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In Silico Study, Synthesis and Biological Activity of Chalcone Derivatives as Antibacterial Candidates

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KEYWORDS

In silico. Synthesis, Chalcone derivatives, Antibacterial

ABSTRACT:

Introduction: The growing resistance of many bacteria to current antibiotics underscores the need for new antibacterial agents. 2-Methoxychalcone and its derivatives have been acknowledged for their potential in combating cancer, exhibiting antioxidant properties, as well as antibacterial.

Objectives: This study aims to forecast the antibacterial efficacy of two compounds, namely 2,2',4-Trimethoxychalcone (TMC 1) and 2,4,4'-Trimethoxychalcone (TMC 2). through in silico prediction.

Methods: It was carried through in silico prediction utilizing the Mollegro Virtual Docker method. Additionally, in vitro assays were conducted to assess their antibacterial activity against both Gram-positive *Bacillus subtilis* and Gram-negative *Escherichia coli* bacteria. Synthesis of chalcone derivatives using the Claisen-Schmidt method.

Results: Egestas Molecular docking analyses revealed a superior binding affinity of TMC 2 towards the *Enoyl ACP reductase* inhibitor (PDB.1C14) (docking score: -13.3859 kcal/mol) compared to TMC 1 (docking score: -10.6517 kcal/mol). The positive control utilized was amoxicillin.

Conclusions: Prediction outcomes indicate the potential antibacterial activity of TMC 2 is greater than TMC 1 compounds. Furthermore, in vitro testing demonstrated the antibacterial effectiveness of TMC 2 against both *Bacillus subtilis* (Gram-positive) and *Escherichia coli* (Gram-negative) bacteria is better than TMC 1.

1. Introduction

Drug development has witnessed rapid progress, emerging as a pivotal component in the pharmaceutical sector. Traditional methodologies have long been employed in crafting drug compounds through organic reactions, often necessitating elevated temperatures. Common heat sources, such as oil baths, heating mantles or sand baths, have conventionally been utilized. However, these approaches entail extended reaction durations and may introduce temperature inconsistencies within the reaction mixtures. Furthermore, the localized heating effect within the reaction vessel can induce overheating, consequently compromising the integrity of products, substrates, and accompanying reagents under prolonged exposure. Consequently, there's a quest for alternative methodologies to realize reactions yielding high product yields and expected quality. Microwave-assisted reactions emerge as an innovative choice in the journey of transforming chemical compounds into novel drugs [1,2]. Pathogenic bacteria that have developed resistance to antibiotics pose a significant threat to human health and the global population at large [3]. Enzymes involved in peptidoglycan biosynthesis have emerged as promising targets in attempt to impede the proliferation of bacteria. Inhibiting these enzymes can interfere with the synthesis of peptidoglycan, resulting in the deformation of bacterial cells and ultimately halting their growth and replication [4]. The advancement of computational technology has facilitated the conduct of computational docking simulations, which illustrate the

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hypothetical interaction between proposed compounds and enzyme targets. The ability of 2-methoxychalcone derivatives to selectively and effectively engage with biological targets positions them as strong candidates for antibacterial agents. This approach allows researchers to predict potential interactions between compounds and target enzymes, providing critical insights for developing more potent agents.

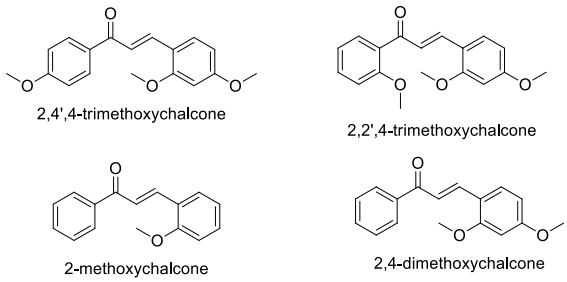

Figure 1. The compounds of 2-methoxychalcone and its derivatives

The compound 2-methoxychalcone is a derivative of chalcone, distinct chemically from the group of antimalarial drugs facing resistance issues [6]. This material exhibits antimalarial efficacy [7], antitubercular [8], anti-inflammatory [9], antioxidant [10,11], antifungal [12], and anticancer activity [13]. The synthesis of 2-methoxychalcone and its derivatives can be achieved through two synthetic pathways: Knövenagel reaction continued by Friedel-Crafts acylation, and Claisen Schmidt condensation [14,15]. The Claisen Schmidt condensation presents a more expedient and practical route for synthesizing 2-methoxychalcone and its derivatives. This condensation can take place under both acidic and basic conditions and requires reagents with alpha hydrogen. Catalysts are used to speed up the reaction and may include either acid or base catalysts, as well as other types of catalysts, like NaOH, Bentonite [16], $TiCl_4$ [14], Potassium chlorida [17], Alumina and MgO [18]. These catalysts offer numerous advantages and have demonstrated effectiveness across various reactions. In this research endeavor, the synthesis of 2-methoxychalcone and its derivatives will be executed employing 2-methoxyacetophenone/ derivatives and 2-methoxybenzaldehyde/ derivatives as reagents (Figure 2) via the aldol Claisen Schmidt condensation, facilitated by NaOH catalyst and microwave irradiation (Green Chemistry). Given the aforementioned context, this study articulates the following inquiry: to perform in silico assessments, followed by the synthesis of 2-methoxychalcone derivatives utilizing the Claisen Schmidt condensation with the aid of microwave, and subsequently assess their antibacterial activity. This investigation holds promise in offering fresh perspectives on the Claisen Schmidt condensation reaction approach, incorporating NaOH catalyst alongside microwave irradiation for the production of 2-methoxychalcone and its derivatives [19] (Figure 1).

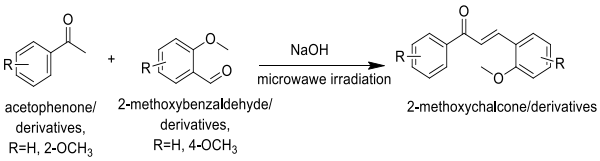

Figure 2. Reaction of 2-methoxychalcone and its derivatives

2. Objectives

n silico study: Prior to the synthesis of selected compounds, an in silico analysis of chalcone derivatives was performed to forecast their potential antibacterial activity. This method aims to enhance the efficiency of designing antibacterial compounds. Synthesis of dhalcone derivatives**:** The design and synthesis of 2-methoxychalcone derivatives involved incorporating a methoxy group into the aromatic ring to investigate structural modifications. The synthesis pathway was refined using microwave irradiation, which is an efficient and rapid technique for compound production, with NaOH as the selected catalyst using the Claisen-Schmidt method. The synthesized 2-methoxychalcone derivatives were analyzed using infrared spectroscopy, UV-Vis spectroscopy, and NMR spectroscopy to verify their structure. To confirm the anticipated antibacterial activity of the chalcone derivatives, in vitro activity tests were performed.

3. Methods

The chemicals employed in study were of p.a./analytical grade purity, unless otherwise indicated. These substances encompassed 2-methoxyacetophenone, 2,4-dimethoxyacetophenone, benzaldehyde, 2-methoxy benzaldehyde, 4-methoxybenzaldehyde, NaOH, silica gel GF_{254}, tetrahydrofuran, chloroform, 96% ethanol, dichloromethane, ethyl acetate, and hexane. The

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instrumentation used comprised standard glassware typically found in chemical laboratories, a Sanyo EM-S800 Watt microwave, a HEWLETT PACKARD 8452A UV-Vis spectrophotometer, a Buck Scientific M 500 IR spectrophotometer, and a JEOL ECS-400 FT-NMR spectrometer. Synthesis procedures were conducted employing the Claisen Schmidt aldol condensation method with NaOH serving as the catalyst. The synthesized product underwent identification through FT-IR, UV-Vis, NMR analyses. Molecular docking studies were carried out utilizing the MVD program. Antibacterial activity assessments were performed via the well method against *Escherichia coli* (Gram-negative) and *Bacillus subtilis* (Gram-positive) bacteria [20]. Synthesis of 2-methoxychalcone and derivatives with microwave irradiation [21,22]. An equal-volume blend comprising acetophenone/ derivatives (1.2 ml, 10 mmol) and 2-methoxybenzaldehyde/ derivatives (2 ml, 20 mmol) was entered into a 25 ml Erlenmeyer, accompanied by the addition of 4.0 ml of tetrahydrofuran solvent and 60% NaOH. The mixture was agitated until achieving uniformity, followed by evaporation. Subsequently, microwave irradiation at 40 Watts was applied for 3 minutes. After cooling to room temperature, the mixture underwent washing. The resulting mixture was subjected to purification via recrystallization. Purity assessment was conducted through m.p. determination and thin-layer chromatography utilizing some different eluents. Characterization procedures involved UV-Vis spectrometry, IR spectrometry, and NMR spectroscopy.

4. Results

Results of physicochemical properties testing, docking molecule in Table I and II.

Table I. Results of physicochemical properties testing

Compounds	Log P	MR (cm^3/ mol)	tPSA	Hidrogen bond acceptors
2-Methoxychalcone (MC)	3.46	74.67	26.30	2
2,4-Dimethoxy chalcone (DMC)	3.33	81.92	35.53	3
2,2’,4-Trimethoxy chalcone (TMC 1)	3.21	89.17	44.76	4
2,4,4’-Trimethoxy chalcone (TMC 2)	3.21	89.17	44.76	4
Amoxicillin (positive control)	-0.58	91.18	132.96	9

Table II. Chalcone derivatives docking molecule results

Compounds	Mol Doc score (kcal/mol)	The amino acid that engages in hydrogen bonding and steric interactions	
		Hydrogen bonding	Steric interactions
2-Methoxy chalcone (MC)	-4.9372	Gly 1013	Asp 1064, Gln 1040, Ala 1196, Ser 1019
2,4-Dimethoxy chalcone (DMC)	-5.8361	Gly 1013	Asp 1064, Gln 1040, Ala 1196, Ser 1019
2,2’,4-Trimethoxy chalcone (TMC 1)	-10.6517	Gly 1013	Asp 1064, Gln 1040, Ala 1196, Ser 1019, Val 1065
2,4,4’-Trimethoxy chalcone (TMC 2)	-13.3859	Gly 1013	Asp 1064, Gln 1040, Ala 1196, Ser 1019, Val 1065
Amoxicillin (positive control)	-15.2035	Gly 1013	Asp 1064, Gln 1040, Ala 1196, Ser 1019, Val 1065, Thr 1194

Docking molecule of 2,2’,4-Trimethoxychalcone and 2,4,4’-Trimethoxychalcone explained on Figure 3 and 4.

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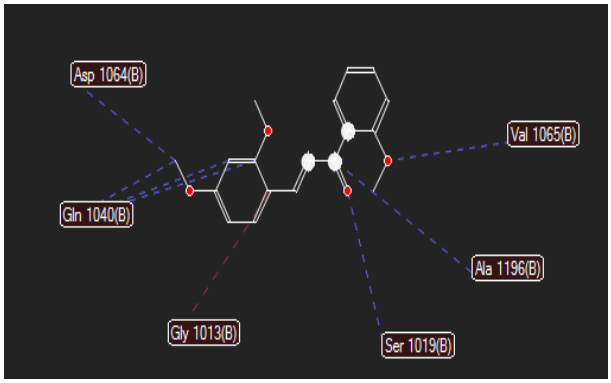

Figure 3. Docking molecule of 2,2',4-Trimethoxy chalcone

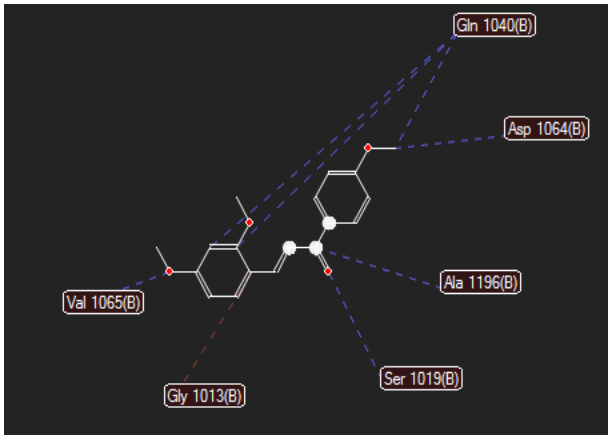

Figure 4. Docking molecule of 2,4,4'-Trimethoxy chalcone

Synthesis chalcone derivatives

Characterization of 2-methoxychalcone

UV-Vis Spectrum: Shows maximum absorbance in methanol at 212 nm and 348 nm. Infrared Spectrum: Absorption bands (cm^{-1}) in KBr pellet observed at 1661 (-C=O); 3061 (aromatic C-H); 1248 and 1033 (C-O-C ether). observed at ^{1}H-NMR spectrum: Chemical shifts (ppm) in $CDCl_3$ solvent; 8.12 ppm, doublet (*J*=16 Hz), 1H from C□; 7.67 ppm, doublet (*J*=12 Hz), 1H from C□; 8.01 ppm, doublet, 2H from (C_6H_5-); 7.63-7.61 ppm, multiplet, 3H from aromatic ring (C_6H_5-); 7.46-7.35 ppm, multiplet, 2H from aromatic ring (C_6H_4-); 7.00-0.98 ppm, multiplet, 1H from aromatic ring (C_6H_4-); 6.94-6.92 ppm, multiplet, 1H from aromatic ring (C_6H_4-); 3.89 ppm, singlet, 3H from -CH_3 [23,24].

Characterization of 2,4-dimethoxychalcone

UV-Vis Spectrum: Shows maximum absorbance in methanol at 212 nm and 348 nm. Infrared Spectrum: Absorption bands (cm^{-1}) in KBr pellet observed at 1650 (-C=O); 3079 (aromatic C-H); 1221 and 1070 (C-O-C ether). ^{1}H-NMR spectrum: Chemical shifts (ppm) in $CDCl_3$ solvent; 7.77 ppm, doublet (*J*=16 Hz), 1H from C□; 7.69 ppm, doublet (*J*=12 Hz), 1H from C□; 7.39-7.37 ppm, multiplet, 3H from aromatic ring (C_6H_5-); 7.52 ppm, singlet, 1H from aromatic ring (C_6H_3-); 7.59-7.53 ppm, multiplet, 2H from aromatic ring (C_6H_3-); 7.59-7.53 ppm, multiplet, 2H from aromatic ring (C_6H_5-); 3.87 ppm, singlet, 1H from -CH_3; 3.84 ppm, singlet, 1H from -CH_3 [23,24].

Characterization of 2,2',4-Trimethoxychalcone

UV-Vis Spectrum: Shows maximum absorbance in methanol at 212 nm and 348 nm. Infrared Spectrum: Absorption bands (cm^{-1}) in KBr pellet observed at 1644 (-C=O); 2996 (aliphatic C-H); 1256 and 1051 (C-O-C ether). ^{1}H-NMR spectrum: Chemical shifts (ppm) in $CDCl_3$ solvent; 8.01 ppm, doublet (*J*=16 Hz), 1H from C□; 7.72 ppm, doublet (*J*=12 Hz), 1H from C□; 6.96-6.94 ppm, multiplet, aromatic ring (C_6H_4-); 6.54 ppm, doublet, 1H from aromatic ring (C_6H_3-); 6.46 ppm, singlet, 1H from aromatic ring (C_6H_3-); 7.58 ppm, doublet, 1H from aromatic ring (C_6H_3-); 3.86 ppm, singlet, 3H from -CH_3; 3.84 ppm, singlet, 3H from -CH_3; 3.81 ppm, singlet, 3H from -CH_3 [23,24].

Characterization of 2,4,4'-Trimethoxychalcone

UV-Vis Spectrum: Shows maximum absorbance in methanol at 212 nm and 348 nm. Infrared Spectrum: Absorption bands (cm^{-1}) in KBr pellet observed at 1647 (-C=O) and 3005 (aromatic C-H). 1250 (C-O-C ether); 828 para-substituent. ^{1}H-NMR spectrum: Chemical shifts (ppm) in $CDCl_3$ solvent, 7.62 ppm, doublet (*J*=16 Hz), 1H from C□; 7.36 ppm, doublet (*J*=16 Hz), 1H from C□□ 6.53-6.45 ppm, multiplet, 2H from monosubstituted aromatic (C_6H_4-); 6.96-6.85 ppm, multiplet, 2H from monosubstituted aromatic ring (C_6H_4-); 7.52-7.49 ppm, multiplet, 2H from disubstituted aromatic ring (C_6H_3-); 7.70 ppm, doublet, 1H from disubstituted aromatic (C_6H_3-); 3.88 ppm, singlet, 3H from -OCH_3; 3.83 ppm, singlet, 3H from -OCH_3; 3.81 ppm, singlet, 3H from -OCH_3 [23,24].

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The results of 2-Methoxychalcone derivatives antibacterial activity on Table III.

Table III. Antibacterial activity of chalcone derivatives results

Compounds	MIC (mg/ml)	
	B. subtilis	***E. coli***
2-Methoxychalcone (MC)	72.6	72.6
2,4-Dimethoxy-chalcone (DMC)	72.6	72.6
2,2’,4-Trimethoxy-chalcone (TMC 1)	31.3	31.3
2,4,4’-Trimethoxy-chalcone (TMC 2)	7.8	7.8
Amoxicillin (positive control)	<3.9	<3.9

5. Discussion

Lipinski's "Rule of Five" is widely used for predicting molecular drug properties through in silico tests. In 1997, Lipinski, a senior pharmaceutical chemist at Pfizer, identified the chemical properties of oral small-molecule pharmaceuticals by comparing candidate drug molecules with common organic compounds, summarizing his findings as the "Rule of Five." The physicochemical properties and compliance with Lipinski's rule of five (also known as Pfizer's rule of five or simply RO5) for 2-methoxychalcone, which exhibits exceptional binding affinity to the PDB.1C14 protein, are detailed in Tables I and II. These compounds have demonstrated potential as antibacterial drug candidates, as they adhere to Lipinski's rules, including a molecular weight under 500 g/mol, fewer than 5 hydrogen bond donors and fewer than 10 hydrogen bond acceptors. Molecular weight reflects the density, size, volume, and mass of therapeutic agents. Hydrogen bond acceptors and donors in the structures of therapeutic agents play a critical role in membrane transport, drug-protein interactions, distribution, and solubility in air. Lipinski's analysis employs physicochemical properties to predict the drug-like characteristics of oral therapeutic agents. The "rule of five" outlines the relationship between these physicochemical properties and pharmacokinetic indices [25].

Orally active, drug-like compounds should adhere to Lipinski's criteria by not violating more than one of the following: having no more than 5 hydrogen bond donors, no more than 10 hydrogen bond acceptors, a molecular weight under 500 g/mol, and an octhanol-water partition coefficient (log P) not exceeding 5. All selected 2-methoxychalcone derivatives meet these criteria. Docking results with the PDB 1C14 receptor showed the lowest docking scores for the compounds 2-methoxychalcone and its derivatives, 2,4',4-trimethoxychalcone and 2,2',4-trimethoxychalcone. As a result, both compounds were synthesized and evaluated for their antibacterial activity against *Bacillus subtilis* (Gram-positive) and *Escherichia coli* (Gram-negative) bacteria. The molecular docking results revealed a lower docking score for TMC 2 (-13.3859 kcal/mol) compared to TMC 1 (-10.6527 kcal/mol), predicting a greater antibacterial activity for TMC 2 than TMC 1 [25].

The synthesized 2-methoxychalcone and derivatives resulted in needle-shaped yellowish crystals. The thin-layer chromatography results, using various eluents, revealed multiple spots compared to the starting materials. The melting point of 2-methoxychalcone, determined with a Fisher John Melting Point apparatus, was 70-71°C, with a yield of 90%. A difference of 1-2°C in melting points across replications indicates the purity of the synthesized compound. For 2,4-dimethoxychalcone, the melting point was recorded at 73-75°C, with a yield of 91%. Compound 2,2’,4-trimethoxychalcone and 2,4,4’-trimethoxychalcone were obtained, each with yields of 95% and 88%, respectively. Purity testing using TLC with three different eluents resulted in a single spot. The melting point test showed 105-106^{o}C for 2,2’,4-trimethoxychalcone and 100-102^{o}C for 2,4,4’-trimethoxychalcone.

The antibacterial activities of the compounds were evaluated against *Escherichia coli* (Gram-negative) and *Bacillus subtilis* (Gram-positive). Amoxicillin served as the positive control. All compounds exhibited antibacterial activity against *E. coli* and *B subtilis*, but with varying minimum inhibitory concentrations (MIC). The results of in vitro testing indicate that 2,4,4’-Trimethoxychalcone (TMC 2) exhibits greater antibacterial activity against *Bacillus subtilis* (Gram-positive) and *Escherichia coli* (Gram-negative) compared to 2,2’,4-Trimethoxychalcone (TMC 1). This is consistent with the predicted docking results, as TMC

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2 demonstrates a lower docking score, suggesting a more stable interaction with the receptor, thus predicting greater activity. Additionally, the presence of two *para*-methoxy groups on the aromatic ring of TMC 2 facilitates interaction with the receptor (Figure 3 and 4).

6. Conclusion

Compound 2,4,4'-trimethoxychalcone exhibits greater antibacterial activity against *Bacillus subtilis* (Gram-positive) and *Escherichia coli* (Gram-negative) compared to 2,2',4-trimethoxychalcone.

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