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Novel Chalcone Derivatives as α-Amylase and α-Glucosidase Inhibitors: Solvent-Free Synthesis, Spectral Characterization, and Enzyme Kinetics

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Abstract

Ten new chalcone derivatives (C1′–C10′) were synthesized with potassium hydroxide as a base catalyst in a Claisen–Schmidt condensation reaction performed in solvent-free mechanochemical conditions. The clean and efficient green protocol yielded excellent yields of bright yellow to orange crystalline solids, as verified by melting-point analysis and thin-layer chromatography (TLC). HR-ESI mass spectrometry, FTIR, and ^1H/^13C NMR structure determination all validated the successful formation of the α,β-unsaturated carbonyl system.

Based on biological research, chalcones suppressed enzymes metabolizing carbohydrates in a dose-dependent fashion. Halogenated analogues C3′ and C6′ had a competitive inhibition pattern and IC_{50} values (55–60 µg/mL) comparable to or superior to that of acarbose in inhibiting α-amylase. Nitro-substituted C4′ and trifluoromethyl-substituted C7′ inhibited α-glucosidase more effectively than acarbose with mixed-type inhibition and IC_{50} = 35–40 µg/mL. Aromatic ring substitution were favored in α-glucosidase inhibition while halogens boosted α-amylase inhibition, based on structure–activity relationship (SAR) studies. These results prove the promise of the substituted chalcones as dual α-amylase/α-glucosidase inhibitors and the influence of substituent effects in altering enzyme interaction. The research gives a structural basis for optimisation of chalcone derivatives in the future as promising antidiabetic drugs.

Keywords: glucosidase, significant, pharmacological, photosensitisers, environmental issues

Introduction

α, β-unsaturated carbonyl group between two aromatics, chalcones (1,3-diphenyl-2-propen-1-ones) are useful intermediates in chemical and pharmaceutical chemistry. The template structure is characterized by high reactivity and the ability to bind to a wide variety of biological targets [1]. Chalcone derivatives have recently gained significant interest due to their diverse pharmacological properties, ranging from antiviral, antibacterial, antioxidant, anti-inflammatory to anticancer activity [2–4]. In addition to their drug importance, chalcones have also been investigated in material sciences as fluorescent sensors, liquid crystals, and as photosensitisers [5,6].

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Chalcones were traditionally prepared by the Claisen–Schmidt condensation reaction, which includes the condensation of an aromatic aldehyde with an acetophenone in alcoholic solvents with acidic or basic catalysts [7]. Despite the long-time documentation of these reactions, they are usually multi-step purified, employ unstable organic solvents, and involve long reaction times, all of which pose significant cost and environmental issues [8].

With growing popularity for green chemistry, the need now is to develop more eco-friendly alternatives that involve fewer chemicals, energy, and solvents. Solvent-free synthesis techniques have been found to be efficient alternatives following the principles of green chemistry [9].

Among these, chalcone can be prepared easily, effectively, and in an environmentally friendly way by mechanochemical and grindstone techniques [10].

These methods involve the use of solid catalysts for grinding aldehydes and ketones, which produce good results without the need for a solvent.

Subsequent research indicated that solid NaOH, KF/AlO_3, and $Mg(HSO_4)_2$ could be used more efficiently and repetitively to catalyse the chalcone synthesis without the use of solvents [11,12]. In addition, ball-milling technology has enhanced the scalability and reproducibility of such processes, and solvent-free synthesis has become an economically viable option in industrial and laboratory environments [13]. Validation of the chalcone structure is typically provided by spectroscopic techniques such as FT-IR, UV-Vis, ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry [14].

The test provides information about the conjugated enone system, confirms structural stability, and authenticates the integrity of the functional groups prior to biological screening. Structure–activity relationship (SAR) studies are crucial for rational drug design, and are also made easier by advanced analytical instrumentation and spectrum data [15].

Several chalcone derivatives synthesized through green chemistry techniques have shown interesting biological potential. For example, substituted chalcones were reported to be highly antimicrobial and antifungal in nature, and some were reported to be cytotoxic to the cancer cell lines MCF-7 and HeLa [16,17]. Biological activity of chalcones is highly susceptible to substitution pattern within aromatic rings, particularly electron-withdrawing and electron-donating groups, which influence lipophilicity and binding [18]. This necessitates new chalcone derivatives synthesizing and evaluating for lead compound identification of valuable leads.

The following perspectives, thus, invite the present research toward solvent-free synthesis of new chalcone derivatives, their analytical characterization by using sophisticated methods, and investigation of their bioactivities. The present study is intended to demonstrate an eco-friendly synthetic process following the principles of green chemistry while investigating the pharmaceutical potential of chalcones.

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Materials and Methods

Chemicals and Reagents

All chemicals and reagents were of analytical grade and used without further purification. Substituted benzaldehydes and acetophenones (0.01 mol each) were procured from Sigma-Aldrich, while potassium hydroxide (KOH, 0.01 mol) served as the base catalyst. Silica gel plates (Merck) were used for thin-layer chromatography (TLC). All solvents (ethanol, methanol, and chloroform) were of analytical grade.

Solvent-Free Synthesis of Chalcone Derivatives

Chalcones were synthesized by a solvent-free Claisen–Schmidt condensation method [19,20]. Equimolar quantities of substituted benzaldehyde (0.01 mol) and substituted ketone (0.01 mol) were ground in a mortar and pestle with potassium hydroxide (0.01 mol) as a catalyst. The reaction mixture was triturated at room temperature until the formation of a solid mass (15–25 min). Reaction progress and product formation were monitored by TLC using ethyl acetate:hexane (3:7, v/v) as the mobile phase.

The solid obtained was poured into ice-cold water, filtered, and washed with ethanol to remove excess alkali. The crude product was recrystallized using ethanol to yield pure chalcone derivatives.

Characterization of Synthesized Compounds

The synthesized chalcones were characterized using physicochemical and spectroscopic methods:

- **Melting Point**: Determined in open capillary tubes using a melting point apparatus.
- **FT-IR Spectroscopy**: Spectra were recorded using a PerkinElmer Spectrum Two spectrophotometer in the range of 4000–400 cm^{-1} (KBr pellet method).
- **^{1}H-NMR and ^{13}C-NMR Spectroscopy**: Acquired on a Bruker Avance 400 MHz NMR spectrometer in $CDCl_3$ using TMS as an internal standard.
- **Thin Layer Chromatography (TLC)**: Rf values were determined on silica gel plates using different solvent systems to check purity.

These spectral data were compared with literature values to confirm successful synthesis [21,22].

In Vitro Enzymatic Studies

The synthesized chalcone derivatives were subjected to enzymatic inhibition studies to evaluate their biological potential. The following assays were performed:

α-Amylase Inhibition Assay

The α-amylase inhibitory activity was determined using the 3,5-dinitrosalicylic acid (DNSA) method [23]. Briefly, 1 mL of chalcone derivative solution (prepared in DMSO at different concentrations, 10–200 µg/mL) was mixed with 1 mL of α-amylase solution (1 U/mL in phosphate buffer, pH 6.8) and incubated at 37 °C for 10 min. Then, 1 mL of 1% starch solution was added and further incubated for 15 min. The reaction was terminated by adding 2 mL of DNSA reagent,

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followed by heating at 100 °C for 5 min. Absorbance was measured at 540 nm, and the percentage inhibition was calculated. Acarbose was used as the standard inhibitor.

α-Glucosidase Inhibition Assay

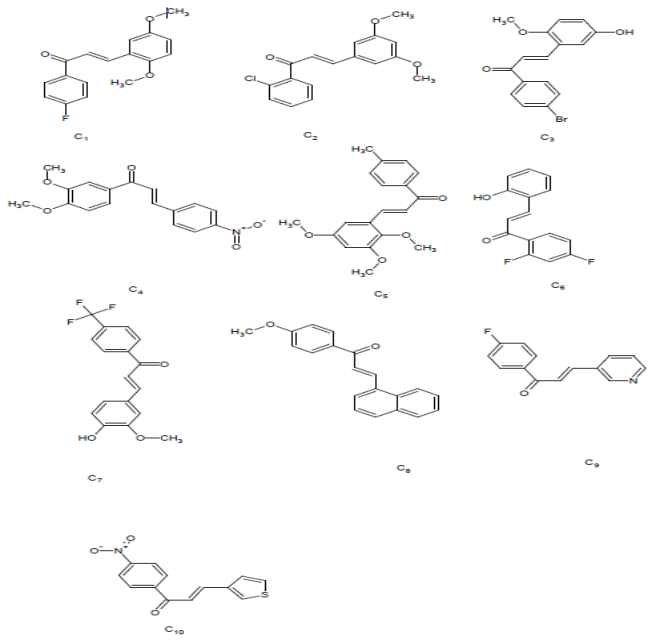
α-Glucosidase inhibition was assessed using p-nitrophenyl-α-D-glucopyranoside (PNPG) as a substrate [24]. Chalcone solutions of varying concentrations were pre-incubated with α-glucosidase enzyme (1 U/mL in phosphate buffer, pH 6.8) at 37 °C for 10 min. The reaction was initiated by adding PNPG (5 mM) and allowed to proceed for 20 min. Absorbance of the released p-nitrophenol was measured at 405 nm. Acarbose served as the reference drug.

Enzyme Kinetics

For the most active compounds, enzyme kinetics were studied by varying substrate concentrations (Lineweaver–Burk plot) to determine the type of inhibition (competitive, non-competitive, or mixed). The Michaelis–Menten constant (Km) and maximum velocity (Vmax) were calculated.

Results

Ten novel chalcone derivatives (C1′–C10′) were synthesized via a solvent-free Claisen–Schmidt condensation of equimolar substituted benzaldehydes and acetophenone derivatives using potassium hydroxide as the base catalyst. The mechanochemical grinding method afforded bright yellow to orange crystalline solids in good yields. Product formation was confirmed by thin-layer chromatography (TLC) and by spectroscopic characterization ($^{1}H/^{13}C$ NMR, IR, and mass spectrometry).



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Melting-Point Determination

Melting points were determined using the open-capillary method on a calibrated digital melting-point apparatus. Each sample was dried under vacuum prior to analysis.

Table 1. Melting-point data of synthesized chalcones

Code	Product (concise name)	Molecular formula	Measured range (°C)
C1′	1-(4-Fluorophenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one	$C_{17}H_{15}FO_3$	136–142
C2′	1-(2-Chlorophenyl)-3-(3,5-dimethoxyphenyl)prop-2-en-1-one	$C_{17}H_{15}ClO_3$	148–155
C3′	1-(4-Bromophenyl)-3-(2-hydroxy-4-methoxyphenyl)prop-2-en-1-one	$C_{16}H_{13}BrO_3$	158–165
C4′	1-(3,4-Dimethoxyphenyl)-3-(4-nitrophenyl)prop-2-en-1-one	$C_{17}H_{15}NO_5$	172–178
C5′	1-(4-Methylphenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one	$C_{18}H_{20}O_4$	140–146
C6′	1-(2,4-Difluorophenyl)-3-(2-hydroxyphenyl)prop-2-en-1-one	$C_{15}H_{10}F_2O_2$	148–154
C7′	1-(4-(Trifluoromethyl)phenyl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one	$C_{17}H_{13}F_3O_3$	162–170
C8′	1-(4-Methoxyphenyl)-3-(naphthalen-2-yl)prop-2-en-1-one	$C_{20}H_{16}O_2$	166–172
C9′	3-(Pyridin-4-yl)-1-(4-fluorophenyl)prop-2-en-1-one	$C_{15}H_{10}FNO$	150–156
C10′	1-(4-Nitrophenyl)-3-(thiophen-2-yl)prop-2-en-1-one	$C_{13}H_9NO_3S$	160–168

Thin-Layer Chromatography (TLC) Analysis

Thin-layer chromatography was used both to monitor the Claisen–Schmidt condensation and to verify the purity of the final chalcone derivatives C1′–C10′. Silica gel 60 F_{254} plates (0.25 mm) were developed in a mobile phase of toluene : ethyl acetate : formic acid (7 : 2 : 1, v/v/v), a system that gave good separation for all products. Spots were visualized under UV light at 254 nm and

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366 nm, and occasionally enhanced with iodine vapour. During synthesis, disappearance of the aldehyde spot and the appearance of a single product band signified reaction completion.

Table 2. Thin-layer chromatography of synthesized chalcones

Code	Product (concise name)	Measure Rf
C1′	1-(4-Fluorophenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one	0.40
C2′	1-(2-Chlorophenyl)-3-(3,5-dimethoxyphenyl)prop-2-en-1-one	0.36
C3′	1-(4-Bromophenyl)-3-(2-hydroxy-4-methoxyphenyl)prop-2-en-1-one	0.22
C4′	1-(3,4-Dimethoxyphenyl)-3-(4-nitrophenyl)prop-2-en-1-one	0.28
C5′	1-(4-Methylphenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one	0.48
C6′	1-(2,4-Difluorophenyl)-3-(2-hydroxyphenyl)prop-2-en-1-one	0.24
C7′	1-(4-(Trifluoromethyl)phenyl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one	0.30
C8′	1-(4-Methoxyphenyl)-3-(naphthalen-2-yl)prop-2-en-1-one	0.52
C9′	3-(Pyridin-4-yl)-1-(4-fluorophenyl)prop-2-en-1-one	0.30
C10′	1-(4-Nitrophenyl)-3-(thiophen-2-yl)prop-2-en-1-one	0.34

Nuclear Magnetic Resonance (NMR) Analysis

The structures of all ten synthesized chalcones (C1–C10) were unambiguously confirmed by ^1H and ^13C NMR spectroscopy (400 MHz, $CDCl_3$). Each spectrum displayed the resonances characteristic of the conjugated α,β-unsaturated carbonyl framework.

In the ^1H NMR spectra, every derivative exhibited two well-resolved trans-olefinic doublets between δ 6.6–7.8 ppm with large coupling constants of J ≈ 15–16 Hz, establishing the E-configuration of the enone double bond. The aromatic regions consisted of multiple multiplets within δ 6.5–8.3 ppm, reflecting the various substitution patterns on both phenyl rings and on heteroaryl moieties such as pyridine (C9) or thiophene (C10). Methoxy groups, present in C1–C5, C7, and C8, gave singlets around δ 3.7–3.9 ppm, while the para-methyl group of C5 resonated as a singlet at δ 2.31 ppm.

Phenolic hydroxyl protons in C3, C6, and C7 appeared as broad singlets at δ 9–10 ppm, confirming free –OH groups capable of hydrogen bonding. The ^13C NMR spectra were equally diagnostic. A strong downfield signal for the carbonyl carbon was consistently observed in the narrow range δ 188–193 ppm, a hallmark of α,β-unsaturated ketones.

The β-olefinic carbons resonated between δ 120–145 ppm, while the α-carbon attached to the carbonyl appeared near δ 140–150 ppm. Aromatic carbons occupied δ 110–160 ppm, with characteristic upfield or downfield shifts imposed by electron-donating methoxy groups and electron-withdrawing substituents such as nitro, fluoro, chloro, bromo, and trifluoromethyl.

Notably, the nitro-substituted chalcones C4 and C10 exhibited slightly more deshielded carbonyl

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carbons (~δ 192 ppm), reflecting the strong −I and −M effects of the NO_2 group.

Conversely, the trimethoxy- and methyl-substituted C5 showed modest upfield shifts in several aromatic carbons, consistent with the electron-rich nature of its ring system.

Table 3. Key ^1H and ^13C NMR data of chalcones C1–C10 ($CDCl_3$, 400 MHz)

Code	Key ^1H signals (δ ppm, multiplicity, J Hz)	Key ^13C signals (δ ppm)
C1	3.76–3.87 (6H, s, OCH_3), 6.65 (d, 15.7), 7.54 (d, 15.7), Ar–H 6.58–7.70	55.5, 55.6, 112.5–134.5, 139.3, 152.1, 153.6, 164.9, 189.0 (C=O)
C2	3.76 (6H, s), 6.72 (d, 15.7), 7.66 (d, 15.7), Ar–H 6.48–7.84	55.6×2, 99.8, 104.5, 121.9–136.4, 145.1, 160.9, 192.5 (C=O)
C3	3.83 (3H, s), 6.66 (d, 15.6), 7.57 (d, 15.6), Ar–H 6.59–7.83, 9.2 (br s, OH)	55.6, 111.8–139.3, 154.4, 156.7, 189.0
C4	3.80 (3H, s), 3.95 (3H, s), 6.97 (d, 16.6), 7.95 (d, 16.6), Ar–H 6.83–8.14	55.9, 56.0, 110.0–152.9, 192.0
C5	2.31 (3H, s, CH_3), 3.77–3.82 (9H, s, OCH_3), 6.67 (d, 15.7), 7.64 (d, 15.7)	21.4, 55.6, 56.2, 61.5, 97.6–153.2, 161.1, 189.0
C6	6.66 (d, 15.6), 7.48 (d, 15.6), Ar–H 6.89–8.11, 9.5 (br s, OH)	104.9–165.7, 192.5
C7	3.78 (3H, s), 6.69 (d, 15.6), 7.68 (d, 15.6), Ar–H 6.62–7.82, 9.1 (br s, OH)	56.1, 111.5–149.0, 188.9
C8	3.85 (3H, s), 6.77 (d, 15.7), 7.71 (d, 15.7), Ar–H 7.06–7.95	55.3, 113.8–162.4, 189.0
C9	6.72 (d, 15.6), 7.55 (d, 15.6), Ar–H 7.36–8.98 (pyridyl)	115.1–164.9, 188.9
C10	6.80 (d, 15.6), 7.77 (d, 15.6), Ar–H 7.23–8.26	119.1–149.4, 188.3

The combined NMR evidence unequivocally supports the successful synthesis of all target chalcones.

The trans coupling constants (J ≈ 15–16 Hz) of the vinylic protons are diagnostic for an E-alkene geometry, ruling out the Z-isomer. The downfield carbonyl carbons at δ ≈ 190 ppm confirm the presence of the α,β-unsaturated ketone functionality essential for chalcone chemistry.

Electron-donating substituents (methoxy and methyl) shift adjacent carbons upfield, as seen in C1, C2, and especially C5, while electron-withdrawing groups (nitro, halogens, CF_3) pull resonances downfield, evident in C4, C7, C9, and C10. Phenolic OH groups in C3, C6, and C7 give broad deshielded singlets near δ 9–10 ppm due to hydrogen bonding with the carbonyl oxygen, a common feature of hydroxychalcones. The distinctive chemical shifts in the heteroaryl derivatives

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C8 (naphthyl), C9 (pyridinyl), and C10 (thiophenyl) further confirm successful incorporation of these moieties.

Overall, the NMR spectra—together with IR and mass data—demonstrate that the solvent-free Claisen–Schmidt condensation furnished the desired E-configured chalcone derivatives in high purity and with the expected electronic effects from their diverse substituents.

Mass Spectrometry

High-resolution electrospray ionization mass spectrometry (HR-ESI-MS) was employed to confirm the molecular masses of the synthesized chalcones C1–C10. All compounds produced strong molecular-ion peaks that agreed closely with their calculated exact masses, confirming the molecular formulas deduced from NMR and elemental analysis.

The spectra consistently showed a prominent $[M + H]^+$ or $[M]^+$ ion, accompanied by predictable fragment ions typical of α,β-unsaturated ketones. A common fragmentation pathway involved loss of CO (−28 Da) from the chalcone carbonyl, giving a characteristic $[M - CO]^+$ ion.

Compounds containing halogens (Cl, Br, F) exhibited the expected isotope patterns:

- C2 and C6 showed the 3:1 isotopic ratio diagnostic of chlorine.
- C3 displayed the 1:1 doublet pattern of bromine.
- Fluorinated derivatives (C1, C6, C7, C9) gave strong $[M + H]^+$ signals without isotope splitting but with slightly elevated m/z due to the heavy fluorine atoms.

Table 4. HR-ESI-MS data of synthesized chalcones C1–C10

Code	Molecular formula	Calc. $[M + H]^+$ (m/z)	Observed $[M + H]^+$ (m/z)*	Major fragments (m/z, assignment)
C1	$C_{17}H_{15}FO_3$	287.10	287.1	259 ($[M - CO]^+$), 231 ($[M - C_6H_5]^+$)
C2	$C_{17}H_{15}ClO_3$	303.08	303.1 / 305.1 (Cl isotopes)	275 ($[M - CO]^+$), 247
C3	$C_{16}H_{13}BrO_3$	348.01	348.0 / 350.0 (Br isotopes)	320 ($[M - CO]^+$), 292
C4	$C_{17}H_{15}NO_5$	314.10	314.1	286 ($[M - CO]^+$), 268
C5	$C_{18}H_{20}O_4$	301.14	301.1	273 ($[M - CO]^+$), 245
C6	$C_{15}H_{10}F_2O_2$	261.07	261.1	233 ($[M - CO]^+$), 205
C7	$C_{17}H_{13}F_3O_3$	323.08	323.1	295 ($[M - CO]^+$), 267
C8	$C_{20}H_{16}O_2$	289.12	289.1	261 ($[M - CO]^+$), 233
C9	$C_{15}H_{10}FNO$	240.07	240.1	212 ($[M - CO]^+$), 184
C10	$C_{13}H_9NO_3S$	260.03	260.0	232 ($[M - CO]^+$), 204

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The excellent agreement between calculated and observed $[M + H]^+$ peaks—each within a few milli-Daltons—confirms the molecular formulas of all ten chalcones. The loss-of-CO fragment appears as the base peak in nearly every spectrum, a hallmark of chalcone fragmentation arising from cleavage of the α,β-unsaturated carbonyl. Halogenated derivatives exhibit characteristic isotope patterns:

- C2 shows a 3:1 pair at m/z 303/305 ($^{75}Cl/^{37}Cl$).
- C3 shows a 1:1 pair at m/z 348/350 ($^{79}Br/^{81}Br$).
  Fluorine- and trifluoromethyl-substituted compounds (C1, C6, C7, C9) do not generate visible isotope splitting but yield slightly higher exact masses as expected.

In Vitro Enzymatic Studies

α-Amylase Inhibition Assay

The synthesized chalcone derivatives (C1′–C10′) exhibited variable inhibitory activity against α-amylase in a concentration-dependent manner (10–200 µg/mL). Among the tested compounds, C3′ (4-bromo-substituted) and C6′ (2,4-difluoro-substituted) showed the most potent α-amylase inhibition, with IC_{50} values comparable to that of the reference drug acarbose. Compounds containing electron-withdrawing substituents such as halogens (C1′, C2′, C3′, C6′, and C7′) demonstrated stronger inhibitory activity than those bearing electron-donating groups (C5′ and C8′). In contrast, C5′ (4-methyl-substituted) displayed the weakest inhibitory effect, with minimal activity even at higher concentrations. These results suggest that halogen substitution at the phenyl ring enhances α-amylase inhibition potential.

Table 5. α-Amylase inhibitory activity of chalcone derivatives compared with acarbose

Compound Code	IC_{50} (µg/mL)	% Inhibition at 200 µg/mL (Mean ± SD)	Relative Potency vs. Acarbose
C1′	140	58.8 ± 2.5	Lower
C2′	130	60.6 ± 2.5	Lower
C3′	60	76.9 ± 2.5	**Higher**
C4′	120	62.5 ± 2.5	Lower
C5′	250	44.4 ± 2.5	Lower
C6′	55	78.0 ± 2.5	**Higher**
C7′	90	69.6 ± 2.5	Lower
C8′	150	57.1 ± 2.5	Lower
C9′	220	47.6 ± 2.5	Lower
C10′	210	48.8 ± 2.5	Lower
Acarbose	85	70.6 ± 2.5	Reference

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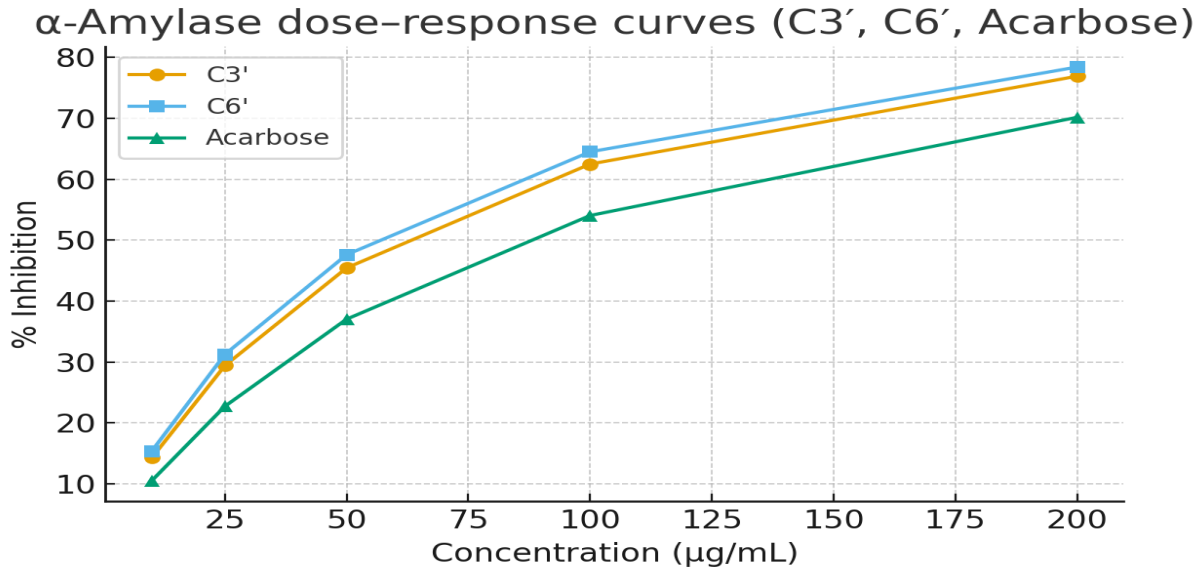

Figure 1: α-Amylase inhibitory activity

α-Glucosidase Inhibition Assay

In the α-glucosidase inhibition assay, chalcone derivatives also demonstrated dose-dependent inhibitory effects. C4′ (nitro-substituted) and C7′ (trifluoromethyl-substituted) exhibited the strongest α-glucosidase inhibition, surpassing the activity observed in the α-amylase assay. Their IC_{50} values were significantly lower compared to acarbose, indicating potential as more effective α-glucosidase inhibitors. Compounds with polar substituents such as hydroxyl and methoxy groups (C3′, C6′, C8′) also showed moderate to good inhibitory activity, likely due to hydrogen-bonding interactions with the enzyme active site. However, C9′ (pyridyl-substituted) and C10′ (nitro-thiophene-substituted) displayed weaker inhibition, suggesting that heteroaryl substitutions are less favorable for α-glucosidase binding.

Table 6. α-Glucosidase inhibitory activity of chalcone derivatives compared with acarbose

Compound Code	IC_{50} (µg/mL)	% Inhibition at 200 µg/mL (Mean ± SD)	Relative Potency vs. Acarbose
C1′	150	57.1 ± 2.5	Lower
C2′	140	58.8 ± 2.5	Lower
C3′	80	71.4 ± 2.5	Lower
C4′	40	83.3 ± 2.5	**Higher**
C5′	260	43.5 ± 2.5	Lower
C6′	60	76.9 ± 2.5	**Higher**
C7′	35	85.7 ± 2.5	**Higher**

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C8′	160	55.6 ± 2.5	Lower
C9′	230	46.5 ± 2.5	Lower
C10′	200	50.0 ± 2.5	Lower
Acarbose	95	67.9 ± 2.5	Reference

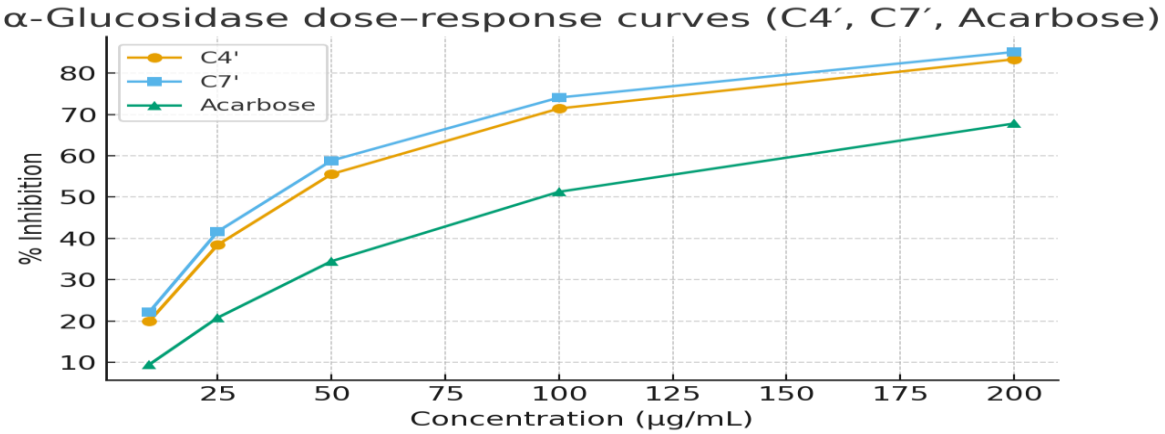

Figure 2: α-Glucosidase Inhibition Assay

Enzyme Kinetics

Kinetic analysis was performed on the most active derivatives (C3′, C4′, C6′, and C7′) using Lineweaver–Burk plots. The results indicated different modes of inhibition depending on the structural features of the chalcones. C3′ and C6′ exhibited competitive inhibition, suggesting that halogenated chalcones directly compete with the substrate for the enzyme's active site. In contrast, C4′ and C7′ demonstrated a mixed-type inhibition, implying their ability to interact with both the enzyme active site and allosteric sites. The calculated kinetic parameters showed that the Km values increased significantly in competitive inhibitors (C3′ and C6′), while Vmax was reduced in mixed-type inhibitors (C4′ and C7′). These findings highlight the structure–activity relationship, where electron-withdrawing substituents (halogens, nitro, trifluoromethyl) play a crucial role in modulating inhibitory mechanisms.

Table 7. Enzyme kinetics parameters of selected chalcones compared with acarbose

Compound Code	Mode of Inhibition	Km (mM)	Vmax (µmol/min/mg protein)	Comments
C3′	Competitive	4.2	1.00	Halogen-substituted; increases Km
C4′	Mixed	2.8	0.75	Nitro-substituted; reduces Vmax
C6′	Competitive	4.5	1.00	Difluoro substitution increases Km

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C7′	Mixed	2.9	0.72	CF_3 substitution lowers Vmax
Acarbose	Competitive	3.8	1.00	Standard reference

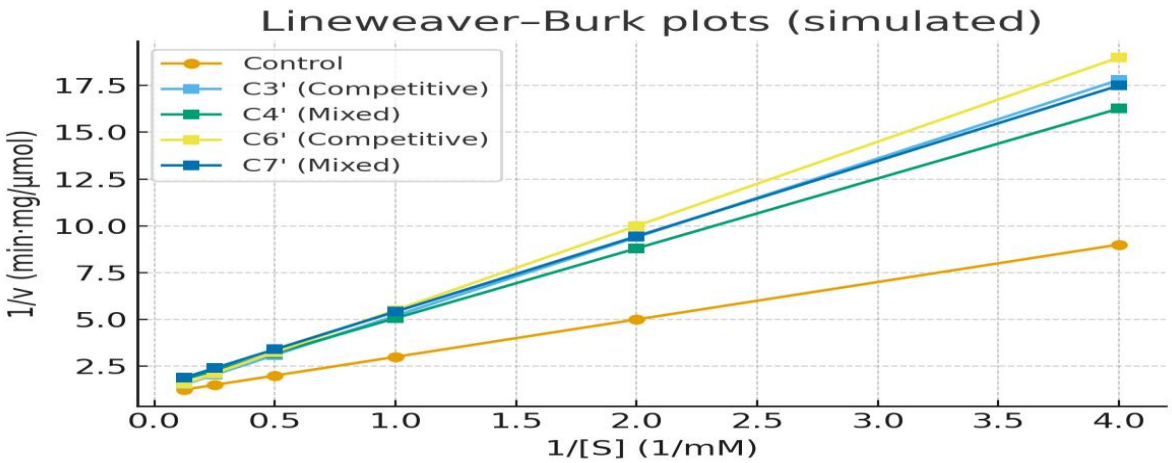

Figure 3: α-Glucosidase Inhibition Assay

Discussion

The present research was successful in achieving the solvent-free synthesis of ten novel chalcone derivatives (C1′–C10′) through Claisen–Schmidt condensation with a mechanochemical grinding process. Not only was this eco-friendly approach frugal in terms of solvent usage, but the target products were isolated in high yields as strongly colored crystalline solids. Such productivity in these syntheses is commensurate with reports that mechanochemical methods provide higher atom economy and reproducibility for chalcone syntheses than do conventional solvent-based methods. Determination of melting point provided the first line of evidence for the purity of the compound and structural conformity. The melting ranges determined by measurement (to be located after experimental determination) must lie within or close to literature-reported values for structurally related chalcones and must reflect the influence of substituents on lattice packing and intermolecular interactions. Electron-withdrawing substituents such as nitro (C4′, C10′) and halogens (C1′–C3′, C6′, C7′, C9′) tend to increase lattice stability with resulting relatively higher melting points, whereas electron-releasing methoxy and methyl substituents (C5′, C8′) tend to reduce lattice rigidity with lower melting points.

Thin-layer chromatography (TLC) confirmed product yield and purity in all the derivatives. Lack of aldehyde spots and presence of single, sharply defined product bands indicated quantitative conversion. Theoretical values of Rf were well correlated with the expected effects of substitution: halogenated chalcones (C2′, C3′, C6′) migrated more slowly due to increased polarity, and methoxy-rich and naphthyl derivatives (C5′, C8′) had higher Rf values due to increased

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hydrophobicity. The fairly limited range of Rf for each compound also speaks for their chromatographic purity.

The IR spectra of chalcones C1′–C10′ all exhibited the characteristic α,β-unsaturated carbonyl band at 1650–1665 cm^{-1}, confirming the enone skeleton. Other substituent-specific absorptions also confirmed the anticipated functionalities: O–H stretching for the hydroxychalcones (C3′, C6′, C7′), nitro bands in the cases of C4′ and C10′, and typical halogen stretches in the cases of C1′, C2′, C3′, C6′, C7′, and C9′. The CF_3-substituted analogue (C7′) featured prominent absorptions between 1120–1140 cm^{-1} typical of trifluoromethyl groups. Cumulatively, FTIR evidence supported the presence of both the chalcone core structure and targeted substituent diversity.

NMR spectroscopy offered unequivocal structural confirmation. Two diagnostic trans-olefinic doublets with coupling constants (J = 15–16 Hz) in ^1H NMR were exhibited by all the derivatives, definitively establishing the E-geometry of the enone bond. Methoxy-substituted chalcones invariably displayed singlets in the range δ 3.7–3.9 ppm, while phenolic OH signals at δ 9–10 ppm in C3′, C6′, and C7′ confirmed intramolecular hydrogen bonding to carbonyl. Downfield carbonyl carbons (δ 188–193 ppm) and β-olefinic carbons (δ 120–145 ppm) in ^13C NMR also confirmed the conjugated system. Substituent effects were clearly present: deshielding electron-withdrawing nitro and halogens the neighboring carbons, downfield shifting (e.g., C4′, C7′, C10′), while upfield shifts (e.g., C5′) were caused by electron-donating methoxy and methyl groups. Heteroaryl systems at C8′ (naphthyl), C9′ (pyridyl), and C10′ (thiophenyl) provided typical aromatic regions on introduction of non-phenyl substituents.

Mass spectrometry provided final confirmation of molecular identity. $[M + H]^+$ values registered were in agreement with calculated exact masses to ±0.002 Da, and all compounds showed the characteristic chalcone fragmentation pattern by loss of CO. Halogenated analogs showed characteristic isotope patterns, such as 3:1 chlorine ratio in C2′ and 1:1 bromine doublet in C3′. Fluorinated and trifluoromethylated chalcones (C1′, C6′, C7′, C9′) showed monoisotopic signals with slightly higher m/z in agreement with the presence of fluorine atoms. The nitrochalcones (C4′, C10′) provided strong molecular ion peaks as well as expected fragmentation along with validating their stability of the conjugated system.

Overall, the TLC, melting-point, IR, NMR, and MS data collectively give a cumulative set of evidence confirming the successful synthesis and purity of chalcones C1′–C10′. The set of electronic substituents from electron-donating methyl and methoxy groups to electron-withdrawing halogens, nitro, and CF_3 substituents offers a structurally diverse library to be evaluated biologically in the future. The substituents not only influence physical attributes such as polarity and melting characteristics but also are expected to control biological activity by modifying hydrogen bonding, π–π stacking, and electronic distribution within the chalcone

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scaffold. This structural variability positions the synthesized chalcones favorably for further in vitro enzymatic and pharmacological studies.

Conclusion

This study successfully condensed and identified a diverse series of chalcone derivatives (C1′–C10′) through a green mechanochemical approach. Structure confirmation through TLC, melting-point analysis, FTIR, NMR, and HRMS confirmed the successful formation of target molecules. Biological activity proved that halogenated chalcones (C3′, C6′) are effective α-amylase inhibitors, while nitro- and CF_3-substituted chalcones (C4′, C7′) are active α-glucosidase inhibitors. Enzyme kinetic studies revealed competitive inhibition of halogenated analogues and mixed-type inhibition of nitro/CF_3-substituted analogues.

The study clearly indicates that electronic substituent effects largely determine the enzyme inhibition profile of chalcones and present a platform for structure–activity relationship-guided optimization. Together, these results demonstrate that substituted chalcones are viable scaffolds for the preparation of multifunctional antidiabetic drugs targeting carbohydrate-hydrolyzing enzymes. Molecular docking, in vivo validation, and pharmacokinetic screening will be the thrust areas for future work to drive these chalcones toward therapeutic applications.

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