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SYNTHESIS AND *IN-SILICO* ASSESSMENT OF CHALCONE DERIVATIVES AGAINST *BACILLUS SUBTILIS*

Partha Pratim Gogoi, Nichan Boruah, Penlisola Longkumer, Mhasilhoutuo Pucho, Upasana Bora Sinha*

Department of Chemistry, Nagaland University, Lumami, Zunheboto, 798627.

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***Corresponding Author: Upasana Bora Sinha**
Department of Chemistry, Nagaland University, Lumami, Zunheboto, 798627.
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ABSTRACT

Chalcones are privileged flavonoid scaffolds with a broad spectrum of antimicrobial activity. In this study, a series of brominated chalcone derivatives (103, 116, 198 and 200) were synthesized by Claisen–Schmidt condensation followed by regioselective bromination using tetrabutylammonium tribromide (TBATB). The chemical structures were confirmed by FTIR, ^{1}H NMR, ^{13}C NMR and mass spectrometry. In-silico molecular docking was done using Molegro Virtual Docker against DNA gyrase sub-unit B (GyrB PDB ID: 6F86) to elucidate the antimicrobial mechanism of the compounds against Bacillus subtilis. The binding affinities calculated for all the synthesized derivatives were better than the standard reference drug ciprofloxacin (MolDock score: -93.74). Among these, Chalcone 116 showed the most promising binding energy (MolDock score: -106.05; Rerank score: -83.44) with important interactions to critical active-site residues such as Asn46, Gly77, Asp73 and Glu50, similar to the native co-crystallized ligand. These results suggest that strategic bromination optimizes target engagement at the GyrB ATP binding site, making Chalcone 116 a strong lead for further empirical validation as an antibacterial agent.

KEYWORDS: Chalcone derivatives; *Bacillus subtilis*; DNA gyrase subunit B; Molecular docking; Antibacterial activity.

INTRODUCTION

Chalcones are naturally occurring organic compounds of an open chain structure, which form a central core for 1,3-diarylprop-2-en-1-one.[1] They are an important class of the flavonoid family. The nucleus of this molecule is a C6-C3-C6 scaffold made up of two aromatic rings connected by a three-carbon α,β-unsaturated carbonyl moiety, which acts as a critical Michael acceptor.[2] Chalcones are important secondary metabolites in the plant kingdom and are widely

distributed in many edible and medicinal plants such as tomato, apple, licorice, and fingerroot.[3] They are important intermediates and key precursors in the biosynthetic pathway of other complex flavonoids and isoflavonoids. Chalcone molecules are characterized by the presence of conjugated double bonds and a system of delocalized π-electrons over the benzene rings, capable of actively participating in electron transfer reactions.[4] Chalcones are known to interact with a wide range of cellular targets due to their relatively simple structural architecture and highly reactive α,β-unsaturated ketone moiety.[5] Thiol groups readily form covalent bonds, with a particular focus on the cysteine residues of certain cellular enzymes and proteins.[6] The chalcone scaffold has drawn much attention in recent medicinal chemistry due to its structural simplicity and easy synthetic modification. These features are often used by researchers to change the biological profile by adding different substituents (hydroxyl, methoxy, prenyl groups) on the aromatic rings.[7] Thus natural and synthetic chalcones and their derivatives are known to be a rich source of biosynthetic utility and also highly privileged template for development of new biologically active compounds with diverse pharmaceutical applications in drug discovery.[8]

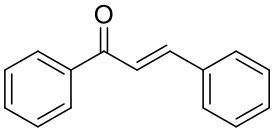


Figure 1: General structure of chalcone.

The main reason for their wide pharmacological significance is the reactive ketoethylenic moiety present in the chalcone structure. These medicinally privileged molecules possess a remarkably wide range of biological activities, thus making them of great value in pharmaceutical development.[9] Chalcones have been associated with potent antioxidant, antibacterial, antiviral, anti-inflammatory and anticancer activity.[10] These compounds have significant antimalarial, antidiabetic and neuroprotective efficacy, amongst. The remarkable ability to modulate their chemical scaffold synthetically allows medicinal chemists to improve their potency, maximize their molecular targeting capabilities and reduce potential toxicity effectively, guaranteeing their enormous importance in rational drug design.[11]

Recent studies have resorted to sophisticated computational methodologies to screen chalcone candidates prior to physical synthesis, in an effort to optimize the development of effective therapeutic agents.[12] In this regard, several promising chalcone derivatives were selected from our previously published literature and subjected to rigorous in-silico evaluations. In silico the pharmacokinetic profiles, drug-likeness according to Lipinski's Rule of Five and Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) parameters of these molecules were evaluated. This computational pre-screening successfully identified compounds with good oral bioavailability, high safety margins and optimal binding affinities, ensuring that only the most suitable, non-toxic derivatives went forward for laboratory synthesis.[13]

Following successful synthesis, these optimized chalcone derivatives were extensively screened *in silico* for their specific antibacterial potential against the Gram-positive bacterium *Bacillus subtilis*. Molecular docking studies were carried out against essential *Bacillus subtilis* target proteins to understand the exact mechanistic basis of their antimicrobial action. The *in-silico* molecular docking analysis provided detailed three-dimensional insights into the ligand-receptor interactions, predicting the binding affinities, favourable conformations and hydrogen-bonding

networks at the active sites of these bacterial enzymes. Structural validation revealed that the synthesized derivatives could anchor effectively to the targeted pathogenic proteins, thus successfully establishing the correlation of the computational predictions with their intrinsic antibacterial capabilities.

This work ultimately closes the gap between computer aided drug design and real-world synthesis of organic compounds, creating a complete pipeline for the discovery of new antibacterial agents. The main contribution of this manuscript is the very integrated dual approach methodology. The integration and application of molecular docking especially to *Bacillus subtilis* proteins provides deep mechanistic insights that cannot be obtained using only standard *in-vitro* screening methodologies.

The synergistic workflow validates the therapeutic utility of the synthesized chalcone analogues and definitively clarifies their exact atomic-level interactions with essential bacterial targets. Given the ongoing threat of antimicrobial resistance (AMR) as a serious global health problem, the discovery of structurally optimized compounds that can directly target critical pathogenic bacterial pathways is of great importance. Thus, the present work provides a detailed and reproducible platform for future medicinal chemistry efforts. The findings will represent a significant addition to the overall pharmacological database and will demonstrate how rational structural modifications based solely on the computational predictive models and validated by the targeted *in-silico* molecular docking can systematically generate highly potent antimicrobial therapeutics.

MATERIALS AND METHOD

Chemicals and instrumentation

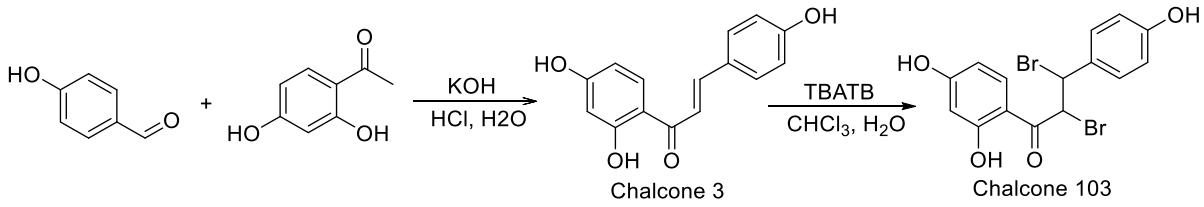
Chemicals, solvents and reagents were purchased from commercial suppliers (Sigma Aldrich, Merck and SD Fine) and were used as such without further purification. The progress of reactions and purity of the compounds was monitored by Thin Layer Chromatography (TLC) on Merck Silica Gel HF_{254} plates and detection of spot was done under 254 nm UV lamp. The chromatographic separations were carried out on columns of silica gel (60-120 mesh). The infrared data were collected on a Perkin Elmer Spectrum Two FT-IR spectrometer to confirm the chemical structures. Furthermore, ^{1}H and ^{13}C NMR spectra were measured in DMSO on a 400 MHz Bruker Advance II FT-NMR spectrometer.

Synthesis of the brominated chalcone derivatives

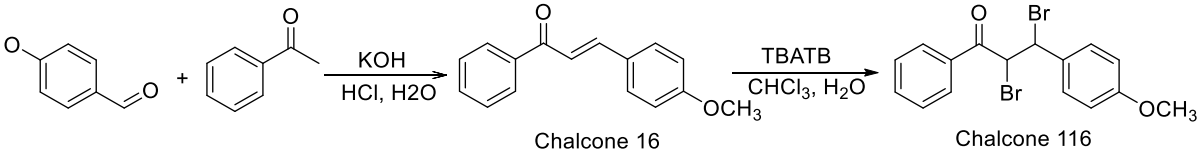
10 mmol acetophenone (or derivatives thereof) and benzaldehyde (or derivatives thereof) were dissolved in 20 mL absolute ethanol in a 100 mL flask. The mixture was stirred for 24 hours above room temperature, with the addition of 5 to 10 drops of 40% KOH as a catalyst. The progress of the reaction was monitored by thin layer chromatography (TLC) on silica gel HF254. After complete reaction of the aldehydes with the ketones to give the chalcone compounds, stirring was stopped. The resultant reaction mixture was poured into a beaker containing cold distilled water and was neutralized with 25% HCl till the indicator showed neutralization. The crystals formed were filtered and dried at room temperature. The chalcone derivatives were prepared by recrystallization in hot ethyl acetate and the final product was purified by column chromatography using ethyl acetate-hexane as solvent system.[14-16]

In the next bromination process, synthesized chalcone derivatives and TBATB were dissolved in a little quantity of dry aprotic solvent in a 1:1 ratio.[17] The mixture was stirred vigorously at room temperature and checked constantly by TLC on silica gel HF254. Reaction times ranged from minutes to hours depending on the particular derivatives. The

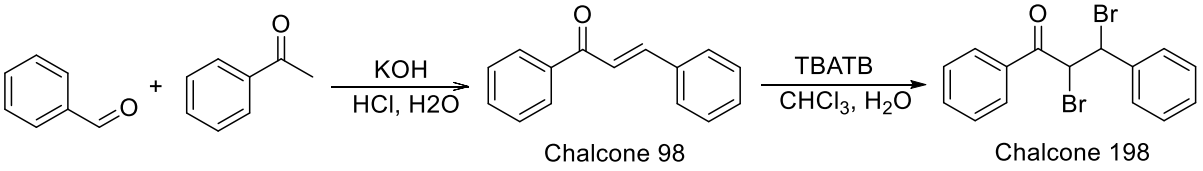
solid precipitate formed was washed with the reaction solvent and purified by recrystallization from ethanol. Finally, the product was obtained by column chromatography in ethyl acetate-hexane solvent system **(Scheme 1 to 4)**.



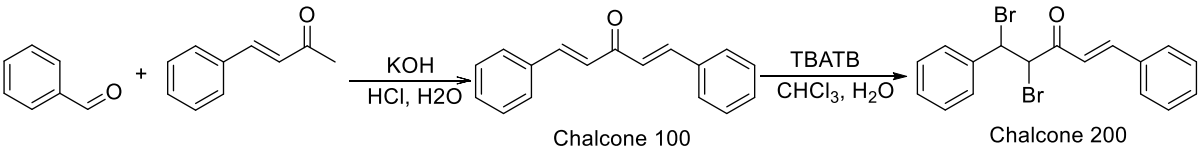
Scheme 1: ***Reaction pathway for Chalcone 103.***



Scheme 2: ***Reaction pathway for Chalcone 116***



Scheme 3: ***Reaction pathway for Chalcone 198.***



Scheme 4: Reaction pathway for Chalcone 200.

***In-silico* studies**

Target protein selection and optimization

The choice of a proper biological target is a key prerequisite for reliable computational drug evaluation. In this study, the main macromolecular target selected was Bacillus subtilis (PDB ID: 6F86) DNA gyrase subunit B (GyrB).[18] DNA gyrase is an essential bacterial type II topoisomerase that catalyses topological changes in DNA during replication, transcription and recombination.[19] The ATPase active site in the GyrB subunit is highly conserved in bacteria and structurally distinct from human topoisomerases. This site is a highly selective and validated target for novel antibacterial discovery.[20]

For our study we selected the particular crystal structure 6F86 due to the excellent crystallographic resolution and the presence of a clearly defined structurally intact ligand binding pocket. This high-quality structure from a well-established Gram-positive model organism provides a comprehensive and accurate geometric scaffold. This allows the subsequent molecular docking simulations to reliably assess the binding affinities, thermodynamic stability, and exact non-covalent interactions (e.g., hydrogen bonding and hydrophobic contacts) of the prepared antibacterial scaffolds within the active site.[21]

The three-dimensional crystal structure of the target protein was prepared for molecular docking using BIOVIA Discovery Studio 2026.[22] In an effort to avoid spatial interference and computational artifacts in the docking simulations, the crystallographic water molecules, extraneous heteroatoms, and non-essential polypeptide chains were removed from the structural model.[23] Subsequently, the polar hydrogen atoms were added to the macromolecule and appropriate protonation states were assigned. This is an essential step for accurate calculation of electrostatic and hydrogen bonding interactions between the receptor and the ligands.[24] The Structure-Based Design (SBD) site feature integrated in the software was used to define the primary ligand-binding pocket and the corresponding spatial docking grid. Following these optimization steps, the fully refined receptor structure was exported and saved in the standard Protein Data Bank (PDB) format, ready for the subsequent virtual screening procedures.

Ligand preparation and optimization

The synthesized chalcone derivatives were drawn in two-dimensional chemical structures using ChemDraw 2D and their respective Simplified Molecular-Input Line-Entry System (SMILES) notations were generated.[25] These SMILES strings were then imported to ChemDraw 3D to convert the two-dimensional topologies to initial three-dimensional spatial geometries. Structural optimization was done to ensure that the ligands were in the most thermodynamically stable and energetically favourable conformation before virtual screening. This was done by energy minimizing the 3D models using the embedded Molecular Mechanics 2 (MM2) force field of the software.[26] The MM2 optimization systematically optimized bond lengths, bond angles and torsional constraints to relieve any internal steric clashes and obtain a local energy minimum.[27] After successful geometry optimization, the optimized structures of ligands were exported and saved in the standard '.mol2' format. We have chosen this particular format because it preserves the three-dimensional coordinates, bond orders and atom types that are strictly necessary for downstream molecular docking calculations.

Molecular docking studies

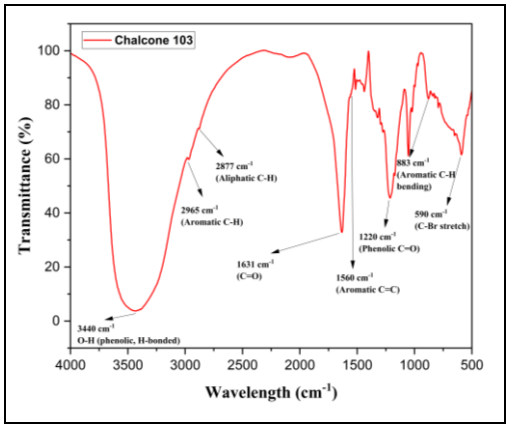
Molegro Virtual Docker (MVD) was used for molecular docking studies. 6.0.[28] The docking protocol was validated by re-docking of the co-crystallized ligand.[29] The protein preparation including missing residue correction was performed using the integrated protein preparation module available in MVD 6.0. The cavity detection module was used to detect optimal binding sites using an expanded van der Waals molecular surface. The grid set for docking against the target protein DNA gyrase subunit B (GyrB) from Bacillus subtilis (PDB ID: 6F86) was set to x: 62.34, y: 30.12 and z: 62.23 with the volume of 15.872 $Å^3$ and surface area of 72.96 $Å^2$ along with radius of 12 Å.

The chalcone derivatives were then imported to the workspace. The scoring function was set to MolDock score with a grid resolution defined as 0.30 Å. MolDock SE was selected as the search algorithm and 30 independent runs were performed for each simulation. Simplex evolution and pose generation parameters were set to default values and the maximum number of poses retained per simulation was five. Finally, the obtained docking conformations were analyzed and visualized using BIOVIA Discovery Studio (DS) Visualizer 2026.

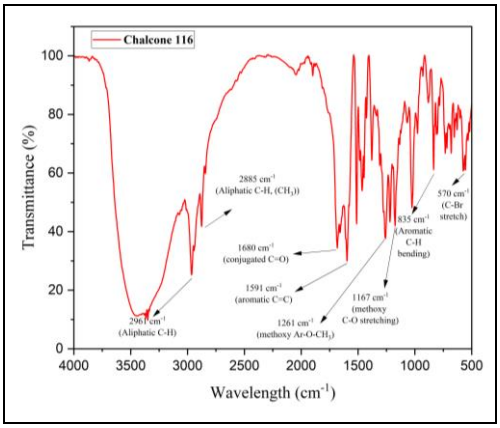
RESULTS AND DISCUSSION

FTIR Spectroscopy

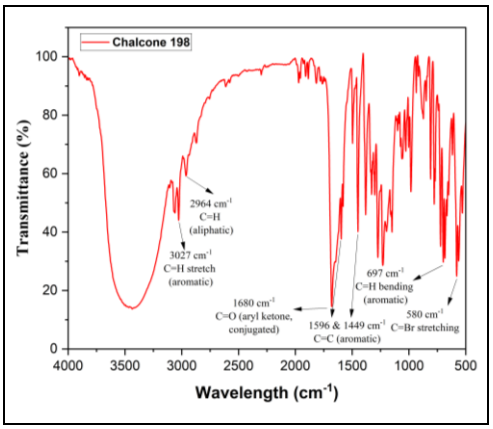
The FTIR spectra of the synthesized chalcone derivatives are presented in Figure 2 below.



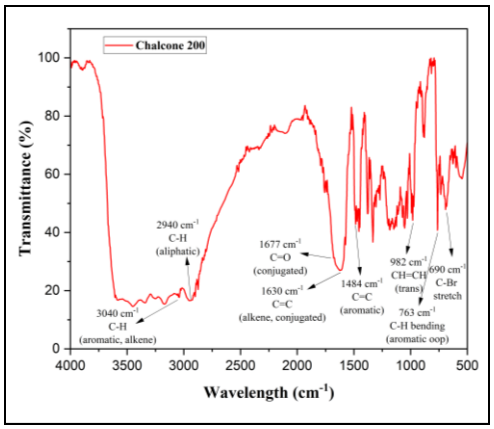
Chalcone 103



Chalcone 116



Chalcone 198



Chalcone 200

Figure 2: ***FTIR spectra of Chalcone 103, 116, 198 and 200.***

Mass Spectroscopy

The Mass spectra of the synthesized chalcone derivatives are provided in Figure 3-6 below.

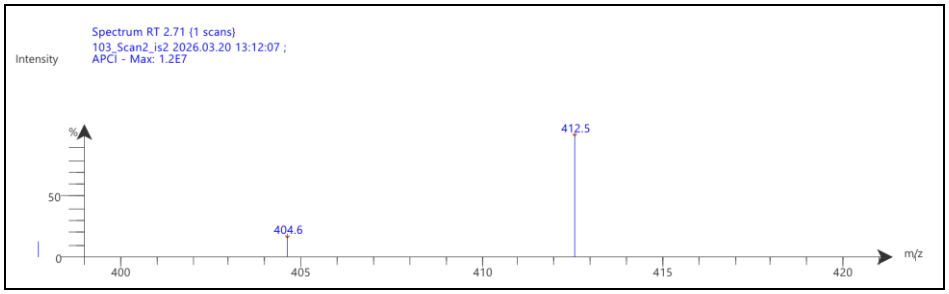


Figure 3: ***Mass spectrum of Chalcone 103.***

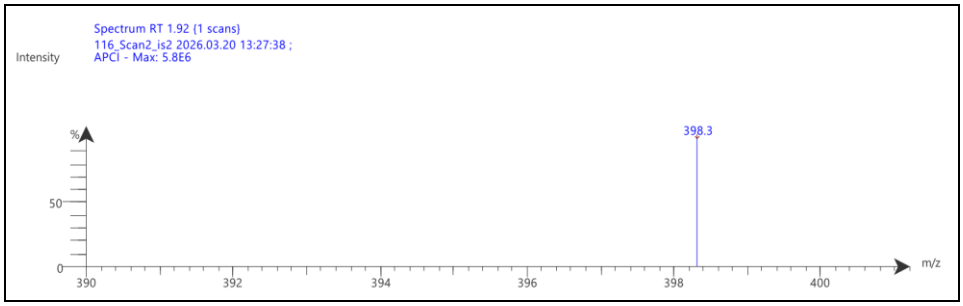


Figure 4: ***Mass spectrum of Chalcone 116.***

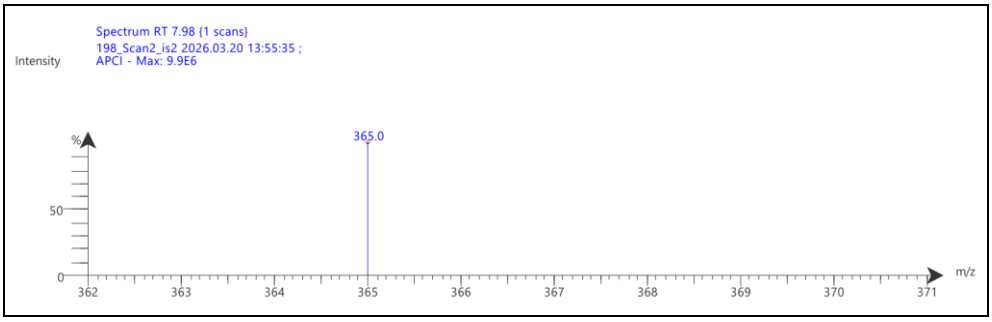


Figure 5: ***Mass spectrum of Chalcone 198.***

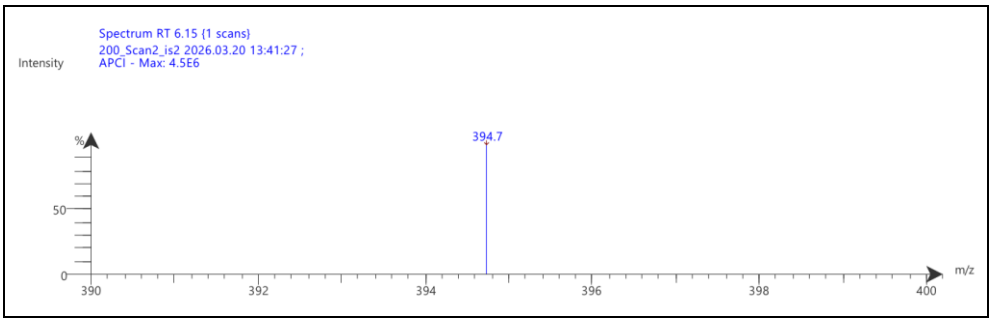


Figure 6: ***Mass spectrum of Chalcone 200.***

Spectral Data

(Chalcone 103) 2,3-dibromo-1-(2,4-dihydroxyphenyl)-3-(4-hydroxyphenyl)propan-1-one: (Yield % 60; MP 210^{o}C) ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 3.66 (s, 1H), 5.74 (d, *J* = 10.4 Hz, 1H), 6.09 (d, *J* = 10.3 Hz, 1H), 6.65 (m, 2H), 6.78 (d, *J* = 7.3 Hz, 2H), 7.10 (d, *J* = 7.5 Hz, 2H), 7.63 (d, *J* = 7.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 50.1, 50.5, 74.3, 104.2, 109.2, 114.2, 116.3, 126.3, 130.9, 133.0, 156.8, 162.9, 164.8, 182.9, 196.8

(Chalcone 116) 2,3-dibromo-3(4-methoxy-phenyl)-1-phenyl-propan-1-one: (Yield % 68; MP 148^{o}C) ^{1}H NMR (400MHz, $CDCl_3$) δ (ppm) 8.15 (m, 2H, ArH), 7.46 (m, 5H, ArH), 6.86 (m, 2H, ArH), 5.28 (d, 1H, -C*H*Br(ArH)-), 5.20 (d, 1H, -C*H*Br(CO)-), 3.8 (s, 3H, -OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 50.1, 52.8, 55.6, 113.9, 114.6, 127.5, 128.2, 128.7, 129.1, 129.4, 131.6, 133.8, 137.2, 159.8, 192.1

(Chalcone 198) 2,3-Dibromo-1,3-diphenyl-propane-1-one: (Yield % 87; MP 160^{o}C) ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 5.65 (d, *J* = 11.4 Hz, 1H), 5.83 (d, *J* = 11.4 Hz, 1H), 7.39 (m, 3H), 7.53 (m, 4H), 7.66 (m, 1H), 8.10 (d, *J* = 8.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 47.2, 50.2, 128.5, 128.7, 129.0, 129.1, 129.2, 129.5, 134.4, 134.6, 138.4, 191.2

(Chalcone 200) 4,5-Dibromo-1,5-diphenyl-pent-1-ene-3-one: (Yield % 76; MP 120^{o}C) ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 5.22 (d, *J* = 11.6 Hz, 1H), 5.49 (d, *J* = 11.6 Hz, 1H), 6.97 (d, *J* = 15.6 Hz, 1H), 7.44 (m, 8H),7.63 (m, 2H), 7.87 (d, *J* = 15.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 49.9, 51.9, 114.8, 122.3, 128.4,129.0, 129.1, 129.2, 129.4, 129.6, 131.4, 134.2, 138.3, 146.3, 189.9

Molecular Docking studies

The output files generated from the molecular docking simulations were analysed by BIOVIA Discovery Studio (DS) Visualizer. Comparative analysis of the interactions of each ligand with amino acid residues in the active site of the

target protein was undertaken. For these interactions, the binding profiles of the test compounds were compared to the co-crystallized ligand and the standard reference drug, Ciprofloxacin.

The specific scores and interacting residues for each ligand are summarized in **Table 1** below.

Table 1: ***Docking scores of the ligands with target protein 6F86.***

Ligand	[a]MolDock Score	[b]Rerank Score	[c]Interaction	[d]Internal	[e]HBond	[f]LE1	[g]LE3
CWW_500	-141.447	-116.777	-145.753	4.30629	-4.90237	-5.23877	-4.32508
116	-106.048	-83.4404	-120.528	14.4799	-2.5	-5.30242	-4.17202
200	-104.146	-79.999	-116.524	12.3781	-1.62951	-5.20732	-3.99995
103	-104.814	-75.2341	-112.747	7.93317	-3.59222	-4.99115	-3.58258
198	-97.6428	-75.1036	-113.465	15.8224	-2.06388	-5.4246	-4.17242
Ciprofloxacin	-93.7423	-62.0997	-118.727	24.9849	-3.58774	-3.90593	-2.58749

[a]The primary empirical scoring function used to evaluate and rank the overall binding energy of the generated ligand-protein pose; [b]A refined, computationally heavier scoring function applied to the top poses to provide a more accurate estimation of binding affinity; [c]The calculated total interaction energy strictly between the docked ligand and the target protein receptor; [d]The internal energy of the ligand's conformation, accounting for intramolecular steric clashes and torsional strain; [e]The specific energetic contribution derived from hydrogen bonding interactions between the ligand and the active site residues; [f]The binding efficiency calculated by normalizing the MolDock Score by the number of heavy atoms in the ligand; [g]The binding efficiency calculated by normalizing the Rerank Score by the number of heavy atoms in the ligand.

Interpretation of docking data

The molecular docking data analysed showed that the chalcone derivatives successfully occupied the same active binding pocket as the co-crystallized ligand (CWW) and the standard reference drug, ciprofloxacin. The chalcones make important binding contacts primarily by conventional hydrogen bonds, carbon-hydrogen bonds, halogen bonds and a large hydrophobic network (alkyl and pi-alkyl bonds etc). The comparative analysis indicates that the synthesized series consistently target the key active site residues, i.e. Val43, Asn46, Gly77, Asp73, Ala47, and Ile78, in close similarity to the binding profiles of the reference compounds. For instance, ciprofloxacin is strongly anchored by interactions with Val43, Asn46, Asp73 and Gly77, while the co-crystallized ligand has an even wider network including these residues plus Glu50, Arg76 and Val71. The ability of the chalcone derivatives to imitate these specific interaction modes points to a very similar mode of target recognition.

A detailed comparison of the interacting residues revealed that Chalcone 116 showed the highest similarity to both the co-crystallized ligand and ciprofloxacin. Chalcone 116 forms a conventional hydrogen bond with Asn46 and a carbon-hydrogen bond with Gly77, directly corresponding to the main hydrogen-bonding scheme employed by both reference compounds. Chalcone 116 also has a halogen interaction with Asp73, and a pi-anion contact with Glu50 which fits perfectly into the critical amino acid pockets targeted by the references. Its hydrophobic interaction profile includes Val71, Ala47, Val43, Ile78 and Pro79 and is almost identical to the alkyl and pi-alkyl interaction that occurs in CWW and ciprofloxacin. Chalcone 198 also shows significant binding similarity but does not interact with Gly77 and Pro79. Therefore, Chalcone 116 is the closest derivative to the well-known reference ligands in the binding mode in the active site of the target protein, due to its comprehensive and overlapping binding signature.

The specific interacting residues for each ligand are summarized below in the **Figure 7** and **Table 2**.

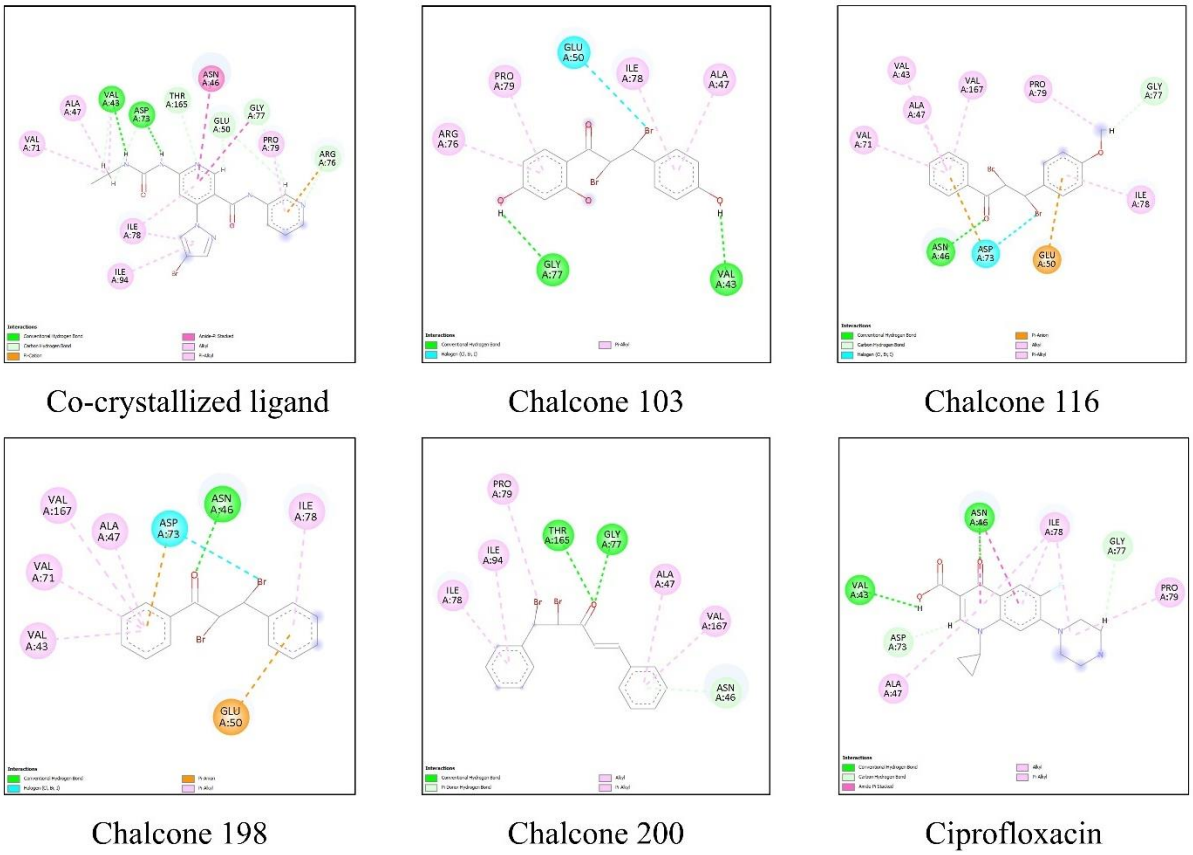


Figure 7: 2D Interaction profile of the chalcone derivatives and reference standard Ciprofloxacin and co-crystallized ligand.

Table 2: Detailed Interaction profile of the chalcone derivatives and reference standard Ciprofloxacin and co-crystallized ligand.

Ligands	Type of interactions	Residues
Co-crystallized ligand (CWW)	Conventional hydrogen bond	Val43, Asp73
	Carbon hydrogen bond	Thr165, Glu50, Gly77, Arg76
	Pi-Cation	Arg76
	Amide Pi-Stacked	Asn46, Gly77
	Alkyl	Val71, Ala47, Val43
	Pi-Alkyl	Ile78, Ile94, Pro79
Chalcone 103	Conventional hydrogen bond	Gly77, Val43
	Halogen	Glu50
	Pi-Alkyl	Arg76, Pro79, Ile78, Ala47
Chalcone 116	Conventional hydrogen bond	Asn46
	Carbon hydrogen bond	Gly77
	Halogen	Asp73
	Pi-Anion	Glu50
	Alkyl	Pro79
	Pi-Alkyl	Val71, Ala47, Val43, Val167, Ile78
Chalcone 198	Conventional hydrogen bond	Asn46
	Halogen	Asp73
	Pi-Anion	Glu50
	Pi-Alkyl	Val43, Val71, Val167, Ala47, Ile78

Chalcone 200	Conventional hydrogen bond	Thr165, Gly77
	Pi-Donor Hydrogen Bond	Asn46
	Alkyl	Pro79
	Pi-Alkyl	Ile78, Ile94, Ala47, Val167
Ciprofloxacin	Conventional hydrogen bond	Val43, Asn 46
	Carbon hydrogen bond	Asp73, Gly77
	Amide Pi-Stacked	Asn46
	Alkyl	Ile78
	Pi-Alkyl	Ala47, Ile78, Pro79

DISCUSSION

Herein, we report the successful synthesis of a series of brominated chalcone derivatives (Chalcones 103, 116, 198, and 200) and their evaluation as potential targeted antibacterial agents against *Bacillus subtilis* DNA gyrase subunit B (GyrB). DNA gyrase is an essential bacterial type II topoisomerase involved in the regulation of DNA topology during replication and transcription. Thus, targeted inhibition of DNA gyrase is a validated and highly effective antimicrobial strategy. Subsequent structural validation of the synthesized scaffolds was performed with detailed FTIR and mass spectrometry followed by successive *in-silico* molecular docking simulations to elucidate their putative pharmacological mechanisms.

The molecular docking data gave valuable information about the binding favourability of the synthesized chalcones in the active site of GyrB. The primary empirical measure of binding energy, MolDock scores, indicated that all the 4 synthesized derivatives showed higher theoretical binding affinities than the standard clinical reference drug, ciprofloxacin (-93.7423). Chalcone 116 was the best candidate among the tested compounds in the series with the most favourable MolDock score (-106.048) and Rerank score (-83.4404). This indicates formation of a very stable protein-ligand complex. Although the native co-crystallized ligand (CWW) showed the highest affinity overall (−141.447), the fact that the brominated chalcone scaffolds could consistently surpass ciprofloxacin underscores their significant potential as viable lead compounds for antibacterial drug development.

The molecular interaction at the atomic level has been compared in detail to clarify the structural basis for these favourable binding energies. Potent GyrB inhibitors bind to the ATP binding pocket through specific hydrogen-bonding and hydrophobic networking pathways. The interaction profiling verified that the synthesized series of chalcones could localize within this very orthosteric pocket mimicking the spatial and interactive signatures of both CWW and ciprofloxacin. Specifically, the derivatives consistently interacted with a set of critical active site residues including Val43, Asn46, Gly77, Asp73, Ala47, and Ile78, suggesting a similar competitive target recognition mechanism.

The Chalcone 116 that obtained the best binding scores showed excellent interactive similarity to the reference standards. Its structural conformation facilitated the formation of a classical hydrogen bond with Asn46 and a carbon-hydrogen bond with Gly77, which corresponded directly to the major anchoring framework for strong GyrB inhibition. Furthermore, the rational positioning of the bromine substituent allowed a key halogen interaction with Asp73, which was further reinforced by a pi-anion interaction with Glu50. These specific electrostatic interactions, along with a large hydrophobic network including Val71, Ala47, Val43, Ile78 and Pro79, are mainly responsible for its improved docking profile. In contrast, the general binding modality of Chalcone 198 was similar, but it had a lower binding affinity (-

97.6428) due to the lack of interaction with Gly77 and Pro79. This mismatch highlights the strict structural requirements of the active site and provides useful structure-activity relationship (SAR) information.

These computational results ultimately suggest that the synthesized brominated chalcones especially chalcone 116 act through direct, competitive inhibition of the GyrB ATP-binding domain in their proposed antibacterial activity. Clearly, the intentional halogenation of the chalcone backbone maximizes target engagement and molecular stability. These insights not only validate the rational design of the compounds but also provide an accurate structural basis for future optimization of chalcone-based antimicrobial therapeutics. Further validation is necessary through *in vitro* and *in vivo* experimentations.

LIMITATIONS OF THE STUDY

The present study is based on chemical synthesis and computational evaluations, especially molecular docking and pharmacokinetic profiling. These in silico methods are useful for the initial screening and selection of potential inhibitors against Bacillus subtilis DNA gyrase subunit B (GyrB, PDB ID: 6F86), but they are only theoretical predictions and not experimental proof of biological activity or actual binding affinity. In addition, mathematical models cannot fully recapitulate the complex nature of dynamic biological systems and thus cannot account for important issues such as in vivo toxicity, cellular permeability, metabolic stability and unintended off-target interactions. So, despite strong theoretical binding affinities of the tested chalcone derivatives to the GyrB target, there is no experimental evidence to date for their real interaction with the enzyme or any effect on the survival of B. subtilis.

Finally, the molecular mechanisms proposed in this work are a preliminary theoretical framework and the computational findings need to be extensively validated in future biochemical, biophysical and microbiological *in vitro* and *in vivo* assays.

CONCLUSION

In this study, we report the successful synthesis and structural characterization of a series of brominated chalcone derivatives and assess their potential as targeted antibacterial agents against Bacillus subtilis. The study established a strong theoretical basis for the antimicrobial activity of these compounds by systematically targeting the DNA gyrase subunit B (GyrB) as a main therapeutic target. The in-silico molecular docking simulations revealed that the complete synthesized series in theory was better than the standard reference drug, ciprofloxacin, in terms of better binding affinities and thermodynamic stabilities.

Among the compounds tested, Chalcone 116 was identified as the most promising lead candidate. It demonstrated an optimal binding profile through a highly complementary interaction network in the GyrB active site, directly mimicking the key anchoring mechanisms of both ciprofloxacin and the native co-crystallized ligand. Computational data show that a strategic bromine substitution is very effective in improving target engagement mainly through unique halogen and pi-anion contacts that stabilize the ligand-receptor complex.

These results ultimately suggest that structurally optimized chalcone scaffolds are potent competitive inhibitors of the GyrB ATP binding domain. The current in-silico models, although providing very promising mechanistic insights and predictive pharmacological profiles, are a basis for a hypothesis. These intriguing theoretical results, particularly those concerning Chalcone 116, need to be translated into practical pharmaceutical applications. This will require extensive

biological evaluations both in vitro and in vivo to definitively demonstrate their empirical antimicrobial efficacy, pharmacokinetic feasibility and clinical safety.

Author Contributions

Partha Pratim Gogoi: Writing the original draft, Reviewing and Editing, Data collection; **Nichan Boruah:** Data collection and Editing; **Penlisola Longkumer:** Validation; **Mhasilhoutuo Pucho:** Visualisation; **Upasana Bora Sinha:** Writing and editing the original draft and Supervision.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

The data substantiating the outcomes of this manuscript are available within the manuscript.

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