

ANTIMICROBIAL ACTIVITIES OF LIGANDS AND ITS METAL COMPLEXES

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ABSTRACT

Synthetic Schiff base ligands and their complexes are known to possess appreciable antibacterial activity. The characteristic property has been related to the ability of the metal ion form complexes with ligands containing nitrogen oxygen donor atoms. A comparative study of the ligand and their metal complexes indicate that most of the metals chelate exhibit higher biological activity than that of the free ligand. The increase antifungal activity of the metal chelate with increase in concentration is due to the effect of metal ion on the normal cell process. Such increased activity of the metal chelate can be explained on the basis of overtone's concept and chelating theory.

KEYWORDS: Cup-plate method, microbial activity, chelate.

INTRODUCTION

The compound bearing C=N group and other groups, derivatives are known to exhibit bacteriostatic, anticancerous, inhibitor of tumor growth^[1-10] and other biochemical values. The common electron donor groups employed in drugs^[11-13] are = N-, -NH₂, = NH, -N=N-, -N-N-, -N=O, C=N-O-, C-O-C-S, -C=S etc. The actual donor group elected by the metals depends upon many factors. These factors include the character of the metal, the nature of each donor group and the type of the supporting solvent. Some carcinogens are capable of acting directly and powerful ligands, either in an aqueous or in non-aqueous environment and the other carcinogens can react indirectly by undergoing metabolism into strong ligand.^[14]

S. Trupti and S. Shahab^[15] observed that certain dyes, having negligible toxicity exhibit a high bacteriostatic index. (This term designates the maximum dilution of a substance in a

nutritive medium at which certain compounds are active against the given species of micro-organisms). It is also reported that certain coumarins are well-known natural products displaying a broad range of biological activities.^[16-22] Owing to their ability to coordinate with metal ions in the body. The Schiff bases were found to have pronounced biological activities.^[23, 24]

A comparative study of the ligands and their complexes indicates that most of the metals chelate exhibit higher antimicrobial activity than that of the free ligand. The increased antifungal activity of metal chelate with increase in concentration is due to the effect of metal ion on the normal cell process.^[25] Such increased activity of the metal chelate can be explained on the basis of overtone's concept^[26] and chelating theory.^[27]

Adipic hydrazides are versatile nitrogen containing heterocyclic compounds, possessing broad spectrum of biological and pharmacological activities such as hypotensive^[28], anticancer, anti-HIV, anti-inflammatory^[29], analgesic, antiviral, antitubercular, antimicrobial, anti-bacterial, antipyretic, antimitotic, anticonvulsant^[30], anticoagulant, anti-fibrillatory, cardiac stimulant and diuretic.^[31,32] The quinoline have been tested successfully against cancer and HIV virus.^[33,34] Their synthetic analogues possess antimalarial, hypolipidemic and antiproliferative activities.^[35,36] The coordination chemistry of adipic hydrazide ligands has received much attention because of its biological implications. The complexes show enhanced antitumor, antifungal and antibacterial activities compared to the free ligand.^[37] Quinolines are opportunistic infections with pneumocystis carinii and toxoplasma gondii are a major cause of morbidity and mortality in patients with the acquired immune deficiency syndrome.^[38] The sensitivity of the gram positive bacterial to the tested quinolines was higher than that of gram negative bacteria.^[39]

2-amino-5-iodo benzoic acid hydrazide derivatives exhibit very potent antifungal and antibacterial activities.^[40] 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^[41,42] and this class of compound showed in vitro selective anti-helicobacter pylori activity.^[43] A series of racemic 2-amino-5-iodo benzoic acid were synthesized as hybrid molecules of the two major prototypical hallucinogenic drug classes, the phenethylamines and the tryptamines/ergolines. Although it was hypothesized that these new agents might possess high affinity for the serotonin receptor subtype, unaccepted affinity for muscarinic receptor was observed.^[44]

The 2-amino-5-iodo benzoic acid hydrazide Schiff bases and complexes show good microbial activities and better adsorption and fluorescence properties. Bromo and methyl quinolines derivatives shows remarkable antimicrobial properties against microorganisms associated with death in patients carrying immune compromised diseases, analgesic activity as well as inhibitory effects for thymidylate synthase and poly polymerase. Quinolines attract much interest in view of their DNA-binding and DNA-photo damaging properties, inhibit dihydrofolate reductase exhibit an important role in clinical medicine as exemplified by the use of methotrexate in neoplastic diseases, inflammatory bowel diseases, rheumatoid arthritis, psoriasis, asthma, antineoplastic activities.^[45]

The antibacterial activity of the Schiff base ligand and their metal complexes against two micro organisms namely *staphylococcus aureus* and *Escherichia Coli* and have assessed the antifungal activity of the Schiff base ligand and their metal complexes against two micro-organisms namely *Aspergillus niger* and *Aspergillus flavus* and found that the compound tested show different ranges of activity.

In view of these factors the metal complexes of the ligand $L^1 = (N^1E, N'^6E)-N^{1'}, N^{6'}$ -bis ((2-hydroxyquinolin-3-yl)methylene)adipohydrazide, $L^2 = (N^1E, N'^6E)-N^{1'}, N^{6'}$ - bis ((6-bromo-2-hydroxyquinolin-3-yl)methylene) adipohydrazide, $L^3 = (N^1E, N'^6E)-N^{1'}, N^{6'}$ -bis ((2-hydroxy 6-methylquinolin-3-yl)methylene)adipohydrazide, $L^4 = N^{1'}-(1-(5-bromo-2-hydroxyphenyl) ethylidene) - 2 - oxo - 2H - chromene - 3 - carbohydrazide$, $L^5 = N^{1'}-(1-(2-hydroxy-5-methylphenyl)ethylidene)-2-oxo-2H-chromene-3-carbohydrazide$, $L^6 = (Z) - 2 - ((2 - hydroxyquinolin - 3 - yl) methyleneamino) - 5 - iodobenzoic acid$, $L^7 = (Z) - 2 - ((6-bromo-2-hydroxyquinolin-3-yl) methyleneamino) - 5 - iodobenzoic acid$, $L^8 = (Z)-2-((2-hydroxy-6-methylquinolin-3-yl)methyleneamino)-5-iodobenzoic acid$ were screened for their antibacterial and antifungal activity against four organism namely *Escherichia Coli*, *Staphylococcus Aureus*, *Aspergillus niger* and *Aspergillus flavus* by the cup-plate method. The method of evaluation of antibacterial and antifungal activity and the results are described in the fallowing sentence.

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.

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 μ l)
- vi. Micro-pipette (1-200 μ l)
- 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.

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.

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 1, 2 and 3.

RESULTS AND DISCUSSION

All the synthesized ligands L^1 , L^2 , L^3 , L^4 , L^5 , L^6 , L^7 and L^8 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.

Table 1: Antimicrobial activity of the ligand (L^1 = HMOHAD) and its metal (II) complexes.

Sl. No.	Compound	Antibacterial Activity Zone of inhibition (in mm)		Antifungal Activity Zone of inhibition (in mm)	
		<i>E.Coli</i>	<i>S.aureus</i>	<i>A.niger</i>	<i>A.flavus</i>
1.	[C ₂₆ H ₂₄ N ₆ O ₄]	09	08	08	10
2.	[Cu(C ₂₆ H ₂₂ N ₆ O ₄)]	10	11	15	12
3.	[Co(C ₂₆ H ₂₂ N ₆ O ₄)]	14	13	14	13
4.	[Ni(C ₂₆ H ₂₂ N ₆ O ₄)]	12	15	17	15
5.	[Mn(C ₂₆ H ₂₂ N ₆ O ₄)]	15	13	20	19
6.	[Cd(C ₂₆ H ₂₂ N ₆ O ₄)]	20	19	16	17
7.	[Hg(C ₂₆ H ₂₂ N ₆ O ₄)]	16	15	18	16
8.	[Zn(C ₂₆ H ₂₂ N ₆ O 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^2 = HMBRAD) and its metal (II) complexes.

Sl. No.	Compound	Antibacterial Activity Zone of inhibition (in mm)		Antifungal Activity Zone of inhibition (in mm)	
		<i>E.Coli</i>	<i>S.aureus</i>	<i>A.niger</i>	<i>A.flavus</i>
1.	[C ₂₆ H ₂₂ Br ₂ N ₆ O ₄]	08	09	09	10
2.	[Cu(C ₂₆ H ₂₀ Br ₂ N ₆ O ₄)]	10	12	15	13
3.	[Co(C ₂₆ H ₂₀ Br ₂ N ₆ O ₄)]	15	114	15	14
4.	[Ni(C ₂₆ H ₂₀ Br ₂ N ₆ O ₄)]	13	14	17	16
5.	[Mn(C ₂₆ H ₂₀ Br ₂ N ₆ O ₄)]	16	13	19	20
6.	[Cd(C ₂₆ H ₂₀ Br ₂ N ₆ O ₄)]	12	14	17	15
7.	[Hg(C ₂₆ H ₂₀ Br ₂ N ₆ O ₄)]	16	14	18	16
8.	[Zn(C ₂₆ H ₂₀ Br ₂ N ₆ O 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^3 = HMCHAD) and its metal (II) complexes.

Sl. No.	Compound	Antibacterial activity zone of inhibition (in mm)		Antifungal activity zone of inhibition (in mm)	
		<i>E.Coli</i>	<i>S.aureus</i>	<i>A.niger</i>	<i>A.flavus</i>
1.	[C ₂₈ H ₂₈ N ₆ O ₄]	09	08	08	10
2.	[Cu(C ₂₈ H ₂₆ N ₆ O ₄)]	10	11	15	12

3.	[Co(C ₂₈ H ₂₆ N ₆ O ₄)]	14	13	14	13
4.	[Ni(C ₂₈ H ₂₆ N ₆ O ₄)]	12	15	17	15
5.	[Mn(C ₂₈ H ₂₆ N ₆ O ₄)]	15	13	20	19
6.	[Zn(C ₂₈ H ₂₆ N ₆ O ₄)]	11	13	16	17
7.	[Cd(C ₂₈ H ₂₆ N ₆ O ₄)]	16	15	18	16
8.	[Hg(C ₂₈ H ₂₆ N ₆ O 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

Table 4: Antimicrobial activity of the ligand (L⁴ = HEBRCC) and its metal complexes.

Sl. No.	Compound	Antibacterial Activity Zone of inhibition (in mm)		Antifungal Activity Zone of inhibition (in mm)	
		<i>E.Coli</i>	<i>S.aureus</i>	<i>A.niger</i>	<i>A.flavus</i>
1.	C ₁₈ H ₁₃ BrN ₂ O ₄	10	08	08	09
2.	[Cu(C ₃₆ H ₂₄ Br ₂ N ₄ O ₈)]	12	13	12	13
3.	[Co(C ₃₆ H ₂₄ Br ₂ N ₄ O ₈)]	13	12	13	12
4.	[Ni(C ₃₆ H ₂₄ Br ₂ N ₄ O ₈)]	17	15	15	16
5.	[Mn(C ₃₆ H ₂₄ Br ₂ N ₄ O ₈)]	16	15	19	18
6.	[Fe(C ₁₉ H ₁₇ BrN ₂ O ₅)Cl ₂ . H ₂ O]	17	16	13	14
7.	[Zn(C ₁₈ H ₁₂ BrN ₂ O ₄)Cl]	18	20	15	16
8.	[Cd(C ₁₈ H ₁₂ BrN ₂ O ₄)Cl]	20	20	17	16
9.	[Hg(C ₁₈ H ₁₂ BrN ₂ O ₄)Cl]	12	13	20	19
10.	Streptomycin	25	24	-	-
11.	Chlotrimazole	-	-	24	26
12.	DMF (Control)	0	0	0	0
13.	Bore size	08	08	08	08

Table 5: Antimicrobial activity of the ligand (L⁵ = HECHCC) and its metal complexes.

Sl. No.	Compound	Antibacterial Activity Zone of inhibition (in mm)		Antifungal Activity Zone of inhibition (in mm)	
		<i>E.Coli</i>	<i>S.aureus</i>	<i>A.niger</i>	<i>A.flavus</i>
1.	C ₁₉ H ₁₆ N ₂ O ₄	10	-	08	09
2.	[Cu(C ₃₉ H ₃₄ N ₄ O ₈)]	13	13	14	13
3.	[Co(C ₃₉ H ₃₄ N ₄ O ₈)]	14	12	12	14
4.	[Ni(C ₃₉ H ₃₄ N ₄ O ₈)]	16	15	16	17
5.	[Mn(C ₃₉ H ₃₄ N ₄ O ₈)]	15	16	19	20
6.	[Fe(C ₁₉ H ₁₇ N ₂ O ₅ Cl ₂).H ₂ O]	15	17	12	14
7.	[Zn(C ₁₉ H ₁₅ N ₂ O ₄)Cl]	20	20	15	16
8.	[Cd(C ₁₉ H ₁₅ N ₂ O ₄)Cl]	18	20	15	16
9.	[Hg(C ₁₉ H ₁₅ N ₂ O ₄)Cl]	13	11	18	19
10.	Streptomycin	25	24	-	-
11.	Chlotrimazole	-	-	24	26
12.	DMF (Control)	0	0	0	0
13.	Bore size	08	08	08	08

Table 6: Antimicrobial activity of the ligand (L^6 = HMOHBA) and its metal complexes.

Sl. No.	Compound	Antibacterial Activity Zone of inhibition (in mm)		Antifungal Activity Zone of inhibition (in mm)	
		<i>E.Coli</i>	<i>S.aureus</i>	<i>A.niger</i>	<i>A.flavus</i>
1.	[C ₁₇ H ₁₁ IN ₂ O ₃]	08	09	10	08
2.	[(CuC ₃₄ H ₂₄ I ₂ N ₄ O ₈)]	10	11	15	13
3.	[(CoC ₃₄ H ₂₀ I ₂ N ₄ O ₆)]	13	14	15	13
4.	[(NiC ₃₄ H ₂₀ I ₂ N ₄ O ₆)]	15	13	17	15
5.	[(MnC ₃₄ H ₂₄ I ₂ N ₄ O ₈)]	12	15	20	19
6.	[(FeC ₃₄ H ₂₂ ICl ₂ N ₄ O ₇)]	13	12	11	12
7.	[(ZnC ₁₇ H ₁₂ IClN ₂ O ₄)]	13	11	17	16
8.	[(CdC ₁₇ H ₁₂ IClN ₂ O ₄)]	15	16	18	16
9.	[(HgC ₁₇ H ₁₂ IClN ₂ O ₄)]	15	18	18	19
10.	Streptomycin	24	23	--	--
11.	Chlotrimazole	--	--	25	26
12.	DMF (Control)	0	0	0	0
13.	Bore size	08	08	08	08

Table 7: Antimicrobial activity of the ligand (L^7 = HMBRBA) and its metal complexes.

Sl. No.	Compound	Antibacterial Activity Zone of inhibition (in mm)		Antifungal Activity Zone of inhibition (in mm)	
		<i>E.Coli</i>	<i>S.aureus</i>	<i>A.niger</i>	<i>A.flavus</i>
1.	[C ₁₇ H ₁₀ IBrN ₂ O ₃]	08	09	10	08
2.	[Cu(C ₃₄ H ₁₈ Br ₂ I ₂ N ₄ O ₆)]	15	14	15	13
3.	[Co(C ₃₄ H ₁₈ Br ₂ I ₂ N ₄ O ₆)]	13	14	18	16
4.	[Ni(C ₃₄ H ₁₈ Br ₂ I ₂ N ₄ O ₆)]	10	11	17	15
5.	[Mn(C ₃₄ H ₂₀ Br ₂ I ₂ N ₄ O ₇)]	13	11	20	19
6.	[Fe(C ₃₄ H ₂₀ Br ₂ I ₂ N ₄ O ₇)Cl]	12	13	17	16
7.	[Zn(C ₁₇ H ₁₁ BrIN ₂ O ₄)Cl]	15	16	11	13
8.	[Cd(C ₁₇ H ₁₁ BrIN ₂ O ₄)Cl]	15	18	18	19
9.	[Hg(C ₁₇ H ₁₁ BrIN ₂ O ₄)Cl]	18	20	17	15
10.	Streptomycin	24	23	--	--
11.	Chlotrimazole	--	--	25	26
12.	DMF (Control)	0	0	0	0
13.	Bore size	08	08	08	08

Table 8: Antimicrobial activity of the ligand (L^8 = HMCHBA) and its metal complexes.

Sl. No.	Compound	Antibacterial Activity Zone of inhibition (in mm)		Antifungal Activity Zone of inhibition (in mm)	
		<i>E.Coli</i>	<i>S.aureus</i>	<i>A.niger</i>	<i>A.flavus</i>
1.	[C ₁₈ H ₁₃ IN ₂ O ₃]	07	09	09	08
2.	[Cu(C ₃₆ H ₂₄ I ₂ N ₄ O ₆)]	11	10	13	15
3.	[Co(C ₃₆ H ₂₄ I ₂ N ₄ O ₆)]	13	14	15	13
4.	[Ni(C ₃₆ H ₂₄ I ₂ N ₄ O ₆)]	16	13	17	15

5.	[Mn(C ₃₆ H ₂₆ I ₂ N ₄ O ₇)]	16	16	20	19
6.	[Fe(C ₃₆ H ₂₆ ClI ₂ N ₄ O ₇)]	12	13	15	17
7.	[Zn(C ₁₈ H ₁₂ ClIN ₂ O ₃)]	13	12	17	15
8.	[Cd(C ₁₈ H ₁₄ ClIN ₂ O ₄)]	12	15	17	16
9.	[Hg(C ₁₈ H ₁₄ ClIN ₂ O ₄)]	16	17	19	18
10.	Streptomycin	24	23	--	--
11.	Chlotrimazole	--	--	25	26
12.	DMF (Control)	0	0	0	0
13.	Bore size	08	08	08	08

The antibacterial and antifungal activities are summarized in the tables

i	Concentration of the test compound : 1 mg/ml in DMF
ii	Quantity of the test compound : 0.1 ml
iii	Diameter of the cup : 8 mm (Antifungal) : 8 mm (Antibacterial)
iv	Standard for antibacterial activity : Streptomycin
v	Standard for antifungal activity : Chlotrimazole
	Key for antibacterial interpretation zone of inhibition in mm
	<i>E. coli</i> and <i>S. aureus</i>
	Less than 10 mm : inactive
	11-13 mm : weakly active
	14-17 mm : moderately active
	18 mm and above : good active
	24-25 mm : Streptomycin
	0 mm : DMF(control)
	Key for antifungal interpretation zone of inhibition in mm
	<i>A. niger</i> and <i>A. flavous</i>
	Less than 10 mm : inactive
	11-13 mm : weakly active
	14-17 mm : moderately active
	18 mm and above : good active
	25-26 mm : Clotrimazole
	0 mm : DMF (Control)

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