Synthesis and Characterization of New benzimidazole-nitrone Derivatives, and Study of Their Effect as Anti-bacterial.

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Abstract

New benzimidazole derivatives (benzimidazole-nitrone) were synthesized from the condensation of o-phenylene diamine with p-amino benzaldehyde in the presence of (P-TsOH\EtOH). Then the benzimidazole compounds that containing an imine group were oxidized to obtain a nitrone group by used peracetic acid and identified by FT-IR, H^1-NMR spectra, and elemental analysis. All compounds applied to the *Escherichia coli* and *Staphylococcus aureus* gave a different result. The compounds which have (OH, Br, Cl, OCH_3) groups appeared activity as anti-bacterial while compound (2) when R=H don't have activity for each type of bacteria that used.

Keywords:

Benzimidazole, nitrone, Escherichia coli, Staphylococcus aureus, anti-bacterial

Introduction

The benzimidazole is a bicyclic nitrogen behavior aromatic heterocyclic ring, structurally it contains benzene ring merged with imidazole ring in the fourth and fifth site of the ring. Chemically it seems like a white crystal, be similar to the structure of purine. An important part has been played in heterocyclic compounds on the metabolism of all the living cells. The nitrogen created heterocyclic compound show vital role for men. Predominantly benzimidazole has the enormous position not only biologically but also industrially among the whole nitrogen built heterocyclic compound. Initial benzimidazole derivative prepared by Hobrecker at 1872[1].

The first paper in pharmacologic properties of benzimidazole have distributed by Goodman and Nancy Hart in 1943. And then Woolley described the anti-bacterial activity of some benzimidazole derivatives compounds in 1944. After that as of acid hydrolysis of Vit. (B-12), Norman GB and Karl Folker in 1949 described 5,6-dimethyl benzimidazole as a degradation product [2].

Nevertheless, reaction of o-phenylenediamine with carbonyl compound derivatives to give benzimidazole is the predictable method that have used commonly for its synthesis [3–8].

Increasingly benzimidazole ring played as a significant complex heterocyclic system by reason of its varied variety of pharmacological action for instance anti-bacterial [9], anti-parasitic [10], anti-fungal [11], anti-inflammatory [12], analgesics [13], anti-viral [14],anti-tubercular [15] , anti-coagulant [16], anti-histaminic [17], anti-oxidant [18], anti-ulcer [19] and anti-cancer [20–23].

Generally heterocyclic compounds are of excessive importance in medicinal chemistry, as they are used as probable goals in contradiction of several bacterial and fungi pathogens [24]. Bacteria are single-cell organisms that have their place in the prokaryotic group where the organisms lack a few organelles and a true nucleus. It was lived in several spaces in our life. There are several types of bacteria, but the most common bacteria are Escherichia coli and Staphylococcus aureus [25].

Materials and Methods:

The melting point was measured with the Electrothermal Melting Point Apparatus. Infra-red spectra have noted by used (KBr disk) on Shimadzu FT-IR-8300 spectrophotometer in Basrah University, Science college, Chemistry department. H^1-NMR spectra were measured in Tehran University (IRAN) on Avance DRX 500 MHz (from Bruker), using dimethylsulphoxide (DMSO) as internal standards.

Preparation of benzimidazole

(1 mmol) 1,2-diaminobenzene with p-aminobenzaldehyde(2 mmol) in presence of p-TsOH in absolute ethanol. After 6 hours the product isolated by filtration and washed with ethanol and was recrystallized by mixture from DMF and ethanol[26].

preparation of Schiff base

(0.01 mol) of benzimidazole derivative (that contain amine groups) dissolve in ethanol abs. in 100 ml conical flask stirring at room temperature. And then add a solution of aldehyde (0.02 mol) with ethanol abs. and continued to stirrer then the product precipitate over (30 min) as a powder. the product isolated by filtration and

washed with small quantities of ethanol abs. to remove any remained starting materials. The product has recrystallized by ethanol abs. [27]

preparation of nitrone

(0.00422 mol) of Schiff bases was add to a mixture of (2.282mL) of H_2O_2% 36 and (5.08ml) of glacial acetic acid in a round. It reacted severely with the emission of intense heat and was left to stir in an ice bath for (6 h). And then leave the output for (24 h) at (0 C^0). [28]

Anti-bacterial test [29] **:**

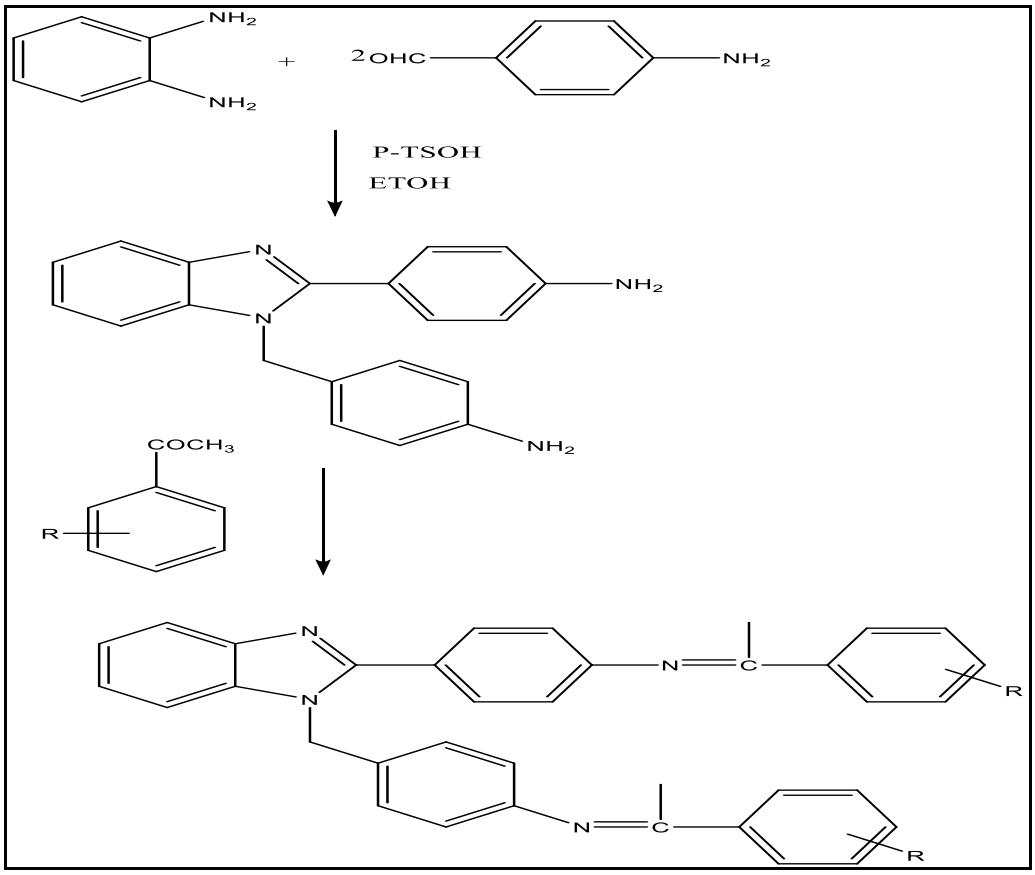
All the prepared compounds were tested, chemical products applied have taken by earlier antimicrobial activity screening make use of a diffusion method against of the two bacterial strains treated through the testing. Aimed at the diffusion method well-variant, a solvent have used dimethyl sulfoxide (DMSO), and then for the lasting methodologies, appropriate solvents have used for the dissolved of the chemical products.

Anti-bacterial activity for products have measured against two bacteria species: *Staphylococcus aureus* and *Escherichia coli*. Overnight cultures have used. Then after (24 h) of incubation, bacterial suspension (inoculum) has diluted by purified physiological solution, for the diffusion test, to 10^8 CFU/ml (turbidity = MCF arland barium sulfate standard 0.5).

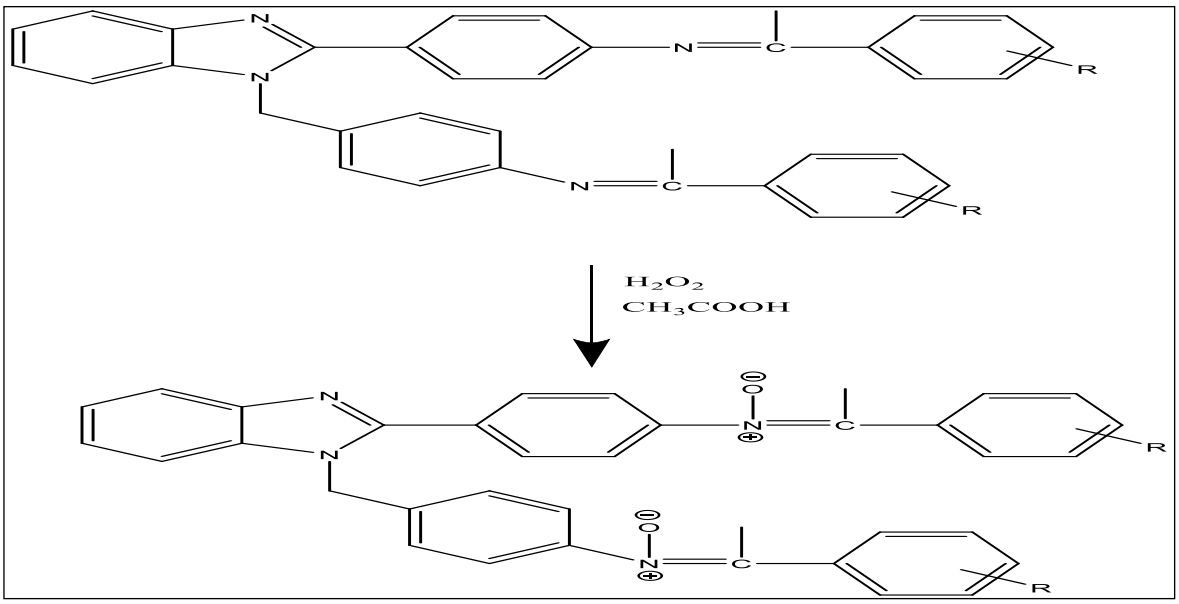
Bacterial inoculums have equivalently distributed by used the sterilized cotton swab on a sterile Petri dish Mueller Hinton Agar (MHA). (50 µl) from (100 mg/ml) concentration of chemical products has added for each well (7 mm diameter holes cut in agar gel, 20 mm apart from one extra). Plates have incubated for (24 h) at (36ºC ± 1ºC) in aerophilic states. Then merging bacterial growth have detected. Inhibition for bacterial growth have measured by mm.

Results and discussion:

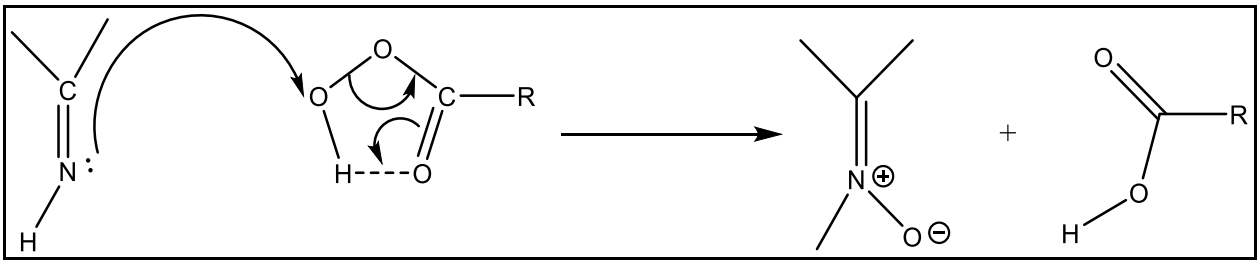
the treatment of 1,2-diaminobenzene (1 mmol) with 4-aminobenzaldehyde (2 mmol) in presence of p-TsOH in absolute ethanol resulted in the formation of 2-aryl 1-arlymethyl-1H-benzimidazole [30] as showe in scheme (1)



The benzimidazole compounds containing an imine group were oxidized to obtain a nitrone group by used peracetic acid. as shown in scheme (2)



The reaction occurs by attacking the free electron pair of the imine group on the acid oxygen, forming the neutron group' as shown in scheme (3)



The physical properties are shown in (Table1). The products were characterized by infra-red (Table2), H-NMR (Table3), and elemental analysis (Table 4).

Table (1) physical properties of synthesized compounds.

No. of comp.	IUBAC name	M.P. C^0	Color	M.wt	Yield %
2 (R=NO_2)	(Z)-1-(4-nitrophenyl)-N-(4-(1-(4-(((Z)-1-(4-nitrophenyl)ethylidene)oxidoazanyl)benzyl)-1H-benzo[d]imidazol-2-yl)phenyl)ethan-1-imine oxide	140-142	Pink	640	49
4 (R=OH)	(Z)-1-(4-hydroxyphenyl)-N-(4-(1-(4-(((Z)-1-(4-hydroxyphenyl)ethylidene)oxidoazanyl)benzyl)-1H-benzo[d]imidazol-2-yl)phenyl)ethan-1-imine oxide	132-133	Yellow	582	52
6 (R=Br)	(Z)-1-(4-bromophenyl)-N-(4-(1-(4-(((Z)-1-(4-bromophenyl)ethylidene)oxidoazanyl)benzyl)-1H-benzo[d]imidazol-2-yl)phenyl)ethan-1-imine oxide	198-200	Brown	708	43
7 (R=OCH_3)	(Z)-N-(4-(1-(4-(oxido((Z)-1-(p-tolyl)ethylidene)azanyl)benzyl)-1H-benzo[d]imidazol-2-yl)phenyl)-1-(p-tolyl)ethan-1-imine oxide	125-128	Brown	578	40
8 (R=Cl)	(Z)-1-(4-chlorophenyl)-N-(4-(1-(4-(((Z)-1-(4-chlorophenyl)propan-2-ylidene)oxidoazanyl)benzyl)-1H-benzo[d]imidazol-2-yl)phenyl)propan-2-imine oxide	177-178	Yellow	647	48

FT-IR spectral data [31]:

The spectra of these compounds that showed in the region (3024-3090) cm^{-1}, which were attributed to the (C-H) aromatic group,and strong bands at (1606-1658) cm^{-1} to the (C=N) stretching, all spectra showed absorption bands in the (1519-1543)cm^{-1} belong to the (C=C) stretching vibration aromatic ring also in the

range (1161-1167)cm^{-1} due to the (N-O) stretching, and the (C-N) stretching appear in (1334-1388)cm^{-1}. The absorption bands data of these compounds are shown in Table (2) and in the Figures (1)-(5) refer to the infrared spectra of the prepared compounds.

Table (2): FT-IR Spectra data for the synthesized compounds

Comp.	C=N Str.	C-N Str.	N-O Str.	C=C Ar. Str.	C-H Ar. Str.	C-H aliph. Str.
2	1631	1377	1161	1519	3066	2951
4	1606	1373	1165	1519	3066	2947
6	1608	1373	1161	1519	3066	2947
7	1631	1377	1161	1519	3066	2951
8	1608	1373	1161	1519	3066	2943

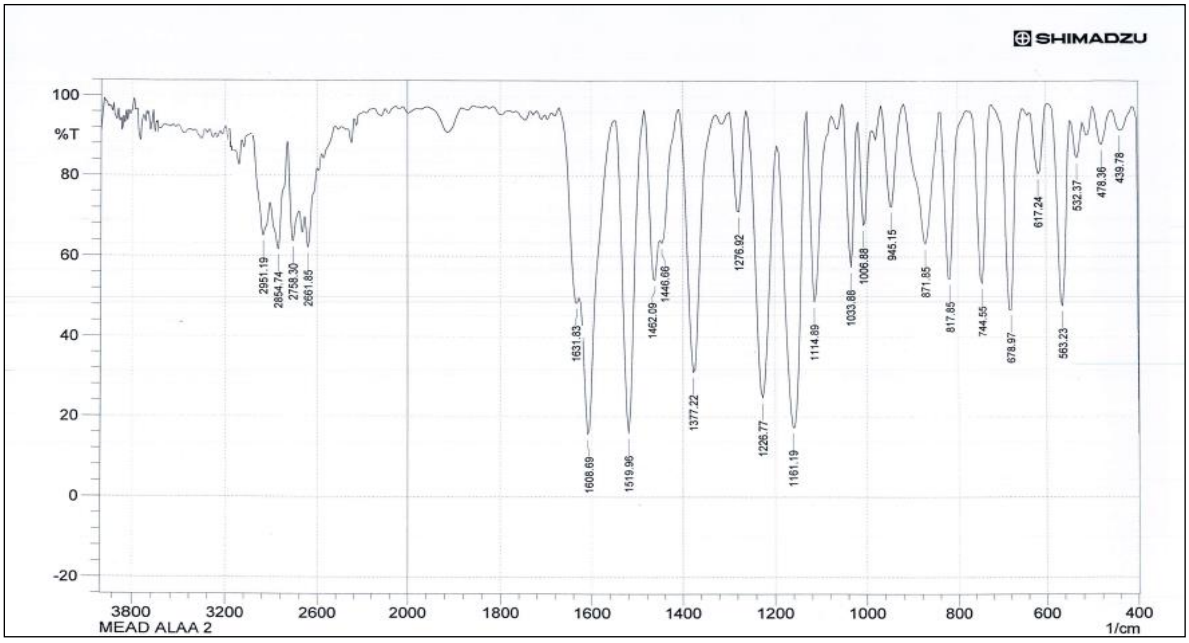


Figure (1): The infra-red Spectrum for the comp. 2

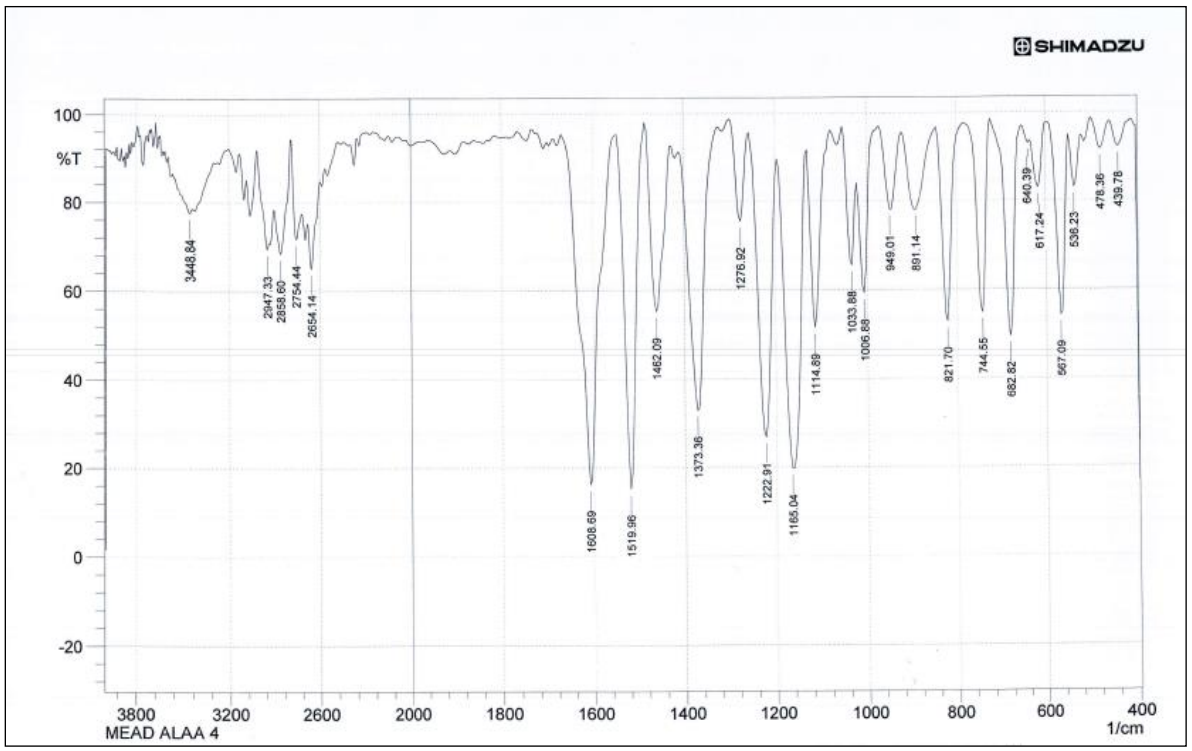


Figure (2): The infra-red Spectrum for the comp. 4

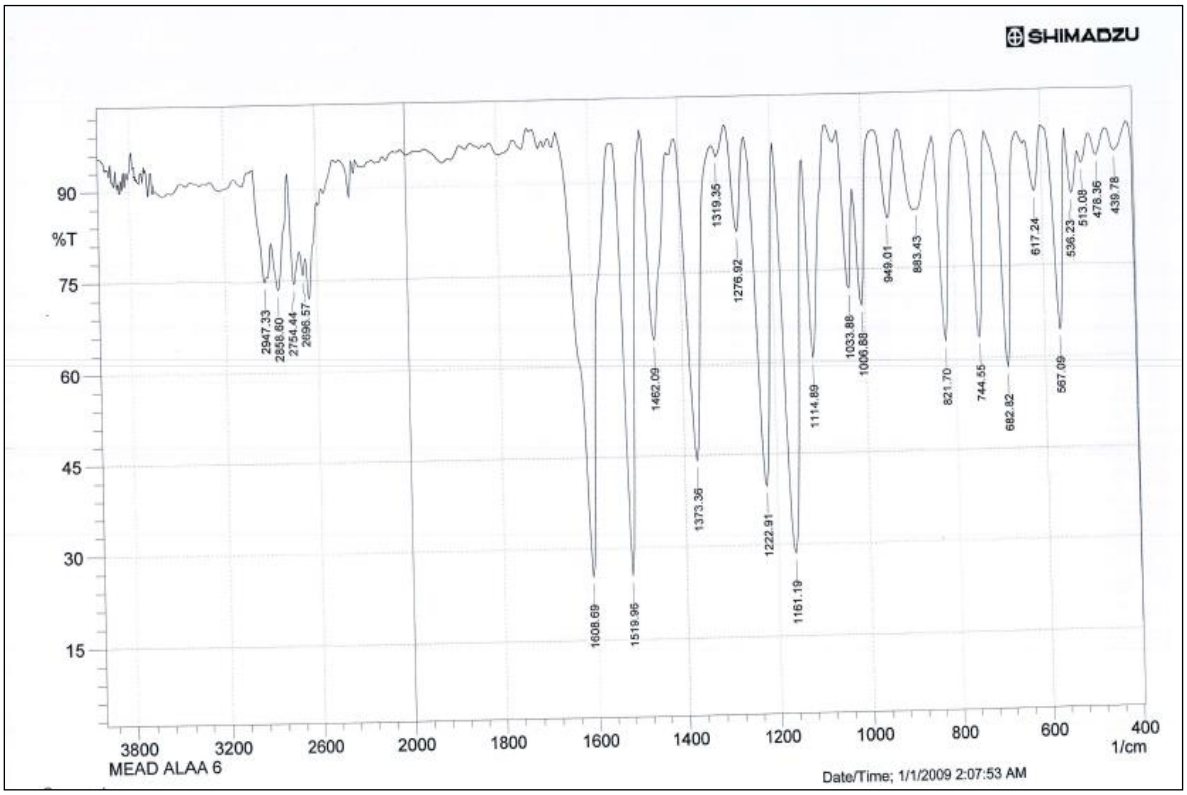


Figure (3): The infra-red Spectrum for the comp. 6

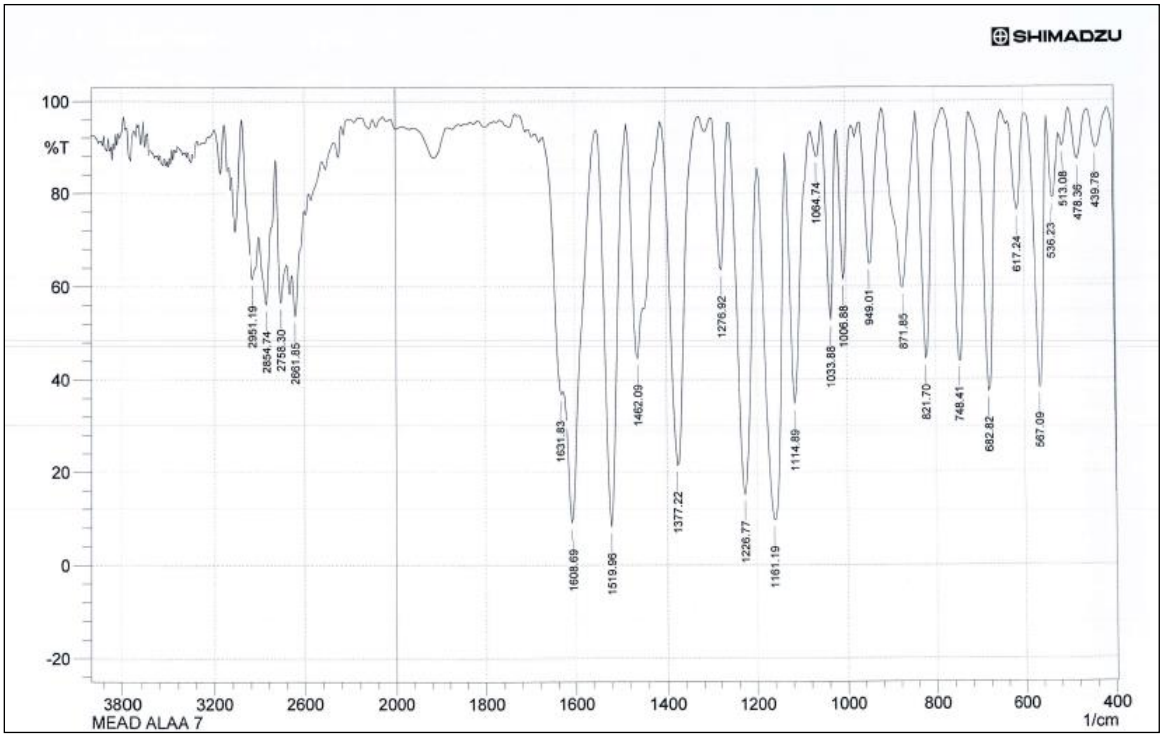


Figure (4): The infra-red Spectrum for the comp. 7

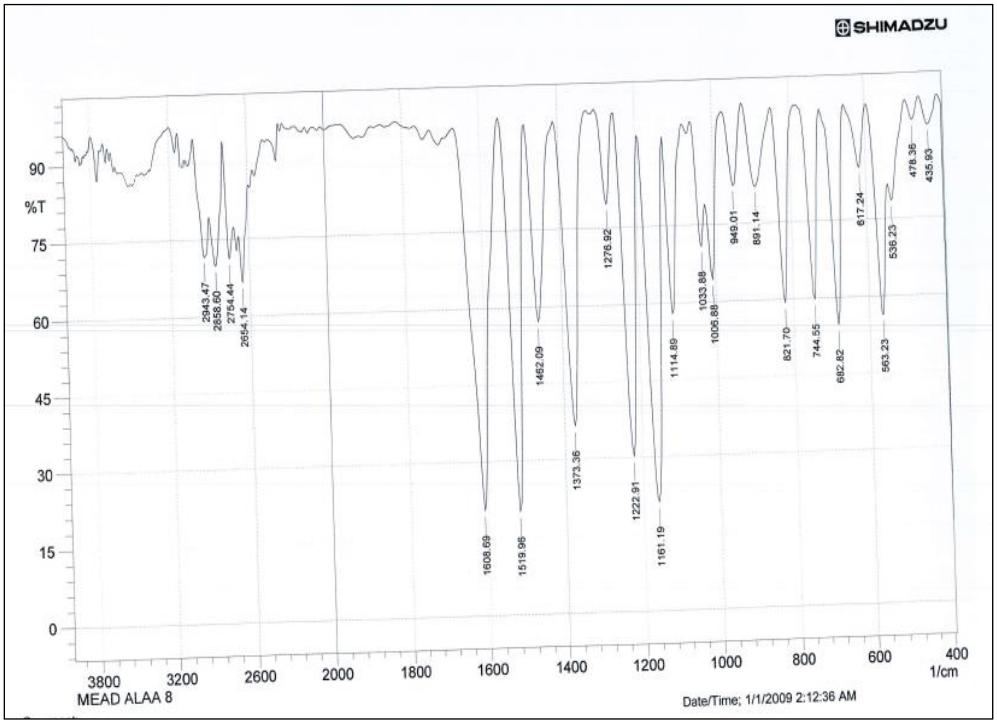


Figure (5): The infra-red Spectrum for the comp. 8

Proton Nuclear Magnetic Resonance (H^1 NMR)

The H^1-NMR spectra of benzimidazole-ketonitrone derivatives compounds showed a singlet signal in δ (2.30) ppm due to the aliphatic methyl of nitrone group. double signals in the region δ (7.5-7.53) ppm, (7.12-7.14) ppm and (8.03-8.06) ppm for the aromatic protons of benzimidazole rings, **d** and **a** respectively.

A Multiplet singles appear at δ (6.96-6.99) ppm belong to **b , H** protons and in the region δ (7.73-7.77)ppm for the aromatic protons **c**(as appears in the Figure(6), also it was observed that bundles of dimethyl sulfoxide DMSO solvent in their specific locations upon displacement (2.50-2.53) ppm, and for the H_2O at (3.10-3.30)ppm.

The chemical shifts of H^1-NMR spectra of the prepared compounds are shown in Table(3), and in Figures (7) - (11)

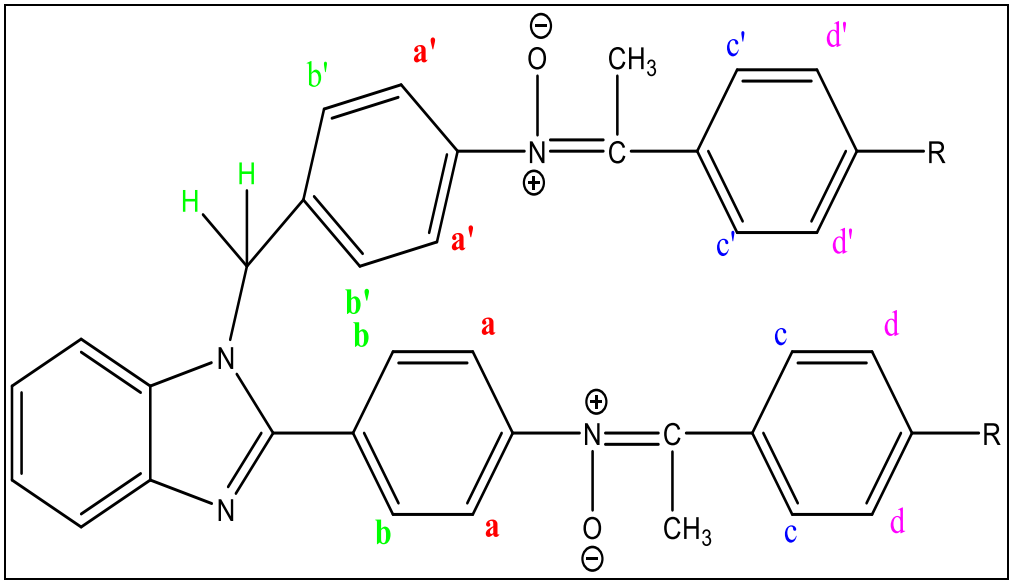


Figure (6): The structure of the synthesized compounds

Table (3): Chemical Shifts H^1.NMR Spectra for the synthesized compounds.

Comp.	a	b	c	d	CH_3	Benzimidazole ring	Others
2	8.04-8.06	6.97-7.00	7.72-7.76	7.11-7.13	2.30	7.48-7.53	
4	8.03-8.06	6.96-6.99	7.73-7.77	7.12-7.14	2.30	7.49-7.53	14.52 OH
6	8.03-8.06	6.65-6.99	7.73-7.77	7.12-7.14	2.30	7.50-7.53	
7	8.03-8.06	6.67-7.00	7.72-7.76	7.12-7.13	2.30	7.48-7.52	
8	8.03-8.06	6.96-6.99	7.73-7.76	7.12-7.13	2.30	7.49-7.53	

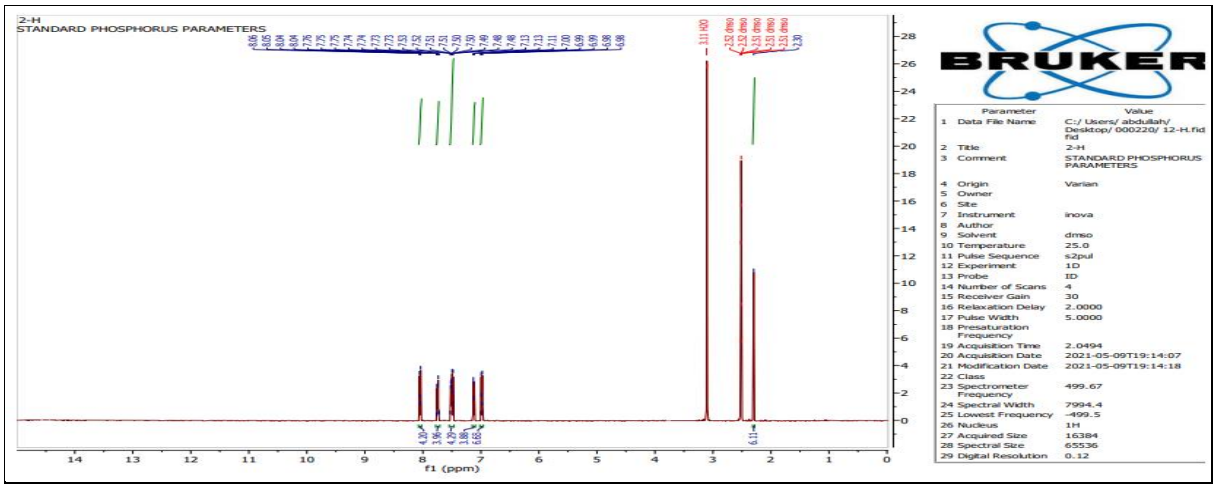


Figure (7): H^1-NMR spectra for comp. 2

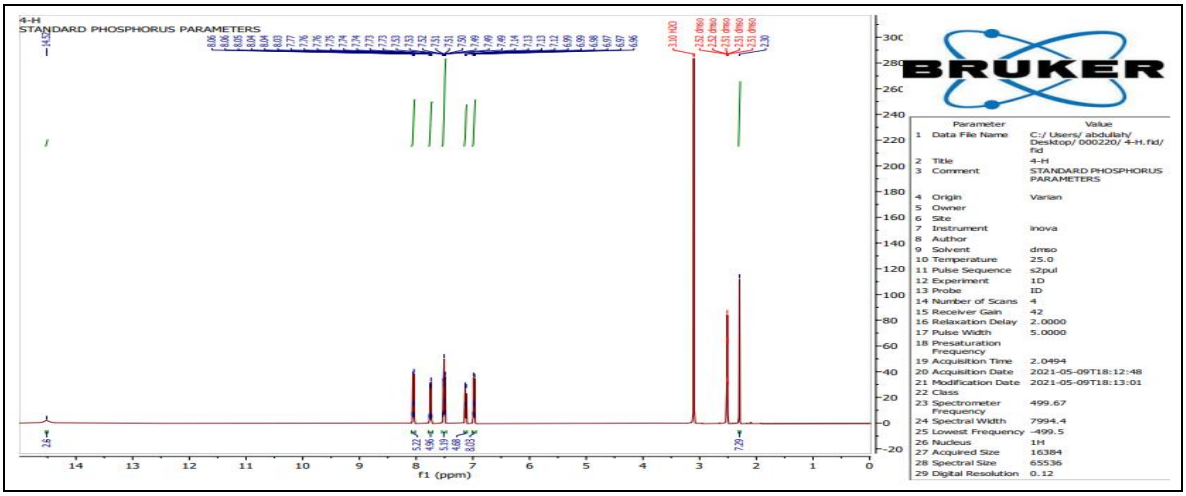


Figure (8): H^1-NMR spectra for comp. 4

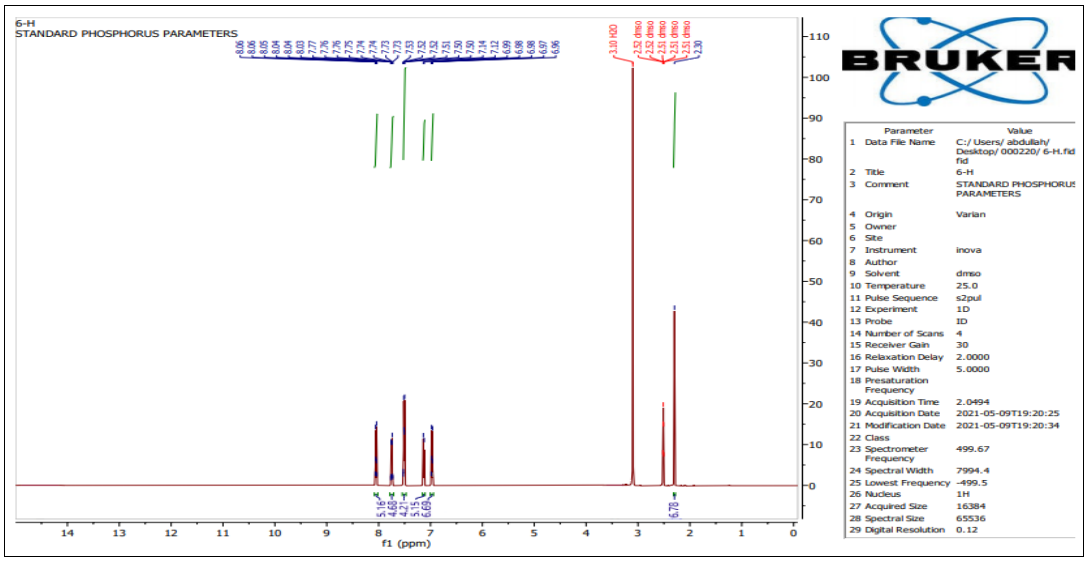


Figure (9): H^1-NMR spectra for comp. 6

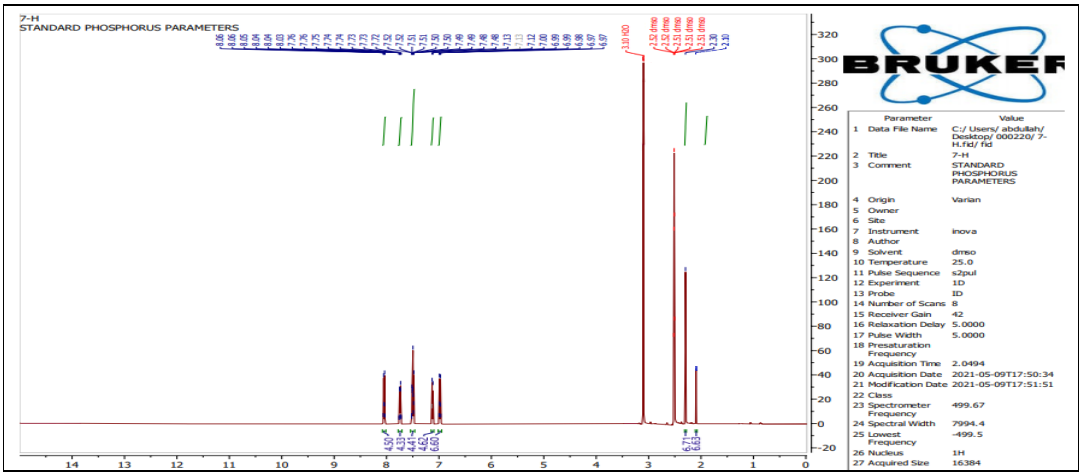


Figure (10): H^1-NMR spectra for comp. 7

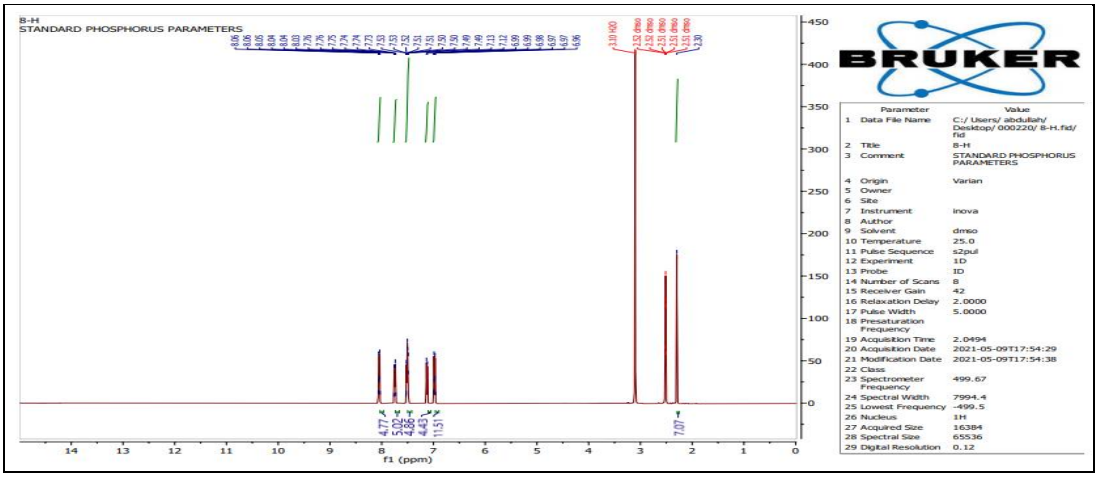


Figure (11): H^1-NMR spectra for comp. 8

Elemental Analysis C H N

An elemental analysis was carried out for the prepared compounds and the table (4) shows the extent of congruence between the practical percentages of the elements C H N and the calculated theoretical ratios for these prepared compounds.

Table (4): Elemental Analysis for the synthesized Compounds

Comp.	Calculated			Observed		
	%C	%H	%N	%C	%H	%N
2	67.49	4.41	13.12	67.51	4.40	13.15
4	74.21	5.19	9.62	74.19	5.21	9.65
6	61.03	3.98	7.91	61.08	4.02	7.93
7	78.87	5.92	9.68	78.85	5.96	9.72
8	70.48	4.98	8.65	70.50	5.01	8.69

Result of Anti-bacterial activity:

Heterocyclic compounds were excessive importance in medicinal chemistry, as they were used as probable goals in contradiction of several bacterial pathogens [32].

All compounds were applied on the positive gram *staphylococcus aureus* bacteria and *Escherichia coli* bacteria, and some gave inhibition zones with varying ratios. as it is shown in Table (5) and figures (12),(13)

Table (5) appears the inhibition zone of all synthesized compounds towards Staph. And E. coli bacteria

Comp.	E. coli bacteria	Staph. Bacteria
2	-	-
4	12mm	17mm
6	-	17mm
7	-	10mm
8	-	16mm

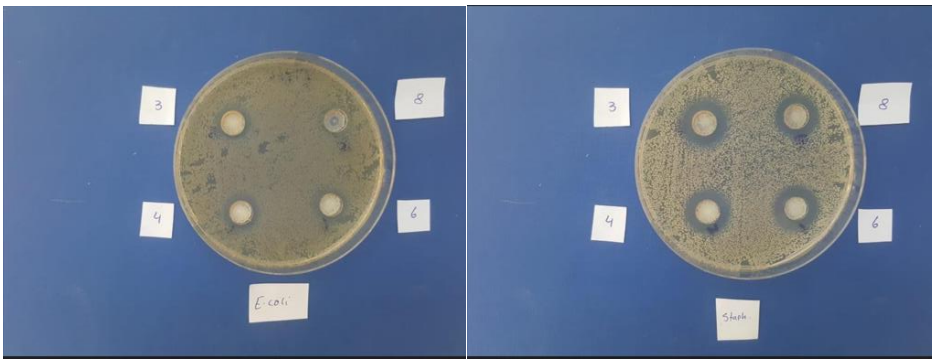


Figure (12) antibacterial activity for compounds (4,6,8)

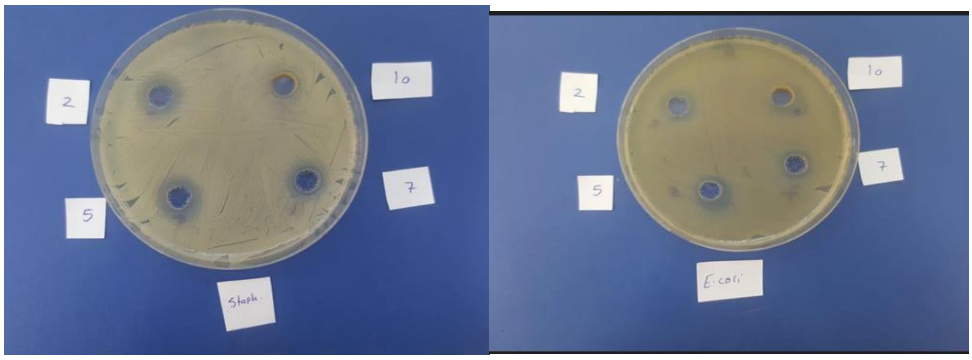


Figure (13) antibacterial activity for compounds (2,7)

The benzimidazole core has antimicrobial potential that explored notably since late 1990s and early 2000s [33]. As well as the nitrone compounds possess numerous beneficial and pharmacological effects such as antibacterial [34]. in addition to the prepared compounds contain (OH, Br, Cl, OCH_3) groups appeared activity as anti-bacterial while compound 2 (R=H) don't have activity for each type of bacteria that used.

Conclusion

All compounds applied on two different gram (- ve) and gram (+ ve) bacteria such as *Escherichia coli* and *Staphylococcus aureus* respectively and gave a different result. The compounds which have (OH, Br, Cl, OCH_3, NH_2) groups appeared activity as anti-bacterial.

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