Gold(III) tetraarylporphyrin phosphonate derivatives as potential anticancer agents

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5-[4-(Dialkyoxyphosphorylamino)]phenyl-10,15,20- triphenylporphyrinato gold(III)chlorides have been synthesised and evaluated for their *in vitro* cytotoxic activity against SMMC-7721 human hepatic and sarcoma 180 mouse cancer cell line panels. 5-[4-(Diisopropoxyphosphorylamino)]phenyl-10,15,20- triphenylporphyrinato gold(III)chloride exhibited significant growth inhibitory properties against sarcoma 180 mouse cancer cells (IC_{50} value = 2.60 μM) and 5-[4-(dipropoxyphosphorylamino)]phenyl-10,15,20-triphenylporphyrinato gold(III)chloride showed significant growth inhibitory properties against SMMC-7721 human hepatic cancer cells (IC_{50} value = 5.10 μM).

Keywords: gold(III) porphyrin derivatives, N-dialkylphosphonate, tetraaryl porphyrins, *in vitro* cytotoxicity

In clinical use, cisplatin and its derivatives carboplatin and oxaliplatin have demonstrated excellent anti-cancer activity in the treatment of several forms of cancer, including ovarian, cervical, head and neck, and non-small-cell lung cancers.[1–4] However, the main problem in using cisplatin and its derivatives is their low selectivity for cancer cells and their often high toxicity to the body.[5,6] Hence, research into new-type of platform complexes or other noble metal complexes with less toxicity has received much attention in recent years. Among these complexes, many gold-based compounds have been synthesised and evaluated successfully as potential anti-cancer agents.[7–10] However, it is difficult to bring some available gold(III) compounds into clinical use because these compounds are effective only at relatively high doses. Moreover, these compounds are usually unstable under physiological conditions, due to the reduction of gold(III) to gold(I).

Based on the above, Che *et al.* have reported the synthesis and antitumour efficacy of gold(III) tetraphenyllporphyrin.[11] Subsequently, great efforts have been made to elucidate the mechanisms of action of gold(III) tetraphenylporphyrin and to synthesise many modified gold(III) tetraphenylporphyrins. These gold(III) porphyrin compounds, which behave as organic lipophilic cations with a planar structure, are stable against demetallation under physiological conditions.

Further *in vitro* and *in vivo* studies revealed that gold(III) porphyrin is a promising anticancer agent for the treatment of colon and liver cancer,[12–20] because the porphyrin ligand in these compounds can efficiently stabilise the gold(III) centre, drastically reducing its redox reactivity and oxidising character. But, although gold(III) tetraphenyl porphyrin shows a high anti-cancer activity, it is not an approved clinical drug. In order to reduce its toxicity and to improve its activity, much work has been carried out to develop more efficient and selective derivatives of gold(III) tetraphenylporphyrin.

Previous reports have shown that altering the substituents on the porphyrin ligand can impact on the electrochemical properties and *in vitro* cytotoxicity of the corresponding gold(III) porphyrins.[11,14,21] Phosphorus substituents regulate important biological functions and molecular modifications involving the introduction of organophosphorus functionalities could increase their biological activity.[22–24] Many studies have shown anti-tumour activities of phosphonate derivatives such as aminophosphonate,[25,26] phosphoramidate monoesters, ether phospholipids,[27–29] and steroidal phosphate.[30] Some studies also indicated that the introduction of phosphodiesters could improve their water solubility and permit a targeting of the drug to a specific organ.

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Literature review reveals that no synthetic analogues of gold(III) tetraarylporphyrin phosphonate derivatives have been reported for the purpose of cytotoxicity evaluation. We were thus encouraged to extend our earlier work[31,32] to design aminophosphonate derivatives of gold(III) tetraarylporphyrin for antitumour evaluation. Here we report the synthesis of novel phosphate derivatives of gold(III) tetraphenyl porphyrin and their cytotoxic activity against SMMC-7721 human hepatic and sarcoma 180 mouse cancer *in vitro* by the standard MTT method.

Experimental

All reagents and solvents were commercial available analytical grade and used as received. Solvents were purified and dried by standard methods and distilled prior to use when necessary. All evaporations of organic solvents were carried out using a rotary evaporator in conjunction with a water aspirator. Melting points were taken on a hot-plate microscope apparatus and are uncorrected. ^{1}H NMR spectra were recorded with a Bruker AV-600 spectrometer. IR spectra were obtained on a Bruker Tensor 27 spectrometer (KBr disc). MS spectra were obtained on a ZAB-HS mass spectrometer with 70 eV; TPPAuCl [5,10,15,20-tetraphenyl porphyrin gold(III) chloride] was synthesised by the reported method.[11,31] UV-Vis spectra were obtained on a UV 2501 PC spectrometer. Elemental analytical data were obtained with a model 240 instrument. Mycoplasma-free newborn calf serum was purchased from Hangzhou Sijiqing Biological Engineering Materials Co., Ltd Cisplatin (Cis) was the product of Jiangsu Hansen Pharmaceutical Co., Ltd RPMI 1640, MTT and DMSO were purchased from Sigma-Aldrich Chemical Co. The phosphate derivatives were tested against SMMC-7721 human hepatic and sarcoma 180 mouse cancer *in vitro* by the standard MTT method.[32]

Cell lines and cell culture

SMMC-7721 human hepatic carcinoma (SMMC-7721) and sarcoma 180 mouse cancer cells were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Science. The cell line was cultured in RPMI 1640 medium with 10% newborn calf serum serum. It was maintained in a humidified incubator with an atmosphere of 95% air and 5% CO_2 at 37 °C. The cells were continuously passaged once every 3–4 days. Growing cells were collected for experiments.

Synthesis of of 5-[4-(dialkyoxyphosphorylamino)]phenyl-10,15,20-triphenyl porphyrins (**5a–e**)

5,10,15,20-Tetraphenylporphyrin (**2**, TPP) was synthesised in 20.1% yield by the reported method.[11,33]

5-(4-Nitro)phenyl-10,15,20-triphenylporphyrin (**3**):[35] A solution of $NaNO_2$ (27.6 mg, 0.40 mmol) in trifluoroacetic acid (2 mL) was added to a solution of **2** (245.7 mg, 0.40 mmol) in trifluoroacetic acid (10 mL) at room temperature for 1 min. The resultant mixture was stirred at room temperature for 40 min and then poured into water (100 mL). The mixture was extracted with CH_2Cl_2 (3 × 50 mL) and the organic phase was washed with saturated aqueous Na_2CO_3 and water, it was then dried over anhydrous Na_2SO_4. After removal of Na_2SO_4, the filtrate was concentrated and the crude product purified by column

chromatography (silica gel, CH_2Cl_2/n-hexane: 2/3 V/V). Recrystallisation from CH_2Cl_2/ethanol gave the pure product. 5,10,15-triphenyl-20-(4-nitro)phenyl porphyrin (**3**) 30.5% yield (80.4 mg): m.p. >250 °C; ^{1}H NMR ($CDCl_3$, 600 MHz), δ (ppm): –2.79 (s, 2H, inner-NH), 7.75–7.81 (m, 9H, Ph-CH), 8.21 (d, *J* = 7.2 Hz, 6H, Ph-CH), 8.40 (d, *J* = 8.4 Hz, 2H, Ph-CH), 8.64 (d, *J* = 8.4 Hz, 2H, Ph-CH), 8.74 (d, *J* = 4.2 Hz, 2H, Por-CH), 8.86–8.90 (m, 6H, Por-CH); IR(KBr): υ 3446(s), 2918(w), 2850(w), 1596(w), 1517(w), 1472(w), 1392(w), 1345(m), 1073(w), 840(w), 798(m), 706(m) cm^{-1}; MS (EI): 660 (M+1, 58%); UV-Vis (CH_2Cl_2) λ_{max}/nm (log ε) 418 (2.28), 514 (1.28), 549 (2.98), 588 (4.85), 645 (4.18).

5-(4-Amino)phenyl-10,15,20-triphenylporphyrin (**4**):[33] $SnCl_2$ (0.27 g, 1.2 mmol) and 36.5% HCl (5 mL) was added to a solution of 5,10,15-triphenyl-20-(4-nitro)phenyl porphyrin (245.7 mg, 0.4 mmol) and 36.5% hydrochloric acid (7 mL) under nitrogen gas. The resultant mixture was stirred at 0 °C for 1 h. After the reaction mixture was treated slowly with ammonia (5 mL), a green precipitate was collected by filtering. The green solid was then dissolved in dichloromethane (25 mL), the organic phase was washed with water, sat. $NaHCO_3$ and brine respectively and dried over Na_2SO_4. After removal of solvent, the crude product was purified by column chromatography (silica gel, DCM) and the purified product **4** was obtained in 87.0% yield (218.9 mg): m.p. >250 °C; ^{1}H NMR ($CDCl_3$, 600 MHz), δ (ppm): –2.74 (s, inner-NH), 4.02 (s, 2H, amino), 7.08 (d, *J* = 8.3 Hz, 2H), 7.78 (m, 9H), 8.00 (d, *J* = 8.2 Hz, 2H), 8.20 (m, 6H), 8.83 (s, 2H), 8.85 (d, *J* = 5.0 Hz, 2H), 8.95 (d, *J* = 5.0 Hz, 2H); IR(KBr): υ 3426(s), 2920(w), 2854(w), 1610(w), 1530(w), 1470(w), 1382(w), 1345(m), 1083(w), 920(w), 810(m), 720(m) cm^{-1}; MS (EI): 630 (M+1, 32%); UV-Vis (CH_2Cl_2) λ_{max}/nm (log ε) 426 (2.10), 516 (1.22), 552 (2.98), 590 (4.80), 648 (4.20); Anal. Calcd for $C_{44}H_{31}N_5$: C, 83.92; H, 4.96; N, 11.12. Found C, 84.08; H, 4.70; N, 11.22%.

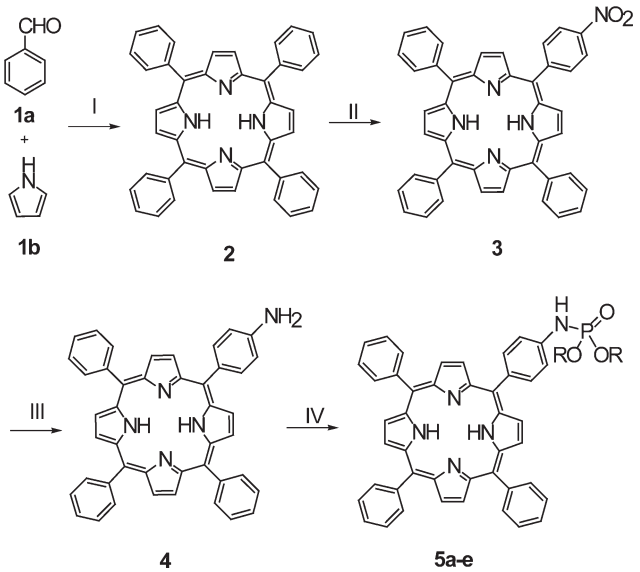
5-[4-(Dimethoxyphosphorylamino)]phenyl-10,15,20-triphenylporphyrin (**5a**): Dimethyl phosphonate (8.91 mg, 0.081 mmol) and CCl_4 (5 mL) was added dropwise to a solution of **4** (20 mg, 0.027 mmol), triethylamine (2 mL) and dioxane (5 mL) at 10 °C for 1 h. The resultant mixture was stirred at 25 °C for 3–6 h. After removal of the solvent, the residues were dissolved in dichloromethane (25 mL). The organic phase was washed with water, saturated $NaHCO_3$ and brine respectively, then dried over Na_2SO_4. After removal of solvent, the crude product was purified by column chromatography (silica gel, CH_2Cl_2/n-hexane/Et_3N: 1/1/0.01 V/V/V) to give the title compound **5a** in 78.0% yield(15.5 mg). ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 8.79 (s, 2H), 8.76 (s, 6H), 8.14 (d, *J* = 6.6 Hz, 6H), 8.04 (d, *J* = 7.8 Hz, 2H), 7.68 (d, *J* = 7.2 Hz, 9H), 7.28 (d, *J* = 7.8 Hz, 2H), 5.30 (d, *J* = 7.2 Hz, 1H), 3.92 (s, 3H), 3.91 (s, 3H), –2.82 (s, 2H); IR (KBr, cm^{-1}): 2956, 2853, 1546, 1447, 1338, 1285, 1018, 966, 799, 700; MS (EI): 737.20 (M+1, 19%); UV-Vis (CH_2Cl_2) λ_{max}/nm (log ε) 424 (2.16), 512 (1.22), 550 (2.90), 592 (4.80), 660 (4.60); Anal. Calcd for $C_{46}H_{36}N_5O_3P$: C, 74.89; H, 4.92; N, 9.49. Found: C, 74.70; H, 4.82; N, 9.58%.

5-[4-(Diethoxyphosphorylamino)]phenyl-10,15,20-triphenylporphyrin (**5b**): Following the above procedure using diethyl phosphonate as the starting material, **5b** was obtained as a purple solid (56.5% yield): ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 8.80–8.77 (m, 8H), 8.13 (d, *J* = 6.0 Hz, 6H), 8.03 (d, *J* = 6.6 Hz, 2H), 7.69–7.65 (m, 9H), 7.28 (d, *J* = 7.8 Hz, 2H), 5.35 (d, *J* = 6.0 Hz, 1H), 4.32–4.25 (m, 2H), 4.16–4.13 (m, 2H), 1.42 (t, *J* = 4.8 Hz, 6H), –2.85 (s, 2H); MS (EI): (M+1): 766.00 (30%); IR (KBr, cm^{-1}): 2953, 1575, 1473, 1439, 1350, 1281, 1026, 965, 799, 700; UV-Vis (CH_2Cl_2) λ_{max}/nm (log ε) 427 (2.09), 518 (1.34), 552 (2.86), 592 (4.83), 650 (4.26); Anal. Calcd for $C_{48}H_{40}N_5O_3P$: C, 75.28; H, 5.26; N, 9.14. Found: C, 75.39; H, 5.03; N, 9.28%.

5-[4-(Diisopropoxyphosphorylamino)]phenyl-10,15,20-triphenylporphyrin (**5c**): Following the above procedure using diisopropyl phosphonate as the starting material, compound **5c** was obtained as a purple solid(58.9% yield): ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 8.87–8.85 (m, 8H), 8.23 (d, *J* = 6.6 Hz, 6H), 8.10 (d, *J* = 7.8 Hz, 2H), 7.79–7.75 (m, 9H), 7.36 (d, *J* = 8.4 Hz, 2H), 5.44 (d, *J* = 7.8 Hz, 1H), 4.09–4.08 (m, 1H), 3.95–3.89 (m, 1H), 1.46 (d, *J* = 6.0 Hz, 12H), –2.73 (s, 2H); MS (EI): 794.44 (M+1, 100%); IR (KBr, cm^{-1}): 3315, 2921, 2850, 1596, 1467, 1399, 1308, 1223, 1153, 990, 964, 873, 752; UV-Vis (CH_2Cl_2) λ_{max}/nm (log ε) 425 (2.12), 522 (1.41), 554 (2.90), 592 (4.85), 640 (4.09); Anal. Calcd for $C_{50}H_{44}N_5O_3P$: C, 75.64; H, 5.59; N, 8.82. Found: C, 75.50; H, 5.66; N, 8.72%.

5-[4-(Dipropoxyphosphorylamino)]phenyl-10,15,20-triphenylporphyrin (**5d**): Following the above procedure using dipropyl phosphonate as the starting material, compound **5d** was obtained as a purple solid(60.2% yield): ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 8.80–8.77 (m, 8H), 8.15 (d, *J* = 6.6 Hz, 6H), 8.03 (d, *J* = 7.8 Hz, 2H), 7.71–7.68 (m, 9H), 7.30 (d, *J* = 7.8 Hz, 2H), 5.51 (d, *J* = 7.8 Hz, 1H), 4.21–4.12 (m, 4H), 1.64–1.77 (m, 4H), 1.00 (t, *J* = 7.2 Hz, 6H), –2.85 (s, 2H); MS (EI): 794.44 (M+1, 100%); IR (KBr, cm^{-1}): 2974, 2938, 1608, 1437, 1397, 1227, 1171, 1000, 965, 851, 798; UV-Vis (CH_2Cl_2) λ_{max}/nm (log ε) 424 (2.10), 519 (1.20), 555 (2.73), 582 (4.89), 656 (4.20); Anal. Calcd for $C_{50}H_{44}N_5O_3P$: C, 75.64; H, 5.59; N, 8.82. Found: C, 75.48; H, 5.38; N, 8.70%.

5-[4-(Dibutoxyphosphorylamino)]phenyl-10,15,20-triphenylporphyrin (**5e**): Following the above procedure using dibutyl phosphonate as the starting material, compound **5d** was obtained as a purple solid (25.4% yield): ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 8.80–8.76 (m, 8H), 8.14 (d, *J* = 7.2 Hz, 6H), 8.02 (d, *J* = 8.4 Hz, 2H), 7.71–7.67 (m, 9H), 7.28 (d, *J* = 8.4 Hz, 2H), 5.47 (d, *J* = 8.4 Hz, 1H), 4.23–4.14 (m, 4H), 1.74–1.71 (m, 4H), 1.46–1.43 (m, 4H), 0.92 (t, *J* = 7.8 Hz, 6H), –2.85 (s, 2H); MS (EI): 822.87 (M+1, 100%); IR (KBr, cm^{-1}): 2972, 2935, 1600, 1435, 1387, 1237, 1169, 1031, 965, 853, 790; UV-Vis (CH_2Cl_2) λ_{max}/nm (log ε) 426 (2.19), 512 (1.28), 553 (2.94), 592 (4.73), 648 (4.22); Anal. Calcd for $C_{52}H_{48}N_5O_3P$ (%): C, 75.99; H, 5.89; N, 8.52. Found: C, 75.82; H, 5.88; N, 8.72%.



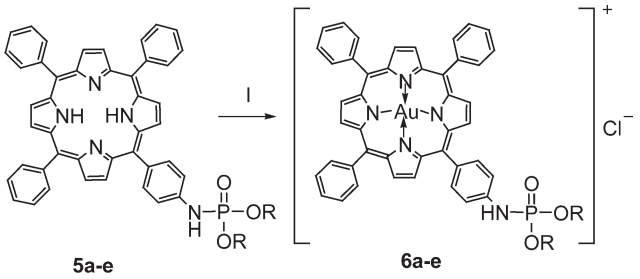
Scheme 1 Preparation of 5-[4-(dialkyoxyphosphorylamino)] phenyl-10,15,20- triphenylporphyrins (**5a–e**). Reaction conditions: (I) propionic acid, reflux, 3h, 20.1%; (II) $NaNO_2$, TFA, rt, 1 min, 30.5%; (III) $SnCl_2{\cdot}2H_2O$/HCl, 87.0%; (IV) $HPO(OR)_2$, 1,4-Dioxane, CCl_4, NEt_3, 3–6 h, 25.4–78.0%.

Table 1 The preparation of substituted tetraarylporphyrins **5a–e**

Compound	R	Time/h	Yield/%
5a	CH_3	6	78.0
5b	CH_3CH_2	6	56.5
5c	$(CH_3)_2CH$	6	58.9
5d	$CH_3CH_2CH_2$	3	60.2
5e	$CH_3CH_2CH_2CH_2$	3	25.4

*Synthesis of 5-[4-(dialkyoxyphosphorylamino)]phenyl-10,15,20-triphenylporphyrinato gold(III)chlorides (**6a–e**)*

5-[4-(Dimethoxyphosphorylamino)]phenyl-10,15,20-triphenyl porphyrinato gold(III) chloride (**6a**): A mixture of **5a** (18.4 mg, 0.025 mmol), $K[Au^{III}Cl_4]$ (28.4 mg, 0.07 mmol) and sodium acetate (20.5 mg, 0.25 mmol) in acetic acid (15 mL) was refluxed for 18–24 h. After completion, the reaction was checked by TLC, the crude product was obtained by removing acetic acid. Next, the solid product was dissolved thoroughly with dichloromethane (20 mL) and the organic phase was washed thoroughly with water and brine respectively, and then dried over Na_2SO_4. Removal of the solvent gave the



Scheme 2 Preparation of gold(III) tetraarylporphyrin derivatives (**6a–e**).
Reagents and conditions: (I) (1) $KAuCl_4 \cdot 2H_2O$, NaOAc, HOAc, reflux 17–24 h; (2) LiCl; 70.5–88.9%.

Table 2 Preparation of gold(III) tetraarylporphyrin derivatives (**6a–e**)

Compound	R	Time/h	Yield/%
6a	CH_3	24	70.5
6b	CH_3CH_2	17	88.9
6c	$(CH_3)_2CH$	19	75.5
6d	$CH_3CH_2CH_2$	20	79.7
6e	$CH_3CH_2CH_2CH_2$	18	72.2

crude product, which was purified by column chromatography (silica gel, DCM/methanol: 5/1 V/V). The purified product was dissolved in acetone and after filtering the mixture, the solution was treated with 10% LiCl (3.5 mL) in aqueous acetone to give analytically pure **6a** in 70.5% yield (16.9 mg) as a purple-mahogany solid. ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 9.32 (s, 2H), 9.19 (s, 6H), 8.17 (d, *J* = 6.0 Hz, 6H), 8.02 (d, *J* = 6.6 Hz, 2H), 7.84–7.79 (m, 9H), 7.67 (d, *J* = 6.0 Hz, 2H), 3.95 (s, 3H), 3.94(s, 3H); MS (EI): 932.53 (M+1-Cl, 100%); IR (KBr, cm^{-1}): 2917, 2849, 1740, 1582, 1464, 1373, 1260, 1181, 1035, 802, 704; UV-Vis (CH_2Cl_2) λ_{max}/nm (log ε) 408 (2.10), 519 (2.22), 552 (2.98), 590 (3.76), 648 (4.02); Anal. Calcd for $C_{46}H_{34}N_5O_3PClAu$ (%): C, 57.06; H, 3.54; N, 7.23. Found: C, 57.22; H, 3.42; N, 7.46%.

5-[4-(Diethoxyphosphorylamino)]phenyl-10,15,20-triphenylporphyrinato gold(III) chloride (**6b**): Following the above procedure using **5b** as a starting material, compound **6b** was obtained as a purple solid(88.9% yield): ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 9.34 (d, *J* = 4.8 Hz, 2H), 9.21–9.18 (m, 6H), 8.16 (d, *J* = 7.8 Hz, 6H), 8.01 (d, *J* = 9.4 Hz, 2H), 7.86–7.78 (m, 9H), 7.68 (d, *J* = 7.8 Hz, 2H), 4.34 (t, *J* = 7.2 Hz, 2H), 4.32 (t, *J* = 7.2 Hz, 2H), 1.42 (t, *J* = 7.2 Hz, 6H); MS (EI): 960.45 (M+1-Cl, 100%); IR (KBr, cm^{-1}): 2919, 2849, 1736, 1606, 1510, 1463, 1361, 1312, 1130, 1036, 800, 723; UV-Vis (CH_2Cl_2) λ_{max}/nm (log ε) 409 (2.10), 520 (2.26), 552 (2.98), 590 (4.01), 650 (3.89); Anal. Calcd for $C_{48}H_{38}N_5O_3PClAu$ (%): C, 57.87; H, 3.84; N, 7.03. Found: C, 57.70; H, 3.82; N, 7.20%.

5-[4-(Diisopropoxyphosphorylamino)]phenyl-10,15,20-triphenylporphyrinato gold(III) chloride (**6c**): Following the above procedure using **5c** as a starting material, compound **6c** (75.5% yield) was obtained as a purple solid: ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 9.29 (s, 2H), 9.20 (s, 6H), 8.17 (d, *J* = 6.6 Hz, 6H), 8.03 (d, *J* = 6.6 Hz, 2H), 7.84–7.79 (m, 9H), 7.56 (d, *J* = 6.0 Hz, 2H), 6.43 (d, *J* = 5.4 Hz, 1H), 4.23–4.21 (m, 1H), 4.08–4.05 (m, 1H), 1.43(d, *J* = 5.4 Hz, 6H), 1.41 (d, *J* = 4.8 Hz, 6H); MS (EI): 988.34 (M+1-Cl, 100%); IR (KBr, cm^{-1}): 2918, 2849, 1739, 1594, 1464, 1361, 1261, 1176, 1104, 1021, 800, 757; UV-Vis (CH_2Cl_2) λ_{max}/nm (log ε) 410 (2.10), 520 (2.35), 547 (2.72), 592 (3.89), 650 (3.88); Anal. Calcd for $C_{50}H_{42}N_5O_3PClAu$ (%): C, 58.63; H, 4.13; N, 6.84. Found: C, 58.78; H, 4.02; N, 6.96%.

5-[4-(Dipropoxyphosphorylamino)]phenyl-10,15,20-triphenylporphyrinato gold(III) chloride (**6d**): Following the above procedure using **5d** as a starting material, compound **6d** (79.7% yield) was obtained as a purple solid: ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 9.32 (d, *J* = 4.8 Hz, 2H), 9.21–9.19 (m, 6H), 8.17 (d, *J* = 7.2 Hz, 6H), 8.02 (d, *J* = 7.8 Hz, 2H), 7.86–7.80 (m, 9H), 7.65 (d, *J* = 7.8 Hz, 2H), 6.89 (d, *J* = 8.4 Hz, 1H), 4.22 (t, *J* = 7.2 Hz, 2H), 4.21 (t, *J* = 7.2 Hz, 2H), 1.82–1.77 (m, 4H), 1.00 (t, *J* = 7.8 Hz, 6H); MS (EI): 988.54 (M+1-Cl, 100%); IR (KBr, cm^{-1}): 2961, 2920, 2850, 1731, 1606, 1511, 1464, 1359, 1260, 1184, 1021, 801, 756; UV-Vis (CH_2Cl_2) λ_{max}/nm (log ε) 408 (2.08), 519 (3.02), 550 (3.02), 591 (3.84), 650 (3.69); Anal. Calcd for $C_{50}H_{42}N_5O_3PClAu$ (%): C, 58.63; H, 4.13; N, 6.84. Found C, 58.66; H, 4.24; N, 6.90%.

5-[4-(Dibutoxyphosphorylamino)]phenyl-10,15,20-triphenylporphyrinato gold(III) chloride (**6e**): Following the above procedure using **5e** as a starting material, compound **6e** was obtained as a purple solid (72.7% yield): ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 9.32 (d, *J* = 5.4 Hz, 2H), 9.21–9.20 (m, 6H), 8.16 (d, *J* = 6.6 Hz, 6H), 8.02 (d, *J* = 8.4 Hz, 2H), 7.81–7.79 (m, 9H), 7.62 (d, *J* = 7.8 Hz, 2H), 4.26–4.23 (m, 4H), 1.77–1.72 (m, 4H), 1.46–1.44 (m, 4H), 0.92 (t, *J* = 7.8 Hz, 6H); MS (EI): 1016.51 (M+1-Cl, 100%); IR (KBr, cm^{-1}): 2967, 2928, 2846, 1730, 1600, 1516, 1460, 1352, 1264, 1174, 1029, 809, 751; UV-Vis (CH_2Cl_2) λ_{max}/nm (log ε) 410 (2.16), 521 (2.52), 550 (3.80), 592 (3.82), 645 (3.88); Anal. Calcd for $C_{52}H_{46}N_5O_3PClAu$ (%): C, 59.35; H, 4.41; N, 6.65. Found: C, 59.18; H, 4.32; N, 6.58%.

Cytotoxicity assay for the effect of gold(III) substituted tetraarylporphyrin chlorides on SMMC-7721 human and sarcoma 180 mouse cancer cell proliferation

Gold(III) substituted tetraarylporphyrin chlorides were prepared in the concentrations 6.4×10^{-5} M, 3.2×10^{-5} M, 1.6×10^{-5} M, 0.8×10^{-5} M, 0.4×10^{-5} M and 2×10^{-6} M respectively. DMSO was used as latent solvent with the highest concentration less than 0.1% in the solution of tetraarylporphyrin. The control groups of cisplatin, blank (1640) and DMSO solvent were set up at the same time. The cytotoxicities of gold(III) substituted tetraarylporphyrin chlorides were determined by MTT cytotoxic assay. SMMC-7721 human hepatic carcinoma cells or sarcoma 180 mouse cancer cells were plated in 96-well plates at 1×10^5/mL and 100 μL/well in complete media. The cells were incubated for 24 hours. Then the prepared and various amounts of gold(III) substituted tetraarylporphyrin chloride and cisplatin were plated in 96-well plates containing SMMC-7721 human hepatic carcinoma cells or sarcoma 180 mouse cancer cells and incubated commonly for 44 h. Then 100 μL supernatant liquid was sucked from each hole, 10 μL of MTT (5 mg mL^{-1}) was added in and cultured for 4h, the media was then removed and hydrochloric acid/isopropanol added at 100 μL/well. Surging for 5 minutes using the micro oscillator dissolved the MTT crystal and OD values were measured at 570nm using a Model Elx 800 Autoplate reader (Bio-Tek Instruments, USA).

Statistical analysis of experimental data

All the data of the experiment were compiled and analysed according to SPSS 15.0 software. Measurement data were expressed as the mean.

Results and discussion

Phosphonate is an important functional group in organic chemistry, biochemistry, and medicine. Phosphonates play crucial roles in the human body and other organisms. Recent studies showed that phosphonates and their analogues have exhibited a wide spectrum of important bioactivities such as an antitumour action.[25–30]

Because of the good affinity of phosphonate toward cells and nucleic acids, the introduction of a phosphonate segment into drugs can facilitate their interactions with cells and tissues and thereby provide a robust strategy to design new drugs or lead compounds. Porphyrin compounds have distinguished themselves from other small molecules because of their profound bioactivities. Some recent examples suggested that the practice of attaching porphyrin skeletons and natural small molecules, such as a sugar and a steroid, onto one molecule has received much attention from synthetic and medicinal chemists attempting the discovery of novel compounds with unknown or improved pharmacological properties. However, the research of phosphonate used in conjunction with a porphyrin segment is rather limited. Thus it is an urgent task to synthesise a new class of porphyrins containing a phosphonate. Based on these previous studies, we have designed and synthesised a series of new gold(III) complexe analogues of the type gold(III) tetraarylporphyrin phosphonate. The desired gold(III) tetraarylporphyrin phosphonates contain three well defined parts: the gold(III) porphyrin core, an aromatic side chain connected by an amine and a linear phosphonate side chain.

To reach the desired target structures a five-step synthesis route was successful. Scheme 1 shows the synthetic routes to the target 5-[4-(dialkyoxyphosphorylamino)]phenyl-10,15,20-triphenylporphyrins. First the porphyrin base core was coupled with the aromatic side chain. Using reaction conditions similar to those reported previously,[11,33] 5,10,15,20-tetraphenylporphyrin (**2**) was prepared from the benzaldehyde and pyrrole (Scheme 1) in 20.1% yield. A mono-nitro group in the aromatic side chain was introduced according to the literature procedure,[35] thus 5-(4-nitro)phenyl-10,15,20- triphenylporphyrin (**3**) was readily obtained by reaction of **2** and sodium nitrite promoted by trifluoroacetic acid in 30.5% yield (Scheme 1). Reduction of **3** by $SnCl_2$–HCl under dinitrogen gave 5-(4-amino)phenyl-10,15,20- triphenylporphyrin (**4**). The 5-[4-(dialkyoxyphosphorylamino)]phenyl-10,15,20- triphenylporphyrins (**5a–e**) were prepared from **4** and the appropriate dialkyl phosphonate in the presence of triethylamine for 3–6 h (Scheme 1, Table 1) in yields 25.4–78.0%. The general synthesis of gold(III) porphyrin compounds (**6a–e**) was achieved through the route outlined in Scheme 2. Firstly, gold(III) porphyrin compounds were synthesised by treatment of $K[Au^{III}Cl_4]$ with the free-base porphyrin ligand in the presence of NaOAc in acetic acid. After purification with column chromatography and metathesis reaction with LiCl in aqueous acetone, gold(III) porphyrin compounds were obtained as chloride salts in 70.5–88.9% yields (Scheme 2, Table 2). ^{1}H NMR, electrospray ionisation (ESI) mass (+ mode) analysis spectrometry, UV-Vis and elemental analysis were used to characterise all the synthesised compounds. Downfield shifts of benzene and porphyrin exocyclic hydrogen resonances are generally observed in the ^{1}H NMR spectrum upon coordination to a gold cation, and this behaviour is found in compounds **6a–e**. The two hydrogen resonances from the porphyrin inner cyclic backbone disappear from *ca* –2.8 ppm once the ligand coordinates to the gold(III) centre. The UV-Vis absorption spectrum of compounds **6a–e** is also similar to previous reports of gold(III) substituted porphyrin complexes.[21]

To analyse the potential of compounds (**6a–e**) as antitumour agents, their cytotoxicity was evaluated (Table 3, Figs 1 and 2) towards SMMC-7721 human hepatic tumour cells and for comparison purposes the cytotoxicity of cisplatin and TPPAuCl were also evaluated under the same experimental conditions. From the data shown in Table 3, the new compounds **6a–e** showed a measurable anti-cancer activity against SMMC-7721 human hepatic and sarcoma 180 mouse cancer cell lines tested.

Although this is a preliminary screening, the results showed that compounds **6a–e** exerted a significant inhibitory effect on the growth of SMMC-7721 human hepatic and sarcoma 180 mouse cancer cells. A dose-dependent relationship was found between 1 and 100 μM and, in general, the cytotoxicities of **6d** against SMMC-7721 human hepatic tumour cells and **6c** against sarcoma 180 mouse cancer cells were higher than cisplatin and TPPAuCl. Compound **6c** had also a better inhibitory effect on SMMC-7721 human hepatic tumour cells, the IC_{50} value was 9.59 μM in SMMC-7721 human hepatic tumour cell line. Compounds **6a** and **6e** had nearly the same inhibitory effect on SMMC-7721 human hepatic tumour cells as cisplatin and **6b** exhibited an IC_{50} value nearly four-fold higher than cisplatin on SMMC-7721 human hepatic tumour cells. From the results of *in vivo* assays, the antitumour activity order was **6d**>TPPAuCl >**6c**> cis **>6a> 6e>6b** with respect to the SMMC-7721 human hepatic tumour cell line. Compared to TPPAuCl, compounds **6d** and **6c** show a significant inhibitory effect on the growth of SMMC-7721 human hepatic tumour cells, but the linear phosphonate side chain of **6d** and **6c** could balance the aqueous solubility with liposolubility and strengthen the affinity toward cells and nucleic acids, which would be helpful in transporting drugs into the target cells to improve the antitumour activity of the complexes.

Table 3 Cytotoxicity of **6a–e** against sarcoma 180 mouse and SMMC-7721 human tumour cell line *in vitro*

Entry	Compound	Maximum inhibition rate/%[a]		IC_{50}/μM	
		S180cell	SMMC-7721cell	S180cell	SMMC-7721cell
I	TPPAuCl	91.49	93.81	3.61	5.83
II	**6a**	91.65	97.62	6.39	10.38
III	**6b**	94.70	90.76	6.75	40.81
IV	**6c**	91.10	88.80	2.60	9.59
V	**6d**	82.54	93.84	15.86	5.10
VI	**6e**	97.50	98.74	4.31	12.60
VII	Cisplatin	89.64	91.91	7.05	9.75

[a]The concentration of the gold complexes is 32 μM.

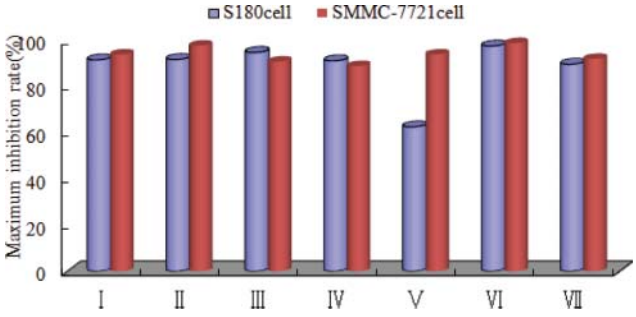


Fig. 1 Maximum inhibition rate of gold(III) substituted tetraarylporphyrin chloride against sarcoma 180 mouse (left bar) and SMMC-7721 human (right bar) tumour cell line *in vitro*.

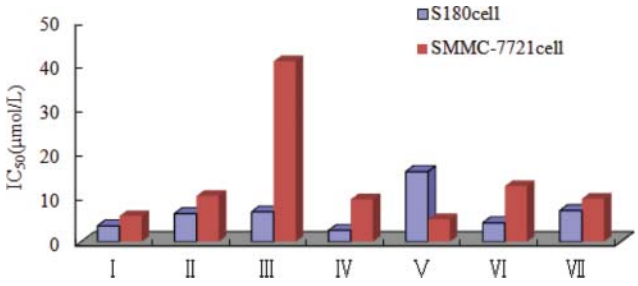


Fig. 2 IC_{50} of gold(III) substituted tetra-aryl porphyrin chloride against sarcoma 180 mouse (left bar) and SMMC-7721 human (right bar) tumour cell line *in vitro*.

Conclusions

A number of new substituted gold(III) tetraarylporphyrin phosphonates have been synthesised by a five-step synthesis route and evaluated for their *in vitro* anti-human hepatic and anti-sarcoma 180 mouse tumour activities. The two new substituted gold(III) tetraarylporphyrins (**6c** and **6d**) were more active than cisplatin and (TPPAuCl). Further *in vivo* tests of the compounds are under way and the anti-tumour mechanism will be studied in the future.

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