EGYPTIAN PHARMACEUTICAL JOURNAL EPJ

Original Article 1

Synthesis, antibacterial activity and molecular docking study of new chalcones derived from 5-formyl-1,4-dihydropyridine

Ali G. Swadi[*1,2], Ali K Mohammed[2], Tahseen A. Alsalim[1],Aymen G. Faisal[3]

[1]Department of Chemistry, College of Education for Pure Sciences, University of Basrah, Basrah, Iraq
[2]Department of Chemistry, Faculty of Pharmacy, University of Basrah, Basrah, Iraq
[3]Department of Applied Marine Sciences, College of Marine Sciences, University of Basrah, Basrah,, Iraq
[*]Corresponding author: Ali G. Swadi , Msc, Department of Chemistry, College of Education for Pure Sciences, University of Basrah, Basrah, Iraq
Email: ali.swadi@uobasrah.edu.iq
ORCID ID: 0000-0002-5828-0565

Received: 24 January 2025
Revised: 2 March 2025
Accepted: 5 March 2025
Published: July 2025

Egyptian Pharmaceutical Journal 2025, 00: 00-00

Background
Chalcones are extremely desirable compounds due to their simple structure, ease of production, and potential biological functions, such as antibacterial, antioxidant, anticancer, etc.

Objective
To synthesis, examine, and select new chalcones derived from 5-formyl-1,4-dihydropyridine, and evaluate the ability as antimicrobials using *Staphylococcus aureus*, *Escherichia coli,* and molecular docking.

Materials and methods
Chalcone derivatives of 1,4-Dihydropyridine (1,4-DHPs) were synthesized by a condensation process. Fourier-transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance (1H NMR), carbon-13 nuclear magnetic resonance (13C NMR), and mass spectrometry were employed to analyze all produced compounds, including ethyl 6-chloro-5-formyl-2-methyl-4-(3-chlorophenyl)-1,4-dihydropyridine-3-carboxylate and its acetophenone derivatives. The efficacy of the produced compounds as antibacterial agents was established through assessment.

Results and conclusion
The multidrug-resistant microorganisms *Staphylococcus aureus* and *Escherichia coli*, chemicals Ch1 and Ch2 demonstrated significant antibacterial efficacy. Compound Ch1 demonstrated a notable 31 mm zone of inhibition against *S. aureus* at a concentration of 70 µg, and a substantial 30 mm inhibition zone against *E. coli* at the identical concentration. The results exceeded the reference drugs Cephalexin 30 µg/ml and Ciprofloxacin 5 µg/ml. The compounds Ch$_1$, Ch$_2$, Ch$_3$ and Ch$_4$ at concentration of (30, 50 and 70) µg exhibited statistically significant ($P > 0.05$). In silico studies were used to analyze the chalcone derivatives under study. Their potential as antibacterial agents were assessed by analyzing their interactions with the receptor proteins for *Staphylococcus aureus* (PDB ID: 1N67) and *Escherichia coli* (PDB ID: 6G9S). These substances were found to bind to the receptor effectively. The compounds with the highest activity, according to molecular docking studies, had the highest binding scores with the *Staphylococcus aureus* protein.

Keywords: 1,4-dihydropyridine, Chalcone, Vilsmeier – Haack reaction, antibacterial activity.

Egypt Pharmaceut J 00:00–00
© 2025 Egyptian Pharmaceutical Journal
1687-4315

Introduction

Global research interest in chalcone chemistry is intensifying. Tambor and Kostanecki (1899) coined the name "chalcone". Chalcone is alternatively referred to as benzylidene acetophenone or benzyl acetophenone. Chalcone is an α, β-unsaturated ketone consisting of two benzenoid rings linked by a three-carbon aliphatic chain. The rings are replaced by unique groups. The linear configuration and pronounced electrophilicity of the α,β-unsaturated ketone system have been shown [1-4]. Chalcones can be manufactured by two reactions: the Claisen-Schmidt condensation and the base-catalyzed aldol condensation involving appropriate aldehydes and ketones, or through an acid-catalyzed process followed by dehydration [5, 6].

The backbone of chalcone is found to be very effectual as intermediate for the production of substances with clinical potential against miscellaneous diseases [7]. Some applications of chalcones have been published for their biological actions [8,9]. Chalcones (α,β-unsaturated ketones) have many proven biological effects[10] such as anticancer[11,12], antifungal[13,14], anti-inflammatory[15], neuroprotective[16], antimalarial[17,18], antibacterial[19], antilipidemic[20], antihyperglycemic [21], antiplatelet[22] and antimycobacterial[23]. Chalcones with an N-heterocyclic moiety such as pyridine, pyrazoline, dihydropiridine, and quinolone scaffolds take the role of space of medicine[24]. Regarding the antimicrobial activity of 1,4-DHPs, some derivatives have also been exhibited to be powerful antitubercular agents [25-28] with capability to inhibit both Gram-negative and Gram-positive bacteria [29,30].

Generally, the importance of having 1,4-DHPs in the structure of synthesized chalcones is that it may improve the biological activity of these synthesized compounds, like the presence of a 1,4-DHPs ring and no substitution at N atom and low weight droop (almost alkyl groups) at the positions C$_2$ and C$_6$, and phenyl ring or substituted phenyl ring. Additionally, chiral center in C$_4$ position as well as presence ester groups in C$_3$ and C$_5$ positions, all these conditions supported sufficient biological activity by their electronic and steric effects [31, 32]. The purpose of this work is to investigate some novel substituted chalcones based on 1,4-DHPs derived from 5-formyl-1,4-dihydropyridine-3-carboxylate. We also investigated the effect of substituents on the biological activity of these compounds.

Materials and methods

Instruments

Chemical substances and solvents were obtained from Merck, Sigma, and Fischer, and were of the utmost purity. The solvent DMSO-d6 and the internal standard Si(CH3)4 (TMS) were utilized. The IR spectra are recorded on a KBr disc with a Shimadzu FTIR (Affinity-1) within the 4000-400 cm-1 range. The Brucker 400 spectrometer was utilized to investigate the 1H and 13C NMR spectra. The Agilent technology (HP) model 5973 was evaluated for EI-MS using electron impact ionization at 70 eV. The melting point was ascertained using the melting point/SMP40 instrument.

Chemistry section Synthesis of ethyl 2-methyl-4-(3-chlorophenyl) -6-oxo-1,4,5,6 tetrahydropyridine -3-carboxylate (A)

One-pot, 3-chlorobenzaldehyde (1.40 g , 10 mmol), Meldrum's acid (1.44 g , 10 mmol), ethyl acetoacetate (1.30 g , 10 mmol) and ammonium acetate (0.809 g, 10.5 mmol) in (10 ml) glacial acetic acid was refluxed for 12 h with monitored by TLC . Mixture of reaction was cooled; the precipitated was collected by filtration, recrystallized from methanol. Yield 60%, and m.p 155-157 °C , ^{1}H-NMR (400 MHz, DMSO-d$_6$) δ 10.07(s, 1H, NH), (8.09 – 7.63)(m, 4H, Ar-H) , 4.30 (broad s, 1H , C-H DHPo ring) , 4.01 (broad s, 2H, O<u>CH$_2$</u>CH$_3$) , 3.02 (dd, 1H, CH$_2$ DHPo ring) , 2.35 (s, 3H, CH$_3$ attached with DHPo ring) , 1.09 (t, 3H, <u>CH$_3$</u>CH$_2$O). ^{13}C-NMR(100 MHz, DMSO-d$_6$) δ, 169.8, 166.6, 104.5, 60.0, 38.17, 37.62, 18.72, 14.5 . IR(υ, cm^{-1}) : 3234 (NH), 3132 (C-H sp^2), 2819 (C-H sp^3), 1697 (C=O ester), 1631 (C=O cyclic amid), 1529 (C=C ring), 1350 (C-N), 1199 (C-O) . MS: EI (70 Ev, m/z), 293.2[M$^+$], 287 (base peak), 259.1, 231.2, 115.2, 102.2.

Synthesis of ethyl 6-chloro-5-formyl-2-methyl-4-(3-chlorophenyl)-1,4-dihydropyridine-3-carboxylate (A-CHO)

phosphoryl chloride POCl$_3$ (1.5 ml, 16 mmol) was added dropwise at room temperature to a mixture of anhydrous N, N-dimethylformamide (1.24 ml, 16 mmol) and dry dichloromethane for 30 min. then a solution compound A (1.216 g, 4 mmol) in 16 ml of dry dichloromethane was added to the reaction mixture; the reaction continued stirring at room temperature for 24 hrs. A solution of sodium acetate (16 g in 24 ml of water) was slowly added. After stirring for 1 hr, the product was extracted by DCM. The solvent was dried and evaporated. the product collected and recrystallized from methanol. Light-yellow powder. Yield 69%, m.p 172-174° C , IR (υ, cm^{-1}) : 3323 (NH), 2854 and 2762 (CHO), 1701 (C=O ester), 1649 (C=O aldehyde) . ^{1}H-NMR (400 MHz, DMSO-d$_6$) δ, 10.5 (S,1H, NH), 9.6 (s, 1H,CHO), 8.0 – 7.5 (m, 4H, Ar-H), 5.0 (s, 1H, CH-DHP ring), 4.0 (q, 2H, *J* =8 ,<u>CH$_2$</u>-O ester) , 2.3 (s, 3H, CH$_3$ attach with DHP ring) , 1.1 (t, 3H, *J* = 8 , <u>CH$_3$</u>-CH$_2$O) . ^{13}C-NMR (100 MHz, DMSO-d$_6$) δ , 187.0, 166.1, 148.2, 134.6, 38.6, 18.3, 14.3 . MS : EI (70 Ev, m/z) , 339.3 [M$^+$], 333.3, 228.2 (base peak), 200.2, 154.2, 118.2, 76.1.

General procedure of synthesis of chalcone analogues Ch(1-4)

A mixture of substituted acetophenone (2 mmol), 5-formyl-1,4-dihydropyridine (A-CHO) (0.678 g, 2 mmol) and ammonium acetate (0.77 g, 15 mmol) in 10 ml absolute ethanol was stirred at 50-60 °C for 12 hrs. The reaction was monitored by TLC. The colored solid was separated, filtered, and recrystallized from ethanol.

Ethyl(E)-6-chloro-5-(3-(2-fluorophenyl)-3-oxoprop-1-en-1-yl)-2-methyl-4-(3-chlorophenyl) - 1,4-dihydropyridine-3-carboxylate : (Ch$_1$)

Light greenish yellow crystal from 2-fluoro acetophenone 0.276 g , yield 70% , m.p 222-224 °C , IR (υ, cm^{-1}) : 3207 (NH), 3050 (CH-sp^2), 2981 (CH-alkene), 2931 (CH-sp^3), 1708 (C=O ester), 1666 (C=O ketone), 1627 (C=C), 1595 (C=C Ar-ring), ^{1}H-NMR (400 MHz, DMSO-d$_6$) δ 10.1 (s, 1H, NH), 8.04 (d, 1H, HC=C trans , *J* = 16 HZ), 7.49 (d,1H, HC-C=O, J = 16 HZ), 5.0 (s, 1H, CH DHP ring), 3.9 (q, 2H, $\underline{CH}_2$-O), 2.3 (s, 3H, CH_3 attach with DHP ring), 1.1 (t, 3H, $\underline{CH}_3CH_2O$) . ^{13}C-NMR (100 MHz, DMSO-d_6) δ, 187.5, 168.8, 166.1, 148.6, 124.9, 60.0, 43.0, 18.5, 14.4 . MS : EI(70 Ev, m/z) , 459.1[M^+], 356.2, 251.2, 209.2 (base peak), 181.1, 163.1, 92.1 .

Ethyl(E)-6-chloro-5-(3-(4-nitrophenyl)-3-oxoprop-1-en-1-yl)-2-methyl-4-(3-chlorophenyl)-1,4-dihydropyridine-3-carboxylate : (Ch_2)

Yellow crystal from 3-nitro acetophenone 0.398 g, yield 72% , m.p 216-218 °C , IR (υ, cm^{-1}) : 3207 (NH), 3053 (CH-sp^2), 2981 (CH-alkene), 2931 (CH-sp^3), 1710 (C=O ester), 1666 (C=O ketone), 1627 (C=C), 1595 (C=C Ar-ring), ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.1 (s, 1H, NH), 8.04 (d, 1H, HC=C trans , J = 16 HZ), 7.54 (d,1H, HC-C=O, J = 16 HZ), 5.0 (s, 1H, CH DHP ring), 3.9 (q, 2H, $\underline{CH}_2$-O), 2.3 (s, 3H, CH_3 attach with DHP ring), 1.1 (t, 3H, $\underline{CH}_3CH_2O$) . ^{13}C-NMR (100 MHz, DMSO-d_6) δ, 188.1, 168.8, 166.1, 148.6, 127.2, 60.0, 43.0, 19.0, 14.6 . MS : EI(70 eV, m/z) , 486.3[M^+], 493.3, 356.3, 330.3, 302.2, 258.2, 209.2 (base peak), 181.1, 163.1, 92.1 .

Ethyl(E)-6-chloro-5-(3-(2-chlorophenyl)-3-oxoprop-1-en-1-yl)-2-methyl-4-(3-chlorophenyl) -1,4-dihydro pyridine-3-carboxylate : (Ch_3)

Yellow crystal from 2-chloro acetophenone 0.308 g , yield 76% , m.p 241-242 °C , IR (υ, cm^{-1}) : 3207 (NH), 3050 (CH-sp^2), 2981 (CH-alkene), 2931 (CH-sp^3), 1708 (C=O ester), 1666 (C=O ketone), 1629 (C=C), 1595 (C=C Ar-ring), ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.1 (s, 1H, NH), 8.04 (d, 1H, HC=C trans, J = 16 HZ), 7.57 (d,1H, HC-C=O , J = 16 HZ), 5.1 (s, 1H, CH DHP ring), 3.9 (q, 2H, $\underline{CH}_2$-O), 2.36 (s, 3H, CH_3 attach with DHP ring), 1.1 (t, 3H, $\underline{CH}_3CH_2O$) . ^{13}C-NMR (100 MHz, DMSO-d_6) δ, 189.5, 168.8, 166.1, 148.6, 125.9, 60.0, 43.0, 18.5, 14.4 . MS: EI(70 eV, m/z) , 475.1[M^+], 356.3, 330.3, 251.2, 209.2(base peak), 181.2, 163.2, 92.1 .

Ethyl(E)-6-chloro-2-methyl-4-(3chlorophenyl) -5-(3-oxo-3-(*m*-tolyl)propo-1-en-1-yl)-1,4-dihydropyridine-3-carboxylate : (Ch_4)

Orange crystal from 3-methyl acetophenone 0.268 g , yield 82% , m.p 215-217 °C , IR (υ, cm^{-1}) : 3255 (NH), 3053 (CH-sp^2), 2981 (CH-alkene), 2931 (CH-sp^3), 1708 (C=O ester), 1666 (C=O ketone), 1629 (C=C), 1595 (C=C Ar-ring), ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.1 (s, 1H, NH), 8.03 (d, 1H, HC=C trans , J = 16 HZ), 7.57 (d,1H, HC-C=O , J = 16 HZ), 5.0 (s, 1H, CH DHP ring), 3.9 (q, 2H, $\underline{CH}_2$-O), 2.35 (s, 3H, CH_3 attach with DHP ring), 2.30 (s, 3H, CH_3 Ar-ring), 1.1 (t, 3H, $\underline{CH}_3CH_2O$) . ^{13}C-NMR (100 MHz, DMSO-d_6) δ, 191.6, 168.1, 166.2, 148.6, 127.5, 60.0, 43.0, 23.7, 18.5, 14.4 . MS: EI(70 eV, m/z) = 455.2[M+], 356.3, 330.3, 251.2, 209.2 (base peak), 181.1, 163.1, and 92.1.

In vitro antibacterial activity

The bactericidal efficacy of the produced chemicals (A, A-CHO, Ch1, Ch2, Ch3, and Ch4) was assessed on Muller Hinton agar medium utilizing the Disc diffusion Bauer-Kirby method. Two clinical isolates were used (*Staphylococcus aureus* and *Escherichia Coli),* obtained from the research laboratory of the college of Pharmacy, University of Basrah, Department of Laboratory Science. The nutrient agar, Mckonky agar and Muller–Hinton agar were used according to the manufacturer's instructions. The isolates were diagnosed by conventional biochemical reactions such as, catalase, oxidase, lactose fermentation, gram stain and microscopic examination [33].

Biological activity assay

Disc diffusion Baueyer – Kirby method was used, where the discs were sterilized by ultraviolet rays. Then, these discs were immersed in synthesized compounds solution at concentration (10 , 30 , 50 and 70) µg/ml of DMSO solvent after were activated in the liquid culture medium by Nutrient Broth for 3 hours . The antibiotic discs (Cephalexin 30 µg/ml and Ciprofloxacin 5 µg/ml) were used as standard drugs from Bioanalysa company. Following the addition of synthetic chemicals and standard medicinal substances to the growth plates, the plates were incubated at 37 ^{0} C for 24 hours. Subsequently, we measured the zones of bacterial growth inhibition for each chemical in millimeters (mm). The IC_{50} values were estimated based on the method by Soothill et al. [34].

Molecular docking studies

The Protein Data Bank (PDB) (https://www.rcsb.org/) was utilized to collect *Staphylococcus aurous* (PDB ID: 1N67) and *Escherichia coli* proteins (PDB ID: 6G9S). The molecular docking was performed using the web-based Swissdock application (https://www. swissdock.ch/docking). The data were scored and ranked by full fitness (kcal.mol^{-1}), and the estimated Gibbs free energy ΔG (kcal.mol^{-1}) was used to indicate spontaneous binding. MD simulations were performed using the Schrodinger-Desmond module. The water-saturated solvated system was created using the Desmond System Builder tool. The simulation box was orthorhombic, with periodic boundary conditions positioned 10 units from the protein's surface. Counter ions were supplied in sufficient quantities to balance the water-saturated solvated system. Prior to starting the simulation, an equilibration approach was used to confirm that the system was stable. The simulation lasted 100 nanoseconds, with a temperature of 310 kelvin and an ambient pressure of 1.013 bar. A simulation interaction diagram was used to thoroughly examine the MD simulation findings. Root Mean Square Deviation (RMSD) was analyzed. The ligand interaction figure, the amino acid residues that interact with the ligand in each trajectory frame, and the trajectory of different ligand attributes [35-37].

Statistical analysis

Statistical analysis was performed to compare the significant and insignificant differences in the microbial inhibition of the synthesized and standard compounds using Analysis of Variance (ANOVA).

Results and discussions

Spectral diagnosis, the complete set of spectral data of studied compounds was gathered in the supplementary materials. In this study, prepared 1,4-dihydropyridine (DHPo) one-pot reaction. the 5-formyl-1,4-dihydropyridine was synthesized by Vilsmeier-Haack reaction. Chalcones were synthesized by Claisen – Schmidt reaction. This reaction entailed the interaction of 5-formyl-1,4-dihydropyridine-3-carboxylate (A-CHO) with several acetophenones in the presence of NH4OAc. The yields of the resultant chalcones range from 66% to 82%. All produced compounds were characterized using spectroscopic techniques including infrared, 1H-NMR, 13C-NMR, and EI-Mass. A medium band within the range of (3323–3207) cm^{-1} was seen in the IR spectra of all produced compounds, attributable to the NH group of the DHPs ring. The carbonyl group of the ester is responsible for the absorption band in the region of 1710–1697 cm⁻¹ [35]. Additionally, IR spectra showed a band at 1649 cm^{-1} back to C=O aldehyde in the A-CHO compound and the disappearance of the band at 1631 cm^{-1} back to C=O cyclic amide. In chalcone compounds (Ch1-Ch4), we show some important bands, such as the band in 1666 cm^{-1} corresponding to C=O and the band in 1627 cm^{-1} corresponding to C=C of chalcones, respectively. The band at 2981 cm-1 indicated the presence of HC=CH of α-H and β-H. The ^{1}H-NMR spectra of all synthesized compounds were appeared singlet signal within the range δ (10.50 – 10.07) ppm attributed to –NH proton of DHPs ring, singlet signal within the range δ (5.1 – 4.3) ppm indicated to –CH proton in DHPs ring[38,39]. The most important feature of the A-CHO compound, show a distinctive signal at δ 9.6 ppm attributed to –CHO proton [40] which disappeared during the synthesis of chalcones. Chalcone molecules display a signal in the region of δ 7.57–7.47 ppm, whereas the signal in the range of 8.04–8.04 ppm is ascribed to α-H and β-H, respectively [41]. This signifies the synthesis of trans chalcones. The chemical shifts of ^{13}C-NMR spectra were displayed in the expected regions. The mass spectra of synthesized compounds showed the peaks of molecular ions at the anticipated m/z values, and the chalcone compounds were characterized by a base peak at 209.2 m/z ethyl 6-chloro-5-ethynylnicotanate $[C_{10}H_8ClNO_2]^+$.

Antibacterial activity results

Chalcones are known to display a variety of biological activities, which are affected by the replacements attached to the chalcone ring [42]. The Bauer-Kirby disc diffusion method was utilized to assess the biological activity of the produced compounds in this research. The zone of inhibition (ZOI) was employed to quantify the outcomes of each drug. The sensitivity of *S.aureus* to the synthesized compounds was tested using different concentrations (10, 30, 50 and 70) µg/ml and show different responses, Fig. 1. In 10 µg/ml concentration, the results of A and A-CHO compounds show no response to inhibition of growth of the mentioned bacteria while, the Ch_1 , Ch_2 , Ch_3 and Ch_4 show a relative inhibition zone. In 30 and 50 µg/ml, the results of the synthesized chalcone derivatives were good results. At a dosage of 70 µg/ml, the Ch1 and Ch2 compounds exhibited the highest antibacterial effectiveness against gram-positive bacteria (*S. aureus*), with a statistically significant difference ($P > 0.05$). Chalcone derivatives at the highest concentration against the examined pathogen which have mighty inhibition zone exceeding both reference drugs, Cephalexin (30 µg/ml) and Ciprofloxacin (5 µg/ml), Fig. 3 and Table 1. The sensitivity of *E. Coli* to the synthesized compounds was examined by using different concentrations (10, 30, 50 and 70) µg/ml and show different results, Fig. 2. In 10 µg/ml concentration, antimicrobial process of A and A-CHO compounds show no response to inhibition of growth of gram negative bacteria (*E. coli*) but, the Ch_1, Ch_2 , Ch_3 and Ch_4 compounds show an important inhibition zone. At concentrations (30, 50) µg/ml,the synthesized chalcone derivatives gave a significant results . Especially, the concentration at 70 µg/ml, the synthesized chalcones show highest potent as antibacterial efficacy against E. coli with a significant difference $P > 0.05$ and the highest value were also for Ch1 and Ch2 compounds, Table 2. Chalcone derivatives at the 70 µg/ml concentration against the tested pathogen which have excellent inhibition zone and more than both reference drugs, Cephalexin (30 µg/ml) and Ciprofloxacin (5 µg/ml), Fig. 3. Soothill et al.'s [34] calculation method was used to estimate IC_{50} values for potential compounds CH1 and CH2. Compound CH1 had IC_{50} values of 23.20 µg/ml for

S. aureus and 20.90 µg/ml for *E. coli*. Compound CH2 had a IC_{50} values of 23.60 µg/ml against *S. aureus* and 20.40 µg/ml against *E. coli*, as shown in Fig. 4. The compound Ch1, which contains an electron-withdrawing fluoro group at position 2 in the aromatic ring (ketone), has been demonstrated to be a robust antibacterial agent [43]. The compound's antibacterial activity is activated by the presence of a fluoro group on either ring in chalcone[44,45]. Additionally, the compound Ch2 exhibited antimicrobial activity due to the electron-withdrawing NO_2 group at position 4 [46,47]. Additionally, the compound Ch_3 , presence chloro group in this synthesized chalcone give a good potent antibacterial agent and great results of efficacy in inhibiting the microbial strains which consistent with previous studies[48]. Konduru et al. (2013) substantiated this information and its consequences by demonstrating that the antibacterial activity of the hybrid Halo-chalcone (Cl, F, Br) had considerable efficiency against various microorganisms [49]. In Ch_4 compound, zone of inhibition against *S. aureus* and *E. coli* was good compared with standard drugs where the composition characterized of Ch_4 by the presence of methyl group in position 3 at aromatic ring of part ketone which give significant antibacterial agents [50].

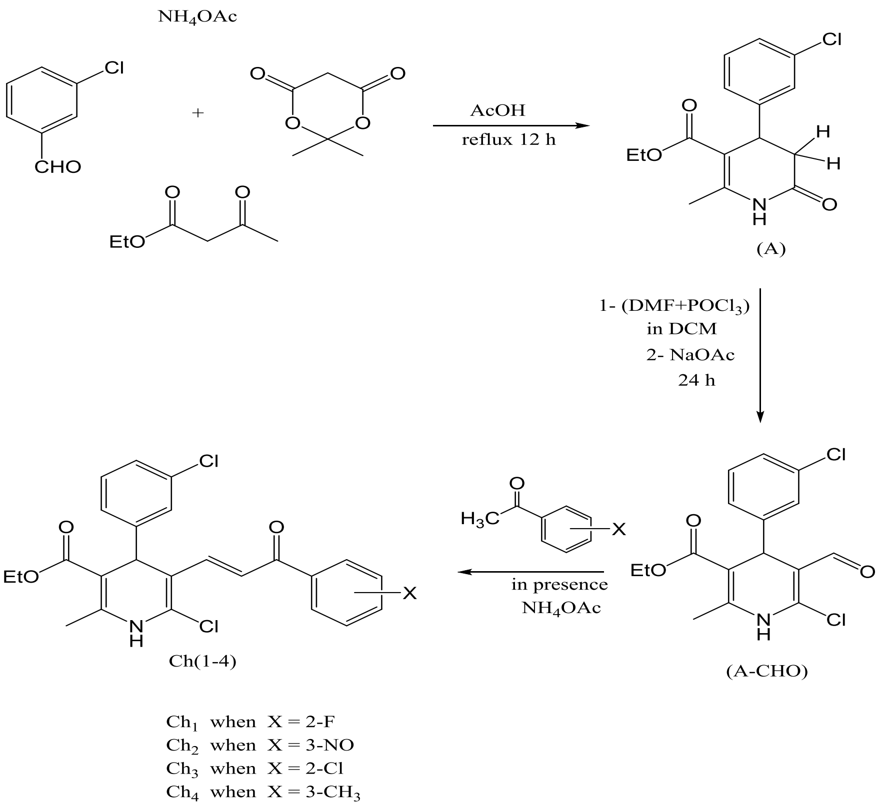
Structure – activity relationship (SAR)

Structure-activity relationship (SAR) studies object to explain the structural properties of chalcone derivatives that are very important for their antibacterial activity and selectivity [51]. SAR studies have disclosed that substitution design on the chalcone scaffold, as well as position and nature of additional chemical moieties, remarkably impact the antibacterial activity of chalcone [52]. For example:

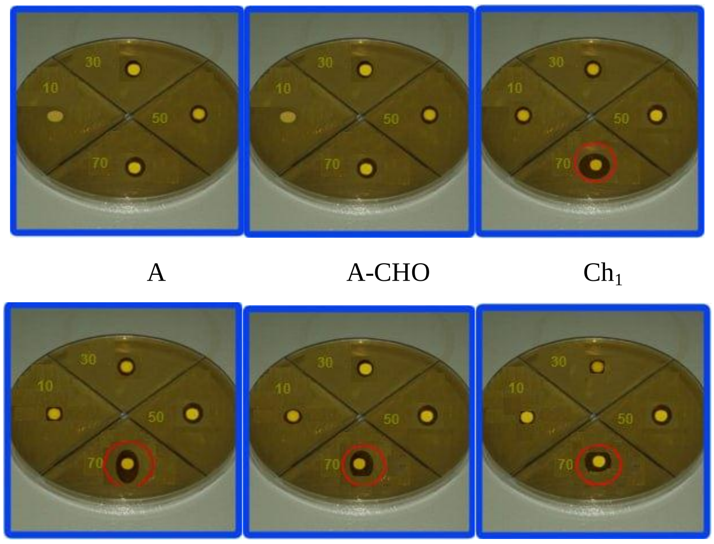
1) Ch_1 (2-fluoro substitution): the fluoro group considered bioisostere, in addition to strengthens hydrogen bonding with key residues which enhances inhibitory potency.
2) Ch_2 (4-nitro substitution): the nitro electron – withdrawing group and strengthens hydrogen bonding often enhance antibacterial potency by improving membrane permeability and binding affinity to microbial target[53].
3) Ch_3 (2-chloro substitution): the chloro group substitution increased toxicity on the chalcone scaffold which enhances inhibitory potency .
4) Ch_4 (3-methyl substitution): the methyl group supports additional hydrophobic interactions and improves electron density, boosting activity[54] (Fig. 5).

Molecular docking results

Currently, molecular bioinformatics docking studies are highly beneficial in the drug development industry due to their emphasis on potential targets and the reduction of the time and cost required to evaluate new compounds [55]. Consequently, molecular docking simulations are one of the most critical methods for predicting the interaction between a substrate and its receptor. By employing molecular docking modeling to ascertain the binding mode of the target proteins (*Staphylococcus aureus* protein PDB ID: 1N67 and *Escherichia coli* protein PDB ID: 6G9S) with chalcone derivatives at the active site, it was possible to determine whether the examined compounds obstruct the proteins and, as a consequence, alter cellular migration [56]. The ADT software was employed to precisely dock the investigated compounds into the active pocket of proteins. The docking study's results indicated a satisfactory match between the binding sites of the two proteins, with affinity energies ranging from 7.59 to 9.31 kJ/mol for the 6G9S protein and 8.39 to 10.13 kJ/mol for the 1N67. Additionally, Tables 3 and 4; Fig. 6 and 7 illustrate the binding interactions between amino acid residues and ligands. The docking data indicated that the *Staphylococcus aureus* protein exhibited the maximum affinity energies with compounds ch1, ch3, and ch4, respectively, at 10.13 kJ/mol, 9.13 kJ/mol, and 9.99 kJ/mol. The docking results between the chalcone and *Escherichia coli* protein indicated that ch2 exhibited the highest affinity energies (9.31 kJ/mol) among the other variants, which was consistent with the antibacterial test results. The results of the antibacterial test are consistent with this. The docking data results of the ligand-*S. aureus* interaction that was investigated are as follows: Compound ch1 formed two hydrogen bonds with amino acid residues (HIS 252A and VAL 450A) and exhibited a number of hydrophobic interactions with amino acid residues, including ASP 385A, ARG 395A, TYR 448A, ASN 284A, and ASP 340A. Compound ch3 formed three hydrogen bonds with amino acid residues (VAL 450A, HIS 252A, and TYR 399A) as a side-chain acceptor. In addition to hydrophobic interactions with amino acid residues, such as GLU 342A, THR 397A, and ASP 385A. Compound ch4 formed two hydrogen bonds with amino acid residues (HIS 252A and VAL 450A) and exhibited a number of hydrophobic interactions with those residues, including ASN 284A, TYR 448A, ILE 339A, and THR 397A. The docking data result of the interaction compound ch2 with the s. aureus protein indicated that three hydrogen bonds were formed as a side-chain acceptor with amino acid residues (GLN 455A, SER 330A, and SER 387A) and multiple hydrophobic interactions were formed with amino acid residues (ASP 389A, LYS 333A, GLY 546A, and THR 529A).



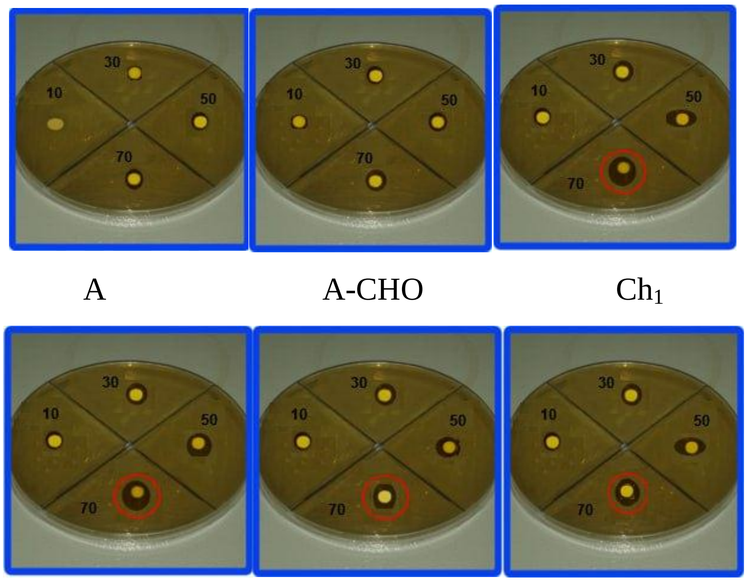
Scheme 1 Synthesis routes of studied compounds Ch (1-4).



A A-CHO Ch_1

Ch_2 Ch_3 Ch_4

Fig. 1 ***Effect of synthesized compounds on S. aureus.***



A A-CHO Ch_1

Ch_2 Ch_3 Ch_4

Fig. 2 Effect of synthesized compounds on *E. coli*.

Table 1 Antimicrobial activity against *S. aureus* of Synthesized compounds and standard drugs

Comp.	**Diameter of inhibition zone (mm)** **_S. aureus_ per 1ml DMSO**				
	5µg	**10µg**	**30µg**	**50µg**	**70µg**
A	R*	R*	7	10	13
A-CHO	R*	R*	8	12	14
Ch_1	R*	12	17	24	31
Ch_2	R*	10	15	21	27
Ch_3	R*	8	13	19	24
Ch_4	R*	6	11	16	22
Ciprofloxacin	20	-	-	-	-
Cephalexin	-	-	R*	-	-

When R* = Resistance; - = without concentration

Table 2 Antimicrobial activity against *E. coli* of synthesized compounds and standard drugs .

Compound	**Diameter of inhibition zone (mm)** **_E. coli_ per 1ml DMSO**				
	5µg	**10µg**	**30µg**	**50µg**	**70µg**
A	R*	R*	6	9	12
A-CHO	R*	R*	7	12	14
Ch_1	R*	11	18	23	29
Ch_2	R*	10	17	22	27
Ch_3	R*	8	14	18	24
Ch_4	R*	7	12	17	23
Ciprofloxacin	21	-	-	-	-
Cephalexin	-	-	R*	-	-

When R* = Resistance; - = without concentration

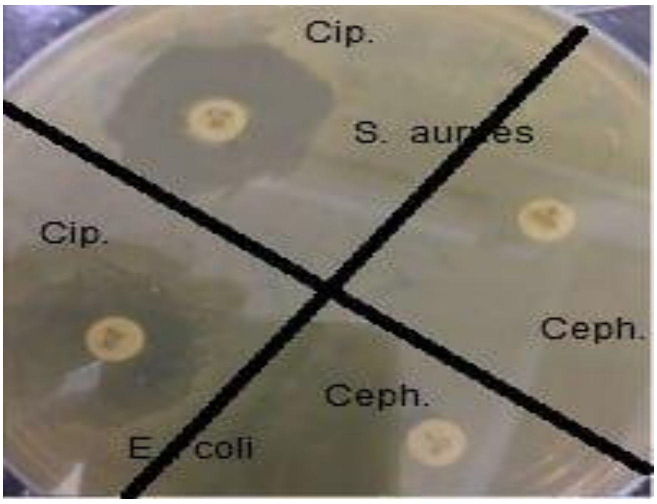


Fig. 3 Effect of standard drugs on *S. aureus* and *E. coli*.

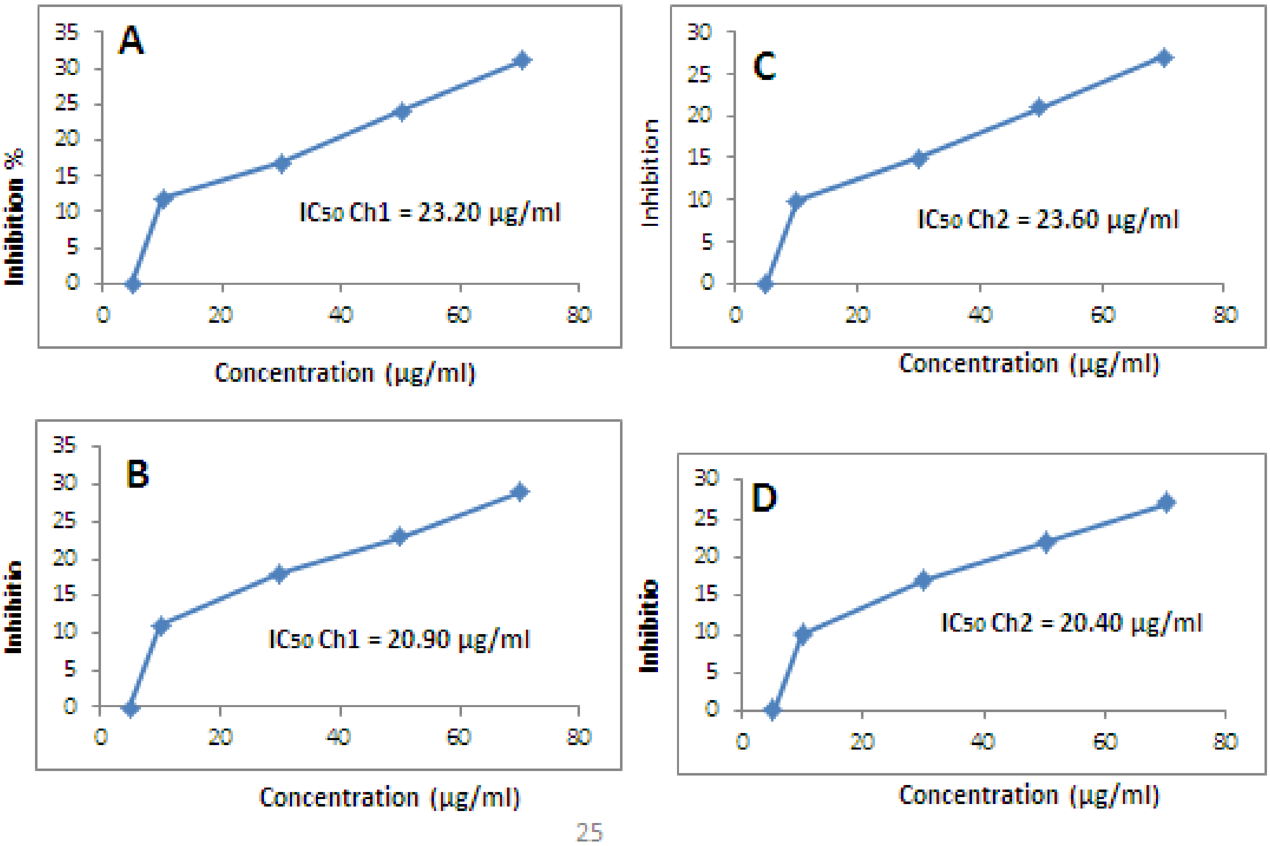


Fig. 4. A: IC_{50} value of Ch1 compound against *S. aureus*. B: IC_{50} value of Ch1 compound against *E. coli*. C: IC_{50} value of Ch2 compound against *S. aureus*. D: IC_{50} value of Ch2 compound against *E. coli* .

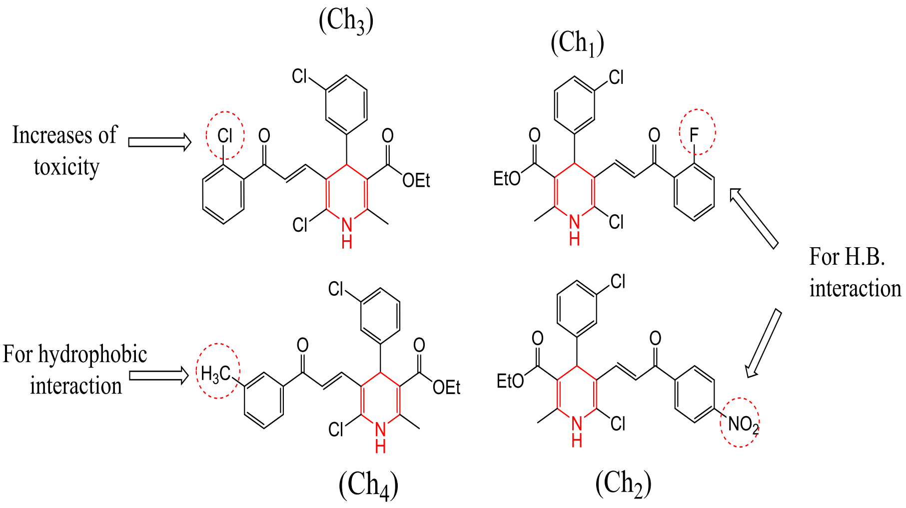


Fig. 5 Structure-Activity Relationship of the potent compounds Ch1-Ch4 .

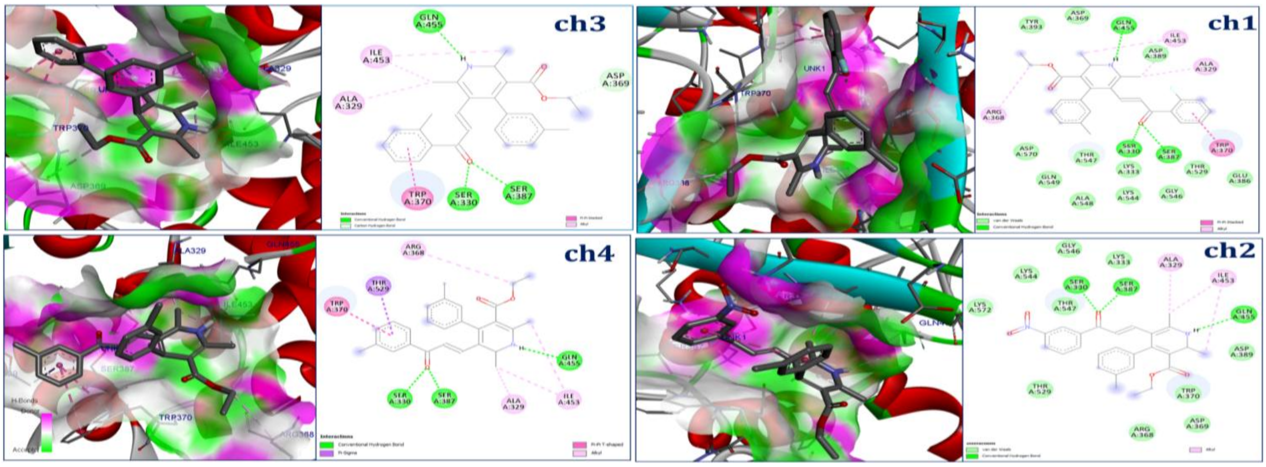


Fig. 6 Hydrogen bonds and other binding interactions with residues in the 1n67 active site are displayed in the interaction profiles of compounds ch1–ch4. The ligands essential functional groups and spatial orientation within the binding pocket are shown in the diagrams.

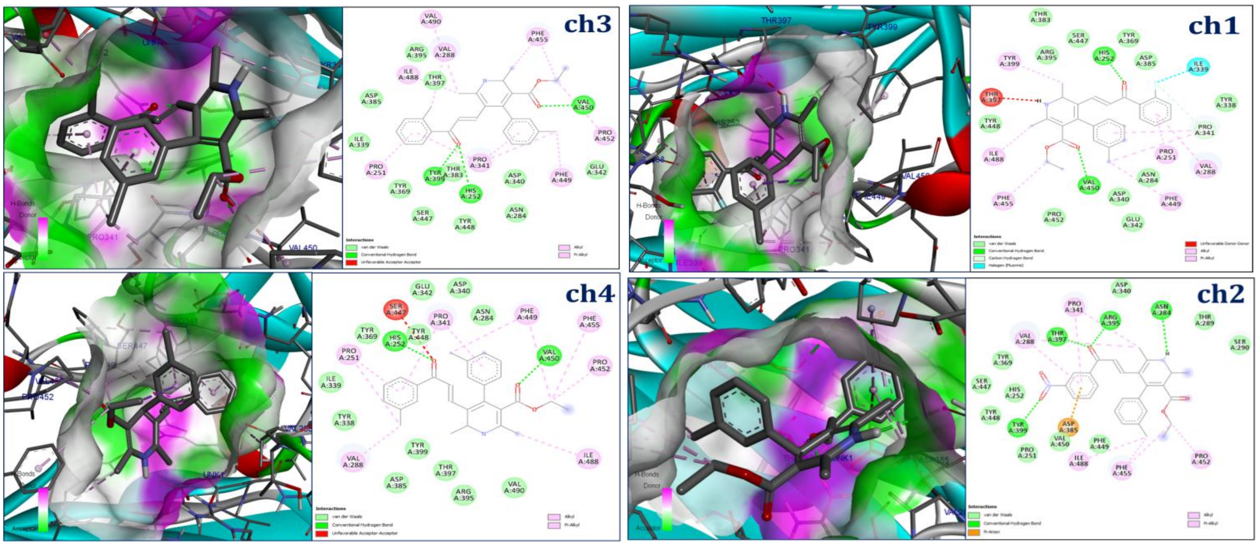


Fig. 7 Hydrogen bonds and other binding interactions with residues in the 6G9S active site are displayed in the interaction profiles of compounds Ch1–Ch4. The ligands essential functional groups and spatial orientation within the binding pocket are shown in the diagrams.

Conclusion

A new chalcone derivatives were synthesized by Claisen – Schmidt condensation reaction with 5-formyl dihydropyridine. The studied compounds were examined for antibacterial activity against Gram-positive *S. aureus* and Gram-negative *E. coli*. Most of them showed a good inhibition than the standard drugs (Cephalexin 30 µg, Ciprofloxacin 5 µg). The study also showed that Ch1 and Ch2 were found to be better than standard drugs. Merit investigations to develop these compounds for future research.

Conflicts of interest

The authors declare there are no conflicts of interest.

Acknowledgment

The authors would like to thank the Department of Chemistry, College of Education for Pure Sciences, University of Basrah, Basrah, and Collage of Pharmacy in the University of Basrah, Basrah, Iraq to support this research project. The authors most grateful to the laboratory staff NMR unit of the Department of Chemistry College of Education for Pure Sciences, University of Basrah. This research is part of the PhD research project and one of the graduation requirements.

Funding

Supported by the Department of Chemistry College of Education for Pure Sciences, University of Basrah

Authors' contributions

AGS and TAA: Designed, synthesis, analysis, molecular docking analysis, and writing the manuscript**. AKM and AGF:** Analysis the results of antibacterial and tables. All authors were approved the final version of manuscript.

Ethical considerations

Not applicable

References

1. Goyal K, Kaur R, Goyal A, Awasthi R, Chalcones: A review on synthesis and pharmacological activities. Journal Applied Pharmacology Sciences, 2021;11 (sup 1):001 – 014. DOI: 10.7324/JAPS.2021.11s101
2. Awasthi SK, Mishra N, Kumar B, Sharma M, Bhattacharya A and Mishra LC, Potent Antimalarial Activity of Newly Synthesized Substituted Chalcone Analogs in Vitro. Medicinal Chemistry Research. 2009;18(6): 407-420. doi:10.1007/s00044-008-9137-9
3. Pereira R, Silva AMS, Ribeiro D, Silva VLM, Fernandes E. Bis-chalcones: A review of synthetic methodologies and anti-inflammatory effects. European Journal of Medicinal Chemistry. 2023;252:115280. doi: 10.1016/j.ejmech.2023.115280.
4. Liu M, Wilairat P, Go ML, Antimalarial Alkoxylated and Hydroxylated Chalones: Structure-Activity Relationship Analysis. Journal of Medicinal Chemistry, 2001;44:4443-4452. Doi:10.1021/jm0101747
5. Morrison RT, Boyd RN, Organic Chemistry. 6th Ed. , Prentice Hall, 2004.
6. Singh P, Anand A, Kumar V. Recent developments in biological activities of chalcones: a mini review. European Journal of Medicinal Chemistry. 2014;85:758-77. doi: 10.1016/j.ejmech.2014.08.033.
7. Marinov R, Markova N, Krumova S, Yotovska K, Zaharieva MM, Genova- Kalou P, Antiviral properties of chalcones and their synthetic derivatives: a mini review. Pharmacia,2020; 67(4):325 – 337. DOI: 10.3897/pharmacia.67.e53842
8. Amine MM, Shaykoon M, Marzouk AA, Beshr EAM, Abuo – Rahman GA, Recent Updates on Synthetic Strategies of Chalcone Scaffold and their Heterocyclic Derivatives. Journal of Advanced Biomedical& Pharmacology Sciences. 2023;6(3):124- 132. DOI: 10.21608/JABPS.2023.198509.1184
9. Valavanidis A, Vlachogianni T, Plant Polyphenols: Recent Advances in Epidemiological Research and Other Studies on Cancer Prevention. Stud. In Natu. Product Chemistry, Chapter 8. 2013; 39:269 – 295. doi:10.1016/B978-0-444-62615-8.00008-4
10. Çelik Onar H, Özden EM, Taslak HD, Gülçin İ, Ece A, Erçağ E. Novel coumarin-chalcone derivatives: Synthesis, characterization, antioxidant, cyclic voltammetry, molecular modelling and biological evaluation studies as acetylcholinesterase, α-glycosidase, and carbonic anhydrase inhibitors. Chemistry Biological Interaction. 2023;383:110655. doi: 10.1016/j.cbi.2023.110655.
11. Li MT, Xie L, Jiang HM, Huang Q, Tong RS, Li X, Xie X, Liu HM. Role of Licochalcone A in Potential Pharmacological Therapy: A Review. Frontier Pharmacology. 2022;13:878776. doi: 10.3389/fphar.2022.878776.
12. **Vlasiou MC, Structural Characterization of Two Novel, Biological Active Chalcone Derivatives, Using Density Functional Theory Studies. Bio interface Research Applied Chemistry. 2021;11(6):15051 – 15057. doi:10.33263/BRIAC116.1505115057**
13. Su W, Qian Q, Li P, Lei X, Xiao Q, Huang S, Huang C, Cui J. Synthesis, characterization, and anticancer activity of a series of ketone-N(4)-substituted thiosemicarbazones and their ruthenium(II) arene complexes. Inorganic Chemistry. 2013;52(21):12440-9. doi: 10.1021/ic401362s. .
14. Piotrowska H, Kucinska M, Murias M. Biological activity of piceatannol: leaving the shadow of resveratrol. Mutant Research. 2012;750(1):60-82. doi: 10.1016/j.mrrev.2011.11.001.
15. Mahapatra DK, Bharti SK, Asati V. Chalcone Derivatives: Anti-inflammatory Potential and Molecular Targets Perspectives. Current Topical Medicine Chemistry. 2017;17(28):3146-3169. doi: 10.2174/1568026617666170914160446..
16. Bertl E, Becker H, Eicher T, Herhaus C, Kapadia G, Bartsch H, Gerhäuser C. Inhibition of endothelial cell functions by novel potential cancer chemopreventive agents. Biochemistry Biophysics Research Communication. 2004;325(1):287-95. doi: 10.1016/j.bbrc.2004.10.032..
17. Jiwrajka M, Phillips A, Butler M, Rossi M, Pocock JM. The Plant-Derived Chalcone 2,2',5'-Trihydroxychalcone Provides Neuroprotection against Toll-Like Receptor 4

Triggered Inflammation in Microglia. Oxidization Medicine Cell. 2016;2016:6301712. doi: 10.1155/2016/6301712.

18. Krishnakumar B, Swaminathan MJ, A recyclable and highly effective sulfated TiO_2-P25 for the synthesis of quinoxaline and dipyridophenazine derivatives at room temperature. Journal of Organometallic Chemistry. 2010;695:2572 – 2577. DOI:10.1016/j.jorganchem.2010.08.055
19. Ünver Y, Tuluk M, Kahriman N, Emirik M, Bektas E, Direkel S, New Chalcone Derivatives with Schiff Base-Thiophene: Synthesis, Biological Activity, and Molecular Docking Studies. Russian Journal of General Chemistry.2019;89(4):794–799. Doi:10.1134/S107036321904025X
20. Koudokpon H, Armstrong N, Dougnon TV, Fah L, Hounsa E, Bankolé HS, Loko F, Chabrière E, Rolain JM. Antibacterial Activity of Chalcone and Dihydrochalcone Compounds from *Uvaria chamae* Roots against Multidrug-Resistant Bacteria. Biomedical Research International. 2018;2018:1453173. doi: 10.1155/2018/1453173.
21. Sun H, Wang D, Song X, Zhang Y, Ding W, Peng X, Zhang X, Li Y, Ma Y, Wang R, Yu P. Natural Prenylchalconaringenins and Prenylnaringenins as Antidiabetic Agents: α-Glucosidase and α-Amylase Inhibition and in Vivo Antihyperglycemic and Antihyperlipidemic Effects. Journal of Agriculture Food Chemistry. 2017;65(8):1574-1581. doi: 10.1021/acs.jafc.6b05445.
22. Fontenele JB, Leal LK, Ferreira MA, Silveira ER, Viana GS, Antiplatelet Effect of Lonchocarpin and Derricin Isolated fromLonchocarpus sericeus. Pharmacology Biology, 2005; 43:726 – 731. doi:10.1080/13880200500387406.
23. Friis-Møller A, Chen M, Fuursted K, Christensen SB, Kharazmi A. In vitro antimycobacterial and antilegionella activity of licochalcone A from Chinese licorice roots. Planta Medicine. 2002;68(5):416-9. doi: 10.1055/s-2002-32087.
24. Mezgebe K, Melaku Y, Mulugeta E. Synthesis and Pharmacological Activities of Chalcone and Its Derivatives Bearing *N*-Heterocyclic Scaffolds: A Review. ACS Omega. 2023;8(22):19194-19211. doi: 10.1021/acsomega.3c01035.
25. Trivedi A, Dodiya D, Dholariya B, Kataria V, Bhuva V, Shah V. Synthesis and biological evaluation of some novel 1,4-dihydropyridines as potential antitubercular agents. Chemistry Biology Drug. 2011;78(5):881-6. doi: 10.1111/j.1747-0285.2011.01233.x.
26. Al-Mathkuri T, Al-Jubori H, Majed A, Moker M, Santali E, Alnajjar R, Synthesis, breast cancer activity, molecular docking and dynamic simulation of 1,4-Dihydropyridine derivatives. Journal of Molecular Structure, 2025;1321(Part 1):139704. doi:10.1016/j.molstruc.2024.139704
27. Desai B, Sureja D, Naliapara Y, Shah A, Saxena AK. Synthesis and QSAR studies of 4-substituted phenyl-2,6-dimethyl-3, 5-bis-N-(substituted phenyl)carbamoyl-1,4-dihydropyridines as potential antitubercular agents. Bioorganic Medicine Chemistry. 2001;9(8):1993-8. doi: 10.1016/s0968-0896(01)00141-9.
28. Kharkar PS, Desai B, Gaveria H, Varu B, Loriya R, Naliapara Y, Shah A, Kulkarni VM. Three-dimensional quantitative structure-activity relationship of 1,4-dihydropyridines as antitubercular agents. Journal of Medicine Chemistry. 2002;45(22):4858-67. doi: 10.1021/jm020217z.
29. Prashantha BR, Kumar M, Pankaj E, Karthikeyan A,Vijayan P, Synthesis of novel Hantzsch dihydropyridines and Biginelli dihydropyrimidines of biological interest: a 3D-QSAR study on their cytotoxicity. Medicine Chemistry Research. 2010;19:344 – 363. DOI:10.1007/s00044-009-9195-7
30. Khan A, Iqbal A, Ahmedi S, Manzoor N, Siddiqui T. Synthesis, Anti-Fungal Potency and In silico Studies of Novel Steroidal 1,4-Dihydropyridines. Chemistry Biodiversity. 2023;20(5):e202300096. doi: 10.1002/cbdv.202300096.
31. Marchalin S, Chudik M, Cvopova K, Kozisek J, Lesko J, Daich A, Conformationally constrained 1,4-DHPs. A convenient route to bis-1,4-DHPs as a novel class of nitrogen compounds. Tetrahedron, 2002;58:5747 – 5754. DOI:10.1016/S0040-4020(02)00542-2
32. Olejníková P, Svorc L, Olšovská D, Panáková A, Vihonská Z, Kovaryová K, Marchalín S. Antimicrobial activity of novel c2-substituted 1,4-dihydropyridine analogues. Science Pharmacology. 2014;82(2):221-32. doi: 10.3797/scipharm.1311-04.
33. Bharathi D, Ranjithkumar R, Chandarshekar B, Bhuvaneshwari V. Preparation of chitosan coated zinc oxide nanocomposite for enhanced antibacterial and photocatalytic activity: As a bionanocomposite. International Journal of Biology Macromolecular. 2019 May 15;129:989-996. doi: 10.1016/j.ijbiomac.2019.02.061.
34. Soothill JS, Ward R, Girling AJ. The IC50: an exactly defined measure of antibiotic sensitivity. J Antimicrob Chemother. 1992 Feb;29(2):137-9. doi: 10.1093/jac/29.2.137.
35. Al-Shawi AAA, El-Arabey AA, Mutlaq DZ, Eltayb WA, Iriti M, Abdalla M. Study on Molecular Anti-tumor Mechanism of 2-Thiohydantoin Derivative based on Molecular Docking and Bioinformatic Analyses. Curr Top Med Chem. 2023;23(6):440-452. doi: 10.2174/1568026623666230106121527.
36. Mutlaq D, Al-Shawi AA, AlAsadi R, Synthesis, characterization, anticancer activity, and molecular docking of novel maleimide–succinimide derivatives. *Egyptian Pharmaceutical J, 2021;*20(4):303-312.| *DOI:* 10.4103/epj.epj_26_21
37. Naser, N.M., Mutlaq, D.Z., Al-Shawi, A.A.A. *et al.* Synthesis, Characterization, Antibacterial, and Molecular Docking and Dynamics Studies of Novel 2-thioxoimidazolidin-4-one Analogue. *Chemistry Africa*, 2024;**7**:4193–4210. https://doi.org/10.1007/s42250-024-01040-y
38. Suarez M, de Armas M, Ramirez O, Alvarez A, Synthesis and structural study of new highly lipophilic 1,4-dihydropyridines. New Journal of Chemistry. 2005;29:1567 – 1576. DOI: 10.1039/B506018D
39. Bade TS, Ebrahimi HP, Alsalim T, Titinchi S, Abbo H, Bolandnazar Z, Ebrahimi A, A novel series of 1, 4-Dihydropyridine (DHP) derivatives bearing thiazolidin-4-one: From synthesis to structure. Journal of Molecular Structure. 2017;1138:136 –148. DOI:10.1016/j.molstruc.2017.03.005
40. Trilleras J, Molina A, Gamboa A, Acost-Gusman P, Quiroga J, Synthesis of pyrimido[4,5-*b*]quinolindiones and formylation: ultrasonically assisted reactions. Research Society Open Science. 2024;11:1 – 14. DOI: 10.1098/rsos.231128
41. Aguiar ASN, Queiroz JE, Firmino PP, Vaz WF, Camargo AJ, de Aquino GLB, Napolitano HB, Oliveira SS. Synthesis, characterization, and computational study of a new heteroaryl chalcone. Journal of Molcular Model. 2020;26(9):243. doi: 10.1007/s00894-020-04506-1.
42. Lim YH, Oo CW, Koh RY, Voon GL, Yew MY, Yam MF, Loh YC. Synthesis, characterization, and anti-cancer activity of new chalcone derivatives containing naphthalene and fluorine moieties. Drug Diversity Research. 2020;81(8):994-1003. doi: 10.1002/ddr.21715.
43. Nawaz T, Tajammal A, Qurashi AW, Nisa MU, Binjawhar DN, Iqbal M. Synthesis, antibacterial, antibiofilm, and docking studies of chalcones against multidrug resistance pathogens. Heliyon. 2024;10(13):e30618. doi: 10.1016/j.heliyon.2024.e30618.
44. Shaik AB, Yejella RP, Shaik S. Synthesis, Antimicrobial, and Computational Evaluation of Novel Isobutylchalcones

as Antimicrobial Agents. International Journal of Medical Chemistry. 2017;2017:6873924. doi: 10.1155/2017/6873924.

45. Amole KL, Bello IA, Oyewale AO, Synthesis, Characterization and Antibacterial Activities of New Fluorinated Chalcones. Chemistry Africa. 2019;2:47–55. DOI:10.1007/s42250-019-00043-4.
46. Shaik AB, Bhandare RR, Nissankararao S, Edis Z, Tangirala NR, Shahanaaz S, Rahman MM. Design, Facile Synthesis and Characterization of Dichloro Substituted Chalcones and Dihydropyrazole Derivatives for Their Antifungal, Antitubercular and Antiproliferative Activities. Molecules. 2020;25(14):3188. doi: 10.3390/molecules25143188.
47. Mukhtar SS, Morsy NM, Hassan AS, Hafez TS, Hassaneen HM, Saleh FM, A Review of Chalcones: Synthesis, Reactions, and Biological Importance. Egyptian Journal of Chemistry. 65(8) (2022) 379 – 395. 10.21608/EJCHEM.2022.112735.5125
48. Özdemir A, Altıntop MD, Sever B, Gençer HK, Kapkaç HA, Atlı Ö, Baysal M. A New Series of Pyrrole-Based Chalcones: Synthesis and Evaluation of Antimicrobial Activity, Cytotoxicity, and Genotoxicity. Molecules. 2017;22(12):2112. doi: 10.3390/molecules22122112.
49. Krawczyk-Łebek A, Żarowska B, Janeczko T, Kostrzewa-Susłow E. Antimicrobial Activity of Chalcones with a Chlorine Atom and Their Glycosides. International journal of Molecular Science. 2024;25(17):9718. doi: 10.3390/ijms25179718.
50. Konduru NK, Dey S, Sajid M, Owais M, Ahmed N. Synthesis and antibacterial and antifungal evaluation of some chalcone based sulfones and bisulfones. European Journal of Medicine Chemistry. 2013;59:23-30. doi: 10.1016/j.ejmech.2012.09.004.
51. Dos Santos ATL, de Araújo-Neto JB, Costa da Silva MM, Paulino da Silva ME, Carneiro JNP, Fonseca VJA, Coutinho HDM, Bandeira PN, Dos Santos HS, da Silva Mendes FR, Sales DL, Morais-Braga MFB. Synthesis of chalcones and their antimicrobial and drug potentiating activities. Microbiology Pathology. 2023;180:106129. doi: 10.1016/j.micpath.2023.
52. Krawczyk-Łebek A, Żarowska B, Janeczko T, Kostrzewa-Susłow E. Antimicrobial Activity of Chalcones with a Chlorine Atom and Their Glycosides. International Journal of Molecular Science. 2024;25(17):9718. doi: 10.3390/ijms25179718.
53. Al-Saheb R, Makharza S, Al-Battah F, Abu-El-Halawa R, Kaimari T, Abu Abed OS. Synthesis of new pyrazolone and pyrazole-based adamantyl chalcones and antimicrobial activity. Bioscience Report. 2020;40(9):BSR20201950. doi: 10.1042/BSR20201950.
54. Abood RG, Abdulhussein HA, Abbas S, Majed AA, Al-Khafagi AA, Adil A, Alsalim TA, Anti-breast cancer potential of new indole derivatives: Synthesis, in-silico study, and cytotoxicity evaluation on MCF-7 cells. Journal of Molecular Structure. 2024;1326:141176. DOI:10.1016/j.molstruc.2024.141176.
55. Zhang J, Li JC, Song JL, Cheng ZQ, Sun JZ, Jiang CS. Synthesis and evaluation of coumarin/1,2,4-oxadiazole hybrids as selective BChE inhibitors with neuroprotective activity. Journal of Asian Natural Product Research. 2019;21(11):1090-1103.

doi: 10.1080/10286020.2018.1492566.

56. Omran HA, Majed AA, Hussein K, Abid DS, Abdel-Maksoud M, Elwahsh A, Kotob MH, Aufy M, Kotob MH,, Anti-Cancer Activity, DFT and molecular docking study of new Bis-Thiazolidine amide. Results in Chemistry 2024;12:101835. doi:10.1016/j.rechem.2024.101835