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Synthesis and evaluation of chalcone derivatives for its alpha amylase inhibitory activity

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ABSTRACT

Chalcones represent a group of compounds with interesting biological activities that are formed from Claisen-Schmidt reaction between a benzaldehyde and an acetophenone in the presence of NaOH as a catalyst and ethanol as a solvent. Four different chalcones were synthesized using various substituted aldehydes as 4-nitrobenzaldehyde, 4-hydroxybenzaldehyde, 4-chlorobenzaldehyde, and 2-furfuraldehyde. The designed molecules were successfully synthesized in the laboratory using literature methods and structures were confirmed by NMR and IR spectroscopy. For anti-diabetic activity detection alpha amylase inhibitory assay was carried out. According to the results obtained, it can be concluded that of the synthesized compound 3-(4-hydroxyphenyl)-1-phenylprop-2-en-1-one has an Alpha amylase inhibitory activity.

INTRODUCTION

Chalcone is a class of open-chain flavonoids that is not only biosynthesized by plants but also can be prepared synthetically. Chalcone is an aromatic ketone that forms the central core for a variety of important biological compounds. Other names for chalcone are benzalacetophenone and phenyl styryl ketone[1]. Chalcones show antibacterial, antifungal, antitumor and anti-inflammatory properties. They are also intermediates in the biosynthesis of flavonoids, which are substances widespread in plants and with an array of biological activities[3]. Chalcones are also intermediates in the Auwers synthesis of flavones[4]. Chalcone can be prepared by an aldol condensation between a benzaldehyde and an acetophenone in the presence of a catalyst. Aldol condensation is also known as Claisen-Schmidt rection[2].

The aldol condensation relies on the reactivity of a carbonyl group to build a new carbon-carbon bond. The aldol reaction is one of the most powerful methods available for forming a carbon-carbon bond. In this reaction, the conjugate base of an aldehyde or ketone adds to the carbonyl group of another aldehyde or ketone to give a β-hydroxyaldehyde or β-hydroxyketone product. This is the intermediate product of the crossed-aldol reaction[5].

Chalcones are popular intermediates for synthesizing various heterocyclic compounds. The compounds with the backbone of chalcones have been reported to possess various biological activities such asantimicrobial, anti-inflammatory, analgesic, antiplatelet, antiulcerative, antimalarial, anticancer, antiviral, antileishmanial, antioxidant, anti-tubercular, anti-hyperglycemic, immunomodulatory, inhibition of chemical mediators release, inhibition of leukotriene B4, inhibition of tyrosinases and inhibition of aldose reductase activities[7]. The presence of a reactive α,β-unsaturated

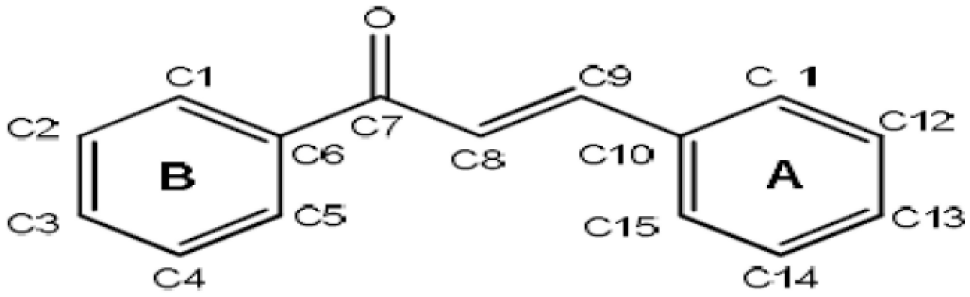

Figure 1 : Chalcone

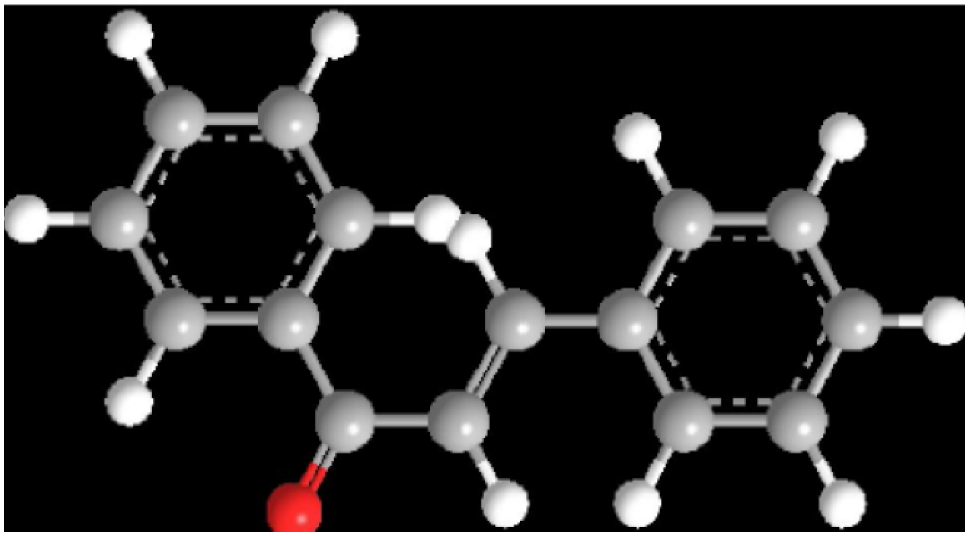

Figure 2 : Energy minimized 3D structure of chalcone

keto function in chalcones is found to be responsible for their biological activities.

Chalcones represent a group of compounds with interesting biological activities that are formed from a Claisen-Schmidt reaction between a benzaldehyde and an acetophenone in the presence of NaOH as a catalyst[6]. In this study Four different chalcones were synthesized. In this synthesis four chalcones was carried out by shaking the benzaldehyde (4-nitro, 4-hydroxy, 4-chloro, Furfural) and acetophenone in the presence of 30% sodium hydroxide and Ethanol as a solvent in iodine flask. Chalcones were obtained in high yields (72-82%) and high purity. The results seemed to indicate the success of the synthesis which is simple, highly efficient and eco-friendly.

The prevalence of diabetes mellitus is on the increase and needs to be addressed appropriately and significant advances have been made in the past few years in the isolation and preparation of several chalcone derivatives. In this study, Alpha amylase inhibitory activity of different chalcone derivatives was evaluated[8].

EXPERIMENTAL WORK

Materials

TABLE 1 : List of materials used in the study

Sr.No	Excipient	Grade	Manufacturer
1	Acetophenone	A.R	S.D. Fine Pvt. Ltd
2	Benzaldehyde	A.R	S.D. Fine Pvt. Ltd
3	4-Hydroxybenzaldehyde	A.R.	S.D. Fine Pvt. Ltd
4	4-Chlorobenzaldehyde	A.R	S.D. Fine Pvt. Ltd
5	2-furaldehyde(Furfural)	A.R.	S.D. Fine Pvt. Ltd
6	4-Nitrobenzaldehyde	A.R.	S.D. Fine Pvt. Ltd
7	sodium hydroxide	A.R	S.D. Fine Pvt. Ltd
8	Dimethyl sulfoxide	A.R	S.D. Fine Pvt. Ltd
9	Chloroform	A.R.	Lobachem
11	Petroleum ether	A.R	*Lobachem*
12	Acetone	A.R.	*Lobachem*
13	Methanol	A.R.	Lobachem
14	Magnesium Bromide	A.R	S.D. Fine Pvt. Ltd
15	Ethanol	A.R	S.D. Fine Pvt. Ltd

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Instruments

TABLE 2 : List of instrument's used in the formulation development

Sr.No	Name of the Equipment	Manufacturer
1	FT-NMR	Shimadzu
2	Magnetic Stirrer	Remi Laboratory
3	Fourier Transform Infrared Spectroscopy	Shimadzu
4	U V Spectroscopy	Shimadzu
6	Melting point apparatus	Veego
7	Hot air oven	Hexatec instruments Pvt. Ltd.

General procedures

Analytical thin-layer chromatography (TLC) was carried out on precoated plates SiO2 (silica gel 60, F 254, Merck).The spots were viewed under ultraviolet (UV) light. FTIR spectra were recorded on Perkin Elmer RX I spectrometer using KBr pellets. All melting points (m.p.) were recorded on VMP-DS (VEEGO Instruments corporation), having oil-heating system and were uncorrected. The NMR spectra were recorded on JEOL AL-300 FT-NMR spectrometer and Schimatzu 60 MHz FT-NMR with CDCl3 / DMSO-D6 as solvent using tetramethylsilane (TMS) as internal reference.

Synthesis of chalcone

The synthesis of chalconewas carried out *via* crossed Aldol or Claisen- Schmidt condensation of commercially available Acetophenone and Benzaldehyde in the presence of NaOH (30%) as a base in ethanol. The reaction was initiated by removal of a proton from the α-carbon of Acetophenone to form a resonance-stabilized enolate ion by the base. This was followed by the nucleophilic enolate attacks on the electrophilic carbonyl carbon of benzaldehyde resulting in a new carbon-carbon bond formation. This reaction joined the α-carbon of acetophenone to the carbonyl carbon of benzaldehyde to form intermediate. The final step of this reaction was protonation and deprotonation by hydroxide ion to form an α,β-unsaturated ketone, chalcone or 1, 3-diphenylpropenone as a light yellow solid in 78.33% yield. The reaction mechanism for chalcone synthesis is illustrated in Scheme 6.1.

Synthesis of designed molecules (Compound 1)

A solution Acetophenone (3.0 mL, 0.025 mole) stirred in ethanol (8 ml) was added with 2-furaldehyde (Furfural) (3.0 g, 0.025 mole) in a conical flask (25 mL) and then NaOH 30% (4 mL) was added drop wise into it. The mixture was stirred in ice cold water bath until it solidified. Then solidified mass is kept in cold condition overnight and after that solidified mass separated and dried on room temperature to give compound.

Synthesis of designed molecules (Compound 2)

A solution Acetophenone (3.0 mL, 0.025 mole) stirred in ethanol (8 ml) was added with 4-Chlorobenzaldehyde (3.0 g, 0.025 mole) in a conical flask and then NaOH 30% (4 mL) was added drop wise into it. The mixture was stirred in ice cold water bath until it solidified. Then solidified mass is kept in cold condition overnight and after that solidified mass separated and dried on room temperature to give compound.

Synthesis of designed molecules (Compound 3)

A solution Acetophenone (3.0 mL, 0.025 mole) stirred in ethanol (8 ml) was added with 4-Hydroxybenzaldehyde (3.0 g, 0.025 mole) in a conical flask and then NaOH 30% (4 mL) was added drop wise into it. The mixture was stirred in ice cold water bath until it solidified. Then solidified mass is kept in cold condition overnight and after that solidified mass separated and dried on room temperature to give compound.

Synthesis of designed molecules (Compound 4)

A solution Acetophenone (3.0 mL, 0.025 mole) stirred in ethanol (8 ml) was added with 4-nitrobenzaldehyde (3.0 g, 0.025 mole) in a conical flask and then NaOH 30% (4 mL) was added drop wise into it. The mixture was stirred in ice cold water bath until it solidified. Then solidified mass is kept in cold condition overnight and after that solidified mass separated and dried on room temperature to give compound.

Enzymatic assay of alpha-amylase

Reagent preparation

(A) Starch solution

0.5% (w/v) Starch Solution (Starch) was prepared in 20 mM Sodium Phosphate Buffer (pH-6.9)and solubilisation of starch was done by heating the starch

solution in a glass beaker directly on a water bath using constant stirring for 15 minutes.

(B) Colour reagent solution

Colour reagent was prepared by addition of Sodium Potassium Tartrate Solution to 96 mM 3, 5-Dinitrosalicylic Acid SolutionWith stirring, and was stored in amber colored bottle and protected from light.

(C) 0.2% (w/v) maltose

Standard Solution was prepared by dissolving maltose monohydrate in to distil water.

(D) Alpha-amylase solution

Immediately before use, alpha amylase solution 2mg/ml in ice cold distil water was prepared.

Procedure

TABLE 3 : Reagents and their quantity for preparation of standard curve

Standard	Std 1	Std 2	Std 3	Std 4	Std 5	Blank
0.2%w/vs maltose solution	0.2	0.4	0.6	0.8	1.0	-
Distilled water	1.8	1.6	1.4	1.2	1.0	2.0
Colour reagent	1.0	1.0	1.0	1.0	1.0	1.0

Standard curve method

A standard curve was plotted by pipetting (in millilitres) the following reagents into a volumetric flaskAnd kept on boiling water bath for exactly 15 minutes, and then cool on ice to room temperature and Water was added 9.00 ml in each flask and Mix by inversion and record the A540nm for the Standards and Standard Blank using a suitable spectrophotometer.

For test incubation, test and blank solutions were prepared.

For test solution preparation 1 ml starch solution was equilibrated at 25°C and 1 ml freshly prepared enzyme solution was added and solution was mixed and incubated at 25°C exactly for 0,1,2,3,6,12 and 24 minutes then 1 ml colour reagent was added and solution was kept on boiling water bath exactly for 15 minutes then cool the solution on ice to room temperature and 9 ml water was added.

For blank solution preparation 1 ml starch solution was equilibrated at 25°C and incubated at 25°C exactly for 3 minutes then 1 ml freshly prepared enzyme solution and 1 ml colour reagent were added and solution was kept on boiling water bath exactly for 15 minutes then cool the solution on ice to room temperature and 9 ml water was added.

Solutions were mixed and absorbance at 540 nm was recorded using uv spectrophotometer.

Calculation

Standard curve

DA540nm Standard = A540nm Std - A540nm Std Blank

Plot the DA540nm of the Standards vs. milligrams of Maltose.

Sample determination

DA540nm Sample = A540nm Test - A540nm Blank

Determine the milligrams of Maltose liberated using the standard Curve.

$$\text{Units/ml enzyme} = \frac{(\text{mg of Maltose released})\,(df)}{1} \quad (1)$$

Where, df = dilution factor; 1 = Volume (in milliliter) of enzyme used;

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

Alpha amylase inhibitory activity assay

Procedure

Crude α –amylase was dissolved in ice-cold distilled water to give a concentration of 4 unit/ml solution. Starch (0.5% w/v) in 20mM phosphate buffer (pH 6.9) containing 6.7mM sodium chloride, was used as a substrate solution. Experiments were performed with three replicate determinations for each experiment.

40 µl of plant extract (20 mg/ml in DMSO), 160µl of distilled water and 400µl of starch were mixed in a screw top plastic tube. The reaction was started by the addition of 200µl of the enzyme solution. The tubes were incubated at 25°C for a total of 3 min. Final concentrations in the incubation mixture were plant extract,1 mg/ml, 0.25% (w/v) starch and 1 unit/ml enzyme. At intervals from addition of enzyme (1, 2 and 3 min), 200µl mixture was removed and added into a separate tube containing 100µl DNS colour reagent solution (96mM 3,5-dinitrosalicylic acid, 5.31M sodium potassium tartarate in 2M NaOH) and placed into a 85°C water bath. After 15 min, this mixture was diluted with 900 µl distilled water and removed from the water bath. α-Amylase activity was determined by measuring the ab-

sorbance of the mixture at 540 nm.

Control incubations, representing 100% enzyme activity were conducted in an identical fashion replacing plant extract with DMSO (40 µl).

For blank incubations (to allow for absorbance produced by the plant extract), the enzyme solution was replaced with distilled water and the same procedure was carried out as above. A separate set of incubations was prepared for the reaction of $t = 0$ min, adding samples to the DNS solution immediately after addition of enzyme.

Calculation

The absorbance (A) due to maltose generated was calculated as:

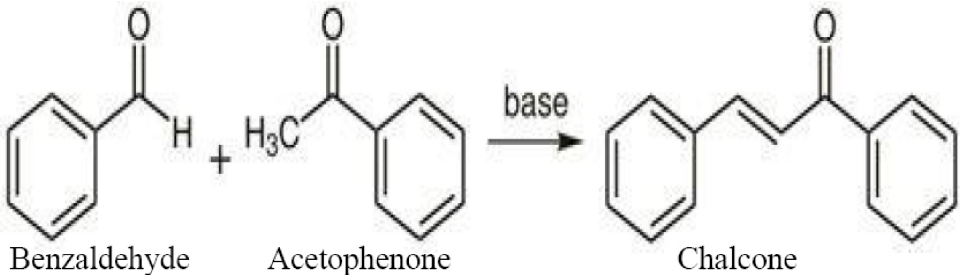
A540nm control or plant extract = A540nm Test - A540nm Blank

From the net absorbance obtained, the % (w/v) of maltose generated was calculated from the equation obtained from the maltose standard calibration curve (0.2% w/v maltose).

Percent of inhibition was calculated as 100 – % reaction at $t = 3$ min whereby the

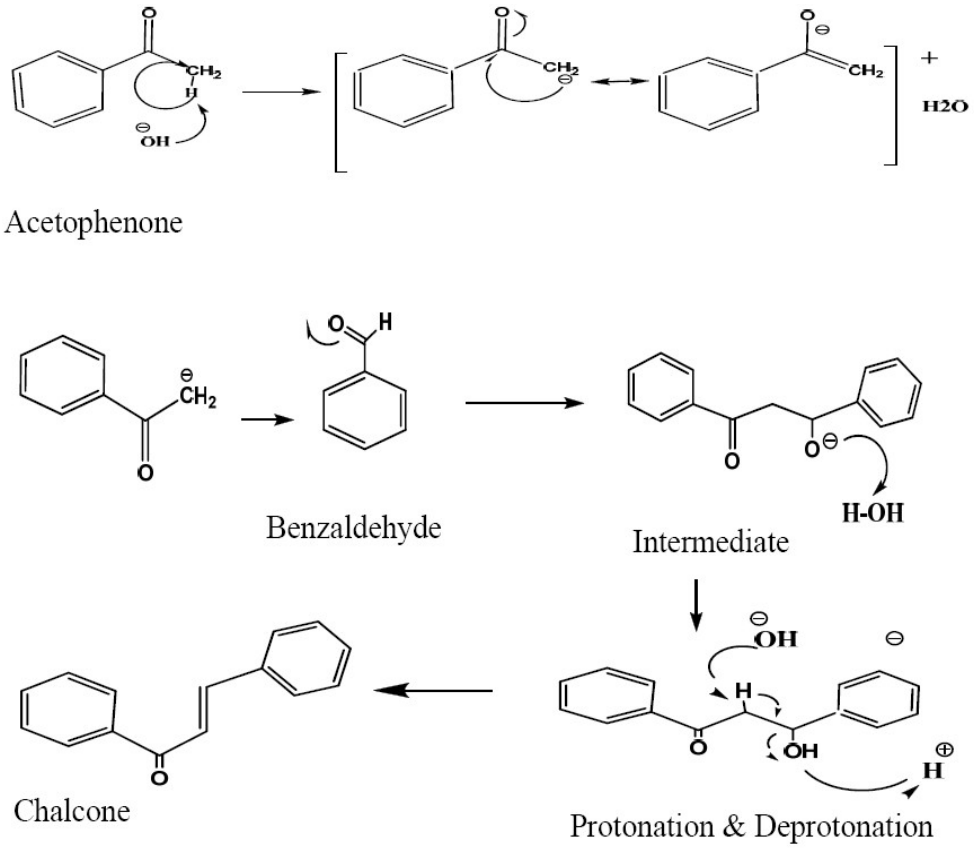
% reaction = (mean maltose in sample/mean maltose in control) ×100

Synthesis of chalcone



Scheme 1 : Synthesis of chalcone by general rocedure

Mechanism



Scheme 2 : Mechanism for Claisen-Schmidt condensation of acetophenone and benzaldehyde

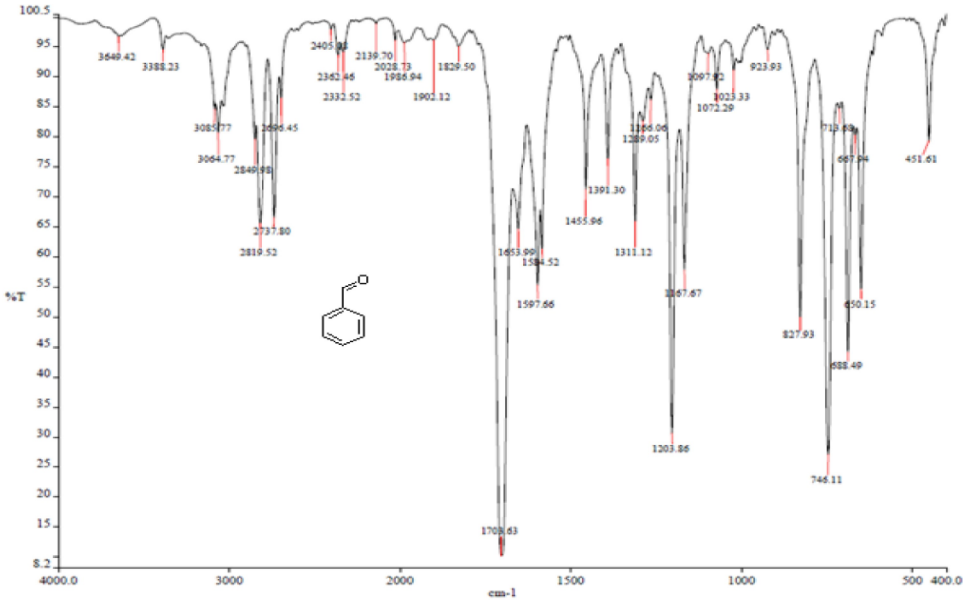


Figure 4 : Representative IR spectra of acetophenone

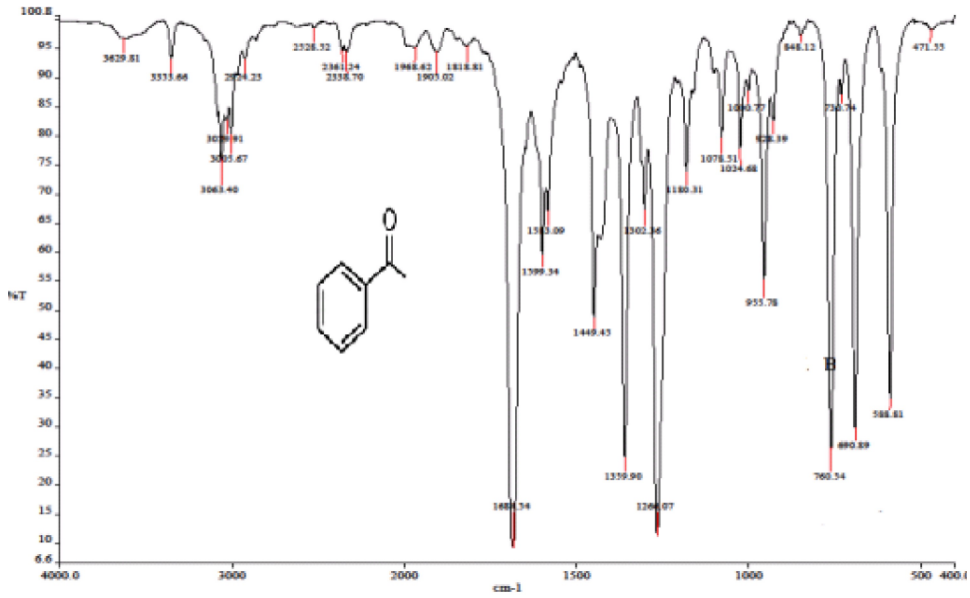


Figure 3 : Representative IR spectra of benzaldehyde

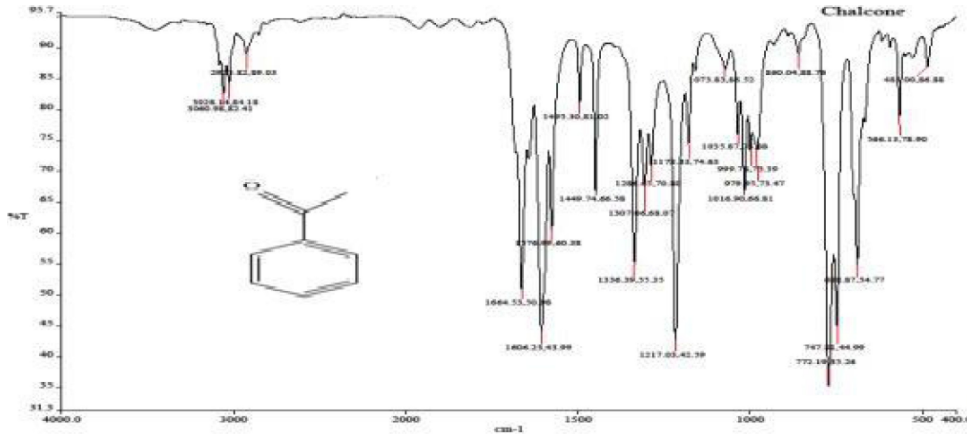


Figure 5 : Representative IR spectra of chalcone

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TABLE 4 : Description for compound synthesized by general procedure

Tests	Inference	
Colour	Yellow	
State	Solid	
Odour	Aromatic	
Yield	82%	
Melting Point	58-60 °C	
UV λmax	306 nm	
IR frequencies (cm-1)	C=O	1664.21
	C=C (Aromatic)	1577.77, 1449.71
	C=C (Olefinic)	1606.26

The representative IR spectra for the starting material & the product were depicted below:

Synthesis of designed molecules (Compound 1)

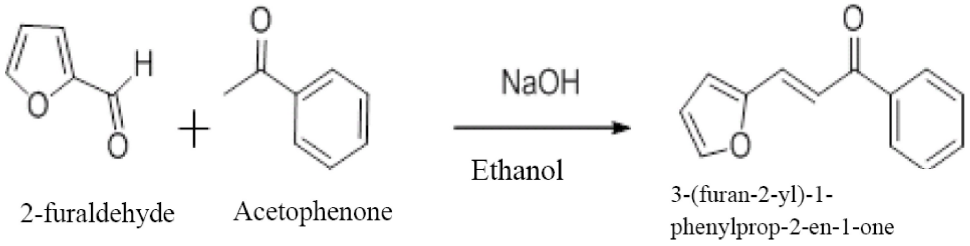
The representative IR spectra for the starting material & the product were depicted below

Synthesis of designed molecules (Compound 2)

The representative IR spectra for the starting material & the product were depicted below

Synthesis of designed molecules (Compound 3)

The representative IR spectra for the starting material & the product were depicted below



Scheme 3 : Synthesis of 3-(furan-2-yl)-1-phenylprop-2-en-1-one by general procedure

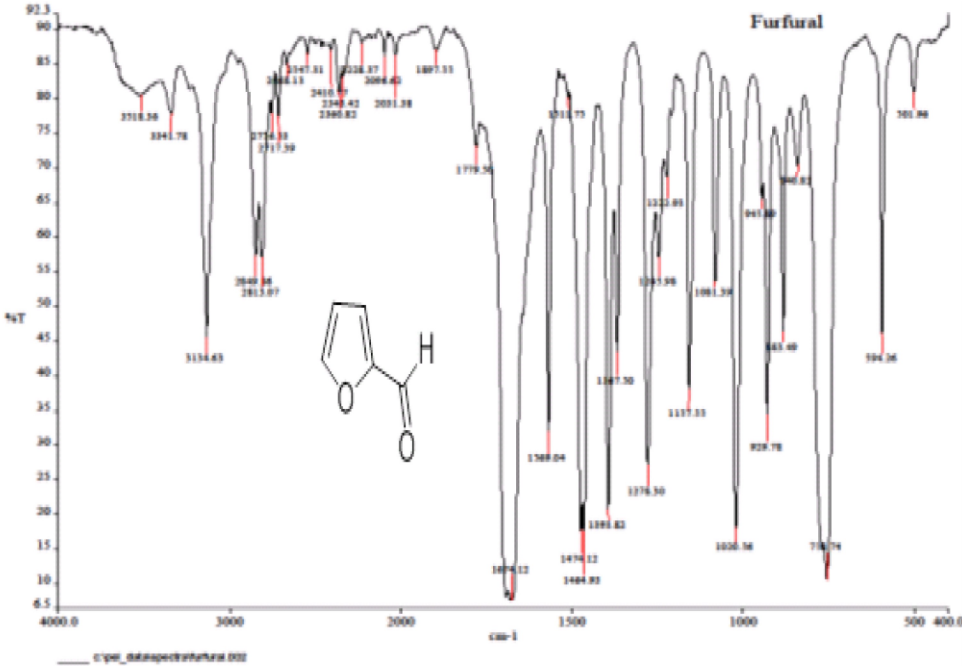


Figure 6 : Representative IR spectra of 2-furaldehyde

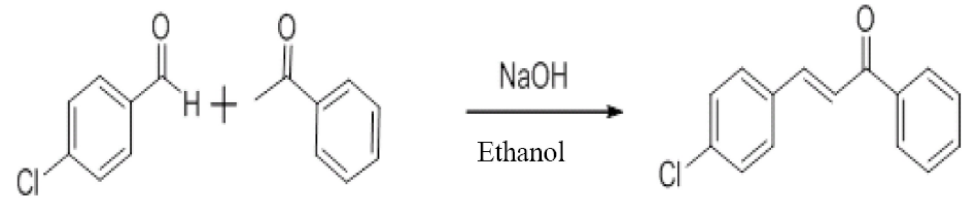
Synthesis of designed molecules (Compound 4)

The representative IR spectra for the starting material & the product were depicted below

^{1}HNMR spectrum of compound is showing signals for both aliphatic and aromatic protons. ^{1}HNMR spectrum showed a pair of doublets corresponding the range of 7.82 to 7.68 ppm and 8.0 to 8.33 ppm respectively. ^{1}HNMR ($CDCl_3$) δ 8.24–8.30 (e, 2H, 5H, 3H), 8.0–8.05 (e, 2H, 2H, 6H), 7.78-7.82 (a, 2H, 6'H, 2'H), 7.6-7.68 (b, 2H, 5'H, 2'H), 7.5-7.56 (c, 3H, 3'H, 4'H, 5'H).

Alpha amylase inhibitory activity assay

TABLE 9 indicates that 3-(4-hydroxyphenyl)-1-phenylprop-2-en-1-one (sample 3) showing inhibition compared to control sample after time interval.



4-Chlorobenzaldehyde Acetophenone

3-(4-chlorophenyl)-
1-phenylprop-2-en-1-one

Scheme 4: Synthesis of 3-(furan-2-yl)-1-phenylprop-2-en-1-one by general procedure

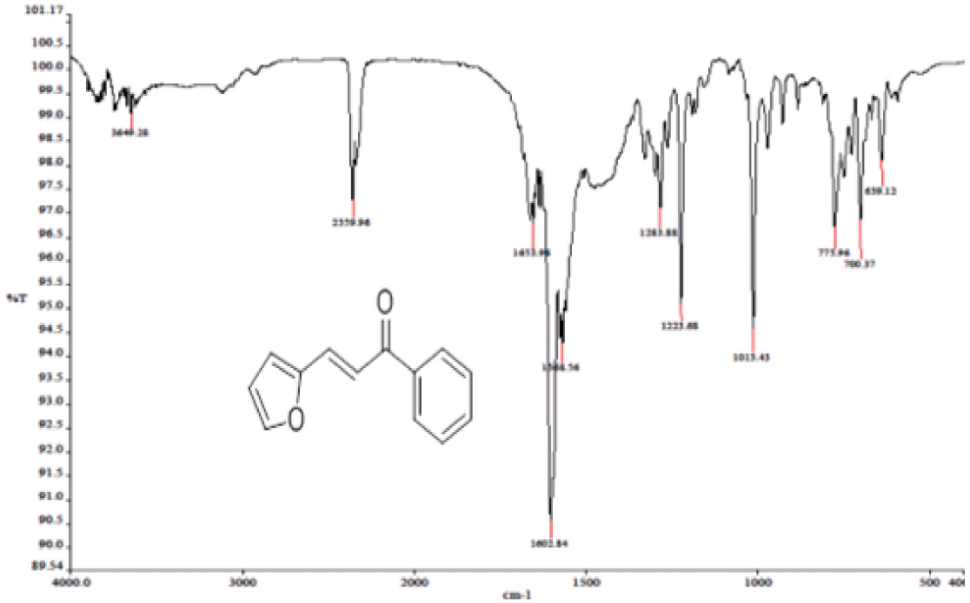


Figure 7 : Representative IR spectra of compound 1

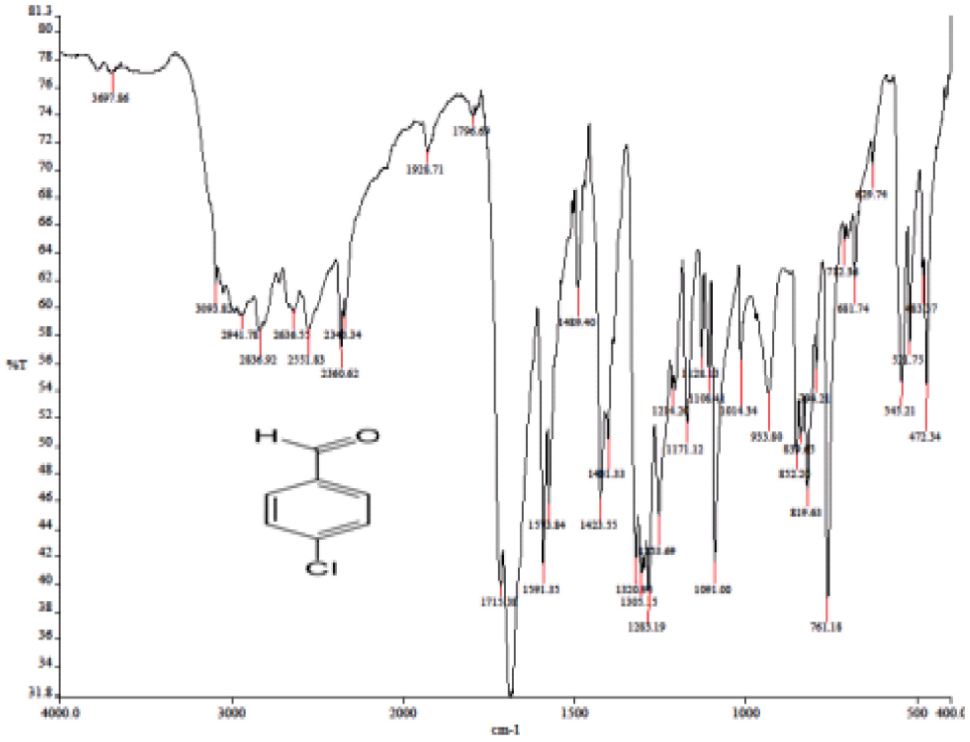


Figure 8 : Representative IR spectra of 4-chlorobenzaldehyde

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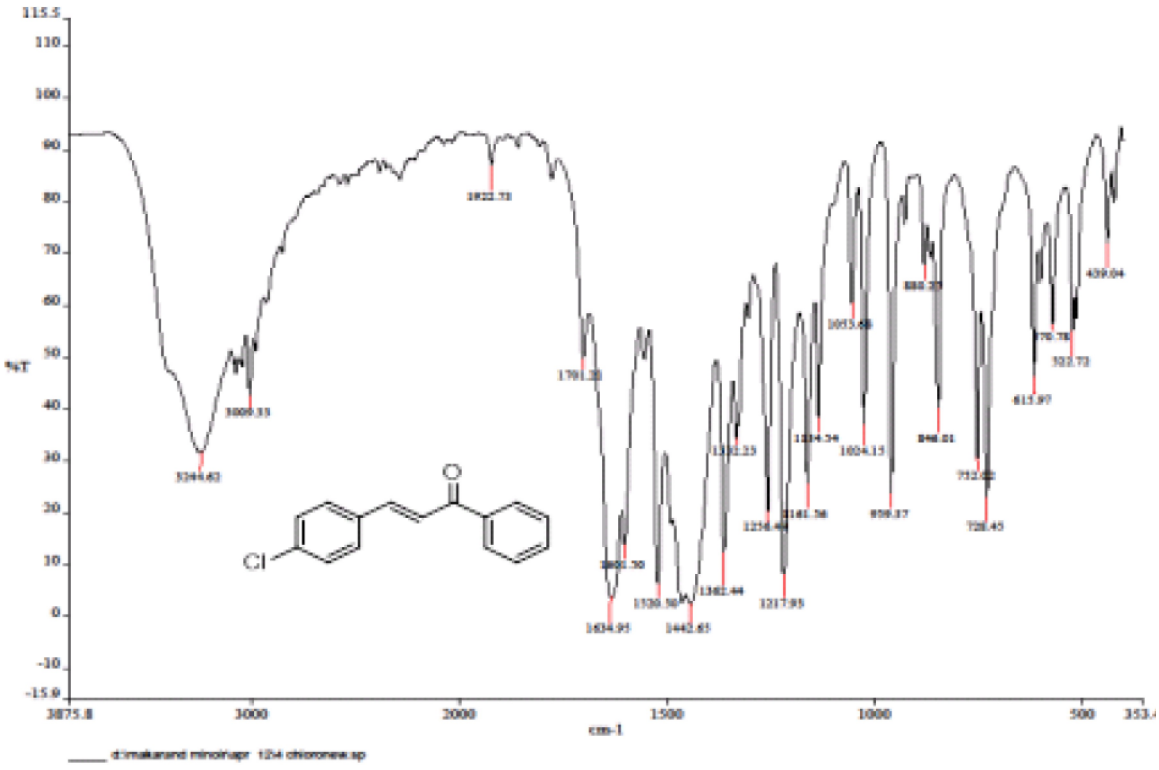


Figure 9 : Representative IR spectra of compound 2

TABLE 5 : Description of compound-1 synthesized by general procedure

Tests		Inference
Colour		Yellowish brown
State		Solid
Odour		Aromatic
Yield		70%
Melting Point		132-135 °C
UV λmax		321 nm
IR frequencies (cm-1):	C=O	1660.45
	C=C	1766.24
	Ar-H	639.41, 700.21

TABLE 6 : Description of compound-2 synthesized by general procedure

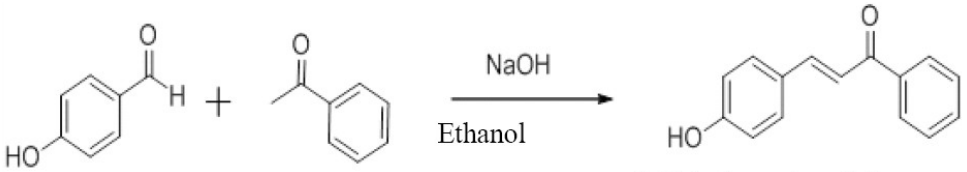
Tests		Inference
Colour		Light Yellow
State		Solid
Odour		Aromatic
Yield		77%
Melting Point		110-113 °C
UV λmax		313.85 nm
IR frequencies (cm-1):	-CL	788.21
	C=C	1190
	C=O	1634.26

TABLE 7 : Description of compound-3 Synthesized by general procedure

Tests		Inference
Colour		Brownish Yellow
State		Solid
Odour		Aromatic
Yield		74%
Melting Point		147-149 °C
UV λmax		345 nm
IR frequencies (cm-1):	-OH	3146
	C=C	1638.5, 1485.8
	C-O	1152.1

TABLE 8 : Description of compound-4 synthesized by general procedure

Tests		Inference
Colour		Brownish Yellow
State		Solid
Odour		Aromatic
Yield		81%
Melting Point		154-157 °C
UV λmax		315.87 nm
IR frequencies (cm-1):	$-NO_2$	1513.21
	C=O	1645.56
	Ar-CH	1449.23



4-Hydroxybenzaldehyde Acetophenone 3-(4-hydroxyphenyl)-1-phenylprop-2-en-1-one

Scheme 5 : Synthesis of 3-(4-hydroxyphenyl)-1-phenylprop-2-en-1-one by general procedure

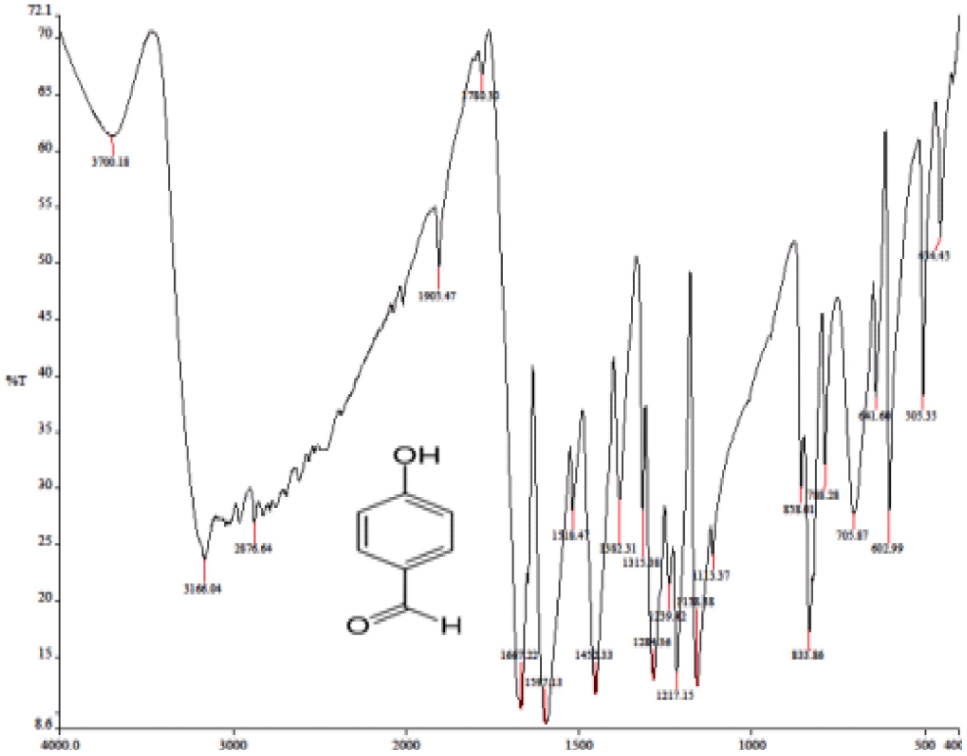


Figure 10 : Representative IR spectra of 4-hydroxybenzaldehyde

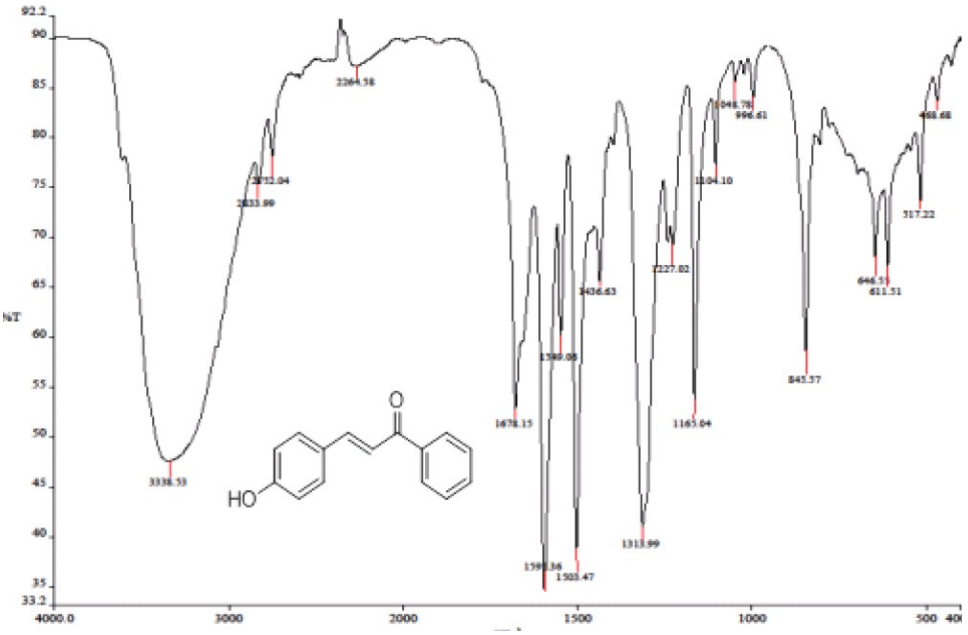


Figure 11 : Representative IR spectra of compound 3

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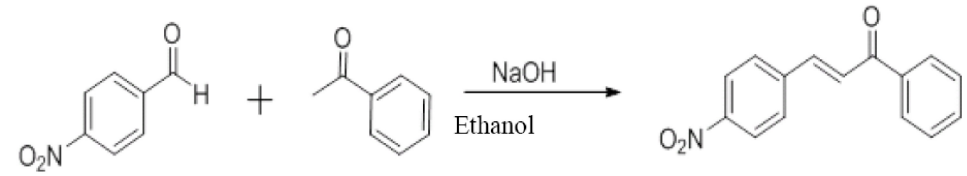

4-nitrobenzaldehyde Acetophenone

3-(4-nitrophenyl)-1-phenylprop-2-en-1-one

Scheme 6 : Synthesis of 3-(4-nitrophenyl)-1-phenylprop-2-en-1-one by general procedure

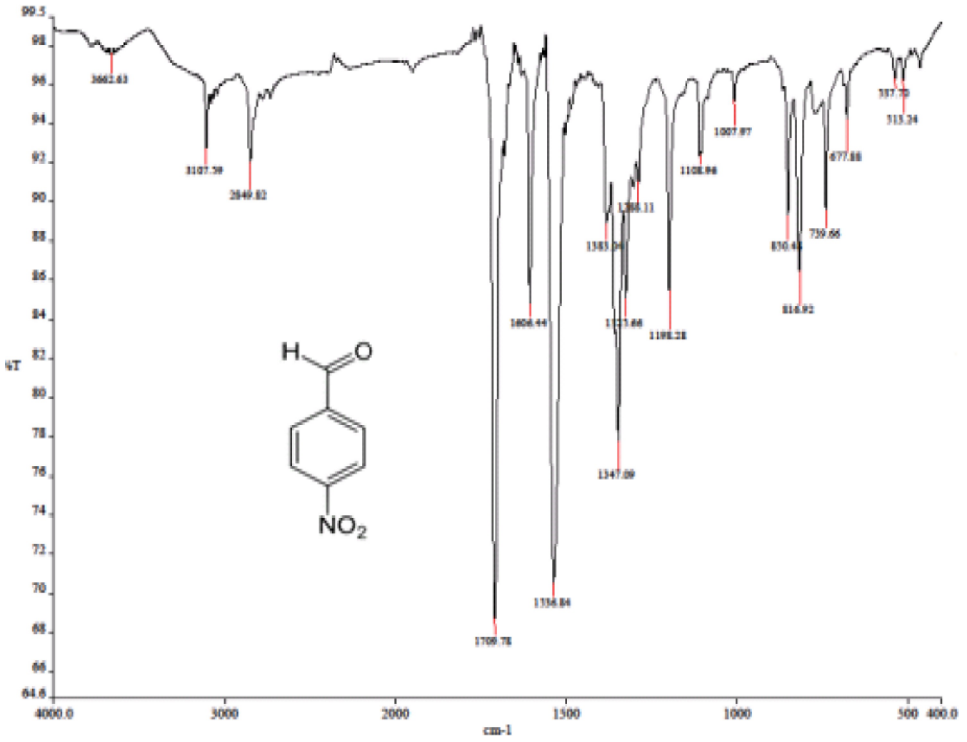

Figure 12 : Representative IR spectra of 4-nitrobenzaldehyde

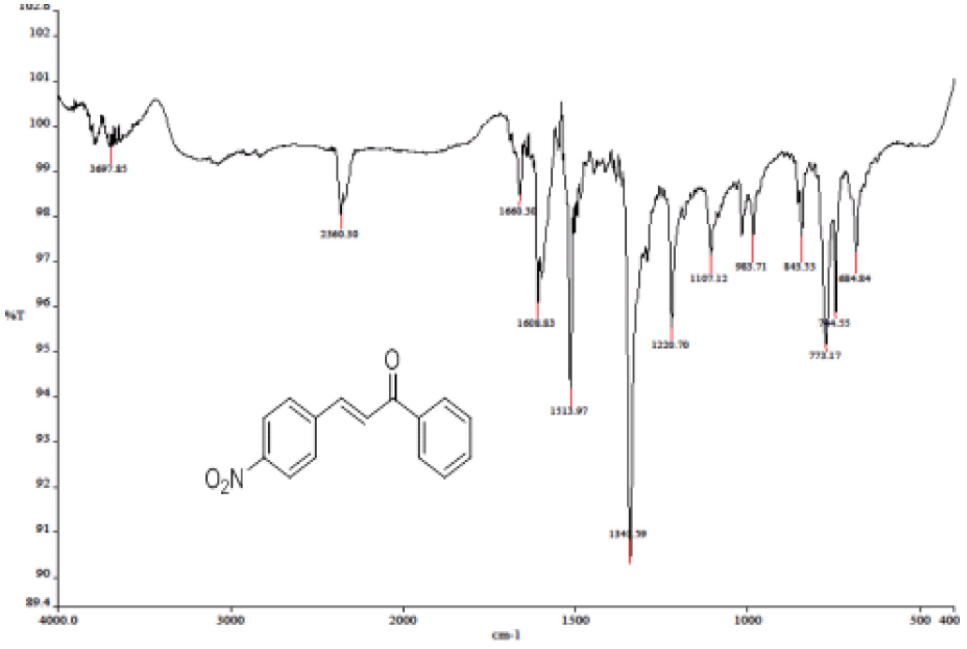

Figure 13 : Representative IR spectra of compound 4

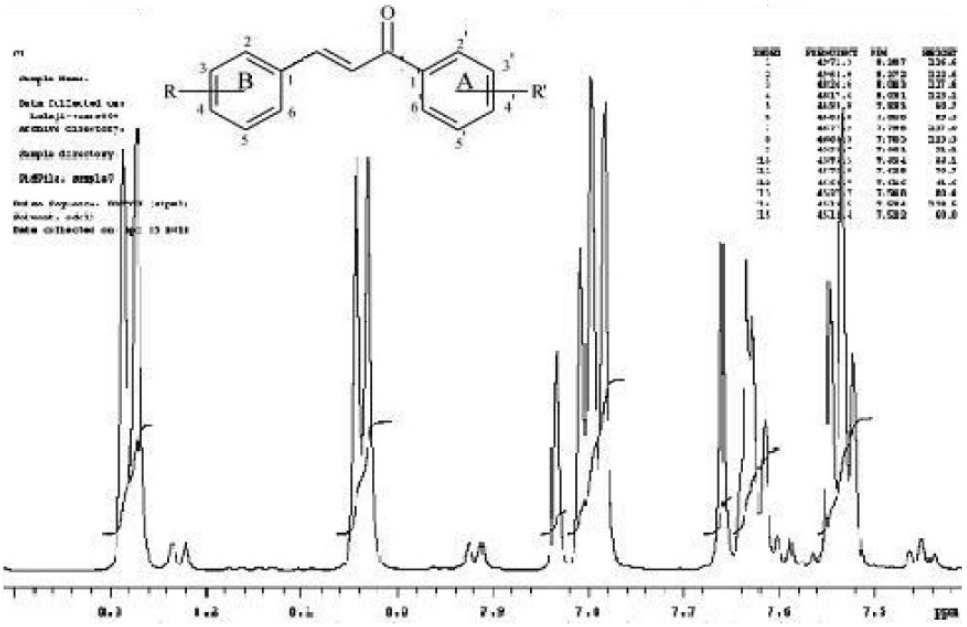

Figure 14 : NMR of compound 4 synthesized by general procedure

TABLE 9 : Alpha amylase inhibitory activity assay compounds

Sample	mg of maltose liberated(mean±SEM)				
Time(Min.)	**0**	**1**	**2**	**3**	**6**
Control	0.54±0.021	0.57±0.011	0.59±0.007	0.58±0.006	0.58±0.013
1	1.35±0.024	1.20±0.013	1.22±0.018	1.28±0.021	1.29±0.02
2	2.35±0.016	2.43±0.024	2.57±0.032	2.72±0.038	2.87±0.032
3	0.2±0.0011	0.22±0.018	0.30±0.067	0.35±0.23	0.35±0.21
4	5.32±0.21	5.45±0.23	5.46±0.21	5.44±0.32	5.23±0.12

SUMMARY AND CONCLUSION

This study describes the usefulness of chalcone and derivatives as valuable agents for various applications in the field of medicinal chemistry. The studies show that they are particularly useful as Anti-diabetic agents

Chalcones represent a group of compounds with interesting biological activities that are formed from Claisen-Schmidt reaction between a benzaldehyde and an acetophenone in the presence of NaOH as a catalyst and ethanol as a solvent.

In this study four different chalcones were synthesized using various substituted aldehydes as 4-nitrobenzaldehyde, 4-hydroxybenzaldehyde, 4-chlorobenzaldehyde, and 2-furfuraldehyde. The designed molecules were successfully synthesized in the laboratory using literature methods and structures were confirmed by NMR and IR spectroscopy.

For anti-diabetic activity detection alpha amylase inhibitory assay was carried out. According to the results obtained, it can be concluded that of the synthesized compound 3-(4-hydroxyphenyl)-1-phenylprop-2-en-1-one has an Alpha amylase inhibitory activity.

However, there are still interesting aspects to explore in this project and some of these aspects are outlined below.

Future perspectives

- Optimization of synthesis by all aspects.
- Complete characterization of synthesized Compounds.
- Synthesis of chalcone by using different ketones.

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