Synthesis, characterization and in vitro anticancer activities of semicarbazone and thiosemicarbazone derivatives of salicylaldehyde and their copper complexes against human breast cancer cell line MCF-7

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Copper complexes of salicylaldehyde semi-thiosemicarbazones are prepared and characterized structurally through their X-ray structures. The chloro compound of the semicarbazone ligand is monomeric having square planar geometry while the nitro complex is dimeric containing phenoxyl bridges. The chloro complex of the thiosemicarbazone derivative is dimeric where individual copper centres possess square pyramidal geometries. The parent ligands are almost inactive against the rapidly dividing human breast cancer cell line MCF-7 while the copper conjugates of semicarbazone ligand are found to be potent anti proliferative agents due to their facile Cu²⁺/Cu⁺ redox couple and can generate considerable intracellular oxidative stress.

**Notes**

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The semi- and thiosemicarbazone ligands have received great impetus in recent years due perhaps to their remarkable potential in inhibiting ribonucleotide reductase, an obligatory enzyme in DNA synthesis. As a consequence, compounds containing these pharmacophores have been evaluated for their antiproliferative properties against a variety of tumors. Besides the antitumor properties, these compounds have also been shown to possess antitubercular, antimalarial, anti-leishmanial and antifungal activities. The structure-activity correlations for the N-substituted thiosemicarbazones have been reviewed recently by Padhye et al. The biological activities of the thiosemicarbazone ligands have been attributed to their trace metal complexing abilities and the metal compounds have been generally found to possess enhanced therapeutic properties. For example, the iron complexes of 2-hydroxy-1,4-naphthoquinone-1-thiosemicarbazone and its C-3 methyl derivative have been shown to be active against P388 leukemia as well as MCF-7 breast cancer cells. Similarly, metal complexes of 3-benzoyl-α-methylbenzene acetic acid thiosemicarbazone have been shown to be effective against human MCF-7 breast cancer cell lines. The role of copper conjugates of thiosemicarbazone ligands has perhaps remained less explored compared to the corresponding iron compounds although remarkable anticancer activities have been noted in case of several copper thiosemicarbazones.

The semi- and thiosemicarbazone derivatives of salicylaldehyde ligand have been reported in literature along with their copper compounds. However, the nature of the geometries of these compounds has remained unresolved due to lack of x-ray crystal structure data.

The structural characterization of copper complexes of salicylaldehyde semi- and thiosemicarbazones including their crystallographic studies are reported here. In view of the anticancer activity observed for an analogous copper complex of salicylaldehyde-β-D-glycoside thiosemicarbazone against human leukemia cells, we have also evaluated the antiproliferative properties of the present compounds against human breast cancer cells, MCF-7, and have explored the underlying differences in their therapeutic potentials.

**Experimental**

Salicylaldehyde and semicarbazide hydrochloride were the products of Chemical Drug House (Mumbai) whereas thiosemicarbazide was obtained from Sisco Research Ltd (Mumbai). Copper chloride dihydrate was procured from Qualigens Chemicals Ltd (Mumbai). All other chemicals and solvents employed for synthesis were of analytical grade and were used as supplied. The instrumental measurements were made as described already.

**Synthesis of ligands**

Salicylaldehyde semicarbazone (L₁) and thiosemicarbazone (L₂) (Scheme 1) were prepared according to known methods. Anal. Calc. for C₉H₉N₂O₂ (L₁): C, 53.63; H, 5.06; N, 23.48. Found: C, 53.36; H, 5.11;
N, 23.38%. IR (cm\(^{-1}\)): 3454 vOH; 1669 vC=O; 1618 vC=N. Anal. Calc. for C\(_{10}\)H\(_4\)N\(_3\)O\(_2\) (L\(^2\)): C, 49.21; H, 4.65; N, 21.52. Found. C, 49.16; H, 5.50; N, 21.21%. IR spectra (cm\(^{-1}\)): 3441 vOH; 857 vC=S; 1613 vC=N.

![Scheme 1](image)

**Synthesis of copper complexes**

The copper-semicarbazonate complex, viz. [Cu(L\(^1\))][Cl\(_2\)I\(_2\)H\(_2\)O] (1), was prepared by refluxing ethanolic solutions of the ligand L\(^1\) and copper chloride dihydrate in 1:1 stoichiometric ratio for 15 min. The obtained precipitate was filtered, washed with ethanol and dried in vacuum\(^{24}\). Anal. Calc. for CuClO\(_4\)N\(_3\)CuH\(_2\): C, 32.42; H, 3.38; N, 13.40; Cu, 20.21%. Found: C, 32.09; H, 3.28; N, 13.52; Cu, 19.74. IR (cm\(^{-1}\)): 1652 vC=O; 1602 vC=N.

The analogous complex having general formula [Cu\(_2\)(L\(^1\))\(_2\)(H\(_2\)O)\(_2\)](NO\(_3\))\(_2\) (2) was prepared by employing copper nitrate and following a similar procedure. The green prismatic crystals of the resulting product suitable for single crystal X-ray diffraction studies were obtained from the reaction mixture on slow evaporation. Anal. Calc. for C\(_{13}\)H\(_{32}\)Cu\(_2\)N\(_3\)O\(_7\): C, 29.83; H, 3.10; N, 17.40; Cu, 19.74; C, 29.83. Found: C, 30.09; H, 3.28; N, 17.56; Cu, 19.21%. IR (cm\(^{-1}\)): 3475 vOH; 1653 vC=O; 1603 vC=N.

The copper-thiosemicarbazonate complex (3), [Cu\(_2\)(L\(^2\))\(_2\)(H\(_2\)O)\(_2\)], was synthesized by refluxing the methanolic solutions of the ligand L\(^2\) and copper chloride dihydrate in 1:1 metal to ligand molar ratio for 2 h. The obtained green precipitate was filtered, washed with methanol and dried in vacuum. Anal. Calc. for C\(_{13}\)H\(_{32}\)N\(_7\)S\(_2\)Cu\(_2\): C, 31.78; H, 2.97; N, 13.90; Cu, 21.03. Found. C, 31.45; H, 3.10; N, 13.52; Cu, 20.41%. IR (cm\(^{-1}\)): 1606 vC=N; 845 vC=S.

**Anticancer activity**

The cell viability of MCF-7 breast cancer epithelial cells was determined using DMSO solutions of the test substances (final concentration not exceeding 1%) by the MTT assay following a procedure described previously\(^25\).

**X-ray crystallography of 2**

The crystallographic data were measured using a Stoe IPDS area detector diffractometer with graphite-monochromatised Mo-K\(_\alpha\) radiation; wherein the crystals were cooled to 200 K with an Oxford Cryostream low-temperature attachment. The structures were solved by the direct method and refined against \(R^2\) (all data, anisotropic thermal parameters for all non-H atoms, all H atoms located and fully refined with isotropic thermal parameters), using the SHELXTL software suite\(^{36}\).

**Results and discussion**

The compositional data on the synthesized copper complexes reveal a 1:1 metal to ligand stoichiometry for the present compounds. Compounds 2 and 3 exhibit diamagnetic behaviour at room temperature possibly arising out of dimeric associations promoting antiferromagnetic exchange interactions through the phenoxide bridges between the two copper atoms\(^{27}\).

An ORTEP view of the dimeric complex 2 is shown in Fig. 1 while the crystallographic parameters and selective bond distances and bond angles are summarized in Tables 1 and 2 respectively. The crystal structure of the complex 2 consists of a centrosymmetric dimer consisting of two copper atoms bridged by the phenoxide oxygens. The ligand L\(^1\) in this compound acts as a monoaonic, tridentate moiety coordinating to the central metal through phenolate oxygen, azomethine nitrogen and carbonyl oxygen of the semicarbazide side chain. The two copper centers (Cu\(_1\)--Cu\(_2\)) are separated by a distance of 3.0049(8) Å which is similar to that found for an analogous copper compound, viz. [Cu(HL)(H\(_2\)O)\(_2\)]NO\(_3\), where HL = salicylaldehyde acetylhydrazone anion\(^{28}\). The ONO donor atoms of the ligand constitute the basal plane in the present complex with the bond distances of Cu-O (average) 1.956(3) Å, Cu-N = 1.942(3) Å and Cu-O = 1.982(2) Å, respectively. The fifth axial position is occupied by a water molecule with the copper-oxygen distance being 2.245 Å.

All bond angles around the copper center are unequal and consequently the coordinated water molecule is not exactly perpendicular to the chelate rings surrounding copper but is slightly tilted towards one of them. The geometry around the copper atoms can be best described as the distorted square pyramidal one. It is clear that the nitrate ions are not directly coordinated to the copper atom but are
Table 1—Selected crystallographic data for [Cu$_2$L$_1$(H$_2$O)$_2$](NO$_3$)$_2$ (2)

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<th>Property</th>
<th>Value</th>
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<td>$c = 18.3617(15)$ Å</td>
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<td></td>
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<tr>
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<td>Goodness-of-fit on $F^2$</td>
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<td>R indices (all data)</td>
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<tr>
<td>R indices [I$&gt;$2sigma(I)]</td>
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<tr>
<td>Largest diff. peak and hole</td>
<td>1.187 and -0.512 e.Å$^{-3}$</td>
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</table>

present in the crystal lattice and are involved in a hydrogen-bonding network.

In the IR spectrum of the ligand L$^1$, the stretching vibrations due to the semicarbazide carbonyl and the imine functions are observed at 1669 and 1618 cm$^{-1}$ respectively which are shifted to 1653 and 1603 cm$^{-1}$ in the corresponding copper complexes indicating their involvement in metal coordination. The presence
of the coordinated water molecule in the complex 2 is confirmed by the hydroxyl stretching frequency at 3475 cm$^{-1}$ which is absent in case of compound 1.

The phenolic hydroxyl peak appearing at 3441 cm$^{-1}$ in the spectrum of the ligand L$^2$ is found to be absent in the corresponding copper complex while the thiocarbonyl absorption at 857 cm$^{-1}$ in the ligand is found to be shifted to the lower wavenumbers 845 cm$^{-1}$ indicating involvement of the corresponding donor atom centre in metal coordination. The involvement of the azomethine nitrogen in the coordination is indicated by shifting of the imine vibrational band from 1613 cm$^{-1}$ to 1606 cm$^{-1}$ in the corresponding copper complex.

The electronic spectra of all copper compounds recorded in the DMF solvent exhibit the charge transfer bands in the region 26000-36500 cm$^{-1}$, while an intense band observed at 25400 cm$^{-1}$ for the compound 3 can be ascribed to S→Cu$^{II}$ transition as suggested by West et al. The broad band seen in the visible region of these complexes around 14300-15600 cm$^{-1}$ is typical of the copper compounds having square pyramidal geometry as reported by several researchers. The proposed structure for the complex 3 is shown in Fig. 2.

The cyclic voltammetric profiles of the synthesized complexes show an irreversible ligand-based peak corresponding to the reduction of the azomethine C=N linkage at $-0.94$ volts. The reversible Cu$^{II}$/Cu$^{I}$ redox couple for the present compounds is observed in the range $+0.28$ to $+0.40$ volts indicating a facile reduction of the copper center in compounds 1 and 2 which may be relevant to their subsequent biological activities.

The antiproliferative activities of the ligands and their metal complexes were examined on human breast cancer cell line MCF-7 (Fig. 3), in the concentration range 1-6 μM which reveal that the metal complexes have more pronounced antiproliferative activities than the corresponding ligands. The ligand L$^2$ as well as its copper complex 3 is inactive at the tested concentrations which can be correlated to its lower Cu(II)/Cu(I) redox couple. On the other hand, the copper complexes 1 and 2 show IC$_{50}$ values of 3.6 μM and 3.2 μM, respectively. Similar five-coordinated dimeric complexes have been shown to possess antiproliferative activity. The remarkable enhancement observed in the antiproliferative activities of the copper compounds can be explained on the basis of their facile Cu(II)/Cu(I) redox couple which can generate considerable intracellular oxygen by the reduced cuprous centres. MCF-7 Breast cancer cells have been shown to be very sensitive to such additional oxidative stress due to their down-regulated antioxidant defense enzymes leading generally to apoptotic death.

The present work has thus shown that how copper complexation of two known chelating ligands like salicylaldehyde semi-thiosemicarbazone ligands is advantageous in designing active anticancer agents whose antiproliferative activities can be modulated following their Cu(II)/Cu(I) redox couples.

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References


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