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Synthesis, spectroscopic characterization and biological application of copper complex of 5-carbethoxy-2-thiouracil

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Abstract

5-carbethoxy-2-thiouracil (eitotH$_2$) reacts with CuX (X= Cl, Br, I) halides to give the formula [CuX(eitotH)$_2$]$_2$ dinuclear complexes, while the formula [CuX(PPh$_3$)$_2$(eitotH)$_2$] mononuclear mixed ligand complexes result when reaction is carried out in the presence of two equivalent of triphenylphosphine (PPh$_3$). The new copper (I) complexes were studied against two tumor cell lines, A549 (human pulmonary carcinoma cell line) and HeLa (human epithelial carcinoma cell line) and one regular immortalized cell line, MRC5 (human fetal lung fibroblast). In comparison to the phosphine free ones that hindered cell proliferation only at relatively high concentration, the mixed ligand complexes with triphenylphosphine were found to be extremely cytotoxic.

Keywords: Copper (I), 5-carbethoxy-2-thiouracil (eitotH$_2$), Triphenylphosphine, in vitro cytotoxicity, carcinoma cell lines

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INTRODUCTION:

The main biochemical function of copper is well known both as an important trace metal to many metalloenzymes and as a component of exogenously administered compounds in humans, primarily in the complexes that can interact with biomolecules. Numerous copper enzymes and proteins, most of them in their active site possessing a bimetallic copper nucleus, play Important features in biological processes [1]. Therefore, there is a particular interest in bimetallic copper complexes since they can act as templates for a variety of important biological systems. For example, in the biological binding, activation and reduction of dioxygen, the role of dicopper sites in electron transfer processes has been the subject of intensive research over the last two decades [2-5]. Copper complexes are interesting because of their possible use as antimicrobial agents [6-8], anti-inflammatory agents [9,10] antitumor agents [11-14], but copper (II) complexes are most of the studied compounds. Similar studies are less popular on copper(I) derivatives, and the complexes typically contain planar aromatic chelating ligands [15-17] or ligands capable of stabilizing the aqueous media`s low oxidation state of the metal ion.

Orotic acid (6-carboxyuracil) is a biologically very essential molecule and is the only active precursor for nucleic acid production of the pyrimidine bases [18, 19]. Orotic acid plus its derivatives and metal complexes have been the focus of extensive studies for the reason [20-23]. Differently relatively less consideration has been given its anticancer, antibacterial, and anti-hypertensive properties, isoorotic acid (5-carboxyuracil) [24, 25]. Copper complex of isoorotic acid show antibacterial properties, [26] and platinum complex show antibacterial, antiviral and antitumor properties [27].

5-carbethoxy-2-thiouracil prefer the ester contrary to the free 5-carboxy-2-thiouracil we wanted to assure the well-known coordination of thione-S to the soft copper (I) ion, preventing any potential side effect, such as metal oxidation. 5-carbethoxy-2-thiouracil (eitotH$_2$) has received little attention so far, considering its biological significance [28], as there are literally no references to its co-ordinating action. The synthesis and characterization of [CuX(eitotH$_2$)$_2$]$_2$ and [CuX(eitotH$_2$)(PPh$_3$)$_2$] copper (I) halide complexes and the structural characterization of representative compounds of each form are recorded in this work (where eitotH$_2$ = 5-carbethoxy-2-thiouracil). We also researched the cytotoxic activity of the new compounds against tumor cell lines, A549

(cell line of human pulmonary carcinoma) and HeLa (cell line of epithelial carcinoma) and one normal immortalized cell line, MRC5 (human foetal lung fibroblast)

EXPERIMENTAL SECTION

Material for synthesis

Copper (I) halides, triphenylphosphine and 5-carbethoxy-2-thiouracil, commercially available were bought as reagent grade and used as materials, while the solvents were filtered. According to guideline Infra-red spectra with Nicolet FTIR 6700 spectrophotometer were obtained in KBr discs in the range of 4000-200 Cm^{-1}, while a Shimadzu 160A spectrophotometer was used to obtain the electronic absorption spectra.

Synthesis of complexes 1-3: A solution of 200.2 mg (1mmol of 5-carbethoxy-2-thiouracil was applied to a a solution of (0.5 mmol) copper (I) halide (49 mg for CuCl, 71.7 mg for CuBr, 95.2 mg for CuI) in 30 cm^3 of dry acetonitrile in 20cm^3 of methanol and the mixture was stirred for 1 h at ambient temperature. The resulting bright yellow solution was filtered and left to stand in the atmosphere, depositing yellow crystals that was filtered off and vacuum dried.

*$[CuCl(eitotH_2)_2]_2$ (**1**):* Yellow powder (112 mg, 45 %), m.p. 270°C; Anal. Calc. For

$C_{28}H_{32}Cu_2Cl_2N_8O_{12}S_4$: C, 33.67; H, 3.23; N, 11.22. Found: C, 33.47; H, 3.12; N, 11.08. IR (cm^{-1}): 3119m, 3064m, 2934m, 1715vs, 1747vs, 1617s, 1562vs, 1464s, 1372m, 1293s, 1140vs, 1061m, 1010m, 802s, 746m, 584s; UV-Vis (λmax, log ε): 264 (4.23), 311 (4.37).

*$[CuBr(eitotH_2)_2]_2$ (**2**):* Yellow crystals (207 mg, 70 %), m.p. 271°C; Anal. Calc. For

$C_{28}H_{32}Cu_2Br_2N_8O_{12}S_4$: C, 30.92; H, 2.97; N, 10.30. Found: C, 31.17; H, 3.03; N, 10.18. IR (cm^{-1}): 3142m, 3050m, 2999m, 1733vs, 1622vs, 1552vs, 1529vs, 1460s, 1395s, 1302vs, 1210vs, 1145vs, 1010m, 886m, 792m, 602s, 510m; UV-Vis (λmax, log ε): 263 (4.41), 310 (4.67).

*$[CuI(eitotH_2)_2]_2$ (**3**):* Yellow crystals (221 mg, 75 %), m.p. 274°C; Anal. Calc. For

$C_{28}H_{32}Cu_2I_2N_8O_{12}S_4$: C, 28.46; H, 2.73; N, 9.48. Found: C, 28.55; H, 2.78; N, 9.41. IR (cm^{-1}):

3129m, 3050m, 2911m, 1733vs, 1617vs, 1556vs, 1524vs, 1460vs, 1390vs, 1302vs, 1210vs, 1176s, 1149vs, 1010m, 886m, 866s, 792s, 602vs, 593vs, 514vs; UV-Vis (λmax, log ε): 264 (3.94), 311 (4.11).

Synthesis of complex 4-6: Copper (I) halide solution (0.25mmol) (24.5 mg for copper (I) halide solution in 30 cm^3 of dry acetonitrile, triphenylphosphine (131mg, 0.5mmol) was added to CuCl, 38.5 mg for CuBr, 47.6mg for CuI, and the solution was stirred until a white precipitate was observed. A solution of 50 mg (0.25 mmol) of 5-carbethoxy-2-thiouracil was added and added to 20 cm^3 of methanol. The mixture was stirred at room temperature for 1 hr.

*$[CuCl(PPh_3)_2(eitotH_2)].0.5\ CH_3C(O)CH_3$ (**4**)*: Yellow crystals (134 mg, 65 %), m.p. 147°C; Anal. Calc. For $C_{43.5}H_{40}CuClN_2P_2O_{3.5}S$: C, 62.67; H, 4.85; N, 3.28. Found: C, 62.47; H, 4.82; N, 3.32. IR (cm-1): 3050m, 2984m, 1752vs, 1723vs, 1695vs, 1616s, 1565, 1540 vs, 1480s, 1461vs, 1435vs, 1410s, 1369s, 1296vs, 1226vs, 1147vs, 1093vs, 1061m, 1030m, 796s, 742vs, 694vs, 590s, 517vs, 501vs; UV-Vis (λmax, log ε): 262 (4.48), 310 (4.39).

*$[CuBr(PPh_3)_2(eitotH_2)].CH_3CN$ (**5**):* Yellow crystals (173 mg, 80 %), m.p. 154°C; Anal. Calc. For $C_{45}H_{41}CuBrN_3P_2O_3S$: C, 59.44; H, 4.54; N, 4.62. Found: C, 59.17; H, 4.61; N, 4.64. IR (cm^{-1}): 3148m, 3049m, 2936m, 1752vs, 1730vs, 1619s, 1566, 1556vs, 1482vs, 1458s, 1435vs, 1407s,

1367s, 1293vs, 1223vs, 1150vs, 1097vs, 1061m, 1026m, 846m, 745vs, 694vs, 587s, 517vs, 498vs; UV-Vis (λmax, log ε): 263 (4.56), 308 (4.49).

*$[CuI(PPh_3)_2(eitotH_2)]$ (**6**):* Yellow powder (176 mg, 77 %), m.p. 191 °C; Anal. Calc. For

$C_{43}H_{38}CuIN_2P_2O_3S$: C, 56.43; H, 4.18; N, 3.06. Found: C, 56.15; H, 4.10; N, 3.01. IR (cm^{-1}):

3050m, 2932m, 1755vs, 1736vs, 1616s, 1556vs, 1477vs, 1457s, 1435vs, 1401s, 139v0s, 1293vs,

1226vs, 1144vs, 1093s, 1024m, 886m, 846m, 799s, 742vs, 694vs, 587s, 517vs, 501vs, 489s; UV-Vis (λmax, log ε): 264sh (5.11), 309 (4.88).

Spectroscopic analysis: -

Complex 1-6 electronic spectra, recorded in acetonitrile at room temperature, show two extreme large bands with a maximum of 264 and 310 nm. The high energy band can be used with reference to the absorption spectrum of the uncoordinated 5-carbethoxy-2-thiouracil intraligand transition on the thione ligand are assigned to π→ π* , while the lower energy band may be considered to be thione originating CT transitions on the thione ligand are assigned to π→ π*, while the lower energy band may be considered to be a thione originating CT transition at the C=S bond [29,30] that may have a partial CT character, as it lies in region where the free ligand absorbs, expressing a free ligand. Small red shift as a result of copper coordination. The absorption due to the intraligand transition within the triphenylphosphine totally overlaps with the high energy band assigned to the intraligand π→ π* transition on the 5-carbethoxy-2-thiouracil ligand in the compound spectrum 4-6, resulting in a wide band of increased intensity compared to that in the compound 1-3 spectrum.

The FTIR spectrum of compound 1-6 recorded in 4000-25 cm^{-1} in which characteristic band of 5-carbethoxy-2-thiouracil ligand with band shift of S coordination. The intense bands appear at 1755 cm^{-1} and 1733 cm^{-1} in the spectrum of free $eitotH_2$, due to ν(C=O) stretching vibrations, appear slightly shifted to higher energies in the spectra of all complexes. "thioamide I" band, appear at having contributions from ν(C–N) and δ(C–H), remains almost unshifted, the very strong band at 1565 cm^{-1} known as "thioamide I" band, having contributions from ν(C–N) and δ(C–H), remains almost unshifted, but the band at 1163 cm^{-1} assigned to the "thioamide III", which involves major contributions from ν(C=S), appears clearly shifted (by ca. 20 cm^{-1}) towards lower energy upon coordination.

MTT cytotoxicity assay:

The growth inhibitory effect of tumor cell line was assessed by means of MTT assay. By tracking the conversion of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to formazan, cell proliferation was assessed. MTT reduction cell is catalyzed by the enzyme mitochondrial dehydrogenase and is thus an indicator of cellular dehydrogenase [31].

1* 10^4 well (A549) and 5 * 10^3 well (HeLa and MRC5) cells were incubated into 96-well plates and treated with various concentration (5,10, 15,20,30 40, 50, 60 and 120 µM of copper complexes and their uncoordinated ligands (5-carbethoxy-2-thiouracil and triphenylphosphine) previously dissolved in DMSO after 24 Hours. The cells which are not

introduced to copper complexes worked as the monitor. Medium removal from each well and replacement with 100 µl of fresh medium and 10 µl of MTT 12Mm (5mg/ml) per well was followed by incubation of complexes with cells for 48 hours. After a further brief incubation at 37°C for 4h, before the purple colored formazan product forms, 100 µl of 10% SDS 10%-HCl 0.01M was applied to the cells and left for 15-18hrs under the same growth conditions.

Cell cultures:

Human cell lines were cultured in DHEM (Dulbecco`s adaptaed Eagle`s medium) from three separate sources namely A549 (human pulmonary cell line), immortalized MRC5 (human foetal lung fibroblast) and HeLa (human epithelial carcinoma cell line)

RESULT AND DISCUSSION

Cytotoxic activity

The antitumor activity of the compounds was estimated by determining their ablity to inhibit tumor cell growth in the culture medium DMEM (Dulbecco Modified Eagle Medium with L-glutamine) complemented by 10% foetal bovine serum and antibiotic penicillin and streptomycin.

Copper compounds 1-6 and the corresponding free ligands eitotH_2 and PPh_3 underwent an MTT assay and their cytotoxic properties were investigated against a panel of two tumor cell lines, A549 (human pulmonary carcinoma cell line) and HeLa (cell line of epithelial carcinoma) and one immortalized normal cell line MRC (humh of MTT complex an foetal lung line) Fiberblasting IC values calculated from the dose survival curve obtained after 48 h of MTT complex solution therapy. In all the cell lines used the free 5-carbethoxy-2-thiouracil eitotH_2 and triphenylphosphine (PPh_3) proved to be very ineffective. In particular, PPh_3 gave recognizable IC_{50} values only at higher concentration. On the contrary, all the complexes examined demonstrated a growth inhibitory potency in the micromolar range against the different cell lines.

The three mixed-ligand complexes namely $[CuI(PPh_3)_2(eitotH_2)]$, $[CuBr(PPh_3)_2(eitotH_2)]$ were significantly more successful against all cell lines and notable against HeLa cell lines with IC_{50} values of 2.55µM, 3.66µM and 3.36µM respectively. Copper complexes of Carbethoxy-2-thiouracil are less active towards HeLa, A549 cancer cell line along with MRC5 non-cancerous cell line, triphenylphosphine counterpart (compound 4-6) even at low temperature.

The cytotoxic activity of the three homoleptic copper (I) halide complexes 1-3 is in the order of $[CuI(eitotH_2)_2]_2$ > $[CuBr(eitotH_2)_2]_2$ > $[CuCl(eitotH_2)_2]_2$ with half the minimum inhibitory concentration (IC_{50}) ranging from 45.5-86.6 µM for HeLa to 54.25-89.56 for MRC5 and 41.3-110 for A549.

The values of half the minimum inhitory concentration (IC_{50}) are slightly lower, ranging from 2.55-3.6 µM for HeLa 4.77-6.84 for MRC and 4.56-5.0 for A549, considering the three heteroleptic complexes 4-6. The in vitro cytotoxicity against A549 of all three complexes exceeds that recently recorded for cisplatin by a factor of approximately 2 [32]. There is no strong pattern in the cell killing effect observed when moving from chlorine to bromo to iodo derivative compared with the case of compound 1-3.

Now a day, Copper complexes examined as a suitable drug for cytotoxicity against tumor cells. [33]. It is well known that phosphine-containing complexes are used as possible anticancer agents [34-36]. In most cases, the presence of phosphine in the gold was observed compared to phosphine-free bacteria, thiolates induced increased cytotoxicity. It is known that copper binding compounds are only successful in binding ubiquinated protein accumulation and apoptosis in tumors' but not in nontransformed cells, as other proteasome inhibitors have been identified. The effects of anti-angiogenesis and the possible use of proteasome inhibitors in cancer therapy have been extensively investigated [37,38]. In general, studies involving DNA breakage or copper replacement of copper containing enzymes, ROS development improvement or alteration in copper metabolism need to be conducted. Our observation, however are promising, along with recent research on copper (I) derivatives, as we may consider these new derivatives as lead compounds to discover new potential cancer drugs.

CONCLUSION

5-carbethoxy-2-thiouracil (eitotH_2) co-ordinates to copper (I) halides entirely via the soft thione S atom forming $[CuX(eitotH_2)_2]_2$ composition dinuclear complexes with the two metal ions in a highly distorted tetrahedral framework, doubly bridged by atoms of thione Sulphur. These dimers are used to generate mononuclear four coordinate phosphine/ thione as precursors for the preparation of mixed ligand $[CuX(eitotH_2)(PPh_3)_2]$ form complexes. These dimers are used to generate mononuclear four coordinate phosphine/thione as precursors for the preparation of mixed –ligand to form complexes $[CuX(eitotH_2)(PPh_3)_2]$. Evaluation of the outcomes of cytotoxicity indicates that triphenylphosphine is present in complexes 4-6 contribute to a large increase in cytotoxic activity relative to activity of the phosphine-free compounds 1-3. In addition, the cytotoxicity of all the above complexes is in contrast to that of each of the free ligands, higher in all the cell lines that were tested. The remarkable antitumor behavior of $[CuCl(PPh_3)_2(eitotH_2)]$, $[CuIPPh_3)_2(eitotH_2)]$, due to the literature data available so far is due to the literature data available so far is due to the PPh_3 presence may be considered to be representative of their capacity to induce apoptosis. A

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