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(54) Title: AIE COMPOUNDS WITH FLUORESCENCE, PHOTOACOUSTIC, AND RAMAN PROPERTIES

(57) Abstract: The present subject matter relates to fluorescent compounds that have aggregation-induced emission (AIE) characteristics. The compounds exhibit boosted fluorescence, photoacoustic (PA) properties, and Raman properties. The compounds, in nanoparticle form, can generate high fluorescence, PA, and Raman signals in aqueous environments. The compounds can be used to identify tumors at different surgical stages and improve cancer surgery outcomes.

AIE Compounds with Fluorescence, Photoacoustic, and Raman Properties

CROSS-REFERENCE

The present application claims priority to provisional United States Patent Application No. 62/919,427, filed March 13, 2019, which was filed by the inventors hereof and is incorporated herein by reference in its entirety.

FIELD

The present subject matter relates generally to a series of fluorescent compounds with aggregation-induced emission characteristics and near infrared absorption and their applications in bioimaging.

BACKGROUND

Molecular motions that play a pivotal role in determining many fundamental physical or chemical processes have great potential for advancing the biomedical field. Controllability and utilization of dynamic molecular motions can lead to functional smart materials with accurately-tunable properties that are particularly desirable for precision medicine and personalized theranostics. In particular, for a chromophore, its intramolecular motions (*e.g.*, rotation, vibration, and twisting) in excited states are associated with energy transition processes, according to the Jablonski diagram, which are of vital importance for determination of the chromophore's role and effectiveness in biomedical applications. For example, recent studies of certain aggregation-induced emission luminogens (AIEgens) clearly demonstrate that intramolecular motions can contribute greatly to photophysical energy dissipation pathways and that restriction of intramolecular motions can significantly promote fluorescence in aggregates. However, so far, there have been few reports on the design of multifunctional bioagents for precision medicine which fully take advantage of active intramolecular motion after light absorption by commanding and unifying microcosmic molecular dynamic behaviors to determine and boost the efficacy of macroscopic biomedical function.

Complete removal of tumor tissues is decisively important for prolonging patients' lifetime and even thoroughly curing cancers. Image-guided cancer surgery that employs molecular imaging techniques to catch and remove all tumor nodules has been used clinically in recent years. Ideal image-guided cancer surgery calls for diverse imaging methods at different stages of cancer operation. Before surgery, basic information such as size, number, and location

of tumors inside the body must be confirmed, which requires imaging techniques with excellent spatial resolution and high sensitivity.

During surgery, it is often necessary to identify tiny tumors (*e.g.*, < 1 mm), the margin between normal and tumorous tissues, as well as to determine whether residual tumors exist post main tumor resection. Hence, an imaging technique with superb sensitivity and prominent signal-to-background ratio intraoperatively is highly desirable. Unfortunately, there is no individual imaging technique which can satisfy all of these requirements throughout the surgery. Taking versatile optical imaging techniques, for example, fluorescence imaging possesses excellent sensitivity, but its penetration capability and spatial resolution are limited.

While photoacoustic (PA) imaging can provide large penetration depth beyond the optical diffusion limit and maintain high spatial resolution, this type of imaging is not sufficiently sensitive. Raman imaging is a complementary optical imaging technique, featuring a cell-silent region (1800-2800 cm^{-1}), which permits high-contrast imaging with zero interference of biological background. Accordingly, Raman imaging holds great potential for precise intraoperative inspection of residual tumors.

The combined advantages of fluorescence, PA, and Raman imaging modalities can improve cancer surgical outcomes, but require highly efficient fluorescence-PA-Raman triple-modality imaging agents.

Traditionally, the most commonly used strategy for preparing multi-modality imaging agents has been to combine various components into one platform (all-in-one strategy) to make use of their respective functions. Although effective, this method is typically hindered by complicated compositions, reduced reproducibility, and uncertain pharmacokinetics. Alternatively, one-for-all organic agents with multiple imaging capacities in one molecule have received more attention than all-in-one agents due to less complexity, simpler preparation, defined structure, and far better reproducibility. However, one-for-all organic agents with simultaneous fluorescence, PA, and Raman imaging capabilities are difficult to achieve since it is difficult to develop a molecular guideline to enable and maximize every optical imaging efficacy.

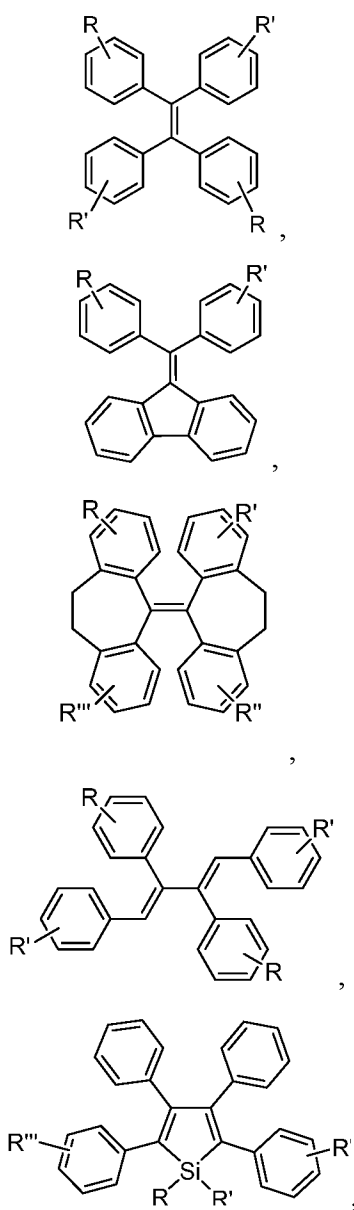
Accordingly, AIEgens with simultaneous fluorescence, PA, and Raman imaging capabilities are highly desirable.

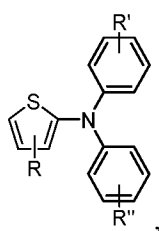
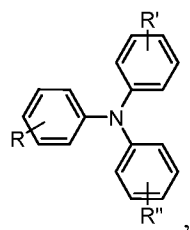
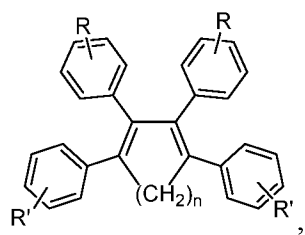
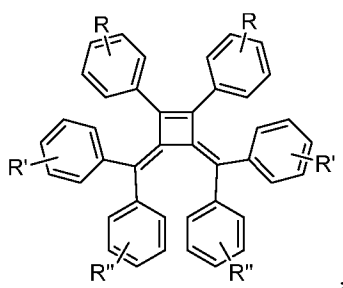
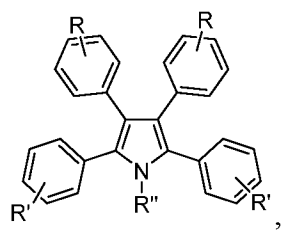
SUMMARY

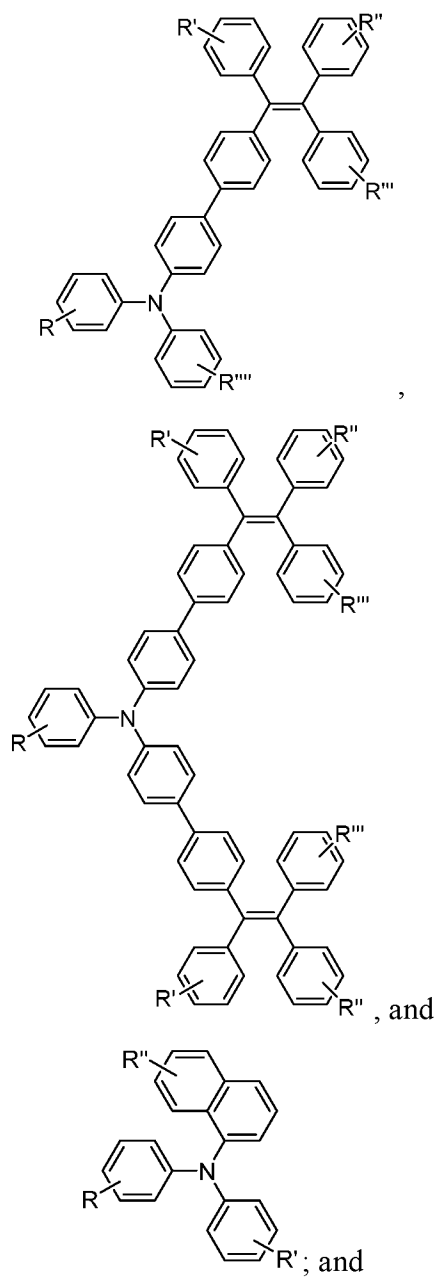
The present subject matter relates to fluorescent compounds that have aggregation-

induced emission (AIE) characteristics. The compounds can exhibit one or all of boosted fluorescence, photoacoustic (PA) properties, and Raman properties. The compounds, in nanoparticle form, can generate one or all of high fluorescence, PA, and Raman signals in aqueous environments. The compounds can be used to identify tumors at different surgical stages and improve cancer surgery outcomes.

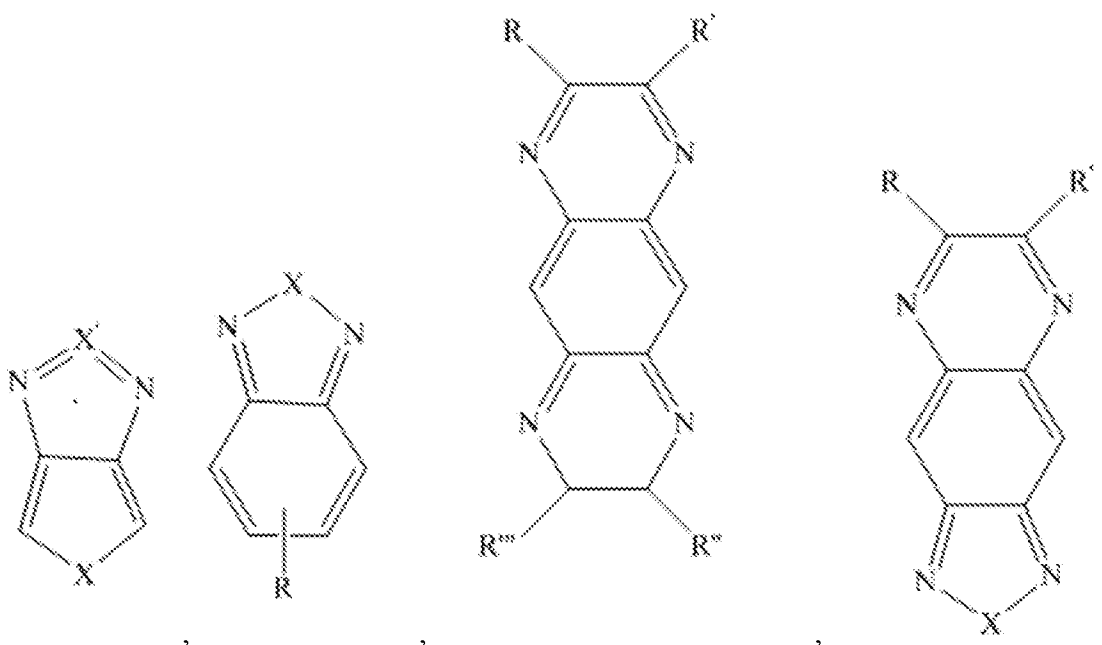
In an embodiment, the fluorescent compounds include a donor unit selected from one or more of the group consisting of:



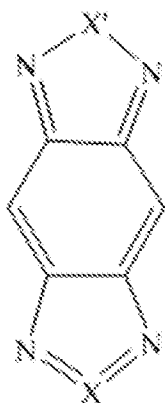




an acceptor unit (A) selected from the group consisting of:



and



wherein the compound has the donor and acceptor units arranged in a form selected from the group consisting of D-A, D-A-D', A-D-A', D-D'-A-D''-D''', A-A'-D-A''-A''', D-D'-A-D''-A', D-D'-A-A'-D'', D-A-D'-A'-A'', A-D-D'-A'-A'', D-A-D'-A'-D'', and A-D-A'-D'-A''

wherein D, D', D'' and D''' can be the same or different and represent the donor unit;

wherein A, A', A'' and A''' can be the same or different and represent the acceptor unit;

wherein each of X and X' is independently selected from the group consisting of O, S, Se,

and Te;

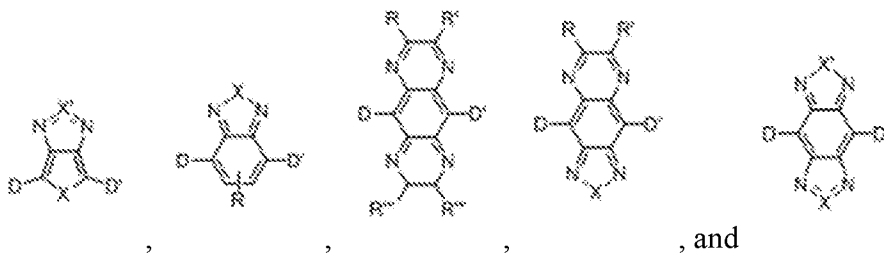
wherein each of R, R', R'', R''', and R'''' is unsubstituted or substituted and is independently selected from the group consisting of F, H, alkyl, unsaturated alkyl, azide, alkynyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, hydrazine, carboxyl group, amino group, sulfonic group, sulfhydryl, alkylthio, nitron, aldehyde, hydroxyl, halide, charged ionic group, and alkoxy group;

wherein at least one of R, R', R'', R''', and R'''' is a terminal functional group independently selected from the group consisting of N₃, NCS, SH, NH₂, COOH, substituted or unsubstituted alkyne, N-hydroxysuccinimide ester, maleimide, hydrazide, nitron group, -CHO, -OH, halide, and a charged ionic group; and

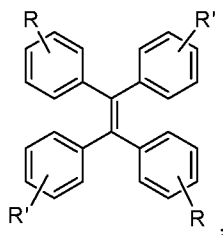
wherein at least one of R, R', R'', R''', and R'''' includes an alkyne group.

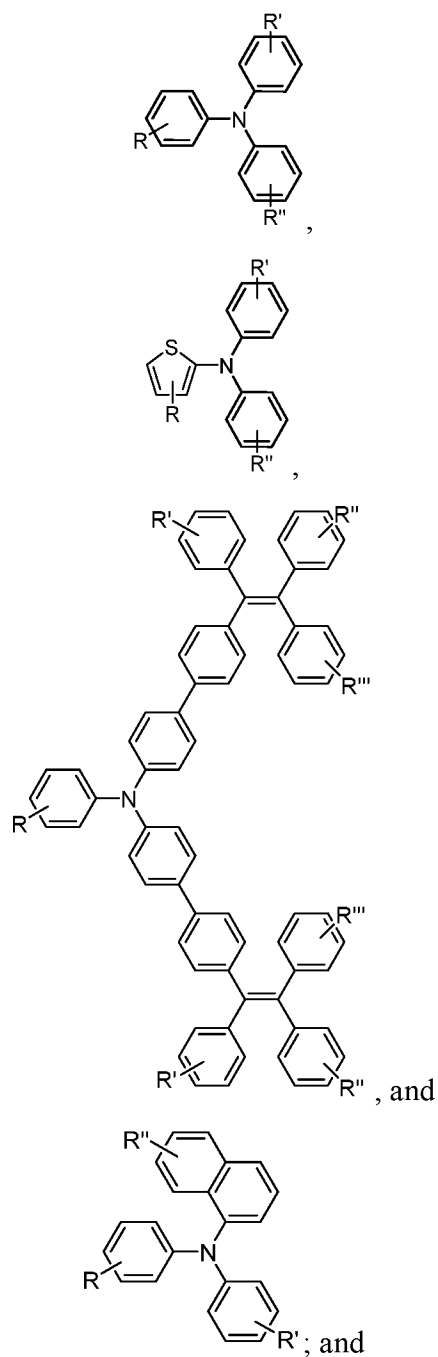
In an embodiment, at least one of R, R', R'', R''', and R'''' is a substituted or unsubstituted alkyne group, or one of the other listed groups substituted with an alkyne-containing group.

In an embodiment, the compound has a backbone structural formula selected from the group consisting of:



wherein each of D and D' is independently selected from the group consisting of





wherein each of R, R', R'', and R''' is unsubstituted or substituted and is independently selected from the group consisting of F, H, alkyl, unsaturated alkyl, azide, alkynyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, hydrazine, carboxyl group, amino group, sulfonic group, sulfhydryl, alkylthio, nitrene, aldehyde, hydroxyl, halide, charged ionic group, and alkoxy group;

wherein at least one of R, R', R'' and R''' is a terminal functional group independently

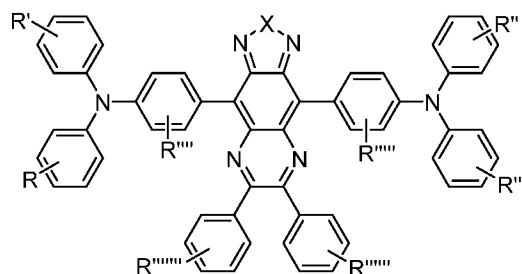
selected from the group consisting of N₃, NCS, SH, NH₂, COOH, substituted or unsubstituted alkyne, N-Hydroxysuccinimide ester, maleimide, hydrazide, nitron group, -CHO, -OH, halide, and charged ionic group;

wherein each of X and X' is independently selected from the group consisting of O, S, Se, and Te; and

wherein at least one of R, R', R'', R''', and R'''' includes an alkyne group.

In an embodiment, at least one of R, R', R'', R''', and R'''' is a substituted or unsubstituted alkyne group, or one of the other listed groups substituted with an alkyne-containing group.

In an embodiment, the compound comprises the following structural formula:



wherein each of R, R', R'', R''', R''', R''', R''', and R'''' is unsubstituted or substituted and is independently selected from the group consisting of F, H, alkyl, unsaturated alkyl, azide, alkynyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, hydrazine, carboxyl group, amino group, sulfonic group, sulfhydryl, alkylthio, nitron, aldehyde, hydroxyl, halide, charged ionic group, and alkoxy group;

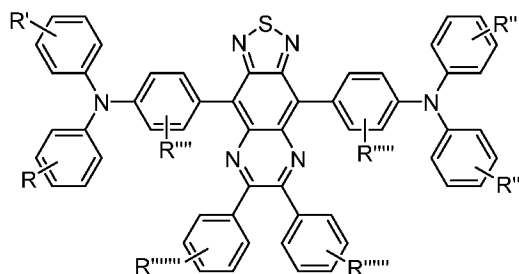
wherein at least one of R, R', R'', R''', R''', R''', R''', and R'''' is a terminal functional group independently selected from the group consisting of N₃, NCS, SH, NH₂, COOH, substituted or unsubstituted alkyne, N-Hydroxysuccinimide ester, maleimide, hydrazide, nitron group, -CHO, -OH, halide, and charged ionic group;

wherein each of X and X' is independently selected from the group consisting of O, S, Se, and Te; and

wherein at least one of R, R', R'', R''', R''', R''', R''', and R'''' includes an alkyne group.

In an embodiment, at least one of R, R', R'', R''', R''', R''', R''', and R'''' is a substituted or unsubstituted alkyne group, or one of the other listed groups substituted with an alkyne-containing group.

In an embodiment, the compound comprises the following structural formula:



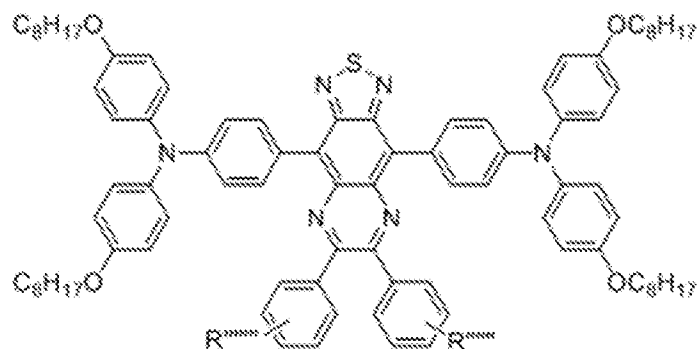
wherein each of R, R', R'', R''', R''', R''', R''', and R'''' is unsubstituted or substituted and is independently selected from the group consisting of F, H, alkyl, unsaturated alkyl, azide, alkynyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, hydrazine, carboxyl group, amino group, sulfonic group, sulfhydryl, alkylthio, nitron, aldehyde, hydroxyl, halide, charged ionic group, and alkoxy group;

wherein at least one of R, R', R'', R''', R''', R''', R''', and R'''' is a terminal functional group independently selected from the group consisting of N₃, NCS, SH, NH₂, COOH, substituted or unsubstituted alkyne, N-Hydroxysuccinimide ester, maleimide, hydrazide, nitron group, -CHO, -OH, halide, and charged ionic group; and

wherein at least one of R, R', R'', R''', R''', R''', and R'''' includes an alkyne group.

In an embodiment, at least one of R, R', R'', R''', R''', R''', and R'''' is a substituted or unsubstituted alkyne group, or one of the other listed groups substituted with an alkyne-containing group.

In an embodiment, the compound comprises the following structural formula:



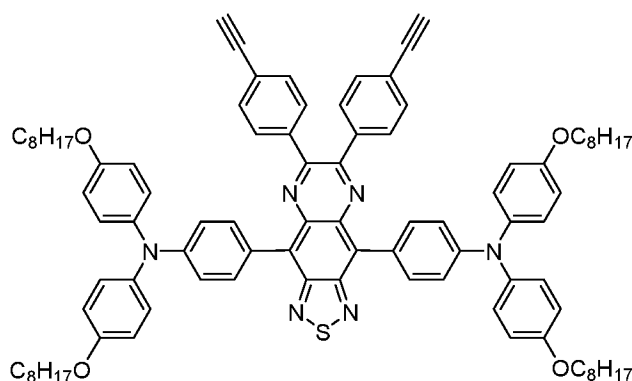
wherein each of R''' and R''' is unsubstituted or substituted and is independently selected from the group consisting of F, H, alkyl, unsaturated alkyl, azide, alkynyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, hydrazine, carboxyl group, amino group, sulfonic group, sulfhydryl, alkylthio, nitron, aldehyde, hydroxyl, halide, charged ionic group, and alkoxy group;

wherein at least one of R''' and R''' is a terminal functional group independently selected from the group consisting of N₃, NCS, SH, NH₂, COOH, substituted or unsubstituted alkyne, N-Hydroxysuccinimide ester, maleimide, hydrazide, nitron group, -CHO, -OH, halide, and charged ionic group; and

wherein at least one of R''' and R''' includes an alkyne group.

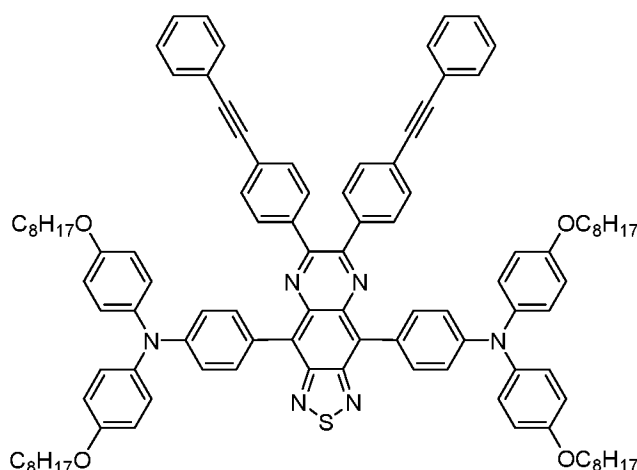
In an embodiment, at least one of R, R', R'', R'', R'', R'', and R''' is a substituted or unsubstituted alkyne group, or one of the other listed groups substituted with an alkyne-containing group.

In an embodiment, the compound is selected from the group consisting of



OTPA-TQ2

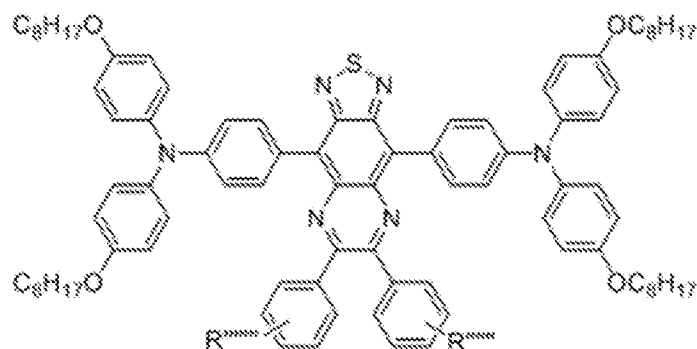
and



OTPA-TQ3.

In an embodiment, a method of locating a tumor site in a patient comprises administering a compound to the patient; contacting the tumor site with the compound; and

locating the tumor site using an imaging method after the tumor site is contacted with the compound, wherein the compound comprises the following structural formula:

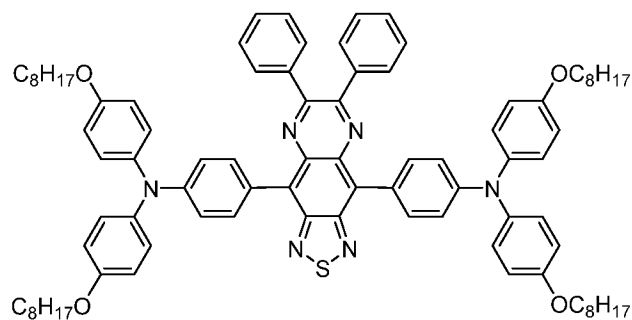


wherein each of R^{''''} and R^{'''''} is unsubstituted or substituted and is independently selected from the group consisting of F, H, alkyl, unsaturated alkyl, azide, alkynyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, hydrazine, carboxyl group, amino group, sulfonic group, sulfhydryl, alkylthio, nitron, aldehyde, hydroxyl, halide, charged ionic group, and alkoxy group; and

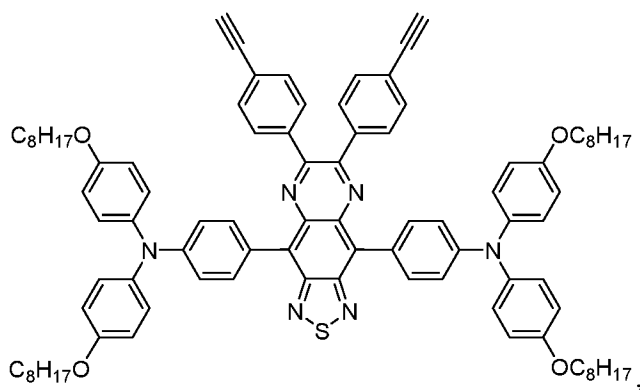
wherein at least one of R^{''''} and R^{'''''} is a terminal functional group independently selected from the group consisting of N₃, NCS, SH, NH₂, COOH, substituted or unsubstituted alkyne, N-Hydroxysuccinimide ester, maleimide, hydrazide, nitron group, -CHO, -OH, halide,

and charged ionic group.

In an embodiment, the compound used for locating a tumor site is selected from the group consisting of

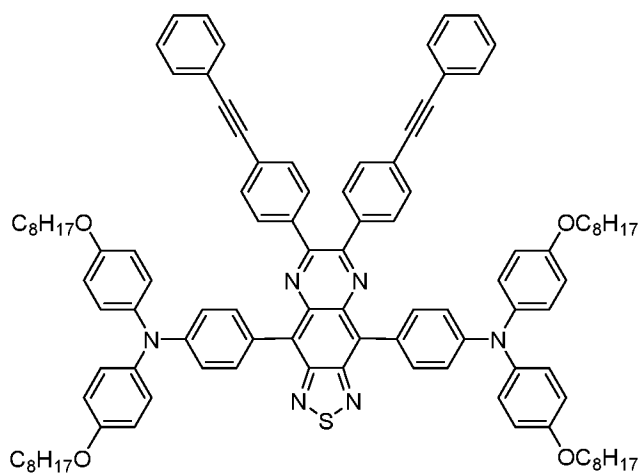


OTPA-TQ1,



OTPA-TQ2

and



OTPA-TQ3.

BRIEF DESCRIPTION OF DRAWINGS

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

Fig. 1 depicts ^1H NMR spectrum of OTPA-TQ1 in CDCl_3 at 298 K.

Fig. 2 depicts ^{13}C NMR spectrum of OTPA-TQ1 in CDCl_3 at 298 K.

Fig. 3 depicts HRMS of OTPA-TQ1.

Fig. 4 depicts ^1H NMR spectrum of OTPA-TQ2 in CDCl_3 at 298 K.

Fig. 5 depicts ^{13}C NMR spectrum of OTPA-TQ2 in CDCl_3 at 298 K..

Fig. 6 depicts HRMS of OTPA-TQ2.

Fig. 7 depicts ^1H NMR spectrum of OTPA-TQ3 in CDCl_3 at 298 K.

Fig. 8 depicts ^{13}C NMR spectrum of OTPA-TQ3 in CDCl_3 at 298 K.

Fig. 9 depicts HRMS of OTPA-TQ3.

Fig. 10A depicts the highest occupied molecular orbital (HOMO) energy level of OTPA-TQ1.

Fig. 10B depicts the lowest unoccupied molecular orbital (LUMO) energy level of OTPA-TQ1.

Fig. 11A depicts the highest occupied molecular orbital (HOMO) energy level of OTPA-TQ2.

Fig. 11B depicts the lowest unoccupied molecular orbital (LUMO) energy level of OTPA-TQ2.

Fig. 12A depicts the highest occupied molecular orbital (HOMO) energy level of OTPA-TQ3.

Fig. 12B depicts the lowest unoccupied molecular orbital (LUMO) energy level of OTPA-TQ3.

Fig. 13A depicts optimized molecular geometries of OTPA-TQ1, OTPA-TQ2, and OTPA-

TQ3.

Fig. 13B depicts absorption spectra of OTPA-TQ1, OTPA-TQ2, and OTPA-TQ3 in THF (20 μ M).

Fig. 13C depicts PL spectra of OTPA-TQ1, OTPA-TQ2, and OTPA-TQ3 in THF solution (20 μ M).

Fig. 13D depicts α_{AIE} value versus water fraction (f_{w}) in THF/water mixtures (α_{AIE} is defined as the ratio of the PL intensities of the compounds in THF/water mixtures and pure THF).

Fig. 14 depicts PL spectra of OTPA-TQ1-3 in THF.

Fig. 15A depicts PL spectra of OTPA-TQ1 in THF/water mixtures with different water fractions (f_{w}) as indicated.

Fig. 15B depicts PL spectra of OTPA-TQ2 in THF/water mixtures with different water fractions (f_{w}) as indicated.

Fig. 16A depicts a schematic illustration of the nanoprecipitation process.

Fig. 16B depicts a representative DLS result and TEM image of OTPA-TQ3 NPs.

Fig. 16C depicts absorption spectra of the NPs (20 μ M).

Fig. 16D depicts PL spectra of the NPs (20 μ M).

Fig. 16E depicts PLE mapping of OTPA-TQ3 NPs in aqueous dispersion.

Fig. 17A depicts representative DLS and TEM results of OTPA-TQ1 NPs

Fig. 17B depicts representative DLS and TEM results of OTPA-TQ2 NPs.

Fig. 18A depicts photographs of OTPA-TQ1 in THF (i) and as NPs (ii).

Fig. 18B depicts photographs of OTPA-TQ2 in THF (iii) and as NPs (iv).

Fig. 18C depicts photographs of OTPA-TQ3 in THF (v) and as NPs (vi).

Fig. 19 depicts PLE mapping of OTPA-TQ1 NPs in aqueous dispersion.

Fig. 20 depicts PLE mapping of OTPA-TQ2 NPs in aqueous dispersion.

Fig. 21A depicts relative PA intensity of OTPA-TQ molecules (50 μM) in DMF/glycerol mixtures with different glycerol fractions.

Fig. 21B depicts PA spectra of OTPA-TQ1-3 NPs (50 μM).

Fig. 21C depicts PA intensity of MB, semiconducting polymer NPs (SPNs), and OTPA-TQ1-3 NPs with the same concentration (50 μM) at 700 nm (the molar concentration of SPNs is based on the repeating unit ($n = 3$ per group)); data are presented as the mean $\pm$ SEM).

Fig. 21D depicts PA amplitudes of OTPA-TQ3 NPs as a function of concentration ($n = 3$ per group) (data are presented as the mean $\pm$ SEM).

Fig. 22A depicts Raman spectra of OTPA-TQ1-3 NPs (100 μM).

Fig. 22B depicts Raman intensity of OTPA-TQ3 (50 μM) in pure THF, THF/water mixture with 10% THF fraction, and encapsulated NPs.

Fig. 23A depicts photostability of the NPs and MB (50 μM) under continuous light (650 nm, 200 mW cm^{-2}) irradiation (I and I_0 are the maximal PL intensity of OTPA-TQs NPs and MB without and with light irradiation)

Fig. 23B depicts plot of I/I_0 versus RONS (ClO^- and $\cdot\text{OH}$, 1 mM) treatment (I and I_0 are the maximal PL intensity of OTPA-TQs NPs and ICG in PBS solutions in the presence and absence of RONS ($n = 3$ per group), respectively; data are presented as the means $\pm$ SEM).

Fig. 24A depicts fluorescence images of tumor-bearing mice after intravenous injection of OTPA-TQ3 NPs (200 μL , 650 μM) at different time points as indicated.

Fig. 24B depicts fluorescence intensity of the tumor site as a function of post-injection time ($n = 3$ per group) (data are presented as the mean $\pm$ SEM).

Fig. 24C depicts fluorescence intensity of tumor and main organs (heart, liver, spleen, lung, and kidneys) resected from the tumor-bearing mice 24 h postinjection ($n = 3$ per group) (data are presented as the mean $\pm$ SEM).

Fig. 24D depicts PA images of tumor site after intravenous administration of OTPA-TQ3 NPs at different time points as indicated ($n = 3$ per group) (data are presented as the mean $\pm$ SEM).

Fig. 24E depicts PA intensity of tumor site after intravenous administration of OTPA-TQ3 NPs at different time points as indicated ($n = 3$ per group) (data are presented as the mean $\pm$ SEM)

Fig. 25 depicts fluorescence images of tumor and main organs (heart, liver, spleen, lung, kidneys) resected from the tumor-bearing mice 24 h post-injection.

Fig. 26A depicts representative fluorescence images of OTPA-TQ3 NPs-treated tumor-bearing mice before and after S1 treatment.

Fig. 26B depicts Raman imaging at the operative site after S1.

Fig. 26C depicts H&E stained tissues at the operative site after S1.

Fig. 26D depicts representative fluorescence images of OTPA-TQ3 NPs-treated tumor-bearing mice before and after S2 treatment.

Fig. 26E depicts Raman imaging at the operative site of S2.

Fig. 26F depicts H&E stained tissues at the operative site of S2.

Fig. 27 depicts survival curves for mice after various treatments as indicated ($n = 10$).

DETAILED DESCRIPTION

The following definitions are provided for the purpose of understanding the present subject matter and for constructing 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.

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.

The term " λ_{ex} " as used herein refers to excitation wavelength.

The phrase "aggregation caused quenching" or "ACQ" as used herein refers to the phenomenon wherein the aggregation of π -conjugated fluorophores significantly decreases the fluorescence intensity of the fluorophores. The aggregate formation is said to "quench" light emission of the fluorophores.

The phrase "aggregation induced emission" or "AIE" as used herein refers to the phenomenon manifested by compounds exhibiting significant enhancement of light-emission upon aggregation in the amorphous or crystalline (solid) states whereas they exhibit weak or

almost no emission in dilute solutions.

“Emission intensity” as used herein refers to the magnitude of fluorescence/phosphorescence normally obtained from a fluorescence spectrometer or fluorescence microscopy measurement; “fluorophore” or “fluorogen” as used herein refers to a molecule which exhibits fluorescence; “luminogen” or “luminophore” as used herein refers to a molecule which exhibits luminescence; and “AIEgen” as used herein refers to a molecule exhibiting AIE characteristics.

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 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). 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 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, “alkenyl” refers to a straight-chain or branched alkyl group having one or more carbon-carbon double bonds. Examples of alkenyl groups 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). 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.

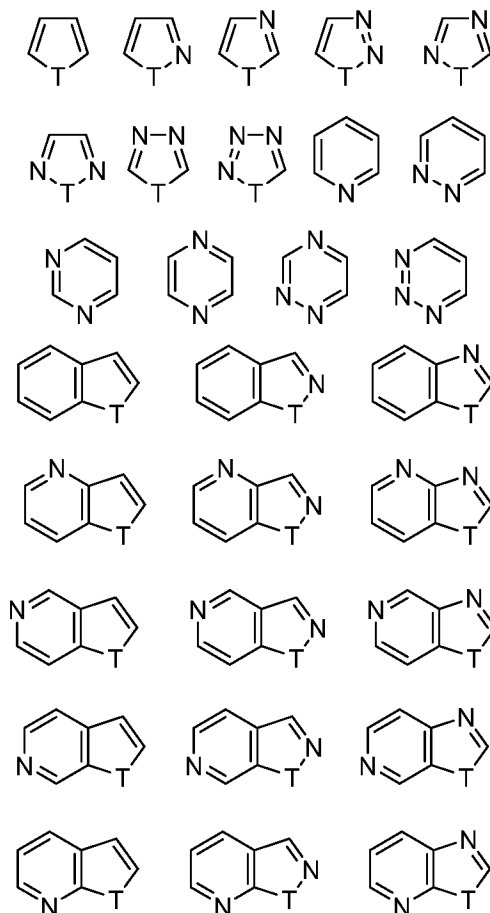
As used herein, “heteroatom” refers to an atom of any element other than carbon or hydrogen and includes, for example, nitrogen, oxygen, silicon, sulfur, phosphorus, and selenium.

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) 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 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 include benzodioxanyl, benzodioxolyl, chromanyl, indoliny 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, “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 those having two or more heteroaryl rings fused together, as well as those having at least one monocyclic heteroaryl ring 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 24 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 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 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, isobenzofuryl, 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, benzofuopyridinyl groups, and the like. In some embodiments, heteroaryl groups can be substituted as described herein.

As used herein, a "donor" material refers to an organic material, for example, an organic nanoparticle material, having holes as the majority current or charge carriers.

As used herein, an "acceptor" material refers to an organic material, for example, an organic nanoparticle material, having electrons as the majority current or charge carriers.

As used herein, a "theranostic agent" refers to an organic material, for example, an organic nanoparticle 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. 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.

Fluorescent Compounds

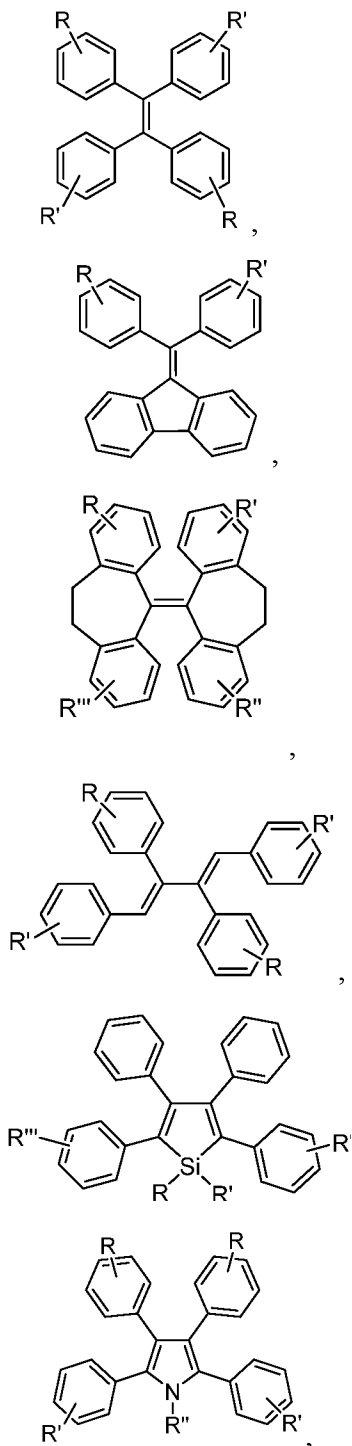
The present subject matter relates to a series of near-infrared (NIR)-absorbing organic fluorescent compounds that have aggregation-induced emission (AIE) characteristics and different sized rotation units. Compounds with a larger intramolecular motion unit can exert a greater impact on these optical properties. The rotation units can include, for example, at least one of phenyl, phenyl-alkyne, and phenyl-alkyne-phenyl. The compounds can be in nanoparticle form (hereinafter, "NPs"). The NPs can be water soluble and can generate the highest fluorescence, PA, and Raman signals in the cell silent region ($1,800\text{--}2,800\text{ cm}^{-1}$) in aqueous environments. The compounds can provide preoperative NIR fluorescence and PA imaging as well as intraoperative NIR fluorescence and Raman imaging. As such, the compounds can provide tumor information at different surgical stages of image-guided cancer surgery and thereby improve cancer surgery outcomes.

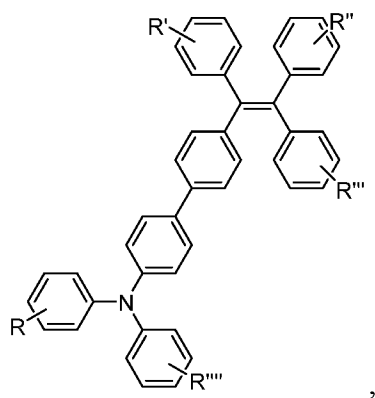
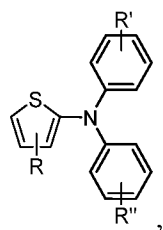
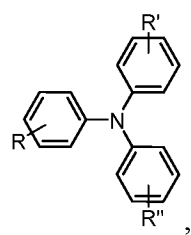
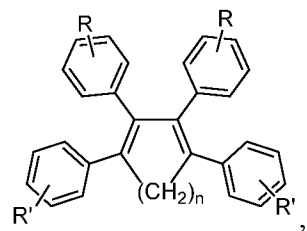
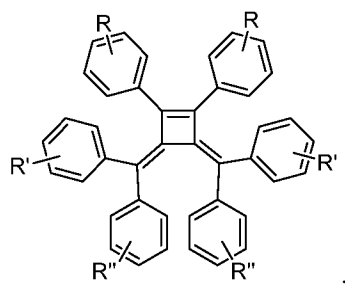
The present compounds can have a push-pull or donor-acceptor (D-A) structure, in which the electron-donating and -withdrawing moieties are alternatively arranged along the conjugated structure. The compounds have a strong D-A interaction, which ensures efficient intramolecular charge transfer (ICT). The ICT can be beneficial to realize small electronic bandgap and, thus, NIR absorption/emission. In an embodiment, the present compounds are NIR chromophores, including alkoxy-substituted triphenylamine (OTPA) as the donor, and thiadiazoloquinoxaline (TQ) as the acceptor. The octyloxy substitutes in the triphenylamine unit can increase electron-donating, as well as endow the resultant compounds with good solubility/processability. The compounds can include long side chains to retain some room between the conjugated backbones, which is favorable for intramolecular motions in the aggregated state.

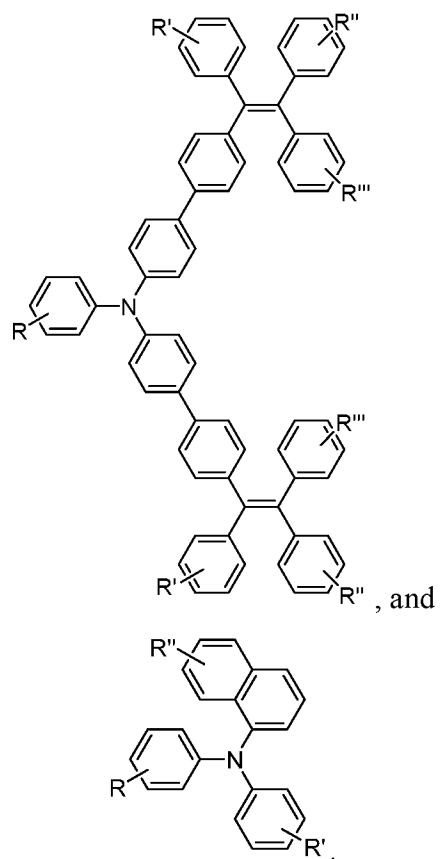
In an embodiment, the compounds include a series of compounds with different substituted groups (*i.e.*, phenyl, phenyl-alkyne, and phenyl-alkyne-phenyl) in a TQ core. The present compounds include donor-acceptor (D-A) type organic molecules with near-infrared (NIR) absorption. In an embodiment the compounds can be in nanoparticle form. In an

embodiment, the nanoparticles can be encapsulated in an amphiphilic matrix, e.g., an amphiphilic lipid-PEG₂₀₀₀ co-polymer.

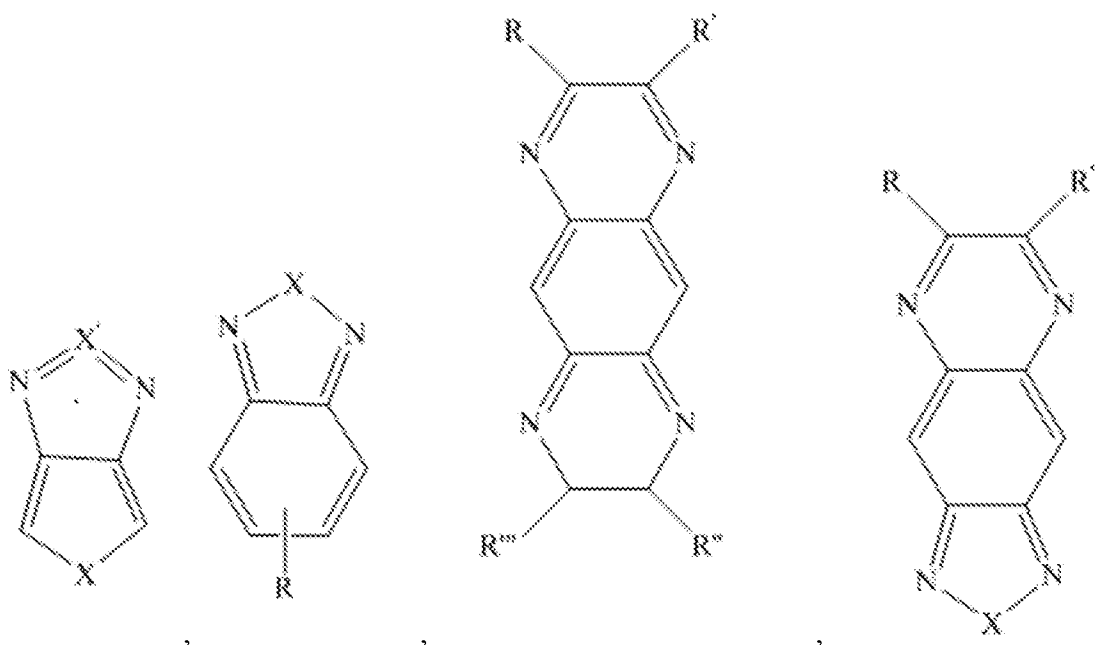
In the “D-A” compounds, the donor unit (D) can be selected from the group consisting of:



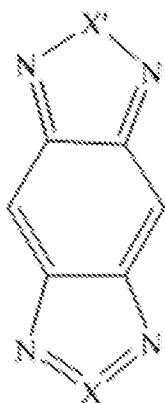




Likewise, the acceptor unit (A) can be selected from the group consisting of:



and



wherein the compound has the donor and acceptor units arranged in a form selected from the group consisting of D-A, D-A-D', A-D-A', D-D'-A-D''-D''', A-A'-D-A''-A''', D-D'-A-D''-A', D-D'-A-A'-D'', D-A-D'-A'-A'', A-D-D'-A'-A'', D-A-D'-A'-D'', and A-D-A'-D'-A''

wherein D, D', D'' and D''' can be the same or different and represent the donor unit;

wherein A, A', A'' and A''' can be the same or different and represent the acceptor unit.

In certain embodiments, each of X and X' is independently selected from the group consisting of O, S, Se, and Te;

each of R, R', R'', R''', and R'''' is unsubstituted or substituted and is independently selected from the group consisting of F, H, alkyl, unsaturated alkyl, azide, alkynyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, hydrazine, carboxyl group, amino group, sulfonic group, sulfhydryl, alkylthio, nitron, aldehyde, hydroxyl, halide, charged ionic group, and alkoxy group;

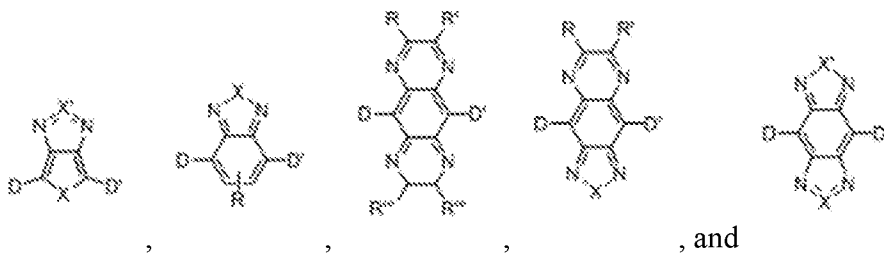
at least one of R, R', R'', R''', and R'''' is a terminal functional group independently selected from the group consisting of N₃, NCS, SH, NH₂, COOH, substituted or unsubstituted alkyne, N-hydroxysuccinimide ester, maleimide, hydrazide, nitron group,

-CHO, -OH, halide, and a charged ionic group; and

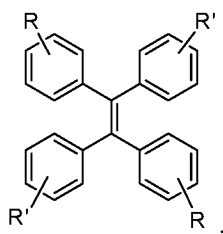
at least one of R, R', R'', R''', and R'''' includes an alkyne group.

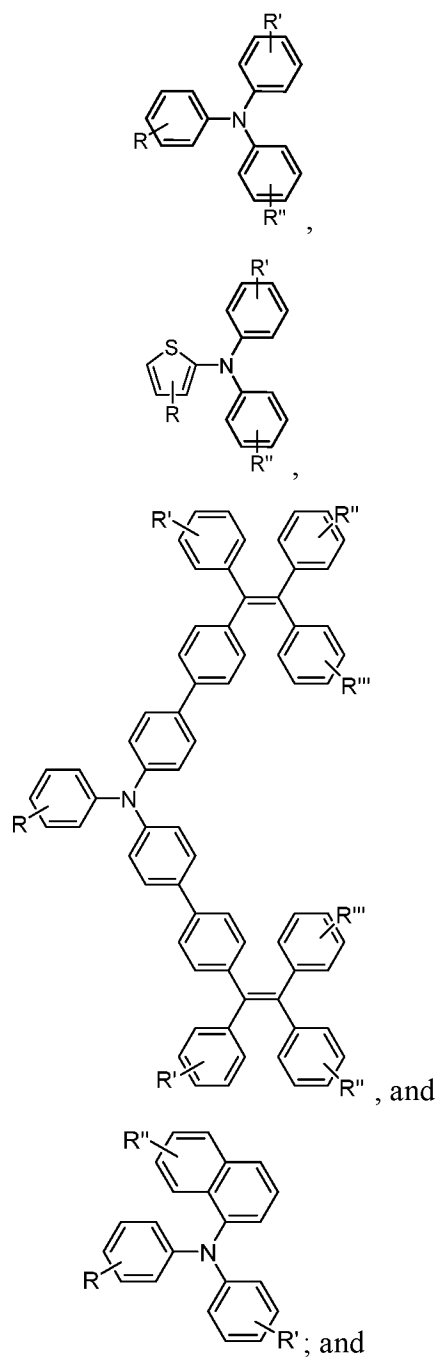
In an embodiment, at least one of R, R', R'', R''', and R'''' is a substituted or unsubstituted alkyne group, or one of the other listed groups substituted with an alkyne-containing group.

In an embodiment, the compound comprises a structural formula selected from the group consisting of:



wherein each of D and D' is independently selected from the group consisting of





wherein each of R, R', R'', and R''' is unsubstituted or substituted and is independently selected from the group consisting of F, H, alkyl, unsaturated alkyl, azide, alkynyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, hydrazine, carboxyl group, amino group, sulfonic group, sulfhydryl, alkylthio, nitrene, aldehyde, hydroxyl, halide, charged ionic group, and alkoxy group;

wherein at least one of R, R', R'' and R''' is a terminal functional group independently

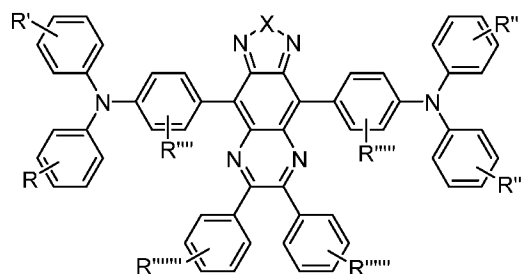
selected from the group consisting of N_3 , NCS, SH, NH_2 , COOH, substituted or unsubstituted alkyne, N-Hydroxysuccinimide ester, maleimide, hydrazide, nitron group, -CHO, -OH, halide, and charged ionic group;

wherein each of X and X' is independently selected from the group consisting of O, S, Se, and Te; and

wherein at least one of R, R', R'', R''', and R'''' includes an alkyne group.

In an embodiment, at least one of R, R', R'', R''', and R'''' is a substituted or unsubstituted alkyne group, or one of the other listed groups substituted with an alkyne-containing group.

In an embodiment, the compound comprises the following structural formula:



wherein each of R, R', R'', R''', R''', R''', R''', and R'''' is unsubstituted or substituted and is independently selected from the group consisting of F, H, alkyl, unsaturated alkyl, azide, alkynyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, hydrazine, carboxyl group, amino group, sulfonic group, sulfhydryl, alkylthio, nitron, aldehyde, hydroxyl, halide, charged ionic group, and alkoxy group;

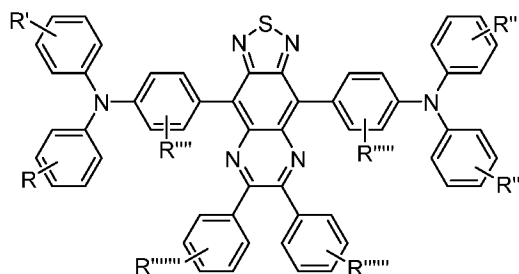
wherein at least one of R, R', R'', R''', R''', R''', R''', and R'''' is a terminal functional group independently selected from the group consisting of N_3 , NCS, SH, NH_2 , COOH, substituted or unsubstituted alkyne, N-Hydroxysuccinimide ester, maleimide, hydrazide, nitron group, -CHO, -OH, halide, and charged ionic group;

wherein each of X and X' is independently selected from the group consisting of O, S, Se, and Te; and

wherein at least one of R, R', R'', R''', R''', R''', R''', and R'''' includes an alkyne group.

In an embodiment, at least one of R, R', R'', R''', R''', R''', R''', and R'''' is a substituted or unsubstituted alkyne group, or one of the other listed groups substituted with an alkyne-containing group.

In an embodiment, the compound comprises the following structural formula:



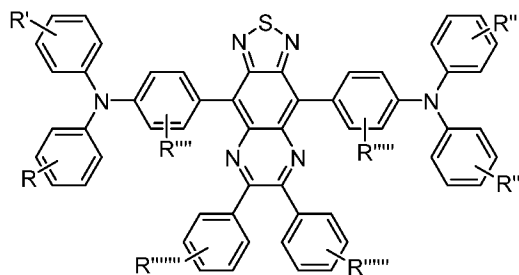
wherein each of R, R', R'', R''', R''', R''', R''', and R'''' is unsubstituted or substituted and is independently selected from the group consisting of F, H, alkyl, unsaturated alkyl, azide, alkynyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, hydrazine, carboxyl group, amino group, sulfonic group, sulfhydryl, alkylthio, nitron, aldehyde, hydroxyl, halide, charged ionic group, and alkoxy group;

wherein at least one of R, R', R'', R''', R''', R''', R''', and R'''' is a terminal functional group independently selected from the group consisting of N₃, NCS, SH, NH₂, COOH, substituted or unsubstituted alkyne, N-Hydroxysuccinimide ester, maleimide, hydrazide, nitron group, -CHO, -OH, halide, and charged ionic group; and

wherein at least one of R, R', R'', R''', R''', R''', and R'''' includes an alkyne group.

In an embodiment, at least one of R, R', R'', R''', R''', R''', and R'''' is a substituted or unsubstituted alkyne group, or one of the other listed groups substituted with an alkyne-containing group.

In an embodiment, the compound comprises the following structural formula:



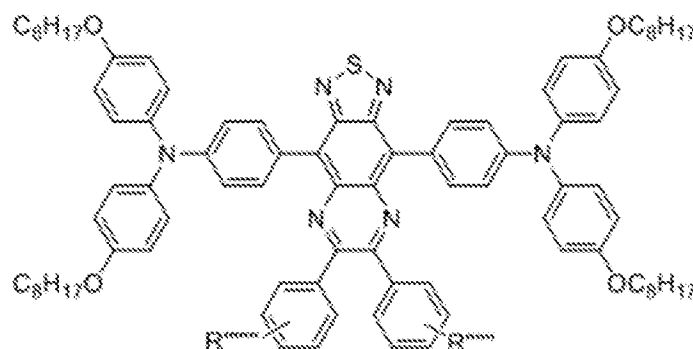
wherein each of R, R', R'', R''', R'''', R''''', and R'''''' is unsubstituted or substituted and is independently selected from the group consisting of F, H, alkyl, unsaturated alkyl, azide, alkynyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, hydrazine, carboxyl group, amino group, sulfonic group, sulfhydryl, alkylthio, nitron, aldehyde, hydroxyl, halide, charged ionic group, and alkoxy group;

wherein at least one of R, R', R'', R''', R'''', R''''', R'''''', and R''''''' is a terminal functional group independently selected from the group consisting of N₃, NCS, SH, NH₂, COOH, substituted or unsubstituted alkyne, N-Hydroxysuccinimide ester, maleimide, hydrazide, nitron group, -CHO, -OH, halide, and charged ionic group; and

wherein at least one of R, R', R'', R''', R''', R''', and R'''''' includes an alkyne group.

In an embodiment, at least one of R, R', R'', R''', R''', R''', and R'''' is a substituted or unsubstituted alkyne group, or one of the other listed groups substituted with an alkyne-containing group.

In an embodiment, the compound comprises the following structural formula:



wherein each of R¹ and R² is unsubstituted or substituted and is independently selected from the group consisting of F, H, alkyl, unsaturated alkyl, azide, alkynyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, hydrazine, carboxyl group, amino group, sulfonic group, sulfhydryl, alkylthio, nitro, aldehyde, hydroxyl, halide, charged ionic group, and alkoxy group;

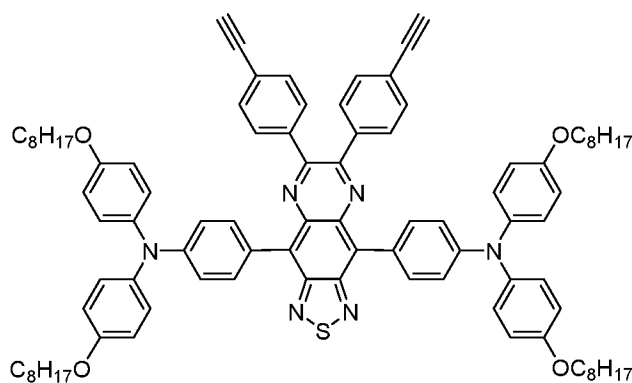
wherein at least one of R¹ and R² is a terminal functional group independently selected from the group consisting of N₃, NCS, SH, NH₂, COOH, substituted or unsubstituted alkyne, N-Hydroxysuccinimide ester, maleimide, hydrazide, nitron group, -CHO, -OH, halide,

and charged ionic group; and

wherein at least one of R^{''''''} and R^{''''''''} includes an alkyne group.

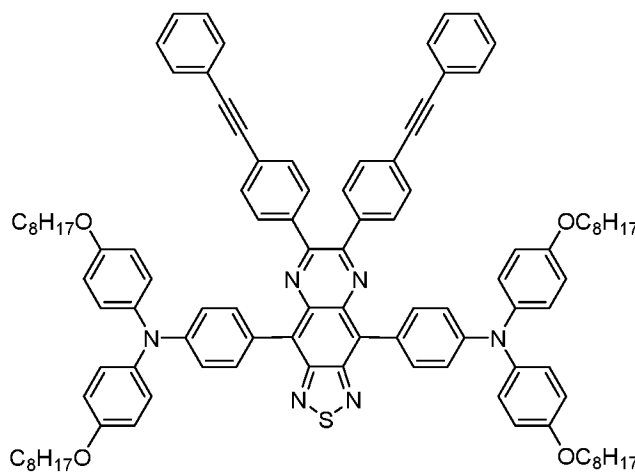
In an embodiment, at least one of R^{''''''} and R^{''''''''} is a substituted or unsubstituted alkyne group, or one of the other listed groups substituted with an alkyne-containing group.

In an embodiment, the compound is selected from the group consisting of



OTPA-TQ2

and



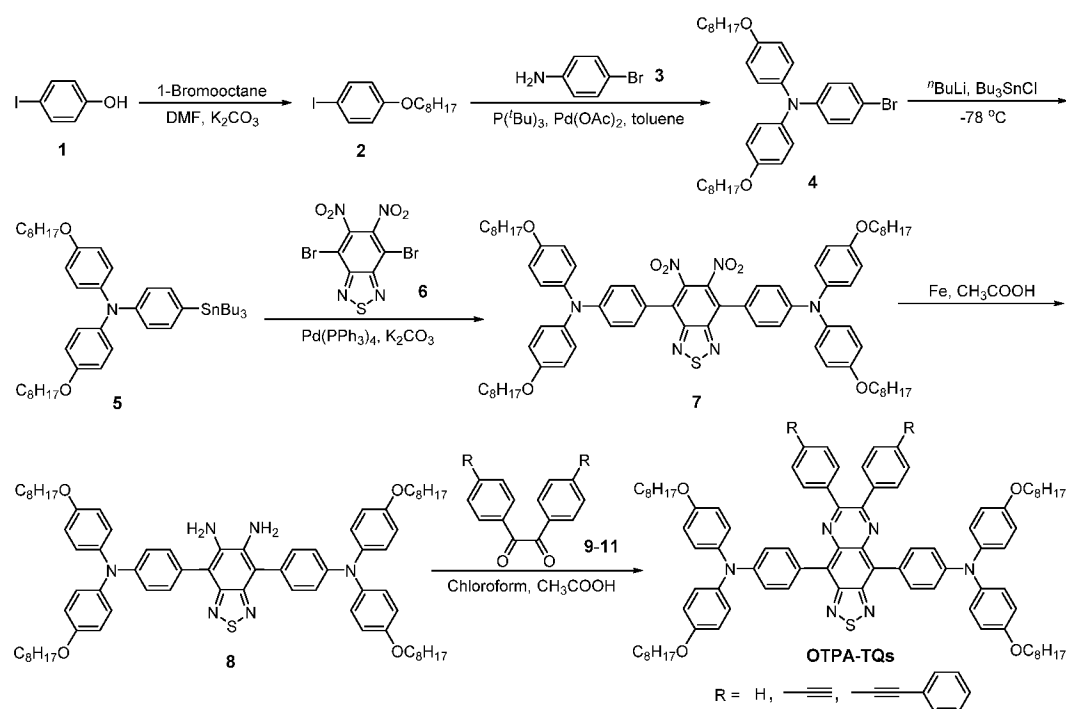
OPTA-TQ3.

The present compounds can include NIR absorbing organic molecules with a donor-acceptor (D-A) structure. The present compounds can include molecular rotors with different substituted groups (*i.e.*, phenyl, phenyl-alkyne, and phenyl-alkyne-phenyl) grafted to a central D-A core. In one embodiment, OTPA-TQ3 has the strongest intramolecular motions in dilute solution, *i.e.*, the free molecule state, but not the nanoparticle form. The intramolecular motions

of OPTA-TQ3 in the nanoparticle form will be more restricted. Accordingly, the OPTA-TQ3 NPs could generate the highest fluorescence because the largest molecular rotors (phenyl-alkyne-phenyl) could restrict the molecular motion in the aggregated state in a more efficient manner than the other two smaller rotors (phenyl and phenyl-alkyne).

According to an embodiment, one or more peptides can be conjugated to the present compounds.

An exemplary reaction scheme for preparing some of the fluorescent compounds is provided below:



Key synthesis steps in the reaction scheme above include a Stille cross-coupling reaction between the tributyltin-substituted OPTA (5) and the dibromo-molecule (6) to produce the dinitro-compound (7) as a dark purple solid, followed by the iron-catalyst nitro reduction and subsequent cyclization with benzils to obtain the final compounds. The benzil derivatives (9-11) with different substitutes were prepared in advance. The intermediates and final compounds were characterized by ^1H NMR, ^{13}C NMR, and high-resolution mass spectrum (HRMS) (Figs. 1-9). Different sizes of intramolecular rotors were synthesized to investigate their impact on optical imaging properties. The sizes of intramolecular rotors significantly affected intramolecular motions and intermolecular interactions. Thus, distinct substituted groups influence the optical properties in terms of fluorescence, PA, and Raman.

Tumor Imaging

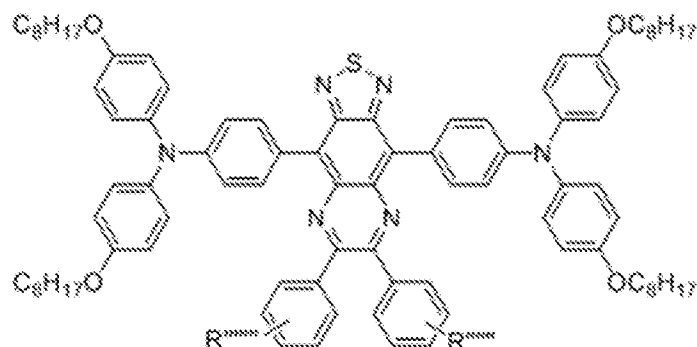
In an embodiment, one or more of the fluorescent compounds can be administered to a patient and an imaging method can be used to locate a tumor site while the compound contacts the tumor site. In an embodiment, the compound can be administered by intravenous injection. In an embodiment, the imaging method can include at least one of fluorescence microscopy, Raman microscopy, and photoacoustic imaging.

In an embodiment, the method can include altering intramolecular motions after light absorption to simultaneously amplify fluorescence-PA-Raman properties in one organic fluorophore. By gradually activating the intramolecular motions (e.g., changing the viscosity of a solution or aqueous environment of the compound and/or forming different kinds of aggregates), the PA and Raman properties can be enhanced. Moreover, the twisted 3D molecular structure and pronounced AIE effects enable high fluorescent brightness in the aggregated form.

In an embodiment, the present compounds can be used *in vivo* to obtain tumor information at different surgical stages and improve cancer surgery outcomes. In the preoperative stage, a combination of fluorescence and PA imaging can provide comprehensive information about tumors. In the intra-operative stage, a combination of fluorescence and Raman imaging can delineate tumor margins in a sensitive, high contrast manner. Each of the present compounds can provide a one-for-all organic molecular agent that allows for accurate cancer imaging and resection, and holds promise for integrated multi-modality imaging applications.

In an embodiment, a method of *in vivo* imaging of an animal can include administering the fluorescent compound to the animal; and obtaining images of the animal while the compound is within the animal using an imaging method. In an embodiment, the imaging method can include at least one of fluorescence microscopy, Raman microscopy, and photoacoustic imaging.

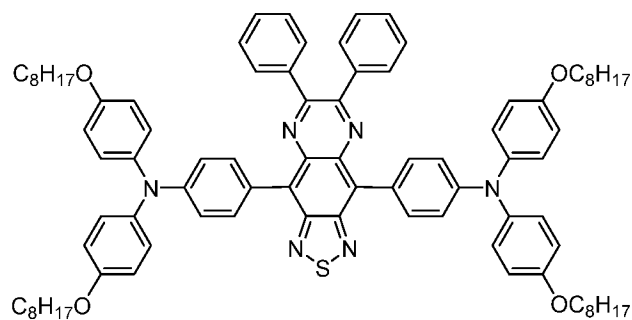
In an embodiment, a method of locating a tumor site in a patient can include administering a compound to the patient; contacting the tumor site with the compound; and locating the tumor site using an imaging method after the tumor site is contacted with the compound, wherein the compound comprises the following structural formula:



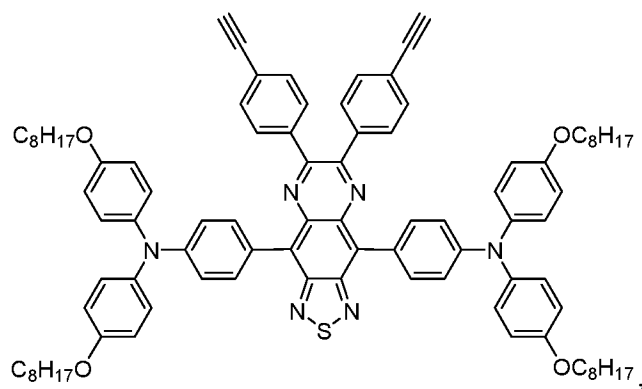
wherein each of R'''' and R'''' is unsubstituted or substituted and is independently selected from the group consisting of F, H, alkyl, unsaturated alkyl, azide, alkynyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, hydrazine, carboxyl group, amino group, sulfonic group, sulfhydryl, alkylthio, nitron, aldehyde, hydroxyl, halide, charged ionic group, and alkoxy group; and

wherein at least one of R'''' and R'''' is a terminal functional group independently selected from the group consisting of N₃, NCS, SH, NH₂, COOH, substituted or unsubstituted alkyne, N-Hydroxysuccinimide ester, maleimide, hydrazide, nitron group, -CHO, -OH, halide, and charged ionic group.

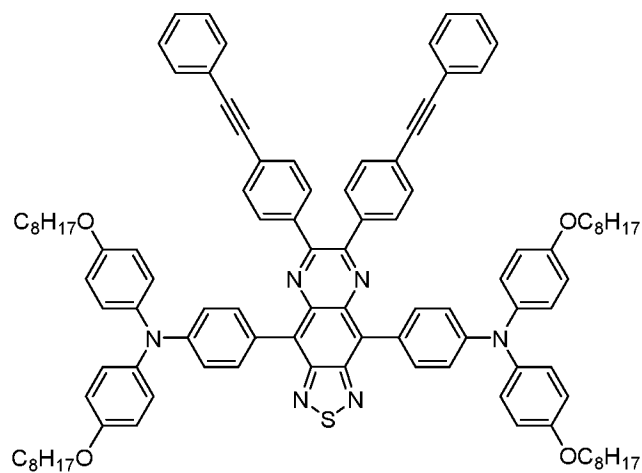
In an embodiment, the compound is selected from the group consisting of



OTPA-TQ1,

**OTPA-TQ2**

and

**OTPA-TQ3.**

Of the three compounds, OTPA-TQ1, OTPA-TQ2, and OTPA-TQ3, OTPA-TQ3 has the largest sized rotation units, i.e., the phenyl-alkyne-phenyl unit, which results in the most twisted molecular structure, the strongest intramolecular motions, and the largest conjugation of alkyne-containing unit. Accordingly, OTPA-TQ3 NPs can generate the highest fluorescence, PA, and Raman signals (in the cell-silent region) in aqueous environments.

EXAMPLES

Materials and Instruments

All the chemicals and reagents were purchased from chemical sources and were used as received. The nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AV 400 spectrometer. High-resolution mass spectra (HRMS) were measured with a GCT premier CAB048 mass spectrometer in matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mode. The geometry optimization was calculated at the level of B3LYP/6-31G* using density functional theory (DFT) method with the Gaussian 09 program package. The UV-vis absorption spectra were performed using a Shimadzu 2550 UV-vis scanning spectrophotometer. The photoluminescence (PL) spectra were conducted on a Horiba Fluorolog-3 spectrofluorometer. Raman spectra were acquired by a confocal Raman microspectroscope with a 532 nm excitation (Renishaw). Dynamic light scattering (DLS) was measured on a 90 plus particle size analyzer. Transmission electron microscopy (TEM) images were obtained from a JEM-2010F transmission electron microscope with an accelerating voltage of 200 kV.

Preparation of the NPs

1 mg of the organic compound and 2 mg of amphiphilic lipid-PEG (DSPE-PEG₂₀₀₀) were dissolved in 1 mL of tetrahydrofuran (THF). The obtained THF solution was poured into 9 mL of deionized water under sonication with a microtip probe sonicator (XL2000, Misonix Incorporated, NY). The mixture was then sonicated for another 1 min and violently stirred in a fume hood overnight at room temperature to evaporate residue THF, and the NPs solution was used directly.

Animal Experiments

All animal studies were conducted under the guidelines set by Tianjin Committee of Use and Care of Laboratory Animals, and the overall project protocols were approved by the Animal Ethics Committee of Nankai University.

Tumor-Bearing Mice

6-Week-old female BALB/c mice were obtained from the Laboratory Animal Center of the Academy of Military Medical Sciences (Beijing, China). To establish the xenograft 4T1 tumour-bearing mouse model, 4T1 breast cancer cells (1×10^6) suspended in 30 μ L of RPMI-1640 medium were injected subcutaneously into the right axillary space of the BALB/c mouse. After about 10 days, mice with tumor volumes of about 80–120 mm³ were used.

Preoperative Fluorescence and Photoacoustic Imaging

The xenograft 4T1 tumor-bearing mice were used for the following fluorescence and PA

imaging experiments. The tumor-bearing mice were anesthetized using 2% isoflurane in oxygen, and OTPA-TQ3 NPs (200 μ L, 650 μ M based on OTPA-TQ3) were intravenously injected into the tumor-bearing mice using a microsyringe, respectively ($n = 3$ mice). Then *in vivo* fluorescence and PA imaging were concurrently carried out to provide comprehensive information about tumors. Fluorescence imaging was performed with the Maestro EX fluorescence imaging system (CRi, Inc.) with excitation at 704 nm and signal collection in the spectral region of 740-950 nm. PA imaging was performed with the same mice as fluorescence imaging on a commercial small-animal opt-acoustic tomography system (MOST, iTheraMedical, Germany). The PA images were acquired at 700 nm at designated time intervals after injection. Fluorescence and PA images at designated time intervals were recorded.

Tumor Resection and Intraoperative Fluorescence-Raman Imaging

Based on the information provided by preoperative fluorescence and PA imaging at 24 h post-injection, the tumors of mice ($n = 3$) were resected. Briefly, the tumor-bearing mice were anesthetized using 2% isoflurane in oxygen. With the experience of a surgeon, the tumor tissues were aseptically prepped and sterile instruments were employed to excise the tumors (S1). NIR fluorescence imaging was conducted on the operative incision site to detect if there were residual tumors left behind. The fluorescence imaging was performed using the same process as the aforementioned *in vivo* fluorescence imaging (Maestro EX fluorescence imaging system with excitation at 704 nm and signal collection in the spectral region of 740-950 nm). The tissues at the operative incision sites were subsequently dissected and sliced, and the frozen sections were used for Raman microscopy and H&E staining. For Raman microscopy, frozen sections were placed on quartz slides (Ted Pella, Inc.) and air-dried. A 50x or 12x objective lens was used, and Raman spectral maps and correlating white light images were acquired using the Renishaw Streamline function (532 nm, mW). The Raman spectra were analyzed by least squares analysis using Wire 2.0 Software (Renishaw). At the same time, H&E staining was performed to confirm if there were tumors in the operative sites or not. The obtained slices were examined by a digital microscope (Leica QWin) to evaluate whether there were residual tumors left behind after surgery. After demonstrating the existence of residual tumors after the first surgery (S1), the second surgery (S2) was performed to remove the residual tumors until there were no fluorescence and Raman signals, as further confirmed by H&E staining.

Statistical Analysis

Quantitative data were expressed as mean $\pm$ standard deviation (SD). Statistical comparisons were made by ANOVA analysis and two-sample Student's t-test. P value < 0.05 was considered statistically significant.

Example 1

Synthesis and Characterization of OTPA-TQ

Synthesis of 1-iodo-4-(octyloxy)benzene (2): 1-Bromooctane (4.24 g, 22 mmol), 4-iodophenol (4.4 g, 20 mmol), and K_2CO_3 (8.3 g, 60 mmol) were added into a 250 mL two-necked round-bottom flask, and the flask was vacuumed and purged with dry nitrogen three times. Then, anhydrous DMF (120 mL) was added, and the mixture was heated to reflux and stirred for 24 h. After cooling down to room temperature, water was added, and the mixture was washed with dichloromethane three times. The organic phase was combined, dried with $MgSO_4$, and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane/hexane (v/v 1:10) as the eluent to afford 1-iodo-4-(octyloxy)benzene as a colorless oil (83% yield). ^1H NMR (400 MHz, $CDCl_3$): δ 7.53 (d, 2H), 6.86 (d, 2H), 3.90 (t, 2H), 1.82-1.70 (m, 2H), 1.48-1.39 (m, 2H), 1.39-1.21 (m, 8H), 0.89 (t, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.02, 138.14, 116.94, 82.38, 68.14, 31.81, 29.34, 29.23, 29.16, 26.01, 22.66, 14.11.

Example 2

Synthesis of 4-bromo-N,N-bis(4-(octyloxy)phenyl)aniline (4)

4-Bromoaniline (1.03 g, 6 mmol), 1-iodo-4-(octyloxy)benzene (4.98 g, 15 mmol), 1,10-phenanthroline (0.18 g, 1 mmol), CuI (2.12 g, 0.19 mmol), and KOH (5.04 g, 90 mmol) were added into a 250 mL two-necked round-bottom flask. Dry toluene (50 mL) was added into the flask under nitrogen atmosphere. Then, the flask was vacuumed and purged with dry nitrogen three times, and the mixture was heated to reflux and stirred for 24 h. After cooling to room temperature, water was added to the mixture, and the mixture was washed with dichloromethane three times. The organic phase was combined, dried with $MgSO_4$, and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane/hexane (v/v 1:6) as the eluent to afford 4-bromo-N,N-bis(4-(octyloxy)phenyl)aniline as a viscous oil (76% yield). ^1H NMR (400 MHz, $CDCl_3$): δ 7.22 (d, 2H), 7.00 (d, 4H), 6.79 (t, 6H), 3.92 (t, 4H), 1.81-1.72 (m, 4H), 1.49-1.41 (m, 4H), 1.36-1.24 (m, 16H), 0.89 (t, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.65, 148.01, 140.36, 131.71, 126.57,

121.86, 115.33, 112.17, 68.28, 31.82, 29.37, 29.34, 29.25, 26.08, 22.66, 14.11.

Example 3

Synthesis of 4-(octyloxy)-N-(4-(octyloxy)phenyl)-N-(4-(tributylstannyl)phenyl)aniline (5)

4-Bromo-N,N-bis(4-(octyloxy)phenyl)aniline (2.32 g, 4 mmol) was added into a 100 mL two-necked round-bottom flask. The flask was then vacuumed and purged with dry nitrogen three times, and anhydrous THF (50 mL) was added. Then, the mixture was cooled with dry ice-acetone mixture to -78 °C, and maintained at this temperature for 15 min, followed by the addition of n-butyllithium (ⁿBuLi, 2.5 M hexane solution, 1.6 mL, 4 mmol). After stirring at this temperature for 2 h, tri-n-butyltin chloride (1.1 mL, 4 mmol) was added, and the mixture was slowly warmed to room temperature, and stirred overnight. Afterwards, water was added to quench the reaction, and the mixture was extracted with dichloromethane three times. The organic phase was combined, dried with MgSO₄, and the solvent was evaporated under reduced pressure. The crude product was used without further purification.

Example 4

Synthesis of 4,4'-(5,6-dinitrobenzo[c][1,2,5]thiadiazole-4,7-diyl)bis(N,N-bis(4-(octyloxy)phenyl)aniline) (7)

4-(Octyloxy)-N-(4-(octyloxy)phenyl)-N-(4-(tributylstannyl)phenyl)aniline (3.16 g, 4 mmol), 4,7-dibromo-5,6-dinitrobenzo[c][1,2,5]thiadiazole (576 mg, 1.5 mmol), and Pd(PPh₃)₄ (58 mg, 0.05 mmol) were added into a 100 mL two-necked round-bottom flask. The flask was then vacuumed and purged with dry nitrogen three times, and anhydrous THF (50 mL) was added. The mixture was heated to reflux and stirred for 24 h. After cooling down to room temperature, water was added, and the mixture was washed with dichloromethane three times. The organic phase was combined, dried with MgSO₄, and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane/hexane (v/v 1:2) as the eluent to afford 4,4'-(5,6-dinitrobenzo[c][1,2,5]thiadiazole-4,7-diyl)bis(N,N-bis(4-(octyloxy)phenyl)aniline) as a blue solid (78% yield). ¹H NMR (400 MHz, CDCl₃): δ 7.35 (d, 4H), 7.15 (d, 8H), 6.95 (d, 4H), 6.87 (d, 8H), 3.94 (t, 8H), 1.83-1.73 (m, 8H), 1.51-1.42 (m, 8H), 1.38-1.23 (m, 32H), 0.89 (t, 12H). ¹³C NMR (100 MHz, CDCl₃): δ 156.48, 153.33, 152.62, 142.12, 139.24, 130.14, 127.91, 120.35, 117.73, 115.50, 68.28, 31.84, 29.38, 29.33, 29.27, 26.09, 22.68, 14.13. HRMS (MALDI-TOF, m/z): [M]⁺ calcd for C₇₄H₉₂N₆O₈S, 1224.6697; found, 1224.6699.

Example 5Synthesis of 4,4'-(5,6-diaminebenzo[c][1,2,5]thiadiazole-4,7-diyl)bis(N,N-bis(4-(octyloxy)phenyl)aniline) (8)

Iron powder (0.84 g, 15 mmol) was added to the mixture of 4,4'-(5,6-dinitrobenzo[c][1,2,5]thiadiazole-4,7-diyl)bis(N,N-bis(4-(octyloxy)phenyl)aniline) (0.613 g, 0.5 mmol) and acetic acid (100 mL) in a 250 mL two-necked round-bottom flask. The mixture was heated to 80 °C, and stirred for 4 h. After cooling down to room temperature, water was added, and the mixture was washed with dichloromethane three times. The organic phase was combined, dried with MgSO₄, and the solvent was evaporated under reduced pressure. The crude product was used without further purification.

Example 6Synthesis of 4,4'-(6,7-diphenyl-[1,2,5]thiadiazolo[3,4-g]quinoxaline-4,9-diyl)bis(N,N-bis(4-(octyloxy)phenyl)aniline) (OTPA-TQ1)

4,4'-(5,6-Diaminebenzo[c][1,2,5]thiadiazole-4,7-diyl)bis(N,N-bis(4-(octyloxy)phenyl)aniline) (0.35 g, 0.3 mmol) and benzil (0.126 g, 0.6 mmol) were dissolved in a mixture of acetic acid (40 mL) and chloroform (40 mL) in a 250 mL round-bottom flask. Then, the mixture was heated to 70 °C, and stirred for 12 h. After cooling down to room temperature, water was added, and the mixture was extracted with dichloromethane three times. The organic phase was combined, dried with MgSO₄, and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane/hexane (v/v 1:2) as the eluent to afford 4,4'-(6,7-diphenyl-[1,2,5]thiadiazolo[3,4-g]quinoxaline-4,9-diyl)bis(N,N-bis(4-(octyloxy)phenyl)aniline) (OTPA-TQ1) as a dark green solid (75% yield). ¹H NMR (400 MHz, CDCl₃): δ 7.92 (d, 4H), 7.65 (d, 4H), 7.34-7.29 (m, 4H), 7.21 (d, 8H), 7.15 (d, 4H), 6.88 (d, 8H), 3.96 (t, 8H), 1.85-1.74 (m, 8H), 1.53-1.42 (m, 8H), 1.41-1.24 (m, 32H), 0.89 (t, 12H). ¹³C NMR (100 MHz, CDCl₃): δ 155.82, 153.25, 152.55, 148.94, 140.46, 138.74, 136.01, 133.93, 130.10, 129.38, 128.42, 128.16, 127.28, 126.27, 118.54, 115.33, 68.32, 31.86, 29.42, 29.29, 26.14, 22.69, 14.14. HRMS (MALDI-TOF, m/z): [M]⁺ calcd for C₈₈H₁₀₂N₆O₄S, 1338.7683; found, 1339.7738.

Example 7Synthesis of 1,2-bis(4-((trimethylsilyl)ethynyl)phenyl)ethane-1,2-dione

4,4'-Dibromobenzil (1.84 g, 5 mmol), Pd(PPh₃)₂Cl₂ (70 mg, 0.1 mmol), CuI (19 mg, 0.1

mmol), and PPh_3 (26 mg, 0.1 mmol) were added into a 500 mL two-necked round-bottom flask. The flask was then vacuumed and purged with dry nitrogen three times, and THF/triethylamine (2:1 v/v) (250 mL) was added. After the compounds were completely dissolved, trimethylsilylacetylene (2.1 mL, 15 mmol) was added into the flask and the mixture was heated to 50 °C and stirred for 24 h. Then the solvents were evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane/hexane (v/v 1:4) as the eluent to afford 1,2-bis(4-((trimethylsilyl)ethynyl)phenyl)ethane-1,2-dione as a light yellow solid (81% yield). ^1H NMR (400 MHz, CDCl_3): δ 7.90 (d, 4H), 7.57 (d, 4H), 0.27 (s, 18H). ^{13}C NMR (100 MHz, CDCl_3): δ 193.13, 132.41, 132.09, 129.96, 129.69, 103.66, 99.91, 0.25.

Example 8

Synthesis of 1,2-bis(4-ethynylphenyl)ethane-1,2-dione (10)

1,2-Bis(4-((trimethylsilyl)ethynyl)phenyl)ethane-1,2-dione (2.01 g, 5 mmol) and THF (50 mL) were added into a 250 mL two-necked round-bottom flask. Then, the mixture of tetrabutylammonium fluoride (TBAF, 1 M THF solution, 15 mL) and acetic acid (0.57 mL) were added into the flask. After stirring at room temperature for 1 h, water was added, and the mixture was extracted with dichloromethane three times. The organic phase was combined, dried with MgSO_4 , and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane/hexane (v/v 1:5) as the eluent to afford 1,2-bis(4-ethynylphenyl)ethane-1,2-dione as a white solid (60% yield). ^1H NMR (400 MHz, CDCl_3): δ 7.93 (d, 4H), 7.62 (d, 4H), 3.33 (s, 2H). ^{13}C NMR (400 MHz, CDCl_3): δ 192.98, 132.66, 132.46, 129.75, 128.93, 82.46, 81.75.

Example 9

Synthesis of 4,4'-(6,7-diphenyl-[1,2,5]thiadiazolo[3,4-g]quinoxaline-4,9-diyl)bis(N,N-bis(4-(octyloxy)phenyl)aniline) (OTPA-TQ2)

4,4'-(5,6-Diaminebenzo[c][1,2,5]thiadiazole-4,7-diyl)bis(N,N-bis(4-(octyloxy)phenyl)aniline) (0.35 g, 0.3 mmol) and 1,2-bis(4-ethynylphenyl)ethane-1,2-dione (0.155 g, 0.6 mmol) were dissolved in a mixture of acetic acid (40 mL) and chloroform (40 mL) in a 250 mL round-bottom flask. The mixture was heated to 70 °C and stirred for 12 h. Then, water was added, and the mixture was extracted with dichloromethane three times. The organic phase was combined, dried with MgSO_4 , and the solvent was evaporated under reduced pressure.

The crude product was purified by column chromatography on silica gel using dichloromethane/hexane (v/v 1:2) as the eluent to afford 4,4'-(6,7-diphenyl-[1,2,5]thiadiazolo[3,4-g]quinoxaline-4,9-diyl)bis(N,N-bis(4-(octyloxy)phenyl)aniline) (OTPA-TQ2) as a dark green solid (71% yield). ¹H NMR (400 MHz, CDCl₃): δ 7.88 (d, 4H), 7.60 (d, 4H), 7.45 (d, 4H), 7.21 (d, 8H), 7.14 (d, 4H), 6.89 (d, 8H), 3.97 (t, 8H), 3.18 (s, 2H), 1.85-1.74 (m, 8H), 1.53-1.42 (m, 8H), 1.41-1.24 (m, 32H), 0.89 (t, 12H). ¹³C NMR (100 MHz, CDCl₃): δ 155.91, 153.33, 151.38, 149.10, 140.29, 138.74, 135.83, 133.87, 132.05, 129.93, 128.60, 127.38, 125.93, 123.24, 118.32, 115.33, 83.26, 79.00, 68.30, 31.85, 29.42, 29.40, 29.28, 26.13, 22.69, 14.14. HRMS (MALDI-TOF, m/z): [M]⁺ calcd for C₉₂H₁₀₂N₆O₄S, 1386.7683; found, 1387.7678.

Example 10

Synthesis of 1,2-bis(4-(phenylethynyl)phenyl)ethane-1,2-dione (11)

4,4'-Dibromobenzil (1.84 g, 5 mmol), Pd(PPh₃)₂Cl₂ (70 mg, 0.1 mmol), CuI (19 mg, 0.1 mmol), and PPh₃ (26 mg, 0.1 mmol) were added into a 500 mL two-necked round-bottom flask. The flask was then vacuumed and purged with dry nitrogen three times, and THF/triethylamine mixture (2:1 v/v) (250 mL) was added. After the compounds were completely dissolved, phenylacetylene (1.65 mL, 15 mmol) was injected into the flask and the mixture was heated to 50 °C and stirred for 24 h. Then, the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane/hexane (v/v 1:5) as the eluent to afford 1,2-bis(4-(phenylethynyl)phenyl)ethane-1,2-dione as a light yellow solid (77% yield). ¹H NMR (400 MHz, CDCl₃): δ 7.97 (d, 4H), 7.65 (d, 4H), 7.59-7.53 (m, 4H), 7.40-7.35 (m, 6H). ¹³C NMR (100 MHz, CDCl₃): δ 193.24, 132.08, 131.92, 131.86, 130.26, 129.88, 129.10, 128.51, 122.40, 94.23, 88.48.

Example 11

Synthesis of 4,4'-(6,7-diphenyl-[1,2,5]thiadiazolo[3,4-g]quinoxaline-4,9-diyl)bis(N,N-bis(4-(octyloxy)phenyl)aniline) (OTPA-TQ3)

4,4'-(5,6-Diaminebenzo[c][1,2,5]thiadiazole-4,7-diyl)bis(N,N-bis(4-(octyloxy)phenyl)aniline) (0.35 g, 0.3 mmol) and 1,2-bis(4-(phenylethynyl)phenyl)ethane-1,2-dione (246 mg, 0.6 mmol) were dissolved in the mixture of acetic acid (40 mL) and chloroform (40 mL) in a 250 mL round-bottom flask. The mixture was heated to 70 °C and stirred for 12 h. After cooling to room temperature, water was added, and the mixture was extracted with

dichloromethane three times. The organic phase was combined, dried with MgSO_4 , and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane/hexane (v/v 1:2) as the eluent to afford 4,4'-(6,7-diphenyl-[1,2,5]thiadiazolo[3,4-g]quinoxaline-4,9-diyl)bis(N,N-bis(4-(octyloxy)phenyl)aniline) (OTPA-TQ3) as a dark green solid (74% yield). ^1H NMR (400 MHz, CDCl_3): δ 7.91 (d, 4H), 7.65 (d, 4H), 7.57-7.53 (m, 4H), 7.50 (d, 4H), 7.38-7.34 (m, 4H), 7.24-7.18 (m, 8H), 7.15 (d, 4H), 6.89 (d, 8H), 3.97 (t, 8H), 1.85-1.74 (m, 8H), 1.52-1.42 (m, 8H), 1.41-1.23 (m, 32H), 0.89 (t, 12H). ^{13}C NMR (100 MHz, CDCl_3): δ 155.90, 153.34, 151.60, 149.09, 140.37, 138.20, 135.91, 133.90, 131.69, 131.49, 130.03, 128.40, 127.36, 126.06, 124.50, 123.04, 118.40, 115.36, 91.37, 89.13, 68.32, 31.84, 29.40, 29.26, 26.12, 22.67, 14.11. HRMS (MALDI-TOF, m/z): $[\text{M}]^+$ calcd for $\text{C}_{104}\text{H}_{110}\text{N}_6\text{O}_4\text{S}$, 1538.8309; found, 1539.8306.

Example 12

Photophysical properties

The absorption spectra of the compounds OTPA-TQ1-3 in tetrahydrofuran (THF) showed strong absorption in the range of 600-750 nm (Fig. 13B), matching well with the excitation light sources of commercially available fluorescence and PA imaging systems. The photoluminescence (PL) maxima of compounds OTPA-TQ1-3 in THF solution were in sequence at 894, 911, and 910 nm, respectively (Fig. 14). The large Stokes shifts of about 200 nm efficaciously avoided overlap between excitation and emission spectra, allowing for efficient utilization of the fluorescent light.

Water (a poor solvent) was added into THF (a good solvent) solution. For all three compounds, the PL intensities slightly decreased when water fraction was gradually increased from 0% to 30% (Figs 13C, 13D and Fig. 15), due to the solvent polarity effect and the transformation to twisted intramolecular charge transfer (TICT) state. The emission intensities largely intensified when further increasing water fraction to 95%, representing a typical AIE feature. Interestingly, the α_{AIE} value (defined as the ratio of maximal PL intensity in aggregate state and solution state) of OTPA-TQ3 in THF/water mixtures with 95% water fraction was higher than the other two derivatives, meaning OTPA-TQ3 has the strongest intramolecular motions in dilute solutions, i.e., the free molecule state. This can be attributed to the large planar conjugated rotors (phenyl-alkyne-phenyl) on OTPA-TQ3, causing a reduction of intermolecular interactions, such as π - π stacking, in the aggregated state, which saves more absorbed energy for

fluorescence emission. Thus, the OTPA-TQ3 NPs could generate the highest fluorescence because the largest molecular rotors (phenyl-alkyne-phenyl) could restrict the molecular motion in the aggregated state in a more efficient manner than the other two smaller rotors (phenyl and phenyl-alkyne). This result reveals that the size of intramolecular rotors significantly influences fluorescence property.

Example 13

Theoretical Calculations

Density functional theory (DFT) calculations were conducted with Gauss 09 program at B3LYP/6-31G(d) level. In order to simplify the calculations, the substituted aliphatic groups were abbreviated as methoxy units. The optimized molecular geometries of the compounds are presented in Fig. 13A. The compounds possess a similar geometry, with largely twisted intramolecular rotors. The largely twisted intramolecular rotors dissipate the excited-state energy as the free high-frequency rotation in solution and are beneficial for realizing AIE effect and bright emission in aggregation/solid state. Moreover, the two phenyl rings-capped alkyne unit (phenyl-alkyne-phenyl) in OTPA-TQ3 possess a highly conjugated planar geometry, which influences the molecular vibrations and thus, Raman intensity. As shown in Figs. 10-12B, the wave function of the highest occupied molecular orbital (HOMO) is distributed along both OTPA and TQ units, whereas the lowest unoccupied molecular orbital (LUMO) is mostly localized on the electron-deficient TQ core, indicating D-A characteristics and efficient ICT.

Example 14

Characterization and Fluorescence Properties

To render the hydrophobic compounds with good *in vivo* biocompatibility, OTPA-TQs were formulated into stable and small-nanosized NPs through the nanoprecipitation method (Fig. 16A) using an amphiphilic lipid-PEG₂₀₀₀ co-polymer as the encapsulation matrix. The size and morphology of the OTPA-TQ1-3 NPs, respectively, were characterized by transmission electron microscopy (TEM) and dynamic light scattering (DLS) measurements. As depicted in Fig. 16B and Figs. 17A-17B, the DLS data suggest that the hydrodynamic diameters of OTPA-TQ1-3 NPs are 141, 142, and 144 nm, respectively, and the TEM images indicate that all of the three NPs possess approximately spherical morphology. The obtained NPs are a green solution with good transparency, similar to the molecules in THF solution (Fig. 18). The absorption maxima of OTPA-TQ1-3 NPs are centered at 684, 701, and 705 nm, respectively (Fig. 16C), which are red-

shifted slightly as compared with those in solution states. On the contrary, the PL spectra of OTPA-TQ1-3 NPs are centered at 880, 897, and 895 nm, respectively (Fig. 16D). The hypsochromic shifts of NP emission spectra for about 15 nm when compared with those in THF solutions might be attributed to the rigidified molecular structures. By using indocyanine green (ICG) as the reference (with a nominal quantum yield (QY) of 13% in dimethyl sulfoxide), the fluorescence QYs of OTPA-TQ1-3 NPs were 2.5%, 1.8%, and 2.7%, respectively. These values are comparable to recent reported bright organic emitters with a similar emission range and are much higher than the widely used carbon nanotubes (~0.4%). The changes of fluorescence QYs exhibited a trend similar to the trend of the α_{AIE} values, suggesting that the AIE characteristic is vital for realizing highly luminescent organic NPs. OTPA-TQ3 NPs exhibited the highest fluorescent brightness and α_{AIE} values. This can be attributed to the OTPA-TQ3 NPs having the most twisted molecular structure due to the large size of phenyl-alkyne-phenyl rotors, which reduces the non-radiative intermolecular interactions within NPs to the greatest extent. PL excitation (PLE) mapping was also performed to gain a deeper understanding about the excitation-emission relationships, and PLE maps of the organic NPs (Fig. 16E and Figs. 19, 20) manifest eligible excitation and fluorescence in the NIR region.

Example 15

In Vitro Photoacoustic Properties of the Compounds and NPs for example:

The structure-PA property relationship of OTPA-TQ molecules and the influence of rotor size in PA signal output was investigated. First, glycerol was employed to elevate the solution's viscosity so as to significantly suppress the intramolecular motions of OTPA-TQ molecules. As shown in Fig. 21A, the PA intensities of all three OTPA-TQ molecules decreased upon increasing the glycerol fraction in N,N-dimethylformamide (DMF)/glycerol mixtures. This result reasonably reveals that the excited-state intramolecular motions, such as rotation, play a key role in PA signal output. As further shown in Fig. 21A, the PA amplitude of OTPA-TQ3 in DMF/glycerol mixture with 90% glycerol fraction is profoundly lower (70%) than the PA amplitude of OTPA-TQ3 in pure DMF. This difference in PA intensity with increasing glycerol fraction was more pronounced for OTPA-TQ3 than for the other two derivatives (40% for OTPA-TQ1 and 50% for OTPA-TQ2). This result suggests that the excited-state intramolecular motions of larger intramolecular rotors contributes to a greater extent on PA generation.

The PA spectra of OTPQ-TQ1-3 NPs were recorded by measuring the PA intensity at

different wavelengths from 680 to 900 nm. As shown in Fig. 21B, the PA spectra of OTPQ-TQ1-3 NPs match well with the absorption profiles (Fig. 21C), indicating that the PA signals are produced from the NIR absorption of the organic molecules. Under irradiation of a 700 nm NIR pulsed laser, for each OTPA-TQ molecule (50 μ M), its NP state ($\sim$ 140 nm by DLS) shows around 2.5-fold higher PA intensity than its bare aggregate (without lipid-PEG₂₀₀₀) state in a THF/water mixture with 95% water fraction (the aggregates possess broad size distribution of $>$ 400 nm measured by DLS). The amphiphilic co-polymer lipid-PEG₂₀₀₀ acting as the surfactant can essentially improve the water-solubility of the hydrophobic OTPA-TQ molecules and provide much higher specific surface area, which permits more effective excited-state intramolecular motions in aqueous media. This result further validates that there is a positive correlation between excited-state intramolecular motion and PA signal output, in good accordance with Fig. 21A.

When comparing the PA amplitudes among the three OTPA-TQ NPs, as depicted in Fig. 21C, OTPA-TQ3 NPs exhibit the highest PA intensity, which is about 1.4-fold higher than the other two counterpart NPs. It is believed that the conjugated phenyl-alkyne-phenyl units having a larger size lead to stronger intramolecular motions, and thus generate a stronger PA signal, agreeing well with Fig. 21A. The PA amplitude of OTPA-TQ3 NPs was also compared with well-known high-performing PA imaging agents including semiconducting polymer NPs (SPNs) and methyl blue (MB). As shown in Fig. 21C, under the same experimental conditions, the PA amplitude of OTPA-TQ3 NPs is $\sim$ 1.7-fold and $\sim$ 2.4-fold higher than that of SPNs and MB, respectively. The data verify that excited-state intramolecular motion is indeed an effective approach to obtain superior PA imaging agents. Furthermore, the PA intensity of OTPA-TQ3 NPs shows a good linear relationship with the molar concentration of OTPA-TQ3 (Fig. 21D), indicating good potential of the NPs for quantitative analysis.

Example 16

In Vitro Raman Properties of the Compounds and NPs

The alkyne group was introduced into OTPA-TQ2 and OTPA-TQ3 molecules as it has been well accepted to produce a Raman signature in the cell-silent region. Fig. 22A displays the Raman spectra of the organic OTPA-TQ1-3 NPs. Noteworthy, the OTPA-TQ3 NPs possess a rather strong and narrow Raman signal at 2215 cm^{-1} , which is in the cell-silent region and refers to the typical carbon-carbon triple bond stretching and vibration signature of alkyne groups. As

compared to OTPA-TQ2 NPs with phenyl-alkyne units, the intense Raman signal of OTPA-TQ3 NPs can be ascribed to the large conjugation of the phenyl-alkyne-phenyl unit. The salient Raman scattering characteristic of OTPA-TQ3 NPs can render OTPA-TQ3 NPs an efficient marker for highly specific Raman imaging with negligible background.

Furthermore, the Raman spectra of OTPA-TQ3 in the form of free molecules (in THF solution), large aggregates (in THF/water mixture with 90% water fraction) and NPs were investigated. As depicted in Fig. 22B, the OTPA-TQ3 NPs show much stronger Raman intensity at 2215 cm^{-1} than the large aggregates, whereas the free molecule form has the highest Raman signal, which is in accord with previous reports that molecular motion brings about Raman scattering. As the polymer surfactant lipid-PEG₂₀₀₀ favors improved solubility of OTPA-TQ3 molecules in water, and thereby promotes intramolecular motions, this result implies that the Raman intensity from phenyl-alkyne-phenyl in the unconfined free-motion form would be stronger than the restricted aggregate. From the basic theory of Raman scattering, after light absorption, the molecules are excited to a virtual energy state. In this case, the wavelength of the incident light (532 nm) could also lead to the electronic transition of the OTPA-TQ3 molecule. Therefore, the result in Fig. 22B suggests that intramolecular motions in the aforementioned high energy state after light excitation would significantly enhance the Raman signal from phenyl-alkyne-phenyl. The data in Figs. 21 and 22 reasonably highlight the importance and necessity of the proposed molecular guideline in this work via regulating intramolecular motions to boost all of the fluorescence-PA-Raman properties in one organic fluorophore.

Example 17

Stability of the NPs

Photostability is of critical importance for optical agents. Hence, the photobleaching resistance capacities of the OTPA-TQ1-3 NPs under light irradiation were investigated. The popularly used MB was used as a control. After exposure to continuous red light (650 nm , 200 mW cm^{-2}) irradiation for 60 min, the absorption and emission properties of all three OTPA-TQ NPs remained constant, whereas MB dye with similar absorption maximum was easily photobleached, as evidenced by the PL intensity decreasing to $\sim 50\%$ of the original value (Fig. 23A). Additionally, reactive oxygen and nitrogen species (RONS) are important signaling molecules closely related to body health, which are also known to overexpress in many diseased regions, *e.g.*, cancer, inflammation, and cardiovascular diseases. As a consequence, stable optical

probes that are resistant to RONS are momentous for *in vivo* disease detection. As presented in Fig. 23B, all of the OTPA-TQ1-3 NPs show excellent resistance to various RONS. In contrast, the FDA-approved ICG is severely destroyed in the presence of ClO^- and $\cdot\text{OH}$, which is likely due to the degradation of alternatively arranged single-double bonds.

Example 18

Preoperative Fluorescence/PA Imaging of Tumors for example

The *in vitro* results reasonably demonstrated that OTPA-TQ3 NPs, with the largest phenyl-alkyne-phenyl units, possess the highest signals in terms of fluorescence, PA and Raman signals in aqueous environments through regulating intramolecular motions. Thus, the OTPA-TQ3 NPs were used for the following *in vivo* experiment to investigate whether such optical agents with excellent fluorescence-PA-Raman properties could be beneficial to precise image-guided cancer surgery. As preoperative imaging requires both good spatial resolution and high sensitivity to reveal the size, number, and location of the tumors *in vivo*, both NIR fluorescence imaging and PA imaging were performed before surgery with 4T1 subcutaneous tumor-bearing mice by intravenous administration of OTPA-TQ3 NPs. Post NPs injection, the tumor-bearing mice were concurrently scanned by IVIS imaging system and small-animal opt-acoustic tomography system (MOST) at designated time intervals.

The time-dependent *in vivo* non-invasive NIR fluorescence images are shown in Fig. 24A and the corresponding fluorescence intensity-time relationship in tumors is depicted in Fig. 24B. As shown in Figs. 24A and 24B, the NIR fluorescence signal at the tumor site becomes intense gradually as time elapses, stemming from the passive enhanced permeability and retention (EPR) effect. The outstanding EPR effect of OTPA-TQ3 NPs *in vivo* can be attributed to their appropriate size and surface chemistry. Besides the tumor site, the reticuloendothelial system (RES) organs including liver and spleen where the NPs are prone to accumulate also light up (Fig. 24C and Fig. 25). At 24 h post-injection, the fluorescence signal at the tumor site reaches the maximum and the tumor signal-to-background (surrounding normal skin autofluorescence) ratio is as high as ~ 9.2 , which is considered to be very high according to the literature.

On the other hand, the same mice were imaged by PA instrument within a 24 hour time period. As shown in Fig. 24D, the PA signal in tumors gradually amplifies over time, and peaks at 24 h post-injection, coinciding well with the trend of time-dependent NIR fluorescence intensity (Figs. 24A-24B). Noteworthy, the average PA signal in tumors at 24 h is ~ 7.0 times

higher than the background (0 h) before NP injection (Fig. 24E), giving better performance than many high-performing PA imaging agents. Both preoperative NIR fluorescence imaging and PA imaging indicate that the OTPA-TQ3 NPs can detect tumors *in vivo* in an extremely high-contrast manner at 24 h post-injection, providing the surgeon with important information to develop an optimal surgical plan.

Example 19

Intraoperative Fluorescence/Raman Imaging of Residual Tiny Tumors

Next, tumor resection surgery was conducted by a surgeon from Tianjin First Central Hospital (Tianjin, China) using information obtained from preoperative imaging. In the clinic, the most challenging issues for the surgeon during surgery are to evaluate whether there are residual tumors left behind post major tumor excision as well as to differentiate the boundaries between normal and tumor tissues. The combination of NIR fluorescence and Raman imaging hold the integrated advantages of fast and real-time mode, excellent sensitivity and super-high signal-to-background ratio, which are desirable during cancer operation. In this case, the first surgery (S1) was performed by the surgeon after the OTPA-TQ3 NPs were intravenously injected into 4T1 tumor-bearing mice for 24 h, which was followed by NIR fluorescence and Raman imaging at the same time. When the tumors were totally removed by the surgeon, as confirmed by hematoxylin and eosin (H&E) histological analyses, fluorescence and Raman signals were not detected at the surgical incision sites. In other cases in which tiny tumors were left behind, a certain degree of fluorescence signal could be observed (Fig. 26A). Although OTPA-TQ3 NPs possess rather high NIR fluorescence, the volume of residual tumors are tiny, leading to only a small amount of NPs in them. Further, auto-fluorescence from normal tissue compromises the signal-to-background ratio as well. Thus, the surgeon may not be able to accurately assess whether the weak fluorescent areas are indeed tumors. Even so, intraoperative fluorescence imaging is quite necessary, as it is fast, sensitive and real-time and can rapidly point out the suspicious areas of residual tumors.

To pursue precise surgical treatment, Raman imaging with microscopic resolution was conducted in the suspicious areas with faint fluorescence. As illustrated in Fig. 26B, the OTPA-TQ3 NPs with strong Raman signal (2215 cm^{-1}) in the cell-silent region can sensitively visualize the residual tumors and their boundaries to normal tissues by Raman imaging with 'yes-or-no' signature. Such excellent effectiveness of residual tumor detection during surgery can be

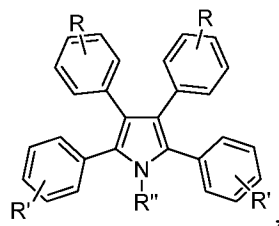
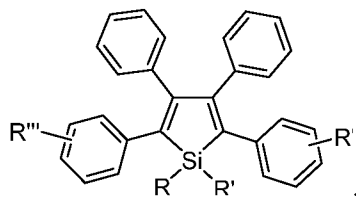
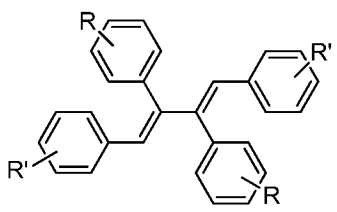
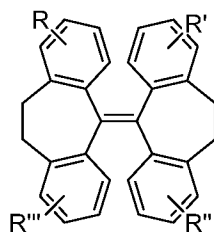
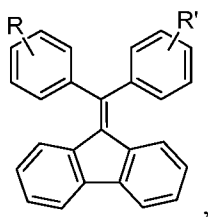
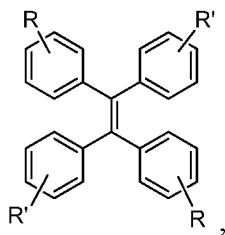
attributed to both the high Raman signal of OTPA-TQ3 NPs and the innate zero background nature of Raman imaging in the cell-silent region. It was noted that 94% of the tested tiny areas with Raman signal were confirmed as tumors by the H&E histological analysis (Fig. 26C). As Raman imaging is much faster than histological analysis, it holds great promise for intraoperative residual tumor inspection. After demonstrating the existence of residual tumors, the surgeon could perform the second surgery (S2) to remove the residual tumors until there were no remaining fluorescence and Raman signals (Figs. 26D-F). The survival rates of mice after S1 (with fluorescence and Raman signals at the surgical incision sites) and S2 (without any signals), respectively, were monitored. As shown in Fig. 27, the mice only undergoing S1 all died during a 40-day study duration post-surgery. In sharp contrast, all of the 10 mice in the S2 group survived 40 days. The result not only indicates that complete removal of tumor tissues is decisive to prolong lifespan, but also suggests that the strategy of using fluorescence-PA-Raman triple-modality OTPA-TQ3 NPs is potent to improve cancer surgery outcomes.

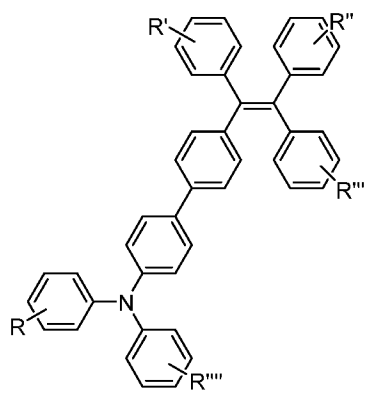
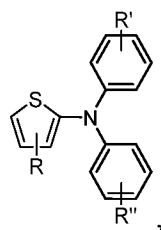
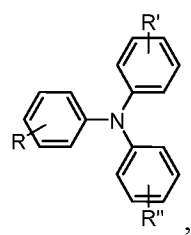
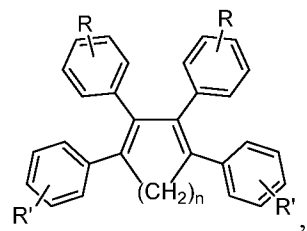
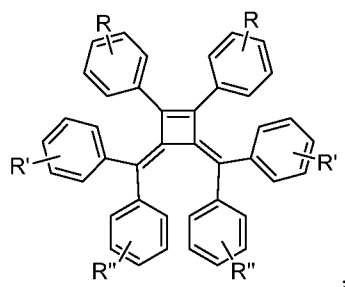
The present subject matter being thus described, it will be apparent that the same may be modified or varied in many ways. Such modifications and variations are not to be regarded as a departure from the spirit and scope of the present subject matter, and all such modifications and variations are intended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

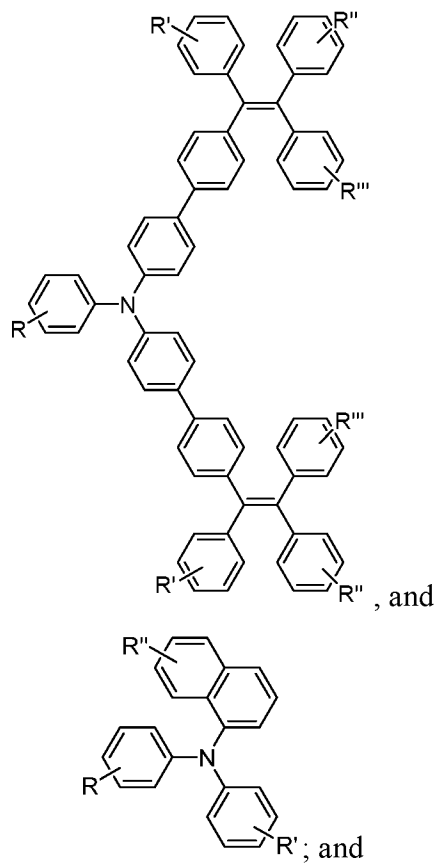
CLAIMS

We claim:

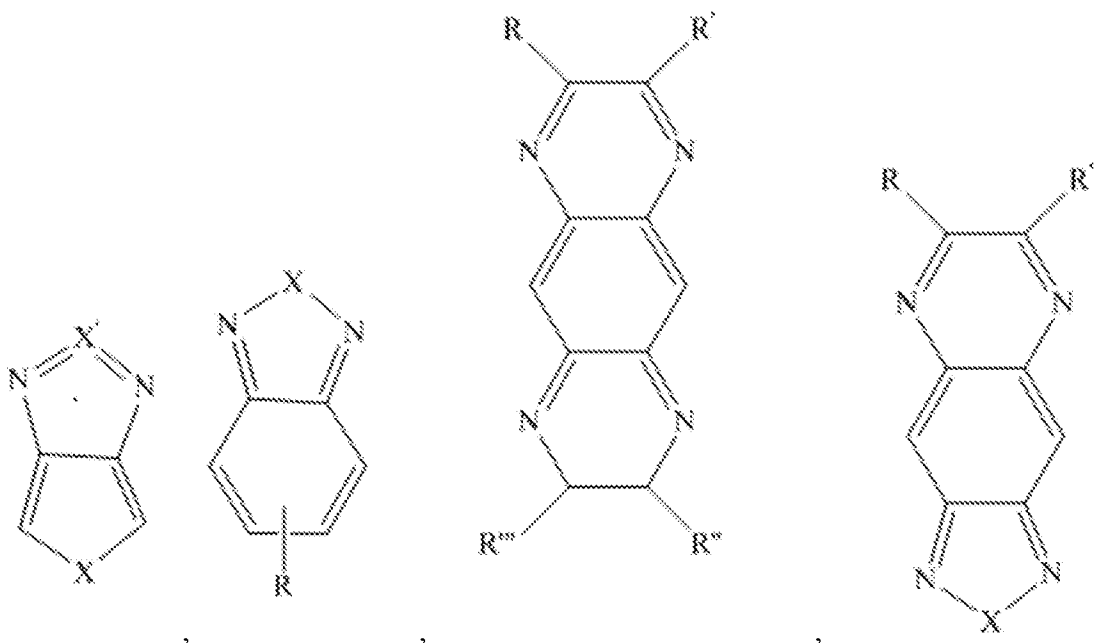
1. A compound having a donor unit selected from one or more of the group consisting of:



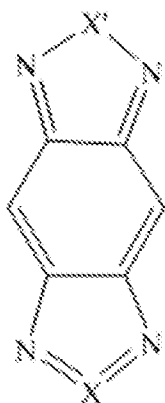




an acceptor unit (A) selected from the group consisting of:



and



wherein the compound has the donor and acceptor units arranged in a form selected from the group consisting of D-A, D-A-D', A-D-A', D-D'-A-D''-D''', A-A'-D-A''-A''', D-D'-A-D''-A', D-D'-A-A'-D'', D-A-D'-A'-A'', A-D-D'-A'-A'', D-A-D'-A'-D'', and A-D-A'-D'-A''

wherein D, D', D'' and D''' can be the same or different and represent the donor unit;

wherein A, A', A'' and A''' can be the same or different and represent the acceptor unit;

wherein each of X and X' is independently selected from the group consisting of O, S, Se, and Te;

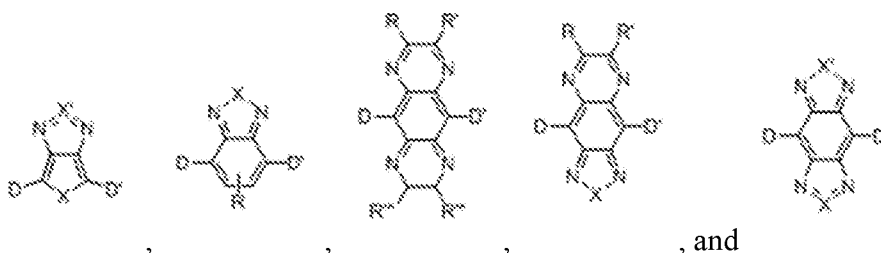
wherein each of R, R', R'', R''', and R'''' is unsubstituted or substituted and is independently selected from the group consisting of F, H, alkyl, unsaturated alkyl, azide, alkynyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, hydrazine, carboxyl group, amino group, sulfonic group, sulfhydryl, alkylthio, nitrene, aldehyde, hydroxyl, halide, charged ionic group, and alkoxy group;

wherein at least one of R, R', R'', R''', and R'''' is a terminal functional group independently selected from the group consisting of N₃, NCS, SH, NH₂, COOH, substituted or unsubstituted alkyne, N-hydroxysuccinimide ester, maleimide, hydrazide, nitrene group, -CHO, -OH, halide, and a charged ionic group; and

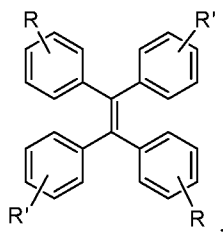
wherein at least one of R, R', R'', R''', and R'''' includes an alkyne group.

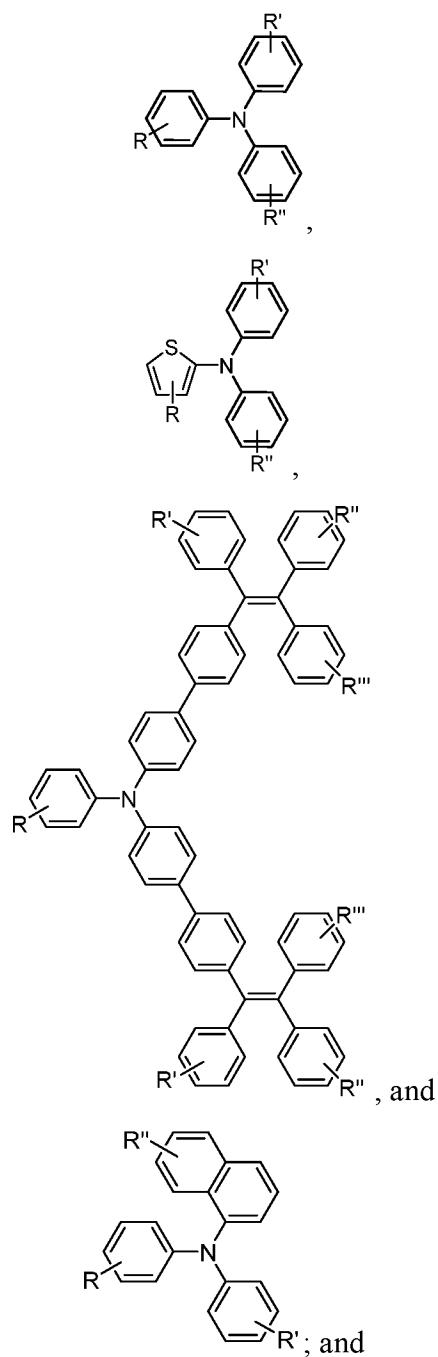
2. The compound according to claim 1, further comprising one or more peptides conjugated thereto.

3. The compound according to claim 1, wherein the compound comprises a structural formula selected from the group consisting of:



wherein each of D and D' is independently selected from the group consisting of





wherein each of R, R', R'', and R''' is unsubstituted or substituted and is independently selected from the group consisting of F, H, alkyl, unsaturated alkyl, azide, alkynyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, hydrazine, carboxyl group, amino group, sulfonic group, sulfhydryl, alkylthio, nitrene, aldehyde, hydroxyl, halide, charged ionic group, and alkoxy group;

wherein at least one of R, R', R'' and R''' is a terminal functional group independently

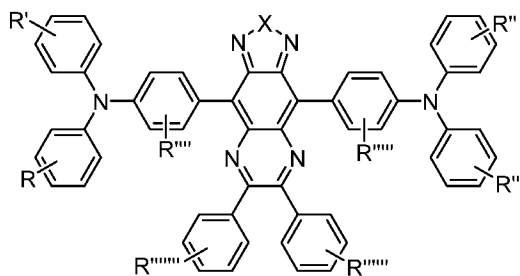
selected from the group consisting of N₃, NCS, SH, NH₂, COOH, substituted or unsubstituted alkyne, N-Hydroxysuccinimide ester, maleimide, hydrazide, nitron group, -CHO, -OH, halide, and charged ionic group;

wherein each of X and X' is independently selected from the group consisting of O, S, Se, and Te; and

wherein at least one of R, R', R'', R''', and R'''' includes an alkyne group.

4. The compound according to claim 3, further comprising one or more peptides conjugated thereto.

5. The compound according to claim 3, comprising the following structural formula:



wherein each of R, R', R'', R''', R''', R''', R''', and R'''' is unsubstituted or substituted and is independently selected from the group consisting of F, H, alkyl, unsaturated alkyl, azide, alkynyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, hydrazine, carboxyl group, amino group, sulfonic group, sulfhydryl, alkylthio, nitron, aldehyde, hydroxyl, halide, charged ionic group, and alkoxy group;

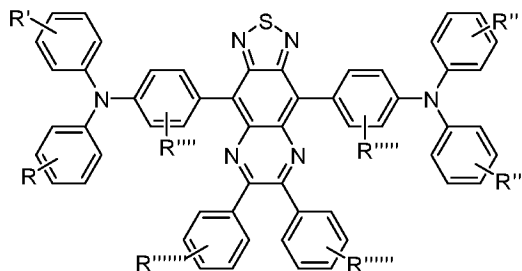
wherein at least one of R, R', R'', R''', R''', R''', R''', and R'''' is a terminal functional group independently selected from the group consisting of N₃, NCS, SH, NH₂, COOH, substituted or unsubstituted alkyne, N-Hydroxysuccinimide ester, maleimide, hydrazide, nitron group, -CHO, -OH, halide, and charged ionic group;

wherein each of X and X' is independently selected from the group consisting of O, S, Se, and Te; and

wherein at least one of R, R', R'', R''', R''', R''', R''', and R'''' includes an alkyne group.

6. The compound according to claim 5, further comprising one or more peptides conjugated thereto.

7. The compound according to claim 5, comprising the following structural formula:



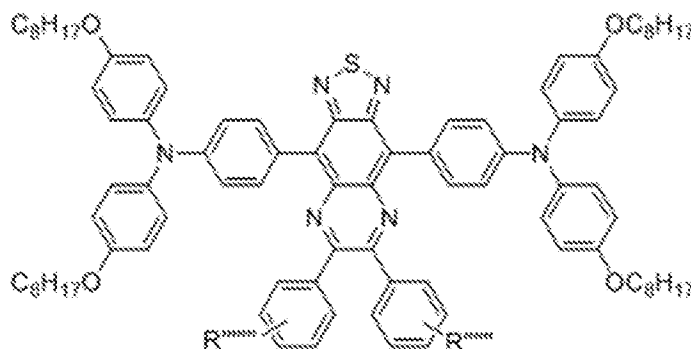
wherein each of R, R', R'', R''', R''', R''', R''', and R'''' is unsubstituted or substituted and is independently selected from the group consisting of F, H, alkyl, unsaturated alkyl, azide, alkynyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, hydrazine, carboxyl group, amino group, sulfonic group, sulfhydryl, alkylthio, nitron, aldehyde, hydroxyl, halide, charged ionic group, and alkoxy group;

wherein at least one of R, R', R'', R''', R''', R''', R''', and R'''' is a terminal functional group independently selected from the group consisting of N₃, NCS, SH, NH₂, COOH, substituted or unsubstituted alkyne, N-Hydroxysuccinimide ester, maleimide, hydrazide, nitron group, -CHO, -OH, halide, and charged ionic group; and

wherein at least one of R, R', R'', R''', R''', R''', and R'''' includes an alkyne group.

8. The compound according to claim 7, further comprising one or more peptides conjugated thereto.

9. The compound according to claim 7, wherein the compound comprises the following structural formula:



wherein each of R'''' and R'''' is unsubstituted or substituted and is independently

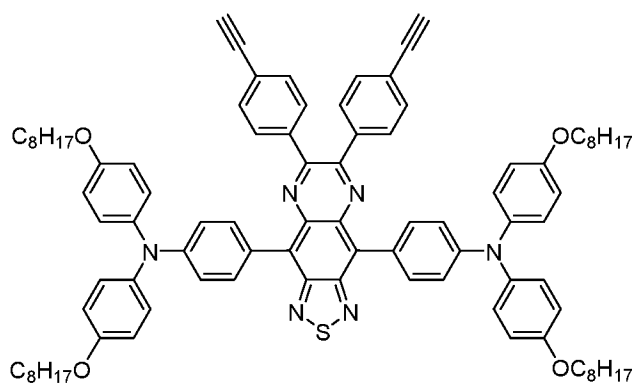
selected from the group consisting of F, H, alkyl, unsaturated alkyl, azide, alkynyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, hydrazine, carboxyl group, amino group, sulfonic group, sulfhydryl, alkylthio, nitron, aldehyde, hydroxyl, halide, charged ionic group, and alkoxy group;

wherein at least one of R^{''''''} and R^{''''''''} is a terminal functional group independently selected from the group consisting of N₃, NCS, SH, NH₂, COOH, substituted or unsubstituted alkyne, N-Hydroxysuccinimide ester, maleimide, hydrazide, nitron group, -CHO, -OH, halide, and charged ionic group; and

wherein at least one of R^{''''''} and R^{''''''''} includes an alkyne group.

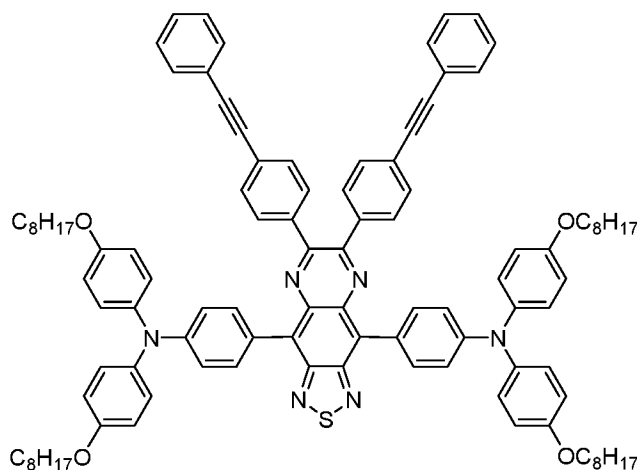
10. The compound according to claim 9, further comprising one or more peptides conjugated thereto.

11. The compound according to claim 9, wherein the compound is selected from the group consisting of



OTPA-TQ2

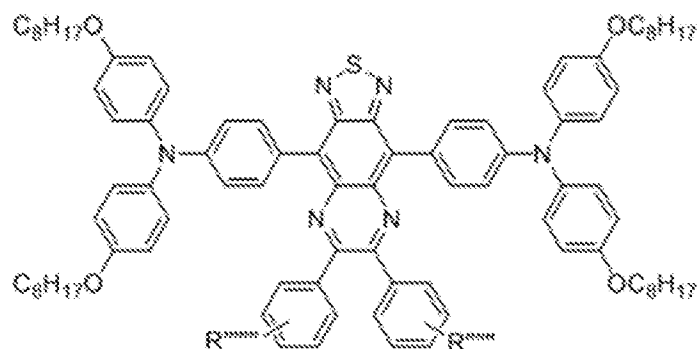
and

**OPTA-TQ3.**

12. The compound of claim 1, wherein the compound is in nanoparticle form.
13. A composition comprising the compound of claim 12 and an amphiphilic matrix encapsulating the compound.
14. A method of locating a tumor site in a patient, comprising:
administering the compound of claim 1 to the patient;
contacting the tumor site with the compound; and
locating the tumor site using an imaging method after the tumor site is contacted with the compound.
15. The method of claim 14, wherein the compound is administered by intravenous injection.
16. The method of claim 14, wherein the imaging method comprises at least one of fluorescence microscopy, Raman microscopy, and photoacoustic imaging.
17. The method of claim 14, further comprising altering intramolecular motions of the compound by changing viscosity of a solution containing the compound or forming different kinds of aggregates.
18. A method of *in vivo* imaging of an animal, comprising:
administering the compound of claim 1 to the animal; and
obtaining images of the animal while the compound is within the animal using an

imaging method.

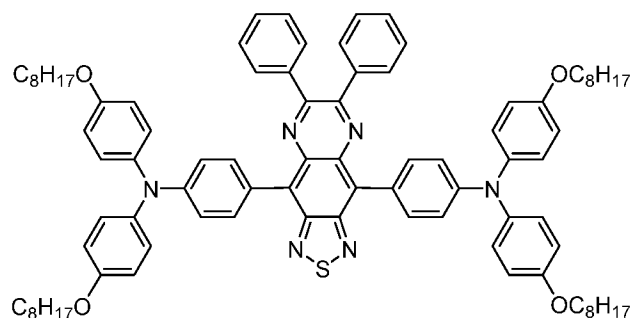
19. A method of locating a tumor site in a patient, comprising:
 administering a compound to the patient;
 contacting the tumor site with the compound; and
 locating the tumor site using an imaging method after the tumor site is contacted with the compound, wherein the compound comprises the following structural formula:



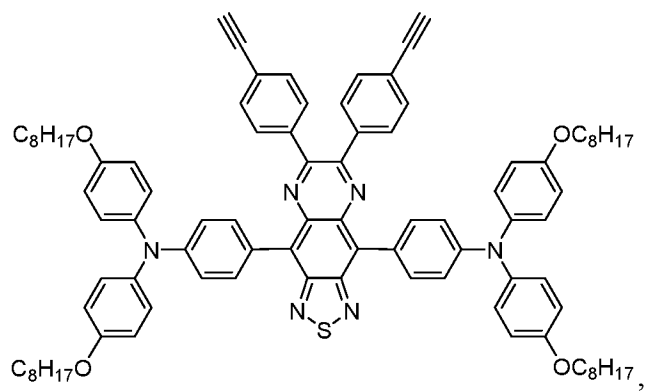
wherein each of R'''' and R'''' is unsubstituted or substituted and is independently selected from the group consisting of F, H, alkyl, unsaturated alkyl, azide, alkynyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, hydrazine, carboxyl group, amino group, sulfonic group, sulfhydryl, alkylthio, nitron, aldehyde, hydroxyl, halide, charged ionic group, and alkoxy group; and

wherein at least one of R'''' and R'''' is a terminal functional group independently selected from the group consisting of N₃, NCS, SH, NH₂, COOH, substituted or unsubstituted alkyne, N-Hydroxysuccinimide ester, maleimide, hydrazide, nitron group, -CHO, -OH, halide, and charged ionic group.

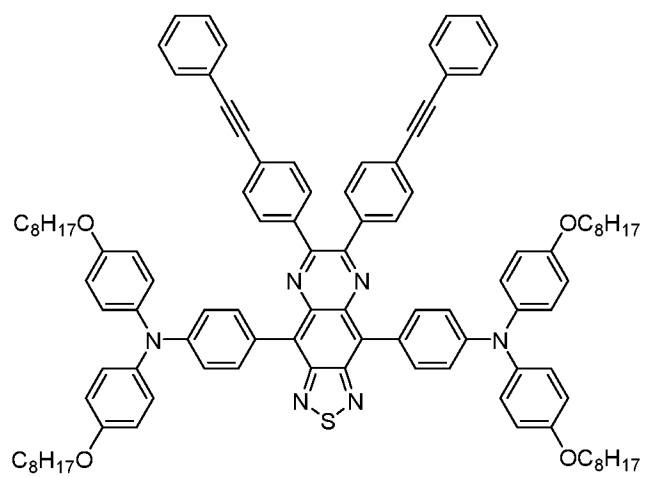
20. The method according to claim 19, wherein the compound is selected from the group consisting of



OTPA-TQ1,

**OTPA-TQ2**

and

**OPTA-TQ3.**

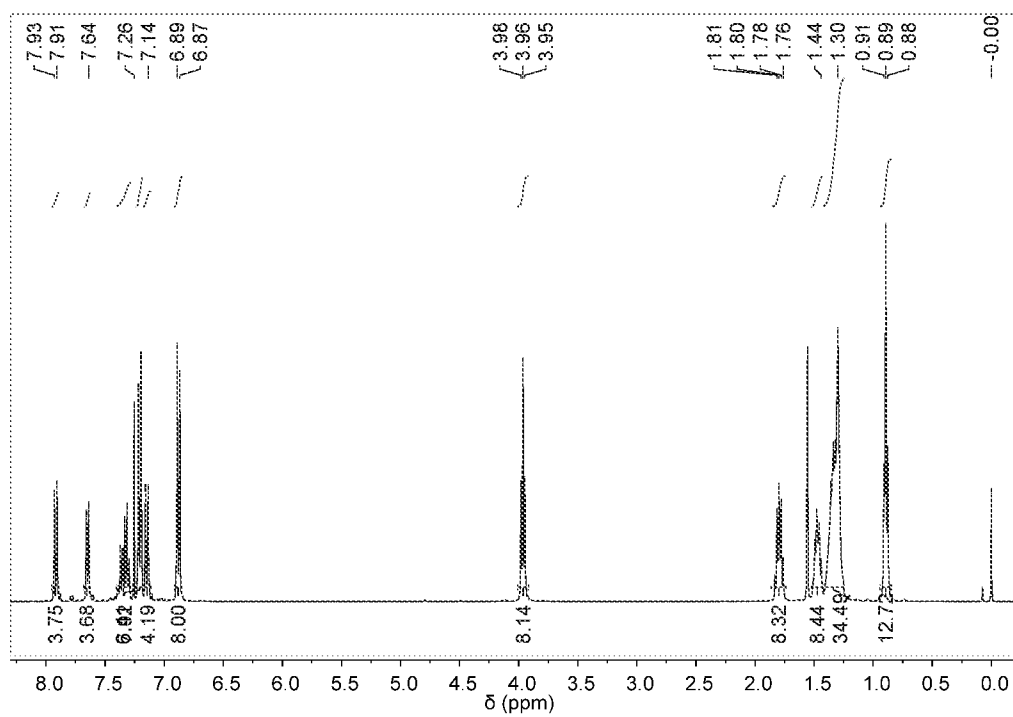


Fig. 1

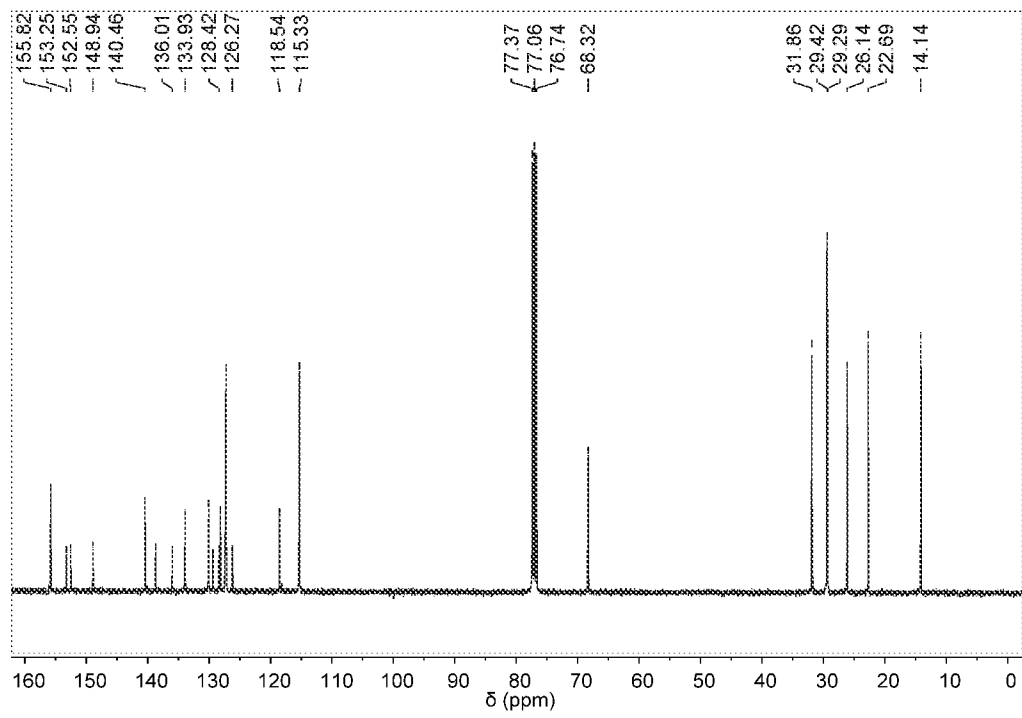


Fig. 2

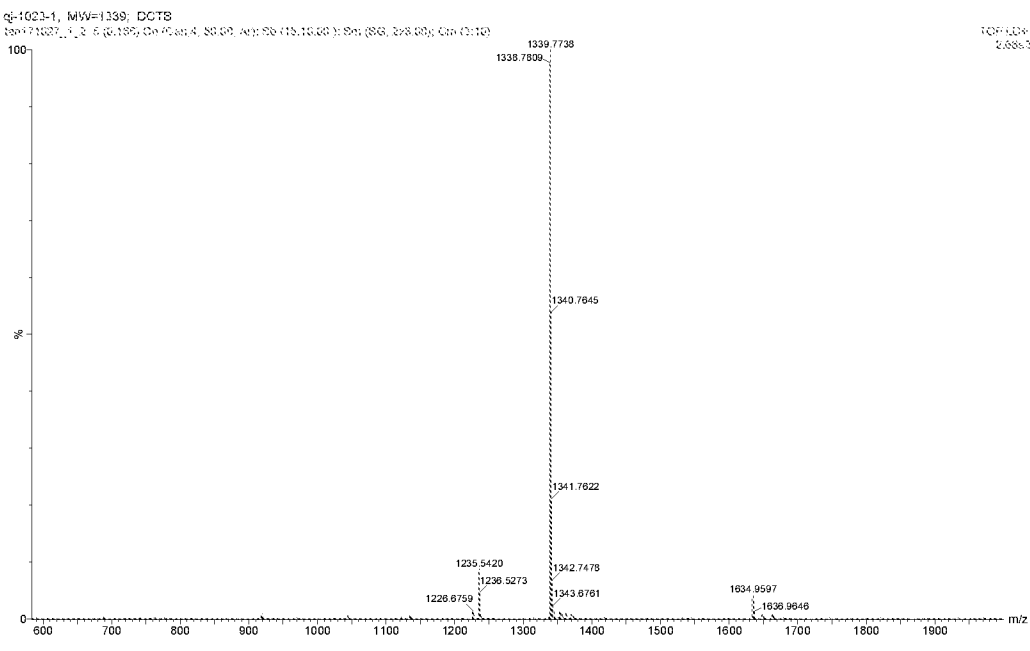


Fig. 3

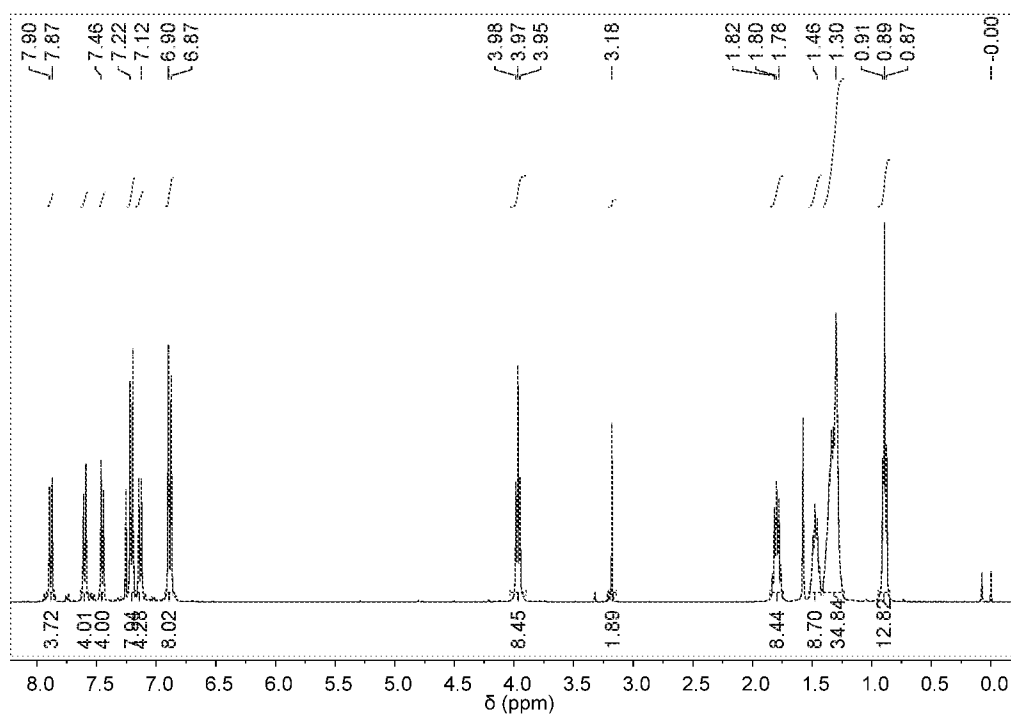


Fig. 4

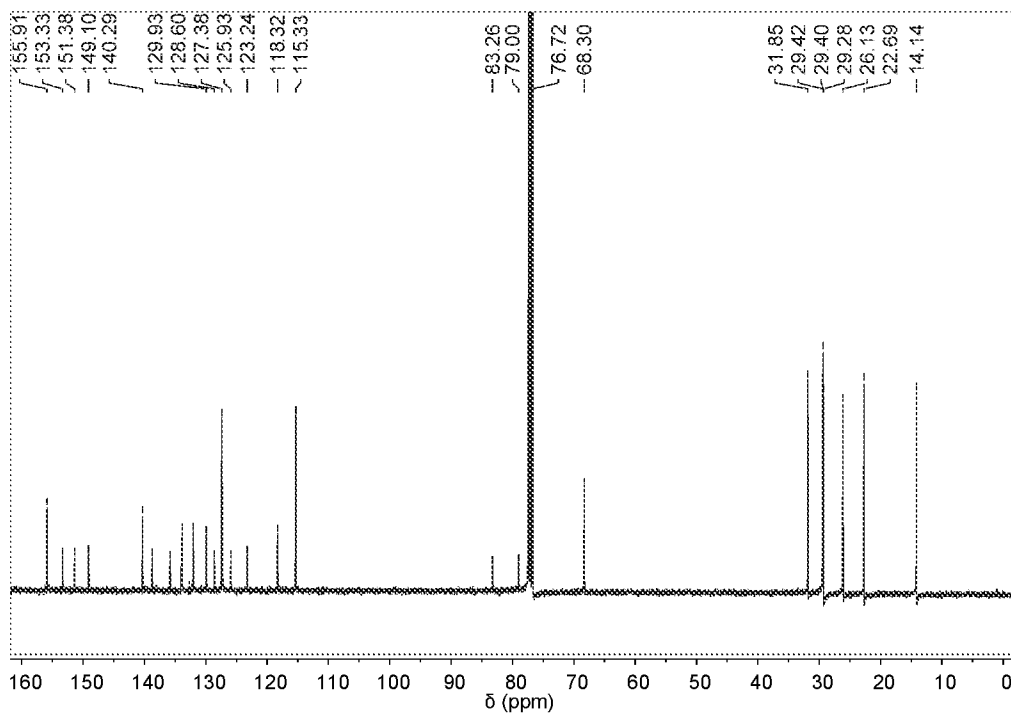


Fig. 5

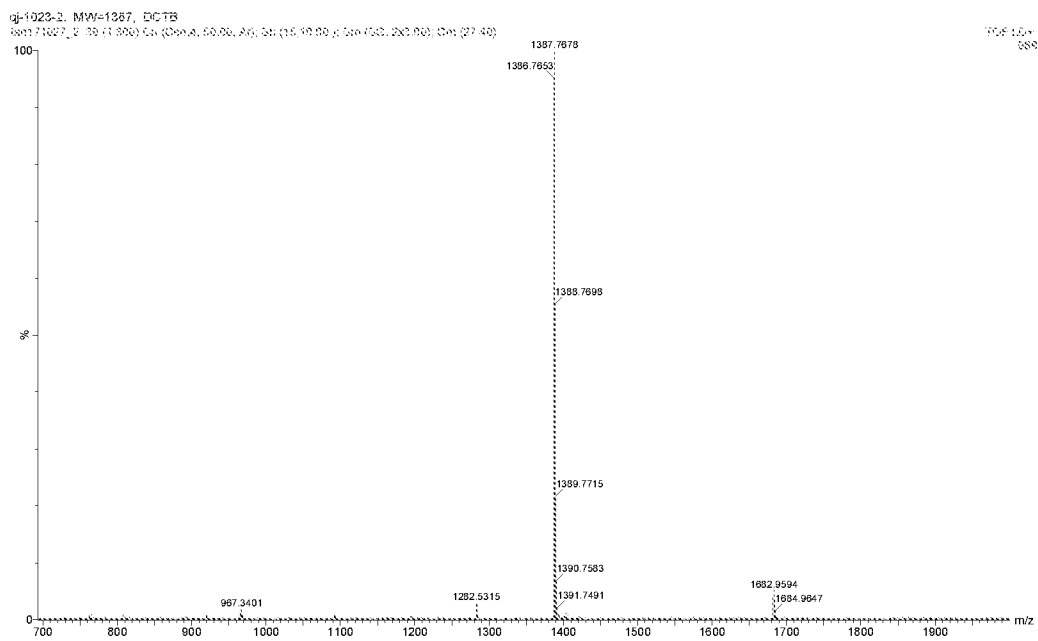


Fig. 6

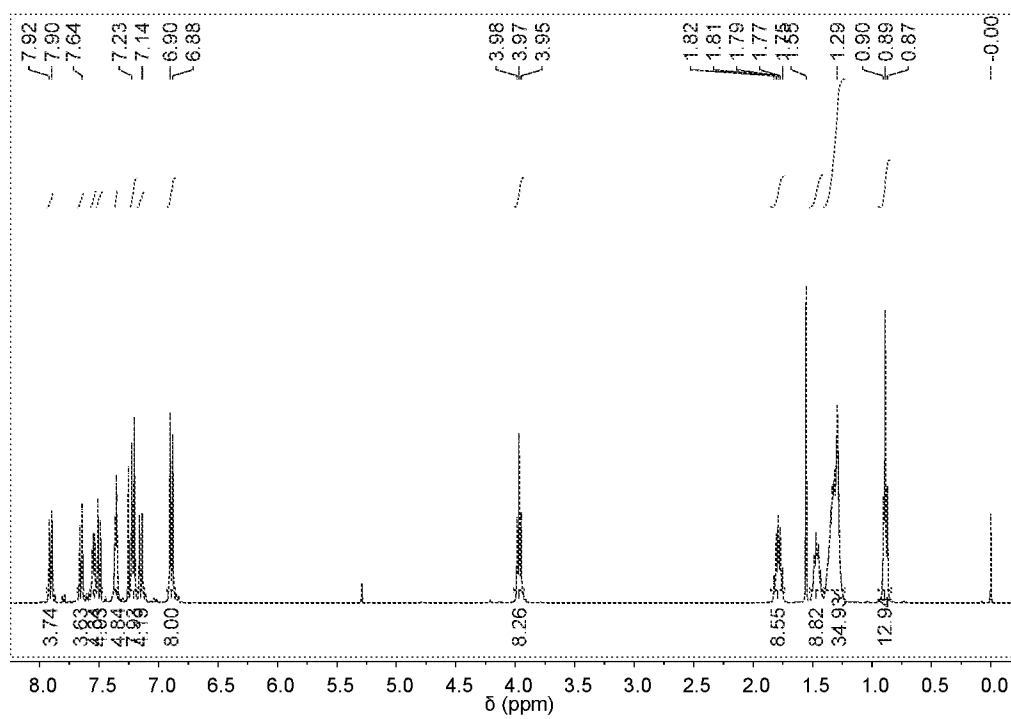


Fig. 7

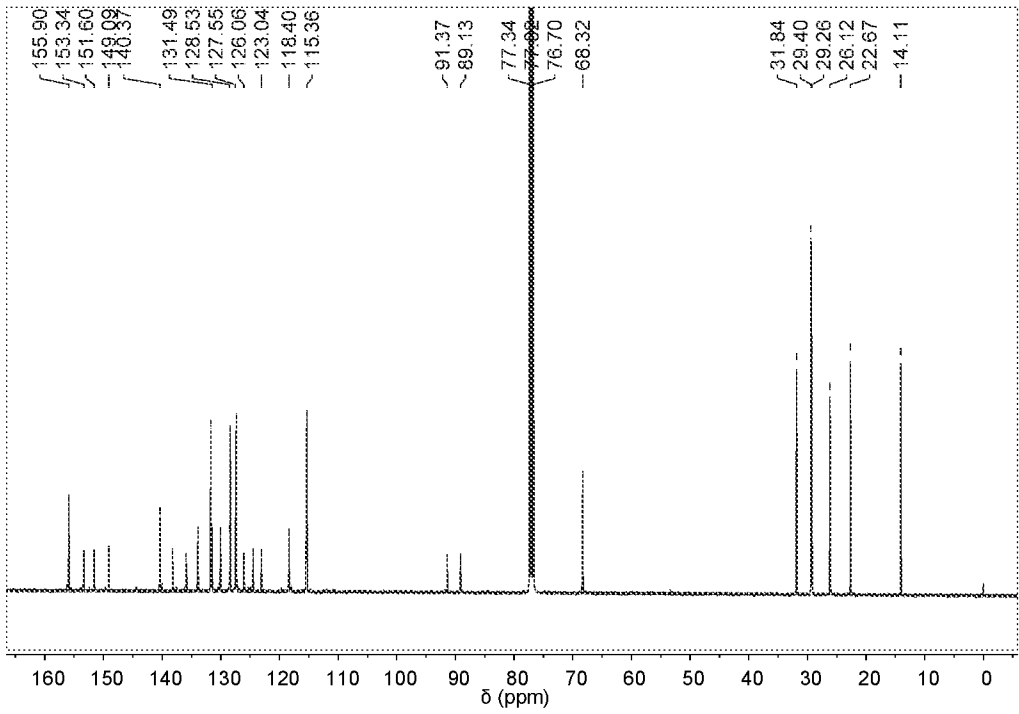


Fig. 8

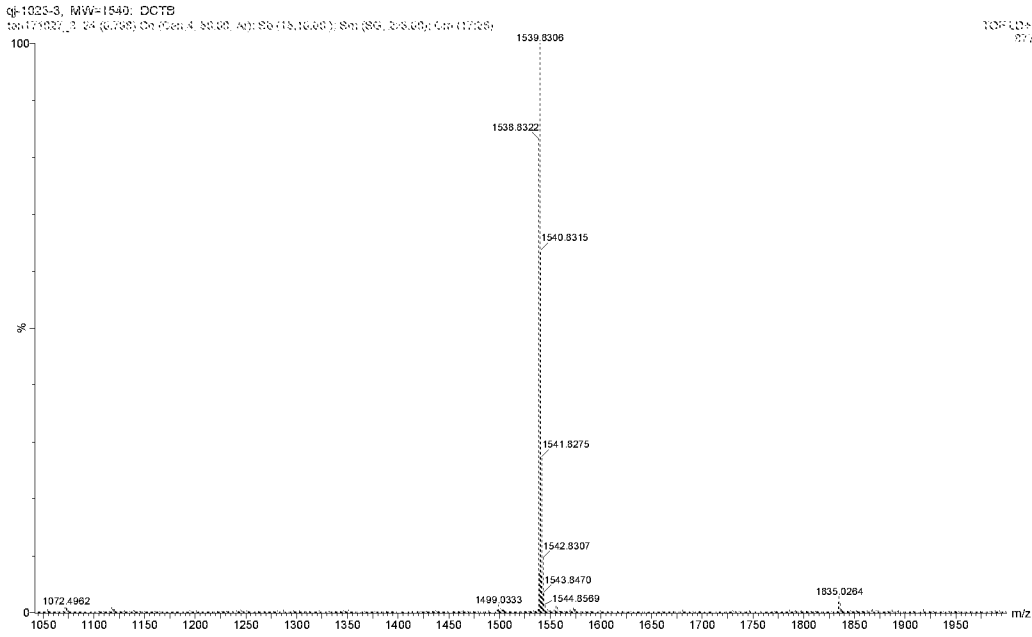
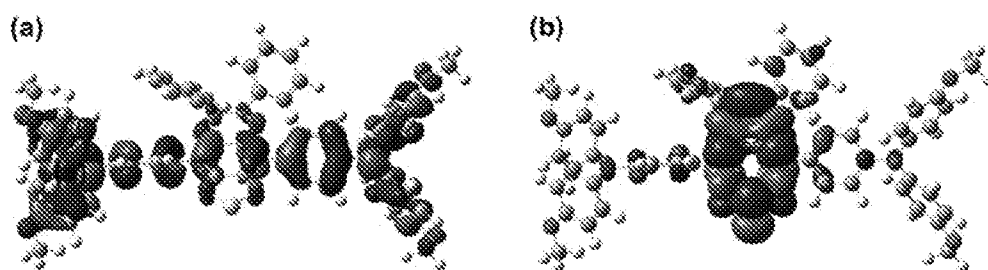
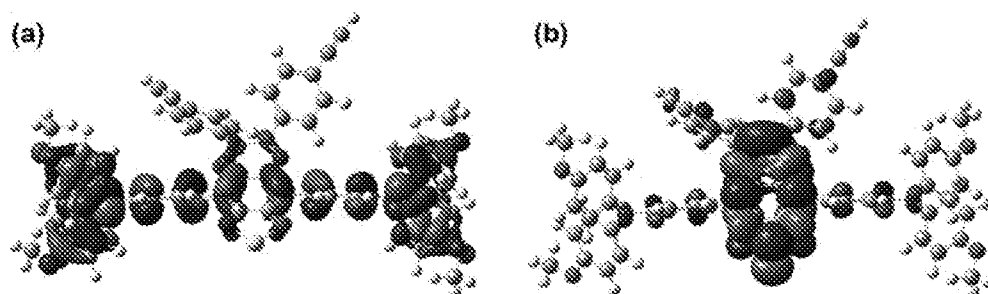


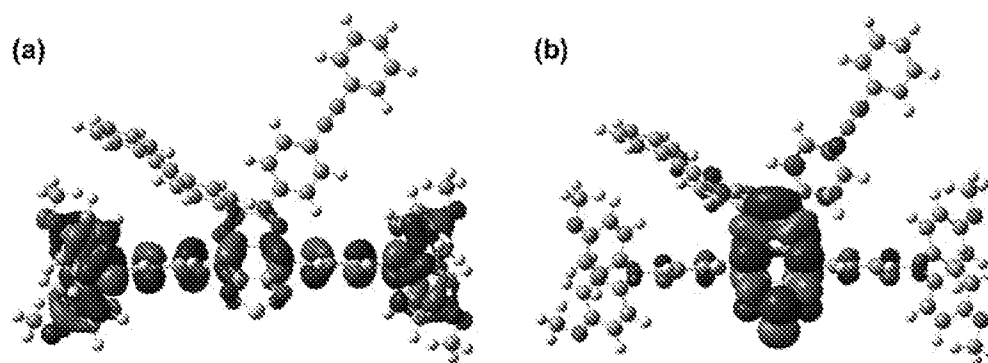
Fig. 9



Figs. 10A-10B



Figs. 11A-11B



Figs. 12A-12B

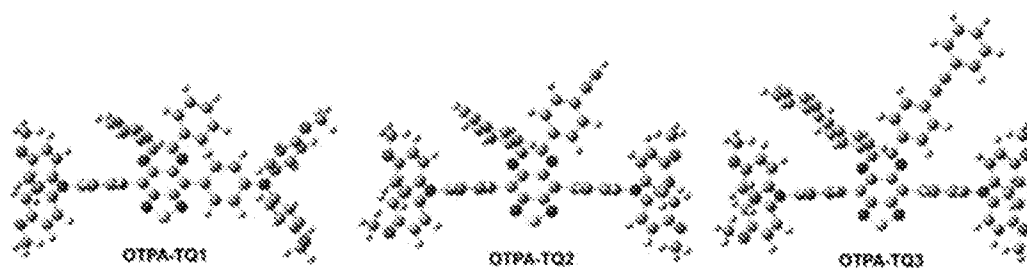


Fig. 13A

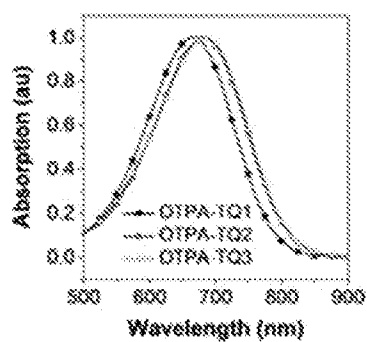


Fig. 13B

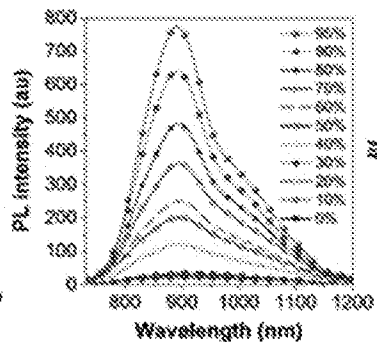


Fig. 13C

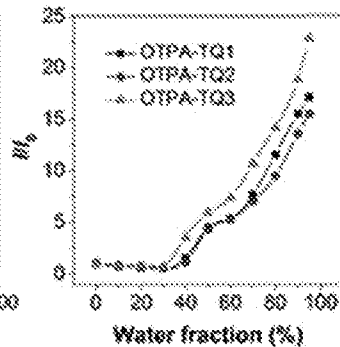


Fig. 13D

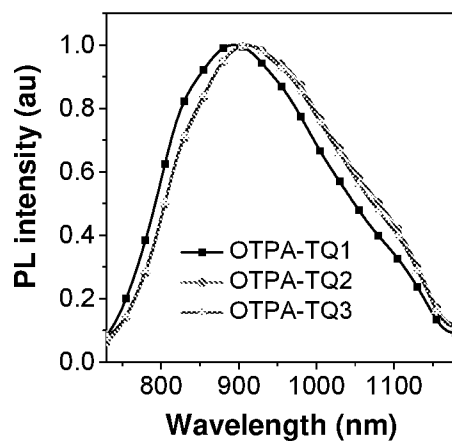
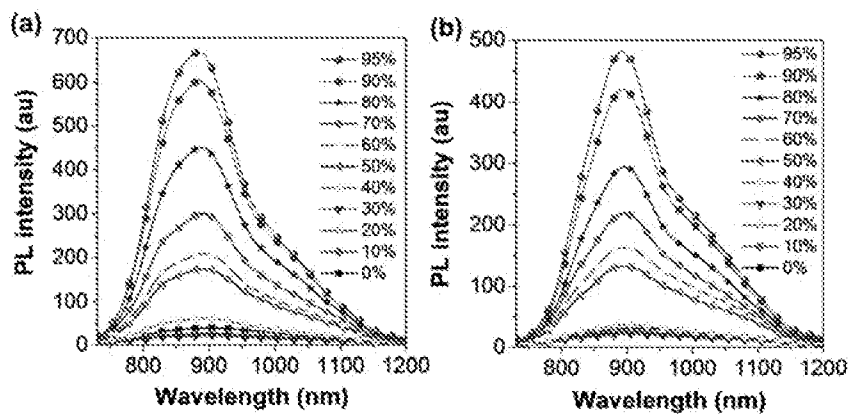
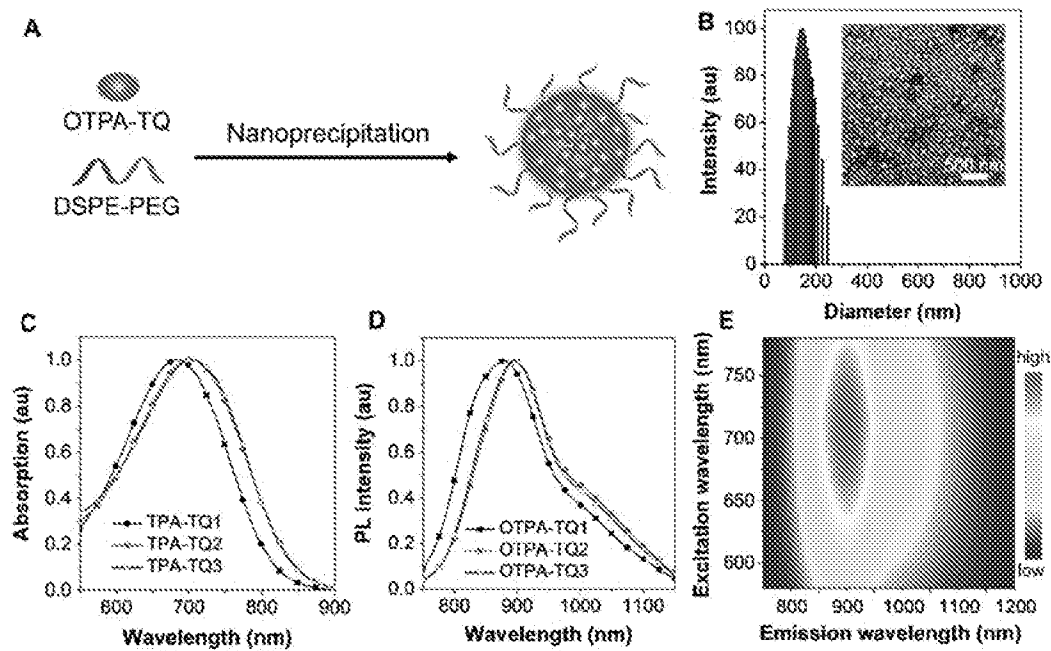


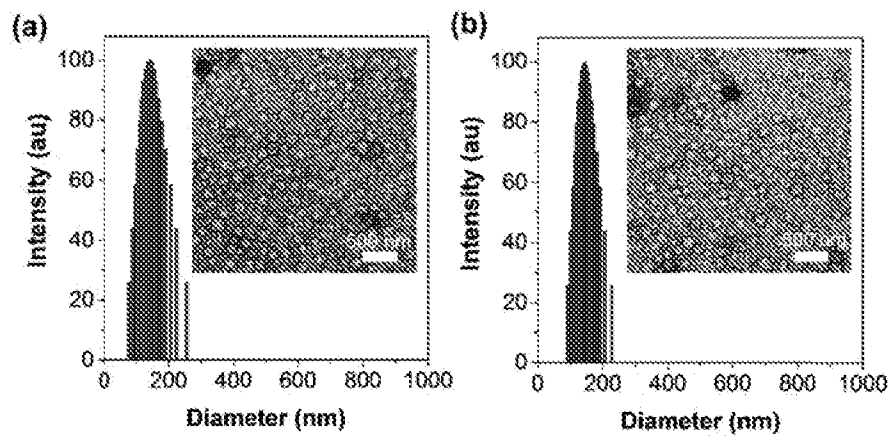
Fig. 14



Figs. 15A-15B



Figs. 16A-16E



Figs. 17A-17B

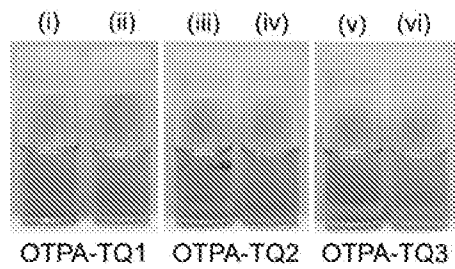


Fig. 18A

Fig. 18B

Fig. 18C

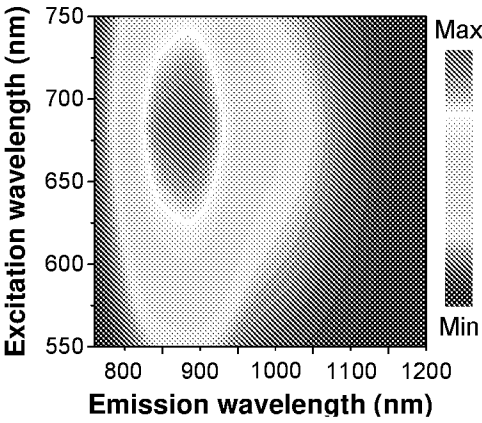


Fig. 19

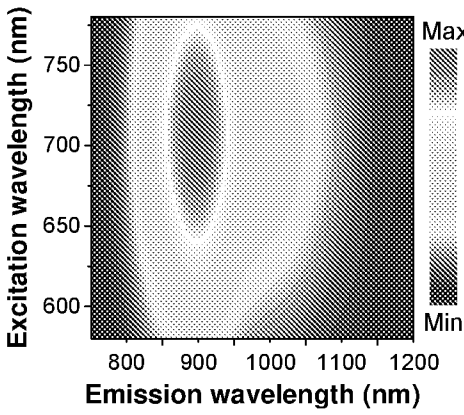
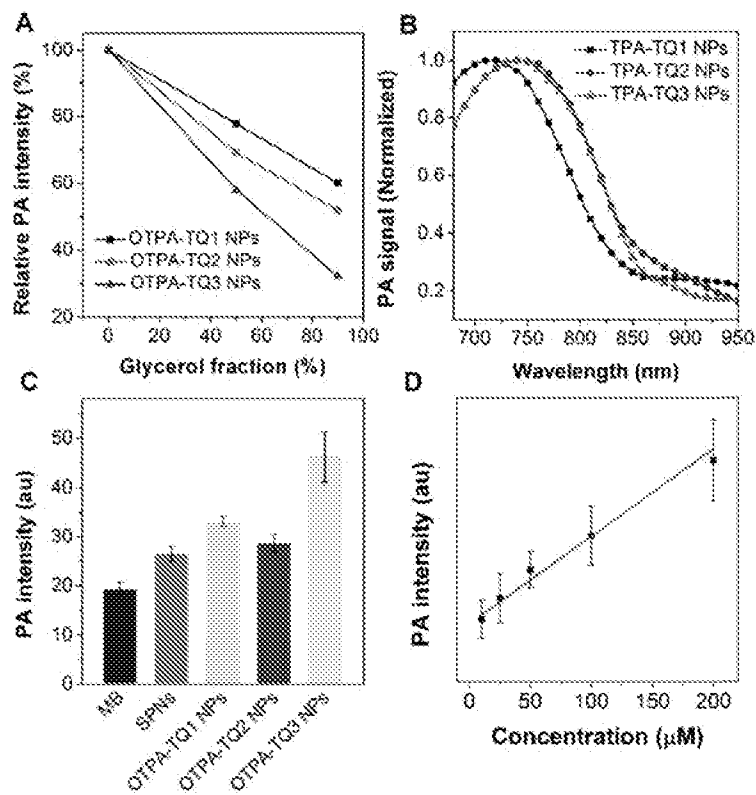
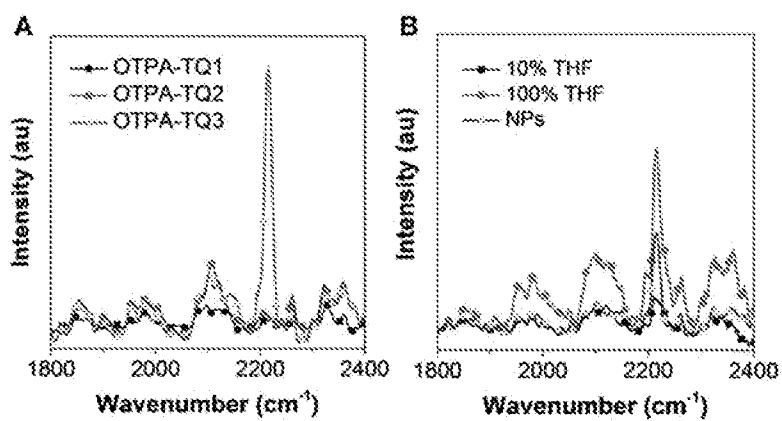


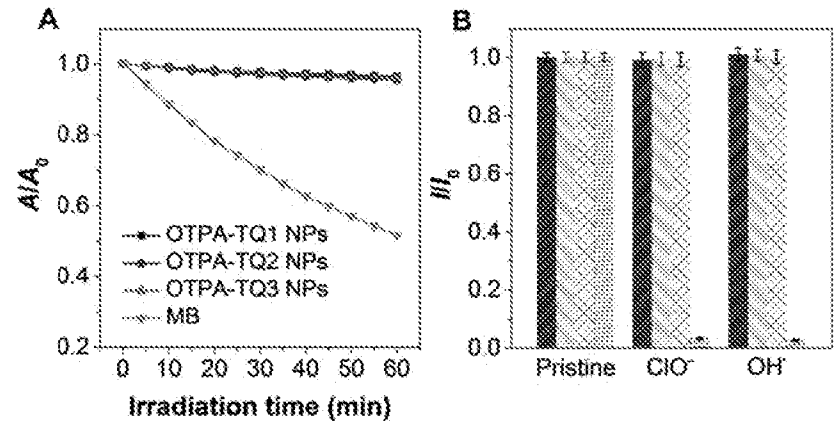
Fig. 20



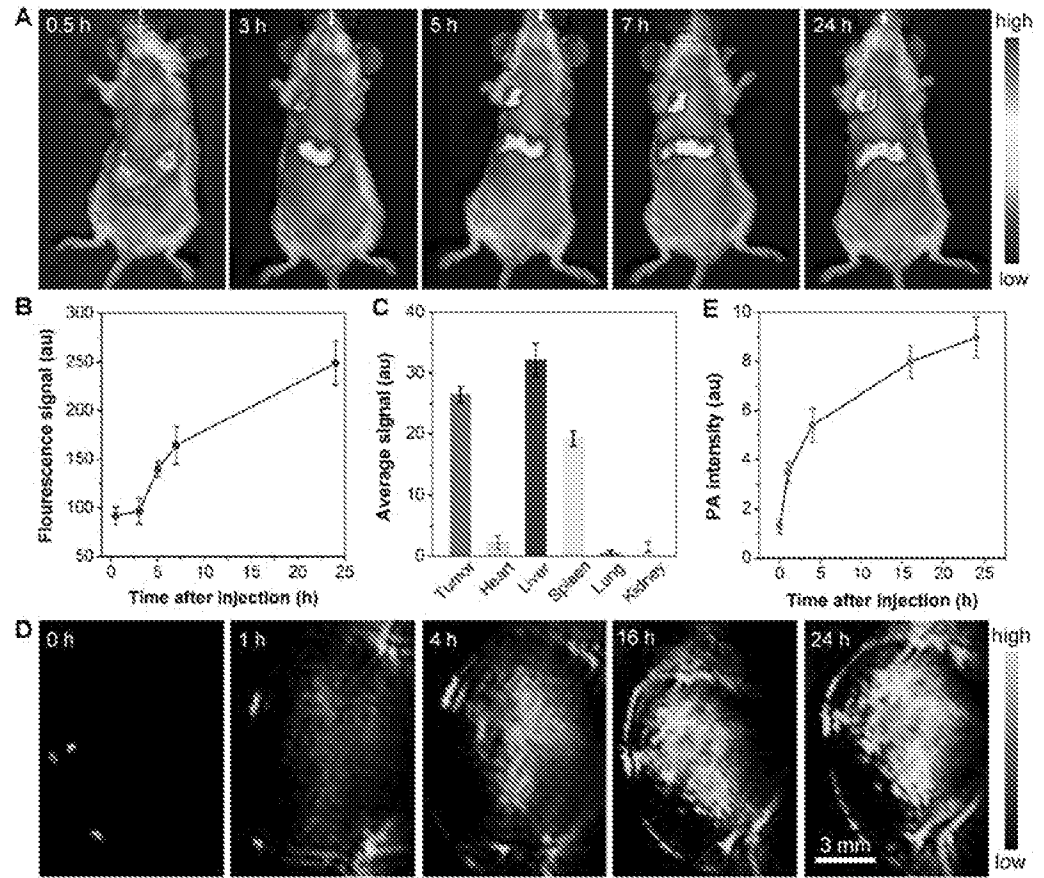
Figs. 21A-21D



Figs. 22A-22B



Figs. 23A-23B



Figs. 24A-24E

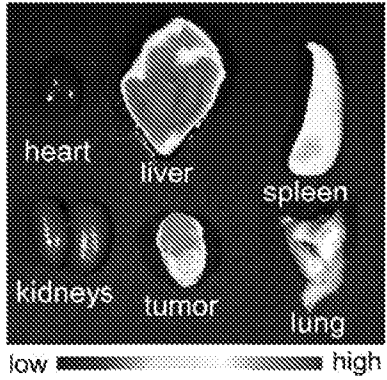
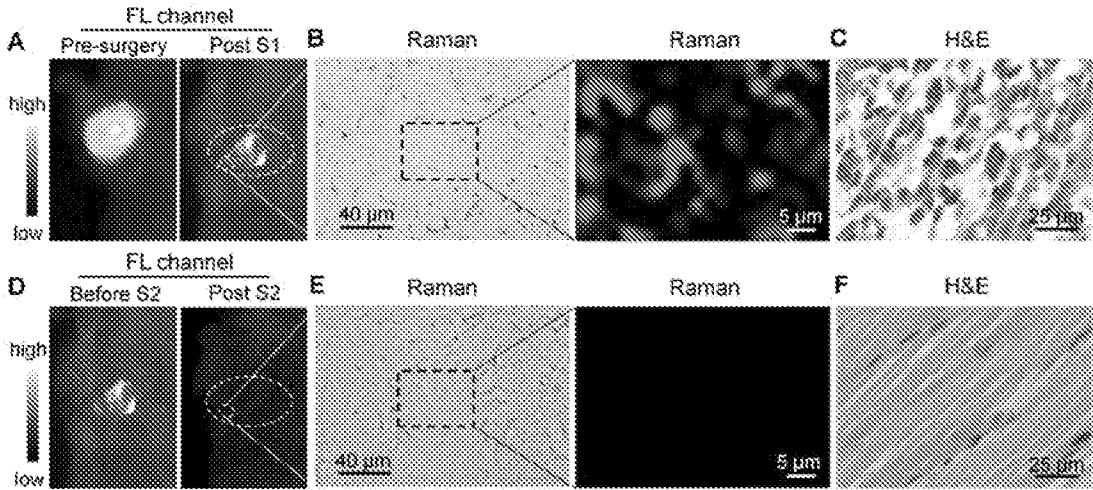


Fig. 25



Figs. 26A-26F

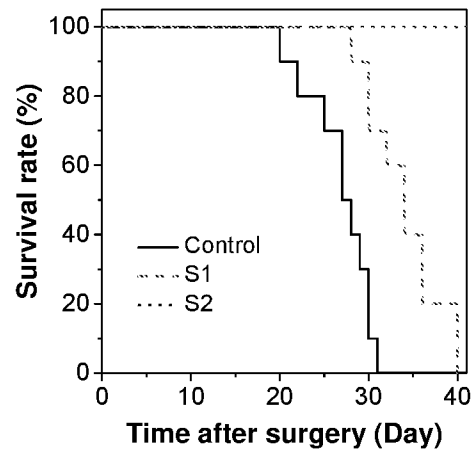


Fig. 27

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2020/078905

A. CLASSIFICATION OF SUBJECT MATTER

C07D 241/24(2006.01)i; C07D 487/02(2006.01)i; C09K 11/00(2006.01)i; H01L 51/50(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)

C07D; C09K; H01L

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)

WPI,EPODOC,CNPAT,CNKI,REGISTRY,CAPLUS:AIE,luminogen,donor,acceptor,aggregat+,biological,emission,probe,sensor,structure

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2018108070 A1 (THE HONG KONG UNIVERSITY OF SCIENCE AND TECHNOLOGY) 21 June 2018 (2018-06-21) see paragraphs [120]-[125], page 39 compound TPE-TQ-A	1-20
A	JP 2018006700 A (KONICA MINOLTA INC) 11 January 2018 (2018-01-11) see the whole document	1-20
A	CN 106831650 A (UNIV. JIANGXI NORMAL) 13 June 2017 (2017-06-13) see the whole document	1-20
A	ISHI-I, T. et al. "Light-emitting properties of donor-acceptor and donor-acceptor-donor dyes in solution, solid, and aggregated states: structure-property relationship of emission behavior" <i>RSD Advances</i> , Vol. 5, 14 October 2015 (2015-10-14), see pages 89171-89187	1-20
A	MCNAMARA, L.E. et al. "Donor-Acceptor-Donor Thienopyrazines via Pd-Catalyzed C-H Activation as NIR Fluorescent Materials" <i>The Journal of Organic Chemistry</i> , Vol. 81, 24 November 2015 (2015-11-24), see pages 32-42	1-20



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

08 May 2020

Date of mailing of the international search report

02 June 2020

Name and mailing address of the ISA/CN

National Intellectual Property Administration, PRC
6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing
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Facsimile No. (86-10)62019451

Authorized officer

HE, Yiqiu

Telephone No. 86-(10)-53962317

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KONO, T.et al. "Synthesis and photo-electrochemical properties of novel thienopyrazine and quinoxaline derivatives, and their dye-sensitized solar cell performance" <i>Organic Electronics</i> , Vol. 13, 04 October 2012 (2012-10-04), see pages 3097-3101 Fig.1	1-20
A	LU, X.et al. "Controlling the Charge Transfer in D–A–D Chromophores Based on Pyrazine Derivatives" <i>J.Org.Chem.</i> , Vol. 13, 04 October 2012 (2012-10-04), see pages 6480-6489 Chart 1	1-20
A	KATO, S.et al. "Strongly red-fluorescent novel donor–p-bridge–acceptor–p-bridge–donor (D–p–A–p–D) type 2, 1, 3-benzothiadiazoles with enhanced two-photon absorption cross-sections" <i>ChemComm</i> , 07 September 2004 (2004-09-07), see pages 2342-2343 compounds4-5, 7	1-20
A	MCNAMARA, L.E.et al. "Donor–Acceptor–Donor Thienopyrazines via Pd-Catalyzed C–H Activation as NIR Fluorescent Materials" <i>The Journal of Organic Chemistry</i> , Vol. 81, 24 November 2015 (2015-11-24), see pages 32-43	1-20
A	QIAN G.et al. "Simple and Efficient Near-Infrared Organic Chromophores for Light-Emitting Diodes with Single Electroluminescent Emission above 1000 nm" <i>Adv.Mater.</i> , Vol. 21, 31 December 2009 (2009-12-31), see pages 111-116 Figure 1	1-20

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2020/078905

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.: **14-20**
because they relate to subject matter not required to be searched by this Authority, namely:

[1] Claims 14-20 are directed to a method for diagnosis of diseases, but the search has been carried out and is based on the use of the claimed compound for manufacturing medicaments having the alleged effects.
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).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2020/078905

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
WO	2018108070	A1	21 June 2018	US	2020079787	A1	12 March 2020
				CN	110312708	A	08 October 2019
JP	2018006700	A	11 January 2018	None			
CN	106831650	A	13 June 2017	None			