**Title:** USE OF ATORVASTATIN LACTOLS AS MEDICAMENTS

**Abstract:** This invention relates to the discovery of novel atorvastatin analogues. More specifically, the invention relates to novel atorvastatin analogues which have utility in treating conditions treatable by the inhibition of HMG-CoA reductase.


**Designated States (unless otherwise indicated, for every kind of regional protection available):** ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Declarations under Rule 4.17:**
- of inventorship (Rule 4A.7(iv))
- with international search report (Art. 21(3))
USE OF ATORVASTATIN LACTOLS AS MEDICAMENTS

The present invention relates to atorvastatin lactols. In particular, the present invention relates to the use of atorvastatin lactols in the manufacture of a medicament for treating certain conditions. Conditions that are treatable using the compounds of the present invention include conditions which are modulated by the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase). Inhibition of the enzyme therefore represents a viable therapy for a number of diseases. The compounds used in the invention are 6-(3- or 4-carboxamido-substituted pyrrol-1-yl)-4-hydroxy-3,5-dihydro-pyran-2-ol derivatives.

Trans-6-[2-(3- or 4-carboxamido-substituted pyrrol-1-yl)alkyl]-4-hydroxy-3,5-dihydro-pyran-2-ol derivatives are lactones which were first disclosed in US 4,681,893. This document also disclosed their corresponding ring opened acid equivalents. The lactones apparently do not have intrinsic activity of their own. However, the corresponding ring-opened acid equivalents are useful as cholesterol biosynthesis inhibitors. Also disclosed in US 4,681,893 are various methods of manufacture for such compounds.

Atorvastatin, which is the R form of the ring-opened acid of trans-5-(4-fluorophenyl)-2-(1-methylethyl)-N,4-diphenyl-1-[2-tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-1H-pyrrole-3-carboxamide, and its use in the inhibition of the biosynthesis of cholesterol was first disclosed in EP 0409281. Atorvastatin both in racemic form, and in the form of its [R-(R',R'')] isomer is a potent inhibitor of HMG-CoA enzyme.

Clin Invest Med, Volume 24, No 5, p258-72, 2001 (Baker and Tamopolsky) discloses that whilst statins having an open, hydroxy acid conformation are active, the lactone, closed-ring analogue is inactive. Hepatic hydrolysis at alkaline pH decyclises and hence activates the lactone prodrugs lovastatin and simvastatin in vivo. However, one problem with such compounds is that extensive first path metabolism leads to rapid clearance of these statins.

Similarly, Trends in Pharmacological Sciences, Volume 19, Issue 1, 1 January 1998, Pages 26-37 discloses that the inactive lactones must be metabolised to their corresponding open hydroxy acid forms in order to inhibit HMG-CoA reductase.
The lactone form, and also the ring opened active form, may suffer problems in terms of stability over an extended period of time. This represents a significant problem during manufacture of an active principal or during extended storage of the same in a pharmacy. For example, loss of the hydroxy group in a dehydration reaction may occur. The resulting decomposition product may have a double bond that is conjugated with the lactone carbonyl group and this may tend to favour formation of the decomposition product. Equally, in the ring opened form, one of the possible decomposition products could also have a conjugated double bond with the acid carbonyl group.

It is therefore an aim of the present invention to provide compounds capable of inhibiting HMG-CoA reductase. Atorvastatin is a very potent inhibitor of HMG-CoA reductase. It is also therefore an aim of the present invention to provide compounds capable of inhibiting HMG-CoA reductase which have an IC50 value comparable to or better than that of atorvastatin. Ideally, these compounds will have good stability and bioavailability relative to atorvastatin. It is thus an aim to provide compounds having improved stability. Ideally, the compounds will have an extended shelf-life. It is thus an aim of the present invention to provide compounds capable of inhibiting HMG-CoA reductase which have increased half-life. It is thus an aim of the present invention to provide further compounds capable of inhibiting HMG-CoA reductase and having improved bioavailability. It is also an aim of the present invention to provide compounds capable of inhibiting HMG-CoA reductase and increasing promotion of high density lipoprotein (HDL). It is also an aim of the present invention to provide compounds capable of reducing low density lipoprotein (LDL) and increasing promotion of high density lipoprotein (HDL). Specifically, it is an aim of the present invention to provide compounds capable of reducing low density lipoprotein (LDL) and increasing promotion of high density lipoprotein (HDL) by more than 10%, preferably up to 15% or higher. The invention thus seeks to provide therapies for inhibiting cholesterol biosynthesis. The invention also aims to treat a range of diseases in which cholesterol formation is inhibited.

This invention provides compounds that achieve one or more of the above aims.

According to one aspect, the present invention provides a use of a compound of Formula I and pharmaceutically acceptable salts and solvates thereof:
in the manufacture of a medicament for treating a condition which is modulated by the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase), wherein:

R¹, R⁴ and one of R² and R³ are independently selected from the group comprising:
hydrogen, halo, C₁-₆ alkyl, C₂-₆ alkenyl, C₃-₆ cycloalkyl, aryl, C₄ alkyl aryl, heterocyclyl, and C₅-₆ alkyl heteroaryl;

the other of R² and R³ is -CONR⁹R¹₀ where R⁹ and R¹₀ are independently selected from the group comprising: hydrogen, C₁-₆ alkyl, C₂-₆ alkenyl, aryl, C₄ alkyl aryl, heteroaryl, C₅-₆ heteroaryl;

R⁵ and R⁶ are independently selected from the group comprising: hydrogen, C₁-₆ alkyl, C₁-₆ haloalkyl, C₂-₆ alkenyl, C₃-₆ cycloalkyl, aryl, C₁-₆ alkyl aryl, C₁-₆ alkanoyl aryl, heteroaryl, C₁-₆ alkanoyl heteroaryl, and C₅-₆ alkyl heteroaryl; provided always that both R⁵ and R⁶ are not hydrogen;

R⁷ and R⁸ are independently selected from the group comprising: H, C₄ alkyl and halo;

X is -(CRᵃRᵇ)ₘ(CRᵃ=CRᵇ)ₙ(CRᵃRᵇ)₀ where Rᵃ and Rᵇ are independently selected from the group comprising: H, methyl, ethyl and halo and m, n, and o are independently 0, 1, 2, or 3 provided that m + n + o is not more than 3; and wherein each of the above groups R¹ to R¹₀ may, where chemically possible, be independently optionally substituted by from 1 to 5 groups chosen independently at each occurrence from the groups comprising: halo, C₃ alkyl, halo C₃ alkyl, C₃ alkoxy, C₃ haloalkoxy, hydroxy, and cyano.

Usually conditions that are modulated by HMG-CoA reductase are conditions that would be treated by the inhibition of the enzyme using a compound of the present invention.
According to another aspect, the present invention provides a compound of Formula I and pharmaceutically acceptable salts and solvates thereof:

\[(\text{I})\]

for use in treating a condition treatable by the inhibition of the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) wherein \(R^1 - R^{10}, R^a, R^b, X, m, n\) and \(o\) are as defined above.

According to another aspect, the present invention provides a method of treating a condition treatable by the inhibition of the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) comprising administering an effective amount of a compound of Formula I:

\[(\text{I})\]

or a pharmaceutically acceptable salt or solvate thereof, wherein \(R^1 - R^{10}, R^a, R^b, X, m, n\) and \(o\) are as defined above.

Compounds have activity in their own right or may in certain cases ring open under physiological conditions to corresponding compounds having inhibitory activity.

Pharmaceutically acceptable salts of the compounds of formula (1) include the acid addition and base salts thereof.

Suitable acid addition salts are formed from acids which form non-toxic salts. Examples include the acetate, aspartate, benzoate, besylate, bicarbonate/carbonate, bisulphate/sulphate, borate, camsylate, citrate, edisylate, esylate, formate, fumarate,
gluceptate, gluconate, glucuronate, hexafluorophosphate, hibenzate, hydrochloride/chloride, hydrobromide/bromide, hydroiodide/iodide, isethionate, lactate, malate, maleate, malonate, mesylate, methylsulphate, naphthylate, 1,5-naphthalenedisulfonate, 2-napsylate, nicotinate, nitrate, orotate, oxalate, palmitate, pamoate, phosphate/hydrogen phosphate/dihydrogen phosphate, saccharate, stearate, succinate, tartrate, tosylate and trifluoroacetate salts.

Suitable base salts are formed from bases which form non-toxic salts. Examples include the aluminium, arginine, benzathine, calcium, choline, diethylamine, diolamine, glycine, lysine, magnesium, meglumine, olamine, potassium, sodium, tromethamine and zinc salts. Hemisalts of acids and bases may also be formed, for example, hemisulphate and hemicalcium salts. For a review on suitable salts, see "Handbook of Pharmaceutical Salts: Properties, Selection, and Use" by Stahl and Wermuth (Wiley-VCH, Weinheim, Germany, 2002).

Pharmaceutically acceptable salts of compounds of formula (1) may be prepared by one or more of three methods:

(i) by reacting the compound of formula (1) with the desired acid or base;
(ii) by removing an acid- or base-labile protecting group from a suitable precursor of the compound of formula (1) or by ring-opening a suitable cyclic precursor, for example, a lactone or lactam, using the desired acid or base; or
(iii) by converting one salt of the compound of formula (1) to another by reaction with an appropriate acid or base or by means of a suitable ion exchange column.

All three reactions are typically carried out in solution. The resulting salt may precipitate out and be collected by filtration or may be recovered by evaporation of the solvent. The degree of ionisation in the resulting salt may vary from completely ionised to almost non-ionised.

The compounds of the invention may exist in both unsolvated and solvated forms. The term 'solvate' is used herein to describe a molecular complex comprising the compound of the invention and a stoichiometric amount of one or more pharmaceutically acceptable solvent molecules, for example, ethanol. The term 'hydrate' is employed when said solvent is water.
Included within the scope of the invention are complexes such as clathrates, drug-host inclusion complexes wherein, in contrast to the aforementioned solvates, the drug and host are present in stoichiometric or non-stoichiometric amounts. Also included are complexes of the drug containing two or more organic and/or inorganic components which may be in stoichiometric or non-stoichiometric amounts. The resulting complexes may be ionised, partially ionised, or non-ionised. For a review of such complexes, see J Pharm Sci, 64 (8), 1269-1288 by Halebian (August 1975).

Hereinafter all references to compounds of formula (1) include references to salts, solvates and complexes thereof and to solvates and complexes of salts thereof.

The compounds of the invention include compounds of formula (1) as hereinbefore defined, including all polymorphs and crystal habits thereof, prodrugs and isomers thereof (including optical, geometric and tautomeric isomers) as hereinafter defined and isotopically-labeled compounds of formula (1).

Before purification, the compounds of the present invention may exist as a mixture of enantiomers depending on the synthetic procedure used. For example, the compounds of the present invention may exist as a mixture of enantiomers having a ratio of between 2:1 and 3:1, though they may also occur in other ratios. The enantiomers can be separated by conventional techniques known in the art. Thus the invention covers individual enantiomers as well as mixtures thereof. When the chemical structures disclosed herein includes an "*", it is intended that the compound is a mixture of enantiomers having a ratio of between 2:1 and 3:1.

For some of the steps of the process of preparation of the compounds of formula (1), it may be necessary to protect potential reactive functions that are not wished to react, and to cleave said protecting groups in consequence. In such a case, any compatible protecting radical can be used. In particular methods of protection and deprotection such as those described by T.W. GREENE (Protective Groups in Organic Synthesis, A. Wiley-Interscience Publication, 1981) or by P. J. Kocienski (Protecting groups, Georg Thieme Verlag, 1994), can be used. All of the above reactions and the preparations of novel starting materials used in the preceding methods are conventional and appropriate reagents and reaction conditions for their performance or preparation as well as procedures for isolating the desired products will be well-known to those skilled
in the art with reference to literature precedents and the examples and preparations hereto.

Also, the compounds of formula (1) as well as intermediate for the preparation thereof can be purified according to various well-known methods, such as for example crystallization or chromatography.

In an embodiment, \( R^1 \) is selected from the group comprising: hydrogen, \( \text{C}_i \text{C}_j \) alkyl, \( \text{C}_2 \text{C}_6 \) alkenyl or \( \text{C}_3 \text{C}_5 \) cycloalkyl. In an embodiment, \( R^1 \) is selected from the group comprising: \( \text{C}_2 \text{C}_6 \) alkenyl or \( \text{C}_3 \text{C}_5 \) cycloalkyl. In an alternative embodiment, \( R^1 \) is \( \text{C}_i \text{C}_j \) alkyl. In an embodiment, \( R^1 \) is methyl, ethyl, propyl or butyl. In an embodiment, \( R^1 \) is \( \text{-} \text{propyl} \).

In an embodiment, \( R^2 \) is -\( \text{CONR} \) \( \text{R}^9 \text{R}^{10} \).

In an embodiment, \( R^3 \) is selected from the group comprising: aryl, \( \text{C}_i \text{C}_j \) alkyl aryl, heteroaryl and \( \text{C}_i \text{C}_j \) alkyl heteroaryl. In an embodiment, \( R^3 \) is selected from the group comprising: aryl and \( \text{C}_i \text{C}_j \) alkyl aryl. In an embodiment, \( R^3 \) is aryl. In an embodiment, \( R^3 \) is phenyl.

In an embodiment, \( R^4 \) is selected from the group comprising: aryl, \( \text{C}_i \text{C}_j \) alkyl aryl, heteroaryl and \( \text{C}_i \text{C}_j \) alkyl heteroaryl, wherein each of the aforementioned groups may be optionally substituted as discussed above in relation to the first aspect. In an embodiment, \( R^4 \) is selected from the group comprising: aryl and \( \text{C}_i \text{C}_j \) alkyl aryl. In an embodiment, \( R^4 \) is aryl. In an embodiment, \( R^4 \) is phenyl. In an embodiment, \( R^4 \) is substituted with halo, optionally wherein the halo is fluorine. In an embodiment, \( R^4 \) is 4-fluorophenyl.

In an embodiment, \( R^5 \) is selected from the group comprising: hydrogen, \( \text{C}_i \text{C}_j \) alkyl, aryl, \( \text{C}_i \text{C}_j \) alkyl aryl, heteroaryl and \( \text{C}_i \text{C}_j \) alkyl heteroaryl. In an embodiment, \( R^5 \) is selected from the group comprising: hydrogen, \( \text{C}_i \text{C}_j \) alkyl aryl and \( \text{C}_i \text{C}_j \) alkyl heteroaryl. In an embodiment, \( R^5 \) is \( \text{C}_i \text{C}_j \) alkanoyl heteroaryl, e.g. methanoyl heteroaryl. In a preferred embodiment, \( R^5 \) is methanoyl pyridyl, e.g. 2-methanolyl pyridine, 3-methanolyl pyridine or 4-methanolyl pyridine, preferably 3-methanolyl pyridine. In an embodiment, \( R^5 \) is hydrogen. In an alternative embodiment, \( R^5 \) is \( \text{C}_i \text{C}_j \) alkyl aryl, e.g. -\( \text{C}_i \) alkyl-Ph, -\( \text{C}_2 \) alkyl-Ph, -\( \text{C}_3 \) alkyl-Ph, or -\( \text{C}_4 \) alkyl-Ph. In an embodiment, \( R^5 \) is benzyl.
In an embodiment, $R^6$ is the same as $R^5$.

In a further embodiment, $R^6$ is selected from the group comprising: $C_{1-6}$ alkyl, $C_{2-6}$ alkenyl, $C_{3-6}$ cycloalkyl and aryl. In a preferred embodiment, $R^6$ is $C_{i-6}$ alkyl. In further preferred embodiment, $R^6$ is methyl, ethyl, n-propyl, i-propyl or t-butyl. In another preferred embodiment, $R^6$ is $C_{2-6}$ alkenyl. In another preferred embodiment, $R^6$ is allyl.

In another preferred embodiment, $R^6$ is $C_{3-6}$ cycloalkyl. In another preferred embodiment, $R^6$ is cyclohexyl. In another embodiment, $R^6$ is aryl, such as optionally substituted phenyl.

In an embodiment, $R^7$ is H.

In an embodiment, $R^8$ is H.

In an embodiment, $R^9$ is hydrogen or $C_{i-4}$ alkyl. In an embodiment, $R^9$ is hydrogen.

In an embodiment, $R^{10}$ is selected from the group comprising: aryl, $C_{i-4}$ alkyl aryl, heteroaryl and $C_{i-4}$ alkyl heteroaryl. In an embodiment, $R^{10}$ is selected from the group comprising: aryl and $C_{i-4}$ alkyl aryl. In an alternative embodiment, $R^{10}$ is selected from the group comprising: heteroaryl and $C_{i-4}$ alkyl heteroaryl. In an embodiment, $R^{10}$ is phenyl.

In an embodiment, $n$ is 0. In an embodiment, $m = 1$, $n = 0$ and $o = 1$ or $m = 2$, $n = 0$ and $o = 0$ or $m = 0$, $n = 0$ and $o = 2$. In an alternative embodiment, $m = 3$, $n = 0$ and $o = 0$.

In an alternative embodiment, $m = 1$, $n = 0$ and $o = 0$. In an alternative embodiment, $m = 1$, $n = 1$ and $o = 0$, or $m = 0$, $n = 1$ and $o = 1$.

In an embodiment, $R^a$ is H at each occurrence.

In an embodiment, $R^b$ is H at each occurrence.

In a further embodiment, each $R^a$ is H, each $R^b$ is H and $m = 2$, $n = 0$ and $o = 0$.

Aryl groups include aromatic ring systems comprising $6, 7, 8, 9, 10, 11, 12, 13, 14, 15$ or $16$ ring carbon atoms. Aryl groups may consist of a single ring but may include a
polycyclic ring system, having two or more rings, at least one of which is aromatic. Aryl groups include: phenyl, naphthyl, fluorenyl, azulenyl, indenyl and anthryl groups.

In an embodiment, the aryl group is phenyl.

Heteroaryl groups include aromatic heterocyclic ring systems having 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 ring atoms with 1 to 4 heteroatoms independently selected from nitrogen, oxygen and sulfur. The group may be a polycyclic ring system, having two or more rings, at least one of which is aromatic, but is more often monocyclic. Preferred heteroaryl groups are monocyclic groups containing 5 or 6 ring atoms. Heteroaryl groups include: pyrrolyl, pyrazolyl, imidazolyl, pyrazinyl, oxazolyl, isoxazolyl, thiazolyl, furyl, thiophenyl, pyridyl, pyrimidyl, benzimidazolyl, indolyl, isoquinolyl, quinoxalinyl and quinolyl.

In an embodiment, the heteroaryl group is selected from the group comprising: pyridine, pyrimidine, pyrazine, pyrazole, and oxazole. Preferably the heteroaryl group is pyridine.

When one or more of the above groups is optionally substituted, each optional substituent is preferably an independently chosen halo atom. Amongst halo, chloro and fluoro are preferred. Preferably, the halo atoms are the same when there are more than one.

In an embodiment, $R_1$ is $C_{1-4}$alkyl, preferably i-propyl, and $R_4$ is optionally substituted aryl, preferably 4-fluorophenyl.

In another embodiment, $R_2$ is -CONR$_9$R$_{10}$ wherein $R_9$ is optionally substituted aryl, preferably phenyl; $R_{10}$ is hydrogen; and $R_3$ is optionally substituted aryl, preferably phenyl.

In a further embodiment, $R_1$ is $C_{i-4}$alkyl, preferably i-propyl; $R_2$ is -CONR$_9$R$_{10}$ wherein $R_9$ is optionally substituted aryl, preferably phenyl, $R_{10}$ is hydrogen; $R_3$ is optionally substituted aryl, preferably phenyl; and $R_4$ is optionally substituted aryl, preferably 4-fluorophenyl.
The relationship between the groups $R^5$ and $R^6$ is important for the activity of the compounds. Thus both $R^5$ and $R^6$ cannot be hydrogen. In one embodiment, $R^5$ is not hydrogen. In one embodiment, $R^6$ is not hydrogen.

In another embodiment, $R^5$ is optionally substituted benzyl and $R^6$ is optionally substituted $C_{1-6}$ alkyl. In this embodiment, preferably the alkyl group is propyl or butyl, preferably isopropyl or tertbutyl. In another embodiment, $R^5$ is optionally substituted benzyl and $R^6$ is optionally substituted $C_{2-6}$ alkenyl. In this embodiment, preferably the alkenyl group is allyl.

In another embodiment, $R^5$ is $H$ and $R^6$ is optionally substituted $d$-$6$ alkyl. In this embodiment, preferably the alkyl group is propyl. In another embodiment, $R^5$ is $H$ and $R^6$ is $c3$-$6$ cycloalkyl. In this embodiment, preferably the cycloalkyl group is cyclohexyl.

In another embodiment, $R^5$ is $H$ and $R^6$ is optionally substituted aryl. In this embodiment, preferably the optionally substituted aryl is optionally substituted phenyl, e.g. phenyl substituted by alkoxy (e.g. methoxy).

In another embodiment, $R^5$ is $C_{1-6}$ alkanoyl heteroaryl and $R^6$ is optionally substituted $C_{1-6}$ alkyl. In this embodiment, the alkyl group is preferably methyl and the $C_{1-6}$ alkanoyl heteroaryl group is preferably methanoyl heteroaryl, (e.g. methanoyl pyridine).

In an embodiment, the compound has a structure selected from:
As mentioned above, statins having an open, hydroxy acid conformation are known to have an inhibitory effect on HMG-CoA reductase. It is also known that the lactone, closed-ring analogue of such hydroxy acids are inactive with respect to inhibiting HMG-CoA reductase and that decyclisation of the lactone is necessary to activate the lactone. However, we have found that functionalised lactols of the present invention have a significant inhibitory effect on HMG-CoA reductase in their own right. This is surprising in view of the fact that these molecules are conformationally constrained in ring closed form.

Examples of conditions that may be treated by the inhibition of HMG-CoA reductase include hypercholesterolemia, atherosclerosis and hyperlipidemia. Statins have been used in the secondary prevention of cardiovascular disease, or in the primary prevention of cardiovascular disease when the risk for cardiovascular disease is significantly raised. It is therefore expected that the compound of the present invention will have utility in the treatment or prevention of cardiovascular diseases due to their inhibitory activity. Example cardiovascular diseases which may be treatable by the compounds of the present invention include: coronary heart disease, myocardial infarction, stroke and peripheral artery disease. In addition, these compounds may also have a beneficial effect in the treatment of inflammation, dementia, cancer, nuclear cataracts, diabetes and hypertension.
The conditions that may be treated by the inhibition of HMG-CoA reductase may be a condition of the human or animal body. These compounds are intended in particular for human patients.

The atorvastatin derivatives of the present invention can be assayed using the following procedure in which the plasma triglyceride level is measured after treating a rat with a compound of the present invention (or atorvastatin). The change in rat plasma triglyceride levels is considered to be a fair test for determining HMG CoA reductase activity.

The procedure used is as follows: male SD rats (Harlan) are housed in groups of 6 under a 12h light dark cycle (lights on 07.00 h) with free access to food (normal laboratory chow) and water. Animals between 148-183 g are allocated to treatment groups of 8 balanced by body weight and treatments are balanced across cages.

Solutions including 5 mg/mL of the atorvastatin analogues (in e.g. 10% PEG300/10% cremophor/80% methyl cellulose (0.5%)) and a suspension including 5mg/kg of atorvastatin (formulated in 0.5% Tween in 0.5% methyl cellulose) are made.

The rat subjects are orally dosed with one of the atorvastatin analogues (25mg/kg) or atorvastatin (25 mg/kg po), BID for 3 or 5 days.

Sixteen hours after the last treatment, terminal plasma samples are taken, stored at -20°C, and transported on dry ice for analysis of triglyceride levels.

Data for each time-point are analysed by 1-way ANOVA and post-hoc Dunnett's test.

Processes for the manufacture of the compounds of the present invention are disclosed in WO2005/012246, in particular, in the examples. The disclosure of WO2005/012246 insofar as the synthetic procedures are concerned forms part of the disclosure of the present invention. In the interests of brevity, the details of these synthetic procedures is not reproduced here but it is intended that this subject matter is specifically incorporated into the disclosure of this document by reference.

The present invention also includes the synthesis of all pharmaceutically acceptable isotopically-labelled compounds of formula (I) wherein one or more atoms are replaced
by atoms having the same atomic number, but an atomic mass or mass number different from the atomic mass or mass number usually found in nature.

Examples of isotopes suitable for inclusion in the compounds of the invention include isotopes of hydrogen, such as $^2$H and $^3$H, carbon, such as $^{11}$C, $^{13}$C and $^{14}$C, chlorine, such as $^{35}$Cl, fluorine, such as $^{18}$F, iodine, such as $^{123}$I and $^{125}$I, nitrogen, such as $^{15}$N and $^{15}$N, oxygen, such as $^{15}$O, $^{17}$O and $^{18}$O, phosphorus, such as $^{32}$P, and sulphur, such as $^{35}$S.

Certain isotopically-labelled compounds, for example, those incorporating a radioactive isotope, are useful in drug and/or substrate tissue distribution studies. The radioactive isotopes tritium, i.e. $^3$H, and carbon-14, i.e. $^{14}$C, are particularly useful for this purpose in view of their ease of incorporation and ready means of detection.

Substitution with heavier isotopes such as deuterium, i.e. $^2$H, may afford certain therapeutic advantages resulting from greater metabolic stability, for example, increased in vivo half-life or reduced dosage requirements, and hence may be preferred in some circumstances.

Substitution with positron emitting isotopes, such as $^{11}$C, $^{18}$F, $^{15}$O and $^{15}$N, can be useful in Positron Emission Topography (PET) studies for examining substrate receptor occupancy.

Isotopically-labelled compounds can generally be prepared by conventional techniques known to those skilled in the art or by processes analogous to those described using an appropriate isotopically-labelled reagent in place of the non-labelled reagent previously employed.

Throughout the description and claims of this specification, the words "comprise" and "contain" and variations of the words, for example "comprising" and "comprises", means "including but not limited to", and is not intended to (and does not) exclude other moieties, additives, components, integers or steps.

Throughout the description and claims of this specification, the singular encompasses the plural unless the context otherwise requires. In particular, where the indefinite
article is used, the specification is to be understood as contemplating plurality as well as
singularity, unless the context requires otherwise.

Features, integers, characteristics, compounds, chemical moieties or groups described
in conjunction with a particular aspect, embodiment or example of the invention are to be understood to be applicable to any other aspect, embodiment or example described herein unless incompatible therewith.

**General Procedure**

All assays were carried out in a reaction buffer containing 100mM K$_2$PO$_4$ at pH 7.2, 1mM EDTA, 500mM KCl and 1mg/ml BSA. The concentrations of NADPH and HMG-CoA were both 200μM. The enzyme concentration used is unknown although this concentration is 10-fold lower than that of the stock solution purchased. Inhibitors were dissolved in 75% DMSO. Where inhibitors were found to be insoluble or only partly soluble in 75% DMSO, 100% DMSO was used. Reactions were activated by the addition of enzyme and agitated for 12 seconds following the addition. Absorbance readings were then taken every 20 seconds for 600 seconds. In initial tests the concentration of each inhibitor was set at 50nM to identify which compounds were the better inhibitors, compared to the known Pravastatin inhibitor. After these were identified, assays were carried out varying their concentrations from 0nM to 50nM allowing IC50 values to be calculated.

**Example 1**

The following procedure was followed using a HMG-CoA Reductase assay kit obtained from Sigma-Aldrich (catalogue number CS1090). The assay is based on the spectrophotometric measurement of the decrease in absorbance at 340 nm of NADPH in solution. A decrease in absorbance is caused by the oxidation of NADPH by the catalytic subunit of HMGR in the presence of the substrate HMG-CoA. Effective inhibition of the HMG-CoA leads to a reduction in oxidation of NADPH which in turn leads to a smaller reduction in the absorbance at 340 nm over time. This is illustrated in the following reaction scheme:

\[
\text{HMG-CoA} + 2\text{NADPH} + 2\text{H}^+ \rightarrow \text{mevalonate} + 2\text{NADP}^- + \text{CoA-SH}
\]
Compounds showing the best inhibitory action are those which reduce the absorbance least.

**Preparation of the assay solution**

Ultrapure water (17 MΩ-cm or equivalent was used for the preparation of reagents and throughout the procedure.

First, an assay buffer solution was prepared using the following method: 0.2 ml of assay buffer, 5 x (catalogue number A5981) was diluted with 0.8 ml of ultrapure water. The resulting buffer solution was kept on ice or stored at -20°C for further use.

Next, 25 mg of NADPH (catalogue number N6505) was reconstituted with 1.5 ml of the buffer solution. The reconstituted NADPH was stored in working aliquots at -20°C.

The HMG-CoA substrate solution (catalogue number S7447), HMG-CoA reductase (catalogue number H8789) and inhibitor solution (e.g. pravastatin, catalogue number I5909) were kept on ice throughout the procedure.

1. Before beginning, the spectrophotometer was set at 37°C and 340 nm, with a kinetic programme: 1 ml sample, read every 20 seconds for up to 10 minutes.

2. The appropriate volumes of the reaction solutions were added according to Table 1 (1 ml assay).

**Table 1**

Reaction volumes for 1 ml samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>1x Assay buffer</th>
<th>Test compound / Pravastatin</th>
<th>NADPH</th>
<th>HMG-CoA</th>
<th>HGMG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>920 µl</td>
<td>-</td>
<td>20 µl</td>
<td>60 µl</td>
<td>-</td>
</tr>
<tr>
<td>Activity</td>
<td>915 µl</td>
<td>-</td>
<td>20 µl</td>
<td>60 µl</td>
<td>5 µl</td>
</tr>
<tr>
<td>Inhibition</td>
<td>910 µl</td>
<td>5 µl</td>
<td>20 µl</td>
<td>60 µl</td>
<td>5 µl</td>
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</tbody>
</table>
The reagents were added to the reaction in the following order:

a. Add a buffer to all samples.
b. Add the inhibitor (test compound/Pravastatin) to the inhibition sample.
c. Add the reconstituted NADPH to all samples.
d. Add Substrate Solution (HMG-CoA) to all samples.
e. Add HMG-CoA Reductase (HMGR) to the Activity and Inhibition samples.
f. Mix the samples thoroughly.

3. The kinetics programme was started immediately. The activity of the product was calculated according to the following equation:

\[
\text{Units/mgP} = \frac{(\Delta A_{340 \text{min sample}} - \Delta A_{340 \text{min control}}) \times TV}{12.44 \times V \times 0.6 \times LP}
\]

where:

12.44 = \epsilon_{\text{MM}} \text{ - the extinction coefficient for NADPH at 340 nm is 6.22 mM}^{-1}\text{cm}^{-1}. 12.44 \text{ represents the 2 NADPH consumed in the reaction.}

TV = total volume of the reaction in ml (1 ml for cuvettes)

V = volume of enzyme used in the assay (ml)

0.6 = enzyme concentration in mg-protein (mgP/ml) (0.55-0.65 mgP/ml)

LP = light path in cm (1 for cuvettes).

**Example 2**

The following table provides IC50 values for particular atorvastatin compounds of the present invention.

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<th>Compound Structure</th>
<th>IC_{50} (nM)</th>
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</tr>
<tr>
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<tr>
<td>4</td>
<td><img src="image" alt="Chemical Structure 4" /></td>
</tr>
</tbody>
</table>
Claims:

1. A compound of Formula I and pharmaceutically acceptable salts and solvates thereof:

   \[
   \text{(I)}
   \]

   for use in treating a condition treatable by the inhibition of the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase), wherein:

   \[R^1, R^4 \text{ and one of } R^2 \text{ and } R^3 \text{ are independently selected from the group comprising: hydrogen, halo, } C_{i-6} \text{ alkyl, } C_{2-6} \text{ alkenyl, } C_{3-6} \text{ cycloalkyl, aryl, } \text{Ci}_{-4} \text{ alkyl aryl, heterocycl}, \]

   \[\text{and } \text{Ci}_{-4} \text{ alkyl heteroaryl};\]

   \[\text{the other of } R^2 \text{ and } R^3 \text{ is } -\text{CONR}^9 \text{R}^{10} \text{ where } R^9 \text{ and } R^{10} \text{ are independently selected from the group comprising: hydrogen, } C_{i-6} \text{ alkyl, } C_{2-6} \text{ alkenyl, aryl, } \text{Ci}_{-4} \text{ alkyl aryl, heteroaryl, } \text{Ci}_{-4} \text{ heteroaryl};\]

   \[R^5 \text{ and } R^6 \text{ are independently selected from the group comprising: hydrogen, } C_{i-6} \text{ alkyl, } \text{Ci}_{-6} \text{ haloalkyl, } C_{2-6} \text{ alkenyl, } C_{3-6} \text{ cycloalkyl, aryl, } \text{Ci}_{-6} \text{ alkyl aryl, } \text{Ci}_{-6} \text{ alkanoyl aryl, heteroaryl, } \text{Ci}_{-6} \text{ alkanoyl heteroaryl, and } \text{Ci}_{-6} \text{ alkyl heteroaryl}; \text{provided always that both } R^5 \text{ and } R^6 \text{ are not hydrogen;}\]

   \[R^7 \text{ and } R^8 \text{ are independently selected from the group comprising: } H, \text{Ci}_{-4} \text{ alkyl and halo;}\]

   \[X \text{ is } -(\text{CR}^a \text{R}^b)_m(\text{CR}^a=\text{CR}^b)_n(\text{CR}^a \text{R}^b)_o \text{ where } R^a \text{ and } R^b \text{ are independently selected from the group comprising: } H, \text{methyl, ethyl and halo and } m, n, \text{ and } o \text{ are independently } 0, 1, 2, \text{ or } 3 \text{ provided that } m + n + o \text{ is not more than } 3; \text{and wherein}\]

   each of the above groups } R^1 \text{ to } R^{10} \text{ may, where chemically possible, be independently optionally substituted by from } 1 \text{ to } 5 \text{ groups chosen independently at each occurrence}
from the groups comprising: halo, $\text{C}_{\text{i-3}}$ alkyl, halo $\text{C}_{\text{i-3}}$ alkyl, $\text{C}_{\text{i-3}}$ alkoxy, $\text{C}_{\text{i-3}}$ haloalkoxy, hydroxy, and cyano.

2. A compound of claim 1, wherein $R^1$ is $\text{C}_{\text{i-6}}$ alkyl.

3. A compound of claim 1 or claim 2, wherein $R^2$ is $-\text{CONR}^9\text{R}^{10}$ where $R^9$ is H and $R^{10}$ is aryl.

4. A compound of any of claims 1 to 3, wherein $R^3$ is aryl.

5. A compound of any preceding claim, wherein $R^4$ is optionally substituted aryl.

6. A compound of any preceding claim, wherein $R^1$ is i-propyl, $R^2$ is $-\text{CONHPh}$, $R^3$ is phenyl and $R^4$ 4-fluorophenyl.

7. A compound of any preceding claim, wherein $R^5$ is selected from the group comprising: hydrogen, aryl, $\text{C}_{\text{i-6}}$ alkyl aryl, $\text{C}_{\text{i-6}}$ alkanoyl aryl, heteroaryl, $\text{C}_{\text{i-6}}$ alkanoyl heteroaryl, and $\text{C}_{\text{i-6}}$ alkyl heteroaryl.

8. A compound of claim 7, wherein $R^5$ is hydrogen.

9. A compound of claim 7, wherein $R^5$ is selected from the group comprising: $-\text{Ci alkyl-Ph}$, $-\text{C}_2\text{alkyl-Ph}$, $-\text{C}_3\text{alkyl-Ph}$, and $-\text{C}_4\text{alkyl-Ph}$.

10. A compound of claim 9, wherein $R^5$ is benzyl.

11. A compound of claim 7, wherein $R^6$ is $\text{C}_{\text{i-6}}$ alkanoyl pyridine.

12. A compound of claim 11, wherein $R^5$ is 3-methanoyl pyridine.

13. A compound of any preceding claim, wherein $R^6$ is selected from the group comprising: hydrogen, $\text{C}_{\text{i-6}}$ alkyl, $\text{C}_{\text{i-6}}$ haloalkyl, $\text{C}_{\text{2-6}}$ alkenyl, $\text{C}_{\text{3-6}}$ cycloalkyl, optionally substituted aryl and $\text{C}_{\text{i-6}}$ alkyl aryl.

14. A compound of claim 13, wherein $R^6$ is selected from the group comprising: $\text{C}_{\text{i-6}}$ alkyl, $\text{C}_{\text{3-6}}$ cycloalkyl, $\text{C}_{\text{2-6}}$ alkenyl and optionally substituted aryl.
15. A compound of claim 14 wherein R^6 is selected from the group comprising: methyl, ethyl, propyl, butyl, cyclohexyl and allyl.

16. A compound of claim 13, wherein R^6 is optionally substituted aryl.

17. A compound of claim 16, wherein R^6 is selected from the group comprising: C_6 alkoxy substituted phenyl.

18. A compound of claim 16, wherein R^6 is 2,4-dimethoxyphenyl.

19. A compound of any of claims 1 to claim 6, wherein R^5 is hydrogen and R^6 is an Ci-6 alkyl, C_3-6 cycloalkyl or an optionally substituted aryl.

20. A compound of any of claims 1 to claim 6, wherein R^5 is an optionally substituted benzyl and R^6 is an optionally substituted Ci-6 alkyl or an optionally substituted C_2-6 alkenyl.

21. A compound of any of claims 1 to claim 6, wherein R^5 is a d-alkanoyl heteroaryl and R^6 is an optionally substituted Ci-6 alkyl.

22. A compound of any preceding claim, wherein R^7 is H and R^8 is H.

23. A compound of any preceding claim, wherein each R^5 is H, each R^6 is H and m = 2, n = 0 and o = 0.

24. A compound of claim 1 which has a structure selected from:
25. A compound of any preceding claim, wherein condition treatable by the inhibition of HMG-CoA reductase is selected from the group comprising: hypercholesterolemia, atherosclerosis and hyperlipidemia.

26. A compound having a structure as defined in any of claims 1 to 24, for use in treating a condition selected from the group comprising: cardiovascular disease, coronary heart disease, myocardial infarction, stroke or peripheral artery disease.

27. A compound having a structure as defined in any of claims 1 to 24, for use in treating a condition selected from the group comprising: inflammation, dementia, cancer, nuclear cataracts, diabetes and hypertension.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/40 A61P9/00 A61P9/10 A61P3/06

B. CLASSIFICATION

A61K31/40 A61P9/00 A61P9/10

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>WO 2005/092867 A2 (AVECIA PHARMACEUTICALS LTD [GB]; MOODY DAVID JOHN [GB]; WIFFEN JONATHA) 6 October 2005 (2005-10-06) page 2, line 7 - line 19 page 5, line 16 - line 19</td>
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Further documents are listed in the continuation of Box C

See patent family annex

Date of the actual completion of the international search

20 April 2010

Date of mailing of the international search report

04/05/2010

Name and mailing address of the ISA/

European Patent Office, P B 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel (+31-70) 340-2040
Fax (+31-70) 340-3016

Authorized officer

Bonzano, Camilla
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<td>WO 2006/122644 A2 (RATIOPHARM GMBH [DE]; TARAROV VITALI [RU]; BOERNER ARMIN [DE]; KOENIG) 23 November 2006 (2006-11-23) page 29; example 7 claim 23</td>
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<td>TATAROV: &quot;Synthesis of the chiral side of chain of statins&quot; EUROPEAN JOURNAL OF ORGANIC CHEMISTRY, 2006, pages 5543-5550, XP8121532 figure 3</td>
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