STUDIES ON COORDINATION CHEMISTRY AND BIOACTIVITY OF COMPLEXES OF A TRIDENTATE ONS SCHIFF BASE WITH SOME HEAVIER TRANSITION METAL IONS

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Abstract

A tridentate Schiff base, S-benzyl-β-N-(2-hydroxyphenyl)methyleneithiocarbazate, (HONSH), with a donor sequence of ONS, was synthesized from the condensation of S-benzylithiocarbazate (SBDTC) with an equimolar amount of 2-hydroxybenzaldehyde in absolute ethanol. The reactions of HONSH with metal ions [La(III), Ce(IV) and Th(IV)] yielded complexes of compositions, [La(ONS)NO3·2H2O], [Ce(ONS)(NO3)2] and [Th(ONS)2]. The ligands and the complexes were characterized from elemental analyses and spectroscopic measurements. The metal complexes were found to be active against colon cancer cell lines with the CD50 values of 27.5, 28.4 and 19.3 µg/ml for the La(III), Ce(IV) and Th(IV) complexes, respectively. The La(III) complex was found to be very active against leukemic cell lines with the CD50 value of 6.8 µg/ml.

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**Introduction**

There has been a lot of interest in the study of coordination chemistry of dithiocarbazic acid, \( \text{NH}_2\text{NHCHS}_2 \), and its derivatives over the past few decades [1-19]. Dithiocarbazic acid and the Schiff bases derived from its S-alkyl esters form an interesting series of ligands. The properties of these ligands can be greatly modified through the introduction of organic substituents into the ligand molecules, thereby inducing different geometries in the resultant metal complexes [1-6, 8-15]. Researchers in this field have been involved in the isolation of Schiff bases of dithiocarbazic acid or its S-alkyl esters with different aldehydes or ketones. The number of ligands synthesized are increasing because different ligands show different biological properties, and also because some are of industrial importance [20], although certain ligands may differ only slightly in their molecular structures [10]. In our continuous effort to contribute to the discovery of biomedical drugs, we became interested to extend our study with a new Schiff base produced from the condensation of SBDTC with 2-hydroxybenzaldehyde. We report herein the syntheses of the organic ligand along with its complexes, and also on their biomedical properties.

**Experimental**

IR spectra were recorded in KBr on a FT-IR Perkin-Elmer 1725X IR spectrometer. All the spectra were run between the range of 400-4000 cm\(^{-1}\). Carbon, hydrogen and nitrogen analyses were carried out at the University of Trieste, Italy. Molar conductance of 1 X 10\(^{-3}\) Molar solutions of the metal complexes in DMSO were measured at 30°C using a Jenway 4310 conductivity meter and a dip-type cell with platinized electrode. The UV/VIS spectra, in DMSO, were recorded on a Shimadzu UV-160 Spectrophotometer over 200-800 nm range. All chemicals were used as received from the suppliers. Biological properties were evaluated at the Animal Cell and Tissue Culture Laboratory, Department of Biotechnology, Universiti Putra Malaysia.

**Culture of Cells and Cytotoxic assay**

The Human cell T-lymphoblastic leukemia (CEM-SS) and colon cancer (HT-29) cell lines were obtained from the National Cancer Institute, Frederick, Maryland, USA. The cells were cultured in RPMI-1640 (Sigma) medium supplemented with 10% fetal calf serum (Flow Lab). Cytotoxicity was determined using the microtitration 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay (MTT) reported by
Mosmann [21]. Solutions of compounds of different concentrations were prepared from the stock solutions (10 mg/mL) by serial dilution in RPMI-1640 to give a volume of 100 µL in each well of a microtiter plate as described by Ali et al. [22]. Each well was filled with 100 µL of cell suspension in complete growth medium (CGM) at 1-2 × 10^5 cells/mL. Cytotoxicity was expressed as the minimum concentration able to reduce the absorbance of treated cells by 50% with reference to the untreated cells (CD50). Tamoxifen was used as a control.

**Qualitative antimicrobial assay**

Eight pathogenic microbials were used to test the biological potential of the Schiff bases. They were *bacillus subtilis* (mutant defective DNA repair - B28), *pseudomonas aeruginosa* (60690), *methicillin resistant staphylococcus* (MRSA), *bacillus subtilis* (wild type - B29), *saccaromyces ceciricaee* (20341), *candida albican* (C. A.), *candida lypolytica* (2075) and *aspergillus ochraceous* (398). Antimicrobial activity of the extracts was qualitatively determined by a modified disc diffusion method [23]. A lawn of microorganisms was prepared by pipetting and evenly spreading 10 µL of inoculum (adjusted turbidometrically to 10^5 - 10^6 CFU/cm^3 [ CFU= colony forming units]) onto agar set in petri dishes, using Nutrient agar (NA) for the bacteria and Potato dextrose agar (PDA) for fungi. Whatman No. 1 filter paper discs of 6 mm diameter were impregnated with dimethylsulfoxide (DMSO) stock solution of the compounds (100 mg/cm^3) and dried under sterile conditions. The dried discs were then placed on the previously inoculated agar surface. The plates were inverted and incubated for 24 h at 37°C for the bacteria and 30°C for the fungi. Antimicrobial activity was indicated by the presence of clear inhibition zones around the discs.

*S-benzyl-β-N-(2-hydroxyphenyl)methylene thiocarbazate (HONS Schiff base)*

A solution of SBDTC (0.1 mol) in absolute EtOH (40 cm^3) was added to a solution of salicyaldehyde (0.1 mol) in the same solvent (25 cm^3). The mixture was heated on a steam bath for 15 min and then cooled to 0°C in an ice-salt bath whereupon yellow crystals formed. These were filtered off, washed with EtOH and dried *in vacuo* over silica gel. Yield 14.7 g, m.p : 184 °C (lit. 185°C) [24].
General method for the synthesis of metal complexes of La(III), Ce(IV) and Th(IV) with S-benzyl-β-N-(2-hydroxyphenyl)methylendithiocarbazate

The metal salts (0.013 mole) were dissolved in absolute ethanol (50 ml). An equimolar quantity of the HONSH Schiff base (0.013 mole) was dissolved in hot absolute ethanol (50 ml) in the presence of KOH. The two hot solutions were mixed and heated on a steam bath for 5-10 min and then it was cooled to room temperature. The precipitate formed was filtered off, washed with a small amount of EtOH and dried in vacuo over P₄O₁₀.

Results and discussion

The analytical results along with other physical properties are given in Table 1. The IR spectral data are given in Table 2. A strong band at 1038 cm⁻¹ in the IR spectrum of the Schiff base is tentatively assigned to the ν(C=S) mode. The Schiff base has a thiketo group (C=S) adjacent to a proton. The thione group is relatively unstable in its monomeric form and tends to give a stable C-S single bond by enethiolisation depending on the availability of a hydrogen atom adjacent to the C=S bond. The enethiolisation is promoted by the formation of a conjugated diene, −CH=NN=CS₂-R, a driving force stabilising thiolate coordination [1-9, 14-17]. The IR spectrum of the Schiff base did not display ν(S-H) at ca. 2568 cm⁻¹, indicating that in the solid state it remains in the thione form. However, in solution, it may remain in equilibrium with the thiol tautomer [1, 24]. The ν(C=S) band, however, disappears in the spectra of the metal complexes, indicating enethiolization in solution, and concomitant thiolate coordination. The IR spectrum of the Schiff base exhibits a strong band at 3105 cm⁻¹, assigned to the ν₅₆(N-H) of the free ligand. This band disappeared in the spectra of all the metal complexes suggesting that the proton attached to the α-nitrogen atom is lost upon complexation. The IR spectrum of the Schiff base showed a band of medium intensity at ca. 3082 cm⁻¹ which was assigned to the phenolic O-H. The absence of this band in the IR spectra of the metal complexes indicated that the phenolic proton is also lost upon complexation. This is further evident from the appearance of bands at ca. 629-631 cm⁻¹, arising from the metal-oxygen stretching modes. The ν(C=N) band of the Schiff base is lowered by 10-15 cm⁻¹ upon complexation, indicating coordination through the nitrogen atom of the azomethine group, thus affording tridentate dinegative chelation with the metal ions. The electronic spectra of the complexes in DMSO showed bands between 315-361 nm which were caused by of charge transfers. The molar conductance values showed that all the compounds were non-electrolytes excepting 1, which exhibited values corresponding to 1:1 electrolytes in DMSO [5], presumably arising because of solvation,
Cytotoxic activity

The cytotoxic screening results showed that only 1 was active against CEM-SS with CD$_{50}$ value of 6.8 µg/ml, while 1, 2 and 3 were found to be active against HT-29 with CD$_{50}$ values of 27.5, 28.4 and 19.3 µg/ml, respectively. Shier [25] suggested that a CD$_{50}$ value of more than 10 - 25 µg/mL will be considered weak cytotoxic while compounds with CD$_{50}$ values of less than 5.0 µg/mL were considered very active. Those having intermediate values, 5.0 - 10.00 µg/mL, were classified as moderately active. All of the present compounds were relatively more active against CEM-SS and HT-29 as compared to Tamoxifen (Table 3).

Antimicrobial activity

Qualitative antimicrobial assays indicated that none of the compounds were active against the microbes (Table 4).

Acknowledgements

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References


Table 1. Analytical data and other properties of the complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Colour</th>
<th>Found (Calc.) (%)</th>
<th>Molar conductance</th>
<th>UV/Vis spectral</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>H</td>
<td>N</td>
<td>(Ω (^{-1}) cm(^2) mol(^{-1}))</td>
</tr>
<tr>
<td>HONSH Schiff base</td>
<td>Yellow</td>
<td>59.57 (59.80)</td>
<td>4.67 (4.33)</td>
<td>9.26 (9.10)</td>
<td>90.4</td>
</tr>
<tr>
<td>(1) [La(ONS)NO(_3).2H(_2)O]</td>
<td>Yellow</td>
<td>33.53 (33.10)</td>
<td>3.00 (2.78)</td>
<td>7.82 (7.54)</td>
<td>6.65</td>
</tr>
<tr>
<td>(2) [Ce(ONS)(NO(_3))(_2)]</td>
<td>Brownish Green</td>
<td>33.91 (33.77)</td>
<td>4.14 (4.66)</td>
<td>9.92 (10.28)</td>
<td>25</td>
</tr>
<tr>
<td>(3) [Th(ONS)(_2)]</td>
<td>Light Yellow</td>
<td>43.26 (43.12)</td>
<td>2.90 (2.89)</td>
<td>6.72 (6.65)</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 2 IR spectral data of the compounds\(^a\)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Bands</th>
<th>(cm(^{-1}))</th>
<th>v(O-H)</th>
<th>v(M-O)</th>
<th>v(N-H)</th>
<th>v(C=N)</th>
<th>v(C=S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HONSH Schiff base</td>
<td></td>
<td></td>
<td>3082m</td>
<td>-</td>
<td>3102s</td>
<td>1610s</td>
<td>1038s</td>
</tr>
<tr>
<td><a href="1">La(ONS)NO(_3).2H(_2)O</a></td>
<td></td>
<td></td>
<td>3446br</td>
<td>631w</td>
<td>-</td>
<td>1600m</td>
<td>-</td>
</tr>
<tr>
<td>[Ce(ONS)(NO(_3))(_2)] (2)</td>
<td></td>
<td></td>
<td>-</td>
<td>632w</td>
<td>-</td>
<td>1597vs</td>
<td>-</td>
</tr>
<tr>
<td>[Th(ONS)(_2)] (3)</td>
<td></td>
<td></td>
<td>-</td>
<td>629w</td>
<td>-</td>
<td>1600m</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\)Relative band intensities are denoted by vs, s, m, w, sh and br, representing very strong, strong, medium, weak and shoulder and broad respectively.
Table 3. Cytotoxic screening against leukemic cells (CEM-SS) and colon cancer cells (HT-29)*

<table>
<thead>
<tr>
<th>Compound</th>
<th>CD$_{50}$ µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CEM-SS</td>
</tr>
<tr>
<td>[La(ONS)NO$_3$.2H$_2$O] (1)</td>
<td>6.8</td>
</tr>
<tr>
<td>[Ce(ONS)(NO$_3$)$_2$] (2)</td>
<td>Inactive</td>
</tr>
<tr>
<td>[Th(ONS)$_2$] (3)</td>
<td>Inactive</td>
</tr>
<tr>
<td>Tamoxifen (standard)</td>
<td>36.0</td>
</tr>
</tbody>
</table>

*CD$_{50}$ (µg/ml) = cytotoxic dose at 50%, i.e. the concentration to reduce growth of cancer cells by 50 %

Table 4. Qualitative Antimicrobial Assay (100 mg/ml)

<table>
<thead>
<tr>
<th>Sample</th>
<th>B28</th>
<th>60690</th>
<th>MRSA</th>
<th>B29</th>
<th>20341</th>
<th>C.A.</th>
<th>2075</th>
<th>398</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>9 mm</td>
<td>6 mm</td>
<td>8 mm</td>
<td>9 mm</td>
<td>11 mm</td>
<td>6 mm</td>
<td>10 mm</td>
<td>9 mm</td>
</tr>
<tr>
<td>(2)</td>
<td>7 mm</td>
<td>7 mm</td>
<td>8 mm</td>
<td>8 mm</td>
<td>6 mm</td>
<td>6 mm</td>
<td>6 mm</td>
<td>6 mm</td>
</tr>
<tr>
<td>(3)</td>
<td>8 mm</td>
<td>7 mm</td>
<td>6 mm</td>
<td>8 mm</td>
<td>8 mm</td>
<td>8 mm</td>
<td>8 mm</td>
<td>8 mm</td>
</tr>
</tbody>
</table>

* Inhibition diameter > 15 mm – strongly active