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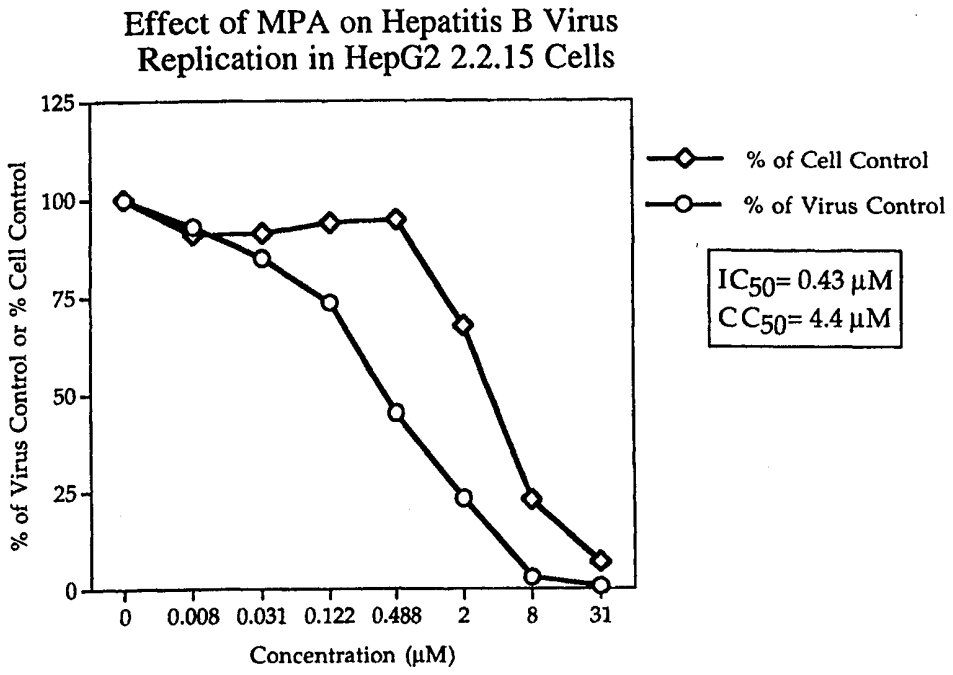

(57) Abstract

The invention relates to methods of treating viral diseases caused by viruses of the family *Flaviviridae*, or by a virus which targets the mammalian liver as a main repository for viral replication. The methods of this invention involve the use of mycophenolic acid or its derivatives, particularly mycophenolate mofetil alone, or in combination with other anti–viral agents. The methods of this invention are particularly useful in treating hepatitis B virus, hepatitis C virus, and dengue virus viral infections.

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METHODS OF TREATING VIRAL DISEASE

TECHNICAL FIELD OF THE INVENTION

The invention relates to methods of treating viral diseases caused by viruses of the family *Flaviviridae*, or by a virus which targets the mammalian liver as a main repository for viral replication. The methods of this invention involve the use of mycophenolic acid or its derivatives, particularly mycophenolate mofetil alone, or in combination with other anti-viral agents. The methods of this invention are particularly useful in treating hepatitis B virus, hepatitis C virus, and dengue virus viral infections.

BACKGROUND OF THE INVENTION

Viral infections caused by virus of the family *Flaviviridae* and viral diseases which target the mammalian liver as the main site of viral replication both present a great health risk to humans around the world.

A virus which falls into both categories, hepatitis C virus ("HCV"), is a major world health problem. HCV infection causes about 10,000 deaths per year. It is estimated that at least 4 million Americans are infected with HCV -- 2 percent of the population. Presently there is no cure for the disease and treatments have just begun to be available.

Hepatitis B virus is an example of a prevalent viral disease which targets the liver. Although a

vaccine is currently available, it is of questionable utility in individuals who are chronically infected with HBV.

Dengue is an example of a virus of the family *Flaviviridae*. Dengue is not as prevalent as HBV or HCV, but is known to break out in large epidemics in warmer climates. Dengue fever is an acute infectious disease caused by the flavivirus (a small RNA virus related to yellow fever, tick-borne encephalitis, St Louis encephalitis, and Japanese encephalitis) which is transmitted by infected mosquitoes. The disease causes headache, fever, muscle pain and rash, but is rarely fatal.

However, a more severe form of the disease seen mostly in children, dengue hemorrhagic fever, causes severe symptoms including fever, shock, hemorrhaging from the nose and mouth, respiratory distress and death. Currently there is no treatment for dengue.

Mycophenolic acid ("MPA"), a naturally occurring antibiotic produced by *Penicillium brevicompactum*, and its derivatives including mycophenolate mofetil ("MMF"; the morpholinoethyl ester of MPA), have recently been described as immunosuppressant drugs. In fact, the Food and Drug Administration recently approved MMF for use in preventing kidney transplant rejection.

Numerous derivatives of MPA and MMF, their synthesis and use in treating various diseases are described in United States Patents 4,686,234; 4,725,622; 4,727,069; 4,748,173; 4,753,935; 4,786,637; 4,808,592; 4,861,776; 4,948,793; 4,952,579; 4,959,386; 4,992,467; 5,247,083; 5,380,879; 5,441,953; 5,444,072; 5,493,030;

5,512,568; 5,525,602; 5,536,747; 5,538,969; 5,554,612; and 5,633,279.

Although, several of the above-identified patents have suggested that MPA, MMF and its derivatives may be useful an anti-viral agents, the viral targets mentioned fall into either the retroviral or the Herpes virus class. Nowhere is there any teaching or suggestion that MPA, MMF and their derivatives would be useful in treating diseases that target the mammalian liver, in particular hepatitis B virus, hepatitis C virus, and dengue virus.

Viral diseases that target the mammalian liver are often widespread and severe. This, coupled with the lack of effective treatment for a subset of these diseases, places a great need on developing new avenues for treatment.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the effect of varying concentrations of MPA on the amount of virion-associated hepatitis B virus DNA present in HepG2-2.2.15 cells and on cell death.

SUMMARY OF THE INVENTION

Applicants have solved the problem set forth above by discovering that MPA, MMF and their derivatives are surprisingly and unexpectedly useful in treating *flavivirus* infections and infections caused by virus that use the mammalian liver as their main site for replication.

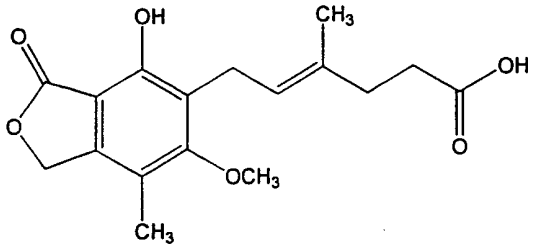
The use of these well-known compounds alone, or in conjunction with one or more other anti-viral agents,

such as interferons, ribavirin, or inhibitors of viral life cycles (such as protease inhibitors, nucleoside analogs, inhibitors of other viral enzymes necessary for viral replication and infection) provides a method for treating such viral diseases.

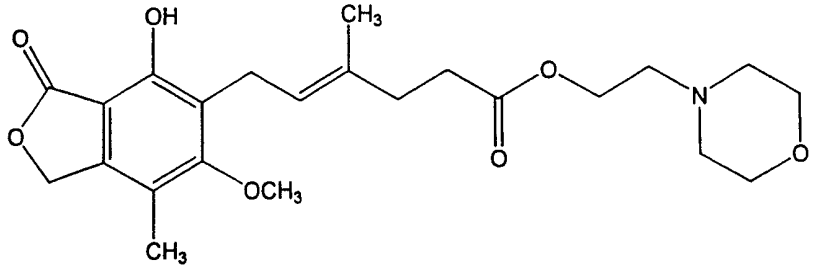
DETAILED DESCRIPTION OF THE INVENTION

According to one embodiment, the present invention provides a method of treating a mammal suffering from a viral disease caused by a member of the family *Flaviviridae* or a viral disease of the liver, wherein said method comprises the step of administering to said mammal an amount of a compound selected from mycophenolic acid, mycophenolate mofetil, or a derivative of either of these compounds effective to lessen the severity of said viral disease, wherein said compound is in a pharmaceutically acceptable composition.

Mycophenolic acid has the structure:



Mycophenolate mofetil has the structure:



Derivatives of these two compounds have been well-described in the literature. For example, 4-amino derivatives of mycophenolic acid are described in United

States Patents 5,380,879 and 5,441,953, and in PCT publication WO 95/22535. Mycophenolic acid derivatives containing a 5-substitution are described in United States Patents 5,493,030 and 5,633,279, and in PCT publication WO 95/22538. Mycophenolic acid derivatives containing a 6-substitution are described in United States Patents 5,444,072 and 5,536,747, as well as in PCT publication WO 95/22536. 4-amino derivatives of mycophenolic acid which contain a 5- or a 6-substitution are described in United States Patents 5,538,969, and 5,554,612, and in PCT publications WO 95/22537 and WO 95/22534. The disclosures of each of the above-cited patents and publications are herein incorporated by reference.

Any of the above-described compounds are useful in the methods of this invention. Preferably, the compound used is mycophenolate mofetil.

The term "viral diseases of the liver" as used herein, means a disease whose causative agent is a virus, wherein the virus targets the mammalian liver as its primary site of replication. Such viruses include, but are not limited to, hepatitis B virus, hepatitis C virus, hepatitis D virus, and hepatitis E virus. Examples of virus from the family *Flaviviridae* may be treated according to this invention are dengue and hepatitis C virus. According to a preferred embodiment, the methods of this invention are used to treat a hepatitis C virus infection.

The efficacy of the methods of this invention may be measured by observing how the treatment lessens the severity of the viral infection. This may be measured by various means. For example, a reduction in

the viral titer in the blood is considered to be a lessening of the severity of infection. Similarly, a decrease in the amount of an enzymatic activity associated with the virus (e.g., viral protease activity; viral helicase activity; viral polymerase activity) or a reduction in the presence of viral proteins may be taken as a measure of efficacy.

Another parameter is a sustained improvement in the patients health and well-being. This may be measured by a reduction or elimination of symptoms associated with the viral infection. One specific measure that may be used is the concentration of liver transaminases, particularly ALT and AST, found in the patient's plasma. It may also be measured, in a human being, by interviewing the patient.

A pharmaceutically acceptable composition according to this invention is one in which the compound is formulated together with a pharmaceutically acceptable carrier for administration to a mammal.

Pharmaceutically acceptable carriers that may be used in these compositions include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes,

polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

According to a preferred embodiment, the compositions of this invention are formulated for pharmaceutical administration to a human being.

Such pharmaceutical compositions of the present invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques. Preferably, the compositions are administered orally.

Sterile injectable forms of the compositions of this invention may be aqueous or oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-

acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as Ph. Helv or similar alcohol.

The pharmaceutical compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents may also be added.

Alternatively, the pharmaceutical compositions of this invention may be administered in the form of suppositories for rectal administration. These can be prepared by mixing the agent with a suitable non-irritating excipient which is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

The pharmaceutical compositions of this invention may also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical application, including diseases of the eye, the skin, or the lower intestinal

tract. Suitable topical formulations are readily prepared for each of these areas or organs.

Topical application for the lower intestinal tract can be effected in a rectal suppository formulation (see above) or in a suitable enema formulation. Topically-transdermal patches may also be used.

For topical applications, the pharmaceutical compositions may be formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, the pharmaceutical compositions can be formulated in a suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

For ophthalmic use, the pharmaceutical compositions may be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions in isotonic, pH adjusted sterile saline, either with our without a preservative such as benzylalkonium chloride. Alternatively, for ophthalmic uses, the pharmaceutical compositions may be formulated in an ointment such as petrolatum.

The pharmaceutical compositions of this invention may also be administered by nasal aerosol or

inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents.

Various pharmaceutical formulations of MPA, MMF and their derivatives have been described in United States Patent 5,455,045; 5,543,408; 5,554,384 and 5,688,529, the disclosures of which are herein incorporated by reference. These formulations, in particular the high dose oral suspensions of MMF described in United States Patent 5,688,529, are preferred in the methods of the present invention.

In a preferred embodiment, the MPA, MMF or a derivative thereof is administered together with at least one other anti-viral agent.

Other anti-viral agents useful in this embodiment include, but are not limited to, α-interferons, β-interferons, γ-interferons, viral protease inhibitors, ribavirin, viral helicase inhibitors, viral polymerase inhibitors, antibodies to viral surface proteins, and antibodies to viral core proteins.

Preferably, the other anti-viral agent used in the methods of this invention is an α-interferon, which is optionally modified by conjugating with polyethylene glycol ("pegylation").

The other anti-viral agent may be administered as part of a single dosage combination with MPA, MMF or a derivative thereof. Alternatively, the other anti-viral agent may be administered as a separate dosage form.

When used as a separate dosage form the other anti-viral agent may be administered prior to, simultaneously with, or following administration of MPA, MMF or a derivative thereof.

Daily dosages of MPA, MMF or derivatives thereof utilized in the methods of this invention should be between about 0.01 to 100.0 mg/kg body weight, preferably between about 0.1 to 70 mg/kg of body weight. If other anti-viral agents are present, they should be administered at daily dosages of between about 70 to 100% of the dosage normally administered when that agent is used in a monotherapy.

The amount of active compound administered will, of course, be dependent upon a number of factors, including the sex of the mammal, the nature and severity of the viral infection, the route and schedule of the administration and the judgment of the prescribing physician.

In order that this invention be more fully understood, the following examples are set forth. These examples are for the purpose of illustration only and are not to be construed as limiting the scope of the invention in any way.

EXAMPLE 1

Effect of MPA on Hepatitis B Virus Infection

We plated HepG2-2.2.15 cells in 96-well microtiter plates at an initial density of 2.5×10^3 cells/100 µl in DMEM medium supplemented with 10% fetal bovine serum. To promote cell adherence, the 96-well plates were pre-coated with collagen prior to cell plating. After incubation at 37°C in a humidified, 5% CO_2

environment for 16-24 hours, the confluent monolayer of HepG2-2.2.15 cells was washed, and the medium was replaced with complete medium containing various concentrations of MPA (0 to 31 μM). On day three, the culture medium was replaced with fresh medium containing the same amount of MPA. Six days following the initial administration of MPA, the cell culture supernatant was collected and clarified by centrifugation (Sorvall RT-6000D centrifuge, 1000 rpm for 5 min).

Samples were then treated with 0.75 mg/ml Pronase for 30 minutes at 37°C and 1 unit DNase for 60 minutes at 37°C to inactivate proteases and degrade any unencapsidated viral DNA. The DNase present in the samples was inactivated by heating the samples to 95°C for 30 minutes. Three microliters of clarified supernatant was then subjected to real-time quantitative PCR using conditions described below.

Virion-associated HBV DNA present in the tissue culture supernatant was PCR amplified using primers derived from HBV strain *ayw*. PCR-amplified HBV DNA was detected in real-time (i.e., at each PCR thermocycle step) by monitoring increases in fluorescence signals that result from exonucleolytic degradation of a quenched fluorescent probe molecule following hybridization of the probe to the amplified HBV DNA. The TaqMan probe molecule, designed with the aid of Primer Express™ (PE-Applied Biosystems) software, is complementary to DNA sequences present in the HBV DNA region.

Routinely, 3 μl of clarified supernatant was analyzed directly (without DNA extraction) in a 50 μl PCR reaction. Reagents and conditions used are per the manufacturers suggestions (PE-Applied Biosystems). For

each PCR amplification, a standard curve was simultaneously generated from several log dilutions of a purified 1.2 kbp HBV *ayw* subgenomic fragment. Routinely, the standard curve ranged from $1x10^6$ to $1x10^1$ nominal copy equivalents per PCR reaction.

The results of this assay are shown in Figure 1. Figure 1 demonstrates that there is a linear decrease in the amount of HBV DNA detected with increasing amounts of MPA administered up to 0.488 μM. Above 0.488 μM, the cells begin to die and therefore the further linear decrease in HBV DNA is difficult to interpret. This assay demonstrates that MPA is effective in reducing hepatitis B viral titer in infected cells. Infections caused by other virus of the *Flaviviridae* family and other virus that target the liver of a mammal as the major site of replication will also be reduced in severity by treatment with MPA.

While we have hereinbefore presented a number of embodiments of this invention, it is apparent that our basic construction can be altered to provide other embodiments which utilize the methods of this invention. Therefore, it will be appreciated that the scope of this invention is to be defined by the claims appended hereto rather than the specific embodiments which have been presented hereinbefore by way of example.

CLAIMS

We claim:

1. A method of treating a mammal suffering from a disease caused by a virus of the family *Flaviviridae* or by a virus that targets the liver of said mammal as the major site of replication, said method comprising the step of treating said mammal with a pharmaceutical composition comprising:

a. an amount of a compound selected from mycophenolic acid, mycophenolate mofetil, or a derivative thereof effective to decrease the severity of said disease; and

b. a pharmaceutically acceptable carrier.

2. The method according to claim 1, wherein said mammal is additionally administered an additional antiviral agent, wherein said antiviral agent is formulated together with said compound in single dosage form or wherein said antiviral agent is administered to said mammal as a separate dosage form.

3. The method according to claim 2, wherein said additional antiviral agent is selected from an α-interferon or a pegylated α-interferon.

4. The method according to any one for claims 1 to 3, wherein said mammal is suffering from a disease caused by a virus selected from hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus,

or dengue virus.

5. The method according to claim 4, wherein said virus is hepatitis C virus and said mammal is a human.

6. The method according to claim 1 or 5, wherein said pharmaceutical composition is formulated for oral administration.

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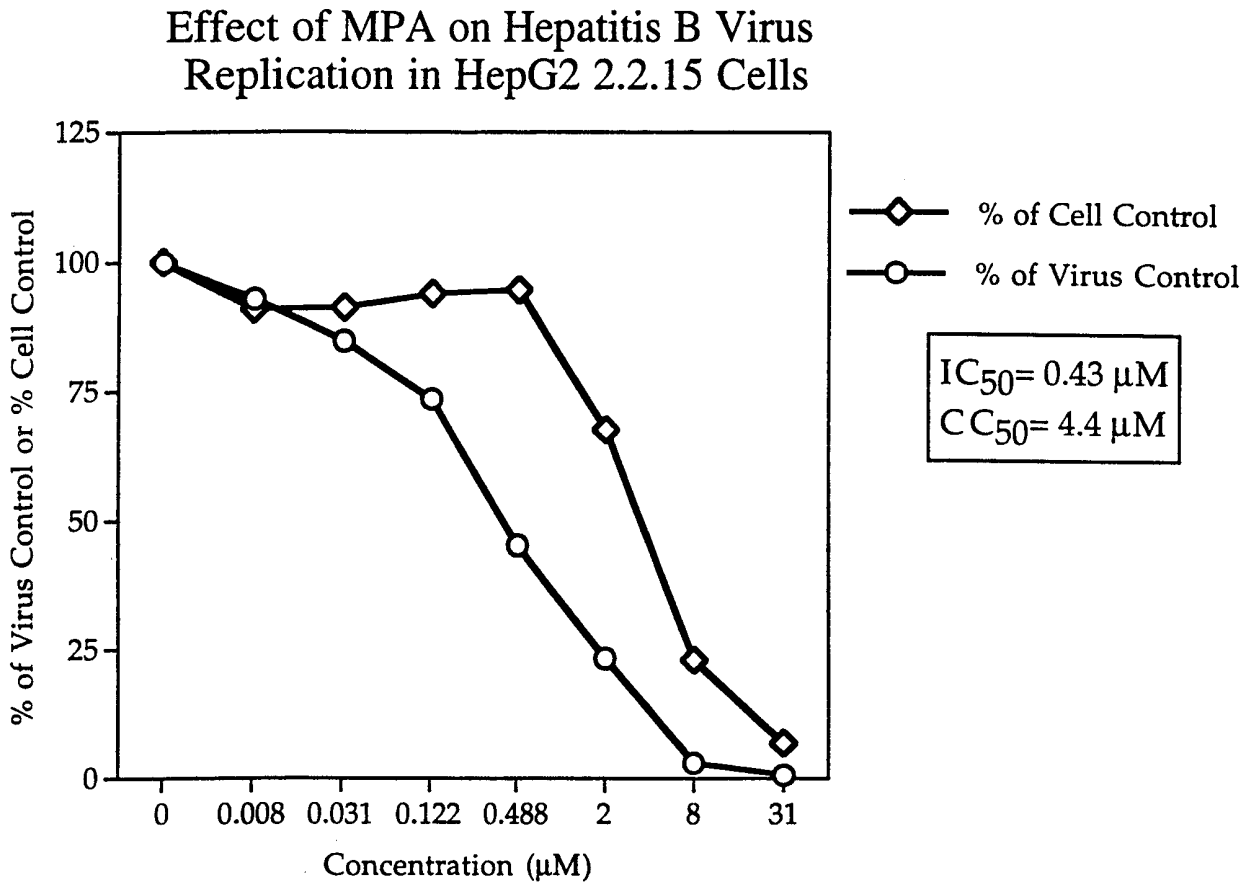
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50
25
0
0
0.008
0.031
0.122
0.488
2
8
31
Concentration (µM)
% of Cell Control
% of Virus Control
IC50= 0.43 µM
CC50= 4.4 µM