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SCREENING STUDIES ON ANTIMICROBIAL AND ANTICANCER ACTIVITY OF CO (II) COMPLEX WITH 2–METHOXY-6-(8-IMINOQUINOLINYL METHYL) PHENOL

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ABSTRACT:
Compounds o-vanillin and8-aminoquinolinewere condensed in 1:1 molar ratio to derive a Schiff base ligand . ThenCo (II)metal complex was prepared using the metal salt and Schiff base ligand in 1:2 ratio. The metal complex was characterized by using elemental thermal and spectral analysis. The compositions of the Schiff base and its metal complex were established by elemental analysis which indicated a metal: ligand ratio of 1:2 and metal complex revealed its general formula as $[ML_2(H_2O)_2]$ where M=Co(II) and L=deprotonated Schiff base ligand. The in-vitro antibacterial activities of the metal complexwere tested using bacterial speciesStaphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa and Escherichia coli. These compounds were also tested for antifungal activities against Candida albicans and Aspergillus niger. The Schiff basewas also screened for its anticancer activities against breast cancer MCF-7 cell line and colon cancer HT-29 cell line by sulforhodamine-B (SRB) assay.The Schiff base and its Co(II) complex showed anticancer activity against breast cancer MCF-7 cell line while Co(II)complex showed no activity against colon cancer HT-29 cell line.

KEYWORDS: *Synthesis, Schiff base ligand , transition metal complex, antimicrobial activity, anticancer activity.*

INTRODUCTION:

Schiff bases are widely used as chelating ligands in coordination chemistry. The metal complexes of Schiff bases prepared from heterocyclic compounds have attracted researchers in recent years. Schiff bases derived from aromatic aldehydes and amines have applications in various fields like biological, inorganic and analytical chemistry. Schiff bases can be synthesized byrelatively by simple procedures enabling us to design compounds which are structurally similar to some substances of biological origin [1-3].

It is well known that N and O atoms play a key role in the co-ordination of metals at the active sites of many metallic-biomolecules [4].Transition metal complexes have been widely studied because they have various properties like antibacterial, antifungal, anticancer, herbicidal, analytical etc. They find wide applications as

catalysts in many synthetic and biological reactions.Besides, they can undergo bonding with metal ions in a variety of ways and the resulting compounds show varied activities. 8-aminoquinoline and o-vanillin are especially known for their antibacterial and antifungal activities and are thus used as synthetic precursors in pharmaceuticals. O-vanillin is flavor and aroma compound in vanilla which is used in food flavoring and pharmaceuticals. Schiff bases containing 8-aminoquinoline and o-vanillin Moiety form stable complexes with significant bioactivities. Considering the distinct biological activities of these compounds, in continuation of our work, we herein put forth an account of the synthesis and characterization of ligand complexes of Co(II) derived from 8-aminoquinolline and o-vanillin and the biological screening of Schiff base ligand and its complexes against different bacteria, fungi and cancer cell lines [5].

2. EXPERIMENTAL:

2.1 Apparatus:

The antibacterial and antifungal activity of Schiff base-4 and its cobalt metal complex was carried out by standard agar well diffusion method and agar ditch method respectively for which pure R and D purpose strains of bacteria and fungi were obtained from National Collection of Industrial Micro-organisms, NCL, Pune. The anticancer activity was determined at ACTREC, Tata Memorial Centre, Navi Mumbai by standard SRB assay.

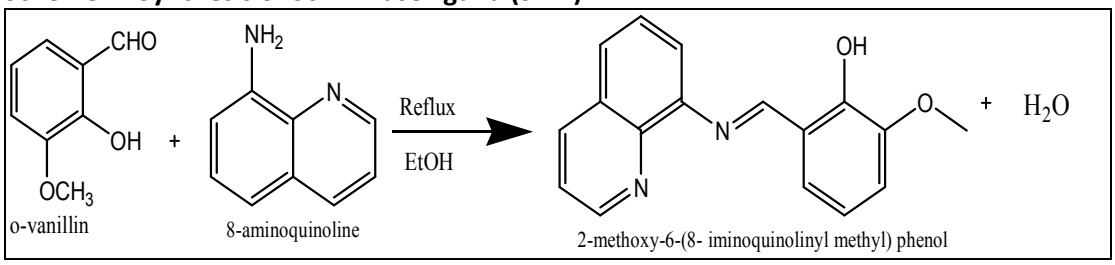
2.2 Reagents:

Analytical grade chemicals - Sigma- Aldrich o-vanillin, 8-aminoquinoline, andStandard Qualigens (SQ) hydrated Cobalt (II) chloride was used for synthesis. The solvents AR grade absolute ethanol,DMSO etc. were used.

2.3 Synthesis of Schiff base ligand (SB-4)

20 mmol (3.04g) of o-vanillin is dissolved in absolute ethanol and added dropwise into 20 mmol (2.88g) of ethanolic solution of 8-aminoquinoline. The mixture was refluxed for 2hours then cooled and filtered [6-7]. Intense red colored crystals of Schiff base SB-4 were formed (yield 89%, 5 g). The crystals were washed with anhydrous diethylether and dried over anhydrous$CaCl_2$[6-10]The synthesis of Schiff base ligand (SB-4) is shown in Scheme-1

Scheme-1: Synthesis of Schiff Base ligand (SB-4)

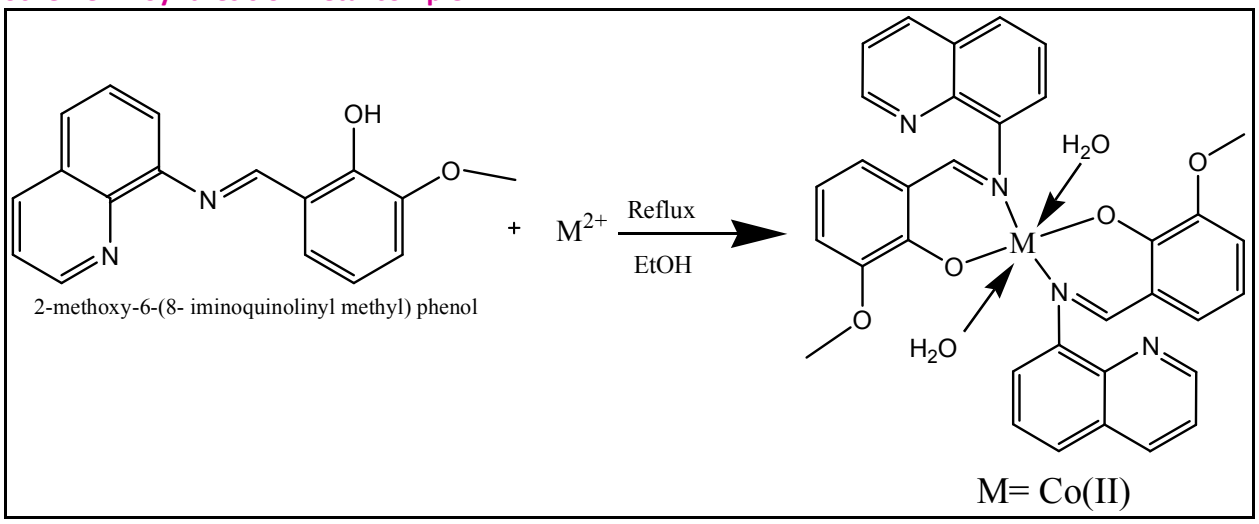


2.4 Synthesis of Schiff base transition metal complex:

10 mmol ethanolic solution (20ml) of metal (II) chloride salt was added to 20 mmol of Schiff base (SB-4) dissolved in ethanol (30ml). The metal-ligand molar ratio taken was 1:2. The mixture was refluxed for 3 hours

when a crystalline metal complex was obtained on cooling. It was filtered, recrystallized from ethanol and dried in desiccator over anhydrous $CaCl_2$. The Co(II) metal complex of Schiff base (SB-4) were prepared by above general method using salts $CoCl_2.6H_2O$[11-19]. The preparation of metal complex is shown in Scheme -2.

Scheme -2: Synthesis of metal complex:



Schiff base (SB-4) and its Co (II) complex synthesized were stable at room temperature and soluble in common solvents like DMSO, methanol etc. The Schiff base and the metal complex were characterized by elemental, thermal and spectral analysis and their molecular formulae were determined. Biological activity namely antibacterial, antifungal and anticancer activity of the ligand and its metal complexwere studied.

3. RESULTS AND DISCUSSION:

3.1 Analytical and Physicochemical data:

The stoichiometry of ligand and their metal complexes is confirmed by the elemental and spectral analysis. The analytical and physicochemical data of Schiff base (SB-4) and its metal complexes is found in good agreement with the proposed structure of ligand and the metal complexes.[20-26] The data being listed in Table-1

Table-1: Analytical and Physicochemical data of Schiff base (SB-4) and its metal complex.

Comp.	Molecular formula	M. p. (°C)	Mol. Weigh t	Color	% Observed (Theoretical)			
					C%	H%	N%	M%
SB-4	$C_{17}H_{14}O_2N_2$	103	278	Red	72.91 (73.38)	4.87 (5.03)	9.98 (10.07)	-
SB-4-Co	$C_{34}H_{26}O_4N_4Co.(H_2O)_2$	>350	648.93	Dark Pink	62.16 (62.87)	4.77 (4.62)	8.47 (8.62)	8.88 (9.08)

3.2. Antibacterial and Antifungal activity:

The antibacterial and antifungal studies of the Schiff base (SB-4) and its metal complexes SB-4-Co were tested on Gram positive bacteria such as *S.aureus* and *B. cereus* and Gram negative bacteria such as

*P.auruginosa*and *E.coli* while their antifungal activities were tested on fungi *C.albicans* and *A.niger*. Well-known agar-well diffusion method was used for studies on antibacterial activity and Agar-Ditch method for studies on antifungal activity [20-25]. The stock solutions of Schiff base and metal complex of concentration 1000 µg/ml were prepared and used to prepare their various concentrations of 100,200,300,400 and 500 µg/ml. The bacteria and fungi were incubated on the surface of Nutrient agar and Sabouraud's agar respectively, the various concentrations of the compounds were incubated in the wells and ditches prepared on the agar plates. The plates were incubated at room temperature for 24 hours for bacteria and 48 hours for fungi. In order to clarify the effect of DMSO for its antimicrobial activity by agar plate assay, separate studies were carried out with DMSO and showed no activity against any bacteria and fungi. The standards Gentamycin and Fluconazole are used for antibacterial and antifungal studies respectively. The results are as summarized in the Table-2. Metal complex in general exhibited better antibacterial and antifungal activity than ligand. Co(II) complex exhibited good antimicrobial activity againstGram positive bacteria*S.aureus* and *B. cereus* and Gram negative bacteria*P.auruginosa.*

Table-2: Antibacterial and Antifungal activities of ligand (SB-4) and its Co (II)Complex

Compound	Concentration µg/ml	S.aureus	B.cereus	P.auruginosa	E.coli	C.albicans	A.niger
SB-4	100	100	5+	5+	14++	3-	2-
	200	200	6+	8+	16++	4-	3-
	300	300	8+	10++	19++	5+	4-
	400	400	10++	11++	22+++	6+	5+
	500	500	12++	13++	23+++	8+	5+
SB-4-Co	100	8+	15++	15++	12++	3-	2-
	200	10++	16++	17++	13++	4-	3-
	300	12++	17++	20+++	15++	5+	5+
	400	16++	19++	23+++	17++	9+	5+
	500	18++	21+++	24+++	20+++	11++	8+
Standard	100	20+++	25+++	21+++	20+++	21+++	22+++
	200	23+++	27+++	28+++	23+++	25+++	25+++
	300	25+++	30+++	30+++	28+++	30+++	29+++
	400	25+++	35+++	35+++	32+++	35+++	34+++
	500	38+++	37+++	40+++	35+++	42+++	40+++
DMSO	100	1-	1-	2-	1-	1-	1-
	200	2-	2-	2-	2-	1-	1-
	300	2-	2-	3-	3-	2-	1-
	400	3-	3-	3-	3-	2-	2-
	500	3-	3-	3-	3-	2-	2-

Activity Scale	- ve= Inactive (Zone of inhibition <5mm)
	+ve= weakly active (5 ≤Zone of inhibition < 10mm)

	++ve= moderatively active (10 ≤ Zone of inhibition < 20mm)
	+++ve= Highly active (Zone of inhibition ≥20mm)
Standard	Gentamycin for study of antibacterial activity.
	Fluconazole for study of antifungal activity.

3.3 Anticancer activity studies:

The anticancer activity of the ligand (SB-4) and its Co(II) Complex was determined by sulforhodamine -B assay on human breast cancer cell line MCF-7 and human colon cancer cell line HT-29 at ACTREC, Tata Memorial Centre, Kharghar, Navi Mumbai. The cell lines were cultured in RPMI 1640 medium, supplemented with 10% fetal bovine serum (FBS) and 2millimolar L- glutamine at 37^0C in a humidified atmosphere of 5% CO_2. About $5X10^3$ cells/well were seeded in 96-well micro titer plate using a culture medium. After 24 hours, Schiff base (SB-4) and its Co(II) , Ni(II) and Cu(II) metal complexes at the concentrations of 10,20,40 and 80 µg/ml were added to respective wells at a single concentration and incubated for 48 hours. After incubation the sulforhodamine-B assay was performed [20-26].

Ligand (SB-4) and its Co(II) complex are showing anticancer activity on human breast cancer cell line MCF-7 in the assay system used with GI50 near or less than 10 µg/ml which is comparable to that of Adriamycin, a standard positive control drug with GI50 value less than 10 µg/ml. Therefore ligand and complex may prove as lead compounds for *in vivo* screening of anticancer activity against malignant breast cancer.

However ligand (SB-4)and its Co(II) complex are resistant to human colon cancer cell line HT-29 with GI50, 45.5µg/ml, and 43.0µg/ml.

The results of cytotoxicity of ligand (SB-4) and its complexes on human breast cancer cell lines MCF-7 and colon cancer cell lines HT-29 are shown in Table 3 and Table 4.

Table -3: Cytotoxicity of Schiff base (SB-4) and its Co (II) metal complex on human breast cancer cell line (MCF-7

Sr.No	Compound	Drug Concentrations (µg/ml)		
		LC 50	TGI	GI-50
1	SB-4	>80	53.35	<10
2	SB-4-Co	NE	NE	23.05
3	ADR	>80	30.11	<10

Table 4: Cytotoxicity of Schiff base (SB-4) and its Co (II) metal complex on human colon cancer cell line (HT-29)

Sr.No	Compound	Drug Concentrations (μg/ml)		
		LC 50	TGI	GI-50
1	SB-4	>80	71.2	45.5
2	SB-4-Co	>80	>80	43.0
3	ADR	NE	<10	<10

Value GI50* of <10 µg/ml- super active 10-15 µg/ml- Moderately active 15-30 µg/ml – Weakly active 30-80 µg/ml – Resistant > 80 µg/ml – Inactive	GI50= Concentration of drug causing 50% inhibition of cell growth TGI= Concentration of drug causing total inhibition of cell growth. LC50= Drug Concentration that kills 50% of the cells NE = Non evaluable data ADR= Adriamycin (Doxorubicin, Positive control drug).

In -vitro testing for anticancer activity in cell lines based on GI50 values shows that ligand SB-4and its Co(II) , Ni(II) and Cu(II) metal complexes are more active against human breast cancer cell line MCF-7 than human colon cancer cell lines HT-29.[27-31] Cytotoxicity of ligandSB-4 and its complexes on human breast and colon cancer cell lines is shown in Figure-1 and Figure-2.

Figure-1 : Cytotoxicity of Schiff bases (SB-4) and its Co(II) metal complex on human breast cancer cell line (MCF-7).

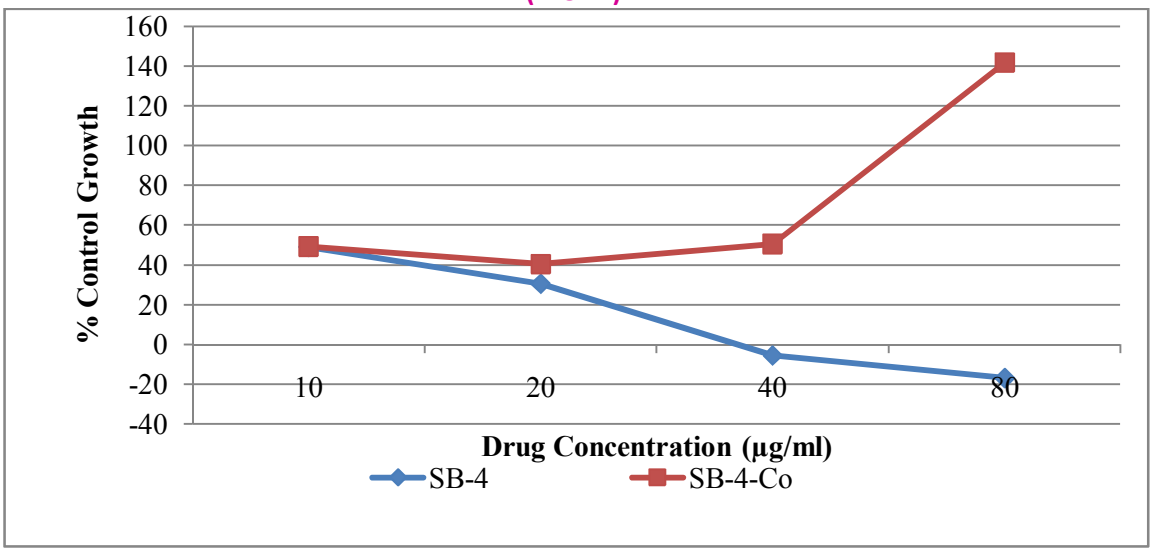
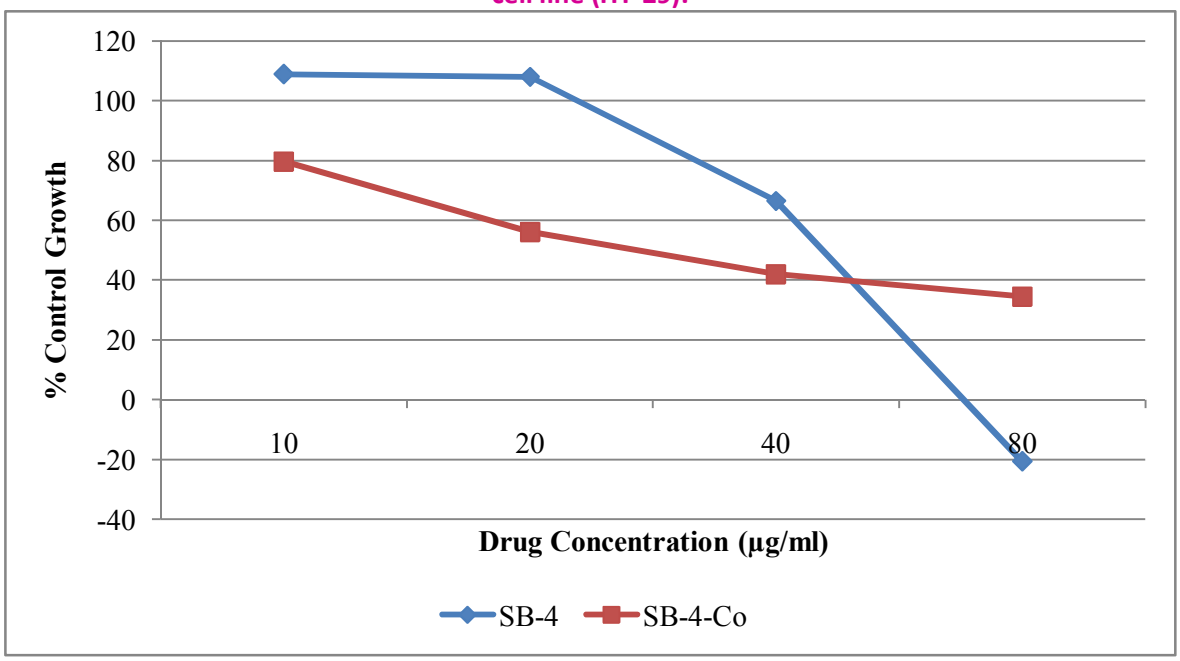


Figure-2 : Cytotoxicity of Schiff bases (SB-4) and its Co (II) metal complex on human colon cancer cell line (HT-29).



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