Title: NOVEL GENIPIN DERIVATIVE HAVING LIVER PROTECTION ACTIVITY

Abstract

The present invention relates to novel genipin derivatives which have an excellent liver protection activity with little cytotoxicity, and these compounds are so stable in vivo that they do not induce any side effects.
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NOVEL GENIPIN DERIVATIVE HAVING LIVER PROTECTION ACTIVITY

TECHNICAL FIELD

The present invention relates to novel genipin derivatives represented by the following formulas (I)a, (I)b, (I)c and (I)d, which have a liver protection activity:

\[
\text{O} \quad \text{OR}_1
\]

\[
\text{R}_3 \quad \text{OR}_2
\]

(I)a

in which

- \(\text{R}_1\) represents lower alkyl;
- \(\text{R}_2\) represents lower alkyl, pyridylcarbonyl, benzyl or benzoyl;
- \(\text{R}_3\) represents formyl, hydroxymethyl, azidomethyl, 1-hydroxyethyl, acetyl, methyl, hydroxy, pyridylcarbonyl, cyclopropyl, aminomethyl substituted or unsubstituted by (1,3-benzodioxolan-5-yl)carbonyl or 3,4,5-trimethoxybenzoyl, 1,3-benzodioxolan-5-yl, ureidomethyl substituted or unsubstituted by 3,4,5-trimethoxyphenyl or 2-chloro-6-methyl-3-pyridyl, thiomethyl substituted or unsubstituted by acetyl or 2-acylamino-2-ethoxycarbonylethyl, oxymethyl substituted or unsubstituted by benzoyl, pyridylcarbonyl or 3,4,5-trimethoxybenzoyl;

provided that \(\text{R}_3\) is not formyl, hydroxymethyl, acetyl, methylaminomethyl, acetylthiomethyl, benzoyloxymethyl or pyridylcarbonyloxymethyl when \(\text{R}_1\) is methyl.
in which

$R_4$ represents lower alkoxy, benzyloxy, benzoyloxy, phenylthio, $C_1 \sim C_{12}$ alkanoyloxy substituted or unsubstituted by t-butyl, phenyl, phenoxy, pyridyl or thieryl;

$R_5$ represents methoxycarbonyl, formyl, hydroxyiminomethyl, methoxyiminomethyl, hydroxymethyl, phenylthiomethyl or acetylthiomethyl;

provided that $R_5$ is not methoxycarbonyl when $R_4$ is acetoxy.

in which

$R_6$ represents hydrogen atom, lower alkyl or alkali metal;

$R_7$ represents lower alkyl or benzyl;

$R_8$ represents hydrogen atom or lower alkyl;

$R_9$ represents hydroxy, lower alkoxy, benzylxoy, nicotinoxyloxy, isonicotinoxyloxy, 2-pyridylmethoxy or hydroxycarbonylmethoxy;

provided that $R_9$ is not hydroxy or methoxy when $R_6$ is methyl and $R_8$ is
hydrogen atom.

in which

$R_{10}$ represents lower alkyl;

$R_{11}$ represents lower alkyl or benzyl;

$R_{12}$ represents lower alkyl, pyridyl substituted or unsubstituted by halogen, pyridylamino substituted or unsubstituted by lower alkyl or halogen, 1,3-benzodioxolanyl;

$R_{13}$ and $R_{14}$ represent each independently hydrogen atom or isopropylidene together;

their pharmaceutically acceptable salts, or stereoisomers.

The present invention also relates to pharmaceutical compositions comprising as an active ingredient of the compound of formulas (I)\textsubscript{a}, (I)\textsubscript{b}, (I)\textsubscript{c} and (I)\textsubscript{d}, which can be effectively used for the liver protection activity.

**BACKGROUND ART**

It has been reported that the known iridoids genipin represented by the following formula (A) and aucubin represented by the following formula (B) are
natural substances, and act as a therapeutic agent for hepatitis B through the mechanism to inhibit the HBV replication (see, Korean Patent Laid-open No. 94-1886).

![Chemical structure of genipin (A) and aucubin (B)]

Said genipin of formula (A) and aucubin of formula (B) have some in vivo activities such as liver-protection, inhibition of biosynthesis of RNA and protein, detoxification as well as antiviral activity. Particularly, it has been disclosed that genipin is also effective as an anti-tumor agent (Japanese Patent Laid-open No. 80/164625). However, these compounds may be decomposed with amino acid residues of proteins such as albumin. Such a series of reactions may induce some color change of urine, faeces, and various internal organs into blue as well as immunotoxicities.

Compounds having a similar structure to the compound according to the present invention include the compound represented by the following formula (C) in addition to genipin and aucubin (see, WO 92/06061 and European Patent Laid-open No. EP-0505572):
in which
R₁ represents benzyl, hydroxy, acetoxy or ethoxyethoxy, and
R₂ represents benzoyloxy, methoxymethyl, t-butyldimethylsilyloxyethyl, carboxy or hydroxymethyl.

It is described in the above literatures that the compound of formula (C) above may be used effectively as a therapeutic agent for hyperlipemia or as a cholangogues.

On the other hand, the present inventors have synthesized a series of novel aucubin and genipin derivatives on the basis of the prior arts as mentioned above in order to develop compounds having a superior activity to the earlier compounds on inhibition against HBV. After the antiviral activity and little cytotoxicity of the novel compounds prepared were identified, the present inventors have filed a patent application on the novel compounds (see, Korean Patent Laid-open No. 97-21072).

**DISCLOSURE OF INVENTION**

The present inventors have continuously and intensively studied to develop novel compounds having more improved properties, and as a result, have succeeded to synthesize new compounds of formulas (I)a, (I)b, (I)c and (I)d
according to the present invention. By determining the antiviral activity and cytotoxicity of the compounds, we have identified that compounds according to the present invention are so stable in vivo that they do not induce any side effects such as change to blue color, etc. and that they may be effectively used for liver protection since they have an excellent liver protection activity with little cytotoxicity.

Therefore, it is an object of the present invention to provide novel genipin derivatives represented by the following formulas (I)a, (I)b, (I)c and (I)d which have an excellent liver protection activity as well as little cytotoxicity:

![Chemical Structure](image)

(1)a

in which

R₁ represents lower alkyl;
R₂ represents lower alkyl, pyridylcarbonyl, benzyl or benzoyl;
R₃ represents formyl, hydroxymethyl, azidomethyl, 1-hydroxyethyl, acetyl, methyl, hydroxy, pyridylcarbonyl, cyclopropyl, aminomethyl substituted or unsubstituted by (1,3-benzodioxolan-5-yl)carbonyl or 3,4,5-trimethoxybenzoyl, 1,3-benzodioxolan-5-yl, ureidomethyl substituted or unsubstituted by 3,4,5-trimethoxyphenyl or 2-chloro-6-methyl-3-pyridyl, thiomethyl substituted or unsubstituted by acetyl or 2-acetylamino-2-ethoxycarbonylethyl, oxymethyl substituted or unsubstituted by benzoyl, pyridylcarbonyl or 3,4,5-trimethoxybenzoyl;
provided that R₃ is not formyl, hydroxymethyl, acetyl, methylaminomethyl, acetylthiomethyl, benzoyloxymethyl or pyridylcarbonyloxymethyl when R₁ is methyl.
in which

\( R_4 \) represents lower alkoxy, benzyloxy, benzoylexy, phenylthio, \( \text{C}_1 - \text{C}_{12} \) alkanoyloxy substituted or unsubstituted by t-butyl, phenyl, phenoxy, pyridyl or thieryl;

\( R_5 \) represents methoxycarbonyl, formyl, hydroxyiminomethyl, methoxyiminomethyl, hydroxymethyl, phenylthiomethyl or acetylthiomethyl;

provided that \( R_5 \) is not methoxycarbonyl when \( R_4 \) is acetyloxy.

in which

\( R_6 \) represents hydrogen atom, lower alkyl or alkali metal;

\( R_7 \) represents lower alkyl or benzyl;

\( R_8 \) represents hydrogen atom or lower alkyl;

\( R_9 \) represents hydroxy, lower alkoxy, benzyloxy, nicotinoyloxy, isonicotinoyloxy, 2-pyridylmethoxy or hydroxycarbonylmethoxy;
provided that R₉ is not hydroxy or methoxy when R₆ is methyl and R₈ is hydrogen atom.

\[
\text{(I)d}
\]

in which
\[R_{10}\] represents lower alkyl;
\[R_{11}\] represents lower alkyl or benzyl;
\[R_{12}\] represents lower alkyl, pyridyl substituted or unsubstituted by halogen, pyridylamino substituted or unsubstituted by lower alkyl or halogen, 1,3-benzodioxolanyl;
\[R_{13}\] and \[R_{14}\] represent each independently hydrogen atom or isopropylidene together;

their pharmaceutically acceptable salts or stereoisomers.

It is another object of the present invention to provide pharmaceutical compositions for the liver protection comprising as active ingredients the compound of formulas (I)a, (I)b, (I)c and (I)d as defined above, together with pharmaceutically acceptable inert carriers.
BEST MODE FOR CARRYING OUT THE INVENTION

Among the compounds of formula (I)a having a potent liver protection activity, the preferred compounds include those wherein R₁ represents methyl, R₂ represents benzyl or methyl and R₃ represents 1-hydroxyethyl, aminomethyl, 3,4,5-trimethoxybenzoylaminomethyl, N-hydroxy-N-methylaminomethyl or 3,4,5-trimethoxyphenylureidomethyl.

Among the compounds of formula (I)b having a potent liver protection activity, the preferred compounds include those wherein R₄ represents acetylxy when R₅ is acetyltiethiomethyl, formyl, hydroxyiminoethyl or methoxyiminoethyl; R₄ represents acetylthio when R₅ is methoxycarbonyl, acetyltiethiomethyl, formyl or methoxyiminoethyl; R₄ represents t-butyacetyloxy when R₅ is methoxycarbonyl, acetyltiethiomethyl or formyl; R₄ represents isonicotinoyloxy when R₅ is methoxycarbonyl or acetyltiethiomethyl; R₄ represents benzyloxy, phenylthio, pyvaroyloxy, lauroyloxy, phenylacetyloxy, hydrosynamoyloxy, phenoxyacetyloxy, thiophenacetyloxy or benzoyloxy when R₅ is methoxycarbonyl.

Among the compounds of formula (I)c having a potent liver protection activity, the preferred compounds include those wherein R₆ represents hydrogen atom, methyl, isopropyl or sodium, R₇ represents methyl or benzyl, R₈ represents hydrogen atom or methyl, and R₉ represents hydroxy, methoxy, t-butoxy, benzyloxy, nicotinoyloxy, isonicotinoyloxy, 2-pyridylmethoxy or hydroxycarbonylmethoxy.

Among the compounds of formula (I)d having a potent liver protection activity, the preferred compounds include those wherein R₁₀ represents methyl, R₁₁ represents methyl or benzyl, R₁₂ represents 3-pyridyl, 2-chloro-6-methyl-3-pyridyl, 3-pyridylamino, 2-chloro-3-pyridylamino, 2-chloro-6-methyl-3-pyridylamino, 5,6-dichloro-3-pyridylamino or 1,3-benzodioxolan-5-yl and R₁₃ and R₁₄ represent each independently hydrogen atom or isopropyliden together.
The compounds of formulas (I)a, (I)b, (I)c and (I)d according to the present invention can form pharmaceutically acceptable salts. Such salts include a salt with pharmaceutically acceptable acids such as asparagic acid, gluconic acid, glutamic acid, hydrochloric acid, p-toluenesulfonic acid or citric acid, etc., and a salt with acids or bases which are generally known and conventionally used in the technical field of iridoid-based compounds. These pharmaceutically acceptable salts can be prepared according to a conventional conversion method.

**Synthetic Process for Preparing Compounds**

The compounds of formulas (I)a, (I)b, (I)c and (I)d of the present invention can be prepared according to the methods described below. However, it should be understood that the process for preparing compounds of formulas (I)a, (I)b, (I)c and (I)d are not limited to those explained below since the compound can be easily prepared by optionally combining the various methods disclosed in prior arts, and such a combination may be conventionally carried out by a person having ordinary skill in the art.

Following is reaction scheme of preparing the compound of formula (I)a.

![Reaction Scheme](image)

The definitions of $R_1$ to $R_3$ are same as described above.

Following is reaction scheme of preparing the compound of formula (I)b.
The definitions of R₄~R₅ are same as described above.

Following is reaction scheme of preparing the compound of formula (I)c.

The definitions of R₆~R₉ are same as described above.

Following is reaction scheme of preparing the compound of formula (I)d.
The definitions of $R_{10}$ to $R_{14}$ are same as described above.

**Efficacy and Toxicity of Compounds of Formulas (I)a, (I)b, (I)c and (I)d**

The efficacy of compounds of formulas (I)a, (I)b, (I)c and (I)d was measured according to the method of carbon tetrachloride model [referred to: Philippe letteron et al., *Biochemical Pharmacology*, 39, 12, 2027–2034, 1990] and

Carbon tetrachloride and D-galactosamine are known as the compounds inducing the severe damage to liver cells, because carbon tetrachloride suppresses the biosynthesis of protein in the liver and induces the necrosis of liver cells, and D-galactosamine also induces the necrosis of liver cells by changing the structure of liver cell membranes.

In the present invention, the compounds of formulas (I)a, (I)b, (I)c and (I)d were orally administered to the rats as experimental animals for 4 days, and then the liver protection effect was examined by measuring the serum ALT or AST values in the experimental animals (referred to: *Biol. Prram. Bull.*, 20, 4, 381–385, 1997; *Toxicology and Applied Pharmacology*, 95, 1–11, 1988).

Following is calculation formula to evaluate liver protection properties of compounds of formulas (I)a, (I)b, (I)c and (I)d:

\[
\left[ 1 - \frac{\text{ALT value of the group administered by the compounds} - \text{ALT value of normal group}}{\text{ALT value of control group} - \text{ALT value of normal group}} \right] \times 100
\]

in above formula
the control group means the group to which carbon tetrachloride or D-galactosamine is administered and the liver cells are impaired;
the normal group means the group to which normal solution is administered.

The liver protection effects of compounds of formulas (I)a, (I)b, (I)c and (I)d are shown to following Table 1 in comparision with known liver protection compound sylimarin.
Table 1.

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</table>

* The compound (I)a represents methyl (7R,3aS,7aS)-1-azidomethyl-7-benzyloxy-3,7,3a,7a-tetrahydro-6-oxaindene-4-carboxylate prepared in example 1.

The compound (I)b represents (2S,2aR,4aS,7aR,7bS)-2-methoxy-5-methoxy-carbonyl-2a,3,4,4a,7a,7b-hexa-hydro-2H-1,7-dioxacyclopent[c,d]indene prepared in example 3.

The compound (I)c represents methyl (1S,5R,6S)-5-benzyloxy-7-(t-buthoxy-iminomethyl)-4-oxa-bicyclo[4.3.0]nona-2,7-dien-2-carboxylate prepared in example 4.

The compound (I)d represents methyl (1S,8S,12S)-2-[(3-pyridyl)ureido]methyl-4,4-dimethyl-3,5,11-trioxo-12-benzyloxy-tricyclo[6.4.0.0<2,6>]dodec-9-en-9-carboxylate prepared in example 7.

On the other hand, the acute toxicity of compounds of formulas (I)a, (I)b, (I)c and (I)d is measured using mouse according to the standard of drug toxicity test. The mouse is selected from the 4 weeks old ICR mouse and each dosage of 250mg/kg, 500mg/kg, 1,000mg/kg and 2,000mg/kg compounds of formulas (I)a, (I)b, (I)c and (I)d are administered after suspending the compounds in the corn oil. Table 2 shows the acute toxicity of the compounds of formulas (I)a, (I)b, (I)c and (I)d.
<table>
<thead>
<tr>
<th>Administration compounds</th>
<th>Dosage(mg/kg)</th>
<th>Number of dead animals/Number of administered animals</th>
<th>Lethal ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Compound (I)a</strong></td>
<td>250</td>
<td>0/5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0/5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0/5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>0/5</td>
<td>0</td>
</tr>
<tr>
<td><strong>Compound (I)b</strong></td>
<td>250</td>
<td>0/5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0/5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0/5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>0/5</td>
<td>0</td>
</tr>
<tr>
<td><strong>Compound (I)c</strong></td>
<td>250</td>
<td>0/5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0/5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0/5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>0/5</td>
<td>0</td>
</tr>
<tr>
<td><strong>Compound (I)d</strong></td>
<td>250</td>
<td>0/5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0/5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0/5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>0/5</td>
<td>0</td>
</tr>
</tbody>
</table>

Therefore, the compounds of formulas (I)a, (I)b, (I)c and (I)d are proved as very safe materials. Further, the inventors have performed cytotoxicity test using the neutral red dye uptake method to determine. As a result, it was identified that the toxicity of compounds of formulas (I)a, (I)b, (I)c and (I)d is much less than that of dideoxy cytidine. Also, from the acute toxicity test using mouse as the test animal, it could be seen that the compound according to the present invention has a superior safety to the known compound genipin.

Consequently, compounds of formulas (I)a, (I)b, (I)c and (I)d according to the present invention are safe and have an excellent therapeutic effect for liver protection. Therefore, it is another object of the present invention to provide pharmaceutical compositions for the liver protection comprising as active ingredients of compounds of formulas (I)a, (I)b, (I)c and (I)d, as defined above, or their pharmaceutically acceptable salts.
When the pharmaceutical compositions according to the present invention are used for clinical purpose, they may be formulated into solid, semi-solid or liquid pharmaceutical preparations for oral or parenteral administration by combining compounds of formulas (I)a, (I)b, (I)c and (I)d with pharmaceutically acceptable inert carriers.

The pharmaceutically acceptable inert carriers which can be used for this purpose may be solid or liquid. It may be one or more selected from the group consisting of diluents, flavouring agents, solubilizing agents, lubricants, suspending agents, binders, swelling agents, etc. Specific example of the solid or liquid carrier which may be suitably used in the present invention includes lactose, starch, mannitol, cottonseed oil, etc.

When the active compounds of formulas (I)a, (I)b, (I)c and (I)d of the present invention are used as medicine for the prevention or protection of the liver, it is preferably administered in an amount of 0.1 to 100mg per kg of body weight per day at the first stage. However, the administration dosage can be varied with the requirement of the subject patient, severity of the infections to be treated, the selected compound and the like. The preferred dosage suitable for a certain condition can be determined by a person skilled in this art according to a conventional manner. In general, the therapeutic treatment is started from the amount less than the optimal dosage of the active compound and then the administration dosage is increased little by little until the optimal therapeutic effect is obtained. As a matter of convenience, the total daily dosage can be divided into several portions and administered over several times.

The present invention will be more specifically explained by the following examples. However, it should be understood that the examples are intended to illustrate but not in any manner to limit the scope of the present invention.
EXAMPLE 1

Synthesis of methyl (7R,3aS,7aS)-1-azidomethyl-7-benzyloxy-3,7,3a,7a-tetrahydro-6-oxaindene-4-carboxylate (Ia)

Methyl (7R,3aS,7aS)-1-hydroxymethyl-7-benzyloxy-3,7,3a,7a-tetrahydro-6-oxaindene-4-carboxylate (3.66g, 0.012mol) was dissolved with 50ml of methylenechloride, and cooled at 0°C under nitrogen atmosphere. Triethylamine (8.1ml, 0.058mol) was added drop by drop to the reaction mixture, and stirred for 30 min. Again, methansulfonylchloride (2.7ml, 0.035mol) was added and the reaction was finished after a lapse of 30 min. Saturated sodiumbicarbonate solution was added to finish the reaction, and the organic solvent layer was separated and washed by normal saline, then, dried, filtered and concentrated with anhydrous magnesium sulfate. The residue was dissolved with 10ml of DMF and sodiumazide (2.26g, 0.035mol) was added and stirred at 50°C for one night. After confirmation of the reaction by TLC, ethylacetate/hexane (1:2, v/v) and saturated saline were added to the reaction mixture. In the organic layer, the material was dried, filtered and concentrated, and the captioned compound was obtained by column chromatography (eluant: hexane/ethylacetate = 1/10, v/v Rf=0.25). White solid phase of captioned compound was obtained (3.16g; yield 80%).

^1H NMR (300MHz, CDCl3) :
\[ \delta 2.14 \text{ (m, 1H)}, 2.70 \text{ (t, 1H, J=7.2Hz)}, 2.94 \text{ (dd, 1H, J=8.5, 16.8Hz)}, 3.28 \text{ (dd, 1H, J=8.1, 16.5Hz)}, 3.76 \text{ (s, 3H)}, 3.89 \text{ (d, 1H, J=14.7Hz)}, 4.00 \text{ (d, 1H, J=14.7Hz)}, 4.66 \text{ (d, 1H, J=11.4Hz)}, 4.70 \text{ (d, 1H, J=8.0Hz)}, 4.99 \text{ (d, 1H, J=11.6Hz)}, 5.91 \text{ (s, 2H)}, 7.37 \]
EXAMPLE 2

Synthesis of methyl (7R,3aS,7aS)-1-aminomethyl-7-benzyloxy-3,7,3a,7a-tetrahydro-
6-oxaindene-4-carboxylate (Ia)

The compound obtained in example 1 (0.557g, 1.63mmol) was dissolved with 5ml of methanol and tinchloride 1 hydrate (0.773g, 4.08mmol) was added. After stirring for 2 hours, the completion of the reaction was confirmed and the solvent was evaporated. Ethylacetate and water were added to the residue, and cooled at 0°C, then sodium hydroxide added to separate the layers. After separating the organic layer, saturated saline was added to wash the layer several times and dried, filtered and concentrated with anhydrous sodium sulfate. The concentrated solution was purified by silica gel column chromatography (eluant: methanol/chloroform/triethylamine = 1/10/0.1, v/v/v, Rf=0.3). Yellow solid phase of captioned compound was obtained (0.411g; yield 80%)

$^1$H NMR (300MHz, CDCl$_3$):

$\delta$ 2.13 (m, 1H), 2.70 (t, 1H, $J$=7.2Hz), 2.90 (dd, 1H, $J$=8.5, 16.8Hz), 3.25 (dd, 1H, $J$=8.1, 16.5Hz), 3.76 (s, 3H), 3.89 (d, 1H, $J$=14.7Hz), 4.10 (d, 1H, $J$=14.7Hz), 4.26 (d, 1H, $J$=12.4Hz), 4.70 (d, 1H, $J$=8.0Hz), 4.59 (d, 1H, $J$=12.6Hz), 5.91 (s, 2H), 7.37 (m, 5H), 7.72 (s, 1H)
EXAMPLE 3

Synthesis of (2S,2aR,4aS,7aR,7bS)-2-methoxy-5-methoxycarbonyl-2a,3,4,4a,7a,7b-hexahydro-2H-1,7-dioxacyclopent[c,d]indene (I)b

10g (44.2mmol) of methyl(4aS,7aS)-1-hydroxy-7-hydroxymethyl-1,4a,5,7a-tetrahydrocyclopenta[c]pyrane-4-carboxylate was dissolved with 600ml of methylene chloride, and 19.06g (88.4mmol) of pyridiniumchlorocromate was added, and stirred for 2 hours. After filtering the reaction mixture, the filtered solution was concentrated and purified by using column chromatography (hexane/ethylacetate = 3/1, v/v), and 8.78g of methyl(4aS,7aS)-7-formyl-1-hydroxy-1,4a,5,7a-tetrahydrocyclopenta[c]pyrane-4-carboxylate was obtained (yield : 89%).

10g (44.6mmol) of obtained compound was dissolved with 300ml of ethanol, and 10% Pd/C (0.5g) was added in room temperature, and stirred for 1 hour in hydrogen atmosphere (1atm). The reaction mixture was filtered and concentrated in reduced pressure. Concentrated residue was purified by using column chromatography (hexane/ethylacetate = 4/1, v/v), and 6.5g of white solid phase of methyl(2S,2aR,4aS,7aR,7bS)-2-hydroxy-2a,3,4,4a,7a,7b-hexahydro-2H-1,7-dioxacyclopent[c,d]indene-carboxylate was obtained (yield : 60%).

2.3g (10.17mmol) of obtained compound was dissolved with 60ml of anhydro methanol and cooled at 0°C. 2.3ml of trifluoroborondiethyl ether (48%) was added and stirred for 2 hours in room temperature. The reaction mixture was cooled at 0°C and neutralized with saturated sodiumbicarbonate solution, and organic solvent was removed in reduced pressure. After extracting the water
layer with ethylacetate twice, the extracted solution was washed with saturated saline and dried and concentrated with anhydrosodiumsulfate. Residue was purified by using column chromatography (hexane/ethylacetate = 4/1, v/v), and 2.1g of oil phase of captioned compound was obtained (yield: 86%).

$^1$H NMR (CDCl$_3$);
$\delta$ 1.01 - 1.13 (m, 1H), 1.69 (m, 1H), 1.85 (m, 1H), 2.26 (m, 2H), 2.54 - 2.75 (m, 1H), 3.38 (s, 3H), 3.71 (s, 3H), 4.56 (d, 1H, J=12.8Hz), 5.73 (d, 1H, J=4.83Hz), 7.53 (s, 1H)

$^{13}$C NMR (CDCl$_3$);
25.99, 30.01, 33.85, 39.73, 51.59, 51.68, 55.60, 99.78, 109.58, 110.51, 150.16, 168.18

EXAMPLE 4
Synthesis of methyl (1S,5R,6S)-5-benzyloxy-7-(t-buthoxyiminomethyl)-4-oxa-bicyclo[4.3.0]nona-2,7-dien-2-carboxylate (I)

Methyl (1S,5R,6S)-5-benzyloxy-7-(t-buthoxyiminomethyl)-4-oxa-bicyclo[4.3.0]nona-2,7-dien-2-carboxylate (0.63g, 2.00mmol) was dissolved with 11ml of mixed solution of methanol and water (10/1, v/v), and 0.34g of t-buthoxylamine hydrochloride (2.71mmol) was added and stirred for 1 hour in room temperature, and reaction mixture was concentrated. Residue was dissolved with ethylacetate, and washed with saturated saline solution, and dried, filtered and concentrated with anhydrous
magnesium sulfate. Residue was purified by using silica gel column chromatography (hexane/ethylacetate = 10/1, v/v), and 0.64g of white solid phase of captioned compound was obtained (yield : 83%).

\(^1\)H NMR (300MHz, CDCl\(_3\)) ;
\[ \delta \]
1.19 (s, 9H), 2.37-2.45 (m, 1H), 2.82-2.92 (m, 1H), 3.38-3.40 (m, 2H), 3.75 (s, 3H), 4.63 (d, 1H, J=12.3Hz), 4.83 (d, 1H, J=12.3Hz), 5.70 (d, 1H, J=2.6Hz), 6.06 (s, 1H), 7.29-7.37 (m, 5H), 7.50 (s, 1H), 7.83 (s, 1H)

\(^13\)C NMR (CDCl\(_3\)) ;
\[ \delta \]
27.84, 33.09, 39.34, 47.54, 51.51, 70.53, 79.13, 97.04, 112.04, 128.19, 128.26, 128.83, 136.79, 137.65, 138.21, 145.09, 152.33, 168.11

MASS : 386 [M+1]^+

EXAMPLE 5

Synthesis of methyl (1S,5R,6S)-5-benzyloxy-7-benzyloxyiminomethyl-4-oxa-bicyclo
[4.3.0]nona-2,7-dien-2-carboxylate (I)c

![Chemical structure](image)

The process was carried out in the same manners of the example 4 except that benzyloxyamine hydrochloride was used instead of t-buthoxylamine hydrochloride (yield : 83%).

\(^1\)H NMR (300MHz, CDCl\(_3\)) ;
\[ \delta \] 2.36-2.43 (m, 1H), 2.86-2.94 (m, 1H), 3.34-3.39 (m, 2H), 3.76 (s, 3H), 4.60 (d, 1H, J=12.1Hz), 4.84 (d, 1H, J=12.1Hz), 5.03 (s, 2H), 5.53 (d, 1H, J=3.9Hz), 6.15 (s, 1H), 7.29-7.40 (m, 5H), 7.52 (s, 1H), 7.94 (s, 1H)

\[^{13}C\] NMR (CDCl\textsubscript{3})

\[ \delta \] 33.52, 39.61, 47.08, 51.51, 70.79, 76.61, 97.72, 111.77, 128.16, 128.23, 128.29, 128.38, 128.76, 136.26, 137.68, 137.92, 139.61, 146.61, 152.44, 168.06

MASS : 420 [M+1]\textsuperscript{+}, 442 [M+23]\textsuperscript{+}

EXAMPLE 6

Synthesis of methyl (1S,5R,6S)-5-benzyloxy-7-hydroxycarbonylmethoxyiminomethyl-4-oxa-bicyclo[4.3.0]nona-2,7-dien-2-carboxylate (I)c

\[ \begin{array}{c}
\text{HO}_2\text{C} \quad \text{N} \\
\text{O} \quad \text{O}
\end{array} \]

The process was carried out in the same manners of the example 4 except that hydroxycarbonylmethoxylamine hydrochloride was used instead of t-buthoxylamine hydrochloride (yield : 44%).

\[^{1}H\] NMR (300 MHz, CDCl\textsubscript{3})

\[ \delta \] 2.31-2.39 (m, 1H), 2.86-2.95 (m, 1H), 3.23-3.27 (m, 1H), 3.31-3.36 (m, 1H), 3.75 (s, 3H), 4.55 (s, 2H), 4.62 (d, 1H, J=12.1Hz), 4.86 (d, 1H, J=12.1Hz), 5.34 (d, 1H, J=4.8Hz), 6.23 (bs, 1H), 7.29-7.36 (m, 5H), 7.52 (s, 1H), 7.98 (s, 1H)

\[^{13}C\] NMR (75 MHz, CDCl\textsubscript{3})
δ 33.94, 39.75, 51.65, 70.56, 70.79, 97.65, 111.49, 128.31, 128.39, 128.77, 128.91, 135.72, 137.35, 141.57, 148.23, 152.63, 168.11, 174.75

MASS: 388 [M+1]^+, 410 [M+23]^+

EXAMPLE 7

Synthesis of methyl (1S,8S,12S)-2-[(3-pyridyl)ureido]methyl-4,4-dimethyl-3,5,11-trioxa-12-benzylxy-tricyclo[6.4.0.0<2,6>]-dodec-9-en-9-carboxylate (1)d

Nicotinic acid hydrochloride (296mg, 2.40mmol) was suspended with 2ml of methylene chloride and 2ml of oxalic chloride was added and refluxed with stirring for 3 hours, then concentrated in reduced pressure. Residue was suspended with 10ml of toluene and sodiumamide (468mg, 7.2mmol) was added and refluxed with stirring for 1 night to form 3-pyridylisocyanate. Methyl (1S,8S,12S)-2-aminomethyl-4,4-dimethyl-3,5,11-trioxa-12-benzylxy-tricyclo[6.4.0.0<2,6>]-dodec-9-en-9-carboxylate was added to above obtained solution, and 2ml of pyridine was added drop by drop and stirred for 2 hours at room temperature. Ethylacetate was added to reaction mixture and washed with saturated sodium bicarbonate and saturated saline solution. After drying and concentrating with anhydrous magnesium sulfate, residue was purified by using silica gel column chromatography (hexane/ethylacetate = 2/1, v/v), and 550mg of captioned compound was obtained (yield : 90%).
\[ ^1H \text{NMR (300 MHz, CDCl}_3) ; \]
\[ \delta \ 1.27 \text{ (s, 3H), 1.37 (s, 3H), 1.98 (m, 1H), 2.10 (m, 1H), 2.39 (m, 1H), 3.09 (m, 1H), 3.20 (d, 1H, J=13.4Hz), 3.67 (s, 3H), 4.05 (dd, 1H, J=9.8, 14.2Hz), 4.29 (d, 1H, J=7.1Hz), 4.56 (d, 1H, J=11.2Hz), 4.69 (d, 1H, J=11.2Hz), 5.42 (d, 1H, J=3.4Hz), 5.55 (d, 1H, J=9.3Hz), 7.21 (m, 6H), 7.34 (s, 1H), 7.72 (s, 1H), 8.06 (d, 1H, J=8.4Hz), 8.19 (brs, 1H), 8.35 (brs, 1H) \]

**EXAMPLE 8**

*Synthesis of methyl (1S,8S,12R)-2-[(5,6-dichloro-3-pyridyl)ureido]methyl-4,4-dimethyl-3,5,11-trioxo-12-benzyloxy-tricyclo[6.4.0.0<2,6>]dodec-9-en-9-carboxylate (I)*

![Chemical Structure](image)

The process was carried out in the same manners of the example 7 except that 5,6-dichloronicotinic acid hydrochloride (691mg, 3.6mmol) was used instead of nicotinic acid hydrochloride, and methyl (1S,8S,12R)-2-aminomethyl-4,4-dimethyl-3,5,11-trioxo-12-benzyloxy-tricyclo[6.4.0.0<2,6>]dodec-9-en-9-carboxylate (467mg, 1.2mmol) was used instead of methyl (1S,8S,12S)-2-aminomethyl-4,4-dimethyl-3,5,11-trioxo-12-benzyloxy-tricyclo[6.4.0.0<2,6>]dodec-9-en-9-carboxylate and. The captioned compound was obtained (yield : 40%).

\[ ^1H \text{NMR (300 MHz, CDCl}_3) ; \]
\[ \delta \ 1.36 \text{ (s, 1H), 1.46 (2s, 6H), 2.28 (m, 1H), 2.48 (dd, 1H, J=6.2, 14.2Hz), 3.05 \]
EXAMPLE 9

Synthesis of methyl (15S,8S,12R)-2-[[2-chloro-3-pyridyl]ureido]methyl-4,4-dimethyl-3,5,11-trioxo-12-methoxy-tricyclo[6.4.0.0<2,6>1]dodec-9-en-9-carboxylate (I)d

Genipin (5g, 22.1mmol) was dissolved with 250ml of methanol and catalytic amount of trifluoroboron diethylether was added, and stirred for 3 hours, then saturated sodium bicarbonate solution was added to finish the reaction. Under reduced pressure, methanol was removed and extracted with ethylacetate, and dried, filtered and concentrated with anhydous magnesium sulfate. Oily phase of methyl (3aS,7aS)-1-hydroxymethyl-7-methoxy-3,7,3a,7a-tetrahydro-6-oxaindene-4-carboxylate (5.26g, 7S : 7R = 1 : 3) was obtained.

The process was carried out in the same manners of the example 7 using obtained compound as starting material except that 2-chloronicotinic acid hydrochloride (567mg, 3.6mmol) was used instead of nicotinic acid hydrochloride (yield : 85%).

$^1$H NMR (300 MHz, CDCl₃)
\[ \delta \text{ 1.43 (s, 3H), 1.48 (m, 1H), 1.49 (s, 3H), 2.31 (m, 1H), 2.50 (dd, 1H, J=6.8, 14.5Hz), 3.33 (m, 2H), 3.58 (s, 3H), 3.75 (s, 3H), 3.90 (m, 1H), 4.43 (d, 1H, J=5.1Hz), 4.59 (d, 1H, J=8.7Hz), 5.71 (d, 1H, J=4.3Hz), 7.10 (bs, 1H), 7.22 (m, 1H), 7.51 (s, 1H), 8.01 (dd, 1H, J=1.6, 4.6Hz), 8.53 (dd, 1H, J=1.6, 8.2Hz) } \]

**EXAMPLE 10**

Inhibitory effect on HBV replication

Test for identifying the anti HBV effect of the compound of the present invention was carried out according to a known assay method (see, Korba and Milman, Antiviral Res., 15, 217, 1991). The assay procedure is briefly described in the following.

A. Cell culture

2.2.15. cell was cultured and preserved in RPM 11640 culture medium containing 5% fetal bovine serum (FBS), 2mM glutamine and 50\(\mu\)g/mL gentamicin sulfate. Resistance to G418 of the cell culture and degree of Mycoplasma contamination were examined according to conventional methods.

Cells (\(1 \times 10^4/\text{cm}^2\)) were inoculated into a multi-well tissue culture plate, confluenly cultured for 7 days, and then kept for 2 or 3 days in confluent condition to stabilize the HBV DNA level. Then, culture medium was replaced 24 hours before cells were exposed to test compound. During the treatment of 9 days, culture medium was replaced and then test compound was added to the fresh culture medium at intervals of 24 hours. Culture medium was collected immediately before the first introduction of test compound, and after 3, 6, 9 days, respectively, and stored at -70°C before HBV DNA analysis. Then, cytolysis was carried out to analyze the intracellular HBV DNA.
B. Extraction of DNA and RNA

To analyze the extracellular HBV DNA, 0.2 mL of culture medium was incubated in 1M NaOH/10×SSC (1×SSC=0.15M NaCl/0.015M sodium citrate, pH 7.2) for 20 minutes at 25°C and then immediately applied to a nitrocellulose membrane presoaked in 20×SSC using a slot blot apparatus. The sample was washed twice with 0.5 mL of 1M Tris/2M NaCl (pH 7.2) and once with 0.5 mL of 20×SSC to neutralize, and then it was washed again with 2×SSC and heated at 80°C for one hour under vacuum. Generally, the cells which have been cultured and preserved in a dish having a diameter of 10 cm are dissolved in 6 mL of lysis buffer, and the extracellular DNA is prepared according to the method of Korba et al., 1991.

C. Electrophoresis in gel

10 μg/lane of cellular DNA sample was digested with restriction enzyme Hind III. Then, the digested sample was applied to 1% agarose gel electrophoresis and transferred to a nitrocellulose membrane.

D. Hybridization analysis of HBV DNA

3.2 kb HBV DNA fragment obtained by EcoRI digestion and purification was labeled with [32P]dCTP using nick translation method. This labeled fragment was used as a hybridization probe. Condition for hybridization and post-washing were controlled by referring to the method of Korba et al., 1991 and HBV nucleic acid content among test sample was determined by Ambis beta scanner. The relative radioactivity of 32P hybridized to the test sample was compared with that of 32P hybridized to the standard amount of HBV DNA which was applied to each nitrocellulose membrane filter (gel or slot blot). From the calibration curve, the amount of HBV DNA corresponding to the relative cpm value was calculated.

Since the content of intracellular and extracellular HBV DNA has some
inherent variations, only inhibition greater than 3.5-fold in the case of HBV virion DNA or 3.0-fold in the case of HBV DNA replication intermediates form the average level of HBV DNA formed in the untreated cell were considered to be statistically significant (P<0.05) in the present experiment. The level of HBV DNA integrated during each cellular DNA preparation (which remains constant per cell in the present experiment) was used to calculate the level of intracellular HBV DNA formed, thereby the technical variations inherent in the blot hybridization analysis can be eliminated. Typical values for extracellular HBV virion DNA in the untreated cells ranged from 50 to 150pg/ml culture medium with an average value of about 75pg/ml. Intracellular HBV DNA replication intermediates (RI) in the untreated cells ranged form 50 to 100pg/µg cellular DNA with an average value of about 74pg/µg. On the basis of the results from the hybridization analysis carried out in the present invention, 1.0pg of intracellular HBV DNA/µg cellular DNA corresponded to 2 to 3 genome copies per cell, and 1.0pg of extracellular HBV DNA/ml culture medium corresponded to $3 \times 10^5$ virus particles.

According to the method as explained above, the inhibitory effect of the compound of the present invention of HBV replication was evaluated. Herein, untreated group was used as a control and ddc (dideoxy cytidine) known as a potent therapeutic agent for hepatitis as well as AIDS was used as a comparative compound. The anti viral activities of the novel genipin derivatives of formulas (I)a, (I)b, (I)c and (I)d, are described in the following Table 3.

EXAMPLE 11
Cytotoxicity test

Cytotoxicity test was carried out in order to determine whether the antiviral effect of the compound according to the present invention is due to the general influence on cell growth or not. In the present experiment, neutral red dye uptake method was used. This is a standard method widely utilized for
examining cell survival, by which the variety of relations between viruses such as HSV or HIV and host organism can be understood.

Cytotoxicity test was performed on a 96-well tissue culture plate. Cells were cultured and treated with test compounds in the same manner as Biological Example 1, and the experiments at 4-kind concentrations were repeated three times, respectively. Since the relative toxicity can be determined from the uptake level of neutral red dye, quantitative analysis was carried out using the absorbance of internalized dye at 510 nm (A_{510}). The test results on cytotoxicity are also described in the following Table 3.

Table 3.
Inhibitory effect of the compounds of formulas (I)a, (I)b, (I)c and (I)d on HBV replication in 2.2.15 cell culture, cytotoxicity, and SI (selectivity Index, IC_{50}/ED_{50})

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>ED_{50} (μ M)</th>
<th>IC_{50} (μ M)</th>
<th>SI</th>
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<tbody>
<tr>
<td>(I)a</td>
<td>80</td>
<td>120</td>
<td>4.5</td>
</tr>
<tr>
<td>(I)b</td>
<td>40</td>
<td>90</td>
<td>3.0</td>
</tr>
<tr>
<td>(I)c</td>
<td>30</td>
<td>180</td>
<td>5.7</td>
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<tr>
<td>(I)d</td>
<td>20</td>
<td>70</td>
<td>2.5</td>
</tr>
<tr>
<td>ddC</td>
<td>15</td>
<td>&gt;30</td>
<td>&gt;2.0</td>
</tr>
</tbody>
</table>

* The compound (I)a represents methyl (7R,3aS,7aS)-1-azidomethyl-7-benzyloxy-3,7,3a,7a-tetrahydro-6-oxaindene-4-carboxylate prepared in example 1. The compound (I)b represents (2S,2aR,4aS,7aR,7bS)-2-methoxy-5-methoxy-carbonyl-2a,3,4,4a,7a,7b-hexa-hydro-2H-1,7-dioxacyclopent[c,d]indene prepared in example 3. The compound (I)c represents methyl (1S,5R,6S)-5-benzyloxy-7-(t-butoxy-iminomethyl)-4-oxa-bicyclo [4.3.0]nona-2,7-dien-2-carboxylate prepared in example 4.
The compound (I)\textsubscript{d} represents methyl (1S,8S,12S)-2-[(3-pyridyl)ureido]methyl-4,4-dimethyl-3,5,11-trioxo-12-benzyloxy-tricyclo[6.4.0.0^{2,6}]dodec-9-en-9-carboxylate prepared in example 7.

As can be seen from the results of Table 3, the compounds of formulas (I)\textsubscript{a}, (I)\textsubscript{b}, (I)\textsubscript{c} and (I)\textsubscript{d} according to the present invention exhibit a potent inhibitory activity on HBV replication and its safety has been remarkably improved compared with the known compound ddC. Therefore, it is expected that the compound of the present invention can be preferably used in the treatment of hepatitis B.
WHAT IS CLAIMED IS:

1. A novel genipin derivatives represented by the following formula (I)a which has a liver protection activity:

```
O
/\       (I)a
\  /          OR3
/ \       OR2
O  R3
```

in which
R1 represents lower alkyl;
R2 represents lower alkyl, pyridylcarbonyl, benzyl or benzoxy;
R3 represents formyl, hydroxymethyl, azidomethyl, 1-hydroxyethyl, acetyl, methyl, hydroxy, pyridylcarbonyl, cyclopropyl, aminomethyl substituted or unsubstituted by (1,3-benzodioxolan-5-yl)carbonyl or 3,4,5-trimethoxybenzoyl, 1,3-benzodioxolan-5-yl, ureidomethyl substituted or unsubstituted by 3,4,5-trimethoxyphenyl or 2-chloro-6-methyl-3-pyridyl, thiomethyl substituted or unsubstituted by acetyl or 2-acetylamino-2-ethoxycarbonylthethyl, oxymethyl substituted or unsubstituted by benzoyl, pyridylcarbonyl or 3,4,5-trimethoxybenzoyl;

provided that R3 is not formyl, hydroxymethyl, acetyl, methylaminomethyl, acetylthiomethyl, benzoyloxyethyl, or pyridylcarbonyloxyethyl when R1 is methyl.

its pharmaceutically acceptable salts, or stereoisomers.

2. A novel genipin derivatives represented by the following formula (I)b which has a liver protection activity:
in which
R₄ represents lower alkoxy, benzyloxy, benzoyloxy, phenylthio, C₁₋C₁₂ alkanoyloxy substituted or unsubstituted by t-butyl, phenyl, phenoxy, pyridyl or thienyl ;
R₅ represents methoxycarbonyl, formyl, hydroxyiminomethyl, methoxyimino- methyl, hydroxymethyl, phenylthiomethyl or acetylthiomethyl ;
provided that R₅ is not methoxycarbonyl when R₄ is acetyloxy.

its pharmaceutically acceptable salts, or stereoisomers.

3. A novel genipin derivatives represented by the following formula (I)c which has a liver protection activity :

in which
$R_6$ represents hydrogen atom, lower alkyl or alkali metal;
$R_7$ represents lower alkyl or benzyl;
$R_8$ represents hydrogen atom or lower alkyl;
$R_9$ represents hydroxy, lower alkoxy, benzyloxy, nicotinoxyloxy, isonicotinoxyloxy, 2-pyridylmethoxy or hydroxycarbonylmethoxy;
provided that $R_9$ is not hydroxy or methoxy when $R_6$ is methyl and $R_8$ is hydrogen atom.

its pharmaceutically acceptable salts, or stereoisomers.

4. A novel genipin derivatives represented by the following formula (I) which has a liver protection activity:

![Chemical Structure](attachment:image)

in which
$R_{10}$ represents lower alkyl;
$R_{11}$ represents lower alkyl or benzyl;
$R_{12}$ represents lower alkyl, pyridyl substituted or unsubstituted by halogen, pyridylamino substituted or unsubstituted by lower alkyl or halogen, 1,3-benzodioxolanyl;
$R_{13}$ and $R_{14}$ represent each independently hydrogen atom or isopropylidene together;
its pharmaceutically acceptable salts, or stereoisomers.

5. The compound of claim 1, wherein $R_1$ represents methyl, $R_2$ represents benzyl or methyl and $R_3$ represents 1-hydroxyethyl, aminomethyl, 3,4,5-trimethoxybenzoylaminomethyl, N-hydroxy-N-methylaminomethyl or 3,4,5-trimethoxyphenylureidomethyl.

6. The compound of claim 2, wherein $R_4$ represents acetyloxy when $R_5$ is acetylthiomethyl, formyl, hydroxyiminomethyl or methoxyiminomethyl ; $R_4$ represents acetyltio when $R_5$ is methoxycarbonyl, acetyltiomethyl, formyl or methoxyiminomethyl ; $R_4$ represents t-butylnacetyloxy when $R_5$ is methoxycarbonyl, acetyltiomethyl or formyl ; $R_4$ represents isonicotinoxyloxy when $R_5$ is methoxycarbonyl or acetyltiomethyl ; $R_4$ represents benzylxioxy, phenylthio, pyvaroxyloxy, lauroxyloxy, phenylacetyloxy, hydrosoynamayoxyloxy, phenoxyacetoxy, thiophenacetyloxy or benzoyloxy when $R_5$ is methoxycarbonyl.

7. The compound of claim 3, wherein $R_6$ represents hydrogen atom, methyl, isopropyl or sodium, $R_7$ represents methyl or benzyl, $R_8$ represents hydrogen atom or methyl, and $R_9$ represents hydroxy, methoxy, t-butoxy, benzyloxy, nicotinoxyloxy, isonicotinoxyloxy, 2-pyridylmethoxy or hydroxycarbonylmethoxy.

8. The compound of claim 4, wherein $R_{10}$ represents methyl, $R_{11}$ represents methyl or benzyl, $R_{12}$ represents 3-pyridyl, 2-chloro-6-methyl-3-pyridyl, 3-pyridylamino, 2-chloro-3-pyridylamino, 2-chloro-6-methyl-3-pyridylamino, 5,6-dichloro-3-pyridylamino or 1,3-benzodioxolan-5-yl and $R_{13}$ and $R_{14}$ represent each independently hydrogen atom or isopropylidene together.

9. A pharmaceutical composition for liver protection comprising the compound disclosed in any one of preceding claims as an active ingredient together with a pharmaceutically acceptable inert carrier.
10. The pharmaceutical composition of claim 9, wherein the inert carrier is one or more selected from a group consisting of lactose, starch, mannitol and cottonseed oil.
**INTERNATIONAL SEARCH REPORT**

**In** national application No.  
PCT/KR 98/00273

### A. CLASSIFICATION OF SUBJECT MATTER

**IPC**: C 07 D 311/94; A 61 K 31/35  
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)

**IPC**: C 07 D 311/94

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

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

DARC

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<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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</table>
| A,P      | WO 98/17 663 A1 (CHOONGWAE PHARMA CORPORATION)  
30 April 1998 (30.04.98), totality. | 1-10                  |
| A        | DE 43 23 567 A1 (CHOONGWAE PHARMACEUTICAL CO., LTD.)  
20 January 1994 (20.01.94), claims 1-5. | 1-10                  |

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 application or patent published on or after the international filing date
- **L** document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- **O** document referring to an oral disclosure, use, exhibition or other means
- **P** document published prior to the international filing date but later than the priority date claimed

Later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

Document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

Date of the actual completion of the international search  
07 December 1998 (07.12.98)

Date of mailing of the international search report  
16 December 1998 (16.12.98)

Name and mailing address of the ISU

Austrian Patent Office  
Kohlmarkt 8-10; A-1014 Vienna  
Facsimile No. 1/53424/535

Authorized officer  
Brus  
Telephone No. 1/53424/519

Form PCT/ISA/210 (second sheet) (July 1998)
WO 98/17663
The present invention relates to a novel genipin derivative represented by formula (1), which has anti hepatitis B virus (HBV) activity, in which $R^1$ represents lower alkyl, benzyl, etc., $R^2$ represents hydroxymethyl, formyl, acetyl, etc., $R^3$ represents methoxycarbonyl, formyl, etc., its pharmaceutically acceptable salts, and stereoisomer.

DE 4323567
Pharmaceutical compositions for treating hepatitis B virus (HBV) infections in humans and animals contains (apart from conventional carriers, diluents and additives) an iridoid glycoside or corresponding aglycone of formula (1) or its salt. In (1) $R^1 = H$ or $OH$; $R^2 = H$ or $COOR^b, R^b = H$ or lower alkyl; $R^3 = H$ or beta-D-glucose; $R^4 = OH, CH_2OH$ or 3-phenyl-propanol; $R^5 = H, OH$, O-beta-D-glucose or O-beta-D-glucose-C$_2$-beta-D-glucose; C$_2$-C$_4$ = single bond, double bond (delta-7) or O(epsilon). (1) are administered orally or parenterally. Pref., (1) is selected from aucubin (1a), geniposide, catalpol, rehmannioside, genipin, harpagoside, harpagide and their aglycones and salts. (1) strongly inhibit HBV-DNA replication. They are natural products with low cytotoxicity and acute toxicity.
<table>
<thead>
<tr>
<th>Document de brevet cité dans le rapport de recherche</th>
<th>Datum der Veröffentlichung</th>
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