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New Chalcone Derivatives: Synthesis, Characterization, Antioxidant, Antimicrobial, and Docking Study Against GLcN-6-P Synthase

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ABSTRACT: ***Background:*** *Chalcone derivatives are well-known for their versatile pharmacological properties, particularly their antimicrobial and antioxidant activities. Their simple chemical structure and ease of synthesis make them attractive candidates for drug discovery, especially in combating microbial resistance and oxidative stress-related disorders.* ***Objective:*** *The study aimed to synthesize and characterize a series of new chalcone derivatives and evaluate their antimicrobial and antioxidant activities, alongside molecular docking studies to assess their potential as therapeutic agents.* ***Methods:*** *The chalcone derivatives were synthesized via Claisen–Schmidt condensation of two methyl ketones (p-aminoacetophenone and cyclopropyl methyl ketone) with p-substitutedphenyl aldehydes under mild conditions. Structural elucidation was performed using FT-IR, ^{1}H-NMR, ^{13}C-NMR, and GC-MS spectroscopy. Antimicrobial activities were tested against five microbial strains: Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumoniae, and Candida albicans, using the agar well-diffusion method. Antioxidant activity was evaluated using the standard DPPH radical scavenging assay. Molecular docking studies were conducted using PyRx software to investigate the binding interactions of the synthesized chalcones with the active site of glucosamine-6-phosphate (GlcN-6-P) synthase, a target enzyme for antimicrobial agents.* ***Results:*** *The synthesized chalcone derivatives were successfully characterized and exhibited varying levels of antimicrobial activity against the tested strains, with some compounds demonstrating significant inhibitory effects. Antioxidant assays revealed promising radical scavenging capabilities for several synthesized chalcones. Docking studies showed favorable binding affinities between the identified compounds and the GlcN-6-P synthase active site, supporting the experimental bioactivity results.* ***Conclusions:*** *This study demonstrates that the newly synthesized chalcone derivatives possess both antimicrobial and antioxidant activities. Their effective interaction with GlcN-6-P synthase suggests potential for further development as lead compounds in antimicrobial drug research.*

KEYWORDS: *Chalcone; Antimicrobial; Antioxidant; Docking; GlcN-6-P synthase*

INTRODUCTION

Chalcones, also known as α,β-unsaturated ketones, are a class of compounds characterized by the presence of a carbonyl functional group with general structure PhCOCH=CHR, where R is typically an aryl or alkyl group. These derivatives are produced from the condensation reaction between methyl ketone and the corresponding aldehyde, typically under mild conditions [1]. Chalcone compounds have garnered significant structure in versatile scientific domains, especially in medicinal chemistry, owing to their skeleton versatility and pronounced pharmaceutical activity[2]. Recently, chalcone derivatives have been recognized as privilege scaffold for development of new therapeutic

agents as drug candidates [3]. Notably, chalcone derivatives have shown broad spectrum of pharmaceutical activities such as antibacterial [4], antifungal [5], antioxidant [6], AChE inhibitor [7], anti-inflammatory [8], and anticancer agents [9]. The incorporation of heteroatoms such as bromine, chlorine, nitrogen, and oxygen into chalcone frameworks has been shown to significantly enhance their pharmacokinetic and pharmacodynamic profiles, rendering them more applicable for medicinal application [10], [11]. The escalating global burden of infectious diseases coupled with the emergence of drug resistance, has intensified the search for new chemotherapeutic agents. Multidrug resistant microbial pathogens, especially those belonging to gram positive, gram negative, and *Candida albicans (C. albicans)* have a substantial threat to public health [12]–[18]. Chalcones represent a promising scaffold as potent antimicrobial agents due to their ability to interfere with critical biomolecular target such as glucosamine-6-phosphate synthase as depicted in several published articles. Several articles have reported chalcones as potent agents against a variety of gram positive and gram-negative bacterial strains as well as fungal species. Their activity mechanisms are generally associated with the inhibition of glucosamine-6-phosphate synthase (GlcN-6-P)4–6. Structural modifications, such as the inclusion electron-donating and electron withdrawing substituents on the aromatic ring, have been demonstrated to modulate the electronic properties of chalcone, thus influencing its biological efficacy [19].

To date, no comprehensive study has been conducted on chalcone derivatives containing both a substituted furan ring and *p*-aminophenyl group, or on those incorporating a cyclopropyl moiety. Furthermore, the *p*-amino group is known for its strong electron-donating behavior, which can enhance the electron density of the chalcone system and influence its reactivity toward biological targets. On the other hand, incorporating a cyclopropyl moiety to chalcone framework will give rigidity and increase the hydrophobic interactions with the target enzyme. Based on this rationale, the present work was designed with the specific aim of synthesizing two sets of chalcone derivatives: the first series including three chalcones bearing a substituted furan moiety and a *p*-aminophenyl group (2a-c), as described previously by our group [4], while the second includes derivatives incorporating a cyclopropyl moiety (3a-c). Our primary hypothesis is that such structural modifications will enhance biological activity by increasing molecular interactions with key microbial enzymes, especially with glucoseamine-6-phosphate synthase enzyme. The main assumptions underlying this hypothesis are: (i) the electron-donating *p*-amino group will improve interaction with nucleophilic or hydrogen-bond-accepting sites; (ii) the furan moiety will facilitate π–π and polar interactions; and (iii) the cyclopropyl group will enhance rigidity and orientation for optimal target binding with target enzyme.

In parallel, the antioxidant activity of chalcone derivatives has attracted significant attention due to their ability to scavenge free radicals and inhibit oxidative stress. Chalcones incorporating heterocyclic moieties often exhibit strong radical scavenging activity, making them promising candidates for therapeutic application targeting oxidative damage. The presence of various functional substituents enhances their electron-donating properties, contributing to their effectiveness as antioxidants [6].

In this study, the chalcone derivatives (2a–c, 3a–c) were synthesized and their structures were confirmed through infrared (IR) spectroscopy, proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR), as well as gas chromatography-mass spectrometry (GC-MS). The antimicrobial potential of these compounds was tested against a range of microorganisms, including *Staphylococcus aureus (S. aureus)*, *Staphylococcus epidermidis (S. epidermidis)*, *Escherichia coli (E. coli)*, *Klebsiella pneumoniae (K. pneumoniae)*, and *C. albicans*. Their antioxidant properties were also examined using the DPPH radical scavenging method. Furthermore, molecular docking simulations were performed with PyRx software tool to explore the binding affinities of the chalcone derivatives (2a–c, 3a–c) with glucosamine-6-phosphate synthase (GlcN-6-P), a crucial enzyme in microbial cell wall formation.

MATERIALS AND METHODS

Materials

All solvents and starting materials utilized in this study were procured from commercial suppliers (BDH, CDH, Fluorochem, Merck, Sigma-Aldrich, and Thomas Baker) and used without further purification. The melting points of the synthesized compounds were measured using an electrothermal capillary melting point apparatus and are reported without correction. Fourier-transform infrared (FT-IR) spectra were recorded using a Bruker ALPHA II FTIR spectrometer (PLATINUM-ATR). Nuclear magnetic resonance (^{1}H and ^{13}C-NMR) spectra were recorded on a Bruker Analytik 300, and 500 MHz spectrometer, with chemical shifts referenced to the residual solvent peak of DMSO-d_6 (δ = 2.50 ppm). GC-Mass analyses were conducted on an AGILENT instrument.

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Synthetic Part

1 Synthesis of 5-Arylfuran-2-carbaldehyde derivatives (1a-c)

The aldehyde derivatives 1a–c were prepared based on a previously established procedure [4]. In summary, para-substituted anilines (0.136 mol) of dissolved in 7 M hydrochloric acid (56.2 mL). The solution was placed in an ice bath to maintain a temperature of 0 °C, and diazotization was carried out at 0–5 °C using sodium nitrite solution (9.5 g, 0.138 mol) in water (25 mL). After stirring the mixture for 10 minutes, it was filtered, and the resulting f iltrate was treated with an aqueous solution of furan (15.4 g, 0.160 mol) in water (50 mL). Subsequently, solution of $CuCl_2 \cdot 2H_2O$ (5.0 g, 0.03 mol) in water (25 mL) was added while maintaining the temperature between 10–15 °C. The reaction mixture was then stirred at 40 °C for 4 hours. The obtained solid was collected via Büchner filtration, washed successively with water, 5% sodium hydrogen carbonate (20 mL), and water (20 mL), then dried and purified by recrystallization from ethanol.

2 5-(4-Bromophenyl)furan-2-carboxaldehyde (1a)

Brown solid, yield 65%, m. p. 150–152 °C; FT-IR (cm^{-1}): 3110 (furan ring C-H), 3056 (aromatic C-H), 2859 (aldehyde group C-H), 1672 (carbonyl group), 1660 (furan ring C=C), 1596 (aromatic ring C=C), 1040 (C-Br). ^{1}H-NMR (500 MHz, DMSO) δ (ppm): 7.35 (d, J = 3.7 Hz, 1H, furan ring C-H), 7.65 (d, J = 3.8 Hz, 1H, furan ring C-H), 7.71 (d, J = 8.6 Hz, 2H, aromatic C-H), 7.81 (d, J = 8.6 Hz, 2H, aromatic C-H), 9.60 (s, 1H, aldehyde group CH).

5-(4-Chlorophenyl)furan-2-carboxaldehyde (1b)

Dark brown solid, yield 58%, m. p. 128–130 °C; FT-IR (cm^{-1}): 3114 (furan ring C-H), 3060 (aromatic C-H), 2846 (aldehyde group C-H), 1681 (carbonyl group), 1663 (furan ring C=C) 1599 (aromatic ring C=C), 1040 (C-Cl). ^{1}H-NMR (500 MHz, DMSO) δ (ppm): 7.33 (d, J = 3.7 Hz, 1H, furan ring C-H), 7.56 (d, J = 8.6 Hz, 2H, aromatic C-H), 7.65 (d, J = 3.7 Hz, 1H, furan ring C-H), 7.87 (d, J = 8.6 Hz, 2H, Aromatic C-H), 9.60 (s, 1H, aldehyde group C-H).

3 5-(4-Nitrophenyl) furan-2-carboxaldehyde (1c)

Yellow solid, yield 69%, mp. 196–200 °C; FT-IR (cm^{-1}): 3116 (furan ring C-H), 3090 (aromatic ring C–H), 2843 (aldehyde group C–H), 1680 (carbonyl group), 1666 (furan ring C=C), 1598 (aromatic ring C=C). ^{1}H-NMR (500 MHz, DMSO) δ (ppm): 7.56 (d, J = 3.7 Hz, 1H, furan ring C-H), 7.69 (d, J = 3.7 Hz, 1H, furan ring C-H), 8.09 (d, J = 8.9 Hz, 2H, aromatic C-H), 8.31 (d, J = 8.9 Hz, 2H, aromatic C-H), 9.66 (s, 1H, aldehyde group C-H).

4 Synthesis of chalcone derivatives 2a-c and 3a-c (General procedure)

Six target chalcones 2a–c and 3a–c were synthesized via a Claisen–Schmidt condensation using a modified approach based on a previously established procedure [20]. In this method, 40% aqueous sodium hydroxide (1.0 mL) was added to the stirred solution of methyl ketone derivative (1.0 mmol) in ethanol (10 mL). The reaction was maintained at room temperature for 30 minutes before introducing an equimolar amount 5-aryl-2-furaldehydes 1a–c (1.0 mmol). Stirring was continued for an additional 12 hours at room temperature. The reaction was monitored by thin-layer chromatography (TLC) using a 1:1 mixture of ethyl acetate and hexane as the mobile phase. Upon completion, the reaction mixture was poured into ice cold water to facilitate product precipitation. The solid was then collected by filtration, rinsed with water (3 × 10 mL), air-dried, and purified by recrystallization from ethanol.

5 (*E*)-1-(4-aminophenyl)-3-(5-(4-bromophenyl)furan-2-yl)prop-2-en-1-one (2a)

Yellow solid, yield 70%, m. p. 173-176 °C; FT-IR (cm^{-1}): 3330, 3207 (amino group NH_2), 3116 (aromatic ring C-H), 2935 (aliphatic C-H), 1626 (carbonyl of C=C–C=O), 1602 (chalcone CH=CH), 1576 (aromatic ring C=C), 950 (C-Br). ^{1}H-NMR (300 MHz, DMSO) δ (ppm): 6.19 (s, 2H, amino group NH_2), 6.61 (d, J = 8.0 Hz, 2H, aromatic C-H), 7.10 (d, J = 3.1 Hz, 1H, furan ring C-H), 7.22 (d, J = 3.4 Hz, 1H, furan ring C-H), 7.45 (d, J = 15.3 Hz, 1H, chalcone C-H), 7.61 – 7.73 (m, 3H, 2 aromatic C-H, chalcone C-H), 7.83 – 7.94 (m, 4H, aromatic ring C-H). ^{13}C-NMR (DMSO) δ (ppm):

109.62, 112.73, 117.97, 119.52, 121.41, 125.24, 126.09, 127.70, 128.62, 130.92, 131.85, 151.52, 153.74, 153.85, 185.09. GC-MS (EI) m/z: 368 M^+ for $C_{19}H_{14}BrNO_2$.

6 (*E*)-1-(4-aminophenyl)-3-(5-(4-chlorophenyl)furan-2-yl)prop-2-en-1-one (2b)

Yellow solid, yield 70%, m.p. 173-176 °C; FT-IR (cm^{-1}): 3336, 3213 (amino group NH_2), 3109 (aromatic C-H), 2900 (aliphatic C-H), 1650 (carbonyl of C=C–C=O), 1627 (chalcone CH=CH), 1597 (aromatic C=C), 1017 (C-Cl). 1H NMR (300 MHz, DMSO) δ (ppm): 6.22 (s, 2H, amino group NH_2), 6.66 (d, J = 8.7 Hz, 2H, aromatic C-H), 7.12 (d, J = 3.6 Hz, 1H, furan ring C-H), 7.22 (d, J = 3.6 Hz, 1H, furan ring C-H), 7.49 (d, J = 15.2 Hz, 1H, chalcone CH), 7.55 (d, j = 8.6 Hz, 2H, aromatic ring C-H), 7.71 (d, J = 15.3 Hz, 1H, chalcone C-H), 7.95 (m, 4H, aromatic ring C-H). ^{13}C-NMR (DMSO) δ (ppm): 110.12, 113.26, 118.63, 119.92, 125.73, 126.41, 128.27, 128.82, 129.51, 131.53, 133.32, 152.01, 154.24, 154.42, 185.60. GC-MS (EI) m/z: 323 $M^+\cdot$ for $C_{19}H_{14}ClNO_2$.

7 (*E*)-1-(4-aminophenyl)-3-(5-(4-nitrophenyl)furan-2-yl)prop-2-en-1-one (2c)

Purple solid, yield 80%, m.p. 216-218 °C; FT-IR (cm^{-1}): 3336, 3217 (amino group NH_2), 3105 (aromatic C-H), 2911 (aliphatic C-H), 1630 (carbonyl of C=C–C=O), 1598 (chalcone CH=CH), 1576 (aromatic C=C), 1510, 1329 (C-NO2). ^{1}H-NMR (300 MHz, DMSO) δ (ppm): 6.23 (s, 2H, amino group NH_2), 6.63 (d, J = 8.5 Hz, 2H, Aromatic C-H), 7.17 (d, J = 3.5 Hz, 1H, furan ring C-H), 7.45-7.50 (m, 2H, chalcone C-H, furan ring C-H), 7.77 (d, J = 15.2 Hz, 1H, chalcone C-H), 7.92 (d, J = 8.3 Hz, 2H, aromatic C-H), 8.16 (d, J = 8.5 Hz, 2H, aromatic C-H), 8.29 (d, J = 8.8 Hz, 2H, aromatic C-H). ^{13}C-NMR (DMSO) δ (ppm):112.75, 112.87, 117.94, 120.93, 124.31, 124.83, 125.13, 127.42, 131.03, 135.14, 146.38, 152.48, 153.05, 153.98, 184.99. GC-MS (EI) m/z: 334 $M^+\cdot$ for $C_{19}H_{14}N_2O_4$.

8 (*E*)-3-(5-(4-bromophenyl)furan-2-yl)-1-cyclopropylprop-2-en-1-one (3a)

Yellow solid, yield 70%, m.p. 82-84 °C; FT-IR (cm^{-1}): 3110 (aromatic C-H), 2859 (aliphatic C-H), 1632 (carbonyl of C=C–C=O), 1593 (chalcone CH=CH), 1474 (aromatic C=C), 970 (C-Br). ^{1}H-NMR (300 MHz, DMSO) δ (ppm): 0.96 (d, J = 5.5 Hz, 4H, cyclopropyl C-H), 2.43 (p, J = 6.2 Hz, 1H, cyclopropyl C-H), 6.92 (d, J = 15.9 Hz, 1H, chalcone C-H), 7.10 (d, J = 3.6 Hz, 1H, furan ring C-H), 7.24 (d, J = 3.6 Hz, 1H, furan ring C-H), 7.50 (d, J = 15.9 Hz, 1H, chalcone C-H), 7.68 (d, J = 8.6 Hz, 2H, aromatic C-H), 7.82 (d, J = 8.6 Hz, 2H, aromatic C-H). ^{13}C-NMR (DMSO) δ (ppm): 11.19, 19.60, 110.29, 119.60, 122.16, 123.88, 126.58, 128.42, 128.97, 132.46, 151.15, 154.70, 199.15. GC-MS (EI) m/z: 316 $M^+\cdot$ for $C_{16}H_{13}BrO_2$.

9 (*E*)-3-(5-(4-chlorophenyl)furan-2-yl)-1-cyclopropylprop-2-en-1-one (3b)

Yellow solid, yield 70%, m.p. 78-79 °C; FT-IR (cm^{-1}): 3090 (aromatic C-H), 2886 (aliphatic C-H), 1632 (carbonyl of C=C–C=O), 1599 (chalcone CH=CH), 1514 (aromatic C=C), 1022 (C-Cl). ^{1}H-NMR (300 MHz, DMSO) δ (ppm): 0.96 (d, J = 6.2 Hz, 4H, cyclopropyl C-H), 2.42 (p, J = 6.2 Hz, 1H, cyclopropyl C-H), 6.90 (d, J = 15.9 Hz, 1H, chalcone C-H), 7.09 (d, J = 3.6 Hz, 1H, furan ring C-H), 7.20 (d, J = 3.6 Hz, 1H, furan ring C-H), 7.44 – 7.58 (m, 3H, 2 aromatic C-H, chalcone C-H), 7.87 (d, J = 8.6 Hz, 2H, aromatic C-H). ^{13}C-NMR (75 MHz, DMSO) δ (ppm): 11.16, 19.57, 110.17, 119.54, 123.84, 126.33, 128.40, 128.63, 129.53, 133.49, 151.12, 154.66, 199.10. GC-MS (EI) m/z: 272 $M^+\cdot$ for $C_{16}H_{13}ClO_2$.

10 (*E*)-3-(5-(4-nitrophenyl)furan-2-yl)-1-cyclopropylprop-2-en-1-one (3c)

Purple solid, yield 94%, m. p. 166-168 °C; FT-IR (cm^{-1}): 3112 (aromatic C-H), 2985 (aliphatic C-H), 1676 (carbonyl of C=C–C=O), 1594 (chalcone CH=CH), 1562 (aromatic C=C), 1501, 1329 (C-NO2). ^{1}H-NMR (300 MHz, DMSO) δ (ppm): 0.97 (d, J = 4.5 Hz, 4H, cyclopropyl C-H), 2.44 (p, J = 6.6 Hz, 1H, cyclopropyl C-H), 6.97 (d, J = 15.9 Hz, 1H, chalcone C-H), 7.16 (d, J = 3.6 Hz, 1H, furan ring C-H), 7.47 (d, J = 3.7 Hz, 1H, furan ring C-H), 7.52 (d, J = 15.9 Hz, 1H, chalcone C-H), 8.08 (d, J = 8.6 Hz, 2H, aromatic C-H), 8.29 (d, J = 8.6 Hz, 2H, aromatic C-H). ^{13}C-NMR (DMSO) δ (ppm): 11.30, 19.64, 113.43, 119.49, 124.89, 125.07, 125.33, 128.19, 135.46, 146.95, 152.64, 153.40, 199.19. GC-MS (EI) m/z: 283 $M^+\cdot$ for $C_{16}H_{13}NO_4$.

In Vitro Assays

1 Antimicrobial Evaluation

The antimicrobial potential of the synthesized chalcones (2a–c and 3a–c) was assessed in vitro through the agar well diffusion technique [21], [22]. This analysis involved testing the compounds against five microbial strains, including two Gram-positive bacteria (*S. aureus* and *S. epidermidis*), two Gram-negative bacteria (*E. coli* and *K. pneumoniae*), and one fungal strain (*C. albicans*). Standard antibiotics—amoxicillin and fluconazole—served as reference drugs. All test samples were prepared at a concentration of 100 mg/mL in dimethyl sulfoxide (DMSO). Müller-Hinton agar was sterilized and poured into Petri dishes. After solidification, the agar surfaces were inoculated with microbial suspensions using sterile cotton swabs. Wells were made in the agar, and 50 μL of each test solution was added after a 15-minute resting period. Plates were incubated at 37 °C for 24 hours, and the diameters of inhibition zones were measured in millimeters.

2 Determination of Minimum Inhibitory Concentration (MIC)

The MIC value for chalcone derivative 2c was identified using the agar dilution method [23]. A series of two-fold dilutions ranging from 0.19 to 100 μg/mL was prepared from the stock solution. Each dilution was added to Müller-Hinton agar maintained at 50 °C, mixed well, and poured into sterile Petri dishes. Once the agar solidified at room temperature, the plates were stored at 4 °C and used within 24 hours. To prepare the bacterial inoculum, 4–5 colonies from an overnight culture were suspended in 5 mL of saline, with turbidity adjusted to match a 0.5 McFarland standard (~1.5×10^8 CFU/mL). An aliquot of 5 μL of this suspension was applied to each plate and allowed to stand for 10 minutes before incubation at 37 °C for 24 hours.

Antioxidant Evaluation

1 Qualitative Analysis via TLC Autographic Method

Each synthesized chalcone (2a–c and 3a–c) and gallic acid (used as a positive control) was dissolved in methanol and spotted on a TLC plate using a capillary tube. Once dried, the plates were sprayed with a 0.2% DPPH methanolic solution and incubated for 30 minutes. The plates were then visualized under UV light. Antioxidant-active compounds were indicated by blue or yellow zones contrasting with the purple DPPH background [24].

2 Quantitative Analysis Using Spectrophotometry

The DPPH free radical scavenging activity of the active synthesized chalcone derivatives (2b, 3a–c) was quantitatively determined using a UV–VIS spectrophotometric method. To perform the assay, 500 μL of DPPH solution was combined with 500 μL of each sample at concentrations of 50, 100, 150, 200, and 250 μg/mL. The total volume was brought to 2 mL with absolute ethanol. Absorbance was measured at 517 nm after adequate incubation to determine radical scavenging efficiency [25].

Docking Study

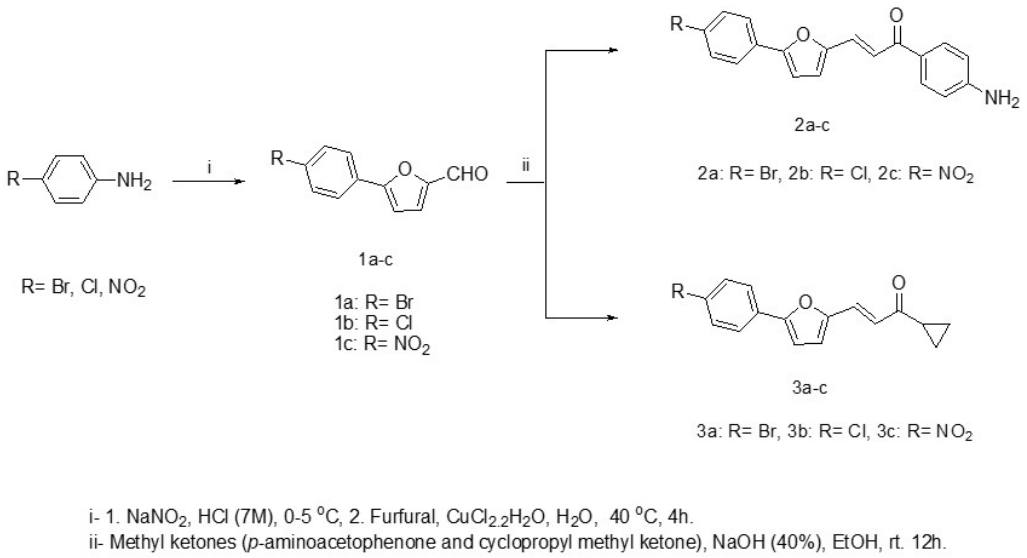
Crystal structure as PDB file of GlcN-6-P synthase (1MOQ) were retrieved from the RCSB Protein Data Bank. Prior to docking, all water molecules were removed and modified amino acid residues were repaired to obtain a clean and reliable target for molecular interaction studies. Molecular docking was carried out using PyRx AutoDock Vina (version 0.8), a widely used approached for predicting ligand–receptor interactions within enzyme binding sites [26], [27]. The three-dimensional structures of the synthesized chalcones (2a-c, 3a-c) were constructed using ChemDraw Ultra 7.0, and the resulting molecular files were converted to PDB format via Open Babel. Glucoseamine-6-phosphate, the substrate in the crystal structures were removed to allow accurate preparation of the binding sites. Docking grids were performed with the known active site of the target enzyme. The grid box was centered at coordinates X = 31.6, Y = 17.6, Z = −1.4 with dimensions of 23.8 × 26.4 × 23.3 Å. The synthesized derivatives were docked within these defined binding sites, and binding affinities (expressed in kcal/mol) were calculated for each docked conformation. The conformation with the most favorable (i.e., lowest) binding energy was considered to represent the optimal binding pose. To validate the docking protocol, redocking of the glucoseamine-6-phosphate, the original

co-crystallized substrate was performed within their respective binding site. The resulting poses were compared to the crystallographic conformations to evaluate the accuracy and reliability of the docking methodology. All docking results and molecular interactions were analyzed and visualized using Discovery Studio 2021. Intermolecular interactions, such as hydrogen bonds and hydrophobic contacts between ligands and target active site, were examined in detail as depicted in the docking study section.

RESULTS AND DISCUSSION

Chemistry

The intermediate aldehyde compounds (1a–c) were synthesized using the Meerwein reaction protocol. Initially, *p*-substituted-arylamines (Ar-NH_2) were diazotized with sodium nitrite in 7 M hydrochloric acid at a temperature of 0–5 °C for 10 minutes. Subsequently, furfural and cuprous chloride were added in an aqueous medium. The temperature was then increased to 40 °C, and the reaction mixture was stirred for 4 hours, leading to the formation of the target intermediates (1a–c) with good, isolated yields. The chalcone derivatives (2a–c, 3a–c) were synthesized through a Claisen–Schmidt condensation reaction involving equimolar amounts of methyl ketones (*p*-aminoacetophenone and cyclopropyl methyl ketone) and the prepared aldehydes (1a-c). The reaction was carried out at room temperature for 12 hours in ethanol with 40% sodium hydroxide as the base, yielding the chalcones (2a–c, 3a–c) in appreciable amounts as described by Scheme 1.



Scheme 1. Synthesis of aldehydes (1a–c) and the chalcone derivatives (2a–c and 3a–c)

The structures of the six synthesized chalcones (2a–c, and 3a–c) were identified and characterized through various spectral analyses, including IR, ^{1}H-NMR, ^{13}C-NMR, and GC-MS spectroscopy (Figure 1 illustrates the IR and the ^{1}H-NMR spectra of the synthesized derivatives). As a representative case, the characterization of compounds 2a, and 3a is detailed here. The IR spectrum of compound 2a displayed absorption at 3330, 3207 cm ^{1}, corresponding to NH_2 stretching, and at 3116 cm ^{1} for aromatic C-H stretching. The absorption at 1626 is related to carbonyl of chalcone framework. The ^{1}H-NMR spectrum showed a singlet at 6.19 ppm integrating for two protons, indicative of an amino group. A doublet at 6.61 integrated two protons assigned to AA'/BB' system of the *p*-substituted phenyl group. The two doublet signals at 7.10 and 7.22 corresponded to two furan protons. Furthermore, the doublet signal at 7.45 ppm is assigned to one proton of the CH=CH–C=O group. The two multiplets from 7.61–7.73 and 7.83–7.94 ppm were related to remaining CH=CH–C=O group proton and aromatic protons. The ^{13}C-NMR δ (ppm) at 109.62, 112.73, 117.97, 119.52, 121.41, 125.24, 126.09, 127.70, 128.62, 130.92, 131.85, 151.52, 153.74, 153.85, and 185.09 confirming the structure elucidation. The GC-MS spectroscopy determines a molecular ion peak at m/z = 367.02, which represents the molar mass of 2a in the positive form (M^+). For compound 3a, IR spectrum shows

absorptions at 3110 cm^{-1} and 2859 cm^{-1} for aromatic and aliphatic C-H, respectively. The absorption band at 1632 cm^{-1} is related to C=O stretching. ^{1}H-NMR spectrum of 3a showed a doublet signal at 0.96 ppm, integrating for four protons, indicative of a cyclopropyl moiety. A quintet signal at 2.43 ppm corresponded to C-H of cyclopropyl ring. The two protons of the CH=CH–C=O group appeared as a doublet at 6.92 and 7.50 ppm. The two doublets at 7.10 and 7.24 ppm are related to C-H of furan ring. Additional doublets at 7.68 and 7.82 ppm were assigned to the AA'/BB' system of the *p*-disubstituted phenyl group. The ^{13}C-NMR and the GC-MS spectroscopy further elucidation the structure of 3a compound (See the experimental part).

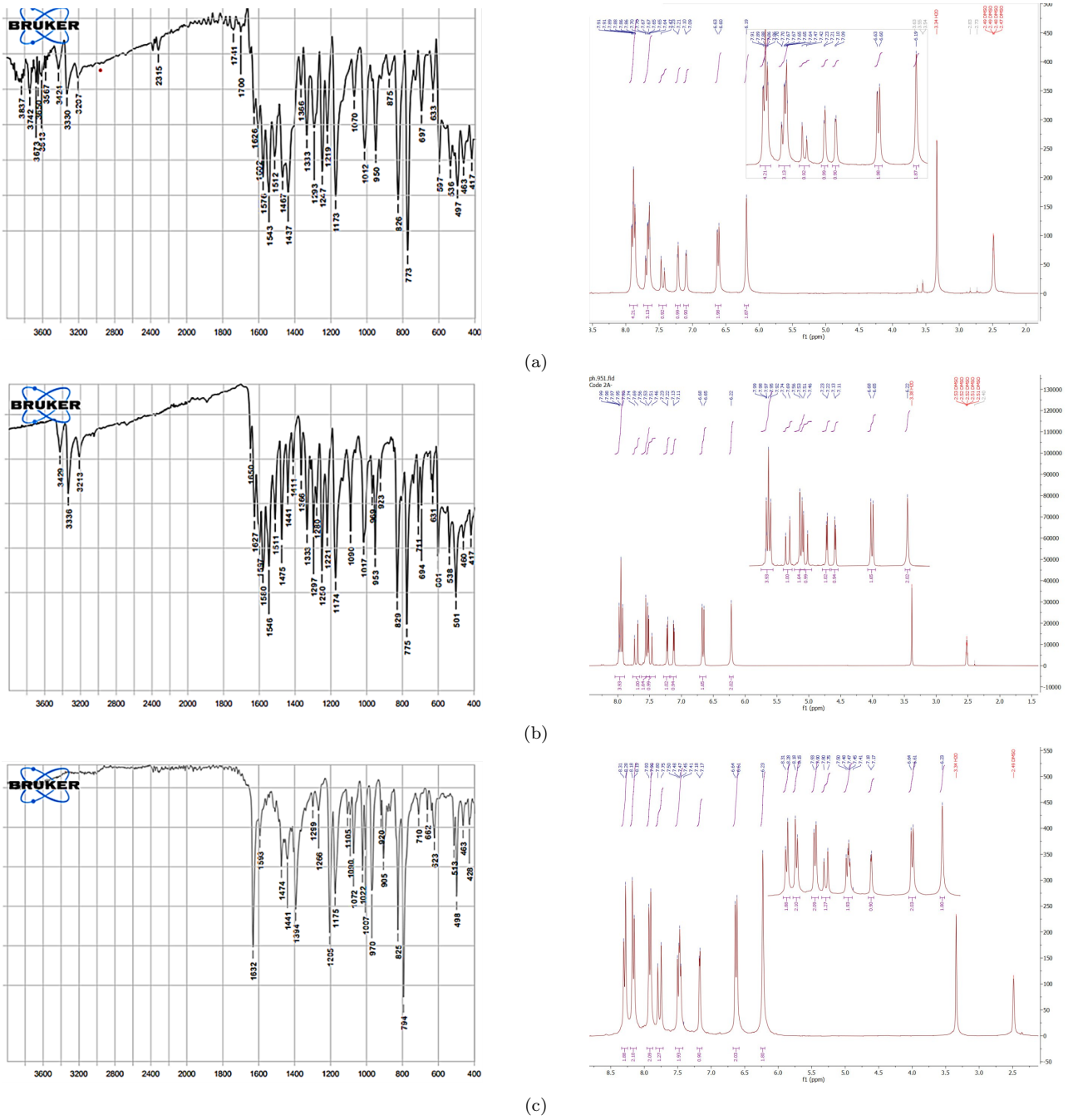
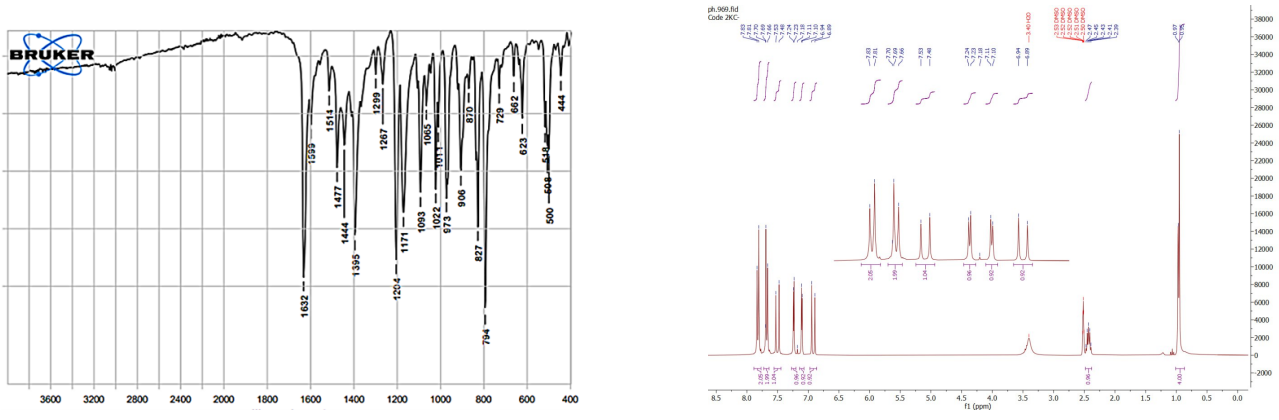
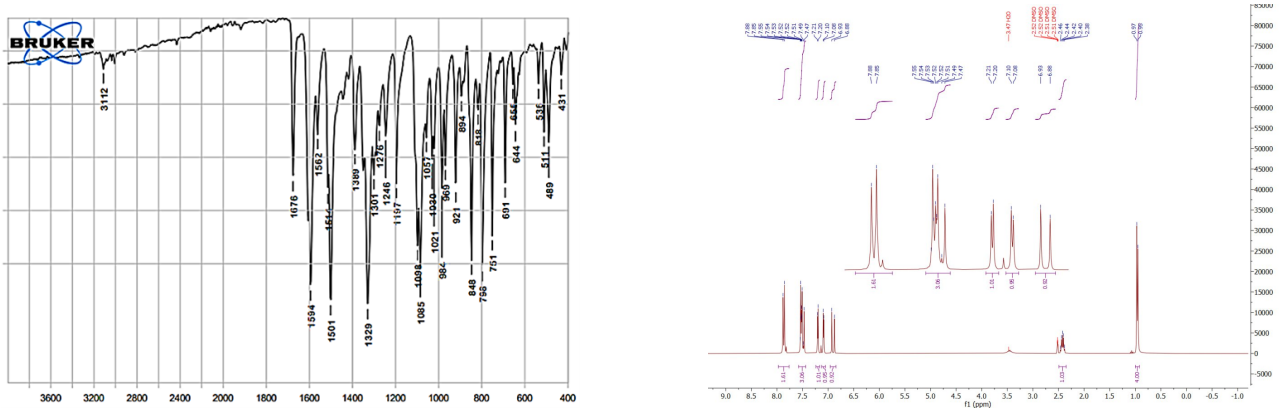


Figure 1. IR and ^{1}H-NMR spectra of the synthesized chalcones: (a) 2a; (b) 2b; (c) 2c; (d) 3a; (e) 3b; (f) 3c



(d)



(e)

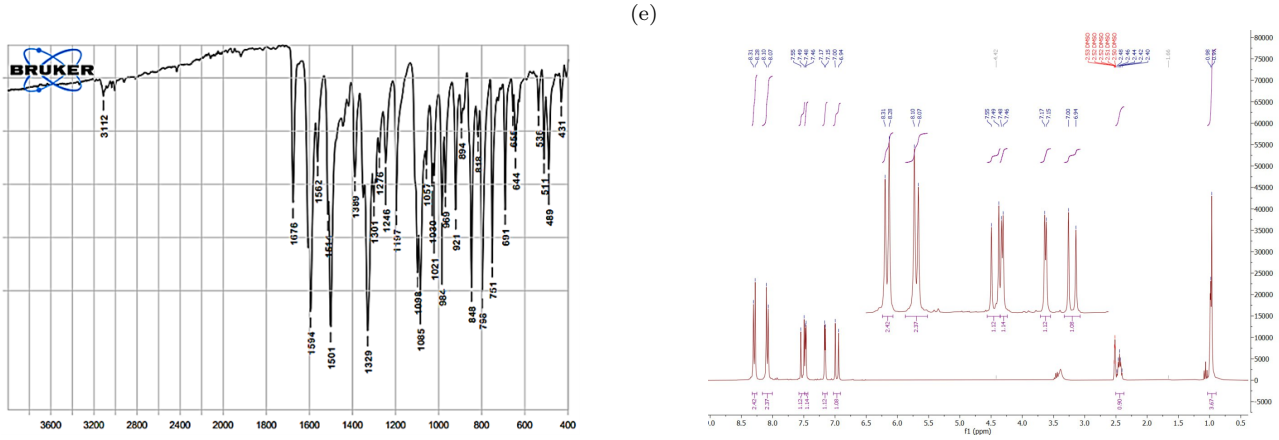


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Figure 1. *Continued*

In Vitro Assay

1 Antimicrobial Assay

The antimicrobial evaluation of all the chalcone derivatives (2a-c, 3a-c) was carried out against several species including *S. aureus*, *S. epidermidis* (Gram-positive), *E. coli*, *K. pneumoniae* (Gram-negative), and the fungal strain *C. albicans* (Table 1). The activity was assessed using both the inhibition zone diameters (in mm) and minimum inhibitory concentration (MIC in μg/mL), where available. Among the tested compounds, chalcone derivative 2c exhibited the most potent and broad-spectrum antimicrobial activity. It exhibited potent activity against all tested strains: *S. aureus* (MIC = 12.5 μg/mL), *S. epidermidis* (12.5 μg/mL), *E. coli* (1.56 μg/mL), *K. pneumoniae* (25 μg/mL), and C.

albicans (6.25 μg/mL). This suggests that compound 2c possesses strong antimicrobial properties, likely due to favorable structural features that enhance cell permeability and target binding as illustrated in the docking study section. Chalcone derivative 2a and 3a showed selective activity against *S. epidermidis* only, with inhibition zones of 15 mm, indicating a narrow spectrum of antibacterial effect limited to certain Gram-positive organisms. Notably, they exhibited no activity against Gram-negative bacteria or C. albicans. Chalcone 2b showed moderate activity against *S. aureus* and *S. epidermidis* with inhibition zones of 10 mm and 13 mm, respectively, but was inactive against Gram-negative bacteria and fungi. This suggests some degree of selectivity for Gram-positive strains, though less potent than compound 2c. Compound 3b displayed a broader activity profile, being effective against *S. aureus* and *S. epidermidis* (15 mm) and moderately active against *K. pneumoniae* (12 mm). This intermediate spectrum indicates its activity as a lead structure for further optimization against both Gram-positive and selected Gram-negative bacteria. Furthermore, chalcone derivative 3c also exhibited moderate activity, with inhibition zones against *S. aureus* (13 mm), *S. epidermidis* (14 mm), and C. albicans (12 mm), suggesting dual antibacterial and antifungal activity, but still less potent than compound 2c. The standard drug amoxicillin used as standard, significantly showed inhibition zones against Gram-positive bacteria (*S. aureus*: 45 mm, *S. epidermidis*: 33 mm), while fluconazole was examined against C. albicans (27 mm). In comparison, synthesized derivative 2c, less potent than the standard drugs, still showed promising efficacy across all tested strains, highlighting its potential for further pharmaceutical development. A comparative evaluation between (*E*)-1-(4-aminophenyl)-3-(5-(4-substituted phenyl)furan-2-yl)prop-2-en-1-one derivatives (2a-c) and (*E*)-3-(5-(4-substitutedphenyl)furan-2-yl)-1-cyclopropylprop-2-en-1-one derivatives (3a-c) revealed that derivatives incorporating the *p*-aminophenyl group (2a-c) exhibited a broader antimicrobial spectrum than those containing the cyclopropyl moiety (3a-c). The first series of chalcone derivatives, particularly compound 2c, demonstrated notable potency against both Gram-positive and Gram-negative bacteria as well as *C. albicans*, whereas the second series chalcone compounds were largely restricted to Gram-positive activity with only occasional and moderate effects on Gram-negative and fungal strains. Within Gram-positive species, all the synthesized chalcones produced comparable inhibition zone diameters (~13–15 mm), but the extended spectrum of 2a-c derivatives suggests that the presence of a second aromatic ring on the -C=C-C=O system enhance target binding and contribute to improved overall antimicrobial efficacy. Finally, these findings suggest that the chalcone derivatives, particularly compound 2c, are promising antimicrobial agents.

Table 1. Inhibition zone and MIC at 100 mg/ml concentration of the synthesized derivatives (2a–c, 3a–c)[a]

Diameter of Inhibition zone (mm), (MIC)* in μg/mL					
Compd.	**Gram positive bacteria**		**Gram negative bacteria**		
	S. aureus	***S. epidermidis***	***E. coli***	***K. pneumoniae***	***C. albicans***
2a	-	15	-	-	-
2b	10	13	-	-	-
2c	(12.5) *	(12.5) *	(1.56) *	(25) *	(6.25) *
3a	-	15	-	-	-
3b	15	15	-	12	-
3c	13	14	-	-	12
Amoxicillin	45	33	-	-	-
Fluconazole	n.m.	n.m.	n.m.	n.m.	27

[a] n.m. not measured

2 Antioxidant Activity

The antioxidant activity of the synthesized chalcones (2a–c, 3a–c) was evaluated using the DPPH radical scavenging assay qualitatively and quantitatively. Qualitative assessment revealed that compounds 2a and 2c exhibited no observable antioxidant activity. Quantitative testing, based on the percentage of DPPH radical scavenging at concentrations ranging from 50 to 250 μg/mL, was conducted for compound 2b, and 3a-c (Figure 2). The results indicate differences in antioxidant efficacy among the active compounds. Chalcone 2b showed the weakest activity among the tested group, with a gradual increase in radical scavenging from 2.8% at 50 μg/mL to 12.35% at 250 μg/mL. Compound

3a exhibited the highest antioxidant activity across all tested concentrations, reaching 35.86% at 250 μg/mL. Chalcone 3b demonstrated a sharp increase in antioxidant activity with concentration, particularly between 100 μg/mL and 150 μg/mL, reaching 37.26% at 250 μg/mL. This steep dose-dependent behavior indicates a threshold concentration above which the compound becomes highly effective, possibly due to aggregation effects or enhanced solubility. Furthermore, chalcone derivative 3c also displayed strong antioxidant activity, increasing from 16.81% at 50 μg/mL to 40.12% at 250 μg/mL, suggesting comparable or even superior efficacy to compound 3b at higher doses. This may point to a more balanced structure capable of consistent radical quenching over a broad concentration range.

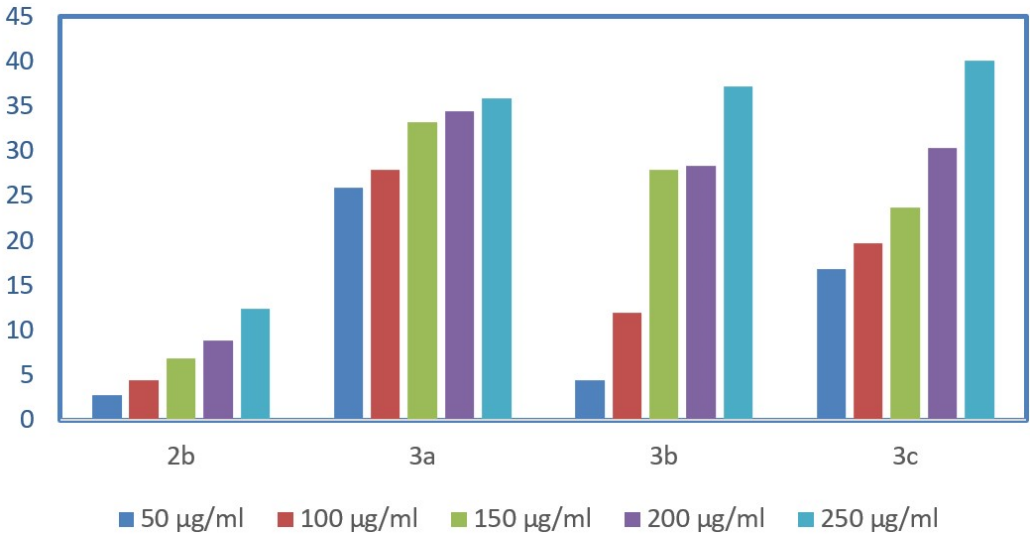


Figure 2. Antioxidant activity of the synthesized chalcone derivatives (2b, 3a–c)

Docking Study

The docking study aimed to evaluate the binding affinity of the synthesized derivatives (2a–b, 3a–b) as well as glucoseamine-6-phosphate, a reference ligand, within the active site of glucoseamine-6-phosphate synthase (GlcN-6-P), a promising target for antimicrobial drug design (Figure 3). The results demonstrate varying degrees of interaction strength between the tested derivatives and the enzyme active site, measured through their binding affinities (in kcal/mol). The binding energies of the compounds were between 8.2 and 6.7 kcal/mol. For comparative purposes, the glucoseamine-6-phosphate, the substrate of the receptor exhibited a binding affinity of –6.5 kcal/mol. Notably, four of the six tested derivatives (2a–c and 3c) exhibited stronger binding affinities than the substrate, with chalcone derivative 2c showing the highest affinity at - 8.2 kcal/mol. This value reflects a significant improvement in binding strength, indicating a more stable interaction with the receptor's substrate site. derivative 2a and 2b, each with binding affinities of - 8.0 kcal/mol, also demonstrate enhanced binding potential compared to the substrate. Chacone derivative 3c, though slightly weaker at - 7.8 kcal/mol, still outperforms the substrate in terms of receptor affinity. In contrast, compounds 3a and 3b both exhibited binding affinities of -6.7 kcal/mol, which are marginally weaker than that of the substrate (-7.4 kcal/mol). These results suggest that although these compounds do bind within the substrate site, their interaction may be less energetically favorable than that of the substrate ligand. The comparative analysis suggests that at least four of the six synthesized derivatives could serve as potential leads for further development due to their enhanced binding properties. The stronger binding interactions may be attributed to improved hydrogen bonding, hydrophobic interactions, or van der Waals forces with key residues within the glucoseamine-6-phosphate synthase binding pocket. In conclusion, this docking study highlights the potential of the investigated synthesized derivatives, particularly 2c as measured by in vitro assay, as promising candidates for antimicrobial drug development targeting the glucoseamine-6-phosphate synthase receptor.

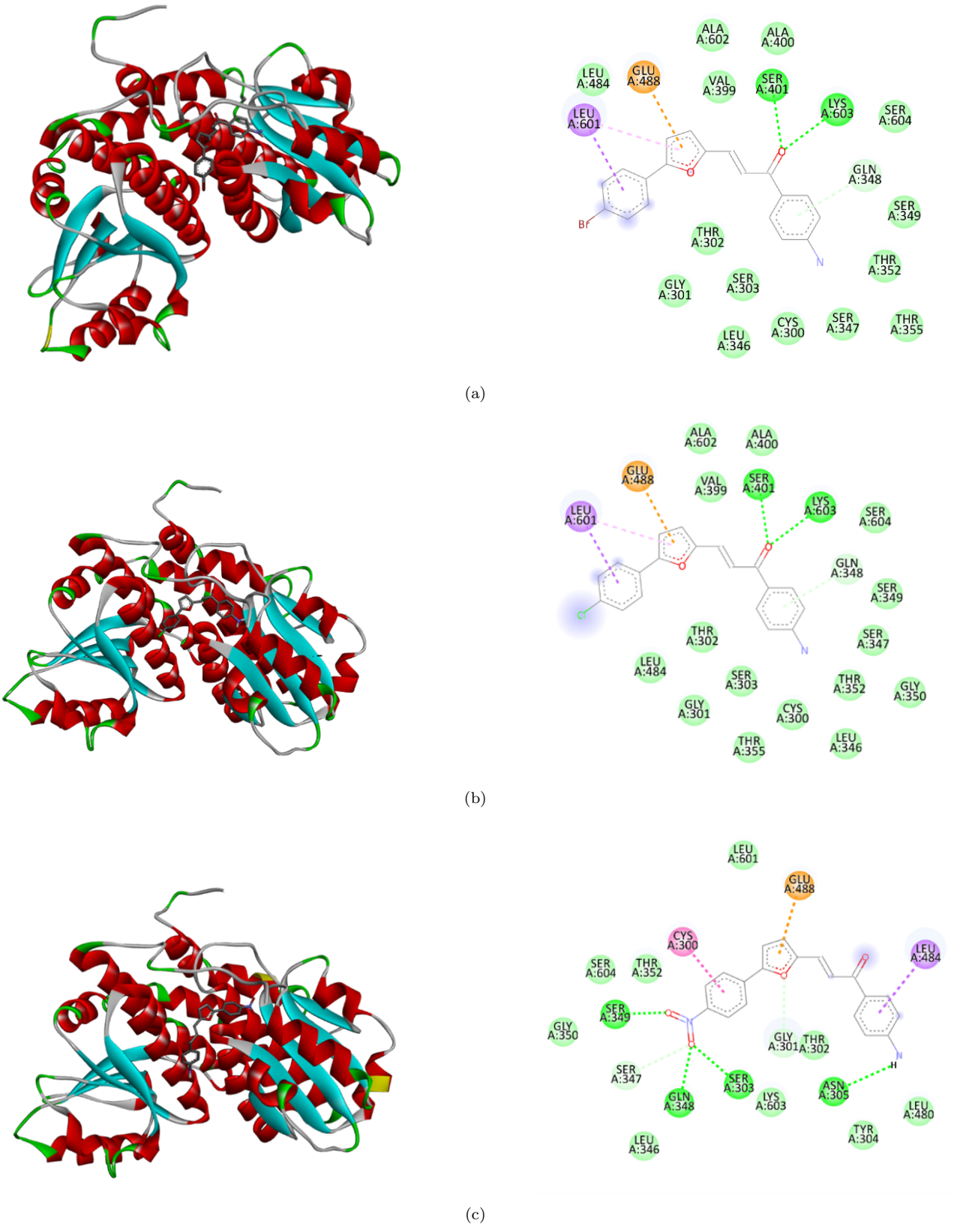
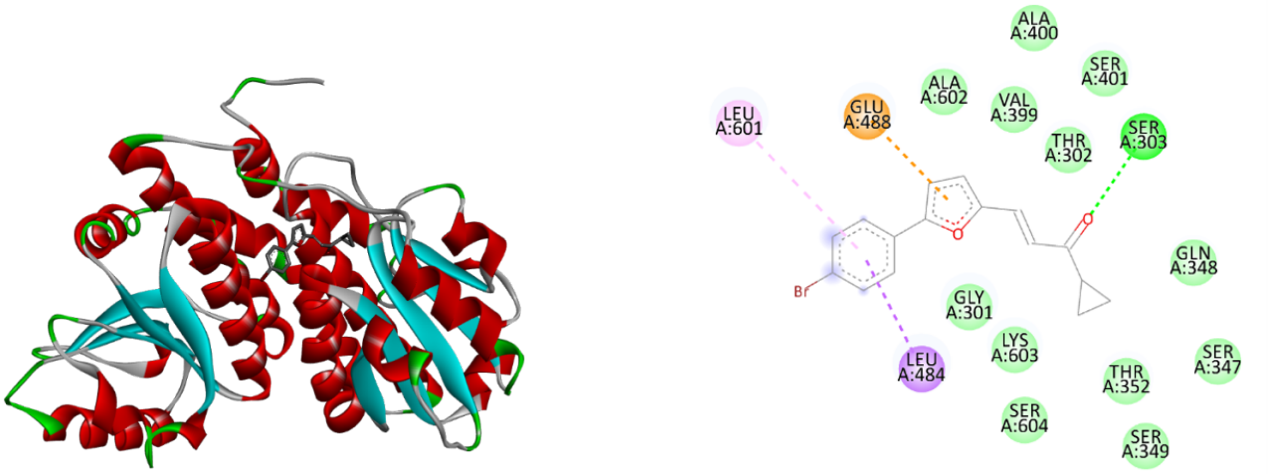


Figure 3. Docking of the synthesized derivatives (2a–c, 3a–c) and glucosamine-6-phosphat inside the GlcN-6-P synthase: (a) 2a inside GlcN-6-P synthase; (b) 2b inside GlcN-6-P synthase; (c) 2c inside GlcN-6-P synthase; (d) 3a inside GlcN-6-P synthase; (e) 3b inside GlcN-6-P synthase; (f) 3c inside GlcN-6-P synthase; (g) glucosamine-6-phosphat inside GlcN-6-P synthase



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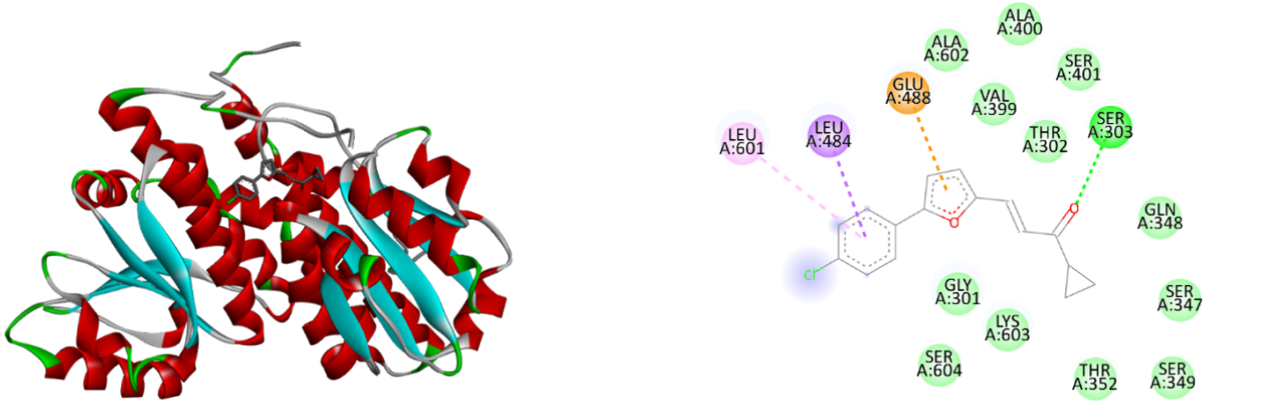

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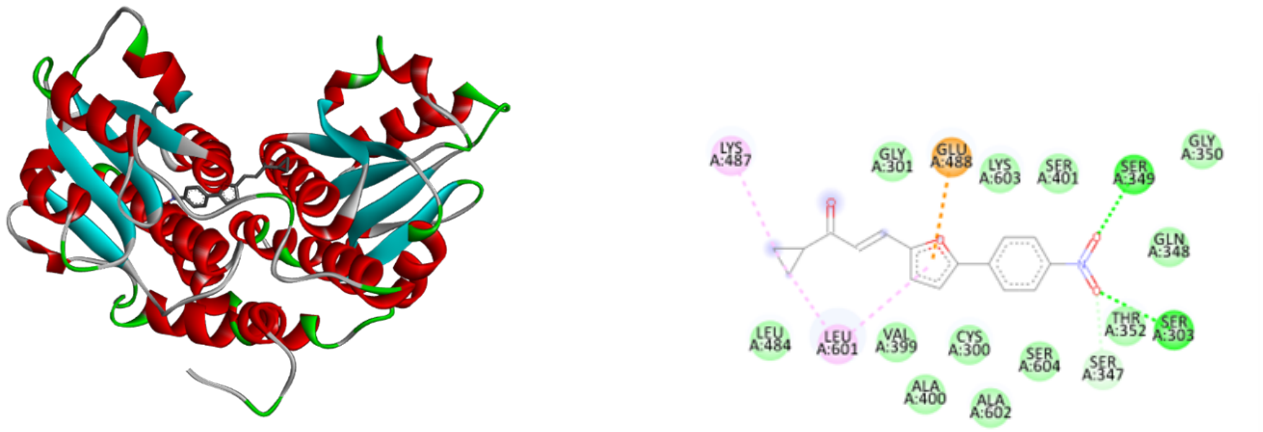

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Figure 3. *Continued*

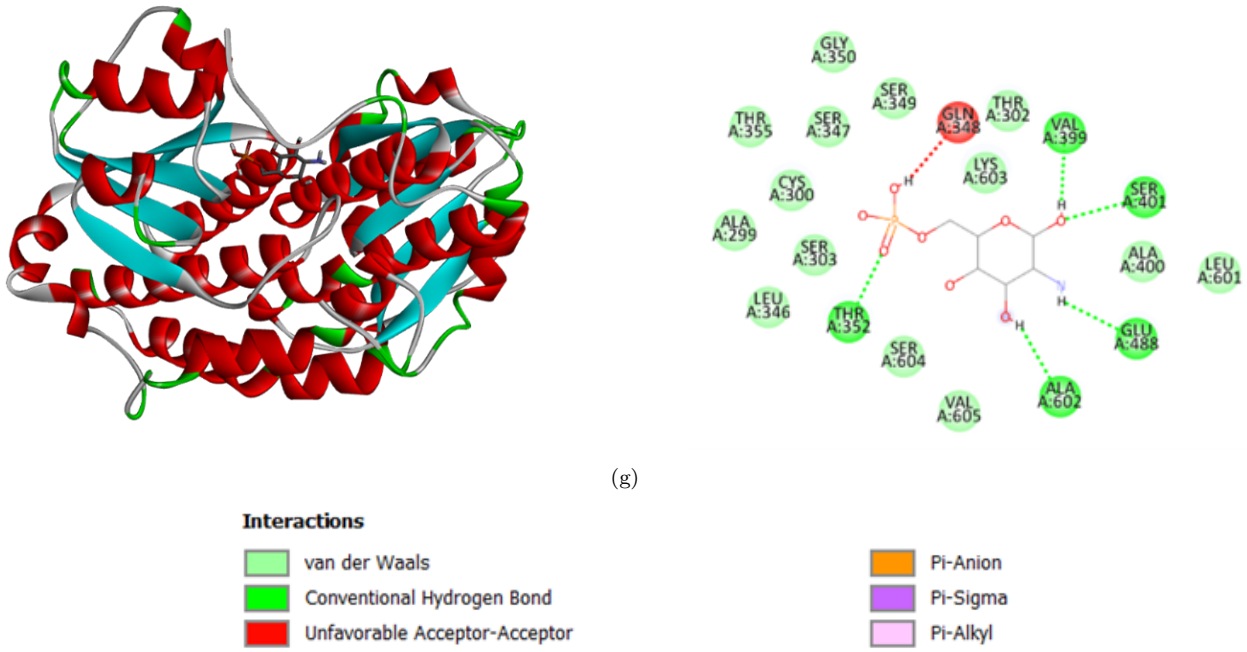

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Figure 3. *Continued*

CONCLUSION

In this study, the chalcone derivatives (2a–c, 3a–c) were successfully synthesized and structurally characterized using IR, ^{1}H-NMR, ^{13}C-NMR, and GC-MS spectroscopic techniques. The in vitro assay revealed that chalcone 2c exhibited significant antimicrobial activity against all tested microbial strains, including two Gram-positive bacteria (*S. aureus*, *S. epidermidis*), two Gram-negative bacteria (*E. coli*, *K. pneumoniae*), and *C. albicans*. The other synthesized compounds (2a, 2b, 3a–c) showed potent to moderate activity against some of selected species. Antioxidant assay using the DPPH method demonstrated that while two derivatives (2a and 2c) lacked radical scavenging activity, the others showed moderate to potent antioxidant potential. Furthermore, molecular docking study using PyRx tool were conducted to investigate the interaction of the synthesized compounds (2a–c, 3a–c) with the active site of GlcN-6-P synthase, the target enzyme associated with antimicrobial activity. Notably, chalcone derivative 2c exhibited strong binding affinity within the binding site, supporting and correlating well with its in vitro antimicrobial efficacy. These findings suggest that compound 2c represents a promising lead for the development of new broad spectrum antimicrobial agents.

SUPPLEMENTARY MATERIAL

None.

AUTHOR CONTRIBUTIONS

Jameel A. M. Al-Duraye: data curation, writing—original draft preparation. Ahmed Mutanabbi Abdula: Conceptualization, methodology, writing—review and editing. Younis Baqi: validation, formal analysis, investigation, supervision.

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DATA AVAILABILITY STATEMENT

Data are available from the authors upon reasonable request.

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CONFLICTS OF INTEREST

The authors, Ahmed Mutanabbi Abdula, managing editor of Al-Mustansiriyah journal of science, and Younis Baqi, editorial board member of the journal, declare a conflict of interest as members of the editorial board. To ensure a fair and transparent review process, neither of them had any role in handling or decision-making related to this submission. The manuscript was handled independently by Yusra Al-Hilali, section editor, and the final acceptance decision was made exclusively by the editor-in-chief, Mohammed Y. Kamil.

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