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Article

Use of Solid Basic Catalysts in the Preparation of Cyclohexenone Derivatives and Evaluation of Their Bacterial Activity

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Abstract: Solid basic catalysts prepared from the reaction of potassium nitrate with alumina were used to synthesize hexagonal rings derived from cyclohexene by reacting the prepared chalcones with ethyl acetoacetate. Using the catalysts as a catalyst, cyclohexene derivatives were prepared. Biological activity was evaluated using two types of bacterial isolates known for their resistance to antibiotics, Gram-negative (Gram -ve) and Gram-positive (Gram +ve). In addition to determining the melting point and purity, physical and spectroscopic methods such as infrared spectroscopy, proton spectroscopy and carbon nuclear magnetic resonance (1H, 13C-NMR) were used to confirm the correct structure of the prepared compounds, and follow-up analysis was carried out by thin layer chromatography (TLC).

Keywords: Heterocyclic, catalyst, Cyclohexenone, biological activity.

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1. Introduction

Heterocyclic are compounds with ring structures containing different atoms, such as oxygen, sulfur or nitrogen. These compounds are widely distributed in nature. They have multiple importance and uses in many fields, including industrial and medical. These compounds enter into the composition of sugars and their derivatives, as well as enzymes, proteins and nucleic acids[1]. **catalyst** Heterogeneous basic catalysts are more common and used than acid catalysts because they are insoluble, easily separated, and can be used multiple times [2]. The effectiveness of these catalysts is evaluated based on physical surface properties such as surface area, interstitial volume, and active group concentration [3].

Cyclohexenone are versatile organic intermediates used in the manufacture of a variety of chemical products such as pharmaceuticals and perfumes which are formed by the condensation of chalcone with ethyl acetoacetate[4]. Cyclohexenone substitutes have shown important biological and medicinal activities, including antioxidant, antibacterial [5], anticancer [6], antifungal [7], and antibiotic activities[8]. This study aims to use solid basic catalysts in cyclization reactions with chalcones.

2. Materials and Methods

2.1. Chemicals Used

Chemicals prepared from Aldrich, BDH Thomas, Fluka, and Merck, were used.

2.2. Devices Used

Melting points were measured with a thermoelectric melter 3900. KBr disj at 400-4000 cm-1 scale, Shimadzu FT-IR 8400S spectrophotometer; Bruker equipment running at 400 MHz for 1H-NMR spectra. Fluka silica gel plates, with a thickness of 0.2 mm, were used in thin-layer chromatography (TLC)

2.3. Preparation of Cyclohexenone derivatives (MH21-MH25).[9,10]

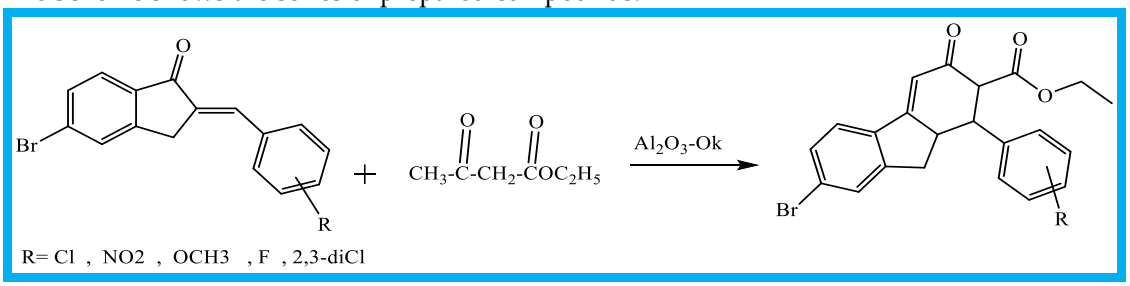
Dissolve (0.0015 mol) chalcone in (20 ml (absolute ethanol)), then add (0.00225 mol, 0.29 ml) ethyl acetoacetate and stir for (10) minutes, then add the catalyst (Al2O3-OK) at a rate of 20% by weight of chalcone and stir in a water bath at 40 °C for 4-5 hours, then filter the solution and get rid of the precipitate and leave the filtrate. The reaction progress was monitored using a TLC plate until it was dried and recrystallized from absolute ethanol.As in TABLE 1

2.4. Biological activity study

Two bacterial isolates were tested and obtained from the Advanced Microbiology Research Laboratory, Department of Life Sciences - Institute of Pure Science Education, Tikrit University, one Gram-negative [Gr-ve] i.e. E. coli, and one Gram-negative E. [Gr-ve], which is Escherichia coli. Positive [Gr + ve][11-15]. This is Staphylococcus aureus. Two colonies of pure bacterial isolates of both Gram-positive and Gram-negative bacteria were transferred from the solid culture medium to test tubes containing (5 ml) distilled water using heat-sterilized holders. The tubes were incubated at 30 °C. (37) °C for 16-20 hours and then diluted with physiological solution until the turbidity reached the standard turbidity level to obtain a cell count of approximately (1.5×108) cells/ml. Insert a sterile cotton swab into the test tube containing the diluted bacterial growth and remove excess inoculum by pressing the swab against the inner wall of the tube[16-20]. Inoculate Mueller Hinton Agar (MHA) with the sterile cotton swab and wipe it over the cultures. Leave the Petri dish aside (10-15 days) for a few minutes to absorb the culture and dry the medium[20-25] .

3. Results

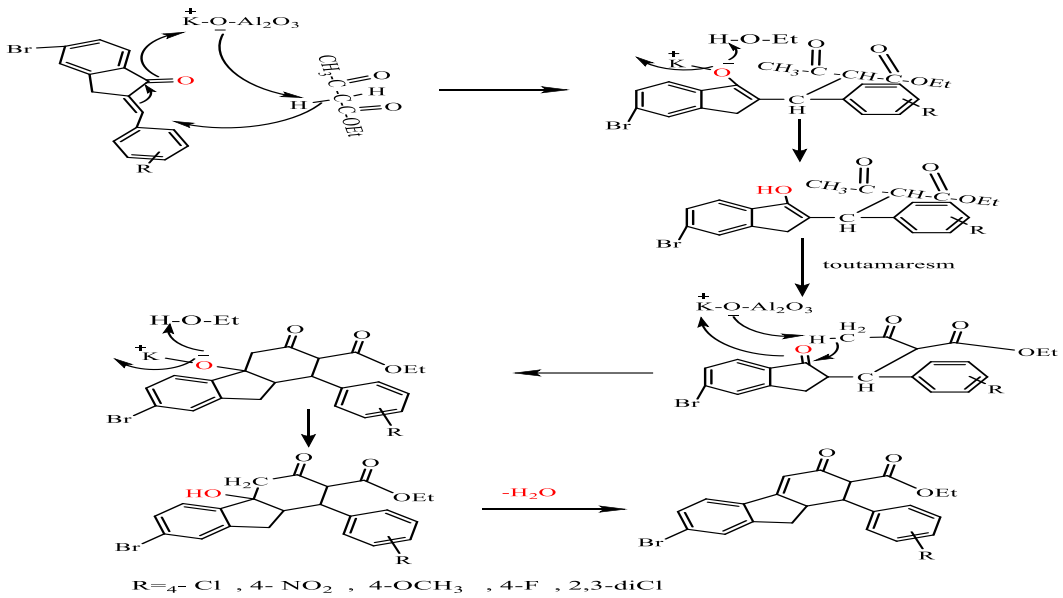
The Scheme shows the series of prepared compounds.



Scheme (1) : Path of the Ready Compounds (MH21-M25)

3.1. Characterization of Cyclohexenone derivatives (MH21-MH25)

In the presence of an Al2O3-OK catalyst, ethanol is used as a solvent, and the prepared chalcone derivative is reacted with ethyl acetoacetate to prepare the cyclohexenone derivative. The mechanism of cyclohexenone derivatives is as follows:



Scheme (2) : Mechanism of Preparation of cyclohexenone derivatives [MH20-MH25]

When studying the infrared spectrum of the compounds [MH21-MH25], it was observed that absorption bands appeared in the range (1714-1738) cm-1, which are due to the stretching of the ester (C=O) bond, and the appearance of absorption bands in the range (1685-1648) cm-1 is due to the stretching of the (C=O) bond of the ketone, and the low value of the carbonyl group is due to the alternation between the carbonyl group and the double bond (C=C) that appeared in the range (1585-1596) cm-1, which leads to a decrease in the value of the force constant for the double bond, which reduces its frequency. It was also observed that two absorption bands appeared in the range (1432-1469) cm-1 and (1510-1535) cm-1, which are due to the stretching of the aromatic (C=C) bond, in addition to absorption bands in the range (3012-3090) cm-1, which are due to the stretching of the (Ar-H) bond. Aromaticity and absorption band at (3157-3112) cm-1 are due to stretching of the olefinic (=C-H) bond [26]. as shown in Table 1 and Figure 1.2 The 1H-NMR spectrum of the compound [MH23] showed the appearance of a quadruple signal at the position ppm (3.21-3.16) due to the proton of the (CH) group adjacent to the double bond in cyclohexenone, and the appearance of a triple signal at the range ppm (3.65-3.67) due to the proton of the (CH) group attached to the aromatic ring, as well as a quadruple signal at the range ppm (3.16-3.21) due to the proton of the (CH) group attached to the (CH2) group, as well as the appearance of a double signal at the positions ppm (3.41-3.43) due to the proton of the (CH) group adjacent to the carbonyl group and a single signal at the positions ppm (6.37) due to the proton of the (=CH) group, as well as a quadruple signal at the range ppm (3.16-3.21) due to the proton of the (CH) group of the cyclohexenone ring, in addition to a triple signal at The ppm sites (1.29-1.34) are due to the protons of the (CH3) group, and a quadruple signal appeared in the range (4.12-4.15) ppm attributed to the protons of the (CH2) group, and a multiple signal appeared in the ppm sites (6.66-7.77) attributed to the protons of the aromatic ring, in addition to the appearance of a signal in the ppm sites (3.80) attributed to the protons of the (OCH_3) group belonging to the benzaldehyde substitute, and the appearance of a signal in the ppm site (2.48) attributed to the protons of the solvent (DMSO-d6) . as in Figure (3). The 13C-NMR spectrum of the compound [MH23] showed a signal at position (195.04) ppm attributed to the carbonyl group (C=O) of the cyclohexene ring, a signal at position (164.84) ppm attributed to the carbonyl group (C=O) of the ester, a signal at position (159.99) ppm attributed to the carbon of the (C=-) group in cyclohexene, a signal at position (158.74) ppm attributed to the carbon of the (C-O) group, in addition to a signal at position (32.24) ppm attributed to the carbon of the (CH) group of cyclohexene attached to the aromatic ring, as well as multiple signals at position (156.14-115.66) ppm attributed to the carbons of the aromatic benzene ring, and a signal at position (159.99) ppm (45.84) is attributed to

the carbon of the (CH2) group of indenone, and the appearance of signals in the ppm range (39.49-40.49) is attributed to the carbons of the solvent (DMSO-d6) , as in Figure (4).

3.2. Evaluation of the Biological Activity of Prepared Compounds

The antibacterial activity of the prepared compounds was tested using the agar diffusion method (121). After inoculating the culture medium with the bacterial isolates, holes were made in the Petri dishes using the cylinder measuring method (according to USP 35)[27-32]. Using a drill: Place the prepared compounds (40 µl) at three concentrations in each well and incubate the dish in an incubator at (37) °C (24 h) before placing the dish in the incubator. The results were read after (24) h and (48) h to indicate the sensitivity of the derivative used, which depends on the inhibitory diameter shown in the Petri dish surrounding the well used, as an increase in the inhibitory diameter means an increase in the inhibitory diameter. Preparation of the inhibitory diameter [33-36].The bioavailability of the compounds was compared with the inhibitory diameter of standard antibiotics, some of which were used as control samples in the form of a solution[37-42] . as shown in Table 3

Table (1) : Some Physical Properties of for Prepared Compunds (MH21-MH25)

Comp. No.	R	Molecular formula	m.p. °C	Yield%	Color
MH_{21}	4-Cl	$C_{22}H_{18}BrClO_3$	170−172	49	Light brown
MH_{22}	4-NO_2	$C_{22}H_{18}BrNO_5$	187−190	51	Brown
MH_{23}	4-OCH_3	$C_{23}H_{21}BrO_4$	187−189	41	Green
MH_{24}	4-F	$C_{22}H_{18}BrO_3F$	178−180	54	Yellow
MH_{25}	2,3-diCl	$C_{22}H_{17}BrCl_2O_3$	188−190	57	Gray

Table (2) : FT-IR absorption result for Prepare compounds (MH21-MH25)

Comp. No.	R	IR (KBr) cm^{-1} ν(C-H) Arom	ν(C-H) Olph.	ν(C-H) .Aliph.	ν(C=C) Olph.	ν(C=O) aster	ν(C=C) Arom.	ν(C=O) keton	Others
MH_{21}	**Cl4-**	3090	3121	2971 2845	1596	1720	1523 1453	1665	ν(C-Br) 621 ν(C-Cl)720
MH_{22}	**4-NO_2**	3012	3123	2927 2852	1585	1728	1535 1463	1648	ν(C-Br) 617 ν(N-O)1311
MH_{23}	**4-OCH_3**	3060	3157	2945 2840	1593	1726	1510 1460	1658	ν(C-Br) 621 ν(C-O)1384
MH_{24}	**4-F**	3018	3141	2918 2832	1587	1738	1526 1432	1685	ν(C-Br)609 ν(C-F) 995
MH_{25}	**2,3-diCl**	3058	3112	2970 2854	1591	1714	1527 1469	1656	ν(C-Br) 680 ν(C-Cl)786

Table (3) : Biological officacy of produced substances and control methods (measured in cm of inhibition)

Comp. No.	E. Coil Conc. mg/ml			Staph. Aureus Conc. mg/ml		
	0.01	0.001	0.0001	0.01	0.001	0.0001
MH_{21}	18	15	3	28	28	15

	MH_{22}	17	13	10	30	27	4
	MH_{23}	35	35	10	20	15	10
	MH_{24}	27	21	5	15	10	5
MH_{25}		25	21	15	26	20	8
Amoxicillin		38	35	18	35	30	20

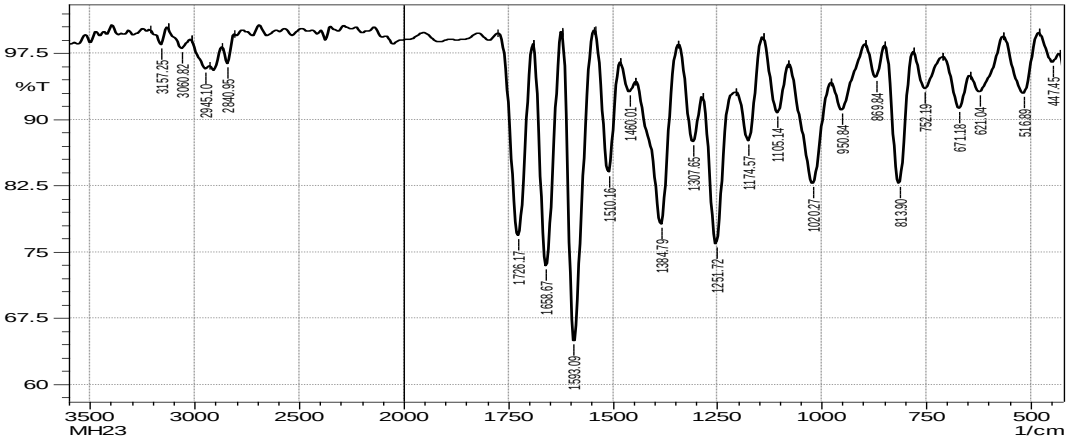


Figure (1) : The compund's FT-IR spectra (MH23)

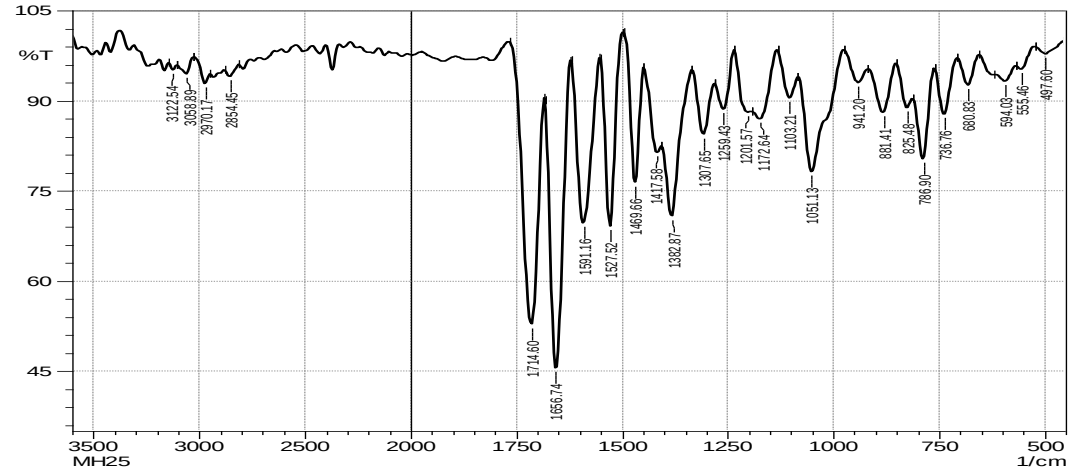


Figure (2) : The compound's FT-IR spectra (MH25)

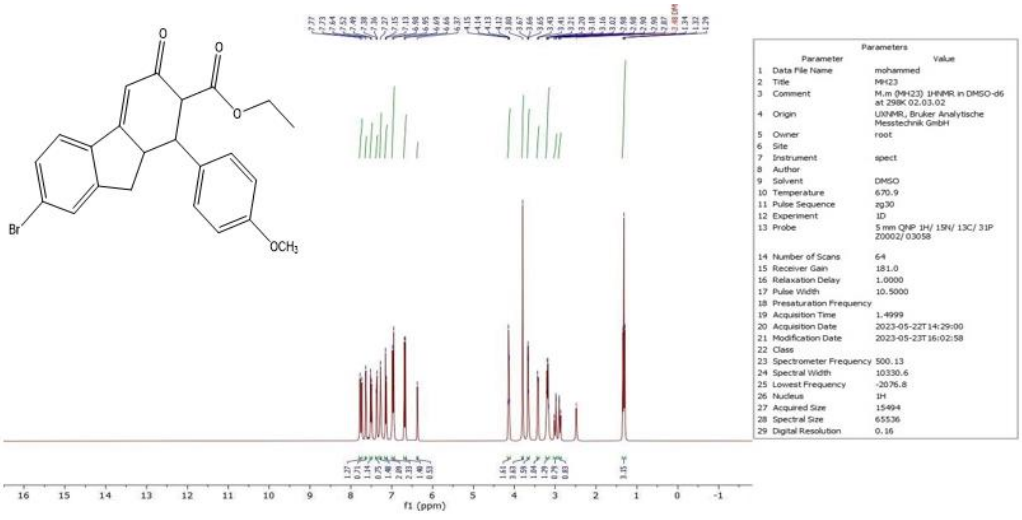


Figure (3) : 1-H NMR spectra of the substance (MH23)

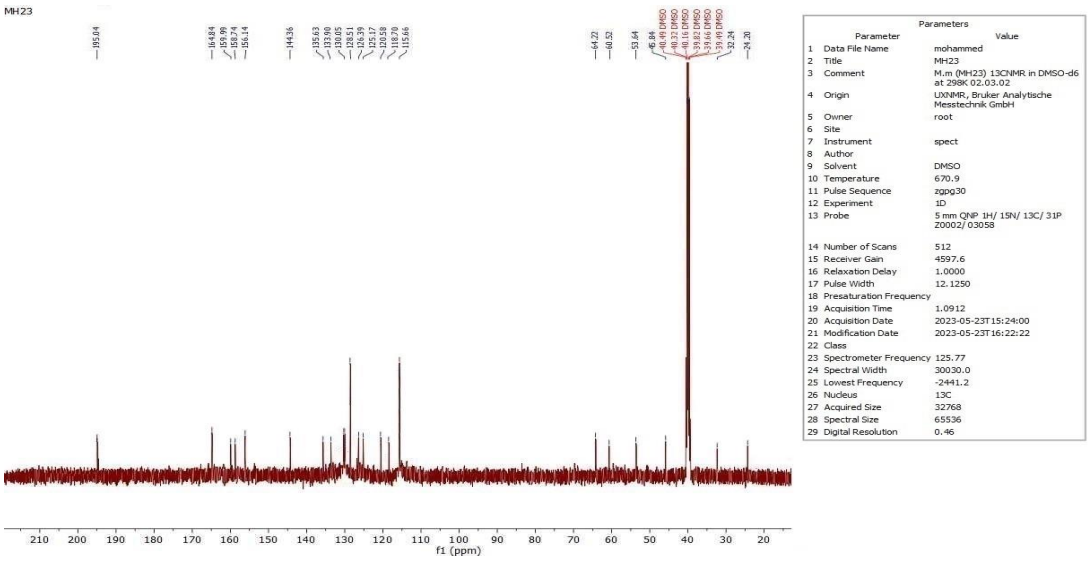


Figure (4) : 13C- NMR spectra of the substance (MH23)

4. Conclusion

The use of catalysts in reactions is a safe, secure and economical method, as spectroscopic measurements have proven the accuracy and precision of the products, as they were of very high purity. The prepared compounds also showed good bacterial activity against the two types of bacteria used, with inhibition rates close to those of the drug used.

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