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(71) Applicant (for all designated States except US): DUKE UNIVERSITY [US/US]; 230 North Building, Research Drive, Box 90083, Durham, NC 27708-0083 (US).

(72) Inventors; and


(54) Title: METHOD OF TREATING HEPATITIS DELTA VIRUS INFECTION

(57) Abstract

The present invention relates, in general, to hepatitis delta virus, and in particular, to a method of treating hepatitis delta virus infection. The method involves inhibiting farnesylation of the delta virus large antigen using an inhibitor of farnesyltransferase.
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METHOD OF TREATING HEPATITIS DELTA VIRUS INFECTION

This invention was made with Government support (NIH Grant No. GM46372). Accordingly, the Government has rights in the invention.

TECHNICAL FIELD

The present invention relates, in general, to hepatitis delta virus, and in particular, to a method of treating hepatitis delta virus infection. The method involves inhibiting farnesylation of the delta virus large antigen using an inhibitor of farnesyltransferase.

BACKGROUND

Hepatitis delta virus (HDV) is a satellite virus of hepatitis B virus that can cause an increase in the incidence and severity of liver disease in individuals infected with both viruses (Rizzetto, (1983) Hepatology 3, 729-737; Lai, (1995) Annu. Rev. Biochem. 64, 259-286). HDV consists of the HDV RNA genome and two HDV encoded proteins, designated as the small (shHDAg) and large (lHDAg) HDV antigens, encapsulated in an envelope composed of hepatitis B surface antigens (Lai, (1995) Annu. Rev. Biochem. 64, 259-286).

shHDAg and lHDAg contain identical deduced amino acid sequences for their first 195 amino acids, with lHDAg containing an additional 19 amino acid C-terminal tail (Lai, (1995) Annu. Rev. Biochem. 64, 259-286.).
2


lHADg is modified by an isoprenoid lipid on a cysteine located near its C-terminus (Glenn et al., (1992) Science 256, 1331-1333), and this modification is necessary for lHADg to facilitate HDV assembly (Glenn et al., (1992) Science 256, 1331-1333; Lee et al., (1994) Virology 199, 169-175). The prenylation motif contained in lHADg is the C-terminal tetrapeptide Cys-Arg-Pro-Gln (CRPQ) (Glenn et al., (1992) Science 256, 1331-1333). This sequence is similar to the conventional prenylation motif which consists of the C-terminal tetrapeptide CaaX, where the cysteine residue is the prenylation site, "a" are generally aliphatic residues, and X can be one of several amino acids.

As prenylation of lHDAg is required for HDV assembly, inhibition of the enzyme responsible for its prenylation provides a treatment of HDV infection. Two
reports have appeared indicating that lHDAg is modified with the geranylgeranyl isoprenoid (Glenn et al, (1992) Science 256, 1331-1333; Lee et al, (1994) Virology 199, 169-175). The present invention results, at least in part, from the realizations that the predominant modification of lHDAg is farnesylation rather than geranylgeranylation, and that the protein is exclusively a substrate for FTase.

SUMMARY OF THE INVENTION

The present invention relates to a method of inhibiting hepatitis delta virus (HDV) assembly. The method comprises contacting an HDV-infected cell with an amount of a farnesyltransferase inhibitor sufficient to inhibit farnesylation of lHDAg and thereby inhibit HDV assembly. The invention further relates to a method of treating a patient infected with HDV. The method comprises administering to the patient an amount of a farnesyltransferase inhibitor sufficient to inhibit farnesylation of lHDAg and thereby effect the treatment.
BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Prenylation of GST-1HDAG by bovine brain cytosol. GST-1HDAG, H-Ras, and H-Ras-CVLL were incubated with bovine brain cytosol in the presence of either 2 μM [³H]FPP or 2 μM [³H]GGPP for 1 h at 37°C. Prenylated proteins were resolved by SDS-PAGE and visualized by fluorography. The gel was exposed for either 2 days (Fig. 1A) or 14 days (Fig. 1B). Samples processed in the various lanes are as follows: 1 and 4, no added substrate protein; 2 and 6, H-Ras; 3 and 7, H-Ras-CVLL; 4 and 8, GST-1HDAG. Data shown are from a single experiment, which is representative of several such experiments.

Figure 2. Prenylation of GST-1HDAG by purified recombinant protein prenyltransferases. GST-1HDAG, H-Ras or H-Ras-CVLL were incubated with FTase (Fig. 2A) or GGTase-I (Fig. 2B) in the presence of either 2 μM [³H]FPP or 2 μM [³H]GGPP for 1 h at 37°C. Prenylated proteins were resolved by SDS-PAGE and visualized by fluorography. Exposure time was 4 days. Samples processed in the various lanes are as follows: 1 and 5, no added substrate protein; 2 and 6, H-Ras; 3 and 7, H-Ras-CVLL; 4 and 8, GST-1HDAG. Data shown are from a single experiment, which is representative of two such experiments.

Figure 3. Kinetics of protein prenyltransferase modification of GST-1HDAG. Saturation curves for
modification of GST-1HDAg, H-Ras, and H-Ras-CVLL by both FTase and GGTase-I were determined. Assays were conducted with either FTase and FPP (Fig. 3A) or GGTase-I and GGPP (Fig. 3B). Reactions were stopped by addition of 2% SDS and prenylated proteins produced determined by filter binding assays. Data shown represent the mean of duplicate determinations from a single experiment, which is representative of several such experiments.

Figure 4. Prenylation of 1HDAg in animal cells.
Fig. 4A. COS cells were transfected with either an expression vector encoding MEV alone (lanes 1 and 3) or with expression vectors for both MEV and 1HDAg (lanes 2 and 4). Detergent-solubilized extracts were prepared and either analyzed directly (lanes 1 and 2) or after immunoprecipitation using anti-1HDAg antibody (lanes 3 and 4). Samples were resolved by SDS-PAGE and prenylated proteins visualized by fluorography. Exposure time was 3 days. Figs. 4B-D. Isoprenoid analysis of metabolically labelled 1HDAg. Samples identical to those in Fig. 4A were subjected to isoprenoid analysis. Solubilized cell extract (Fig. 4B) or samples subjected to immunoprecipitation with anti-1HDAg (Figs. 4C and D) were processed by TCA precipitation and isoprenoid lipids cleaved from prenylated proteins using methyl iodide. Farnesol (C15) and geranylgeraniol (C20) were added to the samples as internal standards, and released isoprenoids were resolved by reverse-phase HPLC. Fractions were analyzed
by liquid scintillation spectroscopy. Isoprenoid analysis was performed on the following samples: Fig. 4B, detergent-solubilized extract of cells expressing both MEV and lHDAg (corresponding to Fig. 4A, lane 2); Fig. 4C, immunoprecipitate from cells expressing both MEV and lHDAg (corresponding to Fig. 4A, lane 4); Fig. 4D, immunoprecipitate from cells expressing only MEV (corresponding to Fig. 4A, lane 3). For all panels, data shown are from a single experiment, which is representative of data obtained from two different transfections.

Figure 5. Inhibition of the farnesylation of lHDAg in vitro. Fig. 5A. SCH56582. Fig. 5B. B581.

DETAILED DESCRIPTION OF THE INVENTION

The prenylation motif at the C-terminus of the HDV large antigen lHDAg is necessary for the protein to interact with hepatitis B surface antigen in vitro (Hwang et al, (1993) J. Virol. 67, 7659-7662). Prenylation of lHDAg is also required for HDV particle formation (Glenn et al, (1992) Science 256, 1331-1333; Lee et al, (1994) Virology 199, 169-175; (Chang et al, (1994) J. Virol. 68, 646-653). It has now been demonstrated that lHDAg is exclusively a substrate for FTase and that the protein is farnesylated. The present invention thus provides a method of preventing viral particle formation and thereby inhibiting HDV infection using inhibitors of FTase.

Compounds that can be used to inhibit farnesylation of 1HDAg by FTase include substrate analogs, farnesyl diphosphate analogs, bisubstrate analogs, non-competitive and other types of inhibitors (see Graham, Review, Oncologic, Endocrine and Metabolic, Exp. Opin. Ther. Patents 5(12):1269-1285 (1995), hereinafter "the Graham review" the contents of which is incorporated herein by reference). (See also Buss et al, (1995) Chemistry & Biology 2:787-791, the contents of which is incorporated by reference)

USP 5352705, WO 9500497, WO 9509001, WO 9509000, WO 9511917 and WO 9512612. The Graham review makes reference to the above-indicated disclosures as well as others from the technical literature (the above disclosures and the literature references cited in the Graham review being incorporated herein by reference).


The Graham review makes reference to these disclosures, which are also incorporated herein by reference, and others from the technical literature, the contents of which are incorporated herein by reference.


Additional compounds suitable for use in the invention include derivatives of pyridobenzocycloheptene described in WO 9510516, WO 9510515, WO 9510514 and in the Graham review (those disclosures being incorporated herein by reference). Further disclosures of suitable compounds can be found in WO 9324643, USP 5436263, USP 5420334, WO 9508542, WO 9318651, USP 5276217, and
USP 5349112. These disclosures and others from the technical literature are cited in the Graham review and are incorporated herein by reference.

Compounds suitable for use in the present invention can be prepared in accordance with the disclosures referenced above.

It will be clear from a review of the above-referenced documents that the amount of compound to be administered to effect inhibition of farnesyl transferase varies with the agent. These disclosures provide ample guidance from which an optimum dose can be selected for a particular patient, given the patient's, age, weight, status and responsiveness. These disclosures also provide guidelines as to administration regimens suitable for use in inhibiting HDAG farnesylation, which will also vary with the agent and the patient.

The farnesyltransferase inhibitors referenced above can be formulated as pharmaceutical compositions in accordance with the details provided in the respective disclosures. Typically, the agent will be combined with a pharmaceutically acceptable carrier or diluent, according to standard pharmaceutical practice. These compounds can be administered as indicated in the respective disclosures, for example, orally, or parenterally, including via intravenous, intramuscular, intraperitoneal, subcutaneous, rectal and topical routes, providing that the route of administration results in distribution of the agent to the liver, the site of HDV infection.
Certain aspects of the present invention are described in greater detail in the non-limiting Examples that follow.

**EXAMPLES**

The following experimental protocols are referenced in the Examples that follow.

**Protein prenyltransferases and protein substrates.**

**Expression and purification of GST-1HDAG.** The GST-1HDAG fusion protein was expressed in E. coli and purified essentially as described by Lee et al., ((1994) Virology 199, 169-175). A 500 ml flask of LB media was inoculated with 1 ml of an overnight culture of JM109 cells that had been transformed with the vector pGEX-2T-
lHDAg (a bacterial expression vector containing the cDNA for a fusion protein between glutathione-S-transferase and the C-terminal 117 amino acids of lHDAg (Lee et al., (1994) Virology 199, 169-175). After incubation for 4 h at 37°C, IPTG was added to 1 mM, and the cells were grown for an additional 4 h. Cells were harvested by centrifugation at 3000 x g for 15 min, resuspended in lysis buffer (PBS containing 10 mM EDTA, 10 mM DTT, 1% Triton X-100, 10% streptomycin sulfate and protease inhibitor mix), and lysed by three passes through a French press. Cell debris was removed by centrifugation at 45000 x g for 1 h, and the supernatant was incubated with glutathione-Sepharose 4B for 1 h at room temperature. The mixture was transferred to a column, and the column was washed with 20 volumes of PBS containing 850 mM NaCl, 10 mM EDTA, 10 mM DTT, and 1% Triton X-100. GST-lHDAg was then eluted from the column with 50 mM Tris-HCl, pH 7.7, containing 5 mM glutathione, and concentrated to 40 mg/ml using an Amicon Centricon-30.

In vitro prenylation reactions. GST-lHDAg (20 μg), H-Ras (2 μg) or H-Ras-CVLL (2 μg) were utilized in prenylation reactions that contained 50 mM Tris-HCl, pH 7.7, 5 mM MgCl₂, 5 μM ZnCl₂, 2 mM DTT and either 2 μM [³H]FPP (8 Ci/mmol) or 2 μM [³H]GGPP (8 Ci/mmol) (Chen et al., (1993) J. Biol. Chem. 268, 9675-9680; Zhang et al., (1994) J. Biol. Chem. 269, 23465-23470). The reactions were initiated by the addition of either bovine brain cytosol (500 μg protein) or purified recombinant FTase
(75 ng) or GGTase-I (75 ng), incubated for 1 h at 37°C, and stopped by addition of SDS-PAGE sample buffer and heating at 65°C for 5 min. Samples were resolved on 12% SDS-PAGE gels. Proteins were visualized by Coomassie Blue staining, and the gels were prepared for fluorography by rinsing in water and soaking for 45 min in 1 M salicylic acid. Prenylated proteins were visualized by exposing the gels to Fuji RX film.

For kinetic determinations, the concentrations of prenylation-competent GST-1HDAg, H-Ras, and H-Ras-CVLL were determined by driving the prenylation reactions to completion in the presence of excess FTase or GGTase-I and the respective prenyldiphosphate, and determining the amount of protein prenylated by the filter binding assay described below. Final concentrations of substrates ranging from 0.1 to 20 μM were utilized in kinetic assays. Assay conditions were identical to those described above, with the exception that the specific activities of [3H]FPP and [3H]GGPP were 3 Ci/mmol. Reactions were initiated by the addition of 75 ng of FTase or GGTase-I, and incubated for 15 min at 37°C. The reactions were stopped by addition of 4% SDS, proteins precipitated by addition of trichloroacetic acid (TCA), and prenylated proteins separated from free isoprenoid by filtration through nitrocellulose filters as described (Reiss et al, (1990) Cell 62, 81-88). The amount of prenylated protein retained on the filters was quantitated by liquid scintillation spectroscopy.
Metabolic labelling of lHDAg with [3H]mevalonate in transfected COS cells. COS-7 cells were grown in DMEM supplemented with 8% calf serum and 2% fetal calf serum. The cells were transfected using a DEAE/Dextran-chloroquine method (Lopatta et al, 1984) Nucleic Acids Res. 12, 5707-5717). DMEM containing 0.25 mg/ml DEAE/Dextran, 5 μg/ml pMEV (a mammalian expression vector containing the cDNA for a mevalonate transporter (Kim et al, 1992) J. Biol. Chem. 267, 23113-23121), and 5 μg/ml pSV-lHDAg (a mammalian cell expression vector containing the cDNA for lHDAg (Ryu et al, 1992) J. Virol. 66, 2310-2315) was added to COS-7 cells (3 X 10⁶ cells/100 mm dish). Following an incubation at 37°C for 1 h, 7 ml of supplemented DMEM containing 52 μg/ml chloroquine was added to the cells. After 6 h, the media was aspirated from the cells, the cells were washed with DMEM, and fresh supplemented DMEM was added. At 41 h post-transfection, lovastatin was added to the media to a final concentration of 15 μM. Following a 1 h incubation at 37°C, the media was aspirated, and supplemented DMEM containing 20 μM lovastatin and 50 μCi/ml [5-3H]mevalonate was added. After an 11 h incubation at 37°C, the media was removed from the cells and the cells were washed with PBS and harvested by scraping. Cells were pelleted by centrifugation, resuspended in 50 mM Hepes, pH 7.4, containing 1 mM EDTA, 1 mM DTT, and protease inhibitor mix, flash frozen in liquid nitrogen, thawed and lysed by passing several times through a 27 gauge needle.
Immunoprecipitation of 1HDAg from COS cell extracts. Cellular proteins were solubilized from the lysed cell extracts by addition of Triton X-100 and NaCl to final concentrations of 1% and 250 mM, respectively. Samples were incubated on ice for 30 min, passed through a 27 g syringe several times, and insoluble material precipitated by centrifugation at 100000 x g for 1 h at 4°C. SDS was added to the solubilized protein to a final concentration of 0.5% and the sample was heated at 65°C for 5 min to denature proteins. The sample was then adjusted to 0.25% SDS, 125 mM NaCl, 1% Triton X-100, and 1% sodium cholate. 1HDAg was immunoprecipitated by incubation of the final extract obtained with rabbit anti-1HDAg (Wang et al, (1992) J. Gen. Virol. 73, 183-188) for 15 min, followed by incubation with protein A-sepharose 4B for 15 min. The protein A-sepharose 4B beads were pelleted by centrifugation, and were washed extensively with 0.1 M Tris, pH 7.7, containing 0.1% Triton X-100. Immunoprecipitated proteins were eluted from the beads either by addition of SDS-PAGE sample buffer (for SDS-PAGE) or by two washes with 0.2 M acetic acid (for isoprenoid analysis). Immunoprecipitation of 1HDAg was monitored by fluorography of SDS-PAGE gels.

Isoprenoid analysis. Isoprenoid analysis was conducted essentially as described by Farnsworth et al. (Farnsworth et al, (1990) Methods: A Companion to Meth. Enz. 1, 231-240). Solubilized COS cell proteins or immunoprecipitated 1HDAg were precipitated in 15% TCA
and the resulting protein pellets washed extensively in acetone at -20°C. Precipitated proteins were subjected to trypsin digestion, and isoprenoids cleaved from the tryptic peptides by treatment with methyl iodide in 2% formic acid. Following cleavage, methyl iodide was removed under mild vacuum and the solution neutralized by addition of sodium carbonate. Isoprenoids were extracted into a solution of chloroform:methanol (9:1), and the extracted isoprenoids dried under nitrogen and resuspended in 50% acetonitrile containing 0.1% phosphoric acid. Farnesol and geranylgeraniol (15 nmol of each) were added to the samples as standards, and isoprenoids resolved by HPLC using a 50%-100% gradient of acetonitrile in 0.1% phosphoric acid. Fractions containing [3H]-labelled isoprenoids were identified by liquid scintillation spectroscopy.

Example 1

In Vitro Prenylation of GST-1HDAg
in Bovine Brain Cytosol

In order to identify the protein prenyltransferase responsible for the modification of 1HDAg, it was first determined whether a fusion protein between glutathione-S transferase and the C-terminal 117 amino acids of 1HDAg (GST-1HDAg) (Lee et al, (1994) Virology 199, 169-175) was a substrate for these enzymes present in bovine brain cytosol. Initial experiments indicated that GST-1HDAg was exclusively farnesylated, with no
evident modification with the geranylgeranyl isoprenoid (Fig. 1A, lanes 4 and 8). Prolonged exposure of the gel revealed that a low level of geranylgeranylation of GST-lHDAg occurred (Fig. 1B, lane 8); the level of geranylgeranylation of GST-lHDAg was approximately 1% of the level of farnesylation. Two recombinant Ras proteins, H-Ras (containing the C-terminus -CVLS, and thus a substrate for FTase) and H-Ras-CVLL (containing the C-terminus -CVLL, and thus a substrate for GGTase-I) were used as control substrates for the two enzymes, and clearly demonstrated the presence of each enzyme in bovine brain cytosol (Fig 1A). These results indicated that lHDAg is a substrate for FTase.

Example 2

In Vitro Prenylation of GST-lHDAg Using Purified Recombinant Protein Prenyltransferases

To further assess the ability of GST-lHDAg to serve as a substrate for FTase, the ability of recombinant FTase and GGTase-I to prenylate GST-lHDAg was examined. FTase efficiently transferred the farnesyl group from FPP to GST-lHDAg, and the enzyme also supported a low level of geranylgeranylation, again approximately 1% of the level of farnesylation (Fig. 2A, lanes 4 and 8). GGTase-I was unable to modify GST-lHDAg using either FPP or GGPP as substrates (Fig. 2B, lanes 4 and 8). These results were confirmed by a more detailed kinetic analysis of the interaction of GST-lHDAg with the two
enzymes (Fig. 3). The fusion protein was found to be an excellent substrate for FTase with an apparent $K_m$ of 0.8 $\mu$M, which compares favorably to that measured for H-Ras (Fig. 3A). GGTase-I, however, did not utilize GST-1HDAg as a substrate over the range concentrations tested (Fig. 3B).

Example 3

Isoprenoid Analysis of 1HDAg
Expressed in Animal Cells

Isoprenoid analysis was performed on 1HDAg expressed in COS cells. COS cells transfected with a vector expressing 1HDAg were incubated with [$^3$H]mevalonate, a precursor for both FPP and GGPP, resulting in the metabolic labelling of prenylated proteins (Schmidt et al, (1984) J. Biol. Chem. 259, 10175-10180). In order to increase the labelling of prenylated proteins in the COS cells, the cells were co-transfected with a second plasmid coding for a mevalonate transport protein (MEV) and also treated with lovastatin. Expression of MEV serves to increase the uptake of exogenous mevalonate (Kim et al, (1992) J Biol Chem 267, 23113-23121), while lovastatin treatment inhibits the de novo synthesis of mevalonate by the cell (Endo, (1992) J. Lipid Res. 33, 1569-1582).

[$^3$H]Isoprenoid-labelled proteins were observed in solubilized COS cell extracts prepared from cells expressing either MEV alone or both MEV and 1HDAg (Fig.
4A, lanes 1 and 2); in the latter condition an additional labelled protein of 27 kDa, the predicted size of lHDAg, could be faintly observed. This 27 kDa protein was readily immunoprecipitated from solubilized COS cell extracts using an anti-lHDAg antibody, confirming its identity as the viral antigen (Fig. 4A, lane 4).

The isoprenoid modifying lHDAg was cleaved from the protein by methyl iodide cleavage, and the liberated lipid identified by HPLC. Isoprenoid analysis was performed both on the total pool of prenylated proteins present in solubilized extracts from COS cells, and on immunoprecipitated lHDAg. For the total pool of prenylated proteins, approximately 20% of the protein-associated isoprenoid was farnesyl and 80% was geranylgeranyl (Fig. 4B), a ratio consistent with previous studies (Rilling et al, 1993) Arch. Biochem. Biophys. 301, 210-215). Isoprenoid analysis of the immunoprecipitated lHDAg, however, revealed that the protein was exclusively modified by the farnesyl isoprenoid (Fig. 4C); the amount of geranylgeranyl lipid in immunoprecipitated lHDAg was essentially identical to that observed when COS cells not expressing lHDAg were subjected to the same procedure (compare Fig. 4C and D). These results are completely consistent with the in vitro data that indicated that lHDAg was a FTase substrate. It is believed that the geranylgeranylation of lHDAg observed by others in vitro (Lee et al, 1994) Virology 199, 169-175) was mediated by FTase.
Example 4

Inhibition of Farnesylation of lHDAg In Vitro

The effect of the farnesyltransferase (FTase) specific inhibitor SCH56582 (which is structurally closely related to SCH44342 described in Buss et al., Chemistry & Biology (1995), 2:787-791) on the farnesylation of a glutathione-S-transferase-lHDAg fusion protein (lHDAg) was examined in vitro. The standard assay included 75 ng FTase, 2 μM farnesyl diprophosphate, and either 1.5 μM lHDAg or 2.5 μM Ha-Ras, a well characterized FTase substrate. SCH56582 was included in these assays at concentrations ranging from 0-500 nM. Assays were initiated by the addition of FTase, and were conducted at 37°C for 15 min.

The results shown in Fig. 5A demonstrate that this inhibitor of farnesyltransferase inhibits farnesylation of lHDAg in vitro with an IC₅₀ of ~8 nm.

The effect of the FTase specific inhibitor B581 (see Garcia et al, (1993) J. Biol. Chem. 268:18415) on the farnesylation of the glutathione-S-transferase-lHDAg fusion protein (lHDAg) was also examined in vitro. The standard assay included 75ng FTase, 2μM farnesyl diprophosphate, and either 1 μM lHDAg or 2 μM Ha-Ras. B581 was included in the assays at concentrations ranging from 0-500nM. Assays were initiated by the addition of FTase, and were conducted at 37°C for 15 min. The
results shown in Fig. 5B demonstrate that B581 inhibits farnesylation of 1HDAg in vitro with an IC$_{50}$ of ~50nM (Ha-Ras-IC$_{50}$ of ~90nM).

* * *

All documents cited above are hereby incorporated in their entirety by reference.

One skilled in the art will appreciate from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention.
WHAT IS CLAIMED IS:

1. A method of inhibiting hepatitis delta virus (HDV) assembly in a cell comprising contacting an HDV-infected cell with an amount of a farnesyltransferase inhibitor sufficient to effect said inhibition.

2. The method according to claim 1 wherein said inhibitor inhibits farnesylation of the lHDAg antigen of HDV.

3. The method according to claim 1 wherein said inhibitor is B581.

4. The method according to claim 1 wherein said inhibitor is a pyridobenzocycloheptene derivative.

5. A method of treating a patient infected with HDV comprising administering to the patient an amount of a farnesyltransferase inhibitor sufficient to effect said treatment.

6. The method according to claim 5 wherein said inhibitor inhibits farnesylation of the lHDAg antigen of HDV.

7. The method according to claim 5 wherein said inhibitor is B581.
8. The method according to claim 5 wherein said inhibitor is a pyridobenzocycloheptene derivative.
FIG. 4A

1 2 3 4

27 kDa

Substitute Sheet (Rule 26)
FIG. 5A

FIG. 5B

SUBSTITUTE SHEET (RULE 26)
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPCL(6): Please See Extra Sheet.
US CL.: 514/108; 435/235.1, 238, 236, 963; 436/86
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S.: 514/108; 435/235.1, 238, 236, 963; 436/86

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS, DIALOG, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tr>
<td>Y</td>
<td>US H1345 H (BILLER ET AL) 02 August 1994, see column 1, paragraphs 1-3, and claim 1</td>
<td>1-8</td>
</tr>
<tr>
<td>X</td>
<td>WO 93/24660 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 09 December 1993, see abstract, page 2 paragraphs 1-2, and claims 1-12.</td>
<td>1, 2, 5, 6</td>
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[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
   "A" document defining the general state of the art which is not considered to be of particular relevance
   "E" earlier document published on or after the international filing date
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Date of the actual completion of the international search: 15 SEPTEMBER 1996
Date of mailing of the international search report: 04.10.1996

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
JAY WILLIAMS
Telephone No. (703) 308-0196

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<tr>
<td>Y</td>
<td>GARCIA et al. Peptidomimetic Inhibitors of Ras Farnesylation and Function in Whole Cells. The Journal of Biological Chemistry. 05 September 1993, Vol. 268, No. 25, pages 18415-18418; see abstract.</td>
<td>3, 7</td>
</tr>
<tr>
<td>Y</td>
<td>WO 95/10515 A1 (SCHERING CORPORATION) 20 April 1995, see entire document.</td>
<td>4, 8</td>
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<tr>
<td>Y</td>
<td>US 5,420,245 A (BROWN ET AL.) 30 May 1995, see column 15.</td>
<td>1, 2, 5, 6</td>
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A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):
A61K 31/66, 31/445; C07D 221/16, 401/04, 401/14, 401/12