Compared to Purpurinimides, the Pyropheophorbide Containing an Iodobenzyl Group Showed Enhanced PDT Efficacy and Tumor Imaging ($^{124}$I-PET) Ability

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Two positional isomers of purpurinimide, 3-[1$^\prime$-(3-iodobenzyloxyethyl)] purpurin-18-N-hexylmide methyl ester 4, in which the iodobenzyl group is present at the top half of the molecule (position-3), and a 3-(1$^\prime$-hexyloxyethyl)purpurin-18-N-(3-iodo-benzylmide) methyl ester 5, where the iodobenzyl group is introduced at the bottom half (N-substitued cyclicimide) of the molecule, were derived from chlorophyll-a. The tumor uptake and phototherapeutic abilities of these isomers were compared with the pyropheophorbide analogue 1 (lead compound). These compounds were then converted into the corresponding $^{124}$I-labeled PET imaging agents with specific activity $>1$ Ci/µmol. Among the positional isomers 4 and 5, purpurinimide 5 showed enhanced imaging and therapeutic potential. However, the lead compound 1 derived from pyropheophorbide-a exhibited the best PET imaging and PDT efficacy. For investigating the overall lipophilicity of the molecule, the 3-O-hexyl ether group present at position-3 of purpurinimide 5 was replaced with a methyl ether substituent, and the resulting product 10 showed improved tumor uptake, but due to its significantly higher uptake in the liver, spleen, and other organs, a poor tumor contrast in whole-body tumor imaging was observed.

INTRODUCTION

Positron emission tomography (PET) has wide appeal for research at the drug development stage as it allows studying the drug distribution noninvasively (1). Dedicated animal PET systems whose resolution could reach near 1 mm have intensified this field by enabling drug studies with murine disease models. In recent years, $^{18}$F-fluorodeoxyglucose ($^{18}$F-FDG) has been the primary PET tracer. It is being used in the evaluation of several neoplasms, both before and after therapy, as well as the planning of the radiotherapy in various cancers. Its use in the assessment of cancer after therapy, including restaging the planning of the radiotherapy in various cancers. Its use in the assessment of cancer after therapy, including restaging and monitoring tumor response has been of particular interest for oncologists. However, $^{18}$F-FDG suffers from pitfalls in cases such as where tumors are not metabolically active enough. Additionally, a short half-life of $^{18}$F-isotope (110 min) limits its use in studies involving antibodies and photosensitizers (PS) related to phorphyrins for use in photodynamic therapy (PDT), which take considerably longer time to accumulate in tumors (2). In this respect, $^{124}$I is a better choice due to its half-life of 4.2 days (3, 4). The labeling technique for $^{124}$I-nuclide is now well established, and this approach is continuously being followed to label a variety of biologically active molecules (5–12). In the past few years, various porphyrin-based photosensitizers have been labeled/
Herein, we report the synthesis of the iodobenzyl substituted purpurinimides 4 and 5 (positional isomers; Figure 1) and their significant difference in PDT efficacy. The comparative biodistribution properties of the corresponding 124I-analogues as well as their tumor imaging (PET) abilities are also discussed.

EXPERIMENTAL PROCEDURES

Chemistry. All chemicals were of reagent grade and used as such. Solvents were dried using standard methods. Reactions were carried out under nitrogen atmosphere and were monitored by precocated (0.20 mm) silica TLC plastic sheet (20 × 20 cm) strips (POLYGRAM SIL N-HR) and/or UV-visible spectroscopy. Silica gel 60 (70–230 mesh, Merck) was used for column chromatography. Melting points were determined on a Fisher–Johns melting point apparatus. UV-visible spectra were recorded on a Varian (Cary-50 Bio) spectrophotometer. 1H NMR spectra were recorded on a Brucker AMX 400 MHz NMR spectrometer at 303 K. Proton chemical shifts (δ) are connected to a computer with HP Chemstation software via an L-4000 UV detector, and a radiation detector. These detectors were carried out under nitrogen atmosphere and were monitored in the characterization section. Reactions were first carried out for 45 min. It was then diluted with dichloromethane (200 mL), washed with aqueous sodium bicarbonate solution (100 mL), and then with water (2 × 200 mL). The dichloromethane layer was dried over anhydrous sodium sulfate, concentrated, and treated with diazomethane. Evaporation of the solvent gave a syrupy residue, which was chromatographed over a silica column using (1:4) ethyl acetate/hexane as eluant to remove excess 3-iodobenzylalcohol, followed by (1:1) ethyl acetate/hexane to yield 110 mg (81%) of the desired compound 4, which is sticky in nature.

Analytical HPLC (Symmetry C18; 99/1; MeOH/H2O): tR = 38.71 min, >96%. UV–vis (MeOH): 701 (4.31 × 104), 545 (2.15 × 104), 508 (7.32 × 103), 414 (1.31 × 103). 1H NMR (CDCl3, 400 MHz): δ 9.72 (s, 1H, meso-H), 9.66 (s, 1H, meso-H); 8.58 (s, 1H, meso-H); 7.75 (s, 1H, ArH); 7.64 (d, J = 9.2, 1H, ArH); 7.29 (d, J = 7.2, 1H, ArH); 7.06 (dt, J = 2.4, 7.4, 1H, ArH); 5.88 (q, J = 6.2, 1H, 3-H); 5.41 (d, J = 8.8, 1H, 17-H); 4.68 (dd, J = 2.6, 12.6, 1H, OCH2Ar); 4.55 (dd, J = 3.2, 12.0, 1H, OCH2Ar); 4.45 (t, J = 6.8, 2H, NCH2(CH2)4CH3); 4.37 (q, J = 7.2, 1H, 18-H); 3.84 (s, 3H, 12-CH3); 3.68 (q, J = 7.6, 2H, 8-CH2CH3); 3.56 (s, 3H, 17-OCH2CH3); 3.31 (split s, 3H, 2-CH3); 3.14 (s, 3H, 7-CH3); 2.12 (s, 3H, 31-CH3). HRMS for C41H43N4O4I: 783.2329 (calculated, M + 1); found, 783.2407. Anal. Calcd. for C41H43N4O4I: C, 62.91; H, 5.54; N, 7.13; 116.45.

Synthesis of 3-[1’-(3-Iodobenzoxlyoxy)ethyl]purpurin-18-N-hexylimide Methyl Ester (4). 30% Hydrobromic acid (HBr) in acetic acid (2 mL) was added to purpurin-18-N-hexylimide methyl ester (100 mg, 0.15mmol) (23), and the reaction was stirred at room temperature for 2 h. After evaporating the acids under high vacuum (0.1 mmHg), excess of 3-iodobenzyl alcohol (0.45 mL, 20-fold excess), dry dichloromethane (5 mL), and anhydrous potassium carbonate (40 mg) were added. The reaction mixture was stirred under nitrogen atmosphere for 45 min. It was then diluted with dichloromethane (200 mL), washed with aqueous sodium bicarbonate solution (100 mL), and then with water (2 × 200 mL). The dichloromethane layer was dried over anhydrous sodium sulfate, concentrated, and treated with diazomethane. Evaporation of the solvent gave a syrupy residue, which was chromatographed over a silica column using (1:4) ethyl acetate/hexane as eluant to remove excess 3-iodobenzylalcohol, followed by (1:1) ethyl acetate/hexane to yield 110 mg (81%) of the desired compound 4, which is sticky in nature.

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Synthesis of 3-[1’-(3-Iodobenzoxlyoxy)ethyl]purpurin-18-N-(3-iodo)benzylimide Methyl Ester (5). Hydrobromic acid (HBr) (30%) in acetic acid (2 mL) was added to purpurin-18-N-(3-iodo) benzylimide methyl ester (100 mg, 0.125 mmol) (23), and the reaction was stirred at room temperature for 2 h. After evaporating the acids under high vacuum (0.1 mmHg), an excess of n-hexanol (0.5 mL, 30-fold excess), dry dichloromethane (5 mL), and anhydrous potassium carbonate (25 mg) were added to the residue. The reaction mixture was stirred under nitrogen atmosphere for 45 min. It was then diluted with dichloromethane (200 mL), washed with aqueous sodium bicarbonate solution (100 mL), and then with water (2 × 200 mL). The dichloromethane layer was dried over anhydrous sodium sulfate,
concentrated, and treated with diazomethane. Evaporation of the solvent gave a syrupy residue, which was chromatographed over a silica column using 1% aceton in dichloromethane as eluant to yield 95 mg (85%) of the desired compound 5, which is sticky in nature. Analytical HPLC (Symmetry C18; 99/1: MeOH/H2O; tR = 48.97 min, >96%). UV–vis (MeOH): 700 (4.31 × 10^3), 545 (2.05 × 10^3), 508 (7.76 × 10^3), 414 (1.29 × 10^3). 1H NMR (CDCl3; 400 MHz): δ 9.75 (splits, 1H, meso-H); 9.62 (s, 1H, meso-H); 8.53 (s, 1H, meso-H); 8.09 (s, 1H, ArH); 7.70 (d, J = 7.6, 1H, ArH); 7.66 (d, J = 8.0, 1H, ArH); 7.60 (d, J = 7.6, 1H, ArH); 7.32 (d, J = 8.4, 1H, ArH); 7.10 (t, J = 7.8, 1H, ArH); 5.77 (q, J = 6.8, 1H, 3H-membered ring); 5.61 (s, 2H, NCH2Ar); 5.37 (d, J = 8.4, 1H, 17-H); 4.35 (q, J = 7.2, 1H, 18-H); 3.81 (s, 3H, 12-H); 1.61 (m, 2H, N(CH2)2CH2(CH2)2CH3); 1.38 (m, 3H, 2-CH3); 1.38 (s, 3H, 7-CH3); 2.52 (s, 9H, Sn(CH3)3); 2.26 (s, 9H, Sn(CH3)3); 1.25 (m, 6H, O(CH2)2(CH2)3CH3); 0.78 (m, 3H, O(CH2)3CH2CH3); 0.27 (s, 9H, Sn(CH3)3); −0.01 (brs, 1H, NH); −0.13 (brs, 1H, NH).

Synthesis of 3-1′-(Methoxymethyl)purpurin-18-N-(3-iodo-benzylimide Methyl Ester (10)). It was prepared by following the method described for compound 8 except the intermediate bromo-derivative was reacted with methanol, instead of n-hexanol. Pure product was obtained by column chromatography over a silica column using 1% aceton in dichloromethane as eluant. Analytical HPLC (Symmetry C18; 99/1: MeOH/H2O; tR = 31.42 min, >98%). UV–vis (CH2Cl2): 700 (4.31 × 10^3), 545 (2.12 × 10^3), 414 (1.29 × 10^3). 1H NMR (CDCl3; 400 MHz): δ 9.66 (s, 1H, meso-H); 9.64 (s, 1H, meso-H); 8.54 (s, 1H, meso-H); 8.08 (s, 1H, ArH); 7.70 (d, J = 8.0, 1H, ArH); 7.60 (d, J = 8.0, 1H, ArH); 7.10 (t, J = 8.0, 1H, ArH); 5.73 (q, J = 6.9, 1H, 3H-membered ring); 5.63 (s, 2H, NCH2Ar); 5.38 (dd, J = 1.6, 8.4, 1H, 17-H); 4.35 (q, J = 7.2, 1H, 18-H); 3.84 (s, 3H, 12-CH3); 3.67 (q, J = 7.4, 2H, 8-CH2CH3); 3.56 (s, 3H, 172-CO2CH3); 3.54 (d, J = 2.8, 3H, OCH3); 3.32 (s, 3H, 2-CH3); 3.19 (s, 3H, 7-CH3); 2.62–2.72 (m, 1H, 17-H); 2.30–2.45 (m, 2H, 172-H and 17-H); 2.06 (dd, J = 2.2, 6.6, 3H, 3′-CH2); 1.92–2.02 (m, 1H, 172-H); 1.76 (d, J = 6.0, 3H, 18-CH3); 1.68 (t, J = 7.6, 3H, 8-CH2CH3); 0.03 (s, 1H, NH); −0.07 (brs, 1H, NH). HRMS for C41H34N2O3I: 826.2385 (calculated, M + 1); found, 826.2470.

Synthesis of 3-1′-(Methoxymethyl)purpurin-18-N-(3-trimethylstannyl)benzylimide Methyl Ester (11). The title compound was synthesized following the procedure described above for compound 7 from the respective compound 3-1′-(methoxymethyl)purpurin-18-N-(3-iodo)benzylimide methyl ester (10). Yield: 80%. Analytical HPLC (Symmetry C18; 99/1: MeOH/H2O; tR = 35.78 min, >98%). 1H NMR (CDCl3; 400 MHz): δ 9.68 (s, 1H, meso-H); 9.65 (s, 1H, meso-H); 8.56 (s, 1H, meso-H); 7.90 (s, 1H, ArH); 7.68 (d, J = 7.6, 1H, ArH); 7.40 (d, J = 6.8, 1H, ArH); 7.34 (t, J = 7.2, 1H, ArH); 5.60–5.80 (m, 3H, 3′-H and NCH2Ar); 5.38 (dd, J = 2.0, 6.8, 1H, 17-H); 4.36 (q, J = 7.2, 1H, 18-H); 3.84 (s, 3H, 12-CH3); 3.67 (q, J = 7.4, 2H, 8-CH2CH3); 3.56 (s, 3H, 172-CO2CH3); 3.55 (d, J = 2.8, 3H, OCH3); 3.33 (s, 3H, 2-CH2); 3.20(s, 3H, 7-CH3); 2.62–2.72 (m, 1H, 17-H); 2.30–2.50 (m, 2H, 172-H and 17-H); 2.08 (dd, J = 2.2, 6.6, 3H, 3′-CH2); 1.92–2.02 (m, 1H, 172-H); 1.76 (d, J = 6.0, 3H, 18-CH3); 1.68 (t, J = 7.6, 3H, 8-CH2CH3); 0.28 (s, 9H, Sn(CH3)3); −0.03 (brs, 1H, NH); −0.12 (brs, 1H, NH).

Radioactive Labeling. 124I-analouges of 4, 5, and 10 were prepared from the corresponding trimethylstannyl analogues 6, 7, and 11, respectively, by following the procedure as described below for the 124I-analogue of compound 4.

Synthesis of 124I-Labeled Analogue of 3-[1′-(3-iodobenzoyloxy)ethyl]purpurin-18-N-(3-trimethylstannyl)benzylimide Methyl Ester (4). The trimethyltin analogue 6 (50 µg) was dissolved in 50 µL of 5% acetic acid in methanol. Then, 100 µL of 5% acetic acid in methanol was added to Na124I in 10 µL of 0.1 N NaOH. The two solutions were mixed, and an IODOGEN bead (Pierce Biotechnology, Inc., Rockford, IL 61016) was added. The reaction mixture was incubated at room temperature for 15 min, the iodobead was removed, and the reaction mixture was injected on an HPLC column (Symmetry C18 5 µm, 150 × 4 mm), which was eluted with an isocratic 99/1 MeOH/H2O at a flow rate of 1 mL/min. The UV detector was set at 254 nm wavelength. The labeled product (4) eluted at 46.7 min was collected, and the solvent was evaporated to dryness under a stream of N2 at 60 °C. The product was formulated in saline containing 10% ethanol for in vivo experiments. RadioTLC confirmed the radiochemical purity (>95%) of the product. A standard curve was generated between peak area versus mass by injecting known mass of carrier 4 onto the column. The mass...
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associated with the labeled product was calculated by relating the peak area of the UV absorbance peak of 4 in the labeled product to the standard curve. The specific activity was obtained by dividing the activity of the labeled product collected by the compound mass in micromoles. Specific activity of the radio labeled product was >1 Ci/μmol. The radiochemical yield was found to be 40%.

**PET Imaging.** Mice were imaged in the microPET FOCUS 120, a dedicated 3D small-animal PET scanner (Concorde Microsystems Incorporated) at State University of New York at Buffalo (south campus) under the Institutional Animal Care and Use Committee (IACUC) guidelines. The C3H mice were subcutaneously injected with 3 × 105 RIF cells in 30 μL complete α-MEM (into the axilla), and tumors were grown until they reached 4–5 mm in diameter (approximately 5 days). All tumors C3H mice were injected via the tail vein 50–200 μCi of 1, 8, 9, and 12.

After 24, 48, 72, and 96 h postinjection, the mice were anesthetized by inhalation of isoflurane/oxygen, placed head first prone for imaging, and the acquisition time was set for 30 min. Radioiodine uptake by the thyroid or stomach was not blocked.

**Biodistribution Studies.** All studies were performed as per IACUC guidelines. The mice were injected with 50–200 μCi of 1, 8, 9, and 12 via tail vein, and 3 or 4 mice each at 24, 48, 72, and 96 h time interval were sacrificed and body organs (tumor, heart, liver, spleen, kidney, lung, muscle, etc.) removed immediately. After weighing, the amount of radioactivity in the tumor (50–150 mg), body organs, and blood was measured by a gamma well counter. Radioactivity uptake was calculated as the percentage of the injected dose per gram of the tissue (%ID/g). Statistical analyses and data (%ID/g vs time point) were plotted using Microsoft Excel.

**In Vitro Photosensitizing Efficacy.** The photosensitizing activity of 4, 5, and 10 was determined in the RIF tumor cell line. The RIF tumor cells were grown in α-MEM with 10% fetal calf serum, l-glutamine, penicillin, streptomycin, and neomycin. Cells were maintained in 5% CO2, 95% air, and 100% humidity. For determining the PDT efficacy, these cells were plated in 96-well plates at a density of 5 × 103 cells/well. After a 4 h incubation at 37 °C, the photosensitizers were added at variable concentrations and incubated at 37 °C for 24 h in the dark. Prior to light treatment, the cells were replaced with drug-free complete media. Cells were then illuminated with an argon-pumped dye laser set at 700 nm at a dose rate of 3.2 mW/cm2 for 0–6 J/cm2. After PDT, the cells were incubated for 48 h at 37 °C in the dark. Following the 48 h incubation, 10 μL of 5.0 mg/mL solution of 3-[4,5-dimethylthiazol-2-yl]-2,5-diaryl tetrazolium bromide (MTT) dissolved in PBS (Sigma, St. Louis, MO) was added to each well. After a 4 h incubation at 37 °C, the MTT and media were removed, and 100 μL DMSO was added to solubilize the formazin crystals. The 96-well plate was read on a microtiter plate reader (Miles Inc. Titrtek Multiscan Plus MK II) at an absorbance of 560 nm. The results were plotted as percent survival of the corresponding dark (drug no light) control for each compound tested, and each experiment was done with 4 replicate wells.

**In Vivo Photosensitizing Efficacy.** The in vivo PDT experiments were performed in C3H mice when tumors grew to 4–5 mm in diameter (approximately day 5 post inoculation). The day before laser light treatment, all hair was removed from the inoculation site, and the mice were injected intravenously with varying photosensitizer concentrations. At 24 h postinjection, the mice were restrained without anesthesia in Plexiglas holders and then treated with laser light from an argon-pumped dye laser tuned to emit drug-activating wavelengths as set by the monochromator (665 nm for 1, 705 nm for 4, 5, and 10). The compounds were treated with light under similar treatment parameters under the fluence rate of 75 mW/cm2 with a light dose of 135 J/cm2. The mice were observed daily for signs of weight loss, necrotic scabbing, or tumor regrowth. If tumor growth appeared, the tumors were measured using two orthogonal measurements L and W (perpendicular to L), and the volumes were calculated using the formula V = (L × W2)/2 and recorded. Mice were considered cured if there was no sign of tumor regrowth by day 60 post-PDT treatment.

**RESULTS AND DISCUSSION**

**Chemistry.** For the synthesis of desired compounds, methylophoribide-a, isolated from *Spirulina pacifica* was converted into purpurnin-18 methyl ester by following the known methodology (44). For the preparation of 3-(1’-m-iodobenzyloxyethyl) analogue 4, it was first reacted with m-hexylamine, the intermediate amide derivative (isomeric mixture) so obtained in intramolecular cyclization under basic reaction conditions, and gave 2 in excellent yield. Further reaction of purpurinimide 2 with HBr/AcOH at room temperature produced the intermediate bromo-analogue, which was dried under vacuum and immediately reacted with m-iodobenzyl alcohol to afford 4 as a mixture of methyl ester and the corresponding carboxylic acid, which on treating with diazomethane produced the methyl ester derivative 4 in >70% yield. For the synthesis of the related isomer 5, the purpurin-18 methyl ester was first refluxed with m-iodobenzyl amine, and the intermediate 3 thus obtained on reacting with 1-hexanol by following the approach depicted in Scheme 1 gave the desired photosensitizer in modest yield. Compound 10 was synthesized from 3 following the methodology outlined for 5 and by replacing m-hexanol with methanol. For the preparation of the corresponding 124I-analogues 8, 9, and 12, the trimethylstannyl substituted analogues 6, 7, and 11 on electrophilic aromatic iodination with Na124I in the presence of iodogen beads afforded the 124I-labeled purpurinimides 8, 9, and 12 with >95% radioactive specificity. The purity of the final compounds (4, 5, and 10) was confirmed by NMR (experimental section) and HPLC analysis (see Supporting Information).

**Biological Studies.** Comparative Imaging and Biodistribution of 124I-Labeled Purpurinimide Isomers 8, 9, and 12. The PET imaging and biodistribution study of the radioactive purpurinimides 8 were performed in C3H mice bearing RIF tumors. In a typical experiment, 12 tumor-bearing mice were injected with each compound (50–200 μCi), and 3 mice/group were imaged at 24, 48, 72, and 96 h for 30 min with microPET (Siemens Preclinical Solutions, Knoxville, TN), and finally sacrificed after the 96 h time point. Mice used in longitudinal imaging received higher activity (150–200μCi) compared to the mice used in biodistribution alone (50–100 uCi).

For the biodistribution studies, selected organs [tumor, muscle, kidney, lungs, intestine (gut), stomach, spleen, heart, and lung] were removed, weighed, and measured in a gamma well counter. The tail was also taken into consideration in biodistribution studies to determine the accuracy of the injection. Interestingly, the two isomers showed a remarkable difference in imaging and biodistribution characteristics. The imaging and biodistribution data also correlated well with each other. Between the two isomers, isomer 8 (O-iodobenzyloxyethyl purpurinimide) had a higher background (liver and spleen), and the tumor was not visualized. Although the tumor was also not significantly visualized with isomer 9 (N-(3-iodobenzyl purpurinimide), compared to compound 8, the background images were not as high (Figure 2). If compared with lead compound 1 (124I-labeled), purpurinimide 9 also exhibited higher tumor uptake at 72 and 96 h PI, and unfortunately, higher background uptake negated tumor visualization (Figure 3). However, in
comparison with the $^{125}$I-analogue of pyropheophorbide I, both purpurinimide isomers 8 and 9 showed exceptionally high liver (32-fold and 8-fold, respectively) and spleen uptake (85-fold and 3-fold, respectively) at 24 h PI. The significantly high uptake of purpurinimides 8 and 9 in the liver and spleen resulted in nonvisualization of the tumor and thus produced a poor contrast in whole body PET-imaging. Among isomers 8 and 9, isomer 9 was selected for further modifications. The 1′-hexyloxyethyl group present at the top half of the molecule (position-3) was replaced with a methyl substituent. The resulting product 10, with a reduced overall lipophilicity, was labeled with $^{125}$I (compound 12) and the PET imaging biodistribution data were performed in C3H mice bearing RIF tumors. The results obtained from the biodistribution studies suggest that the reduction in the overall lipophilicity of the molecule substantially reduces the uptake of purpurinimide 12 in the spleen and liver and therefore enhances its tumor imaging capability at 96 h postinjection. The biodistribution and whole-body PET imaging results obtained from compounds 1, 8, 9, and 12 suggest that for an efficient tumor imaging agent it is of utmost important to have a high uptake of the contrast agent in the tumor with a faster clearance profile from other organs. This characteristic possibly explains the improved imaging capability of lead compound 1 over that of the other agents studied so far.

Comparative in Vitro PDT Efficacy. The in vitro photosensitizing ability of the structural isomers 4 and 5 was compared

**Figure 2.** Comparative microPET emission images (coronal view) of C3H mice with RIF tumors at 48 h PI of $^{125}$I-124 labeled purpurinimides 8 (A), 9 (B), 12 (C), and the lead compound 1 (D).
at variable experimental conditions (MTT assay; see Experimental Procedures) in RIF cells. Both isomers were ineffective in vitro at lower light and drug doses. However, as can be seen from Figure 4, at 24 h postincubation and higher light dose (6.0 J/cm², drug concentration (1.0 µM and higher), isomer 5 was more effective than the structural isomer 4. Reducing the overall lipophilicity of 5, by replacing the O-hexyl group at position-3 with an O-methyl group 10 produced enhanced efficacy over isomers 4 and 5.

**Figure 3.** Comparative biodistribution of (124I-labeled) pyropheophorbide-1 and the purpurimides-8, 9, and 12 at 24, 48, 72, and 96 h PI in C3H mice bearing RIF tumors (4 mice/time point). Note: compounds 8, 9, and 12 are 124I-labeled 4, 5, and 10, respectively.

**In Vivo PDT Efficiency of Pyropheophorbide-a 1 and Purpurinimide Isomers 4 and 5.** The in vivo PDT efficacy of isomers 4 and 5 was determined at three doses (1.0, 2.0, and
it can be seen that compound was measured daily. From the results summarized in Figure 5, compound long-term tumor response. Under the same treatment parameters, at the lower half of the molecule did not give any significant same dose, the isomer at the top half of the molecule, at a dose of 1.0 µmol/kg gave more effective at a dose of 1.5 (e.g., 2 µmol/kg) 4 tumor free on day 60. Among isomers 4 and 5, compound 5 containing an N-iodobenzyl group introduced at the bottom half of the purpurinimide showed improved imaging and phototherapeutic abilities than 4 where the iodobenzyl group was present at position-3 of the molecule. Decreasing the overall lipophilicity of compound 5 by substituting the hexyl ether with a methyl ether group (compound 10) further improved its PET imaging ability and PDT efficacy. However, for establishing a correlation between the overall lipophilicity and tumor imaging potential, it is necessary to investigate a series of compounds within a particular system, and these studies are currently in progress.

CONCLUSIONS

Our results suggest that the nature and the position of the substituents in purpurinimides make a significant difference in tumor uptake, which also reflects their imaging and PDT potential. Between the two structural isomers 4 and 5, compound 5 containing an N-iodobenzyl group introduced at the bottom half of the purpurinimide showed improved imaging and phototherapeutic abilities than 4 where the iodobenzyl group was present at position-3 of the molecule. Decreasing the overall lipophilicity of compound 5 by substituting the hexyl ether with a methyl ether group (compound 10) further improved its PET imaging ability and PDT efficacy. However, for establishing a correlation between the overall lipophilicity and tumor imaging potential, it is necessary to investigate a series of compounds within a particular system, and these studies are currently in progress.

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Supporting Information Available: The 1H NMR spectra of compounds 4–7, 10, 11, and the HPLC chromatograms of compounds 1, 4, 5, and 10. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED


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