ORIGINAL ARTICLE

Antimicrobial evaluation of some new nitrone compounds derived from glyoxal

Husam Hamza Salman[1], Munther Abduljaleel Mohammad-Ali[1], Majed Al-Safe[2]

[1]Department of Pharmaceutical Chemistry, College of Pharmacy, University of Basrah, Iraq, [2]Department of Chemistry, College of Science, University of Thi-Qar, Thi-Qar, Iraq

Abstract

Objective: The aim of this work includes the synthesis of nitrone compounds derived from glyoxal by a condensation reaction with substituted arylhydroxylamines and evaluation of their antimicrobial efficacy. **Materials and Methods:** The present work concerned the synthesis of arylhydroxylamine derivatives and subsequently reacted with glyoxal (40%) to synthesize the nitrone compounds. **Results:** The synthesized nitrones in our study, their structures identified with Fourier-transform infrared and ^{1}H-Nuclear magnetic resonance spectroscopies in addition to elemental analysis (C.H.N.). The results support the structures of nitrone compounds. **Conclusion:** Synthesized nitrones obtained in high purity and an excellent yield. The synthesized nitrones investigated for evaluation of their antimicrobial efficacy against Gram-positive (*Staphylococcus aureus*, ATCC 25923) and Gram-negative (*Escherichia coli*, ATCC 25922) bacteria and fungus (*Aspergillus niger and Aspergillus flavus*). The study proved that the synthesized nitrones exhibited significant antimicrobial activity.

Key words: Antimicrobial activity, condensation reactions, glyoxal, nitrones

INTRODUCTION

Nitrone compounds are dipolar (1,3-dipole) and contain an azomethine ($C=N^+-O^-$) group. Nitrones are intermediary compounds of several products of biological importance.[1] Nitrones are interesting intermediates that are used in organic synthesis.[2-4] These compounds used earliest in the trapping of free radicals in biological systems and chemical systems.[1,5-7] Nitrone compounds are useful intermediates in a synthesis of a variety of compounds which contain nitrogen, which applied as pharmaceuticals and as agro-chemicals.[6] Numerous nitrones found as an essential part in structures of important drugs.[7] They have interesting biological activities as anti-inflammatory, antimicrobial, anticonvulsant, and anti-tubercular.[8] Due to antimicrobial studies of nitrones are poor, the aim of our work is the synthesis of nitrone compounds derived from glyoxal by the condensation reaction of N-substituted-arylhydroxylamines with glyoxal and evaluation of their antibacterial (*Escherichia coli* and *Staphylococcus aureus*) and antifungal (*Aspergillus niger* and *Aspergillus flavus*) efficiency. The results of activity compared with standard drugs such as amoxicillin and fluconazole.

MATERIALS AND METHODS

Materials and Instruments

Nitrobenzene, m-nitrotoluene, glyoxal (40%), p-bromonitrobenzene, and p-chloronitrobenzene are purchased from Sigma-Aldrich Company. Ammonium chloride was obtained from BDH Company. All solvents and zinc dust obtained from commercial sources. The melting points of synthesized nitrones recorded using a Gallenkamp Melting Point Apparatus. Fourier transform infrared (FT-IR) spectra recorded using FT-IR 8400S SHIMADZU device as KBr disks at the Department of Pharmaceutical Chemistry, College of Pharmacy, Basrah University. Elemental (CHN) analysis of the nitrones carried out in Jordon/central laboratories/Al-Albayt University. ^{1}H-Nuclear magnetic resonance spectra of nitrones rrecorded by using a device Bruker model ultra-shield 500 MHz (Switzerland), at Tehran University, Iran.

Address for correspondence:
Husam Hamza Salman, Department of Pharmaceutical Chemistry, College of Pharmacy, University of Basrah, Iraq. E-mail: husamhamza1971@gmail.com

Received: 23-02-2019
Revised: 24-03-2019
Accepted: 01-04-2019

Methods

Synthesis of N-substituted arylhydroxylamines 1a and 1b

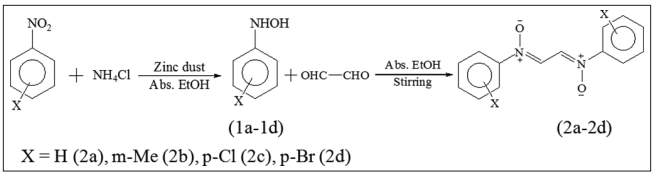
Nitrobenzene or m-nitrotoluene (0.41 mol) was suspended in a solution of NH_4Cl (0.047 mol) and 80 ml of H_2O. With stirring, zinc dust (6.2 g) was added portion wise for 20 min. The stirring continued for additional 20 min. The precipitate zinc oxide filtered off and the filtrate saturated with NaCl. With cooling, in an ice bath salt, the precipitated hydroxylamine [Table 1] filtered by section and purified from toluene and petroleum ether.[9]

Synthesis of N-substituted arylhydroxylamines 1c and 1d

The previous procedure was modified using 70% ethanol instead of H_2O alone [Scheme-1]. In a conical flask (100 ml), 70% EtOH (40 ml), ammonium chloride (0.023 mol), and appropriate halonitrobenzene (0.02 mol) were mixed. With stirring at 10°C, zinc dust (3.1 g) was added portion wise for 15 min. After completion of the addition, the mixture stirred at room temperature (additional 20 min). The mixture filtered off and the filtrate saturated with sodium chloride. The precipitated hydroxylamine [Table 1] filtered and recrystallized from toluene and petroleum ether.[9]

Synthesis of nitrones 2a-2d

In a round bottom flask (50 ml), 0.02 mol of glyoxal (40%) dissolved in absolute ethanol (10 ml). The hot solution of hydroxylamine (1a-1d) (0.04 mol) in 10 ml of absolute EtOH was added portion wise. The mixture was stirred overnight. The desired product [Scheme-1] filtered off and recrystallized from ethanol.[10] Some information about synthesized nitrones are listed in Table 2.



Scheme 1: Synthetic routs of nitrone compounds

ANTIMICROBIAL ACTIVITY

Antibacterial Activity Assay

The disc diffusion method was performed for the testing *in vitro* antibacterial (*S. aureus* ATCC 25923 and *E. coli*, ATCC 25922), activity. Stock solutions of nitrones (1000 µg/ml) with dimethyl sulfoxide (DMSO) as a solvent were prepared. A loop full of the tested strains was grafted into 15 mL of nutrient broth and protected for 24 h in incubator at 37°C to activate the bacterial strain. The 100 mm Petri dish was filled with 20–30 ml of Mueller-Hinton Agar. The Petri dish was prepared in sterile conditions. Steel allowed being dryness and using to assay antibacterial activity. Diameter of Whatman No. 4 (6 mm), impregnated with the solutions of the investigated compounds. The disc then introduced on the surface of the medium using bacteria. Petri dishes incubated at 37°C for 24 h. The antibacterial efficacy was determined by measuring the diameter zone of inhibition in mm. The minimum inhibitory concentration of the prepared nitrones was performed by the serial dilution of the compounds at concentrations ranging from 100 to 750 µg/ml.[1,11-14]

Antifungal Activity

In the antifungal activity, isolated pathogenic strains, *A. niger* and *A. flavus*, are used. The Sabouraud dextrose agar was used. Stock solutions of nitrones (1000 µg/ml) with DMSO as a solvent were prepared. The plates incubated for 3 days.

Table 1: Physical properties of synthesized arylhydroxylamines (1a-1d)

Compound	X	M.p. (°C)	Crystal shape	Yield %
1a	H	81–82	Light yellow needle	68
1b	m-Me	69–71	Pale yellow sheet	71
1c	p-Cl	97–99	White sheet	65
1d	p-Br	89–91	White sheet	60

Table 2: Some information of synthesized nitrones

Compound	X	Name	Mol. formula	Color	M. p. (°C)	Yield (%)
2a	H	*N, N*^{2}-diphenylethane-1,2-diimine N-oxide	$C_{14}H_{12}N_2O_2$	Yellow	181–183	95
2b	m-Me	*N*^{1}, *N*^{2}-bis (3-methylphenyl) ethane-1,2-diimine *N*-oxide	$C_{16}H_{16}N_2O_2$	Yellow	188–190	91
2c	p-Cl	*N*^{1}, *N*^{2}-bis (4-chlorophenyl) ethane-1,2-diimine *N*-oxide	$C_{14}H_{10}Cl_2N_2O_2$	Yellow	212–214	76
2d	p-Br	*N*^{1}, *N*^{2}-bis (4-bromophenyl) ethane-1,2-diimine *N*-oxide	$C_{14}H_{10}Br_2N_2O_2$	Yellow	223–225	80

Then, the inhibition zones formed were measured with in millimeters.[1,12-14]

RESULTS AND DISCUSSION

From Table 3, data for CHN analysis of synthesized nitrones (2a-2d) support the structures of compounds 2a-2d.

Infrared Spectra

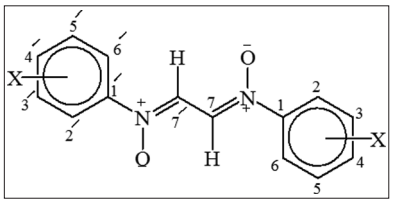
The data of FT-IR spectra of prepared nitrones [Figures 1-4] showed medium absorption bands at 1608–1623 cm^{-1} which attributed to the stretching frequency of the azomethine C=N bond which confirms the founding of ($C=N^{+}O^{-}$) group. In all spectra of the synthesized compounds, the stretching and bending (out of plane) frequencies for aromatic C-H bond appeared at ranges 3055–3088 cm^{-1} and 811–837 cm^{-1}, respectively. The vibrations of aromatic C=C group in the infrared spectra of prepared nitrones appeared as strong bands at 1450–1481 cm^{-1} and 1520–1564 cm^{-1}.[9,10,12] The other vibrations are listed in Table 4.

Table 3: Data of CHN analysis of nitrones

Compound	Calculated (found) %		
	C	H	N
2a	69.99 (69.64)	5.03 (5.23)	11.66 (11.89)
2b	71.62 (71.83)	6.01 (5.89)	10.44 (10.30)
2c	54.39 (54.57)	3.26 (3.37)	9.06 (8.91)
2d	42.23 (41.94)	2.53 (2.61)	7.04 (7.11)

^{1}H-NMR Spectra

The spectra of ^{1}H-NMR [Figures 5-8] of prepared nitrones in our study exhibited a singlet signals for



Scheme 2: Structure of synthesized nitrones for explanation ^{1}H-NMR

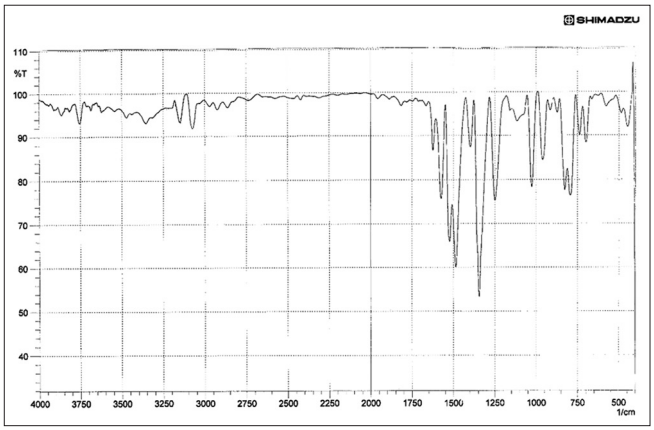


Figure 1: Fourier transform infrared spectrum of nitrone compound 2a

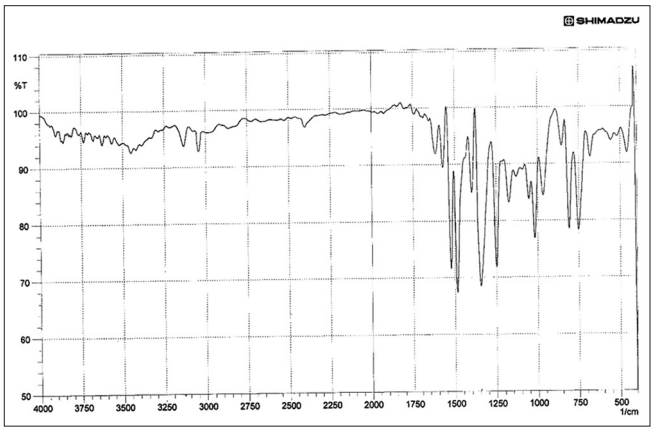


Figure 3: Fourier transform infrared spectrum of nitrone compound 2c

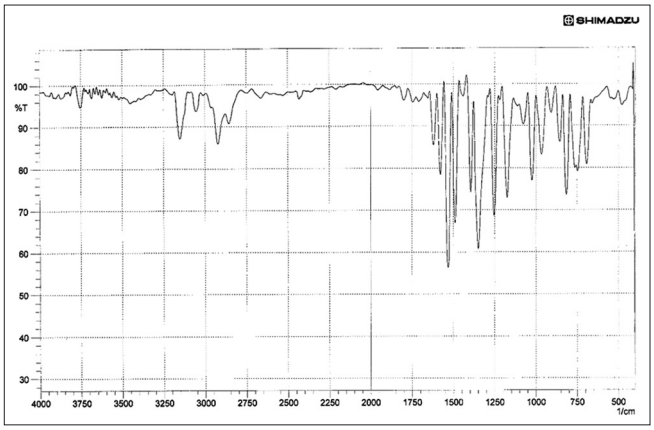


Figure 2: Fourier transform infrared spectrum of nitrone compound 2b

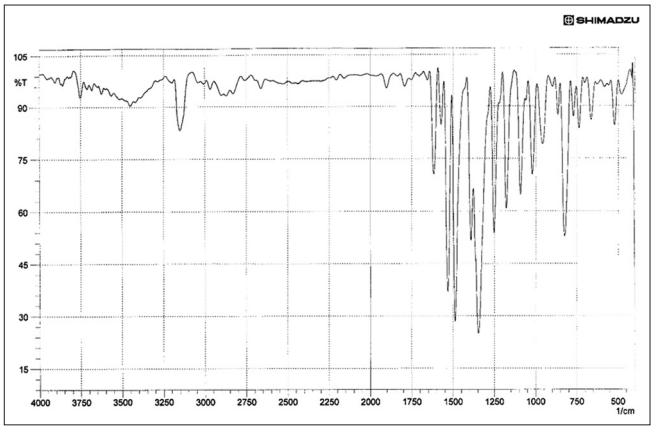


Figure 4: Fourier transform infrared spectrum of nitrone compound 2d

the protons (CH=N^{+}-O^{-}) H-7 and H-7' in the region (8.649–8.8.709 ppm).[9] In case of the ^{1}H-NMR spectrum of nitrone 2b, a singlet signal appeared at 2.407 ppm, which attributed to two methyl groups protons. Protons located ortho to azomethine-N-oxide group for nitrones 2c and 2d appeared as doublet signals at 8.143 ppm and 8.038 ppm, respectively, with ^{3}J= 9 Hz. In addition, the spectra of nitrones 2c and 2d showed doublet signals at 8.282 ppm and 8.182 ppm which attributed for protons ortho to halogen substituents.[9,10,13] The other signals for the aromatic rings are listed in Table 5 [Scheme-2].

Antimicrobial Activity

Nitrones 2a-2d were investigated for their *in vitro* antimicrobial activity against bacteria pathogens *E. col* and *S. aureus* [Figure 9]. The antibacterial activity of [Tables 6 and 7] agents is dependent largely on the morphology of the cell wall of the bacteria, which is the step key in the penetration mechanism action. In addition to that, the activity depends on the lipophilicity of compounds. Depending on the lipophilicity, found that antibacterial potency of synthesized compounds,

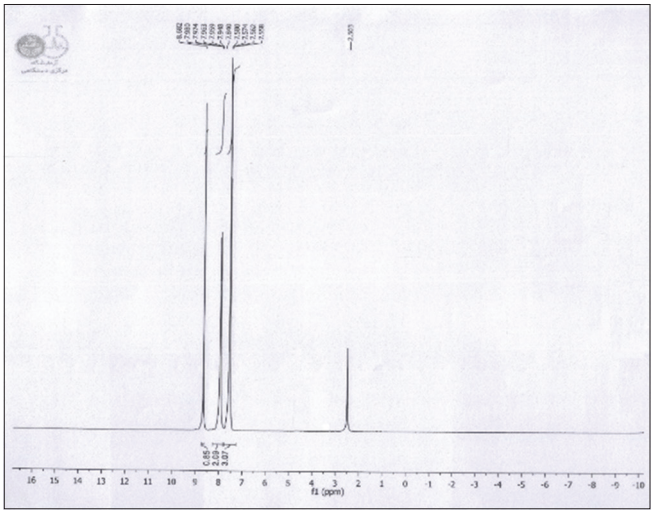


Figure 5: ^{1}H-Nuclear magnetic resonance spectrum of 2a

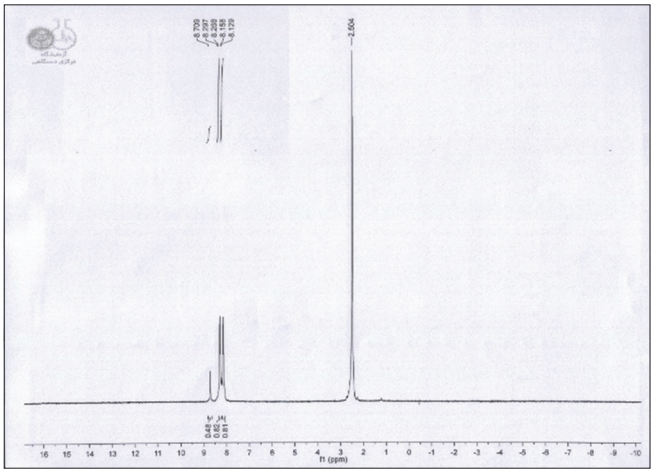


Figure 7: ^{1}H-Nuclear magnetic resonance spectrum of 2c

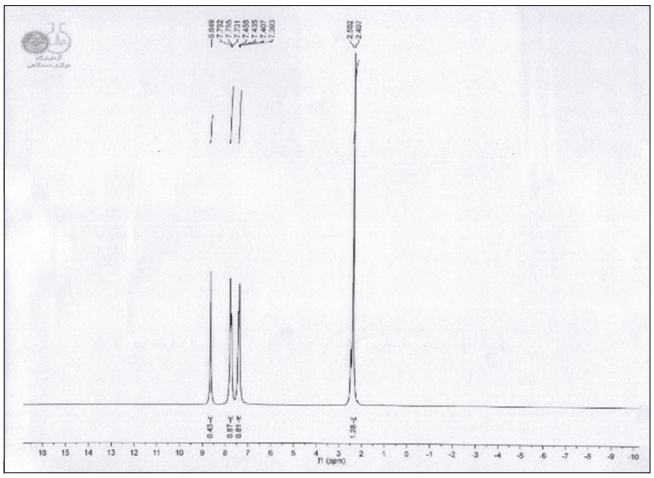


Figure 6: ^{1}H-Nuclear magnetic resonance spectrum of 2b

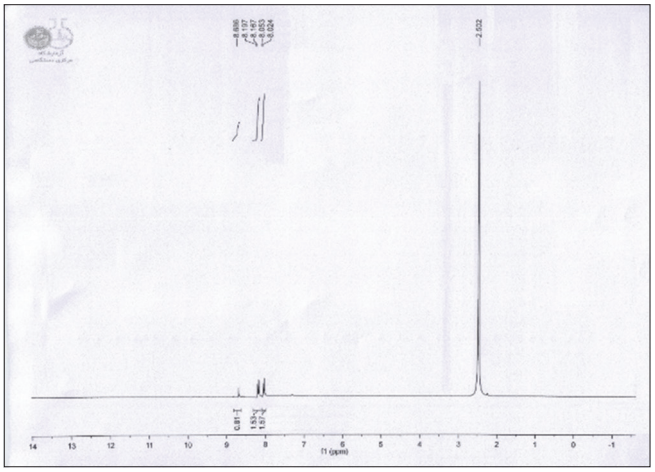


Figure 8: ^{1}H-Nuclear magnetic resonance spectrum of 2d

Table 4: FT-IR information (cm^{-1}) nitrones

Compound	C=N	N-O	C-N	C=C	Aromatic C-H		Aliphatic C-H
					Str.	o. o. p.	
2a	1623	1069	1192	1450 1541	3080	830	------
2b	1618	1077	1185	1457 1564	3067	826	2823 2930
2c	1608	1072	1172	1479 1528	3048	811	------
2d	1611	1072	1180	1481 1520	3110	837	------

o. o. p.= out of plan, FT-IR: Fourier transform infrared

Table 5: Data ^{1}H-NMR for nitrones (2a-2d) in DMSO-d6

Nitrone	X	^{1}H-NMR, δ (ppm), ^{n}J H-H (Hz)		
		(2H,7, 7')	C-H (aliphatic)	C-H (aromatic)
2a	H	8.682	-----	7.558–7.980 (10H,2,3,4,5,6,2',3',4',5',6')
2b	m-Me	8.649	2.407 (6H)	7.393–7.792 (8H,2,3,4,6,2',3',4',6')
2c	p-Cl	8.709	-----	8.143 (4H,2,6,2',6',^{3}J=8.7) 8.282 (4H,3,5,3',5',3J=8.7)
2d	p-Br	8.686	-----	8.038 (4H,2,6,2',6',^{3}J=8.7) 8.182 (4H,3,5,3',5',^{3}J=9)

NMR: Nuclear magnetic resonance

Table 6: *In vitro* antimicrobial efficiency of investigating nitrones (1000 μg/ml)

Microorganism	Diameter of inhibition zone (mm)					
	2a	2b	2c	2d	Amoxicillin	Fluconazole
Escherichia coli	16	18	18	NI	38	-----
Staphylococcus aureus	18	25	18	16	39	-----
Aspergillus niger	17	12	10	15	-----	18
Aspergillus flavus	12	13	9	8	-----	15

Table 7: MIC (μg/ml) of the nitrones in mm

Compound	*Staphylococcus aureus*				*Escherichia coli*			
	750	500	250	100	750	500	250	100
2a	17	13	11	8	14	12	9	NI
2b	23	19	14	11	15	11	8	NI
2c	15	9	7	NI	12	9	9	NI
2d	14	10	11	8	NI	NI	NI	NI

NI: No inhibition, MIC: Minimum inhibitory concentration

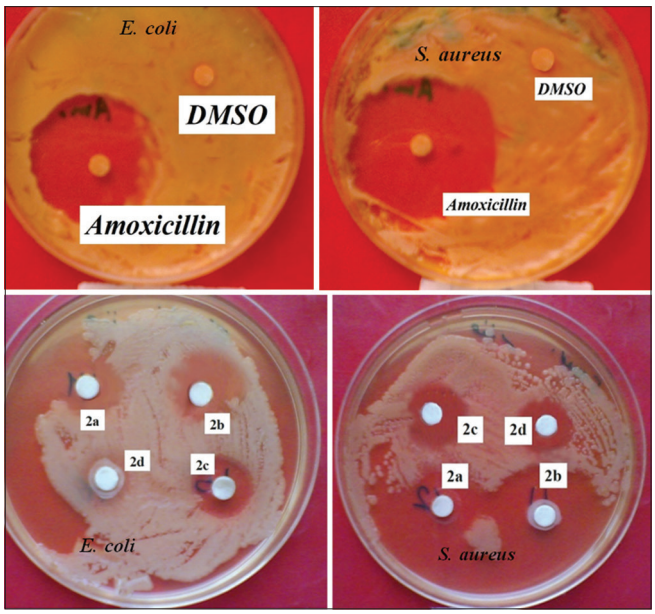


Figure 9: Antibacterial activity of nitrones against *Escherichia coli* and *Staphylococcus aureus* bacteria

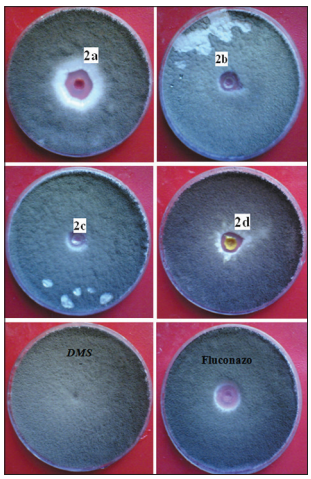


Figure 10: Antifungal activity of nitrones against *Aspergillus niger* fungi

generally, is higher against Gram-positive bacteria than Gram-positive bacteria. In general, the results of *in vitro* antimicrobial properties of investigating nitrones as in Table 6 appeared a

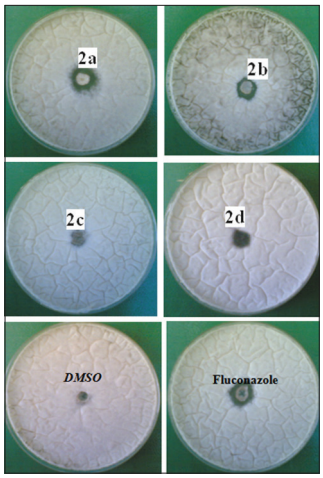


Figure 11: Antifungal activity of nitrones against *Aspergillus flavus* fungi

significant activity against fungi and bacteria. The antifungal activity [Figures 10 and 11] of synthesized nitrones (2a-2d) as shown in Table 6 showed good activity against *A. niger* and *A. flavus* compared with the fluconazole as a standard drug. In the case of antibacterial efficiency, compound 2b showed good activity compared with the other nitrones. In the case of compound 2a, the antifungal activity appeared well compared to the other compounds. In general, all nitrones showed high antifungal activity compared with the antibacterial activity.[14]

CONCLUSION

In our report, nitrones were prepared from condensation of different hydroxylamines with glyoxal. The prepared nitrones identified by element analysis (C. H. N.) as well as FT-IR and ^{1}H-NMR spectroscopies. The results supported structures of prepared nitrones. All compounds exhibited significant *in vitro* antibacterial and antifungal activities. Compound 2b exhibited good antibacterial activity compared with another prepared nitrone. Compounds 2a showed good antifungal activity.

ACKNOWLEDGMENTS

The authors are grateful to the Department of Laboratories Clinical Science, College of Pharmacy, University of Basrah, Iraq, where the standard organisms were obtained.

REFERENCES

1. Atika M, Bouras N. Antimicrobial and antioxidant activities of a series of aromatic nitrones. Algerian J Arid Environ 2018;7:27-36.
2. Mallesha H, Kumar KR, Kumar BK, Mantelingu K, Rangappa KS. Histidine as a catalyst in organic synthesis: A facile *in situ* synthesis of α, N-diarylnitrones. Proc Indian Acad Sci Chem Sci 2001;113:291-6.
3. Maiuolo L, De Nino A, Merino P, Russo B, Stabile G, Nardi M, *et al*. Rapid, efficient and solvent free microwave mediated synthesis of aldo and ketonitrones. Arab J Chem 2016;9:25-31.
4. Bouzid M, Abdennabi R, Damak M, Kammoun M. Synthesis and evaluation of antimicrobial activity of nitrones from derivatives of aryl-substituted dihydroisoquinoline. J Chem 2015;2015:1-6.
5. Goldstein S, Lestage P. Chemical and pharmacological aspects of heteroaryl-nitrones. Curr Med Chem 2000;7:1255-67.
6. Mekheimer RA, Al-Zaydi KM, Al-Shamary A, Sadek KU. Solar thermochemical reactions IV: Unusual reaction of nitrones with acetonitrile derivatives induced by solar thermal energy. Green Sustain Chem 2011;1:176-81.
7. Chen S, Zhao K, Chen G. Synthesis and application of phenyl nitrone derivatives as acidic and microbial corrosion inhibitors. J Chem 2015;2015:1-6.
8. Petkes HI, Gál E, Gaina L, Sabou M, Majdik C, Silaghi-Dumitrescu L. Synthesis and antibacterial properties of new phenothiazinyl-and phenyl-nitrones. Comptes Rendus Chim 2014;17:1050-6.
9. Kriza A, Dianu ML, Stănicã N, Drãghici C, Popoiu M. Synthesis and characterization of some tranzition metals complexes with glyoxal bis-isonicotinoyl hydrazone. Rev Chim 2009;60:555-60.
10. Zitouni CM. Synthesis and microbiological activities of novel acyclic nitronese. Asian J Chem 2016;28:1027-30.
11. Vairalakshmi M, Princessc R, Ranid BK, Rajae SJ. Synthesis, structural elucidation, catalytic, antibacterial and antioxidant activity of thiophene derived mixed ligand metal complexes. J Chil Chem Soc 2018;63: 3844-9.
12. Kumutha R, Krishnan SS, Kumar MT. Computational studies on 2(2-Hydroxyphenyl)-N-phenyl Nitrone, spectroscopic investigation. Orient J Chem 2014;30:1905-12.
13. Taqa AA. Some complexes of N-aryl furfural nitrones with Co (II), Ni (II), Cu (II), Zn (II) and Cd (II) chlorides. Int J Adv Chem 2016;4:10.
14. Ejiah FN, Fasina TM, Familoni OB, Ogunsola FT. Substituent effect on spectral and antimicrobial activity of schiff bases derived from aminobenzoic acids. Adv Biol Chem 2013;3:475-9.

Source of Support: Nil. **Conflict of Interest:** None declared.