INTRODUCTION

Co-infections and super-infections associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) typically arise from either community-acquired bacteria or hospital-acquired multidrug-resistant bacteria and fungi. Among these pathogens, Mycobacterium tuberculosis (MTB), the causative agent of tuberculosis, is capable of causing co-infection in coronavirus disease 2019 (COVID-19). The simultaneous presence of both COVID-19 and tuberculosis complicates the diagnosis and treatment of COVID-19, significantly increasing the risks of mortality and hindered recovery [1-4].

Reports from the World Health Organization (WHO) indicate that the COVID-19 pandemic has reversed years of progress in delivering essential MTB services and reducing the burden of MTB disease. Despite some achievements in specific regions, global MTB targets remain largely unmet, with a significant reduction in the number of newly diagnosed and reported MTB cases worldwide. Treating these co-infections with broad-spectrum empiric antibiotics can increase the likelihood of developing multidrug resistance [5,6]. Furthermore, co-infections and super-infections, particularly in resource-limited regions, are believed to contribute to the relatively higher rates of severe infection and mortality associated with SARS-CoV-2. The presence of bacterial or fungal co-infections alongside SARS-CoV-2 is associated with a higher mortality rate. Although the occurrence of tuberculosis co-infection with COVID-19 is very low, it continues to be a matter of considerable concern, particularly among persons at high risk [7-9].

The chalcone class of enones, also known as 1,3-diaryl-2-propen-1-ones, has played a pivotal role in the fields of organic
and medicinal chemistry for over a century. Heterocyclic chalcones, a subset of synthetic chalcone derivatives, are particularly significant in medicinal chemistry due to the prevalence of heterocyclic scaffolds in biologically active compounds [10-14]. Research has unequivocally established that heteroaromatic hybrid chalcones possess significant medicinal value. They have demonstrated efficacy as anticancer, antimicrobial, antifungal, antituberculous, antiparkinsonian, anti-inflammatory agents, while also serving essential pharmacological functions. Furthermore, these compounds have practical applications in agrochemistry, where they function as photosynthesis inhibitors and in the industrial sector, where they serve as photoinitiators in 3D printing processes [15-25].

In this work, the synthesis and assessment of antitubercular and antibacterial activity for novel five benzo furanyl chalcones viz., 1-(5-chloro-1-benzofuran-2-yl)-3-substituted phenyl prop-2-en-1-ones were carried out. These compounds were generated by introducing various substitutions at the B-ring position of chalcone. The synthesized compounds (3a-e) were also docked against two specific protein targets e.g. enoyl-acyl carrier protein reductase (InhA) in M. tuberculosis (MTB) and topoisomerase IV (Topo IV) in E. coli. The purpose of this dual-target docking analysis was to predict the binding affinity of the synthesized chalcone derivatives toward InhA and Topo IV. This prediction could be instrumental in identifying the potential correlations between binding affinity to these two targets and the minimum inhibitory concentration (MIC) for heteroarylchalcone-based antimicrobial drugs. Additionally, the dual-target docking results can provide valuable insights for subsequent investigations into the structure based drug design of heteroarylchalcone based antimicrobial drugs.

**EXPERIMENTAL**

All chemicals were procured from Sigma-Aldrich Co. (St. Louis, USA), Merck (Whitehouse Station, NJ, USA), Qualigens Fine Chemicals (Mumbai, India), Loba Chemie Pvt. Ltd (Mumbai, India) and Himedia Laboratories Pvt. Ltd (Mumbai, India). To determine the melting points of the synthesized compounds, we utilized a digital melting point apparatus with open capillary tubes and the provided values are uncorrected. Thin layer chromatography technique was used to assess the purity of the compounds. This involved pre-coated silica gel strips and a solvent system consisting of hexane:ethyl acetate (2:1). The spots were then detected using an ultraviolet chamber.

**Characterization:** Infrared spectra were recorded with a Shimadzu FT-IR 4000 instrument, using KBr disks. The CHNO elemental analysis was carried out using the Perkin-Elmer Series II 2400 CHNS/O elemental analyzer. Mass spectra were acquired using a JEOL GC mate II GC-Mass spectrometer at 70 eV, employing the direct insertion probe method. Nuclear magnetic resonance (NMR) spectra were acquired utilizing a Bruker AVIII-500 MHz FT-NMR spectrometer, with tetramethysilane as the internal standard and DMSO as solvent.

**Synthetic procedure:** As shown in Scheme-I, the title compounds were synthesized starting from 5-chlorosalicylaldehyde (1). Initially, 5-chlorosalicylaldehyde (1) under went cyclization to yield the subsequent crucial synthon, 5-chloro-2-acetylbenzofuran (2). Subsequently, the acetyl functionality was transformed into prop-2-en-1-one through Claisen-Schmidt condensation reaction with aromatic aldehydes. This series of chemical transformations led to the formation of the title compounds, namely, 1-(5-chloro-1-benzofuran-2-yl)-3-substituted phenyl prop-2-en-1-ones (3a-e).

**Synthesis of 5-chloro-2-acetyl benzofuran (2):** A mixture containing 5-chlorosalicylaldehyde (1), chloroacetone (4.63 g, 0.05 mol) and anhydrous potassium carbonate (15 g) was gently refluxed in dry acetone (50 mL) for a duration of 12 h. After the completion of the reaction, the reaction product was filtered and the filtrate was subjected to solvent removal under reduced pressure yielding 5-chloro-2-acetylbenzofuran (2) as a light brownish solid. The obtained product was further purified by recrystallization from ethanol, resulting in a yield of 75% having m.p. 83-85 ºC. IR (KBr, νmax, cm⁻¹): 1666 (C=O), 1461 (C=C) and 790 (CCl), MS (m/z, %): 194 (M⁺) and 196 (M+2⁺). 1H NMR (δ ppm): 7.2-7.8 (4Ar-H), 2.6 (3H, s, CH₃).

**General synthesis of 1-(5-chloro-1-benzofuran-2-yl)-3-(substitutedphenyl)-prop-2-en-1-ones (3a-e):** A mixture consisting of 5-chloro-2-acetyl benzofuran (2) (1.94 g, 0.01 mol) and various substituted aromatic aldehydes (0.01 mol), in 50 mL of ethanol was cooled to 5-10 ºC. Aqueous sodium hydroxide (70%, 5 mL) was then added dropwise with constant stirring. The reaction mixture was further stirred for a duration of 2 h and left overnight. Subsequently, it was neutralized with conc. HCl. The solid product that separated out was collected and it was further purified by crystallization from ethanol. The purity of all synthesized compounds was confirmed by thin-layer chromatography (TLC) using a mobile phase consisting of a mixture of n-hexane and ethyl acetate.

![Scheme-I](image-url)
1-(5-Chloro-1-benzofuran-2-yl)-3-phenylprop-2-en-1-one (3a): Yield: 75%; m.p.: 110-114 ºC; FT-IR (KBr, νmax, cm−1): 1650 (C=O), 1438 (C=C), 800 (C-Cl); 1H NMR (DMSO-d6, δ ppm): 6.70 (1H, d), 7.26-7.56 (6H, 7.32 (dd), 7.41 (dd), 7.48 (tt), 7.49 (dd), 6.71-8.30 (3H, 7.63 (dd), 7.72 (dd), 7.74 (dd), 7.98 (1H, dd); MS (m/z, %): 282.12 (M+). Anal. calcd. (found) % for C19H16NO2Cl: C, 70.05 (70.11); H, 4.98 (4.93); Cl, 10.84 (12.89); N, 4.30 (4.36); O, 9.81 (9.87).

Preparation of ligand molecules: In the preparation of ligand molecules, the 2D chemical structures of compounds 3a-e were drawn using Chem Draw Ultra Version 8.0.3 [31] and saved in binary format. These structures were subsequently converted into the SDF format using the Open Babel GUI version 2.4.1, a versatile virtual screening tool designed for Windows [32,33].

Following this, meticulous energy minimization was carried out using the OPLS3e force field with Ligprep. Key considerations included factors such as ionization at a target pH of 7.0 ± 2.0, desalting and the preservation of specified chiralities [34]. To facilitate a comparative assessment of binding affinities, ATPro served as the reference ligand in the docking experiments and the results were comprehensively evaluated by scrutinizing binding interactions and docking scores derived from GLIDE_SP ligand docking.

Antitubercular activity: The synthesized derivatives, namely, 1-(5-chloro-1-benzofuran-2-yl)-3-substituted phenyl prop-2-en-1-ones (3a-e), underwent screening for antitubercular activity using the microplate Alamar blue assay method (MABA). Each of the synthesized compounds was assessed against the M. tuberculosis H37 RV strain, with isonicotinic acid hydrazide (INH) serving as standard drug for comparison.

To set up the assay, 200 µL of sterile deionized water was added to the outer perimeter wells of a sterile 96-well plate to minimize medium evaporation during incubation. Subsequently, 100 µL of Middlebrook 7H9 (MB 7H9) broth was dispensed into the wells and the synthesized compounds were serially diluted directly on the plate. The antitubercular activity of these compounds was assessed at final drug concentrations of 0.2, 0.4, 0.8, 1.6, 3.125, 6.25, 12.5, 25, 50 and 100 µg/mL. The plates were covered and sealed with parafilm, then incubated at 37 ºC for 5 days. After this incubation period, 25 µL of freshly prepared 1:1 mixture of Alamar blue reagent and 10% Tween-80 was added to each well. The plates were then incubated for an additional 24 h. A blue color indicated no bacterial growth, while a pink color was indicative of bacterial growth [35]. The minimum inhibitory concentration (MIC) was determined as the lowest drug concentration that prevented the colour change from blue to pink. Specifically, the MIC was defined as the concentration required to inhibit 90% of the standardized bacterial inoculum.

Antibacterial activity: The antibacterial activity of the synthesized compounds (3a-e) was assessed using the agar cup plate method. Specifically, these compounds were tested against both Gram-negative organisms, namely Escherichia coli and Pseudomonas aeruginosa and Gram-positive organisms, including Staphylococcus epidermidis and Bacillus subtilis. The minimum inhibitory concentration (MIC) method was employed for the evaluation, with ciprofloxacin serving as a reference standard for result comparison.

For the procedure, brain heart infusion agar was maintained at room temperature. Colonies were transferred to the plates and their turbidity was visually adjusted using broth to match the turbidity of a 0.5 McFarland turbidity standard that had been vortexed. To ensure an even distribution, the entire surface of agar plate was swabbed three times, with the plates...
rotated approximately 60° between streaking. Following this, the inoculated plate was allowed to stand for at least 5 min before the application of drugs. A 5 mm hollow tube was heated and pressed onto the inoculated agar plate, developing five wells in the plate, which were then promptly removed. Subsequently, 75, 50, 25, 10 and 5 µL of the synthesized compounds were added into the respective wells on each plate. The plates were then incubated within 15 min and then kept in an incubator at 37 °C for 24 h. The MIC procedure involved repeating the serial dilution up to a 10⁶ dilution for each synthesized compound [36,37].

RESULTS AND DISCUSSION

Traditionally, chalcones can be synthesized via the Claisen-Schmidt reaction, involving the reflux of a ketone and aldehyde in an organic solvent, often in the presence of an acid or base catalyst. In this study, the essential intermediate, 5-chloro-2-acetyl benzofuran (2) was synthesized by condensing 5-chlorosalicylaldehyde (1) with chloroacetone. This pivotal intermediate 2 was then subjected to the Claisen-Schmidt reaction with various aryl aldehydes to obtain the title compounds (3a-e).

The purity of all the synthesized compounds (2 and 3a-e) was confirmed using thin-layer chromatography (TLC), employing a hexane and ethyl acetate mixture as mobile phase. Their identity was further verified by exhibiting a single spot on TLC, sharp melting points and characteristic spectral features.

In the infrared (IR) spectra of the synthesized compounds (2 and 3a-e), a distinct band in the range between 1630 and 1666 cm⁻¹ was observed, confirming the presence of an α,β-unsaturated ketone system. In 1H NMR spectra of these compounds, signals for aromatic protons resonated within the range of 7.2 to 7.9 ppm, while signals for =CH-CO- were observed around 6.6 ppm. Notably, compound 3b exhibited a proton singlet at 9.8 ppm, corresponding to the proton of hydroxy group located at the para-position of the aryl ring attached to the α,β-unsaturated ketone system. Mass spectra further confirmed the presence of the expected molecular ion peak (M⁺) fragments for the synthesized compounds. Remarkably, compound 3b displayed two distinctive peaks at 298 (M⁺) and 300 (M+2)⁺ m/z.

Antimicrobial activity: The synthesized benzofuranyl-chalcones (3a-e) were subjected to test against M. tuberculosis H37Rv in Middlebrook 7H9 broth media (MB 7H9 broth), with isoniazid (INH) employed as standard drug (Table-1). The results of the antitubercular activity screening indicated that compound 3e, featuring p-dimethylamino, compound 3a, which possesses an unsubstituted phenyl ring and compound 3d, featuring p-methoxy on the aromatic moiety at the B-ring position of chalcone, exhibited activity at concentrations of 50 and 100 µg/mL. However, the remaining two compounds, 3b and 3c, did not demonstrate a significant activity in this context. Furthermore, all the synthesized compounds underwent evaluation for their antibacterial activity using the agar cup-plate method, with ciprofloxacin serving as the reference standard. Among these compounds (3a-e), notable antibacterial activity was observed at a dose level of 100 µg, compared to the standard drug (Fig. 1).

In particular, compound 3a exhibited the highest activity against Gram-negative bacterial strains, likely due to the presence of an unsubstituted phenyl ring at the B-ring position of the chalcone. Additionally, all the compounds displayed substantial antibacterial activity against various bacterial strains, which can be attributed to the presence of various substituents at the para-position of the aryl rings, including chloro, hydroxyl, dimethylamino and methoxy groups, in addition to the favourable impact of 5-chlorobenzofuran moiety.

Dual target docking studies: The quality of the 3D target molecule models was assessed using Ramachandran plot calculations with RAMPAGE. The percentage of residues in the favoured region, allowed region and outlier region was determined to be 89.8%, 8.9% and 0.9% for 2PR2 and 94.6%, 5.4% and 0% for 1S14, respectively. Typically, a score close to 100% indicates good model quality, suggesting that the predicted models were of good quality (Fig. 2).

Additionally, these structures were validated by other servers such as ERRAT and Verify 3D. The overall quality factor of the target molecules, as obtained in ERRAT analysis, followed the order of 94.21% (2PR2) < 95.83% (1S14). All these values are either higher or very close to the 95% rejection limit, further confirming the quality of the target protein models. In verify 3D analysis, it was observed that none of the amino acids of 2PR2 had a negative score, but a few residues in 1S14 exhibited marginal negative scores. The percentage of amino acid residues with a 3D-1D score greater than or equal to 0.2 was 99.25% for 2PR2 and 46.43% for 1S14. It is important to observe that compatibility scores above zero indicate an acceptable structural environment (Fig. 3).

The docking scores for each ligand against both target proteins were predicted using Glide, a widely utilized docking software developed by Schrödinger. Figs. 4 and 5 show the docking of compounds 3a-e with the two target protein models.

<table>
<thead>
<tr>
<th>Compound code</th>
<th>Minimum inhibitory concentration</th>
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<tbody>
<tr>
<td></td>
<td>25 µg/mL</td>
</tr>
<tr>
<td>Isoniazid</td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>Resistant</td>
</tr>
<tr>
<td>3b</td>
<td>Resistant</td>
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Fig. 1. Antibacterial activity of synthesized compounds 3a-e

TABLE-1 ANITITUBERCULAR ACTIVITY OF SYNTHESIZED COMPOUNDS (3a-e)
In docking process, ten binding poses were obtained and the binding pose with the highest docking score was selected. Among all the synthesized compounds, compound 3b exhibited the highest docking score (-5.66 1S14). All the compounds displayed interesting docking scores comparable to their respective standards.

A summary of the interactions observed between the synthesized compounds (3a-e) and amino acid residues of the target proteins 2PR2 and 1S14 is presented in Tables 2 and 3. With the exception of compounds 3d and 3e, all the synthesized compounds displayed hydrophobic interactions with 2PR2. Except for 3a, 3d and 3e, compounds 3b and 3c exhibited hydrophobic interactions similar to the standard drug isoniazid. Conversely, compounds 3d and 3e did not demonstrate any hydrophobic interactions with the target 2PR2.

Furthermore, all the synthesized compounds, except compound 3c, engaged in hydrogen bond interactions, albeit at different binding sites. Compounds 3a and 3b were unique in that they interacted with both hydrophobic pockets and hydrogen binding residues, while the remaining compounds interacted...
Fig. 4. Docking of synthesized compounds 3a-e with 2PR2 protein

Fig. 5. Docking of synthesized compounds 3a-e with 1S14 protein
These computational analyses provided insights into their enoyl-ACP reductase and groups on the aromatic ring exhibited strong antimicrobial results. The synthesized compounds with electron-donating tubercular and antibacterial activities revealing the promising impact on diagnosis, treatment and the global burden of tuberculosis, highlighting their anti-infections associated with SARS-CoV-2. In this study, the critical issue of co-infections and super-infections associated with SARS-CoV-2, identifying their potential as antimicrobial agents were carried out. The in vitro antibacterial and antibacterial activities revealing the promising results. The synthesized compounds with electron-donating groups on the aromatic ring exhibited strong antimicrobial activity. In addition to experimental assays, the dual-target docking studies were also conducted to predict the binding affinity of the compounds (3a-e) to Mycobacterium tuberculosis enoyl-ACP reductase and Escherichia coli Topoisomerase IV. These computational analyses provided insights into their potential as lead structures for future drug design.

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**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interests regarding the publication of this article.

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