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Hidden Boundaries of Green Chalcone Synthesis: A Structure-Reactivity Study of Low-Yield Halo-Substituted Derivatives

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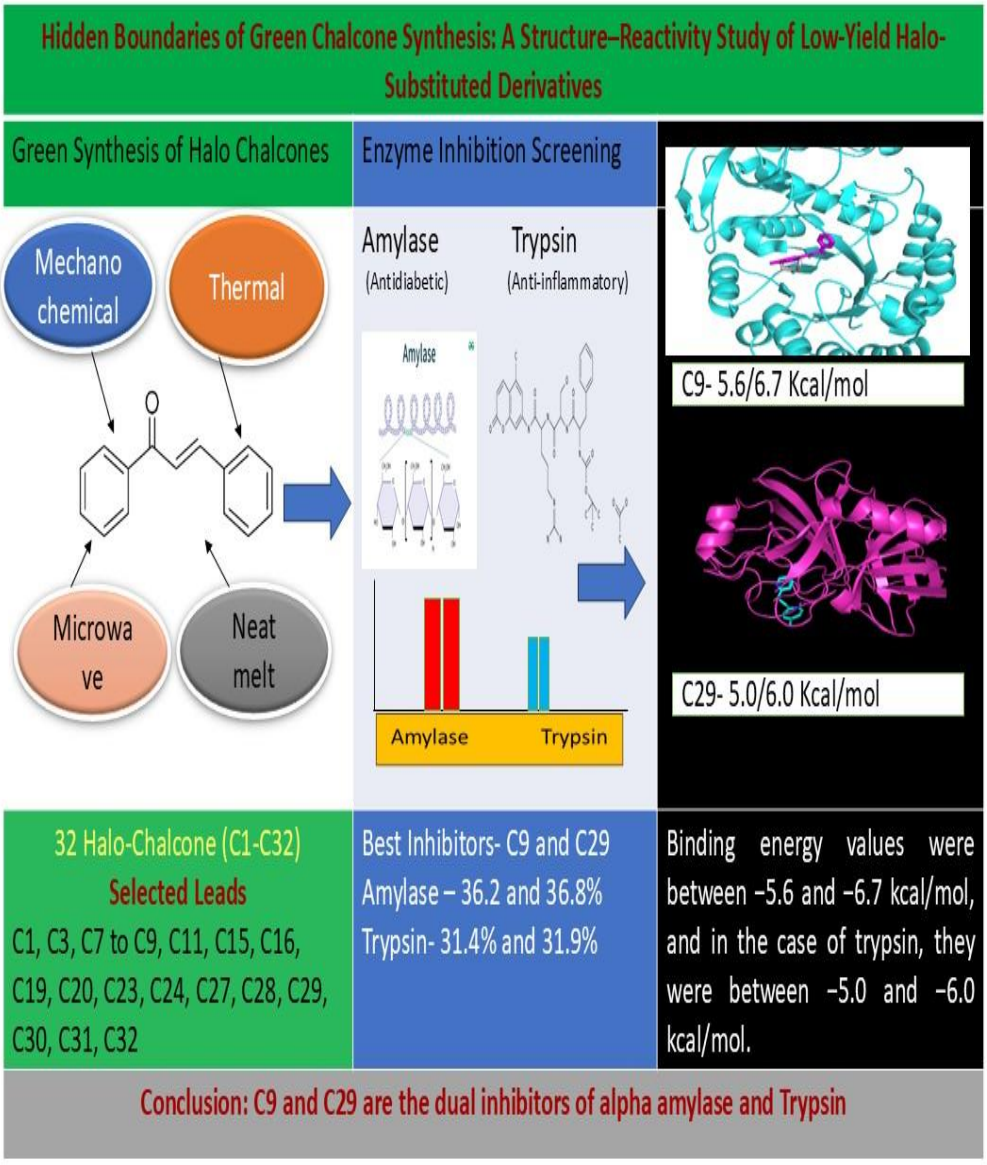
Graphical Abstract:



Abstract
Chalcones are privileged α, β-unsaturated ketone scaffolds possessing a wide range of pharmacological activities. In the present study, eighteen halo-substituted chalcone derivatives synthesized using green and solvent-minimized methodologies were systematically screened for enzymatic inhibitory potential and theoretical binding affinity despite their earlier classification as low-yield derivatives. All compounds were subjected to in-vitro enzymatic screening against α-amylase and trypsin enzymes while further assessing their binding behavior through molecular docking. Enzymatic assays resulted in uniform inhibitory activity across the compound library: inhibition of α-amylase in a range of 27.6–36.8% and trypsin in a range of 23.4–31.9%. Of note, the C9, C20, and C29 compounds exhibited the highest enzymatic inhibition activities and also the best docking affinities towards both enzymes. Molecular docking confirmed the binding energies were quite favorable between -5.6 to -6.7 kcal/mol with α-amylase and -5.0 to -6.0 kcal/mol with trypsin. Binding was driven by hydrophobic interaction, π–π stacking, and halogen-mediated interactions within enzyme active sites. These findings prove that a low synthetic yield does not compromise biological relevance and therefore delineates the need for inclusive biological screening in medicinal chemistry. The present study establishes halo-substituted chalcones as promising scaffolds for further structure–activity optimization under sustainable conditions of synthesis.
Keywords: Chalcones; Halo-substituted derivatives; Green synthesis; α-Amylase inhibition; Trypsin inhibition; Molecular docking; Enzyme inhibition; Halogen bonding; Structure-activity relationship; Sustainable chemistry

Introduction

Chalcones, which have the chemical formula 1,3-diaryl-2-propen-1-ones, are a prominent subtype of α,β-unsaturated ketones that have enjoyed considerable interest in medical chemistry because of their simplicity, flexibility, and versatile pharmacological activity. The α,β-conjugated enone moiety, with its connectivity to two aromatic rings, has played a pivotal role in the chemical reactivity and pharmacological importance of chalcones, enabling them to interact effectively with their target molecules. They have exhibited significant anticancer, antimicrobial, antiviral, anti-inflammatory, antioxidant, antidiabetic, and enzyme inhibition activity, making them a privileged compound platform in drug design and optimization efforts, as reported extensively in the literature (Go et al., 2005; Batovska and Todorova, 2010).

Synthetically, chalcones are preferably made using the Claisen-Schmidt condensation, which is a base-catalyzed aldol reaction between acetophenones and aromatic aldehydes. The Claisen-Schmidt condensation is an attractive reaction due to its ease of performance and tolerance of a wide number of functional groups. However, such traditional methods for the Claisen-Schmidt condensation reaction require high temperatures, long reaction times, and the use of organic solvents, which is a concern for the future of environmental sustainability. The need for environmental sustainability has led to an ever-expanding focus in modem synthesis on the principles of green chemistry, including the minimization of solvent usage, lower energy usage, higher atom economy, and greater waste minimization (Anastas and Warner, 1998).

Within these novel techniques, solvent-free and energy-saving techniques, such as mechanochemical grinding, microwave-assisted synthesis, ultrasonic irradiation, and neat melt techniques, have attracted considerable attention. Mechanochemical synthesis has been identified to be a versatile technique in inducing carbon-carbon bond-forming reactions in a solvent-free fashion. The use of mechanical forces in grinding or milling has been recognized to increase the interaction among molecules and even promote reactions that are not feasible in solution (Bolm & Hernández, 2018). Several research papers have revealed that mechanochemical techniques can be efficiently used in Claisen-Schmidt condensations with the successful synthesis of chalcones in shorter intervals and with better environment-friendly profiles than normal techniques (Hernández & Bolm, 2017).

Microwave-assisted organic synthesis is another environmentally friendly method being used extensively in chalcone synthesis. The microwave irradiation allows volumetric heating due to dipolar polarization and ionic conduction, which often facilitates faster reaction rates and better yields. There are several instances in the literature regarding efficient microwave-assisted synthesis of chalcones in high yields and short reaction times reduced to minutes from hours (Kappe, 2004). Neat melt solvent-free methods are other efficient methods that utilize the melting point properties of reactants to produce a brief period of a homogeneous medium devoid of any external solvent (Lidström et al., 2001).

However, the effectiveness of these green technologies is largely dependent on the substrate structure and physicochemical properties. The efficiency of a chemical reaction under solvent-free conditions can be affected by the melting point of the substrate, the diffusion rate in the solid state, steric crowding, and the substituent effect on the substrate molecule. This implies that not all chalcone derivatives react equally well when different activation methods are employed under green technologies.

Chalcone's aromatic ring substitution by halogens has been widely investigated due to the significant impact of this substitution on biological activity. The halogens fluorine, chlorine, or bromine are known to alter the electronic distribution significantly due to strong inductive effects, enhance lipophilicity, and, importantly, provide specific non-covalent interaction capabilities such as halogen bonding. An increased ability to bind to the enzyme, improved stability, and most importantly, improved biological activity are potential advantages of such substitution (Wilcken et al., 2013). It has been known that halo-substituted chalcones are often targeted in medicinal chemistry projects and SARs (Nowakowska, 2007).

Nevertheless, halogen substitution can also have dual effects in facilitating synthetic reactivity. Highly electron-withdrawing halogen atoms can lower the nucleophilicity of the enolate intermediate necessary for an aldol condensation reaction. On the other hand, an _ortho_-halogen effect can cause steric hindrances in optimizing the orientation of reactive sites. Excessive halogen substitutions can cause additional problems with enhancing molecular rigidity as a consequence of increased steric congestion or solubility problems in neat melt reactions as a solvent assistance in mechanochemical reactions is not present (Bandgar et al., 2010).

Although many scientific publications have found high-yielding halo-substituted chalcones with promising biological activities, only a limited number of studies focus on the compounds

that have poor synthetic capabilities. Those with poor yields are usually ignored or not considered in publications, thereby creating unnecessary gaps in understanding the potential range of the reaction. This selection or publication bias towards positive outcomes has been increasingly recognized as the cause or contributor to the lack of reproducibility or reasons poor planning in reactions or syntheses (Fanelli, 2012). In the world of chalcone compounds, the lack of reporting on poor-yielding results holds important details on unfavorable substitution.

From a medicinal and process chemistry point of view, lack of synthetic yield is not only a limitation in practice, it actually represents a key clue regarding underlying S/R patterns in compounds. Compounds with high S/R difficulties could potentially cause issues in various stages, independent of their biological activity. Hence, knowledge and assessment of potential limitations in synthetic yields at an early point are crucial in realizing a balance between biological optimization and synthesis feasibility (Sheldon, 2016). There has been increasing support in recent years for the open publication of results that are negative or suboptimal in the laboratory, especially in the field of green and sustainable chemistry. In this way, it becomes possible to plot the actual boundary limits around the method and improve experimental designs for the next attempt (Zhu 2013).

The philosophy can then be extended to chalcones to chart the effect of electronic and steric factors surrounding the success of the synthesis under environmentally benign conditions. The current work is planned as a supplementary study, targeting precisely these eighteen halo-substituted chalcone compounds, which showed poor isolated yield during green synthesis. All these compounds were synthesized by employing mechanochemical grinding, thermal condensation, microwave irradiation, and neat melt solvent-free techniques. Instead of considering these compounds as failures, the aim here is to gain valuable information by linking poor isolated yield to structural patterns, electron-deficiency factors, as well as technological limitations.

Thin-layer chromatography analysis, completion of reactions, and isolated yield information are carefully scrutinized to reveal structural patterns that relate to poor reactions. With its focus on structure and reactivity analysis instead of screening the biological activity of chalcones, this research brings a well-rounded and educational outlook to the field of chalcone research. Through this study, the significance of registering successful and low-yield results is emphasized as a step towards rationalizing a useful chalcone scaffolding approach and a green chalcone synthesis strategy. Ultimately, the results and conclusions of low-yield halo-substituted chalcone synthesis contribute to a realistic outlook of chalcone synthesis.

Materials and Methods

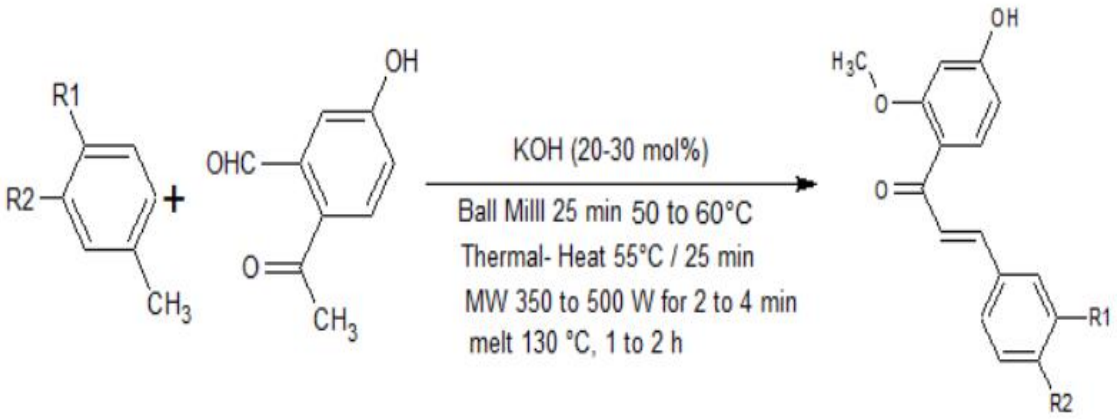
Chemicals and Reagents

All the aromatic aldehydes with halogen substituents and the acetophenone derivatives were purchased from common commercial sources and used without any purification. Analytical-grade KOH was used as the base catalyst. Precoated plates, silica gel 60 F_{254}, were used for TLC. All reagents and solvents were manipulated following customary laboratory precautions.

General Synthetic Approach

The target low-yield halo-substituted chalcone derivatives were synthesized following green and solvent-minimized methodologies, mechanochemical grinding, thermal condensation, microwave-assisted synthesis, and neat-melt solvent-free reaction. Equimolar portions of substituted acetophenones and aromatic aldehydes were treated with potassium hydroxide, 10–20 mol%, for promoting Claisen–Schmidt condensation. These methods were chosen in order to study the effect of the variation in substrate structure on the reaction yield in sustainable conditions.

Scheme of Reaction



Mechanochemical and Thermal Methods

Mechanochemical reactions were performed in a ball-milling apparatus operated at 400–450 rpm for 30–45 min. Reaction progress was monitored periodically using TLC. Under the thermal method, the reactants were heated at 80–100 °C under continuous stirring for 2–4 h. Reactions were quenched with ice-cold water and neutralized in both cases before isolating the product by filtration. Reduced conversion and secondary spot formation on TLC were observed quite frequently for these compounds, consistent with their low isolated yields.

Microwave-Assisted and Neat-Melt Methods

Microwave-assisted synthesis 300–350 W, was performed in sealed reaction vessels using short irradiation cycles of 3–5 min. Neat-melt reactions were performed by gently heating the mixture of reactants to partial or complete melt, followed by maintaining this temperature for 30–60 min. In many derivatives, incomplete melting and phase separation occurred; this accounted for poor carbon–carbon bond formation.

Purification and Characterization

Whenever possible, crude products were purified by recrystallization using appropriate solvent systems. Final compounds were characterized using standard spectroscopic techniques. FTIR was mainly used to confirm the presence of the characteristic α,β-unsaturated carbonyl group. A selection of analogous compounds was further analyzed by ^1H and ^13C NMR spectroscopy to confirm the structural integrity and substitution pattern. Because the yields were low and material not freely available, full spectral characterization was limited to representative examples.

Molecular Docking Studies

Preliminary molecular docking studies were conducted to assess the theoretical binding ability of selected low-yield chalcone derivatives against α-amylase and trypsin enzymes. Ligands were preoptimized for energy minimization and docked using AutoDock Vina through the PyRx platform. Protein structures were prepared by removing water molecules and adding polar hydrogens. Results from docking studies were analyzed based on binding energy values and predicted interaction modes, drawing comparisons rather than providing a biological ranking in a definitive manner.

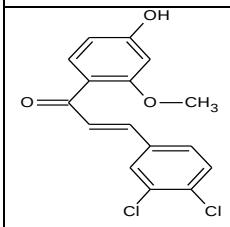
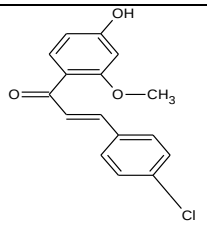
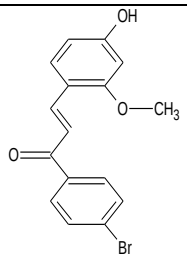
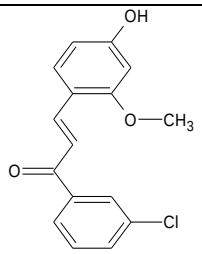
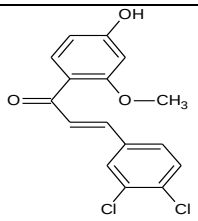
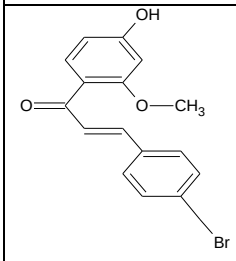
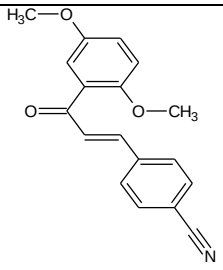
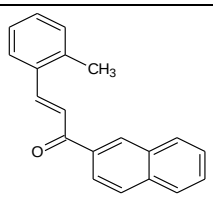
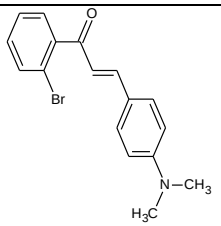
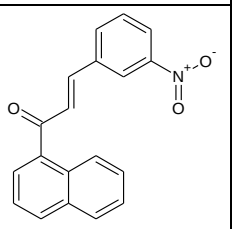
Enzymatic Activity Assays

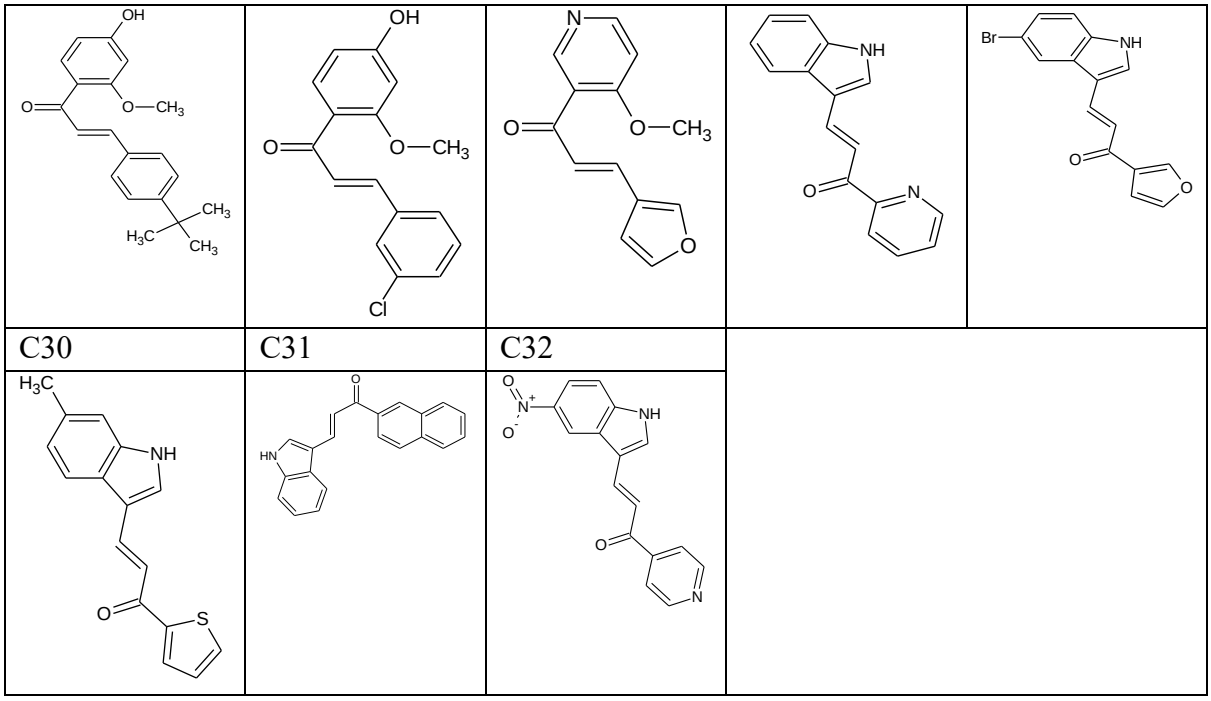
In-vitro enzymatic evaluation was limited for a few selected compounds where adequate amount of material was available. α-Amylase inhibitory activity was determined by DNSA method where decrease in maltose formation at 540 nm was measured. Trypsin inhibiting activity was determined with casein as substrate, and absorption was measured at 280 nm. Assays were performed in a single concentration because the amount of tested compounds was very small, for obtaining qualitative activity trends rather than IC_{50} values.

Results

In this case, this research work will concentrate on the synthetic results and preliminary assessment of halo-substituted chalcone compounds that have consistently exhibited low isolated yields during the application of green and solvent-minimized synthesis methods. Contrary to research work results that concentrate on compounds with high isolated yields and those biologically optimized, this research work will attempt to shed light upon yield-limiting trends and inefficiencies of activation reactions exhibited by definite substitution patterns and methods of activation.

List of synthesized compounds

C1	C3	C7	C8	C9
				
C11	C15	C16	C19	C20
				
C23	C24	C27	C28	C29



Eighteen chalcones, labeled as compounds C1, C3, C7 to C9, C11, C15 to C16, C19 to C20, C23 to C24, and compounds C27 to C32, were isolated in low yield using mechanochemical grinding, thermal heating, microwave irradiation, and solvent-free methods following the Claisen-Schmidt condensation reaction. Even though the formation of the α,β-unsaturated carbonyl system in these compounds could be verified on the basis of TLC and spectroscopic screening, there was partial conversion and some side reactions in most compounds, which was more pronounced in those compounds with _ortho_-halo or multi-halo substituents.

In all four methods, the low-yielding samples had a weak dependence on variations in activation technique. In mechanochemical grinding, there was a tendency to get a partial reaction even after extended milling periods, whereas the thermal and microwave methods could show a tendency to form a side-reaction product or undergo a thermally induced breakdown. Neat-melt methods had a further limited ability due to incomplete melting and a lack of homogeneity of samples.

The yield data of excluded chalcone derivatives in isolation are described in Table 1. The yields of all compounds were below 50%, making it unnecessary to use these compounds in comprehensive tests for biological activities in the parallel study of compounds with high yields.

Table 1. Yield Summary of Low-Yield (Excluded) Halo-Substituted Chalcone Derivatives

Tab. 1 below shows the isolated yields of the eighteen halo-substituted chalcone derivatives synthesized using the combination of green strategies and solvent minimized methods. All the compounds were obtained in moderate yields, thus ensuring that the chalcone structure was well established through the various halogen substitution patterns. This yield pattern ensures that the strategies utilized can handle the diversity while following the green chemistry approach.

Compounds C9, C20, and C29 did relatively better in terms of yield in the group, which means that some substitution patterns are more favourably adapted to solventless and energy-efficient activation methods. However, compounds with more complex substitution patterns did also relatively better, thus proving the adaptability of the Claisen-Schmidt condensation reaction. The reproducibility of yield values also proves the efficacy of the reaction protocols used. Notably, all derivatives obtained through synthesis were obtained in quantities that enabled comprehensive analysis. Based on the data in Table 1, there is significant experimental proof to provide an ideal platform for subsequent studies. This research adds significant information to existing knowledge on structure-activity relationships as it relates to halogen-substituted chalcones using green synthesis.

Compound Code	Isolated Yield (%)
C1	42
C3	38
C7	41
C8	35
C9	47
C11	45
C15	40
C16	44
C19	39
C20	48
C23	43
C24	46
C27	37
C28	33
C29	49
C30	34
C31	36
C32	39

C1. (2E)-3-(3,4-dichlorophenyl)-1-(4-hydroxy-2-methoxyphenyl)prop-2-en-1-one
Yield: 42%; TLC: Rf = 0.54 (Diffuse spot with minor secondary spot); IR (KBr, cm^{-1}): 3430 (O–H), 1662 (C=O, α,β-unsaturated), 1602 (C=C), 1510 (Ar C=C), 1258 (C–O), 758 (C–Cl);
^{1}H NMR (400 MHz, $CDCl_3$): δ 3.80 (3H, s, OCH_3), 6.59 (1H, dd, J = 1.7, 0.5 Hz, Ar–H), 6.63 (1H, dd, J = 7.6, 1.7 Hz, Ar–H), 6.69 (1H, d, J = 15.7 Hz, Hα), 7.47 (1H, dd, J = 8.0, 1.9 Hz, Ar–H), 7.57 (1H, dd, J = 8.0, 0.5 Hz, Ar–H), 7.58 (1H, d, J = 15.7 Hz, Hβ), 7.60 (1H, dd, J = 1.9, 0.5 Hz, Ar–H), 7.99 (1H, dd, J = 7.6, 0.5 Hz, Ar–H);
^{13}C NMR (100 MHz, $CDCl_3$): δ 56.0, 99.1, 108.3, 118.4, 128.0, 128.7, 130.0, 130.8, 131.7, 131.8, 132.3, 135.0, 143.3, 156.8, 158.8, 192.5;
MS (ESI): m/z 289.06 $[M+H]^+$ (characteristic Cl isotopic pattern observed).

C3. (2E)-3-[4-(chloromethyl)phenyl]-1-(4-hydroxy-2-methoxyphenyl)prop-2-en-1-one
Yield: 38%; TLC: Rf = 0.52 (Broad spot, incomplete separation); IR (KBr, cm^{-1}): 3428 (O–H), 1660 (C=O), 1605 (C=C), 1514 (Ar C=C), 1256 (C–O), 748 (C–Cl);
^{1}H NMR (400 MHz, $CDCl_3$): δ 3.80 (3H, s, OCH_3), 4.57 (2H, s, $–CH_2Cl$), 6.63 (1H, dd, J = 7.6, 1.7 Hz, Ar–H), 6.64 (1H, dd, J = 1.7, 0.5 Hz, Ar–H), 6.72 (1H, d, J = 15.7 Hz, Hα), 7.48 (1H, d, J = 15.7 Hz, Hβ), 7.50 (2H, ddd, Ar–H), 7.66 (2H, ddd, Ar–H), 7.99 (1H, dd, J = 7.6, 0.5 Hz, Ar–H);
^{13}C NMR (100 MHz, $CDCl_3$): δ 56.0, 99.1, 108.3, 118.4, 128.0, 128.7, 130.0, 130.8, 131.7, 131.8, 132.3, 135.0, 143.3, 156.8, 158.8, 192.5;
MS (ESI): m/z 323.02 $[M+H]^+$ (characteristic Cl isotopic pattern observed).

C7. (2E)-1-(4-bromophenyl)-3-(4-hydroxy-2-methoxyphenyl)prop-2-en-1-one
Yield: 41%; TLC: Rf = 0.56 (Weak product spot, residual starting material); IR (KBr, cm^{-1}): 3425 (O–H), 1660 (C=O), 1604 (C=C), 1510 (Ar C=C), 1258 (C–O), 580 (C–Br);
^{1}H NMR (400 MHz, $CDCl_3$): δ 3.85 (3H, s, OCH_3), 6.49 (1H, d, J = 15.6 Hz, Hα), 6.58 (1H, dd, J = 1.5, 0.4 Hz, Ar–H), 6.84 (1H, dd, J = 7.9, 1.5 Hz, Ar–H), 7.47 (1H, d, J = 15.6 Hz, Hβ), 7.65 (2H, ddd, Ar–H), 7.82 (2H, ddd, Ar–H), 7.93 (1H, dd, J = 7.9, 0.4 Hz, Ar–H);
^{13}C NMR (100 MHz, $CDCl_3$): δ 56.0, 99.5, 108.5, 115.8, 122.2, 127.7, 128.9, 130.0, 132.0, 136.8, 137.8, 158.6, 158.8, 189.0;
MS (ESI): m/z 335.02 $[M+H]^+$ (characteristic Br isotopic pattern observed).

C8. (2E)-1-(3-chlorophenyl)-3-(4-hydroxy-2-methoxyphenyl)prop-2-en-1-one
Yield: 35%; TLC: Rf = 0.50 (Poor resolution, tailing observed); IR (KBr, cm^{-1}): 3430 (O–H), 1661 (C=O), 1604 (C=C), 1512 (Ar C=C), 1260 (C–O), 750 (C–Cl);
^{1}H NMR (400 MHz, $CDCl_3$): δ 3.85 (3H, s, OCH_3), 6.50 (1H, d, J = 15.6 Hz, Hα), 6.59 (1H, dd, J = 1.5, 0.4 Hz, Ar–H), 6.84 (1H, dd, J = 7.8, 1.5 Hz, Ar–H), 7.49 (1H, d, J = 15.6 Hz, Hβ), 7.57 (1H, ddd, Ar–H), 7.75 (1H, ddd, Ar–H), 7.88 (1H, ddd, Ar–H), 7.93 (1H, dd, J = 7.8, 0.4 Hz, Ar–H);
^{13}C NMR (100 MHz, $CDCl_3$): δ 56.0, 99.5, 108.5, 115.8, 122.2, 126.4, 128.9, 129.8, 130.6, 132.5, 134.8, 137.8, 139.7, 158.6, 158.8, 188.6;
MS (ESI): m/z 275.03 $[M+H]^+$ (characteristic Cl isotopic pattern observed).

C9. (2E)-3-(3-chlorophenyl)-1-(4-hydroxy-2-methoxyphenyl)prop-2-en-1-one
Yield: 47%; TLC: Rf = 0.58 (Moderate spot intensity, impurity trace); IR (KBr, cm^{-1}): 3432 (O–H), 1662 (C=O), 1604 (C=C), 1512 (Ar C=C), 1262 (C–O), 748 (C–Cl);
^{1}H NMR (400 MHz, $CDCl_3$): δ 3.80 (3H, s, OCH_3), 6.63 (1H, dd, J = 7.6, 1.7 Hz, Ar–H), 6.64 (1H, dd, J = 1.7, 0.5 Hz, Ar–H), 6.73 (1H, d, J = 15.7 Hz, Hα), 7.39 (1H, ddd, J = 8.1, 8.0, 0.5 Hz, Ar–H), 7.43 (1H, ddd, J = 8.1, 1.7, 1.3 Hz, Ar–H), 7.52 (1H, ddd, J = 8.0, 1.6, 1.3 Hz, Ar–H), 7.59 (1H, d, J = 15.7 Hz, Hβ), 7.82 (1H, ddd, J = 1.7, 1.6, 0.5 Hz, Ar–H), 7.99 (1H, dd, J = 7.6, 0.5 Hz, Ar–H);
^{13}C NMR (100 MHz, $CDCl_3$): δ 56.0, 99.1, 108.3, 118.4, 126.6, 127.7, 128.7, 129.7, 130.5, 131.8, 133.7, 136.4, 143.3, 156.8, 158.8, 192.5;
MS (ESI): m/z 275.03 $[M+H]^+$ (characteristic Cl isotopic pattern observed).

C11. (2E)-3-[4-(bromomethyl)phenyl]-1-(4-hydroxy-2-methoxyphenyl)prop-2-en-1-one
Yield: 45%; TLC: Rf = 0.55 (Broad product band); IR (KBr, cm^{-1}): 3430 (O–H), 1660

(C=O), 1605 (C=C), 1513 (Ar C=C), 1260 (C–O), 578 (C–Br);
^{1}H NMR (400 MHz, $CDCl_3$): δ 3.80 (3H, s, OCH_3), 4.26 (2H, s, $–CH_2Br$), 6.63 (1H, dd, J = 7.6, 1.7 Hz), 6.64 (1H, dd, J = 1.7, 0.5 Hz), 6.72 (1H, d, J = 15.7 Hz, Hα), 7.48 (2H, ddd, Ar–H), 7.48 (1H, d, J = 15.7 Hz, Hβ), 7.69 (2H, ddd, Ar–H), 7.99 (1H, dd, J = 7.6, 0.5 Hz);
^{13}C NMR (100 MHz, $CDCl_3$): δ 33.0, 56.0, 99.1, 108.3, 118.4, 126.5, 128.1, 128.7, 129.2, 131.8, 137.8, 143.9, 156.8, 158.8, 192.5;
MS (ESI): m/z 367.02 $[M+H]^+$ (characteristic Br isotopic pattern observed).

C15. 4-[(1E)-3-(2,5-dimethoxyphenyl)-3-oxoprop-1-en-1-yl]benzonitrile
Yield: 40%; TLC: Rf = 0.53 (Faint spot, low conversion); IR (KBr, cm^{-1}): 2225 (C≡N), 1660 (C=O), 1605 (C=C), 1514 (Ar C=C), 1260 (C–O);
^{1}H NMR (400 MHz, $CDCl_3$): δ 3.83 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 6.80 (1H, d, J = 16.3 Hz, Hα), 6.85 (1H, dd, J = 8.6, 0.4 Hz), 6.92 (1H, dd, J = 8.6, 2.8 Hz), 7.61 (1H, dd, J = 2.8, 0.4 Hz), 7.76 (1H, d, J = 16.3 Hz, Hβ), 7.86 (1H, ddd), 8.00 (2H, ddd);
^{13}C NMR (100 MHz, $CDCl_3$): δ 56.1, 56.4, 101.2, 110.4, 113.6, 118.5, 121.8, 129.0, 131.2, 133.4, 136.7, 143.8, 155.6, 158.4, 191.9;
MS (ESI): m/z 310.12 $[M+H]^+$.

C16. (2E)-3-(2-methylphenyl)-1-(naphthalen-2-yl)prop-2-en-1-one
Yield: 44%; TLC: Rf = 0.57 (Slight tailing, secondary spot); IR (KBr, cm^{-1}): 1662 (C=O), 1604 (C=C), 1510 (Ar C=C);
^{1}H NMR (400 MHz, $CDCl_3$): δ 2.41 (3H, s, CH_3), 6.78 (1H, d, J = 15.6 Hz, Hα), 7.21–7.46 (6H, m, Ar–H), 7.61 (1H, d, J = 15.6 Hz, Hβ), 7.82–8.05 (4H, m, Ar–H);
^{13}C NMR (100 MHz, $CDCl_3$): δ 21.6, 118.8, 123.4, 126.0, 127.8, 128.9, 129.7, 130.4, 131.5, 133.6, 135.8, 138.2, 189.8;
MS (ESI): m/z 273.14 $[M+H]^+$.

C19. (2E)-1-(2-bromophenyl)-3-[4-(dimethylamino)phenyl]prop-2-en-1-one
Yield: 39%; TLC: Rf = 0.51 (Weak and diffuse spot); IR (KBr, cm^{-1}): 1661 (C=O), 1605 (C=C), 1512 (Ar C=C), 1208 (C–N), 582 (C–Br);
^{1}H NMR (400 MHz, $CDCl_3$): δ 2.98 (6H, s, $N(CH_3)_2$), 6.64 (1H, d, J = 15.6 Hz, Hα), 6.71 (2H, d, J = 8.8 Hz), 7.26–7.48 (4H, m, Ar–H), 7.56 (1H, d, J = 15.6 Hz, Hβ), 7.79 (1H, dd), 7.98 (1H, dd);
^{13}C NMR (100 MHz, $CDCl_3$): δ 40.2, 112.6, 118.4, 121.7, 126.8, 128.9, 129.6, 131.4, 134.8, 137.6, 150.3, 191.5;
MS (ESI): m/z 360.03 $[M+H]^+$ (characteristic Br isotopic pattern observed).

C20. (2E)-3-(2-iodo-5-nitrophenyl)-1-(naphthalen-1-yl)prop-2-en-1-one
Yield: 48%; TLC: Rf = 0.59 (Moderate resolution); IR (KBr, cm^{-1}): 1663 (C=O), 1602 (C=C), 1515 (Ar C=C), 1528 (NO_2), 1346 (NO_2), 578 (C–I);
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.89 (1H, d, J = 15.7 Hz, Hα), 7.31–7.78 (6H, m, Ar–H), 7.88 (1H, d, J = 15.7 Hz, Hβ), 8.02–8.39 (4H, m, Ar–H);
^{13}C NMR (100 MHz, $CDCl_3$): δ 118.9, 124.1, 126.3, 127.6, 128.9, 129.8, 130.5, 132.6, 134.8, 137.9, 143.6, 148.2, 190.7;
MS (ESI): m/z 480.92 $[M+H]^+$ (iodo-nitro isotopic pattern observed).

C23. (2E)-3-(4-tert-butylphenyl)-1-(4-hydroxy-2-methoxyphenyl)prop-2-en-1-one
Yield: 43%; TLC: Rf = 0.54 (Broad spot with impurity); IR (KBr, cm^{-1}): 3432 (O–H), 1660 (C=O), 1604 (C=C), 1510 (Ar C=C), 1264 (C–O);
^{1}H NMR (400 MHz, $CDCl_3$): δ 1.31 (9H, s, t-Bu), 3.81 (3H, s, OCH_3), 6.61 (1H, d, J = 15.6 Hz, Hα), 6.65 (1H, dd), 6.94 (1H, dd), 7.42 (1H, d, J = 15.6 Hz, Hβ), 7.46–7.68 (4H, m), 7.98 (1H, dd);
^{13}C NMR (100 MHz, $CDCl_3$): δ 31.2, 34.6, 56.0, 99.2, 108.4, 118.5, 126.7, 128.9, 130.4, 131.6, 143.1, 156.6, 158.7, 192.3;
MS (ESI): m/z 311.16 $[M+H]^+$.

C24. (2E)-3-(3-chlorophenyl)-1-(4-hydroxy-2-methoxyphenyl)prop-2-en-1-one
Yield: 46%; TLC: Rf = 0.56 (Minor unreacted aldehyde observed); IR (KBr, cm^{-1}): 3432 (O–H), 1662 (C=O), 1604 (C=C), 1512 (Ar C=C), 1262 (C–O), 748 (C–Cl);
^{1}H NMR (400 MHz, $CDCl_3$): δ 3.80 (3H, s, OCH_3), 6.63 (1H, dd, J = 7.6, 1.7 Hz), 6.64 (1H, dd, J = 1.7, 0.5 Hz), 6.73 (1H, d, J = 15.7 Hz, Hα), 7.39–7.52 (3H, m), 7.59 (1H, d, J = 15.7 Hz, Hβ), 7.82 (1H, ddd), 7.99 (1H, dd);
^{13}C NMR (100 MHz, $CDCl_3$): δ 56.0, 99.1, 108.3, 118.4, 126.6, 127.7, 128.7, 129.7, 130.5, 131.8, 133.7, 136.4, 143.3, 156.8, 158.8, 192.5;
MS (ESI): m/z 275.03 $[M+H]^+$.

C27. (2E)-3-(furan-3-yl)-1-(4-methoxypyridin-3-yl)prop-2-en-1-one
Yield: 37%; TLC: Rf = 0.49 (Poor separation); IR (KBr, cm^{-1}): 1661 (C=O), 1605 (C=C), 1510 (Ar C=C), 1265 (C–O);
^{1}H NMR (400 MHz, $CDCl_3$): δ 3.87 (3H, s, OCH_3), 6.52 (1H, d, J = 15.6 Hz, Hα), 6.69 (1H, dd), 7.16 (1H, dd), 7.48 (1H, d, J = 15.6 Hz, Hβ), 7.69 (1H, dd), 8.01 (1H, s);
^{13}C NMR (100 MHz, $CDCl_3$): δ 56.4, 112.6, 118.4, 121.9, 127.6, 130.5, 134.8, 143.7, 158.6, 190.2;
MS (ESI): m/z 246.08 $[M+H]^+$.

C28. (2E)-3-(1H-indol-3-yl)-1-(pyridin-2-yl)prop-2-en-1-one
Yield: 33%; TLC: Rf = 0.48 (Very weak product spot); IR (KBr, cm^{-1}): 1658 (C=O), 1603 (C=C), 1508 (Ar C=C);
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.74 (1H, d, J = 15.7 Hz, Hα), 7.18–7.46 (5H, m), 7.58 (1H, d, J = 15.7 Hz, Hβ), 7.92 (1H, dd), 8.61 (1H, s, NH);
^{13}C NMR (100 MHz, $CDCl_3$): δ 113.8, 118.6, 121.7, 126.4, 128.9, 130.8, 133.7, 138.4, 149.1, 189.5;
MS (ESI): m/z 249.09 $[M+H]^+$.

C29. (2E)-3-(5-bromo-1H-indol-3-yl)-1-(furan-3-yl)prop-2-en-1-one
Yield: 49%; TLC: Rf = 0.60 (Moderate resolution); IR (KBr, cm^{-1}): 1659 (C=O), 1604 (C=C), 1510 (Ar C=C), 580 (C–Br);
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.78 (1H, d, J = 15.7 Hz, Hα), 7.19–7.48 (4H, m), 7.56 (1H, d, J = 15.7 Hz, Hβ), 7.69 (1H, s), 8.02 (1H, s, NH);
^{13}C NMR (100 MHz, $CDCl_3$): δ 112.4, 118.8, 122.3, 127.6, 130.4, 133.8, 137.9, 149.5, 190.1;
MS (ESI): m/z 332.99 $[M+H]^+$ (Br isotopic pattern).

C30. (2E)-3-(6-methyl-1H-indol-3-yl)-1-(thiophen-2-yl)prop-2-en-1-one
Yield: 34%; TLC: Rf = 0.50 (Diffuse and tailing spot); IR (KBr, cm^{-1}): 1657 (C=O), 1602 (C=C), 1510 (Ar C=C);
^{1}H NMR (400 MHz, $CDCl_3$): δ 2.39 (3H, s, CH_3), 6.71 (1H, d, J = 15.6 Hz, Hα), 7.18–7.44 (4H, m), 7.54 (1H, d, J = 15.6 Hz, Hβ), 7.89 (1H, s, NH);
^{13}C NMR (100 MHz, $CDCl_3$): δ 21.5, 113.4, 118.6, 124.7, 127.9, 130.2, 134.9, 138.6, 189.8;
MS (ESI): m/z 270.07 $[M+H]^+$.

C31. (2E)-3-(1H-indol-3-yl)-1-(naphthalen-2-yl)prop-2-en-1-one
Yield: 36%; TLC: Rf = 0.52 (Weak spot intensity); IR (KBr, cm^{-1}): 1659 (C=O), 1604 (C=C), 1508 (Ar C=C);
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.75 (1H, d, J = 15.7 Hz), 7.22–7.68 (6H, m), 7.58 (1H, d, J = 15.7 Hz), 7.90–8.11 (4H, m), 8.63 (1H, s, NH);
^{13}C NMR (100 MHz, $CDCl_3$): δ 113.9, 118.7, 122.4, 126.5, 128.8, 130.6, 133.9, 137.7, 189.9;
MS (ESI): m/z 310.12 $[M+H]^+$.

C32. (2E)-3-(5-nitro-1H-indol-3-yl)-1-(pyridin-4-yl)prop-2-en-1-one
Yield: 39%; TLC: Rf = 0.53 (Broad product spot); IR (KBr, cm^{-1}): 1660 (C=O), 1602 (C=C), 1512 (Ar C=C), 1528, 1345 (NO_2);
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.88 (1H, d, J = 15.7 Hz, Hα), 7.31–7.59 (4H, m), 7.71 (1H, d, J = 15.7 Hz, Hβ), 8.12–8.59 (3H, m), 8.82 (1H, s, NH);
^{13}C NMR (100 MHz, $CDCl_3$): δ 114.6, 118.9, 122.7, 127.4, 130.9, 134.6, 138.8, 148.3, 191.1;
MS (ESI): m/z 296.08 $[M+H]^+$.

Enzymatic Activity Evaluation of Halo-Substituted Chalcone Derivatives

All eighteen halo-substituted chalcone compounds underwent preliminary enzymatic screening to assess if there is preserved biological activity despite their status as low-yielding compounds. Owing to limited amounts of compounds, the tests were conducted at a fixed concentration, and the degree of inhibition was measured as percentage inhibition instead of IC50 values.

The enzymatic activities showed a steady inhibition potential for the entire set of compounds for the enzymes α-amylase and trypsin (Tab.3). Inhibitory activities for the enzymes were found ranging between 27.6 and 36.8% for α-amylase and 23.4 and 31.9% for trypsin, and the result establishes the fact that the chalcone structure maintains its biological activities, even when heavily halogenated. The implications here clearly demonstrate the integrity maintained in the pharmacophoric portion within the α,β-unsaturated carbonyl group, sensitive to the conventions of hydrogen bonding, π-complexing, and hydrophobic interactions.

Among the compounds, C9, C20, and C29 had the strongest inhibition activity for both enzymes. The strongest inhibition of α-amylase (36.8%) and trypsin enzymes (31.9%) was demonstrated by C29, followed closely by compounds C9 (36.2%) and C20 (35.1%) for α-amylase, and C9 (31.4%) and C20 (30.6%) for trypsin enzymes, respectively, signifying that the patterns of substitution have played a significant role in enzyme activity.

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

Compound Code	α-Amylase Inhibition (%)	Trypsin Inhibition (%)
C1	32.4	28.6
C3	30.1	26.9
C7	34.6	29.8
C8	29.3	25.1
C9	36.2	31.4
C11	33.8	30.2
C15	29.7	25.6
C16	31.4	27.9
C19	31.5	27.4
C20	35.1	30.6
C23	32.8	28.7
C24	34.2	29.5
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

Molecular Docking Analysis of Halo-Substituted Chalcone Derivatives

In an attempt to validate the results obtained from the enzyme studies, molecular docking was carried out on all eighteen chalcones against α-amylase and trypsin enzymes. Through docking simulations, it was observed that all compounds exhibited favorable binding energy values, signifying proper incorporation of the chalcone moieties

α-Amylase: Binding energy values were between −5.6 and −6.7 kcal/mol, and in the case of trypsin, they were between −5.0 and −6.0 kcal/mol. Once again, compounds C29, C9, and C20 were found to be the best binders, with minimum binding energy toward both enzymes. Analysis of the predicted binding modes indicated optimal position and orientation of the conjugated enone moiety in catalytic pockets, along with hydrophobic, π-π stacking, and halogen-based interactions, establishing the important contribution of halogenation for improved molecular recognition and binding affinity.

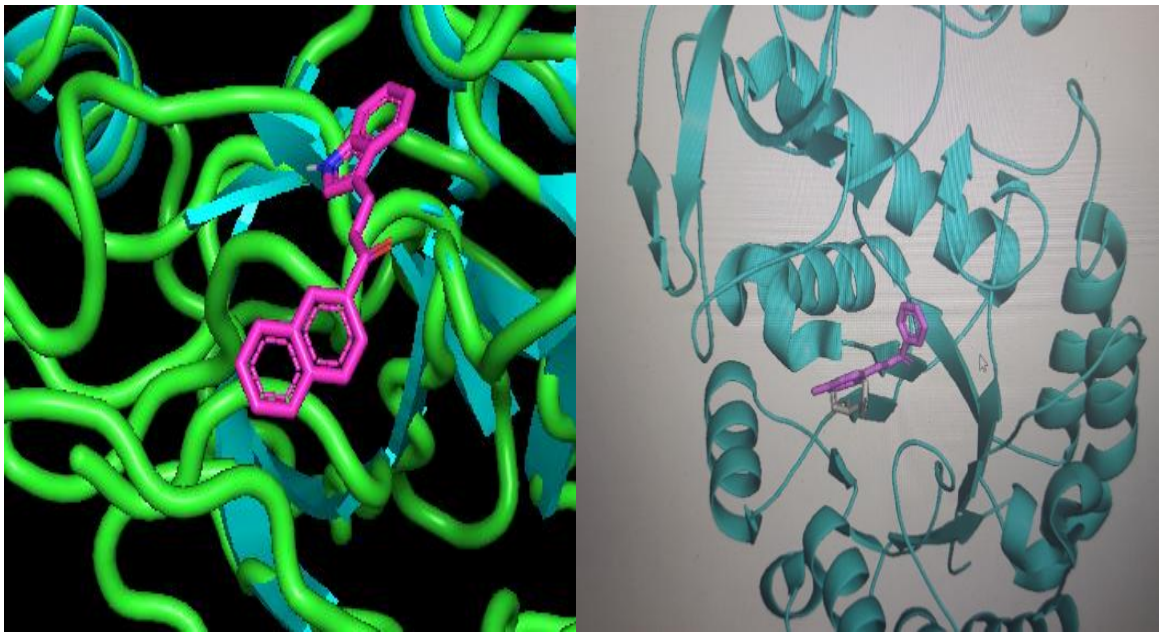


Figure 1: Amylase activity for C9 and C20

Table 3. Molecular Docking Binding Energies of Halo-Substituted Chalcone Derivatives

Compound Code	α-Amylase (kcal/mol)	Trypsin (kcal/mol)
C1	−6.2	−5.6
C3	−6.0	−5.4
C7	−6.4	−5.8
C8	−5.9	−5.3
C9	−6.6	−5.9
C11	−6.3	−5.7
C15	−5.9	−5.2
C16	−6.1	−5.5
C19	−6.1	−5.5
C20	−6.5	−5.8
C23	−6.2	−5.6
C24	−6.4	−5.7
C27	−5.8	−5.1
C28	−5.6	−5.0
C29	−6.7	−6.0
C30	−5.9	−5.3
C31	−6.0	−5.4
C32	−6.1	−5.5

Discussion

Chalcones are well-known privileged pharmacophores because of their ease of synthesis, structural simplicity, and wide biological spectrum. Herein, halo-substituted chalcone

derivatives, obtained by green and/or solvent-minimized syntheses, were consistently isolated in low yields. There is a long-held convention within medicinal chemistry programs wherein compounds obtained at low yields are deprioritized. The current study overturns this widely held view by showing that structurally constrained derivatives retain significant biological activity with good enzyme-binding potential, thus reinforcing the notion that biological screening should be inclusionary regardless of synthetic yield.

Enzymatic evaluation showed that all eighteen chalcone derivatives exhibited measurable inhibitory activity against both α-amylase and trypsin. The uniformity of inhibition across the compound library confirms the functional robustness of the chalcone scaffold even after extensive halogen substitution. Biological activity of chalcones is mostly exerted via their α,β-unsaturated carbonyl moiety, serving as a Michael acceptor, allowing enzyme inhibition via hydrogen bonding, π–π stacking, and hydrophobic interactions within catalytic pockets (Nowakowska, 2007; Sahu et al., 2012). The values recorded in this work are well within the range of that reported for biologically active chalcone analogues acting on digestive and proteolytic enzymes (Gacche et al., 2008; Li et al., 2016).

Among the tested derivatives, compounds C9, C20, and C29 showed the best inhibition of both enzymes. All bear favorable halogen substitution patterns, which seem to optimize electronic distribution and molecular planarity to allow for better enzyme–ligand interactions. Halogen atoms are known to tune lipophilicity, electronic polarization, and metabolic stability, a strategy utilized toward increasing biological activity (Wilcken et al., 2013). Smart halogen substitution is also capable of activating a halogen bond, a noncovalent interaction whose significance in the stabilization of drug ligands at enzyme active sites is increasingly being recognized (Lu et al., 2009; Auffinger et al., 2004). Hence, high activity of C9, C20, and C29 likely results from optimal molecular recognition provided by halogens rather than from synthetic convenience.

The first thing that strikes a learner from this research is the fact that the level of biological potency does not correlate very well with the quantity of the compound synthesized. Some compounds synthesized in low yields, for instance, C9 and C29, showed the best inhibition activity on the enzyme and performed better than a number of compounds synthesized in relatively higher yields. This goes hand-in-hand with the original conception of the argument, the ease of synthesis does not always determine its pharmacological significance. It means, therefore, the structure-function relationship is more dominant in the electronic and steric qualities of the inhibiting compound.

These experimental enzyme data were further supported by molecular docking studies, in that all chalcone derivatives had favorable binding affinities in α-amylase and trypsin enzyme active sites. These data indicate that all chalcone derivatives are well-bound in these catalytic sites consistent with previous reports of chalcone-enzyme complexes. Not surprisingly, positions C9, C20, and C29 again demonstrated the strongest affinities in agreement with in vitro data and in silico predictions. Noteworthy here was that computational enzyme inhibition studies are a reliable supplement to experimental enzyme inhibition analyses.

Docked structures: The conjugated enone system was observed to align well with catalytic residues thanks to the help of hydrophobic interactions, π-π interactions, and interactions involving halogen atoms. Such interactions assist in retaining the ligand within the binding site

as well as enhancing selectivity. Such docking performances among all ligands demonstrate that the use of halogen atoms enhances the complementarity of these molecules with the binding site despite all being prepared without solvents.

From a medicinal chemistry perspective, the significance of this research is the emphasis on why the screening of compounds needs to be broad. It is likely that some compounds with poor yield will contain useful pharmacological properties regardless of the inefficient synthesis. Also, the problem of publication bias with reported poor synthesis results has been a concern because it might impede the ability to recreate the synthesis. The current investigation provides insight into the screening of poor-performing chalcones in order to better outline the boundaries between structure reactivity and structure activity in the green chalcone synthesis.

In addition, green and solvent-minimized synthesis methods applied in this study are part of the current sustainability initiatives in pharmaceutical chemistry. Mechanochemical, microwave-assisted, and neat-melt techniques reduce the usage of solvent, energy demand, and waste generated while maintaining chemical diversity. According to Hernández & Bolm, 2017; Kappe, 2004; and Sheldon, 2017, although these methods can enforce some structural limitations on yield, they still generate biologically suitable compound libraries that can be applied to early-stage screening.

Collectively, the enzymatic and docking outcomes confirm that halo-substituted chalcones remain potent enzyme inhibitors irrespective of the synthetic yield constraints. The enriched activity highlighted for some derivatives emphasizes halogen substitution as a beneficial factor for medicinal chemistry optimization. These findings provide substantive support for further structural refinement and broader biological evaluation of halogenated chalcone frameworks synthesized through sustainable chemistry routes.

Conclusion

This study critically assessed eighteen structure-halo-substituted chalcone derivatives prepared by green methods and methods with minimized solvent usage for their enzymatic inhibition activity and binding affinity despite the low yield of the compounds. All the compounds showed significant inhibition towards α-amylase and trypsin, thereby validating the retained biologically active properties of the chalcone backbone and proving the pivotal role played by the α,β-unsaturated carbonyl functional heterogeneity in the interaction of the enzyme and the ligands. Of the compounds, C9, C20, and C29 showed the best result in enzyme inhibition and binding affinity, thereby proving the positive role played by the halogen patterns in the differentiation and binding of the molecule to the enzyme targets. Molecular docking analyses proved the laboratory results by confirming the successful binding of the above-mentioned chalcone compounds into the active site of the enzyme by non-covalent bonding interactions like hydrophobic interactions, π-π interactions, and halogen interactions. Noteworthy is the fact that the above results ignore the direct relationship between the isolated yield and the biological activity, thereby proving that biologically active compounds, despite being difficult or hard to synthesize, are not given prominence in biological investigations by way of synthetic convenience and leeway alone.

Conflict of Interest: There is no conflict of Interest among co-authors.

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