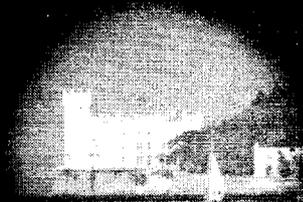




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OF A TRIDENTATE ONS SCHIFF BASE  
WITH LIGHTER AND HEAVIER METAL IONS,  
AND INVESTIGATION INTO THEIR  
BIOLOGICAL PROPERTIES

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WITH LIGHTER AND HEAVIER METALS IONS, AND INVESTIGATION  
INTO THEIR BIOLOGICAL PROPERTIES

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MIRAMARE – TRIESTE

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## Abstract

Several new complexes of a tridentate ONS Schiff base derived from the condensation of S-benzylthiocarbamate with salicylaldehyde have been characterised by elemental analyses, molar conductivity measurements, infrared, and electronic spectral studies. The tridentate Schiff base (HONSH) behaves as a dinegatively charged ligand coordinating through the thio sulphur, the azomethine nitrogen atom and the hydroxyl oxygen atom. It forms mono-ligand complexes of the general formula,  $[M(ONS)X]$ ,  $[M = Ni(II), Cu(II), Cr(III), Sb(III), Zn(II), Zr(IV) \text{ or } U(VI) \text{ and } X = H_2O, Cl]$ . The ligand produced bis-chelated complex with Th(IV) of composition,  $[Th(ONS)_2]$ . Square-planar structures were proposed for the Ni(II) and Cu(II) complexes. Antimicrobial tests indicated that the Schiff base and five of the metal complexes of Cu(II), Ni(II), U(VI), Zn(II) and Sb(III) were found to be strongly active against bacteria. Ni(II) and Sb(III) complexes were the most effective against *Pseudomonas aeruginosa* (gram negative) while the Cu(II) complex proved to be the best against *Bacillus cereus* (gram positive bacteria). Antifungal activities were also noted with the Schiff base and the U(VI) complex. These compounds have shown results against *Candida albicans* fungi. But none of these compounds were effective against *Aspergillus ochraceous* fungi.

## **Introduction**

Although syntheses of these ligands and some complexation were reported a long time ago<sup>3,4</sup>, the evaluation of their biological properties has not been done. Besides, complexation with the heavier metals has not been studied. We undertook a program to extend our studies to both lighter and heavier metals to discover their novel biological properties. We report herein the syntheses of these complexes and report on their potential as antibacterial and antifungal agents.

## **Experimental**

### **Preparation of S-benzylidithiocarbamate (SBDTC)**

This compound was prepared following the literature procedure<sup>4</sup>. A mixture of hydrazine hydrate (0.2 mole) and potassium hydroxide (0.2 mole) in 90% ethanol (70 ml) was cooled down to 0°C in an ice-salt bath. Carbon disulphide (0.25 mole) was added dropwise with constant stirring over a period of an hour. During this time, two layers had formed. The yellow oil in the lower layer was then separated using a separating funnel and dissolved in previously cooled 40% ethanol (60 ml). The mixture was kept in an ice-bath and benzyl chloride (0.2 mole) was added slowly with vigorous stirring of the mixture. After the complete addition of benzyl chloride, stirring was continued for another 15 minutes. The white product which had formed was collected and kept in the freezer. Yield was 17 g, m.p. 124°C<sup>4</sup>.

### **Preparation of S - benzyl - $\beta$ - N - (2 - hydroxyphenyl)methylendithiocarbazate (HONSH Schiff base)**

0.1 mole of SBDTC was dissolved in hot absolute ethanol (200 ml). To this solution was added a solution of salicylaldehyde (0.1 mole) in absolute ethanol (50 ml). The resultant mixture was heated on a steam bath for 10 minutes to reduce the volume to approximately 100 ml. The mixture was cooled to 0°C in an ice-salt bath to get complete crystallization. The crystals were filtered and dried over silica gel. Yield was 15 g. The melting point was 180°C<sup>3</sup>.

### **Attempted reaction of HONSH Schiff base with metal salts in absence of potassium hydroxide**

1 g of metal salt was dissolved in absolute ethanol (100 ml). An equimolar quantity of the HONSH Schiff base was dissolved in hot absolute ethanol (100 ml) which was filtered to get clear solution. The two solutions were mixed and heated on a steam bath for 5 – 10 minutes and then allowed to cool. A layer of yellowish crystals was seen precipitating at the bottom of the beaker. This layer appeared to be the HONSH Schiff base. So the mixture was reheated until the crystals were dissolved and then allowed to cool. The same layer appeared showing that the attempt was unsuccessful.

### **General method for the preparation of metal complexes in presence of potassium hydroxide**

This was prepared by a method similar to the above except that the Schiff base was dissolved in the presence of stoichiometric amount of potassium hydroxide. The

crystals which had formed were filtered off and dried over silica gel. Some metal salts did not yield enough crystals. In those cases, the mixture was treated with about 50 ml of absolute ethanol and reheated in order to concentrate it. Then the mixture was cooled and the crystals formed were filtered and dried over silica gel. The metal salts used were  $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{ZnCl}_2$ ,  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ ,  $\text{SbCl}_3$ ,  $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{ZrOCl}_2 \cdot 8\text{H}_2\text{O}$ ,  $\text{UO}_2\text{Cl}_2 \cdot 4\text{H}_2\text{O}$  and  $\text{Th}(\text{NO}_3)_4 \cdot 8\text{H}_2\text{O}$ .

### Physical measurements and elemental analyses

Elemental analyses were done at the Universiti Kebangsaan Malaysia, and also from the Department of Soil Science, Universiti Putra Malaysia. Infrared spectra of the samples were obtained with a FTIR Perkin Elmer 1750X infrared spectrophotometer. Molar conductance of  $10^{-3}\text{M}$  solutions of the metal complexes in dimethyl sulphoxide (DMSO) were measured at  $30^\circ\text{C}$  using Jenway 4310 conductivity meter and a dip-type cell with platinised electrode. The UV/VIS spectra, in DMSO, were recorded with a Shimadzu UV-160 Spectrophotometer for the range of  $200\text{-}1100\text{ cm}^{-1}$ .

### Antimicrobial Assay

Four pathogenic microbials were used to test the biological potentials of the Schiff base and the metal complexes. They are:

- |      |                               |       |                          |
|------|-------------------------------|-------|--------------------------|
| i)   | <i>Bacillus cereus</i>        | 1447  | (Gram positive bacteria) |
| ii)  | <i>Pseudomonas aeruginosa</i> | 60690 | (Gram negative bacteria) |
| iii) | <i>Aspergillus ochraceous</i> | 398   | (Fungi)                  |
| iv)  | <i>Candida albicans</i>       |       | (Fungi)                  |

The bacterial and fungal stock cultures were maintained on nutrient agar (NA) and potato dextrose agar (PDA) slants respectively, which were stored at 4°C. For the purpose of antimicrobial evaluation, the microorganisms were cultured in the appropriate broths at turbidometrically at a wavelength of 600 nm.

### ***Qualitative Antimicrobial Assay***

Antimicrobial activity of the extracts was qualitatively determined by a modified disc diffusion method<sup>12</sup>. A lawn of microorganisms was prepared by pipetting and evenly spreading 100 µl of inoculum (adjusted turbidometrically to 10<sup>5</sup> - 10<sup>6</sup> CFU/ml [ CFU= colony forming units ]) onto agar set in petri dishes, using NA for the bacteria and PDA for fungi. Whatman No. 1 filter paper discs of 6 mm diameters were impregnated into the stock solution of the complexes (10 - 25 mg/ml) 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 hours at 30°C. Antimicrobial activity was indicated by the presence of clear inhibition zones around the discs.

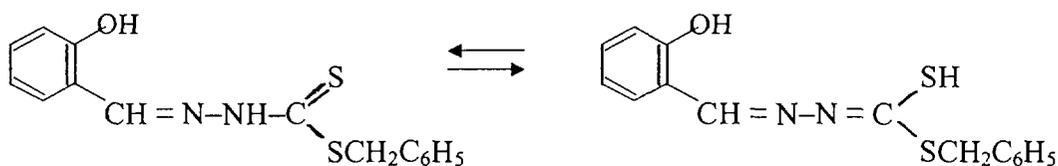
### **Quantitative Antimicrobial Assay**

Extracts that showed positive antimicrobial activity with the disc diffusion assay were subjected to the broth dilution method<sup>13</sup> for the quantitative measurement of microbiostatic (inhibitory) activity. The lowest concentration that completely inhibited visible microbial growth was recorded as the minimum inhibitory concentration (MIC, µg/ml). Both nystatin and kanamycin (Sigma, USA) were used

as positive controls while a negative control, I.E. tube inoculated without the complexes, was also included for each microorganism.

## Results and Discussion

The reaction of S-benzylthiocarbamate with salicylaldehyde yielded the Schiff base ( HONSH ), in good yield, which was soluble in most of the common organic solvents. It exists in tautomeric forms ( **1a**  $\rightleftharpoons$  **1b** ), and in the presence of alkali, the thiol proton undergoes deprotonation thereby coordinating through the thiolato sulfur, the azomethine nitrogen and the hydroxyl oxygen atoms.



( **1a**: thioketo form )

( **1b**: thioenol form)

The addition of potassium hydroxide facilitates formation of a reactive *in situ* ( Fig 2), thereby enhancing the nucleophilicity of the ligand and that the tridentate, dinegative *in situ* readily gives precipitation of complexes with metal ions. The analytical data are given in Table 1. The molar conductance values of  $10^{-3}$  M solutions of the complexes ( Table 2 ) reveal that all of the complexes except **7** and **8** are non- electrolytes. Complexes **7** and **8** show molar conductance corresponding to 1: 1 electrolytes in DMSO<sup>14</sup>.

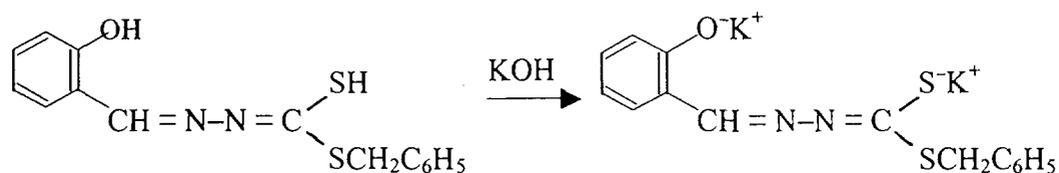
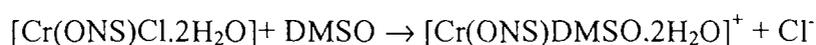
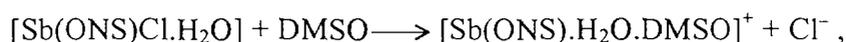


Figure 2

The conductivities arise because of solvation according to the following Schemes.



The analytical and conductance data reveal that Cu(II), Ni(II) and Zn(II) complexes are four- coordinated while the Zr(IV), Th(IV), U(VI) and Sb(III) analogues are hexa-coordinated. The Schiff base has a thioketo group (C=S) adjacent to a proton. The thione group is relatively unstable in monomeric form and tends to give a stable C–S single bond by enethiolisation<sup>1-11</sup> depending on the availability of hydrogen atom adjacent to the C=S bond. The IR spectrum of the Schiff base did not display  $\nu(\text{S–H})$  at *ca.* 2570  $\text{cm}^{-1}$  indicating that in the solid-state it remains in the thione form. However, in solution, it may remain in equilibrium with the thiole tautomeric form<sup>3,4</sup>. The IR spectrum of the Schiff base exhibits a strong band at 3103  $\text{cm}^{-1}$  which can be assigned to the  $\nu(\text{N–H})$  of the free ligand. This band disappeared in the IR spectra of the metal complexes suggesting that the proton on the  $\alpha$ -nitrogen atom is lost upon complex formation. A strong band at 1042  $\text{cm}^{-1}$  in the IR spectrum of the Schiff base is tentatively assigned to the  $\nu(\text{C=S})$  mode. This band disappears in the IR spectra of the metal complexes further supporting the contention of enethiolization, and consequent deprotonation giving the thiolate *in situ*. UV/Vis spectra were recorded in

DMSO to get some understanding on the geometrics of the complexes. The data are shown in Table 3. The electronic spectra of the copper complex shows bands at 409 nm ( ${}^2B_{1g} \rightarrow {}^2A_{1g}$ ) and 323 nm ( ${}^2B_{1g} \rightarrow {}^2E_{1g}$ ), which are characteristic of square-planar stereochemistry of Cu(II)<sup>15,16</sup>. The third band at 295 nm is presumably caused by charge-transfer. The dark-brown Ni(II) complex gives bands at 386 , 366 and 340 nm corresponding to the  ${}^1A_{1g} \rightarrow {}^1A_{2g}$ ,  ${}^1A_{1g} \rightarrow {}^1B_{1g}$  and  ${}^1A_{1g} \rightarrow {}^1E_g$  transitions, respectively, of a square-planar Ni(II) complex<sup>16</sup>. The additional band at 278 nm is probably caused by charge-transfer. For both the complexes, three coordination positions are occupied by the ONS sequence and the fourth coordination site is having been occupied by H<sub>2</sub>O. The chromium complex gives three diagnostic bands at 414, 348 and 300 nm corresponding to the transitions,  ${}^4A_{2g} \rightarrow {}^4T_{2g}$ ,  ${}^4A_{2g} \rightarrow {}^4T_{1g}$  (F) and  ${}^4A_{2g} \rightarrow {}^4T_{1g}$  (P), respectively, of O<sub>h</sub> symmetry of Cr(III) complex<sup>17</sup>. The band at 270 nm is presumably caused by charge-transfer. The complexes having Zr, U, Th, Zn and Sb metals showed bands in the uv region ( 400 nm - 180 nm ) which are supposedly caused by charge-transfer.

### ***Antimicrob Activity***

Preliminary screening for antimicrobial activities of the stock solutions of the Schiff base and the metal complexes were performed qualitatively using the disc diffusion assay ( Table 4 ). The Schiff base, [Cu(ONS).H<sub>2</sub>O], [Ni(ONS).H<sub>2</sub>O], [U(O)<sub>2</sub>(ONS).H<sub>2</sub>O], [Zn(ONS).H<sub>2</sub>O] and [Sb(ONS)Cl.2H<sub>2</sub>O] yielded clear inhibition zones around the discs. These showed significant bio-activity against *Pseudomonas aeruginosa* (gram negative bacteria) and *Bacillus cereus* (gram positive bacteria) while the uranium analogue was effective against *Bacillus cereus* and showed very

weak activity against *albicans* fungi. The Schiff base also displayed very slight activity against *Candida albicans*. None of the compounds showed any activity against the *Aspergillus ochraceous* fungi indicating that the present compounds are more effective against bacteria. The MIC values of the compounds that showed positive results in the screening process were determined for the quantitative measurement of antimicrobial activity and also to distinguish between microbiostatic and microbicidal action ( Table 5 ). The results revealed that the Ni(II) and Sb(III) complexes are the most effective against *Pseudomonas aeruginosa* (only 162.5 µg/ml needed to show results) while the Cu(II) complex proved to be the best against *Bacillus cereus* (79.6 µg/ml). Antifungal activities were noted with the Schiff base and the U(VI) complex. These compounds showed results against *Candida albicans* fungi (10600 µg/ml).

### **Acknowledgements**

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Table 1: Analytical data of the complexes.

Compound	% Carbon		% Hydrogen		% Nitrogen	
	Calc.	Found	Calc.	Found	Calc.	Found
HONSH Schiff base (1)	59.57	59.80	4.67	4.33	9.26	9.10
[Cu(ONS).H <sub>2</sub> O] (2)	47.17	48.90	3.69	3.50	7.33	6.60
[Ni(ONS).H <sub>2</sub> O] (3)	47.78	47.70	3.74	3.20	7.43	7.10
[Zr(O)(ONS). 2H <sub>2</sub> O] (4)	42.13	42.10	3.75	3.54	6.55	6.32
[U(O) <sub>2</sub> (ONS).H <sub>2</sub> O] (5)	30.62	30.40	2.40	2.09	4.76	4.19
[Th(ONS) <sub>2</sub> ] (6)	43.26	42.70	2.90	2.92	6.73	6.80
[Zn(ONS).H <sub>2</sub> O] (7)	46.94	47.10	3.68	3.20	7.30	7.05
[Sb(ONS)Cl.2H <sub>2</sub> O] (8)	36.50	35.80	3.27	2.77	5.67	4.99
[Cr(ONS)Cl.2H <sub>2</sub> O] (9)	42.50	41.90	3.80	3.30	6.61	6.10

Table 2: Molar conductance results

Compound	Colour	$\Lambda_M(\text{ohm}^{-1}\text{cm}^2\text{mole}^{-1})$
[Cu(ONS).H <sub>2</sub> O]	Green	6
[Ni(ONS).H <sub>2</sub> O]	Orange	5
[Zr(O)(ONS). 2H <sub>2</sub> O]	Yellow	9
[U(O) <sub>2</sub> (ONS).H <sub>2</sub> O]	Yellow	6
[Th(ONS) <sub>2</sub> ]	Yellow	2
[Zn(ONS).H <sub>2</sub> O]	Yellow	2
[Sb(ONS)Cl.2H <sub>2</sub> O]	White	40
[Cr(ONS)Cl.2H <sub>2</sub> O]	Yellow	35

Table 3: UV/Vis spectral data

Compound	$\lambda_{\text{max}}$ (nm)
[Cu(ONS).H <sub>2</sub> O]	409, 323, 295
[Ni(ONS).H <sub>2</sub> O]	386, 366, 340, 378
[Zr(O)(ONS). 2H <sub>2</sub> O]	348, 295
[U(O) <sub>2</sub> (ONS).H <sub>2</sub> O]	358, 350, 293
[Th(ONS) <sub>2</sub> ]	360, 340, 295
[Zn(ONS)Cl.2H <sub>2</sub> O]	345, 305
[Sb(ONS)Cl.2H <sub>2</sub> O]	345, 305
[Cr(ONS)Cl.2H <sub>2</sub> O]	414, 348, 300, 270

Table 4: Qualitative antimicrobial assay results

Compound	Weight (mg)	P.A. (mm)	B.C. (mm)	C.A. (mm)	A.O. (mm)
ONS Schiff base	10.6	10.0	11.9	*	-
[Cu(ONS).H <sub>2</sub> O]	20.6	9.2	9.1	-	-
[Ni(ONS).H <sub>2</sub> O]	20.4	7.4	9.7	-	-
[Zr(O)(ONS). 2H <sub>2</sub> O]	10.4	-	-	-	-
[U(O) <sub>2</sub> (ONS).H <sub>2</sub> O]	10.6	-	6.8	*	-
[Th(ONS) <sub>2</sub> ]	10.5	-	-	-	-
[Zn(ONS).H <sub>2</sub> O]	20.6	8.5	5.8	-	-
[Sb(ONS)Cl.2H <sub>2</sub> O]	21.2	15.7	27.1	-	-
[Cr(ONS)Cl.2H <sub>2</sub> O]	10.7	-	-	-	-

Note:

P.A. *Pseudomonas aeruginosa* (gram negative bacteria)

B.C. *Bacillus cereus* (gram positive bacteria)

C.A. *Candida albicans* (fungi)

A.O. *Aspergillus ochraceous* (fungi)

\* Very weak activity

- No activity

Table 5: Quantitative antimicrobial assay results

Compound	Weight (mg)	P.A. ( $\mu\text{g/ml}$ )	B.C. ( $\mu\text{g/ml}$ )	C.A. ( $\mu\text{g/ml}$ )
ONS Schiff base	5.2	1300 <sup>c</sup>	300 <sup>c</sup>	10600
[Cu(ONS).H <sub>2</sub> O]	5.1	318.75 <sup>c</sup>	79.6 <sup>c</sup>	-
[Ni(ONS).H <sub>2</sub> O]	5.2	162.5 <sup>c</sup>	650 <sup>c</sup>	-
[U(O) <sub>2</sub> (ONS).H <sub>2</sub> O]	5.2	-	10600 <sup>c</sup>	10600
[Zn(ONS).H <sub>2</sub> O]	5.1	5100 <sup>c</sup>	5100 <sup>c</sup>	-
[Sb(ONS)Cl.2H <sub>2</sub> O]	5.2	162.5 <sup>s</sup>	325 <sup>s</sup>	-

Note:

P.A. *Pseudomonas aeruginosa* (gram negative bacteria)

B.C. *Bacillus cereus* (gram positive bacteria)

C.A. *Candida albicans* (fungi)

- No activity

c Bactericidal

s Bacteriostatic