

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
21 September 2006 (21.09.2006)

PCT

(10) International Publication Number
WO 2006/097828 A2

- (51) International Patent Classification: Not classified
- (21) International Application Number:
PCT/IB2006/000584
- (22) International Filing Date: 13 March 2006 (13.03.2006)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/662,997 18 March 2005 (18.03.2005) US
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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, HR, HU, ID, IL, IN, IS, JP, KE,
KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV,
LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI,
NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG,
SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US,
UZ, VC, VN, YU, 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, LT, 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:**
— without international search report and to be republished
upon receipt of that report
- For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: ASSAY FOR MYELOSUPPRESSION

(57) Abstract: The invention provides a method of identifying the myelosuppressive liability of antibacterial oxazolidinone com-
pounds.



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ASSAY FOR MYELOSUPPRESSION

FIELD OF THE INVENTION

The invention provides a method of identifying the myelosuppressive liability of antibacterial oxazolidinone compounds.

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BACKGROUND OF THE INVENTION

The oxazolidinones are a new structural class of antibiotics, with activity against several Gram-positive organisms, including several resistant strains. The oxazolidinones inhibit bacterial protein synthesis. Linezolid is a synthetic
10 antibacterial agent of a new class of antibiotics, the oxazolidinones. The in vitro spectrum of activity of linezolid includes aerobic Gram-positive bacteria, certain Gram-negative bacteria and anaerobic bacteria. Clinically, linezolid therapy results in minimal side effects for most patients. However, reversible myelosuppression characterized by anemia, leukopenia, pancytopenia, and
15 thrombocytopenia, has been infrequently observed in patients treated with linezolid for at least 21 days. The mechanism of this effect is not known, but it has been suggested that the myelosuppression may result from linezolid inhibition of mitochondrial protein synthesis.

SUMMARY OF THE INVENTION

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The invention provides a method of identifying the myelosuppressive liability of an antibacterial oxazolidinone compound comprising subjecting a test oxazolidinone compound to analysis in a mitochondrial protein synthesis (MPS) assay and in at least two cell proliferation assays performed with cultures of two
25 different mammalian cells. The IC_{50} of the oxazolidinone test compound in each of the three assays is determined; and compared to the IC_{50} of a reference oxazolidinone in each assay. The test compound has less myelosuppressive

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liability than the reference oxazolidinone if the IC_{50} in at least two of the assays is higher than the IC_{50} of the reference oxazolidinone. In one embodiment, the reference oxazolidinone is linezolid. The mitochondrial protein synthesis assay may be performed on mitochondria isolated from the group consisting of

5 mammalian tissues and mammalian cells. The mammalian tissue may be selected from heart and liver. Alternatively, the mammalian cells are selected from the group consisting of cultured cells and primary cells. For instance, the mammalian cells may be selected from the group consisting of $CD34^+$ cells, K562 cells, CHO cells, HEK cells, and osteosarcoma derived cells. Alternatively, the cells are

10 selected from the group consisting of platelets, neutrophils and red blood cells.

DETAILED DESCRIPTION OF THE INVENTION

The method of the present invention relates to identification of the myelosuppressive liability of antibacterial oxazolidinone compounds.

The term myelosuppression is defined as a decrease in bone marrow

15 activity resulting in lower numbers of red blood cells, white blood cells or platelets in circulation. The term myelosuppressive liability as used herein refers to the capability of a compound to cause myelosuppression. Peripheral blood measurements of red blood cells, hemoglobin, reticulocytes, white blood cells, white blood cell differential and platelets are used to assess myelosuppression in

20 humans. In animal studies, a direct assessment of myelosuppression by measurement of the bone marrow contents can also be performed. The nucleated cell types commonly measured within the bone marrow include erythroid (red blood cell), myeloid (white blood cell) and lymphocyte (white blood cell) progenitor cells, as well as megakaryocytes (platelet producing cells). Decreases

25 in the total number of nucleated cells (TNC) or changes in the ratio of myeloid to erythroid progenitors (M:E ratio) are typically used to assess the degree of myelosuppression as well as the cell types which are affected.

The method of the present invention comprises testing oxazolidinone compounds in at least three assays and comparing the inhibition of protein

30 synthesis in the mitochondrial protein synthesis (MPS) assay (the IC_{50}) with that

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of a reference oxazolidinone, and comparing the IC_{50} of inhibition of cell growth in at least two types of mammalian cells with that of a reference oxazolidinone. The assay of the IC_{50} of the reference oxazolidinone can be done at the same time as the assay of the test compound or the results of the test oxazolidinone may be compared to historical data obtained for a reference oxazolidinone. The reference oxazolidinone may be selected from the group consisting of linezolid, eperezolid and any other antibacterial oxazolidinone. The term IC_{50} refers to the concentration of compound that results in a 50% inhibition of a measured response. Response as used herein refers to, for example, growth of cells or protein synthesis activity.

Methods for measuring mitochondrial protein synthesis are known to those of skill in the art. For instance, McKee et al. describe one method for measuring mitochondrial protein synthesis((1990) Am. J. Physiol. **258**: E503-E510). Mitochondrial protein synthesis may also be measured by measuring the synthesis of individual mitochondrial proteins. Mitochondria useful in the MPS assay may be isolated from any mammalian source. For instance, mitochondria may be isolated from mammalian tissues, such as heart and liver; from cultured mammalian cells, including primary cells or established cell lines; and from blood cells such as platelets, neutrophils or red blood cells.

The methods for the measurement of cell proliferation are well known. Mammalian cell lines may be selected from the group consisting of $CD34^+$ cells, K562 cells, CHO cells, HEK cells, and osteosarcoma derived cells may be utilized and the effect of the test oxazolidinone on cell proliferation determined. The IC_{50} of a test compound is determined by incubation the cells for a period of time of at least 24 hours in the presence and absence of the test compound or the reference oxazolidinone and measuring the viable cell number at the end of the incubation period.

Any method that can be used to determine the number of viable cells may be used. Cell numbers may be directly counted using a hemocytometer or a Coulter counter instrument. Other methods of assessing cell proliferation include measuring the uptake of radioactively labeled compound such as [3H] thymidine uptake measurements, or incorporation of BrdU)5-bromo-2'-deoxyuridine. Another assay for measuring the number of viable cells utilizes MTT (3-[4,5-

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dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) , which is a yellow salt and is cleaved to yield purple formazan crystals by metabolically active, viable cells (Roche Applied Sciences, Penzberg, Germany; Catalog # 1-465-007). The presence of the product (formazan) is measured at 550 nm and background absorbance was measured at 650 nm.

Another commercially available assay for measuring cell proliferation, a CellTiter-Glo Luminescent Cell Viability Assay (Catalog # TB288, Promega Corporation, Madison, WI) measures the ATP present in viable cells, which is a direct correlation to the number of viable cells present in a culture. In this assay, luciferin, in the presence of ATP and the enzyme luciferase, forms a luminescent product, oxyluciferin, which is measured in a luminometer.

For each type of experiment, the concentration of compound versus the cell number quantitation (for example, either direct cell number, or OD reading at 550 nm (for MTT) or the RLU (relative fluorescence units) (for the CellTiter-Glo assay)) is analyzed to yield an IC₅₀ value. An IC₅₀ value is the concentration of compound that inhibits cell growth by 50%. Data is analyzed using GraphPad Prism 4, and fitted to a sigmoidal dose-response (variable slope) curve to generate IC₅₀ values.

EXAMPLES

EXAMPLE 1

Preparation of cell cultures:

The human osteosarcoma-derived cell line 143B (TK-) and its mtDNA-less derivative, 143B206 ρ^0 , were cultured in Dulbeccos's modified Eagle (DME) high glucose medium (GIBCO, Grand Island, NY) supplemented with 10% fetal bovine serum (GIBCO, Grand Island, NY), 1 mM sodium pyruvate, and 50 μ g/ml uridine as described by King and Attardi ((1989) Science **246**: 500-503)). The cells were obtained from G. Attardi and A. Chomyn (California Institute of Technology).

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Chinese hamster ovary (CHO) cells were grown in alpha minimal essential medium (MEM) (GIBCO, Grand Island, NY) supplemented with 10% fetal bovine serum and 4 mM L-glutamine.

Human embryonic kidney (HEK) cells were cultured in DME High Glucose medium supplemented with 10% fetal bovine serum, 4 mM L-glutamine and 1 mM sodium pyruvate.

Human erythroleukemia K562 cells were obtained from ATCC and grown in RPMI 1640 medium (GIBCO, Grand Island, NY) containing 25 mM Hepes buffer, 1.5 mM Glutamax and 10% fetal bovine serum.

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EXAMPLE 2

Assay of Effect on Oxazolidinone Compounds on Cell Proliferation

Cells were incubated at 37°C, in an atmosphere of 5% CO₂ in the presence or absence of an antibacterial oxazolidinone compound. Stock solutions of the oxazolidinone compounds were prepared in 100% dimethyl sulfoxide (DMSO), and the final DMSO concentration in the assay was 0.5%. Following incubation for a period of time of from about 5 to about 10 days, quantitation of proliferation was carried out either by cell counting using a Coulter counter, by using the dye MTT (Roche Cell Proliferation Kit)(Roche Applied Sciences, Germany) or by using a CellTiter-Glo Luminescent Cell Viability Assay (Catalog # TB288, Promega Corporation, Madison, WI). For the MTT method, absorbance was measured at 550 and 650 nm (background) on a SpectraMax spectrophotometer. The difference between the absorbances was calculated and data was analyzed as described above using GraphPad Prism 4, and fitted to a sigmoidal dose-response (variable slope) curve to yield an IC₅₀ value for the oxazolidinone compound being tested.

Human CD34+ cell proliferation assay:

Fresh human CD34+ cells (1M-101b) were purchased from Cambrex Bio Science Walkersville, Inc (Walkersville, MD). Each batch of CD34+ cells was derived from the bone marrow of a healthy donor and was > 95% pure. CD34+ cells were resuspended in HPGM (Hematopoietic progenitor growth medium)

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(Cambrex) medium containing 2 mM glutamine, 200µg/ml transferrin, 50ng/ml Stem Cell Factor (SCF, R & D Systems, Inc., Minneapolis, MN) and 6 units/ ml erythropoietin (EPO, R & D Systems) at a concentration of 20,000 cells/ml. One hundred µl of diluted cells (2000 cells) were plated in 96 well flat bottom plates and 100 µl of a test oxazolidinone compound was added to each well. Compound stocks were prepared in 100% DMSO, and the final DMSO concentration in the assay was 0.1%. Positive control wells contained cells in the absence of any compound, and negative control wells did not have compound, SCF or EPO added. Both positive and negative controls contained 0.1% DMSO. All dilutions of each compound were assayed in duplicate in a given assay. Linezolid (diluted in the same way as other test compounds) was run in every assay as a standard. The plates were placed in a humidified chamber and incubated for 9 to 10 days in 5% CO₂ at 37°C. At the end of the incubation 100µl of CellTiter-Glo Luminescent Cell Viability Assay reagent (Promega Corporation, Madison, WI) was added to each well and mixed. An aliquot (100 µl) from each well was removed and transferred to an opaque 96-well plate, and luminescence was read in a Wallac Victor2 luminometer. Data was analyzed as described above using GraphPad Prism 4 (GraphPad Software, Inc.) using the equation for sigmoidal dose response with variable slope.

Incubation of K562 human leukemia cells with varying concentrations of eperezolid resulted in a time and concentration-dependent inhibition of cell number. There was a 5-10 % difference in cell number for the treated and control cultures at the 24-hour time point. The maximal inhibitory effect on proliferation at each time point was achieved with a concentration of 100 µM. After 96 hr incubation with 100 µM compound, cell growth was significantly slowed. All the cells in the culture were viable throughout the time course, based on trypan blue exclusion.

FACS analysis demonstrated that the cells were not undergoing cell cycle arrest. The compound slowed the growth by increasing the doubling time of the treated cultures. The time course data was used to plot the concentration-dependency curve of growth inhibition. The IC₅₀ for growth inhibition by eperezolid in K562 cells for a 5-day exposure was 12 µM.

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The inhibition of proliferation by eperezolid was reversed upon withdrawal of compound from the growth media. Cells were grown in either 15 μ M or 30 μ M compound for 72 hours followed by removal of the media, washing, and refeeding the cells with media containing either vehicle, 15 μ M or 30 μ M eperezolid. Cells then were cultured for an additional 5 days. After 5 days, the cell number of cultures incubated in 15 μ M compound, then washed, was essentially the same as the untreated cultures. Reversibility was observed for cells treated with 30 μ M compound, although the cell number did not return completely to that of untreated cells at the 5-day time point.

The effect of eperezolid on cell growth of several other cell lines was also investigated. Eperezolid inhibited proliferation of human HEK cells and Chinese hamster ovary (CHO) cells, as well as mouse BB88 cells. The IC_{50} value for eperezolid varied somewhat between the various cell types, and was 63 μ M, and 20 μ M, for CHO and HEK cells, respectively. In all cases, a progressive slowing of growth due to an increase in cell doubling time was observed. These experiments demonstrate that the antiproliferative effect of the oxazolidinones was not cell-type specific.

EXAMPLE 3

Mitochondrial Protein Synthesis (MPS) Assay

Preparation of mitochondria

Rat heart: The procedure for mitochondria preparation and the protein synthesis assay was adapted from that described by McKee et al. Briefly, male Sprague-Dawley rats (~250 grams) were anesthetized by inhalation of isoflurane, injected with heparin IV, and the heart was removed and rinsed in ice-cold MSE buffer (220 mM mannitol, 70 mM sucrose, 50 mM HEPES, 2mM EGTA, pH 7.2) until free of blood. Hearts were minced in MSE buffer containing 10 mg/ml Nalgarse (Sigma), washed once, and homogenized in MSE-Nalgarse buffer using a Brinkman polytron. Protease inhibitor (Complete, Boehringer) then was added to the homogenate. The homogenate was centrifuged at 900 x g for 3 min and the resulting supernatant was centrifuged at 10,000 x g for 10 min. The pellet was resuspended in 1.0 ml of ice-cold MSE buffer at a ratio of 20 ml/gram ventricular

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tissue and centrifuged at a speed of 900 x g for 3 min at 4°C. The resulting supernatant was centrifuged at 10,000 x g for 10 min at 4°C. Pellets were resuspended in MSE buffer and protein was determined (Bio-Rad).

5 **K562 cell:** K562 cells were incubated with compound or vehicle for 5 days and cells were collected, washed once with PBS, and resuspended in 1 ml of TKM buffer (10 mM Tris HCL pH 7.5, 10mM KCL and 0.15 mM NaCl) and homogenized with a hand-held homogenizer (Ultra-Turrax, IKA Labortechnik) at setting 3 for 15 sec. An aliquot (300 ul) of TKM buffer containing 1M sucrose
10 was added and the samples were centrifuged at 1000g for 5 min. The supernatant fraction was respun at 5000g for 20 min. The mitochondrial pellet was resuspended in TKM buffer containing 0.25 M sucrose in 100ul volume. Mitochondrial protein content was measured using the BCA protein assay kit (Pierce).

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Mitochondrial Protein Synthesis (MPS) Assay: Mitochondrial protein synthesis was measured in 50 µl total volume containing 90 mM KCL, 4 mM MgSO₄, 2.5 mM KH₂PO₄, 50 mM HEPES pH 7.2, 0.25 mM amino acids without methionine, 20 mM glutamate, 0.5 mM malate, 2 mM ADP, 1 mg/ml BSA (Sigma
20 A3059), 0.1 mg/ml Cycloheximide (Sigma D-335715), 1 µM cold methionine, ³⁵S-methionine (40 µCi/ml) and mitochondrial protein (1 mg/ml) in the presence or absence of compound. The final concentration of DMSO was 1% in the assay. Incubation was carried out at 30 °C for 90 minutes followed by addition of 200 µl
25 3% sulfosalicylic acid in 0.1 mM EDTA to precipitate proteins. Proteins were harvested onto filtermats (Wallac, 1205-404) using a Tomtec harvester followed by counting in a Wallac BetaPlate scintillation counter. IC₅₀ concentrations were determined by GraphPad Prism software, using a sigmoidal dose response equation with a fixed bottom.

 The oxazolidinones eperezolid and linezolid were tested in an *in vitro*
30 mitochondrial protein synthesis assay. Isolated rat heart mitochondria were incubated with linezolid or eperezolid in the presence of ³⁵S-methionine. Acid-precipitable ³⁵S-methionine and acid-precipitable radioactivity was measured

following a 90-minute incubation. Both compounds inhibited mitochondrial protein synthesis, with IC_{50} values of $9.5 \pm 1.5 \mu M$ ($n=2$) and $16 \pm 2 \mu M$ ($n=2$) for eperezolid and linezolid, respectively.

Western blot analysis of mitochondrial proteins: Mitochondrial proteins (10 μg) were electrophoresed on 10% Criterion gels (Bio-Rad) and transferred to PVD membranes (Bio-Rad). After blocking overnight at $4^{\circ}C$ with 1% BSA and 1% dry milk in Tris-buffered saline (Bio-Rad) containing 0.1% Tween 20, the membranes were incubated with 1 $\mu g/ml$ cytochrome C oxidase subunit 1 (Cox-1) monoclonal antibody (Molecular Probes A-6403) for 3 hrs, followed by chemiluminescent detection (Amersham ECL plus) according to the manufacturer's instructions. Membranes were stripped by RE-blot Plus strong solution (Chemicon 2504) and incubated with 1 $\mu g/ml$ Tom-20 polyclonal antibody (Gift from Dr. B. Wattenberg, Univ. Louisville), followed by chemiluminescent detection. The Cox-1 bands and Tom-20 bands in the X-ray films were scanned by Personal Densitometer SL (Molecular Dynamics) and quantitated by Quantity One (Bio-Rad).

The level of mitochondrial proteins in K562 cells was examined to determine whether oxazolidinones inhibited mitochondrial protein synthesis in intact cells. K562 cells were incubated with varying concentrations of eperezolid for 5 days under the same conditions as eperezolid. The cells were harvested and the mitochondria were isolated. This mitochondrial fraction was run on Western blots as described above and probed with a monoclonal antibody to subunit 1 of cytochrome oxidase, which is one of the 13 proteins encoded by the mitochondrial genome (Figure 6). To normalize for mitochondrial protein loading, after exposure to the Cox-1 antibody the blots were stripped and re-probed with an antibody to Tom 20, an outer mitochondrial membrane protein encoded by the nucleus. Tom 20 protein levels were relatively constant across the gel. However Cox-1 protein expression decreased with increasing concentrations of eperezolid, and 25 μM eperezolid resulted in a greater than 90% decrease in Cox-1 levels. As a control, cells also were treated with 100 μM chloramphenicol, a known mitochondrial protein synthesis inhibitor. Almost complete inhibition of Cox-1 levels was observed with this compound, which also inhibited the proliferation of the cells.

The decreased level of mitochondrial protein was not specific for Cox-1, since ³⁵S-methionine labeling of mitochondria isolated from oxazolidinone-treated cells showed decreased incorporation of radioactivity into all 13 mitochondrial proteins.

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EXAMPLE 4

Rho zero (ρ^0) cells are derived from a human osteosarcoma parent cell line (143B), which was depleted of mtDNA by culturing and selection in the presence of ethidium bromide. These cells cannot carry out oxidative phosphorylation, require pyruvate and uridine for growth, and have glycolysis as their only source of ATP (King). Rho zero and parent 143B cells were treated with various concentrations of eperezolid and cell number was quantitated (Figure 7). 143B cells had a doubling time of 17 ± 1.0 (n=4) hrs, while that of the rho zero cells was longer, 24 ± 0.2 (n=4) hrs. As expected, eperezolid resulted in a time and concentration-dependent decrease in 143B cell number. At the 72 hour time point, the IC₅₀ value was ~ 20 μ M. In contrast, growth of rho zero cells was not inhibited by treatment with any concentration of the two mitochondrial protein synthesis inhibitors, eperezolid or chloramphenicol. As a control, to demonstrate that rho 0 cells are not resistant to all types of growth arrest, cells were treated with etoposide, a topoisomerase II inhibitor, or with cycloheximide. As expected both these compounds inhibited growth of rho 0 cells, as well as the 143B cells.

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EXAMPLE 5

Assessment of bone marrow and hematology parameters in rats

Female Sprague-Dawley rats, 7-8 weeks old, 150-275 g were acclimated for a minimum of six days prior to use in the experiments. Animals were housed individually.

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The effects of repeated oral doses, over a 14-day experiment, of test oxazolidinones were investigated on bone marrow and hematology parameters in rats. Results are compared to historical, preclinical data for linezolid and other oxazolidinones.

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Dosing suspensions were prepared in a vehicle and given by gavage in a dose volume of 5 mL/kg/dose twice a day (10 mL/kg/day) with 6 hours between doses for 14 days.

5 Hematological parameters were evaluated on Day 14 prior to dosing for the day. Blood samples containing EDTA were evaluated for the following parameters using an automated hematology analyzer (e.g. Advia 120):

red cell count	platelet count
hemoglobin	mean platelet volume
hematocrit	white cell count
mean corpuscular volume	white cell differential
mean corpuscular hemoglobin	reticulocyte count
mean corpuscular hemoglobin concentration	red cell distribution width

10 All animals were euthanized using carbon dioxide inhalation. A single femur from each surviving animal was collected and the bone marrow contents were flushed from the bone and resuspended in fetal bovine serum. Cells were washed in PBS and a fraction of the sample was used to determine a total nucleated cell count using an automated hematology analyzer. The remaining sample was stained with either 2'7'-Dichlorofluorescein (DCF) to detect erythroid progenitors, myeloid
15 progenitors, and megakaryocytes or with antibodies specific to lymphocyte progenitor cells. Samples were analyzed by a flow cytometer (e.g. Coulter EPICS XL) and the relative amounts of erythroid progenitors, myeloid progenitors, megakaryocytes and lymphocyte progenitors were determined by the operator using cell size and fluorescence as indicators of cell type.

20 Statistical analyses were conducted for body weight and weight change, food consumption, hematology and bone marrow data. Treatment comparisons were performed on rank-transformed data using a dose-trend test sequentially applied at the two-tailed 1% and 5% significance levels within one-factor analysis of variance (ANOVA). Dunnett's test replaced the sequential trend test if the
25 overall linear trend test was not significant at the 5% level and a quadratic trend is significant at the 1% level.

EXAMPLE 6

Comparison of Myelosuppressive liability of test antibacterial oxazolidinones

For one antibacterial oxazolidinone, Compound A, the IC₅₀s for the MPS, K562, and CD34 assays were 82, 55, and 21 μ M, respectively, compared with
5 IC₅₀s of 20, 14, and 6.2 μ M for linezolid (the reference oxazolidinone). When Compound A was evaluated in rats in vivo for myelosuppression, at exposures comparable to those of linezolid which produced significant decreases in reticulocytes (97%) and RBCs, and an 8.4-fold increase in M:E ratio, Compound A produced less myelosuppression (slight decreases in reticulocytes (35%) and
10 no changes in bone marrow cells or M:E ratio). With another antibacterial oxazolidinone, Compound B, the IC₅₀s for the MPS, K562, and CD34 assays were 81, 248, and 67 μ M, respectively, compared with IC₅₀s of 19, 23, and 11 μ M for linezolid (the reference oxazolidinone). When Compound B was evaluated in rats in vivo for myelosuppression, at exposures comparable to those of linezolid which
15 produced significant decreases in reticulocytes (97%) and RBCs, and an 8.4-fold increase in M:E ratio, Compound B produced no evidence of myelosuppression.

For Compound C oxazolidinone, the IC₅₀s for the MPS, K562, and CD34 assays were 5, 19, and 8 μ M, respectively, compared with IC₅₀s of 17, 16, and 4 μ M for linezolid (the reference oxazolidinone). When Compound C was evaluated
20 in rats in vivo for myelosuppression, at exposures comparable to those of linezolid which produced significant decreases in reticulocytes (97%) and RBCs, and an 8.4-fold increase in M:E ratio, Compound C produced less myelosuppression (decreases in reticulocytes (60%), no decrease in RBCs, and a 4.5-fold increase in M:E ratio).

CLAIMS

What is claimed is:

1. A method of identifying the myelosuppressive liability of an antibacterial
5 oxazolidinone compound, said method comprising:
(a) Subjecting a test compound to analysis in the following assays
a mitochondrial protein synthesis (MPS) assay;
at least two cell proliferation assays performed with mammalian cells;
wherein the IC₅₀ of said compound in each of the above assays is
10 determined; and
(b) Comparing the IC₅₀ of said compound determined in each assay to the
IC₅₀ of a reference oxazolidinone in each assay;
wherein said compound has less myelosuppressive liability than the
reference oxazolidinone if the IC₅₀ in at least two of the assays is higher
15 than the IC₅₀ of the reference oxazolidinone.
2. The method of claim 1 wherein said reference oxazolidinone is linezolid.
3. The method of claim 1 wherein said mitochondrial protein synthesis assay
20 is performed on mitochondria isolated from the group consisting of
mammalian tissues and mammalian cells.
4. The method of claim 3 wherein said mitochondria are isolated from
mammalian tissue.
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5. The method of claim 4 wherein said mammalian tissue is selected from
heart and liver.
6. The method of claim 5 wherein said mammalian cells are selected from the
30 group consisting of cultured cells and primary cells.

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7. The method of claim 6 wherein said mammalian cells are selected from the group consisting of CD34⁺ cells, K562 cells, CHO cells, HEK cells, and osteosarcoma derived cells.
- 5 8. The method of claim 6 wherein said cells are selected from the group consisting of platelets, neutrophils and red blood cells.