(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 December 2013 (27.12.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/189950 A1

(51) International Patent Classification:
C07D 307/83 (2006.01) *A61Q 19/08* (2006.01)
C07D 307/91 (2006.01) *A61P 31/04* (2006.01)
A61K 31/381 (2006.01)

(21) International Application Number:
PCT/EP2013/062664

(22) International Filing Date:
18 June 2013 (18.06.2013)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
MI2012A001082 20 June 2012 (20.06.2012) IT

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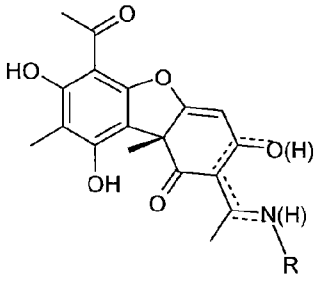
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

(54) Title: DIBENZOFURAN DERIVATIVES WITH ANTIBACTERIAL AND WOUND-HEALING ACTIVITY



(I)

(57) Abstract: Disclosed are dibenzofuran derivatives of usnic acid, compositions thereof and use thereof in dermatological or cosmetic formularions with antimicrobial, regenerative and anti-aging purposes.

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DIBENZOFURAN DERIVATIVES WITH ANTIBACTERIAL AND WOUND-HEALING ACTIVITY

Field of the invention

The present invention relates to dibenzofuran derivatives useful as antibacterial and wound-healing agents and for regenerative or anti-aging treatment of the skin tissues.

Background of the invention

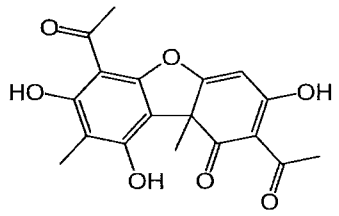
Wound infections represent a significant problem in many post-operative disorders and in burns, and are common in many developing countries due to poor conditions of hygiene. *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa, Streptococcus pneumoniae* and *Klebsiella pneumoniae* are some important micro-organisms that cause wound infections (Mertz P. et al., 1993). Said infections are treated with a number of antibiotics which in many cases fail during the treatment due to the onset of resistance and/or toxicity.

Active ingredients for use on the skin in dermatological or cosmetic formulations with regenerative or anti-aging purposes should ideally have low cytotoxicity on the skin cells, high antibacterial activity against infectious or opportunistic pathogens, and good skin regeneration properties. A large number of wound-healing products of natural, chemical or biotechnological origin are currently on the market. However, in most cases these products are complex mixtures used on an empirical basis, while their action mechanisms and active ingredients have not yet been clearly identified. For example, the best results in the regeneration of chronic or serious wounds are currently obtained in clinical practice with platelet derivatives containing various growth factors and a number of low-molecular-weight compounds (Ranzato et al., 2009).

There is consequently an urgent need to identify new chemical entities

possessing antibiotic and tissue-remodelling properties, for use, either alone or in formulations with other drugs, in the treatment of topical infections such as infected burns, topical otitis (Aronovitz G.H., 2000), haemorrhoids, vaginal lesions, and infections of the oral cavity.

Usnic acid is a dibenzofuran derivative produced by various species of lichen. Its chemical name is 2,6-diacetyl-7,9-dihydroxy-8,9b-dimethyldibenzofuran-1,3(2H,9bH)-dione, corresponding to the following formula:



Usnic acid

The compound exists in nature in the enantiomeric (+) and (-) forms and as a racemic mixture. (+) Usnic acid is commercially available, being easily extracted from lichens of species belonging to the genera *Usnea* and *Ramalina*, wherein it is present in percentages exceeding 26% (Reyim M. et al., 2010).

The antibacterial, antifungal, antiviral and anti-inflammatory properties of usnic acid are known, as are its consequent uses in the cosmetic and pharmaceutical fields, in particular as an active ingredient of creams, deodorants, toothpastes, mouthwashes, antibiotic ointments, vaginal creams, foot creams, powders and shampoos, or as a diet supplement. In Argentina, *Usnea densirotra* tea is known as "Barba della Piedra", and is used for gargling. In these preparations, usnic acid is used as both active ingredient and preservative.

The use of usnic acid in combinatorial synthesis has been described (Tomasi et al., 2006). Other usnic acid derivatives are described in GB 800114.

PCT/EP2009/006960 discloses (+) usnic acid derivatives obtained by conjugating (+) usnic acid with biologically active molecules. The compounds are

useful for the prophylaxis and treatment of infections caused by protozoa of the genus *Plasmodium*.

The potential wound-healing activity of (+)-usnic acid, due to its properties of stimulating the wound closure of human HaCaT keratinocyte monolayers at subtoxic doses, was recently described. The action mechanism was correlated with its ability to promote cell motility (Burlando B. et al., 2009). Earlier studies had indicated (+)-usnic acid sodium salt as a promoter of the wound-healing process, due to the secretion of growth factors and the increased rate of cell migration to a dose-dependent extent (Burlando B. et al., 2009). Experiments effected *in vivo* had confirmed the ability of usnic acid sodium salt to accelerate healing of the epidermis, but this activity was not attributed to stimulation of proliferation (Jin J. et al., 2005). Recent publications report the use of formulations based on usnic acid conveyed by liposomes to heal burn wounds (Nunes P.S. et al., 2011).

Usnic acid is known to has a number of properties which make it useful in the treatment of topical lesions. Lichens belonging to the genus that contains usnic acid have been used all over the world as remedies for systemic and topical use since ancient times.

Usnic acid is universally known as an antibiotic. (+)- and (-) usnic acids are active on sensitive and resistant strains of Gram-positive bacteria (see the review by Ingólfsdóttir K., 2002). Some metal complexes of usnic acid hydrazones have proved more active (Natić M. et al., 2004). Kokubun T. et al., 2007 reports the antibacterial activity of (+) usnic acid on MRSA (methicillin-resistant *S. aureus*) and MDR (multidrug-resistant) strains in addition to mupirocin-resistant strains.

All the results obtained to date confirm a bacteriostatic rather than bactericidal action.

Usnic acid is also a potent antioxidant, and has been extensively used in dermatological products and cosmetics as a preservative (Variati R. et al., 2010; Seifert P. et al., *Cosmetic News*, 1995).

The data regarding the toxicity of usnic acid mainly relate to the systemic use of weight loss preparations containing usnic acid (Guo L. et al., 2008).

Toxicity studies effected at the National Center for Toxicological Research (NCR), USA, have demonstrated that preparations based on Usnea lichen containing equivalent concentrations of usnic acid produce greater toxicity than pure usnic acid.

It has been reported that the contact dermatitis and allergenicity of usnic acid are modest, mainly due to the (-) enantiomer (Hasten B.M. et al., 1993), and probably correlated with its general toxicity.

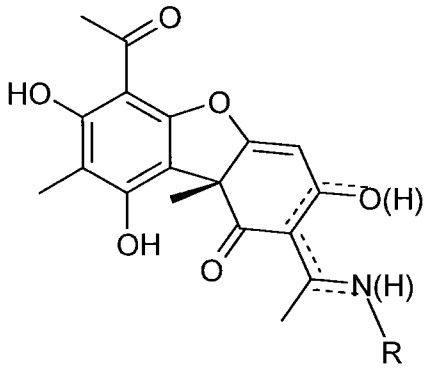
The lipophilia of usnic acid, together with its systemic toxicity, is a characteristic which has limited its use. Usnic acid presents high lipophilia due to the intramolecular hydrogen bonds, which probably contributes to its toxicity, making the molecule permeable to the membranes. The problem of its very low solubility in water has been overcome in cosmetics by using complexes with liposomes or other formulations. (+) usnic acid is therefore a good candidate for investigation of the factors involved in its biological activity: it is easily obtainable and has recognised, proven activities *in vitro*, but presents limitations on use due to its toxicity *in vivo* (Guo L. et al., 2008).

Description of the invention

It has now been found that some (+)-usnic acid derivatives disclosed in PCT/EP2009/006960 and novel derivatives of (+)-usnic acid are useful as antibacterial and wound-healing agents and for regenerative or anti-aging treatment of the skin tissues.

The compounds according to the present invention are (+)-usnic acid derivatives obtained by nucleophilic addition on the triketone moiety of the molecule by amino groups of natural products, in particular amino acids or decarboxyamino acids, preferably those which contain chemical groups involved in cell-cell interaction, such as redox systems or adhesion mechanisms.

The object of the present invention is (+)-usnic acid derivatives having general formula (I):

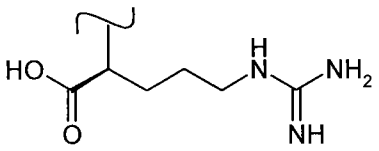
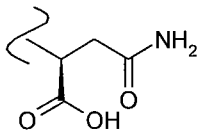
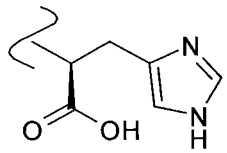
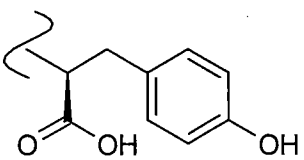


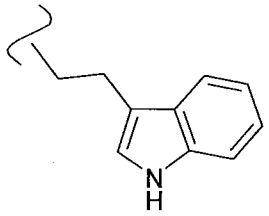
(I)

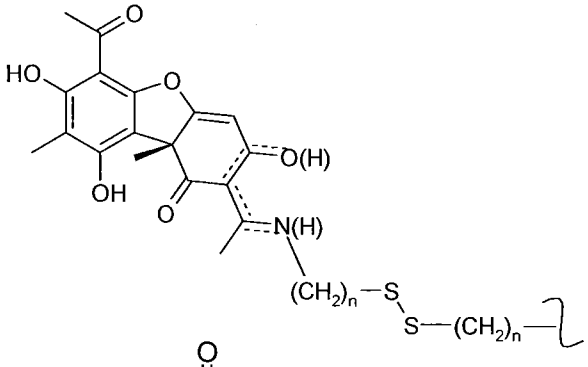
wherein:

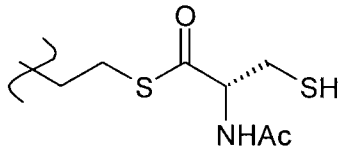
═ represents a single or double bond;

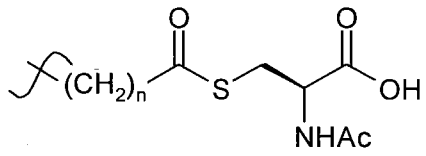
R is a residue selected from:

- H
- $(CH_2)_nX$
- $(CH_2)_nC(O)NH(CH_2)_mX$
- 
- 
- 
- 

- 

- 

- 

- 

wherein the symbol  represents the attachment point of the R group to the N(H) group;

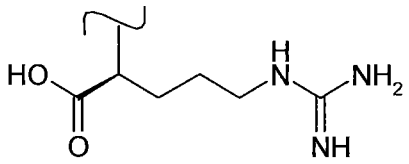
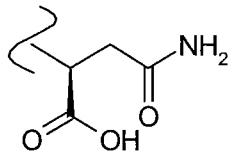
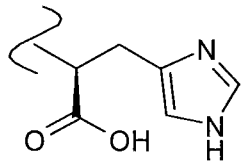
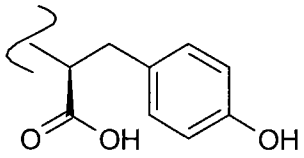
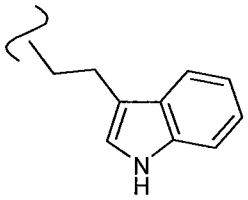
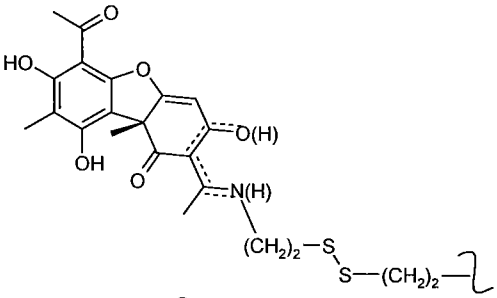
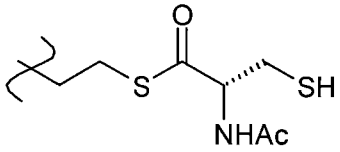
X is selected from OH, SH, COOH and SO_3H;

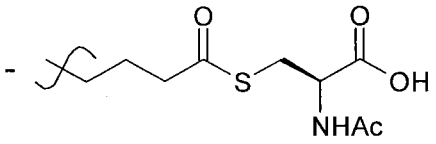
n and m, independently of one another, are an integer from 1 to 10, preferably from 2 to 4; with the proviso that when X is OH or SH, the minimum value of n or m is 2;

their enantiomers and pharmaceutically and cosmeceutically acceptable salts, for use as antibacterial and wound-healing agents and for regenerative or anti-aging treatment of skin tissues.

In one embodiment of the invention, in the compounds of formula (I) the R group is a residue selected from:

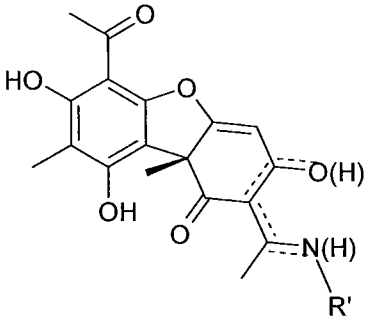
- H
- $(CH_2)_2SH$
- $(CH_2)_2SO_3H$

- CH_2COOH
- $(CH_2)_3COOH$
- $(CH_2)_4OH$
- $(CH_2)_3C(O)NH(CH_2)_3COOH$
- 
- 
- 
- 
- 
- 
- 

- 

In a preferred embodiment, in the compounds of formula (I), R is $(CH_2)_2SO_3H$.

In another embodiment the present invention provides novel compounds of general formula (II)

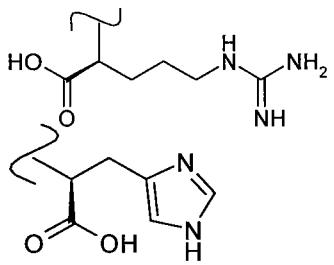
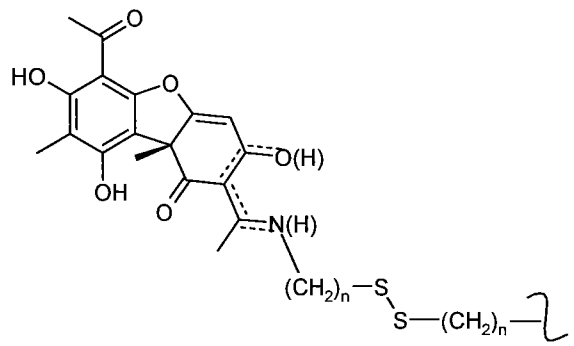


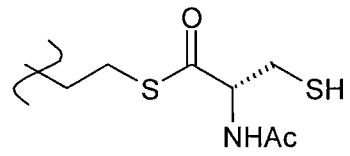
(II)

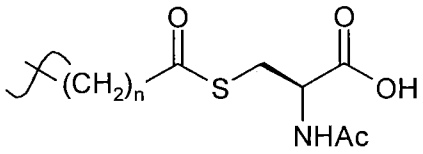
wherein:

$\equiv$ represents a single or double bond;

R' is a residue selected from:

- $(CH_2)_nX'$
- $(CH_2)_nC(O)NH(CH_2)_mX$
- 
- 

- 

- 

wherein the symbol  represents the attachment point of the R' group to the N(H) group;

X is selected from OH, SH, COOH and SO_3H;

X' is selected from OH, SH and COOH;

n and m, independently of one another, are an integer from 1 to 10, preferably from 2 to 4; with the following provisos:

when X or X' is OH or SH, the minimum value of n or m is 2;

when X' is COOH, n is different from 1 or 2;

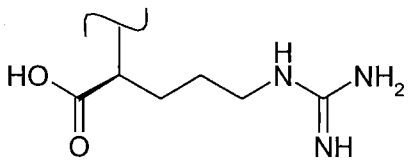
when X' is OH, n is different from 4;

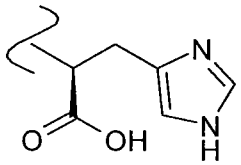
their enantiomers and pharmaceutically and cosmeceutically acceptable salts.

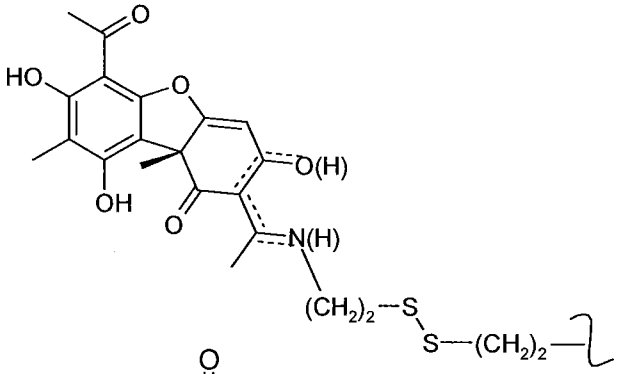
Salts of compounds (I) and (II) bearing acidic functions such as free carboxyl or sulfonyl groups, with Ag^+, Zn^{2+}, Na^+, Mg^{2+} and Ca^{2+} are preferred.

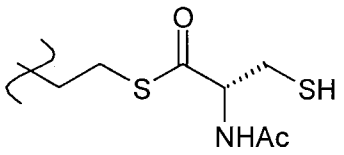
In one embodiment of the invention, the R' group is selected from the group of:

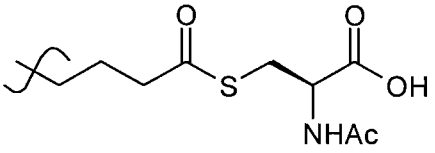
- H
- $(CH_2)_2SH$
- CH_2COOH
- $(CH_2)_3COOH$
- $(CH_2)_4OH$
- $(CH_2)_3C(O)NH(CH_2)_3COOH$

- 

- 

- 

- 

- 

The compounds of the invention of formula (I) and (II) have better antibacterial activity and wound-healing properties than usnic acid. In many cases they also present an overall increase in solubility in water compared with this compound.

The antibacterial activity can be further improved by:

(i) conjugating the compounds (I) or (II) to antibiotic molecules. The conjugation involves suitable moieties present in the usnic acid scaffold of compounds (I) or (II), particularly the R and R' groups.

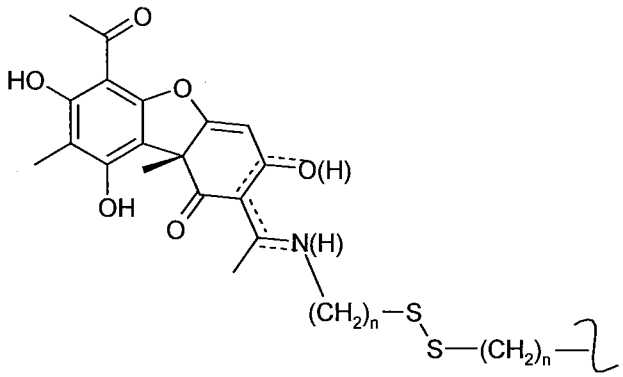
(ii) metal complexation with Zn, Cu, Fe, Au of the compounds of general formula I and II. The formation of complexes involves the enol system and the nitrogen present in the usnic acid scaffold of compounds (I) or (II) .

A further object of the invention is represented by the compounds of

formula (II) for use as medicaments, in particular as antibacterial and wound-healing agents and for regenerative or anti-aging treatment of skin tissues.

The compounds of the invention of formula (I) or (II) can be obtained by reaction between a molar equivalent of (+)-usnic acid and an amount of 1 to 3 molar equivalents of an amine of formula R-NH_2 (III) or R' NH_2, (IV), wherein R, R' are as defined above.

The compounds of the invention wherein R or R' are the residue



can also be prepared by reacting (+)-usnic acid with a diamine of formula H_2N-$(CH_2)_n$-S-S-$(CH_2)_n$-NH_2 (V). In this case, 0.5 molar equivalents of diamine (V) per 1 molar equivalent of (+)-usnic acid are used.

The reaction is generally effected in a solvent such as ethanol, methanol, methylene chloride or mixtures thereof, in the presence of an organic base such as triethylamine or pyridine, or an inorganic base such as potassium hydroxide. The reaction is carried out at the reflux temperature of the solvent mixture, for a time generally ranging from a few hours to a few days. The reaction is preferably carried out with anhydrous reagents and solvents, in an inert gas atmosphere.

The compounds of the invention of formula (I) and (II) have low cytotoxicity on the skin cells, high antibacterial activity against infectious or opportunistic pathogens and skin regeneration properties.

The compounds of the invention of formula (I) and (II) have inhibitory activity against Gram-positive bacteria, and in particular have proved effective

against strains of *S. aureus* and *L. innocua*. Unlike usnic acid, which exhibits a prevalently bacteriostatic effect, the compounds of the invention surprisingly exhibit a bactericidal effect.

In tests effected *in vitro* on human keratinocytes, the compounds of the invention proved significantly less toxic than usnic acid.

In tests effected *in vitro* and *in vivo*, the compounds of the invention proved to have greater wound-healing capacity than usnic acid. In particular, the compounds of the invention are able to promote the reorganisation of a layer of keratinocytes in a way that recalls the epithelial-mesenchymal transition process, similarly to the process functionally described for a platelet lysate (Ranzato et al., 2008).

The compounds of the invention are therefore useful in preparations that stimulate the regeneration of skin wounds or in cosmetic preparations with an anti-aging effect.

Further objects of the present invention are pharmaceutical compositions for topical use or cosmetic preparations containing a compound of formula (I) or formula (II) as defined above, together with compatible excipients for pharmaceutical or cosmeceutical use.

The compositions are preferably administered in the form of semisolid preparations such as creams, salves, ointments or liquids, such as solutions. The dose of active ingredient will depend on the form and administration route, solubility and other pharmacokinetic parameters of the compound. A dose between 0.1 and 1000 mg, preferably between 0.1 and 500 mg, of active ingredient per administration unit is generally acceptable. The daily amount of active ingredient and the duration of the treatment can vary, depending on the severity of the disorder and the characteristics of the patient to be treated.

The pharmaceutical compositions may contain additional active ingredients besides compounds (I) or (II), particularly the antibiotics gentamycin,

erythromycin, clindamycin and oxacillin, which have been found to interact positively with usnic acid (Segatore B, Bellio P, Setacci D, Brisdelli F, Piovano M, Garbarino JA, Nicoletti M, Amicosante G, Perilli M, Celenza G. "In vitro interaction of usnic acid in combination with antimicrobial agents against methicillin-resistant Staphylococcus aureus clinical isolates determined by FICI and ΔE model methods", Phytomedicine. 2012 Feb 15;19(3-4):341-7. doi: 10.1016/j.phymed.2011.10.012. Epub 2011 Nov 25).

Another aspect of the invention are compounds of formula (I) or (II) as defined above or pharmaceutical or cosmetic compositions containing them, for use in regenerative or anti-aging treatment of skin tissues, in particular for treatment of topical infections such as infected burns, topical otitis, haemorrhoids, vaginal lesions, and infections of the oral cavity.

The invention will now be illustrated by the following examples.

Description of Figure

Regeneration of scratch wounds of confluent HaCaT monolayers. The cells, cultured in 12-well plates, were mechanically scratched with the tip of a sterile 0.1-10 μL pipette, and then left to re-epithelialise for 24 h at 37°C in the presence of **usnic acid** (1.7 μg/mL), **ME81** (2.2 μg/mL), **PS8** (2.5 μg/mL), **MB73** (2.1 μg/mL), and **MB56** (2.0 μg/mL). One sample was exposed to 20% platelet lysate (PL) as positive control.

Top panel. Representative image showing HaCaT monolayers subjected to a scratch wound, incubated under control conditions (A) or in the presence of **MB56** (B), and then stained with toluidine blue and observed 24 h after the wound. Scale = 200 μm.

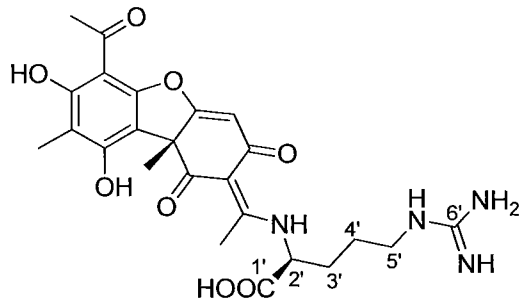
Bottom panel. Chart showing wound-healing measurements, expressed as the difference between the size of the wound after 0 and 6 h. The bars represent the mean±SD of two independent experiments, each with n=20. The mean of the controls was set at 100%. Different letters on the bars indicate groups significantly

different from one another according to the Tukey test ($p < 0.01$).

Examples

Example 1

Synthesis of the conjugate with arginine (PS2)



202 mg (1.16 mmol) of L-arginine was added to a suspension of (+)-usnic acid (400 mg, 1.16 mmol) in absolute EtOH (15 mL) and refluxed under N_2 for 5 h, then left under stirring at room temperature for another 15 h. The solid obtained by concentration under vacuum was crystallised from diisopropyl ether/EtOH 9:1, affording 0.516 g (89%) of a pale yellow solid. M.p.: 232-234°C.

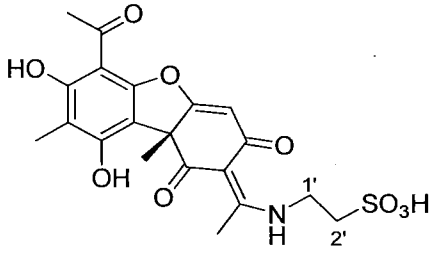
^{1}H NMR 400 MHz (DMSO-d_6) δ 1.58 (5H, m, CH_3-13, CH_2-4') 1.81 (2H, m, CH_2-3') 1.92 (3H, s, CH_3-16) 2.57 (3H, s, CH_3-15) 2.57 (3H, s, CH_3-18) 3.13 (2H, m, CH_2-5') 4.26 (1H, m, CH_2-2'), 5.69 (1H, s, CH-4), 7.62 (3H, br s), 8.89 (1H, br s) 12.43 (1H, br s, OH-10) 13.28 (1H, br s, OH-8) 13.35 (1H, br s, NH).

^{13}C NMR 400 MHz (DMSO-d_6) δ 7.81 (C-16), 19.36 (C-15), 25.14 (C-4'), 30.45 (C-3'), 31.39 (C-18), 32.48 (C-13), 56.68 (C-12), 56.68 (C-5'), 58.87 (C-2'), 101.19 (C-7), 101.97 (C-2), 102.90 (C-4), 105.56 (C-11), 106.81 (C-9), 156.09 (C-6), 157.68 (C-6'), 158.08 (C-10), 163.08 (C-8), 172.54 (C-5), 172.77 (C-1'), 173.03 (C-14), 188.2 (C-3), 197.38 (C-1), 200.95 (C-17).

HRMS (ESI), (positive) m/z 501.1 $[M+H]^+$, calculated for $C_{24}H_{28}N_4O_8$, 500.19.

Example 2

Synthesis of conjugate with taurine (ME81)



375 mg (3 mmol) of taurine was dissolved in aqueous EtOH (10 mL, 1:1), KOH was added to pH~9-10, and the reaction mixture was refluxed for 10 min on a water bath. A suspension of (+)-usnic acid (344 mg, 1 mmol) in absolute EtOH (5 mL) was then added in portions over 30 min, and the mixture was refluxed for 1 day. The reaction mixture was concentrated under low pressure. The crude product was then diluted with CH_2Cl_2 and washed with 1N HCl to pH 4. The aqueous phases were combined, and the solid obtained after concentration under low pressure was washed with a MeOH/H_2O 9:1 solution. After removal of the taurine in suspension by filtration, the filtered solution was concentrated under low pressure, and the desired solid was crystallised from diisopropyl ether/EtOH 9:1, affording 50 mg (11%) of a brown compound.

^{1}H NMR 400 MHz (DMSO-d_6) δ 1.65 (3H, s, CH_3-13), 1.98 (3H, s, CH_3-16), 2.60 (3H, s, CH_3-15), 2.65 (3H, s, CH_3-18), 2.79 (2H, t, J = 6.4 Hz, CH_2-1'), 3.80 (2H, m, CH_2-2'), 5.86 (1H, s, H-4), 7.70 (1H, br s, SO_3H), 12.43 (1H, s, OH-10), 12.92 (1H, br s, OH-8), 13.38 (1H, s, NH).

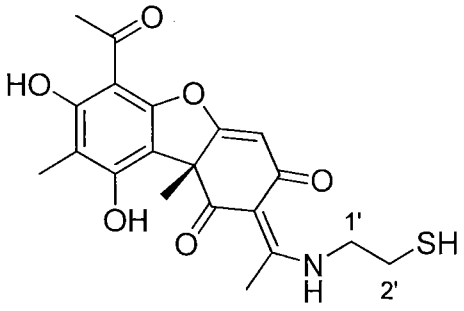
^{13}C NMR 400 MHz (DMSO-d_6) δ 8.0 (C-16), 9.15, 18.5 (C-15), 31.5 (C-18), 32.1 (C-13), 40.7 (CH_2), 50.0 (CH_2), 56.6 (C-12), 101.3 (C-7), 102.2 (C-2), 102.9 (C-4), 105.7 (C-11), 106.7 (C-9), 156.4 (C-6), 158.3 (C-10), 162.3 (C-8), 173.3 (C-5), 175.1 (C-14), 188.7 (C-3), 197.3 (C-1), 201.4 (C-17).

HRMS (ESI), (negative) *m/z* 450.1 $[M-H]^-$, calculated for $C_{20}H_{19}NO_9S$, 451.1.

Example 3

Synthesis of conjugate with cysteamine (MB56)

Procedure A.



A solution of cysteamine (224 mg, 2.90 mmol) in 6 mL of anhydrous MeOH was added under N_2 to a suspension of (+)-usnic acid (1 g, 2.90 mmol) in 6 mL of anhydrous CH_2Cl_2 and 30 mL of anhydrous MeOH. The reaction mixture was refluxed at 65°C for 4 h, and then at room temperature overnight and again at 65°C for 2 h. The solvent was removed under low pressure and the solid obtained was purified by flash-chromatography (toluene/EtOAc 8:2) until the desired product was obtained as a yellow solid (511 mg, 1.27 mmol, 44%). M.p.: 142-143°C.

^{1}H-NMR 400 MHz ($CDCl_3$) δ ppm 1.63 (1H, t, J = 8.6 Hz, SH), 1.71 (3H, CH_3-13), 2.10 (3H, s, CH_3-16), 2.65 (3H, s, CH_3-15), 2.68 (3H, s, CH_3-18), 2.86 (2H, m, CH_2-2'), 3.71 (2H, m, CH_2-1'), 5.80 (1H, s, CH-4), 11.91 (1H, br s, OH-10), 13.36 (1H, s, OH-8), 13.74 (1H, br s, NH).

When D_2O was added, the signal at 2.86 ppm became a triplet (J = 6.4 Hz), and the triplet at 1.63 ppm disappeared.

^{13}C-NMR 100 MHz ($CDCl_3$) δ ppm 8.16 (C-16), 19.13 (C-15), 24.46 (C-2'), 31.95 (C-18), 32.63 (C-13), 47.38 (C-1'), 57.95 (C-12), 102.06 (C-2 + C-7), 103.00 (C-4), 105.67 (C-11), 108.69 (C-9), 156.52 (C-6), 158.88 (C-10), 164.17 (C-8), 174.97 (C-5), 175.65 (C-14), 191.54 (C-3), 199.14

(C-1), 201.33 (C-17).

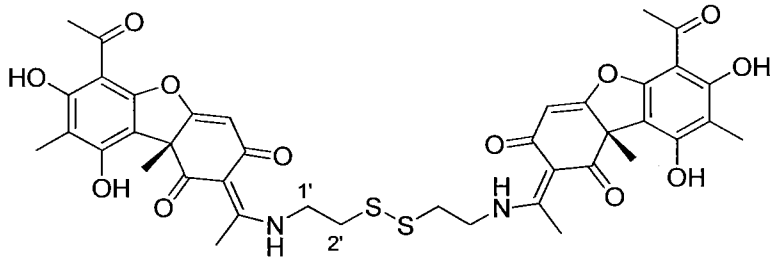
LC-MS (ESI), positive, *m/z* 403.9 $[M + H]^+$, 425.8 $[M + Na]^+$, calculated for $C_{20}H_{21}NO_6S$ 403.1.

Procedure B.

Triphenylphosphine (1.310 g, 4.99 mmol) and 18 mL of water under helium were added to a solution of MB90 of example 4 (2.680 g, 3.33 mmol) in 120 mL of acetone. The reaction mixture was left under stirring at room temperature for 4 days. The solvent was removed under low pressure, and the solid obtained was purified by flash-chromatography (toluene/EtOAc 9.5:0.5) until the desired product was obtained as a yellow solid (1.233 g, 3.056 mmol, 92%).

Example 4

Synthesis of dimer conjugate with cysteamine (MB90)



24 µl of anhydrous pyridine was added to a solution of cystamine dihydrochloride (65 mg, 0.29 mmol) in 3 mL of absolute EtOH. After 30 min stirring at room temperature, a suspension of usnic acid (200 mg, 0.58 mmol) in 3 mL of absolute EtOH was added, and pyridine was then added until completely dissolved. The reaction mixture was refluxed under nitrogen for 8 h. The solvent was evaporated under low pressure, and the solid obtained was acidified with 1N HCl to pH 4, and then extracted twice with EtOAc. The organic phases were combined and concentrated under low pressure, affording 173.2 mg of a pale yellow solid with a quantitative yield.

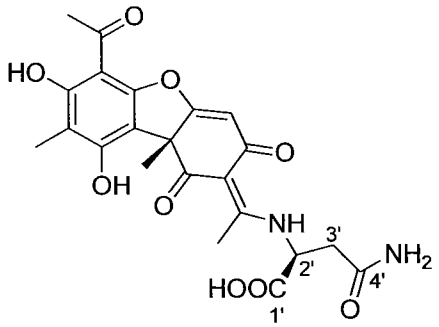
^{1}H-NMR 400 MHz ($CDCl_3$) δ ppm 1.68 (3H, CH_3-13), 2.07 (3H, s, CH_3-16), 2.64 (3H, s, CH_3-15), 2.66 (3H, s, CH_3-18), 3.00 (2H, t, J = 6.5 Hz, CH_2-2'), 3.87 (2H, m, CH_2-1'), 5.76 (1H, s, CH-4), 11.81 (1H, br s, OH-10), 13.33 (1H, s, OH-8), 13.72 (1H, br s, NH).

^{13}C-NMR 100 MHz ($CDCl_3$) δ ppm 8.18 (C-16), 19.14 (C-15), 31.94 (C-18), 32.66 (C-13), 37.26 (C-2'), 42.98 (C-1'), 58.14 (C-12), 102.04 (C-2 + C-7), 102.92 (C-4), 105.58 (C-11), 108.73 (C-9), 156.48 (C-6), 158.80 (C-10), 164.22 (C-8), 174.97 (C-5), 175.69 (C-14), 191.63 (C-3), 199.20 (C-1), 201.30 (C-17).

LC-MS (ESI), positive, m/z 805.2 $[M + H]^+$, calculated for $C_{40}H_{40}N_2O_{12}S_2$ 804.2.

Example 5

Synthesis of conjugate with asparagine (PS5)



79 mg (0.60 mmol) of L-asparagine was added to a suspension of (+)-usnic acid (207 mg 0.60 mmol) in absolute EtOH (15 mL). The reaction mixture was refluxed under N_2 for 4 h, and then at room temperature for a further 15 h. The solid obtained by concentration under vacuum was crystallised from diisopropyl ether/EtOH 9:1, giving 217 g of a yellow solid (79%). Mp: 221-223°C.

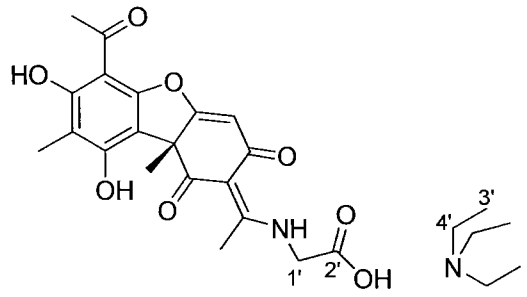
^{1}H NMR 400 MHz (DMSO-d_6) δ ppm 1.65 (3H, s, CH_3-13), 1.96 (3H, s, CH_3-16), 2.72 (3H, s, CH_3-15) 2.73 (3H, s, CH_3-18), 2.76 (1H, m, CH_2-3'), 2.88 (1H, m, CH_2-3'), 4.98 (1H, m, CH-2'), 5.84 (1H, s, CH-4), 12.21 (1H, s, OH), 13.38 (2H, m, OH, NH).

^{13}C NMR 400 MHz (DMSO-d_6): δ ppm 7.8 (C-16), 19.0 (C-15), 30.9 (C-18), 32.1 (C-13), 37.6 (C-3'), 53.4 (C-2'), 56.74 (C-12), 101.3 (C-7), 102.3 (C-2), 102.8 (C-4), 105.63 (C-11), 106.64 (C-9), 156.2 (C-6), 158.2 (C-10), 163.1 (C-8), 171.2 (C-1', C-4'), 173.0 (C-5), 174.7 (C-14), 189.2 (C-3), 198.0 (C-1), 201.3 (C-17).

HRMS (ESI), negative, *m/z* 457.12 [M - H]$^-$, calculated for $C_{22}H_{22}N_2O_9$, 458.13.

Example 6

Synthesis of conjugate with glycine (PS12)



145 mg (1.16 mmol) of L-arginine was added to a suspension of (+)-usnic acid (400 mg, 1.16 mmol), triethylamine (324 µL, 2.32 mmol) in water (3 mL) and absolute EtOH (15 mL); the reaction mixture was refluxed under nitrogen for 4 h, then left under stirring at room temperature for another 15 h. The solid obtained by concentration under vacuum was crystallised from diisopropyl ether/EtOH 9:1, affording 375 g (78%) of a yellow solid. Mp: 210-212°C.

^{1}H NMR 400 MHz (DMSO-d_6) δ ppm 1.16 (9H, t, J = 7.2 Hz, CH_3-3'), 1.62 (3H, s, CH_3-13), 1.94 (3H, s, CH_3-16), 2.54 (3H, s, CH_3-15), 2.61 (3H, s, CH_3-18), 3.01 (6H, q, J = 7.2 Hz, CH^2-4'), 4.06 (2H, m, CH_2-1'), 5.81 (1H, s, CH-4), 12.43 (1H, br s, OH), 12.94 (1H, s, OH), 13.38 (1H, br s, NH).

^{13}C NMR 400 MHz (DMSO-d_6) δ ppm 7.9 (C-16), 8.9 (C-3'), 19.7 (C-15), 31.4 (C-18), 32.23 (C-13), 45.49 (C-4'), 47.9 (C-1'), 56.5 (C-12), 101.2 (C-7), 102.1 (C-2), 103.0 (C-4), 105.7 (C-11), 106.7 (C-9), 156.3 (C-6), 158.2 (C-10), 162.9 (C-8), 169.6 (C-2'), 172.9 (C-5), 173.6 (C-14), 188.4 (C-3), 197.6 (C-1), 201.3 (C-17).

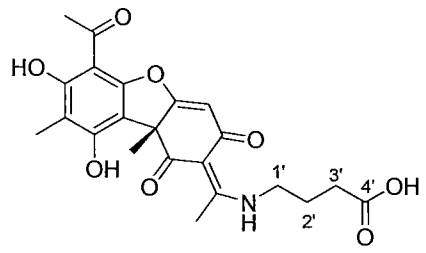
HRMS (ESI), negative, *m/z* 400.10 [M - H]$^-$, calculated for $C_{20}H_{19}NO_8$, 401.11.

Elemental analysis; C 57.75%, H 6.75%, N 5.41%, calculated for

$C_{20}H_{19}NO_8$ x Et_3N x H_2O, C 59.99%, H 6.97%, N 5.38%.

Example 7

Synthesis of conjugate with 4-aminobutyric acid (MB73)



4-aminobutyric acid (1.348 g, 13.07 mmol) was added to a solution of (+)-usnic acid (5.0 g, 14.52 mmol) in 150 mL of 96% EtOH (dried on molecular sieves) and 10 mL of triethylamine, and the reaction mixture was stirred under nitrogen at 80°C for 3 h, and then at room temperature overnight and again at 80°C for 5 h.

The solvent was removed under low pressure and the solid obtained was taken up with CH_2Cl_2 and extracted with 0.1N HCl. The aqueous phase was extracted again with CH_2Cl_2, and the combined organic phases were washed with a NaCl saturated solution, dried on Na_2SO_4, filtered and evaporated under low pressure. The solid obtained was suspended in diisopropyl ether/96% EtOH 9:1 (50 mL) and refluxed for 15 min. The suspension was filtered, and 3.878 g (9.03 mmol, 69%) of a yellow solid was obtained. M.p.: 202-205°C.

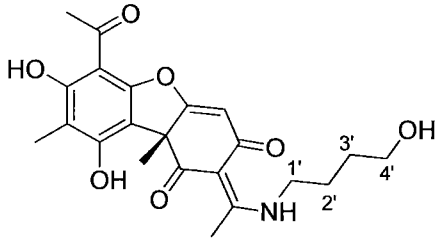
^{1}H-NMR 400 MHz (DMSO-d_6) δ ppm 1.66 (3H, s, CH_3-13), 1.86 (2H, q, J = 7.2 Hz, CH_2-2'), 1.98 (3H, s, CH_3-16), 2.36 (2H, t, J = 7.2 Hz, CH_2-3'), 2.59 (3H, s, CH_3-15), 2.65 (3H, s, CH_3-18), 3.59 (2H, m, CH_2-1'), 5.89 (1H, s, CH-4), 12.23 (1H, br s, COOH), 12.34 (1H, s, OH-10), 13.05 (1H, t, J = 5.2 Hz, NH), 13.42 (1H, s, OH-8).

^{13}C-NMR 100 MHz (DMSO-d_6) δ ppm 7.48 (C-16), 18.08 (C-15), 23.97 (C-2'), 30.66 (C-3'), 30.98 (C-18), 31.73 (C-13), 42.72 (C-1'), 56.30 (C-12), 100.81 (C-7), 101.60 (C-2), 102.28 (C-4), 105.10 (C-11), 106.30 (C-9), 155.69 (C-6),

157.66 (C-10), 162.52 (C-8), 172.92 (C-5), 173.69 (COOH), 174.98 (C-14), 188.95 (C-3), 197.23 (C-1), 200.81 (C-17).

Example 8

Synthesis of conjugate with 4-aminobutanol (BT20)



A solution of 4-amino-1-butanol (800 µL, 774 mg, 8.68 mmol) in 4 mL of EtOH was added to a suspension of (+)-usnic acid (3.0 g, 8.71 mmol) in 100 mL of 96% EtOH (dried on molecular sieves). The reaction mixture was stirred at 80°C for 5 h under N_2. The solvent was then removed under low pressure, and the crude product obtained was purified with a chromatography column (CH_2Cl_2/MeOH 98:2, weight of silica: 180 g, volume of fractions: 60 mL, fractions collected: 28 to 38), the desired product being obtained as a pale yellow solid (2.4 mg, 5.78 mmol, 66%). M.p.: 76°C. $[\alpha]_D^{20}$ +348.4 (*c* 0.40, $CHCl_3$).

^{1}H-NMR 400 MHz ($CDCl_3$) δ ppm 1.52 (1H, m, OH), 1.73 (3H, s, CH_3-13), 1.71-1.78 (2H, m, CH_2-3'), 1.85-1.91 (2H, m, CH_2-2'), 2.12 (3H, s, CH_3-16), 2.66 (3H, s, CH_3-15), 2.70 (3H, s, CH_3-18), 3.57 (2H, m, CH_2-1'), 3.77 (2H, dd, J = 11.0, 5.8), 5.80 (1H, s, CH-4), 11.99 (1H, s, OH-10), 13.38 (1H, s, OH-8 and 1H, s, NH).

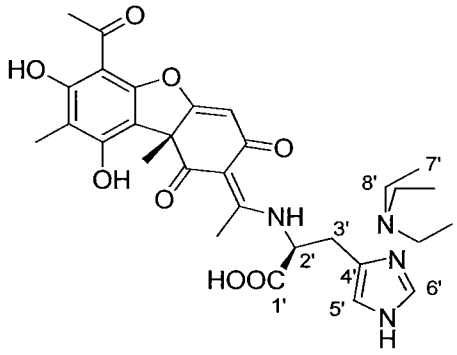
When D_2O was added, the signal at 3.77 ppm became a triplet (J = 6.0 Hz), and the triplet at 1.52 disappeared.

^{13}C-NMR 100 MHz ($CDCl_3$) δ ppm 8.13 (C-16), 18.99 (C-15), 26.34 (C-3'), 30.27 (C-2'). 31.90 (C-18), 32.68 (C-13), 44.50 (C-4'), 57.72 (C-12), 6102.88 (C-2), 103.10 (C-4), 102.62 (C-1'), 102.02 (C-7), 105.76 (C-11), 108.62 (C-9), 156.55 (C-6), 158.94 (C-10), 164.13 (C-8), 174.74 (C-5), 175.54 (C-14), 190.96 (C-3), 198.88 (C-1), 201.34 (C-17).

MS (EI) 415, calculated for $C_{22}H_{25}NO_7$ 415.

Example 9

Synthesis of conjugate with L-histidine (PS6)



0.205 g (0.85 mmol) of L-histidine was added to a suspension of (+)-usnic acid (0.296 g, 0.85 mmol), triethylamine (236 µL, 1.7 mmol) in water (3 mL) and absolute EtOH. The reaction mixture was refluxed under nitrogen for 4 h, and then at room temperature for a further 15 h. The solid obtained by concentration under vacuum was crystallised from diisopropyl ether/EtOH 9:1 affording 0.322 g (78%) of a yellow solid. M.p.: 209-211°C.

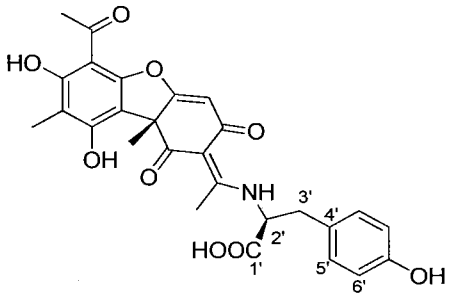
^{1}H NMR 400 MHz (DMSO-d_6) δ ppm 1.16 (9H, t, J = 8.2 Hz, CH_3-7'), 1.61 (3H, s, CH_3-13), 1.94 (3H, s, CH_3-16), 2.50 (3H, s, CH_3-15), 2.61 (3H, s, CH_3-18), 2.95-3.06 (7H, m, CH_2-8', CH_2-3'), 3.18 (1H, m, CH_2-3'), 4.60 (1H, m, CH-2'), 5.80 (1H, s, CH-4), 6.83 (1H, s, CH-5'), 7.69 (1H, d, J = 0.8 Hz, CH-6'), 12.39 (1H, br s, OH), 13.25 (1H, br s, OH) 13.38 (1H, br s, NH).

^{13}C NMR 400 MHz (DMSO-d_6) δ ppm 8.0 (C-16), 8.9 (C-7'), 18.9 (C-15), 31.3 (C-3'), 31.4 (C-18) 32.1 (C-13), 45.6 (C-8'), 56.5 (C-12), 58.8 (C-2'), 101.2 (C-7), 102.0 (C-2), 102.9 (C-4), 105.6 (C-11), 106.6 (C-9), 118.0 (C-5'), 132.8 (C-4'), 135.3 (C-6'), 156.2 (C-6), 158.2 (C-10), 162.9 (C-8), 171.8 (C-1'), 173.0 (C-5), 173.9 (C-14), 188.9 (C-3), 197.9 (C-1), 201.19 (C-17). HRMS (ESI), positive, *m/z* 504.1 $[M + Na]^+$calculated for $C_{24}H_{23}N_3O_8$, 481.15.

Elemental analysis; C 58.73%, H 6.41%, N 8.80%, calculated for $C_{24}H_{23}N_3O_8$ x $2H_2O$ C 58.24%, H 6.84%, N 9.06%.

Example 10

Synthesis of conjugate with L-tyrosine (PS8)



0.157 g (0.87 mmol) of L-tyrosine was added to a suspension of (+)-usnic acid (0.300 g, 0.87 mmol) in absolute EtOH (10 mL) and water (3 mL). The reaction mixture was refluxed under N_2 for 6 h, and then at room temperature for a further 15 h. The solid obtained by concentration under vacuum was crystallised from diisopropyl ether/EtOH 9:1, affording 0.406 g of a yellow solid (92%). M.p.: 183-185°C.

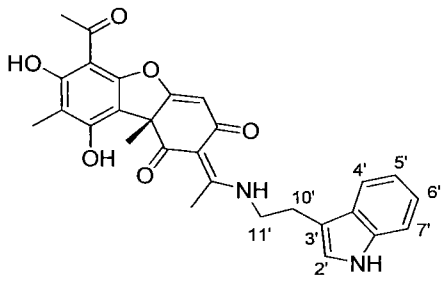
^{1}H NMR 400 MHz (DMSO-d_6) δ ppm 1.63 (3H, s, CH_3-13), 1.96 (3H, s, CH_3-16), 2.36 (3H, s, CH_3-15), 2.62 (3H, s, CH_3-18), 3.01 (1H, dd, J = 7.2, 14 Hz, H-3'), 3.15 (1H, dd, J = 4.8, 14 Hz, H-3'), 4.95 (1H, m, CH-2'), 5.85 (1H, s, CH-4), 6.65 (2H, d, J = 8.4 Hz, CH-5', CH-9'), 6.97 (2H, d, J = 8.4 Hz, CH-6', CH-8'), 12.11 (1H, s, OH), 13.28 (2H, m, OH, NH).

^{13}C NMR 400 MHz (DMSO-d_6) δ ppm 7.7 (C-16), 18.9 (C-15), 31.4 (C-18), 32.0 (C-13), 37.9 (C-3'), 57.1 (C-12), 58.4 (C-2'), 101.2 (C-7), 102.4 (C-2), 103.0 (C-4), 105.6 (C-11), 107.2 (C-9), 116.0 (C-6', C-8'), 126.1 (C-4'), 131.3 (C-5', C-9'), 157.2 (C-7'), 157.3(C-6), 158.60 (C-10), 163.0 (C-8), 171.5 (C-1'), 173.4 (C-5), 174.9 (C-14), 189.2 (C-3), 198.0 (C-1), 201.4 (C-17).

HRMS (ESI), negative, *m/z* 506.14 [M - H]$^-$, calculated for $C_{27}H_{25}NO_9$, 507.15.

Example 11

Synthesis of conjugate with tryptamine (PS9)



0.139 g (0.87 mmol) of tryptamine was added to a suspension of (+)-usnic acid (0.300 g, 0.87 mmol) in absolute EtOH (10 mL). The reaction mixture was refluxed for 5 h, and then at room temperature for another 16 h. The solid obtained after concentration under vacuum was crystallised from diisopropyl ether/EtOH 9:1, affording 0.393 g (93%) of a yellow solid. M.p.: 218-220°C.

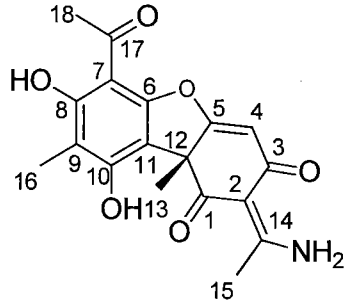
^{1}H NMR 400 MHz ($CDCl_3$) δ ppm 1.69 (3H, s, CH_3-13), 2.12 (3H, s, CH_3-16), 2.55 (3H, s, CH_3-15), 2.69 (3H, s, CH_3-18), 3.22 (2H, m, CH_2-10'), 3.82 (2H, m, CH_2-11'), 5.78 (1H, s, CH 4), 7.17-7.24 (3H, m, CH-5', CH-6', CH-2'), 7.41 (1H, d, J = 8.0 Hz, CH-7'), 7.60 (1H, d, J = 7.8 Hz, CH-4'), 8.38 (1H, s, NH-Ar), 11.98 (1H, br s, OH), 13.36 (2H, m, NH, OH).

^{13}C NMR 400 MHz ($CDCl_3$) δ ppm 7.4 (C-16), 18.5 (C-15), 25.1 (C-10'), 31.1 (C-18), 32.1 (C-13), 44.46 (C-11'), 101.26 (C-7), 102.4 (C-4), 105.16 (C-2), 107.94 (C-11), 110.7 (C-9), 110.9 (C-3'), 111.6 (C-7'), 118.1 (C-6'), 119.7 (C-4'), 122.4 (C-5'), 123.0 (C-2'), 126.7 (C-3'a), 136.4 (C-7'a), 155.7 (C-6), 158.5 (C-10), 163.7 (C-8), 174.9 (C-5), 175.0 (C-14), 188.8(C-3), 198.2 (C-1), 200.7 (C-17).

HRMS (ESI), negative, *m/z* 485.17 [M - H]$^-$, calculated for $C_{28}H_{26}N_2O_6$, 486.18.

Example 12

Synthesis of conjugate with ammonia (BT51)



0.4 mL of concentrated ammonium hydroxide was added to a suspension of (+)-usnic acid (300 mg, 0.87 mmol) in 3 mL of absolute ethanol, and the reaction mixture was heated at 80°C under N_2 for 2 h. After cooling in an ice bath, the yellow solution was concentrated to about a third of the original solution, acidified with 1N HCl and extracted twice with ethyl acetate. The combined organic phases were washed with water and a NaCl saturated solution, dried on Na_2SO_4, filtered and evaporated. The crude product was then crystallised from chloroform to obtain 230 mg (0.67%, 77%) of a pale yellow solid.

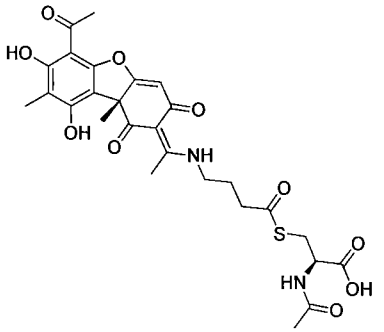
^{1}H-NMR 400 MHz (DMSO-d_6) δ ppm 1.64 (3H, s, CH_3-13), 1.98 (3H, s, CH_3-16), 2.53 (3H, s, CH_3-15), 2.65 (3H, s, CH_3-18), 5.87 (1H, s, C*H*-4), 9.83 (1H, br s, N*H*), 11.54 (1H, br s, N*H*), 12.32 (1H, s, O*H*-10), 13.42 (1H, s, O*H*-8). When D_2O was added, the signals at 9.38 ppm, 11.54 ppm, 12.32 ppm and 13.42 ppm disappeared.

^{13}C-NMR 100 MHz (DMSO-d_6) δ ppm 8.60 (C-16), 25.63 (C-15), 32.11 (C-18), 32.79 (C-13), 57.17 (C-12), 101.95 (C-7), 102.36 (C-2), 103.64 (C-4), 106.21 (C-11), 107.40 (C-9), 156.77 (C-6), 158.77 (C-10), 163.62 (C-8), 174.07 (C-5), 176.94 (C-14), 189.59 (C-3), 198.69 (C-1), 201.94 (C-17).

MS (EI), positive, *m/z* found: 343 [$M^{+\cdot}$], calculated for $C_{18}H_{17}NO_6$: 343.

Example 13

Synthesis of (*R*)-2-acetamido-3-((4-(((*E*)-1-((*R*)-6-acetyl-7,9-dihydroxy-8,9b-dimethyl-1,3-dioxo-1,9b-dihydrodibenzo[*b*,*d*]furan-2(*3H*)-ylidene)ethyl)amino)butanoyl)thio)propanoic acid (BT100).



In an oven dried round bottomed flask, **8** (200 mg, 0.47 mmol) was dissolved in 2.5 mL of anhydrous THF (previously degassed by sparging helium). 4-methylmorpholine (62 µL, 0.56 mmol) and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)morpholine (130 mg, 0.47 mmol) were added and the reaction mixture was stirred at rt for 30 min. *N*-acetyl-L-cysteine (77 mg, 0.47 mmol) was added and the reaction mixture was stirred at rt for 7 h, then overnight. Starting material was still visible, so 4-methylmorpholine (26 µL, 0.235 mmol) and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)morpholine (65 mg, 0.235 mmol) were added and, after 30 min also *N*-acetyl-L-cysteine (38 mg, 0.235 mmol) was added. The reaction mixture was stirred for 2 days. The reaction mixture was diluted with CH_2Cl_2, then washed five times with brine. The combined organic layers were dried over Na_2SO_4, filtered and evaporated to dryness to give 234 mg of crude product as yellow solid. The crude product was purified by column chromatography (CH_2Cl_2/MeOH/EtOH 9:0.5:0.5 to 8:1:1, silica weight 50 g, volume fraction: 30 mL, fractions collected from the 43th to the 49th) and then by reverse phase using Biotage SP1 instrument (SNAP KP-C18-HS 12 g, 35-70 µm, flow rate 15 mL/min, ACN/H_2O 20:80 to 80:20) to obtain 11 mg (0.019 mmol, 4%) of the desired product as yellow solid. $[\alpha]_D^{20}$ +120 (*c* 0.14, MeOH).

^{1}H NMR 400 MHz (DMSO-d_6) δ ppm 1.66 (s, 3H, CH_3-13), 1.80 (s, 3H, $\underline{CH_3}$CONH), 1.89-1.93 (m, 2H, CH_2-3'), 1.98 (s, 3H, CH_3-16), 2.58 (s, 3H, CH_3-15), 2.65 (s, 3H, CH_3-18), 2.64-2.69 (m, 2H, CH_2-2'), 3.04-3.09 (dd, *J*=12.6 Hz, *J*=6.2 Hz, 1H, S$\underline{CH_2}$), 3.37-3.41 (dd, *J*=12.6 Hz, *J*=4.6 Hz, 1H, S$\underline{CH_2}$), 3.55-3.58 (m, 2H, CH_2-4'), 4.02-4.07 (m, 1H, NH$\underline{CH}$), 5.89 (s, 1H, CH-4), 7.51 (d, *J*=6.8 Hz, 1H, $\underline{NH}$CH), 12.27 (br s, 1H), 13.04 (br s, 1H, NH), 13.41 (br s, 1H), 13.45 (s, 1H).

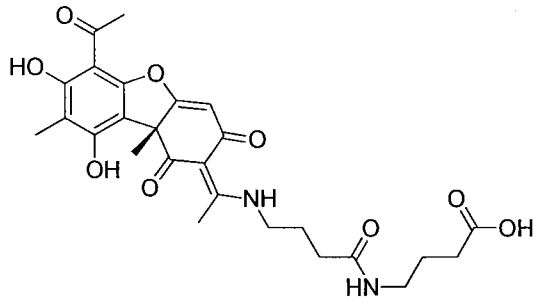
^{13}C NMR 100 MHz (DMSO-d_6) δ ppm 8.65 (C-16), 19.26 (C-15), 23.82 ($\underline{CH_3}$CONH), 25.57 (C-3'), 32.17 (C-18), 32.82 (C-13), 32.92 (S$\underline{CH_2}$), 41.28 (C-2'), 43.62 (C-4'), 53.59 (NH$\underline{CH}$), 57.46 (C-12), 102.01 (C-7), 102.80 (C-2),

103.49 (C-4), 106.31 (C-11), 107.44 (C-9), 156.90 (C-6), 158.84 (C-10), 163.63 (C-8), 169.79 ($CH_3\underline{CO}NH$), 172.42 (COOH), 174.14 (C-5), 176.18 (C-14), 190.09 (C-3), 198.38 (C-1), 199.14 (C-1'), 202.06 (C-17).

LC-MS (ESI), negative, *m/z* found: 572.9 $[M-H]^-$, calcd for $C_{27}H_{30}N_2O_{10}S$: 574.2.

Example 14

Synthesis of (*R*,*E*)-4-(4-((1-(6-acetyl-7,9-dihydroxy-8,9b-dimethyl-1,3-dioxo-1,9b-dihydrodibenzo[*b*,*d*]furan-2(3*H*)-ylidene)ethyl)amino)butanamido)butanoic acid (BT115).



To a solution of **9** (50 mg, 0.12 mmol) in 1.5 mL of anhydrous THF under nitrogen, *N*-methylmorpholine (15 µL, 0.14 mmol) was added and the reaction mixture was stirred at room temperature. After 30 min, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (65 mg, 0.23 mmol)was added and the reaction mixture was stirred at room temperature for 15 min. 4-Aminobutyric acid (12 mg, 0.12 mmol) was added and the reaction mixture was stirred at room temperature for 3 h and 30 min. The solvent was removed under reduced pressure and the solid obtained was diluted with CH_2Cl_2 and extracted twice with 0.1 N HCl. The combined organic layers were washed with brine, dried over Na_2SO_4 and evaporated to dryness to obtain 64 mg of orange solid. The crude product was then purified by flash-chromatography using Biotage SP1 instrument (prepacked 21X55 mm SNAP silica gel cartridge, silica weight 10 g, flow rate 10 mL/min, CH_3OH/CH_2Cl_2 0:100 to 10:90) to obtain 15 mg (0.029 mmol, 25%) of orange

solid (R_f 0.31, eluent: CH_3OH/CH_2Cl_2 1:9). $[\alpha]_D^{20}$ +231 (*c* 0.09, $CHCl_3$).

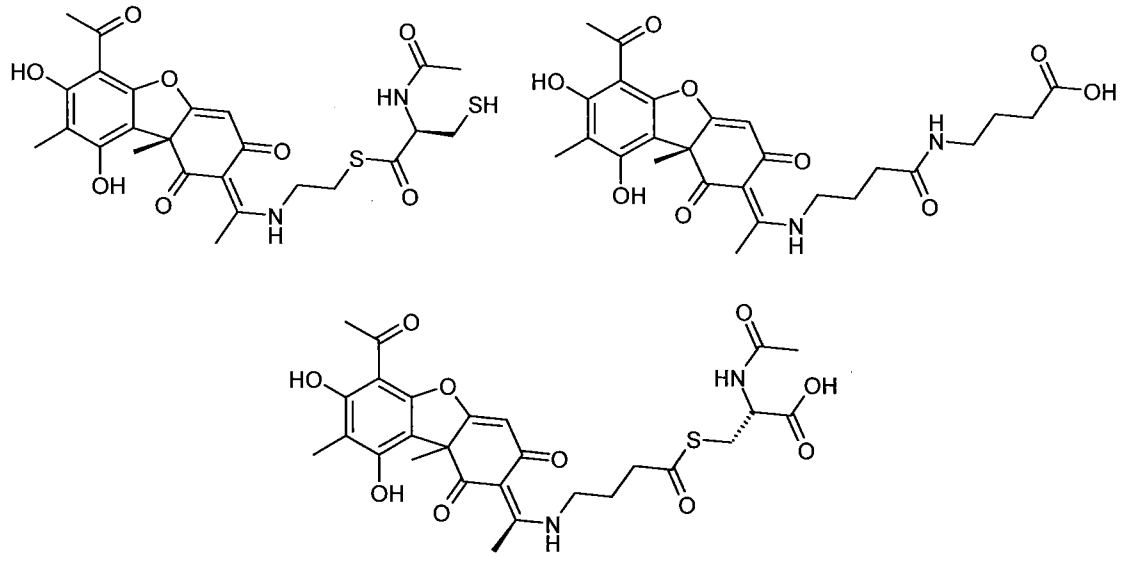
^{1}H NMR 400 MHz (DMSO-d_6) δ ppm 1.58-1.62 (m, 2H, CH_2-3"), 1.66 (s, 3H, CH_3-13), 1.82-1.89 (m, 2H, CH_2-3'), 1.98 (s, 3H, CH_3-16), 2.18-2.22 (m, 4H, CH_2-2" and CH_2-2'), 2.59 (s, 3H, CH_3-15), 2.64 (s, 3H, CH_3-18), 3.03-3.08 (m, 2H, CH_2-4"), 3.53–3.57 (m, 2H, CH_2-4'), 5.88 (s, 1H, CH-4), 7.94 (br s, 1H, NH-CO), 12.32 (s, 1H, OH-10), 13.04 (br s, 1H, NH), 13.41 (s, 1H, OH-8).

^{13}C NMR 100 MHz (DMSO-d_6) δ ppm 8.65 (C-16), 19.25 (C-15), 25.72 (C-3'), 25.80 (C-3), 32.17 (C-18), 32.51 (C-2), 32.84 (C-13), 33.22 (C-2'), 39.14 (C-4), 44.19 (C-4'), 57.45 (C-12), 102.02 (C-7), 102.76 (C-2), 103.49 (C-4), 106.31 (C-11), 107.46 (C-9), 156.92 (C-6), 158.86 (C-10), 163.66 (C-8), 172.12 (C-1'), 174.16 (C-5), 175.72 (C-1"), 176.11 (C-14), 190.08 (C-3), 198.36 (C-1), 202.07 (C-17).

MS (ESI), negative, *m/z* found: 513.4 $[M-H]^-$, calcd for $C_{26}H_{30}N_2O_9$: 514.2.

Example 15

The following compounds were prepared by reacting usnic acid with the suitable amine precursor, substantially following the procedures described in examples 1-12.



Example 16

Antimicrobial efficacy in the agar diffusion test

The antimicrobial assays were effected with the bacterial strains *Staphylococcus aureus* (Biogenetics) and *Listeria innocua (*ATCC 33090). Fresh pure cultures were used in the agar diffusion test. Culture broths maintained for a day in Tryptic Soy Broth (TSB) were seeded evenly on the surface of Muller-Hinton plates according to the Kirby-Bauer method. Usnic acid or derivatives thereof, dissolved in dimethyl sulphoxide (DMSO) (10 µL, 500 µM), was adsorbed on sterile filter paper discs (Whatman No.1, diameter 6 mm) and positioned on the surface of the Muller-Hinton agar plate. DMSO (10 µL) and ampicillin sodium salt (AMP) (10 µL, 500 µM) were used as solvent and control antibacterial respectively. The plates were incubated at 28°C for *L. innocua* and 37°C for *S. aureus*.

The antimicrobial activities of the products tested were evaluated by measuring the diameter of the clear zone (without bacterial growth) round the discs with the absorbed compound. The antibacterial activity was expressed in millimetres of bacterial growth inhibition zone. All the tests were effected in duplicate. The results are set out in Table 1.

Table 1. Antibacterial activity of usnic acid and derivatives thereof according to the disc diffusion test. (ND = no inhibition).

Compounds (example no.)	**Concentration (µg/mL)**	**Diameter of inhibition zone (mm)**	
		Staphylococcus aureus	***Listeria innocua***
Usnic acid	172	12	18
PS2 (1)	257	ND	8
PS8 (10)	254	13	19
PS9 (11)	243	ND	11
PS12 (6)	208	ND	10
ME81 (2)	276	11	13
AMP	197	29	9
DMSO		ND	ND

The compounds of examples 1, 11 and 6 (**PS2**, **PS9** and **PS12** respectively) exhibited an inhibitory effect on *L. innocua*, whereas the compounds of examples 10 and 2 (**PS8 and ME81** respectively) and usnic acid proved effective against both *S. aureus* and *L. innocua*, The results obtained demonstrate the bacterial inhibition activity of the compounds of the invention on Gram-positive bacteria, similarly to usnic acid. Usnic acid and the derivatives thereof presented lower antibacterial activity against *S. aureus* than ampicillin sodium salt, and a similar or greater effect against *L. innocua*.

Example 17

Evaluation of the Minimum Inhibitory Concentration (MIC) and the Minimum Lethal Concentration (MLC).

The compounds of examples 10 and 2 (**PS8** and **ME81**) which exhibited the greatest antimicrobial efficiency in the agar diffusion test of example 16 were selected together with **usnic acid** to evaluate the Minimum Inhibitory Concentration (MIC) and the Minimum Lethal Concentration (MLC). The MIC and MLC were determined by the broth microdilution method (Cosentino et al., 2003; Elo et al., 2007), Briefly, the culture broths maintained overnight were prepared with Nutrient Broth (NB) for *S. aureus* and Tryptic Soy Broth (TSB) for *L. innocua*. **Usnic acid** and the derivatives thereof were diluted at 12 different concentrations in the interval between $34 \cdot 10^3$ and 34 µg/mL (double serial dilutions). A volume of 10 µL was distributed in each of the two test tubes used (1.5 mL). Each test micro-organism was diluted to 10^5 CFU/ml, and the bacterial suspension was added (490 µL) to each test tube. The concentration of the bacterial suspension was confirmed by viable count on Tryptic Soy Agar (TSA). The concentrations of the substances tested were equivalent to 1.95-2000 µM in the test tubes of the final tests.

The test tubes were incubated at 37°C for 24 h, and the MIC and MLC were evaluated. Bacterial growth was revealed by the presence of a pellet on the base of

the test tube. The MICs were determined on the basis of the first test tube without a visible pellet of bacteria, in increasing order of concentration of the compound. A volume (100 µL) from each test tube used to evaluate the MIC was suspended in TSA to confirm the MIC values and establish the MLC. The number of surviving bacteria was determined after incubation.

The MIC value was taken as the lowest concentration of the compound able to produce a significant reduction in viability (>90%), while the MLC value was taken as the concentration at which a percentage ≥ 99.9% of the initial inoculum was killed.

All the measurements were performed in duplicate. The results are set out in Table 2.

Table 2. MIC and MLC of usnic acid and derivatives thereof on *S. aureus* and *L. innocua*

Compound (Example)	Staphylococcus aureus		Listeria innocua	
	MIC (µg/mL)	MLC (µg/mL)	MIC (µg/mL)	MLC (µg/mL)
Usnic acid	5.4	>690	689	>690
PS8 (10)	7.9	507	31.7	1015
ME81 (2)	17.3	276	28.2	903

The difference between the MIC and MLC values of **usnic acid** on *S. aureus* suggests that this compound has a bacteriostatic effect, as previously observed (Lauterwein et al. 1995). The compounds of examples 2 and 10 presented lower MLC values than **usnic acid,** indicating a prevalently bactericidal effect. The MIC values were very similar for all the compounds tested on *S. aureus*, whereas the compounds of examples 2 and 10 presented lower MIC values on *L. innocua*.

Example 18

Evaluation of wound-healing activity *in vitro* on HaCaT keratinocytes

Background

The wound-healing capacity of usnic acid derivatives was tested on an *in vitro* wound-healing model consisting of HaCaT keratinocyte monolayers. Said cells represent an *in vitro* model of keratinocyte proliferation and migration. The HaCaT cell line imitates many properties of normal epidermal keratinocytes, is not invasive and can differentiate under suitable experimental conditions (Petrussevska RT, *et al.* 1988; Schoop VM, et al. 1999). The HaCaT cell line was previously used in studies of the wound-healing process as an *in vitro* model of the system of re-epithelialisation, a phase typical of the wound-healing process (Matsuura K, *et al.* 2007; Ranzato, E., et al. 2008).

Chemical reagents and cell cultures in vitro

All the reagents were supplied by Sigma Chemical Co. unless otherwise indicated. The cells were maintained at 37°C, 5% CO_2 in DMEM culture medium supplemented with 10% foetal bovine serum (FBS, Euroclone, Pero, Italy) and 1% antibiotic mixture.

Cell viability assay

Cell viability was assayed on HaCaT cells and human epithelial carcinoma cells A431. The Neutral Red Uptake (NRU) test was used for this analysis, according to the method reported by Borenfreund et al. (Borenfreund, E., et al. 1989). Said cell viability assay is based on incorporation of the neutral red stain in the lysosomes of the viable cells after incubation with the test agent. Briefly, the cells were seeded in 96-well plates (20,000 cells per well), grown for 24 h before the experiment and then exposed for 24 h to different concentrations of usnic acid or derivatives thereof. After removal of the medium, an 0.05% neutral red solution was added to each well, followed by incubation for 3 h at 37°C. The cells were then washed with PBS, and a 1% solution of glacial acetic acid in 50% ethanol was added to fix the cells and extract the neutral red stain incorporated in the lysosomes. The absorbance of the supernatants was measured at 540 nm with a microplate reader. The estimates of IC50 and IC05 and their 95% confidence

intervals (95% CI) were obtained with a Microsoft Excel® application developed by CSIRO, Australia, based on the logistical function:

$$f = \frac{1}{(1+\exp(-m\cdot(\log(D)-\log(\mathrm{IC50}))}$$

wherein f is cell viability (on a 0-1 scale) dependent on dose D, and m is the slope of the curve or Hill coefficient (Barnes, M., et al., R., and Stevens, D., 2003).

The results are set out in Table 3.

Table 3. *In vitro* toxicity of usnic acid and derivatives thereof on HaCaT keratinocytes, as estimated with NRU endpoint after 24 h exposure.

Compound (Example)	IC50 (µg/mL)	IC05 (µg/mL)
PS2 (1)	**163** (108-244)	**16** (8.0-30)
ME81 (2)	**47** (39-58)	--
ME56 (3)	**155** (116-207)	**43** (18-103)
MB73 (7)	**150** (140-161)	**67** (55-80)
Usnic acid	**24** (18-32)	**0.5** (0.2-1.3)

IC50 = median effective concentration; IC05 = toxicity threshold; the 95% CIs are shown in brackets

The compounds of examples 1, 2, 3 and 7 (**PS2**, **ME81**, **MB56** and **MB73** respectively) proved less toxic than usnic acid.

Scratch wound test

The scratch wound test was effected on confluent HaCaT monolayers by selecting the compounds with the most potent antibacterial activity, namely the compounds of examples 10 and 2 (**PS8** and **ME81** respectively), and those with the lowest toxicity on the keratinocytes, namely the compounds of examples 3 and 7 (**ME56** and **MB73** respectively). The analysis was effected as described in Ranzato et al. (Ranzato, E., et al. 2009a).

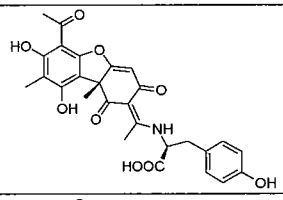
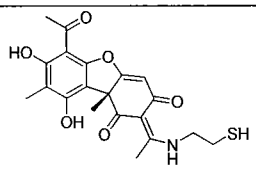
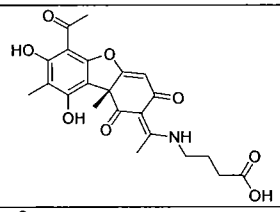
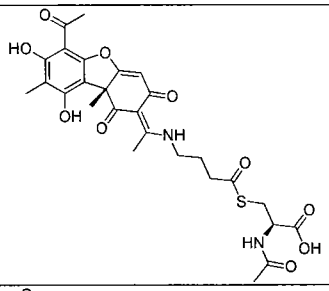
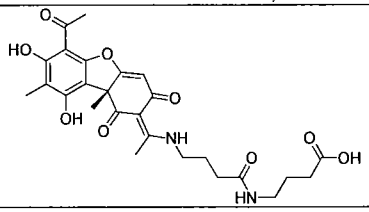
The cells were grown to confluency in multiwell plates, and the cell layers

were then scratched with the tip of a sterile 0.1-10 µL pipette. After washing away the cells in suspension, the cultures were incubated again with the medium and exposed to usnic acid and derivatives thereof, used at the concentration of 5 µM for 24 h. Some samples, used as positive control, were exposed to a dose of 20% (v/v) of platelet lysate (PL) which, according to earlier studies, promotes wound-healing in those cells, and was obtained from blood samples as described in Ranzato et al. (Ranzato, E., et al. 2008).

After the treatments, the cells were fixed with 3.7% formaldehyde in PBS for 30 min, and then stained with 0.1% toluidine blue at room temperature for 30 min (Figure 1, top image). The size of the wound was measured at the time of the lesion and at the end of the treatment, using a Televal inverted microscope (Carl Zeiss Inc., Thornwood, NY) equipped with a digital video camera and NIH ImageJ software. Wound closing was determined as the difference in the size of the wound at 0 and 24 h.

Usnic acid presented a significant wound-closure effect, as previously observed (Burlando, B, et al. 2009), while all the compounds of the invention tested presented greater wound-closing capacity (Figure 1, bottom panel). In particular, the compound of example 7 (GABA-usnic derivative **MB73)** stimulated faster wound-healing, almost equalling the wound-closure capacity of platelet lysate.

Table 4 Wound-closure of keratinocytes subjected to scratch wound assay in the presence of different compounds

Compound		Conc (µM)	(µg/mL)	Wound closure rate
usnic acid		5	1.7	57± 8
ME81		5	2.5	79± 7
PS08		5	2.2	78± 5
MB56		5	2.0	79± 2
MB73		5	2.1	89± 4
BT100		5	2.9	78± 5
BT115		5	2.6	82± 4
Platelet lysate		--	20% (v/v)	100± 3

The would-closure rate is the difference between the rim distance at zero time and after 24hours. Values are expressed as the percent of the would-closure rate obtained with platelet lysate

Example 19

Evaluation of wound-healing activity *in vivo*

Animals

Male Sprague-Dawley rats (160-180 g) and Swiss albino mice (20-25 g) were supplied by the Saki Yenilli animal breeding laboratory (Ankara, Turkey). The animals were maintained under environmental conditions for 3 days for acclimatisation purposes. During the experiments they received a standard diet of pellets, and unlimited access to water. The study was authorised by the Institutional Animal Ethics Committee and performed in compliance with international legislation governing experiments on animals and biodiversity.

Preparation of test samples for the bioassays

Incision and excision wound models were used to evaluate wound-healing activity. For the wound in the *in vivo* models, the test samples were prepared using as base an ointment (carrier) consisting of glycol stearate, propylene glycol and liquid paraffin (3:6:1) at the concentration of 1%. Aliquots of 0.5 g of each ointment to be tested were applied locally to the wound created with a surgical blade.

The animals in the carrier group were only treated with the basic ointment, while the animals in the reference medicament group were treated with 0.5 g of Madecassol® (Bayer, 00001199), which contains 1% of *Centella asiatica* extract.

Linear incision wound model

A total of seven rats per group were anaesthetised with 0.15 cc of Ketalar® (Shetty et al., 2006), and the dorsal region of the rats was shaved and cleaned with 70% alcohol. Two linear incisions 5 cm long were made in the paravertebral region with a sterile blade on the shaven skin at a distance of 1.5 cm from the median dorsal line, on each side. The wounds were closed with three surgical stitches at 1 cm intervals.

The ointments prepared with the test samples, the reference medicament

(Madecassol®) or the basic ointment [glycol stearate: propylene glycol: liquid paraffin (3:6:1)] were applied locally to the wounds in the dorsal region in each group of animals once a day for 9 days. All the stitches were removed on the last day, and the ultimate tensile strength of the previously damaged skin was measured with a tensiometer (Zwick/Roell Z0.5, Germany) (Lodhi et al., 2006; Suguna et al., 2002).

Circular excision wound model

This model was used to monitor the process of contraction of the wound and the wound closure time. Each group of animals (seven animals in each) was anaesthetised with 0.01 cc of Ketalar®.

The fur on the backs of the mice was shaved, creating a circular wound in the interscapular dorsal region of the animals through excision of the skin with a 5 mm biopsy drill (Nopa instruments, Germany); the wounds were left open (Tramontina et al., 2002).

The test samples, the reference medicament (Madecassol®, Bayer) and the ointments used as carrier were applied once a day until the wound had completely healed. The progressive changes in the wound area were monitored with a camera (Fuji, S20 Pro, Japan) every two days. The wound area was then evaluated with the use of the AutoCAD program. The contraction of the wound was measured as the percentage reduction of the wound area. A tissue sample was taken from the healed skin of each group of mice for histopathological examination (Sadaf et al., 2006).

Statistical data analysis

Statistical analysis of the percentage data relating to anti-inflammatory and wound-healing activity was performed with analysis of variance (ANOVA). Values of $p < 0.05$ were considered statistically significant.

The histopathological data were non-quantitative, so no statistical tests were performed.

Results

Tables 5 and 6 show the results of the experiment.

As shown in Table 5, topical application of the ointment prepared with the compound of example 7 (**MB73**) on incised wounds presented the best result in terms of the ultimate tensile strength of the wound: 47.6% ($p < 0.01$) on the tenth day. Moreover, **usnic acid** and the compound of example 2 (**ME81**) generally proved more efficient (31.9% and 29.3% respectively) in the linear incision wound model.

Table 6 shows the contraction values in the development of wound healing on circular excision wound models for the groups treated with the carrier, the negative control, the compounds and the reference medicament. **Usnic acid** and compounds **MB73** and **ME81** exhibited potential wound-healing activity, while the carrier and negative control groups did not exhibit statistically significant wound-healing activity.

The wound contractions, on the eighth and tenth days respectively, were 52.42% ($p < 0.01$) and 82.95% ($p < 0,001$) for the group treated with the compound **MB73**, 30.36% ($p < 0.05$) and 52.19% ($p < 0.01$) for the Group treated with **usnic acid**, 37.55% ($p < 0.05$) and 40.83%($p < 0.01$) for the group treated with compound **ME81,** and were compared with the reference medicament Madecassol® [72.24% ($p < 0.01$) -100% ($p < 0.001$)].

Table 5. Effect of ointments containing **usnic acid** and derivatives thereof on the linear incision wound model

Material (Example)	Statistical Mean ± S.E.M.	(Ultimate tensile strength %)
Carrier	11.75 ± 2.48	10.6
Negative control	10.62 ± 2.63	-
Usnic acid	15.50 ± 2.14	**31.9****
MB73 (7)	17.34 ± 1.92	**47.6****
ME81 (2)	15.19 ± 2.02	**29.3***
Madecassol®	18.87 ± 1.39	**60.6*****

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0,001$; S.E.M.: standard error of the mean

Percentage values of ultimate tensile strength: the group treated with the carrier was compared with the negative control group; the compounds and the reference medicament were compared with the group treated with the carrier.

Table 6. Effect of ointments containing **usnic acid** and derivatives thereof on the circular excision wound model

Material (Example)	Wound area± S.E.M. (% contraction)					
	0	**2**	**4**	**6**	**8**	**10**
Carrier	19.63 ± 2.13	18.02 ±2.61 -	16.02± 2.68 -	13.32 ± 1.90 (3.41)	8.07 ± 1.11 (2.18)	3.87± 1.04 (6.29)
Negative control	19.55 ± 2.10	17.99 ± 2.71	15.86 ± 2.63	13.79 ± 1.76	8.25 ± 1.28	4.13 ± 1.43
Usnic acid	19.59 ± 2.01	17.04 ± 2.16 (5.44)	14.10± 2.12 (11.99)	10.25 ± 1.84 (23.05)	5.62 ± 1.75 **(30.36)***	1.85 ± 0.63 **(52.19)****
MB73 (7)	19.70 ± 2.25	16.03 ± 1.87 (11.04)	12.81 ± 1.36 (20.04)	9.01 ± 1.51 **(32.36)***	3.84 ± 1.12 **(52.42)****	0.66 ± 0.22 **(82.95)*****
ME81 (2)	19.33 ± 2.15	17.74 ± 2.03 (1.55)	14.52± 2.01 (9.36)	11.42 ± 1.38 (14.26)	5.04 ± 1.61 **(37.55)***	2.29 ± 0.58 **(40.83)****
Madecassol®	19.49 ± 2.11	15.21 ± 1.74 (15.59)	10.45 ± 1.17 **(34.77)***	6.47 ± 1.29 **(51.43)****	2.24 ± 0.52 **(72.24)****	0.00± 0.00 **(100.00)*****

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; S.E.M.: standard error of the mean

Percentage values of ultimate tensile strength: the group treated with the carrier was compared with the negative control group; the compounds and the reference medicament were compared with the group treated with the carrier.

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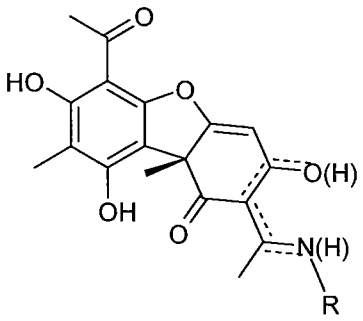
CLAIMS

1. Compound of general formula (I):

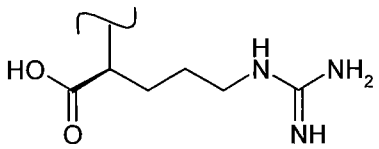


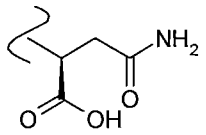
(I)

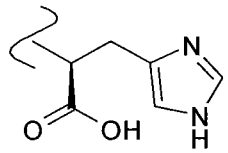
wherein:

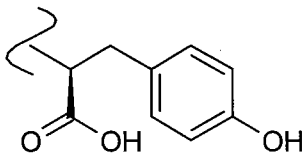
═ represents a single or double bond;

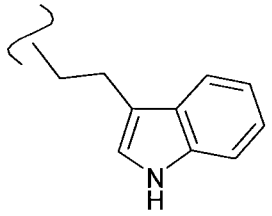
R is a residue selected from:

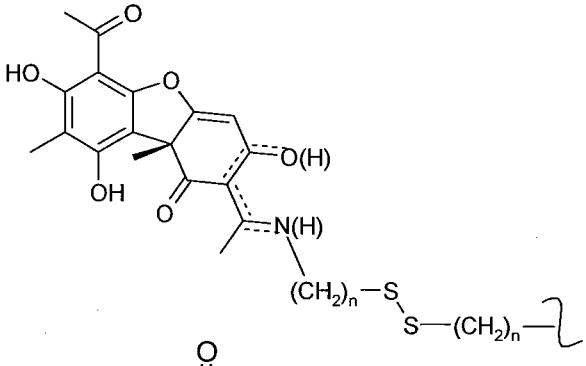
- H
- $(CH_2)_nX$
- $(CH_2)_nC(O)NH(CH_2)_mX$
- 

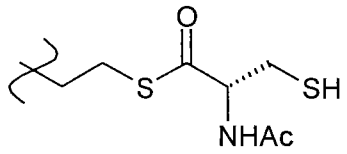
- 

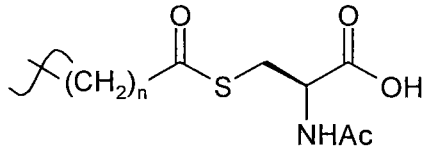
- 

- 

- 

- 

- 

- 

wherein the symbol  represents the attachment point of the R group to the N(H) group;

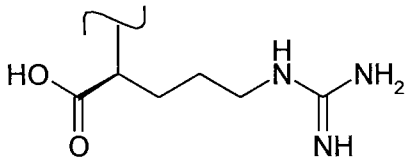
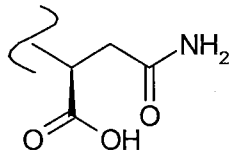
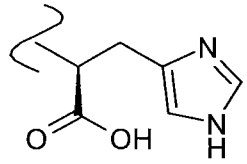
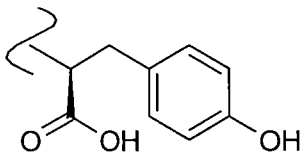
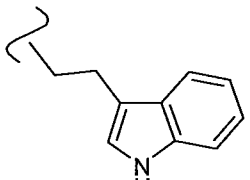
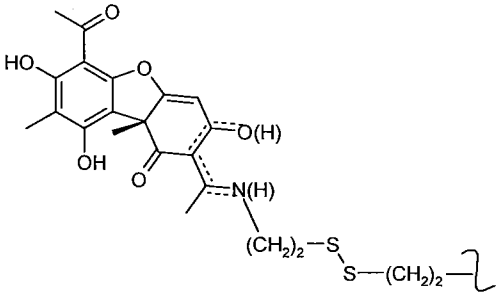
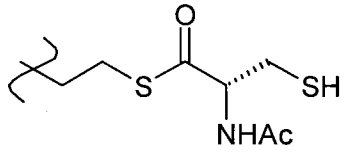
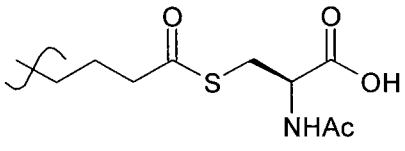
X is selected from OH, SH, COOH, SO_3H;

n and m independently from each other are an integer from 1 to 10, preferably from 2 to 4; with the proviso that when X is OH or SH, the minimum value of n or m is 2;

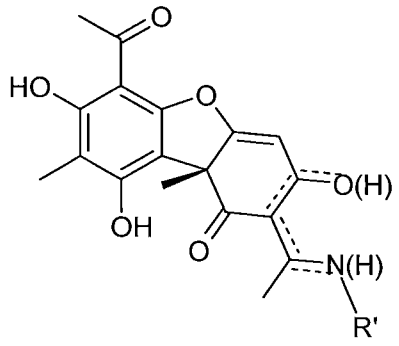
their enantiomers and salts acceptable for pharmaceutical or cosmetic use, for use as antibacterial and wound healing agents.

2. Compound for use according to claim 1, wherein R is a residue selected from:

- H
- $(CH_2)_2SH$
- $(CH_2)_2SO_3H$
- CH_2COOH

- $(CH_2)_3COOH$
- $(CH_2)_4OH$
- $(CH_2)_3C(O)NH(CH_2)_3COOH$
- 
- 
- 
- 
- 
- 
- 
- 

3. Compound of general formula (II)

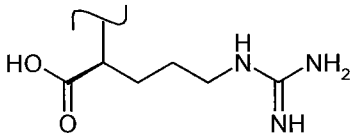
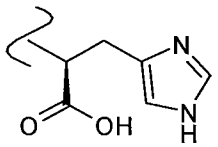
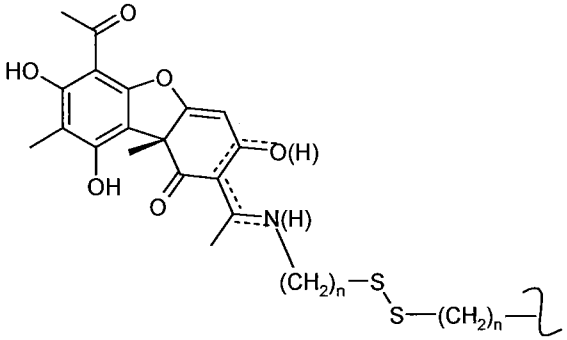
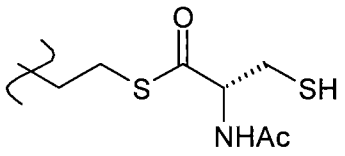


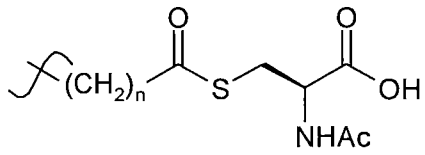
(II)

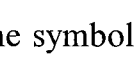
wherein:

═ represents a single or double bond;

R' is a residue selected from:

- $(CH_2)_nX'$
- $(CH_2)_nC(O)NH(CH_2)_mX$
- 
- 
- 
- 

- 

wherein the symbol  represents the attachment point of the R' group to the N(H) group;

X is selected from OH, SH, COOH, SO_3H;

X' is selected from OH, SH, COOH;

n and m independently from each other are an integer from 1 to 10, preferably from 2 to 4;

with the following provisos:

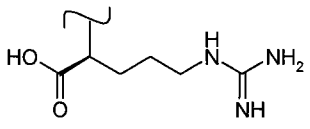
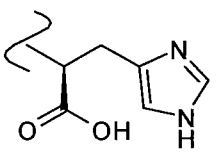
when X or X' is OH or SH, the minimum value of n or m is 2;

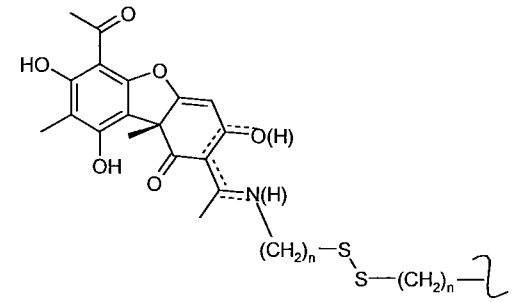
when X' is COOH, n is different from 1 or 2;

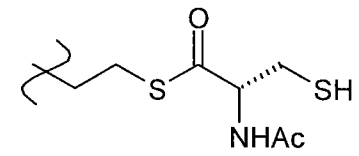
when X' is OH, n is different from 4;

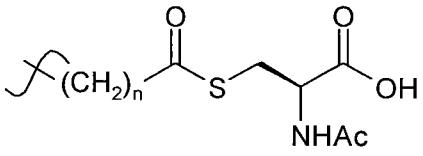
their enantiomers and salts acceptable for pharmaceutical or cosmetic use.

4. Compound according to claim 3 wherein R' is selected in the group of:

- H
- $(CH_2)_2SH$
- CH_2COOH
- $(CH_2)_3COOH$
- $(CH_2)_4OH$
- $(CH_2)_3C(O)NH(CH_2)_3COOH$
- 
- 

- 

- 

- 

5. Compound according to claim 3 or 4 for use as a medicament.

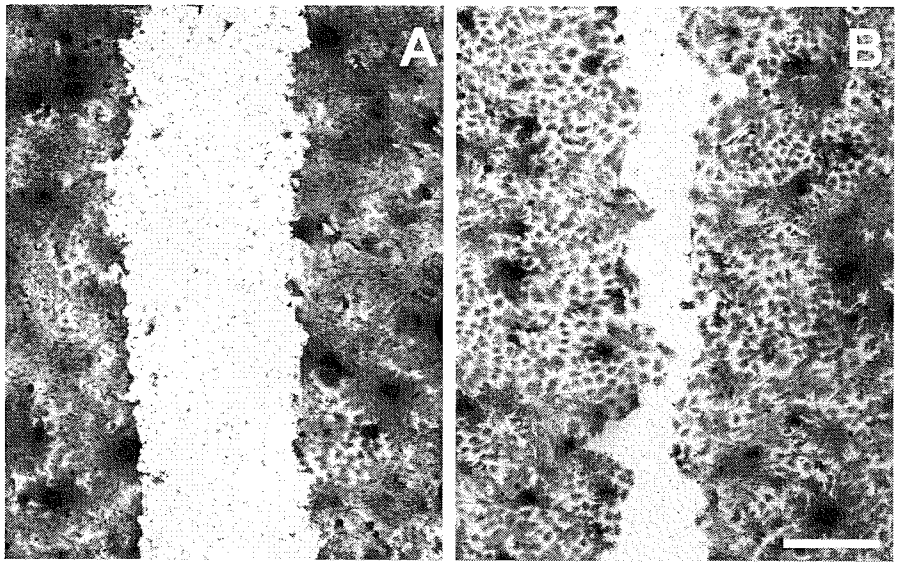
6. Compound according to claim 3 or 4 for use as an antibacterial or wound healing agent.

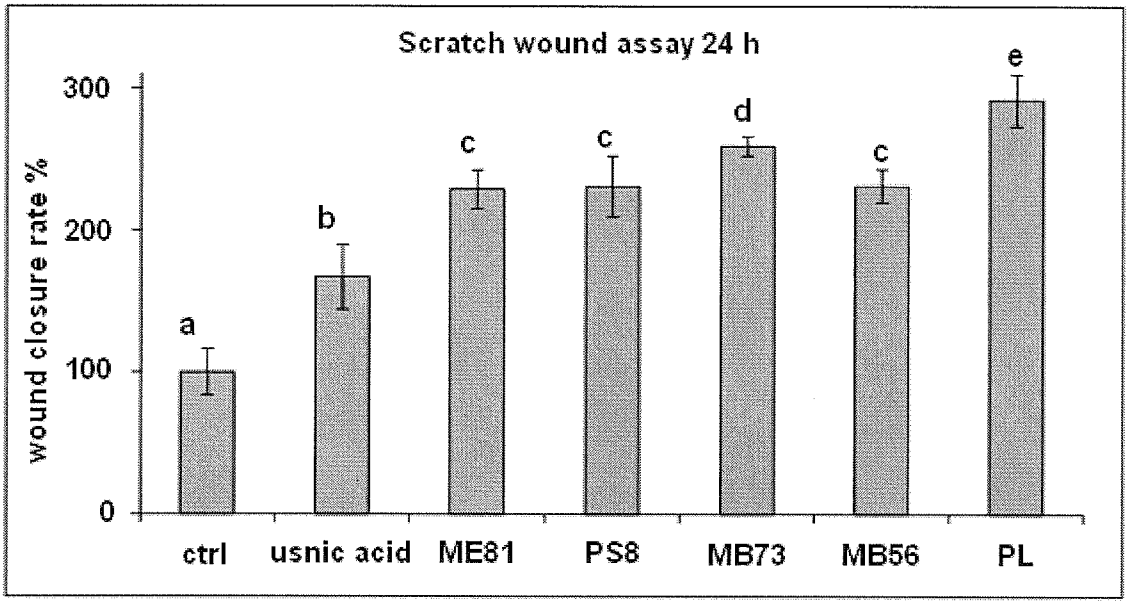
7. Dermatological or cosmetic composition containing a compound according to claims 1-4 together with excipients compatible for pharmaceutical or cosmetic use.

8. Compound according to claims 1-4 or composition according to claim 7 for use in the regenerative or anti-aging treatment of skin tissues.

9. Compound or composition according to claim 8 wherein the regenerative or anti-aging treatment of skin tissues comprises the treatment of topical infections selected from infected burns, topical otitis, haemorrhoids, vaginal lesions and mouth infections.

FIGURE


A
B


Scratch wound assay 24 h
wound closure rate %
300
200
100
0
a
b
c
c
d
c
e
ctrl
usnic acid
ME81
PS8
MB73
MB56
PL

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/062664

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D307/83 C07D307/91 A61K31/381 A61Q19/08 A61P31/04
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D A61K A61Q

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MARC-ANTOINE BAZIN ET AL: "Synthesis and cytotoxic activities of usnic acid derivatives", BIOORGANIC & MEDICINAL CHEMISTRY, vol. 16, no. 14, 1 July 2008 (2008-07-01), pages 6860-6866, XP055039732, ISSN: 0968-0896, DOI: 10.1016/j.bmc.2008.05.069 compound 5 on page 6864 ----- -/--	1-9

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 August 2013	20/08/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bérillon, Laurent

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/062664

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LUZINA O A ET AL: "Chemical modification of usnic acid 2. Reactions of (+)-usnic acid with amino acids", RUSSIAN CHEMICAL BULLETIN, KLUWER ACADEMIC PUBLISHERS-PLENUM PUBLISHERS, NE, vol. 56, no. 6, 1 June 2007 (2007-06-01), pages 1249-1251, XP019556738, ISSN: 1573-9171, DOI: 10.1007/S11172-007-0189-7 compounds 9 and 10 on page 1250	1-9
A	JAMES P. KUTNEY ET AL: "Studies in the usnic acid series. I. The condensation of (+)-usnic acid with aliphatic and aromatic amines", CANADIAN JOURNAL OF CHEMISTRY, vol. 54, no. 17, 1 September 1976 (1976-09-01), pages 2795-2803, XP055039733, ISSN: 0008-4042, DOI: 10.1139/v76-395 compound 2 of figure 1; figure 1	1-9
A	WO 2010/034512 A1 (UNIV DEGLI STUDI MILANO [IT]; VEROTTA LUISELLA [IT]; MONTI DIEGO [IT]) 1 April 2010 (2010-04-01) claims 1,5	1-9
A	CHANGON SEO ET AL: "Usimines A-C, Bioactive Usnic Acid Derivatives from the Antarctic Lichen Stereocaulon alpinum", JOURNAL OF NATURAL PRODUCTS, vol. 71, no. 4, 1 April 2008 (2008-04-01), pages 710-712, XP055039737, ISSN: 0163-3864, DOI: 10.1021/np070464b the whole document	1-9
A	TOMASI S ET AL: "Solid-Phase Synthesis of Polyfunctionalized Natural Products:", JOURNAL OF COMBINATORIAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY, WASHINGTON, US, vol. 8, no. 1, 1 January 2006 (2006-01-01) , pages 11-14, XP009163315, ISSN: 1520-4766 the whole document	1-9

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2013/062664

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010034512 A1	01-04-2010	NONE	