Antimicrobial Activities Of Synthesized Zinc(II) Mixed-Ligand Complexes Derived From 2-Acetylpyridine-4-Phenylsemicarbazone And Nitrogen-Sulphurmonodentate Ligands

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Abstract: The reaction of zinc chloride with 2-acetylpyridine-4-phenylsemicarbazone and nitrogen–sulphurmonodentate ligands such as thiophene, pyridine, picoline ,aniline and ammonia; yielded five novel mixed -ligand complexes: Zn(2-Ac.4-Psc.Th)Cl_2 ,Zn(2-Ac.4-Psc.Py)Cl_2, Zn(2-Ac.4-Psc.Pi)Cl_2, Zn(2Ac.4-Psc.An)Cl_2 and Zn(2-Ac.Psc.Am)Cl_2. These were characterized by elemental analysis,molar conductivity,^{1}H and ^{13}C-NMR, IR and electronic absorption spectroscopic studies.All the complexes possess tetrahedral geometries. The antimicrobial activities were evaluated against *staphylococcus aureus, Bacillus anthracis,Aspergillusnigers* and*Candida albicans*.The result of significant inhibition of growth and proliferation of these microbes by the chelates were obtained particularly with the highest efficiency shown by thethiophene and aniline incorporated complexes

Keywords: mixed ligands, Zinc(II) 2-acetylpyridine-4-phenylsemicarbazone, complexes, semicarbazone, tetrahedral geometry, antimicrobial, activity,

Introduction

Semicarbazones, thiosemicarbazones and their derivatives have aroused much interest in recent times due to their versatility and their interesting pharmacological properties as antibacterial, antifungal(13,6) antivirial, antiamoebic, antimalarial, antitumor, anticonvulsant (14), anti-inflammatory , antineoplastic and corrosive inhibitory activities (15). The chemistry of semicarbazones and thiosemicarbazones has been extremely active and extensively investigated because of these chemotherapeutic and anti-corrosive activities. Secondly, the ease of preparation, diversity in geometrical configurations also added to their valuable applications. As is the case in many structure-activity relationship studies, modifications of the parent molecular structure with various active substituents at the different positions of the moiety and the type of metal ion incorporated in the complex (11, 18) significantly alter the configuration and properties. These alterations often times are done to enhance the therapeutic potency and efficiencies as drugs.

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Though understanding the exact mechanism of the antimicrobial activities of these drugs are yet to be completely ascertained, presently some evidences exist of a correlation between hypophilicity (i.e ease of transport across cell membrane) and the degree of antimicrobial activity in an organism (10). This is deduced from the results of the interaction of the complex with the DNA of the cell, which is known to block it out of normal metabolic activities within the cell. Also the antimicrobial phenomenon can be as a result of interaction of the complex with the cell membrane only (2, 14, 19). All these seem to be more dependent on the metal ions and the type of microbes. This paper describes the synthesis, characterization of mixed ligand complexes of Zinc 2-acetylpyridine 4-phenylsemicarbazones with nitrogen- sulphurmonodentated ligands with particularreference to the antimicrobial activity against *Staphylococcus aureus, Bacillus anthracis, Aspergillusnigers* and *Candida albicans*.

Experimental

Analytical grade chemicals were used. Carbon, hydrogen and nitrogen analyses were obtained using Perkin Elmer PE 240 automatic elemental analyzer. Molar conductivity measurements in DMF solvent were done using a systronic direct reading conductivity bridge with a conventional dip-type black electrode. IR spectra were obtained from a FT-IR Perkin-Elmer 1600 spectrophotometer. ^{1}H and ^{13}C-nmr spectra of the ligand were recorded on a Joel 270 MHz spectrometer with TMS as internal infernal reference.

Preparation of the ligand 4-phenylsemicarbazide

(0.01mole) was dissolved in aqueous ethanol and treated with 5 drops of acetic acid. 2-acetylptridine (0.01mole) was added drop by drop while stirring. The mixture was boiled under reflux for 30minutes and later cooled on ice bath. The precipitate formed was filtered, washed with ethanol and dried in a dessicator. The yield was 82.01%.

Preparation of Mixed Ligand Complexes

2-Acetylpyridine 4-phenylsemicarbazone (0.005mole) was dissolved in ethanol and zinc chloride (0.005moles) also

dissolved in ethanol were mixed together and boiled under reflux for 20 minutes. 20ml of the secondary ligand like ammonia was added and boiled under reflux for 2 hours. At the end of the time, the solution was left to stand overnight. The precipitate formed was filtered with ethanol and dried in adessicator. The process was repeated with thiophene, picoline, aniline and pyridine as secondary ligands. The yield of 66.04% for thiophenecomplex, 45.61% for pyridine complex, 43.48% for picoline complex, 47.83% for aniline complex and 79.84% for ammonia complex were obtained.

Antimicrobial Activity Test

The primary ligand and the mixed ligand complexes were all tested for possible inhibitory action against the growth of two bacteria- *Staphylococcus aureus, Bacillus anthracis,* and two fungi-*Candida albican and Aspergillusniger* using the paper disc method and broth dilution method. The synthesized compounds in DMF solution were applied *in vitro* by paper disc method. All materials used were sterilized in a hot air oven and the colony of each of the tested micro-organisms were subcultured and incubated for about 8 hours before introducing into agar plates (DifcoLabs, Detroit-USA). The discs (7.0mm diameter) were soaked with different test samples (concentration 1000µg/ml) drained and then placed on the agar plate using sterilized forcept. The plates were incubated at 37^{oC} for 48hours. At the end of the incubation period, the zones of inhibition of the different bacteria were carried out by double serial dilution containing 500, 250,125, 62.5, 31.25, 15.625, 7.81, 3.91 and 1.95µg/ml of the test compounds. Ampicillin was used as a reference standard. The antifungal test was done by using broth dilution method for *Candida albican* and agar diffusion method for *Aspergillusniger. Candida albicans* was grown in dextrose broth at 37^{oC} for 48hours. The inoculation was done by suspending the cells in the medium in order to obtain the final concentration. The stock solutions were prepared by dissolving the synthesized compounds, 25mg of each in 1ml of DMF and diluting the solution with 5ml of the dextrose broth. Nistatine was used as the reference.

Table 1: Physical properties and elemental analysis

Compound	Colour	M.pt ^0C	Molar conductivity ($Ohm^{-1}.cm_2.mol^{-1}$)	%C found (calcd)	%H found (calcd)	%N found (calcd)
2-Ac-4-Psc	Pale Yellow	280				
Zn[2-Ac-4-Psc Th]Cl_2	Pale Yellow	290	173.3	48.38 (48.79)	4.85 (5.10)	20.16 (19.96)
Zn[2-Ac-4-Psc Py]Cl_2	Brown	285	174.6	48.38 (48.59)	4.68 (5.10)	20.16 (19.96)
Zn[2-Ac-4-Psc Pi]Cl_2	Yellow	310	177.3	48.10 (48.31)	5.18 (4.82)	20.11 (20.08)
Zn[2-Ac-4-Psc An]Cl_2	Light brown	295	178.1	50.11 (49.82)	4.78 (4.10)	19.38 (19.85)
Zn[2-Ac-4-Psc Am]Cl_2	Yellow	290	175.6	36.86 (37.13)	4.99 (5.10)	25.05 (24.98)

Table: 2. Antimicrobial Activity

Zone of Inhibition; 1000 µg/ml)
(Minimum Inhibitory Conc. µg/ml)

Compound	***S.aureus***	***B.anthracis***	***C. albican***	***A. nigers***
Zn[2-Ac-4-Psc,Th]Cl_2	15.50 (125)	13.00 (250)	14.00 (500)	13.00 (125)
Zn{2-Ac-4-Psc.Py]Cl_2	----	16.00 (500)	5.00 (1000)	--
Zn[2-Ac-4-Psc.Pi]Cl_2	15.00 (31.25)	--- --	----- ---	--- --
Zn[2-Ac-4-Psc.An]Cl_2	17.00	11.00	12.00	6.00
Zn[2-Ac-4-Psc.Am]Cl_2	---	8.00 (1000)	9.00 (500)	17.00 (62.50)
Ampicillin	25.50 (500)	16.60 (250)	13.80 (500)	12.00 (500)

Line indicates that compound was not active

Results and Discussion

The physical and elemental analysis data for the compounds are listed in Table 1. All the analytical data are in good agreement with the empirical formulae of the synthesized compounds. The complexes are stable in air. They have varied colours and are soluble in common coordinated solvents such asdimethylsulfoxide, acetone, dimethylformamide etc. Their molar conductivity taken in DMSO solution indicates a 1:1 electrolytic nature

IRSpectra

Selected vibration bands of ligand and its metal complexes are given in Table 2. These bands provide insight into the bonding mode of the ligand to metal ion. Assignments are based on typical frequencies characterizing absorptions of -NH_2, C=N, N-N and C=O groups respectively. The ligand coordinates through NNO sites indicated by the pyridine ring experiencing shift in the stretching bands to lower frequencies and ring breaking C-H + and (ring) all shifted to higher wave numbers (3). The strong and broad absorption in the 3220-3490cm^{-1} observed in the ligand attributed to vOH is found to disappear on complexation. The vC=O and vC-N +NH bands are found at 1680 cm^{-1} and 1270 cm^{-1} in the ligands. In the solid state the ligand exist in the keto form but in solution it forms an equilibrium mixture of both keto and enol forms (fig.1) (19).

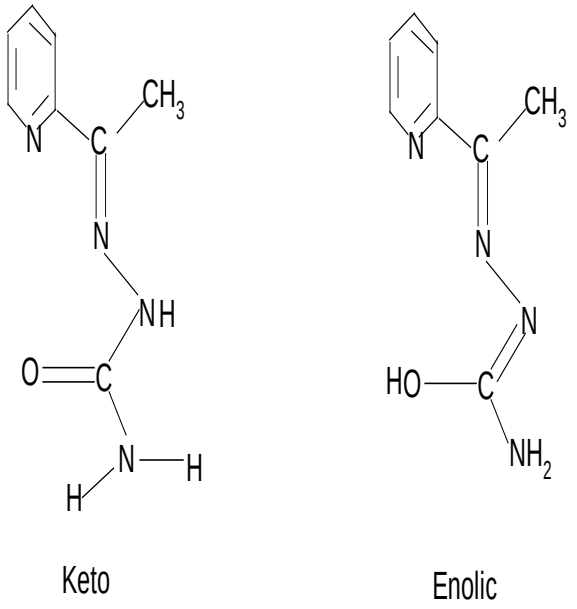

Fig. 1: Proposed keto and enol forms of 2-acetylpyridine semicarbazone

The band considered to have significant contribution from the C-O bond is shifted to the lower frequency by 10 – 15cm^{-1} in the enolic complexes and by 50 -70cm^{-1} in the keto complexes. These confirms coordination via oxygen which is further supported by v(C=N) + δNH_2 bands shifted to higher frequencies of 1293 -1321cm^{-1} (9, 2). The v(C=N) band occurring in the region of 1595 –1600cm^{-1} in the free ligand shifted by 20 – 60cm^{-1} to lower frequency in the complexes thus suggesting that the nitrogen of azomethine group is bonded to the metal ion. This is further affirmed by the appearance of the amide band at 1274cm^{-1} and v(N-N) band at 1151cm^{-1}. The metal – nitrogen and metal – sulphur band of the monodentate secondary ligand at the fourth coordinative site of the metal have been identified in accordance with literature (4, 5, 16) to be within the range 350–244cm^{-1}.

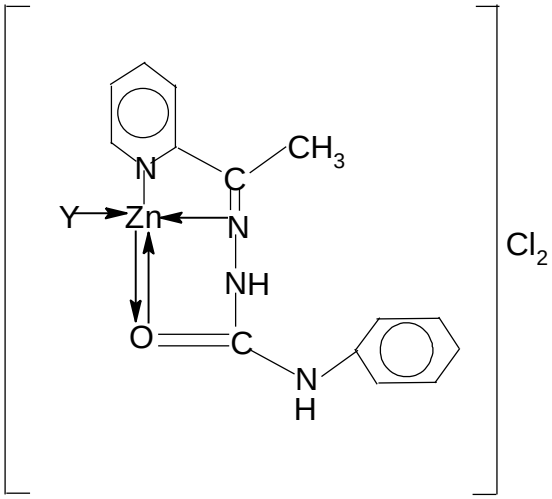

Fig: 2 Tetrahedral zinc(II) mixed ligands complex of 2-acetylpyridine 4-phenylsemicarbazone with N/S donor molecules. (Y=thiophene or pyridine or picoline or aniline or ammonia)

The electronic absorption data of these compounds in DMF are as given below. The spectrum of the ligand shows bands on the 26700-29000cm^{-1} range assignable to n – π* transition of azomethine group in the 4-phenylsemicarbazone moiety. These bands shifted to lower wavelength on complexation suggesting the coordination of nitrogen atom of the azomethine group to metal ion. The bands in the 34000-40000cm^{-1} range are attributed to π – π* transition of the pyridyl ring and carbonyl oxygen. Zinc(II) (a d^{10} ion with no LFSE) also diamagnetic, has in its electronic spectrum bands in the range of 35700 – 40000cm^{-1}. These bands may be due to the n –π* and π – π* transition of pyridyl ring nitrogen and carbonyl oxygen coordinated to the metal. The less intense broad bands at 23000 – 40000cm^{-1} from the complexes, results from the overlap of the low energy π – π* transition localized within the semicabazonechromophore and the LMCT transition from the lone pair electrons of the oxygen donor to the metal ion (Zn^{2+}). Therefore the Zn(II) mixed ligand complexes based on the available IR, far – IR and electronic spectra data are assigned tetrahedral geometry (fig. 2). This also conforms with similar reports by 20, 1 and 8.

Antimicrobial Activity

The *in vitro* antimicrobial properties of the ligand and its zinc mixed ligands complexes were tested against these gram –positive bacteria- *S. aureus, B. anthracis*and the single celled saprophytic fungal pathogen-*A.niger* and *C,aldican* in broth culture. Table 2 dataclearly indicates that the parent ligand does not inhibit the proliferation of the tested microbes and that zinc conjugation synergistically enhances the antimicrobial activity of this parent ligand. Such enhancement may be due to an increase in cell permeability of the lipophilic zinc conjugates which allow for the intracellular chelate accumulation (17). It is also likely that this intracellular accumulation may lead to higher

cytoplasmic concentration of the metal ion which may prove lethal for the bacteria or fungi (16; 17 and 10) and thereby prevent the normal metabolic activities of the cell. As 4-N-substituted chelates, the effectiveness of antimicrobial activities against tested microbes is fairly high compared to the unsubstitutedsemicarbazone chelates as reported by 12. Thereplacement of labile monodentate secondary ligands within the complexes also play their significant roles. This is affirmed by the high efficiency with which the complexes inhibit the growth and proliferation of all the tested microbes especially the aniline containing complex.. In conclusion the present study has shown that the zinc conjugates of 4N- substituted semicarbazone containing nitrogen donor ancillary ligands are advantageous in enhancing antimicrobial

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