Coelenterazine Derivatives and Methods of Using Same

Cross-Reference to Related Applications

[001] This application claims the priority of U.S. Serial No. 61/409,413, filed November 2, 2010, ~~which is incorporated herein by reference~~.

Field of the Invention

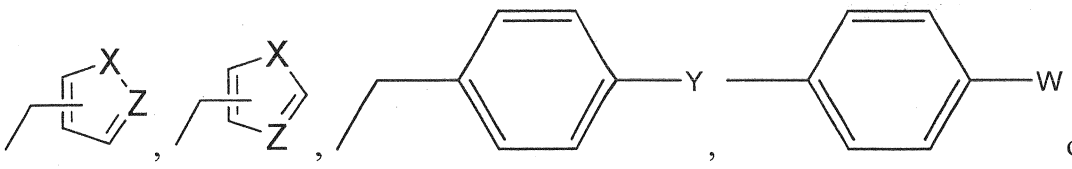
[002] The present invention provides compounds and methods for assaying the presence and activity of enzymes.

Background

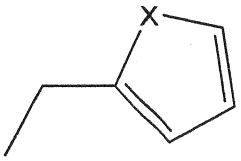
[003] The presence and activity of enzymes can be used to determine the health or metabolic state of a cell. Enzymes can also be markers for a particular cell type since the occurrence and activity of certain enzymes is frequently characteristic of a particular cell. For instance, the activity of certain enzymes can often be used to distinguish cells of bacterial, plant or animal origin or to distinguish the identity of tissue from which the enzyme originates.

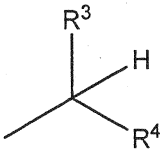
[004] Glycosidases, also know as glycoside hydrolases, catalyze the hydrolysis of the glycosidic linkage to generate two smaller sugars. They are extremely common enzymes with roles in nature including degradation of biomass such as cellulose and hemicellulose, in anti-bacterial defense strategies (e.g. lysozyme), in pathogenesis mechanisms (e.g., viral neuraminidases) and in normal cellular function (e.g. trimming mannosidases involved in N-linked glycoprotein biosynthesis). In bacteria and prokaryotes, glycosidases are found both as intracellular and extracellular enzymes that are largely involved in nutrient acquisition. One of the important occurrences of glycosidases in bacteria is the enzyme beta-galactosidase (LacZ), which is involved in the regulation of expression of the *lac* operon in *E. coli*. In higher organisms, glycosidases are found within the endoplasmic reticulum and Golgi apparatus where they are involved in processing of N-linked glycoproteins, and in the lysozome as enzymes involved in the degradation of carbohydrate structures. Deficiency in specific lysosome glycosidases can lead to a range of lysosomal storage disorders that result in development problems or death. Glycosidases are involved in the biosynthesis and degradation of glycogen in the body. Together with glycosyltransferases, glycosidases form the major catalytic machinery for the synthesis and breakage of glycosidic bonds.

[005] Diaphorases are a ubiquitous class of flavin-bound enzymes that catalyze the reduction of various compounds, which act as hydrogen acceptors, from the reduced form of di- and tri-phosphopyridine nucleotides, i.e., NADH, NADPH. Cellular energy metabolism is a complex process that allows cells to store energy through a series of enzymatic and chemical reactions. One essential aspect of cellular energy metabolism is the reduction-oxidation state of the cell.



or C_{2-5} alkyl; each X is independently –S-, –O- or –NH-; Z is –CH- or –N-; Y is -H or –OH; W is $–NH_2$, halo, -OH, -NHC(O)R, $-CO_2R$; and R is C_{1-7} alkyl.

[0041] In some embodiments, R^2 is 

; and X is O or S. In other embodiments, R^2 is C_{2-5} straight-chain alkyl. In certain embodiments, R^8 is 

, lower cycloalkyl or H, wherein R^3 and R^4 are both H or C_{1-2} alkyl. In other embodiments, R^8 is benzyl.

[0042] In some embodiments, V is S.

[0043] Suitably the compounds of the present invention are derivatives of naturally-occurring ("native") coelenterazines as well as analogs thereof, including coelenterazine-n, coelenterazine-f, coelenterazine-h, coelenterazine-hcp, coelenterazine-cp, coelenterazine-c, coelenterazine-e, coelenterazine-fcp, bis-deoxycoelenterazine ("coelenterazine-hh"), coelenterazine-i, coelenterazine-icp, and 2-methyl coelenterazine, in addition to those disclosed in WO 2003/040100 and U.S. Application Serial No. 12/056,073 (paragraph [0086])~~, the disclosures of which are incorporated by reference herein~~. Additional suitable coelenterazines that may be derivatized according to the present invention include those in FIG. 4.

[0044] In some embodiments, coelenterazine derivatives of the present invention are substrates for glycosidases. Suitable derivatives include those shown in FIG. 5.

[0045] In some embodiments, coelenterazine derivatives of the present invention are substrates for proteases such as caspase 2, caspases 3/7, caspase 6, caspase 8, caspase 9, dipeptidyl peptidase 4 (DPPIV), calpain, chymotrypsin-like proteasome, trypsin-like proteasome, caspase-like proteasome, granzyme B, cathepsins B/L/K, thrombin, trypsin, aminopeptidase, and SARS protease. Suitable peptides and amino acids, and the enzymes for which they are substrates, include those listed in FIG. 6. Additional suitable derivatives include those listed in FIG. 7.

[0046] In some embodiments, coelenterazine derivatives of the present invention are substrates for cytochrome P450 enzymes.

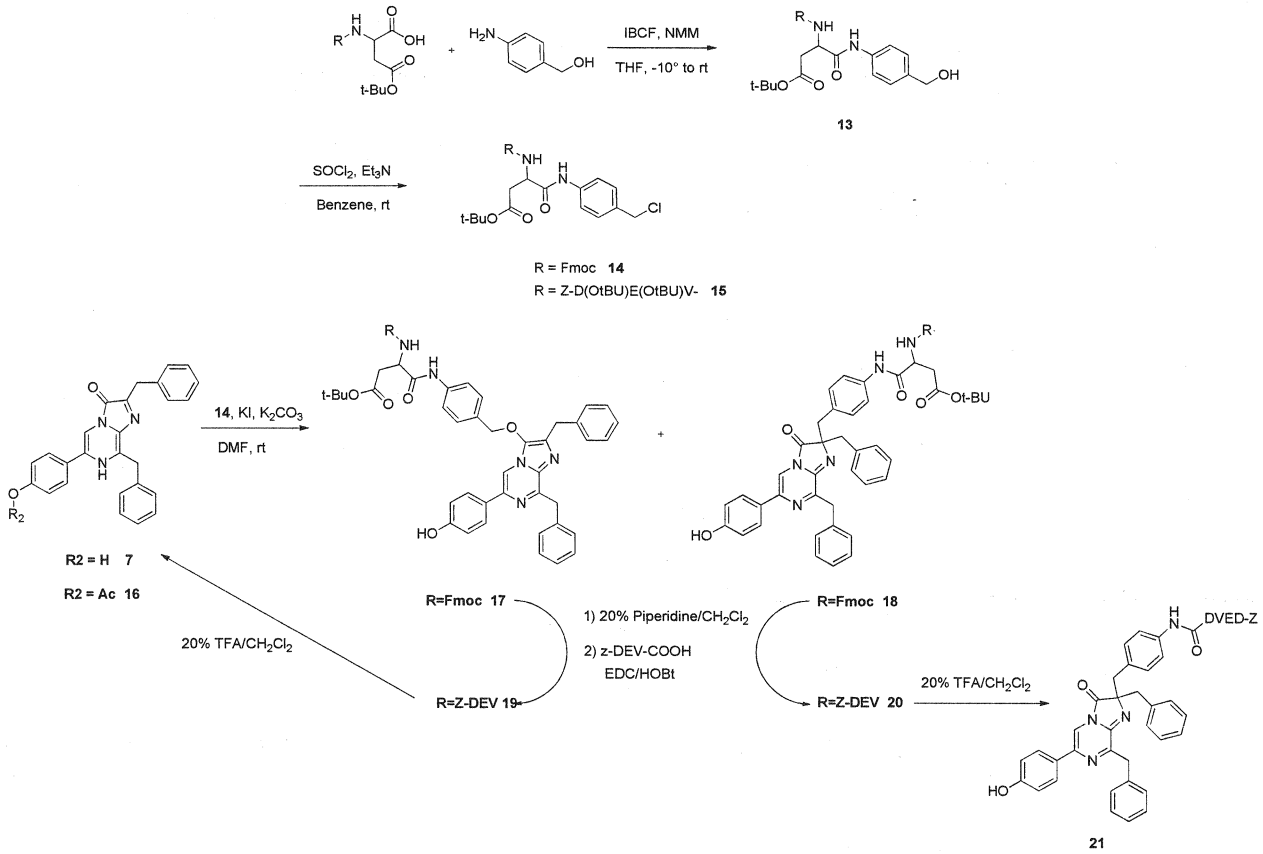
[0047] In some embodiments, coelenterazine derivatives of the present invention are substrates for diaphorase enzymes.

derivative(s), non-luciferase enzyme(s), coelenterazine-dependent luminescent enzyme(s), and reaction buffer(s). The reaction buffers may be present in individual formulations for the non-luciferase enzyme reactions and the luminescent enzyme reactions or in a single formulation for a single step assay. The kits of the present invention may also contain inhibitors, activators and/or enhancers for the non-luciferase enzyme(s). The kits of the present invention may also contain a positive and/or negative control for the assay.

[0058] The invention is further described by the following non-limiting examples.

Examples

Example 1: Synthesis of z-DEVD-coelenterazine-h (compound 21)



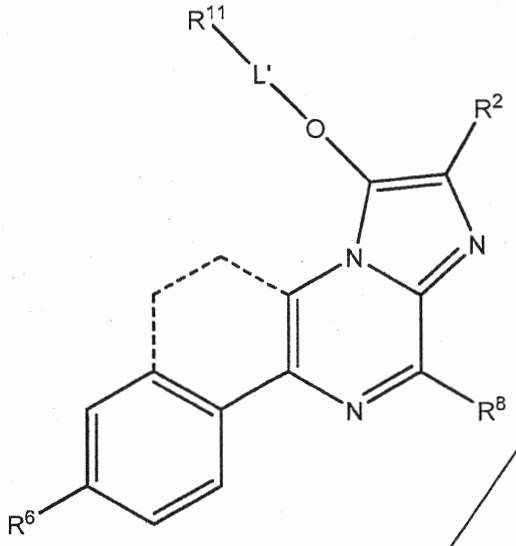
[0059] ***tert-Butyl 3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-((4-(hydroxymethyl)phenyl)amino)-4-oxobutanoate (13)***: A flask was charged with Fmoc-Asp(OtBu)-OH (6.7 g, 16.28 mmol) and 100 ml of dry THF. To this solution, at ambient temperature, neat N-methylmorpholine (1.9 mL, 17.28 mmol) was added, and the solution was cooled to -20 °C. The neat isobutylchloroformate (2.2 mL, 16.96 mmol) was added via syringe and the resulting suspension was stirred for 10 minutes. The amino alcohol (2.0 g, 16.24 mmol) was added in one portion, and the reaction mixture was stirred for 10 minutes before the cold bath was removed and stirring continued for 2 hours at ambient temperature. The reaction mixture was diluted with 300 mL of ethyl acetate, and the mixture was washed sequentially with two 50 mL portions of water, 50 mL of 0.5 M HCl and two 50 mL portions of brine solution.

[0084] For the assay, 10 µL NADH (titrated from 60 µM to 0 µM), 10 µL 12 U/mL diaphorase enzyme in PBS, 10 µL 165 U esterase in PBS and 10 µL 60 µM PBI-4600 in PBS were incubated at room temperature for 1 hour. After incubation, an Oplophorus luciferase detection reagent (100mM MES pH 6, 1mM CDTA, 150mM KCl, 35mM Thiourea, 1mM DTT, 0.5% Tergitol and 0.05% Mazu) containing an Oplophorus luciferase variant (L27V) at a 10^9 dilution was added. Luminescence was measured at 5 minutes and 1 hour (FIG. 9).
The data demonstrates that the compounds of the present invention can be used to measure NADH in a sample by detecting an enzyme which utilizes NADH.

~~**[0085]** All publications, patents and patent applications are incorporated herein by reference. While in the foregoing specification this invention has been described in relation to certain preferred embodiments thereof, and many details have been set forth for purposes of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that certain of the details described herein may be varied considerably without departing from the basic principles of the invention.~~

Claims:

1. A compound of formula (II):

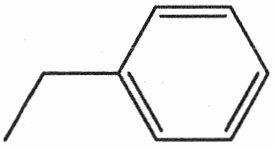


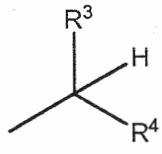
(II)

wherein R^2 is $-(CH_2)_n$-T or C_{1-5} alkyl;

R^6 is selected from the group consisting of -H, -OH , $-NH_2$, -OC(O)R or $-OCH_2OC(O)R$;

R^8 is selected from the group consisting of



, 

, H or lower cycloalkyl;

R^{11} is selected from the group consisting of a peptide, an amino acid, a saccharide, $-O-R^A$, $-OC(O)O-R^A$, $-N(R^B)_2$, or $-NHC(O)OR^A$;

wherein R^3 and R^4 are both H or both C_{1-2} alkyl;

R^A is C_{1-4} alkyl, substituted C_{1-4} alkyl, $-CH_2-R^C$ or $-CH_2-V-R^C$;

each R^B is independently -H or $-R^A$;

R^C is aryl, heteroaryl, substituted aryl or substituted heteroaryl;

L' is a direct bond or a linker;

n is 0 to 3;

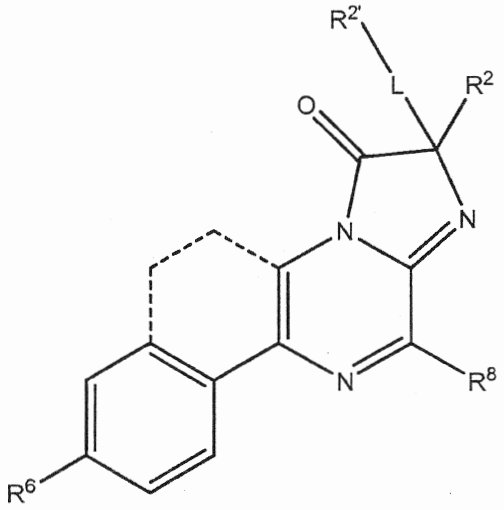
each R is independently a C_{1-7} alkyl;

T is aryl, heteroaryl, substituted aryl, substituted heteroaryl or cycloalkyl;

V is –S- or –O-; and

the dashed bonds indicate the presence of an optional ring, which may be saturated or unsaturated.

2. A compound of formula (I):



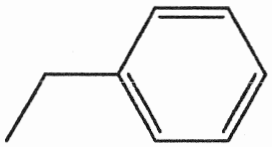
(I)

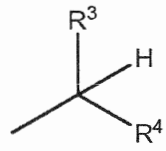
wherein R^2 is $-(CH_2)_n$-T or C_{1-5} alkyl;

$R^{2'}$ is selected from the group consisting of a peptide, an amino acid, a saccharide, $-O-R^A$, $-OC(O)O-R^A$, $-N(R^B)_2$, or $-NHC(O)OR^A$;

R^6 is selected from the group consisting of -H, -OH , $-NH_2$ -OC(O)R or $-OCH_2OC(O)R$;

R^8 is selected from the group consisting of



, , H or lower cycloalkyl;

wherein R^3 and R^4 are both H or both C_{1-2} alkyl;

R^A is C_{1-4} alkyl, substituted C_{1-4} alkyl, $-CH_2-R^C$ or $-CH_2-V-R^C$;

each R^B is independently -H or $-R^A$;

R^C is aryl, heteroaryl, substituted aryl or substituted heteroaryl;

L is a linker;

n is 0 to 3;

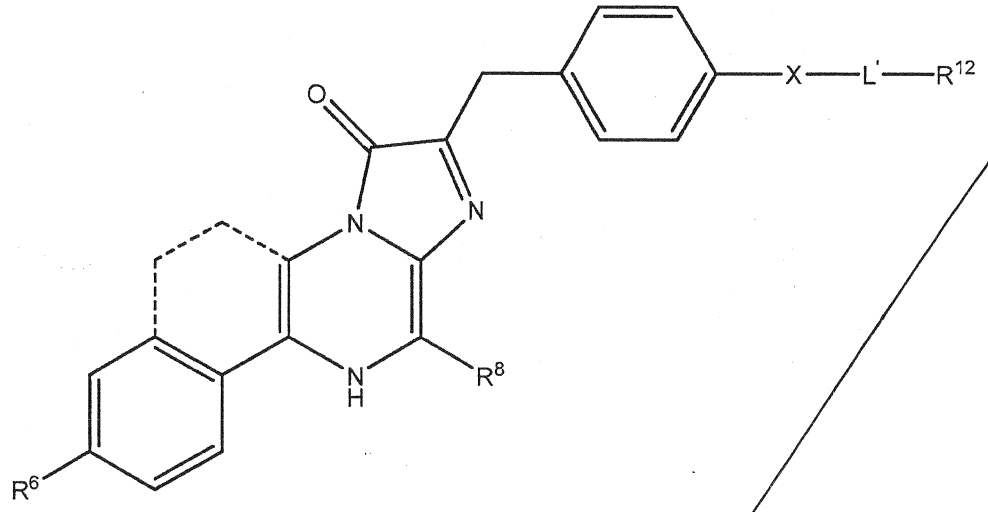
each R is independently a C_{1-7} alkyl;

T is aryl, heteroaryl, substituted aryl, substituted heteroaryl or cycloalkyl;

V is –S- or –O-; and

the dashed bonds indicate the presence of an optional ring, which may be saturated or unsaturated.

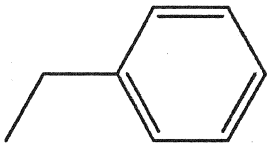
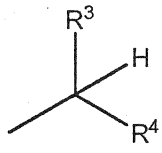
3. A compound of formula (III):



(III)

wherein R^6 is selected from the group consisting of -H, -OH , $-NH_2$ -OC(O)R or $-OCH_2OC(O)R$;

R^8 is selected from the group consisting of

, H or lower cycloalkyl;

R^{12} is selected from the group consisting of a peptide, an amino acid, a saccharide, $-O-R^A$, $-OC(O)O-R^A$, $-N(R^B)_2$, or $-NHC(O)OR^A$;

wherein R^3 and R^4 are both H or both C_{1-2} alkyl;

R^A is C_{1-4} alkyl, substituted C_{1-4} alkyl, $-CH_2-R^C$ or $-CH_2-V-R^C$;

each R^B is independently -H or $-R^A$;

R^C is aryl, heteroaryl, substituted aryl or substituted heteroaryl;

L' is a direct bond or a linker;

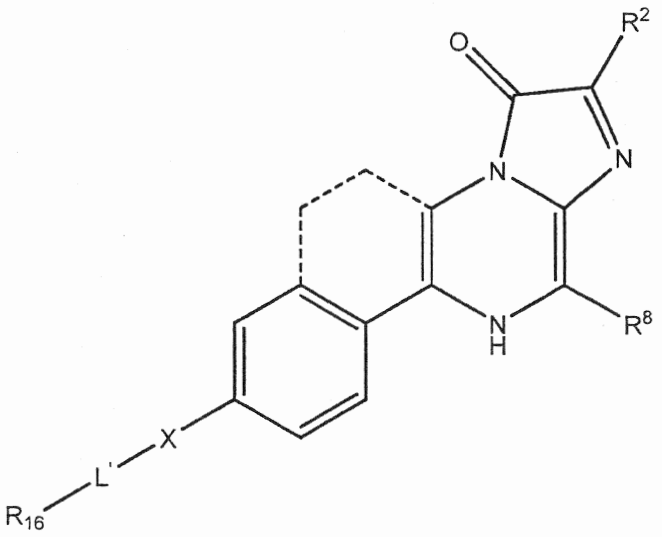
V is –S- or –O-;

each X is independently–S-, –O- or –NH-;

each R is independently C_{1-7} alkyl; and

the dashed bonds indicate the presence of an optional ring, which may be saturated or unsaturated.

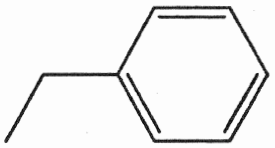
4. A compound of formula (IV):

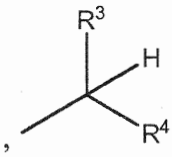


(IV)

wherein R^2 is $-(CH_2)_n$-T or C_{1-5} alkyl;

R^8 is selected from the group consisting of





, H or lower cycloalkyl;

R^{16} is selected from the group consisting of a peptide, an amino acid, a saccharide, $-O-R^A$, $-OC(O)O-R^A$, $-N(R^B)_2$, or $-NHC(O)OR^A$.

wherein R^3 and R^4 are both H or both C_{1-2} alkyl;

R^A is C_{1-4} alkyl, substituted C_{1-4} alkyl, $-CH_2-R^C$ or $-CH_2-V-R^C$;

each R^B is independently -H or $-R^A$;

R^C is aryl, heteroaryl, substituted aryl or substituted heteroaryl;

L' is a direct bond or a linker;

n is 0 to 3;

T is aryl, heteroaryl, substituted aryl, substituted heteroaryl or cycloalkyl;

V is –S- or –O-;

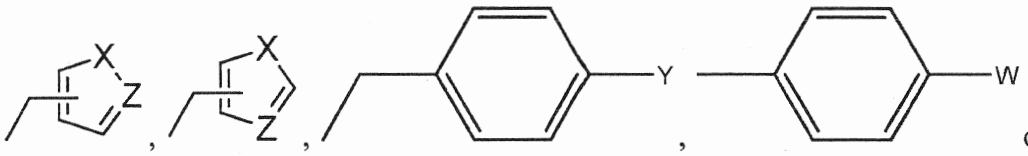
each X is independently –S-, –O- or –NH-;

each R is independently C_{1-7} alkyl; and

the dashed bonds indicate the presence of an optional ring, which may be saturated or unsaturated.

5. A compound according to any one of claims 1, 2 and 4,

wherein R^2 is



or C_{2-5} straight-chain alkyl;

each X is independently –S-, –O- or –NH-;

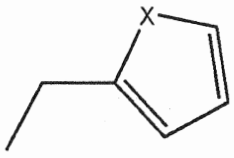
Z is –CH- or –N-;

Y is -H or –OH;

W is $–NH_2$, halo, -OH, -NHC(O)R, $-CO_2R$; and

R is C_{1-7} alkyl.

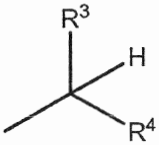
6. A compound according to claim 5, wherein

R^2 is 

; and

X is O or S.

7. A compound according to claim 5, wherein R^2 is C_{2-5} straight-chain alkyl.

8. A compound according to any one of claims 1-7, wherein R^8 is 

, lower cycloalkyl or H, wherein R^3 and R^4 are both H or C_{1-2} alkyl.

9. A compound according to any one of claims 1-7, wherein R^8 is benzyl.

10. A compound according to any one of claims 1-9, wherein V is S.

11. A method of detecting the presence or amount of an enzyme comprising:

contacting a sample suspected of containing the enzyme with a compound according to any one of claims 1 to 10; and

detecting luminescence of the sample.

12. The method of claim 11, wherein the luminescence is quantified.

13. The method of claim 11, wherein the sample is suspected of containing a second enzyme, comprising

contacting the sample with a second compound according to any one of claims 1 to 10, wherein the second compound contains a substrate for the second enzyme; and

detecting the luminescence of the sample.

14. A method of detecting the presence of an enzyme *in vivo* comprising:

administering a compound according to any one of claims 1 to 10 to a transgenic animal; and

detecting luminescence.

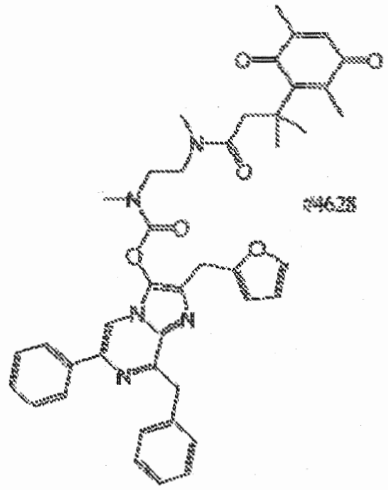
15. A method of detecting the presence of an enzyme comprising:

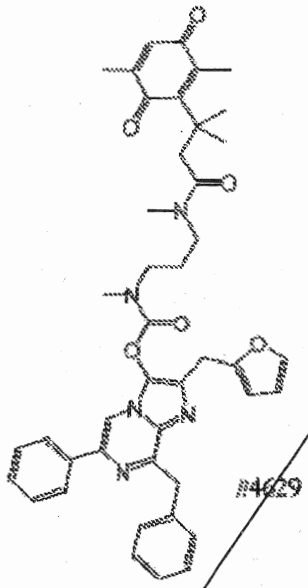
administering a compound according to any one of claims 1 to 10 to an animal;

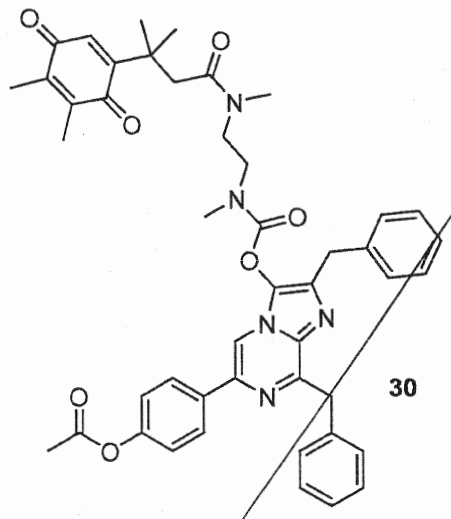
obtaining a sample from the animal; and

detecting luminescence of the sample.

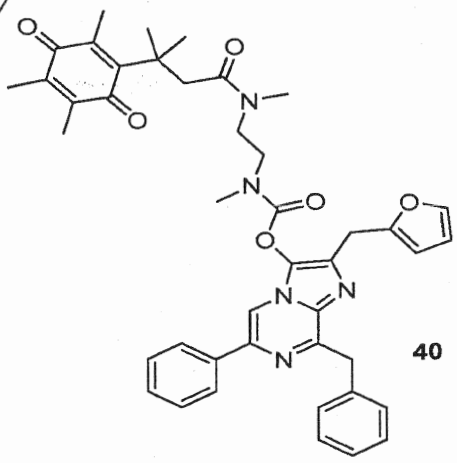
16. A compound selected from the group consisting of:



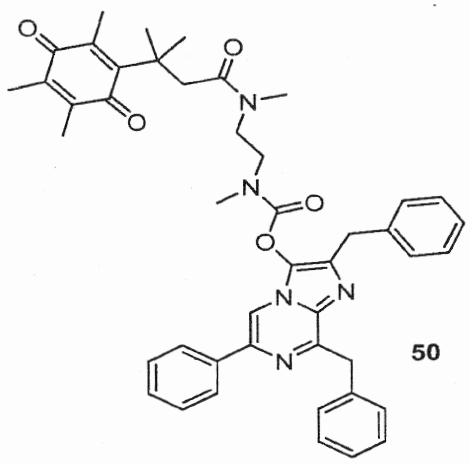




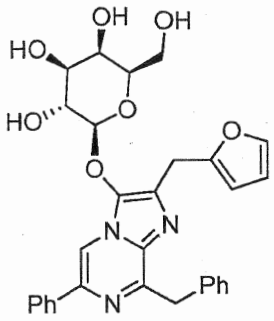
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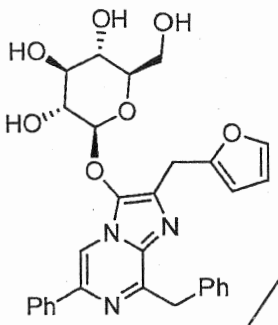
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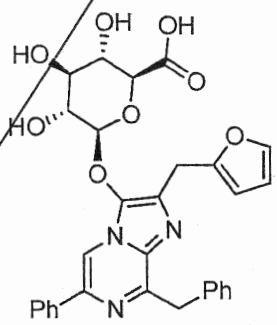
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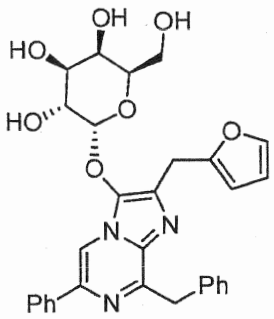
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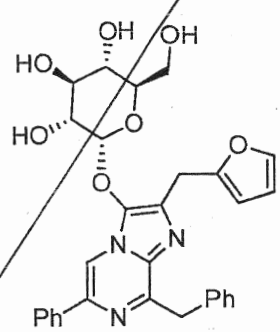
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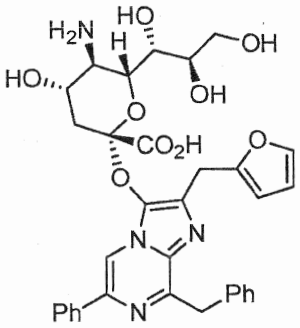
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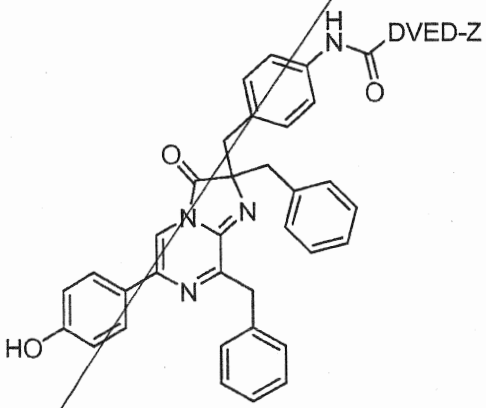
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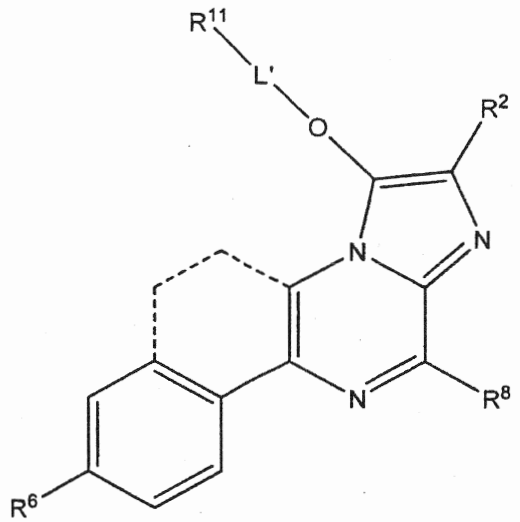


(30)



CLAIMS

1. A compound of formula (II):

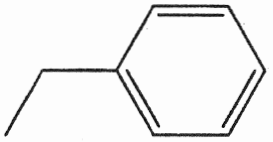


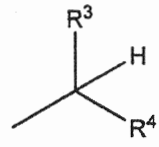
(II)

wherein R^2 is $-(CH_2)_n-T$ or C_{1-5} alkyl;

R^6 is selected from the group consisting of -H, -OH , $-NH_2$, -OC(O)R or $-OCH_2OC(O)R$;

R^8 is selected from the group consisting of



, 

, H or lower cycloalkyl;

R^{11} is selected from the group consisting of a peptide, an amino acid, a saccharide, $-O-R^A$, $-OC(O)O-R^A$, $-N(R^B)_2$, or $-NHC(O)OR^A$;

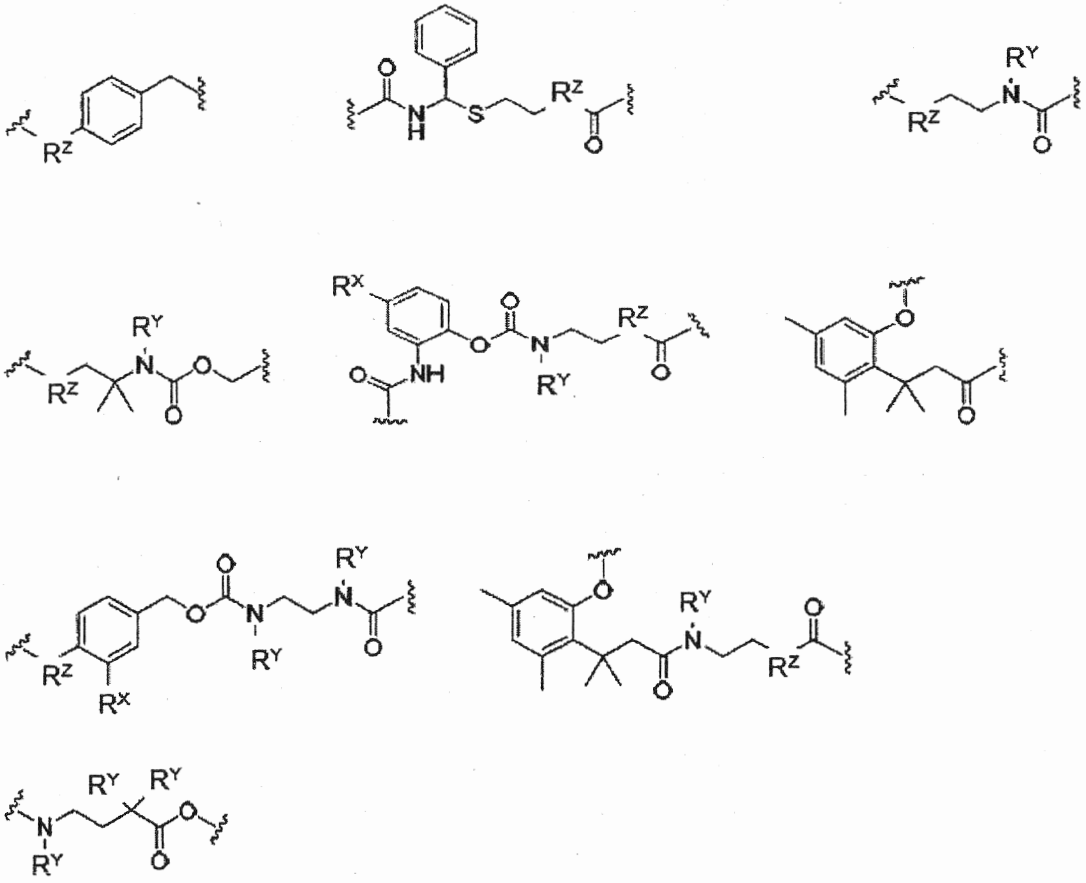
wherein R^3 and R^4 are both H or both C_{1-2} alkyl;

R^A is C_{1-4} alkyl, substituted C_{1-4} alkyl, $-CH_2-R^C$ or $-CH_2-V-R^C$;

each R^B is independently -H or $-R^A$;

R^C is aryl, heteroaryl, substituted aryl or substituted heteroaryl;

L' is a linker selected from



each R^X is independently H, halogen or NO_2

each R^Y is independently H, or Me

each R^Z is independently NH, NMe, O or S

n is 0 to 3;

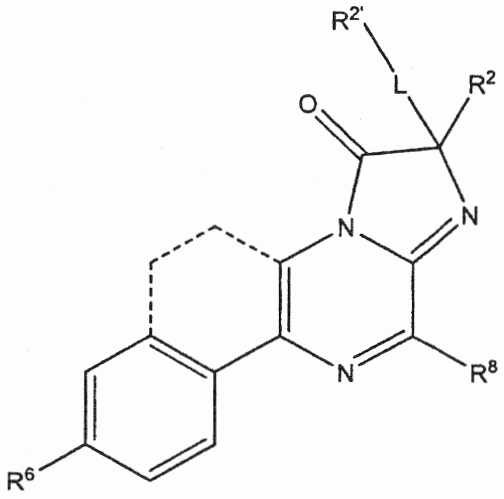
each R is independently a C_{1-7} alkyl;

T is aryl, heteroaryl, substituted aryl, substituted heteroaryl or cycloalkyl;

V is –S- or –O-; and

the dashed bonds indicate the presence of an optional ring, which may be saturated or unsaturated.

2. A compound of formula (I):



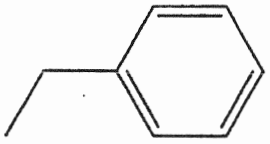
(I)

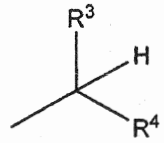
wherein R^2 is $-(CH_2)_n$-T or C_{1-5} alkyl;

$R^{2'}$ is selected from the group consisting of a peptide, an amino acid, a saccharide, $-O-R^A$, $-OC(O)O-R^A$, $-N(R^B)_2$, or $-NHC(O)OR^A$;

R^6 is selected from the group consisting of -H, -OH , $-NH_2$ $-OC(O)R$ or $-OCH_2OC(O)R$;

R^8 is selected from the group consisting of



, 

R^4, H or lower cycloalkyl;

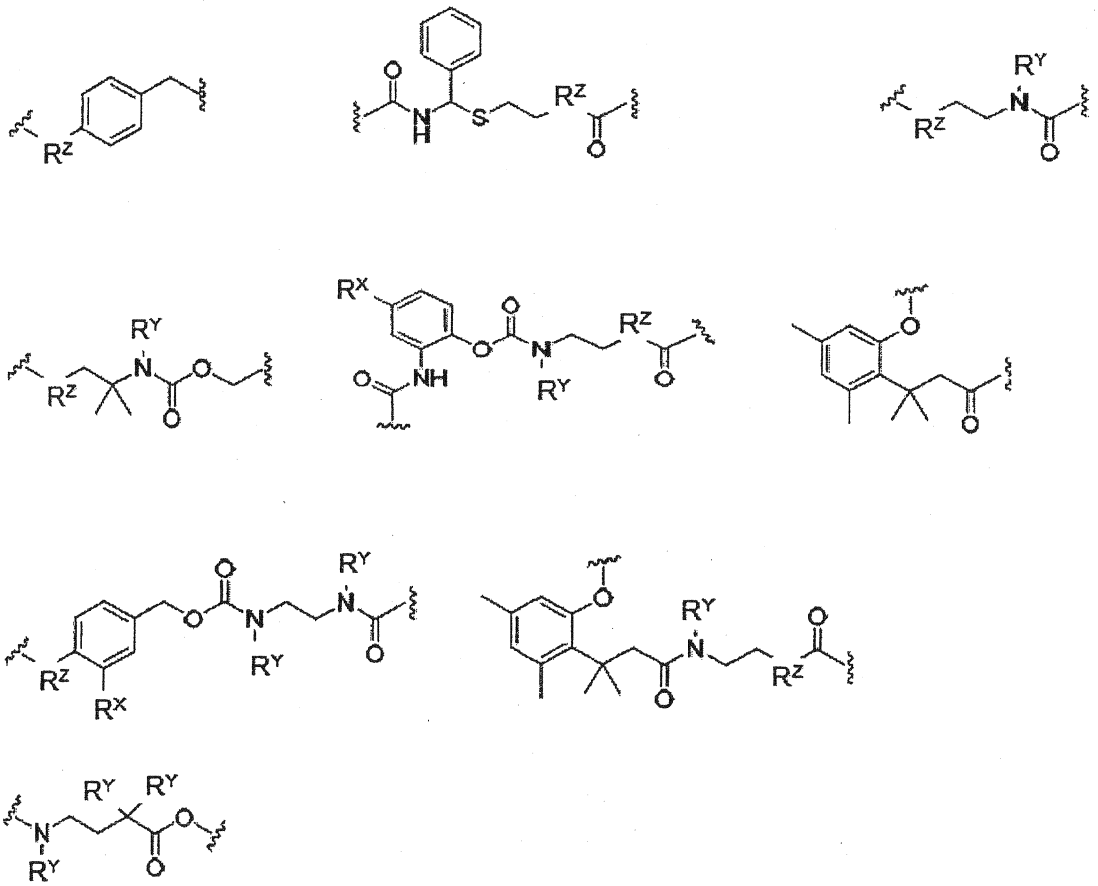
wherein R^3 and R^4 are both H or both C_{1-2} alkyl;

R^A is C_{1-4} alkyl, substituted C_{1-4} alkyl, $-CH_2-R^C$ or $-CH_2-V-R^C$;

each R^B is independently -H or $-R^A$;

R^C is aryl, heteroaryl, substituted aryl or substituted heteroaryl;

L is a linker selected from



each R^X is independently H, halogen or NO_2

each R^Y is independently H, or Me

each R^Z is independently NH, NMe, O or S

n is 0 to 3;

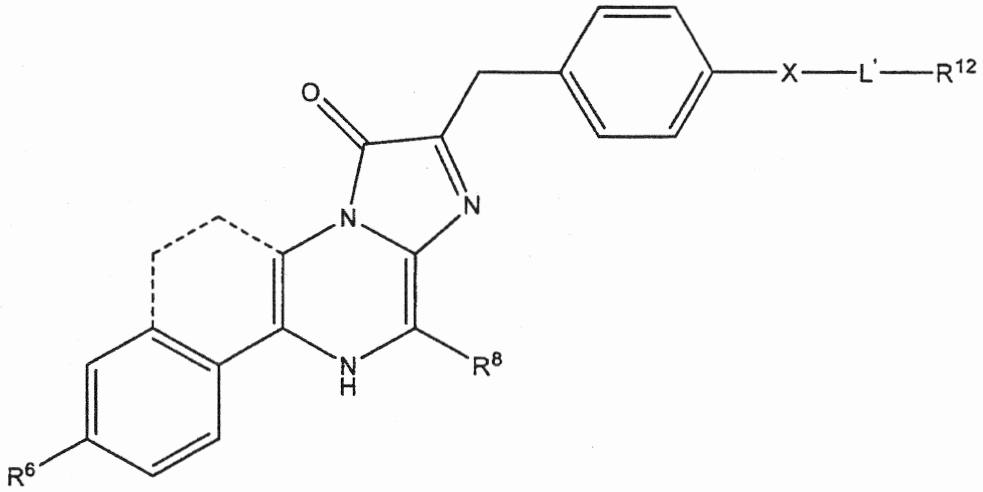
each R is independently a C_{1-7} alkyl;

T is aryl, heteroaryl, substituted aryl, substituted heteroaryl or cycloalkyl;

V is –S- or –O-; and

the dashed bonds indicate the presence of an optional ring, which may be saturated or unsaturated.

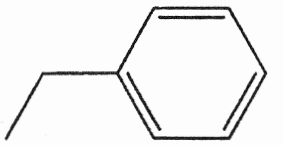
3. A compound of formula (III):

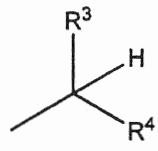


(III)

wherein R^6 is selected from the group consisting of -H, -OH , $-NH_2$ -OC(O)R or $-OCH_2OC(O)R$;

R^8 is selected from the group consisting of



, 

R^4, H or lower cycloalkyl;

R^{12} is selected from the group consisting of a peptide, an amino acid, a saccharide, $-O-R^A$, $-OC(O)O-R^A$, $-N(R^B)_2$, or $-NHC(O)OR^A$;

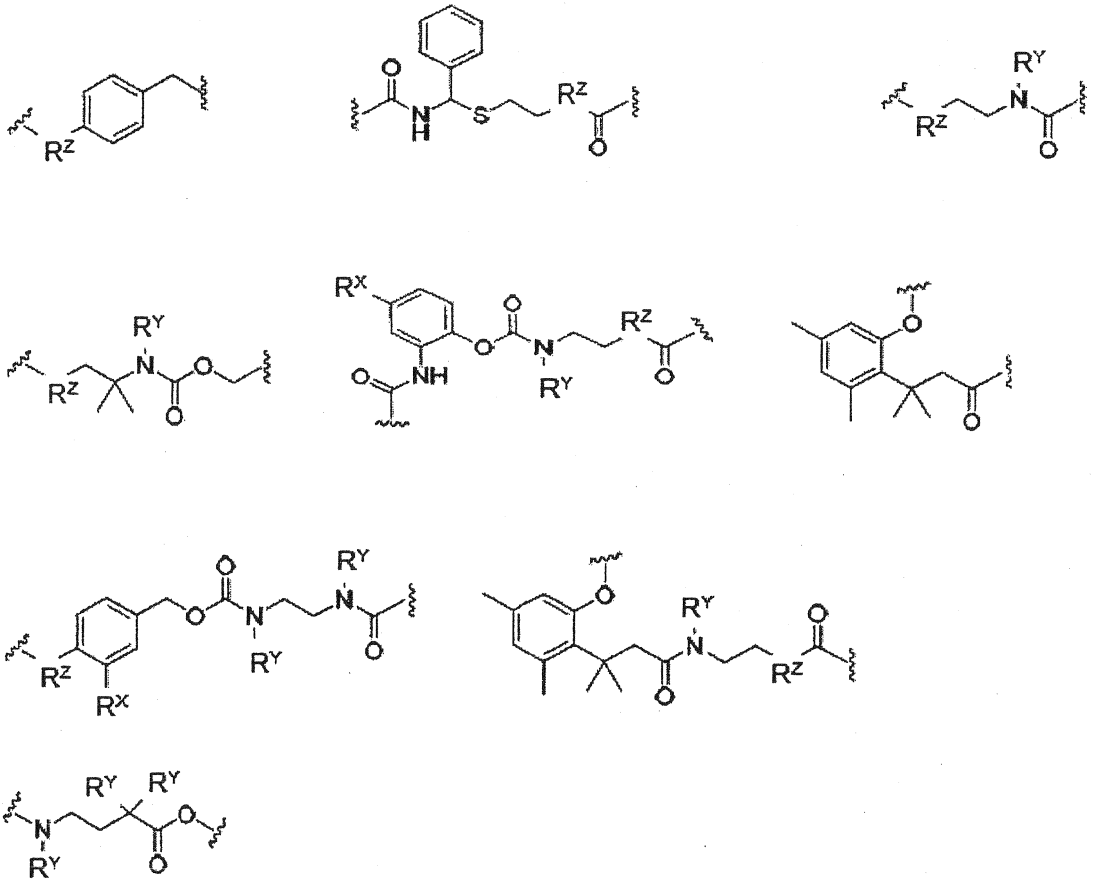
wherein R^3 and R^4 are both H or both C_{1-2} alkyl;

R^A is C_{1-4} alkyl, substituted C_{1-4} alkyl, $-CH_2-R^C$ or $-CH_2-V-R^C$;

each R^B is independently -H or $-R^A$;

R^C is aryl, heteroaryl, substituted aryl or substituted heteroaryl;

L' is a linker selected from



each R^X is independently H, halogen or NO_2

each R^Y is independently H, or Me

each R^Z is independently NH, NMe, O or S

;

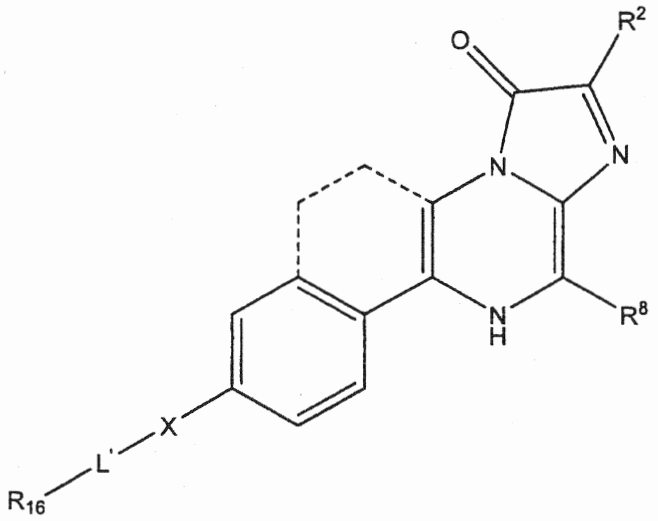
V is –S- or –O-;

each X is independently–S-, –O- or –NH-;

each R is independently C_{1-7} alkyl; and

the dashed bonds indicate the presence of an optional ring, which may be saturated or unsaturated.

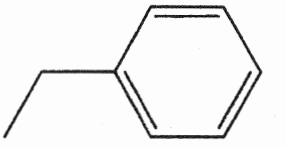
4. A compound of formula (IV):

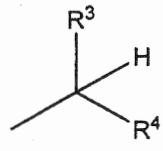


(IV)

wherein R^2 is $-(CH_2)_n$-T or C_{1-5} alkyl;

R^8 is selected from the group consisting of



, 

R^4, H or lower cycloalkyl;

R^{16} is selected from the group consisting of a peptide, an amino acid, a saccharide, $-O-R^A$, $-OC(O)O-R^A$, $-N(R^B)_2$, or $-NHC(O)OR^A$.

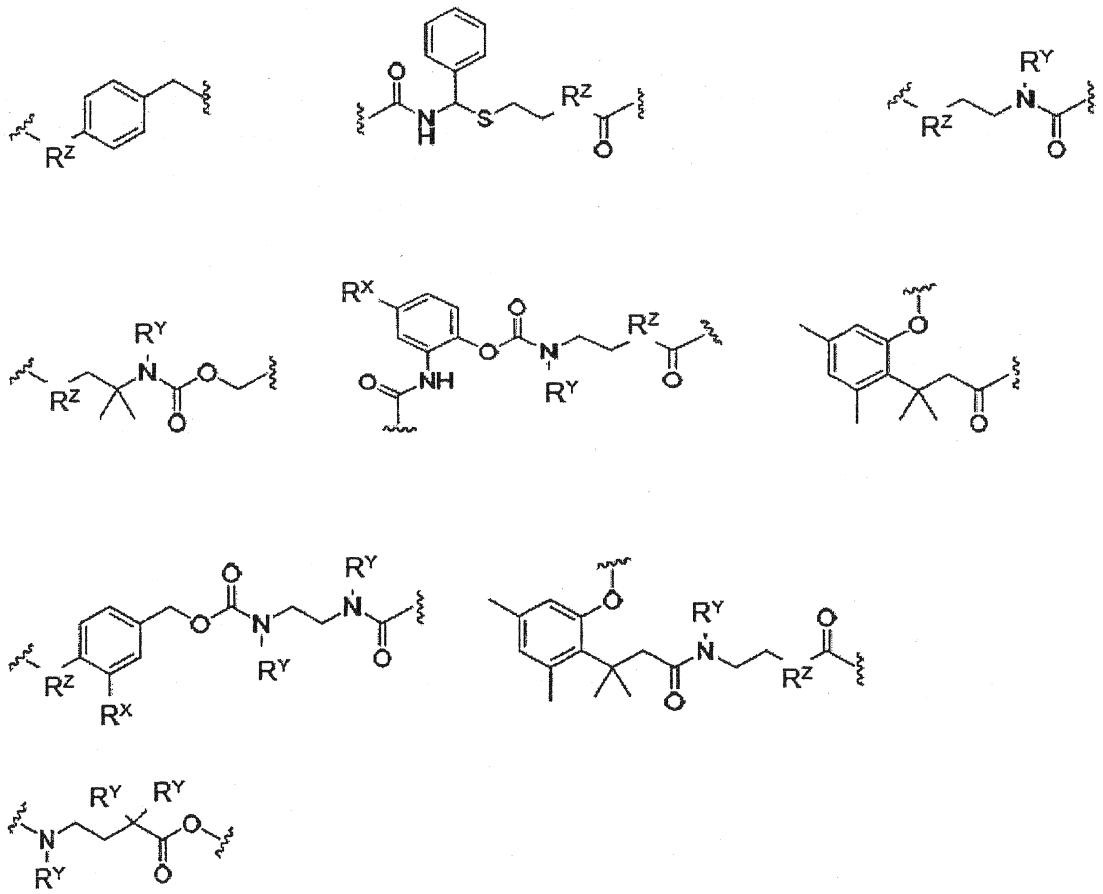
wherein R^3 and R^4 are both H or both C_{1-2} alkyl;

R^A is C_{1-4} alkyl, substituted C_{1-4} alkyl, $-CH_2-R^C$ or $-CH_2-V-R^C$;

each R^B is independently -H or $-R^A$;

R^C is aryl, heteroaryl, substituted aryl or substituted heteroaryl;

L' is a linker selected from



each R^X is independently H, halogen or NO_2

each R^Y is independently H, or Me

each R^Z is independently NH, NMe, O or S

;

n is 0 to 3;

T is aryl, heteroaryl, substituted aryl, substituted heteroaryl or cycloalkyl;

V is –S- or –O-;

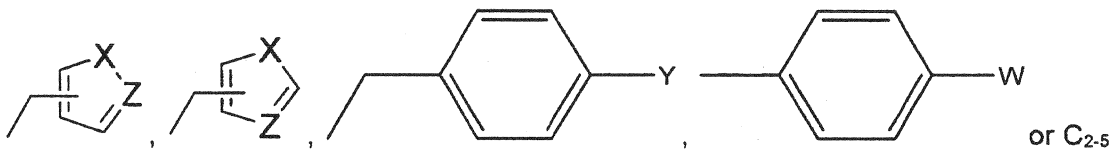
each X is independently –S-, –O- or –NH-;

each R is independently C_{1-7} alkyl; and

the dashed bonds indicate the presence of an optional ring, which may be saturated or unsaturated.

5. A compound according to any one of claims 1, 2 and 4,

wherein R^2 is



or C_{2-5} straight-chain alkyl;

each X is independently –S-, –O- or –NH-;

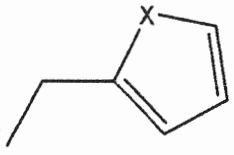
Z is –CH- or –N-;

Y is -H or –OH;

W is $–NH_2$, halo, -OH, -NHC(O)R, $-CO_2R$; and

R is C_{1-7} alkyl.

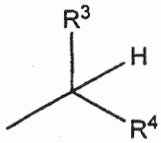
6. A compound according to claim 5, wherein

R^2 is 

; and

X is O or S.

7. A compound according to claim 5, wherein R^2 is C_{2-5} straight-chain alkyl.

8. A compound according to any one of claims 1-7, wherein R^8 is 

, lower cycloalkyl or H, wherein R^3 and R^4 are both H or C_{1-2} alkyl.

9. A compound according to any one of claims 1-7, wherein R^8 is benzyl.

10. A compound according to any one of claims 1-9, wherein V is S.

11. A method of detecting the presence or amount of an enzyme comprising:
contacting a sample suspected of containing the enzyme with a compound according to any one of claims 1 to 10; and
detecting luminescence of the sample.

12. The method of claim 11, wherein the luminescence is quantified.

13. The method of claim 11, wherein the sample is suspected of containing a second enzyme, comprising
contacting the sample with a second compound according to any one of claims 1 to 10, wherein the second compound contains a substrate for the second enzyme; and
detecting the luminescence of the sample.

14. A method of detecting the presence of an enzyme *in vivo* comprising:

administering a compound according to any one of claims 1 to 10 to a transgenic animal; and

detecting luminescence.

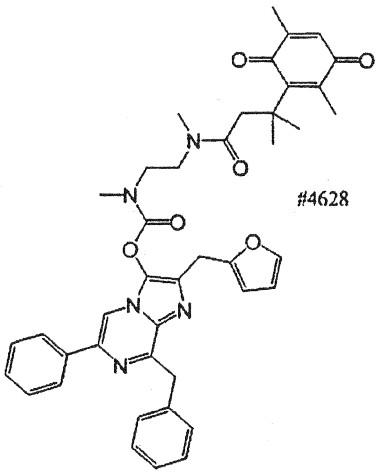
15. A method of detecting the presence of an enzyme comprising:

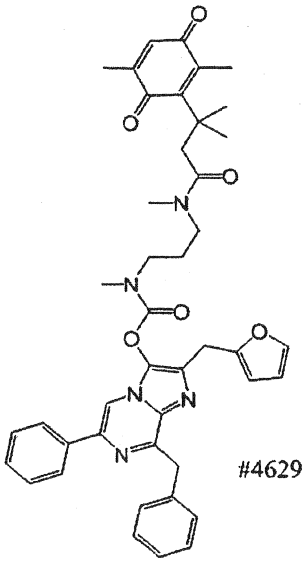
administering a compound according to any one of claims 1 to 10 to an animal;

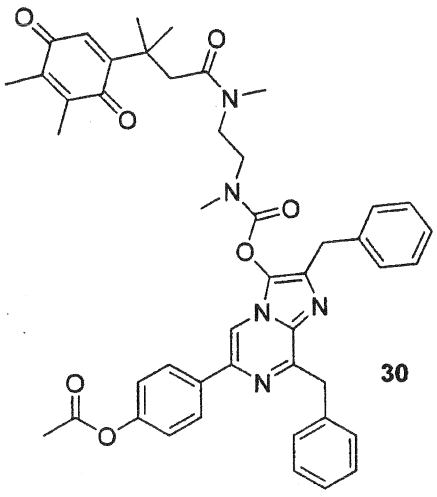
obtaining a sample from the animal; and

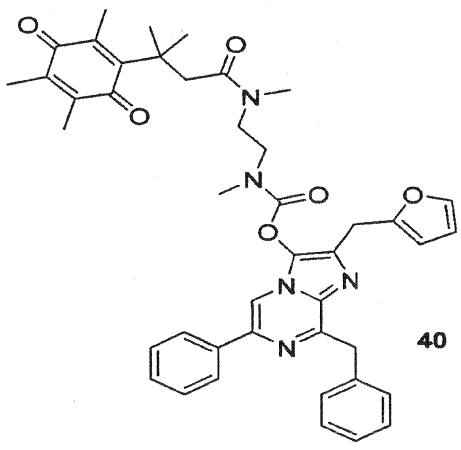
detecting luminescence of the sample.

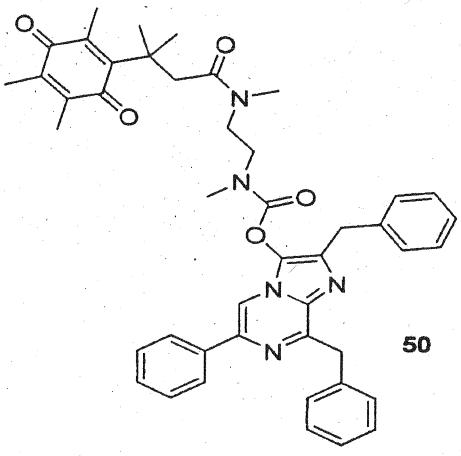
16. A compound selected from the group consisting of:



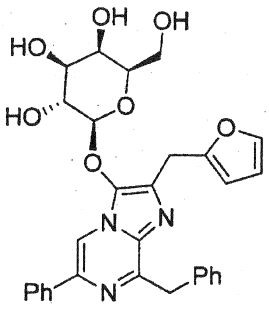




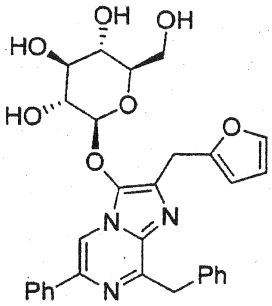




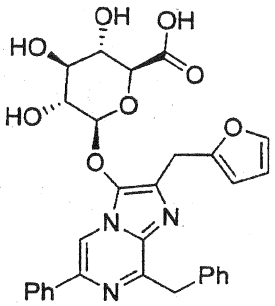
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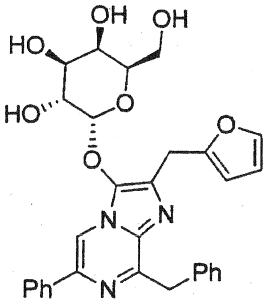
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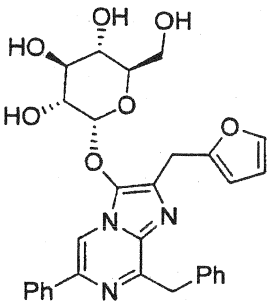
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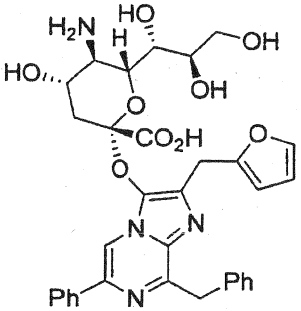
(27)



(28)



(29)



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