INHIBITION OF HISTONE DEACETYLASES FOR THE TREATMENT OF HYPERLIPIDAEMIAS AND PREVENTION OF ATHEROSCLEROSIS AND CARDIOVASCULAR DISEASES

The present invention relates to the use of inhibitors of the histone deacetylases for the preparation of a medicament for the treatment of pathologies caused by increased levels of plasma cholesterol and plasma and hepatic triglycerides. Particularly, the invention relates to the use of said inhibitors for the preparation of a medicament for the treatment of diseases such as hyperlipidaemia, particularly, hypercholesterolaemia, hypertriglyceridaemia, atherosclerosis, cardiovascular and cerebrovascular pathologies, obesity, diabetes metabolic syndromes.
DESCRIPTION

INHIBITION OF HISTONE DEACETYLASES FOR THE TREATMENT OF HYPERLIPIDAEMIAS AND PREVENTION OF ATHEROSCLEROSIS AND CARDIOVASCULAR DISEASES.

The present invention relates to the use of inhibitors of histone deacetylases (HDAC) for controlling plasma cholesterol and triglyceride levels.

Cardiovascular and cerebrovascular diseases represent the principal causes of death or serious disability in industrialised countries, and for this reason the social and economic costs as a result of such diseases are constantly on the rise. Among the risk factors, hyperlipidaemias, particularly hypercholesterolaemia, play a significant role. The currently available treatments for reducing cholesterol levels are mainly represented by inhibitors of the enzyme β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) reductase (statins) and by PPAR nuclear receptor ligands. However, the identification of new molecular targets for the design of novel, and more potent, therapies for the reduction of blood cholesterol levels, and the associated increased cardiovascular risk, is worthwhile. Within this perspective, a promising field is represented by the cholesterol excretion pathways, through its conversion to bile acids. The molecular mechanisms regulating this
metabolic pathway are an ideal target for the development of novel therapeutic approaches.

The biosynthesis of bile acids represents the major cholesterol excretory pathway in mammals. The gene encoding cholesterol 7α-hydroxylase (CYP7A1), the enzyme regulating the conversion of cholesterol into bile acids, is negatively regulated at the transcriptional level by bile acids returning to the liver through the enterohepatic cycle. Bile acids exert their effects by promoting the dissociation of transcription coactivators [e.g. PPARγ-coactivator-1 (PGC-1) and cAMP Response Element Binding protein (CREB) Binding Protein (CBP)] from the promoter of the cholesterol 7α-hydroxylase gene, with the simultaneous recruitment of transcription corepressors and specific histone deacetylases to the cholesterol 7α-hydroxylase gene promoter. The discovery of this novel biochemical mechanism has been experimentally confirmed *in vivo* and *in vitro* by ourselves. We have recently observed that the use of inhibitors of histone deacetylases prevents the bile acid mediated repression of the cholesterol 7α-hydroxylase gene, both in experimental *in vitro* models and also *in vivo* models and, hence, increase hepatic conversion of cholesterol into bile acids, with a consequent overall reduction in plasma cholesterol levels. Particularly,
inhibition of the histone deacetylases results in a marked increase in the level of Cyp7a1 mRNA bringing about an overall reduction in plasma cholesterol, both in normal phenotype (C57BL/6J) mice and in genetically hypercholesterolaemic mice, carrying a mutation in the low density lipoprotein (LDL) receptor gene (Ldl−r/−). Analysis of the lipoprotein fractions indicates that the inhibition of histone deacetylases causes a sharp reduction in the fraction containing the intermediate density (IDL) and low density (LDL) lipoproteins, and hence the HDL/LDL ratio is increased.

It has also been observed in the Ldl−r/− mice that inhibitors of histone deacetylases provides reductions in plasma and hepatic triglycerides, as well as loss of body mass.

The principal classes of inhibitors of histone deacetylases are indicated hereinafter. One first class of inhibitors of histone deacetylases used in the present invention are the compounds of formula (I) and the pharmaceutically acceptable salts and solvates thereof:

\[
\begin{align*}
\text{O} \\
\text{R} \quad \text{OH}
\end{align*}
\]

Formula (I)

wherein \( R \) is linear or branched (C₁₋₁₂) alkyl; linear or branched (C₁₋₁₂) alkenyl; (C₁₋₁₆) arylalkyl
either non substituted or substituted with (C\textsubscript{1-4}) alkyl, 
(C\textsubscript{1-4}) alkoxy, hydroxy, cyano, nitro, halo, trifluoromethyl.

The compounds of formula (I) of the present invention may be optically active, both as R and S enantiomers, or racemic mixtures or mixtures of diastereoisomers.

For example, butyric acid, phenylbutyric acid and valproic acid belong to this category.

Belonging to a second class of molecules are the compounds of formula (II) and the pharmaceutically acceptable salts and solvates thereof:

\[
\begin{array}{c}
\text{A} \\
\text{R}^2 \\
\text{N} \\
\text{OH}
\end{array}
\]

Formula (II)

wherein \( \text{R}^2 \) is linear or branched (C\textsubscript{1-26}) alkyl; linear or branched (C\textsubscript{1-26}) alkenyl; linear or branched carbonyl (C\textsubscript{1-16}) alkyl; linear or branched carbonyl (C\textsubscript{1-16}) alkenyl; alkynyl (C\textsubscript{1-6}) alkyl; alkynyl (C\textsubscript{1-6}) alkenyl; (C\textsubscript{1-16}) alkyl-CO-NH;

A is single ring or condensed ring aryl or heteroaryl, either non substituted or substituted in any position by \( \text{NR}^3\text{R}^4; \text{CO-NH-OH}; \text{NH-SO}_2\text{-R}^5; \text{(C}\textsubscript{1-4})\text{ alkoxy; } \)
linear or branched (C₁₋₄) alkyl; linear or branched (C₁₋₄) alkenyl; halo; cyano; nitro; trifluoromethyl.

Preferably it is a heterocycle selected from:

![Heterocycle structure](image)

thiophene, furan, pyrole, imidazole, thiazole, oxazole, pyridine, pyrimidine, pyrazine, pyridazine, 1,2,4-triazine, 1,2,4,5-tetrazine, either non substituted or substituted with one or more substituent groups selected from halo, hydroxy, nitro, cyano, (C₁₋₄) alkoxy, trifluoromethyl. Even more preferably, it is a group selected from: naphthyl, indolyl, indazolyl, quinolyl, isoquinolyl, quinoliziny, benzimidazolyl, benzofuranyl either non substituted or substituted with one or more substituent groups selected from halo, hydroxy, nitro, cyano, (C₁₋₄) alkoxy, or trifluoromethyl.

R³ and R⁴ are H; linear or branched (C₁₋₆) alkyl; linear or branched (C₁₋₆) alkenyl; saturated or unsaturated 5 or 6 member rings.

R³ is linear or branched (C₁₋₆) alkyl; aryl either non substituted or substituted with (C₁₋₄) alkoxy; linear or branched (C₁₋₄)alkyl; linear or branched (C₁₋₄) alkenyl; halo; cyano; nitro; trifluoromethyl.

The compounds of formula (II) of the present
invention may be optically active, both as R and S enantiomers, or racemic mixtures or mixtures of diastereoisomers.

For example, belonging to this category are the following compounds: tricostatin A, scriptaid, pyroxamide, suberoylanilide hydroxamic acid (SAHA), m-carboxycinnamic acid bis-hydroxamide (CBHA), oxamflatin and:

![Chemical Structure](image)

Belonging to a third class of molecules are the compounds of formula (III) and the pharmaceutically acceptable salts and solvates thereof:

![Chemical Structure](image)

Formula (III)

wherein $R^6$, $R^7$, $R^8$, $R^9$ are: $H$; linear or branched ($C_{1-6}$) alkyl; linear or branched ($C_{1-6}$) alkenyl; ($C_{1-10}$) alkylaryl either non substituted or substituted with ($C_{1-6}$) alkyl; linear or branched ($C_{1-6}$) alkenyl; ($C_{1-10}$) alkylaryl either non substituted or substituted with ($C_{1-6}$) alkyl.
4) alkoxy, linear or branched (C<sub>1-4</sub>) alkyl, linear or branched (C<sub>1-4</sub>) alkenyl, halo, cyano, nitro, trifluoromethyl.

R<sup>11</sup>, R<sup>12</sup>, R<sup>13</sup>, R<sup>16</sup>, R<sup>19</sup> are: H; (C<sub>1-16</sub>) alkylaryl either non substituted or substituted with (C<sub>1-4</sub>) alkoxy, linear or branched (C<sub>1-4</sub>) alkyl; (C<sub>1-16</sub>) alkyl carbonyl epoxy; (C<sub>1-16</sub>) alkyl-CO-NH-OH; linear or branched (C<sub>1-16</sub>) alkyl; linear or branched (C<sub>1-16</sub>) alkenyl; -CH<sub>2</sub>-S-CH<sub>3</sub>; -(CH<sub>2</sub>)<sub>4</sub>-S-CH<sub>3</sub>; (C<sub>1-16</sub>) alkylheterocyclicl (wherein the heterocycle is selected from: thiophene, furan, pyrole, imidazole, thiazole, oxazole, pyridine, indole N-substituted with (C<sub>1-4</sub>) alkoxy, (C<sub>1-6</sub>) alkyl, (C<sub>1-6</sub>) alkenyl); or are the group -R<sup>14</sup>-CO-R<sup>15</sup> wherein R<sup>14</sup> and R<sup>15</sup> are selected from the following groups: linear or branched (C<sub>1-10</sub>) alkyl and linear or branched (C<sub>1-10</sub>) alkenyl.

R<sup>6</sup>, R<sup>7</sup>, R<sup>8</sup>, R<sup>9</sup> may form closed rings with R<sup>12</sup> R<sup>11</sup>, R<sup>13</sup>, R<sup>19</sup> respectively (in the latter case R<sup>16</sup> is H), to give a saturated 5 or 6 member ring. In all the above cases R<sup>10</sup> is a carbon atom.

R<sup>10</sup> may be a carbon atom or is:

```
    CH---CH---O---CO---CH---
    R<sup>17</sup>   R<sup>18</sup>   R<sup>20</sup>
```

wherein R<sup>18</sup> is a (C<sub>6-10</sub>) alkyl or (C<sub>6-10</sub>) alkenyl chain containing a disulphide bond, and is terminated by
bonding to CR\textsuperscript{11};

R\textsuperscript{17} and R\textsuperscript{20} are H, linear or branched (C\textsubscript{1-6}) alkyl; linear or branched (C\textsubscript{1-6}) alkenyl.

The compounds of formula (III) of the present invention may be optically active, both as R and S enantiomers, or racemic mixtures or mixtures of diastereoisomers.

For example, belonging to this category are the following compounds: trapoxin A and B, HC-toxin, chlamydociocin, apicidin, depsipeptide (also known as FK228), dimethyl-depsipeptide (also known as dimethyl FK228), cyclo-(Aoe-D-Tyr(Me)-Ile-Pip) (also known as Cyl-2), wherein Aoe = 2-ammino-9,10-epoxy-8-oxodecanoic acid and Tyr(Me), Ile and Pip are as described hereinafter.

Other compounds belonging to this category are the so-called "CHAPs (cyclic Hydroxamic acid-containing peptide"), trapoxin derivatives wherein the terminal epoxyketone group is replaced with a hydroxamic acid (Furumai et al.; Proc. Natl. Acad. Sci. U.S.A., 2001, 98, 87-92; Komatsu et al.; Cancer Research, 61, 4459-4466). Examples of compounds belonging to the CHAPs family include:

cyclo(Asu(NHOH)-Phe-Phe-D-Pro) (CHAP1),
cyclo(Asu(NHOH)-D-Tyr(Me)-Ile-Pip) (CHAP49),
cyclo(Asu(NHOH)-D-Tyr(Me)-Ile-Pro) (CHAP30),
cyclo(Asu(NHOH)-D-Phe-Leu-Pip) (CHAP53),
cyclo(Asu(NHOH)-Aib-Phe-D-Pro) (CHAP15),
cyclo(Asu(NHOH)-D-Pro-Ala-D-Ala) (CHAP13),
cyclo(-L-Asu(NHOH)-D-Tyr(Me)-L-Ile-D-Pip) (CHAP50),
cyclo(-L-Asu(NHOH)-D-Tyr(Me)-L-Ile-L-Pip) (CHAP49),
cyclo(-L-Asu(NHOH)-D-Tyr-L-Ile-D-Pro) (CHAP77),
cyclo(-L-Asu(NHOH)-D-Tyr(Me)-L-Ala-D-Pro) (CHAP44),
cyclo(-L-Asu(NHOH)-D-Phe-L-Phe-D-Pip) (CHAP57),
cyclo(-L-Asu(NHOH)-D-Phe-L-Ala-D-Pro) (CHAP45),
cyclo(-L-Asu(NHOH)-L-Ala-L-Phe-D-Pro) (CHAP80),
cyclo(-L-Asu(NHOH)-D-Phe-L-Phe-D-Pro) (CHAP27),
wherein Asu = α-aminosuberic acid, Pip = pipecolic acid, Aib = α-aminoisobutyric acid. Amino acid sequences are expressed from the amino terminal end, using the standard three letter codes (Ala, Phe, Pro, Ile); Tyr(Me) is the aminoacid tyrosine in which the phenolic OH group is replaced by OMe. The expression "cyclo" means that the amino terminal end and the COOH-terminal have combined to form an amide bond. NHOH in brackets, means that the hydroxamic acid residue is located at the end of the Asu side chain.

Belonging to a fourth class of molecules are the compounds of formula (IV) and the pharmaceutically acceptable salts and solvates thereof:
Formula (IV)

wherein B is linear or branched (C<sub>1-16</sub>) alkyl; linear or branched (C<sub>1-16</sub>) alkenyl.

$R^{21}$ and $R^{22}$ are

\[
\text{CH} \quad \text{R}^{23}
\]

\[
\text{OH}
\]
or linear or branched (C<sub>1-6</sub>) alkyl; linear or branched (C<sub>1-6</sub>) alkenyl; (C<sub>1-10</sub>) alkylaryl either non substituted or substituted with (C<sub>1-4</sub>) alkoxy, linear or branched (C<sub>1-4</sub>) alkyl, linear or branched (C<sub>1-4</sub>) alkenyl, halo, cyano, nitro, trifluoromethyl.

$R^{23}$ is linear or branched (C<sub>1-6</sub>) alkyl; linear or branched (C<sub>1-6</sub>) alkenyl.

The compounds of formula (IV) of the present invention may be optically active, both as R and S enantiomers, or racemic mixtures or mixtures of diastereoisomers.

For example, depudesin belongs to this category.

Belonging to a fifth class of molecules are the compounds of formula (V) and the pharmaceutically acceptable salts and solvates thereof:
wherein \( R^{25} \) is \( NR^{26}R^{27} \); a hydroxyl group; a carboxylic group; halo; cyano; nitro and

\( R^{26} \) and \( R^{27} \) are H; linear or branched (C\(_{1-6}\)) alkyl; linear or branched (C\(_{1-6}\)) alkenyl; saturated or aromatic 5 or 6 member rings.

\( R^{24} \) is H; linear or branched (C\(_{1-6}\)) alkyl; linear or branched (C\(_{1-6}\)) alkenyl; a saturated or aromatic 5 or 6 member ring; or is COOR\(^{28}\) or COR\(^{29}\) wherein \( R^{28} \) and \( R^{29} \) are selected from linear or branched (C\(_{1-6}\)) alkyl; linear or branched (C\(_{1-6}\)) alkenyl; (C\(_{1-16}\)) alkylaryl; (C\(_{1-16}\)) alkylheteroaryl.

The compounds of formula (V) of the present invention may be optically active, both as R and S enantiomers, or racemic mixtures or mixtures of diastereoisomers.

For example, members of this category include: \( N\)-acetyldinaline (also known as CI-994) and MS-275.

MS-275 has the following structural formula:
Since the histone deacetylases are generally metalloenzymes, with a zinc atom at the active site, compounds possessing the ability to bind zinc, for example carboxylic groups, hydroxamic groups, alpha-ketothio groups etc., are potential HDAC inhibitors.

The formulae of such compounds and their histone deacetylase inhibitory activities are known in the state of the art.

Since histone deacetylases are also involved in the biochemical mechanisms regulating cellular growth, inhibitors thereof are used in the treatment of pathologies such as: various tumour types (leukaemias, solid tumours, haematopoietic tissue tumours etc.), psoriasis, haematological disorders, haemoglobinopathies, spinal muscular atrophy, Huntington's disease, genetic metabolic disorders (cystic fibrosis, adrenoleukodystrophy etc.). In relation to the above, the following documents may be consulted: WO03099789, WO0250244, US6638530, WO02076941, WO03099760, WO03099272, GB2389365, WO03092686, WO03087066, WO03082288.
WO03076430, WO03076422, WO03076395, US2003078216, EP1307784. Other pathologies which may be cured through the administration of HDAC inhibitors are cardiac hypertrophy (US200314434), liver fibrosis and hepatic cirrhosis (WO03076438).

The use of the above mentioned molecules and, histone deacetylase inhibitors in general, for the control of blood cholesterol and triglycerides levels and, hence, for the treatment of pathologies such as hyperlipidaemias (particularly hypercholesterolaemia) atherosclerosis, obesity, diabetes and metabolic syndromes and the prevention of cardiovascular and cerebrovascular diseases (for example, myocardial infarction and stroke) is not known.

In a first aspect, the present invention relates to the use of inhibitors of histone deacetylase (HDAC) for the preparation of a medicament for the control of plasma cholesterol levels.

In a second aspect, the invention relates to the use of inhibitors of histone deacetylase for the preparation of a medicament for the control of plasma and hepatic triglyceride levels.

In a third aspect, the present invention relates to the use of inhibitors of histone deacetylase for the preparation of a medicament for the treatment of
conditions mediated by cholesterol 7α-hydroxylase activation. By the term “cholesterol 7α-hydroxylase activation” is meant, particularly, but not exclusively, stimulation of transcription of the cholesterol 7α-hydroxylase gene.

Preferably, the present invention relates to the use of inhibitors of histone deacetylases (HDAC) for the preparation of a medicament for the treatment of diseases such as hyperlipidaemias (particularly hypercholesterolaemia) atherosclerosis, obesity, diabetes and metabolic syndromes and the prevention of cardiovascular and cerebrovascular pathologies, such as myocardial infarction or stroke.

More preferably, the present invention relates to the use of HDAC inhibitors selected from the compounds belonging to the groups described by formulae (I), (II), (III), (IV) and (V).

The preferred inhibitors belonging to said groups are: tricostatin A, phenylbutyrate, scriptaid, apicidin, pyroxamide, depsipeptide.

Other histone deacetylase inhibitors employed for the uses according to the invention are listed hereinafter:

pivaloyloxymethylbutyrate (also known as AN-9);
cyclosterollettamine, particularly cyclosterollettamine A,
cyclostellitamine G, dehydrocyclostellitamine D and dehydrocyclostellitamine E (Naoya Oku et al.; Bioorganic and Medicinal Chemistry Letters 14, 2004, 2617-2620); hydroxamic acids having the following structural formulae:

(Also known as NVP-LAK974)

(Also known as NVP-LAQ824)
described by Marson et al.; Bioorganic and Medicinal Chemistry Letters 14, 2004, 2477-2481;


Other compounds advantageously used for the purposes of the invention are described by the following general formula (VI):
Formula (VI)

wherein $X = 1, 2$; $n = 1, 2, 3$; $R30 = H, CH_3$.

Examples of compounds belonging to this class include:

- cyclo(-L-Lys(For, OH)-D-Tyr(Me)-L-Ile-L-Pip),
- cyclo(-L-Lys(For, OH)-D-Tyr(Me)-L-Ile-D-Pip),
- cyclo(-L-Hly(For, OH)-D-Tyr(Me)-L-Ile-L-Pip),
- cyclo(-L-Hly(For, OH)-D-Tyr(Me)-L-Ile-D-Pip),
- cyclo(-L-Aoc(For, OH)-D-Tyr(Me)-L-Ile-L-Pip),
- cyclo(-L-Aoc(For, OH)-D-Tyr(Me)-L-Ile-D-Pip),


Another inhibitor, useful for the purposes of the invention is LBH589 which has shown a good level histone deacetylase inhibitory activity (Gorge et al. Blood, vol. 105, 4, 1768-1776).

Furthermore, the invention provides a pharmaceutical
formulation comprising one or more of the previously
described compounds, or a physiologically acceptable
derivative thereof together with one or more
physiologically acceptable carriers and, optionally,
other therapeutic and/or prophylactic components. The
carriers or excipients must be "acceptable" in the sense
that they must be compatible with the other components of
the formula and not harmful to the recipient.

By "physiologically acceptable derivative" is meant
any physiologically acceptable salt or solvate, ester, or
solvate of said ester, of a compound of the invention or
any other compound which, by administration to the
recipient, is capable of providing (either directly or
indirectly) a compound of the invention or an active
metabolite or residue thereof.

The preferred physiologically acceptable derivatives
of the compounds of the invention are the
pharmaceutically acceptable salts thereof.

The pharmaceutically acceptable salts of the
compounds of formula (I) include those derived from
organic or inorganic bases. The base derived salts
include the salts of alkaline metals (for example
sodium), alkaline earth metals (for example magnesium),
ammonium and NR4+ (wherein R is C1-4 alkyl).

The compounds to be used, according to the present
invention, may be formulated for oral, buccal, parenteral, rectal or transdermal administration or in a form which is suitable for administration by inhalation or insufflation (either through the mouth or nose).

For oral administration, the pharmaceutical compositions may be, for example, in the form of tablets or capsules, prepared in the conventional manner with the aid of pharmaceutically acceptable excipients such as binding agents (for example pre-gelatinised corn starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (for example lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (for example magnesium stearate, talc or silica); disintegrants (for example potato starch or sodium starch glycolate); or inhibiting agents (for example sodium dodecyl sulphate). Tablets may be coated, using methods well known in the art. Liquid preparations for oral administration may be, for example, in the form of solutions, syrups or suspensions or may be as lyophilised products to be reconstituted, prior to use, with water or other suitable carriers. Such liquid preparations may be prepared using conventional methods with pharmaceutically acceptable additives such as suspension agents (for example sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifiers (for example lecithin or acacia); non-
aqueous carriers (for example almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (for example methyl- or propyl-p-hydroxybenzoates or sorbic acid). Preparations may also suitably contain flavourings, dyes and sweeteners.

Preparations for oral administration may be suitably formulated to allow the controlled release of the active ingredient.

Compositions for buccal administration may be in the form of conventionally formulated tablets or lozenges.

The compounds according to the present invention may be formulated for parenteral administration by injection. Formulations for injections may be presented in single dose form, for example in vials, with added preservative.

The compositions may be presented in the aforementioned form as suspensions, solutions or emulsions in oily or aqueous carriers and may contain formulary agents such as suspension agents, stabilisers and/or dispersants. Alternatively, the active ingredient may be in powder form for reconstitution, prior to use, using a suitable carrier, for example sterile water.

According to the present invention, the compounds may also be formulated in rectal compositions such as suppositories or retention enemas, for example containing common basic suppository components such as cocoa butter.
or other glycerides.

In addition to the previously described compositions, the compounds may also be formulated as deposit preparations. Such long acting preparations may be administered as implants (for example subcutaneously, transcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds according to the present invention, may be formulated with suitable polymeric or hydrophobic materials (for example in the form of an emulsion in a suitable oil) or ion exchange resin or as sparingly soluble derivatives, for example as a sparingly soluble salt.

According to the present invention the dosage of the compounds suggested for administration in humans (with body weight of approx. 70 Kg) ranges from 0.1 mg to 1 g and, preferably from 1 mg to 100 mg of the active ingredient per unit dose. The unit dose may be administered, for example, from 1 to 4 times daily. The dose will depend on the route selected for administration. It should be considered that it might be necessary to make continual adjustments to the dosage depending on the age and weight of the patient, in addition to the seriousness of the clinical condition to be treated. The exact dose and the administration route will finally be at the discretion of the physician or
veterinarian.

EXPERIMENTAL DATA AND EXAMPLES

Our cellular model assays using the technique known as the "chromatin immunoprecipitation assay" indicate that bile acids cause rapid localised deacetylation of histones (Figure 1A) and the nuclear receptor Hepatocyte Nuclear Factor-4 (HNF-4) (Figure 1B) present in one specific region of the cholesterol 7α-hydroxylase gene promoter. Said localised chromatin deacetylation is mediated by histone deacetylase (HDAC) and causes the specific repression of the cholesterol 7α-hydroxylase gene. Among the 18 mammalian histone deacetylases described to date, histone deacetylases 1, 3 and 7 play specific roles in this type of regulation. Indeed, bile acids cause the selective recruitment of histone deacetylases 1, 3 and 7 to the promoter region of the cholesterol 7α-hydroxylase gene. Particularly, using a confocal microscope, we have observed that when hepatic cells are exposed to physiological concentrations of bile acids, histone deacetylase 7 is translocated from the cytoplasm to the nucleus, where it exerts its inhibitory action. The resulting interpretation is that bile acids cause the intranuclear localisation of histone deacetylase 7, which in turn promotes the recruitment of histone deacetylases 1 and 3, in addition to the
simultaneous recruitment of the transcription corepressors, to the promoter region of the cholesterol 7α-hydroxylase gene. The above events result in the selective transcriptional repression of the cholesterol 7α-hydroxylase gene. Based on the results described above, we have hence hypothesised that histone deacetylase inhibitors, such as valproic acid or tricostatin, may prevent inhibition of transcription of the cholesterol 7α-hydroxylase gene. Experiments using cultured liver cells show that histone deacetylase inhibitors, such as for example valproic acid and tricostatin A, cancel out the inhibitory effect of bile acids on cholesterol 7α-hydroxylase mRNA levels (figure 2), while the phosphoenolpyruvate carboxykinase gene (a key enzyme in hepatic gluconeogenesis), used here as a negative control, continues to be repressed by bile acids, despite the addition of valproic acid or tricostatin A.

The suppression of specific histone deacetylase isoforms using "RNA interference" technology allows the identification of histone deacetylase 7 (HDAC7) as a specific factor playing a key role in the repression of the gene encoding cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme in the pathway for the conversion of cholesterol to bile acids (Figure 3). This
important information will allow focussing further attention on a specific target, and hence the design of molecules with more specific effects. Furthermore, the "small interfering RNA (siRNA)" oligonucleotides, selectively silencing the expression of the histone deacetylases involved in the repression of the cholesterol 7α-hydroxylase gene, may be administered to patients for the treatment of pathologies such as hypercholesterolaemias, hypertriglyceridaemias, atherosclerosis, diabetes, obesity, metabolic syndromes and for the prevention of cardiovascular and cerebrovascular pathologies and Alzheimer’s disease.

IN VIVO TESTS

In vivo experiments have been performed in a hypercholesterolaemia genetic model, the LDL-receptor knockout mouse, which develops hypercholesterolaemia as a result of lacking the low density lipoprotein (LDL) receptor. The HDAC inhibitors used were valproic acid and tricostatin A, two structurally unrelated molecules. The in vivo results obtained using this animal model show a significant increase in cholesterol 7α-hydroxylase mRNA levels, as well as increased bile acid synthesis, measured as faecal excretion, in both valproic acid and tricostatin A treated animals (Figure 4A). Both HDAC inhibitors cause marked reductions in plasma cholesterol
(Figure 4B) and LDL (Figure 4C) levels; analysis of a narrow panel of genes involved in lipid and glucose metabolism has shown that histone deacetylase inhibitors do not significantly alter the levels of mRNA for such genes, hence the hypocholesterolemizing effect is to be principally attributed to the derepression of the cholesterol 7α-hydroxylase gene, responsible for the conversion of cholesterol to bile acids (Figure 4D).

The administration of valproic acid and tricostatin A in the same animals also leads to reductions in plasma and hepatic triglycerides. Such reductions are accompanied by reductions in body weight, even though the animals treated with the two inhibitors consume more food with respect to the animals treated with carrier alone.

It should be noted that animals treated with HDAC inhibitors show increased expression of PGC-1 protein (PPAR γ coactivator-1) in the liver, the increase of which is generally observed in animals deprived of sources of energy (e.g. fasting).

<table>
<thead>
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<th></th>
<th>Plasma triglycerides (mg/dl)</th>
<th>Hepatic triglycerides (mg/g of liver)</th>
<th>Body weight (g)</th>
<th>Food consumption (g/day)</th>
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</thead>
<tbody>
<tr>
<td>Controls</td>
<td>160±22</td>
<td>32±18</td>
<td>22.35±0.35</td>
<td>3.25±0.21</td>
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</tbody>
</table>


<table>
<thead>
<tr>
<th>VPA</th>
<th>103±22</th>
<th>6.5±3.4</th>
<th>20.42±1.87</th>
<th>4.04±0.13</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSA</td>
<td>76±17</td>
<td>10.5±7</td>
<td>16.70±1.36</td>
<td>4.61±0.96</td>
</tr>
</tbody>
</table>

VPA = valproic acid  
TSA = tricostatin

The net increase in cholecyst content, mirroring the increased hepatic conversion of cholesterol to bile acids, should be underlined as additional confirmation that plasma cholesterol levels are reduced following stimulation of the cholesterol 7α-hydroxylase gene. In conclusion, such results clearly demonstrate that valproic acid and tricostatin A possess the ability to prevent the bile acid mediated repression of the cholesterol 7α-hydroxylase gene and, hence, increase the expression of cholesterol 7α-hydroxylase with consequential increased hepatic conversion of cholesterol to bile acids and reduced plasma cholesterol levels. The histone deacetylases 1, 3 and 7 selectively recruited onto the promoter of the cholesterol 7α-hydroxylase gene, ultimately represent novel molecular targets for new molecules having hypocholesterolemizing actions.  

Selective inhibitors of histone deacetylases may assume a role as drugs for preventing atherosclerosis and the associated risk of cardiovascular and cerebrovascular diseases. Furthermore, in the light of the fact that
elevated cholesterol levels play a role in the physiopathology of Alzheimer's disease, such molecules may also have a protective role in relation to this pathology.

As already mentioned, the histone deacetylases are currently the targets for the treatment of various types of tumours. Results from phase I and II clinical trials have shown that their antitumour activity is associated with optimal tolerability and modest side effects at therapeutic doses. Any potential hepatic toxicity described for high doses of valproic acid is to be attributed not to its histone deacetylase inhibitory activity, but to the fact that it accumulates as the Coenzyme A derivative. In our in vivo experiments, valproic acid and tricostatin A have hence been used as pharmacological "tools" to demonstrate that the inhibition of the histone deacetylases, selectively recruited onto the promoter of the cholesterol 7α-hydroxylase gene, constitutes an extremely promising approach for the treatment of hypercholesterolaemia and associated cardiovascular risks. The discovery of this novel property for the histone deacetylase inhibitors represents a potential therapeutic application for reducing blood cholesterol and the associated risk of developing atherosclerosis, cardiovascular and
cerebrovascular diseases.
Legend: HepG2 hepatic cell cultures have been treated with the bile acid chenodeoxycholic acid for the times indicated in the figure. Upon completion of treatment, the acetylation states of histones H3 and H4 in the promoter of the gene encoding cholesterol 7α-hydroxylase (panel A), the amount of HNF-4 associated with the same promoter and its degree of acetylation (panel B) have been assessed using the "chromatin immunoprecipitation assay".
Legend: HepG2 cell cultures have been treated with chenodeoxycholic acid (CDCA), tricostatin A (TSA), valproic acid (VPA), CDCA + TSA (C+T) CDCA + VPA (C+V). Upon completion of treatment cholesterol 7α-hydroxylase mRNA levels have been measured in the various samples.
Legend: cultures of the hepatic cell line HepG2 have been transfected with oligonucleotides which specifically silence HDAC7 (panel A), HDAC1 (panel B) and HDAC3 (panel C). Only the silencing of HDAC7 (panel A) is capable of preventing the reduction in CYP7A1 mRNA levels caused by the bile acid chenodeoxycholic acid (CDCA) (panel A),
while the silencing of HDAC1 (panel B) and HDAC3 (panel C) do not prevent the effect of the bile acid on the level of CYP7A1 mRNA.

**Figure 4**

Legend: *Ldl-r*−/− mice have been treated with intraperitoneal injections of isotonic saline (controls), with 200 mg/kg of body weight twice daily of valproic
acid (VPA), or with 1 mg/kg of body weight once daily of tricostatin A (TSA). The duration of the treatment was 7 days. Upon completion, liver cholesterol 7α-hydroxylase mRNA levels (panel A) and the faecal excretion of bile acids, as an indicator of their synthesis (panel A), total plasma cholesterol levels (panel B), the cholesterol content in the plasma lipoprotein fractions (panel C) and levels of the hepatic mRNA of the genes indicated (panel D) have been measured.
CLAIMS

1. Use of inhibitors of histone deacetylases (HDAC) for the preparation of a medicament for controlling plasma cholesterol levels.

2. The use of inhibitors according to claim 1 for the preparation of a medicament for the treatment of hypercholesterolaemias through the transcriptional activation of the gene encoding cholesterol 7α-hydroxylase.

3. The use according to claims 1 or 2 for the preparation of a medicament for the treatment of diseases such as hypercholesterolaemia and atherosclerosis and the prevention of cardiovascular and cerebrovascular pathologies and Alzheimer’s disease.

4. The use according to claim 3 wherein said cardiovascular and cerebrovascular diseases are: myocardial infarction and stroke.

5. The use of inhibitors of histone deacetylases (HDAC) for the preparation of a medicament for controlling plasma and hepatic triglyceride levels.

6. The use according to claim 5 for the preparation of a medicament for the treatment of diseases such as hypertriglyceridaemias.

7. The use according to claim 5 for the preparation of a medicament for the treatment of diseases such as
diabetes and metabolic syndromes.

8. The use according to claim 5 for the preparation of a medicament for the treatment of obesity.

9. The use according to any of the claims 1 to 8, said inhibitors being compounds of formula (I) and pharmaceutically acceptable salts and solvates thereof, R or S enantiomers, racemic mixtures and diastereoisomeric mixtures:

\[
\begin{align*}
\text{formula (I)} \\
\text{wherein } R^1 \text{ is linear or branched (C}_{1-12} \text{) alkyl; linear or branched (C}_{1-12} \text{) alkenyl; (C}_{1-16} \text{) arylalkyl either non substituted or substituted with (C}_{1-4} \text{) alkyl, (C}_{1-4} \text{) alkoxy, hydroxy, cyano, nitro, halo, trifluoromethyl.}
\end{align*}
\]

10. The use according to claim 9 wherein said inhibitors are selected from: butyric acid, phenylbutyric acid, valproic acid and pharmaceutically acceptable salts and solvates thereof.

11. The use according to any of the claims 1 to 8, said inhibitors being compounds of formula (I) and pharmaceutically acceptable salts and solvates thereof, R or S enantiomers, racemic mixtures and
diastereoisomeric mixtures:

![Chemical Structure Image]

**Formula (I)**

wherein R\(^1\) is linear (C\(_{1-12}\)) alkyl; linear or branched (C\(_{1-12}\)) alkenyl; (C\(_{1-16}\)) arylalkyl either non substituted or substituted with (C\(_{1-4}\)) alkyl, (C\(_{1-4}\)) alkoxy, hydroxy, cyano, nitro, halo, trifluoromethyl.

12. The use according to claim 11 wherein said inhibitors are selected from: butyric acid, phenylobutyric acid and pharmaceutically acceptable salts and solvates thereof.

13. The use according to any of the claims 1 to 8, said inhibitors being compounds of formula (II) and pharmaceutically acceptable salts and solvates thereof, R or S enantiomers, racemic mixtures and diastereoisomeric mixtures:

![Chemical Structure Image]

**Formula (II)**

wherein R\(^2\) is linear or branched (C\(_{1-20}\)) alkyl; linear or branched (C\(_{1-20}\)) alkenyl; linear or branched carbonyl (C\(_{1-16}\)) alkyl; linear or branched carbonyl (C\(_{1-16}\))
alkenyl; alkynyl (C₁₋₆) alkyl; alkynyl (C₁₋₆) alkenyl;
or R² is -(C₁₋₁₆) alkyl-CO-NH;
A is single ring or condensed ring aryl or heteroaryl,
either non substituted or substituted in any position
by NR³R⁴; CO-NH-OH; NH-SO₂-R⁵; (C₁₋₄) alkoxy; linear or
branched (C₁₋₄) alkyl; linear or branched (C₁₋₄)
alkenyl; halo; cyano; nitro; trifluoromethyl.
R³ and R⁴ are H; linear or branched (C₁₋₆) alkyl; linear
or branched (C₁₋₆) alkenyl; saturated or unsaturated 5
or 6 member rings.
R⁵ is linear or branched (C₁₋₆) alkyl; aryl either non
substituted or substituted with (C₁₋₄) alkoxy; linear
or branched (C₁₋₄) alkyl; linear or branched (C₁₋₄)
alkenyl; halo; cyano; nitro; trifluoromethyl.

14. The use according to claim 13 wherein, in said
inhibitors of formula (II), group A is selected from:

and þ thiophene, furan, pyrole, imidazole, thiazole,
oxazole, pyridine, pyrimidine, pyrazine, pyridazine,
1,2,4-triazine, 1,2,4,5-tetrazine, either non
substituted or substituted with one or more
substituent groups selected from halo, hydroxy, nitro, cyano, (C\textsubscript{1-4}) alkoxy, trifluoromethyl.

15. The use according to claims 13 or 14 wherein said group A is selected from: naphthyl, indolyl, indazolyl, quinolyl, isoquinolyl, quinolizinyl, benzimidazolyl, benzofuranyl either non substituted or substituted with one or more substituent groups selected from halo, hydroxy, nitro, cyano, (C\textsubscript{1-4}) alkoxy, trifluoromethyl.

16. The use according to any of the claims 13 to 15 wherein said inhibitors are selected from: tricostatin A, scriptaid, pyroxamide, suberoylanilide hydroxamic acid (SAHA), m-carboxycinnamic acid bis-hydroxamide (CBHA), oxamflatin and pharmaceutically acceptable salts and solvates thereof.

17. The use according to any of the claims 1 to 8, said inhibitors being compounds of formula (III), pharmaceutically acceptable salts and solvates thereof, R or S enantiomers, racemic mixtures, diastereoisomeric mixtures:
formula (III)

wherein $R^6$, $R^7$, $R^8$, $R^9$ are: H; linear or branched (C$_{1-6}$) alkyl; linear or branched (C$_{1-6}$) alkenyl; (C$_{1-10}$) alkylary; either non substituted or substituted with (C$_{1-4}$) alkoxy, linear or branched (C$_{1-4}$) alkyl, linear or branched (C$_{1-4}$) alkenyl, halo, cyano, nitro, trifluoromethyl;

$R^{11}$, $R^{12}$, $R^{13}$, $R^{16}$, $R^{19}$ are: H; (C$_{1-16}$) alkylary; (C$_{1-16}$) alkyl carbonyl epoxyl; linear or branched (C$_{1-16}$) alkyl; linear or branched (C$_{1-16}$) alkenyl; (C$_{1-16}$) alkylheterocycycl (wherein the heterocycle is selected from: thiophene, furan, pyrole, imidazole, thiazole, oxazole, pyridine, indole N-substituted with (C$_{1-4}$) alkoxy, (C$_{1-6}$) alkyl, (C$_{1-6}$) alkenyl); or are the group $-R^{14}-CO-R^{15}$ wherein $R^{14}$ and $R^{15}$ are selected from the following groups: linear or branched (C$_{1-10}$) alkyl and linear or branched (C$_{1-10}$) alkenyl; or $R^{11}$, $R^{12}$, $R^{13}$, $R^{16}$, $R^{19}$ are selected from: (C$_{1-16}$) alkylary either non
substituted or substituted with (C₄₋₄) alkoxy, linear or branched (C₄₋₄) alkyl; (C₁₋₁₆) alkyl-CO-NH-OH, -(CH₂-S-CH₃), -(CH₂)₄-S-CH₃.

R⁶, R⁷, R⁸, R⁹ may form closed rings with R¹¹ R¹², R¹³, R¹⁹ respectively to give a saturated 5 or 6 member ring.

R¹⁰ may be a carbon atom or is:

\[
\begin{array}{c}
\text{CH} \\
R^{17} \\
\text{CH} \\
\text{O} \\
\text{CO} \\
R^{18} \\
\text{CH} \\
R^{20}
\end{array}
\]

wherein R¹₈ is a (C₅₋₁₀) alkyl or (C₆₋₁₀) alkenyl chain containing a disulphide bond, and is terminated by bonding to CR¹¹;

R¹⁷ and R²⁰ are H, linear or branched (C₁₋₄) alkyl; linear or branched (C₁₋₄) alkenyl.

18. The use according to claim 17 wherein said inhibitors are selected from: trapoxin, HC-toxin, chlamydacin, apicidin, depsipeptide and pharmaceutically acceptable salts and solvates thereof.

19. The use according to claim 17, said inhibitors being selected from: dimethyl-depsipeptide (FK228), cyclo-(Aoe-D-Tyr(Me)-Ile-Pip) (Cyl-2) and pharmaceutically acceptable salts or solvates thereof.

20. The use according to claim 17, said inhibitors being compounds belonging to the CHAPs family and pharmaceutically acceptable salts or solvates thereof.
21. The use according to claim 20 wherein said CHAPs are selected from:
\[
\begin{align*}
& \text{cyclo(Asu(NHOH)-Phe-Phe-D-Pro)} \quad \text{(CHAP1)}, \\
& \text{cyclo(Asu(NHOH)-D-Tyr(Me)-Ile-Pip)} \quad \text{(CHAP49)}, \\
& \text{cyclo(Asu(NHOH)-D-Tyr(Me)-Ile-Pro)} \quad \text{(CHAP30)}, \\
& \text{cyclo(Asu(NHOH)-D-Phe-Leu-Pip)} \quad \text{(CHAP53)}, \\
& \text{cyclo(Asu(NHOH)-Aib-Phe-D-Pro)} \quad \text{(CHAP15)}, \\
& \text{cyclo(Asu(NHOH)-D-Pro-Ala-D-Ala)} \quad \text{(CHAP13)}, \\
& \text{cyclo(-L-Asu(NHOH)-D-Tyr(Me)-L-Ile-D-Pip)} \quad \text{(CHAP50)}, \\
& \text{cyclo(-L-Asu(NHOH)-D-Tyr(Me)-L-Ile-L-Pip)} \quad \text{(CHAP49)}, \\
& \text{cyclo(-L-Asu(NHOH)-D-Tyr-L-Ile-D-Pro)} \quad \text{(CHAP77)}, \\
& \text{cyclo(-L-Asu(NHOH)-D-Tyr(Me)-L-Ala-D-Pro)} \quad \text{(CHAP44)}, \\
& \text{cyclo(-L-Asu(NHOH)-D-Phe-L-Phe-D-Pip)} \quad \text{(CHAP57)}, \\
& \text{cyclo(-L-Asu(NHOH)-D-Phe-L-Ala-D-Pro)} \quad \text{(CHAP56)}, \\
& \text{cyclo(-L-Asu(NHOH)-L-Ala-L-Phe-D-Pro)} \quad \text{(CHAP80)}, \\
& \text{cyclo(-L-Asu(NHOH)-D-Phe-L-Phe-D-Pro)} \quad \text{(CHAP27)}. \\
\end{align*}
\]

22. The use according to any of the claims 1 to 8, said inhibitors being compounds of formula (IV), pharmaceutically acceptable salts and solvates thereof, R or S enantiomers, racemic mixtures, diastereoisomeric mixtures:
Formula (IV)

wherein B is linear or branched (C_{1-16}) alkyl; linear or branched (C_{1-16}) alkenyl.

R^{21} and R^{22} are

\[ \text{CH} \overline{\text{R^{23}}} \text{OH} \]

or linear or branched (C_{1-6}) alkyl; linear or branched (C_{1-6}) alkenyl; (C_{1-10}) alkylaryl either non substituted or substituted with (C_{1-4}) alkoxy, linear or branched (C_{1-4}) alkyl, linear or branched (C_{1-4}) alkenyl, halo, cyano, nitro, trifluoromethyl.

R^{23} is linear or branched (C_{1-6}) alkyl; linear or branched (C_{1-6}) alkenyl.

23. The use according to claim 22 wherein said inhibitor is depudesin and pharmaceutically acceptable salts and solvates thereof.

24. The use according to any of the claims 1 to 8, said inhibitors being compounds of formula (V), pharmaceutically acceptable salts and solvates thereof, R and S enantiomers, racemic mixtures, diastereoisomeric mixtures:
Formula (V)

wherein \( n = 0,1 \)

wherein \( R^{25} \) is \( NR^{26}R^{27} \); a hydroxyl group; a carboxylic group; halo; cyano; nitro and

\( R^{26} \) and \( R^{27} \) are \( H \); linear or branched \((C_{1-6}) \) alkyl; linear or branched \((C_{1-6}) \) alkenyl; saturated or aromatic 5 or 6 member rings.

\( R^{24} \) is \( H \); linear or branched \((C_{1-6}) \) alkyl; linear or branched \((C_{1-6}) \) alkenyl; a saturated or aromatic 5 or 6 member ring; or is \( COOR^{28} \) or \( COR^{29} \) wherein \( R^{28} \) and \( R^{29} \) are selected from linear or branched \((C_{1-6}) \) alkyl; linear or branched \((C_{1-6}) \) alkenyl; \((C_{1-16}) \) alkylaryl; \((C_{1-16}) \) alkylheteroaryl.

25. The use according to claim 24 wherein said inhibitors are selected from: \( N \)-acetyldinaline and MS-275 and pharmaceutically acceptable salts and solvates thereof.

26. The use according to any of the claims 1 to 25, said inhibitors being selected from: valproic acid and tricostatin A and pharmaceutically acceptable salts and solvates thereof.

27. The use according to any of the claims 1 to 8, said
inhibitors being selected from:
pivaloyloxymethylbutyrate; cyclostellattamine,
particularly cyclostellattamine A, cyclostellattamine G,
dehydrocyclostellattamine D and

dehydrocyclostellattamine E.

28. The use according to any of the claims 1 to 8, said
inhibitors being selected from hydroxamic acids having
the following structural formulae:
29. The use according to any of the claims 1 to 8, said inhibitors being compounds of formula (VI) and pharmaceutically acceptable salts and solvates thereof, R or S enantiomers, racemic mixtures and diastereoisomeric mixtures:
formula (VI)

wherein X = 1, 2; n = 1, 2, 3; R30 = H, CH₃.

30. The use according to claim 27, said inhibitors being selected from: cyclo(-L-Lys(For, OH)-D-Tyr(Me)-L-Ile-D-Pip), cyclo(-L-Hly(For, OH)-D-Tyr(Me)-L-Ile-L-Pip), cyclo(-L-Hly(For, OH)-D-Tyr(Me)-L-Ile-D-Pip), cyclo(-L-Aoc(For, OH)-D-Tyr(Me)-L-Ile-L-Pip), cyclo(-L-Aoc(For, OH)-D-Tyr(Me)-L-Ile-D-Pip), cyclo(-L-Lys(For, OH)-D-Tyr(Me)-L-Ile-L-Pip).

31. The use according to any of the claims 1 to 28, said inhibitors being LBH589.

32. A hypercholesterolaemia and atherosclerosis treatment method, including positive transcriptional modification of the gene encoding cholesterol 7α-hydroxylase.

33. The treatment method according to claim 32, said modulation comprising the administration of pharmaceutically efficacious doses of histone deacetylase inhibitors to patients.

34. The treatment method according to claim 32, said modulation comprising the administration of
pharmacologically efficacious doses to patients of small interfering RNAs (siRNA) which selectively silence the expression of the histone deacetylases involved in the negative regulation of the cholesterol 7α-hydroxylase gene.

35. A method for the prevention of cardiovascular, cerebrovascular pathologies and Alzheimer’s disease comprising the positive transcriptional modification of the gene encoding cholesterol 7α-hydroxylase.

36. The treatment method according to claim 35, said modulation comprising the administration to patients of pharmacologically efficacious doses of small interfering RNAs (siRNA) which selectively silence the expression of the histone deacetylases involved in the negative regulation of the cholesterol 7α-hydroxylase gene.

37. The treatment method according to claim 35, said modulation comprising the administration of pharmacologically efficacious doses of histone deacetylase inhibitors to patients.

38. The method according to any of the claims 35 to 37 wherein said cardiovascular and cerebrovascular diseases are: myocardial infarction and stroke.

39. A treatment method for hypertriglyceridaemias and obesity, comprising the positive modification of the gene encoding cholesterol 7α-hydroxylase.
40. The treatment method according to claim 39, said modulation comprising the administration of pharmaceutically efficacious doses of histone deacetylase inhibitors to patients.

41. The treatment method according to claim 39, said modulation comprising the administration of pharmaceutically efficacious doses to patients of small interfering RNAs (siRNA) which selectively silence the expression of the histone deacetylases involved in the negative regulation of the cholesterol 7α-hydroxylase gene.

42. A treatment method for diabetes and metabolic syndromes, comprising the positive modification of the gene encoding cholesterol 7α-hydroxylase.

43. The treatment method according to claim 42, said modulation comprising the administration of pharmaceutically efficacious doses of histone deacetylase inhibitors to patients.

44. The treatment method according to claim 42, said modulation comprising the administration of pharmaceutically efficacious doses to patients of small interfering RNAs (siRNA) which selectively silence the expression of the histone deacetylases involved in the negative regulation of the cholesterol 7α-hydroxylase gene.