Synthesis, Alpha Amylase Inhibition, Antimicrobial, Antioxidant and Molecular Docking Studies of *p*-Coumaric Acid Derivatives

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ABSTRACT: A series of *p*-coumaric acid derivatives were designed to yield compounds having antidiabetic, antimicrobial and antioxidant activity. Here, we synthesized derivatives, docked them and evaluated all the synthesized products by biological methods. Results of this study revealed that amongst all the compounds, compound 1 displayed activity against inhibition of pancreatic alpha amylase, antimicrobial, antioxidant and showed maximum binding energy. For docking studies AutoDock4.2 was used, for inhibition test of alpha amylase activity, calorimetric method was adopted. Antimicrobial activity was evaluated by tube dilution method and antioxidant activity was done by DPPH free radical assay. © 2017 iGlobal Research and Publishing Foundation. All rights reserved.

INTRODUCTION

Type 2 diabetes (T2DM) is most common form of diabetes mellitus and characterized by hyperlipidemia, hyperglycemia, insulin resistance, high blood glucose level and glycosuria [15]. T2DM was formerly called adult-onset or non-insulin-dependent, resulting from ineffective insulin used by body [1]. -1,4-Glycosidic bonds are broken down by -amylase which is a glycoside hydrolase enzyme. Hydrolyses of disaccharides to monosaccharide can be done by -glucosidase and absorbed into the blood stream [2]. -amylase and -glucosidase acts as key enzyme for the absorption and digestion of carbohydrates and for the modulation of the pathologic postprandial hyperglycemia, the enzymes have been identified as important therapeutic targets for T2DM suffering patients [1].

Plants are the rich sources of hypoglycemic, hypolipidemic compounds, antioxidants such as phenolic acids and related polyphenols. [3]. *P*-coumaric acid is the most common hydroxycinnamic acid widely distributed in foods, fruit juices and vegetables [4]. *P*-coumaric acid exhibits interesting pharmacological activities including antioxidant [5], antimicrobial [6], antidiabetic [7], anticancer [8], anti-inflammatory [9], antihypertensive [10], antiulcer [11] and antimelanogenic [12].

The aim of the present research is to synthesize various *p*-coumaric acid derivatives and evaluate them for antidiabetic, antimicrobial and antioxidant potential.

MATERIALS AND METHODS

To prove the feasibility of the synthesis, experiments were performed using various solvents like thionyl chloride, toluene, ether and methanol etc. All reagents and solvents used in study were of analytical grade and procured locally. Melting points were determined in open capillary tubes on a Sonar melting point apparatus and are uncorrected. Reaction progress was monitored by thin layer chromatography on silica gel coated glass plate and the purity of the compounds was ascertained by single spot on TLC sheet. H[1] nuclear magnetic resonance (H[1] NMR) spectra were recorded on

Agilent NMR 4300 MHz spectrometer using DMSO-d6 solvent and are expressed in parts per million (, ppm). Infrared (IR) spectra were recorded on a Shimadzu FT-IR spectrophotometer.

General procedure for synthesis of (*E*)-3-(4-hydroxyphenyl) acryloyl *chloride*.

Thionyl chloride (0.15mol) was added gently to *p*-coumaric acid (0.125 mol) in a round bottom flask. Then after addition of thionyl chloride, the mixture was stirred for 4 to 5 hours and heated to 80 ^{0}C for 30- 40 min in water bath. The end point of the reaction was detected by using thin layer chromatography. After completion of the reaction, solid mass was filtered and washed with toluene to obtain (*E*)-3-(4-hydroxyphenyl) acryloylchloride.

General procedure for synthesis of (E)- quinolin-8-yl 3-(4-hydroxyphenyl) acrylate

The solution of 8-hydroxy quinolin (0.005 mol) in ether (7mL) was added to a solution of (*E*)-3-(4-hydroxyphenyl) acryloylchloride (0.15mol) in ether (7mL). Then the reaction mixture was heated to 80- 82^{0}C for 60 min in water bath. The reaction monitoring was done using TLC. The mixture was cooled at 37°C and evaporation of solvent yielded the crude ester which was purified by recrystallization with alcohol.

General procedure for synthesis of amides.

The solution of corresponding amine (0.005 mol) in ether (7 mL) was added dropwise to a solution of (*E*)-3-(4-hydroxyphenyl) acryloyl chloride (0.15mol) in ether (7 mL) and maintained temperature at 0-10 ^{0}C (**Scheme 1**). The reaction mixture was stirred for 50 - 60 min and the precipitated amide was separated out. The crude amide, **2-14** (**Table 1**) was recrystallized with alcohol.
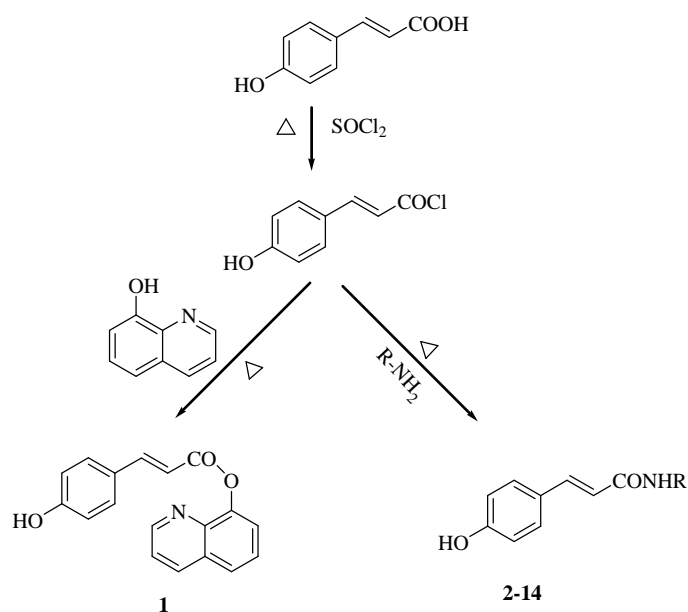
Scheme 1: Synthesis of *p*-coumaric acid derivatives

Table 1: Substituent used for targeted compounds

Comp.	R	Comp.	R
2.	—⟨phenyl⟩—F	**3.**	—⟨phenyl⟩
4.	Cl ⟨phenyl⟩—	**5.**	O₂N ⟨phenyl⟩—
6.	—⟨phenyl⟩—NO₂	**7.**	O₂N ⟨phenyl⟩—NO₂
8.	—⟨phenyl⟩—Cl	**9.**	H₃C ⟨phenyl⟩ H₃C
10.	F ⟨phenyl⟩—	**11.**	H₃CO ⟨phenyl⟩—
12.	OCH₃ ⟨phenyl⟩—	**13.**	—⟨phenyl⟩—OCH₃
14.	H₃C ⟨phenyl⟩—CH₃		

Table 2: Physicochemical properties of synthesized *p*-coumaric acid derivatives (1-14)

Comp.	Mol. Formula	M. Wt.	m.p. (^{0}C)	R_f Value*	% Yield
1.	$C_{18}H_{13}NO_2$	291.13	212-215	0.42	78.40
2.	$C_{15}H_{12}FNO_2$	257.26	217-219	0.59	68.96
3.	$C_{16}H_{15}NO_2$	253.3	219-223	0.45	62.06
4.	$C_{16}H_{14}ClNO_2$	287.74	228-230	0.80	97.77
5.	$C_{16}H_{14}N_2O_4$	298.29	232-234	0.81	53.79
6.	$C_{15}H_{12}N_2O_4$	284.27	237-239	0.21	59.09
7.	$C_{15}H_{11}N_3O_6$	329.26	243-245	0.58	81.37
8.	$C_{15}H_{12}ClNO_2$	273.71	250-252	0.44	70.34
9.	$C_{17}H_{17}NO_2$	267.32	257-258	0.52	50.34
10.	$C_{15}H_{12}FNO_2$	257.26	261-263	0.71	56.81
11.	$C_{16}H_{15}NO_3$	269.3	267-269	0.36	75.55
12.	$C_{16}H_{15}NO_3$	269.3	271-273	0.50	79.44
13.	$C_{16}H_{15}NO_3$	269.3	275-278	0.20	69.65
14.	$C_{17}H_{17}NO_2$	267.32	283-285	0.46	57.95

*TLC mobile phase:-Chloroform:Benzene (3:7)

RESULTS AND DISCUSSION

Physicochemical properties of the synthesized derivatives of p-coumaric acid was tabulated in **Table 2**.

Antidiabetic activity: The inhibition test for alpha amylase was done by calorimetric method [13]. Briefly, the assay mixture contained phosphate buffer pH 6.9 [14]. 1 ml of each synthesized compounds and 1 ml enzyme solution were mixed in a test tube and incubated at 25 ^{0}C for 10 min. To 1 ml of this mixture was added 1 ml of starch solution and the test tube was incubated at 25 ^{0}C for 10 min. Then, 1 ml of colour reagent was added and the closed test tube was placed into water bath at 85 ^{0}C for 10 min. After 15 min, the reaction mixture was removed from the water bath, cooled and diluted with 9 ml distilled water and the absorbance value was determined at 540 nm in UV spectrophotometer. Acarbose solution was used as positive control. Compounds concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted as inhibition percentage against compound concentration [15]. All the synthesized compounds were tested against diastase amylase by using calorimetric method and IC_{50} are shown in **Table 3**. Acarbose was used as a standard in the assay with IC_{50} value of 0.19 µM/ml. Compound 1 (0.21µM/ml), compound 6 (0.24µM/ml) were found to be active on comparison with standard. From the results of -amylase inhibitory activity it was found that compound 1 and 6 had good amylase inhibition activity.

The inhibition percentage of -amylase was calculated using following formula:

$$I\% = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

Where,

$A_{control:}$ Absorbance of the control reaction

A_{sample}: Absorbance of the test compound

Table 3: IC_{50} value of synthesized compounds for -amylase activity

Compound	IC_{50} value µM/ml
1.	0.21
2.	0.25
3.	0.38
4.	0.30
5.	0.29
6.	0.24
7.	0.27
8.	0.32
9.	0.39
10.	0.29
11.	0.35
12.	0.27
13.	0.31
14.	0.32
Acarbose*	0.19

* Standard

Antimicrobial assay: The synthesized derivatives was evaluated against Gram-positive bacteria: *Staphylococcus aureus* MTCC 3160, *Bacillus subtilis* MTCC 441, Gram-negative bacterium: *Escherichia coli* MTCC 443 and fungal strains: *Candida albicans* MTCC 227 and *Aspergillus niger* MTCC 281 using tube dilution method using ciprofloxacin and fluconazole as standard drugs for antibacterial and antifungal activity, respectively [6]. Dilutions of test and standard compounds were prepared in double-strength nutrient

broth-I.P. (bacteria) or sabouraud dextrose broth I.P. (fungi). The samples were incubated at 37°C for 24 hr (bacteria), at 25°C for 7 days (*A. niger*), and at 37°C for 48 hr (*C. albicans*), and the results were recorded in terms of minimum inhibitory concentration (MIC) [16].

All the synthesized compounds (1-14) were investigated for their *in vitro* antibacterial activity against Gram-positive bacteria (*S.aureus, B.subtilis*), Gram-negative bacterium (*E.coli*) and fungal strains (*C.albicans* and *A.niger*) by tube dilution method and the results are reported in **Table 4**. Compounds 1, 2, 8 and 10 showed potent antibacterial activity against the tested strains. In particular, compounds 1 and 8, which possess 8-hydroxy quinoline and 4-chloro groups respectively, were found to be active (MIC: 0.48μM/ml and 0.49μM/ml respectively) against *E.coli* with MIC comparable to Norfloxacin (MIC: 0.47μM/ml). Compound 1, 2 and 10 were potent against *B. subtilis* and *S.aureus* (0.49μM/ml, 0.48μM/ml and 0.48μM/ml respectively) which possess 8-hydroxy quinoline, 4-flouro and 2-flouro respectively, as compared to the standard Norfloxacin (MIC: 0.47μM/ml), whereas in an antifungal activity it was observed that compound 1 and 5 (MIC:0.51μM/ml) having 8-hydroxy quinoline and 2-nitro groups respectively, showed maximum activity against *C.albicans* (MIC:0.50μM/ml). Compound 1 and 6 (MIC: 0.51μM/ml and 0.54μM/ml respectively) having 8-hydroxy quinoline and 3-nitro group showed activity maximum against *A.niger* (MIC:0.50μM/ml). From the overall results of antimicrobial activity it was found that compound 1 had maximum antibacterial as well antifungal activity against *E. coli*, *S. aureus* and *C. albicans*, *A. niger* respectively.

Antioxidant activity: The radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH)-free radical method was used to determine antioxidant activity of compounds (1-14). The DPPH assay based on free radical DPPH, with an odd electron gives a maximum absorption at 517 nm (purple colour). When antioxidants react with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor and is reduced to the DPPH-H and as consequence the absorbance decreased from the DPPH. Radical to the DPPH-H form, results in decolorization (yellow colour) with respect to the number of electrons captured. After incubating at 37°C for 30 min samples were read against blank at 517nm and IC_{50} was calculated from the graph plotted as inhibition percentage against compound concentration [17].

$$I\% = \frac{A\ Control - A\ Sample}{A\ Control} \times 100$$

Where

$A_{control}$ is the absorbance of the control reaction.

A_{sample} is the absorbance of the test compound. [18]

Tests were carried out in triplicate using ascorbic acid as a positive control. From the results of antioxidant activity, it was observed that compounds 1, 4 and 6 have IC_{50} value comparable to that of positive control ascorbic acid.

Table 4: Minimum Inhibitory Concentration (MIC: μM/ml) of (*E*)-3-(4-hydroxyphenyl) acrylic acid derivatives against bacterial and fungal Strains

Comp d.	$MIC_{50(EC)}$	$MIC_{50(BS)}$	$MIC_{50(SA)}$	$MIC_{50(CA)}$	$MIC_{50(AN)}$
1	**0.48**	0.49	**0.49**	**0.51**	**0.51**
2	0.85	**0.48**	**0.48**	0.54	0.58
3	0.98	0.98	0.57	0.98	0.98
4	0.86	0.86	0.83	0.86	0.86
5	0.95	0.83	0.89	**0.51**	0.83
6	0.59	0.87	0.87	0.59	**0.54**
7	0.75	0.57	1.51	0.87	0.75
8	**0.49**	1.82	0.91	0.95	0.91
9	1.46	0.93	1.46	0.93	0.86
10	0.81	**0.48**	0.58	0.97	0.97
11	0.92	0.92	0.92	0.56	0.55
12	0.98	0.98	1.85	1.85	0.57
13	1.85	0.59	1.85	0.92	0.86
14	0.93	1.87	0.93	0.57	0.57
Std	0.47	0.47	0.47	0.50	0.50

[a]Norfloxacin   [b]Fluconazoles, EC=*E. coli,* BS=*B. subtilis*, SA=*S. aureus*, CA=*C. albicans*,   AN=*A. niger*

Table 5: %age Inhibition and IC_{50} value of synthesized compounds (1-14)

Compound	25μg/ml	50 μg/ml	75 μg/ml	100 μg/ml	IC_{50}μM/ml
1.	28.18	50.67	75.98	91.89	0.17
2.	28.19	51.67	67.90	77.87	0.21
3.	29.51	44.76	77.90	87.14	0.20
4.	41.01	50.00	56.19	64.28	0.18
5.	39.18	45.35	64.89	77.89	0.22
6.	45.02	47.61	56.66	68.09	0.18
7.	44.88	47.14	52.85	59.52	0.30
8.	42.65	47.14	51.90	89.52	0.19
9.	36.96	41.90	46.19	50.95	0.36
10.	41.23	45.23	50.47	54.76	0.29
11.	39.81	42.85	64.28	77.61	0.19
12.	39.33	43.33	51.90	68.09	0.23
13.	37.91	42.38	57.61	71.42	0.21
14.	37.44	41.42	52.85	66.19	0.24
Ascorbic acid*	29.08	51.78	78.33	93.78	0.27

Molecular docking studies: Molecular docking studies were analysed by AutoDock version 4.2.5.1 along with AutoDockTool (ADT). The Lamarckian Genetic Algorithm (LGA), considered one of the best docking method in the AutoDockTool was used which is capable of predicting binding orientation, position and conformations of the ligand [18]. The AutoGrid alogorithm was used to create 3D grid boxes which explore the chemistry of macromolecules in terms of binding energy [19]. Ligand PDB were prepared by Chem3D Ultra. AutoGrid is used to calculate detailed study of ligand in the actual docking target site [20]. Conformations of docked compounds were aligned in terms of hydrogen bonding, energy and hydrophobic interaction between ligand and receptor protein. Free binding energy of all synthesized compounds was calculated by docking scores [21]. For display of the receptor with ligand binding site and interaction of compounds with amino acids residues in the active sites PyMol software was used [20]. Acarbose in the crystal structure was docked as reference. Binding profile of docking is shown in Table no 6. Compound 1 shows maximum binding energy (-8.30 kcal/mol).

Table 6: Molecular docking results of target compounds (1-14)

Compound no.	Binding energy (kcal/mol)	Inhibition constant (μM)
1.	-8.03	1.29
2.	-6.81	10.15
3.	-5.94	44.30
4.	-6.43	19.28
5.	-6.52	16.67
6.	-6.71	12.14
7.	-6.28	24.99
8.	-6.54	15.97
9.	-7.44	3.50
10.	-5.86	50.82
11.	-6.53	16.21
12.	-6.14	31.41
13.	-6.52	16.59
14.	-6.88	9.09
Acarbose	**-10.82**	**0.01172**

For docking analysis: The molecular studies was developed by AutoDockTools (ADT) (Sanner, 1999) version 1.5.6 and Autodock version 4.2.5.1programs; (Autodock, Autogrid, Copyright-1989-2012) from the Scripps Research Institute http://www.scripps.edu/mb/olson/doc/autodock. Ligand Compounds 3D structures were drawn using Chemsketch. For input into docking, the structure was saved as pdb file format; ligand bond torsion was aligned and saved in PDBQT file format. Three dimensional structures of Acarbose (PDB id: 1RP8), was obtained from the Protein Data Bank (PDB) http://www.pdb.org. Further, using ADT to remove crystal water, added Kollman and Gagteiger charges to each atom, and merged the non-polar hydrogen atoms to the protein structure. The distance between donor and acceptor atoms that form a hydrogen bond was defined as 1.9 Å with a tolerance of 0.5 Å, and the acceptor–hydrogen–donor angle was not less than 120. The structures must be saved in PDBQT file format for accurately input into docking. [20].
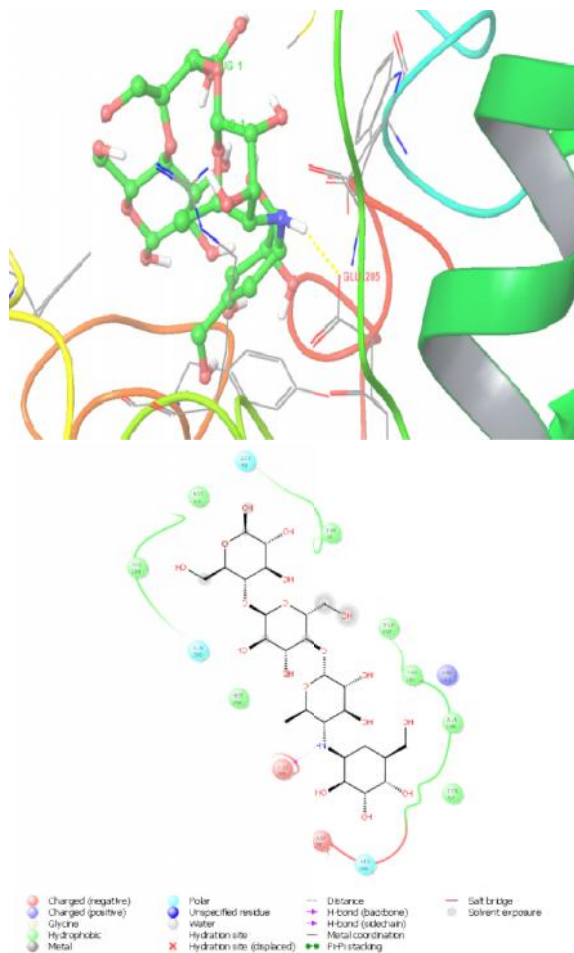

Fig 1: Binding residues of Acarbose

Spectral data

Intermediate **(E)-3-(4-hydroxyphenyl) acryloyl chloride**
IR (KBr pellets) cm^{-1}:3033(C-H str., phenyl nucleus), 1598(C=C skeletal str., phenyl),3376(O-H str., phenol),1631(C=C str., alkene). **^{1}H NMR (DMSO-d$_6$, ppm):** 6.31-7.14 (q, 4H, Ar-H), 6.78 (d, 2H, CH=CH), 7.95 (s,1H, Ar-CH).

*Compound 1***(E)-quinolin-8-yl 3-(4-hydroxyphenyl) acrylate**
IR (KBr pellets) cm^{-1}:3417 (O-H str., phenol), 3034 (C-H str., aromatic ring), 1598 (C=C str., skeletal vibration of phenyl nucleus), 1733 (C=Ostr., ester), 1632 (C=C str., alkene), 1309 (ring str., quinoline), 760 (C-H out of plane bending, quinoline), 1505 (C=N str., quinoline). **^{1}H NMR (DMSO-d$_6$,**

ppm): 6.81-7.14 (q, 4H, Ar-H), 6.81-8.89 (m, 6H, quinoline), 7.67 (d, 2H, CH=CH).

Compound 2 (E)-N-(4-flourophenyl)-3-(4-hydroxyphenyl) acrylamide

IR (KBr pellets) cm^{-1}:3748 (O-H str., phenol), 3035 (C-H str., aromatic ring), 1599 (C=C str., skeletal vibration of phenyl nucleus), 1632 (C=O str., 2° amide), 1736 (C=C str., alkene), 3367 (N-H str., 2° amide), 1015 (C-F str., aromatic), 1506 (N-H bend). **^{1}H NMR (DMSO-d$_6$, ppm):** 6.64-7.64 (m, 8H, Ar-H), 6.55 (d, 2H, CH=CH), 7.08 (s,1H, Ar-CH), 7.94 (s, 1H, NH).

Compound 3 (E)-3-(4-hydroxyphenyl)-N-phenyl acrylamide

IR (KBr pellets) cm^{-1}:3748 (O-H str., phenol), 3035(C-H str., aromatic ring), 1598 (C=C str., skeletal vibration of phenyl nucleus), 1633 (C=O str., 2° amide), 1739 (C=C str., alkene), 3365 (N-H str., 2° amide), 1506 (N-H bend). **^{1}H NMR (DMSO-d$_6$, ppm):** 6.64-7.64 (m, 10H, Ar-H), 6.83 (d, 2H, CH=CH), 7.51 (s,1H, Ar-CH), 7.96 (s, 1H, NH).

Compound 4 (E)-N-(2-chlorophenyl)-3-(4-hydroxyphenyl) acrylamide

IR (KBr pellets) cm^{-1}:3034(C-H str., aromatic ring), 1598 (C=C str., skeletal vibration of phenyl nucleus), 1631 (C=O str., 2° amide), 1733 (C=C str., alkene), 3367(N-H str., 2° amide), 754 (C-Cl str., aromatic), 1505 (N-H bend). **^{1}H NMR (DMSO-d$_6$, ppm):** 6.64-7.60 (m, 8H, Ar-H), 6.84 (d, 2H, CH=CH), 7.60 (s,1H, Ar-CH), 7.96 (s, 1H, NH).

Compound 5 (E)-3-(4-hydroxyphenyl)-N-(2-nitrophenyl) acrylamide

IR (KBr pellets) cm^{-1}:3067 (C-H str., aromatic ring), 1733 (C=C str., alkene),1598 (C=C str., skeletal vibration of phenyl nucleus), 1630 (C=O str., 2° amide), 3365 (N-H str., 2° amide), 1362(NO$_2$sym. str., Ar-NO$_2$), 1505 (N-H bend). **^{1}H NMR (DMSO-d$_6$, ppm):** 6.68-7.97 (m, 8H, Ar-H), 6.84 (d, 2H, CH=CH), 7.59 (s,1H, Ar-CH), 7.97 (s, 1H, NH).

Compound 6 (E)-3-(4-hydroxyphenyl)-N-(3-nitrophenyl) acrylamide

IR (KBr pellets) cm^{-1}:3067 (C-H str., aromatic ring), 1733 (C=C str., alkene), 1598 (C=C str., skeletal vibration of phenyl nucleus), 1631 (C=O str., 2° amide), 1310 (C-N str., aromatic), 3370 (N-H str., 2° amide), 1356 (NO$_2$sym. str., Ar-NO$_2$), 1505 (N-H bend). **^{1}H NMR (DMSO-d$_6$, ppm):** 6.67-8.01 (m, 8H, Ar-H), 6.67 (d, 2H, CH=CH), 7.56 (s,1H, Ar-CH), 8.01 (s, 1H, NH).

Compound 7 (E)-N-(2, 4-dinitrophenyl)-3-(4-hydroxyphenyl) acrylamide

IR (KBr pellets) cm^{-1}:3107 (C-H str., aromatic ring), 1733 (C=C str., alkene), 1599 (C=C str., skeletal vibration of phenyl nucleus), 1630 (C=O str., 2° amide),3336 (N-H str., 2° amide), 1333(NO$_2$sym. str., Ar-NO$_2$), 1501 (N-H bend). **^{1}H NMR (DMSO-d$_6$, ppm):** 7.12-8.80 (m, 7H, Ar-H), 7.24 (s,1H, Ar-CH), 8.14 (s, 1H, NH).

Compound 8 (E)-N-(4-chlorophenyl)-3-(4-hydroxyphenyl) acrylamide

IR (KBr pellets) cm^{-1}:3034 (C-H str., aromatic ring), 1734 (C=C str., alkene), 1598 (C=C str., skeletal vibration of phenyl nucleus), 1631 (C=O str., 2° amide), 3368 (N-H str., 2° amide), 1505 (N-H bend). **^{1}H NMR (DMSO-d$_6$, ppm):** 6.65-7.66 (m, 8H, Ar-H), 6.86 (d, 2H, CH=CH), 7.51 (s,1H, Ar-CH), 7.94 (s, 1H, NH).

Compound 9 (E)-N-(2,6-dimethylphenyl)-3-(4-hydroxyphenyl) acrylamide

IR (KBr pellets) cm^{-1}:3034 (C-H str., aromatic ring), 1734 (C=C str., alkene), 1598 (C=C str., skeletal vibration of phenyl nucleus), 1631 (C=O str., 2° amide), 3365 (N-H str., 2° amide), 2831 (Ar-CH$_3$), 1505 (N-H bend). **^{1}H NMR (DMSO-d$_6$, ppm):** 6. 65-7.13 (m, 7H, Ar-H), 6.83 (d, 2H, CH=CH), 7.51 (s,1H, Ar-CH), 7.93 (s, 1H, NH), 2.41 (m, 6H, Ar-CH$_3$).

Compound 10 (E)-N-(2-flourophenyl)-3-(4-hydroxyphenyl) acrylamide

IR (KBr pellets) cm^{-1}:3034 (C-H str., aromatic ring), 1733 (C=C str., alkene), 1598 (C=C str., skeletal vibration of phenyl nucleus), 1631 (C=O str., 2° amide), 3368 (N-H str., 2° amide), 1015 (C-F str., aromatic), 1505 (N-H bend). **^{1}H NMR (DMSO-d$_6$, ppm):** 6.61-7.63 (m, 8H, Ar-H), 6.83 (d, 2H, CH=CH), 7.52 (s,1H, Ar-CH), 7.95 (s, 1H, NH).

Compound 11 (E)-3-(4-hydroxyphenyl)-N-(2-methoxyphenyl) acrylamide

IR (KBr pellets) cm^{-1}:3035 (C-H str., aromatic ring), 1733 (C=C str., alkene), 1598 (C=C str., skeletal vibration of phenyl nucleus), 1630 (C=O str., 2° amide), 3415 (N-H str., 2° amide), 1280 (C-O-C str., Ar-OCH$_3$), 1505 (N-H bend). **^{1}H NMR (DMSO-d$_6$, ppm):** 6.79-7.52 (m, 8H, Ar-H), 6.84 (d, 2H, CH=CH), 7.52 (s,1H, Ar-CH), 7.95 (s, 1H, NH), 3.75 (t, 3H, OCH$_3$).

Compound 12 (E)-3-(4-hydroxyphenyl)-N-(3-methoxyphenyl) acrylamide

IR (KBr pellets) cm^{-1}:3067 (C-H str., aromatic ring), 1733 (C=C str., alkene), 1598 (C=C str., skeletal vibration of phenyl nucleus), 1631 (C=O str., 2° amide), 3371 (N-H str., 2°

amide), 1280 (C-O-C str., Ar-OCH$_3$), 1505 (N-H bend). ^{1}**H NMR (DMSO-d$_6$, ppm):** 6.65-7.51 (m, 8H, Ar-H), 6.84 (d, 2H, CH=CH), 7.51 (s,1H, Ar-CH), 7.97 (s, 1H, NH), 3.73 (t, 3H, OCH$_3$).

Compound 13 (E)-3-(4-hydroxyphenyl)-N-(4-methoxyphenyl) acrylamide

IR (KBr pellets) cm^{-1}:3035 (C-H str., aromatic ring), 1733 (C=C str., alkene), 1598 (C=C str., skeletal vibration of phenyl nucleus), 1631 (C=O str., 2° amide), 3362 (N-H str., 2° amide), 1280 (C-O-C str., Ar-OCH$_3$),1507 (N-H bend). ^{1}**H NMR (DMSO-d$_6$, ppm):** 6.67-7.56 (m, 8H, Ar-H), 6.83 (d, 2H, CH=CH), 7.56 (s,1H, Ar-CH), 7.94 (s, 1H, NH), 3.75 (t, 3H, OCH$_3$).

Compound 14 (E)-N-(2,4-dimethylphenyl)-3-(4-hydroxyphenyl) acrylamide

IR (KBr pellets) cm^{-1}:3068 (C-H str., aromatic ring), 1744 (C=C str., alkene), 1598 (C=C str., skeletal vibration of phenyl nucleus), 1630 (C=O str., 2° amide), 3416 (N-H str., 2° amide), 2830 (Ar-CH$_3$), 1507 (N-H bend). ^{1}**H NMR (DMSO-d$_6$, ppm):** 6.65-7.41 (m, 7H, Ar-H), 6.85 (d, 2H, CH=CH), 7.51 (s,1H, Ar-CH), 7.96 (s, 1H, NH), 2.36 (m, 6H, CH$_3$-Ar).

CONCLUSION

From the above mentioned biological activities of the *p*-coumaric acid derivatives, the following structure activity relationship can be derived (**Fig. 2**):

1. Substitution with 8-hydroxy quinoline (heteroaryl group) at R position, as indicated in compound (E)-quinolin-8-yl 3-(4-hydroxyphenyl)acrylate **(1)** showed most potent antimicrobial, antioxidant and also alpha amylase inhibitory activity.

2. Substitution with flouro group at phenyl ring attached to hydroxybenzamide ring, as present in compound **2** and **10**, increases antibacterial activity against *B. subtilis* and *S. aureus.*

3. Substitution with nitro group at phenyl ring attached to hydroxybenzamide ring, as present in compound **6** increases antioxidant and alpha amylase inhibitory activity.

4. Substitution with chloro group at phenyl ring attached to hydroxybenzamide ring, as present in compound **4** and **8** increases antioxidant and antibacterial activity against *E. coli.*

From these results, we may conclude that different structural requirements are required for a compound to be effective against different targets.
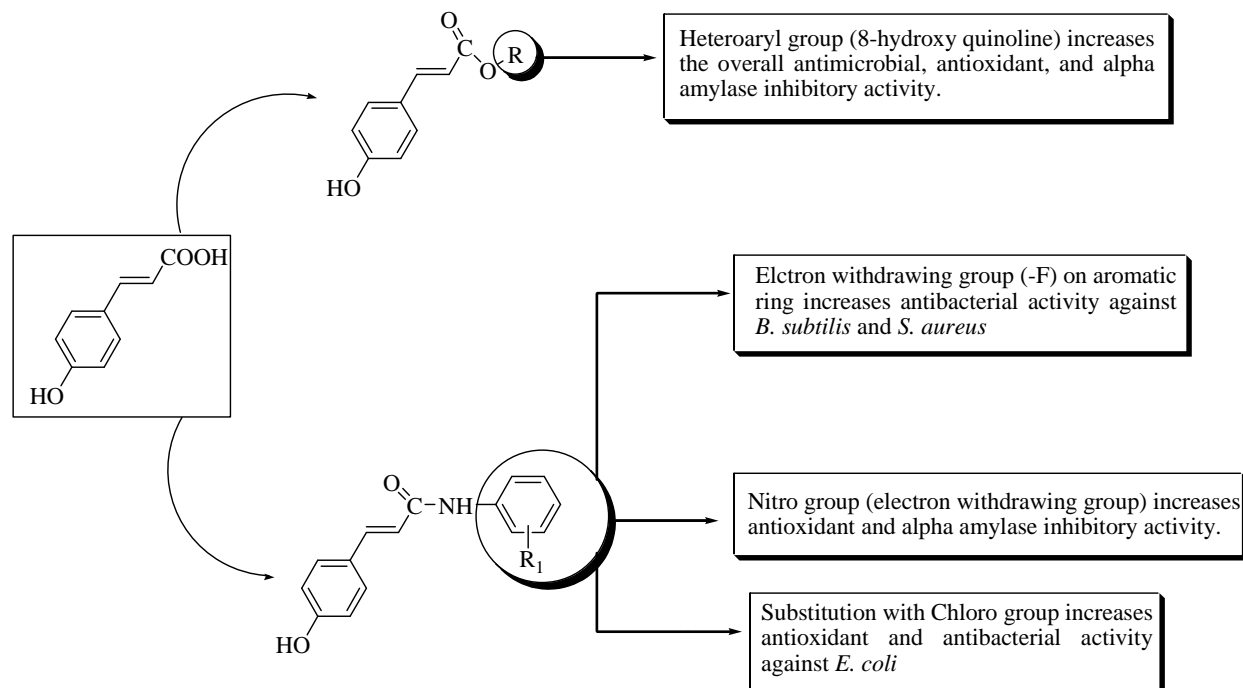

Fig 2: Structure-activity relationship of the *p*-coumaric acid derivatives.

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