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Synthesis and Antimicrobial Evaluation of Some Isoxazolidine Derivatives

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Abstract:

New isoxazolidines were synthesized in a good to an excellent yields by 1,3-dipolar cycloaddition of *N*-phenylmaleimide dipolarphile and nitrones. The structures of the synthesized compounds were confirmed by elemental analysis (C. H. N.), IR and ^{1}H-NMR spectroscopy. The compounds were screened for their *in-vitro* antibacterial activity against *Staphylococcus aureus*, *Esherichia coli* and for their antifungal activity, *Aspergillus niger Aspergillus flavus*. The zone of inhibition was determined by disc diffusion technique. All the synthesized compounds exhibited promising antimicrobial activity against the studied set of microorganisms compared with standard drugs used in this study.

Keywords: Isoxazolidine, Dipolar cycloaddition, Antibacterial activity and Antifungal.

Introduction

The 1,3-dipolar cycloaddition of nitrones and alkenes is a powerful synthetic device that allows up to three new stereogenic centers to be assembled in a stereospecific manner in a single step[1,2]. Among these N and O containing five-membered heterocycles, isoxazolidines and isoxazoline derivatives[3,4] have emerged as important candidates and have been shown to display useful anticancer, antiviral and antibiotic properties[5]. The syntheses of isoxazolidine derivatives are an important subject in organic chemistry because they are found in the structure of most natural compounds and drugs. In recent years, isoxazolidine derivatives have been synthesized in high yield *via* intermolecular cycloaddition of substituted *N*-phenyl-α-phenyl nitrone with olefins and are employed for biological evaluation [6]. These isoxazolidines are used in the syntheses of *β*-lactams which are of value in the treatment of bacterial infections, occur as natural products, serve as versatile synthetic intermediates, and are biologically interesting compounds [7,8].

Materials and Methods:

Experimental chemical part

All chemicals were obtained from commercial sources and purified by distillation or recrystallization before use. All melting points were determined in open capillary tubes using Electrothermal (GallenKamp) apparatus were uncorrected. All the reaction were routinely monitored and purity was determind on thin layer chromatography using coated aluminum plates and spots were visualized by exposing the dry plates

in iodine vapours. IR spectroscopy analyses were recorded on FT-IR 8400S SHIMADZU(Japan) as KBr disk in Petrochemical industry company. ^{1}H-NMR spectra were recorded using Bruker model ultra shield 300MHz (Switzerland) by using DMSO as a solvent and tetra-methylsilane, TMS, as an internal standard in central laboratories in Al-Albayet University/ Jordan. CHN analysis were recorded using EuroVector model EA3000A (Italy) in central laboratories in Al-Albayet University/Jordan.

General procedure preparation of Nitrones. A1-A3 [6]

The starting N-arylhydroxylamines (**1**) were prepared according to the reported procedure[9].To a well stirred solution of an appropriate N-arylydroxylamine (0.1 mole) in benzene (20 ml)) taken in a 100 ml round bottom flask was added an appropriate aldehyde (0.1 mole) and was stirred at room temperature with a magnetic stirrer for 24 hr. After that, excess of the solvent was removed in a rotary evaporator and cooled the solid mass that separated out was filtered and recrystallized from absolute ethanol. The characteristics of nitrones were indicated in Table1.

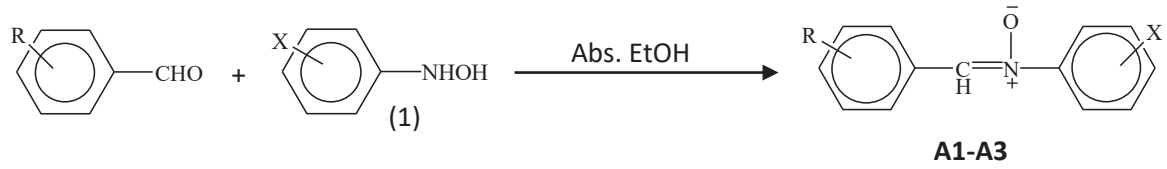


Table (1): physical properties of nitrones

Compound	R	X	M. p. °C	Molecular formula	Name	Yield (%)
A1	*o*-Cl	*m*-Me	110-112	$C_{14}H_{12}ClNO$	α-(*o*-chlorophenyl)-N-(*m*-methylphenyl) Nitrone	87%
A2	*o*-OH	H	145-147	$C_{13}H_{11}NO2$	α -(*o*-hydroxyphenyl)-N-phenyl Nitrone	93%
A3	*p*-F	*m*-Me	169-171	$C_{14}H_{12}FNO$	α -(*p*-chlorophenyl)-N-(*m*-methylphenyl) Nitrone	89%

General preparation of isoxazolidines, B1-B3.

The starting N-phenylmaleimide was prepared according to the procedure [11]. By using 1 equivalent of both N-phenylmaleimide (**2**) and the appropriate nitrone (**A1-A3**) in dry benzene (100ml) was refluxed for 4-7 hrs. The progress of the reaction was monitored by TLC. After refluxing, excess of the solvent was removed and allowing the reaction mixture to cool, the solid is separated out then filtered off and recrystallized several times from toluene and preserved in a desiccator over dried silica gel[10]. The physical properties and names of title compounds are listed in Tables 2 and 3.

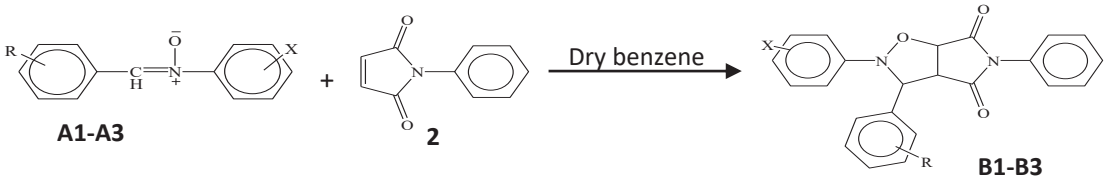


Table (2): Physical properties of synthesized isoxazolidines.

Compound	R	X	M. F.	M. p. °C	yield (%)	R_f value
B1	*o*-Cl	*m*-Me	$C_{24}H_{19}ClN_2O_3$	122-124	72	0.66
B2	*o*-OH	H	$C_{23}H_{18}N_2O_4$	160-162	80	0.71
B3	*p*-F	*m*-Me	$C_{24}H_{19}FN_2O_3$	192-194	70	0.53

Table (3): Symbols and names of isoxazolidines.

Compound	R	X	Name of compound
B1	*o*-Cl	*m*-Me	3-(2-Chloro-phenyl)-5-phenyl-2-m-tolyl-tetrahydro-pyrrolo[3,4-d]isoxazole-4,6-dione
B2	*o*-OH	H	3-(2-Hydroxy-phenyl)-2,5-diphenyl-tetrahydro-pyrrolo[3,4-d]isoxazole-4,6-dione
B3	*p*-F	*m*-Me	3-(4-Fluoro-phenyl)-5-phenyl-2-m-tolyl-tetrahydro-pyrrolo[3,4-d]isoxazole-4,6-dione

Experimental biological part

The synthesized Schiff bases were screened for antibacterial and antifungal activity.

Antibacterial Testing

The *in vitro* antibacterial activity of the isoxazolidines compared with amoxicillin as standard drug, was studied against Gram-positive bacteria, *Staphylococcus aureus* and Gram-negative bacteria, *Escherichia coli* by the standard disc diffusion method. The bacterial cultures were obtained from Department of biology/College of Science/ University of Basrah. Each of the isoxzolidine compounds dissolved in DMSO at a concentration of 1000 µg/ml was prepared. Paper discs of Whatman filter paper No. 1 (6-mm-diameter) were cut and sterilized in an autoclave. The paper discs were saturated with solution of the tested compounds were placed aseptically in the Petri dishes containing Mueller Hinton Agar media inoculated with the above mentioned two bacteria separately. The Petri dishes were incubated at 37 °C and the inhibition zones (in mm) were recorded after 24 hr of incubation [12, 13].

Antifungal testing

The antifungal activity of the synthesized compounds compared with fluconazole, were evaluated against pathogenic strains of *Aspergillus niger, Aspergillus flavus* which were obtained from Department of biology/ College of Science/University of Basrah. Antifungal activity of each compound were evaluated by the agar disc-diffusion method. Sabouraud Dextrose agar is used as growth medium. Sterile, filter paper discs of 6 mm diameter were impregnated with prepared isoxazolidines (1000 µg/ml) and were placed on to the media, seeded with fungus. The plates were then incubated for 72 hrs. At the end of period the inhibition zones formed on media were measured with a zone reader in millimeters[13-15].

Results and discussion

Elemental analysis:

It is clear from the data that the experimental values shown for each of the compound are in good agreement with the theoretical values calculated as shown in Table 4.

Table (4): Elemental analysis of synthesized isoxazolidine

Compound	M. F.	Found			Calculated		
		C %	H %	N %	C %	H %	N %
B1	$C_{24}H_{19}ClN_2O_3$	68.92	4.67	6.74	68.82	4.57	6.69
B2	$C_{23}H_{18}N_2O_4$	71.61	4.79	7.32	71.49	4.70	7.25
B3	$C_{24}H_{19}FN_2O_3$	71.74	4.84	6.98	71.63	4.76	6.96

Infrared spectra:

The FT-IR spectra of the studied compounds as KBr discs were recorded in the range 500-4000 cm^{-1} and the most representative spectra are shown in Table 5. The IR spectra of isoxazolidine compounds (B1-B3) showed the absorption bands related to (N-O), (C-N) and (C-O) stretching in the range between 1097cm^{-1} and 1394 cm^{-1}[16]. The spectra also showed strong absorption bands in the range (1712-1724) cm^{-1} for the (C=O) stretching. General it is clear that the absorption bands (1624 cm^{-1}) which related to the nitrone group (─CH=N→O) is disappear in synthesized compounds and confirm that the reaction was take place the cycloaddition [17]. The other characteristic absorption bands for aromatic rings are summarized in Table 5 the experimental FT-IR spectra are shown in Figures (1-3).

Table (5): FT-IR data of isoxazolidine derivatives

Compoud	C=O (Str.)	O-H Str.	C=C (Str.)	C-O-C (Str.)	C-N C-O N-O (str.)	C-H Aromatic		C-H (str.) Aliphatic
						Str.	Bend.	
B1	1724 vs	--------	1442 s 1492 s	1037 m 1259 m	1097-1391 s	3063 w	850 m	2864 w 2987 w
B2	1712vs	3402	1491 s 1595 s	1029 m 1263 m	1095-1394 s	3066 w	904 m	2975 w 3000 w
B3	1723vs	--------	1455 s 1502 s	1099 m 1263 m	1100-1387 s	3070 w	865 m	2864 w 2995 w

^{1}H-NMR spectra

The ^{1}H-NMR spectra of the isoxazolidines in DMSO shows the following signals given in Table 6. All spectra of synthesized compounds exhibited a significant signals for protons C_3H, C_4H and C_5H at regions (5.503-6.031ppm,$J_{3,4}$=7.8Hz), (4.209-4.277ppm, $J_{4,5}$= 7.2-8.4 Hz) and (5.190-5.493ppm) respectively, these signals confirm the formation of isoxazolidine ring and the reaction of cycloaddition is takes place[18, 19]. The spectra showed the multiple signals for aromatic rings at 6.637- 7.794 ppm. The signal at 10.200 ppm for compound B2 is attributed to the phenolic proton (OH). The other compounds B1 and B3 revealed singlet signal at 2.295 ppm and 2.345 ppm, respectively, is assigned to the methyl group. The ^{1}H-NMR spectrum of isoxazolidne B3 exhibited doublet signal for proton C_3H 5.503 ppm is suggested that protons C_3H and C_4H at *cis*- configuration, while other compounds (B1 and B2) showed that protons C_3H and C_4H are in *trans*-configuration[20, 21]. The experimental ^{1}H-NMR spectra are shown in Figures (4-6).

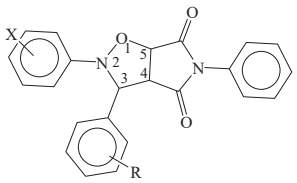
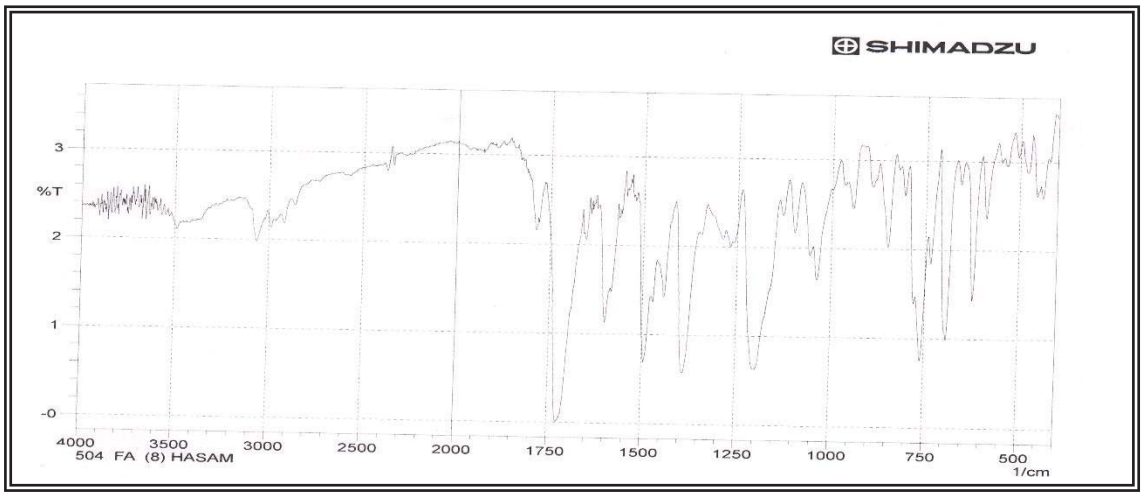
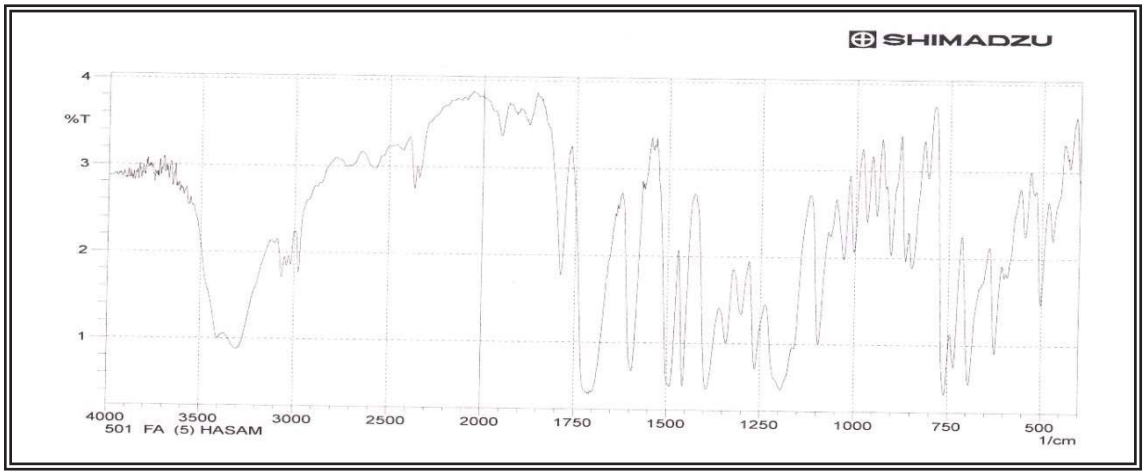


Table (6): Data of ^{1}H-NMR spectra [δ (ppm), J (Hz)] of isoxazolidines

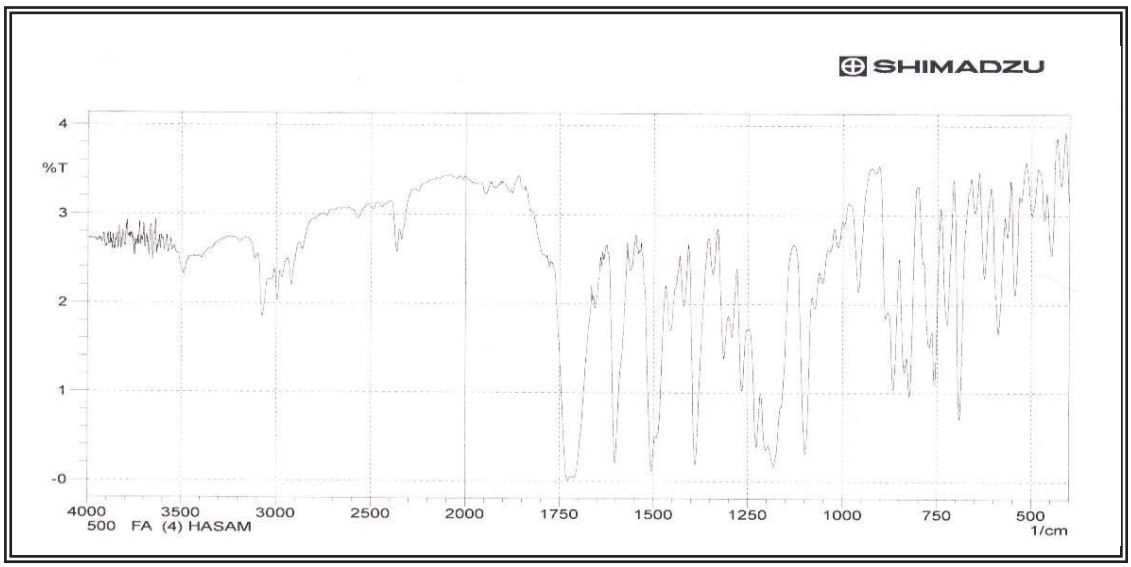
Compound	C_3H	C_4H	C_5H	$J_{3,4}$	$J_{4,5}$	Aromatic C-H	Aliphatic C-H	Phenolic O-H
B1	6.031(s)	4.234 (d)	5.398	----	7.2	6.656-7.794	2.295	--------
B2	6.017 (s)	4.209 (d)	5.493	----	7.5	6.637-7.495	-------	10.200
B3	5.503 (d)	4.277 (t)	5.190	7.8	8.4	6.923-7.597	2.345	-------



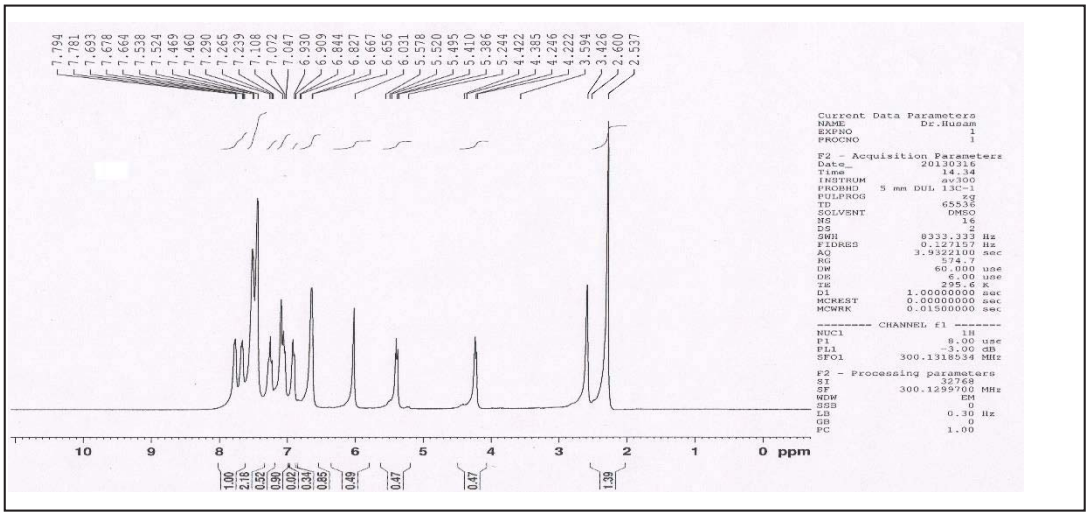
(Figure 1): FT-IR spectrum of compound B1



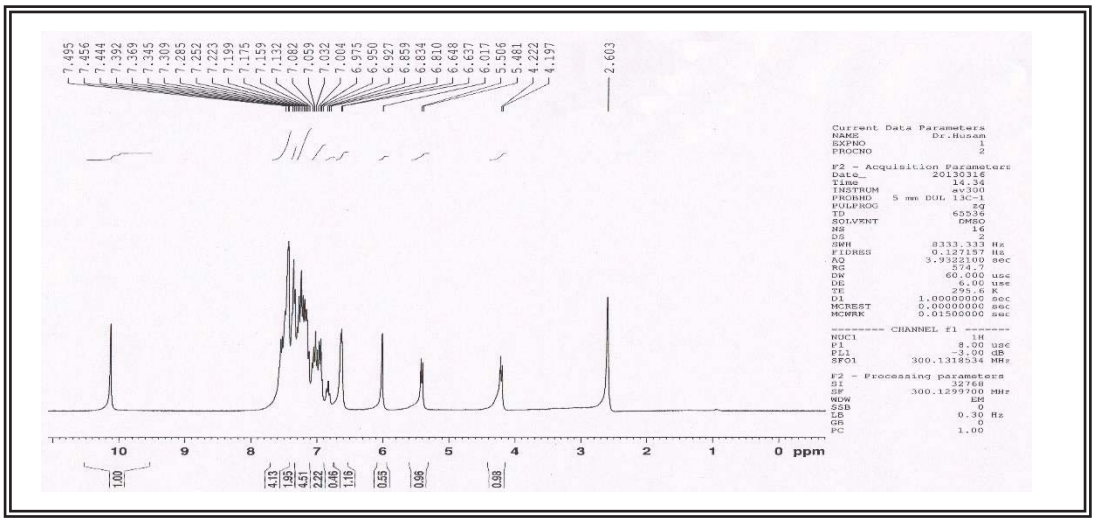
(Figure 2): FT-IR spectrum of compound B2



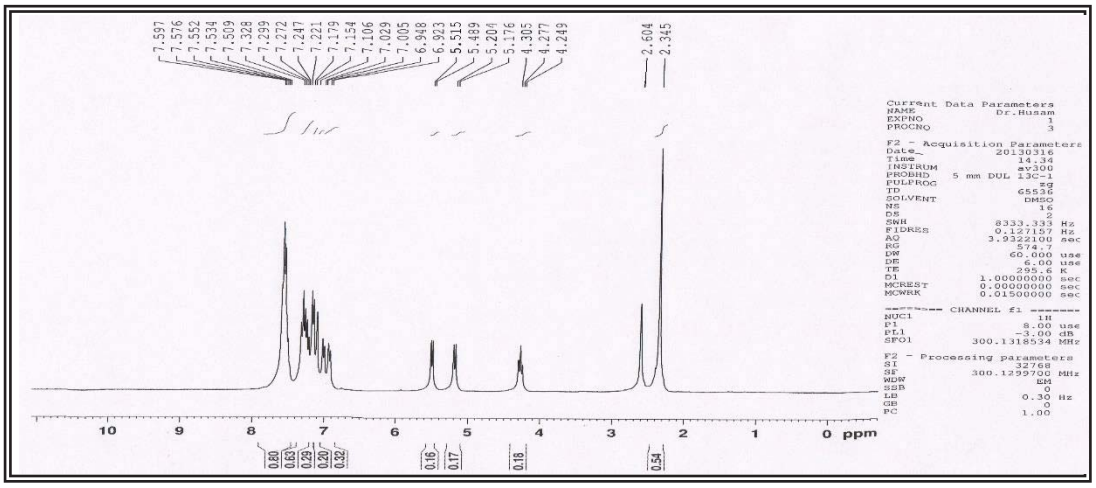
(Figure 3): FT-IR spectrum of compound B3



(Figure 4): ^{1}H-NMR spectrum of compound B1



(Figure 5): ^{1}H-NMR spectrum of compound B2



(Figure 6): ^{1}H-NMR spectrum of compound B3

Antimicrobial activity

Antibacterial activity:

The *in vitro* antibacterial activity of the isoxazolidines have been carried out against the Gram-positive *Staphylococcus aureus* and Gram-negative *Escherischia.Coli* bacteria using disc diffusion method by taking DMSO as solvent. The results of antibacterial actvity summarized in Table 7 . The results revealed that the title compounds exhibited good antibacterial activity against the selected microorganisms as compared with standard antibiotic drug amoxicillin at a concentration of 1000µg/ml. This is probably due the lipophilic nature of the synthesized compounds. Such increased activity can be explained on the basis of Overtone's concept and Tweedy's chelation theory. According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid soluble materials due to which liposolubility is considered to be an important factor that controls the antmicrobial activity[14, 21, 22].

Antifungal activity:

The antifungal activities of the isoxazolidines were tested against pathogenic fungi of *Aspergillus niger* and *Aspergillus flavus* using disc diffusion method. The antifungal activity data (Table 7) indicate that the compounds show an appreciable activity as compared with standard drug fluconazole (*S. nieger*, 18 mm and *A. flavus*, 23 mm). Isoxazolidine B1 shows the highest activity (i.e 12 mm against *A. niger* and 21 mm against *A. flavus*. The other compounds (B2 and B3) obviously show less activity against these fungi than the isoxazolidine B1**.**

Table (7): Antimicrobial activity of synthesized isoxazolidines

Compound Conc. (1000µg/ml)	***In vitro* activity zone of inhibition (mm)**			
	Antibacterial activity		**Antifungal activity**	
	E. coli	***S. aueus***	***A. nieger***	***A. flavus***
B1	20	26	12	21
B2	17	20	9	10
B3	18	21	8	15
Amoxicillin	20	30	NI	NI
Fluconazole	NI	NI	18	23

Conclusion:

This study is included the synthesis of isoxazolidine derivatives and comprises of three stages: firstly synthesis of nitrones, second synthesis of N-phenylmaleimide and third dipolar cycloaddition reactions. The IR- spectra of isoxazolidine compounds (B1-B3), showed strong absorption bands between the range (1712-1724) cm^{-1} for the (C=O) stretching , which means that the cycloaddition of nitrones with olefins

occurred. While the bands attributed to the nitrone group (CH=N→O) disappeared in the spectra of isoxazolidine compounds . All the compounds exhibit parent peaks. The synthesized compounds have good antimicrobial activity as compared with standard drugs.

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