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Synthesis, Characterization and Preliminary Antimicrobial Activity of New Alfa-Beta Unsaturated Ketone of Dibenzo-18-Crown-6

Conflict of interest: nothing to declare.
Authors' contribution: Abdali Z. – conceptualization, methodology, formal analysis, investigation, resources, data curation, writing – original draft; Mahesein R.J. – supervision, conceptualization, data curation, visualization, writing – original draft, writing – review, editing.
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Abstract

Introduction. Crown ethers can be functionalized by a variety of chemical processes due to their high solubility. Thus, numerous crown ether derivatives have been manufactured include the acylation of dibenzo-18-crown-6 which represent a promising approach for the synthesis of alfa-beta unsaturated ketone of dibenzo-18-crown-6. Functionalization for dibenzo-18-crown-6-ether to synthesis acylation dibenzo-18-crown- 6-ether(4,4'-diacetyldibenzo-18-crown-6-ether) to increase the solubility and stability.
Purpose. To synthesized and determined the characterization and antimicrobial activity of Alfa-beta unsaturated ketone of Dibenzo-18-crown-6.
Materials and methods. New alfa-beta-unsaturated ketone of dibenzo-18-crown-6 is obtained in two steps involving: Reaction dibenzo-18-crown-6 with acetic anhydride in the presence of phosphoric acid to synthesize acetyl-dibenzo-18-crown-6. Followed by Claisen-Schmidt condensation reaction with various aromatic aldehydes in strong basic medium (conc. KOH) to form the final product, alfa- beta-unsaturated ketone of dibenzo-18-crown-6 (chalcone). Crown ethers can be functionalized by a variety of chemical processes due to their high solubility. Thus, numerous crown ether derivatives have been manufactured.
Results. Including the dibenzo-18-crown-6 ether represent a promising approach for the synthesis of chalcone and its derivative compounds in two steps. Acylation of dibenzo-18-crown-6 followed by condensation with various aromatic aldehydes to form the corresponding alfa-beta unsaturated ketone of dibenzo-18-crown ether-6. The inhibition zone for the chalcone compounds with different concentration against Streptococcus, Staphylococcus and Proteus. We noticed in the concentrations 50 ppm, 100 ppm, 150 ppm, 200 ppm, 400 ppm, the inhibition zone for chalcone in (400 ppm) against Streptococcus has inhibition zone (22 mm) larger than Azithromycin in (400 ppm) (have inhibition zone (14mm)) that is mean the effect of chalcone has a good antibacterial activity against Streptococcus. Also inhibition zone of chalcone in (400 ppm) against Staphylococcus is (15 mm) while for Azithromycin the inhibition zone is (14 mm). And the inhibition zone for chalcone against Proteus is (13 mm) and for Azithromycin is (16 mm).

Conclusion. The acylation reaction successfully yielded chalcone and its derivatives from the reaction between acylationdibenzo18-crown-6 and benzaldehyde. The synthesis of chalcone holds significance as it represents a novel approach to the development of antibacterial agents. The evaluation of antibacterial activity revealed that the synthesized chalcone exhibited notable inhibitory effects against a range of bacterial strains Streptococcus, Staphylococcus, and Proteus. The results indicated that chalcone and its derivative have the potential to be developed, as an alternative antibiotic agent, which could contribute to addressing the growing issue of antibiotic resistance. The comparative analysis with azithromycin shows strong inhibition into these bacteria, that indicated the potential of chalcone and its derivative as an effective alternative in resisting bacterial infection.

Keywords: crown ethers, alfa beta-unsaturated ketone, chalcone, dibenzo-18-crown-6, Claisen-Schmidt reaction

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Синтез, определение характеристик и предполагаемая антибактериальная активность нового альфа-бета-ненасыщенного кетона дибензо-18-краун-6

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Резюме

Введение. В силу своей высокой растворимости краун-эфиры могут подвергаться функциональным преобразованиям посредством различных химических реакций. В результате функционализации получены многочисленные производные краун-эфиров. Ацилирование дибензо-18-краун-6-эфира представляет собой перспективный подход к синтезу альфа-бета-ненасыщенного кетона дибензо-18-краун-6. Функционализация дибензо-18-краун-6-эфира с последующим синтезом с применением реакции ацилирования дибензо-18-краун-6-эфира (4,4′-диацетилдибензо-18-краун-6-эфира) позволяет улучшить его растворимость и стабильность. Синтез альфа-бета-ненасыщенного кетона (халькона) путем конденсирования ацетилдибензо-18-краун-6 с различными ароматическими альдегидами представляет интерес в силу присущих ему характерных особенностей их фармакологических свойств, в частности антибактериальных и противогрибковых.

Цель. Синтез, определение характеристик и анализ проявлений антибактериальной активности альфа-бета-ненасыщенного кетона дибензо-18-краун-6-эфира.
Материалы и методы. Новый альфа-бета-ненасыщенный кетон дибензо-18-краун-6 получали в два этапа. На первом из них проводили реакцию между дибензо-18-краун-6 и уксусным ангидридом в присутствии фосфорной кислоты для получения ацетил-дибензо-18-краун-6. Вторым этапом была реакция конденсации Клайзена – Шмидта с различными ароматическими альдегидами в сильной основной (щелочной) среде (создаваемой гидратом окиси калия – KOH) с образованием конечного продукта – альфа-бета-ненасыщенного кетона дибензо-18-краун-6 (халькона). Благодаря своей высокой растворимости краун-эфиры могут быть функционализированы с помощью различных химических процессов, что позволяет получать многочисленные производные краун-эфиров.
Результаты. Использование дибензо-18-краун-6-эфира открыло перспективу осуществления двухступенчатого синтеза халькона и его производных. Ацилирование дибензо-18-краун-6-эфира с последующей конденсацией его с различными ароматическими альдегидами позволяет получить соответствующие альфа-бета-ненасыщенные кетоны дибензо-18-краун-6-эфира. Установлена и охарактеризована зона подавления роста Streptococcus, Staphylococcus и Proteus соединениями халькона в растворах разной концентрации. Определено, что при их использовании в концентрации 50 ppm, 100 ppm, 150 ppm, 200 ppm и 400 ppm зона подавления хальконом (400 ppm) роста Streptococcus составляет 22 мм, что превышает зону подавления азитромицином (400 ppm), составляющую 14 мм. То есть халькон проявляет высокую антибактериальную активность в отношении Streptococcus. Помимо этого, показано, что зона ингибирования хальконом (400 ppm) роста стафилококка составляет 15 мм, в то время как у азитромицина она равна 14 мм. Зона подавления роста Proteus составляет для халькона 13 мм, а для азитромицина – 16 мм.
Заключение. В результате реакции ацилирования дибензо-18-краун-6-эфира и бензальдегида был получен халькон и его производные. Разработка технологии синтеза халькона имеет большое значение, поскольку она представляет собой новаторский подход к созданию антибактериальных препаратов. Оценка антибактериальной активности соединения показала, что синтезированный халькон обладает выраженным антибактериальным действием в отношении ряда бактериальных штаммов Streptococcus, Staphylococcus и Proteus. Результаты исследования свидетельствуют о том, что разработка препаратов на основе халькона и его производных может стать перспективной альтернативой антибактериальным препаратам, способствуя решению постоянно возрастающей проблемы антибиотикорезистентности. Оценка эффективности антимикробного действия нового соединения по сравнению с таковой азитромицина показала более выраженное торможение роста указанных микроорганизмов препаратами на основе халькона, что свидетельствует о перспективности использования халькона и его производных в качестве эффективной альтернативы ранее известным препаратам в борьбе с резистентными бактериальными инфекциями.
Ключевые слова: краун-эфиры, альфа-бета-ненасыщенные кетоны, халькон, дибензо-18-краун-6, реакция Клайзена – Шмидта

■ INTRODUCTION

Since Pedersen reported the synthesis and cation complexing properties of a new class of compounds known as crown ethers in 1967. These first neutral synthetic heterocyclic compounds have attracted extensive and continuous attention due to the unusual and powerful noncovalent cation binding properties that they possess. The term "classical crown ethers" refers to a class of macrocyclic polyethers that contain anywhere from three to twenty oxygen atoms, each of which is separated from the next by two or more carbon atoms. However, the macrocyclic oligomers of ethylene oxy units that include 5–10 oxygen atoms are the most efficient complexing agents. These oligomers can be substituted or unsubstituted, depending on their composition. The common names for these macrocycles, which are typically used in place of their systematic names, consist of the number and type of attached hydrocarbon rings (in the substituted derivatives), the number and type of atoms in the polyether ring, the class name "crown" that comes from their molecular shape and ability to "crown" a metal ion upon coordination, and the number of oxygen atoms in the polyether ring. In general, common names are preferred over systematic names. Take, for instance, the compound dibenzo-18-crown-6 [1].

In their common names, the amount and kinds of atoms that make up the polyether ring are indicated. These molecules' binding selectivity for a variety of metal ions is determined by the size of their crown rings; as a result, the 18-crown-6 molecule has a strong affinity for potassium, whereas the 15-crown-5 molecule prefers sodium cations. In order to provide an explanation for this phenomenon, it has been discovered that highly complex stability is linked to a larger penetration of the metal cation into the polyether cavity [2].

Crown ether macrocycles are known in all ring sizes, ranging from 9 to at least 60, which has led to a large deal of structural diversity (exceeding 10,000 examples) [3]. As a result, crown ethers have been the subject of a significant amount of research from a variety of perspectives, such as sensor applications, biological model systems (such as abiotic ion channels) and biological functionality, particularly in regard to their potential anticancer and antimicrobial effects [1, 3]. Because crown ether derivatives work in a manner that is analogous to that of naturally occurring ionophores (such as gramicidin), researchers have been able to utilize them to investigate a variety of biological processes. In particular, researchers have found that crown ether derivatives have the ability to create channels and transport ions through lipid membranes [1]. The potential of macrocycles for drug development has gained a significant amount of attention in recent years. Crown ethers can be functionalized by a variety of chemical processes due to their high solubility. Thus, numerous crown ether derivatives have been manufactured. Conformational flexibility is one of the distinguishing characteristics of crown ethers [4].

In recent years, dibenzo-18-crown-6 has garnered attention in the field of organic synthesis, particularly for its potential as a versatile building block for the synthesis of functionalized compounds. Its unique structural features make it a suitable starting material for various transformations, such as acylation and alkylation [5].

The aromatic rings are acylated in Friedel-Crafts acylation. Typically, acyl chlorides are used as acylating agents. Carboxylic acids and acid anhydrides are both acceptable alternatives. Aluminium trichloride is an example of a Lewis acid catalyst. However, in contrast to the Friedel- Crafts alkylation, in which the catalyst is constantly regenerated, a stoichiometric amount or more of the "catalyst" must generally be used because the

product ketone forms a rather stable complex with Lewis acids such as AlCl3 [6]. The reaction is carried out under conditions analogous to the Friedel-Crafts alkylation. The alkylation reaction loses out to this reaction in various ways. Ketone products are never more reactive than the parent molecule because the carbonyl group acts as an electron-withdrawing group. Furthermore, the positive charge remains on the oxygen, stabilizing the acylium ion in a resonance structure, so no carbocation rearrangements occur.

The Claisen-Schmidt condensation is a reaction between an aldehyde and a ketone that leads to the formation of an α,β-unsaturated carbonyl compound, also known as a α,β-unsaturated ketone or chalcone. The reaction is a type of carbon-carbon bond forming condensation reaction and is an important tool for the synthesis of many organic compounds. The Claisen-Schmidt condensation reaction is a two-step process. In the first step, the base (usually a strong base like sodium hydroxide or potassium hydroxide) deprotonates the ketone to form its enolate anion. The enolate anion then attacks the aldehyde at the electrophilic carbon, forming an intermediate β-hydroxy aldehyde. In the second step, the β-hydroxy aldehyde loses a molecule of water to form the final product, an α,β-unsaturated carbonyl compound, or chalcone. This reaction is a type of aldol condensation and involves the formation of a carbon-carbon double bond. The Claisen-Schmidt condensation is an important reaction in organic synthesis and is widely used to synthesize a variety of compounds, including natural products, pharmaceuticals, and other biologically active molecules [7, 8].

In this experiment, we made compared the chalcone compounds and azithromycin antibiotic which is a macrolide antibiotic.

Macrolides are important antibiotics used in therapy. Macrolide molecules consist of a lactone ring with 10 into 12 or 13 carbon atoms and a deoxy sugar or amino sugar residue. The use of macrolides of the second generation in treatment enhanced the pharmacokinetics and pharmacodynamics of this class of antibiotics; however, this did not resolve the issue of rising antibiotic resistance. Macrolide antibiotics are known for their wide range of efficacy. They are effective against Gram-positive and Gram-negative bacteria in equal measure. Although the action spectrum of macrolides may vary very slightly from one another azithromycin was one of the drugs that was used the most at the onset of the SARS-CoV2 epidemic. Because it encourages the development of drug-resistant bacterial strains. Regarding azithromycin, the main mechanism for increasing bacterial resistance to this antibiotic is changing the target of the active ingredient on the ribosome [9, 10].

■ PURPOSE OF THE STUDY

To synthesized and determined the characterization and antimicrobial activity of Alfa-beta unsaturated ketone of Dibenzo-18-crown-6.

■ MATERIALS AND METHODS

Dibenzo-18-crown-6 ether, Acetic acid, benzaldehyde, KOH, Solvents. The progress of reaction was monitored by TLC (Silica gel 60 F254). IR spectra were recorded on Perkin-Elmer spectrometer. The 1H and NMR were recorded on Bruker 400 Avance II with Chloroform/d/DMSO-d6 as solvent and TMS as internal standard. Melting points were determined in open capillary tube and are uncorrected.

Acylation of dibenzo-18-crown ether-6 [11]

A mixture of dibenzo-18-crown-6 ether (1 g, 2.77 mmole), acetic anhydride (7 ml) and phosphoric acid (3ml) in round-Bottom flask (100ml) with magnetic bar stirring in water bath and condensation for 4 hr, at 60–70 C.

The reaction mixture was left to cool at room temperature after that added ice cube to the mixture reaction which lead to formation white sticky ppt. recrystallized by using DCM then filter it and let it dry at the room temp.

Yield (85%), Melting point (170 C). Purity of compound was checked by using TLC at the time reaction (Rf value = 0.325 in DCM/EtOH 2:1).

Synthesis Unsubstituted chalcone of dibenzo-18-crown -6 (H) [12]

In round bottom flask dissolve (0.222 g, 2.24 mmole) of acytaldibenzo-18-crown-6-ether in 5ml of absolute ethanol then added (3–5 drops) of (60% KOH in ethanol) slowly with good stirring for (4 hour) then added (0.106 g, 2.12 mmole) of benzyldehyde dissolved it in ethanol absolute was added to the mixture slowly with for 2hr.Then added ice crush from deionized water then filter and let dry at room temperature. Recrystallized by using DCM then filter it and let dry at room temp. TLC used to monitor the time of reaction elute used (Rf = 0.71 in DCM/EtOH 2:1) Melting point (260–270 C). IR (KBr): 2926 (CH), 1597–1600 (CO), 1552, 1454.33, 1396.46, 1382.96, 1328.95, 1290.38, 1060,997,705 cm-1; 1H NMR (chloroform-d6, 400 MHz): δ 8.34 (d, J = 7.3 Hz, 1H), 7.64 – 7.51 (m, 3H), 7.24 – 7.14 (m, 9H), 4.50 – 4.39 (m, 21H), 3.25 (s, 2H) (Fig. 1).

Synthesis of P-OH-chalcone (H1) [12]

In round the bottom flask dissolve (0.222 g, 2.24 mmole) of acytaldibenzo-18-crown-6-ether in 10ml of absolute ethanol then added (3-5) drops of (60% KOH in ethanol). Then, dissolved 0.106 g, 2.12 mmole of p-hydroxy-benzaldehyde in ethanol absolute then added to the mixture slowly and let for (4 hour) hour until ppt. form, added ice piece, neutralized

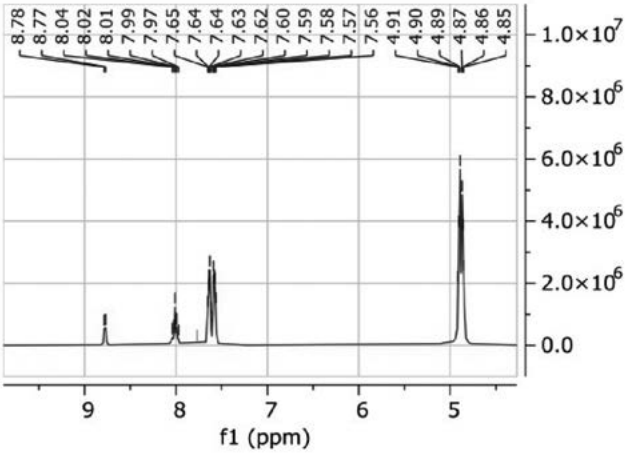

Fig. 1. ^{1}HNMR for chalcone

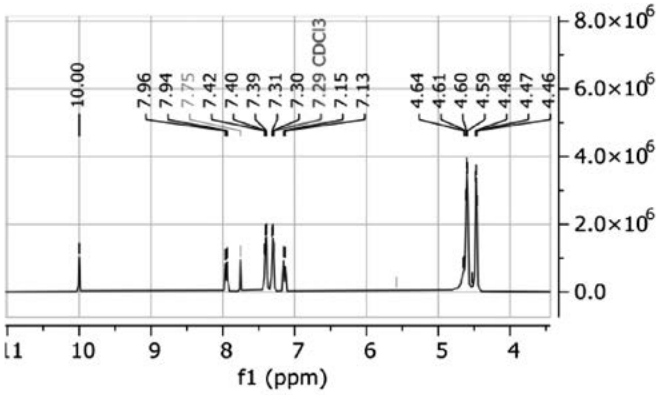

Fig. 2. [1]HNMR for P-OH-Ch.

by HCl then filter and let dry overnight. Then recrystilization by using DCM then filter and let to dry (Rf=0.64) in TLC elute used (DCM/EtOH 2:1) and melting 150 °C. IR (KBr): 2929-2835 (CH), 1595-1649 (CO), 1575- 1452,1377,1359,1253,1132,1060,943,943-742,705,597. [1]H NMR (400 MHz, Chloroform-d) δ 9.80 (s, 1H), 7.76 (d, J = 8.3 Hz, 2H), 7.24 – 7.17 (m, 5H), 7.15 – 7.07 (m, 5H), 6.95 (d, J = 8.3 Hz, 2H), 4.48 – 4.38 (m, 14H), 4.31 – 4.24 (m, 8H) (Fig. 2).

Synthesis of P-NO2-chalcone (H2) [12]

In Round the bottom Flask dissolve (0.222 g, 2.24 mmole) of acytaldibenzo-18-crown-6-ether in 10ml of absolute ethanol then added (3–4) drops of (60% KOH in ethanol), dissolve (0.106 g, 2.12 mmole) of p-nitrobenzaldehyde in ethanol absolute then added to the mixture slowly and let for (4hour) until dark brown ppt. form added ice piece, neutralized by HCl then filter and let dry over night at RT. Recrystallized by using DCM then filter and

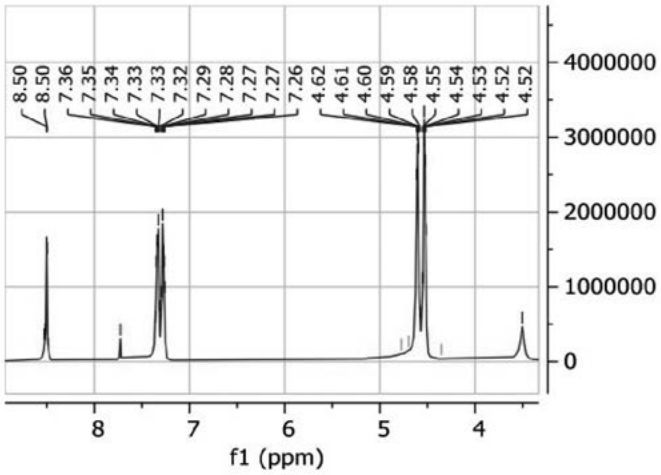

Fig. 3. [1]HNMR FOR P-NO2-Ch.

let dry at RT. Rf=0.7 in TLC elute used (DCM /EtOH 2:1), and melting point (140C-150˚C). IR(KBr): 2924-2854 (CH),1591-1620 (CO),1508-1456,1379,1342,1253,1128,1056.1H NMR (400 MHz, Chloroform-d) δ 8.60 (dd, J =7.9, 2.6 Hz, 1H), 7.40 (ddt, J = 22.3, 6.0, 3.5 Hz, 4H), 4.66 (ddd, J = 31.2, 6.4, 3.4 Hz, 8H), 3.60 (s, 1H) (Fig. 3).

Synthesis P-CL2-Chalcone [12] (H3)

In Round bottom Flask dissolve (0.222 g, 2.24 mmole) of acytaldibenzo-18-crown-6-ether in 10ml of absolute ethanol then added (3-4 drops) of (60% KOH in ethanol), dissolved (0.106 g 2.12mmole) of p-chlorobenzaldehyde in ethanol absolute then added to the mixture slowly and let for (4hour) until ppt. form then added ice piece, neutrilized the product by using HCL, filter and let dry at room temp. TLC used to monitor the time reaction (Rf value = 0.8, in chloroform/ethanol 99%-1%) with melting point (140˚C). IR(KBr):2924-2854 (CH),1595-1600 (CO),1575-1504,1379,1365,1251,1012,945. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.02 (dd., J = 7.9, 2.5 Hz, 1H), 7.31 (d, J = 8.0 Hz, 1H), 7.04 – 6.92 (m, 7H), 4.73 (s, 2H), 4.31 – 4.25 (m, 9H), 4.20 (d, J = 4.9 Hz, 9H) (Fig. 4 and 5).

Antibacterial activity of new chalcone compounds (H, H1, H2, H3)

The antibacterial potential of the prepared Samples (Chalcone. P-OH-Ch, P-NO2-Ch, P-Cl2-Ch. Azithromycin) was investigated against Gram's negative and Gram's positive

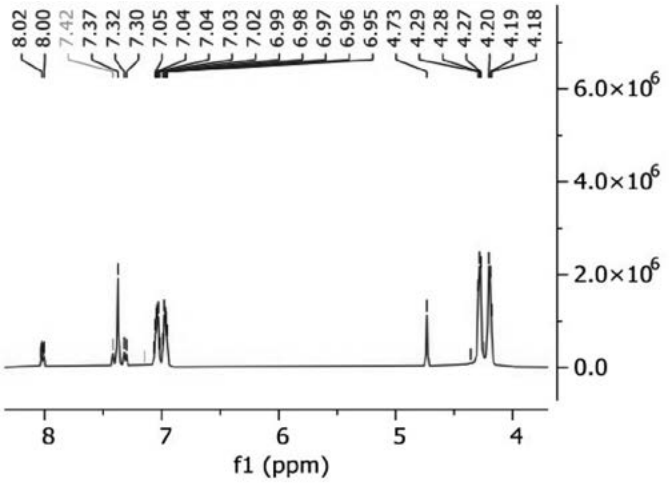

Fig. 4. ^{1}HNMR for p-cl-Ch.

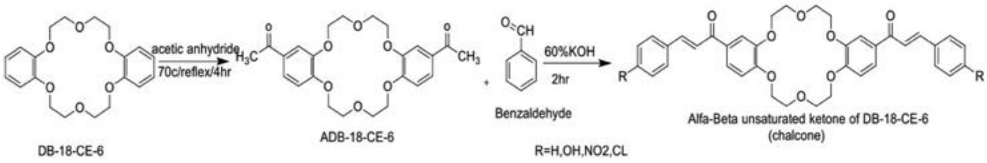

Fig. 5. Mechanism for alfa-beta unsaturated ketone synthesis

bacterial strains using agar well diffusion assay [13]. About 20mL of on Muller–Hinton (MH) agar was aseptically poured into sterile Petri dishes. The bacterial species were collected from their stock cultures using a sterile wire loop. After culturing the organisms, 6 mm-diameter wells were bored on the agar plates using of a sterile tip. Into the bored wells, different concentrations of the Samples (H, H1, H2, H3) were used. The cultured plates containing the Samples (H, H1 H2 H3 Azithromycin), and the test organisms were incubated overnight at 37°C before measuring and recording the average the zones of inhibition diameter [14].

Statistical analysis

Data were statically analysis using Graphpad prism program. Data are represented as mean ± SD of three experiments. Indicate statistically significant difference at $p<0.05$ [15].

■ RESULTS

Crown ethers can be functionalized by a variety of chemical processes due to their high solubility. Thus, numerous crown ether derivatives have been manufactured. Conformational flexibility is one of the distinguishing characteristics of crown ethers. the focus is on the acylation of dibenzo- 8-crown-6 and its subsequent application in the synthesis of chalcone compounds. The goal is to explore the potential antibacterial activity of these compounds. By utilizing dibenzo-18-crown-6 as a precursor, it is expected to obtain chalcone derivatives with enhanced biological activity and potentially novel mechanisms of action.

In this experiment first synthesis acetyl dibenzo-18-crown ether-6 by Friedel-Crafts reaction, was discovered by Friedel and Crafts in (1877). If a Lewis acid is added to an acyl halide in the presence of an aromatic ring, an electrophilic aromatic substitution reaction can occur whereby the acyl group adds to the aromatic ring (with loss of HX) (Fig. 6).

The reaction typically involves the use of an acylating agent, such as acetic anhydride, along with a Lewis acid catalyst, such as phosphoric acid by electrophilic attack. In next step synthesis chalcone by Claisen-Schmidt reactions being carried employing sodium hydroxide as the base in conjunction with benzaldehydes. It is a reaction between

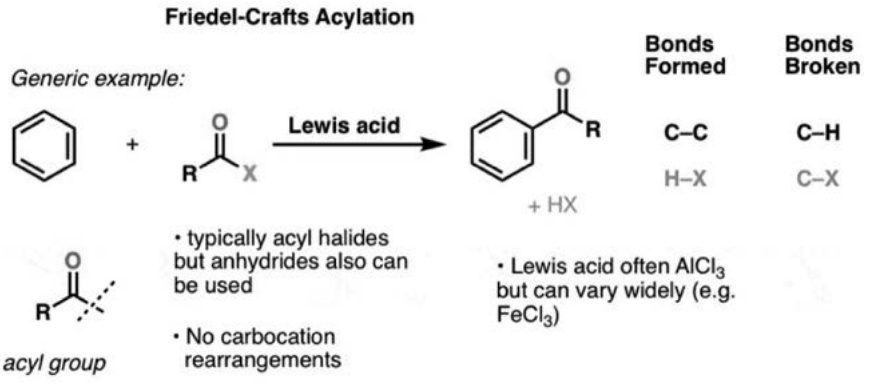

Fig. 6. General mechanism of Friedel-Crafts Acylation

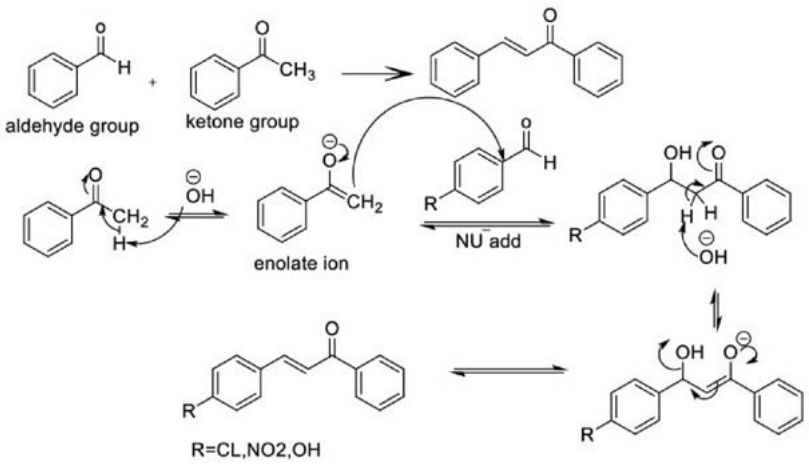

Fig. 7. General mechanism for synthesis of alfa-beta unsaturated ketone

an aldehyde and a ketone that leads to the formation of an α,β-unsaturated carbonyl compound, also known as a α,β-unsaturated ketone or chalcone (Fig. 7).

Table explain the antibacterial activity of chalcone compounds (H, H1, H2, H3) [H=Chalcone, H1=P-OH-Ch, H2=P-NO2-Ch, H3=P-CL2-Ch]. As in the table (1) shown the inhibition zone for the chalcone compounds with different concentration against Streptococcus, Staphylococcus and Proteus. We noticed in the concentrations 50 ppm, 100 ppm, 150 ppm, 200 ppm, 400 ppm, the inhibition zone for chalcone in (400 ppm) against Streptococcus has inhibition zone (22 mm) larger than Azithromycin in (400 ppm) (have inhibition zone (14mm)) that is mean the effect of chalcone have a good

Antibacterial analysis (Zone of inhibition)

Sample		A	B (50 ppm)	C (100 ppm)	D (150 ppm)	E (200 ppm)	F (ppm)
Streptococcus	chal	6	15	17	18	20	22
	P-OH	6	14	17	18	19	20
	P-NO2	6	14.5	15.5	16.5	17.5	18.5
	P-CL2	6	17	19	21	23	25
Azithromycin		6	8	9	10	13	14
Staphylococcus	Chal	6	8	9	10	13	15
	P-OH	6	6	7	9	12	13
	P-NO2	6	7	8	9	12	13
	P-CL2	6	6	7	8	10	13
Azithromycin		6	7	9	10	12	14
Proteus	Chal	6	7	7	9	11	13
	P-OH	6	6.5	7	10	11	12
	P-NO2	6	6	7	9	12	13
	P-CL2	6	6	7	9	12	13
Azithromycin		6	12	13	14	15	16

antibacterial activity against Streptococcus. Also inhibition zone of chalcone in (400 ppm) against Stapholococcus is (15 mm) while for Azithromycin the inhibition zone is (14 mm). And the inhibition zone for chalcone against Proteus is (13 mm) and for Azithromycin is (16 mm).

■ DISCUSSION

The reaction is a type of carbon-carbon bond forming condensation reaction and is an important tool for the synthesis of many organic compounds [8, 9]. The acylation reaction successfully yeilded chalcone compounds from the reaction between dibenzo18-crown ether-6 and benzaldehyde. The progress of reaction was monitored for chalcone compounds:

Chlacone of DB-18-CE-6 by TLC Rf=0.71 elute used (DCM/EtOH 2:1), Melting point (260-270) and spectroscopic data where characterized by. FT-IR: 2926 (CH), 1597-1600 (CO), 1552, 1454.33, 1396.46, 1382.96, 1328.95, 1290.38,1060,997,705 cm-1; 1H NMR (chloroform-d6, 400 MHz): δ 8.34 (d, J = 7.3 Hz, 1H), 7.64 – 7.51 (m, 3H), 7.24 – 7.14 (m, 9H), 4.50 – 4.39 (m, 21H), 3.25 (s, 2H).

P-OH-Ch. TLC Rf=0.64 elute used (DCM/EtOH 2:1), and melting150c. IR(KBr): 2929-2835 (CH), 1595-1649 (CO), 1575-1452,1377,1359,1253,1132,1060,943,943-742,705,597. ^{1}H NMR (400 MHz, Chloroform-d) δ 9.80 (s, 1H), 7.76 (d, J = 8.3 Hz, 2H), 7.24 – 7.17 (m, 5H), 7.15 – 7.07 (m, 5H), 6.95 (d, J = 8.3 Hz, 2H), 4.48 – 4.38 (m, 14H), 4.31 – 4.24 (m, 8H).

P-NO2-Ch, Rf=0.7 TLC elute used (DCM/EtOH 2:1) and melting point(140C-150˚C). IR(KBr):2924-2854 (CH),1591-1620 (CO),1508-1456,1379,1342,1253,1128,1056.1H NMR (400 MHz, Chloroform-d) δ 8.60 (dd, J = 7.9, 2.6 Hz, 1H), 7.40 (ddt, J = 22.3, 6.0, 3.5 Hz, 4H), 4.66 (ddd, J = 31.2, 6.4, 3.4 Hz, 8H), 3.60 (s, 1H).

P-CL2-Ch, TLC used to monitor the time reaction (Rf value=0.8,inDCM/EtOH 2:1), Melting Point (140˚C). IR(KBr):2924-2854 (CH),1595-1600 (CO),1575-1504,1379,1365,1251,1012,945. 1H NMR (400 MHz, Chloroform-d) δ 8.02 (dd., J = 7.9, 2.5 Hz, 1H), 7.31 (d, J = 8.0 Hz, 1H), 7.04 – 6.92 (m, 7H), 4.73 (s, 2H), 4.31 – 4.25 (m, 9H), 4.20 (d, J = Hz, 9H).

The antibacterial potential of the prepared Samples (H, H1, H2, H3, Azithromycin) was investigated against Gram's negative and Gram's positive bacterial strains using agar well diffusion assay. 6 mm-diameter wells were bored on the agar plates using of a sterile tip. Into the bored wells, different concentrations of the Samples (H, H1, H2, H3, Azithromycin) were used. The cultured plates containing the Samples (H, H1, H2, H3, Azithromycin) and the test organisms were incubated overnight at 37°C before measuring and recording the average zones of inhibition diameter.

The rise in antibiotic resistance that can be attributed to a number of different sources has led researchers to explore novel chemicals that are effective against infections that are resistant to several drugs. In this experiment, chalcone-based compounds displayed a diversity of pharmacological properties, and their derivatives possess a high degree of structural variance. As a result, there is a critical demand for the development of novel antibacterial medication candidates [16].

Antibiotic resistance is a rising problem, reducing the effectiveness of macrolides, the first-line treatment for many diseases. Staphylococci frequently exhibit multiple molecular determinants of resistance to macrolides. Research into new macrolide antibiotics could help in the fight against drug-resistant bacteria, hence it's crucial to understand the

processes of macrolide resistance. The comparative analysis with azithromycin indicated the potential of chalcone and its derivative as an effective alternative in resist bacterial infection [9, 17].

We noticed in the concentrations, the inhibition zone for chalcone in (400 ppm) against Streptococcus has greater inhibition zone than Azithromycin in that is mean the effect of chalcone have a good antibacterial activity against Streptococcus. Also inhibition zone of chalcone against Stapholococcus is larger than for Azithromycin whereas the inhibition zone for chalcone against Proteus is smaller than for Azithromycin. Determine the minimum inhibitory concentrations (MICs) of chalcone compounds against various pathogens to assess their potency and efficacy. Comparative studies with known antibiotics like Azithromycin can provide valuable insights into their relative effectiveness [6].

■ CONCLUSION

The acylation reaction successfully yielded chalcone and its derivatives from the reaction between acylationdibenzo18-crown-6 and benzaldehyde. The synthesis of chalcone holds significance as it represent a novel approach to the development of antibacterial agents. The evaluation of antibacterial activity revealed that the synthesized chalcone exhibited notable inhibitory effects against a range of bacterial strains Streptococcus, Staphylococcus, and Protus. The results indicated that chalcone and its derivative have the potential to be developed, as an alternative antibiotic agent, which could contribute to addressing the growing issue of antibiotic resistance. The comparative analysis with azithromycin shows strong inhibition into these bacteria, that indicated the potential of chalcone and its derivative as an effective alternative in resisting bacterial infection.

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