Abstract:
The present invention relates to a pharmaceutical composition and a therapeutic combination comprising a novel cholesterol ester transfer protein (CETP) inhibitor and an adenosine triphosphate citrate lyase inhibitor/adenosine monophosphate-activated protein kinase activator, which may be used in the treatment of subjects suffering from or having an increased risk for cardiovascular diseases, in particular hyperlipidemia or mixed dyslipidemia.
PHARMACEUTICAL COMPOSITION AND THERAPEUTIC COMBINATION COMPRISING A CHOLESTERYL ESTER TRANSFER PROTEIN INHIBITOR AND AN ATP CITRATE LYASE INHIBITOR - AMPK ACTIVATOR

TECHNICAL FIELD OF THE INVENTION

The present invention relates to a pharmaceutical composition and a therapeutic combination comprising a novel cholesteryl ester transfer protein (CETP) inhibitor and an adenosine triphosphate citrate lyase (ACL) inhibitor / adenosine monophosphate-activated protein kinase (AMPK) activator, which may be used in the treatment of subjects suffering from or having an increased risk for cardiovascular diseases, in particular hyperlipidemia or mixed dyslipidemia.

BACKGROUND OF THE INVENTION

Prospective epidemiological studies have clearly established a strong association between low density lipoprotein-cholesterol (LDL-C) levels and risk for cardiovascular disease (CVD). Indeed, the results of the recent FMPROVE-IT trial showed that further LDL-C reductions that exceed the goal still result in better outcomes, and the IMPROVE-IT trial reaffirmed the causal role of LDL-C in CVD. A linear risk reduction in cardiovascular events in comparison to LDL-C levels is therefore expected. Based on this expectation, current thinking about LDL-C lowering treatment is therefore "the lower the LDL-C level, the better the risk reduction for the patient ".

3-Hydroxy-3-methylglutaryl coenzyme A reductase (hereinafter: HMG CoA reductase) is an enzyme in the liver that functions in the production of cholesterol. Inhibition of HMG CoA reductase by HMG CoA reductase inhibitors, commonly referred to as "statins", has been shown to reduce the level of LDL-C in the blood by reducing the production of cholesterol and by accelerating uptake of cholesterol. The application of such statin therapy to decrease the LDL-C level in the blood has resulted in a marked reduction of cardiovascular disease-related morbidity and mortality. However, there are safety issues associated with HMG CoA reductase inhibitors such as statins. Particularly at high doses, statins may cause an increase in liver enzymes and myopathy and occasionally rhabdomyolysis, which may lead to death from acute renal failure. In some trials, as high as about one third of trial participants reported unexplained muscle pain and weakness. Statin dose taken at the maximum approved level is
associated with an increase in the incidence of side effects. Furthermore, only less than 40% of patients achieve their target LDL-C goals, when taking statins. In coronary heart disease patients, only 18% reach their target LDL-C goals. Approximately 12% of patients on statins discontinue therapy, citing myalgia as the primary reason for discontinuation. Already in the USA only, millions of adults are considered to be statin intolerant. Poor statin adherence can lead to worsening of cardiovascular outcomes and increased risk of major adverse cardiac events (MACE).

Partially due to the challenges of statin dose toxicity, hepatic adenosine triphosphate (ATP) citrate lyase inhibitors / hepatic adenosine monophosphate-activated protein kinase (AMPK) activators are currently under clinical investigation as a representative of a new class of anti-hyperlipidemics for the treatment of hypercholesterolemia. The ATP citrate lyase (ACL) inhibitor / AMPK activator has a dual activity, and targets two distinct molecular targets. ACL inhibitor / AMPK activator builds up a class of compounds that inhibits both sterol and fatty acid synthesis and enhances mitochondrial long-chain fatty acid beta-oxidation and produces beneficial effects on pro-atherogenic lipids. The ACL inhibitor / AMPK activators are also mentioned as peroxisome proliferator-activated receptor agonists. ATP citrate lyase is a key enzyme in the cholesterol biosynthetic pathway. AMPK represents an attractive therapeutic target, as it plays a fundamental role in maintaining cellular energy homeostasis via coordinating glucose and lipid metabolism as well as down-regulating pro-inflammatory signaling pathways. Both enzymes ACL and AMPK play significant roles in the synthesis of cholesterol and glucose in the liver. Inhibiting cholesterol synthesis in the liver, causes the liver to take up LDL particles from the blood, which reduces LDL-C levels. ACL inhibitor / AMPK activator has a dual mechanism of action by inhibiting ATP citrate lyase and also activating AMP-activated protein kinase. This way, ACL inhibitor / AMPK activator reduces LDL-C levels.

An intensively studied representative investigational ACL inhibitor / AMPK activator drug compound is the small molecule ETC-1002, or bempedoic acid, previously known as compound "ESP-55016" of Esperion Therapeutics. ETC-1002 is currently under development in the clinic for the treatment of dyslipidemia and coronary artery disease, and other cardiometabolic risk factors. ETC-1002 is in development primarily for patients with hypercholesterolemia and a history of statin intolerance. The molecular formula of ETC-1002 is C19H36O5, the molecular weight of ETC-1002 is 344.5 g/mol. The 2D structure of ETC-1002
is provided below (See Scheme 2). The IUPAC name of ETC-1002 is 8-hydroxy-2,2,14,14-tetramethylpentadecanedioic acid.

ETC-1002 is analyzed in Phase II clinical trials either as a mono-therapy, or in a combination therapy approach together with ezetimibe in statin-intolerant patients. In addition, ETC-1002 is tested in clinical trials in patients already taking a statin and who are not at their LDL-C goal. With every doubling of the dose of a statin taken by a patient, on average the LDL-C level reduces an additional 6%. ETC-1002 used as an add-on to statins in hypercholesterolemia patients already taking a statin yielded a 24% further decrease in LDL-C levels when taking 180 mg ETC-1002 daily, compared to the LDL-C levels reached by the statin monotherapy. The incremental reduction in LDL-C levels was 17% when 120 mg ETC-1002 was incorporated in the combination therapy with the statin. In addition to the effect seen on LDL-C levels, ETC-1002 demonstrated reductions of up to 40 percent in high-sensitivity C-reactive protein, an important marker of inflammation in coronary disease, which reduction is consistently seen in clinical trials with ETC-1002.

Typically, the ETC-1002 dosage form tested in clinical trials is an orally available once daily treatment with 80, 120 or 180 mg ETC-1002. Other dosages tested in clinical trials are 20, 40, 60, 140, 220 and 240 mg, once daily. In several phase II trials, typically, ETC-1002 is dosed at 60, 120 mg or 180 mg ETC-1002 per day for human adult patients. Typically, about 60% of patients receiving ETC-1002 therapy in a clinical trial achieves their LDL-C goal.

ETC-1002 at 120 mg daily dose as a mono-therapy has been shown to moderately reduce LDL-C levels in patients by up to 27% when compared to placebo, in patients with statin intolerance. In a Phase lib study with hypercholesterolemia patients with or without statin intolerance, ETC-1002 treatment for twelve weeks resulted in patients achieving LDL-C levels that were up to 30% decreased. This ability of ETC-1002 to lower LDL-C when used as a monotherapy is less effective than that of several statins available today administered at their most effective dose, e.g. atorvastatin, fluvastatin, lovastatin, pravastatin, rosvastatin and simvastatin at equivalent dosages, which lower LDL-C levels with up to approximately 55%, 40%, 45%, 45%, 60% and 45%, respectively.

Compared to statin monotherapy, ETC-1002 in combination with a statin has been shown to reduce LDL-C by a further 24%, compared to the reduction in LDL-C level achieved with the
stain monotherapy. Based on these findings, pharmacological modulation of ACL and AMPK appears to be a promising therapeutic strategy to lower LDL-C levels in hypercholesterolemic patients who cannot tolerate, or are unable to fully benefit from, statin therapy. The effect of ETC-1002 on clinical outcomes such as coronary heart disease events is unknown. What is established, however, is that ETC-1002 does not influence plasma concentration of high-density lipoprotein (HDL-C), i.e. ETC-1002 does not increase HDL-C levels in patients receiving ETC-1002 in clinical trials.

An elevated level of LDL-C represents a significant modifiable risk factor for CVD, and therefore hypercholesterolemia is currently a primary target for lipid-lowering therapies and for reduction of the risk of MACE. Several studies, now, have shown that also a low plasma concentration of high-density lipoprotein cholesterol (HDL-C) is a powerful risk factor for the development of cardiovascular diseases, in addition to the established strong association between LDL-C levels and risk for CVD.

One new approach now, which enables reduction of LDL-C levels and elevation of HDL-C levels in patients is to inhibit the Cholesteryl Ester Transfer Protein (CETP). CETP is a plasma protein secreted primarily by liver and adipose tissue. CETP mediates the transfer of cholesteryl esters from HDL to apolipoprotein B (ApoB)-containing particles (mainly LDL and VLDL) in exchange for triglycerides (TG), thereby decreasing the cholesterol content in HDL in favor of that in (V)LDL. Hence, CETP inhibition has been hypothesized to retain cholesteryl esters in HDL-C and decrease the cholesterol content of the atherogenic ApoB fraction.

Despite the evidence supporting the potential of CETP inhibition in reducing cardiovascular morbidity, clinical development of CETP inhibitors has not been straightforward. The first compound to progress to Phase III clinical trials was torcetrapib, a small molecule drug candidate, which was dosed at 60 mg. Torcetrapib was shown to increase HDL-C by 72% and decrease LDL-C by 25%, but it was subsequently withdrawn from development owing to safety concerns including an unexpected increase in cardiovascular events and death and little identifiable cardiovascular benefit (despite substantial increases in HDL) when used in combination with the HMG CoA reductase inhibitor atorvastatin in Phase III clinical trials.

Although the mechanism of those events is not fully understood, there is increasing evidence that they might have been due to off-target effects of torcetrapib such as increased blood pressure, changes in electrolytes (increases in sodium and bicarbonate and decreases in potassium) and increases in aldosterone, consistent with mineralocorticoid activity. There is
also some evidence from animal studies that torcetrapib increases expression of endothelin-1, which has been postulated to be have contributed to the apparent (non-significant) increase in cancer deaths in the ILLUMINATE trial. These observations could be related to the relatively high dose of torcetrapib needed.

Subsequently, another CETP inhibitor, dalcetrapib, entered clinical trials. Dalcetrapib was shown to be a weak inhibitor that increased HDL-C by 30-40% with minimal effects on LDL-C concentrations. Dalcetrapib development has also been terminated on the grounds of futility in a Phase III study where the drug was dosed at 600 mg. Lack of efficacy was probably related to modest CETP inhibition.

Two more CETP inhibitors, anacetrapib and evacetrapib, are currently in Phase III clinical trials. Data from Phase II studies suggest that both anacetrapib and evacetrapib are CETP inhibitors without mineralocorticoid activity. Anacetrapib 200 mg once daily has been shown to increase HDL-C by 97% and decrease LDL-C by 36% in fasted healthy subjects and 150 mg once daily anacetrapib has been shown to increase HDL-C by 139% and decrease LDL-C by 40% in patients. Evacetrapib (500 mg once daily monotherapy in patients) has been shown to increase HDL-C by 129% and decrease LDL-C by 36%.

In the Phase III studies, a once daily dose of 100 mg anacetrapib is clinically evaluated, whereas for evacetrapib (LY2484595; Eli Lilly) a once daily dose of 130 mg is being evaluated in the ACCELERATE trial (approximately 12,000 people with high-risk atherosclerotic cardiovascular disease (ASCVD)). Such relatively high amounts of active ingredients may however lead to serious problems. For example, for anacetrapib a concern was raised in relation to the time that the drug remains in people's body after they stop taking it. A report shows that even after 4 years the levels of anacetrapib were still detectable.

A disadvantage of the use of the known CETP-inhibitors is that due to the relatively high dosage which has to be used to obtain CETP-inhibition, more and stronger side effects may occur. This can have a negative influence on both the physical well-being of the patient as well as on patient compliance.

It has thus been difficult to use CETP-inhibitors in combination with other pharmaceutically active compounds. More particularly, in view of the existing side effects of HMG CoA reductase inhibitors and the relatively high dose of the known CETP-inhibitors, combining these inhibitors in a pharmaceutical combination has proven to be problematic, as has been observed when the combination of torcetrapib and atorvastatin was clinically tested. More particularly, aiming at beneficially combining an ACL inhibitor/AMPK activator such as ETC-1002, with the relatively high dose of the known CETP-inhibitors, will also be
problematic, let alone when considering a combination therapy consisting of (high-dose) CETP-inhibitor, ETC-1002 and a statin.

Hence, a continuing need remains to find convenient, safe and effective agents or combination of agents for use in the treatment of subjects suffering from or having an increased risk for cardiovascular diseases.

SUMMARY OF THE INVENTION

A first aspect of the present invention relates to a pharmaceutical composition comprising:

(a) a compound of the formula:

(b) at least one adenosine triphosphate citrate lyase inhibitor / adenosine monophosphate-activated protein kinase activator (hereinafter: ACL inhibitor / AMPK activator), preferably ETC-1002, or a pharmaceutically acceptable salt thereof; and

(c) one or more pharmaceutically acceptable excipients.

A second aspect of the present invention relates to a therapeutic combination comprising said Compound A or a pharmaceutically acceptable salt thereof and at least one ACL inhibitor / AMPK activator, preferably ETC-1002, or a pharmaceutically acceptable salt thereof.

A third aspect of the present invention relates to the use of the pharmaceutical composition or the therapeutic combination in the treatment of subjects suffering from cardiovascular diseases or having an increased risk for cardiovascular diseases.

Clinical studies have shown that compared to other known CETP-inhibitors only a relatively low dose of Compound A is needed to significantly increase the HDL-C
concentration and significantly lower the LDL-C concentration. This makes Compound A particularly suitable to be used in combination with other pharmaceutically active compounds, such as an an ACL inhibitor / AMPK activator, e.g. ETC-1002.

It has further been shown in preclinical studies that combining Compound A with an ACL inhibitor / AMPK activator gives an advantageous effect with respect to the lowering of the LDL-C concentration in blood and that it does not lead to serious side effects. In other words, when Compound A is used in combination with an ACL inhibitor / AMPK activator, such as for example ETC-1002, the LDL-C concentration decreases more than one would have expected.

Hence, the pharmaceutical and therapeutic combination according to the present invention are thus also particularly suitable to be administered to patients which are suffering from cardiovascular diseases or have an increased risk for cardiovascular diseases, but which are statin intolerant.

DEFINITIONS

The term 'pharmaceutical composition' as used herein has its conventional meaning and refers to a composition which is pharmaceutically acceptable.

The term 'pharmaceutically acceptable' as used herein has its conventional meaning and refers to compounds, material, compositions and/or dosage forms, which are, within the scope of sound medical judgment suitable for contact with the tissues of mammals, especially humans, without excessive toxicity, irritation, allergic response and other problem complications commensurate with a reasonable benefit/risk ratio.

The term 'excipient' as used herein has its conventional meaning and refers to a pharmaceutically acceptable ingredient, which is commonly used in the pharmaceutical technology for preparing a granulate, solid or liquid oral dosage formulation.

The term 'salt' as used herein has its conventional meaning and includes the acid addition and base salts of Compound A.

The term 'increased risk' has its conventional meaning and refers to a situation in a subject, preferably human, where in individuals, either male or female, have an LDL-cholesterol level above 2.6 mmol/l (100.54 mg/dL), such that they are exposed at an increased risk of a cardiovascular event, compared to those with lower levels.

The term 'treatment' as used herein has its conventional meaning and refers to curative, palliative and prophylactic treatment.
The term 'cardiovascular disease' as used herein has its conventional meaning and includes arteriosclerosis, peripheral vascular disease, hyperlipidemia, mixed dyslipidemia betalipoproteinemia, hypoalphalipoproteinemia, hypercholesteremia, hypertriglyceridemia, familial-hypercholesteremia, angina, ischemia, cardiac ischemia, stroke, myocardial infarction, reperfusion injury, restenosis after angioplasty, hypertension, cerebral infarction and cerebral stroke.

The term 'HMG-CoA reductase inhibitor' as used herein has its conventional meaning and is used interchangeably with the term 'statins' and refers to compounds which are used to lower LDL-C by inhibiting the enzyme HMG-CoA reductase. Well known HMG-CoA reductase inhibitors are atorvastatin, pravastatin, fluvastatin, simvastatin, lovastatin, rosvastatin and pitavastatin.

The term 'adenosine triphosphate citrate lyase inhibitor / adenosine monophosphate-activatedprotein kinase activator', or in brief 'ACL inhibitor / AMPK activator', as used herein has its conventional meaning and refers to compounds which are used to lower LDL-C by inhibiting both sterol and fatty acid synthesis and enhancing mitochondrial long-chain fatty acid beta-oxidation and producing beneficial effects on proatherogenic lipids. A typical example of an adenosine triphosphate citrate lyase inhibitor / adenosine monophosphate-activated protein kinase activator is ETC-1002.

The term 'unit dosageform' has its conventional meaning and refers to a dosage form which has the capacity of being administered to a subject, preferably a human, to be effective, and which can be readily handled and packaged, remaining as a physically and chemically stable unit dose comprising the therapeutic agent, i.e. Compound A or combination of therapeutic agents, such as Compound A and an ACL inhibitor / AMPK activator.

The term 'fixed dose combination' as used herein has its conventional meaning and refers to a combination of defined doses of two or more drugs or active ingredients presented in a single dosage unit (e.g. a tablet or a capsule) and administered as such.

The term 'free dose combination' as used herein has its conventional meaning and refers to a combination of two drugs or active ingredients administered simultaneously but as two distinct dosage units.

DETAILED DESCRIPTION OF THE INVENTION

A first aspect of the present invention relates to a pharmaceutical composition comprising:
(a) a compound of the formula:

![Chemical Structure](image)

(hereinafter: Compound A) or a pharmaceutically acceptable salt thereof;

(b) at least one ACL inhibitor / AMPK activator or a pharmaceutically acceptable salt thereof;

and

(c) one or more pharmaceutically acceptable excipients.

Compound A as such has already been described in the European patent application EP 1730152, wherein it has been identified as a CETP-inhibitor among many other CETP-inhibitors. The 2D structure of Compound A is provided in Scheme 1, below. The chemical name of Compound A is \( \{4-[(2-[[3,5-bis(trifluoromethyl)benzyl] [(2R,4S)-1-(ethoxycarbonyl)-2-ethyl-6-(trifluoromethyl)-1,2,3,4-tetrahydroquinolin-4-yl]amino]pyrimidin-5-yl]oxy\} \) butanoic acid.

Surprisingly, clinical studies have now shown that compared to other known CETP-inhibitors only a relatively low dose of Compound A is needed to significantly increase the HDL-C concentration and significantly lower the LDL-C concentration. This makes Compound A particularly suitable to be used in combination with other pharmaceutically active compounds.

For example, Compound A has been analysed in a trial with individuals with dyslipidemia. The study investigated the effects of Compound A on a wide range of established CVD biomarkers. The results showed potent effects on the primary endpoint, which was a composite of change from baseline in LDL-C and HDL-C. Five mg of Compound A reduced LDL-C by about 50% and increased FIDL-C by about 160%. Ten mg of Compound A in combination with statin therapy reduced LDL-C by an additional approximate 50%. In addition, Compound A boosted cholesterol efflux significantly.

Compound A was safe and well tolerated without any drug accumulation, as has been reported with other CETP inhibitors.
It has now surprisingly been shown in preclinical studies that combining Compound A with an ACL inhibitor / AMPK activator gives an advantageous effect with respect to the lowering of the LDL-C concentration in blood. In other words, when Compound A is used in combination with an ACL inhibitor / AMPK activator, such as for example ETC-1002, the LDL-C concentration decreases more than when these compounds are used separately.

Moreover, no serious side effects have been observed by using a combination of these compounds.

The pharmaceutical composition according to the present invention is therefore preferably used in the treatment of subjects suffering from cardiovascular diseases or having an increased risk for cardiovascular diseases. Said pharmaceutical composition is more preferably used in the treatment of subjects suffering from hyperlipidemia or mixed dyslipidemia or having an increased risk for hyperlipidemia or mixed dyslipidemia.

Due to the absence of serious side effects, the composition according to the present invention is particularly useful to be used in patients which are statin intolerant.

Compound A is preferably used in combination with the ACL inhibitor / AMPK activator ETC-1002 (also referred to as bempedoic acid), with formula:

![Structural formula of ETC-1002]

or a pharmaceutically acceptable salt thereof.

This ACL inhibitor / AMPK activator ETC-1002 has been analyzed in clinical trials and has shown to be able to significantly reduce the LDL-C concentration in patients.

The pharmaceutical composition according to the present invention preferably comprises 1 to 25 mg of Compound A and 1 to 300 mg of the ACL inhibitor / AMPK activator, more preferably 20 to 240 mg of the ACL inhibitor / AMPK activator, most preferably about 60 to 180 mg of the ACL inhibitor / AMPK activator. The ACL inhibitor/AMPK activator used in said composition is preferably ETC-1002.

In a further embodiment of the pharmaceutical composition according to the present invention the composition comprises 5 to 10 mg of Compound A and 1 to 300 mg of ACL inhibitor / AMPK activator, preferably about 20 to 240 mg ACL inhibitor / AMPK activator, more preferably the composition comprises about 1 to 25 mg of Compound A and about 60 to 180 mg of the ACL inhibitor / AMPK activator, wherein the ACL inhibitor / AMPK activator is preferably ETC-1002.
In a further embodiment, the pharmaceutical composition according to the present invention preferably comprises 1 to 25 mg of Compound A and about 120 mg of the ACL inhibitor / AMPK activator, wherein the ACL inhibitor / AMPK activator is preferably ETC-1002.

Due to the advantageous combined effect between Compound A and ACL inhibitor / AMPK activator, such as ETC-1002, it is now surprisingly also possible to lower the amount of ACL inhibitor / AMPK activator used, thereby lowering the risk for side effects.

The pharmaceutical composition according to the present invention is preferably administered orally to subjects in need thereof. Oral administration may involve swallowing, so that the pharmaceutically active compounds enter the gastrointestinal tract. Specific pharmaceutical preparations, as described below, may be developed which facilitate the oral administration.

The pharmaceutical composition according to the present invention is preferably formulated as an oral free dose combination or as an oral fixed dose combination, more preferably as an oral fixed dose combination. The different pharmaceutically active ingredients may be present in said combinations as granulates. Preferably, the pharmaceutical composition is an oral fixed dose combination, such a combination is very convenient for patients and avoids problems with administering the correct amounts of these compounds.

Solid oral dosage forms which may be used within the context of the present invention include besides tablets and capsules amongst others caplets, lozenges, pills, mini-tablets, pellets, beads and granules. Liquid oral dosage forms which may be used for the pharmaceutical preparation of the present invention include, but are not limited to drinks, solutions, suspensions, syrups, beverages and emulsions.

The oral fixed dose combination or oral free dose combination is preferably formulated as a solid dosage form, such as a tablet or capsule. Generally, the administration of these kinds of formulations is considered to be the most convenient for patients.

In a particularly preferred embodiment the pharmaceutical composition according to the present invention is an oral fixed dose combination comprising 1 to 25 mg of Compound A and 1 to 300 mg of an ACL inhibitor / AMPK activator. Preferably, said ACL inhibitor / AMPK activator is ETC-1002. In an even more preferred embodiment thereof, said pharmaceutical composition comprises 1 to 25 mg of Compound A and 20 to 240 mg of said ACL inhibitor / AMPK activator, preferably ETC-1002, more preferably about 60 to 180 mg of said ACL inhibitor / AMPK activator, which is preferably ETC-1002.
Besides Compound A and the ACL inhibitor / AMPK activators as such, pharmaceutically acceptable salts thereof may also be used in the pharmaceutical composition according to the present invention. Pharmaceutically acceptable salts of Compound A and the ACL inhibitor / AMPK activators include the acid addition and base salts thereof, such as preferably the calcium, potassium or sodium salts. For a review on suitable salts, reference is made “Handbook of Pharmaceutical Salts: Properties, Selection, and Use” by Stahl and Wermuth (Wiley-VCH, Weinheim, Germany, 2002).

A pharmaceutically acceptable salt of Compound A or of ACL inhibitor / AMPK activators may be readily prepared by mixing together solutions of such compounds and the desired acid or base, as appropriate. The salt may precipitate from solution and be collected by filtration or may be recovered by evaporation of the solvent.

The present invention also relates to the use of pharmaceutically acceptable solvates or pro-drugs of Compound A and/or pharmaceutically acceptable solvates or pro-drugs of the ACL inhibitor / AMPK activators in the pharmaceutical composition of the present invention.

In an embodiment of the present invention, the composition according the present invention comprises polyunsaturated fatty acids (PUFAs), preferably omega-3 polyunsaturated fatty acids, more preferably PUFAs chosen from the group consisting of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), acid a-Linolenic acid (ALA) or combinations thereof. Preferably, PUFAs are present in their free acid form, in the pharmaceutical compositions according to the invention.

PUFA's, in particular omega-3 PUFAs, have a specific capacity against triglyceride rich lipoproteins, remnant cholesterol and small dense LDL, whereas CETP-inhibitors have no or little effect against triglyceride rich lipoprotein and remnant cholesterol. Hence, combining ACL inhibitor / AMPK activators, CETP inhibitors and PUFAs in a pharmaceutical composition makes such a composition particularly suitable for the treatment of subjects suffering from or having an increased risk for cardiovascular diseases.

In this regard it is also noted that due to the relatively low amounts of Compound A and ACL inhibitor / AMPK activators, such as ETC-1002, which are needed to obtain a clinically relevant effect, it is possible that oral formulations of the composition according to the present invention comprise relatively high amounts of PUFA's.

The pharmaceutical composition according to the present invention comprises besides Compound A and the at least one ACL inhibitor / AMPK activators also a pharmaceutically acceptable excipient, i.e. a pharmaceutically acceptable ingredient, which is commonly used
in the pharmaceutical technology for preparing granulate, solid or liquid oral dosage formulations.

Examples of categories of excipients include, but are not limited to, binders, disintegrants, lubricants, glidants, fillers and diluents. One of ordinary skill in the art may select one or more of the aforementioned excipients with respect to the particular desired properties of the granulate and/or solid oral dosage form by routine experimentation and without any undue burden. The amount of each excipient used may vary within ranges conventional in the art. The following references which are all hereby incorporated by reference disclose techniques and excipients used to formulate oral dosage forms. See "The Handbook of Pharmaceutical Excipients", 4th edition, Rowe et al., Eds., American Pharmaceuticals Association (2003); and "Remington: The Science and Practice of Pharmacy", 20th edition, Gennaro, Ed., Lippincott Williams & Wilkins (2000).

In one embodiment of the invention, the pharmaceutical composition according to the present invention comprises besides Compound A and the at least one ACL inhibitor / AMPK activator also an HMG CoA reductase inhibitor or a pharmaceutically acceptable salt thereof. In a further embodiment, this HMG CoA reductase inhibitor is selected from the group consisting of atorvastatin, pravastatin, fluvastatin, simvastatin, lovastatin, rosuvastatin and pitavastatin, more preferably from the group consisting of atorvastatin calcium, pravastatin sodium, fluvastatin sodium, simvastatin, lovastatin and rosuvastatin calcium.

In a further embodiment of the invention, the pharmaceutical composition according to the present invention thus comprises Compound A and the at least one ACL inhibitor / AMPK activator, such as ETC- 1002, and an HMG CoA reductase inhibitor, preferably about 1 to 80 mg of an HMG CoA reductase inhibitor. Typically, the pharmaceutical composition according to the present invention comprises about 1 to 25 mg Compound A and about 1 to 300 mg of the at least one ACL inhibitor / AMPK activator, such as ETC- 1002, and about 1 to 80 mg of an HMG CoA reductase inhibitor.

In a further embodiment of the present invention, the pharmaceutical composition according to the present invention comprises about 1 to 25 mg Compound A and about 20 to 240 mg, preferably about 60 to 180 mg of the at least one ACL inhibitor / AMPK activator, such as ETC- 1002, and about 1 to 80 mg of an HMG CoA reductase inhibitor, preferably about 1 to 20 mg of an HMG CoA reductase inhibitor. Most preferably, said ACL inhibitor / AMPK activator is ETC- 1002 according to the invention.

Hence, in one embodiment of the invention, the pharmaceutical composition according to the present invention comprises about 1 to 25 mg Compound A and about 20 to 240 mg,
preferably about 60 to 180 mg of the at least one ACL inhibitor / AMPK activator, e.g. ETC-1002, and further comprises about 1 to 30 mg of a cholesterol absorption inhibitor, e.g. ezetimibe, preferably about 10 mg ezetimibe.

When the pharmaceutical composition of the invention comprises an HMG CoA reductase inhibitor and if PUFAs are comprised in the composition, these PUFAs are preferably present in their free acid form, i.e. not in the form of ethyl esters in which the PUFA species are present in substantially esterified form. When the PUFAs are used in this form, the HMG CoA reductase inhibitors are better soluble in said PUFAs. In this regard reference is made to WO2013/169797, which document is herewith incorporated by reference.

A second aspect of the present relates to a therapeutic combination comprising

(a) a compound of the formula:

(b) at least one ACL inhibitor / AMPK activator or a pharmaceutically acceptable salt thereof.

Preferably, the ACL inhibitor / AMPK activator of said therapeutic combination is ETC-1002 with the formula:

or a pharmaceutically acceptable salt thereof.

In an embodiment the therapeutic combination comprises about 1 to 25 mg of Compound A and about 1 to 300 mg of the ACL inhibitor / AMPK activator, preferably about 20 to 240 mg of ACL inhibitor / AMPK activator, more preferably about 60 to 180 mg ACL
inhibitor / AMPK activator, wherein preferably the ACL inhibitor / AMPK activator is ETC-1002.

In a further embodiment of the therapeutic combination according to the present invention the combination comprises 5 to 10 mg of Compound A and 20 to 240 mg of ACL inhibitor / AMPK activator, preferably about 60 mg to 180 mg of ACL inhibitor / AMPK activator. , preferably ETC-1002 is used.

In another embodiment, the therapeutic combination according to the present invention comprises 1 to 25 mg of Compound A and about 60 mg to 180 mg of the ACL inhibitor / AMPK activator, preferably ETC-1002. Due to the advantageous effect between Compound A and ACL inhibitor / AMPK activator it is now also possible to lower the amount of ACL inhibitor / AMPK activator used, thereby reducing the risk for side effects.

The therapeutic combination according to the present invention is preferably formulated as a fixed dose combination or a free dose combination, preferably a fixed dose combination.

In a further embodiment of the present invention the therapeutic combination comprises a first unit dosage form comprising about 1 to 25 mg of Compound A or a pharmaceutically acceptable salt thereof and a second unit dosage form comprising about 1 to 300 mg of ETC-1002, preferably 20 to 240 mg of ETC-1002, more preferably about 60 to 180 mg of ETC-1002, or a pharmaceutically acceptable salt thereof. The first and second unit dosage forms are in such a case preferably provided as a kit of parts. Preferably said combination comprises in such a case a package comprising said unit dosage forms.

The therapeutic combination according the present invention may comprise polyunsaturated fatty acids (PUFAs), preferably omega-3 polyunsaturated fatty acids, more preferably PUFAs chosen from the group consisting of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), acid a-Linolenic acid (ALA) or combinations thereof. PUFAs are preferably present in their free acid form.

PUFA's, in particular omega-3 PUFAs, have a specific capacity against triglyceride rich lipoproteins, remnant cholesterol and small dense LDL, whereas CETP-inhibitors have no or little effect against triglyceride rich lipoprotein and remnant cholesterol. Hence, combining ACL inhibitor / AMPK activator, CETP inhibitors and PUFAs in a therapeutic combination makes such a combination particularly suitable for the treatment of subjects suffering from or having an increased risk for cardiovascular diseases.

In a further embodiment of the invention, the therapeutic combination comprises Compound A, at least an ACL inhibitor / AMPK activator, such as ETC-1002, and further
comprises at least one HMG CoA reductase inhibitor or a pharmaceutically acceptable salt thereof, according to the invention.

In a further embodiment, this HMG CoA reductase inhibitor is selected from the group consisting of atorvastatin, pravastatin, fluvastatin, simvastatin, lovastatin, rosuvastatin and pravastatin, more preferably from the group consisting of atorvastatin calcium, pravastatin sodium, fluvastatin sodium, simvastatin, lovastatin and rosuvastatin calcium.

In another embodiment of the invention, the therapeutic combination according to the present invention thus comprises Compound A and the at least one ACL inhibitor / AMPK activator and an HMG CoA reductase inhibitor, preferably about 1 to 80 mg of an HMG CoA reductase inhibitor. Typically, the therapeutic combination according to the present invention comprises about 1 to 25 mg Compound A and about 1 to 300 mg of the at least one ACL inhibitor / AMPK activator and about 1 to 80 mg of a HMG CoA reductase inhibitor. More preferably, the therapeutic combination according to the present invention comprises about 1 to 25 mg Compound A and about 20 to 240 mg of the at least one ACL inhibitor / AMPK activator, preferably about 60 to 180 mg ACL inhibitor / AMPK activator, and about 1 to 20 mg of a HMG CoA reductase inhibitor. Preferably, the ACL inhibitor / AMPK activator is ETC-1002.

Hence, in one embodiment of the invention, the pharmaceutical composition according to the present invention or the therapeutic combination according to the present invention comprises about 1 to 25 mg Compound A and about 20 to 240 mg, preferably about 60 to 180 mg of the at least one ACL inhibitor / AMPK activator, e.g. ETC-1002, and further comprises about 1 to 30 mg of a cholesterol absorption inhibitor, such ezetimibe, preferably about 10 mg of ezetimibe.

When the therapeutic combination of the invention comprises an HMG CoA reductase inhibitor and if PUFAs are comprised in the combination, these PUFAs are preferably present in their free acid form, i.e. not in the form of ethyl esters in which the PUFA species are present in substantially esterified form. When the PUFAs are used in this form, the HMG CoA reductase inhibitors are better soluble in said PUFAs. In this regard reference is made to WO2013/169797, which document is herewith incorporated by reference.

A third aspect of the present invention relates to the use of the pharmaceutical composition or the therapeutic combination as described above in the treatment of subjects suffering from cardiovascular diseases or having an increased risk for cardiovascular diseases.
The pharmaceutical composition or the therapeutic combination is preferably for use in the treatment of subjects suffering from hyperlipidemia or mixed dyslipidemia or having an increased risk for hyperlipidemia or mixed dyslipidemia.

Preferably, a subject in need of the pharmaceutical composition or the therapeutic combination according to the present invention is administered by means of said composition or combination 1 to 25 mg per day of Compound A and 1 to 300 mg, preferably about 120 to 180 mg per day ACL inhibitor / AMPK activator, such as ETC-1002. Alternatively, the pharmaceutical composition or therapeutic combination is administered in such amounts that a subject in need thereof receives 1 to 25 mg per day of Compound A and 20 to 240 mg, preferably about 60 to 180 mg per day of the ACL inhibitor / AMPK activator. Preferably, the ACL inhibitor / AMPK activator in said pharmaceutical composition or therapeutic combination is ETC-1002 according to the invention.

In a further embodiment, the pharmaceutical composition or the therapeutic combination as described above are administered to subjects suffering from hyperlipidemia or mixed dyslipidemia or having an increased risk for hyperlipidemia or mixed dyslipidemia in such amount that such a subject receives about 1 to 25 mg per day of Compound A or a pharmaceutically acceptable salt thereof and about 1 to 300 mg of ETC-1002, preferably about 20 to 240 mg, more preferably about 60 to 180 mg of ETC-1002 or a pharmaceutically acceptable salt thereof. In a further embodiment, the subject may receive between about 5 to about 10 mg per day of Compound A in combination with the above mentioned amounts of ETC-1002.

In a further embodiment, the pharmaceutical composition or the therapeutic combination according to the invention are administered to subjects suffering from hyperlipidemia or mixed dyslipidemia or having an increased risk for hyperlipidemia or mixed dyslipidemia in such amount that such a subject receives about 1 to 25 mg per day of Compound A or a pharmaceutically acceptable salt thereof and about 1 to 300 mg of ETC-1002, preferably about 20 to 240 mg of ETC-1002, more preferably about 60 to 180 mg of ETC-1002 or a pharmaceutically acceptable salt thereof and about 1 to 80 mg of at least one HMG CoA reductase inhibitor or a pharmaceutically acceptable salt thereof.

The pharmaceutical composition or therapeutic combination according to the present invention is preferably administered to subjects in need thereof for at least one week, preferably at least three weeks.
The present invention will be illustrated further by means of the following non-limiting examples.

EXAMPLES

EXAMPLE 1

Double blind randomized study of subjects receiving compound A monotherapy, statin monotherapy or placebo

In a double-blind, placebo controlled clinical study the effects of 12 weeks of administration of Compound A alone in patients with mild dyslipidemia was examined in comparison to administration of statin alone.

Patients

This randomised, double-blind, placebo-controlled, parallel-group Phase II trial was conducted in male and female patients (18-75 years) with fasting LDL-C levels >2.5 mmol/L and <4.5 mmol/L, HDL-C levels <1.8 mmol/L and >0.8 mmol/L and tri-glyceride (TG) levels <4.5 mmol/L after run-in, or washout of existing therapies. Key exclusion criteria included patients with clinical manifestation of atherosclerotic vascular disease, type 1 diabetes, uncontrolled type 2 diabetes (haemoglobin A1c ≥8%), uncontrolled hypertension, history of hyperaldosteronism, active muscle disease or persistent creatine kinase >3x the upper limit of normal (ULN), clinically significant renal or hepatic dysfunction, or evidence of any other clinically significant non-cardiac disease. Patients were recruited at 20 sites in the Netherlands and Denmark. The trial was approved by Independent Ethics Committees, and each patient provided written informed consent. The trial was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice Guidelines and the protocol was registered on ClinicalTrials.gov (NCT01970215).

Trial design

The trial consisted of a screening visit, followed by a run-in phase of 4 weeks (or 6 weeks for patients that required a washout of existing lipid-lowering therapy). After the run-in phase, a sub-group of patients was randomly assigned to receive one of the following seven treatments: 1 mg, 2.5 mg, 5 mg or 10 mg of Compound A alone or matching placebo, or 20 mg Atorvastatin alone or 10 mg Rosuvastatin alone. All treatments were to be taken once
daily with food for 12 weeks. During the double-blind treatment phase visits were conducted at baseline (Week 0) and at Weeks 4, 8 and 12. Follow-up visits were conducted 2 and 8 weeks after the end of treatment. Safety was assessed throughout the trial by monitoring adverse events and concomitant medication use, 12-lead electrocardiograms (ECGs), vital signs, laboratory safety assessments and physical examinations. Additional assessments included salivary Cortisol, plasma aldosterone, high-sensitivity C-reactive protein (hsCRP) and endothelin 1.

Efficacy assessments included fasted lipid profiles including total cholesterol (TC), HDL-C, LDL-C, triglycerides, apolipoproteins Al, B and E (ApoAI, ApoB and ApoE), lipoprotein a (Lp[a]) and derived parameters. Exploratory endpoints included assessments of PCSK9, HDL-driven endothelial production of nitric oxide (NO), HDL particle numbers (using nuclear magnetic resonance [NMR] spectroscopy and ion mobility analysis), HDL particle subfractions (using 2D gel electrophoresis), CETP levels and activity, and insulin resistance (based on fasting plasma glucose and insulin levels using homeostasis model assessment insulin resistance [HOMA-IR] method). Blood samples were collected for pharmacokinetic analysis of Compound A.

Analytical methods
Total cholesterol and triglycerides were measured by homogenous enzymatic assay using a Modular analyser (cholesterol oxidase peroxidase-peroxidase aminophenazone phenol [CHOP-PAP]) method and a glycerol phosphate oxidase [GPO-PAP] method, respectively. Apolipoproteins Al, A2, B and E were measured by immunoturbidimetry using reagents from Rolf Greiner Biochemica (Germany) and N apoprotein standard serum from Siemens (Germany). Lp(a) was measured by immunoturbidimetry using reagents and standards from Wako Chemicals (Germany). LDL particle size was determined by gradient gel electrophoresis. HDL fraction was separated by a combined ultracentrifugation-precipitation method (β-quantification). HDL-2 and HDL-3 fractions were then separated by further ultracentrifugation. Total-cholesterol in HDL, HDL-2 and HDL-3 fractions, free cholesterol in HDL fraction, triglycerides in HDL fraction and phospholipids in plasma and HDL-fraction were measured using enzymatic methods and reagents from Diasys Diagnostics (Germany). The measurements were performed on an Olympus AU600 automatic analyzer and were calibrated using secondary standards from Roche Diagnostics (total-cholesterol, triglycerides) and Diasys Diagnostics (free cholesterol, phospholipids), respectively. Esterified cholesterol was calculated as the difference between total-cholesterol and free cholesterol.
Statistical analysis

The co-primary efficacy endpoints were the percentage changes in both HDL-C and LDL-C levels at Week 12 compared to baseline. Secondary and exploratory efficacy endpoints included the percentage changes in other efficacy parameters at Week 12 compared to baseline. The primary and secondary efficacy analyses were performed using an analysis of covariance (ANCOVA) model with treatment and use of statin therapy at randomisation as factors and the baseline value for the respective efficacy variable as a covariate. Least-squares means, standard errors and 2-tailed 95% confidence intervals for each treatment group and for pairwise comparisons between Compound A doses and placebo, between Compound A alone and Atorvastatin alone, and between Compound A alone and Rosuvastatin alone were provided. As there were two co-primary efficacy variables, a closed testing procedure was used in order to control the family-wise error. No interim analyses were planned or conducted.

The sample size of 37 completed patients per treatment group was intended to provide 88% power to detect a 22.5% (standard deviation [SD] 30%) increase in HDL-C compared with statin alone. This sample size with an assumed 10% (SD 15%) greater decrease in LDL-C for the investigational product compared with placebo was expected to provide a power of 80% for a successful study. All tests were 2-sided tests with a significance of 0.05. To allow for a 10-15% drop-out rate, 42 randomised patients per group were planned.

Results

The results of this first clinical study are provided in Table 1 below. From this Table 1 it is apparent that Compound A already at a relatively low dose significantly increases HDL-C concentration, decreases LDL-C concentrations and Lp(a) concentrations. Treatment with the statins alone did not result in an increase of HDL-C levels, and an effect of statin treatment on reducing LP(a) levels was absent. Hence, the administration of Compound A alone already appears to be very beneficial for patients suffering from cardiovascular diseases, in particular dyslipidemia.
Table 1: results of the first clinical study

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>1 mg of Compound A</th>
<th>2.5 mg of Compound A</th>
<th>5 mg of Compound A</th>
<th>10 mg of Compound A</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>40</td>
<td>39</td>
<td>41</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>HDL-C</td>
<td>1.80</td>
<td>76.04</td>
<td>122.28</td>
<td>160.90</td>
<td>180.64</td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.18</td>
<td>-27.05</td>
<td>-34.38</td>
<td>-47.49</td>
<td>-47.26</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>-5.06</td>
<td>-28.81</td>
<td>-26.69</td>
<td>-37.33</td>
<td>-34.86</td>
</tr>
</tbody>
</table>

Table 1: results of the first clinical study (continued)

<table>
<thead>
<tr>
<th></th>
<th>Atorvastatin</th>
<th>Rosuvastatin</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>40</td>
<td>41</td>
<td>362</td>
</tr>
<tr>
<td>HDL-C</td>
<td>1.27</td>
<td>6.16</td>
<td></td>
</tr>
<tr>
<td>LDL-C</td>
<td>-46.73</td>
<td>-47.36</td>
<td></td>
</tr>
<tr>
<td>Lp(a)</td>
<td>-4.46</td>
<td>-7.79</td>
<td></td>
</tr>
</tbody>
</table>

EXAMPLE 2

Preclinical lipid study in hamsters receiving compound A monotherapy, receiving ETC-1002 monotherapy, receiving combination therapy consisting of compound A together with ETC-1002, or receiving placebo

The aim of the preclinical study was to evaluate the effects of the CETP inhibitor Compound A alone or in combination with ACL inhibitor / AMPK activator ETC-1002 or ETC-1002 alone, on low-density lipoprotein cholesterol (LDL-C) profile and on high-density lipoprotein cholesterol (HDL-C) profile in the diet-induced hypercholesterolemic hamster model (reference: Briand F. et al, Liver X receptor activation promotes macrophage-to-feces reverse cholesterol transport in a dyslipidemic hamster model. J Lipid Res 2010, 51:763-70.).
Guidelines

The study was carried out according to the standard operating procedure in place at the test facility. All procedures will be performed in accordance with the Guide for the Care and Use of Laboratory Animals (revised 1996 and 2011, 2010/63/EU) and national laws.

Table 1: Test item Compound A

<table>
<thead>
<tr>
<th>Name</th>
<th>Compound A 1 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration in the formulation</td>
<td>0.1 mg/mL; 10 mL/kg</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.5% carboxymethyl cellulose and 0.025% Tween-20, pH 7-8</td>
</tr>
<tr>
<td>Protocol of preparation</td>
<td>Compound A was ground using a small mortar and pestle then 0.5% CMC solution is added then mixed and sonicated to make a 0.3 mg/mL suspension. Suspension was then diluted to 0.1 mg/mL with 0.5% CMC under stirring with the addition of Tween20 (0.025%) at pH 7-8.</td>
</tr>
<tr>
<td>Conditions of formulation storage</td>
<td>4°C</td>
</tr>
</tbody>
</table>

Table 2: Reference item ETC-1002

<table>
<thead>
<tr>
<th>Name</th>
<th>ETC-1002 10 mg/kg or ETC-1002 30 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration in the formulation</td>
<td>10 mL/kg; 2 mg/mL or 10 mL/kg; 6 mg/mL</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.5% carboxymethyl cellulose and 0.025%, Tween-20, pH 7-8</td>
</tr>
<tr>
<td>Required quantity</td>
<td>2 g</td>
</tr>
<tr>
<td>Packaging</td>
<td>Glass vial</td>
</tr>
</tbody>
</table>
Aspect (powder, color, …) | White powder
---|---
Conditions of storage | Room temperature (powder)
Protocol of preparation | A disodium salt aqueous solution using 2:1 molar ratio of NaOH to ETC- 1002 in water was prepared. Carboxymethyl cellulose (CMC) and Tween-20 was then added to make a final solution containing 0.5% CMC and 0.025% Tween, with a final pH 7-8.
Conditions of formulation storage | 4°C

Table 3: vehicle

<table>
<thead>
<tr>
<th>Name</th>
<th>0.5% carboxymethyl cellulose and 0.025% Tween-20, pH 7-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>0.5%, carboxymethyl cellulose and 0.025% Tween-20</td>
</tr>
<tr>
<td>Conditions of storage</td>
<td>Room temperature</td>
</tr>
<tr>
<td>Protocol of preparation</td>
<td>Dissolve in distilled water</td>
</tr>
<tr>
<td>Conditions of formulation storage</td>
<td>4°C</td>
</tr>
</tbody>
</table>
Test system and management

Specification and justification

Specie : Hamster
Breed : Golden Syrian Hamster
Sex : Male
Age : 6-week old
Number : 60
Weight : 91-100g
Supplier : Janvier Elevage, France
Justification : Golden Syrian hamsters have a similar lipoprotein cholesterol metabolism as compared with humans.

Identification

Animals were identified with electronic chip or ear tags.

Housing

The 60 animals were housed in ventilated and enriched housing cages (29.8 X 30.4 X 18 cm³) throughout the experimental phase. Animals' cages litters were changed at least once a week. Animals were housed in groups of 3-4 animals on a normal 12 hours light cycle (at 08:00 am lights off), 22 ± 2 °C and 50 ± 10 % relative humidity.

Acclimation

At least 5 days of acclimation.

Food and water

During the acclimation period, standard chow diet (Diet#105, Safe Diets) and tap water were provided ad libitum. After the acclimation period, hamster were fed the same standard chow
supplemented with 0.3% cholesterol (diet#105 + 0.3% cholesterol from Safe Diets, ref#U8400) until the end of the experiment.

Treatments

Dosage regimen

Hamsters were treated for 10 days by oral gavage (10 mL/kg), QD, with vehicle, Compound A alone or ETC-1002 alone or in combination, as described below, following the two-weeks period in which the hamsters were fed a hypercholesterolemic diet.

Test groups

Group 1: vehicle, n = 10.
Group 2: ETC-1002, dose is 10 mg/kg, n = 10.
Group 3: ETC-1002, dose is 30 mg/kg, n = 10.
Group 4: Compound A, dose is 1 mg/kg, n = 10.
Group 5: Compound A at 1 mg/kg and ETC-1002 at 10 mg/kg, n = 10.
Group 6: Compound A at 1 mg/kg and ETC-1002 at 30 mg/kg, n = 10.

Methods

Body weight was measured weekly during the whole experimental phase. After the acclimation period, hamsters (n = 60) were kept on a standard chow supplemented with 0.3% cholesterol (Safe Diets, ref#U8400) for 2 weeks prior to treatment, i.e. the hypercholesterolemic diet. After 2 weeks of hypercholesterolemic diet, hamsters were weighed and fasted at 08:00 am then bled by retro-orbital bleeding under isoflurane anesthesia (100 µTEDTA) at -02:00 pm to measure LDL-cholesterol (direct determination), HDL-cholesterol (phosphotungstate / MgCl₂, apolipoprotein B-lipoproteins precipitation method) and total cholesterol. Hamsters were then randomized into 6 homogenous treatment groups (n = 10/group) according to their 1) LDL-cholesterol, 2) HDL-cholesterol, 3) total cholesterol levels and 4) body weight.

After randomization:

- hamsters were treated orally QD for 10 days with vehicle (placebo group), ETC-1002 at 10 mg/kg, ETC-1002 at 30 mg/kg, Compound A at 1 mg/kg, Compound A at 1 mg/kg in
combination with ETC-1002 at 10 mg/kg, or Compound A at 1 mg/kg in combination with ETC-1002 at 30 mg/kg.

- At 10 days of treatment, hamsters were weighed and fasted at 08:00 am, then bled by retro-orbital bleeding under isoflurane anesthesia (maximal blood volume/EDTA) at 02:00 pm to measure LDL-cholesterol (direct determination), HDL-cholesterol (phosphotungstate/MgCl$_2$ apolipoprotein B-lipoproteins precipitation method) and total cholesterol. Hamsters were then sacrificed by cervical dislocation under isoflurane anesthesia, then liver was collected and weighted. Two liver samples (-50 mg) were kept stored at -80°C for till further analysis.

**Parameters**

- body and liver weight (g)
- LDL-cholesterol, HDL-cholesterol and total cholesterol (g/L)

**Data analysis**

Mean ± standard error of mean (sem) have been presented. Statistical analysis using GraphPad Prism 5.01 software were determined according to the data presented.

**Results**

From the preclinical study described above it has become clear that Compound A treatment alone decreased LDL-C and increased HDL-C. This is in line with the clinical data observed in Example 1 above. It was further observed that ETC-1002 treatment alone resulted only in a decreased LDLC-C. This was also in line with prior clinical data observed with ETC-1002. However, it was remarkably found that when Compound A was combined with ETC-1002 a further reduction in LDL-C was observed.

**SCHEME 1**

CHEMICAL NAME AND FORMULA OF COMPOUND A
[4-((2-[[3,5-bis(trifluoromethyl)benzyl]](2R,4S)-L-(ethoxycarbonyl)-2-ethyl-6-(trifluoromethyl)-1,2,3,4-tetrahydroquinolin-4-yl)amino)pyrimidin-5-yl)oxy]butanoic acid
SCHEME 2

CHEMICAL NAME AND FORMULA OF ETC-1002 (BEMPEDOIC ACID)

8-hydroxy-2,2,14,14-tetramethylpentadecanedioic acid
1. Pharmaceutical composition comprising:
   (a) a compound of the formula:

   ![Chemical Structure]

   (hereinafter: Compound A) or a pharmaceutically acceptable salt thereof;
   (b) at least one adenosine triphosphate citrate lyase inhibitor / adenosine monophosphate-activated protein kinase activator or a pharmaceutically acceptable salt thereof; and
   (c) one or more pharmaceutically acceptable excipients.

2. Pharmaceutical composition according to claim 1, wherein the adenosine triphosphate citrate lyase inhibitor / adenosine monophosphate-activated protein kinase activator is ETC-1002 with the formula:

   ![Chemical Structure]

   or a pharmaceutically acceptable salt thereof.

3. Pharmaceutical composition according to claim 1 or 2, wherein the composition comprises about 1 to 25 mg of Compound A and about 1 to 300 mg of the adenosine triphosphate citrate lyase inhibitor / adenosine monophosphate-activated protein kinase activator, preferably ETC-1002.
4. Pharmaceutical composition according to claim 1 or 2, wherein the composition comprises about 1 to 25 mg of Compound A and about 20 to 240 mg of the adenosine triphosphate citrate lyase inhibitor / adenosine monophosphate-activated protein kinase activator, preferably ETC-1002.

5. Pharmaceutical composition according to claim 1 or 2, wherein the composition comprises about 1 to 25 mg of Compound A and about 60 to 180 mg of the adenosine triphosphate citrate lyase inhibitor / adenosine monophosphate-activated protein kinase activator, preferably ETC-1002.

6. Pharmaceutical composition according to any of the previous claims, wherein the composition is formulated as a oral free dose or oral fixed dose combination, preferably as an oral fixed dose combination.

7. Pharmaceutical composition according to the previous claim, wherein the oral fixed dose combination is a solid dosage form, such as a capsule or tablet.

8. Pharmaceutical composition according to any of the previous claims, wherein the composition is an oral fixed dose combination comprising about 1 to 25 mg of Compound A and about 1 to 300 mg of an adenosine triphosphate citrate lyase inhibitor / adenosine monophosphate-activated protein kinase activator, preferably ETC-1002.

9. Pharmaceutical composition according to any of the previous claims, wherein the composition comprises polyunsaturated fatty acids (PUFAs), preferably omega-3 polyunsaturated fatty acids, more preferably PUFAs chosen from the group consisting of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), α-Linolenic acid (ALA) or combinations thereof.

10. Pharmaceutical composition according to the previous claim, wherein the PUFAs are present in their free acid form.
11. Pharmaceutical composition according to any of the previous claims, wherein the pharmaceutical composition further comprises at least one HMG CoA reductase inhibitor or a pharmaceutically acceptable salt thereof.

12. Pharmaceutical composition according to claim 11, wherein the HMG CoA reductase inhibitor is selected from the group consisting of atorvastatin, pravastatin, fluvastatin, simvastatin, lovastatin, rosvastatin and pitavastatin.

13. Pharmaceutical composition according to claim 11 or claim 12, wherein the HMG CoA reductase inhibitor is selected from the group consisting of atorvastatin calcium, pravastatin sodium, fluvastatin sodium, simvastatin, lovastatin and rosvastatin calcium.

14. Pharmaceutical composition according to any of the previous claims 11-13, wherein the composition comprises about 1 to 80 mg of the HMG CoA reductase inhibitor.

15. Therapeutic combination comprising

(a) a compound of the formula:

(b) at least one adenosine triphosphate citrate lyase inhibitor / adenosine monophosphate-activated protein kinase activator or a pharmaceutically acceptable salt thereof.
16. Therapeutic combination according to claim 15, wherein the adenosine triphosphate citrate lyase inhibitor / adenosine monophosphate-activated protein kinase activator is ETC-1002 with the formula:

![Chemical Structure]

or a pharmaceutically acceptable salt thereof.

17. Therapeutic combination according to claim 15 or claim 16, wherein the combination comprises about 1 to 25 mg of Compound A and about 1 to 300 mg of the adenosine triphosphate citrate lyase inhibitor / adenosine monophosphate-activated protein kinase activator, preferably ETC-1002.

18. Therapeutic combination according to claim 15 or claim 16, wherein the combination comprises about 1 to 25 mg of Compound A and about 20 to 240 mg of the adenosine triphosphate citrate lyase inhibitor / adenosine monophosphate-activated protein kinase activator, preferably ETC-1002.

19. Therapeutic combination according to claim 15 or claim 16, wherein the combination comprises about 1 to 25 mg of Compound A and about 60 to 180 mg of the adenosine triphosphate citrate lyase inhibitor / adenosine monophosphate-activated protein kinase activator, preferably ETC-1002.

20. Therapeutic combination according to any of the claims 15-19, wherein the combination is a fixed dose combination or a free dose combination, preferably a fixed dose combination.

21. Therapeutic combination according to any of the claims 15-20, wherein the combination comprises a first unit dosage form comprising about 1 to 25 mg of Compound A or a pharmaceutically acceptable salt thereof and a second unit dosage form comprising about 1 to 300 mg ETC-1002, preferably about 20 to 240 mg ETC-1002, more preferably about 60 to 180 mg of ETC-1002 or a pharmaceutically
acceptable salt thereof.

22. Therapeutic combination according to claim 21, wherein the combination comprises a package comprising said unit dosage forms.

23. Therapeutic combination according to any of the claims 15-22, wherein the composition comprises polyunsaturated fatty acids (PUFAs), preferably omega-3 polyunsaturated fatty acids, more preferably PUFAs chosen from the group consisting of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), α-Linolenic acid (ALA) or combinations thereof.

24. Therapeutic combination according to claim 23, wherein the PUFAs are present in their free acid form.

25. Therapeutic combination according to any of the claims 15 to 24, wherein the therapeutic combination further comprises at least one FIMG-CoA reductase inhibitor or a pharmaceutically acceptable salt thereof.

26. Therapeutic combination according to claim 25, wherein the FDVIG-CoA reductase inhibitor is selected from the group consisting of atorvastatin, pravastatin, fluvastatin, simvastatin, lovastatin, rosuvastatin and pitavastatin.

27. Therapeutic combination according to claim 25 or claim 26, wherein the FDVIG-CoA reductase inhibitor is selected from the group consisting of atorvastatin calcium, pravastatin sodium, fluvastatin sodium, simvastatin, lovastatin and rosuvastatin calcium.

28. Therapeutic combination according to any of the previous claims 25-27, wherein the composition comprises about 1 to 80 mg of the FDVIG-CoA reductase inhibitor.

29. Pharmaceutical composition according to any of the claims 1-14 or the therapeutic combination according to any of the claims 15-28 for use in the treatment of subjects suffering from cardiovascular diseases or having an increased risk for cardiovascular diseases.
30. Pharmaceutical composition or therapeutic combination according to claim 29 for use in the treatment of subjects suffering from hyperlipidemia or mixed dyslipidemia or of subjects having an increased risk for hyperlipidemia or mixed dyslipidemia.

31. Pharmaceutical composition or therapeutic combination for use according to claim 29 or 30, wherein a subject in need thereof is administered about 1 to 25 mg per day of Compound A and about 1 to 300 mg per day adenosine triphosphate citrate lyase inhibitor / adenosine monophosphate-activated protein kinase activator.

32. Pharmaceutical composition or therapeutic combination for use according to any of the claims 29-31, wherein the adenosine triphosphate citrate lyase inhibitor / adenosine monophosphate-activated protein kinase activator is ETC-1002.

33. Pharmaceutical composition or therapeutic combination according to any of the claims 29-32 for use in the treatment of subjects suffering from or having an increased risk for hyperlipidemia or mixed dyslipidemia, wherein said subjects are administered about 1 to 25 mg per day of Compound A or a pharmaceutically acceptable salt thereof and about 1 to 300 mg of ETC-1002, preferably about 20 to 240 mg of ETC-1002 mg or a pharmaceutically acceptable salt thereof.

34. Pharmaceutical composition or therapeutic combination according to claim 33, wherein said subjects are administered about 1 to 25 mg per day of Compound A or a pharmaceutically acceptable salt thereof and about 60 to 180 mg of ETC-1002 or a pharmaceutically acceptable salt thereof.

35. Pharmaceutical composition or therapeutic combination according to claim 33 or claim 34, wherein said subjects are further administered about 1 to 80 mg per day of an HMG CoA reductase inhibitor or a pharmaceutically acceptable salt thereof.

36. Pharmaceutical composition or therapeutic combination for use according to any of the claims 29-35, wherein the composition or combination is to be administered to subjects in need thereof for at least one week, preferably at least three weeks.
# INTERNATIONAL SEARCH REPORT

**A. CLASSIFICATION OF SUBJECT MATTER**

<table>
<thead>
<tr>
<th>Inv.</th>
<th>A61K31/506</th>
<th>A61P3/06</th>
<th>A61P9/00</th>
<th>A61K31/20</th>
<th>A61K31/22</th>
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</thead>
</table>

**ADD.**

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

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

| A61K |

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

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

| EPO-Internal, WPI Data, BIOSIS |

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
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</table>

| X | Further documents are listed in the continuation of Box C. |
| X | See patent family annex. |

* Special categories of cited documents:

- **A** document defining the general state of the art which is not considered to be of particular relevance
- **E** earlier application or patent but published on or after the international filing date
- **L** document which may throw doubts on priority claim(s) one of which is cited to establish the publication date of another citation or other special reason as specified
- **O** document referring to an oral disclosure, use, exhibition or other means
- **P** document published prior to the international filing date but later than the priority date claimed

- **T** later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

- **X** document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

- **Y** document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

- **Z** document member of the same patent family

**Date of the actual completion of the international search**

8 April 2016

**Date of mailing of the international search report**

20/04/2016

**Name and mailing address of the ISA**

European Patent Office, P.B. 5818 Patentlaan 2
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**Uryga-Pol owy, V**
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