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(54) Title: SOLID COMPOSITIONS COMPRISING A PCSK9 INHIBITOR AND A SALT OF N-(8-(2-HYDROXYBENZOYL)AMINO)CAPRYLIC ACID

(57) Abstract: The invention relates to pharmaceutical compositions comprising a PCSK9 inhibitor, such as an EGF(A) peptide, and a salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid. The invention further relates to processes for the preparation of such compositions, and their use in medicine.



WO 2021/089761 A1

SOLID COMPOSITIONS COMPRISING A PCSK9 INHIBITOR AND A SALT OF N-(8-(2-HYDROXYBENZOYL)AMINO)CAPRYLIC ACID

TECHNICAL FIELD OF THE INVENTION

5 The present invention relates to solid compositions comprising a PCSK9 inhibitor and a salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid, their method of preparation and their use in medicine.

INCORPORATION-BY-REFERENCE OF THE SEQUENCE LISTING

10 The Sequence Listing, entitled "SEQUENCE LISTING", is 52 KB and was created on November 4th, 2020 and is incorporated herein by reference.

BACKGROUND

15 High LDL-C (Low Density Lipoprotein cholesterol) levels and dyslipidaemia are well-recognised drivers of cardiovascular disease.

 Statins have been approved for the treatment of dyslipidemia for 25 years. This class has demonstrated substantial and consistent reduction of cardiovascular events with an acceptable safety profile. The best-selling statin, atorvastatin (Lipitor™) was the world's best-selling drug of all time, with more than \$125 billion in sales from 1996 to 2012.

20 Despite the availability and widespread use of statins and other lipid lowering agents, many patients do not reach their target LDL-C levels and remain at high risk for developing cardiovascular disease. PCSK9 (Proprotein Convertase Subtilisin/Kexin type 9) promotes hepatic LDL-R (LDL receptor) degradation, thereby reducing hepatic LDL-R surface expression and consequently clearance of LDL particles. Conversely, blocking of
25 PCSK9 increases the clearance of LDL-C as well as other atherogenic lipoproteins. Indeed, LDL receptors contribute to the clearance of atherogenic lipoproteins other than LDL, such as intermediate-density lipoproteins and remnant particles. Increased intermediate-density lipoproteins and remnant particle clearance may have therapeutic benefits beyond that provided by LDL reduction.

30 Statins increase the expression of both LDL-R and PCSK9 via the SREBP2 transcription factor. The increased expression of PCSK9 may diminish the effect of statins on LDL-C clearance from the circulation.

 By inhibiting the binding of PCSK9 to the LDL-R and thereby preventing LDL-R degradation the efficacy of statins is enhanced. Taken together, PCSK9 inhibition offers a
35 novel approach to lipid management.

Two anti-PCSK9 antibodies, alirocumab/Praluent® and evolocumab/Repatha®, have been approved for the treatment of high LDL-C levels. These are administered by 1 ml subcutaneous injections every two weeks.

The EGF(A) (Epidermal Growth Factor-like domain A) sequence (40 amino acids) of the LDL-R (LDL-R-(293-332)) is well recognized as the site for PCSK9 binding. The isolated wild-type EGF(A) peptide has been shown to inhibit the binding of PCSK9 to the LDL-R with an IC_{50} in the low μM range (Biochemical and Biophysical Research Communications 375 (2008) 69–73). This poor potency has prevented a practical pharmaceutical use of the EGF(A) peptide. Furthermore, the half-life of such peptides would be expected to be too short to be of therapeutic use.

WO2012177741 and J. Mol. Biol. (2012) 422, 685-696 disclose analogues of the EGF(A) and Fc-Fusion thereof.

Alternative EGF(A) peptide based PCSK9 inhibitors with an extended half-life have been disclosed in WO2017/121850. In order to increase the usability of such drugs it is of interest to develop a suitable oral formulation. Oral administration of therapeutic peptides is challenging due to the rapid degradation of such peptides in the gastrointestinal system.

Oral bioavailability of peptide compounds is generally limited but useful results have been obtained for semaglutide as described in WO 2012/080471 and WO 2013/139694.

SUMMARY

The present invention in an aspect relates to a composition comprising a PCSK9 inhibitor and an absorption enhancer or delivery agent and a hydrotrope. The composition according to the invention comprises balanced amounts of the delivery agent and the hydrotrope. The provided compositions display an accelerated dissolution enabling fast and efficient uptake of the active pharmaceutical ingredient.

Described herein are pharmaceutical compositions demonstrating an accelerated dissolution and thus an improved exposure of the PCSK9 inhibitor by oral administration within 15-30 minutes after administration. The inventors have found that the dissolution and absorption of a PCSK9 inhibitor composition occurs faster when the composition is prepared with a hydrotrope.

An aspect of the invention relates to a composition comprising

- i) a PCSK9 inhibitor
- ii) a salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid (NAC) and
- iii) a hydrotrope,

wherein the hydrotrope is capable of increasing the solubility of SNAC at least 2-fold, such as 5-fold or such as at least 10-fold.

In one embodiment the composition comprises:

- i) 0.1-100 mg PCSK9 inhibitor,
- 5 ii) 50-600 mg salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid (NAC), such as the sodium salt of NAC (SNAC) and
- iii) 20-400 mg nicotinamide or resorcinol and
- iv) 0-10 mg lubricant.

In additional embodiments, the composition further includes a lubricant.

10

A further aspect relates to a method for producing a solid pharmaceutical composition comprising the steps of;

- a) obtaining a salt of NAC and a hydrotrope,
- b) co-processing the salt of NAC and hydrotrope of a) and
- 15 c) preparing said solid pharmaceutical composition using the product of b).

In a further aspect the invention relates to a composition or a granule as defined herein for use in medicine, such as for improving lipid parameters and/or preventing and/or treating cardiovascular diseases.

20

In a further aspect the invention relates to a method of improving lipid parameters and/or preventing and/or treating cardiovascular diseases comprising administering the composition as defined herein to a patient in need thereof, wherein said composition is a tablet and is administered orally.

25 BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 shows dose dependent effect of nicotinamide (A) and resorcinol (B) on SNAC solubility at pH 6.

Fig. 2 shows dissolution of test compositions 1, 3, 5, 6 and 7.

30 DESCRIPTION

An aspect of the invention relates to a composition comprising a PCSK9 inhibitor and an absorption enhancer or delivery agent and a hydrotrope. The composition may be in the form suitable for oral administration, such as a tablet, sachet or capsule. In an embodiment the composition is an oral composition, or a pharmaceutical composition, such as an oral pharmaceutical composition.

35

The provided compositions display an accelerated dissolution and thereby enables fast uptake of the active pharmaceutical ingredient.

PCSK9 inhibitor

5 The term "PCSK9 inhibitor" as used herein refers to a compound, which fully or partially prevents PCSK9 from binding to the human Low Density Lipoprotein Receptor (LDL-R).

The EGF(A) LDL-R(293-332) peptide binds PCSK9, but is not considered a PCSK9 inhibitor due to a relatively weak binding to PCSK9. The potential of an EGF(A) analogue to
 10 inhibit PCSK9 may be measured in an ELISA assay (such as Assay I herein) providing the apparent affinity of the EGF(A) analogue or a compound comprising an EGF(A) analogue reported as a K_i . A low K_i is thus characteristic for compounds with a strong inhibitory function as described in WO2017/121850. Based on their ability to inhibit the interaction of PCSK9 with LDL-R, such compounds are referred to as PCSK9 inhibitors. Based on the
 15 findings described in WO2017/121850 a suitable PCSK9 inhibitor has a K_i below 8 nM, such as below 5 nM. In one embodiment the PCSK9 inhibitor has a K_i around 0.5-8 nM, or such as 0.5-5 nM or such as 1.0-4 nM. An assay suited for determining the K_i is described herein in Assay I.

In one embodiment the PCSK9 inhibitor has an inhibitory function at least
 20 comparable to EGF(A) 301L. In one embodiment the PCSK9 inhibitor has an PCSK9 inhibitory function comparable to EGF(A) 301L. In a given assay, such as Assay I described herein, the ratio $\frac{K_i(\text{PCSK9 inhibitor})}{K_i(\text{EGF(A)301L})}$ is thus preferably below 2, such as below 1.5, such as below 1.2. In one embodiment the ratio is at most 1.0, such as at most 0.8, such as at most 0.7, such as at most 0.6 or such as at most 0.5. In one embodiment $\frac{K_i(\text{PCSK9 inhibitor})}{K_i(\text{EGF(A)301L})}$ the
 25 ratio is 2.0-0.2, such as 1.5-0.5 or such as 1.2-0.8.

In one embodiment where the PCSK9 inhibitor has an inhibitory function comparable to EGF(A) 301L. In one embodiment the PCSK9 inhibitor has an improved PCSK9 inhibitory function compared to EGF(A) 301L, 309R, 310K. In a given assay, such as Assay I described herein, the ratio $\frac{K_i(\text{PCSK9 inhibitor})}{K_i(\text{EGF(A)301L,309R,312E})}$ is thus preferably below 2, such as
 30 below 1.5, such as below 1.2. In one embodiment the ratio is at most 1.0, such as at most 0.8, such as at most 0.7, such as at most 0.6 or such as at most 0.5. In one embodiment $\frac{K_i(\text{PCSK9 inhibitor})}{K_i(\text{EGF(A)301L})}$ the ratio is 2.0-0.2, such as 1.5-0.5 or such as 1.2-0.8.

In one embodiment PCSK9 inhibitor comprises an EGF(A) peptide analogue as further described below.

EGF(A) compound

The term "EGF(A) compound" is used herein to generally refer to a compound comprising an EGF(A) peptide, encompassing wt-LDL-R(293-332) as defined by SEQ ID NO: 1 and analogues hereof. The term EGF(A) compound encompasses derivatives of EGF(A) peptide and analogue thereof i.e. EGF(A) peptide analogues with a substituent as described herein is a typical example of an EGF(A) compound.

EGF(A) peptides

The term "peptide", as e.g. used in the context of the invention, refers to a compound which comprises a series of amino acids interconnected by amide (or peptide) bonds. In a particular embodiment the peptide consists of amino acids interconnected by peptide bonds.

The peptide of the invention comprises at least 35, such as 36, 37, 38, 39 or at least 40 amino acids. In a particular embodiment the peptide is composed of 36, such as 38 or 40 amino acids. In an additional particular embodiment, the peptide consists of 35, 36, 37, 38, 39 or 40 amino acids.

In the presence of amino acid additions, referred to herein as N-terminal and C-terminal elongations, the peptide of the invention may comprise up to 140 amino acids. In an embodiment, the peptide of the invention may comprise or consist of 41 amino acid residues. In a particular embodiment, the peptide comprises 40-140, 40-120, 40-100, 40-80, 40-60 or 40-50 amino acids.

The terms "EGF(A) domain of the LDL-R", "LDL-R (293-332)", "native LDL-R (293-332)", "EGF(A) (293-332)", "wild-type EGF(A)", "wt-EGF(A)" or "native EGF(A)" as used herein refer to a peptide consisting of the sequence SEQ ID NO: 1.

SEQ ID NO: 1 is :

Gly-Thr-Asn-Glu-Cys-Leu-Asp-Asn-Asn-Gly-Gly-Cys-Ser-His-Val-Cys-Asn-Asp-Leu-Lys-Ile-Gly-Tyr-Glu-Cys-Leu-Cys-Pro-Asp-Gly-Phe-Gln-Leu-Val-Ala-Gln-Arg-Arg-Cys-Glu.

In this formula the numbering of the amino acid residues follows the numbering for the EGF(A) domain of the LDL-R (LDL-R-(293-332)), wherein the first (N-terminal) amino acid residue is numbered or accorded position no. 293, and the subsequent amino acid residues towards the C-terminus are numbered 294, 295, 296 and so on, until the last (C-

terminal) amino acid residue, which in the EGF(A) domain of the LDL-R is Glu with number 332.

The numbering is done differently in the sequence listing, where the first amino acid residue of SEQ ID NO: 1 (Gly) is assigned no. 1, and the last (Glu) no. 40. The same applies
5 for the other sequences of the sequence listing, i.e. the N-terminal amino acid assigned is no. 1 irrespective of its positioning relative to 293Gly or 293 substituting amino acid residue by reference to LDL-R(293-332). However, herein the numbering of amino acid positions is with reference to LDL-R(293-332), as explained above.

The present invention relates to analogues of the EGF(A) peptide identified by SEQ
10 ID NO:1 and derivatives of such EGF(A) peptide analogues of the wild-type EGF(A) domain of LDLR defined by SEQ ID NO: 1.

The term "analogue" generally refers to a peptide, the sequence of which has one or more amino acid changes when compared to a reference amino acid sequence.

The terms "analogue of the invention", "peptide analogue of the invention", "LDL-R(293-332) analogue", "EGF(A) analogue" or "analogue of SEQ ID NO: 1" as used herein
15 may be referred to as a peptide, the sequence of which comprises amino acid substitutions, i.e. amino acid replacement, relative to sequence SEQ ID NO: 1. An "analogue" may also include amino acid elongations in the N-terminal and/or C-terminal positions and/or truncations in the N-terminal and/or C-terminal positions.

The level of identity to SEQ ID NO.:1 can be calculated by determining the number
20 of amino acids that are not changed relative to SEQ ID NO 1. SEQ ID NO: 1 consists of 40 amino acid residues and if three amino acid substitutions are introduced the level of identity is $37/40\%=92.5\%$. If 5 amino acid residues are changed the level of identity is 87, 5 %. If the peptide is N-terminal or C-terminal elongated that part is usually not included in the
25 comparison, whereas a deletion of one or more amino acids shortens the comparator. For instance, in the examples above, if the N-terminal amino acid is deleted the level of identity is slightly reduced to $36/39 \times 100\%$ and $34/39 \times 100\%$, respectively. When discussing identity of the back-bone sequence of a derivative the amino acid residue of the substituent e.g. the residue to which the substituent is attached, also termed the amino acid residue of the
30 substituent, may be either a wild type (wt) or a substituted amino acid. If the amino acid residue of the substituent is a wild type residue, such as the N-term Gly or 312K this residue is included in the calculation of identity level, whereas a Lys in any other position from 293 to 332 would be an amino acid substitution and not included when calculated amino acid identity to SEQ ID NO.:1.

In one embodiment the EGF(A) peptide analogue has 1-15 amino acid substitutions compared to SEQ ID NO.: 1. In one embodiment the EGF(A) peptide analogue has 1-10 amino acid substitutions compared to SEQ ID NO.: 1. In one embodiment the EGF(A) peptide analogue has 1-8 amino acid substitutions compared to SEQ ID NO.: 1, such as 1-7, 1-6, 1-5 amino acid substitutions compared to SEQ ID NO.: 1. In a particular embodiment, up to 7 amino acid substitutions may be present, for example up to 6, 5, 4, 3, 2 or 1 amino acid substitutions may be present in the EGF-1 peptide analogue.

In one embodiment the analogue of the invention has at least 75 % identity, such as 80 %, such as 85, such as 90 or even 95 % identity to SEQ ID NO.:1 corresponding to up to 10, 8, 6, 4 and 2 amino acid substitutions relative to SEQ ID NO 1, respectively in case of no truncation.

Each of the peptide analogues of the invention may be described by reference to i) the number of the amino acid residue in the native EGF(A) (LDL-R(293-332)) which corresponds to the amino acid residue which is changed (i.e., the corresponding position in native LDL-R(293-332) EGF(A)), and to ii) the actual change.

In other words, the peptide analogues of the invention may be described by reference to the native LDL-R(293-332) EGF(A) peptide, namely as a variant thereof in which a number of amino acid residues have been changed when compared to native LDL-R(293-332) EGF(A) (SEQ ID NO: 1). These changes may represent, independently, one or more amino acid substitutions.

The followings are non-limiting examples of suitable analogue nomenclature:

The EGF(A) peptide incorporated in the derivative of Example 2 in WO2017/121850 is thus referred to as the following LDL-R(293-332) EGF(A) analogue: (301Leu, 309Arg) LDL-R(293-332) EGF(A), or (Leu301, Arg309)-LDL-R(293-332) EGF(A) or (301L,309R) LDL-R(293-332) or (L301,R309) LDL-R(293-332). This means that when this analogue is aligned with native LDL-R(293-332), it has i) a Leu at the position in the analogue which corresponds, according to the alignment, to position 301 in native LDL-R(293-332) EGF(A), ii) an Arg at the position in the analogue which corresponds to position 309 in native LDL-R(293-332) EGF(A).

Analogues "comprising" certain specified changes may comprise further changes, when compared to SEQ ID NO: 1.

In a particular embodiment, the analogue "has" or "comprises" the specified changes. In a particular embodiment, the analogue "consists of" the changes. When the term "consists" or "consisting" is used in relation to an analogue e.g. an analogue consists or consisting of a group of specified amino acid substitutions, it should be understood that the

specified amino acid substitutions are the only amino acid substitutions in the peptide analogue. In contrast an analogue "comprising" a group of specified amino acid substitutions may have additional substitutions.

As is apparent from the above examples, amino acid residues may be identified by their full name, their one-letter code, and/or their three-letter code. These three ways are fully equivalent.

The expressions "a position equivalent to" or "corresponding position" may be used to characterise the site of change in a variant LDL-R(293-332) EGF(A) sequence by reference to the reference sequence native LDL-R(293-332) EGF(A) (SEQ ID NO: 1). Equivalent or corresponding positions, as well as the number of changes, are easily deduced, e.g. by simple handwriting and eyeballing; and/or a standard protein or peptide alignment program may be used, such as "align" which is based on a Needleman-Wunsch algorithm.

In what follows, it may occur that a chemical formula is defined such that two subsequent chemical groups may both be selected to be "a bond". In such instances, the two subsequent chemical groups would actually be absent, and just one bond would connect the surrounding chemical groups.

Amino acids are molecules containing an amino group and a carboxylic acid group, and, optionally, one or more additional groups, often referred to as a side chain.

The term "amino acid" includes proteinogenic (or natural) amino acids (amongst those the 20 standard amino acids), as well as non-proteinogenic (or non-natural) amino acids. Proteinogenic amino acids are those which are naturally incorporated into proteins. The standard amino acids are those encoded by the genetic code. Non-proteinogenic amino acids are either not found in proteins, or not produced by standard cellular machinery (e.g., they may have been subject to post-translational modification). Non-limiting examples of non-proteinogenic amino acids are Aib (α -aminoisobutyric acid, or 2-aminoisobutyric acid), norleucine, norvaline as well as the D-isomers of the proteinogenic amino acids.

In what follows, each amino acid of the peptides of the invention for which the optical isomer is not stated is to be understood to mean the L-isomer (unless otherwise specified).

EGF(A) peptide analogues

An aspect of the invention relates to an analogue of a peptide of SEQ ID NO: 1. The peptide analogues of the invention may be defined as peptides comprising an amino acid sequence which is an analogue of SEQ ID NO: 1. The peptide analogues of the

invention have the ability to bind to PCSK9. In a specific embodiment, the analogues of the invention have an improved ability to bind to PCSK9, for example compared to native LDL-R(293-332) (native EGF-(A)) or to other PCSK9-binding compounds.

5 The peptide analogues of the invention have the ability to inhibit PCSK9 binding to the LDL-R. In one embodiment the peptide is a PCSK9 inhibitor. In one embodiment the peptide inhibits PCSK9 binding to human Low Density Lipoprotein Receptor (LDL-R). Such binding may be assessed using the assay described in Assay I herein. In one embodiment the peptide analogues and peptide derivatives of the invention are PCSK9 inhibitor peptides or simply PCSK9 inhibitors. In one embodiment the invention relates to a peptide analogue of
10 SEQ ID NO.:1, wherein peptide analogue is a capable of inhibiting PCSK9 binding to human Low Density Lipoprotein Receptor (LDL-R).

In one embodiment the peptide analogues, compounds or PCSK9 inhibitors of the invention have an improved ability to bind PCSK9 compared to EGF(A) LDL-R(293-332) (SEQ ID 1).

15 As described above EGF(A) peptide analogues or compounds comprising such are considered PCSK9 inhibitors when such molecules have the ability to inhibit the binding of PCSK9 to LDL-R, by having an improved binding to PCSK9 compared to EGF(A) LDL-R(293-332) (SEQ ID 1).

In one embodiment the K_i of the peptide analogues, compounds or PCSK9 inhibitors
20 as described herein as measured in the PCSK9-LDL-R binding competitive ELISA assay (Assay I) is below 10 nM, such as below 8 nM or such as below 5 nM.

Functionality of EGF-(A) analogues and derivatives hereof may be further characterized by their ability to improve LDL uptake, such as described in WO2017/121850 Example D1.2. In one embodiment the peptide analogues, compounds or PCSK9 inhibitors
25 of the invention increases LDL uptake in the presence of PCSK9. In one embodiment the peptide analogues, compounds or PCSK9 inhibitors of the invention are capable of reversing or reducing PCSK9 mediated reduction of LDL uptake.

In one embodiment the peptide analogues, compounds or PCSK9 inhibitors of the invention have a EC_{50} as measured in the LDL uptake assay of below 1500 nM, such as
30 below 1000 nM or such as below 500 nM.

In an embodiment, a peptide analogue of the invention may be defined as comprising at least 1 amino acid substitution compared to SEQ ID NO: 1, and optionally an elongation. In an embodiment, a peptide analogue of the invention may be defined as comprising up to 15, up to 14, up to 13, up to 12, up to 11, up to 10, up to 9, up to 8, up to 7,
35 up to 6, up to 5, up to 4, up to 3, up to 2 or 1 amino acid(s) substitution(s) compared to SEQ

ID NO: 1, and optionally an elongation. This means that a peptide comprising an elongation in the N-terminal and/or in the C-terminal may comprise up to 15 amino acids substitutions in positions from 293 to 332 in addition to said elongation.

An amino acid "elongation" may also be referred to as "extension". In an embodiment, peptide analogues of the invention comprise an elongation. Said elongation may be an addition of up to 50 amino acid residues in position N-terminal of SEQ ID NO: 1 or an analogue thereof, also referred to as an N-terminal elongation, meaning that a peptide of the invention may comprise up to 50 amino acids from position 292 down to, for example position 242. Additionally, or alternatively, said elongation may be an addition of up to 50 amino acid residues in position C-terminal of SEQ ID NO: 1 or analogue thereof, also referred to as a C-terminal elongation, meaning that a peptide of the invention may comprise up to 50 amino acids from position 333 up to, for example position 383.

Said elongation may be present either in N-terminal, in C-terminal or both. Said elongation may also be of any length between 0 and 50 amino acids on each side, independently of each other. In one embodiment, the peptide analogues of the invention comprise a N-terminal elongation of 1-50, 1-40, 10-40, 1-30, 10-30, 20-30, 20-40, 20-50, 30-50, 1-10, 11-20, 21-30, 31-40 or 41-50 amino acid residues or of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50 amino acid residues. In addition or alternatively, the peptide analogues of the invention may comprise a C-terminal elongation of 1-50, 1-40, 10-40, 1-30, 10-30, 20-30, 20-40, 20-50, 30-50, 1-10, 11-20, 21-30, 31-40 or 41-50 amino acid residues or of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50 amino acid residues.

An elongation may in some situation be referred to a substitution as a new amino acid residue is introduced, such as the 292A, 292Lys or 333Lys exemplified herein.

Minor truncations at the N-terminal and/or C-terminal of the EGF(A) peptide may be present in the EGF(A) peptide analogue.

In one embodiment the EGF(A) peptide comprise at least 35 amino acid residues, such as 36 amino acid residues, such as 37 amino acid residues, such as 38 amino acid residues or such as such as 39 amino acid residues. In one embodiment the EGF(A) peptide analogue according comprises an N-terminal truncation of 1-2 amino acid residues. In one embodiment one or two N-terminal amino acid residues are deleted. In further embodiments the EGF(A) peptide analogue accordingly comprises an N-terminal truncation deleting at least or specifically amino acid 293Gly.

In further embodiments the EGF(A) peptide analogue comprises an N-terminal truncation deleting at least or specifically 293Gly-294Thr.

In one embodiment the EGF(A) peptide analogue comprises a C-terminal truncation of 1 amino acid residue. In one embodiment a single C-terminal amino acid residue is
5 deleted. In one embodiment the peptide analogue comprises a C-terminal truncation deleting specifically amino acid 332Glu.

In addition, or alternatively, a peptide analogue of the invention may comprise at least one amino acid elongation in the N-terminal or the C-terminal for example in position 292 and/or 333.

10 The EGF(A) peptide analogue of the invention comprises the amino acid substitution of amino acid residue 301 from Asn to Leu, also described by Asn301Leu or simply 301Leu. In a specific embodiment, the EGF(A) peptide analogue comprises the substitution 301Leu.

In addition, or alternatively the EGF(A) peptide analogue comprises the amino acid residues 297Cys, 304Cys, 308Cys, 317Cys, 319Cys and 331Cys. Those Cys residues are
15 wild type residues which may be engaged in disulphide bridges, such as the disulphide bridges between 297Cys and 308Cys, between 304Cys and 317Cys and between 319Cys and 331Cys.

In one embodiment, the EGF(A) peptide analogue comprises 301Leu and a number of further amino acid substitutions, as described above.

20 In one embodiment the EGF(A) peptide analogue comprises 301Leu, 310Asp and an amino acid substitution of 312Lys.

In one embodiment, the EGF(A) peptide analogue comprises 301Leu and 310Asp and wherein the peptide analogue does not have a substitution of 299Asp to Glu, Val or His.

In one embodiment the EGF(A) peptide analogue comprises 301Leu, 309Arg and
25 312Glu.

In one embodiment the EGF(A) peptide analogue comprises 301Leu and 309Arg with a proviso that the peptide analogue does not have a substitution of 310Asp to 310Lys or

In one embodiment the EGF(A) peptide analogue comprises 301Leu and 309Arg with a proviso that the peptide analogue does not have a substitution of 299Asp to Glu, Val
30 or His.

In a further embodiment the peptide analogue does not have any of the substitutions D310K, D310N, D310Q, D310Q, D310R and D310A or even any substitution of 310Asp.

In one embodiment the EGF(A) peptide analogue comprises one, two, three or all four wild type residues: 295Asn, 296Glu, 298Leu and 302Gly.

In one embodiment the EGF(A) peptide analogue comprises one, two, three, four or all five wild type residues: 295Asn, 296Glu, 298Leu, 302Gly and 310Asp.

In one embodiment the peptide has 295Asn.

In one embodiment the peptide analogue has 296Glu. In one embodiment the peptide analogue has 298Leu. In one embodiment the peptide analogue has 302Gly. In one embodiment the peptide analogue has 310Asp.

In one embodiment the peptide analogue has two or more of 310Asp, 295Asn and 296Glu. In one embodiment the peptide analogue has all three of 310Asp, 295Asn and 296Glu.

The EGF(A) peptide analogue may comprise further amino acid substitutions as described herein. In one embodiment the analogue of the invention may further comprise one or more amino acid substitution in a position(s) selected from the group of positions: 293, 294, 296, 299, 300, 303, 305, 306, 309, 311, 312, 313, 314, 315, 316, 318, 320, 321, 322, 323, 324, 325, 326, 328, 329, 330 and 332.

In one embodiment the analogue of the invention may further comprise one or more amino acid substitution(s) in a position(s) selected from the group of positions: 293, 294, 299, 300, 303, 305, 306, 309, 311, 312, 313, 314, 316, 318, 321, 322, 323, 324, 325, 326, 328, 329, 330, 331 and 332.

In one embodiment the analogue of the invention may further comprise one or more amino acid substitution(s) in a position(s) selected from the 294, 299, 300, 303, 309, 312, 313, 314, 316, 318, 321, 322, 323, 324, 325, 326, 328, 329, 330 and 332.

In one embodiment the analogue of the invention may further comprise one or more amino acid substitution(s) in a position(s) selected from the 299, 300, 309, 313, 316, 318, 321, 322, 323, 324, 326, 328, 329, 330 and 332.

In one embodiment the analogue of the invention may further comprise one or further amino acid substitution(s) in a position(s) selected from the group of positions: 309, 312, 313, 321, 324, 328 and 332.

In a further embodiment the peptide analogue comprises either the wt amino acid residue or a different residue i.e. an amino acid substitution, in certain specific positions in addition to the amino acid residues specified herein above.

In one such embodiment the analogue of the invention comprises the amino acid residue Gly(G) or Asn(N) in position 293.

In one such embodiment the analogue of the invention comprises the amino acid residue Trp (W), Thr(T) or Gly(G) in position 294.

In one such embodiment the analogue of the invention comprises the amino acid residue Asp(D), Gly(G), Pro(P), Arg(R), Lys(K), Ser(S), Thr(T), Asn(N), Gln(Q), Ala(A), Ile(I), Leu(L), Met(M), Phe(F), Tyr(Y) or Trp(W) in position 299.

5 In one such embodiment the analogue of the invention comprises the amino acid residue Asp(D), Gly(G), Pro (P), Arg(R), Lys(K), Ser(S), Thr(T), Asn(N), Gln(Q), Ala(A), Met(M), Phe(F), Tyr(Y) or Trp(W) in position 299.

In one such embodiment the analogue of the invention comprises the amino acid residue Asp(D), Ser (S), Arg(R), Leu (L), Ala (A), Lys(K) or Tyr(Y) in position 299.

10 In one such embodiment the analogue of the invention comprises the amino acid residue Asp(D) or Ala(A) in position 299.

In one such embodiment the analogue of the invention comprises the amino acid residue His(H) or Asn(N) in position 300.

In one such embodiment the analogue of the invention comprises the amino acid residue Val(V), Ser(S), Thr (T) or Ile (I) in position 307.

15 In one such embodiment the analogue of the invention comprises the amino acid residue Val(V) or Ile (I) in position 307.

In one such embodiment the analogue of the invention comprises Ser (S), Thr (T) or Ile (I) in position 307.

20 In one such embodiment the analogue of the invention comprises Ile (I) in position 307.

In one such embodiment the analogue of the invention comprises the amino acid residue Asn(N), Glu (E), His (H,) Arg (R), Ser (S) or Lys (K) in position 309.

In one such embodiment the analogue of the invention comprises the amino acid residue Asn(N), Arg (R), Ser (S) or Lys (K) in position 309.

25 In one such embodiment the analogue of the invention comprises the amino acid residue Asn(N) , Arg (R) or Ser (S) in position 309.

In one such embodiment the analogue of the invention comprises the amino acid residue Asn(N) or Arg (R) in position 309.

30 In one such embodiment the analogue of the invention comprises the amino acid residue Lys(K) or Arg (R) in position 309.

The EGF(A) peptide analogue may comprise several amino acid substitutions as described herein, such as one or more amino acid substitutions selected from the group of: 299Ala, 307Ile and 321Glu.

In further embodiments, the EGF(A) peptide analogue comprises the amino acid residue Asp(D), Lys (K) or Glu(E) in position 321.

In further embodiments, the EGF(A) peptide analogue comprises the amino acid residue Asp(D) or Glu(E) in position 321.

5 In further embodiments, the EGF(A) peptide analogue comprises the amino acid residue Glu(E) in position 321.

In further embodiments, the EGF(A) peptide analogue comprises the amino acid residue Gln (Q) or Gly (G) in position 324.

10 In further embodiments, the EGF(A) peptide analogue comprises the amino acid residue Arg (R) or His (H) in position 329.

In further embodiments, the EGF(A) peptide analogue does not have a substitution of 300Asn(N) to Pro(P).

15 The EGF(A) domain of LDL-R includes a Lysine in position 312 which may be useful for substitution as described herein. In embodiments where attachment of the substituent to 312 is not wanted 312Lys may be substituted by another amino acid as described herein.

In one embodiment, Lys in position 312 is substituted by an amino acid residue selected from: Gly, Pro, Asp, Glu, Arg, His, Ser, Thr, Asn, Gln, Ala, Val, Ile, Leu, Met, Phe and Tyr. In one embodiment, Lys in position 312 is substituted by an amino acid residue
20 selected from: Gly, Asp, Glu, Ser, Thr, Asn, Ala, Val, Ile, Leu, Phe and Tyr. In one embodiment, Lys in position 312 is substituted by an amino acid residue selected from: Asp, Glu, Thr, Asn, Ile, Leu, Phe and Tyr. In one embodiment, 312Lys is substituted by 312Asp, 312Glu, 312Thr, 312Asn, 312Ile or 312Phe. In one embodiment, 312Lys is substituted by 312Glu, 312Asp, 312Gln or 312Arg.

25 In one embodiment, 312Lys is substituted by 312Glu, 312Thr, 312Asn, 312Ile, 312Phe or 312Tyr. In one embodiment, 312Lys is substituted by 312Glu, 312Asn or 312Ile,

In one embodiment, 312Lys is substituted by 312Glu or 312Arg. In one embodiment 312Lys is substituted by 312Arg. In one embodiment, 312Lys is substituted by 312Glu.

30 To include an option for attaching the substituent in various positions (see further below), a Lys may be introduced by amino acid substitution of a wild type residue of SEQ ID NO.: 1 or by a peptide elongation of SEQ ID NO.: 1, such as a 292Lys or a 333Lys.

In cases where more than one substituent is desired one may be via 312Lys while the second is via a Lys introduced by peptide elongation or substitution in SEQ ID NO.: 1.

35 In one embodiment the peptide analogue of SEQ ID NO: 1 comprises at least one Lys residue in a position selected from the group of: 292Lys, 293Lys, 294Lys, 296Lys,

299Lys, 300Lys, 303Lys, 305Lys, 306Lys, 309Lys, 311Lys, 312Lys, 313Lys, 314Lys, 315Lys, 316Lys, 318Lys, 320Lys, 321Lys, 322Lys, 323Lys, 324Lys, 325Lys, 326Lys, 327Lys, 328Lys, 329Lys, 330Lys, 332Lys and 333Lys.

In one embodiment the peptide analogue of SEQ ID NO: 1 comprises at least one
5 Lys residue in a position selected from the group of: 292Lys, 293Lys, 294Lys, 299Lys, 300Lys, 303Lys, 305Lys, 306Lys, 309Lys, 311Lys, 312Lys, 313Lys, 314Lys, 315Lys, 316Lys, 318Lys, 320Lys, 321Lys, 322Lys, 323Lys, 324Lys, 325Lys, 326Lys, 327Lys, 328Lys, 329Lys, 330Lys, 332Lys and 333Lys.

In one embodiment the peptide analogue of SEQ ID NO: 1 comprises at least one
10 Lys residue in a position selected from the group of: 292Lys, 293Lys, 294Lys, 300Lys, 303Lys, 305Lys, 306Lys, 309Lys, 311Lys, 312Lys, 313Lys, 314Lys, 316Lys, 318Lys, 321Lys, 322Lys, 323Lys, 324Lys, 325Lys, 326Lys, 327Lys, 328Lys, 329Lys, 330Lys, 332Lys and 333Lys.

In one embodiment the peptide analogue of SEQ ID NO: 1 comprises at least one
15 Lys residue in a position selected from the group of: 292Lys, 293Lys, 294Lys, 300Lys, 303Lys, 305Lys, 306Lys, 311Lys, 312Lys, 313Lys, 314Lys, 316Lys, 318Lys, 322Lys, 323Lys, 324Lys, 325Lys, 326Lys, 327Lys, 328Lys, 329Lys, 330Lys, 332Lys and 333Lys.

In one embodiment the peptide analogue of SEQ ID NO: 1 comprises at least one
Lys residue in a position selected from the group of: 292Lys, 293Lys, 294Lys, 300Lys,
20 303Lys, 305Lys, 306Lys, 311Lys, 313Lys, 314Lys, 316Lys, 318Lys, 322Lys, 323Lys, 324Lys, 325Lys, 326Lys, 327Lys, 328Lys, 329Lys, 330Lys, 332Lys and 333Lys.

In addition or alternatively, the peptide analogue of the invention comprises at least one amino acid substitution selected from 292Lys, 293Lys, 294Lys, 295Lys, 296Lys, 298Lys, 299Lys, 301Lys, 302Lys, 303Lys, 305Lys, 306Lys, 307Lys, 309Lys, 310Lys, 311Lys,
25 313Lys, 314Lys, 315Lys, 316Lys, 318Lys, 320Lys, 321Lys, 322Lys, 323Lys, 324Lys, 325Lys, 326Lys, 327Lys, 328Lys, 329Lys, 330Lys, 332Lys and 333Lys.

In a further embodiment, the EGF(A) peptide analogue of the invention comprises at least one amino acid substitution selected from: 292Lys, 293Lys, 294Lys, 295Lys, 296Lys, 298Lys, 299Lys, 302Lys, 303Lys, 305Lys, 306Lys, 307Lys, 309Lys, 311Lys, 313Lys,
30 314Lys, 315Lys, 316Lys, 318Lys, 320Lys, 321Lys, 322Lys, 323Lys, 324Lys, 325Lys, 326Lys, 327Lys, 328Lys, 329Lys, 330Lys, 332Lys and 333Lys.

In a further embodiment, the EGF(A) peptide analogue of the invention comprises at least one amino acid substitution selected from 292Lys, 293Lys, 294Lys, 295Lys, 296Lys, 298Lys, 299Lys, 303Lys, 305Lys, 306Lys, 309Lys, 311Lys, 313Lys, 314Lys, 315Lys,

316Lys, 318Lys, 320Lys, 321Lys, 322Lys, 323Lys, 324Lys, 325Lys, 326Lys, 327Lys, 328Lys, 329Lys, 330Lys, 332Lys and 333Lys.

In a further embodiment, the EGF(A) peptide analogue of the invention comprises at least one amino acid substitution selected from 292Lys, 293Lys, 294Lys, 295Lys, 296Lys, 299Lys, 303Lys, 305Lys, 306Lys, 309Lys, 311Lys, 313Lys, 314Lys, 315Lys, 316Lys, 318Lys, 320Lys, 321Lys, 322Lys, 323Lys, 324Lys, 325Lys, 326Lys, 327Lys, 328Lys, 329Lys, 330Lys, 332Lys and 333Lys.

In a further embodiment, the EGF(A) analogue peptide of the invention comprises at least one amino acid substitution selected from 292Lys, 293Lys, 294Lys, 296Lys, 299Lys, 303Lys, 305Lys, 306Lys, 309Lys, 311Lys, 313Lys, 314Lys, 315Lys, 316Lys, 318Lys, 320Lys, 321Lys, 322Lys, 323Lys, 324Lys, 325Lys, 326Lys, 327Lys, 328Lys, 329Lys, 330Lys, 332Lys and 333Lys.

In a further embodiment, the EGF(A) peptide analogue of the invention comprises at least one amino acid substitution selected from 292Lys, 293Lys, 294Lys, 299Lys, 303Lys, 305Lys, 306Lys, 309Lys, 311Lys, 313Lys, 314Lys, 315Lys, 316Lys, 318Lys, 320Lys, 321Lys, 322Lys, 323Lys, 324Lys, 325Lys, 326Lys, 327Lys, 328Lys, 329Lys, 330Lys, 332Lys and 333Lys.

In a further embodiment, the EGF(A) peptide analogue of the invention comprises at least one amino acid substitution selected from 292Lys, 293Lys, 294Lys, 299Lys, 303Lys, 305Lys, 306Lys, 309Lys, 311Lys, 313Lys, 314Lys, 315Lys, 316Lys, 318Lys, 320Lys, 321Lys, 322Lys, 323Lys, 324Lys, 325Lys, 326Lys, 327Lys, 328Lys, 329Lys, 330Lys, 332Lys and 333Lys.

In a further embodiment, the EGF(A) peptide analogue of the invention comprises at least one amino acid substitution selected from 292Lys, 293Lys, 294Lys, 299Lys, 303Lys, 305Lys, 306Lys, 310Lys, 311Lys, 313Lys, 314Lys, 315Lys, 316Lys, 318Lys, 320Lys, 321Lys, 322Lys, 323Lys, 324Lys, 325Lys, 326Lys, 327Lys, 328Lys, 329Lys, 330Lys, 332Lys and 333Lys.

In a further embodiment, the EGF(A) peptide analogue of the invention comprises at least one amino acid substitution selected from 292Lys, 293Lys, 294Lys, 299Lys, 303Lys, 305Lys, 306Lys, 309Lys, 310Lys, 311Lys, 313Lys, 314Lys, 315Lys, 316Lys, 318Lys, 321Lys, 322Lys, 323Lys, 324Lys, 325Lys, 326Lys, 327Lys, 328Lys, 329Lys, 330Lys, 332Lys and 333Lys.

In a further embodiment, the EGF(A) peptide analogue of the invention comprises at least one amino acid substitution selected from 292Lys, 293Lys, 294Lys, 303Lys, 305Lys, 306Lys, 310Lys, 311Lys, 313Lys, 314Lys, 315Lys, 316Lys, 318Lys, 321Lys, 322Lys,

323Lys, 324Lys, 325Lys, 326Lys, 327Lys, 328Lys, 329Lys, 330Lys, 332Lys and 333Lys. In one embodiment, the peptide analogues of the invention do not comprise any of the following substitutions: 296K, 298K, 301K, 302K and 307K.

In one embodiment, the peptide analogues of the invention do not comprise any of the following substitution: 296K, 298K, 301K, 302K, 307K and 310K.

In one embodiment, the peptide analogues of the invention do not comprise any of the following substitution: 296K, 298K, 301K, 302K, 307, and 295K.

In one embodiment, the peptide analogues of the invention do not comprise any of the following substitution: 296K, 298K, 301K, 302K, 307K and 295D.

In a particular embodiment, the peptide analogue of the invention comprises 1 or 2, of such Lys substitutions.

In addition, or alternatively, the peptide of the invention may comprise 312Lys.

In one embodiment the peptide analogue of the invention comprises two Lys residues. In one embodiment the peptide analogue of the invention comprises two Lys residues selected from the pairs consisting of:

- | | |
|---------------------|------------------------|
| i. 293K and 294K | xiv. 313K and 321K |
| ii. 293K and 312K | xv. 313K and 324K |
| iii. 293K and 333K | xvi. 313K and 328K |
| iv. 309K and 313K | xvii. 313K and 332K |
| v. 309K and 324K | xviii. 313K and 333K |
| vi. 309K and 328K | xix. 314K and 333K |
| vii. 309K and 332K | xx. 321K and 332K |
| viii. 309K and 333K | xxi. 321K and 333K |
| ix. 311K and 313K | xxii. 324K and 333K |
| x. 312K and 333K | xxiii. 324K and 328K |
| xi. 312K and 313K | xxiv. 328K and 333K |
| xii. 312K and 314K | xxv. 330K and 333K and |
| xiii. 313K and 314K | xxvi. 332K and 333K. |

As seen herein above various peptide analogues are provided by the present invention. In a further embodiment the EGF(A) peptide analogue according to the invention comprises at least two amino acid substitutions identified by any of the groups i-xxiv shown below compared to SEQ ID NO.:1.

In a still further embodiment, the EGF(A) peptide analogue of the invention consists of the amino acid substitutions identified by any of the groups i-xxiv as shown below.

In a further embodiment the EGF(A) peptide analogue according to the invention comprises at least two amino acid substitutions identified by any of the groups i-xvi shown below compared to SEQ ID NO.:1.

5 In a still further embodiment, the EGF(A) peptide analogue of the invention consists of the amino acid substitutions identified by any of the groups i-xvi as shown below.

- i. 301Leu and 309Arg
- ii. 301Leu, 309Arg, 312Glu
- iii. 301Leu, 307Ile and 309Arg
- 10 iv. 301Leu, 307Ile, 309Arg and 312Glu
- v. 301Leu, 309Arg and 321Glu
- vi. 301Leu, 309Arg, 321Glu and 312Glu
- vii. 301Leu, 307Ile, 309Arg and 299Ala
- viii. 301Leu, 307Ile, 309Arg, 299Ala and 312Glu
- 15 ix. 301Leu and 309Arg and at least one Lys substitution
- x. 301Leu, 309Arg, 312Glu and at least one Lys substitution
- xi. 301Leu, 307Ile and 309Arg and at least one Lys substitution
- xii. 301Leu, 307Ile, 309Arg and 312Glu and at least one Lys substitution
- xiii. 301Leu, 309Arg and 321Glu and at least one Lys substitution
- 20 xiv. 301Leu, 309Arg, 321Glu and 312Glu and at least one Lys substitution
- xv. 301Leu, 307Ile, 309Arg and 299Ala and at least one Lys substitution or
- xvi. 301Leu, 307Ile, 309Arg, 299Ala and 312Glu and at least one Lys substitution.

25 In a further embodiment the EGF(A) peptide analogue according to the invention comprises at least two amino acid substitutions identified by any of the groups xvii-xx shown below compared to SEQ ID NO.: 1.

In a still further embodiment, the EGF(A) peptide analogue of the invention consists of at the amino acid substitutions identified by any of the groups xvii-xx as shown below.

- xvii. 301Leu and 309Lys
- 30 xviii. 301Leu, 309Lys and 312Glu
- xix. 301Leu and 309Lys and at least one further Lys substitution
- xx. 301Leu, 309Lys and 312Glu and at least one further Lys substitution.

In a further embodiment the EGF(A) peptide analogue according to the invention comprises at least two amino acid substitutions identified by any of the groups xxi-xxiv shown below compared to SEQ ID NO.: 1.

5 In a still further embodiment, the EGF(A) peptide analogue of the invention consists of the amino acid substitution identified by any of the groups xxi-xxiv as shown below

- xxi. 301Leu and 307Ile,
- xxii. 301Leu, 307Ile and 312Glu
- xxiii. 301Leu and 307Ile and at least one further Lys substitution and
- xxiv. 301Leu, 3307Ile and 312Glu and at least one further Lys substitution.

10

In further specific embodiments the peptide analogue or the peptide analogue of the compounds according to the invention comprises or consists of anyone of the amino acid sequences identified by SEQ ID 1 to 114.

15 In one embodiment the peptide analogue comprises or consists of anyone of the amino acid sequences identified by SEQ ID NO.: 2-114.

In one embodiment the peptide analogue comprises or consists of anyone of the amino acid sequences identified by SEQ ID NO.: 2-47 and 49-114.

20 In one embodiment the peptide analogue comprises or consists of anyone of the amino acid sequences identified by anyone of the amino acid sequences SEQ ID NO.: 2-44, 46, 47 and 49-1-114.

In one embodiment the peptide analogue comprises or consists of anyone of the amino acid sequences identified by of SEQ ID NO.: 2-44, 46, 47, 49-53, 55, 58-114.

In one embodiment the peptide analogue comprises or consists of anyone of the amino acid sequences identified by SEQ ID NO.: 2-4, 6-44, 46, 47, 49-53, 55, 58-114.

25 In one embodiment the peptide analogue comprises or consists of anyone of the amino acid sequences identified by SEQ ID NO.: 2-4, 6-19, 21-44, 46, 47, 49-53, 55, 58-114.

In one embodiment the peptide analogue comprises or consists of anyone of the amino acid sequences identified by SEQ ID NO.: 2-4, 6-19, 21-44, 46, 47, 49-53, 55, 58-114.

30 In one embodiment the peptide analogue comprises or consists of anyone of the amino acid sequences identified by SEQ ID NO.: 3-114.

In one embodiment the peptide analogue comprises or consists of anyone of the amino acid sequences identified by SEQ ID NO.: 3-45 and 47-114.

35 In one embodiment the peptide analogue comprises or consists of anyone of the amino acid sequences identified by anyone of the amino acid sequences SEQ ID NO.: 3-45, 47-53 and 55-114.

In one embodiment the peptide analogue comprises or consists of anyone of the amino acid sequences identified by of SEQ ID NO.: 3-45, 47-55, 58-114.

In one embodiment the peptide analogue comprises or consists of anyone of the amino acid sequences identified by SEQ ID NO.: 3-4, 6-45, 47-53, 55, 58-114.

5 In one embodiment the peptide analogue comprises or consists of anyone of the amino acid sequences identified by SEQ ID NO.: 3-4, 6-19, 21-45, 47, 49-53, 55, 58-114.

In one embodiment the peptide analogue comprises or consists of anyone of the amino acid sequences identified by SEQ ID NO.: 3-4, 6-19, 21-45, 47, 49-53, 55, 58-114.

10 In one embodiment the peptide analogue comprises or consists of anyone of the amino acid sequences identified by SEQ ID NO.: 3-4, 6-19, 21-45, 47, 49-53, 55, 58-62, 64-114.

In one embodiment the peptide analogue comprises or consists of anyone of the amino acid sequences identified by SEQ ID NO.: 3, 6 and 81.

15 In one embodiment the peptide analogue comprises or consists of anyone of the amino acid sequences identified by SEQ ID NO.: 4, 8, 11, 15-19, 21, 22, 24, 31-42, 44, 51-53, 70-73, 77-78, 91, 94, 95, 97-102, 104-109, 112-114.

In one embodiment the peptide analogue comprises or consists of anyone of the amino acid sequences identified by SEQ ID NO.: 4, 6, 32, 72, 76, 78, 98, 104 and 105.

20 Intermediate compounds

The present invention also relates to peptide analogues which may be incorporated in the derivatives of the invention. Such peptide analogues may be referred to as "intermediate product" or "intermediate compound". They are in the form of novel LDL-R(293-332) analogues, which as described above can be incorporated in EGF(A) derivatives of the invention as further describe below. Such peptide analogues are as defined in the above section.

In particular, a peptide analogue, or intermediate peptide, according to the present invention may be referred to as a peptide analogue of sequence SEQ ID NO: 1.

30 In one aspect the invention relates to a EGF(A) peptide analogue as described herein for use in the manufacture of a EGF(A) compound, such as a EGF(A) derivative.

Other features, definitions, aspects and embodiments disclosed herein in connection with peptide analogues of the invention may also be applicable to the intermediate products of the invention.

35 EGF(A) derivatives

The peptides analogues of the invention may further comprise a substituent and thereby become derivative compounds.

The term "derivative" generally refers to a compound which may be prepared from a native peptide or an analogue thereof by chemical modification, in particular by covalent
5 attachment of one or two substituents.

The terms "derivative of the invention", "EGF(A) derivative", "EGF(A) derivative or "LDL-R(293-332) derivative" or "derivative of a LDL-R(293-332) analogue" as used herein refers to as a peptide to which one or two substituents are attached. Each of these may, also or alternatively, be referred to as a side chain. In other words, a "derivative of the invention"
10 comprises a peptide i.e. a peptide sequence, which herein is an EGF(A) peptide analogue, and at least one, including such as one or two, substituent(s).

The terms "substituent" is used to describe a moiety covalently bond to the EGF(A) peptide e.g. the substituent is a moiety not part of the EGF(A) peptide itself.

In one embodiment the one or more substituent(s) is/are attached to a nitrogen atom
15 of the EGF(A) peptide analogue. In one embodiment the one or more substituent(s) is/are attached to an amino group of the EGF(A) peptide analogue. In one embodiment the one or more substituent(s) is/are attached to the N-terminal amino acid of the EGF(A) peptide analogue or to a Lys residue of the EGF(A) peptide analogue. In one embodiment the one or more substituent(s) is/are attached to the N-terminal amino acid of the EGF(A) peptide
20 analogue. In one embodiment the one or more substituent(s) is/are attached to the alpha-nitrogen of the N-terminal amino acid residue of the EGF(A) peptide analogue. In one embodiment the one or more substituent(s) is/are attached to a Lys residue in the EGF(A) peptide analogue. In one embodiment the one or more substituent(s) is/are attached to the epsilon-nitrogen of a Lys residue in the EGF(A) peptide analogue.

25 Examples of substituents are various and further described below.

In one aspect, the invention relates to an EGF(A) derivative comprising an EGF(A) peptide analogue and at least one substituent. In one embodiment the substituent of the derivative comprises at least one fatty acid group. For all embodiments the term EGF(A) derivative also encompasses any pharmaceutically acceptable salt, amide, or ester thereof.

30 **Substituents**

A substituent is a moiety attached to an EGF(A) peptide analogue. According to the invention it is preferred that the moiety e.g. the substituent has no or minimal effect on the functionality of the EGF(A) peptide while adding other beneficial properties, such as longer half-life and/or improved exposure after oral dosing.

It follows that the derivatives, as well as the analogues of the invention described above, have the ability to bind to PCSK9. Such binding to PCSK9 inhibits PCSK9 binding to the LDL-R, thereby preventing LDL-R degradation hence increasing the clearance of LDL-C and atherogenic lipoproteins.

5 In a specific embodiment, the derivatives and analogues of the invention have an improved ability to bind to PCSK9, for example compared to native LDL-R(293-332) or to other PCSK9-binding compounds. The analogues and derivatives of the invention can for example be tested for their ability to inhibit PCSK9 binding to LDL-R using the assay described in Assay I herein.

10 In an embodiment the substituent is aimed at improving the functionality of the peptides.

In one embodiment the substituent increase half-life of the peptide analogue in a way that the plasma half-life of a derivative comprising a backbone peptide and a substituent have an increase half-life compared to the half-life of the backbone. Methods for determining half-life in different species are well known in the art and exemplified in WO2017/121850 for mice and dogs (Section D2 and D5).

In one embodiment the EGF(A) derivative according to the invention has a half-life above 4 hours.

20 In one embodiment the EGF(A) derivative according to the invention has a half-life above 6 hours, such as above 8 hours or such as above 10 hours in mice measured after either subcutaneously or intravenously dosing.

In one embodiment the EGF(A) derivative according to the invention has a half-life above 25 hours in dogs.

25 In one embodiment the EGF(A) derivative according to the invention has a half-life above 50 hours, such as above 100 hours or such as above 150 hours in dogs.

In one embodiment, a half-life extending substituent is a protein moiety. In a further such embodiment the protein moiety may include human albumin, an Fc-domain or an unstructured protein extension. In a further embodiment the protein moiety may be fused to the peptide analogue. In a further embodiment, the protein moiety is Fc domain and the Fc domain is fused to the peptide analogue. When an Fc fusion is prepared the resulting compound will usually be divalent as two Fc-polypeptides will form one Fc-domain.

In one embodiment the substituent is not a protein moiety. In one embodiment the substituent is not a protein moiety fused to the EGF(A) peptide analogue. In one embodiment the protein moiety is not an Fc domain.

35 In another embodiment the substituent is a non-protein moiety.

In a particular embodiment, the substituent is capable of forming non-covalent complexes with albumin, thereby promoting the circulation of the derivative within the blood stream, and also having the effect of protracting the time of action of the derivative. In a particular embodiment, the substituent is capable of protracting the time of action of the EGF(A) compound without substantially decreasing its binding capacity to PCSK9.

In one embodiment the EGF(A) derivative comprises a half-life extending substituent. Various half-life extending substituents are well-known in the art and include in particular albumin binders comprising a fatty acid group as described further below, and such albumin binders are non-protein substituents.

The substituent comprises at least one fatty acid group.

In a particular embodiment, the fatty acid group comprises a carbon chain which contains at least 8 consecutive $-\text{CH}_2-$ groups. In one embodiment the fatty acid group comprise at least 10 consecutive $-\text{CH}_2-$ groups, such as least 12 consecutive $-\text{CH}_2-$ groups, at least 14 consecutive $-\text{CH}_2-$ groups, at least 16 consecutive $-\text{CH}_2-$ groups, at least 18 consecutive $-\text{CH}_2-$ groups.

In one embodiment the fatty acid group comprises 8-20 consecutive $-\text{CH}_2-$ groups. In one embodiment the fatty acid group comprises 10-18 consecutive $-\text{CH}_2-$ groups. In one embodiment the fatty acid group comprises 12-18 consecutive $-\text{CH}_2-$ groups. In one embodiment the fatty acid group comprises 14-18 consecutive $-\text{CH}_2-$ groups.

In situations where the derivative comprise two substituents, an increased half-life may be obtained with shorter fatty acid groups, thus in an embodiment where the derivative comprise two substituents the fatty acid groups may comprise at least 8 consecutive $-\text{CH}_2-$ groups, such as least 10 consecutive $-\text{CH}_2-$ groups, such as least 12 consecutive $-\text{CH}_2-$ groups, at least 14 consecutive $-\text{CH}_2-$ groups, at least 16 consecutive $-\text{CH}_2-$ groups.

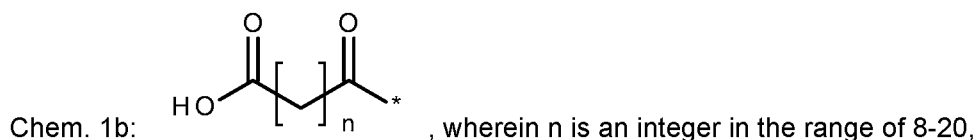
In a further embodiment where the derivative comprises two substituents, the substituents each comprise a fatty acid group comprising 8-18 consecutive $-\text{CH}_2-$ groups. In further such embodiments the fatty acid groups comprise 10-18 consecutive $-\text{CH}_2-$ groups, such as 12-18 consecutive $-\text{CH}_2-$ groups, such as 14-18 consecutive $-\text{CH}_2-$ groups.

The term "fatty acid group" as used herein may be referred to as chemical group comprising at least one functional group being a Brønsted-Lowry acid with a $\text{pK}_a < 7$. Non-limiting examples of such functional groups that are Brønsted-Lowry acids include a carboxylic acid (including also carboxyphenoxy), a sulphonic acid, a tetrazole moiety.

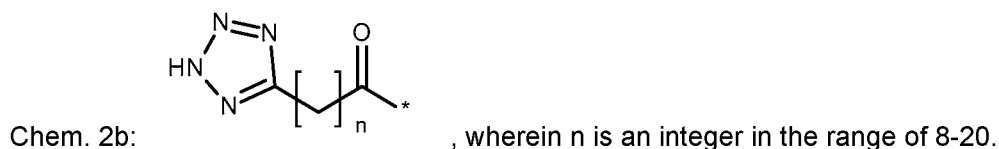
In one embodiment said fatty acid group comprises a functional group selected from a carboxylic acid, a sulphonic acid, a tetrazole moiety, a methylsulfonylcarbamoylamino (MSU) moiety and a 3-Hydroxy-isoxazole/soxazole moiety. Accordingly, the half-life

extending substituent of the invention in an embodiment comprises a carboxylic acid, a sulphonic acid, a tetrazole moiety, a methylsulfonylcarbamoylemino moiety or a hydroxy-isoxazoleisoxazole moiety further including 8-20 consecutive $-\text{CH}_2-$ groups as defined by:

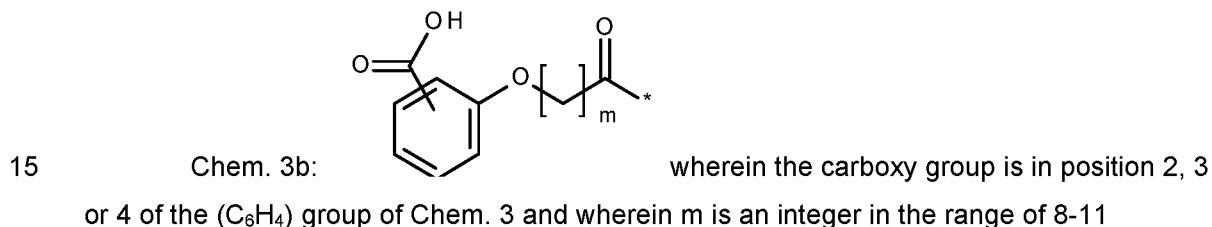
- 5 Chem. 1: $\text{HOOC}-(\text{CH}_2)_n-\text{CO}-^*$ wherein n is an integer in the range of 8-20, which may also be referred to as a $\text{C}(n+2)$ diacid or as



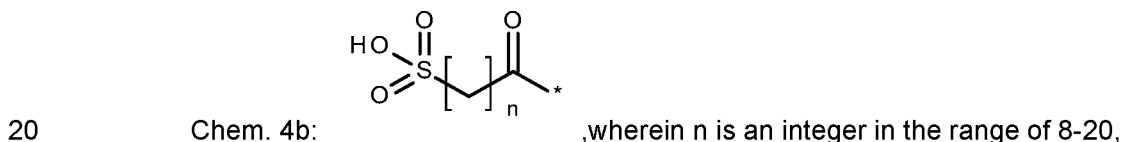
- Chem. 2: 5-tetrazolyl- $(\text{CH}_2)_n-\text{CO}-^*$ wherein n is an integer in the range of 8-20, which
10 may also be referred to as



Chem. 3: $\text{HOOC}-(\text{C}_6\text{H}_4)-\text{O}-(\text{CH}_2)_m-\text{CO}-^*$ wherein n is an integer in the range of 8-20, which may also be referred to as

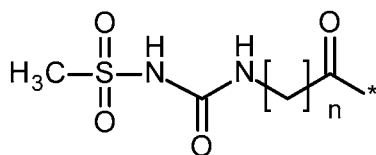


Chem. 4: $\text{HO-S(O)}_2-(\text{CH}_2)_n-\text{CO}-^*$ wherein n is an integer in the range of 8-20, which may also be referred to as



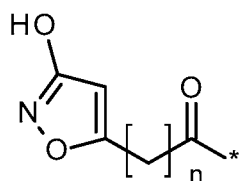
Chem. 5: $\text{MeS(O)}_2\text{NH(CO)NH}-(\text{CH}_2)_n-\text{CO}-^*$ wherein n is an integer in the range of 8-20, which may also be referred to as.

25



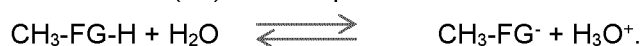
Chem. 5b: , wherein n is an integer in the range of 8-20,

Chem. 6: 3-HO-Isoxazole-(CH₂)_n-CO-* wherein n is an integer in the range of 8-20, which may also be referred to as



Chem. 6b: , wherein n is an integer in the range of 8-20.

The term functional group in its acidic form is referred to as FG-H and its form as conjugated base referred to as FG⁻. The term "functional group with a pKa < 7" as used herein may be referred to as a Brønsted-Lowry acid which in the form of its methyl derivative (CH₃-FG-H) in aqueous solution has a equilibrium pKa of below 7, wherein the pKa is the -log to the equilibrium constant (Ka) of the equilibrium shown below:



Methods for the determination of pKa are well known in the art. Such a method has for example been described by Reijenga et al. in Anal Chem Insights 2013 (2013; 8: 53–71).

Substituents according to the invention in an embodiment comprise one or more linker elements. The linker elements may be linked to the fatty acid group by amide bonds and referred to as Z₂-Z₁₀. As further defined herein below the number of linker elements may be at most 10.

20

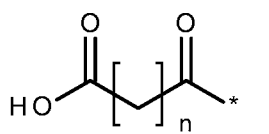
In a specific embodiment, the substituent is of Formula I:

Z₁-Z₂-Z₃-Z₄-Z₅-Z₆-Z₇-Z₈-Z₉-Z₁₀- [I] wherein

Z₁ is selected from:

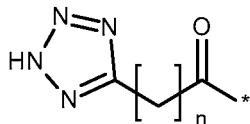
25

Chem. 1: HOOC-(CH₂)_n-CO-* or



Chem. 1b: ,

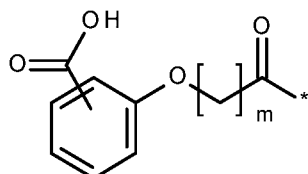
Chem. 2: 5-tetrazolyl-(CH₂)_n-CO-* or



Chem. 2b:

5

Chem. 3: HOOC-(C₆H₄)-O-(CH₂)_m-CO-* or

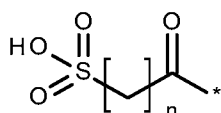


Chem. 3b:

, wherein the carboxy group is in position 2,

3 or 4 of -(C₆H₄)-,

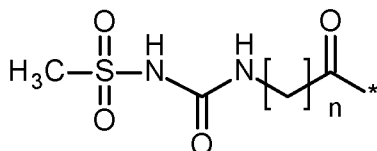
Chem. 4: HOS(O)₂-(CH₂)_n-CO-* or



Chem. 4b:

10

Chem. 5: MeS(O)₂NH₂N(CO)NHN-(CH₂)_n-CO-* or

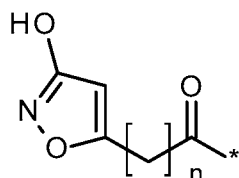


Chem.5b:

and

15

Chem. 6: 3-HO-Isoxazole-(CH₂)_n-CO-* or



Chem. 6b:

wherein n is an integer in the range of 8-20 and m is an integer in the range of 8-11.

In a particular embodiment, n is 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 in Chem. 1 or 1b. In a particular embodiment, n is 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 in Chem. 2 or 2b. In a particular embodiment, n is 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 in Chem. 4 or 4b. In a particular embodiment, m is 8, 9, 10 or 11 in Chem. 3 or 3b.

5 In a particular embodiment, n is 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 in Chem. 5 or 5b.

In a particular embodiment, n is 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 in Chem. 6 or 6b.

10 In a particular embodiment, the symbol * indicates the attachment point to the nitrogen in Z₂. In another embodiment, where Z₂ is a bond, the symbol * indicates the attachment point to the nitrogen of the neighbouring Z element.

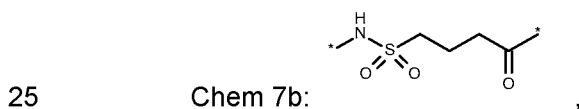
The term "bond" as used in the context of Formula I means a covalent bond. When a component of Formula I (Z₁-Z₁₀) is defined as a bond, it is equivalent to a formula I wherein said component is absent.

15 The indication herein below that any of Z₂-Z₁₀ is a bond may also be read as any of Z₂-Z₁₀ being absent. Logically "a bond" cannot follow "a bond". The indication "a bond" here thus means that the previous Z element is covalently linked to the next Z element that is not "a bond" (or absent).

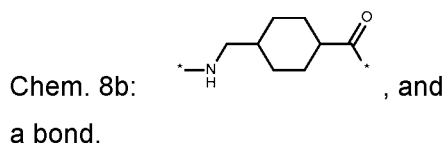
20 The linker elements Z₂-Z₁₀ are selected from chemical moieties that are capable of forming amide bounds, including amino acid like moieties, such as Glu, γGlu (also termed gammal Glu or gGlu and defined by *-NH-CH-(COOH)-CH₂-CH₂-CO-*), Gly, Ser, Ala, Thr, Ado, AEEP, AEEP and TtdSuc and further moieties defined below.

Z₂ is selected from

Chem. 7: *-NH-SO₂-(CH₂)₃-CO-* or



Chem. 8: *-NH-CH₂-(C₆H₁₀)-CO-* or



30

Z₃ is selected from γGlu, Glu, or a bond.

Z₃ is selected from γGlu, Glu, or a bond when Z₂ is Chem. 7 or Chem. 7b.

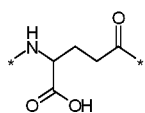
Z_3 is selected from γ Glu, Glu, or a bond, provided that Z_3 is selected from γ Glu, Glu when Z_2 is Chem. 8.

Z_3 is selected from γ Glu and Glu when Z_2 is Chem. 8.

- 5 $Z_4, Z_5, Z_6, Z_7, Z_8, Z_9$ are selected, independently of each other, from Glu, γ Glu, Gly, Ser, Ala, Thr, Ado, Aeep, Aeeep, TtdSuc and a bond.

Glu, Gly, Ser, Ala, Thr are amino acid residues as well known in the art.

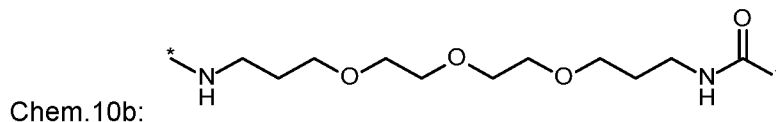
- 10 γ Glu is of formula Chem. 9: $^*\text{-NH-CH(COOH)-(CH}_2\text{)}_2\text{-CO-}^*$ which is the same as Chem. 9b:



and may also be referred to as γ Glu.

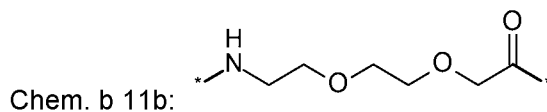
TtdSuc is of formula Chem. 10:

- 15 $^*\text{-NH-(CH}_2\text{)}_3\text{-O-(CH}_2\text{)}_2\text{-O-(CH}_2\text{)}_2\text{-O-(CH}_2\text{)}_3\text{-NHCO}^*$ or
 $^*\text{-NH-CH}_2\text{CH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{CH}_2\text{NHCO}^*$ which is the same as



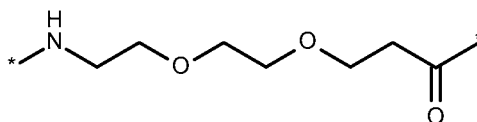
Chem.10b:

- Ado is of formula Chem. 11: $^*\text{-NH-(CH}_2\text{)}_2\text{-O-(CH}_2\text{)}_2\text{-O-CH}_2\text{-CO-}^*$ may also be
 20 referred to as 8-amino-3,6-dioxaoctanoic acid and which is the same as



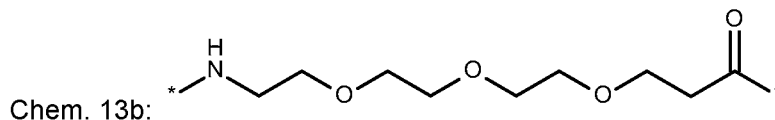
Chem. b 11b:

Aeep is of formula Chem. 12: $^*\text{NH-CH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{CO}^*$, which may also be referred to as

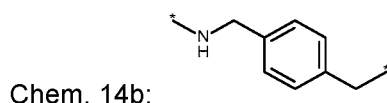


- 25 Chem. 12b:

Aeeep is of formula Chem. 13: $^*\text{NH-CH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{CO}^*$, which may also be referred to as



5 Z_{10} is selected from a bond, and Chem. 14: $^*\text{-NH-CH}_2\text{-(C}_6\text{H}_4\text{)-CH}_2\text{-}^*$, which may also be referred to as



In a particular embodiment, when Z_{10} is Chem. 14, the substituent is attached to the
10 N-terminal amino group of said peptide.

In another embodiment, when Z_{10} is a bond, said substituent is attached to the epsilon position of a Lys residue present in said peptide or to the N-terminal amino acid residue of said peptide.

In one embodiment the derivative comprises two substituents. In one such
15 embodiment the two substituents are identical. In one such embodiment the two substituents are different. In one embodiment the two substituents are attached to nitrogen atoms of the EGF(A) peptide analogue. In one embodiment the two substituents are attached to amino groups of the EGF(A) peptide analogue. In one embodiment the two substituents are attached to the N-terminal amino acid EGF(A) and to a Lys residue of the EGF(A) peptide
20 analogue. In one embodiment, one substituent is attached the alpha-nitrogen of the N-terminal amino acid residue of the EGF(A) peptide analogue and one substituent is attached to a Lys residue of the EGF(A) peptide analogue. In one embodiment two substituents are attached to the N-terminal amino acid of the EGF(A) peptide analogue. In one embodiment the two substituents are attached to different Lys residues of the EGF(A) peptide analogue.
25 In one embodiment the two substituents are attached to the epsilon-nitrogens of different Lys residues in the EGF(A) peptide analogue.

In one embodiment where two substituents are present, Z_{10} is Chem. 14 in one substituent which is attached to the N-terminal amino group of a peptide analogue and Z_{10} is a bond in the other substituent which is attached to the epsilon position of a Lys residue
30 present in said peptide analogue.

In another embodiment where two substituents are present, Z₁₀ is a bond in one substituent which is attached to the N-terminal amino group of a peptide analogue and Z₁₀ is a bond in the other substituent which is attached to the epsilon position of a Lys residue present in said peptide analogue.

5 In another embodiment where two substituents are present, Z₁₀ is a bond in both substituents and each of the two substituents is attached to the epsilon position of different Lys residues present in a peptide analogue.

In a particular embodiment, the derivatives of the invention may be prepared from an EGF(A) peptide analogue by covalent attachment of one or two substituent(s).

10 In a particular embodiment, the two substituents are of Formula I: Z₁-Z₂-Z₃-Z₄-Z₅-Z₆-Z₇-Z₈-Z₉-Z₁₀ [I]. Z₁ to Z₁₀ are as defined above. In a particular embodiment, the two substituents are of formula I and are identical, meaning that selected Z₁ to Z₁₀ are the same in both substituents. In another embodiment, the two substituents are of formula I and are different, meaning that one or more of selected Z₁ to Z₁₀ are different between one
15 substituent and the other.

Specific substituents

As seen above various substituents can be prepared by the persons skilled in the art. The substituents include in the present application are thus not to be considered limiting to the invention.

20 In one embodiment the one or two substituent(s) is/are selected from the group of substituents consisting of:

HOOC-(CH ₂) ₁₈ -CO-gGlu-2xADO
HOOC-(CH ₂) ₁₈ -CO-NH-CH ₂ -(C ₆ H ₁₀)-CO-gGlu-2xADO
HOOC-(CH ₂) ₁₆ -CO-gGlu-2xADO
HOOC-(CH ₂) ₁₆ -CO-gGlu-2xADO-NH-CH ₂ -(C ₆ H ₄)-CH ₂
HOOC-(CH ₂) ₁₆ -CO-gGlu
HOOC-(CH ₂) ₁₆ -CO-NH-CH ₂ -(C ₆ H ₁₀)-CO-gGlu-2xADO
HOOC-(CH ₂) ₁₄ -CO-gGlu-2xADO
HOOC-(CH ₂) ₁₄ -CO-gGlu-
HOOC-(CH ₂) ₁₄ -CO-gGlu-2xADO-
HOOC-(CH ₂) ₁₂ -CO-gGlu-2xADO
4-HOOC-(C ₆ H ₄)-O-(CH ₂) ₁₀ -CO-gGlu-2xADO
4-HOOC-(C ₆ H ₄)-O-(CH ₂) ₁₀ -CO-gGlu-3xADO

4-HOOC-(C ₆ H ₄)-O-(CH ₂) ₁₀ -CO-gGlu
4-HOOC-(C ₆ H ₄)-O-(CH ₂) ₁₀ -CO-2xgGlu
4-HOOC-(C ₆ H ₄)-O-(CH ₂) ₁₀ -CO-gGlu-3xGly
4-HOOC-(C ₆ H ₄)-O-(CH ₂) ₁₀ -CO-2xgGlu-2xADO
4-HOOC-(C ₆ H ₄)-O-(CH ₂) ₁₀ -CO-gGlu-TtdSuc
4-HOOC-(C ₆ H ₄)-O-(CH ₂) ₉ -CO
4-HOOC-(C ₆ H ₄)-O-(CH ₂) ₁₀ -CO-gGlu-4xADO
4-HOOC-(C ₆ H ₄)-O-(CH ₂) ₁₀ -CO-NH-CH ₂ -(C ₆ H ₁₀)-CO-gGlu-2xADO
4-HOOC-(C ₆ H ₄)-O-(CH ₂) ₉ -CO-gGlu-2xADO
3-HOOC-(C ₆ H ₄)-O-(CH ₂) ₉ -CO-gGlu-2xADO
3-HO-Isoxazole-(CH ₂) ₁₂ -CO-gGlu-2xADO
HOS(O) ₂ -(CH ₂) ₁₅ -CO-gGlu-2xADO-NH-CH ₂ -(C ₆ H ₄)-CH ₂
HOS(O) ₂ -(CH ₂) ₁₃ -CO-gGlu-2xADO
Tetrazolyl-(CH ₂) ₁₅ -CO-NH-SO ₂ -(CH ₂) ₃ -CO-ADO-ADO-NH-CH ₂ -(C ₆ H ₄)-CH ₂
Tetrazolyl-(CH ₂) ₁₂ -CO-gGlu-2xADO
Tetrazolyl-(CH ₂) ₁₅ -CO-gGlu-2xADO and
MeS(O) ₂ NH(CO)NH-(CH ₂) ₁₂ -CO-gGlu-2xADO.

In one embodiment, the substituent is of Formula I wherein Z₁ is Chem. 1: HOOC-(CH₂)_n-CO-*, wherein n is 16; Z₂ is a bond; Z₃ is γGlu; two of Z₄, Z₅, Z₆, Z₇, Z₈, Z₉ are Ado and the remaining four are bonds; Z₁₀ is Chem. 14: *-NH-CH₂-(C₆H₄)-CH₂-*.

5 In one embodiment, the substituent is of Formula I wherein Z₁ is Chem. 1: HOOC-(CH₂)_n-CO-*, wherein n is 16; Z₂ is a bond; Z₃ is γGlu; two of Z₄, Z₅, Z₆, Z₇, Z₈, and Z₉ are Ado and the remaining four are bonds; Z₁₀ is a bond.

In one embodiment, the substituent is of Formula I wherein Z₁ is Chem. 1: HOOC-(CH₂)_n-CO-*, wherein n is 14 or 16; Z₂ is a bond; Z₃ is γGlu; and all of Z₄, Z₅, Z₆, Z₇, Z₈ and Z₉ are bonds; Z₁₀ is a bond.

In one embodiment, the substituent is of Formula I wherein Z₁ is Chem. 1: HOOC-(CH₂)_n-CO-*, wherein n is 16 or 18; Z₂ is Chem 8 (Trx); Z₃ is γGlu; two of Z₄, Z₅, Z₆, Z₇, Z₈ and Z₉ are Ado and the remaining four are bonds; Z₁₀ is a bond.

15 In one embodiment, the substituent is of Formula I wherein Z₁ is Chem 2: Tetrazolyl-(CH₂)_n-CO-*, wherein n is 15; Z₂ is Chem 7 (sulfonimide); Z₃ is a bond; two of Z₄, Z₅, Z₆, Z₇, Z₈ and Z₉ are Ado and the remaining four are bonds; Z₁₀ is Chem. 14: *-NH-CH₂-(C₆H₄)-CH₂-*.

In one embodiment, the substituent is of Formula I wherein Z_1 is Chem 2: Tetrazolyl- $(CH_2)_n-CO-^*$, wherein n is 15; Z_2 is a bond; Z_3 is γ Glu; two of Z_4, Z_5, Z_6, Z_7, Z_8 and Z_9 are Ado and the remaining four are bonds; Z_{10} is a bond.

5 In one embodiment, the substituent is of Formula I wherein Z_1 is Chem 2: Tetrazolyl- $(CH_2)_n-CO-^*$, wherein n is 12; Z_2 is a bond; Z_3 is γ Glu; two of Z_4, Z_5, Z_6, Z_7, Z_8 and Z_9 are Ado and the remaining four are bonds; Z_{10} is a bond.

In one embodiment, the substituent is of Formula I wherein Z_1 is Chem. 3: $HOOC-(C_6H_4)-O-(CH_2)_m-CO-^*$, wherein m is 10; Z_2 is a bond; Z_3 is a bond; and all off Z_4, Z_5, Z_6, Z_7, Z_8 and Z_9 are bonds; Z_{10} is a bond.

10 In one embodiment, the substituent is of Formula I wherein Z_1 is Chem. 3: $HOOC-(C_6H_4)-O-(CH_2)_m-CO-^*$, wherein m is 10; Z_2 is a bond; Z_3 is a γ Glu; and all off Z_4, Z_5, Z_6, Z_7, Z_8 and Z_9 are bonds; Z_{10} is a bond.

In one embodiment, the substituent is of Formula I wherein Z_1 is Chem. 3: $HOOC-(C_6H_4)-O-(CH_2)_m-CO-^*$, wherein m is 10; Z_2 is a bond; Z_3 is a γ Glu; and one off Z_4, Z_5, Z_6, Z_7, Z_8 and Z_9 is a γ Glu and the remaining five are bonds; Z_{10} is a bond.

In one embodiment, the substituent is of Formula I wherein Z_1 is Chem. 3: $HOOC-(C_6H_4)-O-(CH_2)_m-CO-^*$, wherein m is 10; Z_2 is a bond; Z_3 is a γ Glu; and one off Z_4, Z_5, Z_6, Z_7, Z_8 and Z_9 is a γ Glu and two are Ado and the remaining three are bonds; Z_{10} is a bond.

20 In one embodiment, the substituent is of Formula I wherein Z_1 is Chem. 3: $HOOC-(C_6H_4)-O-(CH_2)_m-CO-^*$, wherein m is 10; Z_2 is a bond; Z_3 is a γ Glu; and three off Z_4, Z_5, Z_6, Z_7, Z_8 and Z_9 are Gly and the remaining three are bonds; Z_{10} is a bond.

In one embodiment, the substituent is of Formula I wherein Z_1 is Chem. 3: $HOOC-(C_6H_4)-O-(CH_2)_m-CO-^*$, wherein m is 10; Z_2 is a bond; Z_3 is a γ Glu; and two off Z_4, Z_5, Z_6, Z_7, Z_8 and Z_9 are Ado and the remaining four are bonds; Z_{10} is a bond.

25 In one embodiment, the substituent is of Formula I wherein Z_1 is Chem. 3: $HOOC-(C_6H_4)-O-(CH_2)_m-CO-^*$, wherein m is 10; Z_2 is a bond; Z_3 is a γ Glu; and three off Z_4, Z_5, Z_6, Z_7, Z_8 and Z_9 are Ado and the remaining three are bonds; Z_{10} is a bond.

In one embodiment, the substituent is of Formula I wherein Z_1 is Chem. 3: $HOOC-(C_6H_4)-O-(CH_2)_m-CO-^*$, wherein m is 10; Z_2 is a bond; Z_3 is a γ Glu; and four off Z_4, Z_5, Z_6, Z_7, Z_8 and Z_9 are Ado and the remaining two are bonds; Z_{10} is a bond.

30 In one embodiment, the substituent is of Formula I wherein Z_1 is Chem. 3: $HOOC-(C_6H_4)-O-(CH_2)_m-CO-^*$, wherein m is 10; Z_2 is a bond; Z_3 is a γ Glu; and one off Z_4, Z_5, Z_6, Z_7, Z_8 and Z_9 is a TtdSuc and the remaining five are bonds; Z_{10} is a bond.

In one embodiment, the substituent is of Formula I wherein Z_1 is Chem. 3: $\text{HOOC}-(\text{C}_6\text{H}_4)-\text{O}-(\text{CH}_2)_m-\text{CO}-^*$, wherein m is 10; Z_2 is Chem 8 (Trx); ; Z_3 is a γGlu ; and two off Z_4 , Z_5 , Z_6 , Z_7 , Z_8 and Z_9 are Ado and the remaining four are bonds; Z_{10} is a bond.

5 In one embodiment, the substituent is of Formula I wherein Z_1 is Chem. 3: $\text{HOOC}-(\text{C}_6\text{H}_4)-\text{O}-(\text{CH}_2)_m-\text{CO}-^*$, wherein m is 9; Z_2 is a bond; Z_3 is a γGlu ; and one off Z_4 , Z_5 , Z_6 , Z_7 , Z_8 and Z_9 is a TtdSuc and the remaining five are bonds; Z_{10} is a bond.

In one embodiment, the substituent is of Formula I wherein Z_1 is Chem. 3: $\text{HOOC}-(\text{C}_6\text{H}_4)-\text{O}-(\text{CH}_2)_m-\text{CO}-^*$, wherein m is 10; Z_2 is a bond; Z_3 is γGlu ; two of Z_4 , Z_5 , Z_6 , Z_7 , Z_8 and Z_9 are Ado, the remaining four are bonds; Z_{10} is a bond.

10 In one embodiment, the substituent is of Formula I wherein Z_1 is Chem. 3: $\text{HOOC}-(\text{C}_6\text{H}_4)-\text{O}-(\text{CH}_2)_m-\text{CO}-^*$, wherein m is 10; Z_2 is a bond; Z_3 is γGlu ; two of Z_4 , Z_5 , Z_6 , Z_7 , Z_8 and Z_9 are Ado, the remaining four are bonds; Z_{10} is a bond.

In one embodiment, the substituent is of Formula I wherein Z_1 is Chem. 4: $\text{HO-S(O)}_2-(\text{CH}_2)_n-\text{CO}-^*$, wherein n is 15; Z_2 is a bond; Z_3 is γGlu ; two of Z_4 , Z_5 , Z_6 , Z_7 , Z_8 and Z_9 are Ado, the remaining four are bonds; Z_{10} is a bond.

In one embodiment, the substituent is of Formula I wherein Z_1 is Chem. 4: $\text{HO-S(O)}_2-(\text{CH}_2)_n-\text{CO}-^*$, wherein n is 15; Z_2 is a bond; Z_3 is γGlu ; two of Z_4 , Z_5 , Z_6 , Z_7 , Z_8 and Z_9 are Ado, the remaining four are bonds; Z_{10} is Chem. 14: $^*-\text{NH}-\text{CH}_2-(\text{C}_6\text{H}_4)-\text{CH}_2-^*$.

In one embodiment, the substituent is of Formula I wherein Z_1 is Chem. 5:
20 $\text{MeS(O)}_2\text{NH(CO)NH}-(\text{CH}_2)_n-\text{CO}-^*$, wherein n is 12; Z_2 is a bond; Z_3 is γGlu ; two of Z_4 , Z_5 , Z_6 , Z_7 , Z_8 and Z_9 are Ado, the remaining four are bonds; Z_{10} is a bond.

In one embodiment, the substituent is of Formula I wherein Z_1 is Chem. 6: $3\text{-OH-Isoxazole}-(\text{CH}_2)_{12}-\text{CO}-^*$, wherein n is 12; Z_2 is a bond; Z_3 is γGlu ; two of Z_4 , Z_5 , Z_6 , Z_7 , Z_8 and Z_9 are Ado, the remaining four are bonds; Z_{10} is a bond.

25 **Specific substituent combinations:**

In one embodiment, the compound of the invention comprises or has two substituents of Formula I wherein Z_1 is Chem. 1: $\text{HOOC}-(\text{CH}_2)_n-\text{CO}-^*$, wherein n is 16; Z_2 is a bond; Z_3 is γGlu ; two of Z_4 , Z_5 , Z_6 , Z_7 , Z_8 , Z_9 are Ado and the remaining four are bonds; Z_{10} is a bond.

30 In one embodiment, the compound of the invention comprises or has two substituents of Formula I wherein Z_1 is Chem. 1: $\text{HOOC}-(\text{CH}_2)_n-\text{CO}-^*$, wherein n is 14; Z_2 is a bond; Z_3 is γGlu ; two of Z_4 , Z_5 , Z_6 , Z_7 , Z_8 , Z_9 are Ado and the remaining four are bonds; Z_{10} is a bond.

In one embodiment, the compound of the invention comprises or has two substituents of Formula I wherein Z_1 is Chem. 1: $\text{HOOC}-(\text{CH}_2)_n-\text{CO}-^*$, wherein n is 14; Z_2 is a bond; Z_3 is γGlu ; all four of Z_4 , Z_5 , Z_6 , Z_7 , Z_8 , Z_9 are bonds; Z_{10} is a bond.

In one embodiment, the compound of the invention comprises or has two substituents of Formula I wherein Z_1 is Chem. 3: $\text{HOOC}-(\text{C}_6\text{H}_4)-\text{O}-(\text{CH}_2)_m-\text{CO}-^*$, wherein m is 10; Z_2 is a bond; Z_3 is γGlu ; two of Z_4 , Z_5 , Z_6 , Z_7 , Z_8 and Z_9 are Ado, the remaining four are bonds; Z_{10} is a bond.

In one embodiment, the compound of the invention comprises or has two substituents, one being of Formula I wherein Z_1 is Chem. 1: $\text{HOOC}-(\text{CH}_2)_n-\text{CO}-^*$, wherein n is 16; Z_2 is a bond; Z_3 is γGlu ; two of Z_4 , Z_5 , Z_6 , Z_7 , Z_8 , Z_9 are Ado and the remaining four are bonds; Z_{10} is Chem. 14: $^*-\text{NH}-\text{CH}_2-(\text{C}_6\text{H}_4)-\text{CH}_2-^*$; the other substituent being of Formula I wherein Z_1 is Chem. 1: $\text{HOOC}-(\text{CH}_2)_n-\text{CO}-^*$, wherein n is 16; Z_2 is a bond; Z_3 is γGlu ; two of Z_4 , Z_5 , Z_6 , Z_7 , Z_8 , Z_9 are Ado and the remaining four are bonds; Z_{10} is a bond.

In one embodiment, the compound of the invention comprises or has two substituents, one being of Formula I wherein Z_1 is Chem. 1: $\text{HOOC}-(\text{CH}_2)_n-\text{CO}-^*$, wherein n is 16; Z_2 is a bond; Z_3 is γGlu ; two of Z_4 , Z_5 , Z_6 , Z_7 , Z_8 , Z_9 are Ado and the remaining four are bonds; Z_{10} is Chem. 14: $^*-\text{NH}-\text{CH}_2-(\text{C}_6\text{H}_4)-\text{CH}_2-^*$; the other substituent being of Formula I wherein Z_1 is Chem. 3: $\text{HOOC}-(\text{C}_6\text{H}_4)-\text{O}-(\text{CH}_2)_m-\text{CO}-^*$, wherein m is 10; Z_2 is a bond; Z_3 is γGlu ; two of Z_4 , Z_5 , Z_6 , Z_7 , Z_8 and Z_9 are Ado, the remaining four are bonds; Z_{10} is a bond.

In one embodiment, the compound of the invention comprises or has two substituents, one being of Formula I wherein Z_1 is Chem. 1: $\text{HOOC}-(\text{CH}_2)_n-\text{CO}-^*$, wherein n is 16; Z_2 is a bond; Z_3 is γGlu ; two of Z_4 , Z_5 , Z_6 , Z_7 , Z_8 , Z_9 are Ado and the remaining four are bonds; Z_{10} is a bond; the other substituent being of Formula I wherein Z_1 is Chem. 3: $\text{HOOC}-(\text{C}_6\text{H}_4)-\text{O}-(\text{CH}_2)_m-\text{CO}-^*$, wherein m is 10; Z_2 is a bond; Z_3 is γGlu ; two of Z_4 , Z_5 , Z_6 , Z_7 , Z_8 and Z_9 are Ado, the remaining four are bonds; Z_{10} is a bond.

In one embodiment, the compound of the invention comprises or has two substituents, one being of Formula I wherein Z_1 is Chem. 1: $\text{HOOC}-(\text{CH}_2)_n-\text{CO}-^*$, wherein n is 16; Z_2 is a bond; Z_3 is γGlu ; two of Z_4 , Z_5 , Z_6 , Z_7 , Z_8 , Z_9 are Ado and the remaining four are bonds; Z_{10} is a bond; and the other substituent is of formula I wherein Z_1 is Chem. 4: $\text{HOS}(\text{O})_2-(\text{CH}_2)_m-\text{CO}-^*$, wherein m is 15; Z_2 is a bond; Z_3 is γGlu ; two of Z_4 , Z_5 , Z_6 , Z_7 , Z_8 and Z_9 are Ado, the remaining four are bonds; Z_{10} is Chem. 14: $^*-\text{NH}-\text{CH}_2-(\text{C}_6\text{H}_4)-\text{CH}_2-^*$.

In one embodiment, the compound of the invention comprises or has two substituents, one being of Formula I wherein Z_1 is Chem. 3: $\text{HOOC}-(\text{C}_6\text{H}_4)-\text{O}-(\text{CH}_2)_m-\text{CO}-^*$, wherein m is 10; Z_2 is a bond; Z_3 is γGlu ; two of Z_4 , Z_5 , Z_6 , Z_7 , Z_8 and Z_9 are Ado, the remaining four are bonds; Z_{10} is a bond; the other substituent being of Formula I wherein Z_1

is Chem. 4: $\text{HOS(O)}_2\text{-(CH}_2\text{)}_n\text{-CO-}^*$, wherein m is 15; Z_2 is a bond; Z_3 is γGlu ; two of Z_4 , Z_5 , Z_6 , Z_7 , Z_8 and Z_9 are Ado, the remaining four are bonds; Z_{10} is Chem. 14: $^*\text{-NH-CH}_2\text{-(C}_6\text{H}_4\text{)-CH}_2\text{-}^*$.

Peptide and attachment site

5 An EGF(A) derivative or compound according to the invention comprises an EGF(A) peptide analogue of the EGF(A) domain of LDL-R as defined by SEQ ID NO.: 1. Such peptide sequence have been described in details herein above and the peptide of the derivative or compound of the invention may be described and defined by identical terms. The EGF(A) derivative or compound further has at least one substituent as described herein
10 above which is linked to the peptide sequence.

In the compounds of the invention, the substituent is covalently attached to the peptide, meaning to one amino acid residue of the peptide sequence.

In one embodiment the EGF(A) derivative of the invention, comprise a substituent which is not attached to any one of the following positions: 295, 296, 298, 301, 302 and 307.

15 In a further embodiment the substituent is not attached to any one of the following positions: 295, 296, 298, 301, 302, 307 and 310. In further such embodiments, it is also not attached to any one of the following positions: 299 and 320.

In a particular embodiment a substituent is attached via any position from 292 to 333 except in any or the positions 297, 304, 308, 317, 319 and 331.

20 In a particular embodiment a substituent attached via any position from 292 to 333 except in any of the positions 297, 298, 301, 302, 304, 307, 308, 317, 319 and 331.

In a particular embodiment a substituent attached via any position from 292 to 333 except in any of the positions 295, 296, 297, 298, 301, 302, 304, 307, 308, 317, 319 and 331. In a particular embodiment a substituent attached via in any position from 292 to 333
25 except in any of the positions 295, 296, 297, 298, 301, 302, 304, 307, 308, 310, 317, 319, 320 and 331. In a particular embodiment a substituent attached via any position from 292 to 333 except in any of the positions 295, 296, 297, 298, 301, 302, 304, 307, 308, 309, 310, 317, 319, 320 and 331.

In one embodiment, the substituent(s) is/are attached to any one or two of the
30 positions 292, 293, 294, 299, 300, 303, 305, 306, 309, 311, 312, 313, 314, 315, 316, 318, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 332 and 333 of the EGF(A) peptide analogue.

In one embodiment, the substitution(s) is/are attached to any one or two of the positions 292, 293, 294, 300, 303, 305, 306, 309, 311, 312, 313, 314, 315, 316, 318, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 332 and 333 of the EGF(A) peptide analogue.

5 In one embodiment, the substitution(s) is/are attached to any one or two of the positions 292, 293, 294, 300, 303, 305, 306, 311, 312, 313, 314, 315, 316, 318, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 332 and 333 of the EGF(A) peptide analogue.

In one embodiment, the substituent is attached to the N-terminal amino acid of the peptide sequence. In a particular embodiment, the N-terminal amino acid is Gly. In a particular embodiment, the N-terminal amino acid is 293Gly. In a particular embodiment, the N-terminal amino acid is 293Lys. In a particular embodiment, the N-terminal amino acid is 292Lys. It may also be a Lys or a Gly or another amino acid residue in the N-terminal position which may be 293 or any position further down from the N-terminus, such as 294Thr, 294Gly or 294Lys or 295Asn. In a particular embodiment, the substituent is attached to the alpha-nitrogen of the N-terminal amino acid residue of the peptide analogue. In another embodiment, if the N-terminal amino acid residue is Lys, the substituent may be covalently linked to the alpha-nitrogen or to the epsilon amino group of the lysine residue.

15 In a particular embodiment, a substituent is attached to the ϵ -amino group of a Lys residue present in the peptide.

In another embodiment, a substituent is attached to a Lys in C-terminal position which may be position 332, 333 or any position further towards the C-terminus.

20 In embodiments wherein the peptides of the invention comprise an elongation, either in N-terminal or C-terminal, the substituent(s) may be attached to an amino acid residue of said elongation(s). In the presence of a N-terminal elongation, a substituent may be attached to the N-terminal amino acid of said elongation or to a Lys present within the elongation sequence. In the presence of a C-terminal elongation, a substituent may be attached to a Lys residue in C-terminal position or to a Lys present within the elongation sequence.

25 In yet another embodiment, the substituent is attached to an amino acid present in the peptide sequence. In a particular embodiment, the substituent is linked to a lysine residue present in the peptide. In a particular embodiment, the substituent is linked to the epsilon amino group of a lysine residue present in the peptide. The lysine residue to which the substituent is linked may be located in any position of the LDL-R(293-332) EGF(A) analogue including the N-terminal position or C-terminal position of the peptide, any position within or at the N-terminal end residue of a N-terminal elongation if present, any position within or at the C-terminal end residue of a C-terminal elongation if present.

As described herein above the EGF(A) peptide analogue may have one or more Lys residues; and those residues are useful for attachment of substituents.

In a particular embodiment, the lysine(s) to which the substituent(s) is/are linked is selected from the group of: 292Lys, 293Lys, 294Lys, 299Lys, 300Lys, 303Lys, 305Lys,
5 306Lys, 309Lys, 311Lys, 312Lys, 313Lys, 314Lys, 315Lys, 316Lys, 318Lys, 320Lys, 321Lys, 322Lys, 323Lys, 324Lys, 325Lys, 326Lys, 327Lys, 328Lys, 329Lys, 330Lys, 332Lys and 333Lys.

In a particular embodiment, the lysine(s) to which the substituent(s) is/are linked is selected from 293Lys, 294Lys, 295Lys, 296Lys, 298Lys, 299Lys, 301Lys, 302Lys, 303Lys,
10 305Lys, 306Lys, 307Lys, 309Lys, 310Lys, 311Lys, 312Lys, 313Lys, 314Lys, 315Lys, 316Lys, 318Lys, 320Lys, 321Lys, 322Lys, 323Lys, 324Lys, 325Lys, 326Lys, 327Lys, 328Lys, 329Lys, 330Lys, 332Lys and 333Lys.

In a particular embodiment, the lysine(s) to which the substituent(s) is/are linked is/are selected from 293Lys, 294Lys, 300Lys, 303Lys, 306Lys, 309Lys, 311Lys, 312Lys,
15 313Lys, 314Lys, 315Lys, 316Lys, 318Lys, 321Lys, 322Lys, 323Lys, 324Lys, 325Lys, 326Lys, 328Lys, 329Lys, 330Lys, 332Lys and 333Lys.

In another embodiment, the lysine(s) to which the substituent(s) is/are linked is/are selected from 293Lys, 294Lys, 298Lys, 299Lys, 303Lys, 305Lys, 306Lys, 309Lys, 311Lys, 312Lys, 313Lys, 314Lys, 315Lys, 316Lys, 318Lys, 320Lys, 321Lys, 322Lys, 323Lys,
20 324Lys, 325Lys, 326Lys, 327Lys, 328Lys, 329Lys, 330Lys, 332Lys and 333Lys.

In another embodiment, the lysine(s) to which the substituent(s) is/are linked is/are selected from: 292Lys, 293Lys, 294Lys, 299Lys, 300Lys, 303Lys, 305Lys, 306Lys, 309Lys, 311Lys, 313Lys, 314Lys, 315Lys, 316Lys, 318Lys, 320Lys, 321Lys, 322Lys, 323Lys, 324Lys, 325Lys, 326Lys, 327Lys, 328Lys, 329Lys, 330Lys, 332Lys and 333Lys.

25 In another embodiment, the lysine(s) to which the substituent(s) is/are linked is/are selected from: 292Lys, 293Lys, 294Lys, 300Lys, 303Lys, 305Lys, 306Lys, 309Lys, 311Lys, 313Lys, 314Lys, 316Lys, 318Lys, 321Lys, 322Lys, 323Lys, 324Lys, 325Lys, 326Lys, 327Lys, 328Lys, 329Lys, 330Lys, 332Lys and 333Lys.

In another embodiment, the lysine(s) to which the substituent(s) is/are linked is/are
30 selected from: 293Lys, 294Lys, 300Lys, 303Lys, 305Lys, 306Lys, 309Lys, 311Lys, 313Lys, 314Lys, 316Lys, 318Lys, 321Lys, 322Lys, 323Lys, 324Lys, 325Lys, 326Lys, 327Lys, 328Lys, 329Lys, 330Lys, 332Lys and 333Lys.

In another embodiment, the lysine(s) to which the substituent(s) is/are linked is/are selected from: 293Lys, 294Lys, 300Lys, 303Lys, 305Lys, 306Lys, 311Lys, 313Lys, 314Lys,

316Lys, 318Lys, 321Lys, 322Lys, 323Lys, 324Lys, 325Lys, 326Lys, 327Lys, 328Lys, 329Lys, 330Lys, 332Lys and 333Lys.

In embodiments where the substituent is attached to a C-terminal elongation, the lysine to which the substituent is linked may be selected from anyone of 333Lys to 242Lys position and/or to anyone of 333Lys to 383Lys position.

In embodiments where compounds of the invention have two substituents, the substituents may be linked independently of each other as defined above, meaning that either one may be attached to the N-terminal amino acid of the peptide, to the C-terminal amino acid of the peptide, or to an amino acid within the amino acid sequence of the peptide.

In embodiments where a Lys is present in N-terminal position, two substituents may be both linked to the N-terminal Lys of the peptide. One may be linked to the N-terminal alpha-amine of said Lys while the other may be linked to the epsilon nitrogen of said Lys. When two substituents are present, one may be linked to the N-terminal amino acid of the peptide while the other substituent is linked to an amino acid, such as a Lys, within the peptide. Alternatively, one substituent may be linked to a Lys in position C-terminal of the peptide while the other substituent is linked to an amino acid, such as a Lys, in the peptide. Alternatively, one substituent may be linked to an amino acid residue, such as a Lys, within the peptide, including elongations, the other substituent being linked to another amino acid residue, such as a Lys, within the peptide, including elongations.

20

In an embodiment, the compounds of the invention have one substituent, said substituent is linked to the peptide at the N-terminal; or said substituent is linked to the peptide in position 292Lys; or said substituent is linked to the peptide in position 293Lys, or said substituent is linked to the peptide in position 299Lys; or said substituent is linked to the peptide in position 300Lys; or said substituent is linked to the peptide in position 309Lys; or said substituent is linked to the peptide in position 311Lys; or said substituent is linked to the peptide in position 312Lys; or said substituent is linked to the peptide in position 313Lys; or said substituent is linked to the peptide in position 314Lys; or said substituent is linked to the peptide in position 315Lys; or said substituent is linked to the peptide in position 316Lys; or said substituent is linked to the peptide in position 318Lys; or said substituent is linked to the peptide in position 320Lys; or said substituent is linked to the peptide in position 321Lys; or said substituent is linked to the peptide in position 322Lys; or said substituent is linked to the peptide in position 323Lys; or said substituent is linked to the peptide in position 324Lys; or said substituent is linked to the peptide in position 325Lys; or said substituent is linked to the peptide in position 326Lys; or said substituent is linked to the peptide in position 328Lys; or

said substituent is linked to the peptide in position 329Lys; or said substituent is linked to the peptide in position 330Lys; or said substituent is linked to the peptide in position 332Lys; or said substituent is linked to the peptide in position 333Lys.

In an embodiment where the derivative of the invention has two substituents, said substituents may be linked to the peptide via the N-terminal and any of the above mention
 5 Lys positions, such as 293Lys, 309Lys, 313Lys, 324Lys, 328Lys, 330Lys, 332Lys and 333Lys.

In further embodiments where the derivative comprises two substituents, they may be linked to two different Lys residues, such as any of the following pairs of Lys residues

- | | |
|---------------------|------------------------|
| i. 293K and 294K | xiv. 313K and 321K |
| ii. 293K and 312K | xv. 313K and 324K |
| iii. 293K and 333K | xvi. 313K and 328K |
| iv. 309K and 313K | xvii. 313K and 332K |
| v. 309K and 324K | xviii. 313K and 333K |
| vi. 309K and 328K | xix. 314K and 333K |
| vii. 309K and 332K | xx. 321K and 332K |
| viii. 309K and 333K | xxi. 321K and 333K |
| ix. 311K and 313K | xxii. 324K and 333K |
| x. 312K and 333K | xxiii. 324K and 328K |
| xi. 312K and 313K | xxiv. 328K and 333K |
| xii. 312K and 314K | xxv. 330K and 333K and |
| xiii. 313K and 314K | xxvi. 332K and 333K. |

10

In one embodiment the two substituents are attached via 333Lys and a Lys selected from 293Lys, 309Lys, 312Lys, 313Lys, 314Lys, 321Lys, 324Lys, 328Lys, 330Lys and 332Lys.

In one embodiment the two substituents are attached via 333Lys and a Lys selected
 15 from 312Lys, 313Lys, 314Lys, 321Lys, 324Lys, 328Lys and 330Lys.

In one embodiment the two substituents are attached via 333Lys and a Lys selected from 313Lys, 324Lys and 328Lys.

As described above the peptide may have one or more amino acid substitutions
 20 which may be combined with specific amino acid residues in specific positions as described herein. Such specific amino acid residues may be wild type amino acid residues that should be maintained, such as the cysteines which may in a series of preferred embodiments e.g. in

combination with other features described herein, be present in the peptide analogue. In such embodiments the peptide analogue comprises three disulphide bridges in positions 297Cys-308Cys, 304Cys-317Cys and 319Cys-331Cys. In a further example of such embodiments the peptide analogue of a peptide derivative comprises three disulphide
5 bridges in positions 297Cys-308Cys, 304Cys-317Cys and 319Cys-331Cys and at least one substituent, wherein the substituent(s) is not attached to a positions selected from 295, 296, 298, 301, 302 and 307 of said peptide analogue, The skilled person will understand that combinations of peptide sequence information may be combined with information on position and identity of the substituent to define various specific embodiments of the present
10 invention.

In an embodiment, the peptide analogue comprises no Lys in other positions than the positions to which a substituent is linked.

In an embodiment, the compounds of the invention have one substituent, said substituent is linked either in position N-terminal or to a Lys in any position, and the peptide
15 analogue comprises no Lys in all other positions. In an embodiment, the compounds of the invention have one substituent, said substituent is linked to a Lys in any position other than position 312, and the peptide analogue comprises an Arg in position 312Arg.

In an embodiment, the compounds of the invention have two substituents, and the peptide analogue comprises no Lys in positions other than positions to which the
20 substituents are linked.

In one embodiment the EGF(A) derivative according to the invention is selected from the group of EGF(A) derivative consisting of: Examples 1-47, 51-102 and 106-159 disclosed in WO2017/121850.

In further embodiments the EGF(A) derivative according to the invention is
25 individually selected from the group of EGF(A) derivative consisting of: Examples 1-47, 51-102 and 106-159 disclosed in WO2017/121850. .

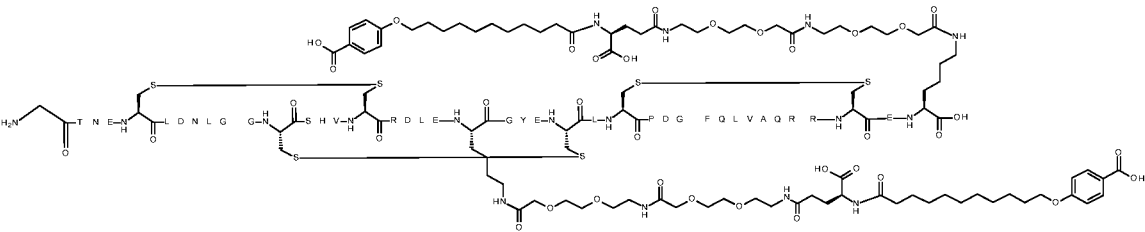
In one embodiment the EGF(A) derivative according to the invention is selected from the group of EGF(A) derivative consisting of: Examples 1-44, 46-47, 51-55, 57, 60-64, 66-69, 71-102 and 106-159 disclosed in WO2017/121850. .

30 In one embodiment the EGF(A) derivative according to the invention is selected from the group of EGF(A) derivative consisting of: Examples 31, 95, 128, 133, 143, 144, 150, 151, 152 and 153 disclosed in WO2017/121850 with the structure shown below.

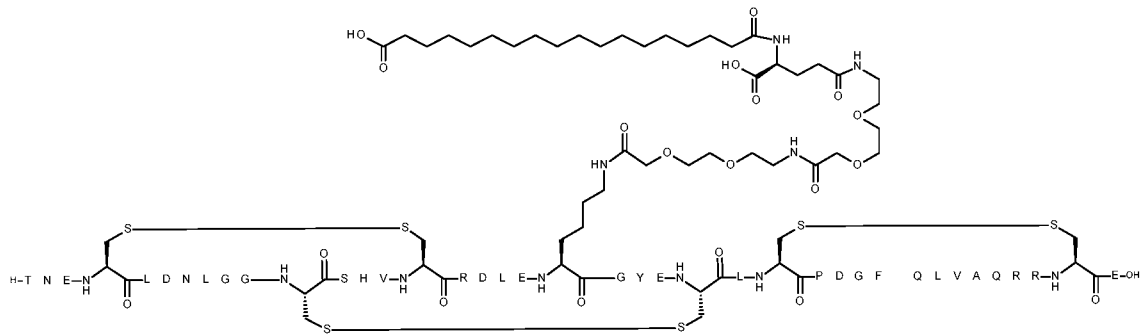
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Structure

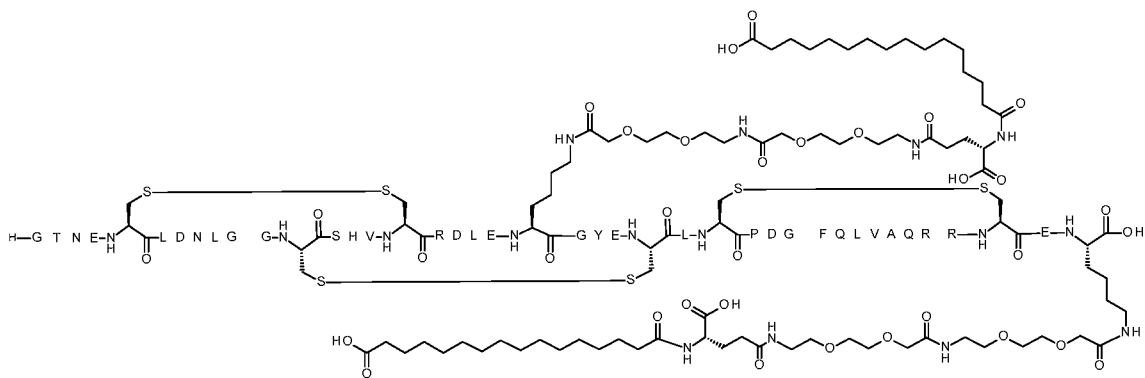
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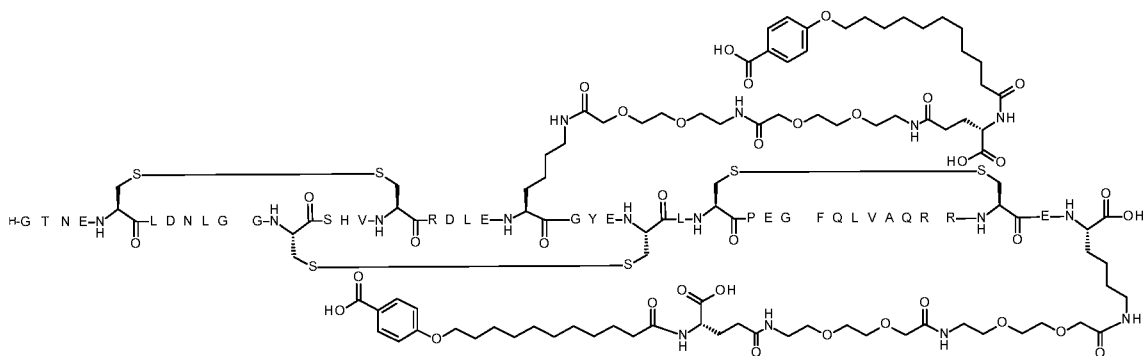
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128



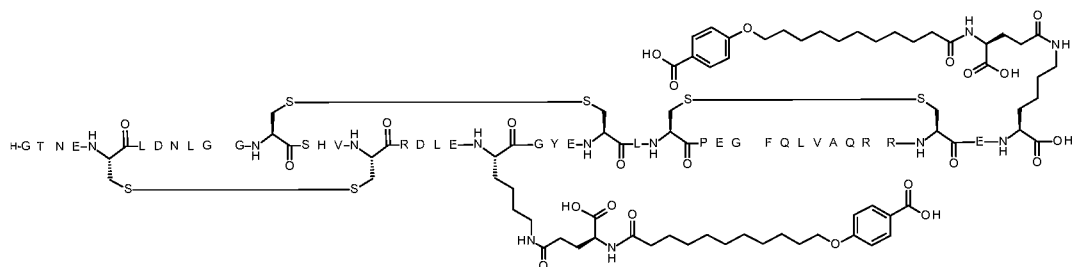
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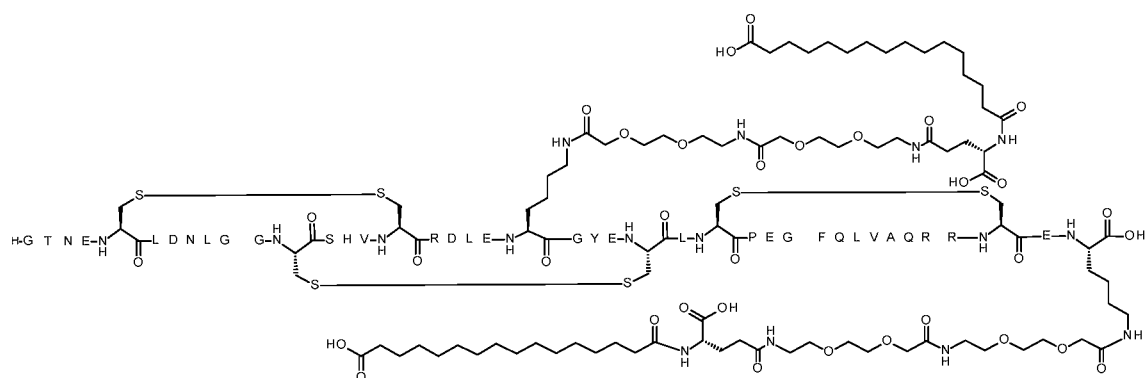
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Structure

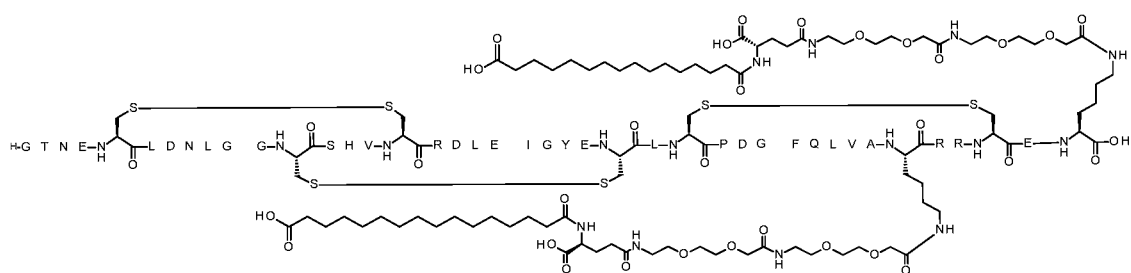
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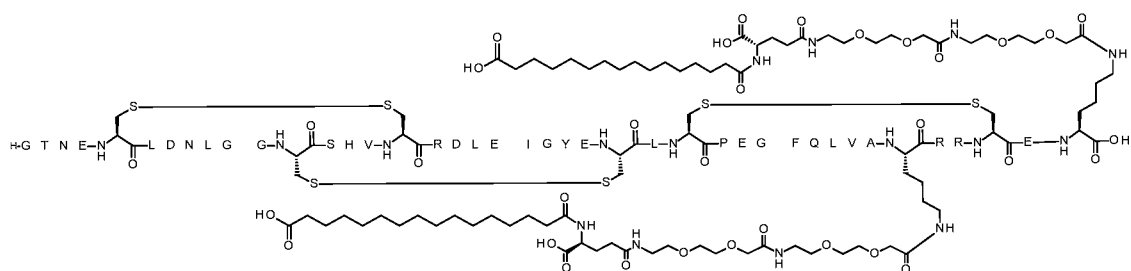
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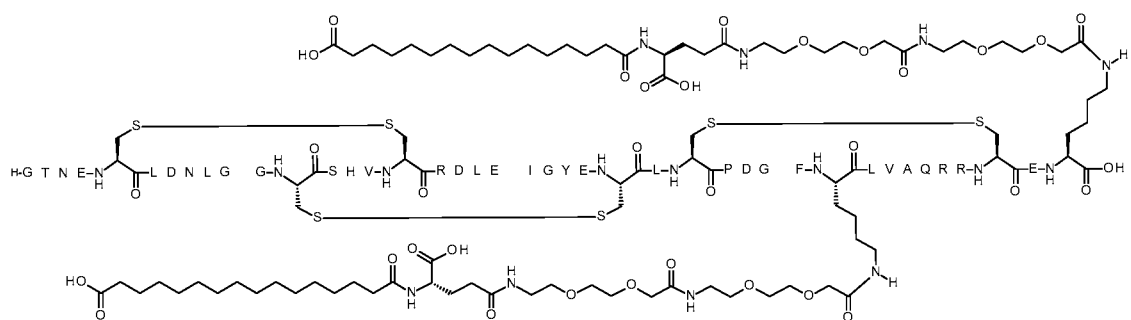
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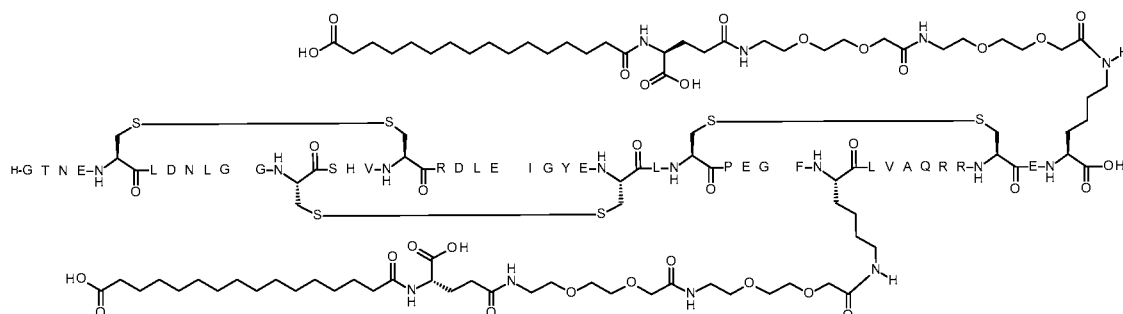


152



Structure

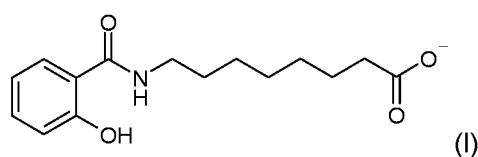
153



Delivery agent

Salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid

The delivery agent used in the present invention is a salt of N-(8-(2-
5 hydroxybenzoyl)amino)caprylic acid (NAC). The structural formula of N-(8-(2-
hydroxybenzoyl)amino)caprylate is shown in formula (I).



In some embodiments the salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid comprises one monovalent cation, two monovalent cations or one divalent cation. In some
10 embodiments the salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid is selected from the group consisting of the sodium salt, potassium salt and/or calcium salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid. In one embodiment the salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid is selected from the group consisting of the sodium salt, potassium salt and/or the ammonium salt. In one embodiment the salt of N-(8-(2-
15 hydroxybenzoyl)amino)caprylic acid is the sodium salt or the potassium salt. In one embodiment the salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid is selected from the group consisting of the sodium salt and the ammonium salt. Salts of N-(8-(2-hydroxybenzoyl)amino)caprylate may be prepared using the method described in e.g. WO96/030036, WO00/046182, WO01/092206 or WO2008/028859.

20 The salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid may be crystalline and/or amorphous. In some embodiments the delivery agent comprises the anhydrate, monohydrate, dihydrate, trihydrate, a solvate or one third of a hydrate of the salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid as well as combinations thereof. In some embodiments

the delivery agent is a salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid as described in WO2007/121318.

In some embodiments the delivery agent is sodium N-(8-(2-hydroxybenzoyl)amino)caprylate (referred to as "SNAC" herein), also known as sodium 8-(salicyloylamino)octanoate.

Composition

The composition or pharmaceutical composition of the present invention is a solid or dry composition suited for administration by the oral route as described further herein below.

In some embodiments the composition comprises at least one pharmaceutically acceptable excipient. The term "**excipient**" as used herein broadly refers to any component other than the active therapeutic ingredient(s) or active pharmaceutical ingredient(s) (API(s)). An excipient may be a pharmaceutically inert substance, an inactive substance, and/or a therapeutically or medicinally non active substance.

The excipients may serve various purposes, e.g. as a carrier, vehicle, filler, binder, lubricant, glidant, disintegrant, flow control agent, crystallization inhibitors, solubilizer, stabilizer, colouring agent, flavouring agent, surfactant, emulsifier, delivery agent, hydrotrope or combinations of thereof and/or to improve administration, and/or absorption of the therapeutically active substance(s) or active pharmaceutical ingredient(s). As described herein the salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid is an excipient acting as a delivery agent. The amount of each excipient used may vary within ranges conventional in the art. Techniques and excipients which may be used to formulate oral dosage forms are described in Handbook of Pharmaceutical Excipients, 8th edition, Sheskey et al., Eds., American Pharmaceuticals Association and the Pharmaceutical Press, publications department of the Royal Pharmaceutical Society of Great Britain (2017); and Remington: the Science and Practice of Pharmacy, 22nd edition, Remington and Allen, Eds., Pharmaceutical Press (2013).

In some embodiments the excipients may be selected from **binders**, such as polyvinyl pyrrolidone (povidone), etc.; **fillers** such as cellulose powder, microcrystalline cellulose, cellulose derivatives like hydroxymethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose and hydroxy-propylmethylcellulose, dibasic calcium phosphate, corn starch, pregelatinized starch, etc.; **lubricants** and/or **glidants** such as stearic acid, magnesium stearate, sodium stearyl fumarate, glycerol tribehenate, etc.; **flow control agents** such as colloidal silica, talc, etc.; **crystallization inhibitors** such as povidone, etc.; **solubilizers** such as pluronic, povidone, etc.; **colouring agents**, including dyes and

pigments such as iron oxide red or yellow, titanium dioxide, talc, etc.; **pH control agents** such as citric acid, tartaric acid, fumaric acid, sodium citrate, dibasic calcium phosphate, dibasic sodium phosphate, etc.; **surfactants** and **emulsifiers** such as pluronic, polyethylene glycols, sodium carboxymethyl cellulose, polyethoxylated and hydrogenated castor oil, etc.;
5 and mixtures of two or more of these excipients and/or adjuvants.

The composition may comprise a **binder**, such as povidone; starches; celluloses and derivatives thereof, such as microcrystalline cellulose, e.g., Avicel PH from FMC (Philadelphia, PA), hydroxypropyl cellulose hydroxyethyl cellulose and hydroxypropylmethyl cellulose METHOCEL from Dow Chemical Corp. (Midland, MI); sucrose; dextrose; corn
10 syrup; polysaccharides; and gelatine. The **binder** may be selected from the group consisting of dry binders and/or wet granulation binders. Suitable dry binders are, e.g., cellulose powder and microcrystalline cellulose, such as Avicel PH 102 and Avicel PH 200. In some embodiments the composition comprises Avicel, such as Avicel PH 102. Suitable binders for wet granulation or dry granulation are corn starch, polyvinyl pyrrolidone (povidone),
15 vinylpyrrolidone-vinylacetate copolymer (copovidone) and cellulose derivatives like hydroxymethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose and hydroxypropylmethylcellulose. In some embodiments the composition comprises povidone.

In some embodiments the composition comprises a **filler**, which may be selected from lactose, mannitol, erythritol, sucrose, sorbitol, calcium phosphate, such as
20 calciumhydrogen phosphate, microcrystalline cellulose, powdered cellulose, confectioner's sugar, compressible sugar, dextrates, dextrin and dextrose. In some embodiments the composition comprises microcrystalline cellulose, such as Avicel PH 102 or Avicel PH 200.

In some embodiments the composition comprises a lubricant and/or a glidant. In some embodiments the composition comprises a **lubricant** and/or a **glidant**, such as talc,
25 magnesium stearate, calcium stearate, zinc stearate, glyceryl behenate, glyceryl dibehenate, behenoyl polyoxyl-8 glycerides, polyethylene oxide polymers, sodium lauryl sulfate, magnesium lauryl sulfate, sodium oleate, sodium stearyl fumarate, stearic acid, hydrogenated vegetable oils, silicon dioxide and/or polyethylene glycol etc. In some embodiments the composition comprises magnesium stearate or glyceryl dibehenate (such
30 as the product Compritol® 888 ATO which consists of mono-, di- and triesters of behenic acid (C22) with the diester fraction being predominant).

In some embodiments the composition comprises a **disintegrant**, such as sodium starch glycolate, polacrillin potassium, sodium starch glycolate, crospovidon, croscarmellose, sodium carboxymethylcellulose or dried corn starch.

The composition may comprise one or more **surfactants**, for example a surfactant, at least one surfactant, or two different surfactants. The term "surfactant" refers to any molecules or ions that are comprised of a water-soluble (hydrophilic) part, and a fat-soluble (lipophilic) part. The surfactant may e.g. be selected from the group consisting of anionic
5 surfactants, cationic surfactants, nonionic surfactants, and/or zwitterionic surfactants.

Hydrotrope

An aspect of the invention relates to a composition comprising a PCSK9 inhibitor, an absorption enhancer or delivery agent and a hydrotrope. The composition of the present
10 invention further comprises one or more hydrotropes. Hydrotropes, like a surfactant, includes both a hydrophilic part and a hydrophobic and can form micelles and self-aggregate, however they solubilize solutes without micellar solubilization. The inventors have found that absorption of the PCSK9 inhibitor and thus the plasma exposure can be increased by including a hydrotrope in the compositions. Without being bound by theory, it is contemplated
15 that the hydrotrope increases the solubility of the delivery agent, such as a salt of NAC, as exemplified by SNAC herein. As shown by Assay VI herein hydrotropes can increase the solubility of SNAC in water.

In one embodiment the hydrotrope is capable of increasing the solubility of SNAC. In one embodiment the hydrotrope is capable of increasing the solubility of a salt of NAC, such
20 as SNAC, at least 2-fold at a concentration of 200 mg/ml at pH 6 at room temperature. In further embodiments, the hydrotrope increases solubility of a salt of NAC, such as SNAC, at least 3-, 4- or 5-fold when measured as described in Assay VI herein. In a further embodiment, the hydrotrope increase the solubility of SNAC at least 5-fold, such as 8-fold or such as 10-fold when measured as described in Assay VI.

25 In one embodiment the hydrotrope or hydrotropes are selected from the group consisting of: Nipecotamide, Nicotinamide, p-hydroxybenzoic acid sodium, N,N dimethyl urea, N,N dimethyl benzamide, N,N diethyl nicotinamide, Sodium salicylate, Resorcinol, Sodium benzoate, Sodium Xylenesulfonate, Sodium p-toluenesulfonate, 1-Methylnicotinamide, Pyrogallol, Pyrocatechol, Epigallocatechin gallate, Tannic acid and
30 Gentisic acid sodium salt hydrate.

In one embodiment the hydrotrope or hydrotropes are selected from the group consisting of: Nicotinamide, p-hydroxybenzoic acid sodium, N,N dimethyl urea, N,N dimethyl benzamide, N,N diethyl nicotinamide, Sodium salicylate, Resorcinol, Sodium benzoate, Sodium Xylenesulfonate, Sodium p-toluenesulfonate, 1-Methylnicotinamide, Pyrogallol,
35 Pyrocatechol, Epigallocatechin gallate and Gentisic acid sodium salt hydrate.

In one embodiment the hydrotrope or hydrotropes are selected from the group consisting of: Nicotinamide, N,N dimethyl benzamide, N,N diethyl nicotinamide, Resorcinol, Sodium benzoate, Sodium Xylenesulfonate, Sodium p-toluenesulfonate, 1-Methylnicotinamide, Pyrogallol, Pyrocatechol and Gentisic acid sodium salt hydrate.

5 In one embodiment the hydrotrope or hydrotropes are selected from the group consisting of: : Resorcinol, Pyrocatechol, N,N dimethyl benzamide, Pyrogallol, Nicotinamide, Tannic acid, Epigallocatechin gallate, N,N diethyl nicotinamide, Gentisic acid sodium salt hydrate, N,N dimethyl urea, 1-Methylnicotinamide, Sodium Xylenesulfonate, Sodium p-toluenesulfonate, Sodium salicylate, Nipecotamide, p-hydroxybenzoic acid sodium, and
10 Sodium benzoate.

In one embodiment the hydrotrope or hydrotropes are selected from the group consisting of: : Resorcinol, Pyrocatechol, N,N dimethyl benzamide, Pyrogallol, Nicotinamide, Tannic acid, Epigallocatechin gallate, N,N diethyl nicotinamide, Gentisic acid sodium salt hydrate, N,N dimethyl urea, 1-Methylnicotinamide, Sodium Xylenesulfonate and Sodium p-
15 toluenesulfonate.

In one embodiment the hydrotrope or hydrotropes are selected from the group consisting of: : Resorcinol, Pyrocatechol, N,N dimethyl benzamide, Pyrogallol, Nicotinamide, Tannic acid, Epigallocatechin gallate and N,N diethyl nicotinamide.

In one embodiment the hydrotrope or hydrotropes are selected from the group consisting of: Resorcinol, Pyrocatechol, N,N dimethyl benzamide, Pyrogallol and
20 Nicotinamide.

In one embodiment the molecular weight of the hydrotrope is at most 400 g/mol or such as at most 250 g/mol.

In one embodiment the molecular weight of the hydrotrope is at least 80 g/mol or
25 such as at least 100 g/mol

In one embodiment the hydrotrope comprises an aromatic ring structure.

In one embodiment the hydrotrope has a similar molecular structure as nicotinamide and Resorcinol, which both comprise an aromatic ring structure.

Included herein are also a physiologically acceptable salt thereof, such as the
30 sodium, potassium, chloride or sulphate salt.

In one embodiment the one or more hydrotrope has the structure of Chem I
Chem I:



, wherein

X is CH or N,

R¹, R² and R³ are independently selected from: -H, -OH, -CO₂H, -CON(R⁴)₂, -SO₃H and -CH₃, wherein R⁴ is -H, -CH₃ or -CH₂-CH₃

or a physiologically acceptable salt thereof.

5

In one embodiment, where the structure is Chem 1,

X is CH or N,

R¹ is selected from -OH, -SO₃H and CON(R⁴)₂, wherein R⁴ is -H, -CH₃ or -CH₂-CH₃,

R² is selected from: -OH and -H and

10 R³ is selected from: -H, -OH and -CH₃ or a physiologically acceptable salt thereof.

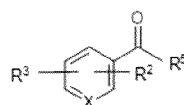
In one embodiment, the hydrotrope has the structure of Chem I, wherein

X is CH,

R¹ is selected from: -OH and -SO₃H,

15 R² and R³ are independently selected from: -H, -OH and -CH₃ or a physiologically acceptable salt thereof.

In one embodiment the one or more hydrotrope has the structure of Chem II
Chem II :



20

, wherein

X is CH or N

R² and R³ are independently selected from: -H, -OH and -CH₃

R⁵ is selected from: -OH and N(R⁴)₂, wherein R⁴ is -H, -CH₃ or -CH₂-CH₃

or a physiologically acceptable salt thereof.

25

In a further embodiment, the one or more hydrotrope has the structure of Chem II
wherein,

X is CH

R⁵ is -OH and

30 R² and R³ are independently selected from: -OH and -H or a physiologically acceptable salt thereof.

In a further embodiment, the one or more hydrotrope has the structure of Chem II as defined above, with the proviso that the hydrotrope is not sodium benzoate.

In a further embodiment, the one or more hydrotrope has the structure of Chem II, wherein

X is N,

5 R^5 is selected from: $-OH$ and $N(R^4)_2$, wherein R^4 is $-H$, $-CH_3$ or $-CH_2-CH_3$

R^2 and R^3 are independently selected from: $-H$, $-OH$ and $-CH_3$ or

a physiologically acceptable salt thereof.

10 In a further embodiment, the one or more hydrotrope has the structure of Chem II, wherein

X is N,

R^5 is NH_2 , and

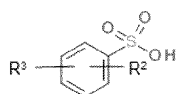
R^2 and R^3 are independently selected from: $-H$, $-OH$ and $-CH_3$ or

a physiologically acceptable salt thereof.

15

In one embodiment the one or more hydrotrope has the structure of Chem III

Chem III:



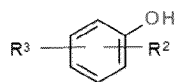
, wherein

R^2 and R^3 are independently selected from $-H$ and $-CH_3$.

20

In one embodiment the one or more hydrotrope has the structure of Chem IV

Chem IV:



, wherein

R^2 and R^3 are independently selected from: $-H$ and $-OH$.

25

In one embodiment the hydrotrope or hydrotropes is/are selected from the group consisting of: Resorcinol, Pyrocatechol, Pyrogallol, Gentisic acid, Xylenesulfonate, p-toluenesulfonate, Nicotinamide, Dimethylbenzamide, Diethylbenzamide, 1-methylnicotinamide, Salicylic acid, P-Hydroxybenzoic acid and Benzoate.

30

In one embodiment the hydrotrope or hydrotropes is/are selected from the group consisting of: Resorcinol, Pyrocatechol, Pyrogallol, Gentisic acid, Xylenesulfonate, p-

toluenesulfonate, Nicotinamide, Dimethylbenzamide, Diethylbenzamide, 1-methylnicotinamide, Salicylic acid and P-Hydroxybenzoic acid.

In one embodiment the hydrotrope or hydrotropes is/are selected from the group consisting of: Resorcinol, Pyrocatechol and Pyrogallol,

5 In one embodiment the hydrotrope or hydrotropes is/are selected from the group consisting of: Xylenesulfonate and p-toluenesulfonate

In one embodiment the hydrotrope or hydrotropes is/are selected from the group consisting of: Nicotinamide, Dimethylbenzamide, Diethylbenzamide and 1-methylnicotinamide,

10 In one embodiment the hydrotrope or hydrotropes is/are selected from the group consisting of: Gentisic acid, Salicylic acid, P-Hydroxybenzoic acid and Benzoate.

In one embodiment the hydrotrope or hydrotropes is/are selected from the group consisting of: Gentisic acid, Salicylic acid and P-Hydroxybenzoic acid.

15 In one embodiment the hydrotrope or hydrotropes are nicotinamide and/or Resorcinol. In one embodiment the hydrotrope is nicotinamide.

In one embodiment the hydrotrope is not sodium benzoate.

As shown in the examples herein, the composition of the invention comprises a PCSK9 inhibitor, a delivery agent and a hydrotrope.

20 The description here below also refers to compositions consisting of specific ingredients, the PCSK9 inhibitor, the delivery agent and the hydrotrope and optionally a lubricant, the term consisting is to be understood to nevertheless encompass trace amounts of any substance with no effect on the function of the composition. Such substances can be impurities remaining in preparation of the PCSK9 inhibitor, from the production of the salt of NAC, the hydrotrope preparation or minimal amounts of any pharmaceutical acceptable
25 excipient that do not affect the quality or absorption of the formulation.

In one embodiment the pharmaceutical composition comprises a balanced amount of the hydrotrope relative to the amount of the delivering agent. The effect of the hydrotrope has been observed over a range of concentrations.

30 In one embodiment the ratio of salt of NAC/hydrotrope (w/w) is at least 0.5, such as at least 0.75 or such as at least 1.

In one embodiment the ratio of salt of NAC/hydrotrope (w/w) is 0.5-10.0 or such as such as 0.5-8 or such as 0.5-5.

In one embodiment the ratio of salt of NAC/hydrotrope (w/w) is 0.5-10.0 or such as 0.75-10.0, 0.5-8.0 or 1-2.0.

In one embodiment the ratio of SNAC/Nicotinamide (w/w) is at least 0.5, such as at least 0.75, such as at least 1.

In one embodiment the ratio of salt of SNAC/Nicotinamide (w/w) is 0.5-10.0 or such as 0.5-8 or such as 0.5-5.

5 In one embodiment the ratio of salt of SNAC/Nicotinamide (w/w) is 0.5-10.0 or such as 0.75-10.0, 0.5-8.0 or 1-2.0.

In one embodiment the ratio of SNAC/Resorcinol (w/w) is at least 0.5, such as at least 0.75, such as at least 1. In one embodiment the ratio of salt of SNAC/Resorcinol (w/w) is 0.5-10.0 or such as 0.75-10.0, 0.5-8.0 or such as 1-2.0.

10 In one embodiment the ratio of hydrotrope/salt of NAC (w/w) is at least 0.1, such as at least 0.2 or such as at least 0.3. In one embodiment the ratio of hydrotrope/salt of NAC (w/w) is 0.1-5.0 or such as 0.1-4.0, 0.2-3.0 or 0.25-2.0.

In one embodiment the ratio of Nicotinamide/SNAC/ (w/w) is at least 0.1-5.0 or such as 0.1-4.0, 0.2-3.0 or 0.25-2.0. In one embodiment the ratio of Nicotinamide/SNAC (w/w) is
15 0.1-5.0 or such as 0.1-4.0, 0.2-3.0 or 0.25-2.0.

In one embodiment the ratio of Resorcinol/SNAC/ (w/w) is at least 0.1-5.0 or such as 0.1-4.0, 0.2-3.0 or 0.25-2.0. In one embodiment the ratio of Resorcinol/SNAC (w/w) is 0.1-5.0 or such as 0.1-4.0, 0.2-3.0 or 0.25-2.0.

In one embodiment the amount of lubricant maybe be considered relative to the total
20 amount of the other excipients, here hydrotrope and delivery agent, and not including the active pharmaceutical ingredient, the PCSK9 inhibitor. Relatively small amounts of the lubricant are usually included, such as less than 5 % of the total weight of the other excipients.

In one embodiment the composition comprises less than 5 w/w % lubricant of the
25 total amount of delivery agent and hydrotrope. In one embodiment the composition comprises 0.15- 5%, such as 0.25-4 w/w % lubricant of the total amount of delivery agent and hydrotrope. In further embodiments the composition comprises 0.15- 5%, such as 0.25-4 w/w % lubricant of the amount of salt of NAC, such as SNAC, and nicotinamide or resorcinol.

30 The pharmaceutical composition according to the invention is preferably produced in a dosage form suitable for oral administration as described herein below. In the following the absolute amounts of the ingredients of the composition of the invention are provided with reference to the content in a dosage unit i.e. per tablet, capsule or sachet.

The pharmaceutical compositions of the invention may in a further embodiment
35 comprise at most 1000 mg of said salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid (NAC)

per dose unit. In one embodiment the invention relates to a composition wherein a dose unit comprises at most 600 mg of said salt.

In some embodiments the amount of the salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid per dose unit is at least at least 0.05 mmol, such as at least 0.075 mmol, such as at least 0.1 mmol, such as at least 0.125 mmol, such as at least 0.15 mmol, such as selected from the group consisting of at least 0.20 mmol, at least 0.25 mmol, at least 0.30 mmol, at least 0.35 mmol, at least 0.40 mmol, at least 0.45 mmol, at least 0.50 mmol, at least 0.55 mmol and at least 0.60 mmol.

In some embodiments the amount of the salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid per dosage unit of the composition is up to 3 mmol, such as up to 2.75 mmol, such as up to 2.5 mmol, such as up to 2.25 mmol, such as 2 mmol, such as up to 1.5 mmol, up to 1 mmol, up to 0.75 mmol, up to 0.6 mmol, up to 0.5 mmol, up to 0.4 mmol, up to 0.3 mmol and up to 0.2 mmol.

In some embodiments the amount of the salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid per dose unit of the composition is in the range of 0.05-3 mmol, 0.10- 2.5 mmol, 0.15- 2.0 mmol, 0.20 – 1.5 mmol, , 0.25-1.0 mmol 0.30-0.75 mmol or such as 0.45-0.65 mmol.

In some embodiments the amount of SNAC in the composition is at least 20 mg, such as at least 25 mg, such as at least 50 mg, such as at least 75 mg, at least 100 mg, at least 125 mg, at least 150 mg, at least 175 mg, at least 200 mg, at least 225 mg, at least 250 mg, at least 275 mg and at least 300 mg per dose unit.

In some embodiments the amount of SNAC in the composition is up to 1000 mg, such as up to 800 mg, such as up to 600 mg, such as up to 575 mg, such as up to 550 mg, up to 525 mg, up to 500 mg, up to 475 mg, up to 450 mg, up to 425 mg, up to 400 mg, up to 375 mg, up to 350 mg, up to 325 mg per dose unit, or up to 300 mg per dose unit.

In some embodiments the amount of SNAC in the composition is in the range of 20-800 mg, such as 25-600 mg, such as 50-500 mg, such as 50-400 mg, such as 75-400 mg, such as from 80-350 mg, such as from around 100 to around 300 mg per dose unit.

In one embodiment, where the salt of NAC is SNAC, the amount of SNAC is in the range of 20-200 mg, such as 25-175 mg, such as 75-150 mg, such as 80-120 mg such as around 100 mg per dose unit.

In one embodiment, where the salt of NAC is SNAC, the amount of SNAC is in the range of 200-800 mg, such as 250-400 mg, such as 250-350 mg, such as 275-325 mg, such as around 300 mg per dose unit.

In an embodiment, a dose unit of the pharmaceutical compositions of the invention comprises 0.1-150 mg, 0.1-100 mg or 0.2 to 100 mg of the PCSK9 inhibitor.

In some embodiments a dose unit of the composition comprises an amount of PCSK9 inhibitor is in the range of 0.5-150 mg, 0.5-120 mg, 0.5-100 mg, 1-80 mg, 1-70 mg, 1-60, 1-50 mg or 1- 40 mg.

In some embodiments a dose unit of the composition comprises an amount of PCSK9 inhibitor is in the range of 0.1 – 50 mg, 0.2 to 50 mg, 0.5 to 50 mg or 1 to 40 mg.

In some embodiments a dose unit of the composition comprises an amount of PCSK9 inhibitor is in the range of 0.1 – 50 mg, 0.1 – 40 mg, 0.1 – 30 mg or 0.1 – 20 mg.

In some embodiments a dose unit comprises 1-50 mg of the PCSK9 inhibitor, such as 0.75- 40 mg, such as 10, 15, 20, 25 or 30 mg or 35, 40, 45 mg, such as 10-30 or 30-50 mg of the PCSK9 inhibitor per dose unit.

In some embodiments a dose unit comprises 20 to 150 mg of the PCSK9 inhibitor, such as 20-120 mg, such as 20-100 mg, such as 20-80 mg, such as 20, 30, 40, 50, 60, 70 or 80 mg, such as 20, 30, 40 or 50 mg, or such as 80, 85, 90, 95 or 100 mg, or such as 50 mg or such as 75 mg of the PCSK9 inhibitor per dose unit.

In some embodiments a dose unit comprises 5 to 50 mg of the PCSK9 inhibitor, such as 10-45 mg, such as 20, 30 or 40 mg, or such as 25, 35, or 45 mg, or such as 30-50 mg or such as 20-40 mg of the PCSK9 inhibitor per dose unit.

In some embodiments a dose unit comprises 2 to 20 mg of the PCSK9 inhibitor, such as 2-15 mg, such as 2, 3, 4 or 5 mg, or such as 8, 10, 12 or 14 mg, such as 15 mg or such as 20 mg of the PCSK9 inhibitor per dose unit.

In some embodiments a dose unit comprises 0.5-5 mg of the PCSK9 inhibitor, such as 0.75- 4 ½ mg, such as 1, 1 ½, 2, 2 ½ or 3 mg or 3 ½, 4, 4 ½ mg, such as 1-3 or 3-5 mg of the PCSK9 inhibitor per dose unit.

The amount of PCSK9 inhibitor may be varied depending on identity of the PCSK9 inhibitor and the effect desired.

In a preferred embodiment a unit dose of the composition comprises 0.5-50 mg magnesium stearate, such as 0.10-25 mg, such as 0.25-10 mg, such as 0.5-8 mg or such as 0.5-5 mg magnesium stearate.

As described above the amount of the hydrotrope is to be balanced with the amount of the delivering agents, such as SNAC, but in general a dose unit of the compositions of the invention comprises 10-600 mg of the hydrotrope.

In one embodiment a dose unit comprises 20-400 mg, such as 40-300, such as 50-200 mg, such as 50-175 mg of the hydrotrope.

In one embodiment a dose unit comprises 100-600 mg, such as 100-500, such as 150-400 mg, such as 150-300 mg of the hydrotrope.

5 In further such embodiments, a unit dose of the composition according to the invention comprises 50-600 mg nicotinamide and/or resorcinol.

In one embodiment a dose unit comprises 50-400 mg, such as 50-300, such as 50-200 mg, such as 50-175 mg nicotinamide and/or resorcinol.

10 In further such embodiments a unit dose of the composition according to the invention comprises 50-600 mg nicotinamide. In one embodiment a dose unit comprises 50-400 mg, such as 50-300, such as 50-200 mg, such as 50-175 mg nicotinamide.

15 In a preferred embodiment the amount of magnesium stearate is determined relative to the amount of the salt of NAC, such as SNAC, such that a unit dose of the composition comprises 0.25-10 mg, such as 0.25-8 mg, such as 0.5-5 mg or such as 1-3 mg magnesium stearate per 100 mg salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid, such as SNAC.

In a preferred embodiment a unit dose of the composition comprises 20-1000 mg SNAC, 0.5-100 mg PCSK9 inhibitor, 10-600 mg hydrotrope and 0.10-50 mg lubricant.

20 In a preferred embodiment a unit dose of the composition comprises 20-800 mg SNAC, 1.0-80 mg PCSK9 inhibitor, 10-500 mg hydrotrope and 0.10-40 mg lubricant.

In a preferred embodiment a unit dose of the composition comprises 40-800 mg SNAC, 2-50 mg PCSK9 inhibitor, 20-400 mg hydrotrope and 0.10-40 mg lubricant.

In a preferred embodiment a unit dose of the composition comprises 20-600 mg SNAC, 5-50 mg PCSK9 inhibitor, 10-600 mg nicotinamide and 0.10-30 mg lubricant.

25 In a preferred embodiment a unit dose of the composition comprises 20-500 mg SNAC, 5-50 mg PCSK9 inhibitor, 10-500 mg nicotinamide and 0.10-25 mg lubricant.

In a preferred embodiment a unit dose of the composition comprises 40-500 mg SNAC, 5-50 mg PCSK9 inhibitor, 20-400 mg nicotinamide and 0.10-25 mg lubricant.

30 In one embodiment a unit dose of the composition according to the invention comprises:

- i) 0.1-100 mg PCSK9 inhibitor
- ii) 20-800 mg salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid (NAC), such as the sodium salt of NAC (SNAC) and
- 35 iii) 20-600 mg hydrotrope, such as 50-200 mg, nicotinamide or resorcinol and

- iv) 0-20 mg lubricant.

In one embodiment a unit dose of the composition according to the invention comprises:

- 5 i) 0.1-100 or 0.1-75 mg PCSK9 inhibitor
 ii) 50-600 mg salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid (NAC), such as the sodium salt of NAC (SNAC) and
 iii) 50-500 mg, such as 50-400 mg, nicotinamide or resorcinol and
 iv) 0-20 mg lubricant.

10

The amount of PCSK9 inhibitor may be varied depending on identity of the PCSK9 inhibitor and the effect desired.

In a preferred embodiment a unit dose of the composition comprises 200-400 mg
15 SNAC, 0.5-75 mg PCSK9 inhibitor, 50-400 mg hydrotrope and 0.25-3 mg lubricant.

In a preferred embodiment a unit dose of the composition comprises 200-400 mg SNAC, 1½ -50 mg PCSK9 inhibitor, 50-400 mg hydrotrope and 0.5-5 mg lubricant.

In a preferred embodiment a unit dose of the composition comprises 200-400 mg SNAC, 5-25 mg PCSK9 inhibitor, 50-400 mg hydrotrope and 1-5 mg lubricant.

20

In one embodiment a unit dose of the composition according to the invention comprises:

- i) 0.1-75 mg PCSK9 inhibitor
 ii) 25-400 mg salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid (NAC), such as the
25 sodium salt of NAC (SNAC) and
 iii) 20-300 mg nicotinamide or resorcinol and
 iv) 0-15 mg lubricant.

In a preferred embodiment a unit dose of the composition comprises 200-400 mg
30 SNAC, 0.5-75 mg PCSK9 inhibitor, 50-400 mg nicotinamide and 0.25-8 mg lubricant.

In a preferred embodiment a unit dose of the composition comprises 200-400 mg SNAC, 1- 50 mg PCSK9 inhibitor, 50-400 mg nicotinamide and 0.5-8 mg lubricant.

In a preferred embodiment a unit dose of the composition comprises 200-400 mg SNAC, 5-25 mg PCSK9 inhibitor, 50-400 mg nicotinamide and 1-8 mg lubricant.

35

The amount of PCSK9 inhibitor may be varied depending on identity of the PCSK9 inhibitor and the effect desired.

In a preferred embodiment a unit dose of the composition comprises 80-120 mg SNAC, 0.5-50 mg PCSK9 inhibitor, 20-200 mg hydrotrope and 0.25-5 mg lubricant.

5 In a preferred embodiment a unit dose of the composition comprises 80-120 mg SNAC, 1 ½ -40 mg PCSK9 inhibitor, 20-200 mg hydrotrope and 0.5-5 mg lubricant.

In a preferred embodiment a unit dose of the composition comprises 80-120 mg SNAC, 5-25 mg PCSK9 inhibitor, 20-200 mg hydrotrope and 0.5-5 mg lubricant.

10 In a preferred embodiment a unit dose of the composition comprises 80-120 mg SNAC, 0.5-50 mg PCSK9 inhibitor, 20-200 mg nicotinamide and 0.25-5 mg lubricant.

In a preferred embodiment a unit dose of the composition comprises 80-120 mg SNAC, 1 ½ -40 mg PCSK9 inhibitor, 20-200 mg nicotinamide and 0.5-5 mg lubricant.

15 In a preferred embodiment a unit dose of the composition comprises 80-120 mg SNAC, 5-25 mg PCSK9 inhibitor, 20-200 mg nicotinamide and 0.5-5 mg lubricant.

In one embodiment the pharmaceutical composition of the invention has a fast disintegration or dissolution *in vitro*. Disintegration or dissolution may be tested as known in the art such as by using Assay II or Assay III described herein.

20 The dissolution or release may be expressed as the amount of the PCSK9 inhibitor measured in solution after a given period relative to the total content of the PCSK9 inhibitor of the composition. The relative amount may be given in percentage.

In one embodiment the release of the PCSK9 inhibitor from the pharmaceutical composition of the invention is at least 85 % within 15 minutes or at least 95 % within 30 minutes. In one such embodiment the release is measured at pH 6.8.

25 In one embodiment the pharmaceutical composition comprises

- i) a PCSK9 inhibitor,
- ii) a salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid and
- iii) a hydrotrope

30 wherein the release of the PCSK9 inhibitor reaches 85 % within 15 minutes or 95 % within 30 minutes. In one embodiment the release is measured at pH 6.8.

Experiments have demonstrated that a composition according to the invention displays fast dissolution and plasma exposure similar to what has previously been observed for Semaglutide and other GLP-1 receptor agonists (PCT/EP2019/061502). The improved

35

plasma exposure of a PCSK9 inhibitor using a composition according to the invention compared to a SNAC/PCSK9 inhibitor composition according to WO 2012/080471 and WO 2013/139694 is seen in the examples herein.

5 Dosage form

The composition may be administered in several dosage forms, for example as a tablet; a coated tablet; a sachet or a capsule such as hard or soft shell gelatine capsules and all such compositions are considered solid oral dosage forms.

10 The composition may further be compounded in a drug carrier or drug delivery system, e.g. in order to improve stability and/or solubility or further improve bioavailability. The composition may be a freeze-dried or spray-dried composition.

The composition may be in the form of a dose unit, such as a tablet. In some embodiments the weight of the unit dose is in the range of 50 mg to 1000 mg, such as in the range of 50-750 mg, or such as in the range of 100-600 mg.

15 In some embodiments the weight of the dose unit is in the range of 75 mg to 400 mg. In an embodiment the weight of the unit dose is in the range of 100-400 mg, such as in the range of 100-300 mg or such as in the range of 150-350 mg.

In an embodiment the weight of the dose unit is in the range of 300 mg to 800 mg, such as in the range of 400-700 mg or such as in the range of 500-600 mg.

20 In some embodiments the composition may be granulated prior to being compacted and i.e. compressed into tablets. The composition may comprise a granular part and/or an extragranular part, wherein the granular part has been granulated and the extragranular part has been added after granulation.

The granular part may comprise the delivery agent and/or the hydrotrope and/or a PCSK9 inhibitor. In an embodiment the granular part may comprise a further excipient, such as a lubricant and/or a glidant.

In an embodiment the intragranular part comprises the delivery agent and a lubricant and/or a glidant.

30 In an embodiment the hydrotrope is included in the granular part, the extra-granular part or both.

In an embodiment the granular part includes the delivery agent and the hydrotrope.

In some embodiments the extragranular part comprises the PCSK9 inhibitor, and/or a lubricant and/or a glidant, such as magnesium stearate. In some embodiments the extragranular part comprises the PCSK9 inhibitor.

In some embodiments the extragranular part comprises an excipient, such as a lubricant and/or glidant, such as magnesium stearate.

In a further embodiment the granular part comprises the delivery agent and the hydrotrope while the extragranular part comprises the PCSK9 inhibitor and the lubricant
5 and/or a glidant.

Preparation of composition

Preparation of a composition according to the invention may be performed according to methods known in the art.

To prepare a dry **blend** of tableting material, the various components are optionally
10 delumped or sieved, weighed, and then combined. The mixing of the components may be carried out until a homogeneous blend is obtained.

The terms "granulate" and "granules" are used interchangeably herein to refer to particles of composition material which may be prepared as described above. The term refers broadly to pharmaceutical ingredients in the form of particles, granules and aggregates
15 which are used in the preparation of solid dose formulations. Generally, granules are obtained by processing a powder or a blend to obtain a solid which is subsequently broken down to obtain granules of the desired size.

If granules are to be used in the tableting material, granules may be produced in a manner known to a person skilled in the art, for example using **wet granulation** methods
20 known for the production of "built-up" granules or "broken-down" granules. Methods for the formation of **built-up granules** may operate continuously and comprise, for example simultaneously spraying the granulation mass with granulation solution and drying, for example in a drum granulator, in pan granulators, on disc granulators, in a fluidized bed, by spray-drying, spray-granulation or spray-solidifying, or operate discontinuously, for example
25 in a fluidized bed, in a rotary fluid bed, in a batch mixer, such as a high shear mixer or a low shear mixer, or in a spray-drying drum. Methods for the production of **broken-down granules**, which may be carried out discontinuously and in which the granulation mass first forms a wet aggregate with the granulation solution, which is subsequently comminuted or by other means formed into granules of the desired size and the granules may then be dried.
30 Suitable equipment for the wet granulation step is, but not limited to, planetary mixers, low shear mixers, high shear mixers, extruders and spheronizers, such as an apparatus from the companies Loedige, Glatt, Diosna, Fielder, Collette, Aeschbach, Alexanderwerk, Ytron, Wyss & Probst, Werner & Pfleiderer, HKD, Loser, Fuji, Nica, Caleva and Gabler. Granules may also be formed by **dry granulation** techniques in which one or more of the excipient(s)

and/or the active pharmaceutical ingredient is compressed to form relatively large moldings, for example slugs or ribbons, which are comminuted by grinding, and the ground material serves as the tableting material to be later compacted. Suitable equipment for dry granulation is, but not limited to, roller compaction equipment from Gerteis such as Gerteis
5 MICRO-PACTOR, MINI-PACTOR and MACRO-PACTOR.

To **compact** the tableting material into a solid oral dosage form, for example a tablet, a tablet press may be used. In a tablet press, the tableting material is filled (e.g. force feeding or gravity feeding) into a die cavity. The tableting material is then compacted by a
10 set of punches applying pressure. Subsequently, the resulting compact, or tablet is ejected from the tablet press. The above-mentioned tableting process is subsequently referred to herein as the "compaction process". Suitable **tablet presses** include, but are not limited to, rotary tablet presses and eccentric tablet presses. Examples of tablet presses include, but are not limited to, the Fette 102i (Fette GmbH), the Korsch XL100, the Korsch PH 106 rotary
15 tablet press (Korsch AG, Germany), the Korsch EK-O eccentric tableting press (Korsch AG, Germany) and the Manesty F-Press (Manesty Machines Ltd., United Kingdom).

In an embodiment the invention relates to a composition comprising
i) a PCSK9 inhibitor,
20 ii) a salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid (NAC) and
iii) a hydrotrope
wherein the composition comprises a granulate of ii) and iii).

In general, granulates may be prepared by wet, melt or dry granulation. Granules
25 comprising i, ii and/or iii may thus be obtained by dry granulation of a blend hereof, such as by roller compaction. In an alternative embodiment wet granulation may be used to obtain the granules. In an alternative embodiment hot melt extrusion may be used to obtain the granules. Such material can then be used directly or further refined to obtain the final granules.

30 In an embodiment the composition comprises at least one granulate. In an embodiment the composition comprises one type of granulate. The composition may alternatively comprise two types of granulates.

In embodiments where the granular part comprises both the delivery agent and the hydrotrope these excipients may be co-processed prior to or in the preparation of the
35 granules.

The granulation maybe be obtained by various methods as described above, wherein ii) and iii) are initially mixed either as powders or by the preparation of a solution comprising both ingredients. In an alternative embodiment, granules of ii) and iii) are obtained by feeding both ingredients separately into the process stream, such as into an
5 extruder.

Granules of ii) and iii) may then be obtained by dry granulation of the blend, such as by roller compaction. In an alternative embodiment the ingredients ii) and iii) may be hot melt extruded to obtain an extrudate which is optionally subsequently milled to obtain the granules. This material can then be used directly or in dry granulation/roller compaction
10 process to obtain the final granules.

In one embodiment a solution of ii) and iii) is prepared and subject to spray granulation whereby granules are directly obtained. In an alternative embodiment, solution of ii) and solution of iii) is prepared separately and subjected to spray granulation. Alternatively, the solution can be used in a fluid bed spray granulation process. In one embodiment spray
15 drying can be used followed by dry granulation/roller compaction to obtain the granules.

In some embodiments the invention relates to a method of preparation a composition according to the invention. In an embodiment the method of preparing a tablet comprises;

- 20 a) granulation of the delivery agent and the hydrotrope
 b) blending of the granulates of a) with a PCSK9 inhibitor, and
 c) compression of the blend into tablets.

The granulation may be a wet, hot or dry granulation. As described above a lubricant such as magnesium stearate or glyceryl behenate may be included in steps a), b)
25 and/or c).

In one embodiment the invention relates to a method for producing a solid pharmaceutical composition comprising the steps of;

- a) obtaining a salt of NAC and a hydrotrope,
 b) co-processing said salt of NAC and hydrotrope of a) and
30 c) preparing said solid pharmaceutical composition using the product of b).

In one embodiment the invention relates to a method for producing a solid pharmaceutical composition comprising the steps of;

- a) obtaining a salt of NAC and a hydrotrope,
 b) co-processing said salt of NAC and hydrotrope of a) and
35 c) preparing said solid pharmaceutical composition using the product of b).

In one embodiment the method is for producing a solid pharmaceutical composition comprising the steps of;

- a) obtaining a salt of NAC and a hydrotrope,
- b) hot melt extruding said salt of NAC and hydrotrope of a) and
- 5 c) preparing said solid pharmaceutical composition, such as tablets, using the extrudate of b).

The method may as described herein include further steps, such as a step of admixing the extrudate of b) with an active pharmaceutical ingredient and optionally any
10 further excipients and preparing said solid pharmaceutical composition using the mixture.

Pharmaceutical Indications

In one aspect the invention relates to the use of an PCSK9 inhibitor, such as an EGF(A) peptide analogue or an EGF(A) derivative for use in the manufacture of a
15 pharmaceutical composition as described herein.

In one aspect the invention relates to a composition comprising a PCSK9 inhibitor, such as an EGF(A) peptide analogue or an EGF(A) derivative, for use as a medicament and/or in a method of treatment.

In one embodiment the composition is for use in a method of treatment, such as for
20 (i) improving lipid parameters, such as prevention and/or treatment of dyslipidaemia, lowering total serum lipids; lowering LDL-C, increasing HDL; lowering small, dense LDL; lowering VLDL; lowering triglycerides; lowering cholesterol; lowering plasma levels of lipoprotein a (Lp(a)); inhibiting generation of apolipoprotein A (apo(A)) ; (ii) the prevention and/or the treatment of cardiovascular diseases, such as cardiac syndrome X, atherosclerosis,
25 myocardial infarction, coronary heart disease, reperfusion injury, stroke, cerebral ischemia, an early cardiac or early cardiovascular disease, left ventricular hypertrophy, coronary artery disease, hypertension, essential hypertension, acute hypertensive emergency, cardiomyopathy, heart insufficiency, exercise intolerance, acute and/or chronic heart failure, arrhythmia, cardiac dysrhythmia, syncope, angina pectoris, cardiac bypass and/or stent
30 reocclusion, intermittent claudication (atherosclerosis obliterans), diastolic dysfunction, and/or systolic dysfunction; and/or the reduction of blood pressure, such as reduction of systolic blood pressure; the treatment of cardiovascular disease. Dyslipidaemia may be such as a high plasma concentration of cholesterol also called hypercholesterolaemia referring to a situation where the plasma cholesterol concentrations is above the normal range of a total

cholesterol ≥ 5.0 mmol/l. In one embodiment the compound or composition of the invention may be used for treatment of hypercholesterolaemia.

Method of treatment

5 The invention further relates to a method of treating a subject in need thereof, comprising administering a therapeutically effective amount of a composition according to the present invention to said subject. In one embodiment the method of treatment is for (i) improving lipid parameters and/or (ii) preventing and/or treating cardiovascular diseases and/or the further indications specified above.

10 In some embodiments, a method is described comprising administering to a subject in need thereof a therapeutically effective amount of a pharmaceutical composition comprising a PCSK9 inhibitor, a salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid (NAC), a hydrotrope, and optionally, a lubricant.

15 In some embodiments, a method for treating diabetes is described comprising administering to a subject in need thereof a therapeutically effective amount of a pharmaceutical composition comprising

- i) 0.1-100 mg of a PCSK9 inhibitor,
- ii) 25-600 mg of salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid (NAC)
- iii) 20-600 mg, such as 50-200 mg, nicotinamide or resorcinol and
- 20 iv) 0.25-15 mg lubricant as described herein above.

Various examples of a lubricant are described, including magnesium stearate. The composition is administered orally and is in a form of a table, capsule or a sachet.

25 In a further such embodiments one or more dose units may be administered to said subject in need.

Combination treatment

Treatment with a PCSK9 inhibitor according to the present invention may be combined with treatment with one or more additional pharmacologically active substances, e.g. selected from anti-diabetic agents, anti-obesity agents, appetite regulating agents, 30 antihypertensive agents, agents for the treatment and/or prevention of complications resulting from or associated with diabetes and agents for the treatment and/or prevention of complications and disorders resulting from or associated with obesity.

Examples of such pharmacologically active substances are: GLP-1 receptor agonists, insulin, DPP-IV (dipeptidyl peptidase-IV) inhibitors, amylin agonists, anti-

inflammatory, triglyceride-lowering agents and leptin receptor agonists. Particular examples of such active substances are the GLP-1 receptor agonists liraglutide and semaglutide and insulin degludec.

- 5 The invention as described herein is, without limitation hereto, further defined by the embodiments described here below and the claims of the document.

EMBODIMENTS

1. A pharmaceutical composition comprising
 - i) a PCSK9 inhibitor
 - 5 ii) a salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid and
 - iii) a hydrotrope.
2. The pharmaceutical composition according to any of the previous embodiments, wherein the hydrotrope is selected from the group of hydrotropes consisting of: Nipecotamide,
10 Nicotinamide, p-hydroxybenzoic acid sodium, N,N dimethyl urea, N,N dimethyl benzamide, N,N diethyl nicotinamide, Sodium salicylate, Resorcinol, Sodium benzoate, Sodium Xylenesulfonate, Sodium p-toluenesulfonate, 1-Methylnicotinamide, Pyrogallol, Pyrocatechol, Epigallocatechin gallate, Tannic acid and Gentisic acid sodium salt hydrate.
15
3. The pharmaceutical composition according any of the previous embodiments, wherein the hydrotrope is selected from the group of hydrtropes consisting of: Nipecotamide, Nicotinamide, p-hydroxybenzoic acid sodium, N,N dimethyl urea, N,N dimethyl benzamide, N,N diethyl nicotinamide, Sodium salicylate, Resorcinol, Sodium
20 Xylenesulfonate, Sodium p-toluenesulfonate, 1-Methylnicotinamide, Pyrogallol, Pyrocatechol, Epigallocatechin gallate, Tannic acid and Gentisic acid sodium salt hydrate.
4. The composition according to any of the previous embodiments, wherein the hydrotrope
25 comprises an aromatic ring structure.
5. The composition according to any of the previous embodiments, wherein the hydrotrope is not sodium benzoate.
- 30 6. The composition according to any of the previous embodiments, wherein the hydrotrope has a molecular weight of less than 400 g/mol.
7. The composition according to any of the previous embodiments, wherein the hydrotrope has a molecular weight of at least 80 g/mol.
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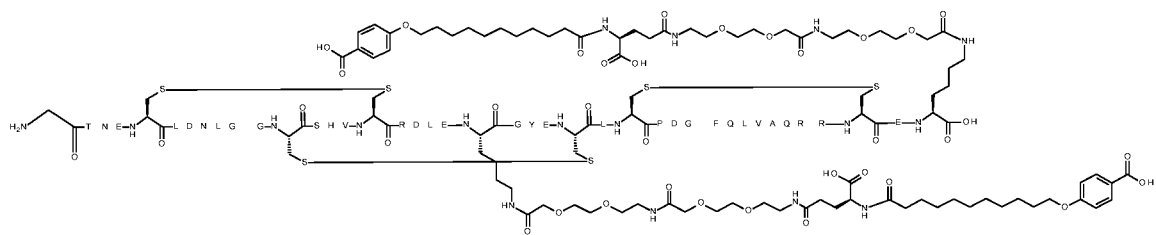
8. The composition according to any of the previous embodiments, wherein the hydrotrope increases the solubility of SNAC at least 2-fold.
9. The composition according to any of the previous embodiments, wherein the hydrotrope is increases the solubility of SNAC at least 5-fold.
10. The composition according to any of the previous embodiments, wherein the solubility is measured at a concentration of 200 mg/ml of the hydrotrope at pH 6.
11. The composition according to any of the previous embodiments, where in the solubility is measured as room temperature.
12. The composition according to any of the previous embodiments, where in the solubility is measured as described in Assay VI herein.
13. The composition according to any of the previous embodiments, wherein the hydrotrope is Nicotinamide or Resocinol.
14. The composition according to any of the previous embodiments, wherein the hydrotrope is Nicotinamide.
15. The composition according to any of the previous embodiments, wherein the ratio of salt of NAC/hydrotrope (w/w) is at least 0.5.
16. The composition according to any of the previous embodiments, wherein the ratio of salt of NAC/hydrotrope (w/w) is 0.5-10.0 or such as 0.75-10.0, 0.5-8.0 or 1-2.0.
17. The composition according to any of the previous embodiments, wherein the ratio of hydrotrope/salt of NAC (w/w) is at least 0.1.
18. The composition according to any of the previous embodiments, wherein the ratio of hydrotrope/salt of NAC (w/w) is 0.1-5.0 or such as 0.1-4.0, 0.2-3.0 or 0.25-2.0.
19. The composition according to any of the previous embodiments, wherein the composition further comprises at least one lubricant.

20. The pharmaceutical composition according to any of the previous embodiments wherein the composition further comprises magnesium stearate.
- 5
21. The pharmaceutical composition according any embodiment 19, wherein the composition comprises 0.15-25 mg, such as 0.25-10 mg, such as 2-5 mg or such as 2-3 mg magnesium stearate per 100 mg salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid.
- 10
22. The pharmaceutical composition according to any of the previous embodiments wherein said salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid and hydrotrope constitutes at least 60 w/w % of the composition.
23. The pharmaceutical composition according to any of the previous embodiments wherein said salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid and hydrotrope constitutes at least 60 w/w % of the excipients of the composition.
- 15
24. The pharmaceutical composition according to any of the previous embodiments, wherein the salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid is selected from the group consisting of the sodium salt, potassium salt and/or ammonium salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid.
- 20
25. The pharmaceutical composition according to any of the previous embodiments, wherein the salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid is sodium N-(8-(2-hydroxybenzoyl)amino)caprylate (SNAC).
- 25
26. The pharmaceutical composition according to any of the previous embodiments, wherein a dose unit comprises at most 1000 mg of said salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid.
- 30
27. The pharmaceutical composition according to any of the previous embodiments, wherein a dose unit comprises at most 500 mg of said hydrotrope.
28. The pharmaceutical composition according to any of the previous embodiments, wherein a dose unit comprises 0.1-100 mg of the PCSK9 inhibitor.
- 35

29. The pharmaceutical composition according to any of the previous embodiments, wherein the PCSK9 inhibitor has an inhibitory function at least comparable to EGF(A) 301L.
- 5 30. The pharmaceutical composition according to any of the previous embodiments, wherein the apparent binding affinity (K_i) for the PCSK9 inhibitor is equal or below the apparent binding affinity (K_i) for EGF(A) 301L.
- 10 31. The pharmaceutical composition according to any of the previous embodiments, wherein the PCSK9 inhibitor has an inhibitory function at least comparable to EGF(A) 301L, 309R, 312E.
- 15 32. The pharmaceutical composition according to any of the previous embodiments, wherein the apparent binding affinity (K_i) for the PCSK9 inhibitor is equal to or below the apparent binding affinity (K_i) for EGF(A) 301L, 309R, 312E.
33. The pharmaceutical composition according to any of the previous embodiments, wherein $\frac{K_i(\text{PCSK9 inhibitor})}{K_i(\text{EGF(A)301L})}$ is below 2.
- 20 34. The pharmaceutical composition according to any of the previous embodiments, wherein $\frac{K_i(\text{PCSK9 inhibitor})}{K_i(\text{EGF(A)301L,309R,312E})}$ is below 2.
- 25 35. The pharmaceutical composition according to any of the previous embodiments, wherein the PCSK9 inhibitor has an apparent binding affinity (K_i) below 10 nM, such as below 8 nM, such as below 5 mM.
- 30 36. The pharmaceutical composition according to any of the previous embodiments 14-20, wherein the apparent binding affinity (K_i) is measured in a competitive ELISA as described in Assay I.
37. The pharmaceutical composition according to any of the previous embodiments, wherein the PCSK9 inhibitor has $T_{1/2}$ of at least 24 hours in mini pigs

38. The composition according to any of the previous embodiments, wherein the PCSK9 inhibitor has T $\frac{1}{2}$ of at least 2 hours in rats.
39. The composition according to any of the previous embodiments, wherein the PCSK9 inhibitor has a molar mass of at most 50 000 g/mol.
40. The composition according to any of the previous embodiments, wherein the PCSK9 inhibitor is an EGF(A) peptide or an EGF(A) derivative.
41. The composition according to any of the previous embodiments, wherein the EGF(A) derivative according to embodiment 42 comprises an albumin binding substituent.
42. The composition according to any of the previous embodiments, wherein the EGF(A) derivative according to embodiment 42 or 43 comprises a fatty acid or a fatty diacid.
43. The composition according to any of the previous embodiments, wherein the EGF(A) derivative according to embodiment 42, 43 or 44 comprises a C16, C18 or C20 fatty acid or a C16, C18 or C20 fatty diacid.
44. The composition according to any of the previous embodiments 42-45, wherein the EGF(A) peptide or EGF(A) derivative comprises an EGF(A) peptide analogue having 1-8 amino acid substitutions compared to the EGF(A) domain of LDL-R defined by SEQ ID NO 1.
45. The composition according to embodiment 46, wherein the EGF(A) peptide analogue comprises 301Leu.
46. The pharmaceutical composition according to any of the previous embodiments, wherein the PCSK9 inhibitor is selected from the group consisting of: EGF(A) derivatives s# 31, 95, 128, 133, 143, 144, 150, 151, 152 and 153 with the following structures:

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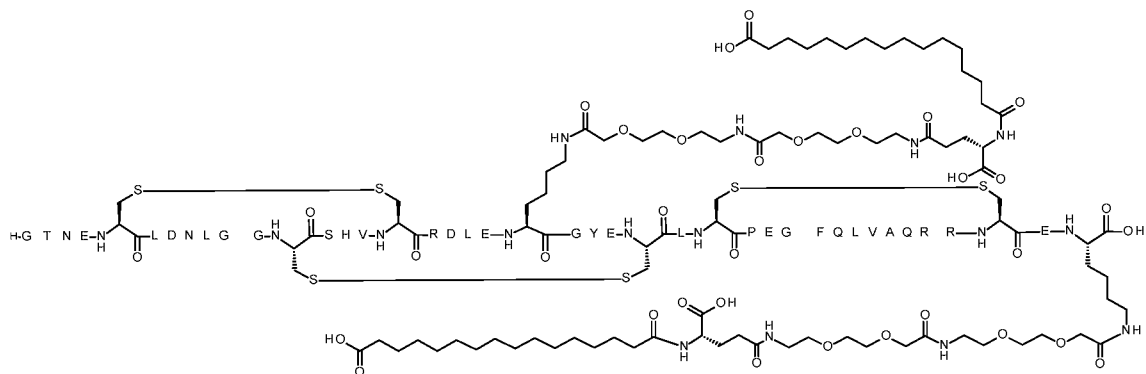
The chemical structure shows a protein complex with a central protein backbone and several attached groups. The protein backbone is represented by a series of amino acid residues: H-G-T-N-E-N-H, L-D-N-L-G, G-H-S-H-V-H, R-D-L-E-N-H, G-Y-E-N-H, L-P-D-G, F-Q-L-V-A-Q-R, R-N-H, and E-N-H. The residues are connected by peptide bonds. There are four disulfide bonds (S-S) connecting the protein backbone to the lipid and linker groups. The lipid groups are long-chain fatty acids (HO-C(=O)-(CH2)14-C(=O)-) and the linker groups are poly(ethylene glycol) (PEG) chains. The structure is shown in a 3D representation with various bonds and angles indicated.

The chemical structure shows a protein chain with two disulfide bonds (S-S) connecting different parts of the sequence. The sequence is: H-G T N E-NH-C(=O)-L D N L G -G-NH-C(=O)-S H V-NH-C(=O)-R D L E-NH-C(=O)-G Y E-NH-C(=O)-L-NH-C(=O)-P E G F Q L V A Q R R-NH-C(=O)-E-NH-C(=O)-OH. The structure includes several modifications: a long alkyl chain attached to the first cysteine (S), a long alkyl chain attached to the second cysteine (S), a long alkyl chain attached to the third cysteine (S), a long alkyl chain attached to the fourth cysteine (S), a long alkyl chain attached to the fifth cysteine (S), a long alkyl chain attached to the sixth cysteine (S), a long alkyl chain attached to the seventh cysteine (S), a long alkyl chain attached to the eighth cysteine (S), a long alkyl chain attached to the ninth cysteine (S), a long alkyl chain attached to the tenth cysteine (S), a long alkyl chain attached to the eleventh cysteine (S), a long alkyl chain attached to the twelfth cysteine (S), a long alkyl chain attached to the thirteenth cysteine (S), a long alkyl chain attached to the fourteenth cysteine (S), a long alkyl chain attached to the fifteenth cysteine (S), a long alkyl chain attached to the sixteenth cysteine (S), a long alkyl chain attached to the seventeenth cysteine (S), a long alkyl chain attached to the eighteenth cysteine (S), a long alkyl chain attached to the nineteenth cysteine (S), a long alkyl chain attached to the twentieth cysteine (S), a long alkyl chain attached to the twenty-first cysteine (S), a long alkyl chain attached to the twenty-second cysteine (S), a long alkyl chain attached to the twenty-third cysteine (S), a long alkyl chain attached to the twenty-fourth cysteine (S), a long alkyl chain attached to the twenty-fifth cysteine (S), a long alkyl chain attached to the twenty-sixth cysteine (S), a long alkyl chain attached to the twenty-seventh cysteine (S), a long alkyl chain attached to the twenty-eighth cysteine (S), a long alkyl chain attached to the twenty-ninth cysteine (S), a long alkyl chain attached to the thirtieth cysteine (S), a long alkyl chain attached to the thirty-first cysteine (S), a long alkyl chain attached to the thirty-second cysteine (S), a long alkyl chain attached to the thirty-third cysteine (S), a long alkyl chain attached to the thirty-fourth cysteine (S), a long alkyl chain attached to the thirty-fifth cysteine (S), a long alkyl chain attached to the thirty-sixth cysteine (S), a long alkyl chain attached to the thirty-seventh cysteine (S), a long alkyl chain attached to the thirty-eighth cysteine (S), a long alkyl chain attached to the thirty-ninth cysteine (S), a long alkyl chain attached to the fortieth cysteine (S), a long alkyl chain attached to the forty-first cysteine (S), a long alkyl chain attached to the forty-second cysteine (S), a long alkyl chain attached to the forty-third cysteine (S), a long alkyl chain attached to the forty-fourth cysteine (S), a long alkyl chain attached to the forty-fifth cysteine (S), a long alkyl chain attached to the forty-sixth cysteine (S), a long alkyl chain attached to the forty-seventh cysteine (S), a long alkyl chain attached to the forty-eighth cysteine (S), a long alkyl chain attached to the forty-ninth cysteine (S), a long 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the ninety-eighth cysteine (S), a long alkyl chain attached to the ninety-ninth cysteine (S), a long alkyl chain attached to the hundredth cysteine (S).

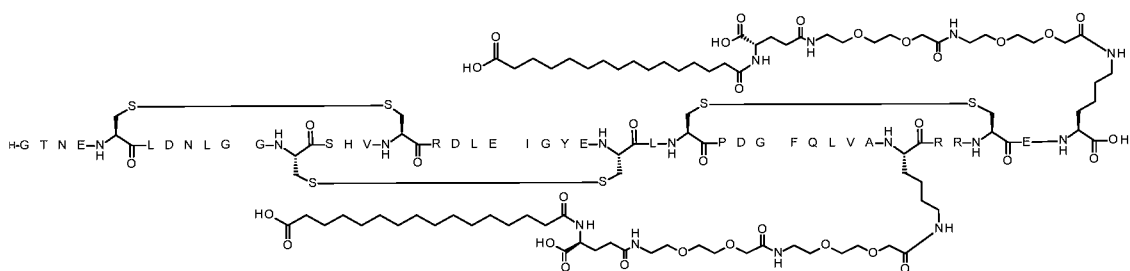
The chemical structure of the 120 kDa protein is a large, complex polypeptide chain. It features a long, continuous backbone with several disulfide bonds (S-S) connecting different parts of the chain. The sequence of amino acids is represented by single-letter codes: H-G-T-N-E-N-H, L-D-N-L-G, G-N-H, S-H-V-N-H, R-D-L-E-N-H, G-Y-E-N-H, L-N-H, P-E-G, F-Q-L-V-A-Q-R, R-N-H, E-N-H, and C-O-H. The chain is heavily modified with various side chains, including a long, branched chain with a carboxylic acid group (HO-C(=O)-) and a long, branched chain with a carboxylic acid group (HO-C(=O)-). The structure is highly complex and represents a large, multi-domain protein.

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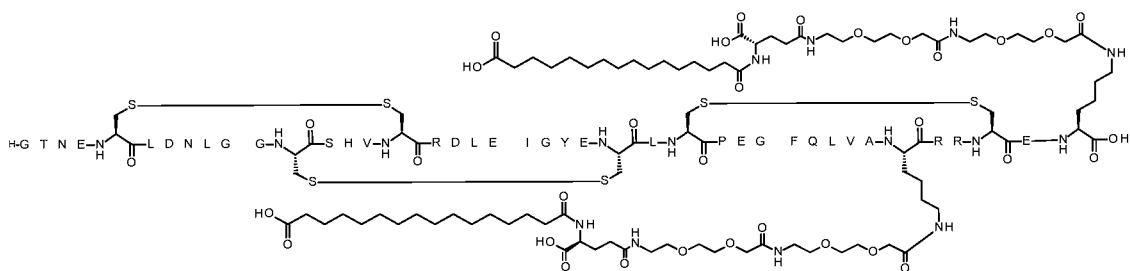
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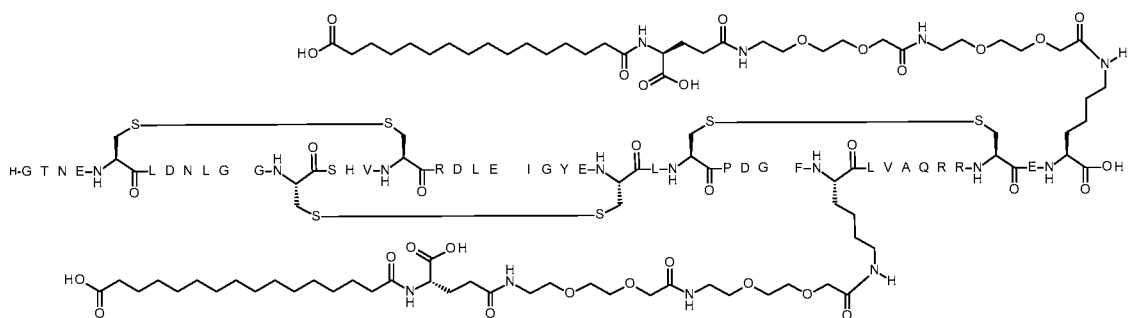
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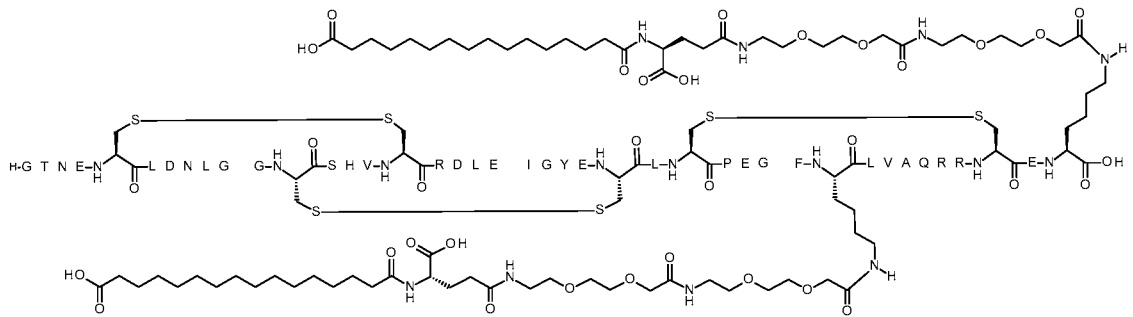
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and

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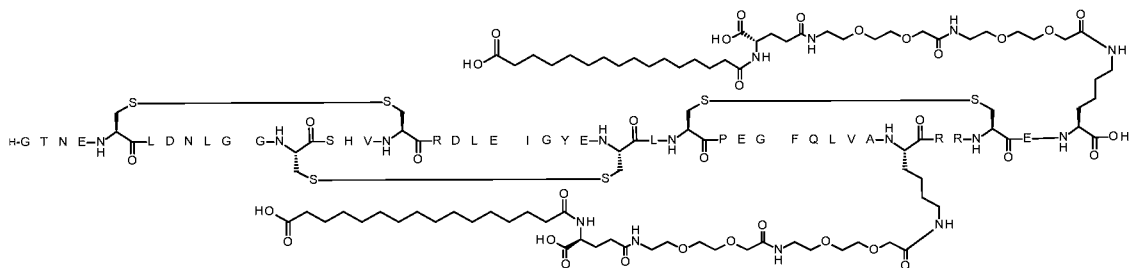
153



47. The pharmaceutical composition according to any of the previous embodiments, wherein the PCSK9 inhibitor is selected from the group consisting of EGF(A) derivative shown as Examples 150, 151, 152 and 153 in WO2017/121850.

5

48. The pharmaceutical composition according to any of the previous embodiments, wherein the PCSK9 inhibitor is:



10

49. The pharmaceutical composition according to any of the previous embodiments, wherein the composition comprises at least one granulate.

50. The pharmaceutical composition according to previous embodiment 49, wherein the at least one granulate comprises the salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid.

15

51. The pharmaceutical composition according to any of the previous embodiments 49-50, wherein the at least one granulate further comprises the hydrotrope, such as nicotinamide or resorcinol.

20

52. The pharmaceutical composition according to any of the previous embodiments 49-51, wherein the at least one granulate further comprises a lubricant, such as magnesium stearate.
- 5 53. The pharmaceutical composition according to any of the previous embodiment 49-52, wherein the at least one granulate is prepared by dry granulation, such as by roller compaction.
- 10 54. The pharmaceutical composition according to any of the previous embodiment 49-52, wherein the at least one granulate is prepared by hot melt extrusion or spray granulation.
55. The pharmaceutical composition according to any of the previous embodiment 49-53, wherein the composition comprises an extra-granular part.
- 15 56. The pharmaceutical composition according to any of the previous embodiment 49-55, wherein the extra-granular part of the composition comprises a lubricant or glidant, such as magnesium stearate and/or the PCSK9 inhibitor.
- 20 57. A pharmaceutical composition comprising
- i) 0.1-100 mg of a PCSK9 inhibitor,
 - ii) 20-800 mg, such as 25-700, such as 50-600 mg of a salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid and
 - iii) 10-600 mg nicotinamide.
- 25 58. A pharmaceutical composition comprising
- i) 1-100 mg, such as 1-50 mg of a PCSK9 inhibitor,
 - ii) 50-800 mg of a salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid and
 - iii) 10-600 mg nicotinamide.
- 30 59. A pharmaceutical composition comprising
- i) 1-100 mg, such as 1-50 mg of a PCSK9 inhibitor,
 - ii) 75-600 mg of a salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid and
 - iii) 25-400 mg nicotinamide.
- 35 60. A pharmaceutical composition comprising

- i) 1-100 mg, such as 1-25 mg of a PCSK9 inhibitor,
 - ii) 75-400 mg of a salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid and
 - iii) 25-400 mg nicotinamide.
- 5 61. A pharmaceutical composition comprising
 - i) 1-100 mg, such as 1-25 mg of a PCSK9 inhibitor,
 - ii) 100-400 mg of a salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid and
 - iii) 25-400 mg nicotinamide.
- 10 62. A pharmaceutical composition comprising
 - i) 1-100 mg, such as 1-25 mg of a PCSK9 inhibitor,
 - ii) 200-600 mg of a salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid and
 - iii) 25-400 mg nicotinamide.
- 15 63. A pharmaceutical composition comprising
 - i) 5-100 mg, such as 5-25 mg of a PCSK9 inhibitor,
 - ii) 250-500 mg of a salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid and
 - iii) 25-400 mg nicotinamide.
- 20 64. The pharmaceutical composition according to any of the embodiments 57-63, further comprising 0.2-25 mg lubricant, such as magnesium stearate.
- 65. The pharmaceutical composition according to any of the embodiments 57-63, further comprising 15-10 mg, such as 0.20-10 mg, such as 0.2-5 mg or such as 0.2-3 mg
- 25 magnesium stearate per 100 mg salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid.
- 66. The pharmaceutical composition according to any of the embodiments 57-63, wherein the salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid is sodium N-(8-(2-hydroxybenzoyl)amino)caprylate (SNAC).
- 30 67. The pharmaceutical composition according to any of the previous embodiment, wherein the composition is for oral administration.
- 68. The pharmaceutical composition according to any of the previous embodiments, wherein
- 35 the composition is a solid composition.

69. The pharmaceutical composition according to the previous embodiments, wherein the composition is a solid composition, such as a tablet, a capsule or a sachet.

5 70. A pharmaceutical composition comprising

- i) a PCSK9 inhibitor,
- ii) a salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid and
- iii) a hydrotrope, capable of increasing the solubility of SNAC at least 2-fold, such as 5-fold or such as at least 10-fold

10 wherein the release of the PCSK9 inhibitor reaches 85 % within 15 minutes or 95 % within 30 minutes.

71. A pharmaceutical composition comprising

- i) a PCSK9 inhibitor,
- 15 ii) a salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid and
- iii) a hydrotrope, capable of increasing the solubility of SNAC at least 2-fold, such as 5-fold or such as at least 10-fold

wherein the dose corrected plasma exposure at t=30 min after dosing is increased relative to a test composition 1.

20

72. A pharmaceutical composition comprising

- i) a PCSK9 inhibitor,
- ii) a salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid and
- 25 iii) a hydrotrope, capable of increasing the solubility of SNAC at least 2-fold, such as 5-fold or such as at least 10-fold

wherein the dose corrected plasma exposure (AUC) for t=0-30 min after dosing is increased relative to test composition 1.

73. The pharmaceutical composition according to any of the previous embodiments 1-73,

30 wherein

- a) the release of the PCSK9 inhibitor reaches 85 % within 15 minutes
- b) the release of the PCSK9 inhibitor reaches 95 % within 30 minutes
- c) the dose corrected plasma exposure at t=30 min after dosing is increased relative to test composition 1 herein and/or

- d) the dose corrected plasma exposure (AUC) for t=0-30 min after dosing is increased relative to test composition 1.
74. The pharmaceutical composition according to embodiment 71 or embodiment 73,
5 wherein the dose corrected plasma exposure (AUC) for T=0-30 min is increased at least 1.2 fold, such as 1.5 fold, such as at least 2 fold.
75. The pharmaceutical composition according to any of the embodiment 71-74, wherein the
10 release is determined as in Assay III herein and/or the dose corrected plasma exposures is determined as in Assay V.
76. The pharmaceutical composition according to any of the embodiments 57-66 further defined by the features of one or more of the embodiments 15-56.
- 15 77. The pharmaceutical composition according to any of the embodiments 70-76 further defined by the features of one or more of the embodiments 1-69.
78. A pharmaceutical composition according to any of the previous embodiments for use in
20 medicine.
79. A pharmaceutical composition according to any of the previous embodiments for use in a method of (i) improving lipid parameters and/or (ii) preventing and/or treating cardiovascular diseases.
- 25 80. A method of treatment of a subject in need thereof, wherein the method comprises administering a therapeutically active amount of a composition according to any of the previous embodiments to said subject.
81. A method for producing a solid pharmaceutical composition comprising a PCSK9 inhibitor
30 comprising the steps of;
a) obtaining a salt of NAC and a hydrotrope,
b) co-processing said salt of NAC and hydrotrope of a) and
c) preparing said solid pharmaceutical composition using the product of b) and a PCSK9 inhibitor.
- 35

82. The method according to embodiment 81, comprising the steps of;

- a) obtaining a blend comprising a salt of NAC and a hydrotrope,
- b) co-processing the blend of a) and
- c) preparing said solid pharmaceutical composition using the product of b) and a PCSK9 inhibitor.

83. The method according to embodiment 81, comprising the steps of;

- a) obtaining a blend comprising a salt of NAC and a hydrotrope,
- b) hot melt extruding the blend of a) and
- c) preparing said solid pharmaceutical composition using the extrudate of b) and a PCSK9 inhibitor.

84. The method according to embodiment 81, comprising the steps of;

- a) obtaining a salt of NAC and a hydrotrope,
- b) spray granulation said salt of NAC and hydrotrope of a) and
- c) preparing said solid pharmaceutical composition using the product of b) and a PCSK9 inhibitor.

85. The method according to embodiment 81, comprising the steps of;

- a) obtaining a solution of a salt of NAC and a hydrotrope,
- b) spray granulation said solution of a) and
- c) preparing said solid pharmaceutical composition using the product of b) and a PCSK9 inhibitor.

METHODS AND EXAMPLES

General Methods of Detection and Characterisation

Assay I: PCSK9-LDL-R binding - Competitive (ELISA)

This assay measures the apparent binding affinity to PCSK9 in competition with LDL-R. In particular the assay is used to evaluate the apparent binding affinity of an PCSK9 inhibitor such as an EGF(A) analogue and compounds comprising an EGF(A) analogue

The assay is performed as follows. The day before the experiment, recombinant human Low Density Lipoprotein Receptor (rhLDL-R; NSO-derived; R & D systems # 2148-LD) is

dissolved at 1 µg/ml in 50 mM sodium carbonate, pH 9.6, and then 100 µl of the solution is

added to each well of the assay plates (Maxisorp 96, NUNC # 439454) and coated overnight at 4 °C. On the day of the experiments, 8 point concentration curves of the EGF(A) compounds containing Biotinylated PCSK9 (0.5 ug/ml, BioSite/BPSBioscience cat#71304) are made in duplicate. Test compound and biotinylated PCSK9 mixtures are prepared and incubated for 1 hour at room temperature in assay buffer containing 25 mM Hepes, pH 7.2 (15630-056, 100 ml, 1M), 150 mM NaCl (Emsure 1.06404.1000) 1 % HSA (Sigma A1887-25G) 0.05 % Tween 20 (Calbiochem 655205) 2 mM CaCl₂ (Sigma 223506-500G). The coated assay plates are then washed 4x in 200 µl assay buffer, and then 100 µl of the mixture of test compounds and biotinylated PCSK9 is added to the plates and incubated 2 h at room temperature. The plates are washed 4x in 200 µl assay buffer and then incubated with Streptavidin-HRP (25ng/ml; VWR # 14-30-00) for 1 h at room temperature. The reaction is detected by adding 50 µl TMB-on (KEM-EN-TEC) and incubated 10 min in the dark. Then the reaction is stopped by adding 50 µl 4 M H₃PO₄ to the mixture, added by electronic multi pipetting. The plates are then read in a Spectramax at 450 and 620 nm within 1 h. The 620 nm read is used for background subtraction. IC₅₀ values are calculated using Graphpad Prism, by nonlinear regression log(inhibitor) vs. response-variable slope (four parameters), and converted into Ki values using the following formula: $K_i = IC_{50} / (1 + (Biotin-PCSK9) / (K_d(Biotin-PCSK9)))$, where K_d of the biotin-PCSK9 is 1.096727714 µg/ml and [Biotin-PCSK9] = 0.5 µg/ml.

20

Higher Ki values reflects lower apparent binding affinities to PCSK9 and vice versa. A value above 500 nM, will indicate that the observed binding is not specific.

Ki values for examples of EGF(A) peptide and derivatives thereof are included below, showing that the high affinity of compounds having an EGF(A) peptide including 301L and optionally one or more of 309R, 312E and 321E is very similar also including compounds with one or two substituents attached to the N-terminal or a Lysine residue.

25

EGF(A) peptide	Ki (nM)
EGF(A) LDL-R (293-332)	-
299A, 301L, 307I, 309R, 310K	9.4
299A, 301L, 307I, 309R	0.9
301L, 309R, 310K	7.3

301L, 309R	1.2
301L	2.8
301L, 309R, 312E	1.1

EGF(A) peptide derivatives

Example compound #	EGF(A) peptide	SEQ ID NO	Substituent	Attachment site(s)	Ki (nM)
3	301L, 309R, 312E, 333K	4	HOOC-(CH ₂) ₁₆ -CO-gGlu-2xADO	333K	0.8
8	301L, 309R, 312E	6	HOS(O) ₂ -(CH ₂) ₁₅ -CO-gGlu-2xADO-NH-CH ₂ -(C ₆ H ₄)-CH ₂ -	N-term	1.2
31	301L, 309R, 312E, 313K, 333K	32	4-HOOC-(C ₆ H ₄)-O-(CH ₂) ₁₀ -CO-gGlu-2xADO	313K, 333K	0.5
95	des293, 301L, 309R, 312E, 313K	76	HOOC-(CH ₂) ₁₆ -CO-gGlu-2xADO	313K	1.5
128	301L, 309R, 312E, 313K, 333K	32	HOOC-(CH ₂) ₁₄ -CO-gGlu-2xADO	313K, 333K	1.0
133	301L, 309R, 312E, 313K, 321E, 333K	98	4-HOOC-(C ₆ H ₄)-O-(CH ₂) ₁₀ -CO-gGlu-2xADO	313K, 333K	1.6
143	301L, 309R, 312E, 313K, 321E, 333K	98	4-HOOC-(C ₆ H ₄)-O-(CH ₂) ₁₀ -CO-gGlu	313K, 333K	2.0
144	301L, 309R, 312E, 313K, 321E, 333K	98	HOOC-(CH ₂) ₁₄ -CO-gGlu-2xADO	313K, 333K	2.09
150	301L, 309R, 312E, 328K, 333K	78	HOOC-(CH ₂) ₁₄ -CO-gGlu-2xADO	328K, 333K	2.3
151	301L, 309R, 312E, 321E, 328K, 333K	104	HOOC-(CH ₂) ₁₄ -CO-gGlu-2xADO	328K, 333K	1.8
152	301L, 309R, 312E, 324K, 333K	72	HOOC-(CH ₂) ₁₄ -CO-gGlu-2xADO	324K, 333K	1.9
153	312E, 321E, 324K, 333K	105	HOOC-(CH ₂) ₁₄ -CO-gGlu-2xADO	324K, 333K	2.0

Assay II: Disintegration test

- 5 A standard disintegration test according to the European Pharmacopeia (Ph Eur 2.9.1) may be performed in an appropriate disintegration apparatus e.g. USP disintegration apparatus to measure the disintegration time of the test compositions *in vitro*.

Assay III: Dissolution test

- 10 A standard dissolution test according to the European Pharmacopeia (Ph Eur 2.9.3) may be performed to measure the release of the PCSK9 inhibitor and SNAC from the test compositions *in vitro*.

A dissolution test is performed in an appropriate dissolution apparatus e.g. USP dissolution apparatus 2. More specifically, an apparatus 2 is used in accordance with United States Pharmacopoeia 35 using a paddle rotation speed of 50 rpm. For testing at pH 6.8, the 500 mL dissolution medium of 0.05 M phosphate buffer is used at a temperature of 37 ± 0.5 °C. Dissolution media has a content of 0.1 % Brij®35. Samples are removed at appropriate intervals and sample content is determined using a RP-UHPLC method for dual detection of PCSK9 inhibitor and SNAC.

The sample content is calculated based on the peak area of the PCSK9 inhibitor and SNAC in the chromatogram relative to the peak areas of the PCSK9 inhibitor and SNAC references, respectively. The released amount of PCSK9 inhibitor and SNAC is calculated as percentages of the actual total content in the test compositions. The total content in the tablets is determined using Assay (IV).

Assay IV: Analysis of amount of PCSK9 inhibitor and SNAC

For assay analysis the test compositions are weighed before extraction of the PCSK9 inhibitor and SNAC. Tablets are dissolved in a relevant amount of 0.05 M phosphate buffer, pH 7.4, with 20 % acetonitrile. Extraction time of two hours is used. Samples are centrifuged, and a suitable volume is transferred to a HPLC vial. Standards of relevant PCSK9 inhibitor and SNAC are prepared by using the same diluent as for the samples. UHPLC with an UV-detector is used for dual determination of the PCSK9 inhibitor and SNAC content. The tablet content is calculated based on the peak area of the PCSK9 inhibitor and SNAC in the chromatogram relative to the peak areas of the PCSK9 inhibitor and SNAC and references, respectively.

Assay V: Pharmacokinetic studies in Beagle dogs

Pharmacokinetic (PK) studies in Beagle dogs are conducted to determine the exposure of the PCSK9 inhibitor after peroral administration of different test compositions.

For the pharmacokinetic studies male Beagle dogs are used, 1 to 5 years of age and weighing approximately 10-12 kg at the start of the studies. The dogs are group housed in pens (12 hours light: 12 hours dark) and fed individually and restrictedly once daily with Royal Canin Medium Adult dog (Royal Canin Products, China Branch, or Brogaarden A/S, Denmark). Exercise and group socialising are permitted daily, whenever possible. The dogs are used for repeated pharmacokinetic studies with a suitable wash-out period between successive dosing. An appropriate acclimatisation period is given prior to initiation of the first

pharmacokinetic study. All handling, dosing and blood sampling of the animals are performed by trained and skilled staff. Before the studies the dogs are fasted overnight and from 0 to 4 h after dosing. Besides, the dogs are restricted to water 1 hour before dosing until 4 hours after dosing, but otherwise have ad libitum access to water during the whole period.

5

The tablets containing the PCSK9 inhibitor are administered in the following manner: 10 min prior to tablet administration the dogs are dosed subcutaneously with approximately 3 nmol/kg of SEQ ID NO: 115). The PCSK9 inhibitor tablets are placed in the back of the mouth of the dog to prevent chewing. The mouth is then closed and tap water is given by a syringe or gavage to facilitate swallowing of the tablet.

10

Blood sampling

Blood is sampled at predefined time points for up till 10 hr post dosing to adequately cover the full plasma concentration-time absorption profile of the PCSK9 inhibitor.

15

For each blood sampling time point approximately 0.8 mL of whole blood is collected in a 1.5 mL EDTA coated tube, and the tube is gently turned to allow mixing of the sample with the EDTA. Blood samples (for example 0.8 mL) are collected in EDTA buffer (8 mM) and then centrifuged at 4 °C and 2000 G for 10 min. Plasma is pipetted into Micronic tubes on dry ice and kept at -20 °C until analysis.

20

Blood samples are taken as appropriate, for example from a venflon in the cephalic vein in the front leg for the first 2 hours and then with syringe from the jugular vein for the rest of the time points (the first few drops are allowed to drain from the venflon to avoid heparin saline from the venflon in the sample).

Assay VI: SNAC solubility in combination with selected hydrotropes

25

A series of 18 different hydrotropes were selected for testing. Hydrotropes are weighed off and dissolved in 5 mL ultrapure water (200 mg/mL) and pH was titrated to pH 6 by addition of 2M HCl. Subsequently SNAC (200 mg) is added to the samples and placed on magnetic stirrers (400 rpm). The pH is maintained at pH 6 throughout the experiment by addition of 2M HCl.

30

After 4 hours of incubation at room temperature the samples were filtered through 0.45 µm syringe filters and the concentration of SNAC in solution is determined using a RP-HPLC method for detection of SNAC. The sample content is calculated based on the peak area of the SNAC peak in the chromatogram relative to the peak area of the SNAC references.

Results obtained are presented in table 1, demonstrating that the majority of hydrotropes increase solubility of SNAC significantly.

Table 1 Hydrotropic effect of selected hydrotropes (200 mg/mL) on SNAC solubility at pH 6

Hydrotrope	Replicates (n)	SNAC concentration (mg/mL)	Fold increase in solubility
SNAC control (no hydrotrope)	1	0.59	1
Nipecotamide	1	1.55	2.6
Nicotinamide	5	8.63	14.6
p-hydroxybenzoic acid sodium	1	1.25	2.1
N,N dimethyl urea	1	2.34	4.0
N,N dimethyl benzamide	4	22.46	38
N,N diethyl nicotinamide	1	4.58	7.8
Sodium salicylate	1	1.59	2.7
Resorcinol	6	29.95	51
Sodium benzoate	2	1.25	2.1
Urea	1	0.77	1.3
Sodium Xylenesulfonate	1	2.01	3.4
Sodium p-toluenesulfonate	1	1.78	3.0
1-Methylnicotinamide	1	2.20	3.7
Pyrogallol	4	8.98	15
Pyrocatechol	1	23.95	40.6
Epigallocatechin gallate	1	5.66	9.6
Tannic acid	1	6.31	10.7
Gentisic acid sodium salt hydrate	1	2.72	4.6

Assay VII: SNAC solubility in varying concentrations of nicotinamide and resorcinol

Nicotinamide or resorcinol was weighed off and dissolved in 5 mL ultrapure water to the final concentrations shown in Figure 1A & B and pH was titrated to pH 6 by addition of 2M HCl. Subsequently SNAC (200 mg) was added to the samples placed on magnetic stirrers (400 rpm) and pH is maintained at pH 6 throughout the experiment by addition of 2M HCl. After 4 hours of incubation samples were filtered through 0.45 µm syringe filters and the concentration of SNAC in solution is determined using a RP-HPLC method for detection of SNAC. The sample content is calculated based on the peak area of the SNAC peak in the chromatogram relative to the peak area of the SNAC references. The results are shown in figure 1 demonstrating a concentration dependent effect on SNAC solubility of both hydrotropes.

General methods for tablet preparation**Method 1: Hot melt extrusion**

Hot melt extrusion is carried out on a Thermo Scientific Process 11 twin screw extruder. SNAC and nicotinamide or resorcinol are blended on a Turbula prior to feeding into the extruder. The equipment is operated at process temperatures varying between 200°C to 105°C along the barrel to facilitate the melt extrusion. The screw speed is varied between 50 – 1000 rpm and material is fed into the extruder using a gravimetric feeder at varying feed rates and extruded through a 2 mm diameter circular die. The resulting extrudates are manually sieved into granules using a final mesh screen between 355 and 150 µm.

Method 2: Tablet compression

Tablets are produced on a Kilian Style One simulating a Fette 102i or on a Fette 102i mounted with a single set of punches, resulting in 5.75 × 10 mm or 6.5 × 11 mm or 8.5 × 16 mm, or 7.5 × 13 mm oval tablets having no score. Punch size is chosen according to the total tablet weight. The press speed is set to 20 rpm. Compression forces around 7 to 21 kN are applied to obtain tablets with a crushing strength from 34 to 132 N respective to the tablet size.

Prior to tablet compression the granulates obtained by method 1 are blended with PCSK9 inhibitor and additional excipients on a Turbula mixer (7 min or 20 min, 25 rpm) and any additional optional excipients on a Turbula mixer (2 min, 25 rpm).

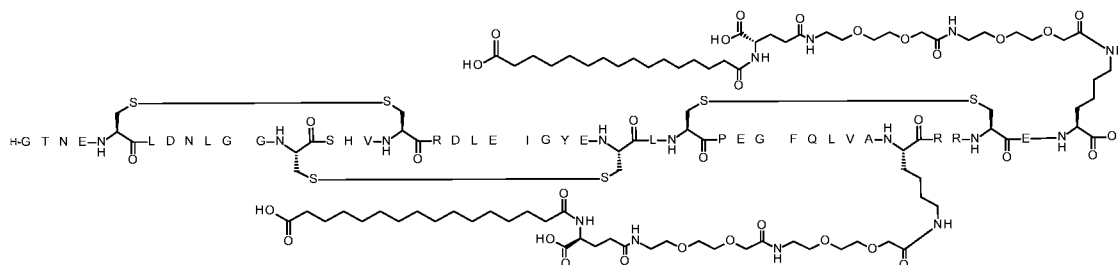
Method 3: Salt exchange

Batches of spray dried EGF(A) derivative material were dissolved in 100mM Tris buffer at neutral pH to a final concentration of 10-20 g/l. The material was subsequently loaded onto a C18 reversed-phase column up to 20 g EGF(A) per litre of resin and washed in the following order: a) with 1 column volume of a solution comprising of 5% w/w ethanol in water followed by b): 10 column volumes of a solution containing 20 mM sodium phosphate and 500 mM sodium chloride at pH 7.5 and c): 10 column volumes of a solution comprising of 5% w/w ethanol. EGF(A) was then eluted from the column by using a 50% w/w ethanol solution. Ethanol was subsequently evaporated by applying a vacuum. The solution was subsequently spray dried providing the EGF(A) derivative as a sodium salt.

EXAMPLES

Example 1 - Preparation of compositions

Test compositions were prepared according to Table 2 below, comprising a peptide
15 based PCSK9 inhibitor. The compound used is a peptide analogue of LDLR293-332
comprising two substituents in the form of fatty diacids attached via a hydrophilic linker
molecule. The EGF(A) derivative is prepared as described in WO 2017/121850 (Example
151/page 161) and has the following structure.



The test compositions were prepared by using a combination of the methods described herein above. Test composition 1 and 6 were produced by granulating a blend of SNAC, magnesium stearate and MCC as described in WO 2013/139694. The granulates were subsequently blended with povidone, the PCSK9 inhibitor and further MCC (optionally granulated (Test 6)) and extra-granular magnesium stearate prior to tablet compression (method 2). Test compositions 2-5 and 7 were produced by blending of SNAC and nicotinamide prior to hot melt extrusion (method 1). The obtained granulates were subsequently blended with PCSK9 inhibitor and additionally with magnesium stearate prior to

tablet compression (method 2). For test compositions 6 and 7 the EGF(A) derivative used in the preparation was obtained as described in method 3.

Table 2. Compositions of PCSK9 inhibitor tablets

	Composition	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6	Test 7
SNAC Granulate	SNAC (mg)	300	100	100	234*	273.4	300	300
	Magnesium stearate (mg)	7.7	-	-	-	-	7.7	-
	MCC (mg)	57	-	-	-	-	57	-
	Nicotinamide	-	66.7	66.7	207	167.6	-	200
Second granule and extra granular ingredients	Compound 151 (mg)	5	5	5	5	5	50	50
	Povidone (mg)	8	-	-	-	-	8	
	MCC (mg)	23	-	-	-	-	23	
	Magnesium stearate (mg)	2	0.8	1.75	2.4	2.4	2	2.5

*Based on actual content measurement (assay IV) while other values are target values used for weighing the material prior to tableting.

Example 2 – Disintegration testing

The objective of the present study was to evaluate the disintegration of the series of the test compositions described in Example 1.

Disintegration was measured according to Assay II using a Pharmatech PTZ auto disintegration tester in accordance with European Pharmacopoeia employing automatic detection. Test compositions 1– 5 were tested in *water R* and considered disintegrated when the automatic detection was deployed. The results are reported as average of 3 tablets. Table 3 shows the results for test compositions prepared according to Example 1 above.

Table 3. Disintegration times

Composition	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6
Disintegration time	15 min 18 s	2 min 51 s	2 min 50 s	4 min 20 s	3 min 32 s	11 min 46 s

The results obtained show that the test compositions 2-5 display a significantly faster disintegration than observed for test composition 1.

Example 3 – Dissolution testing

- 5 The objective of the present study was to evaluate the dissolution of the series of the test compositions described in Example 1.

Dissolution was measured according to Assay III and the amount of the PCSK9 inhibitor and SNAC were measured according to Assay IV. The released amount of PCSK9 inhibitor and
 10 SNAC were calculated as percentages of the actual content in the test compositions i.e. 100 or approximately 300 mg/tablet of SNAC and 5 mg/tablet or 50mg/tablet of PCSK9 inhibitor.

The released amount of PCSK9 inhibitor is reported as average of 3 tablets.

- 15 Table 4 shows the results for test compositions prepared according to Example 1 above, wherein the release is presented as “PCSK9 inhibitor in solution (%)” describing the amount of PCSK9 inhibitor in solution after 15, 30 and 60 min relative to the total amount of PCSK9 inhibitor in the tablet at the start of the experiment. The total content of PCSK9 inhibitor and SNAC in the tablets were determined according to Assay IV.

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Table 4. PCSK9 inhibitor in solution (%)

Composition	PCSK9 inhibitor in solution (%)		
	15 min	30 min	60 min
Test 1	40.2	66.8	92.2
Test 3	Full release	Full release	Full release
Test 5	99.5	Full release	Full release
Test 6	47.9	76.3	95.0
Test 7	Full release	Full release	Full release

The results obtained show that the test compositions 3 and 5 display a faster release of the PCSK9 inhibitor compared to what was observed for test composition 1. The same can be concluded when comparing test composition 7 to test composition 6. A significantly faster release of the PCSK9 inhibitor was observed for the early time points, i.e. at 15 and 30 minutes. The difference in release was less significant after 60 minutes. The amount of SNAC in the test compositions did not influence the release of the PCSK9 inhibitor after 15 min, i.e. test compositions comprising 100 mg SNAC dissolve as fast as test compositions comprising approximately 300 mg SNAC when measured after 15 min or later.

Further data obtained after 5, 10, 15, 20, 30, 45 and 60 min for test compositions 1 to 3 and 6 and 7 are shown in Figure 2, demonstrating that test compositions 3, 5 and 7 are superior to test composition 1 and 6 at every time point.

Example 4 – Oral exposure

The pharmacokinetics of oral administration of the test composition described in Example 1 above were evaluated according to Assay V to evaluate the oral exposure in beagle dogs using 10 ml water for dosing to the dogs. The number of tests performed for each formulation is indicated by n.

Analysis and results

The plasma concentration of the PCSK9i molecule was analysed by LCMS. Individual plasma concentration-time profiles were analysed by a non-compartmental model in WinNonlin v. 5.0 or Phoenix v. 6.2 or 6.3 (Pharsight Inc., Mountain View, CA, USA), or other relevant software for PK analysis. The compound exposure measured at t=30 min was determined and normalized by dose /kg bodyweight.

The area under the plasma concentration versus time curve for the first 30 min (AUC, [time x concentration]) was calculated (by the Pharsight programme) after oral administration and normalized by ((dose/kg bodyweight)*100) to obtain the dose corrected exposure.

Dose corrected exposure of PCSK9i obtained after administration of test composition 1 and 4 was calculated. Data included in table 5 below.

Table 5 – Average exposure measured in dogs after single administration of the test compositions 1, 2 and 7.

Composition	Dose corrected exposure t=30 min (pmol/L)	Dose corrected AUC 0-15 min (arbitrary unit)	Dose corrected AUC 0-30 min (arbitrary unit)	Number of dogs
Test 1	0.18	0.39	3.34	8/8
Test 2	0.33	1.30	7.89	7/8
Test 7	0.39	1.19	8.31	16/16

An accelerated and increased exposure was observed for compositions according to the invention compared to the test 1 composition.

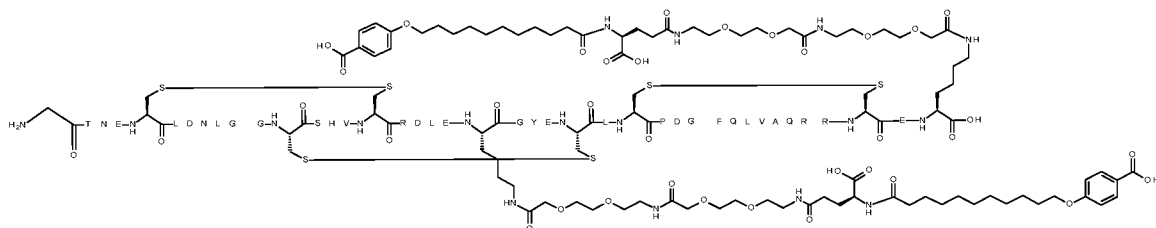
- 5 While certain features of the invention have been illustrated and described herein, many modifications, substitutions, changes, and equivalents will now occur to those of ordinary skill in the art. It is, therefore, to be understood that the appended claims are intended to cover all such modifications and changes as fall within the true spirit of the invention.

CLAIMS

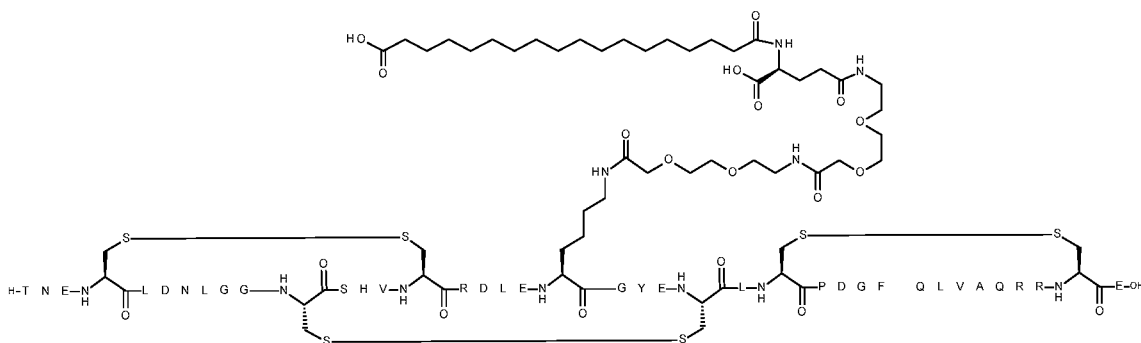
1. A pharmaceutical composition comprising;
 - a) 0.5-100 mg EGF(A) derivative,
 - 5 b) 20-1000 mg, such as 50-600 mg, of a salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid and
 - c) 10-600 mg of a hydrotrope capable of increasing the solubility of SNAC at least 2-fold, such as 5-fold or such as at least 10-fold.
- 10 2. The pharmaceutical composition according to claim 1 wherein the hydrotrope is Nicotinamide or Resorcinol.
3. The pharmaceutical composition according to any of the previous claims, wherein the ratio of salt of NAC/hydrotrope (w/w) is 0.5-10, such as 0.5-8 or such as 0.5-5.
- 15 4. The pharmaceutical composition according to any of the previous claims, wherein the composition further comprises a lubricant selected from magnesium stearate or glyceryl dibehenate.
- 20 5. The pharmaceutical composition according any of the previous claims consisting of;
 - a) an EGF(A) derivative
 - b) a salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid, such as the sodium salt of NAC (SNAC),
 - c) nicotinamide and
 - 25 d) at least one lubricant.
6. The pharmaceutical composition according to any of the above claims, wherein a unit dosage comprises;
 - i) 0.5-100 mg, such as 1.0-50 mg an EGF(A) derivative
 - 30 ii) 50-600 mg salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid (NAC), such as the sodium salt of NAC (SNAC)
 - iii) 20-400 mg nicotinamide and
 - iv) 0-15 mg lubricant.

7. The pharmaceutical composition according to any of the previous claims wherein the EGF(A) derivative is selected from the group of EGF(A) derivatives # 31, 95, 128, 133, 143, 144, 150, 151, 152 and 153 with the following structures:

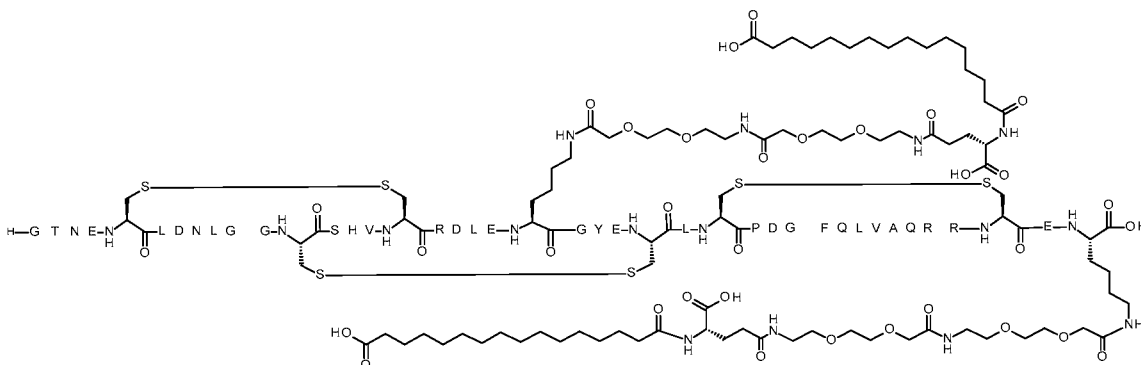
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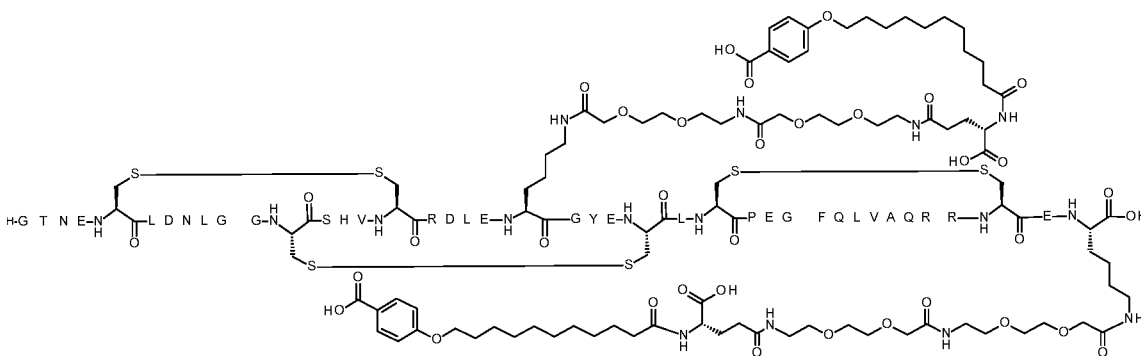
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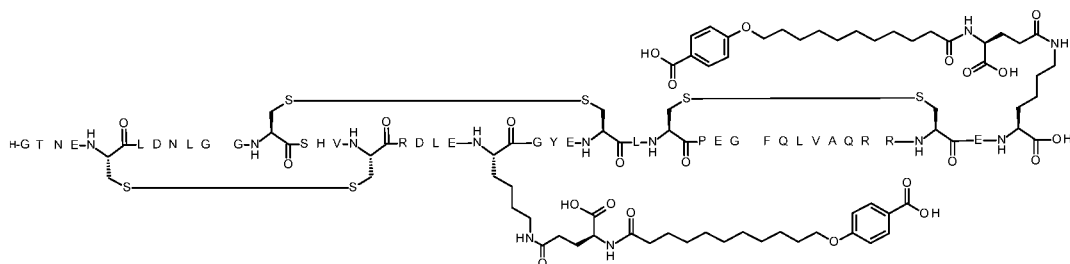


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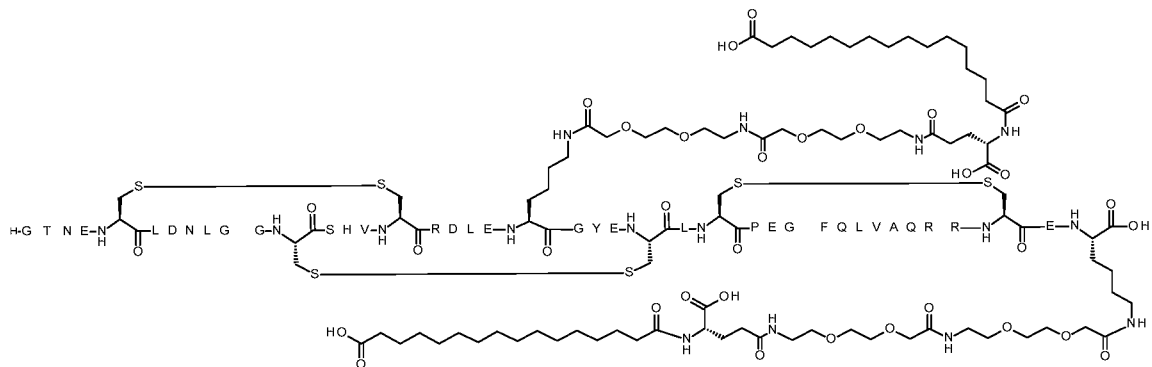


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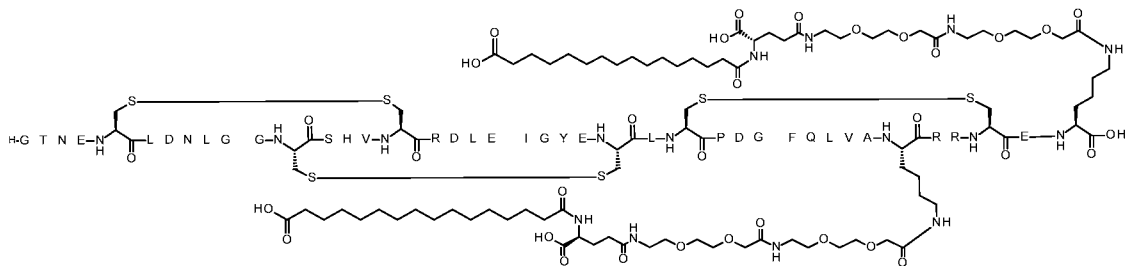
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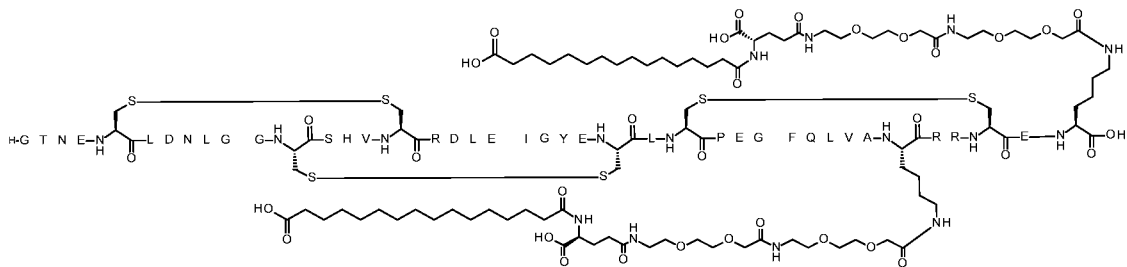
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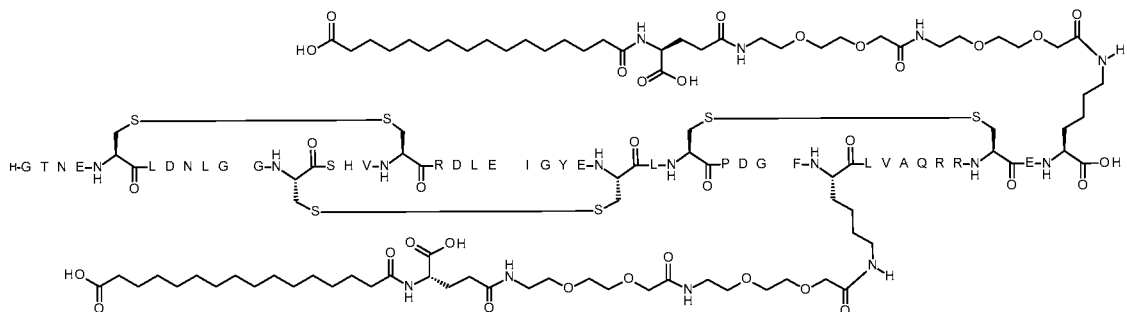
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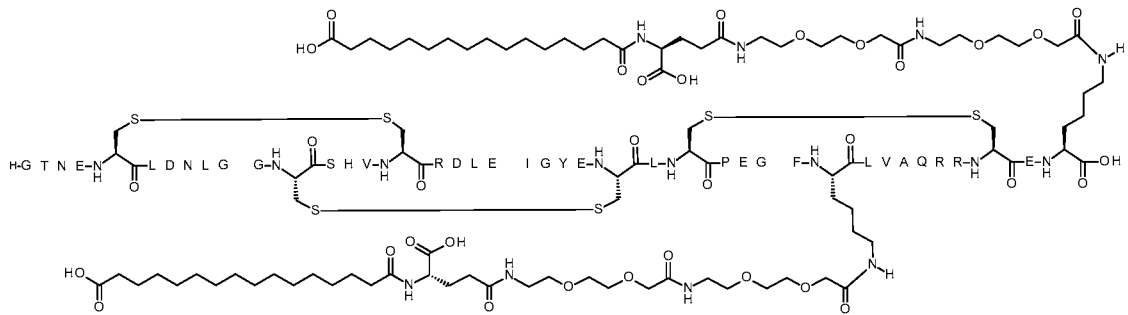
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and

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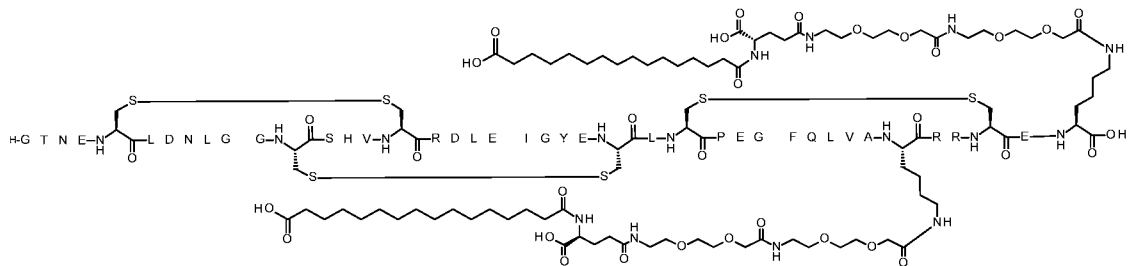
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8. The pharmaceutical composition according to any of the previous claims, wherein the salt of N-(8-(2-hydroxybenzoyl)amino)caprylic acid is sodium N-(8-(2-hydroxybenzoyl)amino)caprylate (SNAC).

5

9. The pharmaceutical composition according to any of the previous claims, wherein the EGF(A) derivative is



10. The pharmaceutical composition according to any of the previous claims, wherein a dose unit comprises;

- 1-100 mg, such as 2-75 mg, such as 3-50 mg or such as 1-25, mg of the EGF(A) derivative,
- 100-600 mg of SNAC,
- 20-400 mg hydrotrope and
- 0.1-30 mg lubricant, such as magnesium stearate.

15

11. The pharmaceutical composition according to any of the previous claims, wherein a dose unit comprises;

- 1-100 mg, such as 2-75 mg, such as 3-50 mg or such as 1-25, mg of the EGF(A) derivative,
- 100-600 mg of SNAC,
- 20-400 mg Nicotinamide and

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d) 0.1-30 mg magnesium stearate.

12. The pharmaceutical composition according to any of the previous claims, wherein the composition is a solid composition, such as a tablet, for oral administration.

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13. A pharmaceutical composition according to any of the previous claims for use in a method of improving lipid parameters and/or preventing and/or treating cardiovascular diseases.

10 14. A method of treatment of a subject in need thereof, wherein the method comprises administering a therapeutically active amount of a composition according to any of the previous claims to said subject.

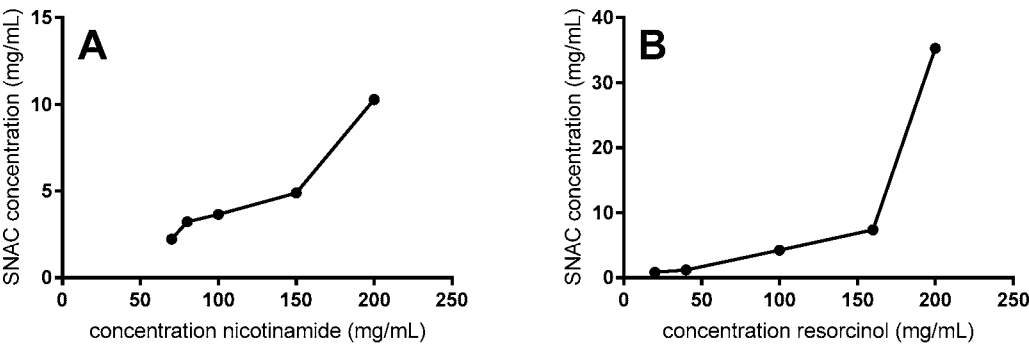
15. A method for producing a solid pharmaceutical composition comprising the steps of;

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- a) obtaining a salt of NAC and a hydrotrope,
- b) co-processing said salt of NAC and hydrotrope of a) and
- c) preparing said solid pharmaceutical composition using the product of b) and an EGF(A) derivative.

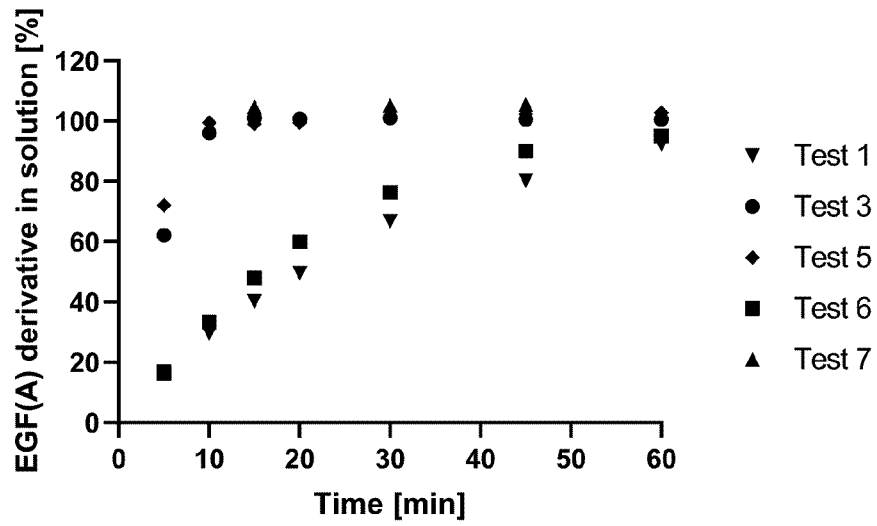
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Fig. 1



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Fig. 2



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2020/081244

A. CLASSIFICATION OF SUBJECT MATTER INV. A61K9/00 A61K9/20 A61K47/10 A61K47/12 A61K47/18 A61K31/00 A61P9/00 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 A61P Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, BIOSIS, EMBASE, WPI Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2017/121850 A1 (NOVO NORDISK AS [DK]) 20 July 2017 (2017-07-20) cited in the application pages 160-161 -----	1-15
☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "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 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 "&" document member of the same patent family		
Date of the actual completion of the international search 19 January 2021		Date of mailing of the international search report 28/01/2021
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 Konter, Jörg

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2020/081244

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