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EFFECT ON THE INHIBITORY ACTIVITY OF POTENTIAL MICROBES ON THE COMPLEXATION OF METHYL ANTHRANILATE DERIVED HYDRAZIDE WITH CU, NI AND ZN(II) METAL IONS

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Abstract: The present report is concerned with the synthesis and coordination compounds of 2-amino-(N-aminobezoyl)benzohydrazide[ABH]. The ligand was charcterized by proton NMR, mass spectrometry, elemental and infrared studies. The ligand has got –NH2 moeity which is capable of chelation. Therefore complexes of Ni, Cu, and Zn were prepared. These complexes were characterized by elemental, infrared, thermal, conductance, and magnetic susceptibility studies. Infrared spectra shows that the ligand form complexes through –NH2 moeity, while the elemental studies suggest M(ABH)X2 composition of the coordination compounds. Knowing about the importance of -N-N- linkage in the biologically active compounds, the synthesized complexes were studied for their biological activities against Gram Negative bacteria which include E. coli, Salmonella typhi, Enterobacter aerogenes, Proteus vulgaris and Pseudomonas aeruginosa, selected Gram Positive bacterial strain is Staph aureus and Fungus like Candida albican. These activities show that the hydrazide containing metal complexes become more potentially resistive to the microbial activities as compared to the sole ligand.

Keywords: aminobenzohydrazine, coordination compounds, bioassay studies.

Introduction

Methyl anthranilate is an aromatic ester, and is widely used in the synthesis of biologically active alkaloids. These alkaloids include quinazoline, quinaxaline, cinnoline, phthalazine and other three or four membered ring azaheterocycles such as, pteridine, alloxazine, isoalloxazine [1]. It is also used as repellents for deterring wildlife entrance into an area. The primary component of synthetic grape flavoring, methyl anthranilate, has been identified to be a powerful avian irritant [2]. It has been successfully tested as a topical repellent, to deter goose grazing on grass [3], to reduce bird damage to blueberries [4], and to repel birds at landfills and standing water on airports [5]. Methyl anthranilate is a substrate reacted with N-

Boc protected amino aldehydes to get the product 5-oxo-1,2,3,4-tetrahydro-5H-1,4-benzodiazepine. The resulting product was further alkylated and acylated by reacting with phenylalanine and tryptophan to get the derived product which show significant selective binding affinity at cholecystokinin CCK1 receptors (IC50=156.5±33.2 nM) [6,7]. Classical benzodiazepines show anxiolytic, sedative, and anticonvulsant activities whereas, several 1,4-benzodiazepine derivatives such as diazepam, triazolam or midazolam, several others have demonstrated activity as antitumor antibiotics, anti-HIV agents, and antiarrhytmics [10,11,12].The synthesis of new indazolone and pyrazolone derivatives starting from methyl anthranilate presents advantageous alternative for the synthesis of the target heterocycles, which implies the use of the environmentally friendly oxidizer PIFA [phenyliodine(III)bis-(trifluoroacetate)] [11] Keeping in view the importance of hydrazides [12-18] and their complexes with Copper, Nickel and Zinc [20,21] we here by reported the hydrazide derived from methyl anthranilate through the condensation with hydrazine, to get a new modified diammine adding to the previous ones we reported so far [22-24]. All these various studies forced us to make complexes of such compounds and to study the effect on their bioassay studies.

ABH Ligand

Experimental

Materials and Methods

All chemicals and solvents used were of Analar grade. Salts of transition metals were obtained from Riedel-de-Haen, Germany and used without further purification. The partial dehydration of the salts was carried out by drying the hydrated salts in a vacuum oven for several hours at 80 – 100o C. Methyl anthranilate was obtained from Across Organics, USA and hydrazine monohydrate from PANREAC Quimica SA, Barcelona, Espania. Solvents were distilled twice before use.

Instrumentation

Infrared spectra were taken in the range of 4000-600 cm on PYE UNICAM Infrared Spectrophotometer in KBr disc. The far IR spectra were examined in KBr discs in the region of 400- 200 cm-1(T- IR SHIMADZU) .

The absorption spectra of solution of complexes in the range of 200- 900 nm using different solvents were obtained on Jasco DEC-1 Spectrophotometer with 1 cm matched quartz –cells. Molar conductances of the solution of the metal complexes were determined with a conductivity meter type HI 8333. All measurements were carried out at room temperature at 30 oC with freshly prepared solution. Magnetic susceptibilities were measured by Gouy method at room temperature using Hg[Co[SCN]4 as a standard [25], magnetic moments were thus calculated. The cations and anions were estimated by using typical analytical procedure [26]. Bioactivities were investigated using agar-well diffusion method [27]. Two to eight hours old bacterial strains in column containing approximately 104–106 colony forming units (CFU)/mL were used in these assays. The wells were dug in the media with the help of a sterile metallic borer with centers at least 24 mm. Recommended concentration (100 µl) of the test sample 1 mg/mL in DMSO was introduced in the respective wells. Other wells supplemented with DMSO and reference antibacterial drug, gentamycin served as negative and positive controls, respectively. The plates were incubated immediately at 37◦C for 20 hours. Activity was determined by measuring the diameter of zones showing complete inhibition (mm). Growth inhibition was compared with the standard drug gentamycin for bacterial strains and mystasin for fungal species. In order to clarify any participating role of DMSO in the biological screening, separate studies were carried out with the solutions alone of DMSO and they showed no activity against any bacterial strains. All these complexes were found to be potentially active against these bacterial strains.

Preparation of Ligand

According to the procedure adopted by Shadia A. Galal [8,9,28], 0.060 mole of methyl anthranilate was mixed with 0.022 mole of hydrazine monohydrate in a round bottom flask, the resulting reaction mixture was refluxed at 80-90 ºC for 1 hour with constant stirring. Needle type crystals of the prepared ligand appeared by keeping the reaction mixture for about two hours in a refrigerator. Crystals were washed with n-hexane and recrystallized with dry ethanol. The yield was 48%. 2.4 General Preparation of the Solid Complexes

All complexes of 2-aminobenzohydrazide (ABH) were prepared using the same general procedure. The required amount of partially dehydrated salts were dissolved in a minimum amount of anhydrous ethanol or methanol. Dehydration of the metal salts was carried out by reacting with the calculated amount of 2,2-dimethoxy propane as dehydrating agent. The solution was stirred up for about half an hour in order to ensure complete dehydration. Dissolved ligand was added slowly to the metal salt solution with constant stirring. The solid complex was formed immediately on mixing the two solutions or in either case complex was obtained by reducing the volume of the solution on a rotary evaporator. The products were filtered through sintered glass crucible, washed several times with n-hexane or dried ethanol and dried under vacuum.

Results and discussions

The ABH is characterized by elemental analyses, mass spectrum and NMR (proton and 13C). The mass spectrum of ABH shows a peak at m/Z 270. This is due to ABH+.

Elemental analytical data of ABH and its complexes are very close to the theoretical values as shown in Table1.

Complexes	Color	C (%)	H (%)	N (%)	Cation (%)	Anion (%)
$C_{14}H_{14}N_4O_2$	White	63.62 (62.21)*	6.08 (5.22)	20.81 (20.73)	---	---
$[Ni(NO_3)_2(C_{14}H_{14}N_4O_2)]$	Grayish white	37.10 (37.12)	4.30 (3.12)	18.90 (18.55)	12.02 (12.96)	27.17 (27.37)
$[NiI_2(C_{14}H_{14}N_4O_2)]$	Grayish yellow	28.50 (28.85)	2.40 (2.42)	10.20 (9.61)	10.00 (10.07)	42.49 (43.55)
$[Cu(NO_3)_2(C_{14}H_{14}N_4O_2)]$	Dark green	36.90 (36.73)	3.21 (3.08)	18.95 (18.36)	13.88 (13.88)	27.67 (27.08)
$[Zn(NO_3)_2(C_{14}H_{14}N_4O_2)]$	Off white	36.40 (36.58)	3.17 (3.07)	18.70 (18.28)	14.90 (14.97)	25.90 (26.97)
$[ZnCl_2(C_{14}H_{14}N_4O_2)]$	White	42.45 (41.36)	2.42 (3.47)	13.97 (13.78)	16.97 (16.08)	17.67 (17.44)

*Calculated values are given in parenthesis

Elemental analysis show that the metal to ligand ratio is 1:1 and the composition of metal complex is M(ABH)X2 (Where X is Cl-, I-, and NO3-).

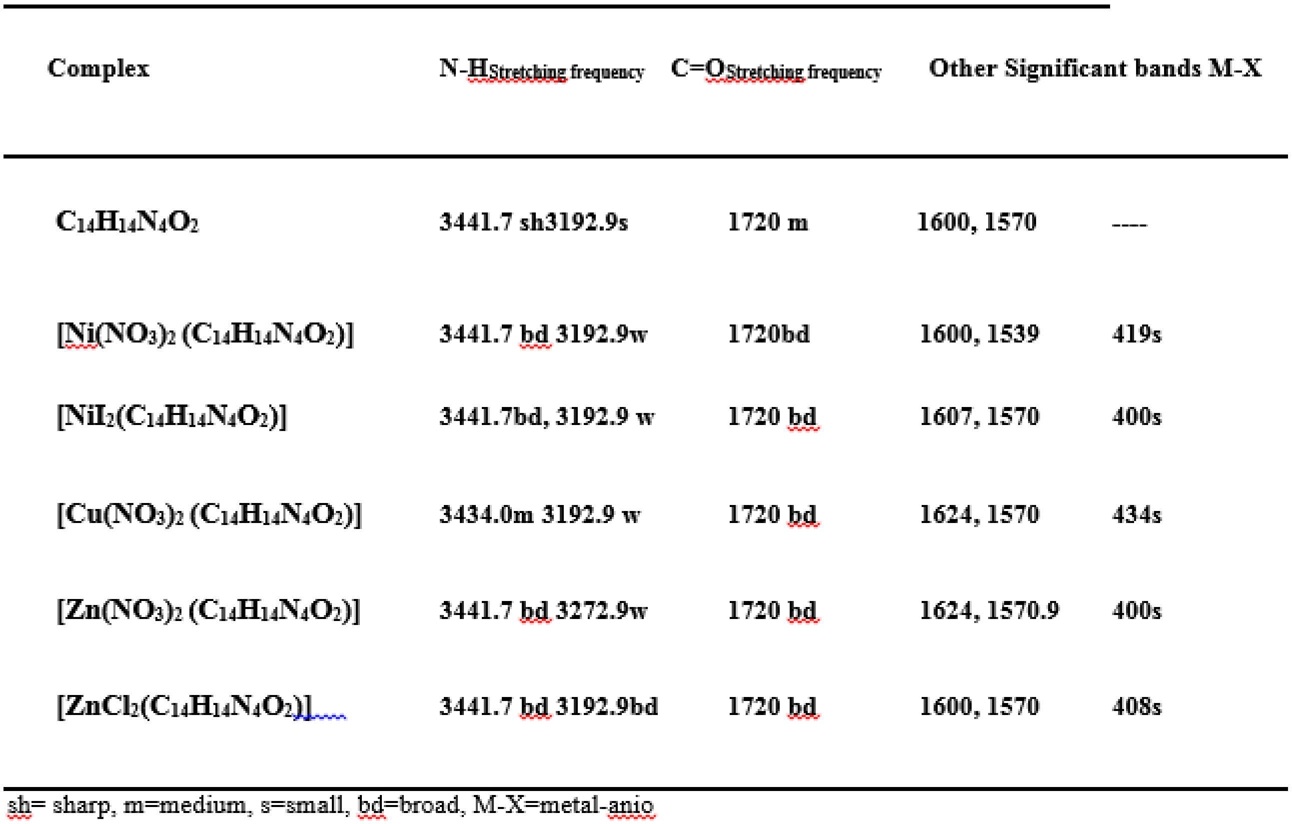
Conductance and melting points of the complexes are given in Table.2, conductance data show that the metal complexes are non-electrolyte indicating the halide or nitrate ions are located inside the coordination sphere. All the complexes are magnetic susceptible except Zn(II) complexes as shown in Table. 2. IR data which is shown in Table. 3 shows broadening of -NH2 peak, which suggest coordination through this site. While the carbonyl peak and of hydrazide –NH remain unaltered.

The 1H-NMR show doublet of doublet for protons located at 3 and 19, 4 and 18, 5 and 17, 6 and 16, which all are observed at 6.7ppm to 7.5 ppm in a very complex form. While the protons located at positions 10 and 11 are observed around 9ppm [28]. While the protons at 7 and 20 positions give a singlet at around 6.3ppm. 13C-NMR show seven peaks for all the seven identical carbons viz., 12 and 8, 1 and 15, 2 and 14, 3 and 19, 4 and 18, 5 and 17, 6 and 16 respectively.

Table 2. Conductance, melting points and magnetic moments data

Complex	Solvent	Melting Point (°C)	Molar Conductance (S/cm)	Cor x M x 10^{-6} (c.g.s)	μ_{eff} (B.M)
$C_{14}H_{14}N_4O_2$	---	109	---	---	---
$[Ni(NO_3)_2(C_{14}H_{14}N_4O_2)]$	DMF	Out of Range	2.49	3866.80	3.05
$[NiI_2(C_{14}H_{14}N_4O_2)]$	DMF	Out of Range	1.91	3972.13	3.09
$[Cu(NO_3)_2(C_{14}H_{14}N_4O_2)]$	DMF	107	14.22	2012.12	2.10
$[Zn(NO_3)_2(C_{14}H_{14}N_4O_2)]$	DMF	204	0.83	----	Dia magnetic
$[ZnCl_2(C_{14}H_{14}N_4O_2)]$	DMF	76	13.91	----	Dia magnetic

Table 3. IR spectra for ABH and its complexes (cm-1) of selected region



Complex	N-H Stretching frequency	C=O Stretching frequency	Other Significant bands	M-X
$C_{14}H_{14}N_4O_2$	3441.7 sh3192.9s	1720 m	1600, 1570	----
$[Ni(NO_3)_2 (C_{14}H_{14}N_4O_2)]$	3441.7 bd 3192.9w	1720bd	1600, 1539	419s
$[NiI_2(C_{14}H_{14}N_4O_2)]$	3441.7bd, 3192.9 w	1720 bd	1607, 1570	400s
$[Cu(NO_3)_2 (C_{14}H_{14}N_4O_2)]$	3434.0m 3192.9 w	1720 bd	1624, 1570	434s
$[Zn(NO_3)_2 (C_{14}H_{14}N_4O_2)]$	3441.7 bd 3272.9w	1720 bd	1624, 1570.9	400s
$[ZnCl_2(C_{14}H_{14}N_4O_2)]$	3441.7 bd 3192.9bd	1720 bd	1600, 1570	408s

sh= sharp, m=medium, s=small, bd=broad, M-X=metal-anio

Nickel Complexes

The visible absorption spectra of Ni(II) complexes (Table 3 and Fig.2) show broad peak around 17,800 cm-1 with shoulder around 16,600 cm-1, assigned to (F)®3T1(P) and 3T1(F)®3T2(F) transition probably indicating tetrahedral geometry. The magnetic moment (3.06 B.M for [Ni(NO3)2 (C14H14N4O2)] and 3.09 B.M for [NiI2(C14H14N4O2)]) and non-electrolytic behavior of these complexes are consistent with distorted tetrahedral symmetry of [Ni(ABH)X2].

Copper Complexes

The complexes of Cu (II) show an absorption band in the region 13000-15000cm-1(Table 3 and Fig.3). The envelopes of these bands are generally unsymmetrical, seeming to encompass several overlapping transitions. This band is similar to absorption maximum at 16,000cm-1 observed in the case of [Cu(daco-diac)2X2], a well known tetrahedral structure [15]. The magnetic moment is around 2.0 B.M; which is very close to the spin only value for the the unpaired electron. The conductance behaviour shows that the complex is non-electrolytic.

Zinc complexes

The conductance data of Zn(II) complexes indicate the non-ionic species. As the complexes of the cyclic diamines have not been reported so far, however, they can be compared with cyclic amidines. In view of the well-known tendencies of Zn(II) to form tetrahedral complexes [6,29,30], same structure may be proposed for Zn(II) ABH complexes with ABH acting as bidentate ligand. Trzaskowski et al observed by density functional calculations that deprotonated serine and cystein, which are close in structural features to ABH, forms tetrahedral geometry around the zinc(II) ion with only four ligands around the metal ion [31]. These calculations are in agreement with the known experimental crystal structures of some zinc-containing complexes. Dudev et al using Continuum Dielectric Calculations showed that zinc monochloride and dichloride tetrahedral complexes were calculated to be more stable than the respective octahedral complexes by -4.5 and -8.6 kcal/mol, respectively, at the B3LYP/6-31++G(2d,2p) level. Furthermore, stationary points for zinc octahedral complexes containing three or four Cl- could not be found since they isomerized into tetrahedral (4 + 2) complexes during optimization [32]. Roe et al also showed that tetrahedral geometry for the zinc+2 complexes are the most stable ones [30]. This geometry would also be consistent with the non-electrolytic behavior of the complexes [32].

Bio-Assay Investigations

The complexes of ABH were investigated for their bioactivity against various available microorganisms. These microorganisms include gram positive and gram negative bacteria along with a selected species of fungi. Gram Negative bacteria include E. coli, Salmonella typhi, Enterobacter aerogenes, Proteus vulgaris and Pseudomonas aeruginosa, selected gram Positive bacterial strain is Staph aureus and fungus like Candida albican.

These studies show that the metal complexes become more biologically active as compared to neat organic moiety. The results are reported in Table 6.

Table 4. Biological activities of complexes against gram negative, gram positive and fungus

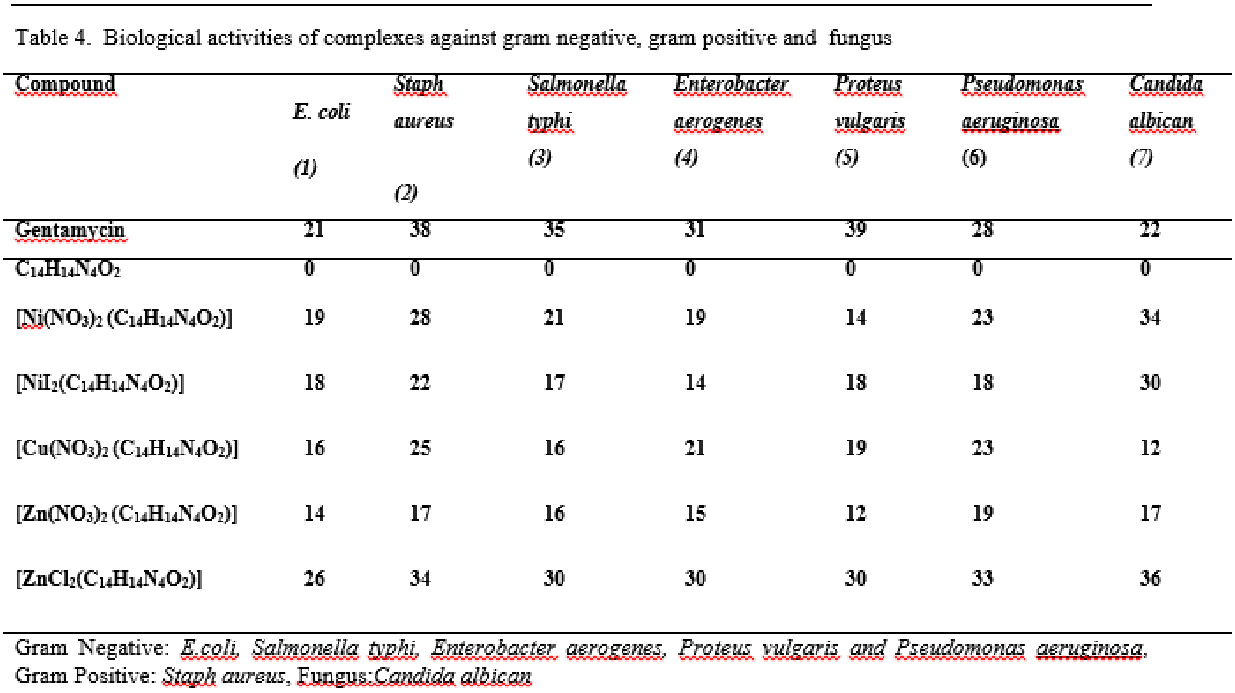


Table 4. Biological activities of complexes against gram negative, gram positive and fungus

Compound	*E. coli* (1)	*Staph aureus* (2)	*Salmonella typhi* (3)	*Enterobacter aerogenes* (4)	*Proteus vulgaris* (5)	*Pseudomonas aeruginosa* (6)	*Candida albican* (7)
Gentamycin	21	38	35	31	39	28	22
$C_{14}H_{14}N_4O_2$	0	0	0	0	0	0	0
$[Ni(NO_3)_2(C_{14}H_{14}N_4O_2)]$	19	28	21	19	14	23	34
$[NiI_2(C_{14}H_{14}N_4O_2)]$	18	22	17	14	18	18	30
$[Cu(NO_3)_2(C_{14}H_{14}N_4O_2)]$	16	25	16	21	19	23	12
$[Zn(NO_3)_2(C_{14}H_{14}N_4O_2)]$	14	17	16	15	12	19	17
$[ZnCl_2(C_{14}H_{14}N_4O_2)]$	26	34	30	30	30	33	36

Gram Negative: *E.coli, Salmonella typhi, Enterobacter aerogenes, Proteus vulgaris and Pseudomonas aeruginosa*, Gram Positive: *Staph aureus*, Fungus:*Candida albican*

Gram Negative: E.coli, Salmonella typhi, Enterobacter aerogenes, Proteus vulgaris and Pseudomonas aeruginosa, Gram Positive: Staph aureus, Fungus:Candida albican

All these complexes were found to be potentially active against these bacterial strains. It is evident that the overall potency of the ligand was enhanced on coordination as shown in comparative graphs in Fig. 1-5.

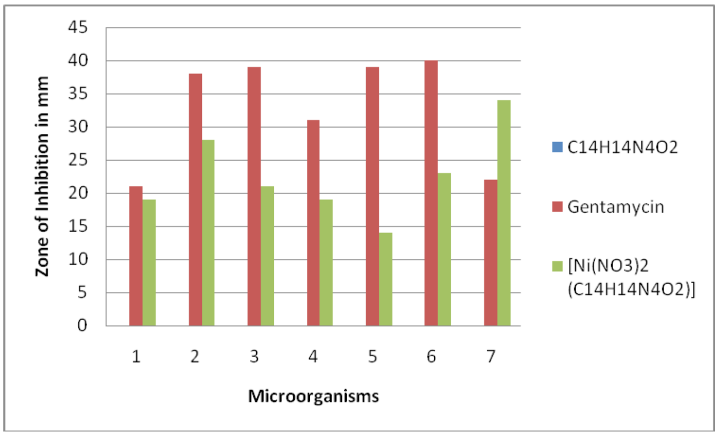


Fig.1 Comparative Graph for Activity shown by [Ni(NO3)2 (C14H14N4O2)] against Microbes

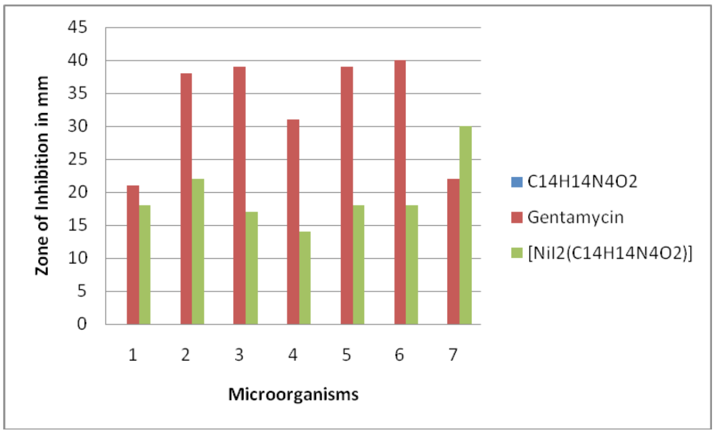


Fig.2 Comparative Graph for Activity shown by [NiI2 (C14H14N4O2)] against Microbes

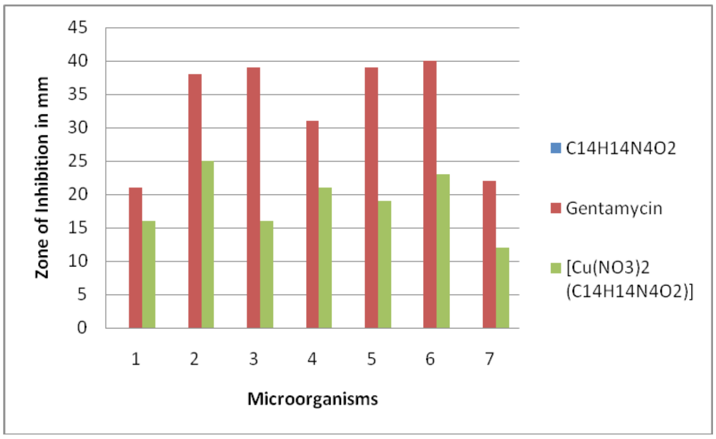


Fig.3 Comparative Graph for Activity shown by [Cu(NO3)2 (C14H14N4O2)] against Microbes

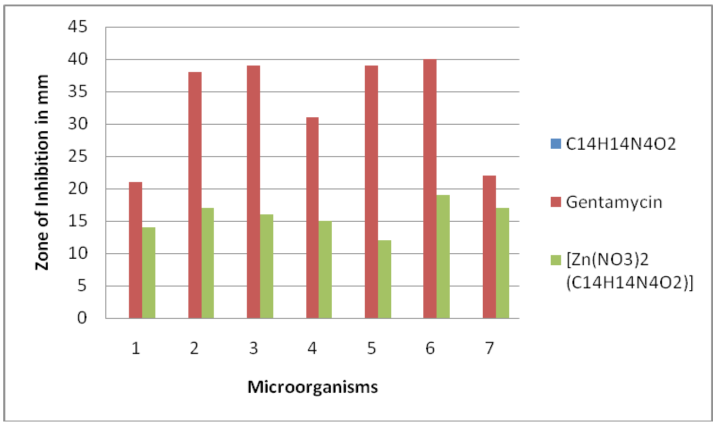


Fig.4 Comparative Graph for Activity shown by [Zn(NO3)2 (C14H14N4O2)] against Microbes

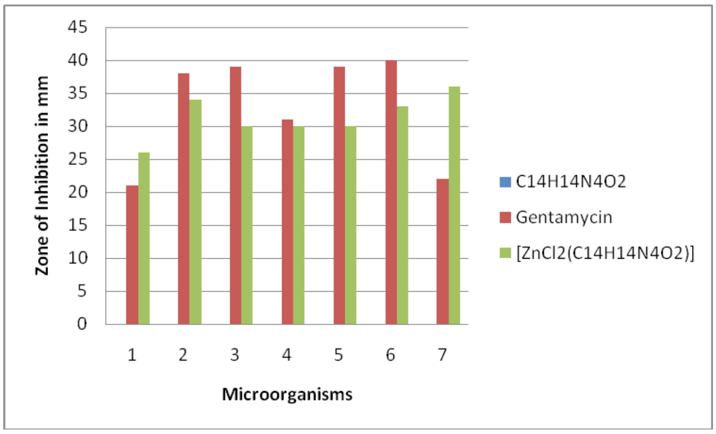


Fig.5 Comparative Graph for Activity shown by [ZnCl2(C14H14N4O2)] against Microbes

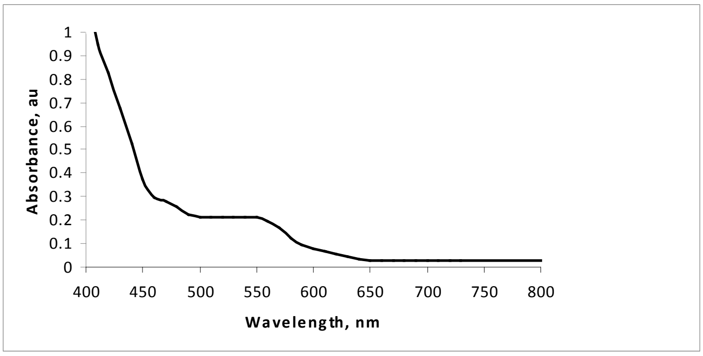


Fig. 6 Visible Spectrum of [Cu(NO3)2 (C14H14N4O2)]

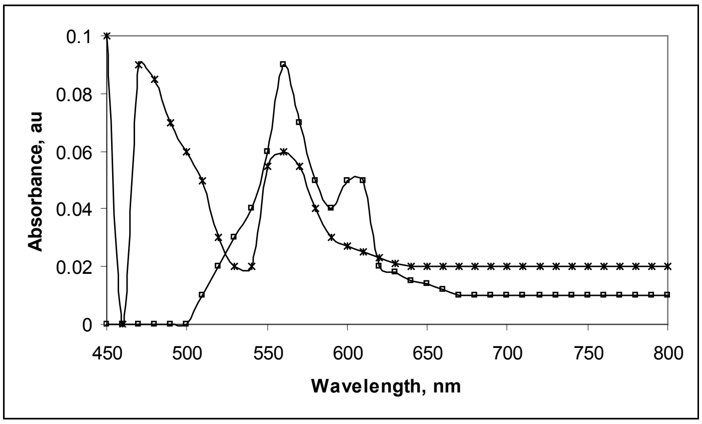


Fig.7 Visible Spectra of Nickel Complexes (x x x x x for [Ni(NO3)2 (C14H14N4O2)] and □ □ □ □ □ for [NiI2 (C14H14N4O2)])

Conclusion

The synthesized complexes of ABH ligand show tetrahedral geometries. Magnetic moment studies prove the assigned geometries. The synthesized hydrazine derived ligand showed antibacterial/ antifungal properties. In comparison, the copper(II), nickel(II), and zinc(II) metal complexes of this compound showed more activity against one or more bacterial/fungal strains, thus introducing a novel class of metal-based bactericidal and fungicidal agents.

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