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Synthesis, Antioxidant and Antibacterial Activities of Some Schiff Bases Containing Hydroxyl and Methoxy Groups

MOHAMMED AL-MAMARY[1,2,*], SIDDIG IBRAHIM ABDELWAHAB[1], HAPIPAH MOHD ALI[3], SALMA ISMAIL[4], MAHMOOD AMEEN ABDULLA[5] and POUYA DARVISH[5]

[1]Department of Pharmacy, Faculty of Medicine, University of Malaya, Petaling Jaya, Kuala Lumpur, Malaysia
[2]Department of Organic Chemistry, Faculty of Pharmacy, Sana's University, Sana'a, Yemen
[3]Department of Chemistry, Faculty of Science, University of Malaya, PetalingJaya, Kuala Lumpur, Malaysia
[4]Department of Microbiology, Faculty of Medicine, University of Malaya, PetalingJaya, Kuala Lumpur, Malaysia
[5]Department of Molecular Medicine, Faculty of Medicine, University of Malaya, PetalingJaya, Kuala Lumpur, Malaysia

*Corresponding author: E-mail: almamarym@hotmail.com; almamarym@um.edu.my

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A series of Schiff bases were synthesized from different aromatic amines and aromatic aldehydes containing hydroxyl and methoxy groups. These compounds were characterized by IR and ^{1}H NMR. All the compounds were screened for *in vitro* antioxidant activity using DPPH method and total reducing power activity based on the ability of compounds to reduce the Fe^{3+}-TPTZ complex to the Fe^{2+}/ferrous. Compounds substituted with hydroxyl and other electron donating groups, such as, methoxy groups showed low to high antioxidant activity. In addition, the compounds have been screened for their antibacterial activity against strains of *Escherichia coli*, Methycillin-resistant *Staphylococcus aureus*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. The tested Schiff bases at 5 mg/disc showed different antibacterial activities depending on the type of the tested bacterial species. However, *E. coli* appeared to be sensitive to seven compounds (**Ia**, **Ib**, **Ic**, **Id**, **If**, **IIb** and **IIe**), while methicillin resistant *Staphylococcus aureus* (MRSA) was affected by **Ic**, **Ie**, **IIc** and IIe compound. On the other hand, the compounds **Ia**, **Ic**, **Id**, **Ie** and **IIh** revealed antibacterial activity against *Klebsiella* spp. The *Pseudomonas aeruginosa* was not sensitive to any of the tested Schiff bases.

Key Words: Antioxidant, DPPH, FRAP, Antibacterial, Schiff bases.

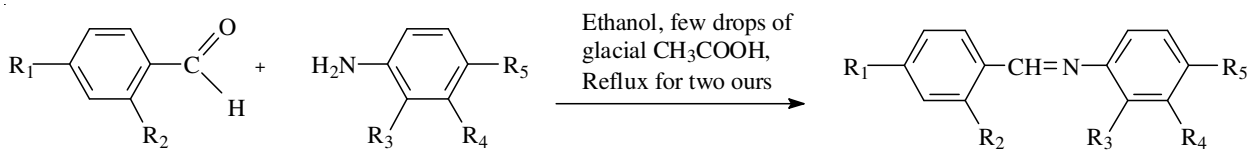
INTRODUCTION

Compounds containing an azomethine group (-CH=N-) known as Schiff bases are formed by the condensation of a primary amine with carbonyl compounds. Schiff bases obtained from aromatic aldehydes and aromatic amines have an effective conjugation system and quite stable. Schiff bases are important compounds owing to their wide range of biological activities and industrial application. They have been found to posses the pharmacological activities such as anticancer, antimicrobial, antifungal, antiviral, antioxidants, antiinflammatory, antiparasitic and antioxidants[1-8]. They also serve as a back bone for the synthesis of various heterocyclic compounds. It seems that the presence of azomethine group is responsible for biological activities expressed by different types of Schiff bases[9]. However, these biological activities can be altered depending upon the types of substituents attached to the aromatic rings[10]. The former reports encouraged us to carry out the synthesis of different Schiff bases containing hydroxyl groups attached to the aromatic rings[11]. Therefore, the main aim of the present work is to find new Schiff bases from different aromatic amines and aromatic aldehydes containing hydroxyl groups and to screen their antioxidant and antibacterial activities.

EXPERIMENTAL

Melting points (ºC) were measured in open glass capillaries using a Barnstead 9001 Electrothermalmelting point apparatus. ^{1}H NMR spectra were obtained on a NMR spectrometer (Jeol) operating at 400 MHz for ^{1}H the chemical shifts are expressed in δ (ppm) downfield from tetramethylsilane (TMS) used as internal standard. The IR spectra were measured as potassium bromide pellets using a Perkin-Elmer 1600 series FTIR spectrometer.

Chemistry: Dissolve 0.02 mol of amino compound in 40 mL of anhydrous ethanol. Add 0.02 mol of salicylaldehyde (drop wise). In some cases, such as, sulfanilamide, *p*-toluidine, aminobenzoic acids (*o*-, *m*- and *p*-aminobenzoic acids), *m*-aminophenol and *p*-aminophenol, precipitate does appear immediately. However, these mixtures were further refluxed



Scheme-I

for *ca.* 20 min. The precipitates were appeared after cooling. Samples were filtered, dried and recrystallized from absolute ethanol.

Antioxidant Activities: The antioxidant activities of the synthesized Schiff bases were measured *in vitro* using two methods, namely: the DPPH free radical scavenging assay and the total reducing power method. All assays were carried out in triplicate and the average value was obtained. All determinations were made spectrophotometrically using the Infinite® 200 PRO plate reader (TECAN, Männedorf, Switzerland).

Free radical scavenging activity using DPPH method: Free radical scavenging activity of the tested compounds were determined by the 1,1-diphenyl picrylhydrazyl (DPPH) assay method[12]. Each of the Schiff bases was dissolved in methanol to obtain concentration of 1 mg/mL. These stock solutions were then diluted to 5, 10, 25, 50 and 100 µg/mL in methanol. Ascorbic acid was prepared in a similar way and used as a positive control. Then, 200 µL of each sample solution or positive control were combined with 50 µL of DPPH (0.3 mmL) in triplicate in a 96-well microtitre plates. Final concentrations of the Schiff bases were 4, 8, 20, 40 and 80 µg/mL. Microtitre plates were incubated for 0.5 h at room temperature. The absorbance of the wells were then determined at 518 nm with the Infinite®200 PRO plate reader (TECAN, Männedorf, Switzerland). The percentage free radical scavenging activity (% inhibition) extrapolated against concentration.

Ferric reducing ability power (FRAP): The FRAP was determined as previously described[13] with modifications. This method is based on a redox reaction in which the antioxidants act as reductants and an easily reduced oxidant (Fe^{3+}) is used in stoichiometric excess, resulting in a blue ferrous complex. The absorbance was then determined spectrophotometrically at 518 nm with the Infinite® 200 PRO plate reader (TECAN, Männedorf, Switzerland). For this a freshly prepared Fe^{3+}-TPTZ complex solution pre-incubated at 37 ºC. This solution (FRAP reagent) was prepared by mixing acetate buffer (300 mmol L^{-1}, pH 3.6), TPTZ (10 mmol L^{-1} in 1.0 mol L^{-1} HCl) and $FeCl_3$ (20 mmol L^{-1}) at 10:1:1 (v/v/v). Then, 200 µL of FRAP reagent solution were combined with 50 µL of Schiff base or positive control solutions in triplicate in a 96-well microtitre plates. The mixtures were shaked and incubated at 37 ºC for 0.5 before absorbance reading at 593 nm. All treatments were run in triplicate. BHT and ascorbic acid (Vit. C) were used as positive controls. The potential of Schiff bases as antioxidants to reduce Fe^{3+}-Fe^{2+} was expressed as Vit. C equivalent (or in µmol Fe^{2+} g^{-1} of Schiff base using a calibration curve of $FeSO_4 \cdot 7H_2O$ (0-800 µg/mL). It was assumed that the higher measured FRAP value, the higher the antioxidant activity of the Schiff base that could reduce the ferric ion to ferrous ion.

Antibacterial activity

Microbial strains: The antibacterial activity of Schiff bases samples was evaluated using Methicillin Resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* 60690, *Escherichia coli* and *Klebsiella pneumoniae*. All bacterial strains were obtained from the Laboratory of Molecular Biomedicine, Faculty of Medicine, University of Malaya.

Disc diffusion method: The screening of the antibacterial effects of Schiff bases was carried out by determining the zone of inhibition using paper disc (6 mm in diameter, Whatman No. 1) diffusion method. The obtained microorganism strains were inoculated in a Petri dish containing nutrient broth at 37 ºC for 24 h and were referred as seeded broth. The density of the bacterial suspension was standardized and the concentrations of the cultures were adjusted turbidometrically at wavelength of 600 nm to 5×10^5-10^6 colony forming unit per mL. Compounds were dissolved in DMSO which was previously tested for antibacterial activity against all tested bacteria and found to have no activity. Compounds were diluted to concentration of 100 mg mL^{-1} and finally sterilized by filtration using 0.45 µm millipore filters. The sterile discs were impregnated with tested solution (0.05 mL from 100 mg mL^{-1}) and placed in inoculated agar. Cloxacillin (30 µg/disc), vancomycin (5 µg/disc) and ampicillin (2 µg/disc) susceptibility discs and methanol impregnated disc were used as positive and negative controls, respectively. After incubation overnight at 37 ºC, inhibition zones were measured and recorded as mean diameter (mm). Antibacterial activity was also expressed as diameter of inhibition zone (mm).

RESULTS AND DISCUSSION

The structures of the synthesized compounds were determined on the basis of their FT-IR and ^{1}H NMR data. IR spectra showed absorption band at 1630-1590 cm^{-1} indicated the stretching vibration of -CH=N- (Schiff-base) and the absence of aldehydic C=O stretching absorption band at *ca.* 1700 cm^{-1} confirming the condensation of reactants. In ^{1}H NMR spectra of the synthesized compounds, the protons of azomethine (-CH=N-) compounds have shown a chemical shift (λ) in the range 8.16-8.20 ppm, which also confirms the condensation of reactants. The IR and ^{1}H NMR analysis data are given below:

Ia: 4-{[(4-Hydroxy-2-methoxyphenyl)methylene] amino}benzoic acid: ^{1}H NMR: 4.48 (s, 3H, -OCH_3); 6.54-7.96 (m, 7H, Ar-H); 8.45 (s, 1H, N=CH); 9.83 (s, 1H, Ar-OH); 12.7 (s, 1H, -COOH). FT-IR (KBr pellet, cm^{-1}): 3504-2600 (-COO-H, str.); 3368 (ArO-H, str.); 1686 (C=O, str.); 1593 (N=C, str.); 1280, 1030 (Ar-O-CH_3, asy. and sym. str.).

Ib: 4-{[(4-Hydroxyphenyl)methylene]amino}benzoic acid: ^{1}H NMR: 6.93-7.70 (m, 8H, Ar-H, 8.01 (s, 1H, -N=CH); 12.7 (s, 1H, Ar-OH); 12.90 (s, 1H, -COOH). FT-IR (KBr pellet,

cm^{-1}): 3500-2563 (-COO-H, str.)); 1681 (C=O, str. in -COOH); 1600 (-N=C, str.); 1280, 1025 (Ar-O-CH_3, asy. and sym. str.).

Ic: 3-{[(4-Hydroxy-2-methoxyphenyl)methylene] amino}benzoic acid: ^{1}H NMR:3.98 (s, 3H, -OCH_3); 6.65-7.93 (m, 7H, Ar-H); 8.62 (s, 1H, -N=CH); 9.82 (s, 1H, Ar-OH); 10.30 (s, 1H, -COOH). FT-IR (KBr pellet, cm^{-1}): 3472-2500 (-COO-H, str.); 3371 (ArO-H, str.); 1693 (C=O, str. in -COOH group); 1623 (N=C, str.); 1280, 1209 (Ar-O-CH_3, asy. and sym. str.).

Id: 4-{[(2-Hydroxyphenyl)methylene]amino}benzoic acid: ^{1}H NMR: 6.95-7.56 (m, 8H, Ar-H); 8.70 (s,1H, -N=CH); 12.65 (s, 1H, Ar-OH); 12.88 (s, 1H, -COOH). FT-IR (KBr pellet, cm^{-1}): 3450-2570 (-COO-H, str.); 3361 (ArO-H, str.); 1678 (C=O, str. in -COOH group); 1623 (N=C, str.).

Ie: 2-{[(4-Hydroxy-2-methoxyphenyl)methylene] amino}benzoic acid: ^{1}H NMR: 3.98 (s, 3H, -OCH_3); 7.03-7.49 (m, 7H, Ar-H); 8.39 (s, 1H, -N=CH); 9.83 (s, 1H, Ar-OH); 12.62 (s, 1H, -COOH). IFT-IR (KBr pellet, cm^{-1}): 3485-2650 (-COO-H, str.); 3376 (ArO-H, str.); 1659 (C=O, str. in -COOH group); 1602 (N=C, str.); 1310, 1026 (Ar-O-CH_3, asy. and sym. str.).

If: 2-{[(4-Hydroxyphenyl)methylene]amino}benzoic acid: ^{1}H NMR: 6.46 - 7.85 (m, 8H, Ar-H); 8.84 (s, 1H, -N=CH); 10.25 (s, 1H, Ar-OH); 10.69 (s, 1H, -COOH). FT-IR (KBr pellet, cm^{-1}): 3500-3470 (-COO-H, str.); 1686 (C=O, str. in -COOH group); 1618 (-N=C, str.); 1246 (Ar-OH, bend.).

IIa: 4-{[(4-Hydroxy-2-methoxyphenyl)methylene] amino}benzenesulfonamide: ^{1}H NMR: 3.83 (s, 3H, -OCH_3); 5.78 (s, 2H, -NH_2); 7.32-7.49 (m, 7H, Ar-H); 9.84 (s, 1H, Ar-OH). FT-IR (KBr pellet, cm^{-1}): 3470, 3440 (-NH_2, str.); 3344 (ArO-H, str.); 1629 (N=C, str.); 1319, 1026 (Ar-O-CH_3, asy. and sym. str.); 1319, 1149 (-SO_2^-, asy. and sym. str.).

IIb: 4-{[(2-Hydroxyphenyl)methylene]amino} benzenesulfonamide: ^{1}H NMR:3.36 (s, 2H, -NH_2); 6.81-7.41 (m, 8H, Ar-H); 8.61 (s, 1H, -N=CH); 12.61 (s, 1H, Ar-OH). FT-IR (KBr pellet, cm^{-1}): 3347 and 3246 (-NH_2, str.); 1617 (-N=C, str.); 1312, 1167 (-SO_2^-, asy. and sym. str.).

IIc: 2-{[(2-Hydroxyphenyl)imino]methyl}phenol: ^{1}H NMR: 5.87 (s, 1H); 6.95 - 7.44 (m, 8H, Ar-H); 8.69 (s, 1H, -N=CH); 12.29 (s, 1H, Ar-OH); FT-IR (KBr pellet, cm^{-1}): 3380 (AO-H, str.); 1631 (N=C, str.); 1223 (Ar-OH, bend.).

IId: 2-{[3-Hydroxyphenyl)imino]methyl}phenol: ^{1}H NMR: 6.77-7.40 (m, 8H, Ar-H); 8.58 (s, 1H, -N=CH); 9.89 (s, 1H, Ar-OH). FT-IR (KBr pellet, cm^{-1}): 3319 (ArO-H, str.); 1621 (-N=C, str.); 1233 (Ar-OH, str.).

IIe: 2-{[4-Hydroxyphenyl)imino]methyl}phenol: ^{1}H NMR: 6.87-7.39 (m, 8H, Ar-H); 8.58 (s, 1H, -N=CH). FT-IR (KBr pellet, cm^{-1}): 3401 (ArO-H, str.); 1617 (-N=C, str.); 1210 (Ar-OH, str.).

IIf: 2-{[(3-Methoxyphenyl)imino]methyl}phenol: ^{1}H NMR: 3.84 (s, 3H, -OCH_3); 6.81-7.42 (m, 8H, Ar-H); 8.61 (s, 1H, -N=CH); 13.21 (s, 1H, Ar-OH). FT-IR (KBr pellet, cm^{-1}): 3380 (ArO-H, str.); 1600 (-N=C, str.); 1286, 1045 (Ar-O-CH_3, str.); 1221 (Ar-OH, str.).

IIg: 3-Methoxy-4-{[4-methylphenyl)imino]methyl} phenol: ^{1}H NMR: 2.36 (s, 3H, -CH_3); 3.98 (s, 3H, -OCH_3); 6.95-7.32 (m, 7H, Ar-H); 7.61 (s, 1H, -N=CH); 8.35 (s, 1H, Ar-OH). FT-IR (KBr pellet, cm^{-1}): 3370 (ArO-H, str.); 1624 (N=C, str.); 1215 (Ar-OH, str.); 1387, 1367 (-CH_3, aym. and sym. bend.); 1281, 1032 (Ar-O-CH_3, str.).

IIh: 2-{[(4-Methylphenyl)imino]methyl}phenol: ^{1}H NMR: 2.40 (s, 3H, -CH_3); 6.90-7.42 (m, 8H, Ar-H); 8.61 (s, 1H, -N=CH); 13.38 (s, 1H, Ar-OH). FT-IR (KBr pellet, cm^{-1}): 3350 (ArO-H, str.); 1619 (N=C, str.); 1183 (Ar-OH, str.); 1386, 1373 (-CH_3, asy. and sym. ben.).

The physical data and yield (%) of the syntheized compounds are given in Table-1

Antioxidant activity: A series of Schiff bases containing hydroxyl and methoxy groups were synthesized and evaluated for their antioxidant activity, such as, scavenging free radical and total reducing power ability using two methods, namely DPPH and reduction of Fe^{3+}-Fe^{2+} ions. The present results indicated that the tested Schiff bases **Ic**, **Ia**, **IIa**, **IIg**, **Ie**, **IIc**, **Id**, **If**, **IIe** and **IId** have free radical scavenging activity, which increases with the increase of their tested concentrations. However, at the lowest concentration some of these compounds showed comparable and even higher ability to scavenge free radicals from the reaction mixtures in comparison with ascorbic acid, which was used as a positive control. On the other hand, the compound **IIc** has shown to have high free radical scavenging activity at its highest concentrations and this activity was not significantly different from those of the positive controls. Schiff bases with two hydroxyl groups attached to the aromatic rings (**IIc**, **IId** and **IIe**) or having one hydroxyl and other electron donating groups, such as, -OCH_3 and -CH_3(**IIg**) showed high to very high free radical scavenging activity. These results were in agreement with other findings obtained by other researchers when two hydroxyl groups attached to the phenyl rings[14]. The effect of Schiff bases on DPPH radical scavenging ability could be due to their hydrogen donating ability[3]. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule[15]. The decrease in absorbance of DPPH radical caused by Schiff bases, might be resulted due to the reaction between these compounds and DPPH radical, which result in the neutralization of the free electron on DPPH by hydrogen donation as the color of the solution changed from purple to yellow (Table-2).

The reductive capabilities of compounds are assessed by the extent of conversion of the Fe^{3+}-TPTZ complex to the Fe^{2+}/ ferrous form using the method described by Benzie and Strain[13]. The reducing powers of the tested Schiff bases were observed at different concentrations and results were expressed as equivalent to Vit. C. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity to reduce Fe^{3+} ions to Fe^{2+} ions. Results in Table-3 showed that all the tested Schiff bases have the ability to reduce Fe^{3+} ions to Fe^{2+} ions and this ability increased with the increase of their concentrations. The present findings indicated that all tested compounds have greater reducing ability than Vit. C at their lower levels. However, if we exclude compounds **IIc** and **IIe**, which have extraordinary reducing ability, other compounds had either lower or comparable reducing capacity in comparison with Vit. C at the tested higher levels. It seems that these types of Schiff bases, especially thosecontaining hydroxyl and other electron donating groups, such as, **IIc**, **IIe**, **IId** and **IIg**

TABLE-1
COMPOUNDS, PERCENTAGE YIELD AND MELTING POINT

Compound	R1	R2	R3	R4	R5	Yield (%)	m.p. (ºC)
Ia	OH	OCH_3	H	H	COOH	77.08	209-210
Ib	H	OH	H	H	COOH	98.34	267-268
Ic	OH	OCH_3	H	COOH	H	70.71	168-170
Id	H	OH	H	COOH	H	97.3	195-196
Ie	OH	OCH_3	COOH	H	H	97.13	114-115
If	H	OH	COOH	H	H	82.35	213–214
IIa	OH	OCH_3	H	H	SO_2NH_2	93.82	202-204
IIb	H	OH	H	H	SO_2NH_2	98.57	219-220
IIc	H	OH	OH	H	H	78.95	190-191
IId	H	OH	H	OH	H	97.95	120-121
IIe	H	OH	H	H	OH	46.58	142-143
IIf	H	OH	H	OCH_3	H	81.08	64-65
IIg	OH	OCH_3	H	H	CH_3	95.51	123-124
IIh	H	OH	H	H	CH_3	91.4	95-96

TABLE-2
FREE RADICAL SCAVENGING ACTIVITY OF SCHIFF BASES USING DPPH METHOD (% INHIBITION, MEAN ± SD)

Compound No.	4 µg/mL	8 µg/mL	20 µg/mL	40 µg/mL	80 µg/mL
Ia	13.2 ± 0.40	23.7 ± 1.19	31.4 ± 0.63	51.6 ± 3.10	62.6 ± 3.57
Ib	1.2 ± 0.06	3.6 ± 0.21	4.5 ± 0.22	5.5 ± 0.11	8.6 ± 0.43
Ic	11.9 ± 0.36	21.4 ± 1.07	28.7 ± 0.57	47.6 ± 2.86	61.0 ± 3.486
Id	5.3 ± 0.27	8.6 ± 0.43	13.3 ± 0.27	16.5 ± 0.82	27.9 ± 1.59
Ie	13.5 ± 0.41	18.9 ± 0.94	31.3 ± 0.63	43.8 ± 2.63	55.0 ± 3.14
If	4.7 ± 0.24	7.3 ± 0.36	15.1 ± 0.30	20.0 ± 1.00	26.5 ± 1.51
IIa	9.9 ± 0.30	14.5 ± 0.73	28.4 ± 0.57	40.2 ± 2.41	57.5 ± 3.28
IIb	-0.2 ± -0.01	1.1 ± 0.06	2.0 ± 0.04	6.6 ± 0.33	8.9 ± 0.44
IIc	30.2 ± 0.91	66.7 ± 3.34	80.8 ± 1.62	85.6 ± 4.38	87.9 ± 4.90
IId	16.0 ± 0.80	25.4 ± 1.27	42.3 ± 0.85	55.1 ± 3.31	68.7 ± 3.92
IIe	21.2 ± 1.06	27.5 ± 1.37	32.3 ± 0.65	53.8 ± 3.23	71.6 ± 4.08
IIf	-0.2 ± -0.01	0.3 ± 0.01	2.2 ± 0.11	3.5 ± 0.17	3.1 ± 0.18
IIg	26.5 ± 0.80	34.6 ± 1.73	54.9 ± 1.10	69.0 ± 1.74	78.7 ± 4.49
IIh	3.8 ± 0.19	8.4 ± 0.25	11.1 ± 0.22	11.6 ± 0.58	14.0 ± 0.80
Vit.C	22.8 ± 0.68	53.1 ± 1.59	90.4 ± 1.81	92.2 ± 2.77	92.1 ± 2.76

TABLE-3
TOTAL REDUCING ABILITY POWER OF SCHIFF BASES EXPRESSED AS VIT. C EQUIVALENT (MEAN ± SD)

Compound	4 µg/mL	8 µg/mL	20 µg/mL	40 µg/mL	80 µg/mL
Ia	18.52 ± 0.25	19.00 ± 0.76	19.73 ± 0.87	22.82 ± 0.30	28.83 ± 0.97
Ib	19.7 ± 1.47	18.79 ± 0.83	20.30 ± 0.38	19.15 ± 0.08	27.92 ± 6.39
Ic	18.35 ± 0.45	19.43 ± 0.09	21.06 ± 0.36	24.75 ± 1.21	32.68 ± 0.59
Id	18.09 ± 0.36	17.99 ± 0.67	17.80 ± 0.61	18.74 ± 0.24	22.69 ± 0.21
Ie	19.96 ± 0.10	19.85 ± 0.06	22.90 ± 2.45	26.70 ± 2.16	28.025 ± 0.16
If	16.83 ± 0.45	17.86 ± 0.0	18.15 ± 0.08	19.43 ± 0.09	21.82 ± 0.22
IIa	20.56 ± 0.59	20.59 ± 1.56	21.63 ± 1.61	21.80 ± 0.37	24.41 ± 0.14
IIb	18.27 ± 0.42	18.19 ± 0.30	18.33 ± 0.83	18.26 ± 0.28	19.89 ± 0.08
IIc	41.82 ± 0.91	82.59 ± 2.12	117.04 ± 5.75	322.75 ± 0.93	540.69 ± 0.21
IId	25.79 ± 0.18	24.93 ± 0.54	30.90 ± 0.18	41.37 ± 1.13	57.56 ± 1.20
IIe	50.53 ± 1.80	86.36 ± 3.05	134.72 ± 7.43	333.54 ± 9.23	516.22 ± 11.10
IIf	20.49 ± 0.32	21.49 ± 0.21	27.12 ± 0.08	41.12 ± 0.93	58.03 ± 2.91
IIg	21.06 ± 0.40	24.19 ± 0.91	33.9 ± 0.99	55.2 ± 1.50	78.28 ± 4.22
IIh	25.09 ± 0.40	26.96 ± 1.44	37.37 ± 1.66	46.99 ± 2.65	74.93 ± 2.77

can take part in electron transfer reactions. On the bases of the new data, it can be suggested that the presence of hydroxyl and other electron donating groups attached to aromatic rings might be responsible for the antioxidant activity of Schiff bases as measured by the two methods described before.

Antibacterial activity: The ever growing resistance to antibiotics leads to continuous screening for new biologically effective compounds of either natural or synthetic origin[16]. Therefore, the present Schiff bases were also screened for the antibacterial significance. This screening of duly characterized Schiff bases was performed using paper disc method against some pathogenic strains of *E. coli*, methycillin-resistant *Staphylococcus aureus*, *Klebsiella* spp. and *Pseudomonas aeruginosa*. The results in Table-3 showed that not all Schiff bases at 5 mg/disc were active against all of the tested bacterial species. *E. coli* appeared to be sensitive to seven compounds

(**Ia**, **Ib**, **Ic**, **Id**, **If**, **IIb** and **IIe**), while MRSA was affected by **Ic**, **Ie**, **IIc** and **IIe**. On the other hand, the compounds **Ia**, **Ic**, **Id**, **Ie** and **IIh** reveal antibacterial against *Klebsiella* spp. However, the *P. aeruginosa* was not sensitive to any of the tested Schiff bases.

TABLE-4
ANTIBACTERIAL SCREENING OF PREPARED SCHIFF BASES (5 mg/disc, ZONE OF INHIBITION IN mm)

Sample	*E. coli*	MRSA	*Klebsiellaspp*	*P. auriginosea*
Ia	15	0	13	0
Ib	20	0	0	0
Ic	14	11	15	0
Id	15	0	18	0
Ie	0	11	12	0
If	20	20	0	0
IIa	0	0	0	0
IIb	14	0	0	0
IIc	0	10	0	0
IId	0	0	0	0
IIe	13	13	0	0
IIf	0	0	0	0
IIh	0	0	9	0
CN (30 µg/disc)	8	–	–	15
VA (5 µg/disc)	–	15	–	–
Amp. (2 µg/disc)	–	–	12	–

Note: MRSA = *Methicillin-resistant staphylococcus aureus*, CN = Cloxacillin, VA = Vancomycin, Amp. = Ampicillin.

Conclusion

The antioxidant activity of the present Schiff bases could be attributed to the active hydroxyl and other electron donating groups, such as, methoxy and methyl groups. The relation between the molecular structures and bioactivity requires further research. The antimicrobial screening suggests that among the synthesized compounds, the compounds **Ia**, **Ic**, **Id**, **Ie**, **If** and **IIe** exhibited antibacterial activities towards *E. coli*, MRSA and *Klebsiella pneumonia*. This work will be continued to synthesize and test the biological activities of Schiff bases containing more hydroxyl and methoxy groups.

REFERENCES

1. J. Balzarini, M. Stevens, E. De Clercq, D. Schols and C. Pannecouque, *J. Antimicrob. Chemother.*, **55**, 135 (2005).
2. A.A. Bekhit, H.T.Y. Fahmy, S.A.F. Rostom and A.M. Baraka, *Eur. J. Med. Chem.*, **38**, 27 (2003).
3. M. Cacic, M. Molnar, B. Šarkanj, E. Has-Schön and V. Rajkovi, *Molecules*, **15**, 6795 (2010).
4. B.S. Holla, K.V. Malini, B.S. Rao, B.K. Sarojini and N.S. Kumari, *Eur. J. Med. Chem.*, **38**, 313 (2003).
5. B.S. Holla, B. Veerendra, M.K. Shivananda and B. Poojary, *Eur. J. Med. Chem.*, **38**, 759 (2003).
6. D. Kovala-Demertzi, A. Boccarelli, M.A. Demertzis and M. Colucci, *Chemotherapy*, **53**, 148 (2007).
7. P. Rathelot, N. Azas, H. El-Kashef, F. Delmas, C. Di Giorgio, P. Timon-David, J. Maldonado and P. Vanelle, *Eur. J. Med. Chem.*, **37**, 671 (2002).
8. O. Tabarrini, G. Manfroni, A. Fravolini, V. Cecchetti, S. Sabatini, E. De Clercq, J. Rozenski, B. Canard, H. Dutartre, J. Paeshuyse and J. Neyts, *J. Med. Chem.*, **49**, 2621 (2006).
9. I. Vazzana, E. Terranova, F. Mattioli and F. Sparatore, *Arkivoc*, 364 (2004).
10. P.A. Vigato and S. Tamburini, *Coord. Chem. Rev.*, **248**, 1717 (2004).
11. P.E. Hansen, Z. Rozwadowski and T. Dziembowska, *Curr. Org. Chem.*, **13**, 194 (2009).
12. W. Brand-Williams, M.E. Cuvelier and C. Berset, *LWT-Food Sci. Technol.*, **28**, 25 (1995).
13. I.F.F. Benzie and J.J. Strain, *Anal. Biochem.*, **239**, 70 (1996).
14. C.G. Hamaker, O.S. Maryashina, D.K. Daley and A.L. Wadler, *J. Chem. Crystallogr.*, **40**, 34 (2010).
15. J.R. Soare, T.C.P. Dinis, A.P. Cunha and L. Almeida, *Free Radic. Res.*, **26**, 469 (1997).
16. P. Butaye, L.A. Devriese and F. Haesebrouck, *Clin. Microbiol. Rev.*, **16**, 175 (2003).