

Design, Synthesis, Characterization and Comparative Cytotoxic Evaluation of bis-(2-mercaptoacetate) gold (III) chloride

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Abstract

In recent years, the interest in gold (III) species have gained more and more attention for cancer chemotherapy, this was stimulating by the possibility to develop new agents with mode of action and clinical profile different from the established platinum metalodrugs.

With this frame, recently new square planar Au(III) complexes (Au(L)(L')_n); where L=SCH₂COO⁻; L'=HSCH₂COO⁻ had been synthesized with S/O – donor ligands.

In this article and by the aim to replace, one of (L') ligand by anion chloride ligand (which supposedly more relevant for the biodistribution of the compound than for its pharmacodynamic effects), new complex (Au(L')₂Cl) was synthesized:



The prepared complex was characterized by elemental analysis, FTIR, H¹NMR, UV, molar conductance and magnetic susceptibility and its anticancer activity was tested against Hep-2 cell line.

The comparative figure of the cytotoxic activities of all the synthesized complexes against Hep-2 cell line, showed cytotoxic profile variant to the well-known anticancer drug cisplatin, indicated that there is no direct correlation exists between the nature of the ligands (L),(L') and (Cl⁻), their different arrangements around central metal core with their collectives cytotoxic results.

Key words: S/O – donor ligands, gold (III), cytotoxic

تصميم، تحضير، تشخيص وتقييم ضد سرطاني و مقارن للمعقد bis –(2-mercapto acetate) gold (III) chloride

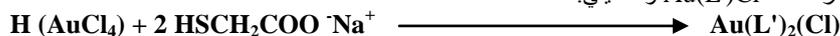
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الخلاصة

لقد حضرت مركبات الذهب (III) في السنين الأخيرة باهتمام أكثر وأكثر كعلاج ضد السرطان وتنتج هذا من خلال تحضير و احتمالية ظهور مركبات جديدة تمتاز بفعالية وصفات سريريته مختلفة لما اثبت سابقا للمركبات البلاتين. ومن هذا الإطار فقد تم مؤخرا¹ تحضير معقدات الذهب (III) بصيغة (Au(L)(L')_n)، عندما L'=HSCH₂COO⁻, L=SCH₂COO⁻ بليكاندات مانحة من نوع S/O.

في هذا البحث ويهدف استبدال, واحدة من ليكاند (L') ببايون الكلوريد (والمقترح أن يكون له علاقة بالانتشار البايولوجي أكثر من تأثيره الدوائي). وعليه فلقد حضر المعقد Au(L')Cl وكما يلي:



تم تشخيص المعقد المحضر بالتحليل الدقيق للعناصر, FTIR, HNMR, UV, التوصيلية المولارية والقابلية المغناطيسية أما فعاليته ضد السرطان فقد اختبرت ضد خلايا Hep-2 السرطانية.

أن الشكل المقارن لفعالية ضد السمية المعقدات ضد خلايا Hep-2, أظهرت صيغة لفعالية مختلفة للدواء المعروف سينربلاتين والمستخدم ضد السرطان, وهذا يؤشر انه لا يوجد علاقة بين طبيعة الليكاند سواء كان (L), (L') أو (Cl) وترتيبهم المختلف حول النواة المركزية Au(III) مع النتائج ضد السمية مجتمعة².

الكلمات المفتاحية: الذهب (III), الدراسة السمية, ليكاندات واهية - S/O.

Introduction

Cancer is a major health problem. Numbers are evident: 10 million cases are diagnosed each year⁽¹⁾, and in 2020, new cancer cases are predicted to have doubled to a 20 million per year⁽²⁾.

In the past, inorganic compounds were applied in an empirical fashion with little attempt to design the compounds used and with little or no understanding of the molecular

basis of their mechanism of action. The development of modern medicinal inorganic chemistry has been made easy by the inorganic chemist's extensive knowledge on coordination and redox properties of metal ions. Then, systematic consideration of specific properties of metal ions, their patterns of tissue uptake and distribution in organisms

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and their preferred coordination in complexes has opened up the possibility for inorganic chemists to contribute to the health and well-being of man.

The unexpected success of cisplatin in treating a fairly wide variety of cancers, however, was slightly obscured by the evidence of serious kidney toxicity and other side effects, natural and acquired resistance to cisplatin and the reduced therapeutic indexes that could be used considering toxicity limitations⁽³⁾.

Consequently the development of novel metallodrugs with pharmacological profile different from that of the platinum drugs in the focus of modern medicinal chemistry and drug design.

Gold therapies have been historically used to treat rheumatoid arthritis which led to the discovery that such treatments also lowered the risk of several types of cancer, and subsequently to increased interest in their application as anticancer therapies⁽⁴⁾. Gold (III) compounds, in particular, are being explored because they are isoelectronic to the Pt(II) ion found in cisplatin⁽⁵⁾. Thus it was originally thought that structural analogues of cisplatin would demonstrate a similar reaction mechanism, however, it has since been demonstrated that gold(III) coordination complexes tend to show a much lower binding affinity to DNA as compared to cisplatin⁽⁶⁻⁷⁾. Despite this, gold compounds appear to be a particularly strong avenue to pursue, since gold complexes tend to have strong cytotoxic potential in the micromolar or even nanomolar ranges⁽⁸⁾. Indeed, gold complexes appear to be more cytotoxic than other analogous, isoelectronic metal complexes⁽⁹⁾. Hence, gold species might give access to a class of non-platinum metal compounds with non-cisplatin-like pharmacodynamic and pharmacokinetic properties, which are major goals of bioinorganic and bioorganometallic medicinal chemistry research⁽¹⁰⁾.

The renewed interest toward gold(III) compounds as potential antitumour agents, that started at the beginning of 1990s, has resulted, until now, in the identification of several gold(III) and organogold (III) compound characterized by a significant structural variety and by encouraging in vitro pharmacological properties. Notably, these gold (III) compounds constitute today an interesting family of new cytotoxic agents that undoubtedly deserve more extensive pharmacological testing and a careful analysis of their mechanism of action⁽¹¹⁾.

Many gold(III) complexes have been evaluated against an *in vitro* panel of human tumour cell lines comprising cells of different tissue types and different responses to cisplatin. Initial *in vitro* studies indicated that the carcinoma cell line are sensitive to these compounds⁽¹²⁻¹⁴⁾.

A rational design of new potential organometallic drugs, with increased biological activities and the potential to overcome resistance, selectivity issues and toxicity, is possible nowadays due to the large diversity of structure and bonding modes (π -coordination, metal-carbon multiple bonds, etc) that can be tuned. The next stage in drugs design has to be the development of high-complex drugs that deal successfully with transport (through membranes), survival in the cell, binding to DNA and excretion mechanisms with minimum side effects where both metal coordination and hydrogen bonding more likely are the key factors⁽¹⁵⁾.

However, developing drugs with metals incorporated in the structure is not an easy mission. It is important to specify which parts of the complex are essential for activity: the metal itself, the ligands, or the entire complex.

Recently, we had synthesized a novel Au (III) complexes of thioglycolate and mercaptoglycolate ligands. The cytotoxic evaluation revealed that the Hep-2 cell line differ in its sensitivity toward the selected complexes compared to cisplatin⁽¹⁶⁾.

In order to better understand the role of ligand or the metal core involvement of biological action of this type of metal-based anticancer compounds, or at least to establish some structure activity relationship that could be applied in the design of more effective drugs, it is paramount involved in the biochemical interaction, in addition the drug design strategy for this novel gold (III) complex was motivated by the aim to replace one of the mercaptoglycolate $L' = HS-CH_2-COO^-$, by chloride ligand (good leaving group) which is supposedly more relevant for the biodistribution of the complex than for its pharmacodynamic effects.

The prepared complex $(Au(L')_2Cl)$ is also designed with a two monodentate ligands (thioglycate and chloride) that could be hydrolyzed and thereby make more than one site for substitution. The potential interest lies mainly in the facility of modifications of the ligand moiety, which could help in the tuning of the biological properties. It is also of major

importance to consider the expensive and highly demanding and time consuming clinical trials of a researcher is truly serious about developing realistically useful drug.

The mentioned complexes essentially correspond to a square planar geometry, since its diamagnetic (Au(III) d^8), while octa- and tetrahedral are paramagnetic (Au(III) and Au^0 are d^{10}), as this confirmed by electronic spectra assigned to $^1A_{1g} \rightarrow ^1A_{2g}$ and $^1A_{1g} \rightarrow ^1E_g$ transition; specifically related to square planar but not octa or tetrahedral geometry. These complexes have different sets of donor atoms. In all cases, of the previous compounds and the new analogue, the remaining donors are O/S – ligands – Now it is clear that multidisciplinary

research is needed to define the main factors involved in the structure-activity relationship of all drugs that later will help in an increasingly purposeful design of new and more effective metal-based therapeutics.

The potential interest lies mainly in the facility of modifications of the ligand moiety, which could help in the tuning of the biological properties.

Thus, the main goal of this article is to enlarge the scope and repertoire of gold (III) complex of potentially interest as anticancer agents through characterization of their chemical structures and preliminary comparative cytotoxic studies.

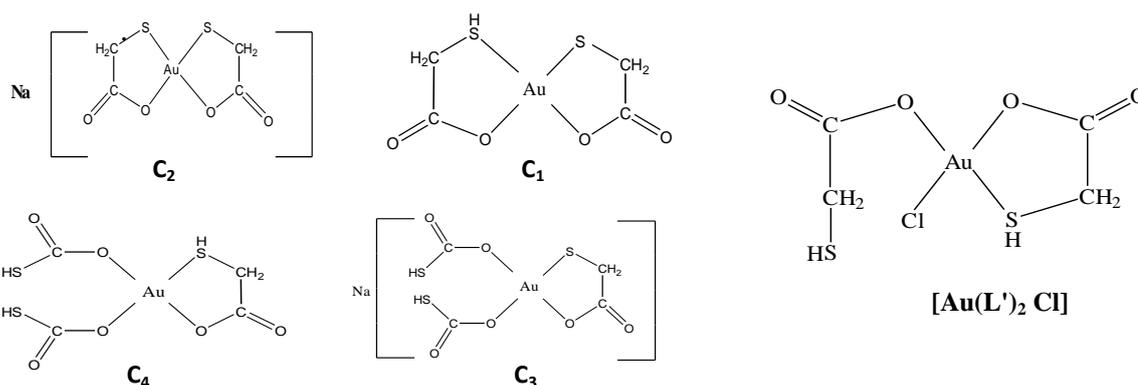


Figure (1) : The chemical structures of the previously synthesized complexes (C1-C4)(16) and the target complex of this article (Au(L')₂Cl).

Experimental

Chemistry

All chemicals were of reagent grade quality and were purchased from commercial sources (BDH and Fluka). They were used without further purification.

IR spectra were recorded on Bruker Tensor 2710 (FTIR) spectrophotometer in the 4000-250 cm^{-1} range using CsI disc. Electronic spectra were recorded on Shimadzu UV 160 spectrophotometer for $10^{-3}M$ solution of the complexes in dimethylformamide using 1 cm quartz cell. The 1H NMR spectra were recorded on Bruker/ Hims University, Syria.

Spectrophotometer in DMSO- d_6 at room temperature conductivity measurements were made on conductivity meter 4070 Jenway. The magnetic measurements were carried out at 25°C on the solid state by Faraday's method using Bruker BM6 instrument. Metal content analyses were made on Shimadzu AA670 atomic absorption spectrophotometer. Elemental analysis (C H S) were carried out using Perkin Elmer 2400 in Al Bait's University / Jordan.

Preparation of disodium 2- thioglycolate (L')

The ligand was prepared according to the following general method. The reaction of an equivalent amount of NaOH (4.00g, 0.01 mol) and mercaptoacetic acid sodium salt (1. 12 g, 0.01 mol) in 30 ml ethanol. The mixture was boiled under reflux for 3h.

Preparation of (Au(OCOCH₂SH)₂Cl)= (Au(L')₂Cl)

A solution of the ligand mercaptoacetic acid sodium salt (0.22g, 0.002 mol.) in 15 ml ethanol was mixed with a solution of H(AuCl₄) (0. 34 g, 0.001 mol.) in 15 ml ethanol in (1:2) molar ratio. The reaction mixture was refluxed for 2 h., the mixture was left 24 h. at room temperature to give a brown precipitate, which was filtered off, washed with ethanol and diethyl ether and then dried under vacuum for several hours.

Stability in Buffer

The stability test was run on the synthesized compound. A minimum amount of dimethyl sulfoxide (DMSO) was used to dissolve the complex, which was then diluted in phosphate buffer (pH 7.4), a solution of concentration $5.0 \times 10^{-5}M$ was made and

observed daily for a period of 7 days. The sample allows to stored in a dark environment throughout the 7-day period. There appears to be no significant shift in the absorption maxima at 320 nm, which is the absorption that arises due to the gold(III) metal ion.

Cytotoxic study

Preliminary cytotoxic test

Human Larynx epidermoid carcinoma (Hep-2) was kindly provided by the Iraqi center for Cancer and Medical Genetics Research ICCMGR). This human cells grew rapidly ,doubling themselves in 2-3 days and were shown to be extremely resistant to ultraviolet Rays ⁽¹⁷⁾. Passages 227-229 were used throughout this study and RPMI-1640 was used in maintaining the cells since they had been adapted at the Iraqi Center for Cancer and Medical Genetics Research to grow on this medium, & were grown in Rosswell Park Memorial Institute (RPMI) 1640 Medium (Gibco ,USA) , which was prepared as follows

- RPMI 1640 medium powder was dissolved in approximately 600 ml of double distilled water (DDW) and then the other components added :
- 0.5 ml ,Streptomycin (1g/5ml)
- Sodium bicarbonate (4.4%) 15 ml to give the final pH of 7.2 Sodium bicarbonate solution was prepared by dissolving 4.4 g. in 100 ml D.W. The solution was autoclaved at 121 °C for 15 minutes and stored at 4°C(18)
- 0.5 ml Benzyl Penicillin G (600 I U /5ml)
- 2.5 mg Amphotericin B.
- 100ml fetal calf serum.

The volume was completed to one liter with DDW. Then the mixture was sterilized using Seitz filter and filtration repeated using 0.2 μm filter unit. The sterilization was done in a sterile environment, then stored at 4 °C for direct use. All antibiotics were freshly prepared.

The growth medium was decanted off and the cell sheet washed twice with phosphate buffered saline (PBS), composed of:

- Sodium chloride (NaCl) 8 g.
- Disodium hydrogen phosphate (Na₂HPO₄) 0.9 g.
- Potassium dihydrogen phosphate (KH₂PO₄) 0.2 g.

After dissolving all components, the solution was autoclaved at 121°C for 15 min and then stored at 4°C prior to any usage, PBS was warmed to 37°C.

Cells were regularly subcultured when monolayers were confluent. Two to three ml of warm trypsin-versene (prepared by mixing 20 ml of trypsin solution, 10 ml of versene

solution and 370 ml PBS and stored at 4°C). Were added to the sheet and the flask rocked gently ⁽¹⁸⁾.

Preparation of 3-(Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium bromide (MTT) solution (Sigma ,USA)

Fifty milligram per ml of MTT dye was used as a final concentration ⁽¹⁹⁾. The solution was filtered through 0.22μ syringe filter to remove any blue formazan product ⁽²⁰⁾, and then stored in sterile , dark ,screw -capped bottles at 4 °C. The solution was used within no longer than 2 weeks of preparation.

Cytotoxic Assay on Hep-2 Cell Line:

This step must be prepared under aseptic condition. The complex was prepared for microtitration assay by dissolving 2 mg. of each compound in 2 ml of solvent (0.2 ml DMSO & 1.8 ml DDW, the stock concentration is 1000μg/ml) and filtered by 0.22 μ Millipore filter .Serial dilutions of each compound (2.50, 1.25, 0.625 & 0.313) μ mol./ml under assay in SFM were added to the well. Three replicates were used for each concentration of either four tested complexes in addition to cisplatin (EBEWE, Austria Europe) as a reference (D: positive control)

When the cells are exactly in the exponential phase in the population doubling time (PDT) , then the cells in full of their activity , the cells were collected after adding trypsin /versin (2-3 ml) not more than 10 min. ,then concentrated into known volume with SFM. Afterwards, 0.2 μ ml of cells in growth medium were added to each well of sterile 96-well micro titration plate. The plate was sealed with a self adhesive filer, placed on CO₂ incubator at 37°C for not more than 24 hrs. (For cell adherence) .After cells attachment, the plate was checked-out for contamination and the media were removed.

Serial concentrations were added and three replicates were used to each concentration and negative control (cell with SFM only), the exposure time was 72hrs.

After the exposure time was finished , the mixtures of analogues and media were removed and a fresh SFM was added to all wells ,and incubated for 24 hrs at 37°C to give chance if the affected cells and not damaged being repaired by self repairing system. Then the media was removed from the plate and washed PBS. A 0.2 ml of MTT working solution dye was added to each well and incubated at 37 °C for 3hrs.

At the end of last incubation period the dye was removed from the plate and the well washed with warm PBS twice, then 0.2 ml DMSO was added to each well to dissolve the

MTT–formazan crystals, during that we added 25 μ l of glycine buffer to each well containing DMSO. Finally the plate became readily for reading by ELISA reader at 570 nm.

Statistical Analysis

Experimental data were analyzed using statistical software SPSS 17.0 for Windows. Significance between control and samples was determined using Students't-test. A P value \leq 0.05 was considered statistically significant.

The results were expressed as percentage of viability which was calculated as the percentage of the mean of absorbance compound to the control.

The IC_{50} , which is the lowest concentration that kill 50% of cells⁽²¹⁾ was calculated according to Wilson⁽²²⁾.

Table(1) : Physical Properties of the complex

Complex	Colour	m.p (°C)	Yield %	Elemental analysis%				Λ_{oh}^{-1} _m	μ_{eff} (B.M)
				Found/(calc.)					
				C	H	S	Au		
(Au(HSCH ₂ COO) ₂ Cl)	Brown	360 ^d	75	11.51 (11.58)	1.42 (1.45)	15.41 (15.44)	47.50 (47.53)	11	Dia

Table(2) : Electronic and Infrared Specification of the complex

UV. VIS λ_{max} (Cm ⁻¹)	IR bands (cm ⁻¹)						
	v assy (coo)	V sym (coo)	Δv (Vas-Vsym)	V (c-s)	V (Au-o)	V (Au-s)	V (Au-cl)
30000, 27248, 44050	1580s	1415s	165	845s	486m	340m	290m

The most important diagnostic feature of IR spectra of the complex was listed in Table 2. The most significant information on the geometry of this complex came from the analysis of caboxylate and thioether absorption region. Stretching frequencies of these functional groups are closely related to the way in which they are coordinated to the metal ion⁽²⁴⁾. The IR spectra of the complex showed broad and intense bands ranging between 1580 and 1415 cm⁻¹ assigned for asym v(COO⁻¹) and for sym. v (COO⁻¹) respectively.(Table 2). The magnitude of Δv ($\Delta v = v$ asym COO⁻¹ - v symCOO⁻¹) were in the range 150-180 cm⁻¹ suggested monodentate bonding of carboxylic group to metal ion⁽²⁵⁾. Further support for this argument came from the IR of the complex which showed a new band at 486 cm⁻¹ attributable v (Au-O). The (C-S) band of the ligand was observed at 845 cm⁻¹, upon coordination with metal ions in complexes it was Shifted to lower frequency values (Table 2). They also showed a band in the region 290 cm⁻¹, which may due to v (Au-Cl) vibration frequency⁽²⁶⁾. The (C-S) band of the ligand

Results and Discussion

Chemistry

The thioglycolate ligands form stable, colored solid and acts as monodentate (O), chloride and bidentate (O/S) with Au(III) ion. The complexe is thermally stable and insoluble in organic solvents. However, fair solubility was attributed in DMF and DMSO. The 10⁻³M solution in DMSO display molar conductance equal to value 11 ohm⁻¹ cm⁻² mol⁻¹ indicating non electrolytic (neutral) nature of thecomplexe⁽²³⁾. This is consistent with stoichiometry for the complexes on the basis of analytical data.

was observed at 860 cm⁻¹, upon coordination with metal ion in complex, it was shifted to lower frequency value (Table 2) . Further support for this coordination has provided from the appearance of new band in the 340cm⁻¹ ranges which are tentatively attributed to v(Au-S)⁽²⁷⁾.

The ¹HNMR spectra of the complex were recorded in DMSO d₆ and show the signal of the coordinated ligand.

For the tested complex, it shows a band at 3.01-3.89 ppm which can be attributed to SCH₂CO group of each ligand and SH proton of 1.67 and 1.71 ppm. Also two bands can be assigned to each OH₂ in the SCH₂CO at 2-258 ppm and the thiol protons of 1.07 and 1.21 ppm,. The diamagnetic nature of the Au(III) complex is consistent with normal square-planar geometry around Au(III) ion⁽²⁸⁾.

Electronic absorption spectra of the complexes in DMSO are listed in Table2. In the spectrum of the ligand the π - π^* transition were observed at 36232 and 33200 cm⁻¹. The spectrum of the complex shows new bands at 2500-26666 and 27248-32845 cm⁻¹ assigned to

$^1A_{1g} \rightarrow ^1A_{2g}$ and $^1A_{1g} \rightarrow ^1E_g$ transition respectively⁽²⁹⁾, these bands correspond fairly well to a square planar geometry around the Au(III) ion. Also the band at 40050 cm^{-1} is tentatively assigned as ligand charge transfer transition. Similar results were found in Pt (II) and Au(III) complexes of the (M(diimine) (dithiolate)) type⁽³⁰⁻³⁴⁾.

Cytotoxic study

Methylthiazolotetrazolium (MTT) assay was employed to assess cell viability. MTT assay was based on the ability of the viable cells to reduce soluble yellow MTT to blue formazan crystals. In this assay, optical density (OD) values represented the absorption of formazan dissolved by DMSO at 570 nm ⁽³⁵⁾.

The comparative cytotoxic properties of synthesized Au(III) complex ($\text{Au(L')}_2\text{Cl}$) and previously synthesized (C_1 - C_4) were tested

against Hep-2 cell line; well-known resisted to cisplatin⁽³⁶⁾. In most cases the investigated compounds showed relevant in vitro anticancer properties with IC_{50} values generally falling in the low μM concentration, additionally, these compounds turned out to overcome largely resistance of Hep-2 cell line to cisplatin (Figure 2 and Figure 3).

The application of T-test, show that the potency parameter (IC_{50}) of the tested complexes (C_1 - C_4), although not significant, it represent high potency compared to the reference compound (Table 3), the lack of cross-resistance suggests that gold (III) induce cytotoxicity through different mechanism. So, S-ligands (L) & (L') are crucial both in stabilizing the Au (III) center and in carrying the metal to its cellular targets⁽³⁷⁾. i.e. to pharmacokinetic.

Table(3): IC_{50} of $C_1 - C_4$ compared to cisplatin

C_1	C_2	C_3	C_4	D (cisplatin)	($\text{Au(L')}_2\text{Cl}$)
0.39	0.45	0.45	0.125	0.325	0.56

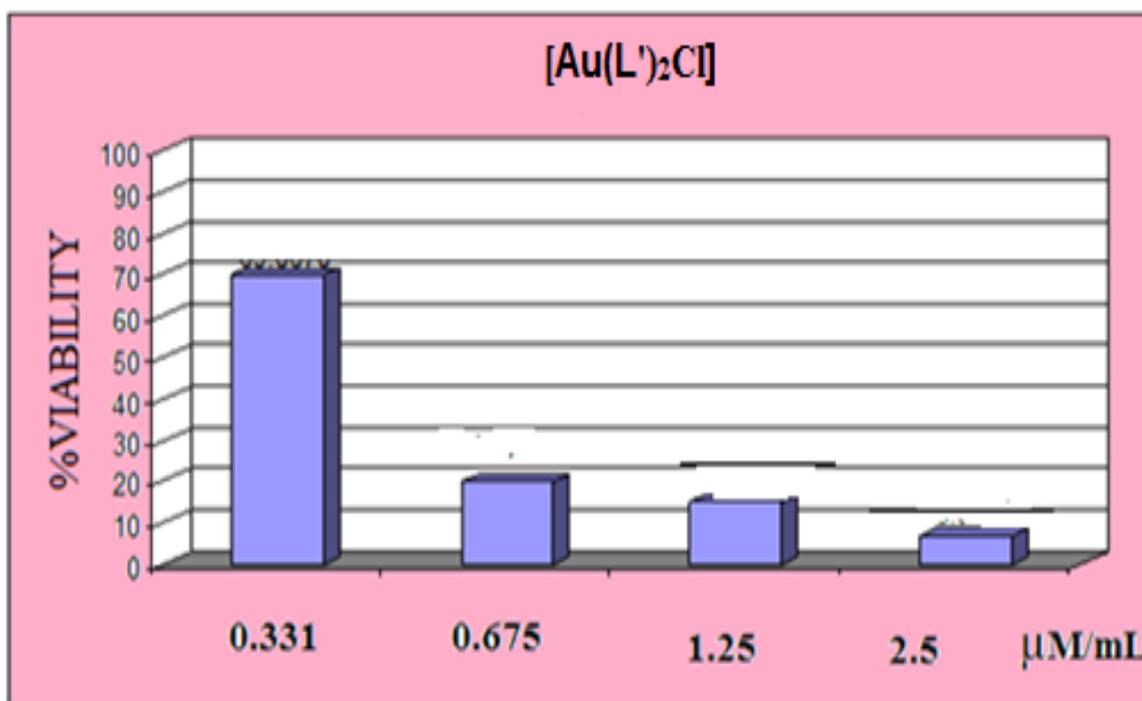


Figure 2: % viability of the [$\text{Au(L')}_2\text{Cl}$]

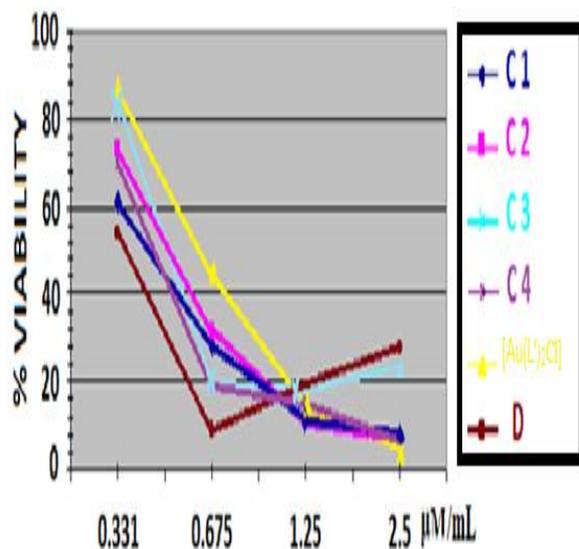


Figure (3) : Comparative cytotoxicities (% viability) of the synthesized complexes ((Au(L)₂Cl).C1-C4) with cisplatin (positive control)

It may be ruled out that there is no correlation between different arrangement of ligands (L) and (L') whether monodentate or bidentate, planar or pentacyclic, and neutral or charged around central metal. Such a finding allows us to state that the presence of H in sulfur-donor ligand is not an essential requirement for cytotoxicity in gold (III) complexes, i.e. to pharmacodynamic⁽¹⁶⁾.

Since, the cytotoxic profile of synthesized complexes is largely differ from cisplatin, it may come in agreement with many literatures stated that Au (III) were found to perturbs greatly the mitochondrial function⁽³⁸⁻³⁹⁾.

The overall of this study pointed out that the cytotoxicity profile of the 2-mercaptoacetate (L), mercaptoglycolate (L') and chloride derived complexes was related to the presence of the gold (III) central atom, the activity was not related to good leaving group, no direct correlation of the antiproliferative effects and the strong stabilization of the gold (III) center (C1 and C2). Cross anticancer activity against the cisplatin resistant cell line was found for one of the complexes C3.

The effect of the targeted complex may related to one of the following mechanisms: (i) formation of monofunctional or bifunctional coordination bonds at the two (liable) cis positions of the gold(III) center; (ii) intercalation of the ligand moiety.

In some cases these compounds exhibit a cytotoxic activity that counteract the Hep-2 cell resistance to cisplatin & being more sensitive. It may be ruled out that there is a direct correlation between the type of the

ligand (halide) & the function of the complex, this finding disagree with the literature⁽⁴⁰⁾. this may come agree that the synthesized complex with mono halide ligand and superior cytotoxic effect compared to the others Au (III) (C1-C4)

Detection of in vitro cytotoxicity has represented, for these metal-based agents, the primary screening criterion in order to assess their potential anti-cancer properties. IC₅₀ values of 10⁻⁵ M or lower were used to indicate a promising, or at least acceptable, anti-tumor efficacy⁽⁴¹⁾.

Analysis of the cytotoxicity data (% viability) permits formulation of some preliminary structure/function relationships that are summarized below:

- The cytotoxicity of these gold (III) complexes is strictly related to the presence of the gold(III) centre, C1-C4 and (Au (L')₂Cl) are significantly more cytotoxic than the corresponding platinum compound.
- The presence of hydrolysable chloride atoms in the gold(III) centre or, in general, of good leaving groups, does not represent an essential requirement for cytotoxicity compared to C1-C4 but showed very pleased unexpected effect compared to cisplatin.
- Attachment of chloride as a ligand may lead to an improvement in their biological activity as a consequence of the hydrophilic nature of chloride that may increase their transportation into biological system.
- The amount of gold (III) that enters in the cells is roughly proportional to the exposure time, at least during the first hours and increased with increase the concentration except for C3.

Conclusion

One of our main duty as medicinal chemists, to provide a complete chemical study of each system that is designed and developed, with the aim to accurately predict the reactivity of these compounds under physiological conditions and more over to predict the reactivity in the biological systems. Unfortunately this is not always possible, because most of the studies are life-time experimental projects.

Tissue targeting is a highly desirable goal for metal-based therapeutics or diagnostics, but it is not always feasible and more specific targeting ligands must be found. Not only the right ligand, but also the right metal-ligand combination, is important.

When studying the biological properties of the coordination compounds, it should be stressed the need of performing biological tests even for the free ligands. This protocol has been omitted often in the biological studies

published in the specialized literature. This useful exercise could reduce or even avoid false positive results.

These studies will also help in the achievement of further fine tuning of the biological properties. Furthermore studies are needed to identify unambiguously the species that are actually responsible for the cytotoxic effects.

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