Synthesis and Antimicrobial Studies of Tridentate Schiff Base Ligands with Pyrazolone Moiety and Their Metal Complexes

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Received 8 September 2010; Revised 23 October 2010; Accepted 2 November 2010

Academic Editor: Feihe Huang

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Synthesis, characterization, and antimicrobial activity of tridentate Schiff base ligands containing pyrazolone moiety (3a and 3b) and their transition metal complexes of VO(II), Cu(II), Fe(III), and Co(II) 4a–h have been investigated. The complexes show enhanced antibacterial activity against S. aureus, E. coli, and S. typhi and antifungal activity against C. albicans, Rhizopus sp., and A. niger compared to the ligands.

1. Introduction

Developing metal-based drugs is emerging as an active area of research. Many organic ligands particularly tridentate Schiff base ligands show enhanced antibacterial, antifungal, and carcinostatic activity when complexed to transition metal ions [1–7]. Heterocyclic systems bearing the pyrazolyl moiety show antibacterial, antifungal, anti-inflammatory and enzyme-inhibitory activity [8–11]. 4-acylpyrazolones are good chelating ligands. Synthesis and characterization of metal complexes of 4-acylpyrazoles are well documented [12–14]. However, biological activity of tridentate Schiff base ligands containing pyrazolone moiety and their transition metal complexes is not explored. Therefore, in the present paper we report the synthesis, characterization, and antimicrobial activity of tridentate Schiff bases of the type ONN (3a) and ONO (3b) containing the pyrazolone moiety and their VO(II), Cu(II), Fe(III), and Co(II) complexes.

2. Results and Discussion

3-Methyl-1-phenylpyrazol-5-one (1) and 4-acetyl-3-methyl-1-phenylpyrazol-5-one were prepared by following the literatures procedure. 4-acetyl-3-methyl-1-phenylpyrazol-5-ones mainly exists as the 3-methyl-1-phenyl-4-acetylpyrazolin-5-ol tautomer (2). Condensing (2) with 1,2-diaminobenzene, and 2-aminophenol resulted in ONN- and ONO-type tridentate ligands (3a and 3b), respectively. Ligands 3a and 3b were complexed with metal salts viz vanadyl sulphate monohydrate, copper(II) nitrate trihydrate, ferric chloride hexahydrate, and cobalt(II) nitrate hexahydrate to obtain, their corresponding complexes (4a–h). Compounds 3a and 3b were characterized by IR, 1H NMR, 13C NMR, and elemental analysis. In addition to these spectral techniques LC-MS was also used for characterization of 3a. 13C NMR and LC-MS data (m/e 307) of 3a indicates the formation of tridentate ligand resulting from condensation of 2 with one amino group of 1,2-diaminobenzene. Though there is a possibility for biscondensation repeated experiments resulted in tridentate ligand (3a) only. Complexes (4a–h) were characterized by IR, UV-Visible, mass, and EPR spectral studies. However, from the data available the structure of complexes could not be fully characterized. The proposed structures of the complexes are given in Table 1. The coordination of methanol in VO(II) complexes (4a and 4e) was verified by thermal analysis. Thermogram of complexes showed a weight loss around 70–130°C ascribable to loss of methanol in the complexes. Further conclusive evidence of the coordination of methanol in these complexes was
shown by the appearance of ν(OH) around 3400 cm\(^{-1}\) in IR spectra.

\[
\text{CH}_3\text{COCl} + \text{Ca(OH)}_2 \rightarrow \text{CH}_3\text{COOH} + \text{CaCl}_2 + \text{H}_2\text{O}
\]

2.1. Antibacterial Studies. The ligands (3a-b) and complexes (4a-h) synthesized were screened for their antibacterial activity by disc-diffusion method [15, 16] against \textit{Staphylococcus aureus} (Gram positive), \textit{Pseudomonas aeruginosa} (Gram negative), and \textit{Salmonella typhi} (Gram negative). The test compounds were prepared at a concentration of 200 \(\mu\text{g/mL}\). Solvent control that is, DMSO was also maintained throughout the experiment simultaneously. Nutrient agar plates were prepared under sterile conditions and incubated overnight to detect contamination. About 0.2 mL of working stock culture was transferred into separate nutrient agar plates and spread thoroughly using a glass spreader. Whatman No. 1 discs (6 mm in diameter) were impregnated in the test compounds and dissolved in DMSO (200 \(\mu\text{g/mL}\)) for about half an hour. Commercially available drug disc (Ciprofloxacin 10 \(\mu\text{g/disc}\)) was used as positive reference standard. Negative controls were also prepared by impregnating disc of same size in DMSO solvent. The discs reference standard. Negative controls were also prepared by impregnating disc of same size in DMSO solvent. The discs were placed on the inoculated agar plates and incubated at 37 ± 1°C for about 18–24 h. Antibacterial activity was evaluated by measuring the zone of inhibition (mm) against the test organism Figure 1.

It is observed that, in general, metal complexes show enhanced antibacterial activity than the corresponding ligands. No general trend in the activity of the ligands or the metal complexes against the bacterial strain is observed. Iron complex 4c showed enhanced activity against all bacterial strains. 4c also showed very low minimum inhibitory concentration (MIC) value (12.5 \(\mu\text{g/mL}\)) of \textit{S. aureus}. The activity of iron complex 4g is less compared to 4c for all bacterial strains.

Among the cobalt complexes 4d and 4h, 4h is more effective against \textit{S. typhi} with low MIC value (25 \(\mu\text{g/mL}\)) compared to the standard ciprofloxacin (50 \(\mu\text{g/mL}\)) as well as its corresponding iron complex.

2.2. Antifungal Studies. The antifungal activity of 3a-b and their complexes (4a-h) were examined with three fungal strains namely \textit{Candida albicans}, \textit{Rhizopus sp.}, and \textit{Aspergillus niger}. Drug amphoterinic B was used as standard. Sabouraud’s dextrose agar (SDA) medium was used for the growth of fungi, and testing was done in Sabouraud’s dextrose broth (SDB) medium. The subculture and the viable count were carried out by the same procedure as done in antibacterial studies except the temperature was maintained at 28 ± 1°C for about 72 h. Similarly for disc diffusion method, the petri dishes were incubated at 28 ± 1°C for about 72 h. The diameters of zone of inhibition (mm) of all the compounds are given in Figure 2.

It is observed that the diameter of zone of inhibition (mm) of cobalt complex 4h shows very high activity with very low MIC value (12.5 \(\mu\text{g/mL}\)) against the fungal strain \textit{A. niger} (MIC of standard amphoterinic B is 50 \(\mu\text{g/mL}\)).

3. Conclusions

4-Acetylpyrazolone predominantly exists as 3-methyl-1-phenyl-4-acetylpyrazolin-5-ol tautomer. The 3-methyl-1-phenyl-4-acetylpyrazolin-5-ol readily condenses with 1,2-diaminobenzene and 2-aminophenol forming tridentate Schiff base ligands of ONN and ONO type. The metal complexes of these ligands with VO(II), Cu(II), Fe(III), and Co(II) show varying geometries. The ligands and metal complexes exhibit varying degree of antibacterial and antifungal activity. In general Fe(III) and Co(II) complexes are more potent than other complexes and ligands.

4. Experimental Section

4.1. General Procedures. \(^1\text{H}\) NMR and \(^{13}\text{C}\) NMR spectra were recorded on a Bruker 400 instrument using tetramethylsilane (TMS) as internal standard and CDCl\(_3\), or DMSO-\(d_6\) as solvent. Chemical shifts are given in parts per million (ppm). IR spectra were recorded on a Nicolet 6700 FT-IR Spectrophotometer in KBr pellet method in the range of 400–4000 cm\(^{-1}\). UV-Vis spectra were obtained from Ocean Optics HR 4000 Spectrophotometer and using DMF as a solvent in a 1 cm quartz cell. Elemental analyses were carried out by the same procedure as done in antibacterial studies except the temperature was maintained at 28 ± 1°C for about 72 h. Similarity for disc diffusion method, the petri dishes were incubated at 28 ± 1°C for about 72 h. The diameters of zone of inhibition (mm) of all the compounds in Figure 2.
Table 1: Proposed structure of complexes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Compound</th>
<th>Structure</th>
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<tbody>
<tr>
<td>4a</td>
<td><img src="image" alt="Structure" /></td>
<td>4c</td>
<td><img src="image" alt="Structure" /></td>
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<td>4b</td>
<td><img src="image" alt="Structure" /></td>
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<td><img src="image" alt="Structure" /></td>
<td>4h</td>
<td><img src="image" alt="Structure" /></td>
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out on a Perkin-Elmer 2400 Series CHNS/O analyzer. The ESI-mass spectra were recorded in QTOF-Micromass-UK by using DMF as solvent, and the LC-MS was taken in Shimadzu LC-MS using acetonitrile as solvent. Thermogravimetric analyses were recorded on TA Instruments Q 600 SDT. The EPR spectra of complexes were recorded in JEOL JES-TE 100 EPR Spectrometer operating at X-band frequency.

3-Methyl-1-phenylpyrazoline-5-one (1). 3-Methyl-1-phenylpyrazoline-5-one was prepared by following the literatures procedure [17].

3-Methyl-1-phenyl-4-acetylpyrazoline-5-ol (2). 3-Methyl-1-phenyl-4-acetylpyrazoline-5-ol was prepared by following the literatures procedure [18].
4.2. General Procedure for the Synthesis of Tridentate Schiff Base Ligands (3a-b). Ethanolic solution of 3-methyl-1-phenyl-4-acetylpyrazolin-5-ol (2 mmol) and 1,2-diaminobenzene (2 mmol) was taken in a round-bottom flask and refluxed for 3 h. The solid product was filtered, washed with cold ethanol and recrystallized from ethanol, and dried under vacuum. A similar procedure was adopted for the reaction between 3-methyl-1-phenyl-4-acetylpyrazolin-5-ol (2 mmol) and 2-aminophenol (2 mmol) to synthesize 3b, but the reaction mixture was refluxed for 8 h and the product was recrystallized from acetonitrile.

4-(1-(2-aminophenylimino)ethyl)-3-methyl-1-phenylpyrazolin-5-ol (3a). Yellow. Yield (75%); m.p. 192°C; IR (KBr, cm⁻¹): 3437 (OH stretch), 3350 (NH stretch), 3036 (aromatic CH stretch), 1621 (azomethine C=N stretch), 1538 (cyclic

Figure 1: Antibacterial activity of (3a-b) and (4a-h).

Figure 2: Antifungal activity of (3a-b) and (4a-h).
C=\text{N} \text{ stretch}) \text{ and 1393 (C–O stretch)}, ^1\text{H} \text{ NMR (CDCl}_3\text{)} ppm: \delta 2.29 (s, 3H, CH\text{)}, 2.42 (s, 3H, CH\text{}), 3.9 (s, 2H, NH\text{)}, 6.77–8.02 (m, 9H, CH aromatic), and 12.58 (s, 1H, OH); \text{ } ^{13}\text{C} \text{ NMR (CDCl}_3\text{)} ppm: \delta 16.6, 17.5, 100.54, 116.31, 118.66, 119.32, 122.17, 124.44, 127.68, 128.75, 129.32, 138.97, 142.6, 147.36, 151.81, 152.38, 167.54, 207.95; \text{ LC-MS m/z 307 } [\text{M}+\text{H}]^+; \text{ analytical calculation for C}_{18}\text{H}_{18}\text{N}_4\text{O}: \text{ C, 70.57; H, 13.89, 14.26, 14.73, 16.55, and 16.58; IR (KBr)} \text{ cm}^{-1} \text{ = 331, 282, 315, 345, 371, and 415, 285, 315, 345, 371, and 415, 285.} 

All the metal complexes have a melting point above 220°C due to the presence of ν(NH\text{2}) varies in the complexes (4a–h) indicates that the paramagnetic ion responsible is VO\text{(II)} having S = 1/2 and I = 7/2. A close look at the spectrum indicates a shoulder on hyperfine lines suggesting that the symmetry of the complex is lower than distorted octahedron. This is not a common observation, as the deviation from axial symmetry is not reported in powder spectrum. The room temperature
Table 3: g and A values for complexes. A values are in units of mT.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Recorded condition</th>
<th>$g_{1}$</th>
<th>$g_{2}$</th>
<th>$A_{11}$</th>
<th>$A_{12}$</th>
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<tr>
<td>4a</td>
<td>LNT Solution</td>
<td>1.937</td>
<td>1.98</td>
<td>18.73</td>
<td>6.49</td>
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<tr>
<td></td>
<td>RT Powder</td>
<td>2.26</td>
<td>2.06</td>
<td>*</td>
<td>*</td>
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<tr>
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<td>LNT Powder</td>
<td>2.24</td>
<td>2.06</td>
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<td></td>
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<tr>
<td>LNT</td>
<td>Solution</td>
<td>$g_{1} = 2.31$, $g_{2} = 2.06$, $g_{3} = 1.29$</td>
<td>15.08</td>
<td>*</td>
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</tr>
<tr>
<td>4c</td>
<td>RT Powder</td>
<td>$g_{1} = 20.44$, $g_{2} = 5.1$, $g_{3} = 1.69$, $g_{4} = 1.29$</td>
<td>*</td>
<td>*</td>
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<tr>
<td>4e</td>
<td>LNT Solution</td>
<td>1.938</td>
<td>1.98</td>
<td>18.70</td>
<td>6.51</td>
</tr>
<tr>
<td></td>
<td>RT Powder</td>
<td>2.26</td>
<td>2.06</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>4f</td>
<td>LNT Solution</td>
<td>$g_{1} = 2.31$, $g_{2} = 2.06$, $g_{3} = 2.04$</td>
<td>14.84</td>
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<td>$g_{1} = 20.4$, $g_{2} = 5.24$, $g_{3} = 2.37$, $g_{4} = 1.7$</td>
<td>*</td>
<td>*</td>
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<tr>
<td>4g</td>
<td>RT Powder</td>
<td>$g_{5} = 1.31$</td>
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</table>

* unresolved.

Powder EPR spectrum of $4b$ was recorded at room temperature and 77 K. The hyperfine lines from copper nucleus are not resolved at room temperature spectrum due to dipole-dipole broadening. In order to obtain the hyperfine values, the complex was dissolved in DMF, and EPR spectrum was recorded at 77 K. The spectrum clearly indicates resonances from copper nucleus ($^{63}$Cu/$^{65}$Cu). The symmetry around the copper is lower than distorted octahedral as reflected from the three $g$ values [20]. Also the three $g$ values suggest that the coordination around the copper is six, which was confirmed by mass spectra (m/z 665).

The powder EPR spectrum of $4c$ at room temperature shows a number of resonances indicating that iron is under the influence of a strong tetragonal distortion. The range of $g$-values suggests that all the three Kramers’ doublets are populated at room temperature. The ESI-Mass of $4c$ complex (m/z 740) suggests that the complex is oxygen bridged bimetallic iron complex. Moreover oxobridged iron complexes are well known in many literatures [21]. The recorded solution EPR spectrum shows a single resonance at $g = 2$. This kind of observation suggests that the dimeric iron complex might have become monomer after dissolution, hence a single line is observed [22]. The complex $4g$ behaves similar to $4c$.

Acknowledgments

G.Vasuki thanks the University Grants Commission and Department of Science and Technology (DST) for financial support. R.Jayarajan thanks the UGC, New Delhi, India for the award of Rajiv Gandhi National Fellowship.
References


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