



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

Elemike E.E.¹, Oviawe A.P.¹ and Otuokere I.E.²

¹Department of Chemistry, University of Benin, NIGERIA

²Department of Chemistry, Michael Okpara University of Agriculture, NIGERIA

Available online at: www.isca.in

(Received 25th July 2011, revised 19th August 2011, accepted 08th September 2011)

Abstract

A Schiff base ligand, 4-benzylimino-2-3-dimethyl-1-phenylpyrazal-5-one have 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 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-aminoantipyrine (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-aminoantipyrine, ethanol, dimethyl sulphoxide (DMSO), dimethyl formamide (DMF), NiCl₂.6H₂O, CuCl₂.2H₂O, CoCl₂.6H₂O, ZnCl₂ and FeCl₂. 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 Shimadzu.

Synthesis of 4-benzylimino-2-3-dimethyl-1-phenylpyrazal-5-one: The Schiff base derived from benzaldehyde and 4-aminoantipyrine was prepared by adding an ethanol solution (25ml) of 4-aminoantipyrine (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 days to crystallize. The crystal formed was recrystallized with hot ethanol and dried in a desiccator over CaCl₂ vacuum⁷. The yield was recorded.

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 FeCl₂.4H₂O (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.

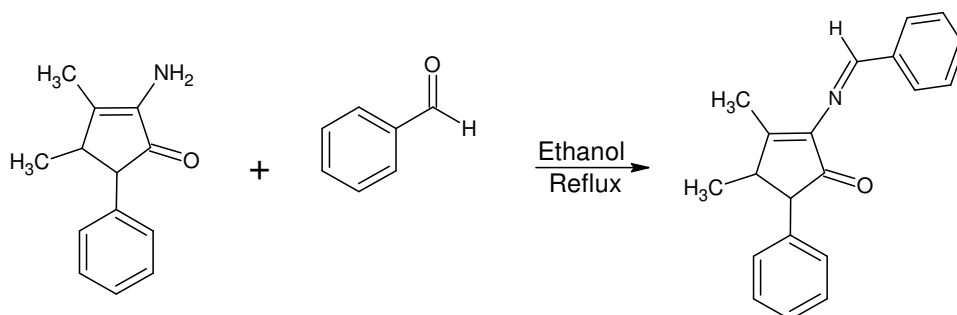
Sensitivity test: The sensitivity tests on the samples were carried out using agar well diffusion method⁸. The nutrient agar was prepared according to the manufacturer's recommendation and was poured into Petri dishes to set. The

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 Macfarland's standards and were inoculated on the surface of the agar⁹. 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 swob stick. The innoculated plates were then incubated at 37°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

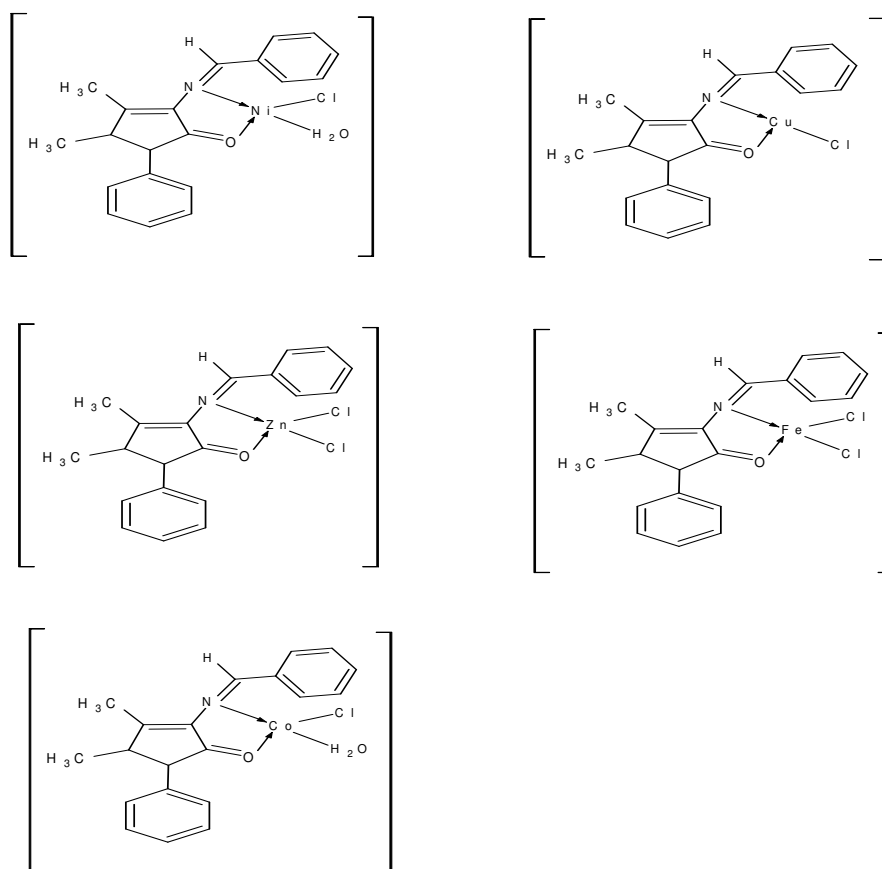


Figure - 1
Proposed structures of the metal complexes

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 complexes 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 complexation¹⁰. 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 $n \rightarrow \pi^*$ (HC=N) transitions¹¹. Two bands were observed in the spectrum of FeL which are 20728 cm^{-1} and 25274 cm^{-1} . These bands have been assigned MLCT and $^5E \rightarrow ^5T_2$ transition and a tetrahedral geometry is therefore proposed¹². For the complex (CoL), three absorption bands are observed 15175 cm^{-1} , 16536 cm^{-1} and 24636 cm^{-1} . These bands are as a result of metal ligand charge transfer (MLCT), d \rightarrow d transfer and $\pi \rightarrow \pi^*$ ligand transfer. The intensity of the band suggested an tetrahedral geometry and their assignments thus $^4T_{1g} \rightarrow ^4T_{2g}$, $^4T_{1g} \rightarrow ^4T_{1g}$ 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 19396 cm^{-1} and 25785 cm^{-1} which represents $^2T_2 \rightarrow ^2E$ 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.

Table – 1
Physical properties of the ligand and complexes

Compound	Colour	Melting point °C	Molecular weight	Yield %
L	Yellow	160	291.01	44.0
FeL	Dark red	146-147	416.12	49.0
CoL	Yellowish brown	134-136	401.77	75.6
NiL	Light green	186-188	401.53	73.7
CuL	Brown	268	388.36	63.9
ZnL	Yellow	279	425.68	70.3

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

Table-2
Infrared data of the ligand and complexes (cm^{-1})

Compounds	$\nu_{\text{OH, H}_2\text{O}}$	$\nu_{\text{C-H aromatic}}$	$\nu_{\text{C=O}}$	$\nu_{\text{C=N}}$	$\nu_{\text{C=C aromatic}}$	$\nu_{\text{M-N}}$	$\nu_{\text{M-Cl}}$
L	-	3042	1649	1569	1481	-	-
FeL	-	3044	1650	1571	1483	585	441
CoL	3425	3043	1649	1570	1483	530	442
NiL	3245	3050	1639	1577	1481	591	447
CuL	-	3171	1623	1620	-	497	387
ZnL	-	3051	1819	1592	-	618	421

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

Table-3
UV/Visible electronic spectra data and possible assignments

Compounds	$\nu_1 \text{cm}^{-1}$	$\nu_2 \text{cm}^{-1}$	Assignment
L	20498	24739	$\pi \rightarrow \pi^*$
FeL	20728	25274	$^5E \rightarrow ^5T_2$
CoL	15175	16536	$^4T_{1g} \rightarrow ^4T_{2g}$, $^4T_{1g} \rightarrow ^4T_{1g}$, $^4T_{1g} \rightarrow ^4T_{2g}$
NiL	25199	28150	$\pi \rightarrow \pi^*$
CuL	19396	25785	$^2T_2 \rightarrow ^2E$
ZnL	20551	24569	$\pi \rightarrow \pi^*$

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

¹H-NMR spectra data: ¹H-NMR 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 δ 2.00ppm 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 linkage¹³. 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 protons¹⁴.

¹³C-NMR: ¹³C-NMR spectra data is presented in table-5. In the ligand, the C=O carbon resonates at 190ppm but in the complexes, C=O 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 complexes¹⁵. 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
¹H-NMR Chemical shifts of the different compounds (all values in ppm)

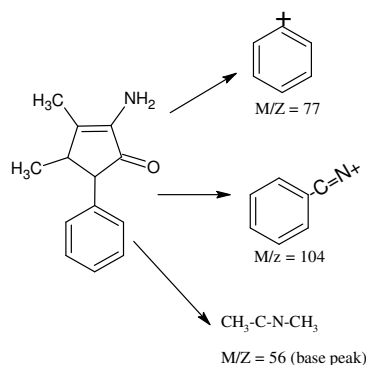
Compound	HC=N	Aromatic H	CH ₃ -N	CH ₃ -C
L	2.53	7.12-7.87	2.49	2.00
FeL	2.73	6.68-7.08	2.44	1.80
CoL	2.78	7.02-7.28	2.54	1.60
NiL	3.23	7.78-8.08	2.64	2.00
CuL	3.97	7.48-7.78	2.23	2.00
ZnL	2.33	7.18-7.48	2.29	1.80

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

Table – 5
¹³C-NMR data for the ligand and complexes in ppm

Compound	C=O	C=N	C=C	Aromatic carbons	CH ₃ -N	CH ₃
L	190	151	146,149	112-142	32	18
FeL	204	148	142,145	112-138	28	16
CoL	213	149	141,146	103-135	27	13
NiL	214	149	142,146	111-138	25	16
CuL	211	149	144,146	107-142	32	18
ZnL	218	152	146,149	118-144	22	12

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



Scheme-2
GCMS major fragmentations

Table – 6
Antimicrobial test results (cm)

Compounds	<i>Escheriachia coli</i>	<i>Enterococcus faecalis</i>	<i>Klebsiella pneumoniae</i>	<i>Staphylococcus aureus</i>	<i>Candida albicans(fungus)</i>	<i>Monosporum audonii (fungus)</i>
L	18	10	17	-	15	10
FeL	16	15	16	15	17	14
CoL	23	20	18	17	21	32
NiL	19	15	11	18	18	30
CuL	19	18	20	27	26	23
ZnL	-	-	-	18	30	18
Control	16	11	17	-	15	10

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 have 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, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, 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.

Acknowledgement

We acknowledge Mr Tobias Egemba of City university, London. He helped us in NMR spectra characterization.

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