ANTIBACTERIAL ACTIVITIES OF 4-BENZOYL-5-PRAZOLONE OF 2-AMINO BENZOIC ACID (A SCHIFF BASE LIGAND) AND ITS COMPLEXES WITH SOME METALS IN THE FIRST TRANSITION SERIES

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Abstract

Reports and experience have shown that causative organisms for various illnesses develop resistance to drugs that are used for their treatment with time. This has necessitated research for new and potent drugs. Consequently in this research, a Schiff base ligand (4-benzoyl-5-pyrazolone of 2-amino benzoic acid) and its complexes with Cr, Mn, Fe, Co, Ni and Cu have been synthesized and characterized using Ir, Uv and Mass spectroscopic methods. Results showed that the mole ratio of the ligand to the metal ion in the complex is 1:1. This means that the general formula of the metal complex is M-L, where M is the metal and L is the Schiff base ligand. Results of physical tests (solubility and melting) showed that the Schiff base ligand and its metal complexes were soluble in polar solvents like di methyl sulphur oxide and dimethyl fluoride but insoluble in non-polar solvents like N-hexane and diethyl ether, the metal complexes were more stable to heat than the Schiff base ligand and the metals were comparatively more stable down the group. Results of antibacterial tests showed that the Schiff base ligand and its metal complexes were active against the growth of *Staphylococuss aureus, Bacillus subtiles, Pseudononas aeruginosa* and *Escherichia coil* and the metal complexes were more active against the growth of the organisms than the Schiff base ligand.

Key Words: Pyrazolone, Schiff base, ligands, transition metals, complexes, bacterial growth.

1. 1. Introduction

Increasing resistance of microorganisms to available antimicrobial drugs is a problem to reckon with when it comes to effective treatment of bacterial infections. Consequently, development of new antibacterial drugs is a very important area of research. According to reports, most compounds bearing an azomethine group exhibit antimicrobial properties (Mohini *et al.*, 2013; Shi, *et al.*, 2007). Schiff bases are organic compounds named after a German Chemist (Hugo Schiff) who discovered them (Bryan, 1972). They are aldehydes or Ketone-like compounds in which the carbonyl group is replaced by an imine or azomethine group. Their general structural formula is $R_2C= NR$ (Where $R^1 \neq H$) (Bryan, 1972). They can be considered a sub-class of imines being either secondary ketimines or secondary aldimines depending on their structure. The general structural formula of an imine is as shown in Figure 1 below.

A number of special naming systems exist for these compounds. For instance a Schiff base derived from aniline, where R^3 is a phenyl or a substituted phenyl group can be called an anil while bis-compounds are often referred to as salen-type compounds. The term Schiff base is normally applied to these compounds when they are being used as ligands to form coordination complexes with metal ions.

Schiff base ligands and their metal complexes have a variety of applications in biological, clinical, analytical and industrial fileds (Ejidike and Ajibade, 2016; Misha and Guatam, 2002). Among these, heterocyclic Schiff base ligands and their metal complexes have significant interest because of their pharmacological properties. Furthermore, the interaction of these complexes with DNA has gained much attention due to their possible applications as new therapeutic agents (Nair *et al.*, 2012). Schiff base compounds and their metal complexes have been extensively investigated due to their wide range of applications including catalysis (Gupta and Sutar, 2008; Cozzi. 2004), Medicine (Turkkan *et al.*, 2011, Siji *et al.*, 2011), Crystal engineering (Sharima 2002), anti-corrosion agent (Ahamad *et al., 2010;* Antonijievic and Petrovi, 2008). Schiff bases are studied widely due to their synthetic flexibility, selectivity and sensitivity towards the central metal atom. Also similarities with natural biological compounds and also due to the presence of azomethine group (-N = CH-) which imports in elucidating the mechanisms of transformation and recemization reaction biologically (Sharghi and Nesseri, 2003; Gao and Zhang, 2002). Schiff base having chelates with oxygen, nitrogen or other donor atoms as well as their metal complexes have been used as drugs and reported to possess a wide range of biological activities against bacteria, fungi and certain types of tumors and have many biochemical, clinical and pharmacological properties (Balell, *et al.*, 1998; Isloor *et al.*, 2009; Eswavan *et al.*, 2009; Proybylski *et al.*, 2009).

This present research was carried out to ascertain the efficacy of 4-benzoyl-5-pyrazolone of 2-amono benzoic acid and its complexes with some metals in the first transition series against the growth of bacterial organisms.

2. METHODS OF STUDY

2.1. Synthesis of 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone (HPMB)

This was done by dissolving 7.5g (0.043M) of 1-phenyl-3-methyl-2-pyrazoline-5-one in 80cm^3 of 1-4-dioxane through the application of heat. After the dissolution, 12g of $Ca(OH)_2$ was added followed by dropwise addition of 5cm^3 of benzoyl chloride, over one minute.

The mixture was refluxed for 30 minutes after which it was poured into 100cm^3 of 0.5M HCl in 250cm^3 beaker and allowed to stay for a day. The mixture was then filtered. The precipitate obtained was washed with 1-4-dioxane and recrystallized from ethanol. The product was dried, and the product yield determined after which it was stored in a desiccator. The Equation of Reaction is as shown in Fig. 2 below.

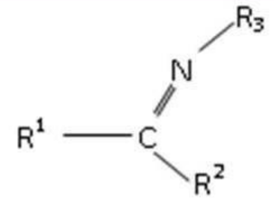


Fig. 1: General structural formula of imines

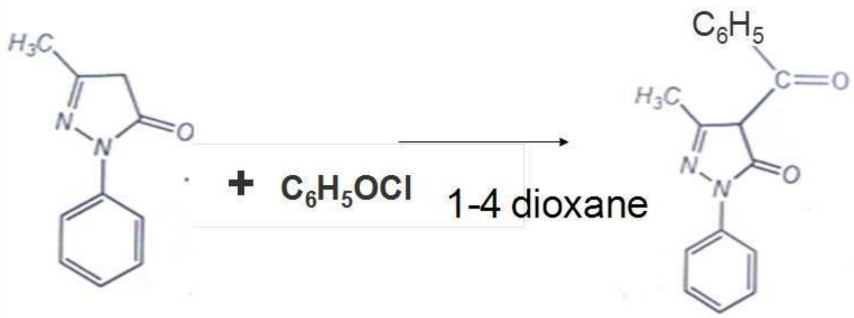


Fig. 2: Synthesis of 1-phenyl-3-methyl-4- benzoyl-5-pyrazolone (HPMB)

2.2. Synthesis of 2-amino benzoic acid (Anthranilic Acid)

In this synthesis, phthalimide was first synthesized by mixing 10g of phthalic anhydride and 2g of urea in a 250cm^3 round bottomed flask. The mixture was heated to 130^{0}C till it melts, froth up and solidified. The mixture was allowed to cool, after which 50cm^3 of water was added to disintegrate the solid. The crude product was filtered, washed with water and then recrystalized from ethanol to obtain the pure product which was dried in a desiccator. Next 7.5g of NaOH was dissolved in 40cm^3 of H_2O and then cooled in ice bath to about 0^{0}C after which 2.1cm^3 of bromine solution was added followed by 6g of phthalimide and 20cm^3 of 10% KOH solution. The mixture was heated till phthalimide dissolved after which it was neutralized with glacial acetic acid. The solution was then heated to 80^{0}C and left to stand until crystallization occurred. The crystals obtained were recrystallized from hot water, washed with cold H_2O and dried in the oven at 100^{0}C. The equation of reaction is as shown in Figure 3.

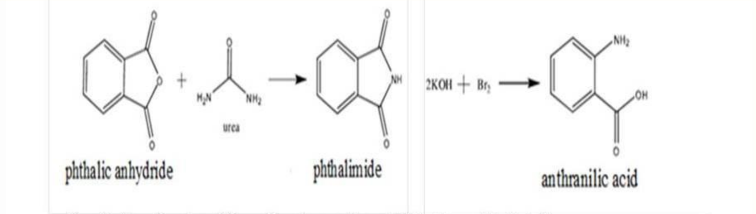


Fig. 3. Synthesis of 2-amino benzoic acid (Anthranilic Acid)

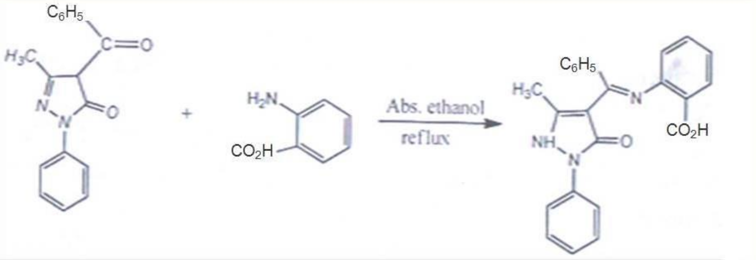


Fig. 4. Synthesis of 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone and 2-amino-benzoic acid Schiff base (L)

2.3. Synthesis of 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone and 2-amino-benzoic acid Schiff base (L)

A mixture of equimolar ethanolic hot solution formed by dissolving 1.37g (0.01M) of 2-amino-benzoic acid in $50cm^3$ of absolute ethanol and that formed by dissolving 2.78g (0.01M) of 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone was refluxed at 70^0c in the presence of five drops of acetic acid for 4hrs. After this, the mixture was cooled and allowed to stand over night at room temperature. The crystals obtained was washed with cold ethanol and then dried in the oven at 70^0C. The product yield was determined and kept for analysis and further experiments. The equation of reaction is as shown in Figure 4.

2.4. Synthesis of First Series Transition Metal Complexes of 4-benzoyl-5-pyrazolone and 2-amino benzoic acid Schiff base (M-L)

Some first series of transition metal complexes of the Schiff base namely: Cr (III), Mn (II), Fe (II), Co(II), Ni(II) and Cu(II) complexes were synthesized by gradually adding $20cm^3$ of a solution formed by dissolving 0.005M of the metal chloride in absolute ethanol to another solution formed by dissolving 1.985g (0.005M) of the Schiff base in $20cm^3$ of absolute ethanol. The mixture was stirred at 60^oc for 2 hours under reflux using magnetic stirrer and then allowed to stand for another 30 minutes after which it was filtered to obtain the crystals produced. The product was kept for analysis after it has been dried and the yield determined.

2.5. Antibacterial Analysis

This was done by disc diffusion method. The discs were prepared by cutting Whatmann No. 1 filter paper to a size of 6mm diameter using a perforator. Solutions each having a concentration of 200mg per $1000cm^3$ were made using the Schiff base ligand and each of the metal complexes with di-Methyl Flouride (DMF) as the solvent. The whatmann's discs were soaked in each solution for 30 minutes after which they were dried and labeled accordingly. Negative control discs were also prepared by soaking the Whatmann's disc in the pure solvent for 30 minutes after which they were dried. Positive control disc, a commercially available drug disc (ciprofloxacin 500mg/disc) was procured.

Nutrient agar plates were prepared and incubated overnight to detect contamination. About $0.2cm^3$ of stock culture (*Staphylococcus aureus*, Bacillus Subtiles, *Pseudomonas aeruginosa* and *Escherichia coil*) were transferred into separate nutrient agar plates and spread thoroughly using a glass spreader.o The discs were placed on the inoculated agar plates and further incubated at 37^0C for 24 hours. Anti-bacterial activity was determined by measuring zone of inhibition.

3. RESULTS DISCUSSION

Results of the analyses are presented in Tables 1-7 below

Table 1: Physical Analysis of 1-Phenyl-3-methyl-4-benzoyl-5-pyrazolone (HPMBP), Schift Base ligand (L) and Transition Metal Complexes.

Compound	% Yield	Colour	Melting Point (^{0}C)
2-amino benzoic acid (Anthranilic acid)	92	White	146-148
4-benzoyl-5-pyrazolone and 2-amino-benzoic acid Schiff base (L)	72	Light brown	224-226
Cr (iii) complex of 2-amino-benzoic acid and 4-benzoyl-5-pyrazolone Schiff base (Cr-L)	52	Gray	272-280
Mn(ii) complex of 2-amino benzoic acid and 4-benzoyl-5-pyrazolone Schiff base (Mn-L)	74	Brown	256 – 258
Fe(ii) complex of 2-amino-benzoic acid and 4-benzoyl-5-pyrazolone Schiff base (Fe-L)	56	Brown	400 – 405 (decompose)
Co(ii) complex of 2-amino benzoic acid and 4-benzoyl-5-pyrazolone Schiff base (Co-L)	68	Light brown	254 – 256
Ni(ii) complex of 2-amno benzoic acid and 4-benzoyl-5-pyrazolone Schiff base (Ni-L)	63	Yellowhish green	302 – 306
Cu(ii) complex of 2-amino benzoic acid and 4-benzoyl-5-pyrazolone Schiff base (Cu-L)	77	Light green	710 – 715 (decompose)

Table 2: Solubility Date of the Schiff Bases and their Metal Complexes

Compound	Solvent			
	DMSO	DMF	Diethylether	N-hexane
L2	Soluble	Soluble	Insoluble	Insoluble
Cr-L	Soluble	Soluble	Insoluble	Insoluble
Mn-L	Soluble	Soluble	Insoluble	Insoluble
Fe-L	Soluble	Soluble	Insoluble	Insoluble
Co-L	Soluble	Soluble	Insoluble	Insoluble
Ni-L	Soluble	Soluble	Insoluble	Insoluble
Cu-L	Soluble	Soluble	Insoluble	Insoluble

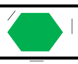
Table 3. IR spectral data of 2-amino benzoic acid (Anthranilic acid)	
Absorption peak cm-^{1}	Type of bond/functional group
3417.95	O-H bond stretch
2314.00	O-H bond
1735.99	C=O stretch
1558.54	Benzene ring stretch
1411.94	 Stretch
1149.61	C-O stretch
1041.00	C-H in plane deformation
709.83	C-H out of plane deformation

Table 4: IR Spectral Data of 4-benzoyl-5-pyrazolone (HPMBP), its Schiff base with 2-aminobenzoic acid (L) and complexes of the Schiff base ligand with some metals in the first transition series.						
	L	Mn-L	Ni-L	Co-L	Fe-L	Cu-L
O-H Stretch (cm^{-1})	3410.26					
N-H Stretch (cm^{-1})	-	3385.20	3391.00	3390.54	3388.23	3395.11
C=O Stretch (cm^{-1})	1730.20	1640.45	1645.69	1642.54	1643.41	1646.69
C=N Stretch (cm^{-1})	1627.97	1565.50	1578.21	1576.25	1570.00	1579.54
Aromatic ring Stretch (cm^{-1})	1481.38	1456.34	1461.74	1449.66	1451.38	1473.88
C-O Stretch (cm^{-1})	1141.91	1149.61	1149.91	1141.60	1149.60	1134.88
C-H plane deformations (cm^{-1})	1026.16	1033.88	1041.60	1026.16	1041.66	1041.61
C–H out of plane deformations (cm^{-1})	846.44	854.54	856.42	825.56	864.14	856.42
M–O Stretch (cm^{-1})	-	524.26	541.12	535.10	548.12	551.15
M-N Stretch (cm^{-1})	-	429.54	475.11	468.55	462.93	482.11

Table 5: Mass spectral analysis of anthranilic acid, 4-benzoyl-5-pyrazolone (HPMBP), its Schiff Base with 2-amino benzoic acid and complexes of the Schiff base with some metals in the first transition series.		
Complex	$^m/_z$ of molecular ion	$^m/_z$ of some fragment ions
Anthranilic acid	137.0	82,78,45
HPMBP	278.0	201, 124, 77
L	398.0	321, 244, 137, 77
Mn–L	451.9	372.9, 298.9, 137,77
Fe–L	453.6	376.8, 299.8, 163.8, 77
Co–L	456.7	379.7, 302.7, 167,77
Ni–L	456.7	379.7, 298.9, 137.7,77
Cu–L	460.5	383.5, 307, 137, 77
Note: m = mass; z = charge ; L = ligand		

Table 6: U.V. Spectral Analysis of 4-benzoyl -5-pyrazolone, its Schiff Base with 2-Amino-benzoic acid and complexes of the Schiff Base with some metals in the first transition series		
Compound	λMax (nm)	Absorptivity (cm^{-1})
Anthranilic acid	205	4.063
HPMBP	205	10.00
Fe-L	218	5.571
Mn-L	218	4.737
Ni-L	219	8.215
Co-L	217	5.174
Cu-L	220	8.125
L	210	4.964

Table 7: Antibacterial Activity of Schiff Base Ligands (L) and their complexes with some metals in the First Transition Series				
	Zone of inhibition against Bacterial Growth (mm)			
	S. aureus (Gram +ve)	B. subtiles (Gram +ve)	E. coil (Gram -ve)	P. aeruginosa (Gram -ve)
L	4	3	4	5
Cr – L	5	4	6	6
Mn – L	6	6	8	7
Fe – L	6	5	8	7
Co – L	5	5	8	6
Ni – L	4	4	8	7
Cu – L	4	4	8	7
Ciprofloxacin (500mg drug disc)	10	10	12	12
DMSO (negative control)	0	0	0	0

4. Discussion

Results of physical tests (Tables 1 and 2) showed that the metal complexes were generally more stable to heat than the Schiff base ligand and that both the Schiff base ligand and the metal complexes were soluble in dimethyl sulphur oxide and Dimethyl floride (which are polar solvents) but insoluble in Diethyl either and N-hexane (which are non-polar solvents).

The Ir spectral data of 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone (HPMBP; Fig 2) showed a broad peak at 3420cm^{-1} which was assigned to O = H stretching vibrations of enols (Paula, 2007).

The peak at 1735cm^{-1} was assigned to C=O stretching vibrations while the band at 1481cm^{-1} was assigned to aromatic ring stretching vibrations. The peak at 1141cm^{-1} was assigned to C – O stretch while the ones at 1055cm^{-1} and 864cm^{-1} were assigned to C – H deformations. (Okafor 1981).

The spectral data of the Schiff base ligand (Fig. 4) also showed a broad peak at 3410cm^{-1} which was assigned to O – H stretching vibrations. The band at 1735cm^{-1} was assigned to C = O stretching vibrations. A new peak was observed at 1617cm^{-1} which was assigned to C = N stretching vibrations.

In the IR spectral data of the transition metals complexes of the Schiff base (M–L) the broad peaks that occurred at the frequency range of 3380cm^{-1} to 3387cm^{-1} were assigned to the stretching vibrations of the N – H bond at the pyrazolone ring (Paula, 2007; Williams and Anthony, 1996; El-Bindary, *et al.*, 1999). The absorption band which occurred at 1735cm^{-1} in the Schiff base ligand (L) was observed at a lower frequency range of 1620 cm^{-1} to 1634 cm^{-1} showing that C = O was involved in coordination with the metal ion. Likewise the peak which occurred at 1617 cm^{-1} in the Schiff base ligand (L) shifted to lower frequencies ranging from 1558 cm^{-1} to 1669 cm^{-1} indicating that the bond C = N responsible for the peak was co-ordinated to the metal ion. Two bands, one of the frequency range of 500cm^{-1} ^{to} 400 cm^{-1}. were observed and were assigned to M – O and M – N bonds respectively (Varshney *et al.*, 2013; Hossain *et al.*, 2017).

These two peaks confirmed that C = N and C = O were involved in bonding and that both N and O atoms were coordinated to the metal ion.

Results UV analysis (Table 6) showed the wavelength of maximum absorption of the Schiff base ligand was 208nm while those of the metal complexes ranged from 216nm to 218nm. The increase in wavelength of maximum absorption could be as a result of the metal ion in the metal complex. The absorptions were attributed to $\pi \rightarrow \pi^*$ transition.

Results of mass spectral analysis of anthranilic acid showed that the value of m/z of its molecular ion was 137. This value corresponds to its molecular mass as calculated from its molecular formula ($C_7H_7NO_2$), hence its confirmation. The results also showed that m/z of the molecular ion of 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone (HPMPB) was 278. This also corresponds to the value of its molecular mass as calculated from its molecular formula ($C_{17}H_{14}N_2O_2$), hence its confirmation. The result obtained with the Schiff base ligand showed that the value of m/z of its molecular ion (398) corresponds to the mass of one mole of the ligands, this served as its confirmation. The results obtained with the metal complexes showed that each complex had m/z value of the molecular ion corresponding to the mass of one molecule of the complex. This showed that the ligand/metal mole ratio of each complex was 1:1, in other words, the complexes can be represented by the general formula, M – L, where M is the transition metal and L is the ligand.

Results of antibacterial tests (Table 7) showed that the Schiff base and its transition metal complexes inhibited the growth of *Staphylococcus aureus*, *Bacillus subtiles*, *Pseudomonas aeruginosa* and *Escherichia coil.* The results also showed that the metal complexes showed greater inhibition against the growth of the organisms tested.

5. **Conclusion**

Schiff base ligand derived from 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone and 2-amino benzoic acid has antibacterial property. It inhibited the growth of *Staphylococcus aureus*, *Bacillus subtiles*, *Escherichia coil* and *Pseudononas aeruginosa*. It also forms stable complexes with metals in the first transition series which have enhanced inhibition against the growth of bacterial organisms.

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