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Review

The anti-cancer properties of gold(III) compounds with dianionic porphyrin and tetradentate ligands

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ABSTRACT

Gold(III) compounds have long been investigated for anti-cancer treatment. However, clinical use has often been hampered by their poor stability in solution. A reduction of gold(III) to gold(I) is usually the case for most cytotoxic gold(III) compounds. Previously, we made use of planar tetradentate dianionic ligands, including porphyrin, Schiff base and bis(pyridyl)carboxamide ligands, to prepare a series of monocationic gold(III) compounds. These gold(III) compounds behave as organic lipophilic cations with a planar structure, are stable under physiological conditions, and possess certain promising anti-cancer activities. In this review, the synthesis, stability, and anti-cancer properties of these gold(III) compounds are presented. We shall detail the *in vitro* and *in vivo* anti-cancer efficacy, as well as the anti-cancer mechanisms of the most potent gold(III) porphyrin compound [Au^{III}(TPP)]Cl (**1a**).

1. Introduction

Gold is known to exist in oxidation states ranging from −I to +V. Apart from the elemental and colloidal forms of gold(0), gold(I) and gold(III) are the most common forms of gold compounds to exist in aqueous media. As far back as 3500 years ago, elemental gold was used to ward off disease or "evil spirit" [1]. In the past century, the medicinal activities of gold compounds have gradually come to be uncovered, starting with the discovery of $K[Au^{I}(CN)_2]$ for the treatment of tuberculosis by Koch in 1890 [2]. In addition, gold(I) compounds have long been used as medicinal agents for the treatment of rheumatoid arthritis [1,3,4]. Forestier first demonstrated the anti-arthritic properties of gold(I) compounds in 1935 [5], and this finding subsequently triggered the discovery of clinically useful gold(I) thiolate compounds including triethylphosphine(2,3,4,6-tetra-*O*-

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acetyl-β-1-D-thiopyranosato-*S*)gold(I) (Auranofin), sodium gold(I) thiomalate (Myochrysin), and gold(I) thioglucose (Solgonal) for the treatment of rheumatoid arthritis [1,6–8]. Lorber and co-workers in 1979 reported that auranofin could inhibit the *in vitro* proliferation of HeLa cells, which subsequently triggered extensive interests in search for novel gold compounds as potential anti-cancer treatments [9]. A variety of auranofin analogues and phosphinogold(I) compounds containing S-donor ligands have been developed and shown to possess potent cytotoxic activities [1,10,11]. Berners-Price and co-workers recently demonstrated that a series of gold(I) compounds with carbene ligands, which could be regarded as a class of lipophilic cations, induce apoptotic cell death *via* interacting with mitochondrial components [12,13]. Among the reported cytotoxic gold(I) compounds, bis(diphosphino)gold(I) compounds have been demonstrated to have promising *in vivo* anti-cancer activities [14]. However, these compounds also exhibited severe cardiotoxicity in animal studies, rendering them unfavorable for further pre-clinical and clinical evaluations [1,15].

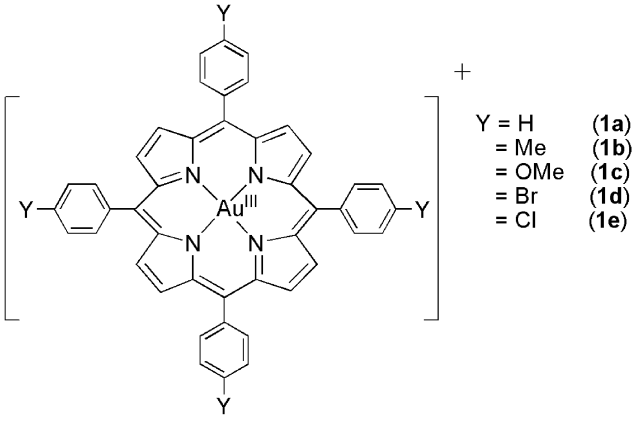
Besides gold(I), gold(III) compounds have long been studied for anti-cancer treatment. Gold(III) ion forms square-planar compounds, and some of which can be viewed as structural analogues of the metallointercalator $[Pt(terpy)Cl]^+$ (where terpy = 2,2′:6′,2″-terpyridine) [1,16–20]. As early as the 1970s, gold(III) compounds were thought to interact with bio-molecules such as DNA and proteins in a manner similar to that of cisplatin. Bordignon and co-workers demonstrated the oxidation of the amino acid methionine by $HAuCl_4$ in 1973 and again in 1976 [21,22]. By means of UV/Vis spectrophotometry and NMR spectroscopy, Isab and Sadler demonstrated the interaction of ribonuclease A and methionine with gold ions in aqueous solutions [23]. In 1980, Shaw et al. first reported the oxidation of disulfides by gold(III) compounds in aqueous media [24]. *In vitro* studies by Mirabelli and co-workers revealed that gold(III) ions could induce DNA strand breaks [25]. Hollis and Lippard [26] and Che and co-workers [27] proposed and demonstrated, respectively, that gold(III) compounds with tridentate terpy ligands (where terpy = 2,2′:6′,2″-terpyridine) could bind DNA via intercalation. Recent reports revealed that gold(III) compounds can bind to or interact with proteins and small peptides including human serum albumin (HSA), thioredoxin reductase (TrxR) and gluthatione (GSH) *in vitro* [28–32].

In the early 1980s, Sadler et al. reported that dimethylgold(III) compounds possess modest anti-cancer activities with a 20% increase in life span (ILS) of mice bearing P388 leukemia [33]. This report was subsequently followed by the discovery of a variety of gold(III) compounds containing monodentate, bidentate, or tridentate ligand(s) exhibiting favorable anti-cancer properties comparable to that of cisplatin. Selected examples of these compounds are discussed below. In 1996, Parish, Buckley, Elsome and co-workers reported a series of cytotoxic gold(III) compounds with tridentate damp ligands (where damp = 2-[(dimethylamino)methyl]phenyl) [34]. Among these compounds, $[Au(acetate)_2(damp)]$ and [Au(malonato)(damp)] are moderately active *in vivo* against human carcinoma xenografts [35]. A series of cytotoxic gold(III) compounds with monodentate [e.g., trichloro(2-pyridylmethanol)gold(III)], bidentate [e.g., dichloro(*N*-methylsalycilaldiminate)gold(III)], and tridentate ligands {e.g., $[Au(terpy)Cl]Cl_2$} [36–41] have been developed by Orioli, Messori and co-workers since 1997. More recently, Messori and co-workers developed a novel class of gold(III) compounds with tridentate bipyridyl ligands, which are stable in the absence of ascorbate and GSH [42–44]. These workers also identified a series of cytotoxic dinuclear gold(III) oxo compounds having a common Au_2O_2 motif [45,46]. Che and co-workers recently prepared 20 examples of cyclometalated gold(III) compounds $[Au_m(C\tilde{N}\hat{C})_mL]^{n+}$ (where m = 1–3; n = 0–3; HC$\tilde{N}\hat{C}$H = 2,6-diphenylpyridine) [47]. These compounds are stable in solution, and some exhibit potent cytotoxicity and induce apoptosis in nasopharyngeal cancer cells, with IC_{50} values as low as ~50 nM. A number of bi- and tri-nuclear anti-cancer gold(III) compounds containing $[Au(C\tilde{N}\hat{C})]^+$ motifs connected by bridging phosphine ligands were prepared by Che and co-workers. Guo and co-workers reported that some gold(III) compounds with 1,4,7-triazacyclononane and aminoquinoline ligands are highly cytotoxic [48,49]. Gold(III) dithiocarbamate compounds were found by Fregona and co-workers to trigger both apoptotic and necrotic cell death, to induce ROS generation, and to make cytosolic and mitochondrial thioredoxin reductase inactive [50–53]. Notably, treatment of MDA-MB-231 breast tumor-bearing mice with dibromo(*N*,*N*-dimethyldithiocarbamato-$\kappa S,\kappa S'$)gold(III) resulted in significant inhibition of tumor growth, associated with proteasome inhibition and massive apoptosis induction *in vivo* [54].

Unlike platinum(II) compounds, the development of gold(III) compounds as clinically useful anti-cancer therapeutics has been hampered by the relatively poor stability of gold(III) compounds in solution [1]. This instability is believed to eventually lead to the gold(I)-associated toxicity *in vivo* [15]. In the literature, reduction of gold(III) to gold(I), and/or covalent binding of gold ions to bio-molecular target(s) have been proposed to account for the cytotoxic actions of gold(III) compounds. Gold(III) compounds with strongly chelating tetradentate ligands have previously been conceived to not be active towards cancer cells, presumably due to the over-stabilization of gold(III) ions against reduction and demetalation. This proposition received support from a report of a gold(III) compound of cyclam (where cyclam = 1,4,8,11-tetraazacyclotetradecane) with low cytotoxicity towards a series of cancer cells, having IC_{50} values of >100 μM [40]. Consequently, although tetradentate ligands have widely been used to stabilize electrophilic and/or oxidizing metal ions, the cytotoxicity of most gold(III) compounds with tetradentate ligands had remained an unexplored area prior to our report on the promising anti-cancer activities of gold(III) porphyrin compounds in 2003 [55].

We hypothesized that gold(III) ions would form lipophilic planar cations in coordination with tetradentate dianionic ligands. In the literature, planar lipophilic cations have been suggested as potential anti-cancer drug candidates [56]. The anti-cancer properties of planar aromatic organic terephthalanilide and its derivatives have had a long history [57]. Rhodamine 123 (Rh123) and dequalinium chloride (DECA) can be viewed as examples of this class of organic cations and have been subjected to substantial *in vitro* and *in vivo* studies [57], and notably, MKT-077, a water-soluble rhodacyanine, is the first lipophilic cation which had been advanced to clinical trials [58]. The synthesis and structural modification of planar π-conjugated organic cations can be a formidable undertaking. Recently, Berners-Price and co-workers studied $[Au^I(dppe)_2]^+$ [where dppe = 1,2-bis(diphenylphosphino)ethane] and its derivatives as lipophilic cations and found that these complex cations exhibited potent *in vitro* and *in vivo* anti-cancer activities via the mitochondrial-mediated apoptotic pathway [12,13].

Coordination of tetradentate dianionic ligand with gold(III) gives mono-cationic gold(III) compounds having a planar geometry. We hypothesized that gold(III) compounds with porphyrin, tetradentate Schiff base and bis(pyridyl)carboxamide ligands would behave similarly to lipophilic organic cations. In this review article, we summarize our observations on the synthesis, stability analysis and anti-cancer activities of gold(III) compounds containing dianionic tetradentate ligands. The *in vivo* anti-cancer activity and molecular mechanism for the anti-cancer action of a physiologically stable gold(III) *meso*-tetraphenylporphyrin compound ($[Au^{III}(TPP)]Cl$, **1a**) are presented.

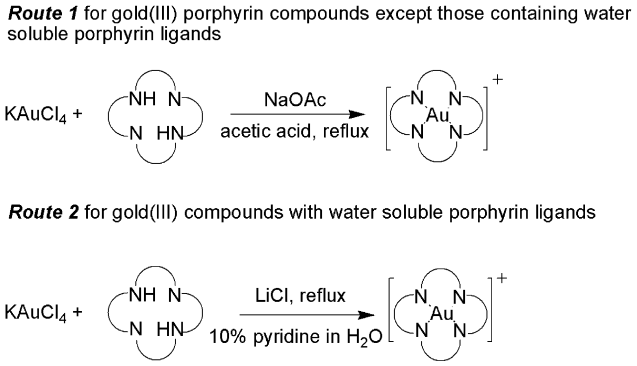


Scheme 1. Schematic drawings of gold(III) porphyrin compounds.

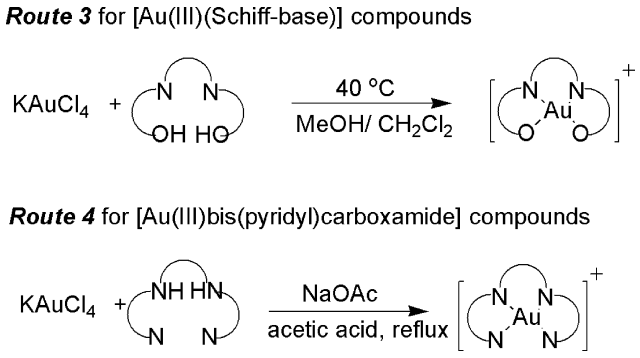
2. Gold(III) compounds with tetradentate dianionic ligands

2.1. Synthesis

Gold(III) compounds with porphyrin [55,59–65], Schiff base [66–68] and bis(pyridyl)carboxamide [67,69] ligands are well documented in the literature, and the groups of Fleischer, Jurisson and Che have contributed to the synthesis of these gold(III) compounds. Gold(III) porphyrin compounds (**1a–e**, Scheme 1) can be prepared by the treatment of $K[Au^{III}Cl_4]$ or $nBu_4N[Au^{III}Cl_4]$ with the free-base porphyrin ligand in the presence of NaOAc in acetic acid (Scheme 2, route 1) [55,59]. After purification with column chromatography and metathesis reaction with LiCl in aqueous acetone, analytically pure gold(III) porphyrin compounds are obtained as chloride



Scheme 2. Synthetic routes for the gold(III) porphyrin compounds.



Scheme 3. Synthetic routes for the gold(III) compounds of Schiff-base (*route 3*) and bis(pyridyl)carboxamide (*route 4*) ligands.

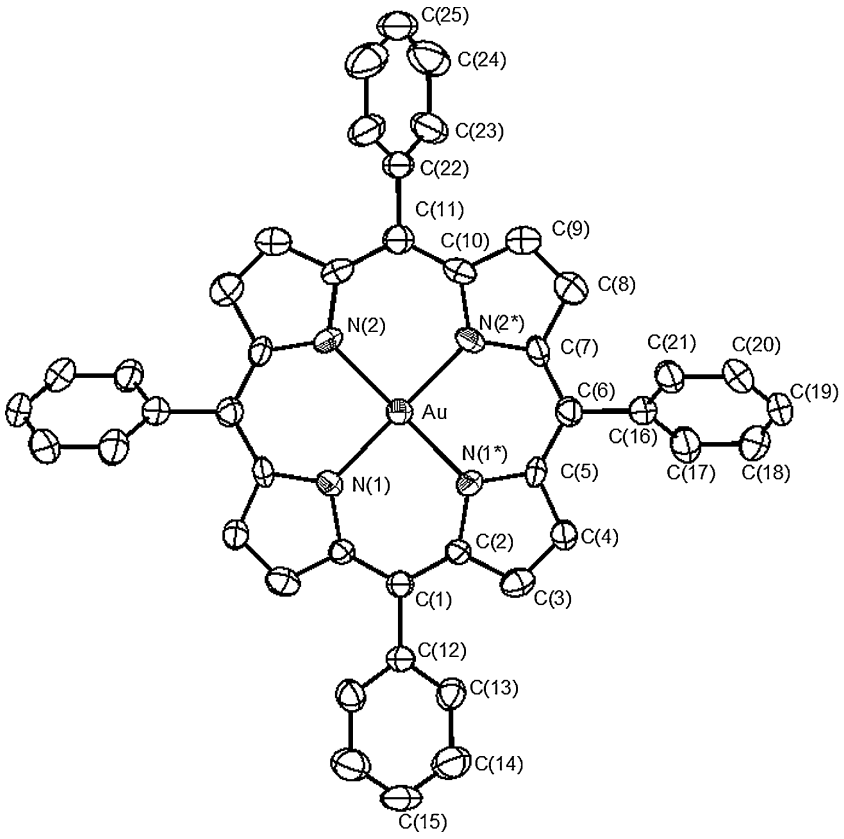


Fig. 1. ORTEP drawing of $[Au^{III}(TPP)]^+$ with atom-numbering scheme. Hydrogen atoms and the perchlorate ion are omitted for clarity. Thermal ellipsoids are drawn at 30% probability level [Ref. [55]].

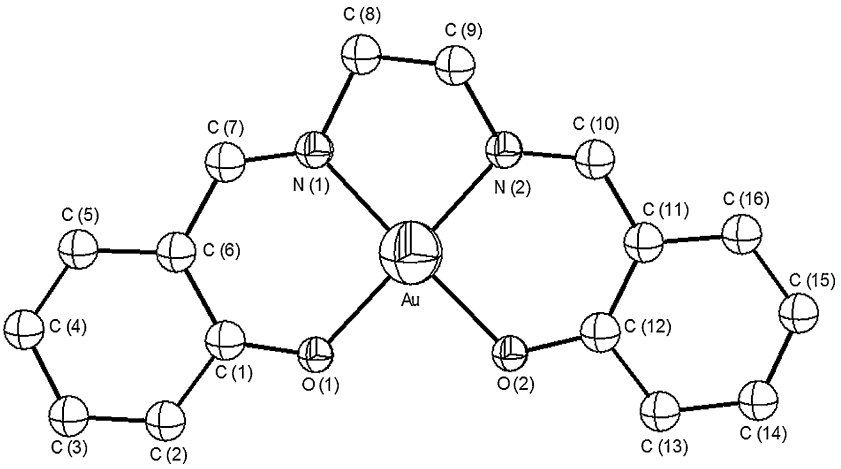


Fig. 2. The ORTEP drawing of $[Au^{III}(salen)]^+$ with thermal ellipsoids at 30% probability level [Ref. [70]].

salts in 60–70% yields. For water-soluble gold(III) porphyrin compounds such as $[Au(TMPyP)Cl_4]Cl$ (**1f**, where $[H_2TMPyP]^{4+}$ = *meso*-tetrakis(*N*-methyl-4-pyridyl)porphyrin) and $Na_4[Au(TPPS)Cl]$ (**1g**, where $[H_2TPPS]^{4-}$ = *meso*-tetrakis(4-sulfonatophenyl)porphyrin), a reported procedure by Harriman and co-workers can be used and these two compounds obtained using 10% aqueous pyridine as the solvent (Scheme 2, route 2) [65].

Gold(III) compounds with tetradentate Schiff base ligands, including salicylideneimines and dimethylglyoxine, have been reported [66,68]. Treatment of the free Schiff-base ligands with $K[Au^{III}Cl_4]$ in a CH_2Cl_2/MeOH mixture affords the gold(III) Schiff-base compounds in ~60% yields (Scheme 3, route 3). Reports of gold(III) compounds with tetradentate bis(pyridyl)carboxamide ligands are relatively sparse in the literature [66–68]. Reaction of $K[Au^{III}Cl_4]$ with bis(pyridyl)carboxamide ligands in the presence of NaOAc in acetic acid or in methanol gave gold(III) bis(pyridyl)carboxamide compounds isolated as chloride salts (Scheme 3, route 4) [67,69].

2.2. X-ray crystal structures

The X-ray crystal structures of several gold(III) compounds with dianionic tetradentate ligands have been reported in the literature [55,60,66]. Previously we have also established the structures of $[Au(TPP)]^+$ (**1a**), $[Au(salen)]^+$ (**2**, where H_2salen = N,N-ethylenebis (salicylideneimine)) and $[Au(dcbpb)]^+$ (**3a**, where H_2dcbpb = 4,5-dichloro-1,2-bis(2-(4-*tert*-butylpyridine)carboxamido)benzene) by X-ray crystallography [70], and their perspective views are depicted in Figs. 1–3, respectively. Fig. 4 depicts all the bond distances and angles associated with the gold atom in the compounds **1a**, **2** and **3a**. For **1a**, its structure features a nearly perfect square-planar geometry with almost four identical N–Au–N bond angles (89.8–90.1°) and Au–N bond lengths (2.032–2.033 Å) (Fig. 5). The robust dianionic porphyrin ligand [Por] is a strong chelator and provides a rigid scaffold, thus rendering $[Au(Por)]^+$ stable against demetalation and reduction under physiological conditions.

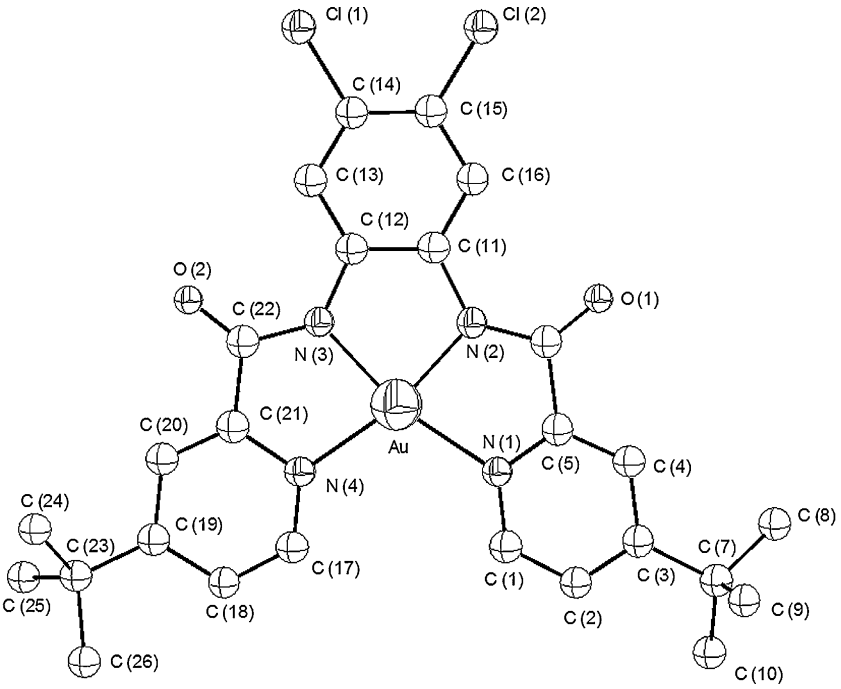


Fig. 3. The ORTEP drawing of $[Au^{III}(dcbpb)]^+$ with thermal ellipsoids at 30% probability level [Ref. [70]].

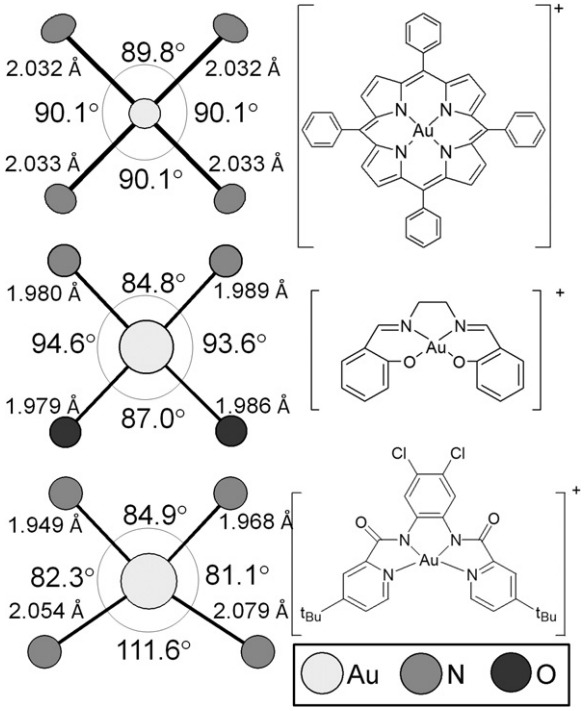


Fig. 4. Bond distances and angles associated with the gold atom in the compounds **1a**, **2** and **3a**.

Unlike the gold(III) porphyrin compounds, the gold(III) ions in both **2** and **3a** have a distorted planar geometry, with the bond angles being 84.8–94.6° and 81.1–111.6°, respectively (Fig. 4). The distortion of the bond angles from 90° is attributed to the bite angles of the coordinated chelating ligands (especially **3a**), which cannot accommodate gold(III) ion in a perfectly square geometry.

2.3. Electrochemistry

The redox properties of the gold(III) porphyrin compounds in non-aqueous medium (0.1 M nBu$_4$NPF$_6$ in CH$_3$CN) were studied by cyclic voltammetry with glassy carbon and platinum as the working and counter electrodes, respectively. The cyclic voltammogram of **1a** is characterized by the reversible reduction waves at E° = −1.34 V and −1.97 V *vs.* NHE (Fig. 6) [55]. The highly reversible redox couples reveals that no demetalation reaction of gold(III) porphyrin takes place upon electrochemical reduction. The electrochemical data of the other gold(III) porphyrin compounds are listed in Table 1. Both

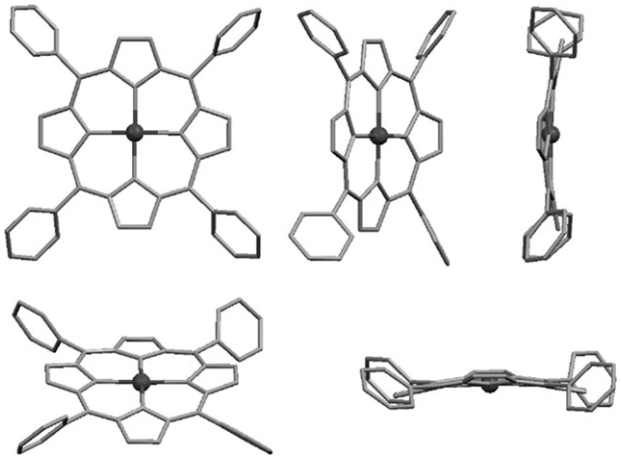


Fig. 5. Different perspective views of the crystal structure of **1a** [Ref. [77]].

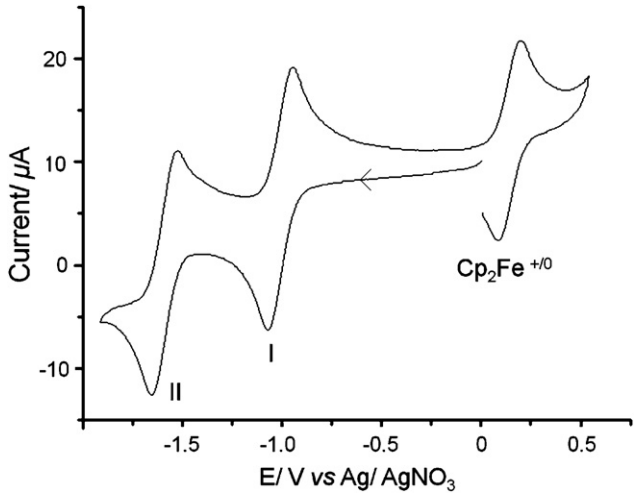


Fig. 6. Cyclic voltammogram of **1a** in CH$_3$CN [V *vs.* 0.1 M Ag/AgNO$_3$ in CH$_3$CN] at a scan rate of 100 mV s^{-1}.

of these two reduction couples show modest sensitivity (±40 mV) to the *para*-substituent (MeO, Me, H, Br and Cl) of the *meso*-aryl groups. Electron-donating groups (MeO and Me) shift the reduction potentials negatively, and inversely with electron-withdrawing substituents (Br and Cl). Compared to the large E° value (1.36 V) for the reduction of free gold(III) ion to gold(I) ion, the coordination of gold(III) ion with a porphyrinato ligand markedly stabilizes the gold(III) ion against reduction, as manifested by the large and negative reduction potentials of [Au(Por)]$^{+/0}$ couple.

The nature of the two reduction waves for gold(III) porphyrins remains a matter of controversy. With reference to previous work by Jamin and Iwamoto on the electrochemistry of [Au^{III}(TPP)]Cl (**1a**), the reduction waves are assigned as coming from a stepwise reduction of the porphyrin ring leading to π-anion radicals [61]. Similar conclusions were also reached by Harriman and co-workers, based on their electrochemical studies of [Au^{III}(TMPyP)]Cl$_5$ and Na$_4$[Au^{III}(TPPS)]Cl in aqueous medium [65]. Recently Kadish et al. reported that [*meso*-tetrakis(3,5-di-*tert*-butylphenyl)porphyrinato]gold(III) hexafluorophosphate underwent reduction to give a gold(II) porphyrin based on the results of spectroelectrochemical and electron spin resonance studies [63].

The electrochemical properties of the non-porphyrin gold(III) complexes **2** and **3a** have also been examined by cyclic voltammetry. Both **2** and **3a** undergo irreversible reduction at −0.41 V and −0.39 V *vs.* Cp$_2$Fe$^{+/0}$, respectively. The electrochemical reaction likely involves a reduction of gold(III) to gold(0), as indicated by the deposition of gold metal at the surface of the platinum electrode [70].

2.4. Stability in solution

Gold(III) porphyrin compounds (**1a**–**g**) are stable in dimethyl sulfoxide (DMSO) and acetonitrile (CH$_3$CN) as well as in phosphate-

Table 1
Reduction potentials of [Au^{III}(*para*-Y-TPP)]Cl in CH$_3$CN [V *vs.* NHE] at a scan rate of 100 mV s^{-1}).

Compound	Y	Couple I[a]/V	Couple II[a]/V
1e	Cl	−1.36	−1.84
1d	Br	−1.37	−1.85
1a	H	−1.40	−1.88
1b	Me	−1.41	−1.89
1c	MeO	−1.42	−1.91

[a] Couple I and couple II are the first and the second reduction couples of [Au^{III}(*para*-Y-TPP)]Cl, respectively.

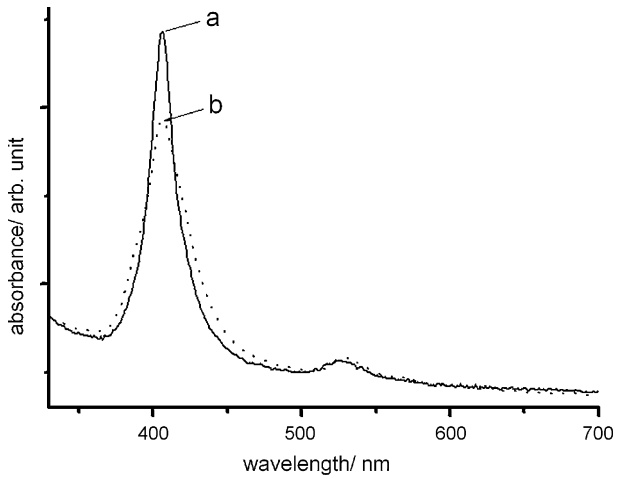


Fig. 7. UV–visible absorption spectra of **1a** in GSH (2 mM) TBS/CH_3CN (19:1) at (a) time = 0 h and (b) time = 48 h [Ref. [55]].

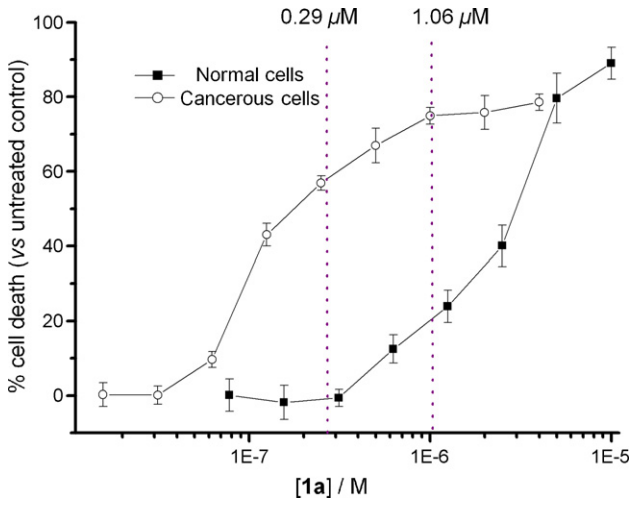


Fig. 8. Cytotoxic profiles of **1a** toward nasopharyngeal carcinoma (SUNE1) and normal peripheral blood mononuclear cells (PBMCs) [Ref. [72]].

buffered saline (PBS) and Tris-buffered saline (TBS) containing 5% DMSO [55]. By UV–visible spectrophotometry, there was a less than 5% change in absorbance of the solution of each of these gold(III) compounds upon standing for 48 h. The solution stability of **1a** in the presence of the biological reductant glutathione (GSH) was also examined. No significant spectroscopic shift and formation of new absorption bands, including that of the free-base porphyrin (H_2TPP, λ_{max} = 514, 548, 590 and 640 nm) were found, and thus demetalation was excluded (Fig. 7). The **1a**/GSH mixture in D_2O/CD_3CN mixture (9:1 v/v) was also examined by ^{1}H NMR spectroscopy. No significant spectroscopic change was observed over a period of 48 h at room temperature, revealing that reduction of gold(III) to gold(I) or gold(0) by GSH is untenable under this condition. Similarly, the water-soluble gold(III) porphyrin **1f** was also stable in GSH solution, as its UV–visible absorption spectrum did not exhibit any significant changes over 48 h.

Unlike the gold(III) porphyrins, compounds **2** and **3a** in TBS/CH_3CN (19:1) solutions showed approximately a 10% decrease in absorbance at 345 and 402 nm, respectively, after standing for 4 h [67]. The addition of glutathione to solutions of **2** and **3a** in TBS/CH_3CN (19:1) resulted in spontaneous spectroscopic changes with dramatic decrease in absorbance at λ_{max} and concomitant band broadening. FAB-MS analysis of the precipitates revealed molecular ions of the free H_2salen (m/z = 267) for **2** and H_2dcbpb (m/z = 498) for **3a**. And yet, ESI-MS analysis of the remaining solutions did not reveal the molecular ions of **2** (m/z = 463) and **3a** (m/z = 694). These observations suggest that both **2** and **3** in solutions undergo extensive demetalation and reduction of gold(III) upon treatment with GSH.

2.5. *In vitro anti-cancer properties*

By means of MTT assay [71], **1a** displayed promising anti-cancer activities toward a panel of human cancer cell lines including nasopharyngeal carcinoma (SUNE1, CNE1, CNE2 and C666-1), promyelocytic leukemia (HL-60), hepatocellular carcinoma (HepG2), cervical epithelioid carcinoma (HeLa) and oral epidermoid carcinoma (KB-3-1 and KB-V1) [55,72,73]. Its IC_{50} values (concentration causing 50% inhibition of cellular growth) ranged between 0.11 and 0.73 μM. Importantly, **1a** is equally cytotoxic to cisplatin-resistant nasopharyngeal cancer cell lines CNE1, CNE2 and C666-1 with IC_{50} values being 0.17, 0.14 and 0.11 μM, which correspond to ca. 240-, 640- and 1680-fold higher potency than cisplatin, respectively.

Compound **1a** also shows significant cytotoxicity against KB-3-1 and its multi-drug resistant (KB-V1) variant. The latter possesses a high level of membrane P-glycoproteins, which exclude drugs such as vinblastine and doxorubincin [74]. The lack of cross-resistance to **1a** suggests that the P-glycoproteins are ineffective towards **1a**.

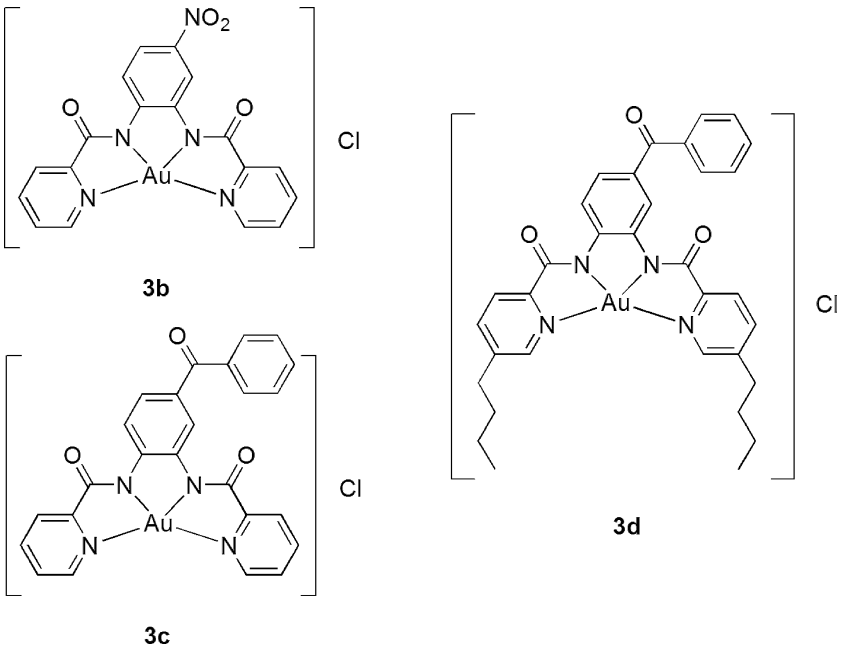
To investigate the cytotoxic effect of **1a** on normal/non-cancerous cells, the cytotoxicity of **1a** toward peripheral blood mononuclear cells (PBMCs) from healthy individuals and CCD-19Lu cells (a fibroblast cell line derived from normal lung) were examined. Results by MTT assay revealed that **1a** exhibits at least 10-fold higher cytotoxicity to cancer cells than normal cells (Fig. 8), suggesting a reasonable safety concentration window of 0.1–1 μM for **1a** to efficiently kill tumor cells while preserving the survival of most of the normal or non-cancerous cells.

The presence of gold(III) ions is critical for the observed *in vitro* anti-cancer properties, since [Zn^{II}(TPP)] is at least 100-fold less cytotoxic (IC_{50} > 50 μM) than [Au^{III}(TPP)]Cl under the same conditions. Yet the porphyrin ligand is essential for the anti-cancer activities, as $KAuCl_4$ and nBu$_4$N[$Au^{III}Cl_4$] are *ca.* 30–90 times less effective than **1a** in killing cancer cells. The gold(III) ion is unstable under physiological conditions as it undergoes reduction to colloidal gold. The porphyrin ligand stabilizes the gold(III) ion and we propose that [Au(TPP)]$^+$ acts as a stable lipophilic planar cation for binding to bio-molecular target(s) through non-covalent interactions.

The anti-cancer activities of certain metal-based drugs, including cisplatin, are diminished in the presence of serum proteins [75,76]. We found that the cytotoxicity of cisplatin was significantly reduced by increasing the concentration of fetal bovine serum (FBS) from 0 to 10% in the solution, while the cytotoxicity of **1a** was unaffected [77]. The difference in plasma protein binding may have a profound effect on both the disposition and activity of metal-based drugs, especially cisplatin, which can covalently bind serum proteins. This might be one of the reasons to account for the higher cytotoxic activity of **1a** than cisplatin *in vitro*.

Since porphyrin and its metal compounds have long been sought for use in photodynamic therapy (PDT) [78,79], the cytotoxicity of **1a** in the presence of light was examined. We found that the cytotoxicity of **1a** did not change under exposure to different doses of light, while the cytotoxicity of hypericin, a known PDT agent, was enhanced with an increasing dose of light exposure. This result indicated that the cytotoxic effect of gold(III) porphyrin **1a** does not correlate with photosensitizing activity.

A comparison of the IC_{50} values of a series of [Au^{III}(*para*-Y-TPP)]Cl (Y = H, Me, OMe, Br and Cl, **1a**–**e**, respectively) compounds showed the para-substituent of the *meso*-phenyl rings has little



Scheme 4. Schematic drawings of the gold(III) compounds containing bis(pyridyl)carboxamide ligands.

effect on the cytotoxicity [55]. Nevertheless, gold(III) porphyrin compounds containing polar ionizable functional groups such as [Au(TMPyP)]Cl_5 (**1f**) and [Na_4Au^{III}(TPPS)]Cl (**1g**) are relatively non-cytotoxic (IC_{50} > 50 μM) [70]. This may be attributed to the polar and hydrophilic nature of the compounds, which induces them to interact differently with biomolecules within cells as compared to other cytotoxic [Au^{III}(*para*-Y-TPP)]$^+$ compounds.

The cytotoxic properties of gold(III) compounds containing Schiff base (**2**) and bis(pyridyl)carboxamide (**3a**) ligands were also evaluated by MTT assay, revealing that they exhibit comparable cytotoxicity to cisplatin (10–30 μM) [70]. Compounds **2** and **3a** show similar potency to the following cytotoxic gold(III) compounds reported in the literature: [$Au^{III}(en)_2$]Cl_3 where en = 1,2-ethylenediamine [IC_{50} (human ovarian carcinoma A2780, 48 h) = 8.4 μM] [40]; [Au^{III}(esal)]Cl_2 where esal = *N*-ethylsalicylaldiminate [IC_{50} (human ovarian carcinoma A2780, 72 h) = 2.1 μM] [37]; [$Au^{III}Cl_2$(damp)] where damp = 2-((dimethylamino)methyl)phenyl [IC_{50} (human colon carcinoma SW620, 72 h) = 50 μM] [35], and Na[$Au^{III}Cl_4$] [IC_{50} (human ovarian carcinoma A2780, 72 h) = 11 μM] [34].

Besides **3a**, the cytotoxicity of other gold(III) bis(pyridyl) carboxamide analogues (Scheme 4) were also examined. Among the gold(III) compounds (**3b**–**3d**), only **3c** shows a significant cytotoxicity towards SUNE1 (IC_{50} = 21.1 μM), which is 3-fold higher than that in normal CCD-19Lu cells (IC_{50} = 64.9 μM). For **3b** and **3d**, their cytotoxicities towards cancer cells (HeLa and SUNE1) (IC_{50} = 9.6–16.2 μM) and normal CCD-19Lu cells [IC_{50} = 19.9 μM (**3b**); 10.1 μM (**3d**)] are similar.

2.6. *In vivo* anti-cancer properties

Although the *in vitro* anti-cancer activities of gold(III) compounds have been documented for more than three decades, very few demonstrate promising *in vivo* anti-cancer activities. Among the gold(III) compounds in the literature, both [Au(acetato)$_2$(damp)] and [Au(malonato)(damp)] (where damp = 2-[(dimethylamino)methyl]phenyl) give ~40% reduction of tumor volume under *in vivo* conditions [35]. Recently, Dou and Fregona reported that a gold(III) dithiocarbamate compound inhibited ~50% growth of breast cancer 29 days after the first injection [54]. Apart from *in vitro* activities, [Au^{III}(TPP)]Cl (**1a**) also shows promising *in vivo* anti-cancer activity. We have recently confirmed the *in vivo* efficacy of **1a** against various types of nasopharyngeal carcinoma (NPC) cells including that of cisplatin-resistant CNE2 and C666-1 cells [72]. Intraperitoneal injection of **1a** (3 mg/(kg week)) effectively suppressed tumor growth in NPC-implanted nude mice without observable side-effects. Tumor growth resumed upon termination of the **1a**-treatment after 4 weeks (Fig. 9). Regular body-weight measurement showed that the mice receiving 1.5 or 3 mg/(kg week) **1a** had no significant weight loss (less than 5%). Using cisplatin as a control, we found that only ~35% inhibition of SUNE1 tumor growth was achieved, and no apparent inhibitory effect (less than 5% growth inhibition) was found for the cisplatin-resistant CNE2 and C666-1 cells under the same conditions.

In addition to NPC, **1a** also exhibited prominent anti-cancer activity toward hepatocellular carcinoma (HCC). Using an orthotopic rat HCC model, **1a** significantly prolonged the survival of HCC-bearing rats (Fig. 10) [80]. Noteworthy is the fact that the body weight of the **1a**-treated rats was not appreciably affected after treatment for 14 days until the death of the rats. Liver biochemistry revealed that the plasma AST level in the **1a**-treated mice was lowered, indicating that **1a** did not inflict damage of normal liver cells and tissues.

The single dose acute toxicology of **1a** was investigated by using a nude mouse model [16]. After single dose injection of **1a** (3.0,

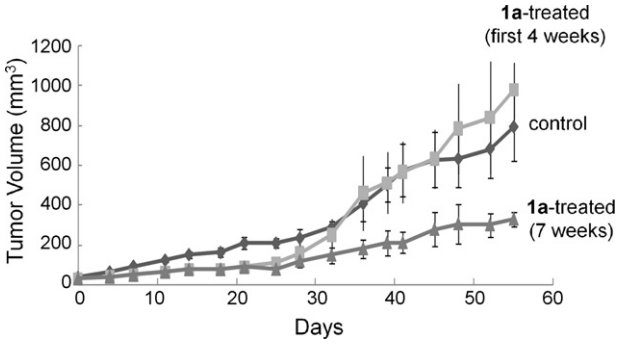


Fig. 9. *In vivo* anti-cancer activity of **1a** toward NPC[Ref. [72]].

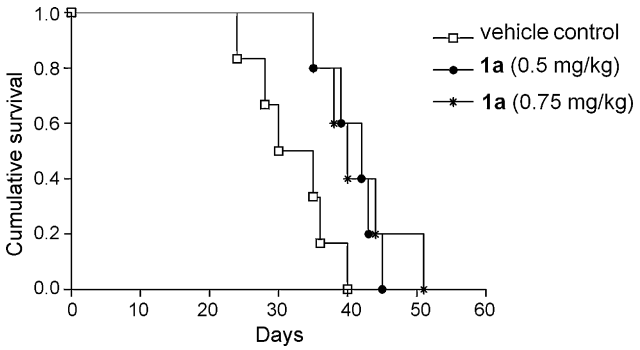


Fig. 10. *In vivo* anti-cancer activity of **1a** toward HCC [Ref. [80]].

3.2, 4.0, 5.0 or 6.25 mg/kg), the mice were sacrificed and dissected immediately after death or on day 14 after treatment. In this experiment, the LD_{50} (a dose at which 50% of subjects will die) of **1a** was determined to be 4.4 mg kg^{-1}, and no observable damage was found in the kidney, lung, heart, reproductive organs and brain, revealing that **1a** does not impose evident toxicity on these major organs.

3. Cellular mechanism of gold(III) porphyrin [Au^{III}(TPP)]Cl (1a)-induced cell death

3.1. Induction of apoptosis in NPC cells

Apoptosis, which was designated by Kerr, Wyllie and Currie in 1972, is characterized by an ordered series of biochemical and biophysical reactions that are regulated by various genes [81]. In contrast to necrosis, another form of cell death, apoptosis does not trigger inflammatory tissue reactions, and thus is advantageous for cytotoxic chemotherapeutic agents able to induce apoptotic cell death [82,83]. Compound **1a**-induced cytotoxicity in NPC cells via an apoptotic pathway. By means of confocal imaging, typical apoptotic morphological changes were detected, including the formation of apoptotic bodies, chromatin condensation and DNA fragmentation [55,73]. Flow cytometric analysis revealed that the percentage of apoptotic cells after **1a** treatment for 24 h was dramatically higher than that found in a control experiment. The **1a**-induced apoptosis was also confirmed by the oligonucleosomic degradation of cellular DNA, as this type of chromatin degradation is characteristic of apoptosis [84]. Furthermore, **1a** caused proteolytic cleavage of poly(ADP-ribose)polymerase-1 (PARP-1) between Asp^{216} and Gly^{217}, and in turn cleaved this 116-kDa native enzyme into 89- and 36-kDa fragments [85]. Taken these altogether, all of these experiments confirmed that **1a**-induced apoptotic cell death in NPC cells.

Apart from apoptosis, a previous study has shown that cisplatin can induce senescence-like growth arrest in NPC cells [86]. Nevertheless, this mechanism is insignificant in the **1a**-induced cancer cell death, since senescence-associated β-galactosidase activity was not observed in **1a**-treated NPC cells [72].

3.2. Mitochondria-mediated apoptotic pathway

Functional proteomic studies revealed an alteration in the expression of several cytoplasmic proteins in NPC cells after **1a** treatment (Fig. 11). The altered proteins include those participating in energy production (e.g., 3-phosphoglycerate dehydrogenase) and in cellular redox balance (e.g., thioredoxin peroxidase) [73,87]. Several clusters of proteins, including stress-related chaperones (e.g., peroxiredoxin 1) and proteins that mediate cell proliferation or differentiation (e.g., Ras-related nuclear protein), were also been identified. Alteration of these protein expressions indicated that mitochondria may play a pivotal role in the **1a**-induced apoptosis.

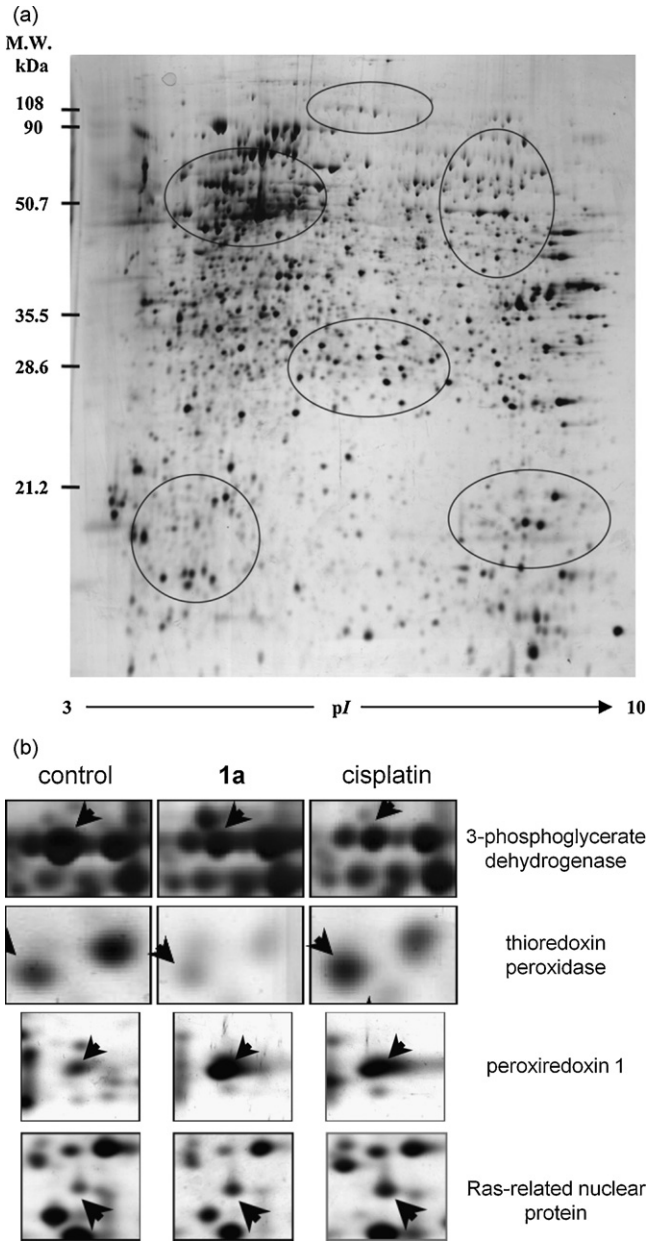


Fig. 11. (a) Representative 2-D gel images for **1a**-treated NPC cells. Areas where significant and consistent alterations of protein expression were identified are circled. (b) Detailed alterations of selected proteins in **1a**- and cisplatin-treated NPC cells [Ref [87]].

Further examination including flow cytometric analysis revealed that there was a quick reduction of mitochondrial membrane potential ($\Delta\psi_m$) after **1a**-treatment [73]. This attenuation of $\Delta\psi_m$ was observed within 3 h, consistent with the hypothesis that the mitochondrion is affected early in the apoptotic process. Western blot analysis revealed that bcl-2 expression was suppressed shortly after **1a**-treatment, suggesting that this suppression may facilitate the attenuation of $\Delta\psi_m$. The loss of $\Delta\psi_m$ was associated with the release of cytochrome *c* and apoptosis-inducing factor (AIF) from mitochondria into the cytoplasm, and both of these signaling proteins hence trigger **1a**-induced caspase-dependent (activation of caspase 3 and caspase 9) and caspase-independent (nuclear translocation of AIF) apoptosis.

Mitogen-activated protein kinases (MAPKs), including the ERK, $p38^{MAPK}$, and JNK, are important intermediates that convert extracellular signals into intracellular responses [88]. Prior exposure of

NPC cells to a $p38^{MAPK}$ inhibitor abrogated **1a**-induced apoptosis, as indicated by the inhibition of $\Delta\psi_m$ depletion as well as the activation of caspase 3, caspase 9 and PARP-1 [73,89]. Meanwhile, **1a** did not activate $p38^{MAPK}$ in mitochondrial-DNA deficient (Rho^0) HONE1 cells. In contrast, JNK and transient activation of ERK were shown not to affect the cytotoxicity of **1a**. Taken together, activation of $p38^{MAPK}$, but not that of ERK and JNK, is imperative for the cytotoxicity of the gold(III) porphyrin **1a**.

3.3. Role of reactive oxygen species

Accumulation of reactive oxygen species (ROS) is often associated with the loss of $\Delta\psi_m$. The action mechanism of **1a** had previously been examined by evaluating the effects of **1a**-induced depletion of $\Delta\psi_m$ in the presence of agents which can regulate the ROS level and mitochondrial permeability transition (MPT). The results showed that the **1a**-induced $\Delta\psi_m$ depletion is mediated, at least in part, by cellular H_2O_2 and/or thiol oxidation [73]. In addition, NPC cells pretreated with MPT inhibitors such as trifluoperazine partially prevented the **1a**-induced $\Delta\psi_m$ depletion. This result suggests that the **1a**-induced mitochondrial permeabilization is not directly mediated by its production of ROS, but that a reduction of intracellular ROS did at least partially decrease mitochondrial permeabilization, thus in turn affecting the cytotoxicity of **1a**.

3.4. Molecular mechanism of *1a*-mediated apoptosis in HCC cells

The molecular mechanism of **1a**-induced apoptosis in HCC cells was evaluated and cDNA microarray was employed to identify genes differentially expressed in response to **1a** [80]. In general, **1a** upregulated genes that induce apoptosis, stabilize *p53* and inhibit proliferation, while downregulating genes that play roles in angiogenesis, invasion and metabolism. Two genes, *Gadd34* and *Gadd153* from the Gadd gene family, were upregulated in response to **1a** at the mRNA level, reflecting their roles in the molecular pathways of **1a**-induced growth arrest or apoptosis. Moreover, the **1a**-induced cell death and apoptosis were greatly reduced after siRNA suppression of either *Gadd34* or *Gadd153*, suggesting that the **1a**-induced cytotoxicity was achieved at least in part through the up-regulation of these two genes.

4. Summary

In summary, gold(III) porphyrin compounds are stable under physiological conditions. Notably, $[Au^{III}(TPP)]Cl$ (**1a**) exhibits prominent stability in solution even in the presence of biological reductants such as glutathione and ascorbic acid. *In vivo*, **1a** inhibited tumor growth in NPC-bearing mice and prolonged survival in HCC-bearing rats. *In vitro*, **1a** exhibited prominent cytotoxicity towards a panel of cancer cell lines and to 1680-fold higher potency than the clinically used cisplatin against NPC cells. Based on the results obtained, a model for the cellular mechanism of gold(III) porphyrin **1a** in NPC cells is proposed (Fig. 12). Compound **1a** activates $p38^{MAPK}$ phosphorylation and causes depletion of mitochondrial membrane potential ($\Delta\psi_m$) shortly after the cellular uptake of **1a** along with suppression of the Bcl-2 anti-apoptotic protein. Loss of $\Delta\psi_m$ triggers the release of cytochrome *c* (Cyt *c*) and AIF from mitochondria. Cyt *c* activates the caspase cascade, and AIF activates the process of nuclear condensation, hence the activation of apoptosis.

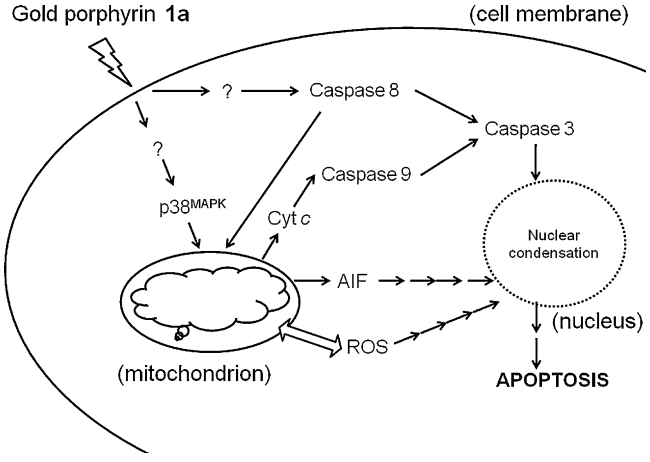


Fig. 12. Proposed model for the cellular mechanism of **1a**-induced apoptosis in NPC cell.

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