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(54) Titre : COMPLEXES DE METALLOGLYCOPROTEINES ET LEUR UTILISATION COMME COMPOSES CHIMIOTHERAPEUTIQUES

(54) Title: METAL-GLYCOPROTEIN COMPLEXES AND THEIR USE AS CHEMOTHERAPEUTIC COMPOUNDS

(57) Abrégé/Abstract:
Compositions of the invention include glycoproteins, such as transferrin, and metal-based coordination complexes, which are preferably chemotherapeutic compounds and more preferably tunable photodynamic compounds. The compositions are useful as in vivo diagnostic agents, and as therapeutic agents for treating or preventing diseases including those that involve hyperproliferating cells in their etiology, such as cancer. Compositions of the invention are further capable of destroying microbial cells, such as bacteria, fungi, and protozoa, and destroying viruses.

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METAL-GLYCOPROTEIN COMPLEXES AND THEIR USE AS CHEMOTHERAPEUTIC COMPOUNDS

BACKGROUND OF THE INVENTION

1. FIELD OF INVENTION

[0001] This invention relates to metal-based coordination complexes, and more particularly to metal-glycoprotein complexes that are particularly useful as therapeutic and diagnostic agents,

2. DESCRIPTION OF RELATED ART

[0002] Photodynamic therapy (PDT) is currently an active area of research for the treatment of diseases associated with hyperproliferating cells such as cancer and non-malignant lesions. The development of new photodynamic compounds (PDCs or photosensitizers, PSs) for photodynamic therapy (PDT) has been increasingly focused on metallosupramolecular complexes derived from metals. For example, WO 2013158550 A1 and WO 2014145428 A2 disclose metal-based PDCs useful as *in vivo* diagnostic agents, as therapeutic agents for treating or preventing diseases that involve unwanted and/or hyperproliferating cell etiology, including cancer, as agents for treating infectious diseases, and as agents for pathogen disinfection and/or sterilization. US 6962910, US 7612057, US 8445475 and US 8148360 disclose supramolecular metal complexes capable of cleaving DNA when irradiated low energy visible light with or without molecular oxygen.

[0003] Delivery of metal-based coordination complexes and PDCs to biological targets can pose a challenge, which many have attempted to address.

[0004] For example, US 20120264802 discloses photosensitizer compounds based on functionalized fullerenes useful in targeted PDT, and methods of use thereof.

[0005] WO 2013020204 A1 discloses biodegradable polymeric nanoparticles comprising an inner core formed of a photodynamic agent capable of being activated to generate cytotoxic singlet oxygen. These nanoparticles have anti-cell proliferation activity and are useful in treating both cancerous and non-cancerous conditions including actinic keratosis, psoriasis and acne vulgaris. Preferably, the photodynamic agent is a hypocrellin B derivative while the polymeric nanoparticle comprises polyglycolic acid, polylactic acid or poly(lactide-co-glycolide). Hypocrellin- comprising nanoparticles are demonstrated to be activated by light or hydrogen peroxide.

[0006] US20110288023 discloses modified Transferrin (Tf) molecules and conjugates of the Tf molecules with a therapeutic agent. Also disclosed are methods of treating cancer wherein the therapeutic agents are chemotherapeutic agents. The modified Tf molecules improve the delivery of the conjugated therapeutic agent to a target tissue.

[0007] WO 2002094271 A1 discloses a homogeneous conjugate for targeting and treating diseased cells wherein the conjugate comprises an anti-cancer drug and a targeting protein, wherein said anti-cancer drug is selected from the group consisting of heat sensitizers, photosensitizers and apoptosis inducing compounds, a method

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[0007] WO 2002094271 A1 discloses a homogeneous conjugate for targeting and treating diseased cells wherein the conjugate comprises an anti-cancer drug and a targeting protein, wherein said anti-cancer drug is selected from the group consisting of heat sensitizers, photosensitizers and apoptosis inducing compounds, a method for making such a conjugate, and methods for using the conjugate. The targeting protein is preferably transferrin.

[0008] US 7001991 discloses a homogeneous conjugate for targeting and treating diseased cells wherein the conjugate comprises an anti-cancer drug and a targeting protein, wherein said anti-cancer drug is selected from the group consisting of heat sensitizers, photosensitizers and apoptosis inducing compounds, a method for making such a conjugate, and methods for using the conjugate. The targeting protein is preferably transferrin.

[0009] US 7809428 discloses PDT methods for treatment of vulnerable plaques by selectively targeting and/or eliminating the inflammatory components of vulnerable plaques. In a preferred embodiment, photosensitizer compositions are coupled to macromolecular carriers that target T cells of vulnerable plaques. These macromolecular carriers can be targeted to, for example, IL-10, receptor, monocyte inflammatory protein-1 and receptors thereof and transferrin. Such macromolecular carriers can be, for example, antibodies against these biomolecules, ligands binding the same or analogs thereof, including, but not limited to monoclonal antibodies that recognize CD1, CD2, CD3, CD4, CDS, CD6, CD7, CDB, CD25, CD28, CD44, CD71 or transferrin.

[0010] Large (>500 Da) PSs are difficult to apply topically. Non-selectivity of delivery is another problem. Various patch- and film-based topical application formulations and methods of enhanced delivery of PSs directly into cancer cells have been proposed to overcome both difficulty of delivery of large (>500 Da) PS molecules and non-selectivity of the delivery. They include various patch- and film-based topical application formulations (Donnelly et al., "Drug delivery systems for photodynamic therapy" (2009). Recent patents on drug delivery & formulation 3 (1), 1-7), redox activation (Graf, Lippard, 2012), receptor-mediated delivery (Nkepang et al., 2014), photoinduced delivery (Chen et al., 2014; Yin et al., 2014; Sardar et al.,

2014), liposomes (Temizel et al., 2014; Muehlmann L. A., Joanitti, G. A., Silva, J. R., Longo, J. P. F., & Azevedo, R. B. (2011). Liposomal photosensitizers: potential platforms for anticancer photodynamic therapy. Brazilian Journal of Medical and Biological Research, 44(8), 729-737), and delivery using nanoparticles including fullerenes (Biju, 2014; Yuan, Liu, 2014; Zhen et al., 2014; Wong et al., 2013; Yang et al., 2014; He et al., 2014). Combining of transferrin with fullerenes is also proposed (Zhang et al., 2015) as well as conjugation of PS-loaded liposomes with many molecules (folate, growth factors, glycoproteins such as transferrin, glycolipids) receptors for which are upregulated in cancer cells (Muehlmann et al., 2011; Nkepang et al., 2014). PEGylated AlPcS4-loaded liposomes conjugated with transferrin were used against cervical cancer cells (Gijsens et al., 2002). Exploration of Tf conjugation on the efficiency of liposome-encapsulated PS Foscan, a chlorine-based photosensitizer, in PDT of esophageal cancer was, however, not successful, likely due to the destabilization of the liposomes (Paszko et al., 2013).

[0011] Protein-based delivery systems include systems based on albumin (nanoparticles system), small heat shock protein, viral capsid and apoferritin (protein cage systems) used for doxorubicin delivery, soy protein (film-based system) for methylene blue delivery (MaHam et al., 2009). Apoferritin (i.e. Ferritin that is not combined with iron, a protein of 450 kDa) was used to encapsulate various cytostatic anticancer drugs: doxorubicin, carboplatin, cisplatin, daunorubicin although immune response to apoferritin may be a drawback (Heger et al., 2014). Among PCs, encapsulation of Methylene blue into apo-ferritin allowed increasing singlet oxygen production and enhancement of cytotoxic effects on cells (Heger et al., 2014, review).

[0012] The use of transferrin with liposomes containing aluminum phthalocyanine tetrasulfonate (LiposomesAlPcS$_4$) is disclosed by Derycke A. S., & de Witte, P. A. (2004). Liposomes for photodynamic therapy. Advanced drug delivery reviews, 56(1), 17-30; Gaspar et al., 2012.

[0013] None of the foregoing references explicitly propose the use of transferrin in combination with metal-based photosensitizers.

[0014] Despite the foregoing developments, it is still desired to provide improved compositions and methods for delivering PDCs to biological targets. It is further desired to provide increased efficacy of selective uptake of PDCs by biological targets. It is further desired to improve intracellular uptake of Ruthenium, Ruthenium-Rhodium and Osmium-based

photosensitizers predominantly by cancer cells and tumor tissues. It is further desired to increase PDC efficacy at longer wavelengths. It is further desired to improve absorbance, ROS production and PDT effect of the Ruthenium, Ruthenium-Rhodium and Osmium-based photosensitizers. It is further desired to improve the PDT effect in hypoxia.

X is selected from the group consisting of Cl^-, PF_6^-, Br^-, BF_4^-, ClO_4^-, $CF_3SO_3^-$, and SO_4^{-2};

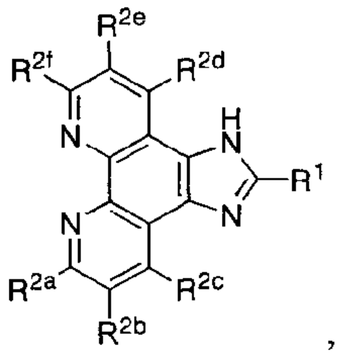
n = 0, 1, 2, 3, 4, or 5;

q is independently at each occurence 0, 1, or 2;

y is independently at each occurence 0, 1, or 2;

z is independently at each occurence 1, 2, or 3;

Lig^1 is a bidentate ligand that at each occurrence is each independently selected from the group consisting of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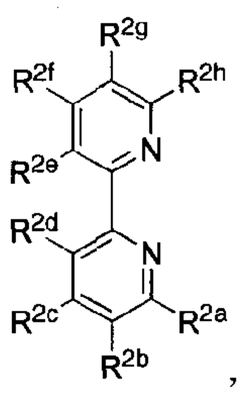
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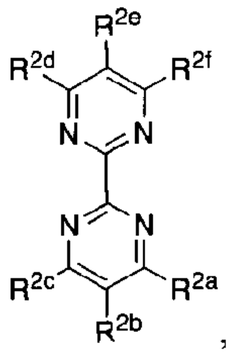
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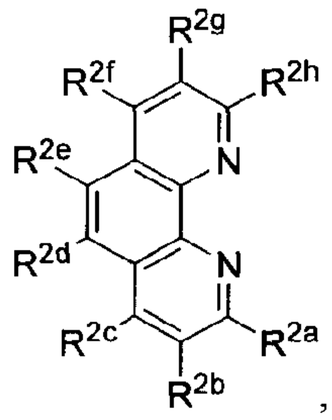
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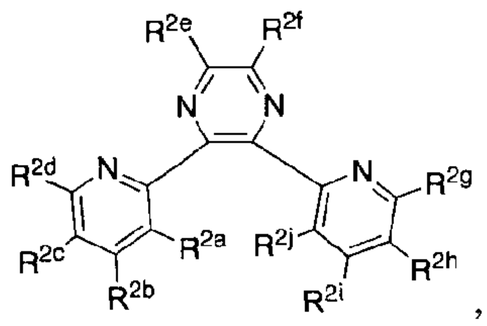
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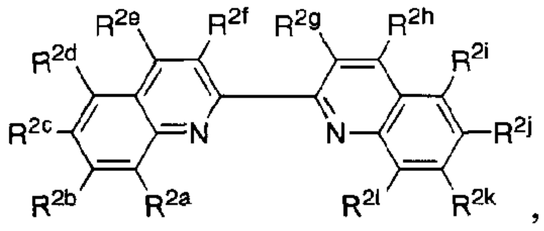
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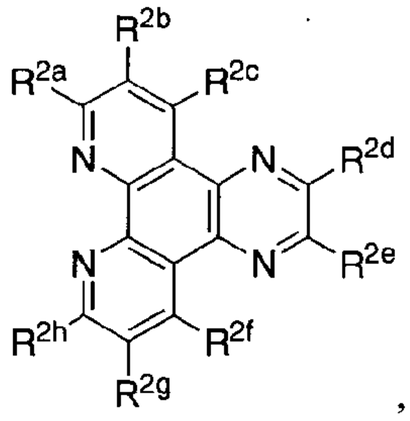
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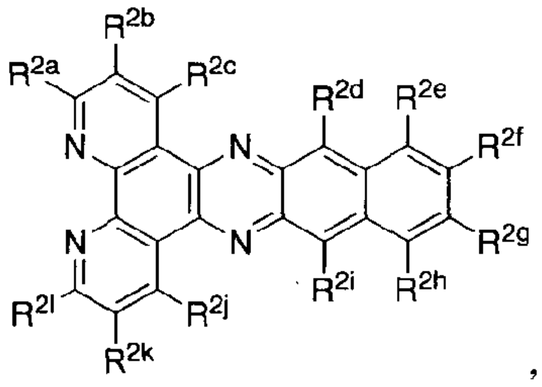
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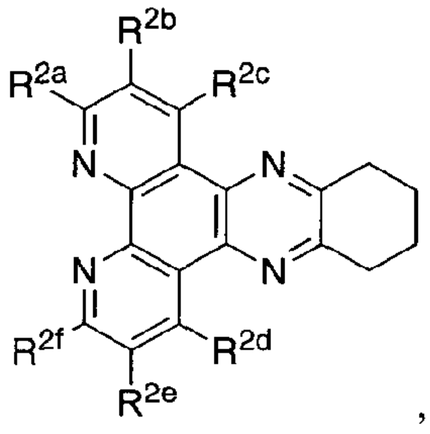
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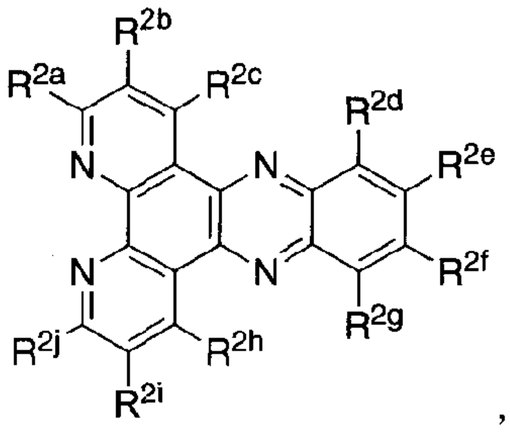
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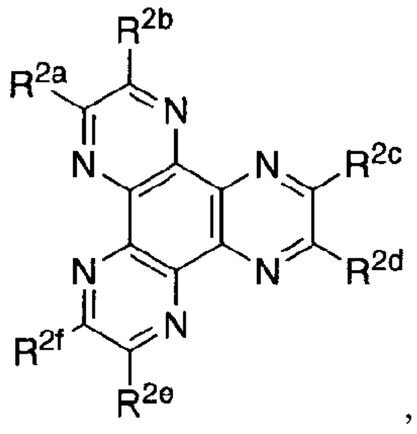
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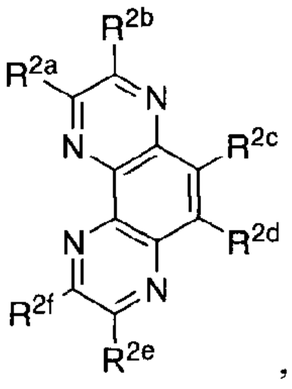
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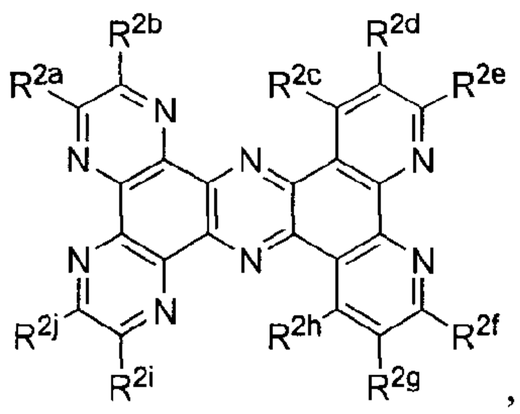
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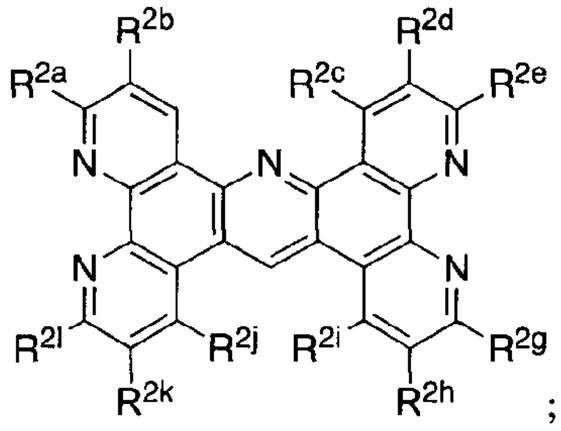
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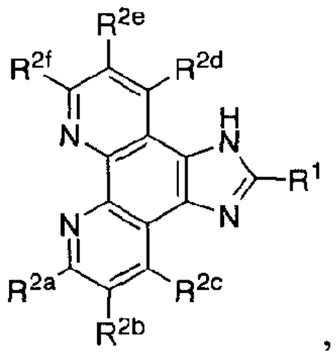
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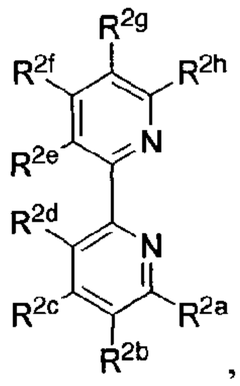
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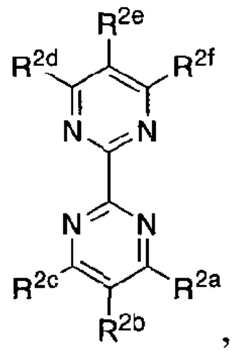
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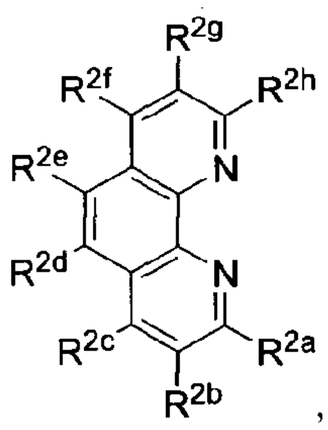
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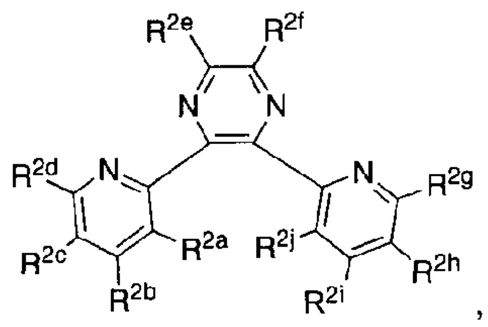
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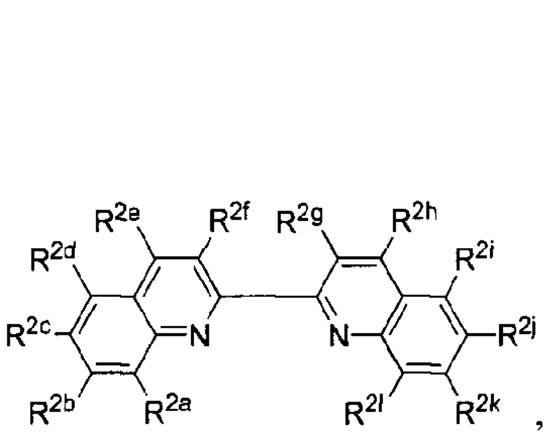
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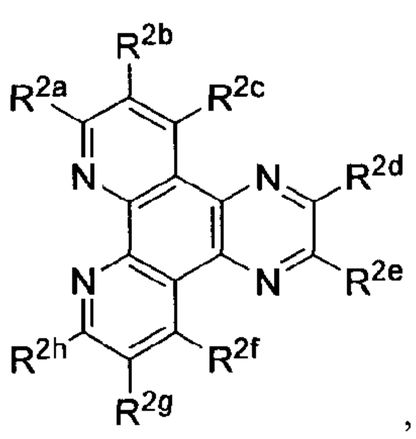
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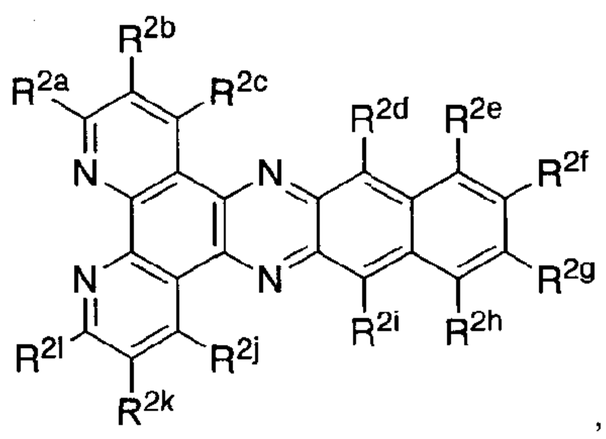
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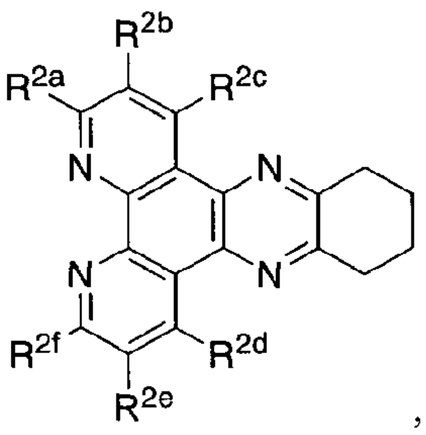
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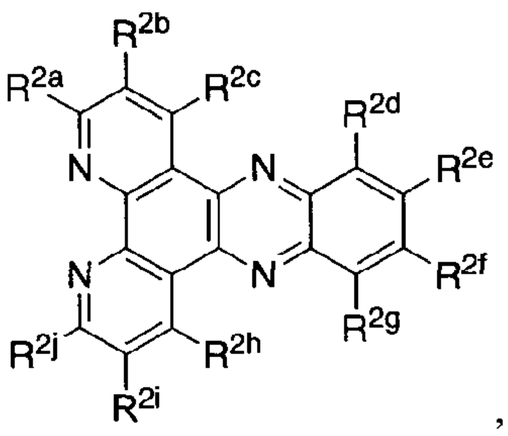
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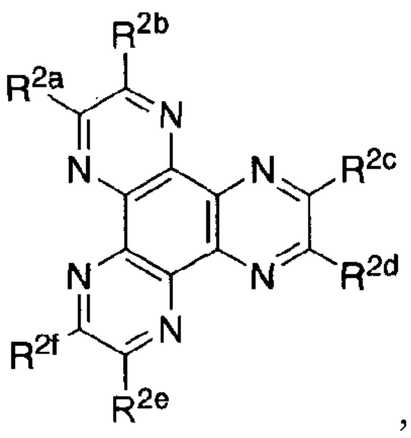
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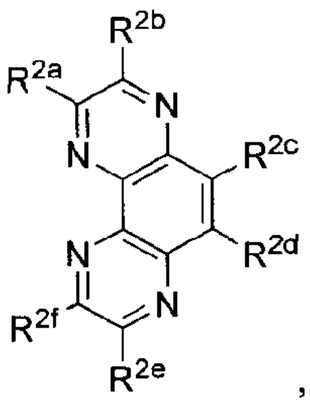
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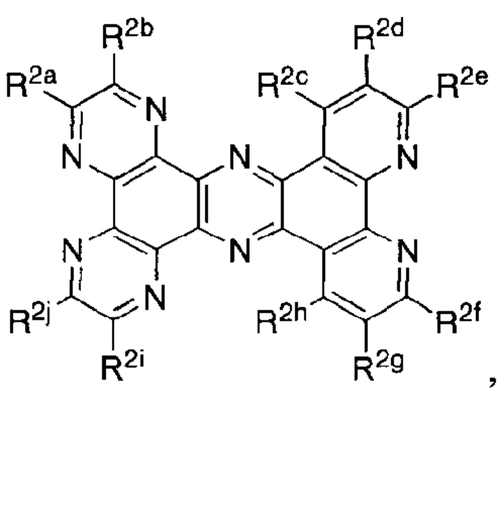
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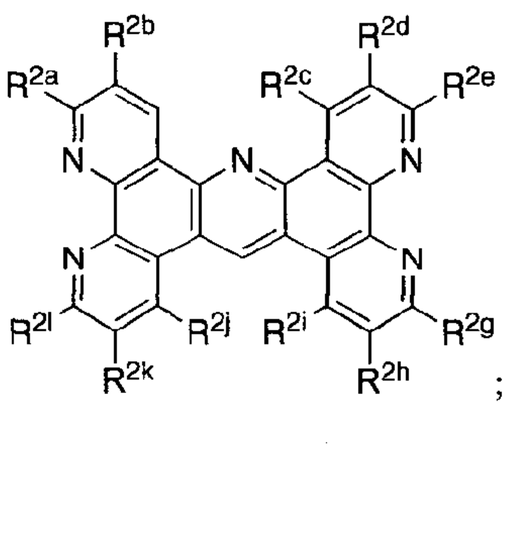
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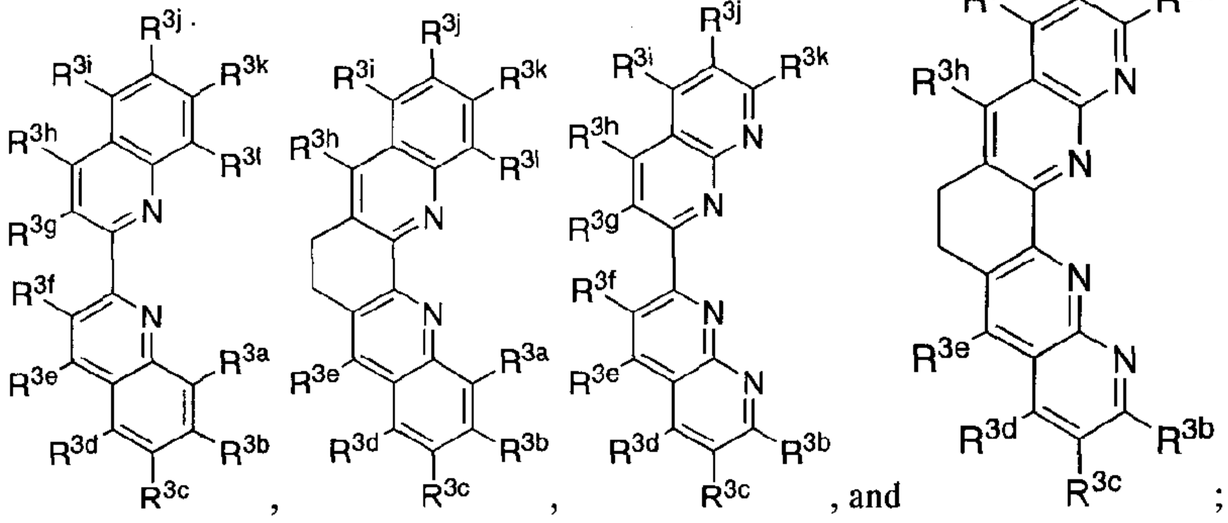
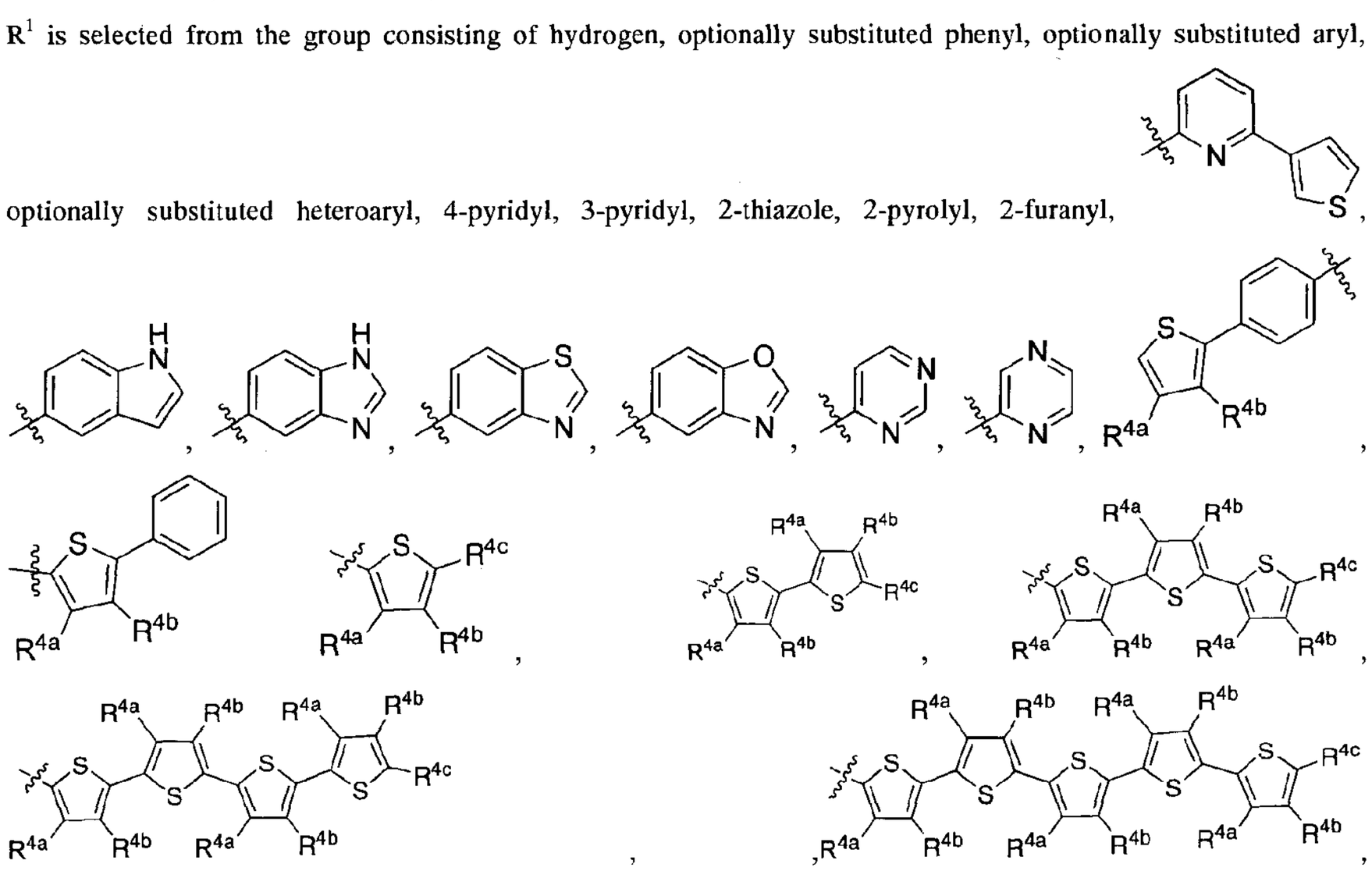
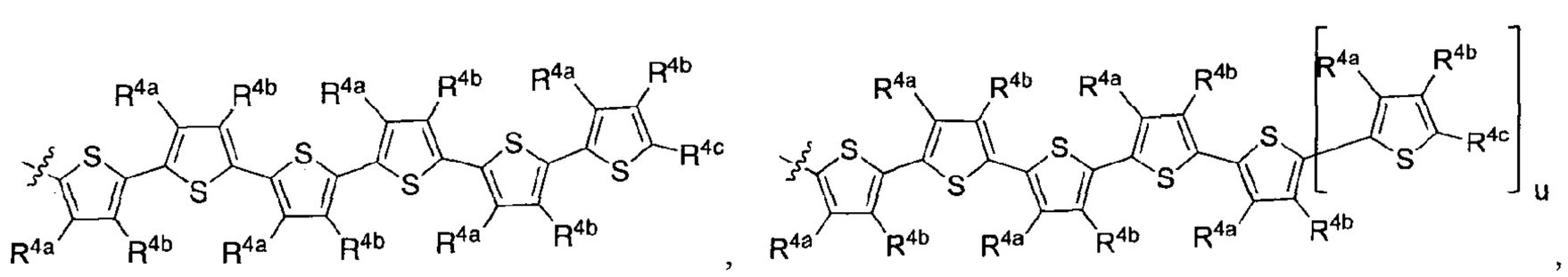
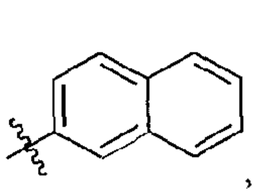
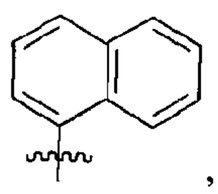
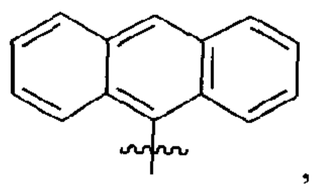
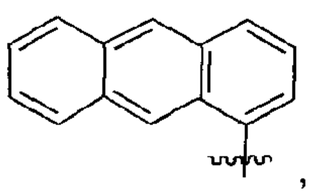
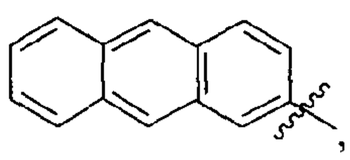
Lig^3 is a bidentate ligand that at each occurrence is each independently selected from the group consisting of


, and ;

R^1 is selected from the group consisting of hydrogen, optionally substituted phenyl, optionally substituted aryl, optionally substituted heteroaryl, 4-pyridyl, 3-pyridyl, 2-thiazole, 2-pyrolyl, 2-furanyl,



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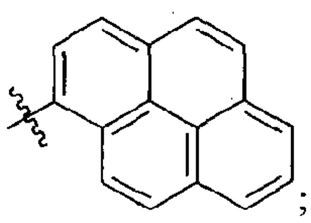

;

u is an integer;

R^{2a}, R^{2b}, R^{2c}, R^{2d}, R^{2e}, R^{2f}, R^{2g}, R^{2h}, R^{2i}, R^{2j}, R^{2k}, and R^{2l} at each occurrence are each independently selected from the group consisting of hydrogen, C_{1-6} optionally substituted alkyl, C_{1-6} optionally substituted branched alkyl, C_{3-7} optionally substituted cycloalkyl, C_{1-6} optionally substituted haloalkyl, C_{1-6} optionally substituted alkoxy, CO_2R^5, $CONR^6_2$, NR^7_2, SO_3H, sulfate, sulfonate, optionally substituted aryl, optionally substituted aryloxy, optionally substituted heteroaryl, and optionally substituted heterocycle;

R^{3a}, R^{3b}, R^{3c}, R^{3d}, R^{3e}, R^{3f}, R^{3g}, R^{3h} R^{3i}, R^{3j}, R^{3k}, and R^{3l} at each occurrence are each independently selected from the group consisting of hydrogen, C_{1-6} optionally substituted alkyl, C_{1-6} optionally substituted branched alkyl, C_{1-6} optionally substituted haloalkyl, C_{1-6} optionally substituted alkoxy, optionally substituted phenyl, and CO_2R^8;

R^{4a}, R^{4b}, and R^{4c} at each occurrence are each independently selected from the group consisting of hydrogen, C_{1-6} optionally substituted alkyl, C_{1-6} optionally substituted branched alkyl, C_{1-6} optionally substituted cycloalkyl, C_{1-6} optionally substituted haloalkyl, C_{1-6} optionally substituted alkoxy, CO_2R^5, $CONR^6_2$, NR^7_2 , sulfate, sulfonate, optionally substituted aryl, optionally substituted aryloxy, optionally substituted heteroaryl, and optionally substituted heterocycle;

R^{4a} and R^{4b} at each occurrence on a thiophene ring are taken together with the atom to which they are bound to form an optionally substituted ring having from 6 ring atoms containing 2 oxygen atoms;

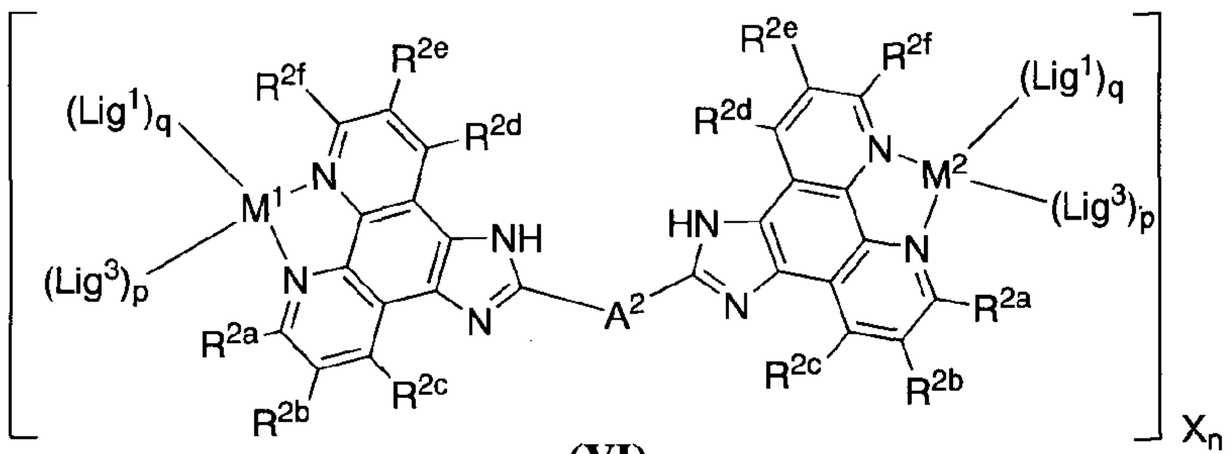
R^5 at each occurrence are each independently selected from the group consisting of hydrogen and optionally substituted alkyl;

R^6 at each occurrence are each independently selected from the group consisting of hydrogen and optionally substituted alkyl;

R^7 at each occurrence are each independently selected from the group consisting of hydrogen and optionally substituted alkyl; and

R^8 at each occurrence are each independently selected from the group consisting of hydrogen and optionally substituted alkyl.

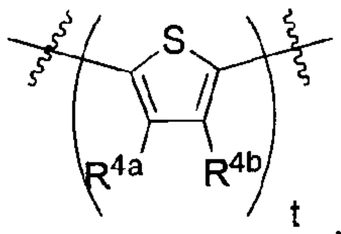
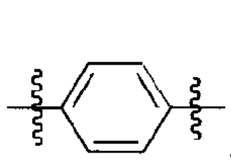
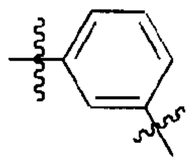
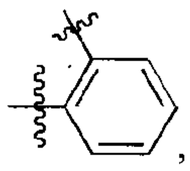
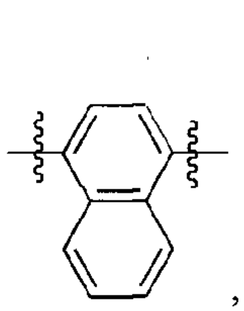
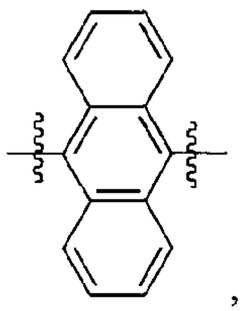
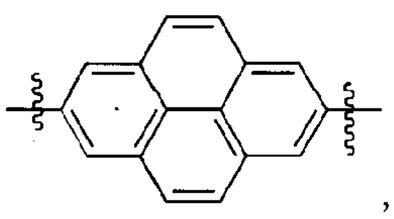
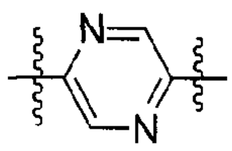
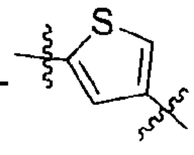
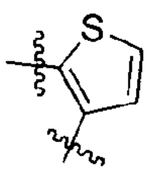
[0017] In certain embodiments, the glycoprotein is transferrin and the chemotherapeutic compound has the formula (VI):



(VI)

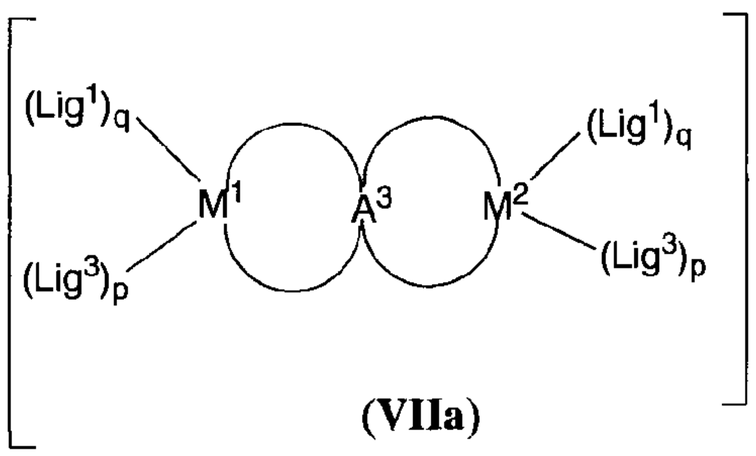
including hydrates, solvates, pharmaceutically acceptable salts, prodrugs and complexes thereof wherein;

M^1 and M^2 at each occurrence is independently a transition metal, and is preferably independently selected from the group consisting of osmium, manganese, molybdenum, rhenium, ruthenium, iron, cobalt, rhodium, iridium, nickel, platinum, and copper;

A^2 is selected from the group consisting of  t ,  ,  ,  ,  ,  ,  ,  ,  , and 

t is an integer.

[0018] In certain embodiments, the glycoprotein is transferrin and the chemotherapeutic compound has the formula (VIIa)



(VIIa)

including hydrates, solvates, pharmaceutically acceptable salts, prodrugs and complexes thereof wherein:

M^1 and M^2 at each occurrence is independently a transition metal, and is preferably independently selected from the group consisting of osmium, manganese, molybdenum, rhenium, ruthenium, iron, cobalt, rhodium, iridium, nickel, platinum, and copper;

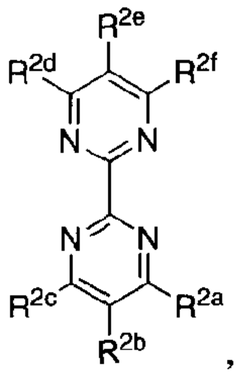
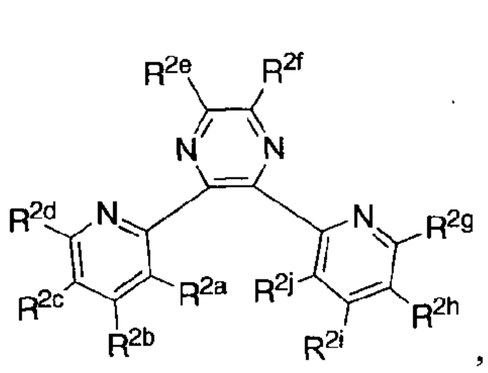
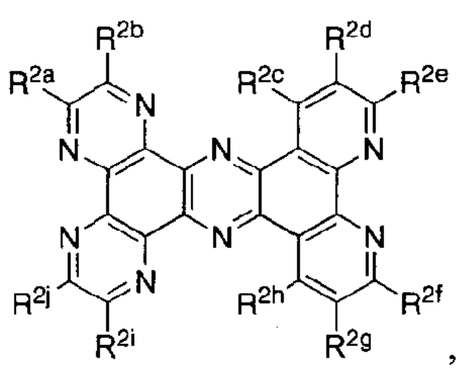
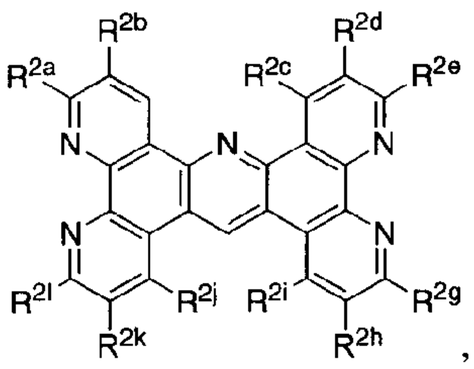
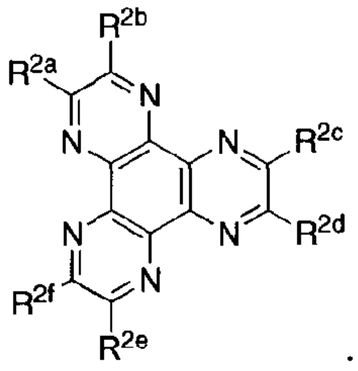
Lig^1 is a bidentate ligand that at each occurrence is each independently selected from the group defined above;

Lig^3 is a bidentate ligand that at each occurrence is each independently selected from the group defined above;

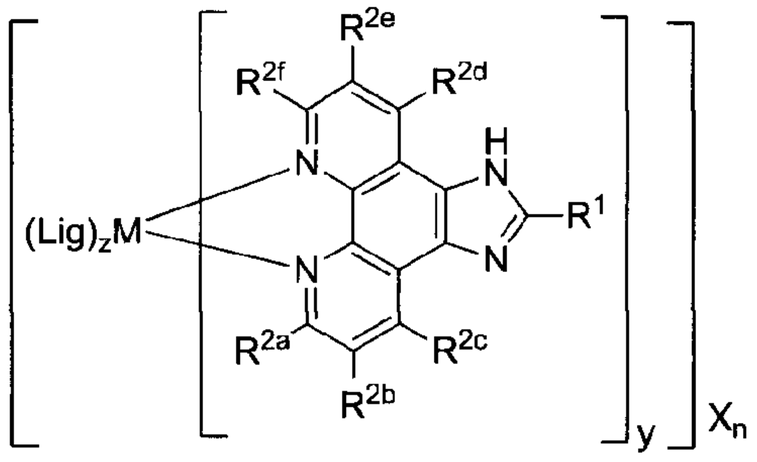
p is independently at each occurence 0, 1, or 2;

q is independently at each occurence 0, 1, or 2;

n is 0, 1, 2, 3, 4, or 5; and

A^3 is selected from the group consisting of  ,  ,  ,  , and  .

[0019] In certain embodiments, the glycoprotein is transferrin and the chemotherapeutic compound has the formula (II)



(II)

including hydrates, solvates, pharmaceutically acceptable salts, prodrugs and complexes thereof, wherein:

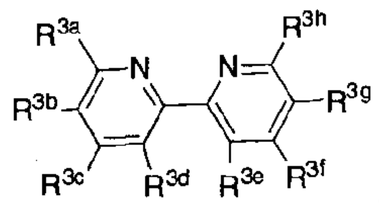
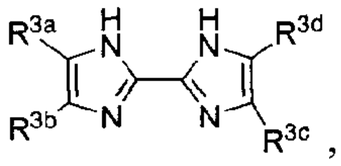
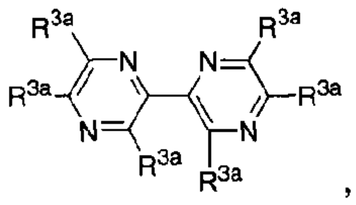
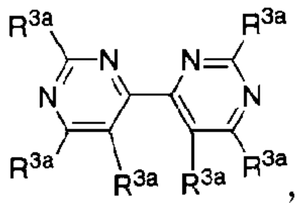
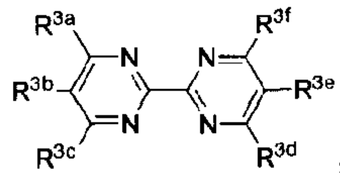
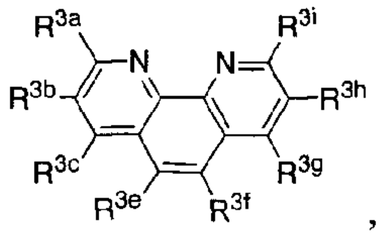
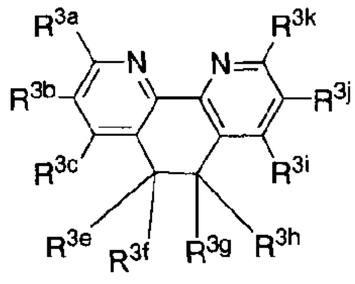
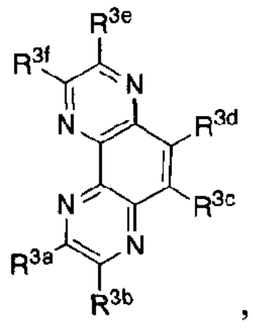
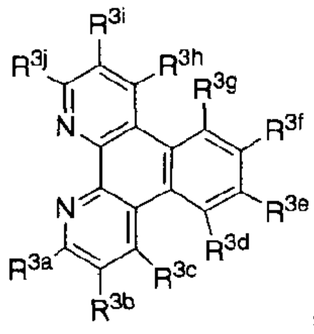
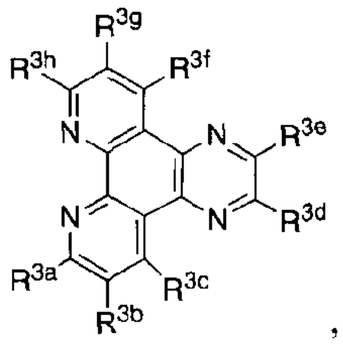
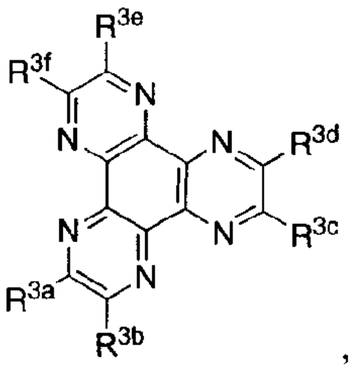
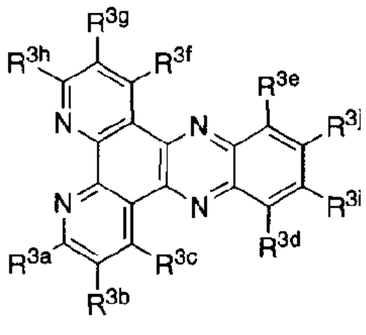
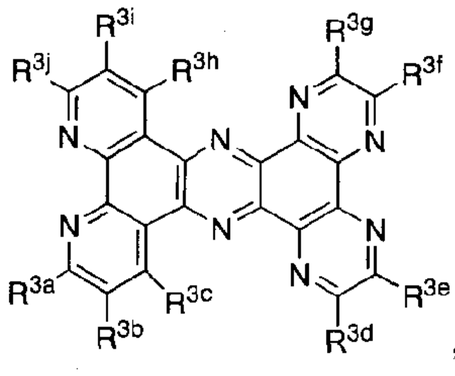
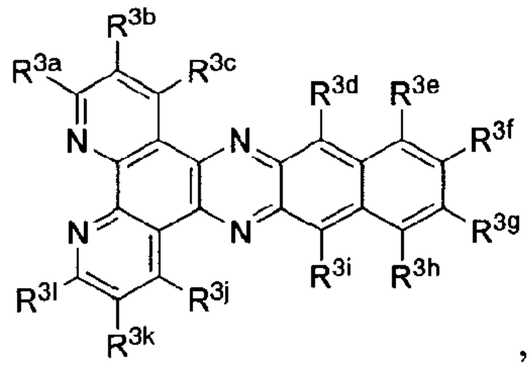
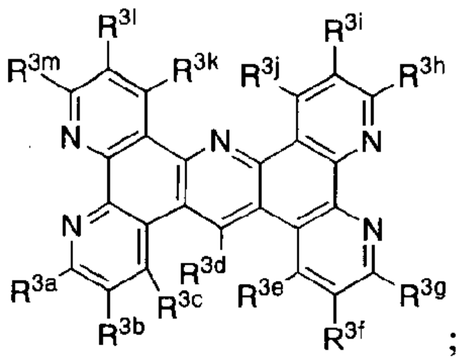
M is a transition metal preferably selected from the group consisting of manganese, molybdenum, rhenium, iron, ruthenium, osmium, cobalt, rhodium, iridium, nickel, platinum, and copper;

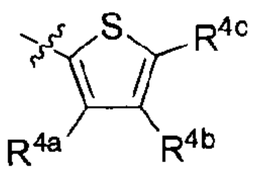
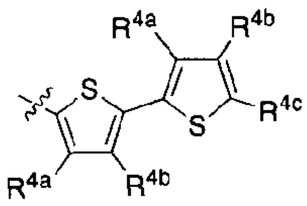
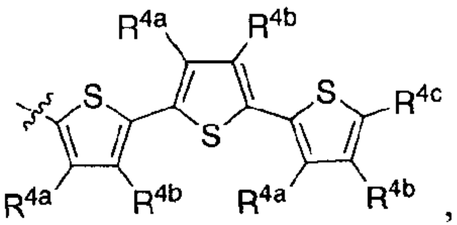
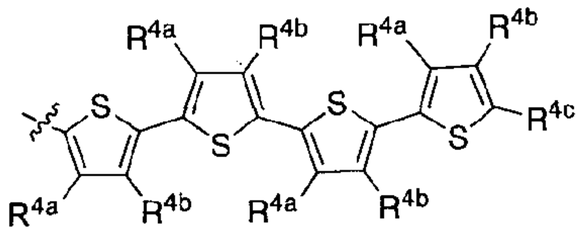
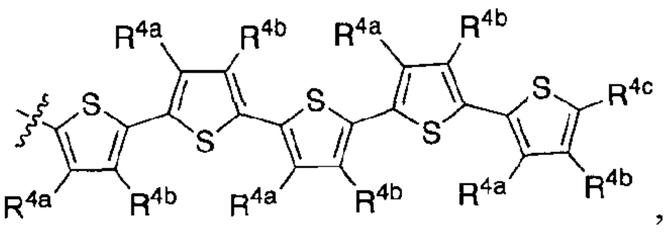
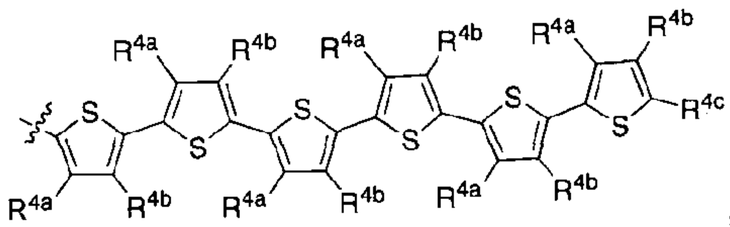
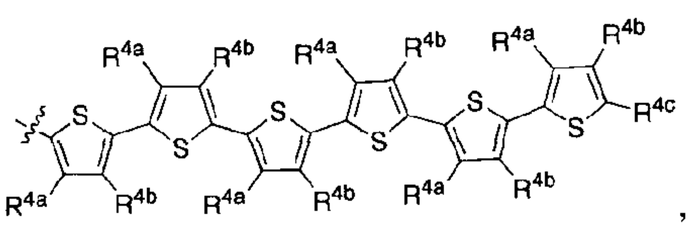
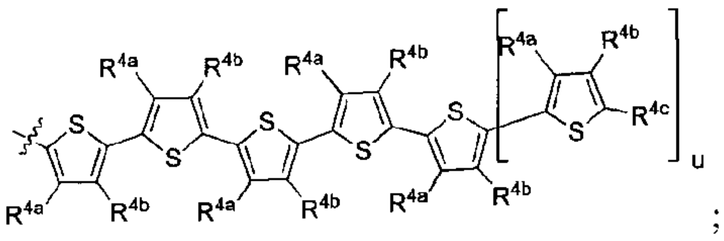
X is selected from the group consisting of Cl^-, PF_6^-, Br^-, BF_4^-, ClO_4^-, $CF_3SO_3^-$, and SO_4^{-2};

n = 0, 1, 2, 3, 4, or 5;

y = 1, 2, or 3;

z = 0, 1, or 2;

Lig at each occurrence is independently selected from the group consisting of  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  , and  ;

R^1 is selected from the group consisting of  ,  ,  ,  ,  ,  ,  , and  ;

u is an integer;

R^{2a}, R^{2b}, R^{2c}, R^{2d}, R^{2e}, and R^{2f} at each occurrence are each independently selected from the group consisting of hydrogen, C1-6 optionally substituted alkyl, C1-6 optionally substituted branched alkyl, C3-7 optionally substituted cycloalkyl, C1-6 optionally substituted haloalkyl, C1-6 optionally substituted alkoxy, CO_2R^5, $CONR^6_2$, NR^7_2 , sulfate, sulfonate, optionally substituted aryl, optionally substituted aryloxy, optionally substituted heteroaryl, and optionally substituted heterocycle;

R^{3a}, R^{3b}, R^{3c}, R^{3d}, R^{3e}, R^{3f}, R^{3g}, R^{3h} R^{3i}, R^{3j}, R^{3k}, R^{3l}, and R^{3m} at each occurrence are each independently selected from the group consisting of hydrogen, C1-6 optionally substituted alkyl, C1-6 optionally substituted branched alkyl, C1-6 optionally substituted haloalkyl, C1-6 optionally substituted alkoxy, and CO_2R^8;

R^{4a}, R^{4b}, and R^{4c} at each occurrence are each independently selected from the group consisting of hydrogen, C1-6 optionally substituted alkyl, C1-6 optionally substituted branched alkyl, C1-6 optionally substituted cycloalkyl, C1-6 optionally substituted haloalkyl, C1-6 optionally substituted alkoxy, CO_2R^5, $CONR^6_2$, NR^7_2 , sulfate, sulfonate, optionally substituted aryl, optionally substituted aryloxy, optionally substituted heteroaryl, and optionally substituted heterocycle;

R^{4a} and R^{4b} at each occurrence on a thiophene ring are taken together with the atom to which they are bound to form an optionally substituted ring having from 6 ring atoms containing 2 oxygen atoms;

R^5 at each occurrence is independently selected from the group consisting of hydrogen and optionally substituted alkyl;

R^6 at each occurrence is independently selected from the group consisting of hydrogen and optionally substituted alkyl;

R^7 at each occurrence is independently selected from the group consisting of hydrogen and optionally substituted alkyl; and

R^8 at each occurrence is independently selected from the group consisting of hydrogen and optionally substituted alkyl.

[0020] A second aspect of the invention comprises a method for treating a condition associated with hyperproliferating cells, said method comprising:

administering to a subject having the condition an effective amount of the composition according to the invention; and

irradiating the subject with light effective to activate the composition so as to treat the condition.

[0021] A third aspect of the invention comprises a method for destroying a microbial cell, said method comprising:

contacting the microbial cell with an effective amount of the composition according to the invention; and

irradiating the microbial cell with light effective to activate the composition so as to destroy the microbial cell.

[0022] These and other objects, features, and advantages will become apparent to those of ordinary skill in the art from a reading of the following detailed description and the appended claims. All percentages, ratios and proportions herein are by weight, unless otherwise specified. All temperatures are in degrees Celsius (°C) unless otherwise specified. The citation herein of any document is not to be construed as an admission that it is prior art with respect to the present invention.

BRIEF DESCRIPTION OF SEVERAL VIEWS OF THE DRAWINGS

[0023] The invention will be described in conjunction with the following drawings in which like reference numerals designate like elements and wherein:

[0024] FIGS. 1A, 1B and 1C show graphs of absorbance/absorbance increase against wavelength.

[0025] FIGS. 2A, 2B and 2C show graphs of absorbance against wavelength.

[0026] FIGS. 3A and 3B show graphs of fluorescence against wavelength.

[0027] FIGS. 4A and 4B show graphs of fluorescence against wavelength.

[0028] FIGS. 5A and 5B show graphs of fluorescence against wavelength.

[0029] FIGS. 6A, 6B and 6C show graphs of absorbance ratio against incident photons.

[0030] FIGS. 7A, 7B and 7C show graphs of absorbance ratio against incident photons.

[0031] FIGS. 8A, 8B and 8C show graphs of absorbance ratio against incident photons.

[0032] FIGS. 9A, 9B and 9C show graphs of absorbance ratio against incident photons.

[0033] FIGS. 10A, 10B and 10C show graphs of cellular uptake of metals by control and test samples.

[0034] FIGS. 11A, 11B and 11C show graphs of cellular uptake of metals by control and test samples.

[0035] FIGS. 12A and 12B show graphs of cell kill with and without transferrin.

[0036] FIGS. 13A and 13B show graphs of cell kill with and without transferrin.

[0037] FIGS. 14A and 14B show graphs of cell kill with and without transferrin.

[0038] FIGS. 15A and 15B show graphs of cell kill with and without transferrin.

[0039] FIGS. 16A, 16B and 16C show graphs of cell kill with and without transferrin.

[0040] FIGS. 17A, 17B, 17C and 17D show graphs of cell kill with and without transferrin.

[0041] FIGS. 18A, 18B, 18C and 18D show graphs of cell kill with and without transferrin.

[0042] FIGS. 19A, 19B, 19C and 19C show graphs of cell kill with and without transferrin.

[0043] FIGS. 20A and 20B show graphs of cell kill with and without transferrin and with and without antibodies.

[0044] FIGS. 21A and 21B show graphs of cell kill with and without transferrin and with and without antibodies.

[0045] FIGS. 22A and 22B show graphs of cell kill with and without transferrin and with and without antibodies.

[0046] FIGS. 23A and 23B show graphs of uptake of metals over time by muscle and tumor tissue with and without transferrin.

[0047] FIGS. 24A and 24B show graphs of uptake of metals over time by muscle and tumor tissue with and without transferrin.

[0048] FIGS. 25A and 25B show graphs of uptake of metals over time by muscle and tumor tissue with and without transferrin.

[0049] FIG. 26 shows a graph of percent survival against days elapsed after PDT.

[0050] FIG. 27 shows a graph of percent survival against days elapsed after PDT.

[0051] FIG. 28 shows a graph of molar extinction coefficient against wavelength.

[0052] FIG. 29 shows a graph of molar extinction coefficient against wavelength.

[0053] FIG. 30 shows a graph of optical density ratio against incident photon number.

[0054] FIG. 31 shows graphs of optical density against wavelength.

[0055] FIG. 32 shows graphs of molar extinction coefficient ratio against energy.

[0056] FIG. 33 shows graphs of optical density against wavelength.

[0057] FIG. 34 shows graphs of optical density against wavelength.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS OF THE INVENTION

[0058] *Glossary*

[0059] Throughout the description, 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 also consist essentially of, or consist of, the recited components, and that the processes of the present teachings also consist essentially of, or consist of, the recited processing steps.

[0060] 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 and can be selected from a group consisting of two or more of the recited elements or components.

[0061] The use of the singular herein includes the plural (and vice versa) unless specifically stated otherwise. In addition, where the use of the term "about" is before a quantitative value, the present teachings also include the specific quantitative value itself, unless specifically stated otherwise.

[0062] 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 can be conducted simultaneously

[0063] For the purposes of the present invention the terms "compound," "analog," and "composition of matter" stand equally well for the inventive compounds described herein, be they photodynamic or not, including all enantiomeric forms, diastereomeric forms, salts, and the like, and the terms "compound," "analog," and "composition of matter" are used interchangeably throughout the present specification.

[0064] Compounds described herein can contain an asymmetric atom (also referred as a chiral center), and some of the compounds can contain one or more asymmetric atoms or centers, which can thus give rise to optical isomers (enantiomers) and diastereomers. The present teachings and compounds disclosed herein include such enantiomers and diastereomers, as well as the racemic and resolved, enantiomerically pure R and S stereoisomers, as well as other mixtures of the R and S stereoisomers and pharmaceutically acceptable salts thereof. Optical isomers can be obtained in pure form by standard procedures known to those skilled in the art, which include, but are not limited to, diastereomeric salt formation, kinetic resolution, and asymmetric synthesis. The present teachings also

encompass cis and trans isomers of compounds containing alkenyl moieties (e.g., alkenes and imines). It is also understood that the present teachings encompass all possible regioisomers, and mixtures thereof, which can be obtained in pure form by standard separation procedures known to those skilled in the art, and include, but are not limited to, column chromatography, thin-layer chromatography, and high-performance liquid chromatography.

[0065] Pharmaceutically acceptable salts of compounds of the present teachings, which can have an acidic moiety, can be formed using organic and inorganic bases. Both mono and polyanionic salts are contemplated, depending on the number of acidic hydrogens available for deprotonation. Suitable salts formed with bases include metal salts, such as alkali metal or alkaline earth metal salts, for example sodium, potassium, or magnesium salts; ammonia salts and organic amine salts, such as those formed with morpholine, thiomorpholine, piperidine, pyrrolidine, a mono-, di- or tri-lower alkylamine (e.g., ethyl-tert-butyl-, diethyl-, diisopropyl-, triethyl-, tributyl- or dimethylpropylamine), or a mono-, di-, or trihydroxy lower alkylamine (e.g., mono-, di- or triethanolamine). Specific non-limiting examples of inorganic bases include $NaHCO_3$, Na_2CO_3, $KHCO_3$, K_2CO_3, Cs_2CO_3, LiOH, NaOH, KOH, NaH_2PO_4, Na_2HPO_4, and Na_3PO_4. Internal salts also can be formed. Similarly, when a compound disclosed herein contains a basic moiety, salts can be formed using organic and inorganic acids. For example, salts can be formed from the following acids: acetic, propionic, lactic, benzenesulfonic, benzoic, camphorsulfonic, citric, tartaric, succinic, dichloroacetic, ethenesulfonic, formic, fumaric, gluconic, glutamic, hippuric, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, malonic, mandelic, methanesulfonic, mucic, napthalenesulfonic, nitric, oxalic, pamoic, pantothenic, phosphoric, phthalic, propionic, succinic, sulfuric, tartaric, toluenesulfonic, and camphorsulfonic as well as other known pharmaceutically acceptable acids.

[0066] When any variable occurs more than one time in any constituent or in any formula, its definition in each occurrence is independent of its definition at every other occurrence (e.g., in $N(R^6)_2$, each R^6 may be the same or different than the other). Combinations of substituents and/or variables are permissible only if such combinations result in stable compounds.

[0067] The terms "treat" and "treating" and "treatment" as used herein, refer to partially or completely alleviating, inhibiting, ameliorating and/or relieving a condition from which a patient is suspected to suffer.

[0068] As used herein, "therapeutically effective" and "effective dose" refer to a substance or an amount that elicits a desirable biological activity or effect.

[0069] As used herein, the term "photodynamic therapy" refers to a treatment for destroying cells or modulating immune function, including immune response, of cells and tissue through use of a drug that can be activated by light of a certain wavelength and dose.

[0070] As used herein the term "chemotherapeutic compound" refers to a chemical compound with prophylactic, ameliorative and/or curative properties with respect to one or more conditions or diseases.

[0071] As used herein, the term "photodynamic compound" refers to a compound that provides photodynamic therapy. Photodynamic compounds are a subset of chemotherapeutic compounds as defined herein.

[0072] Except when noted, the terms "subject" or "patient" are used interchangeably and refer to mammals such as human patients and non-human primates, as well as experimental animals such as rabbits, rats, and mice, and other animals. Accordingly, the term "subject" or "patient" as used herein means any mammalian patient or subject to which the compounds of the invention can be administered. In an exemplary embodiment of the present invention, to identify subject patients for treatment according to the methods of the invention, accepted screening methods are employed to determine risk factors associated with a targeted or suspected disease or condition or to determine the status of an existing disease or condition in a subject. These screening methods include, for example, conventional work-ups to determine risk factors that may be associated with the targeted or suspected disease or condition. These and other routine methods allow the clinician to select patients in need of therapy using the methods and compounds of the present invention.

[0073] As used herein, the term "biological target" refers to an organ, tissue and/or cell of an organism and/or to the organism itself.

[0074] ***Advantages of the Invention***

[0075] The invention is based in part on the unexpected discovery that Ru, Rh and Os based PDCs, particularly those disclosed in WO 2013158550 A1, WO 2014145428 A2, US 6962910, US 7612057, US 8445475 and US 8148360, are significantly improved in their chemical, physical and biomedical properties when they are administered in combination with metal-binding glycoproteins of the invention.

[0076] Ruthenium and osmium are in the same group of the periodic table of elements as Iron, Group 8, and share many of its characteristics. Rhodium is in neighboring Group 9. As Ruthenium and Osmium are in the same group as Iron, they share many characteristics. For example, electronically, Ru (II) and OS (II) molecules readily bond with nitrogen and sulphur donor (this mechanisms is also relevant for induction of NO (Chatterjee D, Shome S, Jaiswal N, Banerjee P. Nitrite reduction mediated by the complex Ru III (EDTA). Dalton Trans. 2014 Sep 28;43(36):13596-600) and may play a role in switching the Type II to Type I photochemistry) molecules that are abundantly found in many proteins within the body. For this reason transition metal complexes are able to take advantage of the body's ability to efficiently transport and uptake of iron (Antonarakis ES, Emadi A. Ruthenium-based chemotherapeutics: are they ready for prime time? Cancer Chemother Pharmacol. 2010 May;66(1):1-9). The PSs are transported inside cancer cell (mitochondria) by binding to endogenous transferrin (a glycoprotein mainly produced in the liver) via transferrin receptor (TfR); Bergamo A, Sava G. Ruthenium anticancer compounds: myths and realities of the emerging metal-based drugs. Dalton Trans. 2011 Aug 21;40(31):7817-23.

[0077] Rapidly dividing tumor cells have an increased demand for iron and the levels of TfRs found on these cancerous cells are greatly increased. The receptor increase on cancerous cells has been document as two to twelve times that of healthy cells (Antonarakis ES, Emadi A. Ruthenium-based chemotherapeutics: are they ready for prime time? Cancer Chemother Pharmacol. 2010 May;66(1):1-9). This greatly increases the selectivity of the PSs as the majority of the dose is sequestered in cancerous tissues, bypassing most healthy cells. This effect contributes to the lower toxicity that is associated to the ruthenium drugs in comparison to platinum (Bruijnincx, Pieter C.A.; Sadler,

Peter J. (2009). "Controlling platinum, ruthenium, and osmium reactivity for anticancer drug design". Advances in Inorganic Chemistry 61. p. 1).

[0078] Because iron is required for growth of cancer cells, we see an additional benefit of competitive binding of PDCs to the transferrin receptor on a tumor cell. In addition to the active uptake of PDCs, the use of this mechanism in inhibiting both cell proliferation and HIF-1α. Angiogenesis under normoxic and hypoxic conditions may be of additional therapeutic use. Moreover, as cancer cells are generally growing and multiplying much more rapidly than normal healthy cells, this creates an environment that is less oxygen rich due to the raised metabolic rate. When this is paired with the tendency of cancerous cells to contain higher levels of glutathione and a lower pH, a chemically reducing environment is created. Indeed, our data have shown an additive and even potentially synergistic role of glutathione in the mechanisms of cancer cells kill by PDCs. The glutathione-mediated reduction is thought to occur by mitochondrial proteins or microsomal single electron transfer proteins, though it may also occur by trans-membrane electron transport systems which reside outside the cell, implying that, due to local administrations of PSs, the PS is still effective even if some quantity of the PS may leak/escape into intracellular space. In theory, it is also possible for the Ruthenium compounds to be oxidized back to their inactive form if they leave the cancerous environment and, hence, in addition to a very strong photostability of PSs, this phenomenon may also contribute to additional treatment efficacy and safety.

[0079] During light activation in preferred embodiments of the invention, in addition to PDT-induced inflammation, there is modifcation of tumor cell death and antigen presenting cells ("APC") activation via the danger-associated molecular patterns ("DAMPs"). The recognition of molecules released or expressed by dead, dying, injured, or stressed "antigenic"-apoptotic cells can elicit potent and tumor-specific immune responses. PDT-induced DAMPs emitted by dying cancer cells can elicit cancer antigen-directed anti-tumor short-term effects (6 to 8 weeks) and a long-term anti-tumor effect (>10 months) immunity. DAMP's stimulate immune responses through dialogue with T lymphocytes ("Th") cells, Natural Killer ("NK") cells and APSs. Certain APSs, such as dendritic cells and macrophages are stimulated and actively trafficked during PDT-induced "immunologic" cell death ("ICD") by danger signalling pathways, which are instigated and regulated by a complex interplay between cellular stress signaling, reactive oxygen species ("ROS") production and certain metabolic/biosynthetic processes (i.e., autophagy, caspase activity and secretory pathway: calreticulin, Adenosine Tri-Phosphate ("ATP"), Heat Shock Proteins, High Mobility Group Box 1, cytokines, etc).

[0080] ***Methods of the Invention***

[0081] Methods of the invention comprise the use of metal-binding glycoproteins as delivery vehicles for metal-based PDCs so as to facilitate delivery of the PDCs into a biological target. The methods are intended to provide enhanced safety and/or efficacy relative to the delivery of metal-based PDCs without the addition of exogenous metal-binding glycoproteins. PDCs delivered via the inventive methods and compositions enjoy enhanced biophysical, biochemical and biomedical properties, such as the efficacy, tolerability, therapeutic efficacy and diagnostic properties of the PDCs in multi-wavelength photodynamic therapy in normoxic and hypoxic

conditions.

[0082] Hence, methods of the invention comprise combining metal-containing PDCs with metal-binding glycoproteins and administering the combination to, e.g., a patient in need of PDT, or an organism to be destroyed.

[0083] The present invention further relates to a method for preparing the compounds of the present invention.

[0084] Compounds of the present teachings can be prepared in accordance with the procedures outlined herein, from commercially available starting materials, compounds known in the literature, or readily prepared intermediates, by employing standard synthetic methods and procedures known to those skilled in the art. Standard synthetic methods and procedures for the preparation of organic molecules and coordination complexes and functional group transformations and manipulations can be readily obtained from the relevant scientific literature or from standard textbooks in the field. It will be appreciated that where typical or preferred process conditions (i.e., reaction temperatures, times, mole ratios of reactants, solvents, pressures, etc.) are given, other process conditions can also be used unless otherwise stated. Optimum reaction conditions can vary with the particular reactants or solvent used, but such conditions can be determined by one skilled in the art by routine optimization procedures. Those skilled in the art of organic and inorganic synthesis will recognize that the nature and order of the synthetic steps presented can be varied for the purpose of optimizing the formation of the compounds described herein.

[0085] The preparation methods described herein can be monitored according to any suitable method known in the art. For example, product formation can be monitored by spectroscopic means, such as nuclear magnetic resonance spectroscopy (e.g., 1H or 13C), infrared spectroscopy, spectrophotometry (e.g., UV-visible), mass spectrometry, or by chromatography such as high pressure liquid chromatography (HPLC), gas chromatography (GC), gel-permeation chromatography (GPC), or thin layer chromatography (TLC).

[0086] Metal-binding glycoproteins suitable for use in the invention are capable of binding transition metals and delivering to a biological target said metals and other materials complexed with said metals. The metal-binding glycoproteins are preferably capable of binding Group 8 metals and/or Group 9 metals, and most preferably Ru, Os and Rh. Most preferred are the iron-binding glycoproteins transferrin, lactoferrin, ovotransferrin and melanotransferrin and variants thereof, with transferrin being most preferred. The glycoprotein can be purified from natural sources or can be from artificial sources. Thus, for example, the glycoprotein in certain embodments is a recombinant transferrin, such as Apo-Transferrin or OPTIFERRIN, a recombinant human transferrin available from InVitria, a division of Ventria Bioscience. See US 20120088729 A1, Zhang et al., "Expression, purification, and characterization of recombinant human transferrin from rice (Oryza sativa L.)." Protein Expr Purif. 2010 Nov;74(1):69-79. Epub 2010 May 4, and Steere et al., "Biochemical and structural characterization of recombinant human serum transferrin from rice (Oryza sativa L.)." J Inorg Biochem. 2012 Jul 11;116C:37-44. OPTIFERRIN is a particularly preferred glycorprotein as it increases the targeting and reduces the photobleaching of the metal-glycoprotein complexes of the invention.

[0087] Biological targets of the invention are organisms, organs, tissues and/or cells amenable to treatment with, and/or detection by, the metal-glycoprotein complexes of the invention. The targets are preferably

hyperproliferating cells, such as cancer and non-malignant lesions.

[0088] To our knowledge, the direct binding between Ru (II) and/or Os (II) and transferrin (Tf) has never been disclosed, as well as binding of photoactive compounds (photosensitizers or PSs) to Tf. Tf solution pre-mixed with PS solution (Ruthenium, Ruthenium-Rhodium and Osmium-based PSs) demonstrates evidence of metal-specific binding of the PS to Tf. The resulting complex has increased absorbance/molar extinction coefficient at long wavelengths (>600 nm), increased ROS production (generation of hydroxyl radical is potentiated to a much greater extent than that of singlet oxygen suggesting a switch to Type I photoreaction in the presence of transferrin), increased and preferential uptake by cancer cells, increased efficacy of in vitro PDT accompanied by a decrease in dark toxicity and consequently by increased therapeutic ratio, increased and selective uptake by cancer cells and tumor tissues and increased efficacy of in vivo PDT in visible and NIR light. In certain embodiments, increased absorbance/molar extinction coefficient at wavelengths at or below 600 nm are achieved, which embodiments are particularly suitable for excitation with green, blue, UV and X-ray radiation.

[0089] Ruthenium, Ruthenium-Rhodium and Osmium-based photosensitizers bind to transferrin demonstrating characteristic binding signatures.

[0090] Binding to transferrin changes chemical, physical and biomedical characteristics of metal-based molecules and/or formulations, and induces absorption by Ruthenium, Ruthenium-Rhodium and Osmium-based photosensitizers in red and NIR wavelengths where their absorption without transferrin is negligible.

[0091] Irradiation-induced fluorescence of Ruthenium, Ruthenium-Rhodium and Osmium-based photosensitizers is increased in the presence of transferrin, which may play a role in using metal-transferrin complexes in diagnostics.

[0092] Binding of Ruthenium, Ruthenium-Rhodium and Osmium-based photosensitizers to transferrin will increase their resistance to photobleaching.

[0093] Binding of Ruthenium, Ruthenium-Rhodium and Osmium-based photosensitizers to transferrin will increase production of reactive oxygen species in a cell free environment. This effect will increase with the increase of transferrin concentration. Increased production of hydroxyl radical suggests that the photosensitizers' photoeffect is switched from Type II to Type I, which is essential for PDT treatment of bulky hypoxic tumors.

[0094] Binding of Ruthenium, Ruthenium-Rhodium and Osmium-based photosensitizers to transferrin will increase their preferential uptake by cancer cells.

[0095] In the presence of additional transferrin, Ruthenium, Ruthenium-Rhodium and Osmium-based photosensitizers will demonstrate lesser dark toxicity in vitro (using cancer cell lines); PDT efficacy will increase. This will result in the increase in therapeutic ratios of the photosensitizers.

[0096] Binding of Ruthenium, Ruthenium-Rhodium and Osmium-based photosensitizers to additional transferrin will demonstrate PDT efficacy in hypoxic conditions absent without transferrin.

[0097] Blocking transferrin receptors with specific antibodies will eliminate facilitating effect of transferrin on PDT effect.

[0098] Incubation of Ruthenium, Ruthenium-Rhodium and Osmium-based photosensitizers with transferrin prior to i.p. injection in vivo will increases MTD50 indicating a decrease in the PSs toxicity.

[0099] Incubation of Ruthenium, Ruthenium-Rhodium and Osmium-based photosensitizers with transferrin prior to IP injection will increase in vivo PDT efficacy in NIR (808nm) and red (625nm).

[00100] The invention will enable:

- Selective delivery of Ruthenium, Ruthenium-Rhodium and Osmium -based photosensitizers;
- Increase in efficacy of the PS, especially in wavelength ranges where the photosensitizers are otherwise ineffective (red, NIR);
- PDT efficacy in hypoxia when the PSs are not effective without Tf;
- Improved safety, tolerability and efficacy of all metal-based medicinal formulations that could be linked to Tf and/or Tf-based substances, including beads and or liposomes; and
- Improved diagnostic properties of metal based molecules.

[00101] The invention further encompasses the use of metal-glycoprotein complexes of the invention to enhance uptake by cells of metal-based pharmaceutical agents that are not light activated (e.g., RAPTA, NAMI, KP1019, RM-175).

[00102] ***Compositions of the Invention***

[00103] The compositions of the invention comprise a metal-binding glycoprotein and a chemotherapeutic compound (e.g., a photodynamic compound) containing at least one transition metal, which is preferably a Group 8 or 9 metal and is most preferably at least one of Ru, Rh and Os.

[00104] The chemotherapeutic compound is preferably at least one such compound disclosed in WO 2013158550 A1, WO 2014145428 A2, US 6962910, US 7612057, US 8445475 or US 8148360. Other chemotherapeutic compounds suitable for use in the invention include but are not limited to RAPTA, NAMI, KP1019 and analogs thereof.

[00105] In certain embodiments, compositions of the invention comprise transitional metal-based combinations that are optionally surrounded (directly and/or indirectly binded) by various ligands, such as in coordination compounds. In some of these embodiments, bridging ligands are included, which are highly electron donating to the metal(s).

[00106] In all of the embodiments provided herein, examples of suitable optional substituents are not intended to limit the scope of the claimed invention. The compounds of the invention may contain any of the substituents, or combinations of substituents, provided herein.

[00107] Properties of the compounds of the invention:

[00108] The compositions of the invention are useful for the treatment and diagnosis of disease states, particularly for the destruction of infectious organisms, hyperproliferating cells, and tumor cells. The compositions preferably included PDCs which (i) are metal-based coordination complexes, (ii) absorb ultraviolet (UV), visible, and infrared (IR) (particularly, near infrared (NIR)) light and are activated by wavelengths from UV to IR,

particularly >800 nm, (iii) kill human cancer cells in culture and in animals, and (iv) destroy bacteria and antibiotic-resistant bacteria.

[00109] Compositions of the invention are also capable of destroying microorganisms, such as *Staphylococcus aureus* (SA) and methicillin-resistant *S. aureus* (MRSA), with activation by UV to IR light, particularly red and NIR light.

[00110] The invention will be illustrated in more detail with reference to the following Examples, but it should be understood that the present invention is not deemed to be limited thereto.

[00111] The Examples provided below provide representative methods for preparing exemplary compounds of the present invention. The skilled practitioner will know how to substitute the appropriate reagents, starting materials and purification methods known to those skilled in the art, in order to prepare the compounds of the present invention.

EXAMPLES

[00112] Example 1

[00113] Transferrin (0.2mg/mL) was incubated with different photosensitizers (10uM) and the absorbance spectra were obtained. FIG. 1A shows an increase in absorption by Ruthenium (TLD1433). FIG. 1B shows Ruthenium-Rhodium (TLD143310) based PSs and FIG. 1C shows Osmium (TLDOsH2dppn) based PSs in the presence of transferrin. For each photosensitizer, the left plot shows the absolute difference in absorbance with transferrin vs. without transferrin. The right plot shows relative increase in absorbance with transferrin vs. without transferrin. Note that a large relative increase in absorbance may be accompanied by a small absolute difference and vice versa.

[00114] The binding of the photosensitizers to transferrin was accompanied by an increased absorption in visible and NIR range, especially at longer wavelengths. It is noteworthy that negligible absorption of the photosensitizers in red & NIR range became substantial upon binding of these photosensitizers to transferrin.

[00115] Example 2: Binding Signatures

[00116] Transferrin (0.2mg/mL) was incubated with the photosensitizers (10uM) and absorption spectra were obtained. FIGS. 2A, 2B and 2C show transferrin binding to Ruthenium (TLD1433, FIG. 2A), Ruthenium-Rhodium (TLD143310, FIG. 2B) and Osmium (TLDOsH2dppn, FIG. 2C) based photosensitizers.

[00117] Binding of Ruthenium and Ruthenium-Rhodium-based photosensitizers to transferrin is characterized by a characteristic signature: an increased peak of absorption at 280nm and a new peak in 400-500nm range. Osmium-based photosensitizers demonstrate different binding signature: new peaks in 300-400nm and 500-600nm ranges.

[00118] Example 3: Fluorescence

[00119] Fluorescence of TLD1433 (10 uM) was measured quartz cuvettes without Tf (in water) and in the presence of 0.2 mg/mL Tf (in phosphate buffer + 100 mM NaCl, pH=7.0). Fluorescence was measured at different excitation wavelengths (380, 400, 450, 470, 500 nm).

[00120] TLD1433 demonstrates measurable fluorescence that is intensified in the presence of transferrin. With no transferrin (FIG. 3A), maximal fluorescence was evoked at excitation wavelengths 380, 400 and 470 nm (emission maximum 624 nm). In the presence of transferrin (FIG. 3B), fluorescence was strongly increased, especially at 470 nm excitation, with emission maximum slightly shifted towards shorter wavelengths (619 nm).

[00121] Detectable fluorescence of TLD1433 makes it useful for diagnostic purposes (detection of selective uptake of TLD1433, preferably by cancerous tissues).

[00122] Example 4 (Prophetic)

[00123] FIGS. 4A and 4B

[00124] Fluorescence of TLD1433 (10 uM) was measured quartz cuvettes without Tf (in water) and in the presence of 0.2 mg/mL Tf (in phosphate buffer + 100 mM NaCl, pH=7.0). Fluorescence was measured at different excitation wavelengths (380, 400, 450, 470, 500 nm).

[00125] TLD143310 demonstrates measurable fluorescence that is intensified in the presence of transferrin. With no transferrin (FIG. 4A), maximal fluorescence was evoked at excitation wavelengths 380, 400, 470 nm (emission maximum 650 nm). In the presence of transferrin (FIG. 4B), fluorescence was strongly increased, especially at 470 nm excitation, with emission maximum slightly shifted towards shorter wavelengths (637 nm). Detectable fluorescence of TLD143310 makes it useful for diagnostic purposes (detection of selective uptake of TLD143310, preferably by cancerous tissues).

[00126] Example 5 (Prophetic)

[00127] Fluorescence of TLDOsH2dppn (10 uM) was measured quartz cuvettes without Tf (in water) and in the presence of 0.2 mg/mL Tf (in phosphate buffer + 100 mM NaCl, pH=7.0). Fluorescence was measured at different excitation wavelengths (380, 400, 450, 470, 500 nm).

[00128] TLDOsH2dppn demonstrates measurable fluorescence that is intensified in the presence of transferrin. With no transferrin (FIG. 5A), maximal fluorescence was evoked at excitation wavelengths 380, 400, 470 nm (emission maximum 660 nm). In the presence of transferrin (FIG. 5B), fluorescence was strongly increased, especially at 470 nm excitation, with emission maximum slightly shifted towards shorter wavelengths (650 nm).

[00129] Detectable fluorescence of TLDOsH2dppn makes it useful for diagnostic purposes (detection of selective uptake of TLDOsH2dppn, preferably by cancerous tissues).

[00130] Example 6: Photobleaching

[00131] Photobleaching was measured: (a) at wavelength in visible range maximal for each photosensitizer. This allows to estimate dynamics of amount of unbleached photosensitizer during irradiation; and (b) at 530, 635 and 808 nm. This allows estimating dynamics of unbleached photosensitizer available to exert PDT effect at these wavelengths as practically useful for PDT treatments.

[00132] Photosensitizers (10uM) were dissolved in phosphate buffer + 100 mM NaCl (pH=7.0) alone or in a buffer with 0.8mg/mL of transferrin at a total volume of 1mL. The mixture was then exposed to a 525nm laser source (130mW) and the absorbance at the maximal absorbance in visible range (432nm for TLD1433, 425nm for TLD143310 and 562nm for TLDOsH2dppn) was measured at specific time points. The plot represents the absorbance ratio (absorbance of exposed samples / absorbance of unexposed sample) as a function of the number of incident photons per cm^{-2}. Decreasing absorbance ratio signifies bleaching of the photosensitizer.

[00133] Effect of Transferrin on bleaching of Ruthenium (TLD1433, FIG. 6A), Ruthenium-Rhodium (TLD143310, FIG. 6B - Prophetic) and Osmium (TLDOsH2dppn, FIG. 6C - Prophetic)-based compounds at 525nm.

[00134] In the presence of transferrin, bleaching of the photosensitizers was reduced.

[00135] Example 7 (Prophetic)

[00136] Photosensitizers (10uM) were dissolved in phosphate buffer + 100 mM NaCl (pH=7.0) alone or in a buffer with 0.8mg/mL of transferrin at a total volume of 1mL. The mixture was then exposed to a 525nm laser source (130mW) and the absorbance at 530 nm was measured at specific time points. The plot represents the absorbance ratio (absorbance of exposed samples / absorbance of unexposed sample) as a function of the number of incident photons per cm^{-2}. Decreasing absorbance ratio signifies bleaching of the photosensitizer.

[00137] Effect of Transferrin on bleaching of Ruthenium (TLD1433, FIG. 7A), Ruthenium-Rhodium (TLD143310, FIG. 7B) and Osmium (TLDOsH2dppn, FIG. 7C)-based compounds: absorbance at 525nm. In the presence of transferrin, bleaching of the photosensitizers was reduced. Transferrin partially protects photosensitizers from photobleaching.

[00138] Example 8 (Prophetic)

[00139] Photosensitizers (10uM) were dissolved in phosphate buffer + 100 mM NaCl (pH=7.0) alone or in a buffer with 0.8mg/mL of transferrin at a total volume of 1mL. The mixture was then exposed to a 525nm laser source (130mW) and the absorbance at 635 nm was measured at specific time points. The plot represents the absorbance ratio (absorbance of exposed samples / absorbance of unexposed sample) as a function of the number of incident photons per cm^{-2}. Decreasing absorbance ratio signifies bleaching of the photosensitizer.

[00140] Effect of Transferrin on bleaching of Ruthenium (TLD1433, FIG. 8A), Ruthenium-Rhodium (TLD143310, FIG. 8B) and Osmium (TLDOsH2dppn, FIG. 8C) based compounds: absorbance at 635 nm. In the presence of transferrin, bleaching of the photosensitizers was reduced. Transferrin partially protects photosensitizers form photobleaching.

[00141] Example 9 (Prophetic)

[00142] Photosensitizers (10uM) were dissolved in phosphate buffer + 100 mM NaCl (pH=7.0) alone or in a buffer with 0.8mg/mL of transferrin at a total volume of 1mL. The mixture was then exposed to a 525nm laser source (130mW) and the absorbance at 808 nm was measured at specific time points. The plot represents the absorbance ratio (absorbance of exposed samples / absorbance of unexposed sample) as a function of the number of incident photons per cm^{-2}. Decreasing absorbance ratio signifies bleaching of the photosensitizer.

[00143] Effect of Transferrin on bleaching of Ruthenium (TLD1433, Panel A), Ruthenium-Rhodium (TLD143310, Panel B) and Osmium (TLDOsH2dppn, Panel C) based compounds: absorbance at 808 nm. In the presence of transferrin, bleaching of the photosensitizers was reduced. Transferrin partially protects photosensitizers form photobleaching.

[00144] Example 10: ROS production (Prophetic)

[00145] Table 1 shows singlet oxygen production by the Ruthenium (TLD1433), Ruthenium-Rhodium (TLD143310) and Osmium (TLDOsH2dppn)-based PS at 530 nm & 1092 J cm-2 incident energy.

[00146] Table 1

	TLD1433	TLD143310	TLDOsH2dppn
Phosphate buffer			
no transferrin	22471	21068	25937
0.8 mg/mL transferrin	45568	32816	42816
1.6 mg/mL transferrin	61568	55934	56372
3.2 mg/mL transferrin	82652	751407	77219
Incomplete DMEM			
no transferrin	32398	21936	36391
0.8 mg/mL transferrin	53549	42138	35127
1.6 mg/mL transferrin	73657	52384	43927
3.2 mg/mL transferrin	95368	71573	61734

[00147] Example 11: (Prophetic)

[00148] Table 2 shows hydroxyl radical production by the Ruthenium (TLD1433), Ruthenium-Rhodium (TLD143310) and Osmium (TLDOsH2dppn)-based PS at 530 nm & 1092 J cm-2 incident energy.

[00149] Table 2

	TLD1433	TLD143310	TLDOsH2dppn
Phosphate buffer			
no transferrin	31358	28030	25290
0.8 mg/mL transferrin	112047	70107	41037
1.6 mg/mL transferrin	130308	121038	83034
3.2 mg/mL transferrin	165023	153096	120307
Incomplete DMEM			
no transferrin	41034	30348	32934
0.8 mg/mL transferrin	150237	60108	70308
1.6 mg/mL transferrin	182037	130025	132309
3.2 mg/mL transferrin	190608	162027	164301

[00150] Example 12: (Prophetic)

[00151] Table 3 shows singlet oxygen production by the Ruthenium (TLD1433), Ruthenium-Rhodium (TLD143310) and Osmium (TLDOsH2dppn)-based PS at 635 nm & 649 J cm-2 incident energy.

[00152] Table 3

	TLD1433	TLD143310	TLDOsH2dppn
Phosphate buffer			
no transferrin	91374	10289	10392
0.8 mg/mL transferrin	53124	41301	42837
1.6 mg/mL transferrin	57621	68027	51832

3.2 mg/mL transferrin	72218	75280	72938
Incomplete DMEM			
no transferrin	19929	9507	15109
0.8 mg/mL transferrin	39824	31937	53931
1.6 mg/mL transferrin	82438	70218	75293
3.2 mg/mL transferrin	95341	75317	81807

[00153] Example 13 (Prophetic)

[00154] Table 4 shows hydroxyl radical production by the Ruthenium (TLD1433), Ruthenium-Rhodium (TLD143310) and Osmium (TLDOsH2dppn)-based PS at 635 nm & 649 J cm-2 incident energy.

[00155] Table 4

	TLD1433	TLD143310	TLDOsH2dppn
Phosphate buffer			
no transferrin	12608	15000	8804
0.8 mg/mL transferrin	71907	50328	25280
1.6 mg/mL transferrin	82093	81867	45372
3.2 mg/mL transferrin	101293	85361	72316
Incomplete DMEM			
no transferrin	18107	15280	12093
0.8 mg/mL transferrin	98928	43623	32531
1.6 mg/mL transferrin	119307	72901	42806
3.2 mg/mL transferrin	152704	93150	73624

[00156] Example 14 (Prophetic)

[00157] Table 5 shows singlet oxygen production by the Ruthenium (TLD1433), Ruthenium-Rhodium (TLD143310) and Osmium (TLDOsH2dppn)-based PS at 808 nm & 4000 J cm-2 incident energy.

[00158] Table 5

	TLD1433	TLD143310	TLDOsH2dppn
Phosphate buffer			
no transferrin	10621	9000	8000
0.8 mg/mL transferrin	41934	20000	10000
1.6 mg/mL transferrin	56305	30000	30000
3.2 mg/mL transferrin	62861	50000	40000
Incomplete DMEM			
no transferrin	0	0	11907
0.8 mg/mL transferrin	1204	1245	25384
1.6 mg/mL transferrin	22394	12307	55631
3.2 mg/mL transferrin	36027	25193	62804

[00159] Example 15

[00160] Table 6 shows hydroxyl radical production by the Ruthenium (TLD1433), Ruthenium-Rhodium (TLD143310) and Osmium (TLDOsH2dppn)-based PS at 808 nm & 4000 J cm-2 incident energy.

[00161] Table 6

	TLD1433	TLD143310	TLDOsH2dppn
Phosphate buffer			

no transferrin	15610	12305	8931
0.8 mg/mL transferrin	22967	15034	13408
1.6 mg/mL transferrin	56390	31384	20392
3.2 mg/mL transferrin	61832	63904	56094
Incomplete DMEM			
no transferrin	503	427	14237
0.8 mg/mL transferrin	701	714	42305
1.6 mg/mL transferrin	20357	12397	77024
3.2 mg/mL transferrin	41395	23297	102864

[00162] Example 16 (Prophetic)

[00163] The photosensitizers were diluted in phosphate buffer (pH=7.0) + 100 mM NaCl or incomplete DMEM. The irradiation was performed in 96-well plates (100 uL working volume). Generation of singlet oxygen was measured by fluorescence signal of SOG indicator, generation of hydroxyl radical by fluorescence signal of HPF indicator. The signal presented is a result of subtraction of the signal in the presence of scavengers (NaN_3 for singlet oxygen and DMTU for hydroxyl radical) from the total fluorescence signal.

[00164] Ruthenium (TLD1433), Ruthenium-Rhodium (TLD143310) and Osmium (TLDOsH2dppn) based photosensitizers produce reactive oxygen species (ROS) under 525 nm (0.250 mW cm-2), 650 nm (0.119 mW cm-2) and NIR (808 nm, 747 mW cm-2) irradiation.

[00165] In the presence of transferrin, generation of both singlet oxygen and hydroxyl radical is potentiated in a dose-dependent manner: higher concentration of transferrin induces greater generation of ROS. Generation of hydroxyl radical is potentiated to a much greater extent than that of singlet oxygen suggesting a switch to Type I photoreaction in the presence of transferrin.

[00166] Example 17: Intracellular Uptake (Prophetic)

[00167] Cell culture (U87 cells) was exposed to 200uM of the photosensitizer without additional transferrin and in the presence of 0.8 mg/mL transferrin for 4 hours. The incubation mix was washed away and the cells were collected, counted, and lysed. Lysed solution was dissolved in nitric acid and appropriate metals concentration was measured using inductively coupled plasma mass spectrometry. The metals concentration is directly proportional to the concentration of the photosensitizer.

[00168] FIGS. 10A, 10B and 10C show transferrin facilitated uptake of Ruthenium (TLD1433, FIG. 10A), Ruthenium-Rhodium (TLD143310, FIG. 10B) and Osmium (TLDOsH2dppn, FIG. 10C) based photosensitizers into cancer cells.

[00169] Cellular accumulation of the photosensitizers was higher in the presence of transferrin. Since tumor cells have been shown to have higher expression of membrane transferrin receptor, these results show that the mentioned photosensitizers can preferentially accumulate in tumor cells.

[00170] Example 18: Uptake by Normal Cells (Prophetic)

[00171] Cell culture (normal human fibroblasts) was exposed to 200uM of the photosensitizer without additional transferrin and in the presence of 0.8 mg/mL transferrin for 4 hours. The incubation mix was washed away and the cells were collected, counted, and lysed. Lysed solution was dissolved in nitric acid and appropriate metals concentration was measured using inductively coupled plasma mass spectrometry. The metals concentration is directly proportional to the concentration of the photosensitizer.

[00172] FIGS. 11A, 11B and 11C show transferrin facilitated uptake of Ruthenium (TLD1433, FIG. 11A), Ruthenium-Rhodium (TLD143310, FIG. 11B) and Osmium (TLDOsH2dppn, FIG. 11C) based photosensitizers into normal cells. Cellular accumulation of the photosensitizers was higher in the presence of transferrin. However, the effect of transferrin on the uptake is to a much lesser extent than for cancer cells indicating uptake improvement preferentially in cancer cells.

[00173] Example 19: In Vitro – Dark Toxicity

[00174] The photosensitizer was pre-mixed with 0.4 mg/mL human transferrin and incubated for 1 hour at 37°C. In control group (no additional transferrin), equivalent volume of no-transferrin medium was added. The cells were subsequently incubated with the pre-mixes for 30 minutes (FIG. 12A) or 90 minutes (FIG. 12B). After that, the medium was replaced with a fresh one (without photosensitizer and transferrin). On the next day (21 hours post-irradiation), viability of the cells was measured using Presto Blue viability assay, and percent of cell kill was calculated.

[00175] The presence of transferrin decreases dark toxicity (photosensitizer alone) of Ruthenium-based photosensitizers (TLD1433) on AY27 cancer cell line. Transferrin decreases dark toxicity of TLD1433. This contributes to an increased safety of PDT treatment in the presence of transferrin. Together with the evidence of binding of TLD1433 to transferrin, these results suggest facilitated uptake of TLD1433 into cells in the presence of transferrin. Increase in PDT efficacy together with the decrease in dark toxicity suggests an increase in therapeutic ratio of TLD1433 when it is used mixed with transferrin.

[00176] Example 20 (Prophetic)

[00177] The photosensitizer was pre-mixed with 0.4 mg/mL human transferrin and incubated for 1 hour at 37°C. In control group (no additional transferrin), equivalent volume of no-transferrin medium was added. The cells were subsequently incubated with the pre-mixes for 30 minutes. After that, the medium was replaced with a fresh one (without photosensitizer and transferrin). On the next day (21 hours post-irradiation), viability of the cells was

measured using Presto Blue viability assay, and percent of cell kill was calculated.

[00178] The presence of transferrin decreases dark toxicity (photosensitizer alone) of Ruthenium-Rhodium (TLD143310, FIG. 13A) and Osmium (TLDOsH2dppn, FIG. 13B) based photosensitizers on AY27 cancer cell line. Transferrin decreases dark toxicity of the photosensitizers. This contributes to an increased safety of PDT treatment in the presence of transferrin.

[00179] Example 21: In Vitro PDT Effect

[00180] The photosensitizer was pre-mixed with 0.4 mg/mL human transferrin and incubated for 1 hour at 37°C. In control group (no additional transferrin), equivalent volume of no-transferrin medium was added. The cells were subsequently incubated with the pre-mixes for 30 minutes (FIG. 14A) or 90 minutes (FIG. 14B). After that, the medium was replaced with a fresh one (without photosensitizer and transferrin) and the cells were irradiated. On the next day (21 hours post-irradiation), viability of the cells was measured using Presto Blue viability assay, and percent of cell kill was calculated. The PDT effect shown is a result of subtraction of "photosensitizer alone" and "light alone" cell kill from the total PDT cell kill.

[00181] The presence of transferrin increases PDT effect (635 nm, 90 J cm-2) of Ruthenium-based photosensitizers (TLD1433) on AY27 cancer cell line. Transferrin potentiates PDT effect of the photosensitizer even at short loading time (30 minutes). This allows for safer PDT treatment due to shorter treatment time and the use of lower concentrations of the photosensitizers. Together with the evidence of binding of TLD1433 to transferrin, these results suggest facilitated uptake of TLD1433 into cells in the presence of transferrin.

[00182] Example 22 (Prophetic)

[00183] The photosensitizer was pre-mixed with 0.4 mg/mL human transferrin and incubated for 1 hour at 37°C. In control group (no additional transferrin), equivalent volume of no-transferrin medium was added. The cells were subsequently incubated with the pre-mixes for 30 minutes. After that, the medium was replaced with a fresh one (without photosensitizer and transferrin) and the cells were irradiated. On the next day (21 hours post-irradiation), viability of the cells was measured using Presto Blue viability assay, and percent of cell kill was calculated. The PDT effect shown is a result of subtraction of "photosensitizer alone" and "light alone" cell kill from the total PDT cell kill.

[00184] The presence of transferrin increases PDT effect (635 nm, 90 J cm-2) of Ruthenium-Rhodium (TLD143310, FIG. 15A) and Osmium (TLDOsH2dppn, FIG. 15B) based photosensitizers on AY27 cancer cell line.

[00185] Transferrin potentiates PDT effect of the photosensitizers even at short loading time (30 minutes). This allows for safer PDT treatment due to shorter treatment time and the use of lower concentrations of the photosensitizers. Together with the evidence of binding of the photosensitizers to transferrin, these results suggest facilitated uptake of the photosensitizers into cells in the presence of transferrin.

[00186] Increase in PDT efficacy together with the decrease in dark toxicity suggests an increase in therapeutic ratio of Ruthenium (TLD1433), Ruthenium-Rhodium (TLD143310) and Osmium (TLDOsH2dppn) based photosensitizers when they are pre-mixed with transferrin.

[00187] Example 23 (Prophetic)

[00188] The photosensitizer was pre-mixed with 0.4 mg/mL human transferrin and incubated for 1 hour at 37°C. In control group (no additional transferrin), equivalent volume of no-transferrin medium was added. The cells were subsequently incubated with the pre-mixes for 30 minutes. After that, the medium was replaced with a fresh one (without photosensitizer and transferrin) and the cells were irradiated. On the next day (21 hours post-irradiation), viability of the cells was measured using Presto Blue viability assay, and percent of cell kill was calculated. The PDT effect shown is a result of subtraction of "photosensitizer alone" and "light alone" cell kill from the total PDT cell kill.

[00189] The presence of transferrin increases PDT effect (530 nm, 90 J cm-2) of Ruthenium-Rhodium (TLD143310, Panel A) and Osmium (TLDOsH2dppn, Panel B) based photosensitizers on AY27 cancer cell line. Transferrin potentiates PDT effect of the photosensitizers even at short loading time (30 minutes). This allows for safer PDT treatment due to shorter treatment time and the use of lower concentrations of the photosensitizers. Together with the evidence of binding of the photosensitizers to transferrin, these results suggest facilitated uptake of the photosensitizers into cells in the presence of transferrin.

[00190] Example 24

[00191] Pre-mixing with transferrin increases therapeutic ratio of Ruthenium (TLD1433), Ruthenium-Rhodium (TLD143310) and Osmium (TLDOsH2dppn) based photosensitizers, due to the increase in PDT efficacy together with the decrease in dark toxicity. See Table 7.

[00192] Table 7. Therapeutic Ratio

	HT1376 cells		U87 cells		AY27 cells	
	No transferrin	Transferrin	No transferrin	Transferrin	No transferrin	Transferrin
530nm, 90 J cm-2						
TLD1433	19024.1 (Prophetic)	50938.4 (Prophetic)	27557.1	65002.3 (Prophetic)	17356.2 (Prophetic)	57324.1 (Prophetic)
TLD143310	421.9 (Prophetic)	835.7 (Prophetic)	495.1	1022.6 (Prophetic)	473.1 (Prophetic)	934.5 (Prophetic)
TLDOsH2dppn	1548.2 (Prophetic)	4963.8 (Prophetic)	1307.3	5238.0 (Prophetic)	1639.8 (Prophetic)	5531.9 (Prophetic)
650nm, 90 J cm-2						
TLD1433	50.4 (Prophetic)	100.8 (Prophetic)	102.6	405.1 (Prophetic)	79.2 (Prophetic)	393.7 (Prophetic)
TLD143310	28.1 (Prophetic)	52.7 (Prophetic)	16.3	50.2 (Prophetic)	25.1 (Prophetic)	59.4 (Prophetic)
TLDOsH2dppn	37.7	61.9 (Prophetic)	15.3	50.3 (Prophetic)	28.7 (Prophetic)	61.7 (Prophetic)

[00193] Example 25: In Vitro Hypoxia (Prophetic)

[00194] The cells were incubated for 90 minutes in premix of the photosensitizer and 0.4 mg/mL human

transferrin (or without transferrin, as a control group) as described above for in vitro PDT. Dark toxicity and PDT effect of the photosensitizers on AY27 cell line under 635 nm (90 J cm-2) irradiation in the absence and presence of transferrin in normoxic (FIGS. 17A, 17B) and hypoxic (0.5-0.1 % O_2, FIGS. 17C, 17D) conditions. The cells were incubated with the photosensitizer and transferrin for 90 minutes. After that, the cells were irradiated and the medium was replaced with a fresh one (without photosensitizer and transferrin). On the next day (21 hours post-irradiation), viability of the cells was measured using Presto Blue viability assay, and percent of cell kill was calculated. The PDT effect shown is a result of subtraction of "photosensitizer alone" and "light alone" cell kill from the total PDT cell kill.

[00195] The presence of transferrin ensured PDT effect (635 nm, 90 J cm-2) of Ruthenium (TLD1433) based photosensitizers on AY27 cancer cell line under hypoxic conditions. Transferrin induces PDT activity in hypoxia, which is not observed in the absence of transferrin. The presence of transferrin, therefore, allows for a greater efficacy of the Ruthenium-based photosensitizers during PDT treatment of bulky hypoxic tumors.

[00196] Example 26 (Prophetic)

[00197] The cells were incubated for 90 minutes in premix of the photosensitizer and 0.4 mg/mL human transferrin (or without transferrin, as a control group) as described above for in vitro PDT. Dark toxicity and PDT effect of the photosensitizers on AY27 cell line under 635 nm (90 J cm-2) irradiation in the absence and presence of transferrin in normoxic (FIGS. 18A, 18B) and hypoxic (0.5-0.1 % O_2, FIGS. 18C, 18D) conditions. The cells were incubated with the photosensitizer and transferrin for 90 minutes. After that, the cells were irradiated and the medium was replaced with a fresh one (without photosensitizer and transferrin). On the next day (21 hours post-irradiation), viability of the cells was measured using Presto Blue viability assay, and percent of cell kill was calculated. The PDT effect shown is a result of subtraction of "photosensitizer alone" and "light alone" cell kill from the total PDT cell kill.

[00198] The presence of transferrin ensured PDT effect (635 nm, 90 J cm-2) of Ruthenium-Rhodium (TLD143310) based photosensitizers on AY27 cancer cell line under hypoxic conditions. Transferrin induces PDT activity in hypoxia, which is not observed in the absence of transferrin. The presence of transferrin, therefore, allows for a greater efficacy of the Ruthenium-Rhodium-based photosensitizers during PDT treatment of bulky hypoxic tumors.

[00199] Example 27 (Prophetic)

[00200] The cells were incubated for 90 minutes in premix of the photosensitizer and 0.4 mg/mL human transferrin (or without transferrin, as a control group) as described above for in vitro PDT. Dark toxicity and PDT effect of the photosensitizers on AY27 cell line under 635 nm (90 J cm-2) irradiation in the absence and presence of transferrin in normoxic (FIGS. 19A, 19B) and hypoxic (0.5-0.1 % O_2, FIGS. 19C, 19D) conditions. The cells were incubated with the photosensitizer and transferrin for 90 minutes. After that, the cells were irradiated and the medium was replaced with a fresh one (without photosensitizer and transferrin). On the next day (21 hours post-irradiation), viability of the cells was measured using Presto Blue viability assay, and percent of cell kill was

calculated. The PDT effect shown is a result of subtraction of "photosensitizer alone" and "light alone" cell kill from the total PDT cell kill.

[00201] The presence of transferrin ensured PDT effect (635 nm, 90 J cm-2) of Osmium (TLDOsH2dppn) based photosensitizers on AY27 cancer cell line under hypoxic conditions.

[00202] Transferrin induces PDT activity in hypoxia, which is not observed in the absence of transferrin. The presence of transferrin, therefore, allows for a greater efficacy of the Osmium-based photosensitizers during PDT treatment of bulky hypoxic tumors.

[00203] Example 28: Transferrin receptor antibodies (Prophetic)

[00204] The cells were incubated with anti-transferrin receptor antibody. The photosensitizer was pre-mixed in parallel with 0.4 mg/mL human transferrin and incubated for 1 hour at 37°C. In control group (no additional transferrin), equivalent volume of no-transferrin medium was added. The cells were washed of the excess of antibody (the cells incubated in medium without antibody served as comparison groups) and incubated with the pre-mixes for 30 minutes. After that, the medium was replaced with a fresh one (without photosensitizer and transferrin) and the cells were irradiated. On the next day (21 hours post-irradiation), viability of the cells was measured using Presto Blue viability assay, and percent of cell kill was calculated. The PDT effect shown is a result of subtraction of "photosensitizer alone" and "light alone" cell kill from the total PDT cell kill. Dark toxicity is shown on FIG. 20A, PDT effect is shown on FIG. 20B.

[00205] Blocking of transferrin receptors of AY27 cancer cells prevents decrease in dark toxicity and facilitation of PDT effect (635 nm, 90 J cm-2) of Ruthenium (TLD1433) based photosensitizers. The results support the role of transferrin-mediated uptake of Ruthenium-based photosensitizers in decrease of their dark toxicity and facilitation of their PDT effect.

[00206] Example 29 (Prophetic)

[00207] The cells were incubated with anti-transferrin antibody. The photosensitizer was pre-mixed in parallel with 0.4 mg/mL human transferrin and incubated for 1 hour at 37°C. In control group (no additional transferrin), equivalent volume of no-transferrin medium was added. The cells were washed of the excess of antibody (the cells incubated in medium without antibody served as comparison groups) and incubated with the pre-mixes for 30 minutes. After that, the medium was replaced with a fresh one (without photosensitizer and transferrin) and the cells were irradiated. On the next day (21 hours post-irradiation), viability of the cells was measured using Presto Blue viability assay, and percent of cell kill was calculated. The PDT effect shown is a result of subtraction of "photosensitizer alone" and "light alone" cell kill from the total PDT cell kill. Dark toxicity is shown on FIG. 21A, PDT effect is shown on FIG. 21B.

[00208] Blocking of transferrin receptors of AY27 cancer cells prevents decrease in dark toxicity and facilitation of PDT effect (635 nm, 90 J cm-2) of Ruthenium-Rhodium (TLD143310) based photosensitizers. The results support the role of transferrin-mediated uptake of Ruthenium-Rhodium based photosensitizers in decrease of their dark toxicity and facilitation of their PDT effect.

[00209] Example 30 (Prophetic)

[00210] The cells were incubated with anti-transferrin antibody. The photosensitizer was pre-mixed in parallel with 0.4 mg/mL human transferrin and incubated for 1 hour at 37°C. In control group (no additional transferrin), equivalent volume of no-transferrin medium was added. The cells were washed of the excess of antibody (the cells incubated in medium without antibody served as comparison groups) and incubated with the pre-mixes for 30 minutes. After that, the medium was replaced with a fresh one (without photosensitizer and transferrin) and the cells were irradiated. On the next day (21 hours post-irradiation), viability of the cells was measured using Presto Blue viability assay, and percent of cell kill was calculated. The PDT effect shown is a result of subtraction of "photosensitizer alone" and "light alone" cell kill from the total PDT cell kill. Dark toxicity is shown in FIG. 22A, PDT effect is shown on FIG. 22B.

[00211] Blocking of transferrin receptors of AY27 cancer cells prevents decrease in dark toxicity and facilitation of PDT effect (635 nm, 90 J cm-2) of Osmium (TLDOsH2dppn) based photosensitizers. The results support the role of transferrin-mediated uptake of Osmium-based photosensitizers in decrease of their dark toxicity and facilitation of their PDT effect.

[00212] Example 31: In Vivo Tissue Uptake (Prophetic)

[00213] Transferrin was dissolved in 40% PG in phosphate buffer (pH=7.0)+100 mM NaCl (0.1 mg/100 uL) and added to the photosensitizer solution (to achieve 10 mg/kg dose). The mixture was incubated for 1 hour prior to injection, and 100 uL of the mixture was injected i.v. to each animal.

[00214] Mixing of the Ruthenium based photosensitizers (TLD1433) with transferrin (1 mg/kg) increases uptake of the photosensitizer into tissues. The presence of transferrin considerably improves selectivity of the uptake into tumors as compared to normal muscle tissue (FIGS. 23A and 23B).

[00215] Example 32 (Prophetic)

[00216] Transferrin was dissolved in 40% PG in phosphate buffer (pH=7.0)+100 mM NaCl (0.1 mg/100 uL) and added to the photosensitizer solution (to achieve 10 mg/kg dose). The mixture was incubated for 1 hour prior to injection, and 100 uL of the mixture was injected i.v. to each animal.

[00217] Mixing of the Ruthenium-Rhodium based photosensitizers (TLD143310) with transferrin (1 mg/kg) increases uptake of the photosensitizer into tissues. The presence of transferrin considerably improves selectivity of the uptake into tumors as compared to normal muscle tissue (FIGS. 24A and 24B).

[00218] Example 33 (Prophetic)

[00219] Transferrin was dissolved in 40% PG in phosphate buffer (pH=7.0)+100 mM NaCl (0.1 mg/100 uL) and added to the photosensitizer solution (to achieve 10 mg/kg dose). The mixture was incubated for 1 hour prior to injection, and 100 uL of the mixture was injected i.v. to each animal.

[00220] Mixing of the Osmium based photosensitizers (TLDOsH2dppn) with transferrin (1 mg/kg) increases uptake of the photosensitizer into tissues. The presence of transferrin considerably improves selectivity of the uptake into tumors as compared to normal muscle tissue (FIGS. 25A and 25B).

[00221] Example 34: In Vivo Toxicity

[00222] Mice were injected (i.p.) with escalating doses of the photosensitizers with and without transferrin. Mixing of the photosensitizers with transferrin increased MTD which suggests a decrease in toxicity of the photosensitizers. For Ruthenium-based TLD1433, complete mouse survival was observed at 125mg/kg; higher doses are currently being tested.

[00223] Maximum tolerated doses (MTD) of Ruthenium (TLD1433), Ruthenium-Rhodium (TLD143310) and Osmium (TLDOsH2dppn) based photosensitizers increases with transferrin are shown in Table 8 below.

[00224] Table 8

	MTD Dose (mg/kg)	
Photosensitizer	No transferrin	With transferrin (1 mg/kg)
TLD1433	100	>125
TLD143310	150 (Prophetic)	200 (Prophetic)
TLDOsH2dppn	50	100 (Prophetic)

[00225] Considering the results of in vitro and in vivo experiments combined Ruthenium, Ruthenium-Rhodium and Osmium-based photosensitizers must be mixed with transferrin for patients treatment not only to improve PDT effect but also to decrease toxicity of the photosensitizers.

[00226] Example 35: In Vivo PDT Effect

[00227] Subcutaneous tumors were grown in mouse thighs and PDT (808nm, 600Jcm^{-2}) was performed when tumor reached 5x5mm in size. Mice were sacrificed if tumor continued to grow after PDT treatment and reached 10x10mm. Controls had a maximum survival of 10 days.

[00228] Transferrin increases mouse survival after PDT treatment with Ruthenium-based photosensitizer (TLD1433). The number of tumor free animals greatly increased when transferrin was added to Ruthenium-based TLD1433 before injection (in our data currently 8 days after PDT treatment, previous data has shown tumors will not reappear after this time, 70 days is assumed to remain tumor free).

[00229] Example 36 (Prophetic)

[00230] Subcutaneous tumors were grown in mouse thighs and PDT (808nm, 600Jcm^{-2}) was performed when tumor reached 5x5mm in size. Mice were sacrificed if tumor continued to grow after PDT treatment and reached 10x10mm. Controls had a maximum survival of 10 days.

[00231] Transferrin increases mouse survival after PDT treatment with Ruthenium-Rhodium-based photosensitizer (TLD143310) and Osmium-based (TLDOsH2dppn) photosensitizers. The number of tumor free animals greatly increased when transferrin was added to Ruthenium-Rhodium-based photosensitizer (TLD143310)

and Osmium-based (TLDOsH2dppn) photosensitizers before injection. See Table 9 below.

[00232] Table 9

		Survival by day 80	
		Without transferrin	**With transferrin**
Mixed metal (Ruthenium-Rhodium)-based	**TLD143310**	**35%** (Prophetic)	**70%** (Prophetic)
Osmium-based	**TLDOsH2dppn**	**40%** (Prophetic)	**80%** (Prophetic)

[00233] Example 36

[00234] Subcutaneous tumors were grown in mouse thighs and PDT (635nm, 192Jcm^{-2}) was performed when tumor reached 5x5mm in size. Mice were sacrificed if tumor continued to grow after PDT treatment and reached 10x10mm. Controls had a maximum survival of 10 days.

[00235] Transferrin increases mouse survival after PDT treatment. The number of tumor free animals greatly increased when transferrin was added to TLD1433 before injection (expected results). See FIG. 27.

[00236] Example 37

[00237] Subcutaneous tumors were grown in mouse thighs and PDT (635nm, 192Jcm^{-2}) was performed when tumor reached 5x5mm in size. Mice were sacrificed if tumor continued to grow after PDT treatment and reached 10x10mm. Controls had a maximum survival of 10 days.

[00238] Transferrin increases mouse survival after PDT treatment with Ruthenium-Rhodium-based photosensitizer (TLD143310) and Osmium-based (TLDOsH2dppn) photosensitizers. The number of tumor free animals greatly increased when transferrin was added to Ruthenium-Rhodium-based photosensitizer (TLD143310) and Osmium-based (TLDOsH2dppn) photosensitizers before injection. See Table 10.

[00239] Table 10

		Survival by day 80	
		Without transferrin	**With transferrin**
Mixed metal (Ruthemium-Rhodium)-based	**TLD143310**	**41%** (Prophetic)	**65%** (Prophetic)
Osmium-based	**TLDOsH2dppn**	**35%** (Prophetic)	**75%** (Prophetic)

[00240] Example 38

[00241] Subcutaneous tumors were grown in mouse thighs and PDT (525nm, 90Jcm^{-2}) was performed when tumor reached 5x5mm in size. Mice were sacrificed if tumor continued to grow after PDT treatment and reached 10x10mm. Controls had a maximum survival of 10 days.

[00242] Transferrin increases mouse survival after PDT treatment with Ruthenium-Rhodium-based photosensitizer (TLD143310) and Osmium-based (TLDOsH2dppn) photosensitizers. The number of tumor free animals greatly increased when transferrin was added to Ruthenium-Rhodium-based photosensitizer (TLD143310)

and Osmium-based (TLDOsH2dppn) photosensitizers before injection. See Table 11.

[00243] Table 11

		Survival by day 80	
		Without transferrin	**With transferrin**
Mixed metal (Ruthenium-Rhodium) – based	**TLD143310**	**48%** (Prophetic)	**85%** (Prophetic)
Osmium-based	**TLDOsH2dppn**	**37%** (Prophetic)	**78%** (Prophetic)

[00244] Example 39

[00245] The optical density of TLD1433 was measured before and after the addition of either OPTIFERRIN (available from InVitria, a division of Ventria Bioscience) or human apo-transferrin (Sigma). As shown in FIG. 28, the TLD1433-OPTIFERRIN complex showed a substantial increase in optical density. Furthermore, the complex showed novel absorption in the red (600nm) and near infrared (800nm) wavelengths. The optical density of the OPTIFERRIN complex was comparable to that of the human apo-transferrin complex. This finding highlights the potential of using OPTIFERRIN to increase the efficacy of Theralase's TLD1433 photosensitizer. The increase in optical density will translate to a higher production of reactive oxygen species during PDT treatment. The absorption in red and near infrared light will allow for novel treatment applications for different tumors.

[00246] Example 40

[00247] The optical density of Optiferrin and human apo-transferrin was measured before and after TLD1433 addition. Transferrin binding to iron is characterized by an increase in absorption at 275nm and 450nm. Much like human apo-transferrin, Optiferrin shows this characteristic optical density increase after TLD1433 addition, showing direct binding of TLD1433 to Optiferrin. See FIG. 29. Direct binding of Optiferrin to TLD1433 can be utilized in the treatment of transferrin receptor rich tumors, where the Optiferrin+TLD1433 complex can be administered to achieve preferential uptake of TLD1433 in cancer cells.

[00248] Example 41

[00249] The bleaching of TLD1433 alone and incubated with Optiferrin or human apo-transferrin in response to 525nm (green) light was measured. Bleaching was measured by the decrease in the optical density at 425nm in response to irradiation to 525nm light, which was normalized by dividing it by the 425nm optical density of the unexposed sample. Optical density ratio of 1 signifies no bleaching. Bleaching of TLD1433 results in the ratio decreasing towards 0. Both Optiferrin and human apo-transferrin reduced the bleaching of TLD1433, with Optiferrin having a stronger effect. See FIG. 30.

[00250] Example 42 - Optiferrin binding with TLD1433 (14C TH2, GS6-22, and GS6-81G) and TH1 (Sky Blue)

[00251] Experiments demonstrated that the following photosensitizers mimic the binding signatures of Fe+Tf binding, but with Optiferrin: TLD1433 GS6-22, GS6-81G, TH2 QR-100, and TH1 lot no. 1411459022. See Fig. 31.

[00252] Example 43 - Photobleaching prevention over 200J with TLD 1433

[00253] 10uM TLD1433 GS6-22 was photobleached with 200J of green light (525nm) and its OD was measured at 0, 1, 2, 5, 10, 20, 50, 100, and 200 Joules. See Fig. 32, which shows the ratio of Molar Extinction Coefficient increase or decrease at 350nm, 400nm, and 600nm between the initial time with 0 Joules, and the final time with 200 Joules.

[00254] Example 44 - TLD1433 infrared absorbance stability

[00255] Experiments demonstrated that the OD of TLD1433 is better maintained in the infrared with the addition of 0.8g/L (or 10uM) Optiferrin over time. See Fig. 33.

[00256] Example 45 - Optiferrin incubation and the maintenance of photosensitiser TLD1433 absorbance

[00257] The incubation duration was examined to see how well a dark sample of 10uM TLD1433 would absorb with the addition of Optiferrin. See Fig. 34. As seen previously this undoubtedly helps prevent TLD1433 from photobleaching.

[00258] While the invention has been described in detail and with reference to specific examples thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope thereof.

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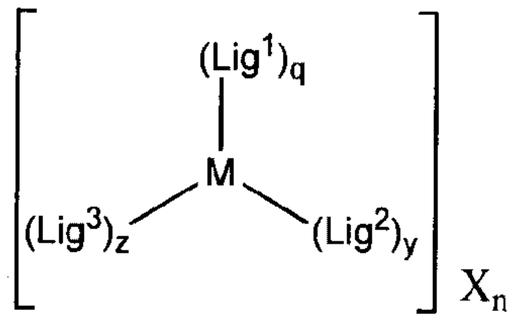
CLAIMS:

1. A composition comprising:

a metal-binding glycoprotein; and

a chemotherapeutic compound having the formula:

a) formula (I)



(I)

including hydrates, solvates, and pharmaceutically acceptable salts thereof, wherein:

M is osmium, ruthenium or rhodium;

X is Cl^-, PF_6^-, Br^-, BF_4^-, ClO_4^-, $CF_3SO_3^-$, or SO_4^{-2};

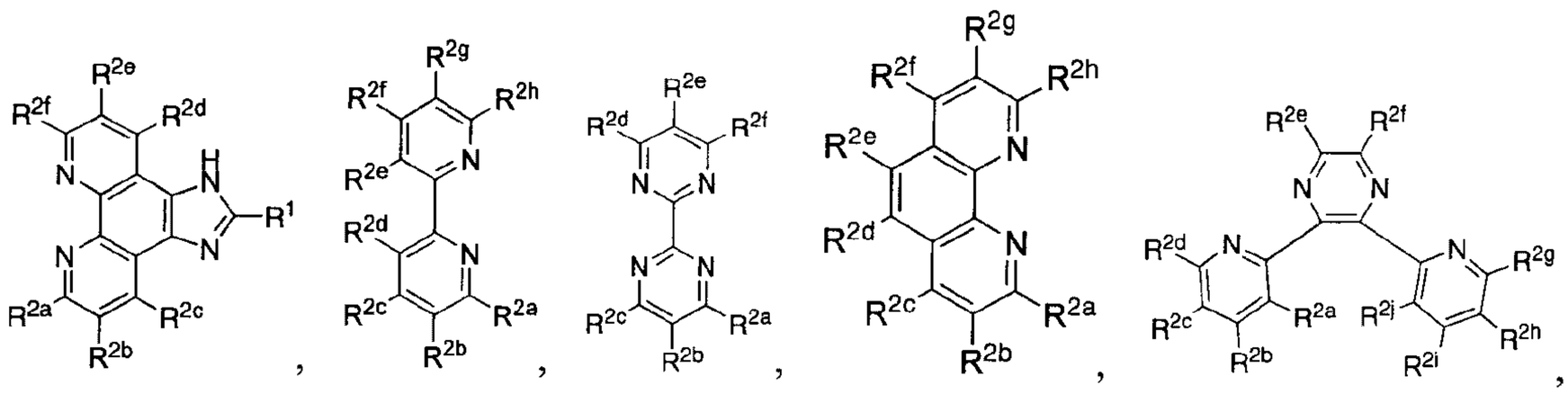
n = 0, 1, 2, 3, 4, or 5;

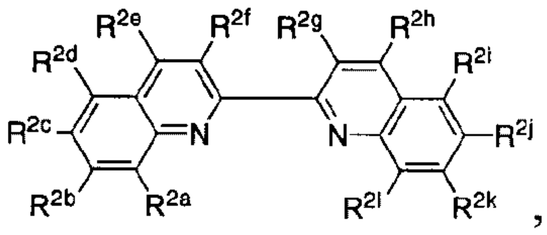
q is 0, 1, or 2;

y is 0, 1, or 2;

z is 1, 2, or 3;

Lig^1 is a bidentate ligand that at each occurrence is independently

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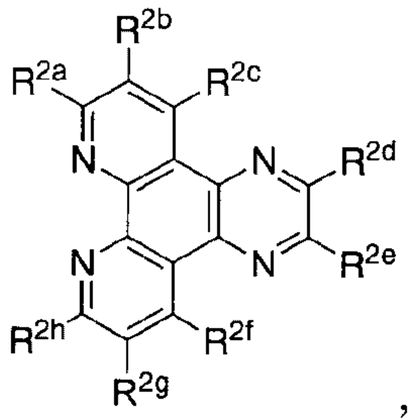
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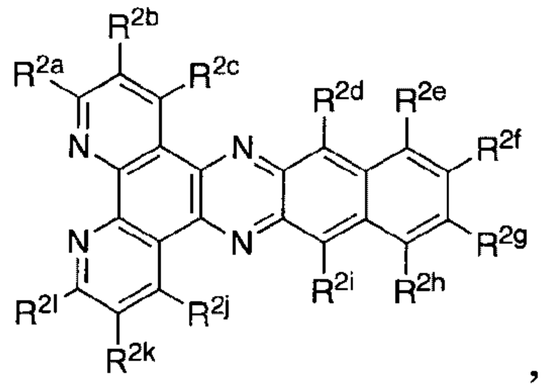
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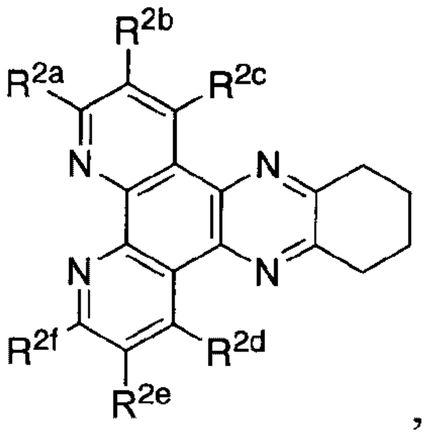
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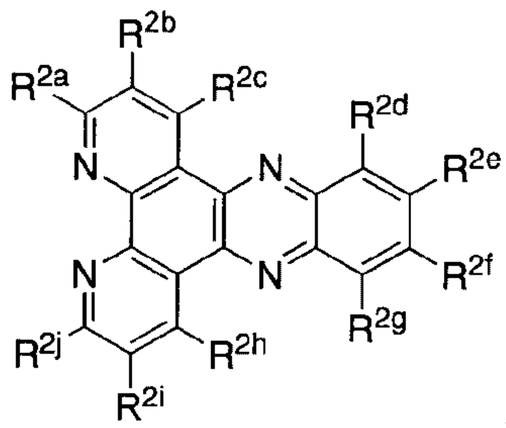
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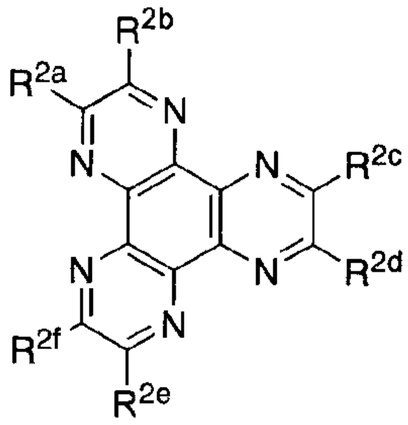
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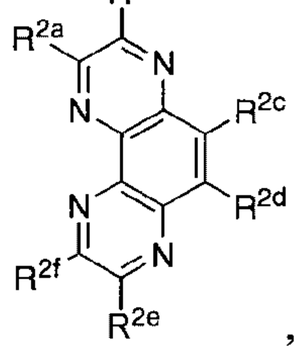
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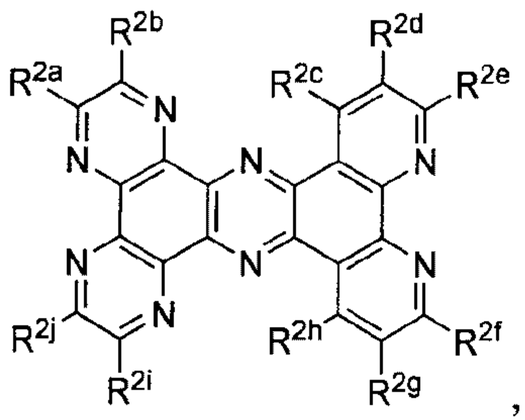
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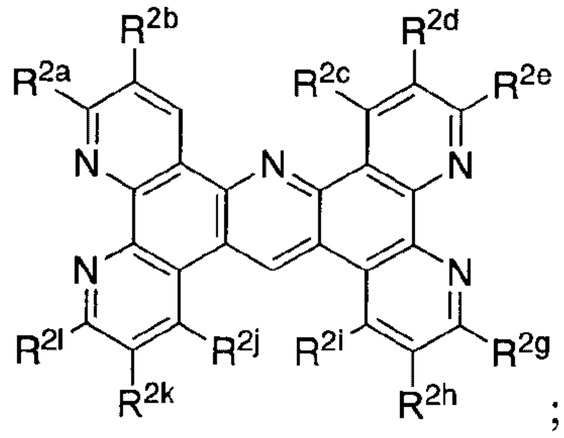
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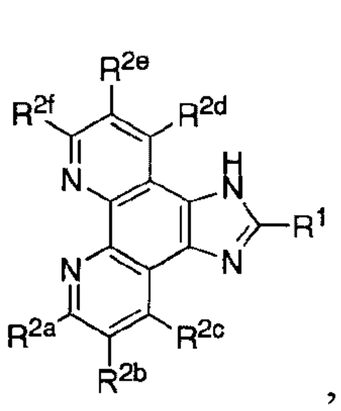
Lig^2 is a bidentate ligand that at each occurrence is independently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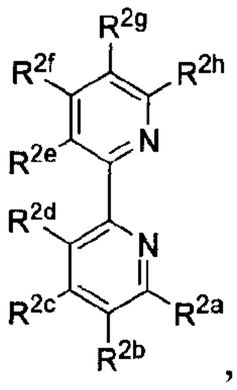
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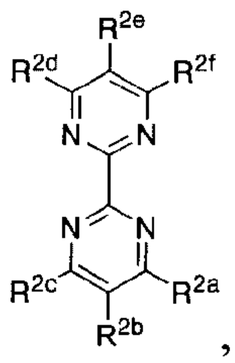
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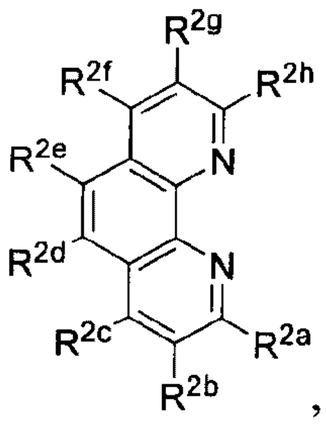
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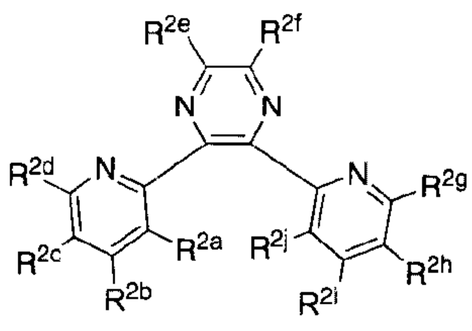
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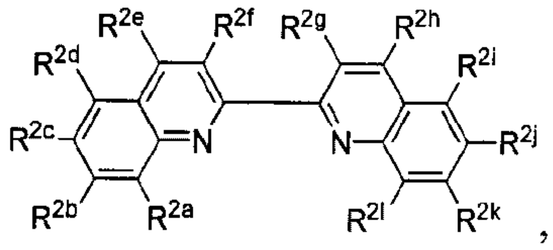
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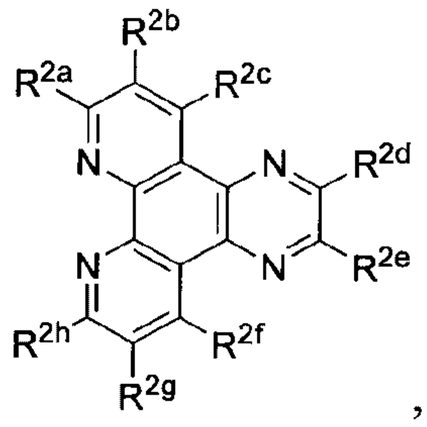
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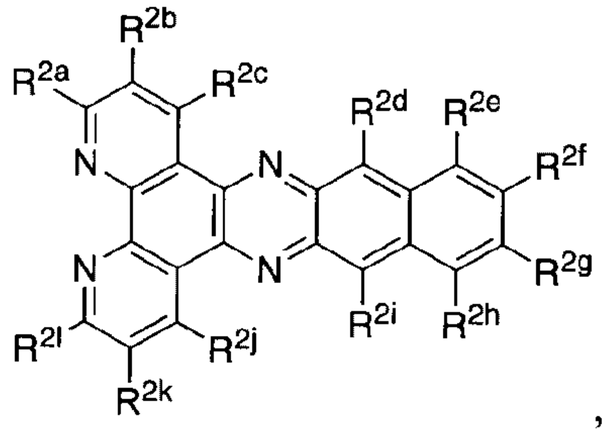
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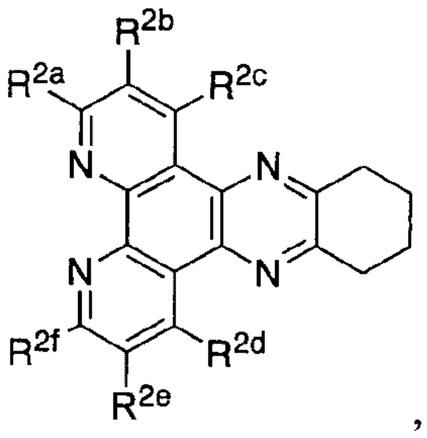
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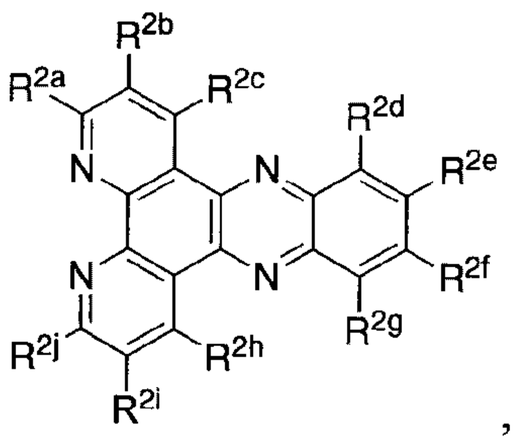
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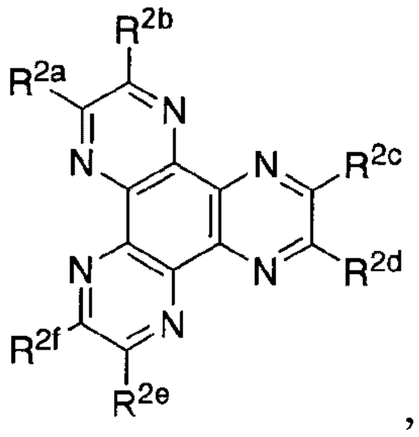
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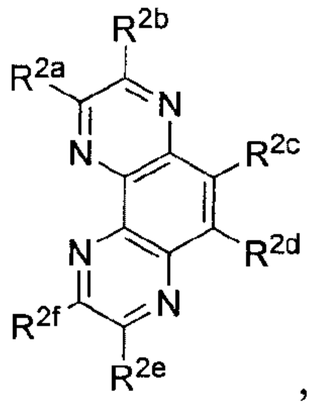
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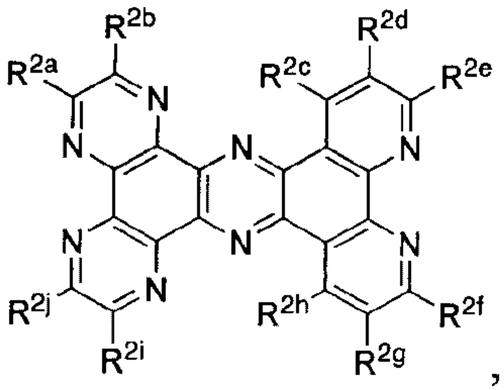
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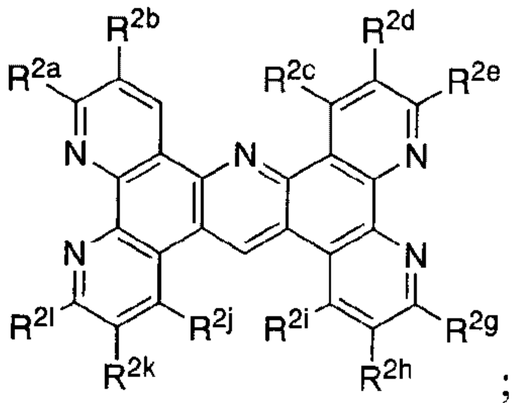
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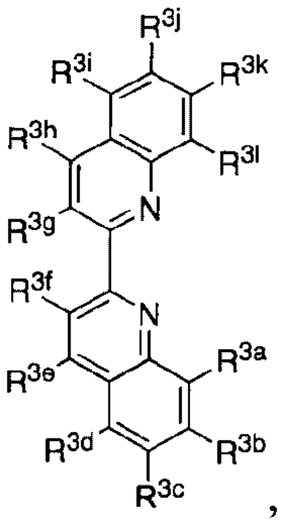
Lig^3 is a bidentate ligand that at each occurrence is independently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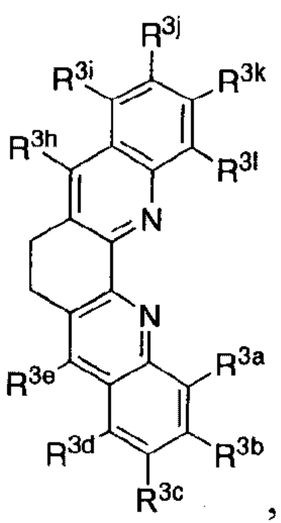
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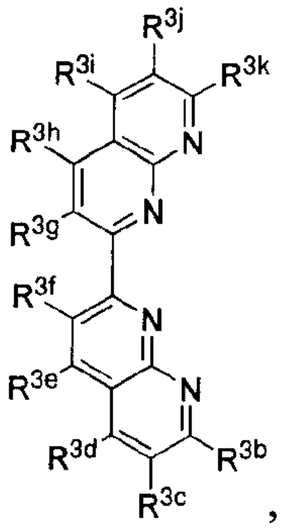
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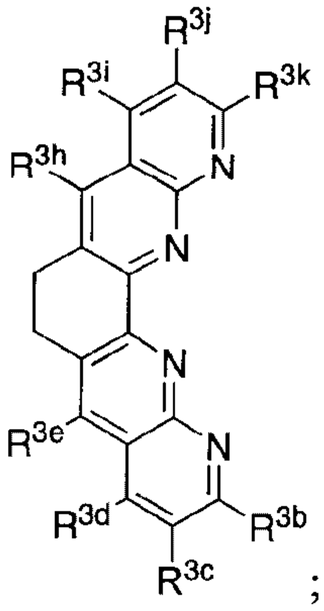
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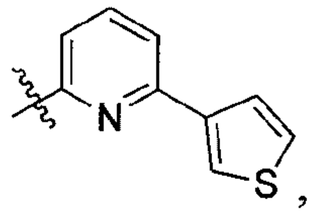
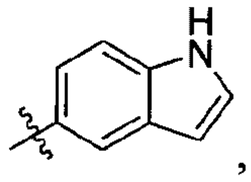
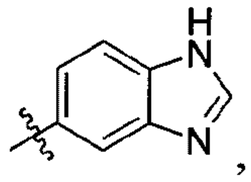
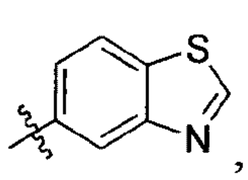
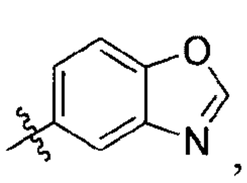
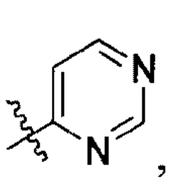
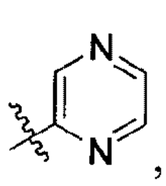
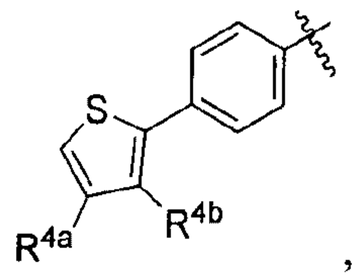
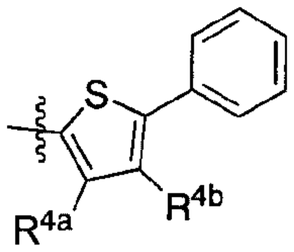
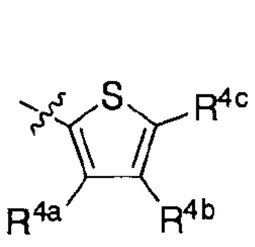
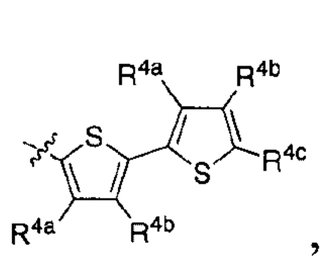
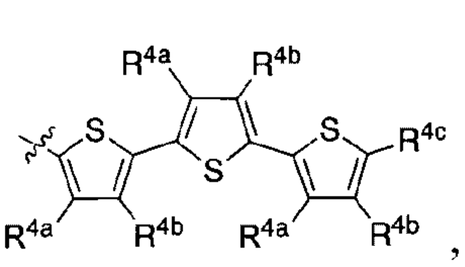
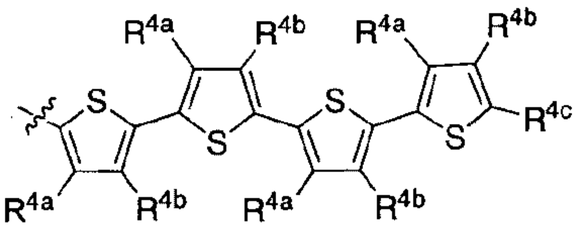
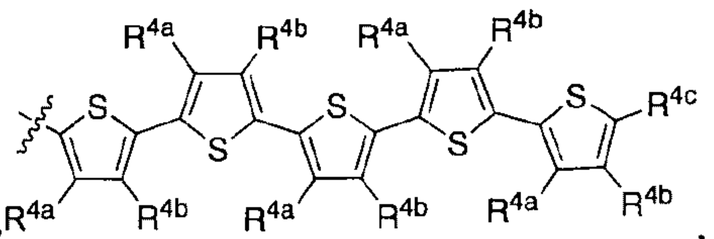
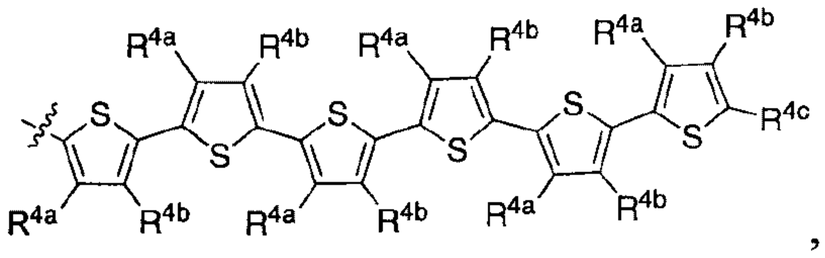
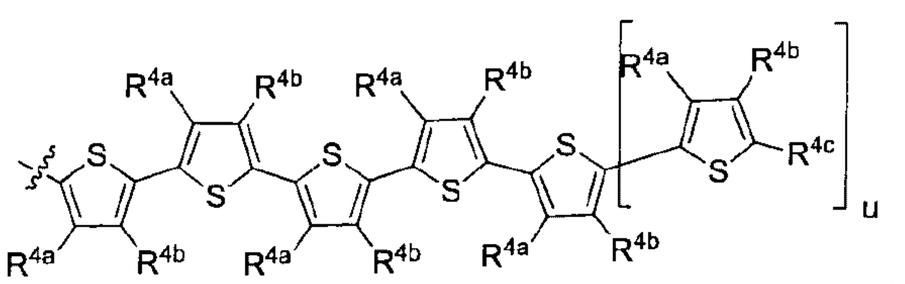
R^1 is hydrogen, optionally substituted phenyl, optionally substituted aryl, optionally

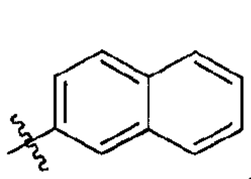
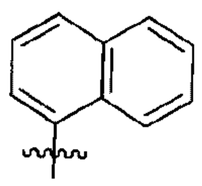
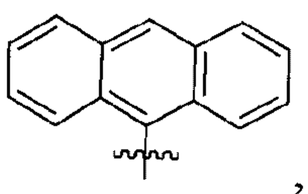
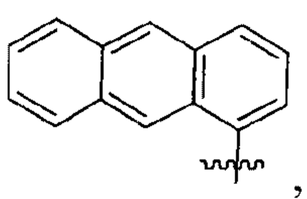
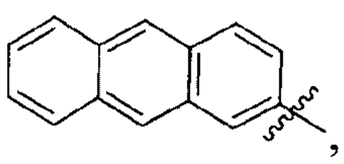
substituted heteroaryl, 4-pyridyl, 3-pyridyl, 2-thiazole, 2-pyrolyl, 2-furanyl,  ,

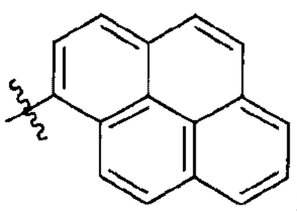
 ,  ,  ,  ,  ,  ,  ,

 ,  ,  ,  ,

 ,  ,

 ,  ,

 ,  ,  ,  ,  , or

 .

u is an integer;

R^{2a}, R^{2b}, R^{2c}, R^{2d}, R^{2e}, R^{2f}, R^{2g}, R^{2h}, R^{2i}, R^{2j}, R^{2k}, and R^{2l} at each occurrence are each independently hydrogen, C_{1-6} optionally substituted alkyl, C_{1-6} optionally substituted branched alkyl, C_{3-7} optionally substituted cycloalkyl, C_{1-6} optionally substituted haloalkyl, C_{1-6} optionally

substituted alkoxy, CO_2R^5, $CONR^6_2$, NR^7_2, SO_3H, sulfate, sulfonate, optionally substituted aryl, optionally substituted aryloxy, optionally substituted heteroaryl, or optionally substituted heterocycle;

R^{3a}, R^{3b}, R^{3c}, R^{3d}, R^{3e}, R^{3f}, R^{3g}, R^{3h} R^{3i}, R^{3j}, R^{3k}, and R^{3l} at each occurrence are each independently hydrogen, C_{1-6} optionally substituted alkyl, C_{1-6} optionally substituted branched alkyl, C_{1-6} optionally substituted haloalkyl, C_{1-6} optionally substituted alkoxy, optionally substituted phenyl, or CO_2R^8;

R^{4a}, R^{4b}, and R^{4c} at each occurrence are the same or different members selected from the group consisting of hydrogen, C_{1-6} optionally substituted alkyl, C_{1-6} optionally substituted branched alkyl, C_{1-6} optionally substituted cycloalkyl, C_{1-6} optionally substituted haloalkyl, C_{1-6} optionally substituted alkoxy, CO_2R^5, $CONR^6_2$, NR^7_2 , sulfate, sulfonate, optionally substituted aryl, optionally substituted aryloxy, optionally substituted heteroaryl, and optionally substituted heterocycle, wherein R^{4a} and R^{4b} at each occurrence on a thiophene ring are optionally taken together with the atom to which they are bound to form an optionally substituted ring having from 6 ring atoms containing 2 oxygen atoms;

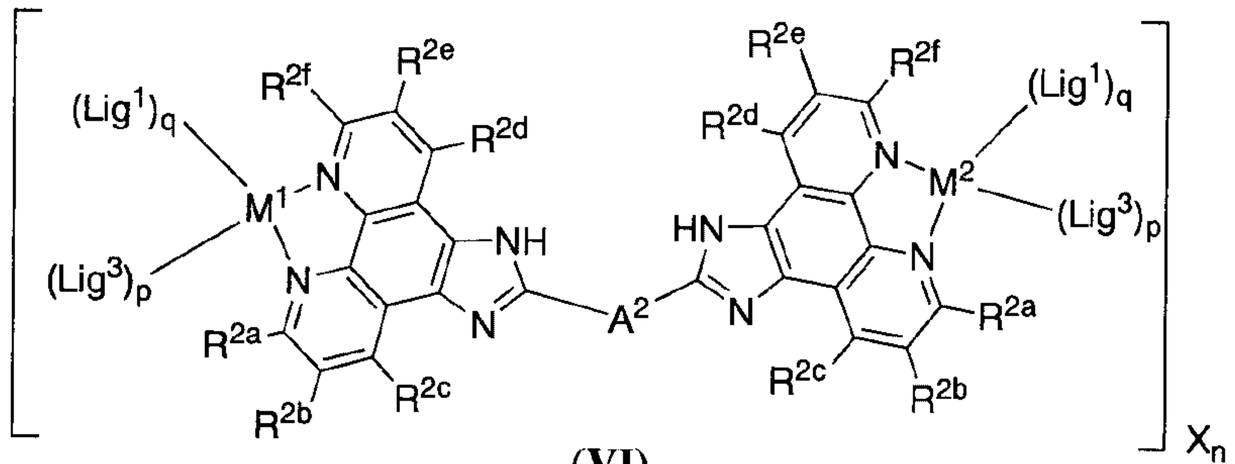
R^5 at each occurrence are each independently hydrogen or optionally substituted alkyl;

R^6 at each occurrence are each independently hydrogen or optionally substituted alkyl;

R^7 at each occurrence are each independently hydrogen or optionally substituted alkyl; and

R^8 at each occurrence are each independently hydrogen or optionally substituted alkyl;

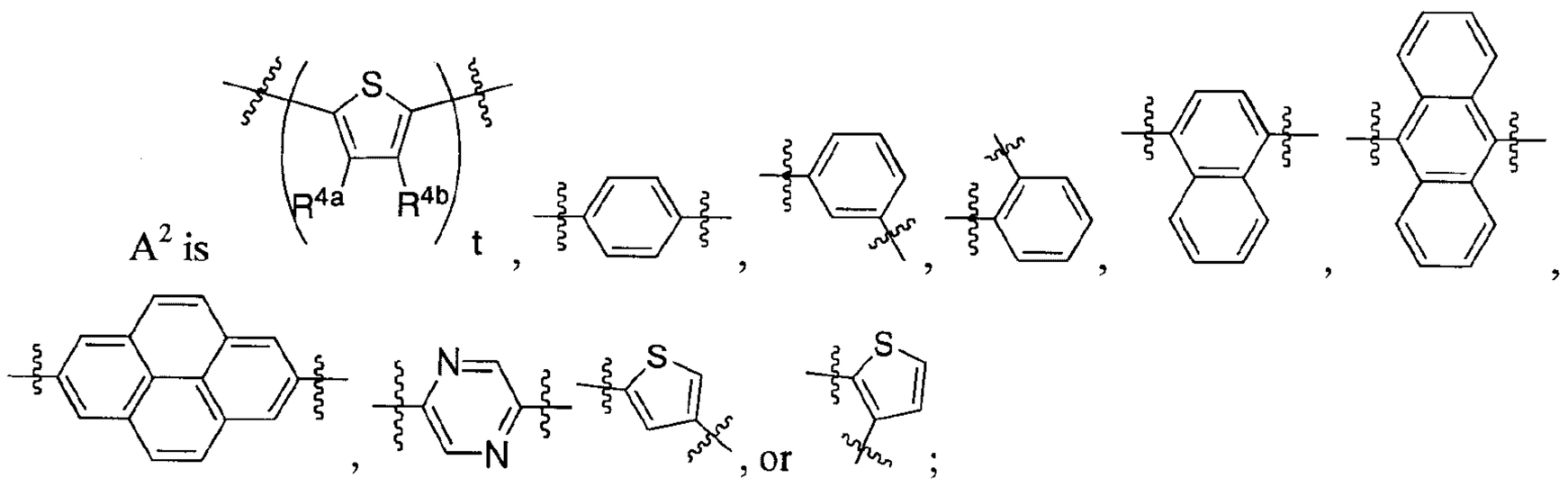
b) formula (VI):



(VI)

including hydrates, solvates, and pharmaceutically acceptable salts thereof wherein;

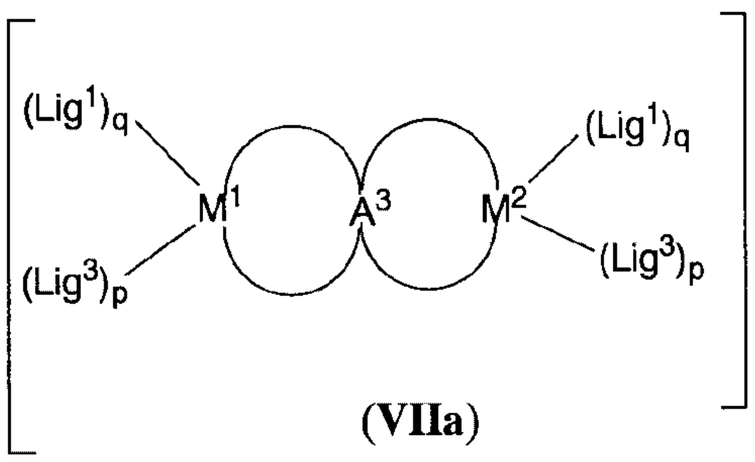
M^1 and M^2 at each occurrence is independently osmium, manganese, molybdenum, rhenium, ruthenium, iron, cobalt, rhodium, iridium, nickel, platinum, or copper;

A^2 is  ;

t is an integer; and

R^{2a}, R^{2b}, R^{2c}, R^{2d}, R^{2e}, R^{2f}, Lig^1, Lig^3, X, n, p and q are as defined above with respect to formula (I);

c) formula (VIIa)

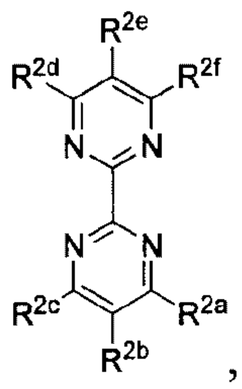
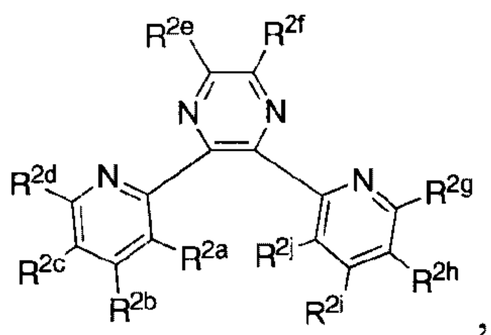
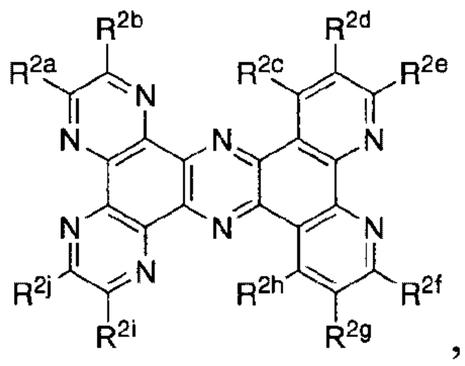
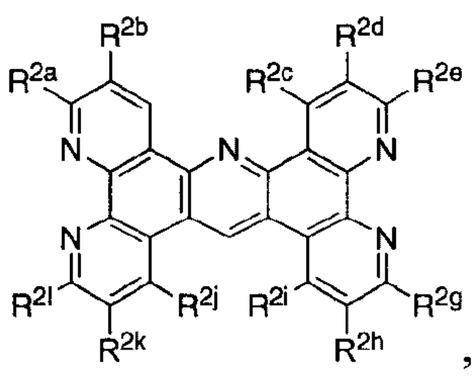
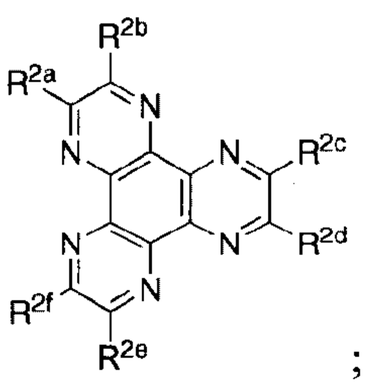


(VIIa)

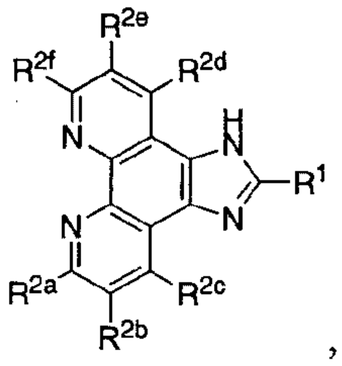
including hydrates, solvates, and pharmaceutically acceptable salts thereof wherein:

M^1 and M^2 at each occurrence is independently osmium, manganese, molybdenum, rhenium, ruthenium, iron, cobalt, rhodium, iridium, nickel, platinum, or copper;

X is Cl^-, PF_6^-, Br^-, BF_4^-, ClO_4^-, $CF_3SO_3^-$, or SO_4^{-2};

A^3 is  ,  ,  ,  , or  ;

Lig^1 is a bidentate ligand that at each occurrence is each independently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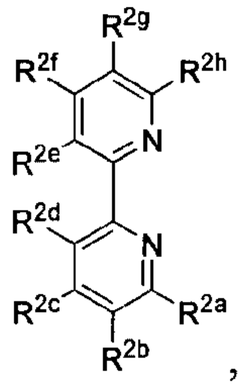
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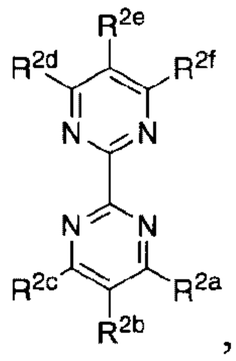
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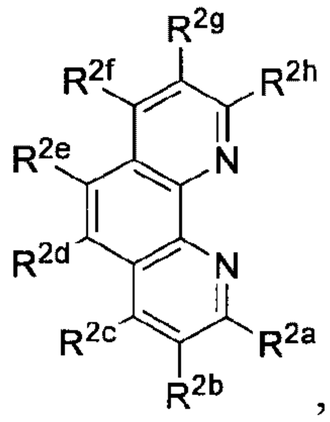
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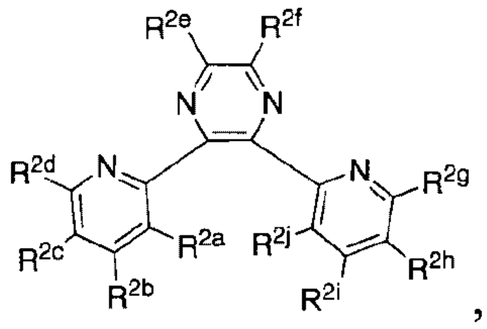
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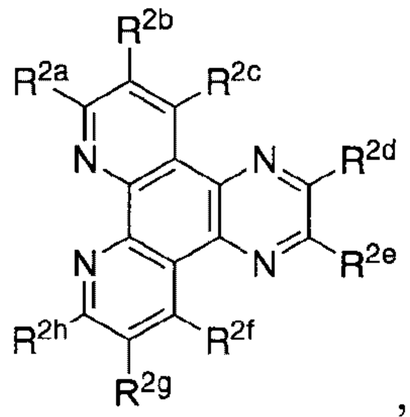
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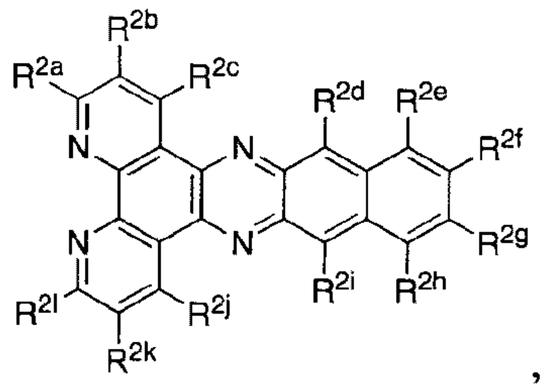
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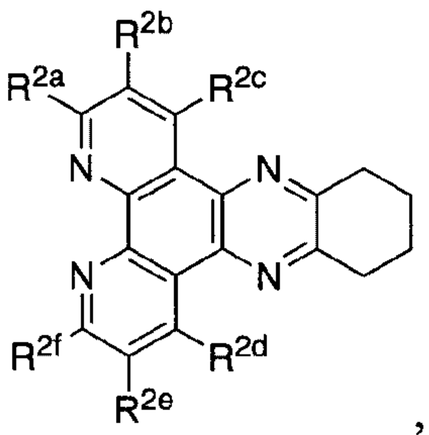
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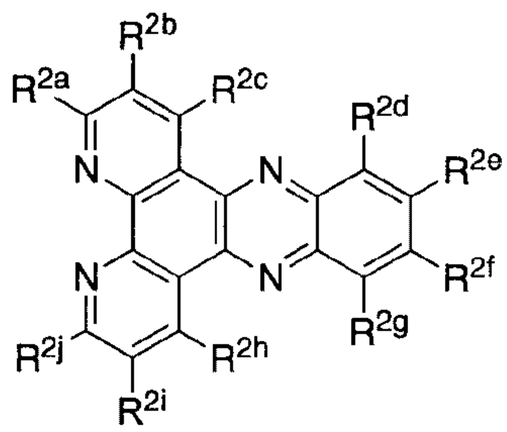
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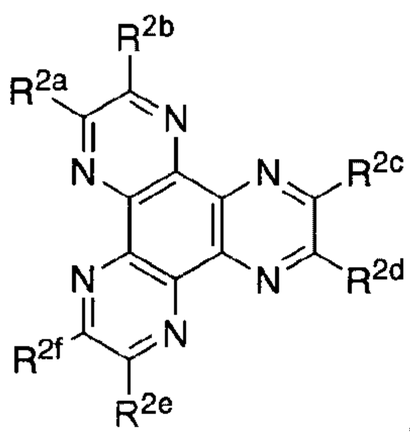
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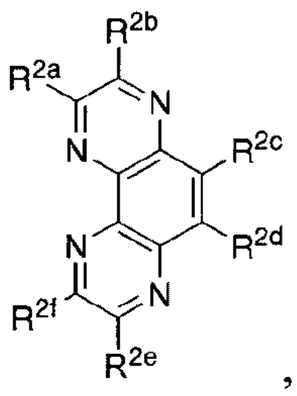
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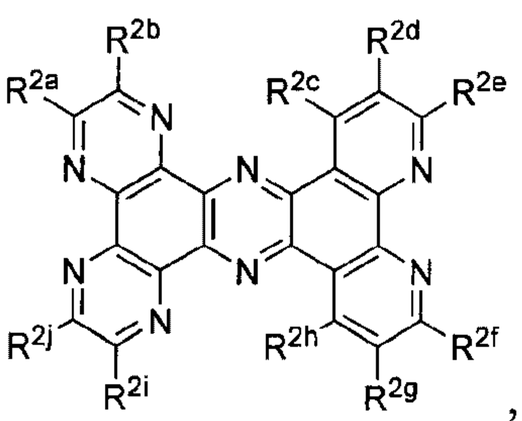
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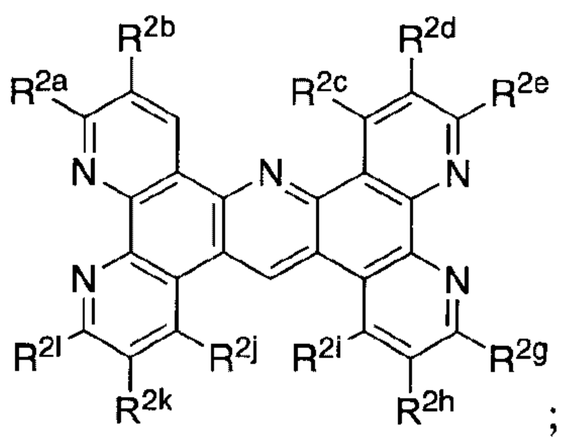
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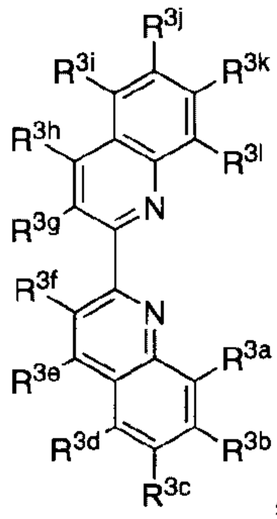
Lig^3 is a bidentate ligand that at each occurrence is each independently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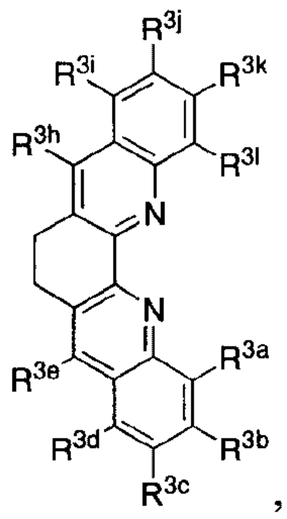
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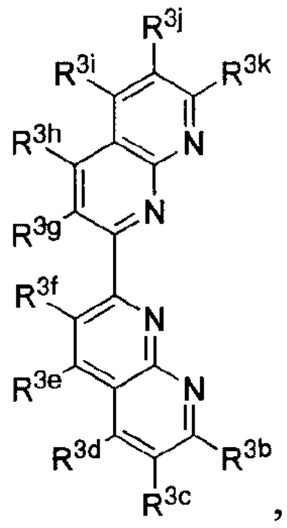
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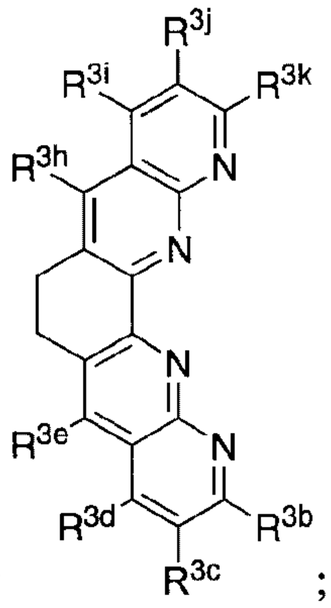
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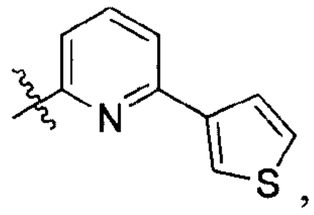
R^1 is hydrogen, optionally substituted phenyl, optionally substituted aryl, optionally substituted heteroaryl, 4-pyridyl, 3-pyridyl, 2-thiazole, 2-pyrolyl, 2-furanyl, 
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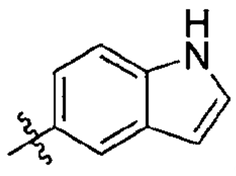
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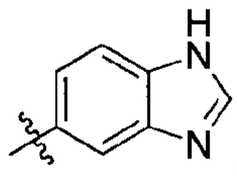
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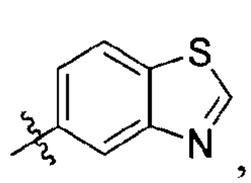
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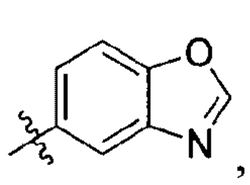
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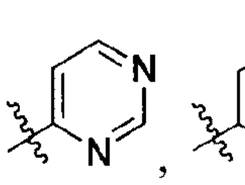
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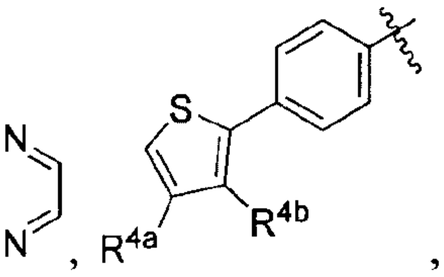
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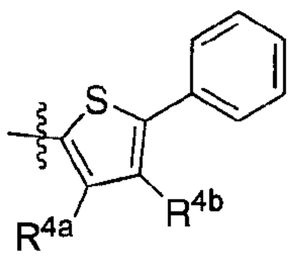
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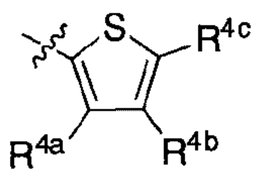
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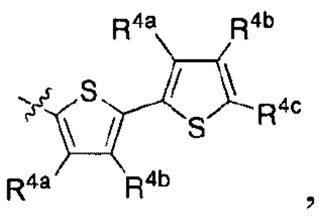
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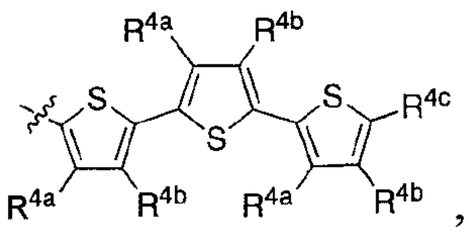
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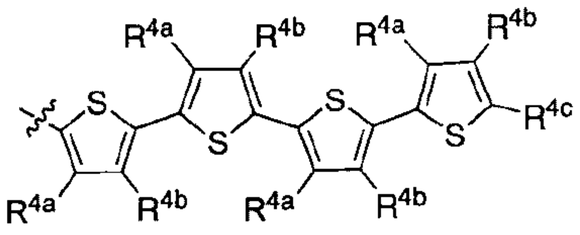
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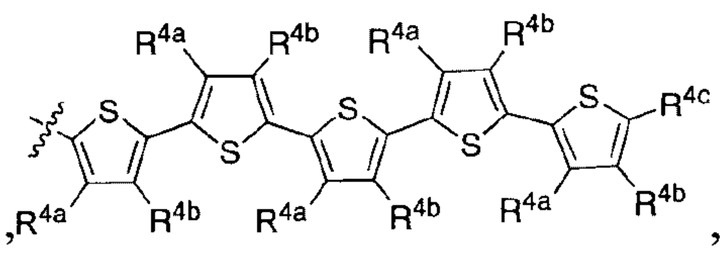
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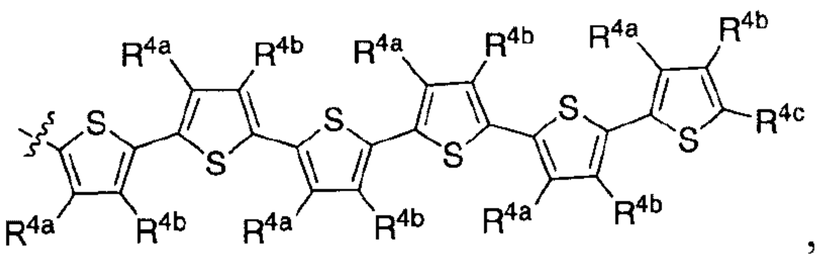
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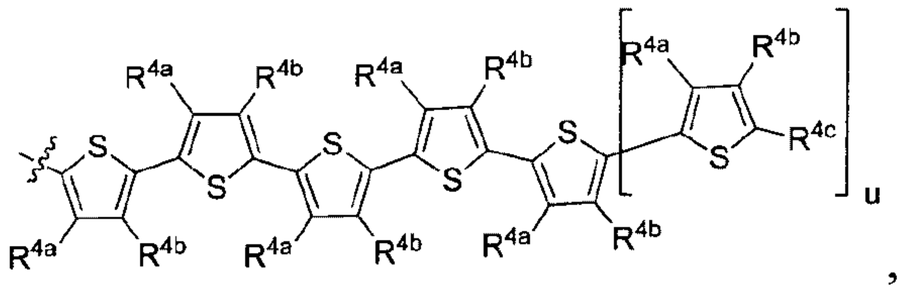
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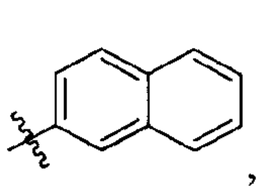
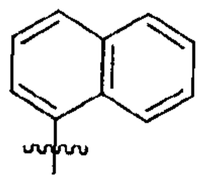
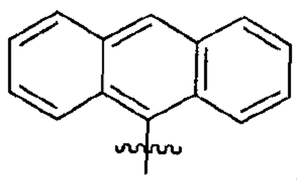
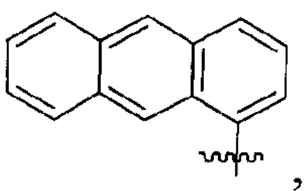
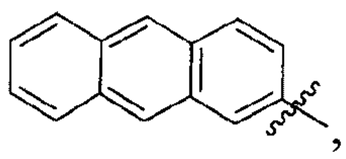
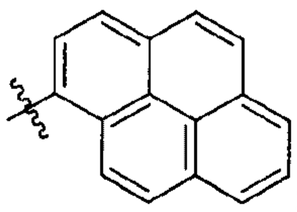
 ,  ,  ,  ,  , or  ;

u is an integer;

R^{2a}, R^{2b}, R^{2c}, R^{2d}, R^{2e}, R^{2f}, R^{2g}, R^{2h}, R^{2i}, R^{2j}, R^{2k}, and R^{2l} at each occurrence are each independently hydrogen, C_{1-6} optionally substituted alkyl, C_{1-6} optionally substituted branched alkyl, C_{3-7} optionally substituted cycloalkyl, C_{1-6} optionally substituted haloalkyl, C_{1-6} optionally substituted alkoxy, CO_2R^5, $CONR^6_2$, NR^7_2, SO_3H, sulfate, sulfonate, optionally substituted aryl, optionally substituted aryloxy, optionally substituted heteroaryl, or optionally substituted heterocycle;

R^{3a}, R^{3b}, R^{3c}, R^{3d}, R^{3e}, R^{3f}, R^{3g}, R^{3h} R^{3i}, R^{3j}, R^{3k}, and R^{3l} at each occurrence are each independently hydrogen, C_{1-6} optionally substituted alkyl, C_{1-6} optionally substituted branched alkyl, C_{1-6} optionally substituted haloalkyl, C_{1-6} optionally substituted alkoxy, optionally substituted phenyl, or CO_2R^8;

R^{4a}, R^{4b}, and R^{4c} at each occurrence are the same or different members selected from the group consisting of hydrogen, C_{1-6} optionally substituted alkyl, C_{1-6} optionally substituted branched alkyl, C_{1-6} optionally substituted cycloalkyl, C_{1-6} optionally substituted haloalkyl, C_{1-6} optionally substituted alkoxy, CO_2R^5, $CONR^6_2$, NR^7_2 , sulfate, sulfonate, optionally substituted aryl, optionally substituted aryloxy, optionally substituted heteroaryl, and optionally substituted heterocycle, wherein R^{4a} and R^{4b} at each occurrence on a thiophene ring are optionally taken together with the atom to which they are bound to form an optionally substituted ring having from 6 ring atoms containing 2 oxygen atoms;

R^5 at each occurrence are each independently hydrogen or optionally substituted alkyl;

R^6 at each occurrence are each independently hydrogen or optionally substituted alkyl;

R^7 at each occurrence are each independently hydrogen or optionally substituted alkyl; and

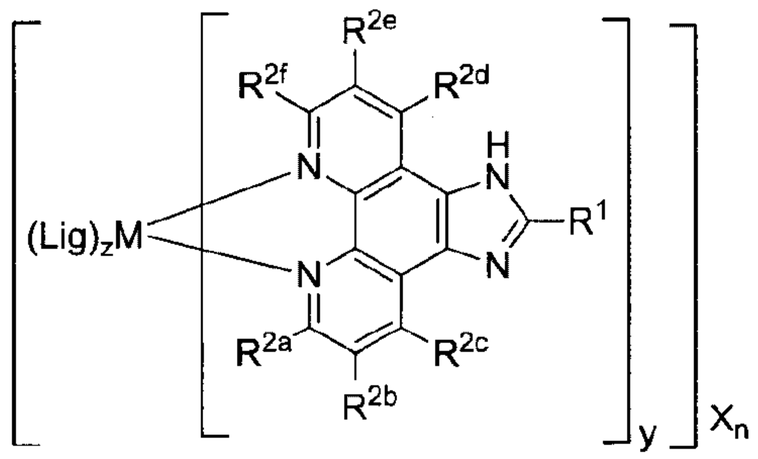
R^8 at each occurrence are each independently hydrogen or optionally substituted alkyl

p at each occurrence is the same or different and is 0, 1, or 2;

q at each occurrence is the same or different and is 0, 1, or 2; and

n is 0, 1, 2, 3, 4, or 5; or

d) formula (II)



(II)

including hydrates, solvates, and pharmaceutically acceptable salts thereof, wherein:

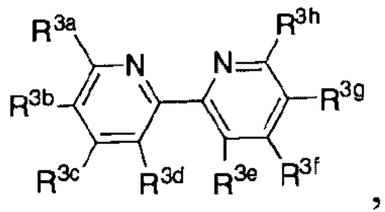
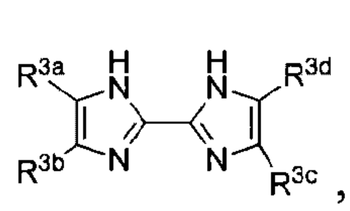
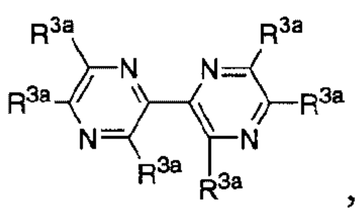
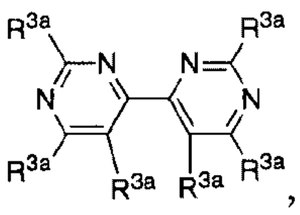
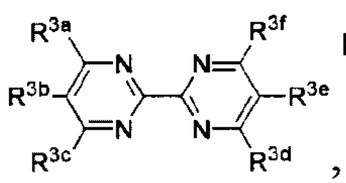
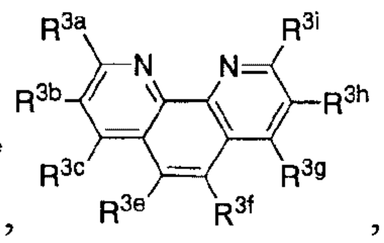
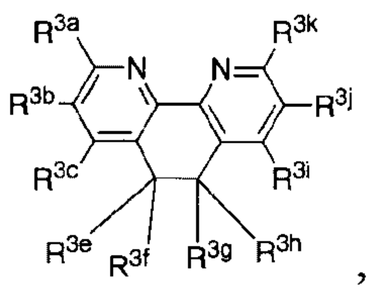
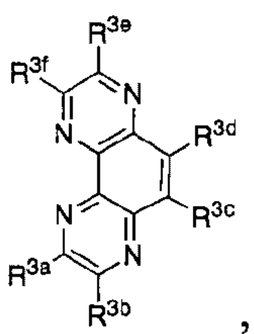
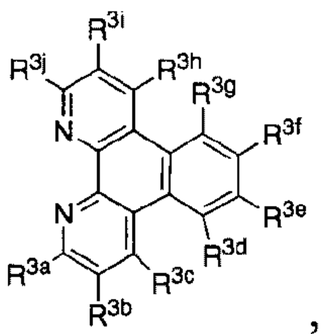
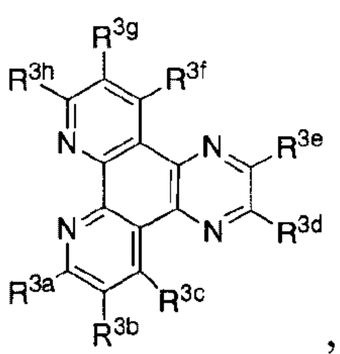
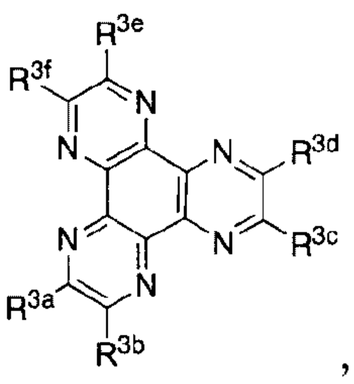
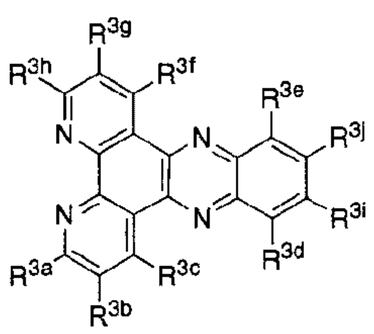
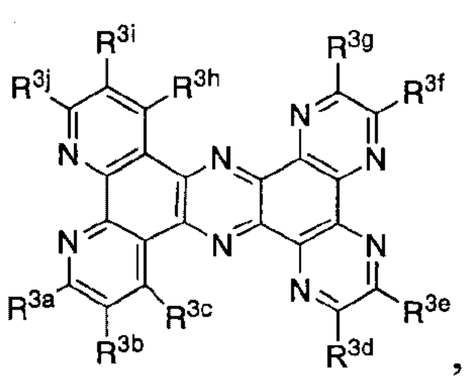
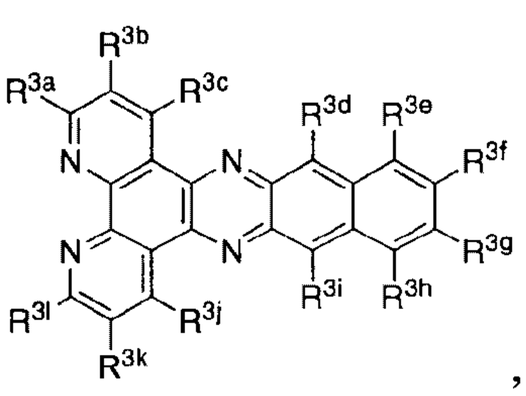
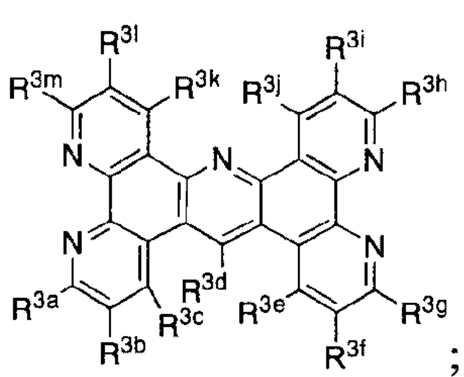
M is manganese, molybdenum, rhenium, iron, ruthenium, osmium, cobalt, rhodium, iridium, nickel, platinum, or copper;

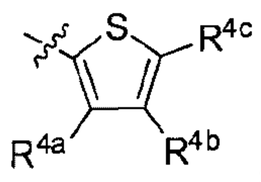
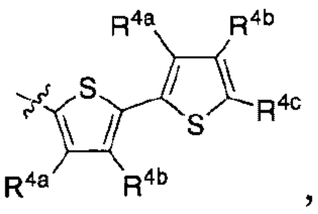
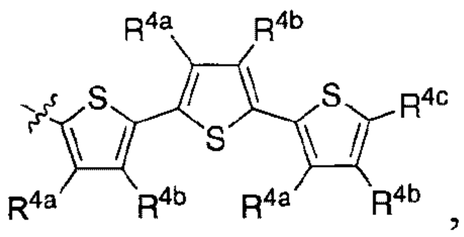
X is Cl^-, PF_6^-, Br^-, BF_4^-, ClO_4^-, $CF_3SO_3^-$, or SO_4^{-2};

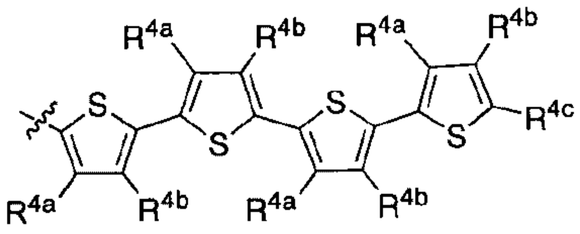
n = 0, 1, 2, 3, 4, or 5;

y = 1, 2, or 3;

z = 0, 1, or 2;

Lig at each occurrence is independently  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  , or  ;

R^1 is  ,  ,  ,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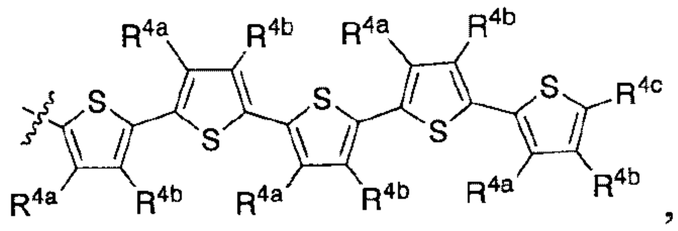
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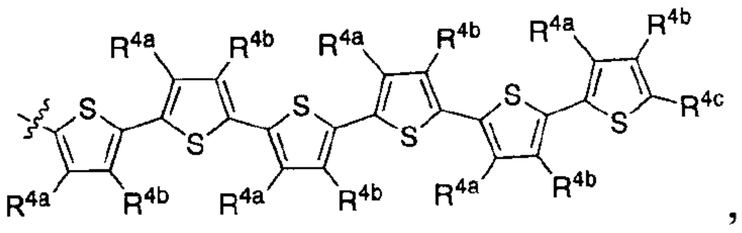
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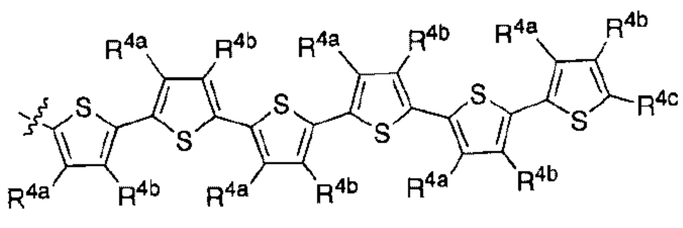
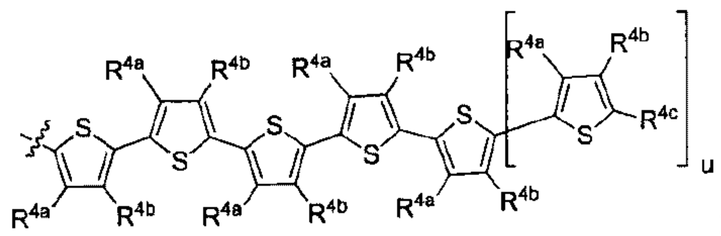

, or

.

u is an integer;

R^{2a}, R^{2b}, R^{2c}, R^{2d}, R^{2e}, and R^{2f} at each occurrence are each independently hydrogen, C1-6 optionally substituted alkyl, C1-6 optionally substituted branched alkyl, C3-7 optionally substituted cycloalkyl, C1-6 optionally substituted haloalkyl, C1-6 optionally substituted alkoxy, CO_2R^5, $CONR^6_2$, NR^7_2 , sulfate, sulfonate, optionally substituted aryl, optionally substituted aryloxy, optionally substituted heteroaryl, or optionally substituted heterocycle;

R^{3a}, R^{3b}, R^{3c}, R^{3d}, R^{3e}, R^{3f}, R^{3g}, R^{3h} R^{3i}, R^{3j}, R^{3k}, R^{3l}, and R^{3m} at each occurrence are each independently hydrogen, C1-6 optionally substituted alkyl, C1-6 optionally substituted branched alkyl, C1-6 optionally substituted haloalkyl, C1-6 optionally substituted alkoxy, or CO_2R^8;

R^{4a}, R^{4b}, and R^{4c} at each occurrence are the same or different members selected from the group consisting of hydrogen, C1-6 optionally substituted alkyl, C1-6 optionally substituted branched alkyl, C1-6 optionally substituted cycloalkyl, C1-6 optionally substituted haloalkyl, C1-6 optionally substituted alkoxy, CO_2R^5, $CONR^6_2$, NR^7_2 , sulfate, sulfonate, optionally substituted aryl, optionally substituted aryloxy, optionally substituted heteroaryl, and optionally substituted heterocycle, wherein R^{4a} and R^{4b} at each occurrence on a thiophene ring are optionally taken together with the atom to which they are bound to form an optionally substituted

ring having from 6 ring atoms containing 2 oxygen atoms;

R^5 at each occurrence is independently hydrogen or optionally substituted alkyl;

R^6 at each occurrence is independently hydrogen or optionally substituted alkyl;

R^7 at each occurrence is independently hydrogen or optionally substituted alkyl; and

R^8 at each occurrence is independently hydrogen or optionally substituted alkyl,

wherein the composition has at least one of the following enhanced properties relative to the chemotherapeutic compound without the glycoprotein: (a) increased uptake by cancer cells; (b) increased uptake by tumors; (c) increased efficacy at wavelengths longer than 600 nm; (d) increased efficacy at wavelengths less than or equal to 600 nm; (e) improved absorbance at wavelengths longer than 600 nm; (f) improved absorbance at wavelengths less than or equal to 600 nm; (g) increased production of reactive oxygen species; (h) increased photodynamic therapy effect under non-hypoxic conditions; (i) increased photodynamic therapy effect under hypoxic conditions; (j) increased LD50; (k) increased maximum tolerated dose; (l) increased photostability; and (m) increased shelf-life.

2. The composition of claim 1, wherein the metal-binding glycoprotein is transferrin and the chemotherapeutic compound has the formula (I).

3. The composition of claim 1, wherein the metal-binding glycoprotein is transferrin and the chemotherapeutic compound has the formula (VI).

4. The composition of claim 1, wherein the metal-binding glycoprotein is transferrin and the chemotherapeutic compound has the formula (VIIa).

5. The composition of claim 1, wherein the metal-binding glycoprotein is transferrin and the

chemotherapeutic compound has the formula (II).

6. The composition of any one of claims 1, 2 or 5, wherein each of M, M^1 and M^2 is independently Ru, Rh or Os.

7. The composition of any one of claims 1 to 6, wherein the metal-binding glycoprotein is a recombinant human transferrin.

8. A use of the composition according to any one of claims 1 to 7 in the treatment of a disease associated with hyperproliferating cells.

9. A method for destroying a microbial cell in vitro, said method comprising:

contacting the microbial cell with an effective amount of the composition according to any one of claims 1 to 7; and

irradiating the microbial cell with light effective to activate the composition so as to destroy the microbial cell.

10. A method for destroying a microbial cell in vitro, said method comprising contacting the microbial cell with an effective amount of the composition according to any one of claims 1 to 7 to destroy the microbial cell.

11. The composition of any one of claims 1, 3 or 4, wherein each of M^1 and M^2 is independently Ru, Rh or Os.

FIG. 1A

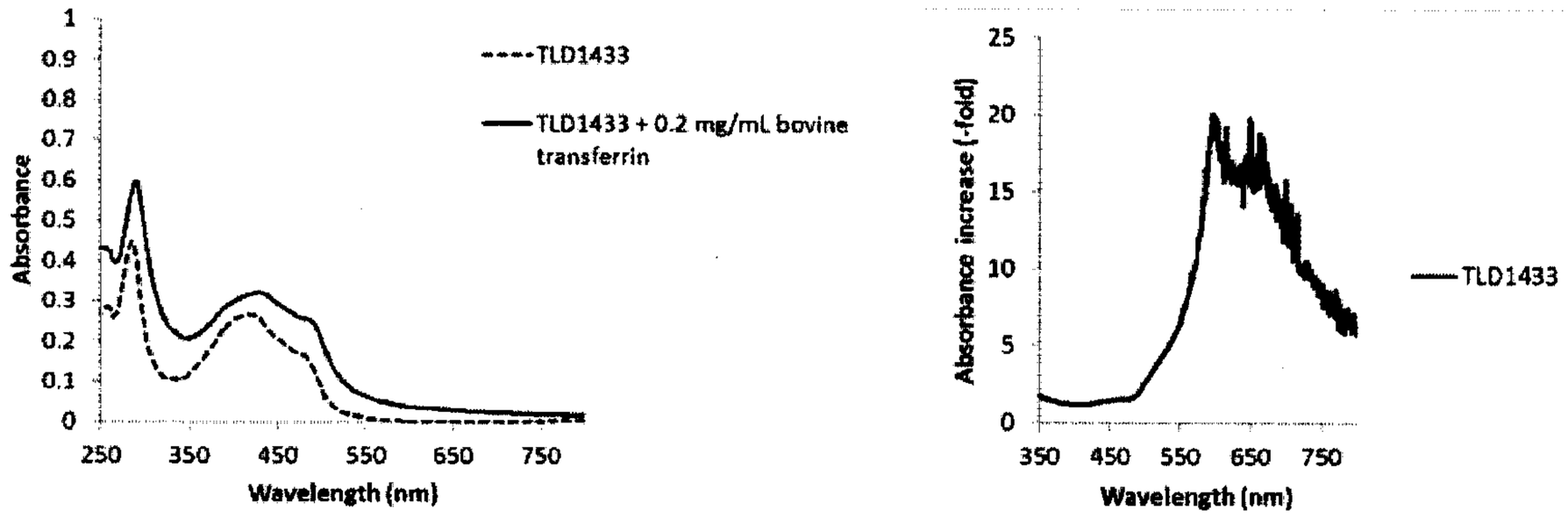


FIG. 1B

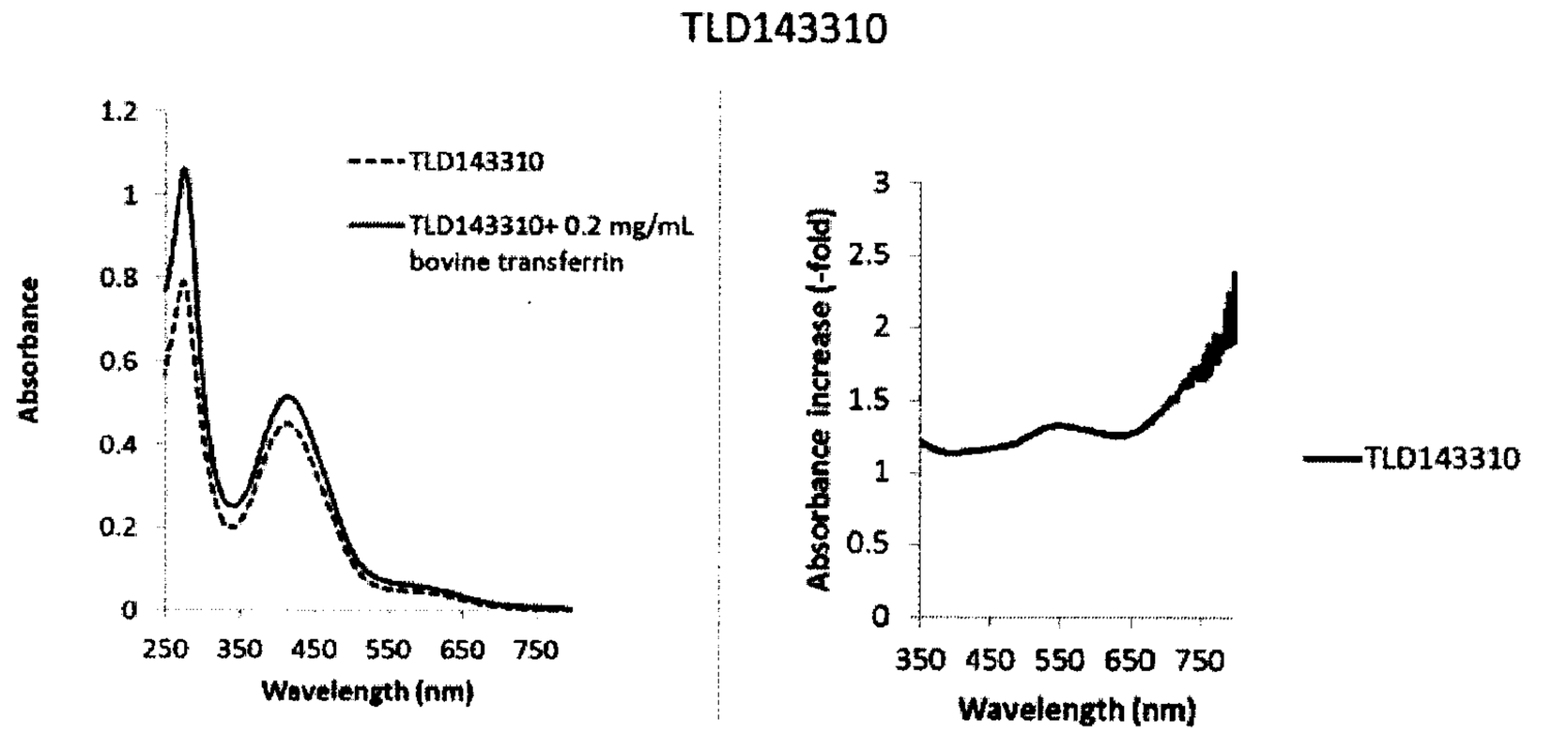


FIG. 1C

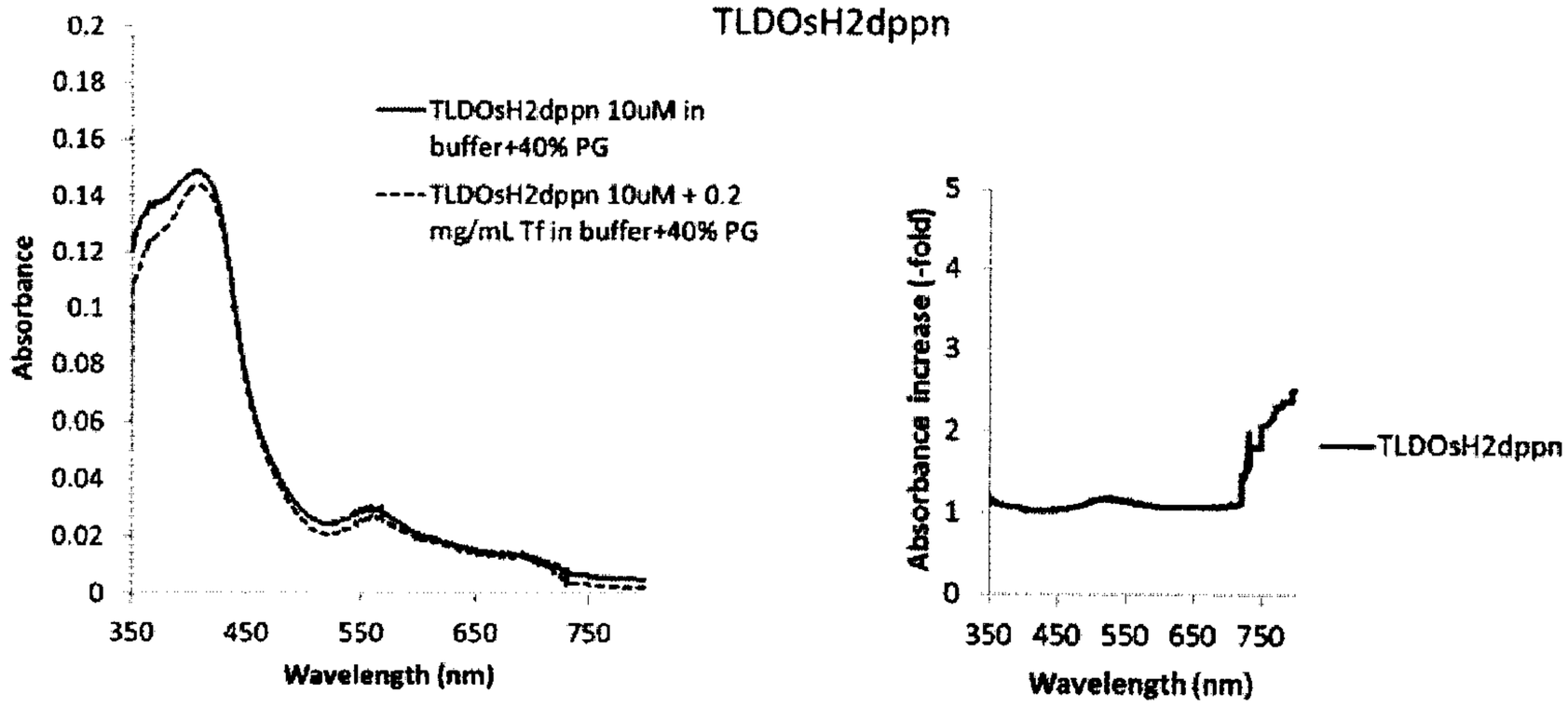


FIG. 2A

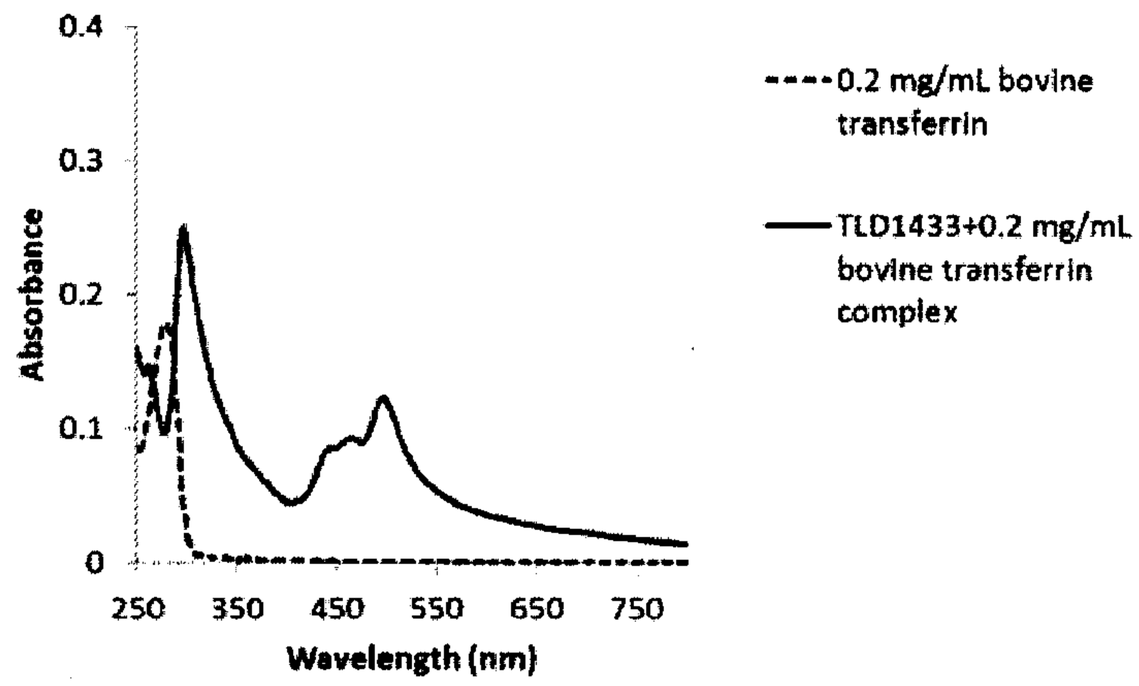


FIG. 2B

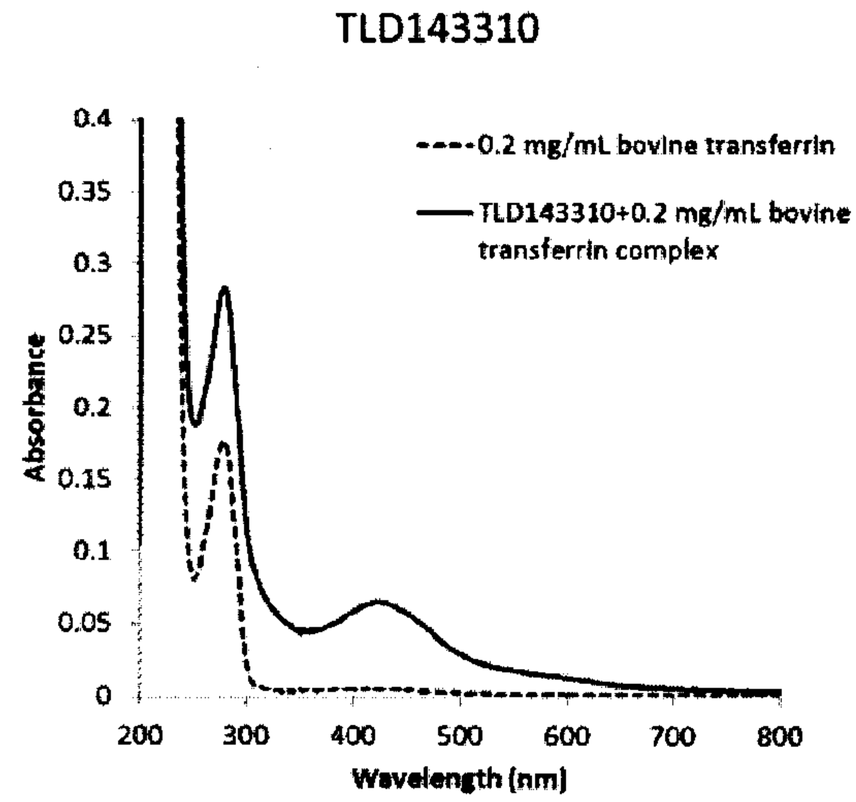


FIG. 2C

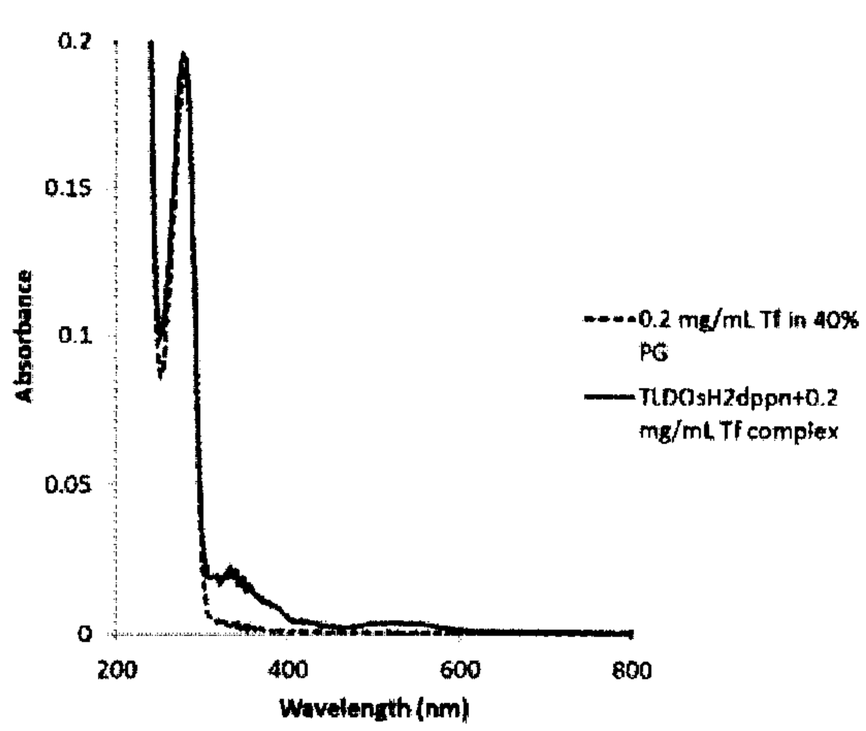


FIG. 3A

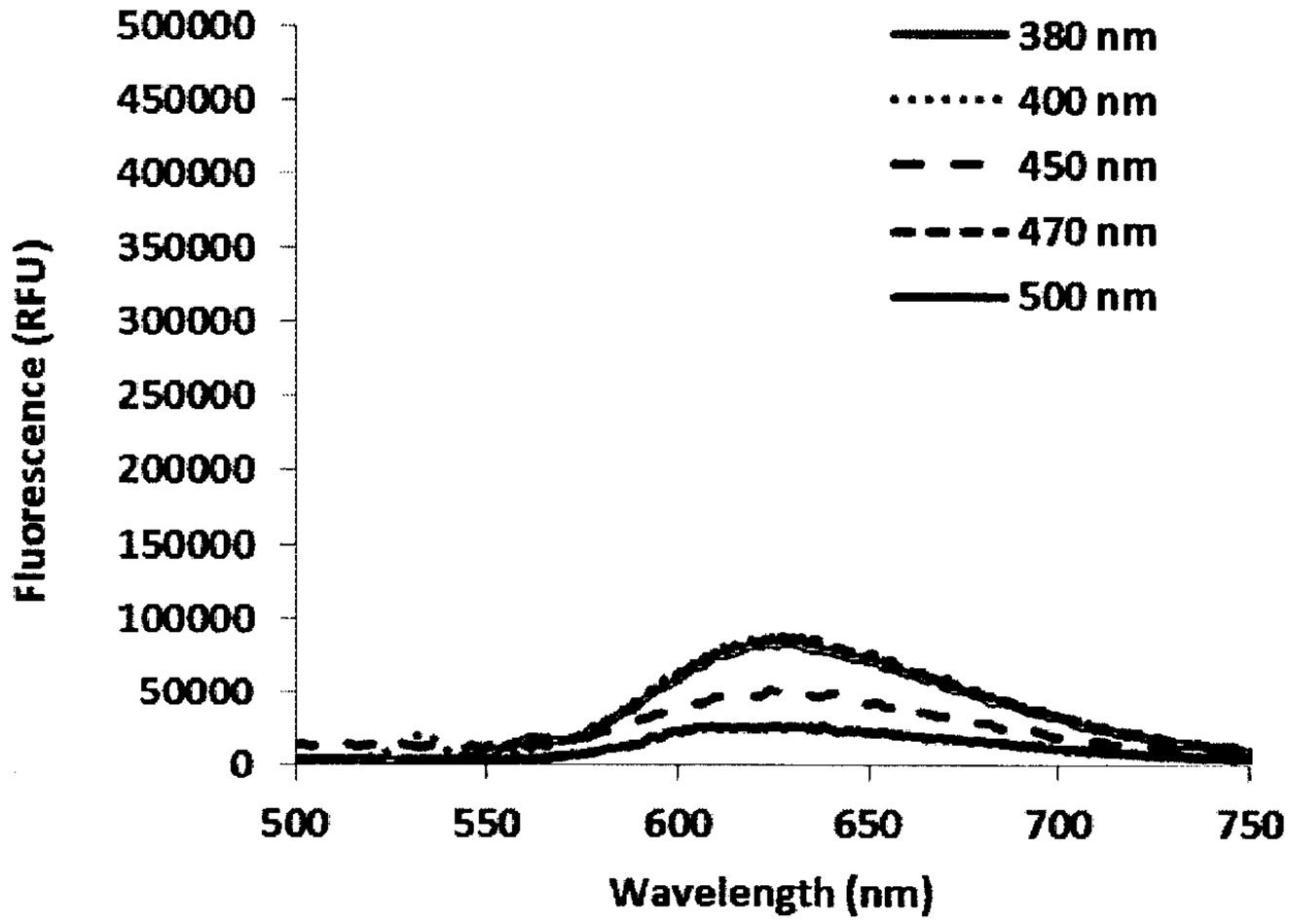

500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
Fluorescence (RFU)
500
550
600
650
700
750
Wavelength (nm)
380 nm
400 nm
450 nm
470 nm
500 nm

FIG. 3B

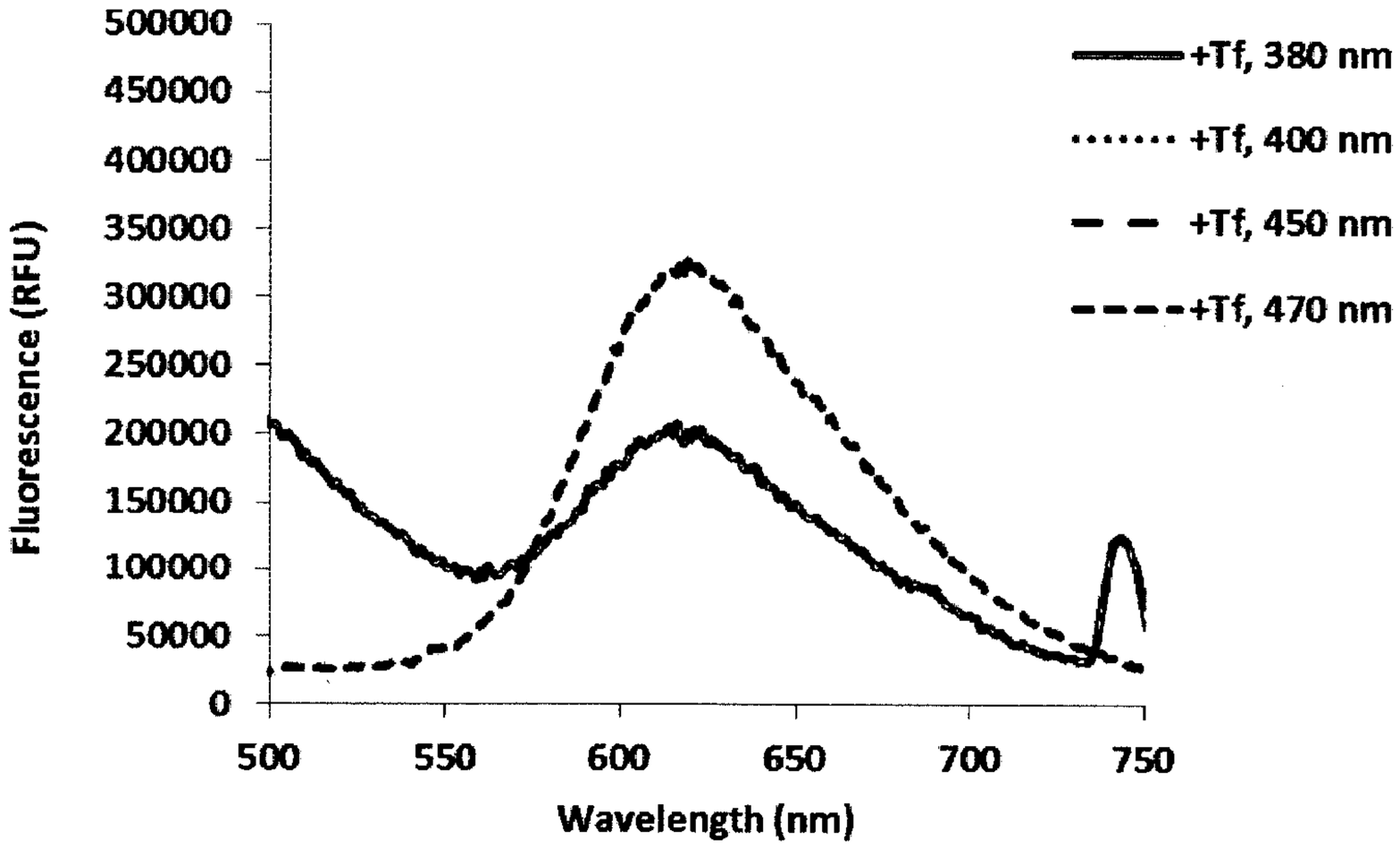

500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
Fluorescence (RFU)
500
550
600
650
700
750
Wavelength (nm)
+Tf, 380 nm
+Tf, 400 nm
+Tf, 450 nm
+Tf, 470 nm

FIG. 4A

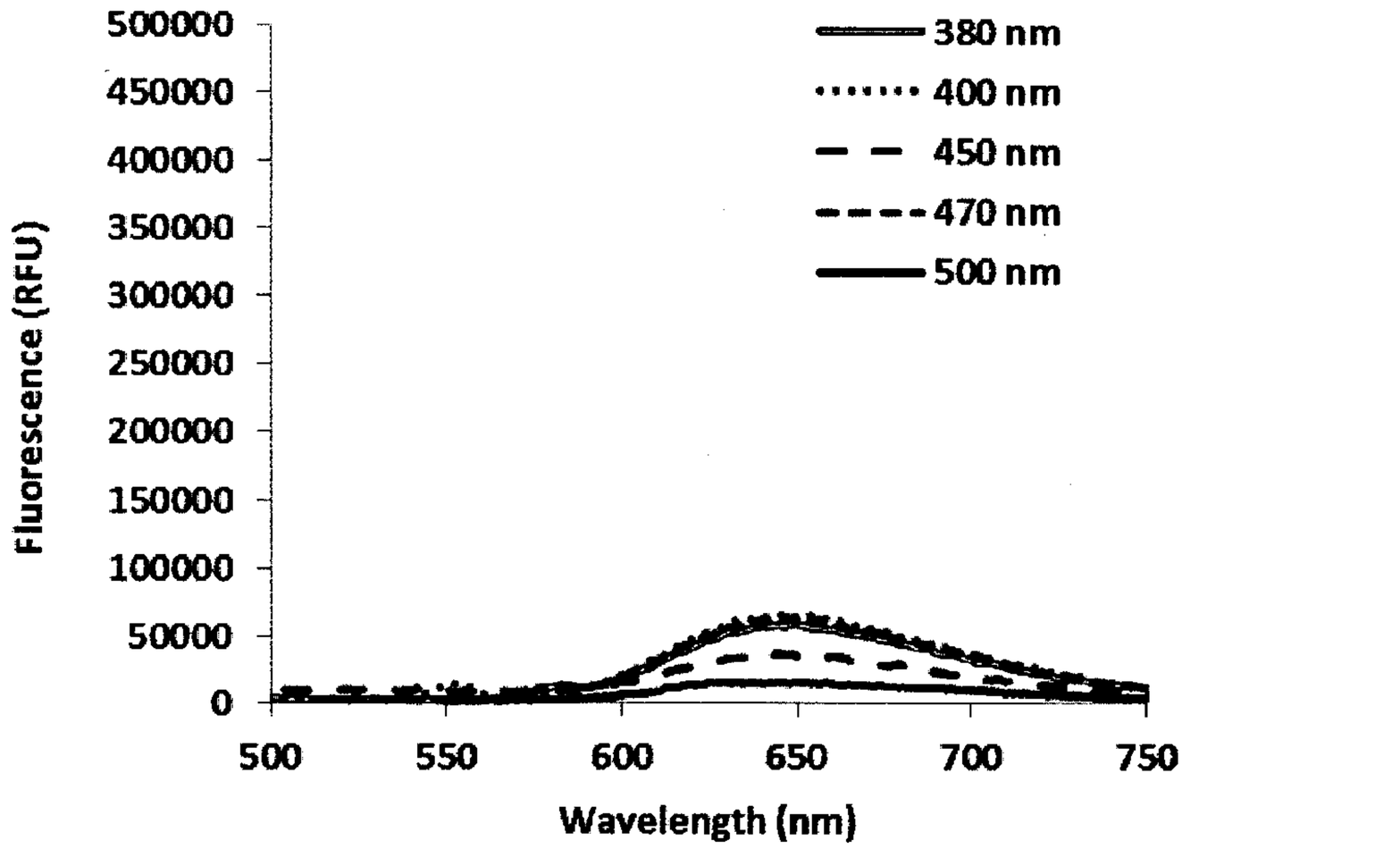

380 nm
400 nm
450 nm
470 nm
500 nm
Fluorescence (RFU)
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
500
550
600
650
700
750
Wavelength (nm)

FIG. 4B

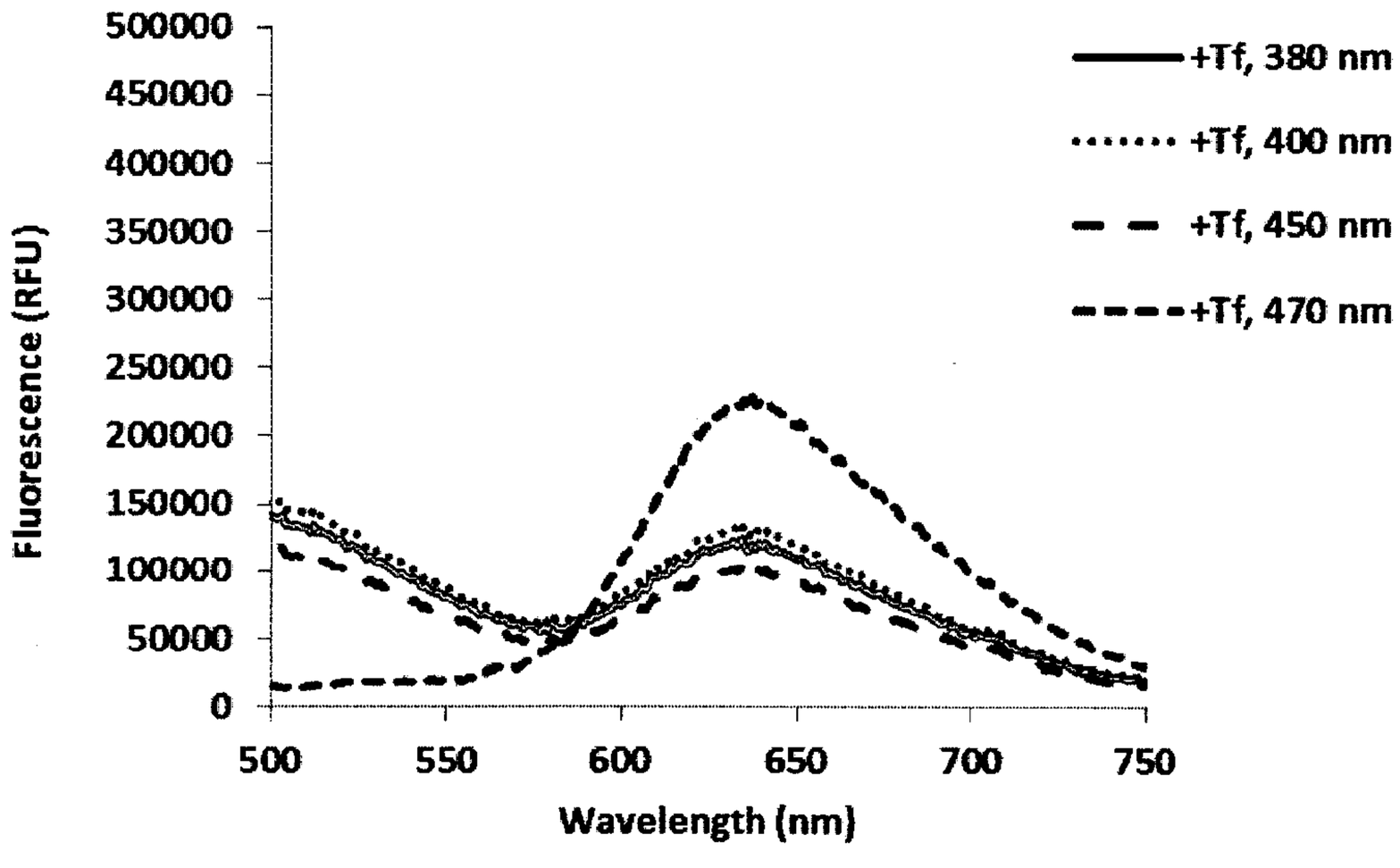

+Tf, 380 nm
+Tf, 400 nm
+Tf, 450 nm
+Tf, 470 nm
Fluorescence (RFU)
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
500
550
600
650
700
750
Wavelength (nm)

FIG. 5A

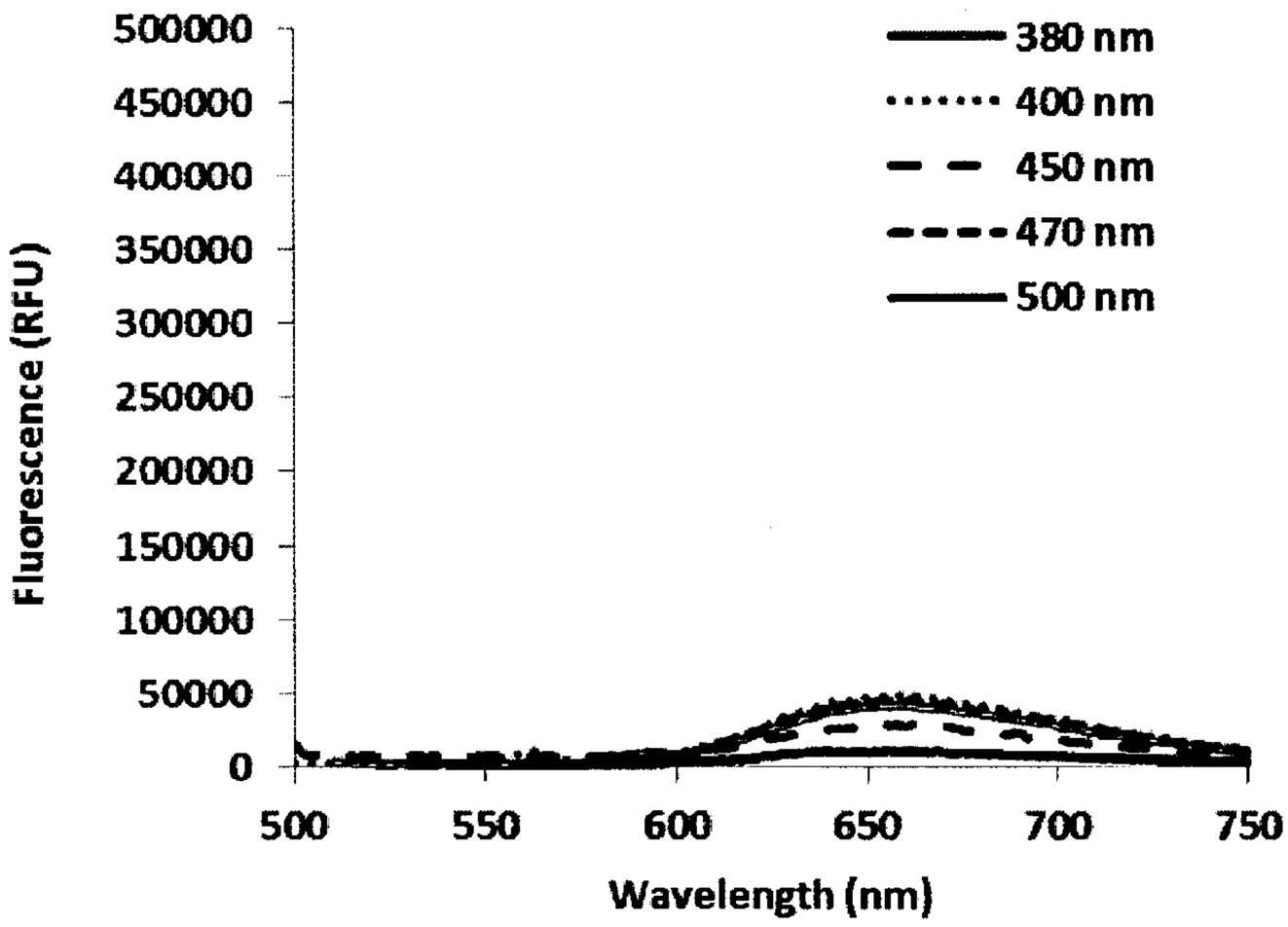

500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
Fluorescence (RFU)
500
550
600
650
700
750
Wavelength (nm)
380 nm
400 nm
450 nm
470 nm
500 nm

FIG. 5B

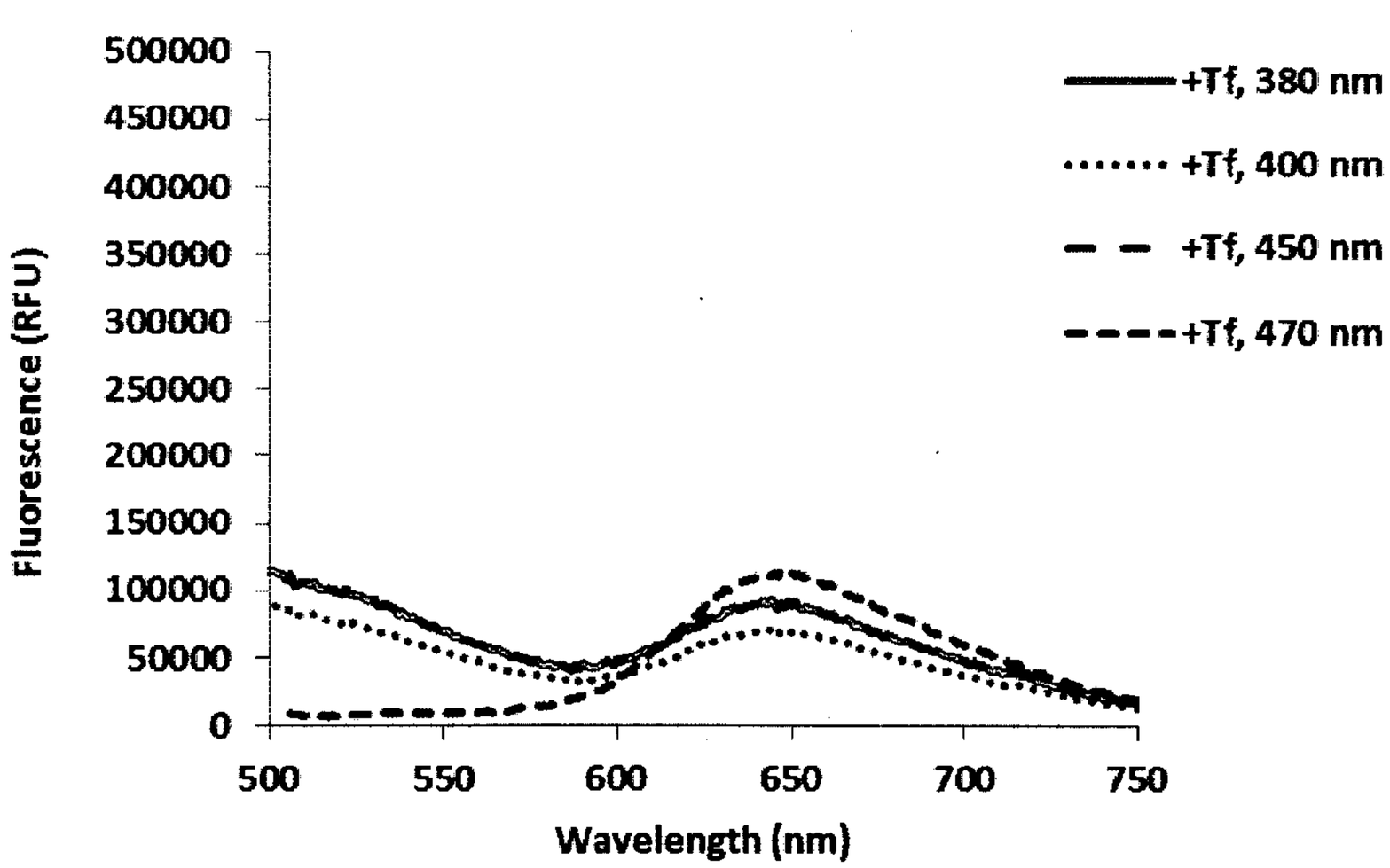

500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
Fluorescence (RFU)
500
550
600
650
700
750
Wavelength (nm)
+Tf, 380 nm
+Tf, 400 nm
+Tf, 450 nm
+Tf, 470 nm

FIG. 6A

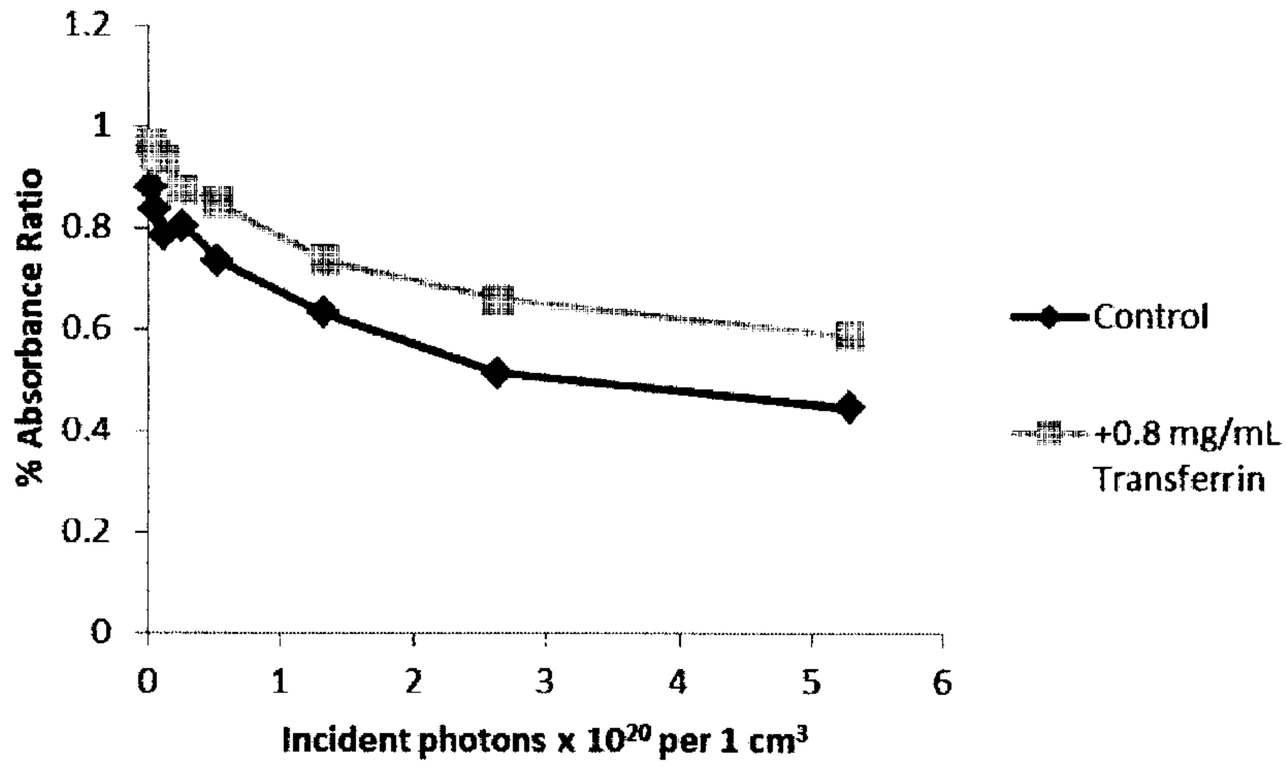


FIG. 6B

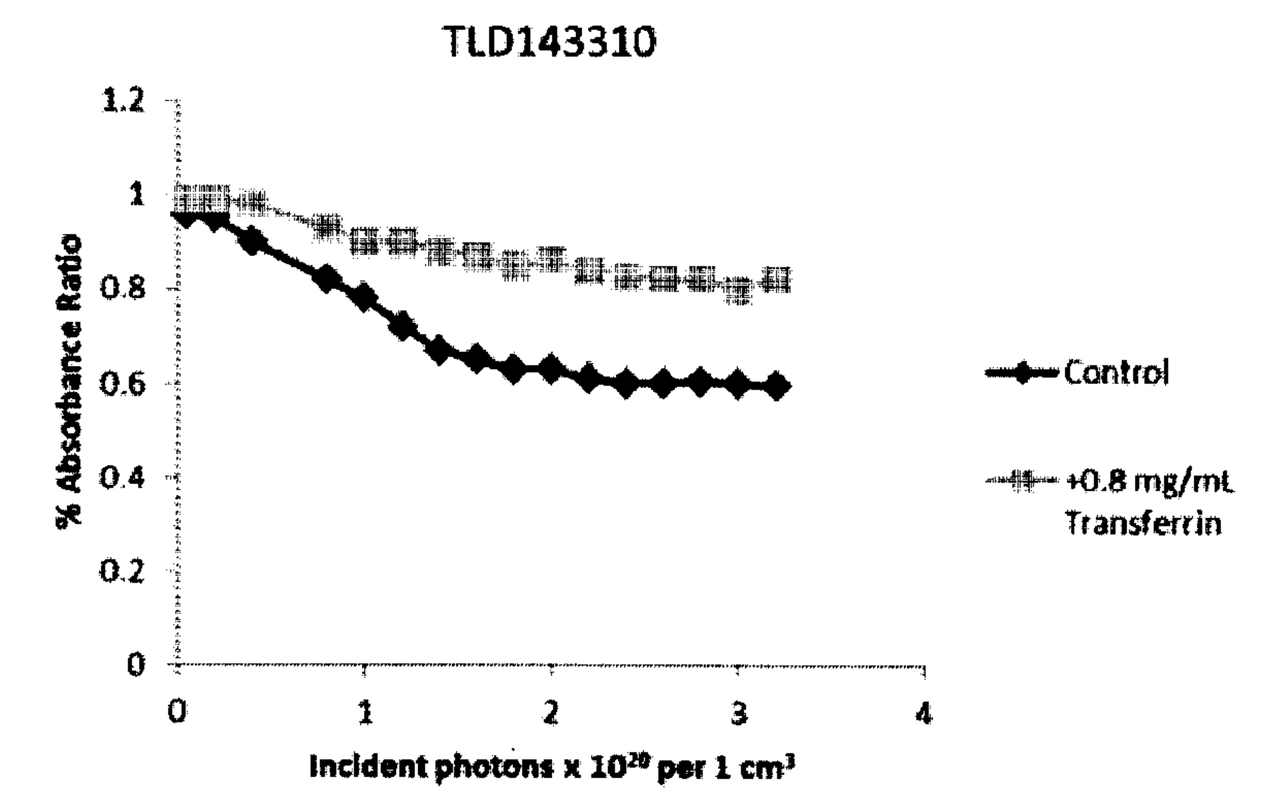


FIG. 6C

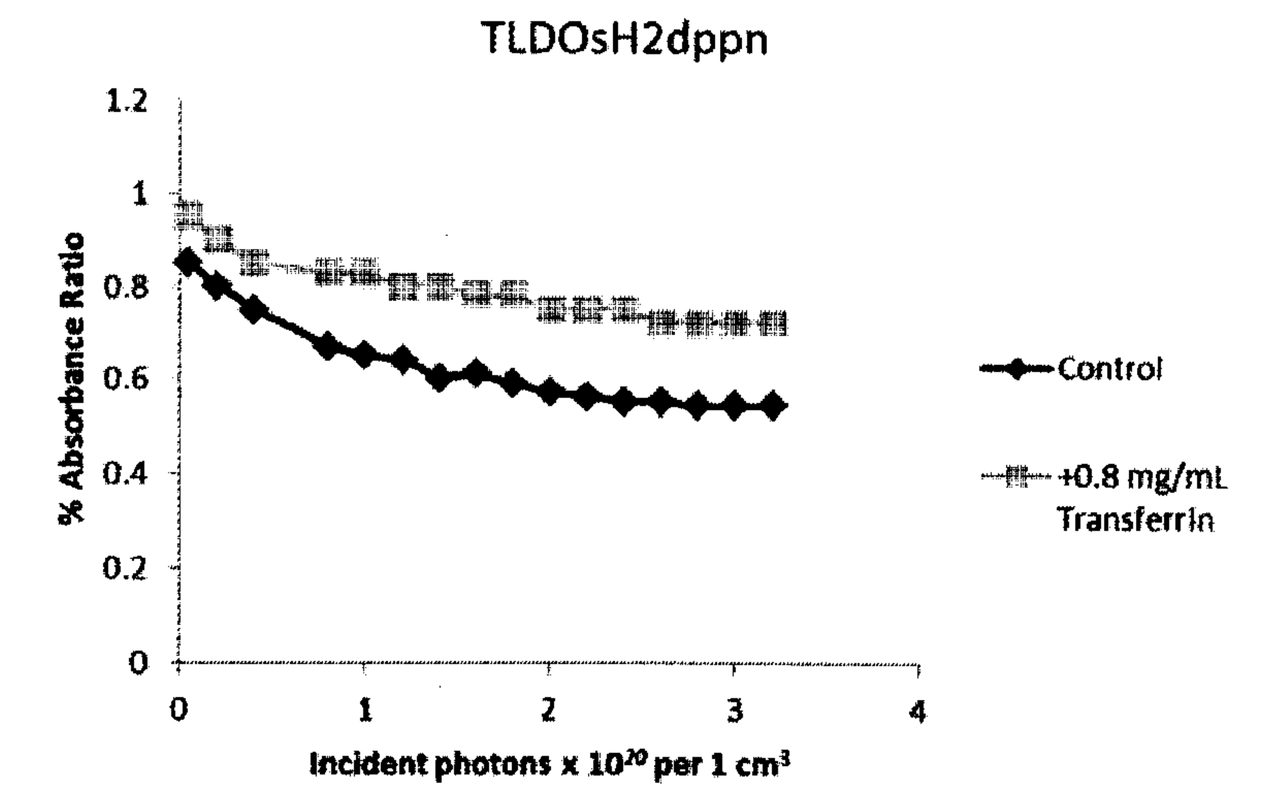


FIG. 7A

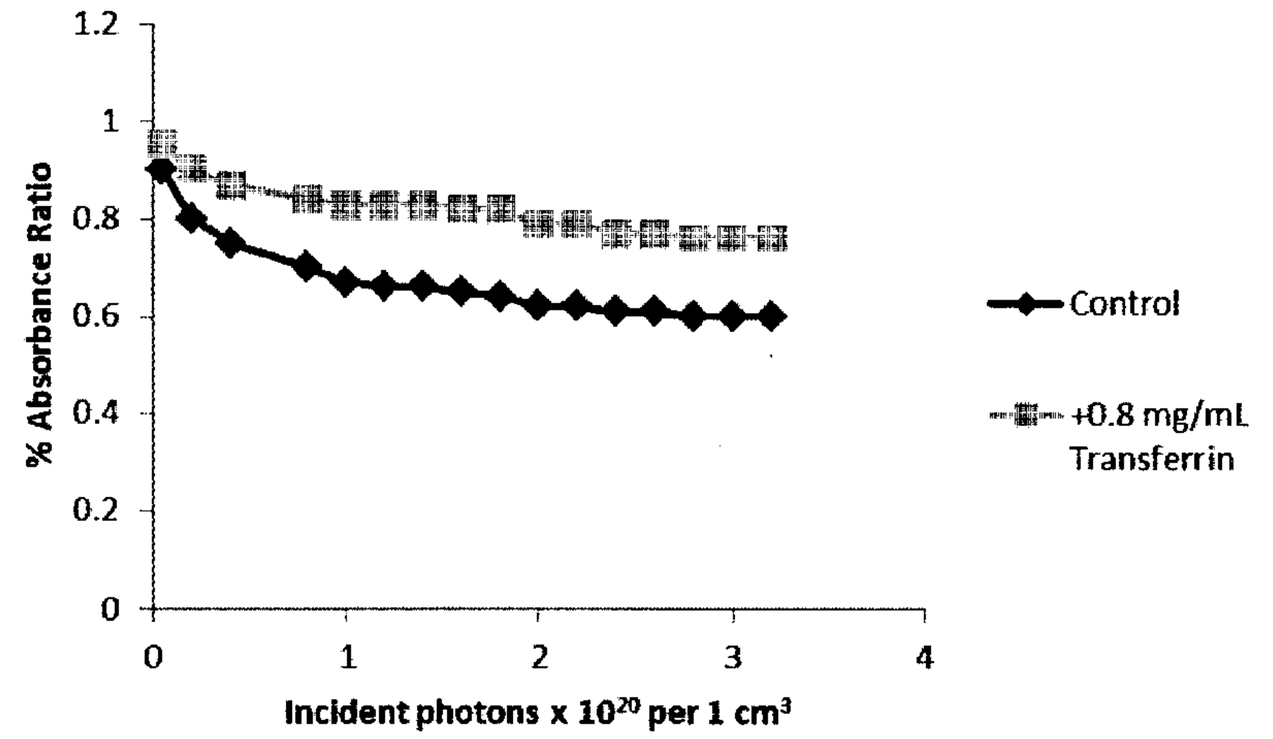


FIG. 7B

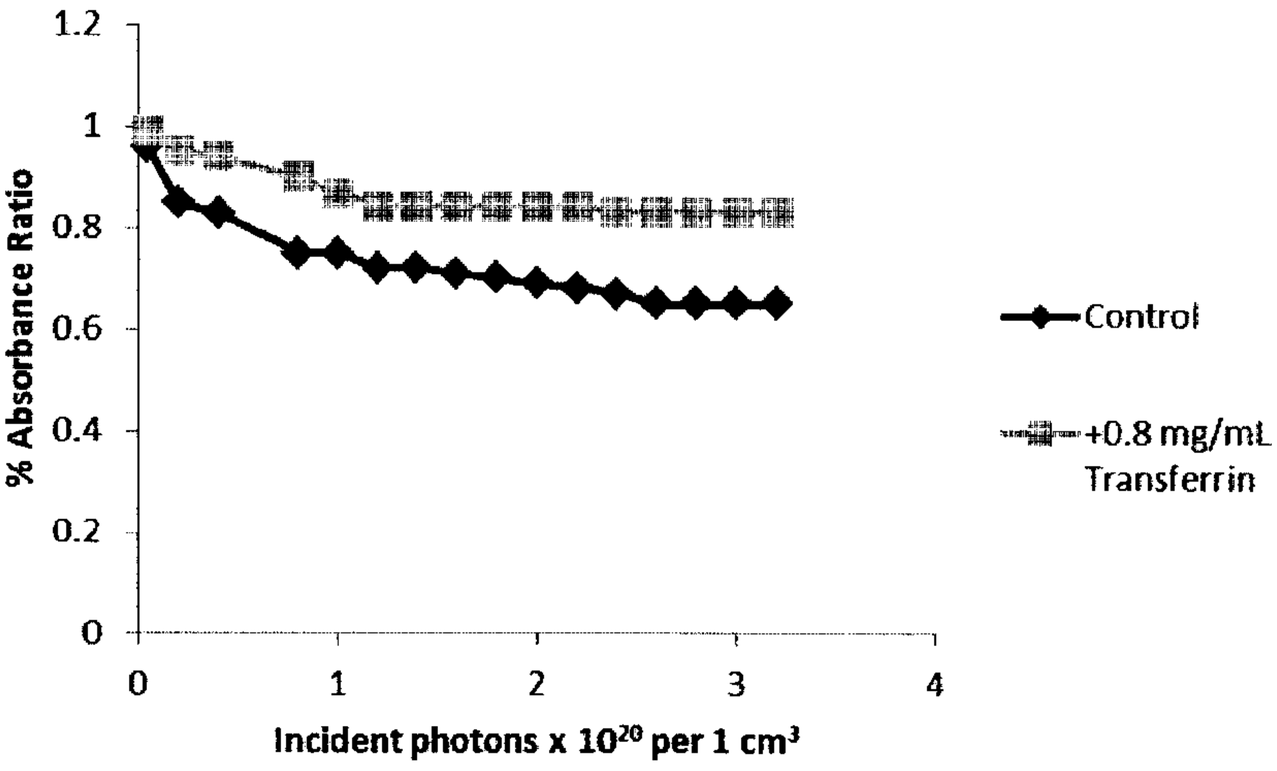


FIG. 7C

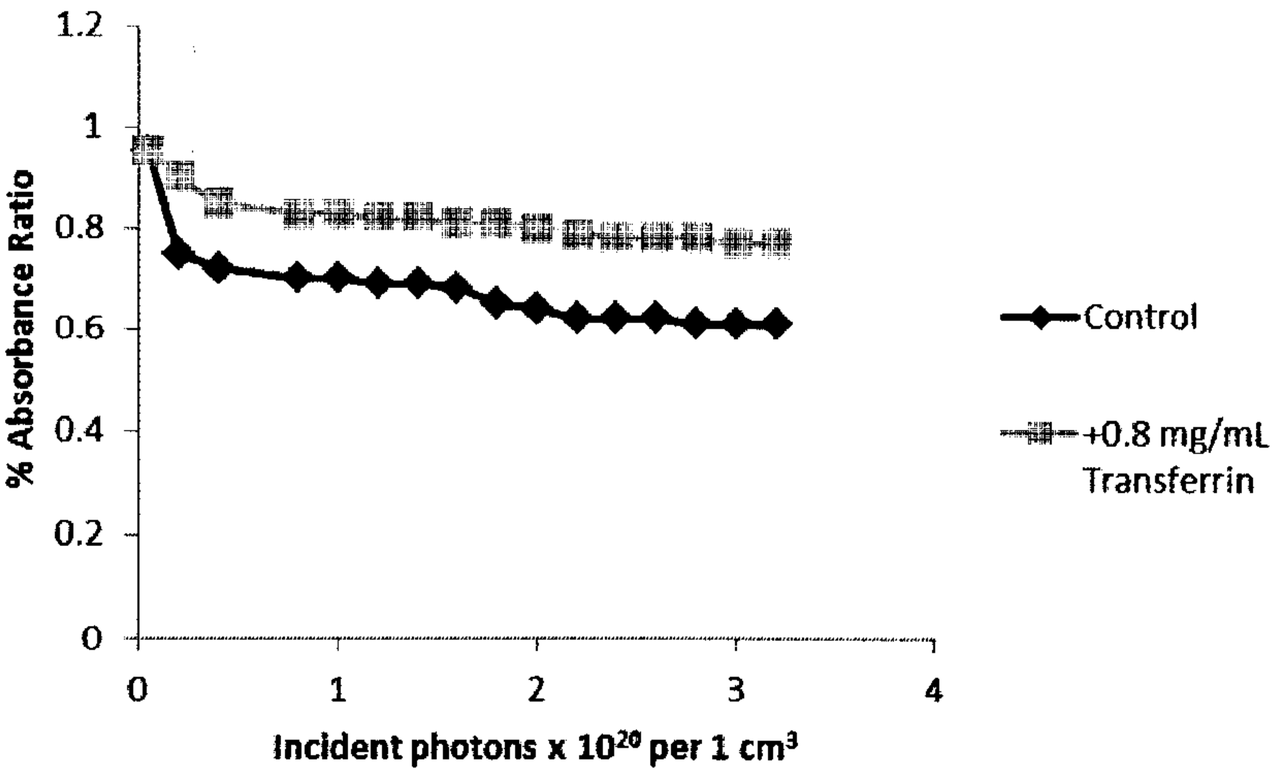


FIG. 8A

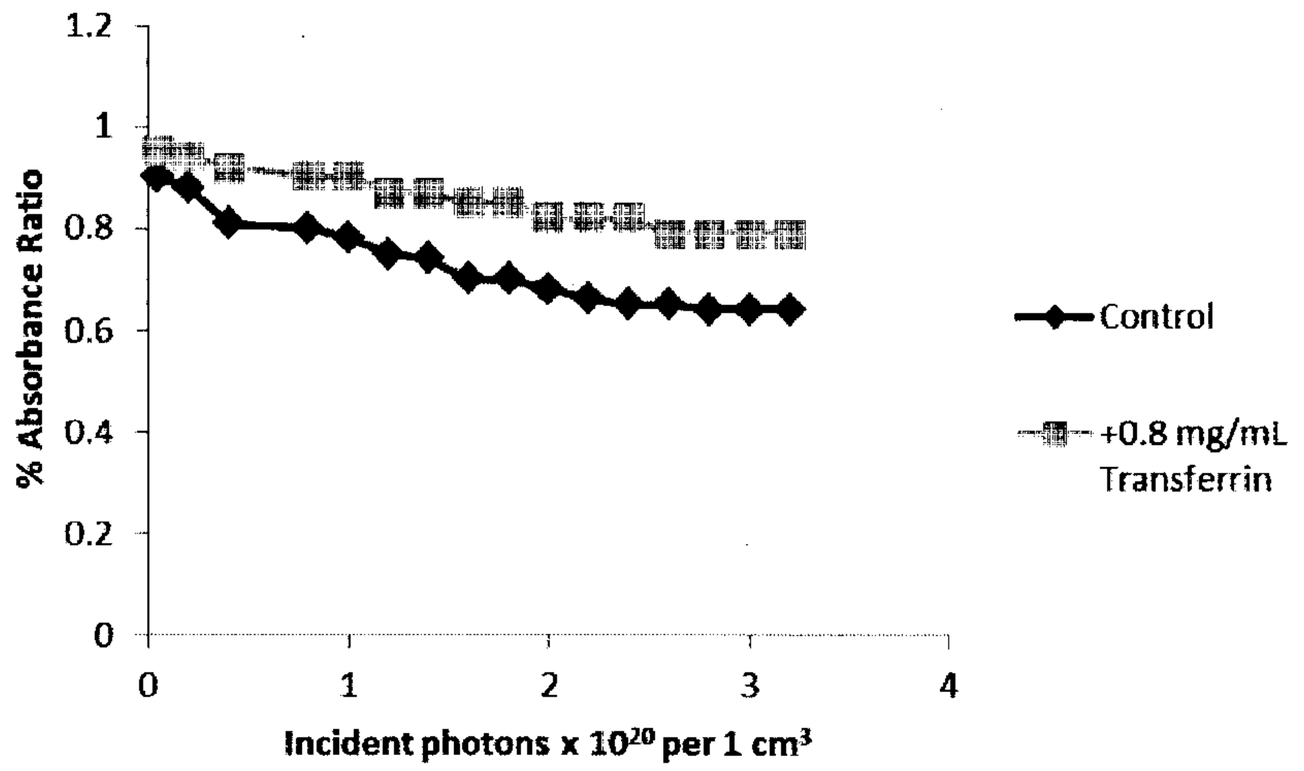


FIG. 8B

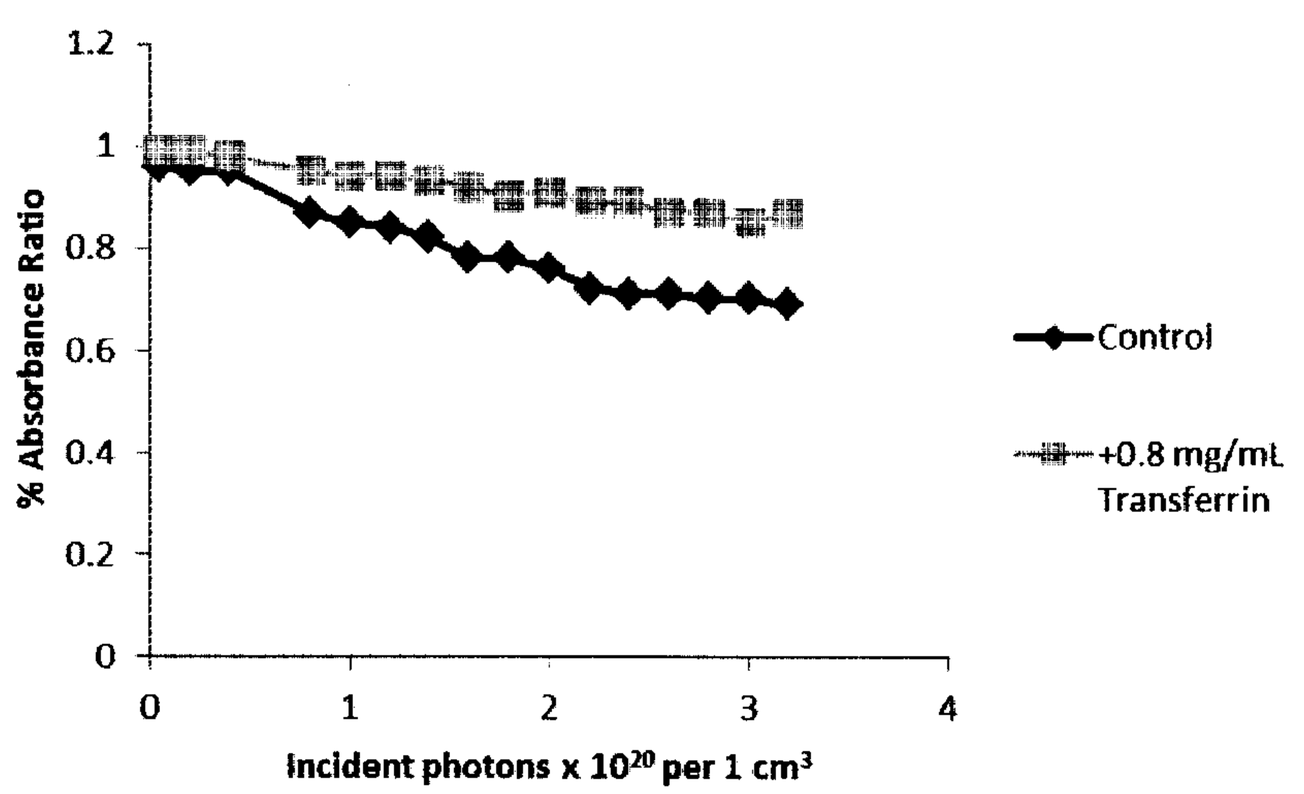


FIG. 8C

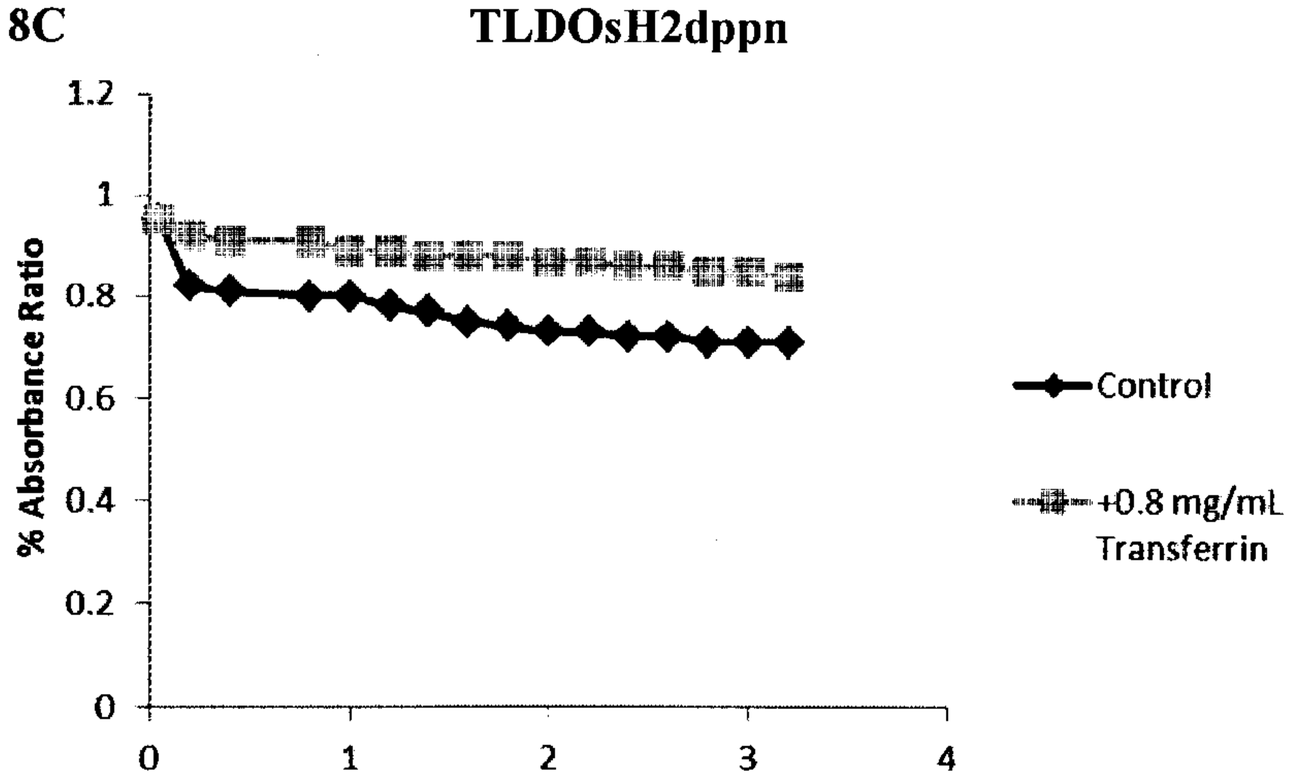


FIG. 9A

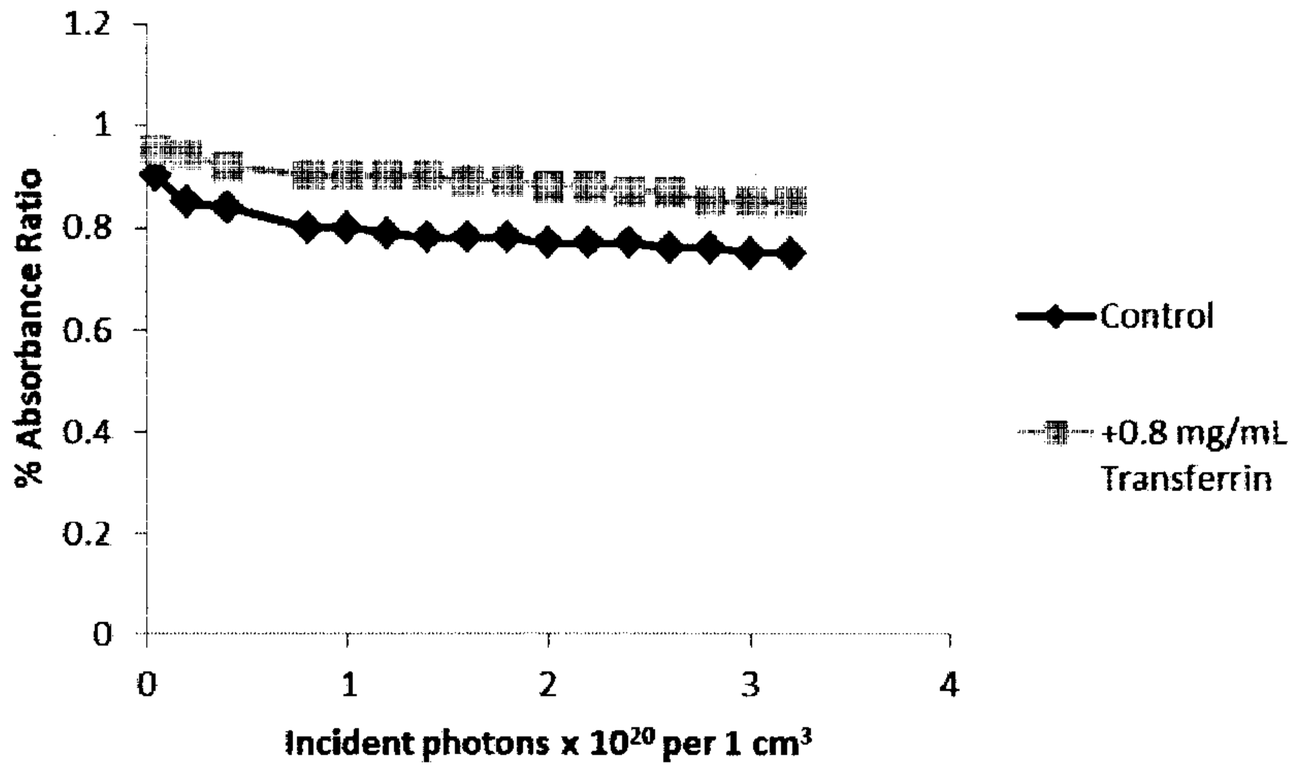


FIG. 9B

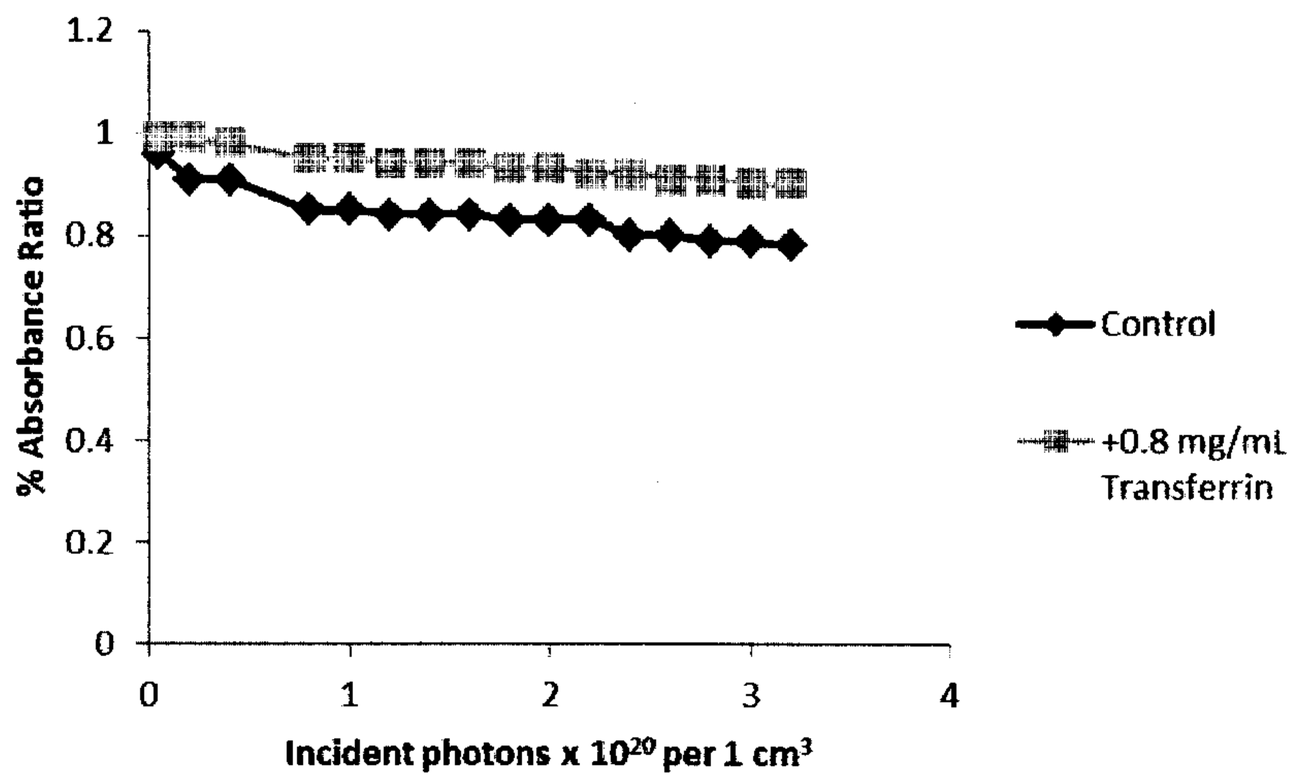


FIG. 9C

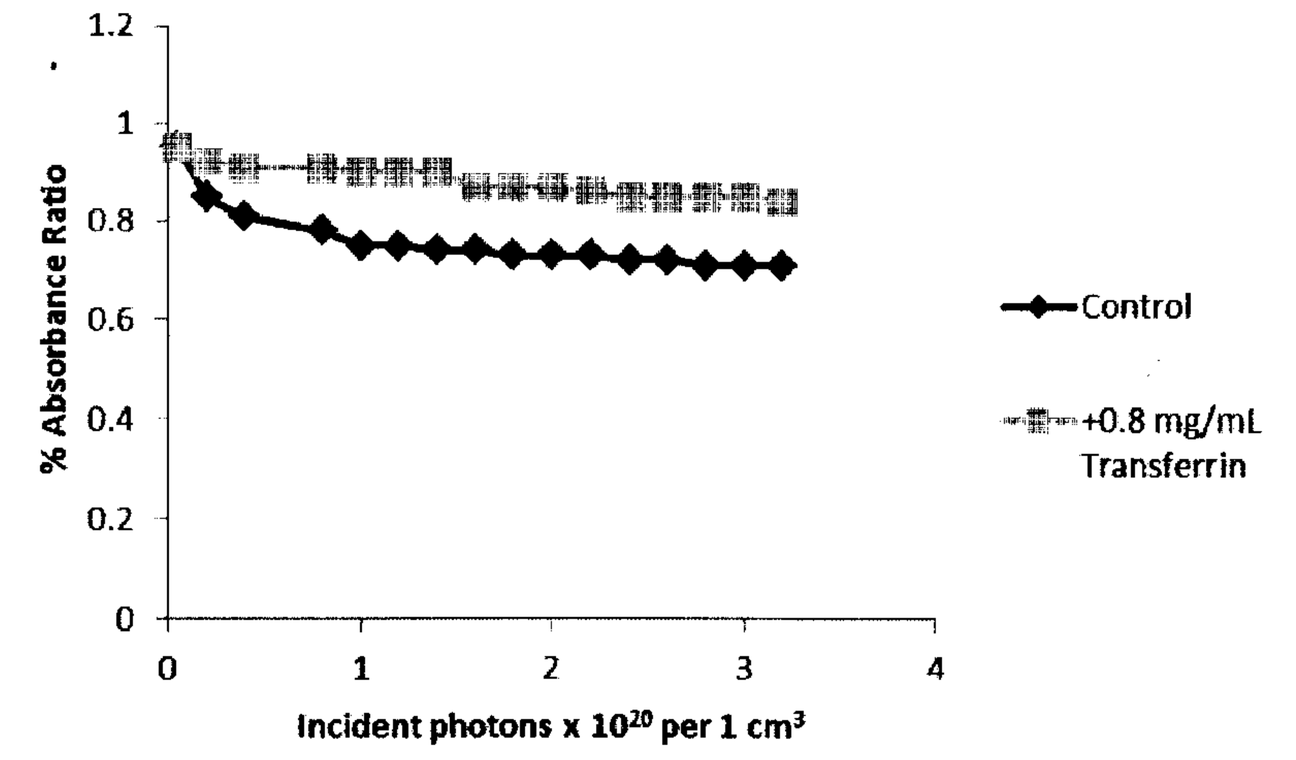


FIG. 10A

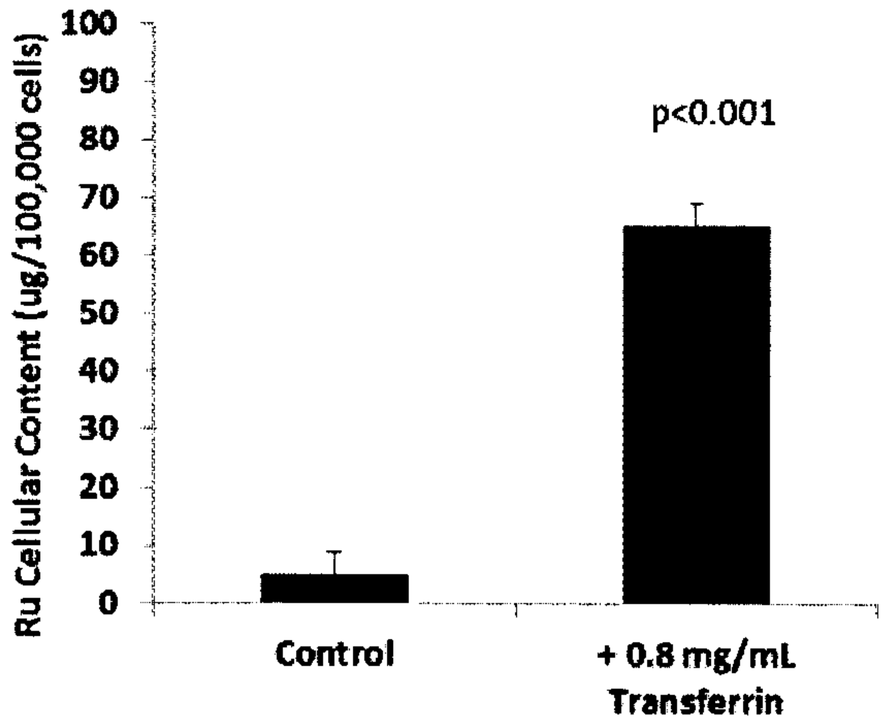


FIG. 10B

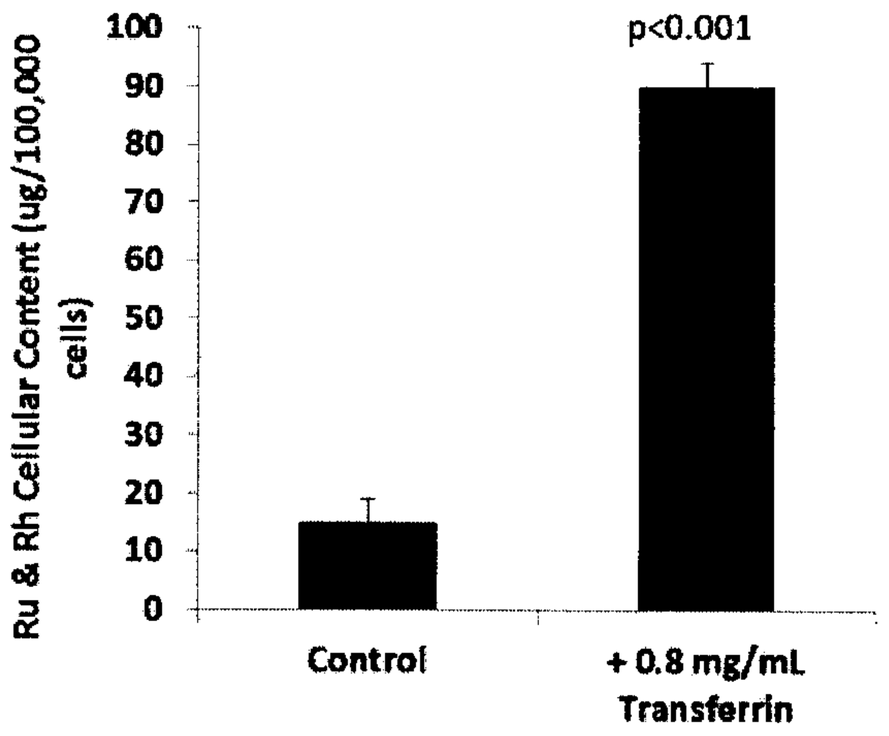


FIG. 10C

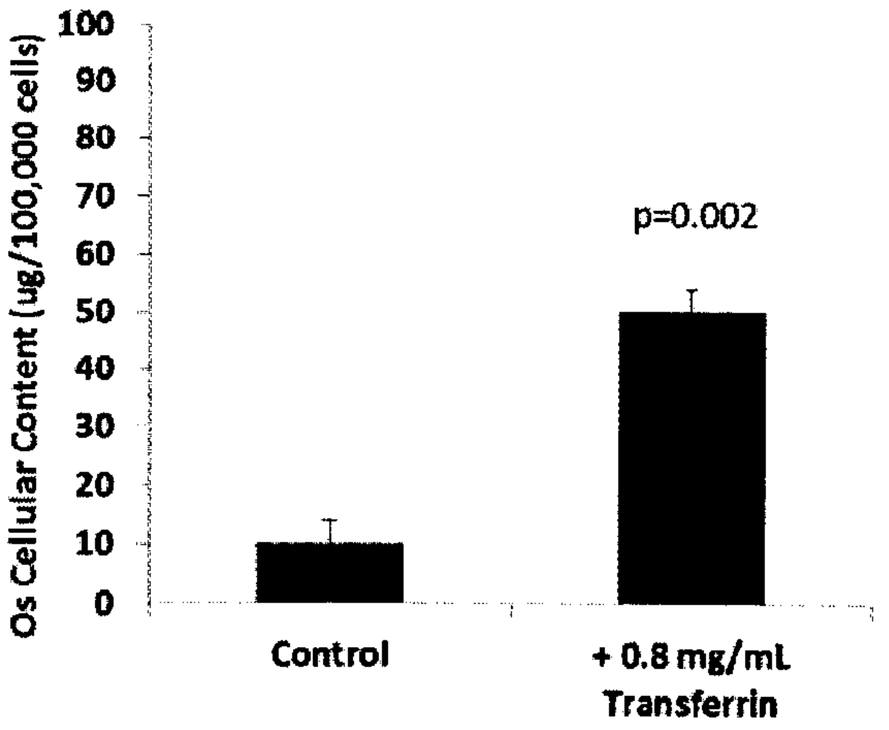


FIG. 11A

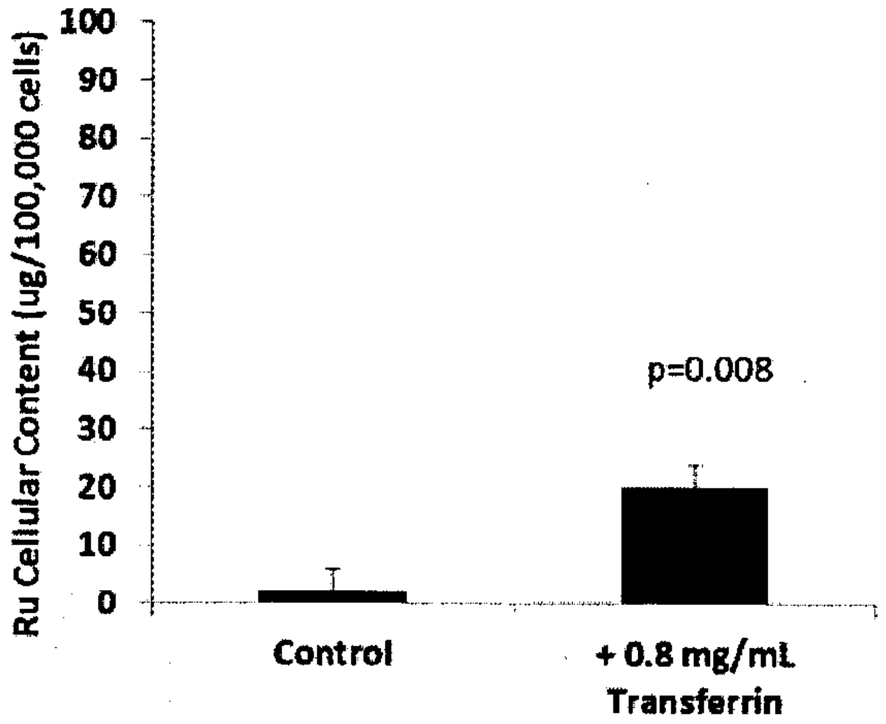


FIG. 11B

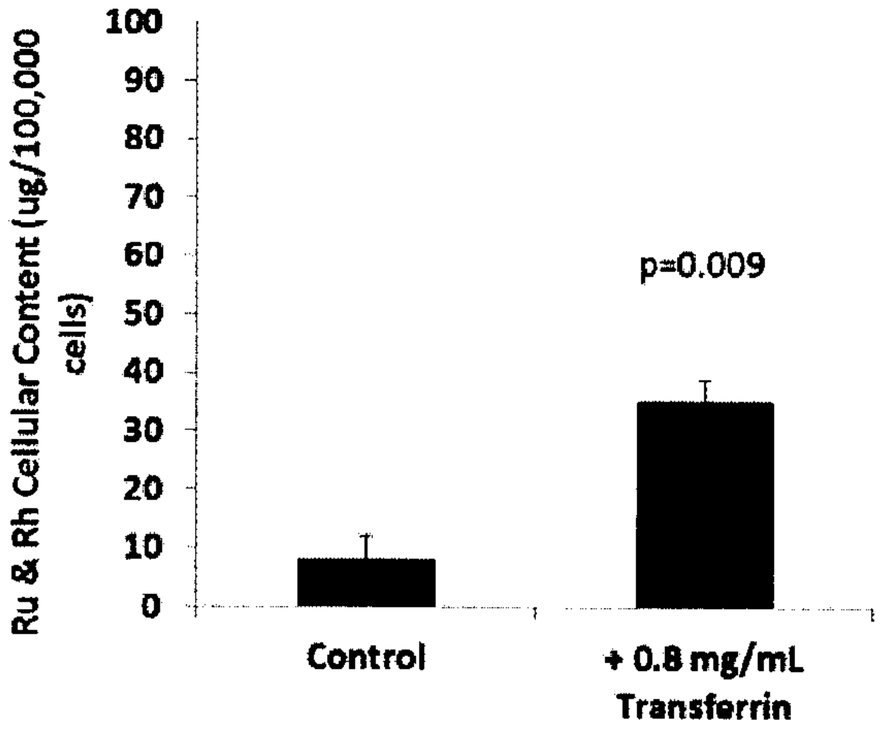


FIG. 11C

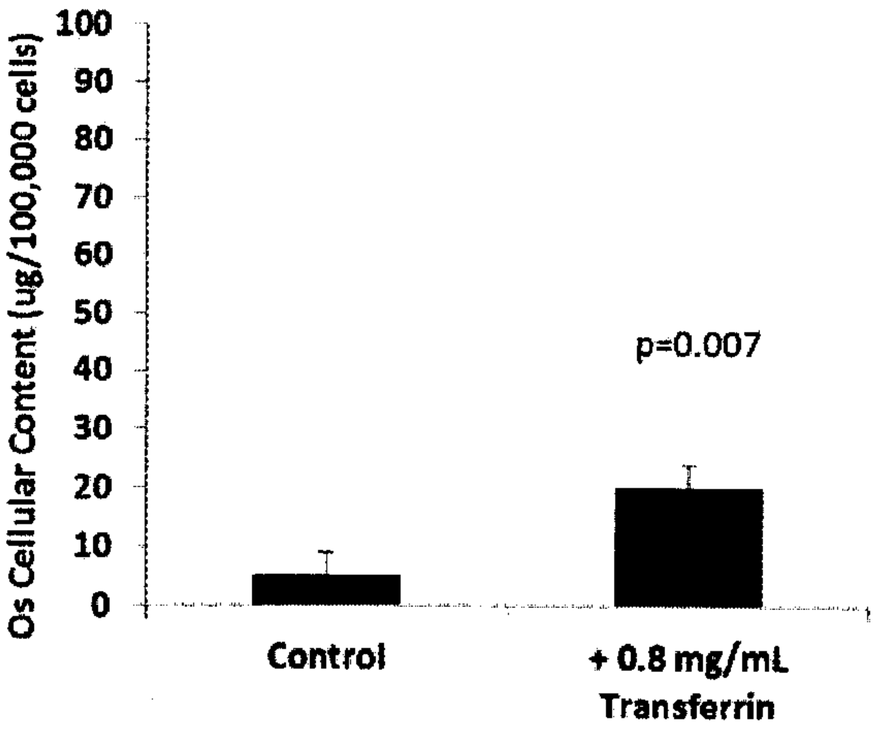


FIG. 12A

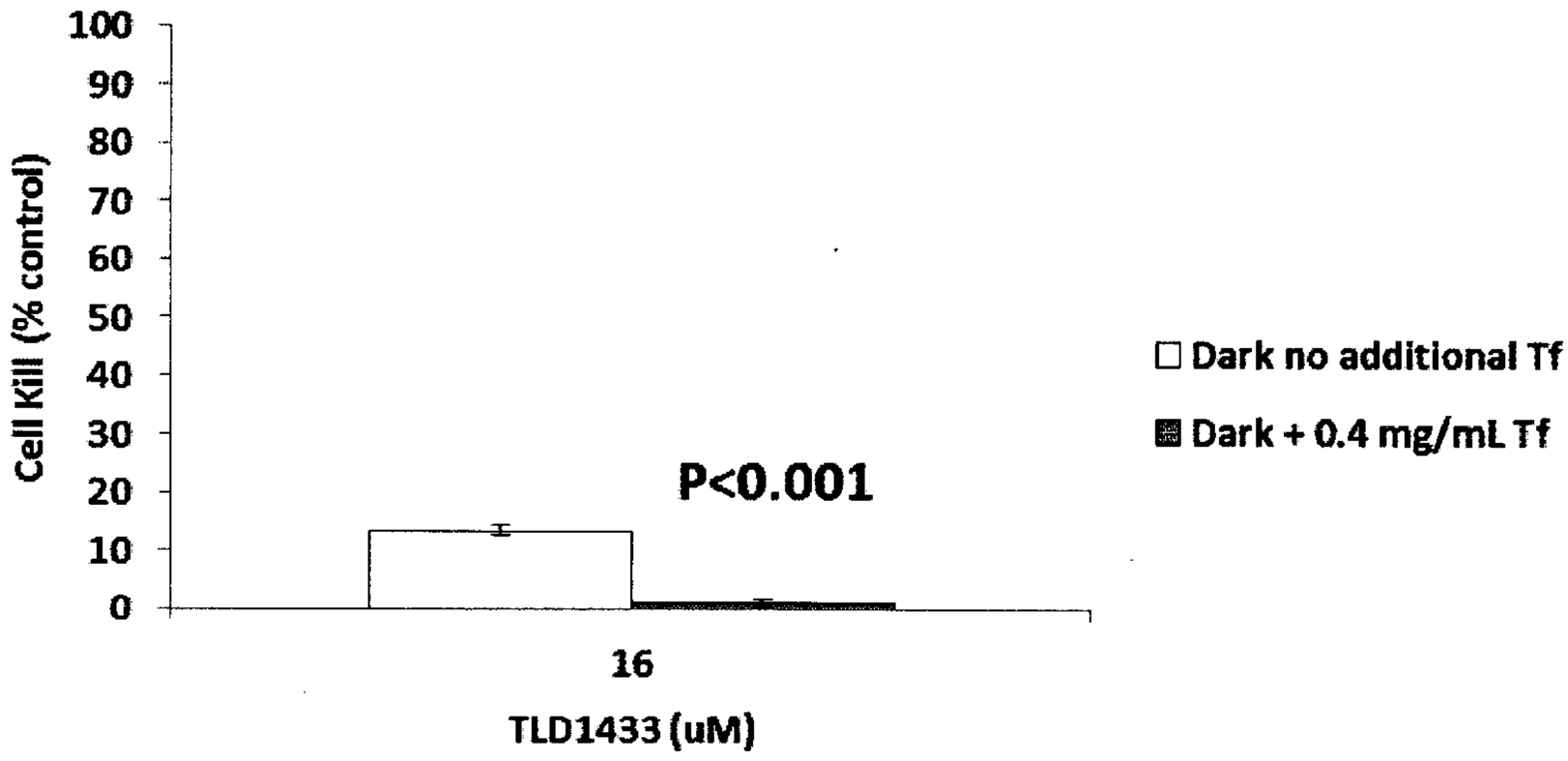


FIG. 12B

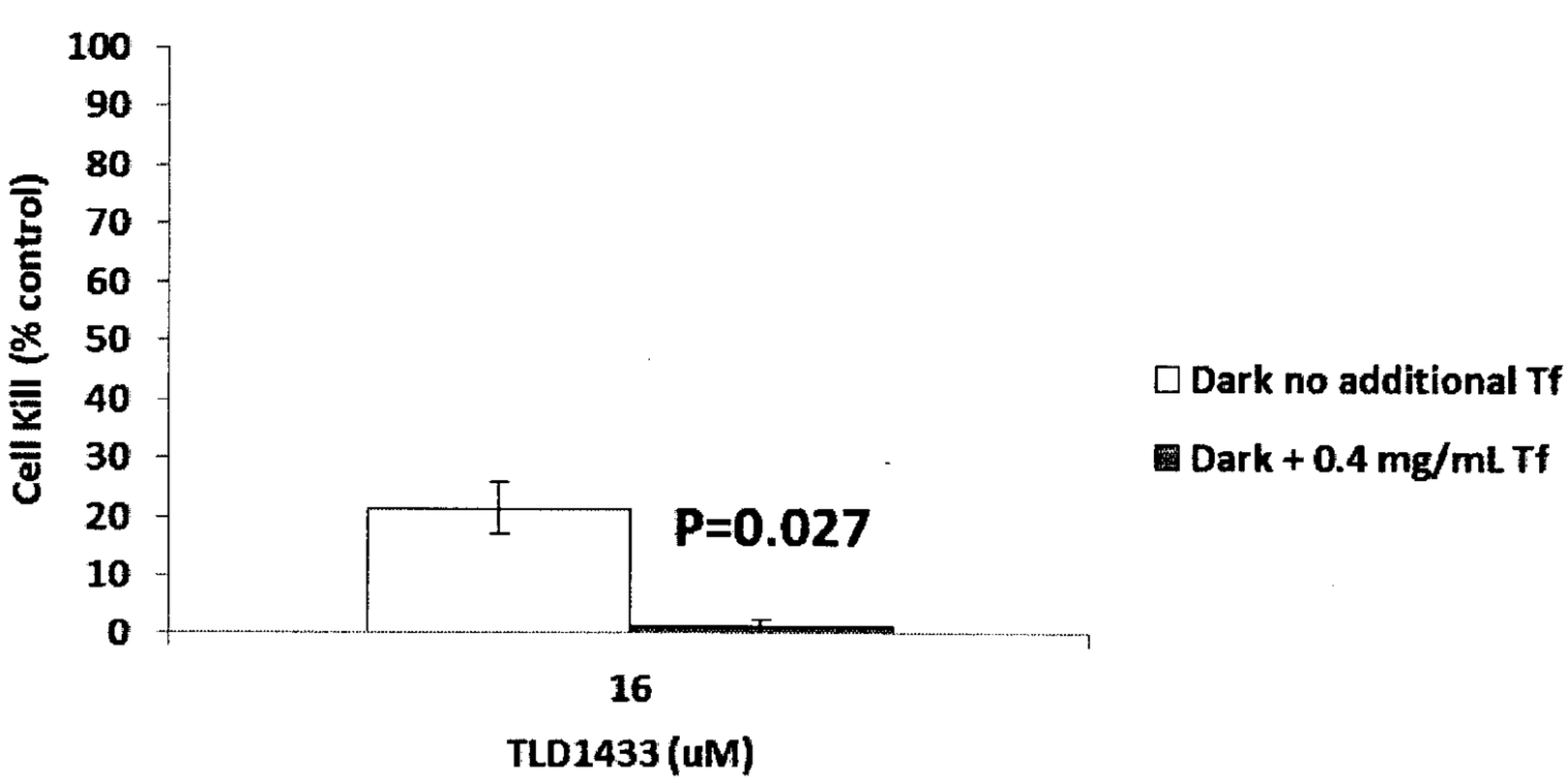


FIG. 13A

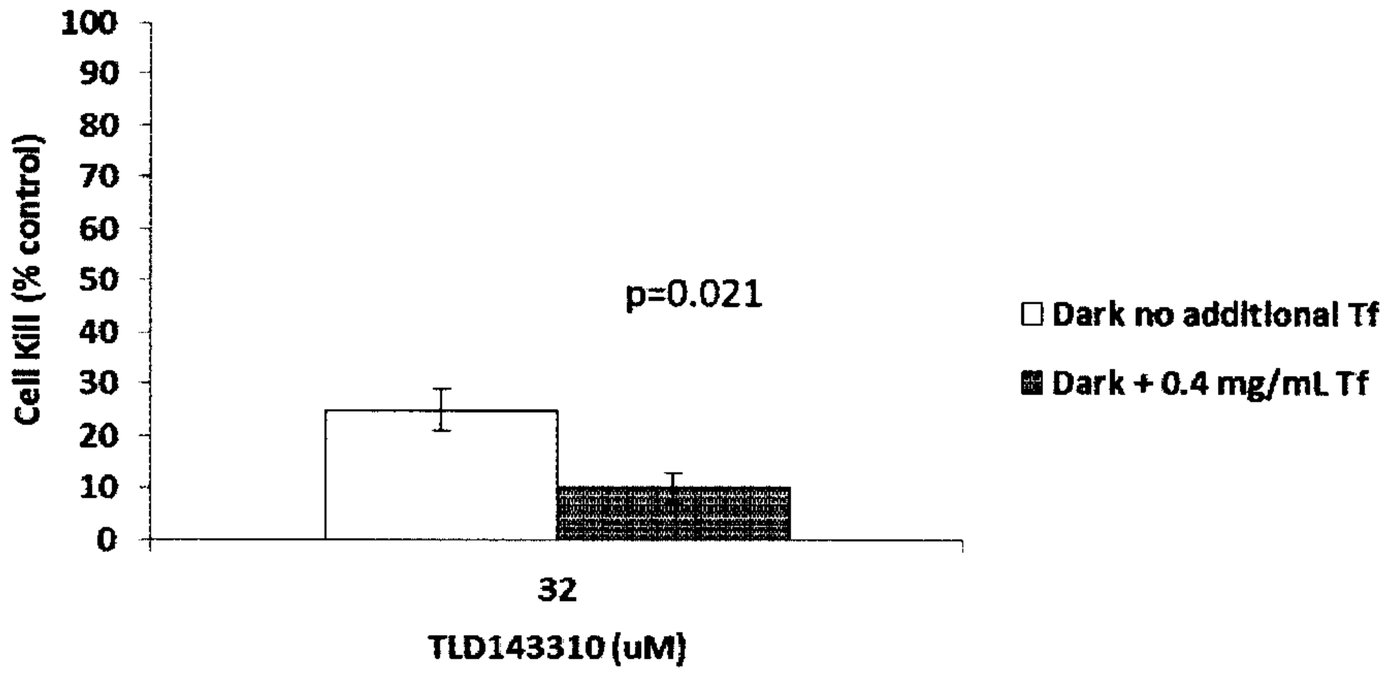


FIG. 13B

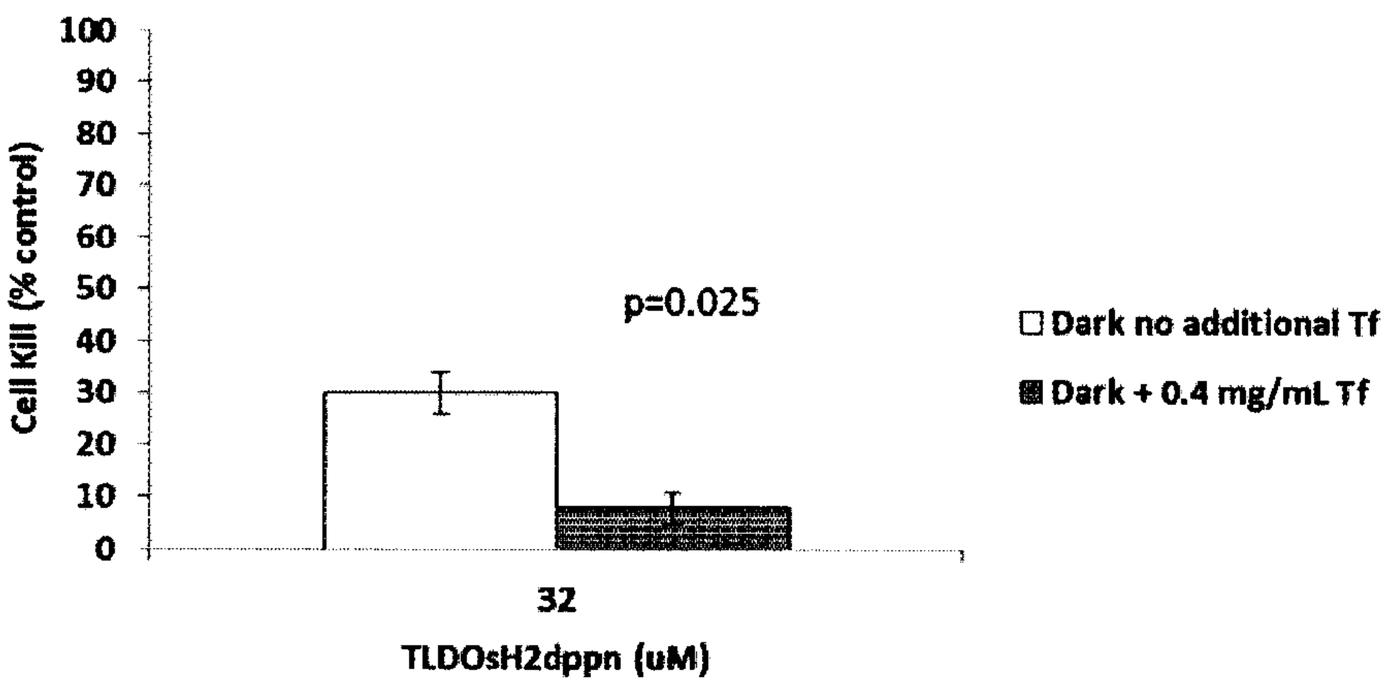


FIG. 14A

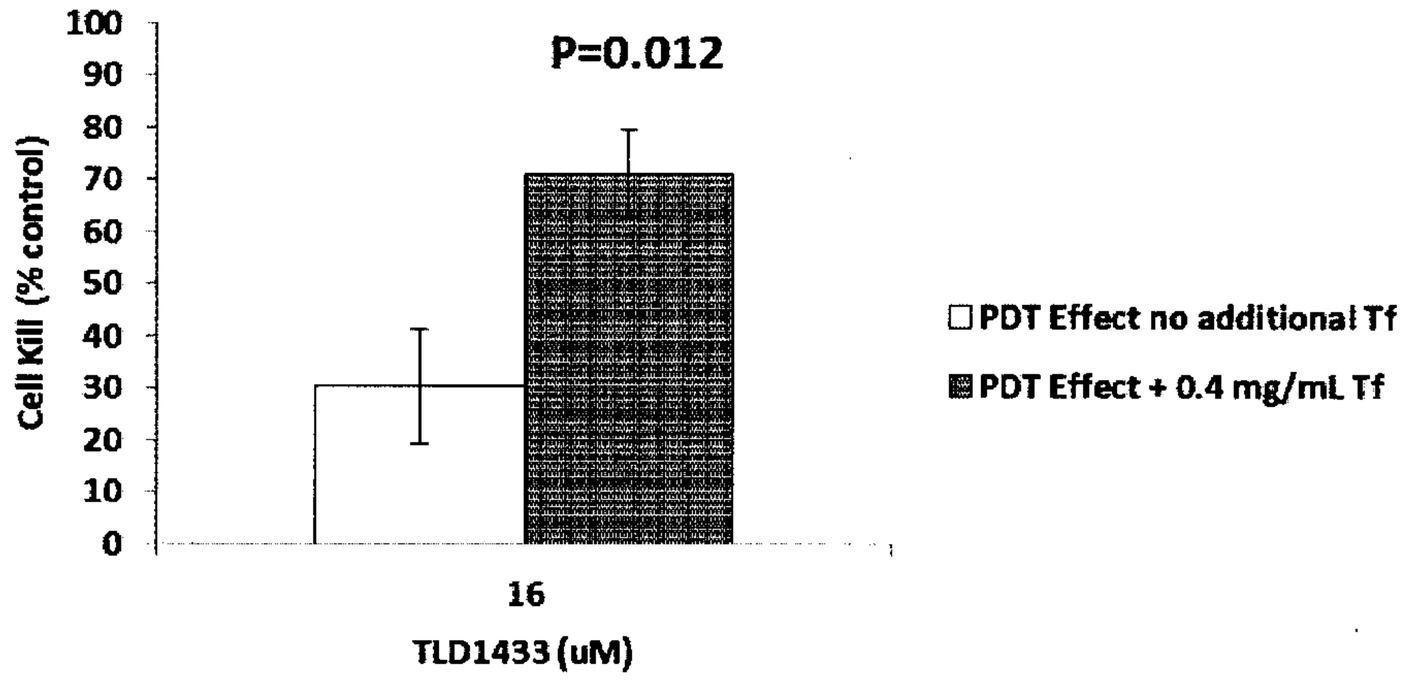


FIG. 14B

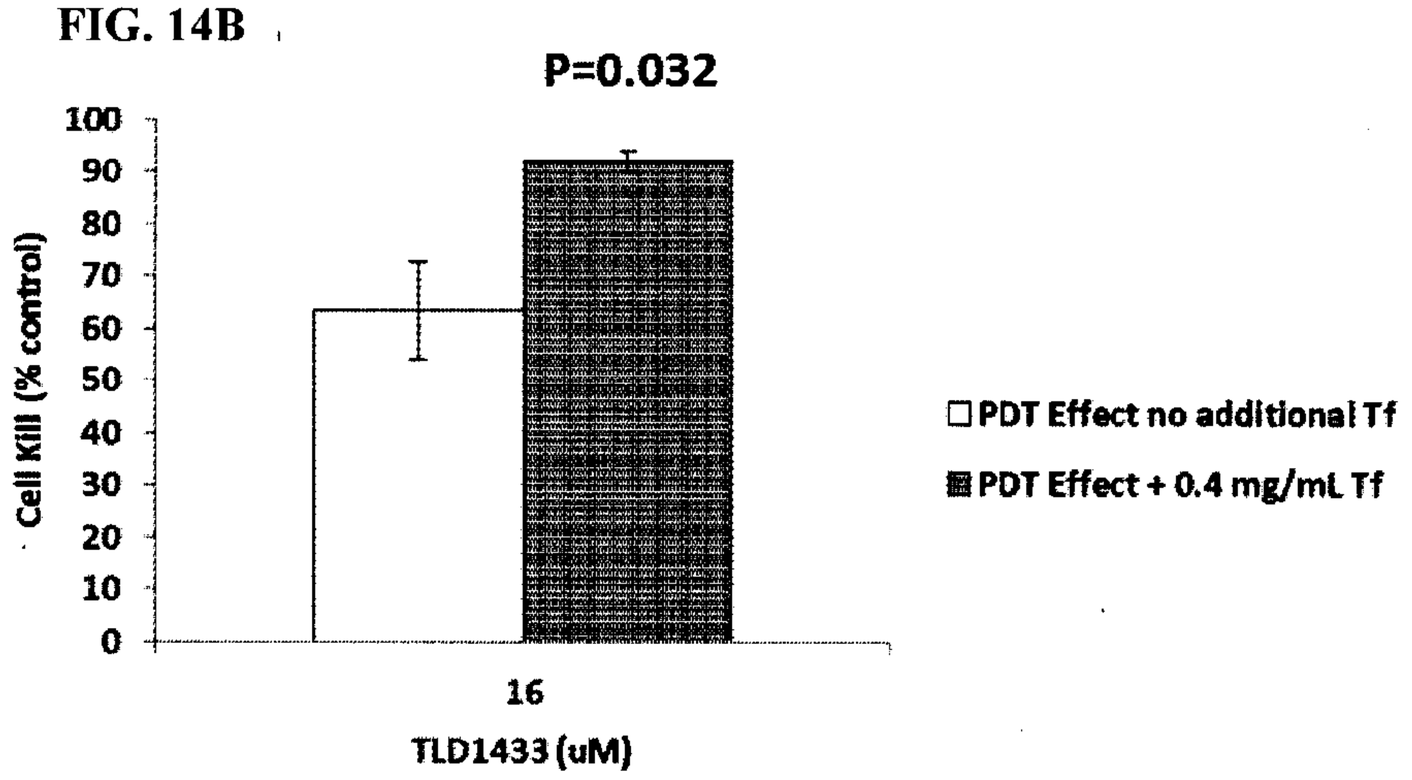


FIG. 15A

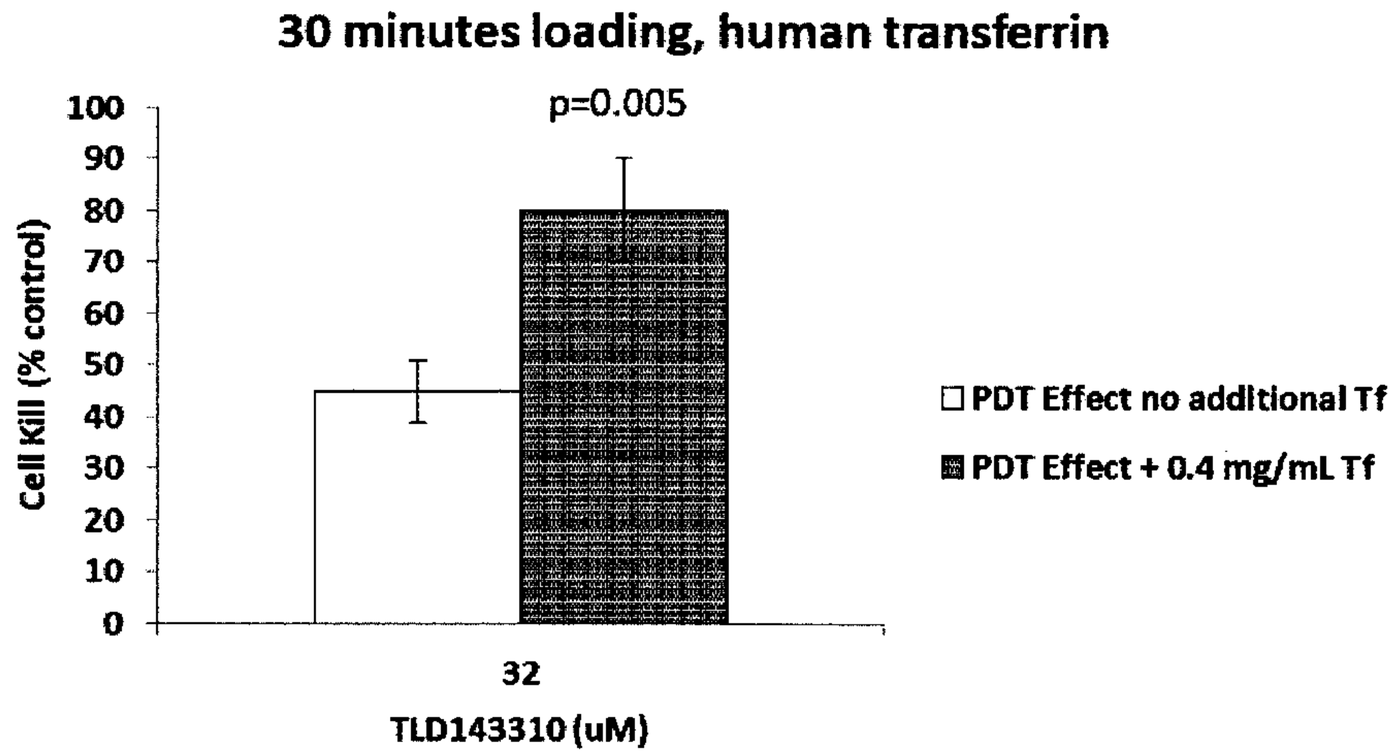


FIG. 15B

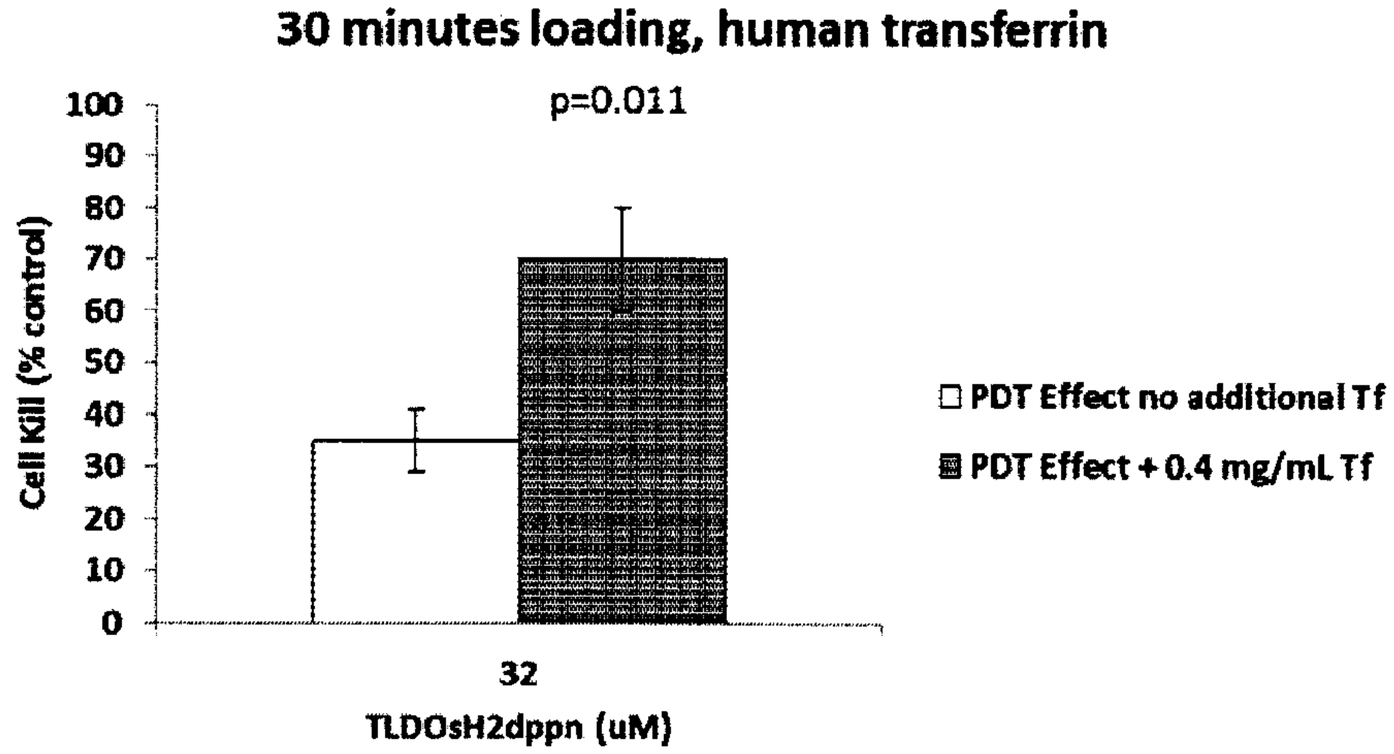


FIG. 16A

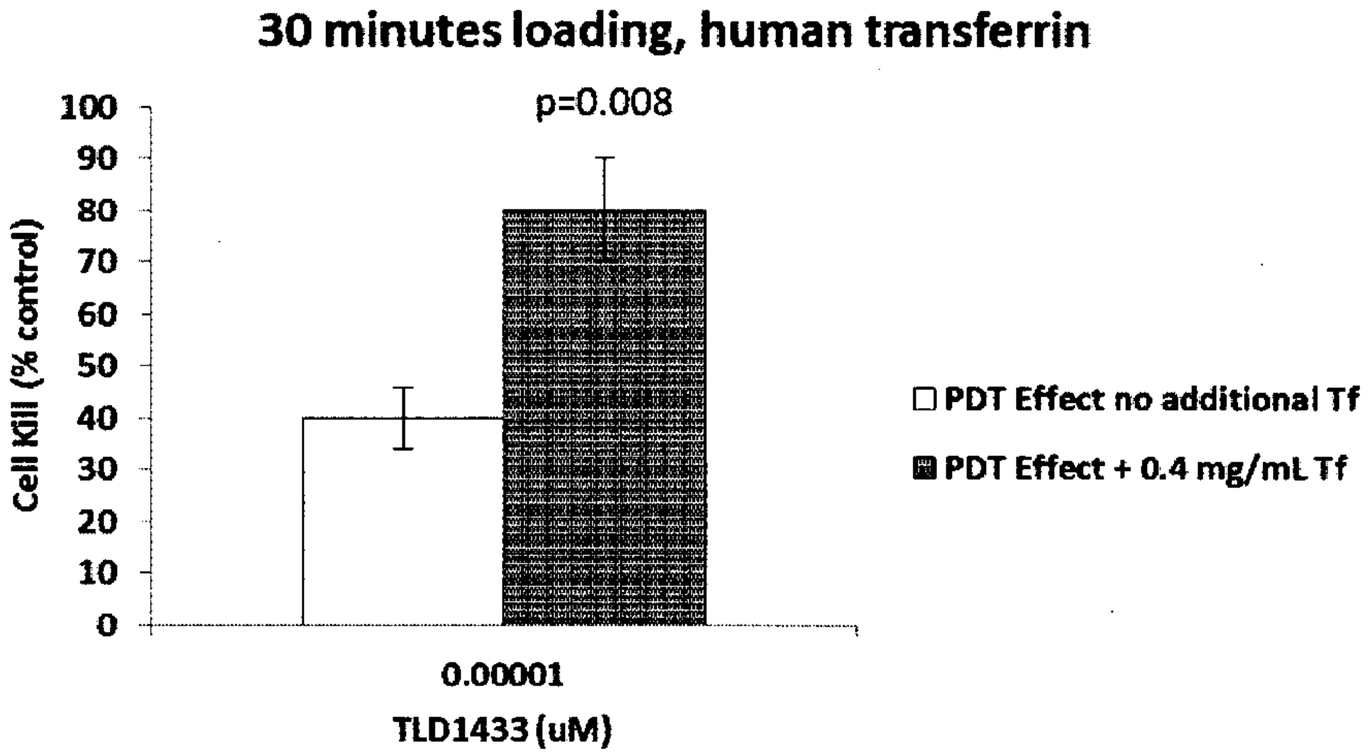


FIG. 16B

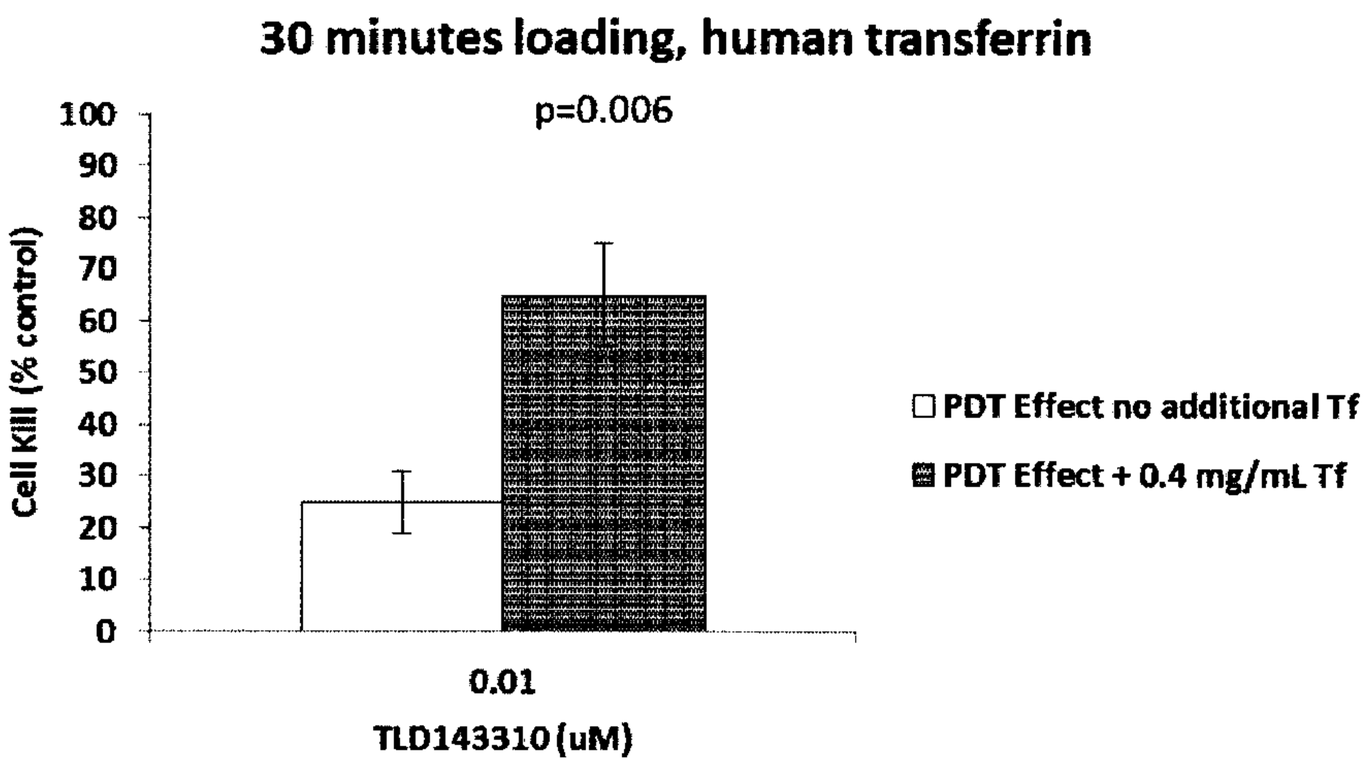


FIG. 16C

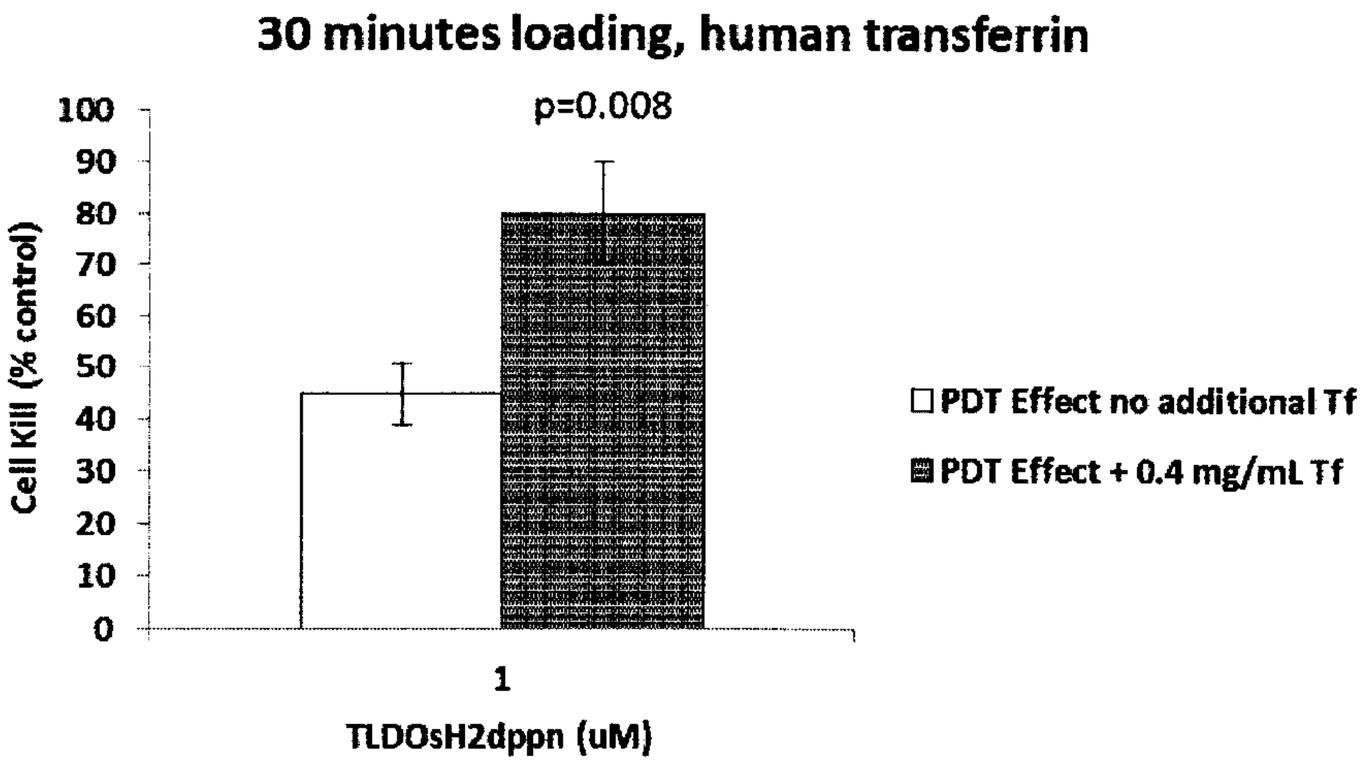


FIG. 17A

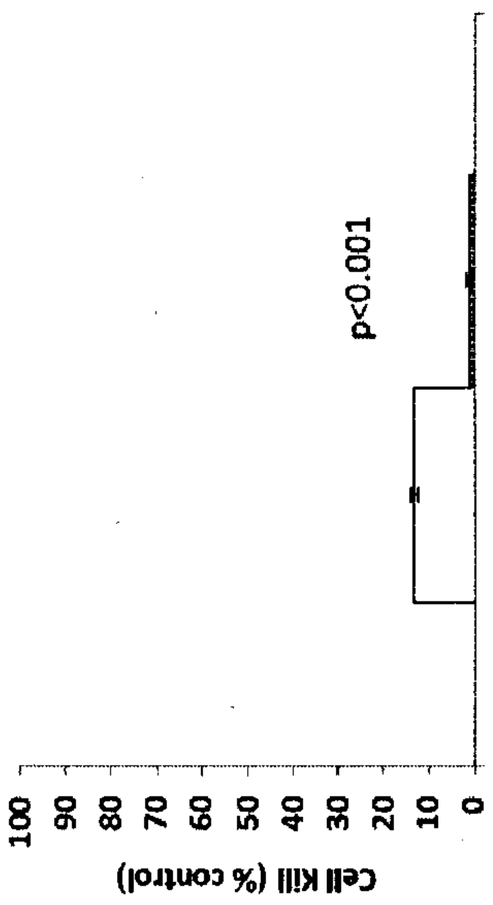


FIG. 17B

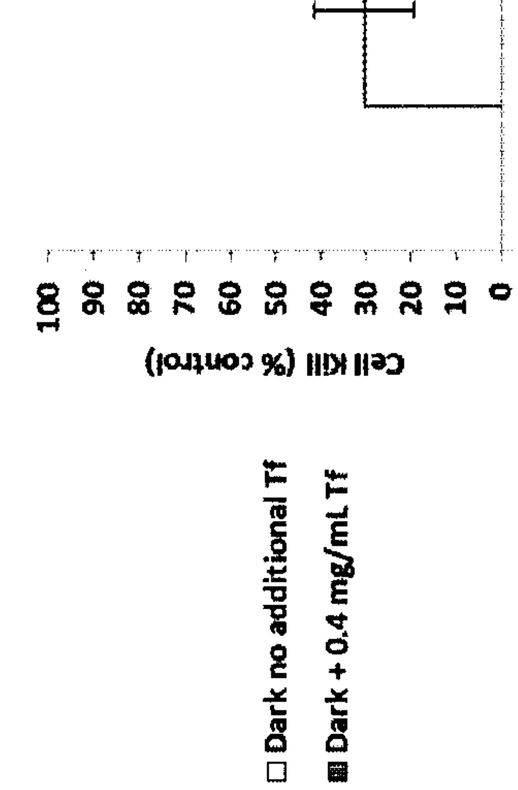


FIG. 17C

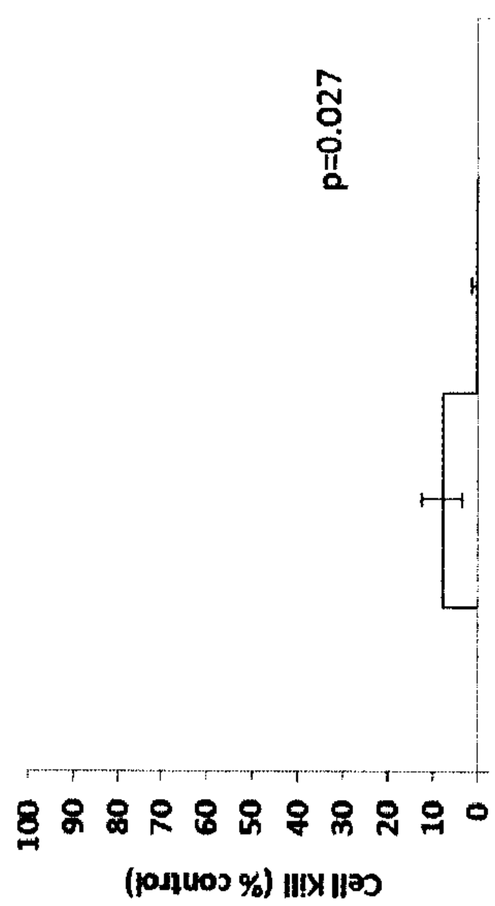
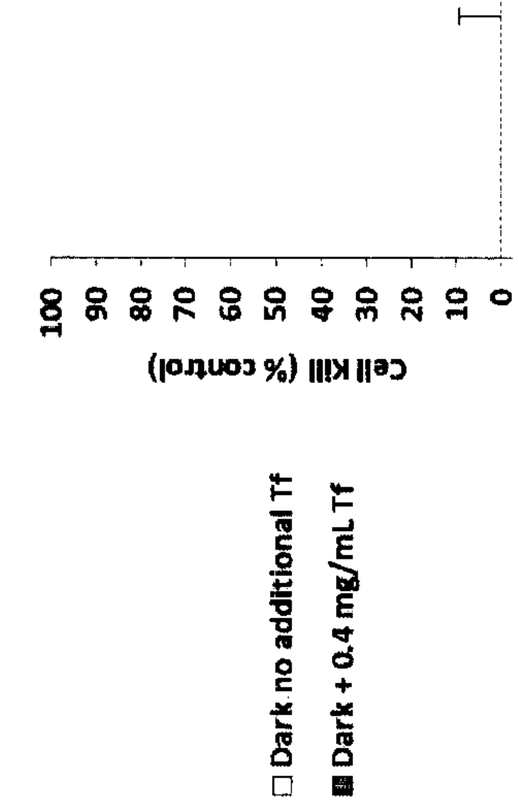


FIG. 17D



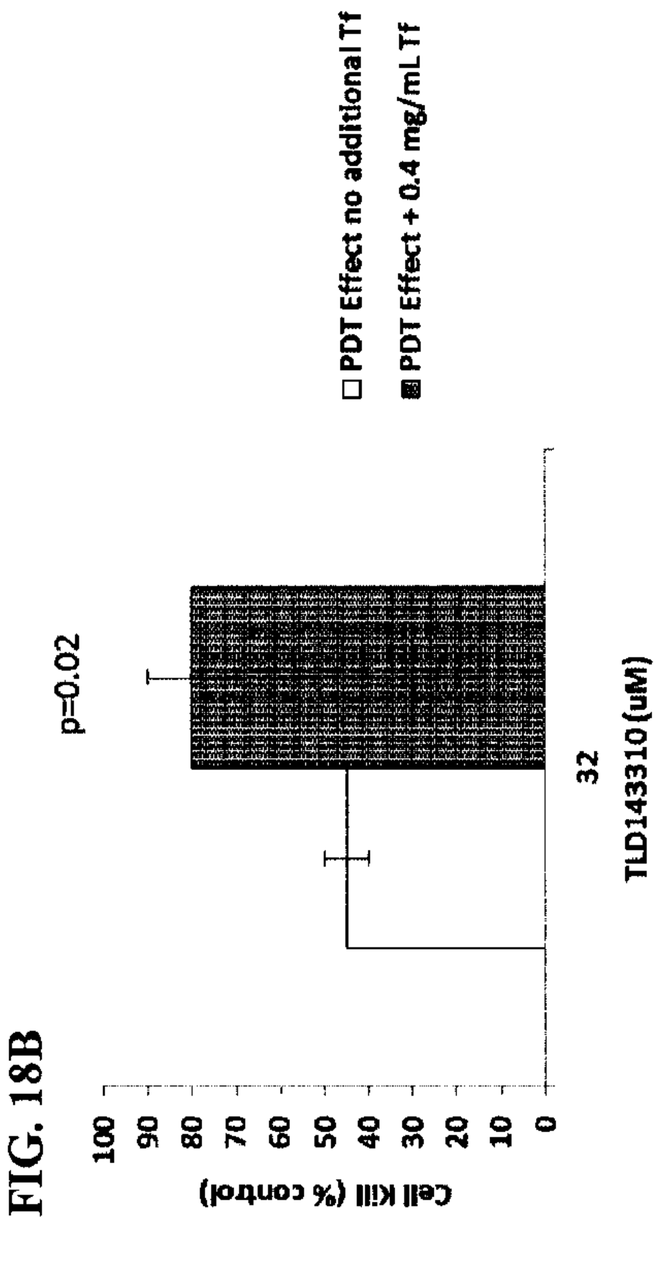


FIG. 18A

FIG. 18B

FIG. 18C

FIG. 18D

FIG. 19A

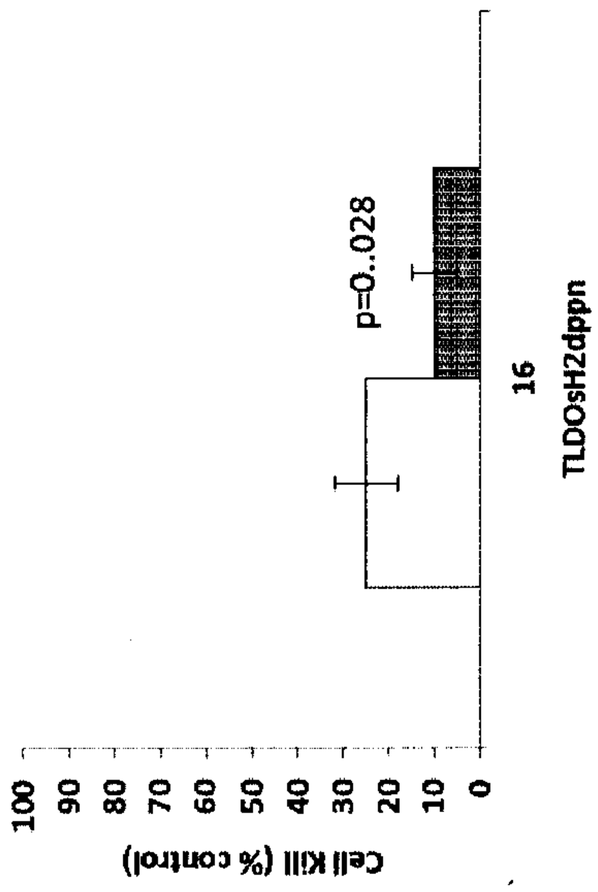


FIG. 19B

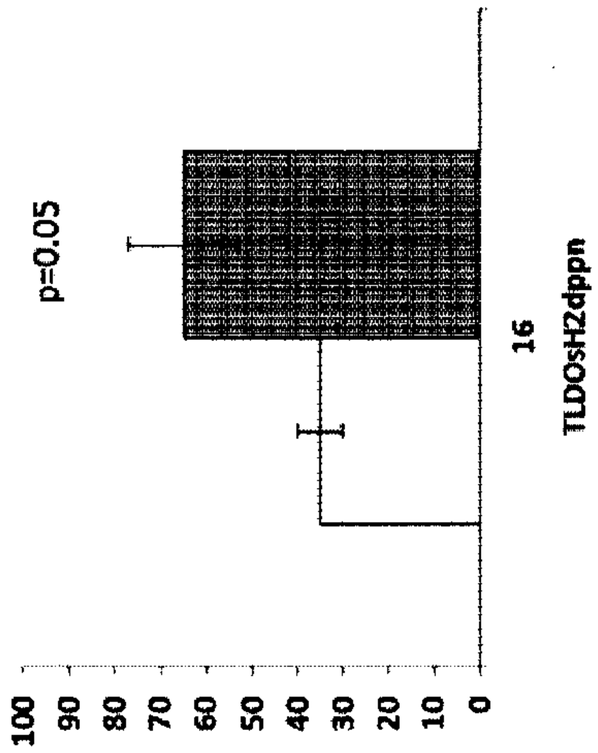


FIG. 19C

FIG. 19D

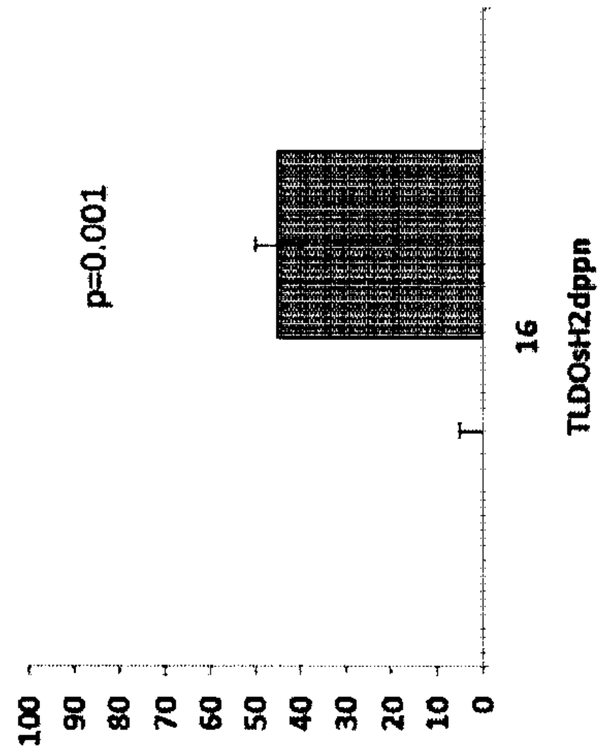


FIG. 20A

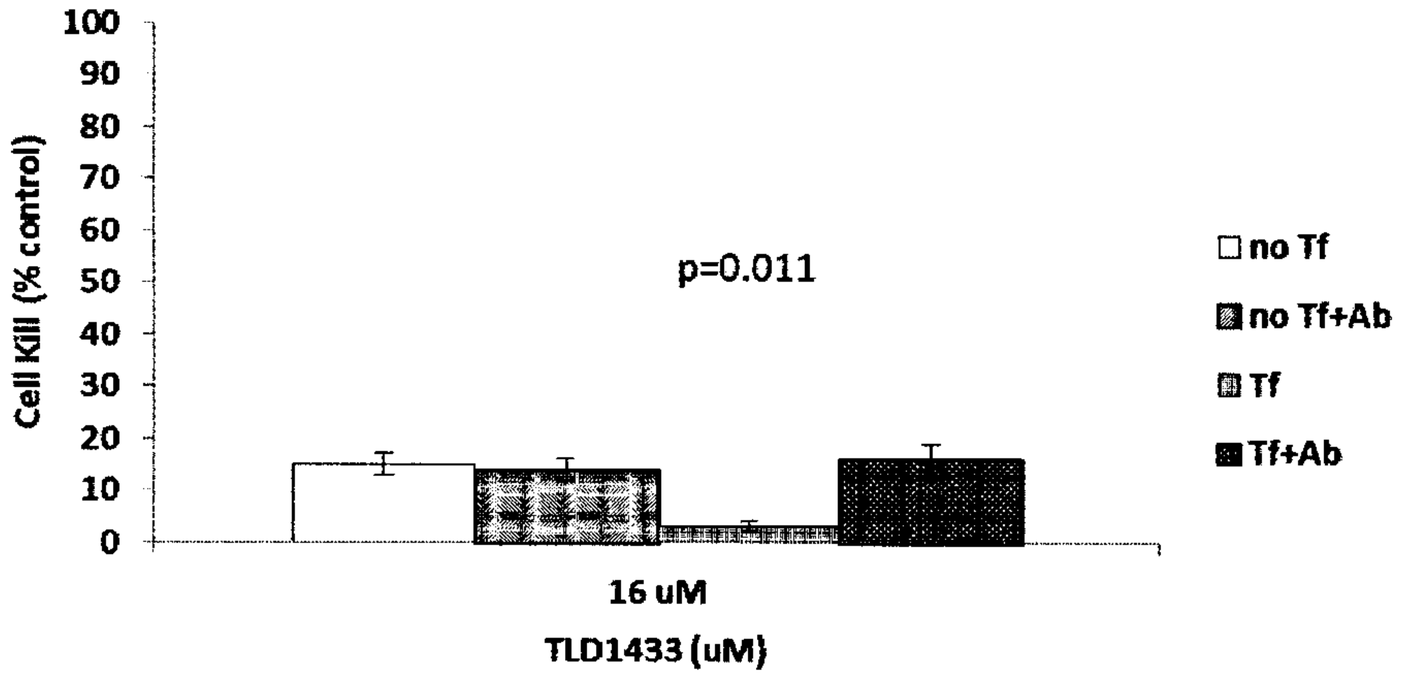


FIG. 20B

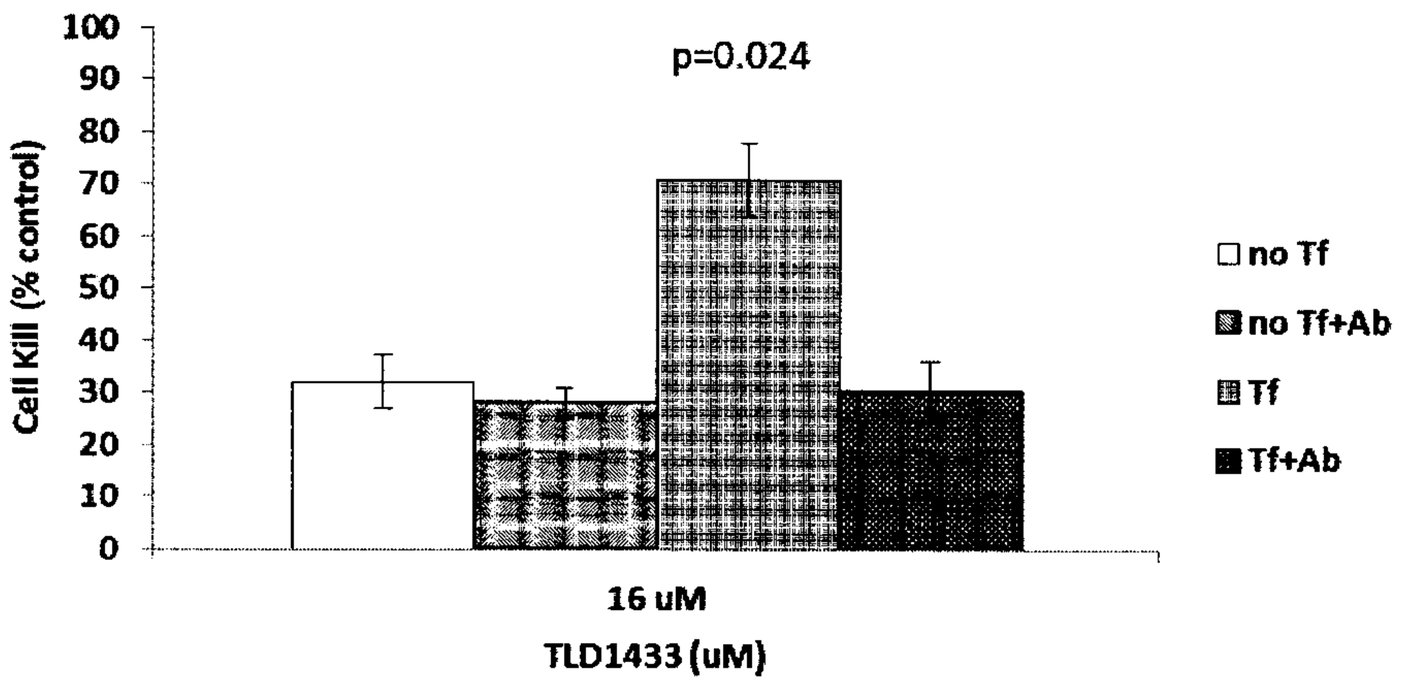


FIG. 21A

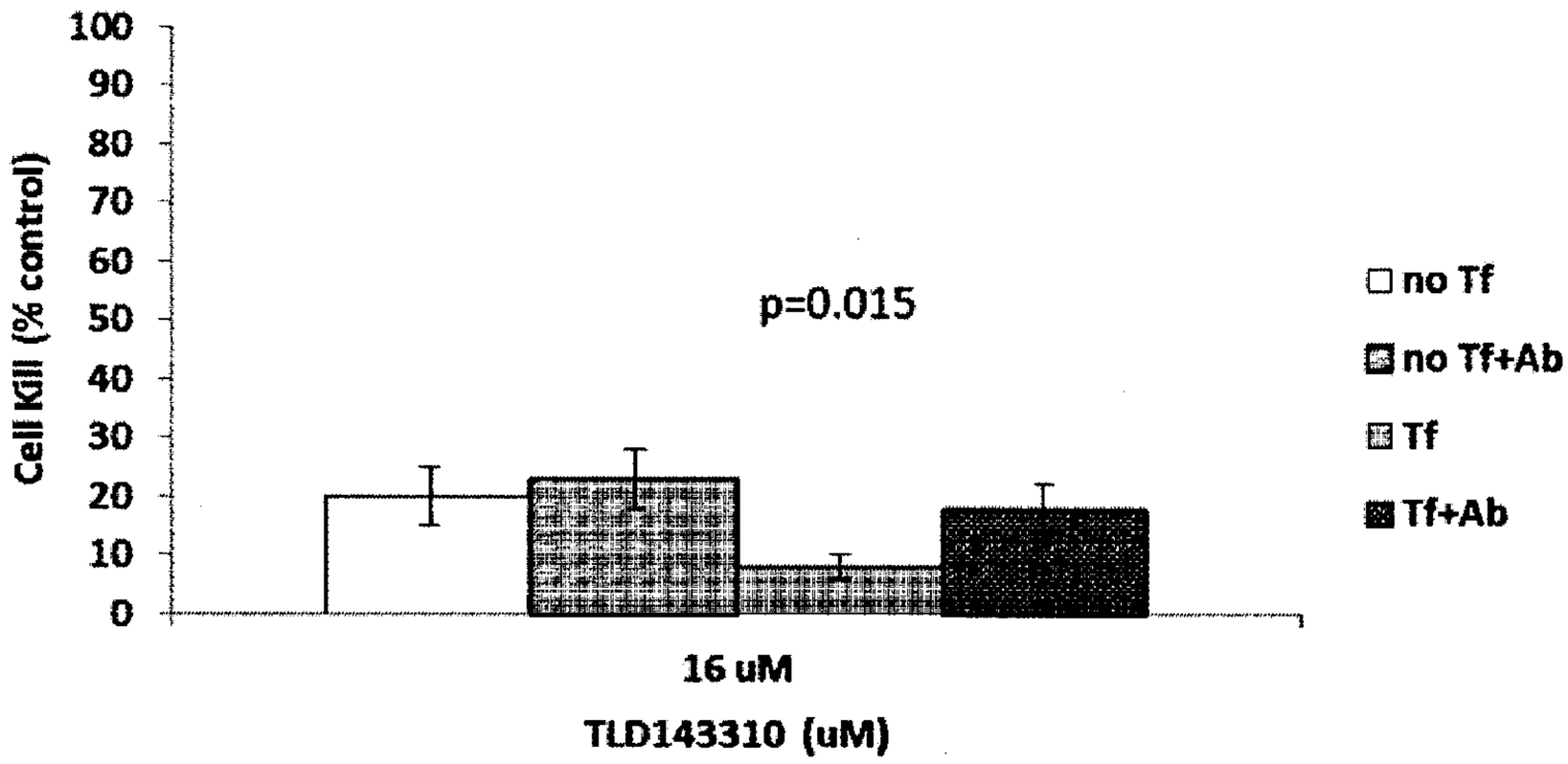


FIG. 21B

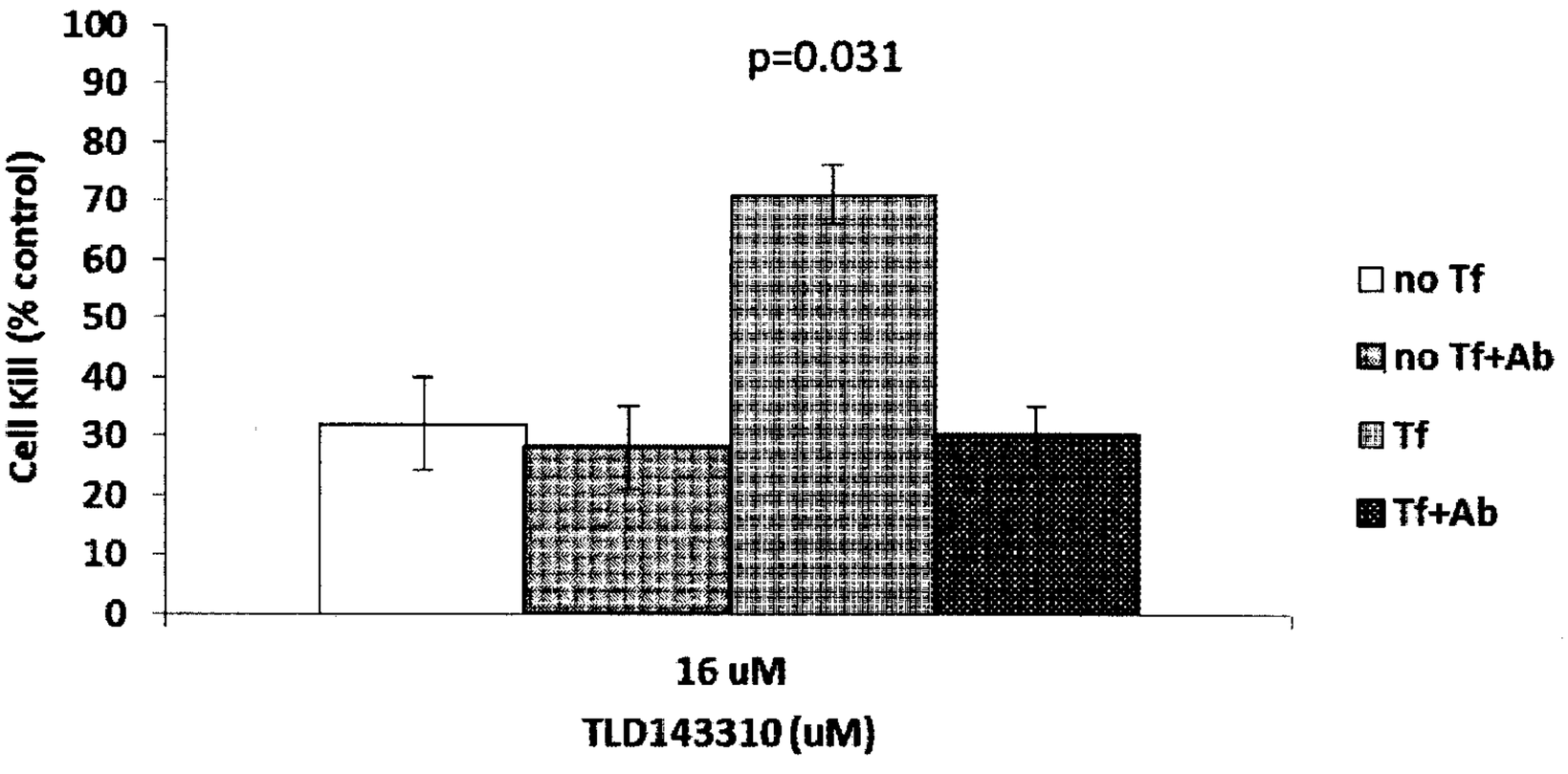


FIG. 22A

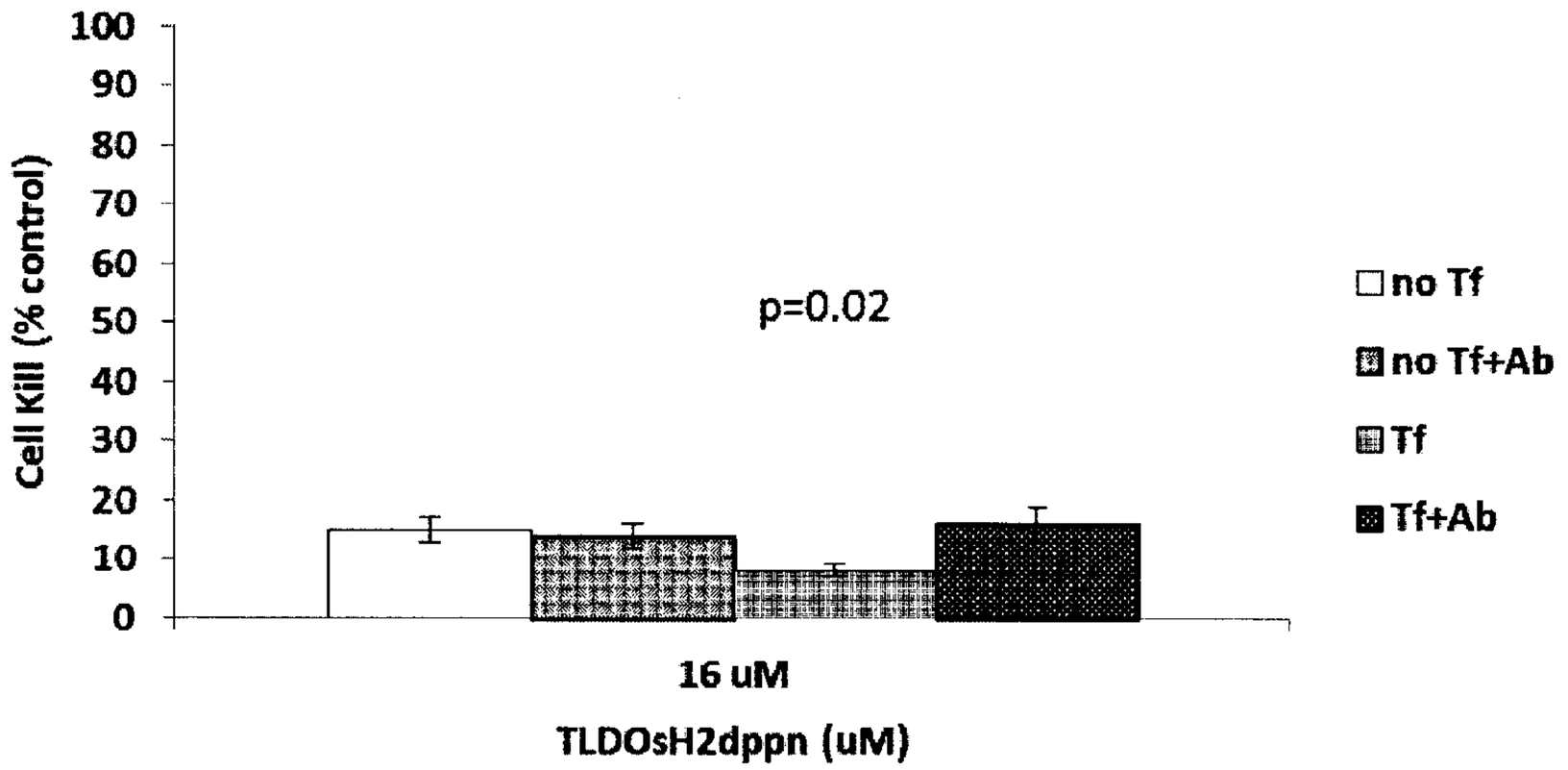


FIG. 22B

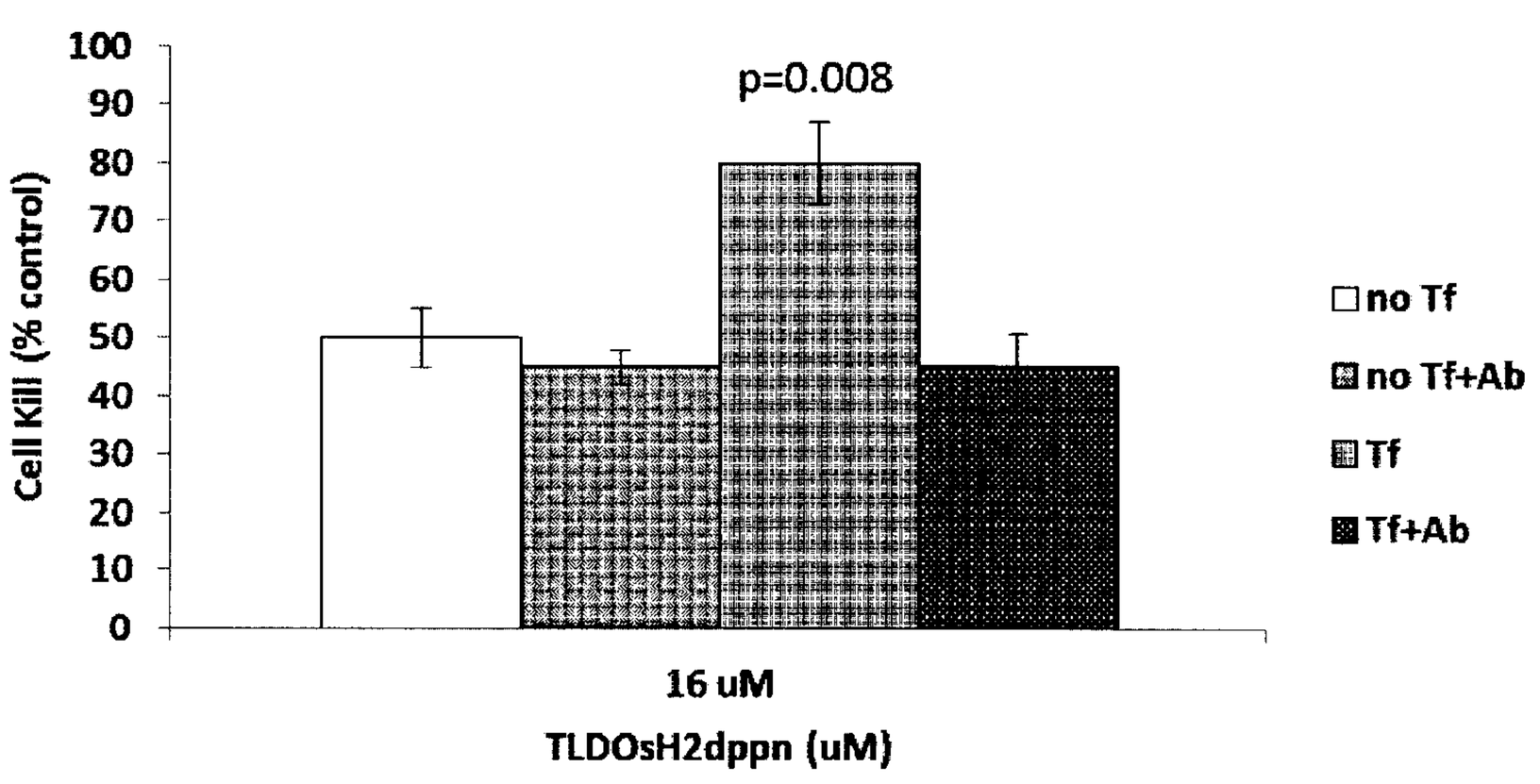


FIG. 23A

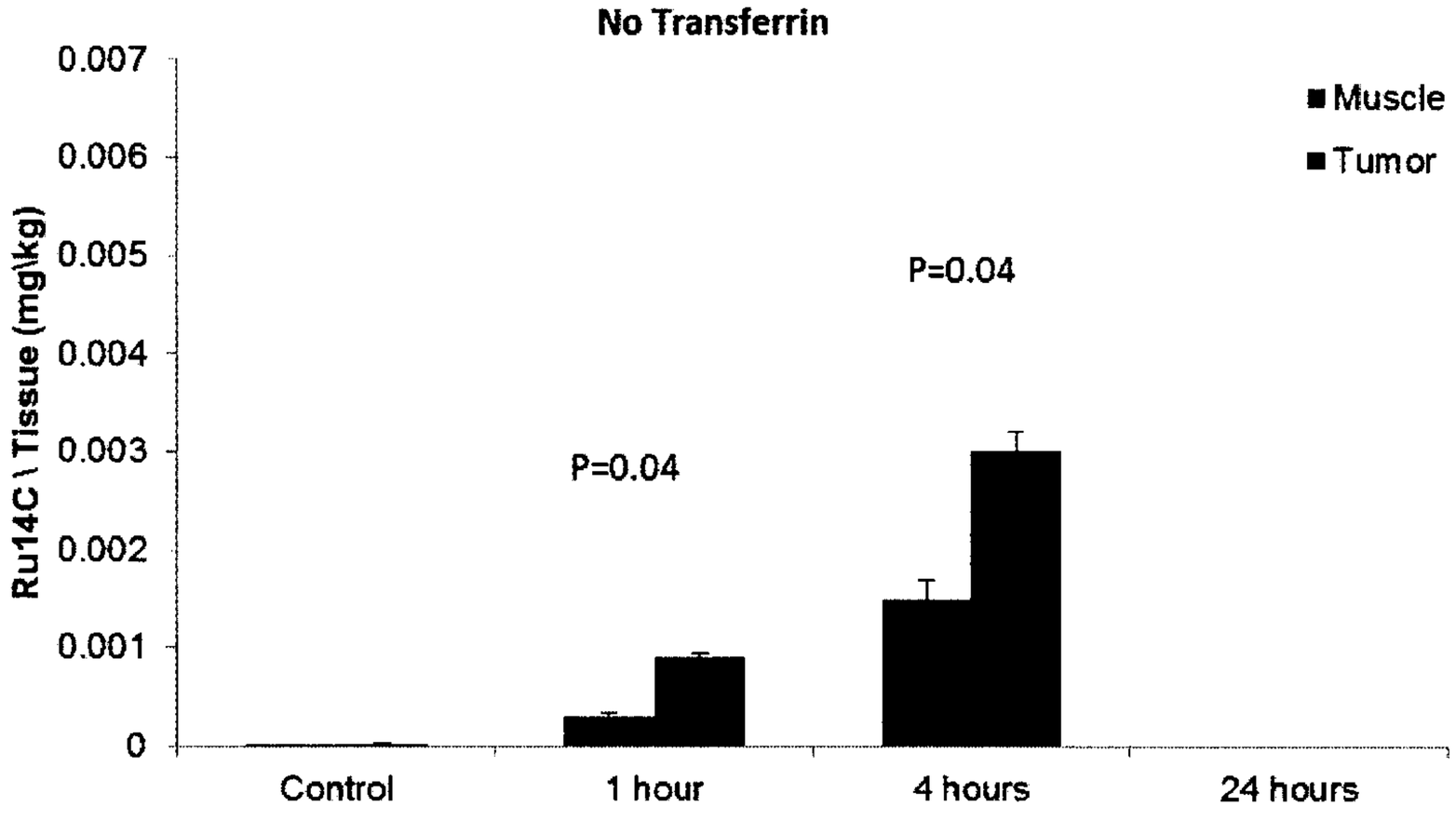


FIG. 23B

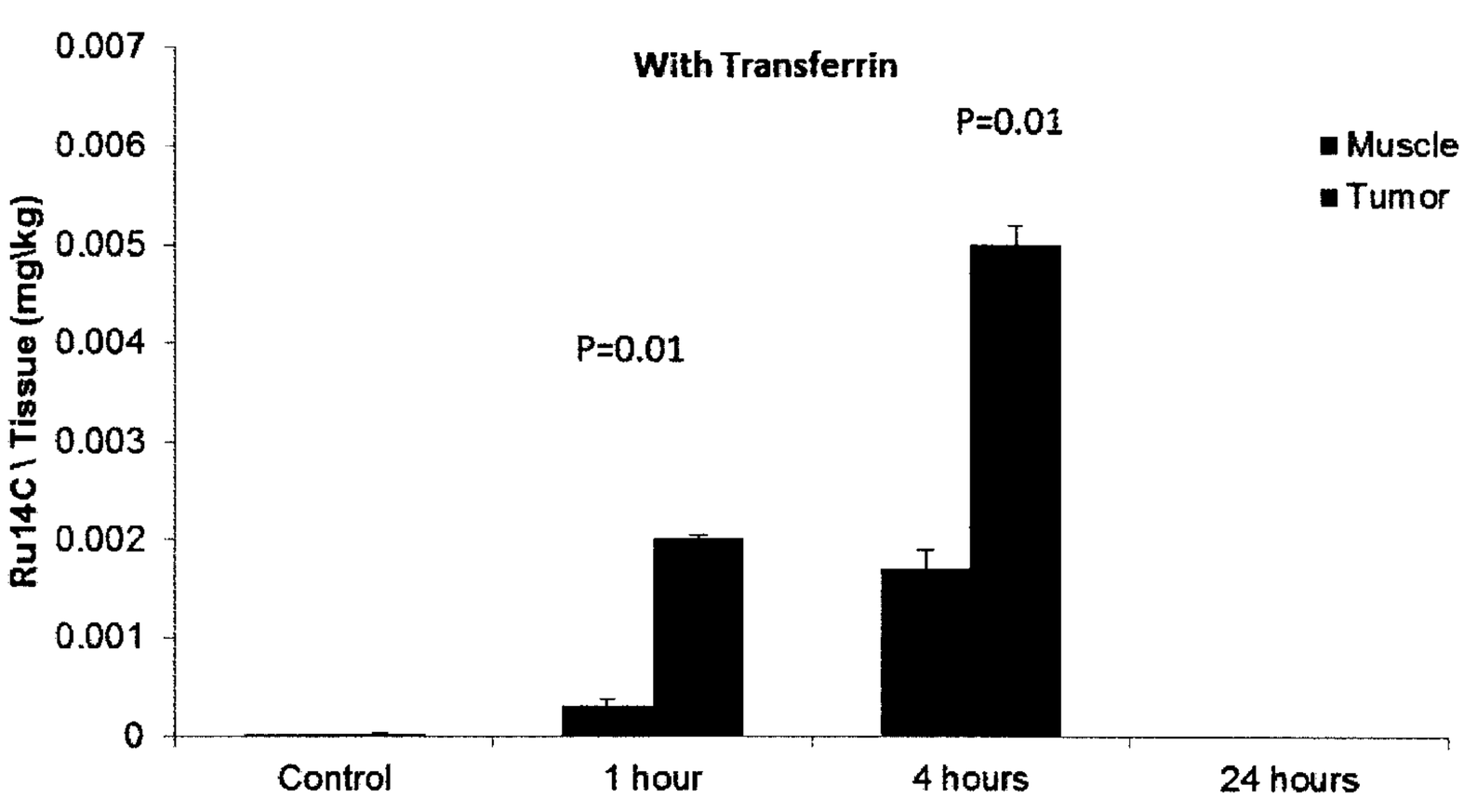


FIG. 24A

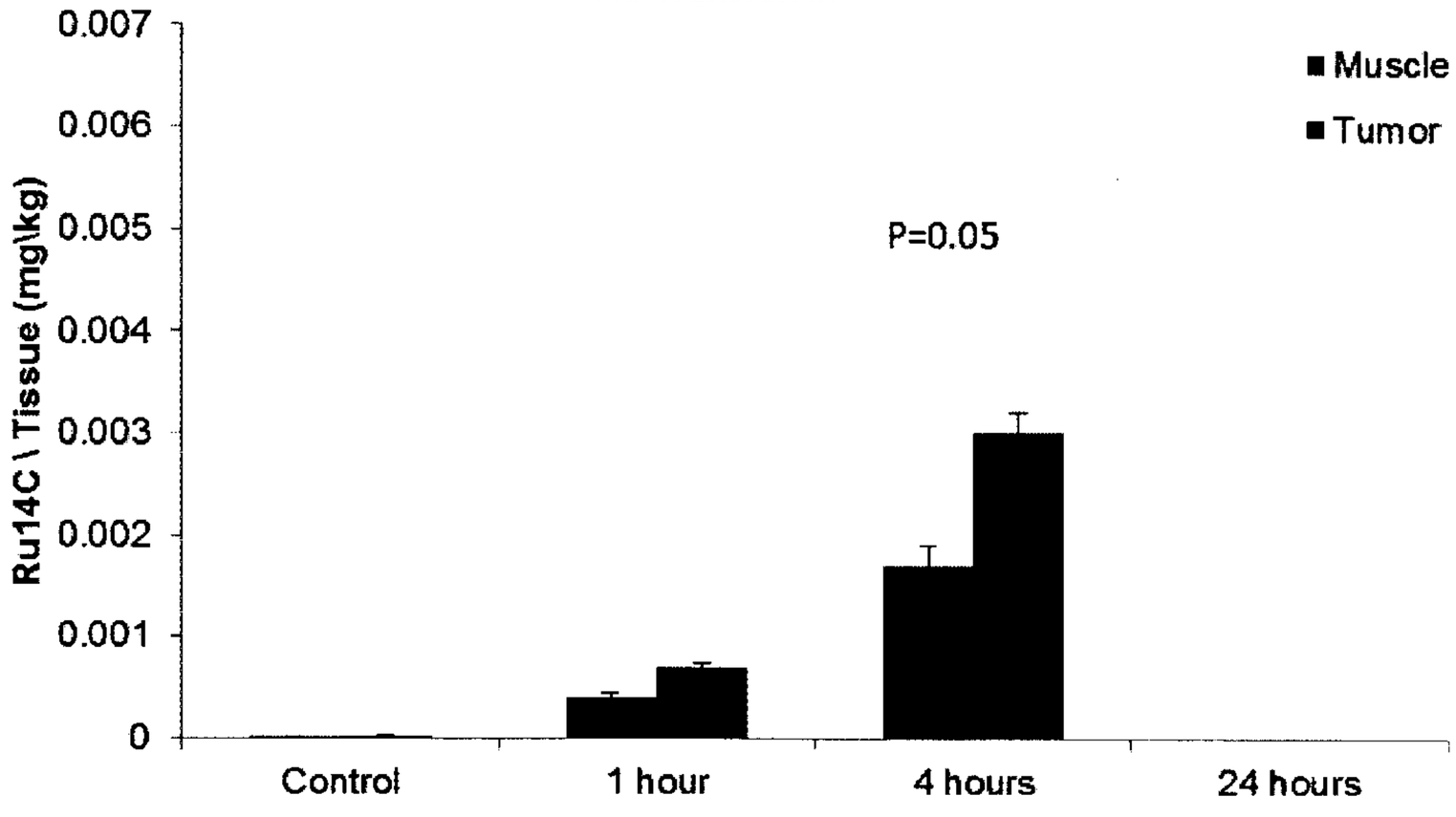


FIG. 24B

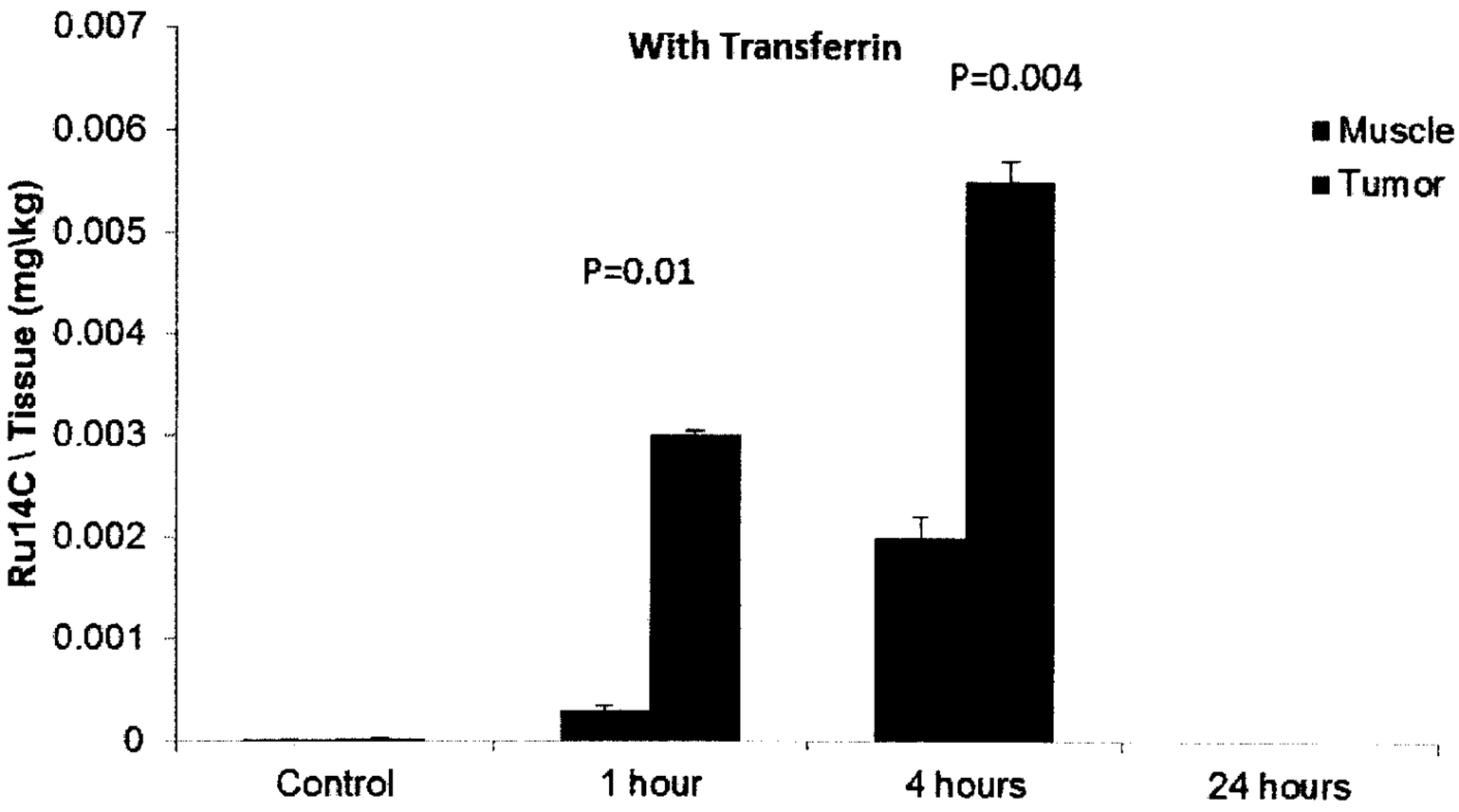


FIG. 25A

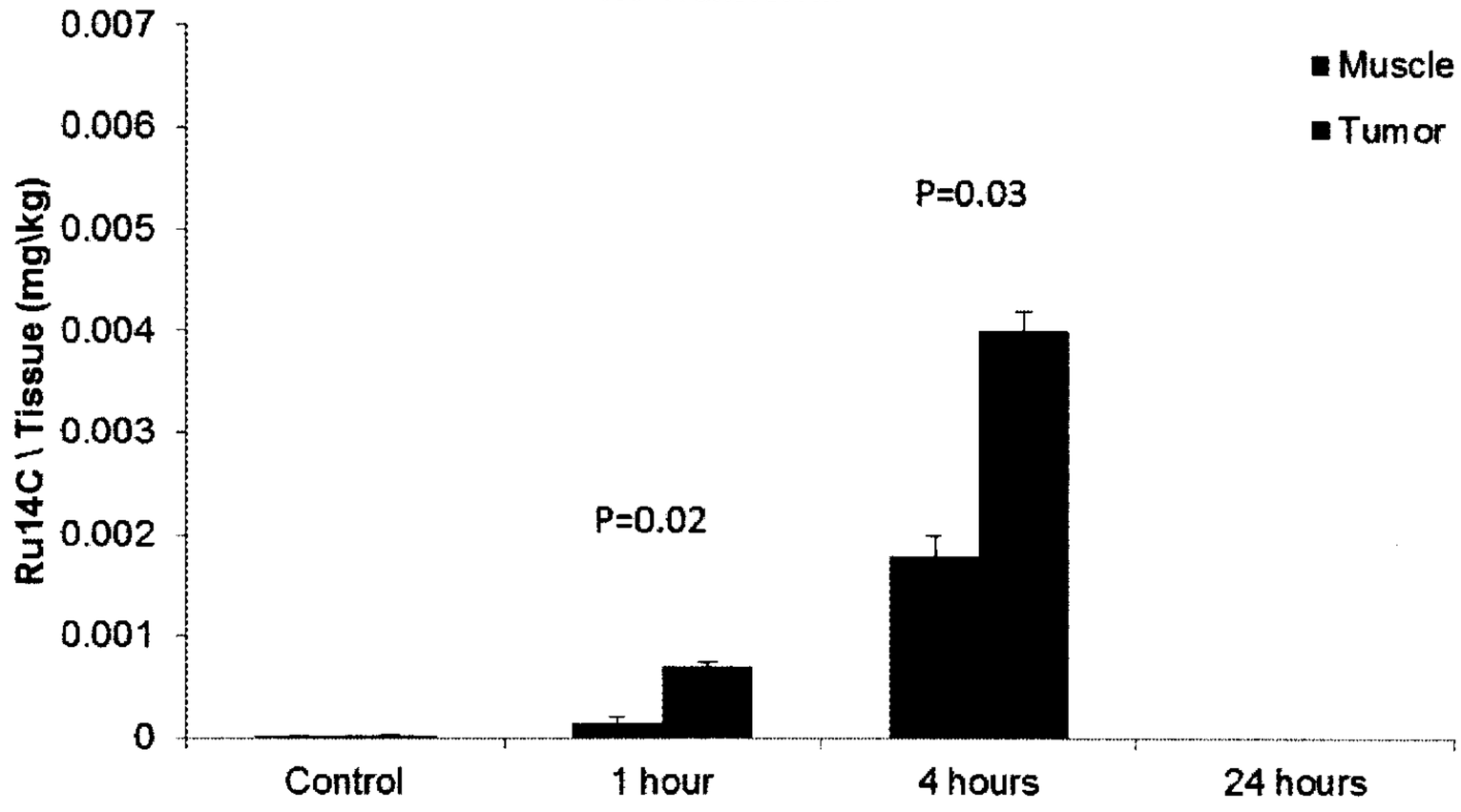


FIG. 25B

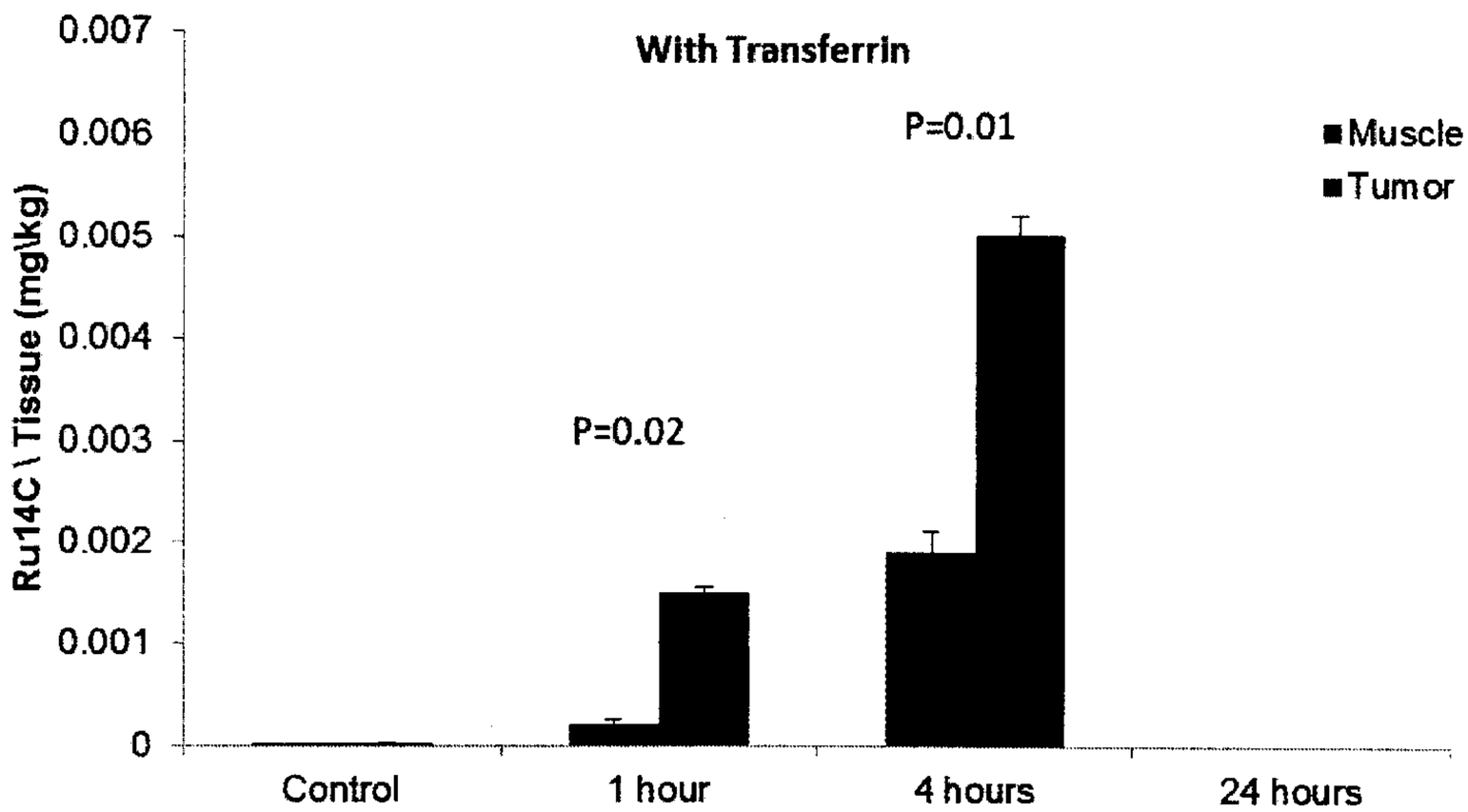


FIG. 26

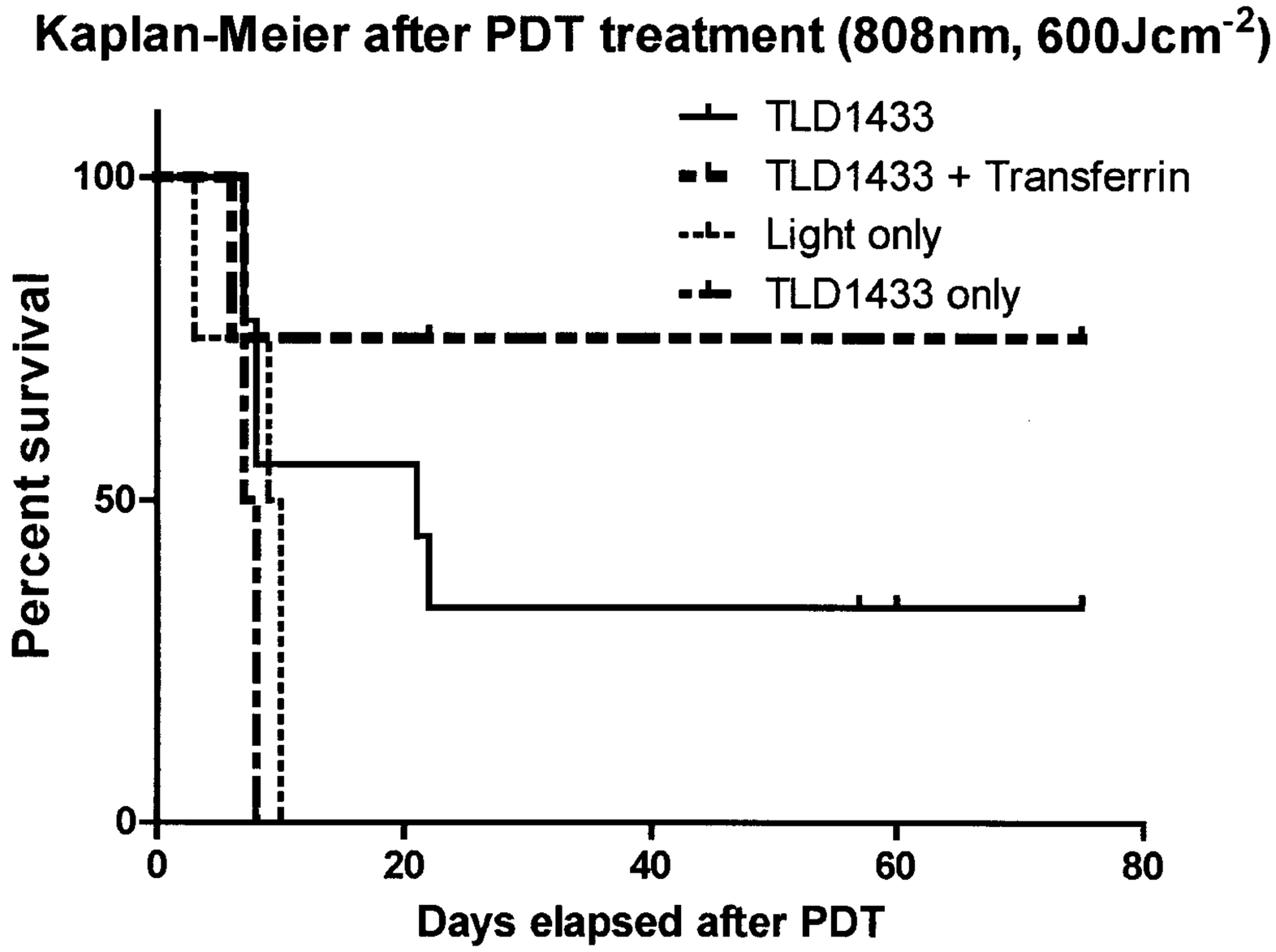

Kaplan-Meier after PDT treatment (808nm, 600Jcm-2)
TLD1433
TLD1433 + Transferrin
Light only
TLD1433 only
Percent survival
100
50
0
0
20
40
60
80
Days elapsed after PDT

FIG. 27

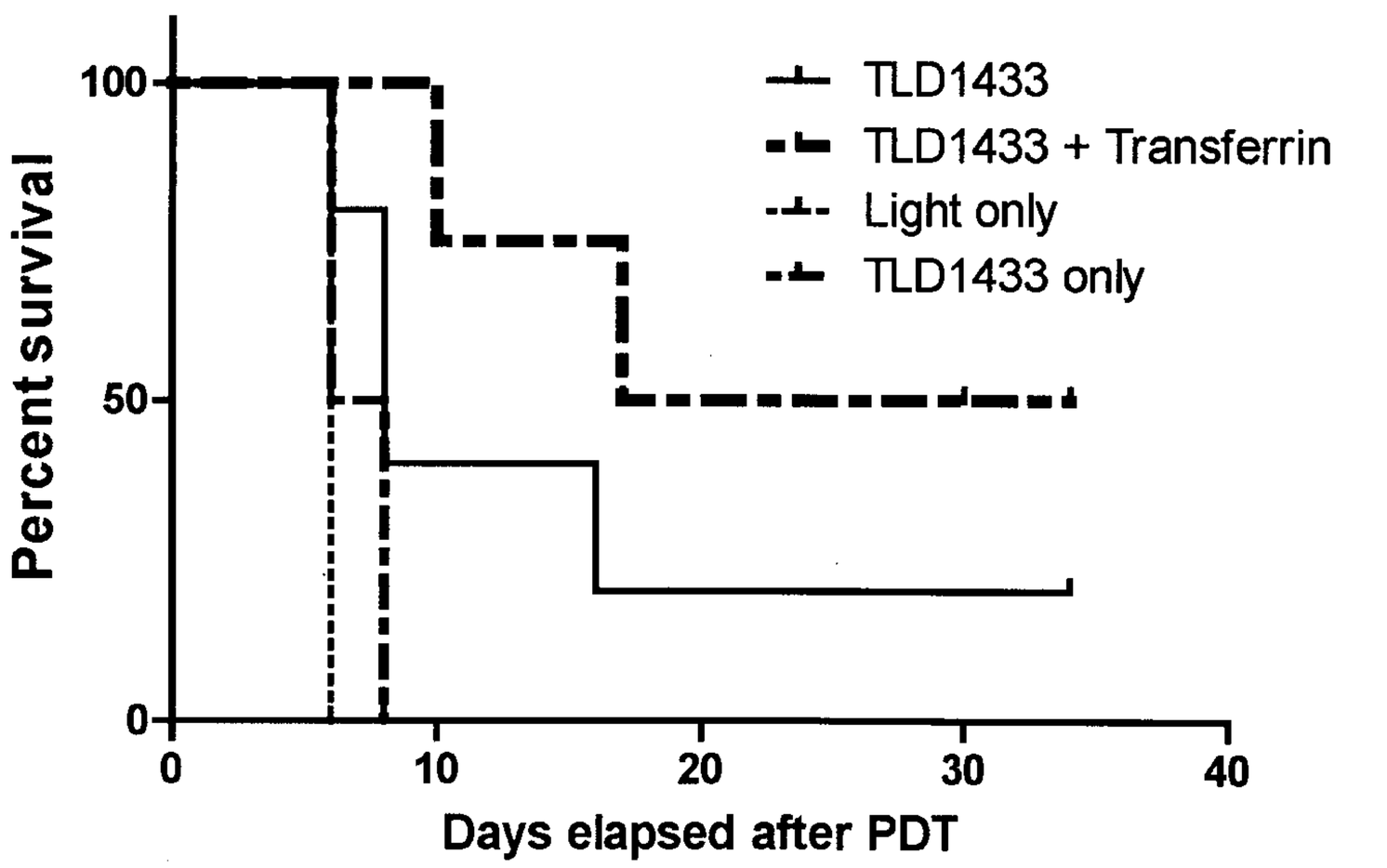

Kaplan-Meier after PDT treatment (635nm, 192Jcm-2)
TLD1433
TLD1433 + Transferrin
Light only
TLD1433 only
Percent survival
100
50
0
0
10
20
30
40
Days elapsed after PDT

FIG. 28

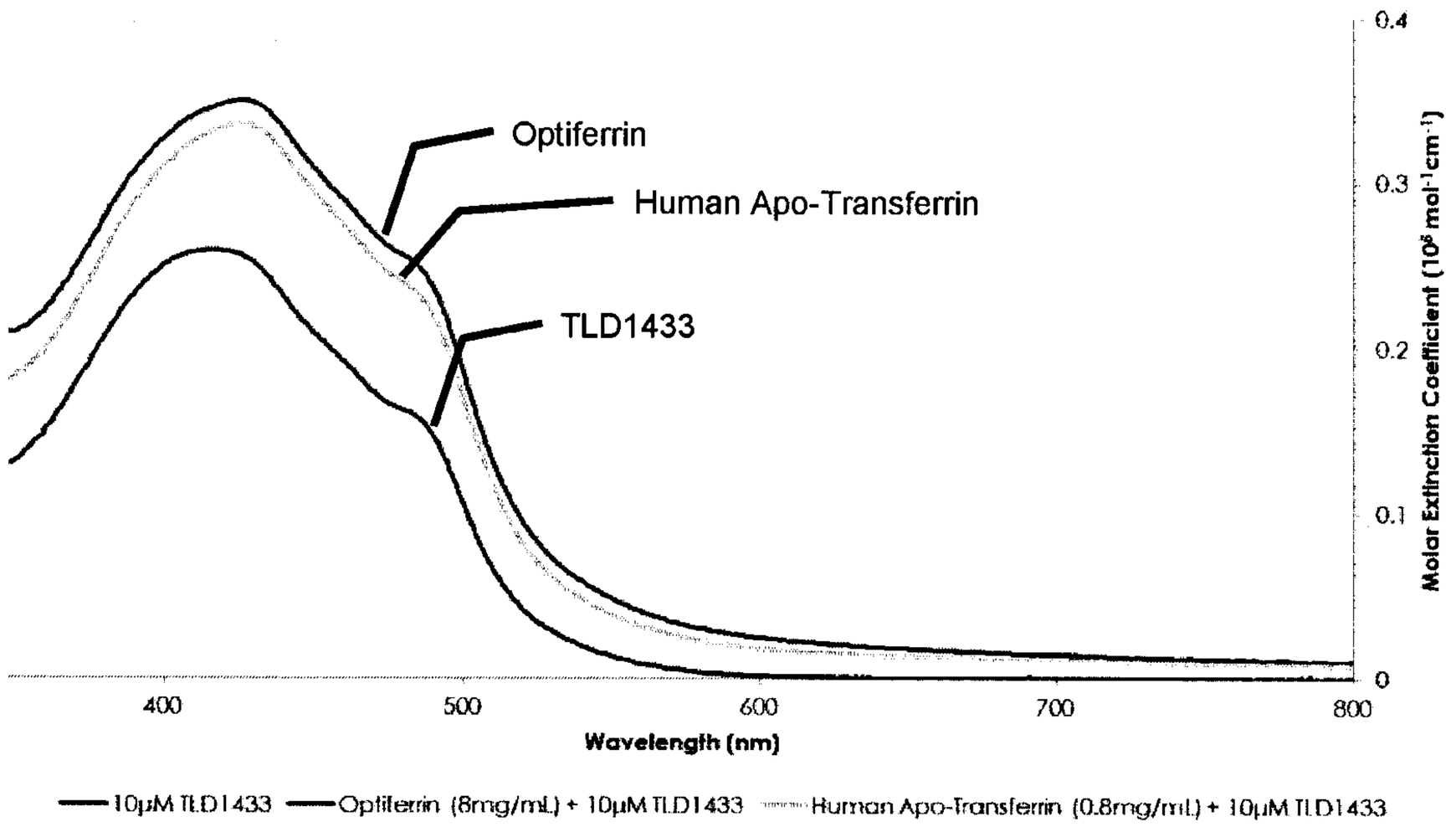

Optiferrin
Human Apo-Transferrin
TLD1433
0.4
0.3
0.2
0.1
0
Molar Extinction Coefficient (10⁵ mol⁻¹cm⁻¹)
400
500
600
700
800
Wavelength (nm)
10µM TLD1433
Optiferrin (8mg/mL) + 10µM TLD1433
Human Apo-Transferrin (0.8mg/mL) + 10µM TLD1433

FIG. 29

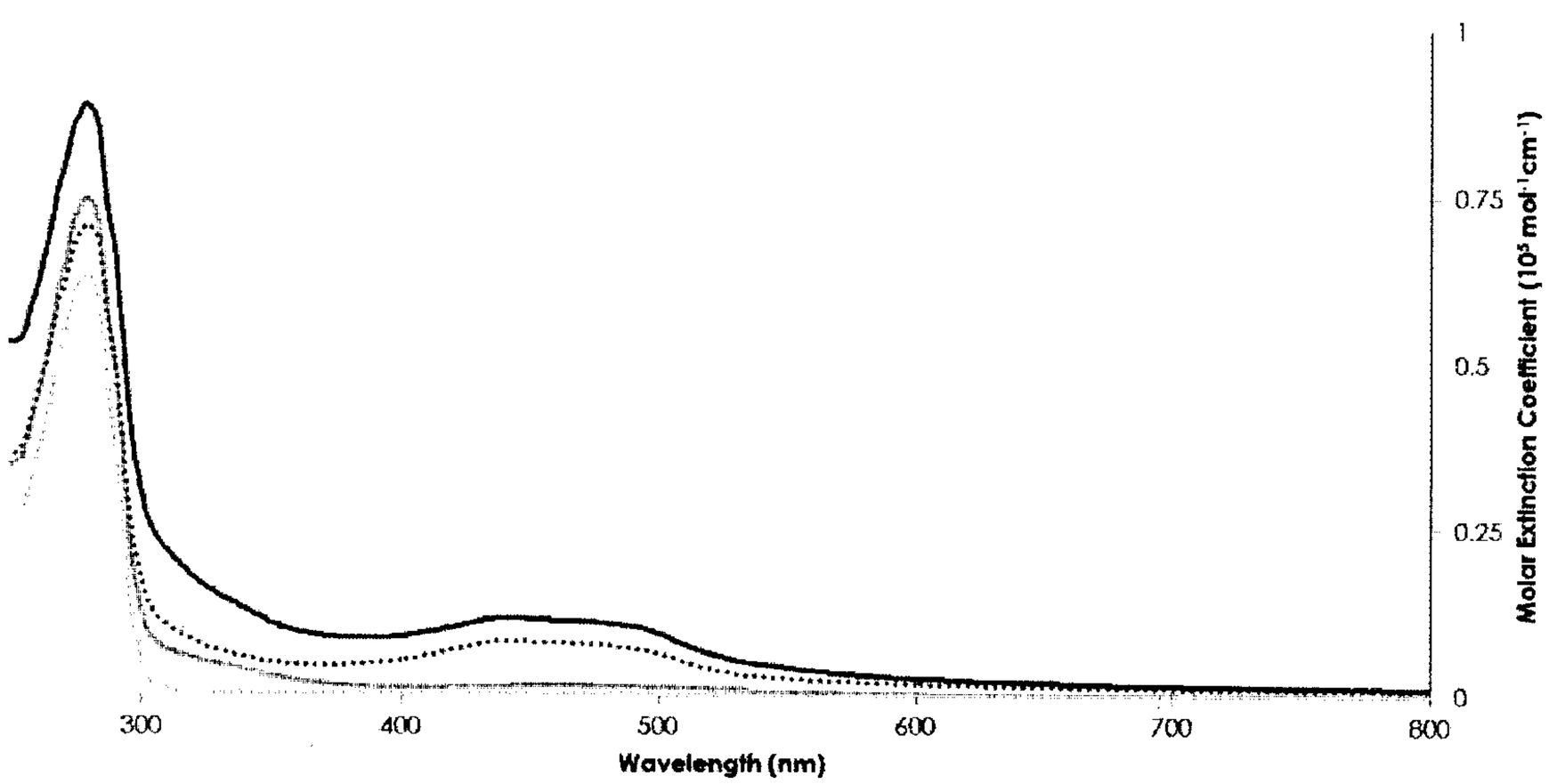

1
0.75
0.5
0.25
0
Molar Extinction Coefficient (10⁵ mol⁻¹cm⁻¹)
300
400
500
600
700
800
Wavelength (nm)
Optiferrin (0.8mg/mL)
Human Apo-Transferrin(0.8mg/mL)
Optiferrin (0.8mg/mL) + 10µM TLD1433
Human Apo-Treansferrin (0.8mg/mL) + 10µM TLD1433

FIG. 30

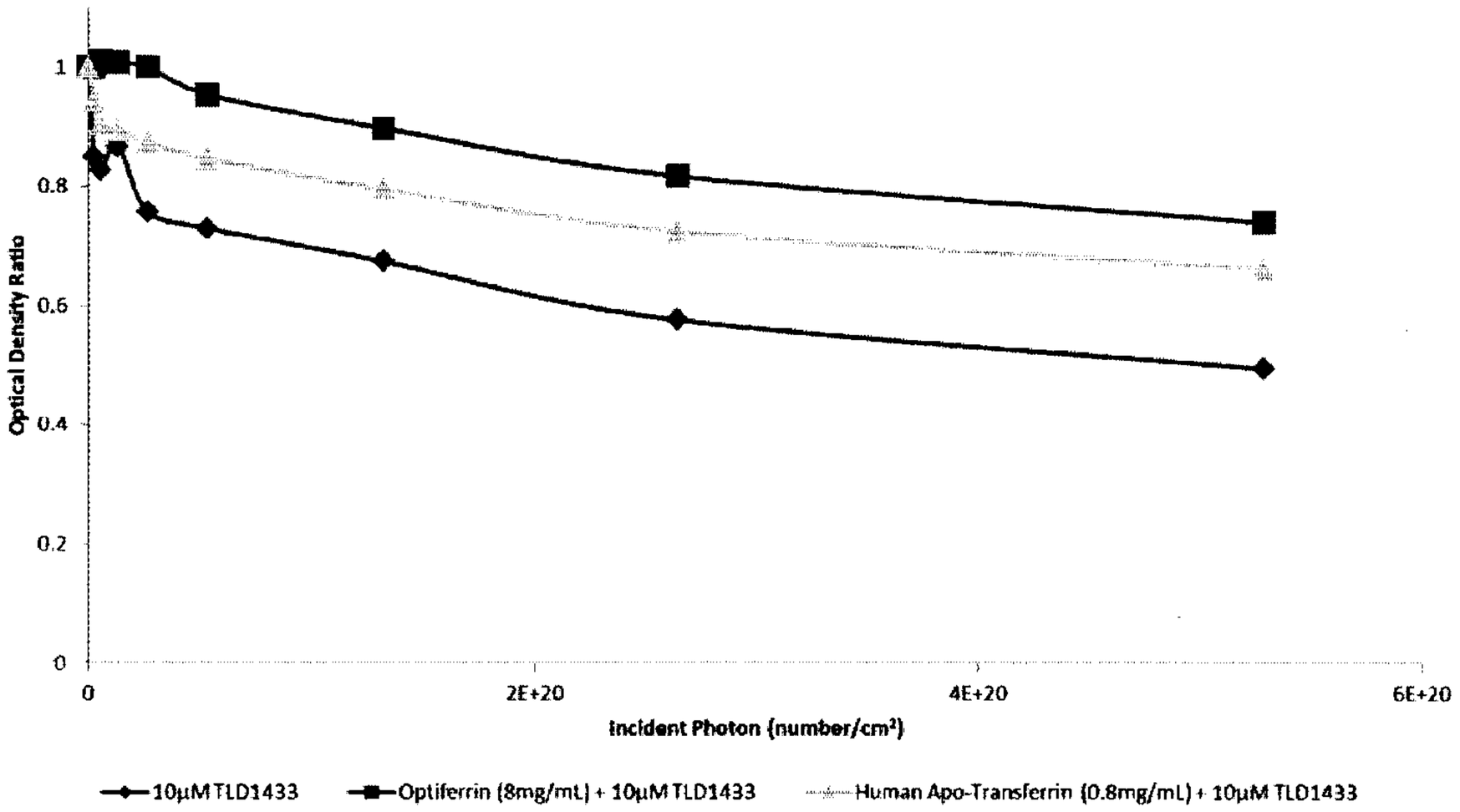


FIG. 31

10μM photosensitisers binding with 10μM Optiferrin in pH 7.4 HPO_4 buffer

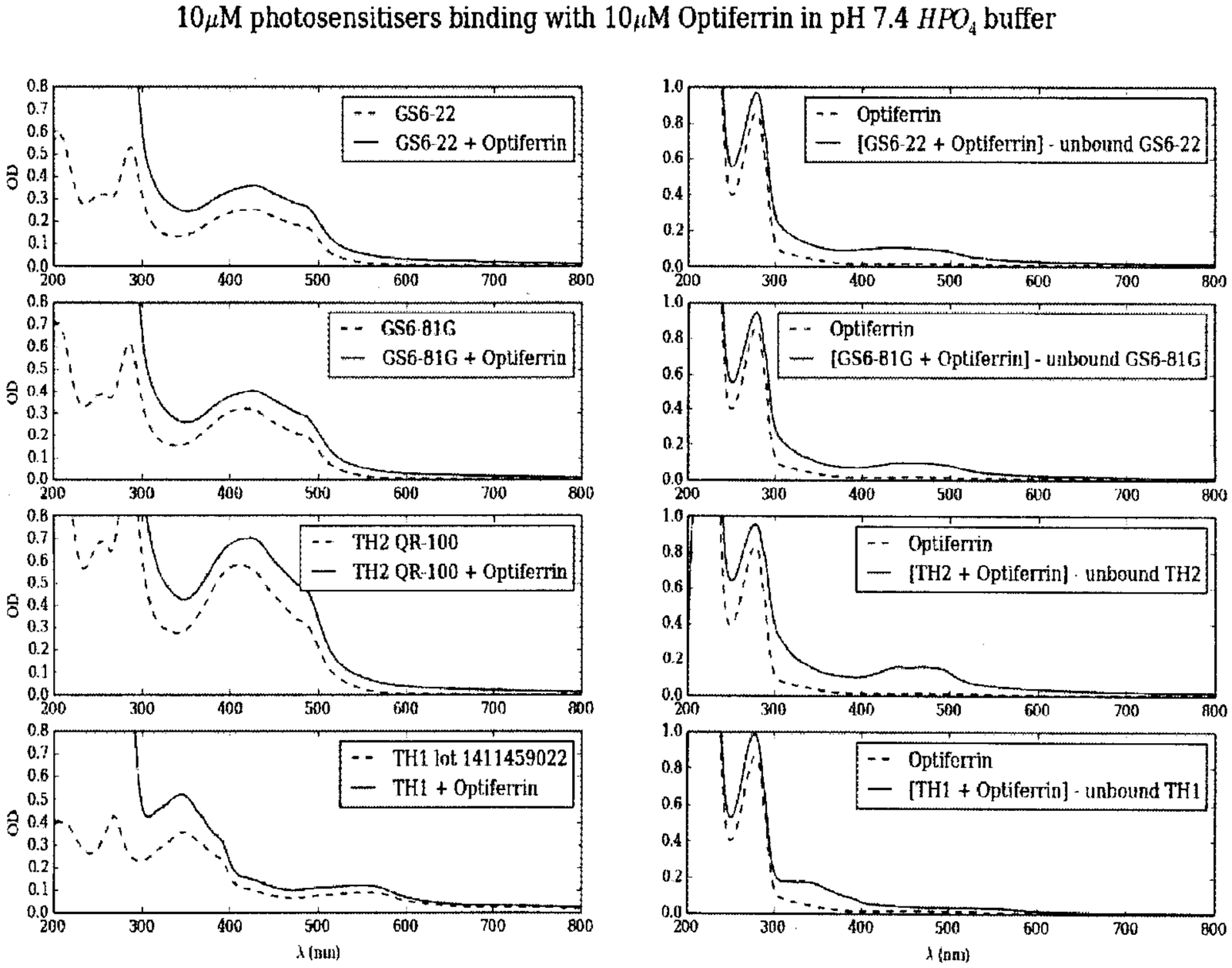


FIG. 32

Photobleaching 10μM TLD1433 with 200 Joules +/- 0.8g/L Optiferrin

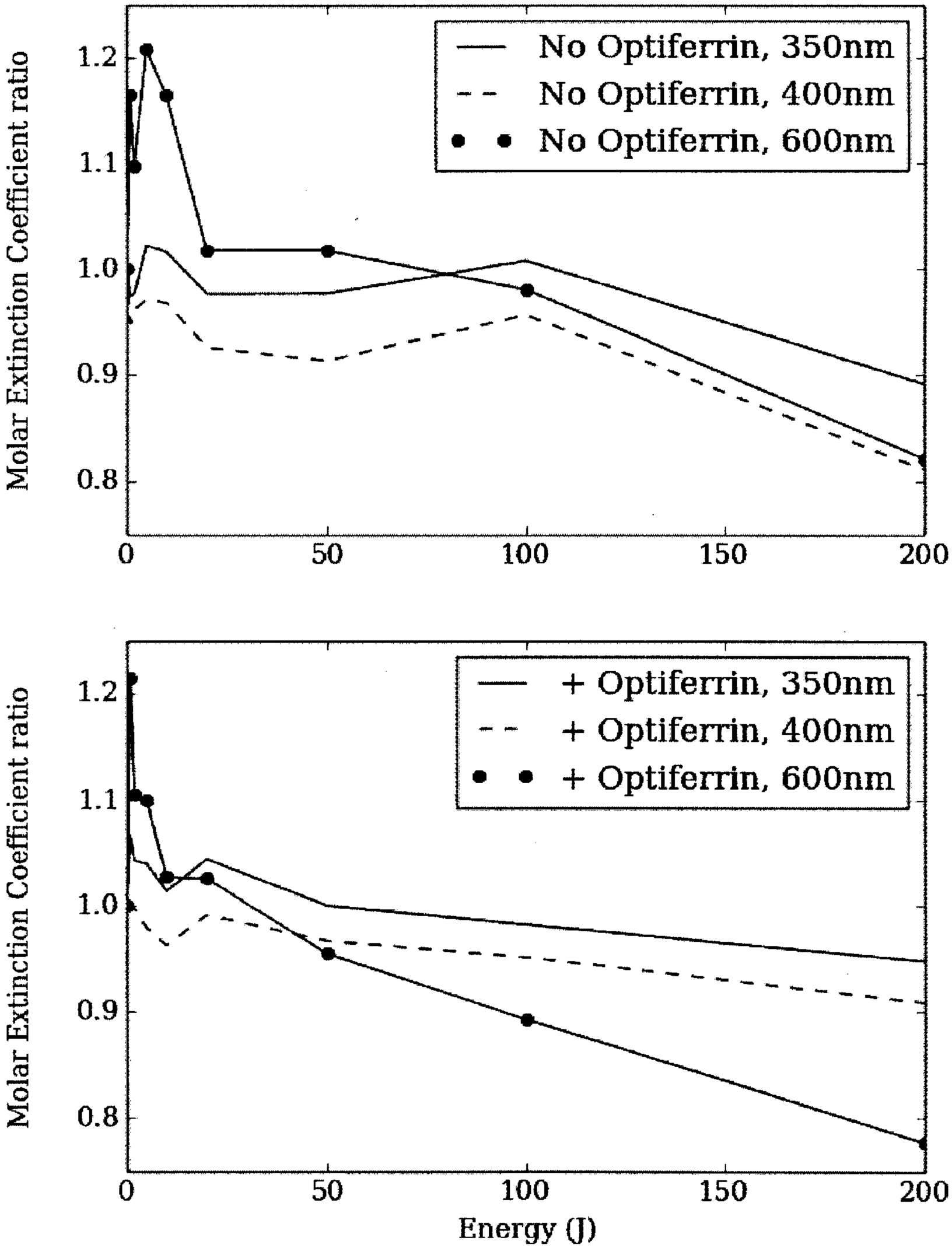


FIG. 33

Infrared absorbance stability of 10μM TLD1433 GS6-81G in pH 7.4 HPO_4 buffer, 10μM Optiferrin, and 10μM apo-hTf

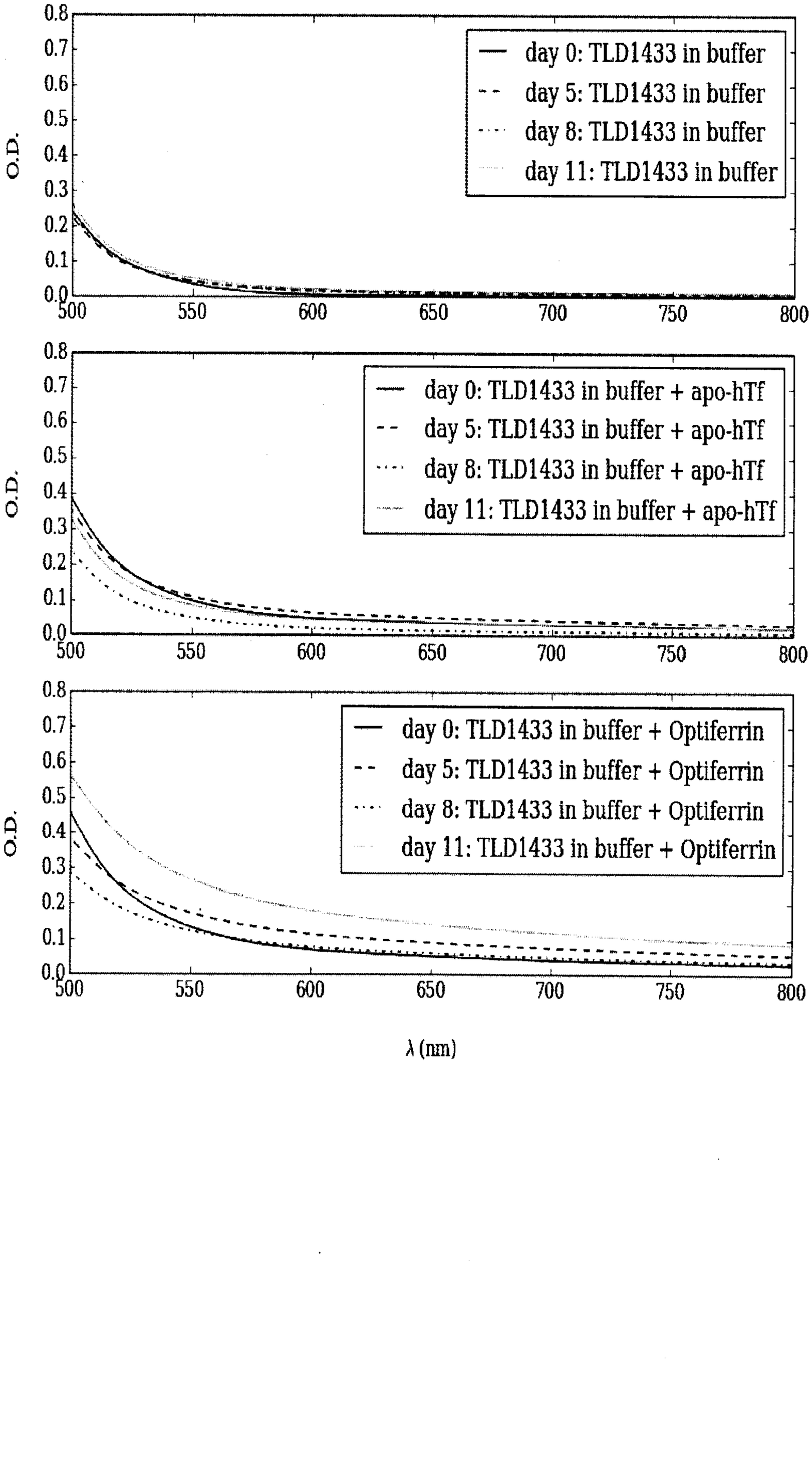
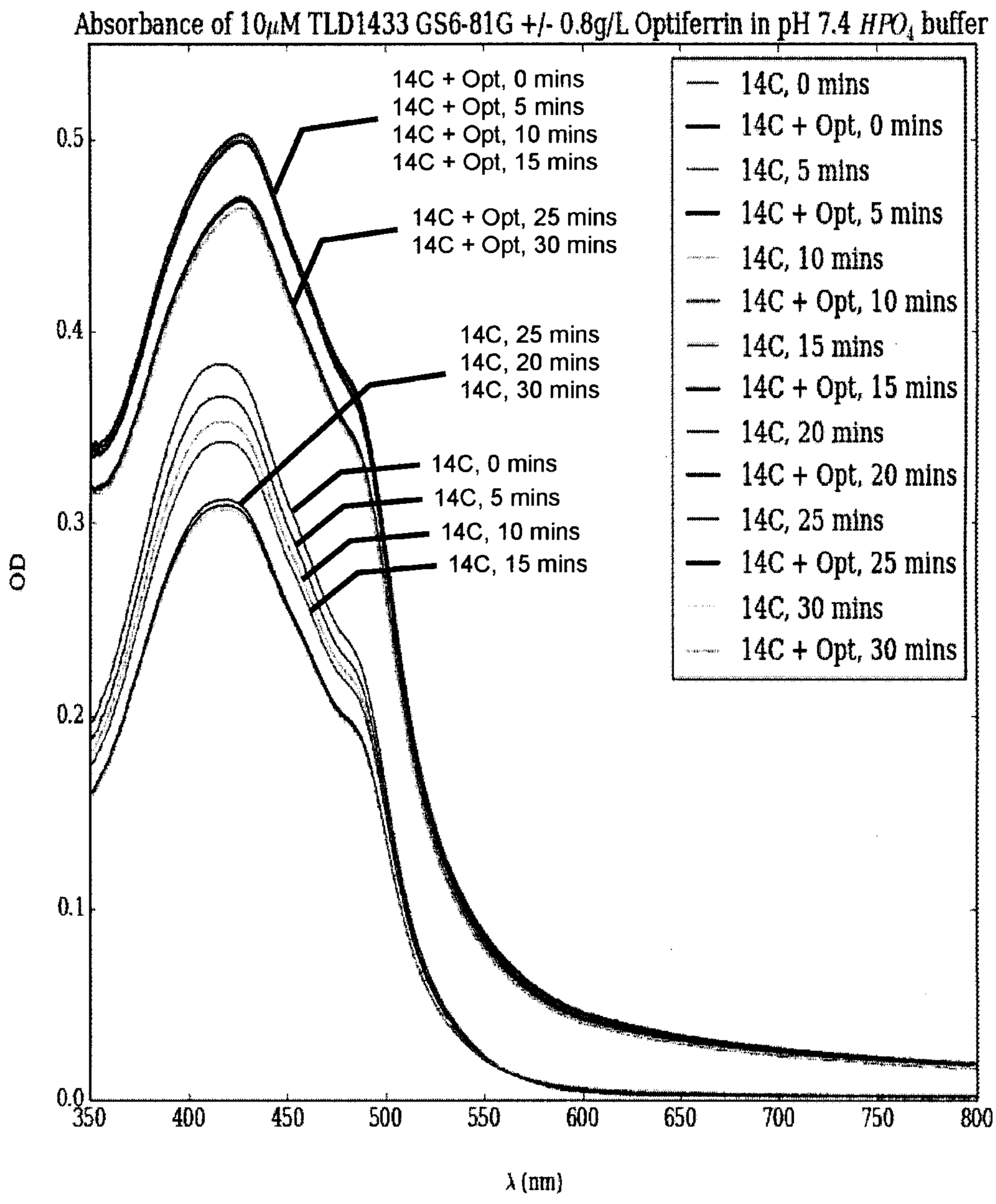


FIG. 34


Absorbance of 10μM TLD1433 GS6-81G +/- 0.8g/L Optiferrin in pH 7.4 HPO_4 buffer
14C + Opt, 0 mins
14C + Opt, 5 mins
14C + Opt, 10 mins
14C + Opt, 15 mins
14C + Opt, 25 mins
14C + Opt, 30 mins
14C, 25 mins
14C, 20 mins
14C, 30 mins
14C, 0 mins
14C, 5 mins
14C, 10 mins
14C, 15 mins
14C, 0 mins
14C + Opt, 0 mins
14C, 5 mins
14C + Opt, 5 mins
14C, 10 mins
14C + Opt, 10 mins
14C, 15 mins
14C + Opt, 15 mins
14C, 20 mins
14C + Opt, 20 mins
14C, 25 mins
14C + Opt, 25 mins
14C, 30 mins
14C + Opt, 30 mins
OD
0.0
0.1
0.2
0.3
0.4
0.5
350
400
450
500
550
600
650
700
750
800
λ (nm)