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(71) Applicant: THE HONG KONG UNIVERSITY OF SCIENCE AND TECHNOLOGY [CN/CN]; Clear Water Bay, Kowloon, Hong Kong (CN).

(72) Inventors: TANG, Benzong; C/o The Hong Kong University Of Science And, Technology, Clear Water Bay, Kowloon, Hong Kong (CN). LI, Qiyao; C/o The Hong Kong University Of Science And, Technology, Clear Water Bay, Kowloon, Hong Kong (CN). WANG, Jiaguo; C/o The Hong Kong University Of Science And, Technology, Clear Water Bay, Kowloon, Hong Kong (CN).

(74) Agent: TEE&HOWE INTELLECTUAL PROPERTY ATTORNEYS; Suite 1, 6-12, 5th Floor, Tower W1, The Tower Offices, Oriental Plaza, No.1 East Chang'an Avenue, Dongcheng District, Beijing 100738 (CN).

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(54) Title: AIE-ACTIVE PHOTSENSITIZER FOR SELECTIVE BACTERIAL ELIMINATION AND CANCER CELL AB-LATION

(57) Abstract: An AIE-based photosensitizer (PS) can be used for selective killing of cancer cells and elimination of bacterial pathogens under white light irradiation. The PS exhibits moderate water solubility, high ROS efficiency with bright emission, good biocompatibility, high specificity to targets, low dark toxicity, significant light toxicity, and efficient singlet oxygen (1O_2) generation. The PS shows aggregation-induced ROS generation (AIROSG) effect.



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AIE-ACTIVE PHOTSENSITIZER FOR SELECTIVE BACTERIAL ELIMINATION AND CANCER CELL ABLATION

FIELD

The present subject matter relates generally to a photosensitizer for photodynamic therapy and, particularly, to an AIE-active photosensitizer for eliminating bacteria and killing cancer cells.

BACKGROUND

Pathogen infections cause severe illness and significant mortality globally. For many decades since the first discovery of penicillin in 1928, antibiotics have been employed predominately to treat diseases caused by bacteria. However, antibiotics suffer from confined targets and common side effects such as diarrhea, nausea and upset stomach. Additionally, after a long period of abuse of antibiotics, the emergence of drug-resistant bacteria poses a severe threat to human health. A recent study reported that about 70% of normal *E. coli* became drug resistant only after 3 hours of exposure to antibiotics. Further, development of new antibiotics fails to keep pace with the generation of drug-resistant bacteria. Therefore, developing alternative antibacterial therapeutics is of utmost urgency and great importance to clinical application.

Cancer, meanwhile, is another major public health problem. Globally, about 1 in 6 deaths are due to cancer. In 2019, about 1,762,450 new cancer cases and about 606,880 cancer-related deaths were expected in the United States. To date, although various therapeutic modalities such as surgery, chemotherapy and radiotherapy have been exploited, each of these modalities is associated with risks and/or harmful side effects. Specifically, surgery can be risky as it is invasive to the human body. Further, surgery often fails to ablate tumors completely and precisely without causing harm to healthy tissues. Chemotherapy drugs, such as Cisplatin, can lead to nephrotoxicity, increased risk of infection, and drug resistance. Patients who receive radiotherapy treatment, on the other hand, may suffer from fatigue and loss of hair in the treatment area.

Recently, photodynamic therapy (PDT) has emerged as a promising method to combat pathogenic bacteria as well as a powerful clinical protocol for cancer treatment. PDT employs photosensitizers (PSs) to produce destructive singlet oxygen ($^1\text{O}_2$) or other reactive oxygen species (ROS) from endogenous molecular oxygen under light irradiation. Thus, combining the

merits of a PS and light irradiation, PDT exhibits several conspicuous advantages including noninvasiveness, no drug resistance, low cytotoxicity, selective targeting, spatiotemporal precision and synergistic effect over conventional therapeutic modalities. Among major achievements in PDT advancement, fluorescence-guided PDT (FL-PDT), with the unique advantage of real-time monitoring, has attracted increasing attention. PS, which serves as the key component of FL-PDT, plays a decisive role in therapeutic effects. Most conventional organic PSs, however, encounter the problem of aggregation-caused quenching (ACQ) in biosystems, resulting in undesired weak emission and poor ROS generation. Luckily, the discovery of the aggregation-induced emission (AIE) phenomenon offers a convenient solution. Unlike ACQ luminophores, AIE luminogens are weakly emissive in dilute solutions. However, they show strong emission and ROS generation in the aggregate state, owing to the mechanism of restriction of intramolecular motion (RIM). Thus, AIE-based PSs hold promising potential in PDT applications. Although research on AIE-based PSs in anticancer and antibacterial treatments proceeds rapidly, various problems remain to be solved. One common problem is that to realize selective binding, specific targeting ligands must be introduced to the PSs. Such a method is not only tedious in synthesis but also is of high cost. Another big challenge is that for most existing PSs, it is quite difficult to manipulate their targets by merely adjusting the external conditions without changing their molecular structure.

Therefore, an AIE PS without additional modification, efficient $^1\text{O}_2$ generation, and pathogen and cancer cell selectivity is highly desirable.

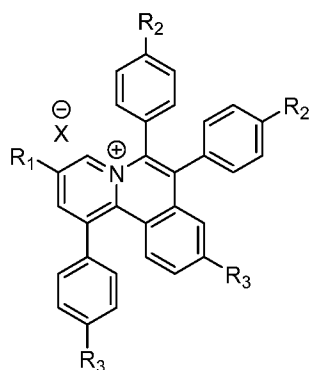
SUMMARY

The present subject matter relates to an AIE-based photosensitizer (PS) that can be used for the selective killing of cancer cells and elimination of bacterial pathogens under white light irradiation. The PS can exhibit moderate water solubility, high ROS efficiency with bright emission, good biocompatibility, high specificity to targets, low dark toxicity, significant light toxicity, and efficient singlet oxygen ($^1\text{O}_2$) generation. The PS can show an aggregation-induced ROS generation (AIROSG) effect.

The PS can recognize and eliminate different targets under different conditions without modification of the structure of the PS. For example, when the PS is incubated with a first target for a first period of time, the PS can kill or eliminate the first target under white light irradiation.

In addition, when the PS is incubated with a second target for a second period of time, the PS can kill or eliminate the second target under white light irradiation. For example, in the presence of white light irradiation, the PS can eliminate drug-resistant *E. coli* infection efficiently, leaving host tissues unaffected. By lengthening the incubation time to 12 hours, the PS can kill cancer cells with only slight harm to normal cells.

In an embodiment, the photosensitizer (PS) comprises a compound having the following backbone structural formula:

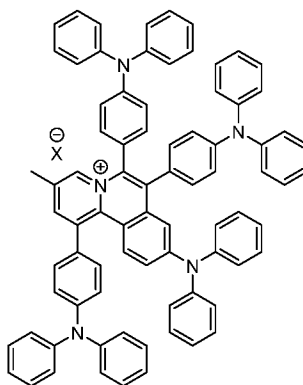


wherein R_1 is selected from the group consisting of H and an alkyl group;

wherein each R_2 and R_3 is independently selected from the group consisting of H, alkyl, unsaturated alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, and diphenyl amine; and

X^- is selected from the group consisting of PF_6^- , BF_4^- , CH_3COO^- , CF_3COO^- , ClO_4^- , F^- , Cl^- , Br^- , I^- , $(F_3CSO_2)N^-$, and PO_4^{3-} .

In an embodiment, the compound is:



In an embodiment, a method of generating singlet oxygen can include irradiating the

compound with white light.

In an embodiment, a method of killing cancer cells can include incubating a target cancer cell with the compound and subjecting the target cancer cell to white light irradiation while the compound is contacting the target cancer cell to kill the target cancer cell.

In an embodiment, a method of killing a target pathogen can include incubating the target pathogen with the compound and subjecting the target pathogen to white light irradiation while the compound is contacting the target pathogen to kill the target pathogen.

BRIEF DESCRIPTION OF DRAWINGS

Various embodiments will now be described in detail with reference to the accompanying drawings.

Figs. 1A-1B depict 1(A) PL spectra of 4TPA-BQ in DMSO/water mixtures with different water fractions (f_w); 1(B) plot of the relative emission intensity (I/I_0) versus f_w . Inset: fluorescence images of 4TPA-BQ in solution ($f_w = 0\%$) and aggregate ($f_w = 70\%$) states under irradiation from a hand-held UV lamp. Excitation wavelength: 380 nm; Concentration: 10 μ M.

Fig. 2 depicts UV-vis spectra of 4TPA-BQ in DMSO solution.

Fig. 3 depicts a plot of particle size distribution of 4TPA-BQ aggregates in DMSO/water mixtures with $f_w = 70\%$.

Figs. 4A-4D depict 4(A) anion- π^+ and F-H interactions; 4(B) dihedral angles; 4(C) intermolecular hydrogen bonding; and 4(D) C-H... π interactions in the single crystal structure of TTPABQ. aE is defined as interaction energy between π^+ and anion calculated based on the single-crystal structure by single-point calculations using M062X/6-31+G(d,p) method.

Fig. 5 depicts crystal stacking of 4TPA-BQ.

Figs. 6A-6E depict 6(A) UV-vis spectra of DHPA without PS; 6(B) for 4TPA-BQ in solution state; 6(C) for Ce6; 6(D) for Rose Bengal; and 6(E) for 4TPA-BQ in aggregate state under white light irradiation in DMSO/water mixtures with $f_w = 99\%$. $[PS] = 9 \times 10^{-6}$ M, $[DHPA] = 4.5 \times 10^{-5}$ M.

Figs. 7A-7D depict 7(A) UV-vis spectra spectrum of RB and 4TPA-BQ; 7(B) UV-vis spectra of DHPA with RB; 7(C) 4TPA-BQ under white light irradiation in DMSO/water mixtures with $f_w = 99\%$; and 7(D) Decomposition rate of DHPA with 4TPA-BQ and RB, respectively. $[DHPA] = 5 \times [PS]$, recording time interval: 30 s.

Figs. 8A-8B depict 8(A) nanosecond transient absorption (ns-TA) spectra of 4TPA-BQ in argon-saturated water solution; and 8(B) triplet lifetime of 4TPA-BQ in argon-saturated water solution. Excitation wavelength: 355 nm.

Figs. 9A-9D depict 9(A) plot of relative absorbance of DHPA without and with different PSs under white light irradiation, where A_0 and A are the absorbance of DHPA at 378 nm before and after white light irradiation, respectively. $[PS] = 9 \times 10^{-6}$ M, $[DHPA] = 4.5 \times 10^{-5}$ M; 9(B) decomposition rate of DHPA with 4TPA-BQ aggregates and RB, respectively; 9(C) calculated energy level diagram between singlet and triplet states; and 9(D) the natural transition orbitals (NTOs) of 4TPA-BQ.

Fig. 10 depicts CLSM images of *E. coli* and *S. epiderimidis* incubated with 5 μ M 4TPA-BQ for 15 min.

Fig. 11 depicts CLSM images of COS-7 cells and HLF cells incubated with 5 μ M 4TPA-BQ for 15 min.

Fig. 12 depicts morphology of *E. coli* and *S. epiderimidis* incubated with 5 μ M 4TPA-BQ with and without white light irradiation. Control: no treatments; Dark: treated with 4TPA-BQ only; PDT: treated with 4TPA-BQ and white light irradiation.

Figs. 13A-13F depict 13(A) killing efficiency of 4TPA-BQ on *E. coli*; 13(B) killing efficiency of 4TPA-BQ on *S. epiderimidis*; 13(C) killing efficiency of 4TPA-BQ on ampicillin-resistant *E. coli*; 13(D) photographs of *E. coli* and *S. epiderimidis* cultured on agar plate supplemented with 5 μ M 4TPA-BQ; and 13(E)-13(F) cell viability of COS-7 and HLF cells incubated with 4TPA-BQ for 15 min.

Fig. 14 depicts CLSM images of *E. coli* and HLF cells and *S. epiderimidis* and HLF cells stained with PI after incubation with 5 μ M 4TPA-BQ under white light irradiation. The dash lines indicate HLF cells.

Figs. 15A-15D depict antibacterial activity against ampicillin-resistant *E. coli* infection *in vivo*. 15(A) depicts photographs of bacterial infected mice burn wounds treated with PBS only (Control group), 4TPA-BQ only (Dark group) and 4TPA-BQ + white light irradiation (PDT group), respectively. 15(B) depicts changes of the infected wound size subjected to different treatments; 15(C) depicts plate photographs of bacterial amount of skin wound and organs at day 5 in 100-fold dilution; and 15(D) depicts hemotoxylin and eosin stain of the infected skin slices of day 5 and day 10. B: blood vessel; H: hair folliculus.

Figs. 16A-16B depict CLSM images of 16(A) COS-7 cells and 16(B) HeLa cells incubated with 10 μ M 4TPA-BQ under different staining time.

Figs. 17A-17B depict CLSM images of 17(A) COS-7 cells and 17(B) HeLa cells incubated with 10 μ M 4TPA-BQ under different staining time followed by white light irradiation.

DETAILED DESCRIPTION

The following definitions are provided for the purpose of understanding the present subject matter and for construing the appended patent claims.

Definitions

It should be understood that the drawings described above or below are for illustration purposes only. The drawings are not necessarily to scale, with emphasis generally being placed upon illustrating the principles of the present teachings. The drawings are not intended to limit the scope of the present teachings in any way.

Throughout the application, where compositions are described as having, including, or comprising specific components, or where processes are described as having, including, or comprising specific process steps, it is contemplated that compositions of the present teachings can also consist essentially of, or consist of, the recited components, and that the processes of the present teachings can also consist essentially of, or consist of, the recited process steps.

In the application, where an element or component is said to be included in and/or selected from a list of recited elements or components, it should be understood that the element or component can be any one of the recited elements or components, or the element or component can be selected from a group consisting of two or more of the recited elements or components. Further, it should be understood that elements and/or features of a composition, an apparatus, or a method described herein can be combined in a variety of ways without departing from the spirit and scope of the present teachings, whether explicit or implicit herein

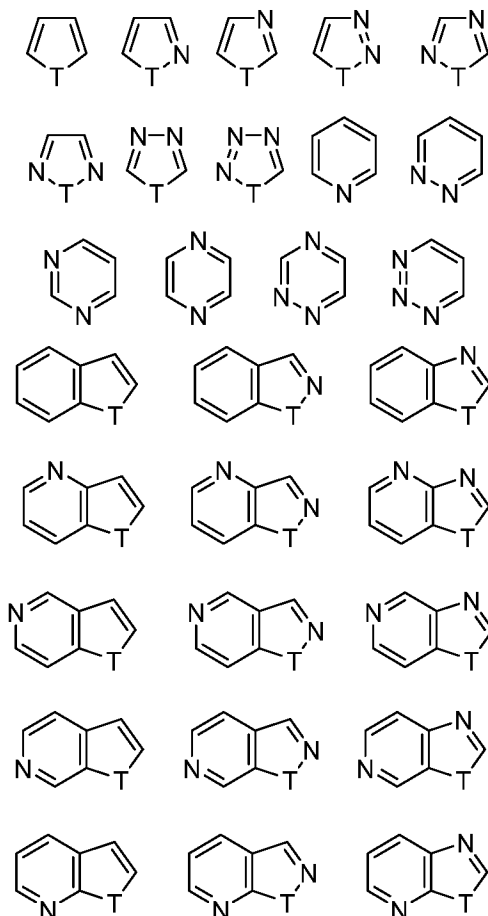
The use of the terms "include," "includes", "including," "have," "has," or "having" should be generally understood as open-ended and non-limiting unless specifically stated otherwise.

The use of the singular herein includes the plural (and vice versa) unless specifically stated otherwise. In addition, where the use of the term "about" is before a quantitative value, the present teachings also include the specific quantitative value itself, unless specifically stated

otherwise. As used herein, the term "about" refers to a $\pm 10\%$ variation from the nominal value unless otherwise indicated or inferred.

It should be understood that the order of steps or order for performing certain actions is immaterial so long as the present teachings remain operable. Moreover, two or more steps or actions may be conducted simultaneously.

As used herein, "heteroaryl" refers to an aromatic monocyclic ring system containing at least one ring heteroatom selected from oxygen (O), nitrogen (N), sulfur (S), silicon (Si), and selenium (Se) or a polycyclic ring system where at least one of the rings present in the ring system is aromatic and contains at least one ring heteroatom. Polycyclic heteroaryl groups include two or more heteroaryl rings fused together and monocyclic heteroaryl rings fused to one or more aromatic carbocyclic rings, non-aromatic carbocyclic rings, and/or non-aromatic cycloheteroalkyl rings. A heteroaryl group, as a whole, can have, for example, 5 to 22 ring atoms and contain 1 -5 ring heteroatoms (i.e., 5-20 membered heteroaryl group). The heteroaryl group can be attached to the defined chemical structure at any heteroatom or carbon atom that results in a stable structure. Generally, heteroaryl rings do not contain O—O, S—S, or S—O bonds. However, one or more N or S atoms in a heteroaryl group can be oxidized (e.g., pyridine N-oxide, thiophene S-oxide, thiophene S,S-dioxide). Examples of heteroaryl groups can include, for example, the 5- or 6-membered monocyclic and 5-6 bicyclic ring systems shown below:



where T is O, S, NH, N-alkyl, N-aryl, N-(arylalkyl) (e.g., N-benzyl), SiH₂, SiH(alkyl), Si(alkyl)₂, SiH(arylalkyl), Si(arylalkyl)₂, or Si(alkyl)(arylalkyl). Examples of such heteroaryl rings can include pyrrolyl, furyl, thienyl, pyridyl, pyrimidyl, pyridazinyl, pyrazinyl, triazolyl, tetrazolyl, pyrazolyl, imidazolyl, isothiazolyl, thiazolyl, thiadiazolyl, isoxazolyl, oxazolyl, oxadiazolyl, indolyl, isoindolyl, benzofuryl, benzothienyl, quinolyl, 2-methylquinolyl, isoquinolyl, quinoxalyl, quinazolyl, benzotriazolyl, benzimidazolyl, benzothiazolyl, benzisothiazolyl, benzisoxazolyl, benzoxadiazolyl, benzoxazolyl, cinnolinyl, 1H-indazolyl, 2H-indazolyl, indoliziny, isobenzofuy, naphthyridinyl, phthalazinyl, pteridinyl, purinyl, oxazolopyridinyl, thiazolopyridinyl, imidazopyridinyl, furopyridinyl, thienopyridinyl, pyridopyrimidinyl, pyridopyrazinyl, pyridopyridazinyl, thienothiazolyl, thienoxazolyl, thienoimidazolyl groups, and the like. Further examples of heteroaryl groups include 4,5,6,7-tetrahydroindolyl, tetrahydroquinolyl, benzothienopyridinyl, benzofuropyridinyl groups, and the like. In some embodiments, heteroaryl groups can be substituted as described herein.

As used herein, "halo" or "halogen" refers to fluoro, chloro, bromo, and iodo.

As used herein, "alkyl" refers to a straight-chain or branched saturated hydrocarbon group. Examples of alkyl groups can include methyl (Me), ethyl (Et), propyl (e.g., n-propyl and z'-propyl), butyl (e.g., n-butyl, z'-butyl, sec-butyl, *tert-butyl*), pentyl groups (e.g., n-pentyl, z'-pentyl, -pentyl), hexyl groups, and the like. In various embodiments, an alkyl group can have 1 to 40 carbon atoms (i.e., C1-40 alkyl group), for example, 1-30 carbon atoms (i.e., C1-30 alkyl group), 1-20 carbon atoms, or 1-10 carbon atoms. In some embodiments, an alkyl group can have 1 to 6 carbon atoms, and can be referred to as a "lower alkyl group." Examples of lower alkyl groups can include methyl, ethyl, propyl (e.g., n-propyl and z'-propyl), and butyl groups (e.g., n-butyl, z'-butyl, *sec-butyl*, *tert-butyl*). In some embodiments, alkyl groups can be substituted as described herein. An alkyl group is generally not substituted with another alkyl group, an alkenyl group, or an alkynyl group.

As used herein, "cycloalkyl," refers to a saturated, non-aromatic, monovalent mono- or polycarbocyclic radical of three to ten, preferably three to six carbon atoms. This term can further be exemplified by radicals such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, norbornyl, and adamantyl.

As used herein, "heterocycloalkyl" refers to a monovalent saturated 5- to 9-membered monocyclic or bicyclic ring system containing one, two or three ring heteroatoms selected from N, O and S, the remaining ring atoms being carbon atoms. In case of monocyclic heterocycloalkyl, the ring is preferably 5- or 6-membered, in case of bicyclic heterocycloalkyl, the bicyclic ring is preferably 7-, 8- or 9-membered. "Heterocycloalkyl" is unsubstituted or substituted as described herein. Examples for substituents on a heterocycloalkyl can independently be selected from alkyl, hydroxy, hydroxyalkyl, benzyl, oxo, —C(O)Oalkyl, cycloalkyl, alkylene-O-alkyl, —C(O)haloalkyl, —C(O)-alkylene-O-alkyl, cyanoalkyl, alkylene-S(O)_x-alkyl, -alkylene-C(O)N(alkyl)₂, halo, haloalkyl and alkoxy, wherein x is 0, 1, or 2.

As used herein, "heteroalkyl," refers to an alkyl group, as defined herein, in which one or more of the constituent carbon atoms have been replaced by nitrogen, oxygen, or sulfur. In some embodiments, the heteroalkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein for alkyl groups. Examples of heteroalkyl substituent groups can include an "alkoxy" which, as used herein, refers to alkyl-O— (e.g., methoxy and ethoxy). A heteroalkylene is a divalent heteroalkyl group.

As used herein, "alkenyl" refers to a straight-chain or branched alkyl group having one or more carbon-carbon double bonds. Examples of alkenyl groups can include ethenyl, propenyl, butenyl, pentenyl, hexenyl, butadienyl, pentadienyl, hexadienyl groups, and the like. The one or more carbon-carbon double bonds can be internal (such as in 2-butene) or terminal (such as in 1-butene). In various embodiments, an alkenyl group can have 2 to 40 carbon atoms (i.e., C2-40 alkenyl group), for example, 2 to 20 carbon atoms (i.e., C2-20 alkenyl group) or 2 to 20 carbon atoms (i.e., C1-10 alkenyl group). In some embodiments, alkenyl groups can be substituted as described herein. An alkenyl group is generally not substituted with another alkenyl group, an alkyl group, or an alkynyl group.

As used herein, a "fused ring" or a "fused ring moiety" refers to a polycyclic ring system having at least two rings where at least one of the rings is aromatic and such aromatic ring (carbocyclic or heterocyclic) has a bond in common with at least one other ring that can be aromatic or non-aromatic, and carbocyclic or heterocyclic. These polycyclic ring systems can be highly p-conjugated and optionally substituted as described herein.

As used herein, "heteroatom" refers to an atom of any element other than carbon or hydrogen and includes, for example, nitrogen, oxygen, silicon, sulfur, phosphorus, and selenium.

As used herein, "aryl" refers to an aromatic monocyclic hydrocarbon ring system or a polycyclic ring system in which two or more aromatic hydrocarbon rings are fused (i.e., having a bond in common with) together or at least one aromatic monocyclic hydrocarbon ring is fused to one or more cycloalkyl and/or cycloheteroalkyl rings. An aryl group can have 6 to 24 carbon atoms in its ring system (e.g., C6-24 aryl group), which can include multiple fused rings. In some embodiments, a polycyclic aryl group can have 8 to 24 carbon atoms. Any suitable ring position of the aryl group can be covalently linked to the defined chemical structure. Examples of aryl groups having only aromatic carbocyclic ring(s) can include phenyl, 1-naphthyl (bicyclic), 2-naphthyl (bicyclic), anthracenyl (tricyclic), phenanthrenyl (tricyclic), pentacenyl (pentacyclic), and like groups. Examples of polycyclic ring systems in which at least one aromatic carbocyclic ring is fused to one or more cycloalkyl and/or cycloheteroalkyl rings can include, among others, benzo derivatives of cyclopentane (i.e., an indanyl group, which is a 5,6-bicyclic cycloalkyl/aromatic ring system), cyclohexane (i.e., a tetrahydronaphthyl group, which is a 6,6-bicyclic cycloalkyl/aromatic ring system), imidazoline (i.e., a benzimidazoliny group, which is a 5,6-bicyclic cycloheteroalkyl/aromatic ring system), and pyran (i.e., a chromenyl group, which is

a 6,6-bicyclic cycloheteroalkyl/aromatic ring system). Other examples of aryl groups can include benzodioxanyl, benzodioxolyl, chromanyl, indolinyl groups, and the like. In some embodiments, aryl groups can be substituted as described herein. In some embodiments, an aryl group can have one or more halogen substituents, and can be referred to as a "haloaryl" group. Perhaloaryl groups, i.e., aryl groups where all of the hydrogen atoms are replaced with halogen atoms (*e.g.*, -C₆F₅), are included within the definition of "haloaryl." In certain embodiments, an aryl group is substituted with another aryl group and can be referred to as a biaryl group. Each of the aryl groups in the biaryl group can be substituted as disclosed herein.

As used herein, a "theranostic agent" refers to an organic material having both diagnostic and therapeutic capabilities.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which the presently described subject matter pertains.

Where a range of values is provided, for example, concentration ranges, percentage ranges, or ratio ranges, it is understood that each intervening value, to the tenth of the unit of the lower limit, unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the described subject matter. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and such embodiments are also encompassed within the described subject matter, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the described subject matter.

Throughout the application, descriptions of various embodiments use "comprising" language. However, it will be understood by one of skill in the art, that in some specific instances, an embodiment can alternatively be described using the language "consisting essentially of" or "consisting of".

For purposes of better understanding the present teachings and in no way limiting the scope of the teachings, unless otherwise indicated, all numbers expressing quantities, percentages or proportions, and other numerical values used in the specification and claims, are to be understood as being modified in all instances by the term "about". Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached

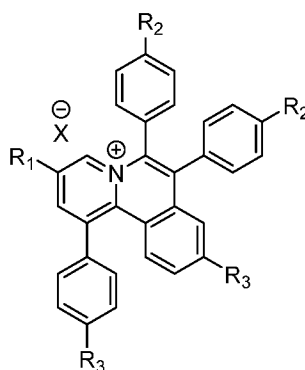
claims are approximations that may vary depending upon the desired properties sought to be obtained. At the very least, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

Photosensitizers

The present subject matter relates to a photosensitizer that includes a compound exhibiting aggregation-induced ROS generation (AIROSG) effects. Based on single crystal analysis, it is believed that anion- π^+ interactions as well as the highly twisted conformation of the compound are responsible for the AIE effect (FIGs. 4A-4D). The inherent positive charge endows the molecule with modest water solubility for bioapplications. Further, the compound exhibits a high $^1\text{O}_2$ quantum yield of up to unity.

The photosensitizer can be used in photodynamic therapy (PDT) to selectively eliminate different targets without altering the structure of the compound. For example, time-dependent photodynamic therapy (PDT) can be achieved by controlling the incubation time of the PS with the different targets. The compound can generate reactive oxygen species (ROS) in photodynamic therapy (PDT) for both selective bacterial elimination and cancer cell ablation. For example, the compound can be used as a photosensitizer in PDT to generate singlet oxygen ($^1\text{O}_2$) with a high efficiency.

In an embodiment, the photosensitizer comprises a compound having the following backbone structural formula:



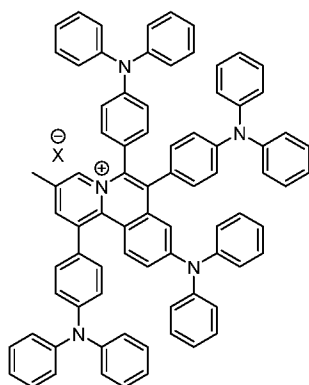
wherein R_1 is selected from the group consisting of H and an alkyl group;

wherein each R_2 and R_3 is independently selected from the group consisting of H, alkyl, unsaturated alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, and diphenyl amine; and

X^- is selected from the group consisting of PF_6^- , BF_4^- , CH_3COO^- , CF_3COO^- , ClO_4^- , F^- , Cl^- , Br^- , I^- , $(F_3CSO_2)N^-$, and PO_4^{3-} .

With respect to the structural formula of the compound provided above, it should be understood that each instance of R_2 may be the same or different and each instance of R_3 may be the same or different. Further, R_2 and R_3 may be the same or different from each other.

In an embodiment, the compound is:



4TPA-BQ.

A sufficiently small energy gap between the excited singlet state and the excited triplet state (ΔE_{ST}) can play a dominate role in the intersystem crossing (ISC) process, desired for 1O_2 generation. The present compounds can exhibit extremely high 1O_2 quantum yields in the aggregate form compared to commercial photosensitizers (PSs) (Figs. 6A-6E and Figs. 7A-7D). For example, 4TPA-BQ exhibited a 1O_2 quantum yield of about 98.7% in the aggregate form.

Selectively Killing Cancer Cells and Eliminating Pathogens

As set forth in detail herein, the present compounds can selectively target cancer cells over normal cells upon incubation with the cancer cells. The present compounds can further provide extremely high reactive oxygen species, e.g., singlet oxygen, generation efficiency upon exposure to white light irradiation. The present compounds can, thereby, act as photosensitizers and provide selective cytotoxicity to the cancer cells. In an embodiment, the present compounds can be used for cancer cell-selective ablation. One or more of the present compounds can be an effective photosensitizer in image-guided PDT.

In an embodiment, a method of killing cancer cells can include incubating a target cancer cell with one or more of the present compounds and subjecting the target cancer cell to white

light irradiation while the compound contacts the target cancer cell to kill the target cancer cell. In an embodiment, the cancer cell is incubated with the compound for about 12 hours. In an embodiment, subjecting the target cancer cell to white light irradiation can include using a power lamp with an irradiation power of about 60 mW cm^{-2} for about 30 minutes. In an embodiment, the target cancer cell is within a living animal.

For example, incubation of the cancer cells with 4TPA-BQ for 12 hours specifically killed cancer cells with low toxicity to normal cells via photodynamic therapy PDT (Figs. 11, 16A-16B and Figs. 17A-17B).

In an embodiment, a method of killing a target pathogen can include incubating the target pathogen with one or more of the present compounds and subjecting the target pathogen to white light irradiation while the compound is contacting the target pathogen to kill the target pathogen. The target pathogen can be selected from at least one of Gram-negative and Gram-positive bacteria. The target pathogen can be incubated with the compound for about 15 minutes. In an embodiment, subjecting the target cancer cell to white light irradiation can include using a power lamp with an irradiation power of about 60 mW cm^{-2} for about 30 minutes. The target pathogen can be within a living animal. The animal can be a human or a mammal.

For example, a broad-spectrum and potent antibacterial activity was attained after incubating 4TPA-BQ with bacteria for 15 min (Fig. 10). Upon light irradiation, 4TPA-BQ targeted and killed both Gram-negative and Gram-positive bacteria at a low concentration of $2 \mu\text{M}$, leaving normal cells unaffected (Fig. 12). Both *in vitro* and *in vivo* experiments demonstrated that 4TPA-BQ has outstanding bacterial eliminating performance against ampicillin-resistant *E. coli* infection (Figs. 13A-13F). It is believed that this time-dependent PDT was achieved by collaboration of electrostatic attraction and hydrophobic effect between 4TPA-BQ and the corresponding targets.

The present teachings are illustrated by the following examples.

EXAMPLES

Materials and Instruments

Characterizations: ^1H and ^{13}C NMR spectra were measured on a Bruker ARX 400 NMR spectrometer using chloroform, MeOD or DMSO as solvent and tetramethylsilane (TMS, $\delta = 0$) as internal reference. High-resolution mass spectra (HRMS) were recorded on a Finnigan MAT

TSQ 7000 Mass Spectrometer System operated in a MALDI-TOF mode. Absorption spectra were recorded on a Shimadzu UV-3600 spectrophotometer. Photoluminescence (PL) spectra were recorded on a Fluorolog[®]-3 spectrofluorometer. Single-crystal X-ray diffraction measurements were conducted on a Bruker-Nonius Smart Apex CCD diffractometer with graphite monochromated Mo K α radiation. The photoluminescence quantum yields were measured using a Hamamatsu absolute PL quantum yield spectrometer C11347 Quantaaurus-QY. Transient PL at room temperature was measured using Quantaaurus-Tau fluorescence lifetime measurement system (C11367-03, Hamamatsu Photonics Co., Japan). Powder and film X-ray diffraction was performed using a Philips PW 1830 X-ray Diffractometer.

Example 1

Synthesis of Compound 1

To an oven-dried 25 mL round-bottom flask were added Pd(PPh₃)₂Cl₂ (0.078 mmol, 58 mg), CuI (0.13 mmol, 24.8 mg), and 4-Bromotriphenylamine (1.3 mmol, 421.5mg), and the flask was purged with argon. Argon-sparged anhydrous toluene (8 mL) and DBU (7.8 mmol, 1.17 mL) were added successively by syringe. Ice-chilled trimethylsilylethyne (0.65 mmol, 92 μ L) was then added by syringe, followed immediately by distilled water (0.52 mmol, 9.36 μ L). The reaction flask was covered by aluminum foil and stirred at a high rate of speed for 20 h at 80 °C. Then the reaction mixture was partitioned in DCM and distilled water. The organic layer was washed with 10% HCl and brine and dried over MgSO₄. The crude product was purified by silica gel column chromatography with hexane/ethyl acetate (5:1, v/v) in 65% yield. ¹H NMR (400 MHz, CD₂Cl₂), δ (ppm): 7.39 – 7.31 (m, 4H), 7.34 – 7.23 (m, 8H), 7.18 – 7.02 (m, 12H), 7.03 – 6.94 (m, 4H). ¹³C NMR (100 MHz, CD₂Cl₂), δ (ppm): 147.08, 146.58, 131.59, 129.00, 128.73, 124.33, 123.14, 122.90, 121.78, 121.59, 115.71, 88.06. HRMS (MALDI-TOF): m/z: [M+H]⁺ calcd for C₃₈H₂₈N₂: 512.2252; found: 512.2235.

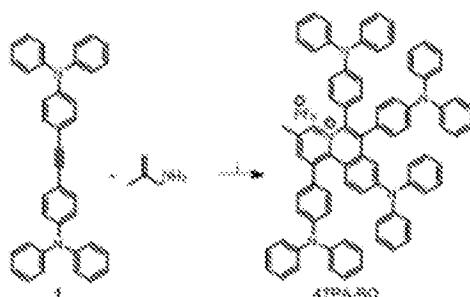
Example 2

Synthesis of 4TPA-BQ

To a 25 mL pressure vial were added 2-methylallylamine (0.15 mmol, 14.03 mg), 4,4'-(1,2-ethynediyl)bis[N,N-diphenylbenzenamine] (0.45 mmol, 229 mg), copper acetate (0.75 mmol, 149.7 mg), [Cp*RhCl₂]₂ (0.0075 mmol, 4.63 mg), tetrafluoroboric sodium (0.225 mmol, 24.7 mg) and methanol. The resulting solution was stirred at 130 °C overnight and dried over

anhydrous MgSO_4 . The crude product was purified by silica gel column chromatography with $\text{DCM}:\text{MeOH}$ (25:1, v:v) in 81% yield. ^1H NMR (400 MHz, CD_2Cl_2), δ (ppm): 8.60 (s, 1H), 7.97 (s, 1H), 7.90 (d, $J = 9.6$ Hz, 1H), 7.42 – 7.27 (m, 21H), 7.21 – 7.03 (m, 24H), 6.98 – 6.96 (m, 5H), 6.89 – 6.84 (m, 4H), 2.50 (s, 3H). ^{13}C NMR (100 MHz, CD_2Cl_2), δ (ppm): 152.03, 150.03, 149.72, 148.12, 147.91, 147.56, 147.30, 145.33, 143.39, 142.17, 139.33, 139.06, 137.01, 136.34, 133.37, 133.08, 132.35, 131.79, 131.28, 131.19, 130.50, 130.34, 130.23, 130.10, 129.95, 128.37, 127.24, 126.88, 126.15, 125.82, 125.13, 124.98, 124.60, 123.96, 123.57, 123.12, 122.10, 120.31, 118.24, 112.78, 18.94. HRMS (MALDI-TOF): m/z : $[\text{M-PF}_6]^+$ calcd for $\text{C}_{80}\text{H}_{60}\text{N}_5^+$: 1090.4843; found: 1090.4847.

An exemplary reaction scheme for preparing the 4TPA-BQ compound can include a one-step synthesis method, as provided below:



Example 3

Photophysical Properties of 4TPA-BQ

4TPA-BQ was fully characterized by NMR, high resolution mass spectrometry and single crystal X-ray diffraction analyses (Table 1). The data obtained was in good agreement with the proposed structure (Figs. 1-3, 5).

Table 1. Photophysical properties of 4TPA-BQ.

	Solution	Aggregate	Solid
λ_{ex} (nm)	490	594	570
Φ (%)	0.30	6.70	15.30
τ (ns) ^a	4.39	5.61	3.10
$k_r (\times 10^7 \text{ s}^{-1})^b$	0.07	1.19	4.93
$k_{nr} (\times 10^7 \text{ s}^{-1})^c$	22.74	16.64	27.28

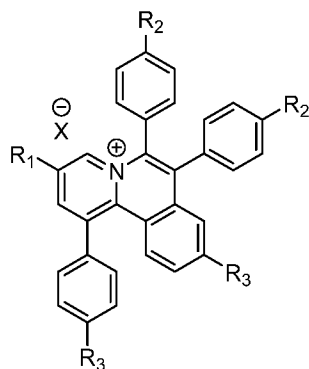
^a τ is defined as average fluorescence lifetime calculated by $\tau = \Sigma A_i(\tau_i)^2 / \Sigma A_i\tau_i$,

where A_i is the pre-exponential factor for lifetime τ_i . ^b $k_r = \Phi/\tau$. ^c $k_{nr} = 1/\tau - k_r$.

The present subject matter being thus described, it will be apparent that the same may be modified or varied in many ways. Such modifications and variations are not to be regarded as a departure from the spirit and scope of the present subject matter, and all such modifications and variations are intended to be included within the scope of the following claims.

We Claim:

1. A photosensitizer, comprising a compound having the following backbone structural formula:

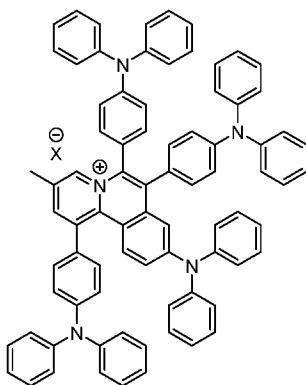


wherein R_1 is selected from the group consisting of H and an alkyl group;

wherein each R_2 and R_3 is independently selected from the group consisting of H, alkyl, unsaturated alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, and diphenyl amine; and

X^- is selected from the group consisting of PF_6^- , BF_4^- , CH_3COO^- , CF_3COO^- , ClO_4^- , F^- , Cl^- , Br^- , I^- , $(F_3CSO_2)N^-$, and PO_4^{3-} .

2. The photosensitizer according to claim 1, wherein the compound is:



3. A method of generating singlet oxygen, comprising irradiating the compound of claim 1 with white light.

4. A method of killing cancer cells, comprising:
incubating a target cancer cell with the compound of claim 1; and
subjecting the target cancer cell to white light irradiation while the compound is contacting the target cancer cell to kill the target cancer cell.

5. The method of claim 4, wherein the cancer cell is incubated with the compound for about 12 hours.

6. The method of claim 4, wherein the target cancer cell is within a living animal.

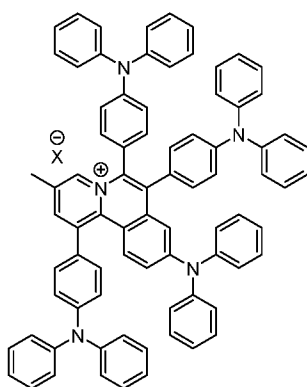
7. A method of killing a target pathogen, comprising:
incubating the target pathogen with the compound of claim 1; and
subjecting the target pathogen to white light irradiation while the compound is contacting the target pathogen to kill the target pathogen.

8. The method of claim 7, wherein the target pathogen comprises at least one of Gram-negative and Gram-positive bacteria.

9. The method of claim 7, wherein the target pathogen is incubated with the compound for about 15 minutes.

10. The method of claim 7, wherein the target pathogen is within a living animal.

11. A photosensitizer, comprising a compound having the formula:



12. A method of generating singlet oxygen, comprising irradiating the compound of claim 11 with white light.

13. A method of killing cancer cells, comprising:
incubating a target cancer cell with the compound of claim 12; and
subjecting the target cancer cell to white light irradiation while the compound is contacting the target cancer cell to kill the target cancer cell.

14. The method of claim 13, wherein the cancer cell is incubated with the compound for about 12 hours.

15. The method of claim 13, wherein the target cancer cell is within a living animal.
16. A method of killing a target pathogen, comprising:
incubating the target pathogen with the compound of claim 12; and
subjecting the target pathogen to white light irradiation while the compound is contacting the target pathogen to kill the target pathogen.
17. The method of claim 16, wherein the target pathogen comprises at least one of Gram-negative and Gram-positive bacteria.
18. The method of claim 16, wherein the target pathogen is incubated with the compound for about 15 minutes.
19. The method of claim 16, wherein the target pathogen is within a living animal.

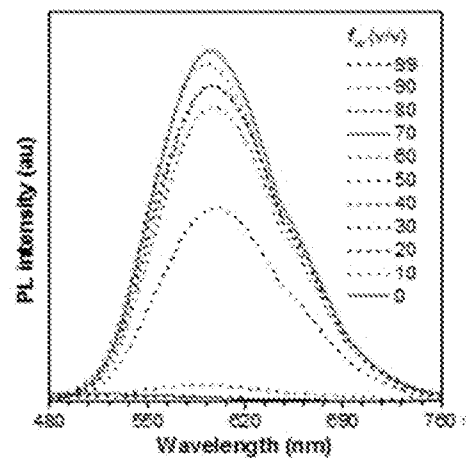


Fig. 1A

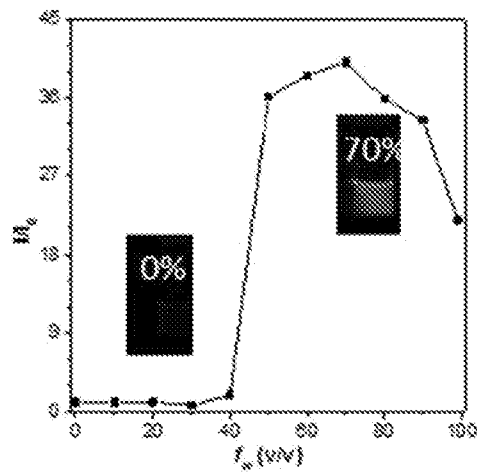


Fig. 1B

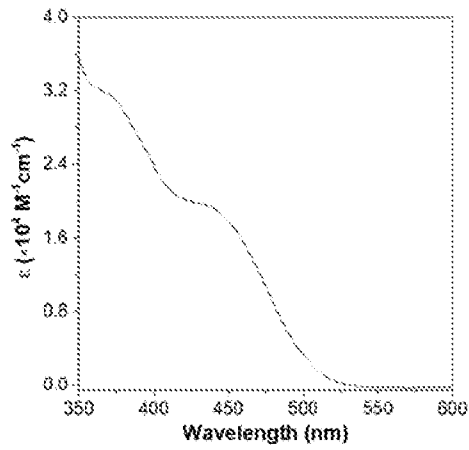


FIG. 2

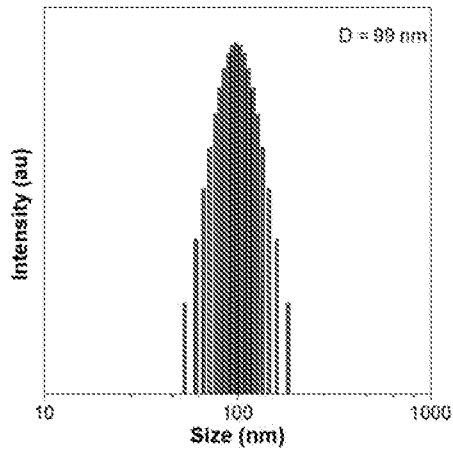


FIG. 3

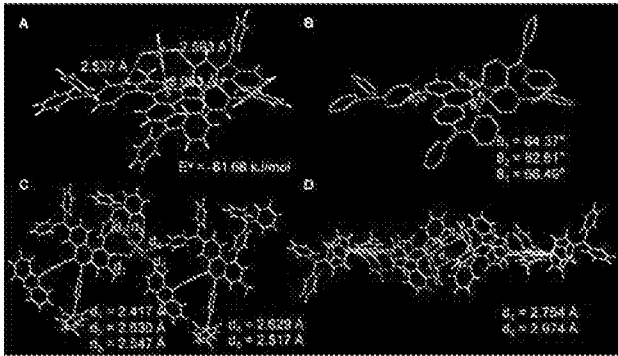


FIG. 4

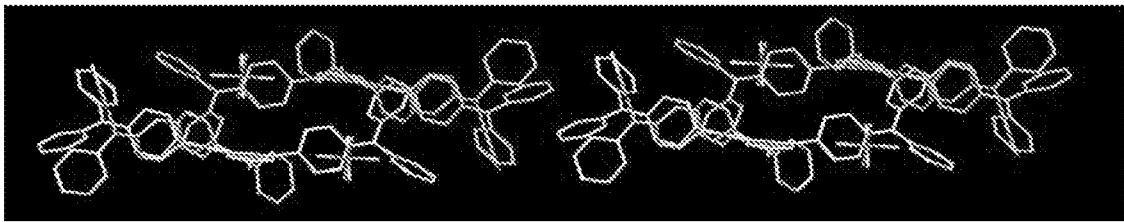
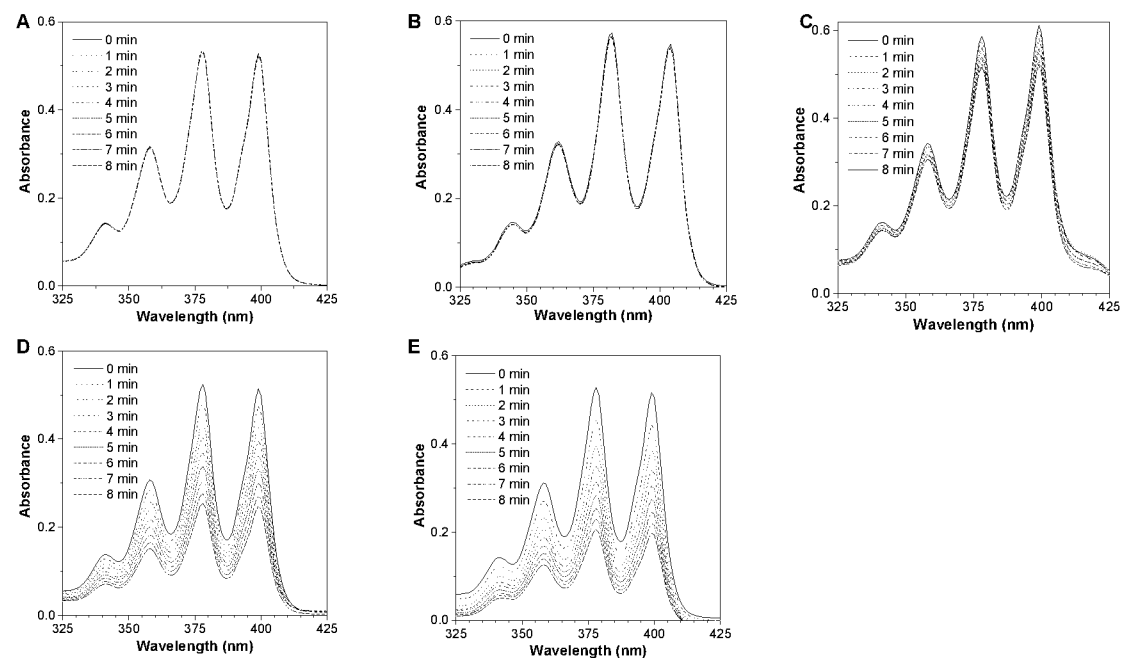
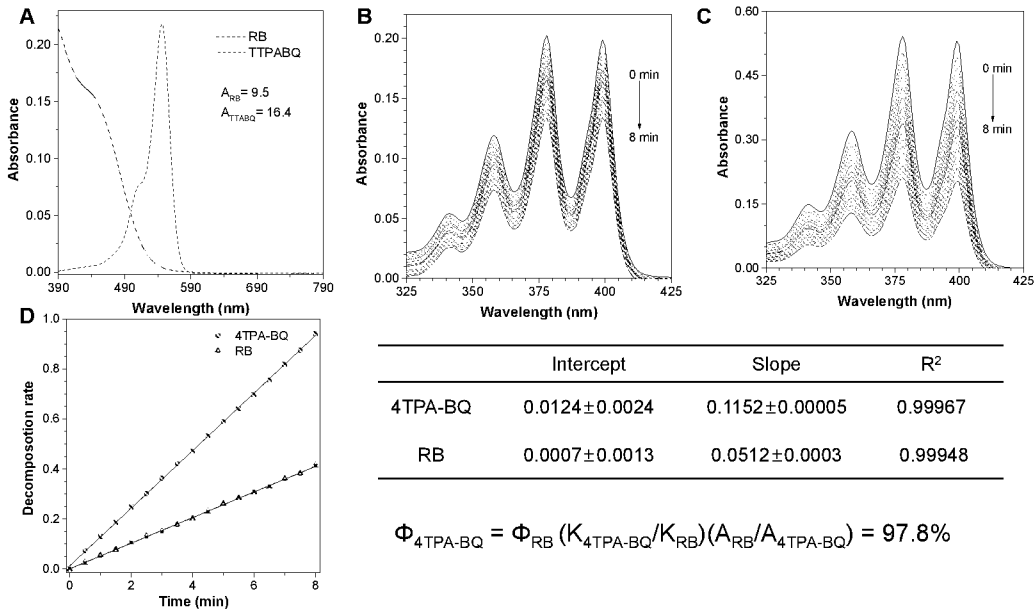


FIG. 5

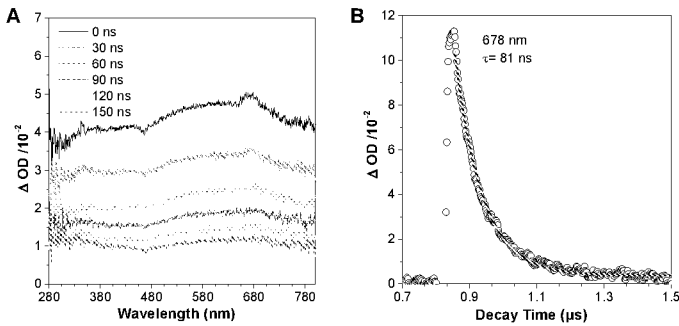


FIGs. 6A-6E

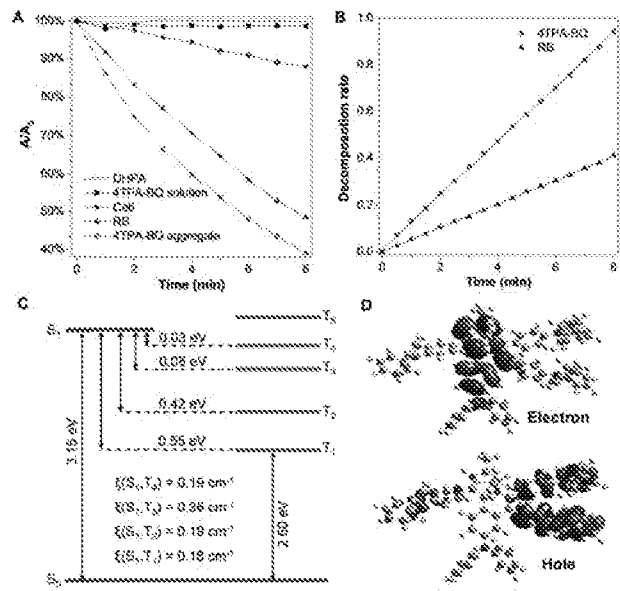


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FIGs. 7A-7D



FIGs. 8A-8B



Figs. 9A-9D

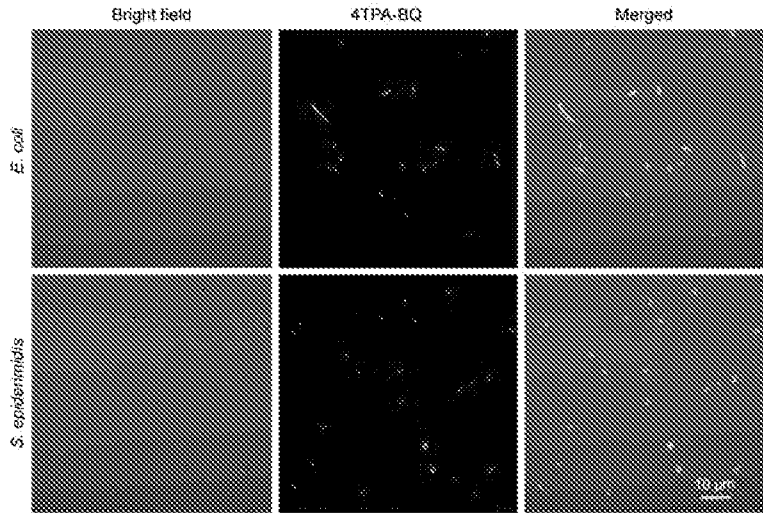


FIG. 10

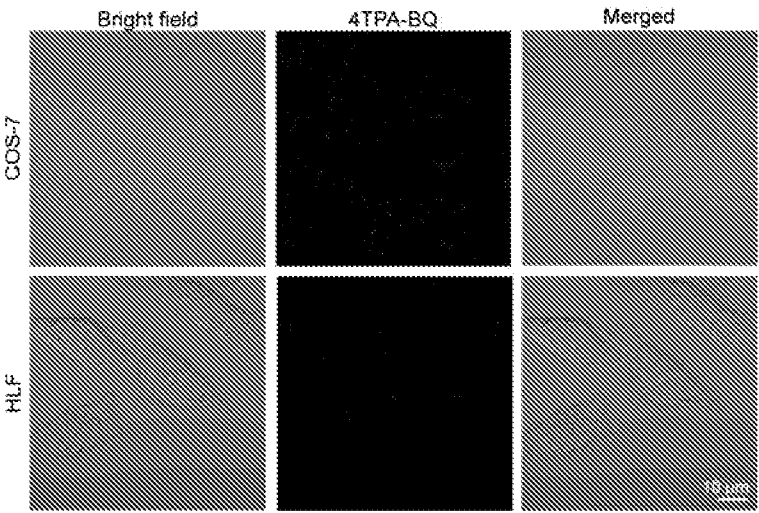


FIG. 11

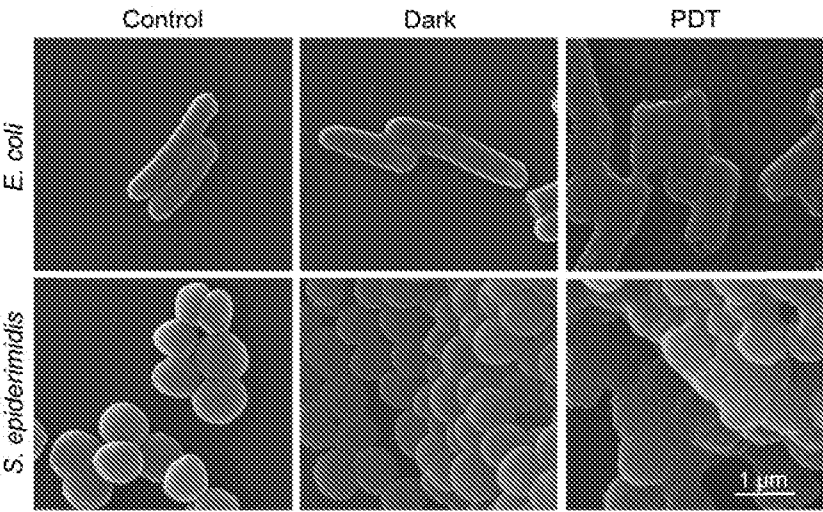
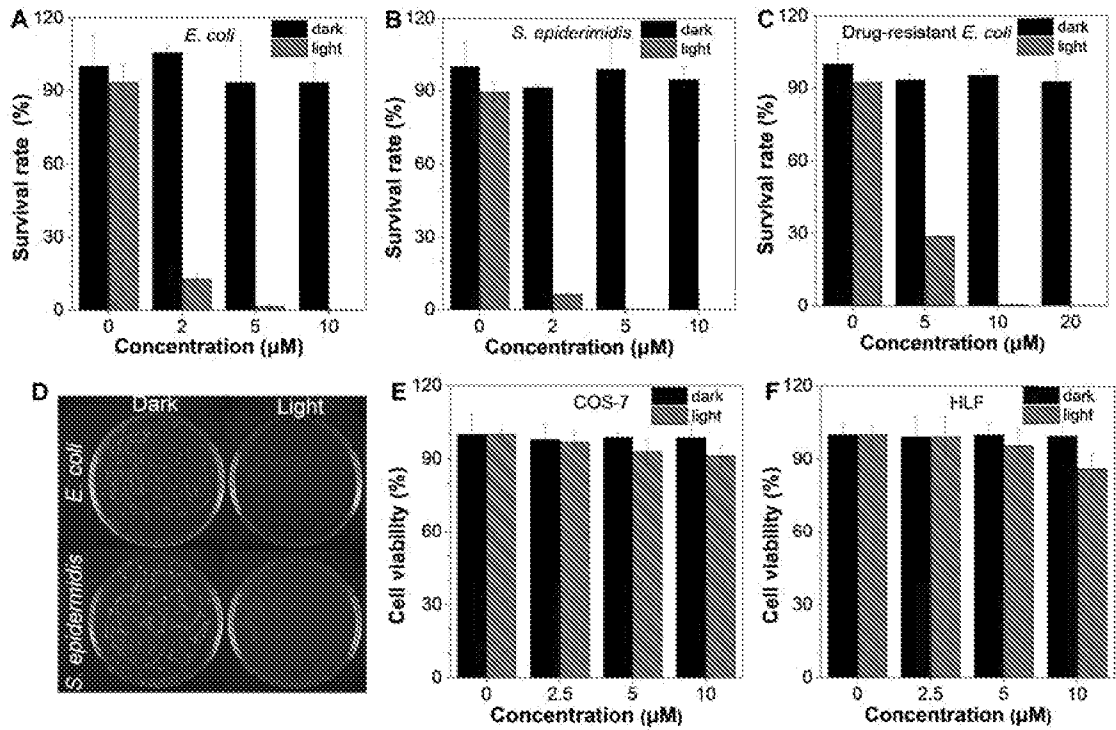


FIG. 12



FIGs. 13A-13F

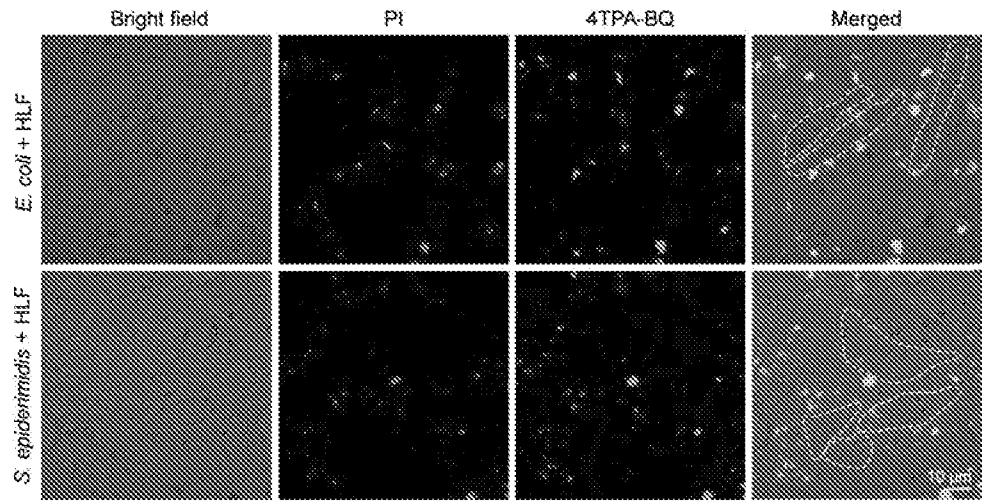
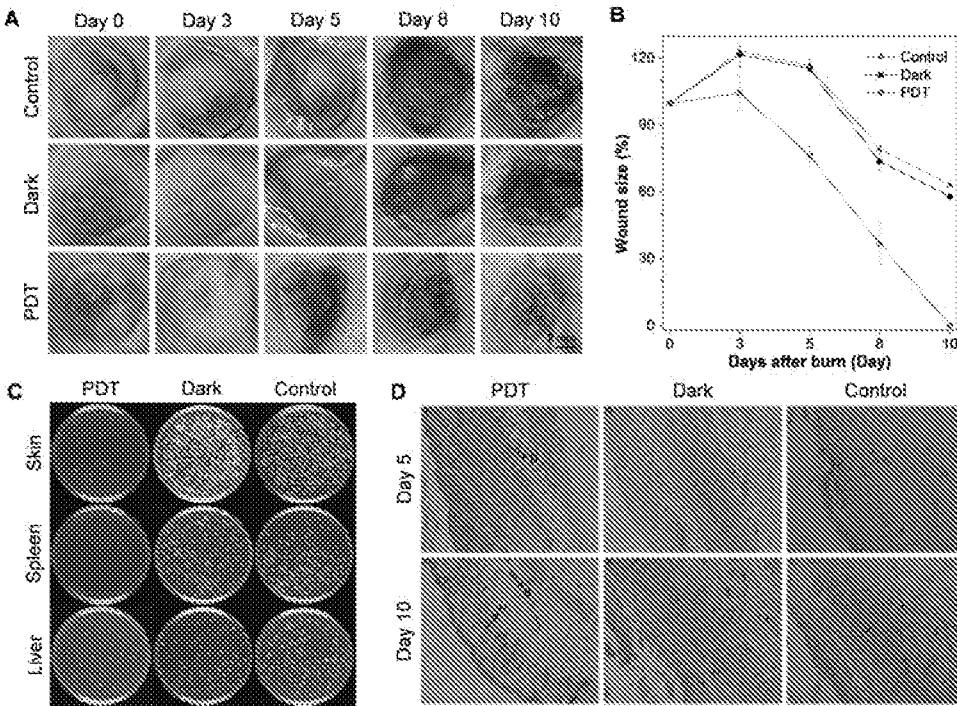


FIG. 14



FIGs. 15A-15D

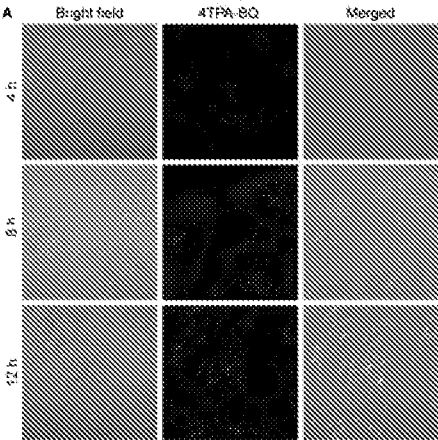


FIG. 16A

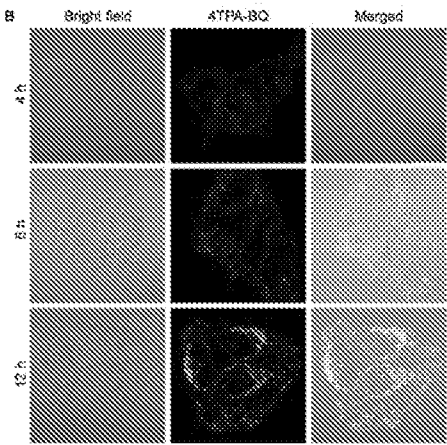


FIG. 16B

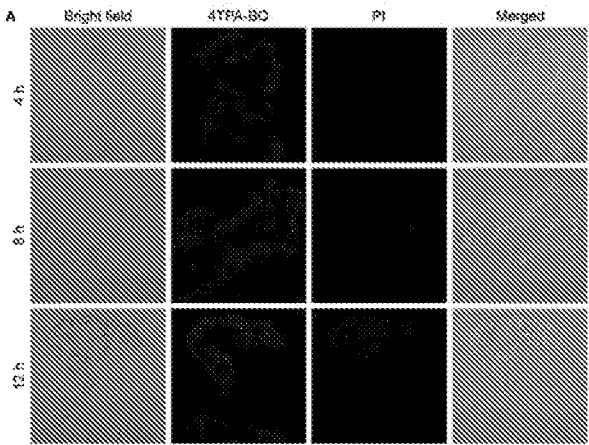


FIG. 17A

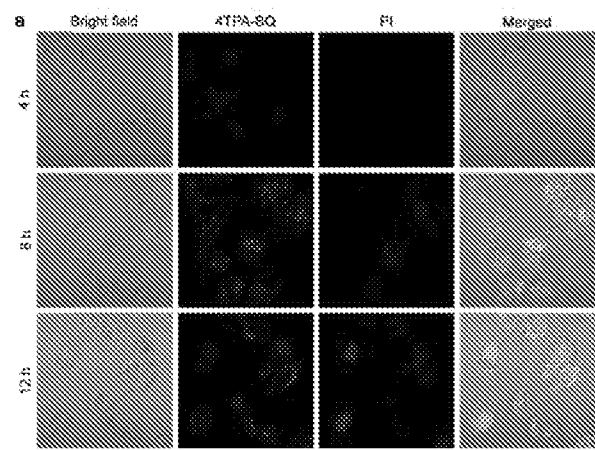


FIG. 17B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2020/103957

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/4375(2006.01)i; A61P 31/04(2006.01)i; A61P 35/00(2006.01)i

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; A61P

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)

CNABS;DWPI;SIPOABS;VEN;CNTXT;EPTXT;USTXT;WOTXT;CNKI; China Medicinal Patent Database;Baidu Scholar; ISI-WEB OF SCIENCE;Pubmed;STN: structure search on the basis of claim 1, 2, 11, photosensitizer, ROS, singlet oxygen, photodynamic therapy, cancer, tumor, tumour, bacteria, pathogen, infection

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	LI, Qiyao, et al. "Time-Dependent Photodynamic Therapy for Multiple Targets: A Highly Efficient AIE-Active Photosensitizer for Selective Bacterial Elimination and Cancer Cell Ablation." <i>Angew. Chem. Int. Ed.</i> , Vol. 59, No. 24, 21 October 2019 (2019-10-21), ISSN: 1433-7851, pages 9470–9477	1-19
X	LIU, Xiaolin, et al. "In Situ Generation of Azonia-Containing Polyelectrolytes for Luminescent Photopatterning and Superbug Killing." <i>J. Am. Chem. Soc.</i> , Vol. 141, No. 28, 20 June 2019 (2019-06-20), ISSN: 0002-7863, pages 11259–11268	1-19
X	HAN, Ye Ri, et al. "Synthesis of Benzoquinolizinium Salts by Rh(III)-Catalyzed Cascade Double N-Annulation Reactions of Allylamines, Diarylacetylenes, and HBF ₄ ." <i>Org. Lett.</i> , Vol. 19, No. 28, 12 May 2017 (2017-05-12), ISSN: 1523-7052, pages 2941–2944	1



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

"&" document member of the same patent family

Date of the actual completion of the international search

13 October 2020

Date of mailing of the international search report

29 October 2020

Name and mailing address of the ISA/CN

National Intellectual Property Administration, PRC
6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing
100088
China

Facsimile No. (86-10)62019451

Authorized officer

CHEN,Lei

Telephone No. (86-10)62411158

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2020/103957**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: **3-10,12-19**
because they relate to subject matter not required to be searched by this Authority, namely:

[1] The subject matter of claims 3-10,12-19 relates to a method of generating singlet oxygen, killing cancer cells, or killing a target pathogen, and therefore does not warrant an international search according to the criteria set out in Rule 39.1(iv). Thus, the search is based on the subject matter that could reasonably be expected to be claimed later in the procedure, namely the use of the photosensitizer in the manufacture of medicament for corresponding therapeutic uses.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).