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Short Communication

Microplate assay for screening the antibacterial activity of Schiff bases derived from substituted benzopyran-4-one

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ABSTRACT

Schiff bases (SB_1–SB_3) were synthesized from the condensation of 6-formyl-7-hydroxy-5-methoxy-2-methylbenzopyran-4-one with 2-aminopyridine (SB_1), p-phenylenediamine (SB_2) and o-phenylenediamine (SB_3), while Schiff bases (SB_4–SB_6) were synthesized by condensation of 5,7-dihydroxy-6-formyl-2-methylbenzopyran-4-one with 2-aminopyridine (SB_4), p-phenylenediamine (SB_5) and o-phenylenediamine (SB_6). Schiff bases were characterized using elemental analysis, IR, UV–Vis, ^{1}H NMR, ^{13}C NMR and mass spectroscopy. These compounds were screened for antibacterial activities by micro-plate assay technique. *Escherichia coli* and *Staphylococcus capitis* were exposed to different concentrations of the Schiff bases. Results showed that the antibacterial effect of these Schiff bases on Gram-negative bacteria were higher than that on Gram-positive bacteria moreover, the Schiff bases containing substituent OCH_3 on position five have higher antibacterial activity than that containing hydroxy group on the same position.

1. Introduction

Microbial infections remain a leading cause of mortality and morbidity all over the world. Many infections, and in particular those caused by bacteria, are treated with antibiotics. Unfortunately, due to the excessive use of antibiotics, there are a growing number of microorganisms resistant to multiple classes of antibiotics. This increase in antibiotic resistance amongst pathogenic bacteria is a serious public health problem, where bacteria replicate very rapidly and obtain a mutation that helps a microbe to survive in the presence of an antibiotic. Antimicrobial resistance is a factor in virtually all hospital-acquired (nosocomial) infections and physicians are concerned that several bacterial infections soon may become untreatable [1,2]. These concerns have led to major research efforts to discover new antibacterial agents that could be used to combat bacterial infections one of which are the Schiff bases.

Schiff bases having the general structure R–CH=N–Ar where R and Ar are aliphatic or aromatic groups result from the condensation of primary amines with aldehydes or ketones [3]. They are characterized by –N=CH– (imine) groups which have biological activities such as antimicrobial [4–6], antifungal [7] and anticancer [8].

Tetradentate Schiff bases especially those with a N_2O_2 donor set, resulting from the condensation of the aliphatic diamines such as ethylenediamine or trimethylenediamine with benzopyran-4-one derivatives have an antibacterial effect on both gram negative and gram positive bacteria possibly due to the presence of azomethine groups (imines) which may interfere with bacterial growth [9].

Micro-plate assay could be used as a rapid and sensitive method for detection of bacterial growth inhibition. This technique allowed real time monitoring compared to classical methods for studying antimicrobial effects [10–12]. The use of micro-plate readers for quantitative analysis of bacterial cell growth is advantageous because of the potential of real time monitoring compared to classical methods for studying antimicrobial effects [13,14]. The aim of the present study, is to prepare Schiff bases which are based on the condensation of a 6-formyl-7-hydroxy-5-methoxy-2-methylbenzopyran-4-one and 5,7-dihydroxy-6-formyl-2-methylbenzopyran-4-one with aromatic amines and investigate their effect on pathogenic strains of Gram-positive and Gram-negative bacteria.

2. Experimental

2.1 Materials and reagents

All materials and solvents used in the present study are chemically pure grade. They included 2-aminopyridine, p-phenylenediamine, o-phenylenediamine, visnagin (Memphis Company for Pharmaceutical and Chemical Industry, Cairo, Egypt), potassium dichromate ($K_2Cr_2O_7$), sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), hydrochloric and sulfuric acids (El-Nasr Pharmaceutical

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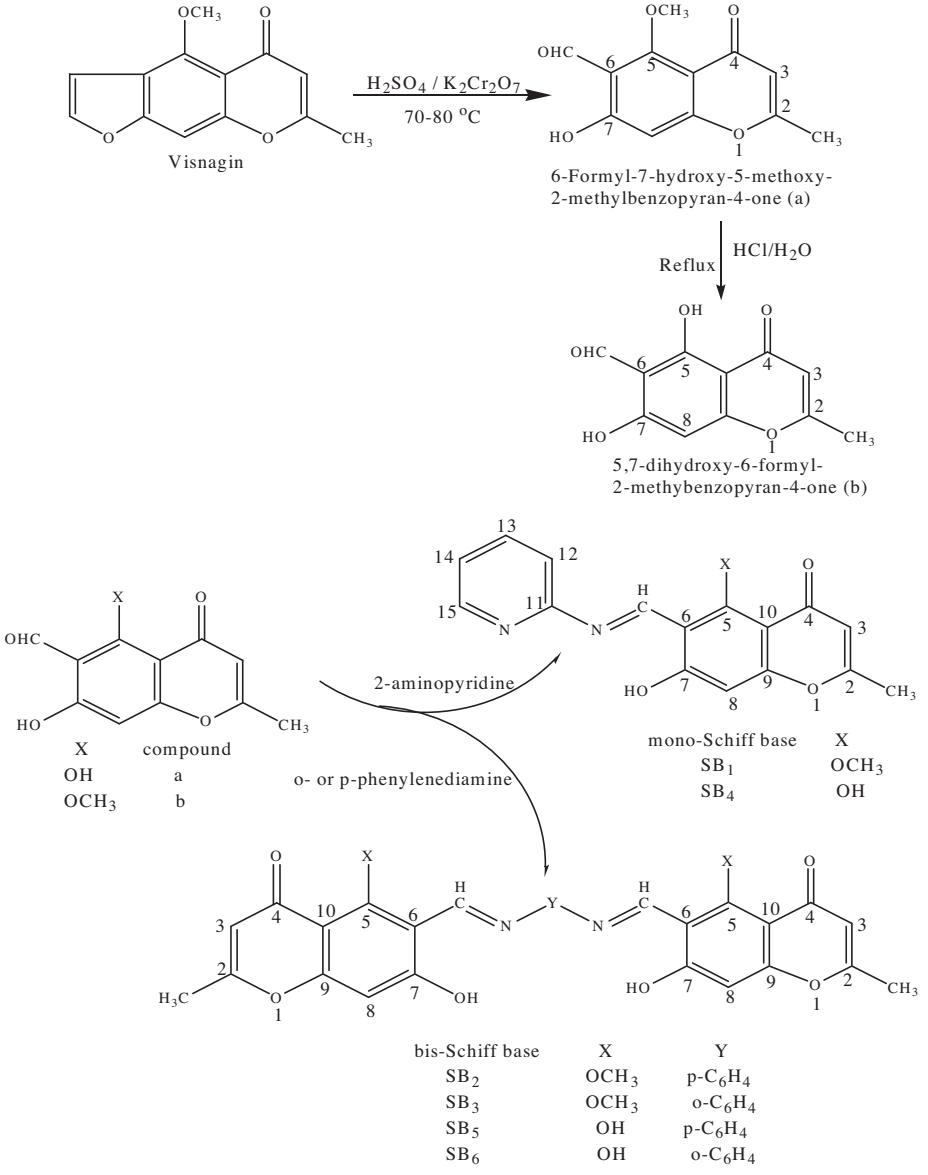
Chemicals, Adwic, Egypt). The organic solvents used included ethanol, dimethylformamide (DMF) and toluene. All these solvents were either spectroscopic pure grade solvents from BDH or purified with the recommended methods [15].

2.2. Analysis and physical measurements

Carbon, hydrogen and nitrogen were analyzed by standard microanalysis methods at Microanalytical center, Cairo University, Giza, Egypt. Infrared spectra (4000–400 cm^{-1}) were obtained with KBr disc technique using test scan Shimadzu FTIR spectrometer. ^{1}H NMR spectra were recorded using Varian 300 MHz NMR Spectrometer, using tetramethylsilane (TMS) as an internal standard in DMSO-d^6 and D_2O. ^{13}C NMR spectra were recorded using Jeol ECA-500, 125 MHz NMR Spectrometer, all the spectra were reported by employing TMS as internal reference and deuterated dimethylsulfoxide (DMSO) as solvent at ambient temperature. Mass spectra of the Schiff bases were recorded with the aid of Q 1000 EX GC–MS Shimadzu (Japan) spectrometer at 70 eV and 100 μA energy using a direct insertion probe at temperature 90–110 °C. The electronic absorption spectra were scanned with UV/Vis-NIR 3101 PC Shimadzu spectrophotometer.

2.3. Synthesis of the Schiff-bases

6-Formyl-7-hydroxy-5-methoxy-2-methylbenzopyran-4-one (a) was synthesized by oxidation of visnagin as previously mentioned [16]. The product was filtered washed several times with water until a white product was obtained. Recrystallization from hot ethanol produced a white solid with melting point 189 °C [lit. m.p. 189]. One gram of this produced solid was hydrolyzed by refluxing with 25 ml 1:1 hydrochloric acid for about 1 h. The yellow orange product of 5,7-dihydroxy-6-formyl-2-methylbenzopyran-4-one (b) was filtered, dried and recrystallized from toluene till constant melting point (195 °C) [lit. m.p. 195] [17]. The target Schiff bases (SB_1–SB_6) were prepared by addition of 2-aminopyridine (10 mMole), p-phenylenediamine or o-phenylenediamine (5 mMole) in 20 ml ethanol drop wise with continuous stirring to a solution of (a) or (b) in ethanol (20 ml, 10 mMole) (Scheme 1). The mixture was stirred at room temperature for at least 30 min



Scheme 1. Preparation of the Schiff bases.

Table 1
Preparation of bacterial suspension with different concentrations of Schiff bases in total volume of 100μL.

Schiff bases conc. (M)	Schiff bases (μL)	LB broth (μL)	Bacterial cells (μL)
10×10^{-6}	10	85	5
20×10^{-6}	20	75	5
30×10^{-6}	30	65	5
40×10^{-6}	40	55	5
50×10^{-6}	50	45	5

and the solids obtained were filtered off, washed with ethanol and recrystallized from dimethylformamide.

2.4. Bacteria

Escherichia coli XL-1 blue, as an example of gram negative bacteria and *Staphylococcus capitis* ATCC 2784 as an example of gram positive bacteria were used as target organisms. Bacterial cells were grown for 24 h on LB agar plates at 37 °C. The cells were suspended in LB broth media to an optical density of 0.5 at 595 nm (OD), and diluted with LB broth media to yield a starting inoculum's of approximately 10^6 colony forming units per ml (CFU/ml), which was confirmed by plate counts. Cells were exposed to the prepared Schiff bases with different concentrations starting from 10×10^{-6} M up to 50×10^{-6} M. The effect of different concentrations of the Schiff bases and the solvent DMF on the bacterial growth was studied. Also, the bacterial cells were exposed to different concentrations of DMF alone.

2.5. Growth profiles

From an overnight confluent culture of a single colony, 1% inoculum was given in Luria Bertani (LB) broth medium in a shake flask. LB broth was prepared with deionized water. Sterile, polystyrene, flat-bottom, 96-well tissue culture plates were used. Growth kinetics in the 96-well microplates were monitored (as turbidity) with a microplate reader (Tecan infinite 200). Growth of the cultures was monitored on a spectrophotometer by measuring the optical density at 700 nm (OD_{700}). Schiff bases were diluted according to Table 1 in a volume of 100 μL; each concentration was prepared in four replicates. Samples were analyzed under a temperature of 37 °C, shaking between measurements and data recording each 30 min. Survival was calculated from the last point in the growth curves, related to the control value.

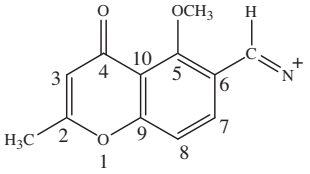
3. Results and discussion

In the present investigation, the newly prepared Schiff bases were investigated and their structures were characterized by elemental analysis, IR, UV–Vis, ^{1}H NMR, ^{13}C NMR and mass spectroscopy. The C, H and N contents of both theoretically calculated and measured values are in accordance with the tentative formula of the Schiff bases. The analytical and physical data of Schiff bases were shown in (Table 2).

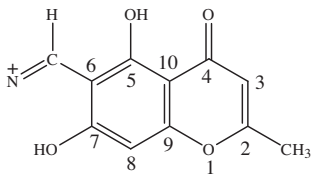
3.1. Characterization of the Schiff bases

3.1.1 Mass spectra of the Schiff bases

The molecular ion peaks are in good agreement with their empirical formula as indicated from elemental analyses. The other peaks represented fragments of the molecular ion. The spectra of the investigated Schiff bases showed molecular ion peaks at (M + 1) (at m/z value representing the molecular weight + 1) for Schiff base SB_3 (m/z 541), SB_4 (m/z 297), SB_5 (m/z 513) and SB_6 (m/z 513) and at M (at m/z values corresponding to the molecular weight) for Schiff bases SB_1 (m/z 310) and SB_2 (m/z 540). It was observed that the most abundant fragment (base peak) of Schiff bases SB_1–SB_3 at m/z 216. Such peak represents the 5-methoxy-2-methylbenzopyran-4-one ring attached to the azomethine group. The peak at 218 m/z in the mass spectra of SB_4–SB_6 Schiff bases represented 5,7-dihydroxy-2-methylbenzopyran-4-one ring attached to the azomethine group. This means that the fragmentation process occurred by breaking of the bond between nitrogen atom and benzene or pyridine ring. According to this pattern of fragmentation a peak at (m/z 78) was found in the spectra of SB_1 and SB_4 Schiff bases due to the pyridine ring. Most of the fragmentation patterns of Schiff bases were ended with aliphatic products [18].



$C_{12}H_{10}NO_3$
m/z 216



$C_{11}H_8NO_4$
m/z 218

3.1.2 IR spectra of the Schiff bases

Table 3 shows the IR band assignments of the investigated Schiff bases. The broad band at 3364–3433 cm^{-1} indicates that the OH groups of these Schiff bases are involved in intramolecular hydrogen bonds [19]. The carbonyl stretching vibration νC=O was observed as singlet at 1666 and 1658 cm^{-1} in the IR spectra of mono Schiff bases SB_1 and SB_4, respectively. This band appeared as doublet at 1617–1662 cm^{-1} for bis-Schiff base SB_2, SB_3, SB_5, and SB_6. The IR spectra of all Schiff bases displayed shoulder at 1584–1609 cm^{-1} which could be assigned to C=N stretching [20] of azomethine groups. The Schiff bases SB_1 and SB_4 contains a pyridine nucleus, the shoulders at 1625 and 1600 cm^{-1}, respectively, could be assigned to C=N stretching of pyridine [21]. There are two strong absorption bands in the lower frequency region [22,23] which can be attributed to the C–OH stretching arises in the region 1170–1225 cm^{-1} and the O–H deformation in the range 1093–1120 cm^{-1}.

3.1.3. ^{1}H NMR and ^{13}C NMR spectra of Schiff bases

A further support for the conclusions obtained from IR spectra of the investigated Schiff bases is gained by the consideration of their proton nuclear magnetic resonance (^{1}H NMR) spectra, Fig. 1. The chemical shifts of the different types of protons in the Schiff bases are recorded in Table 4. ^{13}C NMR spectroscopy was not suitable to all Schiff bases due to poor solubility so it was recorded only for SB_1 and SB_3 as complementary technique for confirming their structure.

The ^{1}H NMR spectra of bis-Schiff bases SB_2, SB_3, SB_5 and SB_6 exhibited doublet at 2.50 ppm, with integration values corresponding to six protons which could be assigned to the aliphatic CH_3 groups in position 2 of the two benzopyran-4-one moieties [24]. This signal was observed at 2.49 and 2.31 ppm in the ^{1}H NMR spectrum of mono-Schiff base SB_1 and SB_4, respectively, with integration value equivalent for only three protons. The proton of C_3 (proton of the pyrone ring) has a marked effect on the shape of the signals due to CH_3 proton in position two which is shortened and broadened in most cases and has been doubled in all Schiff bases. The effect was attributed to long range coupling between C_3 proton and C_2 methyl group [25]. The multiplet signal observed at 6.10–7.51 ppm is assigned to the aromatic protons [24]. The ^{1}H NMR spectra of all Schiff bases show a resonance for CH=N at

Table 2
Analytical and physical data of Schiff bases.

Schiff base	Formula	Color	Melting point	Yield (%)	MW	C% Calc. (Found)	H% Calc. (Found)	N% Calc. (Found)
SB_1	$C_{17}H_{14}N_2O_4$	Yellow	230	95	310.31	65.80 (65.40)	4.55 (4.47)	9.03 (9.29)
SB_2	$C_{30}H_{24}N_2O_8$	Yellow	250	93	540.52	66.66 (66.32)	4.48 (4.40)	5.18 (4.95)
SB_3	$C_{30}H_{24}N_2O_8$	Orange	300	95	540.52	66.66 (66.69)	4.48 (4.43)	5.18 (5.40)
SB_4	$C_{16}H_{12}N_2O_4$	Yellow	210	95	296.28	64.86 (65.00)	4.08 (3.99)	9.46 (9.60)
SB_5	$C_{28}H_{20}N_2O_8$	Yellow	Charring at 250	96	512.47	65.62 (65.69)	3.93 (3.79)	5.47 (5.37)
SB_6	$C_{28}H_{20}N_2O_8$	Orange	Charring at 300	96	512.47	65.62 (65.89)	3.93 (3.84)	5.47 (5.29)

Table 3
Assignment of IR bands of the Schiff bases.

SB_1	SB_2	SB_3	SB_4	SB_5	SB_6	Band assignment
3419 b	3433 b	3432 b	3430 b	3364 b	3430 b	υOH
2930 w	2954 w	2923 w	2924 w	2954 w	2922 w	Asym. CH_3 def.
2843 w	2874 w	2843 w	2850 w	2874 w	2874 w	Sym. CH_3 def.
1666 s	1659, 1662 d	1658, 1608 d	1658 s	1655, 1617 d	1656, 1632 d	υC=O benzopyrone
1609 s	1602 s	1636 s	1625 s	1617 s	1632 s	υC=N azomethine
1529 s	1602 s	1584 s	1560 s	1544 s	1632 s	υC=C
1299 m	1339 m	1340 m	1339 w	1342 w	1341 m	υC–N
1085 m	1078 m	1082 m	1078 w	1086 w	1090 m	Asym. C–O–C stre.
1195 w	1194 m	1188 m	1198 w	1174 w	1172 m	δC–H in-plane def.
1112 m	1109 m	1111 m	1119 m	1116 m	1115 m	δO–H def.
1228 m	1194 w	1167 w	1170 m	1170m	1171 m	δC–OH
844 m	860 w	866 w	819 m	828 w	837 m	γC–H out-of-plan def.

b: broad, s: strong, m: medium and w: weak.

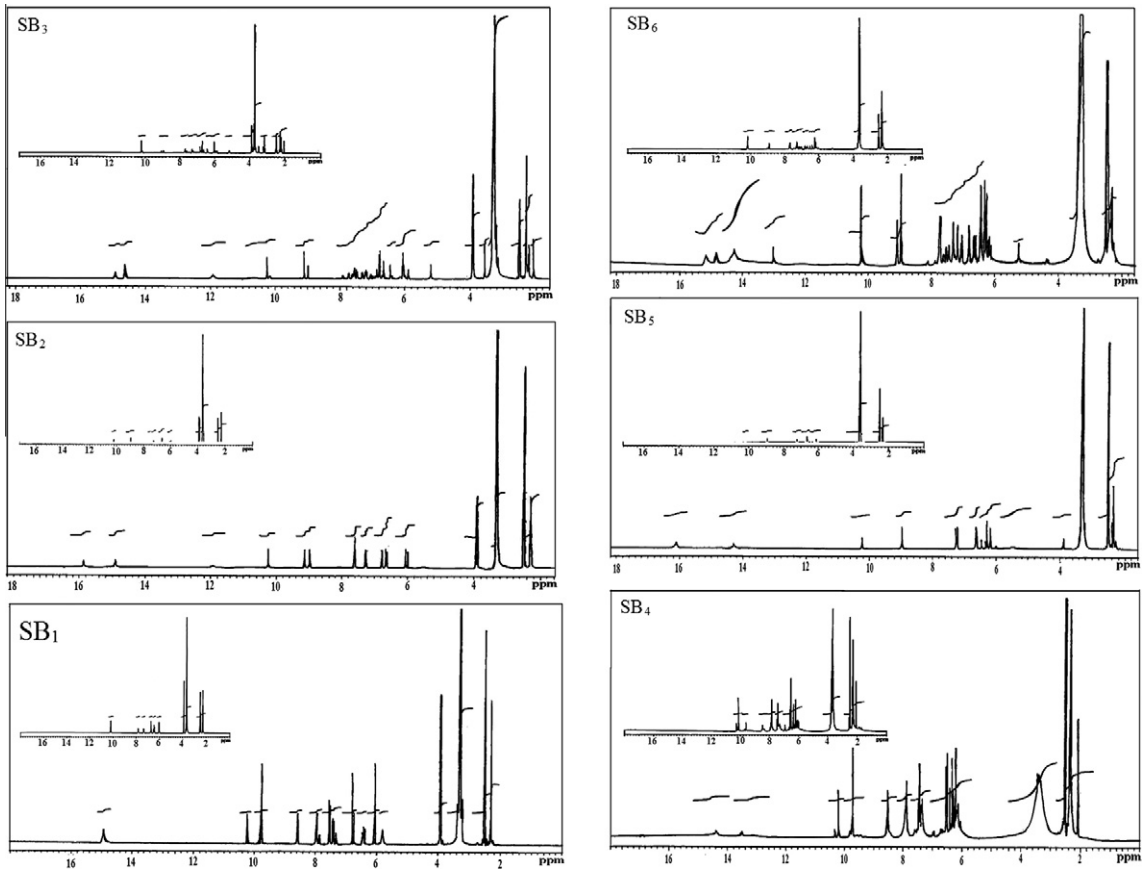


Fig. 1. ^1H NMR spectra of Schiff bases in DMSO and DMSO + D_2O.

Table 4
Proton NMR spectral data of the Schiff bases.

Compound	Chemical shift (ppm)	Assignment
SB_1	14.96	s, 1H, C_7–OH
	10.24	s, 1H, CH=N
	6.44–7.51	m, aromatic CH
	2.49	d, 3H, CH_3 in pos. 2
SB_2	15.86, 14.91	s, 1H, C_7–OH (two benzopyrone moieties)
	10.25	s, 2H, CH=N
	6.10–7.29	m, aromatic CH
	2.50	d, 6H, CH_3 in pos. 2
SB_3	14.93, 14.64	s, 1H, C_7–OH (Two benzopyrone moieties)
	10.25	s, 2H, CH=N
	6.44–7.30	m, aromatic CH
	2.50	d, 6H, CH_3 in pos. 2
SB_4	14.45	s, 1H, C_5–OH
	13.63	s, 1H, C_7–OH
	10.21	s, 1H, CH=N
	6.41–7.40	m, aromatic CH
	2.31	d, 3H, CH_3 in pos. 2
SB_5	16.10	s, 2H, C_5–OH (Two benzopyrone moieties)
	14.30	s, 2H, C_7–OH (Two benzopyrone moieties)
	10.24	s, 2H, CH=N
	6.33–7.26	m, aromatic CH
	2.50	d, 6H, CH_3 in pos. 2
SB_6	15.20, 14.84	s, 1H, C_5–OH (Two benzopyrone moieties)
	14.26, 13.04	s, 1H, C_7–OH (Two benzopyrone moieties)
	10.23	s, 2H, CH=N
	6.32–7.31	m, aromatic CH
	2.50	d, 6H, CH_3 in pos. 2

δ = 10.21–10.25 ppm. The downfield shift of the OH protons may be due to the OH groups that are involved in hydrogen bonding. In all Schiff bases, the OH protons in position seven of the benzopyran-4-one moiety (C_7–OH) are hydrogen bonded to the azomethine (CH=N) groups. So, the ^{1}H NMR spectra of mono-Schiff bases SB_1 and SB_4 showed the signal of OH in position seven of benzopyran-4-one ring at 14.96 and 13.60 ppm, respectively, with integration value equal to one. For bis Schiff bases SB_2, SB_3 and SB_6, the two OH protons in position seven of the two benzopyran-4-one moieties appeared as two singlets at 13.04–15.86 ppm with integration value corresponding to one proton, Table 4, this clearly indicated that the magnetic environment was not equivalent for such protons. On the other hand, the ^{1}H NMR spectrum of Schiff base SB_5 showed only one singlet at 14.30 ppm with integration value corresponding to two protons due to the two C_7–OH protons of the two benzopyran-4-one moieties which are in the same magnetic environment.

For Schiff bases SB_4, SB_5 and SB_6, the OH protons in position five (C_5–OH) are more shifted downfield than C_7–OH protons this is may be due to their involvement in hydrogen bonding with the carbonyl (C=O) groups in position four of the benzopyran-4-one ring. The ^{1}H NMR spectrum of Schiff base SB_4 showed singlet at 14.45 ppm due to C_5–OH proton. In the ^{1}H NMR spectrum of SB_5, the signal due to OH protons in position five appeared as one signal at 16.10 ppm with integration value corresponding to two protons this confirms that they are in the same magnetic environment. These signals appeared as singlet at 15.20 and 14.84 ppm with integration values equivalent to one proton in the spectrum of SB_6 due to the different magnetic environment. The disappearance of the signal of OH protons on deutration confirms that they are ionizable protons.

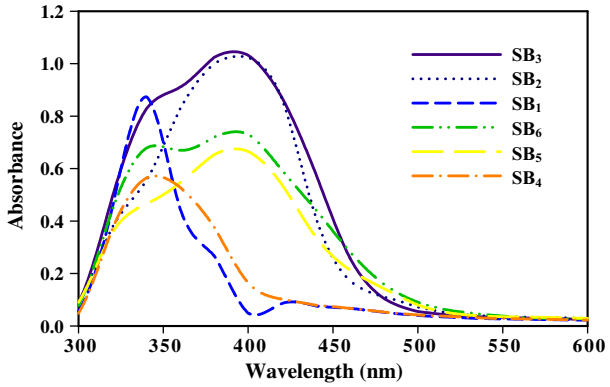


Fig. 2. Electronic absorption spectra of Schiff bases under investigation in DMF.

Table 5
UV–Vis spectral data of Schiff bases under investigation in DMF.

SB_1	SB_2	SB_3	SB_4	SB_5	SB_6	Assignment	
340	325	345	340	325	346	λ_{max}	π–π^* of C=N group
1.74	0.90	1.75	1.18	0.80	1.44	ε_{max}	
380	395	390	–	392	400	λ_{max}	Charge transfer
0.54	2.20	2.20		1.32	1.36	ε_{max}	band
3.27	3.14	3.18	–	3.17	3.10	E_{CT} (eV) from Eq. (1)	
7.73	7.62	7.66	–	7.65	7.59	I_{P1} (eV)	
7.70	7.60	7.63	–	7.62	7.57	I_{P2} (eV)	
7.40	7.31	7.34	–	7.33	7.30	I_{P3} (eV)	
7.61	7.51	7.54	–	7.53	7.49	Mean I_P (eV) values	
3.31	3.21	3.24	–	3.23	3.19	E_{CT} from Eq. (2) [C = −5.6 eV]	

λ_{max} (nm) and ε_{max} ($Lmol^{-1}cm^{-1} \times 10^4$).

In ^{13}C NMR spectrum of SB_1, the number of signals of sharp peaks represents the number of carbons of the compound which are chemically non-equivalent. For Schiff base SB_3 the sharp peaks represents the number of carbons show that these carbons are equivalent. The spectra of SB_1 and SB_3 exhibit the azomethine CH=N carbon at 164 ppm, the carbonyl (C=O) carbon at 175 ppm, methoxy (OCH_3) carbon at 60 ppm and methyl (CH_3) carbon at 20 ppm. The ^{13}C NMR spectra of two Schiff bases show downfield peaks at 166, 155, 164 and 158 ppm corresponding to C_2, C_5, C_7 and C_9 of benzopyran-4-one ring. The downfield shift of these signals is due to the descreening effect of electronegative oxygen atom. The downfield peaks at 144 and 159 ppm in the spectrum of Schiff base SB_1 is attributed to C_{15} and C_{11} of pyridine ring however C_{12}, C_{13} and C_{14} appeared at 124, 135 and 127, respectively. The ^{13}C NMR spectra of both Schiff bases are characterized by very sharp peaks at 112, 119, 101 and 126 ppm corresponding to C_3, C_6, C_8 and C_{10} of the aromatic benzopyran-4-one ring, respectively.

3.1.4 Electronic absorption spectra of the Schiff bases

The electronic absorption spectra of Schiff bases in dimethylformamide (DMF) are shown in Fig. 2, For Schiff bases SB_2, SB_3, SB_5 and SB_6 a shoulder within the 325–346 nm range can be assigned due to the π–π^* transition within the azomethine (CH=N) group and a band at 390–400 nm due to an intramolecular charge transfer (CT transition) involving the whole molecule. The electronic absorption spectra of Schiff base SB_4 exhibits only one band at 340 nm and that of Schiff base SB_1 shows band at 340 nm and shoulder at 380 nm, Table 5.

The charge transfer originates from benzoyran-4-one ring as origin to the C=N group as a sink. This can be confirmed by determining the energy of this charge transfer band from λ_{max} values using the relation:

$$E_{CT} = 1241.6/\lambda_{max}^{CT} \text{ (eV)} \quad (1)$$

Then the values obtained are compared with those calculated using the Briegleb relation [26]:

$$E_{CT} = (I_P - E_A) + C \quad (2)$$

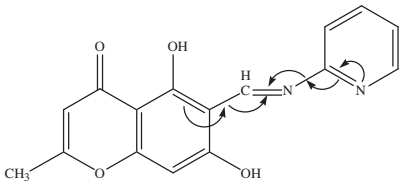
where I_P is the ionization potential of the donor part, E_A is the electron affinity of the C=N acceptor group (−1.3 eV) and C is the columbic force between the electron transferred and the positive hole left behind ($C = -5.6$ eV). The data calculated for E_{CT} from Eq. (2) using the value of C are in satisfactory agreement with the E_{CT} obtained from the experimental data using Eq. (1), Table 5.

The ionization potentials of the Schiff bases under study were determined from the electronic absorption spectra by applying the relation:

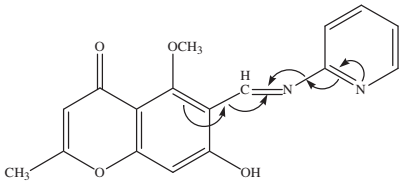
$$I_P = a + bE_{CT} \quad (3)$$

where a and b are constants having the values (4.93 and 0.857) [27], (5.156 and 0.778) [28] or (5.11 and 0.701) [29]. The calculated values together with the mean values are depicted in Table 5.

The charge transfer band was not observed in the electronic spectra of SB_4 ligand. This behavior may be due to the attachment of pyridine ring in the amine moiety, so it can acts as an electron donor group and antagonizes the charge transfer from the benzopyrone ring to the C=N group as represented by the following:



For the ligand SB_1 the charge transfer band intensity is lowered and appeared as a shoulder at 380 nm this may be due to the presence of the electron donating OCH_3 group in position five which intensify the charge transfer from benzopyrone ring to C=N group. Thus, the antagonized effect of pyridyl ring lowers the intensity of the charge transfer that does not compensate it completely.



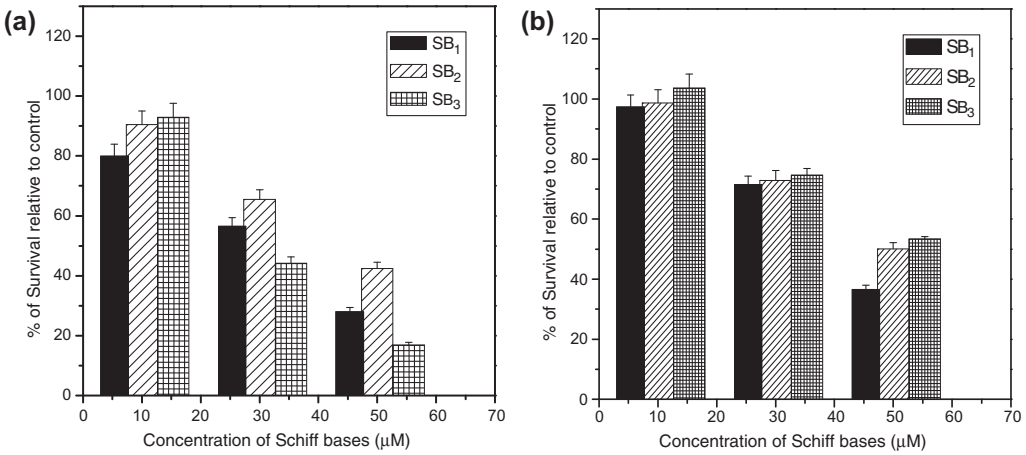


Fig. 3. Effect of different concentrations of Schiff bases SB_1, SB_2 and SB_3 on bacterial cells; (a) *E. coli* and (b) *S. capitis*.

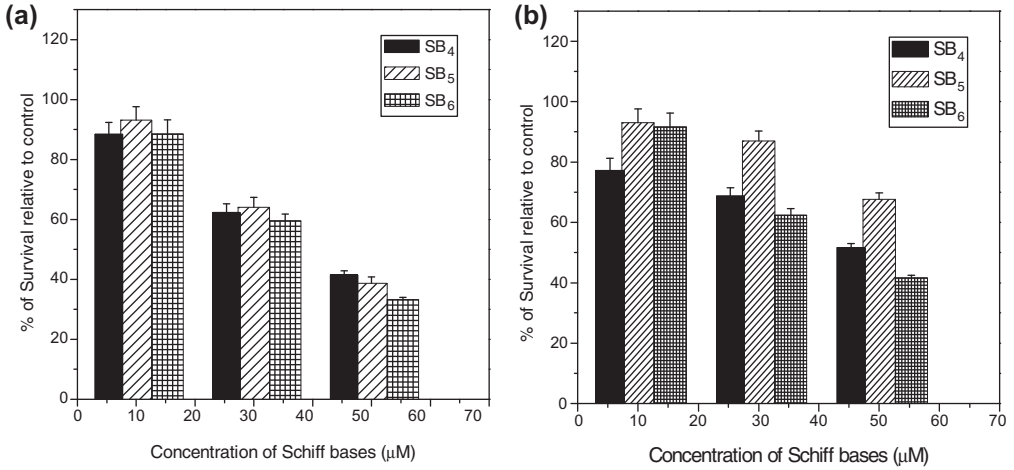


Fig. 4. Effect of different concentrations of Schiff bases SB_4, SB_5 and SB_6 on bacterial cells; (a) *E. coli* and (b) *S. capitis*.

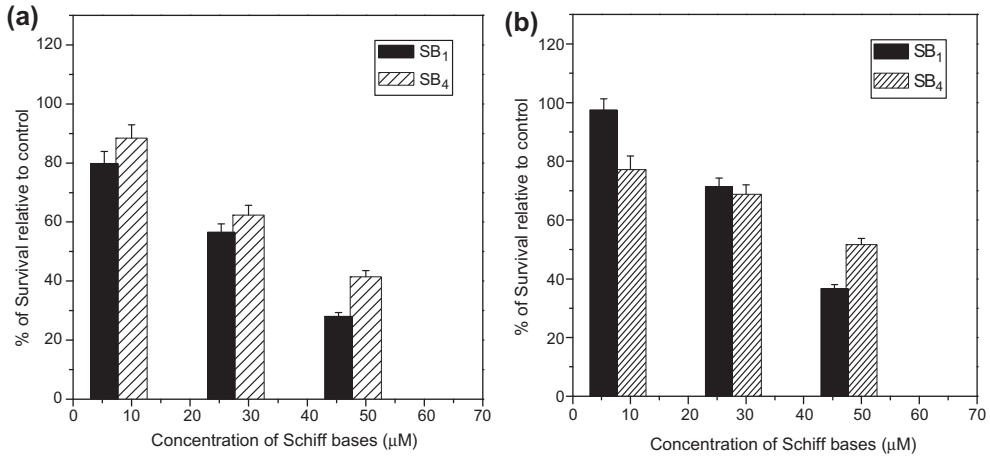


Fig. 5. Effect of different concentrations of Schiff bases SB_1 and SB_4 on bacterial cells; (a) *E. coli* and (b) *S. capitis*.

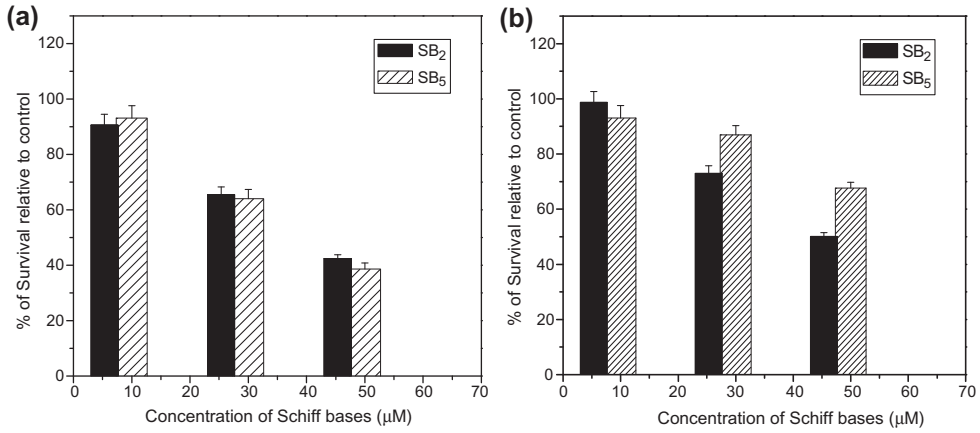


Fig. 6. Effect of different concentrations of Schiff bases SB_2 and SB_5 on bacterial cells; (a) *E. coli* and (b) *S. capitis*.

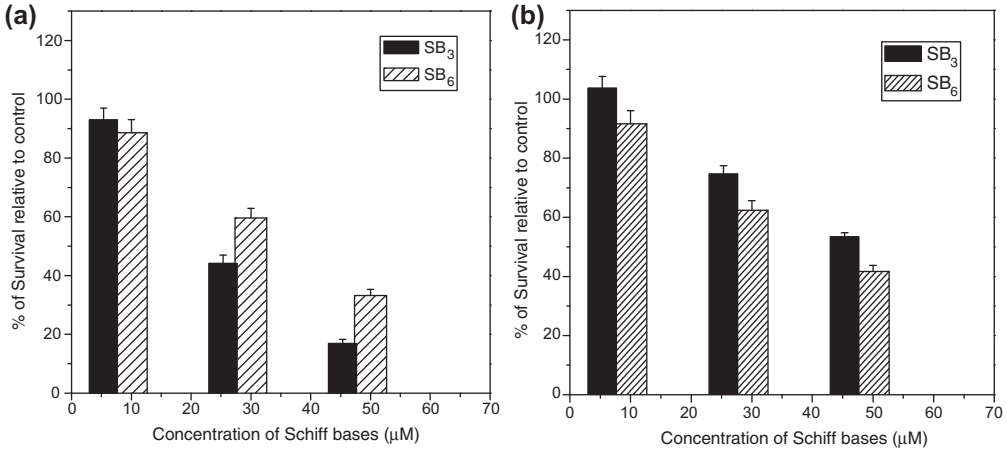


Fig. 7. Effect of different concentrations of Schiff bases SB_3 and SB_6 on bacterial cells; (a) *E. coli* and (b) *S. capitis*.

3.2. Antibacterial activity using microplate assay

All the synthesized compounds were screened for antibacterial activity against *S. capitis* and *E. coli* by the turbidimetric method using a microplate reader. The antibacterial effect of DMF alone (solvent) was determined previously [9] where DMF alone has no significant inhibitory effect at the concentration below 0.5 M so that the concentration of DMF at all prepared Schiff bases-with different concentrations – did not exceed 0.64 M to avoid its inhibitory effect.

The effect of the Schiff bases SB_1, SB_2 and SB_3 on *E. coli* were demonstrated in Fig. 3a. Analysis of the data of the growth curves showed that with increasing concentrations of these Schiff bases up to 50×10^{-6} M, the survival decreases and the percentage of

inhibited cells follow the order 40%, 70% and 84% for SB_1, SB_2 and SB_3, respectively.

By the same way, the effect of Schiff bases SB_1, SB_2 and SB_3 on *S. capitis* were determined, Fig. 3b. Analysis of the data of the growth curves showed that with increasing concentrations the survival decreases until to a concentration of 50×10^{-6} M, where almost up to 50% of bacterial cells were killed.

The antibacterial effect of the Schiff bases SB_4, SB_5 and SB_6 on *E. coli* were shown in Fig. 4a. Analysis of the data of the growth curves showed that with increasing concentrations of these Schiff bases up to 50×10^{-6} M, about 60% of bacterial cells were killed. Also, it was observed that Schiff bases SB_4, SB_5 and SB_6 had almost the same inhibitory effect on *E. coli* at the same concentrations.

In addition, the effect of Schiff bases SB_4, SB_5 and SB_6 on *S. capitis* were determined, Fig. 4b. Analysis of the data of the growth curves showed that with increasing concentrations the survival decreases until to a concentration of 50×10^{-6} M, the survival decreases and the percentage of inhibited cells were about 50%, 30% and 60% for SB_4, SB_5 and SB_6, respectively.

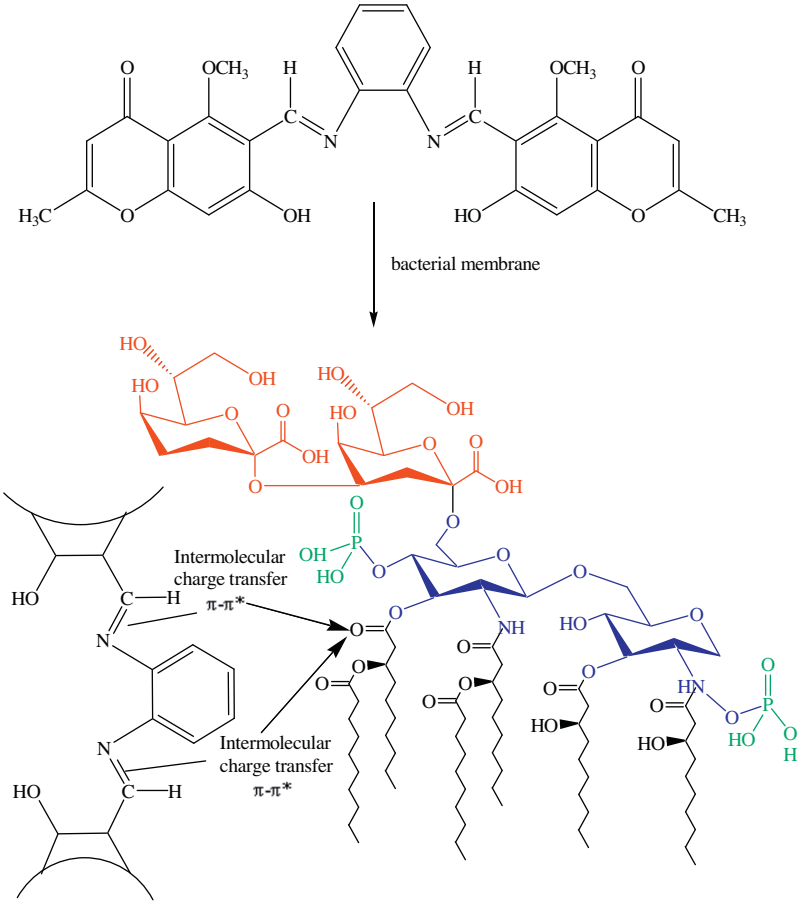


Fig. 8. Schematic diagram representing the charge transfer from Schiff base SB_3 to LPS layers in Gram-negative bacteria.

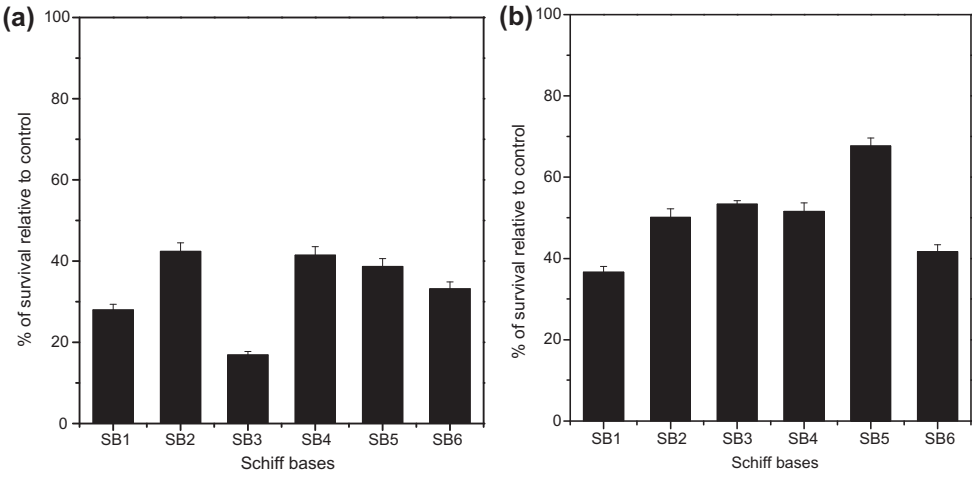


Fig. 9. Effect of 50 μM of Schiff bases SB_1, SB_2, SB_3, SB_4, SB_5 and SB_6 on bacterial cells; (a) *E. coli* and (b) *S. capitis*.

In general, the six Schiff bases had an antibacterial effect on both *E. coli* and *S. capitis*, possibly due to the presence of azomethine groups (imines) which have chelating properties. These properties may affect the metal transport across the bacterial membranes or bind the bacterial cells at specific site and thus inhibit their growth. This is in agreement with Huheey et al. who mentioned that antibiotics such as streptomycin, aspergillic acid, and tetracycline which had chelating properties were able to compete successfully with metal-binding agents of bacteria and thus interfering with their growth [30–32].

Comparison between Schiff bases containing substituent OCH_3 on position 5 (SB_1,SB_2 and SB_3) and those containing hydroxy group at the same position (SB_4, SB_5 and SB_6) on both bacterial starins were studied according to the order SB_1 with SB_4, SB_2 with SB_5 and SB_3 with SB_6 as shown in Fig. 5–7 respectively. Analysis of the data of the growth curves showed that SB_1 had higher antibacterial effect than SB_4 on both gram nagative and gram positive bacteria. In addition, SB_2 showed higher antibacterial effect than SB_5 on gram positive bacteria (*S. capitis*), while had no significant difference on gram negative (*E. coli*). Moreover, SB_3 showed higher antibacterial effect than SB_6 on gram negative (*E. coli*), while there was no significant difference on gram positive bacteria (*S. capitis*). Therefore, the Schiff bases containing substituent OCH_3 on position 5 could enhance the antibacterial activity on gram negative (*E. coli*). This finding is in agree with the others who mentioned that, electron releasing substituent such as methoxy group enhance the antibacterial and antiviral activity [33,34].

It was observed that, although the examined six Schiff bases were used at the same concentrations (10×10^{-6}–50×10^{-6} M) and the number of cells at the beginning of the experiment was the same (10^6 CFU/ml), it is demonstrated that the Schiff bases showed a higher killing effect on gram negative bacteria than on gram positive bacteria. This could be due to gram status, where it was known that the membrane of Gram-negative bacteria is surrounded by an outer membrane containing lipopolysaccharides. These bases seem to be able to combine with the lipophilic layer in order to enhance the membrane permeability of the Gram-negative bacteria. The lipid membrane surrounding the cell favors the passage of only lipid-soluble materials; thus the lipophilicity is an important factor that controls the antimicrobial activity. Also the increase in lipophilicity enhances the penetration of Schiff bases into the lipid membranes and thus restricts further growth of the organism [35,36]. This could be explained by the charge transfer interaction between the Schiff bases molecule and the lipopolysaccharides molecules which lead to the loss of permeability barrier activity of the membrane; Fig. 8. Moreover, results showed that, Schiff base SB_3 has the highest killing effect on *E. coli* among the prepared Schiff bases; Fig. 9.

In general it was observed that aromatic Schiff bases had a higher antibacterial effect than aliphatic Schiff bases which were studied before [9] and this is in agreement with the findings of the others [37].

4. Conclusions

S. capitis as an example of gram positive bacteria is less sensitive to damage than *E. coli* as an example of gram negative bacteria concerning all Schiff bases under study. Moreover, the Schiff bases containing substituent OCH_3 on position 5 have higher antibacterial activity than that containing hydroxy group at the same position. In addition, aromatic Schiff bases had a higher antibacterial effect than aliphatic Schiff bases. Our research is a preliminary step toward developing newly synthesized antimicrobial agents from Schiff base derivatives in order to enhance their effect on bacterial cells. As a next step, the sensitivity of eukaryotic cells to these Schiff bases and their complexes should be examined.

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