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Synthesis and characterization of water-insoluble and water-soluble dibutyltin(IV) porphinate complexes based on the tris(pyridinyl)porphyrin moiety, their anti-tumor activity in vitro and interaction with DNA

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Abstract

The water-insoluble and water-soluble organotin(IV)porphinate complexes based on the tris-(4-pyridinyl)porphyrin and tris(*N*-methyl-4-pyridiniumyl)porphyrin moieties were synthesized and characterized by elemental analysis, ^{1}H NMR, IR and electrospray ionization mass spectra. The in vitro activity of the compounds against P388 leukemia and A-549 was determined. The results show that the anti-tumor activities of organotin(IV)porphinate is related to the water solubility of the compounds and the central ion in the porphyrin ring. The interaction between the water-soluble dibutyltin(IV) porphinate (**7** and **10**) complexes and DNA has been investigated. The result shows that compounds **7** and **10** cause DNA hypochromism measured by A_{260}, a slight increase in the viscosity of the DNA, and an increase in the melting point of DNA by 2.9 and 1.6 °C, respectively at $DNA_{base}/Drug_{Por}$ ratios of 60. The binding constants to DNA were $1.35\pm0.16\times10^7$ M^{-1} (**7**) and $1.45\pm0.12\times10^6$ M^{-1} (**10**) determined using EB competition method based on the porphyrin concentration, which is 20 and five times greater than that of precursor porphyrins [5-*p*,*o*-(carboxy)methoxyphenyl-10,15,20-tris(*N*-methyl-4-pyridiniumyl)] porphyrin (*p*,*o*-tMPyPac) to DNA. Electrophoresis test shows that the compounds cannot cleave the DNA. According to the electrophoresis test result and all the above results, the cytotoxic activity against P388 and A-549 tumor cells appears not to come from the cleavage of DNA caused by the compounds but from the high affinity of compounds to DNA.

Keywords: Dibutyltin(IV); Porphyrin; Anti-tumor activity; DNA

1. Introduction

Since the first observation of the preferential accumulation of hematoporphyrin in neoplastic tissues by Policard in 1924, a number of investigators have used the fluorescence of porphyrins for the detection and destruction of tumors [1–5]. Water-insoluble and water-soluble porphyrinyl-nucleosides containing adenosine and thymidine, have strong tumoricidal activity against human malignant melanoma [6–8]. Cationic manganese-porphyrin complexes are among the efficient DNA cleavers, which alone or linked to 9-methoxylellipticine exhibit anti-HIV or anti-tumor activity [9,10]. In most of the papers metal atoms are coordinated to the donor atoms of the porphyrin ring. Recently, several papers have been published in which metallic or organometallic moieties are coordinated to side-chain carboxylate or sulfate groups of the porphyrins [11–15]. Both the organotin-[*meso*-4-(carboxyphenyl)] porphinate and diorganotin chloro protoporphyrin IX derivatives have been tested for their cytotoxicity, towards immortalized mouse embryonic fibroblasts (NIH-313) and towards early-developing embryos of *Anilocra physodes*, showing that cytotoxicity of the parent organotin halide may be modulated by the use of appropriate ligands. Furthermore, platinum porphyrin complexes have been synthesized and chemically characterized. Their anti-tumor activity in vivo toward MDA-MB 231 mammary carcinoma was tested [14,15]. The study of organotin complexes as potential anti-tumor drugs has been an interesting field [16–22]. In this paper we report the synthesis and characterization of water-insoluble and water-soluble organotin(IV) porphinate complexes based on the tris(pyridinyl)porphyrin and tris(pyridiniumyl)porphyrin moieties, and their activity against P388 and A-549 tumor cell lines and their interactions with DNA.

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2. Experimental

All chemicals were reagent-grade and used without further purification. Pyrrole was purchased from Fluka and freshly distilled before use, *p,o*-(ethoxycarbonylmethoxy)benzaldehyde was prepared from *p,o*-hydroxybenzaldehyde and ethyl bromoacetate [23]. Calf thymus DNA, plasmid pBR_{322} DNA and agarose (DNA grade) were purchased from Beijing Sino-American Biotechnology Company. The solution of DNA was prepared by dissolving DNA in 0.05 M NaCl aqueous solution ($A_{260}/A_{280}>$ 1.85). The concentration of the prepared DNA stock solution was expressed as DNA (P).

^{1}H NMR were recorded on a Bruker-DRX 300 spectrometer at 300.13 MHz, UV–visible spectra and melting point measurement of DNA were carried out on a Hewlett Packard 8453E single beam diode array recording spectrophotometer with a thermostatic cell compartment. Fluorescence measurements were carried out on a PE LS-50B fluorescence spectrometer. Elemental analyses were carried out on a PE-2000 instrument. Positive ion ESI-MS spectra were obtained with a Finnigan LCQ electrospray ionization mass spectrometer with MeOH as matrix. The viscometric measurement was carried out with a Ubbelhde viscometer, the viscometer was thermostated to 20 °C in a constant temperature bath. The electrophoresis experiments were carried out on an electrophoresis system using TBE buffer, and the gel was stained with EB for 0.5 h after electrophoresis, and then photographed (Scheme 1).

2.1. Preparation of [5-(ethoxycarbonyl)methoxy)phenyl-10,15,20-tris(4-pyridinyl)]porphyrin

A mixture [24,25] of *p*- or *o*-(ethoxycarbonyl)methoxybenzaldehyde (5.7 g, 28 mmol), 4-pyridinecarboxaldehyde (7.50 ml, 81 mmol) and propionic acid (250 ml) was heated at 110–120 °C with stirring. To this solution pyrrole (7.50 ml, 114 mmol) was slowly added. The resulting mixture was heated at reflux for 2.5 h, and the solvent was removed under reduced pressure. The residue was washed with ammonia and then with water. The crude material was extracted into chloroform and purified by column chromatography on silica gel with a mixture of chloroform and methanol ($v_{MeOH}/v_{CHCl3}=1/50$). Evaporation of the solvent afforded the desired compound as purple powder.

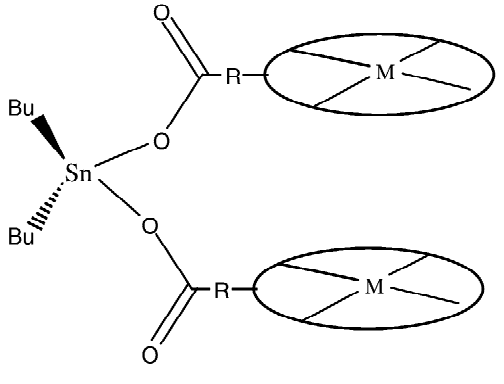
[5-*p*-(ethoxycarbonyl)methoxyphenyl-10,15,20-tris(pyridinyl)]porphyrin (*p*-triPyPPE), yield (2%). Anal. Calcd. for $C_{45}H_{33}N_7O_3$: C 75.10, H 4.58, N 13.62; Found: C 74.74, H 4.56, N 13.12. ^{1}H NMR ($CDCl_3$), δ (ppm): 9.05 (d, 6H, 2,6-pyridine), 9.01 (d, 2H, β-pyrrole), 8.95 (m, 6H, β-pyrrole), 8.17 (d, 6H, 3,5-pyridine), 8.12 (d, 2H, 2,6-phenyl), 7.33 (d, 2H, 3,5-phenyl), 4.94 (s, 2H, OCH_2), 4.44 (q, 2H, $COOCH_2$), 1.26 (t, 3H, CH_3), −2.92 (s, 2H, NH pyrrole). IR (KBr pellet): 3318 cm^{-1} (m, NH-pyrrole), 1756 cm^{-1} (m, C=O).

[5-*o*-(ethoxycarbonyl)methoxyphenyl-10, 15, 20-tris(pyridinyl)]porphyrin (*o*-triPyPPE) yield (1.8%) Anal. Calcd. for $C_{45}H_{33}N_7O_3$: C 75.10, H 4.58, N 13.62; Found: C 74.68, H 4.58, N 13.23. ^{1}H NMR ($CDCl_3$), δ (ppm): 9.09 (s, 6H, 2,6-pyridine), 9.01(s, 2H, β-pyrrole), 8.87 (d, 6H, β-pyrrole), 8.24 (d, 6H, 3,5-pyridine), 8.02 (d, 1H, phenyl), 7.81(m, 1H, phenyl), 7.44 (m, 1H, phenyl), 7.23(d, 1H, phenyl), 4.56 (s, 2H, OCH_2), 4.23 (q, 2H, $COOCH_2$), 1.24 (t, 3H, CH_3), −2.78 (s, 2H, NH-pyrrole). IR (KBr pellet): 3317 cm^{-1} (m, NH-pyrrole), 1756 cm^{-1} (m, C=O).

The metalloporphyrins were synthesized in DMF using an excess of metallic acetate reacted with the appropriate porphyrins according to literature procedure [24]. The hydrolysis of the porphyrins and their metallic derivatives was carried out in potassium hydroxide solution of DMF/H_2O for 12–24 h at room temperature [24]. The product was dried in vacuo at 100 °C for 5 h.

2.2. Synthesis of the dibutyltin porphinate

A mixture of the appropriate porphyrin or metallopor-



= tris (4-pyridinyl)porphyrin

R= *p* -PhOCH$_2$-

1 M=2H, **2** M=Cu, **3** M=Ni

R= *o* -PhOCH$_2$-

4 M=2H, **5** M=Cu, **6** M=Ni

= tris(*N*-methyl-4-pyridiniumyl)porphyrin

R= *p* -PhOCH$_2$-

7 M=2H, **8** M=Cu, **9** M=Ni

R= *o* -PhOCH$_2$-

10 M=2H, **11** M=Cu, **12** M=Ni

Scheme 1.

phyrin and dibutyltinoxide in the molar ratios of 2:1 was heated at reflux for 6 h in dry methanol. The mixture was cooled to room temperature and the precipitate was recovered by filtration.

Dibutyltin [5-*p*-(carboxy)methoxyphenyl-10,15,20-tris(4-pyridinyl)]porphinate (**1**) Anal. Calcd. for $C_{94}H_{74}N_{14}O_6Sn$: C 69.93, H 4.59, N 12.15; Found: C 69.50, H 4.56, N 11.86. ^{1}H NMR ($CDCl_3$) δ (ppm): 9.10 (d, 12H, 2,6-pyridine), 9.04, 8.88 (16H, β-pyrrole), 8.22 (d, 12H, 3,5-pyridine), 8.16 (d, 4H, 2,6-phenyl), 7.43 (d, 4H 3,5-phenyl) 5.10 (s, 4H, $-OCH_2-$), 1.93 (m, 8H, $SnCH_2CH_2$), 1.41 (m, 4H, CH_2), 0.92 (t, 6H, CH_3), −2.94 (s, 4H, NH-pyrrole). IR (KBr pellet): 2923–2856 cm^{-1}, Butyl; 1596 cm^{-1} C=O. λ_{max} ($CHCl_3$): 648, 588, 549, 515, 419 nm (Soret).

Dibutyltin Copper [5-*p*-(carboxy)methoxyphenyl-10,15,20-tris(4-pyridinyl)]porphinate (**2**) Anal. Calcd for $C_{94}H_{70}N_{14}O_6Cu_2Sn$: C 64.98, H 4.06, N 11.29; Found: C 64.57, H 4.11, N 11.05. IR (KBr pellet): 2954–2862 cm^{-1}, SnBu; 1638 cm^{-1}, C=O.

Dibutyltin Nickel [5-*p*-(carboxy)methoxyphenyl-10,15,20-tris(4-pyridinyl)]porphinate (**3**) Anal. Calcd. for $C_{94}H_{70}N_{14}O_6Ni_2Sn$: C 65.38, H 4.08, N 11.35; Found: C 65.01, H 4.12, N 10.97. ^{1}H NMR ($CDCl_3/F_3COOD$) δ (ppm): 9.30 (s, 12H, 2,6-pyridine), 8.97–8.75 (16H, β-pyrrole), 8.67(s, 12H, 3,5-pyridine), 7.98 (d, 4H, 2,6-phenyl), 7.33 (d, 4H, 3,5-phenyl), 5.05 (s, 4H, $-OCH_2-$), 1.89 (m, 8H, $SnCH_2$), 1.43 (m, 4H, CH_2-), 0.95 (t, 6H, $-CH_3$). IR (KBr pellet): 2955–2863 cm^{-1}, SnBu; 1638 cm^{-1}, C=O.

Dibutyltin [5-*o*-(carboxy)methoxyphenyl-10,15,20-tris(4-pyridinyl)]porphinate (**4**) Anal. Calcd. for $C_{94}H_{74}N_{14}O_6Sn$: C 69.93, H 4.59, N 12.15; Found: C 69.53, H 4.46, N 11.76. ^{1}H NMR ($CDCl_3$) δ (ppm): 9.01 (s, 12H, 2,6-pyridine), 8.87–8.69 (m, 16H, β-pyrrole), 8.05 (s, 12H, 3,5-pyridine), 7.71 (m, 4H, phenyl), 7.34 (m, 2H, phenyl), 7.16 (m, 2H, phenyl), 4.65 (s, 4H, $-OCH_2-$), 1.83 (m, 8H, $SnCH_2CH_2$), 1.41 (m, 4H, CH_2), 0.96 (t, 6H, CH_3); −2.91 (s, 4H, NH-pyrrole). IR (KBr pellet): 2955–2866 cm^{-1}, SnBu; 1630 cm^{-1}, C=O. λ_{max} ($CHCl_3$): 648, 588, 549, 515, 419 (Soret).

Dibutyltin Copper [5-*o*-(carboxy)methoxyphenyl-10,15,20-tris(4-pyridinyl)]porphinate (**5**) Anal. Calcd. for $C_{94}H_{70}N_{14}O_6Cu_2Sn$: C 64.98, H 4.06, N 11.29; Found: C 64.84, H 3.98, N 10.89. IR (KBr pellet): 2956–2861 cm^{-1}, SnBu; 1637 cm^{-1}, C=O.

Dibutyltin Nickel [5-*o*-(carboxy)methoxyphenyl-10,15,20-tris(4-pyridinyl)]porphinate (**6**) Anal. Calcd. for $C_{94}H_{70}N_{14}O_6Ni_2Sn$: C 65.38, H 4.08, N 11.35; Found: C 64.99, H 4.19; N, 10.88. ^{1}H NMR ($CDCl_3/F_3COOD$) δ (ppm): 9.30 (s, 12H, 2,6-pyridine), 8.97, 8.75 (m, 16H, β-pyrrole), 8.67 (s,12H, 3,5-pyridine), 7.83 (m, 4H, phenyl), 7.40 (m, 4H, phenyl), 4.70 (s, 4H, $-OCH_2-$), 1.89 (m, 8H, $SnCH_2$), 1.43 (m, 4H, CH_2-), 0.95 (t, 6H, $-CH_3$). IR (KBr pellet): 2954–2862 cm^{-1}, SnBu; 1638 cm^{-1}, C=O.

2.3. Methylation of dibutyltin(IV)porphinate complexes

The dibutyltin porphinate complexes were methylated in dry chloroform with a large excess of methyl iodide (120 equiv.) for 24 h at room temperature and the product was recovered by filtration. The products were purified by addition of ether to a concentrated methanolic solution of dibutyltin(*N*-methyl-4-pyridiniumyl) porphinate. The more water-soluble product was synthesized by treatment of the dibutyltin(*N*-methyl-4-pyridiniumyl) porphinate iodide with equal molar ratios of silver trifluoromethanesulfonate in dry methanolic solution.

Dibutyltin [5-*p*-(carboxy)methoxyphenyl-10,15,20-tris(*N*-methyl-4-pyridiniumyl) porphinate] hexaiodide (**7**) Anal. Calcd. for $C_{100}H_{92}N_{14}O_6SnI_6.6H_2O$: C 46.64, H 4.07, N 7.62; Found: C 46.21, H 4.12, N 7.53. ^{1}H NMR (DMSO-d_6) δ (ppm): 9.43 (d, 12H, 2,6-pyridine), 8.97 (b, 28H, β-pyrrole and 3,5-pyridine), 8.12 (d, 4H, 2,6-phenyl), 7.35 (d, 4H, 3,5-phenyl), 4.99 (s, 4H, $-OCH_2-$), 4.77 (s, 18H, N^+-CH_3), 1.69–1.56 (m, 8H, $SnCH_2CH_2$), 1.35 (m, 4H, CH_2), 0.87 (t, 6H, CH_3), −3.03 (s, 4H, NH-pyrrole). IR (KBr pellet): 2923–2856 cm^{-1} (m, butyl). 1637 cm^{-1} C=O+C=N$^+$. λ_{max} (CH_3OH): 648, 591, 552, 515, 418 (Soret). ESI-MS: 383.4 (M-5I-H_2O).

Dibutyltin Copper [5-*p*-(carboxy)methoxyphenyl-10, 15, 20-tris (*N*-methyl-4-pyridiniumyl) porphinate] hexaiodide (**8**) Anal. Calcd. for $C_{100}H_{88}N_{14}O_6Cu_2SnI_6.6H_2O$: C 44.53, H 3.74, N 7.27; Found: C 44.25, H 3.79, N 7.25. IR (KBr pellet): 2955–2864 cm^{-1} (m, butyl); 1637 cm^{-1} C=O+C=N$^+$. ESI-MS: 414.1 (M-5I).

Dibutyltin Nickel [5-*p*-(carboxy)methoxyphenyl-10,15,20-tris (*N*-methyl-4-pyridiniumyl) porphinate] hexaiodide (**9**) Anal. Calcd. for $C_{100}H_{88}N_{14}O_6Ni_2SnI_6.6H_2O$: C 44.69, H 3.75, N 7.30; Found: C 45.08, H 3.81, N 7.21. ^{1}H NMR (DMSO-d_6) δ (ppm): 10.74 (m, br, 16H, β-pyrrole), 9.44 (s, 12H, 2,6-pyridine), 8.87 (s, 12H, 3,5-pyridine), 7.98 (s, 4H, phenyl), 7.35 (s, 4H, phenyl), 4.91 (s, 4H, $-OCH_2-$), 4.69 (s, 18H, N^+-CH_3), 1.64 (m, 4H, $SnCH_2$), 1.33 (m, 8H, CH_2), 0.89 (t, 6H, CH_3), IR (KBr pellet): 2954–2863 cm^{-1} (m, butyl); 1636 cm^{-1}, C=O+ C=N$^+-CH_3$. ESI-MS: 411.5 (M-5I).

Dibutyltin [5-*o*-(carboxy)methoxyphenyl-10,15,20-tris(*N*-methyl-4-pyridiniumyl) porphinate] hexaiodide (**10**) Anal. Calcd. for $C_{100}H_{92}N_{14}O_6SnI_6.6H_2O$: C 46.64, H 4.07, N 7.62; Found: C 47.12, H 4.08, N 7.49. ^{1}H NMR (DMSO-d_6) δ (ppm): 9.48 (s, 12H, 2,6-pyridine), 9.15–9.03 (b, 28H, β-pyrrole and 3,5-pyridine), 7.90 (m, 4H, phenyl), 7.40 (m, 4H, phenyl), 4.72 (s, 18H, N^+-CH_3), 4.47 (s, 4H, $-OCH_2-$), 1.48 (m, 4H, $SnCH_2$), 1.16 (m, 8H, CH_2), 0.73 (t, 6H, CH_3), −3.02 (s, 4H, NH-pyrrole). IR (KBr pellet): 2953–2865 cm^{-1} (m, butyl); 1637 cm^{-1} C=O+C=N$^+$. λ_{max} (CH_3OH): 648, 591, 552, 515, 418 nm (Soret). ESI-MS: 383.5 (M-5I-H_2O).

Dibutyltin Copper [5-*o*-(carboxy)methoxyphenyl-10, 15, 20-tris (*N*-methyl-4-pyridiniumyl)porphinate] hexaiodide (**11**) Anal. Calcd. for $C_{100}H_{88}N_{14}O_6Cu_2SnI_6.6H_2O$: C

44.53, H 3.74, N 7.27; Found: C 44.41, H 3.68, N 7.31. IR (KBr pellet): 2954–2865 cm^{-1} (m, butyl); 1638 cm^{-1}, C=O+C=N^{+}–CH_3. ESI-MS: 413.8 (M-5I).

Dibutyltin Nickel [5-*o*-(carboxy)methoxyphenyl-10,15,20-tris (*N*-methyl-4-pyridiniumyl) porphinate] hexaiodide (**12**) Anal. Calcd. for $C_{100}H_{88}N_{14}O_6Ni_2SnI_6.6H_2O$: C 44.69, H 3.75, N 7.30; Found: C 44.51, H 3.84, N 7.19. ^{1}H NMR (DMSO-d_6) δ (ppm): 10.24–10.12(m, br, 16H, β-pyrrole), 9.39 (s, 12H, 2,6-pyridine), 8.83 (s, 12H, 3,5-pyridine), 7.75 (m, 4H, phenyl), 7.37 (m, 4H, phenyl), 4.65 (s, 22H, N-CH_3+OCH_2–), 1.53 (m, 4H, $SnCH_2$), 1.21 (m, 8H, CH_2), 0.73 (t, 6H, CH_3), IR (KBr pellet): 2956–2864 cm^{-1} (m, butyl); 1637 cm^{-1}, C=O+C=N^{+}–CH_3. ESI-MS: 411.5 (M-5I).

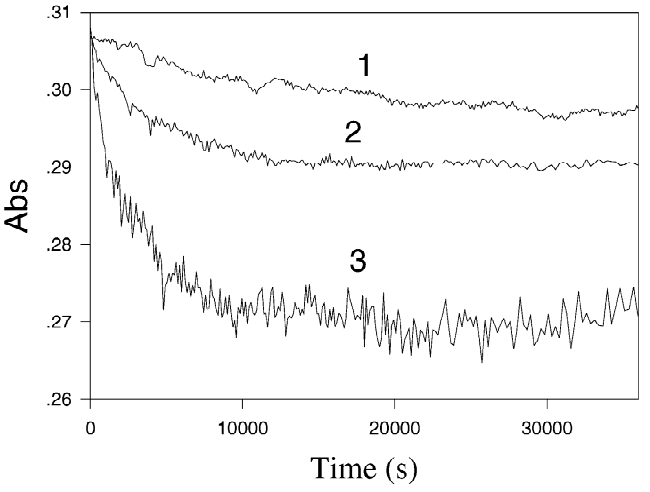


Fig. 1. The time-trace of the interaction of compound **7** with DNA. (1) c_7/c_{DNA}=1:10, (2) c_7/c_{DNA}=1:5, (3) c_7/c_{DNA}=1:2.

3. Results and discussion

3.1. Anti-tumor activity in vitro

Drug activity was determined by using MTT and SRB methods against human A-549 tumor cells and P388 leukemia, as described previously [19].

The results given as inhibitory (%) in Table 1, clearly show that these compounds have strong activity in vitro against P388 and A-549, except **5**–**7** have weak effect against A-549, and *p*-tmPyPac, Nickel [5-*o*-(ethoxycarbonyl) methoxyphenyl-10,15,20-tris(*N*-methyl-4-pyridiniumyl)] porphyrin (*o*-NimPyP), Copper [5-*o*-(ethoxycarbonyl)methoxyphenyl-10,15,20-tris(*N*-methyl-4-pyridiniumyl)]porphyrin (*o*-CumPyP) and compounds **1, 4** have no effect against P388 and A-549. The activities decrease in the order of Cu≈Ni>2H for water-insoluble compounds; the water-insoluble compounds (compounds **1** and **4**) without activity against P388 and A-549 have activity against the two tumors cells when they become water-soluble, and the activities of compounds **5**–**7** against A-549 have a weak to strong effect when their porphyrin groups have been methylated.

3.2. Kinetics of the interactions between DNA and varying concentrations of 7

Fig. 1 shows the time trace in A_{260} from a series of reactions between DNA and three different concentrations of compound **7**. It can be seen from Fig. 1 that a hypochromic effect occurs in the initial period of the reaction. The larger the concentration of the drug, the

Table 1
The inhibitory effects (%)[a] of compound against P388 and A-549 tumor cells in different concentrations

Compound	*C*/*M*											
	10^{-4}		10^{-5}		10^{-6}		10^{-7}		10^{-8}		Evaluation[b]	
1	100	(59.2)	67.3	(9.4)	22.1	(8.8)	5.5	(5.0)	11.5	(9.3)	N.E.	(N.E.)
2	39.7	(78.0)	97.5	(96.4)	100	(88.9)	19.2	(23.4)	35.0	(16.0)	S.E.	(S.E.)
3	52.8	(38.1)	98.2	(95.6)	100	(98.7)	79.1	(24.5)	43.8	(14.1)	S.E.	(S.E.)
4	88.1	(–)	100	(39.3)	100	(9.1)	62.1	(–)	14.0	(–)	N.E.	(N.E.)
5	100	(100)	100	(88.8)	100	(22.7)	100	(15.9)	87.5	(1.1)	S.E.	(W.E.)
6	100	(92.1)	100	(98.9)	100	(57.4)	22.6	(0.8)	7.0	(–)	S.E.	(W.E.)
7	100	(78.0)	100	(90.1)	100	(16.2)	65.3	(0.1)	18.9	(8.9)	S.E.	(W.E.)
8	–	(97.8)	85.2	(98.4)	97.9	(92.4)	87.6	(8.9)	78.0	(0.1)	S.E.	(S.E.)
9	18.6	(95.5)	88.3	(97.8)	98.7	(99.0)	96.9	(12.9)	70.0	(1.8)	S.E.	(S.E.)
10	100	(86.9)	100	(91.8)	100	(93.1)	100	(51.4)	32.4	(19.0)	S.E.	(S.E.)
11	100	(95.8)	100	(97.4)	100	(96.9)	100	(39.8)	37.3	(11.4)	S.E.	(S.E.)
12	46.1	(95.7)	94.0	(97.3)	100	(96.4)	100	(8.9)	70.2	(1.5)	S.E.	(S.E.)
p-tMPyPac	56.7	(65.2)	28.9	(18.2)	5.7	(5.5)	–	(3.9)	–	(0.9)	N.E.	(N.E.)
o-NimPyP[c]	43.9	(49.4)	38.2	(–)	0.3	(–)	5.3	(7.4)	12.3	(2.6)	N.E.	(N.E.)
o-CumPyP[c]	90.3	(86.0)	69.8	(0)	12.7	(0.2)	18.6	(4.0)	45.1	(8.5)	N.E.	(N.E.)

[a] Data in parentheses are against A-549 tumor cell.
[b] N.E., W.E. and S.E. represent not, weakly, and strongly effective, respectively.
[c] *o*-NimPyP and *o*-CumPyP represent [5-*o*-(ethoxycarbonyl)methoxyphenyl-10,15,20-tris(*N*-methyl-4-pyridiniumyl)] porphyrin Nickel and Copper complex, respectively.

stronger the hypochromic effect. In the whole period of the reacting process, hyperchromism does not appear. 'Hypochromic effect' and 'hyperchromic effect' are the spectral features of DNA concerning its double-helix structure [26]. Cations can cause the hypochromism of DNA by binding to the phosphate group of the DNA backbone and thus many contractions in the helix axis of DNA, and the destruction of duplex structure of DNA can cause the hyperchromism [27,28]. The cationic porphyrin could interact with DNA by electrostatic and van der Waals forces, and the predissociated dibutyltin(IV) could also bind to nucleotides via the phosphate group. Moreover, the affinity of Sn(IV) with the dinegative phosphate group is very strong because of its hard Lewis acidic property. In fact, Sn(IV)-phosphate binding has been detected [29]. Therefore, the above results indicate that the drug binds to the phosphate group, neutralizing the negative charge of the DNA and causing a contraction and conformation change in the DNA. This result is similar to that of the 'salt effect'. No hyperchromism indicates the drug cannot cause destruction of the duplex structure of DNA.

3.3. Viscosity study

As a means of further exploring the interaction between compounds **7** and **10** with DNA, we carried out viscosity studies. The viscosity measurement is based on the flow rate of DNA solution through a capillary viscometer. The specific viscosity values of the DNA, measured after the binding agent reacted with DNA for 20 h are summarized in Table 2. The results indicate that the presence of **7** or **10** increases the viscosity of the DNA slightly. It is notable that the free porphyrin *p*-tmPyPac and *o*-tmPyPac decrease the viscosity of DNA slightly [30]. The organotin(IV) compounds, such as Et_2SnCl_2 increase the viscosity of DNA [31] when they bind to the phosphate group of the DNA. The time-traces of viscosity have also been determined, and the results show that in the initial period of the reacting process, the specific viscosity value of DNA is less than 1.0, but becomes larger than 1.0 after the reaction continues for 30 min. From the above, we can conclude that the viscosity increase in the DNA caused by compounds **7** or **10** comes from the binding of the dissociated organotin group of the drug to phosphate of DNA.

3.4. Apparent binding constant

Apparent binding constants are measured by competition with EB as described by Le Pecq et al. [32,33] for other compounds. This method uses the competition between EB and the studied compound for binding to DNA and measures the decrease in fluorescence of EB bound to DNA in the presence of the compound to be studied. This method can be used for all compounds having a good affinity for DNA whatever their mode of binding may be as it only measures the ability of a compound to prevent intercalation of EB into DNA. We use this method because cationic porphyrins do not quench the fluorescence of EB.

Table 2
The effect of compounds **7** and **10** on the relative viscosity of DNA

$R(C_7/C_{DNA})$	0.000	0.005	0.010	0.025
DNA relative viscosity	1.00	1.010	1.020	1.040
$R(C_{10}/C_{DNA})$	0.000	0.005	0.010	0.050
DNA relative viscosity	1.00	1.010	1.043	1.070

In pH 7.40 buffer containing 0.10 M NaCl and 0.05 M Tris–HCl, different concentrations of drug (as porphyrin concentration) were mixed with DNA, 0.1 mg/ml EB solution is used to titration the mixture when they were kept for 48 h in the dark at 25 °C. The apparent binding constants of compounds **7**and **10** binding to DNA are $1.35\pm0.16\times10^7$ M^{-1} and $1.45\pm0.12\times10^6$ M^{-1}, respectively, which is 20 and five times greater than that of *p*-tmPyPac ($7.65\pm0.56\times10^5$ M^{-1}) and *o*-tmPyPac ($3.30\pm0.23\times10^5$ M^{-1}) to DNA. We think such a great difference results from the binding of Sn(IV) atom to phosphate of DNA as well as the interaction of the cationic porphyrin ion with DNA by phosphate and van der Waals [34].

3.5. Melting point measurement

Compounds **7** and **10** increase the melting point of DNA by 2.9 and 1.6 °C, respectively at a $DNA_{base}/Drug_{Por}$ ratio of 60, which is greater than that of *p*-tmPyPac (0.8 °C) and *o*-tmPyPac (0 °C) interaction with DNA. The results suggest that compounds **7** and **10** stabilize the DNA structure more than *p*-tmPyPac and *o*-tmPyPac [34].

3.6. Fluorescence studies

*3.6.1. The fluorescence quenching of EB–DNA caused by **7** and **10***

The fluorescence intensity of EB–DNA containing varying concentrations of **7** or **10** has been measured after they are mixed, and reacted in the dark for different time intervals at 25 °C. The plots of relative fluorescence intensity F_0/F (F_0 and F represent the fluorescence intensity of EB–DNA without drug and containing drug, respectively) versus the concentration of quencher (as porphyrin concentration) are shown in Fig. 2. The results show that the slopes of the curves for **7** and **10** are less than that of curves for *p*-tmPyPac and *o*-tmPyPac. These results indicate that **7** and **10** binding to DNA is not stronger than that of their precursor porphyrin *p*-tmPyPac and *o*-tmPyPac. However the binding constant studies suggested that **7** and **10** binding to DNA is stronger than that of *p*-tmPyPac and *o*-tmPyPac. This result may indicate that the conformation of DNA changes when dibutyltin(IV) binds to DNA, therefore the cationic por-

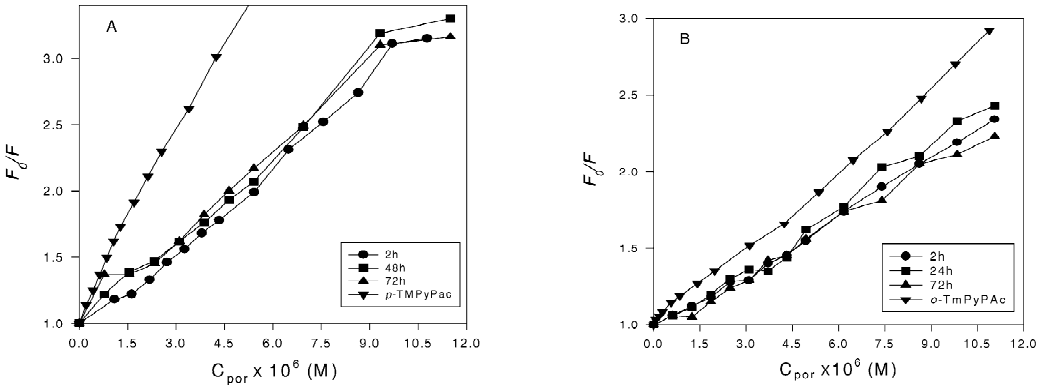


Fig. 2. The quenching relativity fluorescence of EB–DNA by three concentrations of dibutyltin porphyrinate. (A) Compound **7**; (B) compound **10**.

phyrin cannot quench the fluorescence of EB–DNA effectively.

*3.6.2. The effects of phosphate group and ion strength on the binding of **7** and **10** to DNA*

The fluorescence intensity of the EB–DNA complex in the presence of **7** or **10** and varying concentrations of KH_2PO_4 has been measured after they are mixed and reacted in the dark for 48 h at 25 °C (Table 3). The results are reported as intensities relative to the fluorescence intensity of the pure EB–DNA complex (100%). The fluorescence intensity of the Et_2SnCl_2(phen)–EB–DNA complex increases when KH_2PO_4 is added to the system because the fluorescence quenching of the EB–DNA caused by Et_2SnCl_2(phen) is weakened by the competing binding of phosphate of KH_2PO_4 and DNA. The fluorescence of compound **7** and **10**-EB–DNA complexes are not weakened by addition of KH_2PO_4, indicating that phosphate of KH_2PO_4 cannot inhibit the binding of **7** and **10** to DNA effectively.

Table 3
The effect of the phosphate group on the relative fluorescence intensity (*F*, %) of drug–EB–DNA system

	KH_2PO_4, 100 μM					
	0.00	1.00	5.00	10.0	50.0	100
a1	26.0	25.2	24.4	24.2	27.0	26.1
b1	10.0	9.1	9.8	9.3	12.1	9.3
a2	47.1	45.3	46.2	49.0	48.1	45.4
b2	10.1	11.2	12.3	11.5	17.4	14.1
c	59.1	61.9	63.7	67.7	78.2	93.9

a1, **7**–EB–DNA, 0.10 M NaCl, pH 7.4 0.05 M HCl–Tris; a2, **10**–EB–DNA, 0.10 M NaCl, pH 7.4 0.05 M HCl–Tris; b1, **7**–EB–DNA, 0.01 M NaCl, pH 7.4 0.05 M HCl–Tris; b2, **10**–EB–DNA, 0.01 M NaCl, pH 7.4 0.05 M HCl–Tris; c, $H_2PO_4^{1-}$ effect on the fluorescence of Et_2SnCl_2(phen)–EB–DNA, see Ref. [28].

Table 4
The effect of ionic strength on the relative fluorescence intensity (*F*, %) of drug–EB–DNA system

	NaCl (M)				
	0.01	0.02	0.05	0.10	0.50
a	10.0	14.0	13.5	27.7	26.8
b	10.1	15.6	23.6	33.6	28.4
c	59.1	62.0	66.3	70.3	45.3

The fluorescence of EB–DNA quenched by (a) compound **7** in pH 7.4 0.05 M HCl–Tris buffer, (b) **10** in pH 7.4 0.05 M HCl–Tris buffer, (c) Et_2SnCl_2(phen) see Ref. [28].

*3.6.3. The effect of ionic strength on the binding of **7** and **10** to DNA*

This is tested by the same method as above and the ionic strength is adjusted with NaCl (Table 4). The dramatic increase in fluorescence intensity at low ionic strength in the Et_2SnCl_2(phen)–EB–DNA system indicates that the increase in ion strength weakens the binding of Et_2SnCl_2(phen) to DNA. But in our system, the increase in ionic strength can only increase the fluorescence of the system slightly. This result indicates that the increase in ionic strength only slightly weakens the binding of **7** and **10** to DNA because the ion can weaken the binding of cationic pophyrins [35] and organotin to DNA [28].

3.7. Electrophoresis test

The compound **7** (or **10**) was mixed with plasmid DNA pBR322 at different molar ratios, after at least 5 h of incubation in the dark at 37 °C. Then electrophoresis experiments were carried out on an electrophoresis system and the gels were stained with EB for 0.5 h after electrophoresis, and then photographed (Fig. 3). No evidence was found for DNA cleavage by the compounds at molar ratio as high as ($C_{drug}/C_{DNA\,base}$) 2.1:1. This result is similar to that of literature [12].

From all the above results, we can conclude that the anti-tumor activity of dibutyltin porphinate complexes based on the tris(pyridinyl)porphyrin and tris(*N*-methyl-4-pyridiniumyl) porphyrin (M=Cu, Ni, 2H) is related to the water solubility of these compounds and the central ion in the porphyrin ring. The binding of compounds **7** and **10** to

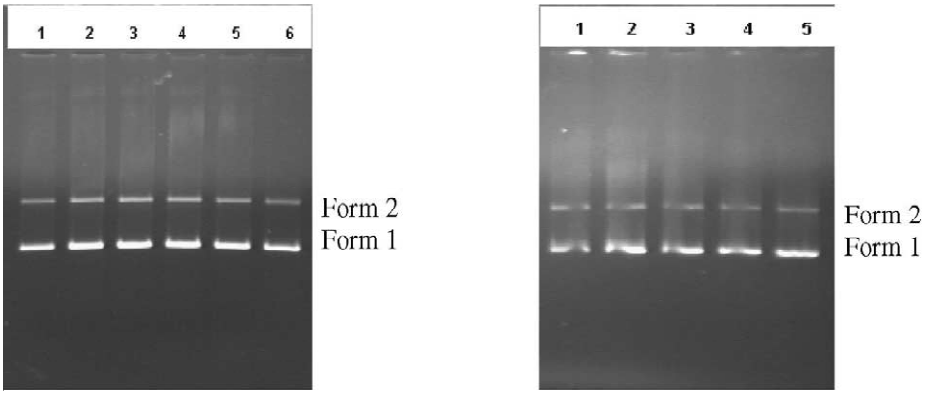


Fig. 3. Agarose gel electrophoresis patterns for the cleavage of PBR322 DNA by *p,o*-tmPyPSn. The ratios of C_{drug}/C_{DNA} are (left) 0.00, 0.053, 0.16, 0.27, 0.43, 0.53 for lanes 6–1, respectively for compound **7**, and (right) 0.00, 0.26, 0.80, 1.35, 2.15, for lanes 5–1, respectively for compound **10**.

DNA is so strong that increasing the concentration of KH_2PO_4 cannot affect their interaction with DNA and increasing ion strength can only weaken their binding to DNA slightly. The electrophoresis test shows that the compounds cannot cleave the DNA. So we can conclude that the activity against P388 and A-549 tumor cells seems not to come from the damage to the DNA cause by the compounds but from the high affinity of compounds to DNA.

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