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Short communication

Synthesis and cancer cell cytotoxicity of water-soluble gold(III) substituted tetraarylporphyrin

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ABSTRACT

The synthesis of novel substituted gold(III) tetraarylporphyrins with aqueous solubility has been carried out. The analogs ClAuTPP($CH_3Py^+ \cdot I^-$), ClAuTCPPNa, ClAuTPPCO$_2$Na, ClAuTSPPNa and ClAuTPPNH$_2$·HCl were evaluated for their in vitro cytotoxic activity against sarcoma 180 mouse tumor and SGC-7901 human gastric cancer cell line panel. Compound ClAuTCPPNa exhibited significant growth inhibitory properties against sarcoma 180 mouse tumor and SGC-7901 human gastric cancer cell examined, and afforded IC_{50} values <25 μM for 66.63% of the cell lines in the panel. Compound ClAuTPPNH$_2$·HCl was an effective inhibitor of sarcoma 180 mouse tumor and SGC-7901 human gastric cancer cell growth, but generally less effective as a cytotoxic agent. Thus, the substituted gold(III) porphyrin ClAuTCPP-Na$^+$ and ClAuTPPNH$_2$·HCl with aqueous solubility were regarded as useful lead compounds for further structural optimization.

1. Introduction

New metal-containing anticancer drugs with preferential anticancer activity have received special attention as promising candidates for new anticancer drugs since the introduction of *cis*-diamminedichloroplatinum(II) (cisplatin) into clinical use [1,2]. However, although cisplatin is the better anticancer agent so far, it has several disadvantages, including obvious toxic side effects such as neurotoxicity, nephrotoxicity, and emesis. In addition, some cancers exhibit inherent resistance to cisplatin, whereas others develop resistance after initial treatment, thereby limiting its clinical use [3,4]. These particular disadvantages have driven the search for new compounds exhibiting high cytotoxic activity along with reduced side effects and no cross-resistance.

In recent years research has increasingly focused on the potential of gold(III) complexes as anticancer drug candidates, because gold(III) possesses the same isoelectronic configuration (d^8) of the platinum(II) ion; accordingly, the dominant coordination geometry for gold(III) complexes is square planar tetra-coordination [5–10]. Some studies have proven that polydentate ligands often enhance the stability of gold(III) complexes in biological environments, and although the fact gold(III) complexes are often structural analogs of cisplatin, they are widely thought to impart tumor cell death via a different mechanism [11–14].

However, although there have been important developments in the field of gold(III) complexes for anticancer chemotherapy, which gold(III) complexes exhibit strong cytotoxic activity in vitro against tumor cells and have tumor-inhibiting properties in vivo [15–18], a major problem hindering the development of gold(III) complexes for medicinal application is their poor stability in aqueous solutions. Consequently, some improved tetradentate ligands chelating gold(III) strongly were designed and synthesized. However, these gold(III) complexes are low cytotoxicity towards selected cancer cells with IC_{50} values of >100 μM [19].

The structural analysis indicated that gold(III) complexes with strongly chelating tetradentate ligands are difficult to carry the metal to the cellular targets due to the over-stabilization of gold(III) ions against reduction and demetalation.

Recently Che and co-workers have designed and synthesized some modified tetraphenyllporphyrin ligands to chelate gold(III). The gold(III) porphyrin compounds which behave as organic lipophilic cations with a planar structure, are stable against demetalation 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 [19–32], because the porphyrin ligand in the complex can efficiently stabilize the gold(III) center, drastically reduces its redox reactivity and oxidizing character. The use of gold(III) porphyrin complexes may provide an advantage for clinical application.

Although it has been demonstrated that various gold(III) porphyrin compounds have favorable anticancer properties, some gold(III) porphyrins exhibit 30-fold to 100-fold stronger cytotoxicity than cisplatin in several human cancer cell lines, including a cisplatin-resistant human nasopharyngeal carcinoma cell line, in colon and liver cancer. The major limitation of current gold(III) porphyrin complexes is that few exhibit good solubility under physiological conditions for clinical use [24–36]. Thus, there is an urgent need for the

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development of gold(III) porphyrin complexes with good solubility and good stability under physiological conditions as new anticancer drug candidates.

As the part of a drug discovery program to discover and develop modified gold(III) porphyrins as potential anticancer agents, we were interested in the substituted phenyl or pyridinyl with hydrophilic group replacement of the phenyl moiety in the tetraphenylporphyrin core skeleton. In continuation of our work on the design and synthesis of substituted tetraphenylporphyrins and their gold(III) complexes we focused on novel water-soluble gold(III) substituted tetraphenylporphyrin complexes that incorporated electron donating and electron withdrawing substituents in the aromatic ring of the tetraphenylporphyrin moiety.

To investigate the effect of changing the water-soluble substituent to gold(III) substituted tetraphenylporphyrin on bioactivity, different aldehydes were used to synthesize the target ligands according to the method described in Scheme 1 and Table 1. The synthesis of target water-soluble gold(III) substituted tetraphenylporphyrin complexes was described in Scheme 2 and Table 2.

2. Materials and methods

2.1. Materials and reagents

All reagents and solvents were commercially available with analytical grade and used as received. Further purification and drying by standard method were employed and distilled prior to use when necessary. All evaporations of organic solvents were carried out with 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). UV–Vis spectra were obtained on a UV 2501 PC spectrometer. The MS spectra were obtained on a ZAB-HS mass spectrometer with 70 eV. Elemental analytical data were obtained by using a model 240 elementary instrument. 5, 10, 15, 20-tetraaryl porphyrins 1–4 were synthesized by the reported method [20].

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, DMSO were purchased from Sigma-Aldrich Chemical Co.

2.2. Cell lines and cell culture

Sarcoma 180 mouse tumor cell line (S180) and SGC-7901 human gastric carcinoma cell line (SGC-7901) 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. 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 on experiments.

Table 1
Preparation of substituted porphyrins (**1–6**).

R^1	R^2	X	Ligands (yield, %)	
H	H	C	23.0	(**1**)
H		N	6.5	(**2**)
CO_2CH_3	CO_2CH_3	C	22.0	(**3**)
H	CO_2CH_3	C	9.0	(**4**)
SO_3H	SO_3H	C	64.2	(**5**)
H	NO_2	C	74.9	(**6**)

2.3. Procedure for the preparation 5,10,15,20-substitutedphenyl porphyrins 1–6

A mixture of benzaldehyde (3.18 g, 30.0 mmol), substituted aromatic aldehyde (10.0 mmol) and freshly distilled pyrrole (2.68 g, 40.0 mmol) in propionic acid (140 mL) was refluxed for 3 h. After completion of the reaction was checked by TLC, the mixture was cooled in an ice-water bath and the crude product was filtered and the filter cake was washed thoroughly with methanol (100 mL) and dichloromethane (10 mL) respectively. The resulting purple crystals are dried under air, the crude product was purified by column chromatography (silica gel, DCM/n-hexane: 1/5 V/V), re-crystallization from ethanol gave pure products **1–4** (Table 1).

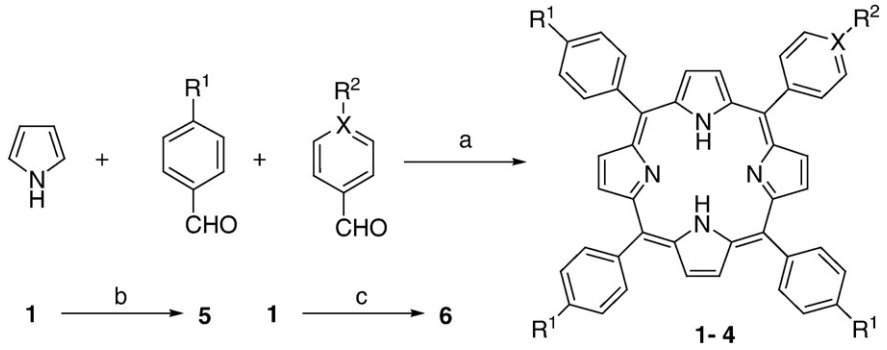
The data of substituted porphyrin ligands were given:

5,10,15,20-Tetraphenylporphyrin (compound **1**) [33]: mp > 250 °C; ^{1}H NMR ($CDCl_3$, 600 MHz), δ (ppm): −2.74 (s, 2H, inner-NH), 7.72–7.78 (m, 12H, Ph–CH), 8.21 (d, J = 6.6 Hz, 8H, Ph–CH), 8.83 (s, 8H, Por-CH); IR (KBr): υ 3438(s), 1560(w), 1472(w), 1437(w), 1348(w), 1070(w), 967(w), 797(m), 731(m), 697(w) cm^{-1}; MS (EI): 615 (M + 1, 46%).

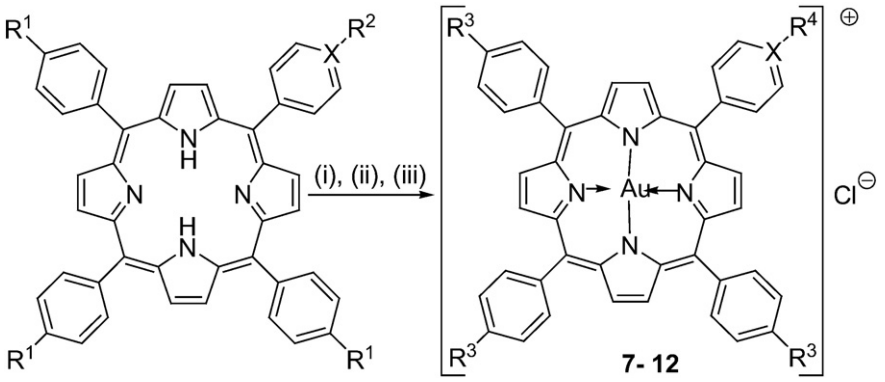
5,10,15-Triphenyl-20-(4-pyridinyl)porphyrin (compound **2**): mp > 250 °C; ^{1}H NMR ($CDCl_3$, 600 MHz), δ(ppm): −2.82 (s, 2H, inner-NH), 7.75–7.80 (m, 9H, Ph-CH), 8.17 (d, J = 5.4 Hz, 2H, Py-CH), 8.21 (d, J = 6.6 Hz, 6H, Ph-CH), 8.79 (d, J = 4.2 Hz, 2H, Py-CH), 8.86–8.90 (m, 6H, Por-CH), 9.03 (s, 2H, Por-CH); IR (KBr): υ 3446(s), 1634(w), 1590(w), 1473(w), 1396(w), 1351(w), 1070(w), 970(w), 798(m), 710(m), 657(w) cm^{-1}; MS (EI): 616 (M + 1, 38%); UV–Vis (CH_2Cl_2) λmax/nm (log ε) 416 (3.72), 513 (sh), 548 (2.37), 586 (4.38), 644 (4.20).

5,10,15,20-Tetra(4-methoxycarbonyl)phenylporphyrin (compound **3**): mp >250 °C; ^{1}H NMR (600 MHz, $CDCl_3$), δ(ppm): −2.79 (s, 2H, inner-NH), 4.11 (s, 12H, –$COOCH_3$), 8.29 (d, J = 7.8 Hz, 8H, Ph-CH), 8.44 (d, J = 7.8 Hz, 8H, Ph-CH), 8.81 (s, 8H, Por-CH); IR (KBr): υ 3425(m), 2919(w), 1724(s), 1607(w), 1435(w), 1383(w), 1277(m), 1108(m), 965(w), 803(w), 762(w) cm^{-1}; MS (EI): 735 (M + 1, 87%); UV–Vis (CH_2Cl_2) λmax/nm (log ε) 417 (3.08), 515 (sh), 549 (2.48), 588 (4.35), 645 (4.22).

5,10,15-Triphenyl-20-(4-methoxycarbonyl)phenyl porphyrin (compound **4**): mp >250 °C; ^{1}H NMR ($CDCl_3$, 600 MHz), δ(ppm): −2.79 (s, 2H, inner-NH), 4.11 (s, 3H, –$COOCH_3$), 7.74–7.79 (m, 9H, Ph-CH), 8.21 (d, J = 6.6 Hz, 6H, Ph-CH), 8.31 (d, J = 7.8 Hz, 2H, Ph-CH), 8.44 (d,



Scheme 1. Reagents and conditions: (a) propionic acid, reflux, 2–4 h; (b) H_2SO_4, 120 °C, 4 h; (c) $NaNO_2$, CF_3CO_2H, r.t. 1 min.



Scheme 2. Reagents and conditions: (i) $KAuCl_4$, NaOAc, HOAc, reflux, 3 h from **2** to **4**, **6** ; $KAuCl_4$, H_2O, reflux, 12 h then NaOH from **5** to **11** ; (ii) LiCl; (iii) CH_3I, reflux, 3 h for **8**; NaOH, DMF, r.t. 2 h for **9**, **10**; $SnCl_2$, HCl, r.t. 24 h for **12**.

J = 7.8 Hz, 2H, Ph-CH), 8.79 (d, J = 4.2 Hz, 2H, Por-CH), 8.85 (s, 6H, Por-CH); IR (KBr): υ 3442(s), 3319(w), 2924(w), 2853(w), 1812(m), 1722(s), 1604(w), 1472(w), 1437(w), 1394(w), 1353(w), 1279(s), 1182(w), 1106(w), 800(m), 739(w) cm^{-1}; MS (EI): 645 (M + 1, 88%); UV–Vis (CH_2Cl_2) λmax/nm (log ε) 416 (3.12), 513 (sh), 548 (2.52), 589 (4.40), 646 (4.18).

5,10,15,20-Tetra(4-sulfo)phenylporphyrin (compound **5**)

The mixture of 5,10,15,20-tetraphenylporphyrin (compound **1**) (1.0 g, 1.63 mmol) and 100% sulfuric acid (20 mL) was stirred at 120 °C for 4 h After the reaction mixture was cooled to room temperature, the mixture was poured into ice-water (60 mL). The resulting mixture was neutralized with 10 M NaOH to pH: 5, following concentrated to 20 mL. Then the mixture was cooled under 0 °C, the mixtures were filtrated and the solid was washed with methanol (60 mL). The filtrate was diluted with methanol (100 mL) and the solid Na_2SO_4 was removed by filter. The Na_2SO_4 was removed by repeating the foregoing operation three times and the resultant solution was concentrated to give a crude product. The product was purified by re-crystallizing with a mixture solvent of methanol and acetone. 5,10,15,20-Tetra(4-sulfo)phenylporphyrin (compound **5**): mp > 250 °C; ^{1}H NMR (600 MHz, DMSO-d_6), δ(ppm): −2.98 (s, 2H, inner-NH), 7.96 (d, J = 6.0 Hz, 8H, Ph-CH), 8.08 (d, J = 6.0 Hz, 8H, Ph-CH), 8.76 (s, 8H, Por-CH); IR (KBr): υ 3449(m), 1635(w), 1448(s), 1385(w), 1192(m), 1126(w), 1042(w), 873(w), 800(w), 739(w), 638(w) cm^{-1}; MS (EI): 935 (M + 1, 19%); UV–Vis (CH_2Cl_2) λmax/nm (log ε) 412 (2.54), 515 (1.68), 551 (2.68), 578 (4.39), 633 (4.08).

5,10,15-Triphenyl-20-(4-nitro)phenyl porphyrin (compound **6**)

To the solution of 5,10,15,20-tetraphenylporphyrin (compound **1**) (245.7 mg, 0.40 mmol) in trifluoroacetic acid (10 mL) was added the solution of $NaNO_2$ (27.6 mg, 0.40 mmol) in trifluoroacetic acid (2 mL) at room temperature in 1 min. The resultant mixture was stirred at room temperature for 40 s and was poured into water (100 mL). The mixture was extracted with CH_2Cl_2 (3 × 50 mL), the organic phase was washed with sat. Na_2CO_3 and water. The organic phase was dried over anhydride Na_2SO_4. After removal of Na_2SO_4, the filtrate was concentrated and the crude product was purified by column chromatography (silica gel, CH_2Cl_2/n-hexane: 2/3 V/V), re-crystallization from CH_2Cl_2/ethanol gave pure product. 5,10,15-triphenyl-20-(4-nitro)phenyl porphyrin (compound **6**): mp > 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) λmax/nm (log ε) 418 (2.28), 514 (1.28), 549 (2.98), 588 (4.85), 645 (4.18).

Table 2
Preparation of gold(III) substituted porphyrins (**8–12**).

Ligands	R^3	R^4	X	Gold(III) complex (yield, %)
2	H	CH_3	N^+I^-	90.1 (**8**)
3	$CO_2^-Na^+$	$CO_2^-Na^+$	C	61.3 (**9**)
4	H	$CO_2^-Na^+$	C	88.1 (**10**)
5	$SO_3^-Na^+$	$SO_3^-Na^+$	C	70.1 (**11**)
6	H	$NH_3^+Cl^-$	C	82.0 (**12**)

2.4. Procedure for the preparation gold(III) substituted porphyrins 8–12

5,10,15-Triphenyl-20-(iodide N-methyl-4-pyridiniumyl) porphyrinato gold(III) chloride **(8)**:A mixture of 5,10,15-triphenyl-20-(4-pyridinyl)porphyrin (compound **2**) (1.54 g, 2.5 mmol), $K[Au^{III}Cl_4]$ (2.82 g, 7.5 mmol) and sodium acetate (2.05 g, 25 mmol) in acetic acid (5 mL) was refluxed for 3 h. After completion of the reaction was checked by TLC, the crude product was obtained by removing acetic acid. Next the solid product was washed thoroughly with dichloromethane and water respectively. Then, the crude product was purified by column chromatography (silica gel, DCM/methanol: 5/1 V/V), the purified product was dissolved in acetone, after filtering the mixture solution was treated with LiCl in aqueous acetone, 5,10,15-triphenyl-20-(4-pyridinyl) porphyrinato gold(III) chloride was obtained. The resultant solution of 5,10,15-triphenyl-20-(4-pyridinyl)porphyrinato gold(III) chloride in chloroform (5 mL) was diluted with nitromethane (5 mL). To the mixture was added iodomethane (635 mg, 5.0 mmol), the reaction mixture was refluxed under nitrogen for 6 h. After solvents and excess iodomethane were removed by distillation, the residues were washed with chloroform (10 mL). The crude product was purified by re-crystallizing to give analytically pure gold(III) porphyrin compound **8** in 90.1% yield.

The data of compound **8** were given: mp 212–214 °C; ^{1}H NMR ($CDCl_3$, 600 MHz), δ(ppm): 4.85(s, 3H),7.86–7.94 (m, 9H, Ph-CH), 8.27 (d, J = 7.8 Hz, 6H, Ph-CH), 9.01 (s, 2H, Ph-CH), 9.42 (d, J = 4.2 Hz, 2H, Ph-CH), 9.32 (d, J = 5.4 Hz, 2H, Por-CH), 9.34 (d, J = 5.4 Hz, 2H, Por-CH), 9.72 (s, 2H, Por-CH), 9.79 (s, 2H, Por-CH); IR (KBr): υ 3393(s), 2924(m), 2853(w), 1637(w), 1460(w), 1359(w), 1317(w), 1261(w), 1078(w), 1033(w), 1021(w), 800(w), 755(w), 702(w) cm^{-1}; MS: 952 (M, 100%); UV-Vis (CH_2Cl_2) λmax/nm (log ε) 407 (3.86), 519 (4.66); Anal. calcd. for $C_{44}H_{30}ClIN_5Au$ (%):C, 53.49; H, 3.06; N, 7.09; Found: C, 53.20; H, 3.00; N, 6.88.

5,10,15,20-Tetra(sodium benzoate-4-yl)porphyrinato gold(III) chloride (**9**): A mixture of 5,10,15,20-tetra(4-methoxycarbonyl)phenylporphyrin (compound **3**) (2.12 g, 2.5 mmol), $K[Au^{III}Cl_4]$ (2.82 g, 7.5 mmol) and sodium acetate (2.05 g, 25 mmol) in acetic acid (5 mL) was refluxed for 3 h. After completion of the reaction was checked by TLC, the crude product was obtained by removing acetic acid. Next the solid product was washed thoroughly with dichloromethane and water

respectively. Then, the crude product was purified by column chromatography (silica gel, DCM/methanol: 5/1 V/V), the purified product was dissolved in DMF (15 mL). To the foregoing solution was added NaOH (0.60 g, 15 mmol), the resultant mixture was stirred at room temperature for 2 h. After the reaction mixture was diluted with water (35 mL), the mixture was extracted with CH_2Cl_2 (3×10 mL), then the water phase was neutralized with 1 M HCl to pH: 4. The acidic solution was extracted with EtOAc (3×15 mL) and the EtOAc phase was washed with water and brine, dried over anhydride Na_2SO_4. After removal of solvent, the residue was dissolved in methanol (5 mL) and the solution was neutralized carefully with 1 M NaOH to pH: 8. Solvents of above solution were removed by reduced pressure to give a crude product. The crude product was purified by re-crystallizing using methanol as solvent to give analytically pure gold(III) porphyrin compound **9** in 61.3% yield. Compound **9**: mp >250 °C; ^{1}H NMR (600 MHz, DMSO-d_6), δ (ppm): 8.40 (d, J=6.0 Hz, 8H, Ph-CH), 8.47 (s, 8H, Por-CH), 9.34 (d, J=8.4 Hz, 8H, Ph-CH); IR (KBr): υ 3432(m), 2924 (w), 2364(w), 1709(s), 1607(m), 1384(w), 1264(m), 1175(w), 1110(w), 1024(w), 869(w), 792(w), 708(w) cm^{-1}; MS: 1074.7 (M+1-Cl$^-$, 100%); UV–Vis (DMF) λmax/nm (log ε) 411 (3.62), 524 (4.85); Anal. calcd. for $C_{48}H_{24}ClN_4O_8Na_4Au$ (%): C, 51.98; H, 2.18; N, 5.05; Found: C, 51.83; H, 2.06; N, 4.86

5,10,15-Triphenyl-20-(sodium benzoate-4-yl)porphyrinato gold(III) chloride (**10**):

Using the procedure for the preparation of 5,10,15,20-tetra(sodium benzoate-4-yl)porphyrinato gold(III) chloride (**10**) with 5,10,15-triphenyl-20-(4-methoxycarbonyl)phenyl porphyrin (compound **4**) as a starting material to give 5,10,15-triphenyl-20-(sodium benzoate-4-yl) porphyrinato gold(III) chloride (**10**) in 88.1% yield. Compound **10**: mp >250 °C; ^{1}H NMR ($CDCl_3$, 600 MHz), δ (ppm): 7.84–7.90 (m, 9H, Ph-CH), 8.24 (d, J=6.0 Hz, 6H, Ph-CH), 8.51 (s, 2H, Ph-CH), 8.72 (d, J=6.0 Hz, 2H, Ph-CH), 9.13 (s, 2H, Por-CH), 9.25–9.28 (m, 6H, Por-CH); IR (KBr): υ 3448(s), 2922(m), 2853(w), 2371(w), 1713(w), 1632(m), 1384(m), 1260(w), 1089(w), 1028(w), 803(w), 706(w) cm^{-1}; MS: 875.7 (M+1-Cl$^-$, 100%); UV–Vis (CH_2Cl_2) λmax/nm (log ε) 412 (3.58), 523 (4.78); Anal. calcd. for $C_{45}H_{27}ClN_4O_2NaAu$ (%): C, 59.32; H, 2.99; N, 6.15; Found: C, 59.19; H, 2.78; N, 6.26.

5,10,15,20-Tetra(sodium benzenesulfonate-4-yl)porphyrinato gold(III) chloride **(11):** A mixture of 5,10,15,20-tetra(4-sulfo)phenylporphyrin (compound **5**) (2.34 g, 2.5 mmol), K[Au^{III}Cl$_4$] (2.82 g, 7.5 mmol) and NaOH (0.40 g, 10 mmol) in deionized water (10 mL) was refluxed for 12 h. After completion of the reaction was checked by TLC, the crude product was obtained by removing water. The product was purified by re-crystallizing using acetone as solvent to give analytically pure gold(III) porphyrin compound **11** in 70.1% yield. Compound **11**: mp >250 °C; ^{1}H NMR (600 MHz, D_2O), δ(ppm): 7.65 (d, J=6.0 Hz, 8H, Ph-CH), 8.22 (d, J=7.2 Hz, 8H, Ph-CH), 8.56 (s, 8H, Por-CH); IR (KBr): υ 3436(s), 2923(w), 1633(w), 1507(w), 1390(w), 1190(m), 1123(m), 1039(m), 1007(w), 810(w), 740(w), 640(w) cm^{-1}; MS: 1218.7 (M+1-Cl$^-$, 100%); UV-Vis (water) λmax/nm (log ε) 404 (2.79), 518 (4.80); Anal. calcd. for $C_{44}H_{24}ClN_4O_{12}S_4Na_4Au$ (%): C, 42.17; H, 1.93; N, 4.47; Found: C, 42.22; H, 1.75; N, 4.60

5,10,15-Triphenyl-20-(chloride anilinium-4-yl)porphyrinato gold(III) chloride (**12**): A mixture of 5,10,15-triphenyl-20-(4-nitrophenyl)porphyrin (compound **6**) (1.65 g, 2.5 mmol), K[Au^{III}Cl$_4$] (2.82 g, 7.5 mmol) and sodium acetate (2.05 g, 25 mmol) in acetic acid (5 mL) was refluxed for 5 h. After completion of the reaction was checked by TLC, the crude product was obtained by removing acetic acid. Next the solid product was washed thoroughly with dichloromethane and water respectively. Then, the crude product was purified by column chromatography (silica gel, DCM/methanol: 5/1 V/V) to give 5,10,15-Triphenyl-20-(4-nitrophenyl)porphyrinato gold(III) chloride. The resultant solution of 5,10,15-triphenyl-20-(4-nitrophenyl) porphyrinato gold(III) chloride, $SnCl_2$ (4.73 g, 25 mmol) and 36.5% HCl (5 mL) in dichloromethane (25 mL) was stirred at room temperature for 24 h. After the reaction mixture was diluted with dichloromethane (25 mL), the organic phase was washed water, sat. $NaHCO_3$ and brine respectively, dried over anhydride Na_2SO_4. Then, removal of solvent, the crude product was purified by column chromatography (silica gel, DCM/methanol: 5/1 V/V), the purified product was dissolved in acetone, after filtering the mixture solution was treated with LiCl in aqueous acetone, analytically pure gold(III) porphyrin compound **12** are obtained as chloride salts in 82.0% yield. Compound **12**: mp 180–181 °C; ^{1}H NMR ($CDCl_3$, 600 MHz), δ (ppm): 7.20 (d, J=8.4 Hz, 2H, Ph-CH), 7.87–7.92 (m, 9H, Ph-CH), 7.96 (d, J=7.8 Hz, 2H, Ph-CH), 8.22 (d, J=6.6 Hz, 6H, Ph-CH), 9.26–9.27 (m, 6H, Por-CH), 9.44 (d, J=5.6 Hz, 2H, Por-CH); IR (KBr): υ 3433(s), 2925 (w), 1603(m), 1492(w), 1442(w), 1383(w), 1360(w), 1247(w), 1180(w), 1082(w), 1031(m), 804(w), 758(w), 705(w) cm^{-1}; MS: 825.7 (M+1-Cl$^-$–HCl, 100%); UV–Vis (CH_2Cl_2) λmax/nm (log ε) 406 (3.50), 524 (4.78); Anal. calcd. for $C_{44}H_{30}Cl_2N_5Au$ (%): C, 58.94; H, 3.37; N, 7.81; Found: C, 58.82; H, 3.13; N, 7.98.

2.5. Cytotoxicity assay for Gold(III) substituted tetraarylporphyrin chloride on the effect of S180 and SGC-7901 human cell proliferation [37]

Gold(III) substituted tetraarylporphyrin chloride was prepared at the concentrations of 6.4×10^{-5} M, 3.2×10^{-5} M, 1.6×10^{-5} M, 8×10^{-6} M, 4×10^{-6} M and 2×10^{-6} M respectively. DMSO was used as latent solvent with the highest concentration less than 0.1% in solution of tetraarylporphyrin. The control groups of cisplatin, blank (1640) and DMSO solvent were set up at the same time. The cytotoxicity of gold(III) substituted tetraarylporphyrin chloride was determined by MTT cytotoxic assay [37]. S180 cells or SGC-7901 human gastric carcinoma cells were plated in 96-well plates at 1×10^5/mL and 100 μL/well in complete media. Then the prepared and various amounts of gold(III) substituted tetraarylporphyrin chloride and cisplatin were plated in 96-well plates contain S180 cells or SGC-7901 human gastric carcinoma cells and incubated commonly for 44 h. 100 μL supernatant liquid was sucked from each hole, 10 μL of MTT (5 mg/mL) was added in and cultured for 4 h, the media was then removed and add hydrochloric acid isopropanol at 100 μL/well. Surging for 5 min using the micro oscillator to dissolve MTT crystal and OD value was measured at 570 nm using a Model Elx 800 Autoplate reader (Bio-Tek Instruments, U.S.A.).

2.6. Statistical analysis of experimental data

All the data of the experiment were compiled and analysized according to SPSS 15.0 software. Measurement data were expressed as the mean ± S. D.

3. Results and discussion

The tetraarylporphyrins **1**, and **3** (Scheme 1, Table 1) were synthesized in 22–23% yield by treating the appropriately freshly distilled pyrrole with benzaldehyde or various substituted benzaldehyde in propionic acid utilizing the reported method [33,34].

Using the similar procedure, the mono-substituted tetraarylporphyrins **2**, and **4** were obtained in 6.5–9.0% yield by treating the freshly distilled pyrrole with benzaldehyde/various substituted benzaldehyde (3/1, mol/mol) in propionic acid.

Sulfphonation of tetraphenylporphyrin (**1**) with 100% H_2SO_4 at 120 °C for 4 h afforded tetrakis(4-sulfonatophenyl)porphyrin (**5**) (64.2%). Triphenyl(4-nitrophenyl) porphyrin (**6**) was readily obtained by reaction of the tetraphenylporphyrin (**1**) and sodium nitrite promoted by trifluoroacetic acid in 74.9% yield (Scheme 1, Table 1).

The general synthesis of water-soluble gold(III) porphyrin compounds (**8**–**12**) was achieved through the route outlined in Scheme 2. Firstly, gold(III) porphyrin compounds were synthesized by the treatment of K[Au^{III}Cl$_4$] with the free-base porphyrin ligand in the presence of NaOAc in acetic acid [20,30]. After purification with column chromatography and metathesis reaction with LiCl in aqueous acetone, gold(III)

Table 3
The water-solubility of Gold(III) substituted tetra-aryl porphyrin chloride.

Symbol	Compound	Water-solubility
7	TPPAuCl[16]	−
8	AuClTPPPy$^+$CH$_3$·I$^-$	++
9	ClAuTCPPNa	+++
10	ClAuTPPCO$_2$Na	++
11	ClAuTSPPNa	+++
12	ClAuTPPNH$_2$·HCl	++
Cis	Cisplatin	−

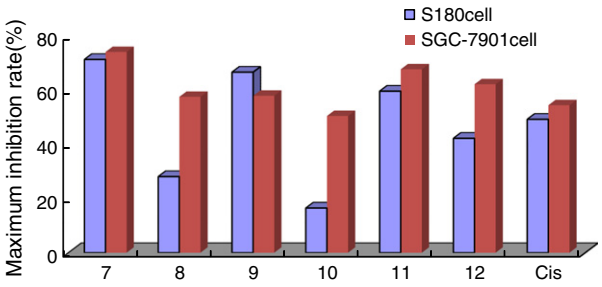


Fig. 1. Maximum inhibition rate of gold(III) substituted tetra-aryl porphyrin chloride against sarcoma 180 mouse (left bar) and SGC-7901 human (right bar) tumor cell line in vitro.

porphyrin compounds are obtained as chloride salts in 60–70% yields. Next, water-soluble gold(III) porphyrin compound **8** was obtained by treatment of gold(III) triphenylpyridinylporphrin with methyl iodide. Treatment of gold(III) tetrakis(4-sulfonatophenyl)porphyrin with NaOH gave water-soluble gold(III) porphyrin compound **11** [31]. Hydrolysis of gold(III) tetrakis(4-methoxycarbonylphenyl)porphyrin and gold(III) triphenyl (4-methoxycarbonylphenyl)porphyrin in the presence of NaOH gave water-soluble gold(III) porphyrin compound **9** and **10** respectively (Scheme 2, Table 2). Gold(III) triphenyl(4-nitrophenyl)porphyrin was reduced by $SnCl_2$ in the presence of 36% HCl to give gold(III) triphenyl(4-aminophenyl)porphyrin, which was treated with HCl to form water-soluble gold(III) porphyrin compound **12** (Scheme 2, Table 2) in 18% yield. Purification was achieved by flash chromatography on silica gel. ^{1}H NMR, electrospray ionization (ESI) mass (+ mode) analysis spectrometry, and elemental analysis were used to characterize all the synthesized compounds. The water-solubility for these synthesized substituted gold(III) porphyrin compounds **7**–**12** and cisplatin was tested (Table 3). The results illustrated that the compounds **9** and **11** with four ion groups possess exceptionally aqueous solubility. The compounds **8**, **10** and **12** with an ion group respectively have low aqueous solubility, but the TPPAuCl and Cisplatin don't dissolve very nearly in water.

To analyze the potential of the compounds **8**–**12** as anti-tumor agents, their cytotoxicity was evaluated (Table 4) towards the sarcoma 180 mouse and SGC-7901 human tumor and for comparison purposes the cytotoxicity of cisplatin and the TPPAuCl [20] was also evaluated under the same experimental conditions. Because of low aqueous solubility, the test compounds such as **7**, **8**, **10**, **12** and **cis** were dissolved in DMSO first and then serially diluted in complete culture medium such that the effective DMSO content did not exceed 1%.

The results showed that compounds **9** and **11** exerted significant inhibitory effect on the growth of S180 and SGC-7901, and the dose-dependent relationship was found between 1 and 32 μM in general, while IC_{50} values of **9** was less than 25 μM in S180 cell line, and IC_{50} values of **11** was less than 50 μM in S180 and SGC-7901 cell lines. **8**, **10** and **12** had weaker inhibitory effect on S180 cells, IC_{50} value was more than 50 μM in S180 cell line, while IC_{50} values of **8**, **9** and **12** were less than 50 μM in SGC-7901 cell line, compound **12** exhibits an IC_{50} value nearly two-fold lower than cisplatin on one cell line (cell line SGC-7901) (Table 4, Figs. 1 and 2). The results showed that aqueous ion groups could balance the aqueous solubility with liposolubility, which was helpful to transport drugs into target cells to improve the anti-tumor activity of complexes. From the results of in vivo assays, the anti-tumor activity order was **7**>**9**>cis>**11**>**12**>**8**>**10** in S180 cell line and **7**>**12**>**9**>**8**>cis>**11**>**10** in SGC-7901.

As presented in Table 4, compounds **7**, **9** and **11** exhibited significant cytotoxic activity against sarcoma 180 mouse tumor cell line, confirming the importance of water-soluble groups on the aromatic ring for gold(III) substituted tetraphenylporphyrin complexes to cytotoxicity in the condition of clinical use. Comparison of the activity of **8**, **10** and **12** suggests that just one water-soluble group including acidic anion and ammonium or pyridinium salt of gold(III) substituted tetraphenylporphyrin complexs decreases this activity. In addition, a carboxylic acid anion group plays an important role in the anti-tumor activity of these compounds. Sarcoma 180 mouse tumor cells seemed more sensitive to compounds with symmetrical water-soluble groups on the gold(III) substituted tetraphenylporphyrin complexs in the para-position, as shown in compounds **7**, **9** and **11**. A single *para*-substituted analog had lower anti-tumor potency compared with these of the other same water-soluble group compounds. But SGC-7901 human gastric carcinoma cell line (SGC-7901) seemed more sensitive to compounds with cation water-soluble groups on the gold(III) substituted tetraphenylporphyrin complexes in the para-position, as shown in compounds **8** and **12**. These results suggested that symmetrical water-soluble groups and cation water-soluble groups on the gold(III) substituted tetraphenylporphyrin complexs may be crucial for anti-tumor activity.

To compare the potential of the compound ClAuTCPPNa (**9**) and its ligand TCPPNa as anti-tumor agents, their cytotoxicity was evaluated towards the sarcoma 180 mouse and SGC-7901 human tumor under the same experimental conditions. Compound ligand TCPPNa exhibited a little growth inhibitory properties against sarcoma 180 mouse tumor and SGC-7901 human gastric cancer cell examined, and afforded IC_{50} values = 79 μM for 43.22% and 91 μM for 40.28% respectively. The result showed that the anti-tumor activity of ligand TCPPNa is lower than its gold(III) complex.

4. Conclusion

In summary, a number of new substituted gold(III) tetraarylporphyrin with aqueous solubility were synthesized and evaluated for their in vitro

Table 4
Cytotoxicity of gold(III) substituted tetra-aryl porphyrin chloride against sarcoma 180 mouse and SGC-7901 human tumor cell line in vitro.

Symbol	Compound	Maximum inhibition rate (%)*		IC_{50} (μM)	
		S180cell	SGC-7901cell	S180cell	SGC-7901cell
7	TPPAuCl[16]	71.32	74.09	15.39 ± 0.20	3.19 ± 0.52
8	AuClTPPPy$^+$CH$_3$I$^-$	28.11	57.56	183.58 ± 3.16	38.86 ± 1.31
9	ClAuTCPPNa	66.63	57.94	23.40 ± 0.37	38.79 ± 0.86
10	ClAuTPPCOONa	16.46	50.47	389.27 ± 0.98	106.21 ± 1.36
11	TSPPAuCl	59.65	67.68	34.83 ± 0.26	59.13 ± 0.39
12	ClAuTPPNH$_2$·HCl	42.21	62.22	56.23 ± 0.68	23.27 ± 0.98
Cis	Cisplatin	49.24	54.45	27.70 ± 0.26	41.69 ± 0.37

* The concentration of the gold complexes is 32 μM.

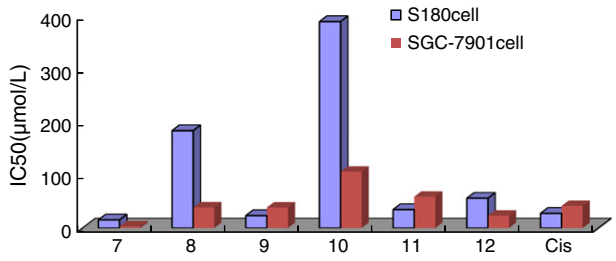


Fig. 2. IC_{50} of gold(III) substituted tetra-aryl porphyrin chloride against sarcoma 180 mouse (left bar) and SGC-7901 human (right bar) tumor cell line in vitro.

anti-tumor activity and the three new substituted gold(III) tetraarylporphyrin (**9**, **11**, **12**) were more active than cisplatin and were close to lead compound gold(III) tetraphenylporphyrin (**7**). The result showed that water-solubility increased significantly while the anti tumor activity maintained well. Especially ClAuTPPNa with four carboxylates substituted groups and $ClAuTPPNH_2 \cdot HCl$ have great potential for anti-tumor drugs, further in vivo tests of the compounds are under way and the anti-tumor mechanism of which would be studied in the future.

5. Abbreviations

TLC	Thin Layer Chromatography
MS	Mass Spectroscopy
IR	Infrared Spectroscopy
NMR	Nuclear Magnetic Resonance
UV-Vis	Ultraviolet-visible Spectroscopy
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
DCM	Dichloromethane
DMF	Dimethylformamide N,N-Dimethylformamide
DMSO	Dimethyl sulfoxide
TPP	5,10,15,20-tetraphenylporphyrin
TCPP	5,10,15,20-tetra(4-carboxy)phenylporphyrin
TSPP	5,10,15,20-tetra(4-sulfo)phenylporphyrin
Cis	*cis*-diamminedichloroplatinum(II); cisplatin

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