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Research Article

Antimicrobial activity of some pyrazolidin-3-one Schiff base derivatives and their complexes with selected metal ions

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Abstract

In spite of the fact that the structural similarity of pyrazolidin-3-one based compounds with the triazole, the antimicrobial activity of pyrazolidin-3-one compounds, have been investigated by many research groups to achieve potent antimicrobial agents. As result of the emerged drug resistance for many antimicrobial agents, we aimed in the current study to develop new potent and safe antimicrobial agents. In this study, a series of novel antimicrobial pyrazolidin-3-one based compounds and their transition metal complexes with Fe (II), Mn (II), Co(II), Ni(II), Zn(II), Cd(II), Cu(II), Pt(II) and Mo(II) ions were synthesized in good yields using microwave irradiation. All synthesized compound (free ligands and their metal complexes) were fully characterized by several spectroscopic techniques such as molar conductance, infrared, UV/Visible electronic, ^{1}H NMR and ^{13}C NMR spectra. In addition, the elemental analyses and conductivity investigations were done and combined with other spectroscopic data to determine the ligand: metal [M: L or L:M] ratio. The free unattached ligands and their complexes were screened for their in vitro antimicrobial activity against *Staphylococcus aureus* (ATCC-29213) *Escherichia coli* (ATCC-25922), and *Candida albicans* (ATCC-10231). The achieved results indicated that some complexes are more potent than their free ligands and metal ions (Fe (II), Pt (II), Co(II), and Ni(II)), and some of the complexes are more potent than the standard antifungal *amphoteracine-B*. In conclusion, the biological activity results indicated the enhancement of the antimicrobial activity of some imine derivatives of pyrazolidin-3-one will open the field for scientists to develop more effective and safe drugs.

Keywords

Antifungal activity, antimicrobial, Schiff base, complexation, 4-aminoantipyrine.

Introduction

Schiff bases and their complexes are important compounds because of their broad variety of biological activities. They have been found to posses pharmacological activities such as anticancer [1-3], antimalarial [4-6], antimicrobial[7-10], antitubercular[11,12], and anti-inflammatory [13-15]. The Schiff bases are characterized by the presence of azomethine (C-N=CHR) group and achieved by the condensation of chelation with transition aldehyde or ketone with primary amines. Owing to their selectivity, as well as the sensitivity toward the metal ions, Schiff bases have increased interest in the field of bioinorganic chemistry. In addition, they form stable complexes which have been generally recognized to serve as models for biologically important species [16-18]. It has been reported that complexes of aromatic Schiff base could catalyze some reactions as hydrolysis, oxygenation, decomposition, and electro-reduction [19,20]. In order to discover new compounds with an improved pharmacological profile, researchers oriented their work towards the complexation of Schiff bases, where some of them showed increased activity upon

chelation with metal ions. The Schiff bases of 4-aminoantipyrine and their complexes are recognized for their variety of therapeutic intentions as they were reported to have anti-inflammatory, analgesic, antiviral, antitumor, antifungal and antibacterial properties [21-26].

The investigation has been done for deoxyribonucleic acid cleavage and the antimicrobial activity of transition metal complexes of 4-aminoantipyrine Schiff base with 3-hydroxy-4-nitrobenzaldehyde, where the metal complexes found to have higher antimicrobial activity than the ligand itself [19,20]. The copper complexes cleave DNA and other complexes are not effective. In the same manner, the synthesis and antimicrobial activities of metal complexes of Schiff base derived from 4-aminoantipyrine, salicylaldehyde and o-phenylenediamine were reported, and most of these complexes have superior antibacterial and antifungal activities in comparison with the free ligand [19,20].

Potentiating of the antimicrobial activity of Schiff bases by metal chelation has been synthesized by the condensation of 4-aminoantipyrine with benzaldehyde and were tested biologically. The biological activity were weak with the ligand alone but were increased significantly when were complexed with metals [27,28]. Recently, salicylaldehyde and α-aminoindole-3-propionic acid were used to synthesize new Schiff base with 4-aminoantipyrine and series of transition metal complexes were reported and their biological activities were investigated against the tested microbial strains reveals, that they showed improved biological activity when compared to the free ligands [29]. Leelavathy and Arul have synthesized metal(II) complexes from 2-aminobenzothiazole and furfurylidene-4-aminoantipyrine and biologically screened against bacterial species *K. pneumoniae, S. aureus, P. vulgaris, P. aeruginosa* and *E. coli* and fungal species *R. stolonife,r A. niger, R. bataicola, C. albicans and A. flavus* and they found that the DNA binding and cleavage activity of the ligand is less than its complexes [30]. In addition, the metal complexes of cobalt(II), nickel(II), copper(II) and zinc(II) have been isolated with ligand derived from the condensation of 4-amino-3-mercapto-6-methyl-5-oxo-1,2,4-triazine with 2-acetylpyridine (L(1)) had marked antimicrobial effect than of the free ligand [31]. Since the quality research always demands thoughtfully framed sample and data collection techniques. In the current paper, two 4-aminoantipyrine based compounds and their transition metal complexes (Fe (II), Pt (II), Co (II), Cu (II), Ni (II), Zn (II), Mo (II), Cd (II), and Mn (II) ions) were synthesized using microwave, and their antimicrobial activity were investigated.

Experimental protocols

Materials and solvents

The solvents and chemicals that are used in this chemical synthesis of Schiff bases and their complexes were purchased from Fluka analytical company, UK or Sigma/Aldrich chemical company, UK. They were of highest purity and used without further purification. Charcoal system and ion exchange (Millipore Ltd, UK) were used to distil and purify the water, which is doubly distilled, for chemical and biological procedures. Double distilled water was always used. Phosphate buffer was prepared from high grade analytical reagent materials in concurrency to standard formulas. Most experiments were performed using 0.01M phosphate buffer (pH 7.4) containing 0.1M NaCl.

Instruments

Microwave closed system (milestone start E-2450 MHz, Italy) was used to perform synthesis of all compounds. TLC (Thin layer chromatographic) analysis was carried out on pre-coated aluminium plates (Fluka analytical, UK). Thin layer chromatographic spots were visualized with both long (366 nm) and short (254 nm) UV light. Melting points were determined using Electrothermal SMP-30 melting point apparatus (Stuart, UK) in open capillary tubes. A bench-top L and R transistor/ultrasonic bath were utilised to assist dissolution of various compounds. Values of pH were determined using a Hanna-instruments (code HI-9321) microprocessor pH meter, standardized with standard buffers obtained from Sigma-Aldrich, UK at 20°C. Perkin-Elmer 240C (USA) elemental analyser was utilised to perform the elemental analysis of the Schiff base ligands and their metal complexes. The infra-red spectra of samples were recorded by Varian FT-IR spectrophotometer-660 (Varian, Australia) in the region of 4000-400 cm^{-1}. UV/Visible electronic spectra were recorded spectrophotometrically using Cary 5000 UV/Visible/ spectrophotometer (Varian, Australia), in 1cm quartz *cuvettes*. The molar conductance measurements carried out on a BC-3020 Professional Bench-Top Conductivity Meter and measured in DMSO solutions. ^{1}H-NMR and ^{13}C-NMR spectra (400 MHz) recorded by Bruker Advance 400 MHz NMR Spectrometer (Bruker, France) in dimethyl sulfoxide using tetramethylsilane (TMS) as an internal standard. The following abbreviations for the NMR exploited are: singlet (s), doublet (d), triplet (t), quartet (q), multiple t(m).

Procedure for microwave synthesis of free ligands; L^1 and L^2

Equimolar quantities (0.01 M) of both starting materials (aldehyde and4-aminoantipyrine) were weighed and triturated in clean and dry Teflon vessels to form homogeneous mixture. The reaction mixture was exposed to microwave irradiation at 350-600 Watt power for around 0.5-2 minutes with maximum heating of sixty degree centigrade. The best reaction time was established based on the reaction completion utilising thin layer chromatography and the suitable solvent system. The reaction mixture was left to cool and then the crude product mostly solid was gathered by vacuum filtration and extracted with three volumes of chloroform (3x50 ml). Then the product was dried over anhydrous magnesium sulphate, evaporated and finally recrystallized from proper solvent. The attained crystals were completely dried and their melting points were allocated. The chemical purity of the products was determined using Waters HPLC (UK) using mobile phases of chloroform/ methanol

(80:20) or acetonitrile/methanol (90:10). The chemical structure for all the synthesized products were explicated using numerous techniques chiefly; ^{1}H-NMR, ^{13}C-NMR, FT-IR, and UV/visible spectrophotometries.

4-((2-Hydroxybenzylidene)amino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one (L^1)

Compound L^1 has been synthesized by subjecting a mixture of 4-aminoantipyrine and salicylaldehyde to irradiation using microwave at 350-Watt power for 1 minute. The pure crystals of MCS04 were attained following three recrystallizations of the crude product from a mixture of ethanol hexane (9: 1). FT-IR (cm-1): 3271 (OH, str.), 2861 (C-H aromatic), 1610 (C=N, str.), 1692 (C=O, str.), 1584 (C=C aromatic). ^{1}H-NMR (400 MHz, DMSO-d6): δ [ppm] 9.22 (s, 1H, HC=N), 8.32 - 7.21 (m, 9 H, Ar-H), 2.28 (s, 3H, *CH3-C=C), 3.67 (s, 3H, CH_3-N),. ^{13}C-NMR (400 MHz, DMSO-d6): δ [ppm] 164.43 (1C, *C=N), 159.25 (1C, C=O), 162.25 (1C, Ar, C-OH), 152.37 (1C, pyrazolone ring, CH=*C-N), 140.61 (1C, Ar, C-N), 133.65 (1C, Ar), 130.02 (1C, Ar), 128.86 (2C, Ar), 126.45 (2C, Ar), 123.23 (1C, Ar), 119.72 (1C, Ar), 120.73 (1C, Ar, *C-C=N), 118.16 (1C, Ar), 109.84 (1C, pyrazolone ring, *CH=C-N), 40.52 (1C, CH3-N), 16.47 (1C, CH3). M.P = 221-222oC, % Yield = 85-90 %.

4-(((2-Hydroxynaphthalen-1-yl) methylene) amino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one (L^2)

Compound L^2 has been synthesized by subjecting a mixture of 4-aminoantipyrine and 2-hydroxynaphthaldehyde irradiation using microwave at 600-Watt power for two minutes. The pure crystals of MCS02 were achieved after three times of recrystallization of the crude product from ethanol hexane mixture (7:3). FT-IR (cm-1); 3392 (OH, str.), 2860 (C-H aromatic), 1647 (C=O, str.), 1635 (C=N, str.), 1583 (C=C aromatic). ^{1}H-NMR (400 MHz, DMSO-d6): δ [ppm] 9.32 (s, 1H, HC=N), 8.22-7.16 (m, 11H, Ar-H), 2.28 (s, 3H, *CH_3-C=C), 3.67 (s, 3H, CH_3-N). 13C-NMR (400 MHz, DMSO-d6):δ[ppm] 169.63 (1C, Ar, C-OH), 159.25 (1C, C=O), 164.21 (1C, *C=N), 152.37 (1C, pyrazolone ring, *CH-C=N), 138.81 (1C, Ar, C-N), 133.65 (1C, Ar), 134.34 (1C, Ar), 130.02 (1C, Ar), 129.45 (1C, Ar), 128.86 (2C, Ar), 127.35 (1C, Ar), 128.01 (1C, Ar), 126.45 (2C, Ar), 123.23 (1C, Ar), 119.72 (1C, Ar), 121.87 (1C, Ar), 115.47 (1C, Ar, *C-C=N), 109.84 (1C, pyrazolone ring, *CH=C-N), 40.52 (1C, CH3-N), 16.47 (1C, CH3). M.P = 230-231oC, % Yield = 85-95 %.

Microwave methods for the synthesis of metal ions complexes

The metal complexes were synthesised by weighing and triturating of equimolar amounts (0.01M) of Schiff base ligand and divalent sulphate or chloride or nitrate metal salts of Fe (II), Mn (II), Co (II), Ni (II), Zn (II) Cu (II), and Cd (II) using dry and clean Teflon vessels. The reaction mixture was microwaved at 600 - 800 Watt for about three to five minutes utilising 0.5 ml 100% ethanol as a solvent. The best reaction time was allocated depending on reaction completion and this confirmed by using TLC with appropriate solvent systems. The obtained crude product was washed many times with ether and ethanol and eventually recrystallized with pure ethanol.

Antimicrobial activities testing

The antimicrobial activity (antibacterial and antifungal) of the entire synthesized Schiff base ligands and their complexes were tested utilising agar diffusion method against numerous strains of microorganisms. *Staphylococcus aureus* (ATCC-29213) and *Escherichia coli* (ATCC-25922), *Candida albicans* (ATCC-10231) who were identified and obtained based on the American type of cell culture collection (ATCC) at the concentration level of 5 µM. Standard manufacturer's procedure was used to prepare the nutrient agar. The synthesized Schiff bases (ligands) and their complexes were dissolved in DMSO and introduced into the inoculated agar following sterile procedure, which then incubated overnight at 37°C. Standard antibacterial (ciprofloxacin) and antifungal (ketoconazole) were used as references and the resulting inhibition zones (diameter, mm) were recorded as listed in Table 4. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of experimental compounds that lead to a decrease in absorbance compared with that of the control (without experimental compounds). The MIC was determined *in vitro* in triplicate by broth dilution method [32] and average of them was considered. The reference drugs used were Ciprofloxacin as an antibacterial standard and fluconazole as an antifungal standard as shown in Figure 5.

Statistical analysis

All biological experiments were conducted in triplicate and data are obtainable as mean values ± standard deviation of at least triplicate determination. Analysis of variance (ANOVA) followed by Dunet's test were used to allocate the significant difference between samples using SPSS version 13 and p value should be less than <0.05.

Results and Discussion

Synthesized Chemicals

Microwave irradiation is highly effective heating source in chemical reactions, and has been shown to accelerate the reaction rate, significantly reduce reaction times and increase product yields. In addition, it has been reported that microwaves assisted synthesis could enhance the product purities and offer better reproducibility of the reactions via the uniform and selective heating compared to conventional heating methods. The advantages mentioned above for the microwave assisted synthesis have been widely exploited in the organometallic chemistry, early in 1991, the microwave synthesis of organometallic stannous complexes was reported with shorter reaction time[33-36]. Comparison of microwave assisted synthesis of manganese(II) complexes of some amide containing ligands with conventional method has been reported, the microwave method is easier, convenient and offered improved yield [37]. Fast and efficient synthesis of Schiff

bases and transition metal ions complexes for benzenetetracarboxylic acid using microwaves assisted method has been reported [38]. Recently, the description of the microwaves synthesis preformed for some essential metal ions complexes with 4-aminoantipyrine based ligands with significant reduction in the reaction time, improved product yields and enhanced purity of the final products [39].

In the present work, the synthesis of L^1 and L^2 Schiff bases derivatives of 4-aminoantipyrine and their complexes is shown in scheme-1 with high yields of the final product (86-97%); the proposed structures of the L^1 and L^2 complexes are presented in (Figure 1). The physical properties (melting point, solubility and colour) and the analytical data for the free ligands L^1 and L^2 and their complexes are as shown in Table-1 and Table-2, respectively. The formation of 2:1 ratio [L:M] where concluded based on the elemental analysis of the Schiff bases and their complexes. The metal complexes are non-hygroscopic, stable at room temperature and soluble in DMSO and methanol but insoluble in water and ethanol. The analytical data of the metal complexes indicated the formation of the targeted compounds of general formula $[ML_2]X$, where M = Fe(II), Mn(II), Ni(II), Co(II), Cu(II), Zn(II), Mo(II), Cd(II) and Pt(II); L = Ligand; X = 2Cl, SO_4 or NO_3 this achievement were based on molar conductance, infrared, UV/Visible electronic and ^{13}C-NMR spectra and ^{1}H-NMR.

The obtained molar conductance values were relatively low for most complexes (8-19 Ohm-1cm2mol-1) by using DMSO as solvent which are shown in (Table-1) and (Table-2), which indicates the existence of an electrolytic behaviour of the complexes.

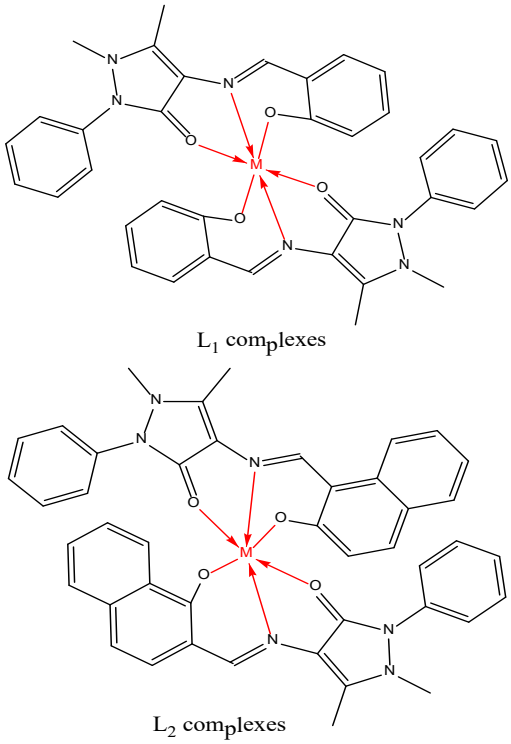
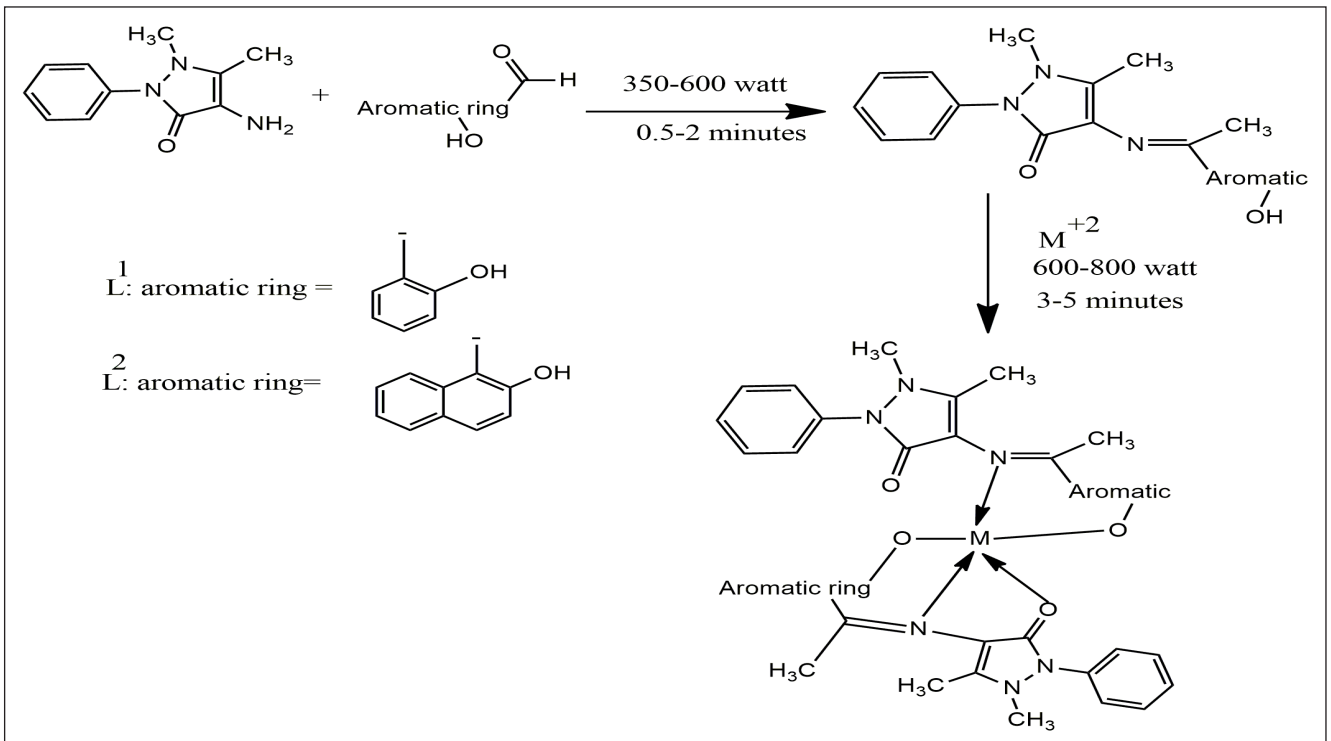


Figure-1: Proposed structures of the L^1 and L^2 complexes (metal ligand interaction appears in red colour).



Scheme-1: Microwave assisted synthesis of L^1 and L^2 Schiff bases derivatives of 4-aminoantipyrine and their complexes

Ligand / complexes	Colour	Yield (%)	MP °C	Found (Calcd) (%)			Molar conductance
				C	H	N	$Ohm^{-1}cm^2mol^{-1}$
L^1	Red	90 %	121 - 222 °C	70.34 (70.11)	5.58 (5.43)	13.67 (13.55)	-
$[Mn(L^1)_2]Cl_2$	Pale brown	88 %	168 - 169 °C	59.67 (59.38)	4.73 (4.98)	11.60 (10.93)	8
$[Fe(L^1)_2]Cl_2$	Dark red	94 %	111 - 112 °C	59.53 (59.31)	4.72 (4.98)	11.57 (10.92)	19
$[Co(L^1)_2]Cl_2$	Red	97 %	116 - 117 °C	59.02 (59.08)	4.68 (4.96)	11.47 (10.88)	14
$[Ni(L^1)_2]Cl_2$	Green	94 %	179 - 180 °C	59.06 (59.09)	4.68 (4.96)	11.48 (10.88)	9
$[Cu(L^1)^2]Cl_2$	Brown	94 %	238 - 239 °C	58.29 (58.72)	4.62 (4.93)	11.33 (10.81)	10
$[Zn(L^1)_2]Cl_2$	Pale brown	94 %	235 - 236 °C	58.00 (58.58)	4.60 (4.92)	11.27 (11.00)	12
$[Cd(L^1)^2]Cl_2$	yellow	92 %	168 - 169 °C	55.50 (55.25)	4.08 (4.64)	10.01 (10.79)	11

Table-1: Physical data, elemental analysis and molar conductance of the Schiff base (L^1) ligand and its complexes

Ligand / complexes	Colour	Yield (%)	MP °C	Found (Calcd) (%)			Molar conductance
				C	H	N	$Ohm^{-1}cm^2mol^{-1}$
L^2	Red	96 %	230 - 231 °C	73.93 (73.61)	5.36 (5.00)	11.76 (11.53)	-
$[Mn(L^2)_2]Cl_2$	Pale brown	92 %	215 - 216 °C	64.08 (63.60)	4.64 (4.87)	10.19 (9.67)	13
$[Fe(L^2)_2]Cl_2$	Dark red	93 %	228 - 229 °C	63.47 (63.53)	4.60 (4.87)	10.09 (9.66)	16
$[Co(L^2)_2]Cl_2$	Red	89 %	260 - 261 °C	63.50 (63.31)	4.60 (4.85)	10.10 (9.63)	10
$[Ni(L^2)_2]Cl_2$	Green	87 %	266 - 267 °C	62.77 (63.33)	4.55 (4.85)	9.98 (9.63)	18
$[Cu(L^2)^2]Cl_2$	Brown	91 %	247 - 248 °C	62.50 (62.98)	4.53 (4.83)	9.94 (9.58)	17
$[Zn(L^2)_2]Cl_2$	Pale brown	91 %	234 - 235 °C	62.24 (62.84)	4.08 (4.82)	8.94 (9.56)	19
$[Cd(L^2)^2]Cl_2$	yellow	84 %	241 - 242 °C	59.83 (59.65)	4.47 (4.57)	8.61 (9.07)	12

Table-2: Physical data, elemental analysis and molar conductance of the Schiff base ligand (L^2) and its complexes

IR spectra and mode of bonding

The infrared spectra of the L^1 and L^2 ligands as shown in the Table-3, exhibited a broad medium intensity stretching bands around 3271 and 3392 cm^{-1} (phenolic hydroxyl group, Ar–OH), characteristic stretching bands at 1610 and 1635 cm^{-1} (imine group, C=N) and strong bands at 1692 and 1647 cm^{-1} (carbonyl group of pyrazolone ring, C=O) of L^1 and L^2 respectively. All infra red spectra of the synthesized complexes showed significant shift in the absorption of the three mentioned groups which indicate their involvement in the formation of coordinate bonds with metals. The reduction of double bond nature of the carbon-nitrogen bond of the imine group leads to lower or higher shift in the frequencies of their bands at (1625-1593

cm^{-1}) and (1649-1622 cm^{-1}) for L^1 and L^2 complexes. The stretching frequency of phenolic hydroxyl groups of all complexes are shifted to higher regions (L^1 = 3283-3326) cm^{-1} and L^2 = 3436-3462 cm^{-1}) in comparison with the free ligands. Additionally, participation of phenolic oxygen (C–O) in the coordination with the metal is supported by the shifting its bands in all complex's spectra to higher frequencies (1374-1394 cm^{-1}). Broad band appears in metal complexes spectra (2931-3419 cm^{-1}), which states the existence of water molecules in the complexes crystals which may overlap with the position of the hydroxyl group peaks. The sharp strong peak of the carbonyl group of pyrazolone ring which appears at 1692 and 1647 cm^{-1} in the free L^1 and L^2 Schiff base ligands spectra respectively, was shifted to lower or higher frequencies (L^1 = 1675-1719 cm^{-1}) and L^2 = 1626-1683 cm^{-1}) in the entire complexes, which indicates the participation of the carbonyl oxygen in the coordination of the complex. Presence of new weak band in the IR spectra of L^1 complexes at 472-518 cm^{-1} and 419- 452 cm^{-1}, which are distinctive to the structure of M-N and M-O coordination bond in these complexes. For L^2 complexes, these coordination bonds appear in the region 513 to 536 cm^{-1} and 419 to 434 cm−1 respectively.

UV/Visible electronic spectra

The UV/Visible spectra of the L^1 and L^2 Schiff base ligands and their metal complexes are recorded in DMSO at the wavelength in the range 200-800 nm. The UV/Visible spectra of L^1 and L^2 showed three bands at 317, 336 and 343 nm which may be attributed to $n\rightarrow\pi^*$ and $\pi\rightarrow\pi^*$ transitions. The incorporation of the active groups (carbonyl, azomethine and the hydroxyl group) in the formation of complexes may leads to a shifting of the three bands to higher wavelength (328-336, 348-352 and 368-372 nm). Additional bands were detected in the UV/Visible spectra of the complexes at display bands at 421-427, 482-490 and 528-533 nm; they may be attributed to the electronic transition within the complexes of d→d type.

^{1}H-NMR and ^{13}C-NMR spectra

^{1}H-NMR spectra of the L^1 and L^2 Schiff base ligands and their metal complexes have four types of protons as shown in the experimental part of this paper, azomethine group is the most affected with the complexation as it was detected as singlet peak at 9.22 ppm and 9.32 ppm for free L^1 and L^2 Schiff base ligands respectively. Downfield shift of azomethine peak chemical shifts were observed in all complexes which may be attributed to the deshielding of the nitrogen atom electrons. ^{13}C-NMR spectra showed the peaks of both carbons of azomethine and carbonyl at 164 ppm and 159 ppm for both ^{L1} and L^2 ligands, these are the most affected peaks upon the complex formation and shifted downfield in all complexes spectra.

Antimicrobial activity

The achieved biological results which showed the zone of inhibition in mm for the free L^1 and L^2 free Schiff bases and their complexes are listed in Table 4. The entire synthesized compounds were screened for both antibacterial and antifungal activity at concentration 5 µM against one Gram (-) bacterial strain (*Escherichia coli*) and one Gram (+) bacterial strain (*Staphylococcus aureus*). In addition to the antibacterial screening the synthesized compounds were also screened on fungi strain called *Candida albican*) utilising agar diffusion method.

In general, both L^1 and L^2 ligands showed lower activity against all tested bacteria and fungi than standard drugs (Ciprofloxacin and Ketoconazole), while the metal complexes are more active compared to the parent ligands and standard drugs. Comparing the antimicrobial results of the free ligands and the synthesized complexes showed that complexation of the ligand with metals has enhanced their antimicrobial potential and the P value was <0.05 indicating a significant statistical difference between the free ligands and the synthesized complexes.

Free ligand/ Metal ions	IR (cm^{-1})) for L^1 complexes				IR (cm^{-1})) for L^2 complexes					
	OH	C=N	C=O	M-N	M-O	OH	C=N	C=O	M-N	M-O
L	3271	1610	1692			3392	1635	1647		
Mn	3310	1613	1693	492	436	3439	1636	1650	513	419
Fe	3323	1619	1711	472	419	-	-	-		
Co	3296	1625	1684	498	440	3451	1649	1636	521	423
Ni	3299	1598	1675	506	446	3441	1638	1626	530	427
Cu	3283	1593	1719	496	438	3436	1622	1683	527	421
Zn	3326	1603	1691	518	452	3462	1629	1645	536	434
Cd	3321	1617	1705	502	442	3446	1642	1668	528	425
Mo	3290	1596	1698	489	430	-	-	-		
Pt	3319	1622	1702	511	447	3452	1640	1676	533	428

Table-3: Infrared data (cm^{-1}) of the L^1 and L^2 ligands and their metal complexes

Free ligand/ Metal ions	L^1 complexes*			L^2 complexes*		
	E. coli ATCC-25922	S. aureus ATCC-29213	C. albicans ATCC-10231	E. coli ATCC-25922	S. aureus ATCC-29213	C. albicans ATCC-10231
L	23	19	18	27	24	21
Mn	26	23	19	29	27	26
Fe	20	16	12	25	23	20
Co	30	24	22	36	26	24
Ni	22	21	18	27	26	21
Cu	25	28	24	28	33	28
Zn	27	25	27	29	29	29
Cd	29	27	24	33	28	26
Mo	28	27	21	34	30	27
Pt	23	20	26	31	22	30
Ciprofloxacin	31	26	-	31	26	-
Ketoconazole	-	-	23	-	-	23

***P<0.05**

Table-4: Biological activity results in terms of diameter zone of inhibition in mm of the L^1 and L^2 ligands and their metal complexes

Free ligand/ Metal ions	L^1 complexes (MIC_{50} µM)			L^2 complexes (MIC_{50} µM)		
	E. coli ATCC-25922	S. aureus ATCC-29213	C. albicans ATCC-10231	E. coli ATCC-25922	S. aureus ATCC-29213	C. albicans ATCC-10231
L	0.07 ± 0.003	2.20 ± 0.003	0.042 ±0.008 µM	0.071 ± 0.004	1.52 ± 0.004	
Mn	0.068± 0.006µM	2.03± 0.006	0.039 ±0.008	0.070± 0.005	2.33± 0.10	0.024 ±0.007
Fe	0.082± 0.002µM	2.35± 0.002	0.041 ±0.007	0.082± 0.002	2.35±0.021	0.041 ±0.005
Co	0.061± 0.004	1.32± 0.004	0.029±0.007	0.064± 0.004	1.32± 0.004	0..007
Ni	0.079± 0.008	2.30± 0.008	0.030 ±0.002	0.082± 0.008	2.00± 0.012	0.032 ±0.004
Cu	0.072± 0.002	1.52± 0.002	0.028±0.002	0.074± 0.003	1.52± 0.022	0.029 ±0.002
Zn	0.071± 0.007	1.82± 0.007	0.027 ±0.007	0.073±0.007	1.35±0.021	0.027±0.004
Cd	0.064± 0.005	1.94± 0.005	0.029 ±0.002	0.066±0.004	1.42± 0.011	0.021 ±0.005
Mo	0.063± 0.010	1.96± 0.010	0.031 ±0.008	0.060± 0.011	1.52±0.12	0.030±0.008
Pt	0.091± 0.002	1.42± 0.002	0.029 ±0.009	0.071± 0.006	1.43± 0.012	0.018±0.004
Ciprofloxacin	0.06 ± 0.004	1.62±0.004	-	0.06 ± 0.004	1.62±0.004	-
Ketoconazole	-	-	0.026 ±0.002	-	-	0.026 ±0.002

Table-5: Biological activity results in terms MIC50 µM of the L^1 and L^2 ligands and their metal complexes

The enhancement was more pronounced in case of Cd (II) and Co (II) against *Escherichia coli*, Cu (II) and Mo (II) against *Staphylococcus aureus*, and Pt (II) and Zn (II) against *Candida albicans*.

These differences in the biological activities of the metal complexes for different metals could be related to the nature of the metal ions and the donor sequence of the ligands. In addition, different ligands exhibit different biological properties and the complexes of each metal could adopt a distorted octahedral geometry around the metal ion. The polarity of the metal ion will be reduced upon the complexation due to the partial sharing of positive charges with donor groups that increases the lipophilicity of the complexes. The enhanced lipophilicity increases the diffusion of the complexes through the lipid membranes and consequently blocking the allocated metal binding sites in the targeted enzymes of the microorganisms [39]. *Abdur et al.* have synthesized many Schiff base, 3-(((4-chlorophenyl)imino)

methyl)benzene-1,2-diol (HL1) and Schiff base 3-(((4-bromophenyl)imino)methyl)benzene-1,2-diol (HL2) and both of the Schiff bases were used to synthesize their zinc (II) and cobalt (II) complexes. They screened these compounds for enzyme inhibition, cytotoxic and antibacterial activities. In addition, they tested them for in vivo antidiabetic activities and they found some of these compounds were active. Their results indicated that Zn Schiff base complex was a good inhibitor of alkaline phosphatase enzyme and possess highest potential against blood cholesterol level, diabetes and cancer cells. The results obtained by the *Abdur et al*. confirmed the biological properties [40].

Conclusion

The enhancement of the antimicrobial activity of Schiff bases derivatives of 4-aminoantipyrine by metal chelation has been studied. Two structurally related Schiff base ligands (L^1 and L^2) have been synthesized by the condensation of salicylaldehyde and 2-hydroxynaphthaldehyde with 4-aminoantipyrine. Several divalent metal complexes were also synthesized based on both L^1 and L^2 ligands. FT-IR, molar conductivity, UV/visible, ^{1}H-NMR and ^{13}C-NMR are the main spectroscopic tools used for the structure elucidation of the free ligands and their complexes. The ligands act as tridentate donor by using their carbonyl, hydroxyl and azomethine moieties as binding sites for the metals. The octahedral structures were proposed for most complexes, while the Cu (II) complex showed tetragonal geometry. The biological activity investigation shows the enhancement of the antimicrobial activity of the free ligands and the synthesized complexes against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans via* complexation.

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