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Original Article

Synthesis, Characterization, Antibacterial Screening And Molecular Docking Study Of Chalcones Containing Thiophene Motif

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ABSTRACT

Bacterial resistance to conventional therapeutic regimens represents a critical global health challenge, necessitating the development of novel antimicrobial agents. In this study, a targeted series of 5-bromothiophene-based chalcones was synthesized via Claisen-Schmidt condensation and structurally characterized. The synthesized compounds were screened *in vitro* for their antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* using the agar well diffusion method, with Streptomycin serving as the standard reference drug. Among the tested derivatives, 1-(5-bromothiophen-2-yl)-3-(2-nitrophenyl) prop-2-en-1-one and 1-(5-bromothiophen-2-yl)-3-(4-hydroxyphenyl) prop-2-en-1-one demonstrated the highest antibacterial potency, exhibiting zones of inhibition up to 12 mm against *S. aureus* and 11 mm against *E. coli*. To rationalize the observed biological outcomes and elucidate the potential mechanism of action, blind molecular docking simulations were performed against glucosamine-6-phosphate (GlcN-6-P) synthase (PDB ID: 1MOQ) using the CB-Dock 2 server. Key ligand-protein interactions, including hydrogen bonding and hydrophobic contacts within the enzymatic binding pocket, were analyzed and visualized using Discovery Studio Visualizer. The docking scores and binding modes correlated well with the experimental antibacterial activity, highlighting these 5-bromothiophene chalcones as valuable lead structures for further structural optimization and antimicrobial drug discovery.

Keywords: Chalcones, 5-Bromothiophene, Antibacterial activity, Molecular Docking.

INTRODUCTION

The rapid and continuous evolution of multidrug-resistant (MDR) bacterial pathogens stands as one of the most formidable threats to global public health and modern clinical medicine [1,2]. Common bacterial strains, encompassing both Gram-positive species like *Staphylococcus aureus* and Gram-negative species like *Escherichia coli* and *Pseudomonas aeruginosa*, have engineered sophisticated mechanisms to evade conventional antibiotic regimens [3,4]. This ongoing resistance crisis sharply compromises the efficacy of standard clinical treatments, leading to heightened mortality rates, prolonged hospitalizations, and escalated healthcare costs [5.6]. Because the misuse and over-prescription of antibiotics have accelerated this selective evolutionary pressure, the identification and structural development of novel, low-toxicity chemical scaffolds capable of bypassing existing bacterial resistance networks remain an urgent imperative for medicinal chemists worldwide [7].

Among the privileged structural frameworks heavily explored in drug discovery, chalcones have emerged as highly attractive core templates [8.9]. Structurally, chalcones consist of two aromatic rings linked by a three-carbon enone system, which serves as an extraordinarily flexible framework for diverse chemical modifications [10,11]. This reactive Michael acceptor system allows chalcones to readily interact with various sulfhydryl groups or nucleophilic residues within bacterial target proteins [12]. Beyond their structural utility, chalcones possess an impressive spectrum of biological profiles, including potent anti-inflammatory, antioxidant, anticancer, and crucially, antimicrobial actions [13,14]. Several natural and synthetic chalcone derivatives have demonstrated

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success in inhibiting bacterial cell wall biosynthesis, disrupting membrane integrity, and impeding nucleic acid synthesis [15].

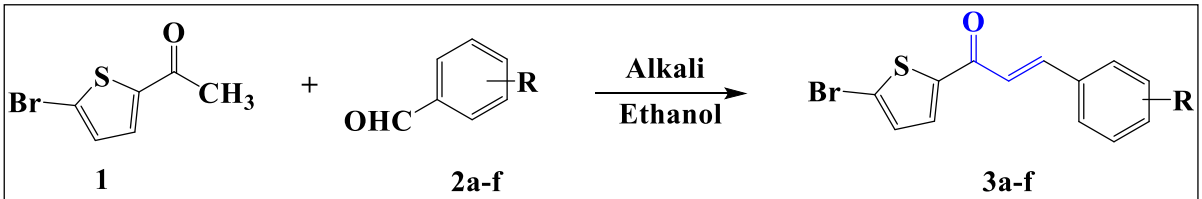
To maximize therapeutic efficacy and address cell wall permeability issues particularly in notoriously defensive Gram-negative bacteria modern structural modifications focus heavily on molecular hybridization [16]. Merging the chalcone template with specialized heterocyclic matrices has proved to be an exceptionally efficient strategy for generating highly potent candidates [17]. In this context, the thiophene ring a sulfur containing five membered heterocycle is highly valued in medicinal chemistry due to its unique electronic characteristics, stability, and bio-isosteric [18]. Thiophene derivatives have historically formed the structural backbone of several notable clinical agents, including the anti-inflammatory drug Tenoxicam and various cephalosporin antibiotics. Incorporating a thiophene core into a molecular architecture enhances overall lipophilicity, which significantly facilitates the passive diffusion of molecules across complex lipid bilayers and bacterial cell membranes, ultimately optimizing target binding inside the cell [19].

Recent literature highlights the immense success of this hybrid approach. For instance, investigations into newly synthesized heteroaryl based chalcones demonstrated exceptional *in vitro* growth inhibition profiles against aggressive pathogenic bacteria, supported by computational models showing robust binding capacity to essential cellular targets like DNA gyrase and Topoisomerase IV [1]. Similarly, recent efforts focused on functionalized chalcone hybrids revealed profound efficacy, where molecular docking studies validating robust binding dynamics either matched or outperformed standard commercial therapeutics like Ciprofloxacin and Amoxicillin [20].

Spurred by these collective insights, the objective of the current investigation is to build upon the synergistic potential of both the chalcone and thiophene moieties. In this work, we report the design, synthesis, and spectroscopic characterization of a series of chalcone derivatives bearing a core thiophene scaffold. The newly synthesized targets were systematically screened for their *in vitro* antibacterial performance against a representative panel of Gram-positive and Gram-negative bacterial strains. To gain deeper insight into their molecular mechanism, molecular docking studies were carried out against glucosamine-6-phosphate synthase, the target enzyme to reveal key ligand-receptor interactions.

MATERIALS AND METHODS:

All substances and solvents were of a high analytical standard and sourced from SDFCL. The melting points were evaluated in open capillary tubes and remain unmodified. The progress and purity of compounds was evaluated by thin-layer chromatography utilizing with F-252 silica gel precoated aluminum plates using petroleum ether-ethyl acetate (9:1) as a developing solvent and spots were visualized by exposing the plates in iodine vapors. Infrared spectra were measured on a Shimadzu spectrophotometer employing the KBr pellet technique (λ max in cm^{-1}). ^1H Nuclear magnetic resonance spectra were recorded on BRUKER ADVANCE (400 FT- NMR) spectrophotometer using dimethyl sulfoxide (DMSO- d6) as a solvent and tetramethyl silane as internal reference (chemical shifts, δ in ppm). The Waters UPLC-TQC Mass Spectrometer was utilized to observe mass spectra.



Compound	3a	3b	3c	3d	3e	3f
R	2-Cl	3-Cl	2-NO_2	3-NO_2	4-NO_2	4-OH

Scheme

General Procedure

Preparation of 1-(-5-bromothiophen-2-yl)-3-aryl-prop-2-en-1-one. (3a-f):

The substituted chalcones were prepared by reacting equimolar quantity of aromatic aldehydes (0.001mol) and 2-acetyl-5-bromothiophene in ethanol. 40% KOH was added to the reaction mixture drop wise with constant stirring at room temperature. After the completion of reaction, the precipitate thus obtained was separated by filtration, washed with cold ethanol, dried and purified by recrystallization from ethanol.

Spectral data of some of the newly synthesized chalcones (3a-f)

1-(5-bromothiophen-2-yl)-3-(2-chlorophenyl) prop-2-en-1-one (3a): 1H-NMR (DMSO-D6, 400MHz, δ in ppm): 7.53(d, 1H, Ar—H), 8.31 (d, 1H, Ar—H), 7.10 (d, 1H, Ar—H), 7.02 (d, 1H, Ar—H), 7.60 (m, 1H, Ar—H), 7.37 (d, 1H, H— C═C), 7.80 (d, 1 H, ═CH—C═O). IR (KBr, cm-1): 3100 cm-1 (C—H Aromatic), 1641 cm-1 (C═O), 1560 cm-1 (C═C Aromatic), 1403 cm-1(C═C). Mass (ESI-MS): 326 (M+1).

1-(5-bromothiophen-2-yl)-3-(4-nitrophenyl) prop-2-en-1-one (3e): 1H-NMR (DMSO-D6, 400MHz, δ in ppm): 8.17 (d, 2H, Ar—H), 8.30 (d, 2H, Ar—H), 7.32 (d, 1H, Ar—H), 7.57 (d, 1H, Ar—H), 7.80 (d, 1H, H— C═C), 8.02 (d, 1 H, ═CH—C═O). IR (KBr, cm-1): 3099 cm-1 (C—H Aromatic), 1643 cm-1 (C═O), 1489 cm-1 (C═C Aromatic), 1407 cm-1(C═C). Mass (ESI-MS): 338 (M+2).

S. N.	Entry	Ar	Colour	% Yield	Melting Point in ^{0}C
	3a	—2Cl—C_6H_5	Yellow	92%	100
	3b	—3Cl—C_6H_5	Pale Green	86%	125
	3c	—2NO_2—C_6H_5	Green	90%	121
	3d	—3NO_2—C_6H_5	Pale Brown	82%	112
	3e	—4NO_2—C_6H_5	Brown	88%	148
	3f	—2OH—C_6H_5	Dark Brown	76%	120

Table-I: Physical Data of Newly Synthesized Chalcones

Antibacterial Assay

All the synthesized chalcones were screened for antimicrobial activity by using two organisms namely *Escherichia coli* and *Staphylococcus aureus,* using streptomycin as a standard drug. Agar well-diffusion method was followed to determine the antimicrobial activity. Bacterial cultures were grown in exponential phase in nutrient broth at 37 ºC for 8 h and adjusted to a final concentration 0.5 McFarland turbidity standard. The nutrient agar plate (thickness 4 to 5 mm) surface is inoculated by spreading a volume of the microbial inoculums over the entire agar surface. A hole with a diameter 6 to 8 mm has punched aseptically with a sterile stainless-steel borer. 30 µl of the chemical agent of desired concentration was introduced into the well with the help of micropipette and allowed to diffuse at room temperature for 1 h. The plates were incubated at 37 ºC for 18 h for bacterial pathogens. The diameter of the inhibition zone (mm) was measured with zone reading scale. The antibacterial activity of the synthesized chalcones against mention organisms is given in **Table-II**.

S. N.	Compound	Antibacterial Activity (Zone of Inhibition in mm)	
		E. Coli	S. Aureus
	3a	11	11
	3b	10	--
	3c	11	12
	3d	10	11
	3e	--	--
	3f	11	12
	Streptomycin	18	19

Table-II: Antibacterial Activity of Chalcone

Molecular Docking Methodology

To elucidate the binding modes, ligand–protein interactions, and binding affinities of the synthesized 5-bromothiophene-based chalcone derivatives, automated molecular docking simulations were performed using the CB-Dock 2 server and visualized using Dassault Systèmes BIOVIA Discovery Studio Visualizer. The co-crystallized ligand, GLP (*2-amino-2-deoxy-6-O-phosphono-α-D-glucopyranose*), served as the reference standard. The binding affinities (docking scores in kcal/mol) and detailed ligand receptor interactions (hydrogen bonding, hydrophobic contacts, and unfavorable interactions) are summarized in **Table-III**.

S. N.	Compound	Docking Score (kcal/mol)	Key Hydrogen-Bonded Residues	Major Non-Covalent Interactions & Unfavorable Contacts
	GLP	-6.5	SER303, SER347, THR352, VAL399, ALA602	**Unfavorable Donor–Donor:** SER349 **vdW:** CYS300, GLY301, GLN348, GLY350, SER401, GLU488, LEU601, LYS603, SER604, VAL605
	3a	-6.6	LYS603	**π–Anion:** GLU488 **Alkyl / π–Alkyl:** CYS300, LEU601 **π–Donor H-bond:** SER401, SER604
	3b	-6.5	THR302	**Alkyl / π–Alkyl:** CYS300, LEU601, VAL605 **π–Donor H-bond:** GLN348, SER303

	3c	-7.3	SER347, GLN348, SER349, THR352, VAL605	**Alkyl / π–Alkyl:** LEU601 **Carbon H-bond / π–Donor:** LYS603, SER604
	3d	-7.3	LYS487	**Unfavorable Positive–Positive:** LYS487 **π–Sigma / π–Alkyl:** LEU480, LEU484
	3e	-7.4	SER347, SER349, THR352	**Alkyl / π–Alkyl:** CYS300, VAL605 **π–Donor H-bond:** SER303
	3f	-7.0	SER347, GLN348, SER349, THR352	**Alkyl / π–Alkyl:** CYS300, VAL399, VAL605 **π–Donor H-bond:** SER303

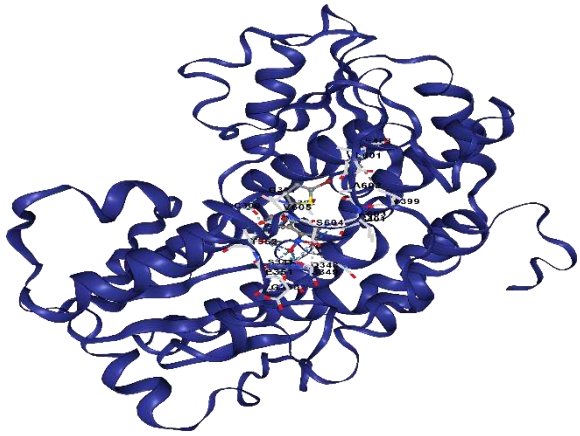
Table-III: Molecular docking scores and interaction profile of GLP and synthesized chalcone derivatives.

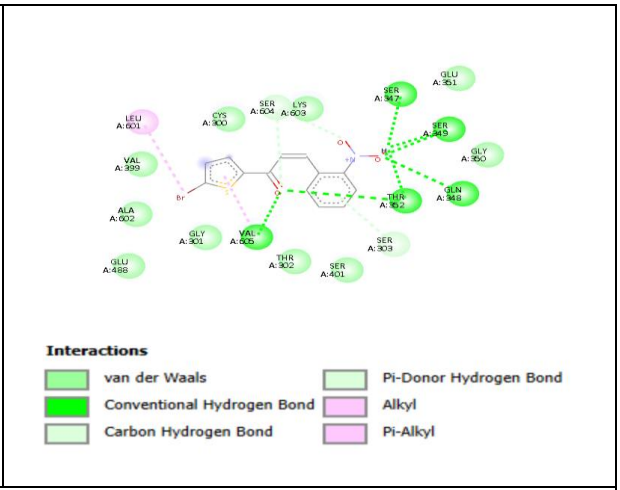
RESULTS AND DISCUSSION:

Molecular Docking Analysis

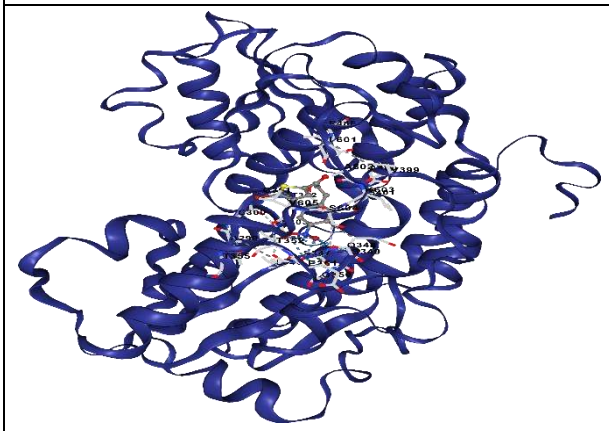
To evaluate the binding efficacy and explore the molecular mechanisms of the synthesized 5-bromothiophene-based chalcone derivatives, automated molecular docking simulations were conducted using CB-Dock 2, and the resulting non-covalent interactions were visualized using Discovery Studio Visualizer. The reference co-crystallized ligand, GLP (*2-amino-2-deoxy-6-O-phosphono-α-D-glucopyranose*), was docked under identical conditions to establish a baseline binding score (-6.5 kcal/mol).

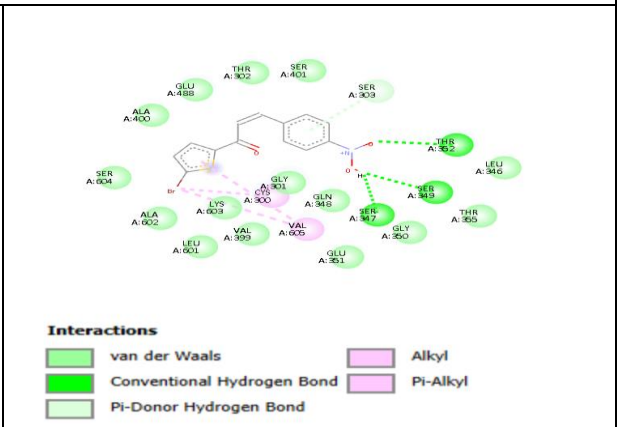
Among the series, the nitro-substituted derivatives exhibited superior binding affinities over GLP, with compound 3e (4-NO_2, −7.4 kcal/mol), compound 3c (2-NO_2, −7.3 kcal/mol), and compound 3d (3-NO_2, −7.3 kcal/mol) showing the highest binding potency, driven primarily by strong conventional hydrogen bonds with polar active-site residues SER347, SER349, and THR352. The phenolic hydroxyl derivative, compound 3f (4-OH), also demonstrated significant binding enhancement (−7.0 kcal/mol) through key hydrogen-bonding interactions with SER347, GLN348, SER349, and THR352. In contrast, the chloro-substituted derivatives 3a (2-Cl, −6.6 kcal/mol) and 2b (3-Cl, −6.5 kcal/mol) displayed binding scores comparable to GLP, relying predominantly on hydrophobic π–alkyl interactions with CYS300, LEU601, and VAL605 with minimal hydrogen bonding. Across all synthesized analogues, the hydrophobic 5-bromothiophene core reliably anchored within the binding pocket through hydrophobic contacts with CYS300 and LEU601. While compound 3d introduced an unfavourable positive–positive interaction with LYS487, compounds 3e and 3f formed favourable, unobstructed interaction networks without electrostatic penalties, establishing them as the most promising lead candidates among the evaluated chalcones.



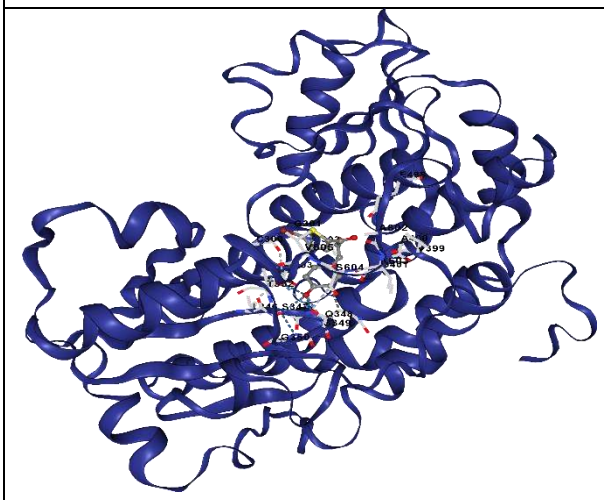


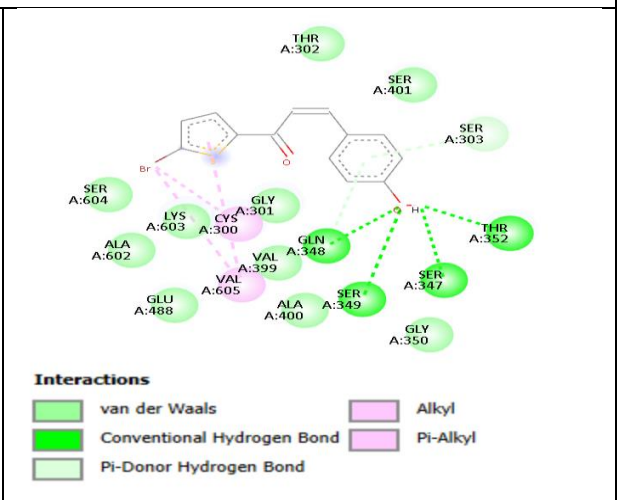
2D and 3D molecular docking interaction of 3c with glucosamine-6-phosphate (GlcN-6-P) synthase





2D and 3D molecular docking interaction of 3e with glucosamine-6-phosphate (GlcN-6-P) synthase





2D and 3D molecular docking interaction of 3f with glucosamine-6-phosphate (GlcN-6-P) synthase

Antibacterial Activity

The *in vitro* antibacterial activity of the synthesized 5-bromothiophene chalcone derivatives (3a–3f) was evaluated against *Escherichia coli* and *Staphylococcus aureus* using the agar well diffusion assay, with Streptomycin (18 mm against *E. coli* and 19 mm against *S. aureus*) serving as the positive standard. Among the evaluated series, compounds 3c and 3f demonstrated the highest antibacterial potency, producing inhibition zones of 11 mm against *E. coli* and 12 mm against *S. aureus*, followed closely by 3a (11 mm against both) and 3d (10 mm against *E. coli*, 11 mm against *S. aureus*). In contrast, compound 3b showed weak, strain-selective activity against *E. coli* (10 mm) with no inhibition against *S. aureus*, while compound 3e proved completely inactive against both tested bacterial strains. Importantly, these experimental antibacterial findings strongly align with the theoretical predictions obtained from molecular docking simulations. The superior antibacterial performance of 3c and 3f directly correlates with the top-ranked *in silico* docking profiles—particularly those of the 4-NO_2 and 4-OH derivatives—which exhibited strong binding affinities (-7.0 to -7.4 kcal/mol) driven by extensive hydrogen bonding with active-site residues SER347, SER349, and THR352. Conversely, the diminished or complete lack of bioactivity observed for 3b and 3e mirrors the lower docking scores (-6.5 kcal/mol) and unfavourable electrostatic/steric contacts observed during computational modelling. Consequently, the experimental bioassay results validate the docking models, establishing the most active 5-bromothiophene chalcones as promising candidate scaffolds for future antibacterial optimization.

CONCLUSION

In conclusion, a series of 5-bromothiophene-based chalcone derivatives was successfully synthesized and structurally elucidated using standard spectroscopic techniques (FTIR, ^{1}H NMR and mass spectrometry), which unequivocally confirmed the characteristic α,β-unsaturated enone linkage ($-CH = CH-$) and target molecular frameworks. Subsequent *in silico* molecular docking simulations demonstrated that electron-withdrawing nitro derivatives (4-NO_2, -7.4 kcal/mol) and electron-donating hydroxyl derivatives (4-OH, -7.0 kcal/mol) exhibited superior binding affinities compared to the benchmark standard GLP (-6.5 kcal/mol). These high docking scores were stabilized by extensive hydrogen-bonding networks with key active-site residues (SER347, SER349, and THR352) and hydrophobic anchoring via the 5-bromothiophene core. Aligning well with these computational predictions, the *in vitro* bioassay against *E. coli* and *S. aureus* identified compounds 3c and 3f as the most active analogues within the series, displaying maximum inhibition zones of 11–12 mm. The strong agreement between spectral verification, molecular docking scores, and experimental antibacterial activities validates the proposed binding mode and highlights compounds 3c and 3f as viable lead scaffolds for further chemical optimization and antibacterial drug development.

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