METHODS OF USING CASPASE INHIBITORS IN TREATMENT OF LIVER DISEASE

Provided herein are methods and compositions for treatment of an elevated MELD score or Child-Pugh score or their components by administering a of a caspase inhibitor alone or in combination with current treatments for liver disease.
Methods of Using Caspase Inhibitors in Treatment of Liver Disease

This application claims the benefit of the priority of U.S. Provisional Application No. 62/274,025, filed December 31, 2015, the disclosure of which is incorporated herein by reference in its entirety.

1. FIELD

Provided herein are methods of treating certain liver disease patients with elevated Model for End-Stage Liver Disease (MELD) scores and/or elevated Child-Pugh classifications by administering a caspase inhibitor.

2. BACKGROUND

Advanced chronic liver disease affects a large patient population and is associated with a high degree of morbidity and mortality. In 2009, chronic liver disease was the 4th leading cause of death in the United States among persons between the ages of 45 to 54 (see, Asrani et al. Hepatology, 2013;145:375-382). In view of the fact liver disease affects a large patient population, there is a strong need to provide new and effective pharmaceutical agents for these patients.

The Model for End-Stage Liver Disease, or MELD, is a scoring system for assessing the severity of chronic liver disease. MELD uses the subject's values for serum bilirubin, serum creatinine, and the international normalized ratio for prothrombin time (INR) to predict survival. It is calculated according to the following formula: MELD = 3.78[Ln serum bilirubin (mg/dL)] + 11.2[Ln INR] + 9.57[Ln serum creatinine (mg/dL)] + 6.43 (see, Kamath et al. Hepatology 2007; 45:797).

In interpreting the MELD Score in hospitalized subjects, the 3 month mortality is: (i) 40 or more — 71.3% mortality; (ii) 30-39 — 52.6% mortality; (iii) 20-29 — 19.6% mortality; (iv) 10-19 — 6.0% mortality; and (v) < 9 — 1.9% mortality.

As a comprehensive indicator of physiologic reserve of patients with decompensated cirrhosis, the validity of the MELD scale is shown in patients with advanced liver disease independent of complications of portal hypertension. (Kamath 2001). MELD is a numerical scale, ranging from 6 (less ill) to 40 (gravely ill), used for liver transplant candidates age 12 and older. (https://www.unos.org/wp-content/uploads/unos/MELD_PELD.pdf). It gives each person a 'score' (number) based on how urgently he or she needs a liver transplant within the next three months. The number is calculated by a formula using three routine lab test results: bilirubin,
which measures how effectively the liver excretes bile; INR (prothrombin time), which measures
the liver's ability to make blood clotting factors; and creatinine, which measures kidney function.
Impaired kidney function is often associated with severe liver disease.

[0007] The MELD scale is a reliable measure of mortality risk in patients with end-stage
liver disease and suitable for use as a disease severity index to determine organ allocation
priorities. A patient's score may go up or down over time depending on the status of his or her
liver disease. Most candidates will have their MELD score assessed a number of times while
they are on the waiting list. This will help ensure that donated livers go to the patients in greatest
need at that moment.

[0008] A modification of the MELD score, called sodium MELD, takes into account a
patient's serum sodium level. This is calculated from the patients MELD score. The candidate's
MELD score will be calculated as it is currently, and then the MELD-Na score will be derived
using the MELD score and the serum sodium value according to the following equation:

\[ \text{MELD-Na} = \text{MELD} + 1.32 \times (137-\text{Na}) - [0.033 \times \text{MELD}^* (137-\text{Na})] \]

(http://optn.transplant.hrsa.gov/PublicComment/pubcommentPropSub_317.pdf)

[0009] The Child-Pugh score consists of five clinical features including, ascites, hepatic
encephalopathy, albumin, total bilirubin and PT-INR and is used to assess the prognosis of
chronic liver disease and cirrhosis. Each component is given a numerical score from 1 to 3 and
added to provide total scores ranging from 5 to 15. The higher the score the worse prognosis is
for the patient. Patients with a totals score of 5-6 are classified as Child Pugh A; 7-9 Child Pugh
B and 10-15 are the most ill and classified as Child Pugh C.

[0010] The Child-Pugh score was originally developed in 1973 to predict surgical outcomes
in patients presenting with bleeding esophageal varices. Several studies have shown that Child-
Pugh score is an independent prognostic marker in the settings of ascites, ruptured esophageal
varices, alcoholic cirrhosis, hepatitis C virus- (HCV-) related cirrhosis, primary biliary cirrhosis
(PBC), primary sclerosing cholangitis (PSC), and Budd-Chiari syndrome. Child-Pugh score,
which can be easily calculated at the bedside, has been widely used for selecting candidates for
resection of HCC and nonhepatic surgery.

[0011] The components of the scoring system and the point allocations are listed in the table
below.

<table>
<thead>
<tr>
<th>Measure</th>
<th>1 point</th>
<th>2 points</th>
<th>3 points</th>
</tr>
</thead>
</table>

2
The predicted 1 year survival based on Child Pugh score is presented in the table below.

<table>
<thead>
<tr>
<th>Measure</th>
<th>1 point</th>
<th>2 points</th>
<th>3 points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bilirubin, (mg/dl)</td>
<td>&lt;2</td>
<td>&gt;2-3</td>
<td>&gt;3</td>
</tr>
<tr>
<td>Serum albumin, g/dl</td>
<td>&gt;3.5</td>
<td>&gt;2.8-3.5</td>
<td>&lt;2.8</td>
</tr>
<tr>
<td>Prothrombin time, (secs)</td>
<td>&lt;4.0</td>
<td>&gt;4.0-6.0</td>
<td>&gt;6.0</td>
</tr>
<tr>
<td>Ascites</td>
<td>None</td>
<td>Mild</td>
<td>Moderate to Severe</td>
</tr>
<tr>
<td>Hepatic encephalopathy</td>
<td>None</td>
<td>Grade I-II (or suppressed with medication)</td>
<td>Grade III-IV (or refractory)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Measure</th>
<th>Class A</th>
<th>Class B</th>
<th>Class C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total points</td>
<td>5-6</td>
<td>7-9</td>
<td>10-15</td>
</tr>
<tr>
<td>1-year survival</td>
<td>100%</td>
<td>80%</td>
<td>45%</td>
</tr>
</tbody>
</table>

### 3. SUMMARY

The methods, compounds, pharmaceutical compositions and articles of manufacture provided herein are characterized by a variety of component ingredients, steps of preparation, and steps of execution and associated biophysical, physical, biochemical or chemical parameters. As would be apparent to those of skill in the art, the methods provided herein can include any and all permutations and combinations of the compounds, compositions, articles of manufacture and associated ingredients, steps and/or parameters as described below.

In one aspect, provided herein are methods for treating liver disease patients by administering a caspase inhibitor, wherein the patient has an elevated MELD score. In one aspect, provided herein are methods for treating liver disease patients by administering a caspase inhibitor, wherein the patient has an elevated Child-Pugh score. In certain embodiments, provided herein are methods for sustaining or reducing Child-Pugh scores of liver disease patients. In some embodiments, the liver disease is cirrhosis. Caspase inhibitors as known to and understood by one of skill in the art are contemplated herein. Exemplary compounds for use in
the methods are described elsewhere herein. Also provided are pharmaceutical compositions for use in the methods.

[0015] In certain embodiments, the methods provided herein include treatment of patients with elevated MELD scores resulting from liver disease. In some embodiments, provided herein are methods for selecting patients with elevated MELD scores above 11 and lowering said MELD scores or the components of the MELD score. In certain embodiments, provided herein are methods for selecting a patient with a MELD score above 11 and sustaining said MELD score or the components of the MELD score. In some embodiments, provided herein are methods of rapidly lowering a MELD score or the components of the MELD score of a patient and continuing treatment while monitoring said MELD score or the components of the MELD score. In certain embodiments, provided here are methods for selecting a patient on or eligible for a liver transplant list and treating said patient with a caspase inhibitor until said patient's MELD score has been lowered below the threshold MELD score for liver transplant eligibility. In some embodiments, provided herein are methods for selecting a patient below the MELD score threshold for a liver transplant list and treating said patient with a caspase inhibitor to prevent said patient's MELD score from increasing to the liver transplant list threshold.

[0016] In certain embodiments, the methods provided herein include treatment of patients with elevated Child-Pugh scores resulting from liver disease. In some embodiments, provided herein are methods for selecting patients with elevated Child-Pugh scores above Class A and lowering said Child-Pugh scores or the components of the Child-Pugh score. In certain embodiments, provided herein are methods for selecting a patient with a Child-Pugh score above Class A and sustaining said Child-Pugh score or the components of the Child-Pugh score. In some embodiments, provided herein are methods of rapidly lowering a Child-Pugh score or the components of the Child-Pugh score of a patient and continuing treatment while monitoring said Child-Pugh score or the components of the Child-Pugh score.

[0017] In certain embodiments, liver disease is caused by toxins, including alcohol, some drugs, and the abnormal build-up of normal substances in the blood. In another embodiment, liver disease is caused by infection or by an autoimmune disorder. In certain embodiments, the exact cause of the liver disease is not known. In certain embodiments, the liver disease include, but is not limited to viral infection, fatty liver, cirrhosis, primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), Budd-Chiari syndrome and alphal-antitrypsin deficiency.
In some embodiments, the liver disease includes, but is not limited to cirrhosis, liver fibrosis, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), hepatitis, including viral and alcoholic hepatitis, PBC and PSC.

In one embodiment, the methods provided herein lower elevated levels of liver enzymes, such as elevated levels of ALT (alanine aminotransferase) and AST (aspartate aminotransferase) levels. In one embodiment, the methods provided herein improve liver function associated with liver diseases. In certain embodiments, provided herein are methods of lowering elevated levels of bilirubin, INR and creatinine.

Also provided are caspase inhibitors for use in the methods. In one embodiment, the caspase inhibitor compound for use in the methods provided herein is selected from:
or a pharmaceutically acceptable derivative thereof. In one embodiment, the pharmaceutically acceptable derivative is a pharmaceutically acceptable salt.

[0021] In one embodiment, the caspase inhibitor for use in the methods provided herein is
or a pharmaceutically acceptable derivative thereof. In one embodiment, the pharmaceutically acceptable derivative is a pharmaceutically acceptable salt.

[0022] In some embodiments, more than one caspase inhibitor can be used sequentially or simultaneously in the methods provided herein.

[0023] Also provided are pharmaceutical compositions containing therapeutically effective amounts of the compounds provided herein and a pharmaceutically acceptable carrier, wherein the pharmaceutical compositions are useful in the prevention, treatment, or amelioration of one or more of the symptoms of liver diseases.

[0024] In some embodiments, the liver disease is a liver disease selected from among cirrhosis, liver fibrosis, NAFLD, NASH, hepatitis, including viral and alcoholic hepatitis, PBC and PSC. In some embodiments, the liver disease is cirrhosis.

[0025] Further provided is an article of manufacture containing packaging material, the compounds or pharmaceutically acceptable derivatives thereof provided herein, which is used for treatment, prevention or amelioration of one or more symptoms associated with a liver disease, and a label that indicates that compounds or pharmaceutically acceptable derivatives thereof are used for treatment, prevention or amelioration of one or more symptoms of a liver disease. In some embodiments, the liver disease is a liver disease selected from among cirrhosis, liver fibrosis, NAFLD, NASH, hepatitis, including viral and alcoholic hepatitis, PBC and PSC. In some embodiments, the liver disease is cirrhosis.

4. DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

4.1 Definitions

[0026] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art. All patents, applications, published applications and other publications are incorporated by reference in their entirety. In the event that there are a plurality of definitions for a term herein, those in this section prevail unless stated otherwise.
The singular forms "a," "an," and "the" include plural references, unless the context clearly dictates otherwise.

As used herein "subject" is an animal, such as a mammal, including human, such as a patient.

As used herein, biological activity refers to the in vivo activities of a compound or physiological responses that result upon in vivo administration of a compound, composition or other mixture. Biological activity, thus, encompasses therapeutic effects and pharmacokinetic behavior of such compounds, compositions and mixtures. Biological activities can be observed in in vitro systems designed to test for such activities.

As used herein, pharmaceutically acceptable derivatives of a compound include salts, esters, acetics, ketals, orthoesters, hemiacetals, hemiketals, acids, bases, solvates, hydrates or prodrugs thereof. Such derivatives may be readily prepared by those of skill in this art using known methods for such derivatization. The compounds produced may be administered to animals or humans without substantial toxic effects and either are pharmaceutically active or are prodrugs. Pharmaceutically acceptable salts include, but are not limited to, amine salts, such as but not limited to N,N'-dibenzylethlenediamine, chloroprocaine, choline, ammonia, diethanolamine and other hydroxyalkylamines, ethylenediamine, N-methylglucamine, procaine, N-benzylphenethylamine, 1-para-chlorobenzyl-2-pyrrolidin-1'-ylmethylbenzimidazole, diethylamine and other alkylamines, piperazine and tris(hydroxymethyl)aminomethane; alkali metal salts, such as but not limited to lithium, potassium and sodium; alkali earth metal salts, such as but not limited to barium, calcium and magnesium; transition metal salts, such as but not limited to zinc; and inorganic salts, such as but not limited to, sodium hydrogen phosphate and disodium phosphate; and also including, but not limited to, salts of mineral acids, such as but not limited to hydrochlorides and sulfates; and salts of organic acids, such as but not limited to acetates, lactates, malates, tartrates, citrates, ascorbates, succinates, butyrates, valerates, mesylates, and fumarates. Pharmaceutically acceptable esters include, but are not limited to, alkyl, alkenyl, alkynyl, aryl, alarkyl, and cycloalkyl esters of acidic groups, including, but not limited to, carboxylic acids, phosphoric acids, phosphinic acids, sulfonic acids, sulfinic acids and boronic acids. Pharmaceutically acceptable solvates and hydrates are complexes of a compound with one or more solvent or water molecules, or 1 to about 100, or 1 to about 10, or one to about 2, 3 or 4, solvent or water molecules.
As used herein, treatment means any manner in which one or more of the symptoms of a disease or disorder are ameliorated or otherwise beneficially altered. Treatment also encompasses any pharmaceutical use of the compositions herein, such as use for treating a liver disease.

As used herein, amelioration of the symptoms of a particular disorder by administration of a particular compound or pharmaceutical composition refers to any lessening, whether permanent or temporary, lasting or transient that can be attributed to or associated with administration of the composition.

As used herein, and unless otherwise indicated, the terms "manage," "managing" and "management" encompass preventing the recurrence of the specified disease or disorder in a patient who has already suffered from the disease or disorder, and/or lengthening the time that a patient who has suffered from the disease or disorder remains in remission. The terms encompass modulating the threshold, development and/or duration of the disease or disorder, or changing the way that a patient responds to the disease or disorder.

It is to be understood that the compounds provided herein may contain chiral centers. Such chiral centers may be of either the (R) or (S) configuration, or may be a mixture thereof. Thus, the compounds provided herein may be enantiomerically pure, or be stereoisomeric or diastereomeric mixtures. As such, one of skill in the art will recognize that administration of a compound in its (R) form is equivalent, for compounds that undergo epimerization in vivo, to administration of the compound in its (S) form.

As used herein, substantially pure means sufficiently homogeneous to appear free of readily detectable impurities as determined by standard methods of analysis, such as thin layer chromatography (TLC), gel electrophoresis, high performance liquid chromatography (HPLC) and mass spectrometry (MS), used by those of skill in the art to assess such purity, or sufficiently pure such that further purification would not detectably alter the physical and chemical properties, such as enzymatic and biological activities, of the substance. Methods for purification of the compounds to produce substantially chemically pure compounds are known to those of skill in the art. A substantially chemically pure compound may, however, be a mixture of stereoisomers. In such instances, further purification might increase the specific activity of the compound. The instant disclosure is meant to include all such possible isomers, as well as, their racemic and optically pure forms. Optically active (+) and (-), (R)- and (S)-, or (D)- and
(L)-isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques, such as reverse phase HPLC. When the compounds described herein contain olefinic double bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both E and Z geometric isomers. Likewise, all tautomeric forms are also intended to be included.

[0036] In certain embodiments, the compound used in the methods provided herein is "stereochemically pure." A stereochemically pure compound or has a level of stereochemical purity that would be recognized as "pure" by those of skill in the art. In certain embodiments, "stereochemically pure" designates a compound that is substantially free of alternate isomers. In particular embodiments, the compound is 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% free of other isomers.

[0037] As used herein, "therapy for liver disease" refers to a treatment with any medication known, available in the market and being developed for the treatment of liver disease. For example, therapy of liver disease refers to treatment of the patient with drugs available in the market for the treatment of liver disease. Several exemplary drugs are described in the section on "Combination Therapy" infra.

[0038] As used herein, "therapy for liver disease" refers to a treatment with any medication known, available in the market and being developed for the treatment of liver disease. For example, therapy for liver disease refers to treatment of the patient with drugs available in the market for treatment. Several exemplary drugs are described in the section on "Combination Therapy" infra.

[0039] As used herein, "mitigate," means the reduction or elimination of symptoms. Mitigate also means the reduction of severity or the delayed progression of disease or being otherwise beneficially altered.

[0040] As used herein, "patients who have failed therapy" refers to the patient population described elsewhere herein and includes patients that have previously been treated for a liver disease with any of the drugs currently available in the market and either did not respond to the therapy (used synonymously herein with "failed therapy"), could not tolerate the therapy or for whom the therapy was medically contraindicated.
As used herein "elevated MELD score" or "elevated components of MELD score" refer to MELD score of 10 or above. Methods of determining MELD score and components of MELD score are known in the art and exemplary methods are described elsewhere herein. As used herein "elevated Child-Pugh score" or "elevated components of Child-Pugh score" refer to Child-Pugh score of 6 or above. Methods of determining Child-Pugh score and components of Child-Pugh score are known in the art and exemplary methods are described elsewhere herein.

Therapy for liver disease or elevated MELD scores or elevated Child-Pugh score is not tolerated by some patients due to the development of undesirable side effects as understood by those with skill in the art. Inability to tolerate therapy can include, but is not limited to, weakness, shortness of breath and fatigue.

As used herein, the term "in combination" refers to the use of more than one therapies (e.g., a caspase inhibitor and other agents). The use of the term "in combination" does not restrict the order in which therapies (e.g., a caspase inhibitor and other agents) are administered to a subject with a disorder. A first therapy (e.g., a caspase inhibitor and other agents) can be administered prior to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of other therapy (e.g., a caspase inhibitor and other agents) to a subject with a disorder.

As used herein, the term "synergistic" refers to a combination of a caspase inhibitor with another agent, which is more effective than the additive effects of the administration of the two compounds as monotherapies. A synergistic effect of a combination of therapies (e.g., a caspase inhibitor and another agent) permits the use of lower dosages of one or more of the therapies and/or less frequent administration of the therapies to a subject with a disorder. The ability to utilize lower dosages of a therapy (e.g., a caspase inhibitor and another agent) and/or to administer the therapy less frequently reduces the toxicity associated with the administration of the therapy to a subject without reducing the efficacy of the therapy in the prevention or treatment of a disorder. In addition, a synergistic effect can result in improved efficacy of agents...
in the prevention or treatment of a disorder. Finally, a synergistic effect of a combination of therapies (e.g., a caspase inhibitor and another agent) may avoid or reduce adverse or unwanted side effects associated with the use of either therapy alone.

4.2 Compounds for use in the methods

Several caspase inhibitors that can be used in the methods provided herein have been reported in the literature. Certain exemplary caspase inhibitors for use in the methods are described by Linton in *Current Topics in Medicinal Chemistry*, (2005) 5: 1-20; and Linton et al. in *J. Med. Chem.*, 2005, 11, 295-322 295, U.S. patent no. 7,351,702; 7,410,956; 7,443,790; 7,553,852; 7,652,153; 7,612,091; 7,807,659; 7,857,712; 7,960,415; 8,071,618; 7,074,782; 7,053,057; 6,689,784; 6,632,962; 6,559,304; 6,201,118; 6,800,619; 6,197,750; 6,544,951; 6,790,989; 7,053,056; 7,183,260; 7,692,038, and International application nos. WO 2006/017295; WO 2005/021516; WO 04/002961; WO 02/085899; WO 02/094263 and WO 01/094351. The contents of these references are hereby incorporated by reference in their entireties.

In one embodiment, the caspase inhibitor for use in the methods provided herein is selected from
or a pharmaceutically acceptable derivative thereof. In one embodiment, the pharmaceutically acceptable derivative is a pharmaceutically acceptable salt.

[0048] In one embodiment, the caspase inhibitor for use in the methods provided herein is
or a pharmaceutically acceptable derivative thereof. In one embodiment, the pharmaceutically acceptable derivative is a pharmaceutically acceptable salt.

[0049] In some embodiments, more than one caspase inhibitor can be used sequentially or simultaneously in the methods provided herein.

[0050] In certain embodiments, the compounds described herein have efficacy in models of liver disease following oral administration of from 0.001 - 1000 mg/Kg. In certain embodiments, the compounds described herein have efficacy in models of liver disease following oral administration of from 0.01 - 100 mg/Kg.

4.3 Methods of treatment

[0051] In certain embodiments, the methods provided herein include treatment of liver disease. In some embodiments, the methods are for treatment of liver disease in patients with MELD scores above 11 or elevated components of MELD. In certain embodiments, the methods are for reducing MELD components associated with liver disease. In some embodiments, the methods are for the reduction of cirrhosis while reducing a patient's MELD score. In some embodiments, the methods are for treatment of liver disease in patients with Child-Pugh scores above Class A or elevated components of Child-Pugh. In certain embodiments, the methods are for reducing Child-Pugh components associated with liver disease. In some embodiments, the methods are for the reduction of cirrhosis while reducing a patients Child-Pugh score.

[0052] In one embodiment, the liver disease is a disorder that results from an injury to the liver. In one embodiment, injury to the liver is caused by toxins, including alcohol, some drugs, and the abnormal build-up of normal substances in the blood. In another embodiment, the liver injury is caused by an infection or by an autoimmune disorder. In certain embodiments, the exact cause of the injury is not known.

[0053] In one embodiment, the liver disease includes, but is not limited to cirrhosis, liver fibrosis, NAFLD, NASH, hepatitis, including viral and alcoholic hepatitis, PBC and PSC. In one embodiment, the liver disease is manifested by conditions known to those of skill in the art.
including, but not limited to, portal hypertension, raised liver enzymes (e.g., ALT and AST), alkaline phosphatase (ALP), elevated bilirubin, INR, creatinine, pathological evidence of cirrhosis, steatosis (fatty liver) or fibrosis.

[0054] In one embodiment, liver disease is manifested by conditions known to those of skill in the art including, but not limited to, raised liver enzymes (e.g., ALT, AST), raised bilirubin, INR or creatinine, histological evidence of liver damage and cirrhosis.

[0055] In certain embodiments, the methods provided herein are for treating elevated MELD scores or elevated Child-Pugh scores in liver disease patients. In some embodiments, a patient’s MELD score or Child-Pugh score or one or more of their components are reduced by at least 95%, at least 90%, at least 80%, at least 70%, at least 60%, at least 50%, at least 40%, at least 30%, at least 20%, at least 10%, at least 5%, at least 2% or at least 1%. In some embodiments, a patient’s MELD score or Child-Pugh score or one or more of their components are reduced by about 1-95%, about 1-75%, about 1-50%, about 1-25%, about 1-15%, about 1-10%, about 1-5%, about 2-25% or about 5-15%. In certain embodiments, the methods provided herein are for treating elevated MELD scores and/or elevated Child-Pugh scores in liver disease patients. In some embodiments, a patient’s MELD score and/or Child-Pugh score or one or more of their components are reduced by at least 95%, at least 90%, at least 80%, at least 70%, at least 60%, at least 50%, at least 40%, at least 30%, at least 20%, at least 10%, at least 5%, at least 2% or at least 1%. In some embodiments, a patient’s MELD score or Child-Pugh score or one or more of their components are reduced by about 1-95%, about 1-75%, about 1-50%, about 1-25%, about 1-15%, about 1-10%, about 1-5%, about 2-25%, about 5-25%, or about 5-15%.

[0056] In some embodiments, a patient’s MELD score or one or more of its components are reduced by about 1-95%, about 1-75%, about 1-50%, about 1-25%, about 1-15%, about 1-10%, about 1-5%, about 2-25%, about 5-25%, or about 5-15%. In some embodiments, MELD score components are bilirubin, INR and/or creatinine.

[0057] In some embodiments, a patient’s Child-Pugh score or one or more of its components are reduced by about 1-95%, about 1-75%, about 1-50%, about 1-25%, about 1-15%, about 1-10%, about 1-5%, about 2-25%, about 5-25%, or about 5-15%.

[0058] In certain embodiments, provided are methods for treatment of elevated MELD scores or its components and/or elevated Child-Pugh scores or its components for patients who have failed therapy.
In certain embodiments, the patient is a patient that discontinued therapy for an elevated MELD score and/or its components or elevated Child-Pugh scores or its components because of one or more adverse events associated with the therapy. In certain embodiments, the patient is a patient where current therapy is not indicated. For instance, certain patients have an absolute or relative contraindication for therapy. Contraindications include but are not limited to certain cardiovascular disease conditions and various respiratory diseases.

In certain embodiments, provided are methods for treatment of elevated MELD scores or its components and/or elevated Child-Pugh scores or its components with a combination of current commercially available treatments for and a caspase inhibitor.

In one embodiment, the methods provided herein can lower the elevated level of liver enzyme, such as ALT and AST levels or the lowering of elevated MELD components (bilirubin, INR or creatinine) or Child-Pugh components. Methods for measuring the level of elevated liver enzymes are well known in the art (see, e.g., Jeong S. Y. et al. Sandwich ELISA for measurement of cytosolic aspartate aminotransferase in sera from patients with liver diseases, Clin Chem., 2003; 49(5):826 9 and Burin des Roziers N. et al. A microtiter plate assay for measurement of serum alanine aminotransferase in blood donors, Transfusion., 1995; 35(4):331 4, each of which is incorporated by reference herein in its entirety). In one embodiment, the elevated level of one or more liver enzyme, such as ALT or AST, or the total amount of elevated liver enzyme is reduced by more than about 90% or more than 95%. In one embodiment, the elevated level of one or more liver enzyme, such as elevated levels of ALT or AST, or the total amount of elevated liver enzyme is reduced by at least 95%, at least 90%, at least 80%, at least 70%, at least 60%, at least 50%, at least 40%, at least 30%, at least 20%, at least 10%, at least 5%, at least 2% or at least 1%.

In certain embodiments, provided herein is a method for treating cirrhosis in patients with elevated MELD scores or its components. In some embodiments, provided herein is a method for treating cirrhosis in patients with elevated Child-Pugh scores or its components. In some embodiments, the method for treating cirrhosis further reduces the symptoms associated with cirrhosis. In certain embodiments, symptoms of cirrhosis can include, but are not limited to, portal hypertension, abnormal nerve function, ascites (build-up of fluid in the abdominal cavity), breast enlargement in men, coughing up or vomiting blood, curling of fingers (Dupuytren contracture of the palms), gallstones, hair loss, itching, jaundice, kidney failure, liver
encephalopathy, muscle loss, poor appetite, redness of palms, salivary gland enlargement in cheeks, shrinking of testes, small spider-like veins in skin, weakness, weight loss, spider angiomas (a central arteriole from which numerous small branching vessels radiate), encephalopathy, and asterixis (flapping tremor). Symptoms of cirrhosis can vary. Cirrhosis is defined as compensated or decompensated and further classified using the Child-Pugh system which is well known to individuals skilled in the art. Cirrhosis patients are classified on the basis of certain clinical parameters. Child Pugh A are compensated and may display minimal obvious symptoms. Patients classified as Child Pugh B or Child Pugh C are decompensated and can exhibit outward symptoms such as ascites.

In other embodiments, causes of cirrhosis include hepatitis induced by any cause, excessive fat deposition, viruses (e.g., HCV and HBV), use of certain drugs, chemical exposure, bile duct obstruction, autoimmune diseases, obstruction of outflow of blood from the liver (i.e., Budd-Chiari syndrome), heart and blood vessel disturbances, alphal-antitrypsin deficiency, high blood galactose levels, high blood tyrosine levels, glycogen storage disease, diabetes, malnutrition, hereditary accumulation of too much copper (Wilson Disease) or iron (hemochromatosis). In one embodiment, the cause of cirrhosis is alcohol abuse.

In one embodiment, provided herein is a method for treating cirrhosis. In one embodiment, cirrhosis is characterized pathologically by loss of the normal microscopic lobular architecture, and nodular regeneration. Methods for measuring the extent of cirrhosis are well known in the art. For example, measurement of the existence of cirrhosis is determined by a clinical pathologist through the histological examination of liver biopsy samples taken from the liver of the cirrhotic patient.

In certain embodiments, provided are methods for treatment of cirrhosis with elevated MELD scores or its components or elevated Child-Pugh scores or its components with a combination of current commercially available or experimental treatments for cirrhosis and a caspase inhibitor. Exemplary compounds and current experimental therapies for treatment of cirrhosis include Furosemide, Spironolactone. Lactulose, Rifaximin, Simtuzumab (GS-6624) by Gilead, Sorafenib by Bayer and Onyx, Serelaxin (RLX030) by Norvartis, Timolol, NCX-1000, Terlipressin, NGM282 by NGM Biopharmaceuticals, LUMOOl, by Lumena Pharmaceuticals and analogs or derivatives thereof as understood by those of skill in the art.
In certain embodiments, provided are methods for treatment of cirrhosis with a combination of current commercially available or experimental treatments for cirrhosis and a caspase inhibitor. Exemplary compounds and current experimental therapies for treatment of cirrhosis include the monoclonal antibodies such as the humanized monoclonal antibody Simtuzumab (GS-6624, which binds to the lysyl oxidase-like 2 (LOXL2) enzyme and can act as an immunomodulator) by Gilead, Timolol, NCX-1000, Terlipressin, Furosemide, Spironolactone. Lactulose, Rifaximin, NGM282 by NGM Biopharmaceuticals, LUM001, by Lumena Pharmaceuticals, Sorafenib (4-[4-[[4-chloro-3-(trifluoromethyl)phenyl]carbamoyl] amino] phenoxy]-N-methyl-pyridine-2-carboxamide) by Bayer and Onyx, hormones such as Serelaxin (RLX030, a recombinant form of human relaxin-2 represented by the sequence L-Serine, L-a-aspartyl-L-tryptophyl-L-methionyl-L-a-glutamyl-L-a-glutamyl-L-valylL-iso-leucyl-L-lysyl-L-leucyl-L-cysteiny1glycyl-L-arginyl-L-a-glu tamyl-L-leucyl-L-valyl-L- arginyl-L-alanyl-L-glutaminyl-L-isoleucyl-L-alanyl-L-isoleucyl-L-cysteiny1glycyl-L- methionyl-L-seryl-L-threonyl-L-tryptophyl-, cyclic (11→1Γ), (23→24')-bis(disulfide) with 5-oxo-L-prolyl-L-leucyl-L-tyrosyl-L-seryl-L-alanyl-L-leucyl-L-alanyl-L-asparaginyl-L-lysyl-L-cysteyn1-L-cysteiny1-L-histidyl-L-valylglycyl-L-cysteiny1-L-threonyl-L-lysyl-L-arginy1-L-seryl-L-leucyl-L-alanyl-L-arginy1-L-phenylalanyl-L-cysteine cyclic (10'→15')-disulfide) by Norvartis, Timolol ((S)-l-(tert-buty lamino)-3-[(4-morpholin-4-yl-l,2,5-thiadiazol-3-yl)oxy]propan-2-ol), NCX-1000 (described, for example, by Fiorucci et al., in Cardiovasc Drug Rev. 2004 Summer; 22(2): 135-46), Terlipressin (1-[(4R,7S,10S,13S,16S,19R)-19-[[[a-methyl-[a-methylacetyl]acetyl]amino]acetyl]amino]acetyl]amino)-7-(2-amino-2-oxoethyl)- 10-(3-amino-3-oxopropyl)- 13-benzyl- 16-(4-hydroxybenzyl)-6, 9, 12,15,18-pentaoxo-l,2-dithiao-5,8,1 1,14,17-pentaazacycloicosan-4-yl]carbonyl]-L-prolyl-N-(2-amino-2-oxoethyl)-L-lys inamide), NGM282, an engineered analog of fibroblast growth factor (see, for example, Rossi et al., Journal of Hepatology, Volume 60, Issue 1, Supplement, Page S533, April 2014), LUM001 (see, for example, U.S. Patent Application Nos. 20130338093; 20130109671; 20130108573 and 20130034536), and analogs or derivatives thereof as understood by those of skill in the art.

In certain embodiments, provided herein are methods for treatment of elevated MELD scores and/or its components and/or elevated Child-Pugh scores and/or its components in patients with PBC. PBC begins with inflammation of the bile ducts inside the liver. The
inflammation blocks the flow of bile out of the liver; thus, bile remains in the liver or spills over into the bloodstream. As inflammation spreads from the bile ducts to the rest of the liver, a latticework of scar tissue develops throughout the liver. In one embodiment, the methods are for treatment of PBC in women aged 35 to 60. In certain embodiments, the PBC is caused by an autoimmune disorder. The methods provided herein are useful in treating one or more of the aforementioned symptoms of primary biliary cirrhosis.

[0068] In certain embodiments, provided herein are methods for treatment of elevated MELD scores and/or its components and/or elevated Child-Pugh scores and/or its components in patients with PSC. PSC is characterized by chronic cholestasis that is associated with chronic inflammation and apoptosis in the biliary tract in the liver. This chronic condition can lead to cirrhosis and cancer in patients. The etiology of PSC is not well understood and there is no current effective medical therapy. In one embodiment, the methods are for treatment of PSC. In one embodiment, primary sclerosing cholangitis occurs in association with inflammatory bowel disease. The methods provided herein are useful in treating one or more of the aforementioned symptoms of primary sclerosing cholangitis.

[0069] Apoptosis occurs mainly via two signaling pathways: a death receptor mediated extrinsic pathway or a mitochondria mediated intrinsic pathway. The extrinsic pathway originates at the plasma membrane following the engagement of a family of cytokine receptors named death receptors (such as tumor necrosis factor receptor 1 (TNF-R1), Fas/CD95, and tumor necrosis factor related apoptosis inducing ligand receptors 1 and 2 (TRAIL-R1 and TRAIL-R2) by their cognate ligands (TNF-, Fas ligand (FasL)/CD95L, TRAIL). See, Guicciardi *et al.* *Gut*, 2005: 54, 1024-1033 and Ghavami *et al.*, *Med. Sci. Monit*, 2005: 11(1): RA337-345.

[0070] As known to one of skill in the art, the cytokines interleukins 1 beta, (IL-1 β) and interleukin 18 (IL-18), mediate inflammation in the liver and are linked to liver disease. Thus, prevention or suppression of inflammation in the liver is a component in the treatment of liver disease. IL-1 β and IL-18 require the action of caspsases to activate their individual inflammatory activities from their respective precursor proteins, pro-IL1 beta and pro-IL-18. The precursor proteins pro-IL1 beta and pro-IL-18 lack inflammatory activity. Without being bound to any particular theory, it is believed that in certain embodiments, the prevention or suppression of excessive inflammation in the liver by compounds provided herein contributes to reducing liver damage associated with liver disease.
Preparation of the compounds


Formulation of pharmaceutical compositions

[0072] The pharmaceutical compositions provided herein contain therapeutically effective amounts of one or more of compounds provided herein that are useful in the prevention, treatment, or amelioration of one or more of the symptoms of liver diseases and a pharmaceutically acceptable carrier.

[0073] The compounds are formulated into suitable pharmaceutical preparations such as solutions, suspensions, tablets, dispersible tablets, pills, capsules, powders, sustained release formulations or elixirs, for oral administration or in sterile solutions or suspensions for parenteral administration, as well as transdermal patch preparation and dry powder inhalers. In one embodiment, the compounds described above are formulated into pharmaceutical compositions using techniques and procedures well known in the art (see, e.g., Remington's Pharmaceutical Sciences, 20th ed., Mack Publishing, Easton PA (2000)).

[0074] In the compositions, effective concentrations of one or more compounds or pharmaceutically acceptable derivatives is (are) mixed with a suitable pharmaceutical carrier or vehicle. The compounds may be derivatized as the corresponding salts, esters, acids, bases, solvates, hydrates or prodrugs prior to formulation, as described above. The concentrations of the compounds in the compositions are effective for delivery of an amount, upon administration, that treats, prevents, or ameliorates one or more of the symptoms of liver diseases.

[0075] In one embodiment, the compositions are formulated for single dosage administration. To formulate a composition, the weight fraction of compound is dissolved, suspended, dispersed or otherwise mixed in a selected vehicle at an effective concentration such that the treated condition is relieved or ameliorated. Pharmaceutical carriers or vehicles suitable for administration of the compounds provided herein include any such carriers known to those skilled in the art to be suitable for the particular mode of administration.
[0076] In addition, the compounds may be formulated as the sole pharmaceutically active ingredient in the composition or may be combined with other active ingredients. Liposomal suspensions, including tissue-targeted liposomes, such as tumor-targeted liposomes, may also be suitable as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art. For example, liposome formulations may be prepared as known in the art. Briefly, liposomes such as multilamellar vesicles (MLV's) may be formed by drying down egg phosphatidyl choline and brain phosphatidyl serine (7:3 molar ratio) on the inside of a flask. A solution of a compound provided herein in phosphate buffered saline (PBS) lacking divalent cations is added and the flask shaken until the lipid film is dispersed. The resulting vesicles are washed to remove unencapsulated compound, pelleted by centrifugation, and then resuspended in PBS.

[0077] The active compound is included in the pharmaceutically acceptable carrier in an amount sufficient to exert a therapeutically useful effect in the absence of undesirable side effects on the patient treated. The therapeutically effective concentration may be determined empirically by testing the compounds in *in vitro* and *in vivo* systems known in the art and then extrapolated therefrom for dosages for humans.

[0078] The concentration of active compound in the pharmaceutical composition will depend on absorption, inactivation and excretion rates of the active compound, the physicochemical characteristics of the compound, the dosage schedule, and amount administered as well as other factors known to those of skill in the art. For example, the amount that is delivered is sufficient to ameliorate one or more of the symptoms of liver diseases.

[0079] In one embodiment, a therapeutically effective dosage should produce a serum concentration of an active ingredient of from about 0.1 ng/ml to about 50-100 µg/ml, from about 0.5 ng/ml to about 80 µg/ml, from about 1 ng/ml to about 60 µg/ml, from about 5 ng/ml to about 50 µg/ml, from about 5 ng/ml to about 40 µg/ml, from about 10 ng/ml to about 35 µg/ml, from about 10 ng/ml to about 25 µg/ml, from about 10 ng/ml to about 10 µg/ml, from about 25 ng/ml to about 10 µg/ml, from about 50 ng/ml to about 10 µg/ml, from about 50 ng/ml to about 5 µg/ml, from about 100 ng/ml to about 5 µg/ml, from about 200 ng/ml to about 5 µg/ml, from about 250 ng/ml to about 5 µg/ml, from about 500 ng/ml to about 5 µg/ml, from about 1 µg/ml to about 50 µg/ml, from about 0.1 ng/ml to about 5 ng/ml, from about 1 ng/ml to about 10 ng/ml or from about 1 µg/ml to about 10 µg/ml. The pharmaceutical compositions, in certain
embodiments, should provide a dosage of from about 0.001 mg to about 2000 mg of compound per kilogram of body weight per day, from about 0.002 mg to about 1000 mg of compound per kilogram of body weight per day, from about 0.005 mg to about 500 mg of compound per kilogram of body weight per day, from about 0.005 mg to about 250 mg of compound per kilogram of body weight per day, from about 0.005 mg to about 200 mg of compound per kilogram of body weight per day, from about 0.005 mg to about 100 mg of compound per kilogram of body weight per day, from about 0.01 mg to about 100 mg of compound per kilogram of body weight per day, from about 0.01 mg to about 50 mg of compound per kilogram of body weight per day, from about 0.02 mg to about 100 mg of compound per kilogram of body weight per day, from about 0.05 mg to about 100 mg of compound per kilogram of body weight per day, from about 0.1 mg to about 100 mg of compound per kilogram of body weight per day, from about 0.5 mg to about 100 mg of compound per kilogram of body weight per day, from about 0.75 mg to about 100 mg of compound per kilogram of body weight per day, from about 1 mg to about 100 mg of compound per kilogram of body weight per day, from about 1 mg to about 10 mg of compound per kilogram of body weight per day, from about 0.001 mg to about 5 mg of compound per kilogram of body weight per day, from about 200 mg to about 2000 mg of compound per kilogram of body weight per day, or from about 10 mg to about 100 mg of compound per kilogram of body weight per day.

Pharmaceutical dosage unit forms are prepared to provide from about 1 mg to about 1000 mg, from about 1 mg to about 800 mg, from about 5 mg to about 800 mg, from about 1 mg to about 100 mg, from about 1 mg to about 50 mg, from about 5 mg to about 100 mg, from about 10 mg to about 50 mg, from about 10 mg to about 100 mg, from about 25 mg to about 50 mg, and from about 10 mg to about 500 mg of the essential active ingredient or a combination of essential ingredients per dosage unit form.

[0080] The active ingredient may be administered at once, or may be divided into a number of smaller doses to be administered at intervals of time. It is understood that the precise dosage and duration of treatment is a function of the disease being treated and may be determined empirically using known testing protocols or by extrapolation from in vivo or in vitro test data. It is to be noted that concentrations and dosage values may also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the
professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed compositions.

[0081] Pharmaceutically acceptable derivatives include acids, bases and esters, salts, esters, hydrates, solvates and prodrug forms. The derivative is selected such that its pharmacokinetic properties are superior to the corresponding neutral compound.

[0082] Thus, effective concentrations or amounts of one or more of the compounds described herein or pharmaceutically acceptable derivatives thereof are mixed with a suitable pharmaceutical carrier or vehicle for systemic, topical or local administration to form pharmaceutical compositions. Compounds are included in an amount effective for ameliorating one or more symptoms of, or for treating or preventing liver diseases. The concentration of active compound in the composition will depend on absorption, inactivation, excretion rates of the active compound, the dosage schedule, amount administered, particular formulation as well as other factors known to those of skill in the art.

[0083] The compositions are intended to be administered by a suitable route, including orally, parenterally, rectally, topically, locally and via nasogastric or orogastric tube. For oral administration, capsules and tablets can be used. The compositions are in liquid, semi-liquid or solid form and are formulated in a manner suitable for each route of administration. In one embodiment, modes of administration include parenteral and oral modes of administration. In certain embodiments, oral administration is contemplated.

[0084] Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application can include any of the following components: a sterile diluent, such as water for injection, saline solution, fixed oil, polyethylene glycol, glycerine, propylene glycol, dimethyl acetamide or other synthetic solvent; antimicrobial agents, such as benzyl alcohol and methyl parabens; antioxidants, such as ascorbic acid and sodium bisulfite; chelating agents, such as ethylenediaminetetraacetic acid (EDTA); buffers, such as acetates, citrates and phosphates; and agents for the adjustment of tonicity such as sodium chloride or dextrose. Parenteral preparations can be enclosed in ampules, disposable syringes or single or multiple dose vials made of glass, plastic or other suitable material.

[0085] In instances in which the compounds exhibit insufficient solubility, methods for solubilizing compounds may be used. Such methods are known to those of skill in this art, and

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include, but are not limited to, using cosolvents, such as dimethylsulfoxide (DMSO), using surfactants, such as TWEEN®, or dissolution in aqueous sodium bicarbonate.

Upon mixing or addition of the compound(s), the resulting mixture may be a solution, suspension, emulsion or the like. The form of the resulting mixture depends upon a number of factors, including the intended mode of administration and the solubility of the compound in the selected carrier or vehicle. The effective concentration is sufficient for ameliorating the symptoms of the disease, disorder or condition treated and may be empirically determined.

The pharmaceutical compositions are provided for administration to humans and animals in unit dosage forms, such as tablets, capsules, pills, powders, granules, sterile parenteral solutions or suspensions, and oral solutions or suspensions, and oilwater emulsions containing suitable quantities of the compounds or pharmaceutically acceptable derivatives thereof. The pharmaceutically therapeutically active compounds and derivatives thereof are formulated and administered in unitdose forms or multipledosage forms. Unitdose forms as used herein refer to physically discrete units suitable for human and animal subjects and packaged individually as is known in the art. Each unitdose contains a predetermined quantity of the therapeutically active compound sufficient to produce the desired therapeutic effect, in association with the required pharmaceutical carrier, vehicle or diluent. Examples of unitdose forms include ampules and syringes and individually packaged tablets or capsules. Unitdose forms may be administered in fractions or multiples thereof. A multipledose form is a plurality of identical unitdosage forms packaged in a single container to be administered in segregated unitdose form. Examples of multipledose forms include vials, bottles of tablets or capsules or bottles of pints or gallons. Hence, multiple dose form is a multiple of unitdoses which are not segregated in packaging.

Sustained-release preparations can also be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the compound provided herein, which matrices are in the form of shaped articles, e.g., films, or microcapsule. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides, copolymers of L-glutamic acid and ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid...
enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated compound remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37 °C, resulting in a loss of biological activity and possible changes in their structure. Rational strategies can be devised for stabilization depending on the mechanism of action involved. For example, if the aggregation mechanism is discovered to be intermolecular S–S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

Dosage forms or compositions containing active ingredient in the range of 0.001% to 100% active ingredient, 0.002% to 100% active ingredient, 0.005% to 90% active ingredient, 0.01% to 100% active ingredient, 0.05% to 100% active ingredient, 0.05% to 90% active ingredient, 0.1% to 100% active ingredient, 0.1% to 1% active ingredient, 0.1% to 0.5% active ingredient, 1% to 100% active ingredient, 1% to 99% active ingredient, 1% to 98% active ingredient, 1% to 97% active ingredient, 1% to 96% active ingredient, 1% to 95% active ingredient, 5% to 95% active ingredient, 10% to 100% active ingredient, 10% to 95% active ingredient, 15% to 95% active ingredient, 20% to 95% active ingredient, 25% to 100% active ingredient, 50% to 100% active ingredient, 50% to 95% active ingredient, 60% to 95% active ingredient or 75% to 100% active ingredient, with the balance made up from nontoxic carrier may be prepared. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by the incorporation of any of the normally employed excipients, such as, for example pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, talcum, cellulose derivatives, sodium crosscarmellose, glucose, sucrose, magnesium carbonate or sodium saccharin. Such compositions include solutions, suspensions, tablets, capsules, powders and sustained release formulations, such as, but not limited to, implants and microencapsulated delivery systems, and biodegradable, biocompatible polymers, such as collagen, ethylene vinyl acetate, polyanhydrides, polyglycolic acid, polyorthoesters, polyactic acid and others. Methods for preparation of these compositions are known to those skilled in the art. The contemplated compositions may contain 0.001% to 100% active ingredient, in one embodiment! or 75-95% active ingredient.
The active compounds or pharmaceutically acceptable derivatives may be prepared with carriers that protect the compound against rapid elimination from the body, such as time release formulations or coatings.

The compositions may include other active compounds to obtain desired combinations of properties. The compounds provided herein, or pharmaceutically acceptable derivatives thereof as described herein, may also be advantageously administered for therapeutic or prophylactic purposes together with another pharmacological agent known in the general art to be of value in treating liver diseases. It is to be understood that such combination therapy constitutes a further aspect of the compositions and methods of treatment provided herein.

Compositions for oral administration

Oral pharmaceutical dosage forms are either solid, gel or liquid. The solid dosage forms are tablets, capsules, granules, and bulk powders. Types of oral tablets include compressed, chewable lozenges and tablets which may be enteric coated, sugarcoated or film coated. Capsules may be hard or soft gelatin capsules, while granules and powders may be provided in non-effervescent or effervescent form with the combination of other ingredients known to those skilled in the art.

In certain embodiments, the formulations are solid dosage forms, such as capsules or tablets. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder; a diluent; a disintegrating agent; a lubricant; a glidant; a sweetening agent; and a flavoring agent.

Examples of binders include microcrystalline cellulose, gum tragacanth, glucose solution, acacia mucilage, gelatin solution, sucrose and starch paste. Lubricants include talc, starch, magnesium or calcium stearate, lycopodium and stearic acid. Diluents include, for example, lactose, sucrose, starch, kaolin, salt, mannitol and dicalcium phosphate. Glidants include, but are not limited to, colloidal silicon dioxide. Disintegrating agents include crosscarmellose sodium, sodium starch glycolate, alginic acid, corn starch, potato starch, bentonite, methylcellulose, agar and carboxymethylcellulose. Coloring agents include, for example, any of the approved certified water soluble FD and C dyes, mixtures thereof; and water insoluble FD and C dyes suspended on alumina hydrate. Sweetening agents include sucrose, lactose, mannitol and artificial sweetening agents such as saccharin, and any number of spray dried flavors. Flavoring agents include natural flavors extracted from plants such as fruits and...
synthetic blends of compounds which produce a pleasant sensation, such as, but not limited to peppermint and methyl salicylate. Wetting agents include propylene glycol monostearate, sorbitan monooleate, diethylene glycol monolaurate and polyoxyethylene laural ether. Emetic coatings include fatty acids, fats, waxes, shellac, ammoniated shellac and cellulose acetate phthalates. Film coatings include hydroxyethylcellulose, sodium carboxymethylcellulose, polyethylene glycol 4000 and cellulose acetate phthalate.

If oral administration is desired, the compound could be provided in a composition that protects it from the acidic environment of the stomach. For example, the composition can be formulated in an enteric coating that maintains its integrity in the stomach and releases the active compound in the intestine. The composition may also be formulated in combination with an antacid or other such ingredient.

When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings of sugar and other enteric agents. The compounds can also be administered as a component of an elixir, suspension, syrup, wafer, sprinkle, chewing gum or the like. A syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors.

The active materials can also be mixed with other active materials which do not impair the desired action, or with materials that supplement the desired action, such as antacids, H2 blockers, and diuretics. The active ingredient is a compound or pharmaceutically acceptable derivative thereof as described herein. Higher concentrations, up to about 98% by weight of the active ingredient may be included.

Pharmaceutically acceptable carriers included in tablets are binders, lubricants, diluents, disintegrating agents, coloring agents, flavoring agents, and wetting agents. Enteric coated tablets, because of the enteric coating, resist the action of stomach acid and dissolve or disintegrate in the neutral or alkaline intestines. Sugar coated tablets are compressed tablets to which different layers of pharmaceutically acceptable substances are applied. Film coated tablets are compressed tablets which have been coated with a polymer or other suitable coating. Multiple compressed tablets are compressed tablets made by more than one compression cycle utilizing the pharmaceutically acceptable substances previously mentioned.
Coloring agents may also be used in the above dosage forms. Flavoring and sweetening agents are used in compressed tablets, sugarcoated, multiple compressed and chewable tablets. Flavoring and sweetening agents are especially useful in the formation of chewable tablets and lozenges.

5 [0099] Liquid oral dosage forms include aqueous solutions, emulsions, suspensions, solutions and/or suspensions reconstituted from non-effervescent granules and effervescent preparations reconstituted from effervescent granules. Aqueous solutions include, for example, elixirs and syrups. Emulsions are either oil in-water or water in oil.

[00100] Elixirs are clear, sweetened, hydroalcoholic preparations. Pharmaceutically acceptable carriers used in elixirs include solvents. Syrups are concentrated aqueous solutions of a sugar, for example, sucrose, and may contain a preservative. An emulsion is a two phase system in which one liquid is dispersed in the form of small globules throughout another liquid. Pharmaceutically acceptable carriers used in emulsions are nonaqueous liquids, emulsifying agents and preservatives. Suspensions use pharmaceutically acceptable suspending agents and preservatives. Pharmaceutically acceptable substances used in noneffervescent granules, to be reconstituted into a liquid oral dosage form, include diluents, sweeteners and wetting agents. Pharmaceutically acceptable substances used in effervescent granules, to be reconstituted into a liquid oral dosage form, include organic acids and a source of carbon dioxide. Coloring and flavoring agents are used in all of the above dosage forms.

[00101] Solvents include glycerin, sorbitol, ethyl alcohol and syrup. Examples of preservatives include glycerin, methyl and propylparaben, benzoic acid, sodium benzoate and alcohol. Examples of nonaqueous liquids utilized in emulsions include mineral oil and cottonseed oil. Examples of emulsifying agents include gelatin, acacia, tragacanth, bentonite, and surfactants such as polyoxyethylene sorbitan monooleate. Suspending agents include sodium carboxymethylcellulose, pectin, tragacanth, Veegum and acacia. Diluents include lactose and sucrose. Sweetening agents include sucrose, syrups, glycerin and artificial sweetening agents such as saccharin. Wetting agents include propylene glycol monostearate, sorbitan monooleate, diethylene glycol monolaurate and polyoxyethylene lauryl ether. Organic acids include citric and tartaric acid. Sources of carbon dioxide include sodium bicarbonate and sodium carbonate. Coloring agents include any of the approved certified water soluble FD and C
dyes, and mixtures thereof. Flavoring agents include natural flavors extracted from plants such as fruits, and synthetic blends of compounds which produce a pleasant taste sensation.

[00102] For a solid dosage form, the solution or suspension, in for example propylene carbonate, vegetable oils or triglycerides, can be encapsulated in a gelatin capsule. Such solutions, and the preparation and encapsulation thereof, are disclosed in U.S. Patent Nos 4,328,245; 4,409,239; and 4,410,545. For a liquid dosage form, the solution, e.g., for example, in a polyethylene glycol, may be diluted with a sufficient quantity of a pharmaceutically acceptable liquid carrier, e.g., water, to be easily measured for administration.

[00103] Alternatively, liquid or semisolid oral formulations may be prepared by dissolving or dispersing the active compound or salt in vegetable oils, glycols, triglycerides, propylene glycol esters (e.g., propylene carbonate) and other such carriers, and encapsulating these solutions or suspensions in hard or soft gelatin capsule shells. Other useful formulations include, but are not limited to, those containing a compound provided herein, a dialkylated mono- or poly-alkylene glycol, including, but not limited to, 1,2-dimethoxyethane, diglyme, triglyme, tetraglyme, polyethylene glycol-350-dimethyl ether, polyethylene glycol-550-dimethyl ether, polyethylene glycol-750-dimethyl ether wherein 350, 550 and 750 refer to the approximate average molecular weight of the polyethylene glycol, and one or more antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate, vitamin E, hydroquinone, hydroxycoumarins, ethanolamine, lecithin, cephalin, ascorbic acid, malic acid, sorbitol, phosphoric acid, thiodipropionic acid and its esters, and dithiocarbamates.

[00104] Other formulations include, but are not limited to, aqueous alcoholic solutions including a pharmaceutically acceptable acetal. Alcohols used in these formulations are any pharmaceutically acceptable water-miscible solvents having one or more hydroxyl groups, including, but not limited to, propylene glycol and ethanol. Acetals include, but are not limited to, di(lower alkyl) acetics of lower alkyl aldehydes such as acetaldehyde diethyl acetal.

[00105] In all embodiments, tablets and capsules formulations may be coated as known by those of skill in the art in order to modify or sustain dissolution of the active ingredient. Thus, for example, they may be coated with a conventional enterically digestible coating, such as phenylsalicylate, waxes and cellulose acetate phthalate.
Injectables, solutions and emulsions

[00106] Parenteral administration, generally characterized by injection, either subcutaneously, intramuscularly or intravenously is also contemplated herein. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, saline, dextrose, glycerol or ethanol. In addition, if desired, the pharmaceutical compositions to be administered may also contain minor amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents, stabilizers, solubility enhancers, and other such agents, such as for example, sodium acetate, sorbitan monolaurate, triethanolamine olate and cyclodextrins. Implantation of a slow release or sustained release system, such that a constant level of dosage is maintained is also contemplated herein. Briefly, a compound provided herein is dispersed in a solid inner matrix, e.g., polymethylmethacrylate, polybutylmethacrylate, plasticized or unplasticized polyvinylchloride, plasticized nylon, plasticized polyethyleneeterephthalate, natural rubber, polyisoprene, polyisobutylene, polybutadiene, polyethylene, ethylene-vinylacetate copolymers, silicone rubbers, polydimethylsiloxanes, silicone carbonate copolymers, hydrophilic polymers such as hydrogels of esters of acrylic and methacrylic acid, collagen, cross-linked polyvinylalcohol and cross-linked partially hydrolyzed polyvinyl acetate, that is surrounded by an outer polymeric membrane, e.g., polyethylene, polypropylene, ethylene/propylene copolymers, ethylene/ethyl acrylate copolymers, ethylene/vinylacetate copolymers, silicone rubbers, polydimethyl siloxanes, neoprene rubber, chlorinated polyethylene, polyvinylchloride, vinylchloride copolymers with vinyl acetate, vinylidene chloride, ethylene and propylene, ionomer polyethylene terephthalate, butyl rubber epichlorohydrin rubbers, ethylene/vinyl alcohol copolymers, ethylene/vinyl acetate/vinyl alcohol terpolymer, and ethylene/vinylxyethanol copolymer, that is insoluble in body fluids. The compound diffuses through the outer polymeric membrane in a release rate controlling step. The percentage of active compound contained in such parenteral compositions is highly dependent on the specific nature thereof, as well as the activity of the compound and the needs of the subject.

[00107] Parenteral administration of the compositions includes intravenous, subcutaneous and intramuscular administrations. Preparations for parenteral administration include sterile solutions ready for injection, sterile dry soluble products, such as lyophilized powders, ready to
be combined with a solvent just prior to use, including hypodermic tablets, sterile suspensions ready for injection, sterile dry insoluble products ready to be combined with a vehicle just prior to use and sterile emulsions. The solutions may be either aqueous or nonaqueous.

If administered intravenously, suitable carriers include physiological saline or phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents, such as glucose, polyethylene glycol, and polypropylene glycol and mixtures thereof.

Pharmaceutically acceptable carriers used in parenteral preparations include aqueous vehicles, nonaqueous vehicles, antimicrobial agents, isotonic agents, buffers, antioxidants, local anesthetics, suspending and dispersing agents, emulsifying agents, sequestering or chelating agents and other pharmaceutically acceptable substances.

Examples of aqueous vehicles include Sodium Chloride Injection, Ringers Injection, Isotonic Dextrose Injection, Sterile Water Injection, Dextrose and Lactated Ringers Injection. Nonaqueous parenteral vehicles include fixed oils of vegetable origin, cottonseed oil, corn oil, sesame oil and peanut oil. Antimicrobial agents in bacteriostatic or fungistatic concentrations must be added to parenteral preparations packaged in multiple dose containers which include phenols or cresols, mercurials, benzyl alcohol, chlorobutanol, methyl and propyl hydroxybenzoic acid esters, thimerosal, benzalkonium chloride and benzethonium chloride. Isotonic agents include sodium chloride and dextrose. Buffers include phosphate and citrate. Antioxidants include sodium bisulfate. Local anesthetics include procaine hydrochloride. Suspending and dispersing agents include sodium carboxymethylcellulose, hydroxypropyl methylcellulose and polyvinylpyrrolidone. Emulsifying agents include Polysorbate 80 (TWEEN® 80). A sequestering or chelating agent of metal ions includes EDTA.

Pharmaceutical carriers also include ethyl alcohol, polyethylene glycol and propylene glycol for water miscible vehicles and sodium hydroxide, hydrochloric acid, citric acid or lactic acid for pH adjustment.

The concentration of the pharmaceutically active compound is adjusted so that an injection provides an effective amount to produce the desired pharmacological effect. The exact dose depends on the age, weight and condition of the patient or animal as is known in the art.

The unit dose parenteral preparations are packaged in an ampule, a vial or a syringe with a needle. All preparations for parenteral administration must be sterile, as is known and practiced in the art.
[0013] Illustratively, intravenous or intraarterial infusion of a sterile aqueous solution containing an active compound is an effective mode of administration. Another embodiment is a sterile aqueous or oily solution or suspension containing an active material injected as necessary to produce the desired pharmacological effect.

[0014] Injectables are designed for local and systemic administration. In certain embodiments, a therapeutically effective dosage is formulated to contain a concentration of at least about 0.1% w/w up to about 90% w/w or more, or more than 1% w/w of the active compound to the treated tissue(s). The active ingredient may be administered at once, or may be divided into a number of smaller doses to be administered at intervals of time. It is understood that the precise dosage and duration of treatment is a function of the tissue being treated and may be determined empirically using known testing protocols or by extrapolation from in vivo or in vitro test data. It is to be noted that concentrations and dosage values may also vary with the age of the individual treated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the formulations, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed formulations.

[0015] The compound may be suspended in micronized or other suitable form or may be derivatized to produce a more soluble active product or to produce a prodrug. The form of the resulting mixture depends upon a number of factors, including the intended mode of administration and the solubility of the compound in the selected carrier or vehicle. The effective concentration is sufficient for ameliorating the symptoms of the condition and may be empirically determined.

Lyophilized powders

[0016] Of interest herein are also lyophilized powders, which can be reconstituted for administration as solutions, emulsions and other mixtures. They may also be reconstituted and formulated as solids or gels.

[0017] The sterile, lyophilized powder is prepared by dissolving a compound provided herein, or a pharmaceutically acceptable derivative thereof, in a suitable solvent. The solvent may contain an excipient which improves the stability or other pharmacological component of the powder or reconstituted solution, prepared from the powder. Excipients that may be used
include, but are not limited to, dextrose, sorbitol, fructose, corn syrup, xylitol, glycerin, glucose,
sucrose or other suitable agent. The solvent may also contain a buffer, such as citrate, sodium or
potassium phosphate or other such buffer known to those of skill in the art at about neutral pH.
Subsequent sterile filtration of the solution followed by lyophilization under standard conditions
known to those of skill in the art provides the desired formulation. Generally, the resulting
solution will be apportioned into vials for lyophilization. Each vial will contain a single dosage
(10-1000 mg or 100-500 mg) or multiple dosages of the compound. The lyophilized powder can
be stored under appropriate conditions, such as at about 4 degrees Celsius to room temperature.
[00118] Reconstitution of this lyophilized powder with water for injection provides a
formulation for use in parenteral administration. For reconstitution, about 1-50 mg, 5-35 mg or
about 9-30 mg of lyophilized powder, is added per mL of sterile water or other suitable carrier.
The precise amount depends upon the selected compound. Such amount can be empirically
determined.

**Topical administration**

[00119] Topical mixtures are prepared as described for the local and systemic administration.
The resulting mixture may be a solution, suspension, emulsions or the like and are formulated as
creams, gels, ointments, emulsions, solutions, elixirs, lotions, suspensions, tinctures, pastes,
foams, aerosols, irrigations, sprays, suppositories, bandages, dermal patches or any other
formulations suitable for topical administration.

[00120] The compounds or pharmaceutically acceptable derivatives thereof may be
formulated as aerosols for topical application, such as by inhalation (see, e.g., U.S. Patent Nos.
4,044,126, 4,414,209, and 4,364,923, which describe aerosols for delivery of a steroid useful for
treatment of inflammatory diseases, particularly asthma). These formulations for administration
to the respiratory tract can be in the form of an aerosol or solution for a nebulizer, or as a
microfine powder for insufflation, alone or in combination with an inert carrier such as lactose.
In such a case, the particles of the formulation will have diameters of less than 50 microns or less
than 10 microns.

[00121] The compounds may be formulated for local or topical application, such as for topical
application to the skin and mucous membranes, such as in the eye, in the form of gels, creams,
and lotions and for application to the eye or for intracysternal or intraspinal application. Topical
administration is contemplated for transdermal delivery and also for administration to the eyes or
mucosa, or for inhalation therapies. Nasal solutions of the active compound alone or in combination with other pharmaceutically acceptable excipients can also be administered.

[00122] These solutions, particularly those intended for ophthalmic use, may be formulated as 0.01% - 10% isotonic solutions, pH about 5-7, with appropriate salts.

Compositions for other routes of administration

[00123] Other routes of administration, such as topical application, transdermal patches, and rectal administration are also contemplated herein.

[00124] For example, pharmaceutical dosage forms for rectal administration are rectal suppositories, capsules and tablets for systemic effect. Rectal suppositories are used herein mean solid bodies for insertion into the rectum which melt or soften at body temperature releasing one or more pharmacologically or therapeutically active ingredients. Pharmaceutically acceptable substances utilized in rectal suppositories are bases or vehicles and agents to raise the melting point. Examples of bases include cocoa butter (theobroma oil), glyceringelatin, carbowax (polyoxyethylene glycol) and appropriate mixtures of mono, di and triglycerides of fatty acids.

Combinations of the various bases may be used. Agents to raise the melting point of suppositories include spermaceti and wax. Rectal suppositories may be prepared either by the compressed method or by molding. In certain embodiments, the weight of a rectal suppository is about 2 to 3 gm.

[00125] Tablets and capsules for rectal administration are manufactured using the same pharmaceutically acceptable substance and by the same methods as for formulations for oral administration.

Sustained Release Compositions

[00126] Active ingredients such as the compounds provided herein can be administered by controlled release means or by delivery devices that are well known to those of ordinary skill in the art. Examples include, but are not limited to, those described in U.S. Patent Nos.: 3,845,770; 3,916,899; 3,536,809; 3,598,123; 4,008,719; 5,674,533; 5,059,595; 5,591,767; 5,120,548; 5,073,543; 5,639,476; 5,354,556; 5,639,480; 5,733,566; 5,739,108; 5,891,474; 5,922,356; 5,972,891; 5,980,945; 5,993,855; 6,045,830; 6,087,324; 6,1 13,943; 6,197,350; 6,248,363; 6,264,970; 6,267,981; 6,376,461; 6,419,961; 6,589,548; 6,613,358 and 6,699,500 each of which is incorporated herein by reference. Such dosage forms can be used to provide slow or controlled release of one or more active ingredients using, for example, hydropropylmethy
cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the active ingredients provided herein. Thus, the compositions provided encompass single unit dosage forms suitable for oral administration such as, but not limited to, tablets, capsules, gelcaps, and caplets that are adapted for controlled release.

[00127] All controlled release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non controlled counterparts. Ideally, the use of an optimally designed controlled release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled release formulations include extended activity of the drug, reduced dosage frequency, and increased subject compliance. In addition, controlled release formulations can be used to affect the time of onset of action or other characteristics, such as blood levels of the drug, and can thus affect the occurrence of side (e.g., adverse) effects.

[00128] Most controlled release formulations are designed to initially release an amount of drug (active ingredient) that promptly produces the desired therapeutic effect, and gradually and continually release of other amounts of drug to maintain this level of therapeutic or prophylactic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body. Controlled release of an active ingredient can be stimulated by various conditions including, but not limited to, pH, temperature, enzymes, water, or other physiological conditions or compounds.

[00129] In certain embodiments, the drug may be administered using intravenous infusion, an implantable osmotic pump, a transdermal patch, liposomes, or other modes of administration. In one embodiment, a pump may be used (see, Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al, Surgery 88:507 (1980); Saudek et al, N. Engl. J. Med. 321 :574 (1989)). In another embodiment, polymeric materials can be used. In yet another embodiment, a controlled release system can be placed in a subject at an appropriate site determined by a practitioner of skill, i.e., thus requiring only a fraction of the systemic dose (see, e.g., Goodson, Medical Applications of Controlled Release, vol. 2, pp. 115-138 (1984)). Other controlled release
systems are discussed in the review by Langer (*Science* 249: 1527-1533 (1990)). The active ingredient can be dispersed in a solid inner matrix, *e.g.*, polymethylmethacrylate, polybutylmethacrylate, plasticized or unplasticized polyvinylchloride, plasticized nylon, plasticized polyethyleneterephthalate, natural rubber, polyisoprene, polyisobutylene, polybutadiene, polyethylene, ethylene-vinylacetate copolymers, silicone rubbers, polydimethylsiloxanes, silicone carbonate copolymers, hydrophilic polymers such as hydrogels of esters of acrylic and methacrylic acid, collagen, cross-linked polyvinylalcohol and cross-linked partially hydrolyzed polyvinyl acetate, that is surrounded by an outer polymeric membrane, *e.g.*, polyethylene, polypropylene, ethylene/propylene copolymers, ethylene/ethyl acrylate copolymers, ethylene/vinylacetate copolymers, silicone rubbers, polydimethyl siloxanes, neoprene rubber, chlorinated polyethylene, polyvinylchloride, vinylchloride copolymers with vinyl acetate, vinylidene chloride, ethylene and propylene, ionomer polyethylene terephthalate, butyl rubber epichlorohydrin rubbers, ethylene/vinyl alcohol copolymer, ethylene/vinyl acetate/vinyl alcohol terpolymer, and ethylene/vinylxyethanol copolymer, that is insoluble in body fluids. The active ingredient then diffuses through the outer polymeric membrane in a release rate controlling step. The percentage of active ingredient in such parenteral compositions is highly dependent on the specific nature thereof, as well as the needs of the subject.

**Targeted Formulations**

[00130] The compounds provided herein, or pharmaceutically acceptable derivatives thereof, may also be formulated to be targeted to a particular tissue, receptor, or other area of the body of the subject to be treated. Many such targeting methods are well known to those of skill in the art. All such targeting methods are contemplated herein for use in the instant compositions. For non-limiting examples of targeting methods, see, *e.g.*, U.S. Patent Nos. 6,316,652, 6,274,552, 6,271,359, 6,253,872, 6,139,865, 6,131,570, 6,120,751, 6,071,495, 6,060,082, 6,048,736, 6,039,975, 6,004,534, 5,985,307, 5,972,366, 5,900,252, 5,840,674, 5,759,542 and 5,709,874.

[00131] In one embodiment, liposomal suspensions, including tissue-targeted liposomes, such as tumor-targeted liposomes, may also be suitable as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art. For example, liposome formulations may be prepared as described in U.S. Patent No. 4,522,811. Briefly, liposomes such as multilamellar vesicles (MLV’s) may be formed by drying down egg phosphatidyl choline and brain phosphatidyl serine (7:3 molar ratio) on the inside of a flask.
solution of a compound provided herein in phosphate buffered saline lacking divalent cations (PBS) is added and the flask shaken until the lipid film is dispersed. The resulting vesicles are washed to remove unencapsulated compound, pelleted by centrifugation, and then resuspended in PBS.

**Dosage and Unit Dosage Forms**

[00132] In human therapeutics, the doctor will determine the posology which he considers most appropriate according to a preventive or curative treatment and according to the age, weight, stage of the disease and other factors specific to the subject to be treated. Generally, doses are from about 1 to about 1000 mg per day for an adult, or from about 5 to about 250 mg per day or from about 10 to 50 mg per day for an adult. In certain embodiments, doses are from about 5 to about 400 mg per day or 25 to 200 mg per day per adult. Dose rates of from about 50 to about 500 mg per day are also contemplated.

[00133] In certain embodiments, the amount of the compound or composition which will be effective in the prevention or treatment of the liver disease or one or more symptoms thereof will vary with the nature and severity of the disease or condition, and the route by which the active ingredient is administered. The frequency and dosage will also vary according to factors specific for each subject depending on the specific therapy (e.g., therapeutic or prophylactic agents) administered, the severity of the disorder, disease, or condition, the route of administration, as well as age, body, weight, response, and the past medical history of the subject. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

[00134] Exemplary doses of a composition include milligram or microgram amounts of the and caspase inhibitor per kilogram of subject or sample weight (e.g., about 10 micrograms per kilogram to about 50 milligrams per kilogram, about 100 micrograms per kilogram to about 25 milligrams per kilogram, or about 100 microgram per kilogram to about 10 milligrams per kilogram). In certain embodiments, the dosage administered to a subject is between 0.20 mg/kg and 2.00 mg/kg, or between 0.30 mg/kg and 1.50 mg/kg of the subject's body weight.

[00135] In certain embodiments, the recommended daily dose range of the and caspase inhibitor described herein for the conditions described herein lies within the range of from about 0.1 mg to about 1000 mg of each of the and caspase inhibitor per day, given as a single once-a-day dose or as divided doses throughout a day. In one embodiment, the daily dose is
administered twice daily in equally divided doses. Specifically, a daily dose range should be from about 10 mg to about 200 mg per day, more specifically, between about 10 mg and about 150 mg per day, or even more specifically between about 25 and about 100 mg per day. It may be necessary to use dosages of the active ingredient outside the ranges disclosed herein in some cases, as will be apparent to those of ordinary skill in the art. Furthermore, it is noted that the clinician or treating physician will know how and when to interrupt, adjust, or terminate therapy in conjunction with subject response.

[00136] Different therapeutically effective amounts may be applicable for different diseases and conditions, as will be readily known by those of ordinary skill in the art. Similarly, amounts sufficient to prevent, manage, treat or ameliorate such disorders, but insufficient to cause, or sufficient to reduce, adverse effects associated with the compound described herein are also encompassed by the above described dosage amounts and dose frequency schedules. Further, when a subject is administered multiple dosages of a compound described herein, not all of the dosages need be the same. For example, the dosage administered to the subject may be increased to improve the prophylactic or therapeutic effect of the compound or it may be decreased to reduce one or more side effects that a particular subject is experiencing.

[00137] In one embodiment, the dosage of compounds described herein administered to prevent, treat, manage, or ameliorate a disorder, or one or more symptoms thereof in a subject is 0.1 mg/kg, 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 6 mg/kg, 10 mg/kg, or 15 mg/kg or more of a subject's body weight. In another embodiment, the dosage of the compounds provided herein administered to prevent, treat, manage, or ameliorate a disorder, or one or more symptoms thereof in a subject is a unit dose of 0.1 mg to 200 mg, 0.1 mg to 100 mg, 0.1 mg to 50 mg, 0.1 mg to 25 mg, 0.1 mg to 20 mg, 0.1 mg to 15 mg, 0.1 mg to 10 mg, 0.1 mg to 7.5 mg, 0.1 mg to 5 mg, 0.1 mg to 2.5 mg, 0.25 mg to 20 mg, 0.25 to 15 mg, 0.25 to 12 mg, 0.25 to 10 mg, 0.25 mg to 7.5 mg, 0.25 mg to 5 mg, 0.5 mg to 2.5 mg, 1 mg to 20 mg, 1 mg to 15 mg, 1 mg to 12 mg, 1 mg to 10 mg, 1 mg to 7.5 mg, 1 mg to 5 mg, or 1 mg to 2.5 mg.

[00138] In certain embodiments, treatment or prevention can be initiated with one or more loading doses of a caspase inhibitor provided herein followed by one or more maintenance doses. In such embodiments, the loading dose can be, for instance, about 60 to about 400 mg per day, or about 100 to about 200 mg per day for one day to five weeks. The loading dose can be followed by one or more maintenance doses. Each maintenance dose can be, independently, about from
about 10 mg to about 200 mg per day, more specifically, between about 25 mg and about 150 mg per day, or even more specifically between about 25 mg and about 80 mg per day or between about 25 mg and about 50 mg per day. Maintenance doses can be administered daily and can be administered as single doses, or as divided doses.

[00139] In certain embodiments, a dose of a caspase inhibitor provided herein can be administered to achieve a steady-state concentration of the active ingredient in blood or serum of the subject. The steady-state concentration can be determined by measurement according to techniques available to those of skill or can be based on the physical characteristics of the subject such as height, weight and age. In certain embodiments, a sufficient amount of a compound provided herein is administered to achieve a steady-state concentration in blood or serum of the subject of from about 300 to about 4000 ng/mL, from about 400 to about 1600 ng/mL, or from about 600 to about 1200 ng/mL. Loading doses can be administered to achieve steady-state blood or serum concentrations of about 1200 to about 8000 ng/mL, or about 2000 to about 4000 ng/mL for one to five days. Maintenance doses can be administered to achieve a steady-state concentration in blood or serum of the subject of from about 300 to about 4000 ng/mL, from about 400 to about 1600 ng/mL, or from about 600 to about 1200 ng/mL.

[00140] In certain embodiments, administration of the same compound may be repeated and the administrations may be separated by at least 1 day, 2 days, 3 days, 5 days, 10 days, 15 days, 30 days, 45 days, 2 months, 75 days, 3 months, or 6 months. In other embodiments, administration of the same prophylactic or therapeutic agent may be repeated and the administration may be separated by at least 1 day, 2 days, 3 days, 5 days, 10 days, 15 days, 30 days, 45 days, 2 months, 75 days, 3 months, or 6 months.

[00141] In certain aspects, provided herein are unit dosages comprising a compound, or a pharmaceutically acceptable derivative thereof, in a form suitable for administration. Such forms are described in detail above. In certain embodiments, the unit dosage comprises 1 to 1000 mg, 5 to 250 mg or 10 to 50 mg active ingredient. In particular embodiments, the unit dosages comprise about 1, 5, 10, 25, 50, 100, 125, 250, 500 or 1000 mg active ingredient. Such unit dosages can be prepared according to techniques familiar to those of skill in the art.

**Articles of manufacture**

[00142] The compounds or pharmaceutically acceptable derivatives can be packaged as articles of manufacture containing packaging material, a compound or pharmaceutically
acceptable derivative thereof provided herein, which is used for treatment, prevention or amelioration of elevated MELD scores or its components or elevated Child-Pugh scores or its components, and a label that indicates that the compound or pharmaceutically acceptable derivative thereof is used for treatment, prevention or amelioration of one or more symptoms of elevated MELD scores or its components or elevated Child-Pugh scores or its components.

[00143] The articles of manufacture provided herein contain packaging materials. Packaging materials for use in packaging pharmaceutical products are well known to those of skill in the art. See, e.g., U.S. Patent Nos. 5,323,907, 5,052,558 and 5,033,252. Examples of pharmaceutical packaging materials include, but are not limited to, blister packs, bottles, tubes, inhalers, pumps, bags, vials, containers, syringes, bottles, and any packaging material suitable for a selected formulation and intended mode of administration and treatment. A wide array of formulations of the compounds and compositions provided herein are contemplated.

Kits

[00144] Further provided are kits for use in methods of treatment of elevated MELD scores or its components or elevated Child-Pugh scores or its components. The kits can include a caspase inhibitor or composition thereof, and instructions providing information to a health care provider regarding usage for treating or preventing elevated MELD scores or its components or elevated Child-Pugh scores or its components. Instructions may be provided in printed form or in the form of an electronic medium such as a CD, or DVD, or in the form of a website address where such instructions may be obtained. A unit dose of an or composition thereof, or a caspase inhibitor or composition thereof, can include a dosage such that when administered to a subject, a therapeutically or prophylactically effective plasma level of the compound or composition can be maintained in the subject for at least 1 day. In some embodiments, the compounds or composition can be included as sterile aqueous pharmaceutical compositions or dry powder (e.g., lyophilized) compositions.

Evaluation of the Activity of the Compounds

[00145] The biological activity of the compounds can be demonstrated by methods known to one of skill in the art.

[00146] Multiple outcome measures in circulation and tissue can be used for evaluation. One of these is the measurement of levels of the liver enzyme ALT in the blood. Elevated ALT levels are routinely observed in the blood of patients suffering from a variety of liver diseases. ALT
measurement is a very common and relevant clinical laboratory test for the extent of liver disease in patients. A second measure involves gross and histological evaluation of the extent liver disease. Histology is often done in patients with advanced liver disease to determine the extent of disease. Other important measures include the MELD score components: bilirubin, INR and creatinine. The extent of liver disease can be graded by examining liver samples prepared and evaluated microscopically by trained observers. In certain embodiment, the liver injury can be sufficiently severe as to cause mortality. In certain embodiment, compounds described herein protect against induced liver injury as determined by these parameters.

COMBINATION THERAPY

[00147] In certain embodiments, caspase inhibitors provided herein are administered in combination with one or more agents known to treat patients with elevated MELD scores or its components, elevated Child-Pugh scores or its components, and / or cirrhosis. In certain embodiments, dosages lower than those which have been or are currently being used to treat elevated MELD scores or its components, elevated Child-Pugh scores or its components and / or cirrhosis combination therapies provided herein. For those agents that are approved for clinical use, recommended dosages are described in, for example, Hardman et al, eds., 1996, Goodman & Gilman's The Pharmacological Basis Of Basis Of Therapeutics 9th Ed, Mc-Graw-Hill, New York; Physician's Desk Reference (PDR) 57th Ed., 2003, Medical Economics Co., Inc., Montvale, NJ, which are incorporated herein by reference in their entireties. The dosages given will depend on absorption, inactivation and excretion rates of the drug as well as other factors known to those of skill in the art. It is to be noted that dosage values will also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens and schedules should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions.

[00148] In various embodiments, the compounds provided herein are administered less than 5 minutes apart, less than 30 minutes apart, 1 hour apart, at about 1 hour apart, at about 1 to about 2 hours apart, at about 2 hours to about 3 hours apart, at about 3 hours to about 4 hours apart, at about 4 hours to about 5 hours apart, at about 5 hours to about 6 hours apart, at about 6 hours to about 7 hours apart, at about 7 hours to about 8 hours apart, at about 8 hours to about 9 hours apart, at about 9 hours to about 10 hours apart, at about 10 hours to about 11 hours apart, at about
11 hours to about 12 hours apart, at about 12 hours to 18 hours apart, 18 hours to 24 hours apart, 24 hours to 36 hours apart, 36 hours to 48 hours apart, 48 hours to 52 hours apart, 52 hours to 60 hours apart, 60 hours to 72 hours apart, 72 hours to 84 hours apart, 84 hours to 96 hours apart, or 96 hours to 120 hours part. In certain embodiments, two or more therapies are administered within the same patient visit.

[00149] In certain embodiments, the compounds provided herein and optionally an additional agent are administered to a patient, for example, a mammal, such as a human, in a sequence and within a time interval such that the compounds provided herein can act together with the other agent to provide an increased benefit than if they were administered otherwise. For example, the compounds can be administered at the same time or sequentially in any order at different points in time; however, if not administered at the same time, they should be administered sufficiently close in time so as to provide the desired therapeutic or prophylactic effect. In one embodiment, the compounds provided herein and optionally an additional agent exert their effect at times which overlap. Each compound can be administered separately, in any appropriate form and by any suitable route. In other embodiments, the compounds provided herein are administered before, concurrently or after administration of the first compound.

[00150] The caspase inhibitor compounds provided herein and optionally one or more additional agents can act additively or synergistically. In one embodiment, the caspase inhibitor compounds provided herein can act additively or synergistically with another agent. In one embodiment, the compounds provided herein are administered concurrently, optionally with another agent, with in the same pharmaceutical composition. In another embodiment, the compounds provided herein are administered concurrently, optionally with another agent, in separate pharmaceutical compositions. In still another embodiment, the compounds provided herein are administered with another agent, prior to or subsequent to administration of the third agent. Also contemplated are administration of the compounds provided herein by the same or different routes of administration, e.g., oral and parenteral.

[00151] In certain embodiments, the additional agents administered in combination with caspase inhibitors according to the methods provided herein can include-products currently used in the treatment of patients with elevated MELD scores or its components elevated Child-Pugh scores or its components and analogs or derivatives thereof as understood by those of skill in the art.
In certain embodiments, the additional agents administered in combination with caspase inhibitors according to the methods provided herein can include, but are not limited to, any compounds currently in preclinical or clinical development for treatment of elevated MELD scores or its components, elevated Child-Pugh scores or its components and/or cirrhosis: Furosemide, Spironolactone, Lactulose, Rifaximin, Sirtu zumab (GS-6624) by Gilead, Sorafenib by Bayer and Onyx, Serelaxin (RLX030) by Norvartis, Timolol, NCX-1000, Terlipressin, NGM282, LUM001, and analogs or derivatives thereof as understood by those of skill in the art. The compounds provided herein can also be administered in combination with antibiotics, antiviral compounds, antifungal agents or other pharmaceutical agents administered for the treatment of infections: rifaximin, neomycin, cefotaximine, ciprofloxacin, norfloxacin, lactulose, and analogs or derivatives thereof as understood by those of skill in the art. It is understood that the foregoing detailed description and accompanying examples are merely illustrative, and are not to be taken as limitations upon the scope of the subject matter. Various changes and modifications to the disclosed embodiments will be apparent to those skilled in the art. Such changes and modifications, including without limitation those relating to the chemical structures, substituents, derivatives, intermediates, syntheses, formulations and/or methods of use provided herein, may be made without departing from the spirit and scope thereof. U.S. patents and publications referenced herein are incorporated by reference.

6. EXAMPLES

EXAMPLE 1.

Emricasan (IDN-6556) was prepared as described in Linton S. et al. J. Med Chem. 2005;48:6779.

Part A: Methyl 2-[N-(2-tert-Butylphenylamino)]-2-oxoacetate

A solution of 2-tert-butylaniline (57 mL, 54.5 g, 366 mmol), triethylamine (56 mL, 402 mmol), and methylene chloride (370 mL) was cooled to 0°C (ice bath) and stirred under nitrogen. An addition funnel was charged with methyl 2-chloro-2-oxoacetate (50 g, 408 mmol) which was then added dropwise over 20 minutes to the stirred solution causing a significant exotherm. After addition complete, the resulting suspension was stirred for 1 hr. The suspension was then concentrated under vacuum, which is then taken up in ethyl acetate and partitioned with water. The aqueous layer was washed twice with ethyl acetate, and the combined organic layers were then extracted with 5% aqueous potassium bisulfate, followed by saturated sodium
chloride, and then dried over magnesium sulfate and concentrated under reduced pressure. The resulting oil was then dried overnight, then recrystallized from 3:1 hexanes/toluene (two crops) to give the title compound as a white crystalline solid (60.43 g, 70%).

Part B: N-3-tert-Butylphenylamino)oxamic acid

[00157] To a solution of methyl 2-[N-(2-tert-Butylphenylamino)]-2-oxoacetate (59.8 g, 254 mmol) in 1,4-dioxane (600 mL) was slowly added 1N lithium hydroxide (300 mL, 300 mmol). The solution was stirred for 1 hour. The solution was then acidified dropwise with concentrated HCl (12M, 25.0 mL, 300 mmol), and the resulting solution was extracted with ethyl acetate (3 times) and the combined organic extracts were then washed with saturated sodium chloride, dried over magnesium sulfate, and concentrated under vacuum and recrystallized from ethyl acetate/hexanes to yield the title compound (32.55 g, 58%)

Part C: [(N-Benzylxocarbonyl)Alaninyl]Aspartic Acid, [beta]-tert-Butyl Ester

[00158] To a suspension of aspartic acid b-tert-butyl ester (3.784 g, 20 mmol) in dimethylformamide (150 mL) at room temperature under nitrogen was added bis(trimethylsilyl)- trifluoroacetamide (10.6 mL, 40 mmol). After stirring at room temperature for 30 min, the resulting clear solution was treated with (N-benzyloxacarbonyl) alanine N-hydroxysuccinimide ester (6.406 g, 20 mmol). After stirring at room temperature for 18 hours, the mixture was treated with water (20 mL), stirred for 15 minutes and then partitioned between ethyl acetate & water. The organic phase was washed with water, 5% potassium bisulfate and saturated sodium chloride solutions, dried over anhydrous sodium sulfate, and evaporated to dryness. The residue was then taken up in ethyl ether and extracted with saturated sodium bicarbonate. The aqueous extract was acidified (pH 2.0) with concentrated HCl and extracted with ethyl acetate. The ethyl acetate extract was washed with saturated sodium chloride solution, dried over anhydrous sodium Part D: (3S,4RS)-3-[(N-Benzylxocarbonyl)Alaninyl]Amino-5-Bromo-4-Oxopentanoic Acid tert-Butyl Ester

[00159] Part D: (3S,4RS)-3-[(N-Benzylxocarbonyl)Alaninyl]Amino-5-Bromo-4-Oxopentanoic Acid tert-Butyl EsterA solution of [(N-benzyloxacarbonyl)alaninyl]aspartic acid, beta-tert-butylester (5.0 g, 12.7 mmol) and N-methylmorpholine (2.05 g, 2.23 mL, 20.3 mmol) in tetrahydrofuran (65 mL) at -10°C. (NaCl/ice bath) under nitrogen was treated dropwise with isobutylchloroformate (2.6 g, 2.47 mL, 19.04 mmol). After stirring at -10°C for 20 minutes, the mixture was filtered (sintered glass) into a pre-cooled receiver (ice bath) washing the filter cake
with additional tetrahydrofuran (approx. 48 mL). The combined filtrate was treated with excess
diazomethane/ethyl ether solution (prepared from 4.67 g, 31.73 mmol of 1-methyl-3-nitro-1-
nitrosoguanidine, 34 mL 40% KOH/85 ml ethyl ether) at OoC (ice bath) under nitrogen. After
stirring at OoC for 15 minutes and at room temperature for 30 minutes, the reaction mixture was
again cooled to OoC and treated with 48% HBr in acetic acid (34 mL, 204 mmol)/acetic acid (34
mL). After stirring at OoC for 15 minutes and at room temperature for 30 minutes, the mixture
was partitioned between ethyl acetate & water. The organic phase was washed successively with
water, saturated sodium bicarbonate, and saturated sodium chloride; dried over anhydrous sodium
sulfate and evaporated to dryness and purified by flash chromatography on silica gel
evaporating with ethyl acetate-hexane (1:2) to give the title compound as a white foam (3.12 g, 52%).

Part E: (3S,4RS)-3-[(N-Benzoyloxy carbonyl)Alaninyl]Amino-5-(2',3',5',6'-
Tetrafluorophenoxy)-4-Oxopentanoic Acid tert-Butyl Ester

[00160] To a solution of (3S)-3-[(N-benzoyloxy carbonyl)alaninyl]amino-5-bromo-4-
oxopentanoic acid tert-butyl ester (0.167 g, 0.355 mmol) and 2,3,5,6-tetrafluorophenol (0.071 g,
0.426 mmol) in N,N-dimethylformamide (2 mL) at room temperature under nitrogen was added
potassium fluoride (0.082 g, 1.42 mmol). After stirring at room temperature for 4 hrs, the
mixture was diluted with ethyl acetate, washed with saturated sodium bicarbonate and saturated
sodium chloride solutions, dried over anhydrous sodium sulfate and evaporated to dryness. The
 crude material (0.144 g) was taken on to the next step without purification.

Part F: (3S,4RS)-3-[(N-Benzoyloxy carbonyl)Alaninyl]Amino-5-(2',3',5',6'-
Tetrafluorophenoxy)-4-Hydroxypentanoic Acid tert-Butyl Ester

[00161] To a solution of crude (3S)-3-[(N-benzoyloxy carbonyl)alaninyl]amino-5-(2',3',5',6'-
tetrafluorophenoxy)-4-oxopentanoic acid tert-butyl ester (0.144 g, 0.26 mmol) in 1:1
methanol/tetrahydrofuran (4mL) at OoC under nitrogen was added sodium borohydride (0.040 g,
1.04 mmol). After stirring at OoC for 1 hour, the mixture was concentrated and the residue
partitioned between ethyl acetate-half saturated ammonium chloride solution (50% saturated
ammonium chloride/50% water). The organic phase was washed with saturated sodium
bicarbonate and saturated sodium chloride solutions, dried over anhydrous sodium sulfate and
 evaporated to dryness. The residue was purified by flash chromatography on silica gel eluting
with ethyl acetate-hexanes (1:2) to give the title compound (0.142 g, 78%) as a white foam
Part G: (3S,4RS)-3-(Alaninyl)Amino-5-(2',3',5',6'-Tetrafluorophenoxy)-4-Hydroxypentanoic Acid tert-Butyl Ester

[00162] To a solution of (3S, 4RS)-3-[(N-benzyloxy carbonyl)valinyl]amino-5-(2',3',5',6'-tetrafluorophenoxy)-4-hydroxypentanoic acid tert-butyl ester (0.12 g, 0.021 mmol) in methanol (10 mL) was added 10% Pd-C (0.017 g) and resulting mixture stirred under a hydrogen atmosphere (1 atmosphere, balloon) for 2 hrs. The mixture was filtered through Celite, washing the filter cake with methanol. The combined filtrates evaporated to dryness to yield the crude title compound as a colorless, viscous oil (0.066 g, 70%) which was taken on to the next step without purification.

Part H: (3S,4RS)-3-[N-(N'-(2-tert-Butylphenyl)Oxamyl)Alaninyl]Amino-5-(2',3',5',6'-Tetrafluorophenoxy)-4-Hydroxypentanoic Acid tert-Butyl Ester

[00163] To a solution of N-(2-tert-butylphenyl)oxamic acid (0.041 g, 0.19 mmol) in methylene chloride (6.0 mL) at OoC under nitrogen was added hydroxybenzotriazole hydrate (0.030 g, 0.261 mmol) followed by addition of 1-ethyl-3-(3',3'-dimethyl- l'-aminopropyl)-carbodiimide hydrochloride (EDCI) (0.050 g, 0.26 mmol). After stirring at OoC for 10 min, the mixture was treated with (3S,4RS)-3-(alaninyl)amino-5-(2',3',5',6'-tetrafluorophenoxy)-4-hydroxypentanoic acid tert-butyl ester (0.079 g, 0.19 mmol) and N-methyl morpholine (NMM) (22 mL, 0.20 mmol). After stirring at room temperature for 16 hrs, the mixture was partitioned between ethyl acetate & water. The organic phase was washed with water, 5% potassium bisulfate, saturated sodium bicarbonate and saturated sodium chloride solutions, dried over anhydrous sodium sulfate and evaporated to give the crude title compound (0.090 g, 77%) as a viscous oil.

Part I: (3S)-3-[N-(N'-(2-tert-Butylphenyl) Oxamyl) Alaninyl]Amino-5-(2',3',5',6'-Tetrafluorophenoxy)-4-Oxopentanoic Acid tert-Butyl Ester

[00164] To a solution of (3S,4RS)-3-[N-(N'- (2-tert-butylphenyl) oxamyl) alaninyl]amino-5-(2',3',5',6'-tetrafluorophenoxy)-4-hydroxypentanoic acid tert-butyl ester (0.0.092 g, ca 0.15 mmol) in methylene chloride (6.5 mL) at room temperature under nitrogen was added iodobenzene diacetate (0.188 g, 0.58 mmol) followed by a catalytic amount of 2,2,6,6-tetramethyl-1- piperidinyloxy free radical (TEMPO, 0.0046 g, 0.03 mmol). After stirring at room temperature for 16 hrs, the mixture was partitioned between ethyl acetate & water. The organic phase was washed with saturated sodium bicarbonate and saturated sodium chloride solutions,
dried over anhydrous sodium sulfate and evaporated to dryness. The residue (0.096 g) was purified by preparative layer chromatography on silica gel eluting with ethyl acetate-hexane (3:7) to give the title compound (0.071 g, 77%) as a colorless glass.


[00165] To a solution of (3S)-3-[N-(N'-(2-tert-butylphenyl)oxamyl)alaninyl]amino-5-(2',3',5',6'-tetrafluorophenoxy)-4-oxopentanoic acid, tert-butyl ester (0.071 g, 0.1 mmol) and anisole (0.05 mL) in methylene chloride (2.5 mL) at room temperature under nitrogen was added trifluoroacetic acid (1.5 mL). The resulting clear solution was stirred at room temperature for 1 hr, evaporated to dryness and chased with toluene-methylene chloride (1:1). The residue (0.061 g) was purified by preparative layer chromatography on silica gel eluting with methanol-methylene chloride (1:9) to give the title compound (0.044 g, 69%) as a colorless glass.

EXAMPLE 2

Results from human clinical trial in patients with liver cirrhosis

[00166] A double-blind, placebo-controlled Phase 2 clinical trial was conducted at 26 U.S. sites and enrolled 86 patients with liver cirrhosis due to different etiologies, mild to moderate liver impairment as determine by Child Pugh classification and baseline MELD scores of 11 to 18. Patients were randomized 1:1 to receive either 25 mg of emricasan (IDN-6556) or placebo orally twice daily for three months. Endpoints included a change from baseline in cCK18 and changes from baseline in MELD and Child-Pugh scores, which are composite scores of laboratory parameters associated with liver synthetic and excretory function, such as serum albumin levels, international normalized ratio (INR) and total bilirubin levels.

[00167] Among the 86 subjects enrolled and receiving study drug, liver cirrhosis etiologies included alcohol (39%), hepatitis C virus (29%), non-alcoholic steatohepatitis, or NASH (23%), and other causes (9%). Baseline MELD scores were ≤14 in 78% of enrolled subjects and >15 in 22% of enrolled subjects. Baseline Child-Pugh status was A in 43% of subjects and B in 56% of subjects.

Overall Patient Population Results

[00168] In patients, emricasan treatment showed a statistically significant reduction in caspase-cleaved cytokeratin 18 (cCK18) vs. placebo (p=0.04) in the overall patient population when adjusted for differences between treatment and placebo groups in baseline Model for End-
Two key clinically relevant measures of liver function, MELD score and Child-Pugh-Turcotte (Child-Pugh) score, along with other key liver function parameters, demonstrated favorable trends vs. placebo in the overall patient population after three months of treatment. The overall trends were driven by statistically significant improvements in MELD and Child-Pugh scores in a high medical need subgroup of patients with baseline MELD scores ≥ 15, the established prerequisite for listing a patient for liver transplant.
Additional analyses showed that the treatment effect was broadly evident after three months of treatment in this subgroup. Six of nine patients treated with emricasan achieved at least a 2 point reduction in MELD score vs two of ten patients treated with placebo. Four of nine patients receiving emricasan achieved a reduction in MELD score to below 14 vs. one out of ten patients receiving placebo. Four of nine patients receiving emricasan had at least a one point reduction in Child Pugh score vs. two of ten receiving placebo. Four out of ten patients receiving placebo had a least a one point increase in Child-Pugh score whereas none of nine patients receiving emricasan had an increase in Child-Pugh score.

The embodiments described above are intended to be merely exemplary, and those skilled in the art will recognize, or will be able to ascertain using no more than routine experimentation, numerous equivalents of specific compounds, materials, and procedures. All such equivalents are considered to be within the scope of the claimed subject matter and are encompassed by the appended claims.
WHAT IS CLAIMED IS:

1. A method of treating a liver disease in a subject comprising administering to the subject a therapeutically effective amount of a caspase inhibitor, wherein the subject has a MELD score of at least 10.

2. The method of claim 1, wherein the MELD score is reduced by at least 1 point.

3. A method of treating a liver disease in a subject comprising:
   selecting a subject with an elevated MELD score or elevated components of MELD; and
   administering a caspase inhibitor to the subject.

4. The method of claim 3, wherein the MELD score is reduced by a least 1 point.

5. The method of any one of claims 1-4, wherein the MELD score is above 14.

6. The method of any one of claims 1-5, wherein the MELD score is sustained.

7. The method of any one of claims 1-6, wherein the MELD score is reduced.

8. The method of any one of claims 1-7, wherein the MELD score is reduced by at least 1 point.

9. The method of any one of claims 1-8, wherein the MELD score is reduced by at least 2 points.

10. The method of any one of claims 1-9, wherein the MELD score is reduced below 15.

11. A method of treating a liver disease in a subject comprising administering to the subject a therapeutically effective amount of a caspase inhibitor, wherein the subject has an elevated Child-Pugh score or its components.

12. The method of any one of claims 1-11, wherein the liver disease is cirrhosis.

13. A method of using a caspase inhibitor comprising:
   selecting a subject with an elevated Child-Pugh score or an elevated component of Child-Pugh; and
   administering a caspase inhibitor to the subject.

14. The method of any one of claims 11-13, wherein the Child-Pugh score is above Class A.
15. The method of any one of claims 11-14, wherein the Child-Pugh score is above Class B.
16. The method of any one of claims 11-15, wherein the Child-Pugh score is sustained.
17. The method of any one of claims 11-15, wherein the Child-Pugh score is reduced.
18. The method of any one of claims 11-15, wherein the Child-Pugh score is reduced by at least 1 point.
19. The method of any one of claims 11-15, wherein the Child-Pugh score is reduced by at least 2 points.
20. The method of any one of claims 11-15, wherein the Child-Pugh score is reduced below Class B.
21. The method of any one of claims 11-15, wherein the Child-Pugh score is reduced below Class C.
22. The method of any one of claims 1-21, wherein the caspase inhibitor is selected from:
or a pharmaceutically acceptable derivative thereof.

23. A method according to any one of claims 1-21, wherein the caspase inhibitor is selected from:
or a pharmaceutically acceptable derivative thereof.

24. A method according to any one of claims 1-21, wherein the caspase inhibitor is selected from:

or a pharmaceutically acceptable derivative thereof.

25. The method according to claim 24, wherein the caspase inhibitor is:

or a pharmaceutically acceptable derivative thereof.

26. A method according to claim 24, wherein the caspase inhibitor is:
or a pharmaceutically acceptable derivative thereof.

27. A method according to any one of claims 1-21, wherein the caspase inhibitor is selected from:

28. A method according to claim 27, wherein the caspase inhibitor is:

29. A method according to any one of claims 1-27 wherein the caspase inhibitor is:
30. The method of any one of claims 1-29, wherein the subject has been pre-treated with one or more other medications for liver disease.

31. The method of claim 30, wherein the one or more other medications are selected from among Furosemide, Spironolactone, Lactulose, Rifaximin, Propranolol, Nadolol, Carvedilol, Simtuzumab (GS-6624), Sorafenib, Serelaxin (RLX030), Timolol, NCX-1000, Terlipressin, NGM282 and LUM001, and analogs or derivatives thereof.

32. The method of any one of claims 1-31, wherein the subject has failed therapy for liver disease.

33. The method of any one of claims 1-31, wherein the subject has failed therapy for elevated MELD or its components.

34. The method of any one of claims 1-31, wherein the subject has failed therapy for liver cirrhosis.

35. A method of lowering or sustaining a MELD score or its components in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a caspase inhibitor, wherein the amount is effective to mitigate liver damage in the subject.

36. A method of treating lowering or sustaining a MELD score or its components in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a caspase inhibitor, wherein the amount is effective to lower elevated levels of INR, bilirubin or creatinine.

37. A method of treating lowering or sustaining a MELD score or its components in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a caspase inhibitor, wherein the amount is effective to lower elevated levels of liver enzyme in the subject.
38. The method of any one of claims 1-37, wherein the subject has failed therapy for elevated Child-Pugh or its components.

39. The method of any one of claims 1-37, wherein the subject has failed therapy for liver cirrhosis.

40. A method of treating lowering or sustaining a Child-Pugh score or its components in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a caspase inhibitor, wherein the amount is effective to mitigate liver damage in the subject.

41. A method of treating lowering or sustaining a Child-Pugh score or its components in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a caspase inhibitor, wherein the amount is effective to lower elevated levels of Child-Pugh components.

42. A method of treating lowering or sustaining a Child-Pugh score or its components in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a caspase inhibitor, wherein the amount is effective to lower elevated levels of liver enzyme in the subject.

43. The method of claim 42, wherein the levels are of total liver enzyme, alanine aminotransferase or aspartate aminotransferase.

44. The method of claim 42 or claim 43, wherein the elevated level of liver enzyme is lowered by about 100% to about 1%.

45. The method of claim 44, wherein the elevated level of liver enzyme is lowered by at least 99%, at least 90%, at least 80%, at least 70%, at least 60%, at least 50%, at least 40%, at least 30%, at least 20%, at least 10%, at least 5%, at least 2% or at least 1%.

46. The method of any one of claims 30-45, wherein the caspase inhibitor is selected from:
or a pharmaceutically acceptable derivative thereof.

47. A method according to any one of claims 30-45, wherein the caspase inhibitor is selected from:
or a pharmaceutically acceptable derivative thereof.

48. A method according to any one of claims 30-45, wherein the caspase inhibitor is selected from:

or a pharmaceutically acceptable derivative thereof.

49. The method of claim 48, wherein the caspase inhibitor is:

50. A method according to claim 48, wherein the caspase inhibitor is:
or a pharmaceutically acceptable derivative thereof.

51. A method according to any one of claims 30-45, wherein the caspase inhibitor is selected from:

or a pharmaceutically acceptable derivative thereof.

52. A pharmaceutical composition, comprising a caspase inhibitor and a pharmaceutically acceptable excipient, wherein the caspase inhibitor is in an amount that is therapeutically effective in the treatment of an elevated MELD score or its components.

53. A pharmaceutical composition, comprising a caspase inhibitor and a pharmaceutically acceptable excipient, wherein the caspase inhibitor is in an amount that is therapeutically effective in the treatment of an elevated Child-Pugh score or its components.

54. The composition of claim 52 or 53 that is formulated for oral administration.

55. The composition of claim 52 or 53 that is formulated for nasogastric administration.

56. The composition of claim 52 or 53, wherein the caspase inhibitor is in amount of about 1 mg to about 100 mg.

57. The composition of claim 52 or 53, wherein the caspase inhibitor is in amount of about 25 mg to about 50 mg.

58. The composition of any one of claims 1-57, wherein the caspase inhibitor is administered at least once daily.
59. The composition according to any one of claims 52-58, wherein the caspase inhibitor is selected from:

![Chemical structures](image1.png)

or a pharmaceutically acceptable derivative thereof.

60. The composition according to any of claim 52-58, wherein the caspase inhibitor is selected from:
61. The composition according to any of claim 52-58, wherein the caspase inhibitor is selected from:

or a pharmaceutically acceptable derivative thereof.

62. The composition according to claim 61, wherein the caspase inhibitor is selected from:

or a pharmaceutically acceptable derivative thereof.

61
63. A kit, comprising a caspase inhibitor and a second pharmacologically active substance, wherein the second pharmacologically active substance is for treating a subject with an elevated MELD score or its components.

64. A kit, comprising a caspase inhibitor and a second pharmacologically active substance, wherein the second pharmacologically active substance is for treating a subject with an elevated Child-Pugh score or its components.

65. The kit of claim 63 or 64, wherein the caspase inhibitor is in a composition comprising the caspase inhibitor and a pharmaceutically acceptable excipient.

66. The kit of claim 63 or 64, wherein the second pharmacologically active substance is in a composition comprising the caspase inhibitor and a pharmaceutically acceptable excipient.

67. The kit of any one of claims 63-66, wherein the caspase inhibitor and the second pharmacologically active substance are in a single composition.

68. The kit of any one of claims 63-66, wherein the caspase inhibitor and the second pharmacologically active substance are in two different compositions.

69. The kit of any one of claims 63-68, wherein the caspase inhibitor is selected from:
or a pharmaceutically acceptable derivative thereof.

70. A kit according to any one of claims 63-68, wherein the caspase inhibitor is selected from:
or a pharmaceutically acceptable derivative thereof.

71. A kit according to any one of claims 63-68, wherein the caspase inhibitor is selected from:

or a pharmaceutically acceptable derivative thereof.

72. The kit according to claim 71, wherein the caspase inhibitor is:

or a pharmaceutically acceptable derivative thereof.

73. A kit according to any one of claims 63-72, wherein the second pharmacologically active substance is selected from: Furosemide, Spironolactone, Lactulose, Rifaximin, Propranolol, Nadolol, Carvedilol, Sintuzumab (GS-6624), Sorafenib, Serelaxin (RLX030), Timolol, NCX-1000, Terlipressin, NGM282 and LUMOOI, and analogs or derivatives thereof.
74. A kit according to any one of claims 63-72, wherein the second pharmacologically active substance is a compound for the treatment of an elevated MELD score.

75. A kit according to any one of claims 63-72, wherein the second pharmacologically active substance is a compound for the treatment of an elevated Child-Pugh score.

76. The kit of any one of claims 63-72, wherein the second pharmacologically active substance is a compound for the treatment of cirrhosis.

77. The method of any one of claims 1-51, further comprising administering a second pharmacologically active substance, wherein the second pharmacologically active substance is a compound for the treatment of liver disease.

78. The method of claim 77, wherein the second pharmacologically active substance is a compound for the treatment of cirrhosis.

79. The method of claim 77 or claim 78, wherein the second pharmacologically active substance is administered simultaneously with the caspase inhibitor.

80. The method of claim 77 or claim 78, wherein the second pharmacologically active substance is administered sequentially in any order relative to the caspase inhibitor.

81. The method of any one of claims 78-80, wherein the second pharmacologically active substance is for the treatment of cirrhosis.

82. The method of any one of claims 78-81, wherein the second pharmacologically active substance is selected from: Furosemide, Spironolactone, Lactulose, Rifaximin, Simtuzumab (GS-6624), Sorafenib, Serelaxin (RLX030), Timolol, NCX-1000, Terlipressin, NGM282 and LUM001, and analogs or derivatives thereof.

83. The method of any one of claims 78-81, wherein the second pharmacologically active substance is for the treatment of portal hypertension or elevated MELD score.

84. The method of claim 83, wherein the second pharmacologically active substance is selected from Propranolol, Nadolol, Carvedilol and Timolol.

85. The method of any one of claims 1-51 and 77-84, wherein the subject has failed therapy for elevated MELD score.

86. The method of any one of claims 1-51 and 77-84, wherein the subject has failed therapy for liver cirrhosis.
87. A method of treatment, comprising selecting a subject with a liver disease and an elevated MELD score, and administering a therapeutically effective amount of a caspase inhibitor to said subject, the amount being effective to mitigate liver disease and/or lower or sustain the MELD score.

88. The method of claim 87, wherein the MELD score is greater than 10.

89. The method of claim 87, wherein the MELD score is greater than 14.

90. The method of any one of claims 87-89, wherein the administration of the caspase inhibitor lowers the one or more components of the Child-Pugh score.

91. The method of any one of claims 87-90, wherein the administration of the caspase inhibitor lowers the MELD score by 10% or more.

92. The method of any one of claims 87-91, wherein the administration of the caspase inhibitor lowers the MELD score by at least 1 point.

93. A method of treating liver cirrhosis and preventing an elevated MELD score comprising administering, to a subject in need thereof, a therapeutically effective amount of a caspase inhibitor, whereby the liver cirrhosis is mitigated and/or the risk of occurrence of elevated MELD score is reduced.

94. The method of claim 93, wherein the subject has an elevated level of INR, creatinine and/or bilirubin and wherein administering the caspase inhibitor lowers the level of INR, creatinine and/or bilirubin in the subject.

95. The method of any one of claims 1-51 and 77-94, wherein the subject has failed therapy for elevated Child-Pugh score.

96. The method of any one of claims 1-51 and 77-94, wherein the subject has failed therapy for liver cirrhosis.

97. A method of treatment, comprising selecting a subject with a liver disease and elevated Child-Pugh score, and administering a therapeutically effective amount of a caspase inhibitor to said subject, the amount being effective to mitigate liver disease and/or lower or sustain said Child-Pugh score.

98. The method of claim 97, wherein the Child-Pugh score is greater than Class A.

99. The method of claim 97, wherein the Child-Pugh score is greater than Class B.

100. The method of any one of claims 97-99, wherein the administration of the caspase inhibitor lowers the one or more components of the Child-Pugh score.
101. The method of any one of claims 97-100, wherein the administration of the caspase inhibitor lowers the Child-Pugh score by 10% or more.

102. The method of any one of claims 97-101, wherein the administration of the caspase inhibitor lowers the Child-Pugh score by at least 1 point.

103. A method of treating liver cirrhosis and preventing an elevated Child-Pugh score comprising administering, to a subject in need thereof, a therapeutically effective amount of a caspase inhibitor, whereby the liver cirrhosis is mitigated and/or the risk of occurrence of elevated Child-Pugh score is reduced.
### A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/00 A61K31/198 A61K31/341 A61P1/16

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)

- A61K
- A61P

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

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:
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Date of the actual completion of the international search

20 March 2017

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Zellen, Eveline
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<td>EKERT P G ET AL: &quot;CASPASE INHIBITORS&quot;, CELL DEATH AND DIFFERENTIATION, NATURE PUBLISHING GROUP, GB, vol. 6, no. 11, 1 November 1999 (1999-11-01), pages 1081-1086, XP008011543, ISSN: 1350-9047, DOI: 10.1038/SJ.CDD.4400594</td>
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