



(86) Date de dépôt PCT/PCT Filing Date: 2007/02/28  
(87) Date publication PCT/PCT Publication Date: 2008/09/12  
(85) Entrée phase nationale/National Entry: 2009/08/27  
(86) N° demande PCT/PCT Application No.: US 2007/062968  
(87) N° publication PCT/PCT Publication No.: 2008/108842

(51) Cl.Int./Int.Cl. *C07D 237/32* (2006.01),  
*A61K 31/502* (2006.01)  
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(54) Titre : ANALOGUES DE LISOFYLLINE, ET PROCEDES D'UTILISATION  
(54) Title: LISOFYLLINE ANALOGS AND METHODS FOR USE

(57) Abrégé/Abstract:

Analogs of a Lisofylline (LSF), and synthetic methods for the preparation of such analogs are provided. The analogs of LSF provided have the ability to protect cell viability, particularly the ability to protect pancreatic  $\beta$ -cells.

## (12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau(43) International Publication Date  
12 September 2008 (12.09.2008)

PCT

(10) International Publication Number  
**WO 2008/108842 A1**

- (51) International Patent Classification:  
C07D 237/32 (2006.01) A61K 31/502 (2006.01)
- (21) International Application Number:  
PCT/US2007/062968
- (22) International Filing Date:  
28 February 2007 (28.02.2007)
- (25) Filing Language: English
- (26) Publication Language: English
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- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LI, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:  
— with international search report

WO 2008/108842 A1

(54) Title: LISOFYLLINE ANALOGS AND METHODS FOR USE

(57) Abstract: Analogs of a Lisofylline (LSF), and synthetic methods for the preparation of such analogs are provided. The analogs of LSF provided have the ability to protect cell viability, particularly the ability to protect pancreatic  $\beta$ -cells.

## LISOFYLLINE ANALOGS AND METHODS FOR USE

### Government Funding

[0001] The invention described herein was made with government support  
5 under Grant Number DK 63521 awarded by the National Institute of Health.  
The United States Government has certain rights in the invention.

### Background of the Invention

[0002] Type 1 diabetes is an autoimmune disorder which results from the  
immune-mediated inflammatory destruction of insulin-producing  $\beta$ -cells in  
10 pancreatic islets. Although the specific pathogenic mechanisms in Type 1  
diabetes are not known, it is believed that activated T cells and macrophages are  
required for the initiation. Once activated, macrophages secrete several  
inflammatory cytokines, such as interleukin 1 $\beta$ (IL-1 $\beta$ ), interleukin 12 (IL-12)  
and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), and trigger interferon- $\gamma$  (IFN- $\gamma$ ) production  
15 from activated T cells (see Z. D. Yang, M. Chen, R. Wu, M. McDuffie, J. L.  
Nadler, *Diabetologia*, **2002**, 45, 1307-1314). These cytokines are reported to be  
cytotoxic to  $\beta$  cells and enhance Th1-mediated inflammatory responses, which  
are believed to be responsible for the  $\beta$  cell destruction (see M. Chen, Z. D.  
Yang, R. Wu, J. L. Nadler, *Endocrinology*, **2002**, 143(6), 2341-2348).

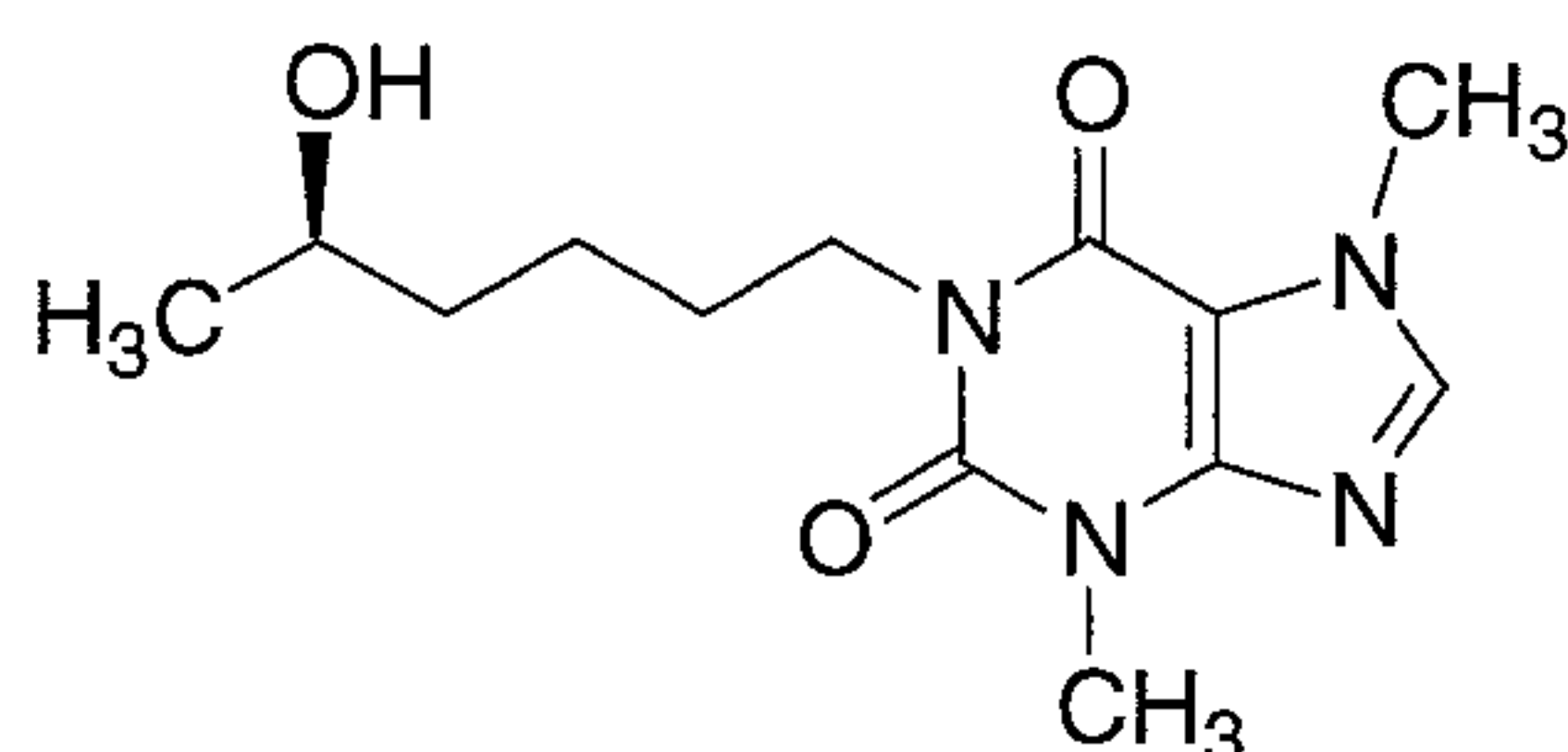
20 [0003] The anti-inflammatory compound Lisofylline (LSF; 1-(5-R-hydroxy-  
hexyl)-3,7-dimethylxanthine)) has been shown to be able to protect  $\beta$ -cells from  
multiple inflammatory cytokine-mediated injuries by its ability to maintain  
insulin secretory capability and cell viability.

[0004] Agents such as Lisofylline may have clinical utility in preventing  $\beta$ -  
25 cell damage during the development of Type 1 diabetes. This hypothesis is  
supported by the studies that showed Lisofylline could significantly reduce  
spontaneous Type 1 diabetes development in the non-obese diabetic (NOD)  
mouse (see Yang). However, the disadvantages of Lisofylline may limit its  
clinical development because it is not orally bioavailable and has relatively weak  
30 potency. The structure of LSF is Formula I.

[0005] Currently, there is a need for novel, potent, and selective agents based on the Lisofylline backbone which have enhanced potency, selectivity, and oral bioavailability.

### Summary

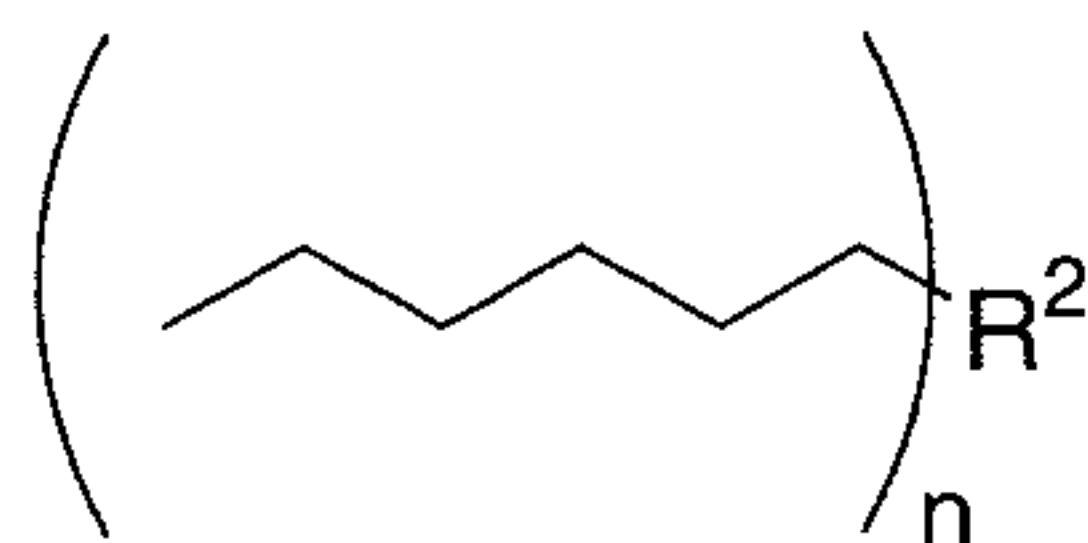
5 [0006] The present invention provides analogs of a Lisofylline (LSF), and synthetic methods for the preparation of such analogs. The analogs can have greater potency and oral bioavailability than LSF. The analogs have the active side chain moiety (5-R-hydroxyhexyl) of LSF. The invention also includes derivatives of LSF. LSF has formula I:



10

### I.

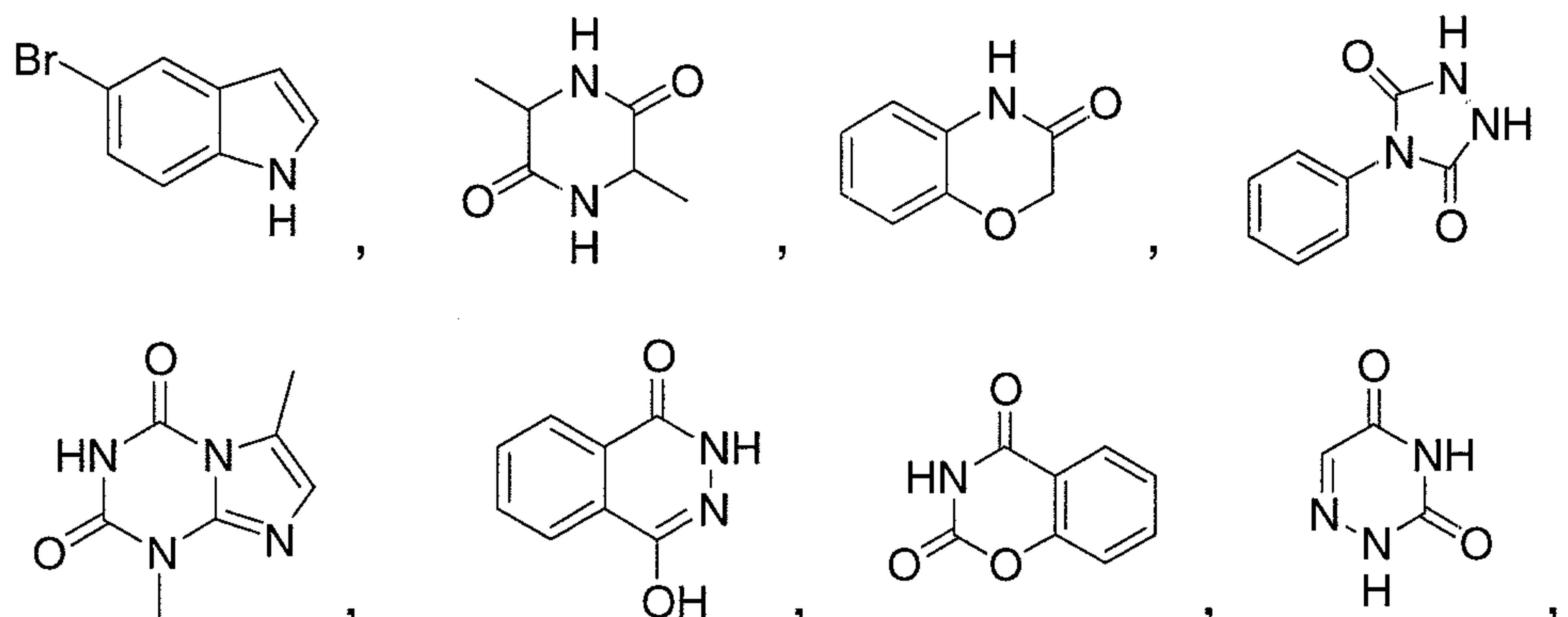
[0007] The disclosed compounds can be substituted with a variety of nitrogen-contained heterocyclic compounds or on the side chain. Accordingly, the invention provides compounds having formula II:

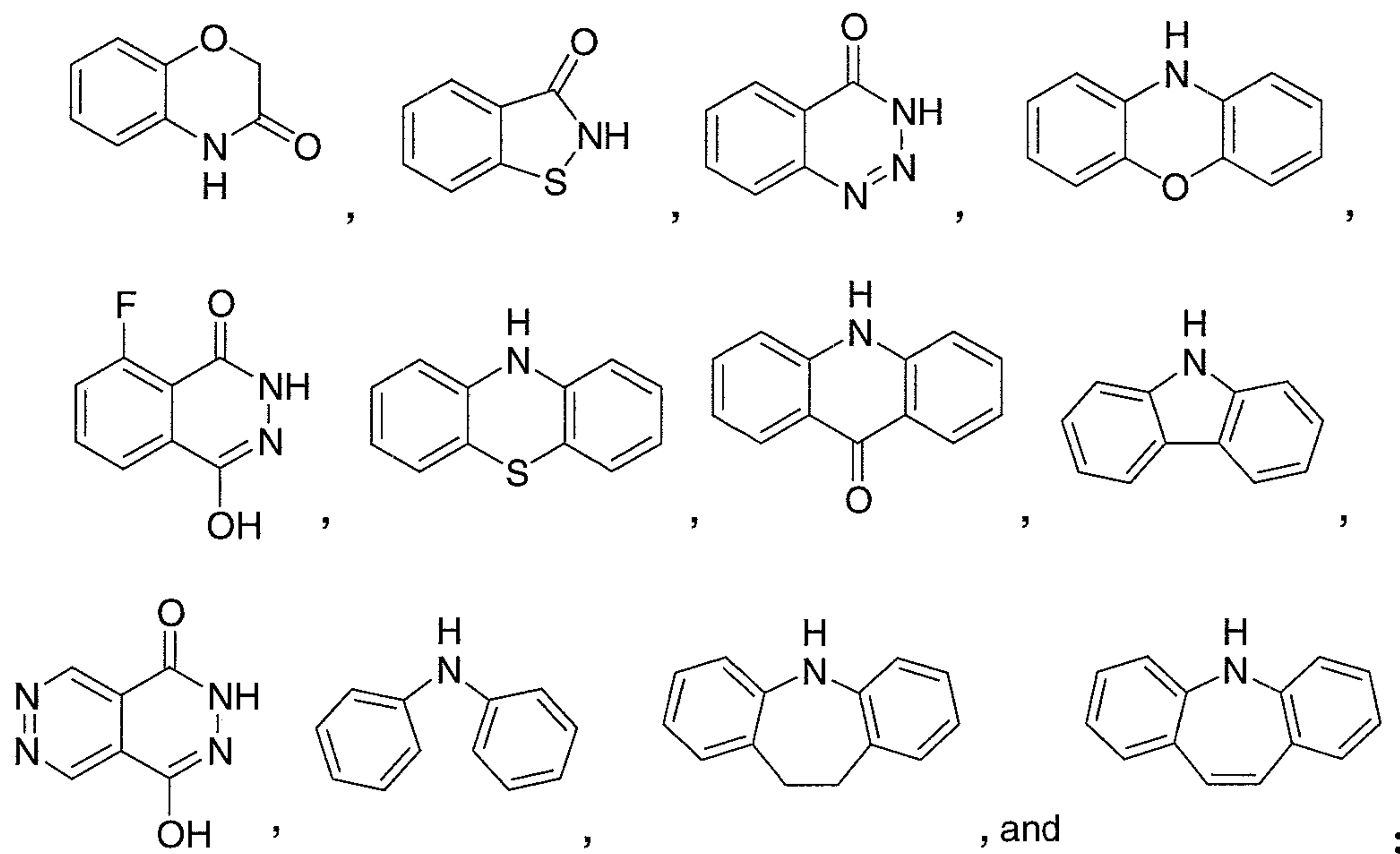


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### II

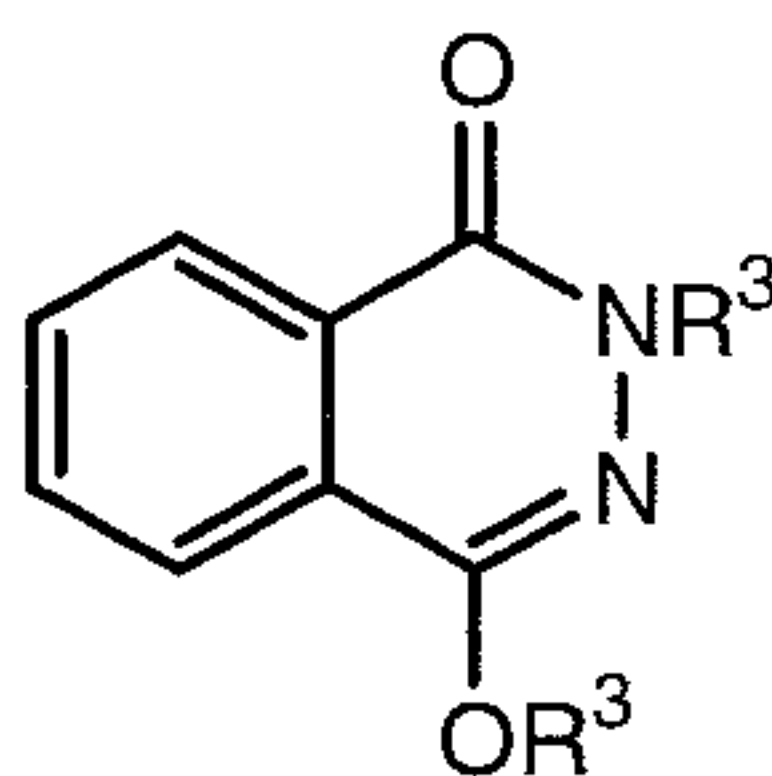
where R<sup>2</sup> is selected from the group consisting of





and n is 1 or 2, or a pharmaceutically acceptable salt thereof.

- 5 [0008] In another embodiment, the present invention provides analogs having formula III:



**III**

- 10 where each  $R^3$  is independently hydrogen,  $C_{1-22}$ alkyl,  $C_{2-22}$ alkenyl or  $C_{2-22}$ alkynyl, provided that at least one  $R^3$  group is not hydrogen, and pharmaceutically acceptable salts thereof.

- 15 [0009] In another embodiment, the present invention provides analogs of LSF having the ability to protect cell viability, particularly the ability to protect pancreatic  $\beta$ -cells. Thus, the analogs of the invention can allow the pancreas to maintain its insulin secretory capability.

[0010] In another embodiment, the present invention provides analogs of LSF which are effective in treating Type 1 diabetes. In another embodiment, the present invention provides analogs of LSF which can inhibit the development of Type 1 diabetes.

- 20 [0011] In another embodiment, the present invention provides analogs of LSF which are effective in treating Type 1 diabetes. In another embodiment, the

present invention provides analogs of LSF which can lead to reversal of type I diabetes by allowing the body to regenerate beta cells.

[0012] In another aspect, the present invention also provides:

5 a pharmaceutical composition comprising a compound of formula II, formula III, or pharmaceutically acceptable salts thereof, and a pharmaceutically acceptable carrier or excipient (the composition preferably comprises an effective amount of the compound or salt);

10 a method of treating or preventing Type 1 diabetes, comprising administering to a mammal (*e.g.*, a human) in need of such treatment, a compound of formula II, formula III, or pharmaceutically acceptable salts thereof;

a method for protecting cell viability, particularly the ability to protect pancreatic  $\beta$ -cells comprising contacting (*in vitro* or *in vivo*) the cells with an effective protective amount of a compound of formula II, formula III, or a pharmaceutically acceptable salt thereof;

15 a compound of formula II, formula III, or a pharmaceutically acceptable salt thereof for use in medical treatment (*e.g.*, the treatment of Type 1 diabetes); and

the use of a compound of formula II, formula III, or a pharmaceutically acceptable salt thereof to prepare a medicament for treating Type 1 diabetes in a mammal (*e.g.*, a human).

20 [0013] The disclosed compounds can be useful in the treatment of inflammatory and autoimmune diseases. Non-limiting examples of such diseases include atherosclerosis, type 2 diabetes, disorders associated with visceral obesity such as non-alcoholic steatohepatitis (NASH), multiple sclerosis, inflammatory bowel disease, psoriasis, rheumatoid arthritis, Alzheimer's disease  
25 and the like.

[0014] The invention also provides methods for testing the activity of the disclosed compounds. Methods not disclosed are known to those of ordinary skill in the art. One of ordinary skill will appreciate that many techniques are available to determine whether the disclosed compounds produce the desired  
30 result.

[0015] The invention also provides a kit for administering the disclosed compounds.

[0016] The invention also provides novel intermediates and processes disclosed herein that are useful for preparing analogs of formula II, or formula  
5 III, including the generic and specific intermediates as well as the synthetic processes described in the Charts and Examples herein.

#### Brief Description of the Drawings

[0017] Figure 1 depicts specific examples of the disclosed compounds.

#### Detailed Description

10 [0018] In describing and claiming the invention, unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any materials and methods similar or equivalent to those described herein can be used in the practice or testing of the present invention, the  
15 preferred materials and methods are described herein. Each of the terms below has the meaning associated with it in this specification. Specific and preferred values listed below for radicals, substituents, and ranges are for illustrations only; they do not exclude other defined values or other values within defined ranges for the radicals and substituents.

20 [0019] The terms “a,” “an,” “the,” “at least one,” and “one or more” are used interchangeably. Thus, for example, a composition that comprises “an” element means one element or more than one element.

[0020] The term “affected cell” refers to a cell of a subject afflicted with a disease or disorder, which affected cell has an altered phenotype relative to a  
25 subject not afflicted with a disease or disorder.

[0021] Cells or tissue are “affected” by a disease or disorder if the cells or tissue have an altered phenotype relative to the same cells or tissue in a subject not afflicted with a disease or disorder.

[0022] A disease or disorder is “alleviated” if the severity of a symptom of  
30 the disease or disorder, the frequency with which such a symptom is experienced by a patient, or both, is reduced.

[0023] The terms “compound” refers to any type of substance or agent that is commonly considered a drug, or a candidate for use as a drug, combinations, and mixtures of the above, as well as polypeptides and antibodies of the disclosed compounds.

5 [0024] An “analog” of a chemical compound may also include a compound that, by way of example, resembles another in structure but is not necessarily an isomer (*e.g.*, 5-fluorouracil is an analog of thymine).

[0025] The terms “cell,” “cell line,” and “cell culture” may be used interchangeably.

10 [0026] A “control” cell, tissue, sample, or subject is a cell, tissue, sample, or subject of the same type as a test cell, tissue, sample, or subject. The control may, for example, be examined at precisely or nearly the same time the test cell, tissue, sample, or subject is examined. The control may also, for example, be examined at a time distant from the time at which the test cell, tissue, sample, or  
15 subject is examined, and the results of the examination of the control may be recorded so that the recorded results may be compared with results obtained by examination of a test cell, tissue, sample, or subject. The control may also be obtained from another source or similar source other than the test group or a test subject, where the test sample is obtained from a subject suspected of having a  
20 disease or disorder for which the test is being performed.

[0027] A “test” cell, tissue, sample, or subject is one being examined or treated.

[0028] A “pathoindicative” cell, tissue, or sample is one that, when present, is an indication that the animal in which the cell, tissue, or sample is located (or  
25 from which the tissue was obtained) is afflicted with a disease or disorder. By way of example, the presence of one or more breast cells in a lung tissue of an animal is an indication that the animal is afflicted with metastatic breast cancer.

[0029] A tissue “normally comprises” a cell if one or more of the cell are present in the tissue in an animal not afflicted with a disease or disorder.

30 [0030] A “derivative” of a compound refers to a chemical compound that may be produced from another compound of similar structure in one or more steps, such as replacement of hydrogen by an alkyl, acyl, or amino group.

[0031] A “disease” is a state of health of an animal wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal's health continues to deteriorate.

[0032] In contrast, a “disorder” in an animal is a state of health in which the animal is able to maintain homeostasis, but in which the animal's state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the animal's state of health.

[0033] The use of the word “detect” and its grammatical variants is meant to refer to measurement of the species without quantification, whereas use of the word “determine” or “measure” with their grammatical variants are meant to refer to measurement of the species with quantification. The terms “detect” and “identify” are used interchangeably herein.

[0034] A “functional” molecule (*e.g.*, compound or analog) is a molecule in a form in which it exhibits a property by which it is characterized. By way of example, a functional enzyme is one that exhibits the characteristic catalytic activity by which the enzyme is characterized.

[0035] The term “inhibit” refers to the ability of a disclosed compound to reduce or impede a described function. Preferably, inhibition is by at least 10%, more preferably by at least 25%, even more preferably by at least 50%, and most preferably, the function is inhibited by at least 75%.

[0036] “Instructional material” includes a publication, a recording, a diagram, or any other medium of expression that can be used to communicate the usefulness of the disclosed compounds in the kit for effecting alleviation of the various diseases or disorders recited herein. Optionally, or alternately, the instructional material may describe one or more methods of alleviating the diseases or disorders in a cell or a tissue of a mammal. The instructional material of the kit may, for example, be affixed to a container that contains a disclosed compound or be shipped together with a container that contains the identified compound. Alternatively, the instructional material may be shipped separately from the container with the intention that the instructional material and the compound be used cooperatively by the recipient.

[0037] The term “parenteral” means not through the alimentary canal but by some other route such as subcutaneous, intramuscular, intrathecal, or intravenous.

[0038] The term “pharmaceutically acceptable carrier” includes any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water and emulsions such as an oil/water or water/oil emulsion, and various types of wetting agents. The term also encompasses any of the agents approved by a regulatory agency of the U.S. Federal government or listed in the U.S. Pharmacopeia for use in animals, including humans. Examples of these and other pharmaceutically acceptable carriers are described in Remington’s Pharmaceutical Sciences (1991, Mack Publication Co., New Jersey).

[0039] The term “purified” and similar terms relate to the isolation of a molecule or compound in a form that is substantially free (at least 75% free, preferably 90% free, and most preferably at least 95% free) from other components normally associated with the molecule or compound in a native environment. The term “purified” does not necessarily indicate that complete purity of the particular molecules achieved during the process. A “very pure” compound refers to a compound that is greater than 90% pure. A “highly purified” compound refers to a compound that is greater than 95% pure.

[0040] A “sample” refers preferably to a biological sample from a subject, including, but not limited to, normal tissue samples, diseased tissue samples, biopsies, blood, saliva, feces, semen, tears, and urine. A sample can also be any other source of material obtained from a subject, which contains cells, tissues, or fluid of interest. A sample can also be obtained from cell or tissue culture.

[0041] The term “standard,” refers to something used for comparison. For example, a standard can be a known standard agent or compound that is administered or added to a control sample and used for comparing results when measuring said compound in a test sample. Standard can also refer to an “internal standard,” such as an agent or compound that is added at known amounts to a sample and is useful in determining such things as purification or recovery rates when a sample is processed or subjected to purification or extraction procedures before a marker of interest is measured.

[0042] A “subject” of analysis, diagnosis, or treatment is an animal. Such animals include mammals, preferably a human.

[0043] A “therapeutic” treatment is a treatment administered to a subject who exhibits signs of pathology for the purpose of diminishing or eliminating  
5 those signs.

[0044] A “therapeutically effective amount” of a compound is that amount of compound that is sufficient to provide a beneficial effect to the subject to which the compound is administered.

[0045] The term “treating” or “treatment” includes prophylaxis of the  
10 specific disorder or condition, or alleviation of the symptoms associated with a specific disorder or condition or preventing or eliminating said symptoms.

[0046] A “prophylactic” treatment is a treatment administered to a subject who does not exhibit signs of a disease or exhibits only early signs of the disease for the purpose of decreasing the risk of developing pathology associated with  
15 the disease.

[0047] A “therapeutic” treatment is a treatment administered to a subject who exhibits signs of pathology for the purpose of diminishing or eliminating those signs.

[0048] An “effective amount” means an amount sufficient to produce a  
20 selected effect.

[0049] As used herein, a “therapeutically effective amount” of a compound or analog is an amount of compound which is sufficient to provide a beneficial effect to the subject to which the compound is administered.

[0050] The values listed below for radicals, substituents, and ranges, are for  
25 illustration only; they do not exclude other defined values or other values within defined ranges for the radicals and substituents. The disclosed compounds include compounds of formula II, or formula III having any combination of the values, specific values, more specific values, and preferred values described herein.

[0051] As used herein, the term “halogen” or “halo” includes bromo, chloro,  
30 fluoro, and iodo. The term “haloalkyl” as used herein refers to an alkyl radical

bearing at least one halogen substituent, for example, chloromethyl, fluoroethyl or trifluoromethyl and the like.

[0052] The term “halogen” or “halo” includes bromo, chloro, fluoro, and iodo. The term “haloalkyl”, refers to an alkyl radical bearing at least one halogen substituent, non-limiting examples include, but are not limited to, chloromethyl, fluoroethyl or trifluoromethyl and the like.

[0053] The term “(C<sub>1</sub>-C<sub>22</sub>)alkyl” refers to a branched or linear alkyl group having from one to twenty-two carbons. Non-limiting examples include, but are not limited to, methyl, ethyl, n-propyl, iso-propyl, butyl, iso-butyl, sec-butyl, tert-butyl, pentyl, hexyl, heptyl, octyl and the like. The term “lower alkyl” refers to alkyl groups having from one (1) to (6) carbon atoms. Typically, C<sub>1</sub>-C<sub>6</sub> alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, iso-propyl, butyl, iso-butyl, sec-butyl, tert-butyl, pentyl, hexyl, and the like.

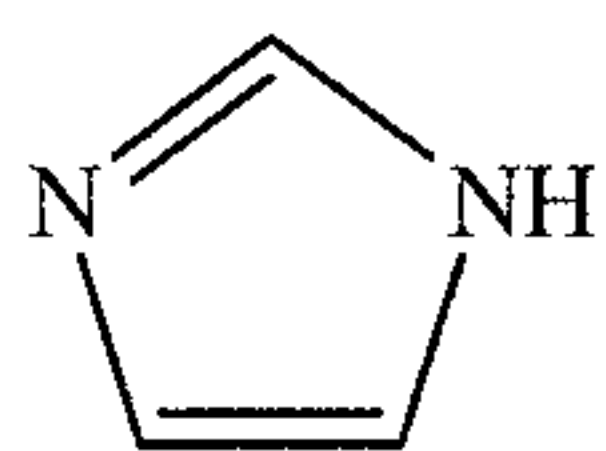
[0054] The term “(C<sub>2</sub>-C<sub>22</sub>)alkenyl”, refers to an olefinically unsaturated branched or linear group having from two to twenty-two carbon atoms and at least one double bond. Typically, (C<sub>2</sub>-C<sub>22</sub>)alkenyl groups include, but are not limited to, 1-propenyl, 2-propenyl, 1,3-butadienyl, 1-butenyl, hexenyl, pentenyl, hexenyl, and the like. Similarly, (C<sub>2</sub>-C<sub>22</sub>)alkynyl can be ethynyl, 1-propynyl, 2-propynyl, 1-butylnyl, 2-butylnyl, 3-butylnyl, 1-pentylnyl, 2-pentylnyl, 3-pentylnyl, 4-pentylnyl, 1-hexynyl, 2-hexynyl, 3-hexynyl, 4-hexynyl, or 5-hexynyl. The carbon atoms of the alkenyl or alkynyl groups that are not multiply bonded are considered alkyl carbon atoms for purposes of substitution or replacement.

[0055] It will be appreciated by those skilled in the art that the disclosed compounds having chiral centers may exist in and be isolated in optically active and racemic forms. It is to be understood that the disclosed compounds encompass any racemic, optically active or stereoisomeric form, or mixtures thereof, of the compound that possess the useful properties described, such as the *S,R*; *S,S*; *R,R*; or *R,S* diastereomers. It is well known in the art how to prepare such optically active forms (for example, by resolution of the racemic form by recrystallization techniques, by synthesis from optically-active starting materials, by chiral synthesis, or by chromatographic separation using a chiral stationary phase) and how to determine cADPR agonist or antagonist activity using the

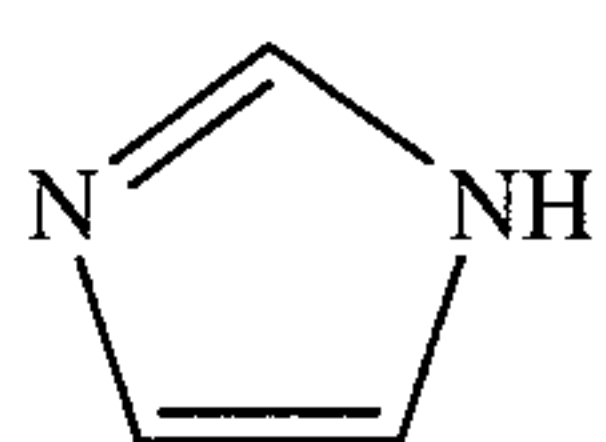
standard tests described herein, or using other similar tests which are well known in the art. In addition, some compounds may exhibit polymorphism.

[0056] The disclosed compounds may exist in tautomeric forms and the invention includes both mixtures and separate individual tautomers. For

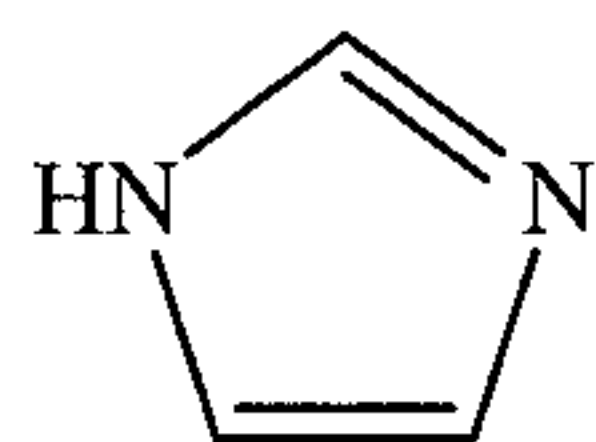
5 example, the following structure:



is understood to represent a mixture of the structures:



as well as



[0057] The terms 16:0, 18:0, 18:1, 20:4 or 22:6 hydrocarbon refers to a branched or straight alkyl or alkenyl group, wherein the first integer represents  
10 the total number of carbons in the group and the second integer represent the number of double bonds in the group.

[0058] The term “pharmaceutically-acceptable salt” refers to salts that retain the biological effectiveness and properties of the disclosed compounds and which are not biologically or otherwise undesirable. In many cases, the disclosed  
15 compounds are capable of forming acid or base salts by virtue of the presence of amino or groups similar thereto.

[0059] In cases where the disclosed compounds are sufficiently basic or acidic to form stable nontoxic acid or base salts, preparation and administration of the compounds as pharmaceutically acceptable salts may be appropriate.  
20 Examples of pharmaceutically acceptable salts are organic acid addition salts formed with acids that form a physiologically acceptable anion, for example, tosylate, methanesulfonate, acetate, citrate, malonate, tartarate, succinate, benzoate, ascorbate,  $\alpha$ -ketoglutarate, and  $\alpha$ -glycerophosphate. Inorganic salts may also be formed, including hydrochloride, sulfate, nitrate, bicarbonate, and  
25 carbonate salts.

[0060] Pharmaceutically acceptable salts may be obtained using standard procedures well known in the art, for example by reacting a sufficiently basic compound such as an amine with an acid affording a physiologically acceptable

anion. Alkali metal (for example, sodium, potassium or lithium) or alkaline earth metal (for example calcium) salts of carboxylic acids can also be made.

**[0061]** Pharmaceutically-acceptable base addition salts can be prepared from inorganic and organic bases. Salts from inorganic bases, include but are not limited to, sodium, potassium, lithium, ammonium, calcium and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary, secondary and tertiary amines, such as alkyl amines, dialkyl amines, trialkyl amines, substituted alkyl amines, di(substituted alkyl) amines, tri(substituted alkyl) amines, alkenyl amines, dialkenyl amines, trialkenyl amines, substituted alkenyl amines, di(substituted alkenyl) amines, tri(substituted alkenyl) amines, cycloalkyl amines, di(cycloalkyl) amines, tri(cycloalkyl) amines, substituted cycloalkyl amines, disubstituted cycloalkyl amine, trisubstituted cycloalkyl amines, cycloalkenyl amines, di(cycloalkenyl) amines, tri(cycloalkenyl) amines, substituted cycloalkenyl amines, disubstituted cycloalkenyl amine, trisubstituted cycloalkenyl amines, aryl amines, diaryl amines, triaryl amines, heteroaryl amines, diheteroaryl amines, triheteroaryl amines, heterocyclic amines, diheterocyclic amines, triheterocyclic amines, mixed di- and tri-amines where at least two of the substituents on the amine are different and are alkyl, substituted alkyl, alkenyl, substituted alkenyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heteroaryl, or heterocyclic and the like. Also included are amines where the two or three substituents, together with the amino nitrogen, form a heterocyclic or heteroaryl group. Non-limiting examples of amines include, isopropylamine, trimethyl amine, diethyl amine, tri(iso-propyl) amine, tri(n-propyl) amine, ethanolamine, 2-dimethylaminoethanol, tromethamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, N-alkylglucamines, theobromine, purines, piperazine, piperidine, morpholine, N-ethylpiperidine, and the like. It should also be understood that other carboxylic acid derivatives would be useful, for example, carboxylic acid amides, including carboxamides, lower alkyl carboxamides, dialkyl carboxamides, and the like.

**[0062]** Pharmaceutically acceptable acid addition salts may be prepared from inorganic and organic acids. Salts derived from inorganic acids include hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid,

and the like. Salts derived from organic acids include acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluene-sulfonic  
5 acid, salicylic acid, and the like.

[0063] The present invention is also includes pharmaceutical compositions comprising the disclosed compounds. More particularly, such compounds can be formulated as pharmaceutical compositions using standard pharmaceutically acceptable carriers, fillers, solubilizing agents and stabilizers known to those  
10 skilled in the art. For example, a pharmaceutical composition comprising the compound, or analog, derivative, or modification thereof, as described herein, is used to administer the appropriate compound to a subject.

[0064] The disclosed compounds are useful for treating a disease or disorder including administering to a subject in need thereof of a therapeutically  
15 acceptable amount of the compound or a pharmaceutical composition comprising a therapeutically effective amount of the compound and a pharmaceutically-acceptable carrier.

[0065] In another embodiment, the present invention provides kits for use in administering or using the disclosed compounds.

20 [0066] Specific and preferred values listed below for radicals, substituents, and ranges, are for illustration only; they do not exclude other defined values or other values within defined ranges for the radicals and substituents.

[0067] The disclosed compounds are generally named according to the IUPAC or CAS nomenclature system. Abbreviations that are well known to one  
25 of ordinary skill in the art may be used (*e.g.*, "Ph" for phenyl, "Me" for methyl, "Et" for ethyl, "h" for hour or hours, "rt" for room temperature, and "*rac*" for racemic mixture).

[0068] The invention also provides compounds of formula II or formula III for use in medical therapy.

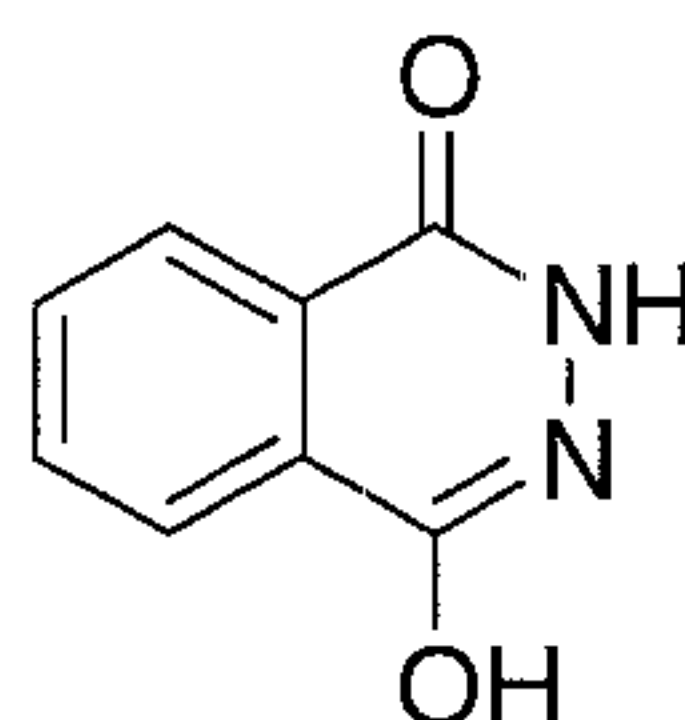
30 [0069] Preferred values for R<sup>3</sup> are hydrogen, methyl, ethyl, propyl, isopropyl, butyl, pentyl, 3-pentyl, hexyl, octyl, decyl, dodecyl, or hexadecyl, branched chains such as 2-methyl-hexyl, 3-methyl-hexyl, 4-methyl-octyl, 5-ethyl-octyl, 5-

phenyl-octyl, as well as unsaturated chains such as 2-hexenyl, 3-hexenyl, 4-hexenyl, 2-hexynyl, 3-hexynyl.

[0070] More preferred values for  $R^3$  are hydrogen, ethyl, butyl, hexyl, octyl, decyl, dodecyl, or hexadecyl. In some compounds the  $R^3$  group can be substituted with methyl.

[0071] Even more preferred values for  $R^3$  are hydrogen, ethyl, butyl, hexyl, or octyl.

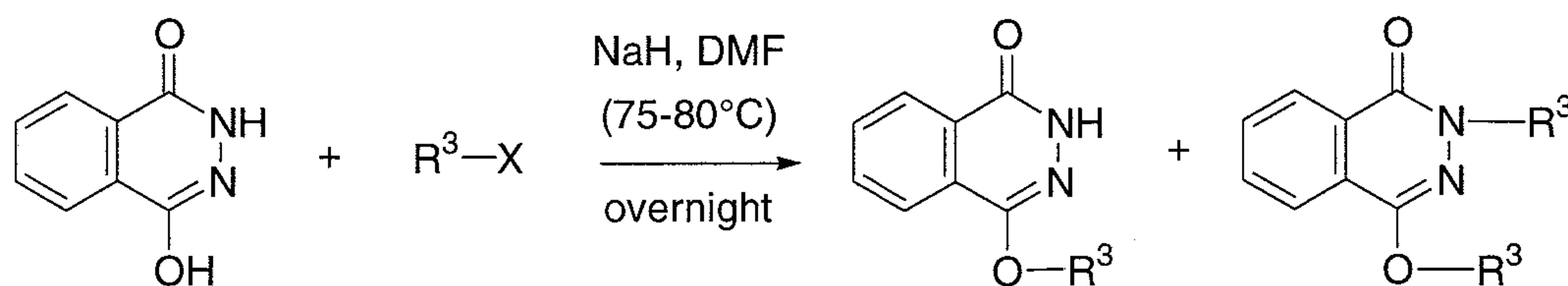
[0072] A preferred  $R^2$  is



10 [0073] In one aspect, the disclosed compounds can have the formulas depicted in Figure 1 and pharmaceutically acceptable salts thereof.

[0074] Sodium hydride (about 1.2 eq.) is added into a solution of phthalhydrazide (about 1.0 eq.) in anhydrous DMF. After stirring at 0°C for 30 minutes, alkyl or alkenyl halides (about 0.9 eq.) and tetrabutylammonium iodide  
15 (about 0.1 eq.) are added. The mixture is stirred at room temperature or about 70-80°C overnight. The reaction is quenched by the addition of water and extracted with ethyl acetate. The combined extracts are washed with water, saturated aqueous sodium chloride solution and dried. The solvent is removed at reduced pressure. The products can be purified by column chromatography (*e.g.*,  
20 silica, ethyl acetate). A general scheme for preparing the disclosed compounds having formula III is provided in Scheme 1:

**Scheme 1**



[0075] Processes for preparing analogs of formula III, are provided as further  
25 embodiments and are illustrated by the following procedures in which the meanings of the generic radicals are as given above unless otherwise qualified.

[0076] The disclosed compounds can be formulated as pharmaceutical compositions and administered to a mammalian host, such as a human patient in a variety of forms adapted to the chosen route of administration, *e.g.*, orally or parenterally, by intravenous, intramuscular, topical or subcutaneous routes.

5 [0077] Thus, the disclosed compounds may be systemically administered, *e.g.*, orally, in combination with a pharmaceutically acceptable vehicle such as an inert diluent or an assimilable edible carrier. They may be enclosed in hard or soft shell gelatin capsules, may be compressed into tablets, or may be incorporated directly with the food of the patient's diet. For oral therapeutic  
10 administration, the active compound may be combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of active compound. The percentage of the compositions and preparations may, of course, be varied and  
15 may conveniently be between about 2 to about 60% of the weight of a given unit dosage form. The amount of active compound in such therapeutically useful compositions is such that an effective dosage level will be obtained.

[0078] The tablets, troches, pills, capsules, and the like may also contain the following: binders such as gum tragacanth, acacia, corn starch or gelatin;  
20 excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring may be added. When the unit dosage form is a capsule, it may contain, in  
25 addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac or sugar and the like. A syrup or elixir may contain the active compound, sucrose or fructose  
30 as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and

substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and devices.

5 [0079] The active compound may also be administered intravenously or intraperitoneally by infusion or injection. Solutions of the active compound or its salts can be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

10 [0080] Exemplary pharmaceutical dosage forms for injection or infusion can include sterile aqueous solutions or dispersions (suspensions) or sterile powders comprising the active ingredient which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. In all cases, the ultimate dosage form should be  
15 sterile, fluid and stable under the conditions of manufacture and storage. This dispersion or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents, or suspending agents. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example,  
20 water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and mixtures thereof. Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono- or di-glycerides. The proper fluidity can be maintained,  
25 for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable  
30 to include isotonic agents, for example, sugars, buffers or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0081] Sterile solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilization. In the case of sterile powders for the preparation of sterile solutions, the preferred methods of preparation are vacuum drying and the freeze drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions. The sterile solutions or powders can be combined with carriers and administered via injection. In addition, the sterile solutions or powders can be combined with carriers or propellants and administered via inhalation.

[0082] For topical administration, the present compounds may be applied in pure form, *e.g.*, when they are liquids. However, it will generally be desirable to administer them to the skin as compositions or formulations, in combination with a dermatologically acceptable carrier, which may be a solid or a liquid.

[0083] Useful solid carriers include finely divided solids such as talc, clay, microcrystalline cellulose, silica, alumina and the like. Useful liquid carriers include water, alcohols or glycols or water-alcohol/glycol blends, in which the present compounds can be dissolved or dispersed at effective levels, optionally with the aid of non-toxic surfactants. Adjuvants such as fragrances and additional antimicrobial agents can be added to optimize the properties for a given use. The resultant liquid compositions can be applied from absorbent pads, used to impregnate bandages and other dressings, or sprayed onto the affected area using pump-type or aerosol sprayers.

[0084] Thickeners such as synthetic polymers, fatty acids, fatty acid salts and esters, fatty alcohols, modified celluloses or modified mineral materials can also be employed with liquid carriers to form spreadable pastes, gels, ointments, soaps, and the like, for application directly to the skin of the user.

[0085] Useful dosages of the compounds of formula II or formula III can be determined by comparing their *in vitro* activity, and *in vivo* activity in animal models. Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art; for example, see U.S. Pat. No. 4,938,949.

[0086] Generally, the concentration of the compound(s) of formula II or formula III in a liquid composition, such as a solution or suspension, will be from about 0.1-25 wt %, preferably from about 0.5-10 wt %. The concentration in a semi-solid or solid composition such as a gel or a powder will be about 0.1-5  
5 wt %, preferably about 0.5-2.5 wt %.

[0087] The amount of the compound, or an active salt or derivative thereof, required for use in treatment will vary not only with the particular salt selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will be ultimately at the discretion of  
10 the attendant physician or clinician.

[0088] In general, however, a dose can be in the range of from about 0.5 to about 20 mg/kg, *e.g.*, from about 1 to about 18 mg/kg of body weight per day, such as 3 to about 16 mg per kilogram body weight of the recipient per day, preferably in the range of 6 to 14 mg/kg/day, most preferably in the range of 9 to  
15 11 mg/kg/day.

[0089] The compound is conveniently administered in unit dosage form; for example, containing 5 to 1000 mg, conveniently 10 to 750 mg, most conveniently, 50 to 500 mg of active ingredient per unit dosage form.

[0090] Ideally, the active ingredient should be administered to achieve peak  
20 plasma concentrations of the active compound of from about 100 nM to about 50  $\mu$ M, preferably, about 90 nM to about 60  $\mu$ M, most preferably, about 70  $\mu$ M to about 80 nM. This may be achieved, for example, by the intravenous injection of a 0.05 to 5% solution of the active ingredient, optionally in saline, or orally administered as a bolus containing about 1-100 mg of the active ingredient.  
25 Desirable blood levels may be maintained by continuous infusion to provide about 0.01-5.0 mg/kg/hr or by intermittent infusions containing about 0.4-15 mg/kg of the active ingredient(s).

[0091] The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three,  
30 four or more sub-doses per day. The sub-dose itself may be further divided, *e.g.*, into a number of discrete loosely spaced administrations; such as multiple inhalations from an insufflator or by administration of a plurality of tablets.

[0092] Compounds which are identified using any of the methods described below may be formulated and administered to a subject for treatment of any of the diseases and disorders described above. However, the use of the disclosed compounds should not be construed to include only the diseases and disorder  
5 described. Preferably, the subject is a human.

[0093] The formulations of the pharmaceutical compositions described may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with a carrier or one or more other  
10 accessory ingredients, and then, if necessary or desirable, shaping or packaging the product into a desired single- or multi-dose unit.

[0094] Although the descriptions of pharmaceutical compositions provided are principally directed to pharmaceutical compositions which are suitable for ethical administration to humans, it will be understood by the skilled artisan that  
15 such compositions are generally suitable for administration to animals of all sorts.

[0095] Modification of pharmaceutical compositions for human administration in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary  
20 pharmacologist can design and perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions is contemplated include, but are not limited to, humans and other primates, and mammals, including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, and dogs.

[0096] A pharmaceutical composition may be prepared, packaged, or sold in bulk, as a single unit dose, or as a plurality of single unit doses. A "unit dose" is a discrete amount of the pharmaceutical composition comprising a  
25 predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would  
30 be administered to a subject or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

[0097] The relative amounts of the active ingredient, the pharmaceutically acceptable carrier, and any additional ingredients in a pharmaceutical composition will vary, depending upon the identity, size, and condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient.

[0098] In addition to the active ingredient, a pharmaceutical composition may further comprise one or more additional pharmaceutically active agents. Particularly contemplated additional agents include anti-emetics and scavengers such as cyanide and cyanate scavengers.

[0099] Controlled- or sustained-release formulations of a pharmaceutical composition of the disclosed compounds may be made using conventional technology.

[00100] In some cases, the dosage forms to be used can be provided as slow or controlled-release of one or more active ingredients therein using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, or microspheres or a combination thereof to provide the desired release profile in varying proportions. Exemplary controlled-release formulations are known to those of ordinary skill in the art, including those described below, can be readily selected for use with the pharmaceutical compositions of the disclosed compounds. Exemplary single unit dosage forms for oral administration such as tablets, capsules, gelcaps, and caplets that are adapted for controlled-release are encompassed and contemplated.

[00101] Most controlled-release formulations are designed to initially release an amount of drug that promptly produces the desired therapeutic effect, and gradually and continually release of other amounts of drug to maintain this level of therapeutic 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.

[00102] Controlled-release of an active ingredient can be stimulated by various inducers, for example pH, temperature, enzymes, water, or other physiological conditions or compounds.

[00103] Powdered and granular formulations of a pharmaceutical preparation  
5 of the disclosed compounds may be prepared using known methods. Such formulations may be administered directly to a subject, used, for example, to form tablets, to fill capsules, or to prepare an aqueous or oily suspension or solution by addition of an aqueous or oily vehicle thereto. Each of these formulations may further comprise one or more of dispersing or wetting agent, a  
10 suspending agent, and a preservative. Additional excipients, such as fillers and sweetening, flavoring, or coloring agents, may also be included in these formulations.

[00104] Formulations of pharmaceutical compositions suitable for oral administration may be prepared, packaged, or sold in the form of a discrete solid  
15 dose unit including, but not limited to, tablets, hard or soft capsules, cachets, troches or lozenges, each containing a predetermined amount of the active ingredient. Other exemplary formulations for oral administration include, but are not limited to, a powdered or granular formulation, an aqueous or oily suspension, an aqueous or oily solution, a paste, a gel, a toothpaste, a  
20 mouthwash, a coating, an oral rinse, or an emulsion. The terms oral rinse and mouthwash are used interchangeably.

[00105] A tablet comprising the active ingredient may, for example, be made by compressing or molding the active ingredient, optionally with one or more additional ingredients. Compressed tablets may be prepared by compressing, in  
25 a suitable device, the active ingredient in a free flowing form such as a powder or granular preparation, optionally mixed with one or more of a binder, a lubricant, an excipient, a surface-active agent, and a dispersing agent. Molded tablets may be made by molding, in a suitable device, a mixture of the active ingredient, a pharmaceutically acceptable carrier, and at least sufficient liquid to moisten the  
30 mixture. Pharmaceutically acceptable excipients used in the manufacture of tablets include, but are not limited to, inert diluents, granulating and disintegrating agents, binding agents, and lubricating agents. Known dispersing agents include, but are not limited to, potato starch and sodium starch glycollate.

Known surface-active agents include, but are not limited to, sodium lauryl sulphate. Known diluents include, but are not limited to, calcium carbonate, sodium carbonate, lactose, microcrystalline cellulose, calcium phosphate, calcium hydrogen phosphate, and sodium phosphate. Known granulating and  
5 disintegrating agents include, but are not limited to, corn starch and alginic acid.

Known binding agents include, but are not limited to, gelatin, acacia, pre-gelatinized maize starch, polyvinylpyrrolidone, and hydroxypropyl methylcellulose. Known lubricating agents include, but are not limited to, magnesium stearate, stearic acid, silica, and talc.

10 **[00106]** Tablets may be non-coated or they may be coated using known methods to achieve delayed disintegration in the gastrointestinal tract of a subject, thereby providing sustained release and absorption of the active ingredient. By way of example, a material such as glyceryl monostearate or glyceryl distearate may be used to coat tablets. Further by way of example,  
15 tablets may be coated using methods described in U.S. Patent Nos. 4,256,108; 4,160,452; and 4,265,874 to form osmotically-controlled release tablets. Tablets may further comprise a sweetening agent, a flavoring agent, a coloring agent, a preservative, or some combination of these in order to provide for pharmaceutically elegant and palatable preparation.

20 **[00107]** Hard capsules comprising the active ingredient may be made using a physiologically degradable composition, such as gelatin. Such hard capsules comprise the active ingredient, and may further comprise additional ingredients including, for example, an inert solid diluent such as calcium carbonate, calcium phosphate, or kaolin.

25 **[00108]** Soft gelatin capsules comprising the active ingredient may be made using a physiologically degradable composition, such as gelatin. Such soft capsules comprise the active ingredient, which may be mixed with water or an oil medium such as peanut oil, liquid paraffin, or olive oil.

**[00109]** Exemplary liquid formulations of a pharmaceutical composition for  
30 oral administration may be prepared, packaged, and sold either in liquid form or in the form of a dry product intended for reconstitution with water or another suitable vehicle prior to use.

[00110] Injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampules or in multi dose containers containing a preservative. Formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations. Such formulations may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In one embodiment of a formulation for parenteral administration, the active ingredient is provided in dry (*e.g.*, powder or granular) form for reconstitution with a suitable vehicle (*e.g.*, sterile pyrogen free water) prior to parenteral administration of the reconstituted composition.

[00111] A pharmaceutical composition may be prepared, packaged, or sold in a formulation for buccal administration. Such formulations may, for example, be in the form of tablets or lozenges made using conventional methods, and may, for example, 0.1 to 20 % (w/w) active ingredient, the balance comprising an orally dissolvable or degradable composition and, optionally, one or more of the additional ingredients described above. Alternately, formulations for buccal administration may comprise a powder or an aerosolized or atomized solution or suspension comprising the active ingredient. Such powdered, aerosolized, or aerosolized formulations, when dispersed, preferably have an average particle or droplet size in the range from about 0.1 to about 200 nanometers, and may further comprise one or more of the disclosed additional ingredients.

[00112] The term "additional ingredients" include, but are not limited to, one or more of the following: excipients; surface active agents; dispersing agents; inert diluents; granulating and disintegrating agents; binding agents; lubricating agents; sweetening agents; flavoring agents; coloring agents; preservatives; physiologically degradable compositions such as gelatin; aqueous vehicles and solvents; oily vehicles and solvents; suspending agents; dispersing or wetting agents; emulsifying agents, demulcents; buffers; salts; thickening agents; fillers; emulsifying agents; antioxidants; antibiotics; antifungal agents; stabilizing agents; and pharmaceutically acceptable polymeric or hydrophobic materials. See Genaro, ed., 1985, Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA, which is incorporated herein by reference.

[00113] The compound can be administered to a subject as frequently as several times daily, or it may be administered less frequently, such as once a day, once a week, once every two weeks, once a month, or even less frequently, such as once every several months or even once a year or less. The frequency of the  
5 dose will be readily apparent to the skilled artisan and will depend upon any number of factors, such as, but not limited to, the type and severity of the disease being treated, the type, and age of the subject, etc.

[00114] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the  
10 pharmaceutical compositions. In accordance with one embodiment, a kit is provided for treating a subject in need of immuno-modulation. Preferably, the subject is a human. In one embodiment, the kit comprises one or more of the disclosed compounds able to protect cell viability, particularly the ability to protect pancreatic  $\beta$ -cells. These pharmaceuticals can be packaged in a variety  
15 of containers, *e.g.*, vials, tubes, microtiter well plates, bottles, and the like. Other reagents can be included in separate containers and provided with the kit; *e.g.*, positive control samples, negative control samples, buffers, cell culture media, etc. Preferably, the kits will also include instructions for use.

[00115] Although any methods and materials similar or equivalent to those  
20 presently described can be used in the practice or testing of the disclosed compounds, the preferred methods and materials are those described.

[00116] In accordance with the present invention, as described above or as discussed in the Examples below, there can be employed conventional clinical, chemical, cellular, histochemical, biochemical, molecular biology, microbiology,  
25 and recombinant DNA techniques which are known to those of ordinary skill in the art. Such techniques are fully explained in the literature.

#### Examples

[00117] The invention is now described with reference to the following  
Examples and Embodiments. Without further description, it is believed that one  
30 of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the disclosed compounds and practice the claimed methods. The following working examples therefore, are provided for

the purpose of illustration only and specifically point out some embodiments, and are not to be construed as limiting in any way the remainder of the disclosure. Therefore, the examples should be construed to encompass any and all variations which become evident as a result of the teaching provided below.

5 Example 1: Synthesis of 4-Butoxy-2H-phthalazin-1-one (CPW29) and 4-Butoxy-2-butyl-2H-phthalazin-1-one (CPW30).

[00118] Sodium hydride (60mg, 95%, 2.4 mmol) was added into a solution of phthalhydrazide (330mg, 2.0 mmol) in 10 ml anhydrous DMF. After stirring at 0°C for 30 minutes, 1-bromobutane (0.2ml, 1.8 mmol) and catalytic amount of tetrabutylammonium iodide were added and the mixture was stirred at room  
10 temperature overnight. The reaction was quenched by addition of water and the reaction mixture was extracted with ethyl acetate. The combined extracts were washed with water, saturated aqueous sodium chloride solution, and dried over anhydrous magnesium sulfate. The solvent was removed at reduced pressure.  
15 The products were purified by column chromatography (silica, ethyl acetate) to afford CPW29 (30 mg) and CPW30 (25 mg).

[00119] CPW29: <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>) δ 1.01 (t, 3H), 1.53 (sextet, 2H), 1.84 (p, 2H), 4.32 (t, 2H), 7.81 (m, 2H), 8.02 (m, 1H), 8.42 (m, 1H), 10.85 (s, 1H). MS (ESI) m/z 218.3 [MH]<sup>+</sup>

20 [00120] CPW30: <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>) δ 0.97 (t, 3H), 1.01 (t, 3H), 1.41 (sextet, 2H), 1.54 (sextet, 2H), 1.81 (m, 4H), 4.12 (t, 2H), 4.31 (t, 2H), 7.76 (m, 2H), 7.96 (m, 1H), 8.40 (m, 1H)

Example 2: Synthesis of 4-Ethoxy-2H-phthalazin-1-one (CPW27) and 4-Ethoxy-2-ethyl-2H-phthalazin-1-one (CPW28).

25 [00121] The title compounds were synthesized via the procedure of Example 1 using bromoethane (0.9 mmol). Yield: CPW27 (25 mg) and CPW28 (30 mg).

[00122] CPW27: <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>) δ 1.48 (t, 3H), 4.38 (q, 2H), 7.02 (m, 2H), 8.03 (m, 1H), 8.43 (m, 1H), 11.01 (s, 1H). MS (ESI) m/z 190.3 [MH]<sup>+</sup>

30 [00123] CPW28: 1.39 (t, 3H), 1.48 (t, 3H), 4.18 (q, 2H), 4.38 (q, 2H), 7.75 (m, 2H), 7.99 (m, 1H), 8.40 (m, 1H).

Example 3: Synthesis of 4-Hexyloxy-2H-phthalazin-1-one (CPW31) and 2-Hexyl-4-hexyloxy-2H-phthalazin-1-one (CPW32).

[00124] The title compounds were synthesized via the procedure of Example 1 using 1-chlorohexane (1.8 mmol). Yield: CPW31 (25 mg) and CPW32 (27  
5 mg).

[00125] CPW31:  $^1\text{H}$  NMR (300MHz,  $\text{CDCl}_3$ )  $\delta$  0.91 (t, 3H), 1.37 (m, 4H), 1.49 (p, 2H), 1.84 (p, 2H), 4.31 (t, 2H), 7.81 (m, 2H), 8.02 (m, 1H), 8.43 (m, 1H), 11.11 (s, 1H).

[00126] CPW32:  $\delta$  0.87 (t, 3H), 0.91 (t, 3H), 1.32 (m, 8H), 1.46 (m, 4H), 1.81  
10 (m, 4H), 4.10 (t, 2H), 4.28 (t, 2H), 7.74 (m, 2H), 7.94 (m, 1H), 8.38 (m, 1H).

Example 4: Synthesis of 4-Octyloxy-2H-phthalazin-1-one (CPW33).

[00127] The title compound was synthesized via the procedure of Example 1 using 1-bromooctane (0.9 mmol). Yield: CPW33 (40 mg).

[00128] CPW33:  $^1\text{H}$  NMR (300MHz,  $\text{CDCl}_3$ )  $\delta$  0.88 (t, 3H), 1.29 (m, 8H),  
15 1.49 (p, 2H), 1.85 (p, 2H), 4.31 (t, 2H), 7.81 (m, 2H), 8.02 (m, 1H), 8.42 (m, 1H), 11.19 (s, 1H). MS (ESI)  $m/z$  274.5  $[\text{MH}]^+$

Example 5: Synthesis of 4-Decyloxy-2H-phthalazin-1-one (CPW34).

[00129] The title compound was synthesized via the procedure of Example 1 using 1-bromodecane (0.9 mmol). Yield: CPW34 (50 mg).

20 [00130] CPW34:  $^1\text{H}$  NMR (300MHz,  $\text{CDCl}_3$ )  $\delta$  0.88 (t, 3H), 1.28 (s, 12H), 1.50 (p, 2H), 1.85 (p, 2H), 4.31 (t, 2H), 7.82 (m, 2H), 8.02 (m, 1H), 8.42 (m, 1H), 11.05 (s, 1H). MS (ESI)  $m/z$  302.5  $[\text{MH}]^+$

Example 6: Synthesis of 4-Dodecyloxy-2H-phthalazin-1-one (CPW35).

[00131] The title compound was synthesized via the procedure of Example 1  
25 using 1-bromododecane (0.9 mmol). Yield: CPW35 (67 mg).

[00132] CPW35:  $^1\text{H}$  NMR (300MHz,  $\text{CDCl}_3$ )  $\delta$  0.88 (t, 3H), 1.26 (s, 16H), 1.49 (p, 2H), 1.85 (p, 2H), 4.31 (t, 2H), 7.81 (m, 2H), 8.02 (m, 1H), 8.43 (m, 1H), 10.95 (s, 1H). MS (ESI)  $m/z$  330.5  $[\text{MH}]^+$

Example 7: Synthesis of 4-Hexadecyloxy-2H-phthalazin-1-one (CPW36).

[00133] The title compound was synthesized via the procedure of Example 1 using 1-bromohexadecane (0.9 mmol). Yield: CPW36 (110 mg).

[00134] CPW36: <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>) δ 0.87 (t, 3H), 1.25 (s, 24H), 1.46 (p, 2H), 1.84 (p, 2H), 4.29 (t, 2H), 7.81 (m, 2H), 8.03 (m, 1H), 8.41 (m, 1H), 10.19(s, 1H). MS (ESI) m/z 386.8 [MH]<sup>+</sup>

Example 8: Synthesis of 4-Eicosyloxy-2H-phthalazin-1-one (CPW37) and 2-Eicosyl-4-eicosyloxy-2H-phthalazin-1-one (CPW39).

[00135] The title compounds were synthesized via the procedure of Example 1 using 1-chlorohexane (0.9 mmol). Yield: CPW37 (82 mg) and CPW39 (115 mg).

[00136] CPW37: <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>) δ 0.88 (t, 3H), 1.26 (s, 32H), 1.49 (p, 2H), 1.85 (p, 2H), 4.29 (t, 2H), 7.82 (m, 2H), 8.04 (m, 1H), 8.43 (m, 1H), 10.02 (s, 1H). MS (ESI) m/z 443.0 [MH]<sup>+</sup>

[00137] CPW39: <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>) δ 0.88 (t, 6H), 1.26 (s, 62H), 1.50 (p, 4H), 1.85 (p, 4H), 4.11 (t, 2H), 4.30 (t, 2H), 7.76 (m, 2H), 7.99 (m, 1H), 8.42 (m, 1H).

Example 9: Synthesis of 4-Docosyloxy-2H-phthalazin-1-one (CPW38).

[00138] The title compound was synthesized via the procedure of Example 1 using 1-bromodocosane (0.9 mmol). Yield: CPW38 (146 mg).

[00139] CPW38: <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>) δ 0.88 (t, 3H), 1.26 (s, 36H), 1.50 (p, 2H), 1.85 (p, 2H), 4.29 (t, 2H), 7.83 (m, 2H), 8.04 (m, 1H), 8.41 (m, 1H), 9.97(s, 1H). MS (ESI) m/z 471.9 [MH]<sup>+</sup>

Example 10: Biological Evaluations of Disclosed Compounds

[00140] The disclosed compounds (CPW27-39) are evaluated in pancreatic β cell lines for apoptosis protection after treatment with inflammatory cytokines (reflected by reduced OD450) and insulin release. For the protective effect of β-cells, some compounds can show comparative potency with LSF and additional analogs are able to protect cells at low concentrations (nm and lower). For the

insulin release assay, some compounds may demonstrate effects similar to those of LSF in response to glucose.

Example 11: Biological Evaluations of LSF Analogs in Human islets

[00141] The disclosed compounds can be evaluated on human islets for the induction of insulin secretion, intracellular ATP concentrations and the effect of reducing cell death exposed to cytokines (combination of human IL-1 $\beta$ , IFN- $\gamma$ , and TNF- $\alpha$ ).

Example 12: Effects of LSF and Analogs on  $\beta$ -cells

[00142] The effects of the disclosed compounds on  $\beta$ -cells in the mouse insulin-secreting INS-1 cell line can be investigated. Cells are maintained in RPMI 1640 medium (Life Technologies, Inc., Gaithersburg, MD) supplemented with 10% heat-inactivated FBS, 10 mM HEPES, 200  $\mu$ M L-glutamine, 1 mM sodium pyruvate, 5 mM 2-mercaptoethanol, 50 U/ml penicillin, and 50  $\mu$ g/ml streptomycin at pH 7.4. The cells are cultured at 37 °C in a humidified incubator supplied with 5% carbon dioxide. Fresh medium is replaced every 2 days. The cells are plated at a density of 10<sup>5</sup>/cm<sup>2</sup>. Culture vessels are coated with poly-D-lysine and gelatin (Sigma, St. Louis, MO) to retain detached and dead cells so that seeding cell numbers reflect the actual cell numbers after all treatment conditions. INS-1 cells are treated with the combination of recombinant mouse IL-1 $\beta$  (5 ng/ml), IFN $\gamma$  (100 ng/ml), and TNF $\alpha$  (10 ng/ml; R&D Systems, Inc., Minneapolis, MN) suspended in complete RPMI medium. LSF (provided by Cell Therapeutics, Inc., Seattle, WA) and the test compounds can be added simultaneously with the cytokines in complete RPMI medium. The treatments are performed for 18 hours.

25 Example 13: Effects of LSF and Analogs on Insulin Secretion and Apoptosis

[00143] The effects of LSF analogs on insulin secretion in INS-1 cells with and without inflammatory cytokines can be determined.  $\beta$ -Cells are treated with apoptosis detecting dye for 2-3 hours at room temperature. Apoptotic cells can be recognized with purple-red color under a microscope. After washing to eliminate free dye and adding dye release reagent, color density can be quantified by reading at OD 450 nm. At the end of treatment, cells are washed with Krebs-

Ringer-bicarbonate-HEPES buffer (KRB) containing 134 mm NaCl, 4.7 mm KCl, 1.2 mm KH<sub>2</sub>PO<sub>4</sub>, 1.2 mm MgSO<sub>4</sub>, 1.0 mm CaCl<sub>2</sub>, 10 mm HEPES, and 0.1% BSA at 37 °C, pH 7.4. The cells are preincubated in the same buffer for 30 min, followed by 60-min incubation in KRB supplemented with 15 mM D-  
5 glucose (J. T. Baker, Phillipsburg, NJ). The supernatant is harvested and subjected to centrifugation to eliminate residue cells. Insulin secreted into the supernatant can be measured by RIA using mouse insulin as a standard. The cells are maintained in basal (3 mM) and glucose-stimulated (28 mM).

Example 14: Mouse-insulin secreting cell line  $\beta$ -TC6

10 Cell preparation

[00144] The  $\beta$ -TC6 cell line is maintained in RPMI 1640 medium (Life Technologies, Rockville, MD) supplemented with 10% heat inactivated fetal bovine serum, 10 mM HEPES, 200 pM L-glutamine, 50 units/ml penicillin and 50  $\mu$ g/ml streptomycin at pH 7.4. The cells are cultured in a 37°C, humidified  
15 incubator supplied with 5% carbon dioxide. Fresh media is replaced every two days. The cells are plated at a density of about 10<sup>5</sup>/cm<sup>2</sup>. Culture vessels (dishes and chamber slides) used for experiments are coated with poly-D-lysine and gelatin (Sigma, St. Louis, MO) to retain detached and dead cells so that seeding cell numbers reflect the actual cell numbers after all treatment conditions.

20 Cytokine and LSF analog treatment of  $\beta$ -TC6 cells.

[00145]  $\beta$ -TC6 cells are treated with vehicle alone or with the combination of recombinant mouse IL-1 $\beta$  (5 ng/ml), IFN- $\gamma$  (100 ng/ml) and TNF- $\alpha$  (10 ng/ml) (R&D Systems, Minneapolis, MN) suspended in complete RPMI medium. LSF (Cell Therapeutics, Inc. Seattle, WA) or the analogs are added simultaneously  
25 with the cytokines in complete RPMI medium in the concentrations ranging from 1 nM to 20 pM. All treatments are conducted for 18 hours.

Example 15: Static insulin secretion.

[00146] At the end of treatment, cells are washed with Krebs-Ringer-bicarbonate-HEPES buffer (KR13) containing in mM: 134 NaCl, 4.7 KCl, 1.2  
30 KH<sub>2</sub>PO<sub>4</sub>, 1.2 MgSO<sub>4</sub>, 1.0 CaCl<sub>2</sub>, 10 HEPES, and 0.1 % bovine serum albumin at

37°C, at a pH of 7.4. The cells are pre-incubated in the buffer for 30 min followed by 60-min incubation in KRB supplemented with 15 mM D-glucose (J.T. Baker, Phillipsburg, NJ). The supernatant is harvested and subjected to centrifugation to eliminate residue cells. Insulin secreted into the supernatant is  
5 measured using EIA with mouse insulin as a standard.

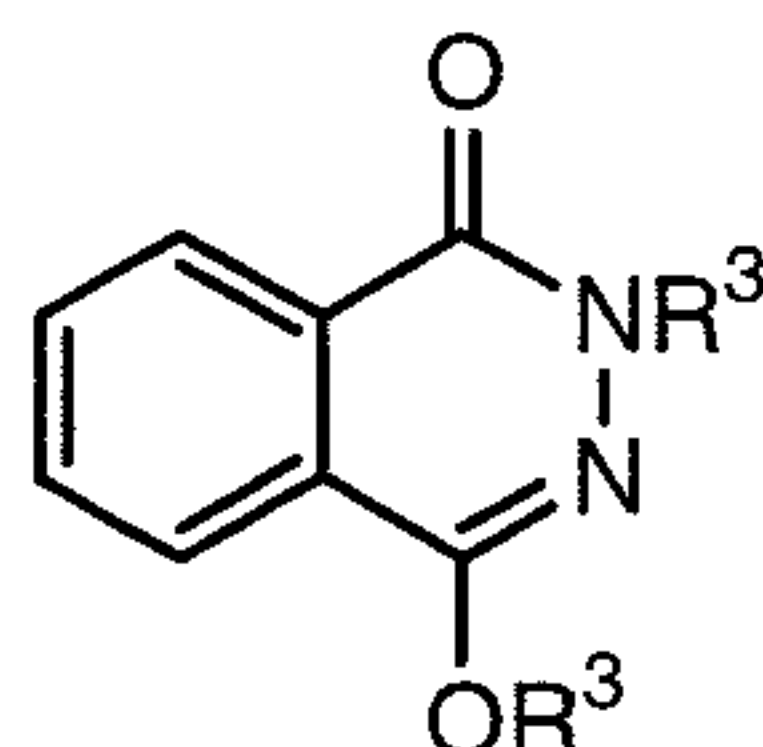
Example 16: STAT4 Phosphorylation

[00147] Murine splenocytes are equally plated in 96-well plates and are treated with or without LPS (1.0 ng/ml) supplemented with LSF or a series of test compounds for 18 hours. The protein lysates are transferred onto the  
10 Hybond-P membranes, and subsequently probed with a polyclonal antibody against phosphorylated STAT4. The hybridized membranes are subjected to ECL and autoradiography. Samples are repeated in triplicate.

[00148] The abbreviations used in the present specification have their  
15 conventional meaning within the chemical and biological arts. All publications, patents, and patent documents cited in the specification are incorporated by reference herein, as though individually incorporated by reference. In the case of any inconsistencies, the present disclosure, including any definitions therein will prevail. The invention has been described with reference to various specific and  
20 preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

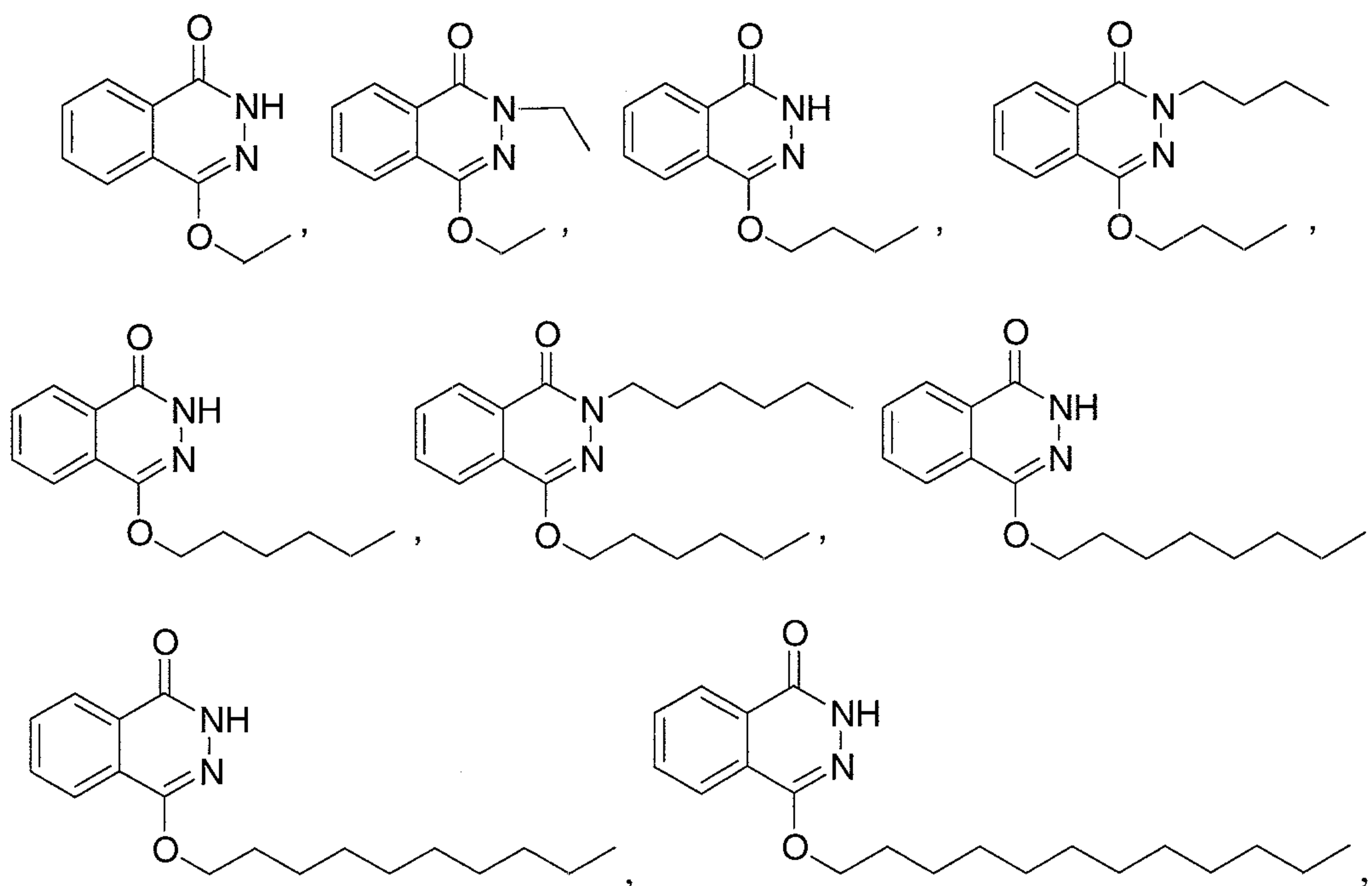
What is claimed is:

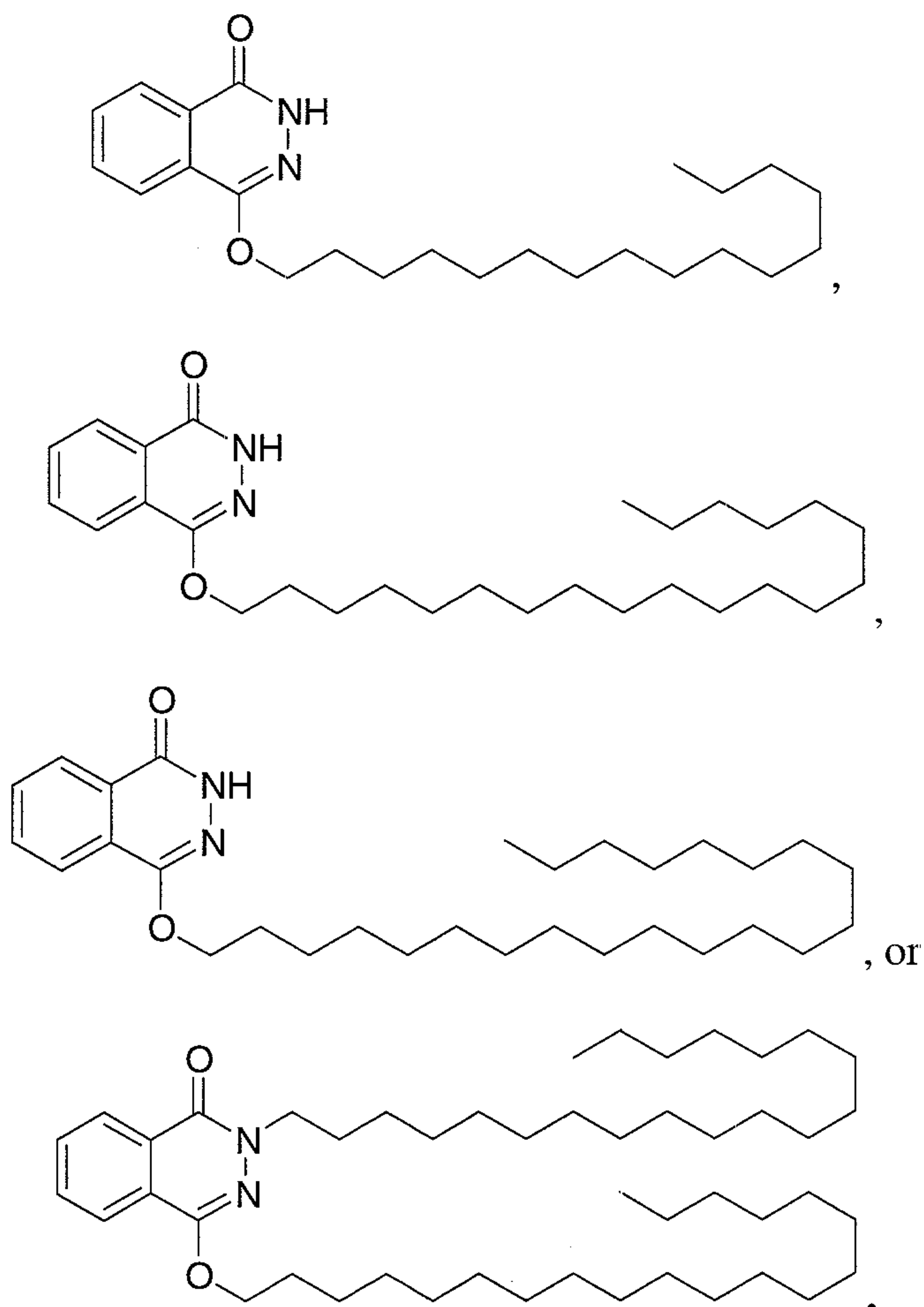
1. A compound having formula III:



where each  $R^3$  is independently hydrogen,  $C_{1-22}$ alkyl,  $C_{2-22}$ alkenyl or  $C_{2-22}$ alkynyl, provided that at least one  $R^3$  group is not hydrogen, or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1, wherein each  $R^3$  independently is hydrogen, ethyl, butyl, hexyl, octyl, decyl, dodecyl, hexadecyl, 2-methyl-hexyl, 3-methyl-hexyl, 4-methyl-octyl, 5-ethyl-octyl, 5-phenyl-octyl, 2-hexenyl, 3-hexenyl, 4-hexenyl, 2-hexynyl, or 3-hexynyl.
3. The compound of claim 2, wherein each  $R^3$  independently is hydrogen, ethyl, butyl, hexyl, octyl, decyl, dodecyl, or hexadecyl.
4. The compound of claim 3, wherein each  $R^3$  independently is hydrogen, ethyl, butyl, hexyl, or octyl.
5. The compound of any of claims 1-4, having the formula:





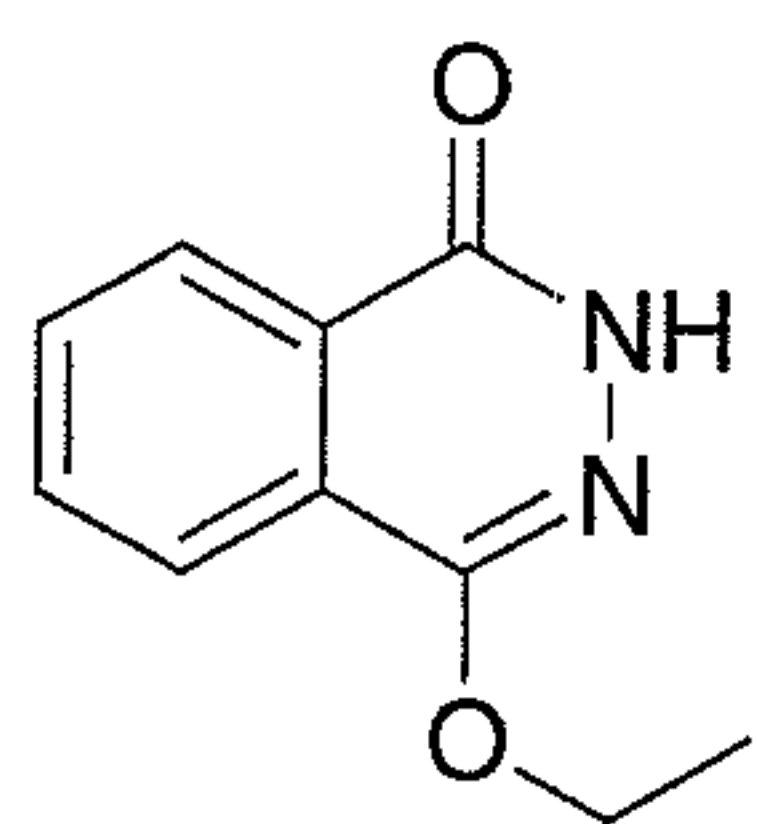
and pharmaceutically acceptable salts thereof.

6. A pharmaceutical composition comprising a compound of any of claims 1-5, in combination with a pharmaceutically acceptable carrier.
7. A method for preventing or treating a pathological condition or symptom in a mammal, wherein the protection of  $\beta$ -cells from Th1 cytokine-induced dysfunction or the onset of Type 1 diabetes can be reduced, and such activity is desired, comprising administering to said mammal an effective amount of a compound of any of claims 1-5, or pharmaceutically acceptable salt thereof.
8. The method of claim 7, wherein the pathological condition or symptom is Type 1 diabetes.
9. The method of claim 7, wherein the mammal is a human.
10. A method for preventing or treating a pathological condition or symptom in a mammal, wherein the protection of pancreatic  $\beta$ -cells is desired comprising

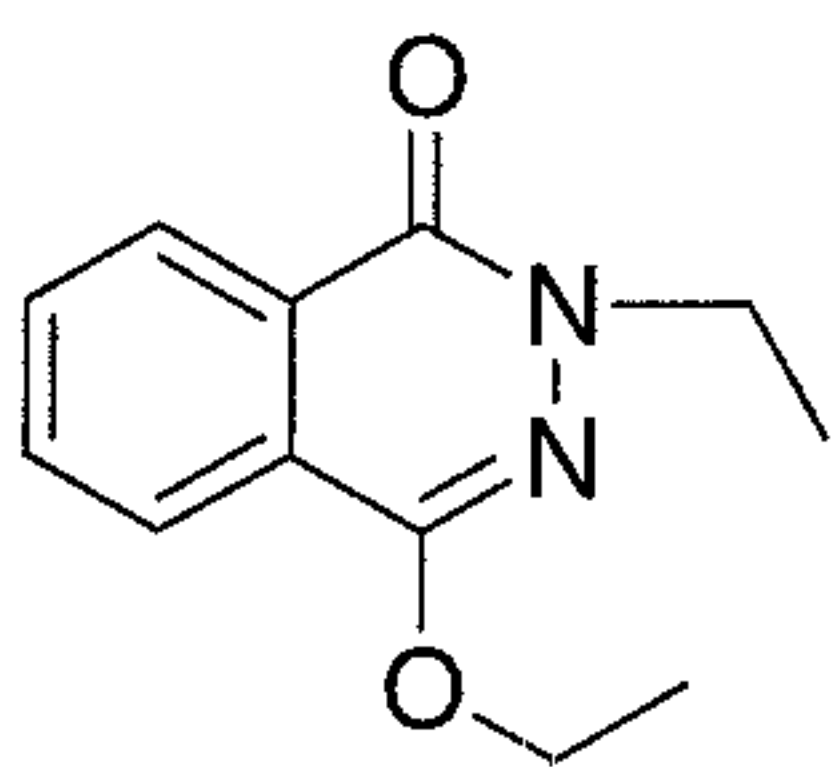
- contacting the cells with an effective protective amount of a compound of any of claims 1-5, or a pharmaceutically acceptable salt thereof.
11. The method of claim 10, wherein the mammal is a human.
  12. A method for preventing or treating a pathological condition or symptom in a mammal wherein treatment of an inflammatory and autoimmune condition is desired comprising administering to said mammal an effective amount of a compound of any of claims 1-5, or pharmaceutically acceptable salt thereof.
  13. The method of claim 12, wherein the inflammatory or autoimmune condition is atherosclerosis, type 2 diabetes, disorders associated with visceral obesity, multiple sclerosis, inflammatory bowel disease, psoriasis, rheumatoid arthritis, or Alzheimer's disease.
  14. The method of claim 13, wherein the mammal is a human.
  15. A compound of any of claims 1-5, for use in medical therapy.
  16. The compound of claim 15, wherein the medical therapy is prevention or treatment of Type 1 diabetes.
  17. The use of compound of any of claims 1-5, to prepare a medicament for prevention or treatment of Type 1 diabetes.
  18. The use of claim 17, wherein the medicament includes a physiologically acceptable carrier.
  19. The use of claim 18, wherein the medicament comprises a liquid carrier.
  20. The use of claim 18, wherein the medicament comprises a solid carrier.
  21. Use of a compound of any of claims 1-5, to prepare a medicament useful for prevention or treatment of a pathological condition or symptom in a mammal, wherein the pathological condition or symptom is an inflammatory or autoimmune condition.
  22. The use of claim 21, wherein the medicament includes a physiologically acceptable carrier.
  23. The use of claim 22, wherein the medicament comprises a liquid carrier.

24. The use of claim 22, wherein the medicament comprises a solid carrier.

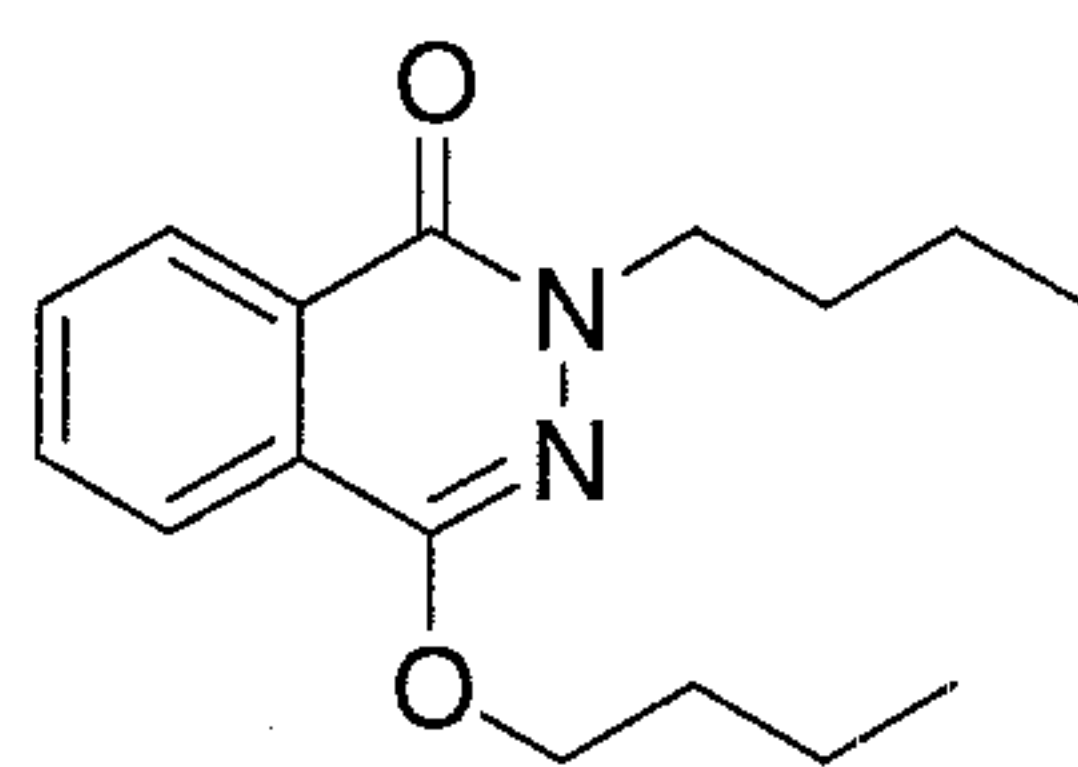
Figure 1



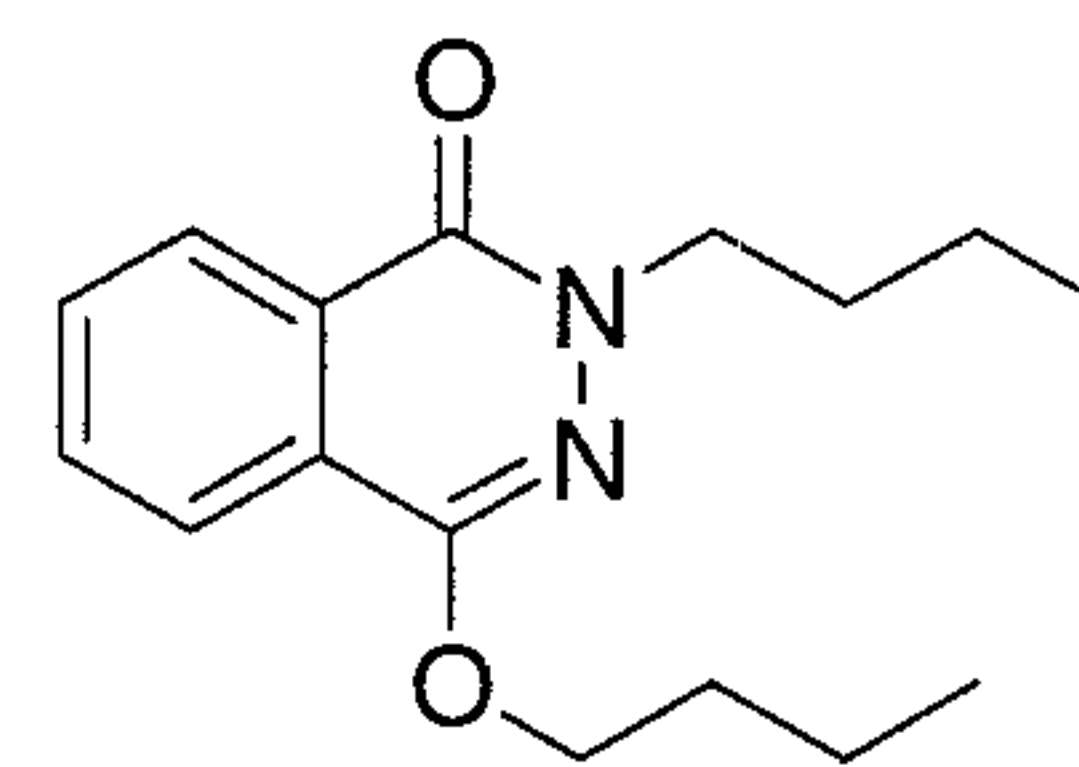
CPW27



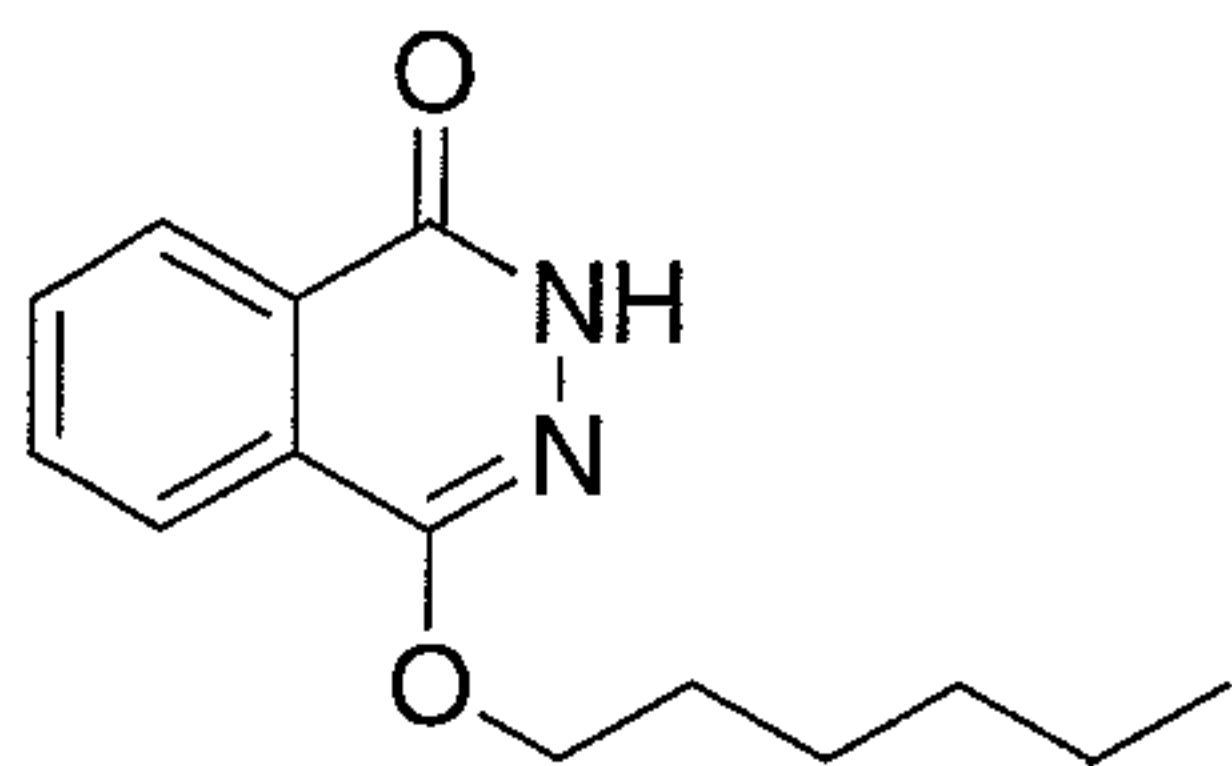
CPW28



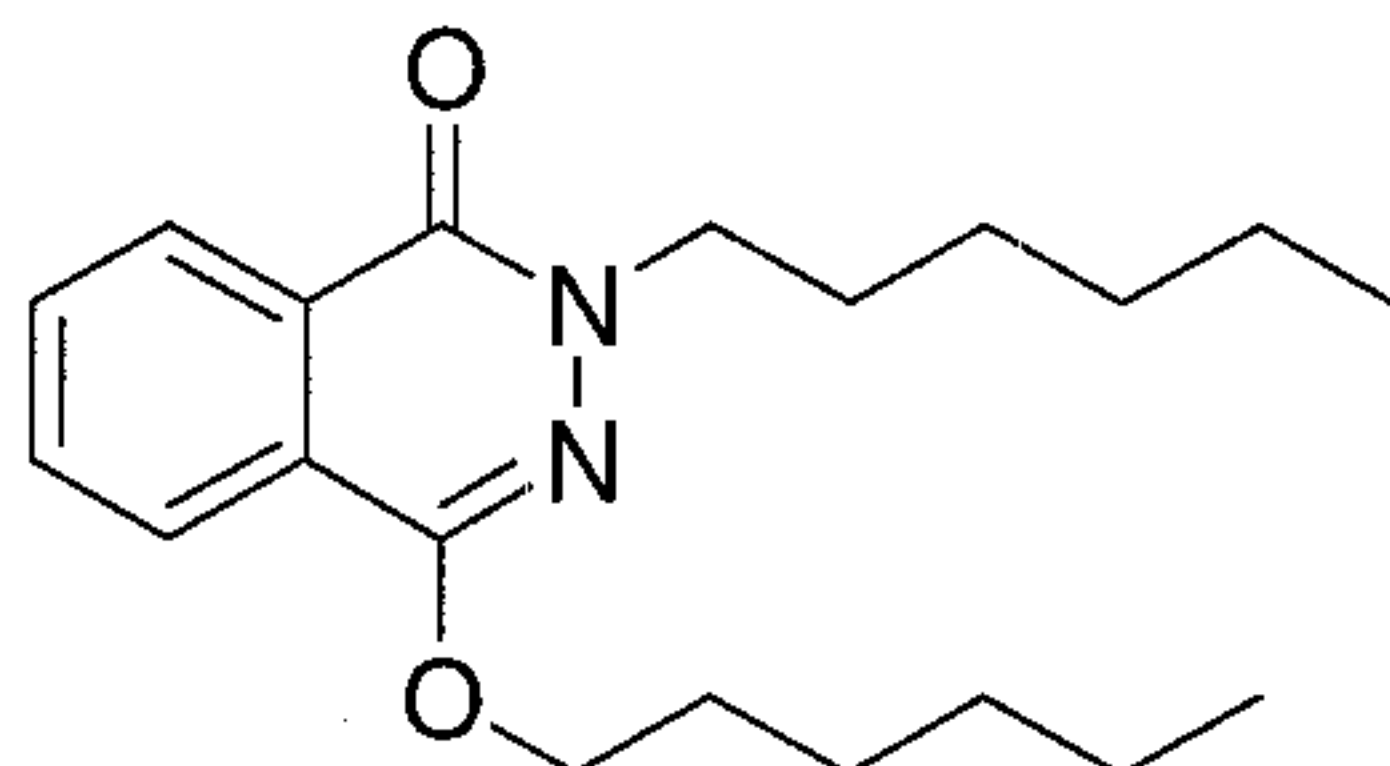
CPW29



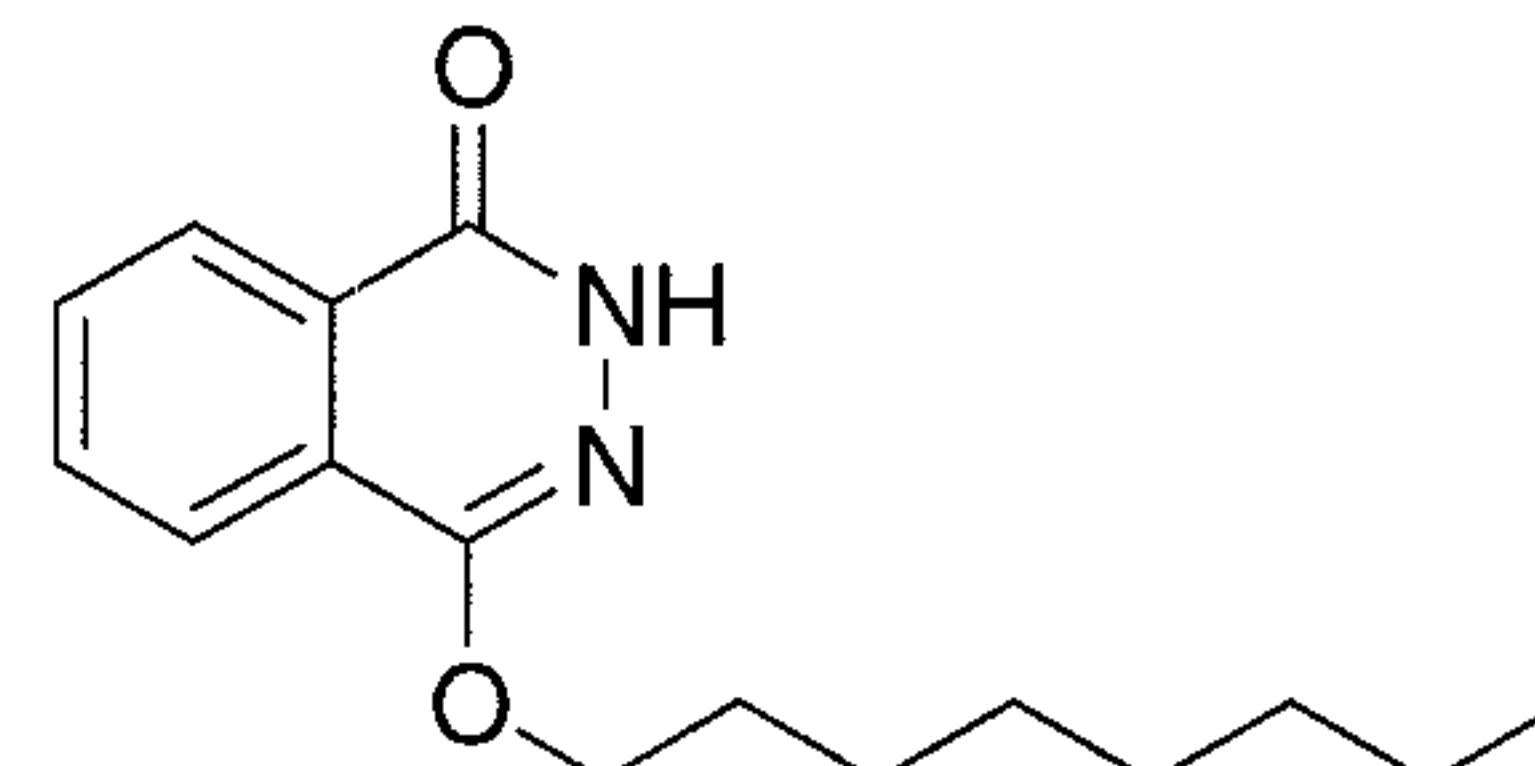
CPW30



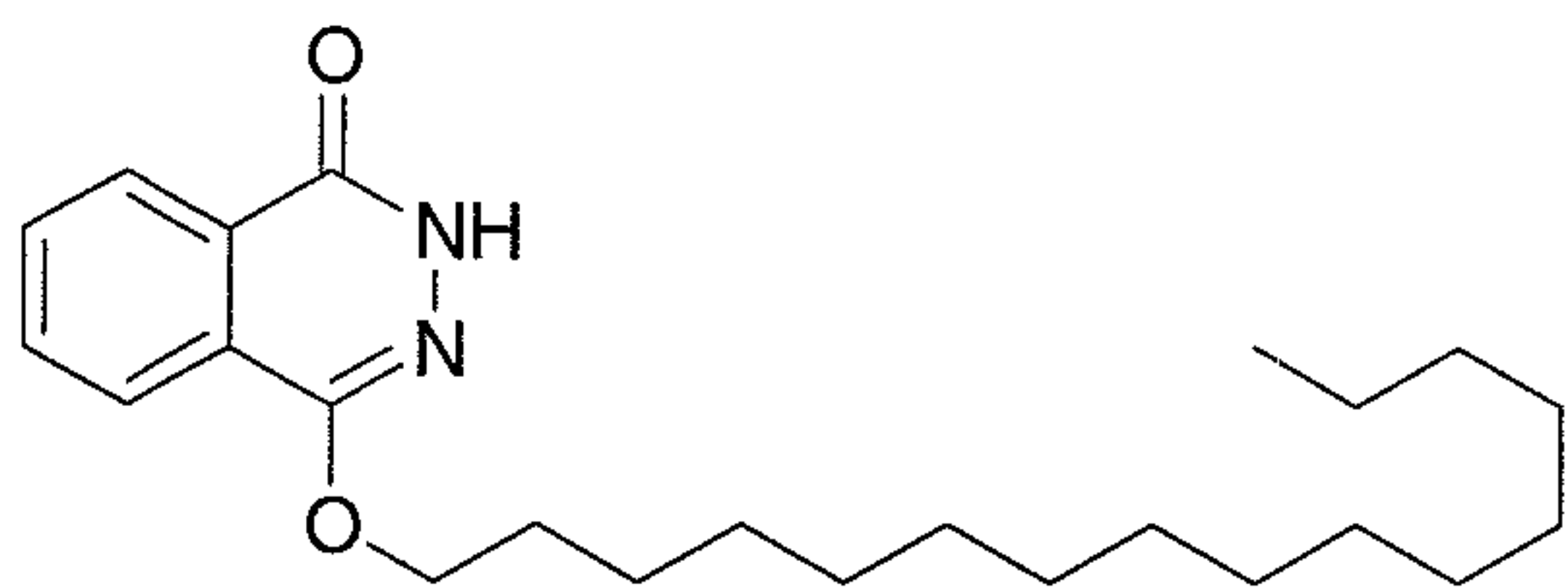
CPW31



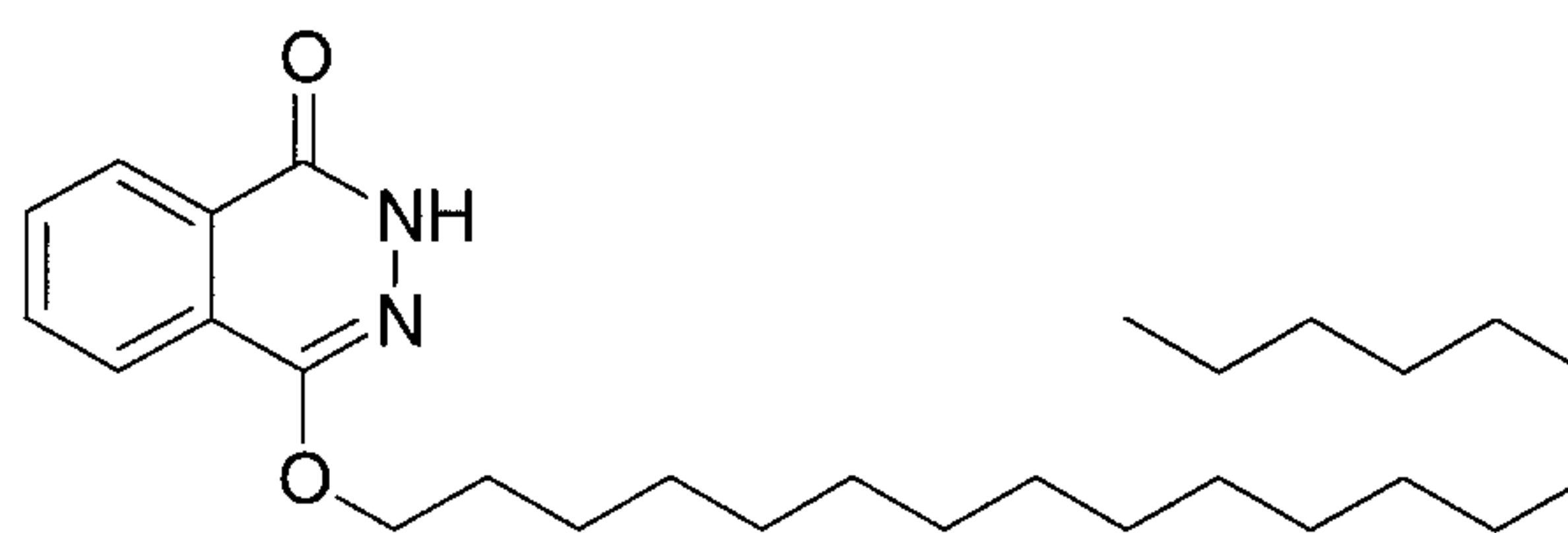
CPW32



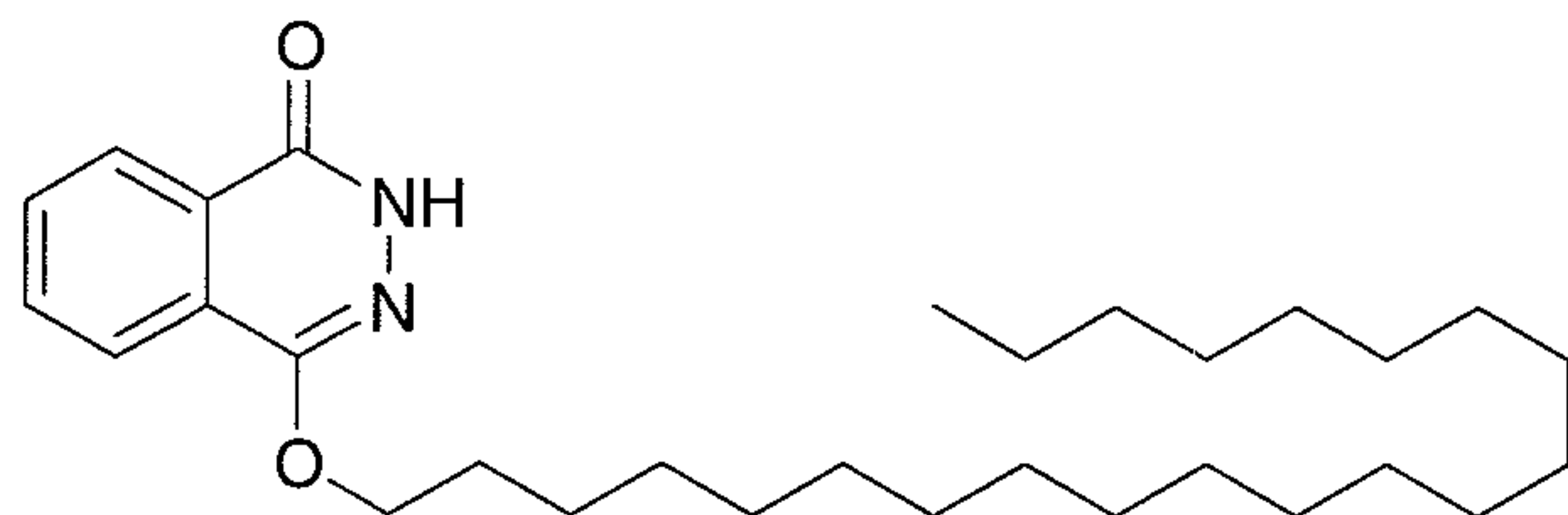
CPW33



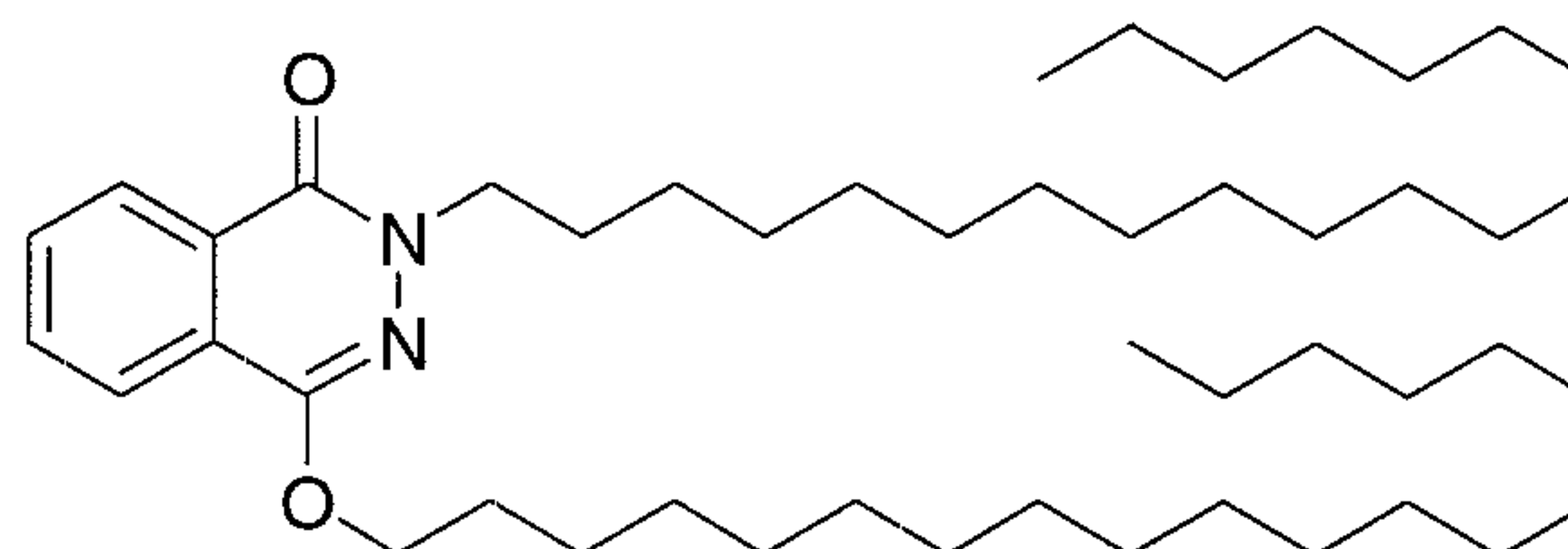
CPW36



CPW37



CPW38



CPW39