Chemico-Biological Interactions 231 (2015) 53–60

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Magnetic property, DFT calculation, and biological activity of bis[(μ^2-chloro)chloro(1,10-phenanthroline)copper(II)] complex

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ARTICLE INFO

Article history:
Received 8 November 2014
Received in revised form 15 February 2015
Accepted 26 February 2015
Available online 7 March 2015

Keywords:
Copper(II) phenanthroline
Magnetism
Thermal
DFT
In vitro cytotoxicity
Anticancer

ABSTRACT

The dinuclear complex bis[(μ^2-chloro)chloro(1,10-phenanthroline)copper(II)] (**1**) was synthesized, and characterized by X-ray, FTIR and thermal analysis. The fitting of magnetic susceptibility and magnetization curve of (**1**) indicates the occurrence of weak antiferromagnetic exchange interaction between copper(II) ions. The electronic structure has been also determined by density functional theory (DFT) method. Complex (**1**) displayed potent anticancer activity against B16 (Melanoma), MDA-MB-32 (Breast Adenocarcinoma), A549 (Lung Adenocarcinoma), HT-29 (Colon Adenocarcinoma) and SF (Astrocytoma) cell lines with an average IC_{50} value of 0.726 μg/ml compared to 4.88 μg/ml for cisplatin. Complex (**1**) has a better therapeutic index and toxicological profile than cisplatin, and has demonstrated a potential chemotherapeutic property.

1. Introduction

Metal-based inorganic complexes have gained popularity in the pharmaceutical industry in the past three decades as new chemotherapeutic drugs [1]. The discovery of *cis*-diamminedichloroplatinum(II), known as cisplatin, was a breakthrough in cancer therapy in the 20th century. It is highly effective in treating a variety of cancers, especially testicular cancer, with a remission rate exceeding 90% [2]. However, cisplatin suffers from severity of side effects such as nephrotoxicity and neurotoxicity [3]. This drawback has stimulated an extensive search for safer alternative metal based antitumor inorganic complexes, with broader spectrum and improved clinical efficacy [4].

Copper complexes are important non-platinum anti-tumor agents for several reasons [5,6]. Copper is a trace element found in all living organisms. It plays a pivotal role as a cofactor for several enzymes and proteins which are involved in energy metabolism, respiration and DNA synthesis [7]. Researchers assumed that the essential element copper is less toxic than the non-essential metal platinum [8]. Several copper complexes with various ligands and donor atoms were synthesized. Among these, copper phenanthroline complexes were found to be several times more cytotoxic than cisplatin against some cervical cancer cell lines [9–11]. Such complexes were reported to inhibit the growth of PC3 human prostate cancer cells and HL-60 human myeloid leukemia cancer cells [12]. Reports on cytotoxicity of copper complexes have shown that the mechanism of action is based on intercalation and cleavage of DNA [13,14] or apoptosis induction [15,16].

On the other hand, organic–inorganic hybrid copper halides have received extensive attention in recent years owing to their electrical, magnetic and optical properties and due to their importance in areas of molecular adsorption and catalysis [17–21]. The copper halides complexes with N-containing organic ligands exhibited one dimensional (1D) chain [22,23] and two dimensional (2D) layer structures [24,25]. The basic copper halide skeletons exhibit several geometrical motifs: cyclic Cu_2X_2 dimeric [21,25], trinuclear Cu_3X_3 [21], cubanetetrameric [26], stair step oligomeric [25], zigzag polymeric [26], and hexagonal [Cu_3Cl_3] 1D polymeric [27] structures.

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http://dx.doi.org/10.1016/j.cbi.2015.02.023
0009-2797/

The present study aims to elucidate the synthesis and characterization of a dinuclear complex (1) bis[(μ^2-Chloro)chloro (1,10-phenanthroline)copper(II)], [CuCl(phen)μ-Cl]$_2$, determines its magnetic and thermal properties [28–30], study its electronic structure by DFT calculation [31–33] and evaluate its *in vitro* cytotoxic activity against several human cancer cell lines [6,34].

2. Materials and methods

2.1. Physical measurements

Infrared spectra were recorded on a Jasco FTIR 410 instrument by using pressed KBr plates in the range 500–4000 cm^{-1}. Thermogravimetric – differential scanning calorimetry (TG-DSC) curves were recorded on a Setaram Labsys thermal analyzer, at a heating rate of 3°C/min, in the 25–800 °C temperature range, and under nitrogen flow. The magnetic susceptibility of crystalline samples was measured on an Oxford Maglab 2000 magnetic measurement system. Diamagnetic corrections were applied using Pascal's constants. X-ray Analysis was done with a Bruker X8 APEX single-crystal diffractometer using Graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) and Bruker APEX II software suite.

2.2. Synthesis of bis[(μ^2-Chloro)chloro(1,10-phenanthroline)copper(II)] (**1**) [CuCl(phen)μ-Cl]$_2$

Blue powder of complex (1) was obtained by adopting the following experiment: Acetonitrile (30 mL) was added onto $CuCl_2 \cdot 2H_2O$ (1.03 gm, 6.04 mmol) and 1,10-phenanthroline (1.09 gm, 6.06 mmol). The reaction mixture was stirred for 5 h at room temperature. The blue powder precipitate was then collected by filtration and washed with acetonitrile giving 1.68 g of compound (1) (% yield = 88.4%). Crystals of [CuCl(phen)μ-Cl]$_2$ (**1**) has been obtained by the reaction of copper(II) chloride hydrate and phenanthroline ligand as described previously [35,36,21,37]. The crystal were analyzed by single crystal X-ray analysis confirming the formula of complex (**1**). The structural data was summarized on a cif mercury file.

2.3. DFT Computational details

The electronic structure has been determined by the density functional theory (DFT) method. DFT method has shown to be sufficient for geometry optimization and calculation of spectral properties of large molecules [31–33]. The geometry of complex (**1**) has been optimized using B3LYP density functional model [38]. In these calculations, we used the 3-21G* basis set for C and H atoms and the 6-31G* basis for N atoms. For Cu and Cl atoms, the LanL2DZ valence and effective core potential functions were used [39]. All DFT calculations were performed using Gaussian 98 R-A.9 package [40]. X-ray structures were used as input geometries when available.

2.4. Biological activity

2.4.1. Cell culture

B16 (Melanoma), MDA-MB-32 (Breast Adenocarcinoma), A549 (Lung Adenocarcinoma), HT-29 (Colon Adenocarcinoma) and SF (Astrocytoma) cell lines were grown in Dulbecco's modified Eagle's medium (DMEM, Sigma), supplemented with 10% fetal bovine serum and 0.5% penicillin–streptomycin (10,000 units penicillin and 10 mg streptomycin/mL, Sigma). The plates were maintained at 37 °C in a humidified incubator containing 5% CO_2.

Freshly isolated human bone marrow stem cells were grown in Roswell Park Memorial Institute medium (RPMI, Sigma) supplemented with 10% fetal bovine serum, 0.5% penicillin–streptomycin (10,000 units penicillin and 10 mg streptomycin/mL, Sigma) and maintained in an incubator containing 5% CO_2 at 37 °C.

2.4.2. Cell proliferation assay

The cells were harvested by trypsin to obtain a single-cell suspension, counted using Trypan-blue and diluted in the respective media. A volume of 100 μL of 10^5 cells/mL was plated in a 96-well plate and incubated for 24 and 48 h. Cells were then treated with various concentrations (0.5, 1.0, 2.5 and 5.0 μg/ml) of complex (**1**) in DMSO. Cisplatin (Ebewe Pharma, Austria) was used as a positive control throughout the experiments.

Cell proliferation was measured after 24 and 48 h of incubation. Cell viability was measured by water-soluble tetrazolium salt-1 (WST-1) a cell proliferation reagent (Roche Applied Science Penzberg, Germany). This salt is cleaved by mitochondrial dehydrogenases in metabolically active cells, forming formazan. Formazan formation was quantified spectrophotometrically at 440 nm using a microplate reader (Thermo Scientific – Multiskan FC). Experiments were done in triplicates for all cell lines.

2.4.3. Determination of median lethal dose (LD_{50})

The LD_{50} of complex (**1**) and cisplatin was determined using the Dixon's up and down method (Dixon, 1965). Adult male Wistar rats weighing 200–250 g were housed at a constant temperature (22 ± 2 °C) and light (12:12 h light:dark) controlled room. A solution of complex (**1**) was prepared in DMSO to reach the test dose concentration. A single dose of the test chemical was administered intra-peritoneally to 6 rats. The survival or death of animal at the used concentration determine the next dose to be either increased or decreased. Survival was depicted by "o" and death as "x" and the score was made accordingly. The animals were observed after 48 h for survival. Dixon's formula was then applied in order to determine the LD_{50}. The formula used the logarithmic (log) value of the doses administered in mg/kg body weight and is depicted by 'Log (LD50) = $X_f + kd$', where X_f is the last dose administered, k is the tabular value and d is a constant equal to the interval between doses.

3. Results and discussion

3.1. Structure of complex (**1**)

The structure of complex (**1**) was determined by single X-ray diffraction. The basic unit structure is presented in Fig. 1. The solid-state structure of complex (**1**) consists of centrosymmetric

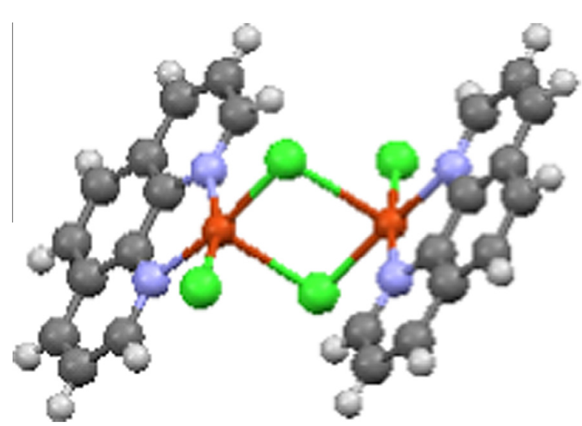

Fig. 1. Ortep figure of the crystal structure of complex (**1**) [CuCl(phen)μ-Cl]$_2$.

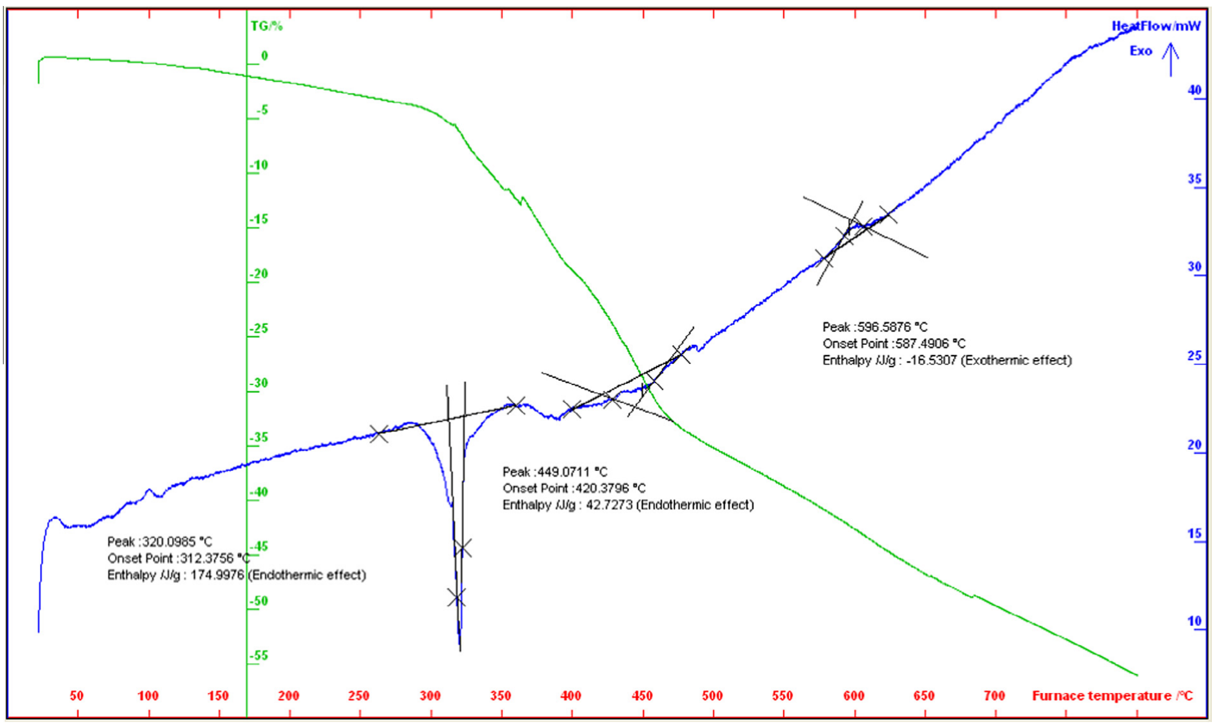

Fig. 2. Thermal analysis of complex (**1**).

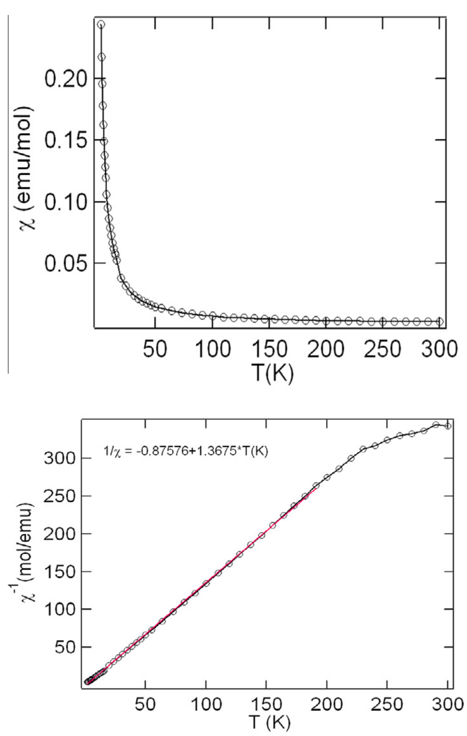

Fig. 3. Temperature dependence of χ_M and χ_M^{-1} for complex (**1**). B = 500 Oe; μ_{eff} = 1.72 μ_B per Cu^{2+} (very close to isolated S = 1/2 spins).

$[CuCl(phen)\mu\text{-}Cl]_2$ dimers bridged by chloride anion. The 1,10-phenanthroline ligand, terminal Cl, and bridging Cl are coplanar and the square pyramidal Cu atom is displaced slightly (0.023 Å) out of this basal plane, towards the axial bridging chloride. The dimeric structure of (**1**) is analogous to the previously reported $[CuCl(phen)\mu\text{-}Cl]_2$ [35,36,21,37].

Table 1
Magnetic data of complex (**1**) and relevant complexes.

Complex	J value (cm^{-1})	Refs.
$[CuClPhen(\mu\text{-}Cl)]_2$	−0.65	This work
$[Cu(Phen)_2Cl][OH].(CH_3OH)_2(H_2O)_6$	−1.9	[43]
$[(Phen)_2Cu(\mu\text{-tdga})Cu[(Phen)]\ (NO_3)_2{\cdot}5H_2O$	−2.4	[44]
$[Cu_4(Phen)_4(\eta^2\text{–}HPO_4)$ $(\eta^2\text{–}H_2PO_4)_2(H_2PO_4)_2]{\cdot}2H_2O$	0.12; −1.32; 0.1	[45]

3.2. Thermal analysis

Thermal analysis plays an important role in studying the structure and the properties of transition metal complexes [6,28,30,41]. Complex (1) was studied by thermo gravimetric analysis from room temperature to 800 °C in nitrogen atmosphere. The TG curves were drawn as % mass loss vs. temperature (TG) curve. Heat flow curve were drawn vs. temperature as Differential Scan Calorimetry (DSC) curves. Typical TG – DSC curve is presented in Fig. 2.

Thermogravimetric analysis proves that complex (**1**) possesses high thermal stability. The TG curve of complex (**1**) indicates that it is stable in the temperature range 20 °C to 250 °C. A major weight loss (39%) occurs between 370 and 550 °C, corresponding to the release of 1,10-phenanthroline and chloride ligands. Two endothermic peaks are associated with this process one sharp at T = 320.1 °C with enthalpy change ΔH = +175.0 J/g, and a second broader one at T = 449.1 °C with ΔH = +42.7 J/g. A further gradual and minor loss 18% occurs between 550 and 800 °C with an associated exothermic process (ΔH = −16.53 J/g, and peak T = 596.6 °C).

3.3. Magnetic measurements

The temperature evolution of magnetization and magnetic susceptibility measurements of complex (**1**) were analyzed by least-squares fitting of the experimental data to the classical Bleany–Bowers equation based on the Hamiltonian $H = -2JS_AS_B$ ($S_A = S_B$ = ½) [42].

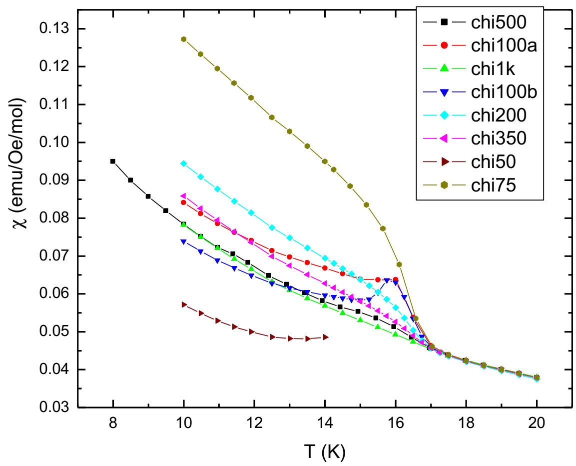

Fig. 4. χ vs. T °K at different chi.

Table 2
Selected bond lengths [Å] for $[CuCl(phen)\mu\text{-}Cl]_2$. Experimental data belong to the solid phase, whereas the calculated data correspond to the isolated molecule in gas-phase.

	Experimental	Theoretical
Cu1-N1	2.041	2.08335
Cu1-N2	2.040	2.07794
Cu1-Cl1	2.267	2.38176
Cu1-Cl2	2.263	2.34149
Cu1-Cl3	2.682	2.80513
Cu1-Cu2	3.549	3.51639
Cu2-N3	2.041	2.08351
Cu2-N4	2.031	2.07783
Cu2-Cl1	2.829	2.80522
Cu2-Cl4	2.259	2.34146
Cu2-Cl3	2.288	2.38181

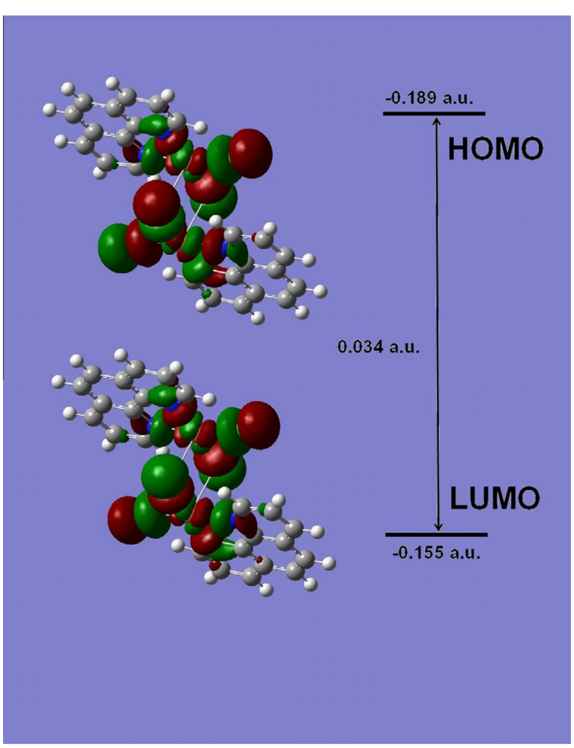

Fig. 5. Frontier molecular orbitals for $[CuCl(phen)\mu\text{-}Cl]_2$ (1).

Table 3
Experimental FT-IR frequencies (cm^{-1}) for $[CuCl(phen)\mu\text{-}Cl]_2$, compared with the theoretical frequencies obtained from DFT calculations.

$[CuCl(phen)\mu\text{-}Cl]_2$	Calculated
3079.5	3201.07
3045.5	3197.30
3011.4	3197.29
1585.4	1545.67
1558.4	1530.53
1515.9	1494.97
1423.4	1444.75
1348.1	1358.15
1147.6	1178.76
1107.1	1120.52
854.4	868.75
721.3	739.01
647.7	655.29

The variation of molar magnetic susceptibility increases continuously with decreasing temperature from room temperature down to 2 K. Experimental data corrected for diamagnetism were fitted to Bleany–Bowers Eq. (1):

$$\chi = \frac{Ng^2\beta^2}{k_BT\left(3+\exp\left(\frac{-J}{k_BT}\right)\right)} \tag{1}$$

where N, β and k_B are Avogadro's number, Bohr magneton and Boltzman constant, respectively. Experimental data corrected for diamagnetism is shown in Fig. 3 and fitted to Eq. (1) where both Landé factor g and exchange parameter J are fitting parameters.

The magnetization measurement (M) was also measured as a function of magnetic field at $T = 2$ K. The experimental data of M were fitted to Eq. (2):

$$M = N\beta g\frac{\sum_{s=0}^{1}\left(S+\frac{1}{2}\right)\exp\left[\frac{J(S(S+1)-3/2)}{k_B}\right]\cosh\left(\frac{g\beta\left(S+\frac{1}{2}\right)B}{k_BT}\right)}{\sum_{s=0}^{1}\exp\left[\frac{J(S(S+1)-3/2)}{k_B}\right]\sinh\left(\frac{g\beta\left(S+\frac{1}{2}\right)B}{k_BT}\right)} - \frac{1}{2}\cosh\left(\frac{g\beta(S+\frac{1}{2})B}{k_BT}\right) \tag{2}$$

The fitting of magnetic susceptibility and magnetization curves indicate the presence of very weak antiferromagnetic exchange interaction between copper ions (II) with best fitting values $g = 2.05 \pm 0.02$ and exchange coupling constants $J = -0.65 \pm 0.02\ cm^{-1}$. The present J value is compared with relevant copper(II) complexes containing phenanthroline or phenanthroline-derived ligands, Table 1, [43–45]. The observed J values in the literature suggest the occurrence of ferromagnetic and antiferromagnetic interactions controlled by a number of structural and electronic factors. For instance, Mao el al. [43] investigated the magnetic properties of $[Cu(phen)_2Cl][OH].(CH_3OH)_2\cdot(H_2O)_6$; they found a weak antiferromagnetic interaction ($J = -1.9\ cm^{-1}$) between copper ions, they interpreted the weak interaction because of the presence of C—H···Cl hydrogen bonding which does not promote the exchange between magnetic centers. In the same manner, Kopel et al. [44] also found similar results in the dimeric compound $[(phen)_2Cu(\mu\text{-tdga})Cu(phen)]^{2+}$, the intradimer exchange coupling was found to be antiferromagnetic with $J = -2.4\ cm^{-1}$; however, they have also reported a relatively strong interdemic exchange coupling but with some uncertainty on the number of nearest neighbor interacting centers. Moreover, such weak exchange coupling was also found in tetranuclear copper compounds; Phuengphai et al. presented the magnetic study on $[Cu_4(phen)_4(\mu_3,\eta^2\text{-}HPO_4)_2(\mu,\eta^2\text{-}H_2PO_4)_2\ (H_2PO_4)_2]\cdot 2H_2O$ [45]; they found dominant exchange coupling within the square pyramidal arrangement of the four copper centers to be weak

($J = -1.32\ cm^{-1}$) and antiferromagnetic. It is also noted that the magnetic interaction present in phenanthroline-bridged dinuclear copper(II) system is much weaker than in other organic bridging systems such as carboxylato or oxalate bipyridine copper(II) complexes [46]. Fig. 4, also shows χ vs. T °K curves at different chi.

3.4. DFT calculations

The calculated structural parameters are listed in Table 2. It should be noted that the experimental data belong to the solid phase, whereas the calculated data correspond to the isolated molecule in gas-phase. The experimental and computational data in Table 2 clearly show that they differ very slightly from each other. For example, the largest difference between experimental and calculated Cu1-Cl1 length is about 0.1146 Å. As a result, the calculated geometrical parameters represent a good approximation.

The NBO charges of Cu(II) and of coordinated atoms were also calculated. The positive charge of the Cu(II) ions was 1.974. The charges of nitrogen atoms of "phen" ligands were −0.333 and −0.334. The two chloride anions have similar charges: Cl1 & Cl2: −0.582 and −0.486 and Cl3 & Cl4: −0.581 and −0.485. The calculations indicate that complex (**1**) has 127 occupied molecular

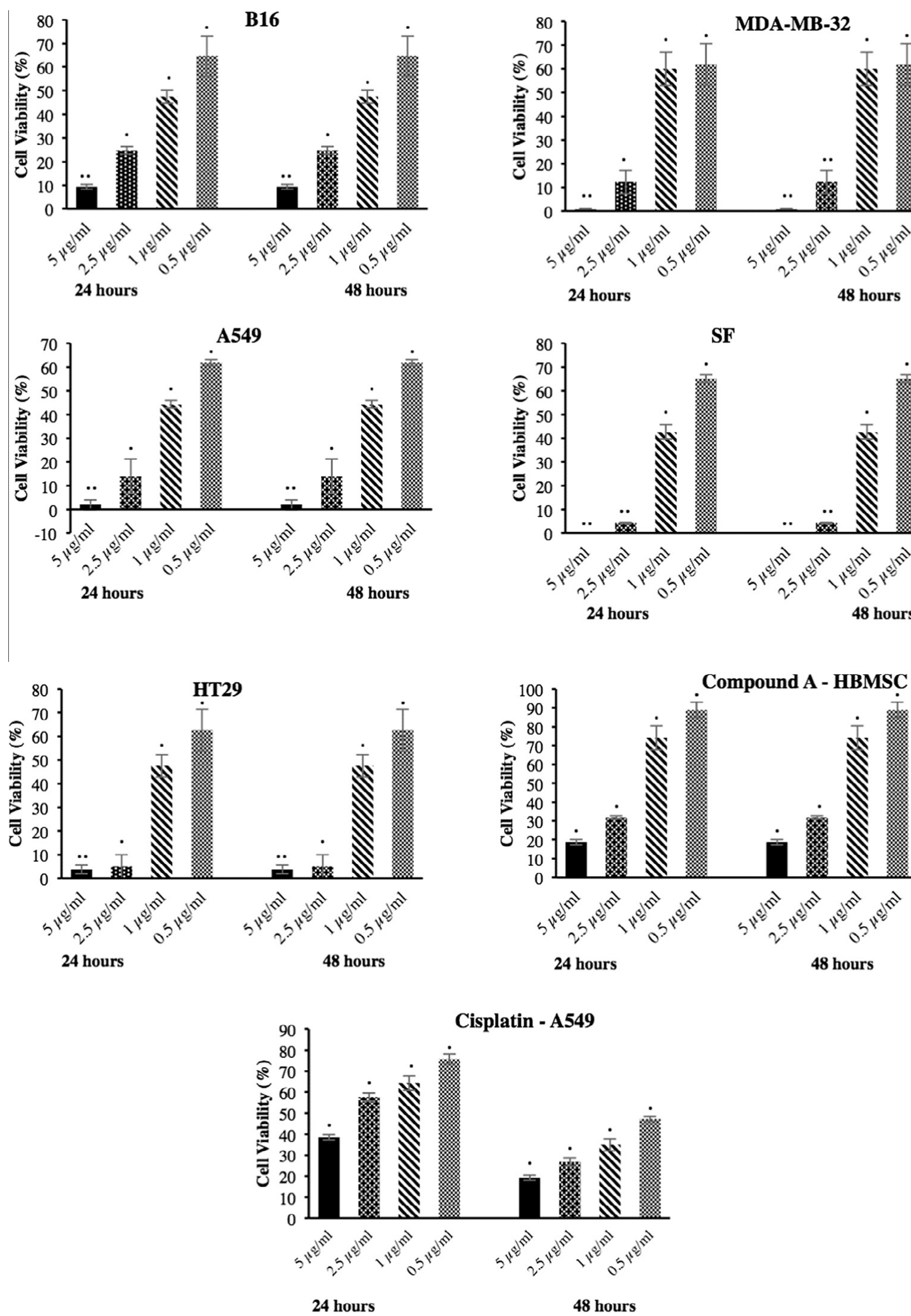

Fig. 6. Effects of complex (**1**) and cisplatin on cell proliferation. Cells were treated at concentrations range from 0.5, 1.0, 2.5 and 5.0 μg/ml for 24 and 48 h. Inhibition of cell proliferation was assessed by using WST-1 assay. Data are expressed as % proliferation of cells. Cisplatin was used as a positive control. Data are the mean ± SEM from three experiments. $^{*}p < 0.05$ and $^{**}p < 0.005$ compared with the group treated with DMSO. HBMSC stands for human bone marrow stem cells.

Table 4
Average IC_{50} values at 48 h of complex (**1**), selected copper (II)-phenanthroline complexes and cisplatin against specific cell lines as reported in literature (PC3, human prostate cancer; MCF-7, human breast cancer cells; HepG2, liver cancer cells; HeLa, cervical cancer; A549, small cell cancer; MDA-MB-32, B16F10, melanoma; human mammary adenocarcinoma; brain SF, brain cancer; HCT-8 colon cancer and HT-29, colon cancer cell lines). $Cu(DCA)(phen)]_n{\cdot}nH_2O$: Polymeric demethylcantharidato (DCA) bridged copper(II) phenanthroline (phen) complex; $[Cu(ph)(phen)_2]$: bis-phenanthroline copper(II) phthalate complexes.

Compound	IC_{50} (μg/mL)	Cell lines	Refs.
Complex (1): $[CuCl(phen)\mu\text{-}Cl]_2$	0.726	MDA-MB-32, HT-29, A549, SF, B16F10	Present study
$[Cu(DCA)(phen)]_n{\cdot}nH_2O$	0.568	HCT-8, HeLa, HepG2, MCF-7	[58]
$Cu(C_{10}\,H_8O_2N)_2(phen)$	1.26	PC3	[52]
$[Cu(ph)(phen)_2]$	10.45	HT-29, MCF-7	[59]
Cisplatin	8.7	HT-29 and A549	[16]

Table 6
Effect of different doses of complex (**1**) on survival of rats for 48 h. ('x' indicates death; 'o' indicates survival).

Complex (1)*			Cisplatin**		
Dose (mg/kg bw)	Log dose	Score	Dose (mg/kg bw)	Log dose	Score
50	1.7	X	8	0.9	x x
25	1.4	o x	5	0.7	o o
13	1.1	o o	3.2	0.5	o
7.5	0.8	O	2	0.3	o

* Final score is oooxxo; with $N = 6$ mice and $k = 1.139$ (value from Dixon's table).Log $(LD_{50}) = X_f + kd$, where X_f is the last dose administered and d is the interval between doses.Log $(LD_{50}) = 1.1 + 1.139(0.3) = 1.447$. LD_{50} = Antilog (1.447) = 27.6 mg/kg bw.
** Final score is oooxox; with $N = 6$ mice and $k = -0.432$ (value from Dixon's table).Log $(LD_{50}) = 0.90 + 0.2(-0.432) = 1.447$. LD_{50} = 6.51 mg/kg bw.

orbitals (MOs). The value of the energy separation between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) was calculated as seen in Fig. 5. The HOMO of the title complex is principally localized on four nitrogen atoms of two "phen" ligands, four Cl anions and two Cu(II) ions, whereas the LUMO is approximately delocalized on these atoms and ions. The calculated HOMO–LUMO gap is 0.034 a.u. (0.092 eV).

The IR spectra show the characteristic absorption bands of "phen" ligand, Table 3. The relatively weak band at 3011 cm^{-1} is attributed to the absorption of aromatic C—H bonds, and the bands at 1423, 1515 and 1585 cm^{-1} is attributed to the absorption of the >C=C< and >C=N— aromatic rings bonds [47].

3.5. Anticancer activity

Complex (**1**) was tested for anti-proliferative effects against B16 (Melanoma), MDA-MB-32 (Breast Adenocarcinoma), A549 (Lung Adenocarcinoma), HT-29 (Colon Adenocarcinoma) and SF (Astrocytoma) cell lines. Fig. 6 showed a potent dose-dependent inhibitory effect of complex (**1**) against five tested cancer cell lines at 24 and 48 h post-treatment. Compared to pure DMSO, cells treated with complex (**1**) exhibited a sharp decline in cell survival, especially at doses 2.5 and 5 μg/mL. Complex (**1**) did not cause significant increase in cytotoxicity after 24 h, unlike cisplatin, which showed significant time-dependent anti-cancer effects. The IC_{50} values for complex (**1**) reported in Table 4, reveals greater selective toxicity against HT-29 and MDA-MB-32 cells. Table 4 also shows that complex (**1**) IC_{50} values for all cell lines at 24 h and 48 h were respectively 30–40 times and 6–8 times more potent than cisplatin. Hence, with a faster onset of action and less time-dependent effect, complex (**1**) clearly exhibits a better cytotoxic profile than cisplatin against cancer cells when tested *in vitro*. Literature search reveals that many experimental prodrugs in the form of copper complexes can cause proteasome inhibition through delivering copper ions into tumor cells leading to cell death [48–51]. In addition, it was shown that 1,10-phenanthroline ligands in copper complex are the best for easy copper ion delivery into target cells [52]. Furthermore, $[Cu(phen)]^{2+}$ type complexes are known to bind to DNA via intercalation and non-intercalation fashion in addition to their potent oxidative nuclease activity (Hirohama et al. [53]). Therefore, it is possible that complex (1) may be sharing a similar mode of action as mentioned above. On the other hand, Table 4 shows that complex (1) exhibits comparable and even better cytotoxic profile than some copper-phenanthroline complexes and cisplatin.

Bone marrow stem cells are used to assess toxicities of chemotherapeutic drugs against normal cells [54]. Freshly isolated human bone marrow stem cells were used in the present study to evaluate the tumor-specific toxicity of both complex (**1**) and cisplatin. Complex (**1**) displayed a significant cytotoxic selectivity against the tested cancer cells compared to normal bone marrow stem cells, with a 2–3 folds increase in normal cell survival at both time intervals. However, cisplatin showed a very modest selectivity especially after 24 h post-treatment (Table 5). Bone marrow is also one of the most sensitive tissues to anticancer agents due to the continuous proliferation of its stem cells, hence they are susceptible to dose-limiting myelotoxcitiy [55]. In this study, the higher selectivity of complex (**1**) can be attributed to its mode of action, which is preferentially cell-cycle specific manner, in contrast to cisplatin which works in a non-phase specific way.

Dixon's up and down method is a well-accepted technique to determine the acute toxicity of drugs *in vivo* using small numbers of animals [56]. In the present study, the calculated LD_{50} for cisplatin (Table 6) was 6.51 mg/kg (21.7 μmol/kg) body weight, and was comparable to that reported by the Material Safety Data Sheet [57]. Table 6 also reveals that the LD_{50} of complex (**1**) was 27.9 mg/kg (44.3 μmol/kg) body weight, which is 4 times higher than that of cisplatin. Therefore, complex (**1**) proved to be superior to cisplatin in terms of (*in vivo*) safety and (*in vitro*) selectivity and efficacy.

Table 5
IC50 values of complex (**1**) and cisplatin after 24 h and 48 h of treatment of MDA-MB-32 (Breast Adenocarcinoma), HT-29 (Colon Adenocarcinoma), A549 (Lung Adenocarcinoma), SF (Astrocytoma), B16F10 (Melanoma) and HMBSC (Human Bone Marrow Stem Cells) cells. Each value represents the mean of 3 experiments.

IC_{50} ± SEM (μg/mL)				
Cell line	Complex 1 (24 h)	Complex 1 (48 h)	Cisplatin (24 h)	Cisplatin (48 h)
MDA-MB-32	0.651 ± 0.105[a,b]	0.639 ± 0.137[a,b]	27.902 ± 1.021	4.950 ± 0.571
HT-29	0.606 ± 0.163[a,b]	0.591 ± 0.201[a,b]	28.051 ± 0.315[a]	5.109 ± 0.438
A549	0.853 ± 0.315[a,b]	0.803 ± 0.082[a,b]	26.913 ± 3.410	3.939 ± 0.412
SF	0.721 ± 0.072[a,b]	0.711 ± 0.06[a,b]	27.609 ± 2.141	4.621 ± 1.501
B16F10	0.915 ± 0.210[a,b]	0.903 ± 0.237[a,b]	30.108 ± 0.682	5.804 ± 0.631
HMBSC	1.901 ± 0.262[b]	1.87 ± 0.30[b]	32.540 ± 0.594	6.276 ± 0.542

[a] $p < 0.05$ compared with HBMSC.
[b] $p < 0.05$ compared with the group treated with cisplatin.

4. Conclusion

In conclusion, the present study reports the synthesis and characterization of a novel copper complex, bis[(μ^2-chloro)chloro (1,10-phenanthroline)copper(II)], and its anticancer activity against selected cell lines. The structure of complex (**1**) was established using single X-ray diffraction, FTIR and thermal analysis. The electronic structure has also been determined by density functional theory (DFT) method. The fitting of magnetic susceptibility and magnetization curve indicates a weak antiferromagnetic exchange interaction between copper ions. Complex (**1**) exhibited a potent anticancer activity against B16, MDA-MB-32, A549, HT-29 and SF cell lines, with an average IC_{50} value of 0.726 μg/ml (1.15 μM), compared to 4.88 μg/ml (16.3 μM) for cisplatin. Additionally, complex (**1**) showed a better selectivity against cancer cells compared to human bone marrow stem cells and less toxicity on rats as compared to cisplatin, rendering it a potential chemotherapeutic agent. The anticancer activity of complex (**1**) could be attributed to the adequate delivery of copper to tumor cells with the aid of phenanthroline, leading to the inhibition of proteasome and elevation of intracellular oxidative damage. Further studies are needed to evaluate the *in vivo* anti-tumor effect of complex (**1**) and elucidate its mode of action.

Transparency Document

The Transparency document associated with this article can be found in the online version.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgement

Prof. Ulie Kortz and Dr Michael Dickman, Department of Chemistry, Jacob's University, Bremen, Germany are thanked for providing X-ray analysis.

Appendix A. Supplementary data

Cif file for bis[(μ^2-chloro)chloro(1,10-phenanthroline)copper(II)]. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cbi.2015.02.023.

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