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## SYNTHESIS, CHARACTERIZATION OF COPPER COMPLEXES AND EFFECT OF GAMMA IRRADIATION ON ANTICANCER ACTIVITY

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### ABSTRACT

Cu(II) complexes of ligand 2-[2-(4-chlorophenylamino acetyl-N-phenyl hydrazine carbothioamide)(H<sub>2</sub>L)] have been synthesized and characterized by using IR spectra, <sup>1</sup>H NMR, elemental analyses, molar conductance, UV-Visible spectra. The study revealed that the ligand behaves as a neutral bidentate or monobasic tridentate and coordination takes place via NH, C=O, >C=N and C-S. The anticancer activities of these compounds were investigated against solid tumor induced in mice by injection of Ehrlich Ascites Carcinoma (EAC) cell line. Results revealed that tested compounds significantly reduced the tumor size. Gamma-irradiated compounds showed potent antitumor activities when compared to that of non-irradiated compounds. In addition, tested compounds exhibited stimulatory effect on the level of catalase and superoxide dismutase activities and glutathione content in liver of tumor bearing mice, while the level of lipid peroxidation was significantly reduced. It is concluded that thiosemicarbazone complexes and ligand are considered as promising anticancer drugs candidate. Moreover, the  $\gamma$ -irradiation evokes the antitumor activity of the tested compounds. Keywords: Complexes, IR, UV, Antitumor activity, Antioxidant enzymes

1. Introduction Thiosemicarbazone derivatives form an important class of organic compounds due to their structural chemistry and biological activities, such as antibacterial, and antiviral activities. Heterocyclic sulfur and oxygen containing thiosemicarbazones have been the subjects of extensive investigation because of their use in different biological applications 1, 2. These compounds have received great interest because of their bonding modes, biological implications, structural diversity, and ion-sensing ability<sup>3</sup>. The biological applications and properties of metal complexes differ from those of either ligands or metal ions, and increased (and/or) decreased biological activities of transition metal complexes like Cu (II) are reported in the literature 4–6. The copper (II) complexes of two salicylaldehyde semicarbazones, HOC<sub>6</sub>H<sub>4</sub>CH NNHCONR<sub>2</sub> [H<sub>2</sub>Bnz<sub>2</sub> (R = CH<sub>2</sub>Ph) and H<sub>2</sub>Bu<sub>2</sub> (R = Bu)], were evaluated for their DNA binding and cleavage properties by spectrophotometric DNA titration, ethidium bromide displacement assay and electrophoretic mobility shift assay. The complexes show similar DNA cleavage activity, which is reflected in the similarity of their frontier molecular orbital energies calculated by density functional theory. These results are discussed in relation to the anticancer properties of the complexes 7.

2. Experimental 2.1. Materials and methods Reagent grade chemicals were used without further purification. 2.2. Synthesis of ligand the ligand 2-phenylaminoacetyl-N-phenylhydrazine carbothioamide (H<sub>2</sub>L) was prepared by mixing equimolar amount of desired hydrazide (0.01mol) in 10ml of absolute ethanol with 4-chloro phenyl isothiocyanate (0.01mol) in 10 ml of absolute ethanol 8, 9. The reaction mixture was refluxed for 3 hrs. The reaction mixture was recrystallized several times from ethanol. 2.3. Synthesis of the metal complexes Copper(II) complexes of the ligand were prepared by adding stoichiometric amount of the copper(II) chloride, bromide, acetate and nitrate in EtOH to 2-[2-(4- chlorophenylamino acetyl-N-phenyl hydrazine carbothioamide)(H<sub>2</sub>L)] in EtOH in a 1:1 molar

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ratio. The reaction solution was stirred magnetically at 60°C for 5 hrs. The resulting solids were filtered off, washed several times with EtOH and dried under vacuum over P4O10. 2.4. Physical measurements Elemental analyses (C, H and Cl) were performed by Microanalytical unit of the Cairo University, Egypt. IR absorption spectra were recorded using KBr discs and a Perkin-Elmer 1430 recording spectrophotometer. <sup>1</sup>H NMR spectra were recorded in d<sub>6</sub>-DMSO using 300 MHz Varian NMR spectrometer. The electronic spectra were carried out as solution (10-3M) in DMF using a Perkin- Elmer Lambda 4B spectrophotometer. The molar conductivity measurements were made in DMF solution (10-3M) using a Tacussel conductometer type CD6N. The fluorescence spectra were carried out using LS 45 PerkinElmer Fluorescence Spectrometer. Magnetic susceptibilities were measured at 27°C using a modified Gouy method with Johnson Matthey balance.  $\gamma$ -Irradiation of Complexes Energetic  $\gamma$ -irradiation exposure was undertaken using a  $\gamma$ -Co60 unit at Atomic Energy Establishment, Egypt; at an accumulated dose of 1 Mega rad in air. 2.5. Preparation of compounds solutions Tested complexes were dissolved in DMSO/H<sub>2</sub>O (7:3) to give a final concentration of 1mM and kept at 4°C. 2.6. Induction of solid tumor in experimental animals A model of solid tumor was induced in female Swiss albino mice, weighing 18 to 20 g, by injecting of 1x10<sup>6</sup> Ehrlich Ascetic Carcinoma (EAC) cell line subcutaneously into the right thigh of the lower limb of the mice A total of 60 mice were injected with EAC and divided into 10 groups, 6 animals per group. Animals of groups 1 to 8 were I.P. injected with 0.1 mM of tested compounds daily for 15 days after tumor implantation. Animals of group 9 were I.P. injected with dissolving solution (DMSO/H<sub>2</sub>O) daily for 3 days after tumor implantation and served as sham group. Group 10 served as tumor control group. In addition to normal control group 2.7. Determination of solid tumor size Tumor size was estimated according to the method of Geran et al. 10. The resultant solid tumor was considered to be prelate ellipsoid with one long axis and two short axes. The two short axes were measured with vernier caliper. The tumor size was calculated using the following formula: Size = Length (cm) × width<sup>2</sup> (cm)/2 2.8. Preparation of liver homogenate A tissue sample from a known portion of the liver was accurately weighed and homogenized (Potter-Elvehjem) in a 10-fold volume of ice-cold (20mM) tris-HCl buffer pH 7.4. The homogenates were divided into aliquots kept at 20°C for future measurements. 2.9. Estimation of hepatic oxidative status Level of hepatic lipid peroxidation was estimated colorimetrically by measuring malondialdehyde (MDA) using the method of Ohkawa et al. 11 and by following the manufacturer's procedure (Biodiagnostics, Egypt). Hepatic catalase activity was estimated by the method of Aebi 12 and according to the manufacturer's procedure (Biodiagnostics, Egypt). Superoxide dismutase activity in liver homogenate was estimated according to the procedure of Nishikimi et al. 13 and by following the manufacturer's procedure (Biodiagnostics, Egypt). Level of reduced glutathione in liver homogenate was estimated by using the method mentioned by Beutler et al. 14. 2.10. Lymphoproliferation assay Polymorphonuclear cells (PMNC) were isolated from spleen according to the method described by Om Ali et al. 15. Lymphoproliferation assay was conducted according to the method of 16. Briefly, cells were suspended in complete RPMI media supplemented with 5% human AB serum and cultured in 96-well round-bottom plates at 3x10<sup>5</sup> per well. Cells were stimulated with phytohemagglutinin (PHA; Sigma, St Louis, MO). Cells were incubated in 5% CO<sub>2</sub> incubator at 37°C for 72 hr. Eighteen hours before the end of incubation, 20 mM of BrdU solution (Pharmlingen, San Diego, CA) was added to the cells. A BrdU proliferation kit (5-bromo-20-deoxy-uridine (BrdU) Labeling and Detection Kit III, Roche) was used according to the manufacturer's instructions and the reaction was quantified by reading in a microplate photometer at a test wavelength of 405 nm. Absorbances (optical density, OD) were determined. The proliferation index to PHA was calculated by dividing OD of PHA-stimulated cells by OD of unstimulated cells. 2.11. Statistical analysis all data were expressed as Mean values ± SD. t-test was used for comparison between groups. P values less than 0.05 were regarded as statistically significance. 3. Results and discussion the ligand 2-[2-(4- chorophenylamino acetyl)-N-phenyl hydrazine carbothioamide(H<sub>2</sub>L) was confirmed by



elemental analysis (Table1), infrared (Table2) and <sup>1</sup>H NMR spectroscopy. The stoichiometries of the isolated complexes of thiosemicarbazones are shown in table (1). Copper complexes of the neutral ligand 2, 3 are formed with bromide and acetate. Copper complex of the monobasic ligand is formed with nitrate. The reaction of the ligand H2L with different salts of Cu(II)chloride, bromide, acetate and nitrate produce complexes of the general formulae [Cu(H2L) (HL)Cl(H2O)], Cu(HL)Br(H2O), Cu(H2L)(OAc)<sub>2</sub> and Cu(HL)(NO<sub>3</sub>)<sub>2</sub>. These air stable complexes are non-hygroscopic, partially soluble in most organic solvents, but freely soluble in DMF and DMSO. Values of molar conductivities in DMF (10<sup>-3</sup>M) solution (Table1) show that the complexes are nonelectrolytes, indicating coordination of the ligand anions 12.

3.1. Spectral studies 3. 2. <sup>1</sup>H NMR The ligand 2-[2-(4- chlorophenylamino acetyl-N-phenyl hydrazine carbothioamide (H2L) was confirmed by elemental analysis (Table 1), infrared (Table 2) and <sup>1</sup>H NMR spectroscopy. The <sup>1</sup>H NMR spectrum of H2L in chloroform, which would produce more information concerning intra molecular hydrogen bonding was not possible due to their low solubility, so they have recorded as d<sub>6</sub> -DMSO solution. The resonance for the amido N(4)H attached to phenyl group is located in the 9.8 ppm spectral region, including that hydrogen bonding with d<sub>6</sub> -DMSO does not occur, in agreement with previous results 17,18 The <sup>1</sup>H signals due to the hydrazido group for N(1)H occurs at at 10.2 ppm indicating the involvement of these hydrogens through intramolecular hydrogen bonding with the carbonyl oxygen of – C-NH group. The other hydrazido group N (2) H appears at 9.6 ppm. A singlet at 3.8 ppm and multiplet at 7.5 ppm are attributed to the protons CH<sub>2</sub> and aryl groups, respectively. 3.3. The infrared spectra of the ligand and copper complexes Selected IR spectral bands for the ligand and copper complexes are given in table (2). The IR spectrum of the free ligand is characterized mainly by two strong bands at 1670 and 765 cm<sup>-1</sup> assigned to ν(C=O) and ν(C=S) vibrations. The absence of any bands above 3500 cm<sup>-1</sup> or the region 2600-2550 cm<sup>-1</sup> due to the bands of ν(OH) and ν(SH), respectively and the lack of any signals in the NMR spectra of the free ligands due to the protons of the-OH or SH, confirms that the ligand exist entirely in the keto form. The three bands at 3345, 3305 and 3235 cm<sup>-1</sup> in the spectra of the ligand are assigned to ν(N(4)-H), ν(N(2)-H) and ν(N(1)-H), while the ν(N-N)19 vibration is observed at 925 cm<sup>-1</sup> as medium sharp band. Also the bands at 1500, 1440 and 1280 cm<sup>-1</sup> may be due to ν(N-C=S) 20. These bands are assigned as coupled modes consisting principally of ν(NH) and ν(CN). The IR spectra of the complexes B1 and B2 show that the bands corresponding to ν(C=O) and (N-(2) H) shift to lower frequency as compared of free ligand. These low shift, may be ascribed to the coordination of the C=O, N (2) H groups to the metal ion. While in complex B3 show that new bands appear at 1525, 690 assigned to ν(C=N), ν(S-C) and ν(C=O) shift to lower frequency, indicating that the ligand behaves as monobasic tridentate, coordinating via ν(C=N), (S-C) 21 and (-C=O) in complex. The new bands appeared at 543-470 cm<sup>-1</sup>, 480-385 cm<sup>-1</sup>, and 340,354 cm<sup>-1</sup> in complexes (2) and (3) assigned to ν(Cu-O), ν(Cu-N) 22, 23 , ν(Cu-Cl) and ν(Cu-S), respectively. While in complex (2) appear broad peaks at 4420–3447cm<sup>-1</sup> indicating coordinated water<sup>24</sup>. Table 1. Elemental analyses and molar conductivities of 2-[2-(4- choro phenyl amino acetyl-N-phenylhydrazine carbothioamide(H2L, C<sub>15</sub>H<sub>16</sub>N<sub>4</sub>O<sub>2</sub>S) ligand and copper metal complexes. <sup>Λ</sup><sub>m</sub> =molar conductivity ohm<sup>-1</sup> cm<sup>2</sup> mol<sup>-1</sup> in 10<sup>-3</sup>M DMF Table 2. Infrared spectral bands (cm<sup>-1</sup>) 2-[2-(4- chorophenylamino acetyl-N-phenyl hydrazine carbothioamide ligand and copper (II) complexes. 3.4. Electronic absorption spectra and magnetic moment. The electronic spectral bands of the copper (II) complexes as well as the spectra of the ligand in solution DMF are shown in table (3), (Fig.1). The π - π\* transition band is No. Compound Colour Yield (%) Mol. Wt. Found (Calc.) % μ<sub>eff</sub> (B.M) C H Cl <sup>Λ</sup><sub>m</sub> H<sub>2</sub> L Pale brown (65) 334. 5 53.6(53.8) 4.6(4.8) \_\_\_ B1 Cu(H<sub>2</sub>L)(OAc)<sub>2</sub> Dark Green (70) 516 44.1(43.8) 4.2(4.3) - 21 1.80 B2 Cu (HL)Br(H<sub>2</sub>O) green (60) 496 36.3(36.7) 3.4(3.4) - 30 1.71 B3 Cu(HL)(NO<sub>3</sub>) Green (60) 459 36.3(36.7) 3.4(3.4) - 19 1.72 No Compound ν(N4- H) ν (N2- H) ν (N1-H) ν (C=O) ν (C=S) ν (CuN) ν (Cu-O) (CuS) H<sub>2</sub>L 3345 3305 3235 1670 765 - - 2 Cu(H<sub>2</sub>L)( OAc)<sub>2</sub> 3240 3165 3100 1595 760 470 440 - 3 Cu (HL)Br(H<sub>2</sub>O) 3285 3240 3120



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1665 690 490 385 340 4 Cu(HL)(NO<sub>3</sub>) 3292 3210 3140 1595 758 543 480 345 observed at 32850cm<sup>-1</sup> for H<sub>2</sub>L. Compared to the free ligand, in the copper (II) complexes, this band is shifted to longer wave length (Red shift) which is consistent with an increase in the degree of Pi-cloud conjugation 25 . The electronic spectra of copper (II) complex display one broad band at the 15560 cm<sup>-1</sup> range due to the 2B<sub>2g</sub> → 2A<sub>2g</sub> transition with a square planar geometry<sup>26</sup>, the band at 14560, 15380 and 15870 cm<sup>-1</sup>, assigned to d-d transition.. The magnetic moment values for Cu (II) complexes are 1.72-1.8 B.M.<sup>27</sup>. Table 3. Solution DMF electronic spectra (cm<sup>-1</sup>) of H<sub>2</sub>L and their Cu (II) complexes. Compounds Intraligand and charge transfer d-d bands  
H<sub>2</sub> L 32850 Cu(H<sub>2</sub>L)( OAc)<sub>2</sub> 30960 24720 15380 Cu(H<sub>2</sub>L)( OAc)<sub>2</sub> 31950 26320 14560  
Cu(HL)(NO<sub>3</sub>) 32050 25970 15870 H<sub>2</sub>L )1( )2( NH CH<sub>2</sub> C O N H H N N H C S Cl NH CH<sub>2</sub> O  
Cu NH C NH N H C S Cl OAc OAc NH CH<sub>2</sub> C O NH Cl HN NH Cu S C Br H<sub>2</sub>O ( 3) Scheme  
.1. Chemical structures of ligand and their copper complexes. 3.5. Effect of copper (II) complexes of thiosemicarbazone on tumor size Results show that the treatment of tumor bearing mice with Cu (II) complexes of thiosemicarbazone resulted in a varied inhibitory effect on the tumor growth. As shown in table (4), significant decreases in solid tumor size were observed after treatment tumor bearing mice with non-irradiated complexes for 15 days as compared to that of tumor bearing mice group. Yousof et al. 28 reported that thiosemicarbazide complexes exhibited antitumor activity against EAC in mice. In contrast to nonirradiated complexes, γ-irradiated complexes exhibited more potent inhibitory action on the tumor size. Table 4: Effect of Cu (II) complexes of thiosemicarbazone before and after irradiation on tumor size Tumor size  
Tumor bearing mice 1.8±0.2 Tumor + DMSO/H<sub>2</sub>O 1.7±0.4 Tumor bearing mice + B 1.1±0.3 b  
+A 0.7±0.2 b,c + B1 1.8±0.7 b +A1 0.6±0.1 b,c + B2 1.1±0.4 b +A2 0.4±0.1 b,c C O Cu S N C  
H N NH H<sub>2</sub> C N H NO<sub>3</sub> + B3 1.3±0.3 b +A3 0.4±0.1 b,c (b) Significant when compared with tumor bearing mice group. (c) Significant change when compared with its non-irradiated complex. 3.6. Effect of copper (II) complexes of thiosemicarbazone on the oxidative status Table (5) demonstrates the effect of γ-irradiated and non-irradiated thiosemicarbazone complexes on some oxidative status parameters in liver of mice bearing malignant tumors. Results showed that all complexes exhibited antioxidative activities where, the levels of lipid peroxidation, measured as MDA, were markedly decreased (p).