

CHROMANOL COMPOUNDS FOR TREATMENT OR PROPHYLAXIS OF AGEING AND AGEING-ASSOCIATED DISORDERS

Field of the Invention

5 [0001] The invention relates generally to chromanol compounds and derivatives thereof for treatment or prophylaxis of ageing, or ageing-associated disorders. In particular, the invention relates to chromanol compounds and derivatives thereof for treatment or prophylaxis of aging in general, and vascular ageing and/or reduced kidney function due to ageing in particular.

10 Description of the Related Art

[0002] The average human life expectancy in most developed countries has doubled in the last 200 years as a consequence of the improved quality of water, food, hygiene, housing and lifestyle, immunization against infectious disease, antibiotics and medical care conditions. However, longer human lives have led to a global burden of late-life disease. About half of
15 human deaths are attributed to ageing-associated disorders or diseases, most prominently cardiovascular disease (CVD), diabetes, chronic obstructive pulmonary disease (COPD), stroke, Alzheimer disease, chronic kidney diseases (CKDs) and cancer, among others (Chang, A., Skirbekk, V., Tyrovolas, S., Kassebaum, N. and Dieleman, J., 2019. Measuring population ageing: an analysis of the Global Burden of Disease Study 2017. *The Lancet Public Health*, 4(3), pp.e159-e167).
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[0003] One of the most predominant ageing-associated diseases affecting developed countries is cardiovascular disease (CVD). CVD is triggered by vascular alterations characterized by impaired vasodilation and the overproduction of inflammatory markers (Camici, G., Savarese, G., Akhmedov, A. and Lüscher, T., 2015. Molecular mechanism of
25 endothelial and vascular aging: implications for cardiovascular disease. *European Heart Journal*, 36(48), pp.3392-3403). Yet, this article also shows the complexity of aging, as many factors are involved.

[0004] It is thought that ageing cells and organisms accumulate increased levels of oxidant-damaged nuclear and mitochondrial DNA. The increasing damage to mitochondrial DNA is
30 thought to lead to compromised mitochondrial function and integrity. Consequently, damaged mitochondria trigger the release of more reactive oxygen species (ROS) setting in motion a vicious cycle of increasing DNA damage leading to increased ROS production that leads to

more DNA damage. Although the role for oxidants and accumulated DNA damage in the pathogenesis of many age-related diseases is widely accepted, the clinical use of antioxidant therapy has been at best equivocal.

- [0005] Studies in accelerated aging animal models show that chronic treatment with sGC
5 activator, PDE1 inhibitor or the PDE5 inhibitor, sildenafil, were not able to improve
endothelial dependent relaxation (Golshiri, K., Ataei Ataabadi, E., Rubio-Beltran, E., et al.,
2021. Selective Phosphodiesterase 1 Inhibition Ameliorates Vascular Function, Reduces
Inflammatory Response, and Lowers Blood Pressure in Aging Animals. *Journal of*
Pharmacology and Experimental Therapeutics, 378(2), pp.173-183.; Golshiri, K., Ataei
10 Ataabadi, E., Brandt, R., et al., 2020. Chronic Sildenafil Treatment Improves Vasomotor
Function in a Mouse Model of Accelerated Aging. *International Journal of Molecular*
Sciences, 21(13), p.4667).
- [0006] AT1 receptor blockade with losartan did not have a beneficial effect on the
vasomotor function of accelerated aging animal models (Wu, H., van Thiel, B., Bautista-
15 Niño, P., et al., 2017. Dietary restriction but not angiotensin II type 1 receptor blockade
improves DNA damage-related vasodilator dysfunction in rapidly aging *Ercc1Δ/-* mice.
Clinical Science, 131(15), pp.1941-1953).
- [0007] The anticancer drug rapamycin was not able to preserve vasodilator function in
accelerated aging animal models (Birkisdóttir, M., Jaarsma, D., Brandt, R., et al., 2021 *Aging*
20 *Cell*, 20(2)). Unlike dietary restriction, rapamycin fails to extend lifespan and reduce
transcription stress in progeroid DNA repair-deficient mice.
- [0008] To date, only dietary restriction has successfully improved vasomotor function in
accelerated aging animal models. However, dietary restriction as a human lifestyle
intervention has limited scope, given the degree of self-restraint required.
- 25 [0009] There remains a need for new compounds for the effective treatment or prophylaxis
of ageing or ageing-associated disorders.
- [0010] WO2006/105806A1 relates to the use of a complex mixture, comprising at least
combination of a statin, a compound suppressing angiotensin production or activity, an anti-
inflammatory agent and at least one antioxidant, for the prevention and/or treatment of ageing
30 process itself and the disorders caused by ageing.

[0011] WO2015/148522A1 relates to compositions comprising nicotinamide mononucleotide for the treatment of age-associated vascular dysfunction by improving endothelial function and reducing large elastic artery stiffness.

[0012] WO2012/116985A1 relates to pharmaceutical compositions comprising at least one renin-angiotensin-aldosterone system inhibitor in a subtherapeutic daily dose and at least one HMG-CoA reductase inhibitor in a subtherapeutic daily dose for use in the prevention, reduction or reversal of arterial aging in apparently healthy subjects.

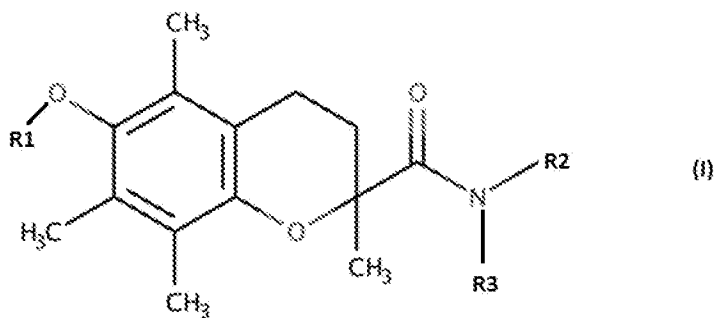
[0013] All these references describe actives with side effects which are unwanted, and/or complex mixtures.

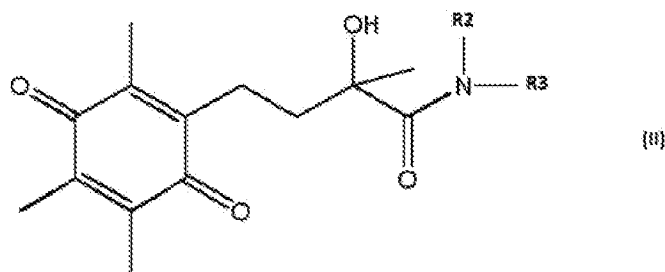
[0014] It is an object of the present invention to provide compounds for the treatment or prophylaxis of ageing, and/or ageing-associated disorders, and in particular to provide compounds for the treatment or prophylaxis of vascular aging and/or of reduced kidney function because of aging.

Brief summary of the invention

[0015] One or more of the above objects is met by providing certain chromanol, quinone or hydroquinone compounds for use in such treatment.

[0016] One or more of the above objects is more in particular met by the present invention by providing compounds according to formula (I), (II), the hydroquinone analogue of formula (II), or a pharmaceutically acceptable salt thereof, for use in the treatment or prophylaxis of aging or ageing-associated disorders;





- wherein R1 represents a hydrogen or prodrug moiety that can be removed in living tissue
- and wherein either

- o R2 and R3 together with the N atom to which they are attached form a saturated or unsaturated, non-aromatic, optionally substituted, 5-8 membered ring, having one to four N, O, or S atoms, wherein R2 and R3 together contain 3-12 carbon atoms;
- o or R2 is a hydrogen atom, or an alkyl group with 1-6 carbon atoms, and R3 is an alkyl group, optionally substituted with nitrogen or oxygen, wherein the alkyl group comprises 3-12 carbon atoms, and wherein the alkyl group in R3 comprises one or more non-aromatic cyclic structures that may comprise nitrogen or oxygen atoms in the ring, and may contain linear and/or branched substituted groups, and one or more ethylenic unsaturations.

[0017] For the present invention, the compound according to formula (II) includes the hydrogenated quinone (i.e. the hydroquinone) analogue, although the quinone derivative is preferred in view of stability.

[0018] In a preferred embodiment, the nitrogen can be amine, quaternary amine, guanidine or imine and oxygen is hydroxyl, carbonyl or carboxylic acid; and/or oxygen and nitrogen together may form amide, urea or carbamate groups.

[0019] In a preferred embodiment of either compounds according to formula (I) or according to formula (II), R2 and R3 together with the N atom to which they are attached form a saturated ring incorporating an additional N atom, which ring is unsubstituted or substituted with an alcohol, or alkanol group having 1-4 carbon atoms, such as ethylol.

[0020] In another preferred embodiment R2 is a hydrogen atom and R3 comprises a saturated cyclic structure having 4-7 carbon atoms and having one nitrogen atom, which ring may be substituted with an alkyl group, alcohol group, or with a group with 1-4 carbon atoms that may comprise an oxygen, carboxylic acid or amine group.

[0021] In another preferred embodiment the compound is a compound according to formula II and R2 is a hydrogen atom and R3 comprises a cyclic structure having 4-6 carbon atoms and having one nitrogen atom which ring is unsubstituted or substituted with an alcohol, or alkanol group having 1-4 carbon atoms, such as ethylol, and preferably is optionally substituted with methyl, ethyl, or alcohol substituted methyl or ethyl.

[0022] In another preferred embodiment, the compound is a compound according to formula I, R2 is a hydrogen atom and R3 comprises a saturated cyclic structure having 4-7 carbon atoms and having one nitrogen atom, which ring is unsubstituted or substituted with an alcohol, or alkanol group having 1-4 carbon atoms, such as ethylol, and preferably is optionally substituted with methyl, ethyl, or alcohol substituted methyl or ethyl.

[0023] According to yet another preferred embodiment, the compound is either (6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)(piperazin-1-yl)methanone (SUL-121), ((S)-6-hydroxy-2,5,7,8-tetramethyl-N-((R)-piperidin-3-yl)chroman-2-carboxamide hydrochloride (SUL-13), or (6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)(4-(2-hydroxyethyl)piperazin-1-yl)methanone (SUL-109) or a pharmaceutically acceptable salt thereof, as racemic mixture or as one of its enantiomers.

[0024] In a most preferred embodiment, the compound is the S-enantiomer of SUL-109, namely S-(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)(4-(2-hydroxyethyl)piperazin-1-yl)methanone (SUL-138).

Brief description of the drawings

[0025] The features and advantages of the invention will be appreciated upon reference to the following drawings, in which:

[0026] FIG. 1: Kaplan-Meier curves displaying the survival of *Drosophila* treated with varying concentrations of the SUL109: control (purple), 10uM (blue), 100uM (green) and 1000uM (grey). The continuous lines represent females and the dotted lines males.

[0027] FIG. 2: Kaplan-Meier curves displaying the survival of *Drosophila* treated with varying concentrations of the SUL121: control (purple), 10uM (blue), 100uM (green) and 1000uM (grey). The continuous lines represent females and the dotted lines males.

[0028] FIG. 3: Effect of chronic SUL138 treatment on ED relaxation in LM vs. EC-KO mice. Acetylcholine (Ach) dose response curve relative to U46619 pre-constriction. Average

is presented as mean $\pm$ SEM per group. *: significant effect of genotype ($p < 0,05$); #: significant effect of treatment ($p < 0,05$); $p = 0,054$ EC-KO control vs. EC-KO treated.

[0029] FIG. 4: Pathway contribution to ACh response in LM vehicle animals. Average is presented as mean $\pm$ SEM per group. *: significant effect of inhibitor ($p < 0,05$).

5 [0030] FIG. 5: Pathway contribution to ACh response in KO vehicle animals. Average is presented as mean $\pm$ SEM per group. *: significant effect of inhibitor ($p < 0,05$).

[0031] FIG. 6: Pathway contribution to ACh response in KO SUL138 treated animals. Average is presented as mean $\pm$ SEM per group. *: significant effect of inhibitor ($p < 0,05$).

[0032] FIG. 7: Albumin and creatinine levels in urine from 22 week old mice. Average is
10 presented as mean $\pm$ SEM per group. *: significant effect of genotype ($p < 0,05$), (*): $p = 0,0871$.

[0033] FIG. 8: Albumin/creatinine ratio in urine and plasma creatinine levels (22 week old mice). Average is presented as mean $\pm$ SEM per group. *: significant effect of genotype ($p < 0,05$).

15 [0034] FIG. 9: Creatine levels in blood plasma (22 week old mice). Average is presented as mean $\pm$ SEM per group. *: significant effect of genotype ($p < 0,05$).

[0035] FIG. 10: Creatinine levels in 24 h urine from 21 week old mice. Average is presented as mean $\pm$ SEM per group. *: significant effect of genotype ($p < 0,05$), (*): $p = 0,088$.

20 Detailed description of the invention

[0036] One or more of the objects of the present invention, to provide compounds for the treatment or prophylaxis of ageing, and/or ageing-associated disorders is met by providing compounds according to formula (I) or (II), as shown above, or a pharmaceutically acceptable salt thereof.

25 [0037] R1 can be a substituent that is easily removed in the human body, such that the compound is a prodrug. R1 can be for example an amino acid derivative or ester derivative, and generally has a molecular weight lower than 100 dalton.

[0038] In a preferred embodiment, R1 in formula (I) is hydrogen or forms together with the 6-oxygen an ester group with 2-6 carbon atoms. The ester can comprise one or more ether or
30 alcohol groups. Suitable esters are acetate, butyrate, 3-hydroxy butyrate and the like.

[0039] In a preferred embodiment of either compounds according to formula (I) or according to formula (II), R2 and R3 together with the N atom to which they are attached

form a saturated ring having 3-6 carbon atoms and incorporating one additional N atom, which may be substituted with 1-4 carbon atoms that may comprise an oxygen, carboxylic acid or amine group.

5 [0040] More preferably, R2 and R3 together with the N atom to which they are attached form a 5-7 membered ring comprising one additional amine group, which ring is optionally substituted with methyl, ethyl, or alcohol substituted methyl or ethyl.

[0041] In another preferred embodiment, R2 is a hydrogen atom and R3 comprises a cyclic structure having 3-6 carbon atoms and having one nitrogen atom.

10 [0042] More preferably, R2 is a hydrogen atom, and R3 comprises a 5-7 membered ring comprising one additional amine group, which ring is attached to the amide-nitrogen, and which ring is optionally substituted with methyl, ethyl, or alcohol substituted methyl or ethyl.

[0043] In either case, the ring (the cyclic structure formed by R2 and R3, or of R3 alone) may be unsubstituted or substituted with an alkyl having 1-4 carbon atoms, alcohol, or alkanol group having 1-4 carbon atoms, such as ethylol.

15 [0044] In a preferred embodiment according to the invention, the compound either according to formula (I) or according to formula (II) has a molecular weight lower than 500 Da.

[0045] In a preferred embodiment, the compound for use according the present invention is a chromanol compound according to formula I.

20 [0046] Certain chromanol compounds have been described in WO2014/098586. The compounds described in detail have abbreviations, referring to SUL-XXX (XXX being a 2 or 3 digit number). Many of these compounds are racemic mixtures, although some enantiomers have been tested as well. Suitable methods to prepare chromanol compounds according to the present invention are described in WO2014/098586 or WO2014/011047.

25 [0047] WO 2017/060432 A1 discloses amide-derivatives of 2-hydroxy-2-methyl-4-(3,5,6-trimethyl-1,4-benzoquinon-2-yl)-butanoic acid and methods of making such compounds.

[0048] Hydrogenated quinone derivatives can be easily prepared by hydrogenation of the quinone structure.

30 [0049] According to yet another preferred embodiment, the compound is either (6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)(piperazin-1-yl)methanone (SUL-121), ((S)-6-hydroxy-2,5,7,8-tetramethyl-N-((R)-piperidin-3-yl)chroman-2-carboxamide hydrochloride (SUL-13), or (6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)(4-(2-hydroxyethyl)piperazin-1-

yl)methanone (SUL-109) or a pharmaceutically acceptable salt thereof, as racemic mixture or as one of its enantiomers.

[0050] In a most preferred embodiment, the compound is the S-enantiomer of SUL-109, namely S-(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)(4-(2-hydroxyethyl)piperazin-1-yl)methanone (SUL-138).

[0051] The counterion in the pharmaceutically acceptable salt can be a counterion as known in the art. Preferably, the compounds have at least one basic nitrogen, an amine, which can be protonated. The counterion preferably is a halogen such as chloride, sulphate, citrate, formate or the like, and most preferably chloride.

[0052] The compounds are effective as a racemic mixture or in a substantially pure enantiomeric form. The compounds have one or more chiral centers, generally one or two.

[0053] Preferably, the compound is a substantially enantiomerically pure compound.

Substantially enantiomerically pure is about 95% enantiomeric excess or more, more preferably about 98% enantiomeric excess, and most preferably about 99% or more enantiomeric excess. Also, in case the compound contains more than one chiral center, these amounts apply.

[0054] The compounds are preferably used in effective amounts, to achieve treatment or prophylaxis of ageing or ageing-associated disorders.

[0055] The wording treatment or prophylaxis includes amelioration of the symptoms of ageing and/or reduction in progress of ageing, including improvement of vascular and renal function.

[0056] The term 'treatment' encompasses reduction in progress of the disorder and/or improvement in symptoms of the disorder.

[0057] In one preferred embodiment of the invention, treatment of ageing includes increasing the life-span of a mammal, preferably human.

[0058] Preferably, the compounds according to the invention are for use of treatment or prophylaxis of ageing-associated disorders in mammals, wherein the mammal is preferably human.

[0059] Ageing related disorders include vascular ageing, reduced kidney function, loss of memory, reduced lung-function and the like. These disorders are idiopathic, or just ageing related, but have no specific underlying disease like asthma, COPD, Alzheimer or the like.

Hence, administering the compounds of the present invention increase the quality of life and/or life span of the ageing mammals.

5 {0060} In a more preferred embodiment according to the invention, the compound the compound either according to formula (I) or according to formula (II) is for use for treating or prophylaxis of vascular ageing.

{0061} The present invention provides compounds for use in treatment or prophylaxis of ageing disorders associated with a deterioration of the mitochondrial function and health.

10 {0062} The present invention provides compounds for use in treatment and prophylaxis of disease conditions associated with a reduced endothelium-dependent NO-cGMP vasodilation. Preferably, the present invention provides compounds for use in the preservation of vascular function through EDHF rescue.

{0063} Even more preferably, the compound the compound either according to formula (I) or according to formula (II) is for use for treating or prophylaxis of cardiovascular disease (CVD).

15 {0064} In a further embodiment, the compound the compound either according to formula (I) or according to formula (II) is for use for treating or prophylaxis of reduced kidney function because of ageing.

20 {0065} Effects generally are observed with amounts of about 1 μ M in body fluid, but preferably higher amounts are used. Preferred amounts are concentrations in vivo or in vitro of about 10 μ M or higher, more preferably about 20 μ M or higher. Generally, a concentration in human of about 200 μ M or lower should be sufficient and safe.

25 {0066} For human use, this would mean – assuming a 30 L distribution volume, 100% availability and a concentration of about 1 μ M – a dosage of about 10 mg or more. Preferred amounts would result in a concentration of about 10 μ M – for which a dosage of about 100 mg or more would be suitable. Hence, preferably, dosage forms of about 20 mg or more, preferably 50 mg or more, preferably 100 mg or more are suitable.

{0067} Generally, solid, oral dosage forms contain as a maximum about 500 mg compound, preferably about 450 mg or less, to allow for excipients.

30 {0068} With parenteral administration, such as for example i.v. other liquid forms of administration, larger amounts can be administered.

{0069} Examples of dosages which can be used are an effective amount of the compounds of the invention of a dosage of 0.2 mg/kg or higher, such as preferably within the range of

about 1 mg /kg to about 100 mg/kg, or within about 2 mg /kg to about 40 mg/kg body weight, or within about 3 mg/kg to about 30 mg/kg body weight, or within about 4 mg/kg to about 15mg/kg body weight. Compounds of the present invention may be administered in a single daily dose, or the total daily dosage may be administered in divided dosage of two, three or
5 four times daily.

[0070] The prophylaxis or treatment of ageing or ageing related disorders generally requires chronic administration of the active compound, i.e. administration of the active over a long period of time. Preferably, the chronic administration generally will comprise administration of the active compound for at least 2 months, preferably for at least 4 months,
10 and even more preferred for more than 6 months. In a further preferred embodiment, the administration will comprise administration of the active compound over a period of 1, 2, 3, 4, 5 or 6 years or more, like up to 10, 20 or 30 years. Administration of the active may be over a period of 60 years or less.

[0071] The treatment over a relatively long period of several months, several years or even
15 longer may comprise daily administration of one or two or more orally taken dosage forms, like tablets or liquids. Preferably, one oral dosage form is administered once daily. However, alternative administration regimens can be efficacious, like every other day, the use of drug holidays such as for example three weeks daily administration and one week no active administered, for at least 4 or 6 months or longer like several years.

[0072] Thus, according the present invention, chronic treatment comprises administration of the active over several months or longer, preferably several years or longer, wherein an effective amount of compound is administered regularly or intermittently for the whole of the period.

[0073] The compounds described herein can be formulated as pharmaceutical compositions
25 by formulation with additives such as pharmaceutically or physiologically acceptable excipients carriers, and vehicles.

[0074] Suitable pharmaceutically or physiologically acceptable excipients, carriers and vehicles include processing agents and drug delivery modifiers and enhancers, such as, for example, calcium phosphate, magnesium stearate, talc, monosaccharides, disaccharides,
30 starch, gelatin, cellulose, methyl cellulose, sodium carboxymethyl cellulose, dextrose, hydroxypropyl-P-cyclodextrin, polyvinylpyrrolidone, low melting waxes, and the like, as well as combinations of any two or more thereof. Other suitable pharmaceutically acceptable

excipients are described in "Remington's Pharmaceutical Sciences, " Mack Pub. Co. , New Jersey (1991).

[0075] A pharmaceutical composition preferably comprises a unit dose formulation, where the unit dose is a dose sufficient to have a therapeutic effect. The unit dose may be a dose administered periodically in a course of treatment or suppression of a disorder.

[0076] The compounds of the invention may be administered enterally, orally, parenterally, sublingually, by inhalation (e. g. as mists or sprays), rectally, or topically in dosage unit formulations containing conventional nontoxic pharmaceutically or physiologically acceptable carriers, adjuvants, and vehicles as desired. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intratarsal injection, or infusion techniques. The compounds are mixed with pharmaceutically acceptable carriers, adjuvants, and vehicles appropriate for the desired route of administration.

[0077] Generally, oral administration is a preferred route of administration, and formulations suitable for oral administration are preferred formulations.

[0078] The compounds described for use herein can be administered in solid form, in liquid form, in aerosol form, or in the form of tablets, pills, powder mixtures, capsules, granules, injectables, creams, solutions, suppositories, enemas, colonic irrigations, emulsions, dispersions, food premixes, and in other suitable forms. The compounds can also be administered in liposome formulations.

[0079] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions, may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in propylene glycol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

[0080] Suppositories for rectal administration of the drug can be prepared by mixing the drug with a suitable non-irritating excipient such as cocoa butter and polyethylene glycols

that are solid at room temperature but liquid at the rectal temperature and will therefore melt in the rectum and release the drug.

[0081] Solid dosage forms for oral administration may include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound may be admixed with at least one inert diluent such as sucrose, lactose, or starch. Such dosage forms may also comprise additional substances other than inert diluents, e.g., lubricating agents such as magnesium stearate. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings.

[0082] Liquid dosage forms for oral administration may include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art, such as water. Such compositions may also comprise adjuvants, such as wetting agents, emulsifying and suspending agents, cyclodextrins, and sweetening, flavouring, and perfuming agents.

[0083] The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host to which the active ingredient is administered and the particular mode of administration. The unit dosage chosen is usually fabricated and administered to provide a defined final concentration of drug in the blood, tissues, organs, or other targeted region of the body. The effective amount for a given situation can be readily determined by routine experimentation and is within the skill and judgment of the ordinary clinician or skilled person.

[0084] The present invention will be further illustrated using the examples below. In the examples, reference is made to figures.

Examples

Example 1 – Study of the effect of SUL compounds on the lifespan of *Drosophila* model

Experimental methodology

Fly stocks and food supplementation

[0085] W1118 flies were bred and housed at 25 °C with a 12h:12h light/dark-cycle. Flies were kept in vials containing approximately 5 mL of Bloomington food consisting of sucrose,

yeast suspension, agar, Nigapin and fresh yeast. Flies kept as a stock were flipped to fresh vials weekly. Food was stored at 18°C and warmed at room temperature before use.

[0086] SUL109 and SUL121 were used to assess the effect of chromanol compounds on the lifespan of the fly stocks. The compounds were independently tested. Either SUL109 or
5 SUL121 were added to the food to a final concentration of 10µM, 100µM and 1000µM.

Drosophila lifespan

[0087] W1118 flies were anesthetized in small groups using CO₂. Subsequently, flies were collected at a density of 5 males and 5 females per tube.

[0088] At day 10 and 11 eclosing adults were anesthetized and housed at a density of 20
10 males and females per tube. Ten vials were used per study condition (total of 100 males and 100 females).

[0089] During the lifespan, flies were transferred onto new vials containing fresh food 3 times a week, and the number and sex of dead flies was recorded.

Results

15 [0090] W1118 flies were exposed to different concentrations of chromanol-based compounds (SUL109 and SUL121) in their food since larvae state until the moment they died. Results for both SUL components show flies that were exposed to the higher doses of the compounds have a better survival than the controls (see Figure 1 for SUL109; Figure 2 for SUL121).

20 [0091] In the current experimental set up, the effect of two of those compounds, SUL109 and SUL121 on the lifespan of *Drosophila* was tested. Results show an increased lifespan associated with the treatment with SUL compounds.

Example 2 - Study of the effect of SUL compounds on the vascular and renal function of *Ercc1*^{+/-} (EC-KO) mice models

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Background

[0092] The effectiveness of the compounds according to the invention for treatment or prophylaxis of ageing-associated diseases was studied in *Ercc1*^{+/-} (EC-KO) mice models.

[0093] *Ercc1*^{+/-} (EC-KO) mice models possess a Endothelial cell (EC)-specific deletion of
30 DNA repair endonuclease *Ercc1*, which leads to accelerated vascular aging features, marked by reduced endothelium-dependent NO-cGMP vasodilation at 22 weeks of age.

[0094] Therefore, EC-KO mice are excellent animal models to study ageing-associated diseases, in particular vascular ageing.

Experimental methodology

Study design

5 [0095] EC-KO mice and corresponding littermates (LM) received either SUL138 treated (30 mg SUL138/ kg BW/ day) or vehicle treated (0,015 % [v/v] ethanol) chow for 8 weeks (14-22 weeks of age).

[0096] At the age of 21 weeks, animals were put in metabolic cages. After 10 h of acclimatization, 24 h urine was collected. At the age of 22 weeks, mice were euthanized,
10 urine, blood plasma and tissue were collected.

Vascular function

[0097] Segments of thoracic aorta were isolated and mounted in wire-myograph containing Krebs Henseleit buffer (at 37 °C, oxygenated with 95 % O₂ and 5 % CO₂) to assess endothelium-dependent (ED) relaxation.

15 [0098] Contraction of the thoracic aorta isolates was induced with 100 mM KCl (final concentration) to evaluate vessel viability.

[0099] A stable synthetic analog of the endoperoxide prostaglandin PGH₂, namely U46619, was used for pre-constriction. Thoracic aorta segments were washed and subsequently pre-constricted with U46619 (30-60 nM final concentration; 75-100 % of the response to 100
20 mM KCl), followed by a dose response curve with ED vasodilator acetylcholine (ACh) (10⁻⁹-10⁻⁵ M final concentration).

[00100] To evaluate the contribution of different pathways to vasodilator response, segments were incubated with inhibitors for 15 minutes prior to pre-constriction with U46619. L-NAME (10⁻⁴ M) was added to assess nitric oxide (NO) contribution to vasodilator response.
25 L-NAME, apamin (100 nM) and TRAM-34 (10 µM) were added to assess ED hyperpolarization factor (EDHF) contribution to vasodilator response.

[00101] The acute effect of SUL138 was evaluated by pre-incubating with 100 µM for 30 minutes.

Renal function

30 [00102] Creatine levels in urine were measured in the samples collected at the age of 21 weeks and at the time euthanasia (22 weeks). QuantiChrom Creatinine Assay Kit (1:2 sample dilution) was used according to manufacturer's instructions.

[00103] Albumin levels in urine were measured in the samples collected at the time euthanasia (22 weeks). Mouse albumin ELISA kit ab108792 (1:400 sample dilution) was used according to manufacturer's instructions.

5 [00104] Blood plasma creatinine was measured in the samples collected at the time euthanasia (22 weeks). Samples were undiluted. QuantiChrom Creatinine Assay Kit (1:2 sample dilution) was used according to manufacturer's instructions.

Results

10 [00105] FIG. 3 shows the effect of chronic treatment of LM and EC-KO mice with SUL138. Chronic treatment with SUL138 restored ED relaxation in EC-KO mice compared to LM control levels.

[00106] FIG. 4 shows the pathway contribution to ACh response in LM control (vehicle treated) mice. It is demonstrated that ED relaxation in LM control mice is partly NO (see L-NAME curve in FIG. 4) and partly EDHF (see L-NAME/apamin/TRAM34 curve in FIG. 4)
15 driven.

[00107] The pathway contribution to ACh response was also tested in EC-KO mice (vehicle treated). Result curves are shown in FIG. 5. The dysfunctional endothelium in the vehicle treated EC-KO led to a shift towards mainly EDHF-driven ED relaxation (see L-NAME curve in FIG. 5).

20 [00108] The results of the pathway contribution to ACh response in EC-KO mice treated with SUL138 showed that the effect of such chronic treatment results from an increased EDHF-driven ED relaxation, as shown in FIG. 6 (see L-NAME curve).

[00109] However, restoration of ED relaxation in EC-KO mice only occurred after chronic treatment with SUL138. There is no effect on ED relaxation following acute treatment of
25 mice with SUL138.

[00110] FIG. 7 shows the albumin and creatin levels in urine in 22 week old mice. Vehicle treated EC-KO mice displayed a trend of albuminuria and decreased creatinine filtration compared to vehicle treated LM at the age. The results show that chronic treatment with SUL138 significantly improved creatinine filtration in EC-KO mice.

30 [00111] FIG. 8 shows the albumin/creatinine ratio in urine and blood plasma in 22 week old mice. It is shown that albumin/creatinine ratio in urine was significantly elevated in vehicle

treated EC-KO mice compared to vehicle treated LM mice. Chronic treatment with SUL138 led to a significant improvement of albumin/creatinine ratios in EC-KO mice.

[00112] No effects could be however identified in the blood plasma creatinine content in vehicle treated EC-KO mice compared to vehicle treated LM mice (FIG. 9).

5 [00113] FIG. 10 shows the creatine levels in 24h urine from 21 weeks old mice. Results obtained from the analysis of urine taken at 21 weeks of age, namely 24h urine, showed the same trend of decreased creatinine filtration in vehicle treated EC-KO mice compared to vehicle treated LM mice. Such trend was significantly improved by chronic treatment of EC-KO mice with SUL138.

10 Conclusion

[00114] It is often observed that EDHF replaces NO in models of progressive endothelial dysfunction. In the present study, *Ercc1*-deficient mouse models are strongly aged at 16 weeks of age, such that they have partially lost EDHF and totally lost NO.

[00115] Our results show that SUL138 delays endothelial aging. The increase of EDHF
15 instead of NO following chronic treatment with SUL138 suggests a compensation for loss of NO. Alternatively, chronic treatment with SUL138 might increase EDHF as a direct effect.

[00116] In summary, chronic treatment with SUL138 resulted in full preservation of vascular function through EDHF rescue in isolated tissue from *Ercc1*-deficient mouse models.

20 [00117] Moreover, chronic treatment with SUL138 also resulted in the protection of renal function in *Ercc1*-deficient mouse models.

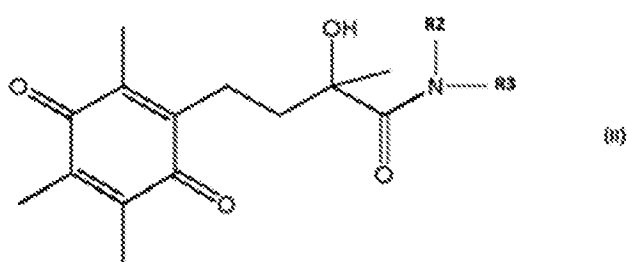
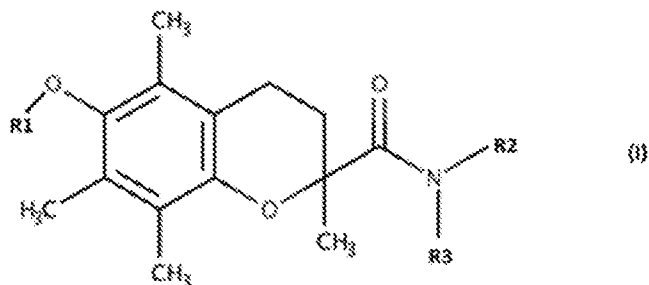
[00118] To date, only dietary restriction was able to improve vasomotor function in *Ercc1*-deficient, accelerated aging, mouse models (Wu et al., 2017, CI Sci). Contrary to the effect of other drugs in endothelial dysfunction models, SUL138 results in an attenuation of the
25 accumulation of persistent DNA damage, similar to the proven effects of dietary restriction.

[00119] Thus, the invention has been described by reference to certain embodiments discussed above. It will be recognized that these embodiments are susceptible to various modifications and alternative forms well known to those of skill in the art.

[00120] Further modifications in addition to those described above may be made to the
30 structures and techniques described herein without departing from the spirit and scope of the invention. Accordingly, although specific embodiments have been described, these are examples only and are not limiting upon the scope of the invention.

CONCLUSIES

1. Verbinding volgens formule (I) of (II), het hydrochinonanalogon met formule (II) of een farmaceutisch aanvaardbaar zout daarvan voor gebruik bij de behandeling of profylaxe van veroudering of met veroudering geassocieerde aandoeningen;



- waarbij R1 een waterstof of prodrug-groep die in levend weefsel kan worden verwijderd
- en waarbij ofwel
 - o R2 en R3 samen met het N-atoom waaraan ze bevestigd zijn een verzadigde of onverzadigde, niet-aromatische, optioneel gesubstitueerde, 5-8-ledige ring, met één tot vier N-, O- of S-atomen, vormen waarbij R2 en R3 samen 3-12 koolstofatomen bevatten;
 - o ofwel R2 een waterstofatoom, of een alkylgroep met 1-6 koolstofatomen is, en R3 een alkylgroep is, optioneel gesubstitueerd met stikstof of zuurstof, waarbij de alkylgroep 3-12 koolstofatomen omvat, en waarbij de alkylgroep in R3 één of meer niet-aromatische cyclische structuren omvat die stikstof- of zuurstofatomen in de ring kunnen omvatten, en lineaire en/of vertakte gesubstitueerde groepen, en één of meer ethyleen-onverzadigingen kunnen bevatten.

2. Verbinding voor gebruik volgens conclusie 1, waarbij R1 waterstof is of samen met de 6-zuurstof een estergroep met 2 - 6 koolstofatomen vormt.

3. Verbinding voor gebruik volgens één van conclusies 1-2, waarbij de stikstof in R2 en/of R3 amine, quaternaire amine, guanidine of imine kan zijn en de zuurstof in R2 en/of R3 hydroxyl, carbonyl of carbonzuur kan zijn; en/of zuurstof en stikstof in R2 en/of R3 samen amide-, ureum- of carbamaatgroepen kunnen vormen.

4. Verbinding voor gebruik volgens één van conclusies 1-3, waarbij in elke verbinding volgens formule (I) of volgens formule (II), R2 en R3 samen met het N-atoom waaraan ze bevestigd zijn een verzadigde ring vormen waarin een extra N-atoom is opgenomen, welke ring ongesubstitueerd of gesubstitueerd met een alcohol, of alkanolgroep met 1-4 koolstofatomen is.

5. Verbinding voor gebruik volgens één van conclusies 1 - 4, waarbij de verbinding een verbinding volgens formule I is.

6. Verbinding voor gebruik volgens conclusie 5, waarbij R2 en R3 samen met het N-atoom waaraan ze bevestigd zijn een 5-7-ledige ring omvattende één additionele aminegroep vormen, welke ring optioneel gesubstitueerd is met methyl, ethyl, of met alcohol gesubstitueerd methyl of ethyl.

7. Verbinding voor gebruik volgens één van conclusies 1-3, waarbij R2 een waterstofatoom is en R3 een verzadigde cyclische structuur met 4-7 koolstofatomen en met één stikstofatoom omvat, welke ring gesubstitueerd kan zijn met een alkylgroep, alcoholgroep, of met een groep met 1-4 koolstofatomen die een zuurstof-, carbonzuur- of aminegroep kan omvatten.

8. Verbinding voor gebruik volgens conclusie 7, waarbij de verbinding een verbinding volgens formule I is, en waarbij R2 een waterstofatoom is, en R3 een cyclische structuur met 4-6 koolstofatomen en met één stikstofatoom omvat, welke ring optioneel gesubstitueerd is met methyl, ethyl, of met alcohol gesubstitueerd methyl of ethyl.

9. Verbinding voor gebruik volgens conclusie 1, waarbij de verbinding (6-hydroxy-2,5,7,8-tetramethylchromaan-2-yl)(piperazine-1-yl)methanon (SUL-121), ((S)-6-hydroxy-2,5,7,8-

tetramethyl-N-((R)-piperidine-3-yl)chromaan-2-carboxamide-hydrochloride (SUL-13) of (6-hydroxy-2,5,7,8-tetramethylchromaan-2-yl)(4-(2-hydroxyethyl)piperazine-1-yl)methanon (SUL-109) of een farmaceutisch aanvaardbaar zout daarvan, als een racemisch mengsel of als één van de enantiomeren is.

5

10. Verbinding voor gebruik volgens conclusie 9, waarbij de verbinding de S-enantiomeer van SUL-109: S-(6-hydroxy-2,5,7,8-tetramethylchromaan-2-yl)(4-(2-hydroxyethyl)piperazine-1-yl)methanon (SUL-138) of een farmaceutisch aanvaardbaar zout daarvan is.

10

11. Verbinding voor gebruik volgens één van conclusies 1-8, waarbij de verbinding volgens formule (I) of formule (II) een molecuul massa lager dan 500 Da heeft.

15

12. Verbinding voor gebruik volgens één van de voorgaande conclusies, waarbij het gebruik voor de behandeling of profylaxe van vasculaire veroudering is.

13. Verbinding voor gebruik volgens één van conclusies 1-11, waarbij het gebruik voor het vertragen van veroudering is.

20

14. Verbinding voor gebruik volgens één van conclusies 1-11, waarbij het gebruik voor de behandeling of profylaxe van verminderde nierfunctie als gevolg van veroudering is.

25

15. Verbinding voor gebruik volgens één van de voorgaande conclusies, waarbij de verbinding chronisch wordt toegediend, bij voorkeur omvattende toediening van de werkzame stof gedurende meerdere maanden of langer, met meer voorkeur meerdere jaren of langer, waarbij een werkzame hoeveelheid verbinding regelmatig of met tussenpozen gedurende de gehele periode wordt toegediend.

FIGURE 1

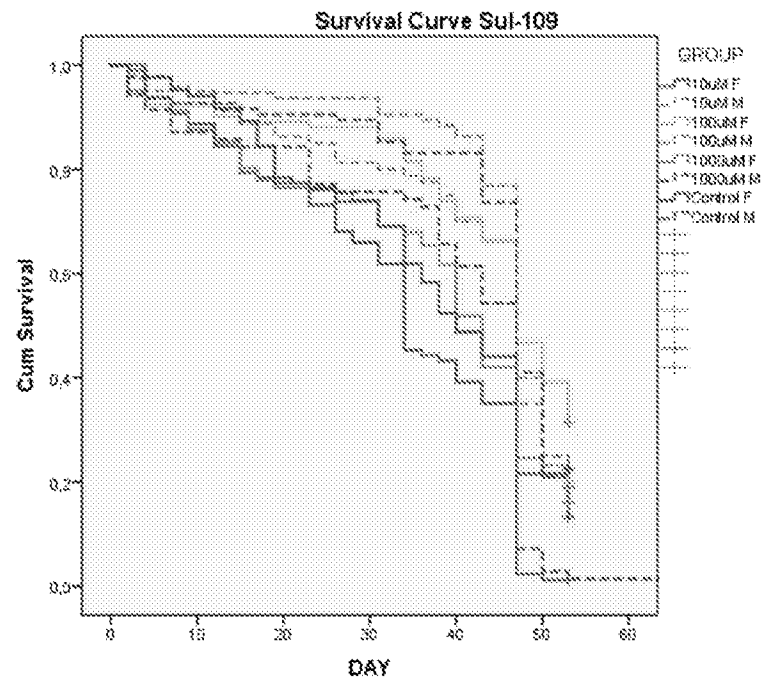


FIGURE 2

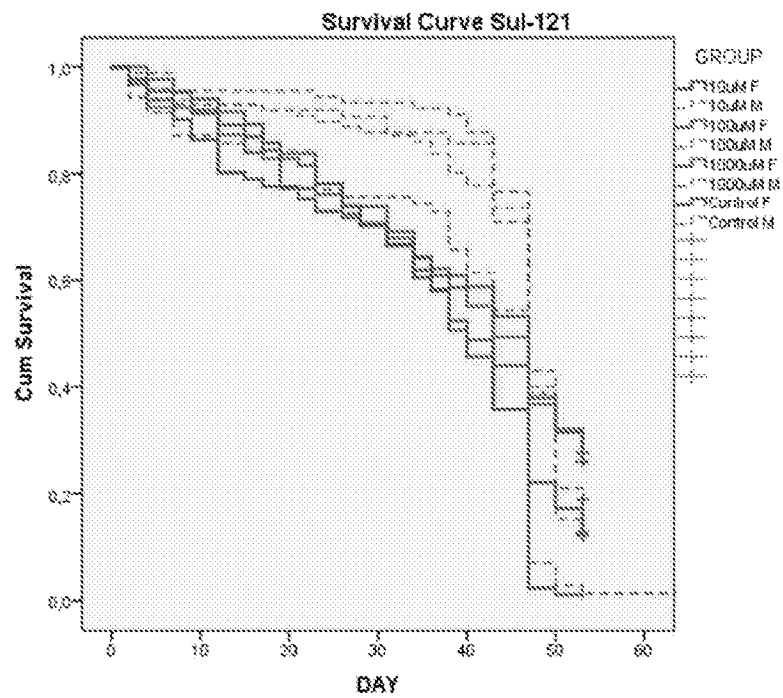


FIGURE 3

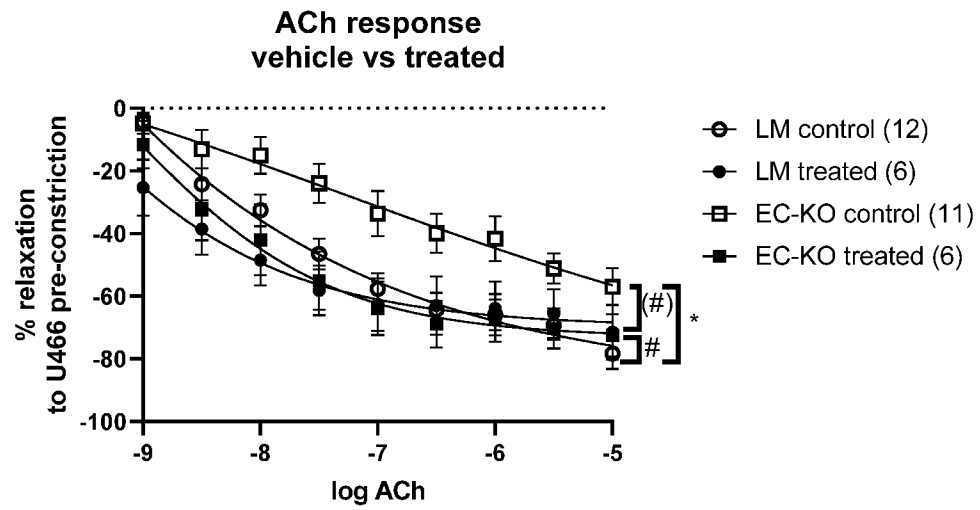


FIGURE 4

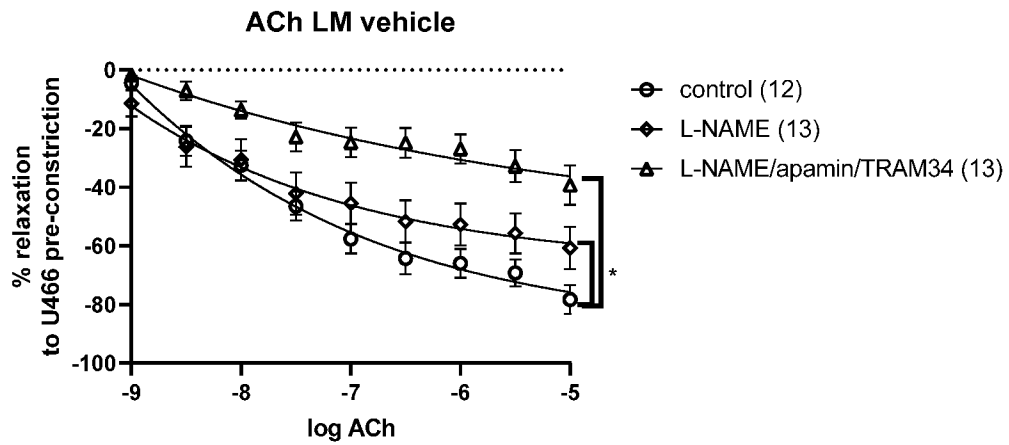


Figure 4

FIGURE 5

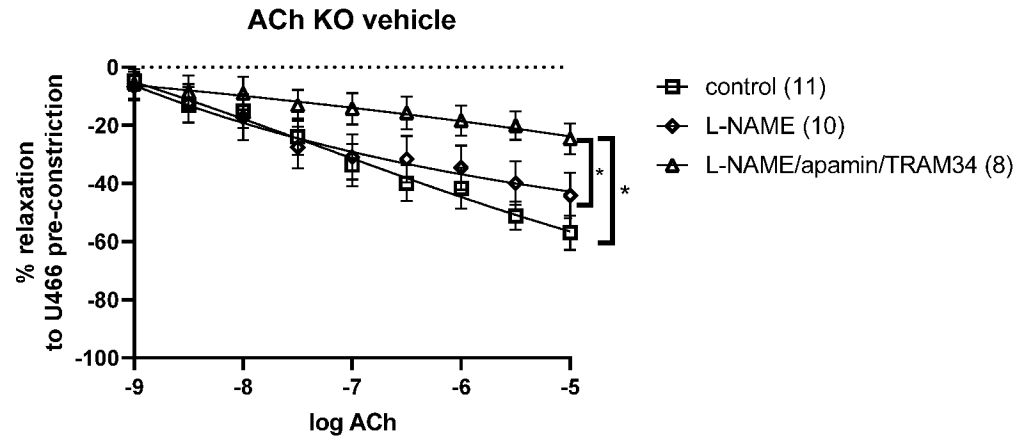


FIGURE 6

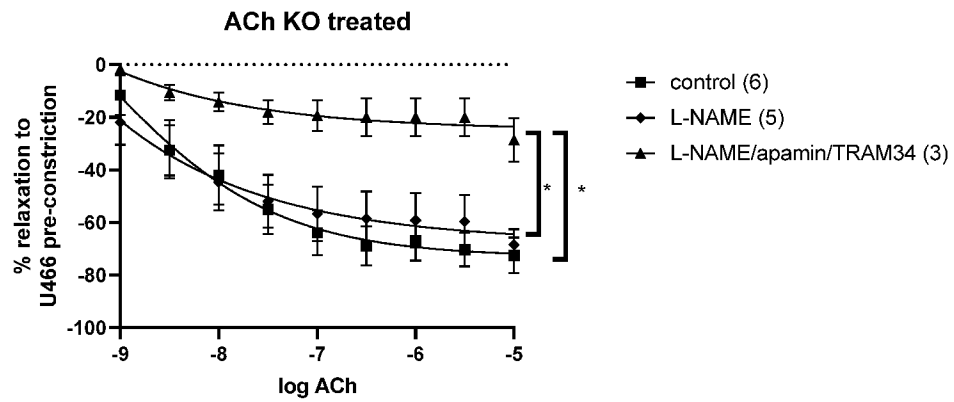


FIGURE 7

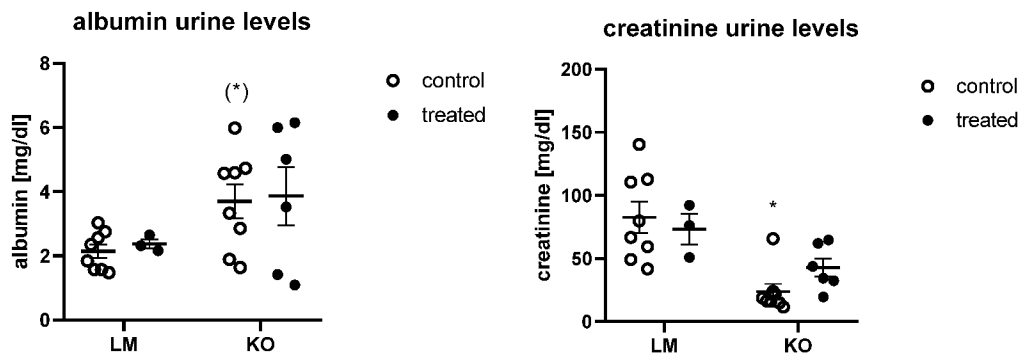


FIGURE 8

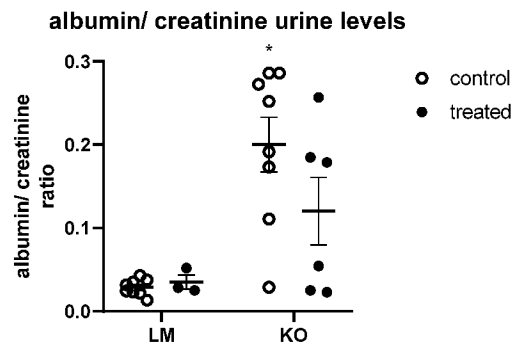


FIGURE 9

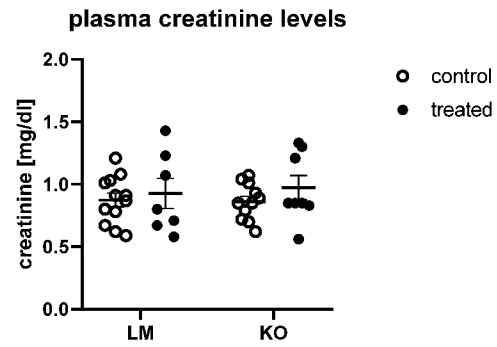
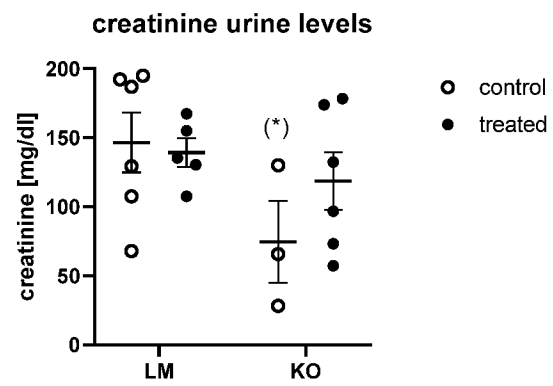


FIGURE 10



SAMENWERKINGSVERDRAG (PCT)

RAPPORT BETREFFENDE NIEUWHEIDSONDERZOEK VAN INTERNATIONAAL TYPE

IDENTIFICATIE VAN DE NATIONALE AANVRAGE		KENMERK VAN DE AANVRAGER OF VAN DE GEMACHTIGDE	
Nederlands aanvraag nr. 2031091		Indieningsdatum 28-02-2022	
		Ingeroepen voorrangsdatum	
Aanvrager (Naam) Sulfateq B.V.			
Datum van het verzoek voor een onderzoek van internationaal type 17-09-2022		Door de Instantie voor Internationaal Onderzoek aan het verzoek voor een onderzoek van internationaal type toegekend nr. SN82147	
I. CLASSIFICATIE VAN HET ONDERWERP (bij toepassing van verschillende classificaties, alle classificatiesymbolen opgeven)			
Volgens de internationale classificatie (IPC) Zie onderzoeksrapport			
II. ONDERZOCHETE GEBIEDEN VAN DE TECHNIEK			
Onderzochte minimumdocumentatie			
Classificatiesysteem	Classificatiesymbolen		
IPC	Zie onderzoeksrapport		
Onderzochte andere documentatie dan de minimum documentatie, voor zover dergelijke documenten in de onderzochte gebieden zijn opgenomen			
III.	GEEN ONDERZOEK MOGELIJK VOOR BEPAALDE CONCLUSIES		(opmerkingen op aanvullingsblad)
IV.	GEBREK AAN EENHEID VAN UITVINDING		(opmerkingen op aanvullingsblad)

**ONDERZOEKSRAPPORT BETREFFENDE HET
RESULTAAT VAN HET ONDERZOEK NAAR DE STAND
VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE**

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NL 2031091

A. CLASSIFICATIE VAN HET ONDERWERP INV. A61K31/16 A61K31/165 A61K31/353 A61P9/14 A61P13/12 A61P43/00 ADD. Volgens de Internationale Classificatie van octrooien (IPC) of zowel volgens de nationale classificatie als volgens de IPC.		
B. ONDERZOCHETE GEBIEDEN VAN DE TECHNIEK Onderzochte minimum documentatie (classificatie gevolgd door classificatiesymbolen) A61K A61P		
Onderzochte andere documentatie dan de minimum documentatie, voor dergelijke documenten, voor zover dergelijke documenten in de onderzochte gebieden zijn opgenomen		
Tijdens het onderzoek geraadpleegde elektronische gegevensbestanden (naam van de gegevensbestanden en, waar uitvoerbaar, gebruikte trefwoorden) EPO-Internal		
C. VAN BELANG GEACHTE DOCUMENTEN		
Categorie °	Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages	Van belang voor conclusie nr.
X	WO 2019/038360 A1 (SULFATEQ BV [NL]; UNIV GRONINGEN [NL]) 28 februari 2019 (2019-02-28) * figuur 4 * * bladzijde 3, regels 7-21 * ----- -/-	1-9, 11, 13, 15
☒ Verdere documenten worden vermeld in het vervolg van vak C. ☒ Leden van dezelfde octrooifamilie zijn vermeld in een bijlage		
° Speciale categorieën van aangehaalde documenten "A" niet tot de categorie X of Y behorende literatuur die de stand van de techniek beschrijft "D" in de octrooiaanvraag vermeld "E" eerdere octrooi(aanvraag), gepubliceerd op of na de indieningsdatum, waarin dezelfde uitvinding wordt beschreven "L" om andere redenen vermelde literatuur "O" niet-schriftelijke stand van de techniek "P" tussen de voorrangsdatum en de indieningsdatum gepubliceerde literatuur		
"T" na de indieningsdatum of de voorrangsdatum gepubliceerde literatuur die niet bezwarend is voor de octrooiaanvraag, maar wordt vermeld ter verheldering van de theorie of het principe dat ten grondslag ligt aan de uitvinding "X" de conclusie wordt als niet nieuw of niet inventief beschouwd ten opzichte van deze literatuur "Y" de conclusie wordt als niet inventief beschouwd ten opzichte van de combinatie van deze literatuur met andere geciteerde literatuur van dezelfde categorie, waarbij de combinatie voor de vakman voor de hand liggend wordt geacht "&" lid van dezelfde octrooifamilie of overeenkomstige octrooipublicatie		
Datum waarop het onderzoek naar de stand van de techniek van internationaal type werd voltooid 9 januari 2023		Verzenddatum van het rapport van het onderzoek naar de stand van de techniek van internationaal type
Naam en adres van de instantie European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		De bevoegde ambtenaar Rodrigues Neibecker

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C.(Vervolg). VAN BELANG GEACHTE DOCUMENTEN		
Categorie °	Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages	Van belang voor conclusie nr.
X	LAMBOOY S. P. H. ET AL: "The Novel Compound Sul-121 Preserves Endothelial Function and Inhibits Progression of Kidney Damage in Type 2 Diabetes Mellitus in Mice", SCIENTIFIC REPORTS, deel 7, nr. 1, 11 september 2017 (2017-09-11), bladzijden 1-13, XP093011112, DOI: 10.1038/s41598-017-11582-6 Gevonden op het Internet: URL:https://www.nature.com/articles/s41598-017-11582-6> * samenvatting * * Title * * bladzijde 10, alinea 3rd * -----	1-9, 11-15
A	FANG YUDONG ET AL: "The ageing kidney: Molecular mechanisms and clinical implications", AGEING RESEARCH REVIEWS, ELSEVIER, AMSTERDAM, NL, deel 63, 22 augustus 2020 (2020-08-22), XP086266729, ISSN: 1568-1637, DOI: 10.1016/J.ARR.2020.101151 [gevonden op 2020-08-22] * section 4.3; bladzijde 6 * * figuur 3 * * sections 4.1 and 4.2; bladzijde 5 * -----	1-15
X	VOGELAAR PIETER C ET AL: "The 6-hydroxychromanol derivative SUL-109 ameliorates renal injury after deep hypothermia and rewarming in rats", NEPHROLOGY DIALYSIS TRANSPLANTATION, deel 33, nr. 12, 11 april 2018 (2018-04-11), bladzijden 2128-2138, XP093011113, GB ISSN: 0931-0509, DOI: 10.1093/ndt/gfy080 * Conclusions; bladzijde 2128 * * bridging paragraph; bladzijde 2128 - bladzijde 2129 * -----	1-11,13, 14
3	X WO 2021/118359 A1 (SULFATEQ BV [NL]; UNIV GRONINGEN [NL]) 17 juni 2021 (2021-06-17) * conclusies 1-12 * * bladzijde 12, regels 5-9 * ----- -/--	1-13,15

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C.(Vervolg). VAN BELANG GEACHTE DOCUMENTEN		
Categorie °	Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages	Van belang voor conclusie nr.
A	UNGVARI ZOLTAN ET AL: "Mechanisms of Vascular Aging", CIRCULATION RESEARCH, deel 123, nr. 7, 13 september 2018 (2018-09-13), bladzijden 849-867, XP093011253, US ISSN: 0009-7330, DOI: 10.1161/CIRCRESAHA.118.311378 * bladzijde 853, linker kolom; figuur 1 * * bladzijde 857, rechter kolom * * bladzijde 855, rechter kolom * -----	1-15
X	WO 2014/098586 A1 (SULFATEQ BV [NL]) 26 juni 2014 (2014-06-26) * bladzijde 11; tabel 1 * * bridging paragraph; bladzijde 5 - bladzijde 6 * * bladzijde 8, regel 31 - bladzijde 9, regel 16 * -----	1-14
X	WO 2017/060432 A1 (KHONDRION IP B V [NL]) 13 april 2017 (2017-04-13) * table, column 1, row 3; bladzijde 57 * * bladzijde 29, regels 20-30; conclusie 14 * -----	1-9, 11-14
X	WO 2019/038361 A1 (SULFATEQ BV [NL]; UNIV GRONINGEN [NL]) 28 februari 2019 (2019-02-28) * bladzijde 3, regels 30-35; conclusie 7 * -----	1-9, 11-13,15
X	WO 2015/193365 A1 (SULFATEQ BV [NL]; UNIV GRONINGEN [NL]) 23 december 2015 (2015-12-23) * bladzijden 11-12 * * bladzijde 1, regels 5-10, 25-26 * -----	1-11,13
X	VOGELAAR PC ET AL: "Towards prevention of ischemia-reperfusion kidney injury: Pre-clinical evaluation of 6-chromanol derivatives and the lead compound SUL-138*", EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES, ELSEVIER AMSTERDAM, NL, deel 168, 3 oktober 2021 (2021-10-03), XP086895294, ISSN: 0928-0987, DOI: 10.1016/J.EJPS.2021.106033 [gevonden op 2021-10-03] * samenvatting * * bladzijde 3, linker kolom * -----	1-11,13, 14
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C.(Vervolg). VAN BELANG GEACHTE DOCUMENTEN		
Categorie °	Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages	Van belang voor conclusie nr.
X	US 2021/113520 A1 (BEYRATH JULIEN DAVID [NL] ET AL) 22 april 2021 (2021-04-22) * alineas [0025], [0031], [0253] * -----	1-9, 11-14
E	NL 2 026 511 B1 (SULFATEQ BV [NL]; UNIV GRONINGEN [NL]) 24 mei 2022 (2022-05-24) * conclusies 1-11; tabel 2 * -----	1-13
E	WO 2022/058620 A1 (SULFATEQ BV [NL]; UNIV GRONINGEN [NL]) 24 maart 2022 (2022-03-24) * conclusies 1-11; tabel 2 * -----	1-13

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In het rapport genoemd octrooigescrift	Datum van publicatie	Overeenkomend(e) geschrift(en)	Datum van publicatie
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			31-08-2017

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		WO 2022058620 A1	24-03-2022

WRITTEN OPINION

File No. SN82147	Filing date (<i>day/month/year</i>) 28.02.2022	Priority date (<i>day/month/year</i>)	Application No. NL2031091
International Patent Classification (IPC) INV. A61K31/16 A61K31/165 A61K31/353 A61P9/14 A61P13/12 A61P43/00			
Applicant Sulfateq B.V.			

This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☒ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the application
- ☒ Box No. VIII Certain observations on the application

	Examiner Rodrigues Neibecker
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WRITTEN OPINION

Box No. I Basis of this opinion

1. This opinion has been established on the basis of the latest set of claims filed before the start of the search.
2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the application, this opinion has been established on the basis of:
 - a. type of material:
 - ☐ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material:
 - ☐ on paper
 - ☐ in electronic form
 - c. time of filing/furnishing:
 - ☐ contained in the application as filed.
 - ☐ filed together with the application in electronic form.
 - ☐ furnished subsequently for the purposes of search.
3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

Box No. V Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty	Yes: Claims	
	No: Claims	1-15
Inventive step	Yes: Claims	
	No: Claims	1-15
Industrial applicability	Yes: Claims	1-15
	No: Claims	

2. Citations and explanations

see separate sheet

WRITTEN OPINION

Application number
NL2031091

Box No. VI Certain documents cited

☒ Certain published documents

see the Search Report

☐ Non-written disclosures

Box No. VIII Certain observations on the application

see separate sheet

1 **Re Item V**

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1.1 Reference is made to the following documents:

- D1 WO 2019/038360 A1 (SULFATEQ BV [NL]; UNIV GRONINGEN [NL]) 28 februari 2019 (2019-02-28)
- D2 Lambooy S. P. H. ET AL: "The Novel Compound Sul-121 Preserves Endothelial Function and Inhibits Progression of Kidney Damage in Type 2 Diabetes Mellitus in Mice", Scientific Reports, deel 7, nr. 1, 11 september 2017 (2017-09-11), bladzijden 1-13, XP093011112, DOI: 10.1038/s41598-017-11582-6
Gevonden op het Internet:
URL:<https://www.nature.com/articles/s41598-017-11582-6>
- D3 FANG YUDONG ET AL: "The ageing kidney: Molecular mechanisms and clinical implications", AGEING RESEARCH REVIEWS, ELSEVIER, AMSTERDAM, NL, deel 63, 22 augustus 2020 (2020-08-22), XP086266729, ISSN: 1568-1637, DOI: 10.1016/J.ARR.2020.101151 [gevonden op 2020-08-22]
- D4 Vogelaar Pieter C ET AL: "The 6-hydroxychromanol derivative SUL-109 ameliorates renal injury after deep hypothermia and rewarming in rats", Nephrology Dialysis Transplantation, deel 33, nr. 12, 11 april 2018 (2018-04-11), bladzijden 2128-2138, XP093011113, GB
ISSN: 0931-0509, DOI: 10.1093/ndt/gfy080
- D5 WO 2021/118359 A1 (SULFATEQ BV [NL]; UNIV GRONINGEN [NL]) 17 juni 2021 (2021-06-17)

- D6 Ungvari Zoltan ET AL: "Mechanisms of Vascular Aging",
Circulation research,
deel 123, nr. 7, 13 september 2018 (2018-09-13), bladzijden
849-867, XP093011253,
US
ISSN: 0009-7330, DOI: 10.1161/CIRCRESAHA.118.311378
- D7 WO 2014/098586 A1 (SULFATEQ BV [NL]) 26 juni 2014
(2014-06-26)
- D8 WO 2017/060432 A1 (KHONDRION IP B V [NL]) 13 april 2017
(2017-04-13)
- D9 WO 2019/038361 A1 (SULFATEQ BV [NL]; UNIV GRONINGEN
[NL]) 28 februari 2019 (2019-02-28)
- D10 WO 2015/193365 A1 (SULFATEQ BV [NL]; UNIV GRONINGEN
[NL]) 23 december 2015 (2015-12-23)
- D11 VOGELAAR PC ET AL: "Towards prevention of ischemia-
reperfusion kidney injury: Pre-clinical evaluation of 6-chromanol
derivatives and the lead compound SUL-138*",
EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES,
ELSEVIER AMSTERDAM, NL,
deel 168, 3 oktober 2021 (2021-10-03), XP086895294,
ISSN: 0928-0987, DOI: 10.1016/J.EJPS.2021.106033
[gevonden op 2021-10-03]
- D12 US 2021/113520 A1 (BEYRATH JULIEN DAVID [NL] ET AL) 22
april 2021 (2021-04-22)
- D13 NL 2 026 511 B1 (SULFATEQ BV [NL]; UNIV GRONINGEN [NL])
24 mei 2022 (2022-05-24)
- D14 WO 2022/058620 A1 (SULFATEQ BV [NL]; UNIV GRONINGEN
[NL]) 24 maart 2022 (2022-03-24)

1.2 **Novelty**

- 1.2.1 The present application does not meet the criteria of patentability, because the subject-matter of claims 1-15 is not new.

- 1.2.2 D1 mentions that 2S enantiomers of chromanols (Figure 4), such as SUL-151 (S enantiomer present in the racemic mixture SUL-121) are excellent pharmaceutical active compounds for treating disorders that require long systemic treatment *a.o.* diabetes, COPD and kidney disorders (page 3, lines 7-21). In line with point 3.1 below, these disorders are considered ageing-related.

Therefore claims 1-9, 11, 13 and 15 are not novel over D1.

- 1.2.3 D2 reports in the context of diabetic nephropathy (which is a vascular complication of diabetes) that SUL-121 limits the progression of diabetic kidney damage via a mechanism that preserves endothelial function and reduces oxidative stress (title, abstract and page 10, 3rd paragraph). For the sake of completeness it is noted that diabetic nephropathy can be considered an ageing-related disorder (see point 3.1 below). Another evidence of this interpretation can be found in D3, which mentions that cell senescence is involved in the pathogenesis of diabetic nephropathy (page 6, section 4.3). In D2, the mice received SUL-121 from age 10 to 18 weeks (abstract).

Therefore claims 1-9, 11-15 are not novel over D2.

- 1.2.4 D4 reports that SUL-109 and SUL-121 ameliorate kidney injury during hypothermia/rewarming by preserving mitochondrial mass, function and ATP levels. Furthermore, both compounds limit DNA damage (page 2128, Conclusions). As explained on pages 2128-2129 (bridging paragraph), hypothermia/rewarming offers a model in which the contribution of mitochondrial dysfunction (and as a result *a.o.* oxidative stress, DNA damage) to renal disease can be studied in an acute setting. Since these mechanisms also underlie senescence of kidney cells (see Figure 3 of D3), the animal of D4 is also considered an animal model for a kidney disease in the context of an ageing kidney.

Therefore claims 1-11, 13 and 14 are not novel over D4.

- 1.2.5 D5 relates to the same compounds as defined in the present claims including SUL-121, SUL-13, SUL-109 and SUL-138 for use in the treatment of Alzheimer's disease (D5, claims 1-12). In the examples of D5 (page 12, lines 5-9), mice were treated with SUL-138 for 3 months (chronic treatment). In line with point 3.1 below, Alzheimer's disease is an ageing-related disorder. Furthermore, as explained in D6 (e.g. figure 1; page 853, left-hand column, 1st paragraph; page 857, right-hand column) Alzheimer's disease is one pathophysiological manifestation of vascular ageing.

Therefore claims 1-13 and 15 are not novel over D5.

- 1.2.6 D7 relates to compounds as defined in the present claims and specifically mentions SUL-121, SUL-109 and SUL-138 (page 11, Table 1) for use in the treatment of diseases related to ageing *a.o.* stroke, thrombosis, cardiovascular disease, renal failure and kidney diseases (pages 5-6, bridging paragraph and pages 8-9, lines 31-16).

Therefore claims 1-14 are not novel over D7.

- 1.2.7 D8 relates to compounds as defined in the present claims. Specifically, SUL-13 is shown on page 57 (table, column 1, row 3). According to D8, the compounds described therein are suitable for use in the treatment of conditions associated with mitochondrial dysfunction such as *a.o.* ageing (D8, claim 14), cardiovascular and coronary artery disease (both vascular disorders) and chronic kidney disease (page 29, lines 20-30). For completeness, it is noted that D3 mentions chronic kidney disease as one possible manifestation of kidney ageing (page 5, section 4.2).

Therefore claims 1-9 and 11-14 are not novel over D8.

- 1.2.8 D9 relates to compounds as defined in the present claims. Specifically, D9 mentions that SUL-121 (racemic mixture) and its R enantiomer SUL-150 are able to inhibit vascular constrictions (page 3, lines 30-35). According to D9, said compounds can be used in the treatment of vasoconstriction related disorders or conditions selected from *a.o.* pulmonary arterial hypertension, heart failure with preserved ejection fraction and cardiac hypertrophy (D9, claim 7). In line with point 3.1 below, these are considered ageing-related disorders.

Therefore, claims 1-9, 11-13 and 15 are not novel over D9.

- 1.2.9 D10 relates to compounds as defined in the present claims. Specifically, D10 mentions SUL-109, SUL-121 and SUL-138 (pages 11-12) as suitable for treating COPD (page 1, lines 5-10), which is an ageing-related disorder as explained on page 1, lines 25-26 (see point 3.1 below).

Therefore, claims 1-11 and 13 are not novel over D10.

- 1.2.10 D11 relates to the use of 6-chromanol derivatives in a model for acute kidney injury (abstract). The complete investigation is carried out with SUL-138, but D11 also mentions SUL-121 and SUL-109 (page 3, left-hand column) as possible drug candidates. SUL-138 was found to safeguard kidney function and decrease renal injury. According to D3 acute kidney injury is a possible manifestation of kidney ageing (D3, page 5, section 4.1).

Therefore claims 1-11, 13 and 14 are not novel over D11.

- 1.2.11 D12 relates to compounds for use in the treatment of a mitochondrial disorder or a condition associated with mitochondrial dysfunction such as *a.o.* ageing, renal tubular acidosis (par. 0025) and cardiovascular disease (par. 0031). The representative compound tested in the examples (par. 0235) is ((S)-6 - hydroxy -2,5,7,8-tetramethyl-N-((R)-piperidin-3-yl)chroman-2-carboxamide hydrochloride (i.e. SUL-13).

Therefore claims 1-9, and 11-14 are not novel over D12.

1.3 **Inventive Step**

- 1.3.1 Since the question of the presence of an inventive step only arises when the subject-matter is new, a more detailed analysis of the compliance of the present application with this requirement is postponed until the objections above are overcome.
- 1.3.2 Applicant's attention is drawn to the fact that, in case the above explained novelty objections are overcome any one of D1, D2, D4, D5 and D7-D12 may be considered the closest prior art in the assessment of inventive step. Said documents relate to the same compounds and same diseases as the presently claimed subject-matter and are therefore suitable starting points for the skilled person working in the field.

2 **Re Item VI**

Certain documents cited

- 2.1 Applicant is informed that D13 (a national Dutch prior right), having filing date 21 September 2020 and Publication date 24 May 2022 discloses the same compounds as presently claimed for use in the treatment of heart failure with a reduced ejection fraction. D13 also mentions SUL-121, SUL-13, SUL-109 and SUL-138 as compounds according to the invention (D13, claims 1-11 and Table 2). Heart failure is an ageing-related vascular condition (see point 3.1 below). Another evidence of this can be found in D6 (page 855, right-hand column), which mentions that endothelial senescence has been implicated in the pathogenesis of heart failure. Therefore, D13 appears to anticipate the subject-matter of claims 1-13.
- 2.2 Applicant's attention is also called to D14, which is an international application belonging to the same family as D13 and having similar content. This document may become relevant for the assessment of novelty for the same reasons explained in relation to D13.

3 **Re Item VIII**

Certain observations on the application

- 3.1 Claims 1-11 and claim 15 are not clear, because the medical use has been vaguely and ambiguously defined as *treatment and prophylaxis of ageing and ageing-associated disorders*. The requirement of clarity is not met if the public is left in doubt as to which subject-matter is covered and which is not. The skilled person should be able to establish the demarcation of the scope of the claim (and consequently its extent of protection) without undue burden. As presently formulated, it is not clear what conditions are encompassed by the claims. In fact, *ageing* is a natural process and not a medical condition as such. Therefore it cannot be the object of a medical treatment or prophylaxis.

With respect to *ageing-associated disorders*, it is not clear how a certain disease differs in its *ageing-associated* and *non-ageing-associated* versions. The feature *ageing-associated* does not allow for a clearly distinguishable selection of a specific pathological condition of a certain disease. Therefore, any disclosure relating to diseases that typically occur in advanced age (*e.g.* cardiovascular disease, diabetes, COPD, stroke, Alzheimer's disease, kidney diseases and cancer; see par. 0002 of the present application) are considered as serving the same medical or therapeutic purpose of the present claims.

- 3.2 Claim 15 lacks clarity because the expressions *several months or longer* and *several years or longer* are imprecise and leaves the reader in doubt as to the duration of the treatment. Claim 15 also lacks clarity because the expression *regelmatig of met tussenpozen* (*regularly or intermittently*; see description par. 072) is based on relative terms. It is not possible to define what dosage schemes are encompassed by these definitions and how a regular intake differs from an intermittent intake.
- 3.3 Claims 1-15 do not fulfil the requirement of sufficiency of disclosure. To meet this requirement, the application has to disclose the suitability of the claimed compounds for the claimed therapeutic application. Furthermore, as a rule, protection conferred by a patent should be commensurate with the range of compounds for which the claimed effect has been adequately demonstrated, including obvious variants thereof. In this respect, the number of claimed variants has to be justified by the contribution to the art. In this respect and in view of the examples, the following is noted:

Present claims 1-8 and 11-15 relate to an extremely large number of possible compounds and the examples have been performed only with SUL109, 121 and 138. All according to formula (I) and having similar structure (note that all 3 have a piperazine ring).

With respect to the demonstrated effects, it is noted that example 1 (performed with SUL109 and SUL121) shows at best that the compounds helped the flies to endure the conditions of the experiment. A correlation between fly survival in said experiment and the claimed medical uses of prophylaxis of ageing (claim 1) or reduction in progress of ageing (claim 13) appear to be far-fetched. Example 2 (performed with SUL138) appears to provide plausibility for the treatment of vascular ageing (claim 12) and reduced renal function (claim 14) only with respect to conditions marked by increase vasoconstriction or reduced tissue perfusion i.e. conditions in which a vasodilation effect may be useful. It is noted that such conditions do not appear to have been specified in the application as filed.

In addition to what has been explained above, it is noted that D1, mentions that SUL-121 (a racemic mixture of the S enantiomer SUL-151 and the R enantiomer SUL-150) has been investigated with respect to inhibition of vascular constrictions and that SUL-151 was not active (pages 2-3, bridging paragraph). D1 also mentions that SUL-151 and analogous S-chromanol compounds are excellent pharmaceutical active compounds for treating a variety of diseases or disorders, while keeping the hemodynamics completely unaltered (page 3, lines 7-16). Also D4, which relates to SUL-109 and SUL-121 mentions that haemodynamics throughout the experiment were unaffected by said compounds (page 2131, left-hand column). Thus, even considering only the effect plausibly demonstrated in example 2 of the present application (*i.e.* vasodilation), it is doubtful that this effect can be extrapolated to all claimed compounds.

Therefore, claims 1-15 do not meet the requirement of sufficiency of disclosure.