

acceptable salts thereof, are also useful for inhibiting the synthesis of saponified fatty acids.

### 12. Example: Measurement of the Cytotoxicity of Illustrative Compounds of the Invention

To evaluate the effects of illustrative compounds of the invention on cytotoxicity, monolayer hepatocyte cultures were exposed to increasing concentrations of up to 250  $\mu$ M Compound A, B, C, or D in DMEM+ for 24 hours. Control cells were exposed to the same media lacking a test compound. All cells were exposed to 0.1% DMSO. The measure of cytotoxicity, release of lactate dehydrogenase (LDH) from the cytosolic compartment of hepatocyte monolayer cultures, reflects damage to the plasma membrane. The assay, based on the method of Wroblewski and LaDue, 1955, *Proc. Soc. Exp. Biol. Med.* 90:210-213; see also Ulrich et al., 1995, *Toxicol. Lett.* 82/83:107-115, describing the use of hepatocytes as models for hepatic toxicity), measures the LDH activity in tissue culture medium and a cell homogenate. Briefly, all the media were removed from plates and transferred to a separate plate. Following removal of media, attached cells were lysed with a hypotonic Tris/Glycerol/EDTA buffer (0.1 M Tris, 20% glycerol, 1 mM EDTA pH 7.3). Activity of LDH in medium and cells was measured spectrophotometrically by monitoring the rate of pyruvate reduction to lactate, coupled with oxidation of NADH; the rate of absorbance change was measured at 340 nm. Cytotoxicity was expressed as ratio using the following equation: (LDH in medium/(LDH in medium+LDH in solubilized hepatocytes))=R.

FIG. 19 shows the results of these experiments. At all concentrations tested, none of Compounds A, B, C, or D resulted in the secretion of more than approximately 25-30% of total LDH in the medium. For Compound A, toxicity was assayed at 2.5-fold the compound's therapeutically effective concentration. These experiments indicate that the toxicity of the compounds of the invention is low. Accordingly, Compounds A, B, C and D, and pharmaceutically acceptable thereof, are potentially suitable for human use without toxic side effects.

### 13. Example: Insulin Sensitization Effects of Compound A

The effects of Compound A on rate of differentiation of 3T3-L1 cells from a "committed pre-adipocyte" to an "adipocyte" phenotype in the absence or presence of insulin is tested. The differentiation of 3T3-L1 cells to an adipocyte-like phenotype is highly dependent upon insulin. This insulin-dependent changes in cellular morphology and metabolism, including: expression of adipocyte-specific genes, greatly increased levels of glucose uptake and metabolism, induction of GLUT4 (and increased expression of GLUT1) glucose transporters, greatly increased lipid synthesis and deposition of intracellular lipid droplets. In this assay the degree of differentiation was a reflection of the rate of lipid synthesis, as measured through incorporation of  $^{14}$ C-acetate over 2 hours. Thus the ability of a compound to stimulate a submaximal insulin response would suggest an insulin-sensitizing activity (Kletzein et al., 1991, *Molecular Pharm.* 41:393-398).

3T3-L1 stem cells were induced to differentiate with dexamethasone, isobutylmethylxanthine and insulin (Green and Kehinde, 1975, *Cell* 5:19-27). Cells were plated in Dulbecco's modified Eagle medium containing 10% calf serum and grown to confluence. Cells were then refreshed with 10% fetal calf serum, and treated with 0.5 mM isobu-

tylmethylxanthine and 250 nM dexamethasone, but no additional insulin, for 48 hours. This treatment induced the differentiation of 3T3-L1 cells into pre-adipocytes. Conversion of preadipocytes to adipocyte phenotype requires the removal of dexamethasone and the presence of insulin, which stimulates differentiation of preadipocytes into adipocytes in a concentration- and time-dependent manner. A maximal insulin effect occurs at about 100 nM insulin, and leads to nearly complete (95-100%) conversion to adipocytes within 4 days.

The preadipocytes were then treated for 4 days with various concentrations of Compound A in 5% fetal calf serum in Dulbecco's modified Eagles medium, with or without a submaximal concentration of insulin (30 nM). Following this four-day treatment, the preadipocytes were pulsed with 0.1  $\mu$ Ci  $^{14}$ C-acetate per well for 2 hours. Cell were then washed with phosphate buffered saline, lysed with 0.1 N NaOH, and  $^{14}$ C-acetate incorporation into lipids was determined using phase separation and liquid scintillation counting.

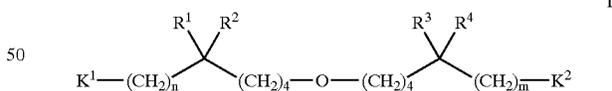
FIG. 20 shows the results of these experiments. Data are represented as the mean +/-one standard deviation for three measurements. Without Compound A,  $^{14}$ C-acetate incorporation into lipids was 4201 DPM in the presence of insulin and 545 DPM in the absence of insulin. In the presence of Compound A,  $^{14}$ C-acetate incorporation increased by approximately 40%, indicating that Compound A potentiates the insulin-dependent increase in acetate incorporation. Accordingly, Compound A or a pharmaceutically acceptable salt thereof is suitable for use as an insulin sensitizer.

The present invention is not to be limited in scope by the specific embodiments disclosed in the examples which are intended as illustrations of a few aspects of the invention and any embodiments which are functionally equivalent are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art and are intended to fall within the appended claims.

A number of references have been cited, the entire disclosures of which are incorporated herein by reference.

What is claimed is:

1. A method for treating or preventing a cardiovascular disease in a patient, comprising administering to a patient in need of such treatment or prevention a therapeutically effective amount of a compound of a formula I:



or a pharmaceutically acceptable salt thereof; and a pharmaceutically acceptable vehicle, wherein:

$R^1$ ,  $R^2$ ,  $R^3$ , and  $R^4$  are independently selected from the group consisting of ( $C_1$ - $C_6$ )alkyl, ( $C_2$ - $C_6$ )alkenyl, ( $C_2$ - $C_6$ )alkynyl, phenyl, and benzyl; or  $R^1$ ,  $R^2$ , and the carbon to which they are attached are taken together to form a ( $C_3$ - $C_7$ )cycloalkyl group; or  $R^3$ ,  $R^4$ , and the carbon to which they are attached are taken together to form a ( $C_3$ - $C_7$ )cycloalkyl group; or  $R^1$ ,  $R^2$ , and the carbon to which they are attached and  $R^3$ ,  $R^4$ , and the carbon to which they are attached are taken together to form a ( $C_3$ - $C_7$ )cycloalkyl group, with the proviso that none of  $R^1$ ,  $R^2$ ,  $R^3$ , or  $R^4$  is  $-(CH_2)_{0-4}C\equiv CH$ ;

n and m are independent integers ranging from 0 to 4;