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(54) Title: INHIBITION OF GLUTATHIONE TRANSFERASE ZETA

(57) Abstract: The present invention relates to a method for treating a metabolic condition in a subject, comprising administering a compound to said subject that inhibits the expression or activity of GSTZ or promotes degradation of GSTZ. The compound may inhibit or disrupt an interaction between GSTZ and one or more molecules, compounds or cellular components.



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Inhibition of Glutathione Transferase Zeta

Technical Field

The present invention relates to inhibition of glutathione transferase zeta (GSTZ). In particular the present invention relates to the inhibition of GSTZ and weight gain. The invention further relates to methods of screening for compounds that inhibit GSTZ activity.

Background

Adipogenesis is a complex pathway where fat cells (adipocytes) differentiate and store fat. Excess adipogenesis leads to obesity a major clinical problem world wide, particularly in developed countries. There is considerable interest in understanding the adipogenic pathway and devising therapeutic agents that can prevent the development of obesity, treat obesity, or facilitate weight loss.

The glutathione transferases (GSTs) are a family of enzymes that are generally considered to contribute to the phase II class of detoxification reactions. In this role they conjugate the tripeptide glutathione (GSH) to a wide range of electrophilic and generally hydrophobic compounds that may be of endogenous or exogenous origin. Examples of exogenous compounds known to be substrates include carcinogenic metabolites of benzo[a]pyrene, aflatoxin B1 and anti-cancer drugs such as chlorambucil, melphalan and busulfan. Endogenous substrates include the products of lipid peroxidation and maleylacetoacetate a product of tyrosine catabolism. A number of more recent studies have implicated GSTs in a range of other physiological roles including the regulation of cell signalling kinases such as JNK and ASK1, the modulation of ryanodine receptor Ca^{2+} ion channels and the activation of interleukin 1β .

The GST gene family can be subdivided into a number of different classes based on their sequence similarity. In mammals, seven classes of cytosolic GST have been described (Alpha, Mu, Omega, Pi, Sigma, Theta and Zeta). Unlike some GST classes there is only a single Zeta class gene in humans and mice.

Fyn kinase (Fyn) is a proto-oncogene tyrosine kinase which has been demonstrated to play a key role in lipid homeostasis and has other roles in lymphocyte signaling, cell cycle

entry and cellular growth and proliferation. Fyn is a member of the Src family of nonreceptor tyrosine kinases that share a conserved structure consisting of three Src homology domains (SH1, SH2 and SH3). The SH1 and SH2 domains are involved with the regulation of Fyn kinase phosphorylation of target proteins in signaling pathways while the SH3 domain is known to mediate protein-protein interactions with proline-rich motifs in target proteins, making the kinase domain of Fyn accessible.

The present invention is predicated on the discovery that glutathione transferase zeta (GSTZ), also known as maleylacetoacetate isomerase (MAAI) plays a role in lipid homeostasis and further that GSTZ interacts with Fyn.

Summary

In a first aspect there is provided a method for treating a metabolic condition in a subject, comprising administering to said subject a compound that inhibits the expression or activity of GSTZ and/or promotes degradation of GSTZ.

In one embodiment the compound may inhibit or disrupt an interaction between GSTZ and one or more molecules, compounds or cellular components.

In a second aspect there is provided a method for treating a metabolic condition in a subject, comprising administering to said subject a compound that inhibits or disrupts the interaction of GSTZ with one or more molecules, compounds or cellular components.

In a third aspect there is provided a method for modulating the metabolic rate of a subject, comprising administering to said subject a compound that inhibits the expression or activity of GSTZ and/or promotes degradation of GSTZ.

In one embodiment the compound may inhibit or disrupt an interaction between GSTZ and one or more molecules, compounds or cellular components.

In a fourth aspect there is provided a method for modulating the metabolic rate of a subject, comprising administering to said subject a compound that inhibits or disrupts the interaction of GSTZ with one or more molecules, compounds or cellular components.

The modulation of the metabolic rate may be an increase in metabolic rate.

In a fifth aspect there is provided a method for modulating lipid homeostasis and/or fatty acid oxidation in a subject comprising administering to said subject a compound that inhibits the expression or activity of GSTZ and/or promotes degradation of GSTZ. For example, the

modulation may comprise increasing the rate of lipolysis, inhibiting adipogenesis or both.

In one embodiment the compound may inhibit or disrupt an interaction between GSTZ and one or more molecules, compounds or cellular components

In a sixth aspect there is provided a method for modulating lipid homeostasis and/or fatty acid oxidation in a subject comprising administering to said subject a compound that inhibits or disrupts the interaction of GSTZ with one or more molecules, compounds or cellular components. For example, the modulation may comprise increasing the rate of lipolysis, increasing the metabolic rate, inhibiting adipogenesis or any combination thereof.

In a seventh aspect there is provided a method for treating insulin resistance in a subject, comprising administering to said subject a compound that inhibits the expression or activity of GSTZ and/or promotes degradation of GSTZ.

In one embodiment the compound may inhibit or disrupt an interaction between GSTZ and one or more molecules, compounds or cellular components.

In an eighth aspect there is provided a method for treating insulin resistance in a subject, comprising administering to said subject a compound that inhibits or disrupts the interaction of GSTZ with and one or more molecules, compounds or cellular components.

In a ninth aspect there is provided the use of a compound that inhibits the expression or activity of GSTZ and/or promotes degradation of GSTZ for the manufacture of a medicament for the treatment of a metabolic condition.

In one embodiment the compound may inhibit or disrupt an interaction between GSTZ and one or more molecules, compounds or cellular components.

In a tenth aspect there is provided the use of a compound that inhibits or disrupts the interaction of GSTZ with one or more molecules, compounds or cellular components for the manufacture of a medicament for the treatment of a metabolic condition

In an eleventh aspect there is provided the use of a compound that inhibits the expression or activity of GSTZ and/or promotes degradation of GSTZ for the manufacture of a medicament for the inhibition of adipogenesis.

In a twelfth aspect there is provided the use of a compound that inhibits or disrupts the interaction of GSTZ with one or more molecules, compounds or cellular components for the manufacture of a medicament for the inhibition of adipogenesis.

In a thirteenth aspect there is provided the use of a compound that inhibits the expression or activity of GSTZ and/or promotes degradation of GSTZ for the manufacture of a medicament for increasing the rate of lipolysis and/or increases the rate of fatty acid oxidation.

In a fourteenth aspect there is provided the use of a compound that inhibits or disrupts the interaction of GSTZ with a cellular component for the manufacture of a medicament for increasing the rate of lipolysis and/or increases the rate of fatty acid oxidation.

In a fifteenth aspect there is provided the use of a compound that inhibits the expression or activity of GSTZ and/or promotes degradation of GSTZ for the manufacture of a medicament for the treatment of insulin resistance.

In a sixteenth aspect there is provided the use of a compound that inhibits or disrupts the interaction of GSTZ with a cellular component for the manufacture of a medicament for the treatment of insulin resistance.

In a seventeenth aspect, there is provided a method of screening for a compound that inhibits the expression or activity of GSTZ and/or promotes degradation of GSTZ, the method comprising contacting a cell expressing GSTZ or a cell expressing a reporter protein under the control of a GSTZ promoter-; incubating the cell and the compound for a period of time under conditions suitable to enable expression of the GSTZ or the reporter protein, and assaying for activity or presence of the GSTZ or the reporter protein.

In an eighteenth aspect, there is provided a method of screening for a compound that inhibits or disrupts the interaction of GSTZ with one or more molecules, compounds or cellular components, the method comprising contacting the GSTZ with a candidate compound under conditions suitable to enable interaction of the candidate compound and the GSTZ and assaying for the presence of an interaction between GSTZ and one or more of said molecules, compounds or cellular components.

The following embodiments relate to any one of the first to sixteenth aspects.

In one embodiment, inhibiting the expression or activity and/or promoting degradation of GSTZ comprises degrading or blocking the synthesis of said GSTZ.

In one embodiment, the molecule, compound or cellular component may comprise a protein with at least one SH3 domain, for example Fyn Kinase.

The GSTZ may comprise human GSTZ. In particular embodiments the GSTZ may be GSTZ1 or GSTZ1-1.

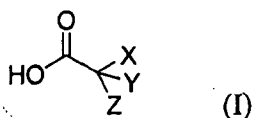
The metabolic condition may be obesity, excess fat, insulin resistance, type II diabetes or metabolic syndrome.

Compounds to be administered may comprise organic compounds, peptides, peptide derivatives, peptidomimetics, inhibitory nucleic acids such as oligonucleotides, inhibitory RNA (such as siRNA or RNAi) or PNA (peptide nucleic acids), antibodies or antibody fragments.

Organic compounds that may be used to inhibit the activity, expression and or promote the degradation of GSTZ or inhibit or disrupt the interaction of GSTZ with one or more molecules, compounds or cellular components include C₂-C₆ carboxylic acids comprising at least two leaving groups, including salts and prodrugs thereof, wherein reference to C₂-C₆ does not include carbon atoms present in the leaving groups.

The at least two leaving groups may be located on the α -carbon relative to the carboxyl group.

The C₂-C₆ carboxylic acids comprising at least two leaving groups, wherein reference to C₂-C₆ does not include carbon atoms present in the leaving groups, may have the following structure (I):



wherein X, Y and Z are independently selected from the group consisting of: C₁-C₄ alkyl, hydroxy, leaving group and hydrogen, provided that at least two of X, Y and Z are leaving groups.

In an alternative embodiment X, Y and Z are independently selected from the group consisting of: C₁-C₄ alkyl, leaving group and hydrogen, provided that at least two of X, Y and Z are leaving groups.

The leaving groups may be independently selected from the group consisting of: chloride, bromide, fluoride, iodide, triflate, tosylate, mesylate, diazonium salts, sulfonates and tetraalkyl ammonium salts.

In one embodiment the leaving groups are chloride, iodide, bromide or any combination thereof.

The C₂-C₆ carboxylic acid comprising at least two leaving groups may be selected from the group consisting of: dichloroacetic acid, bromochloroacetic acid, dibromoacetic acid, diiodoacetic acid, iodochloroacetic acid and iodobromoacetic acid, including salts and prodrugs thereof.

The organic compound may be a glutathione analogue.

Brief Description of the Drawings

A preferred embodiment of the present invention will now be described, by way of example only, with reference to the accompanying drawings wherein:

Figure 1 illustrates the protein sequence of human glutathione transferase zeta 1 isoform 1 (GSTZ1-1) identified by Genbank (<http://www.ncbi.nlm.nih.gov/Genbank/>) accession number NP_665877.

Figure 2 shows the aligned human (SEQ ID NO: 1) and mouse (SEQ ID NO: 2) GSTZ1 amino acid sequences and highlight the two PXXP motifs of GSTZ1 that expected to be the binding site for the SH3 domain of Fyn kinase.

Figure 3 is a schematic diagram of the formation of an inactive GSTZ1-substrate complex after reaction with DCA (dichloroacetate). This figure is reproduced from H.F. Tzeng, A.C. Blackburn, P.G. Board, and M.W. Anders, Polymorphism- and species-dependent inactivation of glutathione transferase zeta by dichloroacetate. *Chem Res Toxicol* 13 (2000) 231-6.

Figure 4A is a Western blot of liver samples and adipose samples from mice receiving 1.5mg/ml DCA (dichloroacetate) in drinking water compared with normal controls not receiving DCA. β -Actin is shown as a loading control.

Figure 4B illustrates a deficiency of mouse liver GSTZ1-1 activity with chlorofluoroacetic acid as a substrate after treatment with DCA (dichloroacetate). N=4 +/- SD.

Figure 5 illustrates the blood glucose levels following an intraperitoneal injection of 250mg/kg glucose in both normal mice (represented by solid squares) and GSTZ1^{-/-} mice (represented by solid triangles). N=5 $\pm$ SD.

Figure 6 indicates the weight gain of normal mice (represented by solid squares) and GSTZ1^{-/-} mice (represented by solid triangles) fed a high fat diet (HF, 23% fat) for five weeks (weeks 5-10) after 5 weeks on normal chow (weeks 1-5). Wild type N=9; GSTZ1^{-/-} N=7; +/-

SEM.

Figure 7A shows the abdominal fat in a wild type mouse (left panel) and a GSTZ1^{-/-} mouse (right panel) after both mice were fed a diet containing 23% fat for 20 weeks.

Figure 7B shows that adipocytes of normal mice (GSTZ1^{+/+}) are larger than those of GSTZ1^{-/-} mice.

Figure 8 illustrates that normal mice fed normal chow or a high fat diet for 8 weeks exhibited significantly reduced total body fat (Fig. 8A) when drinking water was supplemented with DCA although total lean mass does not change on DCA treatment (Fig. 8B).

Figure 9 shows a Western blot probed with antibodies against both FLAG and HA. Lane 1 shows lysate from cells transfected with GSTZ1-HA and identifies GSTZ1. Lane 2 shows lysate from cells transfected with Fyn-FLAG and identifies Fyn. Lane 3 shows an anti HA immunoprecipitation of untransfected cells. Lane 4 shows an anti HA immunoprecipitation of cells transfected with GSTZ1-HA and GSTZ1-HA is detected. Lane 5 shows an anti HA immunoprecipitation of cells transfected with Fyn-FLAG, nothing is detected. Lane 6 shows an anti HA immunoprecipitation of cells transfected with GSTZ-HA and Fyn-FLAG. In this case Fyn is present because it was Co-immunoprecipitated in association with GSTZ.

Figure 10 shows that phosphorylated acetyl-CoA carboxylase (p-ACC) is increased in adipose tissue of GSTZ1^{-/-} mice and total acetyl-CoA carboxylase (ACC) is relatively unchanged.

Definitions

As used in this application, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "an adipocyte" also includes a plurality of adipocytes.

As used herein, the term "comprising" means "including." Variations of the word "comprising", such as "comprise" and "comprises," have correspondingly varied meanings. Thus, for example, a polynucleotide "comprising" a sequence encoding a protein may consist exclusively of that sequence or may include one or more additional sequences.

As used herein, the term "lipid homeostasis" refers to the balance between the pathways

which are involved in the storage of fat in adipocytes and lipolysis where fat stored in adipocytes is processed into its constituent fatty acids and glycerol.

As used herein, the term "activity" as it pertains GSTZ means any enzymatic activity, cellular function, action, effect or influence exerted by GSTZ or any fragment or derivative thereof. The, cellular function, action, effect or influence, for example modulation of lipid homeostasis and/or fatty acid oxidation, may be effected by GSTZ or any fragment or derivative thereof either directly or indirectly.

As used herein the term "inhibiting" and variations thereof such as "inhibition" and "inhibits" in the context of GSTZ do not necessarily imply the complete inhibition of GSTZ activity or expression. Rather, the inhibition may be to an extent, and/or for a time, sufficient to produce the desired effect. Inhibition may be prevention, retardation, reduction, inactivation (either partial or complete) or other hindrance of GSTZ activity or expression. Such inhibition may be in magnitude and/or be temporal in nature. Inhibition of GSTZ activity includes the inactivation of GSTZ, for example by covalent binding of an entity to GSTZ such that the GSTZ is partially, substantially or completely inactive.

As used herein the term "expression" in the context of GSTZ may refer to expression of a polypeptide or protein, or to expression of a polynucleotide or gene, depending on the context. Expression of a polynucleotide may be determined, for example, by measuring the production of RNA transcript levels. Expression of a protein or polypeptide may be determined, for example, by immunoassay using an antibody(ies) that bind with GSTZ or a fragment or derivative thereof.

The term "subject" as used herein refers to mammals and includes human, primates, livestock animals (e.g. sheep, pigs, cattle, horses, donkeys), laboratory test animals (e.g. mice, rabbits, rats, guinea pigs), performance show animals (e.g. horses, livestock, dogs, cats), companion animals (e.g. dogs, cats) and captive wild animals. Preferably the mammal is a human or a laboratory test animal. Even more preferably, the mammal is human.

The term " C_2 - C_6 carboxylic acid(s) comprising at least two leaving groups" means a compound comprising a carboxylic acid or carboxylate functional group, at least two leaving groups and a total of between two and six carbon atoms, not including carbon atoms present in the leaving groups.

The term " C_1 - C_4 alkyl" is taken to include straight chain and branched chain monovalent

saturated hydrocarbon groups having between 1 and 4 carbon atoms, for example, methyl, ethyl, propyl, isopropyl and butyl.

The term "C₁-C₆ alkyl" is taken to include straight chain and branched chain monovalent saturated hydrocarbon groups having between 1 and 6 carbon atoms, for example methyl, ethyl, propyl, isopropyl, butyl, isobutyl, *sec*-butyl, *t*-butyl, pentyl, hexyl and the like.

The term "C₂-C₆ alkenyl" is taken to include straight chain and branched chain monovalent hydrocarbon groups having between 2 and 6 carbon atoms and at least one carbon-carbon double bond, for example vinyl, propenyl, 2-methyl-2-propenyl, butenyl, pentenyl and the like.

The term "C₂-C₆ alkynyl" is taken to include straight chain and branched chain monovalent hydrocarbon groups having between 2 and 6 carbon atoms and at least one carbon-carbon triple bond, for example ethynyl, propynyl, butynyl, pentynyl, hexynyl and the like.

The term "C₃-C₈ cycloalkyl" is taken to include cyclic alkyl groups having between 3 and 8 carbon atoms, for example cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl.

The term "C₃-C₈ cycloalkenyl" is taken to include cyclic hydrocarbon groups having between 3 and 8 carbon atoms and at least one carbon-carbon double bond, for example cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclohexenyl, cycloheptenyl and the like.

Any description of prior art documents herein, or statements herein derived from or based on those documents, is not an admission that the documents or derived statements are part of the common general knowledge of the relevant art.

For the purposes of description all documents referred to herein are incorporated by reference in their entirety unless otherwise stated.

Detailed Description

The inventors have identified that GSTZ1-1 is expressed highly in liver, kidney, adipose tissue and adipose cells and that mice genetically deficient in GSTZ1-1 fail to accumulate weight, in particular abdominal fat when fed a high fat diet as compared to wild-type mice.

Further, the inventors have shown that pharmacological inactivation of GSTZ1-1 in mice

results in loss of GSTZ1-1 from liver tissues, and prevents accumulation of adipose tissue without a significant effect on lean body mass.

Accordingly, the present invention provides compositions and methods for the treatment of metabolic diseases such as obesity. The invention also provides compositions and methods for modulating lipid homeostasis which include methods for weight loss, inhibiting adipogenesis and/or increasing the rate of lipolysis. Further, the invention provides methods of screening for compounds to inhibit adipogenesis, increase weight loss or for the treatment of obesity.

Glutathione Transferases

The cytosolic GSTs are small dimeric enzymes with subunits ranging in size between 23 and 29kD. The catalytic activity of GSTs is enabled by the stabilisation of glutathione as a thiolate ion by the formation of a hydrogen bond from the hydroxyl side chain of a tyrosine or serine in the active site. However, some recently discovered physiological roles such as the modulation of signalling kinases such as JNK and the modulation of ryanodine receptor Ca^{2+} channels are non-catalytic and rely on protein-protein interactions.

The GST gene family can be subdivided into a number of different classes based on their sequence similarity. In mammals, seven classes of cytosolic GST have been described (Alpha, Mu, Omega, Pi, Sigma, Theta and Zeta). In humans, the genes for a particular class are located in clusters on distinct chromosomes (Alpha 6p12, Mu 1q13, Pi 11q13, Theta 22q11, Zeta 14q24.3 Omega 10q24.3). A representative human GSTZ protein sequence is identified by accession number NP_665877 which is reproduced in Figure 1 (SEQ ID NO: 1). GSTZ is also known as maleylacetoacetate isomerase (MAAI). Representative sequences of GSTZ from other species are *Mus musculus* GSTZ identified by accession number NP_034493.1, *Rattus norvegicus* GSTZ identified by accession number NP_001102915.1, *Bos Taurus* GSTZ identified by accession number NP_001069154, *Equus caballus* GSTZ identified by accession number XP_001493411, *Oryctolagus cuniculus* GSTZ identified by accession number XP_002719675 and *Canis familiaris* GSTZ identified by accession number XP_547928.

In some embodiments the GSTZ may be a GSTZ fragment, variant or derivative.

The term "fragment" as it relates to GSTZ refers to an amino acid sequence that comprises a subset of the full length GSTZ amino acid sequence. A fragment of GSTZ can be

a polypeptide in which amino acid residues are deleted as compared to GSTZ itself, but where the remaining amino acid sequence is typically identical to the corresponding positions in GSTZ. Such deletions can occur at the amino-terminus or the carboxy-terminus of GSTZ, or alternatively at both termini. Fragments are typically at least 25 amino acids long, at least 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150 or 200 amino acids long.

The term "derivative" as it relates to GSTZ, refers to GSTZ or a fragment thereof and one or more amino acid substitutions, deletions and/or additions wherein the derivative will display at least about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% similarity or identity to a reference GSTZ polypeptide sequence for example as set forth in Figure 6. The term "derivative" as it relates to GSTZ also refers to GSTZ or a fragment thereof having one or more amino acid residues chemically modified, e.g., by alkylation, a esterification or amidation and/or one or more amino acid residues biologically modified e.g. by lipidoylation such as myristoylation, palmitoylation, farnesylation, steroylation, geranylation, glutionylation, glycation, glycosylation, phosphorylation, acetylation, acylation, methylation, hydroxylation, by biotinylation or ubiquitinylation.

The term "variant" as used herein in the context of GSTZ refers to a substantially similar sequence. In general, two sequences are "substantially similar" if the two sequences have a specified percentage of amino acid residues or nucleotides that are the same (percentage of "sequence identity"), over a specified region, or when not specified, over the entire sequence. Accordingly, a "variant" of a polynucleotide and polypeptide sequence disclosed herein may share at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 83%, 85%, 88%, 90%, 93%, 95%, 96%, 97%, 98% or 99% sequence identity with the reference sequence.

The term "sequence identity" or "percentage of sequence identity" may be determined by comparing two optimally aligned sequences or subsequences over a comparison window or span, wherein the portion of the polynucleotide sequence in the comparison window may optionally comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Thus, a "percentage of sequence identity" may be calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, I) or the identical amino acid residue

(e.g., Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity.

The term "Similarity" refers to the percentage number of amino acids that are identical or constitute conservative substitutions, the nature of conservative substitutions will be understood to those skilled in the art. Similarity may be determined using sequence comparison programs such as GAP (Deveraux *et al.* 1984, *Nucleic Acids Research* **12**, 387-395). In this way, sequences of a similar or substantially different length to those cited herein might be compared by insertion of gaps into the alignment, such gaps being determined, for example, by the comparison algorithm used by GAP.

The *GSTZ* gene and several polymorphisms in the coding sequence and the promoter have been characterised. In addition the crystal structure of human GSTZ is known and its catalytic mechanism has been investigated with a variety of substrates. Unlike some other GST classes there is only a single Zeta class gene in humans and mice. The Zeta class GSTs are the most conserved GST class occurring in all animals and plants studied so far and in some microbes. The high level of conservation has been attributed to their maleylacetoacetate isomerase activity that constitutes an essential step in the catabolism of tyrosine. *GSTZ* knockout mice (*GSTZ1*^{-/-}) show that the electrophilic intermediates that accumulate as a result of the block in the tyrosine degradation pathway (maleylacetoacetate, maleylacetoacetone) cause constitutive oxidative stress and susceptibility to oxidants and drugs that consume glutathione such as acetaminophen. So far no confirmed cases of GSTZ deficiency have been identified in humans. Little is known about the regulation of GSTZ expression although two promoter region SNP's (single nucleotide polymorphisms) have been identified that appear to influence GSTZ expression. GSTZ also plays a role in the glutathione dependent biotransformation of α -halo acids such as dichloroacetic acid and chlorofluoroacetic acid.

Cellular components that interact with GSTZ

As exemplified herein GSTZ interacts with Fyn Kinase as demonstrated by co-immunoprecipitation experiment using Flag-tagged Fyn and HA-tagged GSTZ and is

postulated to function via a pathway comprising Fyn Kinase. Accordingly the activity of GSTZ may be inhibited by disruption or inhibition of the interaction between GSTZ and one or more molecules, compounds or cellular components, for example Fyn Kinase.

Fyn kinase is known to comprise a SRC Homology 3 Domain (SH3 domain) which is known to interact with poly-proline motifs with the sequence PXXP in target proteins, where X is any amino acid. GSTZ has previously been cloned and its crystal structure solved Board *et al* (1997) *Biochem J.* 328: 929-935 and Polekhina *et al* (2001) *Biochemistry.* 40: 1567-1576. The Eucaryotic Linear Motif resource (<http://elm.eu.org/>) indentifies two poly-proline (PXXP) sequences in GSTZ, specifically these motifs are ⁸⁷RLLPQDP⁹³ and ²⁰⁵CRQPDTP²¹¹ (Figure 7). On investigation of the GSTZ crystal structure it is apparent that these PXXP motifs occur on external (solvent accessible) loops of GSTZ1 and thus are expected to be available for interaction with the Fyn kinase SH3 domain.

It is expected that GSTZ may interact with cellular components comprising at least one SH3 domain.

Inhibition and inactivation of GSTZ

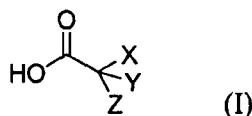
In accordance with the present disclosure, the expression or activity of GSTZ may be inhibited, and/or the degradation of GSTZ promoted by organic compounds, peptides, peptide derivatives, antibodies or fragments thereof such as Fab fragments, peptidomimetics, inhibitory nucleic acids such as oligonucleotides, inhibitory RNA (siRNA or RNAi) or PNA (peptide nucleic acids). It will be understood that inhibition of GSTZ may inactivate and/or promote degradation of the enzyme. Suitable inhibitory agents may be generated or synthesized by methods known in the art.

Organic compounds that may be used to inhibit GSTZ include C₂-C₆ carboxylic acids comprising at least two leaving groups, including salts and prodrugs thereof, wherein reference to C₂-C₆ does not include carbon atoms present in the leaving groups.

The at least two leaving groups may be located on the α -carbon relative to the carboxyl group.

In one embodiment the C₂-C₆ carboxylic acids comprising at least two leaving groups, wherein reference to C₂-C₆ does not include carbon atoms present in the leaving groups, have

the following structure (I):



wherein X, Y and Z are independently selected from the group consisting of: C₁-C₄ alkyl, hydroxy, leaving group and hydrogen, provided that at least two of X, Y and Z are leaving groups.

In an alternative embodiment X, Y and Z are independently selected from the group consisting of: C₁-C₄ alkyl, leaving group and hydrogen, provided that at least two of X, Y and Z are leaving groups.

In an alternative embodiment, X, Y and Z are independently selected from the group consisting of: -CH₂CH₃, -CH₃, hydrogen and leaving group, provided that at least two of X, Y and Z are leaving groups.

In an alternative embodiment, X, Y and Z are independently selected from the group consisting of: methyl, hydrogen and leaving group, provided that at least two of X, Y and Z are leaving groups.

Suitable leaving groups are well known to those skilled in the art and include, but are not limited to: chloride, bromide, fluoride, iodide, triflate, tosylate, mesylate, diazonium salts, sulfonates and tetraalkyl ammonium salts. The at least two leaving groups may be the same or different. The leaving groups may be leaving groups other than fluoride. In one embodiment the at least two leaving groups are chloride, iodide, bromide or any combination thereof.

In another embodiment, the following compounds are specifically excluded: difluoroacetate, 2,2-difluoropropanoate and 3,3-dichloropropanoate.

The C₂-C₆ carboxylic acid comprising at least two leaving groups may be selected from the group consisting of: dichloroacetic acid, bromochloroacetic acid, dibromoacetic acid, diiodoacetic acid, iodochloroacetic acid and iodobromoacetic acid, including salts and prodrugs thereof.

The compounds of structure (I) may have one or more chiral centres. The present invention extends to the use of all enantiomers and diastereomers, as well as mixtures thereof in all ratios.

The C₂-C₆ carboxylic acids comprising at least two leaving groups include salts and prodrugs thereof. Suitable salts include, for example, alkali metal and alkali earth metal salts. The salts may be pharmaceutically acceptable salts. In this context "pharmaceutically acceptable" means that the salt to which it refers is suitable for use in contact with tissues of the body without undue toxicity, incompatibility, instability, irritation, allergic response and the like, commensurate with a reasonable benefit/risk ratio.

Prodrugs include C₂-C₆ carboxylic acids comprising at least two leaving groups which have been derivatised via a covalent bond which is cleaved under *in vivo* conditions to liberate the active C₂-C₆ carboxylic acid comprising at least two leaving groups. Thus, "prodrug" is intended to include any covalently bonded carrier that releases an active C₂-C₆ carboxylic acid comprising at least two leaving groups *in vivo* following administration to a subject. Prodrugs can be used, for example, with the objective of improved chemical stability, improved patient acceptance and compliance, improved bioavailability, prolonged duration of action, improved selectivity, and/or decreased side effects. Prodrugs can be readily prepared using methods known in the art, such as those described by *Burger's Medicinal Chemistry and Drug Chemistry*, Fifth Ed., Vol. 1, pp. 172-178, 949-982 (1995). Examples of prodrugs include, without limitation, esters, thioesters and amides, among others.

In one embodiment the prodrugs are esters, for example alkyl esters, alkenyl esters, alkynyl esters, cycloalkyl esters, cycloalkenyl esters, glycerol esters and the like. The glycerol esters may be mono-, di- or tri-esters. Non-limiting examples of glycerol esters include: 2,3-dihydroxypropyl 2,2-dichloroacetate (Glyceryl 1-monodichloroacetate), 3-hydroxypropane-1,2-diyl bis(2,2-dichloroacetate) [Glyceryl 1,2-di(dichloroacetate)], 2-hydroxypropane-1,3-diyl bis(2,2-dichloroacetate) [Glyceryl 1,3-di(dichloroacetate)] and Propane-1,2,3-triyl tris(2,2-dichloroacetate) [Glyceryl 1,2,3-tri(dichloroacetate)]. Non-limiting examples of alkyl, alkenyl, alkynyl, cycloalkyl and cycloalkenyl esters include C₁-C₆ alkyl esters, such as methyl, ethyl, propyl, isopropyl, and the like, C₂-C₆ alkenyl esters such as vinyl and the like, C₂-C₆ alkynyl esters such as propargyl and the like, C₃-C₈ cycloalkenyl esters such as cyclohexenyl, cyclopentenyl and the like, and C₃-C₈ cycloalkyl esters such as cyclohexanyl and the like.

One example of an organic compound that may be used to inactivate GSTZ is the GSTZ substrate dichloroacetic acid (DCA) which can either be dechlorinated to glyoxylate or act as a

mechanism-based inactivator of GSTZ (Figure 3, reproduced from H.F. Tzeng, A.C. Blackburn, P.G. Board, and M.W. Anders, Polymorphism- and species-dependent inactivation of GSTZ by dichloroacetate. *Chem Res Toxicol* 13 (2000) 231-6.). In the inactivating reaction the S-(α -chlorocarboxymethyl)-glutathione intermediate forms a covalent bond with Cysteine 16 adjacent the active site residues Serine 14 and Serine 15 and effectively blocks access to the site and prevents further reaction. In addition some genetically polymorphic forms of GSTZ are more prone to inactivation by DCA than others.

DCA has been used clinically as an experimental drug to lower blood lactic acid levels in cases of metabolic acidosis and has been proposed as a potential anti cancer agent. DCA achieves these functions by inhibiting pyruvate dehydrogenase kinase (PDK) to enhance metabolism of pyruvate through the Krebs cycle.

As exemplified herein GSTZ inactivated by DCA appears to be degraded and removed from the cell (Figure 4A and 4B) as western blots show a substantial reduction in GSTZ protein in the liver of mice treated with DCA. This inactivation and degradation may be advantageous as a therapy as it is not yet known if the adipogenic action of GSTZ is mediated via its presently known enzymatic active site or by some other "protein-protein" interaction. If GSTZ is acting via an uncharacterized mechanism, simple inhibitors of the known enzymatic activity that do not eliminate the protein may be ineffective. The person skilled in the art will appreciate that it is not necessary for inhibition of GSTZ to inactivate or degrade the enzyme in all embodiments.

While DCA is considered to be of low toxicity in humans the discovery that DCA inactivation of GSTZ retards weight gain provides basis for the use of GST, particularly GSTZ, as targets to be used to identify other modulators of GST activity.

This discovery also provides basis for the use of DCA to inhibit, inactivate and/or degrade GSTZ in the modulation of lipid homeostasis and/or fatty acid oxidation, inhibition of adipogenesis, treatment of insulin resistance, metabolic syndrome, type II diabetes and obesity. It is expected that compounds structurally similar to DCA will also be useful in those methods. In addition other GSTZ inhibitors such as dibromoacetic acid (DBA), bromochloroacetic acid (BCA) or dichloropropionic acid (DCPA) and/or structurally related compounds, or glutathione analogues may also be useful.

Another means of inhibiting GSTZ activity or promoting GSTZ degradation may be

achieved using analogues of glutathione. Glutathione (GSH) is a tripeptide of the formula: γ -Glu-Cys-Gly and is a substrate of GSTZ. Thus analogues of glutathione which are not capable of being conjugated to an electrophilic center by GSTZ but nevertheless interact with the enzyme may be useful in the inhibition of GSTZ.

GSH analogues may include tripeptide analogs of GSH such as those described in Adang, et al., *Biochem J* (1990) **269**:47-54, which interact with GSTZ and are modified forms of GSH in which at least one of the glycine, cysteine, or glutamine residues is replaced by an alternate amino acid residue.

Additional GSH analogues have been disclosed, for example, by Principato, *Enzyme* (1989) **41**:175-180, the tripeptide analogue has formula γ -Glu-p-chlorophenylcarbonylmethyl-Cys-Ser. Morris, *Biochem J* (1960) **76**:349-353, described the synthesis of γ -Glu-benzyl-Cys-Val. Other GSH analogues known in the art contain a substitution for only one or more of the three GSH amino acids.

It is contemplated that any GSH analogue may be useful for the inhibition of GSTZ activity or promotion of GSTZ degradation.

Embodiments of the invention may utilise antisense technology to inhibit the expression of a GSTZ nucleic by blocking translation of the encoded polypeptide. Antisense technology takes advantage of the fact that nucleic acids pair with complementary sequences. Suitable antisense molecules can be manufactured by chemical synthesis or, in the case of antisense RNA, by transcription *in vitro* or *in vivo* when linked to a promoter, by methods known to those skilled in the art.

Binding of the antisense oligonucleotide to their complementary cellular nucleotide sequences may interfere with transcription, RNA processing, transport, translation and/or mRNA stability. Suitable antisense oligonucleotides may be prepared by methods well known to those of skill in the art and may be designed to target and bind to regulatory regions of the nucleotide sequence or to coding (gene) or non-coding (intergenic region) sequences. Typically antisense oligonucleotides will be synthesized on automated synthesizers. Suitable antisense oligonucleotides may include modifications designed to improve their delivery into cells, their stability once inside a cell, and/or their binding to the appropriate target. For example, the antisense oligonucleotide may be modified by the addition of one or more

phosphorothioate linkages, or the inclusion of one or morpholine rings into the backbone (so-called 'morpholino' oligonucleotides).

GSTZ gene expression may be reduced or inhibited by a homologous antisense nucleic acid. Accordingly, the activity of GSTZ may be reduced thereby providing a means of inhibiting GSTZ. Therapeutic or prophylactic use of such nucleic acids of at least 5 nucleotides, generally up to about 200 nucleotides, that are antisense to a gene of complementary DNA (cDNA) encoding GST zeta protein is also provided herein. Such an antisense nucleic acid may be capable of hybridising to a portion of the RNA precursor (generally mRNA) of a GSTZ protein, by virtue of some sequence complementarity, and generally under high stringency conditions. The antisense nucleic acid may be complementary to a coding and/or non-coding region of the RNA precursor of the GSTZ protein. Absolute complementarity to the full RNA precursor of the GSTZ protein is not required. Antisense nucleic acids in this form have utility as therapeutics that reduce or inhibit mast cell activation, and can be used in the treatment or prevention of disease states as described herein.

The antisense nucleic acids complementary to the RNA precursor of the GSTZ protein may be of at least five nucleotides and are generally oligonucleotides which range in length from 5 to about 200 nucleotides. For example, the anti-sense oligonucleotide is at least 10 nucleotides, at least 15 nucleotides, at least 100 nucleotides, at least 125 nucleotides, at least 150 nucleotides, or at least 175 nucleotides. The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded.

The anti-sense nucleic acid complementary to the RNA precursor of the GSTZ protein can be modified at any position on its structure using substituents generally known in the art. The anti-sense nucleic acid may include at least one modified base moiety which is selected from the group including, but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, 2,2-dimethylguanine, 2-methyl-adenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxymethylaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, pseudouracil, 2-thiocytosine, 5-methyl-2-thiouracil, 2-

thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), queosine, wybutoxosine, 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, and 2,6-diaminopurine.

The anti-sense nucleic acid complementary to the RNA precursor of the GSTZ protein may include at least one modified sugar moiety, such as arabinose, 2-fluoroarabinose, xylulose and hexose. The antisense nucleic acid may also include at least one modified phosphate backbone selected from a phosphorothioate, phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof. The anti-sense nucleic acid can be conjugated to another molecule, such as a peptide, hybridisation triggered cross-linking agent, transport agent or a hybridisation- triggered cleavage agent.

Expression of the sequence encoding an anti-sense nucleic acid complementary to the RNA precursor of the GSTZ protein can be achieved by any promoter known in the art to act in mammalian, including human, cells, and may include inducible or constitutive promoters.

RNA interference (RNAi) (see, for example, Chuang et al. (2000) PNAS 97: 4985) can be employed to inhibit the expression of a gene encoding a GST zeta protein. Interfering RNA (RNAi) fragments, particularly double-stranded RNAi, can be used to generate loss of GSTZ protein. RNAi refers to a means of selective post- transcriptional gene silencing by destruction of specific mRNA by small interfering RNA molecules (siRNA). The siRNA is generated by cleavage of double stranded RNA, where one strand is identical to the message to be inactivated. Double-stranded RNA molecules may be synthesised in which one strand is identical to a specific region of the GSTZ mRNA transcript and introduced directly. Alternatively corresponding dsDNA can be employed, which, once presented intracellularly is converted into dsRNA. Methods for the synthesis of suitable molecule for use in RNAi and for achieving post-transcriptional gene silencing are known to those of skill in the art. Methods relating to the use of RNAi to silence genes in organisms are known, for instance, Fire *et al.* (1998) Nature **391**: 806-811 ; Hammond, *et al.* (2001) Nature Rev, Genet. **2**: 110-4119; Hammond *et al.* (2000) Nature **404**: 293-296; Bernstein *et al.* (2001) Nature **409**: 363-366; Elbashir *et al.* (2001) Nature **411**: 494-498), the disclosures of which are incorporated herein by reference.

A further means of inhibiting expression may be achieved by introducing catalytic antisense nucleic acid constructs, such as ribozymes, which are capable of cleaving GSTZ mRNA transcripts and thereby preventing the production of wild type protein. Ribozymes may be targeted to and anneal with a GSTZ sequence by virtue of two regions of sequence complementarity to the target flanking the ribozyme catalytic site. After binding the ribozyme cleaves the target in a site-specific manner. The design and testing of ribozymes which specifically recognise and cleave sequences of interest can be achieved by techniques well known to those in the art (see for example Lieber and Strauss, 1995, *Molecular and Cellular Biology*, 15:540-551).

GSTZ may be inhibited by proteins or peptides either based on the GSTZ sequence or based on the sequence of proteins or peptides that interact with GSTZ. A skilled person will understand that protein or peptide based inhibitors of GSTZ may be based on this sequence or any other known sequence of GSTZ or variants thereof. These proteins or peptides may be considered to be "competitor peptides" in that their sequence corresponds at least in part to some or all of GSTZ and they function to inhibit the enzymatic function of GSTZ or to inhibit the ability of GSTZ to interact with other cellular components or GSTZ substrates. It will be understood that the term "competitor peptides" includes variants and fragments thereof.

In general, the properties and characteristics of the competitor peptides described herein may be modified in order to attempt to improve suitability for a particular therapeutic application. Non-limiting examples of such properties and characteristics that may be improved include but are not limited to solubility, chemical and biochemical stability, cellular uptake, toxicity, immunogenicity and excretion of degradation products. Methods and approaches by which the characteristics and properties of the peptides described herein may be improved are well known in the art. For example, one approach is to search for and identify particular amino acid residues that are either negative or positive determinants for a particular property. This may be achieved, for example, by using the technique of side-chain amputation, in which amino acids are substituted one at a time by the prototypic residue, L-alanine, along the sequence of a peptide. Ascertaining key determinant loci provides a basis for generating and testing variants with both naturally occurring and unnatural amino acid substitutions at the loci identified. Lead competitor peptides that exhibit desirable features may be used as templates for the design of peptidomimetic molecules with improved stability profiles and

pharmacokinetic properties. This approach employs structural modifications guided by rational design and molecular modelling. These include but are not limited to conformationally restricted building blocks and peptide bond isosteres (see, for example, Vagner *et al*, 2008, "*Peptidomimetics, a synthetic tool of drug discovery*", Current Opinion in Chemical Biology, 12: 292-296).

Peptide nucleic acids (PNA) are similar to oligonucleotides and oligonucleotide analogs and can mimic DNA and RNA. The deoxyribose backbone of DNA encoding GSTZ is replaced in PNA by a pseudo-peptide backbone (see for example Nielsen *et al.*, Science, 1991, 254, 1475;). Each subunit or monomer has a naturally occurring or non-naturally occurring nucleobase attached to the backbone. One such backbone consists of repeating units of N-(2-aminoethyl)glycine linked through amide bonds. In the context of the present invention PNA hybridizes to complementary nucleic acids in the DNA encoding GSTZ through Watson and Crick base pairing and helix formation results. The Pseudo-peptide backbone provides superior hybridization properties and resistance to enzymatic degradation.

PNA binds both DNA and RNA to form PNA/DNA or PNA/RNA duplexes. PNA/DNA or PNA/RNA duplexes are bound with greater affinity than corresponding DNA/DNA or DNA/RNA duplexes, as determined by T_m . In addition to increased affinity, PNA has also been shown to hybridize to DNA with increased specificity compared to DNA/DNA duplexes. Furthermore, homopyrimidine PNA oligomers form extremely stable PNA₂-DNA triplexes with sequence-complementary targets in DNA or RNA oligomers. PNAs may also bind to double-stranded DNA or RNA by helix invasion.

One advantage of PNA compared to oligonucleotides is the nuclease and protease resistance of the PNA polyamide backbone. As PNA is not recognized by nucleases or proteases and it is not susceptible to cleavage. Consequently, PNAs are resistant to enzymatic degradation. In antisense applications, PNA bound to GSTZ mRNA or DNA may cause steric hindrance of DNA and RNA polymerases, reverse transcriptase, telomerase and ribosomes thus inhibiting expression of GSTZ.

Inhibition of GSTZ Interactions

Without being bound by any one mechanism it is postulated that GSTZ functions in

lipid homeostasis via a mechanism which involves control of GSTZ expression by PPAR γ , GSTZ in turn interacts with Fyn kinase and triggers the phosphorylation of LKB1 that prevents the phosphorylation of AMPK and acetyl-CoA carboxylase (ACC) that remains active and inhibits fatty acid oxidation and stimulates lipogenesis. In the absence of Fyn kinase or GSTZ the opposite occurs. Accordingly, inhibition of, or prevention of, the interaction of GSTZ with one or more molecules, compounds or cellular components, for example Fyn kinase is expected to prevent accumulation of adipose tissue in a subject. The terms molecules, compounds or cellular components in the context of GSTZ interactions refer to any proteins, lipids, nucleic acids, organelles, membrane constituents, metabolites, extracellular matrix and/or any combinations thereof.

The interactions of GSTZ with other cellular components may be inhibited by any means known in the art for example organic chemicals, peptides, peptide derivatives, peptidomimetics, inhibitory nucleic acids such as oligonucleotides, inhibitory RNA (siRNA or RNAi) or PNA (peptide nucleic acids). Further, the interaction of GSTZ with a cellular component may be inhibited or disrupted by antibodies or fragments thereof which bind GSTZ and/or the cellular component which it binds.

Compounds useful for inhibiting or disrupting the interaction of GSTZ with one or more molecules, compounds or cellular components include those compounds useful for inhibition of GSTZ *per se* and those that directly or indirectly inhibit the interaction of GSTZ with another cellular component. Such compounds include but are not limited to, polypeptides, peptides, peptidomimetics, antibodies, nucleic acids (e.g., RNA and DNA), organic or inorganic molecules as described above.

In one embodiment, peptides having an amino acid sequence corresponding to the portion of GSTZ that binds one or more molecules, compounds or cellular components may be used to compete with native GSTZ and therefore may be inhibit the interaction between GSTZ and a cellular component. Similarly, peptides having an amino acid sequence corresponding to the domain of the one or more molecules, compounds or cellular components that binds to GSTZ may be also used to inhibit the interaction of GSTZ with the one or more molecules, compounds or cellular components. Such peptides may be synthesized chemically or produced via recombinant DNA technology using methods well known in the art (e.g., Creighton, 1983, *Proteins: Structures and Molecular Principles*, W. H. Freeman & Co., N.Y., pp. 34-49;

Sambrook, et al., 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories Press, Cold Spring Harbor, N.Y.). In some embodiments, D-isomers of amino acids or amino acid analogs are included in the inhibitory peptide or polypeptide. Examples of amino acid analogs include α -amino isobutyric acid, 4-aminobutyric acid, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β -alanine, β -methyl amino acids, C α -methyl amino acids and N α -methyl amino acids. Further, in some embodiments inhibitory polypeptides or peptides can be chemically modified by any chemical modification techniques known to those of skill in the art. The modification may be by way of chemical cleavage, chemical modification, enzymatic cleavage, chemical modification or biological modification. Examples of chemical cleavage include cleavage by cyanogen bromide. Examples of enzymatic cleavage include cleavage by trypsin, chymotrypsin, papain or V8 protease. Examples of chemical modification include modifications such as alkylation, esterification or amidation. Examples of biological modifications include the modification of one or more amino acid by for example lipidoylation such as myristoylation, palmitoylation, farnesylation, steroylation or geranylation, glutionylation, glycation, glycosylation, phosphorylation, acetylation, acylation, methylation, hydroxylation, biotinylation or ubiquitinylation. Biological modification of inhibitory peptides of polypeptides may be performed in vivo or in vitro.

Antibodies or antigen binding fragments thereof may also be used to inhibit the interaction of GSTZ with one or more molecules, compounds or cellular components. For example, antibodies that are specific or selective for the binding domains of either or both of GSTZ or the one or more molecules, compounds or cellular components with which the GSTZ interacts may be used. Such antibodies may be generated using standard techniques against GSTZ or the one or more molecules, compounds or cellular components with which the GSTZ interacts or against peptides corresponding to the binding domains of GSTZ or the one or more molecules, compounds or cellular components with which the GSTZ interacts. In other embodiments, antibodies to GSTZ or the one or more molecules, compounds or cellular components with which the GSTZ interacts may be used to interactions of GSTZ with the one or more molecules, compounds or cellular components. Such antibodies include but are not limited to polyclonal, monoclonal, Fab fragments, single chain antibodies, chimeric

antibodies, etc.

In addition, antisense nucleic acids, RNAi, catalytic nucleic acids, competitor peptides and peptidomimetics and peptide nucleic acids may be used to disrupt a or inhibit the interaction of GSTZ with a cellular compound by inhibiting the expression GSTZ *per se*, as described above or by inhibiting the expression of the cellular component with which GSTZ interacts.

Screening for GSTZ inhibitors

The present invention provides methods of screening for a compound that modulates the activity of one or more glutathione transferases. In general these methods comprise contacting the glutathione transferase, typically GSTZ with a candidate compound under conditions suitable to enable or allow interaction of the candidate compound and the GSTZ and assaying for activity of the GSTZ.

GSTZ, and variants, fragments and derivatives are useful for the screening and identification of compounds and agents that interact with these molecules. In particular, desirable compounds are those that modulate the activity of these polypeptides. Such compounds may exert a modulatory effect by activating, stimulating, increasing, inhibiting or preventing expression or activity of GSTZ or variants, fragments or derivatives. Further, GSTZ and variants, fragments and derivatives thereof are useful for the screening and identification of compounds and agents that promote degradation of GSTZ. Suitable compounds may exert their effect by virtue of either a direct (for example binding) or indirect interaction. Alternatively or additionally suitable compounds may exert their effect by modulating the interaction of GSTZ, variants fragments and analogues thereof with other proteins or peptides.

Compounds which bind or otherwise interact with GSTZ, variants, fragments and derivatives, and specifically compounds which modulate their activity or promote their degradation, may be identified by a variety of suitable methods. Non limiting methods include the two-hybrid method, co-immunoprecipitation, immunological based detection methods such as western blotting, affinity purification, mass spectroscopy, tandem affinity purification, phage display, label transfer, DNA microarrays/gene coexpression and protein microarrays.

Further, the ability of a candidate compound that binds or otherwise interacts with GSTZ or variants, fragments and derivatives thereof and which modulates the activity or promote degradation of GSTZ may be assessed by the effect of those candidate compounds on the enzymatic activity of GSTZ or variants, fragments and derivatives thereof. Such enzymatic activity may be measured according to any method known in the art, for example using chlorfluoroacetic acid and malenylacetoacetone as described in Tong, Board and Anders (1998) *Biochem J* **331**, 371-374 and Tong, Board and Anders (1998) *Chem Research in Toxicology* **11**, 1332-1338. Another example of a method of measuring the enzymatic activity of GST zeta using substrates maleylacetone and BNPP (($\pm$)-2-bromo-3-(4-nitrophenyl)propionic acid) is described in Board, Taylor, Coggan, Parker, Lantunum and Anders. (2003) *Biochem J* **374**, 731-737. Alternatively or additionally the degradation or continued presence of GSTZ may be detected by immunological based detection methods such as western blotting, co-immunoprecipitation or by, for example affinity purification or mass spectroscopy.

The present invention also provides methods of screening for a compound that modulates the expression of one or more glutathione transferases, for example GSTZ. In such a method a compound or plurality of compounds would be contacted with a cell expressing GSTZ or a GSTZ promoter-reporter gene construct. After a period of time level of expression the GSTZ or the reporter gene product is determined.

A two-hybrid assay may be used to determine whether a candidate agent or plurality of candidate agents interacts or binds with GST zeta polypeptide of the invention or a variant, fragment or derivative thereof. The yeast two-hybrid assay system is a yeast-based genetic assay typically used for detecting protein-protein interactions (Fields and Song, *Nature* **340**: 245-246 (1989)). The assay makes use of the multi-domain nature of transcriptional activators. For example, the DNA-binding domain of a known transcriptional activator may be fused to a polypeptide of the invention or a variant, fragment or derivative thereof, and the activation domain of the transcriptional activator fused to the candidate agent. Interaction between the candidate agent and the polypeptide of the invention or a variant or derivative thereof, will bring the DNA-binding and activation domains of the transcriptional activator into close proximity. Subsequent transcription of a specific reporter gene activated by the

transcriptional activator allows the detection of an interaction.

In a modification of the technique above, a fusion protein may be constructed by fusing the polypeptide of the invention or a variant, fragment or derivative thereof to a detectable tag, for example alkaline phosphatase, and using a modified form of immunoprecipitation as described by Flanagan and Leder (Flanagan and Leder, *Cell* 63:185-194 (1990)).

Alternatively, co-immunoprecipitation may be used to determine whether a candidate agent or plurality of candidate agents interacts or binds with GSTZ or variants, fragments derivatives thereof. Using this technique a solution or suspension of candidate agents may be incubated a GSTZ or a variant, fragment or derivative thereof and an antibody specific for GSTZ, variant fragment or derivative thereof and immunoprecipitated from the solution, for example by capture with an antibody-binding protein attached to a solid support. Immunoprecipitation of the GSTZ or a variant, fragment or derivative thereof by this method facilitates the co-immunoprecipitation of an agent associated with that protein. The identification an associated agent can be established using a number of methods known in the art, including but not limited to SDS-PAGE, western blotting and mass spectrometry.

Alternatively, the phage display method may be used to determine whether a candidate agent or plurality of candidate agents interacts or binds with a polypeptide of the invention or a variant, fragment or derivative thereof. Phage display is a test to screen for protein interactions by integrating multiple genes from a gene bank into phage. Under this method, recombinant DNA techniques are used to express numerous genes as fusions with the coat protein of a bacteriophage such the peptide or protein product of each gene is displayed on the surface of the viral particle. A whole library of phage-displayed peptides or protein products of interest can be produced in this way. The resulting libraries of phage-displayed peptides or protein products may then be screened for the ability to bind GSTZ or a variant, fragment or derivative thereof. DNA extracted from interacting phage contains the sequences of interacting proteins.

Alternatively, affinity chromatography may be used to determine whether a candidate agent or plurality of candidate agents interacts or binds with GSTZ or a variant, fragment or derivative thereof. For example, GSTZ or variants, fragments and derivatives thereof, may be immobilised on a support (such as sepharose) and cell lysates passed over the column. Proteins or other compounds binding to the immobilised GSTZ polypeptide or a variant,

fragment or derivative thereof may then be eluted from the column and identified, for example by N-terminal amino acid sequencing or sequencing by mass spectrometry.

In this way, proteins or other compounds that do not directly interact with GSTZ or variants fragments or derivatives thereof may be identified. For example, a compound that interacts directly with GSTZ may in turn be associated with an agent that may modulate GSTZ activity without direct interaction with GSTZ. The interaction of the complex of the compound and associate agent with the immobilised GSTZ polypeptide or a variant, fragment or derivative thereof will facilitate identification of compounds that may modulate GSTZ activity and thus lipid homeostasis without direct interaction with GSTZ.

Potential modulators of the activity of GSTZ or variants, fragments or derivatives thereof may be generated for screening by the above methods by a number of techniques known to those skilled in the art. For example, methods such as X-ray crystallography and nuclear magnetic resonance spectroscopy may be used to model the structure of GSTZ or variants, fragments or derivatives thereof, thus facilitating the design of potential modulating agents using computer-based modeling. Various forms of combinatorial chemistry may also be used to generate putative modulators.

GSTZ or, variants, fragments or derivatives thereof can be used in high-throughput screens to assay candidate compounds for the ability to bind to, or otherwise interact therewith. These candidate compounds can be further screened against functional GSTZ or variants, fragments or derivatives thereof to determine the effect of the compound on enzyme activity.

The present invention also contemplates compounds which may exert their modulatory effect on polypeptides of the invention by altering expression of the polypeptide. In this case, such compounds may be identified by comparing the level of expression of the polypeptide in the presence of a candidate compound with the level of expression in the absence of the candidate compound.

It will be appreciated that the methods described above are merely examples of the types of methods that may be utilised to identify agents that are capable of interacting with, or modulating the activity of GSTZ or variants, fragments or derivatives thereof. Other suitable methods, such as surface plasmon resonance, fluorescence resonance electron transfer

(FRET), isothermal titration calorimetry (ITC), saturation transfer NMR and inhibition of cross linking will be known by persons skilled in the art and are within the scope of this invention.

Screening for inhibitors of GSTZ interactions

The present invention provides methods of screening for a compound that disrupts or inhibits the interaction of GSTZ with one or more molecules, compounds or cellular components. In general these methods comprise contacting the GSTZ with at one or more molecules, compounds or cellular components known to interact with GSTZ under conditions suitable to enable or allow interaction of the GSTZ with the one or more molecules, compounds or cellular components in the presence of a compound putatively able to disrupt or inhibit the interaction between the GSTZ and the one or more molecules, compounds or cellular components. The presence, absence or extent of an interaction between GSTZ and the one or more molecules, compounds or cellular components is then determined by a suitable assay. In some embodiments this may involve quantitatively or qualitatively measuring the amount of a complex of GSTZ and the one or more molecules, compounds or cellular components either directly or indirectly.

GSTZ and variants fragments and derivatives thereof are useful for the screening and identification of compounds that disrupt or inhibit the interaction of these molecules with one or more cellular components. The agents may exert a modulatory effect by activating, stimulating, increasing, inhibiting or preventing the formation or activity of a complex between GSTZ or variants, fragments or derivatives thereof and a cellular component. Further, GSTZ or variants, fragments and derivatives thereof are useful for the screening and identification of compounds that promote degradation of a complex of GSTZ and one or more molecules, compounds or cellular components. Suitable agents may exert their effect by virtue of either a direct interaction (for example binding of one either GSTZ or the one or more molecules, compounds or cellular components) or indirect interaction.

Agents which bind or otherwise interact with the one or more molecules, compounds or cellular components, GSTZ or variants, fragments or derivatives thereof, and specifically compounds which modulate the activity or promote the degradation of the one or more molecules, compounds or cellular components or GSTZ, may be identified by a variety of

suitable methods. Non limiting methods include measuring the inhibition or disruption of an interaction in a two-hybrid assay, a co-immunoprecipitation, and immunological based detection methods such as western blotting for the presence or absence of a complex of GSTZ with one or more molecules, compounds or cellular components, affinity purification of such a complex, mass spectroscopy, affinity purification and phage display.

A two-hybrid assay may be used to determine whether a candidate compound or plurality of candidate compounds inhibits or disrupts the interaction of GSTZ or a variant, fragment or derivative thereof with one or more molecules, compounds or cellular components. The yeast two-hybrid assay system is described above and in a modification of that technique, the DNA-binding domain of a known transcriptional activator may be fused to GSTZ or a variant, fragment or derivative thereof, and the activation domain of the transcriptional activator fused to the one or more molecules, compounds or cellular components which interacts with the GSTZ or a variant, fragment or derivative thereof. Interaction between the GSTZ or a variant, fragment or derivative thereof and the one or more molecules, compounds or cellular components will bring the DNA-binding and activation domains of the transcriptional activator into close proximity. Subsequent transcription of a specific reporter gene activated by the transcriptional activator allows the detection of an interaction. Therefore, the expression of the reporter gene and/or the activity of the reporter protein encoded by that reporter gene is indicative of the interaction between the GSTZ or a variant, fragment or derivative thereof and the one or more molecules, compounds or cellular components. In the presence of a compound that inhibits or disrupts the interaction, the level of transcription of the reporter gene and/or activity of its corresponding protein is altered. In some embodiments the level of transcription of the reporter gene and/or activity of its corresponding protein is reduced in the presence of a compound that inhibits or disrupts the interaction between the GSTZ or a variant, fragment or derivative thereof and the cellular component compared the level in the absence of that compound. In other embodiments the level of transcription of the reporter gene and/or activity of its corresponding protein is not detectable in the presence of a compound that inhibits or disrupts the interaction between the GSTZ or a variant, fragment or derivative thereof and the one or more molecules, compounds or cellular components compared to the

level in the absence of that compound.

Alternatively, co-immunoprecipitation may be used to determine whether a candidate compound or plurality of candidate compounds inhibits or disrupts an interaction between GSTZ or variants or fragments thereof and a one or more molecules, compounds or cellular components. Using this technique a solution or suspension of at least one candidate compound may be incubated a GSTZ or a variant, fragment or derivative thereof, the one or more molecules, compounds or cellular components and an antibody specific for the GSTZ or a variant, fragment or derivative thereof and the complex (of the GSTZ or variant, fragment or derivative thereof and the one or more molecules, compounds or cellular components) can immunoprecipitated from the solution, for example by capture with an antibody-binding protein attached to a solid support. Immunoprecipitation of the complex by this method facilitates the co-immunoprecipitation of the cellular component that interacts with the GSTZ or a variant, fragment or derivative thereof. The presence of the one or more molecules, compounds or cellular components can be established using a number of methods known in the art, including but not limited to SDS-PAGE, western blotting and mass spectrometry. When the candidate compound is one that inhibits or disrupts the interaction of the GSTZ or a variant, fragment or derivative thereof with the one or more molecules, compounds or cellular components immunoprecipitation using an antibody specific for the GSTZ or a variant, fragment or derivative thereof will result in the absence or reduced amount of co-precipitated cellular component.

Alternatively, the phage display method may be used to determine whether a candidate compound or plurality of candidate compounds inhibits or disrupts the interaction of GSTZ or a variant, fragment or derivative thereof with one or more molecules, compounds or cellular components. In the context of screening for compounds that disrupt or inhibit an interaction phage display is used to screen for protein interactions by integrating a gene into a phage. Using this method, recombinant DNA techniques are used to express nucleic acids coding for e.g. either the GSTZ or a variant, fragment or derivative thereof or the one or more molecules, compounds or cellular components with the coat protein of a bacteriophage such the peptide or protein product of the nucleic acid is displayed on the surface of the viral particle. The phage-displayed GSTZ or variant, fragment or derivative thereof or one or more molecules, compounds or cellular components, may then be contacted with the cellular

component or the GSTZ or a variant, fragment or derivative thereof, respectively in the presence of a candidate compound. The phage or GSTZ or variant, fragment or derivative thereof or one or more molecules, compounds or cellular components may be bound to a solid support. Thus, under conditions that facilitate or allow interaction of the phage-displayed GSTZ or a variant, fragment or derivative thereof, the phage-displayed cellular component can be detected on the solid support by any means known in the art, for example by an immunoassay. In the presence of a candidate compound which inhibits or disrupts the interaction the phage will not be detectable or be detectable at a lower level compared to the absence of the candidate compound.

Affinity chromatography may be used to determine whether a candidate compound or plurality of candidate compounds inhibits or disrupts the interaction of GSTZ or a variant, fragment or derivative thereof with one or more molecules, compounds or cellular components. For example, a complex of the one or more molecules, compounds or cellular components and the GSTZ or a variant, fragment or derivative thereof, may be immobilised on a support (such as sepharose) either via the GSTZ portion of the complex or via the one or more molecules, compounds or cellular components of the complex. Solutions of a candidate compound or a plurality of candidate compounds may then incubated with the immobilized complex. In the presence of a compound that inhibits or disrupts the interaction the portion of the complex not immobilized to the support may be partially or completely eluted and identified by any means known in the art, for example by enzyme assay, immunological methods, or by mass spectrometry.

Metabolic conditions

As exemplified herein loss of GSTZ inhibits weight gain of mice fed a high fat diet (Figures 1- 5). Thus inhibition of GSTZ, in particular GSTZ1-1 is useful in the treatment of metabolic conditions which may be characterised by excess adipose tissue.

Of particular note amongst such conditions is obesity. The World Health Organization (WHO) defines "obesity" as a body mass index (BMI - weight in kilograms divided by the square of the height in meters (kg/m^2) equal to or more than 30. Obesity is a pathologic disorder resulting from excess fat accumulation in various tissues and is associated with a high incidence of other conditions including adverse psychological development,

reproductive disorders such as polycystic ovarian disease, dermatological disorders such as infections, varicose veins, Acanthosis nigricans, eczema, exercise intolerance, diabetes mellitus, insulin resistance, hypertension, hypercholesterolemia, cholelithiasis, osteoarthritis, orthopedic injury, thromboembolic disease, cancer and coronary heart disease. Other conditions include excess adipose tissue in individuals that do not meet the definition of obese.

Another metabolic condition which may be treated by GSTZ inhibition is insulin resistance or glucose intolerance which is a condition characterized by the inability to properly use insulin or blood sugar. Insulin resistance is often associated with obesity. In insulin resistance, normal amounts of insulin do not produce a normal insulin response from fat, muscle or liver cells. Insulin resistance in fat cells results in hydrolysis of stored triglycerides, which elevates free fatty acids in the blood plasma. Insulin resistance in muscle reduces glucose uptake whereas in liver it reduces glucose storage, both of which serve to elevate blood glucose. High plasma levels of insulin and glucose due to insulin resistance lead to obesity, atherogenic dyslipidemia, elevated blood pressure, prothrombotic state (e.g., high fibrinogen or plasminogen activator inhibitor-1 in the blood) and proinflammatory state (e.g. elevated C-reactive protein in the blood).

As exemplified herein loss or inhibition of GSTZ prevents ameliorates insulin resistance (Figure 5), thus compounds that inhibit or promote degradation of GSTZ can be used to treat or prevent insulin resistance. For subjects with impaired glucose tolerance which may border between healthy subjects and Type II diabetics, inhibition of GSTZ may be useful to prevent the onset of Type II diabetes. Thus inhibition of GSTZ either by inhibition of enzymatic activity, promotion of degradation or inhibition of expression is useful in the treatment or prevention of Type II Diabetes.

Insulin resistance is typically compensated for by increased insulin secretion. However, when the limit of insulin secretion increase is reached, a relative insulin deficiency arises and a concomitant blood sugar level increase is seen. Onset and progression Type II diabetes (also known as non-insulin dependent diabetes) is caused primarily by impaired insulin secretion and progressive increase in insulin resistance.

Another metabolic condition associated with insulin resistance and obesity is metabolic syndrome which is viewed as a cluster of risk factors where insulin resistance plays the role

of the underlying pathophysiological defect. Clinical identification of metabolic syndrome is based on measures of abdominal obesity, atherogenic dyslipidemia, raised blood pressure and glucose intolerance. Metabolic syndrome is viewed as a precursor to type II diabetes and a strong risk factor for coronary heart disease, and stroke. Schulze M B and Frank B H. *Diabetes Care* 27:613-614, 2004.

Metabolic syndrome has been defined by the World Health Organization in 1998 as including insulin resistance, abdominal obesity, elevated blood pressure, and lipid abnormalities (i.e., elevated levels of triglycerides and low levels of high-density lipoprotein [HDL] cholesterol). Diagnostic criteria for metabolic syndrome include insulin resistance plus two of the following components: abdominal/central obesity, hypertriglyceridemia, low HDL cholesterol, high blood pressure, high fasting glucose, and microalbuminuria. Alberti K G, Zimmet P Z. *Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus, provisional report of a WHO consultation. Diabet Med* 1998; 15:539-53.

As exemplified herein loss of GSTZ, or pharmacological inhibition of GSTZ expression or activity inhibits weight gain of mice fed a high fat diet (Figures 3 to 8). Pharmacological inhibition of GSTZ in normal BALBc mice significantly reduces body fat thus inactivation of GSTZ alters lipid homeostasis. In addition, inhibition or loss of GSTZ ameliorates insulin resistance (Figure 5). Thus inhibition of GSTZ in particular GSTZ1-1, is also useful in the treatment of metabolic syndrome. Further, with reference to Example 6, loss of GSTZ in mice increases the metabolic rate compared to normal mice. Thus inhibition of GSTZ in particular GSTZ1-1 is useful for increasing the metabolic rate of a subject. A deficiency of active GSTZ may be generated by treatment of a subject with DCA or an organic compound as described herein. Alternatively or in addition a deficiency of active GSTZ may occur if the interaction between GSTZ and fyn kinase is blocked.

Pharmaceutical compositions and routes of administration

The pharmaceutical compositions of the present invention may be administered therapeutically. In a therapeutic application, the compositions may be administered to a patient already suffering from a metabolic disease or condition in an amount sufficient to cure, or at least partially arrest the disease or condition and its complications. Single or

multiple administrations of the pharmaceutical compositions can be carried out with dose levels and pattern being selected by the treating physician.

The therapeutically effective dose level for any particular patient will depend upon a variety of factors including: the severity of the metabolic disease or condition, the composition employed, the age, body weight, general health and diet of the patient, the time of administration, the route of administration, the duration of the treatment, drugs used in combination or coincidental with the composition, together with other related factors well known in medicine.

One skilled in the art would, by routine experimentation, be able to determine an effective, non-toxic amount of this treatment regime which would be required to treat the metabolic condition with the synergistic combination of the present invention.

In the methods of the invention, the amounts of the inhibitors administered may be in the range of about 0.0001 mg to about 1000 mg per kg body weight; for example, about 0.001 mg to about 750 mg per kg body weight; about 0.01 mg to about 500 mg per kg body weight; about 0.1 mg to about 500 mg per kg body weight; about 0.1 mg to about 250 mg per kg body weight; or about 1.0 mg to about 250 mg per kg body weight. More suitably, an effective dosage per 24 hours may be in the range of about 1.0 mg to about 200 mg per kg body weight; about 1.0 mg to about 100 mg per kg body weight; about 1.0 mg to about 50 mg per kg body weight; about 1.0 mg to about 25 mg per kg body weight; about 5.0 mg to about 50 mg per kg body weight; about 5.0 mg to about 20 mg per kg body weight; or about 5.0 mg to about 15 mg per kg body weight.

Further, it will be apparent to one of ordinary skill in the art that the optimal quantity and spacing of individual dosages of the compositions of the present invention will be determined by the nature and extent of the metabolic disease or condition, the form, route and site of administration, and the nature of the particular patient being treated. Also, such optimum conditions can be determined by conventional techniques.

It will also be apparent to one of ordinary skill in the art that the optimal course of treatment, such as the number of doses of the composition of the present invention given per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

In general, pharmaceutical compositions of the present invention may be prepared

according to methods which are known to those of ordinary skill in the art, and accordingly may include a pharmaceutically acceptable carrier, diluent and/or adjuvant.

The compositions may be administered by standard routes. In general, the compositions may be administered by the parenteral (e.g., intravenous, intraspinal, subcutaneous or intramuscular), oral or topical route. Administration may be systemic, regional or local. The compositions are preferably administered by the oral route.

The route of administration to be used in any given circumstance will depend on a number of factors, including the severity and extent of the metabolic disease or condition, the required dosage, the compounds being delivered and any potential side effects of the compounds.

The carriers, diluents and adjuvants must be "acceptable" in terms of being compatible with the other components of the composition, and not deleterious to the recipient thereof. Examples of pharmaceutically acceptable carriers or diluents are demineralised or distilled water, saline solution, vegetable based oils such as peanut oil, safflower oil; olive oil, cottonseed oil, maize oil, sesame oils such as peanut oil, safflower oil, olive oil, cottonseed oil, maize oil, sesame oil, arachis oil or coconut oil; silicone oils, including polysiloxanes, such as methyl polysiloxane, phenyl polysiloxane and methylphenyl polysiloxane; volatile silicones, mineral oils such as liquid paraffin, soft paraffin or squalane; cellulose derivatives such as methyl cellulose, ethyl cellulose, carboxymethylcellulose, sodium carboxymethylcellulose, or hydroxypropylmethylcellulose, lower alkanols, for example ethanol or iso-propanol; lower aralkanols, lower polyalkylene glycols or lower alkylene glycols, for example polyethylene glycol, polypropylene glycol, ethylene glycol, propylene glycol, 1,3-butylene glycol or glycerine, fatty acid esters such as isopropyl palmitate, isopropyl myristate or ethyl oleate, polyvinylpyrrolidone, agar, carrageenan; gum tragacanth or gum acacia, and petroleum jelly. The carrier or carriers may form from between 10% to 99.9% by weight of the compositions.

The pharmaceutical compositions of the invention may be in the form of a composition in a form suitable for administration by oral ingestion (such as capsules, tablets, caplets and elixirs, for example), in the form of an ointment, cream or lotion suitable for topical administration, in a form suitable for delivery as an eye drop, in an aerosol form suitable for administration by inhalation, such as by intranasal inhalation or oral inhalation, in a form

suitable for parenteral administration, that is subcutaneous, intramuscular or intravenous injection.

For administration *as* an injectable solution or suspension, non-toxic parenterally acceptable diluents or carriers can include Ringer's solution, isotonic saline, phosphate buffered saline, ethanol and 1,2-propylene glycol.

Some examples of suitable carriers, diluents, excipients and adjuvants for oral use include peanut oil, liquid paraffin, sodium carboxymethylcellulose, methylcellulose, sodium alginate, gum acacia, gum tragacanth, dextrose, sucrose, sorbitol, mannitol, gelatine and lecithin. In addition, these oral formulations may contain suitable flavouring and colourings agents. When used in capsule form the capsules may be coated with compounds such as glyceryl monostearate or glyceryl distearate which delay disintegration.

Adjuvants typically include emollients, emulsifiers, thickening agents, preservatives, bactericides and buffering agents.

Solid forms for oral administration may contain binders acceptable in human and veterinary pharmaceutical practice, sweeteners, disintegrating agents, diluents, flavourings, coating agents, preservatives, lubricants and/or time delay agents. Suitable binders include gum acacia, gelatine, corn starch, gum tragacanth, sodium alginate, carboxymethylcellulose or polyethylene glycol. Suitable sweeteners include sucrose, lactose, glucose, aspartame or saccharine. Suitable disintegrating agents include corn starch, Methylcellulose, polyvinylpyrrolidone, guar gum, xanthan gum, bentonite, alginic acid or agar. Suitable diluents include lactose, sorbitol, mannitol, dextrose, kaolin, cellulose, calcium carbonate, calcium silicate or dicalcium phosphate. Suitable flavouring agents include peppermint oil, oil of wintergreen, cherry, orange or raspberry flavouring. Suitable coating agents include polymers or copolymers of acrylic acid and/or methacrylic acid and/or their esters; waxes, fatty alcohols, zein, shellac or gluten. Suitable preservatives include sodium benzoate, vitamin E, alpha-tocopherol, ascorbic acid, methyl paraben, propyl paraben or sodium bisulphite. Suitable lubricants include magnesium stearate, stearic acid, sodium oleate, sodium chloride or talc. Suitable time delay agents include glyceryl monostearate or glyceryl distearate.

Liquid forms for oral administration may contain, in addition to the above agents, a liquid carrier. Suitable liquid carriers include water, oils such as olive oil, peanut oil, sesame

oil, sunflower oil, safflower oil, arachis oil, coconut oil, liquid paraffin, ethylene glycol, propylene glycol, polyethylene glycol, ethanol, propanol, isopropanol, glycerol, fatty alcohols, triglycerides or mixtures thereof.

Suspensions for oral administration may further comprise dispersing agents and/or suspending agents. Suitable suspending agents include sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethyl-cellulose, poly-vinyl-pyrrolidone, sodium alginate or acetyl alcohol. Suitable dispersing agents include lecithin, polyoxyethylene esters of fatty acids such as stearic acid, polyoxyethylene sorbitol mono- or di-oleate, -stearate or -laurate, polyoxyethylene sorbitan mono- or di-oleate, -stearate or -laurate and the like.

Pharmaceutical compositions of the present invention may be prepared by blending, grinding, homogenising, suspending, dissolving, emulsifying, dispersing and/or mixing the selected inhibitors with the selected excipient(s), carrier(s), adjuvant(s) and/or diluent(s).

One type of pharmaceutical composition of the present invention in the form of a tablet or capsule may be prepared by (a) preparing a first tablet or a capsule comprising a first inhibitor, together with any desired excipient(s), carrier(s), adjuvant(s) and/or diluent(s), and (b) preparing a second tablet or a capsule, wherein the second tablet or the capsule includes a second inhibitor and the first tablet or capsule.

Another type of pharmaceutical composition of the present invention in the form of a capsule may be prepared by (a) preparing a first capsule comprising a first inhibitor together with any desired excipient(s), carrier(s), adjuvant(s) and/or diluent(s), and (b) preparing a second capsule, wherein the second capsule includes a second inhibitor and the first capsule.

A further type of pharmaceutical composition of the present invention in the form of a tablet may be prepared by (a) preparing a capsule comprising an inhibitor together with any desired excipient(s), carrier(s), adjuvant(s) and/or diluent(s), and (b) preparing a tablet, wherein the tablet includes the second inhibitor and the capsule.

The emulsions for oral administration may further comprise one or more emulsifying agents. Suitable emulsifying agents include dispersing agents as exemplified above or natural gums such as guar gum, gum acacia or gum tragacanth.

Methods for preparing parenterally administrable compositions are apparent to those skilled in the art, and are described in more detail in, for example, Remington's

Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pa., hereby incorporated by reference herein.

The topical compositions of the present invention, comprise an inhibitor(s) together with one or more acceptable carriers, and optionally any other therapeutic ingredients. Compositions suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin to the site of where treatment is required, such as liniments, lotions, creams, ointments or pastes, and drops suitable for administration to the eye, ear or nose.

Drops according to the present invention may comprise sterile aqueous or oily solutions or suspensions. These may be prepared by dissolving the inhibitor(s) in an aqueous solution of a bactericidal and/or fungicidal agent and/or any other suitable preservative, and optionally including a surface active agent. The resulting solution may then be clarified by filtration, transferred to a suitable container and sterilised. Sterilisation may be achieved by: autoclaving or maintaining at 90°C-100°C for half an hour, or by filtration, followed by transfer to a container by an aseptic technique. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

Lotions according to the present invention include those suitable for application to the skin or eye. An eye lotion may comprise a sterile aqueous solution optionally containing a bactericide and may be prepared by methods similar to those described above in relation to the preparation of drops. Lotions or liniments for application to the skin may also include an agent to hasten drying and to cool the skin, such as an alcohol or acetone, and/or a moisturiser such as glycerol, or oil such as castor oil or arachis oil.

Creams, ointments or pastes according to the present invention are semi-solid formulations of the inhibitor(s) for external application. They may be made by mixing the active ingredient in finely-divided or powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid, with a greasy or non-greasy basis. The basis may comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic soap; a mucilage; an oil of natural origin such as almond, corn, arachis, castor or olive oil; wool fat or its derivatives, or a fatty acid such as stearic or oleic acid together with an alcohol such as

propylene glycol or macrogols.

The compositions may incorporate any suitable surfactant such as an anionic, cationic or non-ionic surfactant such as sorbitan esters or polyoxyethylene derivatives thereof. Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as siliceous silicas and other ingredients such as lanolin may also be included.

The compositions may also be administered or delivered to target cells in the form of liposomes. Liposomes are generally derived from phospholipids or other lipid substances, and are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Specific examples of liposomes used in administering or delivering a composition to target cells are synthetic cholesterol (Sigma), the phospholipid 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC; Avanti Polar Lipids), the PEG lipid 3-*N*-[(-methoxy poly(ethylene glycol)2000)carbamoyl]-1,2-dimyrestyloxypropylamine (PEG-cDMA) and the cationic lipid 1,2-di-*o*-octadecenyl-3-(*N,N*-dimethyl)aminopropane (DODMA) or 1,2-dilinoleyloxy-3-(*N,N*-dimethylaminopropane (DLinDMA) in the molar ratios 55:20:10:15 or 48:20:2:30, respectively, PEG-cDMA, DODMA and DLinDMA. Any non-toxic, physiologically acceptable and metabolisable lipid capable of forming liposomes can be used. The compositions in liposome form may contain stabilisers, preservatives, excipients and the like. The preferred lipids are the phospholipids and the phosphatidyl cholines (lecithins), both natural and synthetic. Methods to form liposomes are known in the art, and in relation to this specific reference is made to: Prescott, Ed., *Methods in Cell Biology*, Volume XIV, Academic Press, New York, N.Y. (1976), p. 33 *et seq.*, the contents of which is incorporated herein by reference.

The compositions may also be administered in the form of microparticles. Biodegradable microparticles formed from polylactide (PLA), polylactide-co-glycolide (PLGA), and epsilon-caprolactone (ϵ -caprolactone) have been extensively used as drug carriers to increase plasma half life and thereby prolong efficacy (R. Kumar, M., 2000, *J Pharm Pharmaceut Sci.* **3**(2) 234-258). Microparticles have been formulated for the delivery of a range of drug candidates including vaccines, antibiotics and DNA. Moreover, these formulations have been developed for various delivery routes including parenteral subcutaneous injection, intravenous injection and inhalation.

The compositions may incorporate a controlled release matrix that is composed of sucrose acetate isobutyrate (SAIB) and organic solvent or organic solvents mixture. Polymer additives may be added to the vehicle as a release modifier to further increase the viscosity and slow down the release rate. SAIB is a well known food additive. It is a very hydrophobic, fully esterified sucrose derivative, at a nominal ratio of six isobutyrate to two acetate groups. As a mixed ester, SAIB does not crystallize but exists as a clear viscous liquid. Mixing SAIB with a pharmaceutically accepted organic solvent such as ethanol or benzyl alcohol decreases the viscosity of the mixture sufficiently to allow for injection. An inhibitor(s) may be added to the SAIB delivery vehicle to form SAIB solution or suspension formulations. When the formulation is injected subcutaneously, the solvent diffuses from the matrix allowing the SAIB-drug or SAIB-drug-polymer mixtures to set up as an in situ forming depot.

Those skilled in the art will appreciate that in accordance with the methods of the present invention the compositions may be administered alone or in conjunction with one or more additional agents as a combination therapy. For example, a composition of the invention may be administered together with one or more additional agents suitable for the treatment of metabolic diseases or conditions.

For such combination therapies, each component of the combination therapy may be administered at the same time, or sequentially in any order, or at different times, so as to provide the desired effect. Alternatively, the components may be formulated together in a single dosage unit as a combination product. When administered separately, it may be preferred for the components to be administered by the same route of administration, although it is not necessary for this to be so.

Combination regimens

Therapeutic advantages may be realized through combination regimens. In combination therapy the respective inhibitors and any other agents may be administered simultaneously or sequentially in any order. Accordingly, methods of treatment according to the present invention may be applied in conjunction with conventional therapy, such as anti-obesity medications. Examples of anti-obesity agents include central nervous system agents that affect neurotransmitters or neural ion channels such as antidepressants (e.g. bupropion),

noradrenaline reuptake inhibitors (e.g. GW320659), selective 5-hydroxytryptamine 2c receptor agonists, antiseizure agents (e.g. topiramate, zonisamide), dopamine antagonists, cannabinoid-1 receptor antagonists, (e.g. rimonabant). Other anti-obesity agents include leptin/insulin/central nervous system pathway agents such as leptin analogues, leptin transport and/or receptor promoters, ciliary neurotrophic factor (e.g. axokine), neuropeptide Y antagonists, agouti-related peptide antagonists, proopiomelanocortin promoters, cocaine and amphetamine regulated transcript promoters, alpha melanocyte-stimulating hormone analogues, melanocortin-4 receptor agonists. Anti-obesity agents may also include agents that affect insulin metabolism and/or activity such as protein-tyrosine phosphatase-1B inhibitors, peroxisome proliferator activated receptors γ receptor antagonists, short-acting bromocriptine (e.g. ergoset), somatostatin agonists (e.g. octreotide), and adiponectin or adipocyte complement-related protein of 30kDa (Actp30, e.g. Famoxin). Further examples of anti-obesity agents include gastrointestinal-neural pathway agents including agents that increase Cholecystikinin-A and Protein YY3-36 activity. Agents that increase GLP-1 activity (e.g. extendin 4, liraglutide, DPP IV inhibitor) and agents that decrease ghrelin activity e.g. Amylin (pramlinitide) may also be used as anti-obesity agents. Further examples of anti-obesity agents include selective 13-3 stimulators or agonists, uncoupling protein homologues, thyroid receptor agonists, melanin concentrating hormone antagonists, phytostanol analogues, amylase inhibitors, growth hormone fragments, synthetic analogues of dehydroepiandrosterone sulfate (e.g. fluasterone), antagonists of adipocyte 11 β -hydroxysteroid dehydrogenase type 1 activity, corticotrophin releasing hormone agonists, carboxypeptidase inhibitors, inhibitors of fatty acid synthesis (e.g. cerulenin and C75), indanones, indanols, aminosterols (e.g. trodusquemine, trodulamine), gastrointestinal lipase inhibitors (e.g. ATL962).

The invention will now be described with reference to specific examples, which should not be construed as in any way limiting the scope of the invention.

Examples

Example 1: Experimental Details

The experiments described in the following Examples were carried out with wild type male BALB/c and male BALB/c GSTZ1^{-/-} mice. The high fat diet was obtained from Speciality Feeds in Western Australia and contained 23% fat. Mice were weighed weekly.

Glucose tolerance tests were undertaken by injecting glucose *ip* at 250mg/kg and determining blood glucose at 15 minute intervals. Blood glucose was determined by the use of Roche Accucheek strips.

Total body fat and lean composition was measured by dual-energy x-ray absorptiometry (DEXA) scanning.

Mice treated with dichloroacetic acid (DCA) received 1.5mg/ml (DCA) in drinking water.

Example 2: Fat Deposition

Wild type and GSTZ1^{-/-} mice were fed normal chow for five weeks. Figure 6 shows that the wild type mice gained weight at a rate the same as that of the GSTZ1^{-/-} mice (no statistically significant difference) over that period. For the following 5 weeks the mice were fed a high fat (23% fat) diet. After 5 weeks on the high fat diet the wild type mice weighed significantly more than the GSTZ1^{-/-} mice (Figure 6). Postmortem examination of the mice after 20 weeks (Figure 7A) revealed that wild type mice had accumulated extensive fat deposits. In contrast the GSTZ1 deficient mice (GSTZ1^{-/-}) presented little evidence of fat deposition. GSTZ^{-/-} mice have significantly smaller adipocytes when compared with wild type controls (Figure 7B), a phenotype that is associated with favorable metabolic parameters.

Example 3: Glucose Tolerance

Glucose tolerance tests on both wild-type and GSTZ1^{-/-} mice were performed after the mice had consumed the high fat diet for 5 weeks. Figure 3 shows that the wild type mice developed insulin resistance. Glucose levels in wild type mice peaked 15 minutes after the glucose injection and remained above the initial levels after 60 minutes. In contrast the GSTZ1^{-/-} mice showed a rapid response and after peaking at 15 minutes blood glucose rapidly declined after 30 minutes and returned to pre injection levels after 45 minutes.

Example 4: Pharmacological Inactivation of GSTZ1-1

The GSTZ1-1 substrate dichloroacetic acid (DCA) can either be dechlorinated to glyoxylate or act as a mechanism-based inactivator (Figure 4, reproduced from H.F. Tzeng, A.C. Blackburn, P.G. Board, and M.W. Anders, Polymorphism- and species- dependent

inactivation of glutathione transferase zeta by dichloroacetate. *Chem Res Toxicol* 13 (2000) 231-6.) (Anderson, *et al.* (1999) *Chem Res. Toxicol.* 12: 1144-1149)

Wild type mice were treated with DCA in drinking water at the rate of 1.5mg/ml. This achieves a dose of about 180mg/kg/day for 8 days. Mice were euthanised and GSTZ1-1 levels from liver investigated by Western blot and enzymatic assay. Inactivated GSTZ1-1 enzyme appears to be degraded and removed from liver tissue and adipose tissue (Figure 4A) as western blots show a significant reduction in GSTZ1-1 protein in the liver and adipose tissue of mice treated with DCA. Similar results (not shown) were obtained by the addition of 250mg DCA/kg body weight for 5 days.

Extracts of mouse liver from wild-type mice treated with DCA were assayed for GSTZ1-1 activity using chlorfluoroacetic acid as a substrate. The assay method is described in Tong, Board and Anders (1998) *Biochem, J*, 331, 371-374 and Tong, Board and Anders (1998) *Chem Research in Toxicology* 11, 1332-1338).

Extracts of mouse liver from wild-type mice that were not treated with DCA were also assayed. Figure 4B shows that DCA treatment significantly reduces GSTZ1-1 activity.

Normal BALBc mice were fed normal chow or a high fat diet and provided with plain water or water containing DCA at a rate of 1.5mg/ml. After 8 weeks total body fat was significantly lower in mice receiving DCA (Figure 8). The total lean mass was not significantly different.

Example 5: Interaction of GSTZ1-1 with Fyn Kinase

The physical association between Fyn and GSTZ1-1 was demonstrated in a co-immunoprecipitation experiment where Flag-tagged Fyn and HA-tagged GSTZ1 were co-transfected into HEK293T cells. As shown in Figure 9, lane 6, Fyn was co-immunoprecipitated by antiserum to HA-GSTZ1.

Immunoprecipitation was carried out in HEK293T cells that were transfected with plasmids which express GSTZ1 with a HA-tag (GSTZ-HA) and Fyn with a FLAG-tag (Fyn-FLAG). Immunoprecipitation was undertaken with anti-HA and the precipitates were fractionated by PAGE and subjected to western blotting with anti-HA and anti-FLAG antibodies.

Example 6: Metabolic rate increase associated with GSTZ1-1 deficiency

Energy expenditure was determined in mice receiving a high fat diet by measuring oxygen consumption. The results obtained from two wild type mice (W) and two GSTZ deficient mice (Z) indicate that the GSTZ deficient mice (GSTZ^{-/-}) have a greater metabolic rate. This is consistent with the inactivation of fyn kinase and a subsequent increase in fatty acid oxidation that is proposed to occur in the absence of GSTZ.

	W*	Z*
Avg VO ₂ 2pm-5pm	81.66	110.45
Avg VO ₂ 5pm-5am	87.60	112.91
Avg VO ₂ 5am-2pm	72.83	75.90
Avg RQ 2pm-5am	0.83	0.90
Avg RQ 5pm-5am	0.85	0.90
Avg RQ 5am-5pm	0.89	0.93

*2 mice for each strain, on high fat diet

VO₂=oxygen consumption, indicative of energy expenditure (ml/kg/min)

RQ=respiratory quotient (Also called RER) (V_{CO_2}/V_{O_2}) - 0.7=fat oxidation;
1=carbohydrate oxidation

Claims

1. A method for treating a metabolic condition in a subject, comprising administering to said subject a compound that inhibits the expression or activity of GSTZ and/or promotes degradation of GSTZ.
2. A method for treating a metabolic condition in a subject, comprising administering to said subject a compound that inhibits or disrupts the interaction of GSTZ with one or more molecules, compounds or cellular components.
3. A method for modulating the metabolic rate of a subject, comprising administering to said subject a compound that inhibits the expression or activity of GSTZ and/or promotes degradation of GSTZ.
4. A method for modulating the metabolic rate of a subject, comprising administering to said subject a compound that inhibits or disrupts the interaction of GSTZ with one or more molecules, compounds or cellular components.
5. The method of claim 3 or claim 4, wherein the modulation of the metabolic rate is an increase in metabolic rate.
6. A method for modulating lipid homeostasis and/or fatty acid oxidation in a subject comprising administering to said subject a compound that inhibits the expression or activity of GSTZ and/or promotes degradation of GSTZ.
7. A method for modulating lipid homeostasis and/or fatty acid oxidation in a subject comprising administering to said subject a compound that inhibits or disrupts the interaction of GSTZ with one or more molecules, compounds or cellular components.
8. The method of claim 6 or claim 7, wherein the modulation of lipid homeostasis is increasing the rate of lipolysis, increasing the rate of fatty acid oxidation, inhibiting adipogenesis or any combination thereof.
9. A method for treating insulin resistance in a subject, comprising administering to said subject a compound that inhibits the expression or activity of GSTZ and/or promotes degradation of GSTZ.
10. A method for treating insulin resistance in a subject, comprising administering to said subject a compound that inhibits or disrupts the interaction of GSTZ with one or more

molecules, compounds or cellular components.

11. The method of any one of claims 1, 3, 6 or 9, wherein the compound inhibits or disrupts an interaction between GSTZ and one or more molecules, compounds or cellular components.

12. The method of any one of claims 1 to 10, wherein inhibiting the expression or activity and/or promoting degradation of GSTZ comprises degrading or blocking the synthesis of said GSTZ.

13. The method of any one of claims 2, 4, 7 or 10, wherein, the molecule, compound or cellular component comprises a protein with at least one SH3 domain.

14. The method of claim 13, wherein the SH3 domain containing protein is Fyn Kinase.

15. The method of any one of claims 1 to 10, wherein the GSTZ is GSTZ1-1.

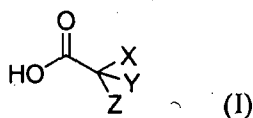
16. The method of claim 1 or claim 2, wherein the metabolic condition is selected from the group consisting of: obesity, excess fat, insulin resistance, type II diabetes or metabolic syndrome.

17. The method of any one of claims 1 to 10, wherein the compound is selected from the group consisting of: organic compounds, peptides, peptide derivatives, peptidomimetics, siRNA, RNAi, PNA, antibodies and antibody fragments.

18. The method of claim 17, wherein the organic compound is a C₂-C₆ carboxylic acid comprising at least two leaving groups, including salts and prodrugs thereof, wherein reference to C₂-C₆ does not include carbon atoms present in the leaving groups.

19. The method of claim 18, wherein the at least two leaving groups are located on the α -carbon relative to the carboxyl group.

20. The method of claim 18 or claim 19, wherein the C₂-C₆ carboxylic acid comprising at least two leaving groups, wherein reference to C₂-C₆ does not include carbon atoms present in the leaving groups, has the following structure (I):



wherein X, Y and Z are independently selected from the group consisting of: C₁-C₄ alkyl, hydroxy, leaving group and hydrogen, provided that at least two of X, Y and Z are leaving

groups.

21. The method of claim 20, wherein X, Y and Z are independently selected from the group consisting of: C₁-C₄ alkyl, leaving group and hydrogen, provided that at least two of X, Y and Z are leaving groups.

22. The method of any one of claims 18 to 21, wherein the leaving groups are independently selected from the group consisting of: chloride, bromide, fluoride, iodide, triflate, tosylate, mesylate, diazonium salts, sulfonates and tetraalkyl ammonium salts.

23. The method of claim 22, wherein the leaving groups are chloride, iodide, bromide or any combination thereof.

24. The method of claim 20, wherein the C₂-C₆ carboxylic acid comprising at least two leaving groups is selected from the group consisting of: dichloroacetic acid, bromochloroacetic acid, dibromoacetic acid, diiodoacetic acid, iodochloroacetic acid and iodobromoacetic acid, including salts and prodrugs thereof.

25. The method of claim 17, wherein the organic compound is a glutathione analogue.

26. A method of screening for a compound that inhibits the expression or activity of GSTZ, and/or promotes degradation of GSTZ, the method comprising;

(a) contacting a cell expressing GSTZ or a cell expressing a reporter protein under the control of a GSTZ promoter;

(b) incubating the cell and the compound for a period of time under conditions suitable to enable expression of the GSTZ or the reporter protein; and

(c) assaying for activity or presence of the GSTZ or the reporter protein.

27. A method of screening for a compound that inhibits or disrupts the interaction of GSTZ with one or more molecules, compounds or cellular components, the method comprising;

(a) contacting the GSTZ with a candidate compound under conditions suitable to enable interaction of the candidate compound and the GSTZ; and

(b) assaying for the presence of an interaction between GSTZ and one or more of said molecules, compounds or cellular components.

FIGURE 2

humanGSTZ1	1	MQAGKPILYSYFRSSCSWRVRIALALKGIDYKTVPINLIKDGQQQFSKDF	50
mouseGSTZ1	1	MQAGKPILYSYFRSSCSWRVRIALALKGIDYEIVPINLIKDGQQQFTEEF	50
humanGSTZ1	51	QALNPMKQVPTLKIDGITIHQSLAII EYLEETRPT PRLLPQDP KKRASVR	100
		. . . : . : .	
mouseGSTZ1	51	QTLNPMKQVPALKIDGITIVQSLAIMEYLEETRPI PRLLPQDP QKRAIVR	100
humanGSTZ1	101	MISDLIAGGIQPLQNL SVLKQVGEEMQLTWAQNAITCGFNALEQILQSTA	150
		. . : .:. . . :	
mouseGSTZ1	101	MISDLIASGIQPLQNL SVLKQVQENQMQWAQKVITSGFNALEKILQSTA	150
humanGSTZ1	151	GIYCVGDEVMTADLCLVPQVANAERFKVDLTPYPTISSINKRLLVLEAFQ	200
		. : : : : : : : : : : . . .	
mouseGSTZ1	151	GKYCVGDEVSMADVCLVPQVANAERFKVDLSPYPTISHINKELLALEVFQ	200
humanGSTZ1	201	VSH P CRQPD T PTELRA	216 (SEQ ID NO: 1)
		. . .	
mouseGSTZ1	201	VSH P RQPD T PAELRT	216 (SEQ ID NO: 2)

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FIGURE 3

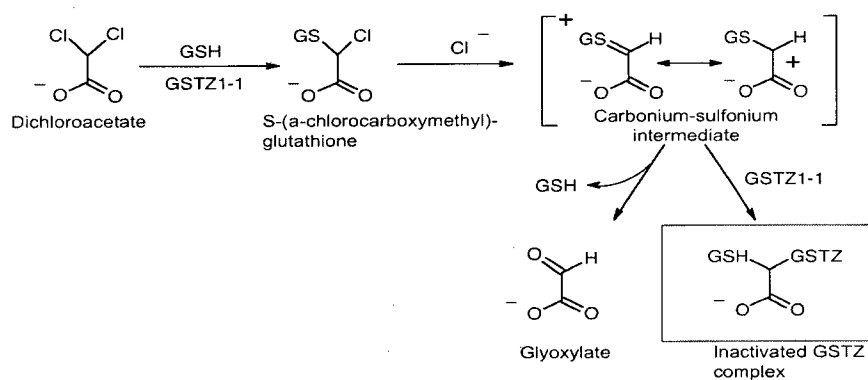
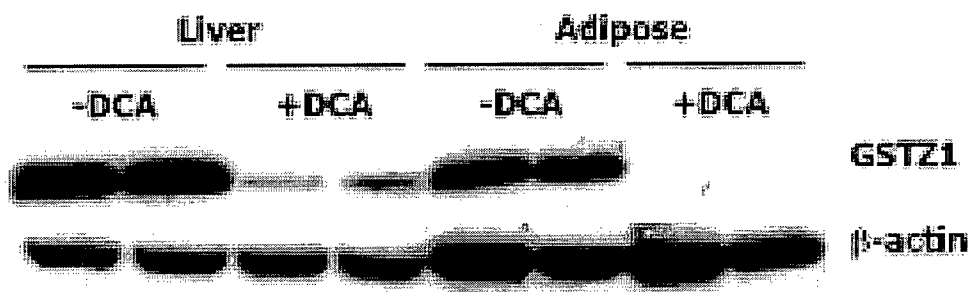


FIGURE 4A



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FIGURE 4B

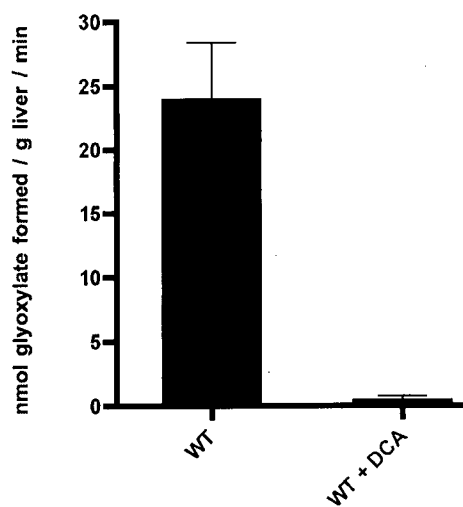
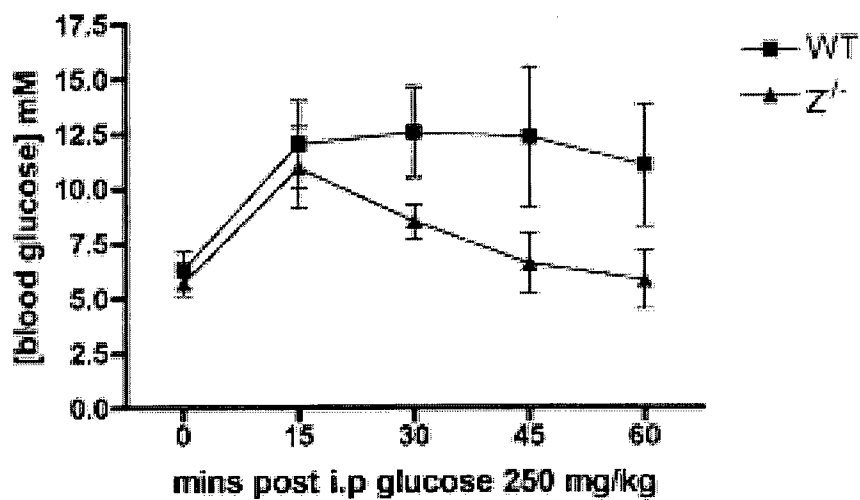
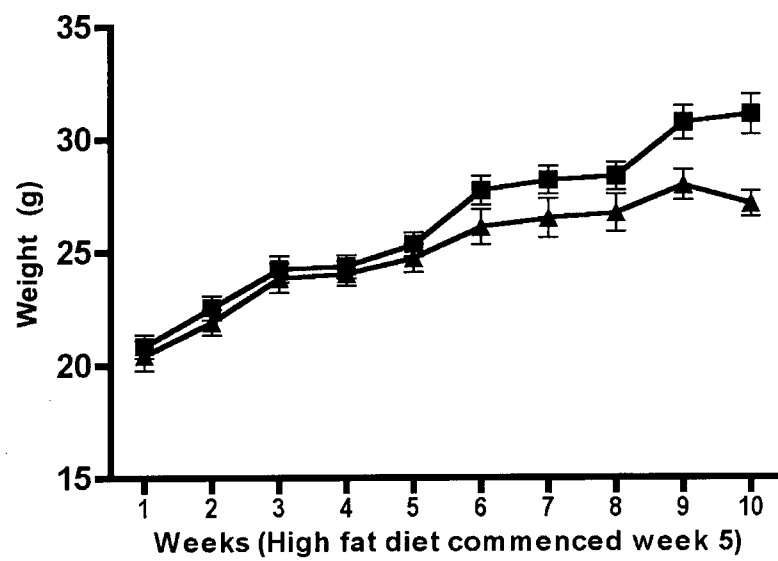


FIGURE 5



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FIGURE 6

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FIGURE 7A

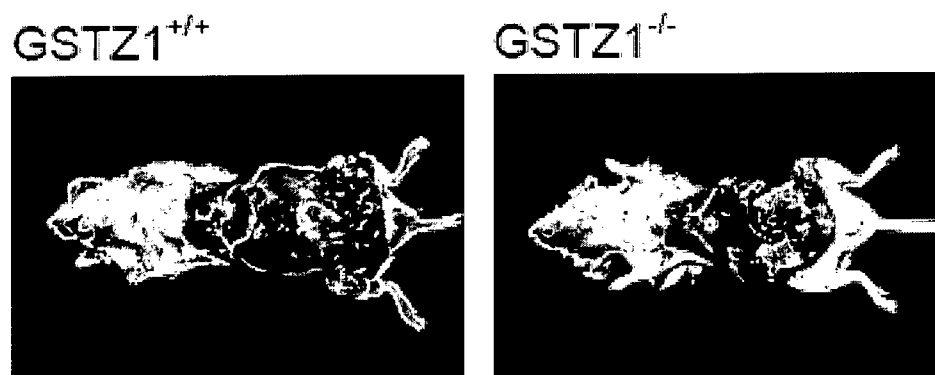


FIGURE 7B

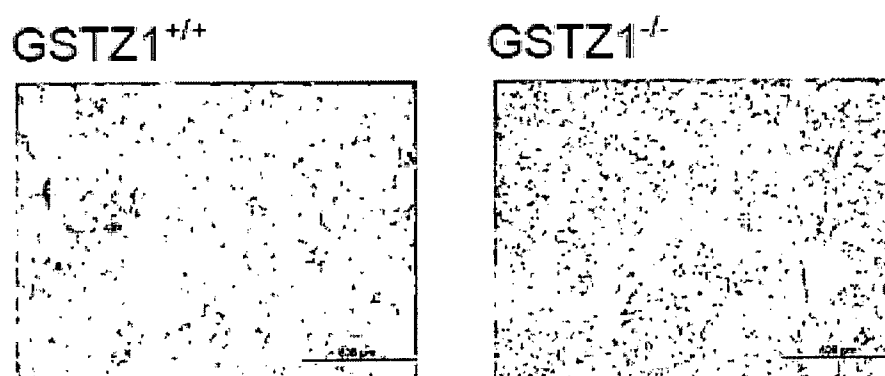


FIGURE 8A

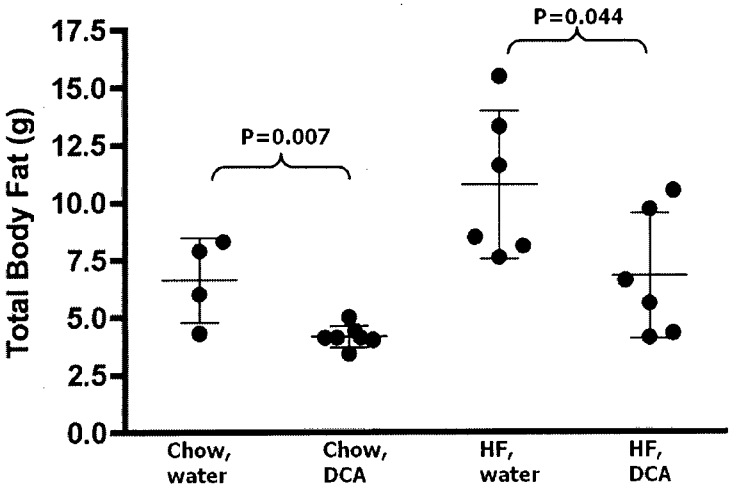
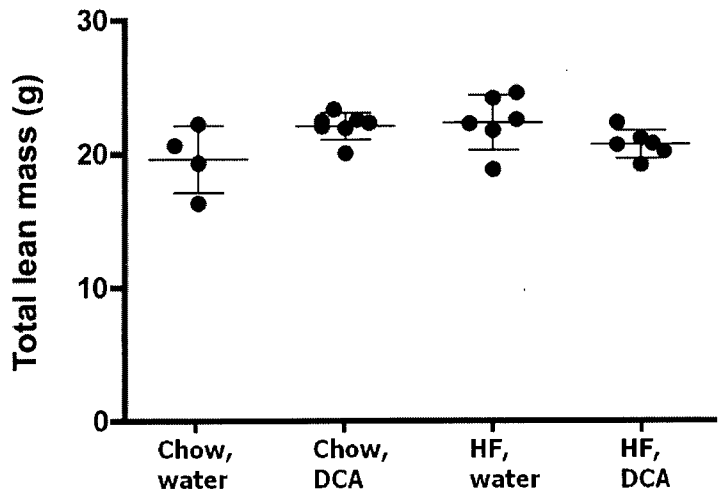


FIGURE 8B



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FIGURE 9

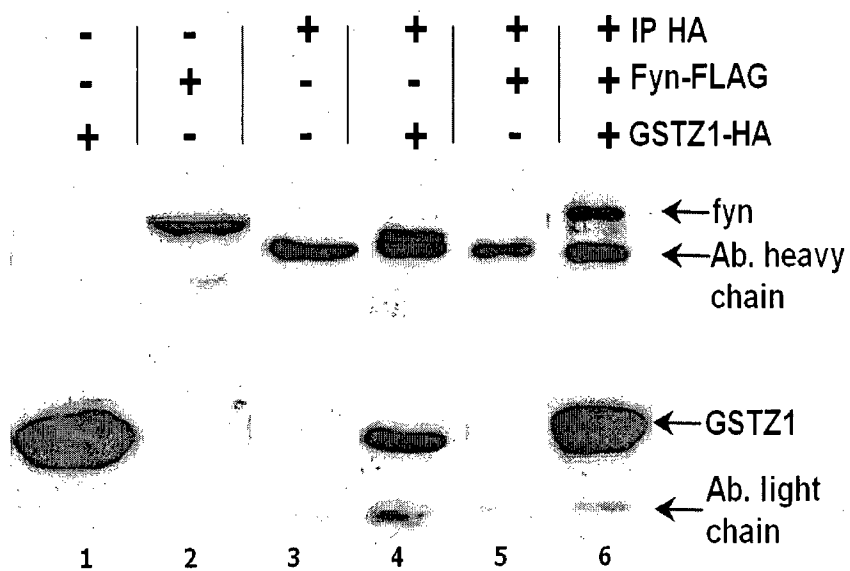


FIGURE 10



INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2011/000276

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

A61K 31/19(2006.01) *A61P 3/06* (2006.01) *G01N 1/18* (2006.01) *G01N 33/483* (2006.01)
A61P 3/04 (2006.01) *A61P 3/10* (2006.01) *G01N 3/50* (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPODOC, WPI, MEDLINE: GSTZ, Glutathione Transferase Zeta, MAAI, Maleylacetoacetate, Degradation, Inhibit, Disruption

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	WO 1996/011679 A1 (STACPOOLE) 25 April 1996	
X	Abstract and Pages 4-5	1-27
	Tzeng et al, "Polymorphism and Species Dependent Inactivation of Glutathione Transferase Zeta by Dichloroacetate, Chemical Research in Toxicology, Volume 13, Pages 231-236, April 2000	
X	Introduction, Pages 231 and 235	1-12 and 15-24
	Board et al, "Human Glutathione Transferase Zeta", Methods in Enzymology, Volume 401, Page 61-77, 2005	
X	Abstract and Pages 66-67	1-27



Further documents are listed in the continuation of Box C



See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
18 April 2011

Date of mailing of the international search report **24 MAY 2011**

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2011/000276

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:
 - a. (means)
☐ on paper
☐ in electronic form
 - b. (time)
☐ in the international application as filed
☐ together with the international application in electronic form
☐ subsequently to this Authority for the purposes of search
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

There was a sequence listing originally filed but it was not used for the purposes of this search and opinion

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/AU2011/000276

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member	
WO	9611679	AU	79719/94
		US	5643951
		CA	2202397
		EP	0786993
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.			
END OF ANNEX			