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Lipophilic phosphonium–lanthanide compounds with magnetic, luminescent, and tumor targeting properties

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ABSTRACT

Multifunctional phosphonium–lanthanide compounds that simultaneously possess paramagnetism, luminescence, and tumor mitochondrial targeting properties were prepared by use of a facile method. These compounds were fully characterized by use of ^{1}H, ^{13}C, ^{31}P NMR, FT-IR, and elemental analyses. The thermal properties of these compounds including melting points and decomposition temperatures were investigated using DSC and TGA analyses. In addition, the paramagnetism, luminescence, and tumor targeting properties of these multifunctional compounds were confirmed by respective use of SQUID, fluorescence, and cell cytotoxicity studies. All compounds exhibited paramagnetism at room temperature, which could provide target delivery of these compounds to parts of the body containing tumor cells using a strong external magnetic field. In addition, these compounds display two major characteristic emissions originating from Dy^{3+}, which can be utilized for imaging tumor cells. The IC_{50} values of these compounds measured against normal breast cell line (Hs578Bst) are significantly greater than those measured against the corresponding carcinoma breast cell line (Hs578T), clearly indicating the selective tumor targeting properties of these compounds. Confocal fluorescence microscopy studies were used to confirm the yellowish-green fluorescence corresponding to the emission of dysprosium thiocyanate anion within cancer cells upon exposure of cancer cell lines such as human pancreatic carcinoma cell line (MIAPaCa-2) and human breast carcinoma (MDA-MB-231) to a solution of these phosphonium–dysprosium compounds.

1. Introduction

The aim of drug targeting is to deliver therapeutic compounds to a particular tissue in the body, a strategy which has found tremendous significance in the field of cancer therapy. A targeted delivery approach may overcome major delivery limitations such as low bioavailability, significant toxicity, undesirable side effects, and unrestricted biodistribution of anticancer drugs [1,2]. As one class of major cellular targeting agents, delocalized lipophilic cations (DLCs) accumulate more rapidly in the mitochondria of most carcinoma-derived cells than in most untransformed cells in response to negative inside transmembrane potentials [3–5]. This difference has been attributed to the abnormally high mitochondrial membrane potentials ($\Delta\Psi m$) characteristic of most tumor cells [6–10]. Accumulation of DLCs in mitochondria may inhibit oxidative phosphorylation, an energy-generating process, and thus disrupt the cellular ATP production required to sustain normal cellular functions [3]. The selective inhibition of this process in tumor cells has provided an effective strategy for treatment of cancer. Numerous in vitro and in vivo studies have demonstrated the selective cytotoxicity of DLCs against tumor cells [11–14].

One class of lipophilic compounds based on phosphonium is called cationic lipophilic phosphonium salts (CLPS). These compounds have shown much higher carcinoma selectivity in vitro than clinically used anti-tumor agents such as cisplatin and cytosine arabinoside (ara-C) [15]. Tetraphenylphosphonium chloride (TPP) is the prototype compound of CLPS. Radiolabeled TPP has been used as a molecular probe for tumor imaging because TPP can selectively accumulate in the mitochondrial membrane of tumor cells [16]. TPP-conjugated antioxidants have also been developed for their targeted delivery to mitochondria which could prevent mitochondrial oxidative damage far more effectively than untagged antioxidants [17,18]. Interestingly, the counteranions of all these lipophilic salts reported to date have been exclusively limited to halides, e.g., chloride and bromide. These halides seem to be simply used as counteranions in order to simply balance the positive electric charge of DLCs, without serving any other useful function. For example, anions were even omitted in the illustrations of lipophilic salt molecular structures in a number of

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articles [19–21]. In our view, however, a functional anion introduced by a simple anion-exchange of the original halide anion may afford additional useful properties. This strategy has been employed by us to prepare magnetic chiral room temperature ionic liquids by replacing the Cl^- of an amino acid methyl ester hydrochloride with a paramagnetic anionic species such as $FeCl_4^-$ [22].

Ionic liquids (ILs) have been studied extensively in a great variety of fields owing to their unique and beneficial properties [23–26]. One of the most desirable properties of ILs is their high tunability. By simply replacing the halide anions of organic salts with functional anionic species, one can generate numerous novel multifunctional ionic liquid materials with many attractive properties [27–32]. Consequently, within a single molecule both the cation and anion may possess distinct and desirable properties and functions, which in turn could be used to prepare multifunctional materials more conveniently. Using a similar rationale previously employed for synthesizing functional ILs, we introduce multifunctional anions based on a lanthanide (dysprosium) complex to exchange the bromide anion of aryltriphenylphosphonium salts. The dysprosium–thiocyanate anion is intrinsically paramagnetic and luminescent, which provides great utility for applications such as targeted delivery in the presence of a magnetic field and fluorescence imaging of tumor cells. Simultaneously, the original lipophilic phosphonium cation can serve as a mitochondrial targeting agent, selective for tumor cells.

We herein report on the synthesis and characterization of novel multifunctional phosphonium–lanthanide compounds that simultaneously contain paramagnetism, luminescence, and tumor mitochondrial targeting properties within a single compound. In addition, we report the application of these compounds as tumor-targeting, therapeutic, and imaging agents in human cell cultures.

2. Experimental

2.1. Chemicals and materials

Phosphonium bromides including tetraphenylphosphonium bromide (98.0%), benzyltriphenylphosphonium bromide (97.0%), (4-ethoxybenzyl)triphenylphosphonium bromide (98.0%), (4-nitrobenzyl)triphenylphosphonium bromide (98.0%) were obtained from TCI America (Portland, Oregon, USA). Potassium thiocyanate, dysprosium(III) oxide, and perchloric acid (70%) were obtained from Sigma-Aldrich (Milwaukee, Wisconsin, USA) and used without further purification. Ethanol, methanol, acetonitrile, chloroform, and carbon tetrachloride were of anhydrous grade (Sigma-Aldrich, Milwaukee, WI), and all other solvents such as acetone, hexane, and water are of HPLC grade (J. T. Baker, Phillipsburg, NJ).

Normal human breast fibroblast cells (Hs578Bst, ATCC no. HTB-125), human breast carcinoma cells (Hs578T, ATCC no. HTB-126), hormone-independent human breast carcinoma cells (MDA-MB-231, ATCC no. HTB-26), human pancreatic carcinoma cells (PANC-1, ATCC no. CRL-1469), and human colorectal adenocarcinoma cells (HT-29, ATCC no. HTB-38) were obtained from the American Tissue Culture Collection (ATCC, Manassas, VA) and grown to 90% confluence according to ATCC's instructions.

2.2. Instrumentation and methods

^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were acquired by use of a Bruker Avance 400 NMR spectrometer. ^{31}P NMR (101 MHz) spectra were taken with a 250 Brucker Avance spectrometer. The ^1H and ^{13}C chemical shifts are given in parts per million (δ) with TMS as an internal standard. The chemical shifts of ^{31}P are recorded relative to external 85% H_3PO_4 with broad-band ^1H decoupling. FT-IR was measured using a Bruker Tensor 27 FT-IR spectrometer. Samples were analyzed in pure form by use of a DuraSamp IR apparatus. All spectra were obtained using 32 scans for both sample and background with a resolution of 4 cm^{-1}. Elemental analyses were contracted to Atlantic Microlab (Atlanta, GA, USA).

A TA Q50 thermal gravimetric analyzer (TA Instruments, New Castle, DE) was used to analyze the thermal stability of phosphonium–dysprosium salts and their decomposition behaviors. Samples (about 3–4 mg) were scanned from room temperature to 500 °C under nitrogen flow (50 mL/min) with a heating rate of 10 °C/min. Values for the onset degradation temperature and peak temperature were determined from the derivative TGA curves. Melting points of the products were investigated by use of a Q100 differential scanning calorimeter (TA Instruments, New Castle, DE). The phosphonium–dysprosium salts (about 5 mg) were put in a sealed aluminum crucible and run under a nitrogen flow (50 mL/min). Samples were cooled and heated with the following sequence: isothermal for 3 min at 20 °C; then heated to 120 °C with a rate of 5 °C/min, isothermal for 3 min at 120 °C; then cooled to 20 °C with a rate of 5 °C/min.

Magnetic properties were measured using approximately 80 to 100 mg of the sample in a Quantum Design Superconducting Quantum Interference Device (SQUID) magnetometer (San Diego, CA, USA) at temperatures between 2 and 300 K and fields between −70 000 and 70 000 Oe. Absorbance measurements were performed on a Shimadzu UV-3101PC UV–vis-near-IR scanning spectrometer (Shimadzu, Columbia, MD). Steady-state fluorescence measurements were recorded at room temperature by use of a Spex Fluorolog-3 spectrofluorimeter (model FL3-22TAU3; Jobin Yvon, Edison, NJ) equipped with a 450-W xenon lamp and R928P photomultiplier tube (PMT) emission detector. Fluorescence emission spectra were collected in a 4-mm quartz fluorescence cuvet with slit widths set for entrance exit bandwidths of 4 nm on both excitation and emission monochromators.

2.3. Synthesis and characterization of the phosphonium–lanthanide compounds

A facile two-step synthesis procedure was used to prepare the phosphonium–lanthanide compounds. In the first step, the four intermediate compounds ($[Ph_4P][SCN]$, $[Ph_3PBnOEt][SCN]$, $[Ph_3PBnNO_2][SCN]$, and $[Ph_3PBn][SCN]$) were synthesized by reacting the respective phosphonium bromide (1 equiv.) with KSCN (2 equiv.) in acetonitrile at room temperature for 2 d through anion-exchange reactions [33]. $Dy(ClO_4)_3.6H_2O$ was obtained by dissolving Dy_2O_3 in 70% $HClO_4$ aqueous solution followed by subsequent removal of water by lyophilization. In the second step, the four phosphonium–dysprosium products ($[Ph_4P]_5[Dy(SCN)_8]$, $[Ph_3PBnOEt]_5[Dy(SCN)_8]$, $[Ph_3PBnNO_2]_5[Dy(SCN)_8]$, and $[Ph_3PBn]_5[Dy(SCN)_8]$) were synthesized according to the procedure reported previously with slight modifications [27,28]. A typical synthesis procedure is as follows. A mixture of $[Ph_4P][SCN]$ (5 equiv.), KSCN (3 equiv.), and $Dy(ClO_4)_3.6H_2O$ (1 equiv.) was stirred in anhydrous ethanol at room temperature overnight. The white suspension of $KClO_4$ byproduct was removed by filtration and ethanol was evaporated by use of a rotavapor. The residue was redissolved in anhydrous dichloromethylene and the solution was allowed to stand overnight in a refrigerator. After filtration dichloromethylene was evaporated under vacuum to afford the products which were further dried under vacuum for 48 h. *Caution*: Although we did not experience any problems in this work, *special care should be taken when perchlorates are handled* due to a potential hazard of explosion. Characterization of the four phosphonium–dysprosium compounds by use of ^1H, ^{13}C, and ^{31}P NMR, FT-IR, and C, H, N, S elemental analysis is provided in Supporting information.

2.4. Cell viability assay

The cytotoxicities of the compounds against various cell lines were determined by use of CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega Corporation, Madison, Wisconsin, USA) according to the manufacturer's instructions. Specifically, the cells (5×10^3 cells/well, 100 μL) were incubated with solutions of compounds dissolved in appropriate culture media (containing 2% DMSO). The cells were incubated with various concentrations of the four compounds at 37 °C, in 5% CO_2 atmosphere for 48 h. At the end of the incubation period, the cells were treated with 3-(4,5-di-methylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfo-phenyl)-2H-tetrazolium salt (MTS) (20 μL). After 1 h, the absorbance was measured at 490 nm using a microplate spectrophotometer (Benchmark Plus, Bio-Rad Laboratories, Hercules, CA, USA). Percentage cell viability was calculated as 100 times the ratio of absorbance in the presence of investigated compounds and the absorbance for treatment-free control samples. The concentration at which the growth of 50% of cells was inhibited (IC_{50}) was calculated using the linear fit equation of the linear part of the cytotoxicity graphs (cell survival versus concentration) for each compound. In the case of compounds for which the linear portion of the graph did not include 50% cell growth inhibition, the IC_{50} was reported as higher than the minimum concentration at which a constant cytotoxicity was reached irrespective of the increase in concentration. The values of IC_{50} were used for comparison of the cytotoxicities among the synthesized compounds.

All experiments were performed in triplicate. Statistical analysis was conducted using one-way analysis of variance (ANOVA). Tukey's studentized range test was performed to ascertain the significant difference between treatments within the 95% confidence interval using SAS 9.2 software (SAS, Cary, NC, USA).

2.5. Tumor cell uptake and imaging study by confocal fluorescence microscopy

Human pancreatic carcinoma cell lines (MIAPaCa-2) (1×10^5 cells/well) were incubated with solutions of the phosphonium–lanthanide compounds (10 μM) for 1 h at 37 °C in 5% CO_2 atmosphere. The cell medium was subsequently removed. Treated cells were then washed with prewarmed phosphate buffered saline (PBS) to remove excess fluorescent probes and possible interferences of the medium. As control, non-treated cells containing no phosphonium–lanthanide compound were incubated and imaged using the same protocol as for the treated cells. Confocal fluorescence microscopy images of live cells were acquired using a confocal laser microscope (Zeiss Confocal LSM510, Carl Zeiss MicroImaging Inc.Thornwood, NY, USA) equipped with an argon-krypton laser *Blue Stain*. The sample was illuminated with a 458 nm Argon/Krypton laser for both the fluorescent and transmitted light images. Images were acquired by collecting emitted light at wavelengths from 512 to 663 nm.

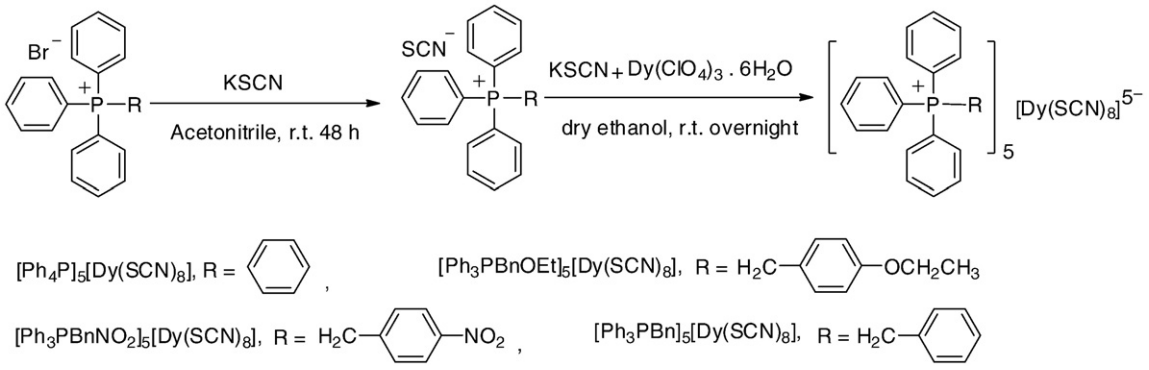
3. Results and discussion

3.1. Synthesis and characterization

Four phosphonium–dysprosium salts were synthesized by following a previously reported method [27,28] with slight modifications (Scheme 1). These compounds were characterized by use of ^1H, ^{13}C, ^{31}P NMR, FT-IR, and elemental analysis. Their thermal properties, including melting points and thermal stabilities, were also investigated by use of differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) (Supporting information). These phosphonium–dysprosium compounds exhibit low solubility in water (Table S1, Supporting information) due to their hydrophobic triphenylarylphosphonium cations. In contrast, as a control compound, $K_5Dy(SCN)_8$ containing a different potassium cation, but the same dysprosium thiocyanate complex anion shows much higher water solubility than the phosphonium-containing compounds. This comparison highlights the significant influence of the cation on the solubility of these compounds. Additional resistivity tests of the aqueous solution containing $[Ph_4P]_5[Dy(SCN)_8]$ and the control compound $K_5[Dy(SCN)_8]$ demonstrated that the saturated aqueous solutions of $[Ph_4P]_5[Dy(SCN)_8]$ had higher resistivity (14.58 versus 8.38 Ω). This comparison of resistivity indicates that the compounds are stable and primarily exist in a non-dissociated state in aqueous solution.

3.2. Paramagnetic properties of the phosphonium lanthanide compounds

All the four compounds contain a magnetically active ion Dy^{3+} with a $4f^9$ electron configuration and exhibit paramagnetic behavior at room temperature. Their magnetic susceptibilities were measured using a superconducting quantum interference device (SQUID) magnetometer. The magnetization at 300 K was measured in a magnetic field ranging from −70 000 to 70 000 Oe and exhibited an expected linear dependence on the applied magnetic field according to Curie's law (Fig. 1a) [29–32]. From the slopes of the fitted lines, the respective molar magnetic susceptibilities of the four phosphonium–dysprosium compounds at 300 K were obtained as $\chi_{mol}=0.0453$, 0.0400, 0.0385, and 0.0353 $cm^3\,mol^{-1}$, respectively, which fit well to the expected value for Dy^{3+}[27]. The effective magnetic moment (μ_{eff}) for Dy^{3+} has been calculated as 10.48 μ_B[27]. The measured μ_{eff} values of the four compounds were close to the calculate → d value (Table 1). All four compounds showed very weak anti-ferromagnetic interactions as observed from their Weiss constants (Table 1). The increase in χ_{mol} with decreasing temperature in the plot of magnetic susceptibility against temperature also proves that $[Ph_3PBnNO_2]_5[Dy(SCN)_8]$



Scheme 1. Synthesis of multifunctional phosphonium–dysprosium compounds.

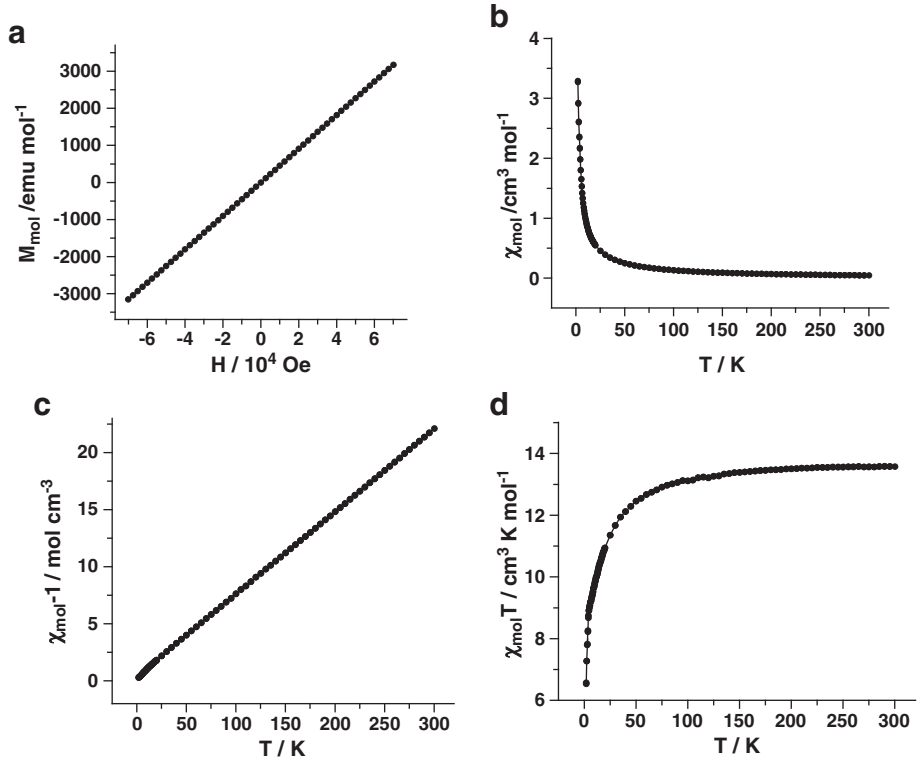


Fig. 1. Magnetic property of $[Ph_3PBnNO_2]_5[Dy(SCN)_8]$. Field dependence of a) the molar magnetization M_{mol} at 300 K. Temperature dependence of b) the static molar magnetic susceptibility χ_{mol}, c) the reciprocal molar magnetic susceptibility χ_{mol}^{-1}, and d) the product of temperature and static molar magnetic susceptibility $\chi_{mol}T$ at a field of 1000 Oe.

is paramagnetic (Fig. 1b). Due to the large anisotropic magnetic moment of Dy^{3+}, all four compounds can be manipulated by a neodymium magnet when the distance between the magnet and compound is less than about 4 mm (Fig. 2). Therefore, the phosphonium–dysprosium compounds could possibly be target delivered under a strong external magnetic field and concentrated in parts of the body containing tumor cells. The field and temperature dependences of the other three phosphonium–dysprosium salts are listed in the Supporting information.

3.3. Luminescence properties of the phosphonium lanthanide compounds

Luminescence properties of the four compounds were studied as well. Dy^{3+} with a $4f^9$ electron configuration is known for its intense luminescent transition, $^4F_{9/2} \rightarrow {}^6H_{15/2}$ in the blue region of visible light spectrum and $^4F_{9/2} \rightarrow {}^6H_{13/2}$ in the yellowish-green spectral region [34]. All four compounds showed the two major characteristic emissions (Fig. 3). The intensity of the hypersensitive transition varies with the environment. The transition $^4F_{9/2} \rightarrow {}^6H_{13/2}$ at 573 nm is the most intense for all the compounds in agreement with their yellowish-green luminescence. It is well known that lanthanide ions have very low absorption coefficients because optical transitions within the 4f subshells of lanthanide ions are partially forbidden [35]. However, energy transfer from an organic antenna chromophore may generate indirect excitation, which allows for excitation at a wavelength where the lanthanide ion does not exhibit significant absorption [36]. In addition, lanthanide ions are usually good quenchers of triplet states so that photobleaching is substantially reduced [36]. For our compounds, the aryltriphenylphosphonium cations may have served as sensitizers (antenna) and are able to activate Dy^{3+} via energy transfer. This bright fluorescence originating from Dy^{3+}

Table 1
Summary of magnetic properties of the compounds.

Compounds	χ_{mol}[a] ($cm^3\ mol^{-1}$)	μ_{eff}[b] (μ_B)	θ[c] (K)
$[Ph_3PBnNO_2]_5[Dy(SCN)_8]$	4.53×10^{-2}	10.4	−4.3
$[Ph_3PBnOEt]_5[Dy(SCN)_8]$	4.00×10^{-2}	10.2	−4.4
$[Ph_3PBn]_5[Dy(SCN)_8]$	3.85×10^{-2}	9.9	−3.7
$[Ph_4P]_5[Dy(SCN)_8]$	3.53×10^{-2}	9.6	−3.1

[a] Molar magnetic susceptibility measured at 300 K
[b] Effective magnetic moment.
[c] Weiss constant.

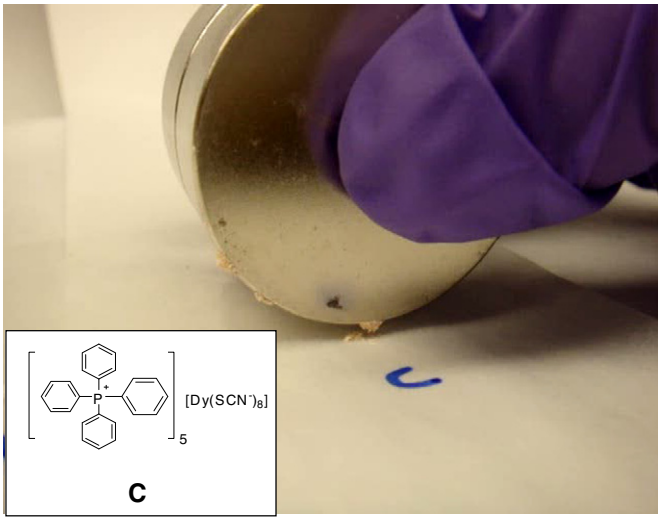


Fig. 2. Response of the pale yellow-colored $[Ph_4P]_5[Dy(SCN)_8]$ to a neodymium magnet.

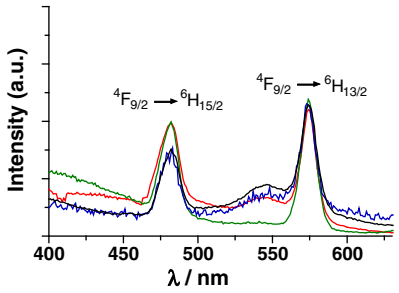


Fig. 3. Emission spectra with transition assignments of $[Ph_4P]_5[Dy(SCN)_8]$ (red), $[Ph_3PBnOEt]_5[Dy(SCN)_8]$ (green), $[Ph_3PBnNO_2]_5[Dy(SCN)_8]$ (black), and $[Ph_3PBn]_5[Dy(SCN)_8]$ (blue) in acetonitrile (1 mM), $\lambda_{ex} = 365$ nm.

was subsequently tested for application as an imaging agent for tumor cells. The excitation spectra obtained by monitoring the emissions at 480 nm and absorption spectra of the four compounds are shown in the Supporting information.

3.4. Selective cytotoxicities of the phosphonium lanthanide compounds against tumor cell lines

These new phosphonium–dysprosium compounds were also investigated for their cytotoxicities against four cancer cell lines and one normal breast cell line. The concentrations (IC_{50}) at which the growth of 50% of cells was inhibited were calculated and used for comparison. In the control tests, the starting material $[Ph_4P][Br]$, a prototype CLPS compound, displayed very low cytotoxicity against all cancer cell lines studied. The IC_{50} values of $[Ph_4P][Br]$ were greater than the highest concentration investigated (400 μM) (Fig. 4). In contrast, the anion-exchange of Br^- with the dysprosium–thiocyanate anion resulted in lower IC_{50} of the four products. It is possible that the increased cytotoxicities of the new compounds are due to a higher amount of active phosphonium per mole of phosphonium–dysprosium compounds as compared with the bromide salt. In addition, therapeutic applications including anticancer agents of lanthanides have been reported [37–40]. Previous studies at the cellular level and animal tests indicated that a high dose of Ln^{3+} can induce apoptosis of cancer cells a result of the damaging effect of cytotoxic behavior.[37] Therefore, it is also possible that the increased cytotoxicities against cancer cell lines were obtained due to the replacement of bromide by the dysprosium-containing anion.

To investigate the selective cytotoxicity of the four compounds against tumor cells, a cell viability study was also performed with the normal breast cell line Hs578Bst as control. The IC_{50} of $[Ph_4P]_5[Dy(SCN)_8]$ against breast cancer cell line Hs578T was 83 μM. Conversely, IC_{50} against normal breast cell line Hs578Bst was greater than 200 μM (Fig. 5a). Our results also show that there was a significant difference between the viability of Hs578T and Hs578Bst cells when treated with $[Ph_4P]_5[Dy(SCN)_8]$ at concentrations between 25 and 200 μM. This comparison demonstrates that $[Ph_4P]_5[Dy(SCN)_8]$ selectively induced cytotoxicity against tumor cells versus normal cells under the same cell culture conditions. The same trend was also observed when $[Ph_3PBnOEt]_5[Dy(SCN)_8]$ was incubated with both the normal and cancer cell lines for 48 h. For example, IC_{50} of $[Ph_3PBnOEt]_5[Dy(SCN)_8]$ against Hs578T was 19 μM, while a much higher IC_{50} of 74 μM against the normal cell line Hs578Bst was observed (Fig. 5b). Our data also indicate that there was at all concentrations of $[Ph_3PBnOEt]_5[Dy(SCN)_8]$ a significant difference in viability between tumor and normal cells, except at 15 μM. Similar results were obtained when cancer and normal cells were treated for 48 h with $[Ph_3PBnNO_2]_5[Dy(SCN)_8]$ (Fig. 5c), and $[Ph_3PBn]_5[Dy(SCN)_8]$ (Fig. 5d). A significant difference was found between cancer and normal cells for treatment with $[Ph_3PBnNO_2]_5[Dy(SCN)_8]$ (5–200 μM) and $[Ph_3PBn]_5[Dy(SCN)_8]$ (5–100 μM). The IC_{50} values of these compounds for Hs578T and Hs578Bst are displayed in Table 2. Altogether, these results demonstrate the selective cytotoxicity of the lipophilic phosphonium–dysprosium salts.

In contrast, most clinical antineoplastic agents such as cisplatin and ara-C do not show appreciable selectivity against tumor-derived versus untransformed cells if the cells are actively proliferating in culture [12]. The anti-carcinoma selectivity of aryltriphenylphosphonium salts has been postulatively ascribed to their different diffusion rates from the extracellular environment into the mitochondria and cytoplasm of tumor cells [15]. Among the four salts investigated, $[Ph_3PBnOEt]_5[Dy(SCN)_8]$ with an electron donating group ($-OCH_2CH_3$) exhibited the highest anticancer activities. Rideout et al. have also reported significantly lower IC_{50} value of a modified aryltriphenylphosphonium chloride that contains an electron donating group ($-N(CH_3)_2$) [15]. IC_{50} values against other tumor cell lines including hormone-independent human breast carcinoma cells (MDA-MB-231), human pancreatic carcinoma cells (PANC-1), and human colorectal adenocarcinoma cells (HT-29) were also investigated (Table 2). The IC_{50} values of $[Ph_3PBnOEt]_5[Dy(SCN)_8]$ against HT-29 and MDA-MB-231 were 86 and 28 μM, respectively. The IC_{50} values of all four compounds against PANC-1 were greater than the concentrations investigated, which suggests that the four compounds have low anticancer activity against the PANC-1 cells after 48 h incubation.

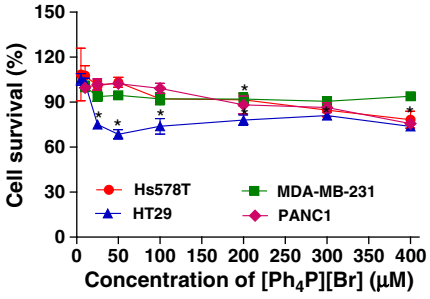


Fig. 4. Cytotoxicity of $[Ph_4P][Br]$ against cancer cell lines, 48 h incubation. All points are mean ± s.d. of triplicate wells of three independent experiments. Data is considered significant when $p < 0.05$.

3.5. Targeted delivery of the phosphonium lanthanide compounds to tumor cells

Confocal fluorescence microscopy has been shown to be effective in observing cellular uptake, intracellular distribution, and localization of fluorescent drugs [41–43]. This technique has also been used in imaging tumor cells [44–48]. In order to demonstrate the potential of these lipophilic phosphonium–dysprosium compounds as contrast agents for imaging tumor cells, we examined the cellular uptake and fluorescence images of $[Ph_4P]_5[Dy(SCN)_8]$ using the human pancreatic carcinoma cell line (MIAPaCa-2). In this study, the cell line MIAPaCa-2 was used as a model cell of tumor cells. The potential application of these phosphonium–dysprosium compounds as tumor cell imaging markers was confirmed using a confocal fluorescence microscope. We observed that upon exposure of MIAPaCa-2 cells to a solution of $[Ph_4P]_5[Dy(SCN)_8]$ for 1 h at 37 °C, the phosphonium–dysprosium compound was internalized. The yellowish-green fluorescence light in Fig. 4c corresponds to the emission of Dy^{3+} inside MIAPaCa-2 cells. The observed fluorescence image is attributed to the significant uptake of $[Ph_4P]_5[Dy(SCN)_8]$ in the tumor cells through highly selective and efficient interactions between phosphonium cations and the mitochondria of the tumor cell [3,4]. In the control experiment, however, no fluorescence emission was observed using the same cell line MIAPaCa-2, but without addition of

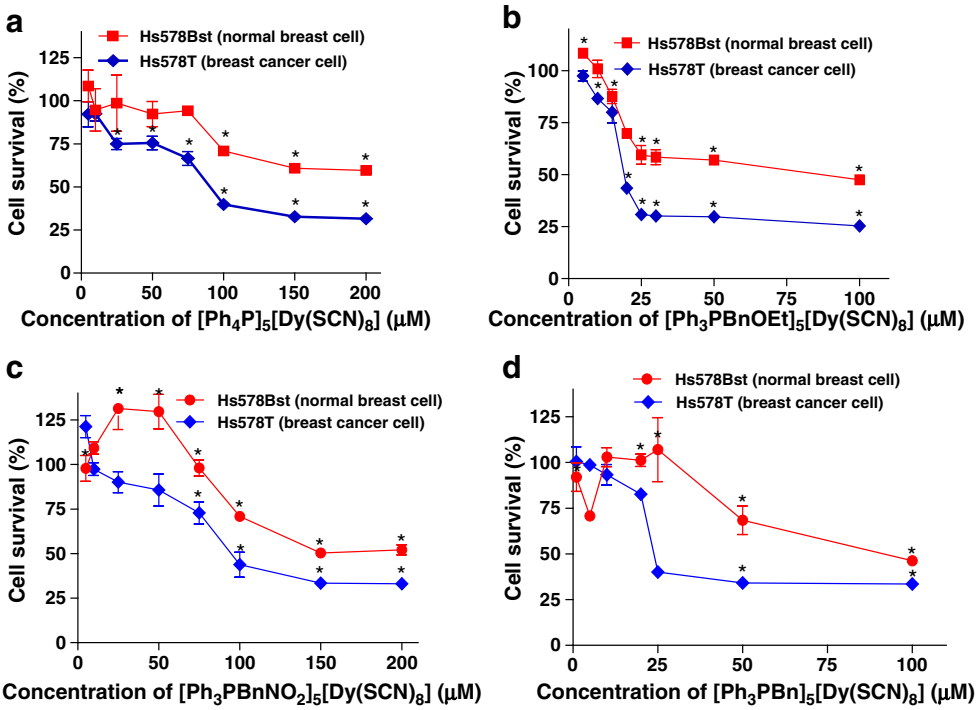


Fig. 5. Cytotoxicity of a) $[Ph_4P]_5[Dy(SCN)_8]$, b) $[Ph_3PBnOEt]_5[Dy(SCN)_8]$, c) $[Ph_3PBnNO_2]_5[Dy(SCN)_8]$, and d) $[Ph_3PBn]_5[Dy(SCN)_8]$ against breast cancer (Hs578T, diamonds) and normal (Hs578Bst, squares) cell lines, 48 h incubation. All points are mean ± s.d of triplicate wells of three independent experiments. Data is considered significant when $p<0.05$.

phosphonium–dysprosium complex (Fig. 6a). Note that the blue fluorescence emission observed in Fig. 6a is due to the treatment of the cell line with a nuclear marker. This comparison clearly demonstrates that the green fluorescence emission observed in Fig. 6c should be ascribed to the fluorescence emission of the $[Ph_4P]_5[Dy(SCN)_8]$. A confocal fluorescence micrograph of a single MIAPaCa-2 cell is also provided (Fig. 6) which clearly demonstrates that $[Ph_4P]_5[Dy(SCN)_8]$ was primarily associated with the cell membrane and can be imaged by use of the greenish fluorescence. These cellular uptake and fluorescence imaging experiments confirm that $[Ph_4P]_5[Dy(SCN)_8]$ has a unique binding (accumulating) property on or in the MIAPaCa-2 cell membrane. In order to further verify the experimental results from fluorescence imaging, we acquired an additional confocal fluorescence microscopic imaging of a different hormone-independent human breast carcinoma cell line (MDA-MB-231) using another phosphonium–dysprosium compound, $[Ph_3PBnNO_2]_5[Dy(SCN)_8]$, as the fluorescence imaging agent (Fig. S42, Supporting information). This cell line has been previously subjected to extensive studies of cellular uptake of different tumor images or therapeutic agents by use of fluorescence microscopy [49–52]. Again, the bright greenish yellow fluorescence emission confirms that $[Ph_3PBnNO_2]_5[Dy(SCN)_8]$ can be internalized by the cancer cell line MDA-MB-231 and visualized by use of confocal laser microscope. As a control test, the image of the cancer cells without adding any phosphonium–lanthanide complex proved that the cell line itself does not produce greenish yellow fluorescence (Fig. S43, Supporting information). Note that the nuclear marker was not used in this test.

Conclusions

We have developed four multifunctional lipophilic phosphonium–dysprosium compounds. Three functionalities were incorporated into a single molecule through a simple and straightforward synthesis. These compounds possess paramagnetic and luminescent properties which arise from the dysprosium-containing anions, as well as carcinoma cell selectivity attributable to the aryltriphenylphosphonium cations. In vitro studies demonstrate that the four compounds selectively inhibit the growth of tumor cells, as compared to normal cells, suggesting that they may be of potential use as cancer therapeutic agents. In addition, these compounds could also be used as fluorescence imaging markers in vital cell cultures.

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Table 2
Inhibitory concentrations (IC_{50}) of the four compounds against cancer cell lines and normal cell line: 48 h assay.

Cell lines	IC_{50} (μM)[a]			
	$[Ph_4P]_5[Dy(SCN)_8]$	$[Ph_3PBnOEt]_5[Dy(SCN)_8]$	$[Ph_3PBnNO_2]_5[Dy(SCN)_8]$	$[Ph_3PBn]_5[Dy(SCN)_8]$
Hs578Bst	>200	74	147	85
Hs578T	83	19	91	22
MDA-MB-231	121	28	141	194
HT29	251	86	303	85
PANC-1	>400	>450	>400	>400

[a] The values for IC50 were calculated from the slopes of cell viability as a function of concentration graphs for each of the investigated compounds and cell lines.

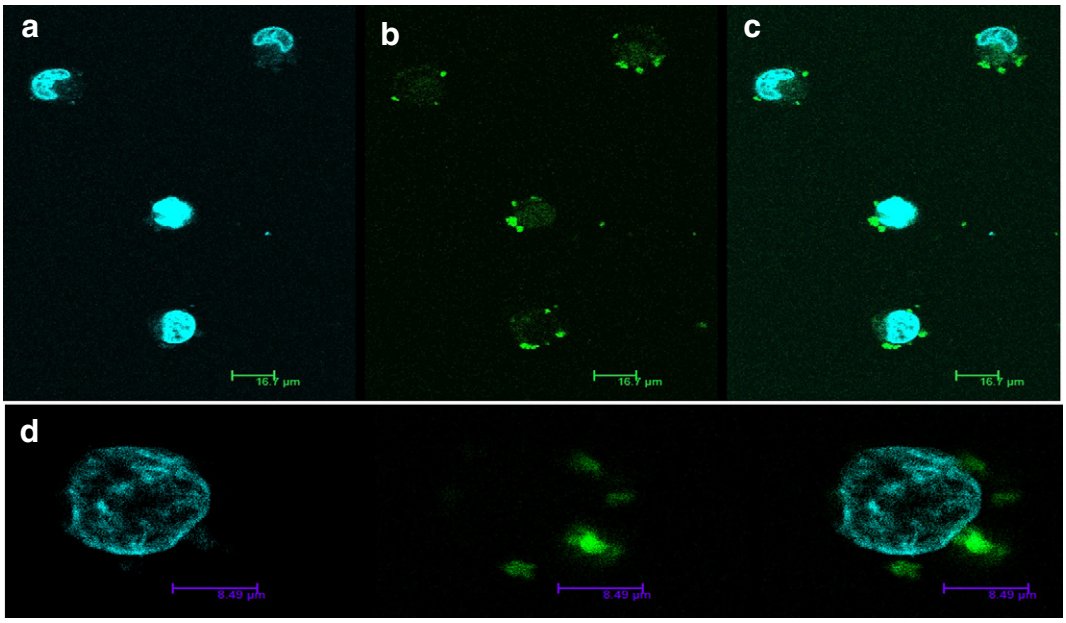

Fig. 6. Visualization of live cancer cells in the presence of $[Ph_4P]_5[Dy(SCN)_8]$ by Confocal Laser Microscopy. Pancreatic cancer cells (MIAPaCa-2) were incubated with $[Ph_4P]_5[Dy(SCN)_8]$ at a concentration of 10 μM for 1 h and washed with PBS to remove excess of $[Ph_4P]_5[Dy(SCN)_8]$ and live cells were visualized under confocal laser microscope equipped with an argon–krypton laser *Blue Stain*:(a) Localization of nucleus (control test showing cell nucleus without using phosphonium–dysprosium compound; *Green Stain*: (b) localization of $[Ph_4P]_5[Dy(SCN)_8]$ only) (c) overlapped (a) and (d) showing $[Ph_4P]_5[Dy(SCN)_8]$ was up-taken by the cells. Excitation at 458 nm. Data clearly indicate that $[Ph_4P]_5[Dy(SCN)_8]$ localizes in the cancer cell membrane.

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.jinorgbio.2011.09.035.

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