Metabolic biomarkers of drug-induced cardiotoxicity

EP 2486404 A1 (text from WO2011044253A1)

ABSTRACT

The invention provides methods and biomarkers for assessing cardiac metabolic response to pharmaceuticals, environmental agents, chemical compounds and biologic therapies. The invention provides methods for identifying cellular metabolites secreted by primary cardiomyocytes, cardiomyocyte precursor cells, clonal cardiomyocytes derived from adult human heart, immortalized cardiomyocytes, human embryonic stem cell (hESC)-derived cardiomyocytes, human induced pluripotent stem cell (iPS)-derived cardiomyocytes, or any cell displaying cardiomyocyte-specific markers in response to exposure to pharmaceuticals, environmental agents, chemical compounds and biologic therapies that are cardiotoxic. Cardiomyocyte-secreted cellular metabolites provide metabolic signatures of cardiotoxicity, and can be used to screen pharmaceutical agents, lead and candidate drug compounds, biologies, and other therapeutics for cardiotoxic effects.

CLAIMS (OCR text may contain errors)

We claim:

1. A method of identifying cellular metabolites differentially produced in cardiomyocyte cells in the presence or absence of a test compound, the method comprising the steps of:

a) contacting cardiomyocyte cells with a test compound;

b) separating a plurality of cellular metabolites of from about 10 to about 1500 Daltons that are secreted from said cardiomyocyte cells; and

c) identifying one or a plurality of cellular metabolites of from about 10 to about 1500 Daltons that are differentially secreted from cardiomyocytes contacted with the test compound compared to cardiomyocytes not contacted with the test compound.

2. The method of claim 1, wherein at least one of the cellular metabolites is produced in greater amounts in cardiomyocytes contacted with the test compound.

3. The method of claim 1, wherein at least one of the cellular metabolites is produced in greater amounts in cardiomyocytes not contacted with the test compound.

4. The method claim 1, wherein the cellular metabolites are separated using a physical separation method.

5. The method according to claim 4, wherein the physical separation method is liquid chromatography/electrospray ionization time of flight mass spectrometry (LC/ESI- TOF-MS).

6. The method according to claim 1, wherein the candidate cellular metabolites are identified by neutral mass. 7. The method of claim 1, wherein the test compound is a cardiotoxic compound.

8. The method of claim 7, wherein the cellular metabolites comprise one or a plurality of cellular metabolites set forth in Tables 2A-2D. 9. The method of claim 7, wherein the cellular metabolites comprise one or a plurality of Triethylamine; NN-Diethylamine; Hexylamine; p- Glucosyloxymandelonitrile; (s)-4-Hydroxymandelonitrilebeta-D-glucoside; 13, 14- dihydro PGE1 (Prostaglandin El); 7-Ketocholesterol; 1,25-Dihydroxyvitamin D3-26,23- lactone; Formononetin 7-0-glucoside-6"-0-malonate; Isochlorogenic acid b; 13- Dicaffeoylquinic acid; 3-Hexaprenyl-4-hydroxy-5-methoxybenzoic acid; 2-Phenylglycine; (E)-4-Hydroxyphenylacetaldehyde-oxime; (Z)-4- Hydroxyphenylacetaldehyde-oxime; Betaine; 2-Ethylhexyl-4-hydroxybenzoate;

Glycerophosphocholme; N-Acetylgalactosamine; CGP52608; Biotin; DL-Homocystine; Ethenodeoxyadenosine; Queuine; N-Acetylaspartylglutamic acid; Tetrahydrocortisone; Cyclic Phosphatidic acid; 2-Methoxyestrone3-glucuronide; Diacylglycerol; Quercetin3- (2G-xylosylrutinoside); Niacinamide; Aspartic Acid; Iminodiacetate; Erythritol; D- Threitol; N-Acetylserine; L-Glutamic acid; L-4-Hydroxyglutamate semialdehyde; 2-Oxo- 4-hydroxy-5 -amino valerate; O-Acetylserine; DL-Glutamate; DL-Glutaminic acid; 2- Aminoglutaric acid; Glutamate; D-Glutamic acid; 3-Pyridinebutanoic acid; Norsalsolinol; D-Phenylalanine; D-alpha-Amino-beta-phenylpropionic acid; L-Phenylalanine; 3-Methylhistidine; 1-Methylhistidine; (R)-N-Methylsalsolinol; (S)-N-Methylsalsolinol; Symmetric dimethylarginine; or Asymmetric dimethylarginine. 10. The method of claim 7, wherein the cellular metabolites identified thereby comprise a metabolic profile characteristic of cardiomyocyte cell response to a cardiotoxic compound.

11. A method according to claim 1 , wherein cellular metabolites identified thereby comprise a metabolic profile characteristic of cardiomyocyte cell response to a test compound.

12. A method for identifying cellular metabolites differentially produced by cardiomyocyte cells in the presence or absence of a plurality of cardiotoxic test compounds, the method comprising the steps of: a) separately contacting each of a plurality of experimental sets of cardiomyocyte cells with a different cardiotoxic test compound;

b) separating a plurality of cellular metabolites of from about 10 to about

1500 Daltons that are secreted from each experimental set of cells;

c) identifying one or a plurality of cellular metabolites of from about 10 to about 1500 Daltons that are differentially secreted from cardiomyocytes contacted with each of the cardiotoxic test compounds compared to cardiomyocytes not contacted with the cardiotoxic test compound; and d) identifying one or a plurality of cellular metabolites differentially produced by substantially all of said experimental sets of cardiomyocyte cells exposed to said test compounds.

13. The method of claim 12, wherein at least one of the cellular metabolites is produced in greater amounts in cardiomyocytes contacted with the test compound.

14. The method of claim 12, wherein at least one of the cellular metabolites is produced in greater amounts in cardiomyocytes not contacted with the test compound.

15. The method claim 12, wherein the cellular metabolites are separated using a physical separation method.

16. The method according to claim 15, wherein the physical separation method is liquid chromatography/electrospray ionization time of flight mass spectrometry (LC/ESI- TOF-MS).

17. The method according to claim 12, wherein the candidate cellular metabolites are identified by neutral mass.

18. The method of claim 12, wherein the cellular metabolites comprise one or a plurality of cellular metabolites set forth in Tables 2A-2D.

19. The method of claim 18, wherein the cellular metabolites comprise one or a plurality of Triethylamine; NN-Diethylamine; Hexylamine; p- Glucosyloxymandelonitrile; (s)-4-Hydroxymandelonitrilebeta-D-glucoside; 13, 14- dihydro PGE1 (Prostaglandin El); 7-Ketocholesterol; 1,25-Dihydroxyvitamin D3-26,23-lactone; Formononetin 7-0-glucoside-6"-0-malonate; Isochlorogenic acid b; 13- Dicaffeoylquinic acid; 3-Hexaprenyl-4-hydroxy-5-methoxybenzoic acid; 2- Phenylglycine; (E)-4-Hydroxyphenylacetaldehyde-oxime; (Z)-4- Hydroxyphenylacetaldehyde-oxime; Betaine; 2-Ethylhexyl-4-hydroxybenzoate;

Glycerophosphocholme; N-Acetylgalactosamine; CGP52608; Biotin; DL-Homocystine; Ethenodeoxyadenosine; Queuine; N-Acetylaspartylglutamic acid; Tetrahydrocortisone; Cyclic Phosphatidic acid; 2-Methoxyestrone3-glucuronide; Diacylglycerol; Quercetin3- (2G-xylosylrutinoside); Niacinamide; Aspartic Acid; Iminodiacetate; Erythritol; D- Threitol; N-Acetylserine; L-Glutamic acid; L-4-Hydroxyglutamate semialdehyde; 2-Oxo- 4-hydroxy-5 -amino valerate; O-Acetylserine; DL-Glutamate; DL-Glutaminic acid; 2- Aminoglutaric acid; Glutamate; D-Glutamic acid; 3-Pyridinebutanoic acid; Norsalsolinol; D-Phenylalanine; D-alpha-Amino-beta-phenylpropionic acid; L-Phenylalanine; 3-Methylhistidine; 1-Methylhistidine; (R)-N-Methylsalsolinol; (S)-N-Methylsalsolinol; Symmetric dimethylarginine; or Asymmetric dimethylarginine. 20. The method of claim 12, wherein the cellular metabolites identified thereby comprise a metabolic profile characteristic of cardiomyocyte cell response to a cardiotoxic compound.

21. The method of claim 20, wherein the test compound is doxorubicin, tamoxifen or paclitaxel.

22. The method of claim 10, wherein the test compound is doxirubicin, tamoxifen or paclitaxel.

23. The method of claims 10 or 20, wherein the cellular metabolites comprise one or a plurality of cellular metabolites set forth in Tables 2A-2D. 24. The method of claims 10 or 20, wherein the cellular metabolites comprise one or a plurality of Triethylamine; NN-Diethylamine; Hexylamine; p- Glucosyloxymandelonitrile;

(s)-4-Hydroxymandelonitrilebeta-D-glucoside; 13, 14- dihydro PGE1 (Prostaglandin El); 7-Ketocholesterol; 1,25-Dihydroxyvitamin D3-26,23- lactone; Formononetin 7-0-glucoside-6"-0-malonate; Isochlorogenic acid b; 13- Dicaffeoylquinic acid; 3-Hexaprenyl-4-hydroxy-5-methoxybenzoic acid; 2-Phenylglycine; (E)-4-Hydroxyphenylacetaldehyde-oxime; (Z)-4- Hydroxyphenylacetaldehyde-oxime; Betaine; 2-Ethylhexyl-4-hydroxybenzoate;

Glycerophosphocholme; N-Acetylgalactosamine; CGP52608; Biotin; DL-Homocystine; Ethenodeoxyadenosine; Queuine; N-Acetylaspartylglutamic acid; Tetrahydrocortisone; Cyclic Phosphatidic acid; 2-Methoxyestrone3-glucuronide; Diacylglycerol; Quercetin3- (2G-xylosylrutinoside); Niacinamide; Aspartic Acid; Iminodiacetate; Erythritol; D- Threitol; N-Acetylserine; L-Glutamic acid; L-4-Hydroxyglutamate semialdehyde; 2-Oxo- 4-hydroxy-5 -amino valerate; O-Acetylserine; DL-Glutamate; DL-Glutaminic acid; 2- Aminoglutaric acid; Glutamate; D-Glutamic acid; 3-Pyridinebutanoic acid; Norsalsolinol; D-Phenylalanine; D-alpha-Amino-beta-phenylpropionic acid; L-Phenylalanine; 3-Methylhistidine; 1-Methylhistidine; (R)-N-Methylsalsolinol; (S)-N-Methylsalsolinol; Symmetric dimethylarginine; or Asymmetric dimethylarginine.

25. A method for identifying cardiotoxic effects in a patient resulting from contact with or administration of a cardiotoxic compound, the method comprising the steps of: a) assaying a biological sample from a patient for the presence of one or a plurality of cellular metabolites having a molecular weight of from about 10 Daltons to about 1500 Daltons; and

b) identifying at least one cellular metabolite present in a metabolic profile of cardiotoxic response.

26. A method of assessing cardiotoxicity of a test compound comprising the steps of: a) contacting cardiomyocyte cells with the test compound;

b) separating a plurality of cellular metabolites of from about 10 to about 1500 Daltons that are secreted from said cardiomyocyte cells; and

c) identifying the test compound as a cardiotoxic compound if at least one one or a plurality of cellular metabolites of from about 10 to about 1500 Daltons that are differentially secreted from cardiomyocytes contacted with the test compound comprise a metabolic profile of cardiotoxicity of claims 10 or 20.

27. The method of claim 26, wherein at least one of the cellular metabolites is produced in greater amounts in cardiomyocytes contacted with the test compound.

28. The method of claim 26, wherein at least one of the cellular metabolites is produced in greater amounts in cardiomyocytes not contacted with the test compound.

29. The method claim 26, wherein the cellular metabolites are separated using a physical separation method.

30. The method according to claim 29, wherein the physical separation method is liquid chromatography/electrospray ionization time of flight mass spectrometry (LC/ESI- TOF-MS).

31. The method according to claim 26, wherein the candidate cellular metabolites are identified by neutral mass.

DESCRIPTION (OCR text may contain errors)

METABOLIC BIOMARKERS OF DRUG-INDUCED CARDIOTOXICITY

This application claims the priority benefit of U.S. provisional patent application Serial No. 61/249,150 filed October 6, 2009, the entirety of which is herein incorporated by reference.

Field of the Invention

This invention provides methods and biomarkers for identifying cardiotoxic effects of pharmaceuticals, biologies, and other chemical compounds and

environmental agents. The invention specifically provides methods for identifying low molecular weight metabolites secreted by cardiomyocytes in response to in vitro exposure to cardiotoxic compounds. Metabolomic methods are provided for identifying candidate biomarkers predictive of cardiotoxicity by measuring low molecular weight metabolites produced and secreted by cardiomyocytes contacted with a chemical compound, pharmaceutical, biologic or

environmental agent. Predictive biomarkers for cardiotoxic effects are also identified and provided herein.

Background of the Invention

Cardiotoxicity has become one of the leading causes of pharmaceutical lead compound attrition and subsequent withdrawal of FDA-approved drugs from the market. The development of screening methods that provide specificity and accuracy for predicting cardiotoxicity are needed to better enable safe drug development and to help reduce soaring financial losses associated with preclinical drug failure.

Currently, cardiotoxicity can only be inferred, predominantly by measuring in vitro alterations to the action potential duration (APD) in cardiomyocytes using patch- clamp procedures. Despite the invaluable knowledge generated by electrophysiology assays, patch clamp procedures are extremely time consuming and low throughput. Briefly, the APD response to pharmaceutical compounds is measured a single cell at a time, and even so-called "high throughput" systems, such as PatchExpress®, only permit recordings of dozens of cells per assay. Most importantly, however, the mechanism of pharmacological cardiotoxicity is not uniform across drugs; thus electrophysiology recordings are limited in their ability to predict the cardiotoxicity of multiple compounds. While certain compounds exert their toxicity primarily by interfering with proper function of cardiac ion channels (which translate into changes to the APD and thus can be detected using conventional assays), others are known disruptors of cardiomyocyte metabolism that are not currently assayed. The primary toxicity of chemotherapies and kinase inhibitors used for

cancer therapy, for example, results in significant changes to metabolic indicators in cardiomyocytes. Independent of the mechanism, cardiotoxicity would ultimately produce changes to the

comprehensive collection of low molecular weight molecules from cardiomyocytes.

Dysregulation of metabolite synthesis, processing and abundance has been associated with cardiotoxicity. Chemotherapeutic and anti-tumor regimens are accompanied by marked changes to mitochondrial function, including interference with oxidative phosphorylation and inhibition of ATP synthesis, myofibrillar structure, and other aspects of energy metabolism. (Takemura & Fugiwara, 2007, Progress in Cardiovascular Diseases 49(5): 330-352). Other metabolic processes that have been implicated in the cardiotoxicity of cancer drugs include lipid peroxidation, oxidation of proteins and DNA, and depletion of glutathione and pyridine nucleotide reducing equivalents. Cardiotoxic side-effects are not limited to pharmaceutical compounds, as cardiotoxicity has been observed with monoclonal antibody therapies and biologies. Therapeutic antibodies such as HER2/ERBB2 monoclonal antibodies and trastuzumab in association with paclitaxel treatment regimen have been shown to have a synergistic negative impact on adult

cardiomyocytes. (Pentassuglia et al., 2007, Experimental Cell Research, 313: 1588- 1601). Detrimental effects of biologies on cardiac safety are prevalent independent of combined therapies: for example, eleven percent of patients on trastuzumab develop cardiac toxicity (Guarneri et al., 2006, Journal of Clinical Oncology, 24: 4107-4115).

There remains a need in this art for in vitro methods for reliably determining cardiotoxicity of pharmaceuticals, biologies, and other chemical compounds and environmental agents.

Summary of the Invention

The present invention provides reagents and methods for identifying a plurality of low molecular weight molecules, preferably secreted by cardiomyocytes or fiESC-derived or human iPS-derived cardiac-specific cells, in response to pharmaceuticals, biologies, and other chemical compounds or environmental agents. In addition, the invention provides reagents and methods for identifying, in certain embodiments, particular metabolites produced by cardiomyocytes in response to a pharmaceutical, biologic, other chemical compound or environmental agent, as well as, in other embodiments, pluralities of cellular metabolites produced by

cardiomyocytes in response to a pharmaceutical, biologic, other chemical compound or environmental agent, thereby also providing metabolic profiles of specific metabolites produced, for example, as the result of cardiotoxicity and that are secreted in response to exposure to particular pharmaceuticals, biologies, and other chemical compounds and environmental agents. The present invention thus provides reagents and methods for predicting cardiotoxic effects of pharmaceuticals, biologies, and other chemical

compounds and environmental agents using profiles of low molecular weight metabolites identified via metabolomic analysis of human cardiomyocytes contacted with such agents in vitro.

Low molecular weight metabolites can be sensitively detected in even low quantities by methods and technologies known in the art, including most particularly variations of liquid chromatography high resolution mass spectrometry (LC-MS) and/or electrospray ionization time of flight mass spectrometry (ESI-TOF). As disclosed herein the sensitivity of applying such methods to detecting metabolites produced by cardiomyocytes in response to pharmaceuticals, biologies, and other chemical compounds and environmental agents, provides improved outcomes for detecting cardiotoxicity compared with less robust methods known in the art.

Advantages of the inventive methods disclosed herein include that they provide direct products of the cardiotoxic response - metabolites produced by cardiomyocytes in response to insults from pharmaceuticals, environmental agents, chemical compounds and/or biologic therapies. The invention disclosed herein also advantageously provides metabolite profiles produced by contacting cardiomyocytes in vitro with specific pharmaceuticals, biologies, and other chemical compounds and

environmental agents. These profiles are comprised of non-limiting collections of candidate biomarkers, providing a biochemical metabolic signature indicative of cardiotoxicity.

In particular embodiments, the invention provides reagents and methods for in vitro screening using cardiomyocytes to detect metabolites associated with cardiotoxicity of specific pharmaceuticals, biologies, and other chemical compounds and environmental agents. The patterns and collections of metabolite biomarkers establish that such cardiomyocytes have a characteristic metabolic response to cardiotoxicity produced by contact with specific pharmaceuticals, biologies, and other chemical compounds and environmental agents.

Practice of the provided methods illustrates the cardiomyocyte metabolome includes potential human biomarkers for disease and cardiotoxic response. These biomarkers are identified by contacting cardiomyocytes with specific

pharmaceuticals, environmental agents, chemical compounds and biologic therapies. The results set forth herein demonstrate that exposure of cardiomyocytes to known cardiotoxic drugs induced significant changes in different metabolic pathways, consistent with known activity as cardiotoxins, and further providing an exemplar for the practice of the inventive methods with uncharacterized pharmaceuticals, biologies, and other chemical compounds and environmental agents to determine the extent of any cardiotoxicity exhibited by these compounds.

Specific embodiments of this invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

Brief Description of the Drawings

These and other objects and features of this invention will be better understood from the following detailed description taken in conjunction with the drawings wherein:

Figure 1 is a photograph of cardiac cells subjected to immunohistochemical (IHC) treatment for cardiac alpha actin. The IHC staining of alpha actin confirmed the cardiac origin of cells exposed to doxorubicin, paclitaxel and tamoxifen. Cardiac cells were subjected to drug treatment for the identification of predictive metabolic biomarkers of cardiotoxicity.

Figure 2 is a graph of percentages of cell death of human cardiomyocytes in response to exposure to anti-tumor drugs as measured by Trypan Blue dye inclusion.

Figure 3 is a Venn diagram of statistically significant mass features, representing different metabolites, in human cardiomyocytes treated with doxorubicin (DOX), paclitaxel (PAC), and tamoxifen (TAM) at 0.05 False Discovery Rates (FDR). Seventy-three features were common to strong cardiotoxicants DOX and PAC.

Figures 4A through 4AG are ion extracted chromatograms (EICs) from statistically significant mass features (i.e., candidate metabolite biomarkers of cardiotoxicity) detected in human cardiomyocytes treated with 26 μM doxorubicin (dotted lines) in comparison to untreated controls (solid black lines) and doxorubicin media (refer to legend in graph for line designations) and cardiomyocytes treated with 15 μM of paclitaxel (dashed lines) in comparison to untreated controls (solid black lines) and media (refer to legend). Doxorubicin (DOX), paclitaxel (PAC), and tamoxifen (TAM).

Figure 5 is a depiction of hierarchical clustering of the metabolomic features following various experimental treatments. The NIPALS Principal Cluster Analysis (PC A) illustrates strong cardiotoxicants (DOX, PAC) exhibiting similar trends (clustering) in comparison to weak cardiotoxicants (TAM). Doxorubicin (DOX), paclitaxel (PAC), tamoxifen (TAM), and Herceptin (HER).

Figure 6 is an ion extracted chromatogram of statistically significant mass feature M203T507 in the cell culture media of cardiac precursor cells treated with doxorubicin (26uM) for 24 hours and then paclitaxel (15 uM) for 48 hours. The EIC demonstrates a statistically significant decrease in the accumulation of Symmetric dimethylarginine or Asymmetric dimethylarginine in treated cardiac precursors. Y- axis is intensity and X-axis is time in seconds.

Figure 7 is an ion extracted chromatogram of mass feature M194T69 in the cell culture media of cardiac precursor cells treated with doxorubicin (26uM) for 24 hours and then paclitaxel (15 uM) for 48 hours. The EIC demonstrates a lack of (R)- N-Methylsalsolinol or (S)-N-Methylsalsolinolin the cell culture media of treated cardiac precursors. Y-axis is intensity and X-axis is time in seconds.

Figure 8 is an extracted ion chromatogram of statistically significant mass feature M192T522 in the cell culture media of cardiac precursor cells treated with doxorubicin (26uM) for 24 hours and then paclitaxel (15 uM) for 48 hours. The EIC demonstrates a statistically significant decrease in the accumulation of 3-Methylhistidine or 1-Methylhistidine in the cell culture media of treated cardiac precursors. Y-axis is intensity and X-axis is time in seconds.

Figure 9 is an ion extracted chromatogram of statistically significant mass feature M188T354 in the cell culture media of cardiac precursor cells treated with doxorubicin (26uM) for 24 hours and then paclitaxel (15 uM) for 48 hours. The EIC demonstrates a statistically significant increase in the accumulation of 3-Pyridinebutanoic acid, Norsalsolinol, or Phenylalanine in the cell culture media of treated cardiac precursors. Y-axis is intensity and X-axis is time in seconds.

Figure 10 is an ion extracted chromatogram of statistically significant mass feature M148T497 1 in the cell culture media of cardiac precursor cells treated with doxorubicin (26uM) for 24 hours and then paclitaxel (15 uM) for 48 hours. The EIC demonstrates a statistically significant increase in the accumulation of N-Acetylserine, Glutamic acid, L-4-Hydroxyglutamate semialdehyde, 2-Oxo-4-hydroxy-5- aminovalerate, or O-Acetylserine in the cell culture media of treated cardiac precursors. Y-axis is intensity and X-axis is time in seconds.

Figure 11 is an extracted ion chromatogram of statistically significant mass feature M145T109 in the cell culture media of cardiac precursor cells treated with doxorubicin (26uM) for 24 hours and then paclitaxel (15 uM) for 48 hours. The EIC demonstrates a statistically significant decrease in the accumulation of Erythritol or Threitol in the cell culture media of treated cardiac precursors. Y-axis is intensity and X-axis is time in seconds.

Figure 12 is an extracted ion chromatogram of statistically significant mass feature M134T504 in the cell culture media of cardiac precursor cells treated with doxorubicin (26uM) for 24 hours and then paclitaxel (15 uM) for 48 hours. The EIC demonstrates a statistically significant decrease in the accumulation of Aspartic Acid or Iminodiacetate in the cell culture media of treated cardiac precursors. Y-axis is intensity and X-axis is time in seconds.

Figure 13 is an extracted ion chromatogram of statistically significant mass feature M134T504 in the cell culture media of cardiac precursor cells treated with doxorubicin (26uM) for 24 hours and then paclitaxel (15 uM) for 48 hours. The EIC demonstrates a statistically significant decrease in the accumulation of Aspartic Acid or Iminodiacetate in the cell culture media of treated cardiac precursors. Y-axis is intensity and X-axis is time in seconds.

Detailed Description of Preferred Embodiments

This invention is more particularly described below and the Examples set forth herein are intended as illustrative only, since numerous modifications and variations therein will be apparent to those skilled in

the art. As used in the description herein and throughout the claims that follow, the meaning of "a", "an", and "the" includes plural reference unless the context clearly dictates otherwise. The terms used in the specification generally have their ordinary meanings in the art, within the context of the invention, and in the specific context where each term is used. Some terms have been more specifically defined below to provide additional guidance to the practioner regarding the description of the invention. In particular, the term "cell" as used herein can be singular or plural, but in a preferred embodiment is plural.

In one embodiment, the invention includes reagents and methods for determining the cellular and/or biochemical effects of exposure to cardiotoxic compounds. The term "cellular metabolite" or the plural form, "cellular metabolites," as used herein refers to a low molecular weight molecule secreted by a cell. In general the size of the metabolites is in the range of about 55 Daltons to about 1500 Daltons. A cellular metabolite may include but is not limited to the following types of low molecular weight molecules: acids, bases, lipids, sugars, glycosides, amines, organic acids, lipids, amino acids, oximes, esters, dipeptides, tripeptides, fatty acids, cholesterols, oxysterols, glycerols, steroids, and/or hormones. In an alternative embodiment, the cellular metabolite is secreted from cardiomyocytes, human embryonic stem cell (hESC)-derived cardiomyocytes or human induced pluripotent stem cell (iPS)-derived cardiomyocytes. In a preferred embodiment, the cellular metabolites include but are not limited to the following low molecular weight molecules: Triethylamine; NN-Diethylamine; Hexylamine; p- Glucosyloxymandelonitrile; (s)-4-Hydroxymandelonitrilebeta-D-glucoside; 13, 14- dihydro PGE1 (Prostaglandin El); 7-Ketocholesterol; 1,25-Dihydroxyvitamin D3- 26,23-lactone; Formononetin 7-0-glucoside-6"-0-malonate; Isochlorogenic acid b; 13-Dicaffeoylquinic acid; 3-Hexaprenyl-4-hydroxy-5-methoxybenzoic acid; 2- Phenylglycine; (E)-4-Hydroxyphenylacetaldehyde-oxime; (Z)-4- Hydroxyphenylacetaldehyde-oxime; Betaine; 2-Ethylhexyl-4-hydroxybenzoate;

Glycerophosphocholine; N-Acetylgalactosamine; CGP52608; Biotin; DL- Homocystine; Ethenodeoxyadenosine; Queuine; N-Acetylaspartylglutamic acid; Tetrahydrocortisone; Cyclic Phosphatidic acid; 2-Methoxyestrone3-glucuronide; Diacylglycerol; Quercetin3-(2G-xylosylrutinoside); Niacinamide; Aspartic Acid; Iminodiacetate; Erythritol; D-Threitol; N-Acetylserine; L-Glutamic acid; L-4-Hydroxyglutamate semialdehyde; 2-Oxo-4-hydroxy-5-aminovalerate; O-Acetylserine; DL-Glutamate; DL-Glutaminic acid; 2-Aminoglutaric acid; Glutamate; D-Glutamic acid; 3-Pyridinebutanoic acid; Norsalsolinol; D-Phenylalanine; D-alpha-Amino-beta- phenylpropionic acid; L-Phenylalanine; 3-Methylhistidine; 1-Methylhistidine; (R)-N- Methylsalsolinol; (S)-N-Methylsalsolinol; Symmetric dimethylarginine; or

Asymmetric dimethylarginine.

The phrase "identifying one or a plurality of cellular metabolites . . .

differentially produced" as used herein includes but is not limited to comparisons of cells exposed to a test compound to untreated (i.e., control) cells. Detection or measurement of variations in low molecular weight molecule populations secreted by a cell, between experimental and control cells are included in this definition. As used herein, the terms "secrete," "secreting," and "secretion" are intended to

encompass any cellular process by which a cellular metabolite produced by a cell is translocated outside the cell. Metabolites or small molecules, particularly those species secreted, excreted or consumed by the cells, or those metabolites that are fluxed through the cells, that participate in functional mechanisms of cellular response to pathological or chemical insult. Metabolites may also be produced as a result of apoptosis or necrosis.

In a preferred embodiment, alterations in cells or cell activity are measured by determining a profile of changes in low molecular weight molecules in treated versus untreated cells. Also included are comparisons between cells treated with different amounts, types or concentrations, durations or intensities of cardiotoxic or potential cardiotoxic compounds.

Alterations in cellular metabolites such as sugars, organic acids, amino acids, fatty acids, and low molecular weight compounds are measured and used to assess the effects of specific pharmaceuticals, environmental agents, chemical compounds and biologic therapies on biochemical pathways in cardiomyocytes. The screened low molecular weight compounds (i.e., metabolites) are secreted in response to a variety of biological activities, including, but not limited to inflammation, anti-inflammation, vasodilation, neuroprotection, fatty acid metabolism, collagen matrix degradation, oxidative stress, antioxidant activity, DNA replication and cell cycle control, methylation, biosynthesis of nucleotides, carbohydrates, amino acids and lipids, among others. Secreted low molecular weight molecules are precursors,

intermediates and/or end products of in vivo biochemical reactions. Alterations in specific subsets of molecules correspond to a particular biochemical pathway and thus reveal the biochemical effects of cardiotoxicity.

The term "cardiomyocyte" or "cardiomyocyte cell(s)" as described herein refers to primary cardiomyocytes, cardiomyocyte precursor cells, clonal

cardiomyocytes derived from adult human heart, immortalized cardiomyocytes, human embryonic stem cell (hESC)-derived cardiomyocytes, human induced pluripotent stem cell (iPS)-derived cardiomyocytes, or any cell displaying cardiomyocyte-specific markers such that a pathologist, scientist, or laboratory technician would recognize the cell to be cardiomyocyte-specific or cardiomyocyte derived.

The term "cardiotoxic" as described herein refers to a substance or treatment, particularly pharmaceuticals, biologies, and other chemical compounds and environmental agents, that induce cardiomyopathy, heart disease, and/or abnormal heart pathology and physiology. Examples of cardiotoxicities encompassed by the definition of the term as used herein include heart abnormalities that would be recognized by a physician, cardiologist, or medical researcher, which could be attributed to or a potential result of a drug-treatment regimen.

In a preferred embodiment the term "compound" or "test compound" includes but is not limited to pharmaceuticals, environmental agents, chemical compounds and biologic therapies, including antibody-based treatments, vaccines, or recombinant proteins and enzymes. In a particularly preferred embodiment, cardiotoxic compounds include tamoxifen, doxorubicin, and paclitaxel. In a further embodiment, potentially cardiotoxic compounds are screened for metabolite similarities to already known cardiotoxic compounds.

The term "cardiomyopathy" refers to heart disease, including but not limited to inflammation of the heart muscle and reduction of heart function. Cardiomyopathy can be classified as primary or secondary and may further include dilated, hypertrophic and restrictive cardiomyopathies. The heart cavity can be enlarged and stretched (e.g., cardiac dilation), and may not pump normally. Abnormal heart rhythms called arrhythmias and disturbances in the heart's electrical conduction also can occur. In this condition, the muscle mass of the left ventricle enlarges or

"hypertrophies." Mass spectrometry-based platforms have been proposed as a means to select peptides and proteins, but not small-molecule metabolites, as candidate biomarkers of cardiotoxicity. For example, brain natriuretic peptide (BNP) and N-terminal proBNP (NTproBNP) are clinical biomarkers of heart failure. BNP hormone and the inactive NTproBNP are predominantly secreted in the ventricles of the heart in response to pressure overload and, consequently, are being investigated as markers of drug-induced cardiac hypertrophy in rat. {See Berna et ah, 2008, Anal Chem 80: 561-566). In addition, myosin light chain 1 (Myl3), a 23-kDa isoform of one of the subunits of myosin and troponin have been proposed as biomarkers of cardiac necrosis to predict drug-induced cardiotoxicity {See Adamcova et ah, 2005, Expert Opinion on Drug Safety 4(3): 457-472). Such peptides and proteins have been recognized in the art as products of the degenerative changes in heart muscle associated with

cardiomyopathies.

Certain of the compounds used herein to demonstrate the usefulness of a metabolomics approach for identifying candidate biomarkers for cardiotoxicity in cardiomyocytes are known cardiotoxic compounds. These compounds are thus illustrative of the reagents and methods for detecting metabolomic markers for cardiotoxicity, and include doxorubicin, paclitaxel and tamoxifen. The assessment of low molecular weight molecule metabolic products secreted by cardiomyocytes in response to exposure to multiple drug-treatment regimens thus provides novel profiles of candidate biomarkers of cardiotoxicity that can be rationalized with these clinical indicia.

The term "control cell(s)" as used herein refers in general to non-cardiac derived cell types. In a preferred embodiment, control cells include human fibroblasts. The term "control cardiomyocytes" as used herein refers to cardiomyocyte or cardiomyocyte-derived cells that are exposed to control conditions.

The term "control sets" as used herein refers to the exposure of a particular cell type to a condition that one of skill in the art would recognize as a control treatment. In a preferred embodiment this includes but

is not limited to the following experimental conditions: the exposure of cardiac cells to non-toxic compounds, or the exposure of non-cardiac cells to cardiotoxic compounds. Conversly, as used herein, an "experimental set" includes cardiac-specific cells exposed to a compound of interest (e.g., test compound), such as specific pharmaceuticals, biologies, and other chemical compounds and environmental agents.

The term "subtracting" as used herein refers to the identification of common cellular metabolites secreted by experimental cells and control cells followed by the selective removal of those metabolites in common from a metabolic signature or biomarker profile of specific cardiotoxic response.

When identifying low molecular weight metabolites that are secreted by cardiomyocytes, a skilled technician or scientist would understand that such metabolites can be measured, for example, those metabolites secreted and/or released into cellular supernatant and/or present in cellular extracts, as well as a variety of other methods available for the assessment of secreted molecules. Identified metabolites may also be waste products excreted by cells.

The phrase "exposure to test compound" may refer to cell samples exposed to an individual compound separately or a plurality of compounds sequentially and/or collectively. In one embodiment, cells are exposed to an individual test compound. In a further embodiment, cells are exposed to multiple compounds. In an alternative embodiment, cells are not exposed to any compound (i.e., control). Cells may be cultured in the presence or absence of test compounds.

The phrase "selecting those with commonality" as used herein refers to secreted metabolites produced in commonality across more than one set of cells. Thus, for example, the metabolites in various cell sets are identified, compared, and those in common may be further selected for commonality.

The term "physical separation method" as used herein refers to any method known to those with skill in the art sufficient to produce a profile of changes and differences in low molecular weight molecules produced by cells exposed to pharmaceuticals, environmental agents, chemical compounds and biologic therapies according to the methods of this invention. In certain embodiments, physical separation methods permit detection of low molecular weight molecules including but not limited to acids, bases, lipids, sugars, glycosides, amines, organic acids, lipids, amino acids, oximes, esters, dipeptides, tripeptides, fatty acids, cholesterols, oxysterols, glycerols, steroids, and/or hormones. In particular embodiments, this analysis is performed by liquid chromatography high resolution mass spectrometry (LC-MS) and/or liquid chromatography/electrospray ionization time of flight mass spectrometry (LC-ESI-TOF-MS), however it will be understood that low molecular weight compounds as set forth herein can be detected using alternative spectrometry methods or other methods known in the art. For example, nuclear magnetic resonance (NMR) is another method that can identify low molecular weight compounds of the invention. Similar analyses have been applied to other biological systems in the art (Want et ah, 2005, Chem Bio Chem 6: 1941-51), providing biomarkers of disease or toxic responses that can be detected in biological fluids (Sabatine et ah, 2005, Circulation 112: 3868-875). It is understood that different

instruments may detect different low molecular weight compounds. Thus, for example, the profile developed by LC-MS and/or LC-ESI-TOF-MS may be the same as or different than the profile developed by NMR.

A "biological sample" includes but is not limited to cells cultured in vitro, a patient sample, or biopsied cells dispersed and cultured in vitro. A "patient" may be a human or animal. A "patient sample" includes but is not limited to blood, plasma, serum, lymph, urine, cerebrospinal fluid, saliva or any other biofluid or waste.

The term "biomarker" as used herein refers, inter alia to low molecular weight compounds as set forth herein that exhibit significant alterations between experimental cell sets and control cell sets, particularly with regard to exposure to cardiotoxic compounds. In certain embodiments, biomarkers are identified as set forth above, by methods including, for example, LC-MS and/or LC-ESI-TOF-MS. In certain embodiments, the following low molecular weight molecules are provided herein, taken alone or in any informative combination, as biomarkers of cardiotoxicity: Triethylamine; NN-Diethylamine; Hexylamine; p-Glucosyloxymandelonitrile; (s)-4-Hydroxymandelonitrilebeta-D-glucoside; 13, 14- dihydro PGE1 (Prostaglandin El); 7-Ketocholesterol; 1,25-Dihydroxyvitamin D3- 26,23-lactone; Formononetin 7-0-glucoside-6"7-0-malonate; Isochlorogenic acid b; 13-Dicaffeoylquinic acid; 3-Hexaprenyl-4-hydroxy-5-methoxybenzoic acid; 2- Phenylglycine; (E)-4-Hydroxyphenylacetaldehyde-oxime; (Z)-4-

Hydroxyphenylacetaldehyde-oxime; Betaine; 2-Ethylhexyl-4-hydroxybenzoate; Glycerophosphocholine; N-Acetylgalactosamine; CGP52608; Biotin; DL- Homocystine; Ethenodeoxyadenosine; Queuine; N-Acetylaspartylglutamic acid; Tetrahydrocortisone; Cyclic Phosphatidic acid; 2-Methoxyestrone3-glucuronide; Diacylglycerol; Quercetin3-(2G-xylosylrutinoside); Niacinamide; Aspartic Acid; Iminodiacetate; Erythritol; D-Threitol; N-Acetylserine; L-Glutamic acid; L-4- Hydroxyglutamate semialdehyde; 2-Oxo-4-hydroxy-5 -amino valerate; O-Acetylserine; DL-Glutamate; DL-Glutaminic acid; 2-Aminoglutaric acid; Glutamate; D-Glutamic acid; 3-Pyridinebutanoic acid; Norsalsolinol; D-Phenylalanine; D-alpha-Amino-beta- phenylpropionic acid; L-Phenylalanine; 3-Methylhistidine; 1-Methylhistidine; (R)-N-Methylsalsolinol; (S)-N-Methylsalsolinol; Symmetric dimethylarginine; or Asymmetric dimethylarginine. In a preferred embodiment, the low molecular weight molecules described herein in Tables 2A-2D taken alone or in any informative combination, are reliable biomarkers of cardiotoxicity. Many of the identified low molecular weight molecules are identified by unique mass feature size or neutral mass, however some molecules are further identified by compound name.

The terms "metabolic signature" and "metabolic profile" as used herein refer to one or a plurality of metabolites identified by the inventive methods. Metabolic signatures and profiles according to the invention can provide a molecular "fingerprint" of the effects of cardiotoxicity and identify low molecular weight compounds significantly altered following exposure to pharmaceuticals, environmental agents, chemical compounds and biologic therapies that are cardiotoxic. In certain embodiments, metabolic signatures or metabolic profiles can be used to predict cardiotoxicity of a compound. In an alternative

embodiment, a metabolic signature or profile may diagnose cardiotoxic effects from drug treatment regimens, pharmaceuticals, environmental agents, chemical compounds or biologic therapies.

In certain embodiments, cardiotoxicity of a test compound can be identified by cardiomyocyte secretion of a single known cardiotoxic biomarker. As an example, a single marker may include Betaine or Glycerophosphocholine. This may include metabolite(s) secreted in response to exposure to a single established cardiotoxic compound (e.g. doxorubicin). In other embodiments, cardiotoxicity is affirmed by detection of a metabolic signature (i.e., one or a plurality of low molecular weight metabolites) commonly produced by cardiomyocytes in response to two or more known cardiotoxic compounds (e.g., doxorubicin and paclitaxel, or doxorubicin, paclitaxel, and tamoxifen). In further embodiments, metabolic signatures of cardiotoxicity comprising one or a plurality of cellular metabolites provided in Tables 2A-2D, or described in the chromatograms of Figures 4A-AG and Figures 6-13 are provided.

Data for statistical analysis were extracted from chromatograms using the

Agilent Mass Hunter software (Product No. G3297AA, Agilent Technologies, Inc., Santa Clara, CA); it will be understood that alternative statistical analysis methods can be used. Masses were binned together if they were within 10 ppm and eluted within a 2 minutes retention time window. A binned mass was considered to be the same molecule across different LC-ESI-TOF-MS analyses (referred to herein as an "exact mass," which will be understood to be ± lOppm). Binning of the data is required for statistical analysis and comparison of masses across the entire

experiment. If multiple peaks with the same mass at the same retention time within a single sample were detected by Mass Hunter, they were averaged to assist data analysis. Masses lacking a natural isotopic distribution or with an absolute height of less than 1000 were removed from the data prior to analysis. It would be understood that the results from this assay provide relative values that are assessed according to annotated values within 10 ppm to provide an identity for the molecular weight detected. Thus, a mass shift within 10 ppm is considered consistent with determining the identity of a specific cellular metabolite previously annotated due to differences in ionization source and instrumentation, e.g. between different experiments or using different instruments.

As used herein, a mass was considered to be the same across LC/ESI-TOF-MS runs using a simple algorithm that first sorts the data by mass and retention time. After sorting, a compound was considered unique if it had an ordered retention time difference of less than or equal to 0.1 minutes and a mass difference less than or equal the weighted formula: consecutive masses did not differ by 10 ppm if under 175 Da, by 7 ppm over the range 175 to 300 Da, and by 5 ppm when greater 300 Da. If a series of measurements fit this definition it was considered to be from the same compound. If either the mass or the retention time varied by more than the limits listed above it was considered to be a different compound and given a new unique designation.

The data from the most reproducible mass features was log base 2 transformed and median centered prior to statistical analysis. Statistical analysis was performed using the open source statistical programming and analysis software R. Statistical significance of individual mass features were performed under the null hypothesis that no difference in abundance exists between control and drug treatment using a permutation based test statistic or a Welch T-test. To test the null hypothesis a one way permutation based t-test assuming a normal approximation of the conditional distribution was used and implement using the Conditional Inference Procedures in a Permutation Test Framework (Coin) library, a contributed package of programming code. Statistics tests were performed without replacement of missing values decrease the degrees of freedom rather than imputing missing values. This oneway test method is ideally suited for analysis of complex data sets where one may not be able to assume that every feature tested will have a normal distribution (Hothorn et al., 2006, Amer. Statistician, 60:257-263). False discovery rates (FDR) were controlled using the Q value estimator (Storey et al, 2003, Proc Natl Acad Sci., 100:9440-5) and implemented using the qvalue library in R (Dabney et al., 2003, qvalue: Q-value estimation for false discovery rate control. R package version 1.10.0., www.CRAN.R- project.org).

In certain embodiments, a cardiotoxic biomarker may reference one or a collection of cellular metabolites produced by cardiomyocytes following exposure to known cardiotoxins. A cardiotoxic metabolic signature can comprise about 1, or about 6, or about 10, or about 20, or about 30 differentially secreted low molecular weight molecules, and while the cardiotoxic signature as disclosed herein comprises from about 1 to about 30 metabolites and includes the low molecular weight molecules set forth in Table 2A-2D herein, said cardiotoxic signature generally comprises a sufficient number of metabolites to independently identify an experimental test compound as being cardiotoxic. It will be understood by those with skill in the art that the differential fold change in metabolite secretion between untreated and treated cells can vary for each metabolite.

Examples

The Examples which follow are illustrative of specific embodiments of the invention, and various uses thereof. They set forth for explanatory purposes only, and are not to be taken as limiting the invention.

Example 1

Verification of Cardiac-Specific Cells and Measurement of Cardiac Cell Death

After Exposure to Cardiotoxic Agents Human cardiomyocytes, clonal cardiomyocytes derived from adult human heart (Celprogen 36044- 15at, San Pedro, CA) or cardiac precursor cells, were treated with varying doses of pharmacological compounds known to have cardiotoxic effects. Cardiomyocytes were treated with doxorubicin and paclitaxel, which are strong toxicants, as well as tamoxifen, a weak toxicant, for 24 or 48 hours. Some combinatorial treatments regimens appeared to exhibit synergistic cardiotoxic effects (e.g., for doxorubicin and trastuzumab combined therapies, see Pentassuglia et ah, 2007, Experimental Cell Research 313: 1588-1601; for paclitaxel and doxorubicin combined therapies, see

Robert, 2007, Cardiovasc Toxicol 7: 135-139)). The cardiac origin of these cells was confirmed by immunohistochemistry using antibodies against cardiac alpha-actin protein (Figure 1). The percentage of cell death, inherently and after drug treatment, was calculated by Trypan Blue staining (Figure 2). Cell death was significantly higher in human cardiomyocytes treated with doxorubicin or paclitaxel (50 - 55%) in comparison to tamoxifen (18%) and untreated controls (7%).

Example 2

Identification of Metabolites Produced by Cardiomyocytes Exposed to

Cardiotoxic Pharmacologics

In order to identify low molecular weight metabolites secreted by cardiomyocytes or cardiac precursors following exposure to cardiotoxic compounds, cells as described above in Example 1 were treated with doxorubicin, paclitaxel, and tamoxifen, for 24 or 48 hours.

The extracellular media from treated and untreated cells was processed as described in Cezar et ah, (2007, Stem Cells Development 16: 869-882, this publication is incorporated by reference), for extraction of low molecular weight molecules (<3kD) for metabolomics analysis. Extracellular low molecular weight molecule preparations were separated by liquid chromatography followed by electrospray ionization time of flight (LC-ESI-TOF-MS) mass spectrometry for ionization and detection of the full spectra of low molecular weight molecules present in each sample. More specifically, the samples were separated using the

ESI_Luna_HILIC_95t06OACN_16min method (HILIC chromatography). Statistical differences were inferred by subsequent bioinformatics and in silico mapping of deisotoped ESI-TOF-MS mass features as described below (also provided in Cezar et al. (2007, id.)).

Briefly, ionization (100m/z-1500 m/z) was acquired on an Agilent 6520

Accurate-Mass Q-TOF in extended dynamic range and positive mode. Mass features were generated using two independent methods. First MassHunter Qualitative Analysis was used to generate mass features using the Molecular Feature Extraction algorithm (MFE). Features generated by MFE were binned in R and analyzed for differential accumulation in response to the drug treatments. The Agilent data files were also converted to mzData file format using Agilent's MassHunter Qualitative Analysis Workstation. The mzData files were analyzed in R using the software library XCMS to find mass feature bins differentially present in the presence of drug. MHD files created by MFE were converted to text files using MassHunterMFE version 44. The MHD text files were loaded into R and meta data corresponding to the file name, cell line (Celprogen Cardiomyocytes or solvent), plate (0, 1, 2 or 3), well (solvent, A, B, or C), experiment replication, cells (supernatant, uncultured media or solvent), cell culture passage number, drug treatment (15uM tamoxifen, 15μM paclitaxel, control, 26μM doxorubicin), feature retention time

group, retention time, feature neutral mass, mass feature mass standard deviation, abundance, saturation, height, number of ions in feature, min charge, max charge, charge number, width, and group feature count were added to each file.

In order to identify metabolites secreted by cardiomyocytes in response to cytotoxic drug treatment, metabolomic analysis was performed on cardiomyocytes from similar cell passages. Statistically-significant features that were common between the cytotoxic drug treatments were identified. Mass features that were present in at least 25% of LC-MS samples of control and drug treated cardiomyocytes were selected. The statistical significance of individual mass features was determined under the null hypothesis that no difference in abundance existed between control and drug treatment using a permutation-based test statistic like Students t-test. A one-way test assuming a normal approximation of the conditional distribution (see Horthon et al., 2006a, The American Statistician 60(3): 257-263) was used to test the null hypothesis and was implemented using the Conditional Inference Procedures in a Permutation Test Framework (Coin) library in R (see Horthon et al, 2006b,

Conditional Inference Procedures in a Permutation Test Framework, R package version 0.4-5, CRAN.R-project.org). Statistics tests were performed on log base two transformed, median normalized abundance values without replacement of missing values reducing the degrees of freedom when a missing value was present. False discovery rates (FDR) were controlled using the Q value estimator (Storey et al. ,

2003, Proc. Natl. Acad. Sci. USA 100: 9440-45) with a lambda of 0 and implemented using the q value library in R (Dabney et al., 2003, qvalue: Q-value estimation for false discovery rate control. R package versions 1.10; CRAN.R-project.org; R

Development Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing: 2007. ISBN 3-900051-07-0; www.R-project.org). After performing statistics, a universe of statistically-significant mass features was created from the comparisons of control to each drug treatment based on FDR-adjusted p values. Boolean logic was utilized to find the statistically significant features in common between the different drug treatments. An intersection of mass features that exhibited statistically significant differences in the drugs affecting cell viability (DOX, PAC), but that were not statistically significant in (TAM) was selected. This intersection represented common mass features that were associated with cardiotoxicity because they exhibited a statistically-significant change in cytotoxic treatments, but no statistically-significant change in non-cytotoxic treatments.

A mass was considered to be the same across LC/ESI-MS runs using a simple algorithm that sorts the data by mass and retention time as performed by the software and methods described above. The criteria used for treated-cells were based on a sliding mass scale to compensate for detector efficiency. Because of flow rate, a mass was considered equivalent if it was within (0.00001 x mass) when under 175 Da, (0.000007 x mass) when 176 Da-300Da, and (0.000005 x mass) when over 300 Da with a retention time difference of 1.5 min. If a series of measurements fit this definition, it was considered to be from the same compound within each experiment. If either the mass retention time varied by more than the limits listed

above, the compound was considered to be a different one and given a different bin description. Specifically, 774,645 features were identified by the MassHunter software with an average of 6455 and a median of 5869 features per LC/MS run. The mass features were then sorted by mass and retention time groupings and feature ID bins were created for each set of mass and retention groupings that did not differ. The neutral exact mass and/or empirical chemical formula of each compound, detected by LC-ESI-TOF-MS, was queried in public searchable databases, METLIN (metlin.crips.edu), The Human Metabolome Database (hmda.ca), Kyoto Encyclopedia of Genes and Genomes (genome.jp/keg), and the Biological Magnetic Resonance Bank (bmrb.wisc.edu/metabolomics) for candidate identities. LC-MS-measured mass signals matched small molecules present in the databases if their exact masses were within 10 parts per million (0.00001 x mass). Exact mass measurements and chemical formulae are generally nonambiguous for small molecules up to a certain size.

Analytical-grade chemical standards were purchased from Sigma for comparative LC- MS. Aliquots of conditioned medium used in experiments were spiked with 1 mM chemical standards followed by standard LC-ESI-TOF-MS, as described above. The neutral exact masses and retention times for standard compounds in spiked

conditioned medium were used to re-extract peaks in experimental samples using Analyst software (Agilent).

The doses for each compound were based upon published standards, equivalent to therapeutic circulating levels whenever possible (see Table 1). Trypan Blue exclusion/cell death assays using the aforementioned concentrations have shown that these doses corroborate published findings, whereby doxorubicin and paclitaxel induced significantly higher cell death as described in Example 1. (Figure 2).

Table 1 : Dosages of Cardiotoxic Pharmacolo;

Compound	Dose	Exposure	Therapeutic levels
Doxorubicin	15mg/kg 26μM (Han *et al.*, 2008 *The Journal of Pharmacology and Experimental*	24 hours	*Maximum cumulative dose 550mg/m^2 (Takemura and Fugiwara, 2007, *Progress in Cardiovascular Diseases* 49(5): 330-352; Kang

Compound Dose Exposure Therapeutic levels

Therapeutics 326(1): et al., 2000, The Journal of 127-134). Biological Chemistry 275(41):

31682-31688; Rahman et al, 2007, International Journal of Nanomedicine 2(4): 567-83).

15μM 48 hours

Paclitaxel (Alloatti ei a/., 1998,

The Journal of

Pharmacology and

Experimental

Therapeutics 284(2):

561-567; Spencer and

Faulds, 1994, Drugs

48(5): 794-847).

15μM 24 hours

Tamoxifen (Daosukho et al,

2007, Free Radical

Biology & Medicine

42: 1818-1825).

Metabolite trends observed in initial studies are shown in Figure 5, wherein strong cardiotoxic compounds exhibit similar mass features (low molecular weight molecules) and thus cluster together upon unsupervised multivariate analysis (NIPALS Principal Cluster Analysis).

Identified features are provided in Table 2A-2D. Specifically, Table 2 A provides identified mass features with commonality between paclitaxel and doxorubicin treatments. Table 2B provides identified mass features with commonality between paclitaxel, doxorubicin, and tamoxifen treatments. Table 2D provides identified mass features secreted from cardiac precursor cells treated with doxorubicin and then paclitaxel.

	A	B	C	D	E	F	G	H	I	J	K	L	M
	cpdID	doxteststat	doxpval	doxqvalues	pacteststat	paipval	paiqvalues	count	RT	MASSavg (neutral mass)	MASSavg_ppmErr or	diffRT	meanAbun
1	109	3.65999	0.000252	0.002824	3.536467	0.000406	0.006242	91	8.69922	103.0998	8.72941	0.545	822635.9
2	2238	-3.3388	0.000841	0.005298	-3.57398	0.000352	0.006242	138	7.375522	151.0591	31.44464	1.149	322511.2
3	2238	-3.3388	0.000841	0.005298	-3.57398	0.000352	0.006242	138	7.375522	151.0591	31.44464	1.149	322511.2
4	2238	-3.3388	0.000841	0.005298	-3.57398	0.000352	0.006242	138	7.375522	151.0591	31.44464	1.149	322511.2
5	2482	3.758323	0.000171	0.002729	3.437757	0.000587	0.006942	45	0.782333	157.0893	5.729226	0.072	122001.7
6	3142	2.874521	0.004046	0.01418	-2.78071	0.005424	0.019249	119	1.34784	173.053	33.51574	0.313	38050.41
7	3597	-2.56464	0.010328	0.026178	-3.00979	0.002614	0.013414	101	8.162436	181.9552	3.022721	0.723	35145.14
8	4472	3.421302	0.000623	0.00433	2.507681	0.012153	0.033821	40	1.26845	201.0769	3.978577	0.031	10576.74
9	4725	4.026689	5.66E-05	0.002729	3.894308	9.85E-05	0.004716	70	0.817114	205.1109	2.193935	0.061	17217.05
10	4822	2.565877	0.010292	0.026178	3.76557	0.000166	0.004717	92	0.770011	207.1273	14.72524	0.083	38689.37
11	5561	3.375031	0.000738	0.004797	2.866626	0.004149	0.017197	68	0.82875	221.1058	2.713632	0.045	32172.62
12	5652	3.957836	7.56E-05	0.002729	3.802377	0.000143	0.004717	68	0.819265	223.1216	5.602326	0.073	34578.25
13	5771	2.533759	0.011285	0.027378	3.711183	0.000206	0.004717	91	0.767385	225.1371	3.775477	0.068	24658.82
14	6879	-2.89811	0.003754	0.013827	-3.07792	0.002085	0.012133	167	1.477719	241.0938	6.63642	0.347	14128.69
15	7026	3.280068	0.001038	0.005742	3.130658	0.001744	0.01122	26	7.097962	244.0398	3.892808	0.214	11948.18
16	7040	3.04579	0.002321	0.009543	2.878176	0.004	0.017197	106	1.517783	244.0935	3.277433	0.053	59825.63

	A	B	C	D	E	F	G	H	I	J	K	L	M
	cpdID	doxtestatat	doxpval	doxqvalues	pactestatat	pacpval	pacqvalues	count	RT	MASSavg (neutral mass)	MASSavg_ppmErr or	diffRT	meanAbun
24	8271	3.823512	0.000132	0.002729	2.690693	0.00713	0.02227	252	1.070321	264.1042	46.57253	0.634	2580424
25	8453	2.760382	0.005773	0.017335	2.302374	0.021314	0.049919	349	9.645029	268.0486	30.77801	2.308	618274.7
26	8468	3.569693	0.000357	0.003093	2.64184	0.008246	0.02468	109	1.107156	268.1284	24.80155	0.073	1178613.5
27	8714	-3.45613	0.000548	0.003874	-2.69271	0.007087	0.02227	103	4.670631	272.1143	2.02121	0.324	664463.4
28	8768	2.897647	0.00376	0.013827	3.359767	0.00078	0.007641	73	1.074288	274.0677	2.371677	0.013	695877.4
29	8845	-3.81137	0.000138	0.002729	-3.55048	0.000385	0.006242	120	7.3087	275.1353	19.62671	0.816	1858271
30	8946	-3.95606	7.62E-05	0.002729	-3.68953	0.000225	0.004842	87	7.465195	277.1152	14.43443	0.6	1033051
31	9204	-3.78777	0.000152	0.002729	-3.01286	0.002588	0.013414	76	3.353303	281.901	2.483141	0.125	368443.5
32	10732	3.310986	0.00093	0.005511	2.800209	0.005107	0.018653	72	1.072306	304.0784	4.110781	0.014	418463.2
33	11282	3.601066	0.000317	0.002907	3.939544	8.16E-05	0.004716	71	7.207735	312.0277	5.12775	0.474	3312666.5
34	11790	-3.16549	0.001548	0.007476	-2.86883	0.00412	0.017197	48	1.164646	319.1002	1.723596	0.018	1418343
35	13631	2.909194	0.003624	0.013648	2.636035	0.008388	0.024762	209	9.137215	348.1376	17.95267	2.016	3545208
36	13631	2.909194	0.003624	0.013648	2.636035	0.008388	0.024762	209	9.137215	348.1376	17.95267	2.016	3545208
37	14684	-3.72252	0.000197	0.002729	-3.34058	0.000836	0.007641	84	7.439631	364.1941	9.47297	0.603	346369.2
38	17842	3.129877	0.001749	0.007919	2.824002	0.020125	0.048287	63	1.049286	416.2036	3.003338	0.022	1401659
39	19693	3.309906	0.000933	0.005511	2.831703	0.00463	0.017818	55	4.195977	447.9792	2.343859	0.165	1398932
40	20903	3.018062	0.002544	0.010334	3.710958	0.000206	0.004717	35	7.063771	472.0395	5.931708	0.238	922852

	N	O	P	Q	R	S	T	U	V	W
		DBid	**annotation**	**formula**	**mass**	**map**	**cellsmedia**	**cellsmedia1**	**cellsmedia2**	**pacfold**
1		no hit	no hit	no hit	no hit	no hit	4.095074	4.643296	5.508201	-1.51728
2	mitochondrial fatty acid beta-oxidation	HMDB02210	2-Phenylglycine; (+/-)-a-phenylglycine; 2-amino-2-phenylacetate; 2-amino-2-phenylacetic acid; 2-phenyl-glycine; DL-2-phenyl-glycine; DL-a-aminophenylacetate; DL-a-aminophenylacetic acid; DL-a-phenylglycine; DL-alpha-aminophenylacetate; DL-alpha-aminopheny	$C_8H_9NO_2$	151.06		242693.5	213500.3	368453.8	2.54239
3		C04350	(E)-4-Hydroxyphenylacetaldehyde-oxime	$C_8H_9NO_2$	151.06		242693.5	213500.3	368453.8	2.54239

	N	O	P	Q	R	S	T	U	V	W
		DBid	**annotation**	**formula**	**mass**	**map**	**cellsmedia**	**cellsmedia1**	**cellsmedia2**	**pacfold**
7		no hit	no hit	no hit	no hit	no hit	1.314752	1.197247	1.325943	1.339736
8		no hit	no hit	no hit	no hit	no hit	101600.2	107976.2	64592.31	-1.34183
9		no hit	no hit	no hit	no hit	no hit	1366553	1768169	578394.8	1.410901
10		no hit	no hit	no hit	no hit	no hit	3717614	3320107	3544193	-2.8368
11		HMDB00212	N-Acetylgalactosamine				233529.4	324223.8	96252.18	1.822035
12		no hit	no hit	no hit	no hit	no hit	2355851	3424140	971475.4	1.611363
13		no hit	no hit	no hit	no hit	no hit	2342748	1999674	2286641	-2.49067
14		no hit	no hit	no hit	no hit	no hit	-1.00465	-1.11906	1.00205	1.743615
15		C15629	CGP52608	$C_8H_{12}N_4OS_2$	244.04		112429.9	113103.3	1	-1.61415
16		HMDB00030	Biotin		244.088	no hit	1.295457	1.348839	1.321124	-1.03152
17		C14716	2-Ethylhexyl-4-hydroxybenzoate	$C_{15}H_{22}O_3$	250.15		6.59162	8.67278	423507.4	2.658541
18		HMDB01983			251.1	C05198	1.503342	1.544133	1.688099	2.771294
19		HMDB00101		$C_{10}H_{13}N_5O_3$	251.1	C00559	1.503342	1.544133	1.688099	2.771294
20		C00559		$C_{10}H_{13}N_5O_3$	251.1	map00230 Purine metabolism	1.503342	1.544133	1.688099	2.771294
21		C05198			251.1		1.503342	1.544133	1.688099	2.771294
22		HMDB00086	Glycerophosphocholine	$C_5H_{12}NO_6P$	257.1028		7.643334	8.083932	5.187342	1.862173
23		no hit	no hit	no hit	no hit	no hit	1.814395	1.651726	1.429755	-2.94027
24		no hit	no hit	no hit	no hit	no hit	2.274744	2.161693	2.763473	-1.38199

	N	O	P	Q	R	S	T	U	V	W
		DBid	**annotation**	**formula**	**mass**	**map**	**cellsmedia**	**cellsmedia1**	**cellsmedia2**	**pacfold**
28		no hit	no hit	no hit	no hit	no hit	658489.4	609487.9	727440.3	-1.47606
29	reactions of DNA with products derived from lipid peroxidation (LPO) and oxidative stress via endogenous pathways	HMDB01786	Ethenodeoxyadenosine		275,101		1.024305	1.293757	-1.56237	1.525743
30	Modified purine	HMDB01495	Queuine		277.11		878124	782870.8	945443.2	3.038284
31		no hit	no hit	no hit	no hit	no hit	353770.8	301136.4	448239.2	-1.00853
32		HMDB01067	N-Acetylaspartylglutamic acid		304.09		372953.6	331557.2	539433.4	-1.65663
33		no hit	no hit	no hit	no hit	no hit	276622.4	303057.4	206406.2	1.099774
34		no hit	no hit	No hit	no hit	no hit	134137.9	102889	203406.5	1.482286
35		no hit	no hit	No hit	no hit	no hit	2.74104	3.100255	146241.9	-1.37095
36		no hit	no hit	no hit	no hit	no hit	2.74104	3.100255	146241.9	-1.37095
37	Membrane component	HMDB00903	Tetrahydrocortisone		364.225	no hit	2.384477	2.710942	205102.5	2.529825
	it is found in tissues subject		Cyclic Phosphatidic							

	N	O	P	Q	R	S	T	U	V	W
		DBid	annotation	formula	mass	map	cellsmedia	cellsmedia1	cellsmedia2	pacfold
43		no hit	no hit	no hit	no hit	no hit	1.556406	1.274636	1.188643	1.133227
44		no hit	no hit	no hit	no hit	no hit	1.332256	1.416453	78984.72	1.152772
45		no hit	no hit	no hit	no hit	no hit	3.021758	3.165466	4.243796	-1.74386
46		no hit	no hit	no hit	no hit	no hit	1.205372	1.263785	1.004284	2.105434
47		no hit	no hit	no hit	no hit	no hit	1.585385	1.618687	90939.43	1.568872
48	synthesized via phosphatidic acid	HMDB07469	Diacylglycerol (42:9)		690.52		526718	451891.1	888373.5	1.076139
49		no hit	no hit	no hit	no hit	no hit	240486	211908.8	450133.4	1.08118
50		no hit	no hit	no hit	no hit	no hit	-1.06731	-1.02311	-1.1846	1.025094
51		C10175	Quercetin3-(2G-xylosylrutinoside)	$C_{32}H_{38}O_{20}$	742.19		1.267	1.147831	1.867214	1.434772
52		no hit	no hit	no hit	no hit	no hit	2.032904	2.233114	1.996657	1.267181
53		no hit	no hit	no hit	no hit	no hit	1.039491	1.052845	1	-1.18461
54		no hit	no hit	no hit	no hit	no hit	-1.08047	-1.03007	-1.12154	1.208073

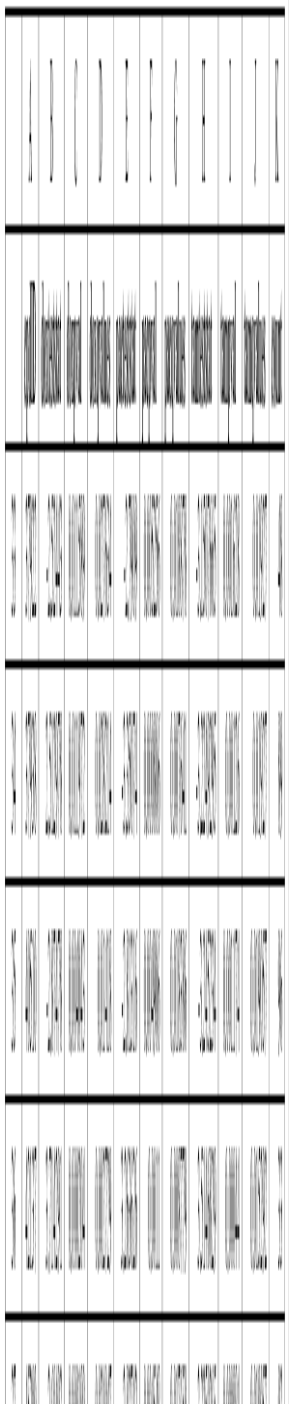
	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH
	tamfold	**doxfold**	**herfold1**	**herfold2**	**doxherfold1**	**doxherfold2**	**X15uM. paclitaxel_ cells_1_10**	**X15uM. paclitaxel_ media_1_10**	**X15uM. tomoxifin _cells_1_ 10**	**X15uM. tomoxifin_me dia_1_10**	**X26uM. doxorubi cin_cells_ 1_10**
1	1.806323	-1.84013	-2.04442	-1.36442	-8.31865	-7.3512	9	1	9	1	9
2	1.667833	1.943857	-3.59384	-1.28855	-399619	-399619	31	0	23	0	24
3	1.667833	1.943857	-3.59384	-1.28855	-399619	-399619	31	0	23	0	24
4	1.667833	1.943857	-3.59384	-1.28855	-399619	-399619	31	0	23	0	24
5	-1.39234	-3.51906	-1.08947	-1.33976	-1.84629	-70521.1	9	0	10	0	7
6	1.976493	1.040279	-3.10976	-1.00834	-4.19629	-3.49593	10	1	10	4	10
7	-1.15474	1.095552	-1.31735	1.061287	-1.83419	-1.5649	8	3	8	3	9
8	-1.24963	-1.49225	-51411.4	1.05172	-51411.4	4.038752	7	0	8	0	9
9	3.434831	1.123107	4.364458	-1.40704	-678606	-678606	9	0	9	0	9
10	1.080332	-1.39233	-1.16321	-1.014	-10.4005	-4.0731	9	0	9	0	9
11	3.24729	1.103918	2.497004	-1.21099	-105106	-105106	9	0	9	0	9
12	2.952069	1.262417	5.999912	-1.41901	-20.6576	-1146124	9	0	4	0	9
13	1.460293	-1.21823	-1.65546	-1.14924	-14.9456	-5.96602	9	0	9	0	9
14	-1.03719	1.804466	-2.18326	-1.65981	-1.85178	-3.73842	15	3	21	7	13
15	1.034898	-1.65549	1	1	1	1	7	0	5	0	8
16	1.050281	-1.04741	-1.00994	-1.00335	-1.37669	-1.20073	9	3	9	3	9
17	1.76098	6.500652	1.197534	-1.36963	-2.12323	2.528179	8	0	9	3	10
18	1.720627	1.857114	-1.65019	1.034298	-2.45923	-1.62846	21	3	22	5	20
19	1.720627	1.857114	-1.65019	1.034298	-2.45923	-1.62846	21	3	22	5	20
20	1.720627	1.857114	-1.65019	1.034298	-2.45923	-1.62846	21	3	22	5	20
21	1.720627	1.857114	-1.65019	1.034298	-2.45923	-1.62846	21	3	22	5	20
22	-1.03329	2.113303	-1.07932	1.032231	-1.36374	-1.11116	8	3	8	3	8
23	-1.11459	-2.3505	1.395382	-1.35627	1.410339	1.503806	65	16	43	16	43
24	-1.13106	-3.88052	-4.66307	-1.59522	-8.15239	-3.83034	24	3	21	9	28
25	-1.49168	-1.37007	1.36971	1.495339	1.347627	1.640189	26	5	32	8	22
26	2.283039	1.271037	-2.56911	1.433367	-5.62144	-3.17335	10	1	9	0	8
27	1.22153	1.759039	-1.22889	1.030843	-1.24031	-1.00867	10	3	9	3	9
28	1.120813	-1.04692	-2.2333	1.023868	-739233	-14.8399	9	0	9	0	9
29	-1.05814	1.667179	1.42771	1.227153	1.944247	2.627888	11	5	10	6	10

	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH
	tamfold	**doxfold**	**herfold1**	**herfold2**	**doxherfold1**	**doxherfold2**	**X15uM. paclitaxel_ cells_1_10**	**X15uM. paclitaxel_ media_1_10**	**X15uM. tomoxifin _cells_1_ 10**	**X15uM. tomoxifin_me dia_1_10**	**X26uM. doxorubi cin_cells_ 1_10**
30	-1.38818	5.482274	1.02779	-1.09895	-2.67136	1.790014	11	0	11	0	9
31	-1.18606	2.192554	-1.39786	1.084111	-472810	-1.38656	8	0	7	0	9
32	-1.30161	-2.00121	-1.95274	-1.14198	-573596	-573596	9	0	9	0	9
33	1.914853	1.254697	-2.70062	-1.5662	-262770	-4.37606	9	0	9	0	9
34	1.125141	1.774099	-206596	-1.02555	-206596	-206596	8	0	6	0	9
35	1.037797	-1.76515	-1.36381	-1.03924	-3.32968	-1.19299	23	1	14	0	14
36	1.037797	-1.76515	-1.36381	-1.03924	-3.32968	-1.19299	23	1	14	0	14
37	-1.58968	3.5843	1.764661	1.09493	-2.64058	-188037	8	2	10	2	9
38	2.06556	-1.83201	-1.9712	-2.81639	-144123	-144123	9	0	9	0	7
39	-1.07797	-1.62021	-187777	1.006976	-187777	-187777	9	0	9	0	9
40	-1.16926	-1.37594	1	1	1	1	8	0	9	0	9
41	-1.01271	-1.82275	1.4989	1.114805	1.9255	1.342812	27	6	25	8	24
42	-1.13279	3.699517	-2.77681	-2.67884	-1.3486	-1.16671	19	6	19	4	21
43	1.023339	1.15108	1.027904	1.170663	1.168296	1.090751	8	3	7	3	7
44	-2.1632	1.185738	2.051372	1.125667	-73352	-73352	8	0	8	1	9
45	-1.75009	-1.45848	1.310577	-1.25896	-3.98183	-1.31392	30	1	24	4	30
46	1.012698	2.471184	1.254055	1.362231	1.16055	2.115293	13	3	7	4	11
47	1.246259	1.910765	-1.36375	-1.13614	-94477.5	-1.02395	10	0	9	0	9
48	-1.00704	1.057886	-1.47705	1.017038	-878502	-878502	9	0	9	0	9
49	-1.01172	1.055024	-1.58889	1.003771	-447153	-447153	8	0	9	0	9
50	1.292738	1.107239	1.067912	-1.10864	-1.09352	-1.0814	9	3	7	3	9
51	1.010477	1.296657	1.25493	1.02807	-1.77178	-1.1245	15	4	26	3	24
52	-1.29959	4.216087	1.101117	-1.38535	1.069377	1.823563	11	2	10	0	9
53	-1.02183	-1.26211	1	1	1	1	7	2	7	2	7
54	1.410131	1.283901	1.050816	1.006325	1.111461	1.05206	9	3	5	3	9
55	-1.16597	-1.51565	-48144	-48144	-48144	-48144	7	0	8	0	8
56	1.552597	1.431377	-1.12464	-118193	-1.64271	-1.21085	9	3	6	3	9
57	1.445509	1.077896	1.104541	-74972.8	-74972.8	-1.13951	9	3	8	3	9
58	1.271828	-1.11465	-1.10581	-1.29256	-123234	-123234	8	0	9	0	9
59	-1.15195	-1.24137	1	1	1	1	9	3	6	3	7

	AI	AJ	AK	AL	AM	AN	AO	AP	AQ	AR	AS
	X26uM. doxorubicin _media_1_1 0	X7.0ug.ml. herceptin_c ells_1_19	X7.0ug.ml. herceptin_c ells_2_20	control_ cells_ 1_10	control_ cells_ 1_19	control_ cells_ 2_20	control_ media_ 1_10	control_ media_ 1_19	control_ media_ 2_20	Dox.Her_ cells_ 1_19	Dox.Her_ cells_ 2_20
1	2	8	11	9	8	8	3	3	2	3	5
2	0	3	12	28	5	12	0	0	0	0	0
3	0	3	12	28	5	12	0	0	0	0	0
4	0	3	12	28	5	12	0	0	0	0	0
5	0	1	3	9	0	3	2	0	0	1	0
6	4	12	12	10	11	12	2	4	3	6	8
7	3	9	9	6	10	9	3	3	2	9	7
8	0	0	4	9	0	2	0	0	0	0	1
9	0	9	8	9	9	8	0	0	0	0	0
10	0	9	10	9	9	10	0	0	0	9	9
11	0	9	8	8	7	9	0	0	0	0	0
12	0	9	9	9	9	9	0	0	0	1	0
13	0	9	10	9	9	9	0	0	0	9	9
14	7	13	13	17	19	11	5	5	2	4	12
15	0	0	0	6	0	0	0	0	0	0	0
16	3	9	9	9	9	9	3	3	2	9	8
17	2	9	5	10	9	6	2	0	0	9	8
18	4	26	21	21	31	25	1	3	2	11	25
19	4	26	21	21	31	25	1	3	2	11	25
20	4	26	21	21	31	25	1	3	2	11	25
21	4	26	21	21	31	25	1	3	2	11	25

	AI	AJ	AK	AL	AM	AN	AO	AP	AQ	AR	AS
	X26nM, doxorubicin_media 1 1_1 0	X7.0ug.ml. herceptin_cells 1 19	X7.0ug.ml. herceptin_cells 2 20	control_cells_1 10	control_cells_1 19	control_cells_2 20	control_media_1 10	control_media 1 19	control_media_2 20	Dox.Her_cells_1 19	Dox.Her_cells_2 20
30	0	9	9	11	8	9	0	0	0	1	9
31	0	9	9	7	9	9	0	0	0	0	9
32	0	9	9	9	9	9	0	0	0	0	0
33	0	9	7	9	9	9	0	0	0	0	1
34	0	0	8	8	0	9	0	0	0	0	0
35	0	16	45	14	23	36	0	0	0	4	19
36	0	16	45	14	23	36	0	0	0	4	19
37	2	7	9	7	9	9	0	2	0	8	0
38	0	6	8	9	8	6	1	0	0	0	0
39	0	0	9	9	0	10	0	0	0	0	0
40	0	0	0	9	0	0	0	0	0	0	0
41	10	28	28	27	29	25	6	4	3	15	19
42	10	16	22	13	10	13	5	6	3	18	27
43	3	8	9	7	6	9	3	3	2	7	8
44	0	6	5	11	9	3	3	0	0	0	0
45	3	22	41	18	23	30	3	0	1	24	32
46	3	10	9	6	9	8	3	3	2	13	10
47	1	4	2	9	9	6	0	0	0	0	4
48	0	9	9	9	9	9	0	0	0	0	0
49	0	9	9	8	9	9	0	0	0	0	0
50	3	5	2	9	9	6	3	2	2	6	2

Table 2B: Doxorubicin, Paclitaxel, and Tamoxifen Common Si natures; DOXPACTAMsi common

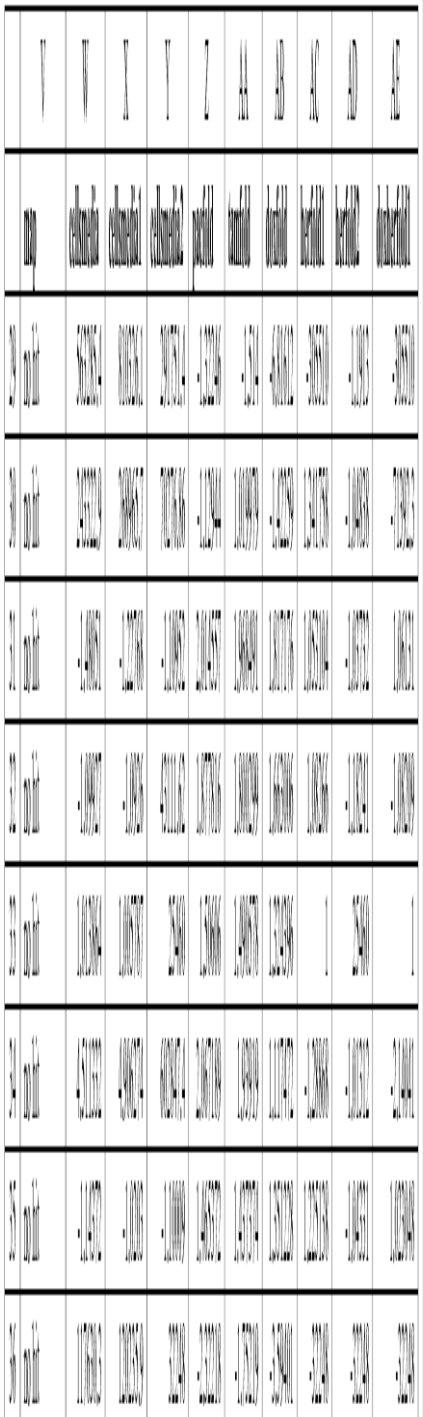


	A	B	C	D	E	F	G	H	I	J	K
	cpdID	doxteststat	doxpval	doxqvalues	pacteststat	pacpval	pacqvalues	tamteststat	tampval	tamqvalues	count
1	36	3.885865	0.000102	0.002729	3.323017	0.00089	0.007807	2.8337946	0.0046	0.031954	76
2	36	3.885865	0.000102	0.002729	3.323017	0.00089	0.007807	2.8337946	0.0046	0.031954	76
3	8902	-2.78786	0.005306	0.016759	-2.7256	0.006418	0.020799	-3.1216186	0.001799	0.01927	119
4	11232	-3.98492	6.75E-05	2.73E-03	-3.96516	7.33E-05	0.004716	-3.0736534	0.002115	0.020401	111
5	11232	-3.98492	6.75E-05	2.73E-03	-3.96516	7.33E-05	0.004716	-3.0736534	0.002115	0.020401	111
6	14190	-3.84625	0.00012	0.002729	-4.09621	4.20E-05	0.004716	-3.97E+00	7.11E-05	0.007404	93
7	14190	-3.84625	0.00012	0.002729	-4.09621	4.20E-05	0.004716	-3.97E+00	7.11E-05	0.007404	93
8	14190	-3.84625	0.00012	0.002729	-4.09621	4.20E-05	0.004716	-3.97E+00	7.11E-05	0.007404	93
9	14190	-3.84625	0.00012	0.002729	-4.09621	4.20E-05	0.004716	-3.97E+00	7.11E-05	0.007404	93
10	15112	-2.74211	0.006105	0.01809	-3.14339	0.00167	0.010906	-2.8663618	0.004152	0.029634	73
11	16504	-3.19062	0.00142	0.007152	-3.36867	0.000755	0.007641	-3.4262479	0.000612	0.016086	92
12	16939	-4.10282	4.08E-05	2.73E-03	-4.10301	4.08E-05	0.004716	-4.10E+00	4.11E-05	0.007198	111
13	17096	3.764629	0.000167	0.002729	2.891704	0.003832	0.017025	3.567149	0.000361	0.01343	45
14	19500	-4.10567	4.03E-05	2.73E-03	-3.98444	6.76E-05	0.004716	-4.11E+00	3.99E-05	0.007198	72
15	19704	-3.10042	0.001932	0.008285	-3.57279	0.000353	0.006242	-2.9106483	0.003607	0.028472	58
16	21926	-3.97991	6.89E-05	2.73E-03	-3.98283	6.81E-05	0.004716	-4.10E+00	4.14E-05	0.007198	103
17	23129	-3.28857	0.001007	0.005716	-2.43349	0.014954	0.040032	-3.1018245	0.001923	0.01927	250
18	23163	-3.77501	0.00016	0.002729	-3.82235	0.000132	0.004717	-3.6333667	0.00028	0.011211	81
19	23163	-3.77501	0.00016	0.002729	-3.82235	0.000132	0.004717	-3.6333667	0.00028	0.011211	81
20	23163	-3.77501	0.00016	0.002729	-3.82235	0.000132	0.004717	-3.6333667	0.00028	0.011211	81
21	24142	-2.61626	0.00889	0.02357	-2.30037	0.021427	0.049919	-3.7236065	0.000196	0.009683	81
22	25445	-2.52979	0.011413	0.027378	-2.94389	0.003241	0.015521	-3.1836372	0.001454	0.01927	33
23	25561	-3.94111	8.11E-05	2.73E-03	-3.89489	9.82E-05	0.004716	-3.1588093	0.001584	0.01927	105
24	25601	-3.72146	0.000198	0.002729	-3.7217	0.000198	0.004717	-3.7291374	0.000192	0.009683	102
25	27363	-3.70878	0.000208	0.002729	-3.70915	0.000208	0.004717	-3.7247001	0.000196	0.009683	101

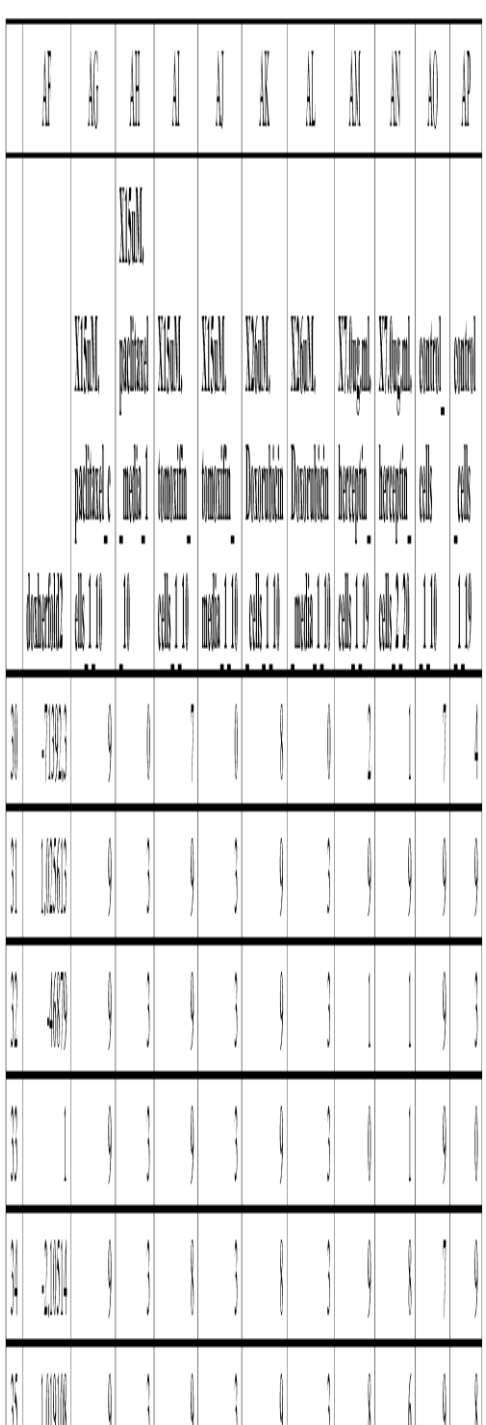
	L	M	N	O	P	Q	R	S	T	U
	RT	MASSavg (neutral mass)	MASSavg _ppmError	diffRT	meanAbun	DBid		annotation	formula	mass
7	1.103957	356.253	23.29805	0.057	2508299	HMDB02689		13,14-dihydro PGE1; prostaglandin E0; PGE0; Dihydroprostaglandin E1; Dihydro-PGE1; 3-hydroxy-2-(3-hydroxyoctyl)-5-oxo-Cyclopentaneheptanoic acid; 13,14-Dihydroprostaglandin E1; 13,14-Dihydro-PGE1; 11a,15-Dihydroxy-9-oxoprostanoic acid; 11,15-Dihydroxy-9-k	$C_{20}H_{36}O_5$	356.25
								Prostaglandin F1alpha; 3,5-dihydroxy-2-(3-hydroxy-1-octenyl)-(8CI)-cyclopentaneheptanoate; 3,5-dihydroxy-2		

	L	M	N	O	P	Q	R	S	T	U
	RT	MASSavg (neutral mass)	MASSavg _ppmError	diffRT	meanAbun	DBid		annotation	formula	mass
1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		Triethylamine; N,N-Diethylethanamine	[illegible]	101.12
2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		Hexylamine	[illegible]	101.12
3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	no hit		no hit	no hit	no hit
4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]	[illegible]

	L	M	N	O	P	Q	R	S	T	U
	RT	MASSavg (neutral mass)	MASSavg _ppmError	diffRT	meanAbun	DBid		annotation	formula	mass
9	1.103957	356.253	23.29805	0.057	2508299	C06475		ProstaglandinF1alpha;(13E15S)-9alpha11alpha-91115-Trihydroxyprost-13-en-1-oicacid	$C_{20}H_{36}O_5$	356.25
10	1.883781	372.0557	1.612662	0.195	1092071	no hit		no hit	no hit	no hit
11	7.25538	393.0913	4.324696	0.505	437721	no hit		no hit	no hit	no hit
12	1.108613	400.2796	32.10256	0.116	2785736	HMDB00501	Major oxidation product of cholesterol	7-Ketocholesterol		
13	6.2306	403.1776	2.728326	0.194	124622	no hit		no hit	no hit	no hit
14	1.106333	444.3034	20.4815	0.088	2711692	HMDB00969	Membrane componente	1,25-Dihydroxyvitamin D3-26,23-lactone		444.2876
15	7.184483	448.1344	19.19067	0.427	156304.5	no hit		no hit	no hit	no hit
16	1.106573	488.3323	28.46422	0.016	1997444	no hit		no hit	no hit	no hit
17	10.44297	515.181	8.152474	1.177	277789.2	no hit		no hit	no hit	no hit
18	7.217222	516.1241	7.556323	0.605	130042.1	C16222		Formononetin7-O-glucoside-6"-O-malonate	$C_{25}H_{24}O_{12}$	516.12



	V	W	X	Y	Z	AA	AB	AC	AD	AE
	map	cellsmedia	cellsmedia1	cellsmedia2	pacfold	tamfold	doxfold	herfold1	herfold2	doxherfold1
1		554109.3	482305.8	655581.4	1.413346	1.889153	-1.18626	-1.77125	1.71092	-591209
2		554109.3	482305.8	655581.4	1.413346	1.889153	-1.18626	-1.77125	1.71092	-591209
3	no hit	2.283468	2.684551	147370.2	2.727537	2.064016	2.438157	-1.20959	-1.48942	1.278284
4		-1.29592	1.008152	-1.26339	4.249579	1.707526	5.193106	-1.14458	-1.2418	1.2543
5	map00350 Tyrosine metabolism	-1.29592	1.008152	-1.26339	4.249579	1.707526	5.193106	-1.14458	-1.2418	1.2543
6	Not Available	-1.01417	1.304255	1.227456	12.07737	11.39781	12.32452	1.137966	-1.17776	-1.04019
7	Not Available	-1.01417	1.304255	1.227456	12.07737	11.39781	12.32452	1.137966	-1.17776	-1.04019
8	C06475	-1.01417	1.304255	1.227456	12.07737	11.39781	12.32452	1.137966	-1.17776	-1.04019
9		-1.01417	1.304255	1.227456	12.07737	11.39781	12.32452	1.137966	-1.17776	-1.04019
10	no hit	887239	681429.4	1885321	-1.02632	-1.26642	-1.24862	-1.40478	1.07942	-2066229
11	no hit	3.432976	2.921983	6.34347	1.219448	1.210027	1.090335	-1.7268	-1.01684	-4.73645
12		1.076731	1.413389	-1.09654	11.76035	10.16336	11.70235	-1.08552	-1.23147	1.199295
13	no hit	1.050517	1.060073	1	-2.02828	-1.83196	-2.02013	1	1	1
14		1.76977	1.918847	-1.30022	17.07824	15.79881	16.23629	4.037686	3.489298	4.396932
15	no hit	137698.1	163339.9	68102.76	2.693743	1.97469	2.122187	-1.23582	-1.27857	-75926.4
16	no hit	1.110932	1.456566	-1.26587	8.466552	7.56426	7.798772	1.089588	-1.11045	1.345859
17	no hit	1.75638	1.803135	1.071783	1.263189	1.412462	1.177707	-1.09643	-1.1782	-1.74796
18	map00943 Isoflavonoid biosynthesis	1.526544	1.870327	76377.25	2.287864	1.749989	2.197596	-1.37132	-1.33822	-94251.9
19		1.526544	1.870327	76377.25	2.287864	1.749989	2.197596	-1.37132	-1.33822	-94251.9
20		1.526544	1.870327	76377.25	2.287864	1.749989	2.197596	-1.37132	-1.33822	-94251.9
21	no hit	486921.5	565998	285200.8	1.806544	2.706438	1.559289	1.081399	-1.08052	-490430
22	no hit	60613.87	60648.85	1	1.258579	1.421176	1.198857	1	1	1



	AF	AG	AH	AI	AJ	AK	AL	AM	AN	AO	AP
	doxherfold2	X15uM. paclitaxel_cells_1_10	X15uM. paclitaxel_media_1_10	X15uM. tomoxifin_cells_1_10	X15uM. tomoxifin_media_1_10	X26uM. Doxorubicin_cells_1_10	X26uM. Doxorubicin_media_1_10	X7.0ug.ml. herceptin_cells_1_19	X7.0ug.ml. herceptin_cells_2_20	control_cells_1_10	control_cells_1_19
1	-5.12427	9	0	9	0	9	0	10	9	9	9
2	-5.12427	9	0	9	0	9	0	10	9	9	9
3	2.132902	14	0	13	2	12	0	11	7	13	16
4	1.569631	10	3	9	3	9	3	10	9	9	10
5	1.569631	10	3	9	3	9	3	10	9	9	10
6	-1.13177	9	3	8	3	7	3	4	9	9	8
7	-1.13177	9	3	8	3	7	3	4	9	9	8
8	-1.13177	9	3	8	3	7	3	4	9	9	8
9	-1.13177	9	3	8	3	7	3	4	9	9	8
10	-12.069	8	0	9	0	9	0	9	9	8	9
11	-2.49296	9	0	9	0	9	0	9	9	9	9
12	1.021101	9	3	9	3	9	3	10	10	9	11
13	1	8	1	9	3	9	3	0	0	9	0
14	4.414748	8	2	9	2	9	2	6	7	9	4
15	-75926.4	9	0	9	0	9	0	1	4	9	9
16	1.116345	8	3	9	3	8	3	9	9	9	9
17	-1.41024	24	2	24	5	25	6	26	19	25	29
18	-1.44377	9	1	9	0	9	0	8	6	13	9
19	-1.44377	9	1	9	0	9	0	8	6	13	9
20	-1.44377	9	1	9	0	9	0	8	6	13	9
21	-5.39965	9	0	9	0	9	0	9	9	9	9

Table 2C:

	AQ	AR	AS	AT	AU	AV
	control_cells_2_20	control_media_1_10	control_media_1_19	control_media_2_20	Dox.Her_cells_1_19	Dox.Her_cells_2_20
17	11	2	4	4	16	28
18	8	2	1	0	0	6
19	8	2	1	0	0	6
20	8	2	1	0	0	6
21	9	0	0	0	0	9
22	0	0	0	0	0	0
23	11	3	3	3	8	9
24	9	1	3	2	9	9
25	9	1	3	1	9	9
26	9	2	3	2	9	9
27	1	3	0	0	4	4
28	0	3	0	0	0	0
29	8	0	0	0	0	0
30	2	0	0	0	0	0
31	9	3	3	2	9	8
32	1	3	0	0	2	0
33	0	2	0	0	0	0
34	8	2	1	0	5	6
35	7	3	3	1	7	8
36	1	0	0	0	0	0
37	6	2	2	2	5	9
38	4	3	2	0	4	5

	AQ	AR	AS	AT	AU	AV
	control_cells_2_20	control_media_1_10	control_media_1_19	control_media_2_20	Dox.Her_cells_1_19	Dox.Her_cells_2_20
1	9	0	0	0	0	3
2	9	0	0	0	0	3
3	12	1	0	0	2	16
4	9	3	3	2	9	10
5	9	3	3	2	9	10
6	7	3	2	1	5	9
7	7	3	2	1	5	9
8	7	3	2	1	5	9
9	7	3	2	1	5	9
10	9	0	0	0	0	3
11	9	0	2	2	9	7
12	9	3	3	2	9	9
13	0	3	0	0	0	0
14	3	3	1	2	3	2
15	8	0	0	0	0	0
16	9	3	3	2	7	9

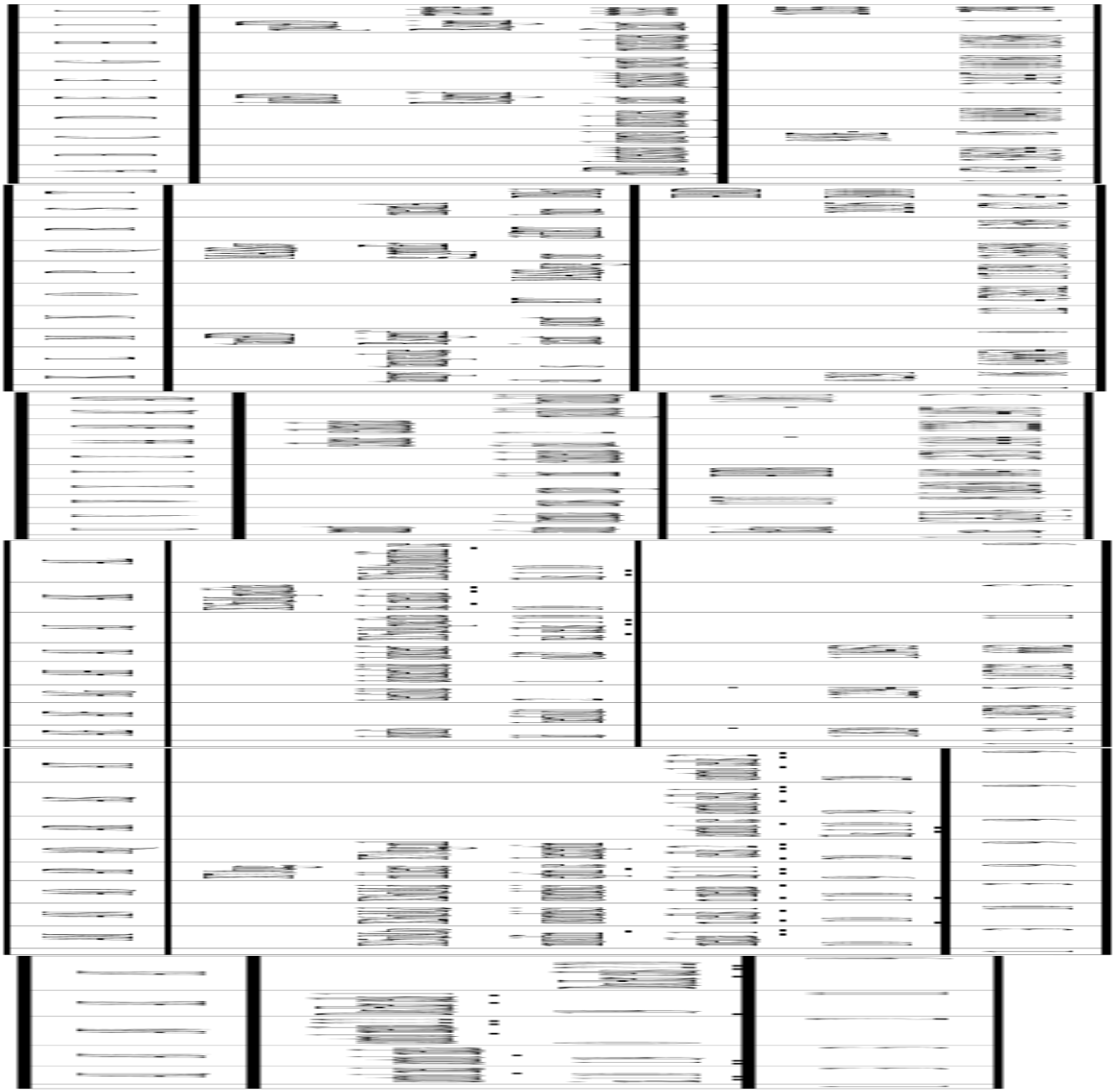


Table 2D:

	A	B	C	D	E	F	G	H
	name	EXP	MASSavg	RT	adductName	Name	KEGG	HMDB
1	M123T94_1	Cardio	123.0555	94.03	M+H	Niacinamide	C00153	HMDB01406
2	M134T504	Cardio	134.0445	504.46	M+H	Aspartic Acid	C00049	HMDB00191
3	M134T504	Cardio	134.0445	504.46	M+H	Iminodiacetate		HMDB11753
4	M145T109	Cardio	145.047	108.64	M+Na	Erythritol	C00503	HMDB02994
5	M145T109	Cardio	145.047	108.64	M+Na	D-Threitol	C16884	HMDB04136
6	M148T497_1	Cardio	148.0605	497.39	M+H	N-Acetylserine		HMDB02931
7	M148T497_1	Cardio	148.0605	497.39	M+H	L-Glutamic acid	C00025	HMDB00148
8	M148T497_1	Cardio	148.0605	497.39	M+H	L-4-Hydroxyglutamate semialdehyde	C05938	HMDB06556
9	M148T497_1	Cardio	148.0605	497.39	M+H	2-Oxo-4-hydroxy-5-aminovalerate	C05941	
10	M148T497_1	Cardio	148.0605	497.39	M+H	O-Acetylserine	C00979	HMDB03011
11	M148T497_1	Cardio	148.0605	497.39	M+H	DL-Glutamate;DL-Glutaminic acid;2-Aminoglutaric acid;Glutamate	C00302	
12	M148T497_1	Cardio	148.0605	497.39	M+H	D-Glutamic acid	C00217	HMDB03339
13	M188T354	Cardio	188.0684	354.105	M+Na	3-Pyridinebutanoic acid		HMDB01007
14	M188T354	Cardio	188.0684	354.105	M+Na	Norsalsolinol		HMDB06044
15	M188T354	Cardio	188.0684	354.105	M+Na	D-Phenylalanine;D-alpha-Amino-beta-phenylpropionic acid	C02265	
16	M188T354	Cardio	188.0684	354.105	M+Na	L-Phenylalanine	C00079	HMDB00159

	I	J	K	L	M	N	O	P
	KeggHuman	HMDBmammal	RADICAL ION TYPE	mzmed	rtmed	Herceptin_fold	Tamoxifen_fold	Valproate_fold
1	1	1		123.0555	94.03	1.135313	1.17988	1.076783
2	1	1		134.0445	504.46	1.682018	1.835062	1.315958
3	0	1		134.0445	504.46	1.682018	1.835062	1.315958
4	0	1		145.047	108.64	-1.72518	-1.11273	-1.36807
5	0	1		145.047	108.64	-1.72518	-1.11273	-1.36807
6	0	1		148.0605	497.39	-1.01305	1.085177	1.118178
7	1	1		148.0605	497.39	-1.01305	1.085177	1.118178
8	1	1		148.0605	497.39	-1.01305	1.085177	1.118178
9	1	0		148.0605	497.39	-1.01305	1.085177	1.118178
10	1	0		148.0605	497.39	-1.01305	1.085177	1.118178
11	1	0		148.0605	497.39	-1.01305	1.085177	1.118178
12	1	1		148.0605	497.39	-1.01305	1.085177	1.118178
13	0	1		188.0684	354.105	1.271909	1.4366	1.17495
14	0	1		188.0684	354.105	1.271909	1.4366	1.17495
15	1	0		188.0684	354.105	1.271909	1.4366	1.17495
16	1	1		188.0684	354.105	1.271909	1.4366	1.17495
17	1	1		192.0721	521.79	2.06004	2.23602	2.82388

	Q	R	S	T	U	V	W
	Doxorubicin_fold	Paclitaxel_fold	HerPac_fold	DoxPac_fold	Herceptin_Wpval	Tamoxifen_Wpval	Valproate_Wpval
1	1.143583	1.069307	1.072776	1.280047	0.000214	0.078556	0.971066
2	1.88419	2.042517	1.830951	2.288645	0.074396	0.020452	0.540049
3	1.88419	2.042517	1.830951	2.288645	0.074396	0.020452	0.540049
4	-2.11453	-1.4969	-1.18321	-2.78391	0.010623	0.211529	0.08865
5	-2.11453	-1.4969	-1.18321	-2.78391	0.010623	0.211529	0.08865
6	1.203785	1.430885	1.392223	1.35825	0.950113	0.952955	0.984808
7	1.203785	1.430885	1.392223	1.35825	0.950113	0.952955	0.984808
8	1.203785	1.430885	1.392223	1.35825	0.950113	0.952955	0.984808
9	1.203785	1.430885	1.392223	1.35825	0.950113	0.952955	0.984808
10	1.203785	1.430885	1.392223	1.35825	0.950113	0.952955	0.984808
11	1.203785	1.430885	1.392223	1.35825	0.950113	0.952955	0.984808
12	1.203785	1.430885	1.392223	1.35825	0.950113	0.952955	0.984808
13	1.524914	1.2233	1.320146	1.393941	0.002743	0.023158	0.724793
14	1.524914	1.2233	1.320146	1.393941	0.002743	0.023158	0.724793
15	1.524914	1.2233	1.320146	1.393941	0.002743	0.023158	0.724793
16	1.524914	1.2233	1.320146	1.393941	0.002743	0.023158	0.724793
17	-7.08308	-3.68456	-2.71895	-1.7823	0.001503	0.017999	0.009218
18	-7.08308	-3.68456	-2.71895	-1.7823	0.001503	0.017999	0.009218

	X	Y	Z	AA	BB	CC	DD
	Doxorubicin_Wpval	Paclitaxel_Wpval	HerPac_Wpval	DoxPac_Wpval	Herceptin_Qval	Tamoxifen_Qval	Valproate_Qval
1	0.162313	0.803929	0.200531	0.001486	0.015568	0.387186	0.987127
2	0.004489	0.002089	0.016868	0.00016	0.373651	0.206845	0.732596
3	0.004489	0.002089	0.016868	0.00016	0.373651	0.206845	0.732596
4	0.000464	0.026414	0.199839	0.00058	0.14146	0.60519	0.292587
5	0.000464	0.026414	0.199839	0.00058	0.14146	0.60519	0.292587
6	0.383297	0.012441	0.004347	0.005881	0.99234	0.997878	0.993376
7	0.383297	0.012441	0.004347	0.005881	0.99234	0.997878	0.993376
8	0.383297	0.012441	0.004347	0.005881	0.99234	0.997878	0.993376
9	0.383297	0.012441	0.004347	0.005881	0.99234	0.997878	0.993376
10	0.383297	0.012441	0.004347	0.005881	0.99234	0.997878	0.993376
11	0.383297	0.012441	0.004347	0.005881	0.99234	0.997878	0.993376
12	0.383297	0.012441	0.004347	0.005881	0.99234	0.997878	0.993376
13	0.014249	0.02065	0.002475	0.002968	0.070701	0.221246	0.849134
14	0.014249	0.02065	0.002475	0.002968	0.070701	0.221246	0.849134
15	0.014249	0.02065	0.002475	0.002968	0.070701	0.221246	0.849134
16	0.014249	0.02065	0.002475	0.002968	0.070701	0.221246	0.849134
17	3.27E-05	0.038105	0.007026	0.054593	0.056481	0.186766	0.111598
18	3.27E-05	0.038105	0.007026	0.054593	0.056481	0.186766	0.111598

	EE	FF	GG	HH
	Doxorubicin_Qval	Paclitaxel_Qval	HerPac_Qval	DoxPac_Qval
1	0.480326	0.973137	0.71285	0.033042
2	0.075791	0.075263	0.230482	0.01161
3	0.075791	0.075263	0.230482	0.01161
4	0.01948	0.29624	0.71285	0.0244
5	0.01948	0.29624	0.71285	0.0244
6	0.717224	0.220901	0.119765	0.077309
7	0.717224	0.220901	0.119765	0.077309
8	0.717224	0.220901	0.119765	0.077309
9	0.717224	0.220901	0.119765	0.077309
10	0.717224	0.220901	0.119765	0.077309
11	0.717224	0.220901	0.119765	0.077309
12	0.717224	0.220901	0.119765	0.077309
13	0.132734	0.276896	0.08987	0.050448
14	0.132734	0.276896	0.08987	0.050448
15	0.132734	0.276896	0.08987	0.050448
16	0.132734	0.276896	0.08987	0.050448
17	0.005382	0.349953	0.163226	0.277833
18	0.005382	0.349953	0.163226	0.277833
19	0.554727	1	0.541304	0.207098
20	0.554727	1	0.541304	0.207098
21	0.501641	0.773714	0.970499	0.033042
22	0.501641	0.773714	0.970499	0.033042

Statistically-significant changes in metabolite secretion can be examined for novel or non-annotated low molecular weight molecules, using the approach reported previously (Cezar et al, 2007, Stem Cells and Development 16: 869-882). Initial experiments have shown that a subset of human metabolites are indeed statistically- significantly altered in response to pharmaceuticals that are strong inducers of cardiomyopathies, namely doxorubicin and paclitaxel, in comparison to weak/moderate inducers such as tamoxifen. (Figure 3).

Data were accrued from n=107 mass spectrometry injections following exposure of human cardiomyocytes (Celprogen 36044- 15at, San Pedro, CA) to three experimental treatments with different degrees of cardiotoxicity: (1.) doxorubicin; (2.) pacitaxel; and (3.) tamoxifen (weak toxicant). Following statistical analysis, with False Discovery Rates (FDR 0.05) adjustments, 187 significant features {e.g., candidate biomarkers), were identified in response to doxorubicin, 185 significant features in response to paclitaxel and 148 significant features in response to tamoxifen. Seventy-three statistically significant features were found to be in common to the strong cardiotoxicants

doxorubicin and paclitaxel as described in the Preferred Embodiments. (Figure 3 and Table 2A).

The putative annotation of the exact neutral masses of such features in chemical databases revealed that several candidate biomarkers map onto energy metabolism pathways, such as $NADPH_2$: oxygen oxidoreductase activity, UDPglucuronate beta-D- glucuronosyltransferase, glycolysis, gluconeogenesis as well as oxidative stress. These results are consistent with published reports on the mechanisms of cardiotoxicity for these particular compounds. Strong robustness and high reproducibility of low molecular weight molecules identified following exposure of human cardiomyocytes to the three established cardiotoxins: paclitaxel, doxorubicin, and tamoxifen was observed. The identification of metabolites secreted by cardiomyocytes in response to two or three cardiotoxins permitted enrichment for candidate biomarkers and provided a metabolic signature of cardiotoxicity.

In addition, the claimed invention is not intended to be limited to the disclosed embodiments. It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications of alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.

PATENT CITATIONS

Cited Patent	Filing date	Publication date	Applicant	Title
WO2007103374A2 *	Mar 6, 2007	Sep 13, 2007	Ceetox Inc	Toxicity screening methods

* Cited by examiner

NON-PATENT CITATIONS

		Reference
1	*	BISTOLA V ET AL: "Long-term primary cultures of human adult atrial cardiac myocytes: Cell viability, structural properties and BNP secretion in vitro", INTERNATIONAL JOURNAL OF CARDIOLOGY, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 131, no. 1, 17 December 2008 (2008-12-17), pages 113-122, XP025690912, ISSN: 0167-5273, DOI: 10.1016/J.IJCARD.2007.10.058 [retrieved on 2008-02-05]
2	*	See also references of WO2011044253A1

* Cited by examiner

CLASSIFICATIONS

International Classification	G01N33/50
Cooperative Classification	G01N33/5038, G01N33/5014, G01N33/5061, G01N2800/32
European Classification	G01N33/50D2D, G01N33/50D2E12, G01N33/50D2F6

LEGAL EVENTS

Date	Code	Event	Description

Date	Code	Event	Description
Aug 15, 2012	17P	Request for examination filed	**Effective date:** 20120330
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