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Novel perfluoroalkyl phthalocyanine metal derivatives: Synthesis and photodynamic activities

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ABSTRACT

Novel perfluoroalkyl phthalocyanine metal derivatives with four perfluorobutyl or four perfluorohexyl groups were synthesized by a two-step procedure starting from 4-iodophthalonitrile; the preparation of the key intermediate 4-(perfluorohexyl)phthalonitrile was optimized. Values of fluorescence quantum yield in ethanol of 0.11 and 0.38 were obtained for zinc(II) tetra (perfluorobutyl)phthalocyanine and zinc(II) tetra (perfluorohexyl) phthalocyanine, respectively. The photodynamic activities of two, zinc (II) phthalocyanine derivatives upon both HL-60 leukemic cells and A375 melanotic cancer cells *in vitro* were investigated revealed that the cell viability was slightly better in the case of HL-60 than A375.

1. Introduction

Phthalocyanines (Pcs) have attracted much attention for many decades because they exhibit excellent photochemical properties [1]. They can be used in jet printing inks, catalysts, display devices, data storage, chemical sensors, solar cells and photonic devices [2,3,4]. Besides the application in such traditional areas, one of their important applications is the utilization as photosensitizers for photodynamic therapy (PDT), which is a recent new technique for treating cancer [5,6,7,8]. Phthalocyanine derivatives exhibit high molar extinction coefficient in the 600–700 nm region of the visible spectrum and a long lifetime of triplet excited state to produce efficiently singlet oxygen.

The solubility of PCs is very important for the investigation of their chemical and physical characteristics. The properties of phthalocyanines are influenced not only by the nature of the substituents (electron donating or electron withdrawing) on the ligand but also by that of the metal ion in the core of the ligand. Thus, the solubility can be improved by introducing different kinds of solubility-enhancing substituents such as alkyl, alkoxyl, phenoxyl and macrocyclic groups into the peripheral of the Pc ring [9,10]. Recently, fluorinated MPcs are currently receiving a great deal of attention [11,12,13] and some fluorine-containing phthalocyanine derivatives substituted by methoxyl group have been reported [14,15]. Among phthalocyanine complexes, fluorinated phthalocyanines were proved to have some advantages over non-fluorinated derivatives as photosensitizer for PDT [16,17].

Based on the literatures above, we synthesized novel perfluoroalkyl phthalocyanine metal derivatives and tried to measure their activity for PDT as photosensitizer. There are several reasons for the synthesis of this structure. Firstly, perfluoroalkyl groups are introduced into phthalocyanines to probably improve their solubility in organic solvents and it will suppress the intermolecular aggregation effect of target phthalocyanines which makes the separation and purification easier [18]. Secondly, fluorine is similar to hydrogen atom in size and can mimic the action of hydrogen in biological environments [19]. Thirdly, the improved solubility of perfluoroalkyl phthalocyanine metal derivatives in most organic solvents renders the designated drugs more amenable for formulation in various vehicles, including water–oil emulsions and nanoparticles and leads to enhanced interaction with membrane [19]. Perfluoroalkyl phthalocyanine metal compounds can be used with perfluorocarbon emulsifying agent F68 for photodynamic actions.

In this paper, we report the synthesis of novel perfluoroalkyl phthalocyanine metal derivatives substituted by either four

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perfluorobutyl or four perfluorohexyl groups, the photodynamic activities of two zinc(II) phthalocyanine derivatives were investigated for photodynamic cytotoxicity *in vitro* on HL-60 leukemic cell and A375 melanotic cancer cell.

2. Experiments

2.1. Materials and instruments

All the solvents were of analytical grade. IR spectra were recorded on a Nicolet Nexus 770 spectrometer, using potassium bromide pellets of solids. ^{1}H NMR spectra with TMS as the internal standard were recorded on a Brucker AV-500 spectrometer. HRMS were recorded on an Ionspec 4.7 Tesla FTMS by The Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences (CAS). Gas chromatography was carried out on 1102G gas chromatograph. Melting points were determined by an X-6 micro-melting point apparatus and are uncorrected. Absorption spectra were determined on PGENRAL TU-1901 UV–vis Spectrophotometer. Fluorescence spectra were measured on a Perkin Elmer LS 50 spectrophotometer.

2.2. Synthesis

2.2.1. Synthesis of active copper

$CuSO_4$ (20 g, 125 mmol) was dissolved in H_2O (70 mL) and zinc powder (7 g, 110 mmol) was added in several small portions. The resulting solution was stirred for 45 min. Hydrochloric acid (1.0 mol L^{-1}, 30 mL) was then added and the mixture stirred for 30 min. The precipitated red copper powder was collected by filtration, washed with H_2O.

Copper powder (5 g, 78 mmol) was added to the solution of iodine in acetone (2%, 50 mL). The resulting solution was stirred for 0.5 h. The precipitated iodine copper was collected by filtration, then it was added to the solution of HClacetone (1:1, 25 mL) and the mixture stirred for 0.5 h. The precipitated red active copper was collected by filtration under reduced pressure, washed with acetone, dried under vacuum at room temperature and kept under N_2.

2.2.2. Synthesis of 4-(perfluorobutyl) phthalonitrile (**1**)

A mixture of 4-iodophthalonitrile (2.0 g, 7.9 mmol), active copper (18.6 g, 290 mmol), purified DMF (40 mL) was stirred for 30 min at room temperature under nitrogen protection, then heated slowly to 115–120 °C, and refluxed for 2.5 h. The mixture was cooled to 75–80 °C; Perfluorobutyl iodide (5.5 g, 16 mmol) was added dropwise in the flask, and stirred for 3 h. After addition of ice-water (75 mL), the mixture was extracted with chloroform (3 × 25 mL), dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residual crude solid product was purified by column chromatography on silica gel with petroleum ether and acetone (8:1–3:1, v/v) as eluent to give a white solid 1.29 g, (47.2%) (compound **1**). m.p. 50–51 °C. HRMS calcd for $C_{12}H_3N_2F_9$: 346.0153, found 346.0149. ^{1}H NMR ($CDCl_3$)δ (ppm): 7.99–8.06 (3H, m, C_4F_9–C_6H_3–$(CN)_2$).

2.2.3. Synthesis of 4-(perfluorohexyl) phthalonitrile (**2**)

The reaction was performed as described above for compound **1**, using 4-iodophthalonitrile (2.0 g, 7.9 mmol) and perfluorohexyl iodide (10.7 g, 24 mmol) as starting materials, to give a white solid 2.0 g (57.1%) (compound **2**). m.p. 84–85 °C. MS *m*/*z* (ion, %): 446.01 (M^+, 2.56), 177.03 (100), 127.03 (2.23). ^{1}H NMR ($CDCl_3$)δ (ppm): 7.98–8.05 (3H, m, C_6F_{13}–C_6H_3–$(CN)_2$). ^{13}C NMR ($CDCl_3$)δ (ppm): 107.09, 107.52, 111.11, 114.86, 115.28, 117.21, 119.11, 119.711, 131.58, 131.653, 131.73, 131.91, 132.00, 132.08.

2.2.4. Synthesis of cobalt tetra (perfluorobutyl)phthalocyaninate (**1a**)

A mixture of 4-(perfluorobutyl) phthalonitrile **1** (0.1 g, 0.29 mmol), cobalt acetate (0.108 g, 0.61 mmol), urea 2.9 g (48.3 mmol), ammonium chloride (0.24 g, 4.5 mmol), ammonium molybdate (0.037 g, 0.03 mmol) was ground and then heated to 70 °C for 30 min, and continued to be heated to 190–200 °C for 4 h at the rate of 10 °C/min. The reaction mixture was cooled to room temperature, hydrochloric acid (1.0 mol L^{-1}, 100 mL) was added and the mixture was refluxed for 1 h. The filtered residue was washed with hot water, and the solid was treated with 10% sodium hydroxide solution. The product was put into a Soxhlet extractor and extracted with CH_2Cl_2 for 8 h. Then the residue was purified twice by column chromatography on silica gel using acetone and petroleum ether (1:5–1:3, v/v) as eluent. The product was gotten as a blue-green solid, 45.1 mg (43%). IR (υ (cm^{-1}), KBr): 3411(=C–H), 1621, 1529(C=C), 1353(CF_3), 1237, 1205, 1135, 1090 (CF_2), 1009 (C–F), 908, 745, 735. HRMS calcd for $C_{48}H_{12}N_8F_{36}Co$: 1442.9942, found: 1442.9948. UV–vis (THF) λ_{max}/nm [log ε/dm^3 mol^{-1} cm^{-1}]: 653.3 (3.78). Fluorescence (THF) λ_{max}/nm (Φ_F): 693.7 (0.032).

2.2.5. Synthesis of copper tetra (perfluorobutyl) phthalocyanine (**2a**)

The synthesis procedure is similar to that of **1a** on a 5 mmol scale, but copper chloride was used as metal salt. **2a** was obtained as a dark blue solid in 44% yield (0.046 g). IR (υ (cm^{-1}), KBr): 3440(=C–H), 1621, 1511(C=C), 1353(CF_3), 1235, 1200, 1134, 1089(CF_2), 1008(C–F), 906, 747, 731. HRMS calcd for $C_{48}H_{12}N_8F_{36}Cu$: 1446.9906, found: 1446.9920. UV–vis (THF) λ_{max}/nm [log ε/dm^3 mol^{-1} cm^{-1}]: 665.0 (5.22).

2.2.6. Synthesis of Zinc(II) tetra (perfluorobutyl)phthalocyanine (**3a**)

The synthesis procedure is similar to that of **1a** on a 5 mmol scale, but zinc chloride was used as salt of metal. **3a** was obtained as a dark blue solid in 13% yield (0.014 g). IR (υ (cm^{-1}), KBr): 3412(=C–H), 1621, 1526(C=C), 1350(CF_3), 1235, 1090(CF_2), 1010(C–F), 904, 741, 732. HRMS calcd for $C_{48}H_{12}N_8F_{36}Zn$: 1447.9901, found: 1447.9881. UV–vis (THF) λ_{max}/nm [log ε/dm^3 mol^{-1} cm^{-1}]: 665.7(4.75). Fluorescence (THF) λ_{max}/nm (Φ_F): 677.2(0.024).

2.2.7. Synthesis of iron tetra (perfluorobuty) phthalocyaninate (**4a**)

The synthesis procedure is similar to that for **1a** on a 5 mmol scale, but iron chloride was used as salt of metal. **4a** was obtained as a dark blue solid in 11% yield (0.011 g). IR (υ (cm^{-1}), KBr): 3445(=C–H), 1622, 1528 (C=C), 1353(CF_3), 1237, 1206, 1135, 1091(CF_2), 1017, 1009(C–F), 908, 871, 803, 767. HRMS calcd for $C_{48}H_{12}N_8F_{36}Fe$: 1439.9959, found: 1439.9920. UV–vis (THF) λ_{max}/nm [log ε/dm^3 mol^{-1} cm^{-1}]: 687.0 (3.70), 653.3 (3.73). Fluorescence (THF) λ_{max}/nm (Φ_F): 693.4 (0.074).

2.2.8. Synthesis of cobalt tetra (perfluorohexyl) phthalocyaninate (**1b**)

A mixture of 4-(perfluorohexyl) phthalonitrile **2** (0.13 g, 0.29 mmol), cobalt acetate (0.108 g, 0.61 mmol), urea 2.9 g (48.3 mmol), ammonium chloride (0.24 g, 4.5 mmol), ammonium molybdate (0.037 g, 0.03 mmol) was ground and then heated to 70 °C for 30 min, and continued to be heated to 190–200 °C for 4 h at the rate of 10 °C/min. The reaction mixture was cooled to room temperature, hydrochloric acid (1.0 mol L^{-1}, 100 mL) was added and the mixture was refluxed for 1 h. The filtered residue was washed with hot water, and the solid was again treated with 10% sodium hydroxide solution. The product was placed in a Soxhlet extractor and extracted with CH_2Cl_2 for 8 h. Then the residue was purified twice by column chromatography on silica gel using acetone: petroleum ether (1:5–1:3, v/v) as eluant. The product was obtained as a blue-green solid, 53.7 mg (40.2%). IR (υ (cm^{-1}), KBr): 3446(=C–H), 1626, 1570, 1552(C=C), 1353(CF_3), 1240, 1203, 1145,

1122(CF_2), 1033, 1019(C–F), 911, 805, 736, 709. HRMS calcd for $C_{56}H_{12}N_8F_{52}Co$: 1842.9687, found: 1842.9647. UV–vis (THF) λ_{max}/nm [log ε/dm^3 mol^{-1} cm^{-1}]: 656.0(4.70). Fluorescence (THF) λ_{max}/nm (Φ_F): 676.2 (0.01).

*2.2.9. Synthesis of copper tetra (perfluorohexyl) phthalocyaninate (**2b**)*

The synthesis procedure is similar to that for **1b** on a 5 mmol scale, but copper chloride was used as salt of metal. **2b** was given as a dark blue solid in 33.6% yield (45.0 mg). IR (υ (cm^{-1}), KBr): 3411(=C–H), 1622, 1512(C=C), 1355(CF_3), 1240, 1205, 1144, 1089(C–F), 910, 804, 746, 708. HRMS calcd for $C_{56}H_{12}N_8F_{52}Cu$: 1846.9651, found: 1846.9637. UV–vis (THF) λ_{max}/nm [log ε/dm^3 mol^{-1} cm^{-1}]: 665.7 (4.27).

*2.2.10. Synthesis of Zinc(II) tetra (perfluorohexyl) phthalocyanine (**3b**)*

The synthesis procedure is similar to that for **1b** on a 5 mmol scale, but zinc chloride was used as salt of metal. **3b** was obtained as a dark blue solid in 34% yield (0.045 g). IR (υ (cm^{-1}), KBr): 3237(=C–H), 1621, 1529(C=C), 1237, 1090(C–F), 911, 839, 709. HRMS calcd for $C_{56}H_{12}N_8F_{52}Zn$: 1847.9646, found: 1847.9622. UV–vis (THF) λ_{max}/nm [log ε/dm^3 mol^{-1} cm^{-1}]: 668.4 (3.29). Fluorescence (THF) λ_{max}/nm (Φ_F): 674.4 (0.68).

*2.2.11. Synthesis of iron tetra (perfluorohexyl) phthalocyanine (**4b**)*

The synthesis procedure is similar to that for 1b on a 5 mmol scale, but iron chloride was used as salt of metal. **4b** was obtained as a dark blue solid in 28% yield (0.037 g). IR (υ (cm^{-1}), KBr): 3444(=C–H), 1532(C=C), 1239, 1204, 1146, 1123, 1091(C–F), 909, 805, 777, 744. HRMS calcd for $C_{56}H_{12}N_8F_{52}Fe$: 1839.9704, found: 1839.9683. UV–vis (THF) λ_{max}/nm [log ε/dm^3 mol^{-1} cm^{-1}]: 663.6 (4.36), 634.6 (4.35). Fluorescence (THF) λ_{max}/nm (Φ_F): 673.9 (0.014).

2.3. The determination of $\Phi_F$values

Fluorescence quantum yields (Φ_F) were determined by the comparative method [20]:

$$\Phi_F = \Phi_{F_{std}} \frac{FA_{std}n^2}{F_{std}An_{std}^2}$$

Where Φ_F is the fluorescence quantum yield, F and F_{std} are the areas under the fluorescence emission curves of the samples and the standard, A and A_{std} are the respective absorbances of the samples and standard at the excitation wavelength, respectively, n and n_{std} are the refractive index of solvents used for the samples and standard. 0.1 M quinine sulfate in 0.1 N sulfuric acid solution was used as the standard (Φ = 0.55) to determine the fluorescence quantum yield [21].

2.4. Drug formulation

(a) Compound **3a** (0.010 g) was dissolved in ethanol (1 mL), F68 (Pluronic F68) (2.0 g) was dissolved in double distilled water (25 mL), the ethanol solution was then slowly added to F68 aqueous solution under violently stirring. The result was solution containing compound **3a** at concentration 357 μg/mL (2.47×10^{-4} mol L^{-1}), containing F68 at concentration 7.1% (w/w). (b) A contrast solution containing F68 and ethyl alcohol was prepared at the same time. (c) The above solutions were sterilized and stored in refrigerator away from light. We prepared solution containing compound **3b** (0.010 g) using the same procedure.

2.5. Photodynamic activity in vitro

HL-60 (acute promyelocytic leukaemia cells) and A375 (melanotic cancer cells) were used. The cells were grown in RPMI-1640 medium containing 10% FCS and supplemented with penicillin (172,000 U l^{-1}) and streptomycin (172 mg L^{-1}1.2 $\times$ 10^{-4} mol L^{-1}). Cells were grown until confluence in 75 mL culture flasks at 37 °C under a water-saturated sterile atmosphere containing 5% CO_2.

For the cytotoxic assays, (a) cell suspensions were seeded at 100 μL/well into 96-well tissue culture plates and then treated for 6 h at 37 °C under a water-saturated sterile atmosphere containing 5% CO_2; (b) The above drug with different concentration was added to every well with 0.100 mL, at the same time 0.100 mL culture medium was added to the blank of contract. The final concentration of cells was 12.5 μg mL^{-1}(6.75 $\times$ 10^{-6} mol L^{-1}), 25 μg mL^{-1}(1.35 $\times$ 10^{-5} mol L^{-1}), 50 μg mL^{-1}(2.7 $\times$ 10^{-5} mol L^{-1}) and 100 μg mL^{-1}(5.4 $\times$ 10^{-5} mol L^{-1}), and the culture plates were put in culture tank with flushing CO_2 gas at 37 °C for 2 h. (c) The cells were then irradiated with light (~610 nm) for 1 h at 0 °C. (Light source: iodine-tungsten lamp, Phillips; optical filter, Jangsu; 15 cm distance from cell flasks). (d) Immediately after irradiation, the culture plates were put in culture tank with flushing CO_2 gas at 37 °C for 24 h (e) 0.10 mL of the MTT (0.5 mg mL^{-1}1.2 $\times$ 10^{-3} mol L^{-1}) was then added to culture plates at 37 °C for 4 h. Blue formazan crystals were then dissolved in DMSO, and the absorbance at 492 nm was determined using a Multiscan spectrometer. These experiments were performed three times with 12 wells per run. The cytotoxic effect of the photosensitizer was evaluated using the following formula:

Cell viability% = (Sham-irradiated cells-irradiated cells)/sham-irradiated cells.

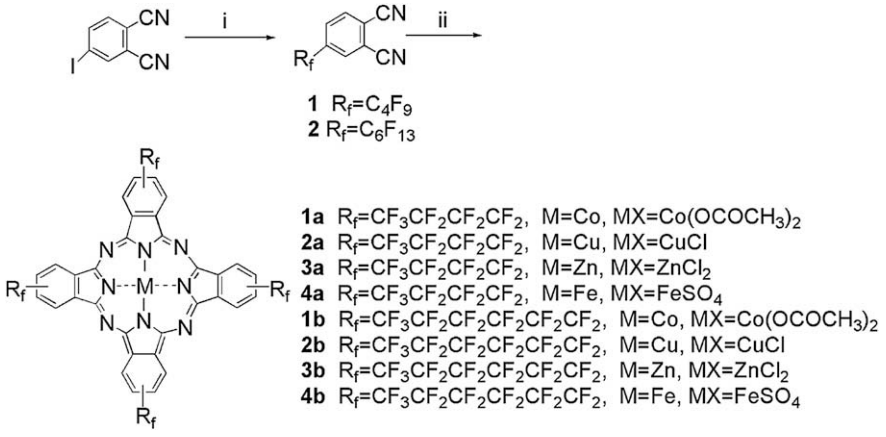

Fig. 1. The structure of synthesized compounds. (i) $CF_3CF_2CF_2CF_2I$ or $CF_3CF_2CF_2CF_2CF_2CF_2I$/Cu, DMF, 125 °C; (ii) H_2NCONH_2, MX, NH_4Cl, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, 200 °C.

Table 1
The influence of starting material's ratio.

Ratio (4-iodophthalonitrile/$C_6F_{13}I$)	1:1	1:2	1:3	1:4
Yield (%)	10	47	60	60

2.6. Morphological image

According to the above procedure described for cytotoxic assays, after the cells were irradiated, the culture plates were put in culture tank with flushing CO_2 gas at 37 °C for 24 h. Then the cells were observed under microscope by assessing morphology changes.

3. Results and discussion

3.1. Synthesis

The desired compounds were prepared according to the route shown in Fig. 1. They were synthesized by a two-step procedure [22,23]. First, compounds **1** and **2** were prepared by the reaction of 4-iodophthalonitrile with perfluorobutyl or perfluorohexyl iodide in the presence of *N*, *N*-dimethylformamide (DMF) using activated copper. **1** and **2** were isolated by column chromatography in 47% and 61% yield, respectively. The mixture of 4-(perfluoroalkyl) phthalonitrile **1** or **2**, metal salt, urea, and ammonium molybdate was heated to 190–200 °C for 4 h. After washed with hydrochloric acid, the product was added in a Soxhlet extractor and extracted with CH_2Cl_2 for 8 h. Then the condensation products were purified by column chromatography to give perfluoroalkyl phthalocyanine metal derivatives as a mixture of constitutional isomers. The structures of final products were identified by IR and HRMS.

In these procedures, the reaction condition of 4-(perfluorohexyl)phthalonitrile (**2**) were optimized because 4-(perfluoroalkyl)phthalonitrile was the key intermediate and its synthesis was difficult. It is important to select solvents for this reaction. Generally, DMSO, DMF or pyridine was suitable, and we selected DMF as solvent in this reaction. In general, there is side reaction in this reaction that phthalonitriles are partly converted to phthalocyanines. We detected phthalycyanines during the whole process of the reaction, but the amount of phthalycyanines is 2% of total yield or so. Because phthalocyanines are insoluble in chloroform, when the reaction mixture was extracted with chloroform, phthalocyanines are easier to be removed, the synthesis of phthalonitrile derivatives is feasible. In addition, the copper powder as the catalyst of this reaction must be activated before used.

In order to find the optimal conditions for synthesis of **2**, trials were performed at different material molar ratio (precursors 4-iodophthalonitrile to perfluorohexyl iodide 1:1, 1:2, 1:3; 1:4, Table 1), reaction time (1.5 h, 2.0 h, 2.5 h, 3.0 h, 3.5 h, Table 2) and reaction temperature (115 °C, 120 °C, 125 °C, 130 °C, Table 3).

From Table 1, 2 and 3, the optimal condition for this reaction was as follows: the ratio of 4-iodophthalonitrile to perfluorohexyl iodide 1:3, reaction time 2.5 h and reaction temperature 125 °C. The yield reached 61%. We prepared compound **1** using the same procedure, the yield was 47%.

Table 2
The influence of reaction time.

Time (h)	1.5	2.0	2.5	3.0	3.5
Ratio (4-iodophthalonitrile/$C_6F_{13}I$)	1:3	1:3	1:3	1:3	1:3
Yield (%)	43	57	58	36	18

Table 3
The influence of temperature.

Temperature (°C)	100–115	115–120	120–125	125–130
Time (h)	2.5	2.5	2.5	2.5
Yield (%)	25	44	61	49

Phthalonitriles are generally converted into phthalocyanines in high-boiling solvents such as hexanol, DMF, quinoline, dimethylamino ethanol, dicyclohexylurea, α-chloro-napthalene, etc. in the presence of a reducing agent. Especially, if N-donor solvents such as DMF, quinoline, or N,N-dimethylamino ethanol are used, no further electron donating reagent is required. When the starting material is a phthalic anhydride derivative, an N-containing reactant such as urea must be used together with the ammonium molybdate as the catalyst. Four approaches were carried out in order to investigate the possibility of this reaction. One approach is using Phthalonitriles and copper Chlorine as reagents and DMF as solvent, without adding urea and ammonium molybdate, Phthalocyanine can not be detected. The second approach is using ammonium molybdate as the catalyst without urea, the reaction did not occur. The third approach is only using urea as N-containing reactant without ammonium molybdate, the reaction did not occur too. The fourth approach is using a mixture of 4-(perfluorobutyl) phthalonitrile, cobalt acetate, urea, ammonium chloride which was ground and baked. In this reaction, urea was used together with ammonium molybdate as the catalyst, phthalocyanine was obtained as our expectance. It means that additional reagents urea should be necessary.

3.2. Solubility of perfluoroalkyl phthalocyanine metal derivatives

It was known that solubility of MPcs is affected by the type of the central metal ions and the peripheral substituents. Generally, fluoroalkyl-substituted compounds are known to have their high solubility in polar solvents. In this paper, the similar results were found that the eight *tert*-fluoroalkyl substituted phthalocyanine metal derivatives (**1a**–**4b**) could be dissolved easily in ethanol, methanol, ethyl acetate and THF, but the solubility in CH_2Cl_2, and benzene was poor. It was also found that the MPcs having peripheral non-fluorinated alkyl groups exhibited high solubility in the lower polar solvents, such as CH_2Cl_2 and benzene [24]. The reason why the solubility increased in polar solvents may be interpreted with the extreme electronegativity of the fluorine atom [25].

3.3. UV–vis absorption spectra and fluorescence spectra

The perfluoroalkyl phthalocyanine metal derivatives have good solubility in common organic solvents, such as ethyl acetate, THF, and ethanol. In order to investigate the photodynamic activity of synthesized compounds, we measured the UV–vis absorption spectra and fluorescence spectra of **3a** and **3b** in ethanol because ZnPcs hold high fluorescence quantum yield in many cases (see Table 4). The toxicity of ethanol is very low

Table 4
The absorption and fluorescence data for **3a** and **3b** in ethanol.

Compound	Solvent	λ_{max} (nm)	log ε (dm^3 mol^{-1} cm^{-1})	λ_{em} (nm)	Φ_F[a]
3a	Ethanol	665	4.97	673	0.11
3b	Ethanol	666	4.63	675	0.38

[a] Fluorescence quantum yield (in general the standard used for determination of Φ_F is 0.1 M quinine sulfate in sulfuric acid ($\Phi_F = 0.55$)).

in biological system according to our former experiment [26]. The absorption spectra of two compounds in ethanol are similar, which are typical spectra of non-aggregated phthalocyanines with an intense and sharp Q-band at 665 nm (for **3a**) or 666 nm (for **3b**) (see Fig. 2). But, their fluorescence emission properties in ethanol are appreciably different. Compound **3a** showed weak fluorescence emission at 673 nm with a fluorescence quantum yield (Φ_F) of 0.11, whereas **3b** exhibited an intense emission at 675 nm while Φ_F value is 0.38. These observations indicate that two compounds exist mainly in monomeric form in ethanol. This non-aggregated property is very important for PDT applications.

3.4. Photodynamic activities on HL-60 leukemic cell and A375 melanotic cancer cell in vitro

In general, the non-fluorinated alkyl substituted phthalocyanines are insoluble in water and soluble in low polar organic solvents such as dichloromethane, benzene and toluene which are high toxicity in biological system. In some case, four hydrophilic sulfonated groups were ever introduced into the periphery of the ring structure in order to improve the solubility of phthalocyanines in water [27]. Compared with these phthalocyanines, ZnPcs **3a** and ZnPcs **3b** were still insoluble in water in our system, but could be easily dissolved in polar organic solvents, e.g. methanol, ethanol, and tetrahydrofuran, and could form a water–oil emulsion with emulsifying agent F68 for photodynamic actions. The preparation of the emulsion was simple and convenient; the emulsion could be sterilized directly and mixed with the culture medium at arbitrary ratio. Because of the low toxicity of ethanol, we prepared the emulsion containing compound **3a** or **3b** in ethanol.

The photodynamic activity of ZnPc **3a** and ZnPc **3b** was investigated and compared *in vitro* using HL-60 leukemic cell and A375 melanotic cancer cell. Cell toxicity induced by the photosensitizers was firstly analyzed in the dark. The HL-60 and A375 cellular cultures were treated with 12.5 μg mL^{-1}(6.75×10^{-6} mol L^{-1}), 25 μg mL^{-1}(1.35×10^{-5} mol L^{-1}), 50 μg mL^{-1}(2.7×10^{-5} mol L^{-1}) and 100 μg mL^{-1}(5.4×10^{-5} mol L^{-1}),of ZnPc **3a** or **3b** at 37 °C in the dark. Under these experimental conditions, sensitizers **3a** and **3b** were not significantly toxic after 24 h of incubation. The cell viability was determined after 2 h incubation and 1 h light exposure. The irradiation system used in these studies is described in Experimental section. The corresponding cell viability values for ZnPc **3a** are shown in Fig. 3. No significant lethality was found for cell cultures without the sensitizer and irradiation. At 100 μg mL^{-1} (5.4×10–5 mol L^{-1}), ZnPc **3a** led to 51.7% cell viability on the HL-60 cell, 65.2% cell viability on A375 cell.

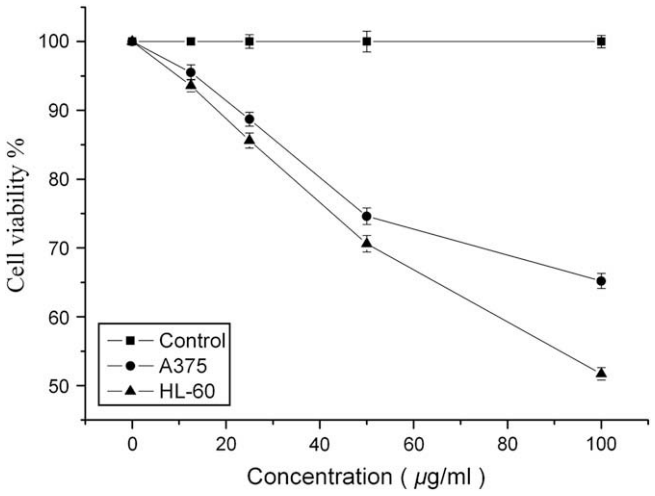

Fig. 3. Cell viability after 1 h light exposure and photosensitization by ZnPc **3a**. Values represent mean ± SD standard deviation of three separate experiments.

The photodynamic effect was higher when using ZnPc **3b** as a photosensitizer, the viability of the HL-60 cell was 50.4% at 100 μg mL^{-1} (5.4×10–5 mol L^{-1}), and the viability of the A375 cell was 60.2% at 100 μg mL^{-1} (5.4×10–5 mol L^{-1}) (Fig. 4).

The image of HL-60 cell undergoing necrosis after photodynamic treatment was determined microscopically by assessing morphology changes in Fig. 5. The typical pattern observed for untreated control HL-60 cells is shown in Fig. 5a. The cell population observed for untreated samples (large nuclei and diffused chromatin) can be identified. When HL-60 cells were irradiated after 24 h incubation with 25 μg mL^{-1} (1.35×10^{-5} mol L^{-1}) ZnPc **3b**, the pattern shown in Fig. 5b was obtained. Some cells began to display nuclear condensation and fragmentation. On the other hand, Fig. 5c and d showed cells with necrotic characteristics. Treatments with concentrations of 50 μg mL^{-1} (2.7×10^{-5} mol L^{-1}) ZnPc **3b** (Fig. 5c) induced a variable amount of perinuclear vacuoles and cell shrinkage. In addition, cells with concentrations of 100 μg mL^{-1} (5.4×10^{-5} mol L^{-1}) ZnPc **3b** showed the typical morphology of cell death by necrosis (Fig. 5d). The observation suggests that PDT treatment can induce both necrosis and apoptotic cell death depending on the concentration of photosensitizer.

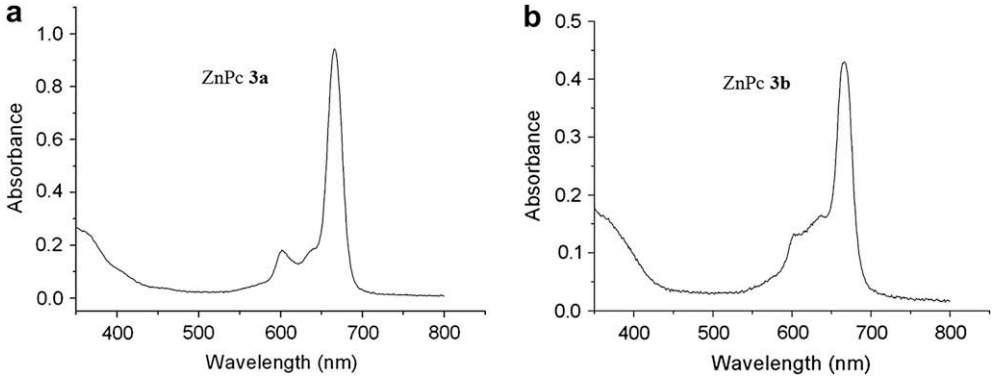

Fig. 2. Absorbance spectra in the visible region of 10^{-5} M solution of ZnPc **3a** and ZnPc **3b** in ethanol.

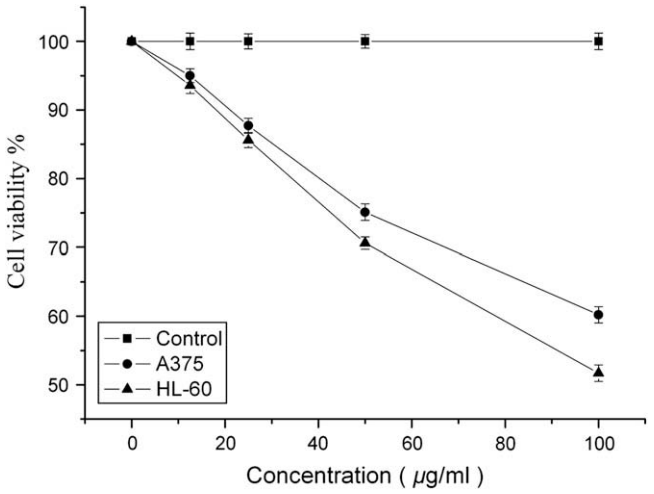

Fig. 4. Cell viability after 1 h light exposure and photosensitization by ZnPc **3b**. Values represent mean ± SD standard deviation of three separate experiments.

In previous studies, Oda et al. reported that zinc tetracarboxyoctafluorophthalocyanine had a remarkable photodynamic for HeLa cells, and the viability of cells was 5% at 1.0×10^{-6} mol L^{-1}. [17]. Tetra-trifluoroethylbenzyloxyl zinc phthalocyanine showed a good photosensitizing efficiency *in vitro* on Hep-2 cells, and the viability of cells was 30% at 1.0×10^{-7} mol L^{-1} [15]. We have reported zinc tetra-trifluoroethoxylphthalocyanine derivatives which displayed photocytotoxicity at concentration of 100 μg mL^{-1} (1.0×10^{-4} mol L^{-1}), and the viability of myeloma cells was 10.8% and the viability of Chinese hamster ovary cells was 2.1% [26]. Compared with the results above, our synthesized Zn tetra (perfluoroalkyl)phthalocyanines showed moderate photosensitizing activity *in vitro* on different cells, which suggested that ZnPc bearing perfluoroalkyl groups could be used as photosensitizer for photodynamic therapy after the molecule were modified further.

4. Conclusion

In summary, the perfluoroalkyl phthalocyanine metal derivatives bearing either four perfluorobutyl or four perfluorohexyl groups have been synthesized by a two-step procedure starting from 4-iodophthalonitrile. The UV–vis absorption spectra and fluorescence spectra of **3a** and **3b** in ethanol was determined. It was found that the absorption spectra of compounds **3a** and **3b** in ethanol are similar and are typical spectra of non-aggregated phthalocyanines with an intense and sharp Q-band at 665 nm (for **3a**) or 666 nm (for **3b**). The result of fluorescence spectra showed that compound **3a** showed weak fluorescence emission at 673 nm with fluorescence quantum yield (Φ_F) 0.11, whereas **3b** exhibited an intense emission at 675 nm while Φ_F value is 0.38. The photodynamic activity of **3a** and **3b** was investigated *in vitro* on HL-60 leukemic cell and A375 melanotic cancer cell. The result suggested that **3a** and **3b** exhibited the potential for the photodynamic treatment. The cell viability of our compounds in the case of HL-60 is slightly better than in the case of A375 melanotic cancer cell.

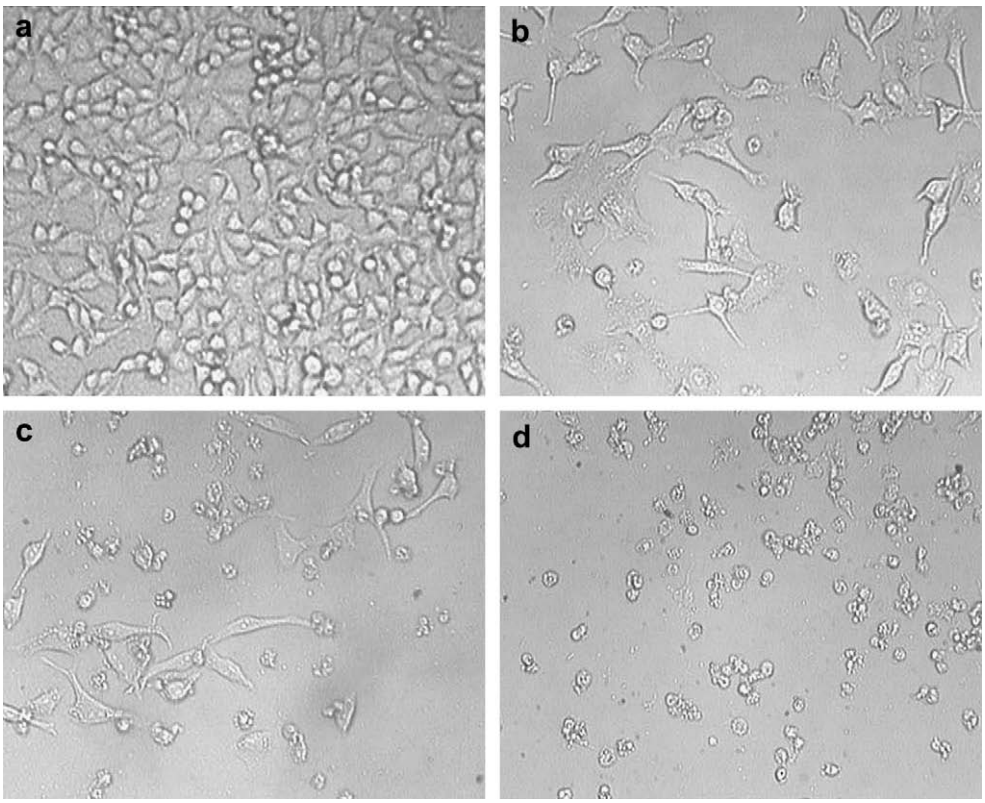

Fig. 5. Images of HL-60 cells. (a) Untreated cells 100×; (b) cells incubated in 25 μg mL^{-1} (calculate 1.35×10^{-5} mol L^{-1}) ZnPc **3b** 100×; (c) in 50 μg mL^{-1} (calculate 2.7×10^{-5} mol L^{-1}) ZnPc **3b** 100×; (d) in 100 μg mL^{-1} (calculate 5.4×10^{-5} mol L^{-1}) ZnPc **3b** 100×.

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