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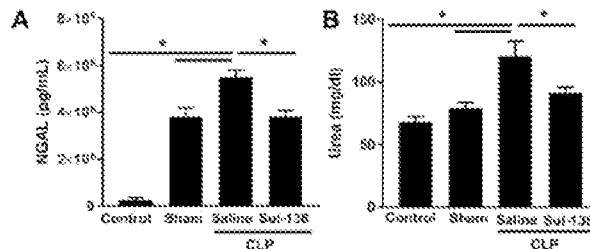
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COMPOUNDS FOR TREATMENT OF SEPSIS

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The invention relates to certain chromanol, quinone or hydroquinone compounds and derivatives thereof for treatment of sepsis and sepsis-induced organ dysfunction. Specifically, the present invention relates to chromanol compounds chosen from S-(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl) (piperazin-1-yl)methanone and S-(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl) (4-(2-hydroxyethyl)piperazin-1-yl)methanone, and pharmaceutically acceptable salts thereof.



COMPOUNDS FOR TREATMENT OF SEPSIS

I. Field of the Invention

The invention relates to chromanol compounds and derivatives thereof for treatment
5 or prophylaxis of sepsis. The invention further relates to chromanol compounds and derivatives thereof for the treatment or prophylaxis of sepsis-induced organ dysfunction.

II. Description of the Background Art

Sepsis is a deleterious systemic inflammatory response to infection. It is the major
10 cause of morbidity and mortality worldwide (Rudd *et al.*, Lancet 2020; 395: 200–211). Sepsis is currently defined as life threatening organ dysfunction caused by a dysregulated host response to infection. In its most severe form, sepsis causes multiple organ dysfunction that can produce a state of critical illness characterized by severe immune dysfunction and catabolism (Gotts & Matthey, BMJ 2016; 353:i1585).

15 The current treatment for sepsis with an emphasis on antibiotics and eradicating the source of infection, supporting blood pressure, organ blood flow, and ventilation has shown only limited efficacy in reducing mortality associated to sepsis. Despite efforts to improve current treatment strategies, the in-hospital mortality rate of sepsis in developed countries still remains around 20% (Seymour *et al.*, N. Engl. J. Med. 2017; 376:2235-2244;
20 Fleischmann-Struzek *et al.* Intensive Care Med. 2018 <https://doi.org/10.1007/s00134-018-5377-4>).

The sepsis response typically begins with a microbial infection. The recognition of microbial components such as lipopolysaccharide (LPS), peptidoglycan, lipoteichoic acid, and unmethylated CpG DNA by toll like receptors (TLRs) leads to the rapid activation of the
25 innate immune response and the release of a variety of humoral mediators, including glucocorticoids, catecholamines, and proximal pro-inflammatory cytokines like tumor necrosis factor α (TNF- α), interleukin-1 β (IL-1 β), and IL-6. This pro-inflammatory state has been defined as being a systemic inflammatory response syndrome (SIRS).

Exaggerated production of pro-inflammatory cytokines and the induction of more
30 distal mediators such as nitric oxide, platelet activation factor, and prostaglandins have been implicated in the endothelial changes and induction of a pro-coagulant state that leads to

hypotension, inadequate organ perfusion, and necrotic cell death associated with multiple organ dysfunction syndrome (MODS).

Multiple-organ dysfunction syndrome (MODS) has been identified as one of the most fatal complications of sepsis. Many agents targeting a variety of steps in the systemic inflammatory response have been developed over the years, but most of these have shown little or no effectiveness in clinical trials.

WO 2019/172766 A1 describes the use of alkaline phosphatases for the prevention, treatment, cure, or amelioration of the symptoms of acute kidney injury caused *e.g.* by sepsis.

WO 2017/220810 A1 describes the use of cilastatin in treating or preventing sepsis in a mammalian subject with the proviso that cilastatin is administered in combination with another drug which is not beta-lactam antibiotic.

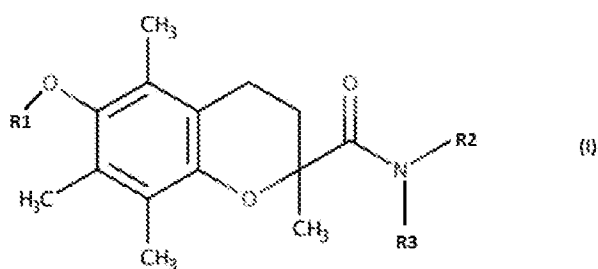
There remains a need for new compounds for treatment of sepsis.

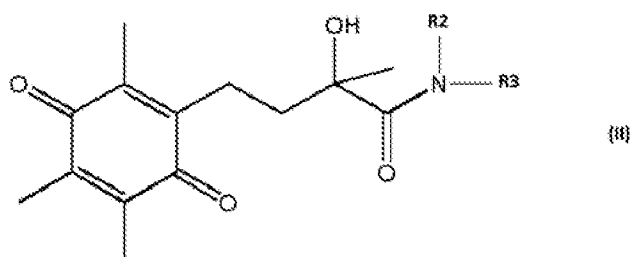
It is an object of the present invention to provide compounds for the treatment or prophylaxis of sepsis, and in particular to provide compounds for the treatment or prophylaxis of organ dysfunction caused by a dysregulated host response to infection such as notably kidney dysfunction.

III. Brief Summary of the invention

The above object is met by providing certain chromanol, quinone or hydroquinone compounds.

The above object is 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 sepsis;





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

- 5
 - 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;
 - or R2 is a hydrogen atom, or an alkyl group with 1-6 carbon atoms, and R3 is
- 10
 - an alkyl group, optionally substituted with nitrogen or oxygen, wherein the alkyl group comprises 3-12 carbon atoms, 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.

15 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.

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

20 together may form amide, urea or carbamate groups.

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.

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

25 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.

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.

5 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.

10 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.

15 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
20 as one of its enantiomers.

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) or the S-enantiomer of SUL-121, namely S-(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)(piperazin-1-yl)methanone (SUL-151) or a pharmaceutically
25 acceptable salt thereof.

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.

In a preferred embodiment according to the invention, the compound either according to formula (I) or according to formula (II) is for use for treating or prophylaxis of sepsis in an
30 organ system, wherein the organ is lung, heart and blood vessels, liver, kidney, brain, or intestines.

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 organ dysfunction caused by a dysregulated host response to infection, and in particular organ disfunction of the kidney, caused by sepsis.

5 The kidney appears to be a key organ in sepsis, as acute kidney injury is most frequently caused by sepsis. In turn, the occurrence of acute kidney injury is strongly associated with failure of other organs and a threefold higher in-hospital mortality rate. Moreover, acute kidney injury leads to a 9-fold increased risk for developing chronic kidney disease after sepsis, associated with an increased risk for developing end-stage renal disease.
10 Hence, preventing or decreasing the severity of acute liver injury is one of the main goals in the treatment of sepsis, which however has to be shown to be very difficult to achieve.

 The compounds for use according to the present invention, in the prophylaxis or treatment of sepsis, generally will be used as adjunct therapy, in addition to the standard therapy of antibiotics and/or other care (see Gotts, cited before).

15 The increased viability of organs may turn out to be very instrumental, not only in the short-term survival of sepsis, but also in the longer term. The long-term survival is of increasing importance: The case fatality rate has decreased during the last decade, which however, leads to an increased number of sepsis survivors at risk for increased long-term morbidity and in particular at major risk for (fatal) cardiovascular events. Consequently, the
20 long-term survival rate after sepsis is less than 50% at 5 years after sepsis.

 The presently invented treatment is expected to substantially improve the long-term survival rate.

IV. Short description of the Figures

25 **Figure 1** shows the xyphoid temperature of mice after the induction of sepsis with the standard cecal ligation and puncture (CLP) model, with and without SUL-138.

Figure 2 shows the plasma levels in mice of cytokines (IL-6, TNF α , and IL12) after induction of CLP with and without treatment with SUL-138.

Figure 3 indicates CLP-induced kidney dysfunction in mice, or the prevention thereof,
30 depending on treatment with SUL-138, as demonstrated by measuring of NGAL and urea in plasma as biomarkers of renal function.

Figure 4 shows CLP-induced kidney inflammation in mice, with or without treatment with SUL-138, as demonstrated by expression of RNA of a number of markers in the kidney.

Figure 5 shows the effect of SUL-151 on survival and on geotaxis after sepsis induction in *Drosophila melanogaster*.

- 5 **Figure 6** demonstrates the effect of SUL-138 on LPS induced mitochondrial dysfunction and cell death *in vitro*.

V. Detailed description of the invention

10 The object of the present invention, to provide compounds for the treatment or prophylaxis of sepsis, and in particular to provide compounds for the treatment or prophylaxis of organ dysfunction caused by a dysregulated host response to infection such as notably kidney dysfunction, is met by providing compounds according to formula (I) or (II), as shown above, or a pharmaceutically acceptable salt thereof for use in the treatment or prophylaxis of sepsis.

15 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 organ dysfunction caused by a dysregulated host response to infection.

Organs, susceptible for damage and/or dysfunction can be one or more of lung, heart and blood vessels, liver, kidney, brain, or intestines.

20 The compounds according to the invention are in particular suitable to treat or prevent organ disfunction of the kidney, caused by sepsis.

The treatment or prophylaxis with the chromanol, quinone or hydroquinone compounds according to the present invention preferably is part of a combination therapy with one or more common other measures to treat sepsis.

25 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.

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.

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.

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.

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.

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.

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.

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.

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

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.

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.

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

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.

5 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) or the S-enantiomer of SUL-121, namely S-(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)(piperazin-1-yl)methanone (SUL-151) or a pharmaceutically acceptable salt thereof.

10 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.

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.

15 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.

20 The compounds are preferably used in effective amounts, to achieve treatment or prophylaxis of sepsis.

The wording treatment or prophylaxis includes amelioration of the symptoms of sepsis and/or reduction in progress of sepsis, including improvement of organ function.

25 Preferably, the compounds according to the invention are for use of treatment or prophylaxis of sepsis in organs in mammals, wherein the mammal is preferably human.

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 organ dysfunction caused by a dysregulated host response to infection.

30 In a most preferred embodiment, the compounds according to the invention are for use of treatment or prophylaxis of kidney dysfunction caused by an infection.

An infection is considered a cause from outside of the body, and is contrasted with for example autoimmune diseases. The general cause for infections is bacterial, fungal, or viral infestation. Bacterial and fungal sources are most common. A recent example of a virus causing a dysregulated host response to infection is COVID-19; upon hospital admission because of respiratory malfunction, the compounds according to the present invention may be administered prophylactically, before sepsis occurs.

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.

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.

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

With parenteral administration, such as for example i.v., or with other liquid forms of administration, larger amounts can be administered.

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 four times daily.

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

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

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.

10 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
15 techniques. The compounds are mixed with pharmaceutically acceptable carriers, adjuvants, and vehicles appropriate for the desired route of administration.

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

As sepsis is often an acute disorder, oral dosage forms can be useful in cases where
20 patients are at risk of developing sepsis, and such oral dosage forms are prophylactically administered to said patients.

However, with sepsis, in particular in acute form, iv injectables and/or continuous iv drip is preferred, because patients often are too ill for oral administration of tablets, pills or the like. Furthermore, sepsis may substantially influence the oral availability of any drug
25 given. Certainty of plasma levels can generally only be achieved with i.v. or other parenteral administration.

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,
30 dispersions, food premixes, and in other suitable forms. The compounds can also be administered in liposome formulations.

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.

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.

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.

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.

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.

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

5

VI. Examples

The effectiveness of the compounds according to the invention for treatment or prophylaxis of sepsis was tested *in vivo* in mice and in drosophila, and *in vitro* in HUVA cells.

10

Example 1: Mice

Experimental

The animal experiments were approved by the Institutional Animal Care and Use Committee of the University Medical Center Groningen (IvD nr. 16593).

Male C57/BL6J mice were housed at room temperature at a light-dark cycle of 12:12 hour. Animals were fed *ad libitum* using standard animal lab chow and they had free access to drinking water at all times.

To induce sepsis, the standard cecal ligation and puncture (CLP) model was used. Animals were anesthetized by subcutaneous injection of xylazine/ketamine (100/10 mg/kg), followed by administration of buprenorphine (0.1 mg/kg) as analgesic. After confirmation of anesthesia by lack of response to paw pinch and eye reflex, the abdomen was shaved, cleaned, and de-germed using a povidone-iodine solution before a 1-cm midline incision was made. The cecum was ligated with a 6-0 suture at half the distance between distal pole and the base of the cecum and punctured once with a 21-Gauge needle ('through-and-through' from mesenteric toward anti-mesenteric direction) which is an accepted model for 'mid-grade' sepsis. A small amount of stool (2-3 mm) was then extruded to ensure wound patency. The cecum was repositioned, thereby taking care not to spill fecal material on the wound edges, followed by closure of the abdomen by running sutures to the abdominal musculature and short interrupted sutures to the skin. Next, 1 ml of saline (warmed, 0.9% NaCl s.c.) was

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administered to compensate for the expected relative volume depletion due to the onset of sepsis.

Mice recovered at 26-28°C. Broad-spectrum antibiotics (imipenem/cilastatine, 100 mg/kg s.c.) were administered at 2 and 10 hours following surgery, together with analgesics (buprenorphine, 0.1 mg/kg body weight, s.c.).

A group of operated animals, in which the cecum was located but not punctured, served as sham.

In addition, a group of animals was included that underwent time-matched anesthesia, but no surgery and served as controls.

Mice in the SUL-138 treated groups were injection with SUL-138 (dissolved in saline, 5 mg/kg, s.c.) at 2 hours before and 8 hours after surgery, while mice in the other groups were injected with an equal volume of saline at these time-points.

The xyphoid temperature of the mice was measured at 8 hr and 24 hr after procedure; results are shown in Fig. 1, discussed below.

Mice were sacrificed 24 hours after the procedure. Upon euthanization, EDTA-anticoagulated blood was separated into plasma by centrifugation at 1,600 g for 10 min and serum by allowing it to clot for 30 min followed by centrifugation at 3,000 g for 10 min.

Plasma, serum and organs were snap-frozen in liquid nitrogen for further analysis.

To quantify the effect of sepsis severity and treatment with SUL-138 on systemic inflammation, levels of TNF α , IL-6 and IL-12 in plasma were measured using Mouse DuoSet ELISAs (DY410, DY406 and D419, respectively, RnD-Systems), according to the manufacturer's instructions. Briefly, ELISA plates (DY990, RnD Systems) were coated overnight with the capture antibody diluted in 100 μ L PBS. Plates were washed three times with wash buffer (0.05% Tween20 in PBS; 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na₂HPO₄, 1.5 mM KH₂PO₄, pH 7.2-7.4), followed by blocking for one hour with 300 μ L of reagent diluent (1% probumin w/v in PBS). Washing was repeated and samples were added to the wells. Plasma samples were diluted 10x for TNF α and IL-12, and 100x for IL-6 in reagent diluent. After incubation for 2 hours at room temperature, plates were washed, followed by adding 100 μ L of detection antibody diluted in reagent diluent to each well. Again, plates were left to incubate for 2 hours at room temperature, followed by washing. Finally, 100 μ L of substrate solution (DY999, RnD Systems) was added and after 20 minutes incubating in

the dark, 50 µL stop solution (2M H₂SO₄) was added. The optical density (OD) was measured using a microplate reader set to 450 nm, while readings at 540 nm were subtracted as correction to increase accuracy. Results are shown in Fig. 2, discussed below.

Furthermore, the amount of NGAL and urea were measured in serum. NGAL and urea are common biomarkers for kidney damage in mice; results are shown in Fig. 3, discussed below.

In order to assess the expression of proinflammatory cytokines and adhesion molecules in the kidney, including IL-6, TNF- α , IL-1 β and ICAM, RNA was isolated from approximately 30 mg kidney tissue using Nucleospin RNA (Machery-Nagel, Düren, Germany), quantified using nanodrop spectrophotometer ND-1000 and converted into copy DNA (cDNA), using 0.5 µg RNA in each sample. For RNA isolation from cells, the same kit was used with slight adaptations: TRIzol and chloroform as lysis buffer, instead of the lysis buffer from the kit. Oligonucleotide primers were designed using NCBI Primer Blast and Clone Manager (see appendix) and validated by assessing the efficiency, melting temperature and curve using qRT-PCR and size on gel electrophoresis of the naive and enzymatically digested products. qRT-PCR amplification was performed using the following thermal profile: 95°C for 2 min, followed by 40 cycli of 95°C for 15 sec, 58°C for 30 sec and 72°C 30 sec. All reactions were carried out in triplicate and a standard curve for each primer was used. Results are shown in Fig. 4, discussed below.

Results

Figure 1 shows the xyphoid temperature of mice after the induction of CLP with and without SUL-138 versus an unchallenged control. Figure 1 demonstrates that CLP procedures result in a reduction of xiphoid temperature after 8 hr and 24 hr, while upon treatment with SUL-138 xiphoid temperature is restored after 24 hours. Significant differences are calculated at 24 hr between unchallenged control versus CLP/saline and CLP/saline versus CLP/SUL-138 using an unpaired one-sided Student's T-test. A '*' in the figures indicates a significant difference.

Figure 2 shows that the plasma levels of the inflammatory cytokines (IL-6, TNF- α and IL12) are decreased by treatment with SUL-138 to levels close to untreated control. Significant differences are calculated between control versus CLP/saline, Sham versus

CLP/saline and CLP/saline versus CLP/Sul-138 using an unpaired one-sided Student's T-test. TNF- α was clearly lower, but the difference did not reach significance in the T test.

Figure 3 indicates that CLP-induced kidney dysfunction is precluded by treatment with SUL-138. Serum urea (A) and NGAL (B) levels are profoundly increased in sepsis, which is precluded by treatment with SUL-138. Serum urea and NGAL levels in animals treated with SUL-138 before CLP were not different from Sham operated animals. Significant differences are calculated between control versus CLP/saline, Sham versus CLP/saline and CLP/saline versus CLP/SUL-138 using an unpaired one-sided Student's T-test.

Figure 4 indicates that CLP-induced kidney inflammation is strongly reduced by treatment with SUL-138. Sepsis induced by CLP upregulated the expression of proinflammatory cytokines and adhesion molecules in the kidney, including IL-6, TNF- α , IL-1 β and ICAM. Treatment of animals before induction of sepsis by CLP with SUL-138 fully prevented the rise in IL-6 expression (see Figure 4A). TNF- α , IL-1 β or ICAM (Figure 4B-D) were lower than in the untreated CLP animals but the difference was not statistically significant using an unpaired one-sided Student's T-test.

Discussion

The reduced body temperature of mice after induction of sepsis illustrates loss of metabolic homeostasis, the restoration after 24 h in mice treated with SUL-138 suggests restoration of metabolic homeostasis despite sepsis.

The increased plasma levels of IL-6 and IL-12 indicate systemic inflammation induced by CLP, while both cytokines are significantly lower after CLP in animals treated with SUL, indicative for reduced levels of inflammation.

The increased levels of NGAL and urea after induction of CLP indicate acute kidney dysfunction. NGAL and urea are biomarkers for renal function in mice. Figure 3 shows that CLP-induced kidney dysfunction in mice is precluded, or at least substantially reduced by treatment with SUL-138.

RNA expression of IL-6, TNF- α , IL-1 β and ICAM in the kidney were increased after induction of CLP, indicating a local inflammatory response in the kidney. Treatment with

Sul-138 significantly reduced expression of IL-6, while other markers were lowered by treatment with SUL-138.

Example 2: *Drosophila Melanogaster*

5

Experimental

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 standard yeast-cornmeal medium. Flies kept as a stock were flipped to fresh vials weekly. The used yeast-cornmeal medium was prepared to the instructions of Stocker and Gallant's 'Drosophila Methods and Protocols' and consisted of 100 grams yeast, 75 grams glucose, 8 grams agar, 55 grams cornmeal, and 10 grams wheat flour per litre of water. After boiling, phosphoric acid and propionic acid were added to the mixture to prevent fungal growth.

15 *Staphylococcus Aureus* (*S. Aureus*) was aerobically cultured overnight in 2.5% tryptic soy broth (TSB) at 37 °C with continuous rotation (200 rounds per minute), from an *S. Aureus* glycerol stock kept at -80 °C. For each experiment, a fresh culture was prepared one day prior to the experiment. For bacterial infection, optical density (OD) was measured the following day at 600 nm. Clean TSB was used as OD=0.00 and the bacterial culture was diluted to OD=2.20 with PBS. Next, 1.0 mL of the bacterial culture (OD=2.20) was

20 centrifuged at 14,000 g for 1 minute, the supernatant was discarded, and the bacterial pellet resuspended in 1.0 mL PBS. Serial dilutions (5x and 25x diluted in PBS with optical densities of approximately 1.05 and 0.25, respectively) were used in the appropriate experiments.

Male flies (three to five days old) were anesthetized on a CO₂ pad just prior to

25 injection. A tungsten needle (0.25mm diameter, Fine Science Tools, 10130-10) was dipped into the bacterial suspension and flies were pinpricked in the lateral side of the thorax (the presutural scutum of the thorax). Sham flies were administered PBS only, control flies were not operated and only anesthetized on the CO₂ pad. Sham flies were injected first to ensure a haemolymph coating on the needle and to prevent accidental bacterial injection of sham flies.

30 Subgroups of flies were treated with antibiotics. Therefore, linezolid was administered orally by dissolving 500 µg of the substance per 1 mL fly medium. After pinpricking intervention

groups with various concentrations of *S. Aureus*, flies were placed in vials containing linezolid-medium.

After 48 hours, the flies were introduced to non-antibiotic containing vials. To verify the efficiency in inducing a bacterial infection, bacterial load was determined by homogenization of ten flies in 250 μ L PBS. After brief centrifugation, the supernatant was plated in 1/10, 1/100, and 1/1000 dilutions onto Luria-Bertani (LB) agar plates and checked 24 hours later.

Linezolid was administered orally by dissolving 500 μ g of the substance per 1 mL fly medium. After pinpricking, control flies, sham-operated flies and the infected intervention groups with various concentrations of *S. Aureus*, flies were placed in vials containing linezolid-medium. After 48 hours, the flies were introduced to non-antibiotic containing vials.

The compound SUL-151 was administered via intrathoracic injection simultaneously with the bacterial injection. The intervention group received 3mM SUL-151 and the vehicle infected flies received 1% DMSO, which were dissolved in the bacterial solution itself just prior to bacterial injection.

Negative geotaxis was studied as a marker of fly health. Therefore, groups of 15 flies were transferred to empty styrene vials (9.5 cm tall). Up to nine vials were then placed into a 3d-printed vial holder designed for negative geotaxis. Flies were allowed to acclimatize for five minutes. The flies were then tapped to the bottom of the vial by tapping the vial holder three times against the worktop. A digital camera was used to take a picture five seconds after the last tap. This experiment was repeated five times, with a one-minute pause between every try. ImageJ was used to determine the distance every fly travelled within five seconds and averaged over five tries per group.

Additionally, fly survival was checked by visual inspection, counting dead flies in the vial, at 24 hours after infection.

Results

Figure 5 shows the effect of SUL-151 on survival (A) and geotaxis (B) after sepsis induction in *Drosophila Melanogaster*. Survival after 24 hr was 80% for SUL-151 treated flies, while only 42% for the flies with sepsis without SUL treatment (A). Geotaxis was

improved both at 24 hr and 48 hr after induction of sepsis in flies pre-treated with SUL (B). Significant differences are calculated between control versus sepsis/DMSO and sepsis/DMSO versus sepsis /SUL-151 using an unpaired one-sided Student's T-test.

5 Discussion

Injection of 3mM SUL-151 at the induction of sepsis lead to improved geotaxis at 24 and 48 h after the induction of sepsis, while mortality was significantly reduced at 24 hr. Both measures indicate efficacy of SUL151 against infection induced inflammation.

10 Example 3: Endothelial cells

Experimental

Human Umbilical Vein Endothelial cells (HUVEC) were obtained from the RuG/UMCG Endothelial Cell Facility. Primary isolates of umbilical cords were mixed and
15 subsequently cultured on HUVEC culture medium, consisted of RPMI 1640 (Lonza, art.nr. BE12-115F) supplemented with 20% heat-inactivated fetal calf serum (ThermoFisher Scientific, art.nr. 10082147), 2 mM l-glutamine (Life Technologies art.nr. 25030), 5 U/ml heparin (Leo Pharmaceutical Products), 1% Penicillin/Streptomycin (Sigma-Aldrich art.nr. P4333), and 50 µg/ml EC growth factor supplement from (Sigma-Aldrich, art.nr. E2759).

20 Primary HUVECs were cultured in 75-cm² tissue culture flasks (Corning, art.nr. 430720U) at 37 °C under 5% CO₂/95% air. HUVECs were used for experiments up to passage 8. Experiments were performed in 6-well (Corning art.nr. 3506) or 96-well culture plates (Corning, art.nr. 3596), at 80% confluency. Cells were stimulated with LPS E. Coli 0111:B4 (Sigma-Aldrich, art.nr. L2630) in different concentrations. Cells were detached with
25 trypsin (Sigma-Aldrich, art.nr. 25300054). All compounds were dissolved in Hanks Balanced Salt Solution (Lonza, art.nr. 10-527F).

HUVECs were pre-incubated with SUL-138 in an amount of 10 microgram/ml, which was added one hour prior to LPS-stimulation. Such pre-incubation is the standard in *in vitro* models.

Membrane potential was measured by using JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl-imidacarbocyanine iodide). Mitochondrial membrane potential was measured after 30 minutes.

Cells and mitochondria were incubated and measured according to manufacturer's protocol in the Synergy H4 micro plate reader (Bio-Tek) at an excitation/emission rate of 548/574 nm.

Results

Figure 6 demonstrates that SUL-138 protects against LPS induced mitochondrial dysfunction and cell death *in vitro*.

Figure 6A demonstrates the HUVECs respiration and shows a reduction in uncoupled state (CCCP) with 10 ug/ml LPS, compared to control. LPS challenged HUVECs treated with SUL-138 almost restores the uncoupled respiration.

Figure 6B shows the LPS-induced increased mitochondrial oxidative stress, measured by MitoSOX. MitoSOX measures ROS production in the mitochondrial, thus SUL-138 reduces LPS induced mitochondrial oxidative stress.

Figure 6C demonstrates that 48 hours with LPS on HUVECs resulted in reduced cell survival, while SUL-138 restores cell survival after 48 hours. Cell death was measured by Cyquant.

Significant differences are calculated between control versus LPS and LPS versus LPS/SUL-138 using an unpaired one-sided Student's T-test.

Discussion

The experiments performed on the LPS-treated endothelial cells demonstrate that sepsis on the cellular level involves mitochondrial dysfunction, which is restored by compounds according to the invention, while whole cell survival increased significantly with the use of SUL-138.

Without being bound by theory, the inventors believe that the compounds according to the invention can be used for the treatment or prophylaxis of sepsis because they protect the cells against LPS-induced mitochondrial dysfunction and cell death.

Conclusions

The examples show SUL-138 to restore the xyphoid temperature after 24 hours after indication of CLP in mice. Treatment with SUL-138 decreases plasma levels of cytokines after sepsis induction. In particular, CLP-induced kidney dysfunction is precluded or
5 significantly reduced by treatment with SUL-138 in mice as shown with the restoration of NGAL and urea function.

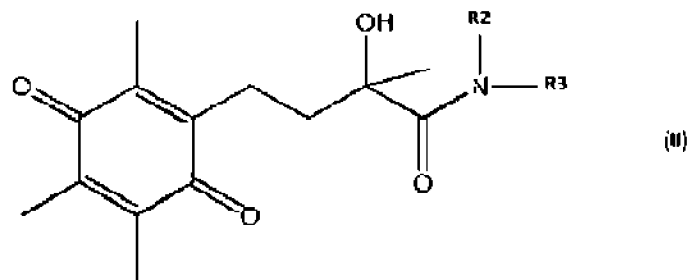
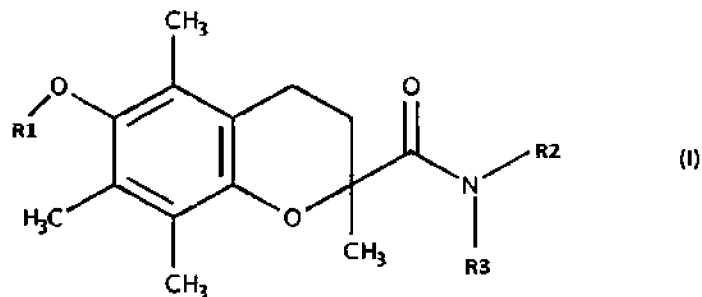
The examples also show that SUL-151 lowers mortality in septic *Drosophila melanogaster* and improves geotaxis at 24h and 48h after the induction of sepsis.

In septic endothelial cells, SUL-138 restores mitochondrial dysfunction.

10 This whole body of *in vivo* and *in vitro* evidence shows that compounds as defined by the present invention show efficacy in treating sepsis, and organ dysfunction caused by infection induced inflammation.

CONCLUSIES

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



- waarbij R1 voor een waterstof of prodrug-groep staat die in levend weefsel kan worden verwijderd
- en waarbij ofwel
 - o R2 en R3 samen met het N-atoom waaraan ze zijn gebonden een verzadigde of onverzadigde, niet-aromatische, optioneel gesubstitueerde 5-8-ledige ring vormen, met één tot vier N-, O- of S-atomen, 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, die optioneel gesubstitueerd is met stikstof of zuurstof, waarbij de alkylgroep 3-12 koolstofatomen omvat, waarbij de alkylgroep in R3 één of meer niet-aromatische cyclische structuren omvat die stikstof- of zuurstofatomen in de ring omvatten en lineaire en/of vertakte gesubstitueerde groepen en één of meer ethylenische onverzadigingen kan 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.
- 5 3. Verbinding voor gebruik volgens een van de conclusies 1-2, waarbij de stikstof amine, quaternaire amine, guanidine of imine kan zijn en zuurstof hydroxyl, carbonyl of carbonzuur is; en/of zuurstof en stikstof samen amide-, ureum- of carbamaatgroepen vormen.
- 10 4. Verbinding voor gebruik volgens een van de conclusies 1-3, waarbij in beide verbindingen volgens formule (I) of volgens formule (II) R2 en R3 samen met het N-atoom waaraan ze zijn gebonden een verzadigde ring vormen met een extra N-atoom, waarbij de ring ongesubstitueerd of gesubstitueerd is met een alcohol of alkanolgroep met 1-4 koolstofatomen.
- 15 5. Verbinding voor gebruik volgens een van de conclusies 1 - 4, waarbij de verbinding een verbinding volgens formule I is.
- 20 6. Verbinding voor gebruik volgens conclusie 5, waarbij R2 en R3 samen met het N-atoom waaraan ze zijn gebonden een 5-7-ledige ring vormen die één extra aminegroep omvat, waarbij de ring optioneel gesubstitueerd is met methyl, ethyl of alcohol gesubstitueerd methyl of ethyl.
- 25 7. Verbinding voor gebruik volgens een van de conclusies 1-3, waarbij R2 een waterstofatoom is en R3 een verzadigde cyclische structuur omvat met 4-7 koolstofatomen en met één stikstofatoom, waarbij de 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 is volgens formule II en waarbij R2 een waterstofatoom is en R3 een cyclische structuur omvat met 4-6 koolstofatomen en met één stikstofatoom waarbij de ring optioneel gesubstitueerd is met methyl, ethyl of alcohol gesubstitueerd methyl of ethyl.

5

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-carboxamidehydrochloride (SUL-13) of (6-hydroxy-2,5,7,8-tetramethylchromaan-2-yl)(4-(2-hydroxyethyl)piperazine-1-yl)methanon (SUL-109) is of een farmaceutisch aanvaardbaar zout daarvan, als een racemisch mengsel of als één van zijn enantiomeren.

10

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) is of een farmaceutisch aanvaardbaar zout daarvan.

15

11. Verbinding voor gebruik volgens conclusie 9, waarbij de verbinding de S-enantiomeer van SUL-121: S-(6-hydroxy-2,5,7,8-tetramethylchromaan-2-yl)(piperazine-1-yl)methanon (SUL-151) is of een farmaceutisch aanvaardbaar zout daarvan.

20

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

25

13. Verbinding voor gebruik volgens een van de voorgaande conclusies, waarbij het gebruik is voor de behandeling of profylaxe van orgaandisfunctie die veroorzaakt wordt door een ontregelde gastheerreactie op infectie.

14. Verbinding voor gebruik volgens conclusie 13, waarbij het orgaan één of meer van long, hart en bloedvaten, lever, nier, hersenen of darmen is, bij voorkeur nier.

30

15. Verbinding voor gebruik volgens een van de conclusies 1 - 14, waarbij de behandeling of profylaxe wordt uitgevoerd in een combinatietherapie met één of meer gebruikelijke maatregelen om sepsis te behandelen.

Figure 1

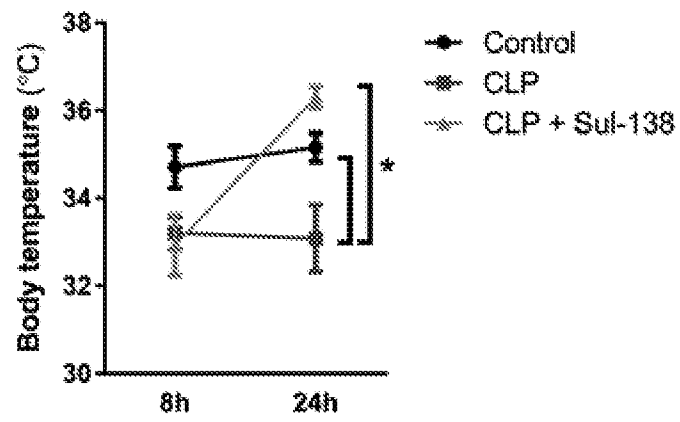


Figure 2

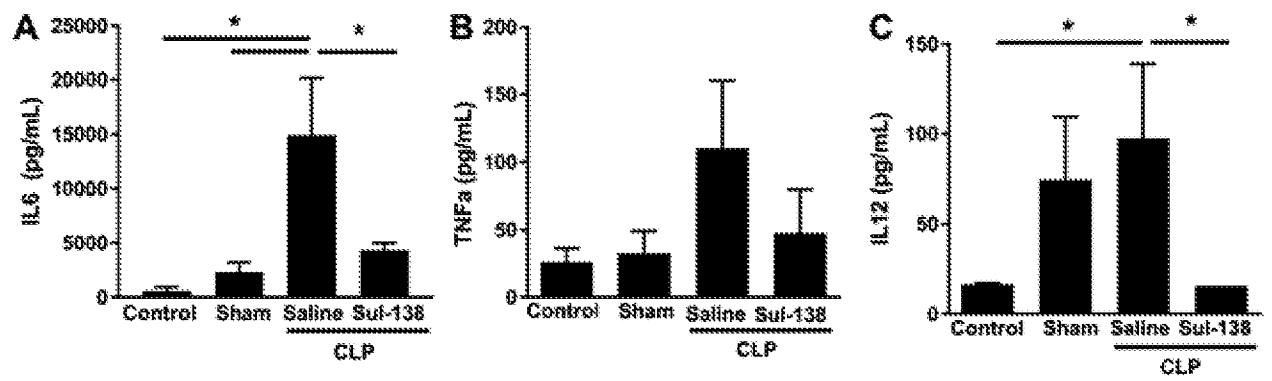


Figure 3

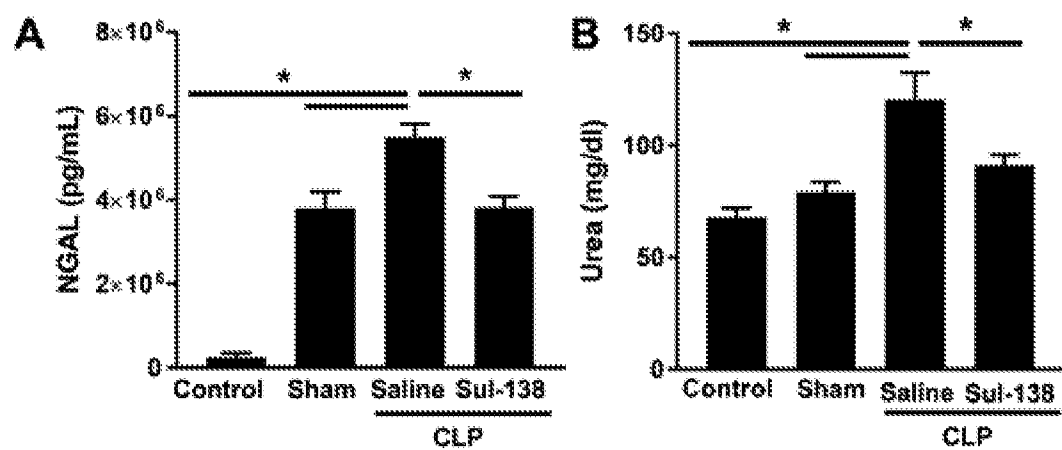


Figure 4

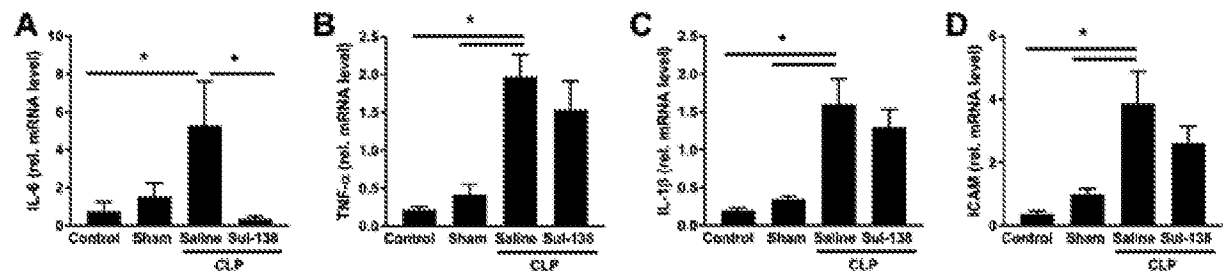


Figure 5

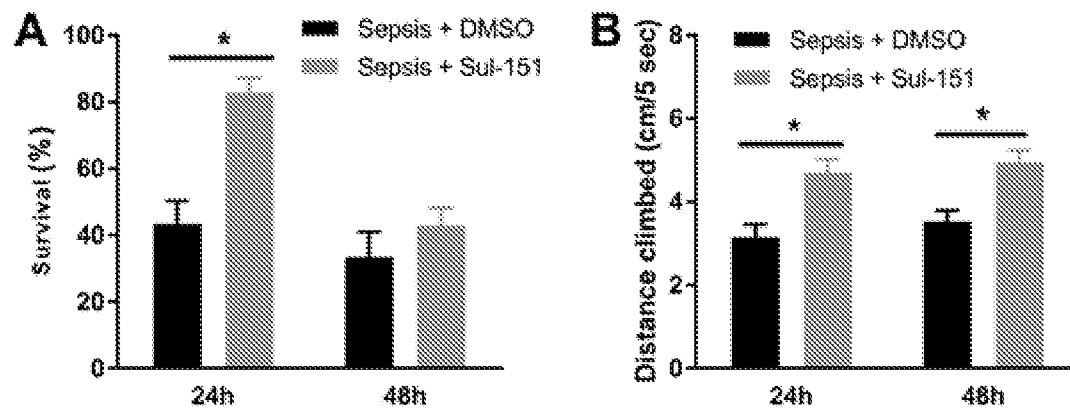
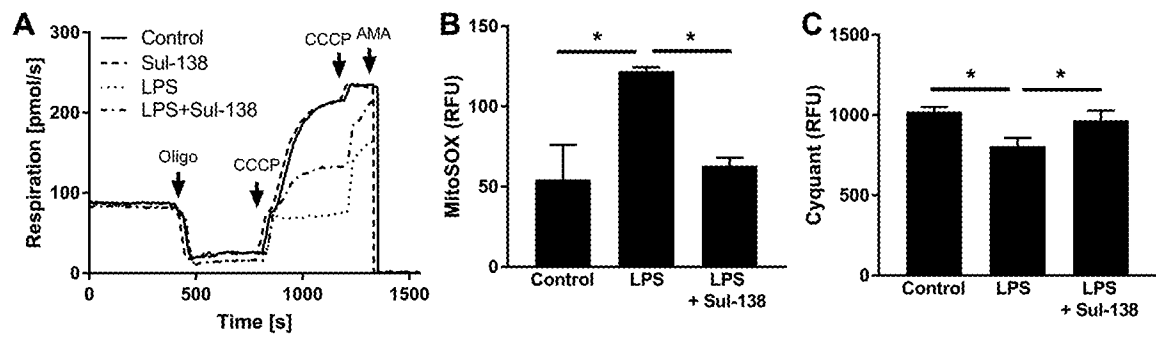


Figure 6



SAMENWERKINGSVERDRAG (PCT)

RAPPORT BETREFFENDE NIEUWHEIDSONDERZOEK VAN INTERNATIONAAL TYPE

IDENTIFICATIE VAN DE NATIONALE AANVRAGE		KENMERK VAN DE AANVRAGER OF VAN DE GEMACHTIGDE	
Nederlands aanvraag nr. 2025730		Indieningsdatum 02-06-2020	
		Ingeroepen voorrangsdatum	
Aanvrager (Naam) Sulfateq B.V., et al			
Datum van het verzoek voor een onderzoek van internationaal type 03-10-2020		Door de Instantie voor Internationaal Onderzoek aan het verzoek voor een onderzoek van internationaal type toegekend nr. SN77001	
I. CLASSIFICATIE VAN HET ONDERWERP (bij toepassing van verschillende classificaties, alle classificatiesymbolen opgeven)			
Volgens de internationale classificatie (IPC) Zie onderzoeksrapport			
II. ONDERZOCHE 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**

Nummer van het verzoek om een onderzoek naar
de stand van de techniek
NL 2025730

A. CLASSIFICATIE VAN HET ONDERWERP		
INV.	A61K31/353 A61K45/06 A61K31/16 A61K31/165 A61K31/453	
	A61K31/496 A61P31/00	
ADD.		
Volgens de Internationale Classificatie van octrooien (IPC) of zowel volgens de nationale classificatie als volgens de IPC.		
B. ONDERZOCHE 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, WPI Data, BIOSIS, CHEM ABS Data, EMBASE		
C. VAN BELANG GEACHTE DOCUMENTEN		
Categorie °	Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages	Van belang voor conclusie nr.
X	US 6 231 894 B1 (STAMLER JONATHAN S [US] ET AL) 15 mei 2001 (2001-05-15) * kolom 10, regel 4 - regel 24 * * kolom 5, alinea 1 * -----	1-9, 12-15
☐ 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		Verzenddatum van het rapport van het onderzoek naar de stand van de techniek van internationaal type
4 februari 2021		
Naam en adres van de instantie		De bevoegde ambtenaar
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Strack, Eberhard

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

Informatie over leden van dezelfde octrooifamilie

Nummer van het verzoek om een onderzoek naar
de stand van de techniek

NL 2025730

In het rapport genoemd octrooigeschrift	Datum van publicatie	Overeenkomend(e) geschrift(en)	Datum van publicatie
US 6231894	B1	15-05-2001	AT
		462434 T	15-04-2010
		AU	778967 B2
			23-12-2004
		CA	2386990 A1
			26-04-2001
		EP	1244460 A1
			02-10-2002
		ES	2343506 T3
			03-08-2010
		US	6231894 B1
			15-05-2001
		WO	0128568 A1
			26-04-2001

WRITTEN OPINION

File No. SN77001	Filing date (<i>day/month/year</i>) 02.06.2020	Priority date (<i>day/month/year</i>)	Application No. NL2025730
International Patent Classification (IPC) INV. A61K31/353 A61K45/06 A61K31/16 A61K31/165 A61K31/453 A61K31/496 A61P31/00			
Applicant Sulfateq B.V., et al			

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 Strack, Eberhard
--	------------------------------

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 and necessary to the claimed invention, 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	2-4, 7-15
	No: Claims	1, 5, 6
Inventive step	Yes: Claims	10, 11
	No: Claims	1-9, 12-15
Industrial applicability	Yes: Claims	1-15
	No: Claims	

2. Citations and explanations

see separate sheet

WRITTEN OPINION

Application number
NL2025730

Box No. VIII Certain observations on the application

see separate sheet

It appears that the subject-matter of claims 10-11 apparently involves an inventive step and is thus patentable. The remaining subject-matter needs to be deleted from the claims and the description.

V.1 The following document (D) is referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1 US 6 231 894 B1 (STAMLER JONATHAN S [US] ET AL) 15 mei 2001
(2001-05-15)

Unless otherwise mentioned, the passages cited in the search report are considered to represent the pertinent passages of said document.

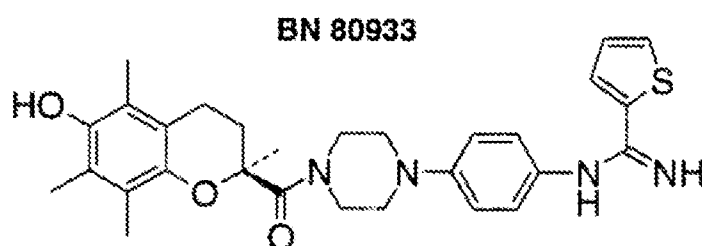
The patentability can be dependent upon the formulation of the claims. The EPO, for example, does not recognise as patentable claims to a method of treatment or the use of a compound in medical treatment, but may allow claims to a product, in particular substances or compositions for use in a first or further medical (therapeutic or prophylactic) treatment.

Patentability, in particular novelty and inventive step, of the claims has been assessed on the basis of a purpose-limited product claim taking into account the alleged effects of the compound/composition.

V.2 Lack of novelty

V.2.1 Claims 1, 5, 6

D1 discloses BN80933 for treating sepsis.



Said compound falls within the scope of said claims in that R2 and R3 form piperazinyl which is further substituted (optional substitution is presently claimed).

Plausibility of said disclosure comes from the fact that the compound is bio-transformed to an NO donor, with NO being commonly known to be involved in the pathophysiology of sepsis.

The subject-matter of said claims is thus not novel.

The remaining dependent claims appear to be novel; their characterising features which constitute a technical difference are discussed below.

V.3 Inventive Step

V.3.1 In as far as the objections of lack of novelty (see Item V) could be overcome, the application would still lack an inventive step over D1, as the subject-matter of the present claims in as far as it could be novel appears to be obvious over said document.

Also, the subject-matter relating to formula II which is insufficiently disclosed as lined out in item VIII below cannot be considered inventive: such subject-matter can only constitute arbitrary modifications of D1 devoid of an inventive step.

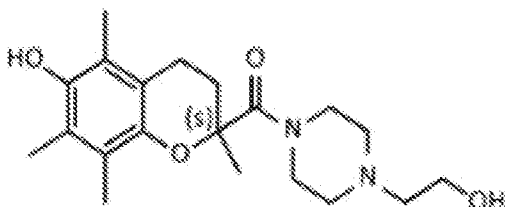
V.3.2 The subject-matter of claims 10-11 seems inventive.

The reasons are as follows:

D1 constitutes the closest prior art. The technical difference for said subject-matter is the substitution of the piperazine ring.

No technical effect is readily apparent from such difference.

The objective technical problem is the provision of an alternative sepsis treatment. Such problem has been solved as evidenced by the data in the application. The compound SUL-138 has been tested by the applicant, indicating pharmacological activity in sepsis treatment:



Also, SUL-151 has been tested, which has the same structure as SUL-138, without the hydroxyethyl substitution of the piperazinyl group.

Starting from D1, the person skilled in the art would not necessarily expect pharmacological activity in treating sepsis from the claimed compounds, because the modification of the substituent of the piperazine group is substantial.

V.3.2 Claim 2-4, 7-9, 12-15, which are dependent on claim 1, also lack an inventive step:

Their characterising features (i.e. claim 2: specific substitutions in R1; claim 3: specific substitutions in R3 for, in particular, oxygen; claim 4: particular substitution of the ring formed by R2 and R3; claims 7-9: particular selections for R2 and R3; claim 12: compounds of a particular molecular mass; claims 13-14: particular disease sub-groups; claim 15: combination therapy) are modifications for which, with regard to a resulting technical effect, no credible information e.g. in form of pharmacological data, is provided.

In the absence of such information, the technical problem to be solved vis-à-vis the cited prior art is the provision of an alternative treatment.

As the technical features of said dependent claims represent minor modifications within the ambit of the skilled person, only, the subject-matter of said claims is regarded as constituting obvious solutions which cannot involve an inventive step.

Item VIII

Importantly, it appears that only compounds falling under claimed formula I have been pharmacologically tested by the applicant (SUL-138 and SUL-151). The application thus provides support and disclosure thus only for compounds of formula I, and not for any of the (structurally fundamentally different) compounds of formula II (let alone its claimed analogues). With the claims encompassing a much broader scope than what is sufficiently disclosed and supported, the application thus lacks disclosure and the claims lack support.