HALOVIR, AN ANTIVIRAL MARINE NATURAL PRODUCT, AND DERIVATIVES THEREOF

Inventors: William Fenical, Del Mar, CA (US); Paul R. Jensen, San Diego, CA (US); David C. Rowley, Wakefield, RI (US)

Correspondence Address:
BROWN, MARTIN, HALLER & MCCLAIN LLP
1660 UNION STREET
SAN DIEGO, CA 92101-2926 (US)

Appl. No.: 10/217,234
Filed: Aug. 9, 2002

Related U.S. Application Data
Continuation-in-part of application No. 09/211,877, filed on Dec. 15, 1998, now Pat. No. 6,458,766.

Publication Classification
Int. Cl. 7 .......................... A61K 38/08; C07K 7/06
U.S. Cl. ............................... 514/17; 530/329; 530/330

ABSTRACT
The invention is a group of compounds named halovirs with antiviral activity that are structurally related to compounds isolated from a marine fungus CNI.240. Halovirs are comprised of a short, amphipathic helical peptide with an extended lipid moiety on the N-terminal end of the peptide. The halovirs have demonstrated activity against herpes simplex virus, types I and II.
FIGURE 1
Halovir A

Halovir B

Halovir C
FIGURE 2

Halovir F

Halovir G

Halovir H
FIGURE 3

% Vero Cell Survival

- Halovir A
- Halovir F
- Halovir G

Concentration (μM)
FIGURE 5

Halovir D

Halovir E
HALOVIR, AN ANTIVIRAL MARINE NATURAL PRODUCT, AND DERIVATIVES THEREOF

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application is a continuation in part of application Ser. No. 09/211,877 filed Dec. 15, 1998, of like title, which is incorporated herein by reference in its entirety.

GOVERNMENT INTEREST

[0002] The invention was made with government support from the National Oceanographic and Atmospheric Administration under grant number NA36RG0537 and the National Cancer Institute under grant number CA 44848. The government has certain rights to this invention.

FIELD OF THE INVENTION

[0003] This invention relates generally to the field of pharmacologically active products derived from marine natural products. More specifically, it relates to peptides derived from a marine fungus that have antiviral activity.

BACKGROUND OF THE INVENTION

[0004] Viral infections have long been and continue to be a major cause of human suffering. The large variety of viruses combined with the diverse types of afflictions continue to challenge endeavors to find and make available agents capable of treating or mitigating the effects of viral infections.

[0005] One family of viruses that is particularly troublesome is the Herpes simplex viruses (HSV). HSV is a relatively common human pathogen which can cause fatal disease in the young or immunocompromised. HSV includes two closely related variants designated type 1 (“HSV-1”) and type 2 (“HSV-2”). These types cross react strongly, but can be distinguished by neutralization titrations. HSV-1 and HSV-2 are responsible for a variety of human diseases, such as skin infection, fever blisters, genital herpes, viral encephalitis, and the like. Both HSV-1 and HSV-2 have been shown to be capable of causing, neonatal infections. HSV-2 genital infections have been linked to the development of cervical cancer.

[0006] Cytomegalovirus (CMV) is another member of the HSV family. CMV infection is the leading cause of congenital viral infections with an incidence averaging 1% of all live births. An additional 5-10% of infants acquire CMV perinatally as a result of mother-to-infant transmission. Although the virus is widely distributed in the population, about 40% of women enter pregnancy without antibodies and are thus susceptible to infection. CMV infections of the eye have resulted in the loss of sight to immunocompromised individuals afflicted with AIDS. CMV infection is also a major concern for organ transplant recipients, especially kidney and liver transplants.

[0007] A major avenue for HSV transmission is through skin to skin contact with an infected area, such as genital to genital contact, and contact with the eye to the hands. It is therefore desirable to administer a potent antiviral, particularly, anti-HSV or anti-CMV agent topically prior to potential viral introduction into an individual.

[0008] Because of these disease conditions, there is a continuing effort made by individual academic investigators, and by small and large pharmaceutical companies to identify new and useful antiviral agents. Various drug discovery strategies have been developed. In some instances, derivatives of known effective drugs are prepared and examined for improved or different by useful characteristics. Another approach is to develop or acquire large libraries of randomly synthesized drug candidates, and screen these compounds for potential efficacy as antiviral agents. Both of these methods have resulted in the identification of potentially useful antiviral agents. Yet another approach has been to identify potentially useful drugs that are produced naturally by living organisms. For example, paclitaxel is a chemical that is produced by the yew tree and, when purified, is effective in treating cancers such as ovarian carcinoma. Applying similar discovery strategy, naturally occurring agents with antiviral activity are being sought and screened for antiviral activity.

[0009] Peptides derived from virus proteins have been found to be useful antimicrobial agents. Some of these peptides correspond to sequences from viral transmembrane proteins, especially lentiviruses (U.S. Pat. No. 5,714,577). The peptides are unmodified, arginine-rich peptides, modeled to have an amphipathic helical structure and are at least 17 amino acids in length. The peptides have a high cytolytic activity towards microorganisms, while being significantly less active in regard to mammalian cells. Another completely different class of peptide antiviral agents are related to glycoproteins present on the surface of viruses (Massi, et al, 1998). Such peptides derived from feline immunodeficiency virus (FIV) were shown to bind specifically to the surface of FIV-permissive cells and act as an inhibitor of viral infection.

[0010] Saturated fatty acids have also been demonstrated to possess antiviral activities (Thornar et al, 1987). Myristic and lauric acids are inhibitors of HSV-1 and vesicular stomatitis virus (VSV) at concentrations of 16 and 10 mM, respectively. Shorter or longer saturated fatty acids are less active. The unsaturated linoleic (18:2) and arachidonic (20:4) fatty acids are somewhat more active at 3.6 and 1.6 mM concentrations. Incubation of intact VSV virions with 0.5 mg/mL of linoleic acid caused leakage of the viral envelopes. However the high concentrations of the fatty acids required for anti-viral activity makes them undesirable as pharmacological agents.

[0011] Using the method of screening natural products, a novel antibiotic, trigochin GA IV, was isolated from the fungus Trichoderma koningii. Trigochin GA IV is a ten amino acid (aa) residue lipopeptaidol blocked in the N-terminus by an n-octanoyl group and in the C-terminus by the 1,2-amino alcohol L-leucinol (Piazza et al., 1999). The molecule exhibits membrane modifying properties which are believed to be responsible for its antibiotic activity. Permeability measurements showed that an appropriate length of the linear acyl chain is a more important characteristic for membrane modifying activity than its position in
the peptide chain. The peptide was determined to have a mixed 3_10^- and α-helical conformation, with the N-terminal region folding into a 3_10^-helix, and the C-terminal portions adopting a mainly α-helical structure. The proposed conformation of the molecule leads to an overall amphiphilic character, with the hydrophobic residues Leu, Ile, Lys, and the lipophilic chain on one side of the helix, and the less hydrophobic Gly residues on the more polar face. Further studies demonstrated that in phosphatidylcholine membranes, trichogin lays parallel to the membrane surface with its hydrophobic face oriented towards the membrane interior (Monaco et al, 1999). These results suggest that trichogin might modify membrane permeability via a carpet-like mechanism, as the peptide chain is too short to span a lipid bilayer and form conductance channels.

Other peptides have been reported to directly inactivate the herpes simplex virus. MCP-1 and MCP-2 are highly cationic, 33 amino acid residue polypeptides isolated from rabbit leukocytes that inactivate several enveloped viruses including HSV-1 and HSV-2, Vascular stomatitis virus, and influenza. Inactivation of HSV-1 by MCP-1 and -2 was dependent upon peptide concentration, ineffective at temperatures below 20°C, and optimum at a pH of 6, which is non-optimal for a pharmacological agent. The magainins are another group of cationic peptides with HSV-1 inactivating capability. Originally isolated from the skin of the African clawed frog Xenopus laevis, synthetic derivatives of this class that are lysine rich show modest virucidal activities between 12.5 and 50 μg/mL.

Synthetic polyhistidine, polylysine, and polyarginine peptides, all of highly cationic character, have also been demonstrated to possess HSV inactivating properties. Polyhistidines require a minimum chain length of at least 24 residues with optimum activities achieved at longer lengths, and activity is highly dependent on a pH between 5 and 6, again an undesirable pH for a therapeutic agent. Polylysine and polyarginine show slightly better activities at higher pH between 7 and 8. The optimum pH difference between these inhibitors likely stems from the differences in the pKs of histidine, lysine, and arginine residues. At neutral pH or higher, the imidazole side chain of histidine, with a pK_a of around 6, would only be minimally charged and would be unable to bind to negatively charged surfaces of an HSV virion. These peptides are only mildly potent, however, displaying inactivating properties at concentrations of 100 μg/mL, far too high for practical use as pharmacological reagents.

SUMMARY OF THE INVENTION

[0014] The present invention provides a compound having the structure:

![Chemical Structure](image)

[0015] wherein,

[0016] R^1 is an alkyl chain comprising at least seven carbons;
[0017] R^2 is lower alkyl;
[0018] R^3 is lower alkyl;
[0019] R^4 and R^5 together form an alkyl bridge selected from the group consisting of propyl, butyl, hydroxypropyl, hydroxybutyl, or acetoxypropyl;
[0020] R^6 is selected from the group consisting of hydrogen or lower alkyl;
[0021] R^7 is selected from the group consisting of hydrogen or lower alkyl;
[0022] R^8 is selected from the group consisting of hydrogen, lower alkyl, or substituted lower alkyl;
[0023] R^9 is selected from the group consisting of hydrogen or lower alkyl;
[0024] R^{10} is selected from the group consisting of —CH=O—R^{11} or —C(O)—R^{11}, wherein R^{11} is independently selected from the group selected from hydrogen or lower alkyl, and R^{12} is independently selected from the group consisting of hydrogen, NH_{2}, methyl, hydroxyl, —OCH_{3}, —OCH_{2}CH_{3} or —OR wherein R^{13} is lower alkyl;
[0025] a pharmaceutically acceptable salt or derivatives thereof, useful for preventing and treating viral and microbial infections.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] The present invention will be better understood from the following detailed description of an exemplary embodiment of the invention, taken in conjunction with the accompanying drawings in which like reference numerals refer to like parts and in which:

[0027] FIG. 1. Halovirs A, B and C.
[0028] FIG. 2. Halovirs F, G and H.
[0029] FIG. 3. Antiviral activity of halovirs.
[0030] FIG. 4. Synthesis of halovir A.
[0031] FIG. 5. Synthetic halovirs D and E.
The present invention provides a compound having the structure:

wherein,

- \( R' \) is an alkyl chain comprising at least seven carbons;
- \( R \) is lower alkyl;
- \( R^3 \) is lower alkyl;
- \( R^4 \) and \( R^5 \) together form a lower alkyl bridge selected from the group consisting of propyl, butyl, hydroxypropyl, hydroxybutyl, or acetoxypropyl;
- \( R^6 \) is selected from the group consisting of hydrogen or lower alkyl;
- \( R^7 \) is selected from the group consisting of hydrogen or lower alkyl;
- \( R^8 \) is selected from the group consisting of hydrogen, lower alkyl, or substituted lower alkyl;
- \( R^9 \) is selected from the group consisting of hydrogen or lower alkyl;
- \( R^{10} \) is selected from the group consisting of \(-\text{CH}_2\text{O}--\text{R}^{11} \) or \(-\text{O}--\text{R}^{12}\), wherein \( R^{11} \) is independently selected from the group selected from hydrogen or lower alkyl, and \( R^{12} \) is independently selected from the group consisting of hydrogen, \( \text{NH}_2 \), methyl, hydroxyl, \(-\text{OCH}_3 \), \(-\text{OCH}_2\text{CH}_3 \) or \(-\text{OR} \) wherein \( R^3 \) is lower alkyl.

- \( R^{13} \) is a pharmaceutically acceptable salt or derivatives thereof, useful for preventing and treating viral and microbial infections.

Definitions

- "alkyl" refers to a branched or straight chain monovalent saturated aliphatic hydrocarbon radical of at least seven carbon atoms. This term is further exemplified by such radicals as n-heptyl, n-decyl, n-tridecyl and the like.

- "lower-alkyl" refers to a branched or straight chain monovalent alkyl radical of one to six carbon atoms. This term is further exemplified by such radicals as methyl, ethyl, propyl, isopropyl, butyl (for example, isobutyl, t-butyl, or n-butyl), pentyl (for example, 2-methylbutyl, 3-methylbutyl), and hexyl (for example, 2-methylpentyl, 2,2-dimethylbutyl and 2,3-dimethylbutyl).

- "substituted alkyl" refers to an alkyl moiety optionally substituted with hydroxy, carbonyl, carboxyl, halide, amidyl, guanidyl, thio, and carbamoyl. Examples of substituted alkyl moieties include but are not limited by, tetradecanoyl, nonylcarbonyl, and 1-chloropentylcarbonyl.

- "substituted lower-alkyl" refers to a lower-alkyl moiety optionally substituted with hydroxy, carbonyl, carboxyl, halide, amidyl, guanidyl, thio, and carbamoyl. Examples of substituted lower-alkyl moieties include but are not limited by, methoxycarbonyl, 4-aminobutyryl, and 4-guanidylpropyl.

- "lower-alkyl bridge" or "substituted lower-alkyl bridge" refers to a lower-alkyl moiety optionally substituted with hydroxy, carbonyl, carboxyl, halide, amidyl, guanidyl, thio, and carbamoyl. Examples of lower-alkyl bridges include but are not limited by, tetradecanoyl, nonylcarbonyl, and 1-chloropentylcarbonyl.

- "isolated" or "substantially pure" means that the compound of the invention is at least about 90%, preferably at least about 95% free of materials with which it normally is associated in a cell, particularly CNI240, and generally is about 90% or 95% free of such materials, particularly at least 99% free of such material.

- "salt" or "pharmaceutically acceptable salt" encompasses those salts that form with the carboxylate anions and includes salts formed with the organic and inorganic cations.

- "halovir" encompasses any compound that is described by the summary of the invention.

The compounds of the instant invention were identified by biosensor guided fractionation of mycelial extract to determine the nature of the antiviral components. The marine fungus CNI240 (deposited with the American Type Culture Collection in Manassas, Va., and has the ATCC designation 74470) was grown in 1 L cultures of sterilized medium consisting of 2 g peptone, 2 g yeast extract and 4 g...
mannitol in one liter of filtered seawater. After approximately 20 days, the white mycelial mats were separated from the broth, lyophilized and extracted with a 1:1 mixture of dichloromethane and methanol. Concentration in vacuo yielded 375 mg crude extract per liter. Assay results indicated a strong antiviral activity at 10-20 μg/ml against HSV infected cells.

Bioassay guided fractionation of the extract using a series of chromatography methods revealed three major compounds, halovir A, B and C as the antiviral components (FIG. 1).

Halovir A was obtained as a colorless, amorphous solid in 12 mg/L yield. A molecular formula of C_{6}H_{10}O_{3}N_{2} was determined based on high resolution ESI mass spectrometry ([M+Na]+ m/z 888.6119; calculated 888.6150) coupled with 1H and 13C NMR data. NMR spectra revealed features consistent with a peptide and at least one aliphatic chain. The planar nature of halovir A was deduced by analysis of homo- and heteronuclear 2D NMR data. The IR spectrum displayed absorptions at 1640 cm^{-1} and 1540 cm^{-1} characteristic of amide carbonyls groups, and a broad absorption at 3290 cm^{-1} consistent with the presence of OH and NH functionalities. The sequence of the peptide was elucidated by correlations observed in HMBC and ROESY experiments. The sequence was confirmed by the analysis of fragment ions, or daughter ions, produced during electrospray mass spectrometry.

Halovir B was isolated in a yield of 2 mg/L and determined to have a molecular formula of C_{9}H_{13}N_{2}O_{6} by high-resolution electrospray ionization mass spectrometry (HREIMS). Carbon NMR DEPT experiments showed that halovir B differed from halovir A by having one less methyl group and methane carbon. Analysis of spin systems from TOCSY and COSY data indicated that halovir B contained alanine in place of the valine unit in 27. HMQC, HMBC, and ROESY NMR data, in addition to ms/ms experiments, all confirmed the presence of alanine and the peptide sequence of halovir B.

Halovir C was isolated in a yield of 1.5 mg/L and determined to have a molecular formula of C_{6}H_{8}N_{2}O_{6} by HREIMS. The difference in the molecular formula of an oxygen from halovir A was attributed to the presence of a proline residue in place of hydroxyproline. The 1H NMR spectra lacked the presence of an OH signal around δ 7.0 found in halovir A and B, and the proline ϕ- and γ-methylene were all appropriately shifted upfield relative to halovir A and B. HMQC, HMBC, ROESY, and DEPT NMR data, in combination with ms/ms experiments, were consistent with the presence of proline and the peptide sequence assigned for halovir C.

During bioassay guided fractionations, it is possible to overlook compounds structurally related to the active components of initial interest because they either lack activity or are masked by the presence of other compounds (e.g. cytotoxic agents). In the case of the fractionation of CNL240 extract, chemical analysis of inactive fractions using C18 HPLC coupled to an electrospray mass spectrometry detector lead to the discovery of three additional halovirs (halovirs F-H, FIG. 2). The fraction yielding these compounds contained metabolites slightly more polar in nature than halovirs A-C and had previously displayed cytotoxic activity in the Vero cell assay. The cytotoxicity was sufficiently potent to prevent the detection of the antiviral activity. When compared with the fractionation pattern observed for pure halovir A-C analyzed under identical conditions, the unknowns clearly contained related peptide fragments. Preparatory-scale purification of the compounds was undertaken and sufficient quantiles of halovir F and G were obtained for further analysis. It was determined that halovir F and G are structurally most similar to halovirs A and C, respectively, containing a lactic acid moiety on the N-terminus.

The halovirs possess potent activity against herpes simplex virus-1 (FIG. 3). Halovirs A, B, and C display IC50 values of approximately 1 μM when added to cells which had been previously infected with HSV-1 for one hour. Halovirs F and G, possessing a slightly shorter lipophilic chain, were slightly less active with IC50’s of 2 and 3 μM, respectively. These values were determined by averaging at least ten replicates. All of these molecules also demonstrated cytotoxicity against uninfected Vero cells, as well as against human colon tumor cells (HCT-116).

Variations of the standard assay were designed and executed in order to investigate the nature of the antiviral activities of these peptides. Halovir A was studied as a representative compound due to its more abundant culture production. The similar data obtained in the preliminary experiments and the similar structures of the halovirs indicate that they most likely have similar mechanisms of action. The various halovirs also indicate that there is flexibility in the molecule. Two initial assays were designed to determine whether the peptides were directly inactivating HSV-1. Infectious HSV-1 was pre-incubated with halovir A prior to addition to cells. A suspension of HSV-1 at 50,000 pfu/mL was exposed to 130 μg/mL of compound for 2.5 hours, then diluted 100-fold and added to Vero cells in microtitre plates. Control experiments included virus processed identically in the absence of halovir A, and media identically handled but with no virus or compound. Additionally, media spiked with halovir A was used to assess compound cytotoxicity, and a “normal assay” was conducted to test for halovir potency on cells pre-infected with virus for one hour. The final assay concentrations of halovir A were 0.85 μM, and the results were monitored after 5 days of incubation. The experiment clearly demonstrated the virucidal effect of halovir A on HSV-1. Nearly 100% cell survival was achieved by pre-exposing the virus to compound. It was found that 0.85 μM of halovir A is insufficient to effectively protect cells pre-infected with HSV-1. Therefore, the major antiviral effects are achieved prior to cell infection. Further, no appreciable cell cytotoxicity was witnessed at this assay concentration.

An additional assay was designed to explore the concentration and time dependence of the viral inactivation process. In this experiment, viral suspensions consisting of 500 pfu/mL of HSV-1 in microtiter plates were treated with serial dilutions of halovir A ranging from 10 down to 0.08 mg/mL. The plates were then incubated for 0, 15, 30, and 60 minutes prior to addition of the virus to cells. The results were again recorded after five days. Halovir A inactivates HSV-1 in a time dependent manner. IC50 values decrease with the longer pre-incubation times, with the maximum effects being reached after approximately one hour.

A variation of the time-dependent inactivation experiment involved the removal of compound and unat-
tached virus after 3 hours. Three hours after the treated viral suspensions were added to the Vero cells, the test wells were aspirated and washed with PBS, aspirated again and overlaid with MEM (1% FBS). The results showed that the antiviral activities were not significantly attenuated in this study. This assay demonstrated that the virus can be inactivated in a short time frame with subsequent removal of the compound, thereby preventing the cytopathic effects.

[0066] Halovir A was found to be active against both HSV-1 and HSV-2. Experiments to test for activity against HSV-2 were conducted at MDS Pharma Services, formerly MDS Panlabs, a biotechnology company. Halovir A was determined to equally inhibit replication of HSV-1 and HSV-2 with an IC50 of 0.3 μM in a standard plaque reduction assay (Mahy and Kangro, 1996).

[0067] These studies demonstrate the utility of the compounds of the instant invention for both the prevention and treatment of infection with HSV-1 and -2. The structural similarity of the compounds, as well as the similarity in the activity of the compounds suggest that they work through similar mechanisms. The ability of the halovir A-C and F-G to have activity in these assays demonstrates the flexibility in the molecule. A number of assays were performed to determine the degrees of flexibility in the halovir. The essential components are the N-terminal alkyll chain, a restriction of rotation of the molecule around the R1 and R3 d-peptide portion, and the lipophilic and helical nature of the molecule. These broad requirements may be fulfilled by a number of related structures that constitute the class of halovir.

[0068] Based upon the antiviral activity of the naturally occurring halovir, a synthetic program was undertaken to determine the amount of flexibility that exists within the molecule, the number of changes that can be made while retaining antiviral activity and to explore the structure-activity relationships of these molecules. These manipulations assisted in the elucidation of the critical structural features necessary for antiviral activity.

[0069] Synthetic halovir were prepared de novo and evaluated in vitro against HSV-1 in order to establish structure-activity relationships relevant to their antiviral properties. The study focused on modifications of the lipophilic N-acetyl substituent, the Aib-Hyp dipeptide segment, and the reduced carboxyl terminus. Also, a total synthesis of halovir A was completed (FIG. 4). Synthetically derived halovir A was identical to the natural substrate in all chemical and biological aspects, thereby giving further evidence of the proposed structure.

[0070] This study demonstrates that the N′-myristoyl and N′-lauroyl chains of the naturally occurring halovir likely play a major role in the antiviral nature of these molecules. Specifically, an N′-acetyl chain of at least fourteen carbons is preferred to maintain the maximum anti-HSV-1 potency. Shorter saturated chains of six or less carbons lost all observable antiviral and cytotoxic activities. Interestingly, N′-acyl chain length modulated the cytotoxic properties analogously to the measured differences in the antiviral properties. These results suggest that it is unlikely that the antiviral mechanism of action involves the heptapeptide portion engaged in a receptor-binding phenomenon. Non-specific interaction of the lipopeptides with the lipid envelope of the virus and cellular membrane remains a plausible explanation.

[0071] Congeners of halovir A incorporating unsaturated lipid chains were found to possess decreased anti-HSV-1 activity. The addition of one point of unsaturation to the N-terminal acyl chain, as demonstrated with the myristoleoyl derivative, only slightly effected HSV inhibition. However, the linoleoyl and linolenoyl targets were two and five fold less active, respectively, than the saturated stearoyl compound. Thus, increases in unsaturation appeared to incrementally decrease the antiviral activity. This raises the possibility that lipid chain flexibility is an important factor in HSV-1 inhibition. These results are in contrast to the antiviral activities of fatty acids themselves, where it was found that unsaturated linoleic (18:2) and arachidonic (20:4) fatty acids were more active against HSV-1 than saturated fatty acids (Thomar et al., 1987).

[0072] The modifications to the Aib-Hyp dipeptide segment in this study nearly abolished the antiviral activity. Substitution of the aminoisobutyric residue with L-alanine greatly diminished the antiviral activity relative to the parent compound. Interestingly, the D-alanine isomer inhibited some HSV induced cell death, although a maximum of only 60% cell survival was achieved (11 μM). Elimination of the cyclic Hyp residue by substitution with a sarcosine also resulted in a nearly inactive peptide. A common effect of these three amino acid substitutions is a reduction in the steric hindrance to rotation between the Aib and Hyp residues. Hindered rotation between the Aib and Hyp residue likely provides a conformational bias in the presence of a lipid membrane leading to observed biological effects. Modification of the Hyp residue itself is tolerated. In the naturally occurring halovir C, the Hyp residue is replaced by a Pro residue. Chemical modification of the residue to Hyp(OAc) as in halovir D (FIG. 5) also resulted in a compound with comparable function to halovir A.

[0073] Modification in the C-terminus of the halovir alters the cellular toxicity of the molecule. Halovir E (FIG. 5), a partial synthesis product of halovir containing a C-terminal methyl ester, was found to be less cytotoxic than halovir A with comparable antiviral activity (IC50=2.3 μM). Modification of the C-terminus to an acetyl ester as in halovir D resulted in a compound with comparable activity and cytotoxicity to halovir A.

[0074] These studies demonstrate degrees of flexibility in the various portions of the halovir. The length of the N-terminal fatty acid chain can vary, with a minimum length of 7 carbons required. An increase in the amount of unsaturation of the lipid results in a decrease in activity. The presence of an Aib-Hyp dipeptide or other group with hindered rotation is required. The steric hindrance provided by this group creates a conformational bias in the molecule that is necessary for antiviral activity. The C-terminus of the molecule is a likely candidate site for modification to alter the pharmacokinetic and pharmacodynamic properties of the molecule. Such changes make the compound amenable for administration via different routes including, but not limited to, topically, orally, parenterally, intravenously, and intramuscularly. Variations exist between the various natural halovir isolated; however, all of them possess anti-viral activity. Depending on the combination of attributes desired in the therapeutic compound (e.g. prophylaxis vs. therapeutic; topical vs. oral availability), the halovir can be modified within the scope of the definition of the halovir in the invention. The spectrum of halovir containing antiviral
compositions may be increased by the use of halovir in conjunction with any of a number of other antiviral agents. Such agents include, but are not limited to: acyclovir, pencyclovir, valacyclovir, famcyclovir, ganciclovir, nonoxynol, docosanol and foscarin.

EXAMPLE

[0075] Synthesis of natural and artificial halovirs. FIG. 4 outlines the construction of the core peptide sequence of Halovir A. Reagents and conditions: (a) EDC, HOBt, DIEA, MeCN, 84%; (b) HCl, EtOH, 0° to room temperature, quant.; (c) N^2-Boc-L-valine, EDC, HOBt, DIEA, MeCN, 87%; (d) N^4-Boc-L-leucine, EDC, HOBt, DIEA, MeCN/DMF, 69%; (e) N^2-Boc-L-trans-4-hydroxyproline, EDC, HOBt, DIEA, MeCN, 88%; (f) N^4-Boc-aminoisobutyric acid, EDC, HOBt, DIEA, MeCN/DMF, 57%; (g) myristic acid, EDC, HOBt, DIEA, MeCN/DMF, 89%; (h) L-BH_4, TIF, 82%. Peptide couplings were accomplished by activation of carboxylic acids with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC), and 1-hydroxybenzotriazole (HOBt) was utilized to suppress racemization. N^4-t-Butyloxycarbonyl (Boc) protected amino acids were cleaved using trifluoroacetic acid (TFA) or ethanolic HCl.

[0076] The total synthesis of halovir A was completed as shown in FIG. 4. The coupling of the amine trifluoroacetate salt of L with myristic acid proceeded in 89% yield. The C-terminal methyl ester of compound 2 was then efficiently reduced with lithium borohydride to yield the desired target. Synthetic halovir A matched the natural substrate in all chemical and biological aspects.

[0077] Although an exemplary embodiment of the invention has been described above by way of example only, it will be understood by those skilled in the field that modifications may be made to the disclosed embodiment without departing from the scope of the invention, which is defined by the appended claims.

REFERENCES


[0081] Piazza, C et al. (1999) Total synthesis and membrane modifying properties of lipopeptoid trikoningin where R^1 is an alkyl chain comprising at least seven carbons; R^2 is lower alkyl; R^3 is lower alkyl; R^4 and R^5 together form an alkyl bridge selected from the group consisting of propyl, butyl, hydroxypropyl, hydroxybutyl, and acetoxypropyl; R^5 is selected from the group consisting of hydrogen and lower alkyl; R^7 is selected from the group consisting of hydrogen and lower alkyl; R^8 is selected from the group consisting of hydrogen, lower alkyl, and substituted lower alkyl; R^9 is selected from the group consisting of hydrogen or lower alkyl; R^10 is selected from the group consisting of —CH—O—R^11 and —C(0)—R^12 wherein R^11 is independently selected from the group consisting of hydrogen or lower alkyl, and substituted lower alkyl and R^12 is independently selected from the group consisting of hydrogen, NH_2, methyl, hydroxyl, and —OR^13 wherein R^13 is lower alkyl.

2. The compound of claim 1, wherein R^12 is selected from the group consisting of methoxy and ethoxy.

3. A composition comprising the compound of claim 1 and a pharmaceutically acceptable carrier.

4. The composition of claim 3, wherein the composition comprises at least a second antiviral agent selected from the group consisting of acyclovir, pencyclovir, valacyclovir, famcyclovir, ganciclovir, nonoxynol, docosanol and foscarin.

5. A method of prevention or treatment of viral infections in a host comprising administering the compound of claim 1 and observing the treated host for amelioration of the infection.

6. The method of claim 5, wherein the viral infection is a herpes virus infection.
7. The method of claim 5, wherein the compound is administered by a route selected from a group consisting of topical, oral, parenteral, intravenous, and intramuscular.

9. A compound of the structure:

R\(^2\) is selected from the group consisting of methyl, 2-propyl, 2-methyl propyl, 2-butyl, and benzyl;

R\(^3\) is selected from the group consisting of methyl, 2-propyl, 2-methyl propyl, 2-butyl, and benzyl;

R\(^4\) and R\(^5\) together form an alkyl bridge selected from the group consisting of propyl, butyl, hydroxypropyl, hydroxybutyl, and aceoxypropyl;

R\(^6\) is selected from the group consisting of hydrogen, methyl, 2-propyl, 2-methyl propyl, 2-butyl, and benzyl;

R\(^7\) is selected from the group consisting of hydrogen, hydroxymethyl, 1-hydroxyethyl, thiomethyl, 4-hydroxyphenylmethyl, aminocarboxylmethyl, 3-propionyl acid amide, carboxyethyl, carboxymethyl, 4-aminobutyl, 3-guanylpropyl, and 4-imidazoyl methyl;

R\(^8\) is selected from the group consisting of hydrogen, methyl, 2-propyl, 2-methyl propyl, 2-butyl, or benzyl;

and

R\(^{10}\) is selected from the group consisting of —CH\(^2\) —O— R\(^{11}\) and —C(=O)—R\(^{12}\), wherein R\(^{11}\) is independently selected from the group selected from hydrogen or alkyl, and R\(^{12}\) is independently selected from the group consisting of hydrogen, NH\(_2\), methyl, hydroxyl, and —OR where R\(^{13}\) is lower alkyl.

10. The compound of claim 1, wherein R\(^{12}\) is selected from the group consisting of methoxy and ethoxy.

11. A composition comprising the compound of claim 9 and a pharmaceutically acceptable carrier.

12. The composition of claim 11, wherein the composition comprises at least a second antiviral agent selected from the group consisting of acyclovir, penciclovir, valacyclovir, famciclovir, ganciclovir, nonoxynol, docosonal, and foscarinet.

13. A method of prevention or treatment of viral infections in a host comprising:

administering the compound of claim 8 and

observing the treated host for amelioration of the infection.

14. The method of claim 13, wherein the viral infection is a herpes virus infection.

15. The method of claim 13, wherein the compound is administered by a route selected from a group consisting of topical, oral, parenteral, intravenous, intramuscular.

16. The method of claim 13, wherein the compound is administered topically.

17. The compound of claim 9, wherein:

R\(^1\) is decanyl;

R\(^2\) is methyl;

R\(^3\) is methyl;

R\(^4\) and R\(^5\) together form a 2-hydroxypropyl bridge;

R\(^6\) is 2-methyl propyl;

R\(^7\) is 2-propyl;

R\(^8\) is 3-propionyl acid amide;

R\(^9\) is 2-methyl propyl; and

R\(^{10}\) is hydroxymethyl

said compound having the name designation halovir F.

18. The compound of claim 9, wherein:

R\(^1\) is decanyl;

R\(^2\) is methyl;

R\(^3\) is methyl;

R\(^4\) and R\(^5\) together form a propyl bridge;

R\(^6\) is 2-methyl propyl;

R\(^7\) is 2-propyl;

R\(^8\) is 3-propionyl acid amide;

R\(^9\) is 2-methyl propyl; and

R\(^{10}\) is hydroxymethyl

said compound having the name designation halovir G.

19. The compound of claim 9, wherein:

R\(^1\) is decanyl;

R\(^2\) is methyl;

R\(^3\) is methyl;

R\(^4\) and R\(^5\) together form a 2-hydroxypropyl bridge;

R\(^6\) is 2-methyl propyl;

R\(^7\) is methyl;

R\(^8\) is 3-propionyl acid amide;

R\(^9\) is 2-methyl propyl; and

R\(^{10}\) is hydroxymethyl

said compound having the name designation halovir H.