(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 January 2009 (08.01.2009)

PCT

(10) International Publication Number
WO 2009/005509 A1

(51) International Patent Classification:
A61K 51/04 (2006.01) *A61P 43/00* (2006.01)
A61B 8/12 (2006.01)

(21) International Application Number:
PCT/US2007/017885

(22) International Filing Date: 14 August 2007 (14.08.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/822,448 15 August 2006 (15.08.2006) US

(71) Applicant *(for all designated States except US)*: **CELLECTAR, LLC** [US/US]; 455 Science Drive, Suite 100, Madison, WI 53711 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **WEICHERT, Jamey** [US/US]; 2765 Rosellen Avenue, Fitchburg, WI 53711-6506 (US). **LONGINO, Marc** [US/US]; 311 Ridge View Trail, Verona, WI 53593-8353 (US). **PINCHUK, Anatoly** [RU/US]; 5212 Nannyberry Drive, Madison, WI 53711-7301 (US). **MUEHLER, Andreas** [US/US]; 13 Laurel Hill Road, Mountain Lakes, NJ 07046 (US).

(74) Agent: **POLYAKOV, Mark**; Wood Phillips, 500 West Madison Street, Suite 3800, Chicago, IL 60661 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

WO 2009/005509 A1

(54) Title: NEAR INFRARED-FLUORESCENCE USING PHOSPHOLIPID ETHER ANALOG DYES IN ENDOSCOPIC APPLICATIONS

(57) Abstract: The present invention provides composition and methods of use of phospholipid dyes for use in detection of neoplastic tissue, typically using the routing procedure of endoscopy and methods of optimizing therapy treatment in a subject.

INVENTION TITLE

NEAR INFRARED-FLUORESCENCE USING PHOSPHOLIPID ETHER ANALOG DYES IN ENDOSCOPIC APPLICATIONS

DESCRIPTION

[Para 1] RELATED FIELD

[Para 2] The invention generally relates to phospholipid ether (PLE) analogs for diagnosis of neoplasia, in particular, the invention relates to use of phospholipid ether dyes in endoscopic application using near infrared fluorescence.

[Para 3] BACKGROUND

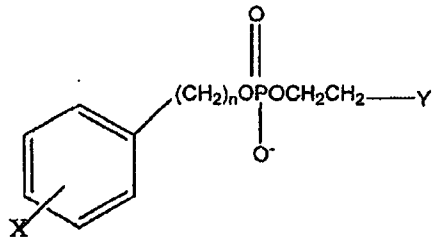
[Para 4] Endoscopy, in particular colonoscopy and bronchoscopy, is utilized to find abnormal growth and tumors protruding into the lumen. A device, called endoscope, is inserted into a body cavity. Traditionally, endoscopes use a daylight channel, i.e. the observer sees all finding at the wavelength of naturally occurring light.

[Para 5] Lately, newer endoscopes have the ability to utilize several channels, i.e. using a daylight channel and one or more additional channels at other light wavelengths. These additional channels are used to monitor either naturally occurring fluorescence or fluorescence of a dye that was either injected into the body or sprayed onto the body cavity surface. One of the possible channels is in the NIR (near infrared) area. The advantage of the NIR area is that the light absorption in the NIR area (usually 600-800 nm) is minimal, and fluorescence can be detected at a depth of a few millimeters to nearly a centimeter beneath the surface of the body cavity. It is believed that this has advantages to detect tumors and lymph node metastases in organs such as colon and lung.

[Para 6] Accordingly, the need exists to further explore the uses of near infrared fluorescence in detecting neoplasia during the endoscopic process.

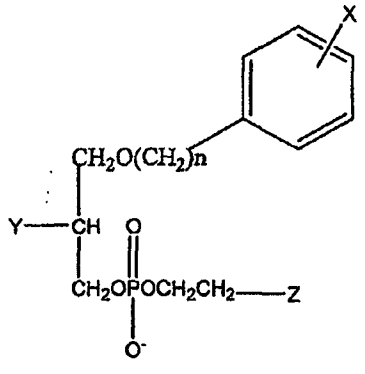
[Para 7] SUMMARY OF THE INVENTION

[Para 8] The invention generally relates to phospholipids ether (PLE) analogs for diagnosis of neoplasia, in particular, the invention relates to use of phospholipid ether dyes in endoscopic application using near infrared fluorescence. In an exemplary embodiment, the present invention provides a phospholipid fluorescent dye, comprising (a) a phospholipid compound of formula I or II



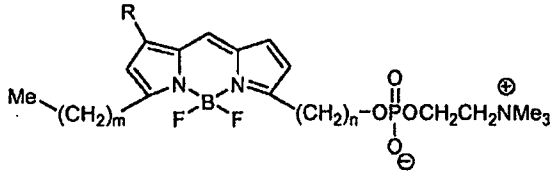
Formula I

[Para 9] where X is a halogen; n is an integer between 8 and 30; and Y is selected from the group comprising NH_2, NR_2, and NR_3, wherein R is an alkyl or arylalkyl substituent or

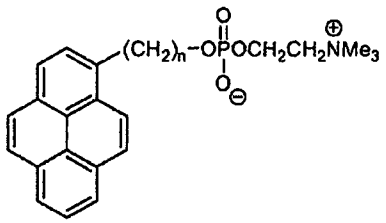


Formula II

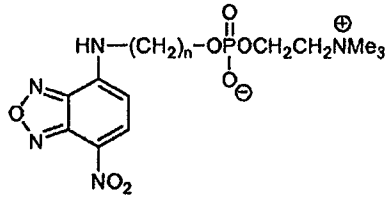
[Para 10] where X is a halogen; n is an integer between 8 and 30; Y is selected from the group consisting of H, OH, COOH, COOR and OR, and Z is selected from the group consisting of NH_2, NR_2, and NR_3, wherein R is an alkyl or arylalkyl substituent; and (b) a fluorescent molecule. In this embodiment, X is selected from the group of radioactive halogen isotopes consisting of ^{18}F, ^{36}Cl, ^{76}Br, ^{77}Br, ^{82}Br, ^{122}I, ^{123}I, ^{124}I, ^{125}I, ^{131}I and ^{211}At. Preferably, the phospholipid compound is18-(p-Iodophenyl)octadecyl phosphocholine, 1-O-[18-(p-Iodophenyl)octadecyl]-1,3-propanediol-3-phosphocholine, or 1-O-[18-(p-Iodophenyl)octadecyl]-2-O-methyl-rac-glycero-3-phosphocholine, wherein iodine is in the form of a radioactive isotope. In yet another exemplary embodiment, the phospholipid dye is selected from the group consisting of



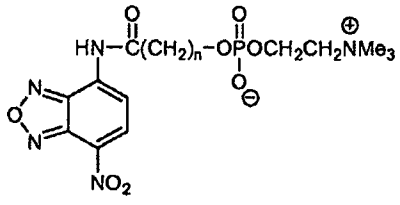
, wherein n is an integer 4 through 21 and m is an integer 0 through17;



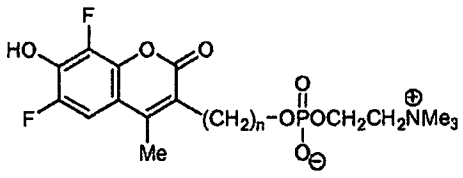
, wherein n is an integer 4 through 22;



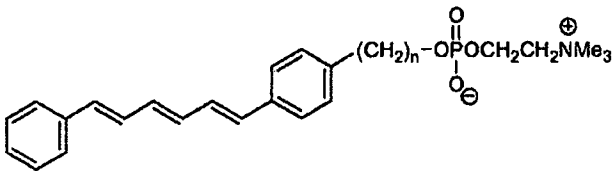
, wherein n is an integer 4 through 22;



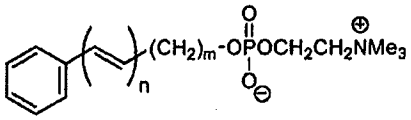
, wherein n is an integer 4 through 21;



, wherein n is an integer 4 through 22;



, wherein n is an integer 3 through 8; and



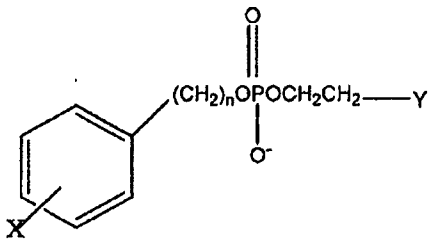
, wherein n is an integer 4 or 5 and m is an integer 4 through 14.

[Para 11] Further, in this embodiment, the fluorescent molecule exhibits fluorescence at a wavelength of about 300 nm to about1000 nm.

[Para 12] Another exemplary embodiment of the invention provides a method for distinguishing a benign structure from a neoplastic tissue in a selected region by using an endoscope have at least two wavelength in a subject comprising the steps of: (a) administering a fluorescently labeled tumor-specific agent to the subject; (b) using a first technique to produce a visualization of the anatomy of the selected region using the first wavelength of an endoscope; (c) using a second technique to produce a visualization of the distribution of fluorescence produced by the fluorescently labeled tumor-specific agent; and (d) comparing the visualization of the anatomy of the selected region by the first wavelength to the visualization of the distribution of fluorescence by the second wavelength produced by the fluorescently labeled tumor-specific agent thereby distinguishing a benign structure from neoplastic tissue. In this embodiment, preferably, the selected region is the gastro-intestinal tract and the respiratory tract.

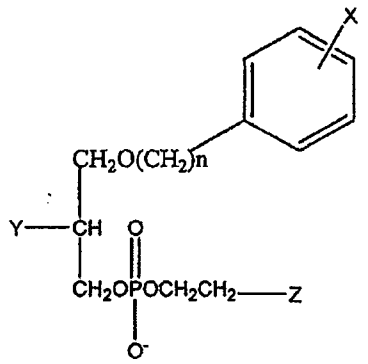
[Para 13] In this embodiment, the first wavelength is about 400 nm to about 800 nm. Also. the second wavelength is about 300 nm to 1000 nm.

[Para 14] Preferably, the fluorescently labeled tumor selective compound is a phospholipid dye, comprising of (a) a phospholipid compound of formula I or II



Formula I

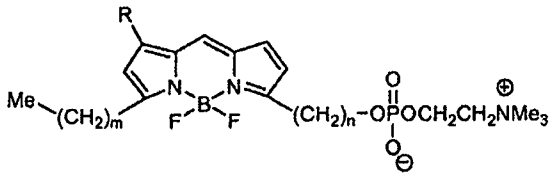
[Para 15] where X is a halogen; n is an integer between 8 and 30; and Y is selected from the group comprising NH_2, NR_2, and NR_3, wherein R is an alkyl or arylalkyl substituent or



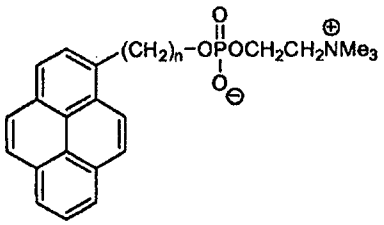
Formula II

[Para 16] where X is a halogen; n is an integer between 8 and 30; Y is selected from the group consisting of H, OH, COOH, COOR and OR, and Z is selected from the group consisting of NH_2, NR_2, and NR_3, wherein R is an alkyl or arylalkyl substituent; and (b) a fluorescent molecule. Further, X is selected from the group of radioactive halogen isotopes consisting of ^{18}F, ^{36}Cl, ^{76}Br, ^{77}Br, ^{82}Br, ^{122}I, ^{123}I, ^{124}I, ^{125}I, ^{131}I and ^{211}At.

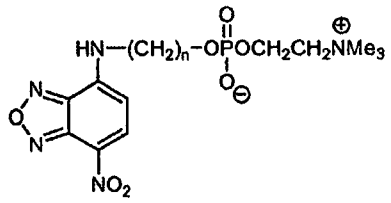
[Para 17] Most preferably, the phospholipid compound is18-(p-Iodophenyl)octadecyl phosphocholine, 1-O-[18-(p-Iodophenyl)octadecyl]-1,3-propanediol-3-phosphocholine, or 1-O-[18-(p-Iodophenyl)octadecyl]-2-O-methyl-rac-glycero-3-phosphocholine, wherein iodine is in the form of a radioactive isotope. Also, preferably, the dye is selected from the group consisting of



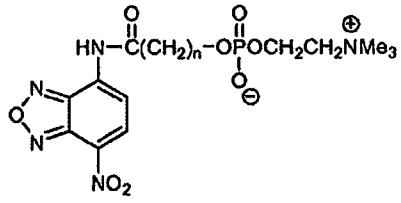
, wherein n is an integer 4 through 21 and m is an integer 0 through17;



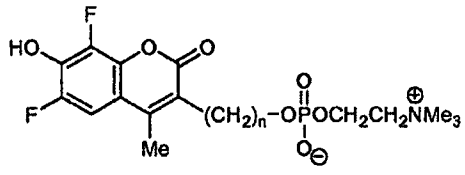
, wherein n is an integer 4 through 22;



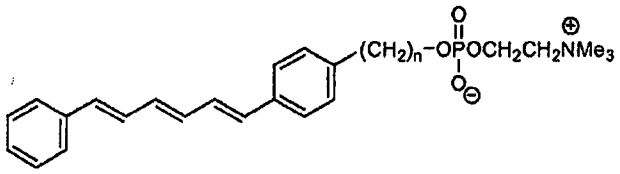
, wherein n is an integer 4 through 22;



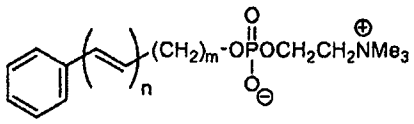
, wherein n is an integer 4 through 21;



, wherein n is an integer 4 through 22;



, wherein n is an integer 3 through 8; and



, wherein n is an integer 4 or 5 and m is an integer 4 through 14.

[Para 18] Further, in this method, the fluorescent molecule exhibits fluorescence at a wavelength of about 300 nm to about1000 nm.

[Para 19] In yet another embodiment, the present invention provides a method of optimizing therapy treatment in a subject, comprising the steps of: (a) providing a radiolabeled phospholipid compound wherein said compound is18-(p-Iodophenyl)octadecyl phosphocholine, 1-O-[18-(p-Iodophenyl)octadecyl]-1,3-propanediol-3-phosphocholine, or 1-O-[18-(p-Iodophenyl)octadecyl]-2-O-methyl-rac-glycero-3-phosphocholine, wherein Iodine is in the form of a radioactive isotope, in a quantity of about 1 millicurie to about 100millicurie; (b) visualizing neoplastic tissue via SPECT or PET imaging; (c) assessing therapy dosage to the subject by quantifying the distribution of the neoplastic tissue.

[Para 20] Another embodiment of the invention provides a method of monitoring tumor therapy response in a subject or effectiveness of a treatment methodology in a subject receiving the treatment for neoplasia, comprising the steps of: (a) providing a radiolabeled phospholipid compound to the subject prior to treatment of neoplasia wherein said compound is18-(p-Iodophenyl)octadecyl phosphocholine, 1-O-[18-(p-Iodophenyl)octadecyl]-1,3-propanediol-3-phosphocholine, or 1-O-[18-(p-Iodophenyl)octadecyl]-2-O-methyl-rac-glycero-3-phosphocholine, wherein Iodine is in the form of a radioactive isotope, in a quantity of about 1 millicurie to about 100millicurie; (b) providing the radiolabeled phospholipid compound to the subject of step (a), after the

treatment of neoplasia in a quantity of about 1 millicurie to about 100millicurie; and (c) assessing difference in accumulation of the phospholipid compound from the pre-treatment of step (a) and the post-treatment of step (b) to determine the response in a subject or effectiveness of the treatment methodology, wherein a greater accumulation of the phospholipid compound in step (a) versus lesser accumulation of phospholipid compound in step (b) indicates a positive response to the treatment in a subject or an effective treatment methodology.

[Para 21] FIGURES

[Para 22] Fig. 1 provides a 2D microCT projection of an excised PIRC rat colon filled with 2% barium (A) and ^{124}I-NM404 microPET image in a PIRC Rat (B) and the fused microPET/microCT image (C). Fiducial marker (M), Tumor (arrow).

[Para 23] DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

[Para 24] The phospholipid ether analogs that can be used for imaging various tumors are defined by formula I and II: wherein in formula I X is a radioactive isotope of a halogen, n is an integer between 8 and 30, Y is selected from the group consisting of H, OH, COOH, O(CO)R, and OR, and Z is selected from the group consisting of NH_2, NR_2, and NR_3, wherein R is an alkyl or aralkyl substituent; and wherein in formula II X is a radioactive isotope of a halogen, n is an integer between 8 and 30, and Y is selected from the group comprising NH_2, NR_2, and NR_3, wherein R is an alkyl or aralkyl substituent.

[Para 25] NM404 and other PLE-based compounds have been known from studies of radiolabeled versions (such as I-124) that these compounds accumulate in malignant tumors, but not in benign tumors such as polyps. An example is given below that the accumulation of NM404 can be used to differentiate benign and malignant tumors. Various PLE-based compounds, such as those described below are also described in various other

patents and patent applications. See U.S. provisional applications 60/521,166 filed on March 2, 2005, 60/521,831 filed in July 8, 2005, 60/593,190 filed on December 20, 2004 and 60/743,232 filed on February 3, 2006; U.S. non-provisional applications 10/906,687 filed on March 2, 2005, 11/177,749 filed on July 8, 2005 and 11/316,620 filed on December 20, 2005, PCT Applications PCT/US05/006681 filed on March 2, 2005, PCT/US05/024259 filed on July 8, 2005 and PCT/US05/047657 filed on December 20, 2005; U.S. Patent Nos. 4,925,649, 4,965,391, 5,087,721, 5,347,030, 5,795,561, 6,255,519 and 6,417,384; Patent publications WO1998/024480 and WO1998/024480; and Canadian Application 2,276,284, all of which are incorporated by reference, as though fully set forth herein.

[Para 26] As depicted in Fig. 1, the left image shows an ex-vivo microCT image of a colon tumor model in rats. Multiple tumors have been detected protruding into the colon lumen. The middle image shows a microPET image using I-124-NM404 of the same colon showing one area of accumulation only. The right image shows a fusion image of MicroCT/microPET that confirms that the accumulation of NM404 was seen only in a tumor that later proved to be an adenocarcinoma. All other colon tumors turned out to be benign polyps and such did not show accumulation of NM404.

[Para 27] It was also previously shown that PLE compounds like NM404 can be labeled with bulky signaling moieties such as fluorescent dyes. See for example, Delgado et al, Fluorescent phenylpolyene analogues of the ether phospholipid edelfosine for the selective labeling of cancer cells, J Med Chem. 2004, 47(22):5333-5.

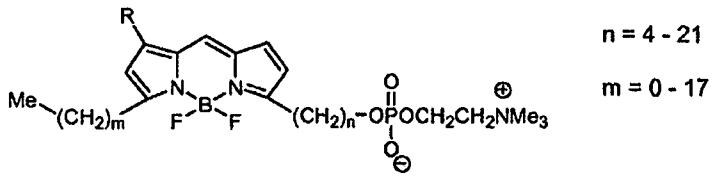
[Para 28] Numerous fluorescent tags are known to one of skill in the art. Methodologies for tagging PLE compounds such as NM404 with fluorescent dyes are also known in the art. Once the PLE compound tagged with a fluorescent dye is prepared by known methodologies, in one exemplary embodiment, the invention describes the use of such PLE compounds such as NM404 labeled with NIR fluorescent moieties (called NIR-PLE dyes).

Such NIR-PLE dye is injected intravenously a few hours before performing endoscopic examinations. An endoscope with at least a daylight and NIR channel is used to examine the body cavity. In operation, the physician may switch between both daylight and NIR channels. The daylight channel is used to detect any abnormal growth or tumors. When those are found, the physician may switch to the NIR channel to determine whether such growth or tumors is malignant or benign. These information can be used for three indications: 1) to diagnose the growth or tumor, 2) to identify the best and most optimal area for a biopsy, or c) to immediately remove (resect) such growth or tumor via minimal surgical methods. Body cavities that the inventions can be used in include, but are not limited to colon, rectum, bronchi, lung, sinus, pancreatic or biliary duct, esophagus, stomach, duodenum, uterus and intra-abdominal cavity.

[Para 29] Fluorescent analogs of NM404

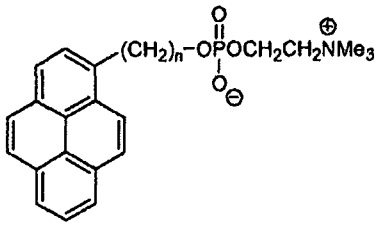
[Para 30] In a exemplary embodiment, several fluorescent analogs of NM404 are provided which may be used as probes as described above. These probes bear structural resemblance to NM404. The fluorophores in these probes are incorporated into hydrophobic alkyl chain of NM404.

[Para 31] In an exemplary embodiment, BODIPY® (500 nm/510 nm) analogs may be used in which the green-fluorescent fluorophores are located within the alkyl chain of NM404:



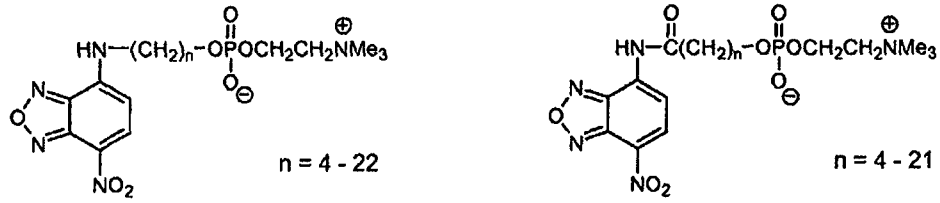
BODIPY® analogs.

[Para 32] In another exemplary embodiment, pyrene analogs (344 nm/378 nm)may be used having 4 to 22 carbons in the alkyl chain:



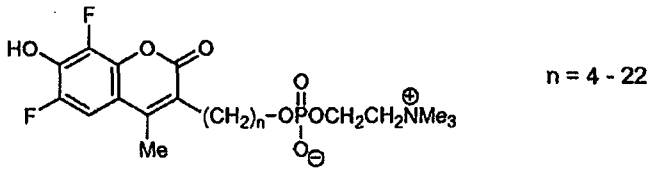
Pyrene analogs.

[Para 33] In yet another exemplary embodiment, NBD (nitrobenzoxadiazole) analogs (463 nm/536 nm) may be used in which fluorophore is attached either via amine or amide bond



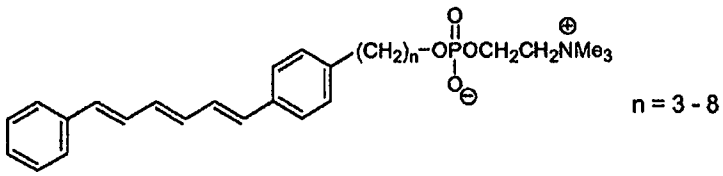
NBD analogs.

[Para 34] In another exemplary embodiment, Coumarin analogs may be used. One example shown below has Marina Blue® (6,8-difluoro-7-hydroxycoumarin) fluorophore (365 nm/460 nm) with 4 to 22 methylene groups:



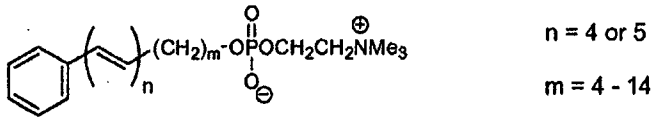
Coumarin analogs

[Para 35] Yet other analogs containing DPH (diphenylhexatriene) fluorophore (350 nm/452 nm) may be used:



DPH analogs

[Para 36] In another exemplary embodiment, group of analogs bearing polyene fluorophore may be used. Fluorophore with n = 4 and m = 7 was described in J Med Chem. 2004; 47 (22): 5333-5 being incorporated into ET-18-OCH_3 analog.



Polyene analog

[Para 37] Other examples and methodologies for synthesizing fluorescent probes are provided in O. Maier et al. Fluorescent lipid probes: some properties and applications (a review), Chem. Phys. Lipids, 2002; 116(1):3-18.

[Para 38] In yet another exemplary embodiment, PLE compounds may be used for tumor therapy response monitoring. Previously, NM404 and other PLE-based compounds were shown to enter and be selectively retained in viable malignant cells. However, cells with impaired status such as those undergoing necrosis were shown to lack significant accumulation of NM404 or other PLE-based compounds. In one exemplary embodiment, the invention provides that this differential property of accumulation in viable and impaired malignant cells can be used to monitor therapy response. Tumor treatments aim to impair the viability of malignant cells in many ways. If an examination with NM404 (or other PLE-based compounds) is performed before and following therapy, the potential difference in

the accumulation of the compound is due to the impairment of metabolism of cancer cells. .f no such difference is found, the therapy has to be regarded non-effective. If a significant drop of accumulation between pre- and post-therapy is found, then the therapy has achieved its goal. The monitoring should ideally be performed with a radioactively labeled PLE compound to be monitored by SPECT or PET imaging, however also fluorescent or NIR methods can be used. This methodology may be useful for measuring not only the response of tumor therapy on a subject, but may also be useful for measuring effectiveness of any treatment methodology in the subject, such as radiation or chemotherapy using PLE or other cancer therapeutic agents.

[Para 39] In yet another exemplary embodiment, PLE compounds may be used in treatment planning for patients receiving the NM404 treatment. NM404 and other PLE-based compounds have been shown to be effective tumor therapies following intravenous injection. However, the effectiveness and effective dose level is known to depend on tumor uptake characteristics, tumor location, tumor perfusion, tumor viability and tumors size. It is difficult to individualize the treatment and inject the most optimal dose with such factors unknown. Nuclear medicine methods like PET or SPECT allow quantitative or at least semi-quantitative assessment of concentration of radioactive tracers. This information can be used to calculate the accumulation of an injected radioactive compound. The invention provides that a tracer dose of radioactive compound such as NM404 or other PLE-based compound may be given to a subject. Such tracer dose (e.g. less than 10 mCi per patient, labels could be I-124 for PET or I-131 for SPECT) determines the individual accumulation characteristics for the tumor to be treated later on with a therapeutic dose of NM404 or another PLE-based compound. Based on these quantitative findings using the "trace dose", the "treatment dose" can be individualized for each patient and treatment.

[Para 40] Typically, radionuclide therapy extends the usefulness of radiation from localized disease to multifocal disease by combining radionuclides with disease-seeking drugs, such as antibodies or custom-designed synthetic agents. DeNardo et al., Cancer Biotherapy & Radiopharmaceuticals, 2002, 17(1): 107-118. Like conventional radiotherapy, the effectiveness of targeted radionuclides is ultimately limited by the amount of undesired radiation given to a critical, dose-limiting normal tissue, most often the bone marrow. Because radionuclide therapy relies on biological delivery of radiation, its optimization and characterization are necessarily different than for conventional radiation therapy. However, the principals of radiobiology and of absorbed radiation dose remain important for predicting radiation effects. Fortunately, most radionuclides emit gamma rays that allow the measurement of isotope concentrations in both tumor and normal tissues in the body. By administering a small "test dose" of the intended therapeutic drug, the clinician can predict the radiation dose distribution in the patient. This can serve as a basis to predict therapy effectiveness, optimize drug selection, and select the appropriate drug dose, in order to provide the safest, most effective treatment for each patient. Although treatment planning for individual patients based upon tracer radiation dosimetry is an attractive concept and opportunity, practical considerations may dictate simpler solutions under some circumstances. There is agreement that radiation dosimetry (radiation absorbed dose distribution, cGy) should be utilized to establish the safety of a specific radionuclide drug during drug development, but it is less generally accepted that absorbed radiation dose should be used to determine the dose of radionuclide (radioactivity, GBq) to be administered to a specific patient (i.e., radiation dose-based therapy). However, radiation dosimetry can always be utilized as a tool for developing drugs, assessing clinical results, and establishing the safety of a specific radionuclide drug. Bone marrow dosimetry continues to be a "work in progress." Blood-derived and/or body-derived marrow dosimetry may be acceptable under specific conditions but clearly do not account for marrow and

skeletal targeting of radionuclide. Marrow dosimetry can be expected to improve significantly but no method for marrow dosimetry seems likely to account for decreased bone marrow reserve.

[Para 41] Various dosimetry determinations may enable a physician to inject a dose or find the individualization of treatment regimen that will provide the most effective treatment regimen (e.g. fractionated dosing) with an optimal treatment effect that produces the least side effects. Such assessment will likely involve a dedicated software to be used to individualize treatment planning.

[Para 42] Radioiodination of NM404 in Preparation for Clinical Use (Prophetic)

[Para 43] A 2-ml glass vial is charged with 10 mg of ammonium sulfate dissolved in 50 µl of deionized water. Six 2 mm glass beads are added, then a Teflon-lined septum and screw cap are added and the vial gently swirled. A solution of 20µg (in 20 µl of ethanol) of stock NM404 is added followed by aqueous sodium iodide (e.g., 125, 131, or 124, 1-5 mCi) in less than 30µl aqueous 0.01 N sodium hydroxide. The isotope syringe is rinsed with three 20 µl portions of ethanol. The resulting reaction vial is swirled gently. A 5-ml disposable syringe containing glass wool in tandem with another 5-ml charcoal nugget filled syringe with needle outlet are attached. The glass wool syringe acts as a condensation chamber to catch evaporating solvents and the charcoal syringe traps free iodide/iodine. The resulting reaction vessel is heated in a heating block apparatus for 45 minutes at 150°C. Four 20 ml volumes of air are injected into the reaction vial with a 25-ml disposable syringe and allowed to vent through the dual trap attachment. The temperature is raised to 160°C. and the reaction vial heated another 30 minutes. After cooling to room temperature, ethanol (200 µl) is added and the vial swirled. The ethanolic solution is then passed through a pre-equilibrated Amberlite IRA 400 resin column to remove unreacted iodide. The eluent volume is reduced to 50 µl via a nitrogen stream (use charcoal syringe trap) and the remaining

volume injected onto a silica gel column (Perkin Elmer, 3 μmX3 cm disposable cartridge column eluted at 1 ml/min with hexane/isopropanol/water (52:40:8)) for purification. Final purity is determined by TLC (plastic backed silica gel-60 eluted with chloroform-methanol-water (65:35:4, Rf=0.1). The HPLC solvents are removed by rotary evaporation and the resulting radioiodinated NM404 solubilized in aqueous 2% Polysorbate-20 and passed through a 0.22 μm filter into a sterile vial.

[Para 44] ^{124}I-NM404-PET Imaging in Patients (Prophetic)

[Para 45] ^{124}I-NM404 maximum dose for human administration is calculated as follows: Animal biodistribution data is generated to determine the percentage of injected dose/organ at varying time points. These animal data are extrapolated to man by means of MIRD formalism (MIRDOSE PC v3.1) using standard conversion factors for differences in organ mass and anatomy between rat and standard man, providing predicted human organ doses. Based on these predicted doses, the permissible mCi dose to be injected into humans is determined using the maximal doses legally permitted by RDRC regulations for specific human tissue as defined in the Federal Register (21CFR Part 361.1). For example, based on the ^{131}I-NM404 data it is expected that the maximum starting dosage for ^{124}I-NM404 should be below 2.0 mCi for pancreatic tumor imaging.

[Para 46] Patients receive SSKI (2 drops three times daily beginning 1 day before and continuing for seven days) in order to minimize uptake of free radioiodide by the thyroid. Patients allergic to iodine may be given potassium perchlorate (200 mg every 8 hours) starting one day before injection and continuing for 3 days post injection. ^{124}I-NM404 is administered intravenously over 5 minutes. A transmission scan using a Ga-68/Ge-68 rotating positron emitting pin source is performed to measure the attenuation. These data are used for attenuation correction of emission data.

[Para 47] The patients are scanned at one or more of the following multiple timepoints following infusion of the ^{124}I-NM-404: 90 minutes dynamic acquisition, 6 hours, 24 hours, 48 hours, and 96 hours.

[Para 48] The PET images are acquired in 2D mode with a BGO based GE ADVANCE PET scanner with an axial field of view of 152 mm. The images are acquired in 256X256 matrix and reconstruction is performed using a Hanning filter. All the images are attenuation corrected using the transmission data.

[Para 49] Before infusion, an intravenous line is established in the upper extremity. The ^{124}I-NM404 dose is measured in a dose calibrator prior to injection. A tracer dose of <2 mCi of ^{124}I-NM04 is infused over 2-5 minutes. The preparation is sterile, pyrogen-free, and contains <5% free iodine by thin layer chromatography (usual syntheses yield free radioiodine of about 1%).

[Para 50] Phantom studies using ^{124}I are performed to determine the calibration factor for the PET scanner and well counter. Phantom studies are performed for the same imaging times and same duration of acquisition.

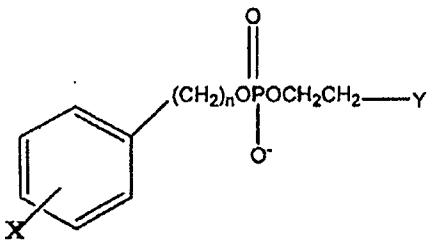
[Para 51] The influx constant of the target region of uptake for any given patient is compared to a background region in the same patient and the lesions are classified as tumor or non-tumor regions based on this comparison. Similar classification of tumor and non-tumor region can also be done by visual analysis.

[Para 52] The present invention is not intended to be limited to the foregoing examples, but encompasses all such modifications and variations as come within the scope of the appended claims.

What is claimed is:

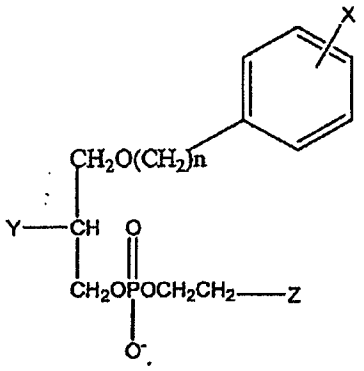
[Claim 1] A phospholipid fluorescent dye, comprising

(a) a phospholipid compound of formula I or II



Formula I

where X is a halogen; n is an integer between 8 and 30; and Y is selected from the group comprising NH_2, NR_2, and NR_3, wherein R is an alkyl or arylalkyl substituent or



Formula II

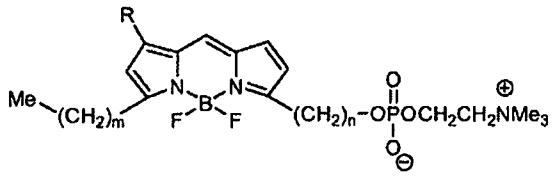
where X is a halogen; n is an integer between 8 and 30; Y is selected from the group consisting of H, OH, COOH, COOR and OR, and Z is selected from the group consisting of NH_2, NR_2, and NR_3, wherein R is an alkyl or arylalkyl substituent; and

(b) a fluorescent molecule.

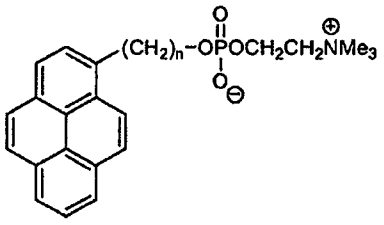
[Claim 2] The phospholipid dye of claim 1, wherein X is selected from the group of radioactive halogen isotopes consisting of ^{18}F, ^{36}Cl, ^{76}Br, ^{77}Br, ^{82}Br,^{122}I, ^{123}I, ^{124}I, ^{125}I, ^{131}I and ^{211}At.

[Claim 3] The phospholipid dye of claim 1, wherein the phospholipid compound is18-(p-Iodophenyl)octadecyl phosphocholine, 1-O-[18-(p-Iodophenyl)octadecyl]-1,3-propanediol-3-phosphocholine, or 1-O-[18-(p-Iodophenyl)octadecyl]-2-O-methyl-rac-glycero-3-phosphocholine, wherein Iodine is in the form of a radioactive isotope.

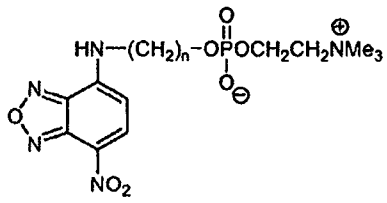
[Claim 4] The phospholipid dye of claim 1, wherein said dye is selected from the group consisting of



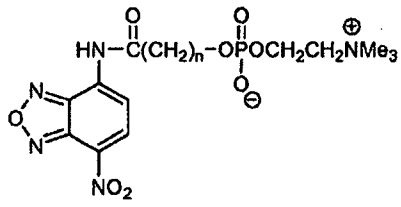
, wherein n is an integer 4 through 21 and m is an integer 0 through17;



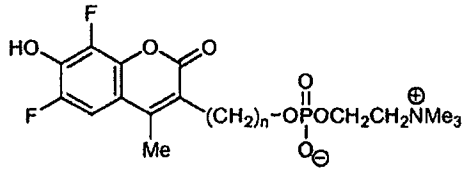
, wherein n is an integer 4 through 22;



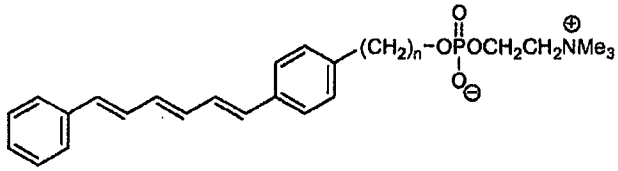
, wherein n is an integer 4 through 22;



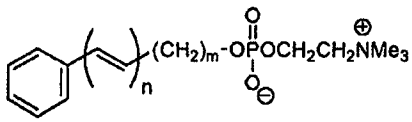
, wherein n is an integer 4 through 21;



, wherein n is an integer 4 through 22;



, wherein n is an integer 3 through 8; and



, wherein n is an integer 4 or 5 and m is an integer 4 through 14.

[Claim 5] The phospholipid dye of claim 1, wherein the fluorescent molecule exhibits fluorescence at a wavelength of about 300 nm to about1000 nm.

[Claim 6] A method for distinguishing a benign structure from a neoplastic tissue in a selected region by using an endoscope have at least two wavelength in a subject comprising the steps of:

(a) administering a fluorescently labeled tumor-specific agent to the subject;

(b) using a first technique to produce a visualization of the anatomy of the selected region using the first wavelength of an endoscope;

(c) using a second technique to produce a visualization of the distribution of fluorescence produced by the fluorescently labeled tumor-specific agent; and

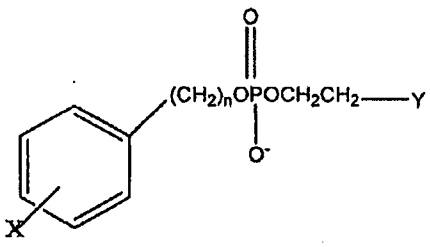
(d) comparing the visualization of the anatomy of the selected region by the first wavelength to the visualization of the distribution of fluorescence by the second wavelength produced by the fluorescently labeled tumor-specific agent thereby distinguishing a benign structure from neoplastic tissue.

[Claim 7] The method of claim 6, wherein the selected region is the gastro-intestinal tract and the respiratory tract.

[Claim 8] The method of claim 6, wherein the first wavelength is about 400 nm to about 800 nm.

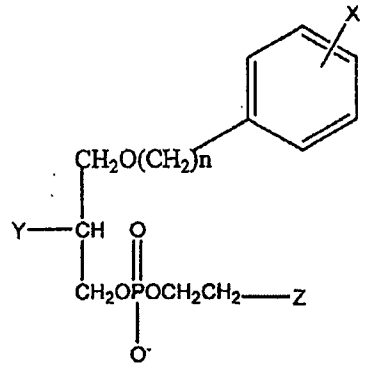
[Claim 9] The method of claim 6, wherein the second wavelength is about 300 nm to 1000 nm.

[Claim 10] The method of claim 6, wherein the fluorescently labeled tumor selective compound is a phospholipid dye, comprising of (a) a phospholipid compound of formula I or II



Formula I

where X is a halogen; n is an integer between 8 and 30; and Y is selected from the group comprising NH_2, NR_2, and NR_3, wherein R is an alkyl or arylalkyl substituent or



Formula II

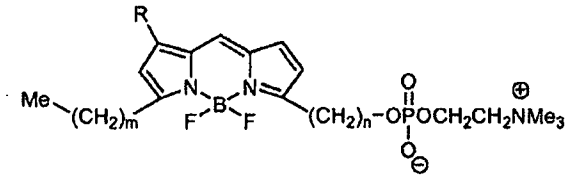
where X is a halogen; n is an integer between 8 and 30; Y is selected from the group consisting of H, OH, COOH, COOR and OR, and Z is selected from the group consisting of NH_2, NR_2, and NR_3, wherein R is an alkyl or arylalkyl substituent; and

(b) a fluorescent molecule.

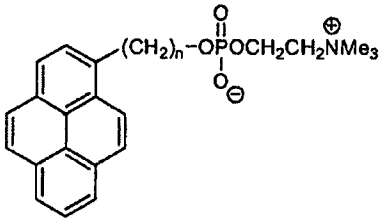
[Claim 11] The method of claim 10, wherein X is selected from the group of radioactive halogen isotopes consisting of ^{18}F, ^{36}Cl, ^{76}Br, ^{77}Br, ^{82}Br,^{122}I, ^{123}I, ^{124}I, ^{125}I, ^{131}I and ^{211}At.

[Claim 12] The method of claim 10, wherein the phospholipid compound is18-(p-Iodophenyl)octadecyl phosphocholine, 1-O-[18-(p-Iodophenyl)octadecyl]-1,3-propanediol-3-phosphocholine, or 1-O-[18-(p-Iodophenyl)octadecyl]-2-O-methyl-rac-glycero-3-phosphocholine, wherein iodine is in the form of a radioactive isotope.

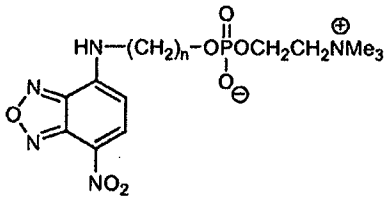
[Claim 13] The method of claim 10, wherein said dye is selected from the group consisting of



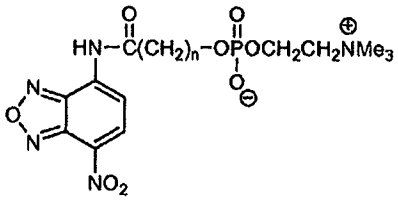
, wherein n is an integer 4 through 21 and m is an integer 0 through17;



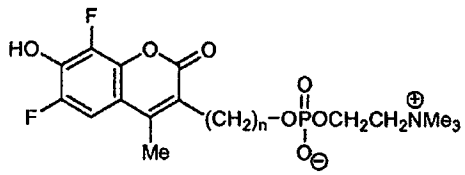
, wherein n is an integer 4 through 22;



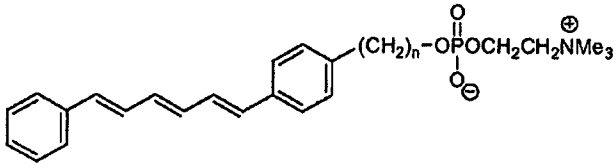
, wherein n is an integer 4 through 22;



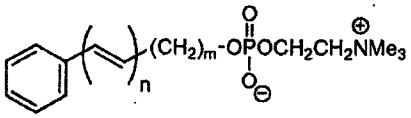
, wherein n is an integer 4 through 21;



, wherein n is an integer 4 through 22;



, wherein n is an integer 3 through 8; and



, wherein n is an integer 4 or 5 and m is an integer 4 through 14.

[Claim 14] The method of claim 9, wherein the fluorescent molecule exhibits fluorescence at a wavelength of about 300 nm to about1000 nm.

[Claim 15] A method of optimizing therapy treatment in a subject, comprising the steps of:

(a) providing a radiolabeled phospholipid compound wherein said compound is18-(p-Iodophenyl)octadecyl phosphocholine, 1-O-[18-(p-Iodophenyl)octadecyl]-1,3-propanediol-3-phosphocholine, or 1-O-[18-(p-Iodophenyl)octadecyl]-2-O-methyl-rac-glycero-3-phosphocholine, wherein iodine is in the form of a radioactive isotope, in a quantity of about 1 millicurie to about 100millicurie;

(b) visualizing neoplastic tissue via SPECT or PET imaging;

(c) assessing therapy dosage to the subject by quantifying the distribution of the neoplastic tissue.

[Claim 16] A method of monitoring tumor therapy response in a subject or effectiveness of a treatment methodology in a subject receiving the treatment for neoplasia, comprising the steps of:

(a) providing a radiolabeled phospholipid compound to the subject prior to treatment of neoplasia wherein said compound is18-(p-Iodophenyl)octadecyl phosphocholine, 1-O-[18-(p-Iodophenyl)octadecyl]-1,3-propanediol-3-phosphocholine, or 1-O-[18-(p-Iodophenyl)octadecyl]-2-O-methyl-rac-glycero-3-phosphocholine, wherein iodine is in the form of a radioactive isotope, in a quantity of about 1 millicurie to about 100millicurie;

(b) providing the radiolabeled phospholipid compound to the subject of step (a), after the treatment of neoplasia in a quantity of about 1 millicurie to about 100millicurie; and

(c) assessing difference in accumulation of the phospholipid compound from the pre-treatment of step (a) and the post-treatment of step (b) to determine the response in

a subject or effectiveness of the treatment methodology, wherein a greater accumulation of the phospholipid compound in step (a) versus lesser accumulation of phospholipid compound in step (b) indicates a positive response to the treatment in a subject or an effective treatment methodology.

DRAWINGS

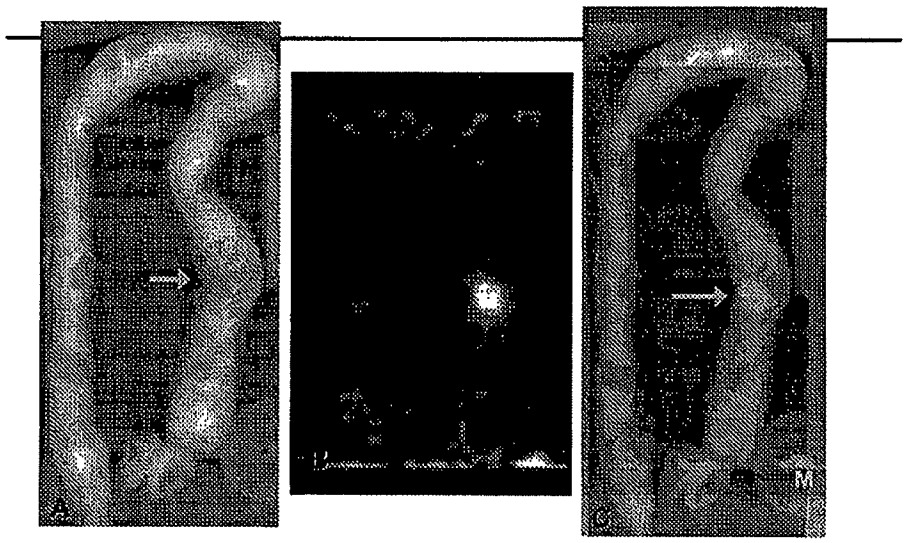


FIG. 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US07/17885

A. CLASSIFICATION OF SUBJECT MATTER
IPC: **A61K 51/04**(2006.01);**A61B 8/12**(2006.01);**A61P 43/00**(2006.01)

USPC: 514/183
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/183

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PLOTZKE. Selective localization of a radioiodinated phospholipid ether analog in human xenografts. 1993. J. Nuclear Medicine, 34, pages 787-792, especiallyl abstract.	1-16
X	US 4,965,391 A (COUNSELL et al) 23 October 1990 (23.10.1990), abstract	1-16
X	WO 2005/084716 A (WEICHERT et al) 15 September 2005 (15.09.2005), abstract and page 1	1-16

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search
31 July 2008 (31.07.2008)

Date of mailing of the international search report
14 AUG 2008

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
Facsimile No. (571) 273-3201

Authorized officer
ANNA PAGONAKIS
Telephone No. 571-270-3505

Form PCT/ISA/210 (second sheet) (April 2007)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US07/17885

Continuation of B. FIELDS SEARCHED Item 3:
EAST (USPat, USPGPub, Derwent, EPO)
STN