(54) COMPOSITIONS AND METHODS FOR TREATING OBESITY, OBESITY RELATED DISORDERS AND FOR INHIBITING THE INFECTIVITY OF HUMAN IMMUNODEFICIENCY VIRUS

(76) Inventors: Sylvia Lee-Huang, New York, NY (US); Paul L. Huang, Boston, MA (US); Philip Lin Huang, Maple Glen, PA (US); Dawei Zhang, Elmhurst, NY (US); John Z.H. Zhang, New York, NY (US); Young Tae Chang, New York, NY (US); Jae Wook Lee, San Diego, CA (US); Ju Bao, Brooklyn, NY (US); Yongtao Sun, Chaanxi Province (CN)

Correspondence Address: KLAUBER & JACKSON 411 HACKENSACK AVENUE HACKENSACK, NJ 07601

(21) Appl. No.: 11/827,135

(22) Filed: Jul. 9, 2007

Related U.S. Application Data

(60) Provisional application No. 60/819,172, filed on Jul. 7, 2006, provisional application No. 60/897,702, filed on Jan. 26, 2007.

Publication Classification

(51) Int. Cl. A61K 36/00 (2006.01) A61K 31/351 (2006.01) A61K 31/70 (2006.01) A61K 38/28 (2006.01)

(52) U.S. Cl. 424/774; 514/460; 514/734; 514/27; 514/636; 514/4; 514/369; 514/342; 514/423; 514/277; 514/419; 514/635; 514/634; 514/331; 514/42; 514/592

(57) ABSTRACT

The present invention relates to methods and pharmaceutical compositions for treating obesity or obesity-related disorders in a subject suffering from or predisposed to developing obesity or an obesity-related disorder, or for inhibiting the infectivity of HIV, by administering oleuropein, an analogue or derivative thereof, or the major metabolites of oleuropein including oleuropein aglycone, hydroxytyrosol, and elenolic acid or their analogues, or derivatives thereof, an iridoid glycoside, or a secosteroid glycoside or analogues or derivatives thereof, or any combination of the foregoing including olive leaf extract. The invention also relates to methods for screening/diagnosing a subject having, or predisposed to having obesity or a related disorder by measuring the expression profiles of an adipogenic gene selected from PPARγ2, LPL and cP2 gene and gene product, or other adipogenic, lipogenic, or lipolytic genes and gene products in an individual. The invention further provides for screening for novel oleuropein analogues.
FIG. 4

Aortic lesions in apoE ko mice fed a Western diet for 4 months
FIG. 10

Oleuropein (glycoside) → \( \beta \)-glucosidase → Oleuropein aglycone + glucose

hydrolysis

Hydroxytyrosal + Elenolic acid
FIG. 11G

OLE hydroxytyrosol  

$\text{c [M-H]}^{-} = 153$

FIG. 11H

OLE olenolic acid  

$\text{b [M-H]}^{-} = 377$
FIG. 12A

Oleuropein LC (280 nm, 230 nm, and MSD)
FIG. 12B
Oleuropein Mass (m/z=539)
FIG. 12C
Oleuropein LC (280 nm, 230 nm, and MSD)
FIG. 12D
Serum Hydroxytyrosol Mass (m/z=153)

c [M-H]⁻ = 153
FIG. 12E
Urine LC (280 nm, 230 nm, and MSDC.)
FIG. 12F

Urine Mass (m/z=100-600)
FIG. 13A

FIG. 13B

[Chemical structure diagram with labels for molecules and molecular weights, including "Ole" and "[M-H] 539".]
FIG. 13E

\[
\begin{align*}
\text{Ole} & \xrightarrow{\beta\text{-Glucosidase}} \text{Ole-AG} + \text{Glucose} \\
\text{HT} & + \text{EA} \\
\end{align*}
\]

FIG. 13F

\[
\begin{align*}
\text{DHPAA} & \xrightarrow{\text{AcCl, MeOH, RT}} \text{DHPAE} \\
\text{DHPAE} & \xrightarrow{\text{LiAlH}_4, \text{THF, } 0^\circ C} \text{HT} \\
\end{align*}
\]
### FIG. 18A

**3'-Processing**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Target</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>U3 5'-ACTGGAAGGGCTAAATTCACTC-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21 mer 3'-TGACCTTCGATTAAGTGAG-5'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV-IN X Ole/HT</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- HT
- Ole

### FIG. 18B

**Strand Transfer**

<table>
<thead>
<tr>
<th>Viral Substrate</th>
<th>Target</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>U3-GT (19 mer)</td>
<td>pCU18</td>
<td>pCU18</td>
</tr>
<tr>
<td>(2.69 kb)</td>
<td></td>
<td>(2.0)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HIV-IN</th>
<th>Ole/HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kb</td>
<td></td>
</tr>
<tr>
<td>9.6</td>
<td>+</td>
</tr>
<tr>
<td>6.6</td>
<td>+</td>
</tr>
<tr>
<td>4.4</td>
<td>+</td>
</tr>
<tr>
<td>2.0</td>
<td>U3-GT</td>
</tr>
</tbody>
</table>

- HT
- Ole

- U3-GT
COMPOSITIONS AND METHODS FOR TREATING OBESITY, OBESITY RELATED DISORDERS AND FOR INHIBITING THE INFECTIVITY OF HUMAN IMMUNODEFICIENCY VIRUS

FIELD OF THE INVENTION

[0001] The present invention relates generally to methods and compositions for modulating the body weight of mammals including animals and humans, and more particularly to materials identified herein as modulators of weight, and the use of such materials for treating obesity and disorders related to obesity and to the diagnostic and therapeutic uses to which such modulators may be put. The present invention also relates to methods and compositions for inhibiting the infectivity of human immunodeficiency virus (HIV).

BACKGROUND OF THE INVENTION

[0002] Obesity, which is defined in general terms as an excess of body fat relative to lean body mass, is now a world wide epidemic, and is one of the most serious contributors to increased morbidity and mortality. Obesity is prevalent in the United States, affecting more than 61% of the total population (Flegal, et al., Overweight and Obesity in the United States: Prevalence and Trends, 1960-1994, Int J Obes 22:39-47, 1998). Obesity is defined more specifically by the United States Centers for Disease Control and Prevention (CDC) as an excessively high amount of body fat or adipose tissue in relation to lean body mass and overweight is defined as an increased body weight in relation to height, when compared to some standard of acceptable or desirable weight. The CDC alternatively defines overweight as a person with a body mass index (BMI) between 25.0 and 29.9 and obesity is defined as a BMI greater than or equal to 30.0. Obesity is often associated with psychological and medical morbidities, the latter of which includes increased joint problems, vascular diseases such as coronary artery disease, hypertension, stroke, and peripheral vascular disease. Obesity also causes metabolic abnormalities such as insulin resistance and Type II diabetes (non-insulin-dependent diabetes mellitus (NIDDM)), hyperlipidemia, and endothelial dysfunction. These abnormalities predispose the vasculature to injury, cellular proliferation and lipid oxidation, with resulting atherosclerosis leading to heart attack, stroke, and peripheral vascular diseases. In 1998, consumers spent $33 billion in the United States for weight-loss products and services with a less than positive outcome (Serdula, et al., Prevalence of Attempting Weight Loss and Strategies for Controlling Weight, JAMA 282:1353-1358, 1999). Thus, obesity and its associated complications continue to be a major problem throughout the worldwide health care system.

[0003] Obesity is clearly an important clinical problem with very broad reaching implications. There is a pressing need for more research on the molecular mechanisms that underlie obesity and its medical consequences, as well as new approaches for its treatment. To date, these approaches have been limited to diet and exercise (therapeutic lifestyle changes), surgical procedures such as gastric bypass, and pharmacologic agents. Drug treatment for obesity has been disappointing since almost all drug treatments for obesity are associated with undesirable side effects that contributed to their termination. A number of monoamines and neuropeptides are known to reduce food intake (Bray, et al., Pharmacological Treatment of Obesity, Am J Clin Nutr 55:151S-319S, 1992). Available pharmacotherapies have included sibutramine (an appetite suppressant), orlistat (a lipase inhibitor), fenfluramine and dexfenfluramine. Although body weight loss is effective, these sympathomimetic drugs cause side effects including pulmonary hypertension, neuroanatomic changes, and a typical valvular heart diseases. For example, fenfluramine and dexfenfluramine were withdrawn from the market in 1997 because of associated cardiac valvulopathy (Connolly, et al., Valvular Heart Disease Associated With Fenfluramine-Phentermine, New Engl J Med 337-581-588, 1997). Thus, nutrition and dietary restriction are most desirable for weight loss. However, long-term success of dietary regulation is low because of noncompliance. The loss of motivation to change dietary habits necessary to consume less fat and fewer calories results in regaining weight.

[0004] There are no real treatments based on the biology of the primary metabolic abnormalities found in obesity and its related conditions, such as metabolic syndrome or atherosclerosis. Accordingly, there is still a need for new compositions and methods that address treating individuals suffering from obesity and obesity-related disorders. There is also a need for new agents and compositions for treating individuals infected with human immunodeficiency virus (HIV). It is toward the development of new compositions and methods for treating obesity and obesity-related disorders and for treating individuals infected with HIV that the present invention is directed.

[0005] The citation of any reference herein should not be construed as an admission that such reference is prior art to the instant invention.

SUMMARY OF THE INVENTION

[0006] In accordance with the broadest aspect of the invention, methods and compositions comprising oleuropein, or an analogue or derivative thereof, or the major metabolites of oleuropein including oleuropein aglycone, hydroxytyrosol, and elenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside or their analogues or derivatives thereof, or any combination of the foregoing including but not limited to olive extract, are disclosed for treating obesity and obesity-related conditions or disorders, as well as for inhibiting the infectivity of HIV. In particular, these compositions modulate adipogenesis, lipodystrophy, reduce fat accumulation and weight gain. These agents also prevent HIV viral fusion/entry into a host cell and bind the catalytic site of the HIV integrase. Thus, these agents provide an advantage over other anti-viral therapies in that both viral entry and integration are inhibited. They exert their effect by modulating adipocyte differentiation (adipogenesis), de-differentiation, transdifferentiation, and by decreasing the number of adipocytes (fat cells), or by modulating adipocyte metabolism (lipid synthesis, storage, accumulation, and utilization) so as to decrease fat accumulation and decrease the size of the fat cell (decrease fat mass), increase fat burning and expenditure. These compositions also have an effect on adipogenic, lipogenic and lipolytic gene/gene product expression, perturbation of pre-adipocyte to adipocyte balance by promoting de-differentiation or transdifferentiation of adipocytes, the end result being a reduction in fat accumulation (adipose mass) and a reduction in weight gain. Accordingly, these compositions are useful for treating obesity and obesity-related disorders. In addition, these compositions have been shown to reduce diet-induced atherogen-
esis, thus allowing for a means of treating one of the major conditions or disorders associated with, or resulting from, obesity. Moreover, the compositions disclosed may also be utilized for treating other obesity-related disorders, including but not limited to, coronary artery disease, hypertension, stroke, peripheral vascular disease, insulin resistance, glucose intolerance, diabetes mellitus, hyperlipidemia, atherosclerosis, cellular proliferation and endothelial dysfunction, diabetic dyslipidemia, HIV-related lipodystrophy, e.g. Highly Active Anti-Retroviral Therapy (HAART)-induced lipodystrophy, and metabolic syndrome, type II diabetes, hyperinsulinemia, diabetic complications including diabetic neuropathy, nephropathy, retinopathy or cataracts, heart failure, hypercholesterolemia, inflammation, thrombosis, congestive heart failure, and other cardiovascular disease related to obesity or an overweight condition, or obesity induced asthma, airway dysfunction and pulmonary disorders. The compositions may also be contemplated for use in the production of lean meat from meat animals, e.g., beef cattle, lambs, hogs, chickens and turkeys.

[0007] Accordingly, a first aspect of the invention provides a method of modulating adipocyte differentiation or adipogenic gene expression, comprising administering a therapeutically effective amount of oleanone or an analogue, or derivative thereof, or oleanone aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside or their analogues, or derivatives thereof, or any combination of any of the foregoing thereof including but not limited to olive leaf extract, to a mammalian subject in need thereof.

[0008] In another embodiment, oleanone or its analogues, or derivatives thereof, or oleanone aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside or their analogues, or derivatives thereof, or any combinations of any of the foregoing including but not limited to olive leaf extract, blocks or down-regulates adipocyte differentiation, regulators, factors and enzymes involved in these pathways.

[0009] In another embodiment, oleanone or an analogue, or derivative thereof, or oleanone aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside or their analogues, or derivatives thereof, or any combinations of any of the foregoing thereof including but not limited to olive leaf extract, enhances de-differentiation of adipocytes and allows for transdifferentiation into osteoblasts, muscle cells, cartilage, and bone as well as regulators and enzymes involved in these pathways.

[0010] In yet another embodiment, the adipogenic gene whose expression is modulated by oleanone or an analogue, or derivative thereof, or oleanone aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside or their analogues, or derivatives thereof, or any combinations of any of the foregoing including but not limited to olive leaf extract, is selected from the group consisting of Peroxisome Proliferator-Activated Receptor γ (PPARγ), lipoprotein lipase (LPL) and the αP2 gene or gene product. Oleanone or its analogues, or derivatives, or oleanone aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside or their analogues, or derivatives thereof, or any combinations of any of the foregoing thereof including but not limited to olive leaf extract, also modulates the expression of all of the three types of PPARs α, γ, and γ resulting in a coordinated regulation of adipocyte differentiation, de-differentiation, transdifferentiation, adipocyte metabolism and energy homeostasis. In yet another embodiment, the lipidogenic, lipolytic and energy uncoupling genes and gene products whose expression is modulated includes, but is not limited to, PPAR γ and its modulated genes and gene products. In yet another embodiment, the lipidogenic, lipolytic and energy uncoupling genes and gene products whose expression is modulated includes, but is not limited to, PPAR α and its modulated genes and gene products.

[0011] A second aspect of the invention provides a method of treating, controlling or preventing obesity, or of reducing body weight, or of inhibiting fat accumulation, or of promoting fat burning and energy uncoupling in vivo, or of treating, controlling or preventing the onset of one or more obesity-related disorders or conditions, comprising administering a therapeutically effective amount of oleanone or an analogue, or derivative thereof, or oleanone aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside or their analogues, or derivatives thereof, or any combinations of any of the foregoing thereof including but not limited to olive leaf extract, to a mammalian subject in need thereof.

[0012] In one embodiment, the one or more obesity-related disorders or conditions are selected from the group consisting of coronary artery disease, hypertension, stroke, peripheral vascular disease, insulin resistance, glucose intolerance, diabetes mellitus, hyperglycemia, hyperlipidemia, hypercholesterolemia, hypertriglyceridemia, hyperinsulinemia, atherosclerosis, cellular proliferation and endothelial dysfunction, diabetic dyslipidemia, HIV-related lipodystrophy and metabolic syndrome, type II diabetes, diabetic complications including diabetic neuropathy, nephropathy, retinopathy or cataracts, heart failure, inflammation, thrombosis, congestive heart failure, any other atheromatous or pathologic disease related to obesity and any other viral infection related diseases and any other cardiovascular disease related to obesity or an overweight condition.

[0013] A third aspect provides a method of treating, controlling or preventing the onset of one or more obesity related disorders or conditions selected from the group consisting of Asthma and related diseases including but not limited to,
Allergy, Atopic Dermatitis (Eczema), Gastroesophageal Reflux Disease, Airway, Pulmonary and Lung disorders, in a mammalian subject in need of treatment, comprising administering a therapeutically effective amount of oleuropein or an analogue, derivative or oleuropein aglycone or their analogues, or derivatives thereof, or hydrotyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside or their analogues, or derivatives, or any component or any combinations of any of the foregoing thereof including but not limited to olive leave extract.

A fourth aspect provides a method of treating, controlling or preventing the onset of one or more obesity related disorders or conditions selected from the group consisting of AIDS and HAART related diseases including but not limited to lipodystrophy in a human and or mammalian subject in need of treatment, comprising administering a therapeutically effective amount of oleuropein or an analogue, derivative or oleuropein aglycone or their analogues, or derivatives thereof, or hydrotyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside or their analogues, or derivatives, or any component or any combinations of any of the foregoing thereof including but not limited to olive leave extract.

A fifth aspect provides a method of treating, controlling or preventing the onset of one or more viral infection related disorders or conditions selected from the group consisting of viral infection related diseases including but not limited to the AIDS virus HIV-1, and other viruses including but not limited to simian immunodeficiency viruses (SIV), Sendai virus, feline immunodeficiency virus (FIV), respiratory syncytial virus (RSV), measles virus, Ebola virus, Nipah and Hendra viruses, the severe acute respiratory syndrome associated coronavirus (SARS-CoV), and the avian flu virus in a human, mammalian, avian, poultry, non-human primates subjects in need of treatment, comprising administering a therapeutically effective amount of oleuropein or an analogue, or derivative, or oleuropein aglycone or their analogues, or derivatives thereof, or hydrotyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside or their analogues, or derivatives, or any component or any combinations of any of the foregoing thereof including but not limited to olive leave extract.

A sixth aspect of the invention provides a method for modulating Peroxisome Proliferator Activation Receptor (PPAR) activity, comprising administering to a mammal need thereof a therapeutically effective amount of oleuropein or an analogue, or derivative thereof, or oleuropein aglycone or their analogues, or derivatives thereof, or hydrotyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside or their analogues, or derivatives thereof, or any component or any combinations of any of the foregoing thereof or an iridoid glycoside, or a secoiridoid glycoside or their analogues, or derivatives, or any component or any combinations thereof including but not limited to olive leave extract.

A seventh aspect provides a method of treating a subject suffering from, or at risk for developing a disease or condition for which PPAR modulation provides a therapeutic benefit, comprising administering to said subject a therapeutically effective amount of oleuropein, or an analogue or derivative thereof or oleuropein aglycone or their analogues, or derivatives thereof, or hydrotyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside or their analogues, or derivatives, or any component or any combinations of any of the foregoing thereof including but not limited to olive leave extract.

In one embodiment, the subject is a human or a non-human mammal. In a preferred embodiment, the subject is a mammal. In another preferred embodiment, the subject is a non-human mammal selected from the group consisting of cows, horses, pigs, sheep, goats, rodents, dogs, cats and other domestic animals or farm animals.

In yet another particular embodiment, the condition for which PPAR modulation provides a therapeutic benefit is selected from the group consisting of obesity, obesity-related disorders and the sequelae thereof. In yet another embodiment, accordingly, the present invention provides for a method of treating inflammatory diseases or conditions comprising administering a PPAR gamma modulator to a subject in need of such therapy. In one particular embodiment, the method provides for treating neurological or neurodegenerative diseases or conditions caused in part by the presence or influx of inflammatory cells, such as for example, multiple sclerosis, stroke or Alzheimer’s disease. The use of a PPAR modulator for treating a nervous system injury is also contemplated, for example, a spinal cord injury or traumatic brain injury. The use of a PPAR modulator for wound healing is also contemplated. In one particular embodiment, the PPAR modulator is a PPAR gamma, alpha, or delta agonist, or a combined agonist.

In yet another particular embodiment, the PPAR is selected from PPAR δ, γ or α, or a dual, or pan PPAR δ, γ and α modulators.

In yet another particular embodiment, the obesity-related disorder or sequelae is selected from the group consisting of coronary artery disease, hypertension, stroke, peripheral vascular disease, insulin resistance, diabetes mellitus, hyperlipidemia, atherosclerosis, cellular proliferation and endothelial dysfunction, type II diabetes, hyperinsulinemia, diabetic complications including diabetic neuropathy, nephropathy, retinopathy or cataracts, heart failure, hypercholesterolemia, inflammation, thrombosis, congestive heart failure, and any other cardiovascular disease related to obesity or an overweight condition.

An eighth aspect of the invention provides a method of reducing or preventing formation of atherothrombotic lesions or preventing diet-induced atherogenesis, comprising administering to a mammal in need thereof a therapeutically effective amount of oleuropein or an analogue or derivative thereof, or oleuropein aglycone or their analogues, or derivatives thereof, or hydrotyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside or their analogues, or derivatives thereof, or any component or any combinations of any of the foregoing thereof or an iridoid glycoside, or a secoiridoid glycoside or their analogues, or derivatives, or any combinations of any of the foregoing thereof.

A ninth aspect of the invention provides a method of modulating endothelial dysfunction comprising administering to a mammal in need thereof a therapeutically effective amount of oleuropein or an analogue, or derivative, or oleuropein aglycone or their analogues, or derivatives thereof, or hydrotyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside or their analogues, or derivatives, or any combinations of any of the foregoing thereof.
hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or enolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside or their analogues, derivatives, or any combinations of any of the foregoing thereof including but not limited to olive leaf extract.

[0024] A tenth aspect of the invention provides a method for treating obesity or obesity related disorders or other disorders for which PPAR modulation provides a therapeutic benefit comprising administering a second agent in combination with oleuropein, or an analogue or derivative thereof, or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or enolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside or their analogues, derivatives, or any combination of any of the foregoing thereof including but not limited to olive leaf extract.

[0025] In one particular embodiment, the second agent is selected from the group consisting of a different PPAR modulating agent, a cholesterol or lipid lowering agent, a biguanide, insulin, an antihyperglycemic agent, GLP-1 or analogues thereof, DPP4 inhibitors, a weight loss agent, and any agent useful for treating metabolic syndrome or type 2 diabetes.

[0026] In another particular embodiment, the different PPAR modulating agent is a thiazolidinedione selected from the group consisting of troglitazone, pioglitazone and rosiglitazone.

[0027] In yet another particular embodiment, the cholesterol or lipid lowering agent is a HMG-CoA reductase inhibitor, and wherein the HMG-CoA reductase inhibitor is a statin selected from the group consisting of atorvastatin,lovastatin, simvastatin,pravastatin and simvastatin. In yet another particular embodiment, the cholesterol or lipid lowering agent is niacin or a fibrate.

[0028] In yet another particular embodiment, the biguanide is selected from the group consisting of metformin, phenformin, and buformin.

[0029] In yet another particular embodiment, the antihyperglycemic is a prandial glucose regulator or an alpha-glucosidase inhibitor.

[0030] In yet another particular embodiment, the prandial glucose regulator is repaglinide or nateglinide.

[0031] In yet another particular embodiment, the alpha-glucosidase inhibitor is selected from the group consisting of acarbose, voglibose and miglitol.

[0032] In yet another particular embodiment, the agent useful for treating metabolic syndrome or type 2 diabetes is a sulfonylurea selected from the group consisting of glimepiride, glibenclamide (glyburide), gliclazide, glipizide, glibiquone, chloropropamide, tolbutamide, acetohexamide, glycopyramide, carbamamide, glibonuride, glisoxepid, glyburiazole, glibuzole, glyhexamide, glymidine, glypinamide, phenbutamide, tolcyamide and tolazamide.

[0033] In yet another particular embodiment, the agent useful for treating AIDs and Highly Active Anti-Retroviral Therapy (HAART)-induced lipodystrophy, metabolic syndrome or type 2 diabetes is a thiazolidinedione (TZD) and a fibrate.

[0034] In yet another particular embodiment, the agent useful for treating HIV-1 infection and AIDS is a component or the full composition of the Highly Active Anti-Retroviral Therapy (HAART), including a protease inhibitor (PI), a nucleotide reverse transcriptase inhibitor (NRTI), and a non-nucleotide reverse transcriptase inhibitor (NNRTI).

[0035] In yet another particular embodiment, the agent useful for treating obesity induced asthma and related disorders is selected from the group consisting of a glucocorticoid, an antileukotriene and an antihistamine.

[0036] An eleventh aspect of the invention provides a pharmaceutical composition comprising a therapeutically effective amount of oleuropein, or an analogue or derivative thereof or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or enolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside or their analogues, derivatives, or any combinations thereof including but not limited to olive leaf extract, and a pharmaceutically acceptable carrier for delivery to a mammal in need of such therapy.

[0037] In one particular embodiment, the pharmaceutical composition may be administered orally, nasally, transdermally, intravenously, intramuscularly, or subcutaneously.

[0038] In another particular embodiment, the subject has or is at risk for unwanted weight gain, obesity or an obesity related disorder, e.g., diabetes or glucose intolerance, insulin resistant states, hypertension, HIV-1 infection, or any of the other disorders disclosed herein. In preferred embodiments, the method includes identifying a subject as being in need of treatment or prevention of unwanted weight gain, obesity or an obesity related disorder. In another particular embodiment, the pharmaceutical composition is formulated for delivery to a human or non-human mammal. In a preferred embodiment, the mammal is a human. In another preferred embodiment, the subject is a non-human mammal selected from the group consisting of cows, horses, pigs, sheep, goats, birds, rodents (including rats, mice and gerbils), dogs, cats and other domestic or farm animals.

[0039] A twelfth aspect of the invention provides a pharmaceutical composition directed to combination therapy, whereby oleuropein or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or enolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside or their analogues, or derivatives, or any combination thereof including but not limited to olive leaf extract, is combined with one or more other therapeutic agents that are useful in the treatment of disorders associated with the development and progression of obesity and obesity related disorders, such as atherosclerosis, hypertension, hyperlipidemias, dyslipidemias, diabetes and other related disorders as described herein.

[0040] In one particular embodiment, the composition for combination therapy comprises oleuropein and at least one other agent. In another particular embodiment, the composition for combination therapy comprises oleuropein and at least two or more other agents.

[0041] In one particular embodiment, the at least one other therapeutic agent is a different PPAR modulating agent other than oleuropein.

[0042] In a more particular embodiment, the different PPAR modulating agent is a thiazolidinedione selected from the group consisting of troglitazone, pioglitazone and rosiglitazone.

[0043] In yet another particular embodiment, the at least one other therapeutic agent is a cholesterol-lowering agent.
[0044] In yet another particular embodiment, the cholesterol lowering agent is a HMG-CoA reductase inhibitor.

[0045] In yet another particular embodiment, the HMG-CoA reductase inhibitor is a statin selected from the group consisting of atorvastatin, bervastatin, cerivastatin, dalvastatin, fluvastatin, itavastatin, lovastatin, mevastatin, nicostatin, niuvastatin, pravastatin and simvastatin.

[0046] In yet another particular embodiment, oleuropein is combined with a therapy useful for the treatment of metabolic syndrome or type 2 diabetes.

[0047] In yet another particular embodiment, the therapy useful for the treatment of metabolic syndrome or type 2 diabetes and its associated complications is selected from the group consisting of a biguanide drug, (including metformin, pantopramide and buformin), insulin (synthetic insulin analogues, amylin) and oral antihyperglycemics.

[0048] In yet another particular embodiment, oleuropein is combined with an oral antihyperglycemic agent, which is a prandial glucose regulator or an alpha-glucosidase inhibitor. In yet another particular embodiment, the prandial glucose regulator is repaglinide or nateglinide. In yet another particular embodiment, the alpha-glucosidase inhibitor is acarbose, voglibose or miglitol.

[0049] In yet another particular embodiment, oleuropein is combined with a sulfonylurea.

[0050] In yet another particular embodiment, the sulfonylurea is selected from the group consisting of glimepiride, glibenclamide (glyburide), gliptizide, glipizide, gliquidone, chloropropamide, tolbutamide, acetohexamide, glycopredynide, glimepiride, glibizide, glyhexamide, glymimid, glibiuzone, glibizamide, tolazamide and tolbutamide.

[0051] In one particular embodiment, the composition comprises a mixture of oleuropein or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside or their analogues or derivatives, or any combination thereof including but not limited to olive leave extract, and the at least one or two other agents, wherein the at least one or two other agents may be administrated prior to, concurrent with, or subsequent to, oleuropein or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside or their analogues or derivatives, or any combination thereof including but not limited to olive leave extract.

[0052] In another particular embodiment, the composition comprises oleuropein or an analogue or derivative thereof or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside or their analogues or derivatives, or any combination thereof including but not limited to olive leave extract, interacts with, e.g., bind to, PPARα, PPARγ and PPARs.

[0053] In yet another particular embodiment, oleuropein or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside or their analogues or derivatives, or any combination thereof including but not limited to olive leave extract, is combined with a therapy useful for the treatment of the AIDS virus HIV-1, and other viruses with a type 1 transmembrane envelope glycoprotein, such as simian immunodeficiency viruses (SIV), Sendai virus, feline immunodeficiency virus (FIV), respiratory syncytial virus (RSV), measles virus, Ebola virus, Nipah virus, Hendra virus, for the severe acute respiratory syndrome associated coronavirus (SARS-CoV), and the avian flu virus H5N1 including but not limited to PI, NRTI, NNRTI, HAART, tamiflu, ribavirin, steroid, recombinant neomote anticoagulant protein c2 (rNAPC2).

[0054] In yet another particular embodiment, oleuropein is combined with a therapy useful for the treatment of obesity induced asthma and related disorders, including but not limited to the PPARs, glucocorticoids, anilteukokrienes and anti-histamines.

[0055] In another particular embodiment, the oleuropein or an analogue or derivative thereof, or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside, or their analogues, or derivatives thereof, or any combination thereof including but not limited to olive leave extract, is targeted to adipose tissue in a subject. The oleuropein or an analogue or derivative thereof or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or any combination thereof including but not limited to olive leave extract, is targeted to adipose tissue in a subject. The oleuropein or an analogue or derivative thereof or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or any combination thereof including but not limited to olive leave extract, is targeted to adipose tissue in a subject. The oleuropein or an analogue or derivative thereof or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or any combination thereof including but not limited to olive leave extract, is targeted to adipose tissue in a subject. The oleuropein or an analogue or derivative thereof or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or any combination thereof including but not limited to olive leave extract, is targeted to adipose tissue in a subject. The oleuropein or an analogue or derivative thereof or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or any combination thereof including but not limited to olive leave extract, is targeted to adipose tissue in a subject. The oleuropein or an analogue or derivative thereof or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or any combination thereof including but not limited to olive leave extract, is targeted to adipose tissue in a subject. The oleuropein or an analogue or derivative thereof or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or any combination thereof including but not limited to olive leave extract, is targeted to adipose tissue in a subject. The oleuropein or an analogue or derivative thereof or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or any combination thereof including but not limited to olive leave extract, is targeted to adipose tissue in a subject. The oleuropein or an analogue or derivative thereof or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or any combination thereof including but not limited to olive leave extract, is targeted to adipose tissue in a subject. The oleuropein or an analogue or derivative thereof or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or any combination thereof including but not limited to olive leave extract, is targeted to adipose tissue in a subject.

[0057] In yet another particular embodiment, the oleuropein or an analogue or derivative thereof, or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxytyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iodized glycoside, or a secoeriodin glycoside or their analogue or derivative, or any combination thereof including but not limited to olive leaf extract, is targeted to a adipocyte and its specific proteins such as pref-1 and C1q to modulate adipocyte differentiation and maturation.

[0058] In yet another particular embodiment, the oleuropein or an analogue or derivative thereof, or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxytyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or a secoeriodin glycoside or their analogues, or derivatives thereof, or any combination of the foregoing including but not limited to olive leaf extract, is targeted to a mesenchymal stem cell (MSC) or an embryonic stem cell. MSCs differentiate into adipocytes, chondrocytes, osteoblasts, and myoblasts. Thus, these stem cells are promising candidates for adipogenesis management by oleuropein and derivatives or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxytyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iodized glycoside, or a secoeriodin glycoside or their analogue or derivative, or any combination thereof including but not limited to olive leaf extract, targeting and/or ex-vivo treatment so as to modulate adipocyte differentiation, adipocyte differentiation and trans-differentiation. The application of oleuropein and derivatives in this system is not only effective in treating obesity and related metabolic syndromes by modulating adipocyte differentiation and development but also provides tools to manipulate adult stem cells for cell-based approaches in regenerative medicine. For example, aged and osteoporotic patients have a high fat to bone ratio in their bones compared with young and healthy counterparts, an outcome possibly due to the conversion of bone to fat cells. Application of oleuropein or an analogue or derivative thereof, or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxytyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iodized glycoside, or a secoeriodin glycoside or their analogue or derivative, or any combination thereof including but not limited to olive leaf extract to mediate transdifferentiation between osteoblasts and adipocytes should be of relevance to the development of therapeutic control of bone loss in osteoporosis. For specific targeting of MSC, these cells express leukemia inhibitory factor, macrophage colony-stimulating factor, and stem cell factor specifically. Thus, these proteins and antibodies specific for or that bind these proteins can be used for targeting.

[0059] In another particular embodiment, the targeting reagent is lipid soluble.

[0060] In another particular embodiment, the administration of the compositions of the invention can be initiated, e.g., (a) when the subject begins to show signs of unwanted weight gain, obesity or an obesity-related disease; (b) when obesity or an obesity-related disease is diagnosed; (c) before, during or after a treatment for obesity or an obesity-related disease is begun or begins to exert its effects; or (d) generally, as is needed to maintain health, e.g., normal weight. The period over which the agent is administered (or the period over which clinically effective levels are maintained in the subject) can be long term, e.g., for six months or more or a year or more, or short term, e.g., for less than a year, six months, one month, two weeks or less.

[0061] In another particular embodiment, the pharmaceutical compositions described herein, including oleuropein, or an analogue or derivative thereof, or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxytyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iodized glycoside, or a secoeriodin glycoside or their analogues, or derivatives thereof, or any combination thereof including but not limited to olive leaf extract and any second agent to be administered with oleuropein or its analogues or derivatives thereof, or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxytyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iodized glycoside, or a secoeriodin glycoside or their analogues or derivatives thereof, or any combination thereof including but not limited to olive leaf extract and any second agent to be administered with oleuropein or its analogues or derivatives thereof, or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxytyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iodized glycoside, or a secoeriodin glycoside or their analogues or derivatives thereof, or any combination thereof including but not limited to olive leaf extract may have an effect on white or brown adipose tissue or a combination thereof. For example, since PPAR delta is involved in thermogenesis and energy uncoupling (the main function of brown fat) and oleuropein has high affinity for PPAR delta, it may also have an effect on brown adipose tissue. Accordingly, oleuropein may be involved in the dissipation of stored fat as heat in brown adipose tissue. Brown fat plays an important role in the control of body weight, and mitochondrial uncoupling proteins may be one of many factors involved in the development of obesity. An interesting demonstration of this is found in a report in which transgenic mice with genetic ablation of brown fat developed obesity in the absence of overeating (Bachman E S, Dhillon H, Zhang C.-Y., et al. BetaAR signaling required for diet-induced thermogenesis and obesity resistance. Science 297:843, 2002).

[0062] In yet another particular embodiment, oleuropein or an analogue, or derivative thereof, or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxytyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iodized glycoside, or a secoeriodin glycoside or their analogues or derivatives thereof, or any combination thereof including but not limited to olive leaf extract may have an effect on white or brown adipose tissue or a combination thereof. For example, since PPAR delta is involved in thermogenesis and energy uncoupling (the main function of brown fat) and oleuropein has high affinity for PPAR delta, it may also have an effect on brown adipose tissue. Accordingly, oleuropein may be involved in the dissipation of stored fat as heat in brown adipose tissue. Brown fat plays an important role in the control of body weight, and mitochondrial uncoupling proteins may be one of many factors involved in the development of obesity. An interesting demonstration of this is found in a report in which transgenic mice with genetic ablation of brown fat developed obesity in the absence of overeating (Bachman E S, Dhillon H, Zhang C.-Y., et al. BetaAR signaling required for diet-induced thermogenesis and obesity resistance. Science 297:843, 2002).

[0063] A thirteenth aspect of the invention provides a method for identifying a candidate compound or an analogue or derivative of oleuropein that modulates adipocyte differentiation, de-differentiation, trans-differentiation, fat accu-
mulation and adipogenic gene expression or for treating obesity or obesity-related disorders, comprising:

- [0064] a. treating a cell with an inducing agent in the presence or absence of a candidate compound;
- [0065] b. determining whether the candidate compound inhibits differentiation of the pre-adipocyte cell to an adipocyte, or whether the candidate compound down-regulates adipogenic and lipogenic gene or gene product expression and/or up-regulates lipolytic and/or an energy uncoupling gene or gene product expression;
- [0066] c. comparing the results obtained with the candidate compound in vitro with the results obtained using oleuropein;
- [0067] d. testing the candidate compound and oleuropein in an animal model of obesity to determine the in vivo effects of both the candidate compound and oleuropein;
- [0068] e. determining whether the candidate compound decreases the amount of adipose tissue in vivo or whether the test compound prevents further fat accumulation in vivo, and
- [0069] f. selecting a candidate compound that has equivalent or better activity than oleuropein.

[0070] In one particular embodiment, the invention relates to a method for identifying a therapeutic agent having analogus activity to oleuropein comprising treating a type of cell that expresses a type of PPAR with a candidate compound/therapeutic; and determining the level of expression of at least one gene selected from the group consisting of PPARα2, lipoprotein lipase (LPL), and cP2 lipid binding protein, wherein a change in the profile of expression of at least one of these genes in the cell treated with the candidate compound/therapeutic relative to a cell that was not treated with the candidate compound/therapeutic indicates that the candidate compound/therapeutic is a therapeutic for treating a disease associated with a PPAR. The “profile,” “profiling” or “profile of expression” refers to both the level of expression of one or more genes and also the activity or function of one or more of these genes. Accordingly, the candidate compound may have an effect not only on the expression of one or more genes, but on the function or activity of one or more genes. In one particular embodiment, an increase in the profile of expression of at least one of these genes following treatment with a candidate compound/therapeutic indicates that the candidate compound/therapeutic is a therapeutic for treating a disease associated with a PPAR. The profile in expression of one or more of these genes relates to the differentiation or maturation of the adipocyte. In another particular embodiment, the inducing agent that is used for in vitro analysis is any compound or molecule that induces differentiation of a pre-adipocyte or a mesenchymal stem cell into a mature adipocyte, e.g., dexamethasone, insulin and methyl xanthine. In another particular embodiment, following contacting/treating a cell containing PPAR with a candidate compound/therapeutic, one may determine the profile of expression of other genes selected from the group consisting of leptin, TNFα, IL-6, PAI-1, adipsin, complement factor C3 and angiotensinogen. In yet another particular embodiment, in addition to classic markers of adipogenesis such as hormone-sensitive lipase (HSL), lipoprotein lipase (LPL), adiponectin, fatty acid-binding protein 4, perilipin and CCAAT enhancer binding protein, and enzymes of energy metabolism such as glyceral-3-phosphate dehydrogenase 1, acetyl-coenzyme A carboxylase, phosphoenolpyruvate carboxykinase and pyruvate dehydrogenase kinase, profiles on the expression of other related genes including PPARα, PPARβ, PPARγ, AP2, MARPK3, SREBP-1, leptin, and GLUT4 are envisioned following treatment of a type of cell with a candidate compound/therapeutic. These are important because they are not only adipocyte “markers” but also important in adipocyte function and pathogenesis. Any agent that affects adipogenesis would modulate the expression of these genes. In one particular embodiment, an adipogenic agent would up-regulate while an anti-adipogenic agent would down-regulate the expression of one or more of these genes. However, physiologically it is not a simple up or down regulation. It involves the regulation of other related genes upstream and downstream as including PPARα, PPARγ, enzymes involved in lipid metabolism, fatty acid oxidation, energy uncoupling as well as genes modulated by the PPARs. In addition to monitoring the up or down regulation of these genes, another particular embodiment provides for measuring the differential expression as well as polymorphisms of these genes. In yet another particular embodiment, the PPAR is selected from the group consisting of α, γ, or γ.

[0071] In another embodiment of the invention, the candidate therapeutic is selected from the group consisting of proteins, peptides, peptidomimetics, antibodies, nucleic acids, including RNA (e.g. siRNA), DNA, derivatives of fatty acids, and small molecules. The small molecules may be synthetic or may be derived from a natural source, such as a plant, animal, microbe, or soil.

[0072] In another embodiment, the disease is obesity or an obesity-related disorder, such as, but not limited to, Type II diabetes.

[0073] In another embodiment, the expression level of at least one of the genes noted above is detected. In another particular embodiment, the expression level of at least two or more of the genes noted above is detected.

[0074] In another embodiment, the composition is an oral capsule or tablet, a liquid suspension, or a chip or wafer for oral delivery. In another embodiment, the composition is formulated for intravenous use, for intramuscular use, for subcutaneous delivery or for intrapertusional injection.

[0075] In another embodiment, the composition comprising oleuropein or an analogue, derivative or oleuropein aglycone or their analogues, or derivatives thereof, or hydroyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or eulenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside, or their analogues, or derivatives thereof, or any combination thereof including but not limited to olive leaf extract may be targeted to e.g., the oleuropein or analogue or derivative thereof can be linked, fused or conjugated to, or enveloped in the adipocyte by attachment of the oleuropein or analogue or derivative thereof to adipose tissue via Resistin or Resistin-Like Molecules (RELMs or FIZZ1-3) or Adipocyte-Specific Secretory Factor (ADSF) Proteins or antibodies to such proteins, as well as Leptin or Leptin Receptor antibodies, AcrP30/Adiponectin or Adipin antibodies, Orexins or Orexin Receptor antibodies, a Glucose Transporter (GLUT1-14) antibody, or an antibody to a Hypoxia Induced Factor (HIF-1alpha, beta).

[0076] In yet another particular embodiment, the composition comprising the oleuropein or an analogue or derivative thereof, or oleuropein aglycone or their analogues, or derivatives thereof, or hydroyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or eulenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a
secoiridoid glycoside, or their analogues, or derivatives thereof, or any combination thereof including but not limited to olive leave extract, is targeted to a prediocyte and its specific proteins such as Preadipocyte factor-1 (Pref-1), which is an epidermal growth factor-like domain-containing transmembrane protein and is implicated in inhibiting preadipocytes differentiation and component of complement (C1) or C1q, which is a serum glycoprotein involved in immune complexes to mediate adipocyte differentiation and maturation.

[0077] In yet another particular embodiment, the composition comprising the oleuropein or an analogue or derivative thereof, or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or a secoiridoid glycoside, or their analogues, or derivatives thereof, or any combination thereof including but not limited to olive leave extract, is targeted to a mesenchymal stem cell (MSC). MSCs differentiate into adipocytes, chondrocytes, osteoblasts, and myoblasts. Accordingly, in yet another embodiment, the invention provides a method of transfurther-iating adipocytes into osteoblasts, myoblasts and chondrocytes, wherein the method comprises administering to a mammal in need thereof a therapeutically effective amount of oleuropein or an analogue, or derivative thereof, or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside, or their analogues, or derivatives thereof, or any combination thereof including but not limited to olive leave extract. Thus, these stem cells are promising candidates for adipogenesis management by oleuropein and derivatives (as mentioned above) targeting and/or ex-vivo treatment so as to modulate adipocyte differentiation, trans-differentiation and de-differentiation. The application of oleuropein and derivatives (as mentioned above) in this system is not only effective in treating obesity and related metabolic syndromes by modulating adipocyte differentiation and development but also provide tools to manipulate adult stem cells for cell-based approaches in regenerative medicine. For example, aged and osteoporotic patients have a high fat to bone ratio in their bones compared with young and healthy counterparts, an outcome possibly due to the conversion of bone to fat cells. Application of oleuropein or an analogue, or derivative thereof, or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside, or their analogues, or derivatives thereof, or any combination thereof including but not limited to olive leave extract to mediate transdifferentiation between osteoblasts and adipocytes should be of relevance to the development of therapeutic control of bone loss in osteoporosis. For specific targeting of MSC, these cells express lym-kaemia inhibitory factor, macrophage colony-stimulating factor, and stem cell factor specifically. Thus these proteins and their antibodies can be used for targeting.

[0078] In another particular embodiment, the targeting reagent is lipid soluble.

[0079] In a fourteenth aspect, the invention provides a method of determining whether a subject is responsive to treatment with a therapeutic such as oleuropein or a therapeutic having analogous activity to oleuropein, comprising determining the level of expression of one or more genes or gene products selected from the group consisting of PPARγ2, LPL, or C2P2 in a cell or a bodily fluid sample of the subject, and/or determining the differential expression of the adipogenic, lipogenic and lipolytic genes or gene products, wherein a change in level of expression of any one or more of these genes or gene products in a cell or bodily fluid sample of the subject relative to that in a cell or bodily fluid sample of a subject that was not treated with oleuropein or a therapeutic having analogous activity to oleuropein, or a change of the profiles and or functions of adipogenic, lipogenic and lipolytic genes, indicates that the subject is responsive to treatment with oleuropein or an oleuropein analogue or derivative.

[0080] A fifteenth aspect of the invention provides a method for determining whether a subject is responsive to treatment with a therapeutic having analogous activity to oleuropein, comprising determining the profile of expression of adipogenic, lipogenic and lipolytic, genes or gene products selected from the group consisting of PPARα, PPARγ and PPARε in cells of the subject, wherein a higher or lower level of expression or function/activity of any one of these genes in the cells of the subject relative to that in cells of a subject that was not treated with a PPAR ligand indicates that the subject is responsive to treatment with the PPAR ligand.

[0081] In one particular embodiment, the cells are obtained from whole blood, e.g. peripheral blood mononuclear cells (PBMC). In another particular embodiment, the cells are adipocytes. In yet another particular embodiment, the cells are pre-adipocytes. In yet another embodiment, the cells are mesenchymal stem cells. In yet another particular embodiment, the bodily fluid sample is plasma or serum.

[0082] In a sixteenth aspect, the invention provides a method for predicting whether a subject would be responsive to treatment with a compound having analogous activity to oleuropein, comprising treating the subject with the candidate or test compound followed by collecting preadipocytes or whole blood of the subject and determining the profile of expression of at least one of the genes selected from the group consisting of PPARγ2, LPL, C2P2, leptin, TNFα, IL-6, PAI-1, adipin, complement factor C3, angiotensin, hormone-sensitive lipase (HSL), lipoprotein lipase (LPL), adiponectin, fatty acid-binding protein 4, perilipin and CCAAT-enhancer binding protein, and enzymes of energy metabolism such as glycero-3-phosphate dehydrogenase 1, acetyl-Coenzyme A carboxylase, phosphoenolpyruvate carboxykinase and pyruvate dehydrogenase kinase, wherein a change in the profile of expression (the level of expression and/or the change in function or activity) of at least one of these genes relative to expression in cells or in a whole blood sample of subjects not treated with oleuropein, indicates that the subject would be responsive to treatment with the compound having analogous activity to oleuropein. In another particular embodiment, a method for predicting whether a subject would be responsive to treatment with a compound having analogous activity to oleuropein, comprises incubating cells of the subject with oleuropein and determining the level of expression of at least one of the genes selected from the group consisting of PPARε and PPARδ as well as their responsive genes in lipid metabolism, lipid utilization and energy uncoupling. The expression of these genes can be profiled in a cellular sample or in a blood sample, for example, whole blood, or plasma or serum. In
addition to PPARα, PPARδ and PPARγ and their responsive genes, the levels of glucose, fasting insulin, insulin AUC, total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride, adiponectin, free fatty acid and TNFα, are also indications of an effect of oleuropein or an analogue or derivative thereof.

A seventeenth aspect of the invention provides a method for purifying biologically active oleuropein, an oleuropein derivative, or an oleuropein metabolite from olive leave extract, the method comprising the steps:

- (0085) a) extracting the oleuropein, or a derivative or metabolite thereof by heating olive leaves to at least 80°C, but not above 85°C, for about 10-12 hours in water, saline or phosphate buffer;
- (0086) b) collecting the liquid from step a) and repeating the extracting procedure of step a) at least one additional time;
- (0087) c) combining the liquid from steps a) and b) and centrifugating at least 20,000g for at least 30 minutes to remove small particulates or insoluble material;
- (0088) d) concentrating the liquid from step c) by lyophilization until dried;
- (0089) e) dissolving the dried extract from step d) into sterile water to a concentration of about 10-20 mg/ml and filter sterilizing;
- (0090) f) distributing the material from step e) into sterile cryotubes under aseptic conditions and storing at a temperature of at least -80°C;
- (0091) g) characterizing and analyzing the extract by a method selected from the group consisting of high pressure liquid chromatography (HPLC), thin layer chromatography (TLC) and liquid chromatography-mass spectrometry (LC-MS); and
- (0092) h) comparing the material obtained in step g) with a known standard.

In one embodiment, the ratio of dried leaves to water, saline or phosphate buffer in step c) above is about 1 gram of dried leaves to about 40 ml of water, saline or phosphate buffer.

In another embodiment, the filter sterilizing is accomplished using a filter of about 0.45 microns.

An eighteenth aspect of the invention provides a method for synthesizing biologically active hydroxytyrosol, or a hydroxytyrosol analogue or derivative, comprising:

- (0096) a) providing 3,4-dihydroxyphenylacetic acid;
- (0097) b) reacting 3,4-dihydroxyphenylacetic acid with acetyl chloride and methanol at stirring at room temperature overnight to yield 3,4-dihydroxyphenylacetic ester;
- (0098) c) purifying the 3,4-dihydroxyphenylacetic ester by column chromatography;
- (0099) d) dissolving the 3,4-dihydroxyphenylacetic ester of step c) in tetrahydrofuran;
- (1000) e) adding 1 molar LiAIH4 into the reaction mixture from step d);
- (1001) f) stirring at 0°C for about 2 hours;
- (1002) g) purifying the hydroxytyrosol, or a hydroxytyrosol analogue or derivative by column chromatography; and
- (1003) h) characterizing the hydroxytyrosol, or a hydroxytyrosol analogue or derivative by liquid chromatography-mass spectrometry.

A nineteenth aspect of the invention provides for the preparation of biologically active hydroxytyrosol, comprising:

- (1004) a) providing oleuropein;
- (1005) b) treating the oleuropein of step a) with betaglucosidase to yield oleuropein aglycone;
- (1006) c) hydrolyzing the oleuropein aglycone to yield hydroxytyrosol and elenolic acid.

In one embodiment, the hydrolyzing of step c) above is accomplished by treating the oleuropein aglycone of step b) with an esterase to yield hydroxytyrosol and elenolic acid.

A twentieth aspect of the invention provides for a method of inhibiting human immunodeficiency virus (HIV) infectivity, comprising administering a therapeutically effective amount of oleuropein or hydroxytyrosol, or a derivative or analogue thereof. In one embodiment, the administering may be in vitro or in vivo.

In another embodiment, the oleuropein or hydroxytyrosol, or a derivative or analogue thereof, prevents the fusion of the virus to the host cell, and/or prevents cell to cell transmission of the virus, and/or prevents viral replication by binding to the active catalytic site of the HIV integrase.

A twenty-first aspect of the invention, the inhibiting of HIV infectivity is the result of the binding of oleuropein or hydroxytyrosol, or a derivative or analogue thereof, to a conserved hydrophobic pocket on the surface of the central trimeric coiled-coil of the HIV gp41 fusion domain.

In yet another embodiment, the oleuropein or hydroxytyrosol, or a derivative or analogue thereof, inhibits the N-terminal heptad repeat (NHR) coiled-coil trimer N36 helices and interferes with the formation of 6HB with the C-terminal heptad repeat (CHR), C34.

In yet another embodiment, the oleuropein or hydroxytyrosol, or a derivative or analogue thereof, inhibits 6HB formation.

In yet another embodiment, the oleuropein or hydroxytyrosol, or a derivative or analogue thereof, binds to both regions I and II of said integrase.

In yet another embodiment, the oleuropein or hydroxytyrosol, or a derivative or analogue thereof, inhibits one or more of the following activities of the integrase:

- (1016) a) inhibition of 3′ processing activity of the HIV integrase;
- (1017) b) inhibition of strand-transfer activity of the HIV integrase; or
- (1018) c) inhibition of the disintegration activity of the HIV integrase.

In yet another embodiment, the human immunodeficiency virus is HIV-1 or HIV-2.

In yet another embodiment, the oleuropein or hydroxytyrosol, or a derivative or analogue thereof, inhibits the fusion or replication of any one of an M tropic or a T tropic strain from different HIV clades.

Other objects and advantages will become apparent from a review of the ensuing detailed description and attendant claims taken in conjunction with the following illustrative drawings. All references cited in the present application are incorporated herein in their entirety.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 Demonstrates that oleuropein modulates adipocyte differentiation
[0123] FIG. 2. Demonstrates that oleuropein de-differentiates adipocytes and allows transdifferentiation.

[0124] FIG. 3. Represents aorta from apoE knock out mice fed a Western diet for 4 months, and then stained with Oil Red 0.

[0125] FIG. 4. Shows aortic lesions in apoE knock out mice fed a Western diet for 4 months.

[0126] FIG. 5. Shows the predicted PPARδ-oleuropein binding structure.

[0127] FIG. 6. Shows the predicted PPARγ-oleuropein binding structure.

[0128] FIG. 7. Shows the predicted PPARα-oleuropein binding structure.

[0129] FIG. 8. Shows the chemical structure of oleuropein.

[0130] FIG. 9. Shows the HPLC elution profile of Olive Leaf Extract (OLE). The numbers represent the identified peaks. The identities of the peaks are shown in Table 5. Peak 6 is oleuropein, and contains the bulk of the biological activity.

[0131] FIG. 10. Shows the chemical structure of oleuropein and its major metabolites.

[0132] FIG. 11. Shows the results of the standardization of olive leaf extract by liquid chromatography-mass spectrometry (LC-MS) based on oleuropein content.

[0133] FIG. 12. Shows the results of the quantitation of oleuropein and metabolites in serum and urine of mice fed oleuropein.

[0134] FIG. 13. LC-MS analysis of Ole and HT, metabolism of Ole and chemical synthesis of HT.

[0135] FIG. 14. LC-MS analysis of Ole and HT, metabolism of Ole and chemical synthesis of HT.

[0136] FIG. 15. Molecular docking of Ole and HT with HIV-1 gp41.

[0137] FIG. 16. The effect of Ole and HT on the formation of 6HB between HIV-1 peptides N36 and C34.

A. Native PAGE was carried out in Tris-glycine 18% gels, at 120 V constant voltages at room temperature for 2 h. The gel was then stained with Coomassie Blue and analyzed by densitometry. Lane 1, molecular weight markers; lane 2, N36; lane 3, C34; lane 4 fusion complex, (N36+C34); lanes 5-8 and 9-12, in the presence of Ole and HT at 25, 50, 75 and 100 nM. B. Western analysis, CD spectra for N36 (Green), C34 (Red), (N36+C34) 6HB (Blue), and N36+C34 in the presence of 25 and 50 nM of Ole or HT.

[0138] FIG. 17. Molecular docking of Ole and HT with HIV-1 integrase.

The predicted binding structures of Ole (A) and HT (B) inside the HIV-1 integrase catalytic site. Integrase is shown as a surface model, while Ole and HT are shown as van der Waals models and the purple sphere represents Mg²⁺. Hydrogen bonds formed by Ole (C) and HT (D) with integrase are indicated as green dotted lines, and the integrase backbone is represented by the cyan ribbon.

[0139] FIG. 18. The effect of Ole and HT on HIV-1 integrase 3' processing, strand transfer and disintegration activities.

A. The Effect of Ole and HT on 141V-1 Integrase 3'-Processing Activity.

[0140] FIG. 19. Left: Schematic representation of the 3'-processing activity of HIV-1 integrase. A 5'-32P-labeled 21-mer of HIV-1 LTR U3 double-stranded (ds) DNA was used as the substrate. Specific cleavage of the dinucleotide GT from the 3' end of the substrate results in the formation of 19-mer 3'-recessed U3-GT.

Right: Inhibition of HIV-1 integrase 3'-processing activity by Ole and HT. Inhibition was monitored by the formation of labeled 19-mer product. Lane 1, the 21-mer substrate, 5'-32P-labeled U3. Lane 2, cleavage of the 3' GT of the 21-mer substrate by HIV-1 integrase results in the formation of the 19-mer 3'-recessed U3-GT. Lanes 3-7 or 8-12, in the presence of 25, 50, 75, 100, and 200 nM Ole or HT.

B. The Effect of Ole and HT on HIV-1 Integrase Strand Transfer Activity.

[0141] FIG. 20. Left: Schematic representation of strand transfer (integration). Pre-cleaved 5'-32P-labeled U3-GT 19-mer was used as the viral substrate and, unlabelled pUC18 DNA (2.69 kb) was used as the heterologous target substrate.

Right: Inhibition of strand-transfer activity of HIV-1 integrase by Ole and HT. Integration was monitored by the conversion of the unlabelled plasmid into labeled DNA. Lanes 1, 5'-32P-labeled size marker, HindIII fragments of β phage DNA. Lanes 2, target substrate; pUC18; because it is unlabelled, it is not seen in the autoradiogram. Lanes 3, the product of integration (ST) by HIV-1 integrase. The integration of the 5'-32P-labeled U3-GT into pUC18 results in the appearance of labeled band at 2.69 kb, corresponding to the size of pUC18. Lanes 4-8, in the presence of 25, 50, 75, 100 and 200 μM Ole or HT.

C. The effect of Ole and HT on HIV-1 Integrase Disintegration Activity.

Left: Schematic representation of the disintegration activity of HIV-1 integrase. The 5' 32P-labeled 38-mer dumbbell was used as the substrate and shown with the predicted secondary structure. Disintegration yields a 32P-labeled 14-mer consisting of the viral sequences in the hairpin stem and a 24-mer unlabelled target sequence that has been repaired.
Right: Inhibition of disintegration activity of HIV-1 integrase by Ole and HT. Lane 1, the 5' 32P-labeled 38-mer dumbbell substrate. Lane 2, treatment with HIV-1 integrase results in the formation of the 5' 32P-labeled 14-mer disintegration product. Lanes 3, 4, 5, 6, 7 disintegration assays in the presence of 25, 50, 75, 100, and 200 nM Ole. Lanes 8, 9, 10, 11, 12, in the presence of 25, 50, 75, 100, and 200 nM HT.

DETAILED DESCRIPTION OF THE INVENTION

[0142] Before the present methods and treatment methodology are described, it is to be understood that this invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for purposes of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only in the appended claims.

[0143] As used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural references unless the context clearly dictates otherwise. Thus, for example, references to "the method" includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

[0144] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference.

DEFINITIONS

[0145] The terms used herein have the meanings recognized and known to those of skill in the art, however, for convenience and completeness, particular terms and their meanings are set forth below.

[0146] The term "adipocyte" refers to a cell existing in or derived from fat tissue which is terminally differentiated. In their differentiated state, adipocytes assume a rounded morphology associated with cytoskeletal changes and loss of mobility. They further accumulate lipid as multiple small vesicles that later coalesce into a single, large lipid droplet displacing the nucleus. The term "human adipocyte" refers to an adipocyte existing in or isolated from human fat tissue. Adipocytes play a critical role in energy homeostasis. They synthesize and store lipids when nutrients are plentiful, and release fatty acids into the circulation when nutrients are required. Numerous adipogenic genes are expressed in functional adipocytes, whereas they are not expressed in preadipocytes in which lipid are not accumulated either. Adipocyte development has been extensively studied in cell culture as well as in animal models. There are several lines of evidence supporting that adipose tissue dysfunction plays an important role in the pathogenesis of type II diabetes mellitus, i.e. failure of adipocyte differentiation is a predisposition to developing diabetes, (see, e.g., Danforth (2000) Nature Genetics 26: 13).

[0147] The term "adipogenic gene expression" refers to the expression of several genes known as "adipogenesis marker genes" and more particularly refers to one or more genes specifically associated with a specific adipogenesis/differentiation stage. Such marker genes are well-known in the art. For example, Peroxisome Proliferator-Activated Receptor y (PPARγ), lipoprotein lipase (LPL), and the adipocyte-selective fatty acid binding protein (the aP2 gene). In addition, other differentiated adipocyte marker genes include glycerol-phosphate dehydrogenase (GPD1), fatty acid synthase, acetyl CoA carboxylase, malic enzyme, G6P 4, and the insulin receptor (see Spiegelman et al. J. Biol. Chem. 268: 6823-6826, 1993, incorporated herein by reference). Preadipocytes also have characteristic marker genes, such as the cell surface antigen recognized by the monoclonal antibody AD-3. Expression level changes of the various isoforms of the CEBP (CCAAT/enhancer-binding proteins) family of transcription factors may also indicate different stages of adipogenesis (see Yu and Hauksson, Exp Cell Res Dec. 15, 1993; 245(2): 343-9). Other genes include the lipolytic genes involved in the mobilization and 1-oxidation of stored fat as well as those in the mitochondria involved in thermogenesis (the uncoupling enzymes, factors and modulators) such as PPAR α, PPARβ, and their related genes, hormone-sensitive lipase (HSL), lipoprotein lipase (LPL), and enzymes of energy metabolism such as glyceral-3-phosphate dehydrogenase 1, acyl-coenzyme A carboxylase, phosphoenolpyruvate carboxykinase and pyruvate dehydrogenase kinase.

[0148] The phrase "essentially pure" refers to a cell population, e.g., a human adipocyte population, that has been isolated from its natural source (e.g., has been isolated or purified from fat tissue, for example, from human fat tissue) and, through a purification step or series of purification steps, has been separated from other cells (e.g., non-adipocyte cells) and cellular debris. An essentially pure cell population, as defined according to the instant invention, is at least 90% pure, i.e., at least 90% of the cells are of the desired cell type (e.g., human adipocytes) and less than 10% are contaminating (e.g. non-adipocyte) cells. In a preferred embodiment, an essentially pure cell population (e.g., an essentially pure adipocyte population) is at least 95% pure. In a more preferred embodiment, an essentially pure cell population (e.g., an essentially pure adipocyte population) is at least 96%, 97%, 98%, 99% or 100% pure).

[0149] The phrase "differentiation-inducing agent" refers to a compound or agent that initiates or stimulates the differentiation of preadipocytes into adipocytes. Preferred "differentiation-inducing agents" include but are not limited to insulin, insulin-sensitizing agents, substrates for lipid synthesis, PPAR ligands (e.g., natural ligands, for example, prostaglandin J2, and synthetic ligands, for example, thiazolidinediones, and the like). The phrase "differentiation-promoting agent" refers to a compound or an agent that enhances or accelerates the differentiation of preadipocytes into adipocytes. Preferred "differentiation-inducing agents" include but are not limited to insulin, insulin-sensitizing agents, substrates for lipid synthesis, PPAR ligands, and the like. "Differentiation-inducing agents" or "differentiation-promoting agents" vary considerably in effectiveness but share common effects on several cellular signaling pathways including, but not limited to: (1) tyrosine kinase pathways (e.g., IGF-1-mediated tyrosine kinase pathway); (2) adenyl cyclase/phosphodiesterase signaling pathways; (3) steroid/thyroid/peroxisome proliferator activated (PPAR)/retinoid nuclear receptors signaling pathways; and (4) protein kinase signaling pathways (Meadowald, O. A. et al. (1995) Annu. Rev. Biochem 64:345-73; Smas, C. M. et al. (1995) Biochem J. 309, 607-710; Come-

[0150] Markers of differentiation include (presented in order of detectable changes in expression): (1) cytoskeletal genes; (2) lipoprotein lipase (LPL) and collagen isoforms; (3) adipocyte fatty acyl binding protein (A2) and glycerol-3-phosphate dehydrogenase (G3PD); and/or (4) the insulin sensitive glucose transporter (GLUT4), angiotensinogen (ang), apolipoprotein E (apoE), leptin, adipin (complement factor D), protein C3, factor B, and other genes occur that contribute to the endocrine/paracrine function of adipose tissue. Increased fat cell mass, which is dependent on the balance between rates of adipogenesis, lipogenesis and lipolysis.

[0151] “Modulation” or “modulates” or “modulating” refers to up regulation (i.e., activation or stimulation), down regulation (i.e., inhibition or suppression) of a response, or the two in combination or apart. As used herein, a fat cell, preadipocyte or adipocyte “modulator” or “modulating” compound or agent is a compound or agent that modulates at least one biological marker or biological activity characteristic of fat cells and/or fat tissue. The term “modulating” as related to adipocyte differentiation or adipogenic gene expression, refers to the ability of a compound or agent to exert an effect on adipocyte differentiation, de-differentiation or transdifferentiation or to alter the expression of at least one gene (as noted above) related to adipogenesis. In addition, compounds or agents of the invention modulate at least one of (1) differentiation-specific gene expression, (2) lipid metabolism (e.g., lipogenesis and/or lipolysis), (3) fatty acid uptake, (4) fat accumulation and/or (5) accumulation of cytoplasmic lipid and (6) modulation of PPAR activity.

[0152] “Differentiation” or “differentiation” as used herein, generally refers to the process by which precursor or progenitor cells differentiate into specific cell types. In the matter of the present invention, the term refers to the process by which pre-adipocytes become adipocytes. Differentiated cells can be identified by their patterns of gene expression and cell surface protein expression. The genes associated with adipocyte differentiation are noted above. As an example, cells of adipocyte lineage typically express the following genes: Ucp, PPARγ and C/EBPβ (see, e.g., Kozak and Kozak, Endocrinology 134(2):906-13 (1994)) and Lee et al., J. Clin. Invest. 111 (4): 453-461 (2003). As used herein, the term “differentiate” refers to having a different character or function from the original type of tissues or cells. Thus, “differentiation” is the process or act of differentiating.

[0153] “De-differentiate” or “dedifferentiation” as used herein, refers to the process by which lineage committed cells reverse their lineage commitment and become precursor or progenitor cells. De-differentiated cells can be identified by loss of patterns of gene expression and cell surface protein expression associated with the lineage committed cells. A loss of expression or decrease in expression levels of one or more of the adipocyte genes noted above indicates that an adipocyte has undergone dedifferentiation.

[0154] “Transdifferentiation” refers to the process by which precursor or progenitor cells pre-committed to cell types of one lineage differentiate into specific cell types of another lineage, e.g., pre-adipocytes can transdifferentiate into osteoblasts and vice versa. Transdifferentiated cells can be identified by their patterns of gene expression and cell surface protein expression. Typically, cells of an osteoblast lineage express genes such as, for example, alkaline phosphatase, collagen type I, osteocalcin, and osteopontin; and bone specific transcription factors such as, for example, Oster1/Runx2, Osx, gsc, Dlx1, Dlx5, Mox1, Car1, Hoxa1, Hoxa2, Hoxa3, Hoxb1, rac28, Isp1, Pax1, Pax3, Pax5, TBX2, TBX4, TBX5, and Brachury (see, e.g., Olsen et al., 2000 supra and Nakashima et al., Cell 108(1):17-29 (2002). The cells can be transdifferentiated within the same progenitors. For example, mesenchymal stem cells or marrow stromal cells (MSCs), are stem cells that can differentiate into osteoblasts, chondrocytes, myocytes, adipocytes, neuronal cells, and, as described latterly, into beta-pancreatic islets cells. Thus, these cells can be cross transdifferentiated under optimal culture conditions and/or growth factors. MSCs cultured in the presence of transformation growth factor (TGF), specifically bone morphogenetic protein (BMP), will differentiate into chondrocytes, whereas MSCs cultured in serum with ascorbic acid, inorganic phosphate and dexamethasone will differentiate into osteoblasts. On the other hand, MSCs cultured under adipogenic conditions in the presence of adenosine, insulin and insulin-methylxanthine will differentiate into adipocytes.

[0155] Adipocyte differentiation is a multistep process controlled by the action of a complex interactive network of transcription factors in mammals. In the fruit fly the serpentine gene is critical for the genesis of the fruit fly fat body, which corresponds to mammalian liver and adipose tissue. The GATA family of transcription factors in mice are the mammalian homologues of the Drosophila serpentine gene. Both GATA-2 and GATA-3 directly bind to specific sites in the proximal promoter of the adipogenic transcription factor, peroxisome proliferator activated receptor-gamma (PPAR-gamma), and negatively regulate its activity. Both GATA-2 and GATA-3 expression are severely defective in the white adipose tissue of several different models of obesity. These results indicate that GATA-2 and GATA-3 are adipocyte markers and play an important role in adipogenesis.

[0156] As used herein, the term “candidate compound” or “candidate therapeutic” or “test compound” or “agent” or “test agent” refers to any compound or molecule that is to be tested. As used herein, the terms, which are used interchangeably, refer to biological or chemical compounds such as simple or complex organic or inorganic molecules, peptides, proteins, antibodies, oligonucleotides, polynucleotides, carbohydrates, or lipoproteins. A vast array of compounds can be synthesized, for example oligomers, such as oligopeptides and oligonucleotides, and synthetic organic compounds based on various core structures, and these are also included.
in the terms noted above. In addition, various natural sources can provide compounds for screening, such as plant or animal extracts, and the like. Compounds can be tested singly or in combination with one another. Agents or candidate compounds can be randomly selected or rationally selected or designed. As used herein, an agent or candidate compound is said to be “randomly selected” when the agent is chosen randomly without considering the specific interaction between the agent and the target compound or site. As used herein, an agent is said to be “rationally selected or designed,” when the agent is chosen on a nonrandom basis which takes into account the specific interaction between the agent and the target site and/or the conformation in connection with the agent’s action. Moreover, the agent may be selected by its effect on the gene expression profile obtained from screening in vitro or in vivo. For example, the gene expression data for primary human preadipocytes and adipocytes can be accessed online through databases including Pub Med, Human Genome Project (HGP), Gene Bank and PDB (Protein Data Bank).

[0157] Biochemically, “adipogenesis” is referred to as the process of fat cell formation. It involves commitment, differentiation and maturation of adipocytes. Adipogenic genes, gene products, enzymes, factors, and pathways are the genes, gene products, enzymes, factors, and pathways for adipocyte differentiation (adipogenesis or fat cell formation), and these genes are described throughout the present application. “Lipogenesis” is referred to as the process of lipid biosynthesis (fat formation), the conversion of carbohydrate or protein to fat and the synthesis of triglycerides from 2-monoglycerol and free fatty acids as well as from glycerol-3-phosphate and fatty acyl CoA. In addition, the synthesis of long chain fatty acids, using acetyl CoA as the primer and malonyl CoA as the addition unit also refers to lipogenesis. Lipogenic genes, gene products, enzymes, factors, and pathways are the genes, gene products, enzymes, factors, and pathways for lipid biosynthesis (fat formation), and these genes are also described throughout the present application. Thus, adipogenesis is different from lipogenesis and the two terms are not synonymous. In addition, “adipogenesis” refers to the process whereby adipose tissue, a mesodermal derivative, develops from preadipocytes. “Adipogenesis” refers to the process by which an undifferentiated precursor cell differentiates into an adipocyte (a fat cell, which is a cell characterized by the cellular function of fat storage e.g., in cytoplasmic lipid droplets). Preadipocytes that are involved in the process of adipogenesis include pre-adipocytes, mesenchymal stem cells and progenitor cells. Generally, diseases associated with adipogenesis include body weight disorders such as obesity and cachexia, and nonsmoking and smoking thermogenesis. Accordingly, in one aspect of the invention, the agents identified as modulators of adipogenesis are potentially useful for modulating body weight-related processes, including, for example, treatment of body weight disorders such as obesity and cachexia, and thermogenesis. Treatment of other obesity related disorders or conditions are also contemplated. Obesity related disorders or conditions may be selected from the group consisting of coronary artery disease/cardiovascular disease, hypertension, cerebrovascular disease, stroke, peripheral vascular disease, insulin resistance, glucose intolerance, diabetes mellitus, hyperglycemia, hyperlipidemia, dyslipidemia, hypercholesterolemia, hypertriglyceridemia, hyperinsulinemia, atherosclerosis, cellular proliferation and endothelial dysfunction, diabetic dyslipidemia, HIV-related lipodystrophy, peripheral vascular disease, cholesterol gallstones, cancer, menstrual abnormalities, infertility, polycytic ovaries, osteoarthritis, sleep apnea, metabolic syndrome (Syndrome X), type II diabetes, diabetic complications including diabetic neuropathy, nephropathy, retinopathy, cataracts, heart failure, inflammation, thrombosis, congestive heart failure, and any other cardiovascular disease related to obesity or an overweight condition. Obesity induced or related asthma, airway dysfunction and pulmonary disorders are also important diseases linked to obesity. “Lipolysis” or “lipolytic” refers to the process of lipid degradation/oxidation (fat burning, utilization, energy production). Lipolytic genes, gene products, enzymes, factors, and pathways are the genes, gene products, enzymes, factors, and pathways for lipid oxidation (fat burning, β-oxidation). “Thermogenesis” refers to the process of heat and energy generation, also known as energy uncoupling. Thermogenic genes, gene products, enzymes, factors, and pathways are the genes, gene products, enzymes, factors, and pathways for body heat generation and energy uncoupling. This process also increases metabolic rate.

[0158] The PPARs play a central role in adipocyte differentiation. PPAR gamma responsive genes in human adipocyte differentiation are briefly discussed below: Affymetrix profiling of gene expression in human adipocytes identified about 1000 genes that were significantly up-regulated subsequent to induction of differentiation and 278 statistically significantly down-regulated genes [Gene: 2006 Mar. 15: 369: 90-9]. In addition, it is also necessary to consider PPAR alpha and delta responsive genes. A recent report (Diabetologia, 2005 September; 48(9):1776-83, Epub 2005 Jul. 30) on microarray gene profiling of isolated abdominal subcutaneous adipocytes from 20 non-obese (BMI 25±3 kg/m²) and 19 obese (BMI 55±8 kg/m²) non-diabetic Pima Indians using Affymetrix HG-U135A GeneChip arrays showed that the most differentially expressed genes in adipocytes of obese individuals consisted of 433 upregulated and 244 downregulated genes. Of these, 410 genes could be classified into 20 functional Gene Ontology categories. The analyses indicated that the inflammation/immune response category was over-represented, and that most inflammation-related genes were upregulated in adipocytes of obese subjects. This study provides evidence supporting the active role of mature adipocytes in obesity-related inflammation. It also provides potential candidate genes for susceptibility to obesity.

[0159] Depending on the desired result, an agent identified to induce adipogenesis is potentially useful for increasing body weight and an agent identified to prevent adipogenesis is potentially useful for decreasing body weight. Adipogenesis in vivo and in vitro is subject to hormonal and transcriptional control, in part mediated by a cascade of transcription factors including members of the CCAAT/enhancer binding protein family, basic helix-loop-helix leucine zipper (bHLH-LZ) family, e.g., ADD1/SREBP1 and peroxisome proliferator activated receptor gamma (PPARgamma) (See, e.g., Wu et al. (1999) Transcriptional activation of adipogenesis Current Opin. Cell Biol 11:689-694. Rosen and Spiegelman (2000) Molecular regulation of adipogenesis Annu Rev Cell Dev Biol 16:145-171. for recent reviews, as well as Kim and Spiegelman (1996) ADD1/SREBP1 promotes adipocyte differentiation and gene expression linkedfatty acid metabolism Genes Dev 10:1096-1107). However details regarding cellular targets of such transcription factors remain largely unde-
terminated, as do the mechanisms underlying their action in physiological and pathological processes.

1060 “Subject” or “patient” refers to a mammal, preferably a human, in need of treatment for a condition, disorder or disease.

1061 The phrase “pharmacologically acceptable” refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a human. Preferably, as used herein, the term “pharmacologically acceptable” means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term “carrier” refers to a diluent, adjuvant, excipient, or vehicle with which the compound is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water or aqueous solution saline solutions and aqueous dextrose and glycerol solutions are preferably employed as carriers, particularly for injectable solutions. Suitable pharmaceutical carriers are described in “Remington’s Pharmaceutical Sciences” by E. W. Martin.

1062 A “therapeutically effective amount” or “an effective amount”, which are used interchangeably, is an amount sufficient to decrease or prevent the symptoms associated with the conditions disclosed herein, including diseases associated with obesity, or an amount to inhibit the infectivity of human immunodeficiency virus (HIV-1 and HIV-2) and other related conditions contemplated for therapy with the compositions of the present invention. The effect on HIV infectivity may be measured by any of the commonly used methods known to those skilled in the art, for example, cell fusion may be measured by assessing the level of syncytia formation. Viral replication may be measured by using PCR procedures to monitor the level of viral nucleic acid in a host cell.

1063 “Metabolic Syndrome” or otherwise known as “Syndrome X” means a disease characterized by spontaneous hypertension, dyslipidemia, insulin resistance, hyperinsulinemia, increased abdominal fat and increased risk of coronary heart disease. As used herein, the terms “Syndrome X”, “Metabolic Syndrome” and “Metabolic Syndrome X” shall mean a disorder that presents risk factors for the development of Type II diabetes mellitus and cardiovascular disease and is characterized by insulin resistance and hyperinsulinemia and may be accompanied by one or more of the following: (a) glucose intolerance, (b) Type II diabetes, (c) dyslipidemia, (d) hypertension and (e) obesity.

1064 “Obesity” refers to a condition in which the body weight of a mammal exceeds medically recommended limits by at least about 20%, based upon age and skeletal size. “Obesity” is characterized by fat cell hypertrophy and hyperplasia. “Obesity” may be characterized by the presence of one or more obesity-related phenotypes, including, for example, increased body mass (as measured, for example, by body mass index, or “BMI”), altered anthropometry, basal metabolic rates, or total energy expenditure, chronic disruption of the energy balance, increased Fat Mass as determined, for example, by DEXA (Dexa Fat Mass percent), altered maximum oxygen use (VO2), high fat oxidation, high relative resting rate, glucose resistance, hyperinsulinemia, insulin resistance, and hyperglycemia. See also, for example, Hopkinson et al. (1997) Am J Clin Nutr 65(2): 432-8 and Butte et al. (1999) Am J Clin Nutr 69(2): 299-307. “Overweight” individuals are generally having a body mass index (BMI) between 25 and 30. “Obese” individuals or individuals suffering from “obesity” are generally individuals having a BMI of 30 or greater. Obesity may or may not be associated with insulin resistance.

1065 An “obesity-related disease” or “obesity related disorder” or “obesity related condition”, which are all used interchangeably, refers to a disease, disorder, or condition, which is associated with, related to, and/or directly or indirectly caused by obesity. The “obesity-related diseases”, or the “obesity-related disorders” or the “obesity related conditions” include but are not limited to, coronary artery disease/ cardiovascular disease, hypertension, cerebrovascular disease, stroke, peripheral vascular disease, insulin resistance, glucose intolerance, diabetes melitus, hyperglycemia, hyperlipidemia, dyslipidemia, hypercholesteremia, hypertriglyceridemia, hyperinsulinemia, atherosclerosis, cellular proliferation and endothelial dysfunction, diabetic dyslipidemia, HIV-related lipodystrophy, peripheral vessel disease, cholesterol gallstones, cancer, menstrual abnormalities, infertility, polycystic ovaries, osteoarthritis, sleep apnea, metabolic syndrome (Syndrome X), type II diabetes, diabetic complications including diabetic neuropathy, nephropathy, retinopathy, cataracts, heart failure, inflammation, thrombosis, congestive heart failure, and any other cardiovascular disease related to obesity or an overweight condition and/or obesity related asthma, airway and pulmonary disorders.

1066 An individual “at risk” may or may not have detectable disease, and may or may not have displayed detectable disease prior to the treatment methods described herein. “At risk” denotes that an individual who is determined to be more likely to develop a symptom based on conventional risk assessment methods or has one or more risk factors that correlate with development of obesity or an obesity-related disease or a disease for which PPAR modulation provides a therapeutic benefit, or an individual who is more likely to acquire HIV through use of shared needles or those individuals who may practice unprotected sexual activity. An individual having one or more of these risk factors has a higher probability of developing obesity or an obesity-related disease, or HIV than an individual without these risk factors. Examples (i.e., categories) of risk groups are well known in the art and discussed herein.

1067 “Development” or “progression” of obesity herein means initial manifestations and/or ensuing progression of the disorder. Development of obesity can be detectable and assessed using standard clinical techniques, such as measurement of increased body mass (as measured, for example, by body mass index, or “BMI”), altered anthropometry, basal metabolic rates, or total energy expenditure, chronic disruption of the energy balance, increased Fat Mass as determined, for example, by DEXA (Dexa Fat Mass percent), altered maximum oxygen use (VO2), high fat oxidation, high relative resting rate, glucose resistance, hyperinsulinemia, insulin resistance, and hyperglycemia. See also, for example, Hopkinson et al. (1997) Am J Clin Nutr 65(2): 432-8 and Butte et al. (1999) Am J Clin Nutr 69(2): 299-307. However, development also refers to disease progression that may be undetectable. For purposes of this invention, development or progression refers to the biological course of the disease state. “Development” includes occurrence, recurrence, and onset.
As used herein “onset” or “occurrence” of obesity includes initial onset and/or recurrence.

[0168] As used herein, “delaying development” of obesity, or an obesity-related disease, means to defer, hinder, slow, retard, stabilize, and/or postpone development of the disease. This delay can be of varying lengths of time, depending on the history of the disorder and/or the medical profile of the individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop detectable disease. A method that “delays” development of disease is a method that reduces the extent of the disease in a given time frame, when compared to not using the method. Such comparisons are typically based on animal or clinical studies, using a statistically significant number of subjects, although this knowledge can be based upon anecdotal evidence. “Delaying development” can mean that the extent and/or undesirable clinical manifestations are lessened and/or time course of the progression is slowed or lengthened, as compared to not administering the agent. Thus the term also includes, but is not limited to, alleviation of symptoms, diminishment of extent of disease, and stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, and remission (whether partial or total) whether detectable or undetectable.

[0169] “Diabetes” refers to high blood sugar or ketoadiposis, as well as chronic, general metabolic abnormalities arising from a prolonged high blood sugar status or a decrease in glucose tolerance. “Diabetes” encompasses both type 1 and type II (Non Insulin Dependent Diabetes Mellitus or NIDDM) forms of the disease. The risk factors for diabetes include the following factors: waistline of more than 40 inches for men or 35 inches for women, blood pressure of 130/85 mmHg or higher, triglycerides above 150 mg/dl, fasting blood glucose greater than 100 mg/dl or high-density lipoprotein of less than 40 mg/dl in men or 50 mg/dl in women.

[0170] The term “hyperinsulinemia” refers to a state in an individual in which the level of insulin in the blood is higher than normal.

[0171] The term “insulin resistance” refers to a state in which a normal amount of insulin produces a subnormal biologic response relative to the biological response in a subject that does not have insulin resistance.

[0172] An “insulin resistance disorder” as discussed herein, refers to any disease or condition that is caused by or contributed to by insulin resistance. Examples include: diabetes, obesity, metabolic syndrome, insulin-resistance syndromes, syndrome X, insulin resistance, high blood pressure, hypertension, high blood cholesterol, dyslipidemia, hyperlipidemia, atherosclerotic disease including stroke, coronary artery disease or myocardial infarction, hyperglycemia, hyperinsulinemia and/or hyperproinsulinemia, impaired glucose tolerance, delayed insulin release, diabetic complications, including coronary heart disease, angina pectoris, congestive heart failure, stroke, cognitive functions in dementia, retinopathy, peripheral neuropathy, nephropathy, hyperuricemia, nephrolithiasis, gout, obstructive sleep apnea and respiratory problems, osteoarthritis, and prevention and treatment of bone loss, e.g., osteoporosis.

[0173] “Alpha-glucosidase inhibitors” act to inhibit alpha-glucosidase, which is an enzyme that converts fructose to glucose, thus these inhibitors delay the digestion of carbohydrates. The undigested carbohydrates are subsequently broken down in the gut, thereby reducing the post-prandial glucose peak. Suitable examples include, but are not limited to, acarbose, voglibose and miglitol.

[0174] “Sulfonylureas” increase insulin production by stimulating pancreatic beta cells, and therefore act as insulin secretagogues. The primary mechanism of action of sulfonylureas is to close ATP-sensitive potassium channels in the beta-cell plasma membrane, initiating a chain of events that result in insulin release. Suitable examples of sulfonylureas include, but are not limited to repaglinide. Agents affecting other components of Glucagon-like Peptide-1 (GLP-1) and its mimetics, Glucagon-like Peptide-1 (GLP-1) and its mimetics, Exendin and it’s mimetics, and Dipeptidyl Peptidase Inhibitors (DPPIV) are also contemplated for use with the invention.

[0175] “Biguanides” are compounds that decrease liver glucose production and increase the uptake of glucose. Suitable examples include, but are not limited to metformin, phenformin and buformin.

[0176] A “peroxisome proliferator activated receptor” or “PPAR” is a member of a family of nuclear receptors, distinguished in alpha (α), delta (δ), and gamma (γ) subtypes as described herein. As used herein the term “PPAR” refers to a peroxisome proliferator-activated receptor as recognized in the art. As indicated above, the PPAR family includes PPAR α (also referred to as PPAR or PPARalpha), PPAR δ (also referred to as PPAR beta, PPARδ or PPARdelta), and PPAR γ (also referred to as PPARγ or PPARgamma). The individual PPARs can be identified by their sequences, where exemplary reference sequence accession numbers are: NM_005036 (cDNA sequence for hPPARα (SEQ ID NO:1)), NP_005027 (protein sequence for PPARa (SEQ ID NO: 2)), NM_015860 (cDNA sequence for hPPARγ isoform 2 (SEQ ID NO: 3)), NP_056593 (protein sequence for hPPARγ isoform 2 (SEQ ID NO: 4)), NM_006238 (cDNA sequence for hPPARδ (SEQ ID NO: 5)), and NP_006229 (protein sequence for hPPARδ (SEQ ID NO: 6)). One of ordinary skill in the art will recognize that sequence differences will exist due to allelic variation, and will also recognize that other animals, particularly other mammals have corresponding PPARs, which have been identified or can be readily identified using sequence alignment and confirmation of activity, can also be used. One of ordinary skill in the art will also recognize that modifications can be introduced in a PPAR sequence without destroying PPAR activity. Such modified PPARs can also be used in the present invention, e.g., if the modifications do not alter the binding site conformation to the extent that the modified PPAR lacks substantially normal ligand binding.

[0177] A “PPAR modulating agent” or “PPAR modulator” refers to any agent that binds to any of the PPAR receptors and acts to either enhance the activity or function of that particular receptor (agonist) or acts to inhibit or depress the activity or
function of that particular receptor (antagonist). The PPAR receptors include the α, γ, and δ receptors. It is also possible to have the following types of PPAR modulators:

[0178] Selective PPAR modulators that serve as selective or partial agonist/antagonist and uncouple therapeutic effects from adverse side effects. For example, a selective partial PPAR γ agonist/antagonist that uncouples the therapeutic effect of insulin sensitizing activity from adipogenic effect (side effect for weight gain during treatment)

[0179] Dual PPAR modulators that act on two PPARs collectively, selectively or partially either as agonists or agonist-antagonists combined. For example, the dual PPARγ/PPARα agonists have two separate therapeutic targets in metabolic pathways in adipose tissue and liver. They may improve both hyperglycemia and atherogenic dyslipidemia and may further reduce the inflammatory component of atherogenesis.

[0180] Pan PPAR modulators that interact with all of the three PPARs collectively, selectively or partially either as agonists or agonist-antagonists combined. For example, the pan PPARγ/PPARα/PPARδ agonists have three separate therapeutic targets—metabolic pathways in adipose tissue, liver and muscle (and other tissues). They may improve hyperglycemia, atherogenic dyslipidemia, inflammatory component of atherogenesis, energy uncoupling and weight reduction.

[0181] “Thiazolidinediones” are insulin sensitizing drugs, which decrease peripheral insulin resistance by enhancing the effects of insulin at target organs and tissues. These drugs bind and activate the nuclear receptor, peroxisome proliferator-activated receptor-gamma (PPAR-gamma) which increases transcription of specific insulin-responsive genes. Suitable examples of PPAR-gamma agonists are the thiazolidinediones which include, but are not limited to rosiglitazone, pioglitazone, troglitazone, isaglitazone (known as MCC-555), 2-(4-hydroxy-2-methyl-3-oxo-1,4- benzoxazin-2-yl)ethoxy)-benzene acetic acid, and the like. Additionally, the non-thiazolidinediones also act as insulin sensitizing drugs, and include, but are not limited to GW2570, and the like.

[0182] The concept of “combination therapy” is well exploited in current medical practice. Treatment of a pathology by combining two or more agents that target the same pathogen or biochemical pathway sometimes results in greater efficacy and diminished side effects relative to the use of the therapeutically relevant dose of each agent alone. In some cases, the efficacy of the drug combination is additive (the efficacy of the combination is approximately equal to the sum of the effects of each drug alone), but in other cases the effect can be synergistic (the efficacy of the combination is greater than the sum of the effects of each drug given alone). As used herein, the term “combination therapy” means the two compounds can be delivered in a simultaneous manner, e.g. concurrently, or wherein one of the compounds is administered first, followed by the second agent, e.g. sequentially. The desired result can be either a subjective relief of one or more symptoms or an objectively identifiable improvement in the recipient of the dosage.

[0183] “Atherogenesis” is a process of forming atheromas, that is, plaques in the inner lining (the intima) of arteries. It is a process in which the immune system appears to take an active part, and refers to the build-up of plaque in the blood vessels. Accordingly, activated lymphocytes have been detected in human and marine plaques, sometimes even preceding the infiltrating lipid-laden macrophages. The atherosclerotic process entails a proliferative phenotype, involving, apart from lymphocytes and macrophages, also smooth muscle cells, which occupy lesions that are relatively more advanced. Furthermore, atherosclerosis is the accumulation of lipid, inflammatory cells, and fibrous tissue in the intima, which causes intimal thickening of large and mid-sized arteries. The clinical manifestations differ depending on the circulatory bed affected. The coronary arteries are particularly susceptible to atherogenesis; atherosclerosis of the coronary arteries may lead to angina pectoris and myocardial infarction. Dyslipidemia is a primary, major risk factor for coronary artery disease (CAD) and may even be a prerequisite for CAD, occurring before other major risk factors come into play. “Diet-induced atherogenesis” refers to the induction or initiation of the atherogenic process by consumption of food that contains a high fat content, which ultimately results in build-up of plaque in the blood vessels.

[0184] “Endothelial dysfunction” is a physiological dysfunction of normal biochemical processes carried out by endothelial cells, the cells that line the inner surface of all blood vessels, arteries and veins. Compromise of normal function of endothelial cells is characteristic of endothelial dysfunction. Normal functions of endothelial cells include mediation of coagulation, platelet adhesion, immune function, control of volume and electrolyte content of the intravascular and extravascular spaces. An important consequence of endothelial dysfunction is the inability of a vessel to dilate in response to physiological stimuli, such as increases in blood flow, reflecting impaired flow-dependent, endothelium-mediated vasodilation (FDD). Accumulating evidence suggests that endothelial dysfunction contributes to exercise intolerance, impaired myocardial perfusion, and left ventricular remodelling in congestive heart failure. Moreover, impaired endothelial function is associated with a number of disease states, including cardiovascular disease (CVD) and its major risk factors. Endothelial dysfunction precedes overt vascular disease by years and may itself be a potentially modifiable CVD risk factor. Although no gold standard for the measurement of endothelial function exists, the measurement of flow-mediated dilatation (FMD) in the brachial artery, assessed with Doppler ultrasoundography, is the most studied method and shows the most promise for clinical application. It is a well-tolerated, noninvasive, and low-risk procedure. Brachial artery FMD is an attractive screening tool for assessing endothelial dysfunction.

[0185] “Treatment” or “treating” refers to therapy, prevention and prophylaxis and particularly refers to the administration of medicine or the performance of medical procedures with respect to a patient, for either prophylaxis (prevention) or to cure (if possible) or reduce the extent of or likelihood of occurrence of the infirmity or malady or condition or event in the instance where the patient is afflicted. More particularly, as related to the present invention, “treatment” or “treating” is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward development of a disease. Treatment can slow, cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease, a symptom of the disease or the predisposition toward disease, e.g., by at least 10%. In the present invention, the treatments using the agents described may be
provided to slow or halt weight gain, or to aid in weight reduction, or to prevent fat accumulation or obesity, or to inhibit adipocyte differentiation or adipogenic gene expression, or to increase the amount or quality of lean muscle mass present in the mammal, or to prevent the infectivity of HIV, or to slow viral spread, or to alleviate one or more symptoms associated with the presence of the viral disease. More preferably, the goal is the treatment of obesity or obesity-related diseases, disorders or conditions and the morbidity associated with such conditions and the treatment of human immunodeficiency virus infections and the symptoms and sequelae associated with the presence of the viral infection.

[0186] “Diagnosis” or “screening” refers to diagnosis, prognosis, monitoring, characterizing, selecting patients, including participants in clinical trails, and identifying patients at risk for or having a particular disorder or clinical event or those most likely to respond to a particular therapeutic treatment, or for assessing or monitoring a patient’s response to a particular therapeutic treatment.

[0187] A “small molecule” refers to a composition that has a molecular weight of less than 3 kilodaltons (kDa), and preferably less than 1.5 kilodaltons, and more preferably less than about 1 kilodalton. Small molecules may be nucleic acids, peptides, polypeptides, peptidomimetics, carbohydrates, lipids or other organic (carbon-containing) or inorganic molecules. As those skilled in the art will appreciate, based on the present description, extensive libraries of chemical and/or biological mixtures, often fungal, bacterial, or algal extracts, may be screened with any of the assays of the invention to identify compounds that modulate a bioactivity. A “small organic molecule” is an organic compound (or organic compound complexed with an inorganic compound (e.g., metal)) that has a molecular weight of less than 3 kilodaltons, and preferably less than 1.5 kilodaltons, and more preferably less than about 1 kilodalton.

[0188] “Disease associated with PPAR γ, δ, or α” or “a disease associated with a PPAR γ, δ, or α receptor” includes diseases treatable with a ligand of any of the above-noted PPAR receptors, such as but not limited to Type II diabetes and obesity, or other obesity related disorders. Diseases related to PPAR expression and/or activity would also be considered as associated with the PPAR receptor, such as obesity or other disorders expected to be affected by alterations in the PPAR’s role in adipocyte differentiation, de-differentiation and transdifferentiation as well as adipocyte functions including fat synthesis, storage, utilization and energy uncoupling. The diseases associated with the modulation of the different PPAR receptors are known to those skilled in the art. For example, possible diseases and/or risk factors associated with PPARs are: the metabolic syndrome and its associated risk factors for atherosclerotic cardiovascular disease (ASCVD) and diabetes, atherogenic dyslipidemia, elevated blood pressure, elevated plasma glucose, a prothrombotic state and a pro-inflammatory state. In addition, other conditions, including fatty liver, polycystic ovary disease, sleep apnea, cholesterol gallstones, asthma and cancer.

[0189] “Propylaphatic” or “therapeutic” treatment refers to administration to the host of one or more of the subject compositions. If it is administered prior to clinical manifestation of the unwanted condition (e.g., disease or another unwanted state of the host animal) then the treatment is prophylactic, i.e., it protects the host against developing the unwanted condition, whereas if administered after manifestation of the unwanted condition, the treatment is therapeutic (i.e., it is intended to diminish, ameliorate or maintain the existing unwanted condition or side effects therefrom).

[0190] “Therapeutic agent” or “therapeutic” refers to an agent capable of having a desired biological effect on a host. Chemotherapeutic and genotoxic agents are examples of therapeutic agents that are generally known to be chemical in origin, as opposed to biological, or cause a therapeutic effect by a particular mechanism of action, respectively. Examples of therapeutic agents of biological origin include growth factors, hormones, and cytokines. A variety of therapeutic agents are known in the art and may be identified by their effects. Certain therapeutic agents are capable of regulating cell proliferation and differentiation. Examples include chemotherapeutic nucleotides, drugs, hormones, non-specific (non-antibody) proteins, oligonucleotides (e.g., antisense oligonucleotides that bind to a target nucleic acid sequence (e.g., mRNA sequence)), peptides, and peptidomimetics. Examples of other therapeutic agents that modulate PPAR gamma, alpha and delta are the thiazolidinediones, fibrates, fatty acids and eicosanoids.

[0191] “Therapeutic effect” refers to a local or systemic effect in animals, particularly mammals, and more particularly humans caused by a pharmacologically active substance. The term thus means any substance intended for use in the diagnosis, cure, mitigation, treatment or prevention of disease or in the enhancement of desirable physical or mental development and conditions in an animal or a human.

[0192] “Agonist” refers to an agent that mimics or up-regulates (e.g., potentiates or supplements) the bioactivity of a protein. An agonist may be a wild-type protein or derivative thereof having at least one bioactivity of the wild-type protein. An agonist may also be a compound that up-regulates expression of a gene or which increases at least one bioactivity of a protein. An agonist may also be a compound which increases the interaction of a polypeptide with another molecule, e.g., a target peptide or nucleic acid. An agonist may also be a compound that increase or up-regulate the activity and/or function of a protein, peptide, an enzyme or biofactor.

[0193] “Antagonist” refers to an agent that down-regulates (e.g., suppresses or inhibits) at least one bioactivity of a protein. An antagonist may be a compound which inhibits or decreases the interaction between a protein and another molecule, e.g., a target peptide or enzyme substrate. An antagonist may also be a compound that down-regulates expression of a gene or which reduces the amount of expressed protein present. An antagonist may also be a compound that decrease or down-regulate the activity and/or function of a protein, peptide, an enzyme or biofactor.

[0194] “Analogue” or “analogues” as used herein, refers to a chemical compound, a nucleotide, a protein, or a polypeptide that possesses similar or identical activity or function(s) as the chemical compounds, nucleotides, proteins or polypeptides having the desired activity and therapeutic effect of the present invention (e.g., to inhibit fat accumulation, or to modulate adipocyte differentiation or adipogenic gene expression or to treat obesity or obesity-related diseases, disorders or conditions), but need not necessarily comprise a compound that is similar or identical to those compounds of the preferred embodiment, or possess a structure that is similar or identical to the agents of the present invention. An agent having activity “analogous to ouluropopein” is an agent that possesses similar functions and activities as ouluropoein, including the effects on the adipogenic and lipolytic genes described herein, and on
adipocyte metabolism, differentiation, de-differentiation, or transdifferentiation or effects on the PPAR receptors described herein.

[0195] “Derivative” refers to the chemical modification of molecules, either synthetic organic molecules or proteins, nucleic acids, or any class of small molecules such as fatty acids, or other small molecules that are prepared either synthetically or isolated from a natural source, such as a plant, that retain at least one function of the active parent molecule, but may be structurally different. Chemical modifications may include, for example, replacement of hydrogen by an alkyl, acyl, or amino group. It may also refer to chemically similar compounds which have been chemically altered to increase bioavailability, absorption, or to decrease toxicity. A derivative polypeptide is one modified by glycosylation, pegylation, or any similar process that retains at least one biological or immunological function of the polypeptide from which it was derived.

[0196] As used herein in connection with the design or development of PPAR ligands, the term “bind” and “binding” and like terms refer to a non-convolent energetically favorable association between the specified molecules (i.e., the bound state has a lower free energy than the separated state, which can be measured calorimetrically). For binding to a target, the binding is at least selective, that is, the compound binds preferentially to a particular target or to members of a target family at a binding site, as compared to non-specific binding to unrelated proteins not having a similar binding site. For example, BSA is often used for evaluating or controlling for non-specific binding. In addition, for an association to be regarded as binding, the decrease in free energy going from a separated state to the bound state must be sufficient so that the association is detectable in a biochemical assay suitable for the molecules involved.

[0197] By “binding pocket” is meant a specific volume within a binding site. A binding pocket is a particular shape, indentation, or cavity in the binding site. Binding pockets can contain particular chemical groups or structures that are important in the non-covalent binding of another molecule such as, for example, groups that contribute to ionic, hydrogen bonding, van der Waals, or hydrophobic interactions between the molecules.

[0198] In the context of target molecules in the present invention, the term “crystal” refers to an ordered complex of target molecule, such that the complex produces an X-ray diffraction pattern when placed in an X-ray beam. Thus, a “crystal” is distinguished from a disordered or partially ordered complex or aggregate of molecules that do not produce such a diffraction pattern. Preferably a crystal is of sufficient order and size to be useful for X-ray crystallography. A crystal may be formed only of target molecule (with solvent and ions) or may be a co-crystal of more than one molecule, for example, as a co-crystal of target molecule and binding compound, and/or of a complex of proteins (such as a holoenzyme).

[0199] By “designing a ligand”, “preparing a ligand”, “discoversing a ligand”, and like phrases is meant the process of considering relevant data (especially, but not limited to, any individual or combination of binding data, X-ray co-crystallography data, molecular weight, clogP, and the number of hydrogen bond donors and acceptors) and making decisions about advantages that can be achieved with resort to specific structural modifications to a molecule, and implementing those decisions. This process of gathering data and making decisions about structural modifications that can be advantageous, implementing those decisions, and determining the result can be repeated as many times as necessary to obtain a ligand with desired properties.

[0200] By “docking” is meant the process of attempting to fit a three-dimensional configuration of a binding pair member into a three-dimensional configuration of the binding site or binding pocket of the partner binding pair member, which can be a protein, and determining the extent to which a fit is obtained. The extent to which a fit is obtained can depend on the amount of void volume in the resulting binding pair complex (or target molecule-ligand complex). The configuration can be physical or a representation of the binding pair member, e.g., an in silico representation or other model.

[0201] In the context of development of modulators using molecular scaffolds, by “ligand” is meant a molecular scaffold that has been chemically modified at one or more chemically tractable structures to bind to the target molecule with altered or changed binding specificity relative to the molecular scaffold. The ligand can bind with a greater specificity or affinity for a member of the molecular family relative to the molecular scaffold. A ligand binds non-covalently to a target molecule, which can preferably be a protein or enzyme.

[0202] By “orientation”, in reference to a binding compound bound to a target molecule is meant the spatial relationship of the binding compound and at least some of its constituent atoms to the binding pocket and/or atoms of the target molecule at least partially defining the binding pocket.

[0203] Binding compounds can be characterized by their affinity for the target molecule as measured by determining the dissociation constant or by measuring their effect on the activity of the target molecule. For example, the IC50 or EC50 is defined as the concentration of compound at which 50% of the activity of the target molecule (e.g., enzyme or other protein) activity being measured is lost (or gained) relative to activity when no compound is present. Activity can be measured using methods known to those of ordinary skill in the art, e.g., by measuring any detectable product or signal produced by occurrence of an enzymatic reaction, or other activity by a protein being measured. For PPAR agonists, activities can be determined as described in the Examples, or using other such assay methods known in the art.

[0204] In addition, the interaction of a ligand with its target structure can be assessed by measurement of the free energy in kcal/mol using approaches that involve AutoDock, Molecular Dynamics (MD) and Molecular Mechanics/Poisson-Boltzmann Solvent Accessible Surface (MM-PBSA) calculations. This is done using the crystal structures of the ligand binding domains of the PPAR receptors. Molecular docking is used to generate several distinct binding orientations. Molecular dynamics simulation is used to further relax the complex. MM-PBSA is then used to estimate the affinity for each binding mode. The binding modes with the lowest free energy are expected to be the most favorable. The energetically most favorable binding mode would provide for a free energy of <0 kcal/mol (negative value), and larger the free energy, the less favorable the interaction. The binding between a ligand and its target is unique in each system and cannot be compared to other systems. However, it can be stated that the smaller the free energy the more favorable the
binding. For favorable interactions, the free energy is negative. The more negative, the more favorable the interaction is.

[0205] The MM/PBSA approach uses a set of structures collected with molecular dynamics to evaluate free energies of binding. This method combines the molecular mechanical energies with the continuum solvent approaches. The molecular mechanical energies are determined based on AMBER force field and represent the internal energy (bond, angle and dihedral), van der Waals and electrostatic interactions. The electrostatic contribution to the solvation free energy is calculated with the Poisson-Boltzmann (PB) method. The hydrophobic contribution to the solvation free energy is determined with solvent-accessible-surface-area dependent terms. And estimates of conformational entropies can be approximated using data from AMBER. Briefly, MM/PBSA methodology involves the following steps:

[0207] 2. Calculate average molecular mechanical and solvation energies from a set of simulation snapshots.
[0208] 3. Use the quasi-harmonic approximation to approximate the entropy of the system.
[0209] 4. By putting energy terms together, free energy of binding can be estimated with the formula

\[
\Delta G = \Delta G(EE) + \Delta G\text{solvent} + \Delta G\text{solvation}
\]

Where \(\Delta G\) = standard free energy (kcal/mol), \(\Delta G(EE)\) = MM energy (kcal/mol), \(\Delta G\text{solvent}\) = free energy of solvent (kcal/mol), \(T\) = temperature (kelvin) and \(\Delta G\text{solvation}\) = entropic (kcal/mol K).


[0211] “Adipocyte-Specific Secretory Factor” (ADSF) is a small cysteine-rich protein secreted from adipose tissue (adipocyte-derived hormones, adipokines) that belongs to a gene family found in inflammatory zone (FIZZ2) or found in resistin-like molecule (RELM). ADSF has been implicated in modulating adipogenesis and insulin resistance. It inhibits adipogenesis, decreases plasma triglyceride and free fatty acid, improves glucose tolerance and insulin sensitivity. (Endocrine. (2006) February; 29(1):81-90; Proc Natl Acad Sci USA. (2004) April 27; 101(17):8780-5. Epub (2004) April 16.)

[0212] “Lepitin” is a 16 kDa adipokine (protein hormone), encoded by the obesity (ob) gene, expressed predominantly by adipocytes. It is important in regulating body weight, metabolism and glucose homeostasis. Smaller amounts of lepitin are also secreted by cells in the epithelium of the stomach and in the placenta. Lepitin receptors are highly expressed in areas of the hypothalamus known to be important in regulating body weight, as well as in neurons that control reproduction. (Int J Obes. (Lond). (2006) May 16)


[0215] “Glucose Transporter” (“Glut1-Glut14”) “Glut 4” is a glucose transporter expressed uniquely in muscle and fat tissues. It moves from intracellular sites to the plasma membrane following insulin stimulation and thus increases the rate of glucose transport into these tissues. GLUT4 expression is absent or low in most cultured cell models, which is a disadvantage for studies of glucose metabolism. However, in conditionally immortalized muscle there is a clear rise in GLUT4 levels following differentiation. (Clin Exp Pharmacol Physiol. 2006 April; 33(4):395-9. Am J Med. 2006 May; 119(5 Suppl 1):S10-S6).

[0216] “Hypoxia-inducible factor-1” (HIF-1) is a transcription factor. It plays a critical role in the transduction of the metabolic response to hypoxia. HIF-1 is composed of α and β subunits, the β-subunit is expressed constitutively whereas the α-subunit is induced by hypoxia. HIF-1 is activated in hypoxia by the stimulation of the expression of the HIF-1α subunit to form the functional transcription factor. HIF-1 are expressed in adipocytes and hypoxia cell culture leads to increased levels of adipokines. Insulin activates hypoxia-inducible factor-1α in human vascular smooth muscle cells via phosphatidylinositol-3 kinase and mitogen-activated protein kinase pathways (HIF-α/β, alpha, Beta, Diabetologia. (2006) May; 49(5):1049-63. Epub (2006) February 28; Biochem Biophys Res Commun. (2006) March 10; 341(2):540-56.) Am J Physiol Endocrinol Metab. (2006) March; 290(3): E591-7. Epub 2005 Oct 18.

[0217] “Pref-1” or Preadipocyte factor-1 is a transmembrane protein with epidermal growth factor-like domain. It is highly expressed in preadipocytes. Pref-1 expression is, however, completely abolished in adipocytes.

[0218] “Adipsin” is a serine protease that is secreted by adipocytes. It is deficient in several animal model of obesity. Adipsin has now been identified as the same protein as complement factor D or C3 convertase activator or properdin factor D. Its expression is induced upon differentiation of preadipocytes.

GENERAL DESCRIPTION

[0219] Olive leaf has a number of constituents, including oleuropein and several types of flavonoids, including rutin, apigenin, luteolin. In the studies presented herein, “ole” refers to oleuropein, and “OLE” refers to Olive Leaves Extract. Recent studies have centered on oleuropein (ole), which is a non-toxic glucoside isolated from olive leaves, which has been shown to have a number of beneficial medicinal properties. For example, early studies by Fleming et al. demonstrated the anti-microbial effects of oleuropein (Fleming, H. P. et al. (1973), Applied Microbiol. 26: 777-782). This work was further supported by the studies of Zanchelli, et al. (Zanchelli, D. et al. (2005) J. Food Prot. 68(7):1492-1496) and Miccol et al. (Miccol, V. et al. (2005), Antiviral Res. 66(2-

Oleuropein is reported herein to be a novel modulator of adipocyte differentiation, de-differentiation, trans-differentiation, and adipogenic and lipolytic genes and gene products expression and also decreases in fat accumulation and increases in fat burning. The oleuropein was prepared from olive leaf extract as reported in Lee-Huang et al. (Lee-Huang S; Zhang L; Huang P L; Chang Y T; Huang P L. “Anti-HIV activity of olive leaf extract (OLE) and modulation of host cell gene expression by HIV-1 infection and OLE treatment”. Biochemical & biophysical research communications. 2003; 307:1029; 307:1029). Commercial oleuropein was used as a standard for the comparison with the oleuropein preparations used in the present studies. In addition, oleuropein has been found to be effective in modulating of endothelial dysfunction and to reduce diet induced atherosclerosis. Moreover, oleuropein and derivatives or analogues thereof reduce the number of fat cells by modulating adipocyte differentiation, de-differentiation and trans-differentiation. Likewise, oleuropein and its derivatives or analogues thereof reduce fat accumulation, decrease the size of the fat cells, increase fat burning and expenditure by modulating adipocyte metabolism in terms of down-regulation of lipogenesis (fat formation), up-regulation of lipolysis (fat burning) and thermogenesis via modulating the lipogenic, lipolytic and thermogenic genes, gene products, enzymes, factors, and pathways. Accordingly, based on the findings presented here, oleuropein is proposed to be useful for treating obesity or obesity related disorders, such as, but not limited to, coronary artery disease/cardiovascular disease, hypertension, cerebrovascular disease, stroke, peripheral vascular disease, insulin resistance, glucose intolerance, diabetes mellitus, hyperglycemia, hyperlipidemia, dyslipidemia, hypercholesterolemia, hypertriglyceridemia, hyperinsulinemia, atherosclerosis, cellular proliferation and endothelial dysfunction, diabetic dyslipidemia, HIV-related lipodystrophy, peripheral vessel disease, cholesterol gallstones, cancer, menstrual abnormalities, infertility, polycystic ovaries, osteoarthritis, sleep apnea, metabolic syndrome (Syndrome X), type II diabetes, diabetic complications including diabetic neuropathy, nephropathy, cataracts, heart failure, inflammation, thrombosis, congestive heart failure, and any other cardiovascular disease related to obesity or an overweight condition. Moreover, while not wishing to be bound by theory it has also been determined that oleuropein binds to all of the PPAR receptors α, β, and γ and modulate their actions coordinately. Whether or not this is the mechanism by which oleuropein acts remains to be determined. Furthermore, with the discovery that oleuropein selectively modulates all of the PPAR receptors, the potential for using high throughput assays to identify other similar/analogous PPAR modulators pharmacologically useful oleuropein-like compounds which modulate these receptors simultaneously is feasible. Such compounds will be useful in the treatment of PPAR-mediated diseases and conditions as well as any for which oleuropein was previously considered to be useful. In addition, oleuropein may be used in diagnostic assays to monitor the progression of disease or to monitor the effectiveness of therapy with agents that are administered to patients suffering from obesity or an obesity related condition, or who are prone to development of such conditions.

Proliferator-Activated Receptors (PPARs)

0221] The Proliferator-Activated Receptors (PPARs) are members of the nuclear receptor superfamily, which upon binding to specific DNA response elements and in response to ligand binding, result in the activation of several genes. The PPARs contain a DNA-binding domain, a ligand-binding domain, and a flexible hinge connecting the two. The PPARs function as ligand-regulated transcription factors that control the expression of target genes by binding to their responsive DNA sequence (DNA response elements or PPRES) as heterodimers with the retinoid X receptor (RXR). The target genes encode enzymes involved in lipid metabolism and differentiation of adipocytes. The PPAR receptor experiences a conformational change upon ligand binding, which results in activation of gene transcription. The term “nuclear receptor” mainly refers to factors which control transcription of a target gene by binding upstream from the target gene promoter in a nuclear receptor ligand-dependent fashion. However, some nuclear receptors lack nuclear receptor ligands. Consequently, even nuclear receptors which lack nuclear receptor ligands are called “nuclear receptors” if their structural and functional homology places them in the nuclear receptor gene superfamily. Nuclear receptors include estrogen receptors (ER), vitamin D receptors (VDR), peroxisome proliferator-activated receptors (PPAR), liver X receptors (LXR), retinoic acid receptors (RAR), retinoid X receptors (RXR), androgen receptors (AR), glucocorticoid receptors (GR), farnesoid x receptors (FXR), mineralocorticoid receptors (MR) and the like for example, but are not limited by these.

0222] There are three known subtypes of PPARs. They include the subtypes PPAR α, PPAR γ, and PPAR δ. Natural agonists of the three types of PPARs include fatty acids, which implicate them as critical regulators in metabolic pathways involving energy storage and utilization. Furthermore, this also suggests that the PPARs may be potential targets for development of therapeutics against disorders such as obesity, and obesity-related disorders, including diabetes and dyslipidemia (Kliewer, et al., Recent Progress in Hormone Research, (2001), 56: 239-63).

0223] PPARα is expressed predominantly in the liver and, to a lesser extent, in cardiac and skeletal muscle. It plays a crucial role in fatty acid oxidation in response to fasting, providing ketone bodies that serve as an energy source for peripheral tissues. PPARα knockout mice cannot meet
energy demands during fasting, and develop hypoglycemia, hyperlipidemia, and fatty liver (Kersten S, Seydoux J, Peters J M, Gonzalez F J, Desvergne B, and Wahli W. Peroxisome proliferator-activated receptor alpha mediates the adaptive response to fasting. J Clin Invest, 1999, 103: p. 1489-98.). PPARα agonists include the fibrates, which are used clinically to treat hypertriglyceridemia.


[0225] PPARδ, also known as PPARβ, is expressed ubiquitously, and regulates expression of genes involved in fatty acid catabolism and adaptive thermogenesis. Transgenic expression of PPARδ results in lean mice that are resistant to obesity and hyperlipidemia, while PPARδ knockout mice show reduced energy uncoupling, and are prone to obesity (Wang Y X, Lee C H, Tep S, Yu R T, Ham J, Kang H, and Evans R M. Peroxisome-proliferator-activated receptor delta activates fat metabolism to prevent obesity. Cell, 2003: 113: p. 159-70.).

[0226] PPARs may modulate vascular effects by affecting insulin resistance, and by expression of adipokines. In addition, PPARs may have direct effects on gene transcription in vascular tissues and endothelial cells.

[0227] The significance of these receptors in physiology and disease is evidenced by the fact that PPAR-γ and PPARα are respective molecular targets for the insulin-sensitizing thiazolidinedione (TZD) and lipid-lowering fibrate drugs that total more than $5 billion in annual sales.

[0228] PPARγ agonist GW501516 (2-methyl-1-(4-(4-methyl-2-(4-trifluoromethylphenyl)-1)-3-thiazol-5-yl)-methyl)safainyl)phenoxyacetic acid) and PPARα, α, γ, pan agonist GW2433 (2-(4-3-(1-(2-(2-chloro-6-fluoro-phenyl)-ethyl)-3-(2,3-dichloro-phenyl)-ureido)-propyl)phenoxy)-2-methyl-propanoic acid) were shown to lower plasma triglyceride levels in obese monkeys while raising high-density lipoprotein levels, prompting the initiation of clinical trials to assess efficacy in hyperlipidemic patients. The medical potential of PPAR δ agonist and PPARα, α, γ, pan agonist is believed to exceed that of both PPARα and PPARγ single agonist or dual agonists.

[0229] The findings presented herein demonstrate that oleuropein interacts with PPARα, PPARγ and PPARδ, and as such, offers a novel application for therapeutic pan modulation of these important transcription factors in the treatment of obesity and related disorders.

[0230] PPAR gamma: Out of the three subtypes, PPAR γ gene has been most extensively studied. It is known to play an important role in the regulation of glucose and lipid homeostasis as well as in adipocyte differentiation (Willson, et al., Journal of Medicinal Chemistry, (2000), 43: 527-550). The PPAR γ gene protein is conserved across several species including mice and humans. One of the first synthetic ligands of PPAR γ gene identified as agonists was a class of antidiabetic compounds known as thiazolidinediones (TZDs). The efficacy of individual TZDs in anti-diabetic therapy appears to correlate with their ability to bind and activate the PPAR γ gene receptor (Auwers, J., Diabetologia, (1999), 42: 1033-1049). This class of compounds has also been shown to induce gene expression in adipocytes, which correlates with lowered glucose levels (Willson, et al.). TZDs have also been shown to reduce lipid and insulin levels. Known thiazolidinediones include Rosiglitazone, Troglitazone, Pioglitazone, and MCC-555. Each of these compounds binds preferentially to PPAR γ gene over the other PPAR subtypes. Pioglitazone and rosiglitazone are Food and Drug Administration approved drugs that are currently sold for the treatment of Type II diabetes. Troglitazone was also FDA approved for Type II diabetes, but has been withdrawn from commercial use due to the occurrence of undesirable side effects.

[0231] Although the advances made with the thiazolidinedione class of antidiabetic agents is significant, there are unacceptable side effects associated with this class of drugs, which have limited their clinical use. Accordingly, there remains a need for potent, selective modulators of PPAR γ gene, of which the activators of PPAR γ gene will be useful for the treatment of NIDDM and other disorders related to lipid metabolism and energy homeostasis. Still further, compounds that block PPAR γ gene activity would be useful for interfering with the maturation of preadipocytes into adipocytes and thus would be useful for the treatment of obesity and obesity-related disorders associated with undesirable adipocyte maturation. In addition, the response of patients to particular TZDs is variable, with about 20-30% of patients using these compounds being classified as non-responders. Accordingly, it is highly desirable to identify compounds for treating diabetes, as well as for treating obesity and other obesity-related disorders that are more therapeutically effective with fewer side effects. There is also a need to develop more accurate methods for predicting whether a subject is likely to respond to a particular treatment as well as methods that determine the extent to which a patient has responded to the treatment.

[0232] PPAR γ gene has been shown to be expressed in an adipocyte tissue-specific manner. Furthermore, it is induced early during the course of differentiation of preadipocytes. Further studies have now demonstrated that PPAR γ gene not only plays a pivotal role in the adipogenic signaling cascade, but also regulates the ob/leptin gene which is involved in regulating energy homeostasis. PPAR γ gene also plays a role in adipocyte differentiation, which has been shown to be
a critical step for targeting therapeutics for treating obesity and obesity-related conditions, such as diabetes.

**[0233]** PPAR gamma ligands also have in vitro anticancer activity against a wide variety of neoplastic cells and in vivo anticancer effects and chemotherapeutic or chemopreventive effects have been seen in animal studies. PPAR gamma ligands may slow the growth of cancer cells and may induce the programmed differentiation of some cancer cells. Overall, much of the literature indicates that PPAR gamma ligands have antiproliferative activity and may be useful in the treatment of cancer, including particularly several common cancers, including those of the colon, prostate, and breast. (See, Koefler H P Clin Cancer Res. 9(1):1-9 (2003)).

**[0234]** Furthermore, there is evidence to suggest that PPAR (e.g., PPAR-gamma) acts by a number of mechanisms to influence the permeability of skin, inhibit the growth of epidermal cells, promote the terminal differentiation of epidermis, and regulate the inflammatory response of skin. Accordingly, PPAR ligands may be useful in the modulation of skin conditions characterized by hyperproliferation, inflammatory infiltrates and abnormal differentiation (e.g., psoriasis), including inflammatory skin diseases (e.g., atopic dermatitis), proliferative skin diseases, acne vulgaris, protease inhibitor associated lipodystrophy and wound healing. (See, Kuenzli S, et al., Br J Dermatol. 149(2):229-36 (2003)).

**[0235]** Additional studies suggest that PPAR gamma plays a role in the pathophysiology of senile osteoporosis. For example, adipogenesis in bone marrow increases with aging. Mesenchymal stem cells expressing a subtype of this receptor (PPAR gamma-2) differentiate into adipocytes. Appropriate modulation of this receptor may promote mesenchymal stem cell differentiation into osteoblasts. Furthermore, activators of PPAR alpha, delta, and gamma have been reported to induce alkaline phosphatase activity and bone matrix calcification. Accordingly, pharmacological use of PPAR activators should promote bone mineral density by modulating osteoblastic maturation. (See, Duque G, Drug News Perspect. 16(6):341-6 (2003); Jackson S M, et al., FEBS Lett. 471(1): 119-24 (2000). Based on the studies presented herein, another aspect of the present invention is the use of oleuropein, or an analogue or derivative thereof for the treatment of osteoporosis, or other diseases or conditions associated with bone loss.

**[0236]** The anti-proliferative and anti-inflammatory effects of PPAR gamma are observed in glial cells and lymphocytes. It has been postulated that activation of such cells may be involved in the pathophysiology of neurological diseases associated with brain inflammation (e.g. Alzheimer’s disease and multiple sclerosis). Studies indicate that PPAR gamma modulators would be therapeutically useful in such diseases. (See, Feinstein D E, Diabetes Technol Ther. 5(1):67-73 (2003)). Accordingly, the present invention provides for a method of treating inflammatory diseases or conditions comprising administering a PPAR gamma modulator to a subject in need of such therapy. In one particular embodiment, the method provides for treating neurological or neurodegenerative diseases or conditions caused in part by the presence or influx of inflammatory cells, such as for example, multiple sclerosis, stroke or Alzheimer’s disease. The use of a PPAR modulator for treating a nervous system injury is also contemplated, for example, a spinal cord injury or traumatic brain injury. In one particular embodiment, the PPAR modulator is a PPAR gamma agonist.

**[0237]** Based on the studies done with the PPAR family of receptors in various disease conditions, it is possible that compounds that interact with the various PPAR receptors may be useful in treating these various diseases. Depending on the biological environment (e.g., cell type, pathological condition of the host, etc.), these compounds can activate or block the actions of PPARgamma. By activating the PPAR gamma receptor, the compounds find use as therapeutic agents capable of modulating conditions mediated by the PPAR gamma receptor. As noted above, an example of such a condition is non-insulin dependent diabetes mellitus (NIDDM). Additionally, the compounds may be useful for the prevention and treatment of complications of diabetes (e.g., neuropathy, retinopathy, glomerulosclerosis, and cardiovascular disorders), and treating hyperlipidemia. Still further, the compounds may be useful for the modulation of inflammatory conditions which most recently have been found to be controlled by PPAR gamma (see, Ricote, et al., Nature, 391:79-82 (1998) and Jiang, et al., Nature, 391:82-86 (1998). Examples of inflammatory conditions include asthma, rheumatoid arthritis and atherosclerosis. Such compounds may also be useful in the treatment of other skin diseases such as acne, atopic dermatitis, psoriasis, photodermatitis, eczema, and seborrhea.

**[0238]** It has also been described in WO 96/33724 that compounds selective for PPAR gamma receptors, such as prostaglandin-J2 or -D2, may be potentially active agents for the treatment of obesity and diabetes. Compounds that act via antagonism of PPAR gamma may be useful for treating obesity, hypertension, hyperlipidemia, hypercholesterolemia, hyperlipoproteinemia, and metabolic disorders.

**[0239]** Compounds which are PPAR gamma agonists or activators by virtue of their anti-inflammatory effects can have neuroprotective effects and find use in the treatment of brain inflammatory conditions such as Alzheimer’s disease and multiple sclerosis. PPAR alpha, PPAR delta and their ligands are important for lipid oxidation and energy uncoupling. They increase fat consumption and decrease fat accumulation, thus they can have weight reduction and anti-obesity effects. A ligand such as oleuropein with the capability to bind all of the three PPARs has the potential to act as a PAN modulator. These are discussed below.

### PPAR Gamma

**[0240]** Owing to their ability to induce gene expression in adipocytes and to enhance adipocyte differentiation, TZDs induce weight gain in often already obese patients. For this reason, efforts are being made to identify new partial agonists or antagonists for PPARgamma in an attempt to combine their anti-diabetic and anti-obesity effects.

### Combined PPARalpha/PPARgamma Agonists

**[0241]** The effects of TZDs on the lipid profile in diabetic patients are not optimal. Given the favorable effect of PPAR activators on plasma lipoprotein metabolism, combined activation of PPARalpha and PPARgamma could lead to a complementary and synergistic action on lipid metabolism, insulin sensitivity and inflammation control. Dual activation of PPARalpha and PPARgamma could, in theory, also limit the occurrence of side effects associated with TZD therapy, such as edema and body-weight gain, although this has not been observed so far in clinical trials with coagulants. Thus, combined PPARalpha/PPARgamma activation has recently emerged as an intriguing concept and spawned the development of co-agonists.
PPAR-Delta and PPARδ Pan Agonist or Modulator

Because of its ubiquitous expression and the paucity of selective ligands, PPAR-delta is the least understood PPAR subtype. Nevertheless, early PPAR-δ-selective agonists were found to elevate HDL-C levels in diabetic mice, an observation that indicated that PPARδ ligands might have beneficial effects on dyslipidemia (Eur J Pharmacol. 2006 Apr 24; 536(1-2):182-91. Epub 2006 Feb. 28). Subsequently, the potent PPARδ agonist GW501516 was shown to increase HDL-C while decreasing elevated TG and insulin levels in obese rhesus monkeys. GW501516 also attenuates weight gain and insulin resistance in mice fed high-fat diets by increasing the expression in skeletal muscle of genes that promote lipid catabolism and mitochondrial uncoupling, thereby decreasing β-oxidation of fatty acids in skeletal muscle (Curr Opin Investig Drugs. 2006 April; 7(4):360-70).

Expected Results of PPAR Pan Modulator Such as OLE/ Oleuropein/Hydroxytyrosol) on Metabolic Endpoints

Our discovery that Oleuropein is able to act as a PPAR pan agonist that targets all of the three isoforms of PPARs, PPARα, PPARδ and PPARγ offers a new class of orally available potential novel drug. A compound targeting all of these PPAR subtypes could provide a combination of triglyceride, LDL and glucose lowering activities, coupled with increases in insulin sensitivity, HDL and reverse cholesterol transport (see Table 1). Its antihyperglycemic, lipid-modulating, insulin-sensitizing activities could be used in the treatment of a variety of metabolic and cardiovascular diseases, including Type II diabetes, impaired glucose tolerance, dyslipidemia, hypertension, metabolic syndrome X, asthma and HIV/AIDS associated lipodystrophies. Furthermore, synergies of such a combination may enable lower dosing and consequently mitigate side effects and toxicities observed with current therapies. Although treatment options for Type II diabetes are available, their usefulness is significantly limited due to their failure to ameliorate concurrent hyperglycemia and hyperlipidemia (e.g. triglycerides, LDL-cholesterol) or to raise HDL, in addition to their side effects.

Use of Oleuropein and/or Hydroxytyrosol for Inhibiting Viral Infectivity

The work presented herein also demonstrates that oleuropein (Ole) and hydroxytyrosol (HT) are a unique class of HIV-1 inhibitors from olive leaf extracts, which are effective against viral fusion and integration. Molecular docking simulation was used to study the interactions of Ole and HT with viral targets. It was determined that Ole and HT bind to the conserved hydrophobic pocket on the surface of the HIV-gp1 fusion domain by hydrogen bonds with Q577 and hydrophobic interactions with 1573, G 572, and I 568 on gp41 N-terminal heptad repeat peptide N36, interfering with formation of the gp41 fusion-active core. To test and confirm modeling predictions, the effect of Ole and HT on HIV-1 fusion complex formation was studied using native polyacrylamide gel electrophoresis and circular dichroism spectroscopy. Ole and HT exhibit dose-dependent inhibition on HIV-1 fusion core formation with EC50 of 66-58 μM, with no detectable toxicity.


**Table 1**

<table>
<thead>
<tr>
<th>Target</th>
<th>Glucose</th>
<th>Insulin</th>
<th>TG</th>
<th>FAA</th>
<th>LDL</th>
<th>HDL</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARα</td>
<td>No effect</td>
<td>No effect</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↑ Ineffective on glucose and insulin sensitivity</td>
</tr>
<tr>
<td>PPARβ</td>
<td>No effect</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↑ Less validation for PPAR</td>
</tr>
<tr>
<td>PPARγ</td>
<td>↓ ↓</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↑ ↑</td>
<td>No effect</td>
</tr>
<tr>
<td>PPARγα</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↑ Edema, weight gain, anemia, LDL-cholesterol ↑, need to monitor liver function</td>
</tr>
<tr>
<td>PPARγδ</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ →↑</td>
</tr>
</tbody>
</table>

Abbreviations: Insulin S, insulin sensitivity; TG, triglycerides; FAA, free fatty acids; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein
*Reported clinical effects in athero, rhesus monkeys and in patients Edema, weight gain, anemia, ↑ LDL, cholesterol, requirement to monitor liver function
**Predicted effect of Oleuropein and derivatives
[0246] New therapeutic approaches include the fusion inhibitor Fuzeon (3-20, enfuvirtide), the non-peptidic PI Tipranavir, the new PI darunavir and the recently approved MCCP Atripla. However, existing experience with HIV-1 highlights the need to use multiple effective agents in combination for maximal and durable effect. Thus, the search for novel anti-HIV agents continues to be of great significance, especially those capable of affecting multiple stages of the viral life cycle.

[0247] We previously reported that olive leaf extract is potent against HIV-1 [S. Lee-I-Huang, L. Zhang, P. L. Huang, and Y. T. Chang, Anti-HIV activity of olive leaf extract (OLE) and modulation of host cell gene expression by HIV-1 infection, Etiology and Treatment of HIV Infections, Biochem Hurpaf Res Comm 307 (2003) 1029-1037]. Subsequent studies as described herein demonstrate that the anti-HIV properties of oleuropein (OLE) and its main metabolite, hydroxytyrosol (HT) are the key anti-HIV components of Olive Leaf Extract. They are active against the multiple stages of the HIV-1 life cycle, inhibiting cell-to-cell HIV-1 transmission and viral core antigen p24 production. Molecular docking simulations indicate that OLE and HT interact with the conserved hydrophobic pocket on the surface of the central trimeric coiled-coil of HIV-gp41 fusion complex, the six helical bundle (6H68), and the catalytic core domain (CCD) of HIV-1 integrase active site.

[0248] Molecular modeling and functional confirmation of OLE and HT binding to HIV-1 integrase was also studied and the results summarized herein. Docking simulations identified two binding regions for OLE within the integrase active site. Region I encompasses the conserved D64-D 116-E 152 motif, while region II involves the flexible loop region formed by amino acid residues 140-149. HT, on the other hand, binds to region II. Both OLE and HT exhibit favorable interactions with important amino acid residues through strong H-bonding and van der Waals contacts, predicting integrase inhibition. To test and confirm modeling predictions, we examined the effect of OLE and HT on HIV-1 integrase activities including 3'-processing, strand transfer and disintegration. OLE and HT exhibit dose-dependent inhibition on all three activities, with EC50 in the nM range. These studies demonstrate that molecular modeling of target-ligand interaction coupled with structural-activity analysis should facilitate the design and identification of innovative integrase inhibitors and other therapeutics.

[0249] HIV-1 integrase is one of three viral enzymes required for viral replication, along with RT and protease [P. O. Brown, Retroviruses, in Coffin, J., Hughes, S., and Var-
mus, H., (Eds.) Cold Spring Harbor Press, Cold Spring Har-
bor, 1998, 161-205; T. K. Chiu, and D. R. Davies, Structure and function of HIV-1 integrase, Curr Top Med Chem 4 (2004) 965-977; K. Zhu, C. Dobard, and S. A. Chow, Requirement for integrase and reverse transcriptase of human immunodeficiency virus type 1 and the effect of cysteine mutations of integrase on its interactions with reverse transcriptase, J Virol 78 (2004) 5045-5055]. Integration of HIV-1 cDNA into the host chromosome is essential for stable maintenance of the viral genome, efficient viral gene expression and productive infection. Thus, viral integrase is a critical target for anti-HIV therapy [Y. Pommier, A. A. Johnson, and C. Marchand, Integrase inhibitors to treat HIV/AIDS, Nat Rev Drug Discov 4 (2005) 236-248; P. A. Sherman, and J. A. Fyele, Human immunodeficiency virus integration protein expressed in Escherichia coli possesses selective DNA cleaving activity, Proc Natl Acad Sci USA 87 (1990) 5119-5123]. The first step leading to viral DNA integration is the binding of viral integrase to HIV long terminal repeat (LTR) sequences. This is followed by three sequential reactions: 1) 3'-processing, the removal of two nucleotides, GT, from the 3'-end of HIV-LTR, 2) strand transfer, a concerted cleavage-ligation reaction, in which the integrase makes a staggered cut in the target DNA and ligates the recessed 3' ends of the viral DNA to the 5' ends of the target DNA, and 3) gap repair, removal of the two unpaired nucleotides at the 5' end of the viral DNA and repair of the gap between the viral and the target DNA. In the presence of a DNA substrate that mimics the product of viral integration, integrase can catalyze the reversal of strand transfer, known as "disintegration" [S. A. Chow, K. A. Vincent, V. Ellison, and P. O. Brown, Reversal of integration and DNA splicing mediated by integrase of human immunodeficiency virus, Science 255 (1992) 723-726]. S. A. Chow, K. A. Vincent, V. Ellison, and P. O. Brown, Reversal of integration and DNA splicing mediated by integrase of human immunodeficiency virus, Science 255 (1992) 723-726], in which viral DNA is released and the target DNA is sealed.

Therapeutic and Prophylactic Compositions and their Use

[0250] Candidates for therapy with the agents identified by the methods described herein are patients either suffering from, or at risk for development of obesity, or an obesity related disease, disorder or condition, including diabetes, dyslipidemia, hypertension and cardiovascular diseases, asthma to name a few. Other obesity related disorders have been described previously. In addition, patients who are obese or are prone to developing obesity or an obesity related disorder based on a genetic predisposition are also considered to be candidates for therapy using oleuropein or a compound having activity analogous to oleuropein. Furthermore, patients who have limited mobility or patients who are bedridden due to a surgical procedure or illness and are not capable of exercise and are thus prone to accumulation of body fat may be candidates for therapy with the agents identified by the methods described. In addition, the present invention contemplates the use of oleuropein, hydroxytyrosol, and their derivatives, analogues or mimics thereof, for inhibiting the growth and/or infectivity of HIV-1 by virtue of the effects of oleuropein and hydroxytyrosol on inhibition of viral fusion and/or by their effects on the viral integrase.

[0251] The invention provides methods of treatment comprising administering to a subject an effective amount of an agent of the invention. In a preferred aspect, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as monkeys, cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human. In one specific embodiment, a non-human mammal is the subject. In another specific embodiment, a human mammal is the subject. Accordingly, the agents identified by the methods described herein may be formulated as pharmaceutical compositions to be used for prophylaxis or therapeutic use to treat these patients. Moreover, the agents of the invention may be useful for administering to non-human mammals to aid in the build-up of lean muscle which may then prove highly beneficial for the meat industry.

[0252] Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, or microcapsules. Methods of introduction can be enteral or parenteral and include but are not limited to intravenous, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, topical and oral routes. The compounds may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may
be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Omnifix reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment.

[0253] Such compositions comprise a therapeutically effective amount of an agent, and a pharmaceutically acceptable carrier. In a particular embodiment, the term “pharmaceutically acceptable” means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term “carrier” refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petrolatum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate; glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as tragacanth. Oral formulation can include standard carriers such as pharmaceutical grades of mannnitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in “Remington’s Pharmaceutical Sciences” by E. W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the subject. The formulation should suit the mode of administration.

[0254] In a preferred embodiment, the composition is formulated in accordance with routine procedures for a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0255] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects (a) approval by the agency of manufacture, use or sale for human administration, (b) directions for use, or both.

[0256] In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved, for example, and not by way of limitation, by local infusion during surgery, by topical application, by injection, by means of a catheter, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers or co-polymers such as Ethix (see Ruam et al., 1992), Proc Natl Acad Sci USA, 89:10872-10876). In one embodiment, administration can be by direct injection by aerosol inhaler.

[0257] In another embodiment, the compound can be delivered in a vesicle, in particular a liposome (see Langer (1990) Science 249:1527-1533; Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, ibid., pp. 317-327; see generally ibid.)


Effective Doses

[0259] Toxicity and therapeutic efficacy of compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the ID<sub>50</sub> (the dose lethal to 50% of the population) and the ED<sub>50</sub> (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio ID<sub>50</sub> / ED<sub>50</sub>. Compounds that exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects can be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to unaffected cells and, thereby, reduce side effects.

[0260] The data obtained from cell culture assays and animal studies can be used in formulating a dose range for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the
ED₃₀ with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to optimize efficacious doses for administration to humans. Plasma levels can be measured by any technique known in the art, for example, by high performance liquid chromatography.

[0261] In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each subject's circumstances. Normal dose ranges used for particular therapeutic agents employed for specific diseases can be found in the Physicians' Desk Reference, 54th Edition (2000).

[0262] While the subject is being treated, the health of the patient may be monitored by measuring one or more relevant indices at predetermined times during a 24-hour period. For example, lipid levels may be monitored during the course of therapy using standard procedures known to those skilled in the art, in order to monitor the effectiveness of therapy. Alternatively, for patients suffering from HIV, blood samples may be obtained during the course of therapy to monitor levels of viral nucleic acid using standard PCR procedures known to those skilled in the art. Treatment, including supplement, amounts, times of administration and formulation, may be optimized according to the results of such monitoring. The patient may be periodically reevaluated to determine the extent of improvement by measuring the same parameters, the first such reevaluation typically occurring at the end of four weeks from the onset of therapy, and subsequent reevaluations occurring every four to eight weeks during therapy and then every three months thereafter. Therapy may continue for several months or even years, with a minimum of one month being a typical length of therapy for humans. Adjustments to the amount(s) of agent administered and possibly to the time of administration may be made based on these reevaluations.

[0263] Treatment may be initiated with smaller dosages which are less than the optimum dose of the compound. Thereafter, the dosage may be increased by small increments until the optimum therapeutic effect is attained.

[0264] The combined use of several compounds of the present invention, or alternatively other therapeutic agents, may reduce the required dosage for any individual component because the onset and duration of effect of the different components may be complimentary. In such combined therapy, the different active agents may be delivered together or separately, and simultaneously or at different times within the day.

[0265] The invention includes use of any modifications or equivalents of the above agents which do not exhibit a significantly reduced activity. The statements of effect and use contained herein are therefore to be construed accordingly, with such uses and effects employing modified or equivalent gene products being part of the invention.

[0266] Oleuropein is a phenolic secoiridoid glycoside with the structure as shown in the figure below. It is an organic molecule that consists of a dihydroxy phenol moiety, a secoiridoid moiety and a glucose moiety. The glucose moiety can be removed by β-glucosidase to yield oleuropein aglycone. Oleuropein aglycone can than be hydrolyzed into hydroxytyrosol and eolectric acid. These are the metabolites of oleuropein and they are all physiologically active (See FIG. 10).

[0267] The present agents that prevent fat accumulation, or that modulate differentiation, de-differentiation and trans-differentiation of adipocytes or that have an effect on the expression of adipogenic, lipogenic and lipolytic genes and gene products can be used as the sole active agent or can be used in combination with other active ingredients

Combination Therapy

[0268] The compounds of the invention may be combined with another therapeutic agent that is useful in the treatment of obesity or disorders associated with the development and progression of obesity and obesity related disorders, such as, diabetes, atherosclerosis, hypertension, hyperlipidemias, dyslipidemias, and cardiovascular disease. The compounds of the invention may be combined with another therapeutic agent that decreases the ratio of LDL:HDL or an agent that causes a decrease in circulating levels of LDL-cholesterol. In patients with diabetes mellitus the compounds of the invention may also be combined with therapeutic agents used to lower blood sugar or to treat complications associated with diabetes, including nephropathy, neuropathy, retinopathy, cardiovascular disease, stroke, or any other complication associated with diabetes.

[0269] Accordingly, the compounds of the invention may be used alongside other therapies for the treatment of metabolic syndrome or type 2 diabetes and its associated complications, these include biguanide drugs, for example metformin, phenformin and buformin, insulin, insulin analogues, amylin and oral antihyperglycemicines (these are divided into prandial glucose regulators and alpha-glucosidase inhibitors). An example of an alpha-glucosidase inhibitor is acarbose or voglibose or miglitol. An example of a prandial glucose regulator is repaglinide or nateglinide. Alternatively, oleuropein and/or hydroxytyrosol may be used in combination with other anti-virals known in the art to be effective against HIV.

[0270] In another aspect of the invention, the agents of the invention, e.g. oleuropein or an analogue or derivative thereof, or a pharmacologically acceptable salt, solvate, solvate of such a salt or a prodrug thereof, may be administered in association with another PPAR modulating agent. PPAR modulating agents include but are not limited to a PPAR alpha, delta and/or gamma agonist, or pharmacaceutically acceptable salts, solvates, solvates of such salts or prodrugs thereof. Suitable PPAR alpha, delta and/or gamma agonists, pharmaceutically acceptable salts, solvates, solvates of such salts or prodrugs thereof are well known in the art. These include the compounds described in WO 01/12187, WO 01/12612, WO 00/628707, WO 99/62872, WO 99/62871, WO 98/57941, WO 01/40170, J Med Chem. 1996, 39, 665, Expert Opinion on Therapeutic Patents, 10 (5), 623-634 and J Med Chem, 2000, 43, 527, which are all incorporated herein by reference in their entirety. Particularly a PPAR alpha and/or gamma agonist refers to NN622/Ragaglitazar, BMS 298585, WY-14643, clofibrate, fenofibrate, bezafibrate, gemfibrozil and ciprofibrate; GW 9578, ciglitazone, troglitazone, pioglitazone, rosiglitazone, eglitazone, pioglitazone, BRL-49634, KRP-297, JTI-501, SB 213068, GW 1929, GW 7845, GW 0207, I-796449, L-156941, GW 2433 and a PPAR alpha and/or gamma agonist such as (S)-1-ethoxy-3-(4-[(4-methanesulphonyloxy)phenyl]ethoxy)-1-phenyl)propanoic acid and pharmaceutically acceptable salts thereof.

[0271] In addition the combination of the invention may be used in conjunction with a sulfonylurea for example: gliclazide, glibenclamide (glyburide), gliclazide, glipizide,
gliptidone, chlorpropamide, tolbutamide, acetohexamide, glycopyramide, carbutamide, glibornuride, glitazepid, glybuthiazole, glibuzole, glyhexamide, glymidine, glynipamide, phenbutamide, tolcyamide and tolazamide. Preferably the sulfonlurea is glimepiride or glipbenclamide (glyburide). Therefore the present invention includes administration of a compound of the present invention in conjunction with one, two or more existing therapies described in this paragraph. The doses of the other existing therapies for the treatment of obesity or an obesity related disorder, such as, type 2 diabetes and its associated complications will be those known in the art and approved for use by regulatory bodies for example the FDA and may be found in the Orange Book published by the FDA. Alternatively smaller doses may be used as a result of the benefits derived from the combination. The present invention also includes a compound of the present invention in combination with a cholesterol-lowering agent. The cholesterol-lowering agents referred to in this application include but are not limited to inhibitors of HMG-CoA reductase (3-hydroxy-3-methylglutaryl coenzyme A reductase). Suitably the HMG-CoA reductase inhibitor is a statin selected from the group consisting of atorvastatin, cerivastatin, cilostazol, dalcavastatin, fluvastatin, itavastatin, lovastatin, mevatastatin, nicosartan, nivastatin, pravastatin and simvastatin, or a pharmaceutically acceptable salt, or a solvate thereof, or a solvate of such a salt. A particular statin is atorvastatin, or a pharmaceutically acceptable salt, solvate, solvate of such a salt or a prodrug thereof. More particular statins are rosuvastatin and atorvastatin calcium salts.

In the present application, the term “cholesterol-lowering agent” also includes chemical modifications of the HMG-CoA reductase inhibitors, such as esters, prodrugs and metabolites, whether active or inactive.

Diabetes mellitus is a syndrome resulting from the interaction of hereditary and environmental factors; it is characterized by disturbances in insulin secretion and other metabolic and vascular abnormalities, i.e. an elevated concentration of glucose in the blood, non-specific accelerated arteriosclerosis, neuropathy and thickening of the capillary basal lamina caused by a degeneration of the kidney and the retina.

According to a modern classification, the diabetes is divided into two main categories:

Insulin-dependent diabetes mellitus (also known as Type 1 diabetes); patients with this type of diabetes literally depend on insulin to prevent ketosis and death. As far as the endogenous insulin secretion is concerned, patients with Type 1 diabetes mellitus exhibit insulinopenia.

Noninsulin-dependent diabetes mellitus (also known as Type 2 diabetes); patients with this type of diabetes do not need insulin to live: they can decide whether using it or not to control the symptoms of the diabetes. As far as the endogenous insulin secretion is concerned, patients with Type 2 diabetes can be further classified into two groups. In the first group, insulin levels are either normal or lower than normal; in the second group, insulin values are higher than normal and patients exhibit insulin resistance.

Accordingly, given the data presented herein, oleuropein may be useful as a stand-alone agent for the treatment of obesity, or for preventing fat accumulation in individuals prone to obesity. However, it is also to be understood that oleuropein may be used as adjunct therapy with other known agents used to treat obesity or obesity related disorders.

**Monitoring a Patient’s Response to the Therapeutic**

**TABLE 2**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Mean Change after 3, 6, or 12 Months or other defined times</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo Group</td>
<td>OLE Group</td>
<td>Placebo Group</td>
</tr>
<tr>
<td>(n = xx)</td>
<td>(n = xx)</td>
<td>(n = xx)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Metabolic characteristics</th>
<th>Placebo Group</th>
<th>OLE Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio of glucose disposal rate to mean serum insulin level at steady state</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting glucose level, mmol/L (mg/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-h glucose level, mmol/L (mg/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting insulin level, pmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin AUC level, pmol/L × 10³ (120 min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol level, mmol/L (mg/dL)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 2-continued

<table>
<thead>
<tr>
<th>Metabolic characteristics</th>
<th>Baseline</th>
<th>Mean Change after 3, 6, or 12 Months or other defined times</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>OLE Group</td>
</tr>
<tr>
<td></td>
<td>(n = xx)</td>
<td>(n = xx)</td>
</tr>
<tr>
<td>LDL cholesterol level, mmol/L (mg/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol level, mmol/L (mg/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglyceride level, mmol/L (mg/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adiponectin level, μg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free fatty acid level, mmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNFα, IL-6, adipokines</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPARα, PPARβ, PPARγ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adipogenic genes and gene products</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipogenic genes and gene products</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipolytic genes and gene products</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Safety issues</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin level, g/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT level, U/L</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* All values are means ± SD. ALT = alanine aminotransferase; AUC = area under the curve; HDL = high-density lipoprotein; LDL = low-density lipoprotein.
† P values for mean change from baseline represent between-group effect from analysis of covariance with baseline value as covariate. For treating AIDS related lipodystrophy one also measures CD4+ counts and Log HIV RNA level, copies/mL.

[0279] The present invention further provides for methods of diagnosing a patient’s response to treatment with oleano- perin or an analogue or derivative thereof. Furthermore, the present invention provides diagnostic methods for evaluating the progression of treatment with oleanoerin or an analogue or derivative thereof. The invention provides a variety of genes and gene products identified as being modulated following treatment with oleanoerin or an analogue or derivative thereof and which may be used to monitor a therapeutic response to treatment with oleanoerin or an analogue or derivative thereof. The genes and gene products, which are up- or down-regulated in response to treatment with oleanoerin may be used diagnostically and prognostically for treatment of a PPAR associated disease, such as obesity or an obesity related disorder or disease such as Type II diabetes, with a PPAR ligand. These genes or gene products (proteins or enzymes) can be monitored from the patient’s blood (whole blood cells, serum or plasma) by enzyme assay, ELISA (antibody), RT-PCR, microarray, and/or proteomics techniques. Exemplary diagnostic tools and assays are set forth below, under followed by exemplary methods for conducting these assays. Diagnostic Tools and Assays and Methods for Identifying Novel Therapeutics for Treating a Disease Associated with a PPAR Receptor.

[0280] One aspect of the invention provides a means of determining whether a subject is responsive to treatment with oleanoerin or an analogue or derivative thereof or a combination of oleanoerin and a second agent used to treat obesity or an obesity related disorder, or of assessing the final outcome of therapy with oleanoerin or an analogue or derivative thereof or a combination of oleanoerin and a second agent used to treat obesity or an obesity related disorder in a subject in need of such therapy. Another aspect of the invention provides for the use of methods for screening for novel therapeutics for treating a disease associated with a PPAR receptor.

The methods described herein are merely exemplary and are not meant to be limiting, and as such, it is to be recognized that one of skill in the art would be cognizant of the various other methodologies that may be used to determine effectiveness of therapy with oleanoerin or analogues or derivatives thereof, or to identify novel analogues or derivatives or metabolites of oleanoerin for use in treating obesity or obesity related disorders.

[0281] In one particular embodiment, the method comprises measuring any one or more of the following clinical parameters: lipid profile, glucose levels, insulin levels, or any one or more cytokines or adipokines present in the serum or plasma of a subject undergoing treatment with oleanoerin, or an analogue or derivative or metabolite of oleanoerin. Any one or more of these sensitive and reliable end-points may be effective at determining whether oleanoerin, or an analogue, or a derivative, or a metabolite thereof, or a combination of oleanoerin or an analogue or derivative or metabolite thereof together with one or more agents useful for treating obesity or an obesity related disorder has proven to be effective in treating these patients suffering from these conditions.

[0282] In another particular embodiment, the effectiveness of therapy with oleanoerin or hydroxytyrosol) or an analogue or derivative thereof in patients infected with HIV may be measured by standard procedures known to those skilled in the art. For example, in the early stages of infection, HIV often causes no symptoms and the infection can be diagnosed only by testing a person’s blood. Two tests are available to diagnose HIV infection—one that looks for the presence of antibodies produced by the body in response to HIV and the other that looks for the virus itself. The first test is an Enzyme Linked Immunosorbent Assay, or ELISA test and it is used to measure antibodies produced by the patient against the virus. When the body is infected with HIV, one looks for such antibodies in blood.
[0283] If antibodies are present, the test gives a positive result, but this must be confirmed by another test called Western Blot or Immunofluorescent Assay (IFA). All positive tests by ELISA may not be accurate and hence Western Blot and repeated tests are necessary to confirm a person's HIV status. A person infected with HIV is termed HIV—positive or seropositive.

[0284] Antibodies to HIV generally do not reach detectable levels in the blood till about three months after infection. This period, from the time of infection till the blood is tested positive for antibodies, is called the Window Period. Some times, the antibodies might take even six months to show up. Even if the tests are negative, during the Window Period, the amount of virus is very high in an infected person. Hence, if a person is newly infected, the risk of transmission is higher.

[0285] If a person is highly likely to be infected with HIV and yet both the tests are negative, a doctor may suggest a repetition of the tests after three months or six months when the antibodies are more likely to have developed.

[0286] The second test is called PCR (Polymerase Chain Reaction), which looks for HIV itself in the blood. This test, which recognizes the presence of the virus' genetic material in the blood, can detect the virus within a few days of infection.

[0287] There are also tests like Radio Immuno Precipitation Assay (RIPA), a confirmatory blood test that may be used when antibody levels are difficult to detect or when Western Blot test results are uncertain. Other available tests are Rapid Latex Agglutination Assay, a simplified, inexpensive blood test that may prove useful in medically disadvantaged areas where there is a high prevalence of HIV infection, and p24 Antigen Capture Assay.

[0288] In another particular embodiment, the responsiveness of a subject to treatment with oleuropein or an analogue or derivative or metabolite thereof can be assessed by measuring body mass index (BMI) or by monitoring the weight of the subject. In a particular embodiment, one would expect to see an improvement in the BMI or a decrease in body weight or at least a stabilization in body mass (e.g. no additional weight gain over a given period of time).

[0289] In another particular embodiment, such a method comprises determining the levels of expression of one or more genes or gene products (proteins) which are modulated in a cell of the subject undergoing treatment with oleuropein (or an oleuropein analogue, derivative, or metabolite thereof) and comparing these levels of expression with the levels of expression of the genes and gene products in a cell of a subject not treated with oleuropein (or an oleuropein analogue, derivative, or metabolite thereof), or of the same subject before treatment with oleuropein (or an oleuropein analogue, derivative, or metabolite thereof), such that the modulation (either up or down-regulation of the gene or gene product) of one or more genes is indicative that the subject is responsive to treatment with oleuropein (or with an analogue, derivative or metabolite thereof). In one embodiment, the cell is obtained from a sample of whole blood, for example, white blood cells, including lymphocytes, monocytes, neutrophils and the like, although other cells expressing these genes are also contemplated for analysis. In one embodiment, the genes may be any of the adipogenic genes or gene products selected from the group consisting of Peroxisome Proliferator-ACTivated Receptor α (PPARα), lipoprotein lipase (LPL), and the adipocyte-selective fatty acid binding protein (the P2 gene). In addition, other differentiated adipocyte marker genes include glycerolphosphate dehydrogenase (GPDH), fatty acid synthase, acetyl CoA carboxylase, malic enzyme, Glut 4, and the insulin receptor (see Spiegelman et al. J. Biol. Chem. 268: 6823-6826, 1993, incorporated herein by reference). Preadipocytes also have characteristic marker genes, such as the cell surface antigen recognized by the monoclonal antibody AD-3. Expression level changes of the various isoforms of the C/EBP (CCAAT/enhancer-binding proteins) family of transcription factors may also indicate different stages of adipogenesis (see Yu and Hausman, Exp Cell Res Dec. 15, 1998; 245(2): 343-9). A person of skill in the art will recognize that in certain diagnostic and prognostic assays, it will be sufficient to assess the level of expression of a single adipogenic, lipogenic or lipolytic gene as noted above and that in others, the expression of two or more is preferred. For example, the level of expression of a gene or gene product (protein) may be determined by a method selected from, but not limited to, cDNA microarray, reverse transcription-polymerase chain reaction (RT-PCR), real time PCR and proteomics analysis. Other means such as electrophoretic gel analysis, enzyme immunoassays (ELISA assays), Western blots, dot-blot analysis, Northern blot analysis and in situ hybridization may also be contemplated for use, although it is to be understood that the former assays that are noted (e.g. microarrays, RT-PCR, real time PCR and proteomics analysis) provide a more sensitive, quantitative and reliable measurement of genes or gene products that are modulated by oleuropein or analogues, derivatives or metabolites thereof. Sequences of the genes or cDNA from which probes are made (if needed) for analysis may be obtained, e.g., from GenBank, other public databases or publications, and are shown here for certain of the exemplary markers shown in Tables 3 and 4. Magnetic resonance imaging may also be used for assessing the effect of oleuropein or analogues or derivatives or metabolites thereof on protein expression.

[0290] In another particular embodiment, novel candidate therapeutics (e.g. oleuropein analogues or derivatives or metabolites thereof) may be tested for activity by measuring their effect on adipocyte differentiation, de-differentiation or trans-differentiation, as described herein. The candidate therapeutics may be selected from the following classes of compounds: proteins, peptides, peptidomimetics, antibiotics, derivatives of fatty acids, nucleic acids, including DNA or RNA, antisense molecules or siRNA molecules, or other small organic molecules, either synthetic or naturally derived. In some embodiments, the candidate therapeutics are selected from a library of compounds. These libraries may be generated using combinatorial synthetic methods.

Use of Microarrays for Determining Gene Expression Levels

[0291] Microarrays may also be used for determining gene expression levels and may be prepared by methods known in the art, or they may be custom made by companies, e.g., Affymetrix (Santa Clara, Calif.) (see www.affymetrix.com). Numerous articles describe the different microarray technologies, (e.g., Shena, et al., Tibtech. (1998), 16: 301; Duggan, et al., Nat. Genet., (1999), 21:10: Bowtell, et al., Nat. Genet., (1999), 21:25; Hughes, et al., Nat. Biotechn. (2001), 19:342). While many of the microarrays utilize nucleic acids and relevant probes for the analysis of gene expression profiles, protein arrays, in particular, antibody arrays or glyco-sylation arrays also hold promise for studies related to protein or glycoprotein expression from biological samples (see for example, RayBiotech, Inc. at www.raybiotech.com/product. html, Panomics at www.panomics.com, Clontech Laboratories, inc. at www.clontech.com, Progonia in Maidenhead, UK and Qiagen at www.qiagen.com). Samples for Analysis

[0292] While the efficacy of oleuropein or an analogue, derivative, or metabolite thereof may be tested in a subject for
its effect on, for example, glucose levels, insulin levels, lipid profile, body mass index or weight loss, it may also be of interest to assess its effects on the modulation of the genes or gene products listed in Table 4. While it may be possible to look at the level of a particular gene in certain cellular samples (whole blood cells or peripheral blood mononuclear cells), a more particular method would involve the analysis of the protein expression in these cell types or in the plasma or serum form the subjects exposed or treated with oleanoprin or an analogue or derivative thereof. For example, protein and nucleic acid prepared from specimens may be obtained from an individual to be tested using either “invasive” or “non-invasive” sampling methods. A sampling methods is said to be “invasive” if it involves the collection of the biosamples from within the skin or organs of an animal (including, especially, a human, a murine, an ovine, an equine, a bovine, a porcine, a canine, or a feline animal). Examples of invasive methods include needle biopsy, pleural aspiration, etc. Examples of such methods are described by Kim, C. H., et al., J. Virol., (1992), 66:3879-3882; Biswas, B. et al., Annals NY Acad. Sci., (1990), 590:582-583; Biswas, B., et al., J. Clin. Microbiol., (1991), 29:2228-2233. Extinction of adipose tissue from individuals used in some embodiments of this invention is well known to those skilled in the art, for example as described by Lonnerblad, et al., Diabetes, (1983), 32:980: 748-54.

In one embodiment the assays of the present invention will be performed on cells including but not limited to whole blood cells, or isolated white blood cells from a mammal, or from adipocyte cultures propagated for laboratory purposes, ST3-L1 adipocytes, cells of skeletal muscle derived from a mammal, skeletal muscle cells propagated for laboratory purposes, C2C12 myotube cells, or mesenchymal stem cells, etc. Primary cultures or cell lines can be used. Alternatively, embryonic stem (ES) cells differentiated into adipocytes can be used, for example, as described in Pollard, et al., Journal of Cell Biology, (1995), 130: 1461-72. Appropriate cell lines that can be obtained for screening purposes are commercially available from the ATCC. In yet another embodiment, a sample of whole blood, blood plasma or serum is obtained for further analysis.

Other Methods for Determining Gene Expression Levels

In certain embodiments, it is sufficient to determine the expression of one or only a few genes, as opposed to hundreds or thousands of genes. Although microarrays may be used in these embodiments, various other methods of detection of gene expression are available.

For example, the modulation of gene expression can be performed using a RT-PCR or Real Time-PCR assay. Total RNA is extracted using the procedures known to those skilled in the art and subjected to reverse transcription using an RNA-directed DNA polymerase, such as reverse transcriptase isolated from AMV, MoMuLV or recombinantly produced. The cDNAs produced can be amplified in the presence of Taq polymerase and the amplification monitored in an appropriate apparatus in real time as a function of PCR cycle number under the appropriate conditions that yield measurable signals, for example, in the presence of dyes that yield a particular absorbance reading when bound to duplex DNA. The relative concentrations of the mRNAs corresponding to chosen genes can be calculated from the cycle midpoints of their respective Real Time-PCR amplification curves and compared between cells exposed to a candidate therapeutic relative to a control cell in order to determine the increase or decrease in mRNA levels in a quantitative fashion.

In addition, a method for high throughput analysis of gene expression is the serial analysis of gene expression (SAGE) technique, first described in Velculescu, et al., Science, (1995), 270, 484-487. Among the advantages of SAGE is that it has the potential to provide detection of all genes expressed in a given cell type, provides quantitative information about the relative expression of such genes, permits ready comparison of gene expression of genes in two cells, and yields sequence information that may be used to identify the detected genes. Thus far, SAGE methodology has proved itself to reliably detect expression of regulated and nonregulated genes in a variety of cell types (Velculescu, et al., (1997), Cell, 88, 243-251; Zhang, et al., Science, (1997), 276, 1208-1272 and Velculescu, et al., Nat Genet, (1999), 23, 387-388. Techniques for producing and probing nucleic acids are further described, for example, in Sambrook, et al., Molecular Cloning: A Laboratory Manual (New York, Cold Spring Harbor Laboratory, 1989).

In other methods, the level of expression of a gene is detected by measuring the level of protein encoded by the gene. In the case of polypeptides which are secreted from cells, the level of expression of these polypeptides may be measured in biological fluids. While methods such as immunoassay, ELISA, Western blot analysis, or immunohistochemical staining using an agent, e.g., an antibody, that specifically detects the protein encoded by the gene may be contemplated, other more sensitive and quantitative methods are preferred, as described below. The invention is not limited to a particular assay procedure, and therefore is intended to include both homogeneous and heterogeneous procedures. General techniques to be used in performing the various immunoassays noted above are known to those of ordinary skill in the art.

Proteomics: Rationale for Use

While genomic profiling provides information about susceptibility to disease, proteomic profiling reflects snapshots of metabolic dynamics, reveals heterogeneous gene expression, identifies biologically relevant phenotypes and generates information on protein structure-function relationships in the severity and prognosis of a disease. Thus, results from proteomic studies should offer insight into the pathology of obesity and effects by oleanoprin therapy. Many forms of protein alterations can be associated with pathophysiological changes and therapeutic treatments. In addition to expression levels and patterns, these include alternative splicing, post-translational modifications, proteolytic processes, co-secretion and protein-protein interactions. Thus, the identification and quantification of proteins alone is not sufficient to understand functional interactions. Changes as small as the addition of a single phosphate, cleavage of a leader peptide, amimidation, or oxidation, can drastically alter the biological function of a protein. Thus, it is important to detect these minute changes using sensitive and accurate proteomic technology.

Sample Preparation

Serum or plasma proteins may be differentially expressed in response to pathophysiological changes in obesity and related diseases and to therapeutic treatments of these disorders. Proteomic study of plasma proteins in normal and obese patients demonstrated significant differences in protein patterns. Plasma will be prepared from blood samples and used in proteomic analyses.

Proteomics: The Use of 2DE, MS, MALDI-TOF, MS/MS, LC/MS, and SELDI-TOF

2-dimensional polyacrylamide gel electrophoresis (2DE) coupled to mass spectrometry (MS) is currently the standard analysis in proteomics. Plasma samples may be subjected to 2DE (first dimension isoelectric focusing, second dimension SDS-PAGE). Selected spots from 2DE may be...
extracted from the gels, digested with trypsin and subjected to MS analysis to determine their identities (Aebesold, R. & Mann, M. Mass spectrometry-based proteomics. Nature 422, 198-207 (2003)). MALDI-TOF (matrix-assisted laser desorption/ionization coupled with time of flight (TOF)) is a method of choice to be used for proteins (Tanaka, K. The origin of macromolecule ionization by laser irradiation (Nobel lecture). Angew Chem Int Ed Engl 42, 3860-70 (2003)). Tandem MS (MS/MS) may be used for selective isolation of peptide fragments to read out the (partial) amino acid sequence, and LC/MS (liquid chromatography coupled to MS) may be used for the identification of small peptides. However, the 2DE/MS detection is restricted to pl between 4 and 10 and proteins within an MW range of 10-200 kDa. Thus, peptides or small proteins (0.5-10 kDa), such as hormones, adipokines and growth factors, which are related to obesity pathogenesis may not be detected by 2DE/MS. Thus, additional initial separation systems such as C/M/MS using HPLC coupled MALDI-TOF for differential small peptide display is contemplated for use (Amelia A., Hodewer, J. H., van Geel, M. H., Lommen, A., Visser, J. P., Hino, J. J. & Hall, R. D. Allignment and statistical difference analysis of complex peptide data generated by multidimensional LC-MS. Proteomics 6, 641-53 (2006). Surface enhanced laser desorption ionization and time of flight (SELDI-TOF) using chromatographic chip surfaces based on amino acid sequence, protein structure, charge and hydrophobicity is also contemplated for use (Weinberger, S. R., Dalmaso, E. A. & Fung, E. T. Current achievements using ProteinChip Array technology, Curr Opin Chem Biol 6, 86-91 (2002)), as well as antibody proteomics based on immunoadfinity (Ingvarsson, J., Lindstedt, M., Borrebaeck, C. A. & Wingren, C. One-step fractionation of complex proteomes enables detection of low abundant analytes using antibody-based microarrays, J Proteome Res 5, 170-6 (2006)).

<table>
<thead>
<tr>
<th>Name of Protein</th>
<th>GenBank Accession Number</th>
<th>DNA/Protein</th>
<th>SEQ I.D. NO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPAR-gamma isoforms 2</td>
<td>NP_056953</td>
<td>Protein</td>
<td>15</td>
</tr>
<tr>
<td>Leptin</td>
<td>D0247154</td>
<td>DNA</td>
<td>16</td>
</tr>
<tr>
<td>P2 Adipocyte Protein</td>
<td>XM_939801</td>
<td>DNA</td>
<td>17</td>
</tr>
<tr>
<td>Adipin</td>
<td>M84526</td>
<td>DNA</td>
<td>18</td>
</tr>
<tr>
<td>Angiotensinogen Protein</td>
<td>AAD34288</td>
<td>Protein</td>
<td>19</td>
</tr>
<tr>
<td>Angiotensinogen DNA</td>
<td>BC011519</td>
<td>DNA</td>
<td>20</td>
</tr>
<tr>
<td>Complement Factor H</td>
<td>AA858049</td>
<td>Protein</td>
<td>21</td>
</tr>
<tr>
<td>Complement Factor D</td>
<td>BC058707</td>
<td>DNA</td>
<td>22</td>
</tr>
<tr>
<td>Lipoprotein Lipase</td>
<td>M13586</td>
<td>DNA</td>
<td>23</td>
</tr>
<tr>
<td>Perilipin</td>
<td>NM_002666</td>
<td>DNA</td>
<td>25</td>
</tr>
<tr>
<td>Glucose Transporter 4 (GLUT4)</td>
<td>NP_010313</td>
<td>Protein</td>
<td>26</td>
</tr>
<tr>
<td>SREBP1</td>
<td>NM_004176</td>
<td>DNA</td>
<td>27</td>
</tr>
<tr>
<td>CCAAT</td>
<td>NM_005194</td>
<td>DNA</td>
<td>28</td>
</tr>
<tr>
<td>Plasminogen Activator Inhibitor-1</td>
<td>NP_009593</td>
<td>Protein</td>
<td>30</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>NM_004797</td>
<td>DNA</td>
<td>31</td>
</tr>
<tr>
<td>Interleukin-6</td>
<td>NM_006090</td>
<td>DNA</td>
<td>32</td>
</tr>
<tr>
<td>Interleukin-6</td>
<td>NP_000591</td>
<td>Protein</td>
<td>33</td>
</tr>
<tr>
<td>TNF-alpha</td>
<td>NP_000885</td>
<td>Protein</td>
<td>34</td>
</tr>
<tr>
<td>PPAR-alpha</td>
<td>NP_000030</td>
<td>DNA</td>
<td>35</td>
</tr>
<tr>
<td>PPAR-alpha</td>
<td>NP_005027</td>
<td>Protein</td>
<td>36</td>
</tr>
<tr>
<td>PPAR-gamma</td>
<td>NM_018699</td>
<td>DNA</td>
<td>37</td>
</tr>
<tr>
<td>PPAR-gamma</td>
<td>NP_056953</td>
<td>Protein</td>
<td>38</td>
</tr>
<tr>
<td>PPAR-delta</td>
<td>NM_006238</td>
<td>DNA</td>
<td>39</td>
</tr>
<tr>
<td>PPAR-delta</td>
<td>NP_062229</td>
<td>Protein</td>
<td>40</td>
</tr>
</tbody>
</table>

**TABLE 3**

<table>
<thead>
<tr>
<th>Gel Lane Gene</th>
<th>Primer sequence (sense/antisense)</th>
<th>Product size (bp)</th>
<th>Gen ID #</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Marker 1 kb plus DNA ladder of 200, 300, 400, 500, 650, 850, and 1000 bp</td>
<td>200-1,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>PPAR2 5'-AAATCTCCTTCTCTTGCAAGA-3' (SEQ ID NO: 1) 630</td>
<td>BC006811</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Adipocytokine 5'-TGCAAGCAATGCTGCTG-3' (SEQ ID NO: 2) 583</td>
<td>BC011353</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>LPL 5'-CAGTTTTCTCTCTGACCC-3' (SEQ ID NO: 3) 276</td>
<td>BC007358</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Adipin 5'-CTGCAATACTTTGCTTCC-3' (SEQ ID NO: 4) 418</td>
<td>BC007580</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>PPAR-delta 5'-GTGAAATGTCCTGCTGTGGCC-3' (SEQ ID NO: 5) 380</td>
<td>BC007580</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Ap2 5'-CGACAGATGCTATGCTG-3' (SEQ ID NO: 6) 310</td>
<td>BC014139</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Ancap 5'-CACCAAGATGCTGCTG-3' (SEQ ID NO: 7) 380</td>
<td>BC007580</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Adipoceptor 5'-ATGCAGCTGCTGCTG-3' (SEQ ID NO: 8) 452</td>
<td>BC007580</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Osteogenic 5'-GGTTGAGTACCTGCTGTGGCC-3' (SEQ ID NO: 9) 589</td>
<td>BC003800</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>OC 5'-CAGCTGCTGCTGCTG-3' (SEQ ID NO: 10) 310</td>
<td>BC007580</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Osteogenic 5'-GCCAGATGCTGCTG-3' (SEQ ID NO: 11) 589</td>
<td>BC003800</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>28S RNA Internal Control 5'-GCAGCTGCTGCTGCTG-3' (SEQ ID NO: 12) 310</td>
<td>BC007580</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 4**

Sequence Identifiers for Relevant Proteins Whose Genes may be Modulated by Exposure to Oleuropein or Analogues or Derivatives therefrom.
EXAMPLES

[0302] The following examples are set forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1
Modulation of Adipogenesis and Lipodystrophy by Oleuropein

Oleuropein Modulates Adipocyte Differentiation

Methods

[0303] Human mesenchymal stem cells (hMSCs) at 10^6 cells/cm² were cultured in MSC growth medium first to confluence. Adipogenesis was induced two days post confluence by culturing the cells in adipogenic medium (AIM) containing 10 μM dexamethasone, 1 μg/ml insulin, and 0.5 mM 3-isobutyl-1-methylxanthine, in the absence and presence of oleuropein at 1, 10 and 100 μM. Fresh culture medium was changed every 3 days. Full differentiation of hMSC to adipocytes was detected by Oil Red O staining at day 12. Gene expression was monitored by RT-PCR, using primers shown in Table 3.

Results

[0304] It was determined that oleuropein down-regulates adipocyte differentiation, fat accumulation and adipogenic differentiation in human mesenchymal stem cells (hMSCs). As seen in FIG. 1, hMSCs grown in control medium do not stain with Oil Red O (counterstained with hematoxylin), while hMSCs grown in AIM contain lipid droplets that stain with Oil Red O. Oleuropein shows dose dependent inhibition of adipocyte differentiation. We are confirming these results using flow cytometry. Oleuropein down-regulates the expression of adipogenic genes PPARγ2, LPL (lipoprotein lipase), and C/EBPγ (lipid binding protein). Oleuropein up-regulates PPARα expression while the expression of the internal control 28S rRNA remains relatively constant.

Oleuropein Decreases Adipocytes and Allows Trans-differentiation into Osteoblasts

Methods

[0305] For transdifferentiation studies, hMSCs were cultured in adipogenic medium for 12 days, allowing full differentiation into adipocytes. Oleuropein (80 nM) was added to the culture medium for 48 hours. De-differentiation of adipocytes was detected by the disappearance of the accumulated lipid by Oil Red O staining. Oleuropein-treated cells were collected by trypsinization and cultured in osteogenic medium containing 10 nM dexamethasone, 10 μM β-glycerophosphate, 50 μg/ml l-ascorbate 2-phosphate, and 10 nM 1α, 25-dihydroxyvitamin D3 for 6 days.

Results

[0306] As seen in FIG. 2, fully differentiated adipocytes from hMSC lost their Oil Red O staining after the addition of oleuropein. Furthermore, treatment with osteogenic medium resulted in transdifferentiation into osteoblasts. In contrast, hMSC-derived adipocytes treated with osteogenic medium, but not with oleuropein, showed little change at day 6. 48 hours after the addition of oleuropein, the expression of adipogenic marker genes PPARγ2, LPL, and C/EBPγ were down-regulated, while PPARα was up-regulated. Upon the switch to osteogenic medium, expression of osteocalcin (OC) and alkaline phosphatase (ALP) are observed.

Summary of Results

[0307] It was determined that oleuropein can modulate adipogenic differentiation of cultured hMSC, and can de-differentiate hMSC-derived adipocytes, allowing transdifferentiation. These results are important because they show that oleuropein can modulate the differentiation and commitment steps in adipogenesis, and offer a possible cellular mechanism for anti-obesity effects.

Example 2
Modulation of Endothelial Dysfunction and Atherosclerosis by Oleuropein

[0308] Oleuropein Reduces Diet-Induced Atherosclerosis in the Western Diet-Fed apoE-ko Mouse

Methods

[0309] It was previously demonstrated that oleuropein and OLE, following administration to mice, could be detected in the blood and urine by LC-MS. To test whether oleuropein reduces diet-induced atherosclerosis, oleuropein was administered to apoE-ko mice at various doses of 0.25 mg/ml, 2.5 mg/ml, and 25 mg/ml in the drinking water followed by feeding them with a Western diet containing 42% of calories from fat (Harlan-Teklad). At the 4 month time-point for the 0.25 mg/ml dose (daily dose 1.25 mg), serum levels of oleuropein achieved with this dose are comparable to those in animals fed a diet supplemented with olive oil.

[0310] The mice were sacrificed, blood and tissues collected. Aortas were dissected from the aortic valve to the iliac bifurcation, opened longitudinally, and pinned to a black wax surface. Atherosclerotic lesions were stained with Oil Red O. The total area of the aorta and the atherosclerotic lesion areas were determined by planimetry using Image Pro software. FIG. 3 shows representative aortas stained with Oil Red O from untreated and oleuropein treated apoE-ko mice.

Results

[0311] As seen in FIG. 4, there was a significant reduction in lesion area at 4 months in both male and female apoE-ko mice that were treated with oleuropein. Female mice had more lesions than male mice in both control and oleuropein-treated groups, consistent with the known gender effects in the apoE-ko model. Error bars indicate SEM; differences between control and oleuropein-treated mice were significant for both genders (p < 0.05).
Summary of these Results

[0312] It was demonstrated that oleuropein modulates diet-induced atherogenesis in the Western diet-fed apoE ko mouse model, at doses comparable to those achievable by diet. These results suggest that the beneficial effects of a Mediterranean diet may not be due solely to effects of olive oil on the lipid profile, but may also be mediated in part by non-lipid components such as oleuropein.

Example 3

Computational Approaches to New Treatments for Obesity

Methods

[0313] Detailed theoretical and computational analysis for binding of oleuropein to PPARγ, PPARα and PPARα was performed, using an approach that combines AutoDock, MD and MM-PBSA calculations. We used the crystal structure of PPARα and PPARα ligand binding domains. The large ligand-binding site is formed by several α-helices, including the C-terminal AF-2 helix. We first used molecular docking to generate several distinct binding orientations, and performed molecular dynamics simulation to further relax the complex. Then, we applied MM-PBSA to estimate the affinity for each binding mode. The binding modes with the lowest free energy are expected to be the most favorable. We then analyzed the detailed interactions based on these binding modes.

Oleuropein Mimics the Binding Mode of High Affinity Ligands for PPARα

[0314] FIG. 5a shows docking of oleuropein to PPARα in the energetically most favorable binding mode (II), with free energy of ~52.73 kcal/mol. In the predicted structure, oleuropein occupies the Y-shaped ligand-binding pocket, identical to two known ligands of PPARα: the synthetic agonist GW2433, which is a high-affinity ligand, and the natural fatty acid eicosapentaenoic acid (EPA), which binds efficiently to all three PPARs.

[0315] FIG. 5b shows an overlay of oleuropein (red and blue), GW2433 (green) and EPA (purple) in their bound configurations. Oleuropein’s sugar group is located in a similar position and orientation as the carboxylic groups in GW2433 and EPA. The two hydroxyls on the sugar ring are oriented toward the AF-2 helix and held in place through a network of hydrogen bonds with Y473, and hydrophobic interactions with L469 and T84, as seen in FIG. 5d. Both Y473 and L469 are part of the AF-2 helix. The same network of hydrogen bonds occurs in the binding of ligands to PPARα and γ, and is believed to be important for ligand-mediated activation of these receptors.

Oleuropein Shows Different Binding Modes than High Affinity Ligands for PPARγ

[0316] FIG. 6a shows docking of oleuropein with PPARγ in the most favorable binding mode (I), with free energy of ~27.09 kcal/mol. In the predicted structure, oleuropein binds into the large PPARγ pocket in an extended (up-down) conformation rather than the U-shaped conformation of other known ligands such as G1262570 and rosiglitazone. FIG. 6b shows an overlay of oleuropein (red and blue), rosiglitazone (yellow) and the PPARγ agonist G1262570 (green). Unlike known ligands, oleuropein does not interact directly with AF-2 helix. At one end of the molecule, the dihydroxy phenol group is directed into the solvent-accessible channel between H3 and the β strands. At the other end, the oleuropein sugar headgroup is inserted 4.0 Å deeper than the phenylxazolone tail of G1262570 into the lipophilic pocket adjacent to the β sheet, forming two strong hydrogen bonds with the carboxyl group of E259 (shown in FIG. 6d).

Oleuropein Shows Different Binding Modes than High Affinity Ligands for PPARα

[0317] FIG. 7a shows the binding structure of PPARα-oleuropein complex with free energy of ~24.71 kcal/mole. The PPARα backbone is represented by the yellow ribbon, and oleuropein is represented with vdW and is color coded as follows: carbon, cyan and oxygen, red. In the predicted structure as shown, oleuropein adopts a Y-shaped configuration when it binds to PPARα, similar to how it binds to PPARα. This is consistent with the similarities between the binding sites of PPARα and PPARα. This conformation differs from the U-shaped conformation of other known ligands of PPARα, such as GW409544. FIG. 7b represents superposition of the structures of GW409544 (green) and oleuropein (red and blue) bound to PPARα. FIG. 7c shows the chemical structures of oleuropein and GW409544. FIG. 7d shows hydrogen bonds formed by oleuropein and the surroundings (indicated as green dotted lines).

Summary of these Results

[0318] The known ligand binding domains of PPAR δ is large and Y-shaped. Our molecular modeling results show binding modes for oleuropein that are as favorable as the currently known ligands. Oleuropein mimics precisely the binding mode of high affinity ligands for PPARδ, indicates that oleuropein is capable of modulating PPARδ activity similar to that of known PPARδ agonist. Oleuropein has the potential to increase fat oxidation, increase energy uncoupling and thermogenesis and thus decrease fat accumulation, reduce body weight and prevent obesity. For PPARγ, on the other hand, in the predicted structure, oleuropein binds to PPARα in an extended (up-down) conformation rather than the U-shaped conformation of other known ligands such as G1262570 and rosiglitazone. Unlike these ligands, oleuropein does not interact directly with AF-2 helix. These results predicate that oleuropein has the potential to interact with PPARγ different from PPARγ known agonists including Tiazolidinediones (TZD). Oleuropein is not on the AF-2 helix of PPARα, the region that involves in adipogenesis, thus suggesting an important mechanism for regulation of PPARγ activity in uncoupling adipogenesis from insulin sensitivity and other activities. For PPARα, in the predicted structure, oleuropein adopts a Y-shaped configuration when it binds to PPARα. This is similar to how it binds to PPARα. This is consistent with the similarities between the binding sites of PPARα and PPARα as well as their functions. Both PPARα and PPARα are involved in fat oxidation and energy utilization. In this context, the energetically favorable Y configuration of oleuropein may offer insight into its biological effects. Studies are underway to confirm the biological significance of these modeling results, and correlating them with in vitro and in vivo data. We are studying the experimental binding and co-crystallization of oleuropein with the ligand binding domains of PPAR α, δ and γ. We are analyzing the expression and activity of PPAR α, δ and γ in tissues from oleuropein-treated apoE ko mice that show reduction in diet-induced atherosclerosis, to identify the importance of these isoforms to the observed biological activity.
Example 4
Preparation of Oleuropein from Olive Leaf Extract

LC-MS Standardization of Olive Leaf Extract (OLE) Containing Oleuropein as the Active Moiety

A. Preparation of Olive Leaf Extract

[0319] Selection cleaning and processing of the olive leaves. Healthy whole olive leaves were selected and cleaned to remove dust, residual insecticides and contaminating materials. The leaves were cut into small pieces or powdered into fine powders and placed in a sterile flask just before extraction, with sterile distilled water and pre-heated to 80°C.

[0320] Extraction media and ratio. A comparison was made between the extraction with water, PBS (Phosphate Buffered Saline, 10 mM sodium phosphate buffer, pH 6.8, containing 0.15% NaCl) and organic solvents (50% methanol, or 40% ethanol). No significant differences were found in biological activity using the different extraction methods. Since the therapeutic ingredients in olive leaf are apparently water-soluble, a decision was made to use water extraction, because it gives stable extracts that can be concentrated with ease. For efficient extraction, it was determined that the optimal ratio is one gram of dry leaves to 40 ml of water.

[0321] Extraction conditions: The extraction mixture was covered and incubated with agitation for 10-12 hours at 80°C in a water bath. It is important that the extraction temperature be kept at 85°C. Heating above 86°C inactivates oleuropein, one of the principle ingredients of OLE. Alternative methods for extraction including but not limited to microwave for two repeats of 5-10 min, or ultrasound for 25 min.

[0322] Concentration of the extract by lyophilization. At the end of 10-12 hours at 80°C, proper extraction results in a medium brown colored OLE with about 70-80% of the original volume. The liquid was poured off and collected. The leaves were extracted again for a second time under the same conditions to ensure complete extraction of the active ingredients. Small pieces of leaves and insoluble material in the extract were removed by centrifugation at 20,000 g for 30 min. The clear supernatant was collected and labeled as Step 1 OLE. Step 1 OLE was concentrated by freeze-drying using a Lyophilizer. The dried OLE was collected, and labeled as Step 2 OLE.

[0323] Sterilization by Millipore filtration. Step 2 OLE was dissolved in sterile water at 10-20 mg/mL, sterilized by Millipore filtration with 0.45 micron filter, distributed at small lots in sterile cryo-tubes under aseptic conditions and stored at -80°C. This is Step 3 OLE and its oleuropein content was quantitated by LC-MS using known oleuropein standard. Standardized OLE was used in all of our experiments.

B. Preparation and LC-MS Standardization of Oleuropein

[0324] Oleuropein was prepared and quantitated by LC-MS as noted above. This process was performed using an HP1100 equipped with diode array detector and ESI-mass spectrometer. LC was done using a C18 column, using a gradient of 5-95% CH3CN—H2O containing 1% acetic acid. The diode array recordings were made at 280 nm and 230 nm, and the ESI mass spectrum was made in negative detection mode. Purified oleuropein was used as a standard. Experiments were optimized by infusion of the standards in negative scan mode to investigate the [M+H] ion of oleuropein glycoside (m/z 539). Oleuropein fractionates as a single peak by HPLC at 1.846 min, and contains predominantly oleuropein glycoside (m/z 539). The oleuropein content in the lyophilized OLE ranges from 20-25% as standardized by LC-MS.

[0326] Preparation and LC-MS Standardization of Hydroxytyrosol

[0327] Hydroxytyrosol was prepared from homogeneous oleuropein isolated from OLE. The procedure involves treatment of oleuropein with β-glycosidase (including but not limited to Sigma G4511) in 80 mM sodium acetate pH 5.0 using 1 Unit of enzyme/mgole of substrate at 37°C, for 1 hr to remove the glucose moiety and to yield oleuropein aglycone. The oleuropein aglycone was then be treated with esterase (including but not limited to Sigma E0089) in sodium phosphate buffer at pH 7.5 using 1 Unit of enzyme/mgole of substrate at 25°C for 1 hr to yield hydroxytyrosol and oleocanalic acid. The final products were resolved and quantitated by LC-MS as described above. Detection and quantification were performed at 280 and 320 nm for hydroxytyrosol at 0.698 min (m/z 153) and oleuropein aglycone at 2.087 min (m/z 377), while 240 nm was used for the detection of oleocanalic acid at 1.846 min (m/z 241).

[0328] LC-MS Standards

Synthesis of Hydroxytyrosol

[0329] Hydroxytyrosol, the major metabolite of oleuropein is biological active. We have designed a unique method for chemical synthesis of hydroxytyrosol. Chemically synthesized hydroxytyrosol is as active as its natural counterpart from OLE. Major steps in chemical synthesis of hydroxytyrosol are briefly outlined below:

[0330] We started our hydroxytyrosol synthesis from 3,4-dihydroxyphenylactic ester. It was prepared by the following procedure. The reaction mixture of 3,4-dihydroxyphenylactic acid 168 mg in CH3OH was treated with Acetyl chloride and CH3OH mixture. The reaction mixture was stirred at room temperature overnight. The reaction mixture was purified by column chromatography. A total of 140 mg of oily compound was obtained.

\[ \text{HO} \quad \text{ArCl} \quad \text{OCH3} \]

\[ \text{HO} \quad \text{MeOH} \quad \text{RT} \]

[0331] Hydroxytyrosol was prepared from 3,4-dihydroxyphenylactic ester by LiAlH4 reduction. 140 mg of 3,4-dihydroxyphenylactic ester was dissolved in tetrahydrofuran (THF). 1M of LiAlH4 (4 mL) solution was added to the reaction mixture. The mixture was stirred at 0°C, for 2 hours. The reaction mixture was purified by column chromatography to give hydroxytyrosol and characterized by LC-MS.

\[ \text{HO} \quad \text{LiAlH4} \quad \text{THF} \quad 0^\circ \text{C} \]
B. HPLC Analysis of OLE Components

[0332] Step 3 OLE was subjected to HPLC using a Waters two solvents delivery system with photodiode array detector. A Symmetry C18 column (5 μm, 3.9x250 mm) column with a Sentry Guard 3.9x20 mm insert was used. Data acquisition and quantitation were performed with Millennium 32 software (version 3.0). The mobile phase was 79% distilled water and 21% acetonitrile (HPLC grade), both acidified to pH 3 with 0.1 M orthophosphoric acid. This solvent system is designed for resolution and quantitation of polyphenolic compounds. The flow rate was 1 ml/min, and the injection volume was 20 μl. Polyphenolic compounds were monitored by absorbance at 280 nm. In the run time was 35 minutes. The structure of oleanuropein is shown in FIG. 8. The elution profile is shown in FIG. 9. A total of seven major peaks were resolved from the included material. The solvent front and excluded material appear before peak 1, with retention time (Rt) less than 6 minutes.

C. Identification of OLE Components

[0333] The major OLE components identified by TLC and HPLC with known standards. TLC was carried out on silica gel 60 F254 (Merck) with chloroform/methanol/acetic acid (70:30:10). Secoisolaurids and flavonoids were detected by visualization under UV light at 254 nm with 10% ferric chloride and 10% aminoethyl diphenylborate spray, using known standards. HPLC verification was conducted by repeated HPLC of peak material with known amounts of standards. The identities of the polyphenolic compounds in each peak are shown in Table 5, along with their relative levels in the OLE and their cytotoxicity.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Rt (min)</th>
<th>Compound</th>
<th>% (w/w)</th>
<th>Cytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>06</td>
<td>Solvent front</td>
<td></td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6.6</td>
<td>Rutin</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>7.8</td>
<td>Verbascoside</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>8.9</td>
<td>Luteolin7-glucoside</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10.0</td>
<td>Apigenin7-glucoside</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>17.0</td>
<td>Flavonol x</td>
<td>0.56</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>22.8</td>
<td>Oleanopin</td>
<td>12.8</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>34.0</td>
<td>Oleuropein</td>
<td>0.51</td>
<td></td>
</tr>
</tbody>
</table>

D. LC-MS Analysis

[0334] Samples: The standard and peak 6 oleanoprene were prepared in sterile water with a final concentration of 1 mg/ml. Step 3 OLE was prepared as described above at a final concentration of 10 mg/ml. All samples were Millipore filtered and stored at -20° C. until use.

[0335] Experimental protocol: LC-MS was performed using an HP 1100 equipped with diodearray detector and ESI-mass spectrometer. LC was done using a C18 column (20x4.0 mm), with 4 minute elution using a gradient of 5-95% CH3CN (containing 1% acetic acid)-H2O (containing 1% acetic acid). The diodearray records were made at 280 nm and 230 nm, and ESI mass spectrum was made in negative detection mode. The standard and peak 6 oleanoprene were diluted to a final concentration of 0.5 mg/ml, and OLE was diluted to 5 mg/ml. The injection volumes were all 5 μl. Experiments were optimized by infusion of the standards in negative scan mode to investigate the [M-H] ion of oleanoprene (m/z 539).

Results

[0336] Peak 6 Oleanoprene Showed Similar Purity to the Oleanoprene Standard by LC

[0337] FIG. 11 shows the LC-MS results of oleanoprene standard (11A), peak 6 oleanoprene (11B) and OLE (11C). As seen in FIGS. 11A-1C and 11B-1C, peak 6 oleanoprene is as pure as the standard. A single major peak was observed at 1.827 min and a minor peak at 1.969 min (less than 1%) in both samples.

[0338] Peak 6 Oleanoprene Showed a Single Mass as the Oleanoprene Standard by MS

[0339] MS of the standard and the peak 6 oleanoprene are seen in FIGS. 11A-1MS and 11B-1MS. A single mass peak at [M-H]−=539 corresponds to oleanoprene was detected in both samples. A minor peaks was observed at mass [M-H]−=1079 it represents [2M-H].

[0340] OLE Contains Several Components by LC

[0341] FIG. 11C-1LC represents the LC profile of OLE. Several peaks were detected. Peaks at 1.82 min is the major peak, peaks B, and C are moderate and minor respectively.

[0342] MS of OLE Identified Oleanoprene, Oleanoprene Glycosyle and Hydroxytyrosol.

[0343] FIGS. 11C-MS of OLE A, OLE B, and OLE C represent MS of LC peaks A, B, C respectively. MS of peaks A, B, and C identified mass peaks at [M-H]−=539, 377 and 153 correspond to oleanoprene, oleanoprene aglycone and hydroxytyrosol respectively.

[0344] Heavy metals, pesticides, fungicides & herbicides were analyzed and not detected in the samples.

Summary and Discussion of Results:

[0345] LC-MS results show that peak 6 oleanoprene consists of a single major peak at [M-H]−=539 corresponds to oleanoprene. The purity of this sample is equal to or even better than the standard. The relative size of the peak [M-H]−=1079 is less in peak 6 oleanoprene than in the standard.

[0346] LC-MS of OLE indicates that in addition to oleanoprene, oleanoprene aglycone and hydroxytyrosol are also present. Oleanoprene is a heterosidic ester of elenonic acid and hydroxytyrosol (3,4-dihydroxy-phenylethanediol), containing a molecule of glucose, upon β-glucosidase action it yields oleanoprene aglycone. Hydrolysis of oleanoprene aglycone yields hydroxytyrosol and elenonic acid. The structural relationships among these compounds are shown in FIG. 10. These molecules are major metabolites of oleanoprene thus their presence in the OLE is expected. Elenoniac acid has no UV absorbance thus it cannot be detected in LCM-MS analysis.

[0347] Oleanoprene can also be prepared from olive leaves by extracting with 50% aqueous methanol. After evaporation of methanol, the aqueous phase can be extracted with chloroform and then saturated with NaCl and filtered. Oleanoprene and other phenolic compounds can be extracted with ethyl acetate. The ethyl acetate phase was totally evaporated to
dryness. Oleuropein can be further purified and characterized by LC-MS as described above.

Example 5

Oleuropein is Bioavailable: it is Absorbed and Well Tolerated in Animals

A. Bioavailability Studies

[0348] It is important to establish that chronic administration of oleuropein results in the absorption of the biologically active material in vivo. In addition, it is necessary to determine the physiologically achievable and relevant concentrations in tissue, blood, and urine, and to compare these with concentrations that show activity in vitro or in cell culture.

[0349] To verify that we can administer oleuropein chronically to mice, we added purified oleuropein to drinking water at concentrations of 5 μg/ml, 50 μg/ml, 500 μg/ml, and 5 mg/ml. The water intake of mice averages 5 ml per mouse per day, and the doses of oleuropein result in daily doses of 0.025 mg, 0.25 mg, 2.5 mg, and 25 mg per mouse per day (equivalent to 1, 10, 100, and 1000 mg/kg body weight). 5 male and 5 female C57BL/6 wild-type mice were used for each dose of oleuropein, and housed them in metabolic cages that allow precise determination of water intake and quantitative urine collection.

B. LC-MS Quantitation

[0350] After seven days to 4 months of chronic steady-state administration, mice blood and urine were collected. 100 μl of serum or urine were extracted with 1 ml ethyl acetate, evaporated to dryness, and reconstituted with 25 μl water and 5 μl was injected for LC-MS analysis.

[0351] LC-MS quantitation was performed using an HP1100 equipped with diode array detector and ESI-mass spectrometer. LC was done using a C18 column (4×20 mm), with a 4 minute elution using a gradient of 5-95% CH3CN—H2O containing 1% acetic acid. The diode array recordings were made at 280 nm and 230 nm, and the ESI mass spectrum was made in negative detection mode. Purified oleuropein, and hydroxytyrosol (synthesized by us) were used as standards. The injection volumes were 5 μl. Experiments were optimized by infusion of the standards in negative scan mode to investigate the [M-H] ion of oleuropein glycoside (m/z 539), oleuropein aglycone (m/z 377), and hydroxytyrosol (m/z 153).

C. Summary of Results

[0352] FIG. 12 shows results from LC-MS analysis of mice blood and urine after 4 months of chronic steady-state administration. Oleuropein standard shows a single peak by LC (12A) with mass of 539 (m/z 539) (12B). In comparison, the LC profile of serum (12C,D) and urine (12E,F) contain mainly hydroxytyrosol (m/z 153) as well as other derivatives and metabolites. Our results show that 1) we can administer oleuropein in a dose-dependent manner chronically to mice, 2) oleuropein is absorbed by the mice and metabolized to hydroxytyrosol, and 3) we can quantify the amount of oleuropein and hydroxytyrosol in the serum and urine of mice by LC-MS.

[0353] In summary, our results show that oleuropein is bioavailable. It is absorbed and well tolerated in animals. It is metabolized to hydroxytyrosol, which is secreted in urine.

Example 6

Inhibition of HIV-1 Fusion by Oleuropein and Hydroxytyrosol

Materials and Methods

Oleuropein (Ole) and Hydroxytyrosol (HT)

[0354] Oleuropein (Ole) was purified from olive leaf extract, characterized, and standardized by liquid chromatography-coupled mass spectrometry (LC-MS) [3]. Hydroxytyrosol (HT) was prepared by stepwise hydrolysis of Ole with β-glucosidase (Sigma G4511) in 80 μM sodium acetate, pH 5.0 using 1 Unit/μmol substrate at 37° C. for 1 hr. This treatment removes the glucose moiety from Ole and yields oleuropein aglycone (Ole-AG). Ole-AG was subsequently hydrolyzed with esterase (Sigma E0887) in 50 mM sodium phosphate buffer at pH 7.5 at 1 Unit/μmol substrate at 25° C. for 1 hr to yield hydroxytyrosol and oleic acid. The mixture was separated by HPLC and standardized by LC-MS. HT was also prepared by chemical synthesis from 3,4-dihydroxyphenylacetic ester (DHPA).

Cell Lines and HIV-1

[0355] Uninfected MT2 and H9 cell lines, and HIV-1,2HR chronically infected H9 (H9/HIV-1,2HR) and HIV-1/HIV-B virus, were obtained through the AIDS Research and Reference Reagent Program, NIH, NH. MT-2 cells [4, 5] were obtained from D. Richman, and H9 and HIV-1,2HR virus stocks [6, 7] from R. Gallo. The cell lines were cultured in RPMI medium 1640 containing 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, and 10% heat-inactivated fetal calf serum. Viral stocks were prepared and standardized as described [8].

Anti-HIV and Cytotoxicity Assays

[0356] The effects of Ole and HT on acute HIV infection and viral replication were measured by assays on syncytial formation in cell-cell HIV-1 transmission and on HIV-1 core protein p24 expression as described [8]. Cytotoxicity was evaluated by the MTT assay [8].

Molecular Modeling

[0357] Molecular modeling was performed by molecular docking, molecular dynamics (MD) simulation and free energy calculations [9,10]. Docking was performed with Autodock version 3.0.5 [11]. The relaxation of docking structure obtained was then implemented under Discovery from Insight II (Accelrys Inc., San Diego, Calif., U.S.A.) using 500 steps of Steepest Descent followed by Conjugate Gradient until the root mean square of the energy gradient reaches a value of 0.01 kcal/mol/Å.

HIV-1 gp41 Fusion Peptides C34 and N36

[0358] HIV-1 gp41 fusion peptides, N36 and C34 were synthesized by solid phase FMOC method (GenMed, CA) and purified by HPLC. The sequences of these peptides are (Ac-SGTVQQQNLRAIAAQQHLLQUIVWgkQ1AR1L-NH2) and (Ac-WMEWDFRFINNYSLLHSL1IESQNNQQKNEQELL-NH2), respectively. Corresponding viral peptides N36 and
C34 prepared from HIV-1 were obtained through the NIH AIDS Research and Reference Reagent Program, NIAID, NIH [12] and used as standards for purification and bioassays.

Fusion Complex Formation

[0359] Fusion complex formation was carried out by incubating equimolar amounts of HIV-1 gp41 fusion peptides N36 and C34 in PBS (Phosphate Buffer Saline, containing 50 mM sodium phosphate, pH 7.2 and 150 mM NaCl) at 10 or 20 μM each at 37°C. Peptide N36 was first incubated either alone or with various concentrations of Ole or HT for 30 min. Next, an equimolar amount of C34 was added and the samples incubated for 30 min.

Native Polyacrylamide Gel Electrophoresis (N-PAGE)

[0360] N-PAGE was carried out as previously reported [13] with modifications that involve fusion peptide concentration, order of reactions, and time of incubation in fusion complex formation. Tris-glycine gels (18%, Invitrogen, Carlsbad, Calif.) were electrophoresed at 120 V for 2 h, stained with Coomassie Blue and analyzed by densitometry.

Circular Dichroism (CD) Spectroscopy

[0361] CD spectra were recorded on a JASCO 620-JS CD spectrometer, using 1 mm sample cells and a fixed temperature of 4°C [8]. Each spectrum is a smoothed average of 10 scans. The bandwidth for each measurement was 1 nm. CD intensities are expressed as mean residue ellipticities [10] (degrees cm²/dmole). Prior to calculation of the final ellipticity, all spectra were corrected by subtracting the reference spectra of PBS without peptides.

Results

Preparation and LC-MS Analysis of Oleuropein (Ole) and Hydroxytyrosol (HT)

[0362] FIGS. 13A-1B and 13C-1D represent LC-MS analysis of Ole and HT. Ole fractionsates as a single peak by HPLC at 1.848 min with m/z of 559 and HT as a single peak by HPLC at 1.108 min with m/z of 153. FIG. 13E shows the major steps in Ole metabolism. These are also the basic reactions in HT preparation from Ole. First, the glucose moiety is removed by β-glucosidase to yield oleuropein aglycone (Ole-AG). Next, Ole-AG is hydrolyzed by esterase to yield HT and 3, 4-dihydroxyphenylacetic acid (DHPAA) as the starting material. Two major steps are involved: 1) acetylation with acetyl chloride (AcCl), and 2) reduction by LiAlH₄ to yield HT. Chemically synthesized HT was purified and characterized by LC-MS, and demonstrates identical biological, chemical and physical properties as natural HT prepared from Ole from olive leaf extract.

Ole and HT Inhibit HIV-1 Infection and Replication, but are not Toxic to Target Cells

[0363] Ole and HT exhibit dose-dependent inhibition of HIV-1 infection and replication as measured by syncytial fusion and p24 production (Table 6). The average EC₅₀ for Ole is 55 μM for syncytial formation and 73 μM for p24 production. The corresponding EC₅₀ for HT are 61 nM and 68 nM for HT. No cytotoxicity was detected, either by MTT assay or trypan blue dye exclusion, over a 10,000-fold concentration range from 1 nM to 10 μM. The EC₅₀ for inhibition on fusion complex, 6HB formation are also presented in Table 6. Ole and HT Bind to the Conserved Hydrophobic Pocket on the Surface of the Central Trimeric Coiled-Coil of HIV-1 gp41

[0364] Inhibition of syncytial formation by Ole and HT reflects effects on early events during viral infection/entry, including CD4 receptor and coreceptor binding as well as viral fusion. To probe anti-HIV mechanisms of Ole and HT, we carried out molecular docking and MD calculations of these small molecules with viral targets. We found that Ole and HT bind to the conserved hydrophobic pocket on the surface of the central trimeric coiled-coil of the HIV-1 gp41 fusion domain.

[0365] HIV-1 envelope glycoprotein (Env) mediates viral entry by fusing virus to target cells. Env is trimeric on the virion surface. Each monomer contains a surface subunit, gp120, for virus binding to CD4 receptor and coreceptors [14-17] and a noncovalently associated transmembrane subunit, gp41, that mediates fusion of the virus with the target cell [18,19]. FIG. 14A shows the structure of HIV-1 gp41. Like other type I transmembrane proteins, HIV-1 gp41 consists of extracellular (ectodomian), transmembrane, and cytoplasmic domains. The ectodomain contains four functional regions: the fusion peptide, N-terminal heptad repeat (NHR), C-terminal heptad repeat (CHR), and a tryptophan-rich region. Binding of gp120 to the cellular receptor CD4 and co-receptor triggers conformational changes in gp41 that induce fusion [20-23]. This increases exposure of two heptad repeat motifs, NHR and CHR, and insertion of the fusion peptide into the target membrane [20-23]. Subsequently NHR and CHR fold in an antiparallel manner to create the six-helix bundle 6HB composed of a trimeric NHR coiled-coil core surrounded by three CHR helices that pack in the grooves of the coiled-coil as seen in FIG. 14B [24-26]. Formation of the 6HB promotes fusion between viral and cellular membranes and is essential for viral entry and infection [25,26]. Ole and HT interact with the NHR coiled-coil trimers N36 helices and interfere with the formation of 6HB with the CHR, C34, as shown in FIGS. 14C and 14D.

[0366] We used the crystal structure of the HIV-1 gp41 fusion complex, PDB code 1AIK [20] as a reference for our modeling work. To provide a ligand binding site, one of the C34 helices was removed from the 6HB (FIG. 15A). FIG. 15B shows the chemical structure of Ole with the 9 free rotatable bonds selected in our modeling interaction.

[0367] Molecular simulations suggest that the conserved hydrophobic cavity of the gp41 N36 trimmer coiled-coil is the most likely binding site for Ole and HT. This cavity is mainly occupied by W628, W631 and neighboring I635 and D632. The predicted binding structures of Ole and HT are shown in FIGS. 15C and 15D respectively. Ole and HT form stable hydrogen bonds with Q577 on the N36 peptide. FIGS. 15E and 15F are ribbon representations of the predicted binding site of Ole and HT. SHB, consisting of three N36-peptides (pink, residues 546-581) and two C34 peptides (green, residues 628-661), is used for docking calculations. Only one groove is exposed for the binding of small molecules. Both Ole and HT occupy the binding site similarly, with the diphenol ring forming stable hydrogen bonds with Q577. This blocks the close contacts between the hydrophobic groove in the gp41 NHR and the indole rings of W631 and W628, thus interfering with the formation of 6HB. In addition to hydro-
gen binding, hydrophobic interactions with I573, G 572, and L 568 also play important roles in the interaction.

Native PAGE Shows That Ole and HT Inhibit HIV-1 Fusion Core 6HB Formation

The effects of Ole and HT on the formation of fusion complex 6HB by electrophoretic mobility shift using native-PAGE (FIG. 16A). The electrodes were connected from cathode (negative terminal on top) to anode (positive terminal on bottom) and the peptides move in the electric field according to their charge and size. Peptides carrying net negative charges, such as C34, moves toward the positive terminal (bottom) whereas peptides carrying net positive charges, such as N36, moves toward the negative terminal or remain at the top of the well. Incubation of N36 and C34 resulted in the formation of the 6HB fusion complex which is larger than C34, moves slower than free C34, and thus migrates to the middle of the gel. Pre-incubation of N36 with Ole or HT results in inhibition of 6HB formation. A dose-dependent disappearance of 6HB band was detected with concomitant appearance of the free C34 band. Total inhibition is achieved at 100 nM Ole or HT with EC50 of around 66 and 58 nM. These results confirm the predictions from molecular modeling.

CD Analysis Indicates that Ole and HT Inhibit HIV-1 Fusion Core 6HB Formation

We also used CD analysis to confirm molecular modeling predictions (FIG. 16B). Because N36 and C34 are single stranded random coils, they do not assume ordered structure in solution, so they display characteristic random coil CD spectra. However, formation of fusion complex 6HB results in a distinctive CD spectrum, including a saddle-shaped negative peak between 210-220 nm in the far UV region and a significant increase in molar ellipticity (0) at 222 nm. Pre-incubation of N36 with Ole or HT interrupts 6HB formation and results in a dose dependent shift of the CD spectra from helical to random coil with EC50 of 62 nM for Ole and 60 nM for HT.

TABLE 6

<table>
<thead>
<tr>
<th>Compound</th>
<th>Syn-EC50 (nM)</th>
<th>Cytosolic EC50 (nM)</th>
<th>Fusion Core Formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ole</td>
<td>55 ± 5</td>
<td>73 ± 8</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>HT</td>
<td>61 ± 6</td>
<td>68 ± 8</td>
<td>&gt;10,000</td>
</tr>
</tbody>
</table>

*EC50, effective concentration at 50% inhibition; IC50, cytotoxicity concentration at 50% inhibition.

**N-PAGE, native polyacrylamide gel electrophoresis; CDS A, circular dichroism spectroscopy analysis.

Values are means ± SD of triplicates in three independent determinations.

Discussion

[3070] Ole and HT are small molecules with molecular weights of 539 and 153 respectively. Their inhibition of the fusion-promoting refolding of gp41 is an excellent example of how small molecules can block formation of protein-protein complexes. We narrowed down the target of binding to a hydrophobic pocket on the gp41 inner core. This pocket is highly conserved among the different HIV clades. Consistent with this, we found that Ole and HT are active against a panel of HIV-1 primary isolates that includes both M and T tropic strains from different clades. Our results suggest that Ole and HT may be useful against other viruses with type I transmembrane envelope glycoprotein, including severe acute respiratory syndrome associated coronavirus [23,27], respiratory syncytial virus, Ebola virus [28], measles virus [29], and avian flu [30,31].

[3071] Fuzeon (T-20 or Enfuvirtide) is the only FDA approved HIV fusion inhibitor [32,33]. It is a peptide derived from the CHR region of gp41 that partially overlaps with the C34 sequence. Fuzeon is commercially produced by chemical synthesis. Because of its large size - it consists of 36 amino acids with a molecular weight of 4492 - it's manufacturing process is very complex, involving 106 chemical steps [34,35]. In contrast, our typical process for chemical synthesis of HT involves only two steps: acetylation and reduction (FIG. 13F). In addition, Ole and HT can also be easily prepared from natural olive leaf extract in only two steps: deglycosylation and oxidation (FIG. 13F). The finding that Ole and HT act both outside and inside of the cellular environment in viral entry and integration offers unique benefits to these small molecules against viral resistance.

REFERENCES


Example 7

Effect of Oleuropein and Hydroxytyrosol on HIV-1 Integrase

Material and Methods

Oleuropein (Ole) and Hydroxytyrosol (HT)

Ole and HT used in this study were prepared and purified from olive leaf extract as described in the preceding article.

Target Cells, HIV-1, Anti-HIV and Cytotoxicity Assays

Target cells MT2, H9, HIV-1_IIIB chronically infected H9 (H9/HIV-1_IIIB) and HIV-1_IIIB virus, were obtained through the AIDS Research and Reference Reagent Program, NIAID, NIH. MT-2 cells were from D. Richman [7,8]. H9 cells and HIV-1_IIIB virus stocks were from R. Gallo [9,10]. The cell lines were cultured in RPMI medium 1640 containing 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM L-glutamine, and 10% heat-inactivated fetal calf serum. Viral stocks were prepared and standardized as described previously [11]. Anti-HIV activity was measured by the microtiter syncytium formation and HIV-1 p24 production assays [11]. Cytotoxicity was determined by the MTT assay [11].

Molecular Modeling

A combination of molecular docking, molecular dynamics (MD) simulation and free energy calculations [12, 13] were performed to prove the interactions of Ole or HT with viral targets. Docking was performed with AutoDock version 3.0.5 [14]. Relaxation of docking structure obtained was carried out by the program Discovery from Insight II (Accelrys Inc., San Diego, Calif., U.S.A.), using 500 steps of...
Steepest Descent followed by Conjugate Gradient until the root mean square (RMS) of the energy gradient reaches 0.01 kcal/mol Å.

HIV-1 Integrase

[0411] HIV-1 integrase was expressed in *E. coli* from pLN (F185H/C280S) and purified according to previously reported method [15]. This recombinant clone makes the integrase protein more soluble and stable without affecting in vitro activity. HIV-1 integrase protein (F185H/C280S) was used as a standard for purification and assay. The integrase clone pLN (F185H/C280S) and the standard integrase protein were obtained through the NIH AIDS Research and Reference Reagent Program, NIAID, NIH from Dr. Robert Craigie [16].

Integrase Substrates

[0412] Oligomericuleate substrates were synthesized and purified as described previously [17]. Three types of substrates were synthesized with sequences that correspond to the U3 and U5 ends of HIV-LTR: (i) the 21-nucleotide minus strand of U3 end HIV-LTR 5'-GAGTGAAATTCACCATT-3' (SEQ ID NO: 41), and the U5 HIV-LTR 5'-GTGAGAAAATCCCTAGCT-3' (SEQ ID NO: 42), as well as their complementary strands, for assaying the 3'-processing reaction; (ii) the 19-mer U3-G1, and U5-G1 (i.e., U3 and U5 minus the 3'-end dinucleotide GT) for assay heterologous integration (strnad transfer); and (iii) a 38-mer dumbbell substrate with the sequence 5'-TGCTATGGTCTAGCAGGC-3' (SEQ ID NO: 43), for assaying the dis-integration.

Radiolabeling and Preparation of the Substrates

[0413] The integrase substrates were 5'-end radiolabeled as reported previously [17]. Briefly, 1 g of the oligonucleotide was 5'-end labeled with 100 μCi of [α-32P]ATP (3000 Ci/mol; 1 Ci=37 GBq; A-mersham) by 20 units of polynucleotide T4 kinase in a final volume of 40 μl of kinase buffer (Boehringer Mannheim) at 37°C for 60 min. The reaction was stopped by EDTA (25 mM) and heat inactivation. Uninorporated label was removed by two passages through a Sephadex G-25 spin column (Boehringer Mannheim). The purified labeled oligonucleotide was then annealed with an equimolar amount of unlabeled complementary strand in 10 mM Tris HCl, pH 7.5/1 mM EDTA/100 mM NaCl at 95°C for 5 min followed by slow cooling. The dumbbell substrate was self-annealed under the same conditions.

Integrase Assays

[0414] Integrase assays were carried out in 20 mM Hepes, pH 7.5/10 mM MgCl2 or MnCl2/10 mM dithiothreitol/0.05% Nonidet P-40 (integrate buffer) with 40 μmol of HIV-1 integrase and 20 ng of 5'-end radiolabeled substrates specific for 3'-processing (21-mer U3), strand-transfer (19-mer U3-GT)) or disintegration (38-mer dumbbell) in the presence or absence of Ole or HT in a final volume of 10 μl at 37°C for 60 min. For 3'-processing and disintegration, the reactions were stopped by the addition of 10 μl of 90% formamide/0.025% bromophenol blue/0.025% xylene cyanol/89 mM Tris/89 mM boric acid/2 mM EDTA, pH 8.0. Samples were heated at 75°C for 3 min, load at 10 μl well onto 18% polyacryla-mide denaturing (7.5 M urea) gels in TBE buffer and electrophoresed at 200 V constant voltage at room temperature for 2 h. The results were visualized by autoradiography of wet gels. For strand-transfer (integration), pUC18 plasmid DNA (50 ng) was used as the target for the integration of viral DNA into heterologous plasmid DNA. The reaction was stopped by 0.1% SDS and integration products monitored on 1% agarose gels at 10 μl well in TBE buffer at 100 V constant voltage at room temperature for 45 min. The results were visualized by autoradiography of dried gels.

Results

Ole and HT Bind to the Catalytic Site of HIV-1 Integrase

[0415] To examine the molecular interactions of Ole and HT with HIV integrase, we performed a series of docking simulations. In these studies, we focused on the catalytic core domain (CCD) of the viral enzyme, because the linkages between the CCD and both the N- and C-terminal domains (NTD and CTD) are flexible and have been not precisely determined. We used the crystal structure 1Q54 (pdb ID) as the starting structure [18], because it is the only structure has an inhibitor (SCITEP) bound in the active site. To keep the binding pocket available, we removed the ligand (SCITEP) from the CCD. Before docking, the missing residues in the loop region and the point mutation at position 185 were restored with AMBER software. The K185 mutation was converted to the native F185.

[0416] FIGS. 17A and 17B represent the predicted bound conformations of Ole and HT within the active site of HIV-1 integrase. Two unique binding regions have been identified within the integrase active site [2, 4], referred to as regions I and II (FIG. 17B). Region I, near the active site center, encompasses the conserved DTE motif, D64-D116-E152 in HIV-1 integrase. These residues are highly conserved in all integrase, retrotransposases and other DNA-processing enzymes (polynucleotide transfersases). Mutation of any of these acidic amino acids abolishes integrase activities and viral replication. D64 and D116 are involved in the formation of coordination complex with divalent metal (Mg2+ or Mn2+). A second metal (Mg2+ or Mn2+) can be coordinated between D116 and E152 once HIV-1 integrase binds its DNA substrates (20, 21). Metal ion coordination with viral integrase and the phosphodiester backbone of the DNA substrates are likely to occur during 3'-processing and strand-transfer reactions. Region II is close to the active catalytic loop (amino acid residues 139-147), and involves the flexible loop formed by amino acid residues 140-149. This loop region has been identified as the DNA binding site which is important for integrase action [19].

[0417] As seen in FIG. 17A, Ole binds to both regions I and II. The β-glycophorosynine moiety of Ole interacts with residues in region II whereas the dihydroxyphenol ring occupies region I (FIGS. 17C and 17D) are ribbon representation of the HIV-integrase CCD showing major strong H-bonding sites with Ole and HT. The flexible loop widens the active site region and allows the sugar ring of Ole to dock with strong H-bond with P142 and Q148 as well as form weak interactions with S147. The dihydroxyphenol moiety of Ole binds to region I with a strong H-bond interaction with D64 and a weak H-bond-network interactions with K156 and K159. This suggests that Ole would be a strong integrase binding inhibitor because it interacts with residues in both regions I and II. On the other hand, the dihydroxyphenol ring of HT binds to region II with strong H bond interaction with I139 and nearby T115, and weak interactions with E138 and Q148. Since the
dihydroxyphenol ring is capable of binding both regions I and II. HT maintains the ability to bind the integrase active site even if mutations occur. Thus the likelihood of resistance development should be less than inhibitors that bind to a single site. Thus, interaction modeling suggests that Ole and HT bind to both regions I and II, so they would be expected to be effective against metal coordination as well as substrate binding. Modeling results therefore predict that Ole and HT would inhibit all of the three HIV-1 integrase activities.

Ole and HT Inhibit 3'-Processing Activity of HIV-1 Integrase

[0418] Modeling predictions that Ole and HT may affect HIV-integrase activity were tested in all of the three activities, namely 3'-processing, strand transfer (integration) and disintegration. Results of HIV-1 integrase inhibitory activities of Ole and HT are summarized in Table 7 with their anti-HIV activities.

[0419] FIG. 18A shows the 3'-processing reaction and results. 5' [32P] labeled 21-mer double-stranded oligonucleotide that mimics the U3-HIV1 LTR was used as a substrate. 3'-processing by HIV-1 integrase, removes the dinucleotide GT from the 3' end of the labeled minus strand of the 21-mer substrate and yields a 3' recessed product (U3-GT) with 19 nucleotides in length. Ole or HT inhibits the 3'-processing activity of HIV-1 integrase. FIG. 18A demonstrates dose-dependent inhibition of 3'-processing by Ole and HT as detected by 7.5 M M urea denaturating polyacrylamide gel electrophoresis. Inhibition of the formation of the 19-mer product from the 21-mer substrate increases with the increase of Ole or HT concentration from 25 to 100 nM. The degree of inhibition depends on the concentrations of the HIV-1 integrase, the substrate and the inhibitor. Under our assay conditions, EC50 of 46 and 54 nM were obtained for Ole and HT respectively, and total inhibition was observed at 100 nM. Substrate U5 HIV-LTR showed similar results.

Ole and HT Inhibit Strand-Transfer Activity of HIV-1 Integrase

[0420] The effect of Ole and HT on the strand-transfer activity of HIV-1 integrase was tested by a quantitative heteroelogous integration assay. FIG. 18B shows the design and results of this assay: U3-GT, a 5' [32P] labeled and 3'-recessed 19-mer was used as the viral substrate. To focus on strand transfer, a supercoiled plasmid DNA of 2.69 kb was used as the heterologous target. Incubation of the 5' [32P] labeled viral substrate with unlabeled target in the presence of HIV-1 integrase results in the integration of the labeled 19-mer viral substrate into the 2.69 kb target plasmid. Integration was monitored by the conversion of unlabeled plasmid to labeled DNA in agarose gel electrophoresis as seen in the figure. Under these conditions, any inhibition detected must be specific for strand transfer and not for 3' processing. Ole and HT demonstrated dose-dependent inhibition of the strand-transfer activity of HIV-1 integrase with EC50 of 56 and 43 nM respectively.

Ole and HT are Effective Against Disintegration Activity of HIV-1 Integrase

[0421] FIG. 18C shows a schematic representation of disintegration reaction and assay results. Disintegration is the reverse of integration and involves concerted strand-cleavage and ligation reactions [20]. Strand cleavage takes place precisely at the junction between the viral and the target sequences and is coupled with the rejoining of the cleaved target sequences. The disintegration substrate is a dumbbell shaped 38-mer oligonucleotide that mimics the recombination intermediate of HIV-1 integration. It contains 5' [32P] labeled virus-specific U5-LTR sequence of 14-mer in the stem of the hairpin loop and arbitrary target DNA sequences of 24-mer in the base of the dumbbell [21,22]. The folded structure of the annealed substrate shown is based on reports obtained from hairpin formation by similar sequences [23, 24]. Dis-integration of the 5' [32P] labeled dumbbell by HIV-1 integrase is expected to give two products, a 5' [32P] labeled 14-mer hairpin loop viral sequence and an unlabeled 24-mer closed circular target DNA. In the presence of HIV-integrase, the production of labeled 14-mer hairpin loop was detected by 7.5 M urea denaturating polyacrylamide gel electrophoresis and autoradiography of the gel, whereas the 24-mer product, the target DNA, was not seen in the autoradiography because it is unlabeled. This product can be detected by UV shadowing of the gel or by the use of 3'-labeled substrate. In the presence of Ole or HT, dose-dependent inhibition on the formation of the labeled 14-mer disintegration product was observed with EC50 of 28 or 18 µM respectively.

<table>
<thead>
<tr>
<th>TABLE 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HIV Activity and Inhibition on HIV-1 Integrase Activity</td>
</tr>
<tr>
<td>Inhibitory</td>
</tr>
<tr>
<td>EC50 (nM)</td>
</tr>
<tr>
<td>Syn-</td>
</tr>
<tr>
<td>Ole</td>
</tr>
<tr>
<td>HT</td>
</tr>
</tbody>
</table>

*EC50: effective concentration at 50% inhibition; IC50: cytotoxicity concentration at 50% inhibition.

Discussion

Several classes of HIV integrase inhibitors have been reported [4,23]; but none is clinically available yet. Lack of structural information for the intact protein, issues regarding different active site conformations dependent on crystal structure, and uncertain oligo-meric character of the enzyme protein have impeded the discovery of a clinically useful HIV-integrase inhibitor [4,23]. Thus, molecular modeling becomes a key component in both the design of new integrase inhibitors and the identification of important protein-ligand interactions. There are 14 crystal structures of HIV-integrase available from the Protein Data Bank (PDB); however, only one has an inhibitor bound in the active site: 1Q84 [18]. We believe that this crystal structure contains the inhibitor would be the most relevant active site conformation on which to conduct the docking simulations with our anti-HIV small molecules. Ole and HT, despite previously reported crystal-packing effects associated with this structure [24].

The docking results reported here show good correlation with experimental data and provide a valuable tool for both evaluating compounds and designing more potent inhibitors. Ole and HT exhibit dose dependent inhibition in all of the three activities of HIV-1 integrase: 3'-processing, strand transfer and disintegration with EC50 all in the nM range.
range. These compounds also showed good antiviral efficacy both in cell-to-cell transmission of HIV-1 as assayed by syncytial formation and in HIV-1 replication as assayed by p24 production. However, they are not toxic in the effective dose ranges and even at the concentration of 1,000 times EC50 (Table 7).

To our knowledge, Ole and HT are the first group of small molecules capable of multiple actions against the AIDS virus, inhibiting both viral entry and integration. To act both outside and inside of the cellular environments represents a great advantage of this novel class of drugs. The structure-functional information described here should facilitate the design of innovative multi-functional HIV-1 inhibitors.

REFERENCES

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOs: 43
<210> SEQ ID NO 1
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer
<400> SEQUENCE: 1
ggatgtcgtg tctgtggega  20

<210> SEQ ID NO 2
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer
<400> SEQUENCE: 2
tgagggagat taccggctcg  20

<210> SEQ ID NO 3
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer
<400> SEQUENCE: 3
gagattttctc tgtatggcac c  21

<210> SEQ ID NO 4
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer
<400> SEQUENCE: 4
cggcaaatga gcacatttctc c  21

<210> SEQ ID NO 5
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer
<400> SEQUENCE: 5
gtacctggga acctgtctcc  20

<210> SEQ ID NO 6
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer
<400> SEQUENCE: 6
gttcaatgca ctacctccacgc  20
<210> SEQ ID NO 7
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 7
gtggaattggc ctgcctccct acac

<210> SEQ ID NO 8
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 8
cagagatga tgcgcagact gatt

<210> SEQ ID NO 9
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 9
tggacgttca gaagtcaccc acca

<210> SEQ ID NO 10
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 10
ttctggttct ctgcagctacc gttc

<210> SEQ ID NO 11
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 11
cctgagaagcc ctcacc

<210> SEQ ID NO 12
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 12
agacagcacac cttagc

<210> SEQ ID NO 13
<211> LENGTH: 23
<212> TYPE: DNA
-continued

<213> ORGANISM: Artificial Sequence
<220> FEATURE: primer
<400> SEQUENCE: 13

gtgcagatct tgtgtgtagt agc  23

<211> SEQ ID NO 14
<212> LENGTH: 24
<213> ORGANISM: Artificial Sequence
<220> FEATURE: primer
<400> SEQUENCE: 14

agagccatc cttaacctga agtt  24

<211> SEQ ID NO 15
<212> LENGTH: 505
<213> ORGANISM: Homo sapiens
<400> SEQUENCE: 15

Met Gly Glu Thr Leu Gly Asp Ser Pro Ile Asp Pro Glu Ser Asp Ser
1   5  10  15
Phe Thr Asp Thr Leu Ser Ala Asn Ile Ser Glu Glu Met Thr Met Val
20  25  30
Asp Thr Glu Met Pro Phe Trp Pro Thr Asn Phe Gly Ile Ser Ser Val
35  40  45
Asp Leu Ser Val Met Gly Asp His Ser His Ser Phe Asp Ile Lys Pro
50  55  60
Phe Thr Thr Val Asp Phe Ser Ser Ile Ser Thr Pro His Tyr Glu Asp
65  70  75  80
Ile Pro Phe Thr Arg Thr Asp Pro Val Val Ala Asp Tyr Lys Tyr Asp
85  90  95
Leu Lys Leu Gin Glu Tyr Gin Ser Ala Ile Lys Val Glu Pro Ala Ser
100 105 110
Pro Pro Tyr Tyr Ser Glu Lys Thr Gin Leu Tyr Asn Lys Pro His Glu
115 120 125
Glu Pro Ser Asn Ser Leu Met Ala Ile Glu Cys Arg Val Cys Gly Asp
130 135 140
Lys Ala Ser Gly Phe His Tyr Gly Val His Ala Cys Glu Gly Cys Lys
145 150 155 160
Gly Phe Phe Arg Thr Ile Arg Leu Lys Leu Ile Tyr Asp Arg Arg Cys
165 170 175
Asp Leu Asn Cys Arg Ile His Lys Ser Arg Asn Lys Cys Gin Tyr
180 185 190
Cys Arg Phe Gin Lys Cys Leu Ala Val Gly Met Ser His Asn Ala Ile
195 200 205
Arg Phe Gly Arg Met Pro Gin Ala Glu Lys Glu Lys Leu Ala Glu
210 215 220
Ile Ser Ser Asp Ile Asp Gin Leu Asn Pro Glu Ser Ala Asp Leu Arg
225 230 235 240
Ala Leu Ala Lys His Leu Tyr Asp Ser Tyr Ile Lys Ser Phe Pro Leu
245 250 255
Thr Lys Ala Lys Ala Arg Ala Ile Leu Thr Gly Lys Thr Thr Asp Lys
260 265 270
Ser Pro Phe Val Ile Tyr Met Met Ser Leu Met Met Gly Glu Asp
275 280 285
Lys Ile Lys Phe His Ile Thr Pro Leu Gin Glu Gln Ser Lys Glu
290 295 300
Val Ala Ile Arg Ile Phe Gln Gly Cys Gln Phe Arg Ser Val Glu Ala
305 310 315 320
Val Gin Glu Ile Thr Gln Tyr Ala Lys Ser Ile Pro Gly Phe Val Asn
325 330 335
Leu Asp Leu Asn Asp Gin Val Thr Leu Leu Lys Tyr Gly Val His Glu
340 345 350
Ile Ile Tyr Thr Met Leu Ala Ser Met Asn Lys Asp Gln Val Leu
355 360 365
Ile Ser Glu Gly Gin Gly Phe Met Thr Arg Glu Phe Leu Lys Ser Leu
370 375 380
Arg Lys Pro Phe Gly Asp Phe Met Glu Pro Lys Phe Glu Phe Ala Val
385 390 395 400
Lys Phe Asn Ala Leu Glu Leu Asp Asp Ser Asp Leu Ala Ile Phe Ile
405 410 415
Ala Val Ile Ile Leu Ser Gly Asp Arg Pro Gly Leu Leu Asn Val Lys
420 425 430
Pro Ile Glu Asp Ile Gin Asp Asn Leu Leu Gin Ala Leu Glu Leu Gin
435 440 445
Leu Lys Leu Asn His Pro Glu Ser Ser Gin Leu Phe Ala Lys Leu Leu
450 455 460
Gln Lys Met Thr Asp Leu Arg Gin Ile Val Thr Glu His Val Gin Leu
465 470 475 480
Leu Gin Val Ile Lys Thr Glu Thr Asp Met Ser Leu His Pro Leu
485 490 495
Leu Gin Glu Ile Tyr Lys Asp Leu Tyr
500 505

<210> SEQ ID NO 16
<211> LENGTH: 225
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 16
atgcagcgcc aggcacagca gctgagcaggg cagctgctgc ggcctgcgggc ccagctgac 60
cgcagcagcc tggacccagg ccctgctcctg cccagggagc tgcgtgagta ggcggagctg 120
ggcctgcag tcagcagcc ggcgcgctca gcctgcgcgc ccgctgctgg gcgcctgagc 180
cgctggggc tggagcttcg ggccggtcgg ttcgcatctg gctga 225

<210> SEQ ID NO 17
<211> LENGTH: 303
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 17
atggtggagc cctttgggg gacccgaaag cttgttcctca tgtgaatact tgtaggattc 60
atgagagac tgtggagtga tttcgccagc cggacactgg caggagtagt gaaaccgaca 120
<210> SEQ ID NO: 19
<211> LENGTH: 118
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 19

Asp Ala His Lys Val Leu Ser Ala Leu Gln Ala Val Gln Gly Leu Leu Leu  
Val Ala Gln Gly Arg Ala Asp Ser Gln Ala Gln Leu Leu Leu Ser Thr  
Val Val Gly Val Phe Thr Ala Pro Gly Leu His Leu Lys Gln Pro Phe  
Val Gln Gly Leu Ala Leu Tyr Thr Pro Val Val Leu Pro Arg Ser Leu  
Asp Phe Thr Gly Arg Asp Val Ala Ala Glu Lys Ile Asp Arg Phe Met  
Gln Ala Val Thr Gly Trp Lys Thr Gly Cys Ser Leu Met Gly Ala Ser
Val Asp Ser Thr Leu Ala Phe Arg Thr Tyr Val His Phe Gln Gly Lys

Ala Arg Leu Ser Ala Gln

95
90
95

<210> SEQ ID NO 20
<211> LENGTH: 1926
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 20
gtttgtctgg gtaacctagc aagaggggta ggggaagcag gcacccacagt ctgactagcc 60
tctctgcggtg gtggcgtcga gggccacat cctctgcgctg tgggtcaggg ctgggctcgcc 120
tgcagggctgc cggggcagca tacaccctct ccaacctcgcc atcacaagg aagtaacttg 180
tgacggaggt gcaccagccg aacgccgga aagccaaagc ccacccttca tacctgcgcc 240
eaacctaggcc agacacctcc gtcgtgtgta gaaggcgcttc cagggcagcg tgggtgctact 300
gctgcaaaaa ctggacagcg aagacaagtt gaggcggcag atggtcgga taggggccaa 360
cctctggggc ttcggtgtat atgctgatca cagttcgaat tgggggttg ccctatgggga 420
cacctgctgc tccacacagcg ctgcttcccgg ccacctg gccctctcctcctggtgctcc 480
gacaccacc gcgcagacgg tcaagcaaat cctgggtgctt cctgagagc agaagacgtc 540
cacctcgggct cttgctggcg acaagttctc ggtgcctgcgc aaggtgcttgct acgggctcgt 600
taggttcgggg ccaggagggc agaagttcttg ccagcgtgctg ctgggtgcgtc 660
gttcgaggc ccagggcagc aacctgaacc gcgggttggtg gcgggtgggc ctctctacct 720
cctctgtgct cttcagcaagt cctgggacct ctgccgaacat gatgggtggt ctgagagata 780	tgacggaggt cagcaggagct ggaggacctg tcctctctcct cggggccagc 840
tgacggaggt cagcaggagct cagcaggagct cagcaggagct gcagcaggagct cagcaggagct 900
cctctctctc gcgcggccct gccggccgat cccgtggtgct gggggccgcc cggggccgat 960
cagcggacg gcggccgat cccgtggtgct gggggccgcc cggggccgat 1020
gcggccgat cccgtggtgct gggggccgcc cggggccgat 1080
tgcctccagc aaggtggggc gtctctccctt ccaagcagcc cccgagacct gcggggccgat 1140
gcggggccgat cccgtggtgct gggggccgcc cggggccgat 1200
gcggccgat cccgtggtgct gggggccgcc cggggccgat 1260
gcggccgat cccgtggtgct gggggccgcc cggggccgat 1320
gcggccgat cccgtggtgct gggggccgcc cggggccgat 1380
gcggccgat cccgtggtgct gggggccgcc cggggccgat 1440
gcggccgat cccgtggtgct gggggccgcc cggggccgat 1500
gcggccgat cccgtggtgct gggggccgcc cggggccgat 1560
gcggccgat cccgtggtgct gggggccgcc cggggccgat 1620
gcggccgat cccgtggtgct gggggccgcc cggggccgat 1680
gcggccgat cccgtggtgct gggggccgcc cggggccgat 1740
gcggccgat cccgtggtgct gggggccgcc cggggccgat 1800
<table>
<thead>
<tr>
<th>Amino Acid Positions</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-20</td>
<td>AACGACACG CTTGGTTTGG AACA AACA GGGTCCCTT TCCAGTTG GAACAAAGT</td>
</tr>
<tr>
<td>19-20</td>
<td>CTTGGTTTGG AATAAAGTTA TAATTTTTG GCATCGAAAAA AAAAAAAAAA</td>
</tr>
<tr>
<td>21-23</td>
<td>AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA</td>
</tr>
</tbody>
</table>

**SEQUENCE: 21**

<table>
<thead>
<tr>
<th>Residue</th>
<th>Position</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leu</td>
<td>1</td>
<td>Met</td>
</tr>
<tr>
<td>Leu</td>
<td>2</td>
<td>Leu</td>
</tr>
<tr>
<td>Leu</td>
<td>3</td>
<td>Ile</td>
</tr>
<tr>
<td>Asn</td>
<td>4</td>
<td>Val</td>
</tr>
<tr>
<td>Val</td>
<td>5</td>
<td>Val</td>
</tr>
<tr>
<td>Ser</td>
<td>6</td>
<td>Thr</td>
</tr>
<tr>
<td>Gly</td>
<td>7</td>
<td>Leu</td>
</tr>
<tr>
<td>Val</td>
<td>8</td>
<td>Ser</td>
</tr>
<tr>
<td>Val</td>
<td>9</td>
<td>Thr</td>
</tr>
<tr>
<td>Thr</td>
<td>10</td>
<td>Val</td>
</tr>
<tr>
<td>Thr</td>
<td>11</td>
<td>Thr</td>
</tr>
<tr>
<td>Thr</td>
<td>12</td>
<td>Ser</td>
</tr>
<tr>
<td>Thr</td>
<td>13</td>
<td>Val</td>
</tr>
<tr>
<td>Thr</td>
<td>14</td>
<td>Val</td>
</tr>
<tr>
<td>Tyr</td>
<td>15</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>16</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>17</td>
<td>Ile</td>
</tr>
<tr>
<td>Asp</td>
<td>18</td>
<td>Glu</td>
</tr>
<tr>
<td>Glu</td>
<td>19</td>
<td>Glu</td>
</tr>
<tr>
<td>Ser</td>
<td>20</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>21</td>
<td>Thr</td>
</tr>
<tr>
<td>Asn</td>
<td>22</td>
<td>Gly</td>
</tr>
<tr>
<td>Gly</td>
<td>23</td>
<td>Pro</td>
</tr>
<tr>
<td>Pro</td>
<td>24</td>
<td>Pro</td>
</tr>
<tr>
<td>Pro</td>
<td>25</td>
<td>Val</td>
</tr>
<tr>
<td>Pro</td>
<td>26</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>27</td>
<td>Val</td>
</tr>
<tr>
<td>Ser</td>
<td>28</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>29</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>30</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>31</td>
<td>Val</td>
</tr>
<tr>
<td>Ser</td>
<td>32</td>
<td>Val</td>
</tr>
<tr>
<td>Glu</td>
<td>33</td>
<td>Glu</td>
</tr>
<tr>
<td>Glu</td>
<td>34</td>
<td>Val</td>
</tr>
<tr>
<td>Val</td>
<td>35</td>
<td>Thr</td>
</tr>
<tr>
<td>Thr</td>
<td>36</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>37</td>
<td>Val</td>
</tr>
<tr>
<td>Val</td>
<td>38</td>
<td>Thr</td>
</tr>
<tr>
<td>Thr</td>
<td>39</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>40</td>
<td>Val</td>
</tr>
<tr>
<td>Ser</td>
<td>41</td>
<td>Thr</td>
</tr>
<tr>
<td>Thr</td>
<td>42</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>43</td>
<td>Val</td>
</tr>
<tr>
<td>Ser</td>
<td>44</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>45</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>46</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>47</td>
<td>Val</td>
</tr>
<tr>
<td>Ser</td>
<td>48</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>49</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>50</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>51</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>52</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>53</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>54</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>55</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>56</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>57</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>58</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>59</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>60</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>61</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>62</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>63</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>64</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>65</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>66</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>67</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>68</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>69</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>70</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>71</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>72</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>73</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>74</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>75</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>76</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>77</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>78</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>79</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>80</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>81</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>82</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>83</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>84</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>85</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>86</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>87</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>88</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>89</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>90</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>91</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>92</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>93</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>94</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>95</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>96</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>97</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>98</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>99</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>100</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>101</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>102</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>103</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>104</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>105</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>106</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>107</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>108</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>109</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>110</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>111</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>112</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>113</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>114</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>115</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>116</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>117</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>118</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>119</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>120</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>121</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>122</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>123</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>124</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>125</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>126</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>127</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>128</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>129</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>130</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>131</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>132</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>133</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>134</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>135</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>136</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>137</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>138</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>139</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>140</td>
<td>Thr</td>
</tr>
</tbody>
</table>

**LENGTH: 330**

**ORIGIN: Homo sapiens**
-continued

<210> SEQ ID NO 22
LENGTH: 878
TYPE: DNA
ORGANISM: Homo sapiens

SEQUENCE:

<210> SEQ ID NO 23
LENGTH: 3649
TYPE: DNA
ORGANISM: Homo sapiens

SEQUENCE:

<210> SEQ ID NO 24
LENGTH: 7513
TYPE: DNA
ORGANISM: Homo sapiens

SEQUENCE:
-continued

tgtaacattg gagaagcct tccgcttggt gcgaatgcag gactggtggt agtgcttgcc 960
tctgtagtgt gctcctagc agcttcgtt ccctcgttct tccctgtggc ttttgaatga 1020
gaaatccac agaggtgact tcaccggagc cttccagttt aaggtcgctg 1080
tgcgggtgag gaaagagcgt ctgcaactt tgcgggtgct agatcataag agtcggagct 1140
aacgggaag cgttaatgtg cctgagagg ctcgttcagc tgcotacca agcgcttcct 1200
taccgaagta agatctcttt ttctggaggc gagaagggcc cccataccca tccaggtttt 1260
gagatgtcttc tgatggcggc gctgagccgg gagagcaacaa tcctcattcc tctgcttgaa 1320
gttctccaa ataaaccgct ctctctccct aattacacag aggtgatatct tggagaacta 1380
tcctagtga agotaccaag gagaaggttt tccatacttt actgctctgg cctgcggagc 1440
agtcgctagc tgctctcaag gagaagcggc gtgggacagc gagaagctcag gaaagaggtg 1500
atcccccttt tggaggagct aggtttctct ctggctgcaag gaaaggggagc agtggttttt 1560
gtgaagctgg atgaagcaagct tctgaatag gcagtctgct gaaactctgg gaacactcag 1620
aacggagaag gcagcttgca aactgtgtgg agagaaggct ttctaaaaaagg 1680
cataaggctt cttgcttggt tcccaaatgg gatgctctgt gactataatt cccaggtcct 1740
ccttctcgag tttttttttg agacgctctt aagcactaa ac agtggtaaa aacacactttat 1800
gggtcttagt tggcagctgct cagcctcaaa aggctcagaa aacacactttat 1860
agctcttctt gcttaagttt cggaaacctg acacacacttt acaggtactag cagttgtgag 1920
cagctgttggt atgagggctc acogctacttt tggatgtaac cttcatttgg aatggaatt 1980
cctgagatct cggagctgag cttccctaaca attactctta agcttcggct tccacagtta 2040
atgccctt gggggaggca ttccctcgctt cttctctttc caaggtcttt acctcgagctg 2100
tggattctct tgcctgtttgc aagtgcgtct cagagctcagc cagaggtcag tatagggtg 2160
agggggagaa aaggggcttg tattagccag cggagttttaa cagctcctac cttgcggcct 2220
ctctgagct aagcctcaaa tccaagggag tattatcttt aagcactata aagcactat 2280
ggcgttacag ttttcttttt cttccttttt tttttctttt acaggtactag cagttgtgag 2340
taataaatg ttttcttttt aggtttcttg aatgcagctg gaaatcttgg ttttcagcttgg 2400
atttgaagtt ggcaaaaag ggctgcttttt ttttacacttt tttttactttt ttctttatag 2460
ttttctcaaa tttaactctta tttcatactt gcaggtactt cttcattttt aggctcatttt 2520
tgtgcgttgg ttcctctcct ttcctctcct ttttttttttt tggcttttttt tggcttttttt 2580
gattttgttg agagctcacttt tgcgtttgta tggccgcacag aagagtttcg aacacacagt 2640
ccccctcaaa gtttaaggggg gtggaggttc ctgggctgga tttcattgaa gtcttggtaa 2700
acccctcttt gtagagttgt tggatgcttt ccacactata gaaaggtcctg catgtgtttt 2760
aacactactt gatgtgatctc aacacactttt ataatagcag tttcttttttt tgcaggttcg 2820
tctctgcttt ataatagcag aatgctttaag aggctcatttt ttcctcagagc cttaatctgg 2880
aacccctcttt gtagagttgt tggatgcttt ccacactata gaaaggtcctg catgtgtttt 2940
gttcgtgac ataatagcag gagaagactt cttgctgcag aagagttcag aacacacagt 3000
gagtagagtaa tgcctttctg ttcggagcag ttcgactgct tccctcagagc gatgtctgct 3060
gctataaat aatgagcagc atgttcctgt taaaactcct gcaagtgtaaa agtgcttttt 3120
cttatasatat aatagagttt tggcttttttt tttcatacttt tttttactttt ttctttatag 3180
ctggatctgg gttgacgcc agggtgcatt accctaaag atccactaaa gcagcactata
3240
gcctcggaac ctcctggccc gacaaccttt cttatatata tcaaggtgtg tctggcttta
3300
cattatat tttacctgtta aatacatgtt tgtgtgtctg aatggagcct gtaactatgtg
3360
gaaaggtcttt tgtgtcttata aaatgggtgt cttaaccgtta tgtgcttta
3420
tcaagtgtg ttcacagac ccacccaaat cttatgaaat tgggtttaac aaaaacaacaa
3480
aggcaagtc atcaatgtgtt gaagaatttg aatacaggcttt taataaattt gacaaccat
3540
tattaccac
3549
<210> SEQ ID NO 24
<211> LENSE: 2315
<212> TYPE: DNA
<213> ORGAN: Homo sapiens
<400> SEQUENCE: 24
gotcagcagc ctataatggt ctgtgaactgc ttcagctggc tcaagagggg cggccgaggga 60
agtggagagc aaggcactcgc ttcagaggtc tttgctgtcct ggccgctgaga cttggcagccg
120
tctccggcgg agggttgcgg cgcgtgacca agaagaagag tttatgcgca tgggaagtttaa 180
atttcctc caaggaactgc aagacacagc tggagcaact tgcgaacctca ttcoggagtt 240
agcgaagctc gtggcaagct gtatttccca ttcacagac ccacccaaat tttatgtgaaat 300
tggggctgcct gtttgcagtc ggagcctgca aaaaaccggc gtagctgctct cctgctgggt 360
caagagaaga ccaccaactc attgttcattt gctgtgagtgg agggttggctgt cctgctggag 420
ttcaccctgtg ttcaggggtct aaccacaccgt gggtggactg cagctggccc gtttttctca 480
tgtgatgtgtc gaggttttta actccctctc gcacaccgct cactcttggt gcattagcct 540
tggaggccct gtctggtgca ttcaggggag ttcagcaact attggaagta cagacattc 600
tggtcgcttg cccagatgct ctaaccctga ttgggtggcc gcccgccgct gttttcttctc 660
tgatgatcct gatttgatcg aactttttgc aaccctgctag cggggctgct gcatttaggt 720
cattggact cagaacccag tgggctagct tgaacccctc cgaataggtg taatttttta 780
ggcagaggtc aacacccctg gactcatcgg cttggtctgtg gatggtgatg 840
ggccagctg tttggatgtgt cccagagcct ctccatggct ttccttcatgc actctcctgt 900
gaattggga agaaccagt gcagcttcct gcagcgtccc aagggagcct tggagccagg 960
gotcagcagc agtagcctgc gagaaccgtt acacatatgg gpgttgtgca ctaaccactg 1020
cagagcctca aagacagcct cagactcttg ttcagatgc cttacaaaagt 1080
tctctacct caggaaaag cgtcttttgg tttggagcat tttggcagcct atccactata 1140
gcactgtggc atttgctgtg gttggatgtg gcatttcgac gccgaggct gcaggtatcct 1200
gctgcttgc ttcactata cagactctgc cttactaatt ttcacagag tggagatatgg 1260
agaactcctc atgtggagag tttgctgctag ttcagttagt gcagcagctg 1320
tgtggagcag ctcctttgctt gcatttgctg gcctactgat aacactgctgg 1380
aaggagcatgc ttcggtctta ggtggtaggt gcctttgctg cagaaagagc aggacactgc 1440
ggatgttagt ctttagcagc cagacctctc ataagagagc ttcaggtgca ctctgggagaa 1500
ttcagacac caagaaaggc atgctgaccc ttggtagaat gagaagga aagtaacctt 1560
tacaacac agaagagcct atgtgacgcc cttggtagaat gagaagga aagtaacctt 1620
-continued

agccctaccc tgggtagtaa tttaggagaa cagctctcaag cactaaagag tgccataattc 1680
aatatatg ggtatatggg caaastaggc acctccacac tctaanaagag aggctgacat 1740
agaaggtgtc ctgtgggagct tgggaagaaga aacaatggtc tggcgccga gtaaanagaag 1800
gcctcccat gttgccattt gggcctagac ctataagtg ttagaaccoc ctattttttc 1860
tggaacttcg gatccctgag accagggcgtc ctctcaactt taototaaagcg ctcccaagt 1920
acacaaagtt ttttttcctcctg gacgaaatcct cacgccttca cacaagcgct atagctggtt 1980
tttgaatgag ttcocctctgg ctagtggaaat gttgctccaga cagtcaccag gacacagtag 2040
cggagcgag gagcgaagaa ggtggctgata aacacagagg ttttaaaacag ttcctccact 2100
tgcgcctgat caggcacagt ttacaaaaat gggagatat aacatcctaga taatatttatt 2160
tttataggg cttctagcttc tattcctttc cctctctttct cctctttcttt tttgcctcaag 2220
attatatag aataatgttc tctgggttagg tggtaggaat gacgctgtaa atccacagctg 2280
acacattat tgaaggtgcg aaaaaaaaaa aaaaa

<510> SEQ ID NO 25
<511> LENGTH: 2022
<512> STRAND: DNA
<513> ORIGIN: Homo sapiens
<560> SEQUENCE: 25

agcctgccgt cttgagacgt gaggctggag tcagcggagag tgaagtgtgg ggctctgggg 60
cactgccccg acatgctggg ctttataaaca tttatggagag aagttggaag cccttgaggcc 120
gaggtgcgcg gcctccacacag gctggatgag gctggcctgag cctgccagaa 180
ttgctcgccg cgcttggcgc gctgatgct ccgcttgccca ccgcttgcgac 240
gacctcaacag cgcctagagc accggccacc cctggtggcc tggcgtgctag ctgcttgtag 300
gaggggggtcg cagagccgg gcggatgggc gttcgctgag caggccgctgg gctgctgagg 360
gcttgccagc cagctccagc gttggcgctgc ggcttcgctg acacgtgcatga 420
gggccagagc cccggccccc ggcagcccccc taaaaagaagg ttccctggcg tgaagacac 480
cattccacg ccctgctgcca gcgctccgcag cctgccctgc ggctccgcatgc 540
agcgacaggg ccctgggctgg gttggcctgct gttgctggct gcctgctggcc atggcggagc 600
cactgccccg agcgccagagc gcggccacctg gctgctggag ggccggtgctt 660
ggccttgccag cggtggccgac cggctttctgg gctgcctcgc ccgctcagcaca 720
agccctgctg cctgggcacag ccagggcgtg gcaagcttggg aagtcgctggc caagcggcctt 780
gcgaggtcg ggggttcgtgcc ccaacaccc cctccgctggt cctgccagca cccggccggc 840
ggccttgccag cgagccagcg ccgctttcgct gcctgcccatgc gcgggctttg cctgctccag 900
cctgggcctgg tgggtgctct cctggcgctcg gcgctttggg ccgctgctggc ggacgcaagt 960
ggcgtctgcccg gtgcgctgac gcggctgcacgc gcccacgggg ggctgcctcg gcagggagctg 1020
gagccgacgg gaggccggag gggagggagt gggtggtgag ggacgagctgg gagaagctgg 1080
cgtgaggtgg ccagccgctgg ggcgtctgcct gcggctgcttg tgggtgttgt gcagacacccct 1140
gcgagacac gcgcttcacac cccttgggg ccgctcagcgc gcgtggctgtc gcgtgtctcg 1200
catggcggggag gggtgctgac gcgcttcacac gcgcctgcttg gcgtgctcaag ccaaggggag 1260
ggcctgctc ccctgctgct gcgtggagtt gcggatggag gcgcctttgg gcgccttggtg ccagcggctt 1320
-continued

gcataaagt gcgctcccca gcgctgctgatggagccat gcggagatc tcggggacat 1380
cgcaccca ccagccgagc tgcagcgcgc gcggcgaggg cgccagcgcgt tgggggccc 1440
gtcggcgccg cgcgagccgg ccgcggtctgc gcacacggcc gcggcgaggc cgcagcgcgg 1500
gcgaggcccc gggcgctggc ccggggcggg cctggagggc gagtgcgcc ccggggcgag 1560
gcccgcggc gcggctccgg cgcgiggccgc cgaggggccag cggggcgcgc cggggcgcgc 1620
cctctctgcc gcgagccgag gcgctgctgc ccgagtgcgcc gcggagaggg ccgggggagc 1680
cagagaaggg tcagctggcg ccacagaggg gcggaggggc gcgggggatt tttcttaaca 1740
aataaaacgc cccggacact ccagccgagc cgcagcgcag ctcagaagttt tctcccggg 1800
agcgatac cactctgtgc atgcgttgcc gcggcactct ttatattttt ttttttttta 1860
tttttttttttttt atgcctcttctt tggggcttca cttcagagc cgatlttatggtt ggggcacacg 1920
agccggacgc tgtgtctggt ctatgggctg tttgtacttt ctaagagctat tctgaacttt 1980
gattttttcat ctttttatcc aaccttcggtt gcggacagata ttgacgcttt tcggaaaatc 2040
agattccgct tcctggagga aataagttgt cgcagatctg aacctgcctgcc accacactga 2100
aggtggcagg cggagaggttt gcacattttc tgcagatgc acctcctttg acattggagt 2160
atctgtccc cctctggtgc acatcottggt atgcatcttg ccotccagcc gcacttttga 2220
agggcagcact gatgtctgcc aagctttttag tgttattat ctaaatctgt gtcaccttttc 2280
ggcgccga ccgctccggt gcgctgatgg cgcagtggctt ctttgtgac gcagctttgc 2340
gctccagcc gcacatcttc catcctcctcg atgcctctcgc ctgggtatgcg cactcttttg 2400
agctggggga ccccaatacg tggtgtattata acagcagact acatcctgcc 2460
acccacctct cttgtggtta tttatattct ataatcctcc aacagtccatt ctcagcatta 2520
cagggttaa atagttcagc agagatgttg ttttttttcc cggagctccgc tgctttctctt 2580
cagcagggc cacaagctcg ccaccttcttg tgcagagcgc gctagcagcc tgcagctggc 2640
tgtgtagcag tccggccttt ctgcttcacag aagcttttgg tgtttgacgc tgtctttgtt 2700
ccctctcttc aatacagcgg tcggagagct ttggtactcc atataaactc ccagcctgctc 2760
ctctctggtgt cccctcaactactc cagctcctgcc aacgctttttt gatttaacta 2820
gtcctctcttc gcgtgctat ccaatgcttg cttttttttt ctttttttgc ttcgcccaac 2880
gtatgatat caaatatcct ctccaaaaa aaaaaaaaaa aa 2922

<210> SEQ ID NO: 26
<211> LENGTH: 509
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<220> SEQUENCE: 26

Met Pro Ser Gly Phe Gln Gln Ile Gly Ser Glu Asp Gly Glu Pro Pro
1      5       10      15
Gln Gln Arg Val Thr Gly Thr Leu Val Ala Val Phe Ser Ala Val
20     25     30
Leu Gly Ser Leu Gln Phe Gly Tyr Asn Ile Gly Val Ala Pro
35     40     45
Gln Lys Val Ile Gln Ser Tyr Asn Glu Thr Trp Leu Gln Gly Arg Gin
50     55     60
Gly Pro Gly Gly Pro Ser Ser Ile Pro Pro Gly Thr Leu Thr Thr Leu

54
-continued

Ala Ala Phe His Arg Thr Pro Ser Leu Leu Glu Gln Glu Val Lys Pro
485 490 495
Ser Thr Glu Leu Glu Tyr Leu Gly Pro Asp Glu Asn Asp
500 505

<210> SEQ ID NO 27
<211> LENGTH: 4194
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 27
acgcagcgtg cggccgggg g acccagttt ccagagaa ct ttcgccgc gcgcggccgc 60
cctgcgctgc cggccggag acagcagcgc ggcggcggag cgcggcagtg gacgggccggg 120
cgcggccgg ccttcctgga gacgagctac gggccggccg gcccggccgg cccttcggag 180
gagccccgct cgcactggac gacccaccct tcaaggggct gcgttgggag cggccggctg 240
gagccccag ccttcctgga gacgagctac tgacgccacat gcaagacatc ctccaggtta 300
tccaacacca agacagctag tccttcggcc tattccaccc accctatgct ggaggcgaggg 360
ccaggcggcc agacagctag cgcgcagata ccaagcttcg accgcgcttg ctcacacctt 420
tgtgctccatt gcgttcacct ttcctgagct tcccagggcg gcggcaagcg gcgttccac 480
ccttccgctgg ttcgccgcgt cgcactggac atcggcgtct gcgttccagct atgctccttt 540
tccctccgct gcgttcacct aagggcggt tgcctgcac gcgttcacct gcgcggccgg 600
cacgccgaccc cggccggag ggcctgctag cgcgttccagct ctcctgagct gcgcggccgg 660
aggtccctgc cgcgcagctgc ttcgccgcgc ttcctgcatt gcgcgggcgt cggccggcag 720
gccctgcct gcgttccagct gcggccggcc gcgcggccgg ctgcgcgcgg ccgcggccgg 780
tgccgctgc cgcgcagctgc ttcctgagct gcggccggcc gcgcggccgg ctgcgcgcgg 840
tgcgcgctgc cgcgcagctgc ttcctgagct gcggccggcc gcgcggccgg ctgcgcgcgg 900
tgcgcgctgc cgcgcagctgc ttcctgagct gcggccggcc gcgcggccgg ctgcgcgcgg 960
agccgcgcgc gcgcgcagctgc ttcctgagct gcggccggcc gcgcggccgg ctgcgcgcgg 1020
tgcgcgcgc gcgcgcagctgc ttcctgagct gcggccggcc gcgcggccgg ctgcgcgcgg 1080
tgcgcgcgc gcgcgcagctgc ttcctgagct gcggccggcc gcgcggccgg ctgcgcgcgg 1140
tgcgcgcgc gcgcgcagctgc ttcctgagct gcggccggcc gcgcggccgg ctgcgcgcgg 1200
tgcgcgcgc gcgcgcagctgc ttcctgagct gcggccggcc gcgcggccgg ctgcgcgcgg 1260
agctgcgtata ggcgtcggag tcggctcatca acgcgcgcgc gcgcggccgg ctgcgcgcgg 1320
tgcgcgcgc gcgcgcagctgc ttcctgagct gcggccggcc gcgcggccgg ctgcgcgcgg 1380
tgcgcgcgc gcgcgcagctgc ttcctgagct gcggccggcc gcgcggccgg ctgcgcgcgg 1440
ggcgtgcagc tcggctcatca gcgcgcgcgc gcgcggccgg ctgcgcgcgg 1500
tgcgcgcgc gcgcgcagctgc ttcctgagct gcggccggcc gcgcggccgg ctgcgcgcgg 1560
ggcgtgcagc tcggctcatca gcgcgcgcgc gcgcggccgg ctgcgcgcgg 1620
tgcgcgcgc gcgcgcagctgc ttcctgagct gcggccggcc gcgcggccgg ctgcgcgcgg 1680
tgcgcgcgc gcgcgcagctgc ttcctgagct gcggccggcc gcgcggccgg ctgcgcgcgg 1740
ggcgtgcagc tcggctcatca gcgcgcgcgc gcgcggccgg ctgcgcgcgg 1800
ggcgtgcagc tcggctcatca gcgcgcgcgc gcgcggccgg ctgcgcgcgg 1860
-continued

tggtgctgt ctctcgggct ctctcctctg ttcagcggct ggccagcaac aaggggcaac 1920
cggccgcgc cgttgacttc tggagacaca gcagacgggc cgtaccgtag cgccgcccgg 1980
gagctctaatt tgcagctctc cagcagctgt ggtgcggtct gcggagacg tgccgcgcgc 2040
tgcccccttc ccagctggggct cttggggtgtata gctctctgct gcagctctcct cgcgtctg 2100
tgccaggcct ctggtgctgg cggagctctg cagcgggggct caggagcctg cagcaggaact 2160
gttcgtctctat agctgagatct aggcgcggag cacgagcgag agcctgtagtc ttacccatcag 2220
tgcacacagct gcacacatgt cgggaagaaca cagggcggtgca tcccaagtgcc acaacacgttg 2280
cgctgagctgc cctgacacctg cagcagctgt gggaggagtc cggctgtcttg ggcagcctgg 2340
cggacagctta tggctccggt gcatttcggag tgaagaccaagt tcctcagccgc gcctccgtcatt 2400
tttcggacagc cttccttcctgt agcagcgccgc gccaaggctg cctggcccaag aagtcgtcctag 2460
tgcctcctct gcatgctttcg ctcgccccag cggctgggga cgcgtttttgc ctggagctggg 2520
acggctctgct gtcgaagtagc ccagctggagac gcctgtacag cttggccggag acaacacgttg 2580
acccccctgg cccagctctcg cagcttctgg cgggaacacat cttagacgga gcaacagact 2640
gtgcaaccc gcacaccccac gcctctgttg cagctgtgcag gcgaagaagaa ttctcgatag 2700
ccctgggattc cctgctgcttg cggagcagctgc ggctctctgc gtcgctctgc 2760
gctgggcccc cgagcagcctgc ggctgatcct gcctggctagc ccctcgaacta 2820
ggagttctat gcacccctgg gcctgctagg ggcagggggc ggcggagcgcgc gggcgccagt 2880
gcctgctgccc gctggtgcagc gactgctgccc gcgggtctgca ggggtgttagc agacccctgg 2940
ccagggcagtct tccagccacc ttcagcggct cgggctgcct gcctggctgt gcacaggcag 3000
agtctgctgc aggcgcagctg acctctgttg aagagggcag tggctactctg cagcagacggc 3060
tggtctcaacc acaccacagc agctcctttg acagcgcaag cagcagcttg ctgtgctgac 3120
tggctttgct gtgtgcacagc gcgtctgtgc gcagacgggc cggccgctgg cgcgggccccg 3180
cggccggcgc ccccaagccgc gcggcccccgg ctccgctcct tgaagctggtg gcgtctacac 3240
gggcactgtg cagcagttgc ggctgtggac agacccctgg gcacgcagat cggaggtcgt 3300
tctctcctct gcgacgctgc ccggctgtag gcggggccgc cccccacacgc acacaccccg 3360
tcccttgacc gcggctgttg ccgggctggag gcggccggcag cagacgacag gcgggtgtgcg 3420
agctgaggtgc gcggccgccc gcgggtggggc aagccggcgtc otctggctcg gcgtctgctg 3480
aacgtccccg cggcggtactct gcggcgtgctg ggccagtgtc gcggtggtcg 3540
cggccacact gcgagaagtt gcggagctgc gcgtgcctgc agactgctcg cagacgtcga 3600	gcgggttagg cagggctggc aacgctagct cccgtctctcc gcgctccgac 3660
aacctgctct ctagctcttg cttcgccgtt gcgctctctg ctgctgctcg agctggtaagg 3720
gcggagagac gcggcagcag cttcgccggt cgcacgcttg cttcgtgctg 3780
tcggccggag gcggccgatc tgcaccctag cgctcagggg gcagcgccggc 3840
gcgctctgct gcgggcccgc cttcgggttg gcggccgcttc gcagtgtgcag 3900
ctgtgctgtgc ggcgcgtgatc ctggctgctgct cacaggtgctg cagactgcag 3960
atttcatcgt gcgctagagc cccggcctgc cttccgctg ggcggcagtgc 4020
cagcagcggcct ctcctcggtgc gcgtggagag gaagctgtac tgaagttggc cagctgtctg 4080
agccctcctc gcctggagtc cttgctctcg ctgcctctcggg cacccttat tttcctgagat 4140
tgagaagt tt ttagagaga ataaaaaagg aaaaaa atatatattt aattttccttt agaa 4194

<210> SEQ ID NO: 29
<211> LENGTH: 1937
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<400> SEQUENCE: 29

gaagccgccgcta cgcgttcgttagctggaggtagggggaggggggaccgagggtt ccagcccaaccgcc acccctctac 60
taatagaggg caacccgggca gcggggcgagc cagcagcgcac gcagcagcggg cgacagctca 120
ggacggggag gccggcccaac cgctgggcccgg gcgggaaggg gcagcccccag gcgccctccc 180
cggcgcgcc cggctctccg acggctgttg gctggggacc cagcagctct ccctctcgccg 240
cgccgcgcgc cggcttttac aacactgagaag ggctcactgt tcctactcga gcgggactgc 300
ttggagctcg cagccgggag cagccgggicc ccaggggcacccc ccgggggaggg cagccggggac 360
cggccgccccc cggccgagcagctggcacc aacagagcgoc catagacttc 420
ageccacgacc tggaggccgctg ggcggcagcgcc gcggcgcagc gccggcgcagc 480
acctccaggg cgctgccccgc cgcgggcccc gcgggccccg cctctcggcc gcggccaccc 540
gctccctct ccagacttctt tccccgcagac tccgggggcc aagactgcac gcagcgggoc 600
ggtccggagcg cccggactctg cggcggcagcc ccggcgcagcc gcggcgcagcc 660
tgtgccgagcg ccgggcccccc acccgccccg cggccgagcg cggccgagcc gcggccagcc 720
ggagggccgctg cagccgggcaag cagagaaggg gcgggagggg gcgggggaggg 780
gggcgagacc tgggggaggg ccctccccgcc gcggctgcgc gcgtatcggcg ccctccgaggg 840
gttcgcgcgc gcggagccgagg aacctccctct ccagcagcagc gcgggccacc 900
cggcgcgcag ctggccagcagc gcggcccgccgc ccggtccgtgt acggccgggg gcggccggccg 960
ccctccgagg tcaagacgac gcggcagagg acggctggcagc acagcgacagc cgggtgcaag 1020
tccgggtagc cggccgggccg ccggagccgtgc gcggccagagc gcagagagag cagagagagc 1080
aactctgggag ccagagccacgc gcggccccgg ccctccgagg tccagccgggg gcgcgagagg 1140
aaggggtggc acggtgctcgg gcgcttcagc accctcgccg atctctccgc gcagctcggcc 1200
ggagccctcgc ccggccacgct cggcagggcc cccgggaggg ccctccctggcc 1260
ggagggggct gcagcggccgg gcggccgggg ccctcccccc gcggccggccg ccgggggagg 1320
cgcgggagggg ccctctcttt ggcagggggg gcggaggggg gcgggggagg 1380
atatatatat atatatatat atatatatat ttggcgccagc caacccgcagc tggagaggg 1440
gcgcggccgc gtggctgcttat ttaagagaga acgctctctag tgctagacagt atctatatac 1500	tcctgtctcgctt ctctccacgg gcggccgggg gcggccgggg gcggaggtgt gcggaggtgt 1560
tgccatggct tttanctctgg cttggccagct gttcgagcgg gcggagcgct gcggaggggt 1620
aactttccg cggccgggct gcgtctgctt atagagagtc ccagcctcgg gcggaggggt 1680
atgtctgcttg tttggtgtttttttgttg tttttggtttt atatatatat atatatatat 1740
atggagagct aggggctgtgt atttttggg aatttttttttt ccagcctcgg gcggaggggt 1800
atatatatat atatatatat atatatatat atatatatat atatatatat atatatatat 1837

<210> SEQ ID NO: 29
<211> LENGTH: 3549
<210> TYPE: DNA
<211> ORGANISM: Homo sapiens
<212> SEQUENCE: 29

```
coccttccc ttccttcocaa gggaagaacr ccacactcta gctgcocctgc cacccctcttt 60
aacagggcat tgtgctacgg ccacagcgc cctccacgcct tcacagcct cccgctacgc 120
cggtcctca cgtggtccgc gcttgcgacg tctcctccag ggacgocccc cgagatgag 180
agccacgcc tcgtctggtc gacttcgagg tgtgatggtc cgcctggttg egcocccccc 240
gagagggctgg cagcagcgcg ccacgaagag gattttatgc acacgaaacat taaattttagc 300
ctagagcccg tgtgagacac agcgtgagac acctggccac ccctctccgg cagcttcagag 360
tcgtgtctca cctggtccatt ctcacagcag acacacacct ccaggttggct ccctggtggtg 420
acgcacagc gatagtgagc gacttggttg ccacaccttg tgtgccgcttc ctgacagaca 480
gacacgaccc caacttgctat tgtgttgccg ctctgccgcg gccctggtcc gacattacca 540
gtccgtccgg cctcccacccg aagagttgag ccagaggtgc ccoctggcttc caacttggtag 600
aggagaggtc tttaacctcc ttcgccacat ctctcctctg tggatacag cctcgagccc 660
catgcgtcgt gcttccgtac ccacgaccgc aataacagag tccacagacat tctgcgctctc 720
gtccggcttg cacatccatt ttgagttgccg caacgccccg ggtgttgatttc gatctctgt 780
gaacagtttt tagagttgtc acacacatcc acacaggtat ccctcgttgtc agccattcca 840
atccagacgc catttgctca gtcgacatt taccgaatag gactttacct tcagccaggg 900
tgtaaccttg gagaagtttt cagcgtttgc cagcagagac gatgcgagc gttggaagtt 960
ctaggtgagt gctccgagac gtctgtcatt ccccttcctta ctgactctgt cttgaagaaa 1020
```

```
gaaactcacc gtaagcagtg ccacgggagga cccctggagaa agggcctgtgc 1080
```

```
tgagtgtaga gaagagacag ccctgcaact ctggcttatt gcagaataa agtcagagcc 1140
```

```
aacagagcg ccaaatgtg cctgtgacag ctgctgccag tggcctacaca acgttccctat 1200
```

```
taccagacat gattttcatt ttcgctggttc gagagcggaa ccaacaccac agcctccttt 1260
```

```
gagacttttc tgtatgccc gctcgccgcag agtcgaacc ccccatcctac tctgcctgaa 1320
```

```
gttctccac ctacacccct cttcttcctta attacccac aggtagatatt tgaggactct 1380
```

```
tcactganctt agctcataat gaggagttt tctactctta gctgttgctag ctcggttggag 1440
```

```
agtccggtct cagacttaata gaaaaagagc gagacgcagc gaaaaatgttctt 1500
```

```
atctcttgag tctagtgtgc aagcagttct acagctcaaa aagctcctaa ctcaatatct 1560
```

```
gtgaatcgc atgacaatgc tctgaatag cagcttgtct gaaactgccc gaaactcagc 1620
```

```
aacaagagac ggcactgtaaa cttctggaag aagctctagg tcattcataaa 1680
```

```
cctacagct ccgtggtggct ttccaaattt gcattttctt caggtatatc ccaacgctca 1740
```

```
cctgtgtaga ttatttttagg aagcagttcct aagcactaara aagctgctaa ttcaatatat 1800
```

```
gggatataag ggcaccaatt cacttcctcc aagctttaaa gagctgagtt gctgaaatgt 1860
```

```
```
```
```
agggacgaag aaggg tgtcg ataaacag aggtttttaa cagtcctac cattggcttg
2220
catcatgaca aagtgacaa tcacagggaga ttcacaactc atgttcaattta
2280
ggcttttag ttatagggtc aatccctcct ccccctctct ttttttgtct aagtttatatat
2340
tataataagg tgtctggtggt aaggtgtgtt gaaatgctctg taatctctag gtgacacata
2400
atttgaagtt gcacagaaaa aaaaaagatac ctgaaattaatt gtttagatt ctccaaatgata
2460
ttttatatc ttaaaatactata tataaatcact cttcagtacct tctcagtttct ctgacctctt
2520
tgcttcatgc tcaattgtaac ttcacatgcag tctctctttgt tcttcgctttt gacatgaaaaa
2580
gataggttct aagtc aactattgt gctgcgcctta cagattttgaa cagagagccg
2640
tttttacta aaaaaggtt tggagggcttc tctggggtgga tttcatttcgaca ggtgtgtctaat
2700
acctctcgtg gccatgagcc gcatacgagta cctagaggtt tggattttgt gtggattttt acag
2760
aactaatacag aagttgagta cacaactttt ataaactagttg ttcctctttct ttcagaatct
2820
tctttcattg ataattatgg aatgataaag atgtcataa tttcatagggct cattagctgg
2880
aacccgactg tgaagagagtg tgcatactgtc gcacactata gaagaagctct gacgaggtgtt
2940
gtcttctagc ataattgtagg aagggaaaaca gctgtatcag ggatgtatgg gacatgtgcttg
3000
aggtaatagtt tgttctgtatgt tgcggccagac ttcagcccttt tctctgagag aagtagctgtct
3060
ggctataata aagtgagggg ataattggtg tgcatacactag ggcgggtgga gagtttcttctat
3120
tttatataa aatgcattgat cattttgtgt ggacctcccc ttatataactt cattaaattt
3180
tttagatatgtt gcgtgcaaccc aaggggtcatc aaatcataaa gcagccata
3240
gcagtgagga ctctggtgcctt gaaatccttt tgtatatata tcaagagatgt tctggcttta
3300
cattttttat attgctgtgt ataagttttg aatggaggtt gatactatttgt
3360
gagggccata tgttgtatctc tgcattatata aatgtggttt gtaacatgtat tgtgtttctta
3420	tctgtgtgtc ttcacagag ccaactcact cttatgataa ggcttttac aacacaagaa
3480
gagaaaagttc ttaactgtgt gcagaaatgtg aactgacgttt tataaaatatt gacacattt
3540	tatttaccac
3549
-continued

<table>
<thead>
<tr>
<th>100</th>
<th>105</th>
<th>110</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glu</td>
<td>Ile</td>
<td>Ser</td>
</tr>
<tr>
<td>115</td>
<td>120</td>
<td>125</td>
</tr>
<tr>
<td>Val</td>
<td>Gln</td>
<td>Gly</td>
</tr>
<tr>
<td>130</td>
<td>135</td>
<td>140</td>
</tr>
<tr>
<td>Lys</td>
<td>Gln</td>
<td>Val</td>
</tr>
<tr>
<td>145</td>
<td>150</td>
<td>155</td>
</tr>
<tr>
<td>Asp</td>
<td>Trp</td>
<td>Val</td>
</tr>
<tr>
<td>165</td>
<td>170</td>
<td>175</td>
</tr>
<tr>
<td>Lys</td>
<td>Gly</td>
<td>Ala</td>
</tr>
<tr>
<td>180</td>
<td>185</td>
<td>190</td>
</tr>
<tr>
<td>Tyr</td>
<td>Phe</td>
<td>Asn</td>
</tr>
<tr>
<td>195</td>
<td>200</td>
<td>205</td>
</tr>
<tr>
<td>Arg</td>
<td>Arg</td>
<td>Leu</td>
</tr>
<tr>
<td>210</td>
<td>215</td>
<td>220</td>
</tr>
<tr>
<td>Met</td>
<td>Ala</td>
<td>Gln</td>
</tr>
<tr>
<td>225</td>
<td>230</td>
<td>235</td>
</tr>
<tr>
<td>Gly</td>
<td>His</td>
<td>Tyr</td>
</tr>
<tr>
<td>245</td>
<td>250</td>
<td>255</td>
</tr>
<tr>
<td>Ser</td>
<td>Met</td>
<td>Phe</td>
</tr>
<tr>
<td>260</td>
<td>265</td>
<td>270</td>
</tr>
<tr>
<td>Leu</td>
<td>Thr</td>
<td>Asn</td>
</tr>
<tr>
<td>275</td>
<td>280</td>
<td>285</td>
</tr>
<tr>
<td>Met</td>
<td>Thr</td>
<td>Arg</td>
</tr>
<tr>
<td>290</td>
<td>295</td>
<td>300</td>
</tr>
<tr>
<td>Thr</td>
<td>Glu</td>
<td>Val</td>
</tr>
<tr>
<td>305</td>
<td>310</td>
<td>315</td>
</tr>
<tr>
<td>Met</td>
<td>Phe</td>
<td>Arg</td>
</tr>
<tr>
<td>325</td>
<td>330</td>
<td>335</td>
</tr>
<tr>
<td>Pro</td>
<td>Leu</td>
<td>His</td>
</tr>
<tr>
<td>340</td>
<td>345</td>
<td>350</td>
</tr>
<tr>
<td>Glu</td>
<td>Ser</td>
<td>Gly</td>
</tr>
<tr>
<td>355</td>
<td>360</td>
<td>365</td>
</tr>
<tr>
<td>Arg</td>
<td>Met</td>
<td>Ala</td>
</tr>
<tr>
<td>370</td>
<td>375</td>
<td>380</td>
</tr>
<tr>
<td>Val</td>
<td>Arg</td>
<td>His</td>
</tr>
<tr>
<td>385</td>
<td>390</td>
<td>395</td>
</tr>
<tr>
<td>Glu</td>
<td>Pro</td>
<td></td>
</tr>
</tbody>
</table>

<210> SEQ ID NO 31
<211> LENGTH: 4592
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 31

```
aggtcatgta ggcctgcgcca ttttttttcc ttcctctctc acctccattc tgaactgcact ctgctgttct
60
gatcccatc cagaggggct cagagagctg ttgctgggag ctgttagttct gcattattgt
120
cgtccgcttc atgaccagga accacgact caagggcccg gatctagctt tctccagccc
180
aaggggggct gccagagttg gatgctgggct atcggagggc atcgggccc taaagggccc
240
cagagccttg atgaccagagta tggacccct ggtgagaagg gtagaagggc agatctacaggt
300
```
| cttatcgttc ctaagggaga cactgtgcca aacggagtac cggggtcgtg aagtcceccga | 360 |
| ggttttcecg gaaacctag cgggagggaa gaacctgcag aagttgcctc tcgatacgcag | 420 |
| tcgacctac gttcggaggt ggaacctacc gttactacgg ccacactgcg cactgcttcttc | 480 |
| acacagactc taccaacctg ccaacacacc tacacagttc ccagcgttaat aaccactgc | 540 |
| aaactctccgt ggtactcata cttggcctac cacaatcacc tcataattgaa ggtctggaag | 600 |
| gtcgctcttc tcgaagagga cagctatcg ctctccccct actctccca ccagggaaat | 660 |
| aagctggacgc cagyctccgg ctctgtgcgc ctgcatcctgg aggigggcga ccagtgctgg | 720 |
| ctcgccccgtg atgggcaag ctgagatagt gcacgactatg tctgatatac cagtgctctc | 780 |
| acctccagc gcttctcttt ctcacagct gcacactgat caccacactgc ctacagcttc | 840 |
| ctcgccccca aacgcgccca aagtccatta aggttcttcga tcaagctgtaa gaacggtatt | 900 |
| attagttagt gcgtggtcgt agtagtttct tcacatcatt ccctacctgt ttacttccttc | 960 |
| attcactagg taaactttaaa taactactat gctagttcccg cgcctcctcag gacgctcaca | 1020 |
| acacgcgcca gtaacgtgac tagaagaggg tagttgcagc tgtctatttg tgcceaatgt | 1080 |
| cttcctcctt gctcactata atcctataag gccacagggaa caagatcttt ccgctttttta | 1140 |
| cagatgtgtcat cttgaggctg agagagttta aagattttctg atcgggcacct acgtgagtct | 1200 |
| gcacgactgt gaaatccaaac cccagcgtgt ggcctttgct cactagactgc ccgctttttta | 1260 |
| tagaggtaca tgttctttgg gcaggtcttg taggttttgtt tttaccatcc caccaggagag | 1320 |
| ccctgaggct tgtctcctcct atgaatttaaa aacccctcgg agaagagcct ccctcagaga | 1380 |
| agttggctcg tgctaggtgg caggttaata cctcaaggca cccagtgctc cctgacaac | 1440 |
| tcacgctctt ctacagtctag cctctctctag ccctctcgag ccacacaggg aacgcccaact | 1500 |
| cctctctctg cgggtcgact caagccctct cctcagcccgc aacgcgagac ggtctttctt | 1560 |
| gatggtctac ccaacccactc tttttttttgg atgggtcttc cccctgctcgc ccaagggagga | 1620 |
| ctcagctggca cagacgctgg ccctactgcag cctctctctc cttggtctca cccatttttg | 1680 |
| tcgctggaac tggagttgag tgtggcctcg gcctgtctcg aacccctggtc cccagccagc | 1740 |
| tgtttttta ctgacacagct gggtctcctgc tgtgtgctgtg gcgcctgcttc aacccctg | 1800 |
| ctagttgac ccacgctgcc ACCCGCGGA ggtgctgtgg tccagctgc gacccctttc | 1860 |
| gcagcccttg tataccttct tttttttttt cccaggagttg agtggctttg tgtgggcaag | 1920 |
| cccgccacca gctgagaggg aacgtcgcag gacagctgt ggtcctccga cctttttaac | 1980 |
| cctcgagatt cctggttaaa cgtcctccga aagcccaatct ctggactctgc atactacctc | 2040 |
| agaatattc ccatctcctcg ctcgctcgtat cccagtattt cctacagcag scatttccct | 2100 |
| tttgatcttc ccctggtttt aagttatcatt cctggtcttc cagcactata gcctattttt | 2160 |
| ccaactgctg tggagttgtc tgtgattttt gacacgcagct ccagatatttt cctcaacgag | 2220 |
| gatggcttg ccaacagatgg gagaacactt ggaacacttt gccctgcttc ttcctccgag | 2280 |
| taccacagg gcagggaggg ctcgacttctt ctaagctttt ttagtttttt tataagcttc | 2340 |
| aggtgttgcg caggttctgt gttactacct cttccctgag acacatactc ctcgactgtt | 2400 |
| cttccacagct ttttctcaca acagacatgta ngtcctcccct atcctatattc ttactttttta | 2460 |
| aacacctttt ctgcttctgc gttttttatt gcacexactt cttctttttc cttgctttcg | 2520 |
| tataactaa gccctttatt tttccacata taagctgctt ctctttttgc tgtgttcttg | 2580 |
tttttaagat ggaatcttccc tctgttggca ggcataaagcc caagagcagc atcttgcttt 2640
tctgcaacc tttgcttccc ggttccaagc attttctctc ttcagttctc cggtagtctg 2700
ggaacctagg tggcttaacc cgtccagggg taatttttttt attttttaga aagacagggg 2760
tttttgcatt tgggcatgct ggcctcagac tttgcaacct tttgagctgc cgtctctcct 2820
tttggtgta tttttgagaa aagatagata tgaggtttag agagggtgtag agaggtgaga 2880
gttaggttgg ggattcttc aggacttggt tggtagttgcc atttttcttc gttttccc 2940
atattattag aactactagtga aagcagaaaa aaaaaaggg aagacccagacctttact 3000
atttttattaa gaaatttccg agggtgcttt cttttgcttt cttttgcttt cttttgcttt 3060
aaaaaactttt gccctttttc gttttccttt cttttgcttt cttttgcttt gttttccttt 3120
ccctacagtgt ttcacagttta tttttctttc gttttccttt cggtagttttc cttttccttt 3180
cctgctccag tttttccttt cctttttttt ctttttcttt ctttttcttt ctttttcttt 3240
aggttagccc ggaatcttccc ggcataaagcc caagagcagc atcttgcttt 3300
ttttttcttt ctttttcttt ctttttcttt ctttttcttt ctttttcttt ctttttcttt 3360
ggaacctagg tggcttaacc cgtccagggg taatttttttt attttttaga aagacagggg 3420
ccctacagtgt ttcacagttta ggaatcttccc ggcataaagcc caagagcagc atcttgcttt 3480
ttttttcttt ctttttcttt ctttttcttt ctttttcttt ctttttcttt ctttttcttt 3540
agacaatgct ggaatcttccc tttttttttt tttttttttt tttttttttt tttttttttt 3600
ggagactctg tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 3660
ggagactctg tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 3720
tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 3780
tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 3840
tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 3900
tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 3960
tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 4020
tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 4080
tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 4140
tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 4200
tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 4260
tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 4320
tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 4380
gggttagttt gagacagcag tttttttttt tttttttttt tttttttttt tttttttttt 4440
atattattag aactactagtga aagcagaaaa aaaaaaggg aagacccagacctttact 4500
atttttattaa gaaatttccg agggtgcttt cttttgcttt cttttgcttt cttttgcttt 4560
tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 4620
tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 4680
<table>
<thead>
<tr>
<th>Residue</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Met</td>
</tr>
<tr>
<td>2</td>
<td>Gly</td>
</tr>
<tr>
<td>3</td>
<td>Leu</td>
</tr>
<tr>
<td>4</td>
<td>Leu</td>
</tr>
<tr>
<td>5</td>
<td>Val</td>
</tr>
<tr>
<td>6</td>
<td>Ala</td>
</tr>
<tr>
<td>7</td>
<td>Phe</td>
</tr>
<tr>
<td>8</td>
<td>Ser</td>
</tr>
<tr>
<td>9</td>
<td>Thr</td>
</tr>
<tr>
<td>10</td>
<td>Ser</td>
</tr>
<tr>
<td>11</td>
<td>Ala</td>
</tr>
<tr>
<td>12</td>
<td>Phe</td>
</tr>
<tr>
<td>13</td>
<td>Phe</td>
</tr>
<tr>
<td>14</td>
<td>Ser</td>
</tr>
<tr>
<td>15</td>
<td>Ser</td>
</tr>
<tr>
<td>16</td>
<td>Ala</td>
</tr>
<tr>
<td>17</td>
<td>Leu</td>
</tr>
<tr>
<td>18</td>
<td>Ala</td>
</tr>
<tr>
<td>19</td>
<td>Ala</td>
</tr>
<tr>
<td>20</td>
<td>Leu</td>
</tr>
<tr>
<td>21</td>
<td>Leu</td>
</tr>
<tr>
<td>22</td>
<td>Ala</td>
</tr>
<tr>
<td>23</td>
<td>Phe</td>
</tr>
<tr>
<td>24</td>
<td>Pro</td>
</tr>
<tr>
<td>25</td>
<td>Val</td>
</tr>
<tr>
<td>26</td>
<td>Val</td>
</tr>
<tr>
<td>27</td>
<td>Phe</td>
</tr>
<tr>
<td>28</td>
<td>Gly</td>
</tr>
<tr>
<td>29</td>
<td>Thr</td>
</tr>
<tr>
<td>30</td>
<td>Ser</td>
</tr>
<tr>
<td>31</td>
<td>Ser</td>
</tr>
<tr>
<td>32</td>
<td>Ala</td>
</tr>
<tr>
<td>33</td>
<td>Phe</td>
</tr>
<tr>
<td>34</td>
<td>Gly</td>
</tr>
<tr>
<td>35</td>
<td>Asp</td>
</tr>
<tr>
<td>36</td>
<td>Ser</td>
</tr>
<tr>
<td>37</td>
<td>Lys</td>
</tr>
<tr>
<td>38</td>
<td>Asp</td>
</tr>
<tr>
<td>39</td>
<td>Ser</td>
</tr>
<tr>
<td>40</td>
<td>Gly</td>
</tr>
<tr>
<td>41</td>
<td>Thr</td>
</tr>
<tr>
<td>42</td>
<td>Asp</td>
</tr>
<tr>
<td>43</td>
<td>Ser</td>
</tr>
<tr>
<td>44</td>
<td>Lys</td>
</tr>
<tr>
<td>45</td>
<td>Asp</td>
</tr>
<tr>
<td>46</td>
<td>Ser</td>
</tr>
<tr>
<td>47</td>
<td>Lys</td>
</tr>
<tr>
<td>48</td>
<td>Asp</td>
</tr>
<tr>
<td>49</td>
<td>Ser</td>
</tr>
<tr>
<td>50</td>
<td>Lys</td>
</tr>
<tr>
<td>51</td>
<td>Ser</td>
</tr>
<tr>
<td>52</td>
<td>Lys</td>
</tr>
<tr>
<td>53</td>
<td>Ser</td>
</tr>
<tr>
<td>54</td>
<td>Lys</td>
</tr>
<tr>
<td>55</td>
<td>Ser</td>
</tr>
<tr>
<td>56</td>
<td>Lys</td>
</tr>
<tr>
<td>57</td>
<td>Ser</td>
</tr>
<tr>
<td>58</td>
<td>Lys</td>
</tr>
<tr>
<td>59</td>
<td>Ser</td>
</tr>
<tr>
<td>60</td>
<td>Lys</td>
</tr>
<tr>
<td>61</td>
<td>Ser</td>
</tr>
<tr>
<td>62</td>
<td>Lys</td>
</tr>
<tr>
<td>63</td>
<td>Ser</td>
</tr>
<tr>
<td>64</td>
<td>Lys</td>
</tr>
<tr>
<td>65</td>
<td>Ser</td>
</tr>
<tr>
<td>66</td>
<td>Lys</td>
</tr>
<tr>
<td>67</td>
<td>Ser</td>
</tr>
<tr>
<td>68</td>
<td>Lys</td>
</tr>
<tr>
<td>69</td>
<td>Ser</td>
</tr>
<tr>
<td>70</td>
<td>Lys</td>
</tr>
<tr>
<td>71</td>
<td>Ser</td>
</tr>
<tr>
<td>72</td>
<td>Lys</td>
</tr>
<tr>
<td>73</td>
<td>Ser</td>
</tr>
<tr>
<td>74</td>
<td>Lys</td>
</tr>
<tr>
<td>75</td>
<td>Ser</td>
</tr>
<tr>
<td>76</td>
<td>Lys</td>
</tr>
<tr>
<td>77</td>
<td>Ser</td>
</tr>
<tr>
<td>78</td>
<td>Lys</td>
</tr>
<tr>
<td>79</td>
<td>Ser</td>
</tr>
<tr>
<td>80</td>
<td>Lys</td>
</tr>
<tr>
<td>81</td>
<td>Ser</td>
</tr>
<tr>
<td>82</td>
<td>Lys</td>
</tr>
<tr>
<td>83</td>
<td>Ser</td>
</tr>
<tr>
<td>84</td>
<td>Lys</td>
</tr>
<tr>
<td>85</td>
<td>Ser</td>
</tr>
<tr>
<td>86</td>
<td>Lys</td>
</tr>
<tr>
<td>87</td>
<td>Ser</td>
</tr>
<tr>
<td>88</td>
<td>Lys</td>
</tr>
<tr>
<td>89</td>
<td>Ser</td>
</tr>
<tr>
<td>90</td>
<td>Lys</td>
</tr>
<tr>
<td>91</td>
<td>Ser</td>
</tr>
<tr>
<td>92</td>
<td>Lys</td>
</tr>
<tr>
<td>93</td>
<td>Ser</td>
</tr>
<tr>
<td>94</td>
<td>Lys</td>
</tr>
<tr>
<td>95</td>
<td>Ser</td>
</tr>
<tr>
<td>96</td>
<td>Lys</td>
</tr>
<tr>
<td>97</td>
<td>Ser</td>
</tr>
<tr>
<td>98</td>
<td>Lys</td>
</tr>
<tr>
<td>99</td>
<td>Ser</td>
</tr>
<tr>
<td>100</td>
<td>Lys</td>
</tr>
<tr>
<td>101</td>
<td>Lys</td>
</tr>
<tr>
<td>102</td>
<td>Lys</td>
</tr>
<tr>
<td>103</td>
<td>Lys</td>
</tr>
<tr>
<td>104</td>
<td>Lys</td>
</tr>
<tr>
<td>105</td>
<td>Lys</td>
</tr>
<tr>
<td>106</td>
<td>Lys</td>
</tr>
<tr>
<td>107</td>
<td>Lys</td>
</tr>
<tr>
<td>108</td>
<td>Lys</td>
</tr>
<tr>
<td>109</td>
<td>Lys</td>
</tr>
<tr>
<td>110</td>
<td>Lys</td>
</tr>
<tr>
<td>111</td>
<td>Lys</td>
</tr>
<tr>
<td>112</td>
<td>Lys</td>
</tr>
<tr>
<td>113</td>
<td>Lys</td>
</tr>
<tr>
<td>114</td>
<td>Lys</td>
</tr>
<tr>
<td>115</td>
<td>Lys</td>
</tr>
<tr>
<td>116</td>
<td>Lys</td>
</tr>
<tr>
<td>117</td>
<td>Lys</td>
</tr>
<tr>
<td>118</td>
<td>Lys</td>
</tr>
<tr>
<td>119</td>
<td>Lys</td>
</tr>
<tr>
<td>120</td>
<td>Lys</td>
</tr>
<tr>
<td>121</td>
<td>Lys</td>
</tr>
<tr>
<td>122</td>
<td>Lys</td>
</tr>
<tr>
<td>123</td>
<td>Lys</td>
</tr>
<tr>
<td>124</td>
<td>Lys</td>
</tr>
<tr>
<td>125</td>
<td>Lys</td>
</tr>
<tr>
<td>Amino Acids</td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td></td>
</tr>
<tr>
<td>Leu Asp Ala Ile Thr Thr Pro Asp Pro Thr Thr</td>
<td>Am Ala Ser Leu Leu</td>
</tr>
<tr>
<td>166</td>
<td>170</td>
</tr>
<tr>
<td>Thr Lys Leu Gln Ala Gin Gin Gin Trp Leu Gin Asp Met Thr Thr</td>
<td>His</td>
</tr>
<tr>
<td>180</td>
<td>185</td>
</tr>
<tr>
<td>Leu Ile Leu Arg Ser Phe Lys Glu Phe Leu Gin Ser Ser Leu Arg Ala</td>
<td></td>
</tr>
<tr>
<td>196</td>
<td>200</td>
</tr>
<tr>
<td>Leu Arg Gln Met</td>
<td></td>
</tr>
<tr>
<td>210</td>
<td></td>
</tr>
</tbody>
</table>

**<210> SEQ ID NO 34**

**<211> LENGTH: 233**

**<212> TYPE: PRT**

**<213> ORGANISM:** Homo sapiens

**<400> SEQUENCE:**

<table>
<thead>
<tr>
<th>1</th>
<th>5</th>
<th>10</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met Ser Thr Glu Ser Met Ile Arg Asp Val Glu Leu Ala Glu Glu Ala</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>25</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Leu Pro Lys Thr Gly Gln Gly Ser Arg Arg Cys Leu Phe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>40</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Leu Ser Leu Phe Ser Phe Leu Ile Val Ala Gly Ala Thr Thr Leu Phe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>55</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Cys Leu Leu His Phe Gly Val Ile Gly Pro Gin Arg Glu Phe Pro</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>70</td>
<td>75</td>
<td>80</td>
</tr>
<tr>
<td>Arg Asp Leu Ser Leu Ile Ser Pro Leu Ala Gin Ser Ser Ser</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95</td>
<td>90</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>Ser Arg Thr Pro Ser Arg Lys Pro Val Ala His Val Val Ala Aen Pro</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>105</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>Gin Ala Glu Gly Gln Leu Gin Trp Leu Aen Gin Arg Gin Ala Aen Ala Leu</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>115</td>
<td>120</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td>Leu Ala Aen Gly Val Glu Leu Arg Asp Gin Gin Leu Val Val Pro Ser</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>130</td>
<td>135</td>
<td>140</td>
<td></td>
</tr>
<tr>
<td>Glu Gly Leu Tyr Leu Ile Tyr Ser Gin Val Leu Phe Lys Gly Gln Gly</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>145</td>
<td>150</td>
<td>155</td>
<td>160</td>
</tr>
<tr>
<td>Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser Arg Ile Ala</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>165</td>
<td>170</td>
<td>175</td>
<td></td>
</tr>
<tr>
<td>Val Ser Tyr Gin Thr Lys Val Asn Leu Leu Ser Ala Ile Lys Ser Pro</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>180</td>
<td>185</td>
<td>190</td>
<td></td>
</tr>
<tr>
<td>Cys Gin Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>196</td>
<td>200</td>
<td>205</td>
<td></td>
</tr>
<tr>
<td>Pro Ile Tyr Leu Gly Val Phe Gin Leu Glu Lys Gly Asp Arg Leu</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>210</td>
<td>215</td>
<td>220</td>
<td></td>
</tr>
<tr>
<td>Ser Ala Glu Ile Asn Gin Gin Pro Gin Asp Phe Ala Gin Ser Gly</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>225</td>
<td>230</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**<210> SEQ ID NO 35**

**<211> LENGTH: 10049**

**<212> TYPE: DNA**

**<213> ORGANISM:** Homo sapiens

**<400> SEQUENCE:**

```
ggcgccgcttcctcggcggtt cggccccacag ggcggcaggg ggccccaggg gcggccaggtc gcggccaggtc gcggccaggtc 60
```

```
gaagtcaggg gcggcgttgcg tctcgtgcac tcaggacgtt gtcagacac aagtgtcagaa 120
```
<table>
<thead>
<tr>
<th>Sequence</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>cttgaaagct gtcaccacag tcctggaggg tgggaagttc aagactaaag tgtccagcaga</td>
<td>180</td>
</tr>
<tr>
<td>ttcagtgctca tggagacag gcgttcatcc tcctatagta agatgtcatt gggactgccg</td>
<td>240</td>
</tr>
<tr>
<td>ggccacacca gcacccacgt gcgtgcatgt ggacccagga aagacccatcc tcgcctctct</td>
<td>300</td>
</tr>
<tr>
<td>cocccactga gcgcggcgtat ctagagaccc cgtatctga agatgtcctg caagaatacg</td>
<td>360</td>
</tr>
<tr>
<td>gaactatcca agagattctg caacctctag gcggagatag ttctggaagc tttggcttta</td>
<td>420</td>
</tr>
<tr>
<td>cggatatacc gattttagga agctgtcttg gtcagagtag ctcgctcttc acgcagca</td>
<td>480</td>
</tr>
<tr>
<td>tttccacacgc ttgagccccc tctccgctga cttaaccctg ggtcctgcgc acgcgtgca</td>
<td>540</td>
</tr>
<tr>
<td>agtccccccag tgggactatg aacatcacat gtagacattg cggggaacag gctctcagct</td>
<td>600</td>
</tr>
<tr>
<td>atctctatac gcgtgagcct cttgagactt ctctgcggga agcattccgc</td>
<td>660</td>
</tr>
<tr>
<td>tcacgctgct gcagcagacgc gcgtcagact ccagaaataa aacgaaaca</td>
<td>720</td>
</tr>
<tr>
<td>aaatgcagct ttggctatt ccaagagcgc ttccttgcttg ggtctcgaca aacccgattc</td>
<td>780</td>
</tr>
<tr>
<td>gtttggcagc aatccgaaga tctggaagaag caaactcaga gaagcagagt tccactgtg</td>
<td>840</td>
</tr>
<tr>
<td>aacagcttacc aaggaattctc aaacactcaac acctccaact tcctggccag</td>
<td>900</td>
</tr>
<tr>
<td>aggcactatc gcactgtaac aacatgacgc cgcttcagtc ctctccagaa</td>
<td>960</td>
</tr>
<tr>
<td>aggcgcgtac aacacccctt ttctgcctcc agtatcggtg gtcgctgacta</td>
<td>1020</td>
</tr>
<tr>
<td>agacgctctg cacggagctg tgcctagcag gcctcagcag cggactgcgc</td>
<td>1080</td>
</tr>
<tr>
<td>tcttccactc ctgctcgctg acgcgctgtg agacgctcag gcgcgagcag gctccagcga</td>
<td>1140</td>
</tr>
<tr>
<td>aggcagatcc aagttccgac ctagcgacaa cgcagataac gtgctagtaa caaaaaatcg</td>
<td>1200</td>
</tr>
<tr>
<td>gatcctgtct gcctgactat cctctctatg gactacagat ggacaaagug ggcgcttgcc</td>
<td>1260</td>
</tr>
<tr>
<td>tagctgccag aatcttgcct atatactcag aacctcatag aaaccgtgctc</td>
<td>1320</td>
</tr>
<tr>
<td>gtctgattct ggcacccag tcctgattct ttctgacagt ggacatcatt gacagtcat</td>
<td>1380</td>
</tr>
<tr>
<td>acactggatc atgcctcttc ccggcgtcgt gtcggtcatt cttagctgct cttgcctttg</td>
<td>1440</td>
</tr>
<tr>
<td>taaatggctg cagcttatgg aagggctggt cagctctgg gtacactcga acagctcagag</td>
<td>1500</td>
</tr>
<tr>
<td>tgcagacagc ccacccgccg gatactcttc tctcctccca aacctctcag aaatgtcgcag</td>
<td>1560</td>
</tr>
<tr>
<td>acctccggca gttttctgag gcactcgagc agctgtgacg ggtcctgaca gcacatcagc aagacggagt</td>
<td>1620</td>
</tr>
<tr>
<td>cgagatgtct gcgttcctgg ctctgctcag gatgtctcag ggcctcctca tgcgttctct</td>
<td>1680</td>
</tr>
<tr>
<td>cgctgactgc acaccccttc caggagtctt ggcctgctca gcacatccag gagaagcgggg</td>
<td>1740</td>
</tr>
<tr>
<td>gcagccacgc atttgccgca aatatacccg aacattaacct tagagcttgg cagctcgtag</td>
<td>1800</td>
</tr>
<tr>
<td>ctgtaggtgtc cggccatatt atccatatcc ttcgctgtca cagctttccgc aagagatcgc</td>
<td>1860</td>
</tr>
<tr>
<td>gacctcaat gcctgagggtg ggcggtctat ccctgagact ggacagatatt gcgcggaat</td>
<td>1920</td>
</tr>
<tr>
<td>gcctcaatgc tttggtctac ctcacccgct gttacacat gcgttcttgg ttaggtctccag</td>
<td>1980</td>
</tr>
<tr>
<td>ttcctgtatt acctattact taacctgttc aacctcaatgt cttggtccttg cttcctcttc</td>
<td>2040</td>
</tr>
<tr>
<td>acctccgttt gtgactattct gtccttcatt aacctcatag cgccttcagct tccttttttc</td>
<td>2100</td>
</tr>
<tr>
<td>aagttgcttat atctctcaag ttagtggtc ttcggctgggc aagatgtcag caacccgca</td>
<td>2160</td>
</tr>
<tr>
<td>aataaatcttt acaagtcct tcctctcggt ctcctgatct ccaggagatag cctctctctcgc</td>
<td>2220</td>
</tr>
<tr>
<td>ttttttaacct tagatgtcct aatatttaaag aagggcttgg gcagcccgac gcgcgctctttca</td>
<td>2280</td>
</tr>
<tr>
<td>ctgctcttc gcagcctttg gcggccgac gcggctgtgt ccaagcttga gcagctcagag</td>
<td>2340</td>
</tr>
<tr>
<td>acacccctgc cccatcattct gcagccctgt gcactacataaa ctaacaaat ttaggcggytg</td>
<td>2400</td>
</tr>
</tbody>
</table>
gtgggtgcac atgcctgtaa tcccgctcag tcgggaagct gagcgaggag aatgtgctta 2460
accaaggagt tgggaacctgc atgagcta gtctgacacc ctcgcatcag gctgctgcag 2520
agacagacag tcggttcatt ataataaacc acaaaaaaaa aactctggtag ataaagtctac 2580
aatttgacg tgggttttttg ttttgtggta actgttttcg tgggtgatac taatatggg 2640
aagccgttac ttcagagtggt gctgtgaaga gccagaaaa cagagttcca agaccccccag 2700
tctggatcgt cctgagttac cacccccttt tggaggggacc agacagacag ccgcagccgg 2760
cacgcttccct gctccctcag gttgcttcgca gtcaccctgaa accaaccagc ctaccagctcc 2820
tgtggaatggt tgtggtttcct tagggacaga cttgacaccct actgttgcaag gtctctccgg 2880
gcccatcttg gactgcttccg tctccttttc tatgtttgtt ttaagagatat gatgtttttat 2940
tgatctttat cctggtgact gtagggtcgg tcggacgacc agacgtagca cattggtttt 3000
cattggtcgg tgtggttttgg gacgacttcg cttggtgacc gcggagagggg ttggagtttt 3060
tctccaattgg cagtttctttt tcaaatggcga gattccacca gattgtgtta ttitttttacaa 3120
tggcttttggtgtatgctct cttttcgttg aataggtgtat tttacccata ctttaaatttt 3180
tgctttaccc tccaaaaacc acttaccaac ctcagcctcag cttgggaaaag agcggtgtatt 3240
atgagagtct tgcgataattattagc gaaaaaccattt ctototctgt ccaactggtta 3300
gataatttgcgg tgtggtgattt gattttctgg gcggactggtg caacgccctt 3360
caggaggagct cggctttgctt gtaggtggct tggagtcctt tccocacatc ttaagaaaat 3420
tggcctttct gaggcttacta tatttttaag aatgattagg attgataaaggt gtccacggac 3480
cagacttacatt aaaaagtagcag agctgaaagg acacagttag gcagacctgg ccaagaaaaag 3540
tgagttagct gccttcctct ttctttttta aaaaatcctaa atttggccag ttttggtttaa 3600
tgattttctc gtagggacag tggctcaacc ctggaaatccg gcacactttgg gggcgaggag 3660
tggggagact acgtgaccc agagttgca gactggtgca gccactcaag cagacccctt 3720
tctgactaca aatatttttt ctgtcctcatt cggggcggcga cactatacgtgt aggaccaga 3780
ttctgacgag tgggaggggg gatggttggc aaccccaagag tggaggtgct cagcgacctg 3840
tgtgctccttt ccctgctctg acgcgtgttcg cagagttgatt atcccacctc tcccaaccoc 3900
gcgcactttaaaaaa aaaaaaagag attgcaacct cgggctgttt gcggagccat gcggagccga 3960
ggggggagct gcgtggccca gttgctcctgtt aggacaaaaag aatatttatt ctttacatat 4020
attgcacgac cccaggggcgg ccctcctcctt atactggggc agaagaagagc tagccttttg tggtttctgta 4080
tgtgctttct tatttttttt aatagattttt cctcttgcag cacaagcaaa aaaaaaagtt 4140
ggggggctag ccccaagcgg gcttgggttt cggccactaca agccctcctg tggccactagt 4200
gatcctaaa gttccctcag cagagaattc gaggggggaa tggagctcct cggattttctc 4260
agagatttcat ttttggtgta cactttctaa aaaaatgtttt aaaaaagact cccgctcag 4320
gttccacgc ttaaacgtgca gttgctcctg atggctgcttg ggcactctacg 4380
cagagttcgg gactctttcttg gtttctctca gggcagcccc gacttcgagc gcacatctgg 4440
gtgtaagaaggc aatgagacca cctgctcact caggctttgc gcggctcagc attggtctct 4500
tgtgctttct ggggagaaaa tcggctaccc ctttcttttt aaaaaaaaag acactaatatt 4560
gccacactct gtgcgtattt ttttggtcctc cttaaatcact attttctcgg gcaaattacc 4620
tcaaatctag ttttttgattc cccaggttca aatgagact aatattacg tggccttttct 4680
-continued

gttgtcggg tttcaacaag cattcagccaa aagacaaaaa gagatgcag gacagattcc 4740
aggggtgcg gagcacaactgt tgggaccccg ctcacctctgg cagcagntgtt aggaaccgcg 4800
caaaatccc cactctccag caagctcgta tttaaaaaag gtttttccttt tttctacaatt 4860
ataggtccaa aaggacacaa agtttctcaag gacactccag ccctgtgctca cctgtcagcc 4920
cagggtcggg gcaggggagg gcagactccag cagactgcgc ccctgtgctca cagcttgctg 4980
tggtctccgg gcagagctcgc gcttcctcatt tgtccagggg agctgtgcggg gcttcctcctc 5040
ctcttcaccc tctggcgacct tccagctggg tgtccacacat gttggattc gcctccacca 5100
cactctccac gcaggggagaa ctcgtcggg ctaagggcgc ccctgtgctca ccctgtgctg 5160
aggggtcctc caagcttcag gcgaggagggc gcagctcggt gcagcttgctg gcagcttgcgg 5220
tgctctttct cccacocctgg tgtccacatgt ccctgtgcgg gcctccacca ctggctagatg 5280
agaggtctaa aagcatctccag taagggcctgt tgtccatata cggactaatag gcagcttgaag 5340
tggtctccgg gcagagctcgc ccctgcacccag cagacccgag gagagctgctc cctgtcattaa 5400
agaggtctgt tttgcttttt tgtcctcattta gaggactgcg agacagcctc tctctgcgct 5460
tgatgctct ctcacgcag gcctttta tgctccaccc acctccagggg aacacgcctc cctgtgacgt 5520
tggtcggccg tgtcttgagg gcagagctcgc ccctgtgctca ccctgtgctg gcagcttgcgg 5580
atggagacttg tgtccacaag gcagctccag ccacactcctc ccctgtgctc ccctgtgctc 5640
ccatgtcaca aatacctgcag ccctgctctgg gaaagcttca cctgtagagct gcagcttgcgg 5700
cctggctttc ctcagctggg gcagacgcctc cttgggttcgc cctgtgctca ccctgtgctc 5760
cctccacca caaggggagaa ctcgtcggg ctaagggcgc ccctgtgctca ccctgtgctg 5820
aggggtcctc caagcttcag gcgaggagggc gcagctcggt gcagcttgctg gcagcttgcgg 5880
hcggggtctgg gcggcttggag gcagagctcgc ccctgtgctca ccctgtgctg gcagcttgcgg 5940
tcctccacca gcctcctcctc ctctccctcctc tctgtcctgg gcgaggagaag cctccgagag 6000
gcagactctc cagagagctc ccacacagtct gcagattgaaa gatagtcaag ccagagcgcg 6060
cagagctggtt cccagcccac ccctgctctttt ccctgtgctg gcgaggaggtt tttgctttcct 6120
tgttcgcttc caagagctcgc ccctgcacccag cctccatattt cccacocctgc tctgtcttgc 6180
cacatctgct acttcoccg gcctatccag attggggttt tttggcttct gctccaggtg 6240
ccactctctc cttggagactt ttcacactgaa ctcctgctttt aaactagtatt ctggtgcttc 6300
acccagcgcgc cccaggttcag ccctgggtcag gcgagcgtctg gctttgtgctgt 6360
gggggtccttc tggggtctttt gcagagctcgc ccctgtgctca ccctgtgctg gcagcttgcg 6420
gtgggtctct cccacttcag gcaggtcttgc cccactccac gcaggtcttgc cccactccac gcaggtcttggc 6480
gtccagcttc gctccctgcc cccacaaaaa atgttcctgtt ctttccttcaca 6540
ccagctggtt cccaggttcag ccctgtgctca ccctgtgctc ccctgtgctg gcagcttgcg 6600
cctgtcgggt ttgggtctttt gcagagctcgc ccctgtgctca ccctgtgctg gcagcttgcg 6660
cctcctggc gtttctgcag gcgaggaggtt cccaggttcag ccctgtgctc ccctgtgctc 6720
cctgagctcgt gcgagctcgc ccctgtgctca ccctgtgctg gcagcttgcg 6780
atgctgtgttc cttgggtctttt gcagagctcgc ccctgtgctca ccctgtgctg gcagcttgcg 6840
aagaggtgta tggagacttc gcagagctcgc ccctgtgctca ccctgtgctg gcagcttgcg 6900
aagaggtgta cccaggttcag ccctgtgctc ccctgtgctg gcagcttgcg 6960
<table>
<thead>
<tr>
<th>Met</th>
<th>Val</th>
<th>Ala</th>
<th>Leu</th>
<th>Asp</th>
<th>Thr</th>
<th>Glu</th>
<th>Ser</th>
<th>Pro</th>
<th>Leu</th>
<th>Cys</th>
<th>Pro</th>
<th>Leu</th>
<th>Ser</th>
<th>Pro</th>
<th>Leu</th>
<th>Glu</th>
<th>Ala</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>11</td>
<td>12</td>
<td>13</td>
<td>14</td>
<td>15</td>
<td>16</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>Gly</td>
<td>Asp</td>
<td>Leu</td>
<td>Gly</td>
<td>Ser</td>
<td>Leu</td>
<td>Ser</td>
<td>Glu</td>
<td>Phe</td>
<td>Leu</td>
<td>Gln</td>
<td>Glu</td>
<td>Met</td>
<td>Gly</td>
<td>20</td>
<td>25</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>Asn</td>
<td>Ile</td>
<td>Gin</td>
<td>Glu</td>
<td>Ile</td>
<td>Ser</td>
<td>Gin</td>
<td>Ser</td>
<td>Ile</td>
<td>Gly</td>
<td>Glu</td>
<td>Asp</td>
<td>Ser</td>
<td>Ser</td>
<td>Gly</td>
<td>Ser</td>
<td>40</td>
<td>45</td>
</tr>
<tr>
<td>Phe</td>
<td>Gly</td>
<td>Phe</td>
<td>Thr</td>
<td>Gly</td>
<td>Tyr</td>
<td>Gln</td>
<td>Tyr</td>
<td>Leu</td>
<td>Gly</td>
<td>Ser</td>
<td>Cys</td>
<td>Pro</td>
<td>Gly</td>
<td>Ser</td>
<td>50</td>
<td>55</td>
<td>60</td>
</tr>
<tr>
<td>Gly</td>
<td>Ser</td>
<td>Val</td>
<td>Ile</td>
<td>Thr</td>
<td>Asp</td>
<td>Thr</td>
<td>Leu</td>
<td>Ser</td>
<td>Pro</td>
<td>Ala</td>
<td>Ser</td>
<td>Pro</td>
<td>Ser</td>
<td>Ser</td>
<td>70</td>
<td>75</td>
<td>80</td>
</tr>
<tr>
<td>Val</td>
<td>Thr</td>
<td>Tyr</td>
<td>Pro</td>
<td>Val</td>
<td>Pro</td>
<td>Gly</td>
<td>Ser</td>
<td>Val</td>
<td>Asp</td>
<td>Glu</td>
<td>Ser</td>
<td>Pro</td>
<td>Ser</td>
<td>Gly</td>
<td>Ser</td>
<td>90</td>
<td>95</td>
</tr>
<tr>
<td>Ala</td>
<td>Leu</td>
<td>Asn</td>
<td>Ile</td>
<td>Glu</td>
<td>Cys</td>
<td>Arg</td>
<td>Ile</td>
<td>Cys</td>
<td>Gly</td>
<td>Asp</td>
<td>Lys</td>
<td>Ala</td>
<td>Ser</td>
<td>Gly</td>
<td>Tyr</td>
<td>100</td>
<td>105</td>
</tr>
<tr>
<td>His</td>
<td>Tyr</td>
<td>Gly</td>
<td>Val</td>
<td>His</td>
<td>Ala</td>
<td>Cys</td>
<td>Glu</td>
<td>Gly</td>
<td>Cys</td>
<td>Lys</td>
<td>Gly</td>
<td>Phe</td>
<td>Phe</td>
<td>Arg</td>
<td>Arg</td>
<td>120</td>
<td>125</td>
</tr>
<tr>
<td>Thr</td>
<td>Ile</td>
<td>Arg</td>
<td>Leu</td>
<td>Lys</td>
<td>Leu</td>
<td>Val</td>
<td>Tyr</td>
<td>Asp</td>
<td>Lys</td>
<td>Cys</td>
<td>Asp</td>
<td>Arg</td>
<td>Ser</td>
<td>Cys</td>
<td>Lys</td>
<td>140</td>
<td>145</td>
</tr>
<tr>
<td>Cys</td>
<td>Leu</td>
<td>Ser</td>
<td>Val</td>
<td>Gly</td>
<td>Met</td>
<td>Ser</td>
<td>His</td>
<td>Ala</td>
<td>Ile</td>
<td>Arg</td>
<td>Phe</td>
<td>Gly</td>
<td>Arg</td>
<td>Met</td>
<td>160</td>
<td>170</td>
<td>175</td>
</tr>
<tr>
<td>Pro</td>
<td>Arg</td>
<td>Ser</td>
<td>Glu</td>
<td>Lys</td>
<td>Ala</td>
<td>Lys</td>
<td>Leu</td>
<td>Ala</td>
<td>Glu</td>
<td>Ile</td>
<td>Leu</td>
<td>Thr</td>
<td>Cys</td>
<td>Glu</td>
<td>190</td>
<td>195</td>
<td>200</td>
</tr>
<tr>
<td>Arg</td>
<td>Ile</td>
<td>Tyr</td>
<td>Glu</td>
<td>Ala</td>
<td>Tyr</td>
<td>Leu</td>
<td>Lys</td>
<td>Arg</td>
<td>Met</td>
<td>Arg</td>
<td>Amn</td>
<td>Leu</td>
<td>Met</td>
<td>Arg</td>
<td>Amn</td>
<td>Lys</td>
<td>Val</td>
</tr>
<tr>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>210</td>
<td>215</td>
<td>220</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ala</td>
<td>Arg</td>
<td>Val</td>
<td>Ile</td>
<td>Leu</td>
<td>Ser</td>
<td>Gly</td>
<td>Lys</td>
<td>Ala</td>
<td>Ser</td>
<td>Amn</td>
<td>Amn</td>
<td>Pro</td>
<td>Pro</td>
<td>Phe</td>
<td>Val</td>
<td>225</td>
<td>230</td>
</tr>
<tr>
<td>Ile</td>
<td>His</td>
<td>Asp</td>
<td>Met</td>
<td>Glu</td>
<td>Thr</td>
<td>Leu</td>
<td>Cys</td>
<td>Met</td>
<td>Ala</td>
<td>Glu</td>
<td>Lys</td>
<td>Thr</td>
<td>Leu</td>
<td>Val</td>
<td>Ala</td>
<td>245</td>
<td>250</td>
</tr>
<tr>
<td>Lys</td>
<td>Leu</td>
<td>Val</td>
<td>Ala</td>
<td>Asn</td>
<td>Gly</td>
<td>Ile</td>
<td>Glu</td>
<td>Asn</td>
<td>Lys</td>
<td>Glu</td>
<td>Ala</td>
<td>Glu</td>
<td>Val</td>
<td>Arg</td>
<td>Ile</td>
<td>260</td>
<td>265</td>
</tr>
<tr>
<td>Phe</td>
<td>His</td>
<td>Cys</td>
<td>Cys</td>
<td>Glu</td>
<td>Cys</td>
<td>Thr</td>
<td>Ser</td>
<td>Val</td>
<td>Glu</td>
<td>Thr</td>
<td>Val</td>
<td>Thr</td>
<td>Glu</td>
<td>Leu</td>
<td>Thr</td>
<td>275</td>
<td>280</td>
</tr>
<tr>
<td>Glu</td>
<td>Phe</td>
<td>Ala</td>
<td>Lys</td>
<td>Ala</td>
<td>Ile</td>
<td>Pro</td>
<td>Gly</td>
<td>Phe</td>
<td>Ala</td>
<td>Asn</td>
<td>Leu</td>
<td>Asp</td>
<td>Leu</td>
<td>Asn</td>
<td>Asp</td>
<td>290</td>
<td>295</td>
</tr>
<tr>
<td>Glu</td>
<td>Val</td>
<td>Thr</td>
<td>Leu</td>
<td>Leu</td>
<td>Lys</td>
<td>Tyr</td>
<td>Val</td>
<td>Tyr</td>
<td>Glu</td>
<td>Ala</td>
<td>Ile</td>
<td>Phe</td>
<td>Ala</td>
<td>Met</td>
<td>305</td>
<td>310</td>
<td>315</td>
</tr>
<tr>
<td>Leu</td>
<td>Ser</td>
<td>Ser</td>
<td>Val</td>
<td>Met</td>
<td>Arg</td>
<td>Lys</td>
<td>Met</td>
<td>Leu</td>
<td>Ala</td>
<td>Val</td>
<td>Tyr</td>
<td>Gly</td>
<td>Amn</td>
<td>325</td>
<td>330</td>
<td>335</td>
<td></td>
</tr>
<tr>
<td>Gly</td>
<td>Phe</td>
<td>Ile</td>
<td>Thr</td>
<td>Arg</td>
<td>Glu</td>
<td>Phe</td>
<td>Leu</td>
<td>Lys</td>
<td>Ser</td>
<td>Leu</td>
<td>Arg</td>
<td>Lys</td>
<td>Pro</td>
<td>Phe</td>
<td>Cys</td>
<td>340</td>
<td>345</td>
</tr>
<tr>
<td>Asp</td>
<td>Ile</td>
<td>Met</td>
<td>Glu</td>
<td>Pro</td>
<td>Lys</td>
<td>Phe</td>
<td>Asp</td>
<td>Phe</td>
<td>Ala</td>
<td>Met</td>
<td>Lys</td>
<td>Phe</td>
<td>Asn</td>
<td>Ala</td>
<td>Leu</td>
<td>355</td>
<td>360</td>
</tr>
<tr>
<td>Glu</td>
<td>Leu</td>
<td>Asp</td>
<td>Asp</td>
<td>Ser</td>
<td>Asp</td>
<td>Ile</td>
<td>Ser</td>
<td>Leu</td>
<td>Phe</td>
<td>Val</td>
<td>Ala</td>
<td>Ile</td>
<td>Ile</td>
<td>Cys</td>
<td>370</td>
<td>375</td>
<td>380</td>
</tr>
<tr>
<td>Cys</td>
<td>Gly</td>
<td>Asp</td>
<td>Arg</td>
<td>Pro</td>
<td>Gly</td>
<td>Leu</td>
<td>Leu</td>
<td>Asn</td>
<td>Val</td>
<td>Gly</td>
<td>His</td>
<td>Ile</td>
<td>Glu</td>
<td>Lys</td>
<td>Met</td>
<td>385</td>
<td>390</td>
</tr>
<tr>
<td>Gln</td>
<td>Glu</td>
<td>Gly</td>
<td>Ile</td>
<td>Val</td>
<td>His</td>
<td>Val</td>
<td>Leu</td>
<td>Arg</td>
<td>Leu</td>
<td>His</td>
<td>Leu</td>
<td>Gln</td>
<td>Ser</td>
<td>Asn</td>
<td>His</td>
<td>405</td>
<td>410</td>
</tr>
<tr>
<td>Pro</td>
<td>Asp</td>
<td>Arg</td>
<td>Ile</td>
<td>Phe</td>
<td>Leu</td>
<td>Phe</td>
<td>Pro</td>
<td>Lys</td>
<td>Leu</td>
<td>Gln</td>
<td>Met</td>
<td>Ala</td>
<td>Asp</td>
<td>420</td>
<td>425</td>
<td>430</td>
<td></td>
</tr>
<tr>
<td>Leu</td>
<td>Arg</td>
<td>Gln</td>
<td>Leu</td>
<td>Val</td>
<td>Thr</td>
<td>Glu</td>
<td>His</td>
<td>Ala</td>
<td>Gln</td>
<td>Leu</td>
<td>Val</td>
<td>Gln</td>
<td>Ile</td>
<td>Ile</td>
<td>Lys</td>
<td>435</td>
<td>440</td>
</tr>
<tr>
<td>Lys</td>
<td>Thr</td>
<td>Glu</td>
<td>Ser</td>
<td>Asp</td>
<td>Ala</td>
<td>Ala</td>
<td>Leu</td>
<td>His</td>
<td>Pro</td>
<td>Leu</td>
<td>Leu</td>
<td>Glu</td>
<td>Ile</td>
<td>Tyr</td>
<td>450</td>
<td>455</td>
<td>460</td>
</tr>
<tr>
<td>Arg</td>
<td>Asp</td>
<td>Met</td>
<td>Tyr</td>
<td>465</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<210> SEQ ID NO: 37
<211> LENGTH: 1863
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 37
actgatgcct tgactctagg gttgattcac aatctctgct acctcaagct tttttcctttt 60
aaccagtaga ttttttgtca gatagagaca aatsctagg gttgattcag gcaascctct 120
atctctggg catctctggg aatcctctggg attctctctc attgaccagc aaagccgttc 180
tctgctcgatt aacgtcttgct acacagatct acacagatct acacagatct acacagat 240
gccatttgg cccaccaact tttggatcag ctcgctgtag ctcgctgtaa tgggaccaca 300
tctctacccc tttgatacct aaagctctaca tactgtgtcct tttccoccaaa cttactcct 360
acactgaag gacatcctata tcatacagca agatcagctt gttgattagc acaagtatt 420
cctagaaactt caagagcata caaagctcaat caaagctcaat cttgctcctc caccttat 480
tttcagagag actctgctct actataagcc tctatgacag ctcattacact ctcattagggc 540
<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>aatgaagtc cgtcgtcgtg gagataaagc ttcgatttt cactatgagag ttcgattt</td>
<td>600</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tcggaggag cacaggttctct ccggagagc actcagagtyg aagctttac catgcagctg</td>
<td>660</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tgattcattaccttct ctcggagatgcc aaaaaaag tagaaatatt gtcagctact gtcggttc</td>
<td>720</td>
<td></td>
<td></td>
</tr>
<tr>
<td>gagattgctgtatgaggtggag tggctctaaa ttggactcaggg ttggagggg aaaagagagggc</td>
<td>780</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cggagggag aagctgctctg ccgagatctc cagtgatctc gaccagcgtga atccagagtc</td>
<td>840</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cgtgagcct ttggggtcgcc caaaccatatt gatgactca tcatatagat ctcctcgcgt</td>
<td>900</td>
<td></td>
<td></td>
</tr>
<tr>
<td>gacaaacgca aagggagagg cgtatgttgcag agaaagaaca acagacaat cccattcgt</td>
<td>960</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tattcatgac atgaattctc taatgatggagg agaagataaa atcaagtt caacacatc</td>
<td>1020</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ccccctcgc gagccagacac aagagttgtcgc catccgcatgt ttcgagggtgc gtcggttgc</td>
<td>1080</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ctcctgtagctgtatgagggct gatagcagaa gatagcggagc atgagagctcgt gtgggttgaat</td>
<td>1140</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tgttaggctt taactctctc caaattaggg gcgcgagac gactacagac aacatcac</td>
<td>1200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aatgctgctttttgtagta ataaagatgcg gctttttcata ttgagggggc aagctttcatt</td>
<td>1260</td>
<td></td>
<td></td>
</tr>
<tr>
<td>gacaaagggag tttttgaaat gctctgcaggg aagtttcttg cgatctttag ggcagcaagtt</td>
<td>1320</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tgaagttgtgc tgcagactctactgctgttgagagctg aagagcttttgatgtaagagc acaatcattt</td>
<td>1380</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tctgtctacttctctggttg ctgacggcgcc aaggtttgctg atgtagcagct tttataaag</td>
<td>1440</td>
<td></td>
<td></td>
</tr>
<tr>
<td>catgcaacag aacgtgctacg aagcctgtgga gtcggcagctg aagcttgacgc acctgtgact</td>
<td>1500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ctcgactctt tttgacagag ctaattcagagc aagagctctgctc acctgagctcctgagga</td>
<td>1560</td>
<td></td>
<td></td>
</tr>
<tr>
<td>acagtggagc ctgtagcagct gttgataaa gcagagagca aagttggtcct tttataaag</td>
<td>1620</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ccctttctctt ttcgtgaggg aacagttgacac aatcacagactc tttcagaaat tttcagaaat</td>
<td>1680</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aagtccatattt aaaaaaaataaagtttagaat tttgcttttct tatattgttatctttatatataa</td>
<td>1740</td>
<td></td>
<td></td>
</tr>
<tr>
<td>agacacattt cacatctactt ttaaatatat aataattcaaa tattttagaaaaa aaaaaaaa</td>
<td>1800</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aaa</td>
<td></td>
<td></td>
<td>1863</td>
</tr>
</tbody>
</table>

<210> SEQ ID NO 38
<211> LEMMTH: 505
<212> TYPHR: PET
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 38

<table>
<thead>
<tr>
<th>Met</th>
<th>Gly</th>
<th>Glu</th>
<th>Thr</th>
<th>Leu</th>
<th>Gly</th>
<th>Aep</th>
<th>Ser</th>
<th>Pro</th>
<th>Ile</th>
<th>Asp</th>
<th>Pro</th>
<th>Glu</th>
<th>Ser</th>
<th>Aep</th>
<th>Ser</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>10</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phe</td>
<td>Thr</td>
<td>Asp</td>
<td>Thr</td>
<td>Leu</td>
<td>Ser</td>
<td>Ala</td>
<td>Asn</td>
<td>Ile</td>
<td>Ser</td>
<td>Gln</td>
<td>Glu</td>
<td>Met</td>
<td>Thr</td>
<td>Met</td>
<td>Val</td>
</tr>
<tr>
<td>20</td>
<td>25</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amp</td>
<td>Thr</td>
<td>Glu</td>
<td>Met</td>
<td>Pro</td>
<td>Phe</td>
<td>Trp</td>
<td>Pro</td>
<td>Thr</td>
<td>Asn</td>
<td>Phe</td>
<td>Gly</td>
<td>Ile</td>
<td>Ser</td>
<td>Ser</td>
<td>Val</td>
</tr>
<tr>
<td>35</td>
<td>40</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amp</td>
<td>Leu</td>
<td>Ser</td>
<td>Val</td>
<td>Met</td>
<td>Glu</td>
<td>Aep</td>
<td>His</td>
<td>Ser</td>
<td>His</td>
<td>Ser</td>
<td>Phe</td>
<td>Asp</td>
<td>Ile</td>
<td>Lys</td>
<td>Pro</td>
</tr>
<tr>
<td>50</td>
<td>55</td>
<td>60</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phe</td>
<td>Thr</td>
<td>Thr</td>
<td>Val</td>
<td>Asp</td>
<td>Phe</td>
<td>Ser</td>
<td>Ser</td>
<td>Ile</td>
<td>Ser</td>
<td>Thr</td>
<td>Thr</td>
<td>Pro</td>
<td>His</td>
<td>Tyr</td>
<td>Glu</td>
</tr>
<tr>
<td>65</td>
<td>70</td>
<td>75</td>
<td>80</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ile</td>
<td>Pro</td>
<td>Phe</td>
<td>Thr</td>
<td>Arg</td>
<td>Thr</td>
<td>Asp</td>
<td>Pro</td>
<td>Val</td>
<td>Val</td>
<td>Ala</td>
<td>Amp</td>
<td>Tyr</td>
<td>Lys</td>
<td>Tyr</td>
<td>Asp</td>
</tr>
<tr>
<td>85</td>
<td>90</td>
<td>95</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu</td>
<td>Lys</td>
<td>Leu</td>
<td>Glu</td>
<td>Tyr</td>
<td>Gln</td>
<td>Ser</td>
<td>Ala</td>
<td>Ile</td>
<td>Lys</td>
<td>Val</td>
<td>Glu</td>
<td>Pro</td>
<td>Ala</td>
<td>Ser</td>
<td>100</td>
</tr>
<tr>
<td>105</td>
<td>110</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro Pro Tyr Tyr Ser Glu Lys Thr Gin Leu Tyr Asn Lys Pro His Glu</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>115</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>125</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu Pro Ser Asn Ser Leu Met Ala Ile Glu Cys Arg Val Cys Gly Asp</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>130</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>135</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>140</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys Ala Ser Gly Phe His Tyr Gly Val His Ala Cys Glu Gly Cys Lys</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>145</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>150</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>155</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>160</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gly Phe Phe Arg Arg Thr Ile Arg Leu Lys Leu Ile Tyr Asp Arg Cys</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>165</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>170</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>175</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asp Leu Asn Cys Arg Ile His Lys Lys Ser Arg Asn Lys Cys Gin Tyr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>180</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>185</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>190</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cys Arg Phe Gin Lys Cys Leu Ala Val Gly Met Ser His Asn Ala Ile</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>195</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>205</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg Phe Gly Arg Met Pro Gin Ala Glu Lys Gln Lys Leu Leu Ala Glu</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>210</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>215</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>220</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ile Ser Ser Asp Ile Asp Gin Leu Asn Pro Glu Ser Ala Asp Leu Arg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>225</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>230</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>235</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>240</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ala Leu Ala Lys His Leu Tyr Asp Ser Tyr Ile Lys Ser Phe Pro Leu</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>245</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>250</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>255</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thr Lys Ala Lys Ala Arg Ala Ile Leu Thr Gly Lys Thr Thr Asp Lys</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>260</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>265</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>270</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ser Pro Phe Val Ile Tyr Asp Met Asn Ser Leu Met Met Gly Glu Asp</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>275</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>280</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>285</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys Ile Lys Phe Lys His Ile Thr Pro Leu Gin Glu Gin Ser Lys Glu</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>290</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>295</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val Ala Ile Arg Ile Phe Gin Gly Cys Gin Phe Arg Ser Val Glu Ala</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>305</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>310</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>315</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>320</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val Gin Glu Ile Thr Glu Tyr Tyr Ala Lys Ser Ile Pro Gly Phe Val Asn</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>325</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>335</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu Asp Leu Asn Asp Gin Val Thr Leu Leu Lys Tyr Gly Val His Glu</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>340</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>345</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>350</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ile Ile Tyr Thr Met Leu Ala Ser Leu Met Asn Lys Asp Gin Val Leu</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>355</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>360</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>365</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ile Ser Gin Gly Gin Lys Gly Gin Met Thr Arg Gin Phe Leu Lys Ser Leu</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>370</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>375</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>380</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg Lys Pro Phe Gin Phe Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>385</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>390</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>395</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>400</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys Phe Asn Ala Lea Gin Leu Gin Asp Ser Asp Leu Ala Ile Phe Ile</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>405</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>410</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>415</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ala Val Ile Ile Leu Ser Gin Asp Arg Pro Gly Leu Leu Leu Gin Val</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>420</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>425</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>430</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro Ile Gin Gin Gin Asp Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>435</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>440</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>445</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu Lys Leu Asn His Pro Gin Ser Ser Gin Leu Phe Ala Lys Leu Leu</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>450</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>455</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>460</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gin Gin Met Thr Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>465</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>470</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>475</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>480</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu Gin Val Ile Lys Lys Thr Gin Thr Gin Gin Gin Gin Gin Gin Gin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>485</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>490</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>495</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu Gin Gin Lys Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>495</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>505</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

-continued
-continued

gggagagtsg tgacgctgag gcggcgaggg aacctggaat taaaggccaa agttttgca 60
ggaagcggag aatttgccgg aagcttgggg aagccgaggg ggcccgcga gaagaggggg 120
aacaattgtg aacgattttt gggtactgaa gttcactca aacgagtgtc ttcgcttcacc 180
aacaagagaa gacaaggtca ccaacaggcc ttgaggggac ccccccgttg agttctcacc 240
gcgctactgg cgacaagctg ccagagctat gactgggctt gcaggtggtg gcccgagggg 300
agatcagca tcggagcgcg acagagggaa gccctctggg tcgggagaga gggyggagaa 360
agggagagcg cgccccccctg gggccgacc cagcgcgac gcctggggcc gctagcagctt 420
cctctcagac gctacaccag ccctctccgg agctcttgcg caocctcact gcctgggaacc 480
cggactcatcg gctctggaag gcctttcatgc gcagccgcct gcggggagct gcggggagct 540
ggggagacag ccctccgctc gtcagcagtt ggctctagtg gctggggcag ccagaggttc 600
ttcgctgctg cgatctgcaat gcagccctgc atcagcaact cctgcagcag ctgcagatgg 660
cagaagagag ccacgactcg ctggccccgc gctcccgttc cagagccgct gcctggggcc 720
agtgccatcg ccagcttggg ttttggccct gccggagcag gcggctgtcg gcctggagct 780
ggcgggcgct ccgctcagcgg tggggagac cagcgcgcac cctgcgagcc gctggggagc 840
ttcacaccag ccctcagcgg agtcttgcct ttcggggagg ttggagttgg ctgcagctgg 900
cacgcgctcc tcacccggccc aagcgcgctc ccgctcaggt ccggccttcc gcacgctcg 960
acattgttgcc agggagcggag ggccggtggt ggtgacgctg tggccggcag ccctgcctcc 1020
tacagagagcg ccctcctctc cctgctgcag gcacacgcct gcagagcttg gcgcagcttg 1080
gggagcctcg ctgctgttcg ccagacacag ccagctgctc gcggccttcc ccctgcgctc 1140
caggtttacct ttctaatgta ttcgctgcaac gggccctctt gctgcgctgt gcgcctcttc 1200
gcgctcagcc gggagctgct gcgtgagcgc gcaggtct gcgtgcttcg gcgtgtccttg 1260
cgcgctctc gcacccctc cagcctagct atgctgctct gcgctctctc gctgcgtcctg 1320
ttcacccgccc tggaaacttga tgcgctggac gctgcgtcct tccagctggt ccctgcgcc 1380
tggtggagcc gcgcgtgatt gcagagcttg ccagggagtt aggctagcct ccagacaccc 1440
cggtgctcg ccagagcttc ccggccggag atggcctgct gcgctctttc gctgccttcg 1500
aacagctgcc tgcacctggg ccacgctgca ccagaggtag ccagagcacc ccaggttccg 1560
cacgccgcca agaagccacc aacgcggact ctgctgcacc ctcggccttg gcagctcagt 1620
aagggagggc actacagccg gcagccggag cctgcctagc acctaaaagt gcggcgcaat 1680
gagggggccc accaccacgc cttttcctct gcacccgttc tggagctccc gcacgggacc 1740
gagccagcgg ccagctgacc gcacgcgctc gctctgctcc tggagctccc 1800
tttccctcc tttcctctct ccagctgccag tctgcgctct cttttcctcc 1860
tctttcctct ttttcctcct cctgtgcctc tttttcttct ttctttctct 1920
tctttcctct cttctctctct tttttctctct ctttcttctct cttttctttct 1980
ttggagatttg ttgggatttt cttacccgcc gcataagac gcacgctgct ttttgagccc 2040
cggagcgagag agaagtaagg gcgggagtgg cttctccctgc ccctcccagc gcacgctgac 2100
-continued

gctagctc tccttgcttt cctctgtcct cagacgaaaa gactagaca cttcaagaa 2160
gactctctag ctttaggtcg gcggctgagg gactgctgag ctgtactgtg cttgacgta 2220
gcccctctata gctctggtct gctttgctca aagagaagtg ggcagaggcc cccagagtga 2280
gagcggagtg ccccccagag actgtcattg cccctcgcatt gctgggagcc cccctcga 2340
cacactgatt ccgtccacga gcacactggc gcacactggc cccctcagtg cccctcagat 2400
tccgtctgt tcgggtgggc aatgtggtgt agtggcgcct cgtgccagcgc ctggaaaggc 2460
tcctccagcg tggaggtcag gcaggggtcc cttccaggtgg gcococaccc cactgaagag 2520
gagcgagttt caagagagag aagggggttt ggctgggtga ggaagcagcag cccctcaattt 2580
tgccctagc actgggggttc tggtctctgg ggcctcaggg ggagaggtcc ggccgagggc 2640
tgccgctgag actccctcctgc ccctcgtgcgc caggagacgc gcagccacctt gttgcagct 2700
cgggctccgg gcggctggct cacatccccc ttcgggcttt tcgtgcagcc gcagccaggg 2760
tcggccaggg actcccctctgg aaagtcgccc cccctccacac acacataagc actgaaatc 2820
cctccctctgc aggcgcctctag cccctcctct cccctccttg gggagggtgag aacagcaga 2880
gagccggtctg caggcagggg gcggcaggtc ctggctgggg gcggcgagcg ctgggggttcg 2940
cagggcggtg ttgggctgag cgcagcctgc ccctgcttggg gcggcgagcgt gtaacagttg 3000
gcgtctcctgt tcgggtgcttc atttgtctca cagctgctgct tcgggtccctt cttctggtta 3060
tcggcagcgt caggtctccc cggaggctgt gtcgcaggtcc cgccttggtt ggcacgggt 3120
tgggagaga cccacagccc gccgctggtgg ccctcggctgc gggggtcgg ccctcggctgc 3180
acctctagc agggccagca cccacagctg gctcccaggt gcagcggagc gcacggccctc 3240
gcggcccctg ccaggtctctg ggccttctgt gcgggtcggc gcggaggtcg gacaagctgg 3300
gccggccgat agggggtctcg gccgctggtgg cccctcctct gcgcaggtcg cccctccctt 3360
gtcgggtatt cggctggtcg ccggccggtg cttcggctct cccagggcgt ggtccggctg 3420
gccgggagc cactgccagt gcggcagggt tctccggcgc cgcctcagcg cagctggagt 3480
gggtgggtgg ggtcctccgt ggcggcaggg taggtgctgg cagccagcct gcctcggcct 3540
ccccctgcgg gcagccctccgc ggcaggcggt gcggcctttgc gctggcggc gaggcagaga 3600
tgcgacctgc tgccggccat cttccggag ctttcttgtgc tagctggtgc gcggccggcc 3660
gttctccgtg cttttatata aataaggtgt aagagctgtga caagagccgg gagaatctta 3720
actataa ccgg

<210> SEQ ID NO: 40
<211> LENGTH: 441
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 40
Met Glu Gin Pro Gin Glu Glu Ala Pro Glu Val Arg Glu Glu Glu Glu 1  5  10  15
Lys Glu Glu Val Ala Glu Ala Glu Glu Ala Pro Glu Leu Asn Gly Gly Gly 20  25  30
Pro Gin His Ala Leu Pro Ser Ser Ser Tyr Thr Thr Asp Leu Ser Arg Ser 35  40  45
 Ser Ser Pro Pro Ser Leu Leu Asp Gin Leu Glu Met Gly Cys Asp Gly 50  55  60
-continued

Ala Ser Cys Gly Ser Leu Asn Met Glu Cys Arg Val Cys Gly Asp Lys
65 70 75 80

Ala Ser Gly Phe His Tyr Gly Val His Ala Cys Gly Gly Cys Lys Gly
85 90 95

Phe Phe Arg Arg Thr Ile Arg Met Lys Leu Glu Tyr Glu Lys Cys Glu
100 105 110

Arg Ser Cys Lys Ile Gln Lys Lys Asn Arg Asn Lys Cys Gln Tyr Cys
115 120 125

Arg Phe Gin Lys Cys Leu Ala Leu Gly Met Ser His Asn Ala Ile Arg
130 135 140

Phe Gly Arg Met Pro Glu Ala Glu Lys Arg Ile Lys Leu Val Ala Gly Leu
145 150 155 160

Thr Ala Asn Glu Gly Ser Gin Tyr Asn Pro Gin Val Ala Asp Leu Lys
165 170 175

Ala Phe Ser Lys His Ile Tyr Asn Ala Tyr Leu Lys Asn Phe Asn Met
180 185 190

Thr Lys Lys Ala Arg Ser Ile Leu Thr Gly Lys Ala Ser His Thr
195 200 205

Ala Pro Phe Val Ile His Asp Ile Glu Thr Leu Trp Gin Ala Glu Lys
210 215 220

Gly Leu Val Trp Lys Gin Leu Val Asn Gly Leu Pro Pro Tyr Lys Glu
225 230 235 240

Ile Ser Val His Val Phe Tyr Arg Cys Gin Cys Thr Thr Val Glu Thr
245 250 255

Val Arg Glu Leu Thr Glu Phe Ala Lys Ser Ile Pro Ser Phe Ser Ser
260 265 270

Leu Phe Leu Asn Asp Gin Val Thr Leu Leu Lys Tyr Gly Val His Glu
275 280 285

Ala Ile Phe Ala Met Leu Ala Ser Ile Val Asn Lys Asp Gly Leu Leu
290 295 300

Val Ala Asn Gly Ser Gly Phe Val Thr Arg Glu Phe Leu Arg Ser Leu
305 310 315 320

Arg Lys Pro Phe Ser Asp Ile Ile Glu Pro Lys Phe Glu Phe Ala Val
325 330 335

Lys Phe Asn Ala Leu Glu Leu Asp Asp Ser Leu Ala Leu Phe Ile
340 345 350

Ala Ala Ile Ile Leu Cys Gly Asp Arg Pro Gly Leu Met Asn Val Pro
355 360 365

Arg Val Glu Ala Ile Glu Asp Thr Ile Leu Arg Ala Leu Glu Phe His
370 375 380

Leu Gin Ala Asn His Pro Asp Ala Gin Tyr Leu Phe Pro Lys Leu Leu
385 390 395 400

Gln Lys Met Ala Asp Leu Arg Gin Leu Val Thr Glu His Ala Gin Met
405 410 415

Met Gin Arg Ile Lys Tyr Thr Glu Thr Glu Thr Ser Leu His Pro Leu
420 425 430

Leu Gin Glu Ile Tyr Lys Asp Met Tyr
435 440

<210> SEQ ID NO 41
<211> LENGTH: 21
1. A method of modulating adipocyte differentiation or adipogenic gene or gene product expression or lipolytic gene or gene product expression, comprising administering a therapeutically effective amount of oleuropein or an analogue, derivative, or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside or their analogue, derivative, or any combination thereof including but not limited to olive leaf extract to a mammalian subject in need thereof.

2. A method of treating, controlling or preventing obesity, or of reducing body weight, or of inhibiting fat accumulation in vivo, or of promoting fat burning in vivo, comprising administering a therapeutically effective amount of oleuropein or an analogue, derivative or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside or their analogue, derivative, or any combination thereof including but not limited to olive leaf extract to a mammalian subject in need thereof.

3. A method of treating, controlling or preventing the onset of one or more obesity related disorders or conditions selected from the group consisting of diabetes mellitus, hyperglycemia, hyperlipidemia, hypercholesterolemia, atherosclerosis, hypertension, hypertriglyceridemia, insulin resistance, or hyperinsulinemia, in a mammalian subject in need of treatment, comprising administering a therapeutically effective amount of oleuropein or an analogue, derivative or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside or their analogue, derivative, or any combination thereof including but not limited to olive leaf extract.

4. A method of treating, controlling or preventing the onset of one or more obesity related disorders or conditions selected from the group consisting of asthma and related diseases including but not limited to, allergy, atopic dermatitis (Eczema), gastroesophageal reflux disease, pulmonary, airway, lung disorders, cholesterol or secoiridoid glycoside or their analogue, derivative, or any combination thereof including but not limited to olive leaf extract.

5. A method of treating, controlling or preventing the onset of one or more obesity related disorders or conditions selected from the group consisting of AIDS and HAART related diseases including but not limited to lipodystrophy in a mammalian subject in need of treatment, comprising administering a therapeutically effective amount of oleuropein or an analogue, derivative or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside or their analogue, derivative, or any combination thereof including but not limited to olive leaf extract.
6. A method of treating, controlling or preventing the onset of one or more viral infection related disorders or conditions selected from the group consisting of viral infection related diseases including but not limited to the AIDS virus HIV-1, and other viruses with type I transmembrane envelope glycoprotein, such as simian immunodeficiency viruses (SIV), Sendai virus, feline immunodeficiency virus (FIV), respiratory syncytial virus (RSV), measles virus, Ebola virus, Nipah and Hendra viruses, the severe acute respiratory syndrome associated coronavirus (SARS-CoV), and the avian flu virus in mammalian, avian, poultry, non human primates subjects in need of treatment, comprising administering a therapeutically effective amount of oleuropein or an analogue, derivative or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside or their analogue, derivative, or any combination thereof including but not limited to olive leaf extract.

7. The method of claim 1, wherein the adipogenic genes and gene products whose expression is modulated includes, but is not limited to, PPARα, PPARγ, LPL, and the αP2 genes and gene products.

8. The method of claim 1, wherein the lipolytic genes and gene products whose expression is modulated including but not limited to PPAR δ and its modulated genes and gene products.

9. The method of claim 1, wherein the lipogenic or lipolytic genes whose expression is modulated including but not limited to PPAR α and its modulated genes and gene products.

10. A method of differentiation and transdifferentiating adipocytes into osteoblasts (bone cells), myoblasts (muscle cells), and chondrocytes (cartilage cells), the method comprising administering to a mammal in need thereof a therapeutically effective amount of oleuropein or an analogue, derivative or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside or their analogue, derivative, or any combination thereof including but not limited to olive leave extract.

11. (canceled)
12. (canceled)
13. (canceled)
14. (canceled)
15. (canceled)
16. (canceled)
17. (canceled)
18. The method of claim 2 further comprising administering a second agent in combination with oleuropein, or an analogue or oleuropein aglycone or their analogues, or derivatives thereof, or hydroxyrosol, or dihydroxy phenol or their analogues, or derivatives thereof, or elenolic acid or their analogues, or derivatives thereof, or an iridoid glycoside, or a secoiridoid glycoside or their analogue, derivative, or any combination thereof including but not limited to olive leave extract.

19. The method of claim 18, wherein the second agent is selected from the group consisting of a different PPAR modulating agent, a cholesterol or lipid lowering agent, a biguanide, insulin, an antihyperglycemic agent and any agent useful for treating metabolic syndrome or type 2 diabetes.

20. The method of claim 19, wherein the different PPAR modulating agent is selected from the group consisting of troglitazone, pioglitazone and rosiglitazone.

21. The method of claim 19, wherein the cholesterol or lipid lowering agent is a HMG-CoA reductase inhibitor, and wherein the HMG-CoA reductase inhibitor is a statin selected from the group consisting of atorvastatin, cerivastatin, fluvastatin, lovastatin, mevastatin, nicotinat, niacin, pravastatin and simvastatin.

22. The method of claim 19, wherein the biguanide is selected from the group consisting of metformin, phenformin, and buformin.

23. The method of claim 19, wherein the antihyperglycemic is a prandial glucose regulator or an alpha-glucosidase inhibitor.

24. The method of claim 23, wherein the prandial glucose regulator is repaglinide or nateglinide.

25. The method of claim 23, wherein the alpha-glucosidase inhibitor is selected from the group consisting of acarbose, voglibose and miglitol.

26. The method of claim 19, wherein the agent useful for treating metabolic syndrome or type 2 diabetes is a sulfonylurea selected from the group consisting of glibenclamide (glyburide), glipizide, glipizide, gliclazide, chloropropamide, tolbutamide, acetohexamide, glycozymamide, carbutamide, glibonuride, glisoxepid, glyburidazole, glibozole, glyhexamide, glymidine, glypinamide, phenbutamide, tolcyclamide and toluazamide.

27. (canceled)
28. (canceled)
29. (canceled)
30. (canceled)
31. (canceled)
32. (canceled)
33. (canceled)
34. (canceled)
35. (canceled)
36. (canceled)
37. (canceled)
38. (canceled)
39. (canceled)
40. (canceled)
41. (canceled)
42. (canceled)
43. (canceled)
44. (canceled)
45. (canceled)
46. (canceled)
47. (canceled)
48. (canceled)
49. (canceled)
50. (canceled)
51. (canceled)
52. (canceled)
53. (canceled)
54. (canceled)
55. (canceled)
56. (canceled)
57. (canceled)
58. (canceled)
59. (canceled)
60. (canceled)
61. (canceled)
62. (canceled)
63. (canceled)  
64. (canceled)  
65. (canceled)  
66. (canceled)  
67. (canceled)  
68. (canceled)  
69. (canceled)