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RESEARCH ARTICLE

SYNTHESIS, DOCKING AND ANTIMICROBIAL ACTIVITY OF TRANSITION METAL COMPLEXES OF PHENYLHYDRAZONES

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ABSTRACT

Zinc complexes have been prepared by reacting metal chloride with ortho chlorobenzaldehyde phenylhydrazone and para dimethyl aminobenzaldehyde phenylhydrazone. Docking study has been carried out for prepared complexes by using autodock software against Biotin Carboxylase (3JZI) as a target protein. The antimicrobial activity have been studied and compared with their ligands against Stapylococcus aureus, Bacillus substilis, Escherichia coli, Salmonilla paratyphi, Candida albicans, Aspergillus fumigates which gave significant results of activity.

Key words: *Synthesis, Transition metal complex, Phenylhydrazone, Antibacterial, Antifungal activity, Antimicrobial activity.*

INTRODUCTION

Synthesis of various phenylhydrazones and their complexes with transition metals are reported in the literature[1-9] and found to be active as antibacterial[1-7], antitubercular[8], antileprotic[10], antiviral[11], antimalarial[12] and active against certain kind of tumours[13, 14]. Considering the importance of transition metal complexes with phenylydrazone derivatives, in the present paper, synthesis, characterization, docking and antimicrobial activity of Zn(II) complexes with ortho chlorobenzaldehyde phenylydrazone and para dimethyl amino benzaldehyde phenylydrazone, are reported, docking and difference in antimicrobial activity between the free ligands and complexes were studied.

EXPERIMENTAL

Melting points were determined in open capillaries and were uncorrected. IR spectra were recorded in KBr on Perkin-Elmer spectrometer. All compounds gave satisfactory analysis. Ortho chlorobenzaldehyde, para dimethyl amino benzaldehyde and Zinc chloride were obtained from sigma- Aldrich Ltd and used without further purification. The docking study of synthesized compounds and free ligands were carried out by using autodock software. Biotin carboxylase (3JZI) was selected as a target protein. All compounds were tested for their anti microbial activity against *Stapylococcus aureus, Bacillus substilis, Escherichia coli, Salmonilla paratyphi, Candida albicans, Aspergillus fumigates* at concentration of each 100 µg/disc by cup-plate method using Ciprofloxacin (10µg/disc) as standard for antibacterial and Clotrimazole (10µg/disc) as standard for antifungal activity.

General method of synthesis of Ortho chlorobenzaldehyde phenylydrazone (4) and Para dimethylamino benzaldehyde phenylydrazone (5)

Ortho chlorobenzaldehyde, Para dimethyl aminobenzaldehyde (0.02 mole) in 15ml ethanol was added to aqueous solution of phenyl hydrazine Hydrochloride (0.08 mole) and sodium acetate (0.1 mole), the mixture was heated at 80-90 ºC for 10 minutes and then left to cool, the precipitate was collected and purified by crystallization from ethanol to give compounds (4-5) as crystals, yields 82.78% and 83.85% respectively. (Fig-1and 3)

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General method of synthesis of Complexes Ortho chlorobenzaldehyde phenylydrazone, Para dimethyl amino benzaldehyde phenylydrazone with Zinc (II) (4a and 5a).
Ortho chlorobenzaldehyde phenylydrazone, Paradimethyl aminobenzaldehyde phenylydrazone, (0.02 Mole) was dissolved in 15ml ethanol and was added to dissolved zinc chloride (0.001) in 15ml ethanol. The mixture was heated at 60ºC for 2-4hrs and then left to cool. The precipitate was collected and purified by crystallization from ethanol to give compounds (**4a &5a**) as crystals, yields 90.04% and 92.45% respectively. (Fig-2 and 4)

Docking studies of Complexes and free ligands into active sites of Biotin carboxylase (3JZI).
Biotin carboxylase (3JZI) was retrieved from the Protein Data Bank (PDB). It is repository for the 3D structural data of large biological molecules such as protein and nucleic acid. It is widely distributed in nature and has important function in fatty acids, cholesterol, and amino acid metabolism, gluconeogenesis, insulin secretion and other cellular processes. It functions as a cofactor that aids in the transfer of CO_2 groups to various target macromolecules. After obtaining PDB id (3JZI), the possible binding sites were searched using computed atlas of surface topography of proteins. These include pockets located on protein surfaces and voids buried in the interior of protein. The inhibitors against the active site of proteins. It is a computational technique. The samples conformation of small molecules in protein binding sites, Scoring functions are used to asses which of these conformations best the protein binding sites. The inhibitor and target protein was geometrically optimized and docked using docking Eigen Autodock-vina and Autodock-4.

In vitro antimicrobial activity of free ligands (4-5) and their metal complexes (4a-5a).
The invitro antibacterial and antifungal activity of free ligands (4-5) and their metal complexes (4a-5a) was carried out against *Stapylococcus aureus, Bacillus substilis, Escherichia coli, Salmonilla paratyphi, Candida albicans, Aspergillus fumigates* using serial dilution technique in double strength nutrient broth for Antibacterial and Sabouraud dextrose broth as medium for antifungal. The ligands and metal complexes were dissolved in DMSO to the concentration of 100µg/disc.

Antibacterial assay- 2 petridishes for Gram-positive organisms (*Stapylococcus aureus* and *Bacillus* substilis) and 2 petridishes for Gram-negative organisms (*Escherichia coli* and Salmonilla *paratyphi*). Each dish is divided into 5 quadrants and name the each quadrant of the disc as 1, 1a, 2, 2a (100µg/disc) and 1 quadrant for standard Ciprofloxacin (10µg/disc). The ligand and metal complexes were placed in each plate with the help of sterile swabs. Then petridishes were placed in refrigerator for diffusion at 4º C for 1 h and incubate at 37ºC for 2hrs. Observe the zone of inhibition produce by free ligand and complexes.

Antifungal assay-2 petridishes for antifungal organisms (*Candida albicans* and *Aspergillus fumigates*). Each dish is divided into 5 quadrants and name the each quadrant of the disc as 4, 4a, 5, 5a (100µg/disc) and 1 quadrant for standard Clotrimazole (10µg/disc). The ligand and metal complexes were placed in each quadrant with the help of sterile swabs. Then petridishes were placed in refrigerator for diffusion at 4º C for 1 h and incubate at 37ºC for 2hrs. Observe the zone of inhibition produce by free ligands and complexes.

RESULTS AND DISCUSSION

Phenylydrazones (4-5) were prepared from ortho chlorobenzaldehyde and para dimethyl aminobenzaldehyde which have a good crystalline yield. The reaction of ortho chlorobenzaldehyde and para dimethyl aminobenzaldehyde with Phenyl hydrazine HCl in ethanol gave a brown crystal in high yield.

In the complexes (4a-5a), the reaction of ortho chlorobenzaldehyde phenylydrazone with Zinc chloride gave pale brown crystals (4a) and the reaction of para dimethyl aminobenzaldehyde phenylydrazone with Zinc chloride gave dark brown crystals (5a). All the compounds are stable at room temperature and insoluble in water. Some physical properties, analytical and spectral data of the compounds are summarized in the table1.

Table-1: Analytical and spectral data of the free ligands and its metal complexes

Compounds No.	Compounds Colour	m.p (ºC)	Key IR band, cm^{-1}	Molecular formula	Mol.wt	%yield
4	Brown	110-112	1609(C=N), 3397(N-H), 3443(C-H aromatic)	$C_{13}H_{11}N_2Cl$	230.5	82.78
5	Brown	174-176	1600(C=N), 3483(N-H), 11034(C-N), 3315(C-H aromatic)	$C_{15}H_{17}N_3$	239.0	83.85
4a	Pale brown	180-182	1579(C=N), 3373(N-H), 3201(C-H aromatic)	$C_{26}H_{20}N_4Cl_2Zn$	524.7	90.04
5a	Dark brown	244-246	1539(C=N), 3460(N-H), 1068(C-N), 3304(C-H aromatic)	$C_{30}H_{32}N_6Zn$	542.0	92.45

The IR spectra of free ligands (4-5) show broad bands 3397 and 3483 cm^{-1}, which corresponds to (N-H) of imino group. The IR spectrum of all the complexes (1a-2a) shows downshift in (N-H) of imino by about 3373-3460cm^{-1}. These may be due to co-ordinate bond formation through nitrogen of imino group [15]. The IR spectral of ligands (4-5) show bands at 1609 and 1600 cm^{-1}, which may be due to (C=N) of phenylhydrazines. The IR spectra of all the complexes (4a-5a) shows downshift (C=N) of phenylhydrazones by 30-61cm^{-1}. These may be due to co-ordinate bond formation through nitrogen of imino group [16-17].

Molecular modeling (docking) study was carried out for ligands (4-5) and their transition metal complexes with Zn (II) (4a-5a). The target protein and inhibitors were geometrically optimized. The 3D structures of a target protein receptor molecule usually a protein, chemical compounds potential affinity toward site are rationally designed with aid of computational methods. All 4 compounds were docked against active site of target proteins. Out of 4 inhibitors analyze all 2 complexes (4a-5a) showed higher binding energy -7.69 kgcal/mole and -6.36 kgcal/mole respectively against the target proteins. The binding energy of all the inhibitors was show in table 2. Figs 5&6 represent the docked structure of ligand and a fig 7&8 represents the docked structure of complexes of the inhibitors to that of target protein.

Table-2: Energy Minimization Table of free ligands and its metal complexes

Compounds Code	Hydrogen bond interaction	H bond length (Aº)	Auto docking Score
4	Asp 17 : O	2.864	-2.8
5	Glu 95 : O	2.796	-4.92
4a	-	-	-7.69
5a	-	-	-6.36

The Table-3 describes the molecular properties of the free ligand (4-5) and its transition metal complexes with Zinc (4a-5a). All complexes satisfy all the criteria of Lipinski rule of five [18-19].

Table-3: Molecular properties of free ligands and its metal complexes

Compounds Code	Mi log p	TPSA	n atoms	n ON	n OHNH	n violations	n rotb	Volume
4	5.393	24.391	16	2	1	1	3	204.651
5	4.800	27.629	18	3	1	0	4	237.021
4a	-0.812	17.872	33	4	0	1	4	421.364
5a	-1.371	24.348	37	6	0	0	6	463.454

The complexation of biologically important metal [Zn (II)] with Phenylhydrazones and free ligands were further exploded with the evaluation of the anti-microbial activity. The ligands (4-5) each 100µg/disc and the metal complexes (4a-5a) each 100µg/disc were evaluated for invitro antibacterial activity against gram (+) ve *stapylococcus aureus* (MTCC2901), *Bacillus substilis* (MTCC2063) and gram (-)ve *Escherichia coli* (MTCC1652), *Salmonilla paratyphi* (MTCC) using Ciprofloxacin (10µg/disc) as standard (Table-4) and invitro antifungal activity against *Candida albicans* (MTCC183*), Aspergillus fumigates* (MTCC). Double strength nutrient broth and Sabouraud dextrose broth[20] were employed as medium for bacterial and fungal growth respectively. Minimum inhibitory concentrations (MIC) were determined by means of serial standard dilution method [21] and are presented in the table-5. All the ligands/ complexes exhibited appreciable invitro activity against the tested strains. The metal complexes (4a-5a) show good antibacterial and antifungal activity. These results indicate that the increases in the size of the transition metal complex with Phenyl hydrazones possess significant anti microbial activity. (Fig.9-12)

Table-4: Invitro antibacterial activity of ligands and its metal complexes (MIC in µg/ml)

Compounds code	Antibacterial activity (Zone of inhibition)			
Standard [Ciprofloxacin (10µg/disc)]	***Staphylococcus aureus***	***Bacillus substilis***	***Escherichia coli***	***Salmonella paratyphi***
	31	34	28	35
4(100 µg/disc)	10	11	9	9
4a (100 µg/disc)	22	23	21	18
5 (100 µg/disc)	11	12	11	09
5a (100 µg/disc)	17	14	12	18

Table-5: Invitro antifungal activity of ligands and its metal complexes (MIC in µg/ml)

Compound code	Antifungal activity (Zone of inhibition)	
	Candida albicans	***Aspergillus fumigates***
Std [Clotrimazole (10µg/disc)]	11	18
4 (100 µg/disc)	21	34
4a(100 µg/disc)	38	46
5 (100 µg/disc)	19	21
5a (100 µg/disc)	26	29

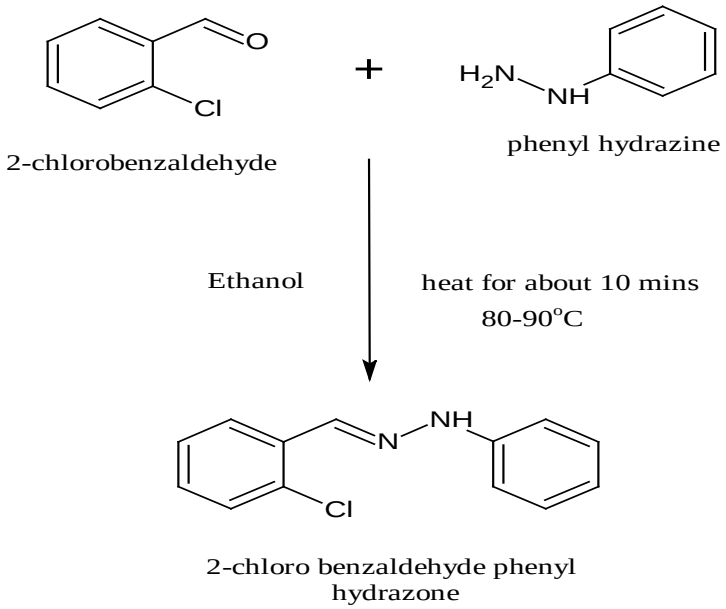

Fig.-1: Synthesis of Compound 1(O-chlorobenzaldehyde phenylhydrazone)

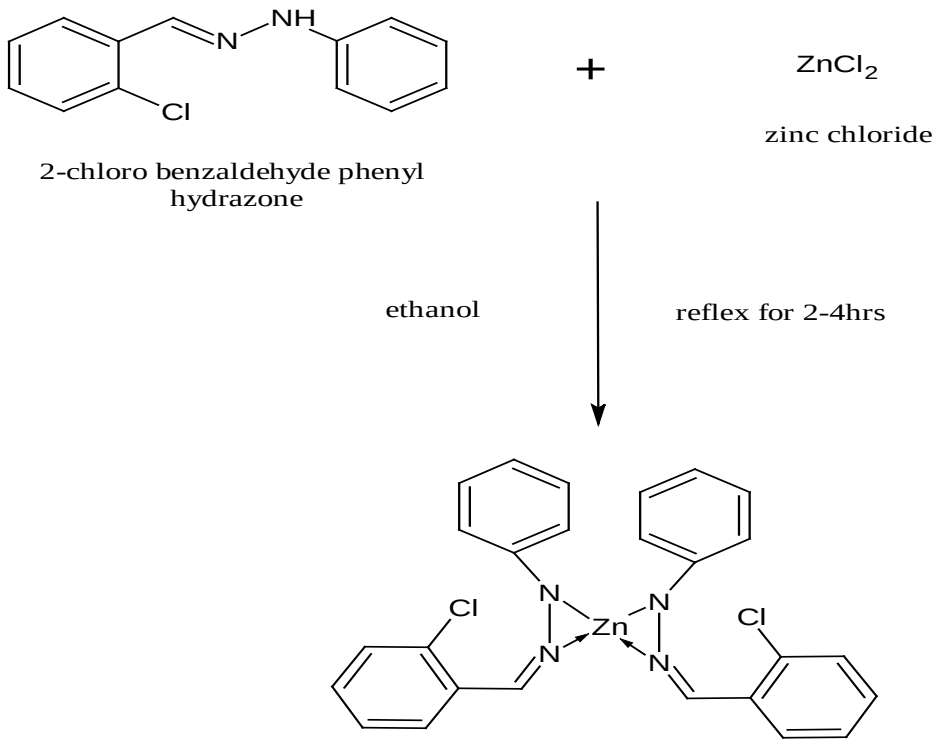

Fig.-2: Synthesis of metal complex of O-chloro benzaldehyde phenylhydrazone (1a)

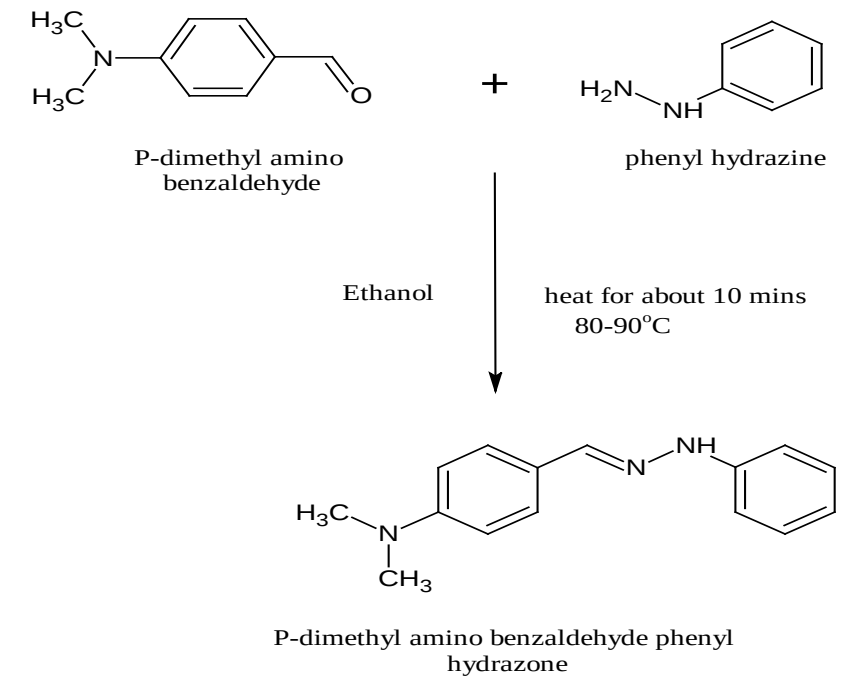

Fig.-3: Synthesis of Compound 2 (P-dimethyl aminobenzaldehyde phenylhydrazone)

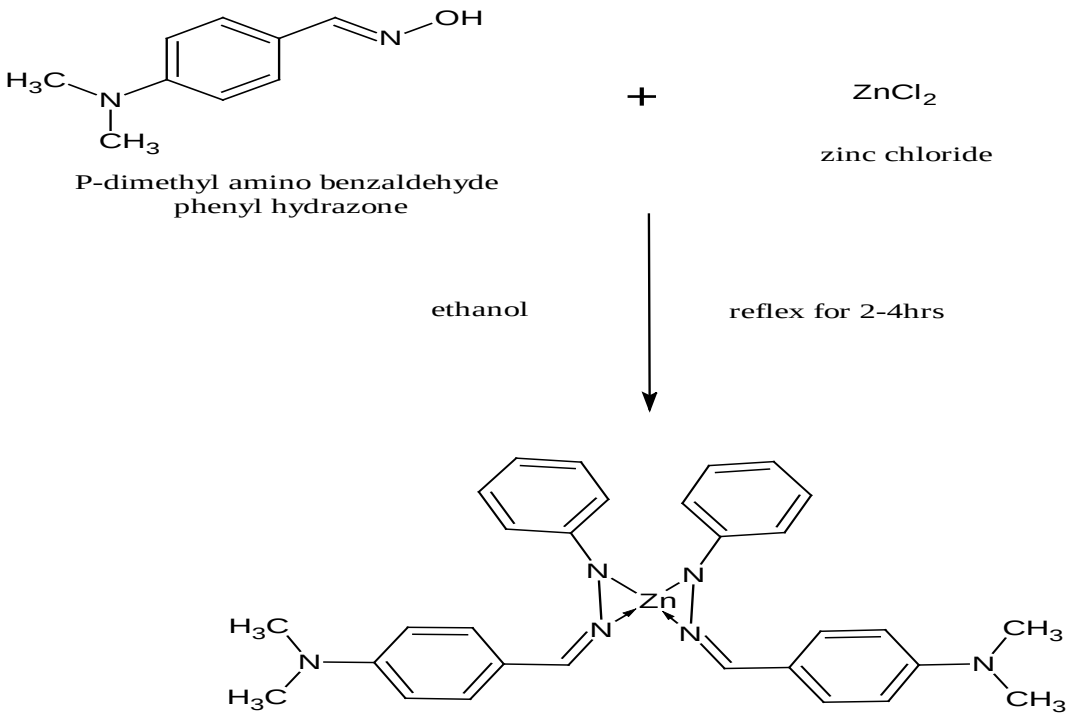

Fig.-4: Synthesis of metal complex of P-dimethyl aminobenzaldehyde phenylhydrazone (2a)

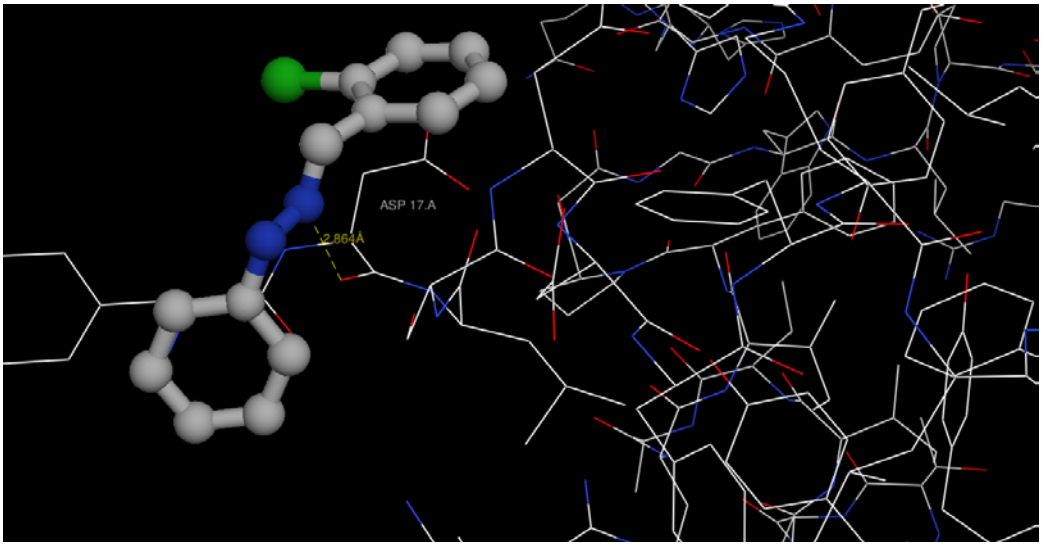

Fig.-5: Dockig structure of free legand (Compound-4)

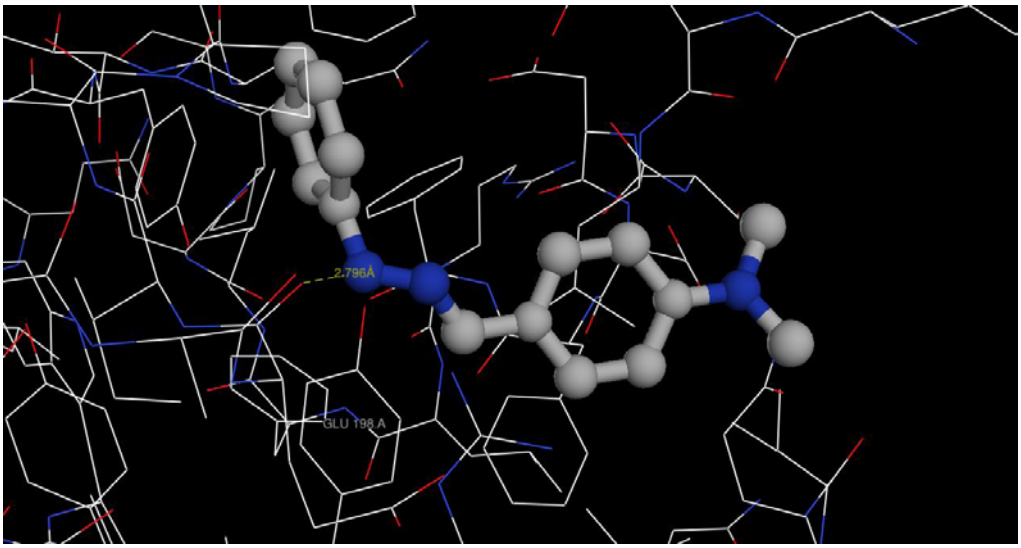

Fig.-6: Dockig structure of free legands (Compound-5)

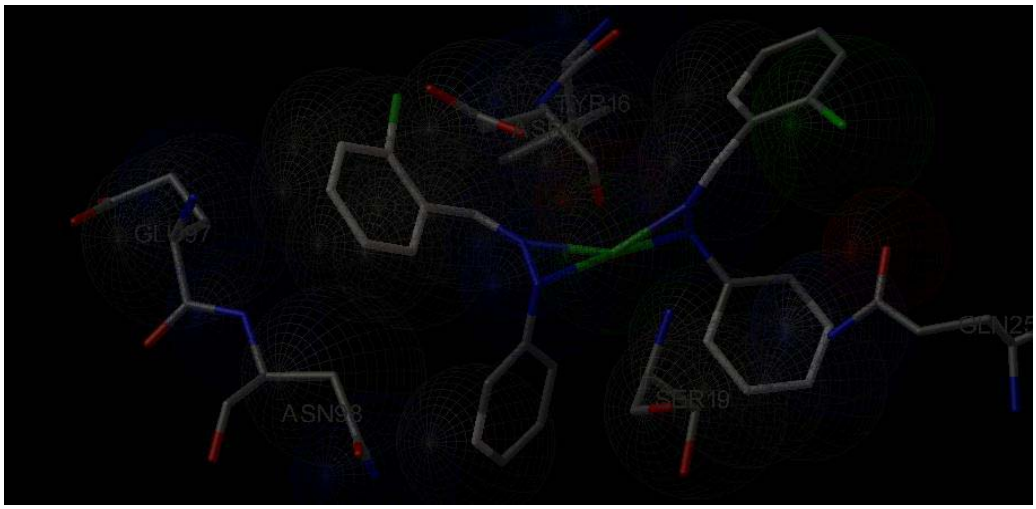

Fig.-7:-Dockig structure of complex (Compound-4a)

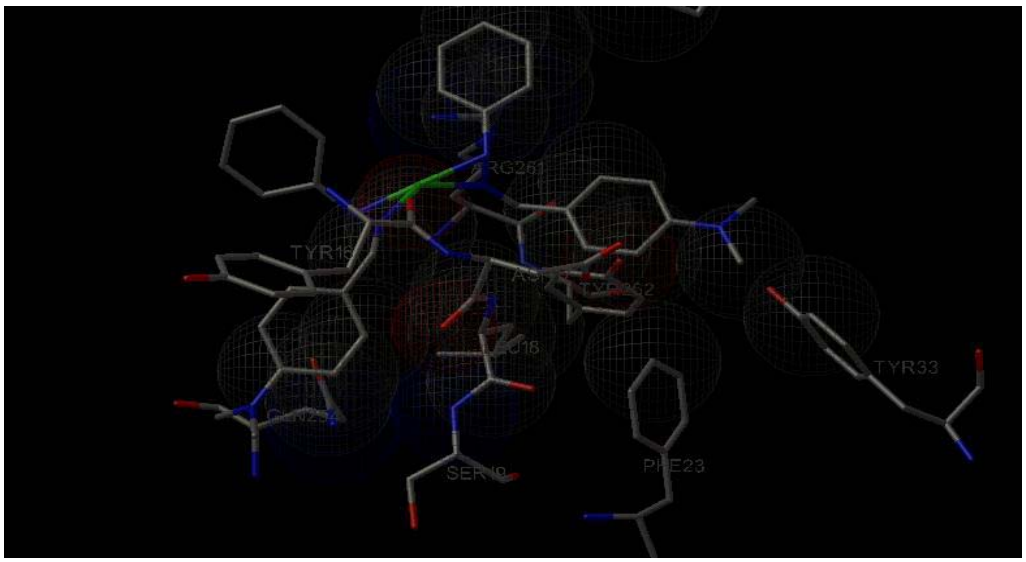

Fig.-8: Dockig structure of complex (Compound-5a)

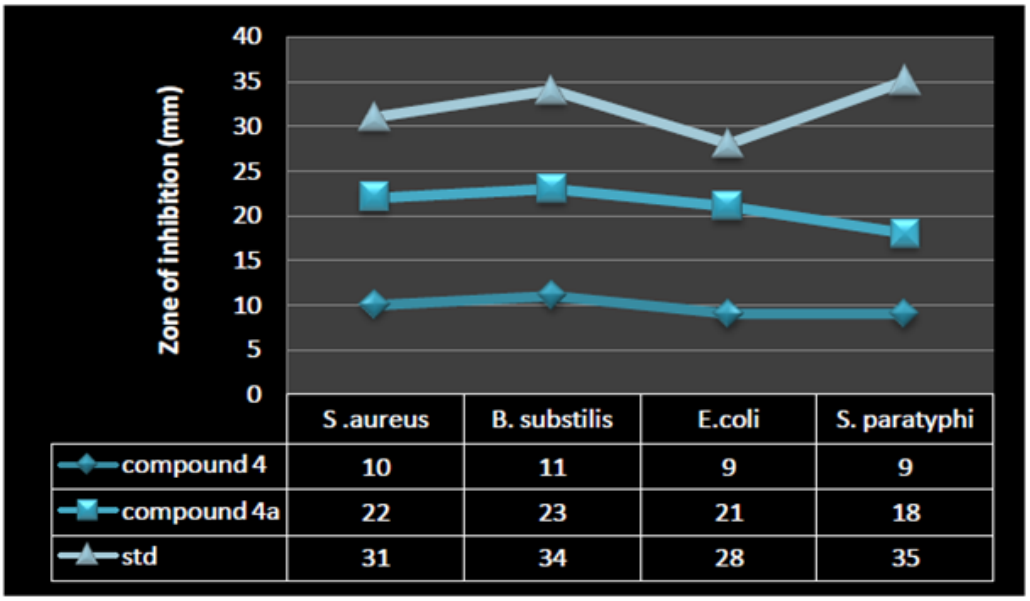

Fig.-9: Anti-bacterial value of free ligand and metal complexs (Compound-4 & 4a)

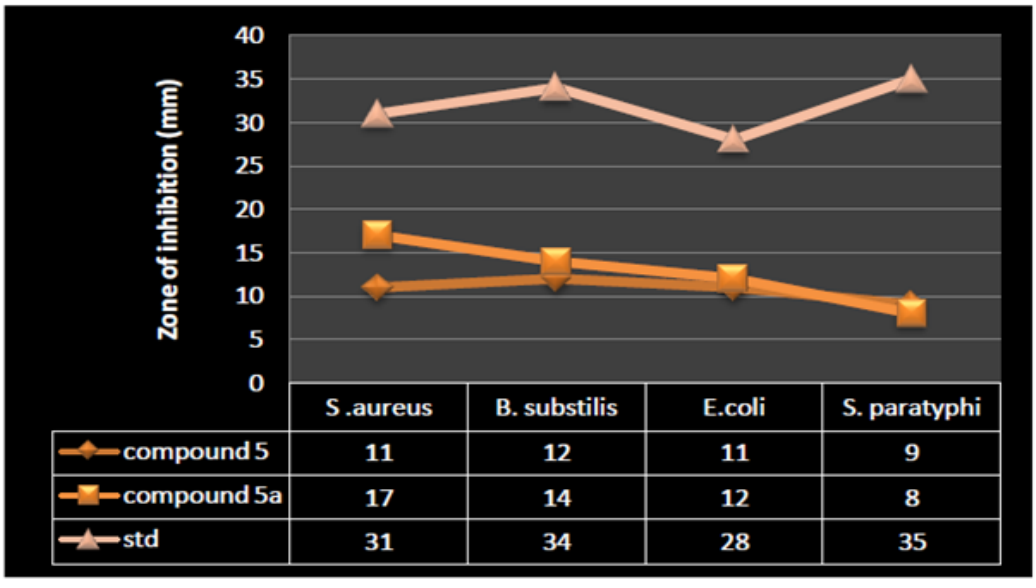

Fig.-10: Anti-bacterial value of free ligand and metal complexs (Compound-5 &5a)

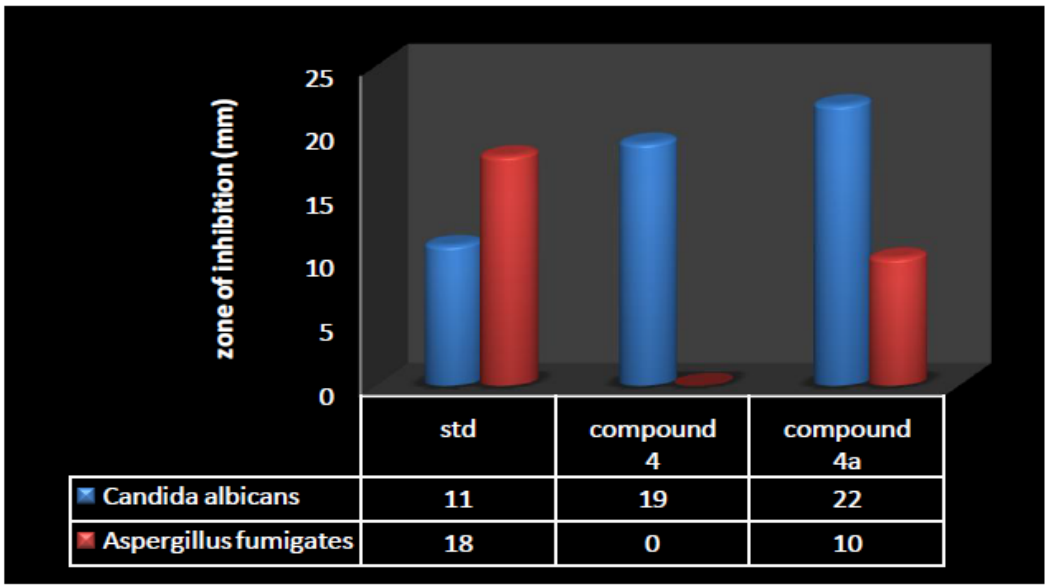

Fig.-11: Anti-fungal value of free ligand and metal complexs (Compound-4 & 4a)

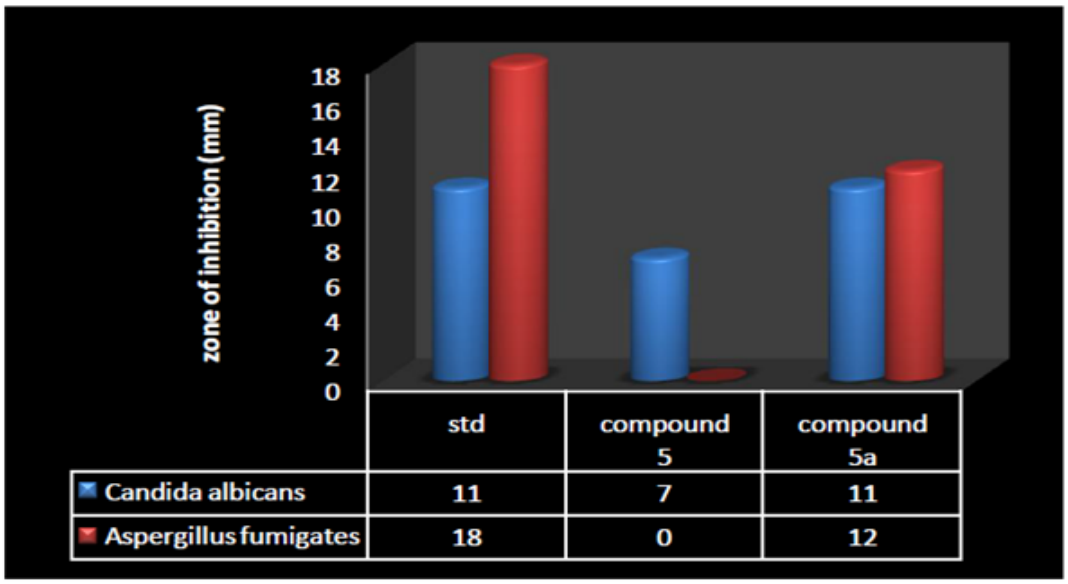

Fig.-12: Anti-fungal value of free ligand and metal complexs (Compound-5& 5a)

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