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Synthesis and Characterization of Crown Ethers

ASKIN KIRAZ†, MUSTAFA YILDIZ* and BASARAN DULGER‡
Department of Chemistry, Faculty of Arts and Sciences,
Çanakkale Onsekiz Mart University, 17100 Çanakkale, Turkey
Fax: (90)(286)2180533; Tel: (90)(286)2180018/1861
E-mail: myildiz@comu.edu.tr; starmustafa45@hotmail.com

Crown ether ligands of Schiff base type (**6-17**) have been synthesized by the reaction of 2-hydroxybenzaldehyde, 2-hydroxy-5-methoxybenzaldehyde 2-hydroxy-5-bromobenzaldehyde, 2-hydroxy-5-chlorobenzaldehyde, 2-hydroxy-5-nitrobenzaldehyde and 2-hydroxy-1-naphthaldehyde with 6,7,8,14-tetrahydrodibenzo[*f,i*][1,5]dioxecine-2,12-diamine (**4**) and 7,9,10,16-tetrahydro-6*H*-dibenzo[*h,k*][1,4,7]-trioxacyclododecine-2,14-diamine (**5**). The structures of Schiff base crown ethers have been investigated by elemental analysis, IR, UV-visible, ^{1}H NMR, ^{13}C NMR and MS spectroscopic data. In solution, all Schiff bases exist as equilibrium mixtures of *enol-imine* and *keto-imine* tautomers. For four Schiff bases (**10, 11, 16, 17**), the *keto-imine* form has been found to be dominant in the DMSO. The antimicrobial activities of the compounds are evaluated using disk diffusion method in dimethyl sulfoxide (DMSO) against 9 bacteria and 5 yeast cultures. The obtained results from disk diffusion method are assessed in side-by-side comparison with those of penicillin-G, ampicillin, cefotaxime, vancomycin, oflaxacin, tetracycline as antibacterial agents and nystatin, ketaconazole and clotrimazole as commercial antifungal agents. In most cases, the compounds show broad-spectrum (Gram-positive and Gram-negative bacteria) activities that are comparatively either, slightly less active or equipotent to, antimicrobial agents in the comparison tests.

Key Words: Crown ether, Schiff base, Antimicrobial activity.

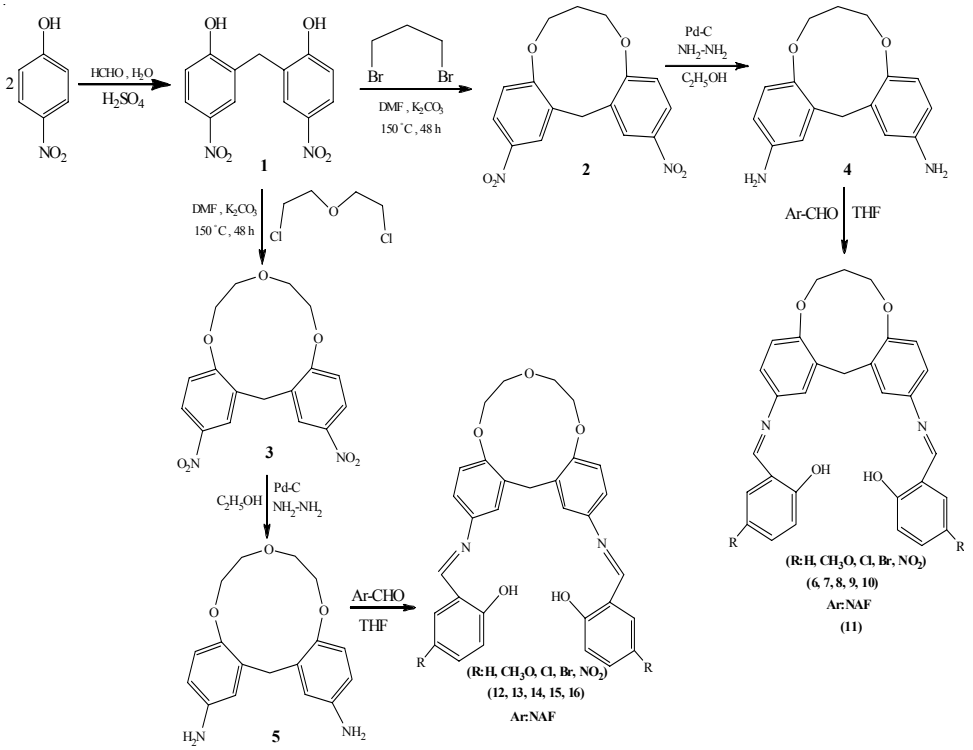
INTRODUCTION

The macrocyclic polyethers has became one of the most popular chemical reagents with a wide area of application in the last century. They are used successfully in chemistry of "host-guest" complexes, extraction, phase transfer catalysis, organic synthesis, analytical chemistry and biology[1-11]. There is also growing interest in the use of crown ethers for their antitumor activity[12]. The properties have been successfully utilized to separate metal ions[13,14] and to develop ion selective electrodes[15,16].

†Near East University, Ataturk Faculty of Education, Nicosia, Mersin, Turkey.
‡Department of Biology, Faculty of Arts and Sciences, Çanakkale Onsekiz Mart University, 17100 Çanakkale, Turkey.

The tautomerism in Schiff bases with OH group in *ortho* position to the imino group both in solution and in solid state were investigated using spectroscopy and X-ray crystallography techniques[17-28]. Schiff bases with OH group in *ortho* position to the imino group are of interest mainly due to the existence of either O–H···N or O···H–N type of hydrogen bond and tautomerism between *enol-imine* and *keto-imine* form. In these compounds, short hydrogen bonds between the OH group in *ortho* position to the imino group and the imine nitrogen is due to the stereochemistry. The rearrangement of these compounds due to the intramolecular proton transfer occurring in the ground or electron excited states was found to be interesting for implementation in various devices and fluorescent chemosensors for metal ions[29-31]. In the latter case, a crown-ether moiety must be attached to the arylimine fragment of the Schiff base. Schiff base type crown ethers were synthesized[32-38] and their structure, spectral, luminescent and complexing properties were studied[38].

In this paper, the new macrocyclic polyether ligands of Schiff base type have been synthesized by the reaction of diamino crown ethers (**4, 5**) with 5-substituted-2-hydroxy-1-benzaldehyde and 2-hydroxy-1-naphthaldehyde (**Scheme-I**). The structures of the synthesized Schiff bases were established by elemental analysis, IR, UV-visible, ^{1}H NMR, ^{13}C NMR and MS spectra. The ligands have been screened techniques *in vitro* againts the microorganisms by disc diffusion method[39,40].



Scheme-I

EXPERIMENTAL

The ^{1}H NMR, ^{13}C NMR spectra were recorded on a Bruker DPX FT-NMR spectrometer operating at 400 and 101.6 MHz. Infrared absorption spectra were recorded on a Perkin-Elmer BX II spectrometer in KBr discs. UV-Vis spectra were recorded on a Shimadzu 1201 series spectrometer. Carbon, hydrogen and nitrogen analyses were performed on a LECO CHNS-932 C, H, N analyzer. LC mass spectra were obtained on a AGILENT 1100 MSD spectrometer with an ion source temperature of 240 °C. Melting points were measured on a Electro Thermal IA 9100 apparatus using a capillary tube, THF, EtOH, DMSO, DMF, $CHCl_3$, hydrazine hydrate, 2-hydroxybenzaldehyde, 5-methoxy-2-hydroxybenzaldehyde, 5-chloro-2-hydroxybenzaldehyde, 5-bromo-2-hydroxybenzaldehyde, 5-nitro-2-hydroxybenzaldehyde, 2-hydroxy-1-naphthaldehyde and Pd-C (10 %) were purchased from Merck (Germany).

Synthesis of 2,2'-methylene*bis*[4-nitrophenol] (1): It was prepared according to the published procedure[41,42].

Synthesis of 2,12-dinitro-6,7,8,14-tetrahydrodibenzo[*f,i*][1,5]dioxecine (2): To 2,2'-methylene*bis*[4-nitrophenol] (**1**) (12.6 g; 4.34×10^{-2} mol) and Na_2CO_3 (5 g; 4.76×10^{-2} mol) dissolved in DMF (500 mL, 99 %) was added 1,3-dibromopropane (8.78 g; 4.34×10^{-2} mol). The mixture stirred at reflux temperature for 48 h and then filtered. The solvent was removed under reduced pressure and the remaining yellow product was washed water. The crude product was recrystallized from methanol. Experimental and analytical data are summarized in Table-1.

TABLE-1
EXPERIMENTAL AND ANALYTICAL DATA

Compd.	m.p. (ºC)	Colour	Yield (%)	Elemental analysis (%): Calcd. (Exp.)		
				C	H	N
2	290-292	Yellow	47	58.18 (58.16)	4.24 (4.21)	8.48 (8.48)
3	269-271	Yellow	21	56.67 (56.66)	4.44 (4.44)	7.77 (7.76)
4	200-202	Black-brown	89	71.11 (71.09)	6.67 (6.65)	10.37 (10.36)
5	146-148	White	89	68.00 (67.96)	6.67 (6.66)	9.33 (9.33)
6	123-124	Yellow	55	75.31 (75.30)	5.44 (5.44)	5.85 (5.85)
7	142-144	Red	60	71.37 (71.35)	5.58 (5.57)	5.20 (5.20)
8	182-184	Yellow	58	65.81 (65.81)	4.38 (4.36)	5.11 (5.11)
9	210-212	Yellow	66	56.60 (56.61)	3.77 (3.78)	4.40 (4.40)
10	178-179	Yellow	80	63.38 (63.37)	4.22 (4.25)	9.86 (9.85)
11	116	White-brown	80	78.89 (78.85)	5.19 (5.18)	4.84 (4.84)
12	214-216	Yellow	49	73.22 (73.21)	5.51 (5.53)	5.51 (5.51)
13	189	Yellow	70	69.71 (69.70)	5.63 (5.63)	4.93 (4.93)
14	264-266	Yellow	61	64.47 (64.47)	4.51 (4.50)	4.85 (4.83)
15	242-244	Yellow	31	55.86 (55.87)	3.90 (3.92)	4.20 (4.20)
16	313-315	Orange	56	62.20 (62.18)	4.35 (4.33)	9.36 (9.35)
17	290-292	Yellow	52	76.97 (76.95)	5.26 (5.26)	4.60 (4.60)

Synthesis of 2,14-dinitro-7,9,10,16-tetrahydro-6*H*-dibenzo[*h*,*k*][1,4,7]-trioxacyclododecine (3): To 2,2'-methylene*bis*[4-nitrophenol] (**1**) (12.6 g; 4.34 × 10^{-2} mol) and Na_2CO_3 (5 g; 4.76 × 10^{-2} mol) dissolved in DMF (500 mL, 99 %) was added diethyleneglycoldichloride (6.20 g; 4.34 × 10^{-2} mol). The mixture stirred at reflux temperature for 48 h and then filtered. The solvent was removed under reduced pressure and the remaining yellow product was washed water. The crude product was recrystallized from methanol. Experimental and analytical data are summarized in Table-1.

Synthesis of 6,7,8,14-tetrahydrodibenzo[*f*,*i*][1,5]dioxecine-2,12-diamine (4): To compound **2** (6 g; 1.8 × 10^{-2} mol) and Pd/C (0.86 g, 5 %) dissolved in ethanol (150 mL, 99 %) was added dropwise hyrazine monohydrate (6.80 mL). The mixture stirred at reflux temperature for 3 h and then filtered through a pad of celite to remove the catalyst. Solvent and excess hydrazine were removed *in vacuo* to give the crude product **4**. The crude product was recrystallized from ethanol. Experimental and analytical data are summarized in Table-1.

Synthesis of 7,9,10,16-tetrahydro-6*H*-dibenzo[*h*,*k*][1,4,7]trioxacyclo-dodecine-2,14-diamine (5): To compound **3** (1.78 g; 4.94 × 10^{-3} mol) and Pd/C (0.24 g, 5 %) dissolved in ethanol (150 mL, 99 %) was added dropwise hyrazine monohydrate (1.88 mL). The compound **5** was obtained as **4**. Experimental and analytical data are summarized in Table-1.

Synthesis of 2,2'-(1*E*,1'*E*)-(6,7,8,14-tetrahydrodibenzo[*f*,*i*][1,5]dioxecine-2,12-diyl)*bis*(azan-1-yl-1-ylidene)*bis*(methan-1-yl-1-ylidene)diphenol (6): To compound **4** (0.13 g; 4.3 × 10^{-4} mol) was added to a dry THF (100 mL) solution of 2-hydroxy-1-benzaldehyde (0.10 g; 8.6 × 10^{-4} mol). The mixture was stirred and heated for 3 h. Compound (**6**) was obtained from the evaporation of THF. Experimental and analytical data are summarized in Table-4.

Other Schiff base crown ethers ligands (**7-17**) were obtained using same method. For all the compounds analytical and experimental details are given in Table-1.

Antimicrobial test: The compounds were dissolved in DMSO to a final concentration of 100 µg/mL. Empty sterilized antibiotic discs having a diameter of 6 mm (Schleicher & Schull No 2668, Germany) were each impregnated with 20 µL of solution. All the bacteria mentioned above were incubated at 30 ± 0.1 °C for 24 h by inoculution into Nutrient Broth (Difco) and the yeasts studied were incubated in Malt Extract Broth (Difco) for 48 h. An inoculum containing 10^6 bacterial cells or 10^8 yeast cells/mL was spread on Mueller-Minton Agar (Oxoid) plates (1 mL inoculum/plate). The discs injected with solutions were placed on the inoculated agar by pressing slightly and incubated at 35 °C (24 h) for bacteria and at 25 °C (72 h) for yeast. On each plate an appropriate reference antibiotic disc was applied depending on the test microorganisms[39,40]. The data reported in Table-3 are the average data of three experiments.

RESULTS AND DISCUSSION

The IR spectra of the compounds are given in Table-2. Vibration bands with the wave numbers of 1624-1612 cm^{-1} ν(C=N), 3072-3033 cm^{-1} ν(C-H, Ar-H), 2870-2917 cm^{-1} ν(C-H, Csp^3-H), 1587-1502 cm^{-1} ν(C=C) and 1036-1288 cm^{-1} ν(C-O-C, Csp^3-O-Csp^3) were observed. The observation of aromatic ν(C-O) at 1338, 1309, 1340 and 1312 cm^{-1} for compounds **10**, **11**, **16** and **17** are the evidence for the existence of the *keto-imine* form N–H···O intramolecular hydrogen bonding only in the solid state. The stretching frequency observed at 2748-2742 cm^{-1} in **5-12** shows the presence of O–H···N intramolecular hydrogen bond[17,18]. The C=N bond which is accountable partially for the existence *enol-imine* form can also be inferred from the IR spectra of compound (**6-9**, **12-15**). The compound with strong band at 1280-1257 cm^{-1} possesses highest percentage of *enol-imine* tautomer due to the stabilization of phenolic C-O bond[43].

TABLE-2
FT-IR SPECTRAL DATA (ν, cm^{-1}) IN THE SOLID STATE (KBr)

Compd.	ν(OH)	ν(C-H) (aromatic)	ν(C-H) (aliphatic)	ν(C=N)	ν(C=C)	ν(C-N)	ν(C-O-C)
6	3432 m	3050 m	2922 s 2848 m	1617 s	1587 m	1498 s	1280 s 1191 s 1107 s 1053 s
7	3389 m	3050 w	2926 s 2833 m	1616 s	1583 s	1492 s	1269 s 1036 s
8	3363 m	3072 w	2920 s 2856 m	1616 s	1553 m	1497 s	1276 s 1180 s 1052 m
9	3411 s	3045 w	2923 m 2870 m	1615 s	-	1477 s	1272 s 1175 s 1054 m
10	3421 s	3072 m	2920 s 2856 m	1621 s	1502 m	1474 s	1239 s 1130 s 1093 m
11	3384 m	3059 m	2933 s 2847 s	1622 s	1539 s	1465 s	1243 s 1175 s 1048 m
12	3429 m	3040 w	2917 s 2848 s	1617 s	1570 s	1480 m	1265 s 1193 s
13	3448 m	3047 w	2926 s 2856 s	1612 m	1581 s	1464 s	1257 s 1128 s
14	3431 m	3055 w	2918 s 2850 s	1618 s	1565 s	1479 s	1259 s 1181 s 1120 s
15	3431 m	3047 w	2920 s 2863 m	1616 s	1563 m	1486 s	1264 s 1124 m
16	3432 s	3070 w	2922 m 2856 w	1623 s	1572 m	1479 m	1288 m 1185 w 1095 m
17	3423 m	3033 w	2922 m 2863 w	1624 s	1577 s	1467 s	1252 s 1158 s

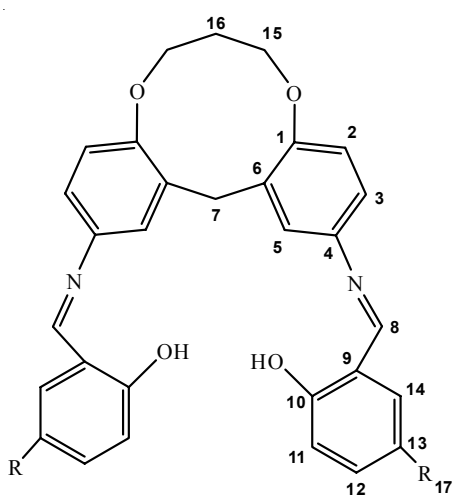
The ^{1}H NMR data for compounds **12-15** show that the tautomeric equilibrium favours the *enol-imine* in DMSO. The compounds **6-11** and **16**, **17** shifts to the *keto-imine* form in the same solvent. The protons of the ethereal group at Ar-OCH_2- also gave a triplet and Ar-OCH_2CH_2-triplet for **6-11**. The ^{1}H NMR data are given in Table-3.

TABLE-3
^{1}H NMR SPECTRAL DATA (δ, ppm, 400 MHz) IN DMSO

Compd.	OH	CH=N	Ar-H	$ArOCH_2$	$ArOCH_2CH_2$	$ArCH_2Ar$	OCH_3
6	13.21(d)	8.83(d) ($^3J_{HH}$=5.48 Hz	7.59 6.81(m)	3.98(t)	3.84(t) ($^3J_{HH}$=6.03 Hz	3.27(s)	
7	12.83(d)	8.88(d) ($^3J_{HH}$=3.38 Hz)	7.66 6.86(m)	4.07(t)	3.96(t) ($^3J_{HH}$=6.20 Hz	3.72(s)	3.74(s)
8	13.18(d)	8.75(d) ($^3J_{HH}$=3.84 Hz)	7.57 6.86(m)	3.98(t)	3.42(t) ($^3J_{HH}$=5.81 Hz)	3.33(s)	
9	13.27(d)	8.89(d) ($^3J_{HH}$=6.84 Hz)	7.86 6.87(m)	3.99(t)	3.50(t) ($^3J_{HH}$=6.19 Hz)	3.36(s)	
10	15.91(d)	10.18(d) ($^3J_{HH}$=11.77 Hz	9.06 6.85(m)	3.99(t)	3.48(t) ($^3J_{HH}$=6.32 Hz)	3.41(s)	
11	15.89(d)	9.52(d) ($^3J_{HH}$=12.74 Hz)	8.84 6.79(m)	4.12(t)	3.98(t) ($^3J_{HH}$=5.91 Hz)	3.87(s)	
12	13.33(s)	8.93(s) ($^3J_{HH}$=3.58 Hz	7.61 6.90(m)	4.11(t)	3.83(t) ($^3J_{HH}$=5.72 Hz	3.48(s)	
13	13.50(s)	8.92(s) ($^3J_{HH}$=3.30 Hz)	7.54 6.86(m)	4.10(t)	3.96(m)	3.48(s)	3.80(s)
14	13.91(s)	8.91(s) ($^3J_{HH}$=2.86 Hz)	7.69 6.95(m)	4.11(t)	3.88(t) ($^3J_{HH}$=4.36 Hz	3.48(s)	
15	13.49(s)	8.42(s) ($^3J_{HH}$=5.85 Hz)	7.49 6.77(m)	4.16(t)	3.87(t) ($^3J_{HH}$=2.47 Hz	3.60(s)	
16	14.69(d)	10.21(d) ($^3J_{HH}$=3.77 Hz	9.04 6.95(m)	4.02(s)	3.80(s)	3.31(s)	
17	13.90(d)	9.90(d) ($^3J_{HH}$=6.74 Hz)	8.59 6.10(m)	4.42(s)	3.79(s)	3.57(s)	

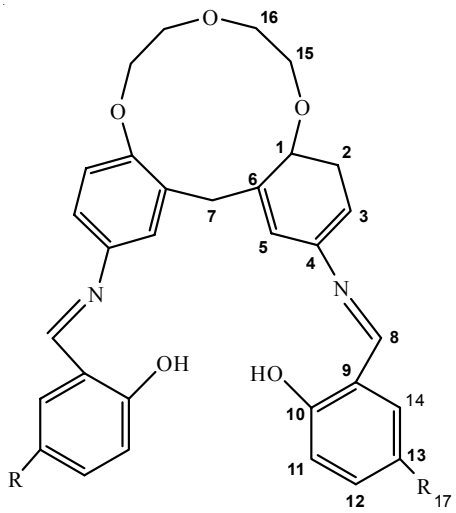
According to the ^{13}C NMR spectra compounds **6**, **8**, **9**, **10**, **12**, **14**, **15**, **16** and **7**, **13** and **11**, **17** have **16**, **17** and **20** signals, respectively showing that the structures in solution are symmetrical. **Scheme-II** shows the numbering of the Schiff base carbons and compound **11**, **17** data. The ^{13}C NMR spectra data of the compounds(**6-10** and **12-16**) are given in Table-4.

The UV-visible spectra of the compounds **6-17** were studied in DMSO. The Schiff bases show absorption in the range greater than 400 nm in polar and non-polar solvents. It is point out that the new band belongs to the *keto-imine* form of the Schiff bases with OH group in *ortho* position to the imino group in polar and non-polar solvents in both acidic and basic media[27-32]. The compounds **6-17**,



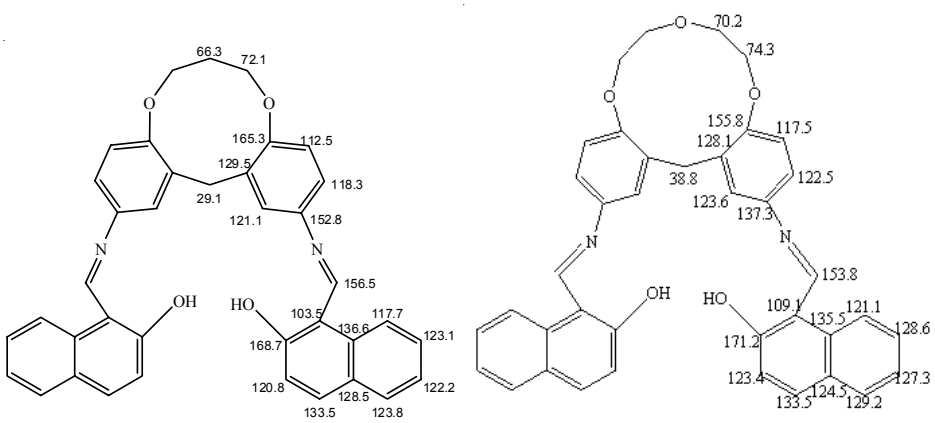
(R:H, CH_3O, Cl, Br, NO_2)

(6, 7, 8, 9, 10)



(R:H, CH_3O, Cl, Br, NO_2)

(12, 13, 14, 15, 16)



Scheme-II

a new band is observed at > 400 nm in the DMSO. The compounds **6-17** are in tautomeric equilibria (phenol-imine, O–H···N $\rightleftharpoons$ *keto-imine*, O···H–N forms) in DMSO (Figs. 1 and 2). This tautomeric equilibria is observed in DMSO for **6-11**, **16** and **17** as supported by UV-visible and ^{1}H NMR data. Only *keto-imine* tautomer is dominant in DMSO for **10**, **11**, **16** and **17**. The *keto-imine* tautomer is 59, 80, 55 and 99 % , respectively, for compounds **10**, **11**, **16** and **17**.

In conclusion, UV-Vis, ^{1}H NMR and ^{13}C NMR results show that in DMSO solution the compounds **10**, **11**, **16** and **17** exist in the *keto-imine* form.

TABLE-4
^{13}C NMR SPECTRAL DATA (δ, ppm, 101.6 MHz) IN DMSO

Compd.	C1	C2	C3	C4	C5	C6	C7	C8	C9
6	142.40	115.59	116.96	132.79	120.79	133.22	32.57	160.63	119.49
7	155.75	110.62	123.04	142.60	128.24	134.25	32.57	161.20	122.53
8	142.08	115.41	118.92	136.15	124.07	127.85	32.55	159.95	122.88
9	138.92	111.17	115.63	134.23	121.74	123.21	32.56	159.71	120.36
10	141.26	112.09	124.82	131.14	124.67	129.10	31.18	162.18	118.05
12	156.12	114.69	119.47	141.28	124.43	133.18	29.01	160.65	119.86
13	150.92	114.75	123.45	141.06	124.20	131.76	26.30	156.12	119.75
14	156.37	114.59	120.52	140.97	124.59	131.33	28.50	159.30	121.12
15	158.16	123.05	129.70	135.92	121.60	111.03	32.00	159.72	110.19
16	164.15	117.88	124.82	140.21	125.44	131.17	30.75	166.14	122.64
	C10	C11	C12	C13	C14	C15	C16	C17	
6	162.24	115.69	123.98	119.86	123.08	73.19	64.98	–	
7	161.82	119.72	120.31	154.65	117.77	73.17	65.32	55.93	
8	160.74	115.59	132.59	123.19	131.33	73.18	64.97	–	
9	160.33	119.39	132.29	110.24	124.05	73.21	65.36	–	
10	166.28	115.30	128.85	140.05	128.40	72.97	68.97	–	
12	161.76	116.96	132.75	120.43	131.75	70.23	69.29	–	
13	161.88	118.90	119.50	148.32	115.64	70.85	69.70	60.43	
14	160.30	118.97	132.63	122.86	131.67	70.31	69.24	–	
15	161.28	120.99	134.14	119.35	132.10	72.95	69.47	–	
16	189.48	118.91	130.11	140.35	128.72	70.52	69.34	–	

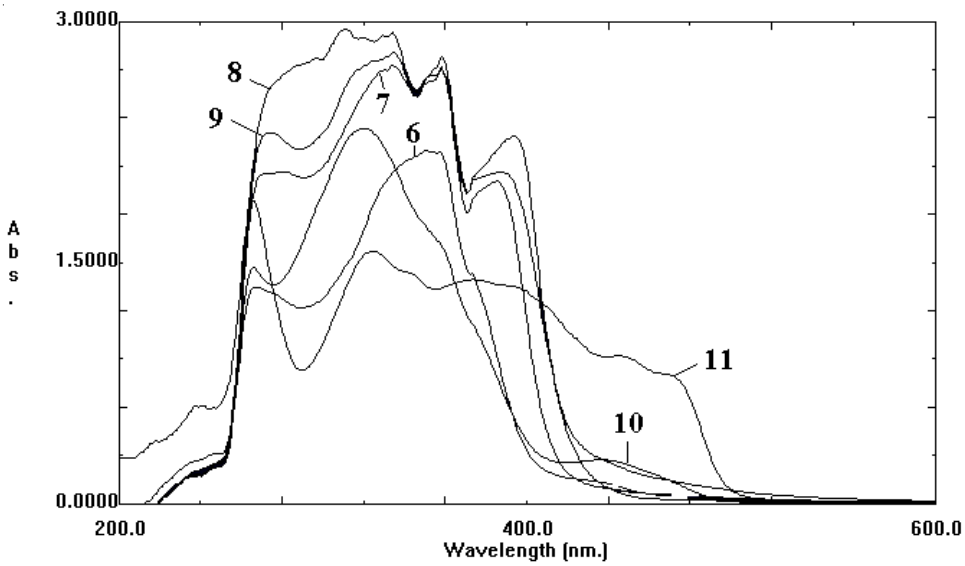


Fig. 1. UV spectra of the compounds **6-11** in the DMSO

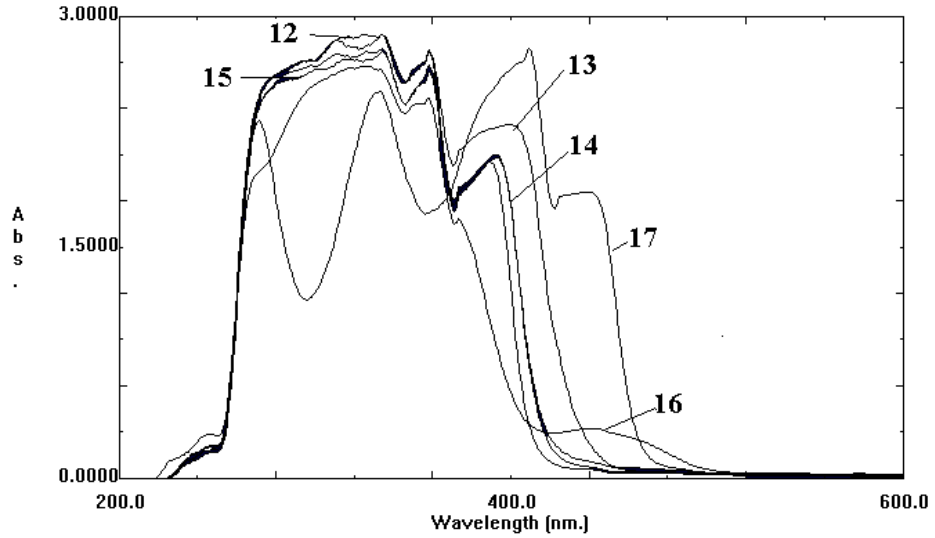


Fig. 2. UV spectra of the compounds **12-17** in the DMSO

Antimicrobial activities: Tables 5 and 6 show antimicrobial activities of the compounds and standard antibiotic discs. As can clearly be seen from Tables 5 and 6, the compounds showed antibacterial activity against both Gram-positive and Gram-negative bacteria and the yeast cultures in this study. In classifying the antibacterial activity as Gram-positive or Gram-negative, it would generally expected that a much greater number would be active against Gram-positive than Gram-negative bacteria[44]. However, in this study, the compounds are active against both types of the bacteria and as well as active against yeasts, which may indicate broad-spectrum properties. Notably, the compounds have stronger antimicrobial activities against the yeast cultures than those of bacteria used in this study.

All the compounds tested exhibit strong or moderate antimicrobial activity in various inhibition zones. The inhibition zones ranged from 11 to 22 mm. The inhibition zone values of the compounds on the bacteria are exceptionally effective compared with most of the reference antibiotics. As an example, *Staphylococcus aureus* is susceptible to the compound **9**, as compared to the standard antibiotics except for OFX5 and TE30. The compounds **7**, **13** and **14** have more antibacterial activity against *Bacillus cereus* than those of some standard antibiotics. In addition all compounds have strong antibacterial effect against *Pseudomonas aeruginosa* than those of the standard antibiotics P10, SAM20 and VA30.

In generally, all compounds have shown high antiyeast activity against the yeast cultures used in this study. While the compound **14** is strong effective against *Kluyveromyces fragilis, Rhodotorula rubra* is more suspectible to the compound **16** compared with most of the reference antibiotics. Notably, the compound **13** have higher antiyeast activity against *Debaryomyces hansenii* than those all standard antibiotics. Also, *Hanseniaspora guilliermondii* and *Candida albicans* are influenced in different levels.

TABLE-5
ANTIMICROBIAL ACTIVITIES OF THE COMPOUNDS AND
SOME STANDARD ANTIBIOTICS

Microorganisms	Inhibition zone (mm)														
	Compounds						Antibiotics								
	6	**7**	**8**	**9**	**10**	**11**	P10	SAM20	CTX30	VA30	OFX5	TE30	Y100	KETO20	CLT10
Escherichia coli	17.0	12.0	15.0	15.0	12.0	16.0	18	12	10	22	30	28	-	-	-
Staphylococcus aureus	13.0	14.0	16.0	18.0	11.0	14.0	13	16	12	13	24	26	-	-	-
Klebsiella pneumoniae	14.0	15.0	12.0	12.0	10.0	18.0	18	14	13	22	28	30	-	-	-
Bacillus cereus	13.0	18.0	15.0	17.0	11.0	16.0	14	12	14	18	30	25	-	-	-
Micrococcus luteus	11.0	16.0	13.0	14.0	14.0	15.0	36	32	32	34	28	22	-	-	-
Proteus vulgaris	16.0	12.0	12.0	13.0	14.0	18.0	10	16	18	20	28	26	-	-	-
Mycobacterium smegmatis	15.0	13.0	12.0	17.0	14.0	18.0	15	21	11	20	32	24	-	-	-
Listeria monocytogenes	13.0	14.0	17.0	18.0	12.0	14.0	10	12	16	26	30	28	-	-	-
Pseudomonas aeruginosa	18.0	15.0	16.0	12.0	14.0	18.0	8	10	54	10	44	34	-	-	-
Kluyveromyces fragilis	12.0	13.0	14.0	15.0	11.0	13.0	-	-	-	-	-	-	18	16	18
Rhodotorula rubra	17.0	13.0	13.0	17.0	15.0	17.0	-	-	-	-	-	-	18	22	16
Candida albicans	13.0	13.0	18.0	18.0	13.0	13.0	-	-	-	-	-	-	20	21	15
Hanseniaspora guilliermondii	17.0	12.0	14.0	12.0	17.0	16.0	-	-	-	-	-	-	21	24	22
Debaryomyces hansenii	12.0	15.0	12.0	14.0	17.0	18.0	-	-	-	-	-	-	16	14	18

P10 = Penicillin G (10 Units), SAM20 = Ampicillin 10 µg, CTX30 = Cefotaxime 30 µg. V30 = Vancomycin 30 µg, OFX 5 = Oflaxacin 5 µg, TE30 = Tetracyclin 30 µg, N100 = Nystatin 100 µg, KETO20 = Ketaconazole 20 µg, CLT10 = Clotrimazole 10 µg.

TABLE-6
ANTIMICROBIAL ACTIVITIES OF THE COMPOUNDS AND SOME STANDARD ANTIBIOTICS

Microorganisms	Inhibition zone (mm)														
	Compounds						Antibiotics								
	12	**13**	**14**	**15**	**16**	**17**	P10	SAM20	CTX30	VA30	OFX5	TE30	Y100	KETO20	CLT10
Escherichia coli	14.0	19.0	16.0	15.0	14.0	15.0	18	12	10	22	30	28	-	-	-
Staphylococcus aureus	12.0	16.0	15.0	13.0	17.0	14.0	13	16	12	13	24	26	-	-	-
Klebsiella pneumoniae	13.0	15.0	17.0	14.0	16.0	15.0	18	14	13	22	28	30	-	-	-
Bacillus cereus	14.0	18.0	18.0	13.0	15.0	15.0	14	12	14	18	30	25	-	-	-
Micrococcus luteus	16.0	15.0	16.0	17.0	17.0	13.0	36	32	32	34	28	22	-	-	-
Proteus vulgaris	16.0	15.0	17.0	13.0	14.0	16.0	10	16	18	20	28	26	-	-	-
Mycobacterium smegmatis	14.0	16.0	15.0	14.0	17.0	15.0	15	21	11	20	32	24	-	-	-
Listeria monocytogenes	17.0	16.0	15.0	13.0	17.0	14.0	10	12	16	26	30	28	-	-	-
Pseudomonas aeruginosa	17.0	16.0	18.0	11.0	14.0	13.0	8	10	54	10	44	34	-	-	-
Kluyveromyces fragilis	15.0	20.0	21.0	15.0	18.0	17.0	-	-	-	-	-	-	18	16	18
Rhodotorula rubra	16.0	20.0	20.0	18.0	22.0	16.0	-	-	-	-	-	-	18	22	16
Candida albicans	15.0	16.0	15.0	18.0	19.0	16.0	-	-	-	-	-	-	20	21	15
Hanseniaspora guilliermondii	15.0	20.0	18.0	18.0	21.0	18.0	-	-	-	-	-	-	21	24	22
Debaryomyces hansenii	17.0	19.0	18.0	15.0	15.0	15.0	-	-	-	-	-	-	16	14	18

P10 = Penicillin G (10 Units), SAM20 = Ampicillin 10 µg, CTX30 = Cefotaxime 30 µg, V30 = Vancomycin 30 µg, OFX 5 = Oflaxacin 5 µg, TE30 = Tetracyclin 30 µg, N100 = Nystatin 100 µg, KETO20 = Ketaconazole 20 µg, CLT10 = Clotrimazole 10 µg.

The compounds differ significantly in their activity against tested microorganisms. These differences may be attributed to fact that the cell wall in Gram-positive bacteria of a single layer, whereas the Gram-negative cell wall is multi-layered structure and the yeast cell wall is quite complex[45].

Bacteria and yeast cultures used in this study were chosen primarily on the basis of their importance as pathogens in humans. Methicillin resistant *Staphylococcus aureus* (MRSA) remains an important nosocomial pathogen. According to the latest report from the National Nosocomial Infection Surveillance System (NNIS), *ca*. 60 % of all *S. aureus* nosocomial infections in intensive care units (ICUs) were methicillin resistant in 2003, representing an 11 % increase in resistance compared to the preceding 5 year period[46]. Notably, *Staphylococcus aureus* is the most sensitive bacterium to the compounds. So, the results of present study indicate that the extracts have the potential to generate novel metabolites. The extracts demonstrating especially antibacterial activity against *Staphylococcus aureus* could result in the discovery of novel antibacterial agents, showing demonstrating broad spectrum activities, this may help to discover new antibiotics that could serve as selective agents against infectious diseases.

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