DNA CLEAVAGE AND BIOLOGICAL STUDIES OF LIGANDS AND ITS METAL (II) COMPLESES

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ITRODUCTION

Adipic hydrazides are very important and wide applications of nitrogen containing heterocyclic compounds, possessing broad spectrum of biological and pharmacological activities such as hypotensive[1], anticancer, anti-HIV, anti-inflammatory[2], analgesic, antiviral, antitubercular, antimicrobial, anti-bacterial, antipyretic, antimitotic, anticonvaulsant[3], anticoagulant, anti-fibrillatory, cardiac stimulant and diuretic.[4] The quinoline have been tested successfully against cancer and HIV virus.[5] Their synthetic analogues possess antimalarial, hypolipidemic and antiproliferative activities[6] . The coordination chemistry of adipic hydrazide ligands has received much attention because of its biological implications. 2-amino-5-iodo benzoic acid hydrazide derivatives exhibit very potent antifungal and antibacterial activities.[7] These 2-amino-5-iodo benzoic acid derivatives are covered the area of biological interest of this compounds have extended recently to various microbial activities such as analgesic, diuretic, anti-inflammatory, anthelmintic, antipruritic activities[8] and this class of compound showed in vitro selective anti-helicobacter pylori activity.

In view of these factors the metal complexes of the ligand L^4= (N'^{1}E, N'^{6}E)-N$^{1'}$,N$^{6'}$-bis ((2 -hydroxyquinolin-3-yl)methylene)adipohydrazide , L^5 = (N'^{1}E, N'^{6}E)-N$^{'1}$, N'^{6} – bis ((6-bromo-2-hydroxyquinolin-3-yl)methylene) adipohydrazide, L^6= (N^1 *E*,N^{6}E)-N$^{1'}$ N$^{6'}$-bis ((2-hydroxy 6-methylquinolin-3-yl)methylene)adipohydrazide.

EXPERIMENTAL

a) Reagents

Dimethyl formamide (DMF) was distilled before use and peptone, pancreatic digest of casein, yeast extract, beef extract, dextrose and agar were used directly.

METHODS OF ANTIBACTERIAL ACTIVITY

The antibacterial activity of purified ligands and complexes have been evaluated for their in vitro growth inhibitory activity against the Gram-positive bacteria, *S. aureus* and another Gram-negative bacteria, *E. coli* using cup-plate methods[9].

Methods: Cup-plate method using nutrient agar
Organism: *Escherichia Coli* (Gram-negative bacteria)
Staphylococcus Aureus (Gram-positive bacteria).

a) The following materials were used

i. Nutrient agar 20-25 ml
ii. Sterilized petri dishes

iii. Bacterial cultures
iv. Sterilized cork borer of 8 mm diameter
v. Sterilized micro tips (1-200 µl)
vi. Micro-pipette (1-200 µl)
vii. Sterile test tubes containing solutions of compounds in the desired concentration.

b) Test organisms

The test organisms were selected from both Gram-positive and Gram-negative organisms to test the antibacterial activity. These organisms were cultured on agar slants and incubated for 24 hrs at 37 °C. From these slants a suspension was made using sterile saline solution (saline solution was prepared by dissolving 0.9 g of sodium chloride in 100 ml distilled water and then sterilized).

c) Preparation of media

The nutrient agar prepared by dissolving bacteriological peptone (1 g/ l), beef extract (5 g/ l), sodium chloride (5 g/ l) in distilled water and the pH of the solution was adjusted to 7.0 by sodium hydroxide (1M) or hydrochloric acid (1M). This solution was filtered and agar (20 g/ l) was added. Then sterilized for 15 minutes at 15 lbs pressure.

d) Preparation of subculture

The organisms used in the present study were obtained from the laboratory stock, two day before testing; the organisms were sub cultured in the sterilized nutrient broth. After incubating the same for 24 hrs, the growth was used as inoculums for the test.

e) Sterilization of media and glass wares

Nutrient agar and nutrient broth were sterilized in a conical flask of suitable capacity by autoclaving the same at 15 lbs/ kg pressure for 15 min. The cork borer and glass wares i.e., Petri dishes, test tubes and micro tips etc., were sterilized by employing autoclave at 15 lbs/ kg pressure for 15 min.

f) Preparation of test solution

It was prepared by dissolving 5 mg of either ligand or metal complexes in 5 ml of dimethyl formamide to give a test concentration 1000 µg/ml.

g) Method of testing

About 15-20 ml of molten nutrient agar was poured into each of the sterilized petri dishes of 3.5 inches diameter. The organisms from the cultured broths were inoculated on to the respectively plates. With the help of sterile cork borer two cups of each with 7 mm diameter were punched and scooped out of the set agar (two cups were numbered for the particular test compound). Each set of the plates were inoculated with the suspension of particular organisms by spread plate technique[10].

The cups of inoculated plate were then filled with 0.1 ml of the test solution, the plates were allowed to stay in them the plates were incubated at 37 °C for 24 hrs. The zone of inhibition developed if any, was then measured for the particular compound with particular organisms[11].

The standard drug streptomycin (100 µl) and the solvent used were also tested independently for their biological activity under the same conditions. The antibacterial results of the ligands and their complexes are tabulated in the tables 6.1, 6.2 and 6.3.

METHODS OF ANTIFUNGAL ACTIVITY

The antifungal activities of the ligands and their metal complexes were tested against *Aspergillus niger* and *Aspergillus flavous*.

Method: Cup-plate method using potato dextrose nutrient agar
Organism: *Aspergillus niger (A. niger)*

Aspergillus flavous. (*A. flavous*)

a) Materials and methods

The following materials were used

i. Potato dextrose agar
ii. Sterilized petri dishes and syringes of 0.1 ml capacity
iii. Fungal culture
iv. Sterilized test tubes containing solutions of the compounds at known concentration

b) Preparation of fungal medium

The media used for antifungal activity was the potato-dextrose agar. It was prepared as follows, potato slices (120 g) were dissolved in 100 ml distilled water by steaming for 30 min. The solution was filtered while hot and the volume was made up to 400 ml. To this solution, dextrose (4 g) and agar (8 g) were added and dissolved by steaming for 30 min. So formed potato-dextrose agar (PDA) media was poured into two separate conical flask and were sterilized, poured into test tubes, kept in slant position and on solidification were inoculated with above fungus using sterile metal inoculation needle[12-15].

c) Preparation of subcultures

The organisms used in the present study were obtained from the laboratory stock four days before testing, the organisms were sub cultured in the sterile Potato-dextrose broth. After incubating the same for 3 days, the growth thus obtained was used as inoculums for the test[15-20].

d) Sterilization of media and glass wares

Potato-dextrose agar was sterilized in conical flask of suitable capacity by autoclaving the same at 15 lbs/ kg pressure for 15 min. The cork borer, glass wares, Petri dishes, test tubes and pipettes were sterilized by employing autoclave at 15 lbs/ kg pressure for 15 min.

e) Preparation of test solution

It was prepared by dissolving 5 mg of the compound in 5 ml of DMF to give the concentration 1000 µg/ ml. This solution (0.1 ml) was used for testing.

f) Method of testing

About 15-20 ml of molten sterilized potato dextrose agar was poured into each of the sterilized Petri dish, inoculated with respective organisms by spread plate technique and with the help of sterile cork borer; two cups of each 7 mm diameter were punched and scooped out from the set PDA medium[20-26].

The wells of inoculated plates were then filled with 0.1 ml of the test solution. Further the plates were incubated at 28 °C (room temperature) for 48 hrs. The zone of inhibition developed if any was then measured for the particular compound and particular organisms[27-30]. The standard drug chlotrimazole and the solvent used were also independently tested data for their activity under the same conditions. The antifungal results of the ligands and their complexes are tabulated in the tables.

All the synthesized ligands **L^4**, **L^5**, **L^6** and their metal complexes such as Cu(II), Co(II), Ni(II), Mn(II), Fe(III), Zn(II), Cd(II) and Hg(II) complexes were tested for their antibacterial activity against the *Escherichia Coli* and *Staphylococcus Aureus*, where as the antifungal activity against *Aspergillus niger* and *Aspergillus flavous*. The results of the antimicrobial studies have been presented in tables.

Table1. Antimicrobial activity of the ligand (L^4 = HMOHAD) and its metal (II) complexes.

Sl. No.	Compound	Antibacterial Activity Zone of inhibition (in mm)		Antifungal Activity Zone of inhibition (in mm)	
		E.Coli	*S.aureus*	*A.niger*	*A.flavus*
1.	$[C_{26}H_{24}N_6O_4]$	09	08	08	10
2.	$[Cu(C_{26}H_{22}N_6O_4)]$	10	11	15	12
3.	$[Co(C_{26}H_{22}N_6O_4)]$	14	13	14	13
4.	$[Ni(C_{26}H_{22}N_6O_4)]$	12	15	17	15
5.	$[Mn(C_{26}H_{22}N_6O_4)]$	15	13	20	19
6.	$[Cd(C_{26}H_{22}N_6O_4)]$	20	19	16	17
7.	$[Hg(C_{26}H_{22}N_6O_4)]$	16	15	18	16
8.	$[Zn(C_{26}H_{22}N_6O_4)]$	13	14	19	18
9.	Streptomycin	24	23	--	--
10.	Chlotrimazole	--	--	25	26
11.	DMF (Control)	0	0	0	0
12.	Bore size	08	08	08	08

Table 2. Antimicrobial activity of the ligand (L^5 = HMBRAD) and its metal (II) complexes

Sl. No.	Compound	Antibacterial Activity Zone of inhibition (in mm)		Antifungal Activity Zone of inhibition (in mm)	
		E.Coli	***S.aureus***	***A.niger***	***A.flavus***
1.	$[C_{26}H_{22}Br_2N_6O_4]$	08	09	09	10
2.	$[Cu(C_{26}H_{20}Br_2N_6O_4)$	10	12	15	13
3.	$[Co(C_{26}H_{20}Br_2N_6O_4)]$	15	114	15	14
4.	$[Ni(C_{26}H_{20}Br_2N_6O_4)]$	13	14	17	16
5.	$[Mn(C_{26}H_{20}Br_2N_6O_4)]$	16	13	19	20
6.	$[Cd(C_{26}H_{20}Br_2N_6O_4)]$	12	14	17	15
7.	$[Hg(C_{26}H_{20}Br_2N_6O_4)]$	16	14	18	16
8.	$[Zn(C_{26}H_{20}Br_2N_6O_4)]$	16	18	18	19
9.	Streptomycin	24	23	--	--
10.	Chlotrimazole	--	--	25	26
11.	DMF (Control)	0	0	0	0
12.	Bore size	08	08	08	08

Table 3. Antimicrobial activity of the ligand (L^6 = HMCHAD) and its metal (II) complexes.

Sl. No.	Compound	Antibacterial activity zone of inhibition (in mm)		Antifungal activity zone of inhibition (in mm)	
		E.Coli	*S.aureus*	*A.niger*	*A.flavus*
1.	$[C_{28}H_{28}N_6O_4]$	09	08	08	10
2.	$[Cu(C_{28}H_{26}N_6O_4)]$	10	11	15	12
3.	$[Co(C_{28}H_{26}N_6O_4)]$	14	13	14	13
4.	$[Ni(C_{28}H_{26}N_6O_4)]$	12	15	17	15
5.	$[Mn(C_{28}H_{26}N_6O_4)]$	15	13	20	19
6.	$[Zn(C_{28}H_{26}N_6O_4)]$	11	13	16	17
7.	$[Cd(C_{28}H_{26}N_6O_4)]$	16	15	18	16
8.	$[Hg(C_{28}H_{26}N_6O_4)]$	15	18	19	18
9.	Streptomycin	24	23	--	--
10.	Chlotrimazole	--	--	25	26
11.	DMF (Control)	0	0	0	0
12.	Bore size	08	08	08	08

RESULTS AND DISCUSSION

a) Antibacterial and Antifungal activity of ligand (L^1 = HMOHAD). The antibacterial activity results revealed that the ligand and its complexes shown weak to good activity (Table I). The ligand and its Cu (II), Hg (II), Ni (II) complexes shows weakly active with the zone of inhibition 10-13 mm against the both organisms when compared to the standard drug streptomycin. The Mn(II), Co(II), Cd(II) shows active and moderate activity as compared to its ligand with zone of inhibition 15 - 17 mm and 18 - 20 when compared to the standard drug streptomycin.

The antifungal activity, results revealed that the ligand and its Cu(II), Co(II), Ni(II), Zn(II), Cd(II),Hg(II) and Mn(II) complexes have exhibited weak to good activity (Table I). The ligand and its Co(II) and Hg(II) complexes shows weak activity with zone of inhibition, the Cu(II), Ni(II), Mn(II), Zn(II) and Cd(II), shows moderate activity as compared to its ligand with the zone of inhibition 15 - 17mm, to good activity with the zone of inhibition of 18 - 20mm when compared to the standard drug chlotrimazole.

b) Antibacterial and antifungal activity of ligand (L^2 = HMBRAD)

The antibacterial activity results revealed that the ligand and its complexes shown weak to good activity (Table II). The ligand and its Cu (II), Zn (II), Ni(II) complexes shows weakly active with the zone of inhibition 10 - 14 mm against the both organisms when compared to the standard drug streptomycin. The Mn(II), Co(II), Cd(II) shows moderate activity as compared to its ligand with zone of inhibition 15 - 17. The Hg(II) is a good activity with zone of inhibition 18-20 when compared to the standard drug streptomycin.

The antifungal activity, results revealed that the ligand and its Cu(II), Co(II), Ni(II), Zn(II), Cd(II), Hg(II), and Mn(II) complexes have exhibited weak to good activity (Table II). The ligand and its Co(II) complexe shows weak activity with zone of inhibition, the Cu(II), Ni(II), Mn(II), Zn(II), Cd(II), Hg(II) shows moderate activity as compared to its ligand with the zone of inhibition 15 - 17mm, to good activity with the zone of inhibition of 18 - 20mm when compared to the standard drug chlotrimazole.

c) Antibacterial and antifungal activity of ligand (L^3 = HMCHAD)

The antibacterial activity results revealed that the ligand and its complexes shown weak to good activity (Table III). The ligand and its Cu(II), Zn(II) and Ni(II) complexes shows weakly active with the zone of inhibition 10 - 13 mm against the both organisms when compared to the standard drug streptomycin. The Mn(II), Co(II) and Cd(II) complexes shows moderate activity as compared to its ligand with zone of inhibition 15 - 17. The Hg(II) complex is a good activity with zone of inhibition 18 - 20 when compared to the standard drug streptomycin.

The antifungal activity, results revealed that the ligand and its Cu(II), Co(II), Ni(II), Zn(II), Cd(II), Hg(II), and Mn(II) complexes have exhibited weak to good activity (Table III). The ligand and its Co(II) complexes shows weak activity with zone of inhibition, the Cu(II), Ni(II), Mn(II), Zn(II), Cd(II), Hg(II) shows moderate activity as compared to its ligand with the zone of inhibition 15 - 17mm, to good activity with the zone of inhibition of 18 - 20mm when compared to the standard drug chlotrimazole.

DNA CLEAVAGE STUDIES

The DNA cleavage activity of Schiff's base, and their Cu(II), Co(II), Ni(II), Zn(II), Cd(II), Hg(II), Mn(II) and Fe(III) complexes were studied by agarose gel electrophoresis method. The gel after electrophoresis clearly revealed that, all the metal complexes have acted on DNA and shows the complete cleavage Mn(II) and Mn(II) appears to bind DNA, due to which the DNA band has significantly shifted its position. It also has shown disrupt band, may be because of partial cleavage. So, Mn(II) analysis is inconclusive. As there was molecular weight difference between the control and the treated DNA samples. This shows that, the control DNA alone does not show any apparent cleavage where as Schiff's base, L^1=HMOHAD and their complexes shown. The results indicated the important role of metal in these isolated DNA cleavage reactions. However, the nature of reactive intermediates involved in the DNA cleavage by the complexes has not been clear. As the compounds were observed to cleave the DNA, it can be concluded that the compounds inhibits the growth of the pathogenic organism by cleaving the genome.

Gel electrophoresis pictures of Schiff's base ligands and their Cu(II), Co(II), Ni(II), Zn(II), Cd(II), Hg(II), Mn(II) and Fe(III) complexes. Photograph showing the effects of transition metal complexes on DNA of Calf-thymus. Lane M (DNA marker), Lane C (Untreated DNA) .

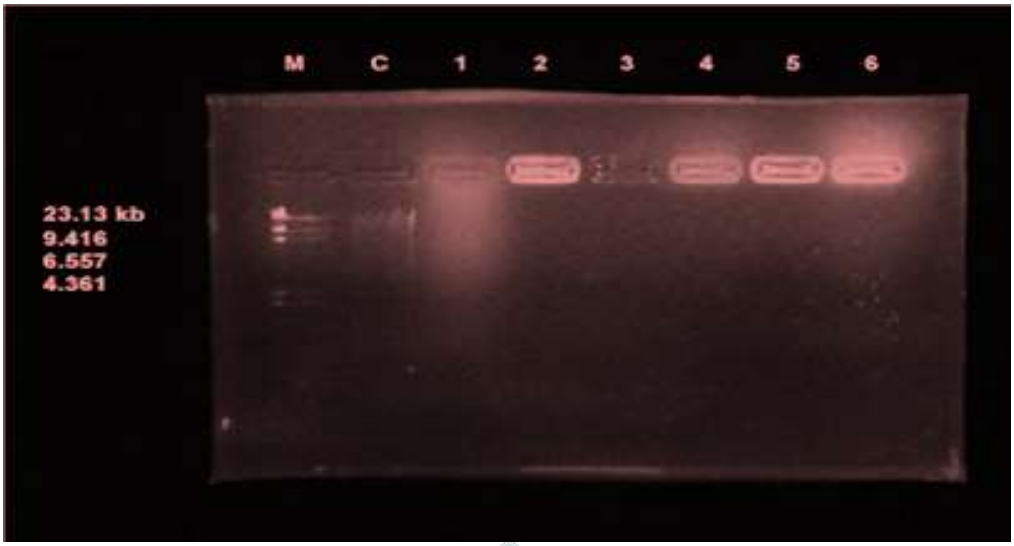

Figure-1. Lane 1 (ligand, L^4), Lane 2 (L^4 Cu(II)), Lane 3 (L^5), Lane 4 (L^5 Co(II)), Lane 5 (L^6), Lane 6 (L^6 Mn(II).

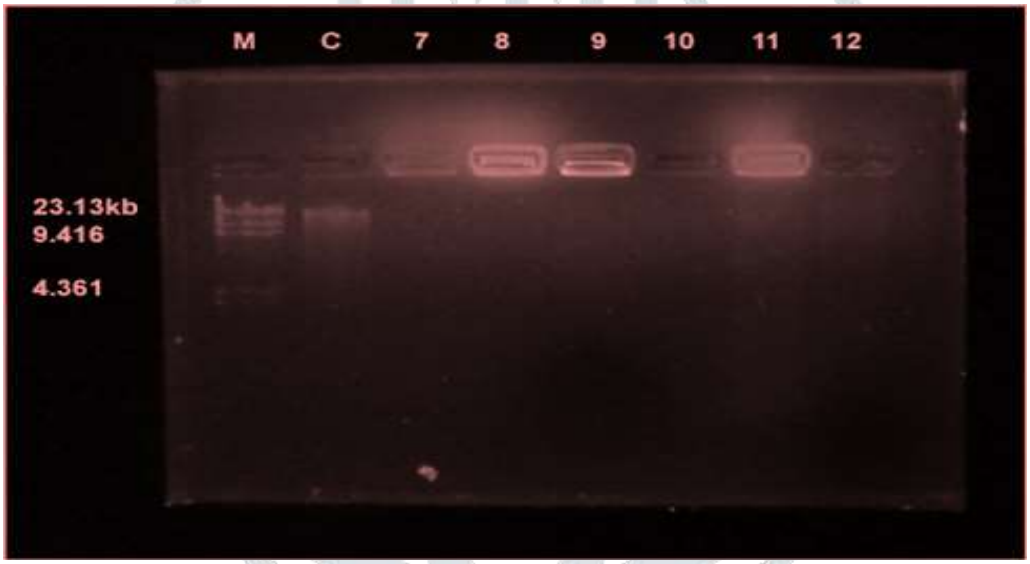

Figure-2. Lane 7 (L^4), Lane 8 (L^4 Ni(II)), Lane 9 (L^5Cd(II)), Lane 10 (L^5 Zn(II)), Lane 11 (L^6 Hg(II)), Lane 12 (L^6 Fe(III))

CONCLUSION

The Schiff's base ligands **L^4, L^5** and **L^6** behaved as a hexadentate ligand and their complexes are isolated in solid state possess 1:1 (M: L) stoichiometry and these are all non-electrolytic behavior of the complexes. The DNA cleavage studies of ligands and its metal complexes that reveals that the results indicated the important role of metal in these isolated DNA cleavage reactions. However, the nature of reactive intermediates involved in the DNA cleavage by the complexes has not been clear. As the compounds were observed to cleave the DNA, it can be concluded that the compounds inhibits the growth of the pathogenic organism by cleaving the genome.

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