Sustainable Health in View: Synthesis, Characterization And Antibacterial Activity of Cobalt Complex of Thiosemicarbazide and 4-Aminoquinoline Mixed Ligand

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ABSTRACT

In the present study complex of Cobalt(II) with mixed ligand of Thiosemicarbazide and 4-Aminoquinoline was reported. The complex was characterized by physical and spectral analysis. Antibacterial activity was carried out on the complex against Salmonella typhi, Shigella specie, E.coli, Klebsiella specie, staphylococcus auerus, Pseudomonas aeruginosa and Neisseria gonorrhoeae. The complex of the type ML_1L_2 [where M=Co(II), L_1=Thiosemicarbazide and L_2=Chloroquine] was formed. The complex was non-electrolyte in methanol. The resulting complex was characterized by conductivity measurements and spectral studies. The mixed ligand used act as a neutral tridentate compound with tentative octahedral geometry with metal ions. Our findings, using the chemical, spectroscopic analysis and physical properties showed that the ligand employed in this work coordinated to metals in 1:1:1 metal-ligand 1-ligand 2. The antibacterial activity was also carried out. The results showed that the complex possess greater biological activity against the bacterial used and the metal complex possess greater biological activities than the parent compounds. Thus they could be promising candidates for novel drugs in pharmaceutical research.

Key word: Mixed ligand, Antibacterial activity, Metal complex, Characterization, Pharmaceutical research

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1. BACKGROUND TO THE STUDY

Life threatening infections and diseases, spreading all over the world, day by day, demand development of new antimicrobial agents, that are highly efficient with lesser side effects and equally cost effective (Joseph *et al*., 2012). Metal complexes are preferred to simple ligands due to an increased activity and several sulphur containing metal chelates have been reported with significant antibacterial and antifungal activities (Bharty *et al*., 2015, Yousef *et al*., 2015). A large majority of Thiosemicarbazones have been extensively studied, because of their antibacterial, antifungal, antitumor, antiviral and anticancer activities (Beraldo & Gambino, 2004). Transition metal complexes of thiosemicarbazone have attracted chemists due to the presence of hard nitrogen and soft sulphur donor atoms which permits coordination with a metal ion in different binding mode yielding stable and intensely coloured complexes(Lobana *et al*., 2006). This versatile nature of the ligand has encouraged us to explore the coordination chemistry further and to make mixed ligand complexes along with a second ligand in such a way that it enhances the biological activity (Brindha and Vijayanthimala, 2016).

For several decades, the 4-aminoquinolines(4A), chloroquine (CQ) and amodiaquine (AQ) were considered the most important drugs for the control and eradication of malaria. The success of this class has been based on excellent clinical efficacy, limited host toxicity, ease of use and simple, cost-effective synthesis. Importantly, chloroquine therapy is affordable enough for use in the developing world. (O'Neill *et al*,). Thiosemicarbazones are an important class among the sulfur-containing compounds, in view of their versatility in the coordination behavior and pharmacological applications. Thiosemicarbazones and their metal complexes are well known for their anticarcinogenic, antibacterial, and antifungal properties.[Quiroga and Raming, 2004].

It has been shown in the literature that the metal derivatives are more effective than the free ligands in anticancer activity. One of the many reasons is that the binding affinity of metals to proteins or enzymes will change the interaction process with DNA, thereby affecting DNA replication and cell proliferation (Saha *et. al*., 2002). In this context, hybrid chelate ligands containing mixed functionalities are widely used as ligands for the isolation of interesting and important metal complexes (Cowley *et al*., 2006). Thiosemicarbazide is a white crystalline powder and is odorless. This material is used as a reagent for ketones and certain metals, for photography and as a rodenticide. It is also effective for control of bacterial leaf blight of rice. Not a registered pesticide in the U.S. It is a chemical intermediate for herbicides and a reagent for detection of metals. (EPA, 1998)

1.1 Statement of Problem

The serious medical problem of bacterial and fungal resistance and the rate at which it develops has led to increasing levels of resistance to classical antibiotics (Daher *et al*., 2011). The discovery and development of effective antibacterial and antifungal drugs with novel mechanism of action have become an urgent task for infectious diseases research programs (Jin *et al*., 2005). Many investigations have proved that binding of drug to metallic element enhances its activity and in some cases, the complexes posseses even more heating properties than the parent drug (Gupta *et al*., 2007). Al-Amiery *et al*., in 2011 reported the synthesis characterization and biological properties of Co(II), Ni(II), and Cu(II) complexes with N-amino quinolone derived from 2-hydroxybenzaldehyde (for L1) and (1-(1H indol -3-yl) ethanone for L2 and Hassan in 2017 reported the preparation , characterization and antimicrobial of some new complexes formed by the reaction of manganese (II), iron (II), cobalt (II), nickel (II), cupper (II) and zinc(II)salt with Schiff bases derived from reaction of N-amino quinolone with benzoin and 4-acetylpyriden.

Also Adekanmbi T. O. in 2015 reported Synthesis, Characterization and Antibacterial Activity of Cobalt (ii) Complex of 8-Hydroxyquinoliine Mixed with Hydrazine and in 2010 Kulkarni *et al*. reported Spectroscopy, Electrochemistry, and Structure of 3d-Transition Metal Complexes of Thiosemicarbazones with Quinoline Core: Evaluation of Antimicrobial Property. The present research reports the preparation , characterization and antimicrobial of new complex formed by the reaction of cobalt (II) salt with Schiff base derived from reaction of 4-aminoquinolone with Thiosemicarbazide.

1.2 Objective

The objectives are:

1. To synthesize mixed ligand of 4-Aminoquinoline and Thiosemicarbazide
2. To complex the mixed ligand with Cobalt(II) salt.
3. To characterize both the ligand and the Cobalt(II) complex.
4. To analyse the antibacterial activity of both the ligand and the complex.

2. METHODOLOGY

2.1 Synthesis of 4-Aminoquinoline-Thiosemicarbazide mixed ligand

4-Aminoquinoline (0.01 mol) in ethanol (100 mL) was treated with Thiosemicarbazide (0.01 mol) in methanol(100mL). The reaction mixture was refluxed for 3–4 hr and the yellow solid separated was filtered, washed two to three times with ethanol, and dried (Scheme 1).(Kulkarni *et al*., 2010)

2.2 Synthesis of Cobalt(II) complex of the mixed ligand

Ligand (0.001 mol) was taken in 35–40 mL of hot ethanol. To this, a hot ethanolic solution of metal chloride (0.001 mol) was added drop-wise with stirring at 60–65^{0}C. After complete addition of metal salt solution, the reaction mixture was stirred for another 30–40 min at the same temperature and refluxed for 3–4 hr in a water bath. The isolated complexes were filtered in hot condition, washed with hot ethanol, and dried (Kulkarni *et al*., 2010)

2.3 Antimicrobial activity

The individual organism were individually grown overnight on nutrient agar slant at 35^{0}C. The suspension was then prepared by washing the growth off with the normal saline solution and further serially diluted with sterile 20ml phosphate buffer to give counts of factor of 1×10^6 cfu/ml. The ligand and its Co(II) complex was studied for their antibacterial activities by agar diffusion method respectively in DMF solvent against Salmonella typhi, Shigella specie, E.coli, Klebsiella specie, staphylococcus auerus, Pseudomonas aeruginosa, Neisseria gonorrhoeae bacterial species using agar nutrient as the medium and gentamycin as the standard drug. The stock solution (10 mg / 10 mL) was prepared by dissolving the compounds in DMF.

A circular well was made at the center of each petri dish with a sterilized steel borer. Then, 0.1mL of each test solution was added to the well using a micropipette and the plate was incubated, 24 h for bacteria growth and the diameter of inhibition zone were noted (Halli *et al*., 2012)

3. RESULT

The physical properties of the products are presented in the table 1 and the result of the solubility tests carried out on the products are presented in table 2. Also the result of the metal content analysis is presented in table 3 and conductivity measurement is presented in 4 while UV-Visible and Infrared spectral analysis result were presented in the tables 5 and 6 respectively. The reaction mechanism is as follows $MX_2.nH_2O$ + L_1L_2 (Where M=Co, n=2, 4, L_1=Thiosemicarbazide, L_2=4-Aminoquinoline

Table 1: Physical properties of ligand and complex

Sample number	Ligand	Colour of product	Nature of product	Percentage yield	Melting point
A	(TSC-4A)	White	Amorphous powder	73%	195^{0}C
B	Co(TSC-4A)	Pink	Crystalline salt	67%	235^{0}C

Table 2: Solubility data of the Ligand and Metal complex

Sample number	Ligand/Complex	Distilled water	Methanol	Ethanol	Acetone	DMF	DMSO	Dil Hcl
A	TSC-4A	SC SH	NSC SSH	SC-SH	SSC SSH	SC SH	SC SH	SC SH
B	Co(TSC-4A)	NSC SSH	NSC SSH	SC SH	SSC SSH	SC SH	SC SH	SSCSH

N.B.: SC-soluble in cold, SH-soluble in hot, NSC-soluble in cold, NSH-not soluble in hot, SSC-sparingly soluble in cold and SSH-sparingly soluble in hot.

Table 3: Result of metal content analysis

Sample number	Complex	Concentration(Mg/L)
B1	Co(TSC-4A)	240.73

Table 4: Result of conductivity measurement of ligand and metal complex

Sample number	Compound molecular formular	Molar mass	Conductivity($\Omega cm^2 Mol^{-1}$)
A	TSC-4A	410.50	-
B	Co(TSC-4A)	504.93	1.6×10^{-4}

Table 5: Ultraviolet-Visible spectra

No	Compound	Wavelength nm			
		Band 1	Band 2	Band 3	Band 4
A	TSC-4A	190	215	280	345
B	Co(TSC-4A)	190	215	255	-

Table 6: Infrared spectra

A – TSC-4A	3362.63	3164.83	-	1616.35	1273.00	-
B – Co(TSC-4A)	3752.74	3129.00	2351.64	1618.67	-	578.34
Tentative Assignment	υ N-H str. of NH2	υ N-H str.	υ S-H str.	SO_2-N	C-N str.	M-L

4. DISCUSSION

The complex obtained in the present study was non-hygroscopic and in the form of amorphous powder. The metal complex is easily soluble in DMSO and DMF and sparingly soluble in ethanol and methanol. Mixed metal complexes were obtained in good yield by the condensation reaction of aqueous/alcoholic solutions of chloroquine and the corresponding Thiosemicarbazide.The mixed ligand (TSC-4A) is mainly white in colour, soluble in water and organic solvents. On heating, especially in the presence of metal ions, due to complexation, their solubility shows a dramatic increase. The molar conductivity of aqueous solution of these ligands corresponds to 1:1:1 of type non-electrolyte.
The complex was obtained by the simple template method i.e., by the reaction of ready-made mixed ligand and metal salt, mainly in warm alcoholic(less often aqueous) solution under an air atmosphere. Considering the metal complex been prepared and investigated, the composition can be represented by the following general formulas

$MCl_n + L_1 + L_2 \rightarrow ML_1L_2$

Where $M=Co^{2+}$, L_1=4-Aminoquinoline and L_2=Thiosemicarbazide

Ligand and Complex formed exhibited various colour characteristics. They vary from white powdery and pink powdery salt. The complex is well soluble in DMF and DMSO, less soluble in MeOH, EtOH and H_2O. The TLC of the metal complex showed a single spot, implying the complex is pure. The spot of the complex and the ligand appear at very closer range, since they have similar functional group. The molar conductance of the solid complex($\lambda m\ \Omega^{-1}\ cm^2\ mol^{-1}$) was calculated. The DMF solubility of the complex made calculations of the molar conductivity (λm) of 10^{-3} mol dm^{-3} solution at 25^0C possible. The data in table 5.4 showed that the molar conductance is of relatively low value for the Co(II) indicating the non-electrolytic nature of this complex.

4.1. Spectra analysis of the complex

4.1.1. UV-Visible Absorption of TSC-4A and its Co(II) complex

The absorption bands for chloroquine-thiosemicarbazide and its Cobalt complex showed that Co complex gave strong absorption bands and within the range of 190nm and 345nm. These bands are tentatively assigned to charge transfer and intra-ligand transitions, based on other relative intensities and positions. UV-Visible spectra of this ligands and its complex have been interpreted in terms of charge transfer transitions from the metals to the antibonding orbital of the ligand and of the $\pi \rightarrow \pi^*$ transitions of the ligand. The ultraviolet spectrum of the free TSC-4A showed five absorption bands at 190nm, 215nm, 250nm, 310nm and 345nm. These transitins involve energies of 52631 cm^{-1}, 46511cm^{-1}, 40000cm^{-1}, 32258cm^{-1} and 28985cm^{-1}. These bands are assigned to $n \rightarrow \sigma^*$ and $n \rightarrow \pi^*$ transition respectively. These bands undergo hypsochromic and bathochromic shifts in the metal complexes due to complexation.

Co-TSC4A complex has an electronic configuration of d7 and a spectroscopic ground state term symbol of 4F. The 4F orbital is split in an octahedral field into sub-energy levels. Three absorption bands was observed in the spectrum of this complex at wavelength 190nm, 215nm and 255nm. Transition involved is 52631nm^{-1}, 46511cm^{-1} and 39215cm^{-1}, they are assigned to charge transfer transitions. However other expected higher transitions for the complex appear to have been submerged by the more intense transition because of their low intensity.

4.1.2. IR-Spectra of the mixed Thiosemicarbazide-4-Aminoquinoline and its metal complex

The important IR spectra bands of both the ligand and the metal complex with their assignment are given in the table 5.6. The IR spectrum of the ligand exhibited a very strong broad bands with a peak maximum at 3364cm^{-1}, 3368cm^{-1}, which are ascribed to the υ(N-H) vibration of amine group and H_2O along with υ(N-H) vibrations. Similar bands observed for the complex but with reduction in intensity and also red shift when compared with the ligand. The increasing and shifts in the absorption frequency of the υ(N-H) band for the complex relative to free ligand is a strong evidence of coordination with one of the N-H bands of thiosemicarbazide. The very strong υ(C-N) band in 4-Aminoquinoline at 1643cm^{-1}. This is close to the value characteristic for the majority of the ligand, in the complexes shifted to higher energies by 10-20cm^{-1}. A number of bands in the range 1433-1150cm^{-1} in the spectra of both ligands and complexes are ascribed to υ(S=O), C=S, δ(NH2) and C=C of the aromatics.

4.2. Discussion of the antimicrobial assay Minimum inhibitory concentration in mm

Generally, the ligands and metal complexes showed antimicrobial effect against the tested organism species.
Neissera gonorrhoea and pseudomonas aeruginosa was the most sensitive organism to the mixed ligand and its metal complex. Metal complex showed comparable activity or greater activity against some of the micro-organisms in comparison to the parent compounds.
For a particular antibacterial, the organism involved is an important factor, Salmonella typhi, Shigella species, Pseudomonas aureginosa sensitive to the metal complex thatn the organism Klebsiella species, Escherichia coli and Staphylococcus aureus.

5. CONCLUSION

5.1 Synthesis and Analysis

Thiosemicarbazide represents a relatively group of tridentate ligands of the Schiff-base type, exhibiting good complexing properties. Depending primarily on the synthesis conditions, pH, and nature of the metal ion, they coordinated with transition metals as single nd mixed liugands. Chloroquine is an antimalarial compound belonging to a group of aminoquionoline compounds. They also posseses good complexing properties with potential binding sites like N and S. Under the different conditions isolated, they were found to be mainly powder and crystalline salts. The compounds have varying colours depending on the metal salts used. The stoichiometries and the structure of the different types of complex reported here are based mainly on the micro analytical and spectroscopic data. The ratio of metal to ligand of the complex is 1:1:1 for double ligand complex. The complex shows 6-coordinated geometry. It was observed from study that thiosemicarbazide coordinate to metal through the sulphur and nitrogen of the hydrazine and amine. While 4-Aminoquinoline through one of the nitrogen of hydrazine and that of the quinolone ring. These were also confirmed from the infrared spectra. The UV-Visible spectra display no d-d transition in the complexes due to very low intensity of the peaks.

The complex showed good solubility in DMF and DMSO solvents but fairly in ethanol, methanol and others.
Attempt to grow single crystal from the powdered sample failed.

5.2 Biological studies

The metal complex studied for its antibacterial activities showed comparable and even higher activity than the parent compounds. In general, the metal complex was most active against N. gonorrhoea.

6. CONTRIBUTIONS TO KNOWLEDGE

Various more effective antimicrobial complexes could be synthesized using transition metals alongside active ligands.

7. RECOMMENDATION

It is suggested that the complex should be tested on virus as well. The ligands should be used to synthesize other metal complex using Copper, Nickel and other transition metals and tested accordingly. The antimicrobial activities should be compared amongst various metal complex of CQ-TSC. The electrochemistry of the complex should be studied to characterize the redox process which may occur in the ligand and complex.

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