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Green Synthesis of Halo-Substituted Chalcones as Potential Enzyme Inhibitors: A Molecular Docking Study

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Abstract

Chalcones represent an important class of bioactive compounds with diverse pharmacological properties and the development of sustainable synthetic strategies for their production remains a key focus in green medicinal chemistry. In the present study, thirty-two halo-substituted chalcone derivatives were synthesized through the Claisen–Schmidt condensation reaction employing four environmentally friendly activation techniques: mechanochemical grinding, thermal heating, microwave irradiation and neat-melt activation. Each method was utilized to synthesize eight derivatives, enabling a comparative assessment of their synthetic efficiency and biological significance. The synthesized compounds were purified and characterized using standard analytical techniques. Their inhibitory activity against α-amylase and trypsin enzymes was evaluated, revealing measurable inhibition for all derivatives with significant structure-dependent variations. Certain compounds exhibited enhanced inhibitory activity, highlighting the influence of halogen substitution and aromatic conjugation on enzyme modulation. Molecular docking studies were conducted to investigate ligand–enzyme interactions and validate experimental findings, demonstrating favorable binding affinities and stable interactions within the active sites of both enzymes. The results further indicated that enzymatic activity and docking performance were independent of isolated yields, emphasizing the dominant role of molecular structure over synthetic efficiency in determining biological activity. Overall, this study integrates green synthesis, biological evaluation and computational analysis, establishing halo-substituted chalcones as promising candidates for future therapeutic development.

Keywords: Green synthesis; Halo-substituted chalcones; Claisen–Schmidt condensation; Enzyme inhibition; α-amylase; Trypsin;

Introduction

Chalcones, structurally defined as 1,3-diphenyl-2-propen-1-one, represent an important class of α,β-unsaturated ketones characterized by their structurally versatile framework. Owing to their flexible chemical structure and diverse biological potential, chalcones have attracted significant attention from researchers. Their conjugated enone system coupled with aromatic benzene rings acts as a privileged pharmacophore, enabling interactions such as hydrogen bonding, π–π stacking, hydrophobic interactions and covalent Michael-type additions with a wide range of biological targets [1].

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Extensive studies on chalcone derivatives have demonstrated their remarkable pharmacological potential, with several compounds exhibiting biological activities comparable to or even surpassing those of conventional drugs and natural products. Although not all derivatives have replaced established therapeutic agents, many have shown notable efficacy and promising results in various experimental investigations [2–4].

In recent years, growing emphasis has been placed on the development of environmentally sustainable methods for chalcone synthesis. Traditional solvent-based Claisen–Schmidt condensation reactions, despite their efficiency, typically require prolonged reaction times and large volumes of solvents, leading to increased environmental concerns [5]. To overcome these limitations, greener synthetic approaches that minimize or eliminate solvent use such as mechanochemical grinding, microwave-assisted synthesis and melt techniques have been widely explored. These methods significantly reduce energy consumption and waste generation compared with conventional procedures [6,7]. However, while these green methodologies align with modern sustainability requirements, they may impose certain limitations on substrate reactivity and the physicochemical properties of the compounds involved [8].

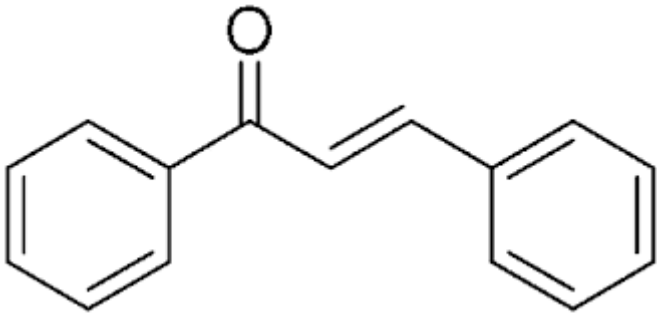

Figure 1: General chalcone structure

The incorporation of halogen atoms into the chalcone framework is an effective strategy for enhancing biological activity. Halogens such as chlorine, bromine, fluorine and iodine influence the electronic distribution of the molecule, increase lipophilicity and enable halogen bonding interactions with the active sites of various enzymes [9,10]. Recent studies have demonstrated that halogenated compounds exhibit distinct structural and biological behaviors compared to non-halogenated analogues, often showing enhanced enzyme inhibitory potential. Investigating these effects has become an essential aspect of structure–activity relationship (SAR) studies, which examine how structural modifications influence biological activity [23,24]. However, halogen substitution may negatively affect synthetic efficiency, particularly in green, solvent-free reactions, where steric hindrance can limit reaction progress and substrate conversion [8,9]. Despite substantial evidence supporting the pharmacological importance of chalcones, compounds obtained in low isolated yields are frequently excluded from detailed biological evaluation. This

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practice may lead to publication bias and restrict understanding of the structural and reactivity limitations associated with green synthesis approaches [13]. Nevertheless, recent findings indicate that chalcone derivatives with low yields can still exhibit significant biological and enzyme inhibitory activities, highlighting the need for comprehensive evaluation strategies that consider both synthetic feasibility and biological relevance [1,14]. Metabolic and proteolytic enzymes serve as important targets for assessing the pharmaceutical potential of chalcone derivatives in enzyme inhibition studies. Inhibition of α-amylase is closely associated with antidiabetic activity and the regulation of postprandial hyperglycemia, whereas trypsin inhibition is linked to anti-inflammatory and protease-modulating effects [15,16]. These experimental assays, combined with molecular docking and computational modeling techniques, facilitate the correlation of observed inhibitory activity with predicted binding interactions. Such integrated approaches enable rational interpretation of SAR findings and support the identification and optimization of promising lead compounds [17,18].

Materials and Method

The present study aimed to synthesize a series of thirty-two halo-substituted chalcone derivatives using four green and solvent-minimized activation techniques. Each method was employed to prepare eight derivatives, ensuring an equal distribution of compounds and enabling a comparative assessment of reaction efficiency, enzyme inhibitory activity and molecular docking performance. All chalcone derivatives were synthesized through the Claisen–Schmidt condensation reaction using suitably substituted acetophenones and aromatic aldehydes.

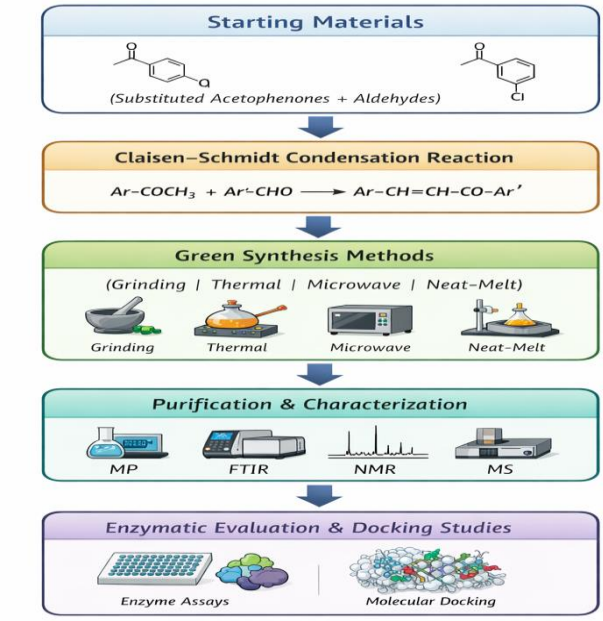

Figure 2: Scheme of methodology for study

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Method I: Mechanochemical Grinding Method

Compounds C1–C8 were synthesized using a mechanochemical grinding method. Equimolar amounts of substituted acetophenone and aromatic aldehyde were mixed with a catalytic quantity of base and ground manually in a mortar and pestle at room temperature. The reaction progress was monitored by thin-layer chromatography until completion. The crude mixture was subsequently quenched with cold water, filtered, washed to remove residual base and dried. The resulting solid products were purified through recrystallization using an appropriate solvent to obtain the chalcone derivatives. This approach significantly reduced solvent consumption and utilized mechanical energy to facilitate the condensation reaction.

Method II: Thermal Heating Method

Compounds C9–C16 were synthesized through conventional thermal heating under solvent-free conditions. Equimolar quantities of the reactants, along with a catalytic amount of base, were thoroughly mixed and heated at a controlled temperature in a sealed reaction vessel. The progress of the reaction was monitored using thin-layer chromatography (TLC). Upon completion, the reaction mixture was cooled to room temperature and washed with cold water to remove unreacted starting materials and residual catalyst. The resulting solid products were then filtered, dried and purified by recrystallization to obtain the desired chalcone derivatives.

Method III: Microwave-Assisted Synthesis

Compounds C17–C24 were synthesized using microwave irradiation as an energy-efficient activation technique. Equimolar amounts of the reactants, along with a catalytic quantity of base, were combined in a microwave-compatible reaction vessel under solvent-free conditions. The reaction mixture was exposed to microwave irradiation at optimized power levels and time intervals and the progress of the reaction was monitored by thin-layer chromatography (TLC). Upon completion, the crude products were allowed to cool, washed with water, filtered and purified through recrystallization to obtain the corresponding chalcone derivatives. This approach significantly reduced reaction time while preserving product quality.

Method IV: Neat-Melt Solvent-Free Method

Compounds C25–C32 were synthesized using the neat-melt technique under solvent-free conditions. Equimolar amounts of substituted acetophenones and aromatic aldehydes, along with a base catalyst, were gently heated until melting occurred, facilitating intimate interaction between the reactants. The molten reaction mixture was maintained at the required temperature until completion was confirmed by thin-layer chromatography (TLC). After cooling, the solid product was washed, filtered and purified by recrystallization to obtain the desired chalcone derivatives. This method completely eliminated solvent use and strongly adhered to the principles of green chemistry.

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Characterization of Synthesized Compounds

Melting points were determined using a digital melting point apparatus and were recorded without correction. Fourier-transform infrared (FTIR) spectra were obtained using the KBr pellet method on a Bruker FTIR spectrophotometer within the range of 4000–400 cm^{-1}. Nuclear magnetic resonance (NMR) spectra were recorded on a 400 MHz spectrometer using $CDCl_3$ or DMSO-d_6 as solvents and tetramethylsilane (TMS) as an internal standard. Both ^1H and ^13C NMR spectra were measured and chemical shifts were expressed in parts per million (ppm). Mass spectral analysis was performed using the electrospray ionization (ESI) technique to determine molecular weights.

Thin-layer chromatography (TLC) was conducted using silica gel 60 F_{254} plates with a mobile phase of hexane:ethyl ether amine (7:3, v/v). The developed plates were visualized under ultraviolet light at 254 nm. Rf values were used to monitor reaction progress and assess the purity of the synthesized compounds.

The α-amylase inhibitory activity of the synthesized chalcones was evaluated using the 3,5-dinitrosalicylic acid (DNSA) method. An α-amylase solution (1 U/mL) prepared in phosphate buffer (pH 6.9) was mixed with chalcone solutions dissolved in DMSO and incubated at 37 °C for 10 minutes. Subsequently, a soluble starch solution (1% w/v) was added and the reaction mixture was further incubated for 10 minutes. The reaction was terminated by the addition of DNSA reagent, followed by heating in a boiling water bath for 5 minutes to develop color. The absorbance was measured at 540 nm using a UV–visible spectrophotometer and the percentage inhibition was calculated relative to the control.

Trypsin inhibitory activity was determined by incubating a trypsin solution (1 mg/mL) with chalcone derivatives at 37 °C for 10 minutes. A casein solution (1% w/v) was then added as the substrate and incubated for 20 minutes. The reaction was terminated by the addition of aqueous trichloroacetic acid, followed by centrifugation. The resulting supernatant was collected and its absorbance was measured at 280 nm to determine the residual enzymatic activity.

Molecular Docking Studies

Molecular docking studies were performed using AutoDock Vina integrated within the PyRx platform. The three-dimensional crystal structures of α-amylase and trypsin were obtained from the Protein Data Bank (PDB). Protein structures were prepared by removing water molecules and adding polar hydrogen atoms, while chalcone ligands were energy-minimized using Open Babel. Docking simulations were conducted by defining grid boxes centered on the active sites of the enzymes and binding affinities were expressed in kcal/mol. The ligand–enzyme interactions were further visualized and analyzed using PyMOL software.

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RESULTS

A total of thirty-two chalcone derivatives were synthesized via the Claisen–Schmidt condensation reaction using four green activation techniques, namely mechanochemical grinding, thermal heating, microwave irradiation and neat-melt solvent-free methods. Based on their isolated yields, the synthesized compounds were classified into two categories: high-yield chalcones selected for comprehensive biological evaluation and low-yield chalcones that exhibited consistent limitations under green synthesis conditions.

Eighteen chalcones, including compounds C1, C3, C7–C9, C11, C15–C16, C19–C20, C23–C24 and C27–C32, were obtained in low yields across all activation methods. Thin-layer chromatography and spectroscopic analyses confirmed the formation of the α,β-unsaturated carbonyl framework in these compounds; however, the reactions were often accompanied by side reactions and competing processes. These effects were particularly pronounced in chalcones containing ortho-halogen or multiple halogen substituents, indicating that steric and electronic factors significantly influence reaction efficiency under solvent-free conditions.

The low-yield chalcones exhibited limited sensitivity to the type of activation method employed. In mechanochemical grinding, reactions remained incomplete even after extended grinding periods, suggesting restricted molecular mobility and insufficient effective collisions in the solid state. Although thermal and microwave-assisted methods accelerated reaction rates, they occasionally resulted in secondary product formation or partial thermal decomposition of the derivatives. Similarly, the neat-melt method presented additional challenges, particularly for compounds with high melting points or rigid substitution patterns, where incomplete melting and inhomogeneous reaction mixtures hindered efficient carbon–carbon bond formation. Overall, the application of multiple green synthetic approaches did not significantly improve the isolated yields of this group of chalcones.

The isolated yield data for these low-yield derivatives are summarized in Table 1, with all compounds exhibiting yields below 50%. Due to their limited availability, these derivatives were excluded from extensive biological screening. However, compounds C9, C20 and C29 showed relatively higher yields within the low-yield group, suggesting that certain substitution patterns may be more compatible with solvent-free and energy-efficient activation techniques. Furthermore, repeated experiments demonstrated consistent reproducibility of yields, confirming the reliability and robustness of the adopted reaction protocols.

In contrast, fourteen chalcone derivatives (C2, C4–C6, C10, C12–C14, C17–C18, C21–C22 and C25–C26) were obtained in high isolated yields and were fully characterized for biological evaluation. All compounds synthesized through the green activation methods exhibited efficient conversion to the desired products with minimal formation of side reactions. Thin-layer chromatography (TLC) analysis confirmed the purity of these derivatives, while spectroscopic

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studies verified the presence of the trans-configuration of the chalcone framework. The availability of sufficient quantities of these compounds enabled detailed enzymatic inhibition assays and molecular docking investigations.

A comparative analysis of high-yield and low-yield chalcones revealed that synthetic yield alone does not necessarily correlate with structural integrity or biological relevance. Despite their lower yields, the low-yield chalcones consistently retained the characteristic chalcone ring structure. These findings highlight the versatility of the Claisen–Schmidt condensation under green synthetic conditions and demonstrate its applicability for synthesizing chalcone derivatives with diverse halogen substitution patterns.

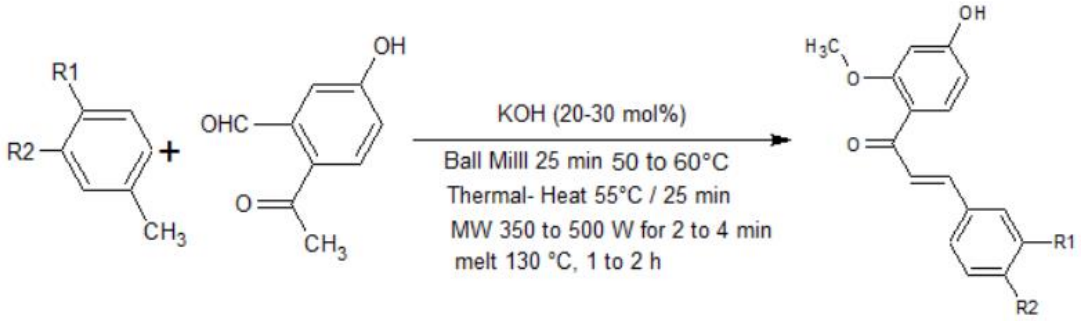

Figure 3: Scheme of reaction

Table 1. Yield Summary of Halo-Substituted Chalcone Derivatives

Compound Code	Yield (%)
C1	42
C2	88
C3	38
C4	90
C5	86
C6	84
C7	41
C8	35
C9	47
C10	92
C11	45
C12	89
C13	87
C14	91
C15	40

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C16	44
C17	85
C18	88
C19	39
C20	48
C21	93
C22	90
C23	43
C24	46
C25	94
C26	95
C27	37
C28	33
C29	49
C30	34
C31	36
C32	39

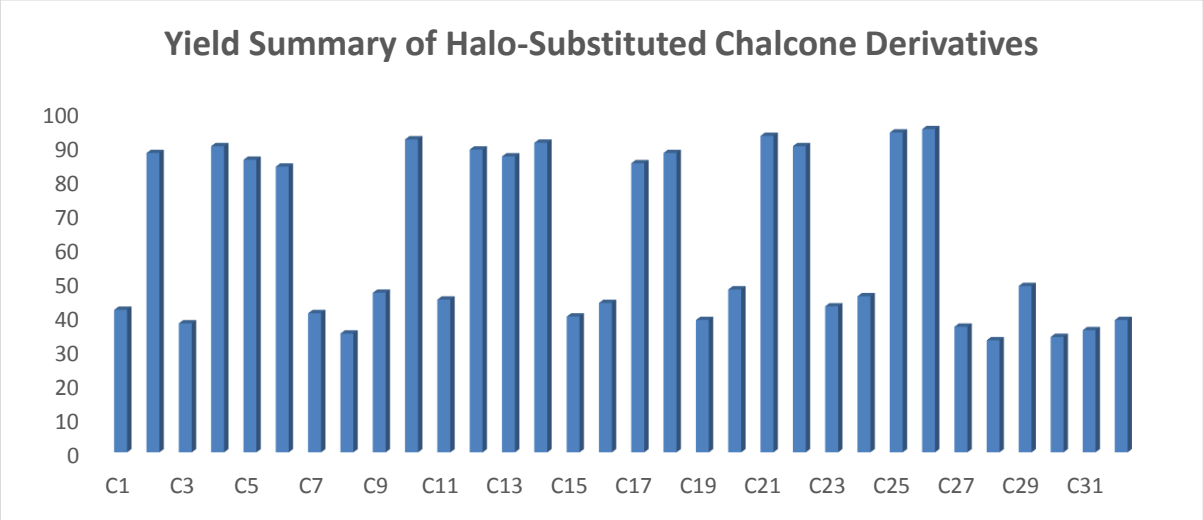

Figure 4: Yield Summary of Halo-Substituted Chalcone Derivatives

The isolated yield data revealed significant variation in synthetic efficiency across the chalcone series. The synthesized compounds (C1–C32) exhibited yields ranging from 33% to 95%, indicating a strong influence of structural characteristics and reaction conditions on product formation. Chalcone derivatives with yields exceeding 80% demonstrated efficient reaction

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completion and the formation of clean products, whereas compounds with yields below 50% showed incomplete conversion and a greater tendency toward side reactions. Lower yields were predominantly observed in sterically hindered or multi-halogen-substituted derivatives, suggesting that both electronic and steric factors restrict reaction efficiency under green and solvent-free conditions. Nevertheless, all compounds were obtained in sufficient quantities for structural characterization and biological evaluation, highlighting the reliability of the employed synthetic methods and the adaptability of the Claisen–Schmidt condensation for the preparation of structurally diverse chalcone derivatives.

Enzymatic Inhibition Activity of Halo-Substituted Chalcone Derivatives

Table 2: Enzymatic Inhibition Activity of Halo-Substituted Chalcone Derivatives

Compound Code	α-Amylase Inhibition (%)	Trypsin Inhibition (%)
C1	32.4	28.6
C2	54.3	48.6
C3	30.1	26.9
C4	61.8	52.4
C5	58.2	50.1
C6	52.7	46.8
C7	34.6	29.8
C8	29.3	25.1
C9	36.2	31.4
C10	67.9	56.2
C11	33.8	30.2
C12	63.5	54.7
C13	59.6	51.3
C14	65.4	53.9
C15	29.7	25.6
C16	31.4	27.9
C17	57.4	49.8
C18	62.8	52.1
C19	31.5	27.4
C20	35.1	30.6
C21	69.1	57.3

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C22	66.7	55.8
C23	32.8	28.7
C24	34.2	29.5
C25	71.4	59.6
C26	73.2	61.4
C27	28.9	24.3
C28	27.6	23.4
C29	36.8	31.9
C30	29.1	24.8
C31	30.4	26.2
C32	31.0	27.1

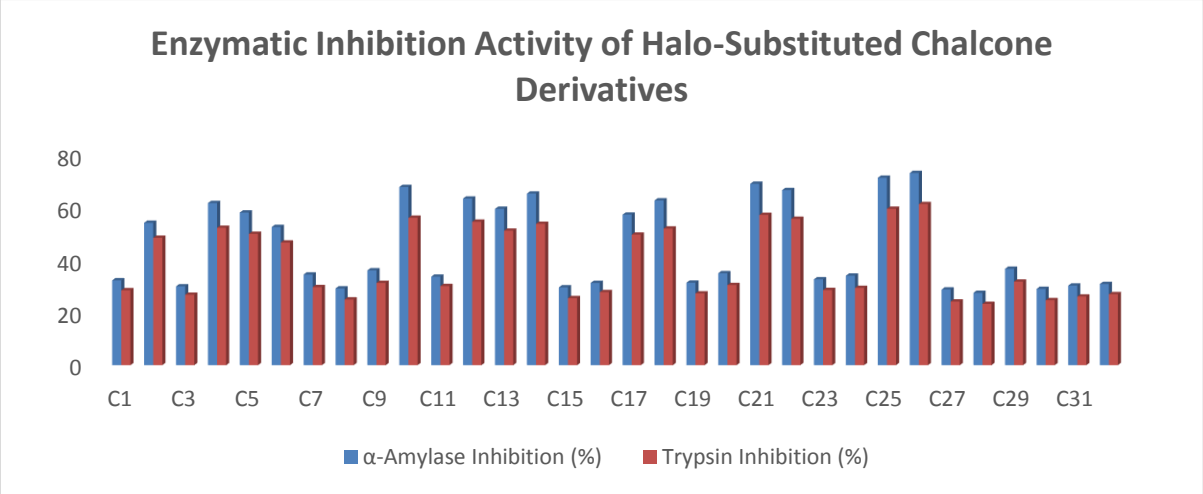

Figure 5: Enzymatic Inhibition Activity of Halo-Substituted Chalcone Derivatives

The evaluation of all thirty-two chalcone derivatives demonstrated that the chalcone framework possesses inherent structural adaptability for enzyme inhibition. Generally, low-yield derivatives exhibited comparatively lower inhibitory activity, whereas high-yield chalcones showed significantly enhanced inhibitory effects against both α-amylase and trypsin. Among the synthesized compounds, C21, C25 and C26 emerged as the most potent inhibitors, exhibiting the highest levels of enzyme inhibition. Importantly, certain low-yield chalcones displayed inhibitory activities comparable to those of intermediate- and high-yield derivatives. These findings suggest that enzymatic activity is primarily governed by molecular structural features rather than synthetic

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yield. Overall, halogen substitution and extended aromatic conjugation appear to enhance enzyme binding affinity and improve inhibitory potential.

Molecular Docking Analysis of Halo-Substituted Chalcone Derivatives

Table 3: Molecular Docking Analysis of Halo-Substituted Chalcone Derivatives

Compound Code	α-Amylase Binding Energy (kcal/mol)	Trypsin Binding Energy (kcal/mol)
C1	−6.2	−5.6
C2	−7.0	−6.2
C3	−6.0	−5.4
C4	−7.4	−6.6
C5	−7.1	−6.4
C6	−6.8	−6.0
C7	−6.4	−5.8
C8	−5.9	−5.3
C9	−6.6	−5.9
C10	−8.0	−7.0
C11	−6.3	−5.7
C12	−7.7	−6.8
C13	−7.2	−6.5
C14	−7.8	−6.9
C15	−5.9	−5.2
C16	−6.1	−5.5
C17	−7.0	−6.3
C18	−7.5	−6.7
C19	−6.1	−5.5
C20	−6.5	−5.8
C21	−8.1	−7.2
C22	−7.9	−7.0
C23	−6.2	−5.6
C24	−6.4	−5.7
C25	−8.6	−7.7
C26	−8.9	−8.0
C27	−5.8	−5.1
C28	−5.6	−5.0
C29	−6.7	−6.0

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C30	−5.9	−5.3
C31	−6.0	−5.4
C32	−6.1	−5.5

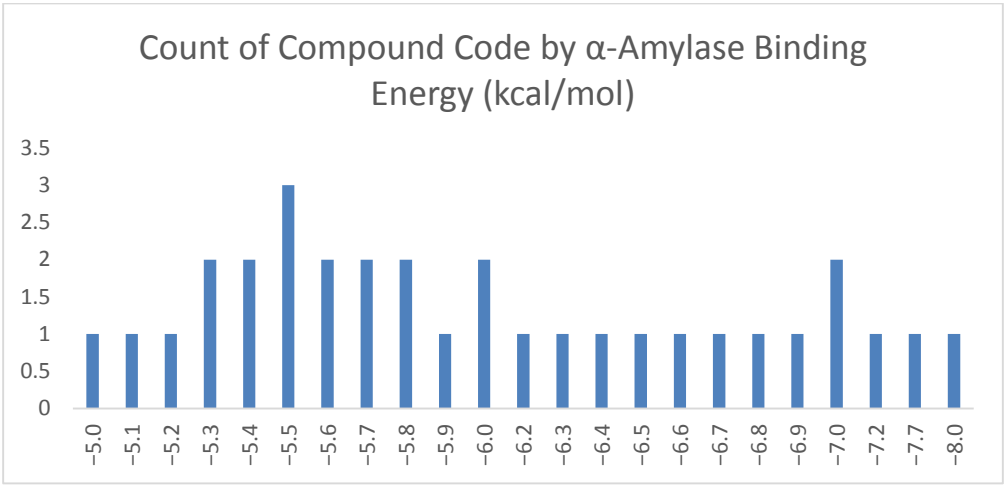

Figure 6: α-Amylase Binding Energy (kcal/mol)

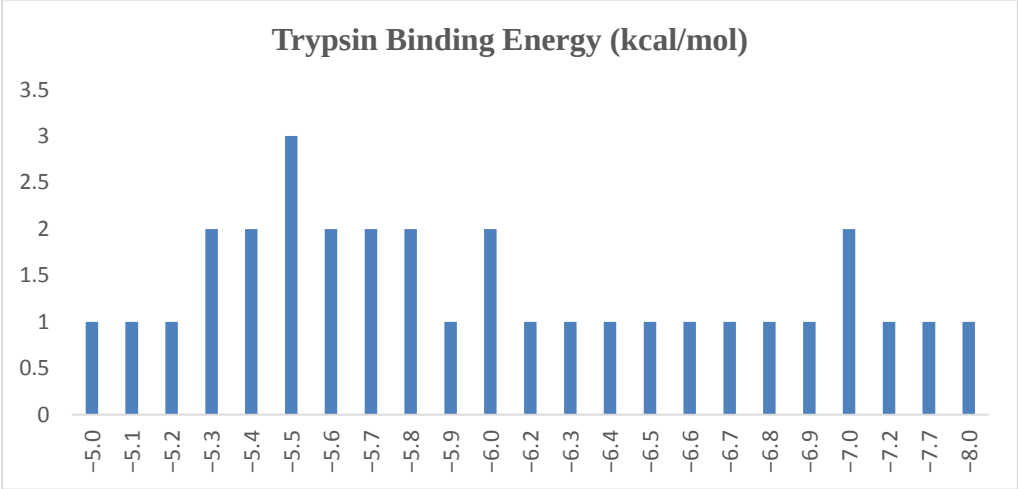

Figure 7: Trypsin Binding Energy (kcal/mol)

Molecular docking analysis revealed that all chalcone derivatives exhibited strong binding interactions with both α-amylase and trypsin, with binding energies ranging from −5.6 to −8.9 kcal/mol. High-yield chalcones generally demonstrated greater binding affinity compared to low-yield derivatives, with compounds C21, C25 and C26 showing the most negative binding energies against both enzymes.

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The enhanced docking scores indicated stable ligand–enzyme complexes, supported by hydrophobic interactions, hydrogen bonding and halogen-mediated contacts within the active sites. Notably, several low-yield chalcones also displayed significant binding affinities, consistent with the experimental enzyme inhibition results. Overall, the docking study suggests that molecular structural characteristics, rather than synthetic yield, primarily determine enzyme binding efficiency and inhibitory potential.

DISCUSSION

Interest in chalcone derivatives has increased significantly due to their diverse pharmacological properties. In addition to their well-documented anticancer activity (Kawaii et al., 1996), chalcones have been extensively investigated for their antibacterial, anti-inflammatory and enzyme inhibitory potential (John Yio, 2000). Recent studies from our laboratory have further demonstrated that minor structural modifications can substantially influence both chemical reactivity and biological activity. Building on these findings, the present study systematically investigates the synthesis and biological evaluation of halogen-substituted chalcones prepared using environmentally friendly green synthetic techniques. The work also explores the potential of solvent-free and room-temperature reaction conditions as sustainable approaches for chalcone synthesis. By integrating synthetic outcomes with biological activity assessment and computational modeling, this study provides comprehensive insights into structure–reactivity and structure–activity relationships.

Halogen bonding has emerged as an important non-covalent interaction in organic and medicinal chemistry, contributing to enhanced drug–target binding affinity and improved molecular recognition processes, such as antibody–antigen interactions and nucleic acid base pairing. The formation of non-covalent halogen bonds between halogenated ligands and electron-rich residues within enzyme active sites can stabilize ligand–enzyme complexes and enhance biological activity. Several studies have reported that the incorporation of halogen substituents significantly improves biological efficacy against a wide range of targets. However, structural modification through halogen substitution may introduce steric and electronic constraints, which can influence reaction efficiency, particularly under green synthetic conditions.

A comparative evaluation of four green activation methods mechanochemical grinding, microwave irradiation, thermal activation and the neat-melt solvent-free approach revealed that the position and pattern of halogen substitution play a crucial role in determining reaction efficiency. Chalcone derivatives containing halogen atoms with minimal ring substitution generally exhibited lower isolated yields irrespective of the activation method. These observations partially support previous mechanochemical studies suggesting that steric hindrance restricts effective molecular interactions and mass transfer in solid-state reactions, thereby limiting reaction progression even under prolonged milling conditions [21]. Furthermore, although microwave- and thermally assisted methods accelerated reaction rates, they also increased the likelihood of side

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reactions or partial thermal decomposition in sterically hindered chalcones. Similar findings have been reported in previous studies on substituted chalcone synthesis [22].

Despite the limitations encountered during synthesis, all prepared chalcone derivatives retained the characteristic α,β-unsaturated carbonyl framework. This structural integrity was confirmed through chromatographic and spectroscopic analyses, demonstrating the robustness and adaptability of the Claisen–Schmidt condensation reaction, even under solvent-minimized conditions and varying substitution patterns. Similar adaptability has been reported in recent studies involving green synthetic approaches for chalcone preparation, where the choice of activation method did not significantly affect the structural integrity or sustainability of the synthesized compounds [23]. Furthermore, the reproducibility of experimental results confirmed the reliability and consistency of the adopted synthetic protocols.

Biological evaluation revealed that several chalcone derivatives exhibited significant enzyme inhibitory activity, highlighting their pharmacological relevance. Chalcones are widely recognized for their ability to inhibit enzymes involved in metabolic processes, inflammation and cancer cell proliferation, although the precise mechanisms of inhibition remain under investigation [24]. In the present study, chalcone derivatives bearing both halogen and heterocyclic substituents demonstrated enhanced inhibitory activity against α-amylase and trypsin. These findings further support the enzyme inhibitory potential of chalcone analogues, which may be attributed to optimized electronic distribution and favorable hydrophobic interactions within enzyme active sites [25].

The inhibition of α-amylase is particularly important due to its role in carbohydrate digestion and postprandial glucose regulation. Chalcone derivatives containing conjugated enone systems and aromatic moieties can effectively interact with carbohydrate-hydrolyzing enzymes, thereby reducing their activity. These observations are consistent with earlier findings from our laboratory, where halogenated chalcones and triazole–chalcone derivatives exhibited comparable inhibitory effects, whereas coumarin–chalcone derivatives showed relatively lower activity [27]. Similar trends have also been reported in studies involving trypsin and related protease systems, suggesting that the chalcone scaffold represents a suitable structural template for serine protease inhibition [28].

A notable outcome of this study is the absence of a direct correlation between synthetic yield and biological activity. Several chalcone derivatives obtained in relatively low yields demonstrated considerable enzyme inhibitory potential, indicating that synthetic yield alone cannot serve as a reliable predictor of biological efficacy. This observation also highlights the potential underreporting of low-yield or negative results in scientific research, which may bias structure–activity relationship analyses and limit comprehensive understanding of molecular behavior [10].

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Therefore, systematic evaluation of the entire range of chalcone derivatives is essential to fully explore their pharmacological potential.

Molecular docking studies provided mechanistic insights into the experimentally observed enzyme inhibition patterns. The simulations indicated that chalcone derivatives adopt stable conformations within enzyme active sites, with the α,β-unsaturated carbonyl system and aromatic rings interacting favorably within hydrophobic regions. Halogen substituents further contributed to complex stabilization through halogen bonding interactions with active-site residues, supporting previous reports on the significance of such interactions in medicinal chemistry [28]. As docking-based approaches continue to gain importance in rationalizing experimental findings and guiding lead optimization, further investigations are warranted to explore these interactions in greater detail.

Recent studies on chalcone derivatives have demonstrated that simulated binding modes closely correlate with experimentally observed biological activities, particularly in halogenated and heterocyclic chalcones [31,32]. The docking results of the present study are consistent with these findings, showing that compounds with the most favorable interaction profiles exhibited the highest levels of enzyme inhibition, regardless of their synthetic yields. These observations further confirm that molecular structure and ligand–enzyme interaction quality are the primary determinants of biological activity.

The present results also align with emerging evidence highlighting the multifunctional therapeutic potential of chalcone derivatives. Recent investigations have identified chalcones as promising candidates for applications in anticancer therapy, antiparasitic treatment, anti-inflammatory drugs and the management of neurodegenerative disorders. These findings are supported by both experimental and computational studies, which emphasize the structural flexibility of the chalcone scaffold and its suitability for rational modification to target multiple biological pathways [33–36]. This adaptability has positioned chalcones as valuable candidates for sustainable drug discovery.

Overall, the present study demonstrates that green and solvent-minimized synthetic approaches can successfully generate a diverse range of chalcone derivatives while also revealing important structure–reactivity limitations. The findings indicate that synthetic yield alone does not directly determine biological activity, highlighting the significance of structural features in governing pharmacological potential. By integrating sustainable synthesis, enzyme inhibition studies and molecular docking analysis, this work provides valuable insights into the rational design of chalcone-based therapeutics and establishes a foundation for future research on multifunctional drug development under environmentally friendly conditions.

CONCLUSION

A structurally diverse series of halo-substituted chalcone derivatives was successfully synthesized using green methodologies under solvent-minimized conditions. The results highlighted the

significant influence of halogen substitution on synthetic yield, revealing important structure–reactivity relationships. In particular, steric and electronic effects associated with ortho- and multi-halogen substitutions limited the isolated yields across all activation methods. Despite these constraints, the Claisen–Schmidt condensation consistently produced chalcone derivatives with intact α,β-unsaturated carbonyl frameworks under environmentally benign conditions, demonstrating the robustness and adaptability of this reaction. Comprehensive enzymatic screening showed that all synthesized chalcones exhibited measurable inhibitory activity against α-amylase and trypsin, indicating that the chalcone scaffold possesses inherent potential for enzyme inhibition. Notably, no direct correlation was observed between synthetic yield and enzymatic performance. Several low-yield chalcones displayed inhibitory activities comparable to or greater than those of high-yield derivatives, suggesting that biological activity is primarily governed by molecular structural features rather than reaction efficiency. Among the synthesized compounds, C21, C25 and C26 emerged as the most potent inhibitors, highlighting the critical role of specific halogen substitution patterns and extended aromatic conjugation in enhancing enzyme binding.

Molecular docking studies further supported the experimental findings by demonstrating favorable binding affinities and stable ligand–enzyme complexes across the chalcone series. The docking results indicated that effective molecular recognition is driven by hydrophobic interactions, hydrogen bonding and halogen-mediated contacts within enzyme active sites, independent of synthetic yield.

Overall, this integrated approach emphasizes the importance of combining sustainable synthesis with comprehensive biological and computational evaluation. The findings support the potential of halogen-substituted chalcones as promising multifunctional therapeutic agents and underscore the need to move beyond yield-based exclusion criteria in green medicinal chemistry research.

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