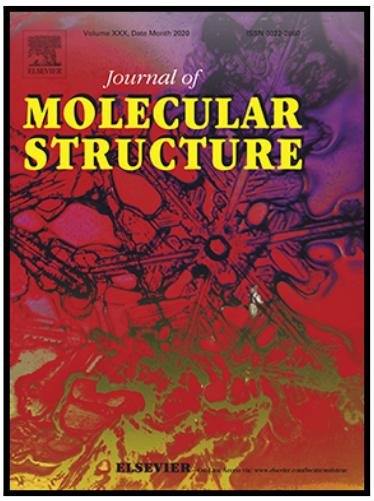

C-H Functionalization of Alkanes, Bactericidal and Antiproliferative Studies of a Gold(III)-Phenanthroline Complex

Dhananjay Dey , Afnan Al-Hunaiti , G. Vinothini , Balaji Perumalsamy , Gowdhami Balakrishnan , Thirumurugan Ramasamy , Dhanasekaran Dharumadurai , Bhaskar Biswas

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Highlights

- Synthesis and structural characterization of a square planar gold(III) complex
- Acts as an efficient catalyst towards C-H activation of a series of alkane in presence of tetrabutyl hydrogen peroxide
- Exhibits good bactericidal properties.
- Shows good cytotoxicity against A549 human lung cancer cell line through mode of apoptosis

C-H Functionalization of Alkanes, Bactericidal and Antiproliferative Studies of a Gold(III)-Phenanthroline Complex

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This research article demonstrates the synthesis, structural characterization, C-H functionalization, bactericidal activity and anti-proliferative studies of a mononuclear Au(III) complex, $[Au(phen)Cl_2]NO_3$ (**1**) [phen = 1,10-phenanthroline]. X-ray structural analysis of **1** reveals that the Au(III) complex crystallises in a monoclinic system with $P2_1/c$ space group and adopts a perfect square planar geometry. The Au(III) complex has been evaluated as an efficient catalytic system towards C-H activation of a series of alkane molecules in presence of TBHP. The catalyst exhibits moderate to excellent reactivity with good selectivity toward aldehyde or ketone when aryl alkanes are used, and ketone is formed when cyclic alkanes are tested. This catalytic reaction recommends the involvement of freely diffusing hydroxyl radicals rather than metal-based oxidant for this course of catalysis. The cytotoxic activity of the Au(III) complex have been investigated against the A549 human lung cancer cell line that induces apoptosis mode of cell death and loss of mitochondrial membrane potential are prominent characteristics as an anti cancer drug as well as antibacterial activity against *Staphylococcus aureus.*

Keywords: Gold(III); Phenanthroline; Crystal structure; C-H activation activity; Antibacterial study; Anticancer activity

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1. Introduction

Selective oxidation of hydrocarbons is an important transformation in organic, medicinal chemistry, and petroleum industry [1-6]. Oxidation of C-H bond appeals substantial attention for it's ability to functionalize natural products in spite of lengthy multistep synthesis and problem of purification. Till date, several methods have been developed for site-selective oxidation. However, very often these methods require the presence of directing groups [7]. Chemoselective oxidation of hydrocarbons has been utilized in the synthesis of various natural products. Several reagents are available for chemoselective oxidation of hydrocarbons which includes Mn, Cu, Co and Fe containing catalysts [8]. Generally, these methods require toxic reagents and very often involve high reaction temperature which is neither environment-friendly nor eco-friendly. In this respect, aryl alkanes oxidation has received monumental appeal in the past few decades in particular benzene, ethylbenzene, toluene, and o-xylene as they are usually found in petroleum derivatives (e.g. gasoline and diesel fuels) [9]. These aromatic volatile organic compounds (VOCs) are also widely functioned as industrial solvents, rubber products, and adhesives [10]. Noteworthy, the prolonged exposure to these VOCs can lead to neurological, respiratory and central nervous system damage and consequently directly influence human health [8-10].

The unselective oxidation of toluene gives a mixture of oxidized products including benzyl alcohol, benzaldehyde, benzoic acid, benzoate ester and phenol [11,12]. In the last few decades, many catalytic routes for toluene oxidation have been introduced; most of which include homogeneous catalysis using metal complexes of manganese, copper, iron(III) containing porphyrins, Schiff bases, chlorins, or triazacyclononane ligand [13]. However, poor selectivity, difficulty in ligand synthesis, tedious separation processes, and low recyclability in the homogeneous protocols made these catalytic routes inefficient for practical/industrial applications. Thus, the key challenges in homogeneous toluene oxidation processes are the large-scale production of aldehydes and benzoic acids. In this regard, and as a continuous search for developing metal dependent catalytic systems in homogeneous and heterogeneous process, gold catalysts have emerged actively in a wide range of oxidation reactions [14,15].

In this context, Hashmi and co-workers put a remarkable effort in developing gold based catalytic systems [16].

Noteworthy, serendipitous discovery of a metallo-drug, cisplatin in 1965, initiated a huge interest to the synthetic inorganic chemists for designing anti-tumour agents [17]. The effective antitumor properties of cisplatin helped to direct a new horizon in which other coordination compounds might be equally useful as anti-cancer drugs [18,19]. In this context, it is commonly observed that Au(III) compounds exhibit isostructural existence as like Pt(II) ion. Thus, square planar Au(III) complexes brought new promises as a suitable alternative of Pt based drug. Although, in comparison to platinum based metallo-drugs, Au(III) compounds seems to be unstable and undergoes effortless reduction to metallic gold which creates some difficulties for their applications in pharmaceutics under physiological conditions [20]. Attempt were already made to explore the antitumor properties of gold(III) complexes consisting of structurally different ligands [21-25]. Literature survey exhibits that the cationic part of this Au(III) complex has been used to explore itself as a metallo-therapeutic agents towards few specific cell lines in drug design perhaps no crystal structure is reported [26a]. Scientific literature shows that Akhmadullina *et al*. published a structurally similar Au(III) complex as $[Au(phen)Cl_2][AuCl_4]$ [26b]. Previously V. Amani *et al.* also synthesized a series of phenanthroline driven Au(III) compounds [26c] and studied cytotoxic behaviour of few substituted polypyridyl Au(III) complexes against skin carcinoma A431, cervical carcinoma HeLa and colon carcinoma HT-29 [26d].

Heterogenous gold have been used before in oxidation of alcohols and aldehydes and even in C-C coupling, but as homogenous catalyst was less studied. In this respect, an effort has been made to account on the emerging evidences of C-H functionalization of a series of alkanes in presence of TBHP by a mononuclear gold(III)-phenanthroline complex, $[Au(phen)Cl_2)]NO_3$. The examined catalytic behaviour in C-H activation phenomenon suggests the involvement of freely diffusing carbon-centred radicals rather than metal based oxidant. Furthermore, cytotoxic activity of the Au(III) complex against A549 human lung cancer cell line induces apoptosis of cell death. Loss of mitochondrial membrane potential is also observed which is a prominent characteristics to behave as an anti cancer drug as well as antibacterial agent.

2. Materials and methods

2.1. Chemicals, solvents and starting materials

High purity 1,10-phenanthroline (E. Merck, India), chloroauric acid (SRL, India), and all reagents were purchased from the respective outlets and used as received.

2.2. Physical measurements

IR spectrum (KBr) of **1** was recorded with a FTIR-8400S SHIMADZU spectrophotometer (Shimadzu, Kyoto, Japan) in the range 400-3600 cm^{-1}. Ground state absorption spectrum of **1** was measured in a JASCO V-730 UV-Vis spectrophotometer spectrophotometer (Jasco, Tokyo, Japan). Electrospray ionization (ESI) mass spectrum was recorded using a Q-tof-micro quadruple mass spectrometer (Waters, Milford, USA). The pH value of the solutions was measured in Systronics pH meter (Systronics, Ahmedabad, India) at room temperature. Elemental analyses were performed on a Perkin Elmer 2400 CHN microanalyzer (Perkin Elmer, Waltham, USA). GC and GC-MS were recorded on Agilent 6890N instrument (Agilent, Santa Clara, USA). GC conversion and yields were determined by GC-FID, HP6890 with FID detector, column HP 530 m × 250 mm × 0.25 μM. ^{1}H, ^{13}C NMR data were recorded on a Bruker ARX 600 spectrometers (Bruker, Massachusetts, USA) using DMSO-d_6, CD_3OD and $CDCl_3$ solvents.

2.3. Synthesis of [Au(phen) Cl_2]NO_3 (1)

An aqueous-acetic acid solution (10 mL) of phen (0.198 g, 1 mM) was added dropwise to an aqueous-acetic acid solution (10 mL) of $HAuCl_4$ (0.30 g, 1 mM) and the resulting mixture becomes turbid. At that moment ceric ammonium nitrate was added portion wise to the reaction mixture (0.550 g, 1 mmol) and kept on magnetic stirrer for 15 minutes. After that, the yellow coloured solution was filtered. The supernatant liquid was kept in air for slow evaporation. After 10-15 days, the fine microcrystalline yellow coloured compound (**1**) was separated out from mother liquor. The compound was washed with hexane and dried in *vacuo* over silica gel indicator. Yield: 0.448 g (63.9% based on metal salt). Anal. Calc. for $C_{12}H_8N_3O_3Cl_2Au$ (**1**): C, 28.26; H, 1.58; N, 8.34. Found: C, 28.20; H, 1.54; N, 8.38. IR (KBr, cm^{-1}): 3089, 3050 (ν_{C-H}), 1606, 1586 ($\nu_{C=N}$), 1384 (ν_{NO3}) (Fig. S1, Table S1); UV-Vis (λ_{max}, nm, MeCN): 271, 321, 357.

2.4. Crystal structure determination and refinement

Single crystal X-ray diffraction data were collected using a Rigaku XtaLABmini diffractometer equipped with Mercury375R (2×2 bin mode) CCD detector. The data were collected with graphite monochromated Mo-Kα radiation (λ=0.71073 Å) at 100(2) K using ω scans. The data were reduced using Crystal Clear suite, and the space group determination

was done using Olex2. The structure was resolved by direct method and refined by full-matrix least-squares procedures using the SHELXL-97 software package using OLEX^2 suite.[27,28]

*2.5. General oxidation procedures of the alkanes by [Au(phen)Cl_2]NO_3 (**1**)*

Conversion of toluene: Toluene (1 mmol) was added to a solution of catalyst (0.018 mmol) in MeCN (1ml) in overall catalyst 1: substrate: oxidant (1.8:100:400). After the addition of TBHP (70% in H_2O; 400 mmol), the reaction mixture was heated at 80^{o}C for 16h. The organic phase was extracted with Et_2O (3×1 ml), washed with brine and dried ($MgSO_4$). After filtration, the solvent of the filtrate was evaporated (rotary evaporator). The remaining mixture was separated by column chromatography (silica gel; diethyl ether: pentane=1:9 as eluent) and the product was analyzed by NMR. The detailed of oxidation of alkanes as well as characterization of oxidation products including calculation of yield are given in Supporting Information.

2.6. Antibacterial activity of Au(III) complex

The *in vitro* antibacterial activity of the Au(III) compound was tested against a range of bacterial pathogens such as *Proteus vulgaris, Staphylococcus aureus* and *Pseudomonas aeruginosa* using well diffusion method [29]. In brief, the bacterial pathogens were subcultured in nutrient broth and incubated for 37°C for 24 h. The lawn of bacterial culture was then prepared by spreading 100µL (10^6 CFU/mL) of the respective indicator strain on nutrient agar plates. The wells were made by boring the agar with a sterile borer and loaded with 100 µL of the Au(III) compound at a concentration of 1 mg/mL. All the plates were incubated for 37°C for 24 h. After incubation, the diameter of zone of inhibition (mm) for each compound was recorded and compared with the antibiotic streptomycin.

2.6.1 Minimum inhibitory concentration by resazurin assay

2.6.1.1 Preparation of resazurin

Resazurin solution was prepared by dissolving 0.015 g resazurin powder in 100 ml of sterile distilled water, vortexed and filter sterilized (0.22 µM filter), and then kept in a brown bottle to prevent exposure to light since it is sensitive to light.

2.6.1.2 Resazurin microtitre assay (RMA)

The minimum inhibitory concentration (MICs) of tested compound was detected by RMA as previously described by Norazah *et al.* (2017) with minor modifications [30]. In brief, 100 µL of Au(III) complex (a stock concentration of 2 mG/mL) was added into the first row of the 96-well round-bottomed sterile micro titre plate. All the other wells were filled with 50 µL of

nutrient broth. Two fold serial dilutions were then performed such that each well had 50 µL of the test compound in serially descending concentrations and finally, a volume of 10 µL of *Staphylococcus aureus* was added to the well in order to achieve a final concentration of 5×10^6 CFU/mL. The experiment had two set of controls: a column with normal saline as negative control and a column with antibiotic (streptomycin) as positive control. Following incubation for 24 h, the plates were stained with 10 µL of resazurin (0.015 %) and were re-incubated for another 2-4 h at 37°C for the observation of colour change. The colour change from blue to pink denoted the reduction of resazurin to resorufin and thereby indicating the presence of bacterial growth. The MIC was defined as the lowest concentration of tested compound which didn't show that colour change.

2.7. Protocol for biological activities

2.7.1 Cell Culture

Human lung cancer cell line A549 was obtained from National Center for Cell Science (NCCS), Pune, India sub-cultured and maintained in DMEM high glucose medium (Sigma-Aldrich, USA), supplemented with 10% fetal bovine serum and 2% of penicillin/streptomycin (Gibco, Thermo Scientific, USA), at 37 °C in a humidified atmosphere of 5% CO_2 in a CO_2 incubator (Thermo Scientific, USA). All experiments were performed using cells from passage 15 or less.

2.7.2 Cell Viability Assay

Stock solution of Au(III) complex was prepared in dimethyl sulfoxide (DMSO). Different concentrations of working solution were prepared from the stock which were added to wells containing 5×10^3 A549 cells per well. DMSO solution was used as the solvent control. After 24 h, 20 µL of MTT solution (5mG/mL in PBS) was added to each well and the plate was wrapped in aluminum foil and incubated for 4h at 37°C. The purple formazan product was dissolved by addition of 100 µL of DMSO to each well. The absorbance was monitored at 570 nm (measurement) and 630 nm (reference) using a 96-well plate reader (Bio-Rad, iMark, USA). Data were collected for three replicates each and used to calculate the respective mean. The percentage inhibition is calculated, from this data, using the formula:

$$\frac{\text{Mean of absorbance of untreated cells (Control)} - \text{Mean of absorbance of treated cells}}{\text{Mean of absorbance of untreated cells (Control)}} \times 100$$

2.8 Acridine orange (AO) and ethidium bromide (EB) staining

Apoptosis, the hallmark of cell death was investigated by fluorescent based staining methods AO/EB and Hoechst 33258 with some modifications [31]. Briefly, the treated cells with an IC_{50} concentration of complex Au(III) complex for 24 h were harvested and washed with cold PBS. Cell suspension was prepared with PBS to a final concentration of 5×10^5 cells/mL was mixed with 25 µL of fluorescent dyes. Working solution of AO/EB and Hoechst stains were prepared as 3.8 µM of AO and 2.5 µM of EB and 1 mG/mL Hoechst dyes respectively. The cells were stained with fluorescent dyes and observed immediately under fluorescent microscope (Carl Zeiss, Axioscope2plus) with UV filter of 450–490 nm for AO/EB and 355-377 nm for hoechst 33258.

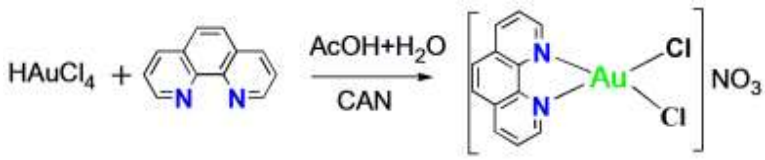
2.9 Assessment of mitochondrial membrane potential (ΔΨm) (JC1 staining)

Mitochondrial trans-membrane potential of Au(III) complex treated cells simultaneously with untreated cells was assessed using the fluorescent probe JC-1. The dye produced orange-red fluorescence when accumulated in the mitochondria of healthy cells but fluoresces green when leached out into the cytosol due to loss of membrane potential resulting in a negative internal potential [32]. The A549 cells were grown in glass coverslips (22 × 22 mm) placed in the wells of 6-well plates and treated with the gold(III) complex, at the 12 h IC_{50} concentration and DMSO was used as a solvent control. The cells were stained with JC-1 dye after 12 h exposure. The mitochondrial depolarization patterns of the cells were observed in the fluorescent microscope at ~590 nm.

3. Results and discussion

3.1. Synthesis and formulation

The mononuclear gold(III) complex is prepared out of reaction between chloroauric acid and 1,10-phenanthroline in water-AcOH medium followed by addition of CAN in the reaction mixture (Scheme 1). CAN is a versatile oxidizing agent and helps to keep the gold ions in +3 oxidation states and supplies nitrate ion in stabilizing the counter cation. The coordination geometry of **1** is determined by mainly single crystal X-ray diffraction study along with different spectroscopic and analytical techniques. The yellow crystals suitable for X-ray data collection are obtained by slow evaporation of resultant reaction mixture. The different formulations are confirmed by elemental analysis, IR, UV-Vis, mass spectral analysis, and crystallographic structural analysis of the compound along with additional DFT data.



Scheme 1. Synthetic route of Au(III) complex

*3.2. Crystal structure of [Au(phen)Cl$_2$]NO$_3$ (**1**)*

The X-ray structural determination of Au(III) complex shows that the mononuclear cationic Au(III) complex crystallizes in monoclinic system with $P2_1/c$ space group and adopts a square planar geometry around Au(III) centre. An ORTEP view of **1** with an atom labelling scheme is shown in Fig. 1. The equatorial plane around Au(III) centre forms by phenanthroline nitrogens (N1/N2) and two chlorine atmos (Cl1/Cl2). The charge of cationic unit is counterbalanced by anionic charge of nitrate molecule. The crystal packing shows the formation of an extended network of 2D sheet through strong to moderate O…H and Cl…H bonding scheme (Fig. 2). The C1-H1…Cl1, C8-H8…Cl2 distances are found as 2.68 Å and 2.61 Å respectively and may be considered as strong H bonds (C1-H1-Cl, 119˚; C8-H8-Cl2, 121˚). However, C-H…O (NO_3) interactive distances are observed in a range from C4-H4…O3, 2.34 Å, C2-H2…O2, 2.43 Å, C6-H6…O1, 2.47 Å, C6-H6…O3, 2.48 Å, C3-H3…O2, 2.57 Å in the formation of self-assembled 2D crystalline network. The crystallographic structural parameters of **1** are listed in Table 1. Selected bond lengths and angles are presented in Table 2.

A comparison of structurally close Au(III)-phenanthroline complex, [Au(phen)Cl$_2$][AuCl$_4$] with our synthesized Au(III) compound, [Au(phen)Cl$_2$]NO$_3$ is also drawn (Table 2). The crystal structure of [Au(phen)Cl$_2$][AuCl$_4$] was previously published by Akhmadullina *et al.* [26b]. In the reported X-ray structure of [Au(phen)Cl$_2$][AuCl$_4$], the average Au-N bond distance was found as 2.033 Å which is slightly longer than average Au-N distance (2.029 Å) of our Au(III) complex. However, average Au-Cl bond distance for both the complexes was identical to 2.25 Å. In both the cationic form of complexes, [Au(phen)Cl$_2$]$^+$, Au(III) centres adopt distorted square planar geometry, although distortion caused in our synthetic Au(III) complex is larger compare to that of the reported structure. This is well evident from the values of bond angles around Au(III) centre which varied from 81.77˚ (N1-Au1-N2) to 175.88˚ (Cl1-Au1-N2) for our Au(III) complex and 82.0˚ (N1-Au1-N1A) to 176.22˚ (Cl1A-

Au1-N1). Cl1-Au1-Cl2 is found perfect (90.17˚) for the square planar geometry of our Au(III) complex but the same slightly deviates from the ideal geometry (89.25˚) for the reported crystal structure. The crystal systems for both the Au(III) complex are monoclinic although space group are different; $P2_1/c$ for our Au(III) complex and $C2/c$ for the reported crystal structure [26b]. Other structural parameters differ from each other as the counter anion is entirely different for the two structures.

*3.3 C-H activation study of [Au(phen) Cl_2]NO_3 (**1**)*

The oxidation of alkanes is tested for series of different alkanes to give the corresponding carbonyl products using 1.8 mol% of catalyst in presence of 4 equivalent TBHP as oxidant. The results are summarized in Table 3. ^{1}H NMR spectra of the oxidation products are also included in Supporting Information. The oxidation of aryl alkanes in presence of the catalyst yields 49-60% acids (Table 3, entries 1-4). Same experiments are performed with secondary aryl alkanes that proceed to produce the corresponding ketones in 57 and 83% yields (Table 3, entries 5 and 6). As the oxidation of aryl alkanes proceeds in good to excellent yields, Noteworthy, in the oxidation of xylenes, the lower transformation of chloro-toluenes can be associated with the presence of an electron withdrawing group (-Cl) that inhibits the oxidation activity of the methyl group. That is, the methyl group of xylenes is more active than that of chloro-toluenes. For the same reason, the selectivity to chlorobenzaldehydes is nearly 100%, whereas in the oxidation of xylenes, the generation of the corresponding acid as byproducts is observed.

The oxidation of cyclic alkanes is further examined using cyclohexane and adamanatane. The catalyst is also capable to oxidize cyclic alkanes and the product yield is 45 and 29% (Table 3, entries 8 and 9) in the present system which, together with some selectivity, suggests its potential use in preparative chemistry. Alcohol and ketone with alcohol/ketone 0.18, entry 8 and in Adamantane Alcohol/ketone 2, entry 9.

Interestingly, Alcohol/ketone ratio of cyclohexane has previously been used to probe the nature of oxidation catalyst species. Indiscriminate hydroxyl radicals typically afford selectivity ratios for A/K oxidation less than 1, whereas more selective oxidants give substantially higher values [8]. In this context, the present catalyst system with a A/Kvalue around 0.18 (after statistical correction) is indicating the involvement of freely diffusing hydroxyl radicals rather than metal-based oxidant.

3.4 In vitro antibacterial activity

The primary screening for antibacterial ability of the compound is performed against a various bacterial pathogens using well diffusion method at a concentration of 1mg/mL. The tested compound exhibits notable antibacterial activity against a wide spectrum of indicator pathogens with varied zone of inhibition. The Au(III) compound (100µg) displays an excellent inhibitory activity (Fig. 3) against the all tested pathogens as compared to the standard drug, streptomycin. It is observed that the Au(III) compound exhibits good activity against the *Staphylococcus aureus*. Hence, the bacterial pathogen *Staphylococcus aureus* is justifiably chosen for further minimum inhibitory concentration assays. The discrepancy in the efficiency of the different compounds against various pathogens depends on different factors including compound property, concentration of the compound, species of bacteria, impermeability of the cell wall of the microbes and differences in ribosome of microbial cells [33].

3.4.1 Minimum inhibitory concentration by RMA

Resazurin is a blue non-fluorescent, non-toxic and oxidation–reduction indicator dye used for the evaluation of cell growth. The metabolically active bacteria cell irreversibly reduces blue dye, resazurin to a pink and highly fluorescent, resorufin. Resorufin is further reduced in to hydroresorufin a colourless and non-fluorescent molecule, by oxidoreductase within viable cells. Such change of colour can be observed visually and therefore spectrophotometer is not needed in this assay as compared to the conventional MA.

In the present study, the resazurin-based MA is adopted to demonstrate the inhibition effects of Au(III) compound against *S. aureus*. The MIC of the compound Au(III) complex against *S. aureus* is 3.125 µG mL^{-1} (Fig. S2, Supporting Information). Column 1 and column 5 represents the negative control (containing phosphate buffered saline, growth medium and bacteria) and the positive control (containing streptomycin, growth medium and bacteria), respectively. The inhibitory activity of the various compounds increase with the increase in the concentration and the inhibitory activity may be assignable for the disturbance in the respiration process of the bacterial cell and blocking the synthesis of proteins restricting further growth of the organism resulting in the growth inhibition [33].

3.5 In vitro cytotoxicity assays for the complex against human lung cancer cell line (A549)

The cytotoxic behavior of the gold complex Au(III) complex has been investigated against the A549 human lung cancer cell line by using MTT assay [34]. The IC_{50} value from

the assay is determined as 48 ± 0.5 µG/mL, in which dose cell survival was reduced half in comparison with untreated cells. Further the results confirm the dose dependent cytotoxicity of Au(III) complex for 24 hr incubation (Fig. 4).

3.6 Morphological observation of apoptosis

Apoptosis is a hallmark of cells that can be measured by morphological changes. Fluorescent staining such as AO/EB and Hoechst will able to determine such morphological changes in the apoptosis induced by the Au(III) complex. The results from AO/EB double-staining assay confirm that the A549 human lung cancer cell line treats with test substance for 24 hr and undergoes both early apoptosis and late apoptosis. The cells treated with Au(III) complex observed as red in color with the morphology of cell shrinkage, chromatin condensation, apoptotic bodies formation were seen mostly. While the control cells more number of green cells with normal cell features of uniform chromatin and intact cell membrane. The same kind result is observed from Hoechst staining. The changes in morphology of the cells upon Au(III) complex treatment, with special reference to cytoplasm and nucleus were observed under fluorescent microscope. The observations revealed that the early apoptotic features such as cell shrinkage, chromatin condensation, and fragmentation have seen mostly in Au(III) complex treated cells and small numbers of necrotic cells are also observed (Fig. 5). Therefore the results from the fluorescent staining procedures conclude that the Au(III) complex induces cell death through apoptosis process.

3.7 Loss of mitochondrial membrane potential (ΔΨm)

Mitochondrial membrane potential in untreated cell was preserved as it produces energy to the cell metabolism and other cellular functions. Thus targeting the mitochondria of cancer cell leads to the collapse of (ΔΨm) and subsequent release of Cytochrome C into the cytosol. In our study, the cationic dye JC-1 was used to detect the ΔΨm of Au(III) complex treated and untreated cells. The JC-1 normally accumulates in healthy mitochondria and emits red color fluorescent. Whereas ruptured mitochondria membrane fluoresce green color due to the mitochondrial membrane depolarization In our study, the complex Au(III) complex induces Loss of ΔΨm as it JC-1 stained cells emits green color under fluorescent microscopic observation. Red color emission was observed whereas staining the untreated cell (Fig. 5). Thus the result concludes in the process of cell death induced by Au(III) complex loss of ΔΨm plays a major role that it requires further elucidation.

4. Conclusions

In summary, the synthesis, spectroscopic characterization, XRD structure, C-H bond activation and in vitro bioactivities of a mononuclear Au(III) complex, $[Au(phen)Cl_2]NO_3$ (**1**) [phen = 1,10-phenanthroline] have been studied. X-ray structural analysis of **1** reveals that this gold compound crystallises in a monoclinic system with $P2_1/c$ space group and adopts a square planar geometry. The Au(III) complex has been evaluated as an efficient catalytic system towards C-H activation of a series of alkane molecules in presence of TBHP. The catalyst shows good to excellent yields when aryl alkanes and moderate reactivity for cyclic alkanes. Furthermore, it exhibits aldehyde/ketone (A/K) value around 0.18 and recommends the involvement of freely diffusing hydroxyl radicals rather than metal-based oxidant for this course of catalysis. The Au(III) complex induces apoptosis mode of cell death and loss of mitochondrial membrane potential is a prominent characteristics as an anti cancer drug. Further studies are required to view more insights about the consideration of gold based metallo-therapeutic agents. Moreover, the compound may be easily promoted to second generation metallo-therapeutic agents by displacement of chlorides with incorporation biological benign oxalate ions in aqueous phase.

Supplementary data

Supplementary crystallographic data are available free of charge from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-1223-336033; E-mail: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk) upon request, quoting deposition number CCDC 1976558.

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AUTHOR STATEMENT

Dhananjay Dey: Conceptualization, Curation, Formal analysis, Methodology, Investigation; **Afnan Al-Hunaiti:** Conceptualization, Formal analysis, Investigation, Writing; **G. Vinothini**: Solution preparation and study for antimicrobial activity; **Balaji Perumalsamy, Gowdhami Balakrishnan, and Thirumurugan Ramasamy**: Detailed study of Antitumor activity, MTT assay, analysis, write-up; **Dhanasekaran Dharumadurai:** Analysis of Antimicrobial activity; **Bhaskar Biswas**: Writing-Reviewing and Editing, Supervision.

Conflict of Interest and Authorship Conformation Form

- All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.
- This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.
- The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript
- The following authors have affiliations with organizations with direct or indirect financial interest in the subject matter discussed in the manuscript:

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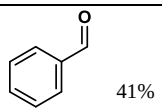
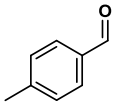
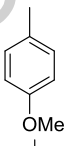
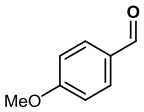
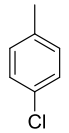
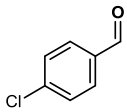
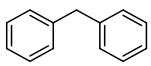
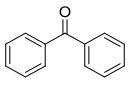
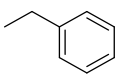
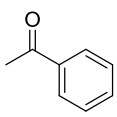
Table 1. Crystallographic refinement parameters of $[Au(phen)Cl_2]NO_3$ (**1**)

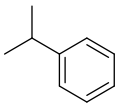
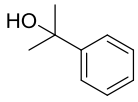
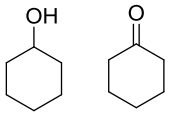
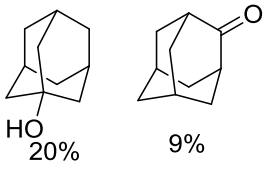
Crystal parameters	**1**
Empirical formula	$C_{12}H_8N_3O_3Cl_2Au$
Formula weight	510.08
Temperature	100 K
Wavelength	0.71075 Å
Crystal system	Monoclinic
Space group	$P2_1/c$
Unit cell dimensions	a = 7.388(3)Å α= 90° b = 13.782(4)Å ß= 98.617(11)° c = 13.269(4)Å ɣ = 90°
Volume	1335.8(8)Å^3
Z	2
Density (calculated)	2.536 Mg/m^3
Absorption coefficient	11.426 mm^{-1}
F(000)	952
Reflections collected	14049
Independent reflections	3044
R(int)	0.113
Goodness-of-fit on F^2	1.05
R indices (all data)	R1 = 0.0335, wR2 = 0.0870
Largest diff. peak and hole	2.71 and -2.53 e. Å^{-3}

Table 2. Selected bond lengths (Å) and bond angles (°) for $[Au(phen)Cl_2]NO_3$ (**1**) and a comparison of bond lengths and bond angles between **1** and cationic $[Au(phen)Cl_2]^+$ in $[Au(phen)Cl_2][AuCl_4]$

Bond lengths (Å)			
$[Au(phen)Cl_2]NO_3$		$[Au(phen)Cl_2]^+$	
Bond	XRD	Bond	XRD
Au1-Cl1	2.2580(15)	Au1-Cl1	2.254(1)
Au1-Cl2	2.2558(15)	Au1-N1	2.033(3)
Au1-N1	2.031(4)		
Au1-N2	2.027(4)		
Bond angles (˚)			
$[Au(phen)Cl_2]NO_3$		$[Au(phen)Cl_2]^+$	
Cl1-Au1-Cl2	90.17(4)	Cl1-Au1-Cl1A	89.25(6)
Cl1-Au1-N1	94.11(12)	N1-Au1-N1A	82.0(2)
Cl1-Au1-N2	175.88(11)	N1-Au1-Cl1A	176.22(9)
Cl2-Au1-N2	93.96(11)	N1 -Au1-Cl1	94.4(1)
N1-Au1-N2	81.77(16)		
Cl2 -Au1-N1	175.46(12)		

Table 3. Series of alkanes oxidation using gold phenanthroline catalyst.[a]

Entry	Substrate	Products yield [%]	Time [hr]	Total yield [%]
1[b]		 41%	16	60
2[b]		 45%	18	56
3	 OMe	 MeO	12	58
4	 Cl	 Cl	20	49
5			19	83
6[c]		 47%	16	77

7[d]			23	82
8[d]			36	45
9[d]			26	29

(a) All reactions were carried out on 1 mmol scale and all yields are isolated product using column chromatography. (b) the only by-product formed was the corresponding acid. (c) The other by-product was formed was benzaldehyde with 20% yield. (d) Yield was GC-MS yield using 1,2-dichlorbenzene as internal standard.

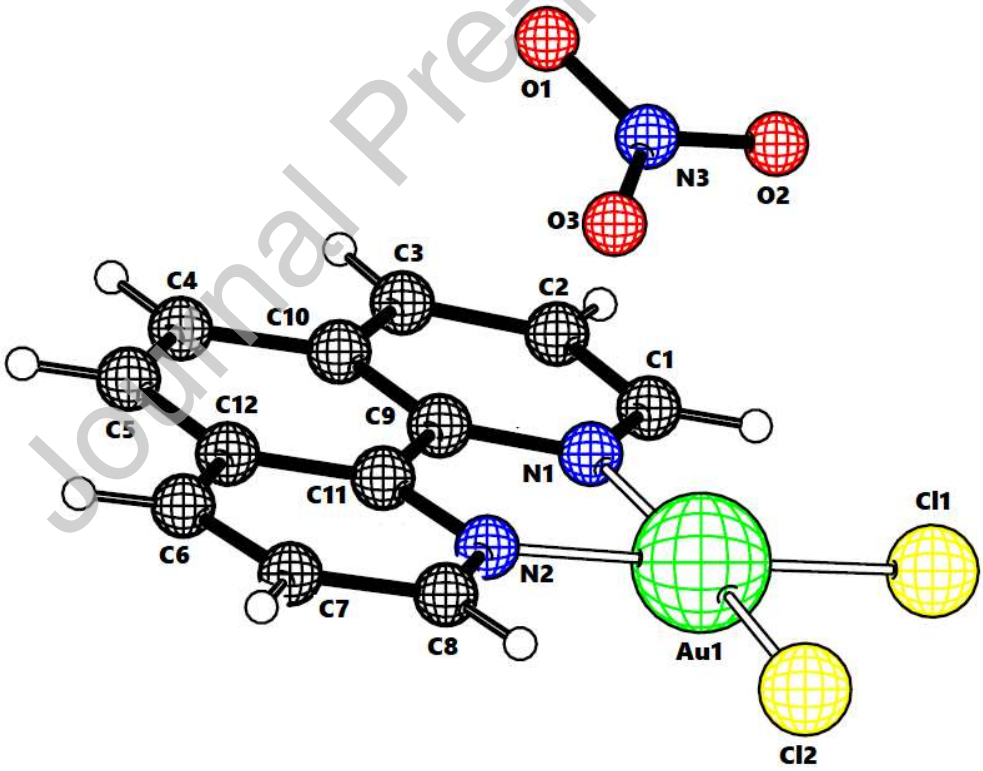


Fig. 1. An ORTEP diagram of $[Au(phen)Cl_2]NO_3$) (**1**) with atom numbering scheme and 30% probability ellipsoids.

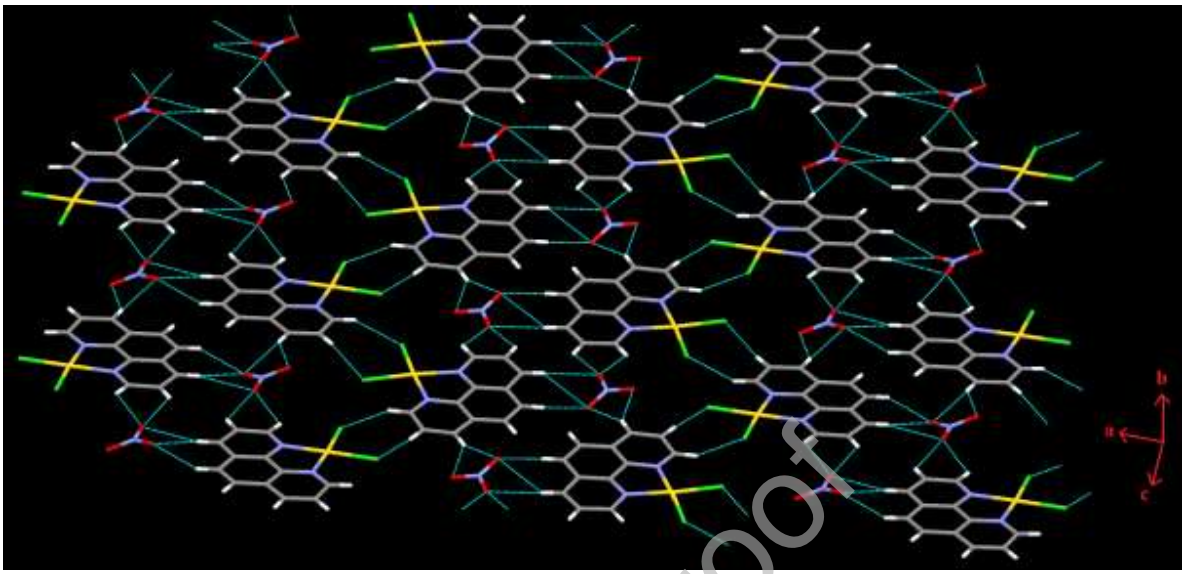


Fig. 2. H-bonded 2D network of Au(III) complex in crystalline phase

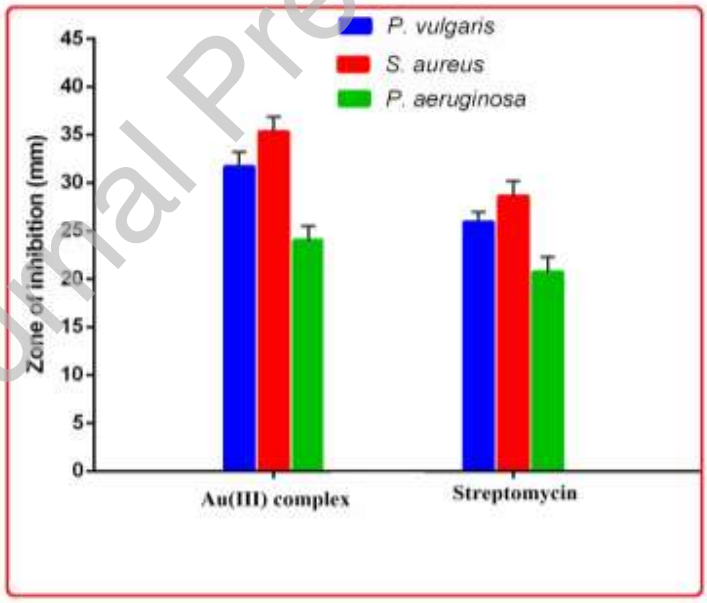


Fig. 3. Inhibitory effects of Au(III) complex and streptomycin using well diffusion assay

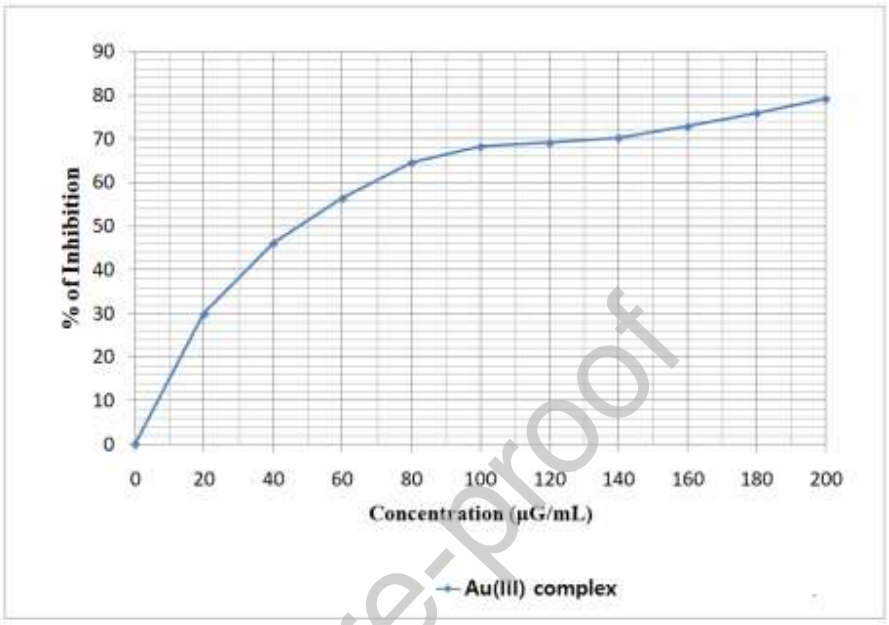


Fig. 4. Cytotoxic effect of the Au(III) complex on A549 cells after exposure for 24 h

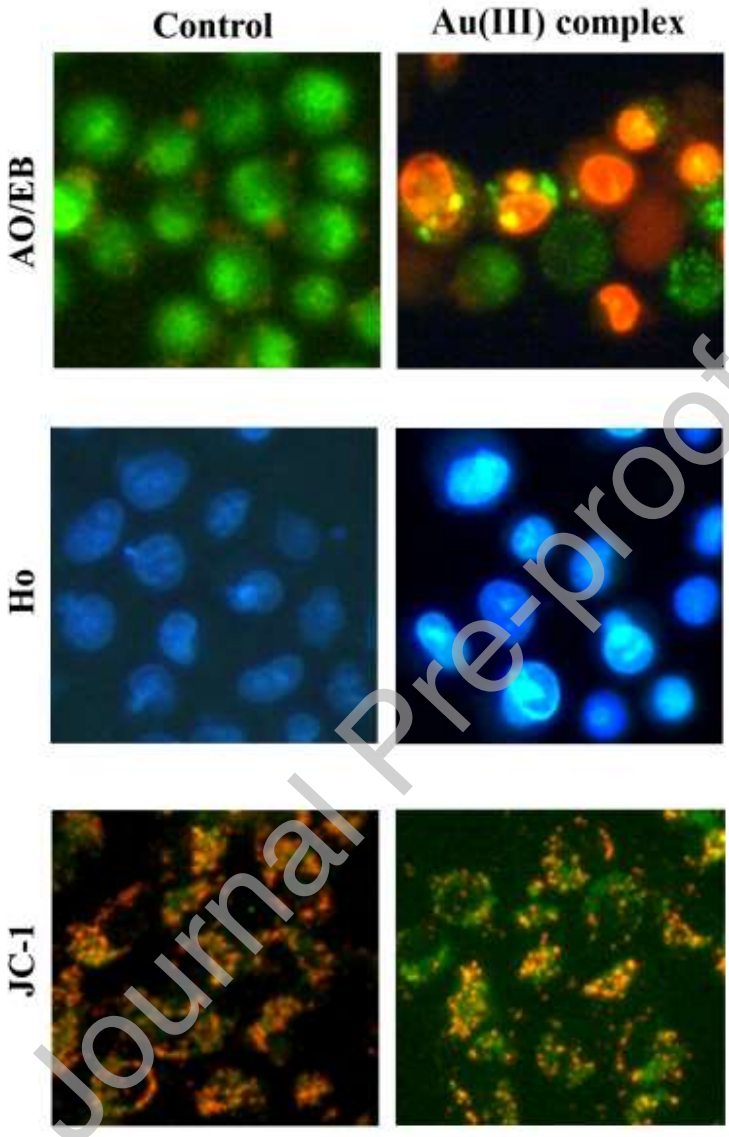


Fig. 5. Morphological changes in the apoptosis induced by Au(III) complex through AO/EB, Hoechst, and JC-1 Staining. Control: Au(III) complex untreated cells

GRAPHICAL ABSTRACT

The present study aims to synthesis, characterization and C-H functionalization of a series of alkanes with good catalytic activity as well as evaluation of antibacterial and anticancer property against A549 human lung cancer cell line by a mononuclear gold(III) complex, $[Au(phen)Cl_2]NO_3$