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Chalcone-related small molecules as potent antibacterial and antifungal agents: Design, synthesis, *In vitro*, and computational approaches

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ABSTRACT

Infectious diseases caused by bacteria and fungi are a global health concern due to resistance to traditional antimicrobial medications. A variety of chalcone-related small molecules have been designed, synthesized, and characterized small molecules using FTIR, NMR, and MS to find antimicrobial agents for treating these infections. These designed compounds (**9, 11, 13**) were evaluated for their potential inhibitory activity against five bacterial strains and one fungal strain using disc diffusion and MIC assays utilizing ampicillin and fluconazole as reference drugs. The MIC values ranged from 2.5 to 160 μg/mL, which can be attributed to improved membrane penetration and increased ligand-protein binding capability. Among them, molecule 9 exhibited a broad spectrum of antibacterial activity against gram-negative bacteria, with an MICs of 40 μg/mL against *P. aeruginosa* and 80 μg/mL against *E. coli*. Compound **11** showed potent activity against gram-positive bacteria and fungi, with a MICs of 40 μg/mL against *S. aureus* and 80 μg/mL against *C. albicans*. Furthermore, similar to *in vitro* study results, molecular docking demonstrated that compounds **9** and **11** had a better binding affinity against gram-positive and gram-negative bacteria and fungal species than reference drugs. Finally, physicochemical and drug-likeness results showed that all the compounds can pass Lipinski's rule of five, are absorbed through the GIT, and are suitable for oral administration.

1. Introduction

Drug-resistant microorganisms are an important cause of concern in the medical community and pose a serious risk to the public's health. Due to antimicrobial resistance, antibiotics are becoming less effective in treating and preventing a variety of microbe-caused illnesses (Yousry et al., 2016; Rajinder Pal and Vikas, 2019). In addition, systemic and skin fungal infections are also significantly increased, especially in people with weakened immune systems, such as cancer patients undergoing chemotherapy and AIDS patients (Yeroushalmi et al., 2020). Although many different antifungal drugs are used to treat fungal infections, some of these drugs cause side effects due to biochemical similarities between human cells and fungal forms (Lee et al., 2021). Among the various strategies used to solve this problem, researchers believe that the development of new medications with increased activity and other mechanisms of action is the best option (Murugaiyan et al., 2022). Several classes of compounds have been studied for their antibacterial and antifungal activities, such as chalcones, phenolics, and imines. Due to their numerous uses in the fields of medical chemistry and material sciences, chalcone derivatives have attracted the greatest interest among these groups of chemicals (Xu et al., 2019; Górniak et al., 2019; Hussen et al., 2024).

Chalcones represent a significant class of organic compounds characterized by their role as the central structural motif in various biologically active molecules. These compounds serve as important precursors for the biosynthesis of flavonoids and isoflavonoids, which are known to exhibit a wide range of biological activities (Abdalla Ali et al., 2024; Al-Maqtari et al., 2024). Structurally, chalcones are substituted aromatic

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ketones that have garnered attention in pharmaceutical research due to their diverse therapeutic potentials (Rammohan et al., 2020). Several FDA-approved drugs, including metochalcone (2), sofalcone (3), isoliquiritigenin (4), xanthohumol (5), and hesperidin-methylchalcone (6) (Fig. 1) (Salehi et al., 2021), exemplify the clinical relevance of chalcones. Their applications extend across a multitude of therapeutic areas, including anti-cancer, antibacterial, antifungal, anti-malarial, antidiabetic, anti-tuberculosis, anti-proliferative, antioxidant, anti-inflammatory, and antiviral activities (Dhaliwal et al., 2022; Constantinescu and Lungu, 2021; Zhuang et al., 2017; Khan et al., 2024). Chalcones have demonstrated a particularly noteworthy protective effect against various microorganisms. Their antimicrobial efficacy is largely attributed to their interaction with the cell membranes of target pathogens. These compounds can disrupt cellular integrity by binding to outer membranes, absorptive proteins, and cell walls, thereby inhibiting microbial growth and proliferation (Henry et al., 2020).Various research groups are involved in identifying the fragment in synthesized or modified natural chalcone molecules that is responsible for previously described activities (Rozmer and Perjési, 2016). Tsukiyama et al. reported the strong antibacterial activity of licochalcone A and licochalcone B. Based on these results, it was assumed that the free phenol group in the ring at position 4 was the key factor for their antibacterial activity (Tsukiyama et al., 2002). Based on the fact that natural bioactive compounds play a crucial role in the development of antimicrobial drugs (Kha and Le, 2021).

Natural compounds with antimicrobial activity indeed have significant pharmacotherapeutic prospects. Many of these compounds, particularly phenolic chemicals, have antibacterial and antifungal effects (Stan et al., 2021; Hussein et al., 2024; Mohammed et al., 2025; Hussen et al., 2025. Given the role of reactive species as a cause of many forms of microorganisms, diets and/or drug treatments, including bioactive compounds with antimicrobial activity, may represent a preventive therapeutic strategy to maintain the patient's well-being. Some of these natural chemicals are found in medicines, dietary supplements and cosmetics (Larsson and Flach, 2022). Vanillin (4-hydroxy-3-methoxybenzaldehyde), the main component of vanilla and a plant secondary metabolite, is a phenolic phenylpropane C6–C1 carbon structure derivative (Walton et al., 2003). It is a crucial flavor and aroma component that is used all over the world. Vanillin is found in a variety of essential plant oils, most notably *Vanilla planifolia, Vanilla tahitensis* and *Vanilla pompona*. It is also commonly found in processed foods, beverages, pharmaceuticals and perfumery. Vanillin possesses antifungal, antibacterial, anticancer, antiviral, anti-inflammatory, antimutagenic, antioxidant and antidiabetic properties (Arya et al., 2021; Olatunde et al., 2022).

Based on the studies discussed and in view of the continuing need to develop potent and selective antimicrobial compounds, we have designed and synthesized a series of derivatives of chalcone-derived vanillin. The compounds were tested for their antimicrobial activity against five different bacterial strains and one fungal strain. Finally, computational analyzes including in silico molecular docking, drug similarity, and pharmacokinetic properties were investigated to streamline the bioassay results.

2. Experimental

2.1. Materials and methods

The chemicals used for the experiment were of analytical grade and were purchased from Sigma-Aldrich and Merck. Synthesized compounds examined by thin layer chromatography (Merck Silica gel 60 F254). A UV lamp was used for the visualization of the TLC spots. Melting points were determined using the Stuart/SMP3 melting point instrument version 5.0 in open capillary tubes. Compound identification was achieved using an IR spectrum recorded on a Shimadzu FT-IR infrared spectrometer, performed with KBr disks, and data interpretation was performed using Origin Pro software. ^{13}CNMR spectra were recorded with Bruker DRX-500 MHz NMR and reported on ppm scale. Mass spectra were obtained using the acquired method and the MS Model 5973 Network Mass Selective Detector.

2.2. General procedure synthesis of some small molecule chalcone derivatives (9,11, and 13)

Aromatic ketones (**8, 10, 12**) (acetaphenone,4-amino-acetaphenone,2-hydroxy acetophenone) (0.01 mol) and vanillin **7** (0.01 mol) were mixed in a round bottom flask. Ethanol (30 mL) is added, followed by 40% potassium hydroxide (15 mL). The mixture was stirred at room temperature for 30 min, and then allowed to stand for 24 h. The mixture was poured into a beaker containing crushed ice to stop the reaction and then neutralized with 10% HCl. The precipitate formed was filtered, washed with distilled water and dried. They were then recrystallized from absolute ethanol to give the desired product (**9, 11,** and **13**), Scheme 1.

2.3. (E)-3-(4-hydroxy-3-methoxyphenyl)-1-phenylprop-2-en-1-one (9)

Chemical formula $C_{16}H_{14}O_3$; yellow powder (71% yield); M.P.

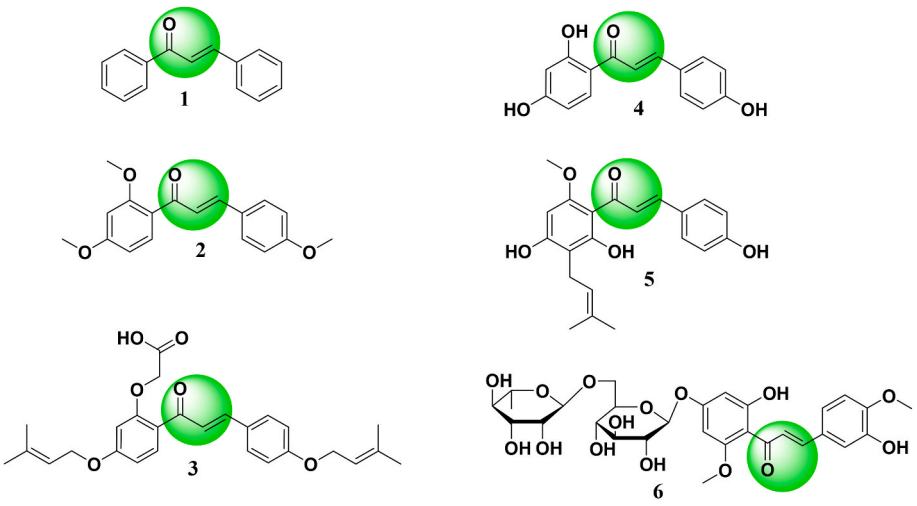
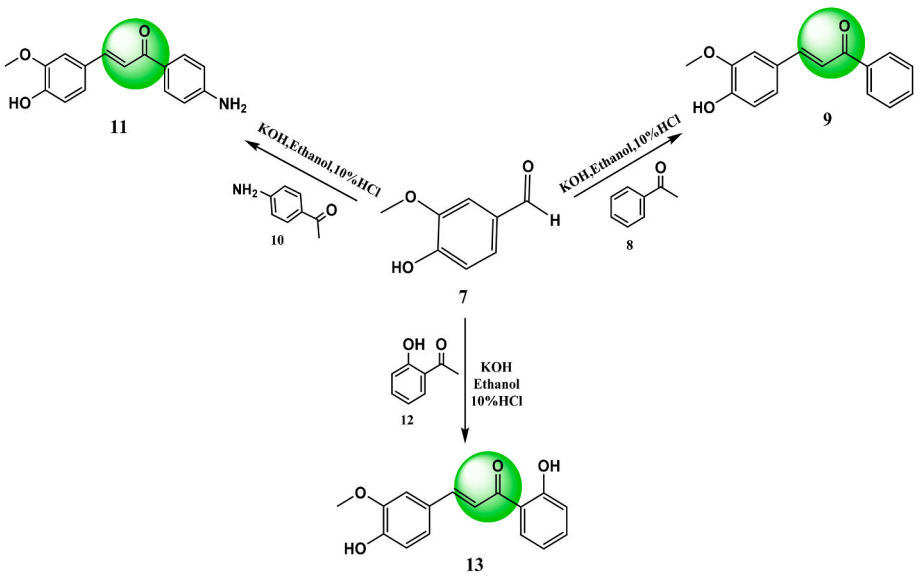


Fig. 1. Chemical structures of approved and clinically tested chalcones: Chalcone (1), Metochalcone (2), Sofalcone (3), Isoliquiritigenin (4), Xanthohumol (5),and Hesperidinmethyl chalcone (6).



Scheme 1. Synthesis of chalcone related small molecules (**9, 11**, and **13**).

251–253 °C; R_f 0.58; IR (with KBr) v (cm-1): 3316 (O–H phenol), 3066 & 3003 (C–H aromatic), 2993 (C–H alkene), 2892 (C–H CH_3), 1677 (C═O chalcone), 1656 (C═C alkene), 1585&1560 (C═C aromatic), 1410 (O–H bending of phenol). ^{13}C NMR (125 MHz, DMSO-d_6): δ 189 (1C, C═O); 150 (1C, C–OCH_3); 148 (1C, phenol); 145 (1C, C═C–C); 138, 133, 129, 128, 126, 124, 119, 116, 112 (11C, aromatic); 56 (1C, CH_3). MS (ESI, *m/z*): 254.1 $[M^+]$ for $C_{16}H_{14}O_3$; 255 $[M+H]^+$ for $C_{16}H_{15}O_3$; 237 $[C_{16}H_{13}O_2^+]$; 223 $[C_{15}H_{11}O_2^+]$; 177.1 $[C_{10}H_9O_3^+]$; 123 $[C_7H_7O_2^+]$; 105 $[C_7H_5O^+]$; 149 $[C_9H_9O_2^+]$; 77 $[C_6H_5^+]$.

2.4. (E)-1-(4-aminophenyl)-3-(4-hydroxy-3-methoxyphenyl) prop-2-en-1- one (11)

Chemical formula $C_{16}H_{15}NO_3$; orange powder (87% yield); M.P. 206–208 °C; R_f 0.62; IR (with KBr) v (cm^{-1}): 3314 (O–H phenol), 3051& 3013 (NH_2), 2990& 2975 (C–H aromatic), 2924 (C–H alkene), 2899 (C–H CH_3), 1672 (C═O chalcone), 1656 (C═C alkene), 1603&1585 (C═C aromatic), 1414 (O–H bending of phenol). ^{13}C NMR (125 MHz, DMSO-d_6): δ 191 (1C, C═O); 153 (1C, C–NH_2); 150 (1C, C–OCH_3); 149 (1C, phenol); 148 (1C, C═C–C); 130.8, 130.2, 129, 126, 121, 115.8, 115.2, 111 (10C, aromatic); 56 (1C, CH_3). MS (ESI, *m/z*): 269 $[M^+]$ for $C_{16}H_{15}NO_3$; 270 $[M+H]^+$ for $C_{16}H_{16}NO_3$; 253.8 $[C_{16}H_{13}O_3^+]$; 252 $[C_{16}H_{14}NO_2^+]$; 238 $[C_{15}H_{12}NO_2^+]$; 205 $[C_{15}H_9O^+]$; 177 $[C_{10}H_9O_3^+]$; 92 $[C_6H_6N^+]$; 76$[C_6H_4^+]$.

2.5. (E)-3-(4-hydroxy-3-methoxyphenyl)-1-(2-hydroxyphenyl) prop-2-en-1- one (13)

Chemical formula $C_{16}H_{14}O_4$; orange powder (74 % yield); M.P. 171–173 °C; IR (with KBr) v (cm^{-1}): 3425 (O–H phenol), 3002& 2972 (C–H aromatic), 2916 (C–H alkene), 2899 (C–H CH_3), 1674 (C═O chalcone), 1633 (C═C alkene), 1589& 1517 (C═C aromatic), 1405 (O–H bending of phenol). ^{13}C NMR (125 MHz, DMSO-d_6): δ 188 (1C, C═O); 161 (1C, phenol); 154 (1C, C–OCH_3); 144 (1C, phenol); 141 (1C, C═C–C); 135, 131.38, 13127, 130, 129, 127, 121, 119, 114, 113 (10C, aromatic); 55 (1C, CH_3). MS (ESI, *m/z*): 270 $[M^+]$ for $C_{16}H_{14}O_4$; 271 $[M+H]^+$ for $C_{16}H_{15}O_4$; 254.2 $[C_{16}H_{14}O_3^+]$; 239 $[C_{15}H_{11}O_3^+]$; 236 $[C_{16}H_{12}O_2^+]$; 149 $[C_9H_9O_2^+]$; 130 $[C_9H_6O^+]$; 123$[C_7H_7O_2^+]$; 121 $[C_7H_5O_2^+]$.

3. Antimicrobial study

3.1. Tested microbial strains

Five bacterial strains (*Staphylococcus aureus*; *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Enterobacter cloacae*) and one fungal strain (*Candida albicans*) derived from hospitalized patients were tested for their antibacterial and antifungal activity. A validation of 0.5 MacFarland turbidity equivalents was performed on the test organisms.

3.2. Disc diffusion method

The disc-diffusion method was used to assess the antimicrobial activity of compounds **9**, **11** and **13** (Balouiri et al., 2016). In this study, an MH agar (Mueller-Hinton) and potato dextrose agar were inoculated in Petri dishes with the five selected bacterial strains and corresponding one fungal strain. The newly synthesized compounds were prepared as stock solutions with a concentration of 2 mg/mL in 5% DMSO solvent. Then, place filter paper plates (approximately 6 mm in diameter), containing the compound to be tested at the desired concentration (15 µL), on the agar surface. The fungal and bacterial strains were incubated at 30 °C and 37 °C, respectively. For all strains of microorganisms studied, inhibition diameter and inhibition rate were measured after 24 h and after 48 hoursi8 for *C. albicans*. The standard drugs used were ampicillin and fluconazole as positive controls and 5% DMSO as negative controls.

3.3. Minimum inhibitory concentration (MIC)

The MICs of compounds **9**, **11** and **13** for five bacterial s and the one fungal strain were determined by micro-dilution technique according to the method described by after 24 h of incubation for bacteria and 48 h for *C. albicans* at 37 °C and 30 °C, respectively and a 200-rpm shaking incubator (LabTech). Serial dilutions of the test compounds were prepared at concentrations of 2.5, 5, 10, 20, 40, 80 and 160 µg/ml in 96-well microtiter plates, which were added to the broth, and microbial suspensions. Each well was adjusted to a total volume of 200 µL. Bacterial growth was determined using a microplate reader at 600 nm. All experiments were performed in triplicate. The lowest concentration inhibiting the growth of bacteria and fungi was considered as the MIC value.

3.4. Statistical evaluation

Quantitative results were expressed as means of triplicate experiments ± SD (standard deviation). The statistical study of the MIC data using linear regression. At $p \leq 0.05$, it was determined that the differences were significant.

4. Computational approaches

4.1. Molecular docking study

MarvinSketch19.9 24 is used to plot the designed compounds. They are sketched mainly as 2D structures and then converted to 3D format (pdb) using the same program. The ligand energy was minimized by applying the Merck Molecular Force Field 94(MMFF94) force field algorithm, and the scaled structures were converted to PDBQT format by using PyRx 0.8 before performing molecular docking analysis. The Protein Data Bank was used to obtain the crystal structure of the penicillin binding protein (PDB ID: 1MWT), penicillin binding protein 3 (PDB ID: 6I1E), and lanosterol 14-alpha demethylase (PDB: 4WMZ) (Salih et al., 2023; Narmin Hamaamin et al., 2024). The Discoverystudio2021 client is used to remove the water molecules, heteroatoms, and co-crystallized ligands. Autodock-Tool-1.5.6 29 was used to add the polar hydrogens and Kollman charges. PyRx was used to convert the PDB file to the PDBQT format. Finally, the PyRx docking tool (Python Prescription 0.8) was used to dock compounds to previously synthesized proteins. The binding site is selected according to the co-crystallized ligands of the target protein. PyRx affinity scores (in kcal/mol) for each chemical were collected and evaluated using the free energy binding theory (more negative values mean higher binding affinity) (Hasan et al., 2022b; Anbukarasi et al., 2023). Protein Plus and PyMOL molecular graphics system (Version 2.3.2 Schrödinger) were used to visualize immobilization (posture) configurations and receptor-ligand interactions at the molecular level (Hamaamin Hussen et al., 2022).

4.2. Physicochemical and pharmacokinetic properties of compounds

The synthesized compounds were characterized using a free online tool called SwissADME. This tool helps analyze the characteristics of the compounds. In the beginning, we drew the chemical structures of the derivatives using a program called Marvin. Then we entered these structures directly on the website to begin the calculation process. For every substance, they used an image of boiled eggs to test how well it can be absorbed by the stomach and how easily it can pass through the blood-brain barrier (Hasan et al., 2022a; Hamaamin Hussen et al., 2022).

5. Results and discussion

5.1. Chemistry

The chalcone derivatives were obtained in good yields (71–87%) by the base-catalyzed Claisen-Schmidt condensation of vanillin **7** with various substituted aromatic ketones (**8**,**10**, and **12**) in absolute ethanol with stirring overnight at room temperature as shown in Scheme 1. The structures of compounds (**9,11, and 13**) were elucidated by FT-IR, ^{13}C NMR and, and mass spectrometry. The FT-IR spectra of all compounds mentioned in the title show almost similar trends. The FT-IR spectrum showed a strong band with peaks at 3425–3314 cm^{-1} assigned to the O–H stretching of the phenol, the two bands at 3051 and 3013 cm^{-1} in compound **11** are for the primary amine. The bands between 1677 and 1672 cm^{-1} in all the derivatives were demonstrating C═O stretching the carbonyl group of the chalcone, while the bands at 1633-1656 cm^{-1} are for the C═C of the alkene. These bands indicate the successful formation of the desired chalcone. The absorbance at 1603-1560 cm^{-1} is distinguished by the C═C stretching of the aromatics. The band between 1414 and 1405 cm^{-1} is attributed to the O–H bending of phenol. All the derivatives showed chemical shifts in the ^{13}C NMR spectrum. The peaks at 191-188 ppm indicated for chalcone a carbonyl group, whereas the peak at 154-150 confirmed the presence of a carbon of methoxy group. The phenol carbon peak appeared at 149-144 ppm in all compounds. All the synthesized compounds showed peak at 148-141 ppm allocated for carbon of alkene group. One peak from compounds **11** observed at 153 ppm, was assigned to carbon attached to a primary amine. Nine signals from compound **9** at 138-112 ppm, were assigned to eleven aromatic carbons. Eight signals from compounds **11** and **13** observed between 135 and 111 ppm, indicate the presence of ten aromatic carbons. The presence of all these peaks in the ^{13}CNMR spectra confirms the formation of chalcone in the derivatives (Narmin Hamaamin, 2023; Hasan et al., 2022b). The derivatives' high-resolution mass spectrometer (HRMS) peak showed up as molecular ions (M^+). The proposed fragmentation patterns [M^+] and relative intensities of the distinctive ion peaks in the mass spectrum assay for all derivatized substances. The remaining signals and peaks from the IR, ^{13}C NMR, and mass spectra are completely consistent with the assigned structures and are listed in the experimental section.

5.2. Antimicrobial activity

The antibacterial and antifungal activities of compounds **9**, **11**, and **13** were assessed *in vitro* against *S. aureus*, *E. coli*, *P. aeruginosa*, *E. cloacae*, *A. baumannii*, and *C. albicans*. The results are presented in Table 1 and Fig. 2. All synthesized derivatives exhibited varying strengths of inhibition zones in disc diffusion tests compared to ampicillin and fluconazole. Most synthetic compounds showed antibacterial effects, particularly against *S. aureus* and *E. coli* when compared to ampicillin. Excluding *P. aeruginosa*, compound **11** demonstrated the highest effectiveness against *S. aureus*, *E. coli*, *E. cloacae*, and *A. baumannii*, with inhibition zones measuring 22 mm, 13 mm, 20 mm, and 12 mm, respectively. In contrast, ampicillin was only effective against *S. aureus* and *E. cloacae*, with inhibition diameters of 13 mm and 10 mm, respectively. Additionally, compound **9** exhibited strong antibacterial activity against *P. aeruginosa*, *E. coli*, and *S. aureus*, with inhibition zones of 28 mm, 21 mm, and 10 mm, respectively. Compound **13** showed activity solely against *S. aureus*, with a 10 mm zone of inhibition. In terms of antifungal activity, compounds **9**, **11**, and **13** demonstrated significant effectiveness against *C. albicans*, as illustrated in Fig. 2, with inhibition zones of 22 mm, 20 mm, and 10 mm, respectively, compared to fluconazole, which had a zone of inhibition of 30 mm. Finally, the MIC analysis was conducted only for compounds that exhibited activity.

The MIC results for the vanillin-chalcone derivatives indicated antibacterial activity against the tested bacterial strains, with values presented in Table 2. Compound **11** demonstrated a broad spectrum of antibacterial activity against both gram-positive and gram-negative species. The most resistant strains among all tested compounds were *P. aeruginosa* and *A. baumannii*. The MIC of compound **11** inhibited

Table 1
The inhibition zones calculated in millimeters for the designed compounds against five bacterial strains and one fungal strain using agar disc diffusion test.

Compounds	Diameter of Zone of Inhibition (mm)					
	Bacterial strains					Fungi
	S. aureus	E. coli	P. aeruginosa	E. cloacae	A. baumannii	C. albicans
9	10	21	28	–	–	22
11	22	13	–	20	12	20
13	10	–	–	–	–	10
Ampicillin	13	–	–	10	–	–
Fluconazole	–	–	–	–	–	30

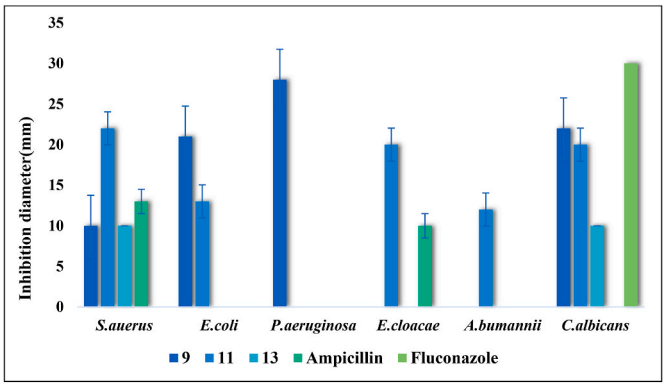


Fig. 2. Antimicrobial activity of some chalcone derivatives (**9,11, and 13**).

S. aureus, *E. coli*, *E. cloacae*, and *A. baumannii* at 40 μg/mL, 160 μg/mL, 80 μg/mL, and 160 μg/mL, respectively. Notably, *S. aureus* had the lowest MIC value and a small standard error of 0.0118 compared to the other bacterial strains.

Compound **9** exhibited maximal activity against *E. coli* and *P. aeruginosa* at 80 μg/mL and 40 μg/mL, respectively, with a lower standard error of 0.012 for *P. aeruginosa*. Additionally, as shown in Table 2, the MICs of compounds **9**, **11**, and **13** against *C. albicans* revealed significant antifungal activity at 160 μg/mL, 80 μg/mL, and 160 μg/mL, respectively, with a standard error of 0.011. Regarding the vanillin-chalcone derivatives (**9,11, and 13**) it was found that the presence of the electron-donating NH_2 on the terminal phenyl ring of the vanillin moiety enhanced the antibacterial activity against gram positive and negative strains (compound **11**). Replacing the amine atom at the para position with hydroxyl group decreased the antibacterial activity against all bacterial strains (Compound **13**), while replacing with hydrogen enhanced the antibacterial activity against *P. aeruginosa* (compound **9**). In addition, with respect to SAR studies of synthesized compounds as antibacterial agents, it was discovered that the activity of electron donating groups of nitrogen is less electronegative than oxygen, making the amine functional group less polar than that of an alcohol and enhanced antibacterial activity to be attributed to the improvement of the physicochemical properties and thus, to the improvement of the permeability of the bacterial cells. Studies using SAR on the antifungal activity of newly synthesized derivatives show that the presence of an electron-donating group in the terminal phenyl ring of the vanillin moiety increases the antifungal activity, and that the antifungal activity of monosubstituted amino derivatives has the para position. has been found to be ideal.

5.3. Molecular docking analysis

Using molecular docking, we determined the mechanism by which the proposed inhibitor binds to the binding sites of three vanillin-chalcone hybrids for penicillin binding protein (PDB ID: 1MWT) (gram-positive bacteria from *S. aureus*) penicillin binding protein 3 (PDB ID: 6I1E) (gram-negative bacteria from *P. aeruginosa*), and lanosterol 14-alpha demethylase (PDB: 4WMZ) (fungal pathogen from C. albicans) (Salih et al., 2023; Narmin Hamaamin et al., 2024). The docking poses obtained were compared to the actual positions of ampicillin and fluconazole. As shown in Table 3, the calculated binding affinity for synthesized compounds (**9, 11**, and **13**) against penicillin binding protein were −7.9, −8.8 and −7.6 kcal/mol, against penicillin binding protein 3 were −7.7, −6.6, and −6.8 kcal/mol, and against lanosterol 14-alpha demethylase were −9.2, −8.3, and −8.0 kcal/mol respectively. It was reported that, the lower the binding affinity value, the better the inhibiting activity (Salih et al., 2022). As a result, compound **9** was the most effective compound against *C. albicans* and gram-negative bacteria, at −9.2 and −7.7 kcal/mol respectively, compared to the reference drugs fluconazole (−7.1 kcal/mol) and ampicillin (−7.3 kcal/mol). Compound **11** showed a greater tendency to inhibit penicillin binding protein against gram positive bacteria at −8.8 kcal/mol, than compounds **9** and **13**. This corresponds to the findings of experimental investigations. Therefore, compounds **9** and **11** were selected for further analysis. As shown in Fig. 3, compound **11** has the ability to bind to specific binding pockets of protein (1MWT) for example creates two hydrogen bonds between the atoms of hydrogen of the ligand's primary amine and phenolic oxygen of Tyr 499 A, and carbonyl oxygen atom of Gln 396 A. Next hydrogen bond was created between phenolic oxygen atom of compound **11** and phenolic hydrogen atom of Tyr 496A. Additionally, similar to ampicillin, compound **11** interact with a hydrophobic pocket created by Leu 286A and Pro 497A. As shown in Figs. 4 and 5, compound **9** was created two hydrogen bonds due to conformations of the residue Ser382 A of 4WMZ. Moreover, compound **9** is surrounded by several protein residues, such as Trp 83A, Tyr 146 A, Lys 197A and Val 203A of 6I1E, as well as Leu 95A, Phe 241A, and Phe 384A from 4WMZ to form hydrophobic interactions.

5.4. The evaluation of physicochemical and pharmacokinetic properties of designed compounds

The Swiss ADME server is a crucial tool for identifying drug-like characteristics in newly developed compounds. It reviews the ADME properties of synthesized compounds to identify safe and promising therapeutic candidates, filtering out those with improper features that may fail in drug development stages (Hasan et al., 2023). We evaluated the pharmacokinetic properties of all synthesized compounds.

According to the Lipinski rule of five, a drug molecule should have a molecular weight of less than 500 g/mol, no more than one lesion, and

Table 3
Using a molecular docking tool, predicted docking scores of designed compounds with target proteins.

Entry	Binding affinity (kcal/mol)		
	1MWT	6I1E	4WMZ
9	−7.9	−7.7	−9.2
11	−8.8	−6.6	−8.3
13	−7.6	−6.8	−8.0
Ampicillin	−7.3	−6.5	–
Fluconazole	–	–	−7.1

Table 2
MIC values (μg/mL) exhibited by the compounds against five bacterial strains and *C. albicans*.

Compounds	MIC values in μg/mL expressed by the test compound					
	Bacterial strains					Fungi
	S. aureus	E. coli	P. aeruginosa	E. cloacae	A. baumannii	C.albicans
9	160	80	40	–	–	160
11	40	160	–	80	160	80
13	80	–	–	–	–	160
Ampicillin	20	–	–	160	–	–
Fluconazole	–	–	–	–	–	10

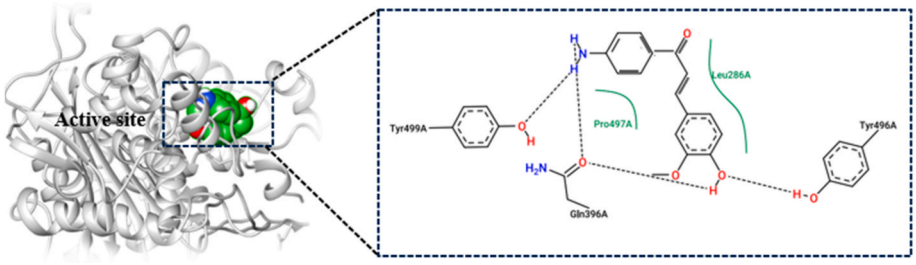


Fig. 3. The binding pattern of compound **11** (Green) in the 1MWT receptor active site and the intermolecular binding interactions were depicted by the Protein Plus program. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

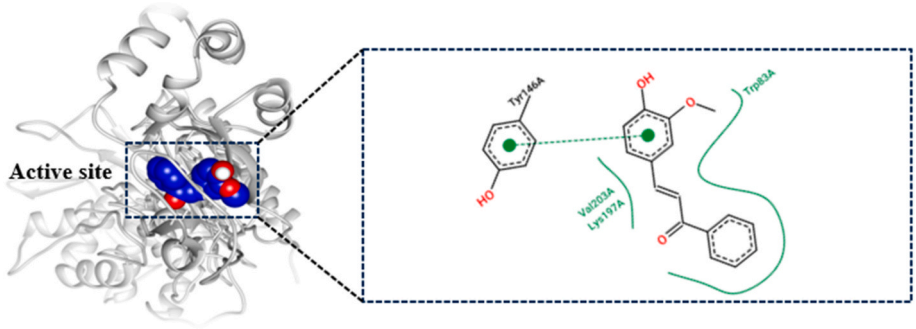


Fig. 4. The binding mode of compound **9** (Blue) at the active site of the 6I1E and the intermolecular binding interactions were demonstrated using the Protein Plus program. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

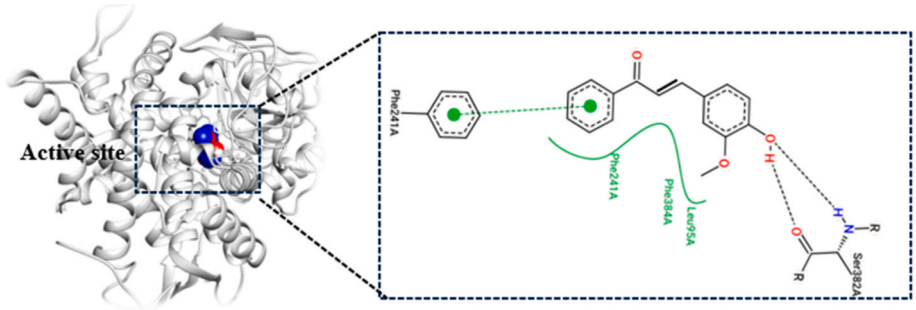


Fig. 5. Binding pattern of compound **9** (Blue) at the 4WMZ receptor active site and intermolecular binding interactions presented by Protein Plus program. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

no more than ten hydrogen bond acceptors. Additionally, the log P value must be 5 or less and hydrogen bond donors must be present. Since compounds **9**, **11**, and **13** all have fewer than 10 hydrogen bond acceptors and five donors, they do not break this condition. Rotatable bonds, non-ring-attached single bonds, are crucial for oral bioavailability. Molecules with fewer than ten rotatable bonds show better oral bioavailability. The Lipinski rule suggests compounds with more rotatable bonds have flexible structures, enhancing interaction with active sites. This study found all compounds with four rotatable bonds, indicating good oral bioavailability with a score of 0.55. The topological polar surface area (TPSA) is a necessary calculation to determine the cellular permeability and intestinal absorption of a drug. The TPSA value of the molecules less than 140 $Å^2$ indicate good transport properties of the drug across the cell membrane and when it is < 60 $Å^2$, it indicates good blood brain penetration. The newly designed compounds exhibit TPSA values ranging from 46.3 $Å^2$ to 72.55 $Å^2$, suggesting their favorable ability to permeate cell membranes. During drug discovery, evaluation of the gastrointestinal (GI) absorption and blood-brain barrier (BBB) properties of a compound are important pharmacokinetic activities evaluated at different stages. different stages of drug development. Evaluation of gastrointestinal absorption and the blood-brain barrier plays a central role in elucidating the permeability properties of synthetic compounds. These measurements offer crucial insights into their potential for effective oral delivery and central nervous system penetration, respectively (Daina and Zoete, 2016). The synthesized derivatives exhibit high gastrointestinal absorption and possess the ability to effectively traverse the blood-brain barrier (BBB), enabling them to potentially serve as therapeutic agents for central nervous system (CNS) infections. Notably, these synthesized compounds demonstrate superior BBB permeability compared to the reference compounds, as they are more lipophilic in nature. Furthermore, it is worth mentioning that none of the synthesized compounds are substrates of the P-glycoprotein, suggesting that they may not be subject to efflux transport mechanisms that could hinder their efficacy in CNS-related treatments (see Table 4).

6. Conclusion

The study aimed to design, synthesize, and characterize small chalcone molecules as potential antimicrobial agents to treat the growing

Table 4
Drug-likeness parameters and pharmacokinetic properties of designed compounds.

Entry	MW[a] (≤500)	Physicochemical parameters					B.S [g]	Lipinski's violation	Absorption Distribution		
		TPSA[b] (Å^2)	HBA[c] (≤10)	HBD[d] (≤5)	RB[e] (≤5)	cLogP[f] (≤5)			GIA[h]	BBB[i]	Pgp[j]
9	254.28	46.53	3	1	4	3.04	0.55	0	High	Yes	No
11	269.3	72.55	3	2	4	2.48	0.55	0	High	Yes	No
13	270.28	66.76	4	2	4	2.8	0.55	0	High	Yes	No
Am	349.4	138	5	3	5	0.08	0.55	0	Low	No	No
Fl	306.27	81.65	7	1	5	0.88	0.55	0	High	No	Yes

Am Ampicillin, Fl Fluconazole.
[a] Molecular weight.
[b] Topological polar surface area.
[c] Number of hydrogen bond acceptor.
[d] Number of hydrogen bond donor.
[e] Number of rotatable bonds.
[f] Consensus of calculated lipophilicity.
[g] bioavailability score.
[h] gastrointestinal absorption.
[i] blood brain barrier.
[j] P-glycoprotein substrate.

global health concern of infections caused by antibiotic-resistant bacteria and fungi.The synthesized compounds showed MIC values ranging from 2.5 to 160 μg/mL, indicating enhanced membrane penetration and ligand-protein binding capabilities. Compound **9** showed broad antibacterial activity against Gram-negative bacteria, with MIC values of 40 μg/mL against *P. aeruginosa* and 80 μg/mL against *E. coli*. Compound **11** showed significant efficacy against Gram-positive bacterial and fungal strains. Molecular docking studies confirmed these findings, showing superior binding affinities for both species. All synthesized compounds adhered to Lipinski's rule of five, suggesting favorable absorption through the gastrointestinal tract and oral administration suitability. The study's findings indicate that developed synthetic derivatives could potentially be used as lead compounds in drug development frameworks.

CRediT authorship contribution statement

Narmin Hamaamin Hussen: Writing – review & editing, Formal analysis, Data curation. **Larin Barzan Hussein:** Writing – original draft, Methodology, Formal analysis. **Aso Hameed Hasan:** Writing – review & editing, Supervision, Investigation. **Shokhan Jamal Hamid:** Validation, Formal analysis, Data curation. **Chawan Othman Abdl:** Writing – original draft, Visualization, Methodology. **Bakhcha Sarkar:** Software, Methodology, Conceptualization. **Kozhin Muhammed:** Formal analysis, Data curation, Conceptualization. **Daroon Muhamad:** Writing – original draft, Validation, Data curation.

Declaration of competing interest

No conflict of interest exists.

We wish to confirm that there are no conflicts of interest associated with this publication, has not been published before and not currently being considered for publication elsewhere.

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