Potentiation of the Antimicrobial Activity of 4-Benzylimino-2, 3-Dimethyl-1-Phenylpyrazal-5-One by Metal Chelation

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Abstract

A Schiff base ligand, 4-benzylimino-2,3-dimethyl-1-phenylpyrazal-5-one have been synthesized by the condensation of Benzaldehyde and 4-aminooantipyrine. Its divalent metal complexes of Fe, Co, Ni, Cu and Zn were also synthesized. The ligand and the complexes were characterized by FTIR, UV/visible, 1H-NMR, 13C-NMR, and GCMS. The ligand behaved as a bidentate donor by using its carbonyl and azomethine N as binding sites for the metals. Tetrahedral structures were proposed for the all complexes excepting the Cu(II) complex. The ligand showed low activity against some microbes but the complexes were remarkably active against the bacteria and fungi species.

Keywords: 4-benzylimino-2,3-dimethyl-1-phenylpyrazal-5-one, benzaldehyde, 4-aminoantipyrine, antimicrobial.

Introduction

Schiff base are derived by condensation reaction of aldehydes or ketones and primary amines. They are compounds containing –N=CHR group. Many Schiff base ligands have been synthesized from heterocyclic compounds1. Schiff base having oxygen, nitrogen, and sulphur donor atoms have been reported by several scientists2. In this work we have synthesized a Schiff base ligand by condensation reaction between benzaldehyde and 4-aminooantipyrine (4-amino-2,3-dimethyl-1-phenylpyrazal-5-one). Benzaldehyde is a typical aromatic aldehyde. It is a colourless oily liquid and a component of complex compound in the seeds of bitter almonds, peach and cherry seeds3. It has been applied in food flavouring, synthetic perfumes, manufacture of cinnamic and benzoic acids and equally as a dye intermediate4. Antipyrine is very much used in medicine5 and it is believed that its amino derivative would equally be of much use in medicine possibly as intermediates in antipyretic and analgesic drugs6.

Material and Methods

All reagents used in this analysis are of analytical grade and obtained from Sigma-Aldrich Chemical Ltd and BDH chemicals. The reagents include: Benzaldehyde, 4-aminooantipyrine, ethanol, dimethyl sulphoxide (DMSO), dimethyl formamide (DMF), NiCl2.6H2O, CuCl2.2H2O, CoCl2.6H2O, ZnCl2 and FeCl3. 4H2O

Instrumental analysis: The melting point was detected using the melting point apparatus, electronic spectra were determined using UNICAM UV 2120 spectrophotometer. IR spectra were also determined using FTIR-8400S spectrophotometer. 1H-NMR and 13C-NMR were recorded in d6-DMSO on a Shimadzu FTNMR spectrometer. The GCMS was performed using GCMS – QP2010 plus Schimadzu.

Synthesis of 4-benzylimino-2,3-dimethyl-1-phenylpyrazal-5-one: The Schiff base derived from benzaldehyde and 4-aminooantipyrine was prepared by adding an ethanol solution (25ml) of 4-aminooantipyrine (2.03g, 0.01mol) to 1.01ml benzaldehyde (0.01mol). The mixture was stirred and refluxed for two hours. This was then filtered and left for 2-4 days to crystallize. The resulting crystals were then dried.

Preparation of the complexes: The complexes were prepared by the reaction of the ligand(0.01mol) with the respective metal (II) salts in ethanol medium (0.01mol NiCl2.6H2O (2.37g); 0.01mol CuCl2.2H2O (1.72g); 0.01mol CoCl2.6H2O (2.78g); 0.01mol ZnCl2 (1.37g) and 0.01mol FeCl3.4H2O (1.98g)). The various 0.01mol of the metal salts were each refluxed with 0.01mol of the ligand in ethanol medium for 2 hours. They were all filtered and washed several times with ethanol after which they were left for 2-4 days to recrystallize7. The resulting crystals were then dried. The yield was recorded.

Sensitivity test: The sensitivity tests on the samples were carried out using agar well diffusion method8. The nutrient agar was prepared according to the manufacturer’s recommendation and was poured into Petri dishes to set.
test organisms (bacteria)-*Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, (fungi)-*Candida albicans* and *Microsporum audoni* were cultured. The overnight bored cultures of the test organisms were properly diluted to the turbidity of Mac-Farland’s standards and were inoculated on the surface of the agar\(^9\). The inoculated agar was left for 20 minutes and holes were bored into it using cork borer. The prepared ligand and complexes were dissolved in DMSO and were then introduced into the agar using sterile swab stick. The inoculated plates were then incubated at 37\(\degree\)C for 18 hours thereafter the resultant zones of inhibition were measured using meter rule and results obtained in centimeters were recorded. Ciprofloxacin and fluconazole which are antibacterial and antifungal agents respectively were used as control drugs.

**Results and Discussion**

The synthesis of 4-benzylimino-2-3-dimethyl-1-phenylpyrazal-5-one ligand is shown in scheme-1. The proposed structures of the complexes are shown in figure-1.

**Scheme-1**

*Synthesis of 4-benzylimino-2-3-dimethyl-1-phenylpyrazal-5-one ligand*

**Figure - 1**

*Proposed structures of the metal complexes*
All the complexes are air stable, colored solids and non-hygroscopic. The physical properties of the compounds are presented in table-1.

**Infra-red spectra:** The infra-red spectra of the ligand and complexes are presented in table-2. The infra-red spectra of cobalt and nickel complex exhibited a broad band at 3425 and 3245 cm\(^{-1}\) respectively. This is due to the presence of water molecules. The sharp bands between 1570 – 1592 cm\(^{-1}\) are due to C=N azomethine vibrations. The free ligand has the C=N vibration at 1569 cm\(^{-1}\) so the shifting of the band to higher frequencies in the complexes indicates complexation. The bands that appeared below 650 cm\(^{-1}\) are assigned to the metal-nitrogen (M-N), metal-oxygen (M-O) and metal chlorine (M-Cl) bonds.

**UV/Visible electronic spectra:** The electronic spectra of the ligand and its complexes were recorded and their assignment given in table-3. The ligand's spectra data displayed two bands at 20498 cm\(^{-1}\) and 24739 cm\(^{-1}\) which results from intra-ligand charge transfer (ILCT), \(\pi\rightarrow\pi^*\) phenyl ring and \(\pi\rightarrow\pi^*\) (HC=N) transitions. Two bands were observed in the spectrum of FeL which are 2072 cm\(^{-1}\) and 2527 cm\(^{-1}\). These bands have been assigned MLCT and \(\pi\rightarrow\pi^*\) ligand transfer. The intensity of the band suggested a tetrahedral geometry and their assignments thus \(^5T_{2g} \rightarrow ^5T_{2g}, \; ^5T_{2g} \rightarrow ^5T_{1g}\) and \(^4T_{1g} \rightarrow ^4A_{2g}\). Ni(II) complex exhibited three bands at 25199 cm\(^{-1}\), 28150 cm\(^{-1}\) and 30827 cm\(^{-1}\). A square planar geometry is suggested for this complex. Copper (II) complex (CuL) has two bands \(2^5T_{2} \rightarrow 2^5E\) transitions and the intensity of the bands suggest trigonal geometry. ZnL complex have two bands at 20551 cm\(^{-1}\) and 24569 cm\(^{-1}\). The absorptions are as a result of intra-ligand charge transfer and a tetrahedral geometry is proposed.
**1HNMR spectra data:** 1HNMR spectra of the ligand and its complexes have four protons environments. The different chemical shifts are shown in Table 4. In the ligand, the first chemical shift appeared at δ2.00ppm indicates the CH₃(alkyl). The azomethine hydrogen (HC=N) appeared at 2.53ppm for the ligand but shifted downfield for the complexes showing complexation through the azomethine linkage. CH₃-N band is shifted lower field compared to the CH₃-C due to the presence of nitrogen group that deshields the electrons. Within the region of δ 7.12 – 7.87ppm, there are multiples of peaks which indicated the presence of aromatic protons.

**13CNMR:** 13CNMR spectra data is presented in table-5. In the ligand, the C=0 carbon resonates at 190ppm but in the complexes, C=0 band shifted downfield due to complexation. The azomethine carbons C=N appeared between 145-152ppm in both the ligands and metal complexes though there was a downfield shift for the complexes. There is a prominent peak that appeared in all the spectra at δ 40 ppm, this resulted from the solvent d₆-DMSO used in the analysis. There are a total of 14 peaks which confirmed the structure of the ligand. There is also a concentration of peaks between δ 102 – 152 ppm which indicated sp² hybridized carbons and they consists of azomethine carbons, C=C carbons, benzylic carbons and aromatic carbons.

### Table – 4

<table>
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<tr>
<th>Compound</th>
<th>HC=N</th>
<th>Aromatic H</th>
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<th>CH₃-C</th>
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<tr>
<td>L</td>
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<td>7.12-7.87</td>
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<tr>
<td>FeL</td>
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<td>6.68-7.08</td>
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<td>CoL</td>
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<td>7.02-7.28</td>
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<tr>
<td>NiL</td>
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<tr>
<td>CuL</td>
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<td>7.48-7.78</td>
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<tr>
<td>ZnL</td>
<td>2.33</td>
<td>7.18-7.48</td>
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</table>

*L = 4-benzylimino-2-3-dimethyl-1-phenylpyrazal-5-one*

### Table – 5

<table>
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<th>Compound</th>
<th>C=0</th>
<th>C=N</th>
<th>C=C</th>
<th>Aromatic carbons</th>
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<td>146,149</td>
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<tr>
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<td>112-138</td>
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<tr>
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<tr>
<td>ZnL</td>
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</table>

*L = 4-benzylimino-2-3-dimethyl-1-phenylpyrazal-5-one*

**Scheme-2**

GCMS major fragmentations
Table – 6
Antimicrobial test results (cm)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Escherichia coli</th>
<th>Enterococcus faecalis</th>
<th>Klebsiella pneumoniae</th>
<th>Staphylococcus aureus</th>
<th>Candida albicans (fungus)</th>
<th>Monosporum audonii (fungus)</th>
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<tr>
<td>L</td>
<td>18</td>
<td>10</td>
<td>17</td>
<td>-</td>
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<tr>
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<tr>
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<td>-</td>
<td>-</td>
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<td>18</td>
</tr>
<tr>
<td>Control</td>
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<td>11</td>
<td>17</td>
<td>-</td>
<td>15</td>
<td>10</td>
</tr>
</tbody>
</table>

L = 4-benzylimino-2,3-dimethyl-1-phenylpyrazal-5-one

GCMS spectra: In the GCMS analysis of ligand and complexes, there were many peaks. The molecular ion peak of the ligand had mass/charge (m/z) ratio of 291 for the ligand which corresponded to the molecular mass of the compound. There was a small peak at (m/z) 292, this is because of the natural abundance of $^{13}C$. The base peak had m/z ratio at 56. Other fragments occurred at 199, 188, 171, 157, 146, 130, 121, 103, 91, 77, 54 and 39. The major fragmentations are represented in scheme-2.

The results in table-6 are zones of inhibition. From the results, we can see that most of the complexes proved potent against some bacteria and fungi. Complexation improved the antimicrobial activities of the ligand. CuL inhibited the growth of *Staphylococcus aureus* more than other complexes. The standard drug used (ciprofloxacin) did not show any inhibition against *Staphylococcus aureus*. CoL, CuL and NiL have shown great antibacterial and antifungal activities than the ligand and other complexes used in this work. The ZnL is not potent against bacteria but showed great activity against fungi.

Conclusion

The potentiation of the antimicrobial activity of 4-benzylimino-2,3-dimethyl-1-phenylpyrazal-5-one by metal chelation has been studied. A Schiff base ligand, 4-benzylimino-2,3-dimethyl-1-phenylpyrazal-5-one has been synthesized by the condensation of Benzaldehyde and 4-aminoantipyrine. Its divalent metal complexes of Fe, Co, Ni, Cu and Zn were also synthesized. The ligand and the complexes have been characterized by FTIR, UV/visible, $^1$H-NMR, $^{13}$C-NMR, and GCMS. The ligand behaved as a bidentate donor by using its carbonyl and azomethine N as binding sites for the metals. Tetrahedral structures were proposed for the all complexes excepting the Cu(II) complex. The ligand showed low activity against some microbes but the complexes were remarkably active against the bacteria and fungi species. We hereby suggest that this ligand and its metal complexes be used as metal based drugs.

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References


