

TOPICAL COMPOSITIONS OF 2-PHENYL-3,4-DIHYDROPYRROLO[2,L-F]
[1,2,4]TRIAZINONE DERIVATIVES AND USES THEREOF

The present invention relates to topical compositions comprising compounds of formula I and thus 2-phenyl-3,4-dihydropyrrolo[2,l-f][1,2,4]triazinone derivatives. The invention also
5 relates to uses of said topical compositions for the treatment of diseases or disorders alleviated by inhibition of PDE5 and/or NO related endothelial dysfunction in a subject, preferably in a human, and particularly, for the treatment of vascular diseases and disorders, in particular peripheral vascular diseases and disorders including for wound and chronic wound healing.

RELATED ART

10 The following discussion of the related art of the disclosure is merely provided to aid the reader in understanding the disclosure and is not admitted to describe or constitute related and art to the present disclosure.

Phosphodiesterases (PDEs) are enzymes that catalyzes the hydrolysis and thus the degradation of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate
15 (cGMP) and thereby regulates intracellular levels of second messengers. Inhibition of PDEs leads to increasing intracellular concentrations of endogenous cAMP/cGMP. Therefore, inhibition of PDE can mediate a variety of physiological mechanisms at different cell and organ levels. Phosphodiesterase type 5 (PDE5) hydrolyses cyclic guanylate monophosphate (cGMP) specifically to 5' GMP. The selective inhibition of PDE5 has been validated as a relevant
20 approach and strategies directed to promote inhibition of PDE5 activity have been applied as therapeutic tools, in particular, in neuronal, vascular and cardiovascular diseases and disorders. Moreover, the introduction of PDE5 inhibitors has revolutionized the treatment of male erectile dysfunction (Andersson KE, British Journal of Pharmacology (2018) 175:2554–2565; Das A et al. Pharmacol Ther. (2015) 147:12–21; Dobhal T et al. Critical Review in Pharmaceutical
25 Sciences (2012) 1(3):13-27). Most prominent examples of PDE5 inhibitors are Sildenafil, Tadalafil and Vardenafil, which have been described among others, for example, in WO 99/24433, WO 01/60825, EP 995'751 and WO 2011/075655. Recently a novel class of PDE 5 inhibitors with dual-pharmacology releasing NO in addition to its PDE 5 inhibition in a more than additive fashion has been described (WO 2017/085056).

30 Systemic sclerosis (SSc, ICD-10 M34) is a rare, complex, multiorgan, autoimmune, chronic connective tissue disease of unknown etiology characterized by vascular abnormalities

and diffuse fibrosis in the skin, joints, and internal organs (especially the oesophagus with gastroesophageal reflux disease (GERD) and dysphagia, lower gastrointestinal tract, lungs, heart, and kidneys). Common symptoms include (secondary) Raynaud's phenomenon (RP), digital ulcers (DU), polyarthralgia, dysphagia, heartburn, and swelling and eventually skin tightening and contractures of the fingers. Lung, heart, and kidney involvement accounts for most deaths (Allanore Y et al. *Nat Rev Dis Primers* (2015) 1:15002; Pearson DR et al. *Clin Dermatol* (2018) 36(4):459-474; Denton CP and Khanna D, *Lancet* (2017) 390:1685-1699). Digital ulcers (DU) that are often located at the fingertips or extensor areas of proximal interphalangeal joints are a frequent clinical manifestation in SSc. Digital ulcers are defined as a loss of epidermal covering with a break in the basement membrane (which separates dermis from epidermis). Clinically digital ulcers in SSc appear as a wound with visible blood vessels, fibrin, granulation tissue and/or underlying deeper structures (e.g., muscle, ligament, fat) or as it would appear on debridement. Digital ulcers substantially contribute to the disease burden in systemic sclerosis also because in general, they are painful and disabling, limiting HRQoL (Amanzi L et al. *Rheumatology (Oxford)* (2010) 49(7):1374-1382; Li W and Frech TM, *J Scleroderma Relat Disord.* (2017) 2(2):69-71; Hughes M et al. *Nat Rev Rheumatol.* (2020) 16(4):208-221). DU derived from digital pitting scars (DPS) are hidden below hyperkeratotic layers of DPS. Digital pitting scars are defined as small hyperkeratotic lesions overlying a cutaneous depression resulting from chronic ischemia. Such ulcers are rather small, superficial (not full thickness wounds) with predilections sites at fingertips and dorsal regions of digits. In the perilesional skin there are signs of inflammation and oedema. DU derived from DPS are associated with spontaneous pain. Such superficial DUs have a mean time to healing of less than one month and in general complications as gangrene or auto-amputation do not occur. Ischemia is assumed to account in part for DU derived from DPS.

Cutaneous signs and symptoms of SSc are of particular importance as they are the earliest, most frequent and most characteristic manifestations and recognized before systemic manifestations, thus allowing earlier initiation of management and treatment (Pearson DR et al. *Clin Dermatol* (2018) 36(4):459-474; Herrick et al. *Exp Dermatol.*(2020) 29:1144-1153). As a general approach, patients should be managed using non-pharmacological interventions such as patient education as first-line treatment, followed by pharmacological and surgical interventions. Regarding pharmacological interventions, systemic vasoactive therapies attempt to address the underlying factors that are implicated in the pathogenesis of SSc such as DU and RP are often used as a first-line therapy, although oral phosphodiesterase type 5 (PDE5) inhibitors such as sildenafil are increasingly used early on in the treatment of SSc and SSc-

associated digital vasculopathy. However, vasodilatory adverse effects are not uncommon with systemic vasoactive therapies and PDE5 inhibitors. For example, oral sildenafil display common adverse drug reactions such as vital signs, namely systemic arterial blood pressure, heart rate and ECG, let alone that these adverse drug reactions are more common in patients receiving higher possible doses than lower possible doses (Hughes M et al. *Nat Rev Rheumatol.* (2020) 16(4):208-22). Moreover, a recent placebo-controlled trial of the PDE5 inhibitor sildenafil observed no difference in ulcer healing between placebo and sildenafil (Hachullan E. et al. *Ann. Rheum. Dis.* (2016) 75, 1009–1015).

Therapies to topically treat SSc Digital Ulcer or Reynolds Phenomena were explored in experimental clinical trials using glyceryl trinitrate in concentrations between 0.9% up to 2% (Hughes M et al. *Microvascular Research* (2017) 111:32–36; Hummers LK, et al. *Ann Rheum Dis* (2013) 72:1962–1967). Excipients in the used water-based formulations were lanolin- or 50% lecithin. The topical formulations were described as unpleasant. Moreover, described treatment side effects of glyceryl trinitrate were headiness, dizziness, vasodilatory side effect and pain. Other experimental clinicals trial for topical treatment of digital ulcers were described using very high concentrations of a formulation of D- α -tocopheryl acetate (96.0%) and micronized silica (4.0%) as gel, or using a topically applied dimethyl sulfoxide solution. However, none of these topical applications are approved for treatment of DU by a Regulatory Authority or are recommended by EULAR recommendations for the treatment of systemic sclerosis (Fiori G et al. *Clinical and Experimental Rheumatology* (2009) 27(Suppl 54):S51-S54; Williams DE et al. *Arthritis Rheum.* (1985) 28(3):308-14; Kowal-Bielecka O et al, *Ann Rheum Dis.* (2017) 76(8):1327-1339).

Thus, there is still a strong and unmet medical need for treatment of vascular diseases and disorders, and in particular peripheral vascular diseases and disorders including for wound and chronic wound healing.

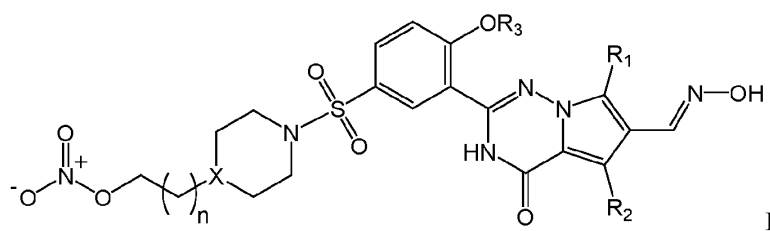
SUMMARY OF THE INVENTION

The inventors have surprisingly found and shown that topically, on wound treatment of the compounds and compositions of the invention demonstrated efficacy and improved wound healing in uPAR deficient mice, a disease-relevant animal model of systemic sclerosis having cutaneous ulcers. Further surprisingly, the inventors were able to provide inventive topical compositions, in particular inventive topical compositions comprising Compound 1, despite the shown de-facto non-solubility of the inventive compounds of formula I, in particular of

Compound **1** in physiological aqueous phosphate buffer (pH 7.5). Moreover, the inventive topical compositions showed efficacy and improved wound healing in the disease-relevant animal model of systemic sclerosis having cutaneous ulcers at surprisingly very low concentrations of the inventive compounds of formula **I**, in particular of Compound **1**.

Moreover, it has been proven that local and topical administration rather than systemic exposure of the inventive compounds caused the improved wound healing. This is particularly advantageous since systemic or oral delivery of the prior art drugs has its limitations not only due to vascular abnormalities and diffuse fibrosis in the skin, but also due to adverse drug reactions observed with the systemic or oral vasodilators. Therefore, suboptimal pharmacokinetics and/or pharmacodynamics associated with systemic drug exposure such as systemic arterial hypotension are avoided or very unlikely to occur by and with the inventive topical administration of compounds and compositions of the invention. Moreover, the topical administration directly to the primary site of action allows application of efficacious therapeutic doses of the inventive compounds to be significantly lower as compared to systemic or oral administration, and thus to integrate local efficacy on wound with minimal systemic exposure. Finally, the topical delivery (at home) is a more convenient mode of administration than intravenous administration or an oral administration (medical ward).

Thus, in a first aspect, the present invention provides a topical composition comprising a compound of formula **I**, preferably an effective amount of a compound of formula **I**, or a pharmaceutically acceptable salt thereof



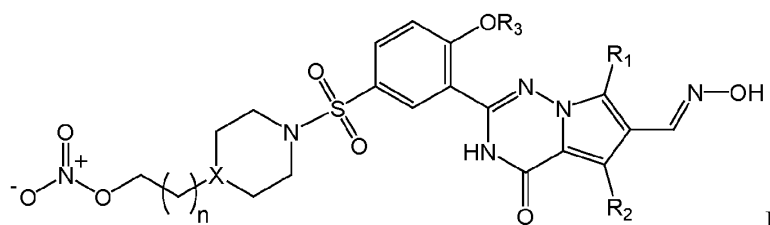
wherein

- R_1 is ethyl or propyl, preferably propyl;
- R_2 is methyl or ethyl, preferably methyl;
- R_3 is ethyl or propyl, preferably propyl;
- X is N or CH, preferably CH;
- n is 1 or 2, preferably n=1.

In another aspect, the present invention provides a topical composition preferably a topical composition for on wound administration, comprising

- (a) a compound of formula I, preferably an effective amount of a compound of formula I, or a pharmaceutically acceptable salt thereof

5



I

wherein

R₁ is ethyl or propyl, preferably propyl;

R₂ is methyl or ethyl, preferably methyl;

10 R₃ is ethyl or propyl, preferably propyl;

X is N or CH, preferably CH;

n is 1 or 2, preferably n=1;

- (b) a solvent; and

- (c) optionally one or more other pharmaceutically acceptable excipients.

15 The topical composition of the present invention can be used for the treatment of diseases or disorders of the skin being mediated by or resulting from PDE5 activity and/or NO related endothelial dysfunction, in particular, such as ischemic skin ulcers, such as digital ulcers (DU) in systemic sclerosis, diabetic foot ulcer, leg ulcer, ischemic arterial ulcers, livedoid vasculopathy, Martorell hypertensive ischemic leg ulcer, thromboangiitis obliterans
20 (Buerger's disease), sickle cell leg ulcer, all of the foregoing preferably for wound healing, further preferably for chronic wound healing.

Thus, in a further aspect, the present invention provides the topical composition of the present invention for use in a method of topically treating a disease or disorder mediated by PDE5 activity and/or NO related endothelial dysfunction, preferably of a disease or disorder of
25 the skin mediated by PDE5 activity and/or NO related endothelial dysfunction, in a subject, preferably in a human. Preferably, said disease or disorder is selected from ischemic skin ulcers, digital ulcers (DU) in systemic sclerosis, diabetic foot ulcer, leg ulcer, ischemic arterial ulcers, livedoid vasculopathy, Martorell hypertensive ischemic leg ulcer, thromboangiitis obliterans (Buerger's disease), sickle cell leg ulcer, all of the foregoing preferably for wound

healing, further preferably for chronic wound healing. Very preferred, said disease or disorder is systemic sclerosis, and wherein preferably said disease or disorder is digital ulcers in systemic sclerosis.

In another aspect, the present invention provides the topical composition of the present invention for use in a method of topically treating a disease or disorder in a subject, preferably in a human, wherein said disease or disorder is selected from ischemic skin ulcers, digital ulcers (DU) in systemic sclerosis, diabetic foot ulcer, leg ulcer, ischemic arterial ulcers, livedoid vasculopathy, Martorell hypertensive ischemic leg ulcer, thromboangiitis obliterans (Buerger's disease), sickle cell leg ulcer, all of the foregoing preferably for wound healing, further preferably for chronic wound healing, and wherein preferably said disease or disorder is systemic sclerosis, and again further preferably said disease or disorder is digital ulcers in systemic sclerosis.

In another aspect, the present invention provides a method for treating a disease or disorder mediated by PDE5 activity and/or NO related endothelial dysfunction, preferably of a disease or disorder of the skin mediated by PDE5 activity and/or NO related endothelial dysfunction, in a subject, preferably in a human, wherein said method comprises topically administering the topical composition of the present invention to said subject, preferably to said human.

In another aspect, the present invention provides a method for treating a disease or disorder in a subject, preferably in a human, wherein said disease or disorder is selected from ischemic skin ulcers, digital ulcers (DU) in systemic sclerosis, diabetic foot ulcer, leg ulcer, ischemic arterial ulcers, livedoid vasculopathy, Martorell hypertensive ischemic leg ulcer, thromboangiitis obliterans (Buerger's disease), sickle cell leg ulcer, all of the foregoing preferably for wound healing, further preferably for chronic wound healing, and wherein preferably said disease or disorder is systemic sclerosis, and again further preferably said disease or disorder is digital ulcers in systemic sclerosis, wherein said method comprises topically administering the topical composition of the present invention to said subject, preferably to said human.

In another aspect, the present invention provides for the use of the topical composition of the present invention for the preparation of a medicament for topically treating a disease or disorder mediated by PDE5 activity and/or NO related endothelial dysfunction, preferably of a disease or disorder of the skin mediated by PDE5 activity and/or NO related endothelial dysfunction, in a subject, preferably in a human, wherein said method comprises topically administering the topical composition of the present invention to said subject, preferably to said

human.

In another aspect, the present invention provides for the use of the topical composition of the present invention for the preparation of a medicament for topically treating a disease or disorder in a subject, preferably in a human, wherein said disease or disorder is selected from
5 ischemic skin ulcers, digital ulcers (DU) in systemic sclerosis, diabetic foot ulcer, leg ulcer, ischemic arterial ulcers, livedoid vasculopathy, Martorell hypertensive ischemic leg ulcer, thromboangiitis obliterans (Buerger's disease), sickle cell leg ulcer, all of the foregoing preferably for wound healing, further preferably for chronic wound healing, and wherein
10 preferably said disease or disorder is systemic sclerosis, and again further preferably said disease or disorder is digital ulcers in systemic sclerosis, wherein said method comprises topically administering the topical composition of the present invention to said subject, preferably to said human.

In a further aspect, the invention provides a kit comprising:

- (i) a first kit component comprising the topical composition of the present
15 invention; and
- (ii) a second kit component comprising a patch, a tape, a dressing, a sheet or a device suitable to maintain contact between said topical composition and the skin of a subject, preferably the skin of a human.

20 In still a further aspect, the present invention provides a method of preparing the topical composition of the present invention, wherein said method comprises dissolving a compound of formula I in a solvent, wherein preferably said solvent comprises PEG400, and further preferably wherein said solvent comprises PEG400 and water.

25 Further aspects and embodiments of the present invention will become apparent as this description continues.

DESCRIPTION OF FIGURES

FIG. 1A: Healing of full thickness, excisional, cutaneous wounds (2 wounds / mouse) was followed over 12-14 days from surgery in uPAR ^{-/-} mice and wild type (WT) mice (mixed C57BL/6 (75%) × 129 (25%) background). Vehicle Composition A was administered once
30 daily, *on wound*, at a volume of 25 µl per wound. Results in the uPAR ^{-/-} mice are from initially 12 wounds, 7 mice. Results in the wild type (WT, uPAR^{+/+}) mice are from initially 11 wounds, 11 mice.

FIG. 1B: Healing of full thickness, excisional, cutaneous wounds (2 wounds / mouse) was followed over 12-14 days from surgery in uPAR $\pm$ mice after repeated, once daily, topical, *on wound* administration of a volume of 25 μ l per wound of Topical Composition **A2** or Vehicle Composition **A**. Results are from initially 12 wounds, 6 mice (Topical Composition **A2**) and 12 wounds, 7 mice (Vehicle Composition **A**). % Wound closure was calculated as the percent ratio of the difference between the ulcer inner diameter at day k ($0 \leq k \leq 14$) and the ulcer inner diameter at day 0 (immediately after surgery) divided by the ulcer inner diameter at day 0. Results are shown as means $\pm$ SEM. Statistical analyses were done by mixed effect analysis, Sidak's multiple comparisons (GraphPad Prism 9.0). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus wild type (FIG. 1A) or vehicle (FIG. 1B). Only wounds with an intact silicone ring and stitches, used to avoid wound contraction, were included in the analysis that explains less evaluable wounds over time (Table 5). In the vehicle groups, some mice received vehicle on one wound and sham at the other wound which explains that the number of wounds may be less than the 2-fold of the number of mice enrolled.

FIG. 2A: For all t, the %BSL_t(Δ PU(Ulcer-CTR)) for Topical Composition **A2**, Topical Composition **A4** or Vehicle Composition **A** were calculated as the ratio between (i) the differences between the PU_t from the ulcer area and the PU_t from the corresponding control area (Δ PU_t = (PU_t (Ulcer) – PU_t (Control))) at time point t and (ii) time point 0 i.e. Δ PU₀ following the equation %BSL_t(Δ PU(Ulcer-CTR)) = Δ PU_t/ Δ PU₀ * 100 %. Graphs depict the means $\pm$ SEM from %BSL_t(Δ PU(Ulcer-CTR)) of 8 mice per group for the ulcer area. For any given t ($6 \leq t \leq 240$) there was no statistically significant difference between %BSL_t(Δ PU(Ulcer-CTR)) for Topical Composition **A2** and Topical Composition **A4** versus Vehicle Composition **A** (2-way ANOVA). Ulcer means the ulcer region of interest (ROI) and CTR means the corresponding control ROI.

FIG. 2B: For all t, $\Delta\Delta$ PU_t(TN53_{corr}-VEH_{corr}) was calculated as the difference between $\Delta\Delta$ PU_{t(0)} = Δ PU_t - Δ PU₀ calculated for (i) Topical Composition **A2** and Topical Composition **A4** and (ii) Vehicle Composition **A** at the ulcer areas. Graphs depict the means $\pm$ SEM from $\Delta\Delta$ PU_t(Cpd **1**_{corr}-VEH_{corr}) of 8 mice per group for the ulcer area. * $p < 0.05$, ** $p < 0.01$ from baseline (zero) by 2-way ANOVA. A letter 't' in subscript means measurements or calculations at time point t after administration of Topical Composition **A2** and Topical Composition **A4** or Vehicle Composition **A**. Perfusion Units (PU) values in ulcers areas corrected for the corresponding values in the control (CTR) areas.}

FIG. 3: Solubility of Compound **1** (provided in mg/mL depicted on x-axis) in relation to the concentration of PEG400 at room temperature.

DETAILED DESCRIPTION OF THE INVENTION

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs.

5 The term "about", as used herein shall have the meaning of +/- 10%. For example about 50% shall mean 45% to 55%. Preferably, the term "about", as used herein shall have the meaning of +/- 5%. For example about 50% shall mean 47.5% to 52.5%. In a preferred embodiment, said term "about" refers to any value of a range of and between - 10% and +10% of the value it refers to. In a preferred embodiment, said term "about" refers to any value of a
10 range of and between - 8% and +8% of the value it refers to. In a preferred embodiment, said term "about" refers to any value of and between a range of - 7% and +7% of the value it refers to. In a preferred embodiment, said term "about" refers to any value of and between a range of - 5% to +5% of the value it refers to. In a preferred embodiment, said term "about" refers to any value of and between a range of - 4% and +4% of the value it refers to. In a preferred
15 embodiment, said term "about" refers to any value of and between a range of - 3% and +3% of the value it refers to. In a preferred embodiment, said term "about" refers to any value of and between a range of - 2% and +2% of the value it refers to.

 The phrase "between value X and value Y", as used herein, shall refer to include the value X and the value Y and any value in between. For example, the phrase "between 0.01 μ mol and
20 50 μ mol" refers to 0.01 μ mol and 50 μ mol and any value in between. The same applies to the phrase "between about value X and about value Y" taking further the variations of the term "about" into account. Analogously, the phrase "from value X to value Y", as used herein, shall refer to include the value X and the value Y and any value in between. For example, the phrase "between 65% and 75%" refers to 65% and 75% and any value in between. The same applies
25 to the phrase "from about value X to about value Y" taking further the variations of the term "about" into account.

 The term "% (w/w)" as used herein refers to (mass of the component / total mass of the composition) x 100. By way of example, 70 wt% PEG400 is 70 g PEG400 per 100 g of the composition.

30 When the terms "a," or "an" are used herein, they mean "at least one" unless indicated otherwise.

 As used herein, the term "topically" and "topical" refers to application of the composition of the present invention to the surface of the skin of a subject, preferably of a human, and thus,

the term "topical administration", as used herein, refers to administration to the skin of a subject, preferably of a human, preferably to on wound administration.

As used herein the term "topical composition" refers to a composition that is suitable for topical administration and may be applied to skin of a subject, preferably of a human.

5 As used herein, the terms "treatment", "treat", "treated" or "treating" refer to prophylaxis and/or therapy. In one embodiment, the terms "treatment", "treat", "treated" or "treating" refer to a therapeutic treatment. In another embodiment, the terms "treatment", "treat", "treated" or "treating" refer to a prophylactic treatment. Preferably, beneficial or desired clinical results of said treatment include, but are not limited to, alleviation of symptoms, diminishment of extent
10 of disease or disorder, stabilized (*i.e.*, not worsening) state of disease or disorder, delay or slowing of disease or disorder progression, amelioration or palliation of the disease or disorder state.

As used herein, the term "effective amount" refers to an amount necessary or sufficient to realize a desired biologic effect. Preferably, the term "effective amount" refers to an amount
15 of a compound of formula I of the present invention that (i) treats or prevents the particular disease or disorder, (ii) attenuates, ameliorates, or eliminates one or more symptoms of the particular disease or disorder, or (iii) prevents or delays the onset of one or more symptoms of the particular disease or disorder, described herein. An effective amount of the inventive compound of formula I, or said topical composition or said pharmaceutical composition, would
20 be the amount that achieves this selected result, and such an amount could be determined as a matter of routine by a person skilled in the art. Further preferably, the term "effective amount", as used herein, refers to an amount necessary or sufficient to be effective to activation of soluble guanylyl cyclase (sGC) and/or increase the inhibition of PDE5. The effective amount can vary depending on the particular composition being applied and the size of the subject. One of
25 ordinary skill in the art can empirically determine the effective amount of a particular the inventive compound of formula I, or said topical composition or said pharmaceutical composition of the present invention without necessitating undue experimentation.

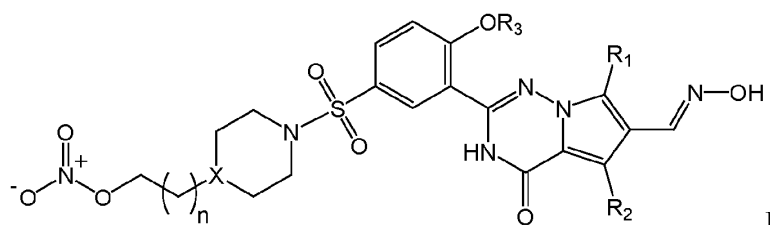
The term "mammal", as used herein, includes, but is not limited to, humans, mice, rats, guinea pigs, monkeys, dogs, cats, horses, cows, pigs, and sheep. The term "mammal", as used
30 herein, preferably refers to humans. The term "subject", as used herein, includes, but is not limited to, humans and mammals. The term "subject", as used herein, preferably refers to humans.

The term "kit" as used herein means that the components comprised in said kit are provided physically separable and distinguishable from one another as different components

but are provided or sold together for the purpose of being administered, or used, together.

Surprisingly, the inventors were able to provide inventive topical compositions, in particular inventive topical compositions comprising Compound 1 despite the shown de-facto non-solubility of the inventive compounds of formula I, in particular of Compound 1 in physiological aqueous phosphate buffer (pH 7.5). Moreover, the inventive topical compositions showed efficacy and improved wound healing in uPAR deficient mice, a disease-relevant animal model of systemic sclerosis having cutaneous ulcers, at surprisingly very low concentrations of the inventive compounds of formula I, in particular of Compound 1.

Thus, in a first aspect, the present invention provides a topical composition comprising a compound of formula I, preferably an effective amount of a compound of formula I, or a pharmaceutically acceptable salt thereof



wherein

R₁ is ethyl or propyl, preferably propyl;

R₂ is methyl or ethyl, preferably methyl;

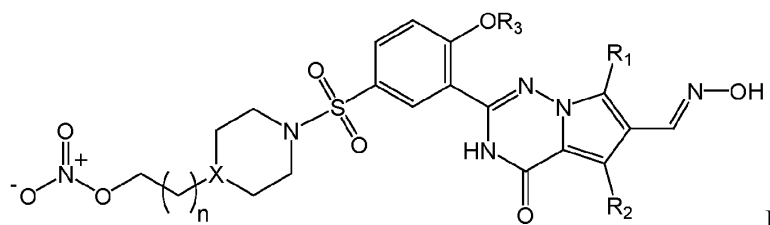
R₃ is ethyl or propyl, preferably propyl;

X is N or CH, preferably CH;

n is 1 or 2, preferably n=1.

In another aspect, the present invention provides a topical composition comprising

(a) a compound of formula I, preferably an effective amount of a compound of formula I, or a pharmaceutically acceptable salt thereof



wherein

R₁ is ethyl or propyl, preferably propyl;

R₂ is methyl or ethyl, preferably methyl;

R₃ is ethyl or propyl, preferably propyl;

5 X is N or CH, preferably CH;

n is 1 or 2, preferably n=1;

(b) a solvent; and

(c) optionally one or more other pharmaceutically acceptable excipients.

10 Compounds of formula **I** comprised in the topical composition of the present invention include pharmaceutically acceptable salts of said compounds. In particular, the term "pharmaceutically acceptable salt" as used herein, refers to pharmaceutically acceptable organic or inorganic salts of a compound of the present invention, in particular acid addition salts. Exemplary salts include, but are not limited to, salts of physiologically acceptable mineral
15 acids, such as hydrochloric acid, sulfuric acid, nitric acid and phosphoric acid, or salts of organic acids, such as methane-sulfonic acid, *p*-toluenesulfonic acid, lactic acid, malic acid, tartaric acid, acetic acid, trifluoroacetic acid, citric acid, succinic acid, fumaric acid, maleic acid and salicylic acid. Further examples of pharmacologically acceptable salts of the compounds of formula **I** are alkali metal and alkaline earth metal salts such as, for example, sodium,
20 potassium, lithium, calcium or magnesium salts, ammonium salts or salts of organic bases such as, for example, methylamine, dimethylamine, triethylamine, piperidine, ethylenediamine, lysine, choline hydroxide, meglumine, morpholine or arginine salts. Further examples of pharmaceutically acceptable salts of the compounds of formula **I** include the hydrochloride, hydrobromide, sulfate, bisulfate, phosphate, hydrogen phosphate, nitrate, acetate, benzoate,
25 succinate, fumarate, maleate, lactate, citrate, benzenesulphonate, *p*-toluenesulphonate or the like.

In one embodiment, said R₁ is propyl. In one embodiment, said R₁ is ethyl. In one embodiment, said R₂ is methyl. In one embodiment, said R₂ is ethyl. In one embodiment, said R₃ is propyl. In one embodiment, said R₃ is ethyl. In one embodiment, said X is N. In one
30 embodiment, said X is CH. In one embodiment, said n is 1. In one embodiment, said n is 2.

In one embodiment, said compound of formula **I** is a compound selected from the group consisting of

2-[1-(3-{6-[(1E)-(hydroxyimino)methyl]-5-methyl-4-oxo-7-propyl-3H,4H-pyrrolo[2,1-f][1,2,4]triazin-2-yl}-4-propoxybenzenesulfonyl)piperidin-4-yl]ethyl nitrate (Compound **1**);

(*E*)-2-(2-ethoxy-5-((4-(2-hydroxyethyl)piperazin-1-yl)sulfonyl)phenyl)-5-ethyl-4-oxo-7-propyl-3,4-dihydropyrrolo[2,1-*f*][1,2,4]triazine-6-carbaldehyde oxime (Compound 2);

(*E*)-2-(4-((4-ethoxy-3-(6-((hydroxyimino)methyl)-5-methyl-4-oxo-7-propyl-3,4-dihydropyrrolo[2,1-*f*][1,2,4]triazin-2-yl)phenyl)sulfonyl)piperazin-1-yl)ethyl nitrate

5 (Compound 3);

(*E*)-2-(4-((4-ethoxy-3-(5-ethyl-6-((hydroxyimino)methyl)-4-oxo-7-propyl-3,4-dihydropyrrolo[2,1-*f*][1,2,4]triazin-2-yl)phenyl)sulfonyl)piperazin-1-yl)ethyl nitrate

(Compound 4);

(*E*)-2-(1-((4-ethoxy-3-(6-((hydroxyimino)methyl)-5-methyl-4-oxo-7-propyl-3,4-

10 dihydropyrrolo[2,1-*f*][1,2,4]triazin-2-yl)phenyl)sulfonyl)piperidin-4-yl)ethyl nitrate

(Compound 5); and

(*E*)-3-(4-((4-ethoxy-3-(6-((hydroxyimino)methyl)-5-methyl-4-oxo-7-propyl-3,4-

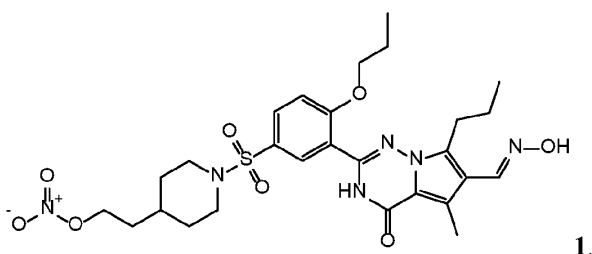
dihydropyrrolo[2,1-*f*][1,2,4]triazin-2-yl)phenyl)sulfonyl)piperazin-1-yl)propyl nitrate

(Compound 6).

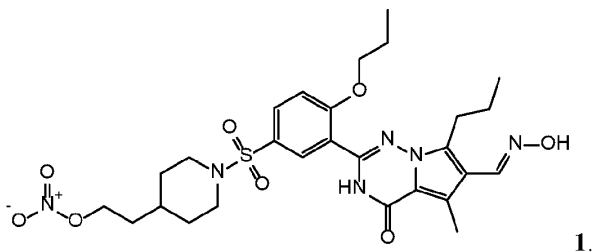
15 In a preferred embodiment, said R_1 is propyl, R_2 is methyl, R_3 is propyl, X is CH and $n=1$.

Thus, in a very preferred embodiment, said compound of formula I is 2-[1-(3-{6-[(1*E*)-(hydroxyimino)methyl]-5-methyl-4-oxo-7-propyl-3*H*,4*H*-pyrrolo[2,1-*f*][1,2,4]triazin-2-yl}-4-propoxybenzenesulfonyl)piperidin-4-yl]ethyl nitrate (Compound 1). In a preferred embodiment, said compound of formula I is Compound 1

20



In a very preferred embodiment, said compound of formula I is Compound 1



In a preferred embodiment, said compound of formula **I**, preferably said Compound **1**, is present from about 0.000006% (w/w) to about 0.03% (w/w), preferably from about 0.00006% (w/w) to about 0.01% (w/w), more preferably from about 0.0001% (w/w) to about 0.01% (w/w), based on the total weight of said topical composition.

5 In a further preferred embodiment, said compound of formula **I** is Compound **1**, and said Compound **1** is present from about 0.000006% (w/w) to about 0.03% (w/w), preferably from about 0.00006% (w/w) to about 0.01% (w/w), more preferably from about 0.0001% (w/w) to about 0.01% (w/w), based on the total weight of said topical composition.

In a further preferred embodiment, said compound of formula **I**, preferably said
10 Compound **1**, is present from about 0.0001% (w/w) to about 0.01% (w/w) based on the total weight of said topical composition. In a further preferred embodiment, said compound of formula **I**, preferably said Compound **1**, is present from about 0.00018% (w/w) to about 0.006% (w/w) based on the total weight of said topical composition. In a further preferred embodiment, said compound of formula **I**, preferably said Compound **1**, is present from about 0.0004% (w/w)
15 to about 0.002% (w/w) based on the total weight of said topical composition. In a further preferred embodiment, said compound of formula **I**, preferably said Compound **1**, is present from about 0.0005% (w/w) to about 0.001% (w/w) based on the total weight of said topical composition. In a further preferred embodiment, said compound of formula **I**, preferably said Compound **1**, is present from about 0.0006% (w/w) based on the total weight of said topical
20 composition. In a further preferred embodiment, said compound of formula **I**, preferably said Compound **1**, is present from about 0.0006% (w/w) to about 0.001% (w/w) based on the total weight of said topical composition.

In a further preferred embodiment, said compound of formula **I** is Compound **1**, and said Compound **1** is present from about 0.0001% (w/w) to about 0.01% (w/w) based on the total
25 weight of said topical composition. In a further preferred embodiment, said compound of formula **I** is Compound **1**, and said Compound **1** is present from about 0.00018% (w/w) to about 0.006% (w/w) based on the total weight of said topical composition. In a further preferred embodiment, said compound of formula **I** is Compound **1**, and said Compound **1** is present from about 0.0004% (w/w) to about 0.002% (w/w) based on the total weight of said topical
30 composition. In a further preferred embodiment, said compound of formula **I** is Compound **1**, and said Compound **1** is present from about 0.0005% (w/w) to about 0.001% (w/w) based on the total weight of said topical composition. In a further preferred embodiment, said compound of formula **I** is Compound **1**, and said Compound **1** is present from about 0.0006% (w/w) based on the total weight of said topical composition. In a further preferred embodiment, said

compound of formula **I** is Compound **1**, and said Compound **1** is present from about 0.0006% (w/w) to about 0.001% (w/w) based on the total weight of said topical composition.

In a further preferred embodiment, said topical composition comprises said compound of formula **I**, preferably said Compound **1**, in an amount between about 0.01 μ mol and about 50 μ mol, preferably between about 0.1 μ mol and about 20 μ mol, further preferably between about 0.2 μ mol and about 15 μ mol.

In a further preferred embodiment, said topical composition comprises said compound of formula **I**, preferably said Compound **1**, in a concentration between about 0.1 μ M and about 535 μ M, preferably between about 1 μ M and about 214 μ M, further preferably between about 2 μ M and about 160 μ M.

In a further preferred embodiment, said compound of formula **I** is Compound **1**, and said topical composition comprises said Compound **1** in an amount between about 0.01 μ mol and about 50 μ mol, preferably between about 0.1 μ mol and about 20 μ mol, further preferably between about 0.2 μ mol and about 15 μ mol.

In a further preferred embodiment, said compound of formula **I** is Compound **1**, and said topical composition comprises said Compound **1** in a concentration between about 0.1 μ M and about 535 μ M, preferably between about 1 μ M and about 214 μ M, further preferably between about 2 μ M and about 160 μ M.

In a further preferred embodiment, said topical composition comprises said compound of formula **I**, preferably said Compound **1**, in an amount between about 0.2 μ mol and about 15 μ mol. In a further preferred embodiment, said topical composition comprises said compound of formula **I**, preferably said Compound **1**, in an amount between about 0.3 μ mol and about 12 μ mol. In a further preferred embodiment, said topical composition comprises said compound of formula **I**, preferably said Compound **1**, in an amount between about 0.3 μ mol and about 10 μ mol. In a further preferred embodiment, said topical composition comprises said compound of formula **I**, preferably said Compound **1**, in an amount between about 0.3 μ mol and about 5 μ mol. In a further preferred embodiment, said topical composition comprises said compound of formula **I**, preferably said Compound **1**, in an amount between about 0.5 μ mol and about 10 μ mol. In a further preferred embodiment, said topical composition comprises said compound of formula **I**, preferably said Compound **1**, in an amount between about 0.5 μ mol and about 5 μ mol. In a further preferred embodiment, said topical composition comprises said compound of formula **I**, preferably said Compound **1**, in an amount between about 0.7 μ mol and about

5 μmol . In a further preferred embodiment, said topical composition comprises said compound of formula **I**, preferably said Compound **1**, in an amount between about $0.8 \mu\text{mol}$ and about $2 \mu\text{mol}$. In a further preferred embodiment, said topical composition comprises said compound of formula **I**, preferably said Compound **1**, in an amount of about $1 \mu\text{mol}$.

5 In a further preferred embodiment, said topical composition comprises said compound of formula **I**, preferably said Compound **1**, in a concentration between about $2 \mu\text{M}$ and about $160 \mu\text{M}$. In a further preferred embodiment, said topical composition comprises said compound of formula **I**, preferably said Compound **1**, in a concentration between about $3 \mu\text{M}$ and about $130 \mu\text{M}$. In a further preferred embodiment, said topical composition comprises said compound
10 of formula **I**, preferably said Compound **1**, in a concentration between about $3 \mu\text{M}$ and about $110 \mu\text{M}$. In a further preferred embodiment, said topical composition comprises said compound of formula **I**, preferably said Compound **1**, in a concentration between about $3 \mu\text{M}$ and about $55 \mu\text{M}$. In a further preferred embodiment, said topical composition comprises said compound of formula **I**, preferably said Compound **1**, in a concentration between about $5 \mu\text{M}$ and about
15 $110 \mu\text{M}$. In a further preferred embodiment, said topical composition comprises said compound of formula **I**, preferably said Compound **1**, in a concentration between about $5 \mu\text{M}$ and about $55 \mu\text{M}$. In a further preferred embodiment, said topical composition comprises said compound of formula **I**, preferably said Compound **1**, in a concentration between about $7 \mu\text{M}$ and about $55 \mu\text{M}$. In a further preferred embodiment, said topical composition comprises said compound
20 of formula **I**, preferably said Compound **1**, in a concentration between about $8 \mu\text{M}$ and about $22 \mu\text{M}$. In a further preferred embodiment, said topical composition comprises said compound of formula **I**, preferably said Compound **1**, in a concentration between about $10 \mu\text{M}$ and about $12 \mu\text{M}$. In a further preferred embodiment said topical composition comprises said compound of formula **I**, preferably said Compound **1**, in a concentration of about $11 \mu\text{M}$.

25 In a further preferred embodiment, said compound of formula **I** is Compound **1**, and said topical composition comprises said Compound **1** in an amount between about $0.2 \mu\text{mol}$ and about $15 \mu\text{mol}$. In a further preferred embodiment, said compound of formula **I** is Compound **1**, and said topical composition comprises said Compound **1** in an amount between about $0.3 \mu\text{mol}$ and about $12 \mu\text{mol}$. In a further preferred embodiment, said compound of formula **I** is
30 Compound **1**, and said topical composition comprises said Compound **1** in an amount between about $0.3 \mu\text{mol}$ and about $10 \mu\text{mol}$. In a further preferred embodiment, said compound of formula **I** is Compound **1**, and said topical composition comprises said Compound **1** in an

amount between about 0.3 μ mol and about 5 μ mol. In a further preferred embodiment, said compound of formula **I** is Compound **1**, and said topical composition comprises said Compound **1** in an amount between about 0.5 μ mol and about 10 μ mol. In a further preferred embodiment, said compound of formula **I** is Compound **1**, and said topical composition comprises said Compound **1** in an amount between about 0.5 μ mol and about 5 μ mol. In a further preferred embodiment, said compound of formula **I** is Compound **1**, and said topical composition comprises said Compound **1** in an amount between about 0.7 μ mol and about 5 μ mol. In a further preferred embodiment, said compound of formula **I** is Compound **1**, and said topical composition comprises said Compound **1** in an amount between about 0.8 μ mol and about 2 μ mol. In a further preferred embodiment, said compound of formula **I** is Compound **1**, and said topical composition comprises said Compound **1** in an amount of about 1 μ mol.

In a further preferred embodiment, said compound of formula **I** is Compound **1**, and said topical composition comprises said Compound **1** in a concentration between about 2 μ M and about 165 μ M. In a further preferred embodiment, said compound of formula **I** is Compound **1**, and said topical composition comprises said Compound **1** in a concentration between about 3 μ M and about 130 μ M. In a further preferred embodiment, said compound of formula **I** is Compound **1**, and said topical composition comprises said Compound **1** in a concentration between about 3 μ M and about 110 μ M. In a further preferred embodiment, said compound of formula **I** is Compound **1**, and said topical composition comprises said Compound **1** in a concentration between about 3 μ M and about 55 μ M. In a further preferred embodiment, said compound of formula **I** is Compound **1**, and said topical composition comprises said Compound **1** in a concentration between about 5 μ M and about 110 μ M. In a further preferred embodiment, said compound of formula **I** is Compound **1**, and said topical composition comprises said Compound **1** in a concentration between about 5 μ M and about 55 μ M. In a further preferred embodiment, said compound of formula **I** is Compound **1**, and said topical composition comprises said Compound **1** in a concentration between about 7 μ M and about 55 μ M. In a further preferred embodiment, said compound of formula **I** is Compound **1**, and said topical composition comprises said Compound **1** in a concentration between about 8 μ M and about 22 μ M. In a further preferred embodiment, said compound of formula **I** is Compound **1**, and said topical composition comprises said Compound **1** in a concentration between about 10 μ M and about 12 μ M. In a further preferred embodiment, said compound of formula **I** is Compound **1**, and said topical composition comprises said Compound **1** in a concentration of about 11 μ M.

In some embodiments, said topical composition can be formulated to different dosage forms, for example, a solution, a suspension, a cream, an ointment, a lotion, a paste, an emulsion, a foam and a gel.

In a preferred embodiment, said topical composition is formulated as a solution or a gel.

5 In a preferred embodiment, said topical composition is in the form of a gel, preferably of an aqueous gel. In a preferred embodiment, said topical composition is a gel, preferably an aqueous gel.

In a preferred embodiment, said topical composition is a liquid topical composition. In a further preferred embodiment, said topical composition is a liquid topical composition, wherein
10 said liquid topical composition is formulated as a solution or a gel. In a further preferred embodiment, said topical composition is a liquid topical composition, wherein said liquid topical composition is formulated as a solution. In a further preferred embodiment, said topical composition is a liquid topical composition, wherein said liquid topical composition is formulated as a gel. In a further preferred embodiment, said topical composition is a liquid
15 topical composition, wherein said liquid topical composition is formulated as an aqueous solution. In a further preferred embodiment, said topical composition is a liquid topical composition, wherein said liquid topical composition is formulated as an aqueous solution, and wherein said solvent comprises water. In a further preferred embodiment, said topical composition is a liquid topical composition, wherein said liquid topical composition is
20 formulated as an aqueous gel. In a further preferred embodiment, said topical composition is a liquid topical composition, wherein said liquid topical composition is formulated as an aqueous gel, and wherein said solvent comprises water.

In a further preferred embodiment, said topical composition is a liquid topical composition, wherein said liquid topical composition is in the form of a solution or a gel. In a
25 further preferred embodiment, said topical composition is a liquid topical composition, wherein said liquid topical composition is in the form of a solution. In a further preferred embodiment, said topical composition is a liquid topical composition, wherein said liquid topical composition is in the form of a gel. In a further preferred embodiment, said topical composition is a liquid topical composition, wherein said liquid topical composition is in the form of an aqueous
30 solution. In a further preferred embodiment, said topical composition is a liquid topical composition, wherein said liquid topical composition is in the form of an aqueous solution, and wherein said solvent comprises water. In a further preferred embodiment, said topical composition is a liquid topical composition, wherein said liquid topical composition is in the form of an aqueous gel. In a further preferred embodiment, said topical composition is a liquid

topical composition, wherein said liquid topical composition is in the form of an aqueous gel, and wherein said solvent comprises water.

In a further preferred embodiment, said topical composition is a liquid topical composition, and wherein preferably said liquid topical composition is in the form of an aqueous gel.

In a preferred embodiment, said topical composition comprises PEG 400. In a preferred embodiment, said topical composition is in the form of a gel, and wherein said topical composition comprises PEG 400.

In a preferred embodiment, said topical composition comprises PEG 400. In a preferred embodiment, said topical composition is in the form of a gel, and wherein said topical composition comprises PEG 400.

In a preferred embodiment, said solvent comprises PEG 400. In a preferred embodiment, said solvent of said topical composition comprises PEG 400, and wherein the amount of said PEG 400 is from about 60% to about 75 % (w/w), preferably from about 65% to about 75 % (w/w), and more preferably from about 68 % to about 72 % (w/w) based on the total weight of the topical composition. In a further preferred embodiment, the amount of said PEG 400 is from about 60% to about 75 % (w/w) based on the total weight of the topical composition. In a further preferred embodiment, the amount of said PEG 400 is from about 65% to about 75 % (w/w) based on the total weight of the topical composition. In a further preferred embodiment, the amount of said PEG 400 is from about 68 % to about 72 % (w/w) based on the total weight of the topical composition. In a further preferred embodiment, the amount of said PEG 400 is from about 60% to about 75 % (w/w) based on the total weight of the topical composition. In a further preferred embodiment, the amount of said PEG 400 is about 65% to 75 % (w/w) based on the total weight of the topical composition. In a further preferred embodiment, the amount of said PEG 400 is from 68 % to 72 % (w/w) based on the total weight of the topical composition. In a preferred embodiment, said solvent comprises PEG 400 and water, and wherein the amount of said PEG 400 is from about 60% to about 75 % (w/w), preferably from about 65% to about 75 % (w/w), and more preferably from about 68 % to about 72 % (w/w) based on the total weight of the topical composition.

In a preferred embodiment, said solvent of said topical composition comprises PEG 400. In a preferred embodiment, said topical composition is in the form of a gel, preferably of an aqueous gel, and wherein said solvent comprises PEG 400.

In a further preferred embodiment, said solvent comprises PEG 400.

In a preferred embodiment, said topical composition comprises PEG 400 and water. In a

very preferred embodiment, said topical composition is in the form of an aqueous gel, wherein said solvent comprises PEG 400 and water.

In a preferred embodiment, said solvent of said topical composition comprises PEG 400, and wherein the amount of said PEG 400 is from about 60% to about 75 % (w/w), preferably from about 65% to about 75 % (w/w), and more preferably from about 68 % to about 72 % (w/w) based on the total weight of the topical composition. In a preferred embodiment, said topical composition is in the form of a gel, preferably of an aqueous gel, and wherein said solvent comprises PEG 400, and wherein the amount of said PEG 400 is from about 60% to about 75 % (w/w), preferably from about 65% to about 75 % (w/w), and more preferably from about 68 % to about 72 % (w/w) based on the total weight of the topical composition.

In a preferred embodiment, said topical composition comprises PEG 400 and water. In a very preferred embodiment, said topical composition is in the form of an aqueous gel, wherein said solvent comprises PEG 400 and water, and wherein the amount of said PEG 400 is from about 60% to about 75 % (w/w), preferably from about 65% to about 75 % (w/w), and more preferably from about 68 % to about 72 % (w/w) based on the total weight of the topical composition.

In a preferred embodiment, said topical composition comprises an aqueous buffer, preferably an aqueous phosphate buffer. In a preferred embodiment, said topical composition is in the form of a gel, preferably in form of an aqueous gel, and wherein said topical composition comprises an aqueous buffer, preferably an aqueous phosphate buffer. In a preferred embodiment, said topical composition is in the form of an aqueous gel, and wherein said topical composition comprises an aqueous phosphate buffer.

In a preferred embodiment, said topical composition comprises PEG 400 and water. In a very preferred embodiment, said topical composition is in the form of an aqueous gel, wherein said solvent comprises PEG 400 and water, and wherein the amount of said PEG 400 is from about 60% to about 75 % (w/w), preferably from about 65% to about 75 % (w/w), and more preferably from about 68 % to about 72 % (w/w) based on the total weight of the topical composition, and wherein said topical composition comprises an aqueous buffer, preferably an aqueous phosphate buffer. In a very preferred embodiment, said topical composition is in the form of an aqueous gel, wherein said solvent comprises PEG 400 and water, and wherein the amount of said PEG 400 is from about 60% to about 75 % (w/w), preferably from about 65% to about 75 % (w/w), and more preferably from about 68 % to about 72 % (w/w) based on the total weight of the topical composition, and wherein said topical composition comprises an aqueous phosphate buffer.

In a preferred embodiment, said topical composition comprises an aqueous buffer, preferably an aqueous phosphate buffer, of a pH of about 6.5 to about 7.5, further preferably an aqueous buffer, preferably an aqueous phosphate buffer, of a pH of about 6.5 to about 7.0, even more preferably an aqueous buffer, preferably an aqueous phosphate buffer, of a pH of about 6.7. In a preferred embodiment, said topical composition is in the form of a gel, preferably in form of an aqueous gel, and wherein said topical composition comprises an aqueous phosphate buffer, wherein preferably said topical composition comprises an aqueous phosphate buffer of a pH of about 6.5 to about 7.5, further preferably an aqueous phosphate buffer of a pH of about 6.5 to about 7.0, even more preferably an aqueous phosphate buffer of a pH of about 6.7.

In a preferred embodiment, said aqueous buffer, preferably said aqueous phosphate buffer, has a molarity of about 10 to about 15 mM, preferably of about 12mM. In a preferred embodiment, said aqueous buffer is an aqueous phosphate buffer, wherein said aqueous phosphate buffer has a molarity of about 10 to about 15 mM, preferably of about 12mM.

In a preferred embodiment, said aqueous buffer, preferably said aqueous phosphate buffer, is present from about 20% (w/w) to about 30% (w/w), preferably about 22% (w/w) to about 28% (w/w), more preferably about 25% (w/w) to about 28% (w/w) and even more preferably of about 27% (w/w) to about 28% (w/w) based on the total weight of said topical composition. In a preferred embodiment, said aqueous phosphate buffer is present from about 20% (w/w) to about 30% (w/w), preferably about 22% (w/w) to about 28% (w/w), more preferably about 25% (w/w) to about 28% (w/w) and even more preferably of about 27% (w/w) to about 28% (w/w) based on the total weight of said topical composition.

In a preferred embodiment, said topical composition comprises an aqueous phosphate buffer, preferably an aqueous phosphate buffer of a pH of about 6.5 to about 7.5, further preferably an aqueous phosphate buffer of a pH of about 6.5 to about 7.0, even more preferably an aqueous phosphate buffer of a pH of about 6.7, and wherein said aqueous phosphate buffer has a molarity of about 10 to about 15 mM, preferably of about 12mM.

In a preferred embodiment, said topical composition comprises an aqueous phosphate buffer, preferably an aqueous phosphate buffer of a pH of about 6.5 to about 7.5, further preferably an aqueous phosphate buffer of a pH of about 6.5 to about 7.0, even more preferably an aqueous phosphate buffer of a pH of about 6.7, and wherein said aqueous phosphate buffer has a molarity of about 10 to about 15 mM, preferably of about 12mM, and wherein said aqueous phosphate buffer is present from about 20% (w/w) to about 30% (w/w), preferably about 22% (w/w) to about 28% (w/w), more preferably about 25% (w/w) to about 28% (w/w) and even more preferably of about 27% (w/w) to about 28% (w/w) based on the total weight of

said topical composition.

In a preferred embodiment, said topical composition comprises PEG 400 and water. In a very preferred embodiment, said topical composition is in the form of an aqueous gel, wherein said solvent comprises PEG 400 and water, and wherein the amount of said PEG 400 is from
5 about 60% to about 75 % (w/w), preferably from about 65% to about 75 % (w/w), and more preferably from about 68 % to about 72 % (w/w) based on the total weight of the topical composition, and wherein said topical composition comprises an aqueous buffer, preferably an aqueous phosphate buffer, wherein said topical composition comprises an aqueous buffer, preferably an aqueous phosphate buffer, of a pH of about 6.5 to about 7.5, preferably an aqueous
10 phosphate buffer of a pH of about 6.5 to about 7.0, and more preferably an aqueous buffer, preferably an aqueous phosphate buffer, of a pH of about 6.7.

In a preferred embodiment, said topical composition comprises PEG 400 and water. In a very preferred embodiment, said topical composition is in the form of an aqueous gel, wherein said solvent comprises PEG 400 and water, and wherein the amount of said PEG 400 is from
15 about 60% to about 75 % (w/w), preferably from about 65% to about 75 % (w/w), and more preferably from about 68 % to about 72 % (w/w) based on the total weight of the topical composition, and wherein said topical composition comprises an aqueous phosphate buffer, wherein said topical composition comprises an aqueous phosphate buffer of a pH of about 6.5 to about 7.5, preferably an aqueous phosphate buffer of a pH of about 6.5 to about 7.0, and
20 more preferably an aqueous phosphate buffer of a pH of about 6.7.

In a preferred embodiment, said topical composition is in the form of an aqueous gel, and wherein said solvent comprises PEG 400 and water.

In a preferred embodiment, the amount of said PEG 400 is from about 60% to about 75 % (w/w), preferably from about 65% to about 75 % (w/w), and more preferably from about 68
25 % to about 72 % (w/w) based on the total weight of the topical composition.

In a preferred embodiment, the solvent is present at a concentration from about 90% w/w to about 99% w/w, preferably about 95% w/w to about 99% w/w, more preferably about 97% w/w to about 98% w/w, based on the total weight of the topical composition.

In one embodiment, said pharmaceutically acceptable excipient is selected from the group
30 consisting of a thickening agent, a stabilizer, an antioxidant, a chelating agent, an oily material, an emulsifier, a penetration enhancer, a pH adjusting agent, a preservative, an antimicrobial agent, an opacifier, a fragrance, a colorant, a gelling agent, a moisturizer, a surfactant, and a combination thereof. In one embodiment, said pharmaceutically acceptable excipient is selected from the group consisting of a thickening agent, a stabilizer, an antioxidant, a pH adjusting

agent, a preservative, an antimicrobial agent, and a combination thereof.

In one embodiment, said topical composition comprises one or more other pharmaceutically acceptable excipients, and wherein said pharmaceutically acceptable excipient is selected from the group consisting of a thickening agent, a stabilizer, an antioxidant, a chelating agent, an oily material, an emulsifier, a penetration enhancer, a pH adjusting agent, a preservative, an antimicrobial agent, an opacifier, a fragrance, a colorant, a gelling agent, a moisturizer, a surfactant, and a combination thereof. In one embodiment, said topical composition comprises one or more other pharmaceutically acceptable excipients, and said pharmaceutically acceptable excipient is selected from the group consisting of a thickening agent, a stabilizer, an antioxidant, a pH adjusting agent, a preservative, an antimicrobial agent, and a combination thereof.

The pharmaceutically acceptable excipients used in the topical composition of the present invention can act in more than one way. For example, a thickening agent can also function as a gelling agent.

In a preferred embodiment, said topical composition comprises one or more other pharmaceutically acceptable excipients, and one of said pharmaceutically acceptable excipients is a thickening agent. In a preferred embodiment, said topical composition comprises a thickening agent.

Thickening agents are known to the skilled person in the art and includes a cellulose derivative, polyvinylpyrrolidone, a carbomer polymer, carbomer derivative, maltodextrin, polydextrose, dextrates, carboxypolymethylene, polyvinyl alcohol, poloxamers and mixtures thereof. Thickening agents may serve, in particular by way of the final viscosity of the topical composition, to ensure and increase the time of stay of the topical composition, for example, on the fingertip wound of the ulcers during the treatment regime.

In a preferred embodiment, said topical composition comprises one or more other pharmaceutically acceptable excipients, and one of said pharmaceutically acceptable excipients is a thickening agent, wherein said thickening agent is selected from the group consisting of carboxymethyl cellulose, hydroxyethyl cellulose, sodium hyaluronate and polyvinylpyrrolidone. In a preferred embodiment, said topical composition comprises a thickening agent, wherein said thickening agent is selected from the group consisting of carboxymethyl cellulose, hydroxyethyl cellulose, sodium hyaluronate and polyvinylpyrrolidone.

In a preferred embodiment, said topical composition comprises one or more excipient selected from a cellulose derivative, polyvinylpyrrolidone, a carbomer polymer, carbomer derivative, maltodextrin, polydextrose, dextrates, carboxypolymethylene, polyvinyl alcohol,

poloxamers or a mixture thereof.

In a preferred embodiment, said topical composition comprises one or more excipient selected from the group consisting of carboxymethyl cellulose, hydroxyethyl cellulose, sodium hyaluronate and polyvinylpyrrolidon. In a preferred embodiment, said topical composition
5 comprises one or more excipient selected from the group consisting of carboxymethyl cellulose, hydroxyethyl cellulose, sodium hyaluronate and polyvinylpyrrolidon.

In a preferred embodiment, said topical composition comprises a thickening agent, wherein preferably said thickening agent is selected from the group consisting of carboxymethyl cellulose, hydroxyethyl cellulose, sodium hyaluronate and polyvinylpyrrolidon.

10 In a preferred embodiment, said topical composition comprises one or more other pharmaceutically acceptable excipients, and one of said pharmaceutically acceptable excipients is a thickening agent, wherein said thickening agent is hydroxyethyl cellulose. In a preferred embodiment, said topical composition comprises a thickening agent, wherein said thickening agent is hydroxyethyl cellulose.

15 In a preferred embodiment, said topical composition comprises one or more other pharmaceutically acceptable excipients, and one of said pharmaceutically acceptable excipients is a thickening agent, wherein said thickening agent is sodium hyaluronate. In a preferred embodiment, said topical composition comprises a thickening agent, wherein said thickening agent is sodium hyaluronate.

20 In a preferred embodiment, said topical composition comprises one or more other pharmaceutically acceptable excipients, and one of said pharmaceutically acceptable excipients is hydroxyethyl cellulose.

In a preferred embodiment, said topical composition comprises one or more other pharmaceutically acceptable excipients, and one of said pharmaceutically acceptable excipients
25 is sodium hyaluronate.

In some embodiments, the thickening agent is present from about 0.01% (w/w) to about 5% (w/w), preferably about 0.05% (w/w) to about 2% (w/w), more preferably about 0.1% (w/w) to about 1% (w/w), based on the total weight of said topical composition.

In a preferred embodiment, said topical composition comprises one or more other
30 pharmaceutically acceptable excipients, and one of said pharmaceutically acceptable excipients is a thickening agent, wherein said thickening agent is hydroxyethyl cellulose, and wherein said hydroxyethyl cellulose is present from about 0.1% (w/w) to about 1% (w/w), preferably about 0.1% (w/w) to about 0.5% (w/w), more preferably about 0.25% (w/w) based on the total weight of said topical composition. In a preferred embodiment, said topical composition comprises a

thickening agent, wherein said thickening agent is hydroxyethyl cellulose, and wherein said hydroxyethyl cellulose is present from about 0.1% (w/w) to about 1% (w/w), preferably about 0.1% (w/w) to about 0.5% (w/w), more preferably about 0.25% (w/w) based on the total weight of said topical composition.

5 In a preferred embodiment, said topical composition comprises one or more other pharmaceutically acceptable excipients, and one of said pharmaceutically acceptable excipients is a thickening agent, wherein said thickening agent is sodium hyaluronate, and wherein said sodium hyaluronate is present from about 0.1% (w/w) to about 1% (w/w), preferably about 0.2% (w/w) to about 0.8% (w/w), more preferably about 0.50% (w/w) based on the total weight
10 of said topical composition. In a preferred embodiment, said topical composition comprises a thickening agent, wherein said thickening agent is sodium hyaluronate, and wherein said sodium hyaluronate is present from about 0.1% (w/w) to about 1% (w/w), preferably about 0.2% (w/w) to about 0.8% (w/w), more preferably about 0.50% (w/w) based on the total weight of said topical composition.

15 In a preferred embodiment, said topical composition comprises hydroxyethyl cellulose. In a preferred embodiment, said topical composition comprises hydroxyethyl cellulose, and wherein said hydroxyethyl cellulose is present from about 0.1% (w/w) to about 1% (w/w), preferably about 0.1% (w/w) to about 0.5% (w/w), more preferably about 0.25% (w/w) based on the total weight of said topical composition.

20 In a preferred embodiment, said topical composition comprises sodium hyaluronate. In a preferred embodiment, said topical composition comprises sodium hyaluronate, and wherein said sodium hyaluronate is present from about 0.1% (w/w) to about 1% (w/w), preferably about 0.2% (w/w) to about 0.8% (w/w), more preferably about 0.50% (w/w) based on the total weight of said topical composition.

25 In a preferred embodiment, said topical composition comprises one or more other pharmaceutically acceptable excipients, and one of said pharmaceutically acceptable excipients is an antioxidant. In a preferred embodiment, said topical composition comprises an antioxidant.

30 Antioxidants are known to the skilled person in the art and includes, for example, butylated hydroxyanisole, butylated hydroxytoluene, vitamin C, vitamin E, vitamin A, lutein, lycopene, retinyl palmitate, potassium metabisulfite, sodium metabisulfite, sodium thiosulfate pentahydrate, 3,4-dihydroxybenzoic acid, propyl gallate, alpha-lipoic acid, ascorbyl palmitate, sodium pyrosulfite, ubiquinone, selenium, or a combination thereof.

In a preferred embodiment, said topical composition comprises one or more other

pharmaceutically acceptable excipients, and one of said pharmaceutically acceptable excipients is an antioxidant, wherein said antioxidant is butylated hydroxytoluene. In a preferred embodiment, said topical composition comprises an antioxidant, wherein said antioxidant is butylated hydroxytoluene.

5 In a preferred embodiment, said topical composition comprises one or more excipient selected from butylated hydroxyanisole, butylated hydroxytoluene, vitamin C, vitamin E, vitamin A, lutein, lycopene, retinyl palmitate, potassium metabisulfite, sodium metabisulfite, sodium thiosulfate pentahydrate, 3,4-dihydroxybenzoic acid, propyl gallate, alpha-lipoic acid, ascorbyl palmitate, sodium pyrosulfite, ubiquinone, selenium, or a combination thereof. In a
10 preferred embodiment, said topical composition comprises butylated hydroxytoluene.

In a preferred embodiment, said topical composition comprises an antioxidant, wherein preferably said antioxidant is butylated hydroxytoluene.

In some embodiments, the antioxidant is present from about 0.001% (w/w) to about 0.1% (w/w), preferably about 0.001% (w/w) to about 0.01% (w/w), more preferably about 0.002%
15 (w/w) to about 0.008% (w/w), based on the total weight of said topical composition.

In a preferred embodiment, said topical composition comprises one or more other pharmaceutically acceptable excipients, and one of said pharmaceutically acceptable excipients is an antioxidant, wherein said antioxidant is butylated hydroxytoluene, and wherein said butylated hydroxytoluene is present from about 0.001% (w/w) to about 0.1% (w/w), preferably
20 from about 0.001% (w/w) to about 0.01% (w/w), more preferably from about 0.002% (w/w) to about 0.008% (w/w), and even more preferably of about 0.006% (w/w) based on the total weight of said topical composition. In a preferred embodiment, said topical composition comprises a an antioxidant, wherein said antioxidant is butylated hydroxytoluene, and wherein said butylated hydroxytoluene is present from about 0.001% (w/w) to about 0.1% (w/w), preferably
25 from about 0.001% (w/w) to about 0.01% (w/w), more preferably from about 0.002% (w/w) to about 0.008% (w/w), and even more preferably of about 0.006% (w/w) based on the total weight of said topical composition.

In a preferred embodiment, said topical composition comprises butylated hydroxytoluene, and wherein said butylated hydroxytoluene is present from about 0.001%
30 (w/w) to about 0.1% (w/w), preferably from about 0.001% (w/w) to about 0.01% (w/w), more preferably from about 0.002% (w/w) to about 0.008% (w/w), and even more preferably of about 0.006% (w/w) based on the total weight of said topical composition.

In a preferred embodiment, said topical composition comprises one or more other pharmaceutically acceptable excipients, and one of said pharmaceutically acceptable excipients

is a preservative. In a preferred embodiment, said topical composition comprises a preservative.

Preservatives are known to the skilled person in the art and includes, for example, benzyl alcohol, benzoic acid, phenol, m-cresol, methyl parabene, propyl parabene, or a combination thereof.

5 In a preferred embodiment, said topical composition comprises one or more other pharmaceutically acceptable excipients, and one of said pharmaceutically acceptable excipients is a preservative, wherein said preservative is benzyl alcohol. In a preferred embodiment, said topical composition comprises a preservative, wherein said preservative is benzyl alcohol.

10 In a preferred embodiment, said topical composition comprises one or more excipient selected from benzyl alcohol, benzoic acid, phenol, m-cresol, methyl parabene, propyl parabene, or a combination thereof. In a preferred embodiment, said topical composition comprises benzyl alcohol.

In a preferred embodiment, said topical composition comprises a preservative, wherein preferably said preservative is benzyl alcohol.

15 In some embodiments, the preservative is present from about 0.01% (w/w) to about 5% (w/w), preferably about 0.1% (w/w) to about 5% (w/w), more preferably about 1% (w/w) to about 3% (w/w), based on the total weight of said topical composition.

In a preferred embodiment, said topical composition comprises one or more other pharmaceutically acceptable excipients, and one of said pharmaceutically acceptable excipients is a preservative, wherein said preservative is benzyl alcohol, and wherein said benzyl alcohol is present from about 0.01% (w/w) to about 5% (w/w), preferably about 0.1% (w/w) to about 5% (w/w), more preferably about 1% (w/w) to about 3% (w/w) and even more preferably of about 2% (w/w) based on the total weight of said topical composition. In a preferred embodiment, said topical composition comprises a preservative, wherein said preservative is benzyl alcohol, and wherein said benzyl alcohol is present from about 0.01% (w/w) to about 5% (w/w), preferably about 0.1% (w/w) to about 5% (w/w), more preferably about 1% (w/w) to about 3% (w/w) and even more preferably of about 2% (w/w) based on the total weight of said topical composition.

20 In a preferred embodiment, said topical composition comprises benzyl alcohol, and wherein said benzyl alcohol is present from about 0.01% (w/w) to about 5% (w/w), preferably about 0.1% (w/w) to about 5% (w/w), more preferably about 1% (w/w) to about 3% (w/w) and even more preferably of about 2% (w/w) based on the total weight of said topical composition.

In a preferred embodiment, said topical composition is in the form of an aqueous gel, and wherein said solvent is a combination of PEG 400 and water, and wherein the amount of said

PEG 400 is from about 60% to about 75 % (w/w), preferably from about 65% to about 75 % (w/w), and more preferably from about 68 % to about 72 % (w/w) based on the total weight of the topical composition; and wherein said topical composition comprises

- 5 (i) an aqueous buffer, preferably an aqueous phosphate buffer, and further preferably an aqueous buffer, preferably an aqueous phosphate buffer, of a pH of about 6.5 to about 7.5;
- (ii) a thickening agent, wherein preferably said thickening agent is selected from the group consisting of carboxymethyl cellulose, hydroxyethyl cellulose, sodium hyaluronate and polyvinylpyrrolidon, and wherein further preferably said thickening agent is
- 10 hydroxyethyl cellulose or sodium hyaluronate, and more preferably wherein said thickening agent is hydroxyethyl cellulose;
- (iii) an antioxidant, wherein preferably said antioxidant is butylated hydroxytoluene; and
- (iv) a preservative, wherein preferably said preservative is benzyl alcohol.

In a preferred embodiment, said topical composition is in the form of an aqueous gel, and

15 wherein said solvent is a combination of PEG 400 and water, and wherein the amount of said PEG 400 is from about 60% to about 75 % (w/w), preferably from about 65% to about 75 % (w/w), and more preferably from about 68 % to about 72 % (w/w) based on the total weight of the topical composition; and wherein said topical composition comprises

- 20 (i) an aqueous buffer, preferably an aqueous phosphate buffer, and further preferably an aqueous buffer, preferably an aqueous phosphate buffer, of a pH of about 6.5 to about 7.5;
- (ii) carboxymethyl cellulose, hydroxyethyl cellulose, sodium hyaluronate or polyvinylpyrrolidon, preferably hydroxyethyl cellulose or sodium hyaluronate, and more preferably hydroxyethyl cellulose;
- 25 (iii) butylated hydroxytoluene; and
- (iv) benzyl alcohol.

In a preferred embodiment, said topical composition is in the form of an aqueous gel, and wherein said solvent is a combination of PEG 400 and water, and wherein the amount of said PEG 400 is from about 60% to about 75 % (w/w), preferably from about 65% to about 75 %

30 (w/w), and more preferably from about 68 % to about 72 % (w/w) based on the total weight of the topical composition; and wherein said topical composition comprises

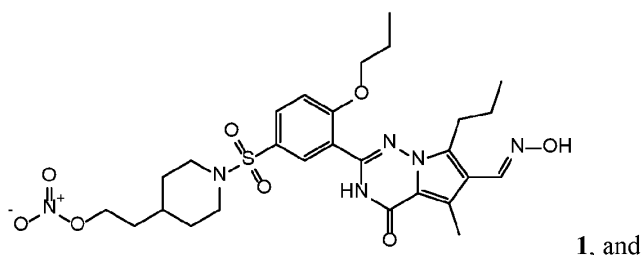
- (i) an aqueous buffer, preferably an aqueous phosphate buffer, and further preferably an aqueous buffer, preferably an aqueous phosphate buffer, of a pH of about 6.5 to about 7.5;

- (ii) a thickening agent, wherein said thickening agent is hydroxyethyl cellulose or sodium hyaluronate, preferably wherein said thickening agent is hydroxyethyl cellulose;
- (iii) an antioxidant, wherein said antioxidant is butylated hydroxytoluene; and
- (iv) a preservative, wherein said preservative is benzyl alcohol.

5 In a preferred embodiment, said topical composition is in the form of an aqueous gel, and wherein said solvent is a combination of PEG 400 and water, and wherein the amount of said PEG 400 is from about 60% to about 75 % (w/w), preferably from about 65% to about 75 % (w/w), and more preferably from about 68 % to about 72 % (w/w) based on the total weight of the topical composition; and wherein said topical composition comprises

- 10 (i) an aqueous buffer, preferably an aqueous phosphate buffer, and further preferably an aqueous buffer, preferably an aqueous phosphate buffer, of a pH of about 6.5 to about 7.5;
- (ii) hydroxyethyl cellulose or sodium hyaluronate, preferably hydroxyethyl cellulose;
- (iii) butylated hydroxytoluene; and
- 15 (iv) benzyl alcohol.

In a preferred embodiment, said compound of formula **I** is Compound **1**,



wherein said topical composition comprises said Compound **1** in an amount between about 0.7 μ mol and about 5 μ mol, preferably in an amount between about 0.8 μ mol and about 2 μ mol, and further preferably in an amount of about 1 μ mol; and

wherein said topical composition is in the form of an aqueous gel, and wherein said solvent is a combination of PEG 400 and water, and wherein the amount of said PEG 400 is from about 60% to about 75 % (w/w), preferably from about 65% to about 75 % (w/w), and more preferably from about 68 % to about 72 % (w/w) based on the total weight of the topical composition;

wherein said topical composition comprises

- (i) an aqueous buffer, preferably an aqueous phosphate buffer, and further preferably an aqueous buffer, preferably an aqueous phosphate buffer, of a pH of about 6.5 to about 7.5, wherein preferably said aqueous buffer, preferably said

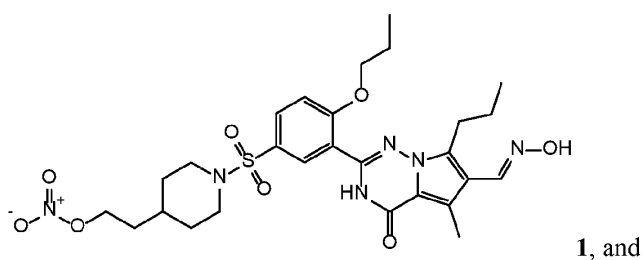
aqueous phosphate buffer has a molarity of about 10 to about 15 mM, preferably of about 12mM, and wherein said aqueous buffer, preferably said aqueous phosphate buffer is present from about 20% (w/w) to about 30% (w/w), preferably about 22% (w/w) to about 28% (w/w), more preferably about 25% (w/w) to about 28% (w/w) and even more preferably of about 27% (w/w) to about 28% (w/w) based on the total weight of said topical composition;

(ii) a thickening agent, wherein said thickening agent is hydroxyethyl cellulose or sodium hyaluronate, preferably wherein said thickening agent is hydroxyethyl cellulose, and wherein preferably the thickening agent is present from about 0.01% (w/w) to about 5% (w/w), preferably about 0.05% (w/w) to about 2% (w/w), more preferably about 0.1% (w/w) to about 1% (w/w), based on the total weight of said topical composition;

(iii) an antioxidant, wherein said antioxidant is butylated hydroxytoluene, and wherein preferably said antioxidant is present from about 0.001% (w/w) to about 0.1% (w/w), preferably about 0.001% (w/w) to about 0.01% (w/w), more preferably about 0.002% (w/w) to about 0.008% (w/w), based on the total weight of said topical composition; and

(iv) a preservative, wherein said preservative is benzyl alcohol, and wherein preferably said benzyl alcohol is present from about 0.01% (w/w) to about 5% (w/w), preferably about 0.1% (w/w) to about 5% (w/w), more preferably about 1% (w/w) to about 3% (w/w) and even more preferably of about 2% (w/w) based on the total weight of said topical composition.

In a preferred embodiment, said compound of formula **I** is Compound **1**,



wherein said topical composition comprises said Compound **1** in a concentration between about 7 μ M and about 55 μ M, preferably in a concentration between about 8 μ M and about 22 μ M, further preferably in a concentration between about 10 μ M and about 12 μ M, and again further preferably in a concentration of about 11 μ M; and

wherein said topical composition is in the form of an aqueous gel, and wherein said

solvent is a combination of PEG 400 and water, and wherein the amount of said PEG 400 is from about 60% to about 75 % (w/w), preferably from about 65% to about 75 % (w/w), and more preferably from about 68 % to about 72 % (w/w) based on the total weight of the topical composition;

5 wherein said topical composition comprises

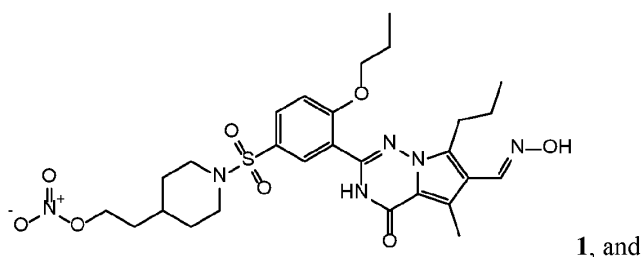
 (i) an aqueous buffer, preferably an aqueous phosphate buffer, and further preferably an aqueous buffer, preferably an aqueous phosphate buffer, of a pH of about 6.5 to about 7.5, wherein preferably said aqueous buffer, preferably said aqueous phosphate buffer has a molarity of about 10 to about 15 mM, preferably of
10 about 12mM, and wherein said aqueous buffer, preferably said aqueous phosphate buffer is present from about 20% (w/w) to about 30% (w/w), preferably about 22% (w/w) to about 28% (w/w), more preferably about 25% (w/w) to about 28% (w/w) and even more preferably of about 27% (w/w) to about 28% (w/w) based on the total weight of said topical composition;

15 (ii) a thickening agent, wherein said thickening agent is hydroxyethyl cellulose or sodium hyaluronate, preferably wherein said thickening agent is hydroxyethyl cellulose, and wherein preferably the thickening agent is present from about 0.01% (w/w) to about 5% (w/w), preferably about 0.05% (w/w) to about 2% (w/w), more preferably about 0.1% (w/w) to about 1% (w/w), based on the total weight of said
20 topical composition;

 (iii) an antioxidant, wherein said antioxidant is butylated hydroxytoluene, and wherein preferably said antioxidant is present from about 0.001% (w/w) to about 0.1% (w/w), preferably about 0.001% (w/w) to about 0.01% (w/w), more preferably about 0.002% (w/w) to about 0.008% (w/w), based on the total weight of said
25 topical composition; and

 (iv) a preservative, wherein said preservative is benzyl alcohol, and wherein preferably said benzyl alcohol is present from about 0.01% (w/w) to about 5% (w/w), preferably about 0.1% (w/w) to about 5% (w/w), more preferably about 1% (w/w) to about 3% (w/w) and even more preferably of about 2% (w/w) based on
30 the total weight of said topical composition.

In a preferred embodiment, said compound of formula **I** is Compound **1**,



wherein said topical composition comprises said Compound **1** in an amount between about 0.7 μ mol and about 5 μ mol, preferably in an amount between about 0.8 μ mol and about 2 μ mol, and further preferably in an amount of about 1 μ mol; and

5 wherein said topical composition is in the form of an aqueous gel, and wherein said solvent is a combination of PEG 400 and water, and wherein the amount of said PEG 400 is from about 60% to about 75 % (w/w), preferably from about 65% to about 75 % (w/w), and more preferably from about 68 % to about 72 % (w/w) based on the total weight of the topical composition;

10 wherein said topical composition comprises

(i) an aqueous buffer, preferably an aqueous phosphate buffer, and further preferably an aqueous buffer, preferably an aqueous phosphate buffer, of a pH of about 6.5 to about 7.5, wherein preferably said aqueous buffer, preferably said aqueous phosphate buffer has a molarity of about 10 to about 15 mM, preferably of about 12mM, and wherein said aqueous buffer, preferably said aqueous phosphate buffer is present from about 20% (w/w) to about 30% (w/w), preferably about 22% (w/w) to about 28% (w/w), more preferably about 25% (w/w) to about 28% (w/w) and even more preferably of about 27% (w/w) to about 28% (w/w) based on the total weight of said topical composition;

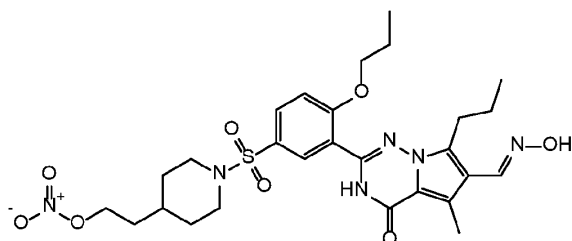
20 (ii) hydroxyethyl cellulose or sodium hyaluronate, preferably hydroxyethyl cellulose, and wherein preferably hydroxyethyl cellulose or sodium hyaluronate is present from about 0.01% (w/w) to about 5% (w/w), preferably about 0.05% (w/w) to about 2% (w/w), more preferably about 0.1% (w/w) to about 1% (w/w), based on the total weight of said topical composition;

25 (iii) butylated hydroxytoluene, and wherein preferably butylated hydroxytoluene is present from about 0.001% (w/w) to about 0.1% (w/w), preferably about 0.001% (w/w) to about 0.01% (w/w), more preferably about 0.002% (w/w) to about 0.008% (w/w), based on the total weight of said topical composition; and

(iv) benzyl alcohol, and wherein preferably said benzyl alcohol is present from

about 0.01% (w/w) to about 5% (w/w), preferably about 0.1% (w/w) to about 5% (w/w), more preferably about 1% (w/w) to about 3% (w/w) and even more preferably of about 2% (w/w) based on the total weight of said topical composition.

In a preferred embodiment, said compound of formula I is Compound 1,



1, and

wherein said topical composition comprises said Compound 1 in a concentration between about 7μM and about 55μM, preferably in a concentration between about 8μM and about 22μM, further preferably in a concentration between about 10μM and about 12μM, and again further preferably in a concentration of about 11μM; and

wherein said topical composition is in the form of an aqueous gel, and wherein said solvent is a combination of PEG 400 and water, and wherein the amount of said PEG 400 is from about 60% to about 75 % (w/w), preferably from about 65% to about 75 % (w/w), and more preferably from about 68 % to about 72 % (w/w) based on the total weight of the topical composition;

wherein said topical composition comprises

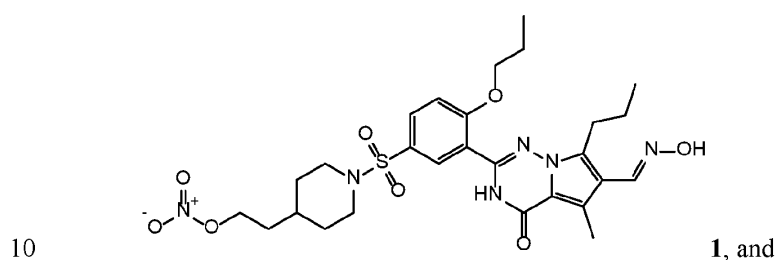
(i) an aqueous buffer, preferably an aqueous phosphate buffer, and further preferably an aqueous buffer, preferably an aqueous phosphate buffer, of a pH of about 6.5 to about 7.5, wherein preferably said aqueous buffer, preferably said aqueous phosphate buffer has a molarity of about 10 to about 15 mM, preferably of about 12mM, and wherein said aqueous buffer, preferably said aqueous phosphate buffer is present from about 20% (w/w) to about 30% (w/w), preferably about 22% (w/w) to about 28% (w/w), more preferably about 25% (w/w) to about 28% (w/w) and even more preferably of about 27% (w/w) to about 28% (w/w) based on the total weight of said topical composition;

(ii) hydroxyethyl cellulose or sodium hyaluronate, preferably hydroxyethyl cellulose, and wherein preferably hydroxyethyl cellulose or sodium hyaluronate is present from about 0.01% (w/w) to about 5% (w/w), preferably about 0.05% (w/w) to about 2% (w/w), more preferably about 0.1% (w/w) to about 1% (w/w), based on the total weight of said topical composition;

(iii) butylated hydroxytoluene, and wherein preferably butylated hydroxytoluene is present from about 0.001% (w/w) to about 0.1% (w/w), preferably about 0.001% (w/w) to about 0.01% (w/w), more preferably about 0.002% (w/w) to about 0.008% (w/w), based on the total weight of said topical composition; and

5 (iv) benzyl alcohol, and wherein preferably said benzyl alcohol is present from about 0.01% (w/w) to about 5% (w/w), preferably about 0.1% (w/w) to about 5% (w/w), more preferably about 1% (w/w) to about 3% (w/w) and even more preferably of about 2% (w/w) based on the total weight of said topical composition.

In a preferred embodiment, said compound of formula **I** is Compound **1**,



wherein said topical composition comprises said Compound **1** in an amount between about 0.7 μ mol and about 5 μ mol, preferably in an amount between about 0.8 μ mol and about 2 μ mol, and further preferably in an amount of about 1 μ mol; and

15 wherein said topical composition is in the form of an aqueous gel, and wherein said solvent is a combination of PEG 400 and water, and wherein the amount of said PEG 400 is from about 60% to about 75 % (w/w), preferably from about 65% to about 75 % (w/w), and more preferably from about 68 % to about 72 % (w/w) based on the total weight of the topical composition;

wherein said topical composition comprises

20 (i) an aqueous phosphate buffer of a pH of about 6.5 to about 7.5, wherein preferably said aqueous phosphate buffer has a molarity of about 10 to about 15 mM, preferably of about 12mM, and wherein said aqueous phosphate buffer is present from about 20% (w/w) to about 30% (w/w), preferably about 22% (w/w) to about 28% (w/w), more preferably about 25% (w/w) to about 28% (w/w) and even

25 more preferably of about 27% (w/w) to about 28% (w/w) based on the total weight of said topical composition;

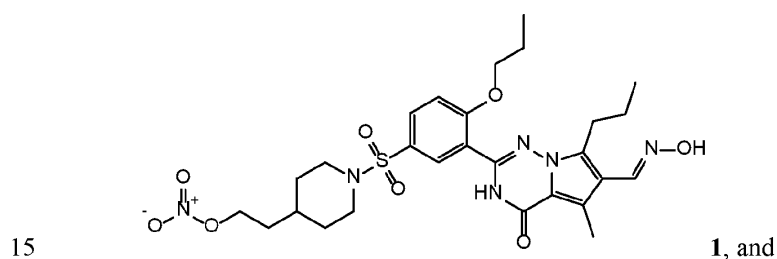
(ii) a thickening agent, wherein said thickening agent is hydroxyethyl cellulose or sodium hyaluronate, preferably wherein said thickening agent is hydroxyethyl cellulose, and wherein the thickening agent is present from about 0.01% (w/w) to

about 5% (w/w), preferably about 0.05% (w/w) to about 2% (w/w), more preferably about 0.1% (w/w) to about 1% (w/w), based on the total weight of said topical composition;

(iii) an antioxidant, wherein said antioxidant is butylated hydroxytoluene, and wherein said antioxidant is present from about 0.001% (w/w) to about 0.1% (w/w), preferably about 0.001% (w/w) to about 0.01% (w/w), more preferably about 0.002% (w/w) to about 0.008% (w/w), based on the total weight of said topical composition; and

(iv) a preservative, wherein said preservative is benzyl alcohol, and wherein said benzyl alcohol is present from about 0.01% (w/w) to about 5% (w/w), preferably about 0.1% (w/w) to about 5% (w/w), more preferably about 1% (w/w) to about 3% (w/w) and even more preferably of about 2% (w/w) based on the total weight of said topical composition.

In a preferred embodiment, said compound of formula **I** is Compound **1**,



wherein said topical composition comprises said Compound **1** in a concentration between about 7 μ M and about 55 μ M, preferably in a concentration between about 8 μ M and about 22 μ M, further preferably in a concentration between about 10 μ M and about 12 μ M, and again further preferably in a concentration of about 11 μ M; and

20 wherein said topical composition is in the form of an aqueous gel, and wherein said solvent is a combination of PEG 400 and water, and wherein the amount of said PEG 400 is from about 60% to about 75 % (w/w), preferably from about 65% to about 75 % (w/w), and more preferably from about 68 % to about 72 % (w/w) based on the total weight of the topical composition;

25 wherein said topical composition comprises

(i) an aqueous phosphate buffer of a pH of about 6.5 to about 7.5, wherein preferably said aqueous phosphate buffer has a molarity of about 10 to about 15 mM, preferably of about 12mM, and wherein said aqueous phosphate buffer is present from about 20% (w/w) to about 30% (w/w), preferably about 22% (w/w) to

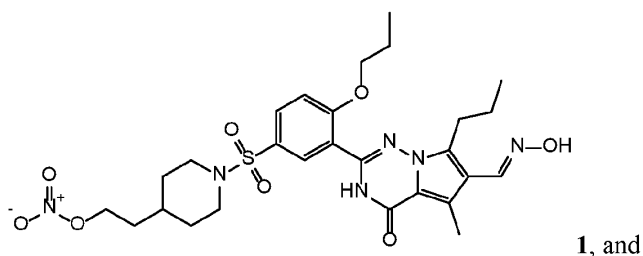
about 28% (w/w), more preferably about 25% (w/w) to about 28% (w/w) and even more preferably of about 27% (w/w) to about 28% (w/w) based on the total weight of said topical composition;

5 (ii) a thickening agent, wherein said thickening agent is hydroxyethyl cellulose or sodium hyaluronate, preferably wherein said thickening agent is hydroxyethyl cellulose, and wherein the thickening agent is present from about 0.01% (w/w) to about 5% (w/w), preferably about 0.05% (w/w) to about 2% (w/w), more preferably about 0.1% (w/w) to about 1% (w/w), based on the total weight of said topical composition;

10 (iii) an antioxidant, wherein said antioxidant is butylated hydroxytoluene, and wherein said antioxidant is present from about 0.001% (w/w) to about 0.1% (w/w), preferably about 0.001% (w/w) to about 0.01% (w/w), more preferably about 0.002% (w/w) to about 0.008% (w/w), based on the total weight of said topical composition; and

15 (iv) a preservative, wherein said preservative is benzyl alcohol, and wherein said benzyl alcohol is present from about 0.01% (w/w) to about 5% (w/w), preferably about 0.1% (w/w) to about 5% (w/w), more preferably about 1% (w/w) to about 3% (w/w) and even more preferably of about 2% (w/w) based on the total weight of said topical composition.

20 In a preferred embodiment, said compound of formula **I** is Compound **1**,



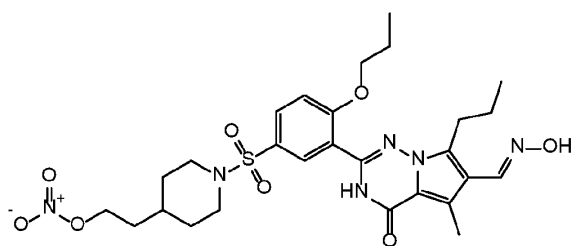
wherein said topical composition comprises said Compound **1** in an amount between about 0.7 μ mol and about 5 μ mol, preferably in an amount between about 0.8 μ mol and about 2 μ mol, and further preferably in an amount of about 1 μ mol; and

25 wherein said topical composition is in the form of an aqueous gel, and wherein said solvent is a combination of PEG 400 and water, and wherein the amount of said PEG 400 is from about 60% to about 75 % (w/w), preferably from about 65% to about 75 % (w/w), and more preferably from about 68 % to about 72 % (w/w) based on the total weight of the topical composition;

wherein said topical composition comprises

- (i) an aqueous phosphate buffer of a pH of about 6.5 to about 7.5, wherein preferably said aqueous phosphate buffer has a molarity of about 10 to about 15 mM, preferably of about 12mM, and wherein said aqueous phosphate buffer is present from about 20% (w/w) to about 30% (w/w), preferably about 22% (w/w) to about 28% (w/w), more preferably about 25% (w/w) to about 28% (w/w) and even more preferably of about 27% (w/w) to about 28% (w/w) based on the total weight of said topical composition;
- (ii) a thickening agent, wherein said thickening agent is hydroxyethyl cellulose or sodium hyaluronate, preferably wherein said thickening agent is hydroxyethyl cellulose, and wherein the thickening agent is present from about 0.01% (w/w) to about 5% (w/w), preferably about 0.05% (w/w) to about 2% (w/w), more preferably about 0.1% (w/w) to about 1% (w/w), based on the total weight of said topical composition;
- (iii) an antioxidant, wherein said antioxidant is butylated hydroxytoluene, and wherein said antioxidant is present from about 0.001% (w/w) to about 0.1% (w/w), preferably about 0.001% (w/w) to about 0.01% (w/w), more preferably about 0.002% (w/w) to about 0.008% (w/w), based on the total weight of said topical composition; and
- (iv) a preservative, wherein said preservative is benzyl alcohol, and wherein said benzyl alcohol is present from about 0.01% (w/w) to about 5% (w/w), preferably about 0.1% (w/w) to about 5% (w/w), more preferably about 1% (w/w) to about 3% (w/w) and even more preferably of about 2% (w/w) based on the total weight of said topical composition.

In a preferred embodiment, said compound of formula **I** is Compound **1**,



1, and

wherein said topical composition comprises said Compound **1** in a concentration between about 7 μ M and about 55 μ M, preferably in a concentration between about 8 μ M and about

22 μ M, and further preferably in a concentration between about 10 μ M and about 12 μ M, and again further preferably in a concentration of about 11 μ M; and

wherein said topical composition is in the form of an aqueous gel, and wherein said solvent is a combination of PEG 400 and water, and wherein the amount of said PEG 400 is
5 from about 60% to about 75 % (w/w), preferably from about 65% to about 75 % (w/w), and more preferably from about 68 % to about 72 % (w/w) based on the total weight of the topical composition;

wherein said topical composition comprises

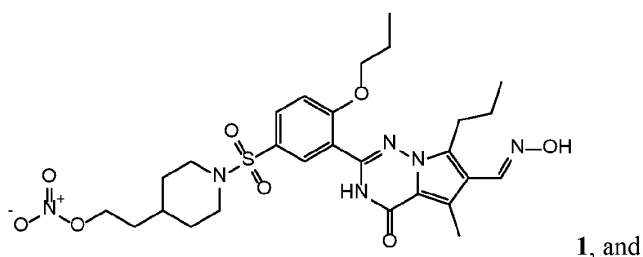
(i) an aqueous phosphate buffer of a pH of about 6.5 to about 7.5, wherein
10 preferably said aqueous phosphate buffer has a molarity of about 10 to about 15 mM, preferably of about 12mM, and wherein said aqueous phosphate buffer is present from about 20% (w/w) to about 30% (w/w), preferably about 22% (w/w) to about 28% (w/w), more preferably about 25% (w/w) to about 28% (w/w) and even more preferably of about 27% (w/w) to about 28% (w/w) based on the total weight
15 of said topical composition;

(ii) a thickening agent, wherein said thickening agent is hydroxyethyl cellulose or sodium hyaluronate, preferably wherein said thickening agent is hydroxyethyl cellulose, and wherein the thickening agent is present from about 0.01% (w/w) to about 5% (w/w), preferably about 0.05% (w/w) to about 2% (w/w), more preferably
20 about 0.1% (w/w) to about 1% (w/w), based on the total weight of said topical composition;

(iii) an antioxidant, wherein said antioxidant is butylated hydroxytoluene, and wherein said antioxidant is present from about 0.001% (w/w) to about 0.1% (w/w), preferably about 0.001% (w/w) to about 0.01% (w/w), more preferably about
25 0.002% (w/w) to about 0.008% (w/w), based on the total weight of said topical composition; and

(iv) a preservative, wherein said preservative is benzyl alcohol, and wherein said benzyl alcohol is present from about 0.01% (w/w) to about 5% (w/w), preferably about 0.1% (w/w) to about 5% (w/w), more preferably about 1% (w/w) to about 3% (w/w) and even more preferably
30 of about 2% (w/w) based on the total weight of said topical composition.

In a preferred embodiment, said compound of formula I is Compound 1,



wherein said topical composition comprises said Compound 1 in an amount between about 0.7 μ mol and about 5 μ mol, preferably in an amount between about 0.8 μ mol and about 2 μ mol, and further preferably in an amount of about 1 μ mol; and

5 wherein said topical composition is in the form of an aqueous gel, and wherein said solvent is a combination of PEG 400 and water, and wherein the amount of said PEG 400 is from about 60% to about 75 % (w/w), preferably from about 65% to about 75 % (w/w), and more preferably from about 68 % to about 72 % (w/w) based on the total weight of the topical composition;

10 wherein said topical composition comprises

(i) an aqueous phosphate buffer of a pH of about 6.5 to about 7.5, wherein preferably said aqueous phosphate buffer has a molarity of about 10 to about 15 mM, preferably of about 12mM, and wherein said aqueous phosphate buffer is present from about 20% (w/w) to about 30% (w/w), preferably about 22% (w/w) to about 28% (w/w), more preferably about 25% (w/w) to about 28% (w/w) and even more preferably of about 27% (w/w) to about 28% (w/w) based on the total weight of said topical composition;

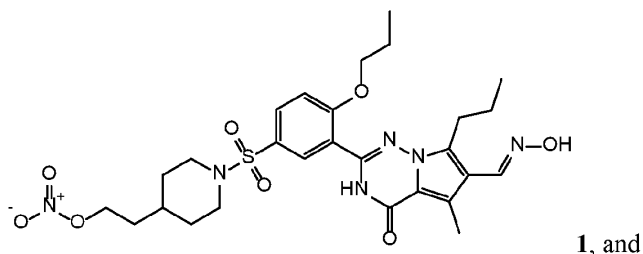
15 (ii) hydroxyethyl cellulose or sodium hyaluronate, preferably hydroxyethyl cellulose, and wherein hydroxyethyl cellulose or sodium hyaluronate is present from about 0.01% (w/w) to about 5% (w/w), preferably about 0.05% (w/w) to about 2% (w/w), more preferably about 0.1% (w/w) to about 1% (w/w), based on the total weight of said topical composition.

20 (iii) butylated hydroxytoluene, and wherein butylated hydroxytoluene is present from about 0.001% (w/w) to about 0.1% (w/w), preferably about 0.001% (w/w) to about 0.01% (w/w), more preferably about 0.002% (w/w) to about 0.008% (w/w), based on the total weight of said topical composition

25 (iv) benzyl alcohol, and wherein said benzyl alcohol is present from about 0.01% (w/w) to about 5% (w/w), preferably about 0.1% (w/w) to about 5% (w/w), more preferably about 1% (w/w) to about 3% (w/w) and even more preferably of about

2% (w/w) based on the total weight of said topical composition.

In a preferred embodiment, said compound of formula **I** is Compound **1**,



wherein said topical composition comprises said Compound **1** in a concentration between
 5 about 7 μ M and about 55 μ M, preferably in a concentration between about 8 μ M and about
 22 μ M, further preferably in a concentration between about 10 μ M and about 12 μ M, and again
 further preferably in a concentration of about 11 μ M; and

wherein said topical composition is in the form of an aqueous gel, and wherein said
 solvent is a combination of PEG 400 and water, and wherein the amount of said PEG 400 is
 10 from about 60% to about 75 % (w/w), preferably from about 65% to about 75 % (w/w), and
 more preferably from about 68 % to about 72 % (w/w) based on the total weight of the topical
 composition;

wherein said topical composition comprises

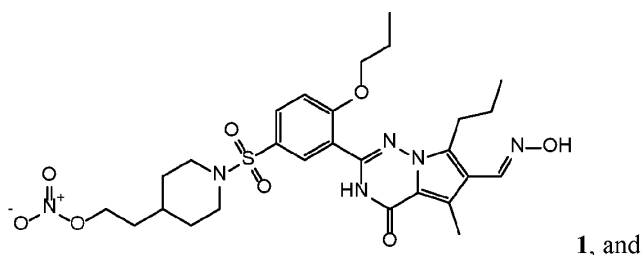
(i) an aqueous phosphate buffer of a pH of about 6.5 to about 7.5, wherein
 15 preferably said aqueous phosphate buffer has a molarity of about 10 to about 15
 mM, preferably of about 12mM, and wherein said aqueous phosphate buffer is
 present from about 20% (w/w) to about 30% (w/w), preferably about 22% (w/w) to
 about 28% (w/w), more preferably about 25% (w/w) to about 28% (w/w) and even
 more preferably of about 27% (w/w) to about 28% (w/w) based on the total weight
 20 of said topical composition;

(ii) hydroxyethyl cellulose or sodium hyaluronate, preferably hydroxyethyl
 cellulose, and wherein hydroxyethyl cellulose or sodium hyaluronate is present
 from about 0.01% (w/w) to about 5% (w/w), preferably about 0.05% (w/w) to about
 2% (w/w), more preferably about 0.1% (w/w) to about 1% (w/w), based on the total
 25 weight of said topical composition.

(iii) butylated hydroxytoluene, and wherein butylated hydroxytoluene is present
 from about 0.001% (w/w) to about 0.1% (w/w), preferably about 0.001% (w/w) to
 about 0.01% (w/w), more preferably about 0.002% (w/w) to about 0.008% (w/w),
 based on the total weight of said topical composition

(iv) benzyl alcohol, and wherein said benzyl alcohol is present from about 0.01% (w/w) to about 5% (w/w), preferably about 0.1% (w/w) to about 5% (w/w), more preferably about 1% (w/w) to about 3% (w/w) and even more preferably of about 2% (w/w) based on the total weight of said topical composition.

5 In a preferred embodiment, said compound of formula I is Compound 1,



wherein said topical composition comprises said Compound 1 in an amount between about 0.8μmol and about 2μmol, preferably in an amount of about 1μmol; and

wherein said topical composition is in the form of an aqueous gel, and wherein said solvent is a combination of PEG 400 and water, and wherein the amount of said PEG 400 is from about 60% to about 75 % (w/w), preferably from about 65% to about 75 % (w/w), and more preferably from about 68 % to about 72 % (w/w) based on the total weight of the topical composition;

wherein said topical composition comprises

15 (i) an aqueous phosphate buffer of a pH of about 6.5 to about 7.5, wherein preferably said aqueous phosphate buffer has a molarity of about 10 to about 15 mM, preferably of about 12mM, and wherein said aqueous phosphate buffer is present from about 20% (w/w) to about 30% (w/w), preferably about 22% (w/w) to about 28% (w/w), more preferably about 25% (w/w) to about 28% (w/w) and even 20 more preferably of about 27% (w/w) to about 28% (w/w) based on the total weight of said topical composition;

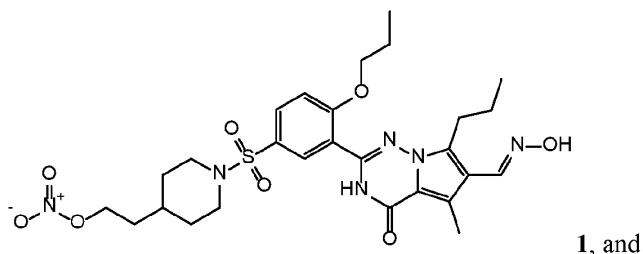
(ii) hydroxyethyl cellulose, wherein hydroxyethyl cellulose is present from about 0.05% (w/w) to about 2% (w/w), preferably about 0.1% (w/w) to about 1% (w/w), based on the total weight of said topical composition.

25 (iii) butylated hydroxytoluene, wherein butylated hydroxytoluene is present from about 0.001% (w/w) to about 0.01% (w/w), preferably about 0.002% (w/w) to about 0.008% (w/w), based on the total weight of said topical composition

(iv) benzyl alcohol, wherein said benzyl alcohol is present from about 0.1% (w/w) to about 5% (w/w), preferably about 1% (w/w) to about 3% (w/w) and even more

preferably of about 2% (w/w) based on the total weight of said topical composition.

In a preferred embodiment, said compound of formula **I** is Compound **1**,



wherein said topical composition comprises said Compound **1** in a concentration between
 5 about 8 μ M and about 22 μ M, preferably in a concentration between about 10 μ M and about
 12 μ M, and further preferably in a concentration of about 11 μ M; and

wherein said topical composition is in the form of an aqueous gel, and wherein said
 solvent is a combination of PEG 400 and water, and wherein the amount of said PEG 400 is
 from about 60% to about 75 % (w/w), preferably from about 65% to about 75 % (w/w), and
 10 more preferably from about 68 % to about 72 % (w/w) based on the total weight of the topical
 composition;

wherein said topical composition comprises

(i) an aqueous phosphate buffer of a pH of about 6.5 to about 7.5, wherein
 preferably said aqueous phosphate buffer has a molarity of about 10 to about 15
 15 mM, preferably of about 12mM, and wherein said aqueous phosphate buffer is
 present from about 20% (w/w) to about 30% (w/w), preferably about 22% (w/w) to
 about 28% (w/w), more preferably about 25% (w/w) to about 28% (w/w) and even
 more preferably of about 27% (w/w) to about 28% (w/w) based on the total weight
 of said topical composition;

(ii) hydroxyethyl cellulose, wherein hydroxyethyl cellulose is present from about
 20 0.05% (w/w) to about 2% (w/w), preferably about 0.1% (w/w) to about 1% (w/w),
 based on the total weight of said topical composition.

(iii) butylated hydroxytoluene, wherein butylated hydroxytoluene is present from
 about 0.001% (w/w) to about 0.01% (w/w), preferably about 0.002% (w/w) to about
 25 0.008% (w/w), based on the total weight of said topical composition

(iv) benzyl alcohol, wherein said benzyl alcohol is present from about 0.1% (w/w)
 to about 5% (w/w), preferably about 1% (w/w) to about 3% (w/w) and even more
 preferably of about 2% (w/w) based on the total weight of said topical composition.

Surprisingly, the inventors were able to provide inventive topical compositions, in particular inventive topical compositions comprising Compound 1, despite the shown de-facto non-solubility of the inventive compounds of formula I, in particular of Compound 1, in physiological aqueous phosphate buffer (pH 7.5). Moreover, the inventive topical compositions showed efficacy and improved wound healing in uPAR deficient mice, a disease-relevant animal model of systemic sclerosis having cutaneous ulcers, at surprisingly very low concentrations of the inventive compounds of formula I, in particular of Compound 1.

As shown in Example 3, Compound 1 shows the profile of an insoluble drug with a determined solubility of 0.0 mg/ml at physiological aqueous phosphate buffer (pH 7.5) and, further, a very pronounced hydrophobicity of calculated logP (clogP) value of 3.79 (See Faller B and Ertl P, Computational approaches to determine drug solubility, Adv Drug Deliv Rev. (2007) 59(7):533-545). Water solubility is an important molecular property for successful drug development as it is a key factor governing drug access to biological membranes. Typically, very poor or no aqueous solubility cannot be compensated by standard formulation developments, in particular due to the unpredictability of the solubility behavior of the very poor or not soluble drug. Poor aqueous solubility is caused by two main factors: i) high lipophilicity and ii) strong intermolecular interactions, which make the solubilization of the solid energetically costly.

As shown further in Example 3, a solubility screening of Compound 1 surprisingly revealed the significant superiority of PEG400, and hereby in particular at very high concentrations of PEG400. Further surprisingly, the use of surfactants for solubilization such as polysorbate 80 and poloxamer 407, typically good solubilization enhancers for lipophilic drugs, showed an 'antagonistic effect' in sense that at 1%, both surfactants lead to an increase in solubility but the amount of drug dissolved did not linearly or not at all increase with higher concentrations of surfactants. This is a very uncommon finding.

Furthermore, and as shown in Example 4, the inventive topical compositions did not demonstrate any negative effect on wound healing properties on an open wound in DU Treatment. This is not only true for the inventive compounds, in particular Compound 1, but as well for the entirety of applied excipients used in combination herewith. This is additionally remarkable since said preferred topical compositions solely comprises excipients approved for topical treatment, in particular for human use, by the relevant authorities. In addition, said usage of regulatorily approved excipients was also in accordance with the accepted defined concentrations. Thus, the inventors were able to provide efficacious topical compositions solely using admissible and allowed excipients for human use within the accepted concentrations.

The topical composition of the present invention can be used for the treatment of diseases or disorders of the skin being mediated by or resulting from PDE5 activity and/or NO related endothelial dysfunction, in particular, such as ischemic skin ulcers, such as digital ulcers (DU) in systemic sclerosis, preferably for wound healing, further preferably for chronic wound healing, diabetic foot ulcer, leg ulcer, ischemic arterial ulcers, livedoid vasculopathy, Martorell hypertensive ischemic leg ulcer, thromboangiitis obliterans (Buerger's disease), sickle cell leg ulcer.

Thus, in a further aspect, the present invention provides the topical composition of the present invention for use in a method of topically treating a disease or disorder mediated by PDE5 activity and/or NO related endothelial dysfunction, preferably of a disease or disorder of the skin mediated by PDE5 activity and/or NO related endothelial dysfunction, in a subject, preferably in a human. Preferably, said disease or disorder is selected from ischemic skin ulcers, digital ulcers (DU) in systemic sclerosis, diabetic foot ulcer, leg ulcer, ischemic arterial ulcers, livedoid vasculopathy, Martorell hypertensive ischemic leg ulcer, thromboangiitis obliterans (Buerger's disease), sickle cell leg ulcer, all of the foregoing preferably for wound healing, further preferably for chronic wound healing. Very preferred, said disease or disorder is systemic sclerosis, and wherein preferably said disease or disorder is digital ulcers in systemic sclerosis.

In another aspect, the present invention provides the topical composition of the present invention for use in a method of topically treating a disease or disorder in a subject, preferably in a human, wherein said disease or disorder is selected from ischemic skin ulcers, digital ulcers (DU) in systemic sclerosis, diabetic foot ulcer, leg ulcer, ischemic arterial ulcers, livedoid vasculopathy, Martorell hypertensive ischemic leg ulcer, thromboangiitis obliterans (Buerger's disease), sickle cell leg ulcer, all of the foregoing preferably for wound healing, further preferably for chronic wound healing, and wherein preferably said disease or disorder is systemic sclerosis, and again further preferably said disease or disorder is digital ulcers in systemic sclerosis.

In another aspect, the present invention provides a method for treating a disease or disorder mediated by PDE5 activity and/or NO related endothelial dysfunction, preferably of a disease or disorder of the skin mediated by PDE5 activity and/or NO related endothelial dysfunction, in a subject, preferably in a human, wherein said method comprises topically administering the topical composition of the present invention to said subject, preferably to said human.

In another aspect, the present invention provides a method for treating a disease or disorder in a subject, preferably in a human, wherein said disease or disorder is selected from ischemic skin ulcers, digital ulcers (DU) in systemic sclerosis, diabetic foot ulcer, leg ulcer, ischemic arterial ulcers, livedoid vasculopathy, Martorell hypertensive ischemic leg ulcer, thromboangiitis obliterans (Buerger's disease), sickle cell leg ulcer, all of the foregoing preferably for wound healing, further preferably for chronic wound healing, and wherein preferably said disease or disorder is systemic sclerosis, and again further preferably said disease or disorder is digital ulcers in systemic sclerosis, wherein said method comprises topically administering the topical composition of the present invention to said subject, preferably to said human.

In another aspect, the present invention provides for the use of the topical composition of the present invention for the preparation of a medicament for topically treating a disease or disorder mediated by PDE5 activity and/or NO related endothelial dysfunction, preferably of a disease or disorder of the skin mediated by PDE5 activity and/or NO related endothelial dysfunction, in a subject, preferably in a human, wherein said method comprises topically administering the topical composition of the present invention to said subject, preferably to said human.

In another aspect, the present invention provides for the use of the topical composition of the present invention for the preparation of a medicament for topically treating a disease or disorder in a subject, preferably in a human, wherein said disease or disorder is selected from ischemic skin ulcers, digital ulcers (DU) in systemic sclerosis, diabetic foot ulcer, leg ulcer, ischemic arterial ulcers, livedoid vasculopathy, Martorell hypertensive ischemic leg ulcer, thromboangiitis obliterans (Buerger's disease), sickle cell leg ulcer, all of the foregoing preferably for wound healing, further preferably for chronic wound healing, and wherein preferably said disease or disorder is systemic sclerosis, and again further preferably said disease or disorder is digital ulcers in systemic sclerosis, wherein said method comprises topically administering the topical composition of the present invention to said subject, preferably to said human.

In a further aspect, the invention provides a kit comprising:

- (i) a first kit component comprising the topical composition of the present invention; and
- (ii) a second kit component comprising a patch, a tape, a dressing, a sheet or a device suitable to maintain contact between said topical composition and the skin of a subject, preferably the skin of a human.

A device suitable to maintain contact between the topical composition of the invention and the skin of the subject include, typically and preferably, a patch including a dressing, a tape, a sheet, an adhesive or non-adhesive patch, or any other form known to those skilled in the art such as microneedles. For example, dressings suitable for the present invention and in particular wound dressings, and hereby preferably for chronic wounds, have been described and reviewed (Dabiri G et al, *Advances in Wound Care* (2016) 5(1):32-41; Shi C et al, *Front. Bioeng. Biotechnol.* (2020) 8:182), the entire disclosures thereof herewith incorporated by way of reference.

Topical dressings are typically designed with the physical placement of a patch with an emphasis on localized delivery. They are designed to be used to alleviate localized conditions in the form of an adhesive or non-adhesive patch. Examples of dressing preparations are hydrogel three-dimensional network of hydrophilic polymers; hydrocolloid hydrogel mixed with synthetic rubber and sticky materials; alginate consisting of polysaccharides derived from brown seaweed; foam consisting of polyurethane or is silicone-based with a semi permeability thermal insulation; films consisting typically of adhesive, porous, and thin transparent polyurethane. The dressing preparation optionally contain the active compound, i.e. the compound of formula I of the present invention, which is disposed on at one side of the backing of the patch. The adhesive or non-adhesive patch is flexible enough to comply to the skin and has reduced skin irritation and high stability.

In one embodiment, said second kit component comprises a wound dressing.

In still a further aspect, the present invention provides a method of preparing the topical composition of the present invention, wherein said method comprises dissolving a compound of formula I in a solvent, wherein preferably said solvent comprises PEG400, and further preferably wherein said solvent comprises PEG400 and water. In a further preferred embodiment, the amount of said PEG 400 is from about 60% to about 75 % (w/w), preferably from about 65% to about 75 % (w/w), and more preferably from about 68 % to about 72 % (w/w) based on the total weight of the topical composition. In another preferred embodiment, the said compound of formula I is Compound 1, and said topical composition comprises said Compound 1 in an amount between about 0.01 μ mol and about 50 μ mol, preferably between about 0.1 μ mol and about 20 μ mol, further preferably between about 0.2 μ mol and about 15 μ mol, further preferably in an amount between about 0.3 μ mol and about 12 μ mol, again further preferably in an amount between about 0.3 μ mol and about 10 μ mol, and again further preferably in an amount between about 0.3 μ mol and about 5 μ mol. In another preferred embodiment, the

said compound of formula I is Compound 1, and said topical composition comprises said Compound 1 in a concentration between about 0.1µM and about 500µM, preferably between about 1µM and about 200µM, further preferably between about 2µM and about 150µM, further preferably in a concentration between about 3µM and about 120µM, again further preferably in a concentration between about 3µM and about 100µM, and again further preferably in a concentration between about 3µM and about 50µM.

EXAMPLES

Materials: The following materials were used to prepare exemplary compositions described herein, all of said materials are known to the skilled person in the art and are further described in detail in Handbook of Pharmaceutical Excipients published jointly by the American Pharmaceutical Association and The Pharmaceutical Society of Great Britain (eds RC Rowe, PJ Sheskey and ME Quinn; Pharmaceutical Press, London, 2009): Hydroxyethyl cellulose (HEC; Natrosol 250H); Butylated hydroxytoluene (Sigma-Aldrich Chemie GmbH); Benzyl alcohol (AppliChem GmbH); PEG 400 (Kollisol PEG E 400; Sigma-Aldrich Chemie GmbH); Sodium Hyaluronate (Fidia farmaceutici S.p.A.); Sodium dihydrogen phosphate anhydrate (Acros Organics); Sodium hydroxide (PanReac).

Synthesis of Compounds of formula I: Compounds of formula I as well as their syntheses are disclosed in WO 2017/085056, the disclosure of which is herewith incorporated by reference in its entirety. In particular Examples 31, 55, 60, 63, 76 and 87 of WO 2017/085056 contain a detailed description of the preparation of preferred compounds of formula I. Specifically Example 31 of WO 2017/085056 discloses the synthesis of the very preferred compound of formula I, 2-[1-(3-{6-[(1E)-(hydroxyimino)methyl]-5-methyl-4-oxo-7-propyl-3H,4H-pyrrolo[2,1-f][1,2,4]triazin-2-yl}-4-propoxybenzenesulfonyl)piperidin-4-yl]ethyl nitrate (Compound 1), used in the following examples.

Analytical HPLC: HPLC-UV was used for the analysis of the exemplary compositions described herein. The content of compound 1 in the compositions was within the predefined range of acceptance.

Equipment: Agilent HP 1100 connected to Waters Empower 3 SR3

Analytical conditions: Column: Waters Acquity XBridge C18 3.5 µm 3.0 x 50 mm, Column temp.: 40°C, Mobile phase A: 0.1% formic acid in water, Mobile phase B: Acetonitrile, Flow: 1.0 mL/min, Injection vol.: 20 µL (single injection), detection: 250 nm wavelength.

Gradient Table - Time [min]	%A	%B
Initial	60.0	40.0
4.00	23.0	77.0
4.01	5.0	95.0
5.00	5.0	95.0
5.01	60.0	40.0

All exemplary compositions were clear gel-like solutions by visual inspection and were stable for at least 2 weeks at RT.

Viscosity measurement: The zero viscosity describes the viscosity of the composition at a plateau, which represents the viscosity at rest. The zero viscosity of the exemplary compositions and composition vehicles for Examples 2A to 2D was in the range of 0.8684 [Pa*s], and for the exemplary compositions described and composition vehicles for Examples 2E and 2F, the zero viscosity was in the range of 3.6784 [Pa*s].

Rheological method: Anton Paar GmbH MCR 102, Software: Rheocompass (Anton Paar GmbH), Cone plate geometry (diameter 50 mm), 5 minutes resting phase. 50 measuring points with varying duration of the measurement point (logarithmic): 100 s start value und 0,1 s end value. Segment duration: 759.862 s.

EXAMPLE 1

Preparation of composition vehicles

A. Preparation of Vehicle Composition A

For 100.0 g of Vehicle Composition **A**, hydroxyethyl cellulose (HEC; 0.25 g) was added in portions to 12 mM phosphate buffer (pH 6.7) and stored below 8°C for dissolution of the thickener. Butylated hydroxytoluene (0.006 g) and benzyl alcohol (2.0 g) were added to PEG 400 (70.0 g), heated to 50°C and cooled down after dissolution. The PEG 400 mixture was added to the aqueous gel (equilibrated to room temperature at phase merging) and stirred with a spatula until a homogenous gel was obtained.

Preparation of phosphate buffer: 12 mM phosphate buffer (pH 6.7) was composed of 2.83 mM Na₂HPO₄ and 9.17 mM NaH₂PO₄ and obtained by adding 402 mg Na₂HPO₄ (MW 142 g / mol) to 1100 mg NaH₂PO₄ (MW 120 g / mol) in demineralized, sterile water to a final volume of 1000 ml.

B. Preparation of Vehicle Composition B

For 100.0 g of Vehicle Composition **B**, sodium hyaluronate (0.5 g) was added in portions to 12 mM phosphate buffer pH 6.7 and stored below 8°C for dissolution of the thickener. Butylated hydroxytoluene (0.006 g) and benzyl alcohol (2.0 g) were added to PEG 400 (70 g),

heated to 50°C and cooled down after dissolution. The PEG 400 mixture was added to the aqueous gel (equilibrated to room temperature at phase merging) and stirred with a spatula until a homogenous gel was obtained.

Preparation of phosphate buffer: 12 mM phosphate buffer (pH 6.7) was composed of 2.83 mM Na₂HPO₄ and 9.17 mM NaH₂PO₄ and obtained by adding 402 mg Na₂HPO₄ (MW 142 g / mol) to 1100 mg NaH₂PO₄ (MW 120 g / mol) in demineralized, sterile water to a final volume of 1000 ml.

EXAMPLE 2

Preparation of inventive compositions

10 A. Preparation of Topical Composition A1

For 100.0 g of Topical Composition **A1**, hydroxyethyl cellulose (HEC; 0.25 g) was added in portions to 12 mM phosphate buffer (pH 6.7) and stored below 8°C for dissolution of the thickener. Butylated hydroxytoluene (0.006 g) and benzyl alcohol (2.0 g) were added to PEG 400 (70.0 g), heated to 50°C and cooled down after dissolution. Compound **1** (0.00018 g; 15 0.3 μmol) was dissolved in the PEG 400 mixture (heated to 50°C for 15 minutes and stirred for 2 hours at room temperature). The PEG 400 mixture was added to the aqueous gel (equilibrated to room temperature at phase merging) and stirred with a spatula until a homogenous gel was obtained.

Preparation of phosphate buffer: 12 mM phosphate buffer (pH 6.7) was composed of 2.83 mM Na₂HPO₄ and 9.17 mM NaH₂PO₄ and obtained by adding 402 mg Na₂HPO₄ (MW 142 g / mol) to 1100 mg NaH₂PO₄ (MW 120 g / mol) in demineralized, sterile water to a final volume of 1000 ml. The composition was all prepared under avoidance of light in a room with minimal UV-light exposure using aluminum foil or amber glass ware.

B. Preparation of Topical Composition A2

25 For 100.0 g of Topical Composition **A2**, hydroxyethyl cellulose (HEC; 0.25 g) was added in portions to 12 mM phosphate buffer (pH 6.7) and stored below 8°C for dissolution of the thickener. Butylated hydroxytoluene (0.006 g) and benzyl alcohol (2.0 g) were added to PEG 400 (70.0 g), heated to 50°C and cooled down after dissolution. Compound **1** (0.00061 g; 1 μmol) was dissolved in the PEG 400 mixture (heated to 50°C for 15 minutes and stirred for 2 30 hours at room temperature). The PEG 400 mixture was added to the aqueous gel (equilibrated to room temperature at phase merging) and stirred with a spatula until a homogenous gel was obtained.

Preparation of phosphate buffer: 12 mM phosphate buffer (pH 6.7) was composed of 2.83 mM Na_2HPO_4 and 9.17 mM NaH_2PO_4 and obtained by adding 402 mg Na_2HPO_4 (MW 142 g / mol) to 1100 mg NaH_2PO_4 (MW 120 g / mol) in demineralized, sterile water to a final volume of 1000 ml. The composition was all prepared under avoidance of light in a room with minimal UV-light exposure using aluminum foil or amber glass ware.

C. Preparation of Topical Composition A3

For 100.0 g of Topical Composition A3, hydroxyethyl cellulose (HEC; 0.25 g) was added in portions to 12 mM phosphate buffer (pH 6.7) and stored below 8°C for dissolution of the thickener. Butylated hydroxytoluene (0.006 g) and benzyl alcohol (2.0 g) were added to PEG 400 (70.0 g), heated to 50°C and cooled down after dissolution. Compound 1 (0.0018 g; 3µmol) was dissolved in the PEG 400 mixture (heated to 50°C for 15 minutes and stirred for 2 hours at room temperature). The PEG 400 mixture was added to the aqueous gel (equilibrated to room temperature at phase merging) and stirred with a spatula until a homogenous gel was obtained.

Preparation of phosphate buffer: 12 mM phosphate buffer (pH 6.7) was composed of 2.83 mM Na_2HPO_4 and 9.17 mM NaH_2PO_4 and obtained by adding 402 mg Na_2HPO_4 (MW 142 g / mol) to 1100 mg NaH_2PO_4 (MW 120 g / mol) in demineralized, sterile water to a final volume of 1000 ml. The composition was all prepared under avoidance of light in a room with minimal UV-light exposure using aluminum foil or amber glass ware.

D. Preparation of Topical Composition A4

For 100.0 g of Topical Composition A4, hydroxyethyl cellulose (HEC; 0.25 g) was added in portions to 12 mM phosphate buffer (pH 6.7) and stored below 8°C for dissolution of the thickener. Butylated hydroxytoluene (0.006 g) and benzyl alcohol (2.0 g) were added to PEG 400 (70.0 g), heated to 50°C and cooled down after dissolution. Compound 1 (0.0061 g; 10µmol) was dissolved in the PEG 400 mixture (heated to 50°C for 15 minutes and stirred for 2 hours at room temperature). The PEG 400 mixture was added to the aqueous gel (equilibrated to room temperature at phase merging) and stirred with a spatula until a homogenous gel was obtained.

Preparation of phosphate buffer: 12 mM phosphate buffer (pH 6.7) was composed of 2.83 mM Na_2HPO_4 and 9.17 mM NaH_2PO_4 and obtained by adding 402 mg Na_2HPO_4 (MW 142 g / mol) to 1100 mg NaH_2PO_4 (MW 120 g / mol) in demineralized, sterile water to a final volume of 1000 ml. The composition was all prepared under avoidance of light in a room with minimal UV-light exposure using aluminum foil or amber glass ware.

E. Preparation of Topical Composition B1

For 100.0 g of Topical Composition **B1**, sodium hyaluronate (0.5 g) was added in portions to 12 mM phosphate buffer pH 6.7 and stored below 8°C for dissolution of the thickener. Butylated hydroxytoluene (0.006 g) and benzyl alcohol (2.0 g) were added to PEG 400 (70 g),
5 heated to 50°C and cooled down after dissolution. Compound **1** (0.00061 g; 1µmol) was dissolved in the PEG 400 mixture (heated to 50°C for 15 minutes and stirred for 2 hours at room temperature). The PEG 400 mixture was added to the aqueous gel (equilibrated to room temperature at phase merging) and stirred with a spatula until a homogenous gel was obtained.

Preparation of phosphate buffer: 12 mM phosphate buffer (pH 6.7) was composed of 2.83
10 mM Na₂HPO₄ and 9.17 mM NaH₂PO₄ and obtained by adding 402 mg Na₂HPO₄ (MW 142 g / mol) to 1100 mg NaH₂PO₄ (MW 120 g / mol) in demineralized, sterile water to a final volume of 1000 ml.

E. Preparation of Topical Composition B2

For 100.0 g of Topical Composition **B2**, sodium hyaluronate (0.5 g) was added in portions
15 to 12 mM phosphate buffer pH 6.7 and stored below 8°C for dissolution of the thickener. Butylated hydroxytoluene (0.006 g) and benzyl alcohol (2.0 g) were added to PEG 400 (70 g), heated to 50°C and cooled down after dissolution. Compound **1** (0.0061 g; 10µmol) was dissolved in the PEG 400 mixture (heated to 50°C for 15 minutes and stirred for 2 hours at room temperature). The PEG 400 mixture was added to the aqueous gel (equilibrated to room
20 temperature at phase merging) and stirred with a spatula until a homogenous gel was obtained.

Preparation of phosphate buffer: 12 mM phosphate buffer (pH 6.7) was composed of 2.83 mM Na₂HPO₄ and 9.17 mM NaH₂PO₄ and obtained by adding 402 mg Na₂HPO₄ (MW 142 g / mol) to 1100 mg NaH₂PO₄ (MW 120 g / mol) in demineralized, sterile water to a final volume of 1000 ml.

25 All prepared compositions were clear and homogeneous low-viscosity gels, wherein Compound **1** was fully dissolved. All the compositions showed shear-thinning behavior which is typical for polymer-based gel compositions. Their consistency allows easy application and spreading on wounds, whilst preventing running off of the composition.

EXAMPLE 3

30

Solubility Studies

A. Solubility of Compound 1 in water.

Hereto, 500 mg of Compound **1** was weighed into separate 10 mL amber glass vials. 5.0

ml of solvent was added to each vial to give a nominal concentration of approximately 100 mg/mL. The samples were stirred for 24 hours under ambient laboratory conditions. After stirring, aliquots of each suspension were filtered through a 1.0 µm PTFE membrane filter, and 0.5 mL of supernatant accurately transferred into separate 250 mL amber volumetric flasks.

5 The flasks were diluted to volume with acetonitrile to give a nominal concentration of 0.2 mg/mL. The concentration of Compound **1** in each of the sample solutions was determined by HPLC. For each solution, the concentration (mg/mL) of Compound **1** was determined using the HPLC system, the observed solubility was back calculated based on the dilution factor.

Preparation of buffers was as follows:

10 Phosphate buffer (pH 7): 100 mL of 0.1M potassium dihydrogen orthophosphate was mixed with 58.2 mL of 0.1 M sodium hydroxide solution and adjusted with 1M sodium hydroxide solution. The pH of the buffer was 7.00.

Phosphate buffer (pH 7.5): 100 mL of 0.1M potassium dihydrogen orthophosphate was mixed with 58.2 mL of 0.1M sodium hydroxide solution and adjusted with 1M sodium hydroxide solution. The pH of the buffer was 7.51.

15

Phosphate buffer (pH 8.5): 100 mL of 0.1 M potassium dihydrogen orthophosphate was mixed with 93.4 mL of 0.1 M sodium hydroxide solution and adjusted with 1M sodium hydroxide solution. The pH of the buffer was 8.50.

Analytical HPLC: HPLC-MS was used for the analysis of the solubility of Compound **1** described herein using as Equipment the Agilent HPLC UV System with the following Analytical conditions: Column: Halo C18, 150 mm * 4.6 mm, 2.7µm. Column temp.: 40°C, mobile phase A: Water/acetonitrile/trifluoro acetic acid (95/5/0.1:v/v/v), mobile phase B: Water/acetonitrile/ trifluoro acetic acid (5/95/0.05:v/v/v), Injection vol.: 5 µL, detection: 250 nm wavelength.

20

25 Table 1. Gradient of mobile phases

Time [min]	% of A	% of B
0.0	100	0
2.00	100	0
27.00	0	100
30.00	0	100
30.1	100	0

Table 2. Solubility for Compound 1 in various aqueous buffers.

Solvent	Determined Solubility (mg/mL)
Aqueous phosphate buffer (pH 7)	0.0
Aqueous phosphate buffer (pH 7.5)	0.0
Aqueous phosphate buffer (pH 8.5)	0.0

Compound 1 shows the profile of an insoluble drug with a determined solubility of 0.0 mg/ml at physiological aqueous phosphate buffer (pH 7.5), and further a very pronounced hydrophobicity of calculated logP (clogP) value of 3.79 (See Faller B and Ertl P, Computational approaches to determine drug solubility, Adv Drug Deliv Rev. (2007) 59(7):533-545).

B. Solubility Screening of Compound 1 in various solvents/solvent systems.

A solubility screening of Compound 1 was effected. Table 3 shows the determined solubility of Compound 1 in the listed solvents or solvent systems. In general, three potential approaches were covered: i) polar organic co-solvents, ii) solubilization by surfactants and iii) oils which may, in particular, serve as a basis of an emulsion gel. A phosphate buffer of pH 6.5 with low molarity was used as this salt and pH is suitable for application on wounds. It is of note that the resulting pH of the screened systems is about 7.3.

Table 3. Solubility Screening for Compound 1

Solvent/Solvent Systems	Determined Solubility (mg/mL)
PEG 400 / PB (30:70, vol:vol)	0.001
PEG 400 / PB (70:30, vol:vol)	0.110
1% polysorbate 80 in PB	0.077
3% polysorbate 80 in PB	0.139
8% polysorbate 80 in PB	0.067
1% poloxamer 407 in PB	0.270
10% poloxamer 407 in PB	0.0232
Medium chain triglycerides (MCT)	0.282
Isopropyl myristate (IPM)	0.093
PEG 400 / PB (30:70, vol:vol) + 3% polysorbate 80	0.020
PEG 400 / PB (30:70, vol:vol) + 10% poloxamer 407	0.054
PEG 400 / PB (30:70, vol:vol) + 3% benzyl alcohol	0.008
8% polysorbate 80 in PB + 10% ethanol	0.043

8% polysorbate 80 in PB + 3% benzyl alcohol	0.056
8% polysorbate 80 in PB + 10% poloxamer 407	0.063
8% polysorbate 80 in PB + 10% MCT	0.037
Octyldodecanol (Kollicream OD)	0.028
Polyethylene glycol PEG 400	10.5

PB = Phosphate buffer 10 mmol/L pH 6.5

As confirmed, Compound **1** shows the profile of an insoluble or very poorly soluble drug with pronounced hydrophobicity. Surprisingly, solely PEG400 led to acceptable solubility data. The use of surfactants for solubilization such as polysorbate 80 and poloxamer 407 were used due to its typically good solubilization potential for lipophilic drugs. At 1%, both surfactants lead to an increase in solubility highlighting the potential of this approach. However, surprisingly, amount of drug dissolved did not linearly or not at all increase with higher concentrations of surfactants. This is a very uncommon finding. As opposed to co-solvents, which typically show sigmoid relationships between solvent concentrations and solubility, surfactants typically show a linear relationship in the concentration range where they form micelles. The observed phenomenon of the lack of solubility increase with surfactant concentration is also referred to as the apparent ‘antagonistic effect’ of different types of cosolvents and/or surfactants. This is, as indicated a very uncommon finding and very rarely seen.

C. Solubility Studies of Compound **1** in solvent systems comprising PEG400.

A solubility study of Compound **1** in solvent systems comprising PEG400 in various concentrations was effected. Table 4 shows the determined solubility of Compound **1** with various solvent systems comprising PEG400.

Table 4. Solubility of Compound **1** in solvent systems comprising PEG400.

Solvent/Solvent Systems (vol:vol)	Determined Solubility (mg/mL)
PEG400:benzyl alcohol (99:1)	12693
PEG400:benzyl alcohol:phosphate buffer (89:1:10)	1768
PEG400:benzyl alcohol:phosphate buffer (79:1:20)	882
PEG400:benzyl alcohol:phosphate buffer (69:1:30)	112
PEG400:benzyl alcohol:phosphate buffer (64:1:35)	37.0
PEG400:benzyl alcohol:phosphate buffer (59:1:40)	12.8

PEG400:benzyl alcohol:phosphate buffer (54:1:45)	5.00
PEG400:benzyl alcohol:phosphate buffer (49:1:50)	2.18
PEG400:benzyl alcohol:phosphate buffer (44:1:55)	0.861

Phosphate buffer 12 mmol/L pH 6.7

The results demonstrates a very sharp increase of solubility of Compound 1 upon higher
5 concentrations of PEG400. The resulting very steep solubility curve is depicted in FIG. 3.

EXAMPLE 4

Topical, on wound administration of inventive compounds and its effects on healing of full thickness excisional cutaneous wounds of uPAR-/- mice *in vivo*

The urokinase-type plasminogen activator receptor deficient (uPAR -/-) mouse that
10 reproduces cardinal features of SSc (dermal) vasculopathy and fibrosis is an animal model of systemic sclerosis that is considered as disease-relevant. The uPAR -/- mouse integrates apoptosis of dermal endothelial cells, a decreased density of dermal microvessels and endothelial to mesenchymal transition reflecting vasculopathy with dermal thickening, collagen accumulation, enhanced myofibroblast counts reflecting skin fibrosis. uPAR-/- induces and
15 develops dermal and pulmonary fibrosis (Manetti M et al. Ann Rheum Dis. (2014) 73:1700-1709; Manetti M et al Ann Rheum Dis. (2017) 76:924-934; Schniering J et al., (2019) *In Atlas of Ulcers in Systemic Sclerosis. Diagnosis and Management*. Matucci-Cerinic M and Denton CP (eds). Springer, Cham, Switzerland, pp. 27-37).

uPAR -/- mice were bred from mating of heterozygotes (uPAR +/-; obtained from
20 Institute for Translational Neurology and Neurology Clinics, University of Münster, Germany). Pups were genotyped and uPAR -/- mice obtained at Mendelian frequency. Two full thickness, excisional cutaneous wounds (6 mm diameter corresponding to an area of 28.3 mm²) were generated, symmetrically to the dorsum midline. To this end, at the day before surgery, mice were anaesthetized with Isoflurane and their dorsum was shaved using Veet[®] depilatory cream
25 and clippers. At the next following day, preoperative analgesia was administered at 30 min before surgery consisting of 0.1 mg / kg of Buprenorphine and 5 mg / kg of Ketoprofen. Animals were anesthetized with Isoflurane. Two full thickness wounds (including epidermis, dermis, hypodermis, panniculus carnosus; depth about 500 µm) of 6 mm diameter each were created using a biopsy punch and scissors each with identical, at least 10 mm distance left or
30 right from the dorsum midline. To minimize wound contraction, wounds were splinted using a silicon splint (Grace Bio-Labs silicone wound splints, red with suture sites, outer diameter ×

inner diameter × thickness 14 mm × 7 mm × 0.5 mm). Splints were kept in place using six interrupted surgical sutures (6/0 polyamide-Ethylon) and Superglue (ethyl 2-cyanoacrylate glue) (Park SA et al. Wound Repair Regen. (2014) 22(3):368-380; Park SA et al. Wound Repair Regen. (2015) 23(2):251-261).

5 The cutaneous ulcers and the splint were protected using a plastic cover maintained using a non-woven adhesive dressing (Hypafix[®] (Smith & Nephew)) and a semioclusive adhesive transparent dressing (Tegaderm[™], (3M[™])). Ketoprofen (5 mg / kg) and Buprenorphine (0.05 mg / kg) were administered for 3 days and then Buprenorphine (0.05 mg / kg) as needed. The estimated volume of a cutaneous ulcer was 28.3 mm² area × 0.5 mm depth = 14.15 mm³ or
10 14.15 µl.

 Based on this calculation a dose volume of 25 µl per wound when the wound area was > 50 % of the original size that was reduced to 12.5 µl from the day the wound size was ≤ 50 % less of the original size was appropriate. Given a mean body weight of about 20 g per mouse the dose per body weight was initially 0.75 µg/kg of Compound **1** (in form of the Topical
15 Composition **A2**) as well as Vehicle Composition **A** followed by 0.37 µg/kg of Compound **1** (in form of the Topical Composition **A2**) as well as Vehicle Composition **A** when the wound size was at least halved.

 The inventive composition and composition vehicle were administered once daily with identical treatment to both wounds of an individual mouse under a short isoflurane general
20 anaesthesia. To this end, following removal of the dressings, the ulcers were cleaned using saline solution and fibrin tissue or eschar removed. The inventive composition and composition vehicle were added by soft pipetting and the wounds were immediately closed using the dressings as described before.

 Groups were (i) uPAR +/+ (wild type) mice with Vehicle Composition **A**, (ii) uPAR -/-
25 mice with Vehicle Composition **A**, (iii) uPAR -/- mice with Topical Composition **A2**.

 Treatment started at the day of surgery defined as day 0 and was continued once daily until wound closure at day 12-14. In order to record potential treatment effects wounds were photographed daily using a digital camera (Sony 6400) fixed on a Stereo-Microscope. Three high resolution pictures were taken per wound per day. The wound area was measured off-line
30 from the recorded high-resolution photographs by Image J software. Image J analyses of each ulcer were analysed at least twice. Per wound and day of observation after surgery a total of four to six Image J analyses for one ulcer area were done.

 The mean of the four to six values was included in the final summary analyses and used for the calculation of % *Wound closure* according to the equation below (Park SA et al. Wound

Repair Regen. (2014) 22(3):368-380):

$$\% \text{ Wound Closure} = \frac{UA_0 - UA_k}{UA_0} * 100 \%$$

wherein UA_0 is ulcer area at observation day 0, UA_k is ulcer area at observation day k ($0 \leq k \leq 14$).

5 Following repeated dosing, once daily, on wound administration both Topical Composition **A2** as well as Vehicle Composition **A** were safe and well tolerated. No treatment emergent local or systemic adverse effects were observed over the 12-14 days treatment period and in terms of body weight changes compared to day 0 there were no significant differences between the groups of Topical Composition **A2** and Vehicle Composition **A** up to and including
10 day 8.

Results of the primary efficacy analysis for wound closure are summarized in FIG. 1A and FIG. 1B and Table 5 showing a delayed healing of cutaneous wounds in uPAR $-/-$ mice and effects of topical, on wound administered Topical Composition **A2**.

The calculated % Wound Closure was less in uPAR $-/-$ mice compared to uPAR $+/+$ wild
15 type mice, both treated once daily, *on wound* with Vehicle Composition **A**. This difference in % Wound Closure between uPAR $-/-$ and uPAR $+/+$ mice was significant at observation day 3 to day 8. Wound healing was delayed. For example, about 46% wound closure was observed at day 5 for uPAR $+/+$ mice but at day 7 for uPAR $-/-$ mice. About 60% wound closure in uPAR $+/+$ mice was found at day 6 versus day 8 in uPAR $-/-$ mice.

20 Following repeated dosing, once daily, topical, *on wound* administration of Topical Composition **A2** (in a dose volume of initially 25 μ l and then 12.5 μ l from the day the wound size was ≤ 50 % less of the original size) to uPAR $-/-$ mice % Wound Closure was significantly improved from observation day 5 through day 8, when compared to Vehicle Composition **A**. Based on the means of % Wound Closure from day 5 to day 8, wound closure appeared
25 accelerated by about 1 day with Compound **1** versus Vehicle (Table 5).

Table 5. Delayed healing of cutaneous wounds in uPAR $-/-$ mice and effects of topical, on wound administered Topical Composition **A2**.

% WOUND CLOSURE			
Mice	uPAR $+/+$ (Wild Type)	uPAR $-/-$	
Treatment	Vehicle (N=11; mice)	Vehicle (N=7; mice)	Compound 1 (A2) 1 μ M (N=6; mice)

Day	Mean	SEM	N Wounds	Mean	SEM	N Wounds	Mean	SEM	N Wounds
0	0.00	0.00	11	0.00	0.00	12	0.00	0.00	12
1	-2.50	3.71	11	-0.66	1.64	12	2.17	1.76	12
2	6.83	3.11	11	3.34	1.51	12	5.82	2.11	12
3	20.17	3.72	11	6.78	2.08	12	12.61	1.86	12
4	31.10	3.63	9	12.88	1.96	12	18.11	2.99	12
5	46.40	3.81	7	21.47	2.61	12	34.35	3.76	12
6	60.91	4.00	7	31.93	3.45	11	49.71	3.39	12
7	75.36	5.59	6	46.90	3.12	11	63.55	5.31	12
8	84.12	3.78	5	61.01	5.16	8	74.76	4.89	12
9	88.52	6.12	3	78.45	5.16	5	86.95	4.61	11
10	93.76	2.85	3	87.01	2.88	4	92.91	2.79	10
11				97.77	1.44	2	95.56	2.41	8
12				99.49	0.51	2	97.84	1.79	5
13				100.00	0.00	1	99.94	0.06	2

Tabulated data from Fig 1. For legend see description of FIG. 1. The means $\pm$ SEM are shown. Only wounds with an intact silicone ring and stitches, used to avoid wound contraction, were included in the analysis that explains less evaluable wounds over time (Table 5). In the vehicle groups, some mice received vehicle on one wound and sham at the other wound which explains that the number of wounds may be less than the 2-fold of the number of mice enrolled.

Finally, based on the data shown in FIG. 1 and Table 5, the area under the effect curves (AUEC) for % wound closure were calculated.

Table 6. Delayed healing of cutaneous wounds in uPAR -/- mice and effects of topical, on wound administered Topical Composition A2, AUEC.

Mice	Treatment	AUEC (d0-7)			Statistics: Post-hoc test with Bonferroni correction	
		Mean	SD	N		
uPAR+/+	HEC/PEG Vehicle	215.77	48.23	6	p=0.032	p=0.0018
uPAR-/-	HEC/PEG Vehicle	99.83	43.35	11		
uPAR-/-	1 μ M Compound 1(A2)	154.54	51.1	12		

AUEC were computed (GraphPad Prism 9.0) based on Data shown in Table 5, FIG. 1. Briefly, AUEC between day 0 and 7 were calculated for each wound using R-Studio (AUEC calculations were limited to day 0-7 to assure that individual AUEC curves from at least 6 wounds per group are included in the analysis). Assumptions of normality were assessed using QQplots and Shapiro test by groups (p. values 0.1933; 0.3633; 0.07 for uPAR -/-, Topical

Composition **A2**; uPAR $-/-$, Vehicle Composition **A**; uPAR $+/+$, Vehicle Composition **A**, respectively) and assumption of homogeneity of variance was performed using Levene test (p. value = 0.702). ANOVA was statistically significant $F(2,26) = 11.765$, $p < 0.001$. Post-hoc test were corrected using Bonferroni correction ($n=3$). P values shown in Table 6 for comparisons
5 (i) between uPAR $+/+$, vehicle and uPAR $-/-$, vehicle and (ii) between uPAR $-/-$, vehicle and uPAR $-/-$, Topical Composition **A2** are from post-hoc tests with Bonferroni correction.

The AUEC for % wound closure was significantly less in uPAR $-/-$ mice compared to uPAR $+/+$ mice, in line with delayed wound healing in the uPAR deficient mice. Second, compared to vehicle, Compound **1** resulted in a significant increase in AUEC for % wound
10 closure in uPAR $-/-$ mice confirming improved wound healing caused by Compound **1**. Numerically, about half of the wound closure delay associated with uPAR deficiency was restored by Compound **1** (Table 6).

In order to address the systemic exposure following topical, *on wound* administration of Compound **1**, in a satellite study, plasma concentrations of Compound **1** was measured. To this
15 end, eight male C57BL/6J mice (12 weeks of age, body weight 26.8 ± 0.4 g (Mean $\pm$ SD)) underwent surgery for generation of one full thickness, excisional, cutaneous wounds, in an identical procedure as described above. The study was performed in C57BL/6J mice due to limited availability of uPAR $-/-$ mice. As described before, Compound **1** (in form of Topical Composition **A2**) was added in a dose volume of 25 μ l to the wounds as a single dose. The dose
20 related to body weight was approximately 0.565 μ g / kg (from a total of 25 μ l of 1 μ M Compound **1** per mouse). Peripheral venous blood was sampled from the tail tip (or by cardiac puncture for the final sampling) in four mice at time points (min) 1, 5, 15, 30, 60 *post* administration of Compound **1** and in another four mice at time points (h) 1, 2, 4, 6, 24 *post* administration of Compound **1**. Plasma was generated and Compound **1** measured by LC-
25 MS/MS. The LLOQ for Compound **1** was at 800 pg / ml corresponding to 1.32 nM. Plasma Compound **1** remained below LLOQ (i.e., < 1.32 nM) at all time points and mice. Considering the plasma protein binding of Compound **1** in mice with unbound fractions (f_u) of 0.08 % for Compound **1** the unbound plasma concentrations should have been < 1.06 pM for Compound **1**, corresponding to < 2.12 pM for Compound **1** when extrapolated to administration to two
30 wounds per mice as this was done in the main efficacy study, under the assumption of dose linearity. These values are much less than the IC₅₀ values of Compound **1** (1.18 nM) and the IC₄₀ values for relaxation of rat aortic rings (Compound **1**, IC₄₀ at 8 pM, as observed in earlier effected measurements. Based on these findings, local rather than systemic exposure should explain the improved wound healing with topical, *on wound* of Compound **1** observed in this

study.

In conclusion, in uPAR^{-/-} mice, an accepted disease-relevant animal model of SSc, the healing of full thickness excisional cutaneous wounds, that was delayed compared to uPAR^{+/+} wild type mice, was improved by once daily, topical administration of Compound 1 in form of the Topical Composition A2. Local rather than systemic exposure accounts for the improved wound healing following topical Compound 1 treatment.

EXAMPLE 5

Topical administration of inventive compounds and its effects on ulcer core perfusion in full thickness excisional cutaneous wounds of C57Bl/6J mice *in vivo*

Effects of a single dose of Topical Composition A2, Topical Composition A4, and Vehicle Composition A, when administered on the perfusion of the ulcer core of full thickness excisional cutaneous wounds in C57Bl/6J mice was investigated. Thus, Topical Compositions A2 and A4 were applied in a dose volume of 25 µl per wound. Each individual mouse had surgery for two wounds located symmetrical to the dorsal midline using 8 mm diameter punch biopsy devices.

Topical Compositions A2 and A4 or Vehicle Composition A were randomly administered to one or the other wound under a blinded protocol. This procedure enabled an intra-individual comparison between Compound 1 and its composition vehicle. Perfusion of the ulcer core as well as of the periwound region was separately measured by Laser Speckle Contrast Imaging (LSCI) / Laser Speckle Contrast Analysis (LASCA) using a Perimed device. LASCA wound perfusion measurements of digital ulcers in SSc were previously shown to correlate with time to healing (Barsotti S et al Clin Rheumatol. (2020) 39(1):69-75).

In detail, a single application of Topical Compositions A2 and A4 or Vehicle Composition A, each at a volume of 25 µl per wound was randomly administered either to the right or left of two full thickness cutaneous wounds per mouse generated by 8 mm punch biopsies and symmetrically to the dorsal midline, at 24 h after surgery. Perfusion was measured by LSCI and recorded as arbitrary perfusion units (PU) before administration of Topical Compositions A2 and A4 or Vehicle Composition A to determine perfusion at baseline (PU₀) and at time points (t) from 6 min to 33 min after administration in 3 min intervals followed by measurements at 60, 120 and 240 min (PU_t with 6 ≤ t ≤ 240).

Results from two types of data analyses are shown in FIG. 2A and FIG. 2B. All compositions applied were well tolerated. Local or systemic adverse events were not reported.

In all analyses there was a trend for a superior ulcer core perfusion with Compound **1** compositions, i.e. Topical Compositions **A2** and **A4** compared to Vehicle Composition A at almost all times of measurements. Significance versus t_0 was found for both Compound **1** compositions tested at 60 and 30 min, respectively in the $\Delta\Delta\Delta\text{PU}(\text{Cpd } \mathbf{1}_{\text{corr}}\text{-VEH}_{\text{corr}})$ analysis but not in any other analyses including calculations of area under the effect curve or from the periulcer area. Taken together, these observations demonstrate that topical, *on wound* administration of Compound **1** formulated in Vehicle Composition **A** increases perfusion of ulcer core from (acute) full thickness excisional cutaneous wounds in healthy C57Bl/6J mice.

EXAMPLE 6

10 **7-day dermal wound GLP toxicity study in Göttingen minipigs with a 7-day recovery using inventive topical compositions**

In a 7-day dermal wound GLP toxicity study with a 7-day recovery, Göttingen minipigs are treated with Compound **1** formulated in Vehicle Composition **A** at concentrations of 6 μM , 24 μM and 96 μM , and Vehicle Composition **A** alone as control. Hereto, the inventive topical composition and vehicle composition are dermally, topically applied once daily for 7 days followed by a 7-day recovery period to a created wound site and to an intact skin site (8 treatment sites).

Anesthesia/Tranquilization/Analgesics. The animals are pre-medicated with analgesics prior to wound creation. The animals are sedated with an injectable anesthetic and may also be supplemented with isoflurane administered via face mask if necessary. Following surgery an Anesthesia Reversal may be administered. Analgesics are administered up to 5 times following the day of wound creation and on an as needed basis during the treatment period. Analgesics may include an opioid and/or a NSAID. An appropriate antibiotic to reduce the potential of infection are administered before wound creation on Day 1, and again on Days 3 and 5. Prior to the start of treatment a full thickness wound will be created on each animal (wound type = 1 cm punch biopsy). Following dermal application, the wound site are covered with a bandage. Bandages are changed at the time of dose administration.

Evaluations and Measurements. Mortality/morbidity (twice daily); cage-side observations (daily); detailed observations (weekly starting 1 week prior to dosing); draize dermal scoring (daily); body weight (weekly); food consumption (daily, qualitative). Wound evaluations (prior to each dose and at termination). Wound areas are assessed at each dressing change for the presence of inflammation, granulation tissue, wound measurements, condition

of skin surrounding the wound, and/or presence of exudate. Inflammation are scored according to modified draize scoring criteria. Photography (prior to each dose and at termination). A digital photo is taken of each study animal's test sites, individually. The study number, animal number, test site and study interval relevant to each photo appears on a cage card or label within the field of the photo along with the date. The digital camera is positioned in such a manner that all animals are photographed from the same distance, same orientation, at the same resolution and with a millimeter ruler in the photograph to provide a gauge for magnification of the image, using a stand to hold the camera. The distance from the camera lens to the punch hole site in each minipig is documented.

10 Toxicokinetic Sample Collection. Days 1 and 7: Assessment of toxicokinetic parameters such as Cmax, tmax, AUC, dose proportionality for a single analyte and 2 metabolites and single dose route. Samples will be taken from all animals at 6 time points.

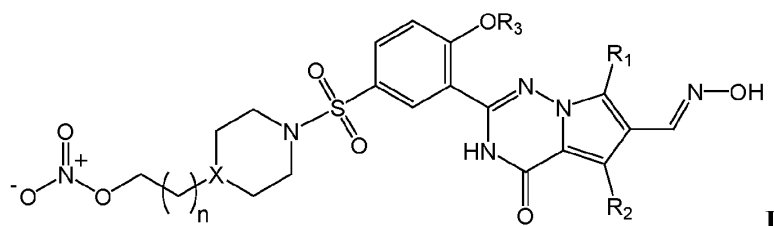
15 Assessments. Day 8 (main) or Day 15 (recovery). Macroscopic examination, organ weights and tissue retention. Moreover, histopathology examination (main and recovery) – all animals (standard tissues including treated and untreated skin sites). Morphometry: Measurements for each punch for each minipig are made to determine the approximate size of the punch hole (including measuring the circumference of the open area, longest diameter of the open punch hole and diameter perpendicular to the longest diameter) using standard parameters. In particular, the area of the wound site (in mm²) remaining open at the time of the photograph is determined. The reduction in measured punch hole area is calculated relative to the Day 1 measurements and expressed as percent closed for each site at each time point. Group means are calculated by treatment group and by time point for measured and calculated parameters.

25

CLAIMS

1. A topical composition comprising a compound of formula **I** or a pharmaceutically acceptable salt thereof

5

**I**

wherein

R₁ is ethyl or propyl, preferably propyl;

R₂ is methyl or ethyl, preferably methyl;

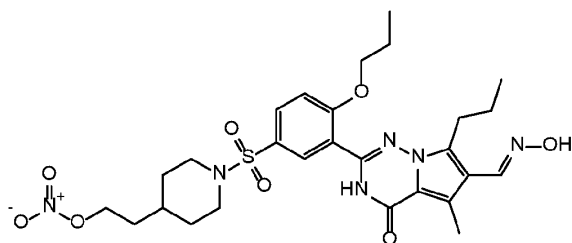
10

R₃ is ethyl or propyl, preferably propyl;

X is N or CH, preferably CH;

n is 1 or 2, preferably n=1.

2. The topical composition of claim 1, wherein said compound of formula **I** is Compound **1**

**1.**

15

3. The composition of claim 1 or claim 2, wherein said compound of formula **I**, preferably said Compound **1**, is present from about 0.000006% (w/w) to about 0.03% (w/w), preferably from about 0.000006% (w/w) to about 0.01% (w/w), more preferably from about 0.0001% (w/w) to about 0.01% (w/w), and again further preferably from about 0.0005% (w/w) to about 0.01% (w/w) based on the total weight of said topical composition.

20

4. The topical composition of any one of the preceding claims, wherein said topical composition is a liquid topical composition, and wherein preferably said liquid topical

composition is in the form of an aqueous gel.

5. The topical composition of any one of the preceding claims, wherein said topical composition comprises a solvent; and wherein said solvent comprises PEG 400.

5

6. The topical composition of any one of the preceding claims, wherein said topical composition comprises a solvent, wherein said topical composition is in the form of an aqueous gel, and wherein said solvent comprises PEG 400 and water.

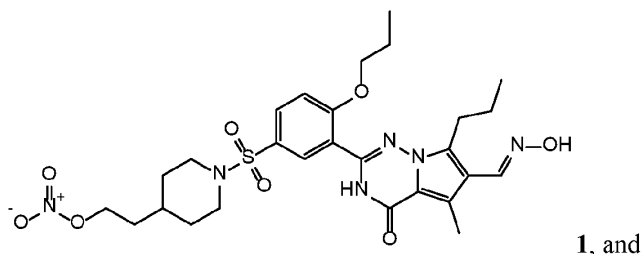
10 7. The topical composition of claim 5 or claim 6, wherein the amount of said PEG 400 is from about 60% to about 75 % (w/w), preferably from about 65% to about 75 % (w/w), and more preferably from about 68 % to about 72 % (w/w) based on the total weight of the topical composition.

15 8. The topical composition of any one of the preceding claims, wherein said topical composition comprises a thickening agent, wherein preferably said thickening agent is selected from the group consisting of carboxymethyl cellulose, hydroxyethyl cellulose, sodium hyaluronate and polyvinylpyrrolidon.

20 9. The topical composition of any one of the preceding claims, wherein said topical composition comprises an antioxidant, wherein preferably said antioxidant is butylated hydroxytoluene.

10. The topical composition of any one of the preceding claims, wherein said topical
25 composition comprises a preservative, wherein preferably said preservative is benzyl alcohol.

11. The topical composition of any one of the preceding claims, wherein said compound of formula **I** is Compound **1**,



wherein said topical composition comprises said Compound 1 in a concentration between about 7 μ M and about 55 μ M, preferably in an amount between about 8 μ M and about 22 μ M, further preferably in a concentration between about 10 μ M and about 12 μ M, and again further preferably in a concentration of about 11 μ M; and

5 wherein said topical composition is in the form of an aqueous gel, and wherein said topical composition comprises a solvent; and wherein said solvent is a combination of PEG 400 and water, and wherein the amount of said PEG 400 is from about 60% to about 75 % (w/w), preferably from about 65% to about 75 % (w/w), and more preferably from about 68 % to about 72 % (w/w) based on the total weight of the topical composition;

10 wherein said topical composition comprises

(i) an aqueous buffer, preferably an aqueous phosphate buffer, of a pH of about 6.5 to about 7.5, wherein preferably said aqueous buffer has a molarity of about 10 to about 15 mM, preferably of about 12mM, and wherein said aqueous buffer is present from about 20% (w/w) to about 30% (w/w), preferably about 22% (w/w) to about 28% (w/w), more preferably about 25% (w/w) to about 28% (w/w) and even more preferably of about 27% (w/w) to about 28% (w/w) based on the total weight of said topical composition;

(ii) hydroxyethyl cellulose or sodium hyaluronate, preferably hydroxyethyl cellulose, and wherein preferably hydroxyethyl cellulose or sodium hyaluronate, preferably hydroxyethyl cellulose, is present from about 0.01% (w/w) to about 5% (w/w), preferably about 0.05% (w/w) to about 2% (w/w), more preferably about 0.1% (w/w) to about 1% (w/w), based on the total weight of said topical composition;

(iii) butylated hydroxytoluene, wherein said butylated hydroxytoluene is present from about 0.001% (w/w) to about 0.1% (w/w), preferably about 0.001% (w/w) to about 0.01% (w/w), more preferably about 0.002% (w/w) to about 0.008% (w/w), based on the total weight of said topical composition; and

(iv) benzyl alcohol, wherein preferably said benzyl alcohol is present from about 0.01% (w/w) to about 5% (w/w), preferably about 0.1% (w/w) to about 5% (w/w), more preferably about 1% (w/w) to about 3% (w/w) and even more preferably of about 2% (w/w) based on the total weight of said topical composition.

12. The topical composition of any one of the claims 1 to 11 for use in a method of topically

treating a disease or disorder mediated by PDE5 activity and/or NO related endothelial dysfunction, preferably of a disease or disorder of the skin mediated by PDE5 activity and/or NO related endothelial dysfunction, in a subject, preferably in a human.

- 5 13. The topical composition for use according to claim 12, wherein said disease or disorder is selected from ischemic skin ulcers, digital ulcers (DU) in systemic sclerosis, diabetic foot ulcer, leg ulcer, ischemic arterial ulcers, livedoid vasculopathy, Martorell hypertensive ischemic leg ulcer, thromboangiitis obliterans (Buerger's disease), sickle cell leg ulcer, all of the foregoing preferably for wound healing, further preferably for chronic wound healing.
- 10
14. The topical composition for use according to claim 12 or claim 13, wherein said disease or disorder is systemic sclerosis, and wherein preferably said disease or disorder is digital ulcers in systemic sclerosis.
- 15 15. A kit comprising:
- (i) a first kit component comprising the topical composition of any one of claims 1 to 11; and
 - (ii) a second kit component comprising a patch, a tape, a dressing, a sheet or a device suitable to maintain contact between said topical composition and the skin of a
- 20 subject, preferably the skin of a human.