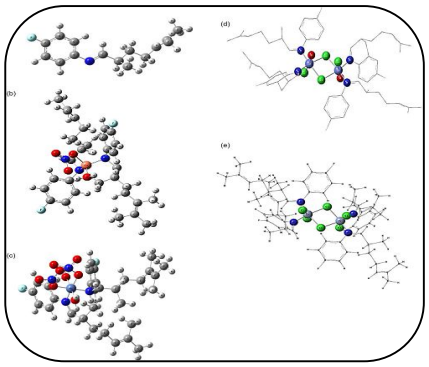
REVIEW OF RESEARCH
IMPACT FACTOR : 5.2331(UIF) UGC APPROVED JOURNAL NO. 48514 ISSN: 2249-894X
VOLUME - 7 | ISSUE - 9 | JUNE - 2018

SCREENING FOR ANTIMICROBIAL AND ANTICANCER ACTIVITY OF CO (II), NI (II) AND CU (II) COMPLEXES WITH 2–METHOXY-6-(6-IMINOQUINOLINYL METHYL) PHENOL



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ABSTRACT :

A Schiff base ligand was synthesized by condensation of o-vanillin with 6-aminoquinoline in 1:1 molar ratio. The Schiff base metal complexes of Co (II), Ni (II) and Cu (II) were prepared using the metal salt and ligand in 1:2 ratio. The metal complexes were characterized byusing elemental, thermal and spectral analysis. The compositions of the Schiff base ligand and the metal complexes were established by elemental analysis which indicated a metal: ligand ratio of 1:2.Thermal and spectral studies indicated that ligand behaved as bidentate coordinating through phenolic oxygen and azomethine nitrogen and formed octahedral metal complexes. The Schiff base SB-3 revealed its molecular formula as $C_{17}H_{14}O_2N_2$and metal complexes revealed their general formula as $[C_{34}H_{26}O_4N_4M(H_2O)_2]$ where M=Co(II),Ni(II) or Cu(II).The in-vitro antibacterial activities of the metal complexes were screened 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. All the Schiff bases were also screened for their anticancer activities against breast cancer MCF-7 cell line and colon cancer HT-29 cell line by sulforhodamine-B (SRB) assay. Interestingly, the Schiff base SB-3and its Co(II),Ni(II) and Cu(II) metal complexes showed active anticancer activity against breast cancer MCF-7 cell line. The Co(II) and Cu(II) metal complexes of Schiff base ligand exhibited superactive anticancer activityagainst breast cancer MCF-7 cell line while Schiff base metal complex of Cu(II) showed superactive anticancer activity against colon cancer HT-29 cell line.

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

1. INTRODUCTION:

Schiff bases which contain an azomethine group (–HC=N–), were first reported by scientistHugo Schiff in 1864. They are prepared by the condensation of primary amine with aldehyde or ketone, resulting into the formation of a new C=N bond [1]. Schiff bases derived from aromatic aldehydes and amines have a wide variety of applications in various fields like biological, inorganic and analytical chemistry. Schiff bases can be synthesized byrelatively simple procedures enabling us to design compounds which are structurally similar to some substances of biological origin [2-3].

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]. Schiff base 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. 6-aminoquinoline and o-vanillin are especially known for theirantibacterial and antifungal activities and are thus used as synthetic precursors in pharmaceuticals.Considering the distinct biological activities of these compounds, we hereinput forth an account of the synthesis and characterization of ligandderived from 6-aminoquinolline and o-vanillinand its Co(II), Ni(II) and Cu(II) complexes and study of their biological screening against different bacteria, fungi and cancers [5-8].

2. EXPERIMENTAL:

2.1 Apparatus:

Standard agar well diffusion method and agar ditch method were used for the study of antibacterial and antifungal activity of Schiff base SB-3 and its metal complexes. 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. An UV-VIS-NIR-3600 recording spectrophotometer(Shimadzu, Japan) was used for the UV spectrum scanning and determination experiments.For FTIR spectral analysis, Bruker, Germany Model 3000 Hyperion Microscope with Vertex 80FTIR system range 400-4000 cm^{-1} (KBr discs) at SAIF, IIT, Bombaywas used. The thermogravimetric analysis of metal complexes was performed on Perkin-Elmer Diamond TG at SAIF, IIT, Bombay.

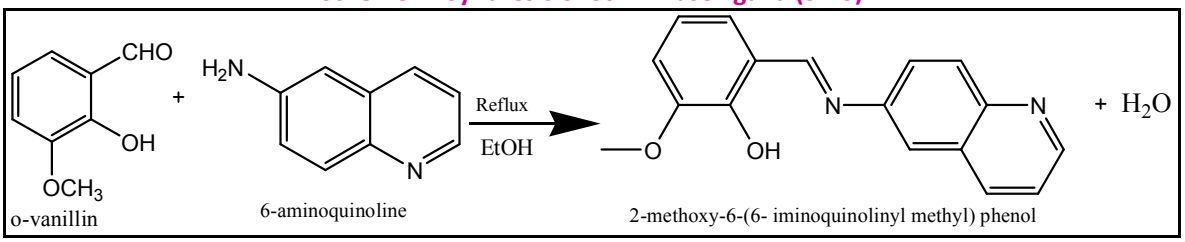
2.2 Reagents:

All the chemicals andreagents used were of AR grade or higher grades. Sigma- Aldrich o-vanillin, 6-aminoquinoline, while Standard Qualigens (SQ)AR grade hydrated Cobalt (II) chloride, Nickel (II) chloride and Copper (II) chloride were used for synthesis. The solvents ethanol,DMSO etc. were used.

2.3 Synthesis of Schiff base ligand (SB-3)

20 mmol (3.04g) of o-vanillin is dissolved in absolute ethanol. Then it was added drop wise into 20 mmol (2.88g) of ethanolic solution of 6-aminoquinoline. The mixture was refluxed for 2hours then cooled and filtered [6-7]. Intense orange colored crystals of Schiff base SB-3 were formed (yield 90%, 5 g). The crystals were washed with cold ethanol and anhydrous diethylether and dried over anhydrous $CaCl_2$[9-17]. The synthesis of Schiff base ligand (SB-3) is shown in Scheme-1

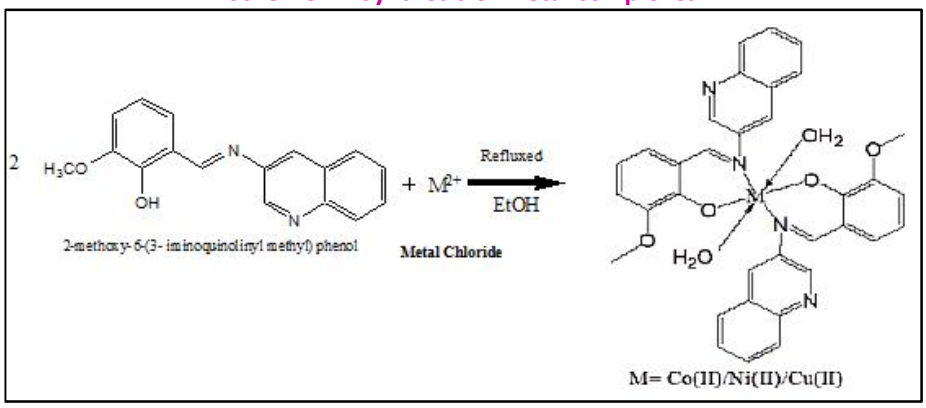
Scheme-1: Synthesis of Schiff Base ligand (SB-3)



2.4 Synthesis of Schiff base transition metal complexes:

20 mmol of Schiff base (SB-3) was dissolved in ethanol (30ml) and added to 10 mmol ethanolic solution (20ml) of metal (II) chloride salt. The metal-ligand molar ratio taken was 1:2. The mixture was refluxed for 3 hours. On cooling, a crystalline metal complex was obtained. It was filtered, recrystallized from ethanol and dried in desiccator over anhydrous $CaCl_2$[9-17]. The Co(II),Ni(II) and Cu(II) metal complexes of Schiff base (SB-3) were prepared by above general method using salts $CoCl_2.6H_2O$, $NiCl_2.6H_2O$ and $CuCl_2.2H_2O$. The preparation of metal complexes is shown in Scheme -2

Scheme -2: Synthesis of metal complexes



Schiff base (SB-3) and its Co (II), Ni (II) and Cu (II) complexes synthesized were stable at room temperature and soluble in common solvents like DMSO, methanol etc. The synthesized ligand and the metal complexes were characterized by elemental, thermal and spectral analysis. Biological activity namely antibacterial, antifungal and anticancer activity of the ligand and its metal complexes were studied. The geometry of the synthesized compounds has been elucidated on the basis of their elemental and spectral studies.

3. RESULTS AND DISCUSSION:

3.1 Analytical and Physicochemical data:

The stoichiometry of ligand and their metal complexes is confirmed by the elemental analysis. The analytical and physicochemical data of Schiff base (SB-3) and its metal complexes is found in good agreement with the proposed structure of ligand and the metal complexes [18-22]. The data being listed in Table-1

Table-1: Analytical and Physicochemical data of Schiff base (SB-3) and its metal complexes

Comp.	Molecular formula	M. P. (°C)	Mol. Weight	Color	% Observed (Theoretical)			
					C%	H%	N%	M%
SB-3	$C_{17}H_{14}O_2N_2$	90	278	Orange	72.82 (73.38)	4.85 (5.03)	9.99 (10.07)	-
SB-3-Co	$C_{34}H_{26}O_4N_4Co.(H_2O)_2$	>350	648.93	Dark Pink	62.10 (62.87)	4.71 (4.62)	8.53 (8.62)	9.01 (9.08)
SB-3-Ni	$C_{34}H_{26}O_4N_4Ni.(H_2O)_2$	>350	648.69	Dark Green	62.41 (62.89)	4.91 (4.62)	8.81 (8.63)	8.89 (9.04)
SB-3-Cu	$C_{34}H_{26}O_4N_4Cu.(H_2O)_2$	>350	653.55	Grey	62.31 (62.42)	4.71 (4.59)	8.351 (8.658)	9.32 (9.72)

3.2. Antibacterial and Antifungal activity:

The antibacterial and antifungalstudies of the Schiff base (SB-3) and its metal complexes SB-3-Co, SB-3-Ni and SB-3-Cu 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 antifungal activities of this Schiff base and its metal complexes were tested on fungi *C.albicans* and *A.niger*. Well-knownagar-well diffusion method was used for studies on antibacterial activity [23-28] and Agar-Ditch method for studies on antifungal activity [23-28]. The stock solutions of Schiff base and metal complexes of concentration 1000 µg/ml were prepared and used to prepare their various concentrations of 100,200,300,400 and 500 µg/ml using DMSO. 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 complexes in general exhibited better antibacterial and antifungal activity than ligand. The Schiff base ligand SB-3and its metal complexes are moderately active to highly active against Gram positive bacteria*S.aureus* and *B. cereus* and Gram negative bacteria*P.auruginosa*and *E.coli*. Co(II) and Cu(II) complexes exhibited highest antimicrobial activity among the compounds tested against *B. cereus.*

Table-2: Antibacterial and Antifungal activities of ligand (SB-3) and itsCo (II), Ni (II) andCu (II)Complexes

Compound	Concentration µg/ml	S.aureus	B.cereus	P.auruginosa	E.coli	C.albicans	A.niger
SB-3	100	10++	23+++	12++	8+	1-	2-
	200	12++	24+++	13++	10++	3-	2-
	300	14++	25+++	15++	13++	5+	3-
	400	16++	26+++	28++	15++	7+	4-
	500	19++	28++	20++	18++	9+	4-
SB-3-Co	100	12++	25+++	14++	10++	2-	2-
	200	16++	26+++	16++	12++	3-	3-
	300	20+++	27+++	17++	15++	6+	4-
	400	23+++	28+++	20+++	17++	10++	5+
	500	26+++	31+++	24+++	21+++	12++	7+
SB-3-Ni	100	10++	23++	13++	9+	2-	1-
	200	12++	25++	14++	11++	4-	2-
	300	18++	27+++	16++	14++	5+	3-
	400	22+++	28+++	18++	16++	8+	4-
	500	24+++	29+++	20+++	19++	10++	5+
SB-3-Cu	100	11++	24+++	13+++	10++	2-	2-
	200	14++	25+++	15++	13++	3-	4-
	300	18++	27+++	17++	15++	6+	5+
	400	21+++	29++	20++	18++	8+	6+
	500	25+++	30+++	23+++	20+++	11++	6+
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-3) and its Co(II), Ni (II) and Cu(II) Complexes 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-3) 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 [29-32].

Ligand (SB-3) and its Co(II), Ni (II) and Cu(II) complexes are active 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.Schiff base ligands SB-3-Co and SB-3-Cu are superactive against MCF-7 cell line. Therefore ligand and complexes may prove as lead compounds for *in vivo* screening of anticancer activity against malignant breast cancer.

Ligand (SB-3) is resistant to human colon cancer cell line HT-29 with GI50, 59.1 µg/ml. However its Cu (II) complex exhibits super in vitro anti-cancer activity against colon cancer cell line HT-29with GI50 value less than 10 µg/ml,which is comparable to Adriamycin with GI50 value less than 10 µg/ml.Hence Cu(II) complex can be considered as lead compound for *in vivo* screening of anticancer activity against malignant colon cancer.

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

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

Sr.No	Compound	Drug Concentrations (μg/ml)		
		LC 50	TGI	GI-50
1	SB-3	>80	98.7	15.3
2	SB-3-Co	>80	67.8	<10
3	SB-3-Ni	>80	67.9	17.4
4	SB-3-Cu	<10	<10	<10
5	ADR	>80	30.11	<10

Table 4: Cytotoxicity of Schiff base (SB-3) and its Co (II), Ni (II) and Cu (II) metal complexes on human colon cancer cell line (HT-29)

Sr.No	Compound	Drug Concentrations (μg/ml)		
		LC 50	TGI	GI-50
1	SB-3	>80	>80	59.1
2	SB-3-Co	>80	>80	75.6
3	SB-3-Ni	>80	>80	68.2
4	SB-3-Cu	20	<10	<10
5	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 and 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. Cytotoxicity of ligand and its complexes on human breast and colon cancer cell lines is shown in growth curves as represented in Figure-1 and Figure-2.

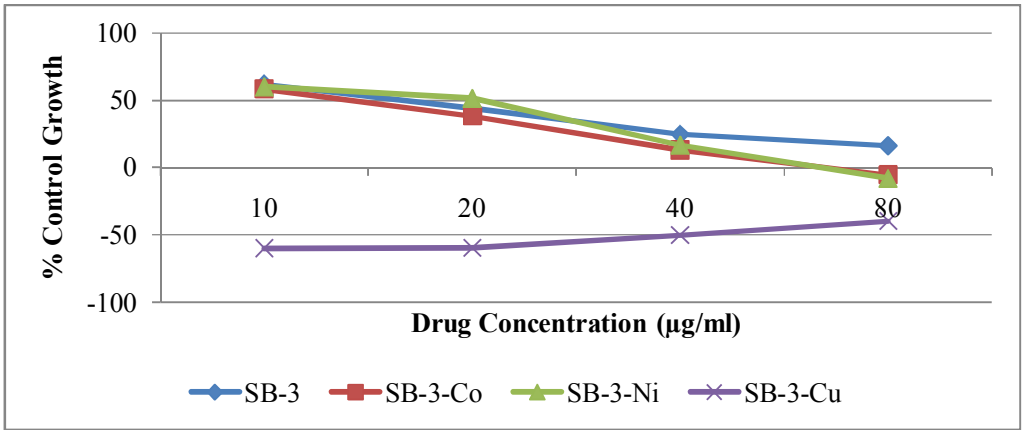


Figure-1 : Cytotoxicity of Schiff bases (SB-3) and its Co(II) , Ni(II) and Cu(II) metal complexes on human breast cancer cell line (MCF-7).

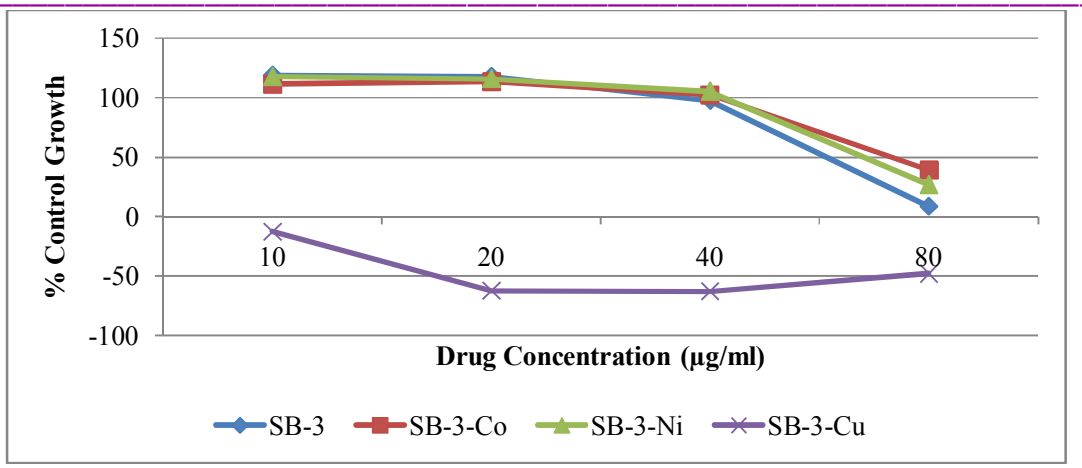


Figure-2:Cytotoxicity of Schiff bases (SB-3) and its Co (II),Ni (II) and Cu (II) metal complexes on human colon cancer cell line (HT-29).

4. ACKNOWLEDGEMENT:

I acknowledge SAIF, Indian Institute of Technology, Mumbai for providing facility ofelemental, spectral and thermogravimetric analysis of sample compounds.I acknowledge, Department of Microbiology, Walchand Centre of Biotechnology, Solapur for providing facility of microbial studies. I am also thankful to ACTREC, Tata memorial Centre, Kharghar, Navi Mumbai for assistance in the study of anticancer activity of sample compounds.

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