Bull. Chem. Soc. Ethiop. **2011**, 25(3), 407-417.
Printed in Ethiopia
ISSN 1011-3924
© 2011 Chemical Society of Ethiopia

SYNTHESIS, SPECTRAL CHARACTERIZATIONS AND ANTIMICROBIAL ACTIVITY OF SOME SCHIFF BASES OF 4-CHLORO-2-AMINOPHENOL

Adem Cinarli[1], Demet Gürbüz[1], Aydin Tavman[1*] and A. Seher Birteksöz[2]

[1]Istanbul University, Faculty of Engineering, Department of Chemistry, 34320, Avcilar, Istanbul, Turkey
[2]Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, 34452, Beyazit, Istanbul, Turkey

(Received September 2, 2010; revised March 3, 2011)

ABSTRACT. A series of 4-chloro-2-[(arylmethylidene)amino]phenols (**1**–**11**) including methoxy group were synthesized using appropriate synthetic route. The structures of the Schiff bases were characterized by FT-IR, UV-Vis, ESI-MS, ^{1}H and ^{13}C-NMR spectroscopic techniques and analytical methods. A relation is observed between melting points and existence of intramolecular hydrogen bonding. IR spectra of the compounds including and not including hydrogen bonding were compared. The compounds **2** and **4** show the characteristic UV bands attributed to the NH-forms. According to the ^{1}H-NMR spectral data the compound **2** has the strongest intramolecular hydrogen bonding and the compound **6** shows two isomeric structure. On the other hand, antibacterial and antifungal activities of the compounds were investigated. Most of the compounds show selective activity toward *S. epidermidis* and *C. albicans*.

KEY WORDS: Schiff base, 4-Chloro-2-aminophenol, Antimicrobial activity

INTRODUCTION

Schiff bases, also known as azomethines due to they have RC=N group, play important roles in biological systems. They are facing a growing interest due to their various applications, e.g. as anticancer [1-5], antibacterial [6-9], antiviral [10-12], antifungal [13-15], and about their other biological properties [16-21]. Intramolecular hydrogen bonding between OH hydrogen and C=N nitrogen atoms of Schiff bases determines the properties of various molecular systems and plays a significant role in many biochemical mechanisms [22]. Also, C=N linkage in the azomethine derivatives is essential for biological activity [23]. Since proton transfer is known to be crucial for physicochemical properties and practical application of Schiff bases, this process has been widely studied in literature [24].

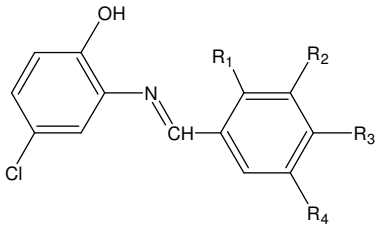
Intramolecular electron transfer is a fundamental chemical phenomenon that relates specifically to redox processes that occur in both natural and synthetic electron-transfer systems [25]. Sterically hindered ligands bearing salicyl parts are known to be effective antioxidants, and are widely used in the rancidification of fats and oil [26]. There has been a steady growth of interest in the synthesis, structure, and reactivity of Schiff bases due to their potential applications in biological modeling, catalysis, design of molecular magnets and materials chemistry [27-29].

On the other hand, Schiff bases have been extensively used as ligands in coordination chemistry because of their excellent donor abilities and chelating agents [30-34]. Schiff bases metal complexes have many industrial uses, especially in catalysis [35-37], dying [38-40] and analytical reagents [41].

In this study, we synthesized eleven Schiff bases derived from 4-chloro-2-aminophenol and various mono- or dimethoxybenzaldehyde derivatives (Scheme 1) and characterized them by using analytical and spectroscopic techniques. Antibacterial and antifungal activities of the Schiff bases were evaluated by the disk diffusion method against six bacteria and *C. albicans* as fungus.

*Corresponding author. E-mail: atavman@istanbul.edu.tr

Compound	R_1	R_2	R_3	R_4
1	OH	OCH_3	H	H
2	OH	H	OCH_3	H
3	OH	H	H	OCH_3
4	OH	OCH_3	H	Br
5	H	OH	OCH_3	H
6	H	OCH_3	OH	NO_2
7	H	OCH_3	OCH_3	H
8	H	OCH_3	H	OCH_3
9	OCH_3	H	H	OCH_3
10	OCH_3	OCH_3	H	H
11	OCH_3	H	OCH_3	H



Scheme 1. Chemical diagram of the Schiff bases under study.

EXPERIMENTAL

All the chemicals and solvents were of reagent grade (Merck, Fluka and Sigma-Aldrich) and were used without further purification. Elemental data were obtained with a Thermo Finnigan Flash EA 1112 analyzer (USA–Thermo Fischer Scientific). Melting points were determined using an Electro thermal melting-point apparatus (The Netherlands). ^{1}H-(499.74 MHz) and ^{13}C-NMR (125.68 MHz) spectra were run on a Varian Unity Inova 500 NMR spectrometer (USA). The residual DMSO-d_6 signal was also used as an internal reference. FT-IR spectra were recorded in KBr disks on a Mattson 1000 FT-IR spectrometer (USA). UV-Visible spectra were performed on a Perkin Elmer Lambda 25 UV/Visible Spectrometer (USA). The Electron Spray Ionization-Mass Spectroscopy (ESI-MS) analyses were carried out in positive ion modes using a Thermo Finnigan LCQ Advantage MAX LC/MS/MS (USA-Thermo Scientific).

Synthesis of the Schiff bases

The Schiff bases were prepared by mixing an ethanolic solution (10 mL) of 4-chloro-2-aminophenol (720 mg; 5 mmol) with appropriate methoxybenzaldehyde (e.g. 4-hydroxy-3-methoxy-5-nitrobenzaldehyde for **6**, 985 mg; 5 mmol) in same volume of ethanol. This mixture was then refluxed with stirring for 3 h and the solution was allowed at the room temperature to crystallize the Schiff bases.

Determination of antimicrobial activity

Antimicrobial activity against *Staphylococcus aureus ATCC 6538*, *Staphylococcus epidermidis ATCC 12228*, *Escherichia coli ATCC 8739*, *Klebsiella pneumoniae ATCC 4352*, *Pseudomonas aeruginosa ATCC 27853*, *Proteus mirabilis ATCC 14153* and *Candida albicans ATCC 10231* were determined by the micro broth dilutions technique strictly following the National Committee for Clinical Laboratory Standards (NCCLS) recommendations [42, 43]. Mueller-Hinton broth for bacteria, RPMI-1640 medium buffered to pH 7.0 with MOPS for yeast strain was used as the test medium. Serial two-fold dilutions ranging from 5000 µg/mL to 4.9 µg/mL were prepared in medium. The inoculum was prepared using a 4–6 h broth culture of each bacteria and 24 culture of yeast strains adjusted to a turbidity equivalent to a 0.5 McFarland standard (corresponds to 10^8 cfu/mL) diluted in broth media to give a final concentration of 5 x

10^5 cfu/mL for bacteria and 0.5 x 10^3 to 2.5 x 10^3 cfu/mL for yeast in the test tray (A McFarland standard is a chemical solution of barium chloride and sulfuric acid. It is used to standardize the approximate number of bacteria in a liquid suspension). The trays were covered and placed in plastic bags to prevent evaporation. The trays containing Mueller-Hinton broth were incubated at 35 ºC for 18–20 h and the trays containing RPMI-1640 medium were incubated at 35 ºC for 46–50 h. The minimum inhibitory concentrations (MIC) were defined as the lowest concentration of compound giving complete inhibition of visible growth. Antimicrobial effects of the solvents were investigated against test microorganisms. The medium with DMSO as solvent was used as a negative control whereas media with ciprofloxacin (standard antibiotic) and fluconazole (standard antifungal drug) were used as the positive controls. The experiments were performed in triplicate.

RESULTS AND DISCUSSION

The analytical data and physical properties of the Schiff bases are summarized in Table 1. As can be seen from Table 1 and Scheme 1, the compounds **1**–**3**, **5** and **7**–**11** are isomers among themselves.

Table 1. The analytical data and physical properties of the compounds **1**–**11**.

Compound No. and molecular formula	Found (calcd) %			Yield %	M.p. ºC	Color
	C	H	N			
1 $C_{14}H_{12}ClNO_3$	60.19 (60.55)	4.64 (4.36)	5.03 (5.04)	87	210	Brick red
2 $C_{14}H_{12}ClNO_3$	60.82 (60.55)	4.46 (4.36)	5.01 (5.04)	85	219	Bright yellow
3 $C_{14}H_{12}ClNO_3$	60.75 (60.55)	4.48 (4.36)	5.00 (5.04)	50	145	Red
4 $C_{14}H_{11}BrClNO_3$	47.28 (47.15)	3.16 (3.11)	4.06 (3.93)	60	232	Orange-red
5 $C_{14}H_{12}ClNO_3$	60.65 (60.55)	4.52 (4.36)	5.37 (5.04)	60	142	Cream
6 $C_{14}H_{11}ClN_2O_5$	52.34 (52.11)	3.61 (3.44)	8.80 (8.68)	96	205	Bright yellow
7 $C_{15}H_{14}ClNO_3$	61.85 (61.76)	4.81 (4.84)	4.92 (4.80)	80	165	Cream
8 $C_{15}H_{14}ClNO_3$	61.82 (61.76)	5.02 (4.84)	5.09 (4.80)	80	124	Cream
9 $C_{15}H_{14}ClNO_3$	61.74 (61.76)	4.97 (4.84)	4.94 (4.80)	81	145	Yellow
10 $C_{15}H_{14}ClNO_3$	61.70 (61.76)	4.88 (4.84)	4.68 (4.80)	65	112	Yellow
11 $C_{15}H_{14}ClNO_3$	61.94 (61.76)	4.78 (4.84)	4.89 (4.80)	65	104	Bright yellow

Melting points

It is expected that there are both inter- and intramolecular hydrogen bondings in compound **1**, **2**, **3** and **4** because of two hydroxy groups. It is known that intermolecular hydrogen bonding increases the melting point of the compounds. Likewise, the melting point of the compound **1**, **2** and **4**, 210, 219 and 232 ºC, respectively, are higher than those of the other Schiff bases. However, melting point of the compound **3** is lower (145 ºC) than that of the other compounds (**1**, **2** and **4**) that include intramolecular hydrogen bonding (Figure 1). The low melting point of **3** may be explained by keto-enol tautomerism. A large number of Schiff bases was structurally determined so far, revealing that crystal structures of compounds of this class were dominated by the O–H···N (enol-imino) tautomeric form [44, 45]. Some of Schiff bases exhibit the N–H···O tautomers [46-51] and their common feature is the presence of a hydroxyl [46, 49-51] or a nitro group [47, 48] at the N-phenyl ring or the phenyl ring of parent aldehyde. It was reported that the tautomeric forms are influenced by the different intermolecular hydrogen bonding patterns of the molecules in the crystalline state. The stabilization of the non-favorable NH form of (Sal-*o*-OH-ph), *N*-(*o*-hydroxyphenyl)-5-methoxysalicylaldimine, is ascribed to stronger intermolecular O–H···O hydrogen bonds in (Sal-*o*-OH-ph) than in *N*-(*m*-

hydroxyphenyl)-5-methoxysalicylaldimine [52]. The compound **3** includes a methoxy group at the *para* position according to the phenolic hydroxy group and this structure is caused to form a keto-enol tautomerism (Scheme 2) [53]. Also, ^{1}H-NMR data support this explanation (See NMR section).

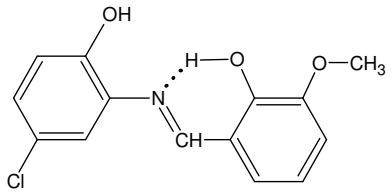
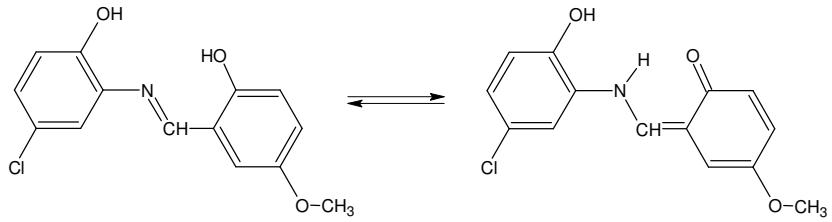


Figure 1. Intramolecular hydrogen bonding at the compound **1**.



Scheme 2. Keto-enol tautomerism at the compound **3**.

High melting point (205 ºC) of the compound **6** can be explained by the strong intermolecular hydrogen bonding that is formed by the nitro and hydroxy groups. Melting points of the Schiff bases that have not included any hydrogen bonding (**7**–**11**) are lower than the others. Their melting points are in the range 104–165 ºC. The individual melting points of the compounds are sharp and uncorrected.

FT-IR spectra

FT-IR spectral data (in KBr pellets) of the compounds are given in Table 2. Also, information of hydrogen bonding existence in Schiff bases is presented in Table 2.

The IR spectra of the Schiff bases shows medium or strong intensity absorption bands at 1615–1650 cm^{-1} assigned to C=N stretching mode. The presence of aromatic rings has been identified by their characteristic ring vibrations at 1500–1400, 1100–1050 and 900–700 cm^{-1} regions. The absence of bands characteristic of ν(C=O), primary amine ν(NH) confirms the formation of the proposed Schiff base framework.

The broad bands between 2800 and 2400 cm^{-1} in the spectra of the compounds **1**, **2**, **3** and **4** demonstrates the formation of the OH···N intramolecular hydrogen bond between the salicyl part OH proton and the nitrogen atoms [52, 54, 55]. This broad band is weaker at the compound **3** than the compounds **1**, **2** and **4** because of the keto-enol tautomerism.

The characteristic ν(C-H) modes of ring residues are observed at near 3050 cm^{-1}. Methoxy and CH=N groups stretching vibrations appear between 3000 and 2800 cm^{-1}. The strong or medium bands between 3428 and 3325 cm^{-1} at the Schiff bases that not including hydrogen bonding can be assigned to ν(OH) [52, 55-58].

The C–O stretching vibrations appear at the 1250–1300 cm^{-1} range as strong bands [59]. The C–Cl stretching vibration is seen at the range 600–650 cm^{-1} as medium or weak bands for the all of the compounds [60]. In the IR spectrum of **6**, the strong or medium bands at 1549 cm^{-1} and at 1332 cm^{-1} can be assigned to the symmetric and asymmetric $\nu(NO_2)$, respectively. The C–Br stretching vibration is seen at 513 cm^{-1} as a medium band for **4** [61].

Table 2. The FT-IR spectral data for **1**–**11**.

Compound	Frequency (cm^{-1}), KBr pellets	H-bonding in the IR spectra
1	3050 m, 2955 m, 2829 m, 1644 s, 1595 m, 1510 s, 1428 m, 1286 m, 1252 m, 1066 m, 901 m, 811 m, 750 m, 573 m	Considerable H-bonding
2	3071 m,br, 2970 m,br, 2909 m,br, 1649 s, 1621 s, 1592 m, 1525 m, 1504 s, 1479 s, 1429 s, 1278 m, 1226 s, 903 m, 831 m, 800 m, 772 m, 656 m	Considerable H-bonding
3	3054 m,br, 3002 m,br, 2930 m,br, 1623 s, 1594 m, 1504 s, 1443 m, 1251 m, 1140 m, 906 m, 827 m, 799 m, 646 m	Weak H-bonding
4	3099 m,br, 3054 m,br, 2939 m,br, 1623 s, 1596 m, 1531 m, 1508 m, 1434 m, 1251 s, 978 m, 854 m, 822 m, 748 m, 569 m, 513 w	Considerable H-bonding
5	3428 m,br, 3317 m,br, 3065 m, 2965 m, 1616 m, 1583 m, 1511 s, 1487 m, 1441 m, 1273 s, 1253 s, 910 m, 863 m, 793 m, 766 m, 541 m	No hydrogen bonding
6	3361 s, 3242 m, 3081 m, 2980 m, 2930 m, 1623 m, 1589 m, 1549 s, 1483 m, 1416 m, 1332 s, 1278 s, 1249 m, 928 m, 876 m, 813 m, 698 m	No hydrogen bonding
7	3350 s, 3082 m, 2962 m, 2838 m, 1623 m, 1583 s, 1513 s, 1491 s, 1420 s, 1273 s, 1246 s, 906 m, 872 m, 799 m, 578 m	No hydrogen bonding
8	3391 m, 3359 s, 3063 w, 3005 m, 2935 m, 2838 m, 1627 m, 1592 s, 1486 s, 1282 m, 1070 s, 915 m, 822 m, 685 m	No hydrogen bonding
9	3420 s, 3072 w, 3007 m, 2966 m, 2941 m, 1621 m, 1585 m, 1501 m, 1434 m, 1285 s, 1045 m, 908 m, 815 m, 725 m, 646 m	No hydrogen bonding
10	3368 m, 3081 w, 3002 m, 2935 m, 2833 m, 1623 m, 1577 m, 1486 s, 1367 m, 1268 s, 1079 m, 915 m, 863 m, 790 m, 751 m, 649 m	No hydrogen bonding
11	3325 m, 3009 m, 2939 m, 2838 m,br, 1614 m, 1594 s, 1486 m, 1461 m, 1292 m, 1260 m, 1158 m, 915 m, 837 m, 804 m, 614 m	No hydrogen bonding

m = medium, s = strong, sh = shoulder, w = weak, br = broad.

UV-Visible spectra

The UV-visible spectral data of the compounds are presented in Table 3. The UV-visible absorption spectra are obtained in methanol at room temperature. The compounds exhibit intense bands in the 200–400 nm region, which may be assigned to $n\rightarrow\pi^*$ and $\pi\rightarrow\pi^*$ transitions in their spectra. The 210–300 nm bands are due to the $\pi\rightarrow\pi^*$ transitions of the aromatic rings. The bands at the 300–350 nm range involve $\pi\rightarrow\pi^*$ transitions of the C=N group. The longer wavelength bands over *ca*. 400 nm can be assigned to the intramolecular charge transfer interactions [62-67]. UV-visible spectroscopy is known to be a very sensitive method for studying tautomeric equilibrium in Schiff bases. The two long-wavelength bands observed in the electronic spectra of the compounds are assigned to the OH- and NH- forms [63-66, 68-70]. The bands at *ca*. 350 nm observed in spectra of the Schiff bases can be assigned to the OH-form. The bands above 370 nm are assigned to the NH-forms [63]. The compounds **2** and **4** have the characteristic bands for NH-forms (Figure 3) in the present study. The UV-Vis spectra of these two Schiff bases indicate the existence of the proton transfer equilibrium.

Table 3. UV-visible spectral data of the compounds.

Compound	Wavelength (λ_{max}, nm) in MeOH (10^{-4} M)
1	462 m,br, 353 m,br, 278 s,br, 220 s
2	446 sh, 426 m,br, 351 s,br, 304 m,br, 285 sh, 242 s,br, 215 sh
3	482 w,br, 373 m,br, 354 sh, 273 s, 252 sh, 236 s,br, 219 sh
4	465 m,br, 378sh, 356 m,br, 276 sh, 269 m, 229 s,br, 212 s
5	350 m,br, 326 sh, 285 m,br, 235 s, 213 sh
6	449 sh, 365 m,br, 293 m,br, 268 m, 247 sh, 229 sh, 214 s
7	370 sh, 350 s, 317 sh, 284 s, 237 s,br, 216 s
8	367 sh, 354 s,br, 282 s,br, 269 s, 261 sh, 232 sh, 214 s
9	406 sh, 368 m,br, 281 sh, 269 m, 262 m, 232 sh, 216 s
10	369 sh, 349 m,br, 278 sh, 269 s, 261 sh, 232 sh, 217 s
11	379 sh, 356 s,br, 322 sh, 282 s, 269 sh, 238 s,br, 215 s

s = strong, m = medium, br = broad, sh = shoulder.

Mass spectra

The ESI-MS spectral data of the compounds are given in Table 4 as molecular ions with the relative abundance. The mole peaks are determined for all the Schiff bases that include chlorine atom. It is known that chlorine has two isotopes: These isotopes, their percent abundance and approximately relative proportions are as follows: ^{35}Cl(75.8%):^{37}Cl(24.2%) (3:1). The isotopic patterns from chlorine atom are identified at the ESI-MS spectra of the all compounds. As expected, a lot of isotopic patterns are observed at the spectrum of the compound **4** that includes both bromine and chlorine atoms. Bromine also has two isotopes as ^{79}Br(50.7%):^{81}Br(49.3%) and their relative proportions are 1:1.

Table 4. The ESI-MS data of the compounds.

Compound and MW (g/mol)	Molecular ions (m/z) with relative abundance (%) and isotopic patterns
1; $C_{14}H_{12}ClNO_3$ 277.5	276.77 (100, $[M-1]^+$ {^{35}Cl}), 277.78 (39.2, $[M]^+$ {^{35}Cl}), 278.71 (86.3, $[M+1]^+$ {^{35}Cl+^{37}Cl}), 279.98 (16.1, $[M+2]^+$ {^{37}Cl})
2; $C_{14}H_{12}ClNO_3$ 277.5	276.67 (77.6, $[M-1]^+$), 278.69 (100, $[M+1]^+$ {^{35}Cl}), 280.16 (30.4, $[M+3]^+$ {^{37}Cl })
3; $C_{14}H_{12}ClNO_3$ 277.5	276.72 (100, $[M-1]^+$), 278.71 (71.3, $[M+1]^+$ {^{35}Cl}), 280.37 (24.4, $[M+3]^+$ {^{37}Cl })
4; $C_{14}H_{11}BrClNO_3$ 356.6	354.57 (45.9, $[M-2]^+$ {^{79}Br+^{35}Cl}), 355.26 (22.8, $[M-1]^+$ {^{79}Br+^{37}Cl}), 356.62 (100, $[M]^+$), 357.27 (31.3, $[M+1]^+$ {^{81}Br+^{35}Cl}), 358.46 (32.6, $[M+2]^+$ {^{81}Br+^{37}Cl}), 359.30 (19.0, $[M+3]^+$)
5; $C_{14}H_{12}ClNO_3$ 277.5	276.39 (100, $[M-1]^+$ {^{35}Cl}), 278.39 (33.1, $[M+1]^+$ {^{37}Cl})
6; $C_{14}H_{11}ClN_2O_5$ 322.7	320.68 (100, $[M-2]^+$, {^{35}Cl}), 322.53 (66.1, $[M]^+$), 323.55 (32.4, $[M+1]^+$, {^{35}Cl+^{37}Cl}), 324.62 (33.2, $[M+2]^+$ {^{37}Cl})
7; $C_{15}H_{14}ClNO_3$ 291.5	290.21 (100, $[M-1]^+$ {^{35}Cl}), 292.22 (31.6, $[M+1]^+$ {^{37}Cl})
8; $C_{15}H_{14}ClNO_3$ 291.5	290.61 (100, $[M]^+$ {^{35}Cl}), 292.73 (29.9, $[M+1]^+$ {^{37}Cl})
9; $C_{15}H_{14}ClNO_3$ 291.5	290.32 (100, $[M]^+$ {^{35}Cl}), 292.43 (29.3, $[M+1]^+$ {^{37}Cl})
10; $C_{15}H_{14}ClNO_3$ 291.5	292.45 (100, $[M+1]^+$ {^{35}Cl}), 293.51 (30.7, $[M+2]^+$ {^{37}Cl})
11; $C_{15}H_{14}ClNO_3$ 291.5	292.50 (100, $[M+1]^+$ {^{35}Cl}), 293.27 (16.6, $[M+2]^+$ {^{35}Cl +^{37}Cl}), 294.23 (33.6, $[M+3]^+$ {^{37}Cl})

NMR spectra

^{1}H-NMR spectral data and their assignments are given in Table 5.

Table 5. ^{1}H-NMR spectral data of the compounds (δ_H, as ppm, in DMSO-d_6).

Compound	Chloro-hydroxyphenyl protons				CH=N	Phenolic protons				
	H3	H4	H6	OH		H2'	H3'	H4'	H5'	H6'
1	7.10 dd,br J=7.8;1.5	7.15 dd J=2.6;8.2	7.48 d J=2.6	10.0 s	8.96 s	13.64 s[a]	3.80 s[b]	7.19 dd,br J=1.3;8.0	6.87 t J=8.3; 7.8	6.95 d J=8.8
2	6.92 d J=8.8	7.10 dd J=2.5;8.8	7.42 d J=2.7	9.99 s	8.86 s	14.01 s[a]	6.41 d J=2.2	3.79 s[b]	6.50 dd J=2.5; 8.8	7.46 d J=8.8
3	6.94 d J=8.8	7.14 dd J=2.9;8.3	7.41 d J=2.9	9.92 s	8.94 s	12.69 s[a]	6.87 d J=9.3	7.02 dd J=3.4;8.8	3.74 s[b]	7.22 d J=3.4
4	6.95 d J=8.3	7.16 dd J=2.6;8.8	7.46 d J=2.6	10.22 s	8.95 s	13.72 s[a]	3.82 s[b]	7.20 d J=2.6	--	7.40 d J=2.1
5	7.03 d J=8.3	7.36 dd J=1.9;8.3	7.49 d J=1.9	9.23 s	8.51 s	7.16 d J=2.9	9.17 s[a]	3.82 s[b]	6.86 d J=8.8	7.05 dd J=2.9;8.8
6; Isomer A (50%)	6.58 dd J=2.4;8.3	7.12 dd J=2.4;8.3	7.59 d J=1.5	9.85 s	8.70 s	7.97 s,br	3.96 s[b]	9.31 s,br[a]	--	8.08 d J=1.95
6; Isomer B (50%)	6.37 dd J=2.4;8.3	6.90 dd J=2.4;8.3	7.32 d J=1.9	9.22 sbr	8.70 s	8.08 d J=1.95	--	9.31 s,br[a]	3.94 s[b]	7.97 s,br
7	6.88 dd J=1.5;8.3	7.47 dd J=8.3;1.5	7.73 d J=1.5	9.15 s	8.61 s	7.24 dd,br J=1.0;1.5	3.84 s[b]	3.83 s[b]	7.07 m	7.07 m
8	6.89 d J=8.8	7.10 dd J=2.4;8.8	7.26 d J=2.4	9.27 s	8.64 s	7.21 d J=2.4	3.81 s[c]	6.65 t J=2.4	3.81 s[c]	7.21 d J=2.1
9	6.88 d J=8.8	7.09 dd J=2.4;8.8	7.72 d J=2.4	9.28 s	8.87 s	3.84 s[b]	7.07 m	7.07 m	3.78 s[b]	7.14 d J=2.4
10	6.89 d J=8.3	7.17 m	7.17 m	9.33 s	8.83 s	3.85 s[b]	3.84 s[b]	7.74 dd J=1.0;7.8	7.09 m	7.22 dd J=1.0;7.8
11	6.85 d J=9.3	6.63 dd J=2.4;9.7	7.07 d J=2.4	9.14 s	8.77 s	3.88 s[b]	6.65 s	3.85 s[b]	7.04 dd J=8.3; 2.4	8.12 d J=7.8

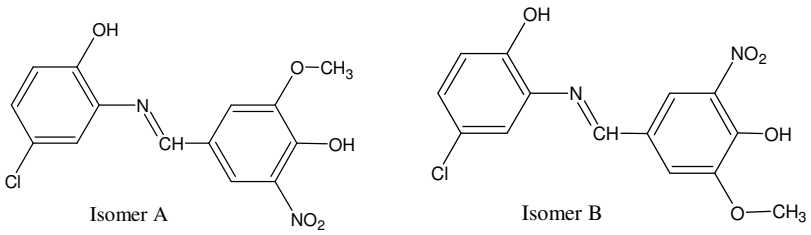
[a] : OH; [b] : 3H (OCH_3); [c] : 6H (2 × OCH_3); s = singlet, d = doublet, dd = doublet of doublets, t = triplet, br = broad, m = multiplet.

The compounds **1**, **2**, **3** and **4** have two hydroxy groups: One of them is located at the salicyl part (R_1 position) and the other one at 5-chloro-2-hydroxyphenyl moiety. It is known that hydrogen bonding shifts the resonance signal of a proton to lower field (higher frequency). The signals at 13.64, 14.01, 12.69 and 13.71 ppm are due to the salicyl part OH protons of the compounds **1**, **2**, **3** and **4**, respectively. Comparing the ^{1}H-NMR data of salicyl part OH protons of the compounds **1** – **4**, it can be said that the strongest intramolecular hydrogen bond (OH···N) is formed in **2**, and the weakest one in **3**. It is interesting that 12.69 ppm value of the compound **3** is lower than those of the other phenolic Schiff bases. This difference means that the phenolic OH proton of **3** has less acidic character and, consequently, the compound **3** has weaker intramolecular hydrogen bonding according to the others. Reason of this is the compound **3** has keto-enol tautomers (Scheme 2). (The phenolic OH proton of **3**, has weaker acidic character than the other OH proton, is more shielded and it appears at lower ppm (higher field) values according to the compounds **1**, **2** and **4**). A similar situation was observed at the melting points of these four Schiff bases: Melting point of **3** is lower than those of the compounds **1**, **2** and **4** (See Melting Point section). These data are in very good agreement with the FT-IR spectra of

these compounds. In the FT-IR spectra of the compounds **1** – **4**, the broad bands at about 2650 cm^{-1} are assigned to the stretching hydrogen motions in the intramolecular OH···N hydrogen bonding.

The OH proton of 5-chloro-2-hydroxyphenyl ring appears between 9.14 and 10.22 ppm. The phenolic OH protons that could not form intramolecular hydrogen bonding (at the 3' and 4' positions of the compounds **5** and **6**, respectively) are observed at 9.17 and 9.22 ppm, respectively. The chemical shifts, observed between 8.50 and 8.96 ppm as singlet, are assigned to the azomethine (CH=N) proton [71]. The peaks at the 6.63 – 8.12 ppm range are assignable to the aromatic protons [64, 72]. The protons of the methoxy groups of the compounds exhibit singlet peaks near 3.80 ppm. According to the ^{1}H-NMR spectral data, the protons of the two methoxy groups of the compound **8**, which gives a singlet at 3.81 ppm, are magnetically equivalent.

The compound **6** has two isomeric structures according to the ^{1}H-NMR spectra in DMSO-d_6. (Table 5; Scheme 3). It is observed that 5-chloro-2-hydroxyphenyl protons are affected considerably by the isomer forming, whereas the salicyl part protons are not.



Scheme 3. The isomeric structures of the compound **6**.

^{13}C-NMR spectral data are given in Table 6 with their assignments. Azomethine carbons of the compounds appear between 156 and 164 ppm. The methoxy carbons are observed at the 55 – 60 ppm range. The carbon atoms bonded to OH oxygen atom (C_2) at 5-chloro-2-hydroxyphenyl ring give signals at the lower ppm value (between 147 and 151 ppm) than the carbon atoms that bonded to the salicyl part OH oxygen (>151 ppm). The carbon atoms connected to the methoxy group are observed at the 151 – 164 ppm range. The carbon atom C_5, bonded to the chlorine atom, appears at *ca.* 124 ppm in all of the compounds [63, 70].

Table 6. ^{13}C-NMR spectral (APT) data of the compounds (δ_C, as ppm, in DMSO-d_6).

Compound	Chloro-hydroxyphenyl carbons						N=CH	OCH_3	Phenolic carbons					
	C_1	C_2	C_3	C_4	C_5	C_6			$C_{1'}$	$C_{2'}$	$C_{3'}$	$C_{4'}$	$C_{5'}$	$C_{6'}$
1	136.7	148.8	118.5	128.0	123.8	119.0	163.8	56.4	120.0	152.2	151.0	116.2	119.9	124.7
2	136.4	150.5	118.3	127.5	124.0	119.7	162.0	56.2	113.8	162.8	101.8	163.6	108.7	134.8
3	137.3	150.8	118.6	128.1	124.0	120.1	163.3	56.2	119.9	157.4	119.7	121.5	152.8	115.9
4	136.0	150.2	118.6	128.4	123.9	119.7	161.8	57.5	120.8	150.8	152.2	118.4	109.5	125.9
5	140.7	147.4	117.8	126.5	123.6	119.7	156.4	56.4	130.0	115.0	150.5	151.8	112.3	123.4
6	143.8	149.9	118.0	127.8	123.7	121.8	159.5	57.8	122.4	114.2	152.5	137.8	138.6	113.0
7	140.1	149.8	117.9	126.8	123.8	119.4	160.8	58.2 [a]	130.0	110.0	152.8	151.0	108.9	125.5
8	139.6	151.2	118.2	127.8	123.8	119.7	161.8	56.2 [a]	140.0	107.7	161.7	104.5	161.7	107.7
9	140.3	150.8	119.9	127.5	124.0	120.3	156.4	56.4, 57.2	125.8	154.0	104.2	108.0	155.2	101.9
10	140.8	150.8	118.2	127.2	124.0	119.7	157.5	56.5, 62.2	130.2	153.6	151.0	120.2	116.6	124.8
11	141.2	150.4	117.9	126.3	123.7	118.8	158.2	58.3, 58.7	117.8	162.0	98.8	164.8	107.5	128.8

[a] 2 × OCH_3.

The compounds **7**–**11** have two methoxy groups. The APT spectral data of the compounds **7** and **8** show that the methoxy carbon atoms are magnetically equivalent. They give only a peak at 58.2 and 56.2 ppm for **7** and **8**, respectively. The methoxy carbon atoms of the other Schiff bases (**9**, **10** and **11**) are magnetically non-equivalent.

Antimicrobial activity

The results concerning *in vitro* antimicrobial activity of the compounds together with MIC values of compared antibiotic and antifungal reagents are presented in Table 7.

Table 7. *In vitro* antimicrobial activity of the compounds (MIC, µg/mL).

Compound	Microorganisms						
	Sa[a]	*Se*[a]	*Ec*[b]	*Kp*[b]	*Pa*[b]	*Pm*[b]	*Ca*
1	–	–	–	–	–	–	–
2	–	–	–	–	–	–	19.5
3	–	156	–	–	–	–	78.0
4	–	–	–	–	–	–	31.2
5	156	156	–	–	–	–	39.0
6	–	625	–	–	–	–	–
7	–	625	–	–	–	–	–
8	–	1250	–	–	–	–	–
9	–	–	–	–	–	–	–
10	–	625	–	–	–	–	–
11	–	625	–	–	–	–	–
Ciprofloxacin	0.125	156	0.0625	0.0625	2.00	0.0312	–
Fluconazole	–	–	–	–	–	–	1.00

Sa = *Staphylococcus aureus* ATCC 6538. *Se* = *Staphylococcus epidermidis* ATCC 12228. *Ec* = *Escherichia coli* ATCC 8739. *Kp* = *Klebsiella pneumoniae* ATCC 4352. *Pa* = *Pseudomonas aeruginosa* ATCC 1539. *Pm* = *Proteus mirabilis* ATCC 14153. *Ca* = *Candida albicans* ATCC 10231. [a] : Gram positive; [b] : Gram negative. – : Antimicrobial activity was not detected.

It is observed that most of the compounds are particularly effective on *S. epidermidis* and *C. albicans*. This observation can be assumed as selective activity. The compound **5** is effective on three different microorganisms: Two of them are *Staphylococcus* type Gram positive bacteria (*S. aureus* and *S. epidermidis*) and the third one is a fungus (*C. albicans*). Antifungal activity of the compound **5** (39 µg/mL) is better than its' antibacterial activity (156 µg/mL). On the other hand, it is an interesting result that the Schiff bases including two methoxy groups (the compounds **7**–**11**) have no any antifungal activity against *C. albicans*. At this stage, it is difficult to find a simple explanation for the antimicrobial activity of the compounds and further studies will be needed to elucidate these observations.

ACKNOWLEDGEMENTS

This work was supported by the Research Fund of Istanbul University. Project number: 1519.

REFERENCES

1. Sinha, D.; Tiwari, A.K.; Singh, S.; Shukla, G.; Mishra, P.; Chandra, H.; Mishra, A.K. *Eur. J. Med. Chem.* **2008**, 43, 160.
2. Crowe, A.J.; Smith, P.J.; Atassi, G. *Chem. Biol. Interact.* **1980**, 32, 171.
3. Wang, M.; Wang, L.F.; Li, Y.Z.; Li, Q.X.; Xu, Z.D.; Qu, D.M. *Transit. Met. Chem.* **2001**, 26, 307.

4. Przybylski, P.; Pyta, K.; Wicher, B.; Gdaniec, M.; Brzezinsk, B. *J. Mol. Struct.* **2008**, 889, 332.
5. Desai, S.B.; Desai, P.B.; Desai, K.R., *Heterocycl. Commun.* **2001**, 7, 83.
6. Dhumwad, S.D.; Gudasi, K.B.; Goudar, T.R. *Indian J. Chem.* **1994**, 33A, 320.
7. Przybylski, P.; Huczynski, A.; Pyta, K.; Brzezinski, B., Bartl, F. *Curr. Org. Chem.* **2009**, 13, 124.
8. Pandeya, S.N.; Sriram, D.; Nath, G.; DeClercq, E. *Pharm. Acta Helv.* **1999**, 74, 11.
9. Karthikeyan, M.S.; Parsad, D.J.; Poojary, B.; Bhat, K.S.; Holla, B.S.; Kumari, N.S. *Bioorg. Med. Chem.* **2006**, 14, 7482.
10. Holla, B.S.; Akberali, P.M.; Shivananda, M.K. *Il Farmaco* **2001**, 56, 919.
11. Jarrahpour, A.; Khalili, D.; De Clercq, E.; Salmi, C.; Brunel, J.M. *Molecules* **2007**, 12, 1720.
12. da Silva, C.M.; da Silva, D.L.; Modolo, L.V.; Alves, R.B.; de Resende, M.A.; Martins, C.V.B.; de Fatima, A. *J. Adv. Res.* **2011**, in press, doi:10.1016/j.jare.2010.05.004
13. Singh, H.; Yadav, L.D.S.; Mishra, S.B.S. *J. Inorg. Nucl. Chem.* **1981**, 43, 1701.
14. Saravanan, G.; Pannerselvam, P.; Prakash, C.R. *J. Adv. Pharm. Techn. Res.* **2010**, 1, 320.
15. Panneerselvam, P.; Nair, R.R.; Vijayalakshmi, G.; Subramanian, E.H.; Sridhar, S.K. *Eur. J. Med. Chem.* **2005**, 40, 225.
16. Singh, N.K.; Singh, S.B. *Indian J. Chem.* **2001**, 40A, 1070.
17. Walsh, O.M.; Meegan, M.J.; Prendergast, R.M.; Nakib, T.A. *Eur. J. Med. Chem.* **1996**, 31, 989.
18. Odabasoglu, M.; Arslan, F.; Ölmez, H.; Büyükgüngör, O. *Dyes and Pigments* **2007**, 75, 507.
19. Vicini, P.; Geronikaki, A.; Incerti, M.; Busonera, B.; Poni, G.; Cabras, C.A.; Colla, P.L. *Bioorg. Med. Chem.* **2003**, 11, 4785.
20. Pandeya, S.N.; Sriram, D.; Nath, G.; DeClercq, E. *Eur. J. Pharm. Sci.* **1999**, 9, 25.
21. Samadhiya, S.; Halve, A., *Orient. J. Chem.* **2001**, 17, 119.
22. Szady-Chelmieniecka, A.; Grech, E.; Rozwadowski, Z.; Dziembowska, T.; Schilf, W.; Kamienski, B. *J. Mol. Struct.* **2001**, 565, 125.
23. Dhanaraj, C.J.; Nair, M.S. *J. Coord. Chem.* **2009**, 62, 4018.
24. Schilf, W.; Kamienski, B.; Dziembowska, T. *J. Mol. Struct.* **2002**, 602-603, 41.
25. Sheldon, R.A.; Kochi, J.R. *Metal Catalysed Oxidation of Organic Compound*, Academic Press: New York; **1981**.
26. Zurita, D.; Menage, S.; Pierre, J.L.; Aman, E.S. *J. Biol. Inorg. Chem.* **1997**, 2, 46.
27. Jones, R.D.; Summerville, D.A.; Basolo, F. *Chem. Rev.* **1979**, 17, 139.
28. Hadjoudis, E.; Mavridis, I.M. *Chem. Soc. Rev.* **2004**, 33, 579.
29. Bhat, K.; Chang, K.J.; Aggarwal, M.D.; Wang, W.S.; Penn, B.G.; Frazier, D.O. *Mater. Chem. Phys.* **1996**, 44, 261.
30. Celik, Ö.; Ulusoy, M.; Tas, E.; Ide, S. *Anal. Sciences* **2007**, 23, x185.
31. Mahmoud, M.R.; El-Gyar, S.A.; Mousrafa, A.A.; Shaker, A. *Polyhedron* **1987**, 6, 1017.
32. Qing-Yu, H.; Zheng-Hua, M.; Ya-Me, Z. *J. Coord. Chem.* **1990**, 21, 199.
33. Cozzi, P.G. *Chem. Soc. Rev.* **2004**, 33, 410.
34. Tarafder, M.T.H.; Jin, K.T.; Crouse, K.A.; Ali, A.M.; Yamin, B.M.; Fun, H.-K. *Polyhedron* **2002**, 21, 2547.
35. Mc Auliffe, C.A.; Parish, R.V.; Abu-El-Wafa, S.M.; Issa, R.M. *Inorg. Chim. Acta* **1986**, 115, 91.
36. Abu-El-Wafa, S.M.; Issa, R.M. *Bull. Soc. Chim. Fr.* **1991**, 128, 805.
37. Boghaei, D.M.; Mohebi, S. *Tetrahedron* **2002**, 58, 5357.
38. Papic, S.; Koprivanac, N.; Grabaric, Z.; Parac-Osterman, D. *Dyes and Pigments* **1994**, 25, 229.

39. Nejati, K.; Rezvani, Z.; Massoumi, B. *Dyes and Pigments* **2007**, 75, 653.
40. Papie, S.; Kaprivanae, N.; Grabarie, Z.; Paracosterman, D. *Dyes and Pigments* **1994**, 25, 229.
41. Tantaru, G.; Dorneanu, V.; Stan, M. *J. Pharm. Biomed. Anal.* **2002**, 27, 827.
42. CLSI, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*, Approved Standard, M7-A5, Wayne, PA, USA, **2006**.
43. CLSI, *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts*, Approved Standard, M27-A2, 2nd ed., Wayne, PA, USA, **2002**.
44. Gavranic, M.; Kaitner, B.; Mestrovic, E. *J. Chem. Crystallogr.* **1996**, 26, 23.
45. Ünver, H.; Polat, K.; Ucar, M.; Zengin, D.M. *Spect. Lett.* **2003**, 36, 287.
46. Puranik, V.G.; Tavale, S.S.; Kumbhar, A.S.; Yerande, R.G.; Padhye, S.B.; Buthcer, R.J. *J. Cryst. Spectrosc. Res.* **1992**, 22, 725.
47. Ogawa, K.; Fujiwara, T. *Chem. Lett.* **1999**, 28, 657.
48. Pizzala, H.; Carles, M.; Stone, W.E.E.; Thevand, A. *J. Chem. Soc., Perkin Trans.* **2000**, 2, 935.
49. Mukherjee, A.K.; De, R.L.; Banerjee, I.; Samanta, C.; Nayak, N.P. *Acta Crystallogr.* **1999**, C55, 407.
50. Kabak, M.; Elmali, A.; Elerman, Y. *J. Mol. Struct.* **1999**, 151, 477.
51. Maciejewska, D.; Pawlak, D.; Koleva, V. *J. Phys. Org. Chem.* **1999**, 12, 875.
52. Popovic, Z.; Pavlovic, G.; Matkovic-Calogovis, D.; Roje, V.; Leban, I. *J. Mol. Struct.* **2002**, 615, 23.
53. Salman, S.R.; Saleh, N.A.I. *Spect. Lett.* **1997**, 30, 1289.
54. Yildiz, M.; Kilic, Z.; Hokelek, T. *J. Mol. Struct.* **1998**, 441, 1.
55. Schilf, W.; Szady-Chelmieniecka, A.; Grech, E.; Przybylski, P.; Brzezinski, B. *J. Mol. Struct.* **2002**, 643, 115.
56. Bui, H.-H.; Lien, E.J.; Ren, S. *J. Food Drug Anal.* **1998**, 6, 679.
57. Mart, H.; Sacak, M.; Yürük, H.; Sahmetlioglu, E.; Vilayetoglu, A.R. *J. Polym. Sci. Part A* **2004**, 42, 1120.
58. Monfared, H.H.; Pouralimardan, O.; Janiak, C. *Z. Naturforsch.* **2007**, 62b, 717.
59. Abd-Elzaher, M.M. *Appl. Organomet. Chem.* **2004**, 18, 149.
60. Sundaraganesan, N.; Anand, B.; Jian, F.-F.; Zhao, P. *Spectrochim. Acta* **2006**, A65, 826.
61. Sundaraganesan, N.; Meganathan, C.; Anand, B.; Lapouge, C. *Spectrochim. Acta* **2007**, A66, 773.
62. Akkus, Ö.N.; Uzman, S. *Spect. Lett.* **2000**, 33, 445.
63. Ambroziak, K.; Rozwadowski, Z.; Dziembowska, T.; Bieg, B. *J. Mol. Struct.* **2002**, 615, 109.
64. Issa, R.M.; Khedr, A.M.; Rizk, H.F. *Spectrochim. Acta* **2005**, A62, 621.
65. Habibi, M.H.; Montazerozohori, M.; Lalegani, A.; Harrington, R.W.; Clegg, W. *J. Fluor. Chem.* **2006**, 127, 769.
66. Heinert, D.; Martell, A.E. *J. Am. Chem. Soc.* **1963**, 85, 183.
67. Bilge, S.; Kilic, Z.; Hayvali, Z.; Hokelek, T.; Safran, S. *J. Chem. Sci.* **2009**, 121, 989.
68. Herzfeld, R.; Nagy, P. *Curr. Org. Chem.* **2001**, 5, 373.
69. Krol-Starzomska, I.; Rospenk, M.; Rozwadowski, Z.; Dziembowska, T. *Pol. J. Chem.* **2000**, 74, 1441.
70. Dziembowska, T.; Jagodzinska, E.; Rozwadowski, Z.; Kotfica, M. *J. Mol. Struct.* **2001**, 598, 229.
71. Tas, E.; Kilic, A.; Konak, N.; Yilmaz, I. *Polyhedron* **2008**, 27, 1024.
72. Tas, E.; Aslanoglu, M.; Ulusoy, M.; Temel, H. *J. Coord. Chem.* **2004**, 57, 677.