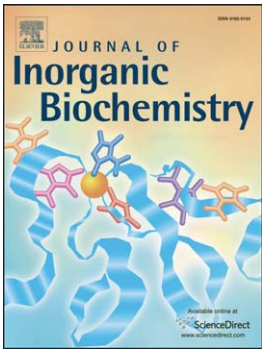


Enhanced anti-cancer activities of a gold(III) pyrrolidinedithiocarbamato complex incorporated in a biodegradable metal-organic framework

Raymond Wai-Yin Sun, Ming Zhang, Dan Li, Mian Li, Alice Sze-Tsai Wong

PII: S0162-0134(16)30183-0
DOI: doi: 10.1016/j.jinorgbio.2016.06.020
Reference: JIB 10022

To appear in: *Journal of Inorganic Biochemistry*

Received date: 7 March 2016
Revised date: 4 June 2016
Accepted date: 14 June 2016

Please cite this article as: Raymond Wai-Yin Sun, Ming Zhang, Dan Li, Mian Li, Alice Sze-Tsai Wong, Enhanced anti-cancer activities of a gold(III) pyrrolidinedithiocarbamato complex incorporated in a biodegradable metal-organic framework, *Journal of Inorganic Biochemistry* (2016), doi: 10.1016/j.jinorgbio.2016.06.020

Article

Enhanced anti-cancer activities of a gold(III) pyrrolidinedithiocarbamato complex incorporated in a biodegradable metal-organic framework

Raymond Wai-Yin Sun,[a,*] Ming Zhang,[a] Dan Li,[a,*] Mian Li,[a] Alice Sze-Tsai Wong

[a] *Department of Chemistry and Key Laboratory for Preparation and Application of Ordered Structural Materials of Guangdong Province, Shantou University, 243 Da Xue Road, Shantou, Guangdong 515063 (P. R. China)*

[b] *School of Biological Sciences, The University of Hong Kong, Pokfulam, Hong Kong (P. R. China)*

*Corresponding authors:

E-mail addresses: rwysun@stu.edu.cn (R. W.-Y. Sun) and dli@stu.edu.cn (D. Li)

Keywords: cancer, carbene, dithiocarbamate, gold, metal-organic framework

Special issue of the *Journal of Inorganic Biochemistry* dedicated to the "1st International Symposium on Clinical and Experimental Metallodrugs in Medicine: Cancer Chemotherapy (CEMM)"

ABSTRACT

An anti-cancer active gold(III) pyrrolidinedithiocarbamato complex [(PDTC)$Au^{III}Cl_2$] (**1**) has been synthesized and characterized by means of X-ray crystallography. Compared to the pyrrolidinedithiocarbamate ligand itself, this gold(III) complex displays an up to 33-fold higher anti-cancer potency towards a panel of cancer cell lines including the cisplatin-resistant ovarian carcinoma cell line (A2780cis). As demonstrated by a set of transwell® assay-based cytotoxicity experiments, incorporating this gold(III) complex in a zinc-based biodegradable metal-organic framework (MOF) displays a significant enhancement in anti-cancer activity towards A2780cis than the gold(III) complex alone.

1. Introduction

Various dithiocarbamates including pyrrolidinedithiocarbamate (PDTC) have been reported to display promising anti-cancer activities and to significantly inhibit nuclear factor kappa-B (NF-kappa B) activation in the past two decades [1–4]. Subsequent studies however revealed that some of these compounds would easily self-aggregate, form complexes with metal ion(s), and/ or non-specifically bind to other biomolecules under physiological conditions [5]. Moreover, specific transportation of dithiocarbamates in biological systems to their biological targets (i.e. cancer cells) is usually not straightforward, since this class of compounds would tightly bind to drug carriers as a result from their relative high reactivity [6]. Thus their medical development and clinical applications have been severely hampered.

Compared to conventional organic moieties, metal coordination complexes often display unique chemical, physical and biological properties, which can be accounted by the wide ranges of coordination numbers, geometries, redox states, kinetics and thermodynamic characteristics arising from the presence of the metal ion(s) [7–13]. In addition to the metal-free dithiocarbamates, a number of metal complexes of dithiocarbamates possessing anti-cancer activities including that of zinc, iron, ruthenium, copper and notably, gold have also been identified in the past twenty years [14–19]. Parish, Buckley and co-workers in 1996 reported the first X-ray crystal structure of a gold(III) dithiocarbamato complex containing a 2-((dimethylamino)methyl)phenyl ligand (damp) [20]. In 2005, Fregona and

co-workers have revealed a series of gold(III) complexes with dimethyldithiocarbamate and S-methyl-*N*,*N*-dimethyldithiocarbamate and ethylsarcosinedithiocarbamate ligands having higher anti-cancer potency than the dithiocarbamate ligands themselves [21]. Unlike the clinically-used platinum(II) drugs which target DNA to execute their cytotoxic activities, many gold(III) dithiocarbamato complexes were found capable to induce cancer apoptotic cell death via inhibition of thioredoxin redox system and proteasomes, and activation of ERK pathway [22–25]. Various peptidomimetic gold(III) dithiocarbamato complexes show enhancing anti-cancer activities and cancer-cell selectivity *in vitro* [26,27]. More recently, the anti-cancer activities and solution behavior of two gold(III)-pyrrolidinedithiocarbamato complexes $[Au^{III}(PDTC)Cl_2]$ and $[Au^{III}(PDTC)Br_2]$ have been examined [28]. These complexes were found to undergo extensive hydrolysis in aqueous medium, which in turn affects their cytotoxic activities against the cancer cells.

One of the major challenges for the medical development of gold(III) complexes, including that of dithiocarbamato complexes, is the stability in aqueous solutions [29–31]. Apart from using strong sigma-donor ligands [31–35], another feasible approach to stabilize the highly oxidizing gold(III) metal center is the utilization of drug carriers or encapsulating materials. To facilitate the transport of anti-cancer active gold(III) complexes in biologically-relevant systems, some recent studies have demonstrated the use of gelatin-acacia [36], polyethylene glycol [37,38],

supramolecular polymer [39] and lipophilic core of micelles produced from surfactant Pluronic® 127 [40]. In the recent decade some bioactive organic compounds incorporated in metal-organic frameworks (MOFs) have been reported [41–43], and we previously have reported the synergism of MOFs on the anti-cancer activity of a dinuclear gold(I) complex [44]. In the present study, we have demonstrated the prominent anti-cancer activities of a gold(III)-pyrrolidinedithiocarbamato complex $[Au^{III}(PDTC)Cl_2]$ (**1**, Fig. 1) towards a panel of cancer cell lines. By using a biodegradable MOF to incorporate this gold(III) complex, and as demonstrated by a set of transwell® assay-based experiments, the *in vitro* cytotoxic activity of **1** was found to be significantly enhanced.

2. Experimental

2.1. Materials and instrumentations

All chemicals, unless otherwise noted, were purchased from Sigma-Aldrich Chemical Co. Solvents (AR and HPLC grades) were purchased from commercial sources and used without further purification. Cell Proliferation Kit I [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay] was purchased from Alfa Aesar. Human ovarian carcinoma cell lines A2780 and its cisplatin-resistant variant A2780cis were purchased from Sigma-Aldrich Chemical Co. Cell lines of human ovarian carcinoma (A2780 and its cisplatin-resistant variant A2780cis), human epithelioid cervix carcinoma (HeLa), human glioblastoma astrocytoma (U-87 MG), mouse melanoma (B16-F10) and a normal Madin-Darby canine kidney (MDCK) cell line were obtained commercially from American Type Culture Collection (ATCC). Cell-culture flasks, 96-well microtitre plates were purchased from Nalge Nunc. Culture medium, other medium constituents, and phosphate-buffered saline (PBS) were from Gibco BRL.

^{1}H NMR spectra were recorded on a Bruker Biospin Avance (400 MHz) NMR spectrometer. UV-vis spectra were recorded with an Agilent 8453 spectrophotometer. The absorbance for MTT assay was measured using a Promega E7031 microplate reader. Powder X-ray diffraction patterns (PXRD) of the bulk samples were measured on a Bruker D8 Advance diffractometer (Mo Kα, λ= 0.71073 Å). The experiments using inductively coupled plasma-atomic emission spectrometry (ICP-AES) were

carried out on ICPE-9000. Energy-dispersive X-ray spectroscopy (EDS) and scanning electron microscope (SEM) analysis was carried out on scanning electron microscopy (JSM-6360LA).

2.2. Synthesis of the gold(III) dithiocarbamato complex

The gold(III) dithiocarbamato complex, dichlorido[*N*-dithiocarboxy-kS,kS'-pyrrolidine]gold(III) ([Au^{III}(PDTC)Cl_2], **1**) was prepared from the reaction between $HAuCl_4$•$4H_2O$ (100.0 mg, 0.24 mmole) and PDTC (80% purity) (48.7 mg, 0.23 mmole) in water (25 mL) at room temperature for 1 h [28]. A yellowish solid was obtained after filtration and the complex was recrystallized from CH_2Cl_2. Yield: 82.5%. ^{1}H NMR (400 MHz, d_6-DMSO), δ 3.83 (s, 4H), 2.06 (s, 4H); Elemental analysis calcd (%) for $C_5H_8AuCl_2NS_2$: C 14.50, H 1.95, N 3.38, S 15.49 ; found: C 14.85, H 1.99, N 3.30, S 15.29.

2.3. X-ray crystallography

Crystals of **1** (CCDC deposition number: 1456336) were mounted with glue at the end of a glass fiber. Data collection was performed on an Oxford Diffraction Gemini E (Enhance Mo X-Ray source, Mo Kα, λ= 0.71073 Å) equipped with a graphite monochromator and ATLAS CCD detector (CrysAlis CCD, Oxford Diffraction Ltd) at room temperature (293 K). Structures were solved by direct methods (SHELXTL-97) and refined on F^2 using full-matrix least-squares (SHELXTL-97) [45]. All non-hydrogen atoms were refined with anisotropic thermal

parameters, and all hydrogen atoms were included in calculated positions and refined with isotropic thermal parameters riding on those of the parent atoms. Crystal data and structure refinement parameters are summarized in *Table S1*. Selected bond lengths and angles are given in *Table S2*. (Supporting Information).

2.4. Cell lines and growth inhibitory assay (MTT assay)

The cell lines were maintained in cell culture media (Eagle's minimum essential medium for U87MG, B16-F10 and MDCK; Dulbecco's Modified Eagle's Medium for HeLa, RPMI-1640 medium for A2780 and A2780cis) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 310 K humidified atmosphere with 5% CO_2. Cell growth inhibitory effects of all the complexes were determined by MTT assay [46]. Briefly, cells were seeded in 96-well plates and incubated for overnight prior to be examined. Complex **1** and PDTC were weighted and dissolved in DMSO. Media in the presence of the tested complexes were added and serially diluted to various concentrations (from 40.0 μM to 0.31 μM), and media containing cisplatin were used as the positive control. The maximum concentration of DMSO in media did not exceed 1 % (v/v). The cells were incubated for 72 h and followed by the addition of MTT solution. After 1 h, the formed formazan was dissolved by adding dimethyl sulfoxide (DMSO) and the absorbance of the solution at 560 nm was measured by using a microtitre plate reader. The IC_{50} values of the complexes (concentrations at which could inhibit cellular growth by 50 % compared to the negative control) were determined from the plots of the cell viability percentage

versus the complex concentration. For each set of data, at least three independent experiments have been conducted.

2.5. Preparation of the zinc-based metal-organic frameworks (Zn-MOFs)

The zinc-based metal-organic frameworks ZnBTCA (BTCA represents benzene-1,3,5-tricarboxyl-adenine) [47], Bio-MOF-1 [48] and PCN-530 (PCN represents porous coordination network) [49] were prepared according to the reported procedures and their phase purities were confirmed by using PXRD.

*2.6. Loading of **1** into Zn-MOFs (**1**@Zn-MOF)*

The procedure of the loading of **1** into three different kinds of Zn-MOFs ZnBTCA, Bio-MOF-1 and PCN-530 was performed as follows: Each kind of Zn-MOF was weighted (approximately 50 mg), rinsed with DMF three times and soaked in a DMF solution containing **1** at 4 mM (soaking solution) for 24 h. The soaking solution was renewed in every 24 h for 3 days. The **1**-loaded Zn-MOF (i.e., **1**@ZnBTCA, **1**@Bio-MOF-1 or **1**@PCN-530) was rinsed with DMF five times and dried under vacuum for a further 24 h.

*2.7. Cumulative release of **1** from **1**-loaded Zn-MOFs*

Cumulative release of **1** from **1**@ZnBTCA, **1**@Bio-MOF-1 or **1**@PCN-530 (~50 mg each) was examined by suspending the **1**-loaded Zn-MOF in water (25 mL) in a closed glass tube (50 mL), which was continuously shaken at a constant rate of 30

rpm or remained stationary. At time = 0, 1, 2, 3, 4, 12, 24, 48 and 72 h, aliquots (<0.2% of the total volume) of the supernatant from the tube were withdrawn and analyzed for both the amount of gold (from **1**) and zinc (from Zn-MOFs) by inductively coupled plasma-atomic emission spectrometry (ICP-AES).

For the ICP-AES experiment, aliquots of nitric acid (30%, 100 μL) were added to the collected solutions and the mixtures were ultrasound for 10 min at room temperature, followed by the addition of double distilled deionized water (3 mL). The mixtures were filtrated by membrane filters with pore size of 0.22 μm, transferred to 10-mL volumetric flasks, and diluted with double distilled deionized water. The zinc and gold concentrations were hence determined by ICP-AES. Calibration curves of zinc and gold were obtained by using a series of solutions with different concentrations of zinc and gold which were prepared by using the commercially available zinc and gold standard solutions, respectively.

2.8. Transwell® assay-based cytotoxicity study

For the *in vitro* cytotoxicity evaluation of **1**@ZnBTCA, A2780cis cells were seeded at a density of 4000 cells/100 μL supplemented with RPMI-1640 (1 mL) in lower chambers of the double-decked transwell® 24-well plates. 200 μL in the up chamber. Solids of **1**@ZnBTCA (0.05 mg or 0.10 mg, equivalent to 4.0 or 8.0 μM of **1**, respectively), or ZnBTCA (0.1 mg) were loaded into the corresponding upper chambers and the whole set of transwell® 24-well plates were kept at 310 K (5% CO_2/ 95% air) incubated for a total incubation time of 24 h. During the incubation

period, upper chambers which contained **1**@ZnBTCA were removed from the transwells® at different time intervals accordingly. As a control experiment, A2780cis cells in other sets of transwells® were incubated with **1** at 8.0 μM for different periods of time. Cell growth inhibitory effects of all the complexes/ **1**@ZnBTCA were determined by MTT cytotoxicity assay when a total incubation time of 24 h had reached.

3. Results and Discussion

3.1. Synthesis and characterization

The gold(III) dithiocarbamato complex, dichlorido[*N*-dithiocarboxy-kS,kS'-pyrrolidine]gold(III) ([Au^{III}(PDTC)Cl_2], **1**) was prepared according to the reported procedure from the reaction between PDTC and $HAuCl_4$•$4H_2O$ with a final yield of 82.5% [28]. The complex was characterized by ^{1}H-NMR spectroscopy and the >99% purity was confirmed by elemental analysis. Crystals of **1** were obtained by slow evaporation of CH_2Cl_2 solutions. The perspective view of its X-ray crystal structure (CCDC deposition number: 1456336) is shown in Fig. 2. The crystallographic data (Table S1), bond angles and bond distances (Table S2) are given in the Supplementary Information. A distorted square-planar geometry is revealed from the *trans* S–Au–Cl angles of 171.69° and 171.91° in **1**. The Au–Cl and Au–S distances are ranging from 2.308 Å to 2.319 Å. The inter-molecular Au–Au distance was found to be 4.432 Å.

3.2. In vitro cytotoxicity

In the past decades, the *in vitro* and/or *in vivo* anti-cancer activities of some gold(III) complexes including that of porphyrins [31–35], carbene [50], terpyridine [51], diphenylpyridine [34], dinuclear oxo-bridged gold(III) complexes [52] and a series of gold(III) dithiocarbamato complexes [25] have been subsequently uncovered. By means of 3-(4,5-dimethylthiazol-2-yl)-2,5-tetrazolium bromide (MTT) assays [46], the *in vitro* cytotoxicity of **1** was evaluated using cancerous cell lines of human

ovarian carcinoma (A2780 and its cisplatin-resistant variant A2780cis), human epithelioid cervix carcinoma (HeLa), human glioblastoma astrocytoma (U-87 MG), mouse melanoma (B16-F10) and a normal Madin-Darby canine kidney (MDCK) cell line. The cytotoxicity (72 h) of **1** towards these cells, in terms of IC_{50} values, was depicted in Table 1. The IC_{50} values span over the range from 1.3 μM to 21 μM towards the carcinoma cell lines. Complex **1** is highly cytotoxic towards the cisplatin-resistant ovarian carcinoma cells (A2780cis) with an IC_{50} value of 1.3 μM, and displays 26-fold and 33-fold higher anti-cancer potencies than cisplatin and PDTC, respectively. The cytotoxicity of **1** towards a non-cancerous origin derived cell line MDCK was also examined and the IC_{50} value was found to be 9.7 μM. Compared to the A2780cis cell line, **1** displays a 7.5-fold selectivity towards the cancerous cells than the normal cells.

A recent study has shown that **1** exhibits a rapid (three-hour) dose-dependent cytotoxic activity towards some human cancer cell lines including the highly aggressive osteosarcoma (SAOS-2) and colorectal carcinoma (HCT116) [28]. Complex **1** undergoes extensive hydrolysis in aqueous solutions and we reckon that the hydrolytic process would affect the cytotoxic activity of this gold(III) complex. As mentioned earlier, the IC_{50} value of **1** of 72-h of incubation towards A2780cis cells was found to be 1.3 μM. In this case, the aqueous stock solution of **1** containing less than 1% DMSO was freshly prepared before the cytotoxicity assay. The corresponding cytotoxic profile was shown in Fig. 3a. However, when the aqueous

stock solution of **1** was prepared 72 h before the cytotoxicity assay, the cytotoxic activity of **1** was found to be significantly affected, with IC_{50} value changed from 1.3 μM to 8.6 μM. UV-vis spectrophotometry revealed that **1** would undergo hydrolysis and is unstable in aqueous solutions especially in the presence of thiol-containing biomolecule glutathione (GSH, Fig. 3b) which the latter is highly expressed in various types of cancer cells. Similar to most gold(III) complexes [31], the instability of **1** in aqueous solutions may hamper its biomedical applications and development.

To enhance the bioavailability and stability of anti-cancer gold(III) complexes, a feasible approach is to use appropriate drug carrier to encapsulate and protect the reactive metal complexes against reduction, hydrolysis and/or reactions in solutions [53]. We recently have reported the use of a class of porous materials coined as metal-organic frameworks (MOFs) having advantages of high surface area and structural tenability to encapsulate various gold(I) complexes and bioactive molecules [44]. In this work, we aimed at improving the stability and bio-activity of the gold(III) complex **1** by employing three recently developed zinc-based MOFs (Zn-MOFs) including ZnBTCA, Bio-MOF-1 and PCN-530 (Fig. 4). All of these contain zinc ions and a biocompatible substance adenine (ad). ZnBTCA is biodegradable (can be decompose in aqueous solutions) and is composed of a benzene-1,3,5-tricarboxylic acid (BTC) linker having a unit-cell formula of $Zn_3(ad)(BTC)_2(H_2O)\cdot(CH_3)_2NH_2\cdot xDMF\cdot yH_2O$ [47]. The framework host of ZnBTCA contains one-dimensional channels with window dimension of ~8.0 × 11.0 Å (Fig. 4a).

Bio-MOF-1 is stable in aqueous solutions and consists of repeating unit of $Zn_8(ad)_4(BPDC)_6O{\cdot}2Me_2NH_2$, 8DMF, $11H_2O$ (whereas BPDC = biphenyldicarboxylate) [48]. It possesses open channels of ~11.0 × 11.0 Å along the *a* axis (Fig. 4b). PCN-530 is another kind of zinc- and adenine-based MOF with single crystal having a molecular formula of $Zn_3[Zn_2(\mu_2\text{-}H_2O)]_3(ad)_6(TATB)_4(DMF)$ (whereas TATB is 4,4',4"-*s*-triazine-2,4,6-triyl-tribenzoate, Fig. 4c) [49]. This Zn-MOF is highly stable in aqueous solution. It has open channels of ~7.4 × 11.9 Å and a solvent accessible volume of 47.8% which indicates its porous nature. All of these Zn-MOFs were prepared according to the literature reported procedures. The crystal lattices and phase purities were confirmed by X-ray crystallography and/or powder X-ray diffraction (PXRD). After mechanical grinding, these Zn-MOFs have average particle dimensions of ~1 μm^3. All these Zn-MOFs are too large to penetrate the membrane of the cancer cells, but may capable to facilitate as anti-cancer drug carriers for intratumoral or intraperitoneal injections.

In the presence of the open channels, **1** was found capable to be loaded to these Zn-MOFs as demonstrated by UV-vis spectrophotometry (Fig. 5). Using ZnBTCA as an example, the color of crystals of the MOF was changed from colorless to yellow upon the loading the gold(III) complex (Fig. 6). PXRD analysis revealed that the crystallinity of ZnBTCA did not significantly alter upon the loading (Fig. 7). Maximum loadings of **1** to all of the three Zn-MOFs were achieved by immersing crystals of Zn-MOFs in a DMF solution containing **1** at 4 mM for 72 h, in which the

DMF solutions were replaced by freshly prepared solutions of **1** in every 24 h. The loading of **1** to these Zn-MOFs were determined by measuring the gold content of the Zn-MOFs by means of inductively coupled plasma atomic emission spectroscopy (ICP-AES). The maximum **1**-loading efficacies of ZnBTCA, Bio-MOF-1 and PCN-530 were determined to be 0.039 g/g, 0.035 g/g and 0.004 g/g, respectively. Compared to the relatively low loading efficiency (~0.004 g/g) of the reported gold(I) pyrrolidinedithiocarbamato complex [44], ZnBTCA was found to be more suitable to act as drug carrier for the gold(III) complex **1**.

Cumulative releases of **1** from the **1**-loaded Zn-MOFs (abbreviated as **1**@Zn-MOFs) in aqueous solutions were examined by suspending them in water (25 mL) in closed glass containers, which were continuously shaken at a constant rate of 30 rpm or remain stationary. After 0, 1, 2, 3, 4, 12, 24, 48 and 72 h, aliquots which are <0.2% v/v of the total volume of the supernatant were withdrawn from the tube and analyzed for the gold contents by ICP-AES. The gold-released profiles from **1**@ZnBTCA with or without shaking were shown in Fig. 8. Less than 30% of gold were released from the **1**@ZnBTCA in the first 12-h incubation in both cases. The longer the incubation time, the more the gold released. Upon 72-h of incubation, ~80% (shaking) and ~60% (without shaking) of gold contents were found to be released. The zinc released from **1**@ZnBTCA was also monitored and was found to have a similar releasing profile to that of gold, suggesting that the release of gold was accompanied by the gradual decomposition of ZnBTCA in water. Unlike ZnBTCA,

both Bio-MOF-1 and PCN-530 are relatively stable in water. Only less than 5% of gold contents were found to be released from **1**@Bio-MOF-1 and **1**@PCN-530 as revealed from the ICP-AES experiments.

Given the promising loading and releasing profiles and also the preferential cytotoxicity of **1** towards the cisplatin-resistant cancerous cells, the *in vitro* cytotoxicity of **1**@ZnBTCA towards A2780cis cells was examined by using a set of transwells® assay-based experiments [44]. To avoid the direct contact of **1**@ZnBTCA with the A2780cis cells, double-chambered transwells® containing semipermeable membranes at the bottom of the upper chambers were employed. In general, A2780cis were seeded in designated lower chambers with each chamber contains culture medium and incubated at 310 K (5% CO_2/ 95% air) for 24 h. ZnBTCA (0.1 mg) and **1**@ZnBTCA (0.05 mg or 0.10 mg, equivalent to 4.0 or 8.0 μM of **1**, respectively) were separately placed inside the designated detachable upper chambers. The presence of the semipermeable membranes allows the released molecules (i.e., **1** from **1**@ZnBTCA) to diffuse to the cancer cells at the lower chambers. The entire sets of transwells® with both the upper and lower chambers were incubated again at 310 K (5% CO_2/ 95% air). Different sets of the upper chambers containing ZnBTCA or **1**@ZnBTCA were detached from the lower chambers after 2, 4, 7 and 24 h of incubation. The lower sets of the chambers which contain A2780cis cells were kept at 310 K (5% CO_2/ 95% air) until a total 24 h of assayed time had been reached. The cell viability in each lower chamber was determined by MTT assay [46]. As a control

experiment, cytotoxicity of **1** towards A2780cis with incubation time of 2, 17, 19 and 24 h at 8.0 μM was also examined.

The 24-h cytotoxic profiles of the A2780cis cells with different co-incubation time with **1**@ZnBTCA (equivalent to **1** at 4 and 8 μM) were shown in Fig. 9. The time-dependent cytotoxic activities of naked **1** (without loaded in ZnBTCA) at 8 μM and ZnBTCA were also examined as references. Percentage survival of the A2780cis cancer cells decreased with increasing co-incubation time of the **1**@ZnBTCA (equivalent to **1** at 4 μM). The cell viabilities were found to be ~70% and ~30% upon incubated with **1**@ZnBTCA for 4 and 24 h, respectively. It should be noted that the cytotoxic profile resembles to that of the naked **1**, indicating that the use of a two-fold lower dose of **1** loaded in ZnBTCA yields similar anti-cancer potency than the naked gold(III) complexes. Moreover, increasing the gold content in ZnBTCA (mass percentage of **1**) resulted in a significant enhanced in cytotoxic activities. As revealed from the cytotoxic profile of **1**@ZnBTCA (equivalent to **1** at 8 μM), this material would offer a rapid (2 h) decrease of cell viability (>75% cell death) of the A2780cis cells. Complex **1** has previously been reported to undergo extensive hydrolysis in aqueous solution [28]. Taken all the results of this work together, we reckon that ZnBTCA could protect the relatively reactive gold(III) complex **1** against hydrolysis/ reduction in culture medium and thus yielded preferential cytotoxic activities towards the A2780cis cells.

4. Conclusion

To sum up, anti-cancer active gold(III) pyrrolidinedithiocarbamato complex [(PDTC)$Au^{III}Cl_2$] (**1**) after metalation was found to display an up to 33-fold higher anti-cancer potency towards cisplatin-resistant ovarian carcinoma cell line (A2780cis). One of the major challenges for the medical development of gold(III) complexes is the stability in aqueous solutions. In this work we found that incorporating this gold(III) complex in a zinc-based biodegradable metal-organic framework (MOF) could significantly enhance its anti-cancer activity. Given the ease in preparing different kinds of MOFs with structural varies and different physical properties, this work provides an example to highlight the prospect in developing MOFs as promising drug carriers for anti-cancer agents which are relatively unstable in aqueous solutions.

Acknowledgements

This work was supported by National Basic Research Programs of China (973 Program, 2012CB821706 and 2013CB834803), National Natural Science Foundation of China (91222202 and 21171114), Shantou University Start-Up Fund, (2013 NTF13005), the General Research Fund (HKU 704812P) administrated by University Grants Council (HKSAR, China) and Novus Life Sciences Ltd. We thank the National Institutes of Health and National Cancer Institute for grant 1R13CA200223-01A1 (Conference Organization support, 1st International Symposium on Clinical and Experimental Metallodrugs in Medicine: Cancer Chemotherapy, CEMM).

References

[1] V. Bala, G. Gupta, V.L. Sharam, Mini-Rev. Med. Chem. 14 (2014) 1021.
[2] H. Riera, V. Afonso, P. Collin, A. Lomri, PLoS One 10 (2015) e0127571.
[3] W. Pfeilschifter, B. Czech, B.P. Hoffmann, M. Sujak, T. Kahles, H. Steinmetz, T.N. Haefelin, J. Pfeilchifter, Neurochem. Res. 35 (2010) 1391.
[4] S. Tahata, B. Yuan, H. Kikuchi, N. Takagi, T. Hirano, H. Toyoda, Int. J. Oncol. 45 (2014) 1749–1759.
[5] L.X. Ye, A. T. Dinkova-Kostova, K.L. Wade, Y.S. Zhang, T.A. Shapiro, P. Talalay, Clin. Chim. Acta 316 (2002) 43–53.
[6] P. Jackson, T.W. McCreary, J. Am. Chem. Soc.189 (1985) 4.
[7] M.J. Cleare, Coord. Chem. Rev. 12 (1974) 349–405.
[8] C.-M. Che, F.-M. Siu, Curr. Opin. Chem. Biol. 14 (2010) 255–261.
[9] I. Ott, R. Gust, Archiv Der Pharm. 340 (2007) 117–126.
[10] R.W.-Y. Sun, D.-L. Ma, E.L.-M. Wong, C.-M. Che, Dalton Trans. (2007) 4884–4892.
[11] P.C.A. Bruijnincx, P.J. Sadler, Curr. Opin. Chem. Biol. 12 (2008) 197–206.
[12] L. Ronconi, P.J. Sadler, Coord. Chem. Rev. 251 (2007) 1633–1648.
[13] G. Sava, A. Bergamo, P.J. Dyson, Dalton Trans. 40 (2011) 9069–9075.
[14] E.M. Nagy, L. Ronconi, C. Nardon, D. Fregona, Mini-Rev. Med. Chem. 12 (2012) 1216–1229.
[15] G. Hogarth, Mini-Rev. Med. Chem. 12 (2012) 1202–1215.
[16] L. Ronconi, C. Marzano, P. Zanello, M. Corsini, G. Miolo, C. Macca, A. Trevisan, D. Fregona, J. Med. Chem. 49 (2006) 1648–1657.
[17] V. Milacic, D. Chen, L. Ronconi, K.R. Landis-Piwowar, D. Fregona, Q.P. Dou, Cancer Res. 66 (2006) 10478–10486.
[18] K.G. Daniel, D. Chen, S. Orlu, Q.C. Cui, F. R. Miller, Q.P. Dou, Breast Cancer Res. 7 (2005) R897–R908.
[19] B. Cvek, V. Milacic, J. Taraba, Q.P. Dou, J. Med. Chem. 51 (2008) 6256–6258.
[20] R.G. Buckley, A.M. Elsome, S.P. Fricker, G.R. Henderson, B.R.C. Theobald, R.V. Parish, B.P. Howe, L.R. Kelland, J. Med. Chem. 39 (1996) 5208–5214.
[21] L. Ronconi, L. Giovagnini, C. Marzano, F. Bettio, R. Graziani, G. Pilloni, D. Fregona, Inorg. Chem. 44 (2005) 1867–1881.
[22] A. Casini, G. Kelter, C. Gabbiani, M.A. Cinellu, G. Minghetti, D. Fregona, H.H. Fiebig, L. Messori, J. Biol. Inorg. Chem. 14 (2009) 1139–1149.
[23] L. Cattaruzza, D. Fregona, M. Mongiat, L. Ronconi, A. Fassina, A. Colombatti, D. Aldinucci, Int. J. Cancer 128 (2011) 206–215.
[24] L. Ronconi, D. Fregona, Dalton Trans. (2009) 10670–10680.
[25] L. Ronconi, D. Aldinucci, Q.P. Dou, D. Fregona, Anti-Cancer Agents Med. Chem. 10 (2010) 283–292.
[26] C. Nardon, S.M. Schmitt, H. Yang, J. Zuo, D. Fregona, Q.P. Dou, PLoS One 9 (2014) e84248.
[27] M.N. Kouodom, G. Boscutti, M. Celegato, M. Crisma, S. Sitran, D. Aldinucci, F. Formaggio, L. Ronconi, D. Fregona, J. Inorg. Biochem. 117 (2012) 248–260.

[28] C. Nardon, F. Chiara, L. Brustolin, A. Gambalunga, F. Ciscato, A. Rasola, A. Trevisan, D. Fregona, ChemistryOpen 4 (2015) 183–191.
[29] C.F.III Shaw, Chem. Rev. 99 (1999) 2589–2600.
[30] I. Ott, Coord. Chem. Rev. 253 (2009) 1670–1681.
[31] T. Zou, C.T. Lum, C.-N. Lok, J.-J. Zhang, C.-M. Che, Chem. Soc. Rev. 44 (2015) 8786–8801.
[32] R.W.-Y. Sun, C.-M. Che, Coord. Chem. Rev. 253 (2009) 1682–1691.
[33] C.-M. Che, R.W.-Y. Sun, Chem. Commun. 47 (2011) 9554–9560.
[34] R.W.-Y. Sun, C.-N. Lok, T.T.-H. Fong, C.K.-L. Li, Z.F. Yang, T. Zou, A.F.-M. Siu, C.-M. Che, Chem. Sci. 4 (2013) 1979–1988.
[35] C.T. Lum, A.S.-T. Wong, M.C.M. Lin, C.-M. Che, R.W.-Y. Sun, Chem. Commun. 49 (2013) 4364–4366.
[36] J.J. Yan, R.W.-Y. Sun, P. Wu, M.C.M. Lin, A.S.-C. Chan, C.-M. Che, Dalton Trans. 39 (2010) 7700–7705.
[37] P. Ringhieri, R. Iannitti, C. Nardon, R. Palumbo, D. Fregona, G. Morelli, A. Accardo, Int. J. Pharmaceutics 473 (2014) 194–202.
[38] P. Lee, R. Zhang, V. Li, X. Liu, R.W.-Y. Sun, C.-M. Che, K.K.Y. Wong, Int. J. Nanomed. 7 (2012) 731–737.
[39] J.-J. Zhang, W. Lu, R.W.-Y. Sun, C.-M. Che, Angew. Chem. Int. Ed. 51 (2012) 4882–4886.
[40] C. Nardon, G. Boscutti, L. Dalla Via, P. Ringhieri, V. Di Noto, G. Morelli, A. Accardo, D. Fregona, Med. Chem. Commun. 6 (2015) 155–163.
[41] R.C. Huxford, J. Della Rocca, W. Lin, Curr. Opin. Chem. Biol. 14 (2010) 262–268.
[42] J. Della Rocca, D. Liu, W. Lin, Acc. Chem. Res. 44 (2011) 957–968.
[43] C.-Y. Sun, C. Qin, X.-L. Wang, Z.-M. Su, Exp. Opin. Drug Delivery 10 (2013) 89–101.
[44] R.W.-Y. Sun, M. Zhang, D. Li, Z.-F. Zhang, H. Cai, M. Li, Y.-J. Xian, S.W. Ng, A.S.-T. Wong, Chem. Eur. J. 21 (2015) 18534–18538.
[45] G.M. Sheldrick, Acta Crystallogr. 64 (2008) 112.
[46] T. Mosmann, J. Immunol. Methods 65 (1983) 55–63.
[47] H. Cai, M. Li, X.-R. Lin, W. Chen, G.-H. Chen, X.-C. Huang, D. Li, Angew. Chem. Int. Ed. 54 (2015) 10454–10459.
[48] J. An, S.J. Geib, N.L. Rosi, J. Am. Chem. Soc. 131 (2009) 8376–8377.
[49] M. Zhang, W. Lu, J.-R. Li, M. Bosch, Y.-P. Chen, T.-F. Liu, Y. Liu, H.-C. Zhou, Inorg. Chem. Front. 1 (2014) 159–162.
[50] J.J. Yan, A.L.-F. Chow, C.-H. Leung, R.W.-Y. Sun, D.-L. Ma, C.-M. Che, Chem. Commun. 46 (2010) 3893–3895.
[51] L. Messori, F. Abbate, G. Marcon, P. Orioli, M. Fontani, E. Mini, T. Mazzei, S. Carotti, T. O'Connell, P. Zanello, J. Med. Chem. 43 (2000) 3541–3548.
[52] A. Casini, M.A. Cinellu, G. Minghetti, C. Gabbiani, M. Coronnello, E. Mini, L. Messori, J. Med. Chem. 49 (2006) 5524–5531.
[53] L. He, T. Chen, Y. You, H. Hu, W. Zheng, W.-L. Kwong, T. Zou, C.-M. Che, Angew. Chem. Int. Ed. 53 (2014) 12532–12536.

Figures and Tables

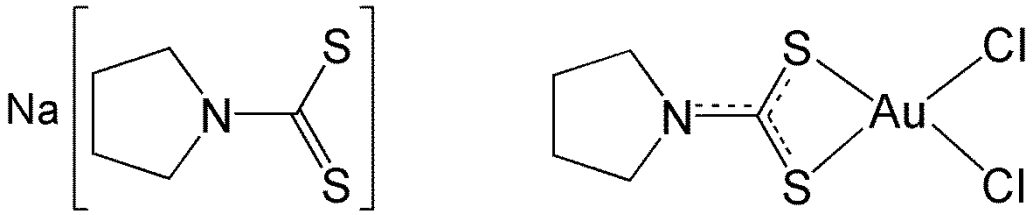


Figure 1. Chemical structures of pyrrolidedithiocarbamate (PDTC), sodium salt (left) and the gold(III) pyrrolidinedithiocarbamato complex (PDTC)$Au^{III}Cl_2$ (**1**, right).

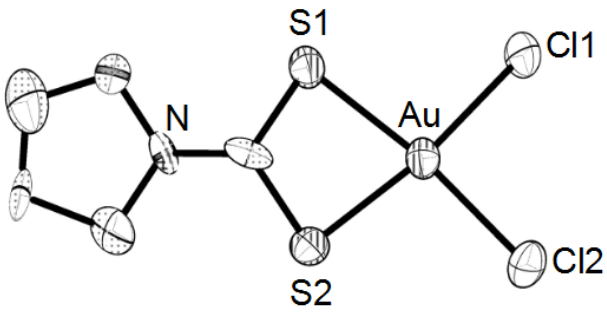


Figure 2. Crystal structure of **1** drawn with 30% probability ellipsoids. H atoms of the complex are omitted for clarity.

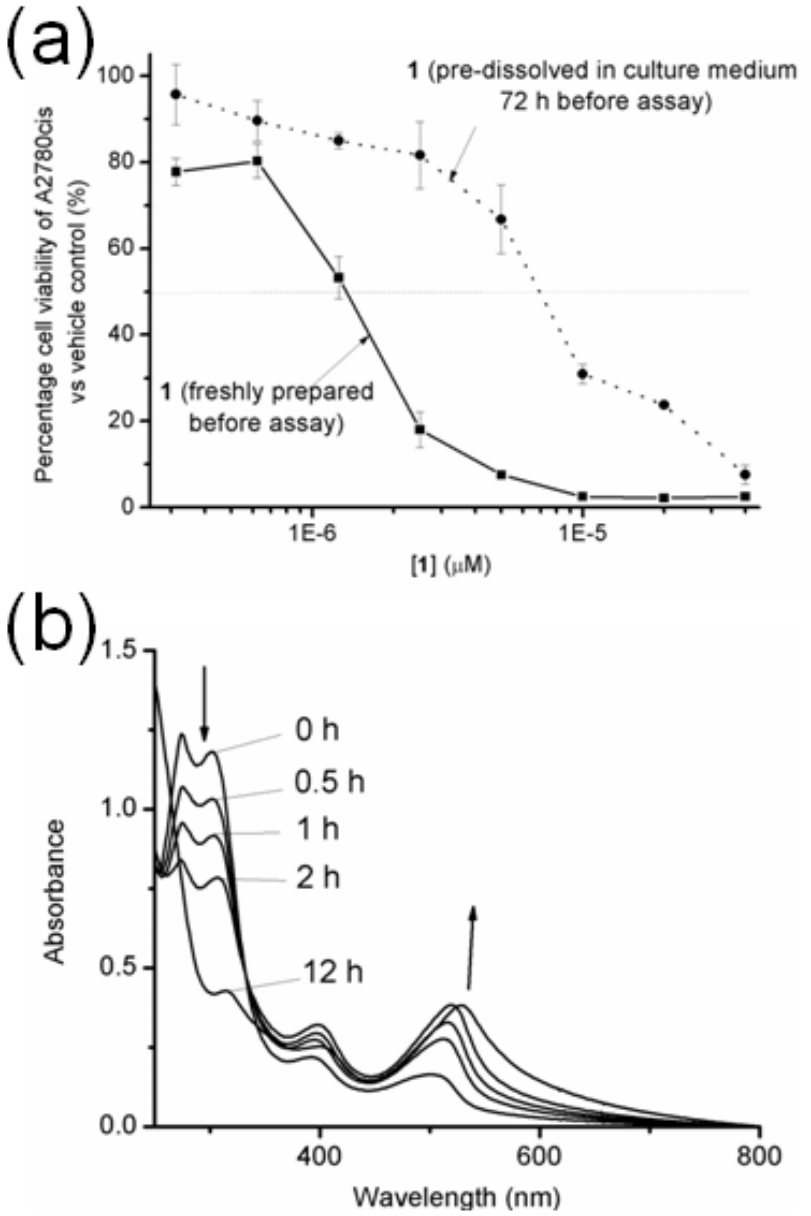


Figure 3. (a) Cytotoxicity profiles showing the change in anti-cancer activity of **1** upon standing in culture medium for 72 h. (b) UV-vis spectral change of **1** in a PBS-DMSO mixture (17: 3, v/v) in the presence of glutathione at 2 mM for 12 h.

.

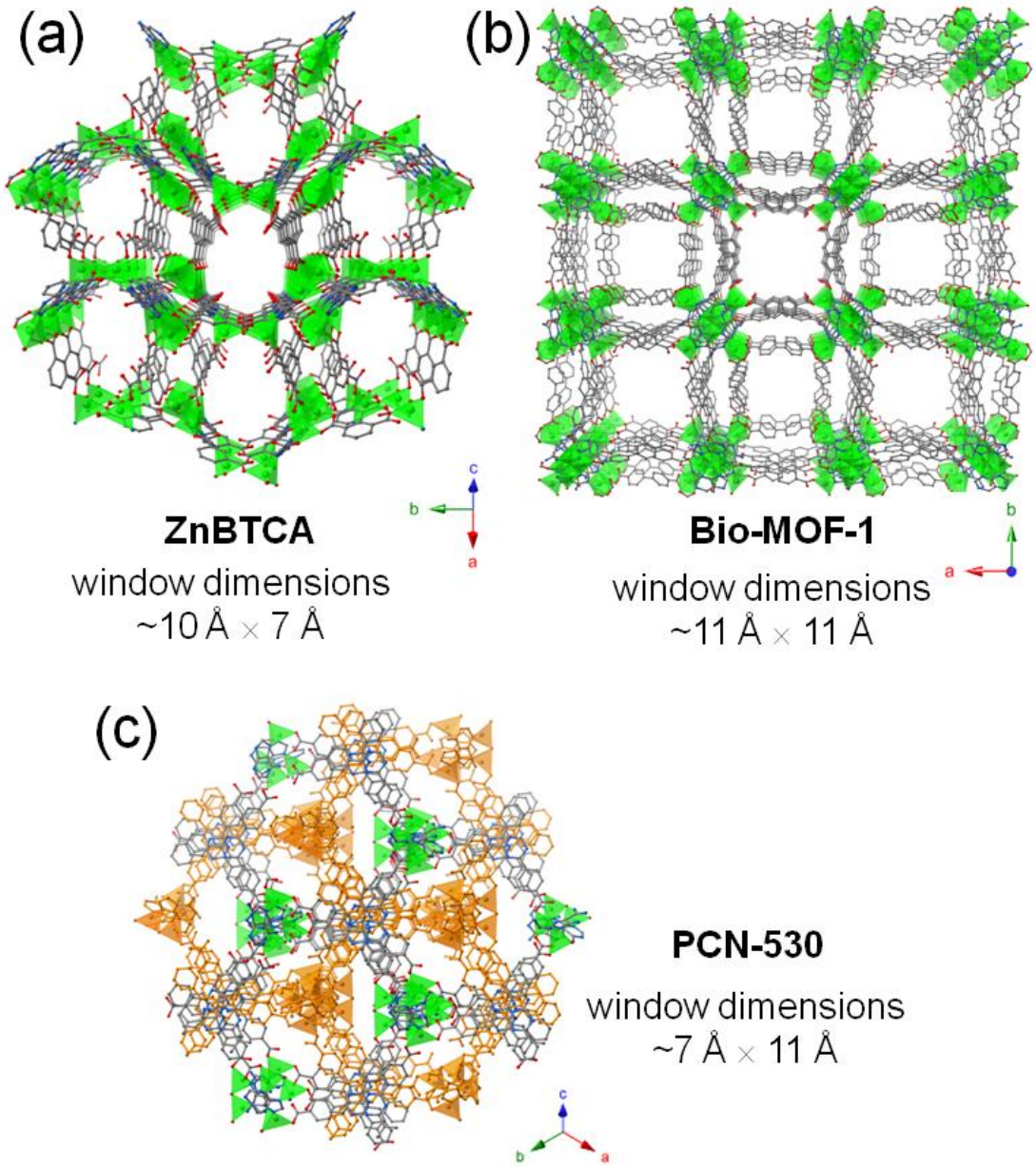


Figure 4. The guest-accessible channels of the Zn-MOFs (a) ZnBTCA [47], (b) Bio-MOF-1 [48], and (c) PCN-530 [49].

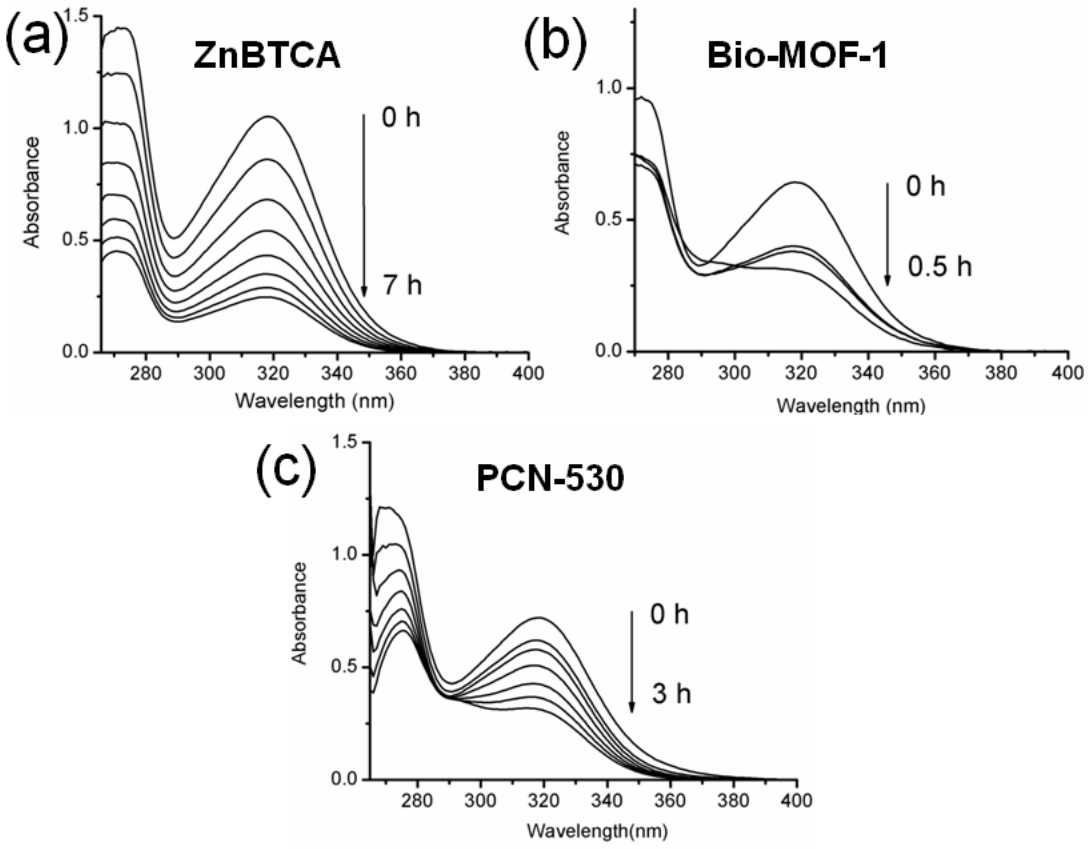


Figure 5. The time-dependent profiles of the loading of **1** to the Zn-MOFs (a) ZnBTCA, (b) Bio-MOF-1, and (c) PCN-530 as determined by UV-vis spectrophotometry. Arrows indicate the changes in absorption intensities of λ_{max} of **1** upon standing in the DMF solutions for the specific time intervals containing different types of Zn-MOFs.

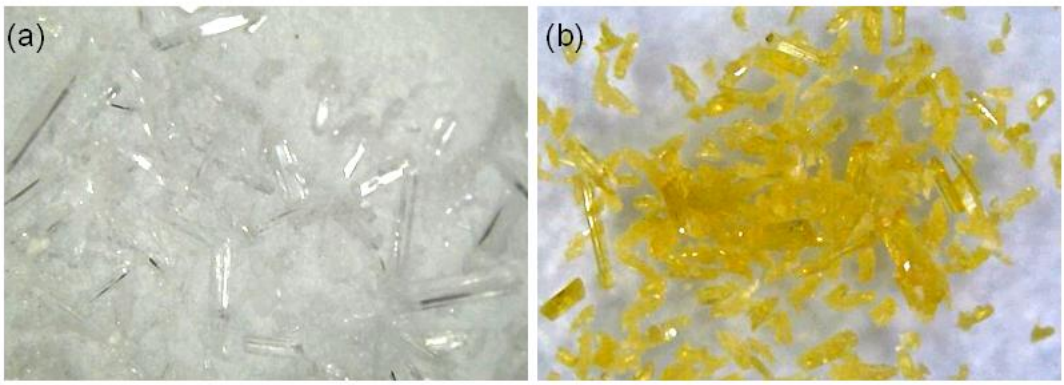


Figure 6. Photographs showing the morphologies and color change of the Zn-MOF ZnBTCA (a) before and (b) after the loading of the gold(III) complex **1**.

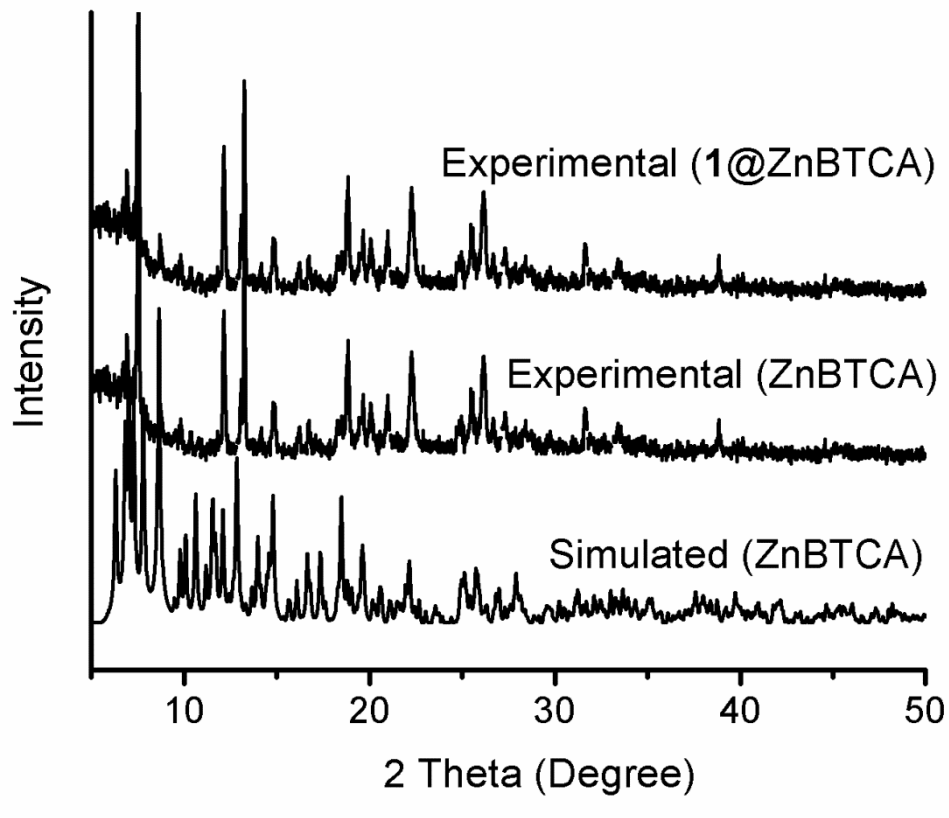


Figure 7. PXRD experiments showing the crystallinity of the Zn-MOF ZnBTCA upon the loading of **1.**

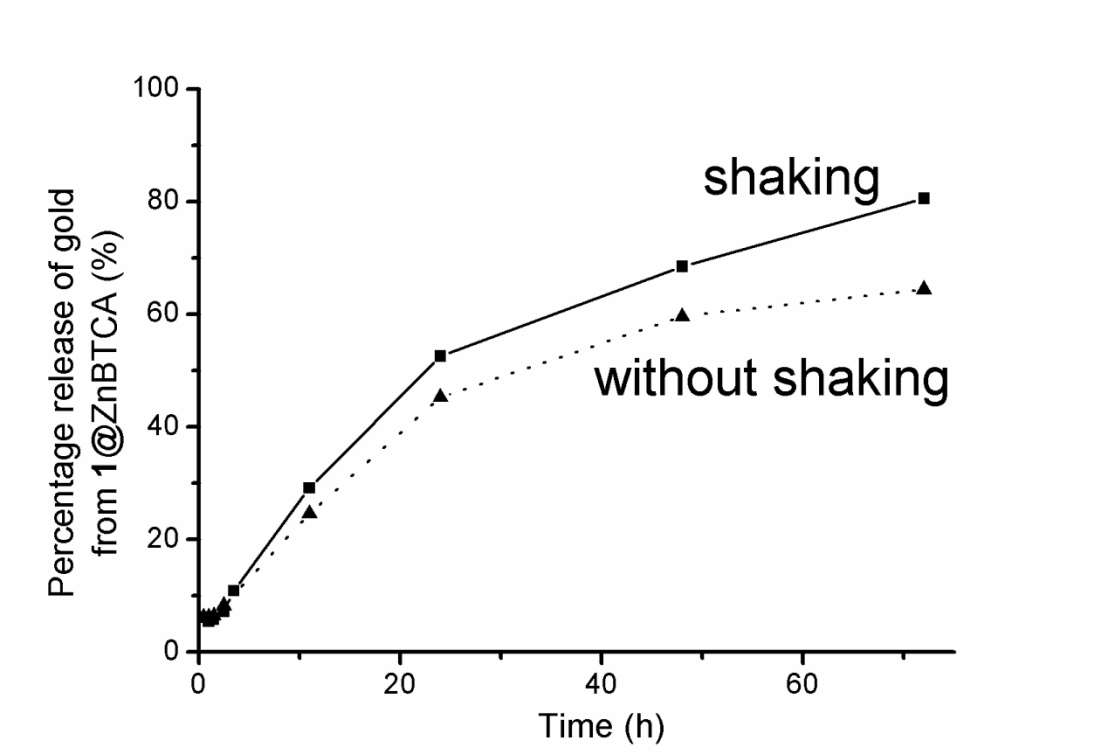


Figure 8. The gold-released profiles from **1**@ZnBTCA with or without shaking at a constant rate of 30 rpm as determined by ICP-AES.

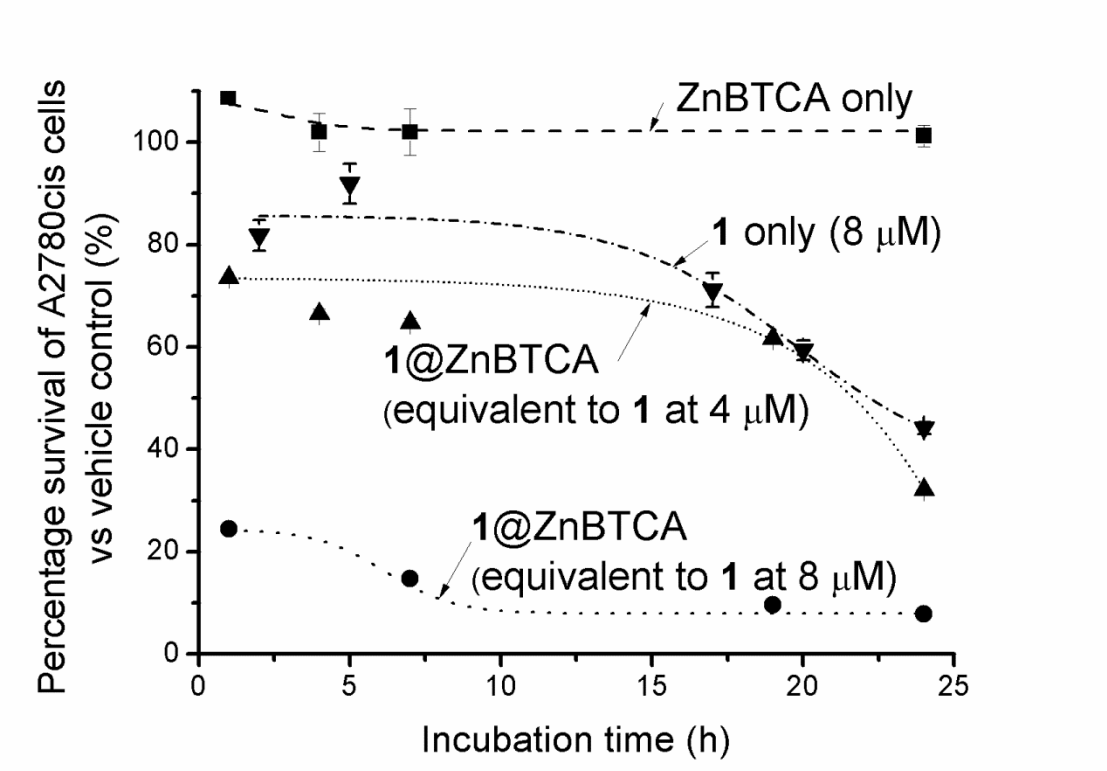


Figure 9. The 24-h cytotoxic profiles of the cells with different co-incubation time with **1**@ZnBTCA. The time-dependent cytotoxic profiles of **1** at 8 μM and ZnBTCA were also shown as references.

Table 1. The IC_{50} values (μM, 72 h) of the gold(III) pyrrolidinedithiocarbamato complex and reference compounds towards selected cancer and normal cell lines.

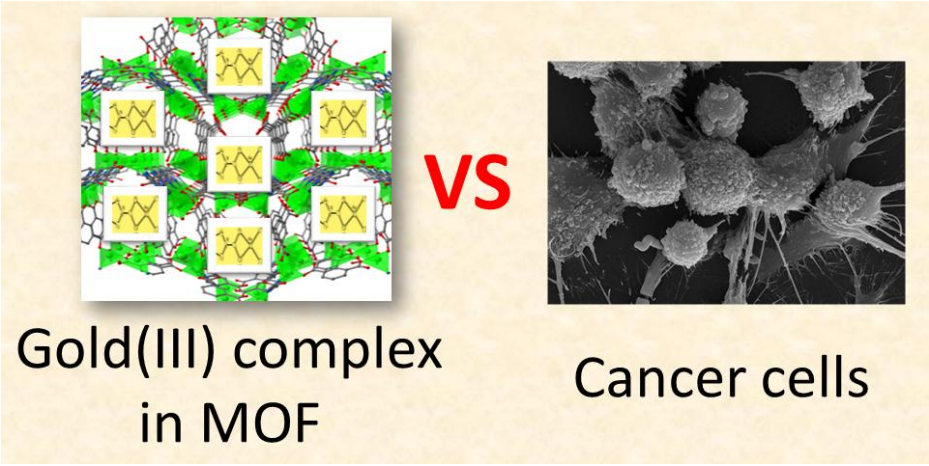
	A2780	A2780cis	HeLa	U-87 MG	B16-F10	MDCK
compound			IC_{50} (72 h) μM[a]			
1	5.1 ± 1.3	1.3 ± 0.1	20 ± 1.0	6.9 ± 0.8	21 ± 2.0	9.7 ± 2.6
cisplatin	3.2 ± 0.6	34 ± 2.4	14 ± 1.5	5.5 ± 1.0	92 ± 2.0	27 ± 3.8
PDTC	47 ± 5.1	43 ± 3.1	41 ± 0.6	30 ± 2	50 ± 1.2	> 100

[a]The IC_{50} values represent the concentrations of the complexes/ compounds causing 50% inhibition of cellular growth. Data are expressed as mean ± standard error μM and are resulted from at least three independent experiments.

TOC Figure

Enhanced anti-cancer activities of a gold(III) pyrrolidinedithiocarbamato complex incorporated in a biodegradable metal-organic framework

Raymond Wai-Yin Sun,* Ming Zhang, Dan Li,* Mian Li, Alice Sze-Tsai Wong



Incorporating this gold(III) complex in a zinc-based biodegradable metal-organic framework (MOF) displays a significant enhancement in anti-cancer activity towards A2780cis than the gold(III) complex alone.

Graphical abstract

Highlights

- Gold(III) dithiocarbamato complex and its X-ray crystal structure
- Promising anti-cancer activity towards cisplatin-resistant ovarian cancer
- Zinc-based biodegradable metal-organic frameworks (MOF) as drug carriers