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Microwave-assisted Synthesis and Molecular Docking Study of Heteroaromatic Chalcone Derivatives as Potential Antibacterial Agents

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In this study, a series of chalcone derivatives (**1a–c**) were synthesized via Claisen-Schmidt condensation, followed by aza-Michael addition to form pyrazoline derivatives (**2a–c** and **3a–c**). The reaction was performed via microwave radiation to give excellent yields (77–93%) in 1–3.5 min. Microwave-assisted reaction of Fischer esterification of pyrazolines (**2a–c** and **3a–c**) afforded heteroaromatic pyrazoline esters (**4a–c**) in high yield (83–96% in <2 min) compared to conventional reflux (55–79% in 30 min). Compounds **1a–c** and **3a–c** demonstrated excellent antibacterial activity against *Staphylococcus aureus* via disc diffusion assay with inhibition zone with 13 and 19 mm compared to a standard drug, ampicillin (11 mm). Structure activity relationship of **1b** and **3b** were visualized via molecular docking interaction against 4pql protein of *S. aureus* with binding free energy −7.0 and −8.0 kcal/mol, respectively. This study is significant in drug discovery process particularly for pharmaceutical industries.

Keywords: Condensation, Acetophenone, Pyrazoline

Introduction

Chalcone is a biologically active molecule derived from natural products. Chalcone and their derivatives can be easily prepared via Claisen-Schmidt condensation.[1] It has a distinctive open chain flavonoid with two aromatic rings and three carbon atoms. Chalcone is a stable intermediate[2] for the synthesis of various heterocyclic compounds such as isoxazole,[3] pyrazoline,[4] epoxide,[5] thiazine,[6] indazole,[7] and pyrimidine[8] valuable in pharmaceutical industries.[9] A recent study disclosed a series of heterocyclic chalcones especially pyrazoline[10] derivatives with remarkable biological properties such as antioxidant,[11] antibacterial,[12] antidepressant,[13] anticancer,[14] antipyretic,[15] antimalarial,[16] antitubercular[17] as well as non-biological applications in solar dyes, chemosensors,[18] photo-conductors,[19] and opto-electronic devices.[20] Pyrazolines are well known heterocyclic compounds with aromaticity. Heterocyclic compounds (*i.e.*, pyrazoles, pyrazolines, oxazoles, pyrrolidinone) are significant in organic chemistry and pharmaceutical industry to achieve unique drugs with excellent potential against microbial diseases.[21]

Utilizing natural product-based compounds as potential antibacterial drugs has grown interest among researchers. The incorporation of chalcone with heterocyclic natural product based compounds such as kojic acid and dihydroartemisinin via ester linkage has been reported to produce kojic ester[22] and dihydroartimisinyl ester,[23] respectively, with excellent antibacterial and anticancer activities. Similarly, chalcone-derived carboxylate pyrazoline[24] has also been reported to cure neurological diseases such as Alzheimer, Huntington, and Parkinson's diseases.[25] Interestingly, the presence of natural product moiety offers strong binding interactions with the target receptors during inhibition due to the availability of numerous active sites in the molecular network.

Various methods have been reported in the synthesis of chalcone derivatives. The nature of reagents and substituents in the chalcone synthesis sometimes requires harsh reaction condition, such as vigorous stirring and heating.[1] The conventional heating method is practically slow and time consuming, which lead to low yield and product decomposition.[26] Microwave-assisted reaction of chalcone derivatives has become a current choice as it provides uniform heating and offers good yield of products in shorter reaction time.[27] Microwave strategy is fascinating for a variety of reactions cycloaddition, condensation and coupling reactions to achieve wide-range of heterocyclic compounds.[28]

Herein, we reported on a green and efficient synthesis of heteroaromatic chalcone derivatives (**2a–c** and **3a–c**) from chalcone (**1a–c**), followed by Fischer esterification to produce (**4a–c**) via microwave-assisted reaction. These compounds were evaluated against *Escherichia coli* and *Staphylococcus aureus*. The effect on Structure Activity Relationship (SAR) of various substituents presence in the molecular network toward biological activities were studied via molecular docking interaction using AutoDock Vina 1.1.2 program and AutoDock Tools 1.5.6.

Experimental

All chemical analytical grade reagents and solvents were used without further purification. Experiments reactions were performed in via microwave radiation using Anton Paar Microwave Synthesis Reactor, Monowave 300. Melting points were analyzed using Stuart SMP3 via open tube capillary method. FTIR spectra were obtained through Perkin Elmer Thermoscientific Smart Omni Transmission Nicolet IS10 FTIR Spectrophotometer. ^{1}HNMR and ^{13}CNMR spectra were recorded on JEOL ECA 500 spectrometer, with chemical shift relative to deuterated dimethyl sulfoxide (DMSO-d_6) as reference. Elemental analysis was recorded by using CHN analyzer THERMOFLASH EA 1112 Series. The transmittance of synthesized compounds was noted through Optima SP-300 Spectrophotometer for antibacterial evaluation. All spectra are reported in the supplementary information (Figure S1–S3 and Table S1).

General Procedure for the Synthesis of Chalcones (1a–c)**.** The synthesis of chalcones (**1a–c**) was performed following Sie[22] with slight modifications. 4-Carboxybenzaldehyde (0.15 g, 0.001 mol) and acetophenone derivatives (0.001 mol) were added to ethanolic solution of sodium hydroxide (0.17 g, 0.001 mol) and the mixture was stirred overnight at room temperature. The reaction completion was monitored by thin-layer chromatography. The mixture was neutralized with hydrochloric solution (3 M) and the precipitate was formed, filtered, and recrystallized from ethanol to afford the title compounds.

General Procedure for the Synthesis of Acetyl Pyrazolines (2a–c)

Via Conventional Method. Chalcone (**1a–c**) (0.0005 mol, 0.146) and hydrazine (20 mmol) in acetic acid (30 mL) were refluxed for 24 h until chalcone consumed into product. The reaction mixture was poured into crushed ice and left overnight. The precipitate was separated by filtration, washed well with water, dried, and recrystallized from ethanol to afford white colored precipitate of **2a–c.**

Via Microwave-assisted Synthesis. A mixture of chalcone (**1a–c**) (0.0005 mol, 0.146) and hydrazine hydrazine (20 mmol) was added in acetic acid (17 mL) and irradiated in microwave for suitable times (reported in Table 1) at 300°C. The reaction mixture was poured into chilled water and stand overnight. The precipitate was formed, filtered, washed well with water, dried and recrystallized with ethanol to afford **2a–c**.

General Procedure for the Synthesis of 1H- and Phenyl Pyrazolines (2d **and** 3a–c)

Via Conventional Method. Sodium hydroxide (0.04 g, 0.001 mol) and chalcone (**1a–c**) (0.001 mol) were added in ethanol (20 mL) and stirred until both dissolved. Hydrazine hydrate or phenyl hydrazine (0.04 mL, 0.01 mol) was added dropwise to the mixture and refluxed for 4 h. The mixture was cooled to room temperature and the solution was poured into ice-cold water and neutralized with HCl (3 M). The precipitate was filtered and purified in ethanol to achieve **3a–c**.

Via Microwave-assisted Synthesis. Chalcone (**1a–c**) (0.001 mol) and sodium hydroxide (0.04 g, 0.001 mol) were dissolved in ethanol (15 mL). Then the reaction mixture was irradiated in the microwave in a suitable reaction time (depicted in Table 1) at 160°C. After the reaction come to completion, the mixture was neutralized with 3 M HCl acid to form a precipitate. The solid was filtered and recrystallized in ethanol to afford **3a–c**.

General Procedure for the Synthesis of Phenyl Pyrazoline Ester (4a–c)

Via Conventional Method. Pyrazoline (**3a–c**) (0.0005 mol) was stirred in methanol (20 mL) at room temperature for 10 min. Sulfuric acid (1 mL) was added into the reaction mixture and refluxed for 30 min. The reaction was cooled to room temperature, neutralized with 2% potassium carbonate, organic layer was extracted with ethyl acetate and dried *in vacuo* and recrystallized in ether.

Via Microwave-assisted Synthesis Method. Pyrazoline (**3a–c**) (0.0005 mol) was stirred at room temperature in methanol (10 mL) for 10 min. Sulfuric acid was added five to six drops and irradiated mixture in the microwave for a suitable time (reported in Table 1) at 120°C. After the completion of reaction, mixture was cool down, neutralized with 2% K_2CO_3, organic layer extracted with ethyl acetate, dried *in vacuo*, and recrystallized in ether.

Antibacterial Study

Kirby Bauer Disc Diffusion Method. Antibacterial screening of **1a–c**, **2a–c**, **3a–c**, and **4a–c** was performed against *E. coli* (ATCC25922) and *S. aureus* (N5923) via Kirby Bauer Disc Diffusion Method by following earlier reported methodology.[29] *E. coli* and *S. aureus* were cultured in Mueller-Hinton Broth (MHB) as inoculum and incubated at 37.5 °C with continuous shaking at 150 rpm for 20 h. Mueller-Hinton Agar (MHA) plates were used to grow bacteria by using bacteria suspension with sterilized cotton-tipped swab. Sterilized filter paper disc placed on the bacteria surface of MHA plate with sterilized forceps and soaked with 10 μL of synthesized compounds in DMSO. The plates were incubated at 37.5°C for 24 h. The zone of inhibition was measured in millimeter to estimate the potency of prepared compounds.

Turbidimetric Kinetic Method. All successfully achieved synthesized compounds **1a–c**, **2a–c**, **3a–c**, and **4a–c** were evaluated against *S. aureus* bacteria. *S. aureus* was cultured on Mueller-Hinton Broth and incubated at 37°C with stirring at 250 rpm overnight. Inoculums were inoculated with a culture medium treated with increasing concentration (50, 80, and 100 ppm) of synthesized compounds in DMSO. The mixture was shaken at 37°C. Inoculum with only DMSO was used as negative control and ampicillin used as positive control. The transmittance (T) was recorded using UV–Visible Spectrophotometer Optima SP-300, Japan after

Table 1. Conventional and microwave methodologies for heteroaromatic chalcones.

	Conventional method			Microwave method	
Codes	Time	Condition	Yield (%)	Time (min)	Yield (%)
1a	18 h	rt	90.6	—	—
1b	18 h	rt	80.2	—	—
1c	18 h	rt	83.3	—	—
2a	24 h	Reflux	84.1	3	89.4
2b	24 h	Reflux	75.5	3	80.8
2c	24 h	Reflux	88.5	3.5	93.8
3a	6 h	Reflux	83.9	2.5	88.7
3b	6 h	Reflux	70.4	3	77.7
3c	6 h	Reflux	79.7	2.5	82.6
4a	30 min	Reflux	55.9	1.5	83.8
4b	30 min	Reflux	64.8	2	95.6
4c	30 min	Reflux	78.9	1.5	96.8

Note: rt = room temperature.

1 h interval. The antibacterial activity was examined by plotting graph of ln Nt vs. time which the ln Nt value describe the number of colony-forming units/mL.[29]

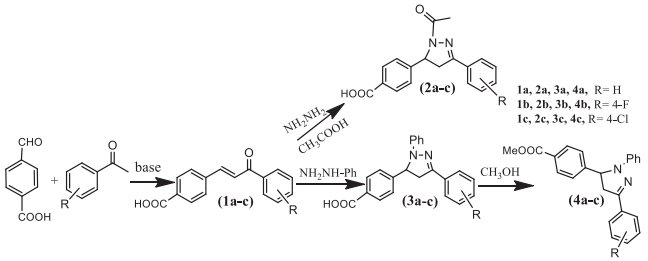
Molecular Docking Study

The molecular docking interaction study was carried out using AutoDock Vina 1.1.2 program, AutoDock Tools is software,[30] The cubic grid box of 40 Å size (x, y, and z) with a spacing of 0.375 Å were centered on the active sites of the protein. The X-ray structure of *S. aureus* DNA binding protein (PDB entry: 4pql) was retrieved from Protein Data Bank.[31]

Results and Discussion

Chemistry. Chalcone derivatives **1a–c** were synthesized via Claisen-Schmidt condensation of 4-formylbenzoic acid with a series of acetophenone derivatives in the presence of ethanolic NaOH. The formation of heterocyclic chalcones namely pyrazolines (**2a–c**, **3a–c**) from the reaction of chalcone **1a–c** with hydrazine derivatives has been successfully performed following aza-Michael reaction with excellent yield (77–93%) in 1–3.5 min. The introduction of ester moiety onto pyrazoline derivatives (**2a–c**, **3a–c**) via Fischer esterification afforded **4a–c** in higher yields (83–96% in <2 min). The synthetic reaction is outlined in Scheme 1. The microwave-assisted reaction gave higher yields and shorter time as compared to conventional method with yields (55.9–88.5%) in longer time (30 min–24 h). The efficiency of microwave-assisted synthesis over conventional method for all compounds is depicted in Table 1. The proposed mechanism is illustrated in Figure 1.

The chemical structures of all synthesized compounds were characterized using FTIR, ^{1}H NMR and ^{13}C NMR. The FTIR spectra of **1a–c** showed characteristic bands at 2988–2944 cm^{-1} attributed to a υ_{CH} while peaks at 1674–



Scheme 1. Synthesis of heteroaromatic chalcone derivatives.

1664 cm^{-1} attributed to $\upsilon_{C=O}$ and $\upsilon_{C=O\ carboxyl}$ in the chalcone network. The presence of sharp peaks was observed at 1601–1583 cm^{-1} attributed to $\upsilon_{C=C}$.[22] ^{1}H NMR spectra displayed aromatic protons of **1a–c** at δ 8.27–7.37 ppm with coupling constant J = 8.8–7.2 Hz, while two doublets of vicinal protons (CHα = CHβ) were observed at δ 7.79–7.76 ppm and 8.06–8.03 ppm with J = 15.5–15.7, respectively, which corresponded to *trans* configuration. ^{13}C NMR spectra displayed sharp signals at 167.3 and 189.6 ppm attributed to (C=O) moiety of carboxylic group and ketone respectively. Although CHα and CHβ appeared at 124.8 and 143.3, respectively.

The FTIR spectra of **2a–c**, **3a–c** showed absorption peaks of $\upsilon_{C=N}$ and υ_{C-N} at 1602–1593 cm^{-1}, while the peaks at 1346–1300 cm^{-1} corresponded to the formation of pyrazoline. The peaks presence at 1669–1716 cm^{-1} were attributed to $\upsilon_{C=O}$ of acetyl moiety in the molecular network. The ^{1}H NMR spectra of **2a–c**, **3a–c** displayed ─CH_2 as three doublet of doublets at 3.02–5.85 ppm, which corresponded to the formation of pyrazolines via cyclization of chalcone derivatives. Compound **2a–c** displayed protons of acetyl group at 2.3 ppm and aromatic region at 8.31–6.70 ppm.[32] The ^{13}C NMR spectra showed prominent signals of 147.7–154.7 ppm and 167.1–167.5 ppm corresponded to C=O and C=N. The resonance observed at 63.09–59.8 ppm attributed to CH pyrazoline and 43.0–

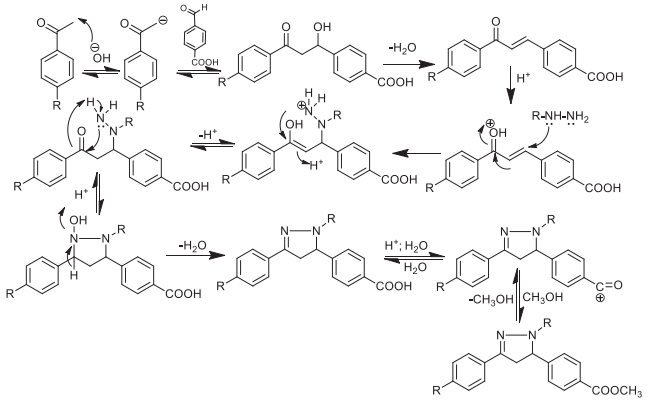


Figure 1. Proposed mechanism for the synthesis of pyrazline ester.

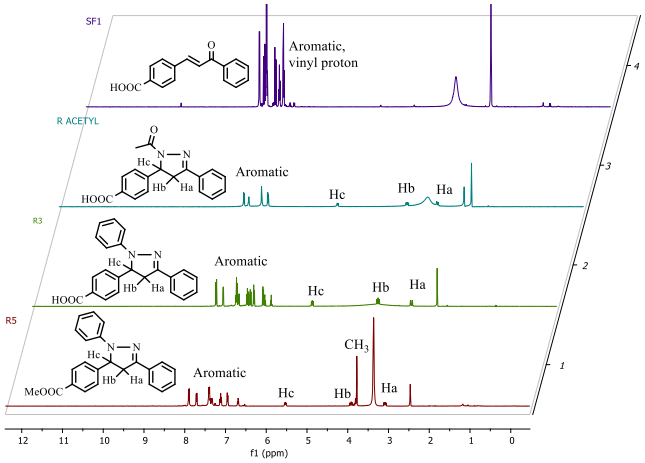


Figure 3. Comparative ^{1}H NMR spectra of **1a**, **2a**, **3a**, and **4a**.

42.4 ppm corresponded to CH_2 in pyrazolines **2a–c** and **3a–c**.

The successful esterification of pyrazolines **2a–c** and **3a–c** has produced **4a–c**, which was confirmed by the presence of strong peaks at 1719 cm^{-1} corresponded to $\upsilon_{C=O}$ of ester group. The ^{1}H NMR spectra of **4a–c** showed resonance as singlet at 3.80 ppm corresponded to CH_3 of ester (Figures 2 and 3). The formation of the desired product was also physically confirmed via sulfuric acid (H_2SO_4) test for chalcone and pyrazoline with reddish brown and green color, respectively. Acetyl pyrazoline, however, did not show green color as of phenyl pyrazoline. This is because the acetyl group in pyrazoline is sensitive toward acid and base and more labile toward cleavage to form complexes with different colors (Table S2).[13]CNMR spectra have also exhibited resonance at 52.4–52.5 ppm (CH_3) and 166.3 ppm (C═OOR ester), which corresponded to the target compounds.

Several strategies were applied for the synthesis 1H-pyrazoline (**2d**), *i.e.*, in base[33] and acid conditions.[34] Many studies have been reported on the formation of (**2d**) under ethanolic base conditions.[34,35] However, attempted synthesis of 1H-pyrazoline (**2d**) was not availed due to the reversible reaction.[34] The presence of free proton in **2d** is believed to form interaction with carboxylic group, which eventually retard the formation of pyrazoline. Acid

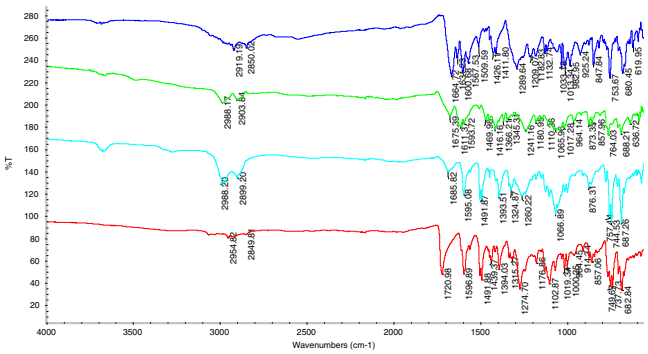


Figure 2. Comparative FTIR results of **1a**, **2a**, **3a**, and **4a**.

neutralization able to protonate nitrogen atom and form unstable quaternary ammonium salt, which eventually caused ring opening in the presence of sodium ethoxide.[36] Hydrazine hydrate is stable in ethanolic base media, but unstable in ethanolic solution due to atmospheric oxidation.[37–39] The hydrazone intermediate was easily cleaved during neutralization due to free proton and reversibility of reaction factors.[40,41]

The presence of carboxylic group and massive Szmant'z solvent shell around hydrazone moiety has caused steric hindrance, therefore it retards the cyclization of hydrazone. This is due to the presence of hydrogen bonding with solvent molecular cage and dimer formation with strong hydrogen bonding (Figure 4). In solution phase, self-assembled units offer informative supramolecular architecture which appeared in the resulting macroscopic crystal structures. Mutual intermolecular hydrogen-bonded dimer of carboxylic acid forms closed packing in crystal in the

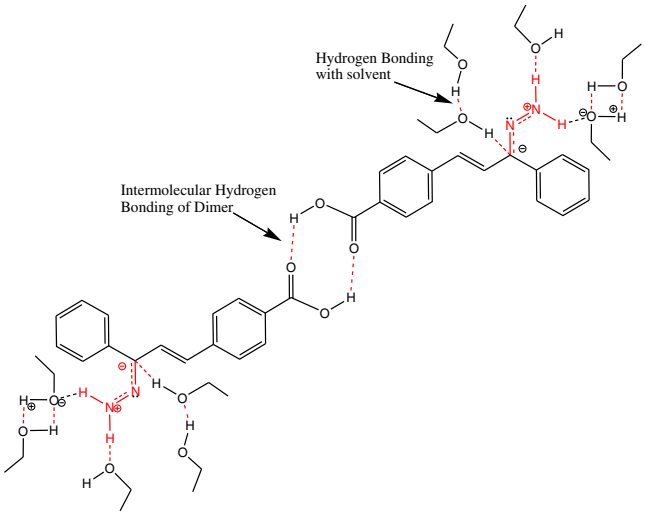


Figure 4. Solvent molecular cage and dimer packing hindrance for 1H-pyrazoline formation.

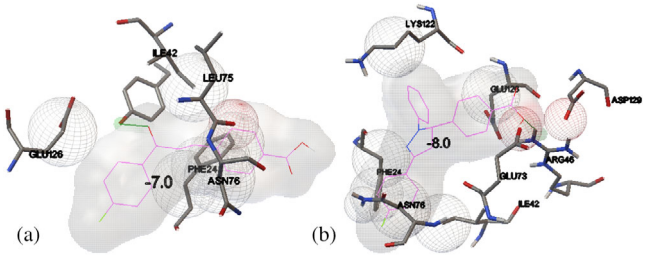


Figure 5. Docking image of **1b** (a), **3b** (b) illustrated binding interaction with targeted protein.

presence of ethanol as solvent.[42] A solute–solvent interaction has also played an important role in the process of internal rearrangements. Fortunately, phenyl pyrazoline derivatives **3a–c** and **4a–c** were stable under basic condition and successfully synthesized via aza-Michael reaction of chalcones and Fischer esterification, respectively.

Antibacterial Activities. Antibacterial activities of all synthesized compounds **1a–c**, **2a–c**, **3a–c**, and **4a–c** have been performed via both disc diffusion method against Gram-positive *S. aureus* and Gram-negative *E. coli*. The ampicillin was used as standard drugs and DMSO as negative control against bacterial strains.

All compounds were evaluated via disc diffusion assay against *S. aureus* and *E. coli*. Zone of inhibitions of all compounds is depicted in Table S3. Compounds **1a–c** and **3a–c** demonstrated excellent zone inhibition with 9–19 mm against *S. aureus*. This phenomenon could be due to the morphology of the bacteria cell wall.[22] The antibacterial potential of the synthesized compounds was varied with the nature and position of substituents, and types of bacterial strain.

The presence of acetyl group in pyrazoline (**2a–c**) and pyrazolines ester (**4a–c**), however, did not show antibacterial activities against *S. aureus* and *E. coli*. This could be no formation of hydrogen bond interaction between acetyl group with the protein receptor.[43,44] Whereas, **4a–c** were inactive in disc diffusion method due to the strong interactions of unexpected solvent cage, which occupy the active and reactive site of the compound with solvent. In short, poor dissolution and improper solvent composition on both ligand-binding and protein stability could also contribute to the antibacterial activity.[45]

Compound **1a–c** demonstrated mild activities with the inhibition zone of 9–13 mm. The presence of hydrophilic moiety (COOH) able to enhance the bacterial activities via hydrogen bonding formation.[43] Compound **1b** showed good inhibition zone (13 mm) compared to **1a** (11 mm) and **1c** (9 mm). Higher electronegative and lithophilic moiety of fluorine substituent in **1b** has contributed to the antibacterial activity.[31] Compound **3a–c** exhibited good antibacterial activities with 16–19 mm inhibition zone. Compound **3b** gave superb activity with 19 mm inhibition zone due to the presence of heterocyclic pyrazoline with N–N moiety and fluoro substituent, that contributed for the enhancement of activity. Phenyl pyrazoline ring **3b** showed higher antibacterial activity compared to chalcone derivatives **1b**.

The antibacterial kinetics studies were also kinetically analyzed against *S. aureus* via turbidimetric kinetics method.[46] The inhibition activity of **1a–c**, **2a–c**, **3a–c,** and **4a–c** was evaluated against *S. aureus* at three different concentrations (50, 80, and 100 ppm).[31] The MIC values were determined by extrapolating concentration toward the zero growth rate (Figures S4 and S5). Compounds **1a–c** and **3a** showed excellent inhibition with MIC values of 88–93 ppm and 80–101 ppm, respectively. Compound **3b** and **3c**, however, showed no antibacterial activity and experienced the formation of precipitate during administration into the media. Similarly, **4a–c** was also precipitated in broth solution due to poor solubility and solvent incompatibility which refrained the kinetic study of antibacterial activity. Overall, the microbial activity via turbidimetric kinetic method was not conclusively promising for comparative analysis in this study compared to disc diffusion method. Solubility is an essential factor in drug discovery process, in addition to other factors such as the nature of compound (lithophilic and hydrophilic sites[47]), dilution rate, temperature, time, and concentration.[48,49]

Molecular Docking

Comprehensive structure activity relationship analysis of the targeted compounds (**1b** and **3b**) with protein docking was performed and visualized using Autodock vina. The selected compounds (**1b** and **3b**) is based on strong inhibition activity against *S. aureus* with diameter of inhibition zone 13 and 19 mm, respectively. *S. aureus N*-Terminal domain of DNA binding protein (PDB entry: 4pql) was selected as the active site to interact with **1b** and **3b**. The docking depicted the molecular interaction between the compound (ligand) and targeted bacteria (receptor) via, *i.e.*, hydrogen bonding, Van der Waal interaction.[50] The strength of interaction between a compound to its target receptor was determined by the binding affinity. Lower binding free energy indicates for stronger, simultaneous, and stable binding interaction.[51] Figure 5 showed the docking modeling result of **1b** and **3b** with the targeted protein.

The interaction of **1b** with *N*-terminal domain of DNA binding protein receptor gave binding free energy of −7.0 kcal/mol (Figure 5(a)). The dominant hydrogen bonding interactions between TRY17 and unsaturated keto moiety of **1b** was observed as represented with green line.[52] Other amino acid residues such as GLU126, PHE24, ASN76, LEU75, and ILE42 appeared to form bonding interaction with aromatic (Ar) group of **1b** via electrostatic interactions and aryl-alkyl interactions.[31,52] Compound **3b** depicted binding free energy of −8.0 kcal/mol (Figure 5 (b)). The docking image of **3b** suggested that a strong hydrogen bond interaction (indicated by green-colored sphere) and hydrophilic interaction occurred between the

–COOH with ARG76 and ASP129 residues, respectively. Hydrogen bond is the most important interactions specifically in biological processes to provide specificity and stabilization of binding between the ligands and enzyme active site.[53] The main interaction appeared to be in contact with the Ar group of **3a** indicating for aryl–aryl and alkyl–aryl interactions with various amino acid residues such as LYS122, GLU126, PHE24, ASN76, GLU73, and ILE42.[52]

Conclusions

Three series of chalcone derivatives **1a–c**, heteroaromatic chalcone derivatives of pyrazoline **2a–c**, **3a–c** and heteroaromatic chalcone esters derivatives **4a–c** have been efficaciously synthesized via conventional and MW radiation in excellent yield. MW-assisted synthesis demonstrated convenience and green route with higher yield and shorter time than the conventional method. Compounds **1a–c**, **3a**, and **3b** exhibited good inhibition activity against *S. aureus*, where **1b** and **3b** demonstrated the highest inhibition zone with 13 and 19 mm, respectively, due to the presence of halogen, carboxylic and pyrazoline (N–N) moieties. Molecular docking supported the experimental results of antibacterial activity via disc diffusion method. This study showed the convenient preparation of chalcones and pyrazoline derivatives and their potential for antibacterial application in pharmaceutical industries.

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Supporting Information. Additional supporting information may be found online in the Supporting Information section at the end of the article.

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