIPMENT NUMBER	63		TEST DATE	06/12/90
STRN	RF2		DATE READ	06/19/90
REAGENT	0.449			
VIRUS CONTROL	0.079			
CELL CONTROL	1.265			
DIFFERENTIAL	1.186			

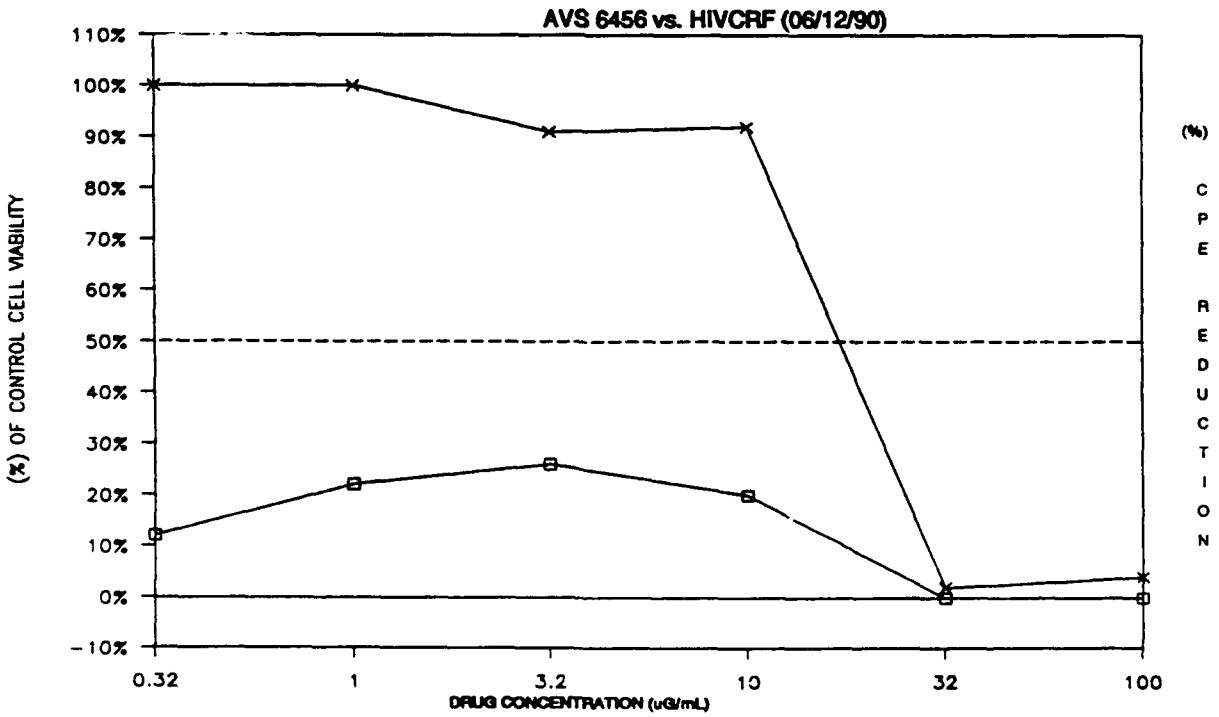
DRUG 6456	25%	50%	95%
TC (uG/mL)	14.28	20.50	31.30
IC (uG/mL)	2.39	——	——
ANTIVIRAL INDEX (AI)	5.92	——	——

DRUG 6456		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% RED. IN CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	0.32	0.137	12%	1.573	100%	0.074
C	1	0.255	22%	1.397	100%	0.007
D	3.2	0.308	26%	1.154	91%	0.006
E	10	0.238	20%	1.167	92%	0.007
F	32	-.102	0%	0.029	2%	-.006
high G *	100	-.086	0%	0.050	4%	-.012

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6456 vs. HIVCRF (06/12/90)



□ DRUG'S ANTIVIRAL EFFECT (% RED. IN VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

PRINTED 08/21/90

SOUTHERN RESEARCH INSTITUTE

PLATE VC5
DRUG 6456

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6456
TAI: 0.00 SI: ------

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.064	0.056	0.057	0.057	0.059	0.058	0.002	0.001	0.002	0.002	0.002	0.002
	tox	cc/vc	drug 6456 experimental			tox					cc/vc	
B	1.137	1.382	0.224	0.255	0.236	1.253					1.348	
C	1.034	1.299	0.235	0.249	0.251	1.061					1.062	
D	0.984	1.077	0.361	0.349	0.350	0.932					1.055	
E	0.772	0.243	0.374	0.289	0.416	0.593					0.234	
F	0.044	0.242	0.035	0.035	0.034	0.037					0.257	
G	0.044	0.233	0.037	0.036	0.033	0.035					0.251	
	drug 6456 colorimetric background											
H	0.040	0.049	0.051	0.051	0.051	0.053						

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

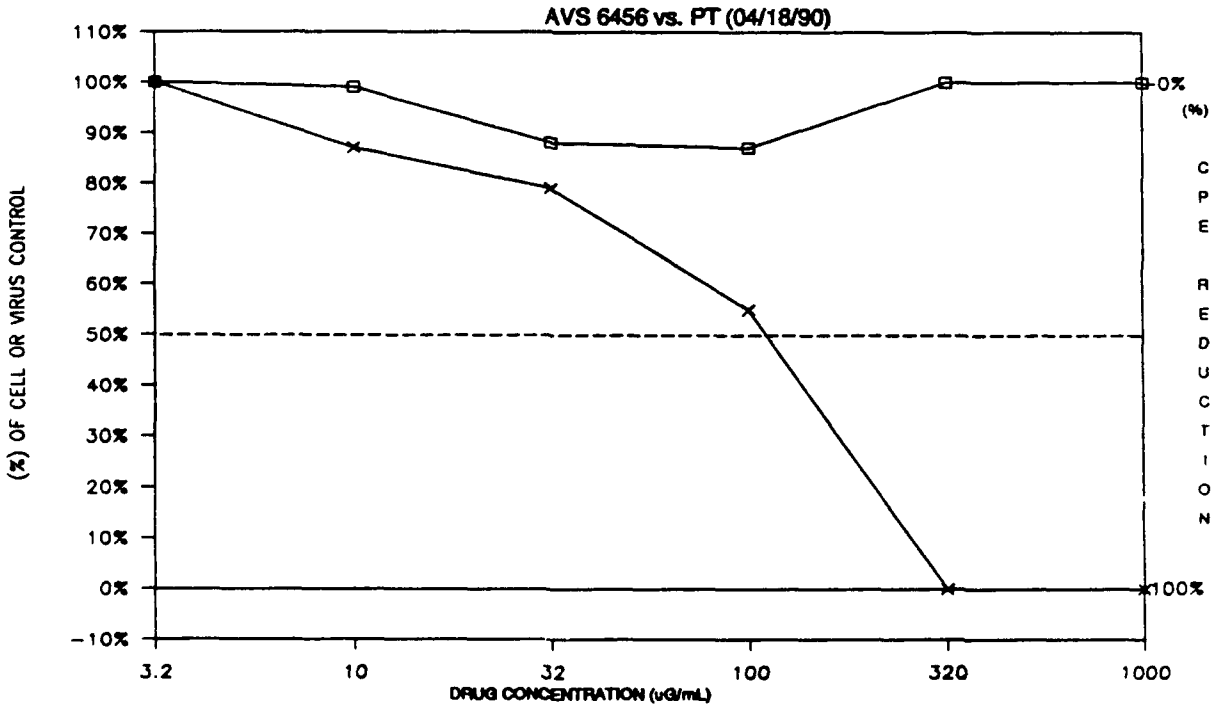
VIRUS	PT		PROJECT #	5975-1
CELLS	VERO	Satisfactory	SPONSOR	USAMRIID
SHIPMENT NUMBER	64	CONFIRMATORY #1 ** NEGATIVE **	TEST DATE	04/18/90
STRN	ADAMES		DATE READ	04/26/90
REAGENT	0.059			
VIRUS CONTROL	0.185			
CELL CONTROL	1.145			
DIFFERENTIAL	0.961			

DRUG 6456	25%	50%	95%
TC (uG/mL)	43.30	120.00	300.00
IC (uG/mL)	------	------	------
ANTIVIRAL INDEX (AI)	------	------	------

DRUG 6456		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	3.2	0.001	100%	1.143	100%	-.006
C	10	0.010	99%	0.997	87%	-.008
D	32	0.118	88%	0.908	79%	-.008
E	100	0.124	87%	0.632	55%	-.008
F	320	-.199	100%	-.008	0%	-.010
high G *	1000	-.189	100%	0.000	0%	-.019

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

PLATE VCL
DRUG 6456

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6456
TAI: 0.00 SI: -----

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.056	0.052	0.051	0.051	0.052	0.052	0.001	0.002	0.001	0.001	0.001	0.001
	tox	cc/vc	drug 6456 experimental			tox					cc/vc	
B	0.906	1.126	0.044	0.043	0.040	1.015					0.781	
C	0.972	1.096	0.067	0.067	0.056	0.958					1.029	
D	1.066	1.047	0.077	0.071	0.061	1.002					0.946	
E	0.644	0.071	0.171	0.183	0.152	0.554					0.069	
F	0.041	0.095	0.038	0.039	0.037	0.038					0.092	
G	0.038	0.070	0.032	0.032	0.031	0.032					0.061	
	drug 6456 colorimetric background											
H	0.038	0.047	0.048	0.053	0.049	0.048						

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	SF		PROJECT #	5975-1
CELLS	VERO	Satisfactory	SPONSOR	USAMRIID
SHIPMENT NUMBER	64	CONFIRMATORY #1 ** NEGATIVE **	TEST DATE	04/18/90
STRN	SICILIAN		DATE READ	04/26/90
REAGENT	0.052			
VIRUS CONTROL	0.024			
CELL CONTROL	0.952			
DIFFERENTIAL	0.928			

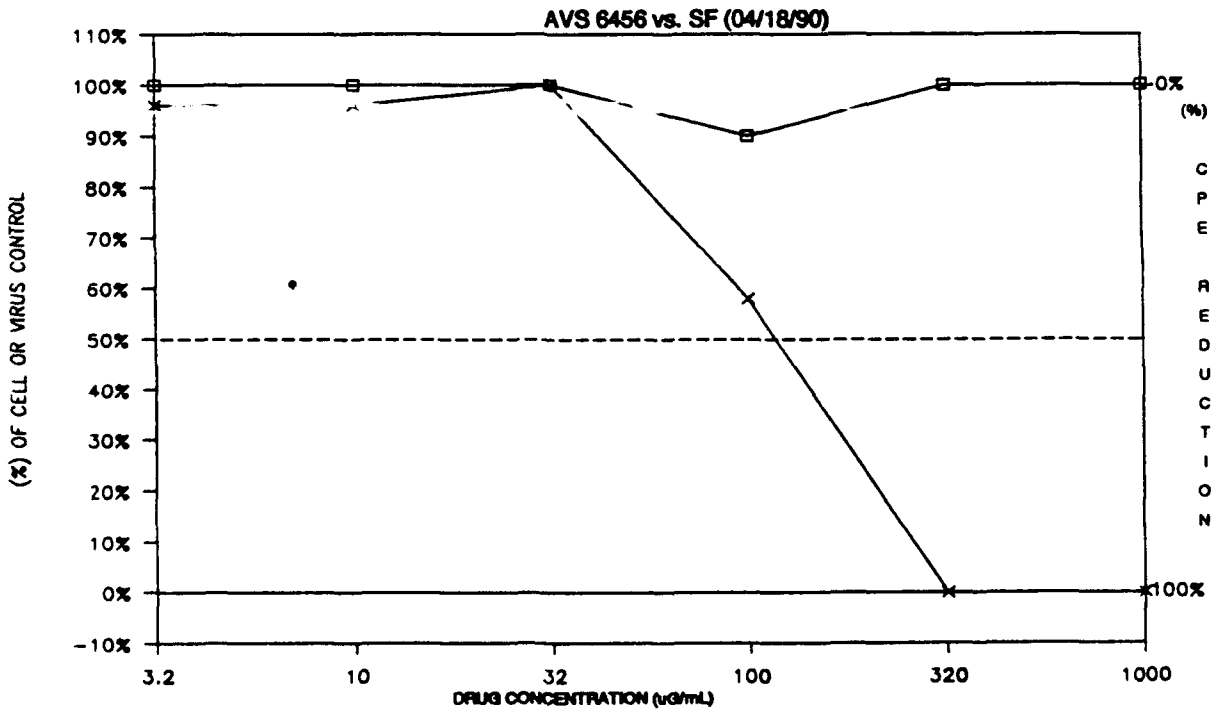
DRUG 6456	25%	50%	95%
TC (uG/mL)	72.50	130.00	301.00
IC (uG/mL)	-----	-----	-----
ANTIVIRAL INDEX (AI)	-----	-----	-----

DRUG 6456		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	3.2	-.030	100%	0.912	96%	-.004
C	10	-.010	100%	0.916	96%	-.003
D	32	-.008	100%	0.981	100%	0.001
E	100	0.096	90%	0.551	58%	-.004
F	320	-.033	100%	-.008	0%	-.005
high G *	1000	-.031	100%	-.003	0%	-.014

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6456 vs. SF (04/18/90)



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

PRINTED 05/08/90

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PLATE VBP
DRUG 6456

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6456
TAI: 0.00 SI: ——

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.086	0.068	0.066	0.068	0.071	0.066	0.001	0.002	0.002	0.001	0.002	0.002
	tox	cc/vc	drug 6456 experimental			tox					cc/vc	
B	1.336	1.491	0.112	0.091	0.111	1.375					1.479	
C	1.312	1.515	0.105	0.115	0.088	1.364					1.465	
D	1.290	1.521	0.083	0.086	0.099	1.282					1.444	
E	1.221	0.117	0.096	0.091	0.093	1.149					0.115	
F	0.410	0.126	0.138	0.196	0.147	0.378					0.120	
G	0.107	0.098	0.092	0.087	0.092	0.095					0.098	
	drug 6456 colorimetric background											
H	0.057	0.073	0.064	0.060	0.062	0.065						

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	VE		PROJECT #	5975-1
CELLS	VERO	Satisfactory	SPONSOR	USAMRIID
SHIPMENT NUMBER	64	CONFIRMATORY #1 ** NEGATIVE **	TEST DATE	04/20/90
STRN	TRINIDAD		DATE READ	04/24/90
REAGENT	0.071			
VIRUS CONTROL	0.042			
CELL CONTROL	1.415			
DIFFERENTIAL	1.374			

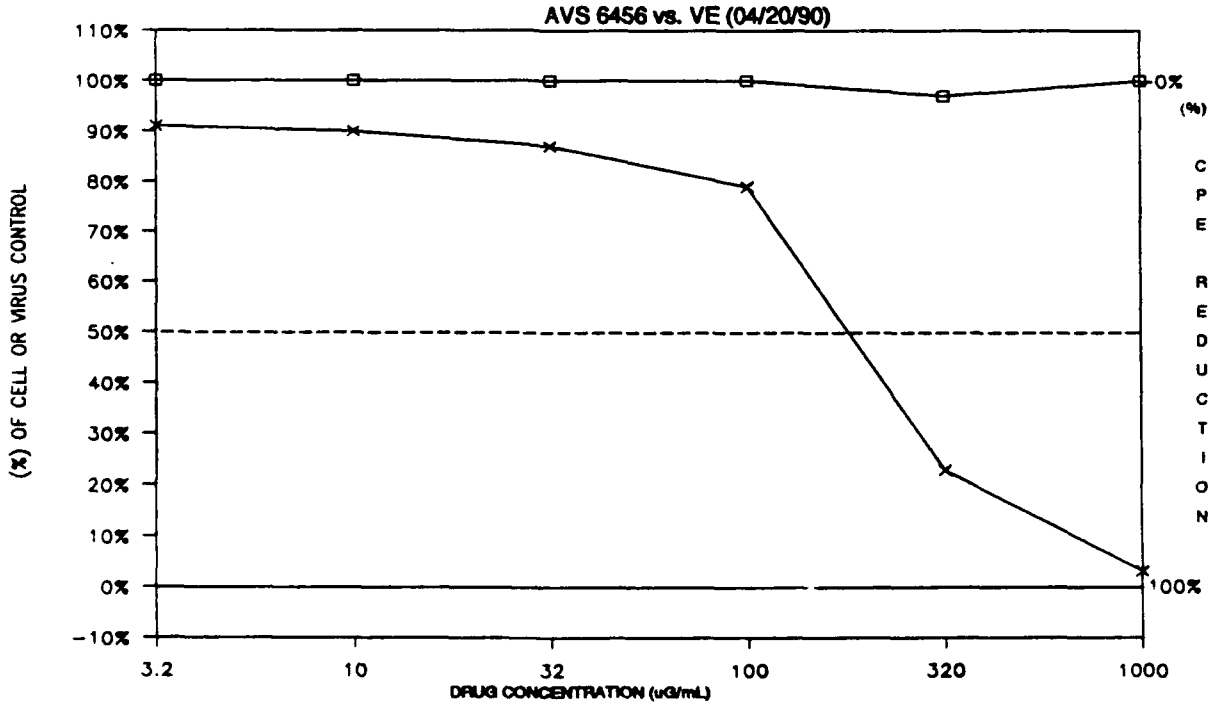
DRUG 6456	25%	50%	95%
TC (uG/mL)	116.00	214.00	932.00
IC (uG/mL)	-----	-----	-----
ANTIVIRAL INDEX (AI)	-----	-----	-----

DRUG 6456		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	3.2	-.002	100%	1.291	91%	-.006
C	10	-.001	100%	1.276	90%	-.009
D	32	-.012	100%	1.226	87%	-.011
E	100	-.012	100%	1.121	79%	-.007
F	320	0.046	97%	0.321	23%	0.002
high G *	1000	-.008	100%	0.044	3%	-.014

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6456 vs. VE (04/20/90)



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

PRINTED 05/08/90

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PLATE VAT
DRUG 6456

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6456
TAI: >15.51 SI: 2.16

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.074	0.076	0.073	0.073	0.073	0.074	0.001	0.001	0.000	0.001	0.001	0.001
	tox	cc/vc	drug 6456 experimental			tox					cc/vc	
B	0.976	1.014	0.301	0.302	0.311	0.917					0.995	
C	0.951	0.907	0.359	0.337	0.348	1.032					0.973	
D	0.954	0.913	0.509	0.517	0.494	0.966					0.989	
E	0.880	0.286	0.761	0.689	0.684	0.802					0.287	
F	0.117	0.292	0.097	0.082	0.079	0.080					0.294	
G	0.056	0.281	0.053	0.051	0.050	0.047					0.287	
	drug 6456 colorimetric background											
H	0.052	0.065	0.069	0.069	0.069	0.068						

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

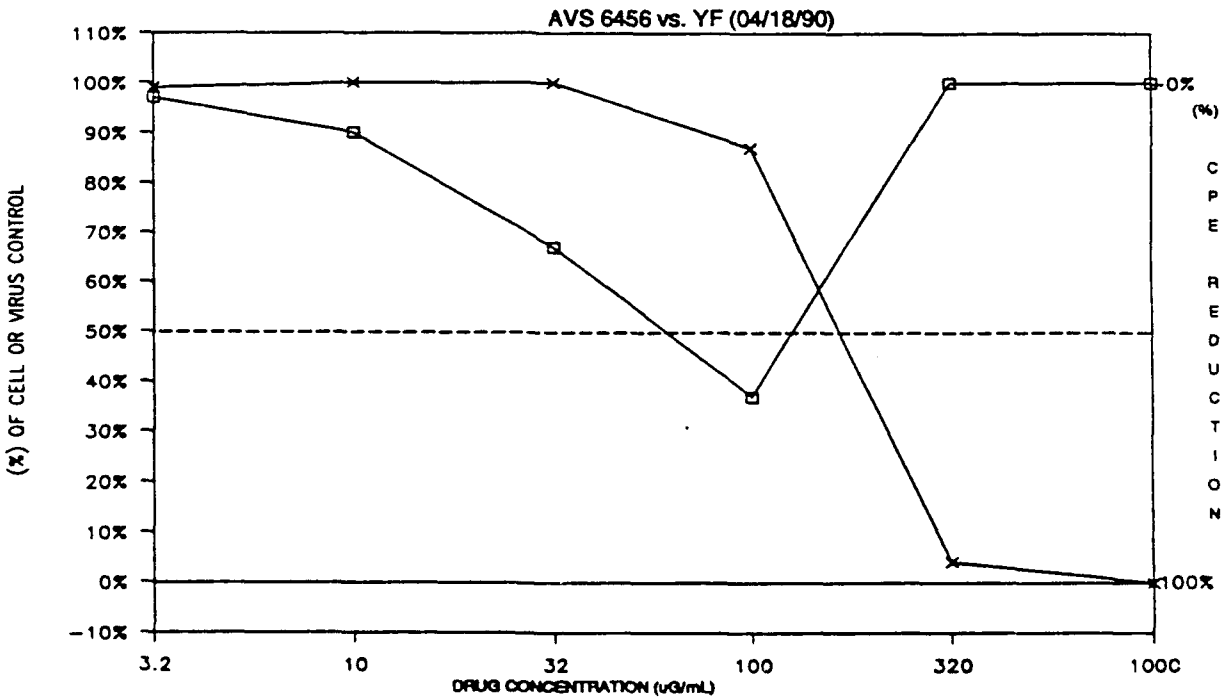
VIRUS	YF		PROJECT #	5975-1
CELLS	VERO	Satisfactory; Active; Retest	SPONSOR	USAMRIID
SHIPMENT NUMBER	64	CONFIRMATORY #1 ** GOOD **	TEST DATE	04/18/90
STRN	ASIBI		DATE READ	04/24/90
REAGENT	0.074			
VIRUS CONTROL	0.214			
CELL CONTROL	0.891			
DIFFERENTIAL	0.677			

DRUG 6456	25%	50%	95%
TC (uG/mL)	132.00	198.00	317.00
IC (uG/mL)	21.40	61.00	----
ANTIVIRAL INDEX (AI)	6.17	3.25	----

DRUG 6456		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	3.2	0.023	97%	0.879	99%	-.006
C	10	0.065	90%	0.923	100%	-.005
D	32	0.224	67%	0.891	100%	-.005
E	100	0.429	37%	0.772	87%	-.005
F	320	-.193	100%	0.034	4%	-.009
high G *	1000	-.215	100%	0.000	0%	-.022

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

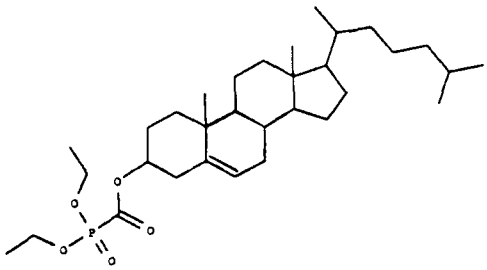
SOUTHERN RESEARCH INSTITUTE

USAMRIID

Antiviral Drug Screening Program

09/21/90

STRUCTURE



SUBMITTER	CTR NO	AVS NO
01141.01	MS-I-44	AVS-006455
DATE RECD	**AMT RECEIVED [mg]**	**MOL WT (au)**
12-28-89	79.20	550.766

HANDLING/STORAGE

SOLUBILITY

STABILITY

ALT NAME

DIETHYL(CHOLESTERYLOXYCARBONYL)-PHOSPHONATE

COMPOUND NAME

DIETHYL(CHOLESTERYLOXYCARBONYL)-PHOSPHONATE

SCREEN INSTRUCTION

PRIORITY=PT>VEE>YF>KHF>PIC>JE>SF>VV>AD2>VSV

IN VIVO TOXICITY [mg/kg]

HOST	VH	RTE	LD50	MTC	LAB PR	DATE

IN VITRO SCREEN [ug/ml]

VIR	VR	VR+	ID50	CELL	MTC	TI	TI+	LAB PRT DATE
HIV			NOT ACT	MT2	> 100	0		SO MTT28-MAR-90
HIV			17.5	CEM	> 100	> 5.71		SO MTT12-JUN-90
JE			NOT ACT	VERO	> 320	0		SO MTT06-MAR-90
PT			NOT ACT	VERO	> 320	0		SO MTT06-MAR-90
SF			NOT ACT	VERO	> 320	0		SO MTT06-MAR-90
VEE			NOT ACT	VERO	> 320	0		SO MTT09-MAR-90
VV			NOT ACT	VERO	> 320	0		SO MTT29-MAR-90
YF			NOT ACT	VERO	> 320	0		SO MTT06-MAR-90

IN VIVO SCREEN [Dose = mg/kg]

VIR	HST	VR	VR+	DOSE	MTC	VEH	RTE	D TOX	SP L	PR	DATE

PLATE 1Q7
DRUG 6455

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6455
TAI: >38.98 SI: >5.71

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.463	0.349	0.539	0.566	0.396	0.388	0.253	0.256	0.399	0.448	0.239	0.199
	tox	co/vo	drug 6455 experimental			tox					co/vo	
B	1.916	1.590	0.861	0.677	0.813	1.889					1.783	
C	1.840	1.486	0.820	0.986	0.873	2.017					1.588	
D	1.800	2.193	0.764	0.557	0.841	1.849					1.641	
E	2.057	0.462	0.856	0.811	1.334	2.016					0.539	
F	1.959	0.636	0.944	1.071	1.242	1.830					0.548	
G	2.003	0.374	1.269	1.478	1.180	1.913					0.607	
	drug 6455 colorimetric background											
H	0.328	0.243	0.480	0.459	0.455	0.448						

tox=cell toxicity co=cell control vo=virus control BOLD = highest drug conc values shown are optical densities

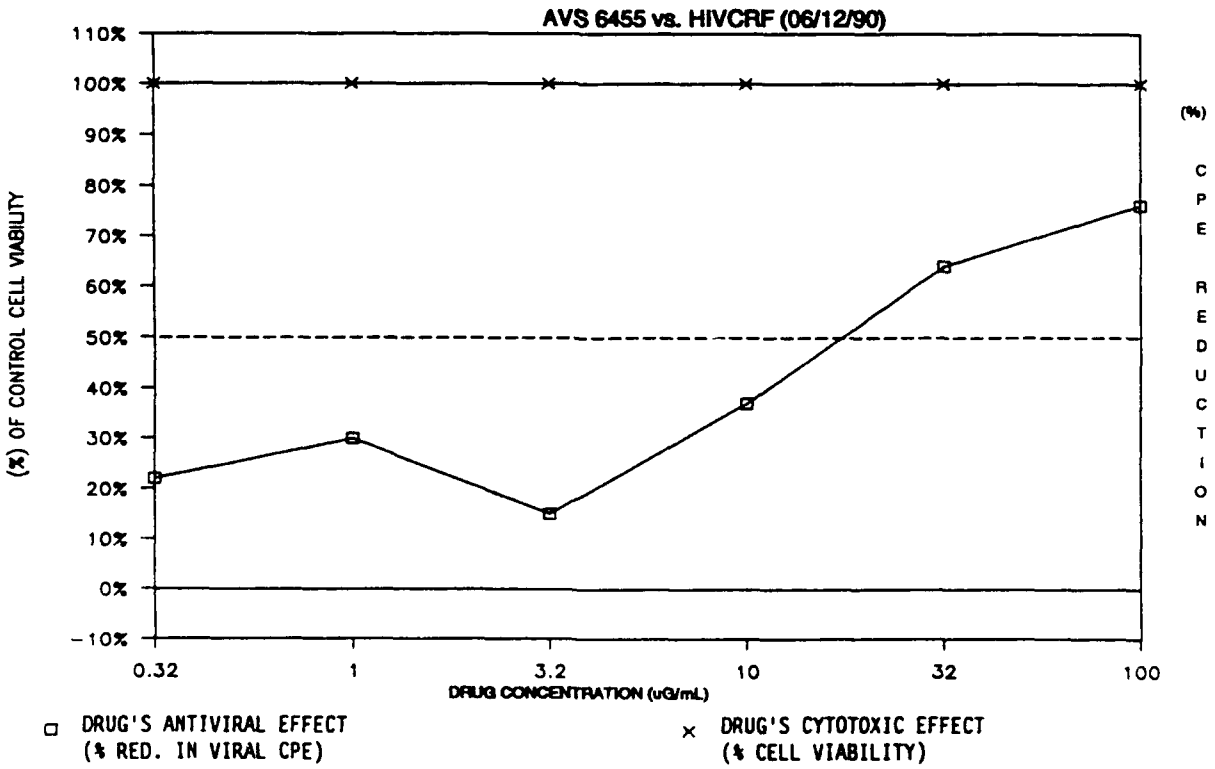
VIRUS	HIVCRF		PROJECT #	6520-2
CELLS	CEM	Satisfactory; Active; Retest	SPONSOR	USAMRIID
SHIPMENT NUMBER	63		TEST DATE	06/12/90
STRN	RF2		DATE READ	06/19/90
REAGENT	0.449			
VIRUS CONTROL	0.079			
CELL CONTROL	1.265			
DIFFERENTIAL	1.186			

DRUG 6455	25%	50%	95%
TC (uG/mL)	> 100.00	> 100.00	> 100.00
IC (uG/mL)	0.49	17.50	-----
ANTIVIRAL INDEX (AI)	> 203.84	> 5.71	-----

DRUG 6455		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% RED. IN VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	0.32	0.256	22%	1.454	100%	0.000
C	1	0.358	30%	1.473	100%	0.007
D	3.2	0.182	15%	1.365	100%	0.011
E	10	0.442	37%	1.557	100%	0.031
F	32	0.764	64%	1.652	100%	-.206
high G *	100	0.902	76%	1.631	100%	-.120

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH



PRINTED 06/21/90

SOUTHERN RESEARCH INSTITUTE

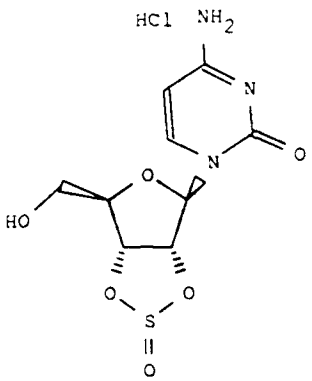
USAMRIID

Antiviral Drug Screening Program

05/18/90

STRUCTURE

CHIRAL



SUBMITTER	CTR NO	AVS NO
01141.01	KN-II-71	AVS-006462
DATE RECD	**AMT RECEIVED [mg]**	**MOL WT (au)**
12-28-89	72.40	325.729

HANDLING/STORAGE

SOLUBILITY

STABILITY

ALT NAME

2',3'-O-SULFINYLCYTIDINE HYDROCHLORIDE

COMPOUND NAME

2',3'-O-SULFINYLCYTIDINE HYDROCHLORIDE

SCREEN INSTRUCTION

PRIORITY=PT>VEE>YF>KHF>PIC>JE>SF>VV>AD2>VSV

IN VIVO TOXICITY [mg/kg]

HOST	VH	RTE	LD50	MTC	LAB PR	DATE

IN VITRO SCREEN [ug/ml]

VIR	VR	VR+	ID50	CELL	MTC	TI	TI+	LAB PRT	DATE
HIV			NOT ACT	MT2	.06	0		SO MTT	
HIV			NOT ACT	MT2	< .32	0		SO MTT	
JE			NOT ACT	VERO	22.4	0		SO MTT	90-03-06
PT			NOT ACT	VERO	38.6	0		SO MTT	90-03-06
SF			NOT ACT	VERO	21	0		SO MTT	90-03-06
VEE			NOT ACT	VERO	9.73	0		SO MTT	90-03-09
VV			1.72	VERO	16.7	14.12		SO MTT	
VV			3.28	VERO	25.7	16.66		SO MTT	
YF			NOT ACT	VERO	44.4	0		SO MTT	90-03-06

IN VIVO SCREEN [Dose = mg/kg]

VIR	HST	VR	VR+	DOSE	MTC	VEH	RTE	D	TOX	SP	L	PR	DATE

PLATE 0SS
DRUG 6462

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6462
TAI: >32.30 SI: 7.85

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.104	0.114	0.112	0.111	0.111	0.112	0.000	0.000	0.000	0.000	0.000	0.000
		cc/vc					tox	drug 6462 experimental			cc/vc	tox
B		1.542					1.492	0.362	0.278	0.427	1.506	1.619
C		1.596					1.491	0.820	0.842	0.963	1.508	1.676
D		1.651					1.650	1.412	1.560	1.551	1.524	1.770
E		0.160					1.040	0.929	0.938	0.852	0.247	1.058
F		0.248					0.573	0.511	0.523	0.524	0.174	0.542
G		0.213					0.320	0.312	0.315	0.305	0.238	0.349
							drug 6462 colorimetric background					
H							0.153	0.123	0.110	0.108	0.110	0.117

tox=cell toxicity cc=cell control vc=virus control **BOLD** = highest drug conc values shown are optical densities

VIRUS	VV		PROJECT #	5975-4
CELLS	VERO	Satisfactory; Active; Retest	SPONSOR	USAMRIID
SHIPMENT NUMBER	63	RETEST AT 100 UG/ML	TEST DATE	03/29/90
STRN	LEDCA		DATE READ	04/04/90
REAGENT	0.111			
VIRUS CONTROL	0.103			
CELL CONTROL	1.444			
DIFFERENTIAL	1.341			

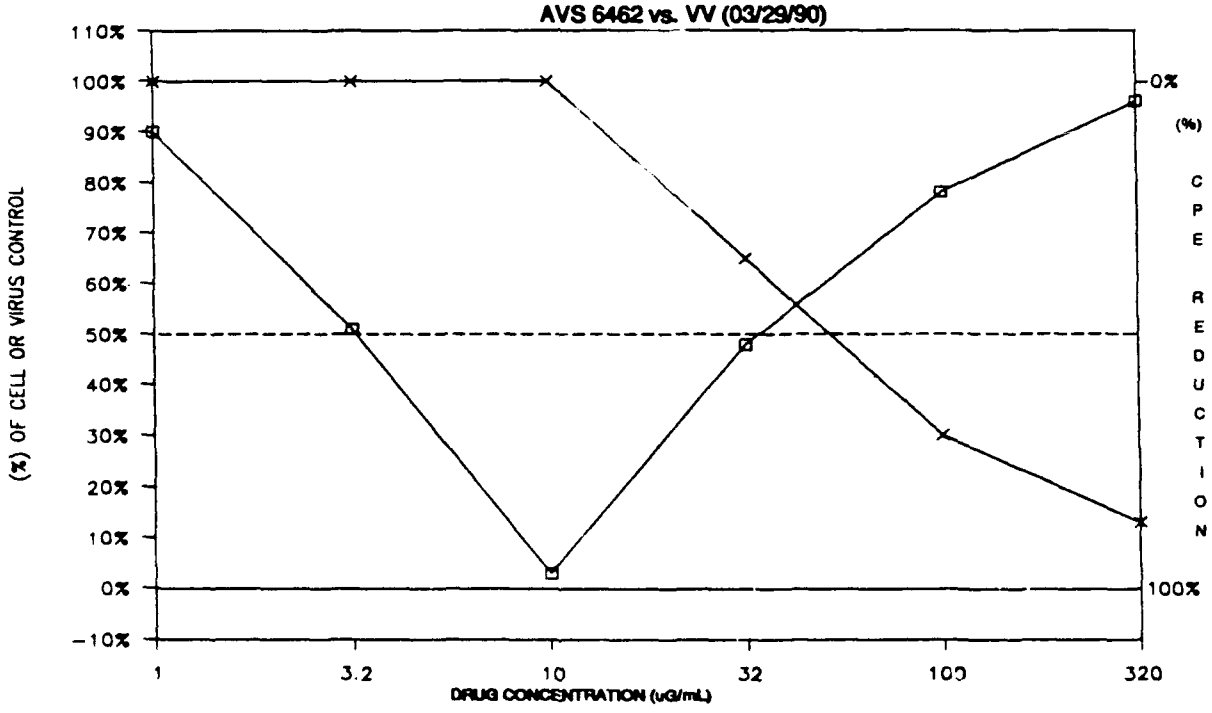
DRUG 6462	25%	50%	95%
TC (uG/mL)	25.70	61.10	> 320.00
IC (uG/mL)	1.56	3.28	9.54
ANTIVIRAL INDEX (AI)	16.44	18.66	> 33.56

DRUG 6462		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	0.136	90%	1.439	100%	0.006
C	3.2	0.663	51%	1.474	100%	-.001
D	10	1.297	3%	1.602	100%	-.003
E	32	0.694	48%	0.939	65%	-.001
F	100	0.294	78%	0.435	30%	0.012
high G *	320	0.055	96%	0.182	13%	0.042

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6462 vs. VV (03/29/90)



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

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PLATE 0U2
DRUG 6462

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6462
TAI: 30.00 SI: 9.69

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.125	0.117	0.119	0.120	0.124	0.130	0.000	0.000	0.000	0.000	0.000	0.000
	tox	cc/vo	drug 6462 experimental			tox					cc/vo	
B	1.284	1.387	0.125	0.155	0.151	1.443					1.322	
C	1.197	1.494	0.453	0.332	0.473	1.315					1.435	
D	1.183	1.466	1.131	1.258	1.282	1.466					1.513	
E	1.311	0.176	1.234	1.216	1.347	1.456					0.278	
F	0.415	0.176	0.359	0.399	0.430	0.446					0.187	
G	0.280	0.197	0.263	0.256	0.255	0.294					0.173	
	drug 6462 colorimetric background											
H	0.124	0.106	0.110	0.108	0.103	0.152						

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	VV		PROJECT #	5975-4
CELLS	VERO	Satisfactory	SPONSOR	USAMRIID
SHIPMENT NUMBER	63	CONFIRMS ORIGINAL ACTIVITY	TEST DATE	04/19/90
STRN	LEDCA		DATE READ	04/25/90
REAGENT	0.123			
VIRUS CONTROL	0.075			
CELL CONTROL	1.314			
DIFFERENTIAL	1.238			

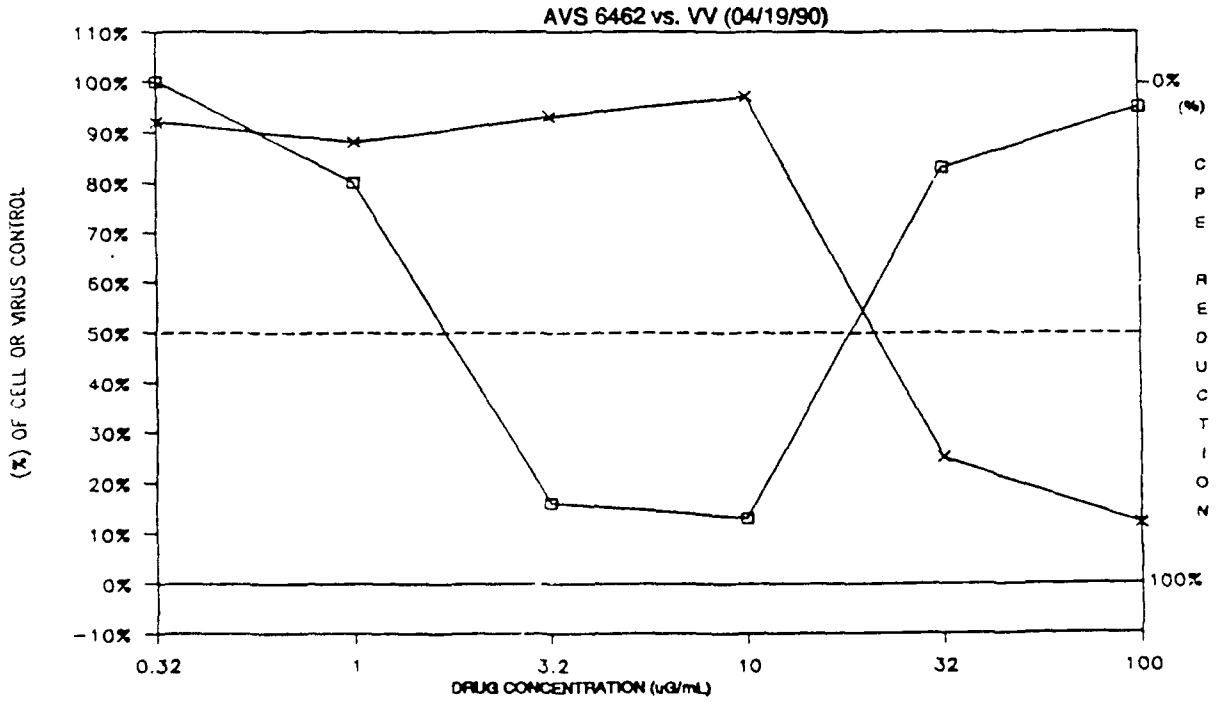
DRUG 6462	25%	50%	95%
TC (uG/mL)	16.70	24.40	> 100.00
IC (uG/mL)	1.10	1.72	----
ANTIVIRAL INDEX (AI)	15.27	14.12	----

DRUG 6462		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	0.32	-.084	100%	1.211	92%	0.029
C	1	0.242	80%	1.154	88%	-.020
D	3.2	1.041	16%	1.217	93%	-.015
E	10	1.081	13%	1.274	97%	-.013
F	32	0.215	83%	0.325	25%	-.017
high G *	100	0.059	95%	0.164	12%	0.001

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6462 vs. VV (04/19/90)



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

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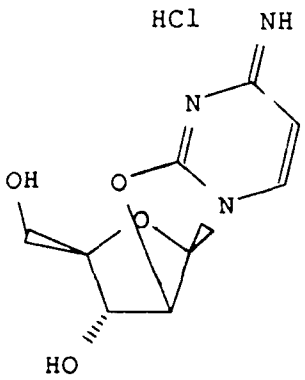
STRUCTURE

CHIRAL



SUBMITTER	CTR NO	AVS NO
01141.01	KN-II-95	AVS-006467
DATE RECD	**AMT RECEIVED [mg]**	**MOL WT (au)**
12-28-89	72.60	261.667

HANDLING/STORAGE

SOLUBILITY

STABILITY

ALT NAME

2',O2-ANHYDROCYTIDINE HYDROCHLORIDE

COMPOUND NAME

2',O2-ANHYDROCYTIDINE HYDROCHLORIDE

SCREEN INSTRUCTION

PRIORITY=PT>VEE>YF>KHF>PIC>JE>SF>VV>AD2>VSV

IN VIVO TOXICITY [mg/kg]

HOST	VH	RTE	LD50	MTC	LAB PR	DATE

IN VITRO SCREEN [ug/ml]

VIR	VR	VR+	ID50	CELL	MTC	TI	TI+	LAB	PRT	DATE
HIV			NOT ACT	MT2	.04	0		SO	MTT	
HIV			NOT ACT	MT2	< .32	0		SO	MTT	
JE			NOT ACT	VERO	.71	0		SO	MTT	90-03-22
JE			NOT ACT	VERO	1.8	0		SO	MTT	90-03-06
PT			NOT ACT	VERO	.48	0		SO	MTT	90-03-22
PT			NOT ACT	VERO	4.99	0		SO	MTT	90-03-06
SF			NOT ACT	VERO	.58	0		SO	MTT	90-03-22
SF			NOT ACT	VERO	2.4	0		SO	MTT	90-03-06
VEE			NOT ACT	VERO	1	0		SO	MTT	90-03-09
VEE			NOT ACT	VERO	.5	0		SO	MTT	90-03-23
VV			.18	VERO	.82	10.53		SO	MTT	
YF			NOT ACT	VERO	.52	0		SO	MTT	90-03-22
YF			NOT ACT	VERO	1.96	0		SO	MTT	90-03-06

IN VIVO SCREEN [Dose = mg/kg]

VIR	HST	VR	VR+	DOSE	MTC	VEH	RTE	D	TOX	SP	L	PR	DATE

PLATE 0U4
DRUG 6467

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6467
TAI: 23.24 SI: 4.65

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.105	0.097	0.123	0.114	0.109	0.119	0.000	0.000	0.000	0.000	0.000	0.000
		cc/vc					tox	drug 6467 experimental			cc/vc	tox
B		1.314					1.488	0.153	0.166	0.134	1.321	1.330
C		1.339					1.510	0.179	0.148	0.205	1.470	1.424
D		1.312					1.553	0.258	0.206	0.325	1.410	1.427
E		0.182					1.415	1.153	1.398	1.302	0.160	1.541
F		0.180					1.058	0.847	0.883	0.733	0.173	0.825
G		0.174					0.450	0.426	0.446	0.427	0.142	0.400
							drug 6467 colorimetric background					
H							0.107	0.110	0.105	0.108	0.114	0.117

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	VV		PROJECT #	5975-4
CELLS	VERO	Satisfactory; Active; Retest	SPONSOR	USAMRIID
SHIPMENT NUMBER	63	RETEST AT 3.2 UG/ML	TEST DATE	04/19/90
STRN	LEDCA		DATE READ	04/25/90
REAGENT	0.111			
VIRUS CONTROL	0.057			
CELL CONTROL	1.250			
DIFFERENTIAL	1.193			

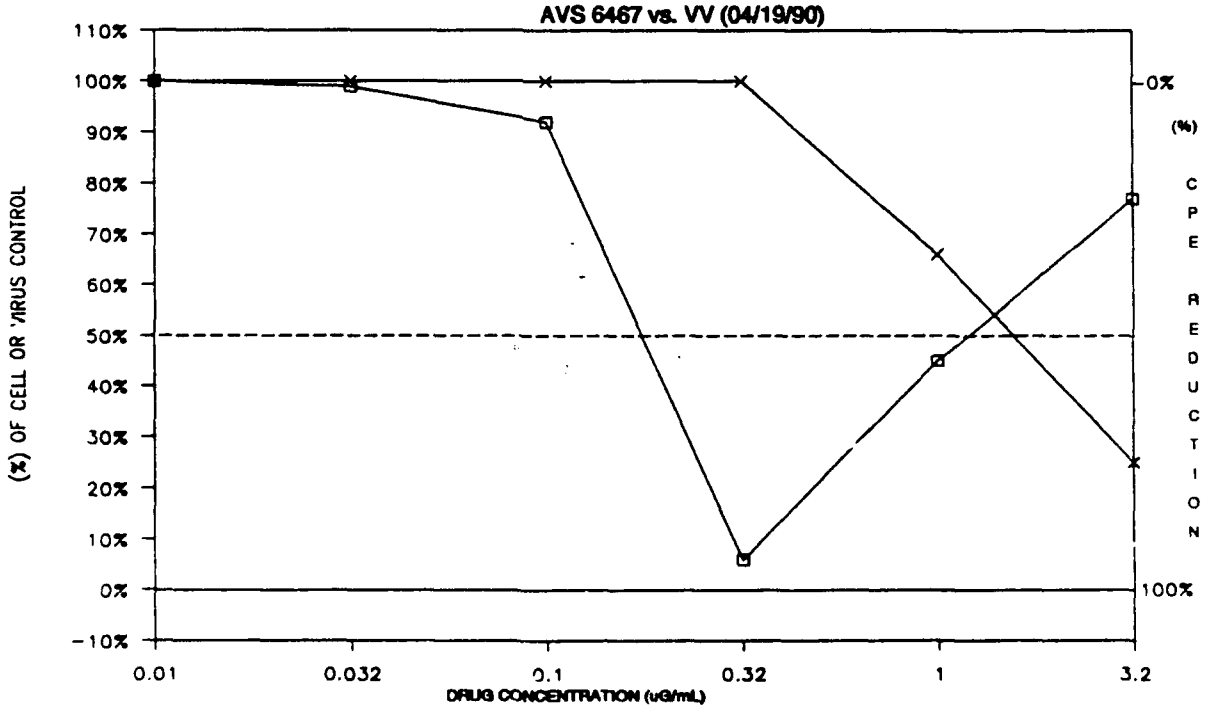
DRUG 6467	25%	50%	95%
TC (uG/mL)	0.82	1.86	> 3.20
IC (uG/mL)	0.13	0.18	------
ANTIVIRAL INDEX (AI)	6.52	10.53	------

DRUG 6467		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	0.01	-.023	100%	1.292	100%	0.006
C	0.032	0.006	99%	1.353	100%	0.003
D	0.1	0.098	92%	1.382	100%	-.003
E	0.32	1.122	6%	1.373	100%	-.006
F	1	0.654	45%	0.831	66%	-.001
high G *	3.2	0.269	77%	0.318	25%	-.004

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6467 vs. VV (04/19/90)



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

PRINTED 06/08/90

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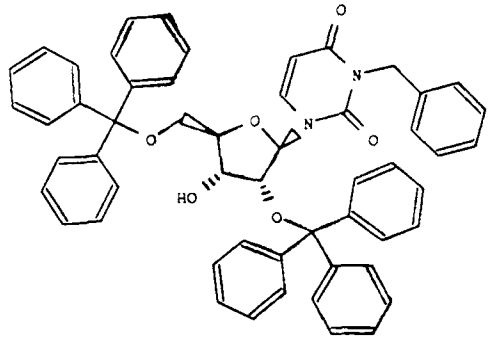
STRUCTURE

CHIRAL



SUBMITTER	CTR NO	AVS NO
01141.01	KN-V-109	AVS-006443
DATE RECD	**AMT RECEIVED [mg]**	**MOL WT (au)**
12-28-89	86.00	818.979

HANDLING/STORAGE

SOLUBILITY

STABILITY

ALT NAME

N3-BENZYL-2',5'-DI-O-TRITYLURIDINE

COMPOUND NAME

N3-BENZYL-2',5'-DI-O-TRITYLURIDINE

SCREEN INSTRUCTION

PRIORITY=PT>VEE>YF>KHF>PIC>JE>SF>VV>AD2>VSV

IN VIVO TOXICITY [mg/kg]

HOST	VH	RTE	LD50	MTC	LAB PR	DATE

IN VITRO SCREEN [ug/ml]

VIR	VR	VR+	ID50	CELL	MTC	TI	TI+	LAB PRT	DATE
HIV			NOT ACT	MT2	> 100	0		SO MTT	90-03-20
JE			NOT ACT	VERO	531	0		SO MTT	90-03-22
JE			NOT ACT	VERO	24.7	0		SO MTT	90-03-01
PT			77.1	VERO	210	> 4.15		SO MTT	90-03-01
PT			NOT ACT	VERO	335	0		SO MTT	
SF			NOT ACT	VERO	441	0		SO MTT	90-03-22
SF			NOT ACT	VERO	> 320	0		SO MTT	90-03-01
VEE			NOT ACT	VERO	773	0		SO MTT	90-03-23
VEE			NOT ACT	VERO	> 320	0		SO MTT	90-03-02
VV			NOT ACT	VERO	124	0		SO MTT	90-03-22
YF			NOT ACT	VERO	427	0		SO MTT	90-03-22
YF			NOT ACT	VERO	320	0		SO MTT	90-03-01

IN VIVO SCREEN [Dose = mg/kg]

VIR	HST	VR	VR+	DOSE	MTC	VEH	RTE	D TOX	SP L	PR	DATE

PLATE UQ9
DRUG 6443

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6443
TAI: >8.74 SI: 0.00

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.061	0.061	0.061	0.059	0.059	0.062	0.001	0.001	0.002	0.002	0.001	0.002
	tox	cc/vc	drug 6443 experimental			tox					cc/vc	
B	1.421	1.475	0.417	0.536	0.571	1.230					1.590	
C	1.514	1.258	0.406	0.411	0.448	0.783					0.962	
D	1.327	1.605	0.658	0.571	0.499	1.230					1.398	
E	1.281	0.452	0.761	0.771	0.763	1.170					0.434	
F	1.222	0.408	0.872	0.841	0.865	1.158					0.366	
G	0.678	0.466	0.284	0.271	0.382	0.582					0.425	
	drug 6443 colorimetric background											
H	0.207	0.089	0.068	0.064	0.064	0.064						

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	YF		PROJECT #	5975-1
CELLS	VERO	Satisfactory; Active; Retest	SPONSOR	USAMRIID
SHIPMENT NUMBER	63	TOXICITY RERUN	TEST DATE	03/22/90
STRN	ASIBI		DATE READ	03/30/90
REAGENT	0.061			
VIRUS CONTROL	0.365			
CELL CONTROL	1.321			
DIFFERENTIAL	0.956			

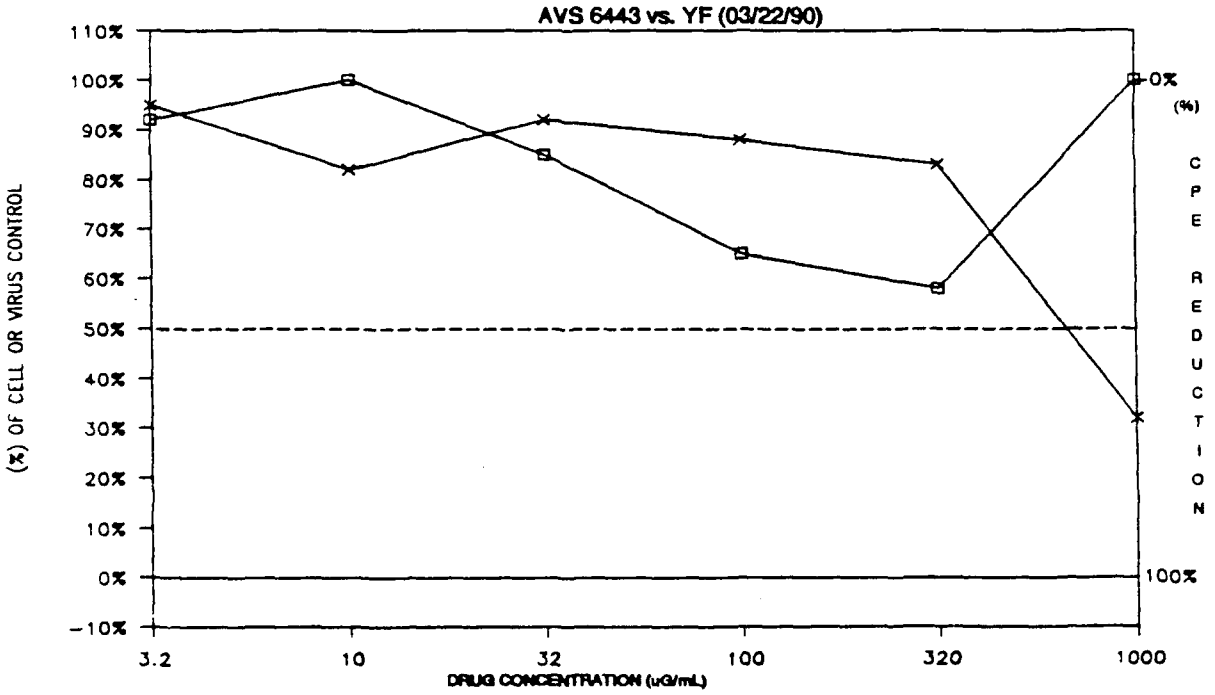
DRUG 6443	25%	50%	95%
TC (uG/mL)	427.00	760.00	> 1000.00
IC (uG/mL)	56.60	-----	-----
ANTIVIRAL INDEX (AI)	7.54	0.00	0.00

DRUG 6443		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	3.2	0.079	92%	1.261	95%	0.004
C	10	-.008	100%	1.084	82%	0.004
D	32	0.147	85%	1.214	92%	0.004
E	100	0.332	65%	1.157	88%	0.008
F	320	0.405	58%	1.101	83%	0.029
high G *	1000	-.260	100%	0.423	32%	0.147

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6443 vs. YF (03/22/90)



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

PRINTED 04/04/90

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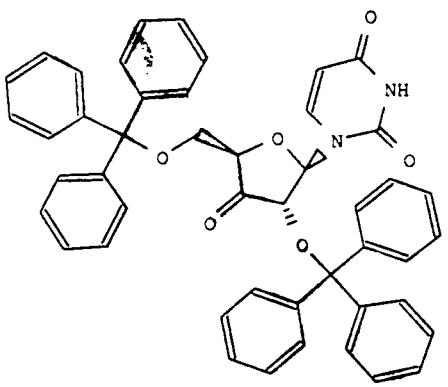
05/18/90

STRUCTURE

CHIRAL



SUBMITTER	CTR NO	AVS NO
01141.01	KN-VII-83	AVS-006444
DATE RECD	**AMT RECEIVED [mg]**	**MOL WT (au)**
12-28-89	79.00	726.837

HANDLING/STORAGE

SOLUBILITY

STABILITY

ALT NAME

3'-DEOXY-2',5'-DI-O-TRITYL-3'-OXOURIDINE

COMPOUND NAME

3'-DEOXY-2',5'-DI-O-TRITYL-3'-OXOURIDINE

SCREEN INSTRUCTION

PRIORITY=PT>VEE>YF>KHF>PIC>JE>SF>VV>AD2>VSV

IN VIVO TOXICITY [mg/kg]

HOST	VH	RTE	LD50	MTC	LAB PR	DATE

IN VITRO SCREEN [ug/ml]

VIR	VR	VR+	ID50	CELL	MTC	TI	TI+	LAB PRT	DATE
HIV			NOT ACT	MT2	> 100	0		SO MTT	90-03-20
JE			NOT ACT	VERO	51	0		SO MTT	90-03-01
JE			NOT ACT	VERO	547	0		SO MTT	90-03-22
PT			79.2	VERO	> 320	> 4.04		SO MTT	90-03-01
PT			NOT ACT	VERO	257	0		SO MTT	
SF			NOT ACT	VERO	30	0		SO MTT	90-03-01
SF			NOT ACT	VERO	365	0		SO MTT	90-03-22
VEE			NOT ACT	VERO	680	0		SO MTT	90-03-23
VEE			NOT ACT	VERO	> 320	0		SO MTT	90-03-02
VV			NOT ACT	VERO	116	0		SO MTT	90-03-22
YF			29.8	VERO	410	24.55		SO MTT	90-03-22
YF			NOT ACT	VERO	> 320	0		SO MTT	90-03-01

IN VIVO SCREEN [Dose = mg/kg]

VIR	HST	VR	VR+	DOSE	MTC	VEH	RTE	D TOX	SP L	PR	DATE

PLATE UQO
DRUG 6444

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6444
TAI: 3.21 SI: ——

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.077	0.073	0.071	0.070	0.072	0.072	0.001	0.001	0.002	0.001	0.002	0.002
		cc/vc					tox	drug 6444 experimental			cc/vc	tox
B		1.519					1.517	0.509	0.525	0.554	1.544	1.671
C		1.478					1.483	0.458	0.481	0.478	1.484	1.577
D		1.487					1.474	0.462	0.467	0.492	1.537	1.384
E		0.607					1.542	0.621	0.651	0.640	0.589	1.615
F		0.615					1.327	0.845	0.897	0.927	0.619	1.517
G		0.626					0.697	0.474	0.515	0.524	0.613	0.680
							drug 6444 colorimetric background					
H							0.121	0.082	0.081	0.078	0.078	0.077

tox=cell toxicity cc=cell control vc=virus control **BOLD** = highest drug conc values shown are optical densities

VIRUS	JE		PROJECT #	5975-1
CELLS	VERO	Satisfactory; Active; Retest	SPONSOR	USAMRIID
SHIPMENT NUMBER	63	TOXICITY RERUN	TEST DATE	03/22/90
STRN	NAKAYAMA		DATE READ	03/29/90
REAGENT	0.073			
VIRUS CONTROL	0.539			
CELL CONTROL	1.436			
DIFFERENTIAL	0.897			

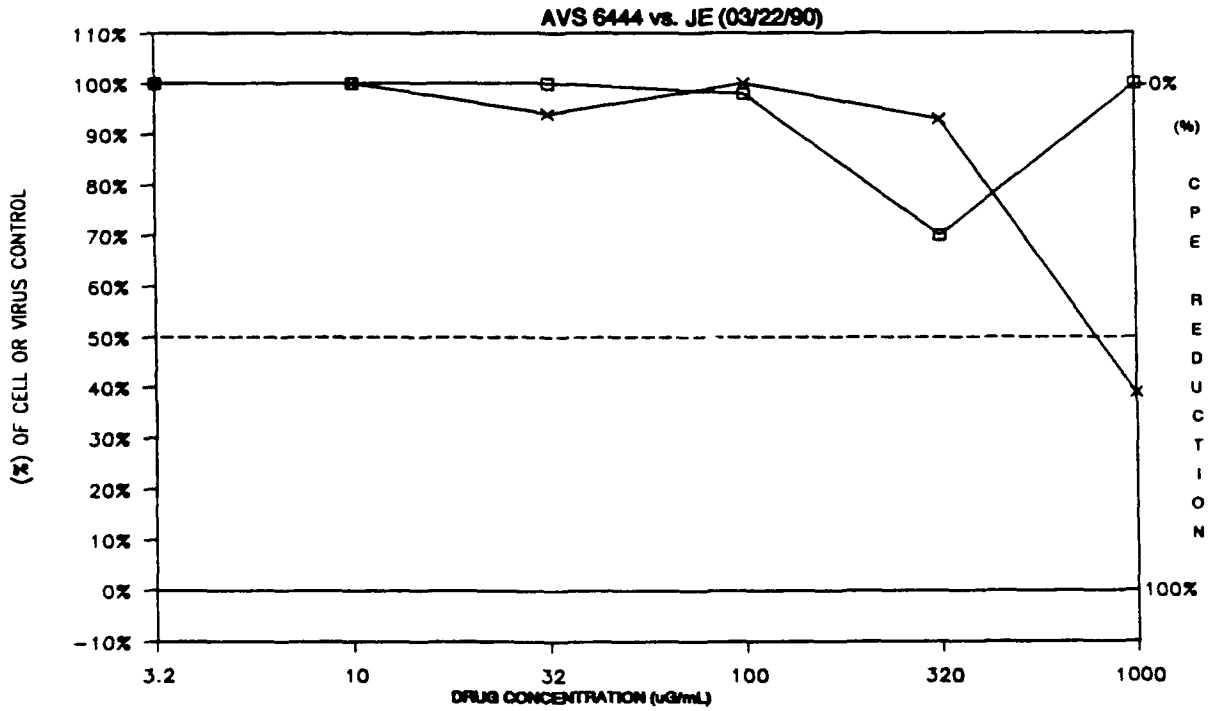
DRUG 6444	25%	50%	95%
TC (uG/mL)	547.00	861.00	> 1000.00
IC (uG/mL)	260.00	-----	-----
ANTIVIRAL INDEX (AI)	2.10	-----	-----

DRUG 6444		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	3.2	-.087	100%	1.517	100%	0.005
C	10	-.145	100%	1.452	100%	0.006
D	32	-.144	100%	1.351	94%	0.006
E	100	0.017	98%	1.497	100%	0.009
F	320	0.268	70%	1.340	93%	0.010
high G *	1000	-.156	100%	0.567	39%	0.049

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6444 vs. JE (03/22/90)



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

PRINTED 04/04/90

SOUTHERN RESEARCH INSTITUTE

PLATE URI
DRUG 6444

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6444
TAI: 3.44 SI: ----

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.054	0.055	0.054	0.051	0.051	0.053	0.002	0.002	0.001	0.001	0.001	0.001
		cc/vc					tox	drug 6444 experimental			cc/vc	tox
B		1.408					1.654	0.253	0.349	0.342	1.305	1.302
C		1.631					1.553	0.377	0.414	0.423	1.539	1.238
D		1.634					1.452	0.477	0.492	0.519	1.485	1.209
E		0.388					1.415	0.593	0.743	0.580	0.418	1.296
F		0.391					1.118	0.988	0.750	0.778	0.386	1.001
G		0.316					0.384	0.422	0.430	0.447	0.403	0.380
							drug 6444 colorimetric background					
H							0.055	0.057	0.056	0.055	0.051	0.049

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	PT		PROJECT #	5975-1
CELLS	VERO	Satisfactory; Active; Retest	SPONSOR	USAMRIID
SHIPMENT NUMBER	63	TOXICITY RERUN	TEST DATE	03/22/90
STRN	ADAMES		DATE READ	03/30/90
REAGENT	0.053			
VIRUS CONTROL	0.331			
CELL CONTROL	1.447			
DIFFERENTIAL	1.117			

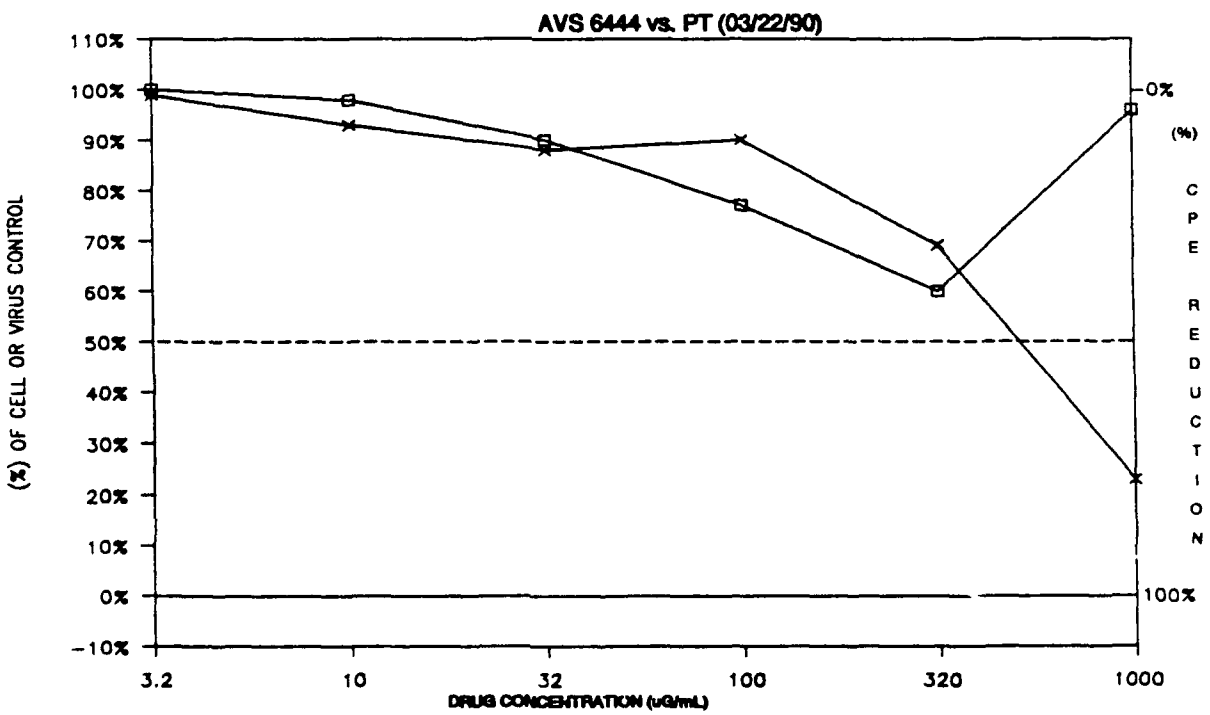
DRUG 6444	25%	50%	95%
TC (uG/mL)	257.00	601.00	> 1000.00
IC (uG/mL)	115.00	-----	-----
ANTIVIRAL INDEX (AI)	2.24	-----	-----

DRUG 6444		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	3.2	-.065	100%	1.429	99%	-.004
C	10	0.023	98%	1.345	93%	-.002
D	32	0.110	90%	1.276	88%	0.002
E	100	0.252	77%	1.300	90%	0.003
F	320	0.451	60%	1.003	69%	0.004
high G *	1000	0.047	96%	0.327	23%	0.002

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6444 vs. PT (03/22/90)



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

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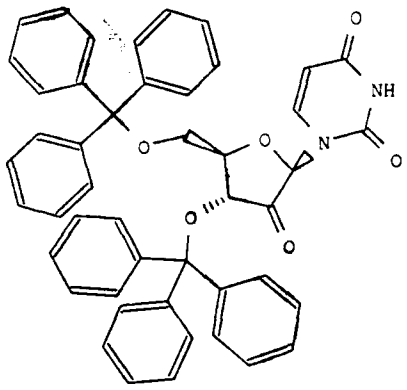
STRUCTURE

CHIRAL



SUBMITTER	CTR NO	AVS NO
01141.01	KN-VII-21	AVS-006445
DATE RECD	**AMT RECEIVED [mg]**	**MOL WT (au)**
12-28-89	74.00	726.837

HANDLING/STORAGE

SOLUBILITY

STABILITY

ALT NAME

2'-DEOXY-3',5'-DI-O-TRITYL-2'-OXOURIDINE

COMPOUND NAME

2'-DEOXY-3',5'-DI-O-TRITYL-2'-OXOURIDINE

SCREEN INSTRUCTION

PRIORITY=PT>VEE>YF>KHF>PIC>JE>SF>VV>AD2>VSV

IN VIVO TOXICITY [mg/kg]

HOST	VH	RTE	LD50	MTC	LAB PR	DATE

IN VITRO SCREEN [ug/ml]

VIR	VR	VR+	ID50	CELL	MTC	TI	TI+	LAB	PRT	DATE
HIV			NOT ACT	MT2	15.5	0		SO	MTT	90-03-20
JE			NOT ACT	VERO	49	0		SO	MTT	90-03-22
JE			NOT ACT	VERO	23.2	0		SO	MTT	90-03-01
PT			22.6	VERO	49	2.92		SO	MTT	90-03-01
PT			NOT ACT	VERO	8.87	0		SO	MTT	90-03-22
SF			NOT ACT	VERO	30.4	0		SO	MTT	90-03-22
SF			NOT ACT	VERO	43.3	0		SO	MTT	90-03-01
VEE			NOT ACT	VERO	32	0		SO	MTT	90-03-02
VEE			NOT ACT	VERO	44	0		SO	MTT	90-03-23
VV			NOT ACT	VERO	34.6	0		SO	MTT	90-03-22
YF			18.9	VERO	48	3.45		SO	MTT	90-03-01
YF			9.06	VERO	29.6	5.83		SO	MTT	90-03-22

IN VIVO SCREEN [Dose = mg/kg]

VIR	HST	VR	VR+	DOSE	MTC	VEH	RTE	D	TOX	SP	L	PR	DATE

PLATE UQA
DRUG 6445

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6445
TAI: >18.33 SI: 3.27

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.063	0.063	0.062	0.060	0.056	0.061	0.001	0.001	0.001	0.001	0.001	0.001
	tox	cc/vc	drug 6445 experimental			tox					cc/vc	
B	1.549	1.409	0.619	0.596	0.557	1.504					1.418	
C	1.442	1.368	0.740	0.805	0.674	1.607					1.392	
D	1.368	1.448	0.998	0.971	0.924	1.488					1.550	
E	1.067	0.526	0.934	0.600	0.848	1.022					0.433	
F	0.062	0.481	0.051	0.050	0.054	0.051					0.380	
G	0.066	0.494	0.066	0.061	0.059	0.065					0.400	
	drug 6445 colorimetric background											
H	0.075	0.071	0.065	0.062	0.064	0.062						

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	YF		PROJECT #	5975-1
CELLS	VERO	Satisfactory; Active; Retest	SPONSOR	USAMRIID
SHIPMENT NUMBER	63	TOXICITY RERUN	TEST DATE	03/22/90
STRN	AS IBI		DATE READ	03/30/90
REAGENT	0.061			
VIRUS CONTROL	0.392			
CELL CONTROL	1.370			
DIFFERENTIAL	0.979			

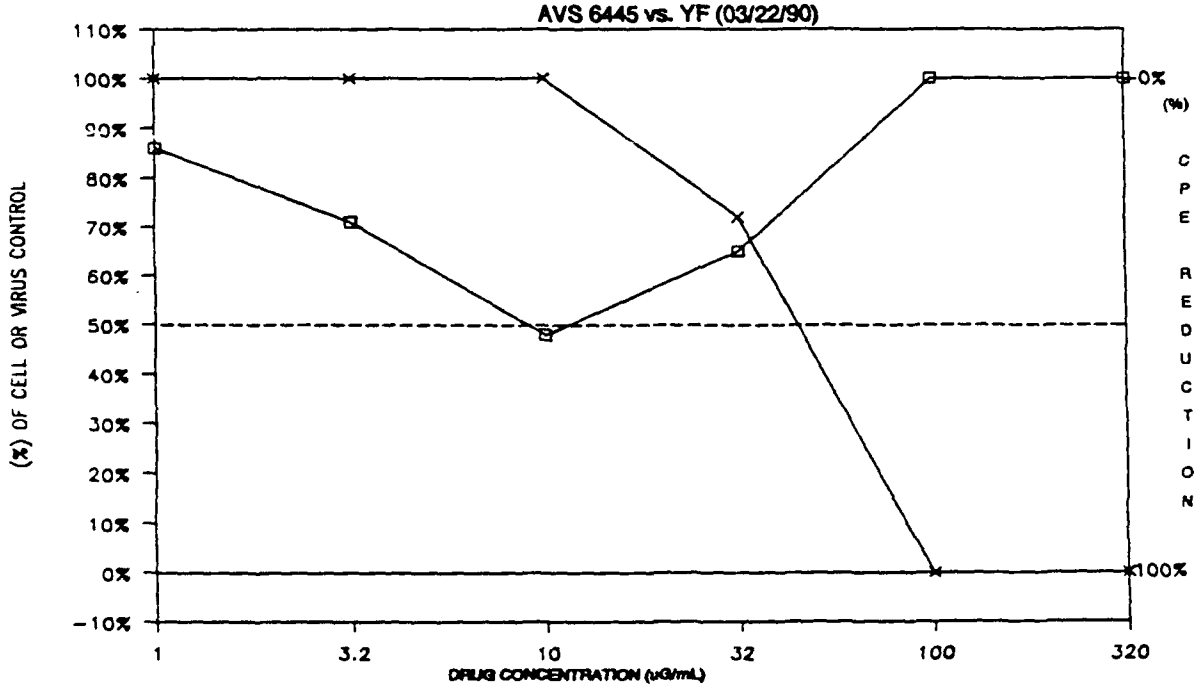
DRUG 6445	25%	50%	95%
TC (uG/mL)	29.60	52.80	95.30
IC (uG/mL)	2.35	9.06	-----
ANTIVIRAL INDEX (AI)	12.63	5.83	0.00

DRUG 6445		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	0.137	86%	1.465	100%	0.001
C	3.2	0.284	71%	1.461	100%	0.003
D	10	0.511	48%	1.366	100%	0.001
E	32	0.338	65%	0.980	72%	0.004
F	100	-.411	100%	-.014	0%	0.010
high G *	320	-.404	100%	-.009	0%	0.014

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6445 vs. YF (03/22/90)



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

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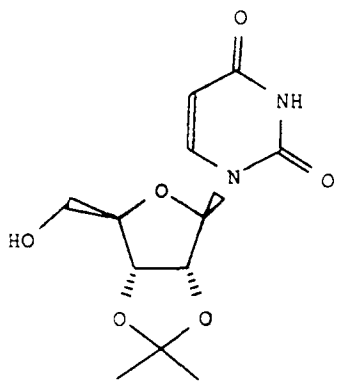
STRUCTURE

CHIRAL



SUBMITTER	CTR NO	AVS NO
01141.01	KN-II-53	AVS-006449
DATE RECD	**AMT RECEIVED [mg]**	**MOL WT (au)**
12-28-89	75.00	284.271

HANDLING/STORAGE

SOLUBILITY

STABILITY

ALT NAME

2',3'-O-ISOPROPYLIDINEURIDINE

COMPOUND NAME

2',3'-O-ISOPROPYLIDINEURIDINE

SCREEN INSTRUCTION

PRIORITY=PT>VEE>YF>KHF>PIC>JE>SF>VV>AD2>VSV

IN VIVO TOXICITY [mg/kg]

HOST	VH	RTE	LD50	MTC	LAB PR	DATE

IN VITRO SCREEN [ug/ml]

VIR	VR	VR+	ID50	CELL	MTC	TI	TI+	LAB	PRT	DATE
HIV			NOT ACT	MT2	> 100	0		SO	MTT	90-03-20
JE			NOT ACT	VERO	466	0		SO	MTT	90-03-22
JE			NOT ACT	VERO	> 320	0		SO	MTT	90-03-01
PT			264	VERO	> 320	> 1.21		SO	MTT	90-03-01
PT			NOT ACT	VERO	363	0		SO	MTT	90-03-22
SF			NOT ACT	VERO	472	0		SO	MTT	90-03-22
SF			NOT ACT	VERO	> 320	0		SO	MTT	90-03-01
VEF			NOT ACT	VERO	251	0		SO	MTT	90-03-02
VEE			NOT ACT	VERO	320	0		SO	MTT	90-03-23
VV			NOT ACT	VERO	195	0		SO	MTT	
YF			NOT ACT	VERO	517	0		SO	MTT	90-03-22
YF			NOT ACT	VERO	> 320	0		SO	MTT	90-03-01

IN VIVO SCREEN [Dose = mg/kg]

VIR	HST	VR	VR+	DOSE	MTC	VEH	RTE	D TOX	SP L	PR	DATE

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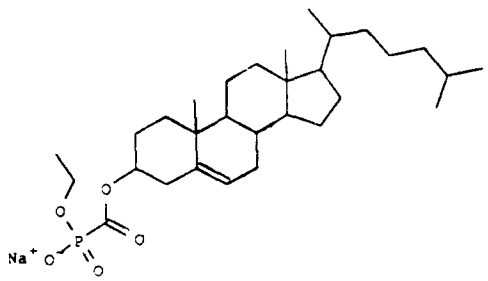
STRUCTURE



SUBMITTER	CTR NO	AVS NO
01141.01	MS-I-47	AVS-006456
DATE RECD	**AMT RECEIVED [mg]**	**MOL WT (au)**
12-28-89	79.20	544.694

HANDLING/STORAGE

SOLUBILITY

STABILITY

ALT NAME

SODIUM ETHYL(CHOLESTERYLOXYCARBONYL)-PHOSPHONATE

COMPOUND NAME

SODIUM ETHYL(CHOLESTERYLOXYCARBONYL)-PHOSPHONATE

SCREEN INSTRUCTION

PRIORITY=PT>VEE>YF>KHF>PIC>JE>SF>VV>AD2>VSV

IN VIVO TOXICITY [mg/kg]

HOST	VH	RTE	LD50	MTC	LAB	PR	DATE

IN VITRO SCREEN [ug/ml]

VIR	VR	VR+	ID50	CELL	MTC	TI	TI+	LAB	PRT	DATE
HIV			NOT ACT	MT2	49	0		SO	MTT	
JE			NOT ACT	VERO	25.6	0		SO	MTT	
JE			NOT ACT	VERO	47.4	0		SO	MTT	90-03-06
PT			NOT ACT	VERO	41.9	0		SO	MTT	90-03-06
PT			NOT ACT	VERO	43.3	0		SO	MTT	
SF			NOT ACT	VERO	50.7	0		SO	MTT	90-03-06
SF			NOT ACT	VERO	72.5	0		SO	MTT	
VEE			NOT ACT	VERO	116	0		SO	MTT	
VEE			NOT ACT	VERO	54.7	0		SO	MTT	90-03-09
VV			NOT ACT	VERO	21.8	0		SO	MTT	
YF			61	VERO	132	3.25		SO	MTT	
YF			NOT ACT	VERO	40.3	0		SO	MTT	90-03-06

IN VIVO SCREEN [Dose = mg/kg]

VIR	HST	VR	VR+	DOSE	MTC	VEH	RTE	D	TOX	SP	L	PR	DATE

PLATE UDN
DRUG 6456

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6456
TAI: 3.19 SI: ——

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.059	0.057	0.063	0.055	0.063	0.054	0.001	0.001	0.002	0.002	0.001	0.002
	tox	cc/vc	drug 6456 experimental			tox					cc/vc	
B	0.936	0.768	0.225	0.228	0.213	0.841					0.941	
C	0.893	0.819	0.172	0.162	0.145	0.951					1.024	
D	0.989	0.750	0.144	0.136	0.135	1.052					0.789	
E	0.964	0.281	0.432	0.408	0.423	0.955					0.268	
F	0.174	0.271	0.053	0.051	0.052	0.077					0.265	
G	0.050	0.280	0.046	0.044	0.044	0.043					0.283	
	drug 6456 colorimetric background											
H	0.050	0.053	0.055	0.057	0.052	0.055						

tox=cell toxicity cc=cell control vc=virus control **BOLD** = highest drug conc values shown are optical densities

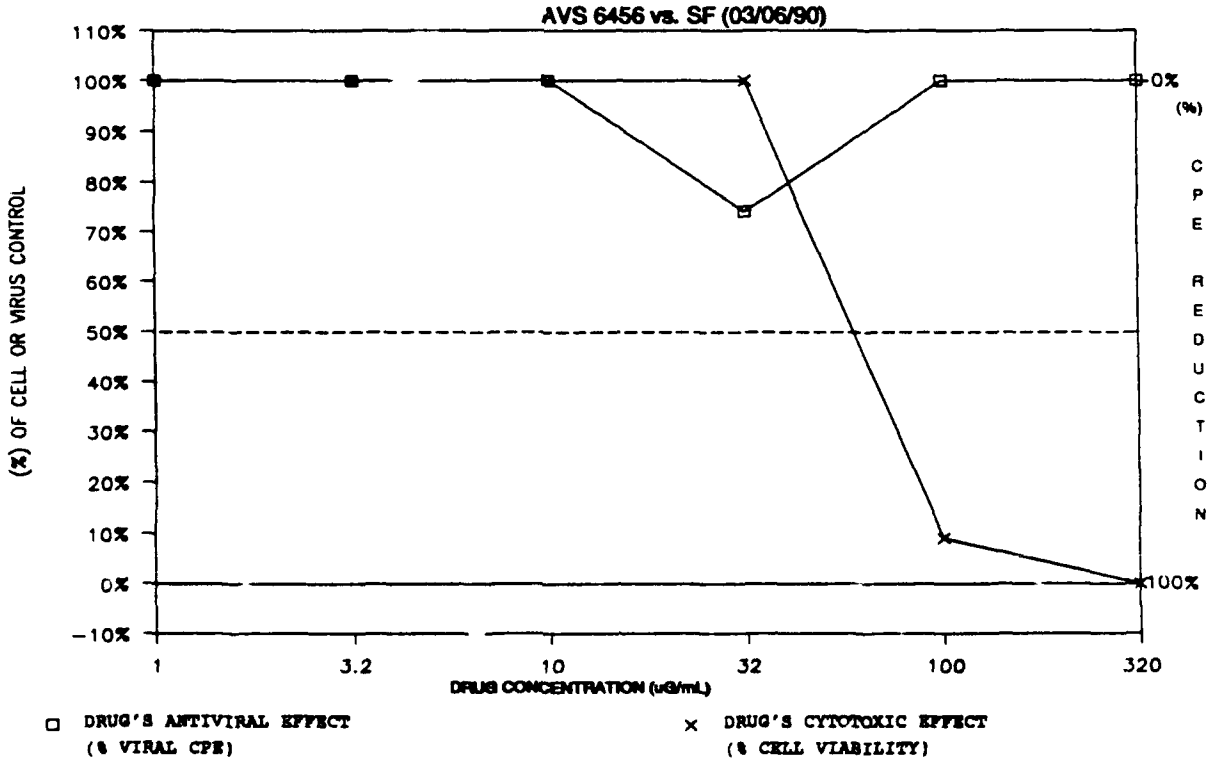
VIRUS	SF		PROJECT #	5975-1
CELLS	VERO	Satisfactory; Active; Retest	SPONSOR	USAMRIID
SHIPMENT NUMBER	63		TEST DATE	03/06/90
STRN	SICILIAN		DATE READ	03/14/90
REAGENT	0.059			
VIRUS CONTROL	0.216			
CELL CONTROL	0.790			
DIFFERENTIAL	0.574			

DRUG 6456	25%	50%	95%
TC (uG/mL)	50.70	69.40	198.00
IC (uG/mL)	30.60	-----	-----
ANTIVIRAL INDEX (AI)	1.66	-----	-----

DRUG 6456		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	-.049	100%	0.834	100%	-.004
C	3.2	-.108	100%	0.871	100%	-.007
D	10	-.135	100%	0.963	100%	-.001
E	32	0.150	74%	0.905	100%	-.004
F	100	-.217	100%	0.073	9%	-.006
high G *	320	-.222	100%	-.004	0%	-.008

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

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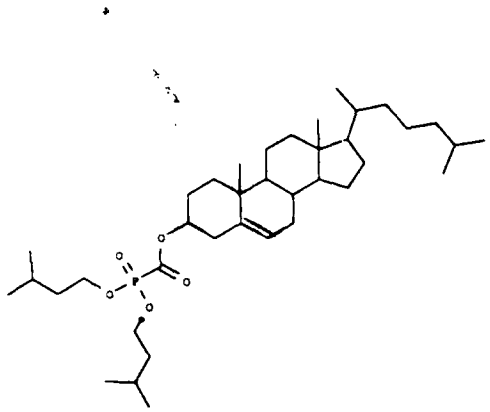
STRUCTURE



SUBMITTER	CTR NO	AVS NO
01141.01	KN-I-105	AVS-006458
DATE RECD	**AMT RECEIVED [mg]**	**MOL WT (au)**
12-28-89	70.40	634.929

HANDLING/STORAGE

SOLUBILITY

STABILITY

ALT NAME

DI-ISOBUTYL(CHOLESTERYLOXYCARBONYL)-PHOSPHONATE

COMPOUND NAME

DI-ISOBUTYL(HOLESTERYLOXYCARBONYL)-PHOSPHONATE

SCREEN INSTRUCTION

PRIORITY=PT>VEE>YF>KHF>PIC>JE>SF>VV>AD2>VSV

IN VIVO TOXICITY [mg/kg]

HOST	VH	RTE	LD50	MTC	LAB PR	DATE

IN VITRO SCREEN [ug/ml]

VIR	VR	VR+	ID50	CELL	MTC	TI	TI+	LAB PRT	DATE
HIV			NOT ACT	MT2	> 100	0		SO MTT	
JE			NOT ACT	VERO	> 320	0		SO MTT	90-03-06
JE			NOT ACT	VERO	> 1000	0		SO MTT	90-03-22
PT			NOT ACT	VERO	> 320	0		SO MTT	90-03-06
PT			NOT ACT	VERO	> 1000	0		SO MTT	90-03-22
SF			NOT ACT	VERO	> 320	0		SO MTT	90-03-06
SF			NOT ACT	VERO	> 1000	0		SO MTT	90-03-22
VEE			NOT ACT	VERO	155	0		SO MTT	90-03-09
VEE			NOT ACT	VERO	> 1000	0		SO MTT	90-03-23
VV			NOT ACT	VERO	> 320	0		SO MTT	
YF			234	VERO	> 1000	> 4.27		SO MTT	
YF			NOT ACT	VERO	> 320	0		SO MTT	90-03-06

IN VIVO SCREEN [Dose = mg/kg]

VIR	HST	VR	VR+	DOSE	MTC	VEH	RTE	D TOX	SP L	PR	DATE

PLATE UC0
DRUG 6458

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6458
TAI: >0.50 SI: ——

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.062	0.059	0.057	0.058	0.057	0.058	0.002	0.001	0.001	0.002	0.001	0.001
	tox	cc/vc	drug 6458 experimental			tox					cc/vc	
B	1.334	1.245	0.411	0.439	0.415	1.113					1.215	
C	1.259	1.243	0.405	0.394	0.392	1.110					1.185	
D	1.298	1.283	0.365	0.422	0.412	1.110					1.211	
E	1.203	0.413	0.454	0.375	0.451	1.054					0.442	
F	1.226	0.403	0.443	0.430	0.497	1.030					0.455	
G	1.024	0.393	0.689	0.669	0.689	0.862					0.446	
	drug 6458 colorimetric background											
H	0.058	0.061	0.061	0.060	0.060	0.061						

tox=cell toxicity cc=cell control vc=virus control **BOLD** = highest drug conc values shown are optical densities

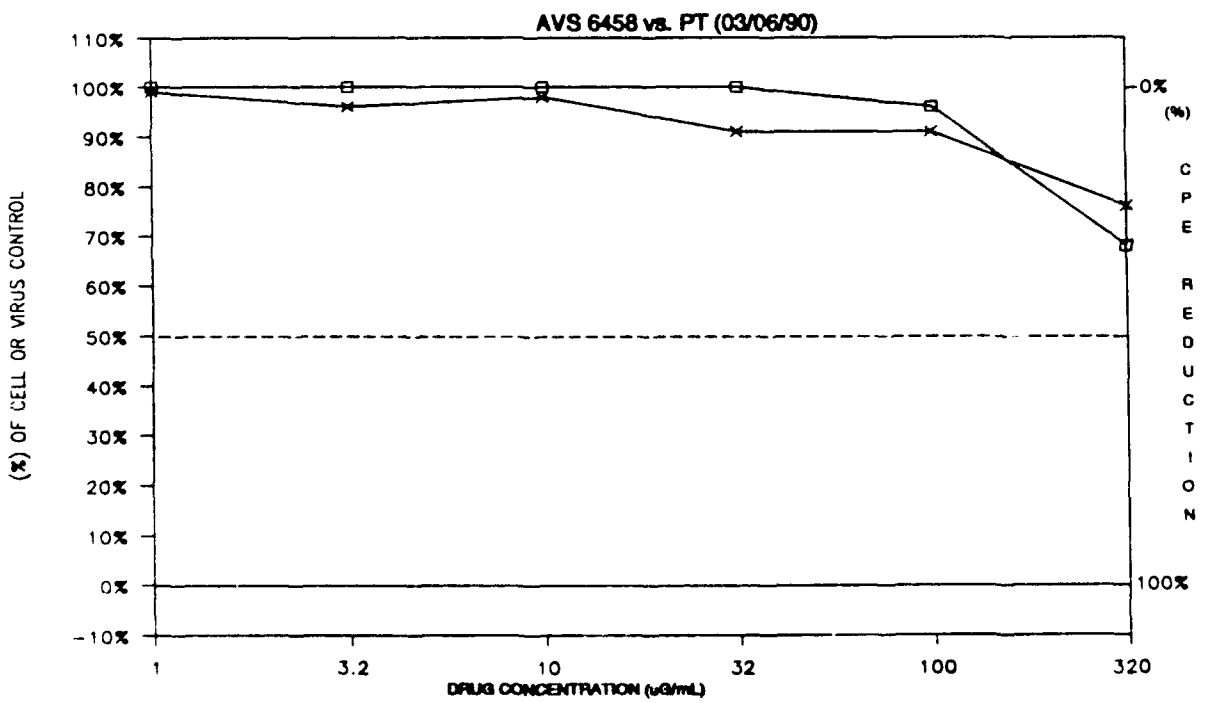
VIRUS	PT		PROJECT #	5975-1
CELLS	VERO	Satisfactory; Active; Retest	SPONSOR	USAMRIID
SHIPMENT NUMBER	63		TEST DATE	03/06/90
STRN	ADAMES		DATE READ	03/16/90
REAGENT	0.059			
VIRUS CONTROL	0.367			
CELL CONTROL	1.172			
DIFFERENTIAL	0.805			

DRUG 6458	25%	50%	95%
TC (uG/mL)	> 320.00	> 320.00	> 320.00
IC (uG/mL)	239.00	-----	-----
ANTIVIRAL INDEX (AI)	> 1.34	-----	-----

DRUG 6458		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	-.006	100%	1.163	99%	0.002
C	3.2	-.029	100%	1.125	96%	0.001
D	10	-.027	100%	1.145	98%	0.001
E	32	-.001	100%	1.068	91%	0.002
F	*100*	*0.029*	*96%*	*1.068*	91%	*0.002*
high G *	320	0.257	68%	0.885	76%	0.000

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

PRINTED 03/22/90

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APPENDIX V

SUMMARY OF COMPOUNDS SHOWING SIGNIFICANT ANTIVIRAL ACTIVITY.

The table summarizes those compounds shown to have significant antiviral activity agaist one or more of the test viruses. The test data together with the I_{c50} are given in the sheets of Appendix 3.

AVS #S OF COMPOUNDS SHOWING SIGNIFICANT ANTI-VIRAL ACTIVITY

HIV	VACCINIA	PUNTA TORO	YELLOW FEVER
6460	6462	6422	6456
6457	6467	6443	6458
6455		6444	6444
6466		6445	6445
		6402	

APPENDIX VI

SUMMARY OF SIGNIFICANT PROJECT ACCOMPLISHMENTS TO DATE AND RECOMMENDATIONS FOR FUTURE EXPLOITATION OF FINDINGS

PROJECT TITLE: SUICIDE INHIBITORS OF VIRAL POLYMERASES AS VIRAL PROPHYLACTICS AND BIOLOGICAL WARFARE ANTIDOTES.

a) Problems to be studied. This project will synthesize new types of antiviral drugs based upon suicide inhibitors of viral polymerases. These compounds will be screened in collaborative studies with the U.S. Army Antiviral Testing Facility for antiviral activity against a spectrum of 10 viruses of interest as military disease hazards and biological warfare agents.

b) Significance and Uniqueness. Suicide and affinity inhibitors of both DNA and RNA viral polymerases will be synthesized. This type of inhibitor contains a latent reactive moiety which selectively and irreversibly inactivates the viral enzyme. In preliminary studies, about 30 compounds with these potentialities have been synthesized and screened for antiviral activity in tissue culture. A number of active compounds including a new family - the nucleoside spiroxiranes - have been identified. Cytotoxicity assays in cultured T-lymphocytes also indicate favorable therapeutic indices for these types of drugs.

Two compounds, 2'3' sulfinyl cytidine hydrochloride and 2',0^2 anhydrocytidine hydrochloride, which have proved to be highly effective against vaccinia virus in tissue culture, will be synthesized in larger quantities for further characterization and *in vivo* studies in the U.S. Army Antiviral Facility, Fort Detrick, MD. Congeners of compounds that have shown moderate activity against Punta Toro and yellow fever viruses will also be developed. Test data on selected compounds are given in the appendix.

Preliminary studies have begun on a series of nucleoside 5' oxaphosphorins and dioxaphospholes that are suicide analogs directed against enzymatic displacement reactions at the 5' α phosphate of the nucleotide substrate. The action mechanism and selectivity of the drugs will be characterized against viral and host nucleotide polymerases *in vitro*.

In the continuing studies, the range and selectivity of the nucleoside spiroxiranes will be extended by synthesizing additional members of the family. The suicide nature of their action and sensitivity will be determined in kinetic studies using viral and cellular DNA and RNA polymerases *in vitro*. Samples (75 mg) of each compound will be supplied to USAMRID for *in vitro* testing. Larger samples (2 g) of compounds showing activity *in vitro* will be supplied for further testing *in vivo*.

c) Relevance to USAMRDC mission studies. Suicide inhibitors represent a new class of antiviral drugs potentially capable of great selectivity. Orally administered antivirals could be militarily useful for temporary viral prophylaxis in emergency troop deployments to environments where advance vaccination is not possible, as antidotes following battlefield exposure to vaccinia-based or other biological warfare vectors, and in other unanticipated epidemic situations.

d) Estimated Project Duration and Personnel. Organic chemist (50% time), 1 or 2 graduate students. Duration 3 years.

e) Animal use. No animal or human use except USAMRDC in-house testing of antivirals supplied as requested.