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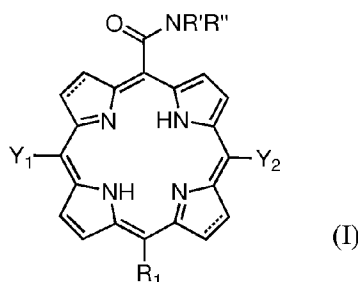
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(54) Title: LOW MOLECULAR WEIGHT DERIVATIVES OF CARBOXAMIDE HALOGENATED PORPHYRINS, NAMELY CHLORINS AND BACTERIOCHLORINS, AND THEIR APPLICATIONS THEREOF



(I)

(57) Abstract: The present invention relates to carboxamide halogenated porphyrin derivatives, in particular bacteriochlorin or chlorin, of formula (I) wherein: represents a carbon-carbon single bond or a carbon-carbon double bond, provided that at least one represents a carbon-carbon single bond; Y₁, Y₂ are each independently chosen from hydrogen, halogenated alkyl or halogenated cycloalkyl with 6 or less carbon atoms, or halogenated phenyl where the halogens are independently chosen from F, Cl and Br, provided that at least one position of the alkyl, cycloalkyl or phenyl is halogenated, and provided that at least one of Y₁, Y₂ is not hydrogen; R₁ is chosen from H, I, Cl, Br or -CONR'R'', where R' and R'' are independently chosen from hydrogen, alkyl with 6 or less carbon atoms, cyclopropyl, cyclobutyl, cyclopentyl, alcohol, primary amine, secondary amine, tertiary amine, positively-charged quaternary amine, carboxylic acid, ether or ester, or pharmaceutically acceptable salts thereof. This invention also relates to the use of the above mentioned carboxamide halogenated porphyrin derivatives, in particular bacteriochlorin or chlorin, of Formula (I) or a pharmaceutically acceptable salt thereof in photodynamic therapy, where the referred derivatives are able to detect and exhibit the presence of hyperproliferative disorders and, in the presence of an adequate lighting, to treat the same. The present invention also describes a pharmaceutical composition comprising one or more of the carboxamide halogenated porphyrin derivatives, in particular bacteriochlorin or chlorin, of Formula (I) or a pharmaceutically acceptable salt thereof, for the treatment of cancer and/or microbial and/or viral infections, in humans or animals.



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DESCRIPTION

“LOW MOLECULAR WEIGHT DERIVATIVES OF CARBOXAMIDE HALOGENATED PORPHYRINS, NAMELY CHLORINS AND BACTERIOCHLORINS, AND THEIR APPLICATIONS THEREOF”

TECHNICAL FIELD

The present application relates to new low-molecular weight carboxamide halogenated porphyrin derivatives, namely chlorin and bacteriochlorin derivatives, and their preparation process and use in photodynamic therapy.

BACKGROUND OF THE INVENTION

Photodynamic therapy (PDT) is a clinically approved treatment based on the administration of a photosensitizing molecule, its accumulation in the target tissue, and then illumination with light selectively absorbed by the photosensitizer. This selectivity is improved using photosensitizers that absorb light in the phototherapeutic window (650-850 nm), where tissues have higher optical penetration depths (e.g., $\delta=2.3$ mm at 750 nm) (1). The absorption of light leaves the photosensitizer in an electronically excited state that reacts with substrate molecules by electron transfer reactions with the formation of superoxide anion and hydroxyl radicals (type I reaction), or to transfer its energy to ground-state molecular oxygen generating singlet oxygen (type II reaction). These photogenerated reactive oxygen species (ROS) trigger biological mechanisms that make PDT an effective anti-cancer procedure (2).

The paradigm of PDT in the treatment of hyperproliferative disorders has been that stable dyes with a stronger absorption of light in the phototherapeutic window and with high ROS quantum yields (Φ_{ROS}) should be better photosensitizers. Additionally, the development of PDT photosensitizers targeting Gram-negative bacteria has been guided by the need to have at least one positive charge present in the photosensitizer (3). Such photosensitizers are usually porphyrin derivatives (chlorins or bacteriochlorins) with molecular weights higher than 600 Da. Although photodynamic inactivation of bacteria suspensions by 5-6 orders of magnitude using micromolar photosensitizer concentrations and light doses ca. 10 J/cm^2 was achieved (4), the transfer to clinical applications has been unsuccessful. A likely reason for the failure to translate the photodynamic inactivation of bacteria suspensions to clinical settings is the incompatibility between the large size of the photosensitizers that absorb infrared light and the small molecular size required for rapid diffusion in the biofilms and uptake by the bacteria. Similar difficulties have been found in the transfer of

PDT photosensitizers to the topical treatment of dermatological disorders. Whereas photosensitizers such as porfimer sodium (trade name Photofrin®) and temoporfin (Foscan®) have obtained approval for cancer indications using intravenous administration, topical applications have not been transferred to the clinical (5). Again, the failure of topical applications of photosensitizers to elicit therapeutic effects is likely related to the difficulty of such photosensitizers to cross the outer layer of the skin, called stratum corneum, and reach their targets. The stratum corneum is the principal barrier to the percutaneous penetration of exogenous molecules.

The best maximum flux ($J_{\max}$) of a drug across the skin after topical application is strongly limited by the molecular weight (MW) of the drug (6),

$$\log J_{\max} = -3.90 - 0.0190 MW$$

For example, drugs with $MW = 600$ or 700 Da should have $J_{\max} = 5 \times 10^{-16}$ or 6×10^{-18} mol/(cm² h), respectively. These calculations show that a modest increase in the molecular weight above 600 Da can lead to a dramatic decrease in the transdermal flux of the photosensitizers. In practical terms, a photosensitizer of 700 Da is likely to take 100 times longer to reach a therapeutic concentration in a subcutaneous target than a photosensitizer of 600 Da. Another critical property that drugs intended for topical applications must meet, is adequate solubility within the lipid domains of the stratum corneum to permit diffusion through this domain whilst still having sufficient hydrophilic nature to allow partitioning into the viable tissues of the epidermis. Drugs that meet this determinant have the logarithm of their *n*-octanol-water partition coefficient ($\log P_{OW}$) between 1 and 3 (7).

The ideal photosensitizer for topical applications of PDT and for photoinactivation of bacteria must have a molecular weight $MW < 700$ Da, a $\log P_{OW}$ between 1 and 3, a high molar absorption coefficient $\epsilon > 30,000 \text{ M}^{-1} \text{ cm}^{-1}$ between 650 and 850 nm, and a ROS quantum yield $\Phi_{ROS} > 0.3$. Additionally, the photostability of the photosensitizer is also critical to the success of PDT (8). The photostability of a photosensitizer can be compared with the turnover of a chemical catalyst: it is related with the number of moles of substrate that a mole of catalyst can convert (i.e., the number of ROS generated) before the catalyst (i.e., photosensitizer) is inactivated (i.e., photodecomposes). The two most widely used photosensitizers for PDT of cancer are porfimer sodium (trade name Photofrin®) and temoporfin (proprietary name Foscan®). Porfimer sodium is a mixture of oligomers formed by ether and ester linkages of up to eight porphyrin units, relatively soluble in aqueous solutions, with $\log POW \approx 0$. Porfimer sodium is not a single molecular entity and does not have a characteristic molecular weight, but the molecular weight of the smallest dimer exceeds 1000 Da. Temoporfin is the very lipophilic 5,10,15,20-tetra (*m*-hydroxyphenyl)chlorin with a molecular weight of 680 Da and $\log POW = 5.5$ at physiological pH. The singlet oxygen quantum

yields of porfimer sodium and temoporfin are 0.36 and 0.43, respectively (8). Their maximum absorption peaks in the red are at $\lambda_{\max}=630$ nm with a molar absorption coefficient $\epsilon_{630}=1170$ M⁻¹ cm⁻¹ for porfimer sodium, and $\lambda_{\max}=650$ nm with $\epsilon_{650}=29600$ M⁻¹ cm⁻¹ for temoporfin. They are relatively photostable, with photodecomposition quantum yields $\Phi_{\text{pd}}=5.5\times 10^{-5}$ and 3.3×10^{-5} for porfimer sodium and temoporfin, respectively. When porfimer sodium or temoporfin are incubated with CT26 (mouse colon adenocarcinoma) cells and, after washing, illuminated with laser light of the wavelength matching their red absorption bands to deliver a light dose of 1 J/cm², it was seen that a porfimer sodium concentration of 18 μM (estimated on the basis of the molecular weight of a porphyrin unit) was necessary to kill 50% of the cells in the culture (IC₅₀=18 μM), whereas for temoporfin the concentration necessary to attain the same toxicity for the same light dose was 0.2 μM (IC₅₀=0.2 μM) (8).

The properties of porfimer sodium are inadequate for the penetration of biological barriers, namely the skin, because of its exceedingly high molecular weight, hydrophilicity and modest light absorption in the phototherapeutic window. Temoporfin partially resolves the issue of the molecular weight but it is exceedingly lipophilic for transdermal delivery and absorbs light just at the limit of the phototherapeutic window. The difficulty of these clinically approved photosensitizers to permeate the biological barriers, namely the skin barrier, is aggravated by the need for relatively large concentrations of these photosensitizers in the biological target to attain the phototoxicity required for PDT to offer a good therapeutic outcome.

It has not been appreciated in earlier uses of photosensitizers for PDT that the ideal properties of a photosensitizer for PDT could be combined in a single molecule with the ideal properties of drugs for topical applications. The ability to rapidly diffuse through biological barriers is critical for the success of, for example, intradermal or transdermal delivery of photosensitizers topically applied in the treatment of dermatological disorders, penetration of the photosensitizers in biofilms for the photoinactivation of bacteria, diffusion of the photosensitizer through nails for the treatment of fungal infections such as onychomycosis. The ability to rapidly diffuse through biological barriers is also critical for the rapid accumulation of the photosensitizer in its biological target, such as the permeation through the outer membrane of eukaryote cells or the membrane of bacterial cells. Such rapid diffusions shorten the time between the administration of the photosensitizer and the illumination of the target, which is advantageous in many applications of photodynamic therapy, and increase the phototoxicity towards the target. It would not be expected by the person skilled in the art that the carboxamide group in at least one meso position of the halogenated porphyrin

derivatives shown in formula (I), in particular bacteriochlorins and chlorins, could contribute to the amphiphilicity and photostability of such bacteriochlorin or chlorin derivatives without compromising the generation of ROS, and with such a small contribution to the molecular weight of the photosensitizer that its diffusion through biological barriers is not impaired.

This invention discloses for the first time photosensitizers for PDT of hyperproliferative disorders and/or for the photoinactivation of bacteria or virus or fungi that meet all the criteria for the ideal photosensitizer and efficiently permeate biological barriers. The present invention also discloses processes to synthesize such photosensitizers and, by the way of examples, illustrates the use of these photosensitizers to kill cancer cells and inactivate bacteria. In a further embodiment of the present invention, the photosensitizers described herein are used for the theranostics of hyperproliferative tissues. Theranostics is a modality of image-guided therapy where the same compound is used to visualize the biological target and to obtain the desired therapeutic effect.

SUMMARY OF THE INVENTION

The purpose of the present invention is to offer new carboxamide halogenated porphyrin derivatives, in particular bacteriochlorins and chlorins, which can be efficiently used to kill bacteria even when present in biofilms, to kill tumor cells even when applied topically, to kill fungi and to inactivate virus. In view of the shortcomings of the current PDT photosensitizers to achieve efficient transdermal delivery or to penetrate biofilms, the present invention discloses new porphyrin derivatives, in particular bacteriochlorins and chlorins, that combine low molecular weights, with strong absorption in the phototherapeutic window, high photostability, high quantum yields of ROS photogeneration, appropriate amphiphilicity and biocompatibility, and that can be produced in large quantities from inexpensive raw materials.

Another aim of the present invention is to offer a medication to be used in photodynamic therapy wherein the target is selected from the group consisting of: a vascular endothelial tissue, a neovasculature tissue, a neovasculature tissue present in the eye, an abnormal vascular wall of a tumor, a solid tumor, a tumor of the skin, a tumor of a head, a tumor of a neck, a tumor of an eye, a tumor of a gastrointestinal tract, a tumor of a liver, a tumor of a breast, a tumor of a prostate, a tumor of a lung, a nonsolid tumor, malignant cells of one of a hematopoietic tissue and a lymphoid tissue, lesions in the vascular system, a diseased bone marrow, and diseased cells in which the disease is one of an autoimmune and an inflammatory disease.

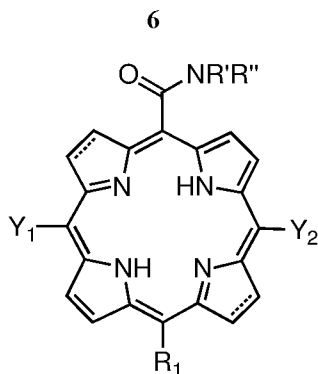
A further aim of the present invention is to offer a medication for the treatment of dermatological disorders such as psoriasis, acne vulgaris and rosacea; gynecological disorders such as dysfunctional uterine bleeding; urological disorders such as condyloma virus; cardiovascular

disorders such as restenosis and atherosclerotic plaques; treatment of fungal infections such as onychomycosis; photodynamic destruction of bacteria or viruses, including multidrug-resistant bacteria; hair removal and cosmetics; inhibition of immune responses following the transplant of organs or tissues. The removal of a superficial layer of cells using the methods of photodynamic therapy with the photosensitizers disclosed herein stimulates the growth of new cells in the underlying skin layers with the subsequent improvement of skin appearance of cosmetic value. Another objective of the present invention is to provide a medication for heart arrhythmia consisting in the selective destruction of cells such as cardiac myocytes, by localized photodynamic therapy, and restoration of the physiological rhythm of the heart.

Finally, it is a further object of the invention to provide methods for the diagnosis of hyperproliferative tissues using new carboxamide porphyrin derivatives, in particular bacteriochlorins and chlorins. Provided that these compounds preferentially accumulate in such tissues, the additional property required for diagnostic purposes is the unambiguous detection of very minute quantities of such compounds. These compounds have very distinct absorption bands in the red and infrared, where the tissues are most transparent. The selective excitation of these compounds leads to distinct fluorescence at wavelengths where biological molecules do not emit. The detection of fluorescence can be made with very sensitive equipment and sub-nanomolar quantities of bacteriochlorins and chlorins can be measured in biological media. The source of irradiation for photodiagnosis and phototherapy is not restricted, but a laser beam is preferable because intensive light rays in a desired wavelength range can be selectively applied. It is necessary that the light rays have sufficient intensity to cause the compounds to emit fluorescence for diagnosis and to exert a cell killing effect for therapy. Additionally, when fluorinated chlorins or bacteriochlorins are employed, fluorine-MRI (Magnetic Resonance Imaging) can detect the accumulation of these compounds in small regions of the body and follow the metabolites formed in its clearance from the body. Moreover, when pulsed lasers are used for excitation, the subsequent radiationless decay processes release heat that generates a photoacoustic wave, and such waves can be detected by means of Photoacoustic Tomography providing further information of interest for the diagnosis of hyperproliferative disorders.

Other aims and technical features will appear in the following description that is given only by way of example and without being limited thereto.

The present invention relates to carboxamide porphyrin derivatives, in particular bacteriochlorin or chlorin, of formula:



Formula (I)

Where:

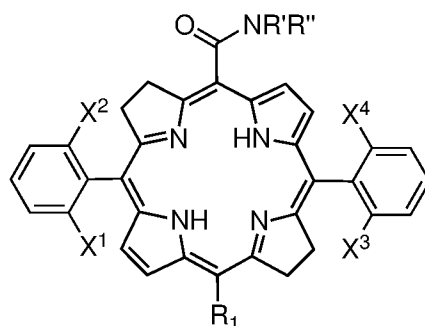
===== represents a carbon-carbon single bond or a carbon-carbon double bond, provided that at least one ===== represents a carbon-carbon single bond;

Y₁, Y₂ are each independently chosen from hydrogen, halogenated alkyl or halogenated cycloalkyl with 6 or less carbon atoms, or halogenated phenyl where the halogens are independently chosen from F, Cl and Br, provided that at least one position of the alkyl, cycloalkyl or phenyl is halogenated, and provided that at least one of Y₁, Y₂ is not hydrogen i.e. at least one of Y₁, Y₂ is halogenated alkyl or halogenated cycloalkyl with 6 or less carbon atoms, or halogenated phenyl where the halogens are independently chosen from F, Cl and Br, provided that at least one position of the alkyl, cycloalkyl or phenyl is halogenated;

R₁ is chosen from H, I, Cl, Br or -CONR'R'';

R' and R'' are independently chosen from hydrogen, alkyl with 6 or less carbon atoms, cyclopropyl, cyclobutyl, cyclopentyl, alcohol, primary amine, secondary amine, tertiary amine, positively-charged quaternary amine, carboxylic acid, ether or ester;
or pharmaceutically acceptable salts thereof.

Hence, the compounds of Formula (I) may be bacteriochlorins of formula



Formula (II)

Wherein:

X^1 , X^2 , X^3 , X^4 are each independently chosen from halogen (F, Cl, Br) and hydrogen atoms, provided that at least X^1 and X^3 are halogens;

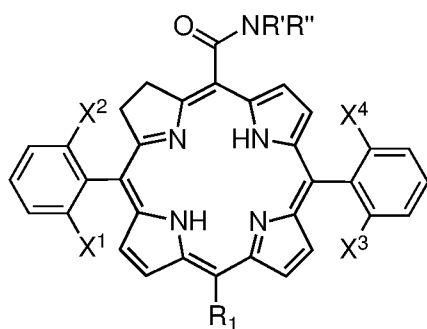
R_1 is chosen from H, F, Cl, Br or $-\text{CONR}'\text{R}''$;

R' and R'' are independently chosen from hydrogen, alkyl with 6 or less carbon atoms, cyclopropyl, cyclobutyl, cyclopentyl, alcohol, primary amine, secondary amine, tertiary amine, positively-charged quaternary amine, carboxylic acid, ether or ester;

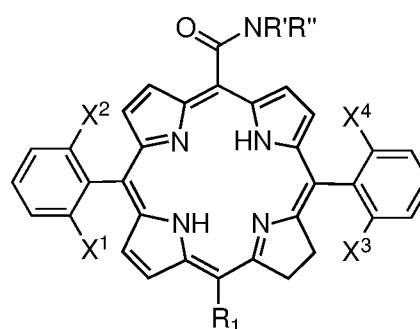
or pharmaceutically acceptable salts thereof.

Specific preferred compounds of the invention include the carboxamide bacteriochlorin of Formula (II) where X^1 , X^2 , X^3 , X^4 are fluorine atoms, R_1 is hydrogen, R' is hydrogen and R'' is methyl.

Alternatively, the compounds of Formula (I) may be chlorins of formula



Formula (IIIa)



Formula (IIIb)

Wherein:

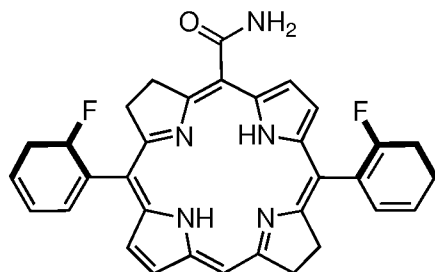
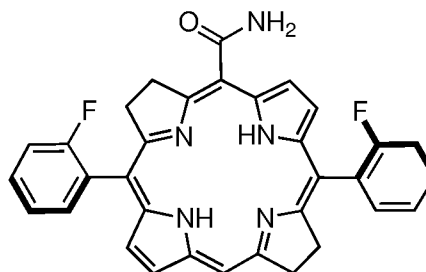
X^1 , X^2 , X^3 , X^4 are each independently chosen from halogen (F, Cl, Br) and hydrogen atoms, provided that at least X^1 and X^3 are halogens;

R_1 is chosen from H, F, Cl, Br or $-\text{CONR}'\text{R}''$;

R' and R'' are independently chosen from hydrogen, alkyl with 6 or less carbon atoms, cyclopropyl, cyclobutyl, cyclopentyl, alcohol, primary amine, secondary amine, tertiary amine, positively-charged quaternary amine, carboxylic acid, ether or ester; or pharmaceutically acceptable salts thereof.

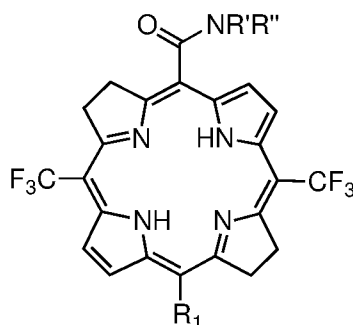
When X^1 is different from X^2 and/or X^3 is different from X^4 , the compounds of Formula (II) or (III) have atropisomers because of the hindered rotation around the phenyl-macrocyclic single bond. In such cases, the atropisomers can be distinguished by the number of heavier atoms on

each side of the plane defined by the macrocycle. Formula IV illustrate two atropisomers of a bacteriochlorin derivative distinguished by the presence of both fluorine atoms on the same side of the macrocycle plane (atropisomer $\alpha\alpha$) or in different sides of the macrocycle plane (atropisomer $\alpha\beta$)

Formula (IV $\alpha\alpha$)Formula (IV $\alpha\beta$)

Where the bold lines indicate that the bolded atoms, and the groups attached thereto, are sterically restricted so as to exist above the plane defined by the macrocycle ring.

The compounds of Formula (I) may also be bacteriochlorins of formula



Formula (XV)

Wherein:

R₁ is chosen from H, I, Cl, Br or -CONR'R'';

R' and R'' are independently chosen from hydrogen, alkyl with 6 or less carbon atoms, cyclopropyl, cyclobutyl, cyclopentyl, alcohol, primary amine, secondary amine, tertiary amine, positively-charged quaternary amine, carboxylic acid, ether or ester; or pharmaceutically acceptable salts thereof.

Specific preferred compounds of the invention include the carboxamide bacteriochlorin of Formula (XV) where R₁ is hydrogen, R' is hydrogen and R'' is methyl.

The invention also provides a pharmaceutical composition, comprising an effective amount a compound described herein and a pharmaceutically acceptable carrier.

Actual dosage levels and time course of administration of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic (or unacceptably toxic) to the patient. In use, at least one compound according to the present invention is administered in a pharmaceutically effective amount to a subject in need thereof in a pharmaceutical carrier by intravenous, intramuscular, subcutaneous, intralesional, or intracerebroventricular injection or by oral administration or topical application. In accordance with the present invention, a compound of the invention may be administered alone or in conjunction with a second, different therapeutic. By "in conjunction with" is meant together, substantially simultaneously or sequentially.

By "pharmaceutically effective amount" as used herein is meant an amount of a compound of the invention, high enough to significantly positively modify the condition to be treated but low enough to avoid serious side effects (at a reasonable benefit/risk ratio), within the scope of sound medical judgment. A pharmaceutically effective amount of a compound of the invention will vary with the particular goal to be achieved, the age and physical condition of the patient being treated, the severity of the underlying disease, the duration of treatment, the nature of concurrent therapy and the specific compound employed. For example, a therapeutically effective amount of a compound of the invention administered to a child or a neonate will be reduced proportionately in accordance with sound medical judgment. The effective amount of a compound of the invention will thus be the minimum amount which will provide the desired effect. Additionally, with photodynamic therapy, the "pharmaceutically effective amount" of the pharmaceutical composition or compound is partially dependent upon other factors such as light dose and oxygen, both of which are required to achieve a therapeutic result. Thus, there will also be an "effective amount" of light as well as amount of oxygen when treating a subject or patient. Other important factors that contribute to the determination of the "pharmaceutically effective amount" of drug, light, and oxygen include drug-to-light intervals (the time between drug administration and illuminating the tissue). Drug-to-light interval is important because, for example, administering a higher drug dose of 50 mg/kg and illuminating the tissue one week later with a light dose of 500 J/cm² may be as inefficient or ineffective as using a drug dose of 0.01 mg/kg and illuminating the tissue 10 minutes after administration at a light dose of 0.1 J/cm². The drug elimination (metabolism) by the organism between the administration of the drug and the illumination may decrease the effectiveness of the therapy when the drug-to-light interval increases (becomes longer). However, increasing the drug-

to-light interval may lead to a more selective therapy and fewer adverse effects. Thus, for at least these reasons, drug-to-light interval is an important factor to consider when determining the “pharmaceutically effective amount” of the compositions of the present invention.

In addition to the factors discussed above that affect the determination of the “effective amount” of drug, light, oxygen, and drug-to-light interval, a person of ordinary skill in the art would also take into account the fluence rate of the light (how many photons are delivered per unit area per unit time). Fluence rate is important because the delivery of too many photons too fast may deplete the oxygen in the tissue and render the therapy inefficient or ineffective.

A decided practical advantage of the present invention is that the compound may be administered in a convenient manner such as by intravenous, intramuscular, subcutaneous, oral, intralesional, or intracerebroventricular injection routes or by topical application, such as in creams or gels. Depending on the route of administration, the active ingredients which comprise a compound of the invention may be required to be coated in a material to protect the compound from the action of enzymes, acids and other natural conditions which may inactivate the compound. In order to administer a compound of the invention by other than parenteral administration, the compound can be coated by, or administered with, a material to prevent inactivation or to improve dissolution.

The present invention also relates to a pharmaceutically composition comprising at least one of the derivatives complying with Formula (I), or pharmaceutically acceptable salts thereof, and a pharmaceutically acceptable carrier, which transiently permeabilizes the skin causing the pharmaceutical composition to be permeable through the various skin layers.

Another object of the present invention is the use of a compound as described herein in the manufacture of a medicament for use in the treatment of a disorder or disease described herein. Another object of the present invention is the use of a compound as described herein for use in the treatment of a disorder or disease described herein.

The recitation of a listing of chemical groups in any definition of a variable herein includes definitions of that variable as any single group or combination of listed groups. The recitation of an embodiment for a variable herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof. The recitation of an embodiment herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

The present invention also relates to the use of the above mentioned derivatives or pharmaceutically acceptable salts thereof and the pharmaceutically composition comprising at least one of the same in the treatment of hyperproliferative disorders and/or cancer and/or bacterial and/or viral or fungi infections. Furthermore the use of the pharmaceutically composition in intradermal and transdermal therapies.

The present invention also relates to the use of the pharmaceutically composition comprising at least one of the above mentioned derivatives or pharmaceutically acceptable salts thereof in theranostics of hyperproliferative disorders, wherein at least one of the derivatives described in claims 1-9 associates with a target tissue is visualized by imaging techniques and optionally through lighting said derivatives elicit the desired therapeutic effect in the target tissue. Where the imaging techniques comprise Magnetic Resonance Imaging (MRI), exposing the derivatives complying with Formula (I), or pharmaceutically acceptable salts thereof, to light of sufficient energy to cause the same to fluoresce, or comprise exposing the derivatives complying with Formula (I), or pharmaceutically acceptable salts thereof, to a light pulse of picosecond or nanosecond duration, of sufficient energy to cause the compound to launch a photoacoustic wave.

Remarkably, the molecular weight of bacteriochlorins or chlorins of Formula (I) range between 425 Da and 700 Da, they are amphiphilic, form few hydrogen bonds and may diffuse efficiently through biological barriers such as the stratum corneum or through biofilms. One of the technical characteristics of these derivatives lies in their low molecular weight and the consequent increased flux through protective biological barriers. Another technical advantage is the preservation of the bacteriochlorin, or chlorin, macrocycle known for its strong absorption of light in the phototherapeutic window and ability to generate ROS in high quantum yields. Yet another advantage is the presence of substituent groups that enhance the photostability of reduced porphyrin derivatives and balance the solubility within lipid domains with biocompatibility with biological and pharmaceutical carriers. A further technical characteristic of the bacteriochlorins or chlorins with Formula (I) is that they are significantly fluorescent, namely with fluorescence quantum yields higher than 0.1, which allows for their non-invasive visualization in the target. This visualization is a desired property because it enables the visualization of the target and the choice of the best timing to start the therapy, for instance when the photosensitizer target-to-surrounding tissue ratio is high. The theranostic use of bacteriochlorins or chlorins with Formula (I) can also be exploited when they contain fluorine atoms, by means of fluorine magnetic resonance imaging (MRI), or

when they launch photoacoustic waves under pulsed laser light, by means of photoacoustic tomography (PAT).

Herein, the meaning of “protective biological barriers” should be understood as barriers to the diffusion of molecular and supramolecular species in the body, such as the skin and more particularly the stratum corneum, barriers of the gastrointestinal tract and also ocular barriers, the nail barrier, the outer membrane of cells and bacteria, and also the biofilms created by microorganisms. It could not be anticipated by the person skilled in the art that carboxamide halogenated porphyrin derivatives, in particular bacteriochlorins and chlorins, could efficiently permeate biological membranes and rapidly become very phototoxic photosensitizers towards malignant cells, bacteria or fungi protected by said biological barriers.

BRIEF DESCRIPTION OF THE DRAWINGS

Without intent to limit the disclosure herein, this application presents attached drawings of illustrated embodiments for an easier understanding.

Figure 1: A) Structures of the molecules of formula (V), (VI), (VII), and (VIII). B) Structures of the molecules of formula (XI), (XII), (XIII), and (XIV).

Figure 2: Structures of the molecules of formula (IX), (Xa), and (Xb).

Figure 3: Absorption spectra of the molecule of formula (IX) in ethanol and DMSO.

Figure 4: Absorption spectra of the molecule of formula (XIV) in dichloromethane.

Figure 5: Phototoxicity of the molecule of formula (IX) against *Propionibacterium acnes* after illumination with 4 J/cm² or with 10 J/cm².

Figure 6: Phototoxicity of the molecule of formula (IX) against cancer lines A549 and CT26 after irradiation (1 J/cm²).

Figure 7. Kaplan-Meier survival curves for mice with subcutaneously implanted CT26 tumors, where the solid line represents the non-treated control group, the dashed line is the group treated

with 0.3 mg/kg of the molecule of formula (IX) and the dotted line is group treated with 0.15 mg/kg of the molecule of formula (IX).

Figure 8. Confocal microscopy of skin samples, cut perpendicular to the surface of the skin, exposed with incubation times of 30, 60 and 90 minutes to topical formulations containing the molecule of formula (IX), where the clearer regions reveal the fluorescence of the photosensitizer in the skin.

DESCRIPTION OF THE EMBODIMENTS

Referring to the drawings, herein are described optional embodiments in more detail, which however are not intended to limit the scope of the present application.

A. Precursor compounds

A.1. 5,15-bis-(2,6-Difluorophenyl)porphyrin precursors

5,15-bis-(2,6-Difluorophenyl)porphyrin (Formula V in Figure 1) was prepared with a modification of a method for the preparation of 5,15-diphenylporphyrins (9a). The equimolar mixture of commercially available 2,2'-dipyrromethane and 2,6-difluorobenzaldehyde was allowed to react in the presence of trifluoroacetic acid (TFA). After oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), workup and purification, V was obtained with 35 % yield in a multigram gram scale (~5 g). The characterization of V is as follows: ¹H NMR: (400 MHz, (CDCl₃) δ ppm: -3.05 (s, 2H, -NH); 7.55-7.79 (t, 4H, Ar-H); 7.98-8.05 (m, 2H, Ar-H); 9.03 (d, *J* = 4.4 Hz, 4H, β-H); 9.41 (d, *J* = 4.5 Hz, 4H, β-H); 10.32 (s, 2H, meso-H); MS (ESI-FIA-TOF): *m/z* calcd for (C₃₂H₁₉F₄N₄) [M+H]⁺ 535.1540; found: 535.1536 [M+H]⁺.

[5,15-bis-(2,6-Difluorophenyl)porphyrinato] zinc (II) (Formula VI) was prepared by the complexation of 5,15-bis(2,6-difluorophenyl)porphyrin with zinc acetate (Zn(OAc)₂) in a mixture of dichloromethane:methanol 1:1. The solution was heated up with magnetic stirring until the starting material was fully consumed. The solution was washed with water, dried with anhydrous sodium sulfate, filtered, and concentrated via rotary evaporation; 4.3 g of isolated product was obtained with 99 % yield. The NMR of the isolated product is as follows: ¹H NMR: (400 MHz, (CDCl₃) δ ppm: 7.41-7.45 (t, 4H, Ar-H); 7.81-7.86 (m, 2H, Ar-H); 9.13 (d, *J* = 4.3 Hz, 4H, β-H); 9.50 (d, *J* = 4.5 Hz, 4H, β-H); 10.37 (s, 2H, meso-H).

[5-bromo-10,20-bis-(2,6-difluorophenyl)porphyrinato] zinc (II) (Formula VII) was synthesized by the reaction of *N*-bromosuccinimide (NBS) (298 mg ± 10 mg) dissolved in dichloromethane (DCM) (100 ± 20 mL) added drop wise to a mixture of 1 g of [5,15-bis-(2,6-difluorophenyl)porphyrinato]

zinc (II) in DCM (400 ± 50 mL) and pyridine (1.35 ± 0.5 mL) at -6 °C. After 1 h the reaction is completed, water was added (50 ± 20 mL) and the organic layer was sequentially washed with a solution of hydrochloric acid 0.1 M (3 times) and water (3 times). The solvent was evaporated and purified by column chromatography with silica gel (DCM/hexane). The [5-bromo-10,20-*bis*-(2,6-difluorophenyl)porphyrinato] zinc (II) was obtained with $70 \pm 5\%$ yield (790 ± 40 mg). ^1H NMR: (400 MHz, (CDCl_3) δ ppm: 7.34-7.38 (m, 4H, Ar-H); 7.76-7.79 (m, 2H, Ar-H); 8.83 (s, 4H, β -H); 9.26 (d, $J = 4.5$ Hz, 2H, β -H); 9.70 (d, $J = 4.7$ Hz, 2H, β -H); 10.13 (s, 1H, *meso*-H).

5-Methylamide-10,20-*bis*-(2,6-difluorophenyl)porphyrin (Formula **VIII**) was synthesized *via* aminocarbonylation reaction. An autoclave steel reactor containing a stirring bar, was charged with 4.0 g (5.9 mmol) of [5-bromo-10,20-*bis*-(2,6-difluorophenyl)porphyrinato] zinc(II), 66.0 mg (0.3 mmol) of palladium acetate ($\text{Pd}(\text{OAc})_2$), 155.0 mg (0.6 mmol) of triphenylphosphine, 0.8 mL (5.9 mmol) of trimethylamine, 14.6 mL (29.5 mmol) of 2 M methylamine solution in tetrahydrofuran (THF), and 60.0 mL of dry THF. The reactor was closed and charged with 5 bar of carbon monoxide. The mixture was stirred at 70 °C and the reaction was allowed to proceed for 15 hours. The reaction mixture was transferred to a round bottom flask and the solvent removed in the rotatory evaporator. The reaction crude was dissolved in DCM and TFA (10 mL) was added. The reaction mixture was stirred at room temperature for 2 hours. The work-up was performed by a liquid-liquid extraction using a saturated sodium bicarbonate solution and distilled water. The organic layer was dried with anhydrous sodium sulfate, filtered and finally the solvent was removed in a rotary evaporator. After column chromatography (silica gel, DCM: ethyl acetate, 20:1) 2.45 g of isolated product was obtained with 70 % yield. The NMR and MS of the isolated product are as follows: ^1H NMR: (400 MHz, (CD_3) $_2\text{CO}$) δ ppm : -3.14(s, 2H, -NH); 3.56 (d, $J = 4.2$ Hz, 3H, -CH $_3$); 7.63(t, $J = 8.2$ Hz, 4H, Ar-H); 8.04-8.11 (m, 2H, Ar-H); 8.49 (bs, 1H, -NH); 9.09 (m, 4H, β -H); 9.63(d, 2H, $J = 4.5$ Hz, β -H), 9.56 (d, 2H, $J = 4.7$ Hz, β -H); 10.60 (s, 1H, *meso*-H). MS (ESI-FIA-TOF): m/z calcd for $\text{C}_{34}\text{H}_{22}\text{F}_4\text{N}_5\text{O}$: 592.1760; found: 592.1751 [$\text{M}+\text{H}$] $^+$.

A2. 5,15-*bis*-(Trifluoromethyl)porphyrin precursors

[5,15-*bis*-(Trifluoromethyl)porphyrinato]zinc(II) (Formula **XI** in Figure 1) was synthesized using a previously described method (9b). The characterization of **XI** is as follows: ^1H RMN: (400 MHz, THF- d_8) δ ppm: 9.67 (d, $J=4.0$, β -H); 9.88 (bs, β -H); 10.57 (s, 2H, *meso*-H).

[5-Bromo-10,20-*bis*-(trifluoromethyl)porphyrinato]zinc(II) (Formula **XII**) was synthesized by the reaction of *N*-bromosuccinimide (NBS) dissolved in dichloromethane, added drop wise to a mixture of [5,15-*bis*-(trifluoromethyl)porphyrinato]zinc(II) in dichloromethane and pyridine at -6 °C. After 1h, the reaction is complete. Water was added and the organic layer was sequentially washed with

a solution of hydrochloric acid 0.1 M (3 times) and water (3 times). The solvent was evaporated to dryness and the compound was used in the next reaction step.

5-Methylamide-10,20-*bis*-(trifluoromethyl)porphyrin (Formula **XIII**) was synthesized *via* aminocarbonylation reaction. An autoclave steel reactor containing a stirring bar, was charged with [5-bromo-10,20-*bis*-(trifluoromethyl)porphyrinato]zinc(II), palladium acetate, triphenylphosphine, triethylamine, methylamine and dried THF. The reactor was closed and charged with a pressure up to 10 bar of carbon monoxide. The mixture was stirred at 70 °C and the reaction was allowed to proceed for 15 hours. The reaction mixture was transferred to a round bottom flask and the solvent removed in the rotatory evaporator. The reaction crude was dissolved in dichloromethane and trifluoroacetic acid was added. The reaction mixture was allowed to stir at room temperature during 2 hours. The work-up was performed by a liquid-liquid extraction using a saturated sodium bicarbonate solution and distilled water. The organic layer was dried with anhydrous sodium sulfate, filtered and finally the solvent was removed in rotary evaporator. After column chromatography (silica gel, dichloromethane: hexane) the product was isolated. The characterization of **XIII** is as follows: ¹H RMN: (400 MHz, THF-d₈) δ ppm : -2.84(s, 2H, -NH); 3.56 (d, *J* = 4.2 Hz, 3H, -CH₃); 8.72 (bs, 1H, -NH); 9.66-9.68 (m, 4H, β-H); 9.80 (bs, 4H, β-H); 10.62 (s, 1H, *meso*-H).

B. Materials and Methods

Elemental analyses were carried out on a Leco TruSpec CHNS elemental analyzer. ¹H-NMR and ¹⁹F-NMR and spectra were recorded on a Bruker Avance 400 MHz. 1H assignments were made using 2D COSY and NOESY experiments, ESI-FIA TOF High Resolution Mass Spectrometry data were acquired using a Micromass Autospec mass spectrometer. HPLC Shimadzu Prominence equipped with a Diode Array (model SPD 20 AV). Separations were followed at 743 nm, 23 °C on a semi-preparative column Inertsil-Phenyl (250*10mm; 5μm).

Optical Absorption: The UV-Vis-NIR optical absorption was recorded with an Agilent Cary5000 UV-Vis-NIR Spectrophotometer in the determination of the molar absorption coefficient and with Shimadzu UV-2100 spectrometer in routine measurements. The absorption spectra were recorded in the wavelengths from 300 nm up to 800 nm.

Fluorescence Emission: The fluorescence emission spectra were recorded in the homemade setup composed of a Horiba-Jobin Fluoromax 4, used to excite the samples, connected to a sample holder through an optical fiber. In the sample holder, perpendicular to excitation fiber, an optical fiber is connected to drive the emission light to the spectrophotometer detector Avantes model SensLine, provided with AvaSoft 7.7.2. The excitation slit was set at 2 mm and the integration time was 3 s, with average number of 3. The spectra were collected from 200 nm up to 1100 nm using standard

cuvettes of 1 cm of optical path. Fluorescence quantum yields (Φ_F) were obtained comparing the integrated fluorescence of the samples with that of a reference fluorimetric compound with known Φ_F .

Fluorescence lifetime: The fluorescence lifetime was determined in homemade equipment composed of a LED that produces a light pulse for exciting the sample, a sample holder, detector and optics. The excitation wavelength was set at 373 nm and the emission collected at 737 nm. The signal was collected using 1024 channels with temporal scale of 28.5 ps per channel.

Transient Absorption: The triplet-triplet transient absorption was recorded in an Applied Photophysics LKS.60 laser flash photolysis spectrometer, with a Hewlett-Packard Infinium Oscilloscope and a Spectra-Physics Quanta-Ray GCR-130 Nd:YAG laser as excitation source. The pulse excitation was set at 355 nm.

Singlet Oxygen Quantum Yield: The experiments were run at room temperature using the Nd-YAG laser Spectra-Physics Quanta-Ray GRC-130. The solutions were excited at 355 nm and the phosphorescence of singlet oxygen collected at 1270 nm in a Hamamatsu R5509-42 photomultiplier, cooled to 193 K in a liquid nitrogen chamber, after selection of the wavelength with a monochromator with 600 lines grading. Phenalenone was used as a reference of singlet oxygen generator. A Newport filter model 10LWF-1000-B was used in the emission to avoid scattering and fluorescence.

Photoacoustic calorimetry: The thermal energy released after electronic excitation was measured by time-resolved photoacoustic calorimetry using a front-face irradiation photoacoustic cell and a EKSPLA OPO model PG-122 pumped by an EKSPLA Nd:YAG. The signal detection was made using a 2.25 MHz Panametrics transducer. The excitation was at 690 nm and azulene was used as photoacoustic calorimetry reference.

n-Octanol:PBS partition ratio: a modification of the shake-flask method was employed to determine the equilibrium concentrations of the photosensitizer in *n*-octanol and in phosphate-buffered saline (PBS) mixed in equal volumes, using the typical fluorescence band of the same photosensitizer and calibration curves.

Photobleaching experiments were conducted in methanol:PBS (3:2) and in organic solvents. The solutions were irradiated in a cuvette with an optical path of 1 cm using a CW laser emitting at 749 ± 3 nm from Omicron Laserage. The total output power was 212 mW or 244 mW. For each compound, the absorbance was collected in time intervals from few minutes up to hours of irradiation.

Phototoxicity towards bacteria was evaluated *in vitro* against *P. acnes* (ATCC® 6919 – Remel, Lenexa, KS, USA) using light with the appropriate wavelength. *P. acnes* bacteria was cultured in Reinforced Clostridial Medium (Oxoid, Basingstok, UK) under anaerobic atmosphere at 37 °C. Anaerobic growth conditions were obtained using an anaerobic jar with a sachet for anaerobic conditions generation (Anaerocult A, Merck, Darmstadt, Germany). *P. acnes* suspension was diluted with culture medium to an optical density at 620 nm of 1.3, corresponding to approximately 1×10^7 CFU/ml (colony-forming units per milliliter). The diluted suspension was centrifuged at 13000 rpm for 10 minutes, and re-suspended in PBS. Test compound stock solutions were dissolved in PEG400:DMSO (propylene glycol 400: dimethyl sulfoxide) (55:45) and were diluted to the appropriate concentrations with PBS. The incubation of the test compounds with *P. acnes* was performed in DB Falcon black 96-well plates with clear flat- bottom (Franklin Lakes, NJ, USA), in the absence of light, during 30 minutes. After the incubation period, the plates were irradiated with a LED light from Marubeni (model L740-66-60-550), emission maximum at 740 nm with FWHM = 25 nm, appropriate to excite bacteriochlorins, for a total light dose of 4 or 10 J/cm². After the irradiation the contents of each well were centrifuged at 13000 rpm for 10 minutes and re-suspended in culture medium. The plate with *P. acnes* was incubated at 37 °C for 24 h, under anaerobic atmosphere. After the incubation period, the viability of *P. acnes* was evaluated. The bacterial suspensions in the plate wells were diluted with culture medium and seeded in Petri dishes with Reinforced Clostridial Agar (Oxoid, Basingstok, UK) for later count of CFU. The petri dishes were incubated at 37 °C for at least 72 h, under anaerobic atmosphere.

Phototoxicity towards cancer cell lines was evaluated *in vitro* using A549 (human lung adenocarcinoma) and CT26 (mouse colon adenocarcinoma) cell lines. The cells were cultured in Dulbecco's modified Eagles's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin. Cells were plated at a density of 20,000/well and 15,000/well respectively, in flat-bottom 96-well plates. On the following day, diluted solutions of the test compound were prepared (1 mM stock) and added to the cells for 30-min incubation. PEG400:DMSO (55:45) concentration in the medium did not exceed 1%. The wells were washed two times with PBS and irradiated after 30-min of incubation using the LED light described above. Light dose was 1 J/cm². The medium was replaced with fresh one after irradiation and the plates were incubated for 24 h, at which time the cellular viability was assessed by resazurin method using a microplate spectrophotometer (Synergy HT Biotek).

Photodynamic therapy of female BALB/c mice bearing tumors was approved by the National Veterinary Authority (DGVA authorization no. 0420/000/000/2011). Mice weighing 18-20 g (Charles River Laboratories, Barcelona, Spain) were kept on a standard laboratory diet with free

access to drinking water. The tumor model was established taking up 350.000 CT26 cells (CRL-2638™, ATCC-LCG Standards, Barcelona, Spain) in 0.1 ml PBS and inoculated the cells subcutaneously in the right thigh of each mouse. The light source used for PDT *in vivo* was a custom-made diode laser, model LDM750.300.CWA.LM with controller 1201-08P and laser head 1201-08D (Omicron, Rodgau, Germany), coupled to an optic fiber with a fixed divergent lens, model FD with a diameter of 2 mm (Medlight, Ecublens, Switzerland).

Skin permeation was evaluated using a topical formulation containing benzyl alcohol (23%), Kolliphor EL (17%), Transcutol (50%) and water (10%). The photosensitizer added to this formulation corresponded to 1.85% of the mass before its addition, and the gelling agent (Aerosil 200) added corresponded to 5% of the mass before its addition. The photosensitizer was first dissolved in Transcutol and exposed to 3 min of vortex and 5 min of ultrasounds. A mixture of benzyl alcohol and Kolliphor EL was then added. Immediately after, it was mixed in an IKA MIXER at 200 rpm for 5 min and water was added drop-by-drop for 10 min. Finally, the gelling agent, Aerosil 200, was mixed in and the microemulsion gel was obtained. Skin permeation studies were performed in pig skin using samples collected from 5 months old pigs. The hair was removed as well as the underlying fatty layer, before the permeation studies.

C. Properties of the compounds

The absorptivities of the compounds were measured at several concentrations, in the μM range, and in all cases were observed to follow the Beer-Lambert law. Additionally, the wavelength of maximum absorption (λ_{max}) in the infrared did not vary in the concentration range studied. This is indicative of negligible aggregation between the molecules, which exist mostly as monomers at these concentrations in the solvents studied. Table 1 presents the infrared molar absorption coefficient (ϵ_{max}) and wavelength maximum in ethanol of a typical carboxamide halogenated bacteriochlorin derivative of Formula (I). The same table also presents triplet lifetimes (τ_{T}) in air and N_2 saturated solutions, fluorescence lifetime (τ_{F}), the quantum yields of fluorescence (Φ_{F}), triplet state formation (Φ_{T}), and singlet oxygen generation (Φ_{Δ}) in ethanol, and photodegradation quantum yield (Φ_{pd}) in methanol:PBS (3:2). Triplet decays were clearly mono-exponential and in air-saturated ethanol the triplet lifetimes were in the range of 200 to 300 nanoseconds. Such values are consistent with diffusion limited energy transfer from the triplet state of the photosensitizer to molecular oxygen through a charge-transfer interaction (8). The absorption intensity of the test compounds in the photobleaching studies followed a mono-exponential decrease as a function of the illumination time.

Table 1: Photophysical and photochemical properties of the carboxamide bacteriochlorin of formula **IX** in ethanol and photodecomposition quantum yield in methanol:PBS (3:2), together with photobiological properties in bacteria (*P. acnes* incubated with 2 μ M and irradiated with 10 J/cm²) and in cancer cell (A549 and CT26 irradiated with 1 J/cm²) cultures.

λ nm	$\varepsilon(\lambda) /$ 10^3 $M^{-1} cm^{-1}$	τ_S ns	Φ_F	τ_T (air) ns	$\tau_T(N_2)$ μs	Φ_Δ	Φ_T	Φ_{pd} $/ 10^{-4}$	Log P_{OW}	log CFU <i>P. Acnes</i>	IC50 / nM	
											A549	CT26
734	69	3.2	0.20	210	71 ± 5	0.38	0.45	1.5	2.9	9	2.5	5.8

The typical photophysical, photochemical and photobiological properties of carboxamide porphyrin derivatives, in particular bacteriochlorins or chlorins, of formula (I) remedy the shortcoming aforementioned of current photosensitizers employed in PDT. In particular, the molecules of Formula (I) can have low molecular weights and may attain high fluxes through biological membranes. The incubation times employed to obtain the phototoxicities illustrated in Table 1 were 30 minutes only, whereas incubation times of 18 h were used to obtain the IC50 values of porfimer sodium and temoporfin discussed above. Moreover the IC50 value of bacteriochlorins or chlorins of formula (I) can be several orders of magnitude lower than those of porfimer sodium or temoporfin, which means that the bacteriochlorins or chlorins of formula (I) attain the same phototoxicity as the clinically approved photosensitizers at orders of magnitude lower concentrations. This will allow such photosensitizers to reach the concentration required to elicit a therapeutic effect within a short period of contact with the protective biological barrier. Moreover, the carboxamide group introduces adequate amphiphilicity for biocompatibility and crossing of biological barriers, namely leading to values of log P_{OW} between 1 and 3. This substituent, together with the halogen atoms in the substituents in the *meso* positions also contributes to enhance the photostability of porphyrin derivatives of Formula (I), which is comparable to that of clinically approved photosensitizers.

The conjugation of photostability, strong absorption in the phototherapeutic window, high yield of ROS and amphiphilicity offers another advantageous technical characteristic to the porphyrin derivatives of formula (I): very high phototoxicity towards bacteria and cancer cells. Table 1 shows an example of a photosensitizer according to Formula (I) that incubated in a 2 μ M concentration with *P. acnes* colonies and illuminated with 10 J/cm² of light absorbed by its red absorption band reduces by 9 orders of magnitude the number of bacterial CFU. The phototoxicity against tumor

cells is equally impressive. The photosensitizer drug doses that kill more than 50% of a population of cancer cells *in vitro* (IC50) under a light dose of 1 J/cm² are below 10 nM.

The ability of porphyrin derivatives, in particular bacteriochlorin or chlorin, of Formula (I) to cross protective biological barriers and diffuse rapidly to they target, combined with their high phototoxicity when illuminated with light in the phototherapeutic window, make these bacteriochlorins or chlorins especially suitable for anti-cancerous and/or antimicrobial and/or antiviral and/or anti-fungi medications for human or animal usage exhibiting as a main active agent one or several porphyrin derivatives described in the present invention. This type of medication, used in particular in PDT, may also contain one or several pharmaceutically acceptable excipients. In PDT a pharmaceutical formulation containing one or several of the compounds described in the present invention is administered either topically, orally or systemically to the subject, and after some time (the drug-to-light interval), the target tissue is illuminated with light absorbed by porphyrin derivatives, preferably bacteriochlorins or chlorins. The percentage of photosensitizer present in the topical formulation may vary from 0.01% to 15%. The dose of light used to activate the photosensitizer applied topically may also vary, and doses between 1 and 100 J/cm² may be required. These light doses may be delivered with light sources that match the absorption band of the photosensitizer in the phototherapeutic window, provided that these light sources have irradiances below the onset of thermal effects, which is close to 250 mW/cm². Alternatively, the light doses can be given over a long period of time, including making use of solar exposure of the areas where the topical formulation was applied. The systemic administration of photosensitizers used in PDT is made using pharmaceutically acceptable carriers, to obtain photosensitizer doses ranging from 0.1 to 10 micromole/kg body mass. After a drug-to-light interval that may range from concomitant with the drug administration to 5 days after the administration, the light dose is delivered to the target. The reactive oxygen species generated by the illuminated photosensitizer molecules trigger a cascade of chemical and biological processes that culminate in the death of the cells and/or bacteria and/or virus.

The compounds of the present invention may also fluoresce with high quantum yields and in the phototherapeutic window. Table 1 presents an example of a photosensitizer with $\Phi_F=0.20$. This typical fluorescence can be used to detect the presence of the compound in the target tissue and offers the possibility of using the compounds of the present invention for the diagnosis of vascular or hyperproliferative disorders.

The compounds of the present invention also loose energy through radiationless processes with high quantum yields. Table 1 presents an example of a photosensitizer with $\Phi_F=0.20$ and $\Phi_T=0.45$

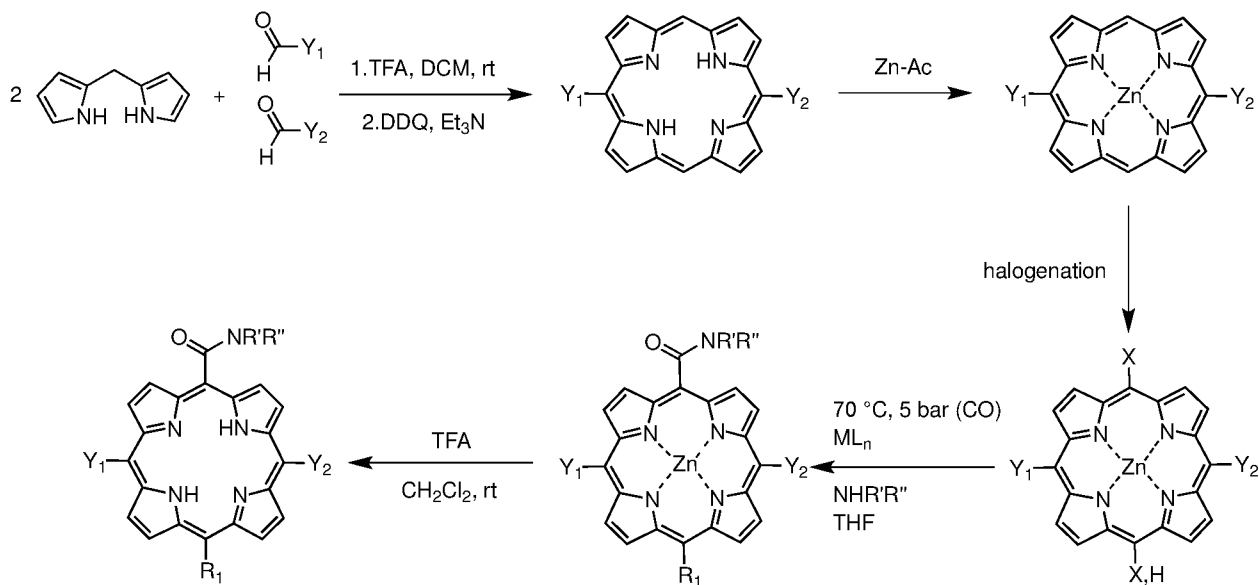
that must have an internal conversion quantum yield of $\Phi_{ic}=0.35$. The thermal energy lost in the 3.2 ns lifetime of the singlet state produces a fast thermoelastic expansion that launches an intense photoacoustic wave. Ultrasonic transducers can be used to detect photoacoustic waves, as described above for photoacoustic calorimetry. Alternatively, they can be detected by means of Photoacoustic Tomography and be used in the diagnosis of vascular or hyperproliferative disorders.

D. Description of methods of preparation of the compounds

Another aim of the present invention consists in the method of preparation of the derivatives described above.

Non-symmetric 5,15-disubstituted porphyrins were prepared with a modification of the method of condensation-cyclization of commercially available 2,2'-dipyrromethane with halogenated aldehydes using TFA as catalyst in DCM under inert atmosphere (10), followed by a step of oxidation of the porphyrinogen to the corresponding porphyrin with DDQ as oxidant. The next steps encompass the metalation of 5,15-disubstituted porphyrins with zinc acetate in DCM/methanol (1:1) solution (11), followed by mono or di-halogenation of 10 or 10,20-porphyrinic positions. The porphyrinate zinc(II) complex was brominated with NBS, chlorinated with *N*-chlorosuccinimide (NCS), iodinated with bis(trifluoroacetoxy)iodobenzene or 2,6-dichloro-1-fluoropyridinium triflate (9a). Although the aminocarbonylation reaction is a standard process for the preparation of carboxamides from aryl halides or aryl triflates and amines, the aminocarbonylation of porphyrins is uncommon (12). Carboxamide porphyrins were prepared by aminocarbonylation of the corresponding halogenated precursors, preferentially the brominated one, with methylamine at low pressures of carbon monoxide (1-10 bar) and temperature between 50-100 °C, in the presence of a base, and using a transition metal complex (ML_n) catalyst. The metal can be chosen from molybdenum, chromium, nickel or, preferentially, palladium. The ligands in the transition metal complex can be chosen from 1,2-bis(diphenylphosphino)ethane (DPPE), 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (Xantphos), 1,2-bis(diphenylphosphino)propane (DPPP), 1,2-bis(diphenylphosphino)butane (DPPB), 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (BINAP), bis-[2-(diphenylphosphino)phenyl] ether (DPEPhos), 2-di-*tert*-butylphosphino-2',4',6'-triisopropylbiphenyl (*t*-BuXpho), tri-*n*-alkylphosphine or, preferentially, triphenylphosphine (PPh₃). The base can be inorganic and selected from carbonates, phosphates or fluorinates. The base can also be organic and selected from amines, preferentially triethylamine. The solvent can be selected from toluene, dioxane, *N*-methyl-2-pyrrolidone (NMP), dimethylformamide (DMF), DCM

or THF. Alternative sources of CO may also be employed, namely $(M(CO)_n)$ where M is Mo or Co. Schematically, the preparation of the porphyrin precursors may be described as follows



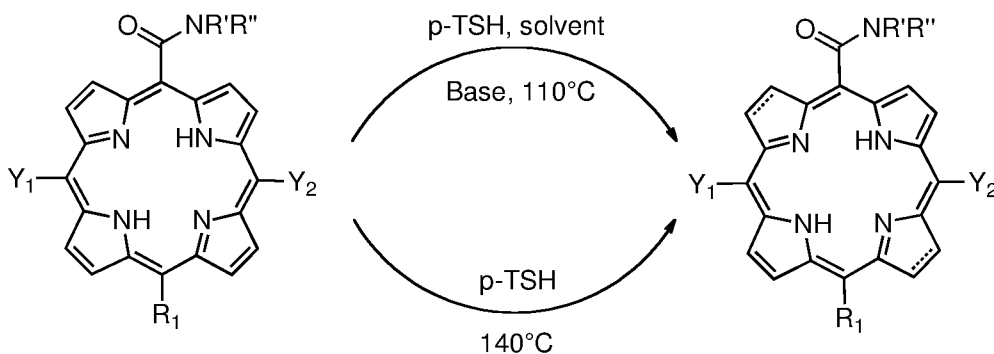
Wherein:

Y_1 , Y_2 are each independently chosen from hydrogen, halogenated alkyl or halogenated cycloalkyl with 6 or less carbon atoms, or halogenated phenyl where the halogens are independently chosen from F, Cl and Br, provided that at least one position of the alkyl, cycloalkyl or phenyl is halogenated, and provided that at least one of Y_1 , Y_2 is not hydrogen;

R_1 is chosen from H, F, Cl, Br or $-CONR'R''$;

R' and R'' are independently chosen from hydrogen, alkyl with 6 or less carbon atoms, cyclopropyl, cyclobutyl, cyclopentyl, alcohol, primary amine, secondary amine, tertiary amine, positively-charged quaternary amine, carboxylic acid, ether or ester.

The carboxamide halogenated porphyrin precursors are then used to obtain the corresponding reduced bacteriochlorins or chlorins. The reduction was based on the diimide reduction method using hydrazide as the hydrogen source, preferably using *p*-toluenesulfonyl hydrazide (p-TSH), inorganic or hindered organic bases, in solvents selected from DMF, toluene, xylene, pyridine and picoline, using a modification of the method disclosed in PCT/EP2005/012212. The reduction can also take place in the absence of solvents and in the absence of bases, using a modification of the method disclosed in PCT/PT2009/000057. The reduction of the carboxamide porphyrin to the corresponding bacteriochlorin or chlorin may be described as follows:



Where: ----- represents a carbon-carbon single bond or a carbon-carbon double bond, provided that at least one ----- represents a carbon-carbon single bond.

EXAMPLES

This invention will now be described in more detail in the following non-limiting EXAMPLES, with reference to the following drawings:

EXAMPLE 1. PROCEDURE FOR THE PREPARATION OF 5-METHYLCARBOXAMIDE-10,20-BIS-(2,6-DIFLUOROPHENYL)CHLORIN

The synthesis of 5-methylcarboxamide-10,20-*bis*-(2,6-difluorophenyl)chlorin was performed by the reaction of *p*-toluenesulphonyl hydrazide (504 ± 10 mg) with 5-methylcarboxamide-10,20-*bis*-(2,6-difluorophenyl)porphyrin (**VIII**) (100 ± 10 mg), potassium carbonate (374 ± 10 mg) and pyridine (15 mL) or alternatively without solvent under inert atmosphere and heating between 100 °C and 150 °C, for 2 hours. After cooling to room temperature, DCM (≈ 50 mL) was added and the organic layer washed with hydrochloric acid solution (0.1 M) (3 times) and water (3 times). The organic phase was dried with anhydrous sodium sulfate, filtered and then concentrated. The solid was dissolved in ethyl acetate (20 mL) and a solution of chloranil (0.6 equiv.) in ethyl acetate (5 mL) was added. The final solution was kept under stirring at 45 °C. The reaction was stopped when the UV-Vis absorption peak of bacteriochlorin (≈ 740 nm) had disappeared. The solvent was evaporated and the crude was dissolved in DCM (50 mL) and then washed with a saturated solution of sodium bicarbonate, with distilled water, and then dried over anhydrous sodium sulfate. The solvent was evaporated and purified by column chromatography with silica gel (DCM). The 5-methylcarboxamide-10,20-*bis*-(2,6-difluorophenyl)chlorin containing the two isomers (formula **Xa** and **Xb**), shown in Figure 2, was obtained with $80 \pm 5\%$ yield (80 ± 5 mg). The NMR and MS of the isolated product are as follows:

Formula **Xa**:

¹H-NMR (400 MHz, (CD₃)CO) δ(ppm): -1.89 (s, 1H, NH); -1.59 (s, 1H, NH); 3.34 (d, *J* = 4.6 Hz, 3H, CH₃); 4.33-4.37 (m, 2H, β-H); 4.76-4.80 (m, 2H, β-H); 7.49-7.55 (m, 4H, Ar-H); 7.89-7.99 (m, 2H, Ar-H); 8.29 (bs, 1H, NH) 8.44 (d, *J* = 4.4 Hz, 1H, β-H); 8.48 (d, *J* = 4.4 Hz, 1H, β-H); 8.82 (d, *J* = 4.2 Hz, 1H, β-H); 8.99 (d, *J* = 4.5 Hz, 1H, β-H); 9.06 (d, *J* = 4.2 Hz, 1H, β-H); 9.25 (s, 1H, *meso*-H); 9.27 (d, *J* = 4.5 Hz, 1H, β-H).

¹⁹F NMR: (376.5 MHz, (CD₃)₂CO) δ ppm: -110.47 (s, 2F, Ar-F); -111.53 (s, 2F, Ar-F).

MS ESI-FIA-TOF: Calculated for (C₃₄H₂₄F₄N₅O) [M+H]⁺: 594.1911, obtained [M+H]⁺: 594.1911.

Formula **Xb**:

¹H-NMR (400 MHz, (CD₃)CO) δ(ppm): -1.79 (s, 1H, NH); -1.63 (s, 1H, NH); 3.37 (d, *J* = 4.6 Hz, 3H, CH₃); 4.33-4.37 (m, 2H, β-H); 4.68-4.72 (m, 2H, β-H); 7.50-7.56 (m, 4H, Ar-H); 7.90-8.04 (m, 3H, Ar-H + NH); 8.46 (d, *J* = 4.4 Hz, 1H, β-H); 8.52 (d, *J* = 4.2 Hz, 1H, β-H); 8.83 (d, *J* = 4.6 Hz, 1H, β-H); 8.97 (d, *J* = 4.6 Hz, 1H, β-H); 9.05 (d, *J* = 4.3 Hz, 1H, β-H); 9.37 (d, *J* = 4.4 Hz, 1H, β-H); 10.05 (s, 1H, *meso*-H).

¹⁹F NMR: (376.5 MHz, (CD₃)₂CO) δ ppm: -110.47 (s, 2F, Ar-F); -111.52 (s, 2F, Ar-F).

MS ESI-FIA-TOF: Calculated for (C₃₄H₂₄F₄N₅O) [M+H]⁺: 594.1911, obtained [M+H]⁺: 594.1912.

EXAMPLE 2. PROCEDURES FOR THE PREPARATION OF 5-METHYLCARBOXAMIDE-10,20-BIS-(2,6-DIFLUOROPHENYL)BACTERIOCHLORIN

Solid state method: The synthesis of 5-methylcarboxamide-10,20-*bis*-(2,6-difluorophenyl)bacteriochlorin (**IX**) was performed by reaction of *p*-toluenesulphonyl hydrazide (2.52 ± 0.05 g) with 5-methylcarboxamide-10,20-*bis*-(2,6-difluorophenyl)porphyrin (0.2 ± 0.05 g), at pressure below 6x10⁻¹ mbar, under heating (140 ± 1 °C) for 60 minutes. After cooling to room temperature the reaction crude was dissolved and purified by chromatography. The 5-methylcarboxamide-10,20-*bis*-(2,6-difluorophenyl)bacteriochlorin was obtained with 80 ± 5% yield (160 ± 20 mg).

Solvent method: The synthesis of 5-methylcarboxamide-10,20-*bis*-(2,6-difluorophenyl)bacteriochlorin (**IX**) was performed by reaction of *p*-toluenesulphonyl hydrazide (12.5 ± 0.05 g) with 5-methylcarboxamide-10,20-*bis*-(2,6-difluorophenyl)porphyrin (1 ± 0.05 g), potassium carbonate (4.6 ± 0.05 g), 2-methylpyridine (20 mL) and toluene (40 mL) under inert atmosphere and heating (110±2 °C) for 3 hours. After cooling to room temperature, DCM (≈400 mL) was added and sequentially washed with a solution of hydrochloric acid (0.1 M) (3 times), water (3 times), sodium hydroxide (0.05 M) (3 times) and water (3 times). The organic phase was dried with anhydrous sodium sulfate, filtrated and then concentrated. The solvent was evaporated

and purified by chromatography. The 5-methylcarboxamide-10,20-*bis*-(2,6-difluorophenyl)bacteriochlorin was obtained with $75 \pm 5\%$ yield (750 ± 50 mg).

The absorption spectra of **IX** in ethanol and DMSO are presented in Figure 3. The NMR, MS and EA of the isolated product are as follows:

¹H-NMR (400 MHz, (CD₃)CO) δ (ppm): -1.52 (s, 1H, NH); -1.56 (s, 1H, NH); 3.30 (d, $J = 4.7$ Hz, 3H, CH₃); 4.11-4.16 (m, 4H, β -H); 4.43-4.45 (m, 2H, β -H); 4.53-4.57 (m, 2H, β -H); 7.44-7.48 (m, 4H, Ar-H); 7.81-7.90 (m, 3H, Ar-H + NH); 8.14-8.16 (m, 1H, β -H); 8.22-8.23 (m, 1H, β -H); 8.63-8.65 (m, 1H, β -H); 8.78-8.80 (m, 1H, β -H); 8.94 (s, 1H, *meso*-H).

¹⁹F NMR: (376.5 MHz, (CD₃)₂CO) δ ppm: -110.70 (s, 2F, Ar-F); -111.76 (s, 2F, Ar-F).

MS ESI-FIA-TOF: Calculated for (C₃₄H₂₆F₄N₅O) [M+H]⁺: 596.2066, obtained [M+H]⁺: 596.2057.

Elemental Analysis (C₃₄H₂₆F₄N₅O.½(H₂O)): calcd. C 67.37, H 4.33, N 11.58, found C 67.37, H 4.13, N 10.99.

EXAMPLE 3. PROCEDURE FOR THE PREPARATION OF 5-METHYLCARBOXAMIDE-10,20-BIS-(TRIFLUOROMETHYL)BACTERIOCHLORIN

The synthesis of 5-methylcarboxamide-10,20-*bis*-(trifluoromethyl)bacteriochlorin (Formula **XIV**) was performed, using the synthetic and purification conditions of the Solvent Method described in Example 2. The absorption spectrum of **XIV** in dichloromethane is presented in Figure 4. The NMR characterization of the isolated product is as follow:

¹H NMR: (400 MHz, CDCl₃) δ ppm : -1.00 (s, 1H, NH); -1.07 (s, 1H, NH); 3.30 (d, $J = 4.7$ Hz, 3H, CH₃); 4.44-4.48 (m, 2H, β -H); 4.52-4.55 (m, 2H, β -H); 4.61-4.65 (m, 2H, β -H); 8.05 (bs, 1H, -NH); 8.75-8.76 (m, 1H, β -H); 8.84-8.86 (m, 1H, β -H); 8.96 (bs, 2H, β -H + *meso*-H); 9.03-9.05 (m, 1H, β -H).

EXAMPLE 4. *IN VITRO* PHOTOTOXICITY TOWARDS *PROPIONIBACTERIUM ACNES* AFTER LIGHT IRRADIATION

This example describes the evaluation of *in vitro* phototoxicity of a carboxamide bacteriochlorin with formula **IX** after light irradiation, and their potential for PDT application in the treatment of acne vulgaris. The phototoxicity was measured according to the description in the Materials and Methods section. The *n*-octanol:PBS partition ratio of **IX** is $P_{OW} = 2.9 \pm 0.5$. An adequate formulation for this carboxamide bacteriochlorin is PEG400:DMSO (55:45). The phototoxicity of the test compound is proportional to the inhibition of *P. acnes* viability relative to the non-treated

control, and is represented in Figure 5 in the form of CFU reduction as a function of photosensitizer concentration for the light doses of 4 and 10 J/cm².

EXAMPLE 5. *IN VITRO* PHOTOTOXICITY TOWARDS A549 AND CT26 CANCER CELL LINES AFTER LIGHT IRRADIATION

This example describes the evaluation of *in vitro* phototoxicity of a carboxamide bacteriochlorin with formula **IX** after light irradiation, and their potential for PDT of cancer. The formulation employed was the same as that of Example 4. The incubation in the dark, for 30 min, of the test compound with the A549 or CT26 cell line showed no signs of toxicity up to 40 µM. The phototoxicity was measured according to the description in the Materials and Methods section. The phototoxicity of the test compound was assessed in terms of the percentage of survival of the cells for various concentrations of the test compound incubated for 30 min, followed by washings with PBS, addition of the culture medium and illumination with a light dose of 1 J/cm². Figure 6 shows that nearly all cells are killed when the concentration of the photosensitizer reaches 50 nM.

EXAMPLE 6. *IN VIVO* ANTITUMOR PDT EFFICACY AGAINST CT26 SUBCUTANEOUS TUMORS IMPLANTED IN BALB/C MICE

This example describes PDT of mice bearing CT26 subcutaneous tumors implanted in the right thigh. The tumors were treated with the carboxamide bacteriochlorin with formula **IX** using a vascular protocol when the largest diameter of the tumor reached 5 mm. The treatment protocol consisted in the intravenous injection of a defined dose of the photosensitizer with formula **IX** in a formulation composed of Kolliphor EL:ethanol:NaCl 0.9% (0.6:3:96.4, v/v/v), followed 15 minutes later by the illumination of the tumor with 749±3 nm laser light. The Materials and Methods section describes the animal model and the laser and optic fiber used in the treatments. The optic fiber was positioned perpendicularly to the tumor surface, in order to illuminate an area of 1.33 cm², concentric with the tumor, to deliver a light dose of 40 J/cm² with an irradiance of 130 mW/cm². The doses of the carboxamide bacteriochlorin with formula **IX** administered were calculated taking into account the purity of the sample. After PDT, the mice were followed to evaluate their response to the therapy until their tumor maximum diameter reached 15 mm. At this point (humanitarian endpoint) the animals were sacrificed. The efficacy results are presented as Kaplan-Meier survival curves in Figure 7. This example shows that the photosensitizers disclosed in this work are extremely phototoxic. Indeed, the photosensitizer dose of 0.3 mg/kg was so phototoxic that the animals died of acute response to the treatment less than 72 h post-PDT. The photosensitizer dose of 0.15 mg/kg used in another treatment group was very well tolerated. The local response in the

illuminated area, in the days following PDT, showed the edema and erythema related to the onset of the acute inflammatory response, accompanied by destruction of the tumor and formation of a necrotic scab. Once the necrotic scab was resolved, it was possible to see that the tumor had a complete regression and a 100% cure was achieved. On the other hand, the tumors grew continuously in the control (untreated group) and all the animals had to be sacrificed within 27 days of the tumor inoculation. The survival curve of the group treated with the 0.15 mg/kg photosensitizer dose is statistically different from that of the non-treated control group, which presents a median survival time of 19 days (Log-rank test, $p < 0.05$).

EXAMPLE 7. SKIN PERMEATION

This example describes the permeation of carboxamide bacteriochlorin with formula **IX** in pig skin. The topical formulation and the animal model were described in the Materials and Methods section. The permeation was assessed both in terms of the amount of photosensitizer in the skin after designated times of contact between the topical formulation and the skin (incubation time) and in terms of the depth of the permeation in the skin.

The assessment of the amount of carboxamide bacteriochlorin with formula **IX** in the skin after various incubation times involved the following steps: (i) application of 0.30 ml of formulation in 1 cm² areas of minipig skin to make 6 independent measurements for the incubation times of 30, 60 and 120 minutes; (ii) cleaning of the surface of the skin at the end of the incubation times; (iii) cutting the skin in small pieces and maceration in 2 ml of dichloromethane with the aid of dispersing machine; (iv) extraction with 10 ml of dichloromethane in a falcon tube for six hours; (v) dilution by a factor of 5 with ethanol. The volume of 200 μ l of the test solution was added in triplicate to 96 well plate and fluorescence intensity was detected using a Synergy HT microplate reader from Biotek (California, USA), with an excitation filter of 508/20 nm and emission filter of 760/35 nm, against a calibration curve. The signal of a blank obtained with the same cleaning method was subtracted from the signal of the sample and the concentration of the photosensitizer in each sample was obtained with the calibration curve. A summary of the results can be seen in Table 2. The flux of the photosensitizer to the skin attains $J_{\max} = 4 \times 10^{-8}$ mol/(cm² h) in 1.5 hours, which is surprisingly high for a photosensitizer with MW = 594 Da. This example shows that the low-molecular weight carboxamide derivatives of halogenated chlorins and bacteriochlorins disclosed in this work are especially capable of crossing biological barriers.

The assessment of the depth of the permeation of carboxamide bacteriochlorin with formula **IX** in the skin after various incubation times involved the following steps: (i) biopsies of tissues incubated with the formulation for 30, 60 and 120 minutes were collected frozen in dry ice; (ii) the frozen

tissues were mounted on a holder with Tissue-Tek O.C.T. Compound (Sakura Finetek Europe B.V., Zoeterwoude, Netherlands) and cut in slices with thicknesses of 25 μm in a cryostat; (iii) the skin slices were collected in microscope slides and kept refrigerated for microscopy. Confocal fluorescence of the bacteriochlorin was performed with a LSM 510 Meta (Carl Zeiss, Jena, Germany) confocal microscope, with a $\times 63$ oil immersion objective (Plan-Apochromat, 1.4 NA; Carl Zeiss), using $\lambda_{\text{ex}} = 514 \text{ nm}$, $\lambda_{\text{em}} \geq 650 \text{ nm}$, laser power at 5% and an amplification $40\times$. Images illustrating the fluorescence of the photosensitizer in the skin after the various incubation times are presented in Figure 8. The control experiments performed in the same conditions but without incubation of the formulation with the skin do not show any fluorescence and in the conditions of Figure 8 are completely black. Increasing the incubation time leads to a deeper penetration of the photosensitizer in the skin that may reach 40 μm depth in 90 minutes and cover most of the epidermis. This example shows that the low-molecular weight carboxamide derivatives of halogenated chlorins and bacteriochlorins disclosed in this work are especially capable of diffusing in biological tissues and rapidly reach their targets.

Table 2. Amount of carboxamide bacteriochlorin with formula **IX** in the skin after various incubation times.

Experiment	Incubation time (min)	Concentration (M)	Concentration average (M)	Mass ($\mu\text{g}/\text{cm}^2$)
#1	30	7.26E-07	9.09E-07	5.41 ± 1.33
#2	30	7.26E-07		
#3	30	4.97E-07		
#4	30	8.35E-07		
#5	30	6.68E-07		
#6	30	2.00E-06		
#1	60	1.06E-06	3.34E-06	19.9 ± 5.12
#2	60	2.73E-06		
#3	60	9.14E-07		
#4	60	4.20E-06		
#5	60	5.40E-06		
#6	60	5.73E-06		
#1	120	3.35E-06	6.26E-06	37.2 ± 7.45
#2	120	2.18E-06		
#3	120	6.16E-06		
#4	120	7.03E-06		
#5	120	8.53E-06		
#6	120	1.02E-05		

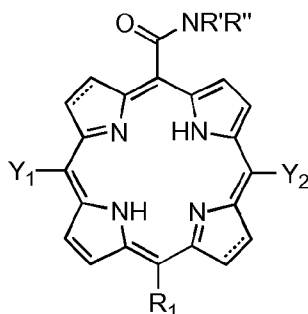
Naturally, the present embodiments are not in any way limited to the embodiments and examples described in this document and a person with average knowledge in the field will be able to predict many possible changes to it without deviating from the main idea, as described in the claims.

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CLAIMS

1. Carboxamide halogenated porphyrin derivatives, in particular bacteriochlorin or chlorin, of formula:



Formula (I)

wherein:

----- represents a carbon-carbon single bond or a carbon-carbon double bond, provided that at least one ----- represents a carbon-carbon single bond;

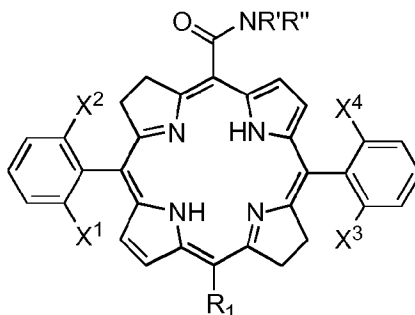
Y_1 , Y_2 are each independently chosen from hydrogen, halogenated alkyl or halogenated cycloalkyl with 6 or less carbon atoms, or halogenated phenyl where the halogens are independently chosen from F, Cl and Br, provided that at least one position of the alkyl, cycloalkyl or phenyl is halogenated, and provided that at least one of Y_1 , Y_2 is halogenated alkyl or halogenated cycloalkyl with 6 or less carbon atoms, or halogenated phenyl where the halogens are independently chosen from F, Cl and Br, provided that at least one position of the alkyl, cycloalkyl or phenyl is halogenated;

R_1 is chosen from H, I, Cl, Br or $-\text{CONR}'\text{R}''$;

R' and R'' are independently chosen from hydrogen, alkyl with 6 or less carbon atoms, cyclopropyl, cyclobutyl, cyclopentyl, alcohol, primary amine, secondary amine, tertiary amine, positively-charged quaternary amine, carboxylic acid, ether or ester;

or pharmaceutically acceptable salts thereof.

2. Carboxamide porphyrin derivatives according to claim 1, in particular bacteriochlorins, of formula:



Formula (II)

wherein:

X^1 , X^2 , X^3 , X^4 are each independently chosen from halogen (F, Cl, Br) and hydrogen atoms, provided that at least X^1 and X^3 are halogens;

R_1 is hydrogen;

R' and R'' are independently chosen from hydrogen, alkyl with 3 or less carbon atoms, cyclopropyl, alcohol, primary amine, secondary amine, tertiary amine, positively-charged quaternary amine, carboxylic acid, ether or ester;

or pharmaceutically acceptable salts thereof.

3. Carboxamide porphyrin derivatives according to claim 2, wherein in Formula (II):

X^1 and X^3 are fluorine atoms;

X^2 and X^4 are hydrogen atoms;

R_1 is hydrogen;

R' , R'' are independently chosen from hydrogen, alkyl with 3 or less carbon atoms.

4. Atropisomer of the carboxamide porphyrin derivatives according to claim 2, wherein in Formula (II):

X^1 and X^3 are fluorine atoms;

X^2 and X^4 are hydrogen atoms;

R_1 is hydrogen;

R' , R'' are independently chosen from hydrogen, alkyl with 3 or less carbon atoms;

where the fluorine atoms are on the same side of the plane defined by the macrocycle.

5. Atropisomer of the carboxamide porphyrin derivatives according to claim 2, wherein in Formula (II):

X^1 and X^3 are fluorine atoms;

X^2 and X^4 are hydrogen atoms;

R_1 is hydrogen;

R' , R'' are independently chosen from hydrogen, alkyl with 3 or less carbon atoms;

where the fluorine atoms are on opposite sides of the plane defined by the macrocycle.

6. Carboxamide porphyrin derivatives according to claim **2**, wherein in Formula (II):

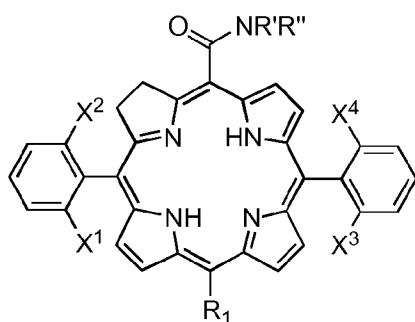
X^1 , X^2 , X^3 and X^4 are fluorine atoms;

R_1 is hydrogen;

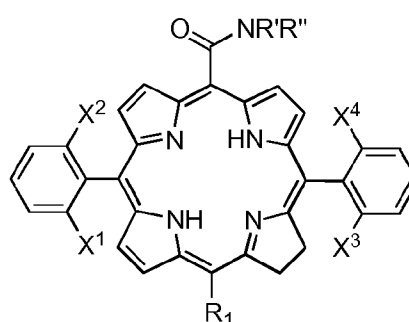
R' is hydrogen,

R'' is methyl.

7. Carboxamide porphyrin derivatives according to claim **1**, wherein the chlorins of formula:



Formula (IIIa)



Formula (IIIb)

wherein:

X^1 , X^2 , X^3 , X^4 are each independently chosen from halogen (F, Cl, Br) and hydrogen atoms, provided that at least X^1 and X^3 are halogens;

R_1 is hydrogen;

R' and R'' are independently chosen from hydrogen, alkyl with 3 or less carbon atoms, cyclopropyl, alcohol, primary amine, secondary amine, tertiary amine, positively-charged quaternary amine, carboxylic acid, ether or ester.

8. Carboxamide porphyrin derivatives according to claim **7**, wherein in Formula (IIIa) or Formula (IIIb):

X^1 and X^3 are fluorine atoms;

X^2 and X^4 are hydrogen atoms;

R_1 is hydrogen;

R' , R'' are independently chosen from hydrogen, alkyl with 3 or less carbon atoms.

9. Atropisomer of the carboxamide porphyrin derivatives according to claim 7, wherein in Formula (IIIa) or Formula (IIIb):

X^1 and X^3 are fluorine atoms;

X^2 and X^4 are hydrogen atoms;

R_1 is hydrogen;

R' , R'' are independently chosen from hydrogen, alkyl with 3 or less carbon atoms;

where the fluorine atoms are on the same side of the plane defined by the macrocycle.

10. Atropisomer of the carboxamide porphyrin derivatives according to claim 7, wherein in Formula (IIIa) or Formula (IIIb):

X^1 and X^3 are fluorine atoms;

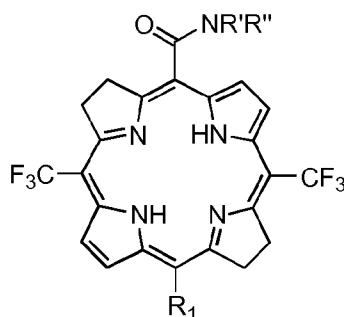
X^2 and X^4 are hydrogen atoms;

R_1 is hydrogen;

R' , R'' are independently chosen from hydrogen, alkyl with 3 or less carbon atoms;

where the fluorine atoms are on opposite sides of the plane defined by the macrocycle.

11. Carboxamide porphyrin derivatives according to claim 1, in particular bacteriochlorins, of formula:



Formula (XV)

wherein:

R_1 is chosen from H, Cl, Br or $-\text{CONR}'\text{R}''$;

R' and R'' are independently chosen from hydrogen, alkyl with 6 or less carbon atoms, cyclopropyl, cyclobutyl, cyclopentyl, alcohol, primary amine, secondary amine, tertiary amine, positively-charged quaternary amine, carboxylic acid, ether or ester;

or pharmaceutically acceptable salts thereof.

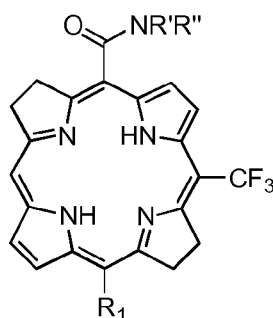
12. Carboxamide porphyrin derivatives according to claim 11, wherein in Formula (XV):

R₁ is hydrogen;

R' is hydrogen

R'' is methyl.

13. Carboxamide porphyrin derivatives according to claim **1**, in particular bacteriochlorins, of formula:



Formula (XVI)

wherein:

R₁ is chosen from H, Cl, Br or -CONR'R'';

R' and R'' are independently chosen from hydrogen, alkyl with 6 or less carbon atoms, cyclopropyl, cyclobutyl, cyclopentyl, alcohol, primary amine, secondary amine, tertiary amine, positively-charged quaternary amine, carboxylic acid, ether or ester;
or pharmaceutically acceptable salts thereof.

14. Carboxamide porphyrin derivatives according to claim **13**, wherein in Formula (XVI):

R₁ is hydrogen;

R' is hydrogen

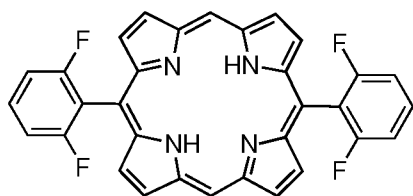
R'' is methyl.

15. Carboxamide porphyrin derivatives described in claims **1-14** for use in the treatment of hyperproliferative disorders including, but are not limited to, cancers or carcinomas, myelomas, psoriasis, macular degeneration.

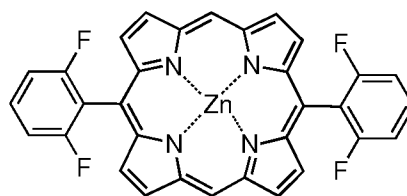
16. Carboxamide porphyrin derivatives described in claims **1-14** for use in the treatment of infectious diseases caused by microorganisms including, but not limited to virus, bacteria, rickettsia, mycoplasma, protozoa, fungi; or parasites including, but not limited to generally microscopic or very small multicellular invertebrates, or ova or juvenile forms thereof.

- 17.** Carboxamide porphyrin derivatives described in claims **1-14** for use in the treatment of cardiovascular diseases including, but not limited to stenosis, atherosclerotic plaques or heart arrhythmia.
- 18.** Carboxamide porphyrin derivatives described in claims **1-14** for cosmetic use in the treatment of skin pigmentation disorders and skin rejuvenation including, but not limited to hyperpigmentation, skin lightening, skin whitening, peeling.
- 19.** A pharmaceutically composition comprising at least one of the derivatives described in claims **1-14** and a pharmaceutically acceptable carrier.
- 20.** A pharmaceutically composition according to claim **19**, wherein the carrier transiently permeabilizes the skin and the pharmaceutical composition is permeable through the various skin layers.
- 21.** The pharmaceutically composition described in claims **19-20**, for use in intradermal and transdermal therapies.
- 22.** The pharmaceutically composition described in claims **19** for use in the treatment of hyperproliferative disorders and/or cancer and/or bacterial and/or viral and/or fungi infections and/or cardiovascular diseases and/or skin pigmentation disorders or skin rejuvenation.
- 23.** The pharmaceutically composition described in claim **19**, for use in theranostics of hyperproliferative disorders, wherein at least one of the derivatives described in claims **1-14** associates with a target tissue, is visualized by imaging technics and optionally through lighting said derivatives elicit the desired therapeutic effect in the target tissue.
- 24.** The pharmaceutically composition according to claim **19** wherein the imaging technics comprise Magnetic Resonance Imaging (MRI), exposing the derivatives described in claims **1-14** to light of sufficient energy to cause the same to fluoresce.
- 25.** The pharmaceutically composition according to claim **19** wherein the imaging technics comprise exposing the derivatives described in claims **1-12** to a light pulse of picosecond or nanosecond duration, of sufficient energy to cause the compound to launch a photoacoustic wave.

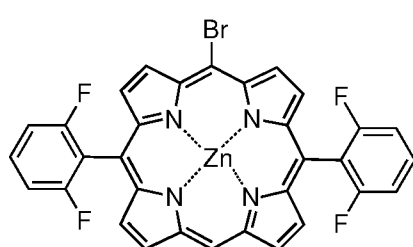
26. A kit comprising a pharmaceutical composition described in claim **19** and instructions for photodynamic therapy/administration of the composition to a subject.



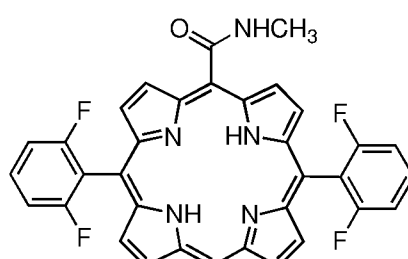
(V)



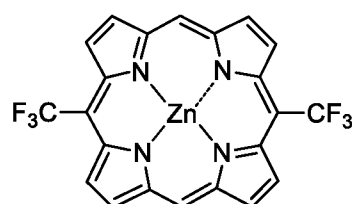
(VI)



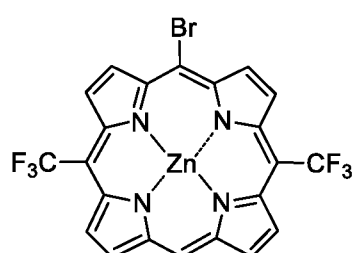
(VII)



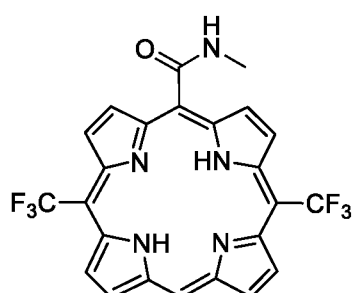
(VIII)



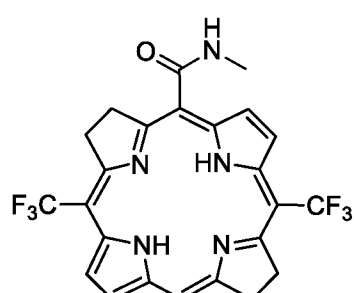
XI



XII

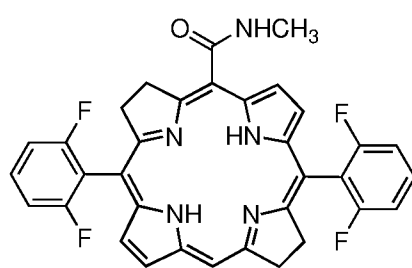


XIII

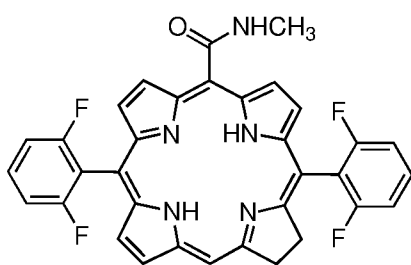


XIV

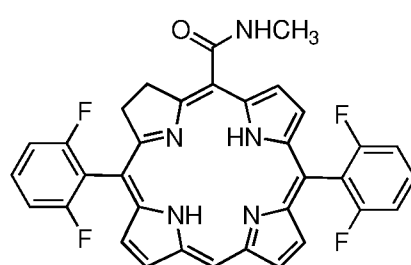
Figure 1



(IX)

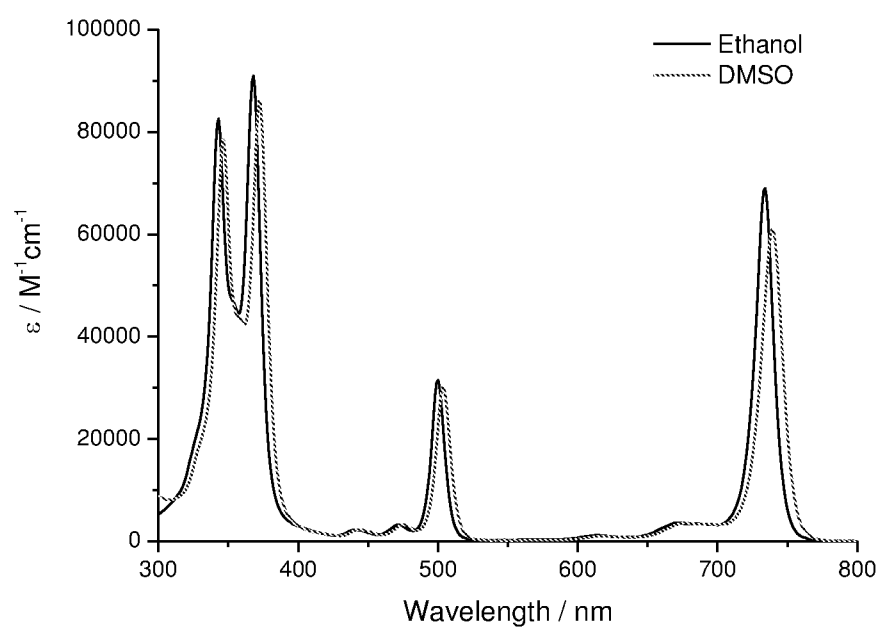


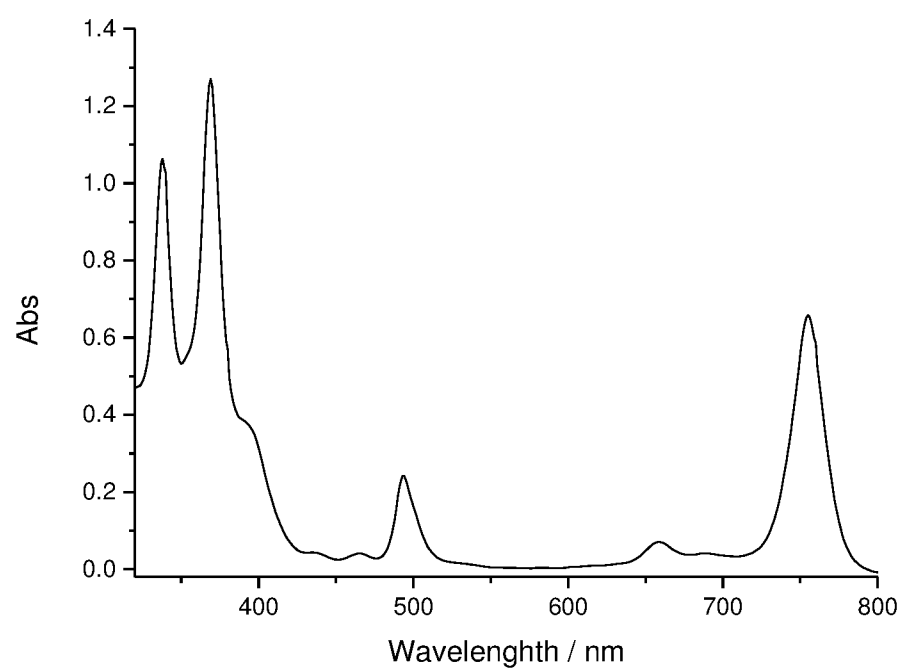
(Xa)

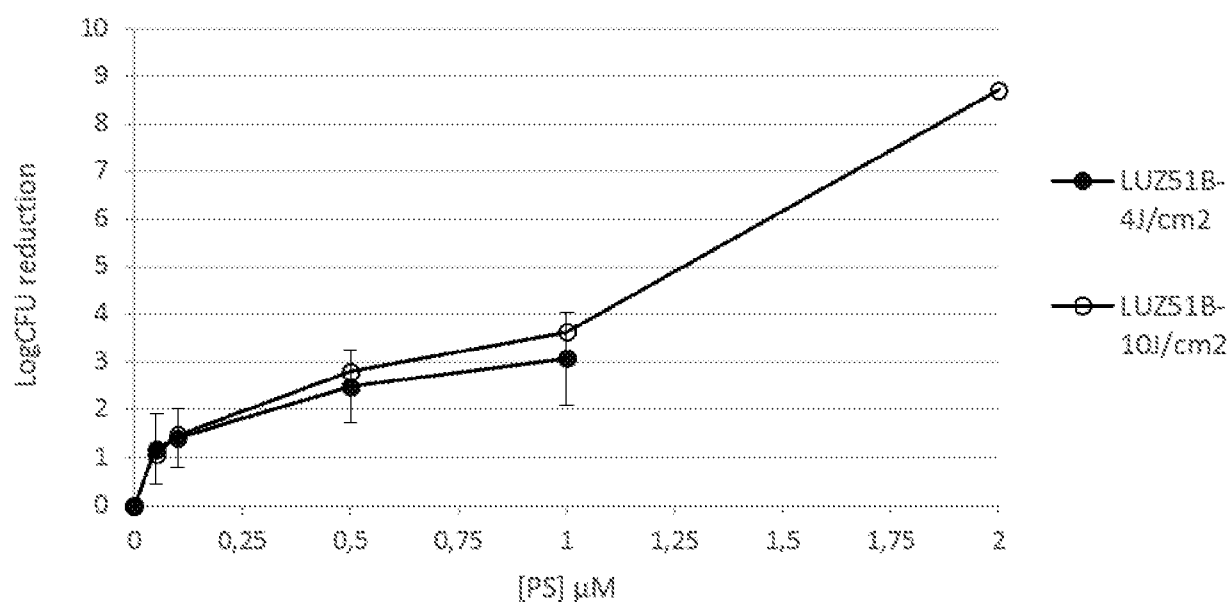


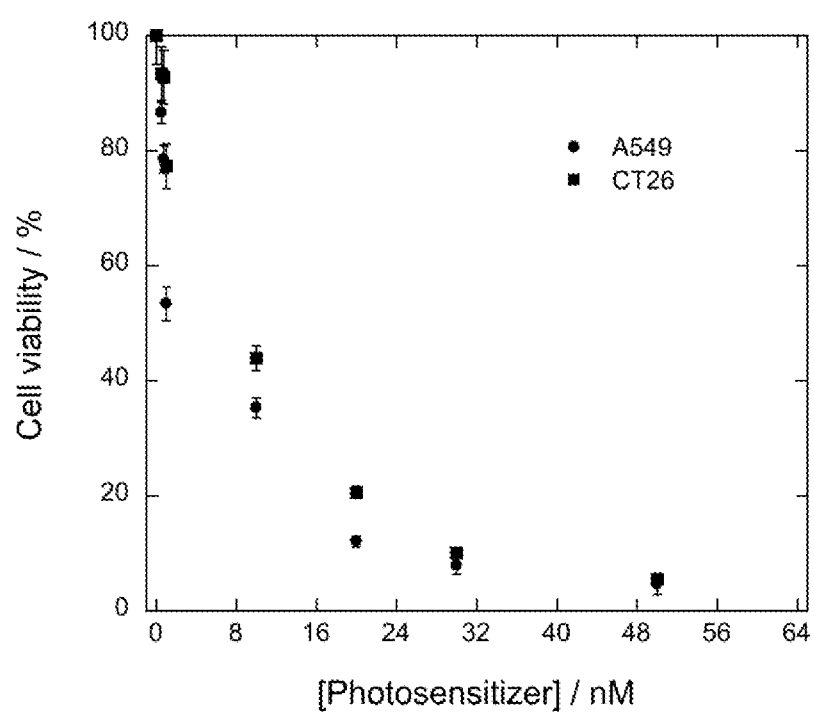
(Xb)

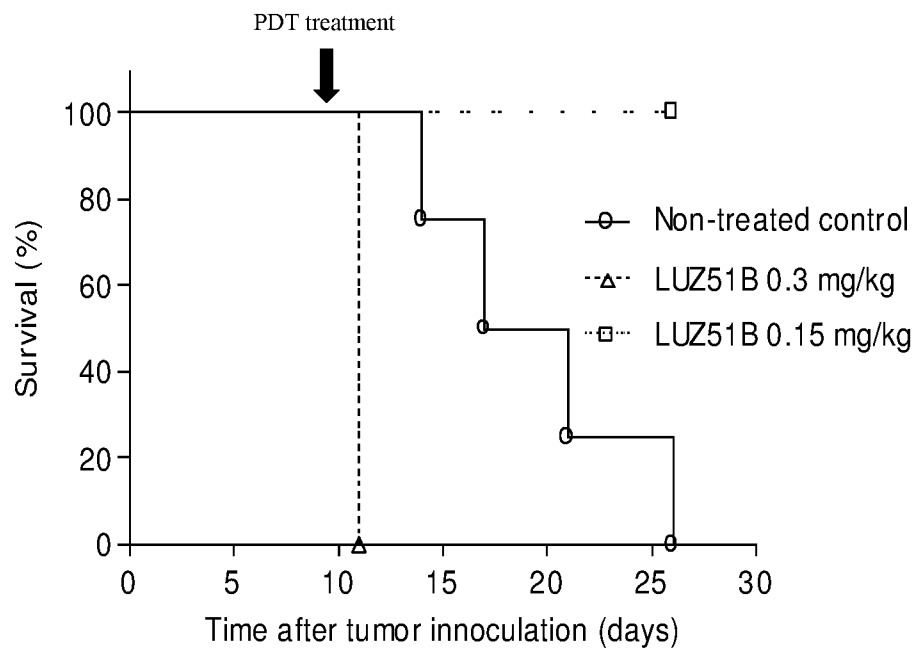
Figure 2

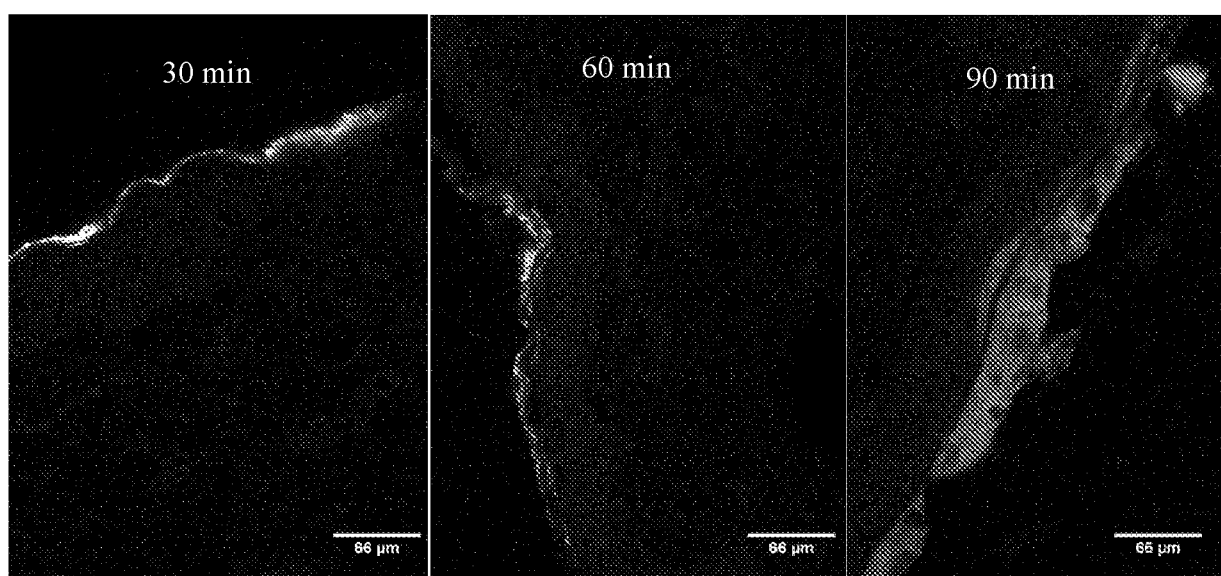
**Figure 3**

**Figure 4**

**Figure 5**

**Figure 6**

**Figure 7**

**Figure 8**

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2016/052606

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07D487/22 A61K31/409 A61P35/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07D

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, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2011/099602 A1 (UNIV NIHON [JP]; NAGASE HIROKI [JP]; TAKAHASHI MOTOICHIRO [JP]; ISHIBA) 18 August 2011 (2011-08-18) paragraph [0010] page 50; compounds (IV), (V) ----- -/--	1-26

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search

14 September 2016

Date of mailing of the international search report

20/09/2016

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
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Fax: (+31-70) 340-3016

Authorized officer

Koch, Kristian

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2016/052606

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TOSHIKATSU TAKANAMI ET AL: "An efficient one-pot procedure for asymmetric bifunctionalization of 5,15-disubstituted porphyrins: a simple preparation of mesoacyl-, alkoxycarbonyl-, and carbamoyl-substituted meso-formylporphyrins", CHEMICAL COMMUNICATIONS - CHEMCOM., no. 1, 1 January 2009 (2009-01-01), pages 101-103, XP055290147, ISSN: 1359-7345, DOI: 10.1039/B817551A first paragraph; page 101, column 1 tables 1-3	1-26
A,P	----- WO 2016/051361 A1 (BIOLITEC UNTERNEHMENS BETEILIGUNGS II AG [AT]; FREIE UNIVERSITÄT BERLIN) 7 April 2016 (2016-04-07) page 1, line 14 - line 17 examples 1-6 claims 1-75 -----	1-26

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2016/052606

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
W0 2011099602	A1	18-08-2011	NONE
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W0 2016051361	A1	07-04-2016	NONE
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L·G·博尔热斯 罗沙

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(51)Int.Cl.

C07D 487/22(2006.01)

A61K 31/409(2006.01)

A61P 9/10(2006.01)

A61P 35/00(2006.01)

A61P 17/00(2006.01)

A61P 31/04(2006.01)

A61P 31/12(2006.01)

A61P 31/10(2006.01)

权利要求书5页 说明书19页 附图5页

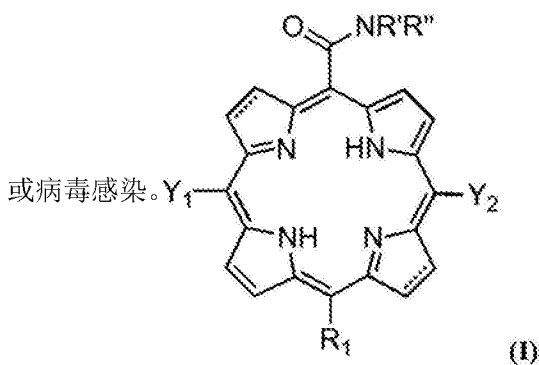
(54)发明名称

甲酰胺卤化的卟啉的低分子量衍生物、即二氢卟吩和菌绿素及其应用

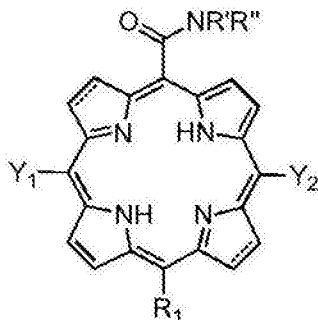
(57)摘要

本发明涉及下式的甲酰胺卤化的卟啉衍生物,尤其是菌绿素或二氢卟吩;式(I)其中:代表碳-碳单键或碳-碳双键,条件是至少一个代表碳-碳单键;Y₁,Y₂各自独立地选自氢,具有6个或更少碳原子的卤化的烷基或卤化的环烷基,或卤化的苯基,其中卤素独立地选自F、Cl和Br,条件是烷基、环烷基或苯基的至少一个位置是卤化的,并且条件是Y₁、Y₂中至少一个不是氢;R₁选自H,I,Cl,Br或-CONR'R",其中R'和R"独立地选自氢,具有6个或更少碳原子的烷基,环丙基,环丁基,环戊基,醇,伯胺,仲胺,叔胺,带正电的季胺,羧酸,醚或酯,或其药学上可接受的盐。本发明也涉及上述甲酰胺卤化的卟啉衍生物,尤其是式(I)菌绿素或二氢卟吩或其药学上可接受的盐在光动力疗法的用途,其中所述的衍生物能够检测

和展示过增殖障碍的存在,并且在适当光照存在下对其进行治疗。本发明也描述药物组合物包含一种或多种甲酰胺卤化的式(I)卟啉衍生物尤其是菌绿素或二氢卟吩或其药学上可接受的盐,用于治疗人或动物中的癌症和/或微生物感染和/



1. 甲酰胺卤化的卟啉衍生物, 尤其是菌绿素或二氢卟吩, 具有下式:



式(I)

其中:

~~~~~代表碳-碳单键或碳-碳双键, 条件是至少一个~~~~~代表碳-碳单键;

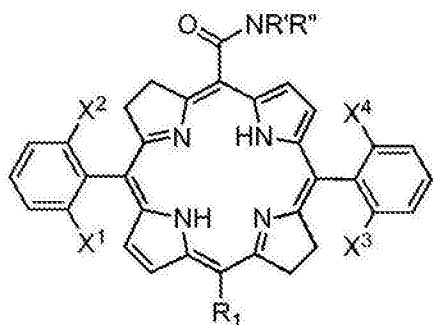
$Y_1, Y_2$ 各自独立地选自氢, 具有6个或更少碳原子的卤化的环烷基或卤化的烷基, 或卤化的苯基, 其中卤素独立地选自F、Cl和Br, 条件是烷基、环烷基或苯基的至少一个位置是卤化的, 并且条件是 $Y_1, Y_2$ 中至少一个是具有6个或更少碳原子的卤化的环烷基或卤化的烷基, 或卤化的苯基, 其中卤素独立地选自F、Cl和Br, 条件是烷基、环烷基或苯基的至少一个位置是卤化的;

$R_1$ 选自H, I, Cl, Br或 $-CONR'R''$ ;

$R'$ 和 $R''$ 独立地选自氢, 具有6个或更少碳原子的烷基, 环丙基, 环丁基, 环戊基, 醇, 伯胺, 仲胺, 叔胺, 带正电的季胺, 羧酸, 醚或酯;

或其药学上可接受的盐。

2. 根据权利要求1的甲酰胺卟啉衍生物, 尤其是菌绿素, 具有下式:



式(II)

其中:

$X^1, X^2, X^3, X^4$ 各自独立地选自卤素 (F, Cl, Br) 和氢原子, 条件是至少 $X^1$ 和 $X^3$ 是卤素;

$R_1$ 是氢;

$R'$ 和 $R''$ 独立地选自氢, 具有3个或更少碳原子的烷基, 环丙基, 醇, 伯胺, 仲胺, 叔胺, 带正电的季胺, 羧酸, 醚或酯;

或其药学上可接受的盐。

3. 根据权利要求2的甲酰胺卟啉衍生物, 其中在式(II)中:

$X^1$ 和 $X^3$ 是氟原子;

$X^2$ 和 $X^4$ 是氢原子;

R<sub>1</sub>是氢；

R', R''独立地选自氢,具有3个或更少碳原子的烷基。

4. 根据权利要求2的甲酰胺卟啉衍生物的阻转异构体,其中在式(II)中:

X<sup>1</sup>和X<sup>3</sup>是氟原子；

X<sup>2</sup>和X<sup>4</sup>是氢原子；

R<sub>1</sub>是氢；

R', R''独立地选自氢,具有3个或更少碳原子的烷基；

其中氟原子位于大环所限定平面的同侧。

5. 根据权利要求2的甲酰胺卟啉衍生物的阻转异构体,其中在式(II)中:

X<sup>1</sup>和X<sup>3</sup>是氟原子；

X<sup>2</sup>和X<sup>4</sup>是氢原子；

R<sub>1</sub>是氢；

R', R''独立地选自氢,具有3个或更少碳原子的烷基；

其中氟原子位于大环所限定平面的对侧。

6. 根据权利要求2的甲酰胺卟啉衍生物,其中在式(II)中:

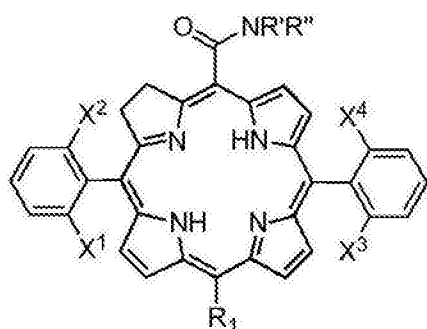
X<sup>1</sup>, X<sup>2</sup>, X<sup>3</sup>和X<sup>4</sup>是氟原子；

R<sub>1</sub>是氢；

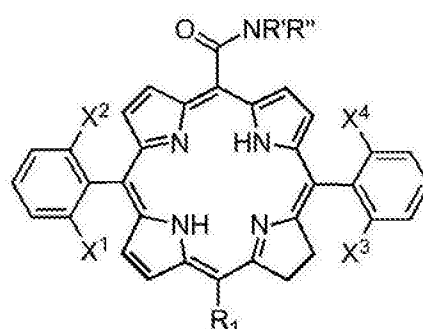
R' 是氢；

R'' 是甲基。

7. 根据权利要求1的甲酰胺卟啉衍生物,其中下式的二氢卟吩:



式(IIIa)



式(IIIb)

其中:

X<sup>1</sup>, X<sup>2</sup>, X<sup>3</sup>, X<sup>4</sup>各自独立地选自卤素(F, Cl, Br)和氢原子,条件是至少X<sup>1</sup>和X<sup>3</sup>是卤素；

R<sub>1</sub>是氢；

R' 和R''独立地选自氢,具有3个或更少碳原子的烷基,环丙基,醇,伯胺,仲胺,叔胺,带正电的季胺,羧酸,醚或酯。

8. 根据权利要求7的甲酰胺卟啉衍生物,其中在式(IIIa)或式(IIIb)中:

X<sup>1</sup>和X<sup>3</sup>是氟原子；

X<sup>2</sup>和X<sup>4</sup>是氢原子；

R<sub>1</sub>是氢；

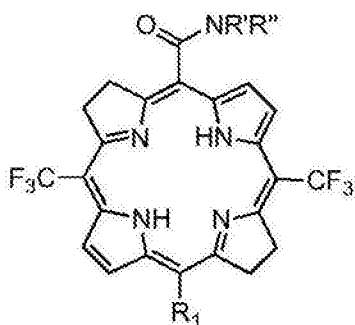
R', R''独立地选自氢,具有3个或更少碳原子的烷基。

9. 根据权利要求7的甲酰胺卟啉衍生物的阻转异构体, 其中在式 (IIIa) 或式 (IIIb) 中:  
 $X^1$  和  $X^3$  是氟原子;  
 $X^2$  和  $X^4$  是氢原子;  
 $R_1$  是氢;  
 $R'$ ,  $R''$  独立地选自氢, 具有3个或更少碳原子的烷基;  
 其中氟原子位于大环所限定平面的同侧。

10. 根据权利要求7的甲酰胺卟啉衍生物的阻转异构体, 其中在式 (IIIa) 或式 (IIIb) 中:

$X^1$  和  $X^3$  是氟原子;  
 $X^2$  和  $X^4$  是氢原子;  
 $R_1$  是氢;  
 $R'$ ,  $R''$  独立地选自氢, 具有3个或更少碳原子的烷基;  
 其中氟原子位于大环所限定平面的对侧。

11. 根据权利要求1的甲酰胺卟啉衍生物, 尤其是菌绿素, 具有下式:



式(XV)

其中:

$R_1$  选自 H, Cl, Br 或  $-CONR'R''$ ;

$R'$  和  $R''$  独立地选自氢, 具有6个或更少碳原子的烷基, 环丙基, 环丁基, 环戊基, 醇, 伯胺, 仲胺, 叔胺, 带正电的季胺, 羧酸, 醚或酯;

或其药学上可接受的盐。

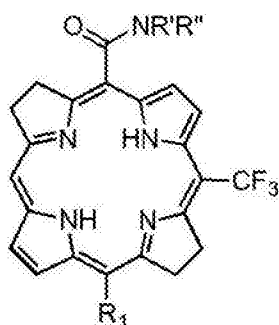
12. 根据权利要求11的甲酰胺卟啉衍生物, 其中在式 (XV) 中:

$R_1$  是氢;

$R'$  是氢;

$R''$  是甲基。

13. 根据权利要求1的甲酰胺卟啉衍生物, 尤其是菌绿素, 具有下式:



式(XVI)

其中:

$R_1$ 选自H, Cl, Br或 $-CONR'R''$ ;

$R'$ 和 $R''$ 独立地选自氢, 具有6个或更少碳原子的烷基, 环丙基, 环丁基, 环戊基, 醇, 伯胺, 仲胺, 叔胺, 带正电的季胺, 羧酸, 醚或酯;

或其药学上可接受的盐。

14. 根据权利要求13的甲酰胺卟啉衍生物, 其中在式(XVI)中:

$R_1$ 是氢;

$R'$ 是氢;

$R''$ 是甲基。

15. 根据权利要求1-14的甲酰胺卟啉衍生物, 用于治疗过增殖障碍, 包括但不限于癌症或肿瘤, 骨髓瘤, 牛皮癣, 黄斑变性。

16. 根据权利要求1-14的甲酰胺卟啉衍生物, 用于治疗由微生物或寄生物引起的传染病, 所述微生物包括但不限于病毒、细菌、立克次体、支原体、原生动、真菌; 而所述寄生物包括但不限于一般微小或很小的多细胞无脊椎动物或其卵或幼小形式。

17. 根据权利要求1-14的甲酰胺卟啉衍生物, 用于治疗心血管疾病, 包括但不限于狭窄, 动脉粥样硬化斑块或心脏心律不齐。

18. 根据权利要求1-14的甲酰胺卟啉衍生物, 用于处理皮肤色素沉着障碍和皮肤年轻化的美容用途, 包括但不限于色素沉着过度, 皮肤美白, 皮肤增白, 剥脱。

19. 药学组合物, 包含至少一种根据权利要求1-14的衍生物和药学上可接受的载体。

20. 根据权利要求19的药学组合物, 其中所述载体短暂地使皮肤透化并且药物组合物可渗透通过各皮肤层。

21. 根据权利要求19-20的药学组合物, 用于真皮内和透皮治疗。

22. 根据权利要求19的药学组合物, 用于治疗过增殖障碍和/或癌症和/或细菌感染和/或病毒感染和/或真菌感染和/或心血管疾病和/或皮肤色素沉着障碍或皮肤年轻化。

23. 根据权利要求19的药学组合物, 用于过增殖障碍的治疗诊断学, 其中与靶标组织结合的至少一种根据权利要求1-14的衍生物通过成像技术可视化和任选地通过光照所述衍生物在靶标组织中引起希望的治疗效果。

24. 根据权利要求19的药学组合物, 其中所述成像技术包括磁共振成像(MRI), 将根据权利要求1-14的衍生物暴露于光, 光的能量足以导致该衍生物发荧光。

25. 根据权利要求19的药学组合物, 其中所述成像技术包括将根据权利要求1-12的衍生物暴露于皮秒或纳秒持续时间的光脉冲, 其能量足以导致化合物发射光声波。

26. 一种试剂盒,包括权利要求19所描述的药物组合物和关于光动力疗法/将组合物给予受试者的指示书。

## 甲酰胺卤化的卟啉的低分子量衍生物、即二氢卟酚和菌绿素及其应用

### 技术领域

[0001] 本申请涉及新的低分子量的甲酰胺卤化的卟啉衍生物,即二氢卟酚(chlorin)和菌绿素(bacteriochlorin)衍生物,及其制备过程和光动力疗法中的用途。

[0002] 发明背景

[0003] 光动力疗法(PDT)是临床上批准的治疗,基于给予光敏化分子,其在靶标组织中蓄积,然后用光敏剂选择性吸收的光进行光照。该选择性用吸收光照治疗窗口中的光(650-850nm)的光敏剂来改善,在该窗口下组织具有更高的光穿透深度(例如 $\delta=2.3\text{mm}$ ,于750nm)(1)。吸收光使得光敏剂处于电子激发态,其通过电子转移反应与底物分子反应,形成超氧化物阴离子和羟基残基(类型I反应),或者将其能量转移至基态分子氧,产生单线态氧(类型II反应)。这些光产生的反应性氧类(ROS)触发生物学机理,其使得PDT成为有效的抗癌程序(2)。

[0004] PDT在治疗过增殖障碍中的范例已经是,在光照治疗窗口中具有更强光吸收和高ROS量子产率( $\Phi_{\text{ROS}}$ )的稳定染料应该是更佳的光敏剂。额外地,靶向革兰氏阴性细菌的PDT光敏剂开发指导是,在光敏剂中需要存在至少一个正电荷(3)。这种光敏剂通常是分子量高于600Da的卟啉衍生物(二氢卟酚或菌绿素)。尽管用微摩尔每升光敏剂浓度和约 $10\text{J}/\text{cm}^2$ 的光剂量实现细菌悬浮液的5-6个数量级的光动力灭活(4),其向临床应用的转用是并不成功的。将细菌悬浮液的光动力灭活转用于临床情况失败的可能原因是在吸收红外光的光敏剂大尺寸与在生物膜中快速扩散并被细胞摄取所需要的小分子尺寸之间的不相容性。相似困难已发现在将PDT光敏剂转用于皮肤病学障碍的局部治疗当中。虽然光敏剂比如卟吩姆钠(商品名**Photofrin®**)和替莫泊芬(**Foscan®**)已获批用于癌症适应症的静脉内给药,但是局部施用还并未转用于临床(5)。同样地,局部施用光敏剂以引起治疗效果的失败可能涉及所述光敏剂跨过皮肤外层(称为角质层)并到达靶标的困难。角质层是外源分子穿透皮肤的主要屏障。

[0005] 药物在局部施用之后跨过皮肤的最佳最大通量( $J_{\text{max}}$ )受到药物(6)分子量(MW)的强烈限制,

[0006]  $\log J_{\text{max}} = -3.90 - 0.0190\text{MW}$

[0007] 例如,MW=600或700Da的药物应分别具有 $J_{\text{max}}=5\times 10^{-16}$ 或 $6\times 10^{-18}\text{mol}/(\text{cm}^2\text{h})$ 。这些计算显示,高于600Da的分子量的适度增加能够导致光敏剂透皮通量的显著下降。在实际情况中,700Da的光敏剂可能需要比600Da的光敏剂更长100倍的时间来达到皮下靶标中的治疗浓度。期望用于局部施用必须符合的药物的又一关键特性是在角质层脂质区域中的适当溶解度从而允许扩散通过该区域,而同时仍具有足够的亲水性质从而允许分配入有活力的表皮组织。符合该决定因素的对数正辛醇-水分配系数( $\log P_{\text{OW}}$ )为1至3(7)。

[0008] 用于局部施用PDT和用于细菌光灭活的理想光敏剂必须具有MW<700Da的分子量,1至3的 $\log P_{\text{OW}}$ ,在650至850nm之间 $\epsilon>30,000\text{M}^{-1}\text{cm}^{-1}$ 的高摩尔吸收系数,和 $\Phi_{\text{ROS}}>0.3$ 的ROS量子产率。额外地,光敏剂的光稳定性也是PDT成功的关键(8)。光敏剂的光稳定性能够与化学催

化剂的翻转比拟:其与在催化剂(即光敏剂)灭活(即光分解)之前1摩尔催化剂能够转化的底物摩尔数(即产生的ROS数)有关。

[0009] 两种最广泛使用的癌症PDT光敏剂是卟吩姆钠(商品名**Photofrin®**)和替莫泊芬(专用名**Foscan®**)。卟吩姆钠是多至8个卟啉单元的醚和酯连接部分形成的低聚物混合物,在水溶液中相对可溶, $\log P_{OW} \approx 0$ 。卟吩姆钠不是单个分子个体并且并不具有特征分子量,但是最小二聚体的分子量超过1000Da。替莫泊芬是很亲油的5,10,15,20-四(间-羟基苯基)二氢卟吩,分子量680Da和在生理学pH的 $\log P_{OW} = 5.5$ 。卟吩姆钠和替莫泊芬的单线态氧量子产率分别是0.36和0.43(8)。它们在红色范围的最大吸收峰是,卟吩姆钠在 $\lambda_{max} = 630\text{nm}$ 的摩尔吸收系数 $\epsilon_{630} = 1170\text{M}^{-1}\text{cm}^{-1}$ ,和替莫泊芬在 $\lambda_{max} = 650\text{nm}$ 的摩尔吸收系数 $\epsilon_{650} = 29600\text{M}^{-1}\text{cm}^{-1}$ 。它们是相对光稳定的,卟吩姆钠和替莫泊芬的光分解量子产率分别是 $\Phi_{pd} = 5.5 \times 10^{-5}$ 和 $3.3 \times 10^{-5}$ 。在将卟吩姆钠或替莫泊芬与CT26(小鼠结肠腺癌)细胞温育、在洗涤之后用匹配其红色吸收带的波长、给出 $1\text{J}/\text{cm}^2$ 的光剂量的激光光照的情况下发现的是,需要 $18\mu\text{M}$ 的卟吩姆钠浓度(基于卟啉单元分子量估计)来灭除培养物中50%的细胞( $\text{IC}_{50} = 18\mu\text{M}$ ),而对于替莫泊芬在相同光剂量获得相同毒性所需的浓度是 $0.2\mu\text{M}$ ( $\text{IC}_{50} = 0.2\mu\text{M}$ )(8)。

[0010] 卟吩姆钠的特性不足以穿透生物学屏障即皮肤,原因是其过度高的分子量、亲水性和在光照治疗窗口中的中等光吸收。替莫泊芬部分解决了分子量问题,但是其对于透皮递送来说过于亲油并且光吸收恰好位于光照治疗窗口的极限。这些临床批准的光敏剂渗透生物学屏障即皮肤屏障的困难被加剧:在生物学靶标中这些光敏剂需要相对大的浓度才能获得PDT提供良好治疗结果所需的光毒性。

[0011] 在将光敏剂用于PDT的早先使用中并未认识到,对于PDT的光敏剂理想特性与用于局部施用的药物理想特性在单个分子中得以结合。快速扩散通过生物学屏障的能力是下述应用成功的关键,例如在治疗皮肤病学障碍中局部施用的光敏剂的真皮内或透皮递送,光敏剂在生物膜中穿透用于细菌光灭活,光敏剂扩散通过甲用于治疗真菌感染比如甲癣。快速扩散通过生物学屏障的能力也是光敏剂在其生物学靶标中快速蓄积的关键,比如渗透通过真核生物细胞外膜或细菌细胞膜。所述快速扩散缩短在给予光敏剂与光照靶标之间的时间,这有利于光动力疗法的许多应用,并且增加对靶标的光毒性。本领域技术人员不会期望的是:示于式(I)的卤化的卟啉衍生物(尤其是菌绿素和二氢卟吩)的至少一个meso位的甲酰胺基团能够有助于所述菌绿素或二氢卟吩衍生物的两亲性和光稳定性,而不损害ROS产生,并且在光敏剂分子量的上述小贡献的情况下其通过生物学屏障的扩散不会受到削弱。

[0012] 本发明首次公开用于过增殖障碍PDT的光敏剂和/或用于细菌或病毒或真菌光灭活的光敏剂,其符合理想光敏剂的全部标准并且有效地渗透生物学屏障。本发明也公开合成所述光敏剂的过程,通过借助实例说明这些光敏剂杀灭癌细胞和灭活细菌的用途。在本发明的又一实施方式中,本文描述的光敏剂用于过增殖组织的治疗诊断学。治疗诊断学是图像引导治疗的一种模式,其中相同化合物用来使得生物学靶标可视化并且获得所希望的治疗效果。

## 发明概要

[0013] 本发明的意图是提供新的甲酰胺卤化的卟啉衍生物,尤其是菌绿素和二氢卟吩,

其能够有效地用来杀灭细菌,甚至当存在于生物膜中时杀灭肿瘤细胞,甚至在局部施用时杀灭真菌和灭活病毒。鉴于目前PDT光敏剂在实现有效透皮递送或穿透生物膜方面的缺点,本发明公开新的卟啉衍生物,尤其是菌绿素和二氢卟酚,其将低分子量、在光照治疗窗口中的强吸收、高光稳定性、ROS光产生的高量子产率、适当两亲性和生物可相容性一起结合,并且其能够从低成本原料大量制备。

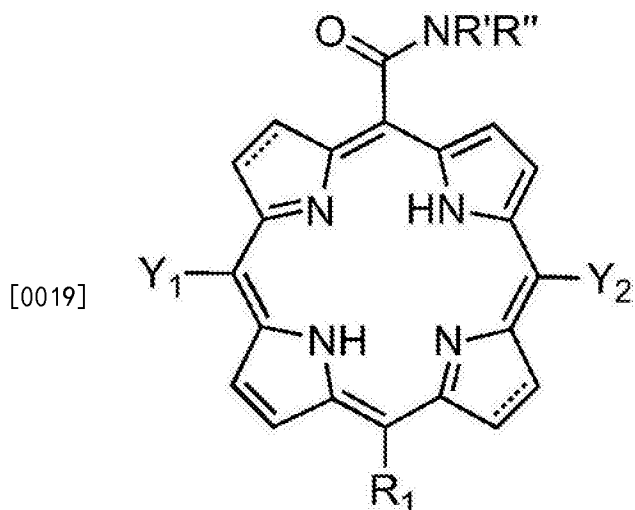
[0014] 本发明的又一目的是提供在光动力疗法中使用的药物,其中靶标选自:血管内皮组织,新血管系统组织,眼内新血管系统组织,肿瘤的异常血管壁,实体性肿瘤,皮肤肿瘤,头肿瘤,颈肿瘤,眼肿瘤,胃肠道肿瘤,肝肿瘤,乳腺肿瘤,前列腺肿瘤,肺肿瘤,非实体性肿瘤,造血组织和淋巴样组织之一的恶性细胞,血管系统中的创伤,患病骨髓,和患病细胞,其中所述疾病是自身免疫和炎症疾病之一。

[0015] 本发明的又一目的是提供药物,其用于下述治疗:皮肤病学障碍比如牛皮癣、寻常痤疮和酒渣鼻;妇科学障碍比如功能不良性子宫出血;泌尿学障碍比如性病湿疣病毒;心血管障碍比如再狭窄和动脉粥样硬化斑块;用于真菌感染比如甲癣的治疗;用于细菌或病毒的光动力破坏,包括对多种药物有抗性的细菌;用于毛发除去和化妆品;用于抑制器官或组织移植后的免疫应答。采用本文公开的光敏剂通过光动力疗法方法除去细胞的表面层刺激下层皮肤的新细胞生长,随后带来具有美容价值的皮肤外观改善。本发明的又一目的是提供用于心律不齐的药物,其在于通过局部光动力疗法选择性破坏细胞比如心肌细胞,并且恢复心脏的生理学节律。

[0016] 最终地,本发明的又一目的是提供用新的甲酰胺卟啉衍生物、尤其是菌绿素和二氢卟酚来诊断过增殖组织的方法。条件是这些化合物优先蓄积在所述组织中,出于诊断意图需要的额外特性是明确检出很小数量的所述化合物。这些化合物在红色和红外区域具有很明显的吸收带,组织在此范围是最为透明的。这些化合物的选择性激发导致明显荧光,其波长是生物学分子不会发出的。荧光检测能够用很敏感的设备进行,并且能够在生物学培养基中测量亚纳摩尔每升量的菌绿素和二氢卟酚。用于光诊断和光治疗的辐射源是不受限的,但优选激光束,原因是能够选择性应用希望波长范围的密集光线。光线需要具有足够强度以导致化合物发出用于诊断的荧光以及发挥用于治疗的细胞杀灭效果。额外地,在使用氟化的二氢卟酚或菌绿素的情况下,氟-MRI(磁共振成像)能够检测这些化合物在身体小区域中的蓄积和跟踪在其从身体清除时形成的代谢物。此外在脉冲激光用于激发的情况下,随后的无辐射衰减过程放热,其产生光声波,而这种波能够通过光声断层摄影检测,提供有关过增殖障碍诊断的进一步信息。

[0017] 在下文的描述中将出现其它目的和技术特征,其仅以示例方式提供而并不对其限制。

[0018] 本发明涉及下式的甲酰胺卟啉衍生物,尤其是菌绿素或二氢卟酚:



### 式(I)

[0020] 其中：

[0021] 代表碳-碳单键或碳-碳双键，条件是至少一个代表碳-碳单键；

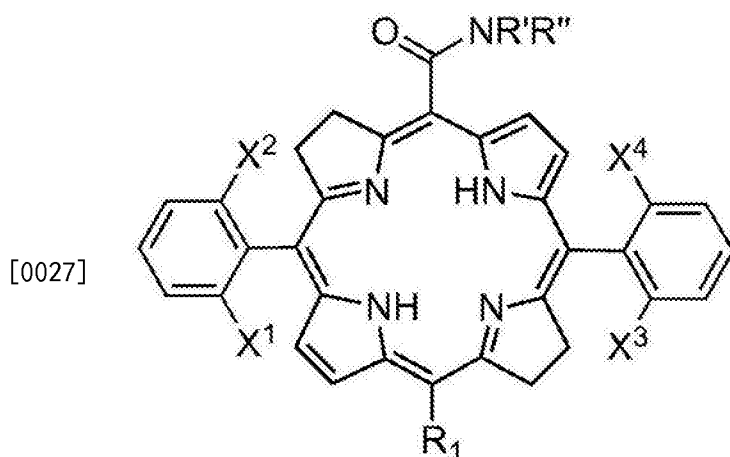
[0022]  $Y_1, Y_2$ 各自独立地选自氢，卤化的烷基或卤化的环烷基，其具有6个或更少碳原子，或者卤化的苯基，其中卤素独立地选自F、Cl和Br，条件是烷基、环烷基或苯基的至少一个位置是卤化的，并且条件是 $Y_1, Y_2$ 中至少一个不是氢，也即 $Y_1, Y_2$ 中至少一个是卤化的烷基或卤化的环烷基，其具有6个或更少碳原子，或者卤化的苯基，其中卤素独立地选自F、Cl和Br，条件是烷基、环烷基或苯基的至少一个位置是卤化的；

[0023]  $R_1$ 选自H, I, Cl, Br或 $-CONR'R''$ ；

[0024]  $R'$ 和 $R''$ 独立地选自氢，具有6个或更少碳原子的烷基，环丙基，环丁基，环戊基，醇，伯胺，仲胺，叔胺，带正电的季胺，羧酸，醚或酯；

[0025] 或其药学上可接受的盐。

[0026] 于是，式(I)化合物可以是下式的菌绿素



### 式(II)

[0028] 其中：

[0029]  $X^1, X^2, X^3, X^4$ 各自独立地选自卤素(F, Cl, Br)和氢原子，条件是至少 $X^1$ 和 $X^3$ 是卤素；

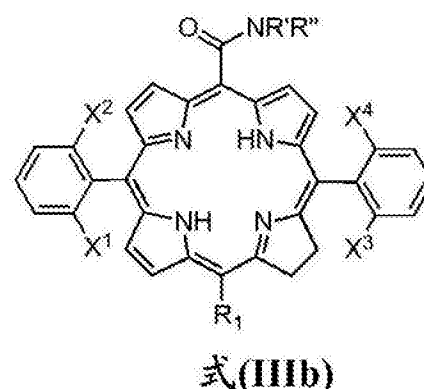
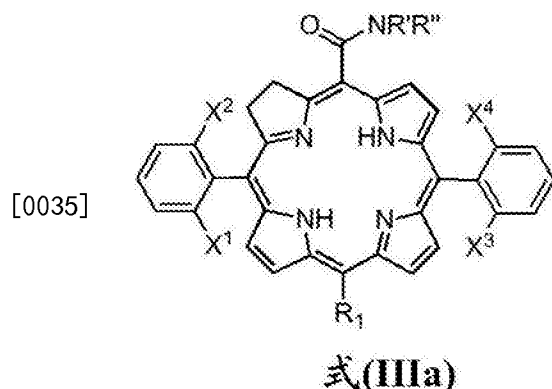
[0030]  $R_1$ 选自H, F, Cl, Br或 $-CONR'R''$ ；

[0031] R' 和R'' 独立地选自氢,具有6个或更少碳原子的烷基,环丙基,环丁基,环戊基,醇,伯胺,仲胺,叔胺,带正电的季胺,羧酸,醚或酯;

[0032] 或其药学上可接受的盐。

[0033] 本发明的特定优选化合物包括式(II)的甲酰胺菌绿素,其中X<sup>1</sup>,X<sup>2</sup>,X<sup>3</sup>,X<sup>4</sup>是氟原子,R<sub>1</sub>是氢,R' 是氢和R'' 是甲基。

[0034] 另选地,式(I)化合物可以是下式的二氢卟吩



[0036] 其中:

[0037] X<sup>1</sup>,X<sup>2</sup>,X<sup>3</sup>,X<sup>4</sup>各自独立地选自卤素(F,Cl,Br)和氢原子,条件是至少X<sup>1</sup>和X<sup>3</sup>是卤素;

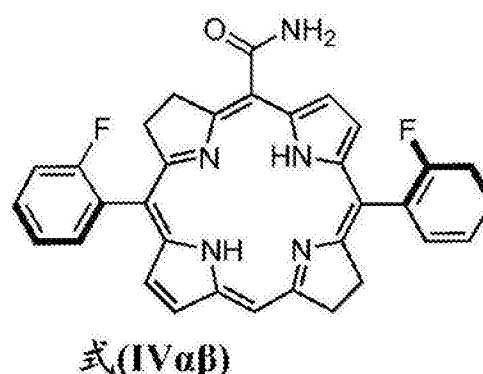
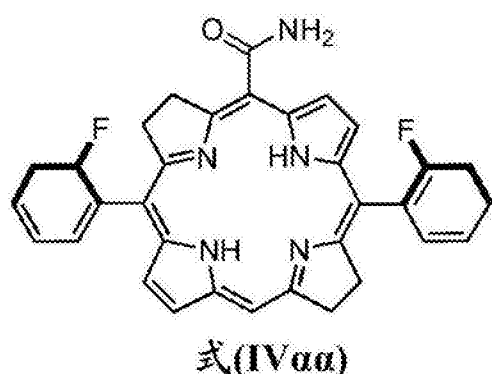
[0038] R<sub>1</sub>选自H,F,Cl,Br或-CONR' R'';

[0039] R' 和R'' 独立地选自氢,具有6个或更少碳原子的烷基,环丙基,环丁基,环戊基,醇,伯胺,仲胺,叔胺,带正电的季胺,羧酸,醚或酯;

[0040] 或其药学上可接受的盐。

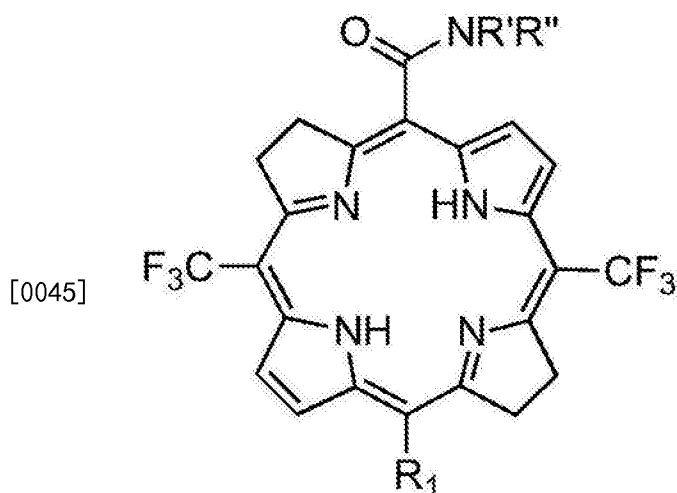
[0041] 在X<sup>1</sup>不同于X<sup>2</sup>和/或X<sup>3</sup>不同于X<sup>4</sup>的情况下,式(II)或(III)化合物具有阻转异构体,原因是围绕苯基-大环单键的受阻旋转。在这种情况下,阻转异构体能够通过在大环所限定平面的各侧更重原子的数量来区分。式IV说明菌绿素衍生物的两个阻转异构体,其区别是两个氟原子出现在大环平面的同侧(阻转异构体αα)或大环平面的异侧(阻转异构体αβ)

[0042]



[0043] 其中加粗线指出的是,加粗的原子和附着于此的基团是立体受限的,以便存在于大环所限定的平面之上。

[0044] 式(I)化合物还可以是下式的菌绿素



式(XV)

[0046] 其中：

[0047]  $R_1$ 选自H, I, Cl, Br或 $-\text{CONR}'\text{R}''$ ；

[0048]  $R'$ 和 $R''$ 独立地选自氢, 具有6个或更少碳原子的烷基, 环丙基, 环丁基, 环戊基, 醇, 伯胺, 仲胺, 叔胺, 带正电的季胺, 羧酸, 醚或酯；

[0049] 或其药学上可接受的盐。

[0050] 本发明的特定优选化合物包括式(XV)甲酰胺菌绿素, 其中 $R_1$ 是氢,  $R'$ 是氢和 $R''$ 是甲基。

[0051] 本发明也提供药物组合物, 包含有效量的本文描述的化合物和药学上可接受的载体。

[0052] 活性成分在本发明药物组合物中的真实剂量水平和给予时间过程可以变化, 以获得活性成分的量(该量有效实现特定患者的希望治疗应答)、组成和给药模式, 同时对患者没有毒性(或无法接受的毒性)。

[0053] 在使用中, 至少一种根据本发明的化合物在药物载体中以药学有效量给予有需要的受试者, 其通过静脉内、肌内、皮下、病灶内、或脑室内注射或通过口服给药或局部施用进行。按照本发明, 本发明化合物可以单独给予或与第二种不同的治疗剂给予。“与…结合”意指一起地, 基本上同时地或依次地。

[0054] “药学有效量”如本文所用意指本发明化合物的量, 其足够高以显著正面地改变所治疗的病症, 但是也足够低以避免严重副作用(合理的益处/风险比), 这属于合理的医学判断范围。本发明化合物的药学有效量随下述因素变化: 所需实现的具体目标, 所治疗患者的年龄和生理条件, 基础疾病的严重性, 治疗持续时间, 同时进行的治疗的性质和所用的特定化合物。比如说, 给予幼儿或新生儿的治療有效量的本发明化合物将按照合理的医学判断成比例地减少。本发明化合物的有效量从而是提供希望效果的最小量。额外地在光动力疗法的情况下, 药物组合物或化合物的“药学有效量”部分取决于其它因素比如光剂量和氧, 两者都需要用来实现治疗结果。从而, 在治疗受试者或患者的情况下还存在光以及氧的“有效量”。有助于确定药物、光和氧的“药学有效量”的其它重要因素包括药物-至-光照间隔(在给予药物与光照组织之间的时间)。药物-至-光照间隔是重要的, 原因是例如给予50mg/kg的更高药物剂量和在1周之后用光剂量500J/cm<sup>2</sup>光照组织可以与使用0.01mg/kg的药物

剂量和在给药10分钟之后用光剂量0.1J/cm<sup>2</sup>光照组织一样效率低下或没有效果。在药物-至-光照间隔增加(变得更长)的情况下,在给予药物与光照之间借助有机体的药物消除(代谢)可以降低治疗的有效性。然而,增加药物-至-光照间隔可以导致更有选择性的治疗和更少的不良作用。从而,出于至少上述的原因,药物-至-光照间隔是在确定本发明组合物的“药理学有效量”时考虑的重要因素。

[0055] 除了上文讨论的影响药物“有效量”确定的因素、光、氧和药物-至-光照间隔之外,本领域普通技术人员还会考虑光的注量率(每单位面积每单位时间递送的光子数)。注量率是重要的,原因是过快过多地递送光子可以耗尽组织中的氧并且使得治疗效率低下或无效。

[0056] 本发明的决定性实际优势是化合物可以以方便的方式给予,比如通过静脉内、肌内、皮下、口服、病灶内、或脑室内注射途径或通过局部施用,比如在霜剂或胶状物中局部使用来进行。取决于给药途径,包含本发明化合物的活性成分可以需要包覆在材料当中以使得化合物避免可以使得化合物灭活的酶、酸和其它天然条件的作用。为了不通过肠胃外给药来给予本发明化合物,化合物能够用材料包覆或与材料一起给予以预防灭活或改善溶解。

[0057] 本发明也涉及药学组合物,其包含至少一种式(I)衍生物或其药学上可接受的盐,和药学上可接受的载体,所述载体短暂地使皮肤透化、使得药物组合物可渗透通过各皮肤层。

[0058] 本发明的又一目的是如本文描述的化合物在制备药物中的用途,所述药物用于治疗本文描述的障碍或疾病。本发明的又一目的是如本文描述的化合物在治疗本文描述的障碍或疾病的用途。

[0059] 在本文变量的任何定义中化学基团列表的描述包括所述变量作为任何单个基团或所列基团的组合的定义。本文变量的实施方式的描述包括,该实施方式作为任何单个实施方式或与任何其它实施方式或其部分的组合。本文实施方式的描述包括,该实施方式作为任何单个实施方式或与任何其它实施方式或其部分的组合。

[0060] 本发明也涉及上述衍生物或其药学上可接受的盐和包含至少一种所述衍生物的药学组合物在治疗过增殖障碍和/或癌症和/或细菌和/或病毒或真菌感染中的用途。另外还涉及药学组合物在真皮内和透皮治疗中的用途。

[0061] 本发明也涉及包含至少一种上述衍生物或其药学上可接受的盐的药学组合物在过增殖障碍的治疗诊断学中的用途,其中与靶标组织结合的至少一种描述于权利要求1-9的衍生物通过成像技术可视化,并且任选地通过光照所述衍生物在靶标组织中引起希望的治疗效果。在成像技术包括磁共振成像(MRI)的情况下,将式(I)衍生物或其药学上可接受的盐暴露于光,所述光的能量足以导致其发荧光,或者包括将式(I)衍生物或其药学上可接受的盐暴露于皮秒或纳秒持续时间的光脉冲,所述光脉冲的能量足以导致化合物发射光声波。

[0062] 显著地,式(I)菌绿素或二氢卟吩的分子量的范围是425Da至700Da,它们是两性的,形成数个氢键,并且可以有效地扩散通过生物学屏障比如角质层或通过生物膜。这些衍生物技术特征之一在于其低分子量和其导致的通过保护性生物学屏障的增加的通量。又一技术优势是在光照治疗窗口中保藏菌绿素或二氢卟吩(已知强烈吸收光的大环),以及以高

量子产率产生ROS能力。还又一优势是存在增强还原的卟啉衍生物的光稳定性的取代基,以及在脂质区域中的溶解度和与生物学载体和药物载体的生物可相容性之间的平衡。式(I)菌绿素或二氢卟吩的又一技术特征是它们是显著荧光的、即荧光量子产率高于0.1,这允许它们在靶标中非侵入性造影。这种造影是希望的特性,原因是其使得靶标造影和选择开始治疗的最佳时机成为可能,例如在光敏剂靶标/周围组织比率较高的情况下。式(I)菌绿素或二氢卟吩的治疗诊断学用途还能够在其含有氟原子的情况下通过氟磁共振成像(MRI)加以利用,或者在其于脉冲激光下发射光声波的情况下通过光声断层摄影(PAT)加以利用。

[0063] 本文中“保护性生物学屏障”的含义应理解为分子和超分子种类在体内扩散的屏障比如皮肤和更特别是角质层,胃肠道屏障以及眼屏障,甲屏障,细胞和细菌外膜,以及微生物产生的生物膜。本领域技术人员无法预计甲酰胺卤化的卟啉衍生物尤其是菌绿素和二氢卟吩能够有效地渗透生物学膜和快速地形成对于所述生物学屏障保护的恶性细胞、细菌或真菌高光毒性的光敏剂。

## 附图说明

[0064] 并不是为了限制本文的公开,本申请展示所举例说明的实施方式的附图,以便于理解。

[0065] 图1:A)式(V),(VI),(VII)和(VIII)分子的结构。B)式(XI),(XII),(XIII)和(XIV)分子的结构。

[0066] 图2:式(IX),(Xa)和(Xb)分子的结构。

[0067] 图3:式(IX)分子在乙醇和DMSO中的吸收谱。

[0068] 图4:式(XIV)分子在二氯甲烷中的吸收谱。

[0069] 图5:在用 $4\text{ J/cm}^2$ 或用 $10\text{ J/cm}^2$ 光照之后,式(IX)分子对痤疮棒杆菌(*Propionibacterium acnes*)的光毒性。

[0070] 图6:在辐射( $1\text{ J/cm}^2$ )之后式(IX)分子对癌系A549和CT26的光毒性。

[0071] 图7.经皮下植入CT26肿瘤的小鼠的Kaplan-Meier生存曲线,其中实线代表未处理对照组,短划线代表用 $0.3\text{ mg/kg}$ 式(IX)分子处理的组而虚线代表用 $0.15\text{ mg/kg}$ 式(IX)分子处理的组。

[0072] 图8.与皮肤表面垂直切割的皮肤样品的共焦显微术,将其暴露于含有式(IX)分子的局部配制剂30、60和90分钟的温育时间,其中较亮区域揭示光敏剂在皮肤中的荧光。

## 具体实施方式

[0073] 参照附图,在此更加详细地描述任选实施方式,然而并不期望限制本申请的范围。

[0074] A.前体化合物

[0075] A.1.5,15-二-(2,6-二氟苯基)卟啉前体

[0076] 5,15-二-(2,6-二氟苯基)卟啉(图1式V)用5,15-二苯基卟啉(9a)制备方法的变型来制备。将可商购2,2'-二吡咯甲烷(dipyrromethane)和2,6-二氟苯甲醛的等摩尔混合物在三氟乙酸(TFA)存在下反应。在用2,3-二氯-5,6-二氧基-1,4-苯醌(DDQ)氧化之后,后处理并纯化,以35%收率获得V,在克天平上称量( $\sim 5\text{ g}$ )。V的表征如下: $^1\text{H NMR}$ : ( $400\text{ MHz}$ ,  $(\text{CDCl}_3)$ )  $\delta\text{ppm}$ :  $-3.05(\text{s}, 2\text{H}, -\text{NH})$ ;  $7.55-7.79(\text{t}, 4\text{H}, \text{Ar-H})$ ;  $7.98-8.05(\text{m}, 2\text{H}, \text{Ar-H})$ ;  $9.03(\text{d},$

$J=4.4\text{Hz}$ ,  $4\text{H}$ ,  $\beta\text{-H}$ ) ;  $9.41$  (d,  $J=4.5\text{Hz}$ ,  $4\text{H}$ ,  $\beta\text{-H}$ ) ;  $10.32$  (s,  $2\text{H}$ , meso-H) ; MS (ESI-FIA-TOF) :  $m/z$  计算值 ( $\text{C}_{32}\text{H}_{19}\text{F}_4\text{N}_4$ )  $[\text{M}+\text{H}]^+$  535.1540; 实测值: 535.1536  $[\text{M}+\text{H}]^+$ 。

[0077] [5,15-二-(2,6-二氟苯基)卟啉基]锌(II) (式VI) 制备如下: 在二氯甲烷: 甲醇 1:1 混合物中, 将 5,15-二-(2,6-二氟苯基)卟啉与乙酸锌 ( $\text{Zn}(\text{OAc})_2$ ) 复合。磁力搅拌加热溶液直至原料完全消耗。溶液用水洗涤, 用无水硫酸钠干燥, 过滤, 经由旋蒸浓缩; 获得 4.3g 的分离产品, 99% 收率。分离产品的 NMR 如下:  $^1\text{H}$  NMR: (400MHz,  $(\text{CDCl}_3)$   $\delta_{\text{ppm}}$ : 7.41-7.45 (t,  $4\text{H}$ , Ar-H) ; 7.81-7.86 (m,  $2\text{H}$ , Ar-H) ; 9.13 (d,  $J=4.3\text{Hz}$ ,  $4\text{H}$ ,  $\beta\text{-H}$ ) ; 9.50 (d,  $J=4.5\text{Hz}$ ,  $4\text{H}$ ,  $\beta\text{-H}$ ) ; 10.37 (s,  $2\text{H}$ , meso-H)。

[0078] [5-溴-10,20-二-(2,6-二氟苯基)卟啉基]锌(II) (式VII) 通过反应合成如下: 将 N-溴代琥珀酰亚胺 (NBS) ( $298\text{mg} \pm 10\text{mg}$ ) 溶于二氯甲烷 (DCM) ( $100 \pm 20\text{mL}$ ), 在  $-6^\circ\text{C}$  滴加至 1g 的 [5,15-二-(2,6-二氟苯基)卟啉基]锌(II) 在 DCM ( $400 \pm 50\text{mL}$ ) 和吡啶 ( $1.35 \pm 0.5\text{mL}$ ) 中的混合物。在 1 小时之后反应完成, 加水 ( $50 \pm 20\text{mL}$ ) 和有机层用 0.1M 盐酸溶液 (3 次) 和水 (3 次) 依次洗涤。蒸发溶剂和通过柱色谱法用硅胶 (DCM/己烷) 纯化。获得 [5-溴-10,20-二-(2,6-二氟苯基)卟啉基]锌(II),  $70 \pm 5\%$  收率 ( $790 \pm 40\text{mg}$ )。  $^1\text{H}$  NMR: (400MHz,  $(\text{CDCl}_3)$   $\delta_{\text{ppm}}$ : 7.34-7.38 (m,  $4\text{H}$ , Ar-H) ; 7.76-7.79 (m,  $2\text{H}$ , Ar-H) ; 8.83 (s,  $4\text{H}$ ,  $\beta\text{-H}$ ) ; 9.26 (d,  $J=4.5\text{Hz}$ ,  $2\text{H}$ ,  $\beta\text{-H}$ ) ; 9.70 (d,  $J=4.7\text{Hz}$ ,  $2\text{H}$ ,  $\beta\text{-H}$ ) ; 10.13 (s,  $1\text{H}$ , meso-H)。

[0079] 5-甲基酰胺-10,20-二-(2,6-二氟苯基)卟啉 (式VIII) 经由氨基羰基化反应合成。向含有搅拌子的高压釜钢反应器加入 4.0g ( $5.9\text{mmol}$ ) 的 [5-溴-10,20-二-(2,6-二氟苯基)卟啉基]锌(II), 66.0mg ( $0.3\text{mmol}$ ) 的乙酸钯 ( $\text{Pd}(\text{OAc})_2$ ), 155.0mg ( $0.6\text{mmol}$ ) 的三苯基膦, 0.8mL ( $5.9\text{mmol}$ ) 的三甲胺, 14.6mL ( $29.5\text{mmol}$ ) 的 2M 甲胺的四氢呋喃 (THF) 溶液, 和 60.0mL 无水 THF。封闭反应器, 充入 5 巴的一氧化碳。在  $70^\circ\text{C}$  搅拌混合物, 允许反应进行 15 小时。将反应混合物转移至圆底烧瓶, 在旋蒸仪中除去溶剂。将粗反应物溶于 DCM, 加入 TFA (10mL)。在室温下搅拌反应混合物 2 小时。后处理通过液-液萃取用饱和的碳酸氢钠溶液和蒸馏水进行。有机层用无水硫酸钠干燥, 过滤和最终在旋蒸仪中除去溶剂。在柱色谱法 (硅胶, DCM: 乙酸乙酯, 20:1) 之后获得 2.45g 的分离产品, 70% 收率。分离产品的 NMR 和 MS 如下:  $^1\text{H}$  NMR: (400MHz,  $(\text{CD}_3)_2\text{CO}$ )  $\delta_{\text{ppm}}$ : -3.14 (s,  $2\text{H}$ , -NH) ; 3.56 (d,  $J=4.2\text{Hz}$ ,  $3\text{H}$ , -CH<sub>3</sub>) ; 7.63 (t,  $J=8.2\text{Hz}$ ,  $4\text{H}$ , Ar-H) ; 8.04-8.11 (m,  $2\text{H}$ , Ar-H) ; 8.49 (bs,  $1\text{H}$ , -NH) ; 9.09 (m,  $4\text{H}$ ,  $\beta\text{-H}$ ) ; 9.63 (d,  $2\text{H}$ ,  $J=4.5\text{Hz}$ ,  $\beta\text{-H}$ ) , 9.56 (d,  $2\text{H}$ ,  $J=4.7\text{Hz}$ ,  $\beta\text{-H}$ ) ; 10.60 (s,  $1\text{H}$ , meso-H) 。 MS (ESI-FIA-TOF) :  $m/z$  计算值  $\text{C}_{34}\text{H}_{22}\text{F}_4\text{N}_5\text{O}$ : 592.1760; 实测值: 592.1751  $[\text{M}+\text{H}]^+$ 。

[0080] A2.5,15-二-(三氟甲基)卟啉前体

[0081] [5,15-二-(三氟甲基)卟啉基]锌(II) (图1式XI) 用预先描述的方法合成 (9b)。XI 的表征如下:  $^1\text{H}$  RMN: (400MHz, THF- $d_8$ )  $\delta_{\text{ppm}}$ : 9.67 (d,  $J=4.0$ ,  $\beta\text{-H}$ ) ; 9.88 (bs,  $\beta\text{-H}$ ) ; 10.57 (s,  $2\text{H}$ , meso-H)。

[0082] [5-溴-10,20-二-(三氟甲基)卟啉基]锌(II) (式XII) 通过反应合成如下: 将 N-溴代琥珀酰亚胺 (NBS) 溶于二氯甲烷, 在  $-6^\circ\text{C}$  滴加至 [5,15-二-(三氟甲基)卟啉基]锌(II) 在二氯甲烷和吡啶中的混合物。在 1h 之后, 反应完成。加水 and 有机层用 0.1M 盐酸溶液 (3 次) 和水 (3 次) 依次洗涤。蒸发溶剂至干, 将化合物用于后续反应步骤。

[0083] 5-甲基酰胺-10,20-二-(三氟甲基)卟啉 (式XIII) 经由氨基羰基化反应合成。向含有搅拌子的高压釜钢反应器加入 [5-溴-10,20-二-(三氟甲基)卟啉基]锌(II), 乙酸钯, 三

苯基膦,三乙胺,甲胺和无水THF。封闭反应器,充入压力多至10巴的一氧化碳。在70℃搅拌混合物,允许反应进行15小时。将反应混合物转移至圆底烧瓶,在旋蒸仪中除去溶剂。将粗反应物溶于二氯甲烷,加入三氟乙酸。在室温下在2小时期间搅拌反应混合物。后处理通过液-液萃取用饱和的碳酸氢钠溶液和蒸馏水进行。有机层用无水硫酸钠干燥,过滤和最终在旋蒸仪中除去溶剂。在柱色谱法(硅胶,二氯甲烷:己烷)之后,分离出产品。XIII的表征如下:<sup>1</sup>H RMN: (400MHz, THF-d<sub>8</sub>)  $\delta$ ppm: -2.84 (s, 2H, -NH); 3.56 (d, J=4.2Hz, 3H, -CH<sub>3</sub>); 8.72 (bs, 1H, -NH); 9.66-9.68 (m, 4H,  $\beta$ -H); 9.80 (bs, 4H,  $\beta$ -H); 10.62 (s, 1H, meso-H)。

#### [0084] B. 材料和方法

[0085] 元素分析在Leco TruSpec CHNS元素分析仪上进行。<sup>1</sup>H-NMR和<sup>19</sup>F-NMR和谱图在Bruker Avance 400MHz上记录。<sup>1</sup>H赋值用2D COSY和NOESY实验进行,ESI-FIA TOF高分辨率质谱数据用Micromass Autospec质谱仪获得。HPLC Shimadzu Prominence配有二极管阵列(模型SPD 20AV)。随后于743nm、23℃在半制备型柱Inertsil-苯基(250\*10mm;5 $\mu$ m)上分离。

[0086] 光吸收:UV-Vis-NIR光吸收用Agilent Cary5000UV-Vis-NIR分光光度计记录、确定摩尔吸收系数,另外用Shimadzu UV-2100光谱仪进行惯例测量。在300nm直至800nm的波长记录吸收谱。

[0087] 荧光发射:荧光发射谱图在自制设备中记录,所述设备由用来激发样品的Horiba-Jobin Fluoromax 4和其通过光纤连接的样品架构成。在样品架中,与激发纤维垂直地连接光纤以驱使发射光进入分光光度计检测器Avantes模型SensLine,提供AvaSoft 7.7.2。激发狭缝设置为2mm而积分时间是3s,平均数是3。从200nm直至1100nm收集谱图,使用1cm光路的标准池。获得荧光量子产率( $\Phi_F$ ):将样品的积分荧光与已知 $\Phi_F$ 的参比荧光化合物的积分荧光比较。

[0088] 荧光寿命:荧光寿命在自制设备中测定,所述设备由产生激发样品的光脉冲的LED,样品架,检测器和光学装置构成。激发波长设为373nm并且于737nm收集发射光。用1024通道,每通道28.5ps的时间尺度收集信号。

[0089] 瞬态吸收:三线态-三线态瞬态吸收在Applied Photophysics LKS.60激光快速光解光谱仪中记录,采用Hewlett-Packard Infinium示波器和Spectra-Physics Quanta-Ray GCR-130Nd:YAG激光作为激发源。脉冲激发设为355nm。

[0090] 单线态氧量子产率:实验在室温下用Nd-YAG激光Spectra-Physics Quanta-Ray GRC-130进行。在用600线分级的单色器选择波长之后,在于液氮室中冷却至193K,于355nm激发溶液,于1270nm Hamamatsu R5509-42光电倍增器中收集单线态氧磷光。非那烯酮用作单线态氧发生物质的参比。在发射中使用Newport滤器模型10LWF-1000-B以避免分散和荧光。

[0091] 光声量热法:在电子激发之后释放的热能通过时间分辨的光声量热法用正面辐射光声池和EKSPLA Nd:YAG泵送的EKSPLA OPO模型PG-122来测量。信号检测用2.25MHz Panametrics换能器进行。于690nm激发,将萘用作光声量热法参比。

[0092] 正辛醇:PBS分配比:用振摇-烧瓶方法的变型来确定光敏剂在等体积混合的正辛醇和磷酸缓冲盐水(PBS)中的平衡浓度,使用相同光敏剂的典型荧光带和校准曲线。

[0093] 光漂白实验在甲醇:PBS (3:2)和在有机溶剂中进行。溶液在池中用1cm光路辐射,使用Omicron Laserage的于749 $\pm$ 3nm发出的CW激光。总输出功率是212mW或244mW。对于各

化合物,以数分钟至数小时的辐射时间间隔收集吸收率。

[0094] 对细菌的光毒性:用适当波长的光对疮疱棒杆菌(ATCC®6919-Remel, Lenexa, KS, USA)进行体外评价。在加强的梭状芽胞杆菌培养基中(Oxoid, Basingstok, UK)在厌氧气氛下在37℃培养疮疱棒杆菌。厌氧生长条件用厌氧罐获得,其中具有产生厌氧条件的小袋(Anaerocult A, Merck, Darmstadt, 德国)。疮疱棒杆菌悬浮液用培养基稀释至光密度于620nm为1.3,相应于大约 $1 \times 10^7$  CFU/ml(菌落形成单元每毫升)。稀释的悬浮液于13000rpm离心10分钟,再悬浮于PBS。将试验化合物储备溶液溶于PEG400:DMSO(丙二醇400:二甲亚砷)(55:45),用PBS稀释至适当浓度。试验化合物与疮疱棒杆菌的温育在平底透明的DB Falcon黑色96-孔板(Franklin湖泊, NJ, USA)、避光、在30分钟期间进行。在温育时间段之后,用LED灯辐射该板,该灯来自Marubeni(模型L740-66-60-550),于740nm的最大发射为FWHM=25nm,其适于激发菌绿素,总光剂量为4或10 J/cm<sup>2</sup>。在辐射之后,将各孔的内容物于13000rpm离心10分钟和再悬浮于培养基。在厌氧气氛下,将具有疮疱棒杆菌的板在37℃温育24小时。在温育时间段之后,评价疮疱棒杆菌的存活率。板孔中的细菌悬浮液用培养基稀释,并接种在具有加强的梭状芽胞杆菌琼脂的培养皿中(Oxoid, Basingstok, UK),用于后续CFU计数。在厌氧气氛下,将培养皿在37℃温育至少72小时。

[0095] 对癌细胞系的光毒性:用A549(人类肺腺癌)和CT26(小鼠结肠腺癌)细胞系进行体外评价。细胞在补充有10%热灭活胎牛血清和1%青霉素的Dulbecco修饰Eagles培养基中培养(DMEM)。细胞分别以20,000/孔和15,000/孔的密度在平底96-孔板中铺板。在后续一天,制备试验化合物的稀释溶液(1mM储备)并加至细胞进行30-min温育。PEG400:DMSO(55:45)的浓度在培养基中不超过1%。各孔用PBS洗涤2次,在30-min温育之后用所述LED灯辐射。光剂量是1 J/cm<sup>2</sup>。在辐射之后将培养基用新鲜培养基替换,将板温育24小时,在此时通过刃天青方法用微量培养板分光光度计(Synergy HT Biotek)评价细胞存活率。

[0096] 光动力疗法:携带肿瘤的雌性BALB/c小鼠的疗法由National Veterinary Authority批准(DGVA批号0420/000/000/2011)。将重18-20g的小鼠(Charles River实验室,巴塞罗那,西班牙)保持在标准实验室膳食,自由获取饮用水。肿瘤模型建立如下:在0.1ml PBS中取350,000 CT26细胞(CRL-2638™, ATCC-LCG标准, 巴塞罗那, 西班牙),在各小鼠的右股经皮下接种细胞。用于体内PDT的光源是模型LDM750.300.CWA.L.M的定制二极管激光,控制器1201-08P和激光头1201-08D(Omicron, Rodgau, 德国),其偶联至具有固定发散透镜的模型FD光纤,直径2mm(Medlight, Ecublens, 瑞士)。

[0097] 皮肤渗透:用含有苯甲醇(23%), Kolliphor EL(17%), Transcutol(50%)和水(10%)的局部配制剂评价。加至该配制剂的光敏剂对应于1.85%的加入前质量,而加入的胶凝剂(二氧化硅气凝胶200)对应于5%的加入前质量。将光敏剂首先溶于Transcutol并暴露至涡旋器3min和超声5分钟。然后加入苯甲醇和Kolliphor EL的混合物。此后立即将其在IKA搅拌器中于200rpm混合5分钟,滴加水10分钟。最终混入胶凝剂二氧化硅气凝胶200,获得微乳剂凝胶。皮肤渗透研究用从5月龄猪收集的样品在猪皮肤中进行。在渗透研究之前,除去毛发以及底部脂肪层。

[0098] C. 化合物的特性

[0099] 化合物的吸收性在μM范围的数个浓度测量,并且在全部情况中都观察到符合比尔-朗伯定律。额外地,红外最大吸收波长( $\lambda_{\max}$ )在研究的浓度范围内不变。这指示可忽视的

分子间团聚,其在所研究的溶剂中在这些浓度下大部分作为单体存在。表1代表典型的式(I)甲酰胺卤化的菌绿素衍生物在乙醇中的红外摩尔吸收系数( $\epsilon_{\max}$ )和最大波长。该表也提供在空气和 $N_2$ 饱和溶液中的三线态寿命( $\tau_T$ ),荧光寿命( $\tau_S$ ),荧光量子产率( $\Phi_F$ ),三线态形成量子产率( $\Phi_T$ ),和乙醇中的单线态氧产生量子产率( $\Phi_\Delta$ ),和甲醇:PBS(3:2)中的光降解量子产率( $\Phi_{pd}$ )。三线态衰减明显是单指数的而在空气饱和的乙醇中三线态寿命的范围是200至300纳秒。所述值符合从光敏剂三线态向分子氧通过电荷-转移相互作用的扩散限制能量转移(8)。试验化合物在光漂白研究中的吸收强度符合单指数的降低,其是光照时间的函数。

[0100] 表1:式IX甲酰胺菌绿素在乙醇中的光物理和光化学特性和在甲醇:PBS(3:2)中光分解量子产率,以及在细菌中(疮疱棒杆菌,与2 $\mu$ M温育和用10J/cm<sup>2</sup>辐射)和在癌细胞(A549和CT26,用1J/cm<sup>2</sup>辐射)培养物中的光生物学特性。

[0101]

| $\lambda$<br>nm | $\epsilon(\lambda)/10^3$<br>M <sup>-1</sup> cm <sup>-1</sup> | $\tau_S$<br>ns | $\Phi_F$ | $\tau_T$<br>(空<br>气)<br>ns | $\tau_T(N_2)$<br>$\mu$ s | $\Phi_\Delta$ | $\Phi_T$ | $\Phi_{pd}$<br>/10 <sup>-4</sup> | Log<br>Pow | log<br>CFU<br>疮疱<br>棒杆<br>菌 | IC50 / nM |      |
|-----------------|--------------------------------------------------------------|----------------|----------|----------------------------|--------------------------|---------------|----------|----------------------------------|------------|-----------------------------|-----------|------|
|                 |                                                              |                |          |                            |                          |               |          |                                  |            |                             | A549      | CT26 |
| 734             | 69                                                           | 3.2            | 0.20     | 210                        | 71 $\pm$ 5               | 0.38          | 0.45     | 1.5                              | 2.9        | 9                           | 2.5       | 5.8  |

[0102] 式(I)甲酰胺卟啉衍生物尤其是菌绿素或二氢卟吩的典型光物理、光化学和光生物学特性弥补了前文所述的PDT中所用的现有光敏剂的缺点。尤其是,式(I)分子能够具有低分子量并且可以获得经过生物学膜的高通量。用于获得表1所述的光毒性的温育时间是仅30分钟,而为了获得上文讨论的卟吩姆钠和替莫泊芬IC50值使用了18小时的温育时间。此外,式(I)菌绿素或二氢卟吩的IC50值能够比卟吩姆钠或替莫泊芬的那些低数个数量级,这意味着式(I)菌绿素或二氢卟吩以低数个数量级的浓度获得与临床批准光敏剂相同的光毒性。该将允许所述光敏剂在接触保护性生物学屏障的短时间段内就达到引起治疗效果需要的浓度。此外,甲酰胺基团为生物可相容性和越过生物学屏障引入了适当两亲性,亦即导致log Pow 1至3的值。该取代基与meso位取代基中的卤素原子一起也有助于增强式(I)卟啉衍生物的可与临床批准光敏剂比拟的光稳定性。

[0103] 光稳定性、在光照治疗窗口中强吸收、ROS高收率和两亲性的结合向式(I)卟啉衍生物提供又一有利的技术特征:对细菌和癌细胞很高的光毒性。表1显示根据式(I)的光敏剂的实例,其以2 $\mu$ M浓度与疮疱棒杆菌菌落温育并用10J/cm<sup>2</sup>光(在红色吸收带被吸收)光照,将细菌CFU数降低9个数量级。对肿瘤细胞的光毒性同样令人惊讶。在1J/cm<sup>2</sup>光剂量下,在体外杀灭大于50%的癌细胞群体的光敏剂药物剂量(IC50)低于10nM。

[0104] 式(I)卟啉衍生物尤其是菌绿素或二氢卟吩跨越保护性生物学屏障和快速扩散至其靶标的能力,与它们在光照治疗窗口中用光光照的高光毒性相组合,使得这些菌绿素或二氢卟吩特别适于人或动物用途的抗癌和/或抗微生物和/或抗病毒和/或抗真菌药物,其将本发明的一种或数种卟啉衍生物用作主要活性剂。该类型药物,尤其在PDT使用的那些,还可以含有一种或数种药学上可接受的赋形剂。在PDT中,含有一种或数种本发明化合物的药物配制剂局部、经口或全身地给予受试者,和在一段时间之后(药物-至-光照间隔),将靶标组织用卟啉衍生物优选菌绿素或二氢卟吩吸收的光光照。光敏剂在局部配制剂中的百分比可以从0.01%变化至15%。用来活化局部施用的光敏剂的光剂量也可以变化,并且可以

需要1至100J/cm<sup>2</sup>的剂量。这些光剂量可以用匹配光敏剂在光照治疗窗口中的吸收带的光源递送,条件是这些光源在热效果开始之下就具有辐照,其接近250mW/cm<sup>2</sup>。另选地,光剂量能够在长时间段给予,包括利用施用局部配制剂的区域的阳光暴露。PDT中使用的光敏剂全身性给药用药学上可接受的载体进行,获得的光敏剂剂量范围是0.1至10微摩尔/kg体重。在范围可以是与给予药物同时直至在给药之后5天的药物-至-光照间隔之后,将光剂量递送至靶标。受光照的光敏剂分子产生的反应性氧类触发化学和生物学过程级联,其结束于细胞和/或细菌和/或病毒的死亡。

[0105] 本发明化合物还可以以高量子产率和在光照治疗窗口中发荧光。表1通过 $\Phi_F=0.20$ 的光敏剂实例。该典型荧光能够用来检测化合物在靶标组织中的存在和提供用本发明化合物诊断血管或过增殖障碍的可能性。

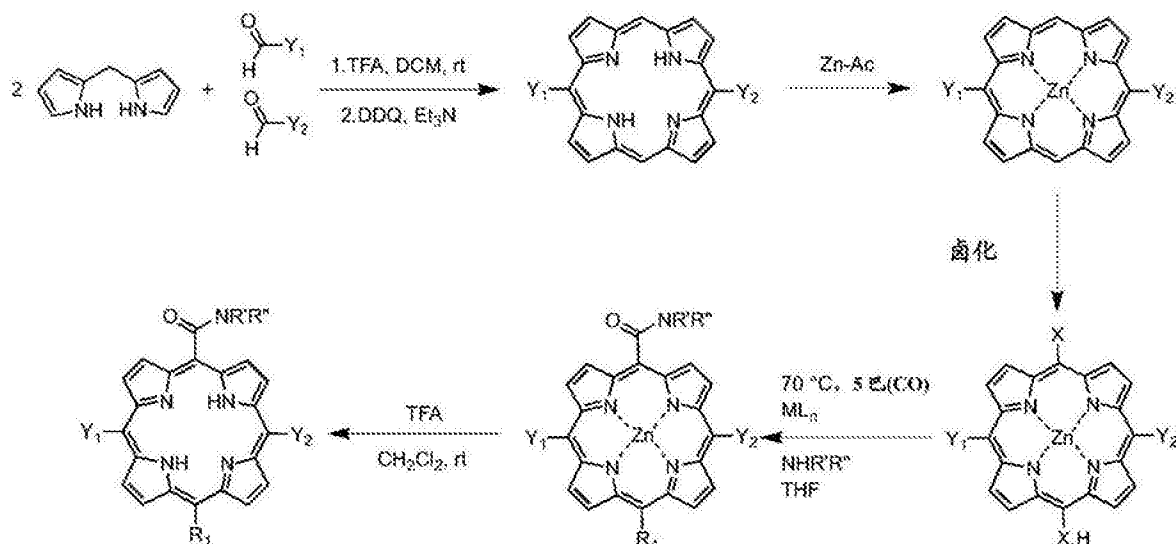
[0106] 本发明化合物也通过高量子产率的无辐射过程失去能量。表1通过 $\Phi_F=0.20$ 和 $\Phi_T=0.45$ 的光敏剂实例,其必须具有 $\Phi_{ic}=0.35$ 的内部转化量子产率。在单线态3.2ns寿命中损失的热能产生快速的热弹性膨胀,其发射强烈的光声波。超声换能器能够用来检测光声波,如上文对光声量热法的描述。另选地,它们能够通过光声断层摄影检测并且用于诊断血管或过增殖障碍。

[0107] D. 化合物制备方法的描述

[0108] 本发明的又一目的在于制备上文描述的衍生物的方法。

[0109] 非对称5,15-二取代的卟啉用修饰下述的方法制备如下:在DCM中,在惰性气氛下,用TFA作催化剂将可商购的2,2'-二吡咯甲烷(dipyrromethane)与卤化醛缩合-环化(10),随后步骤是用DDQ作氧化剂将卟啉原氧化为相应卟啉。后续步骤涵盖5,15-二取代的卟啉与乙酸锌在DCM/甲醇(1:1)溶液中金属化(11),随后进行10或10,20-卟啉位置的一卤化或二卤化。卟啉化锌(II)配合物用NBS溴化,用N-氯代琥珀酰亚胺(NCS)氯化,用二(三氟乙酰氧基)碘苯或2,6-二氯-1-氟吡啶鎓三氟甲磺酸盐碘化(9a)。尽管氨基羰基化反应是从芳基卤或芳基三氟甲磺酸和胺制备甲酰胺的标准过程,卟啉的氨基羰基化是并不常见的(12)。甲酰胺卟啉制备如下:在低压力的一氧化碳(1-10巴)下和50-100℃的温度,在碱存在下,并且使用过渡金属配合物( $ML_n$ )催化剂,用甲胺将相应卤化前体、优选溴化前体氨基羰基化。金属能够选自钼,铬,镍或优选钯。过渡金属配合物中的配体能够选自1,2-二(二苯基膦基)乙烷(DPPE),4,5-二(二苯基膦基)-9,9-二甲基咕吨(Xantphos),1,2-二(二苯基膦基)丙烷(DPPP),1,2-二(二苯基膦基)丁烷(DPPB),2,2'-二(二苯基膦基)-1,1'-联萘(BINAP),二-[2-(二苯基膦基)苯基]醚(DPEPhos),2-二-叔丁基膦基-2',4',6'-三异丙基联苯(叔-BuXpho),三正烷基膦或优选三苯基膦( $PPh_3$ )。碱能够是无机碱和选自碳酸盐,磷酸盐或氟化物。碱还能够是有机碱和选自胺,优选三乙胺。溶剂能够选自甲苯,二噁烷,N-甲基-2-吡咯烷酮(NMP),二甲基甲酰胺(DMF),DCM或THF。还可以使用CO的备择来源,亦即( $M(CO)_n$ ),其中M是Mo或Co。图示地,卟啉前体的制备可以描述如下

[0110]



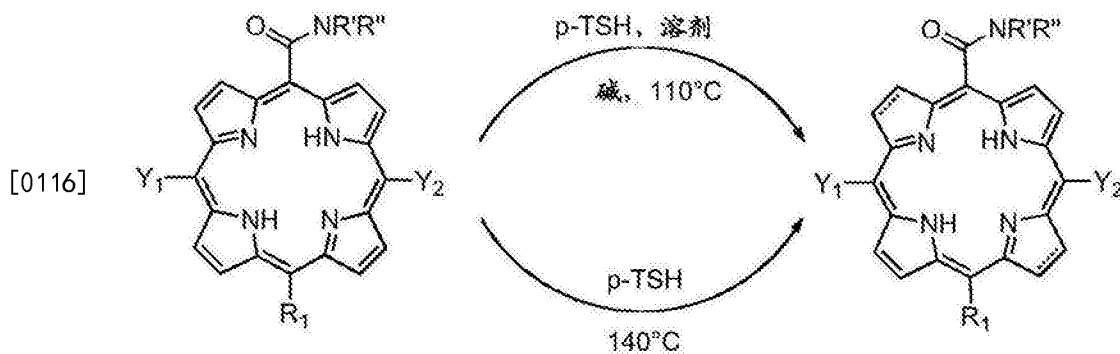
[0111] 其中：

[0112]  $Y_1, Y_2$ 各自独立地选自氢,具有6个或更少碳原子的卤化的烷基或卤化的环烷基,或卤化的苯基,其中卤素独立地选自F、Cl和Br,条件是烷基、环烷基或苯基的至少一个位置是卤化的,和条件是 $Y_1, Y_2$ 中至少一个不是氢；

[0113]  $R_1$ 选自H, F, Cl, Br或 $-CONR'R''$ ；

[0114]  $R'$ 和 $R''$ 独立地选自氢,具有6个或更少碳原子的烷基,环丙基,环丁基,环戊基,醇,伯胺,仲胺,叔胺,带正电的季胺,羧酸,醚或酯。

[0115] 然后将甲酰胺卤化的卟啉前体用来获得相应的还原的菌绿素或二氢卟吩。还原基于二酰亚胺还原方法,其用酰肼优选对甲苯磺酰基酰肼(对-TSH)作为氢源,无机或位阻有机碱,在选自DMF、甲苯、二甲苯、吡啶和吡考啉的溶剂中,用修饰的PCT/EP2005/012212公开的方法进行。还原还能够不存在溶剂和不存在碱的情况下发生,用修饰的PCT/PT2009/000057公开的方法进行。甲酰胺卟啉还原为相应的菌绿素或二氢卟吩可以描述如下：



[0117] 其中： $\text{---}$ 代表碳-碳单键或碳-碳双键,条件是至少一个 $\text{---}$ 代表碳-碳单键。

[0118] 实施例

[0119] 本发明更加详细地描述于下述非限制性实施例中,参考下述附图：

[0120] 实施例1. 制备5-甲基甲酰胺-10,20-二-(2,6-二氟苯基)二氢卟吩的程序

[0121] 合成5-甲基甲酰胺-10,20-二-(2,6-二氟苯基)二氢卟吩进行如下:在惰性气氛下将对-甲苯磺酰基酰肼( $504 \pm 10\text{mg}$ )与5-甲基甲酰胺-10,20-二-(2,6-二氟苯基)卟啉(VIII) ( $100 \pm 10\text{mg}$ ),碳酸钾( $374 \pm 10\text{mg}$ )和吡啶( $15\text{mL}$ )反应,或另选不用溶剂,在 $100^\circ\text{C}$ 至

150℃加热2小时。在冷却至室温后,加入DCM( $\approx 50\text{mL}$ ),有机层用盐酸溶液(0.1M)(3次)和水(3次)洗涤。有机相用无水硫酸钠干燥,过滤然后浓缩。将固体溶于乙酸乙酯(20mL)和加入氯醌(0.6当量)的乙酸乙酯(5mL)溶液。在45℃,将最终溶液保持搅拌。在菌绿素的UV-Vis吸收峰( $\approx 740\text{nm}$ )消失时停止反应。蒸发溶剂,将粗制品溶于DCM(50mL),然后用饱和碳酸氢钠溶液、蒸馏水洗涤,然后在无水硫酸钠上干燥。蒸发溶剂,通过柱色谱法用硅胶(DCM)纯化。获得5-甲基甲酰胺-10,20-二-(2,6-二氟苯基)二氢卟吩,含有示于图2的两种异构体(式Xa和Xb), $80\pm 5\%$ 收率( $80\pm 5\text{mg}$ )。分离产品的NMR和MS如下:

[0122] 式Xa:

[0123]  $^1\text{H-NMR}$  (400MHz,  $(\text{CD}_3)_2\text{CO}$ )  $\delta$  (ppm): -1.89 (s, 1H, NH); -1.59 (s, 1H, NH); 3.34 (d,  $J=4.6\text{Hz}$ , 3H,  $\text{CH}_3$ ); 4.33-4.37 (m, 2H,  $\beta\text{-H}$ ); 4.76-4.80 (m, 2H,  $\beta\text{-H}$ ); 7.49-7.55 (m, 4H, Ar-H); 7.89-7.99 (m, 2H, Ar-H); 8.29 (bs, 1H, NH); 8.44 (d,  $J=4.4\text{Hz}$ , 1H,  $\beta\text{-H}$ ); 8.48 (d,  $J=4.4\text{Hz}$ , 1H,  $\beta\text{-H}$ ); 8.82 (d,  $J=4.2\text{Hz}$ , 1H,  $\beta\text{-H}$ ); 8.99 (d,  $J=4.5\text{Hz}$ , 1H,  $\beta\text{-H}$ ); 9.06 (d,  $J=4.2\text{Hz}$ , 1H,  $\beta\text{-H}$ ); 9.25 (s, 1H, meso-H); 9.27 (d,  $J=4.5\text{Hz}$ , 1H,  $\beta\text{-H}$ )。

[0124]  $^{19}\text{F NMR}$ : (376.5MHz,  $(\text{CD}_3)_2\text{CO}$ )  $\delta\text{ppm}$ : -110.47 (s, 2F, Ar-F); -111.53 (s, 2F, Ar-F)。

[0125] MS ESI-FIA-TOF:  $(\text{C}_{34}\text{H}_{24}\text{F}_4\text{N}_5\text{O})$   $[\text{M}+\text{H}]^+$ 计算值:594.1911,  $[\text{M}+\text{H}]^+$ 实测值:594.1911。

[0126] 式Xb:

[0127]  $^1\text{H-NMR}$  (400MHz,  $(\text{CD}_3)_2\text{CO}$ )  $\delta$  (ppm): -1.79 (s, 1H, NH); -1.63 (s, 1H, NH); 3.37 (d,  $J=4.6\text{Hz}$ , 3H,  $\text{CH}_3$ ); 4.33-4.37 (m, 2H,  $\beta\text{-H}$ ); 4.68-4.72 (m, 2H,  $\beta\text{-H}$ ); 7.50-7.56 (m, 4H, Ar-H); 7.90-8.04 (m, 3H, Ar-H+NH); 8.46 (d,  $J=4.4\text{Hz}$ , 1H,  $\beta\text{-H}$ ); 8.52 (d,  $J=4.2\text{Hz}$ , 1H,  $\beta\text{-H}$ ); 8.83 (d,  $J=4.6\text{Hz}$ , 1H,  $\beta\text{-H}$ ); 8.97 (d,  $J=4.6\text{Hz}$ , 1H,  $\beta\text{-H}$ ); 9.05 (d,  $J=4.3\text{Hz}$ , 1H,  $\beta\text{-H}$ ); 9.37 (d,  $J=4.4\text{Hz}$ , 1H,  $\beta\text{-H}$ ); 10.05 (s, 1H, meso-H)。

[0128]  $^{19}\text{F NMR}$ : (376.5MHz,  $(\text{CD}_3)_2\text{CO}$ )  $\delta\text{ppm}$ : -110.47 (s, 2F, Ar-F); -111.52 (s, 2F, Ar-F)。

[0129] MS ESI-FIA-TOF:  $(\text{C}_{34}\text{H}_{24}\text{F}_4\text{N}_5\text{O})$   $[\text{M}+\text{H}]^+$ 计算值:594.1911,  $[\text{M}+\text{H}]^+$ 实测值:594.1912。

[0130] 实施例2.制备5-甲基甲酰胺-10,20-二-(2,6-二氟苯基)菌绿素的程序

[0131] 固态方法:5-甲基甲酰胺-10,20-二-(2,6-二氟苯基)菌绿素(IX)的合成进行如下:在低于 $6\times 10^{-1}$ 毫巴的压力,在加热( $140\pm 1^\circ\text{C}$ )下,将对-甲苯磺酰基酰肼( $2.52\pm 0.05\text{g}$ )与5-甲基甲酰胺-10,20-二-(2,6-二氟苯基)卟啉( $0.2\pm 0.05\text{g}$ )反应60分钟。在冷却至室温后,将粗反应物溶解,通过色谱法纯化。获得5-甲基甲酰胺-10,20-二-(2,6-二氟苯基)菌绿素, $80\pm 5\%$ 收率( $160\pm 20\text{mg}$ )。

[0132] 溶剂方法:5-甲基甲酰胺-10,20-二-(2,6-二氟苯基)菌绿素(IX)的合成进行如下:在惰性气氛和加热( $110\pm 2^\circ\text{C}$ )下,将对-甲苯磺酰基酰肼( $12.5\pm 0.05\text{g}$ )与5-甲基甲酰胺-10,20-二-(2,6-二氟苯基)卟啉( $1\pm 0.05\text{g}$ ),碳酸钾( $4.6\pm 0.05\text{g}$ ),2-甲基吡啶(20mL)和甲苯(40mL)反应3小时。在冷却至室温后,加入DCM( $\approx 400\text{mL}$ ),用盐酸溶液(0.1M)(3次),水(3次),氢氧化钠(0.05M)(3次)和水(3次)依次洗涤。有机相用无水硫酸钠干燥,过滤然后浓缩。蒸发溶剂,通过色谱法纯化。获得5-甲基甲酰胺-10,20-二-(2,6-二氟苯基)菌绿素, $75\pm 5\%$ 收率( $750\pm 50\text{mg}$ )。

[0133] IX在乙醇和DMSO中的吸收谱示于图3。分离产品的NMR、MS和EA如下:

[0134]  $^1\text{H-NMR}$  (400MHz,  $(\text{CD}_3)_2\text{CO}$ )  $\delta$  (ppm): -1.52 (s, 1H, NH); -1.56 (s, 1H, NH); 3.30 (d,  $J=4.7\text{Hz}$ , 3H,  $\text{CH}_3$ ); 4.11-4.16 (m, 4H,  $\beta\text{-H}$ ); 4.43-4.45 (m, 2H,  $\beta\text{-H}$ ); 4.53-4.57 (m, 2H,  $\beta\text{-H}$ );

7.44–7.48 (m, 4H, Ar-H) ; 7.81–7.90 (m, 3H, Ar-H+NH) ; 8.14–8.16 (m, 1H,  $\beta$ -H) ; 8.22–8.23 (m, 1H,  $\beta$ -H) ; 8.63–8.65 (m, 1H,  $\beta$ -H) ; 8.78–8.80 (m, 1H,  $\beta$ -H) ; 8.94 (s, 1H, meso-H)。

[0135]  $^{19}\text{F}$  NMR: (376.5MHz,  $(\text{CD}_3)_2\text{CO}$ )  $\delta_{\text{ppm}}$ : -110.70 (s, 2F, Ar-F) ; -111.76 (s, 2F, Ar-F)。

[0136] MS ESI-FIA-TOF:  $(\text{C}_{34}\text{H}_{26}\text{F}_4\text{N}_5\text{O})$   $[\text{M}+\text{H}]^+$  计算值: 596.2066,  $[\text{M}+\text{H}]^+$  实测值: 596.2057。元素分析  $(\text{C}_{34}\text{H}_{26}\text{F}_4\text{N}_5\text{O} \cdot \frac{1}{2}(\text{H}_2\text{O}))$ : 计算值 C 67.37, H 4.33, N 11.58, 实测值 C 67.37, H 4.13, N 10.99。

[0137] 实施例3. 制备5-甲基甲酰胺-10,20-二-(三氟甲基) 菌绿素的程序

[0138] 5-甲基甲酰胺-10,20-二-(三氟甲基) 菌绿素 (式XIV) 的合成进行如下: 使用实施例2中描述的溶剂方法的合成和纯化条件。XIV在二氯甲烷中的吸收光谱示于图4。分离产品的NMR表征如下:

[0139]  $^1\text{H}$  NMR: (400MHz,  $\text{CDCl}_3$ )  $\delta_{\text{ppm}}$ : -1.00 (s, 1H, NH) ; -1.07 (s, 1H, NH) ; 3.30 (d, J = 4.7Hz, 3H,  $\text{CH}_3$ ) ; 4.44–4.48 (m, 2H,  $\beta$ -H) ; 4.52–4.55 (m, 2H,  $\beta$ -H) ; 4.61–4.65 (m, 2H,  $\beta$ -H) ; 8.05 (bs, 1H, -NH) ; 8.75–8.76 (m, 1H,  $\beta$ -H) ; 8.84–8.86 (m, 1H,  $\beta$ -H) ; 8.96 (bs, 2H,  $\beta$ -H+meso-H) ; 9.03–9.05 (m, 1H,  $\beta$ -H)。

[0140] 实施例4. 在光辐射之后对疮疱棒杆菌 (*PROPIONIBACTERIUM ACNES*) 的体外光毒性

[0141] 该实施例描述在光辐射之后式IX甲酰胺菌绿素的体外光毒性评价, 以及它们在治疗寻常痤疮中用于PDT应用的潜力。光毒性根据材料和方法部分的描述测量。IX的正辛醇: PBS分配比率是 $\text{P}_{\text{OW}} = 2.9 \pm 0.5$ 。该甲酰胺菌绿素的适当配制剂是PEG400:DMSO (55:45)。试验化合物的光毒性与相对未处理对照的疮疱棒杆菌存活抑制成比例, 并且示于图5中, 其形式为在4和10J/cm<sup>2</sup>的光剂量下作为光敏剂浓度的函数的CFU降低。

[0142] 实施例5. 在光辐射之后对A549和CT26癌细胞系的体外光毒性该实施例描述在光辐射之后式IX甲酰胺菌绿素的体外光毒性评价, 以及它们用于癌症PDT的潜力。所用的配制剂与实施例4相同。将试验化合物与A549或CT26细胞系避光温育30分钟并未显示毒性征兆, 直至40 $\mu\text{M}$ 。光毒性根据材料和方法部分的描述来测量。对于各种浓度的试验化合物, 按照细胞存活百分比评价试验化合物的光毒性: 温育所述试验化合物30分钟, 随后用PBS洗涤, 加入培养基并用1J/cm<sup>2</sup>光剂量光照。图6显示, 在光敏剂浓度达到50nM的情况下, 几乎全部细胞被杀灭。

[0143] 实施例6. 对BALB/C小鼠中植入的CT26皮下肿瘤的体内抗肿瘤PDT效力

[0144] 该实施例描述携带在右股植入的CT26皮下肿瘤的小鼠的PDT。在肿瘤最大直径达到5mm的情况下, 将肿瘤用式IX甲酰胺菌绿素处理, 采用的是血管方案。该治疗方案在于静脉内注射经定义剂量的配制剂中的式IX光敏剂, 所述配制剂包含Kolliphor EL:乙醇:NaCl 0.9% (0.6:3:96.4, v/v/v), 在15分钟后用749 $\pm$ 3nm激光进行对肿瘤的光照。材料和方法部分描述处理所用的动物模型和激光和光纤。光纤与肿瘤表面垂直放置, 以便照亮1.33cm<sup>2</sup>的与肿瘤同心的面积, 递送40J/cm<sup>2</sup>的光剂量, 辐照度130mW/cm<sup>2</sup>。给予的式IX甲酰胺菌绿素的剂量虑及样品纯度来计算。在PDT之后, 跟踪小鼠以评价它们对治疗的应答直至其肿瘤最大直径达到15mm。在该点 (人道主义终点) 将动物处死。效力结果按照Kaplan-Meier生存曲线展示在图7中。该实施例显示该工作中公开的光敏剂是极度光毒性的。实际上, 0.3mg/kg的光敏剂剂量是如此光毒性的, 以致动物在PDT后不到72小时就死于对治疗的急性应答。在另一治疗组中所用的0.15mg/kg光敏剂剂量得到很良好的耐受。在PDT后的天数中, 在光照区

域的局部应答显示水肿和红斑,其涉及急性炎性反应的发作,伴随肿瘤破坏和形成坏死的疮痂。一旦坏死的疮痂消退,就可以观察到肿瘤已完全消退和实现100%的治愈。另一方面,肿瘤在对照(未处理组)中连续生长,肿瘤接种的27天内必须处死全部动物。用0.15mg/kg光敏剂剂量处理的组的生存曲线在统计学上不同于未处理的对照组,后者展示19天的中位数存活时间(Log-排名试验, $p<0.05$ )。

[0145] 实施例7. 皮肤渗透

[0146] 该实施例描述式IX甲酰胺菌绿素在猪皮肤中的渗透。局部配制剂和动物模型描述于材料和方法部分。渗透从两方面评价,一方面是在局部配制剂与皮肤之间经过指定的接触时间(温育时间)之后光敏剂在皮肤中的量,另一方面是在皮肤中的渗透深度。

[0147] 在各温育时间之后评价式IX甲酰胺菌绿素在皮肤中的量牵涉下述步骤:(i) 将0.30ml配制剂施用于 $1\text{cm}^2$ 面积的小型猪皮肤中,对于温育时间30、60和120分钟进行6次独立测量;(ii) 在温育时间终点清洁皮肤表面;(iii) 将皮肤切为小片,在2ml二氯甲烷借助分散设备浸软;(iv) 用10ml二氯甲烷在falcon管中萃取6小时;(v) 用乙醇以5倍因子稀释。将200 $\mu\text{l}$ 体积的试验溶液重复三份加至96孔板,用Biotek (California, USA) 的Synergy HT微读板器参照校准曲线检测荧光强度,其配有508/20nm的激发滤光片和760/35nm发射滤光片。将用相同清洁方法获得的空白信号从样品信号扣除,参照校准曲线获得光敏剂在各样品中的浓度。结果概要能够参见表2。光敏剂向皮肤的通量在1.5小时内达到 $J_{\max}=4\times 10^{-8}\text{mol}/(\text{cm}^2\text{h})$ ,对于MW=594Da的光敏剂来说其令人惊讶地高。该实施例显示,本文公开的卤化二氢卟吩和菌绿素的低分子量甲酰胺衍生物特别能够越过生物学屏障。

[0148] 在各温育时间之后,式IX甲酰胺菌绿素在皮肤中的渗透深度评价牵涉下述步骤:(i) 与配制剂温育30、60和120分钟的组织的活检样品冷冻收集在干冰中;(ii) 在低温恒温器中,将冷冻的组织安装在具有组织-Tek O.C.T.化合物的支架上(Sakura Finetek Europe B.V., Zoeterwoude, 荷兰),切为厚度25 $\mu\text{m}$ 的条片;(iii) 将皮肤切片收集在显微镜载玻片中并保持冷冻,用于显微技术。菌绿素的共焦荧光术用LSM510Meta (Carl Zeiss, Jena, 德国) 共焦显微镜来进行,其具有 $\times 63$ 油浸物镜 (Plan-Apochromat, 1.4NA; Carl Zeiss),使用 $\lambda_{\text{ex}}=514\text{nm}$ 、 $\lambda_{\text{em}}\geq 650\text{nm}$ ,激光功率5%并放大40 $\times$ 。展示在各温育时间之后光敏剂在皮肤中的荧光的图像示于图8。以相同条件但不将配制剂与皮肤温育而进行的对照实验并未显示任何荧光和在图8条件下是全黑色。增加温育时间导致光敏剂在皮肤中的更深穿透,其可以在90分钟内达到40 $\mu\text{m}$ 深度并覆盖绝大部分表皮。该实施例显示,本文公开的卤化二氢卟吩和菌绿素的低分子量甲酰胺衍生物特别能够在生物学组织中扩散并快速达到其靶标。

[0149] 表2. 在各温育时间之后式IX甲酰胺菌绿素在皮肤中的量。

[0150]

| 实验 | 温育时间(min) | 浓度(M)    | 平均浓度(M)  | 质量( $\mu\text{g}/\text{cm}^2$ ) |
|----|-----------|----------|----------|---------------------------------|
| #1 | 30        | 7.26E-07 | 9.09E-07 | 5.41 $\pm$ 1.33                 |
| #2 | 30        | 7.26E-07 |          |                                 |
| #3 | 30        | 4.97E-07 |          |                                 |
| #4 | 30        | 8.35E-07 |          |                                 |
| #5 | 30        | 6.68E-07 |          |                                 |
| #6 | 30        | 2.00E-06 |          |                                 |
| #1 | 60        | 1.06E-06 | 3.34E-06 | 19.9 $\pm$ 5.12                 |
| #2 | 60        | 2.73E-06 |          |                                 |
| #3 | 60        | 9.14E-07 |          |                                 |
| #4 | 60        | 4.20E-06 |          |                                 |
| #5 | 60        | 5.40E-06 |          |                                 |
| #6 | 60        | 5.73E-06 |          |                                 |
| #1 | 120       | 3.35E-06 | 6.26E-06 | 37.2 $\pm$ 7.45                 |
| #2 | 120       | 2.18E-06 |          |                                 |
| #3 | 120       | 6.16E-06 |          |                                 |
| #4 | 120       | 7.03E-06 |          |                                 |
| #5 | 120       | 8.53E-06 |          |                                 |
| #6 | 120       | 1.02E-05 |          |                                 |

[0151] 自然地,本实施方式并不以任何方式局限于本文描述的实施方案和实施例,并且本领域普通技术人员能够预见许多可能的变化但不偏离其主旨,如权利要求中所述。

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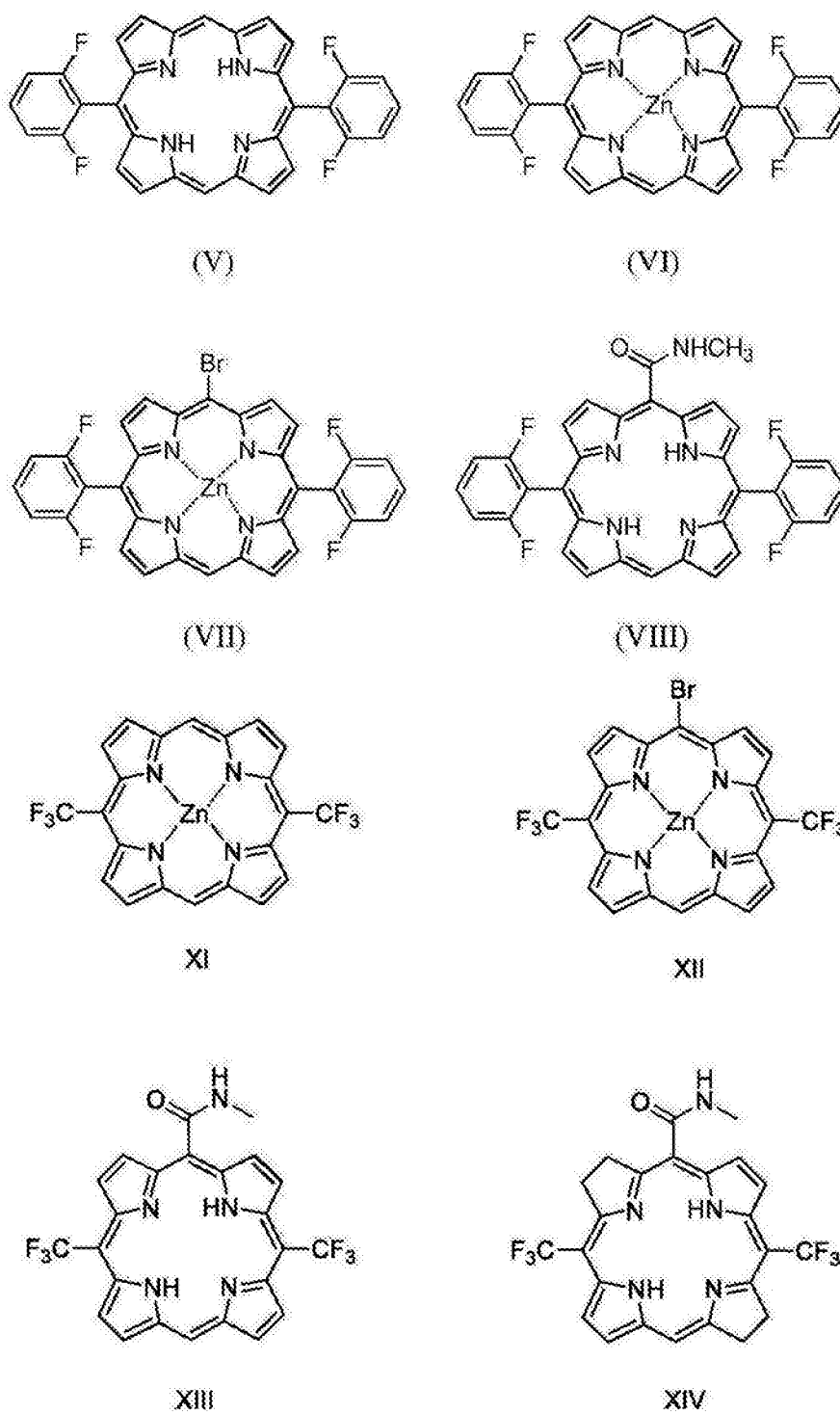


图1

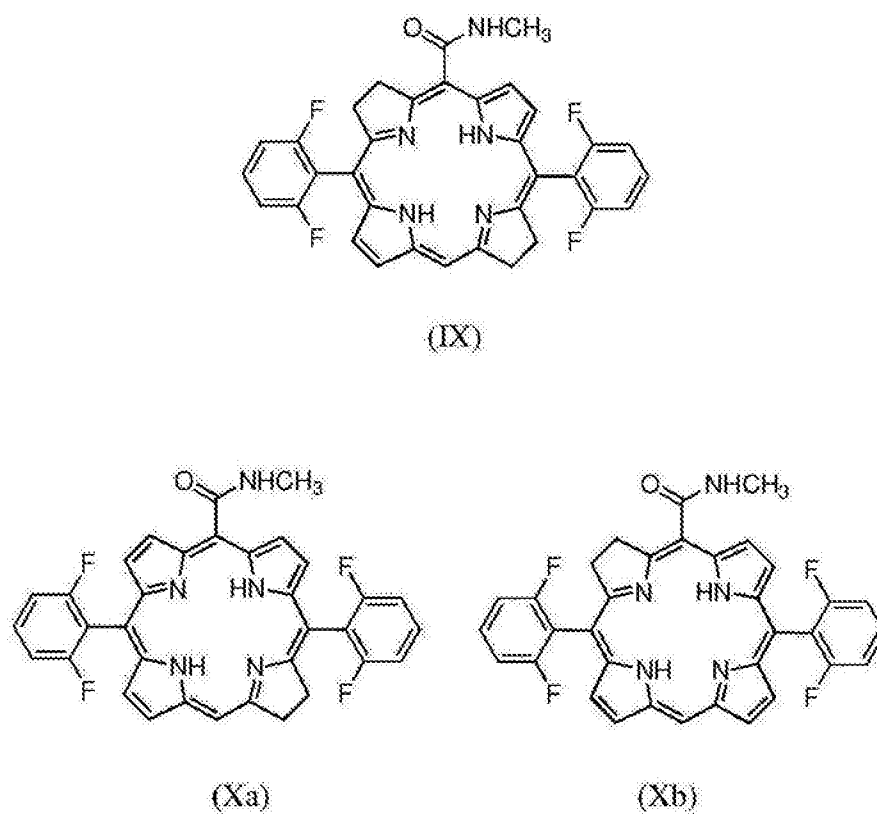


图2

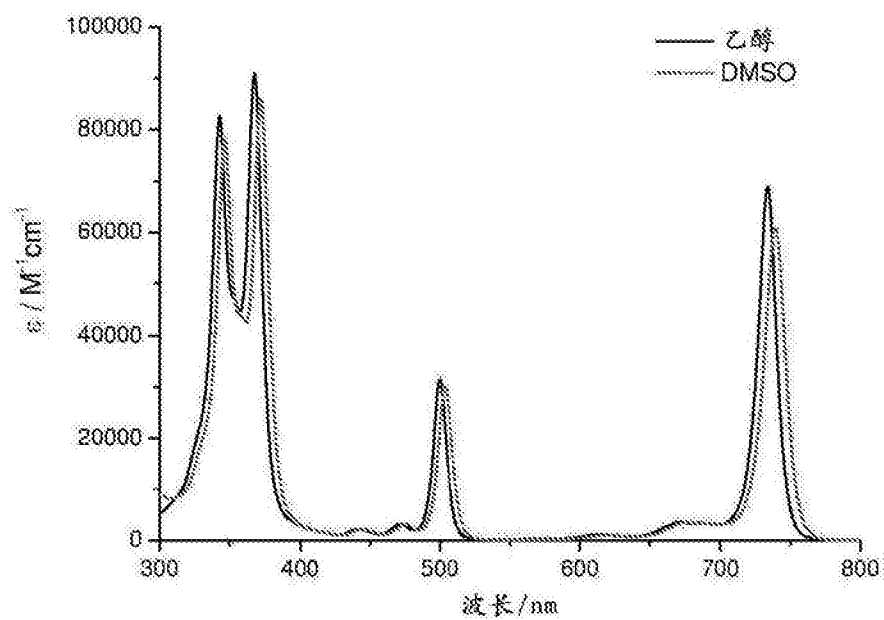


图3

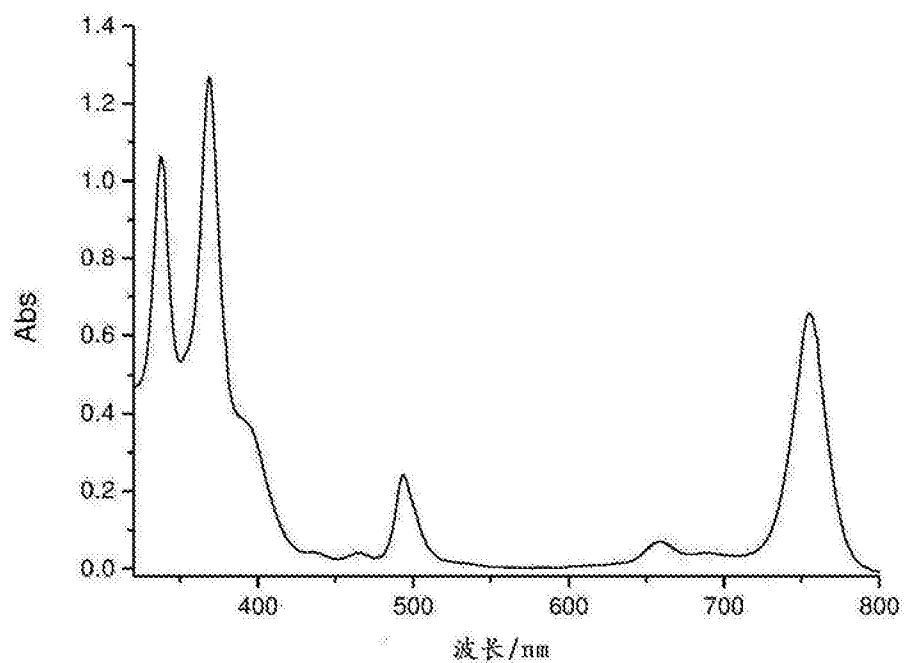


图4

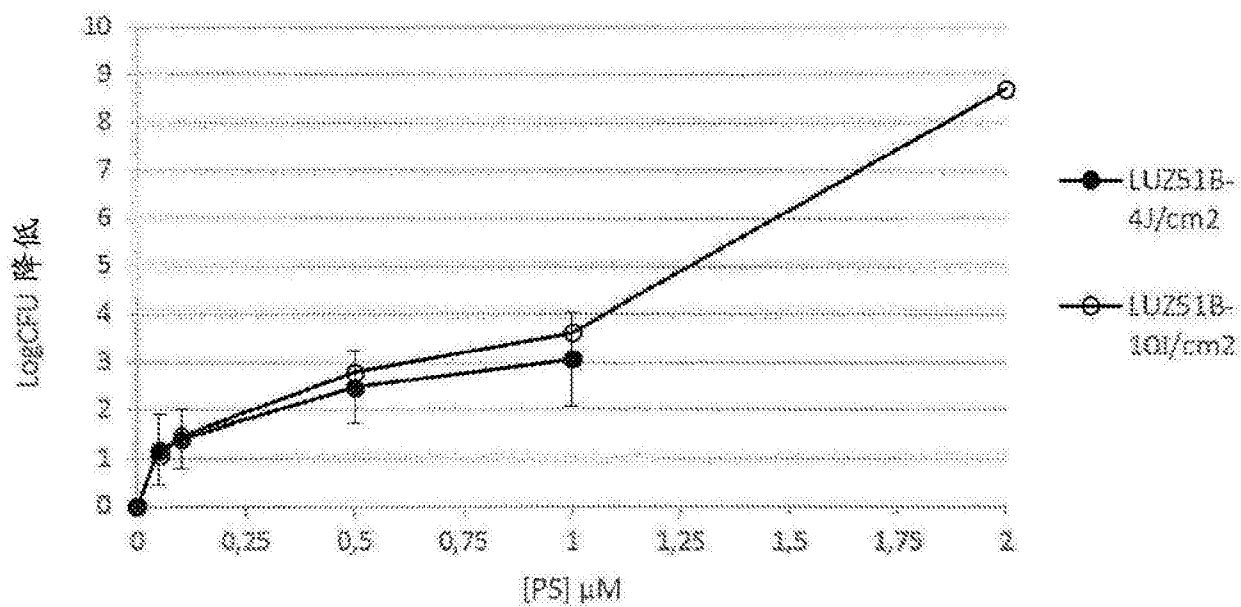


图5

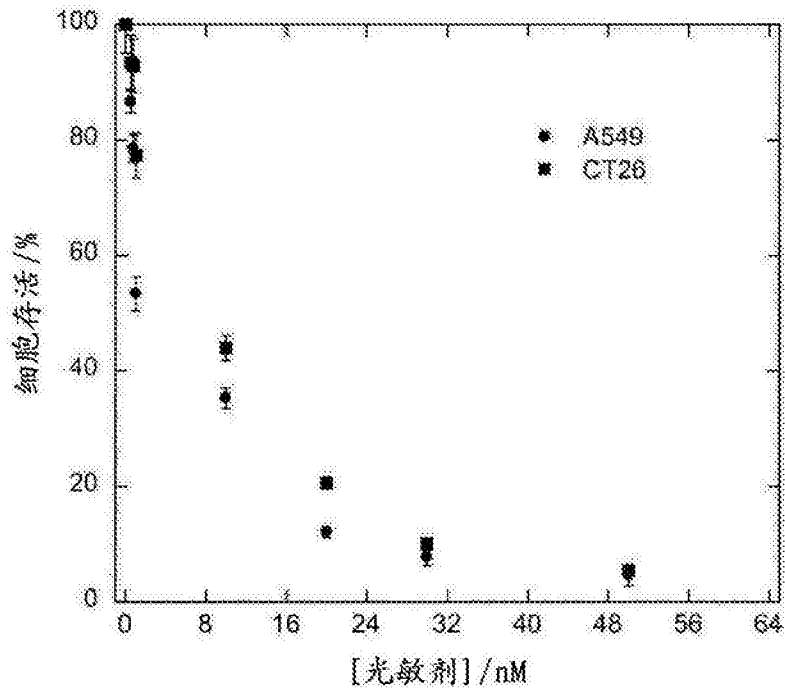


图6

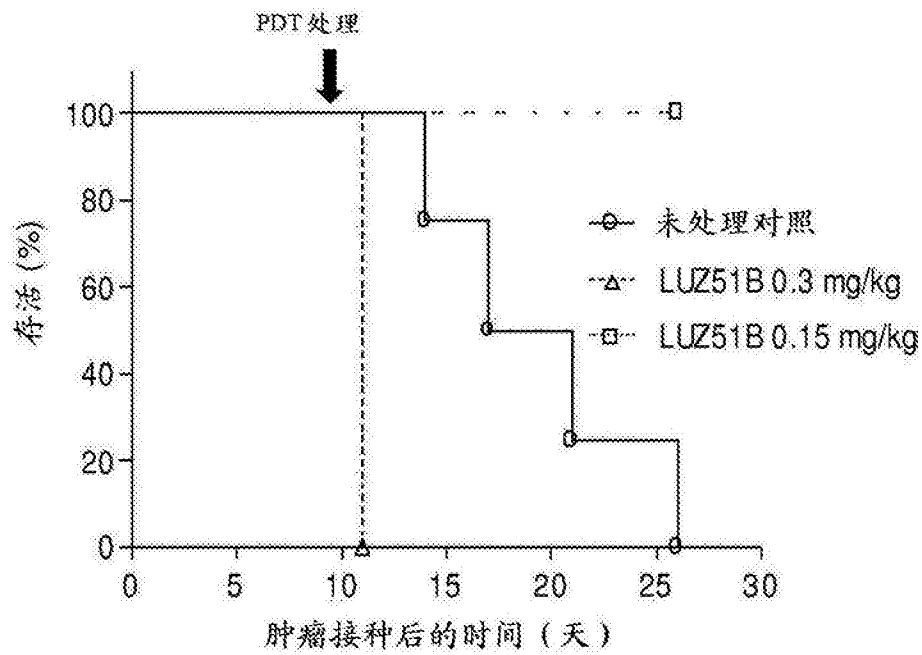


图7

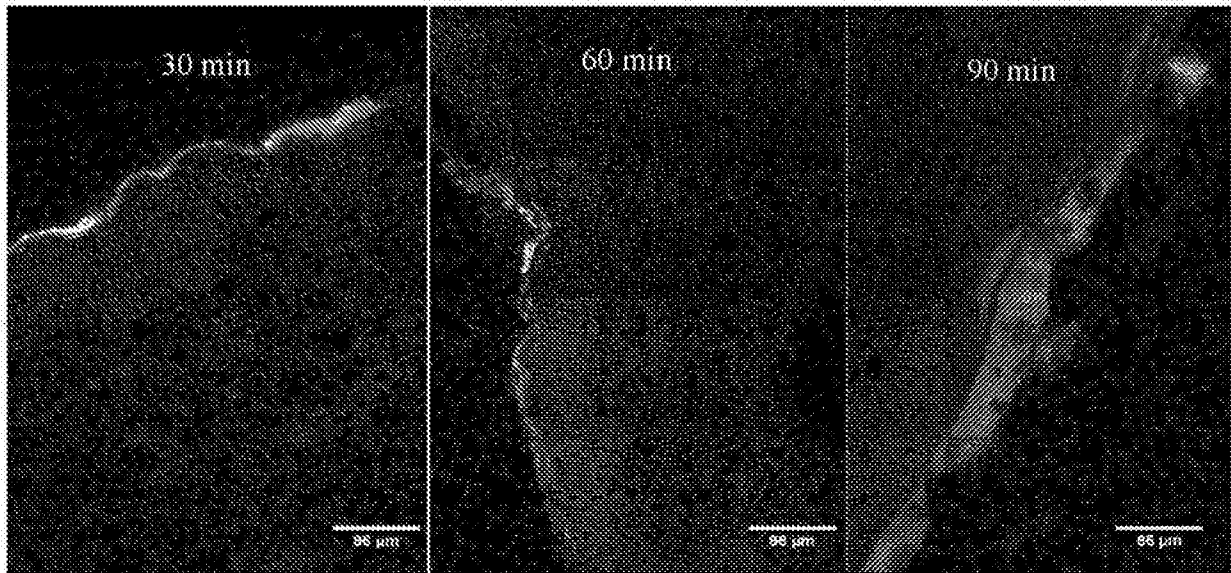


图8