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The NMR Study and Antimicrobial Activity of Some Schiff Bases Derived From Sulphonamide Drug

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ABSTRACT

Some Schiff base compounds derived from sulfonamide drug were synthesized by reaction of 4-aminobenzenesulfonamide with aromatic aldehydes (2-hydroxy-1-naphthaldehyde, 3,4-dihydroxybenzaldehyde and 2-hydroxy benzaldehyde) in good yields. Characterization of synthesized compound was carried by elemental analysis, IR, ^{1}H,^{13}C, HSQC and HMBC- NMR spectroscopy. The synthesized compounds were screened for their antibacterial activity against *Staphylococcus aureus*, *Streptococcus sp*., *Bacillus subtillus, Escherichia coli* and *Klebsiella pneumonia.* Additionally, the compounds were tested for antifungicidal activity against *Candida krusei, Candida tropicalis*, *Aspergillus fumigates* and *Aspergillus niger*.

Key words: Sulfonamide, Schiff base, NMR , Antimicrobial activity, Aldehydes.

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INTRODUCTION

Sulphonamides were the first drugs found to act selectively and could be used systematically as preventive and the therapeutic agents against various diseases [1]. In medicine, the term "sulfonamide" is sometimes used as a synonym for sulfa drug, a derivative or variation of sulfanilamide. The first sulfonamide was discovered in 1932[2]. The condensation products of sulpha drugs with aldehydes and ketones are biologically active [3,4].

Schiff bases are used as pigments and dyes, catalysts, intermediates inorganic synthesis and as polymer stabilizers. A number of Schiff's base molecules show biological activities including antibacterial, antifungal, antidiabetic, antitumour, antiproliferative, anticancer, anti-corrosion and anti-inflammatory activities [5-8]. Sulfa Schiff bases have been subject to thorough studies where a wide diversity of these derivatives have been prepared and used in various biological and pharmacological fields [9-11]. The aim of present work is to synthesis of some Schiff base derived from sulphonamide (Scheme1) and study the biological activity theoretically (Molecular modiling and *in vitro* antimicrobial activity.

EXPERIMANTAL

a- Physical mesurments

Infrared spectra (IR) were recorded as KBr discs in the range of 4000-400 cm^{-1}using FT-IR spectrophotometer Shimadzu model IR. Affinity-1 at the department of Chemistry, College of Education for pure sciences, University of Basrah, Iraq. ^{1}H, ^{13}C, Roesy, HSQC and HMBC NMR spectra were measured on a Brucker at 600 MHz, with TMS as internal reference at Konstanz university, Germany. Microanalysis for carbon, hydrogen and nitrogen were carried out by a Perkin-Elmer 240B Elemental Analyzer. Melting points were measured by a Philip Harris melting point apparatus and uncorrected.

b- Synthesis

General Synthesis of Schiff-bases**5-7**

4–aminobenzenesulfonamide **1** (1.37, 2.00 mmol) and aromatic aldehydes (**2-4**) (2.1 mmol) were dissolved in absolute ethanol followed by addition of catalytic amount of glacial acetic acid dropwise and the mixture was heated under reflux for 4h. The reaction mixture was then cooled in an ice bath and the crude product thus obtained was collected by filtration, further purified by recrystallization from ethanol.

4-{[(2-hydroxynaphthalen-1-yl)methylidene]amino}benzenesulfonamide **5**

Yield,82 %, as a yellow solid, M.p. =276-278^{0}C,FT-IR (KBr, v, cm^{-1}): 3420-3335(OH, NH),3082-3063(CH-aromatic), 1622(C=C), 1602(C=N). ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm):15.46(s,1H, OH), 9.69(s,1H, CH=N), 8.41-7.01(m, 10H, Ar-H), 7.47(s,2H, NH).^{13}C NMR(600 MHz, DMSO-d_6,δ, ppm): 171.5 (C-O), 156.9 (C=N), 147.2-109.3 (C-Ar).

Anal. for $C_{17}H_{14}N_2O_3S$(M.wt 326.3):Calc.C,62.51; H, 4.29; N, 8.58; Found: C, 62.24; H, 3.97; N, 8.31.

4-[(3,4-dihydroxybenzylidene)amino]benzenesulfonamide **6**

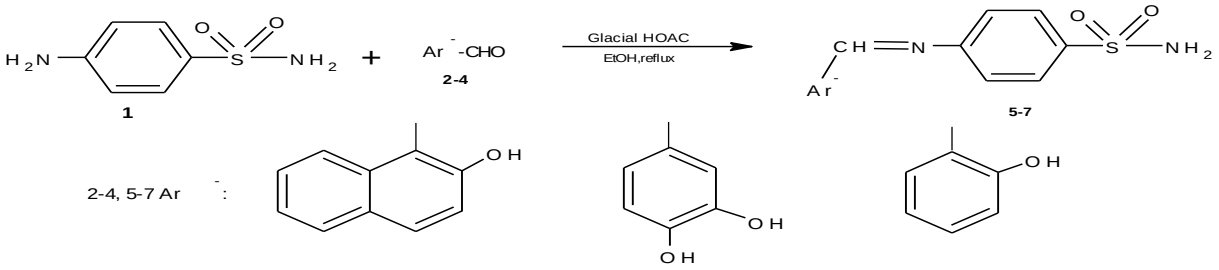
Yield, 78% as a brown solid, M.p.=136-138 ^{0}C,FT-IR (KBr, v, cm^{-1}): 3417-3300(OH, NH), 3074-3052(CH-aromatic), 1610(C=C), 1598(C=N). ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 9.71(s, 1H, CH=N), 8.41-6.59 (m, 10H, Ar-H). ^{13}C NMR(600 MHz, DMSO-d_6,δ, ppm): 162.5 (C=N), 155.3-116.0(C-Ar).

Anal. for $C_{13}H_{12}N_2O_4S$(M.wt 292.3):Calc. C, 53.36; H, 4.10; N, 9.59; Found: C, 52.98; H, 3.97; N, 9.31.

4-[(2-hydroxybenzylidene)amino]benzenesulfonamide **7**

Yield,72% as a yellow solid, M.p. =222-224 ^{0} C,FT-IR (KBr, v, cm^{-1}): 3400-3325(OH, NH) 3080-3067(CH-aromatic), 1618(C=C), 1600(C=N). ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 8.97(s, 1H, CH=N), 8.52-7.01 (m, 10H, Ar-H). ^{13}C NMR(600 MHz, DMSO-d_6,δ, ppm): 170.2 (CH=N), 152.3-112.9 (C-Ar).

Anal. for $C_{13}H_{12}N_2O_3S$(M.wt 276.3):Calc. C,56.46; H, 4.34; N, 10.13; Found: C, 56.14; H, 3.97; N, 9.92.



Scheme 1: Preparation of some Schiff-base of sulphonamide

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c- Antimicrobial activity

The synthesized compounds were screened *in vitro* for their antibacterial activity against:*Staphylococcus aureus*, *Streptococcus sp*., *Bacillus subtillus, Escherichia coli* and *Klebsiella pneumonia.* Additionally, the compounds were tested also for antifungal activity *against Candida albicans, Candida tropicalis*, *Aspergillus fumigates* and *Aspergillus niger* using the paper disc-agar diffusion technique on Muller Hinton agar and Sabouraud dextrose agaras a culture media for antibacterial activity and antifungal activity, respectively [12]. The test compounds were dissolved in DMSO solvent and recommended concentrations (50, 100 and 200 µg/mL) were used in the disc-agar diffusion technique. Antibiotic drug Ampicillin and Nystatin were used as control for bacteria and fungi, respectively. Petri plates containing 20 mL of Mueller Hinton Agar were used for all the bacteria tested. Fungistrains were cultivated in Sabouraud dextrose agar. Sterile Whatman no. 1 filter paper disks (6mm in diameter) impregnated with the solution in DMSO of the test were placed on the Petri plates. A paper disk impregnated with dimethyl sulfoxide (DMSO) was used as negative control. The plates were incubated for 24 h at 37 ^{o}C in the case of bacteria and 72 h that 27 ^{o}C for fungi. The inhibition zone diameters were measured in millimeters. The bacteria and fungi were supplied from department of Microbiology, College of Veterinary Medicine, University of Basrah.

RESULTS AND DISCUSSION

Chemistry

Treatment of 4–aminobenzenesulfonamide**1** with three aldehyde derivatives (2-hydroxy-naphthaldehyde **2**, 3,4-dihydroxybenzaldehyde **3** and 2-hydroxybenzaldehyde **4** in ethanol and catalytic by 3-4 drops of glacial acetic acid under reflux afforded the desired imine derivatives **5-7** in 82, 78 and 72 % yields, respectively (Scheme 1).The structures of the synthesized compounds were assigned by the elemental analysis (CHN),IR and ^{1}H, ^{13}C and 2D NMR. The IR spectra confirm the presence of the azomethine group (-CH=N) stretching with a sharp region for compounds **5-7** at 1602,1598 and 1600 cm^{-1}, respectively. In addition, the bands at the region 1622-1610 cm^{-1} were assigned to the C=C aromatic group. In the ^{1}H NMR spectra of compounds **5**-**7**, the singlets at δ9.69, 9.71 and 8.97 ppm respectively, were assigned for the imine protons (CH=N). The multiplets at the regions *δ* 8.52-6.59 ppm were attributed to the aromatic protons.

In the ^{13}C NMR spectra of compounds **5**-**7**, the resonances at δ155.3-109.3 ppm were assigned for the aromatic carbon atoms (C-Ar). The spectra revealed the presence of CH=N group around 156.9, 162.5 and 170.2 ppm, Figures 1-3 and 6,9,11.

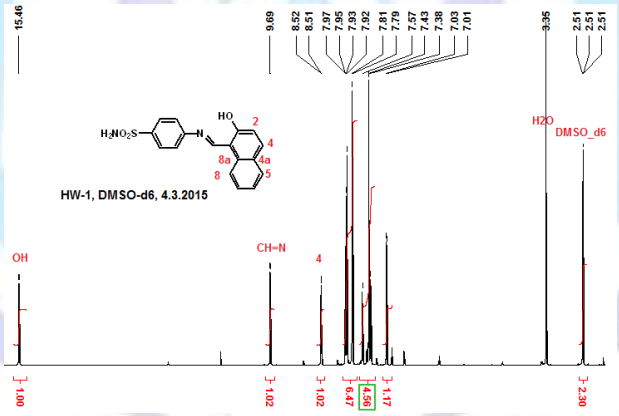

Fig. 1: ^{1}H NMR spectroscopy of compound **5**

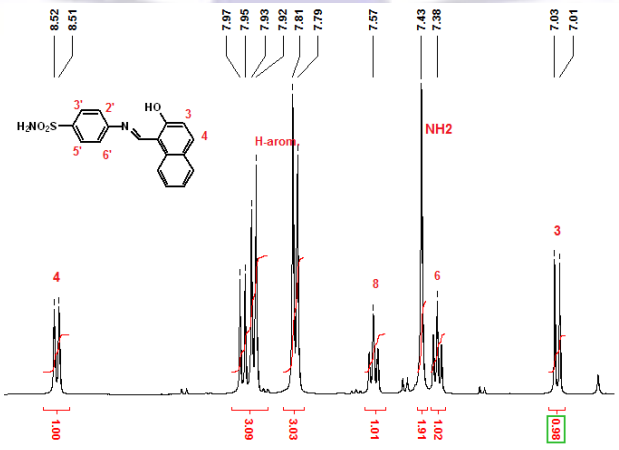

Fig. 2:^{1}H NMR spectroscopy (expantion) of compound **5**

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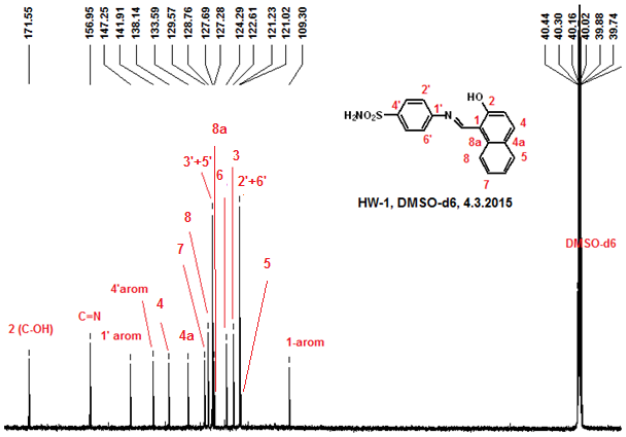
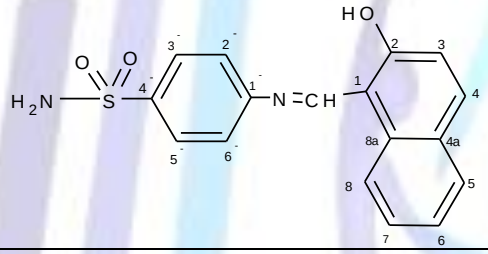


Fig. 3: ^{13}C NMR spectroscopy of compound **5**

The ^{1}H, ^{13}C HSQC NMR spectrum of Schiff base **5** showed a cross peak at δ_H/δ_C = 9.70/156.9 ppm due to azomethine group (N=CH), Thus, the correlation of protons and carbon in aromatic rings such as δ_H/δ_C = 8.52/121.0, 7.95/138.0 ppm and other positions can be assigned to the protons and carbon atoms of the aromatic ring , Table 1, Figure 4.

Table (1): HSQC data for compound **5**

Compound	^{1}H (ppm)	^{13}C(ppm)	Assignment
	9.70	156.9	C,H (CH=N)
	8.50	121.0	C,H (5)
	7.95	138.0	C,H (4)
	7.93	128.0	C,H (3', 5')
	7.81	121.5	C,H (2', 6'))
	7.80	130.0	C,H (7)
	7.55	129.0	C,H (8)
	7.38	125.0	C,H (6)
	7.00	122.0	C,H (3)

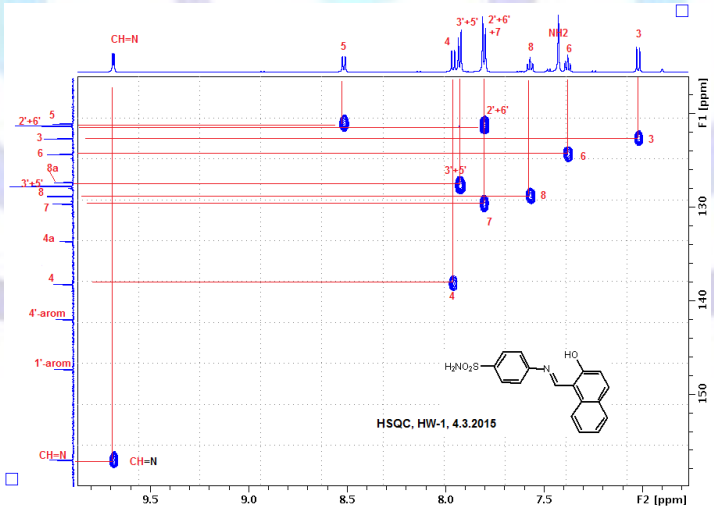


Fig. 4: HSQC- NMR spectroscopy of compound **5**

The gradient-selected ^{1}H,^{13}C, HMBC NMR spectrum of compound **5** revealed two $^{1,3}J_{C,H}$. Thus, the imino proton (CH=N) at δ9.70 ppm showed two $^{1,3}J_{C,H}$ correlations: first one with C-1 of the naphthyl ring at δ160.3 ppm, the second correlation with the aromatic carbon atom C-6' at δ 109.3 ppm and the last one with the aromatic carbon atom C-1' at δ147.2 ppm. Othercorrelations between protons and carbon atoms can be assigned in Figure 5.

The HSQC and HMBC-NMR Spectra of compounds **6** and **7** were supported the structures of synthesized compounds, Figures 7,8,10 and11.

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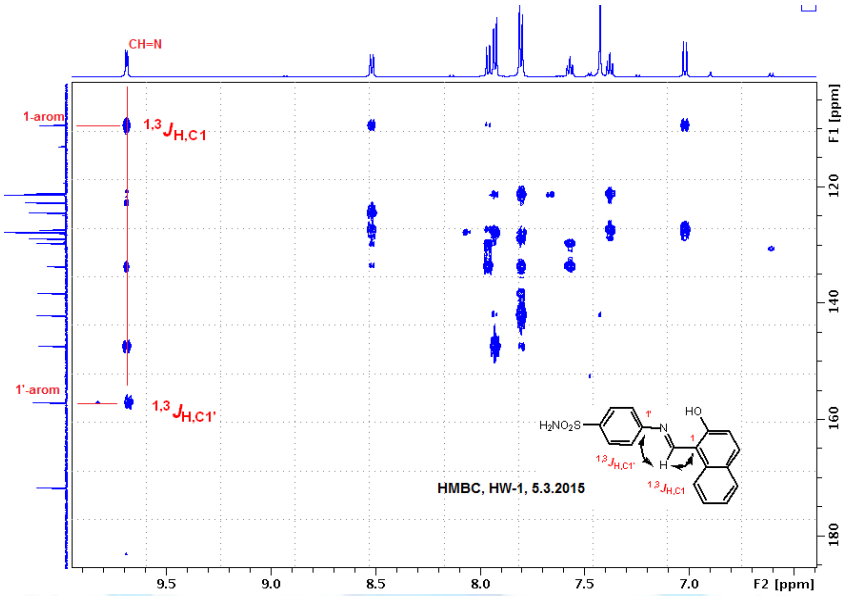

Fig. 5: HMBC- NMR spectroscopy of compound **5**

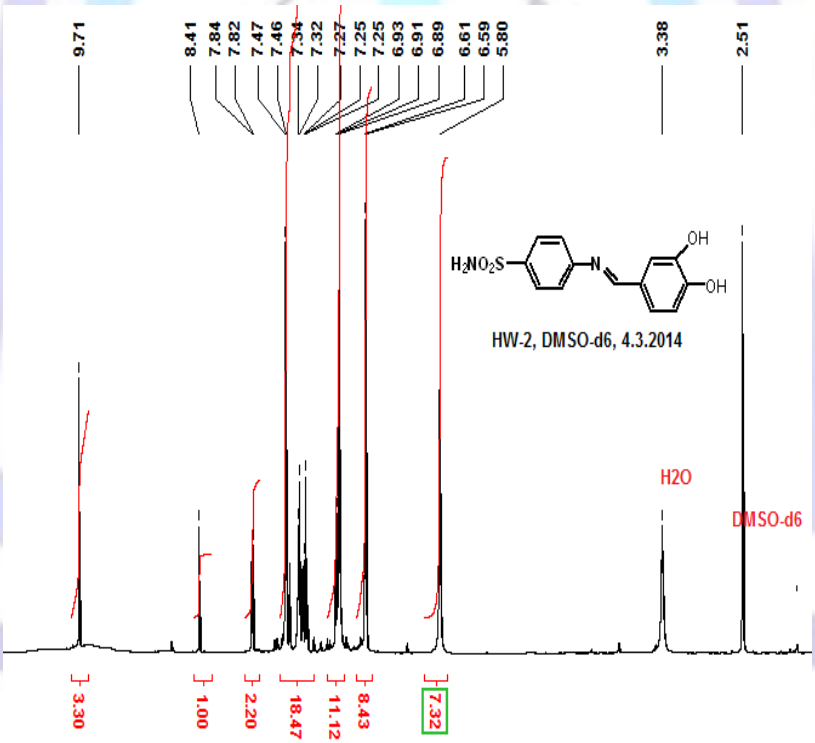

Fig. 6: ^{1}H NMR spectroscopy of compound **6**

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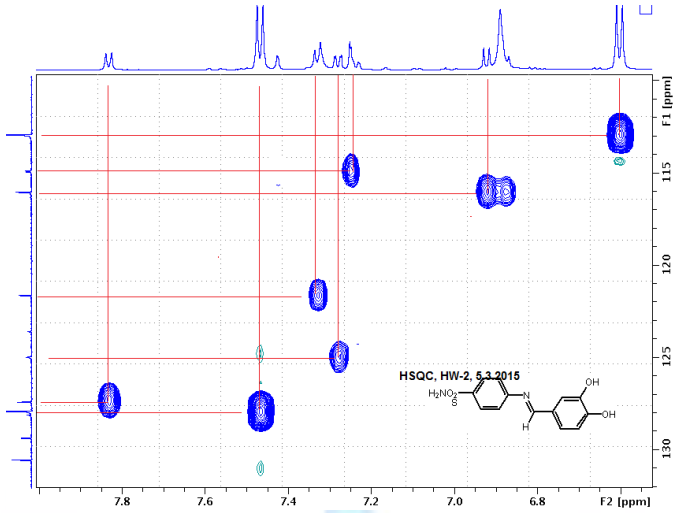

Fig. 7: HSQC- NMR spectroscopy of compound **2**

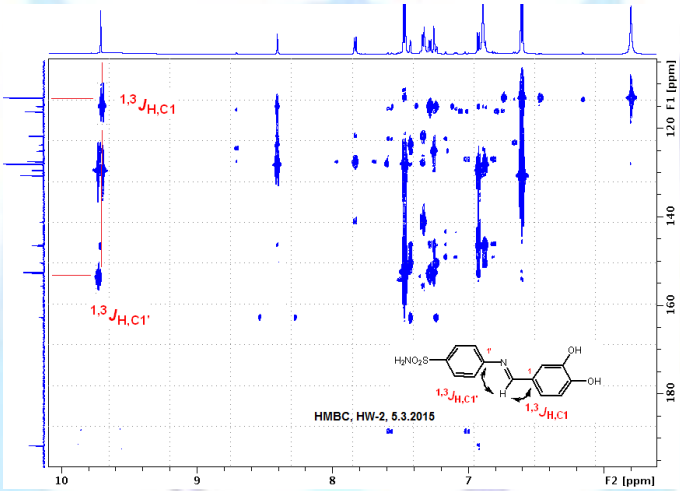

Fig. 8: HMBC- NMR spectroscopy of compound **2**

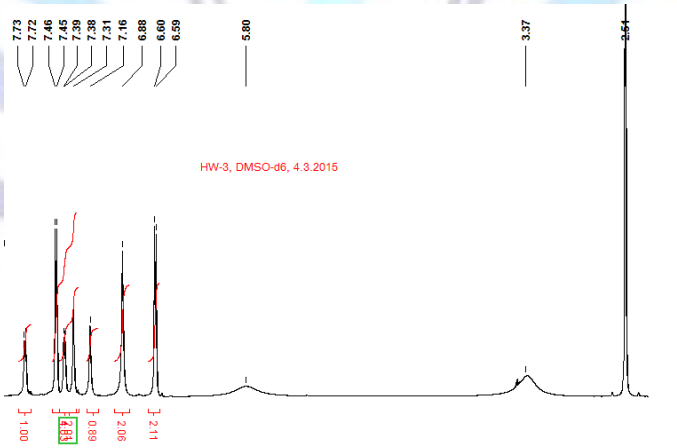

Fig. 9: ^{1}H NMR spectroscopy of compound **3**

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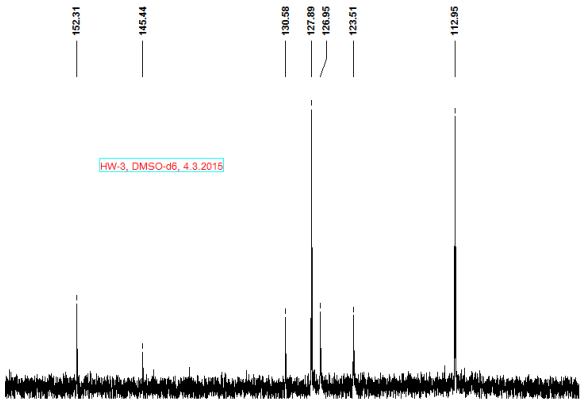

Fig. 10: ^{13}C NMR spectroscopy of compound **3**

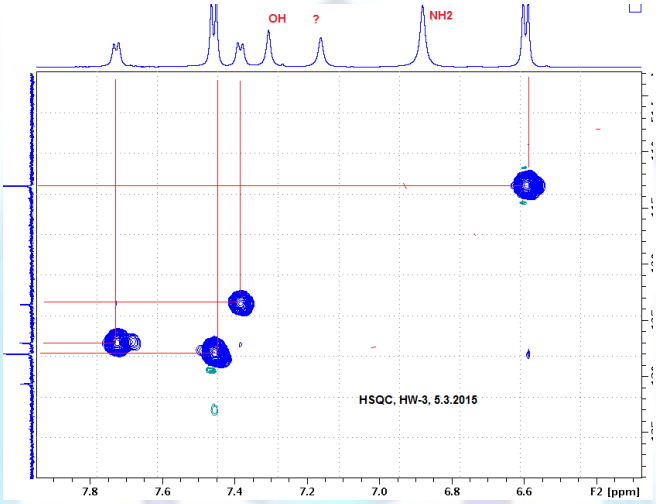

Fig. 11: HSQC- NMR spectroscopy of compound **3**

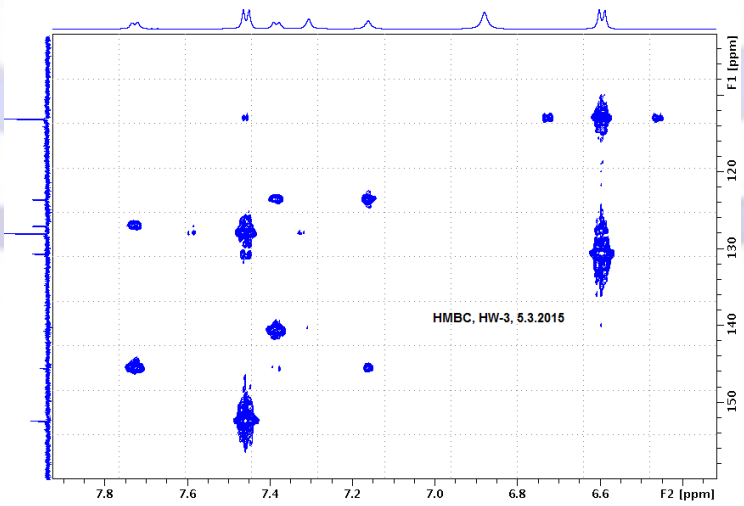

Fig. 12: HMBC- NMR spectroscopy of compound **3**

Antimicrobial activity

The studied compounds have been evaluated *in vitro* for their antibacterial and antifungal activities, using the paper disc-agar diffusion technique [12] by measuring the inhibition zone in mm. Antibiotic drug ampicillin and Nystatin were used as control for bacteria and fungi, respectively. The antibacterial activity of the synthesized compounds were tested against three Gram positive bacteria (*Staphylococcus aureus*,*Streptococcus sp*., *Bacillus subtillus)* and two Gram negative bacteria (*Escherichia coli* and *Klebsiella pneumonia*) at a concentration of 50, 100 and 200µg/ml using DMSO as a

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solvent, which not effected in the growth of microbes. Mueller Hinton agar and Sabouraud dextrose agar were used as culture media for antibacterial and antifungal activity respectively. The results of the antimicrobial activity are shown in Table (1).

The screening results indicate that the activity of compounds increases with an increase in the concentration of the solutions. The synthesized compound **1** show high activity against *E.coli* and *Streptococcus spp* additionally, the compound **1** also exhibit high biological activity against all tested fungi. Whereas the compound **2** show relatively

a good activity against *E.coli* and all fungi. On the other hand, the compound **3** also show a good biological activity against *E.coli* and high antifungal activity against the *Candida tropicalis,* and *Aspergillus niger,* Table 1.

Concerning to these findings, the possible explanations of our results attribute to the fact that different antibiotics, chemical compounds and drugs have different modes of action, owing to the nature of their structure and degree of attraction to certain objective sites within bacterial and fungi cells walls and membranes.

Table 1: Microbial activities of the Schiff-base derivatives of sulphonamide drug

Diameter of inhibition zone in mm for different microbial species

Microorganism	Compound 1			Compound 2			Compound 3			Standard	
	200 µg/ml	100 µg/ml	50 µg/ml	200 µg/ml	100 µg/ml	50 µg/ml	200 µg/ml	100 µg/ml	50 µg/ml	Ampicillin 25 µg/ml	Nystatin 25 µg/ml
Staphylococcus aureus	18	7	-	12	8	3	15	10	7	45	-
Bacillus subtillus	15	11	8	15	-	-	-	-	-	30	-
Streptococcus spp.	18	13	11	15	14	8	20	18	13	20	-
Escherichia coli	22	18	15	25	19	14	25	23	20	20	-
Klebsiella pneumonia	11	9	-	15	11	7	11	10	8	15	-
Aspergillus niger	-	-	-	15	10	4	25	23	18	-	15
Aspergillus fumigatus	18	15	11	25	15	7	-	-	-	-	13
Candida albicans	20	18	15	22	14	10	-	-	-	-	15
Candida tropicalis	13	9	-	20	18	15	21	19	16	-	11

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