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ARTICLE

Synthesis, Spectral, Antibacterial, Antifungal and Anticancer Activity Studies of Schiff Bases Derived from *o*-vanillin and Aminoquinolines

S.N. Battin[1], A.H. Manikshete[1,✉], S.K. Sarasamkar[2], M.R. Asabe[1] and D.J. Sathe[3]

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ABSTRACT

In this work, five varieties of *o*-vanillin Schiff bases *viz*. 2-methoxy-6-(3/5/6/8-iminoquinolinyl methyl)phenol and 2-methoxy-6-(4-iminoquinaldinyl methyl)phenol have been synthesized with the aid of direct reflux technique in ethanol solution. The synthesized compounds were characterized through CHN evalution, UV-visible, FTIR, ^{1}H NMR and Mass spectral studies. The *in vitro* antimicrobial activities of the synthesized Schiff bases were tested using bacterial species such as *Staphylococcus 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.

KEYWORDS

Synthesis, *o*-Vanillin, Schiff base, Spectral studies, Biological activities.

INTRODUCTION

Chemistry and bioactivities of *o*-vanillin and various amino-quinolines prompted us to synthesize a series of new potentially active Schiff bases bearing imine group at various positions in their condensation products. Aromatic Schiff bases constitute a large class of organic compounds, obtained by condensation reaction of aromatic aldehyde and amine [1-3]. The special plane structure of the azomethine -HC=N-group and strong binding ability of the aromatic Schiff bases have the function of antibacterial, antifungal, antitumor and antiviral activities [4-9]. *o*-Vanillin is found to have biological activities such as analgesic, anti-inflammatory, antibacterial, sterilizing and antiviral activities [10-15]. *o*-Vanillin is an useful herbicide, pesticide and bactericide [16-19]. It plays an important role in the process of chemical engineering as a medicine and as a reactant in organic synthesis [20-23]. Hence, *o*-vanillin is used to synthesize various aromatic Schiff bases with biological properties. Quinoline is an azaheterocyclic aromatic weak tertiary base. The quinoline moiety is nontoxic to humans on oral absorption and inhalation. It is found in many compounds, which are pharmacologically active and hence display a wide range of biological activities. In particular, quinoline derivatives

Author affiliations:

[1]Department of Chemistry, Walchand College of Arts and Science, Solapur-413 006, India
[2]Department of Chemistry, Walchand Institute of Technology, Solapur-413 006, India
[3]Department of Chemistry, Kolhapur Institute of Technology, Kolhapur-416 234, India

✉To whom correspondence to be addressed:

Fax: +91 217 2391849
Tel: +91 217 2376371
E-mail: ahmanikshete@gmail.com

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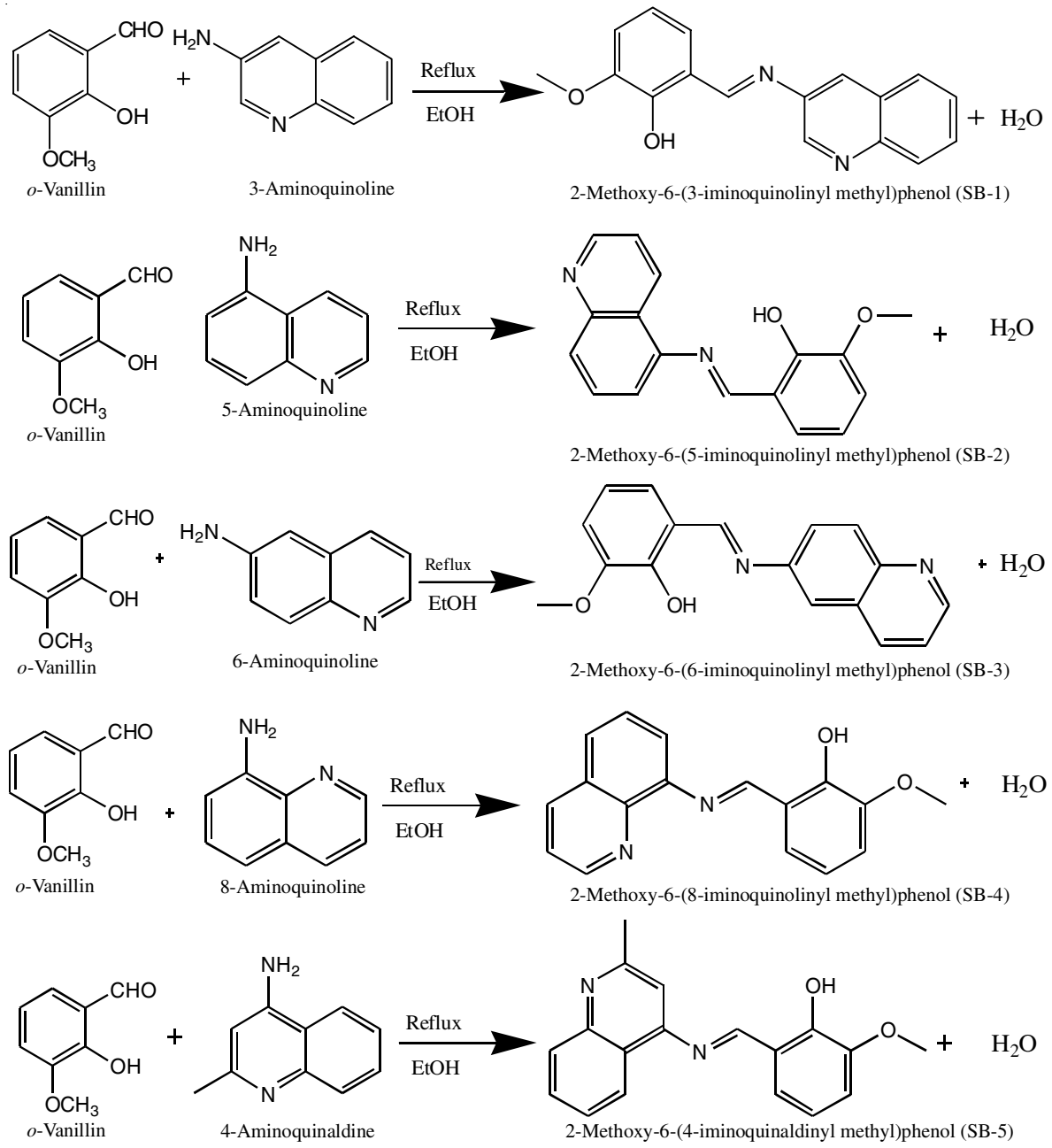
are found to exhibit antimalarial, antibacterial, antiprotozoal, anti-HIV, anticancer and antifungal activities [24-29].

In this paper, we report the synthesis of five varieties (SB-1 to SB-5) of Schiff bases by means of condensation of *o*-vanillin with 3/5/6/8-aminoquinoline and 4-aminoquinaldine. These Schiff bases were synthesized by direct reflux reaction of basic precursors *viz*. *o*-vanillin with 3/5/6/8-aminoquinoline and 4-aminoquinaldine in ethanolic medium as solvent. The synthesized Schiff bases were characterized by CHN analysis, IR, ^{1}H NMR UV-visible, Mass spectral studies and their biological (antibacterial, antifungal and anticancer) activities.

EXPERIMENTAL

All the chemicals/reagents used were of analytical grade (A.R). Sigma-Aldrich *o*-vanillin, 3-aminoquinoline, 5-aminoquinoline, 6-aminoquinoline, 8-aminoquinoline and 4-aminoquinaldine were used. Solvents were dried and distilled before use according to the standard procedures [30,31]. The purity of the compounds was confirmed by TLC using Merck silica gel 60-F254 coated alumina plates and visualized by exposure of iodine vapours.

For synthesis of Schiff bases, 20 mmol (3.04 g) of *o*-vanillin was dissolved in ethanol. Then it was introduced drop wise into 20 mmol (2.88 g) of ethanolic solution of 3-aminoquinoline (1:1 molar proportion). The mixture was refluxed for 2 h and cooled followed by filtration of the formed and coloured solid Schiff base products. Further, it was washed with ethanol and dried over anhydrous $CaCl_2$. The procedure was repeated using *o*-vanillin and 5/6/8-aminoquinoline and 4-aminoquinaldine in 1:1 molar proportion to obtain their respective Schiff bases. The purity of the products was checked by TLC. Reactions involved in the synthesis are shown in **Scheme-I**.



Scheme-I: Synthesis of Schiff bases

TABLE-1
ELEMENTAL AND PHYSICAL CONSTANT DATA OF SCHIFF BASES

S. No	Schiff base	m.f.	m.p. (°C)	m.w.	Colour	Elemental analysis (%): Observed (Theoretical)		
						C	H	N
1	SB-1	$C_{17}H_{14}N_2O_2$	210	278	Red orange	72.79 (73.78)	4.81 (5.03)	9.99 (10.07)
2	SB-2	$C_{17}H_{14}N_2O_2$	162	278	Yellow orange	72.46 (73.38)	4.83 (5.03)	9.94 (10.07)
3	SB-3	$C_{17}H_{14}N_2O_2$	90	278	Orange	72.82 (73.38)	4.85 (5.03)	9.99 (10.07)
4	SB-4	$C_{17}H_{14}N_2O_2$	103	278	Red	72.91 (73.38)	4.87 (5.03)	9.98 (10.07)
5	SB-5	$C_{18}H_{16}N_2O_2$	119	292	Yellow	73.39 (73.97)	5.28 (5.48)	9.45 (9.58)

RESULTS AND DISCUSSION

Electronic spectra were recorded on Shimadzu UV-VIS-NIR-3600 spectrophotometer using ethanol. IR spectra were scanned on Bruker, Germany Model 3000 Hyperion Microscope with Vertex 80FTIR system range 4000-400 cm^{-1} (KBr discs) at IIT Bombay. ^1H NMR spectra were recorded in $CDCl_3$ on Bruker-400 MHz using TMS as internal standard. Mass spectra were recorded on Hewlet Packard 5989B while the CHN elemental analysis was recorded on thermo Finnigan, Italy, Model Flash EA1112 series.

Theoretical and experimentally observed values of elemental analysis of compounds are in good agreement with each other confirming the stoichiometry of synthesized Schiff bases. The elemental and physical constant data of Schiff bases is listed in Table-1.

Electronic spectra of all the Schiff bases are characterized by two bands in the UV-visible region. The UV spectra in Fig. 1 of Schiff bases, reveal that the bands occurring in the range of 220-280 nm are due to low or medium energy $\pi\rightarrow\pi^*$ transition within aromatic moieties. These transitions may originate due to perturbed local excitation of the phenyl group [30-33]. Another intense band in the lower energy region of the spectra between 280-380 nm is assigned to the $n\rightarrow\pi^*$ transition of azomethine group. Electronic spectral data of Schiff bases are presented in Table-2.

The selected vibrational frequencies for the Schiff base ligands are presented in Table-3. A very strong band in the range 1636-1589 cm^{-1} is characteristic of the azomethine nitrogen ν(C=N) present in the Schiff base ligands SB-1 to SB-5 [34]. The ligands showed broad absorption bands at 3433-3297 cm^{-1} which is characteristic of ν(Ar-OH). The absorption bands corresponding to ν(Ar-O) are seen at 1255-1217 cm^{-1}, while the strong bands for ν(C-N) stretching at 1479-1457 cm^{-1} and for ν(O-CH_3) at 3001-2929 cm^{-1} [35]. The appearance of the characteristic infrared absorption peak ν(C=N) at 1636-1589 cm^{-1} for the ligands indicates the formation of Schiff bases.

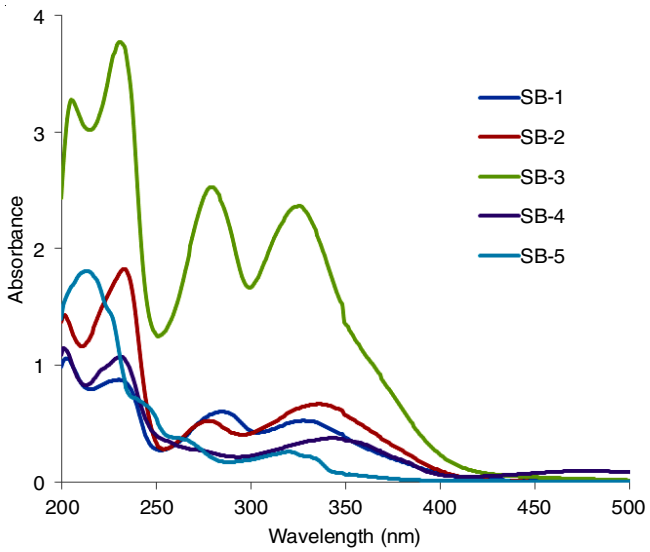


Fig. 1. UV spectra of Schiff bases of SB-1 to SB-5

TABLE-2
ELECTRONIC ABSORPTION SPECTRAL DATA (λ_{max}) VALUES IN nm

S. No.	Schiff base	$\pi\rightarrow\pi^*$	$n\rightarrow\pi^*$
1	SB-1	243	348
2	SB-2	272	337
3	SB-3	270	335
4	SB-4	241	346
5	SB-5	265	327

TABLE-3
RELEVANT INFRARED FREQUENCIES (cm^{-1}) OF THE SCHIFF BASE

Schiff base	ν(O-H)	ν(C=N)	ν(Ar-O)	ν(C-N)	ν(O-CH_3)
SB-1	3426	1622	1250	1465	2929
SB-2	3427	1614	1246	1457	2949
SB-3	3433	1589	1256	1468	2999
SB-4	3428	1618	1244	1459	3001
SB-5	3297	1636	1218	1479	2994

The ^1H NMR spectra of the five synthesized Schiff bases were determined using $CDCl_3$ as a solvent. The ^1H NMR spectrum of the Schiff base ligands, showed D_2O exchangeable singlet in the range 13.258-14.544 ppm, integrating for one proton, is assigned to-OH [36]. From Table-4 it can be seen that the single peaks in the range 8.966-9.945 ppm should be attributed to the proton peaks of -CH=N- bond of Schiff bases. The results indicate that the imino band (-CH=N-) has been

TABLE-4
^1H NMR (δ ppm) SPECTRAL DATA OF SCHIFF BASES IN $CDCl_3$

Schiff base	NMR signals in δ ppm
SB-1	13.258 (s, 1H, Ar-OH); 6.947-8.960 (m, 9H, Ar-H); 8.966 (s, 1H, -CH=N); 3.988 (s, 3H, -OCH_3)
SB-2	13.664 (s, 1H, Ar-OH); 6.958-9.002 (m, 9H, Ar-H); 9.006 (s, 1H, -CH=N); 4.008 (s, 3H, -OCH_3)
SB-3	13.554 (s, 1H, Ar-OH); 6.932-8.937 (m, 9H, Ar-H); 8.941 (s, 1H, -CH=N); 3.986 (s, 3H, -OCH_3)
SB-4	14.544 (s, 1H, Ar-OH); 6.856-8.994 (m, 9H, Ar-H); 8.998 (s, 1H, -CH=N); 3.977 (s, 3H, -OCH_3)
SB-5	13.711 (s, 1H, Ar-OH); 6.541-7.942 (m, 8H, Ar-H); 9.945 (s, 1H, -CH=N); 3.947 (s, 3H, -OCH_3); 2.616 (s, 3H, -CH_3)

formed between *o*-vanillin and aminoquinolines. The multiple signals of the complexes in the range 6.541-9.002 ppm are due to quinoline-H protons and aromatic-H protons. The O-CH_3 group proton is assigned at 3.947-4.008 ppm in all the Schiff base ligands [37].

The spectra of all the synthesized Schiff base ligands exhibited parent peak due to molecular ion (M^+). The proposed molecular formula of these compounds was confirmed by comparing their molecular formula weights with the *m/z* values. The *m/z* values for molecular ion peaks obtained are as follows-279 for SB-1 to SB-4 and 293 for SB-5, which correspond with the proposed molecular formula for these Schiff base ligands. In addition to the peaks due to molecular ions, the spectra exhibited peaks assignable to various fragments arising from the thermal cleavage of the compounds [38].

The synthesized Schiff bases were studied for their antibacterial and antifungal activities using two Gram-positive bacteria *Staphylococcus aureus* and *Bacillus cereus,* two Gram-negative bacteria *Pseudomonas auruginosa* and *Escherchia coli* and two fungi *Candida albicans* and *Aspergillus niger.* Agar well diffusion method was used to evaluate the antibacterial activity [39,40] while the agar ditch method was used for antifungal activity [41,42]. The stock solutions of 1000 µg/mL concentration were prepared using DMSO as solvent which were further used to prepare various concentrations like 100, 200, 300, 400 and 500 µg/mL. The bacteria and fungi were inoculated on the surface of nutrient agar and Sabouraud's agar respectively. The various concentrations of the ligands were inoculated in the wells and ditches prepared on the agar plates. The plates were incubated at room temperature for 24 h for bacteria and 48 h for fungi. In order to clarify the effect of DMSO for its antimicrobial activity by agar diffusion assay, separate studies were carried out with DMSO, which showed no activity against any bacteria or fungi. The antimicrobial activity was determined by measuring the diameter of the zone of inhibition (mm) and thereby they were classified as inactive, weakly active, moderately active and highly active. The results are summarized in Table-5.

In general, Schiff base ligands exhibited better antibacterial activity than antifungal activity. They are active against both Gram-positive and Gram-negative bacteria. The Schiff base 2-methoxy-6-(6-iminoquinal methyl)phenol (SB-3) is highly active against *Bacillus cereus* at lowest concentration of 100 µg/mL.

The anticancer activity of the five Schiff base ligands was determined by sulforhodamine B (SRB) assay on human malignant breast cancer cell line MCF-7 and colon cancer cell line HT-29. The cell lines were cultured in RPMI 1640 medium supplemented with 10 % fetal bovine serum (FBS) and 2 millimolar L-glutamine at 37 °C in a humidified atmosphere of 5 % CO_2. About 5×10^3 cells/well were seeded in 96 well micro titer plate using a culture medium. After 24 h, each drug (Schiff base) was added to the wells at 4 levels of doses (10, 20, 40 and 80 µg/mL) and incubated for 48 h. After incubation, *in vitro* testing was done using SRB assay protocols [43-45]. Adriamycin, a positive control drug was also run in each experiment and each experiment was repeated thrice. The results are given in terms of GI_{50}, TGI and LC_{50} values. Schiff bases SB-1, SB-4 and SB-5 with GI_{50} <10 µg/mL were super active on human breast cancer cell line MCF-7 in the SRB assay

TABLE-5
ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF SCHIFF BASE

Schiff base	Concentration (µg/mL)	*Staphylococcus aureus*	*Bacillus cereus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Candida albicans*	*Aspergillus niger*
SB-1	100	-	-	+	+	-	-
	200	+	-	++	++	-	-
	300	+	-	++	++	+	-
	400	++	+	++	++	+	-
	500	++	+	+++	++	+	-
SB-2	100	-	-	-	-	-	-
	200	-	-	+	+	-	-
	300	+	+	+	+	+	-
	400	+	+	++	++	+	-
	500	++	++	++	++	+	-
SB-3	100	++	+++	++	+	-	-
	200	++	+++	++	++	-	-
	300	++	+++	++	++	+	-
	400	++	+++	++	++	+	-
	500	++	+++	+++	++	+	-
SB-4	100	+	+	++	++	-	-
	200	+	+	++	++	-	-
	300	+	++	++	++	+	-
	400	++	++	+++	++	+	-
	500	++	++	+++	++	+	+
SB-5	100	+	++	++	++	-	-
	200	+	++	++	++	-	-
	300	++	++	++	++	+	-
	400	++	++	++	++	+	-
	500	++	++	++	+++	+	-

'–' Inactive (zone of inhibition < 5 mm); '+' Weakly active (5 mm ≤ zone of inhibition < 10 mm); '++' Moderately active (10 mm ≤ zone of inhibition < 20 mm); '+++' Highly active (zone of inhibition ≥ 20 mm)

TABLE-6
CYTOTOXICITY OF SCHIFF BASES ON HUMAN BREAST CANCER MCF-7 CELL LINE AND COLON CANCER HT-29 CELL LINE

Schiff base	Concentrations (µg/mL)					
	LC_{50}		TGI		GI_{50}	
	MCF-7 cell line	HT-29 cell line	MCF-7 cell line	HT-29 cell line	MCF-7 cell line	HT-29 cell line
SB-1	> 80	> 80	76.5	> 80	< 10	64.3
SB-2	> 80	> 80	> 80	> 80	19.0	> 80
SB-3	> 80	> 80	98.7	> 80	15.3	59.1
SB-4	> 80	> 80	53.35	71.2	< 10	45.5
SB-5	> 80	> 80	56.2	58.0	< 10	27.9
ADR	> 80	NE	30.11	< 10	< 10	< 10

Value GI_{50} if 1) < 10 µg/mL Superactive; 2) 10-15 µg/mL Moderately active; 3) 15-30 µg/mL Weakly active; 4) 30-80 µg/mL Resistant.
GI_{50} = Concentration of drug causing 50 % inhibition of cell growth; TGI = Concentration of drug causing total inhibition of cell growth.
LC_{50} = Concentration of drug causing 50 % inhibition of cell kill; ADR = Adriamycin (doxorubicin, positive control drug).

while the Schiff base SB-2 (GI_{50} = 19 µg/mL) was weakly active and the Schiff base SB-3 (GI_{50} = 15.3 µg/mL) moderately active on the breast cancer cell line MCF-7.

All the Schiff base samples except SB-5 were inactive on human colon cancer cell line HT-29 in the SRB assay system. However SB-5 sample was weakly active on colon cancer cell line HT-29 in the assay system used. The results of the cytotoxicity of Schiff bases on human breast cancer cell line MCF-7 and colon cancer cell line HT-29 are represented in Table-6.

In vitro testing based on GI_{50} values indicate that in general Schiff base compounds are more active on human breast cancer MCF-7 cell line than on human colon cancer HT-29 cell line. Cytotoxicity of the five Schiff bases on MCF-7 cell lines and HT-29 cell lines is represented in Figs. 3 and 4, respectively.

Conclusion

The structure of each synthesized Schiff base was confirmed by CHN analysis, UV-Vis, FTIR, ^{1}H NMR and mass spectral data. All the synthesized Schiff bases are stable in air and soluble in methanol, ethanol and DMSO while insoluble in water. The antimicrobial studies show that the Schiff bases possess better antibacterial activity than antifungal activity. They are active against *Staphylococcus aureus*, *Bacillus cereus, Pseudomonas auruginosa* and *Escherichia coli* while inactive against *Aspergillus niger* and weakly active against *Candida albicans*. The *in vitro* anticancer activity shows that synthesized Schiff bases are more active on human breast cancer MCF-7 cell line than on human colon cancer HT-29 cell line.

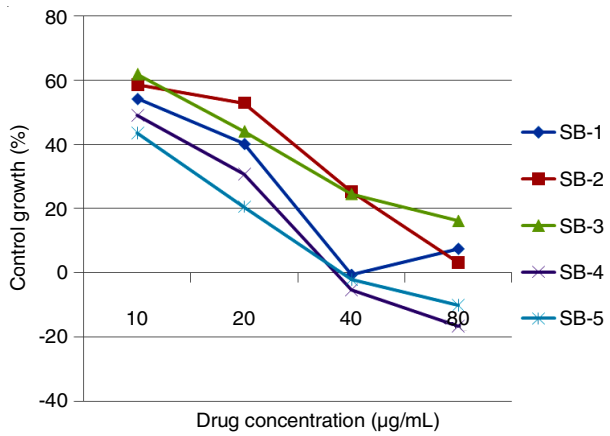


Fig. 2. Cytotoxicity of all the synthesized Schiff bases on human breast cancer MCF-7 cell line

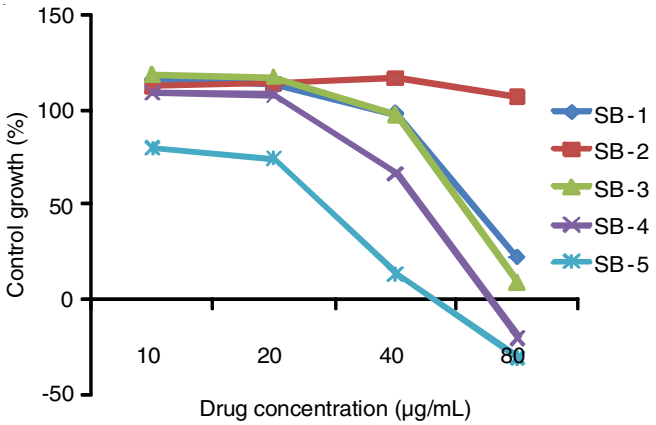


Fig. 3. Cytotoxicity of all the synthesized Schiff bases on human colon cancer HT-29 cell line

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