Synthesis and bioactivity study of 2-((E)-(((E)-2,6-dichlorobenzylidene)hydrazono)methyl) phenol have N,O-Bidentate ligand site and it’s metal complex

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ABSTRACT

N,O-Bidentate ligands were prepared by the condensation of (E)-(2,6-dichlorobenzylidene) hydrazine with substituted Salicylaldehyde and different aromatic aldehydes. New synthesized metal complex are thermally stable and neither air- nor moisture sensitive at RT. Successive ligand and their metal complex are synthesized and characterized using IR, NMR, Elemental, Mass, Physical property. With Bioactivity study of ligand and there metal complexes.

Keywords: N, O-Bi-dentate ligand, Schiff base, Metal complex, IR, NMR, Elemental, Mass, Bioactivity, Antifungal activity, Antimicrobial activity

1. INTRODUCTION

Man is directly influenced by the activities of microorganisms. Microorganisms have shaped our present environment and their activities will greatly influence our future. Control of microbial population is necessary to prevent transmission of disease, Infection, Decomposition, Contamination and spoilage caused by them, Man’s personal comforts and convenience depend to a large extent on the control of microbial population. Recently the application of nitrogen-based ligands species, such as Schiff bases [1-5] chalcones has also consider as a highly
bioactive compound against microorganism [26-30] In present studies have information regard biological activity [31-35] of metal complex and ligand which was synthesized using appropriate 1-4-dioxane as solvents for the reaction. (E)-(2,6-dichlorobenzylidene) hydrazine [5-10] coupling reaction with various Salicylaldehyde derivative and Aldehydes. For coupling reaction Piperidine used as basic catalyst results in formation of 1-((E)-benzylidene)-2-((E)-2,6-dichlorobenzylidene)hydrazine [10-13] and its analogue various derivative [14-20]

Nature of ligand is very important in the metal complex formation. Bulky, electron-rich ligands are promising to metal complex synthesis resulting from their higher donor ability and stabilization effects but a small and electron rich ligand is also suitable.

2. RESULT AND DISCUSSION

2.1. Materials and methods

All the required compounds and solvents were purchased from loba chemie, Merck and spectrochem, and checked by TLC. $^1$H NMR spectra were taken on Bruker NMR spectrometer (400 MHz), using TMS [as internal standard], IR spectra were taken with FT-IR Spectrophotometer (Thermo Scientific). Mass spectra were done on GCMS QP2010 mass spectrometer, Microtof-Q11 System with BRUKER Daltonics APOLLO II ESI, Elemental data was recorded by Carlo Erba EA 1108 elemental analyser.

2.2. General procedure for the synthesis of (RBL-102) as ligand (RBL-101 to 108)

![Reaction Scheme of ligand](Figure 1)
Table 1. Compound details.

<table>
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<tr>
<th>Sr. No.</th>
<th>Ligand Code</th>
<th>Substitution</th>
<th>Colour of Compound</th>
<th>Yield</th>
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<td></td>
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<td></td>
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</tr>
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<td>Ome H H H</td>
<td>Yellow</td>
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</tr>
<tr>
<td>4</td>
<td>RBL-104</td>
<td>H H Br H</td>
<td>Very Light Yellow</td>
<td>69%</td>
</tr>
<tr>
<td>5</td>
<td>RBL-105</td>
<td>H H Cl H</td>
<td>Fancy Intense Yellow</td>
<td>65%</td>
</tr>
<tr>
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<td>RBL-106</td>
<td>H H Ome H</td>
<td>Fancy Intense Yellow</td>
<td>62%</td>
</tr>
<tr>
<td>7</td>
<td>RBL-107</td>
<td>H Ome H H</td>
<td>Fancy Intense Yellow</td>
<td>66%</td>
</tr>
<tr>
<td>8</td>
<td>RBL-108</td>
<td>H OH H H</td>
<td>Faint Yellow</td>
<td>70%</td>
</tr>
</tbody>
</table>

A solution of \(\epsilon\) (2,6-dichlorobenzylidene) hydrazine (0.01 mole) and 2-hydroxybenzaldehyde (Salicylaldehyde) refluxed in presence of Piperidine (0.5 ml) at 80-120 °C for 1 hour and stirring 5 hours at room temperature. The reaction is continuously being monitored by TLC using Hexane: Ethyl acetate (2:1). After completion of the reaction, the product mixture was poured into dilute solution of sodium bisulphate (0.25 molar). Light yellow precipitate fall out and filtered out the separated solid product washed by dry ether for further purification and dried under reduced pressure. This was further crystalizing by chloform as most suitable solvent.

2. 3. Spectral data of ligands (RBL-102)

M.P. 118 °C, Elemental Analytical Calculation for C\(_{14}\)H\(_{10}\)N\(_2\)Cl\(_2\)O (293.15 g/mol): C, 57.36%; H, 3.44%; N, 9.56%; O,5.46%; Cl, 24.19%; Found: C, 57.30%; H, 3.46%; N, 9.54%; O,5.41%; Cl, 24.30%; MS (m/z): 292 (M+1): \(^1\)H-NMR (DMSO-d\(_6\)): δ ppm; 11.58, (s, 1H, -OH); 8.815, (s, 1H, CH=N); 8.767 (s, 1H, CH=N); 7.366 – 7.331 (d, 2H, Ar-H); 7.327 – 7.312 (t, 1H, Ar-H); 7.244 – 7.204 (t, 1H, Ar-H); 6.948 – 6.629 (t, 1H, Ar-H) 7.036 – 7.015 (d, 1H, Ar-H). \(^{13}\)C-NMR (DMSO-d\(_6\)): δ ppm 166.86 (-C-OH); 160.06 (-C-N), 158.21 (-C-N), 135.84 (C-Cl), 132.80 (C=C), 132.50, 130.84, 129.34, 119.64, 116.85, (05 C-Ar). IR, (cm\(^{-1}\)): v (OH) 3128; v(C=O) 1619; v (N-N) 957, 985; v (1,2,3-tri sub. Ar–C–H) 883; v (1,2-di sub. Ar–C–H) 747; v(C-Cl) 711.

2. 4. Spectral data of ligands (RBL-103)

M.P. 98 °C, Elemental Analytical Calculation for C\(_{15}\)H\(_{12}\)N\(_2\)Cl\(_2\)O\(_2\) (323.17 g/mol): C, 55.75%; H, 3.74%; N, 8.67%; O,9.90%; Cl, 21.94%; Found: C, 55.65%; H, 3.74%; N, 8.77%; O,9.94%; Cl, 21.90%; MS (m/z): 322 (M+1): \(^1\)H-NMR (DMSO-d\(_6\)): δ ppm; 11.822, (s, 1H, -
OH); 8.835, (s, 1H, CH=N); 8.788 (s, 1H, CH=N); 7.396-7.376 (d, 2H, Ar-H); 7.284-7.244 (t, 1H, Ar-H); 7.013-6.968 (t, 2H, Ar-H); 6.918-6.878 (t, 1H, Ar-H) 3.929 (s, 3H, C-H)

$^{13}$C-NMR (DMSO-d6): $\delta$ppm 166.63 (C-OH); 158.32 (C-N), 149.96 (C-N), 135.48-135.15 (C-Cl), 131.13 (C=C), 129.96, 129.03, 128.73, 124.21, 119.38, 117.38, 11.21 (67 C-Ar); 56.23, (C-C). IR, (cm$^{-1}$): $\nu$(OH) 2965; $\nu$(C=N) 1617; $\nu$(N-N) 950; $\nu$(1,2,3-tri sub. Ar–C-H) 874; $\nu$(1,2-di sub. Ar–C-H) 777; $\nu$(C-Cl) 728.

2.5. General Preparation of Metal Complexes (RBM-101 to 104)

Copper and Cobalt Coordinate metal complexes [21-25] were prepared by equimolar mixing of ligand in Ethanol and an aqueous solution of the corresponding metal chlorides in 1:1 and 1:2 molar ratio. The reaction mixture was refluxed for 3-4 hrs. on water bath. The completion of the reaction was monitored by TLC using Hexane: Ethyl acetate (1:3) confirming absence of metal by salicylaldoxime as colouring agent. After completion of reaction cooled to room temperature and poured into Distilled water. The solid complexes formed were filtered, washed with hot water (2 times) and ethyl alcohol was finally dried in vacuum desiccators over anhydrous Calcium Chloride. Further recrystallization of Metal complex was done by chloroform as solvent.

Table 2. Compound details.

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<th>Sr. No.</th>
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<td>Wine Red</td>
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<td>68%</td>
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<tr>
<td>3</td>
<td>RBM-103</td>
<td>Light Green</td>
<td>DMF</td>
<td>42%</td>
</tr>
<tr>
<td>4</td>
<td>RBM-104</td>
<td>Dark Brown</td>
<td>Chloroform</td>
<td>70%</td>
</tr>
</tbody>
</table>

6.2.6. Spectral data of Metal complex (RBM-101)

Analytical Calculation for C$_{14}$H$_{11}$N$_{2}$Cl$_{4}$CoN$_{2}$O$_{2}$ (439.99 gm/mol): C, 38.22%; H, 2.52%; N, 6.37%; O, 7.27%; Cl, 32.23%; Co, 13.39 Found: C, 38.18%; H, 2.52%; N, 6.36%; O, 7.31%; Cl, 32.23%; Co, 13.40, MS (m/z): 292 (M+1); IR, (cm$^{-1}$): $\nu$(OH) 3073; $\nu$(N-N) 977; $\nu$(1,2,3-tri sub. Ar–C-H) 885; $\nu$(1,2-di sub. Ar–C-H) 751; $\nu$(C= N) 1612; v(C-Cl) 732.

2.7. Spectral data of Metal complex (RBM-102)

Analytical Calculation for for C$_{28}$H$_{19}$Cl$_{4}$CoN$_{2}$O$_{2}$ (643.21 g/mol): C, 52.29%; H, 2.82%; N, 8.71%; O, 4.97%; Cl, 22.05%; Co, 9.16 Found: C, 52.30%; H, 2.83%; N, 8.69%; O, 4.99%; Cl, 22.05%; Co, 9.13 MS (m/z): 642 (M+1); IR, (cm$^{-1}$): $\nu$(OH) 3075; $\nu$(N-N) 925; $\nu$(1,2,3-tri sub. Ar–C-H) 885; $\nu$(1,2-di sub. Ar–C-H) 775; $\nu$(C=N) 1613; v(C-Cl) 721.
2.8. Spectral data of Metal complex (RBM-103)

Analytical Calculation for C_{14}H_{9}Cl_{3}CuN_{2}O (391.14 g/mol): C, 42.99%; H, 2.32%; N, 8.71%; O, 4.09%; Cl, 27.19%; Cu, 16.25% (Found): C, 42.95%; H, 2.31%; N, 8.74%; O, 4.13%; Cl, 27.20%; Cu, 16.22% MS (m/z): 388 (M+4): IR, (cm$^{-1}$): ν(OH) 3082; ν(N-N) 998; ν (1,2,3-tri sub. Ar–C-H) 883; ν (1,2-di sub. Ar–C-H) 764; ν (C=N) 1623; ν (C-Cl) 719.

2.9. Spectral data of Metal complex (RBM-104)

Analytical Calculation for C_{28}H_{18}Cl_{4}CuN_{4}O_{2} (647.82 g/mol): C, 51.91%; H, 2.80%; N, 8.65%; O, 4.94%; Cl, 21.89%; Cu, 9.81% (Found): C, 51.88%; H, 2.82%; N, 8.65%; O, 4.97%; Cl, 21.89%; Cu, 9.79% MS (m/z): 647 (M): IR, (cm$^{-1}$): ν(OH) 3071; ν(N-N) 995; ν (1,2,3-tri sub. Ar–C-H) 848; ν (1,2-di sub. Ar–C-H) 771; ν (C=N) 1627; ν (C-Cl) 747.

![Reaction Scheme of Metal complex](image_url)

**Figure 2.** Reaction Scheme of Metal complex
Figure 3. Mass spectrum of ligand (RBL-102)
Figure 4. Mass spectrum of ligand (RBL-103)
Figure 5. Mass spectrum of ligand (RBM-101)
Figure 6. Mass spectrum of ligand (RBM--102)
Figure 7. Mass spectrum of ligand (RBM-103)

Figure 8. Mass spectrum of ligand (RBM-104)
Figure 9. IR spectrum of ligand (RBL-102)
Figure 10. IR spectrum of ligand (RBL-103)
Figure 11. IR spectrum of Metal complex (RBM-101)
Figure 12. IR spectrum of Metal complex (RBM-102)
Figure 13. IR spectrum of Metal complex (RBM-103)
Figure 14. IR spectrum of Metal complex (RBM-104)
Figure 15. $^1$HNMR spectrum of ligand (RBL-102)
Figure 16. $^1$HNMR spectrum of ligand (RBL-103)
Figure 17. $^{13}$C NMR spectrum of ligand (RBL-102)
Figure 18. $^{13}$C NMR spectrum of ligand (RBL-103)
3. BIOACTIVITY STUDY

Bacteria are generally unicellular. E.G. Cocci, Bacilli, Etc. Some being sheathed having certain cells specialized for reproduction.

The microorganisms capable of producing diseases in host are known as ‘Pathogenic’. Most of the microorganisms present on the skin and Mucous membrane are non-pathogenic and are often referred to as “Commensals” or if they live on food residues as in Intestine. They may be called “Saprophytes”.

Generally the Pathogenic Cocci and Bacilli are gram positive and the Pathogenic Cocobacilli are gram negative. For evaluation of antibacterial activity in our study, we have used Staphylococcus Aureus and Streptococcus Pyogenes from gram positive group of bacteria and Escherichia Coli and Pseudomonas Aeruginosa from Gram Negative Group of Bacteria. We have used the Broth Dilution Method to evaluate the antibacterial activity. It is one of the non-automated in vitro bacterial susceptibility tests.

This classic method yields a quantitative result for the amount of antimicrobial agents that is needed to inhibit growth of specific microorganisms [36-39]. The determination of minimal inhibition concentrations by micro broth dilution method All MTCC Cultures Were Tested against above Mentioned Known and Unknown Drugs. Mueller Hinton Broth Was Used as Nutrient Medium to Grow and Dilute the Drug Suspension for the Test bacteria. Inoculum Size for Test Strain Was Adjust to 108 CfU [Colonies Forming Unit] per milliliter by comparing the turbidity.

Common standard strains were used for screening of antibacterial and antifungal activities: The strains were procured from Institute of Microbial Technology, Chandigarh. DMSO was used as diluents/vehicle to get desired concentration of drugs to test upon Standard bacterial strains. Serial dilutions were prepared in primary and secondary screening. The control tube containing no antibiotic is immediately sub cultured [before inoculation] by spreading evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37 ºC overnight.

The tubes are then incubated overnight. The MIC of the control organism is read to check the accuracy of the drug concentrations. The lowest concentration inhibiting growth of the organism is recorded as the MIC. The amount of growth from the control tube before incubation [which represents the original inoculum] is compared.

Methods used for primary and secondary screening: Each synthesized drug was diluted obtaining 2000 microgram/ml concentration, as a stock solution.

Primary screen: In primary screening 1000 micro/ml, 500 micro/ml, and 250 micro/ml concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms.

Secondary screen: The drugs found active in primary screening were similarly diluted to obtain 200 micro/ml 100 micro/ml, 50 micro/ml, 25 micro/ml, 12.5 micro/ml, 6.250 micro/ml, and concentrations.

Reading Result the highest dilution showing at least 99 % inhibition zone is taken as MIC. The result of this is much affected by the size of the inoculum. The test mixture should contain 108 organism/ml.
Table 3. Bioactivity table.

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STANDARD DRUG (MICROGRAM/ML)

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4. CONCLUSION

The ligand 2-((E)-((E)-2,6-dichlorobenzylidene)hydrazono)methyl) phenol behaved as a bidentate chelating agent coordinating through one oxo group and one nitrogen atoms of hydrazone. The ligand was so designed that it can secure one metal ions in the close proximity. Analytical data, electronic spectra, IR, $^1$H NMR, FAB mass spectral data reveal mononuclear nature of all the complexes. The mononuclear nature of the complexes was confirmed on the basis of IR data and FAB mass spectral data. The ligand and its Cu(II) and Co(II) complexes were tested for antimicrobial and fungal activity. Ligands were found to be active against the bacteria Staphylococcus Aureus and Streptococcus Pyogenes from gram positive group of bacteria and Escherichia Coli And Pseudomonas Aeruginosa gram negative group along with fungal activity against Candida Albicans and Aspergillus niger. The whole study is showing enhancement of activity of its metal complex then ligand.

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