Synthesis, spectral characterization and biological activity studies of Schiff’s base of 1,5-dimethyl-2-phenyl-2,3-dihydro-1H-pyrazol-4-amine and its metal complexes

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ABSTRACT

N,O,O-donating tridentate ligand was prepared by condensed reaction 1,5-dimethyl-2-phenyl-2,3-dihydro-1H-pyrazol-4-amine with derivative of salicylaldehyde and its metal complexes with Zn, Cd and Hg, which is highly stable at room temperature and characterized by Mass spectra, IR, $^1$H and $^{13}$C NMR, ESI-MASS and TGA-DTA. Comparatively studies of anti-microbial and anti-fungal activity of different ligand and its metal complex carried out.

Keywords: Schiff Bases; anti-microbial activity; Metal complexes; spectral analysis

1. INTRODUCTION

Schiff base complexes are considered to be among the most important stereochemical models in main group and transition metal coordination chemistry due to their preparative accessibility and structural variety$^{[1]}$. Schiff bases are potential anticancer drugs and when administered as their metal complexes, the anticancer activity of these complexes is enhanced in comparison to the free ligand$^{[2]}$. Schiff bases of 4-aminoantipyrine and its complexes present a great variety of biological activity ranging from antitumor, fungicide, bactericide, anti-inflammatory, and antiviral activities$^{[3-5]}$. 
Schiff bases have also been employed as ligands for the complexation of metal ions. Antipyrine (2,3-dimethyl-1-phenyl-5-pyrazolone) and its derivatives exhibit a wide range of biological activities and applications\[^{[6]}\] Nowadays, antipyrine and its derivatives (especially iodine-antipyrine) are widely used in medicine as antiphlogistic species. Antipyrine is also a multifunctional marker drug that is extensively used in studies on the capacity of hepatic oxidative metabolism\[^{[7]}\].

The number of transition metal complexes of Cu(II), Ni(II) and V(IV) with oxygen and nitrogen donor Schiff base derivatives of 4-aminoantipyrine is limited. A small number of papers describe the synthesis and characterization of these compounds based on aminoantipyrine Schiff bases\[^{[8-16]}\]. The complexes and ligands were also tested for their in vitro antibacterial activity against Staphylococcus aureus var. Oxford 6538, Escherichia coli ATCC 10536 and Candida albicans ATCC 10231 strains using the paper disc diffusion method\[^{[17]}\] (for the qualitative determination) and the serial dilutions in liquid broth method\[^{[18]}\] (for determination of MIC).

Thus, the aim of the work is to synthesize and characterize Zn(II), Cd(II), and Hg(II) metal complexes with Schiff base of 1,5-dimethyl-2-phenyl-2,3-dihydro-1H-pyrazol-4-amine and derivative of salicylaldehyde. The Schiff base and its metal complexes were tested for their antibacterial and antifungal activities.

2. MATERIALS AND METHODS

All require chemicals and solvents used in research work are analytical reagent grade (AR). All metal salts were used as their chlorides. All the compounds and solvents were purchased from spectrochem and Thomas baker and checked TLC. IR spectra were taken with Shimadzu IR Affinity-1S FTIR spectrometer. Mass spectra were done on GCMS QP2010 mass spectrometer, \(^1\)H NMR spectra were taken on Bruker NMR spectrometer (400 MHz), using TMS [as internal standard]. Elemental data was recorded by Carlo Erba EA 1108 elemental analyzer.

3. RESULT AND DISCUSSION

3.1. Experimental

3.1.1. General procedure for the synthesis of Schiff base of 1,5-dimethyl-2-phenyl-2,3-dihydro-1H-pyrazol-4-amine

The Schiff base of 1,5-dimethyl-2-phenyl-2,3-dihydro-1H-pyrazol-4-amine was prepared by adding equimolar amounts of 1,5-dimethyl-2-phenyl-2,3-dihydro-1H-pyrazol-4-amine in 30 mL absolute ethanol and different derivative salicylaldehyde with 2-3 drops of glacial acetic acid respectively.

The mixture was stirred at 60-70 °C for 60-90 minute. The reaction was monitored by TLC using hexane: ethyl acetate (2:3). The precipitated products were filtered off and wash with diethyl ether, crystallized from ethanol and dried under vacuum for 30 minutes and finally dried in vacuum desiccators over anhydrous Calcium(II) Chloride.
3. 1. 2. General Procedure for the Synthesis of Metal Complex

Synthesis of the Zn(II) complexes with 4-((2,3-dihydroxybenzylidene)amino)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one. An ethanolic solution (15 mL) of Schiff base ligand BJ-23 was added to ZnCl₂ distilled water solution. This solution was refluxed for 3h and left at room temperature for 2 days. A dull white precipitate was formed. The product was separated by filtration, purified by washing with cold methanol and then with ether. All other complexes were synthesized by the same method. The elemental analysis confirms the molecular formula. The physical and analytical data are presented in Table 1.

![Diagram](image.png)

**Fig. 1.** Scheme for synthesis of metal complex.
Table 1. R₁, R₂ and formula of Metal complexes, colour, molecular weight, M.P. and % yield.

<table>
<thead>
<tr>
<th>No.</th>
<th>Code</th>
<th>R₁, R₂ and Metal complexes</th>
<th>Colour</th>
<th>Mol. Weight</th>
<th>M.P. (°C)</th>
<th>% yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BJ-23</td>
<td>R₁-OH, R₂-H</td>
<td>Light yellow</td>
<td>323</td>
<td>190</td>
<td>80</td>
</tr>
<tr>
<td>2</td>
<td>BJ-24</td>
<td>R₁=2,5-(OH)₂, R₂=CH₃</td>
<td>Dark Yellow</td>
<td>337</td>
<td>202</td>
<td>85</td>
</tr>
<tr>
<td>3</td>
<td>BJ-26</td>
<td>R₁=5-Bromo, Acetyl coumarine, R₂=CH₃</td>
<td>Dark crimish</td>
<td>451</td>
<td>235</td>
<td>75</td>
</tr>
<tr>
<td>4</td>
<td>BJ-29</td>
<td>R₁=4-(diethylamino), R₂=H</td>
<td>Orange</td>
<td>378</td>
<td>220</td>
<td>90</td>
</tr>
<tr>
<td>5</td>
<td>HJ-23</td>
<td>Zn(C₁₈H₁₆N₃O₃)₂</td>
<td>Dull white</td>
<td>710</td>
<td>283</td>
<td>78</td>
</tr>
<tr>
<td>6</td>
<td>HJ-24</td>
<td>Hg(C₁₈H₁₆N₃O₃)₂</td>
<td>Crimish white</td>
<td>845</td>
<td>305</td>
<td>65</td>
</tr>
<tr>
<td>7</td>
<td>HJ-25</td>
<td>Cd(C₁₈H₁₆N₃O₃)₂</td>
<td>white</td>
<td>757</td>
<td>295</td>
<td>72</td>
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</tbody>
</table>

3. 2. Spectral Analysis

3. 2. 1. 4-((2,3-dihydroxybenzylidene)amino)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one [BJ-23]

Elemental Analytical Calculation for C₁₈H₁₇N₃O₃ (323.35 g/mol): C, 66.86%; H, 5.30%; N, 13.00%; O, 14.84%. Found: C, 66.82%; H, 5.25%; N, 12.95%; O, 14.82%. MS (m/z): 323; IR, (cm⁻¹): \(\nu\) (OH) 3253; \(\nu\) (C=O) 1653; \(\nu\) (C=N) 1575; \(\nu\) (N-N) 956; \(\nu\) (Ar-C-H) 2949,3072; \(\nu\) (Ar=C=Ar) 1487; \(^1^H\)-NMR (DMSO-d₆) \(\delta\) ppm: 13.91 (s, 1H, -OH); 9.82 (1H, -OH); 9.77 (1H, N=C); 2.42 (s, 3H CH₃); 3.45 (s, 3H, N-CH₃) 6.03-7.68 (8H Ar-H). \(^1^3^C\)-NMR (DMSO-d₆) \(\delta\) ppm: 160.47 (HC=N); 160.12 (C=O); 10.29 (CH₃); 35.57 (N-CH₃); 147.76, 148.08 (2C-CH₂); 149.54 (N-*C-CH₃), 144.64 (Ar*-C-N) 115.92-134.18 (9 C=C Ar)

3. 2. 2. 4-((1-(2,5-dihydroxyphenyl)ethylidene)amino)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one [BJ-24]

Elemental Analytical Calculation for C₁₉H₁₉N₃O₃ (337.14 g/mol): C, 67.64%; H, 5.68%; N, 12.46%; O, 14.23%. Found: C, 67.62%; H, 5.65%; N, 12.40%; O, 14.21%. MS (m/z): 337; IR, (cm⁻¹): \(\nu\) (OH) 3223; \(\nu\) (C=O) 1631; \(\nu\) (C=N) 1587; \(\nu\) (N-N) 941; \(\nu\) (Ar=C=Ar) 3061; \(\nu\) (Ar=C=Ar) 1487; \(^1^H\)-NMR (DMSO-d₆) \(\delta\) ppm: 14.00 (s, 1H, -OH); 8.99 (1H, -OH); 2.25 (3H, N=C-CH₃); 2.36 (s, 3H CH₃); 3.12 (s, 3H, N-CH₃) 6.73-7.54 (8H Ar-H). \(^1^3^C\)-NMR (DMSO-d₆) \(\delta\) ppm: 172.74 (HC=N); 158.53 (C=O); 154.00, 148.76 (2C-CH₂); 147.66 (N-*C-CH₃), 10.52 (N-C-*CH₃), 18.83 (N=C-*CH₃); 36.07 (N-CH₃); 134.90 (Ar*-C-N) 114.25-129.11 (9 C=C Ar).
3. 2. 3. 4-((1-(6-bromo-2-oxo-2H-chromen-3-yl)ethylidene)amino)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one [BJ-26]

Elemental Analytical Calculation for C_{22}H_{19}BrN_{3}O_{3} (451.05 g/mol): C, 58.42%; H, 4.01%; N, 9.29%; O, 10.61%; Br, 17.67%. Found: C, 58.40%; H, 4.00%; N, 9.26%; O, 10.60%; Br, 17.65%. MS (m/z): 451; IR, (cm⁻¹): ν(C=O) 1716, ν 5-member ring(C=O) 1653, ν(C-O) 1361; ν(C=N) 1641; ν(N-N) 945; ν(Ar–C–H) 3064,2947; ν(Ar–C–Br) 640. \(^1\)H-NMR (DMSO-d₆): δppm 2.22 (3H, N=C-CH₃); 2.41 (s, 3H, CH₃); 3.14 (s, 3H, N-CH₃) 7.23-7.94 (8H Ar-H). \(^13\)C-NMR (DMSO-d₆): δppm 164.65 (HC=N); 158.47, 157.42 (2C=O); 149.71 (N-*CH₃),150.67 (O-C,fused), 118.80 (C-Br), 12.58 (N=C-*CH₃), 14.83 (N=C-*CH₃); 34.70(N-CH₃); 133.72(Ar*C-N), 110.25-135.28 (12 C=C Ar).

3. 2. 4. 4-((4-diethylamino)-2-hydroxybenzylidene)amino)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one [BJ-29]

Elemental Analytical Calculation for C_{22}H_{26}N_{4}O_{2} (378.21 g/mol): C, 69.82%; H, 6.92%; N, 14.80%; O, 8.45%. Found: C, 69.80%; H, 6.88%; N, 14.74%; O, 8.41%. MS (m/z): 378; IR, (cm⁻¹): ν(OH) 3244; ν(C=O) 1670; ν(C=N) 1587; ν(N-N) 975; ν(Ar–C–H) 2899,2980; \(^1\)H-NMR (DMSO-d₆): δppm 13.66 (s, 1H, -OH); 9.56 (s, 1H, HC=N); 6.05-7.36 (8H Ar-H); 1.04-1.08 (t 6H, 2CH₃); 2.22 (s,3H CH₃); 2.95 (s, 3H, CH₃) 3.22-3.27(dd 4H, 2CH₂). \(^13\)C-NMR (DMSO-d₆): δppm 162.83 (HC=N); 160.16 (C=O); 160.75(C=OH); 150.88 (N-*CH₃), 148.78 (N-*C-(CH₃CH₃)₂), 10.25(N-C-*CH₃), 36.10(N-CH₃); 12.73(2CH₃), 44.53(2{*CH₂–CH₃}) 134.90(Ar*C-N) 97.64-133.40(10 C=C Ar).

3. 2. 5. Zinc(II) complex of 4-((2,3-dihydroxybenzylidene)amino)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one [BJ-23]

Elemental Analytical Calculation for C_{36}H_{32}N_{6}O_{6}Zn (708.17 g/mol): C, 60.90%; H, 4.54%; N, 11.84%; O, 13.52%; Zn, 9.21%. Found: C, 60.88%; H, 4.50%; N, 11.81%; O, 13.50%; Zn, 9.20%. ESI-MS (m/z): 708 (ZnL₂); IR( cm⁻¹): ν(C=O)1631; ν(C=N)1556; ν(N-N)941; ν(Ar–C-H)3061.

3. 2. 6. Mercury(II) complex of 4-((2,3-dihydroxybenzylidene)amino)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one [HJ-24]

Elemental Analytical Calculation for C_{36}H_{32}N_{6}O_{6}Hg (846.21 g/mol): C, 51.15%; H, 3.82%; N, 9.94%; O, 11.36%; Hg, 23.73%. Found: C, 51.14%; H, 3.80%; N, 9.92%; O, 11.34%; Hg, 23.71%. ESI-MS (m/z): 846 (HgL₂). IR( cm⁻¹): v(C=O)1628; v(C=N)1552; v(N-N)945; v(Ar–C-H)3056.

3. 2. 7. Cadmium(II) complex 4-((2,3-dihydroxybenzylidene)amino)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one [HJ-25]

Elemental Analytical Calculation for C_{36}H_{32}N_{6}O_{6}Cd (758.14 g/mol): C, 57.11%; H, 4.26%; N, 11.10%; O, 12.68%; Cd, 14.85%. Found: C, 57.10%; H, 4.24%; N, 11.06%; O, 12.65%; Cd, 14.84%. ESI-MS (m/z): 758(CdL₂). IR( cm⁻¹): v(C=O)1634; v(C=N)1557; v(N-N)940; v(Ar–C-H)3053.
Fig. 2. Mass spectra of BJ-23.
Fig. 3. ESI-Mass spectra of HJ-23
Fig. 4. IR spectra of BJ-23.
Fig. 5. IR spectra of HJ-23
Fig. 6. $^1$H NMR spectra of BJ-23.
Fig. 7. $^{13}$C NMR spectra of BJ-23
3. 3. Thermal studies

Thermal stabilities of metal complex of Zn(II), Hg(II) and Cd(II) are carried out in which metal complexes decompose gradually with formation of respectively metal oxide. The Decomposition Data obtain by chemical change with temperature and the percent of metal oxide obtained are given in the Table 2. The spectra of complexes of Zn(II) (HJ-23), Hg(II) (HJ-24) and Cd(II) (HJ-25) decomposes at single step in the region 283, 305 and 295 °C. It is indicates the loss of ligand molecules with respect to the mass loss of 22.60% (Calc: 22.67%), 43.20% (Calc: 43.24%) and 39.80% (Calc: 39.86%), respectively, leaving behind the metal oxide residue. The metal content in all the complexes as done by elemental analysis agrees well with the thermal studies.

Table 2. Thermo gravimetric data of Zn(II), Hg(II), Cd(II) and Pd(II) complexes.

<table>
<thead>
<tr>
<th>Comp. code</th>
<th>Empirical formulae of the complexes</th>
<th>Decompo temp. (°C)</th>
<th>Weight loss (%)</th>
<th>Metal oxide (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Found</td>
<td>Calc</td>
</tr>
<tr>
<td>HJ-23</td>
<td>C_{36}H_{32}N_{6}O_{6}Zn</td>
<td>283</td>
<td>22.60</td>
<td>22.67</td>
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<tr>
<td>HJ-24</td>
<td>C_{36}H_{32}N_{6}O_{6}Hg</td>
<td>305</td>
<td>43.20</td>
<td>43.24</td>
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<tr>
<td>HJ-25</td>
<td>C_{36}H_{32}N_{6}O_{6}Cd</td>
<td>295</td>
<td>39.80</td>
<td>39.86</td>
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</table>

3. 4. Biological activity

3. 4. 1. Antibacterial and antifungal activities Antimicrobial evaluation

The biological activity (antibacterial and antifungal-MIC) in vitro was done by broth dilution method of all the compounds. two Gram-negative bacteria E.coli, Pseudomonas aeruginosa, Two Gram-positive bacteria Staphylococcus aureus, Streptococcus pyogenes and three fungal strains Aspergillus clavatus, Aspergillus Niger, Candida albicans strains were taken. As standard drugs, we have taken ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin, and geseofulvin.

The MIC (minimal inhibitory concentration) values for the synthesized ligands and metal complexes was taken in very low concentration for the preventing the visible growth, it is determined by using broth dilution method with reference to NCCLS standards. Generally dilutions of the reference drugs and novel synthesized compounds were prepared in Mueller-Hinton agar. 10 mg Reference Drugs were dissolved in 1 ml DMSO after that continue dilutions with melted Mueller-Hinton agar. In primary screening set of different 1000, 500, 250 and 100 μg/mL concentrations of the synthesized drugs were taken. The active drugs, which is found in this primary screening was also further tested in the second set of dilution at 200, 100, 50, 25, 12.5 and 6.25 μg/mL concentration against different microorganisms. The tubes were inoculated with 10^8 CFU mL^{-1} (colony forming unit/mL) at 37 °C for 24 h. The minimal inhibitory concentration (MIC) was the minimum concentration of the tested compound that yields no visible growth (turbidity) on the plate. Also onfirm that
DMSO has not any effect on the microorganisms in the concentrations studied. The results given in below table.

Table 3. Antibacterial and antifungal activity.

<table>
<thead>
<tr>
<th>Code</th>
<th>Minimal inhibition concentration (µg mL⁻¹)</th>
<th>Gram-positive</th>
<th>Gram-negative</th>
<th>Fungal species</th>
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<tbody>
<tr>
<td>BJ-23</td>
<td></td>
<td>250</td>
<td>250</td>
<td>100</td>
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<td>BJ-24</td>
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<tr>
<td>Greseofulvin</td>
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</table>

4. CONCLUSION

We have synthesized Schiff base of 1,5-dimethyl-2-phenyl-2,3-dihydro-1H-pyrazol-4-amine with substituted aromatic aldehyde and also synthesized its different metal complexes with Zn(II), Cd(II) and Hg(II). Its obtained in good to moderate yield. All synthesized Schiff base were characterized by Mass spectrometry, IR, ¹H NMR, ¹³C NMR and Elemental Analysis. Its metal complexes were characterized by ESI-Mass, IR, TGA-DTA and Elemental analysis. All the compounds are showing moderate antibacterial and anti-fungal activity with compared to standard Drugs.

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-135-
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Supplimentary Data

Fig. 8. Structure of Ligands
Fig. 9. Mass spectra of BJ-24
Fig. 10. Mass spectra of BJ-26
Fig. 11. Mass spectra of BJ-29
Fig. 12. IR spectra of BJ-26
Fig. 13. IR spectra of BJ-29
Fig. 14. $^1$H NMR spectra of BJ-24
Fig. 15. Expanded $^1$H NMR Spectra of BJ-24
Fig. 16. Expanded $^1$H NMR Spectra of BJ-24
Fig. 17. $^1$H NMR spectra of BJ-29
Fig. 18. Expanded $^1$H NMR spectra of BJ-29
Fig. 19. Expanded $^1$H NMR spectra of BJ-29
Fig. 20. $^{13}$C NMR spectra of BJ-29
Fig. 21. $^{13}$C NMR spectra of BJ-24