The present invention relates to a process of preparing chlorin e4-Zn complex. The present invention also relates to a pharmaceutical composition comprising chlorin e4-Zn complex for the treatment of, for example, cancer. The present invention further relates to methods of treating and diagnosing, for example, cancer by photodynamic therapy or photodynamic diagnosis.
Technical field

The present invention relates to a process of preparing chlorin e4-Zn complex. The present invention also relates to a pharmaceutical composition comprising chlorin e4-Zn complex for the treatment of, for example, cancer. The present invention further relates to methods of treating and diagnosing, for example, cancer by photodynamic therapy or photodynamic diagnosis.

Background art

Porphyrins and their derivatives are known photosensitive chemical compounds, which can absorb light photons and emit them at higher wavelengths. There are many applications for such unique properties and PDT (photodynamic therapy) is one of them.

During light activation, a molecule goes from the ground state ($S_0$) to a singlet excited state ($S_1$), ($S_0 \rightarrow S_1$ transition), and then falls back down to the ground state ($S_0$), ($S_1 \rightarrow S_0$ transition), emitting light at higher wavelengths in the form of fluorescence. There is another energy state known as the triplet state ($T_1$). The triplet state ($T_1$) cannot be efficiently populated by direct excitation. However, intersystem crossing $S_1 \rightarrow T_1$ occurs isoenergetically followed by deactivation to $T_1$. The $S_1 \rightarrow T_1$ change is formally spin-forbidden, but it is a downhill process and in some molecules intersystem crossing occurs so efficiently that fluorescence is quenched. Such compounds are useful for generating excited triplets by excitation transfer (a phenomenon known as sensitization) in molecules where the intersystem crossing is minimal.

At the triplet state ($T_1$) molecules become very unstable and react with oxygen, splitting the latter into two singlet oxygens ($^{1}O_2$). Singlet oxygen ($^{1}O_2$) is known as a "scavenger" and can destroy abnormal biological cells and remove free radicals.
Such unique properties make many photosensitive molecules good photosensitizers for PDT.

Presently, there are two generations of photosensitizers for PDT. The first generation comprises hem porphyrins (blood derivatives), and the second for the most part are chlorophyll derivatives. These compounds are known as chlorins and bacteriochlorins.

Chlorins show photophysical properties similar to those of the porphyrin systems, but have an enhanced, red-shifted Q band (670nm) which makes chlorin-containing systems better candidates for PDT. Chlorophyll a, the green photosynthetic pigment, is one prototype of the chlorin class of natural products. It or its derivatives can be extracted from certain Spirulina Platensis species without any contamination with chlorophyll b, thus avoiding a tedious chromatographic separation.

A water-soluble chlorophyll derivative, known as tri-sodium copper(II) chlorin e6, has been used in the treatment of various human ailments with no evidence of toxicity, skin sensitization or other serious side effects.

Chlorin e4 was studied recently and was shown to display good photosensitive activity. It was indicated that chlorin e4 has a protective effect against indomethacin-induced gastric lesions in rats and TAA- or CCl₄-induced acute liver injuries in mice. It was therefore suggested that chlorin e4 may be a promising new drug candidate for anti-gastrelcosis and liver injury protection.
Summary of the invention

A first aspect of the present invention provides a process of preparing chlorin e4-Zn complex:

comprising the step of reacting chlorin e4 with a zinc compound in the presence of a tetraalkylammonium salt.

Chlorin e4-Zn complex has two chiral centres. The chlorin e4-Zn complex of the present invention is preferably substantially enantiomerically pure, meaning that the chlorin e4-Zn complex comprises less than 10% of other stereoisomers, preferably less than 5%, preferably less than 3%, preferably less than 2%, preferably less than 1%.
Preferably, the process of the present invention comprises the step of adding a solution of the zinc compound to a solution of chlorin e4 and the tetraalkylammonium salt. Preferably, chlorin e4, the tetraalkylammonium salt and the zinc compound are used in a ratio of 0.15-1 : 15-20 : 1-12. Preferably, the zinc compound is a carboxylic acid zinc salt (such as zinc stearate or zinc acetate), a zinc halide (such as zinc fluoride, chloride, bromide or iodide), zinc hydride, or zinc acetylacetonate. Preferably, the zinc compound is zinc stearate. Preferably, the tetraalkylammonium salt is tetrabutylammonium hydroxide. Preferably, the solvent comprises N,N-dimethylformamide (DMF), methanol, chloroform, dimethylsulfoxide (DMSO) or toluene. Preferably, the solvent comprises N,N-dimethylformamide (DMF). In an alternative preferred embodiment, the solvent comprises methanol.

Preferably, the metalation reaction is carried out at a temperature in the range of 25-80°C, preferably in the range of 30-70°C, preferably in the range of 35-50°C. Preferably, the metalation reaction is carried out over 1 to 12 hours, preferably over 2 to 6 hours.

The chlorin e4-Zn complex may be purified by filtering, for example through a 45µm Millipore filter. Alternatively or additionally, the chlorin e4-Zn complex may be purified by chromatography.

A second aspect of the present invention provides chlorin e4-Zn complex when prepared by a process of the first aspect of the present invention.

A third aspect of the present invention provides a pharmaceutical composition, comprising chlorin e4-Zn complex of the second aspect of the present invention, and a pharmaceutically acceptable carrier or diluent.

The third aspect of the present invention also provides a pharmaceutical composition, comprising chlorin e4-Zn complex and a pharmaceutically acceptable carrier or diluent, for the treatment of atherosclerosis, multiple sclerosis, diabetes,
diabetic retinopathy, arthritis, rheumatoid arthritis, a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic infectious disease, HIV, Aids, infection with sars virus, Asian (chicken) flu virus, herpes simplex, herpes zoster, hepatitis, viral hepatitis, a cardiovascular disease, coronary artery stenosis, carotid artery stenosis, intermittent claudication, a dermatological condition, acne, psoriasis, a disease characterised by begin or malignant cellular hyperproliferation or by areas of neovascularisation, a benign or malignant tumour, early cancer, cervical dysplasia, or cancer of the blood, cervix, naso-pharynx, trachea, larynx, bronchi, bronchioles, bladder, esophagus, stomach, rectum, colon, prostate, hollow organs, bile duct, ureter, kidney, uterus, vaginal or other female adnexa. Preferably, the pharmaceutical composition is for the treatment of a disease characterised by begin or malignant cellular hyperproliferation or by areas of neovascularisation. Preferably, the pharmaceutical composition is for the treatment of a benign or malignant tumour.

Finally, the third aspect of the present invention provides a pharmaceutical composition, comprising chlorin e4-Zn complex and a pharmaceutically acceptable carrier or diluent, for use in photodynamic diagnosis. Preferably, the
pharmaceutical composition is for the detection of atherosclerosis, multiple sclerosis, diabetes, diabetic retinopathy, arthritis, rheumatoid arthritis, a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic infectious disease, HIV, Aids, infection with sars virus, Asian (chicken) flu virus, herpes simplex, herpes zoster, hepatitis, viral hepatitis, a cardiovascular disease, coronary artery stenosis, carotid artery stenosis, intermittent claudication, a dermatological condition, acne, psoriasis, a disease characterised by begin or malignant cellular hyperproliferation or by areas of neovascularisation, a benign or malignant tumour, early cancer, cervical dysplasia, or cancer of the blood, cervix, naso-pharynx, trachea, larynx, bronchi, bronchioles, bladder, esophagus, stomach, rectum, colon, prostate, hollow organs, bile duct, ureter, kidney, uterus, vaginal or other female adnexa. Preferably, the pharmaceutical composition is for the detection of an area that is affected by begin or malignant cellular hyperproliferation or by neovascularisation. Preferably, the pharmaceutical composition is for the detection of a begin or malignant tumour. Preferably, the pharmaceutical composition is for the fluorescent or phosphorescent detection of the said diseases, more preferably for the fluorescent or phosphorescent detection and quantification of the said diseases.

Preferably, the pharmaceutical compositions of the third aspect of the present invention are adapted for administration simultaneous with or prior to administration of irradiation or sound, preferably for administration prior to administration of irradiation.

If the pharmaceutical compositions of the third aspect of the present invention are for use in photodynamic therapy or cytoluminescent therapy, then the pharmaceutical compositions are preferably adapted for administration 10 to 100 hours before the irradiation, preferably 50 to 90 hours before the irradiation, preferably about 72 hours before the irradiation.

If the pharmaceutical compositions of the third aspect of the present invention are for use in photodynamic diagnosis, then the pharmaceutical compositions are preferably adapted for administration 3 to 60 hours before the irradiation, preferably 8 to 40 hours before the irradiation.
Preferably, the irradiation is electromagnetic radiation with a wavelength in the range of from 500nm to 1000nm, preferably from 600nm to 900nm, preferably from 620nm to 820nm, preferably from 630nm to 710nm, preferably about 635nm.

Preferably, the pharmaceutical compositions of the third aspect of the present invention are in a form suitable for oral, parental (including intravenous, subcutaneous, intramuscular, intradermal, intratracheal, intraperitoneal, intraarticular, intraabdominal, intracranial and epidural), transdermal, airway (aerosol), rectal, vaginal or topical (including buccal, mucosal and sublingual) administration.

The pharmaceutical composition may be in a form suitable for oral administration. Preferably, the pharmaceutical composition is provided in the form of a tablet, capsule, hard or soft gelatine capsule, caplet, troche or lozenges, as a powder or granules, or as an aqueous solution, suspension or dispersion. Preferably, the pharmaceutical composition is in a form suitable for providing 0.01 to 10 mg/kg/day of the chlorin e4-Zn complex, preferably 0.1 to 5 mg/kg/day.

Alternatively, the pharmaceutical composition may be in a form suitable for parental administration. Preferably, the pharmaceutical composition is in a form suitable for intravenous administration. Preferably, the pharmaceutical composition is an aqueous solution or suspension having a pH of from 6 to 8.5.

A fourth aspect of the present invention provides chlorin e4-Zn complex, for the treatment of atherosclerosis, multiple sclerosis, diabetes, diabetic retinopathy, arthritis, rheumatoid arthritis, a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic infectious disease, HIV, Aids, infection with sars virus, Asian (chicken) flu virus, herpes simplex, herpes zoster, hepatitis, viral hepatitis, a cardiovascular disease, coronary artery stenosis, carotid artery stenosis, intermittent claudication, a dermatological condition, acne, psoriasis, a disease characterised by begin or malignant cellular hyperproliferation or by areas of neovascularisation, a benign or malignant tumour, early cancer, cervical dysplasia, or cancer of the blood, cervix,
naso-pharynx, trachea, larynx, bronchi, bronchioles, bladder, esophagus, stomach, rectum, colon, prostate, hollow organs, bile duct, ureter, kidney, uterus, vaginal or other female adnexa.

The fourth aspect of the present invention also provides chlorin e4-Zn complex, for use in photodynamic therapy or cytoluminescent therapy. Preferably, the chlorin e4-Zn complex is for the treatment of atherosclerosis, multiple sclerosis, diabetes, diabetic retinopathy, arthritis, rheumatoid arthritis, a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic infectious disease, HIV, Aids, infection with sars virus, Asian (chicken) flu virus, herpes simplex, herpes zoster, hepatitis, viral hepatitis, a cardiovascular disease, coronary artery stenosis, carotid artery stenosis, intermittent claudication, a dermatological condition, acne, psoriasis, a disease characterised by begin or malignant cellular hyperproliferation or by areas of neovascularisation, a benign or malignant tumour, early cancer, cervical dysplasia, or cancer of the blood, cervix, naso-pharynx, trachea, larynx, bronchi, bronchioles, bladder, esophagus, stomach, rectum, colon, prostate, hollow organs, bile duct, ureter, kidney, uterus, vaginal or other female adnexa. Preferably, the chlorin e4-Zn complex is for the treatment of a disease characterised by begin or malignant cellular hyperproliferation or by areas of neovascularisation. Preferably, the chlorin e4-Zn complex is for the treatment of a benign or malignant tumour.

The fourth aspect of the present invention further provides chlorin e4-Zn complex, for use in photodynamic diagnosis. Preferably, the chlorin e4-Zn complex is for the detection of atherosclerosis, multiple sclerosis, diabetes, diabetic retinopathy, arthritis, rheumatoid arthritis, a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic infectious disease, HIV, Aids, infection with sars virus, Asian (chicken) flu virus, herpes simplex, herpes zoster, hepatitis, viral hepatitis, a cardiovascular disease, coronary artery stenosis, carotid artery stenosis, intermittent claudication, a dermatological condition, acne, psoriasis, a disease characterised by begin or malignant cellular hyperproliferation or by areas of neovascularisation, a benign or malignant tumour, early cancer, cervical dysplasia, or cancer of the blood, cervix, naso-pharynx, trachea, larynx, bronchi, bronchioles, bladder, esophagus, stomach, rectum, colon, prostate, hollow organs, bile duct, ureter, kidney, uterus, vaginal or
other female adnexa. Preferably, the chlorin e4-Zn complex is for the detection of an area that is affected by begin or malignant cellular hyperproliferation or by neovascularisation. Preferably, the chlorin e4-Zn complex is for the detection of a begin or malignant tumour. Preferably, the chlorin e4-Zn complex is for the fluorescent or phosphorescent detection of the said diseases, more preferably for the fluorescent or phosphorescent detection and quantification of the said diseases.

Preferably, the chlorin e4-Zn complex of the fourth aspect of the present invention is adapted for administration simultaneous with or prior to administration of irradiation or sound, preferably for administration prior to administration of irradiation.

If the chlorin e4-Zn complex is for use in photodynamic therapy or cytoluminescent therapy, then the chlorin e4-Zn complex is preferably adapted for administration to 100 hours before the irradiation, preferably 50 to 90 hours before the irradiation, preferably about 72 hours before the irradiation.

If the chlorin e4-Zn complex is for use in photodynamic diagnosis, then the chlorin e4-Zn complex is preferably adapted for administration 3 to 60 hours before the irradiation, preferably 8 to 40 hours before the irradiation.

Preferably, the irradiation is electromagnetic radiation with a wavelength in the range of from 500nm to 1000nm, preferably from 600nm to 900nm, preferably from 620nm to 820nm, preferably from 630nm to 710nm.

A fifth aspect of the present invention provides use of chlorin e4-Zn complex for the manufacture of a medicament for the treatment of atherosclerosis, multiple sclerosis, diabetes, diabetic retinopathy, arthritis, rheumatoid arthritis, a fungal, viral, chlamydia, bacterial, nanobacterial or parasitic infectious disease, HIV, Aids, infection with sars virus, Asian (chicken) flu virus, herpes simplex, herpes zoster, hepatitis, viral hepatitis, a cardiovascular disease, coronary artery stenosis, carotid artery stenosis, intermittent claudication, a dermatological condition, acne, psoriasis, a disease characterised by begin or malignant cellular hyperproliferation or by areas
of neovascularisation, a benign or malignant tumour, early cancer, cervical dysplasia, or cancer of the blood, cervix, naso-pharynx, trachea, larynx, bronchi, bronchioles, bladder, esophagus, stomach, rectum, colon, prostate, hollow organs, bile duct, ureter, kidney, uterus, vaginal or other female adnexa.

5 The fifth aspect of the present invention also provides use of chlorin e4-Zn complex for the manufacture of a phototherapeutic agent for use in photodynamic therapy or cytoluminescent therapy. Preferably, the phototherapeutic agent is for the treatment of atherosclerosis, multiple sclerosis, diabetes, diabetic retinopathy, arthritis, rheumatoid arthritis, a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic infectious disease, HIV, Aids, infection with sars virus, Asian (chicken) flu virus, herpes simplex, herpes zoster, hepatitis, viral hepatitis, a cardiovascular disease, coronary artery stenosis, carotid artery stenosis, intermittent claudication, a dermatological condition, acne, psoriasis, a disease characterised by benign or malignant cellular hyperproliferation or by areas of neovascularisation, a benign or malignant tumour, early cancer, cervical dysplasia, or cancer of the blood, cervix, naso-pharynx, trachea, larynx, bronchi, bronchioles, bladder, esophagus, stomach, rectum, colon, prostate, hollow organs, bile duct, ureter, kidney, uterus, vaginal or other female adnexa. Preferably, the phototherapeutic agent is for the treatment of a disease characterised by benign or malignant cellular hyperproliferation or by areas of neovascularisation. Preferably, the phototherapeutic agent is for the treatment of a benign or malignant tumour.

The fifth aspect of the present invention further provides use of chlorin e4-Zn complex for the manufacture of a photodiagnostic agent for use in photodynamic diagnosis. Preferably, the photodiagnostic agent is for the detection of atherosclerosis, multiple sclerosis, diabetes, diabetic retinopathy, arthritis, rheumatoid arthritis, a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic infectious disease, HIV, Aids, infection with sars virus, Asian (chicken) flu virus, herpes simplex, herpes zoster, hepatitis, viral hepatitis, a cardiovascular disease, coronary artery stenosis, carotid artery stenosis, intermittent claudication, a dermatological condition, acne, psoriasis, a disease characterised by benign or malignant cellular hyperproliferation or by areas of neovascularisation, a benign or
malignant turnout, early cancer, cervical dysplasia, or cancer of the blood, cervix, naso-pharynx, trachea, larynx, bronchi, bronchioles, bladder, esophagus, stomach, rectum, colon, prostate, hollow organs, bile duct, ureter, kidney, uterus, vaginal or other female adnexa. Preferably, the photodiagnostic agent is for the detection of an area that is affected by begin or malignant cellular hyperproliferation or by neovascularisation. Preferably, the photodiagnostic agent is for the detection of a begin or malignant tumour. Preferably, the photodiagnostic agent is for the fluorescent or phosphorescent detection of the said diseases, more preferably for the fluorescent or phosphorescent detection and quantification of the said diseases.

Preferably, the medicament, phototherapeutic agent or photodiagnostic agent is adapted for administration simultaneous with or prior to administration of irradiation or sound, preferably for administration prior to administration of irradiation.

Preferably, the medicament or phototherapeutic agent is adapted for administration 10 to 100 hours before the irradiation, preferably 50 to 90 hours before the irradiation, preferably about 72 hours before the irradiation.

Preferably, the photodiagnostic agent is adapted for administration 3 to 60 hours before the irradiation, preferably 8 to 40 hours before the irradiation.

Preferably, the irradiation is electromagnetic radiation with a wavelength in the range of from 500nm to 1000nm, preferably from 600nm to 900nm, preferably from 620nm to 820nm, preferably from 630nm to 710nm.

A sixth aspect of the present invention provides a method of treating atherosclerosis, multiple sclerosis, diabetes, diabetic retinopathy, arthritis, rheumatoid arthritis, a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic infectious disease, HIV, Aids, infection with sars virus, Asian (chicken) flu virus, herpes simplex, herpes zoster, hepatitis, viral hepatitis, a cardiovascular disease, coronary artery stenosis, carotid artery stenosis, intermittent claudication, a dermatological condition, acne, psoriasis, a disease characterised by begin or
malignant cellular hyperproliferation or by areas of neovascularisation, a benign or malignant tumour, early cancer, cervical dysplasia, or cancer of the blood, cervix, naso-pharynx, trachea, larynx, bronchi, bronchioles, bladder, esophagus, stomach, rectum, colon, prostate, hollow organs, bile duct, ureter, kidney, uterus, vaginal or other female adnexa, comprising administering a therapeutically effective amount of chlorin e4-Zn complex to a human or animal in need thereof.

The sixth aspect of the present invention also provides a method of photodynamic therapy or cytoluminescent therapy of a human or animal disease, comprising administering a therapeutically effective amount of chlorin e4-Zn complex to a human or animal in need thereof. Preferably, the human or animal disease is atherosclerosis, multiple sclerosis, diabetes, diabetic retinopathy, arthritis, rheumatoid arthritis, a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic infectious disease, HIV, Aids, infection with sars virus, Asian (chicken) flu virus, herpes simplex, herpes zoster, hepatitis, viral hepatitis, a cardiovascular disease, coronary artery stenosis, carotid artery stenosis, intermittent claudication, a dermatological condition, acne, psoriasis, a disease characterised by begin or malignant cellular hyperproliferation or by areas of neovascularisation, a benign or malignant tumour, early cancer, cervical dysplasia, or cancer of the blood, cervix, naso-pharynx, trachea, larynx, bronchi, bronchioles, bladder, esophagus, stomach, rectum, colon, prostate, hollow organs, bile duct, ureter, kidney, uterus, vaginal or other female adnexa. Preferably, the human or animal disease is characterised by begin or malignant cellular hyperproliferation or by areas of neovascularisation, a benign or malignant tumour.

The sixth aspect of the present invention further provides a method of photodynamic diagnosis of a human or animal disease, comprising administering a diagnostically effective amount of chlorin e4-Zn complex to a human or animal. Preferably, the human or animal disease is atherosclerosis, multiple sclerosis, diabetes, diabetic retinopathy, arthritis, rheumatoid arthritis, a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic infectious disease, HIV, Aids, infection with sars virus, Asian (chicken) flu virus, herpes simplex, herpes zoster, hepatitis, viral hepatitis, a cardiovascular disease, coronary artery stenosis, carotid
artery stenosis, intermittent claudication, a dermatological condition, acne, psoriasis, a disease characterised by begin or malignant cellular hyperproliferation or by areas of neovascularisation, a benign or malignant tumour, early cancer, cervical dysplasia, or cancer of the blood, cervix, naso-pharynx, trachea, larynx, bronchi, bronchioles, bladder, esophagus, stomach, rectum, colon, prostate, hollow organs, bile duct, ureter, kidney, uterus, vaginal or other female adnexa. Preferably, the human or animal disease is characterised by begin or malignant cellular hyperproliferation or by areas of neovascularisation. Preferably, the human or animal disease is a begin or malignant tumour. Preferably, the method of photodynamic diagnosis is for the fluorescent or phosphorescent detection of the said diseases, preferably for the fluorescent or phosphorescent detection and quantification of the said diseases.

In any of the methods of the sixth aspect of the present invention, the human or animal is preferably further subjected to irradiation or sound simultaneous with or after the administration of the chlorin e4-Zn complex. Preferably, the human or animal is subjected to irradiation after the administration of the chlorin e4-Zn complex. If the method is a method of photodynamic therapy or cytoluminescent therapy, then the human or animal is preferably subjected to irradiation 10 to 100 hours after administration of the chlorin e4-Zn complex, preferably 50 to 90 hours, preferably about 72 hours.

If the method is a method of photodynamic diagnosis, then the human or animal is preferably subjected to irradiation 3 to 60 hours after administration of the chlorin e4-Zn complex, preferably 8 to 40 hours.

Preferably, the irradiation is electromagnetic radiation with a wavelength in the range of from 500nm to 1000nm, preferably from 600nm to 900nm, preferably from 620nm to 820nm, preferably from 630nm to 710nm.

Preferably, the human or animal is a human.
A seventh aspect of the present invention provides a method of cold sterilising a surgical or other device, comprising the steps of: providing chlorm e4-Zn complex on the device and subjecting the device to irradiation or sound. Preferably, the device is subjected to irradiation or sound simultaneously with or after provision of the chlorm e4-Zn complex on the device, preferably the device is subjected to irradiation after provision of the chlorm e4-Zn complex on the device. Preferably, the irradiation is electromagnetic radiation with a wavelength in the range of from 500nm to 1000nm, preferably from 600nm to 900nm, preferably from 620nm to 820nm, preferably from 630nm to 710nm.

An eighth aspect of the present invention provides chlorm e4-Zn complex, linked or attached to a magnetic element. Preferably, the magnetic element is Gd, Fe or Mn. Preferably, the chlorm e4-Zn complex is for use as an MRI enhancer.

A ninth aspect of the present invention provides a method of carrying out an MRI scan, the method comprising using the chlorm e4-Zn complex of the eighth aspect of the present invention as an MRI enhancer.

A tenth aspect of the present invention provides use of the chlorm e4-Zn complex of the eighth aspect of the present invention as an MRI enhancer.

**Brief description of the drawings**

Figure 1 depicts schematically the metalation and demetalation of a porphyrin ring.

Figure 2 depicts schematically the reaction of chlorm e4 with zinc stearate to form chlorm e4-Zn complex.

Figure 3 shows a UV spectrum of chlorm e4-Zn complex in methanol recorded on a Shimadzu UV-1601 PC spectrophotometer with a 1nm bandwidth and a scanning speed of 120nm/mm.
Figure 4 shows a fluorescence spectrum of chlorin e4-Zn complex recorded on a Perkin Elmer MPF-44B-PC Fluorimeter with a 1nm bandwidth following excitation at 416nm.

Figure 5 shows fluorescence spectra of chlorin e4-Zn complex recorded on a Perkin Elmer MPF-44B-PC Fluorimeter with a 1nm bandwidth following excitation at 320nm, 416nm, 518nm, 561nm, 600nm, 635nm and 645nm.

Figure 6 shows an absorption spectrum (1) and a fluorescence spectrum (2) of chlorin e4-Zn complex.

Figure 7 shows a fluorescence spectrum of tetra-(p-sulfonatophenyl)porphine (TSPP) recorded on a Perkin Elmer MPF-44B-PC Fluorimeter with a 1nm bandwidth following excitation at 416nm.

Figure 8 depicts schematically the reaction of 1,3-diphenylisobenzofuran (DPIBF) with two singlet oxygens ($^1O_2$).

Figure 9 shows absorption spectra of a DPIBF / chlorin e4-Zn complex mixture before light irradiation (1) and after light irradiation (2).

Figure 10 depicts schematically the detection of singlet oxygen.

Figure 11 shows the intensity of singlet oxygen fluorescence from chlorin e4-Zn complex.

Figure 12 is a graph showing the levels of blood parameters ALT, AST and ALP.

Figure 13 is a graph showing the levels of blood parameters RBC, WBC and PLT.
Detailed description of the invention

Metal insertion into the central part of a porphyrin ring is known as metalation and the general chemical process is depicted in Figure 1.

Porphyrins and chlorins can form complexes with a wide variety of metals (M). These metal complexes not only protect the inner nitrogen atoms from electrophilic reagents and strong bases, but also have a pronounced effect on the reactivity of the macrocycles. The most common geometry of these complexes is octahedral, with the metal ions occupying the centre of the N₄ porphyrin plane and the metal ligands being in trans positions (Figure 1).

Metalation can be accomplished by reaction with a metal salt or complex (such as metal acetates, halides, hydrides, acetylacetonates and carbonyls) in an organic solvent, such as chloroform, toluene or JV,N-dimethylformamide (DMF). Demetalation is performed under acidic conditions. The strength of the acid required for the demetalation depends upon the stability of the metal complex.

In accordance with the present invention, chlorin e4-Zn complex was prepared by reacting chlorin e4 with zinc stearate dissolved in JV,N-dimethylformamide (DMF) in the presence of a catalyst such as tetrabutylammonium hydroxide (Figure 2). The yield of the final product was 82%, which is higher compared to known metalation methods (57-63%). The synthesis is easier to perform and can be done at room temperature, stirring the mixture in an open flask.

Since chlorin e4-Zn complex shows low levels of toxicity, no skin photosensitivity (LD₁₀-LD₅₀ acute toxicity tests) and a high yield (0.9) of singlet oxygen (¹O₂) (quantum yield of singlet oxygen (¹O₂)), it is a promising agent for PDT treatments.
Experimental details

Example - general synthetic details

0.15-lmmol of chlorin e4 is dissolved in 60-100mI of IV,IV-dimethylfbrmamide (DMF). To this solution, 15-20ml of tetrabutylammonium hydroxide (IM methanol solution) is added. Separately, 1-12mmol of zinc stearate is dissolved in 40-60ml of N,N-dimethylformamide (DMF), and the latter solution is added to the previous one. The mixture is stirred until the synthesis is complete after about 3 hours. Then the mixture is filtered through a 45µm Millipore filter. The product is purified by flash chromatography with a methanol mobile phase. Then the solution is distilled and lyophilized. In a preferred embodiment of the present invention, the reaction is carried out at a temperature in the range of 25-80°C.

Example - synthesis of chlorin e4-Zn complex

0.5mmol (276mg) of chlorin e4 were dissolved in 60ml of N,N-dimethylformamide (DMF), and the solution was stirred slowly for 10 minutes at 40°C. 15ml of tetrabutylammonium hydroxide (IM methanol solution) was added, and stirring was continued for a further 20 minutes. 1mmol (378mg) of zinc stearate were dissolved in 50ml of N,N-dimethylformamide (DMF), and the solution was stirred at 40°C. The zinc stearate solution was added to the chlorin e4 solution, and the reaction mixture was stirred slowly for 3 hours at 40°C. Then the reaction mixture was filtered through a 45µm Millipore filter. The product was purified by flash
chromatography on a dry silica gel column using a methanol mobile phase. Then the solution was distilled and the product isolated as a dry powder by lyophilization.

Chemical Formula: \( C_{33}H_{34}N_{4}O_{4}Zn \)

Exact Mass: 614.19

Molecular Weight: 616.06

\( m/z \): 614.19 (100.0%), 616.18 (57.4%), 618.18 (39.0%), 615.19 (36.2%), 617.19 (29.6%), 619.19 (15.3%), 616.19 (7.6%), 618.19 (7.2%), 620.19 (2.8%), 620.18 (1.5%), 615.18 (1.5%)

Elemental Analysis: C, 64.34; H, 5.56; N, 9.09; O, 10.39; Zn, 10.62

\(^1\)H NMR: see Table 1

\(^{13}\)C NMR: see Table 2

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<tr>
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<td>5.25</td>
<td>1-ethylene</td>
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Table 1 - \(^1\)H NMR
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<th>Comments</th>
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</tr>
<tr>
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<td>1-imine</td>
</tr>
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</tr>
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<td>aliphatic</td>
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<td>1-ethylene</td>
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<tr>
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<td>15.1</td>
<td>-2.3</td>
<td>aliphatic</td>
</tr>
</tbody>
</table>

Table 2 - $^{13}$C NMR
Example - UV spectrometry

Chlorin e4-Zn complex was dissolved in methanol at a concentration of 1.62mM (lmg/ml). The solution was kept at 2-8°C in an open tube for a period of 2 weeks and UV spectra were taken periodically. During this 2 week period, there were no changes in the recorded UV spectra, showing the stability of the complex and the absence of any oxidation or degradation processes. A Shimadzu UV-1 601 PC spectrophotometer with a 1nm bandwidth and a scanning speed of 120nm/min was used for the spectrum readings.

30µl of chlorin e4-Zn solution was diluted with methanol up to 3ml (total volume) and UV spectra were taken using a cuvette with a lcm bypass. The chlorin e4-Zn complex has two major peaks of absorption within the Sore band at 417nm and 647nm respectively with fluctuations ± 2nm (Figure 3).

Example - fluorimetry

Fluorimetry is one of a few validation methods mostly used to determine the purity of photosensitive compounds. According to Beer-Lambert's law each photosensitive molecule has its own (only one) identifiable fluorescence spectrum, so-called 'finger-print'. If a photosensitive sample consists of two different molecules, this would result in two different fluorescence spectra.

A sample of chlorin e4-Zn complex was dissolved in methanol at a concentration of 1.62mM (lmg/ml), and then 30µl of this solution was diluted with methanol up to 3ml (total volume). First, the chlorin e4-Zn complex solution was excited at its biggest peak of absorption within the Sore band (416nm), using a fluorimetry cuvette with a lcm bypass, and a fluorescence spectrum was taken on a Perkin Elmer MPF-44B-PC Fluorimeter within the 600-750nm range (Figure 4).

Then, the solution sample was excited at all the peaks of absorption present in the spectrometry reading scale (320nm, 416nm, 518nm, 561nm, 600nm, 635nm, 645nm) and fluorescence spectra were taken within the 600-700nm range (Figure 5).
From Figure 6 it can be seen that the peaks in the fluorescence spectrum at 655nm (high peak) and 711nm (small peak) are mirror-like, symmetrical curves of the peaks in the absorption spectrum at 600nm and 647nm respectively, with a small 8nm shift towards the longer wavelength. According to fluorimetry law, such mirror-like absorption/fluorescence spectra (Figure 6) and also identical fluorescence spectra (Figure 5) taken from different excitation wavelengths, indicate the presence of a highly pure chemical compound.

Example - quantum yield of fluorescence

For fluorescence quantum yield studies, tetra-(p-sulfonatophenyl)porphine (TSPP) was used as the standard dye. The fluorescence quantum yield of TSPP is known and constitutes 0.1 ± 0.001 relative units.

Both, chlorin e4-Zn complex and TSPP (standard dye), samples were prepared with an identical optical density of 0.1 relative units. Then the samples were excited at the same wavelength of 416nm. The fluorescence intensity of TSPP is shown in Figure 7.

Comparative results showed that chlorin e4-Zn complex has a fluorescence intensity of 0.4 ± 0.04 compared to TSPP’s 0.1 ± 0.001 (± 5% tolerance), which is four times higher. This proves that chlorin e4-Zn complex is a highly fluorescent dye and emits a large quantity of photons. Hence, chlorin e4-Zn complex can be used efficiently as a fluorescent dye for diagnostic purposes.

Example - quantum yield of singlet oxygen (\(^{1}O_2\))

To identify novel photosensitizers for photodynamic therapy (PDT), the ability of a number of photosensitive dyes to produce singlet oxygen (\(^{1}O_2\)), a potentially cytotoxic compound, in solution was examined. The experiments were performed in N,N-dimethylformamide (DMF) using 1,3-diphenylisobenzofuran (DPIBF) as the scavenger which chemically binds to two singlet oxygens (Figure 8). The chemical
reaction of singlet oxygen with 1,3-diphenylisobenzofuran (DPIBF) caused photobleaching of the latter, lowering its highest peaks of absorption. Two different approaches were used to determine the quantum yield of singlet oxygen.

1. **Indirect calculations**

A mixture of DPIBF and chlorin e4-Zn complex was irradiated with red non-coherent (LED) light at 648nm for 3 minutes. DPIBF had one peak of absorption in methanol solution at 410nm. An absorption spectrum of the DPIBF / chlorin e4-Zn complex mixture was taken before and after light irradiation (Figure 9).

This approach was used to indirectly detect the singlet oxygen ($^1$O$_2$) quantum yield of chlorin e4-2n complex. Calculations were made by means of a comparison of two separate photobleaching processes: (1) DPIBF and (2) DPIBF / chlorin e4-Zn complex mixture. The quantum yield of singlet oxygen for DPIBF is known and comprises 0.7 ± 0.007 relative units. During photoreaction of the DPIBF / chlorin e4-Zn complex mixture, the peak at 410nm became 1.4 smaller as a result of DPIBF oxidation by singlet oxygen, showing that chlorin e4-Zn complex produces 1.4 times more singlet oxygen than DPIBF.

2. **Direct measurements**

The second approach used direct measurements of singlet oxygen fluorescence decay time at 1270nm wavelength after a short laser light flash.

As a light source, a diode laser (650nm) with a 20 nanosecond gap pulse mode was chosen. The diode laser was operated in pulsed mode with a peak output power of 300 mW, which generated a prompt dye fluorescence. Since the lifetime of singlet oxygen is longer than the dye fluorescence, it was possible to detect the singlet oxygen presence after the diode laser was turned off (Figure 10). The singlet oxygen fluorescence was detected with a signal amplifier and optical filtering of singlet oxygen emission at 1270nm.
The intensity of singlet oxygen fluorescence from chlorin e4-Zn complex was recorded (Figure 11), and then compared with the fluorescence intensity of DPIBF, and further calculations were made.

Direct measurements showed again a 1.4 higher singlet oxygen yield from chlorin e4-Zn complex, and this data correlates with the results from the first approach using indirect calculations.

The intensity of singlet oxygen fluorescence from chlorin e4-Zn complex is 0.9 ± 0.05 relative units, which is very high (approaching 1). It shows that chlorin e4-Zn complex can generate a large quantity of singlet oxygen and can be used for PDT treatments.

Example - LD$_{10}$, LD$_{50}$ and LD$_{99}$ trials

In order to evaluate the toxicity levels of chlorin e4-Zn complex (LD$_{10}$, LD$_{50}$), the complex was administered orally to rats. The rats were divided into three experimental groups (1$^{st}$ group (215.9 mg/kg); 2$^{nd}$ group (416.7 mg/kg); 3$^{rd}$ group (875.3 mg/kg)) and one control group for reference (10 animals in each group).

Following administration of the chlorin e4-Zn complex, the rats were exposed to sunlight for 6 hours daily and were observed for 72 hours after administration. The results showed that the mortality rate in each group (1, 2 and 3) was 10%, 30% and 70% respectively.

Final results were obtained through calculations and LD$_{10}$, LD$_{50}$ and LD$_{99}$ doses were calculated as 393.5 mg/kg, 597.4 mg/kg and 907.0 mg/kg respectively.

The histology from different organs between the control and the high dose groups showed no significant changes apart from minor inflammation in tissue slides from heart, liver and lung. No necrotic or markedly affected cells were observed in the high dose experimental group. Significant blood cell changes were observed in the 3$^{rd}$ group.
Materials and Methods:

Animals: Wistar rats (males/females) were used for LD$_{10}$, LD$_{50}$ and LD$_{99}$ studies.

Photosensitizer: Encapsulated dry powder which contained 10mg of chlorin e4-Zn complex.

Administration: The complex was administered orally (directly into the stomach) by a special plunger device. After administration the rats were exposed to sunlight for 6 hours each day and subsequently observed for the next 72 hours.

Calculations: All calculations of LD$_{10}$, LD$_{50}$ and LD$_{99}$ points were based on mathematical equations.

Procedure: 30 rats were divided into 3 groups (10 rats in each group). Administration doses were calculated from the pre-test results. 4 capsules (40mg) were administered orally to each rat from the 1st group, 8 capsules (80mg) to each rat from the 2nd group and 16 capsules (160mg) to each rat from the 3rd group respectively. Before administration, each rat was weighed and average body weights were calculated (see Table 3). The animals were kept during the day under the sunlight for 6 hours after administration and were observed for 72 hours afterwards. Dead rats were counted in each group and mortality rates calculated (see Table 3).

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Average body weight</th>
<th>How many died</th>
<th>Mortality rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>215.9</td>
<td>182</td>
<td>1</td>
<td>10%</td>
</tr>
<tr>
<td>2</td>
<td>416.7</td>
<td>192</td>
<td>3</td>
<td>30%</td>
</tr>
<tr>
<td>3</td>
<td>875.3</td>
<td>182</td>
<td>7</td>
<td>70%</td>
</tr>
</tbody>
</table>

Table 3

The following equation was used to calculate the LD$_{50}$ dose:

$$\text{LD}_{50} = \log - 1[X_{m} - I(\sum p - 0.5)]$$

A linear regression equation was used:

$$\text{Probit (Y)} = -3.3561 + 3.0098 \times \ln(x)$$

Therefore the LD$_{50}$ dose was calculated as:

LD$_{50} = 597.4$ mg/kg
Similar to LD$_{50}$, the LD$_{10}$ and LD$_{99}$ doses were calculated as:

\[
\text{LD}_{10} = 393.5 \text{ mg/kg} \\
\text{LD}_{99} = 907.0 \text{ mg/kg}
\]

**Histology:** For histology assays, tissue samples from the lungs, liver, brain, kidney and heart were taken from dead rats of the high dose group only. Standard H.E. necrosis dye was used to stain microscopy slides. The histology slides showed no marked sign of necrosis or cell damage, but a minor inflammatory process in the liver, heart and lung organs.

**Example - acute toxicity tests**

For acute toxicity tests, the following blood parameters were studied: ALT, AST, ALP, RBC, WBC, and PLT. Blood samples were taken before and 24 hours after chlorin e4-Zn complex administration respectively. Results are presented in Tables 4 and 5 and Figures 12 and 13.

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (u/L)</th>
<th>AST (u/L)</th>
<th>ALP (u/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>171.3±26.9</td>
<td>49.0±5.4</td>
<td>270.0±36.0</td>
</tr>
<tr>
<td>1 (215.9 mg/kg)</td>
<td>178.3±39.0</td>
<td>50.2±14.5</td>
<td>297.2±15.8</td>
</tr>
<tr>
<td>2 (416.7 mg/kg)</td>
<td>215.6±92.3</td>
<td>53.6±22.9</td>
<td>341.7±93.3</td>
</tr>
<tr>
<td>3 (875.3 mg/kg)</td>
<td>380.7±109.6</td>
<td>117.7±70.8</td>
<td>447.3±67.0</td>
</tr>
</tbody>
</table>

**Table 4**

<table>
<thead>
<tr>
<th>Group</th>
<th>RBC (x10$^{12}$/L)</th>
<th>WBC (x10$^9$/L)</th>
<th>PLT (x10$^{12}$/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.26±0.65</td>
<td>11.47±1.32</td>
<td>13.66±1.25</td>
</tr>
<tr>
<td>1 (215.9 mg/kg)</td>
<td>5.77±0.73</td>
<td>9.23±1.04</td>
<td>11.62±1.82</td>
</tr>
<tr>
<td>2 (416.7 mg/kg)</td>
<td>4.56±0.70</td>
<td>5.39±1.18</td>
<td>10.41±1.12</td>
</tr>
<tr>
<td>3 (875.3 mg/kg)</td>
<td>3.93±0.51</td>
<td>3.80±0.40</td>
<td>9.07±1.1</td>
</tr>
</tbody>
</table>

**Table 5**

Acute toxicity tests showed no sign of significant toxic reactions on doses below 215 mg/kg. At the same time, a level of acute toxicity was achieved on doses from
873 mg/kg. A highly toxic dose caused a 37% decrease in red blood cells, a 67% decrease in white blood cells and a 33% decrease in platelet cells.

It will be understood that the present invention has been described above by way of example only. The examples are not intended to limit the scope of the invention. Various modifications and embodiments can be made without departing from the scope and spirit of the invention, which is defined by the following claims only.
Claims

1. A process of preparing chlorin e4-Zn complex:

   comprising the step of reacting chlorin e4 with a zinc compound in the presence of a tetraalkylammonium salt.

2. The process of claim 1, comprising the step of adding a solution of the zinc compound to a solution of chlorin e4 and the tetraalkylammonium salt.

3. The process of claim 1 or 2, wherein chlorin e4, the tetraalkylammonium salt and the zinc compound are used in a ratio of 0.15-1 : 15-20 : 1-12.

4. The process of any one of the preceding claims, wherein the zinc compound is a carboxylic acid zinc salt, a zinc halide, zinc hydride, or zinc acetylacetonate.

5. The process of claim 4, wherein the carboxylic acid zinc salt is zinc stearate or zinc acetate.

6. The process of claim 5, wherein the carboxylic acid zinc salt is zinc stearate.

7. The process of any one of the preceding claims, wherein the tetraalkylammonium salt is tetrabutylammonium hydroxide.
8. The process of any one of the preceding claims, wherein the solvent comprises N,N-dimethylformamide (DMF), methanol, chloroform, dimethylsulfoxide (DMSO) or toluene.

9. The process of claim 8, wherein the solvent comprises N,N-dimethylformamide (DMF) or methanol.

10. The process of any one of the preceding claims, wherein the metalation reaction is carried out at a temperature in the range of 25-80°C.

11. The process of any one of the preceding claims, wherein the metalation reaction is carried out over 1 to 12 hours.

12. The process of any one of the preceding claims, wherein the chlorin e4-Zn complex is purified by filtering.

13. The process of any one of the preceding claims, wherein the chlorin e4-Zn complex is purified by chromatography.

14. Chlorin e4-Zn complex when prepared by a process of any one of claims 1 to 13.

15. A pharmaceutical composition, comprising chlorin e4-Zn complex when prepared by a process of any one of claims 1 to 13, and a pharmaceutically acceptable carrier or diluent.

16. A pharmaceutical composition, comprising chlorin e4-Zn complex and a pharmaceutically acceptable carrier or diluent, for the treatment of atherosclerosis, multiple sclerosis, diabetes, diabetic retinopathy, arthritis, rheumatoid arthritis, a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic infectious disease, HIV, Aids, infection with sars virus, Asian (chicken) flu virus, herpes simplex, herpes zoster, hepatitis, viral hepatitis, a cardiovascular disease, coronary artery stenosis, carotid artery stenosis, intermittent claudication, a dermatological condition, acne,
psoriasis, a disease characterised by begin or malignant cellular hyperproliferation or by areas of neovascularisation, a benign or malignant tumour, early cancer, cervical dysplasia, or cancer of the blood, cervix, naso-pharynx, trachea, larynx, bronchi, bronchioles, bladder, esophagus, stomach, rectum, colon, prostate, hollow organs, bile duct, ureter, kidney, uterus, vaginal or other female adnexa.

17. A pharmaceutical composition, comprising chlorin e4-Zn complex and a pharmaceutically acceptable carrier or diluent, for use in photodynamic therapy or cytoluminescent therapy.

18. The pharmaceutical composition of claim 17, for the treatment of atherosclerosis, multiple sclerosis, diabetes, diabetic retinopathy, arthritis, rheumatoid arthritis, a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic infectious disease, HIV, Aids, infection with sars virus, Asian (chicken) flu virus, herpes simplex, herpes zoster, hepatitis, viral hepatitis, a cardiovascular disease, coronary artery stenosis, carotid artery stenosis, intermittent claudication, a dermatological condition, acne, psoriasis, a disease characterised by begin or malignant cellular hyperproliferation or by areas of neovascularisation, a benign or malignant tumour, early cancer, cervical dysplasia, or cancer of the blood, cervix, naso-pharynx, trachea, larynx, bronchi, bronchioles, bladder, esophagus, stomach, rectum, colon, prostate, hollow organs, bile duct, ureter, kidney, uterus, vaginal or other female adnexa.

19. The pharmaceutical composition of claim 17 or 18, for the treatment of a disease characterised by begin or malignant cellular hyperproliferation or by areas of neovascularisation.

20. The pharmaceutical composition of any one of claims 17 to 19, for the treatment of a benign or malignant tumour.

21. A pharmaceutical composition, comprising chlorin e4-Zn complex and a pharmaceutically acceptable carrier or diluent, for use in photodynamic diagnosis.
22. The pharmaceutical composition of claim 21, for the detection of atherosclerosis, multiple sclerosis, diabetes, diabetic retinopathy, arthritis, rheumatoid arthritis, a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic infectious disease, HIV, Aids, infection with sars virus, Asian (chicken) flu virus, herpes simplex, herpes zoster, hepatitis, viral hepatitis, a cardiovascular disease, coronary artery stenosis, carotid artery stenosis, intermittent claudication, a dermatological condition, acne, psoriasis, a disease characterised by begin or malignant cellular hyperproliferation or by areas of neovascularisation, a benign or malignant tumour, early cancer, cervical dysplasia, or cancer of the blood, cervix, naso-pharynx, trachea, larynx, bronchi, bronchioles, bladder, esophagus, stomach, rectum, colon, prostate, hollow organs, bile duct, ureter, kidney, uterus, vaginal or other female adnexa.

23. The pharmaceutical composition of claim 21 or 22, for the detection of an area that is affected by begin or malignant cellular hyperproliferation or by neovascularisation.

24. The pharmaceutical composition of any one of claims 21 to 23, for the detection of a begin or malignant tumour.

25. The pharmaceutical composition of any one of claims 21 to 24, for the fluorescent or phosphorescent detection of the said diseases.

26. The pharmaceutical composition of claim 25, for the fluorescent or phosphorescent detection and quantification of the said diseases.

27. The pharmaceutical composition of any one of claims 15 to 26, wherein the pharmaceutical composition is adapted for administration simultaneous with or prior to administration of irradiation or sound.

28. The pharmaceutical composition of claim 27, wherein the pharmaceutical composition is adapted for administration prior to administration of irradiation.
29. The pharmaceutical composition of claim 28, wherein the pharmaceutical composition is for use in photodynamic therapy or cytoluminescent therapy, and wherein the pharmaceutical composition is adapted for administration 10 to 100 hours before the irradiation.

30. The pharmaceutical composition of claim 28, wherein the pharmaceutical composition is for use in photodynamic diagnosis, and wherein the pharmaceutical composition is adapted for administration 3 to 60 hours before the irradiation.

31. The pharmaceutical composition of any one of claims 27 to 30, wherein the irradiation is electromagnetic radiation with a wavelength in the range of from 500nm to 1000nm.

32. The pharmaceutical composition of any one of claims 15 to 31, wherein the pharmaceutical composition is in a form suitable for oral, parental (including intravenous, subcutaneous, intramuscular, intradermal, intratracheal, intraperitoneal, intraarticular, intraabdominal, intracranial and epidural), transdermal, airway (aerosol), rectal, vaginal or topical (including buccal, mucosal and sublingual) administration.

33. The pharmaceutical composition of claim 32, wherein the pharmaceutical composition is in a form suitable for oral administration.

34. The pharmaceutical composition of claim 33, wherein the pharmaceutical composition is provided in the form of a tablet, capsule, hard or soft gelatine capsule, caplet, troche or lozenge, as a powder or granules, or as an aqueous solution, suspension or dispersion.

35. The pharmaceutical composition of claim 33 or 34, wherein the pharmaceutical composition is in a form suitable for providing 0.01 to 10 mg/kg/day of the chlorin e4-Zn complex.
36. The pharmaceutical composition of claim 32, wherein the pharmaceutical composition is in a form suitable for parental administration.

37. The pharmaceutical composition of claim 36, wherein the pharmaceutical composition is in a form suitable for intravenous administration.

38. The pharmaceutical composition of claim 36 or 37, wherein the pharmaceutical composition is an aqueous solution or suspension having a pH of from 6 to 8.5.

39. Chlorin e4-Zn complex, for the treatment of atherosclerosis, multiple sclerosis, diabetes, diabetic retinopathy, arthritis, rheumatoid arthritis, a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic infectious disease, HIV, Aids, infection with sars virus, Asian (chicken) flu virus, herpes simplex, herpes zoster, hepatitis, viral hepatitis, a cardiovascular disease, coronary artery stenosis, carotid artery stenosis, intermittent claudication, a dermatological condition, acne, psoriasis, a disease characterised by begin or malignant cellular hyperproliferation or by areas of neovascularisation, a benign or malignant tumour, early cancer, cervical dysplasia, or cancer of the blood, cervix, naso-pharynx, trachea, larynx, bronchi, bronchioles, bladder, esophagus, stomach, rectum, colon, prostate, hollow organs, bile duct, ureter, kidney, uterus, vaginal or other female adnexa.

40. Chlorin e4-Zn complex, for use in photodynamic therapy or cytoluminescent therapy.

41. The chlorin e4-Zn complex of claim 40, for the treatment of atherosclerosis, multiple sclerosis, diabetes, diabetic retinopathy, arthritis, rheumatoid arthritis, a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic infectious disease, HIV, Aids, infection with sars virus, Asian (chicken) flu virus, herpes simplex, herpes zoster, hepatitis, viral hepatitis, a cardiovascular disease, coronary artery stenosis, carotid artery stenosis, intermittent claudication, a dermatological condition, acne, psoriasis, a disease characterised by begin or malignant cellular hyperproliferation or by areas of neovascularisation, a benign or malignant tumour, early cancer, cervical...
dysplasia, or cancer of the blood, cervix, naso-pharynx, trachea, larynx, bronchi, bronchioles, bladder, esophagus, stomach, rectum, colon, prostate, hollow organs, bile duct, ureter, kidney, uterus, vaginal or other female adnexa.

42. The chlorin e4-Zn complex of claim 40 or 41, for the treatment of a disease characterised by benign or malignant cellular hyperproliferation or by areas of neovascularisation.

43. The chlorin e4-Zn complex of any one of claims 40 to 42, for the treatment of a benign or malignant tumour.

44. Chlorin e4-Zn complex, for use in photodynamic diagnosis.

45. The chlorin e4-Zn complex of claim 44, for the detection of atherosclerosis, multiple sclerosis, diabetes, diabetic retinopathy, arthritis, rheumatoid arthritis, a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic infectious disease, HIV, Aids, infection with sars virus, Asian (chicken) flu virus, herpes simplex, herpes zoster, hepatitis, viral hepatitis, a cardiovascular disease, coronary artery stenosis, carotid artery stenosis, intermittent claudication, a dermatological condition, acne, psoriasis, a disease characterised by benign or malignant cellular hyperproliferation or by areas of neovascularisation, a benign or malignant tumour, early cancer, cervical dysplasia, or cancer of the blood, cervix, naso-pharynx, trachea, larynx, bronchi, bronchioles, bladder, esophagus, stomach, rectum, colon, prostate, hollow organs, bile duct, ureter, kidney, uterus, vaginal or other female adnexa.

46. The chlorin e4-Zn complex of claim 44 or 45, for the detection of an area that is affected by benign or malignant cellular hyperproliferation or by neovascularisation.

47. The chlorin e4-Zn complex of any one of claims 44 to 46, for the detection of a benign or malignant tumour.
48. The chlorin e4-Zn complex of any one of claims 44 to 47, for the fluorescent or phosphorescent detection of the said diseases.

49. The chlorin e4-Zn complex of claim 48, for the fluorescent or phosphorescent detection and quantification of the said diseases.

50. The chlorin e4-Zn complex of any one of claims 39 to 49, wherein the chlorin e4-Zn complex is adapted for administration simultaneous with or prior to administration of irradiation or sound.

51. The chlorin e4-Zn complex of claim 50, wherein the chlorin e4-Zn complex is adapted for administration prior to administration of irradiation.

52. The chlorin e4-Zn complex of claim 51, wherein the chlorin e4-Zn complex is for use in photodynamic therapy or cytoluminescent therapy, and wherein the chlorin e4-Zn complex is adapted for administration 10 to 100 hours before the irradiation.

53. The chlorin e4-Zn complex of claim 51, wherein the chlorin e4-Zn complex is for use in photodynamic diagnosis, and wherein the chlorin e4-Zn complex is adapted for administration 3 to 60 hours before the irradiation.

54. The chlorin e4-Zn complex of any one of claims 50 to 53, wherein the irradiation is electromagnetic radiation with a wavelength in the range of from 500nm to 1000nm.

55. Use of chlorin e4-Zn complex for the manufacture of a medicament for the treatment of atherosclerosis, multiple sclerosis, diabetes, diabetic retinopathy, arthritis, rheumatoid arthritis, a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic infectious disease, HIV, Aids, infection with sars virus, Asian (chicken) flu virus, herpes simplex, herpes zoster, hepatitis, viral hepatitis, a cardiovascular disease, coronary artery stenosis, carotid artery stenosis, intermittent claudication, a dermatological condition, acne, psoriasis, a disease characterised by begin or
malignant cellular hyperproliferation or by areas of neovascularisation, a benign or malignant tumour, early cancer, cervical dysplasia, or cancer of the blood, cervix, naso-pharynx, trachea, larynx, bronchi, bronchioles, bladder, esophagus, stomach, rectum, colon, prostate, hollow organs, bile duct, ureter, kidney, uterus, vaginal or other female adnexa.

56. Use of chlorin e4-Zn complex for the manufacture of a phototherapeutic agent for use in photodynamic therapy or cytoluminescent therapy.

57. The use of claim 56, wherein the phototherapeutic agent is for the treatment of atherosclerosis, multiple sclerosis, diabetes, diabetic retinopathy, arthritis, rheumatoid arthritis, a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic infectious disease, HIV, Aids, infection with sars virus, Asian (chicken) flu virus, herpes simplex, herpes zoster, hepatitis, viral hepatitis, a cardiovascular disease, coronary artery stenosis, carotid artery stenosis, intermittent claudication, a dermatological condition, acne, psoriasis, a disease characterised by begin or malignant cellular hyperproliferation or by areas of neovascularisation, a benign or malignant tumour, early cancer, cervical dysplasia, or cancer of the blood, cervix, naso-pharynx, trachea, larynx, bronchi, bronchioles, bladder, esophagus, stomach, rectum, colon, prostate, hollow organs, bile duct, ureter, kidney, uterus, vaginal or other female adnexa.

58. The use of claim 56 or 57, wherein the phototherapeutic agent is for the treatment of a disease characterised by begin or malignant cellular hyperproliferation or by areas of neovascularisation.

59. The use of any one of claims 56 to 58, wherein the phototherapeutic agent is for the treatment of a begin or malignant tumour.

60. Use of chlorin e4-Zn complex for the manufacture of a photodiagnostic agent for use in photodynamic diagnosis.
61. The use of claim 60, wherein the photodiagnostic agent is for the detection of atherosclerosis, multiple sclerosis, diabetes, diabetic retinopathy, arthritis, rheumatoid arthritis, a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic infectious disease, HIV, Aids, infection with sars virus, Asian (chicken) flu virus, herpes simplex, herpes zoster, hepatitis, viral hepatitis, a cardiovascular disease, coronary artery stenosis, carotid artery stenosis, intermittent claudication, a dermatological condition, acne, psoriasis, a disease characterised by begin or malignant cellular hyperproliferation or by areas of neovascularisation, a benign or malignant tumour, early cancer, cervical dysplasia, or cancer of the blood, cervix, naso-pharynx, trachea, larynx, bronchi, bronchioles, bladder, esophagus, stomach, rectum, colon, prostate, hollow organs, bile duct, ureter, kidney, uterus, vaginal or other female adnexa.

62. The use of claim 60 or 61, wherein the photodiagnostic agent is for the detection of an area that is affected by begin or malignant cellular hyperproliferation or by neovascularisation.

63. The use of any one of claims 60 to 62, wherein the photodiagnostic agent is for the detection of a begin or malignant tumour.

64. The use of any one of claims 60 to 63, wherein the photodiagnostic agent is for the fluorescent or phosphorescent detection of the said diseases.

65. The use of claim 64, wherein the photodiagnostic agent is for the fluorescent or phosphorescent detection and quantification of the said diseases.

66. The use of any one of claims 55 to 65, wherein the medicament, phototherapeutic agent or photodiagnostic agent is adapted for administration simultaneous with or prior to administration of irradiation or sound.

67. The use of claim 66, wherein the medicament, phototherapeutic agent or photodiagnostic agent is adapted for administration prior to administration of irradiation.
68. The use of claim 67, wherein the medicament or phototherapeutic agent is adapted for administration 10 to 100 hours before the irradiation.

69. The use of claim 67, wherein the photodiagnostic agent is adapted for administration 3 to 60 hours before the irradiation.

70. The use of any one of claims 66 to 69, wherein the irradiation is electromagnetic radiation with a wavelength in the range of from 500nm to 1000nm.

71. A method of treating atherosclerosis, multiple sclerosis, diabetes, diabetic retinopathy, arthritis, rheumatoid arthritis, a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic infectious disease, HIV, Aids, infection with sars virus, Asian (chicken) flu virus, herpes simplex, herpes zoster, hepatitis, viral hepatitis, a cardiovascular disease, coronary artery stenosis, carotid artery stenosis, intermittent claudication, a dermatological condition, acne, psoriasis, a disease characterised by begin or malignant cellular hyperproliferation or by areas of neovascularisation, a benign or malignant tumour, early cancer, cervical dysplasia, or cancer of the blood, cervix, naso-pharynx, trachea, larynx, bronchi, bronchioles, bladder, esophagus, stomach, rectum, colon, prostate, hollow organs, bile duct, ureter, kidney, uterus, vaginal or other female adnexa, comprising administering a therapeutically effective amount of chlorin e4-Zn complex to a human or animal in need thereof.

72. A method of photodynamic therapy or cytoluminescent therapy of a human or animal disease, comprising administering a therapeutically effective amount of chlorin e4-Zn complex to a human or animal in need thereof.

73. The method of claim 72, wherein the human or animal disease is atherosclerosis, multiple sclerosis, diabetes, diabetic retinopathy, arthritis, rheumatoid arthritis, a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic infectious disease, HIV, Aids, infection with sars virus, Asian (chicken) flu virus, herpes simplex, herpes zoster, hepatitis, viral hepatitis, a cardiovascular disease, coronary artery stenosis, carotid artery stenosis, intermittent claudication, a
dermatological condition, acne, psoriasis, a disease characterised by benign or malignant cellular hyperproliferation or by areas of neovascularisation, a benign or malignant tumour, early cancer, cervical dysplasia, or cancer of the blood, cervix, naso-pharynx, trachea, larynx, bronchi, bronchioles, bladder, esophagus, stomach, rectum, colon, prostate, hollow organs, bile duct, ureter, kidney, uterus, vaginal or other female adnexa.

74. The method of claim 72 or 73, wherein the human or animal disease is characterised by benign or malignant cellular hyperproliferation or by areas of neovascularisation.

75. The method of any one of claims 72 to 74, wherein the human or animal disease is a benign or malignant tumour.

76. A method of photodynamic diagnosis of a human or animal disease, comprising administering a diagnostically effective amount of chlorin e4-Zn complex to a human or animal.

77. The method of claim 76, wherein the human or animal disease is atherosclerosis, multiple sclerosis, diabetes, diabetic retinopathy, arthritis, rheumatoid arthritis, a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic infectious disease, HIV, Aids, infection with sars virus, Asian (chicken) flu virus, herpes simplex, herpes zoster, hepatitis, viral hepatitis, a cardiovascular disease, coronary artery stenosis, carotid artery stenosis, intermittent claudication, a dermatological condition, acne, psoriasis, a disease characterised by benign or malignant cellular hyperproliferation or by areas of neovascularisation, a benign or malignant tumour, early cancer, cervical dysplasia, or cancer of the blood, cervix, naso-pharynx, trachea, larynx, bronchi, bronchioles, bladder, esophagus, stomach, rectum, colon, prostate, hollow organs, bile duct, ureter, kidney, uterus, vaginal or other female adnexa.
78. The method of claim 76 or 77, wherein the human or animal disease is characterised by benign or malignant cellular hyperproliferation or by areas of neovascularisation.

79. The method of any one of claims 76 to 78, wherein the human or animal disease is a benign or malignant tumour.

80. The method of any one of claims 76 to 79, for the fluorescent or phosphorescent detection of the said diseases.

81. The method of claim 80, for the fluorescent or phosphorescent detection and quantification of the said diseases.

82. The method of any one of claims 71 to 81, wherein the human or animal is further subjected to irradiation or sound simultaneous with or after the administration of the chlorin e4-Zn complex.

83. The method of claim 82, wherein the human or animal is subjected to irradiation after the administration of the chlorin e4-Zn complex.

84. The method of claim 83, wherein the method is a method of photodynamic therapy or cytoluminescent therapy, and wherein the human or animal is subjected to irradiation 10 to 100 hours after administration of the chlorin e4-Zn complex.

85. The method of claim 83, wherein the method is a method of photodynamic diagnosis, and wherein the human or animal is subjected to irradiation 3 to 60 hours after administration of the chlorin e4-Zn complex.

86. The method of any one of claims 82 to 85, wherein the irradiation is electromagnetic radiation with a wavelength in the range of from 500nm to 1000nm.

87. The method of any one of claims 71 to 86, wherein the human or animal is a human.
88. A method of cold sterilising a surgical or other device, comprising the steps of: providing chlorin e4-Zn complex on the device and subjecting the device to irradiation or sound.

89. The method of claim 88, wherein the device is subjected to irradiation or sound simultaneously with or after provision of the chlorin e4-Zn complex on the device.

90. The method of claim 89, wherein the device is subjected to irradiation after provision of the chlorin e4-Zn complex on the device.

91. The method of any one of claims 88 to 90, wherein the irradiation is electromagnetic radiation with a wavelength in the range of from 500nm to 1000nm.

92. Chlorin e4-Zn complex, linked or attached to a magnetic element.

93. The chlorin e4-Zn complex of claim 92, wherein the magnetic element is Gd, Fe or Mn.

94. The chlorin e4-Zn complex of claim 92 or 93, for use as an MRI enhancer.

95. A method of carrying out an MRI scan, the method comprising using the chlorin e4-Zn complex of claim 92 or 93 as an MRI enhancer.

96. Use of the chlorin e4-Zn complex of claim 92 or 93 as an MRI enhancer.
Figure 5

Figure 6
Figure 7
Figure 8

Figure 9
Figure 12

Figure 13
### A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/409 A61K41/00 C07D487/22 C07F3/06 A61P35/00 A61K49/00 A61K49/10 A61L2/10

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)
A61K C07D C07F A61L

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 practical, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, WPI Data

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Date of the actual completion of the international search
29 January 2009

Date of mailing of the international search report
06/02/2009

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Authorized officer
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