Chapter 1 : Lalit N. Goswami / Institute Faculty / International Institute of Nano and Molecular Medicine

Bifunctional Agents for Imaging and Therapy. Ravindra K Pandey; View. Show abstract. To develop novel bifunctional agents for tumor imaging (MR) and photodynamic therapy (PDT), certain.

Desirably the compound has the formula: The invention also includes a method of treatment by photodynamic therapy by treatment with light after injecting the compound and a method of imaging by fluorescence after injection of the compound. The above applications are incorporated herein by reference in their entirety. To date, most PS are amphiphilic in nature in that they contain both hydrophilic and hydrophobic substituents. Most of these compounds, therefore, are visibly aggregated in solution, so the challenge remains to be the synthesis of effective water-soluble photosensitizers that accumulate in the tumor, yet clear at a suitable time as to limit toxicity. Unfortunately, these compounds were found to be toxic Therefore, the aim of the present invention was to synthesize effective and non-toxic water-soluble long wavelength absorbing photosensitizers with high singlet oxygen ability, singlet oxygen being a key cytotoxic agent for PDT. Tetrapyrollic compounds, especially porphyrin related compounds, have played a key role in developing a variety of photosensitizers. These approaches have been extremely useful in developing multimodality agents. However, one major drawback with most of these compounds is their limited solubility in water. Therefore, most of the formulations require a biocompatible surfactant, e. An approach for increasing the water solubility is to introduce hydrophilic substituents e. Unfortunately such incorporation can limit biological efficacy. The following references are incorporated by reference as background art. Zheng The Porphyrin Handbook Eds: Kadish, Rodgers and Smith , vol. Nabi, Allan Oseroff and Ravindra K. A Possible See and Treat Approach. Dougherty and Ravindra K. Henderson, Allan Oseroff, Thomas J. Rapid Communication , , 45, The substituted tetrapyrollic compound is usually a chlorin, bacteriochlorin, porphyrin, pyropheophorbide, purpurinimide, or bacteriopurpurinimide. X is an aryl or heteroaryl group. R20 is methyl, butyl, heptyl, dodecyl or 3,5-bis trifluoromethyl -benzyl. R21 is 3,5,-bis trifluoromethyl benzyl. R1a and R2a are each independently hydrogen or substituted or unsubstituted alkyl, or together form a covalent bond. R3 and R4 are each independently hydrogen or substituted or unsubstituted alkyl. R3a and R4a are each independently hydrogen or substituted or unsubstituted alkyl, or together form a covalent bond. R5 is hydrogen or substituted or unsubstituted alkyl. R10 is hydrogen, or substituted or unsubstituted alkyl. Synthetic details for the preparation of examples of water soluble photosensitizers of the invention are depicted in Schemes as follow: All the intermediates and the final products were characterized by NMR and mass spectrometry analyses. The purity was ascertained by analytical TLC. The starting photosensitizers e. HPPH, fluorinated purpurinimide 7 and the N-butyl-purpurinimide 10 were synthesized by following published methodologies that were developed in our laboratory The Synthetic details are as follows: After 10 min of stirring at the same temperature Cbz-Cl 7. Resultant mixture was stirred for 6 hr at room temperature, concentrated partially to remove THF. Organic layers were separated, combined and washed with H2O ml , dried over sodium sulfate and concentrated to give 2 as viscous oil in quantitative yield. Resultant mixture was stirred at room temperature for 16 hr under N2 atm, diluted with DCM ml and washed with brine 50 ml. Organic layer was separated, dried over sodium sulfate and concentrated. The reaction mixture was concentrated and dried under high vacuum to give 6 in quantitative yield. Reaction mixture was concentrated and dried under high vacuum to give 9 in quantitative yield. Reaction mixture was concentrated and dried under high vacuum to give 12 in quantitative yield. The mice were injected s. Three explanations for this may be that 1 the slight charge from the carboxylate groups may be contributing to differing localization sites of PS 16 in comparison to 15 as mentioned above , 2 the PDT-induced mechanism of action may differ in comparison to 16 or 3 the increased PS uptake in the tumor compared to the skin of 16 could be contributing to the enhanced PDT response. The main purpose of these experiments was to determine if the water-soluble PS could be utilized as both a PDT agent and diagnostic imaging tool. The initial in vivo experiments displayed the advantage of the

water-soluble PS over its parent compound, Comparative Photosensitizing Efficacy Water-soluble Photosensitizers 9 and 12 The in vivo photosensitizing efficacy of water-soluble photosensitizers 9 and 12 was determined in BALB-C mice bearing Colo tumors at similar treatment conditions. At 24 h postinjction of the photosensitizer i. The results are summarised in Figure X. As can be seen among the three candidates, compared to 12, compounds 9 and 12 were found to be more effective. In Vivo Fluorescence Imaging With the Water-Soluble Analog 16 Measurement of PS accumulation in the tumor and skin via fluorescence measurements using a non-invasive optical imaging camera system was performed. Therefore, the fluorescence images obtained were not particularly specific for only PS fluorescence. This imaging technology was quite beneficial due to the fact that it was minimally invasive, so that there was no need to sacrifice the animal in order to obtain information about where the PS was localized. Previous studies have involved invasive procedures in which a mouse was sacrificed, the tumor or skin was excised and histological staining was performed on the paraffin blocks. This system was capable of taking qualitative hyperspectral images in the specific range of nm focused on nm. Attached to the small animal images are the spectral properties of the hair yellow , skin blue and tumor red. It is important to remember that these are qualitative images of PS accumulation in the tumor and skin. As a means to determine the exact uptake of the PS in the tumor versus the skin and other organs, a skin-flap excision, as well as, an ex vivo biodistribution study were performed. The organs were homogenized, dissolved in Solvable and read on the Fluoromax II Fluorimeter at nm. This invention describes the successful synthesis of a new long wavelength water-soluble PS. This is the first report of a water-soluble fluorinated purpurinimide being utilized as a dual PDT-imaging agent. An article of manufacture, comprising packaging material and a compound contained within the packaging material or salt thereof wherein the compound or salt thereof is effective in a photodynamic therapy treatment for ameliorating the symptoms of a hyperproliferative disorder; and the packaging material includes a label that indicates that the compound or salt thereof is used in a photodynamic therapy treatment for ameliorating the symptoms of a hyperproliferative disorder and the compound is tetrapyrollic photosensitizer compound having at least one pendant â€”CH2CH2CON CH2CON CH2COOH 2 2 or â€”N CH2COOH 2 group or esters thereof said tetrapyrollic compound being a chlorin, bacteriochlorin, porphyrin, pyropheophorbide, purpurinimide, or bacteriopurpurinimide. An article of wherein the compound has the formula: A method for administering a therapy to a target, comprising: The method of claim 28 , wherein the target is selected from the group consisting of: The method of where the compound has the formula: The method of claim 30 , wherein the target is selected from the group consisting of: The method of claim 28 , further comprising the step of allowing sufficient time for any of the compound that is not preferentially associated to the target tissue to clear from non-target tissue of the subject prior to the step of irradiating. The method of claim 28 where the method is photodynamic therapy for treating hyperproliferative tissue in a subject and the subject is irradiated with light of a wavelength and fluence to sufficiently activate the compound to destroy or impair the hyperproliferative tissue. The method of wherein the compound has the formula: A method for detecting the presence of a hyperproliferative tissue in a subject comprising:

Chapter 2 : Ravindra K. Pandey - Publications

Publications by authors named "Ravindra K Pandey" Nadine S James Ravindra R Cheruku Joseph R Missert Ulas Sunar Bifunctional agents for imaging and therapy.

Desirably the compound has the formula: The invention also includes a method of treatment by photodynamic therapy by treatment with light after injecting the compound and a method of imaging by fluorescence after injection of the compound. The above applications are incorporated herein by reference in their entirety. The government has certain rights in this invention. To date, most PS are amphiphilic in nature in that they contain both hydrophilic and hydrophobic substituents. Most of these compounds, therefore, are visibly aggregated in solution, so the challenge remains to be the synthesis of effective water-soluble photosensitizers that accumulate in the tumor, yet clear at a suitable time as to limit toxicity. Unfortunately, these compounds were found to be toxic Therefore, the aim of the present invention was to synthesize effective and non-toxic water-soluble long wavelength absorbing photosensitizers with high singlet oxygen ability, singlet oxygen being a key cytotoxic agent for PDT. Tetrapyrollic compounds, especially porphyrin related compounds, have played a key role in developing a variety of photosensitizers. These approaches have been extremely useful in developing multimodality agents. However, one major drawback with most of these compounds is their limited solubility in water. Therefore, most of the formulations require a biocompatible surfactant, e. An approach for increasing the water solubility is to introduce hydrophilic substituents e. Unfortunately such incorporation can limit biological efficacy. The following references are incorporated by reference as background art. Zheng The Porphyrin Handbook Eds: Kadish, Rodgers and Smith , vol. Nabi, Allan Oseroff and Ravindra K. A Possible See and Treat Approach. Dougherty and Ravindra K. Henderson, Allan Oseroff, Thomas J. Rapid Communication , , 45, The substituted tetrapyrollic compound is usually a chlorin, bacteriochlorin, porphyrin, pyropheophorbide, purpurinimide, or bacteriopurpurinimide. The target is hyperproliferative tissue that may be selected from vascular endothelial tissue, a neovasculature tissue, a neovasculature tissue present in an eye, an abnormal vascular wall of a tumor, a solid tumor, a tumor of a head, a tumor of a neck, a tumor of an eye, a tumor of a gastrointestinal tract, a tumor of a liver, a tumor of a breast, a tumor of a prostate, a tumors of a lung, a nonsolid tumor, malignant cells of one of a hematopoietic tissue and a lymphoid tissue, lesions in a vascular system, a diseased bone marrow, and diseased cells in which the disease is one of an autoimmune and an inflammatory disease. X is an aryl or heteroaryl group. R20 is methyl, butyl, heptyl, dodecyl or 3,5-bis trifluoromethyl -benzyl. R21 is 3,5,-bis trifluoromethyl benzyl. R1a and R2a are each independently hydrogen or substituted or unsubstituted alkyl, or together form a covalent bond. R3 and R4 are each independently hydrogen or substituted or unsubstituted alkyl. R3a and R4a are each independently hydrogen or substituted or unsubstituted alkyl, or together form a covalent bond. R5 is hydrogen or substituted or unsubstituted alkyl. R10 is hydrogen, or substituted or unsubstituted alkyl. Synthetic details for the preparation of examples of water soluble photosensitizers of the invention are depicted in Schemes as follow: All the intermediates and the final products were characterized by NMR and mass spectrometry analyses. The purity was ascertained by analytical TLC. The starting photosensitizers e. HPPH, fluorinated purpurinimide 7 and the N-butyl-purpurinimide 10 were synthesized by following published methodologies that were developed in our laboratory The Synthetic details are as follows: After 10 min of stirring at the same temperature Cbz-Cl 7. Resultant mixture was stirred for 6 hr at room temperature, concentrated partially to remove THF. Organic layers were separated, combined and washed with H2O ml , dried over sodium sulfate and concentrated to give 2 as viscous oil in quantitative yield. Resultant mixture was stirred at room temperature for 16 hr under N2 atm, diluted with DCM ml and washed with brine 50 ml. Organic layer was separated, dried over sodium sulfate and concentrated. The reaction mixture was concentrated and dried under high vacuum to give 6 in quantitative yield. Reaction mixture was concentrated and dried under high vacuum to give 9 in quantitative yield. Reaction mixture was concentrated and dried

under high vacuum to give 12 in quantitative yield. The mice were injected s. Three explanations for this may be that 1 the slight charge from the carboxylate groups may be contributing to differing localization sites of PS 16 in comparison to 15 as mentioned above , 2 the PDT-induced mechanism of action may differ in comparison to 16 or 3 the increased PS uptake in the tumor compared to the skin of 16 could be contributing to the enhanced PDT response. The main purpose of these experiments was to determine if the water-soluble PS could be utilized as both a PDT agent and diagnostic imaging tool. The initial in vivo experiments displayed the advantage of the water-soluble PS over its parent compound, Comparative Photosensitizing Efficacy Water-soluble Photosensitizers 9 and 12 The in vivo photosensitizing efficacy of water-soluble photosensitizers 9 and 12 was determined in BALB-C mice bearing Colo tumors at similar treatment conditions. At 24 h postinjction of the photosensitizer i. The results are summarised in Figure X. As can be seen among the three candidates, compared to 12, compounds 9 and 12 were found to be more effective. In Vivo Fluorescence Imaging With the Water-Soluble Analog 16 Measurement of PS accumulation in the tumor and skin via fluorescence measurements using a non-invasive optical imaging camera system was performed. Therefore, the fluorescence images obtained were not particularly specific for only PS fluorescence. This imaging technology was quite beneficial due to the fact that it was minimally invasive, so that there was no need to sacrifice the animal in order to obtain information about where the PS was localized. Previous studies have involved invasive procedures in which a mouse was sacrificed, the tumor or skin was excised and histological staining was performed on the paraffin blocks. This system was capable of taking qualitative hyperspectral images in the specific range of nm focused on nm. Attached to the small animal images are the spectral properties of the hair yellow , skin blue and tumor red. It is important to remember that these are qualitative images of PS accumulation in the tumor and skin. As a means to determine the exact uptake of the PS in the tumor versus the skin and other organs, a skin-flap excision, as well as, an ex vivo biodistribution study were performed. The organs were homogenized, dissolved in Solvable and read on the Fluoromax II Fluorimeter at nm. This invention describes the successful synthesis of a new long wavelength water-soluble PS. This is the first report of a water-soluble fluorinated purpurinimide being utilized as a dual PDT-imaging agent. An article of manufacture, comprising packaging material and a compound contained within the packaging material or salt thereof wherein the compound or salt thereof is effective in a photodynamic therapy treatment for ameliorating the symptoms of a hyperproliferative disorder; and the packaging material includes a label that indicates that the compound or salt thereof is used in a photodynamic therapy treatment for ameliorating the symptoms of a hyperproliferative disorder and the compound is a pharmaceutically acceptable tetrapyrollic photosensitizer compound having at least one pendant â€”CH2CH2CON CH2CON CH2COOH 2 2 or â€”N CH2COOH 2 group or esters thereof said tetrapyrollic compound being a chlorin, bacteriochlorin, porphyrin, pyropheophorbide, purpurinimide, or bacteriopurpurinimide wherein the compound has the formula: A method for administering a therapy to a target comprising hyperproliferative tissue, comprising: The method of claim 2 , further comprising the step of allowing sufficient time for any of the compound that is not preferentially associated to the target tissue to clear from non-target tissue of the subject prior to the step of irradiating. The method of claim 2 where the method is photodynamic therapy for treating hyperproliferative tissue in a subject and the subject is irradiated with light of a wavelength and fluence to sufficiently activate the compound to destroy or impair the hyperproliferative tissue. The method of wherein the compound has the formula:

Chapter 3 : Publications Authored by Ravindra K Pandey | PubFacts

Chen Y, Oseroff A, Fenstermaker R, Kadish KM, Fukuzumi S, Erin T, Baumann H, Ciesielski M, Pandey SK, Liu W, Wenbo E, Zhang M, Ohkubo K, Pandey calendrierdelascience.comhysical, electrochemical characteristics and cross-linking of STAT-3 protein by an efficient bifunctional agent for fluorescence image-guided photodynamic therapy.

Published online Aug Part - 1 Nadine S. James Yihui Chen 1. Ohulchanskyy Find articles by Tymish Y. Ohulchanskyy Manivannan Ethirajan 1. The authors have declared that no competing interest exists. This is an open-access article distributed under the terms of the Creative Commons License http: Reproduction is permitted for personal, noncommercial use, provided that the article is in whole, unmodified, and properly cited. This article has been cited by other articles in PMC. Abstract Near-infrared NIR organic dyes have become important for many biomedical applications, including in vivo optical imaging. Conjugation of NIR fluorescent dyes to photosensitizing molecules photosensitizers holds strong potential for NIR fluorescence image guided photodynamic therapy PDT of cancer. Therefore, we were interested in investigating the photophysical properties, in vivo tumor-affinity and fluorescence imaging potential of a series of heterocyclic polymethine dyes, which could then be conjugated to certain PDT agents. For our present study, we selected a series of symmetrical polymethine dyes containing a variety of bis-N-substituted indole or benzindole moieties linked by linear conjugation with and without a fused substituted cyclohexene ring. The N-alkyl side chain at the C-terminal position was functionalized with sulfonic, carboxylic acid, methyl ester or hydroxyl groups. In addition to 3, parent dyes IR and Cypate 6 were also selected and subjected to further modifications by introducing desired functional groups, which could enable further conjugation of the cyanine dyes to an effective photosensitizer HPPH developed in our laboratory. The synthesis and biological studies tumor-imaging and PDT of the resulting bifunctional conjugates are discussed in succeeding paper Part-2 of this study. Introduction Over the past few years, NIR fluorescence imaging techniques have advanced considerably and as such are increasingly becoming important translational tools from bench side research, with the use of small animals, to clinical application 1 - 7. It is being used in challenging applications 8 , on the microscopic and macroscopic platform such as the in vivo imaging of biological targets and diseases 9. Animal models of human cancer and metastasis have been developed to aid in the understanding of disease progression and development of treatment Fluorescent probes have been shown to facilitate in vivo characterization of tumors, significantly advancing tumor visualization 4 , 11 , enable detection and identification of small pre-neoplastic lesions, and metastasis 4. A great number of polymethine cyanine-based fluorophores are being used as exogenous probes for NIR fluorescence imaging. These compounds usually are not tumor-avid therefore, tumor targeting becomes essential. Some of these cyanine dyes have sulfonate groups directly attached to the aromatic benzindolenium or indolenium nucleus. These groups shield the fluorophores from non-specific hydrophobic interaction with other molecules , a process known to affect the emission of many fluorophores in aqueous media Additionally, dyes containing sulfonate or sulfonatoalkyl groups attached to the heterocyclic nucleus, tend to aggregate less Various structural modifications have been made to the polymethine chromophores to enhance its light and chemical stability These modifications usually include rigidization of the polymethine chain in order to inhibit radiationless internal conversion IC and subsequent isomerization The stability of the polymethine chain is lowered as the chain lengthens. However, incorporation of a central ring system such as a cyclohexenyl group enhances the rigidity of the polymethine chain, decreases the efficiency of IC and increases the fluorescence quantum yield. This screening was performed in order to select the optimal fluorophore s for the synthesis of molecule s photosensitizer-near infrared fluorophore conjugate PS-NIRF , see the part 2 related article from the same author within this journal, that can be used as a single unit combining photodiagnosis, fluorescence guided resection and phototherapy Herein, we report results of the evaluation of several polymethine dyes as near-infrared fluorescence probes for tumor imaging in vivo. Materials and Methods In vitro studies: In vitro

tumor cell uptake Colon 26 and U87 cell lines. All compounds used for the synthesis were purchased from Sigma Aldrich and used directly. Purification was done by flash column chromatography. The extinction coefficients of all compounds were determined by weighing a particular amount of solid and dissolving in a 50 ml volumetric flask using methanol as the solvent. First, the molar concentration C of each solution was calculated from its weight and volume. The SPEX M Spectrometer was utilized for measurements in NIR range; a nm line from continuous wave solid state laser Millenia, Spectra Physics or laser diodes emitting at and nm were used as an excitation. The sample placed in a quartz cuvette was positioned directly in front of the entrance slit of the spectrophotometer, and the emission signal was collected at 90o relative to the excitation laser beam. Additional long-pass filters [a LP filter and a AELP filter both from Omega Optical] were used to attenuate the scattered light and fluorescence from the samples. A second harmonic nm from a nanosecond pulsed Nd: In vitro tumor models: The 96 and 6 well plates were purchased from VWR. Animal and tumor models: Prior to commencement of in vivo studies all procedures or protocols were approved by the institutional animal care committee IACUC. The mice were inoculated subcutaneously S. Compounds were imaged using a Maestro GNIR Flex In-vivo imaging system using a broadband excitation at - nm and an nm long pass emission. Tumor uptake in vitro: Colon 26 and U87 cells were seeded at 5. Cells in each well were harvested and placed in 5 ml flow tubes with sieve caps, centrifuged cold at rpm at 10 oC for 10 minutes. A single diode laser excitation at nm was used maximum power 40mw currently at 17mw. Emission was detected using nm long pass LP filter. It was prepared by following the methodology discussed by Strekowski et al 16 - 18 Sodium E E 3- E 1- 5-carboxylatopentyl -3,3-dimethyl3H-indoliumyl vinyl chlorocyclohexenylidene ethylidene -3,3 dimethylindolinyl hexanoate bromide 2: It was prepared by following the methodology discussed by Strekowski et al 16 - 18 Sodium E E 2-chloro E 3,3-dimethyl 4-sulfonato butyl -3H-indoliumyl vinyl cyclohexenylidene ethylidene -3,3-dimethylindolinyl butanesulfonate 3: It was prepared by following the methodology discussed by Strekowski et al 16 - 18 Sodium 2- E E 4-carboxyphenyl thio E 1,1-dimethyl 4-sulfonato butyl -1H-benzo[e]indol-2 3H -ylidene ethylidene cyclohexenyl vinyl -1,1-dimethyl-1H-benzo[e]indoliumyl butane-1 sulfonate 5: In a dry mL round bottom flask rbf , IR mg, 0. It was prepared by following the procedure published by Samuel Achilefu et al 19 , Sodium 2- E E 3-carboxyphenyl thio E 1,1-dimethyl 4-sulfonatobutyl -1H-benzo[e]indol-2 3H -ylidene ethylidene cyclohexenyl vinyl -1,1-dimethyl-1H-benzo[e]indoliumyl butanesulfonate 7: In a dry ml round bottom flask rbf , IR mg, 0. IR 60 mg and 4-aminothiophenol 60 mg was dissolved in dry DMF and stirred overnight. Sodium E E 2- 4-aminophenyl thio E 3,3-dimethyl 4-sulfonatobutyl -3H-indoliumyl vinyl cyclohexenylidene ethylidene -3,3-dimethylindolinyl butanesulfonate 9 IR 60 mg, 0. Sodium E E 2-3-carboxyphenyl thio E 3,3-dimethyl 4-sulfonatobutyl -3H-indoliumyl vinyl cyclohexenylidene ethylidene -3,3-dimethylindolinyl butanesulfonate IR 50 mg, 0. These modified fluorophores 5, were obtained upon functionalizing commercially available IR and IR The fluorescence spectra were acquired for each compound and the fluorescence quantum yields were determined by a comparing each spectrum with that of the indocyanine green ICG , with known fluorescence quantum yield of 0.