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DESIGN, SYNTHESIS AND MOLECULAR DOCKING OF VANILLIC ACID DERIVATIVES AS AMYLOLYTIC ENZYME INHIBITORS

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In the present work, a series of vanillic acid derivatives have been synthesized and tested to exhibit promising amylolytic enzymes inhibition. Structures of the synthesized derivatives were studied by FT-IR, ^{1}H NMR, ^{13}C NMR, EI-MS, and structural analysis. In order to confirm the interaction between ligand and receptor molecule, *in silico* docking was performed. Results indicated that two derivatives (**3a** and **3g**) are promising compounds exhibiting good glide score to the development of selective inhibition of α-amylase and α-glucosidase.

Keywords: alpha-amylase, alpha-glucosidase, *in-silico* studies, vanillic acid derivatives.

1. INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, glycosuria and negative nitrogen balance and it is mainly due to absolute deficiency or diminished effectiveness of insulin. Diabetes is still not completely curable by the present antidiabetic therapy. Insulin therapy is the only satisfactory approach to diabetes mellitus, even though it has several drawbacks like insulin resistance, anorexia, brain atrophy, and fatty liver in chronic treatment [1]. Several drugs such as sulfonylureas and biguanides are presently available to reduce hyperglycemia in diabetes mellitus. Since these drugs demonstrated significant side effects, the search for a new class of compounds is essential to overcome these problems. Therefore, the urgent need to look for new drug scaffolds with minimum side effects is still a challenge to the medicinal chemists [2].

Nowadays, many medicinal plants have been reported to be useful in treating diabetes. Antihyperglycemic activity of natural products is mainly due to their ability to restore the function of pancreatic tissues by causing increase in the insulin output, decrease the absorption of glucose in intestine, or facilitate metabolites in insulin dependent processes. Most of the useful plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc., which frequently exhibit antidiabetic effects [3]. Some natural products obtained from plants can be used as small-molecule drug precursors, which can be converted into compounds of interest by chemical modification or fermentation methods. The semisynthetic approach is usually employed to resolve the shortage of supply due to the low yield of compounds from plants and/or the high cost of total synthesis [4].

Phenolic acids are hydroxylated derivatives of benzoic and cinnamic acids. Vanillic acid (4-hydroxy-3-methoxybenzoic acid, Fig. 1) is a dietary phenolic compound found in plants and fruits and an intermediate in the production of vanillin from ferulic acid. Vanillic acid has been previously reported in scientific investigations to exhibit beneficial effects such as antioxidant, antimutagenic, anti-cancer, anti-inflammatory, and neuroprotective [5, 6]. Moreover, these phytochemicals significantly reduced expression of the enzymes involved in fatty acid and cholesterol synthesis, indicating that they might help prevent the secondary complica-

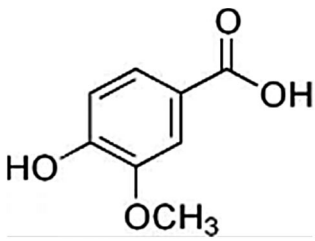

Fig. 1. Chemical structure of vanillic acid.

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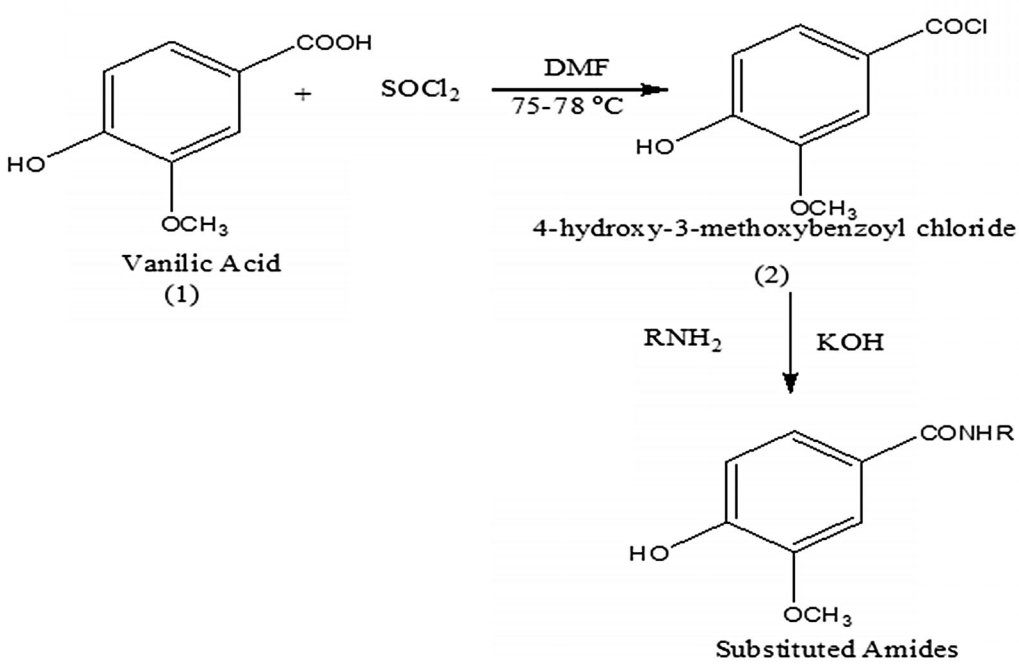


Fig. 2. Synthesis of vanillic acid derivatives.

tions of diabetes [7]. Since a new diabetes management strategy is needed that is more effective and has fewer side effects, we made an attempt to synthesize various derivatives of vanillic acid as potent oral hypoglycemic agents.

2. EXPERIMENTAL CHEMICAL PART

2.1. Chemicals and Instruments

All the chemicals, solvents and reagents used were purchased from Merck, India. Progress of the reaction was monitored by TLC performed on silica gel G and the spots were exposed to iodine vapor and UV lamp for visualization using Chloroform: Ethyl acetate: formic acid (5:4:1) as mobile phase. Fourier Transform Infra-Red spectra were recorded on (Perkin Elmer Spectrum version 10.03.02).Proton (1H) NMR spectra were recorded on JEOL model on 400 MHz. All the spectra were recorded at 25°C using $CDCl_3$ and $DMSO\text{-}d_6$ as deuterated solvents using tetramethylsilane (TMS) as the internal standard. Chemical shift values are given in d (ppm) scale and the signals are described as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet) whereas coupling constants (J) are expressed in Hz. Mass spectra (ESI-MS) were recorded on Waters mass spectrometer (Q-TOF Premier).

*2.2. General Procedure for Synthesis of Compounds **3a-3k***

Chlorination of vanillic acid (**1**) has been carried out by refluxing with thionyl chloride to 4-hydroxy-3-methoxybenzoyl chloride (**2**). Chloride (**2**) was further treated with substituted amines in presence of base to produce corresponding amides. These reactions led to the formation of compounds **3a-3k** (Fig. 2). The separated solids were filtered under reduced pressure and washed thoroughly with water [8].

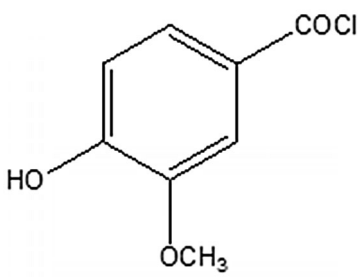


Fig. 3. Structure of 4-hydroxy-3-methoxybenzoylchloride (**2**).

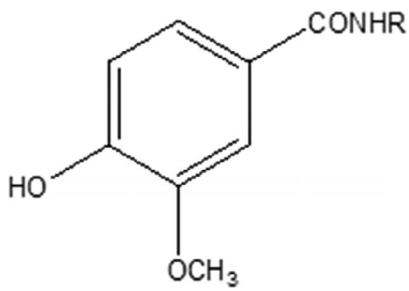


Fig. 4. Structure of substituted amides.

Synthesis of 4-hydroxy-3-methoxybenzoyl chloride (2). 4-Hydroxy-3-methoxybenzoic acid **1** (1.5 gm), 25 mL thionyl chloride, and a drop of dimethyl formamide (DMF) were refluxed in a boiling water bath for 2 h to yield chloride **2** (Fig. 3).

Synthesis of vanillic acid analogs 3a-3k. 4-hydroxy-3-methoxybenzoyl chloride **2** (0.055 mol) was added in small portion to a solution of amine (0.11 mol) and 60 mL of 10% potassium hydroxide with constant stirring and intermittent cooling. The separated solid was then filtered at the vacuum pump and washed thoroughly with water. The crude product was filtered, washed with water and then recrystallized using chloroform.

*2.3. Spectral Data of Compounds **3a-3k***

4-hHdroxy-3-methoxybenzohydrazide(3a). Molecular formula: $C_8H_{10}O_3N_2$; % Yield: 1.37 g (85%); Color: dark yellowish powder; Solubility: soluble in acetone, methanol and butanol; insoluble in water, chloroform, carbon tetrachloride; M. P.: 291 – 295°C; IR spectrum (ν_{max}, cm^{-1}): 1550 – 1510 (CO-NH), 1600 – 1700 (-CO stretch), 3500 (bonded –OH stretch alcohol), 1200 – 1275 (ether –CO stretch), 1600 (aromatic C-C ring stretch). MS (ESI), *m/z*: 183.236, 151.035, 154, 128.015; ^{1}H NMR spectrum in DMSO (δ, ppm): 6.83 – 7.66 (Ar-H), 3.93 (d, H), 3.80 (s, 3H), 2.5 (m), 2.49 – 2.53 (s, Ar-C-H)

4-Hydroxy-3-methoxy-N'-phenylbenzohydrazide (3b). Molecular formula: $C_{14}H_{14}O_3N_2$; Yield: 2.07 g (90%); Color: golden yellow crystals; Solubility: soluble in acetone, methanol and butanol; insoluble in water, chloroform, carbon tetrachloride; M. P.: 282 – 290°C; IR spectrum (ν_{max}, cm^{-1}): 1550 – 1510 (CO-NH), 1600 – 1700 (-CO stretch), 3500 (bonded –OH stretch alcohol), 1200 – 1275 (ether –CO stretch), 1600 (ArC-C ring stretch).MS (ESI), *m/z*: 259.248, 133.146, 149.145, 163.186, 177.093; ^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 7.72 (d, H), 7.69 (d, H), 7.58 – 7.59 (dd, o/m), 7.261 (s, $CDCl_3$), 6.95 – 7.72 (Ar-H), 6.95 (s, H), 6.98 (s, H), 3.95 (d, H), 3.80 (s, 3H), 1.25 (monosubstituted benzene ring).

4-Hydroxy-3-methoxy-N′-(2,4-dinitrophenyl)benzohydrazide (3c). Molecular formula: $C_{14}H_{11}O_7N_3$; Yield: 2.61 g (88%); Color: brown amorphous powder; Solubility: soluble in water, acetone; insoluble in butanol, methanol, chloroform, carbon tetrachloride; M. P.: 300 – 302°C; IR spectrum (ν_{max}, cm^{-1}): 1550 – 1510 (CO-NH), 1550 (N-O stretch), 1600 – 1700 (-CO stretch), 3500 (bonded –OH stretch alcohol), 1200 – 1275 (ether –CO stretch), 1600 (aromatic C-C ring stretch). MS (ESI), *m/z*: 329.118, 222.120, 204.973, 282.336, 154.815, 125.113, 245.005, 181.076; ^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 7.261 (s, $CDCl_3$), 3.93 (d, H), 3.49 (s, OCH_3), 1.56 (s, R_3CH), 1.25 – 1.33 (s, monosubstituted benzene ring), 0.82 – 0.88 (shielded H).

4-Hydroxy-3-methoxy-N-phenylbenzamide (3d). Molecular formula: $C_{14}H_{13}O_3N$; Yield: 1.96 g (91%); Color: brown amorphous powder; Solubility: soluble in water, chloroform, carbon tetrachloride, methanol https://www.rcsb.org/structure/3IJ8.; insoluble in acetone, butanol; M. P.: 282 – 285°C; IR spectrum (ν_{max}, cm^{-1}): 1550 – 1510 (CO-NH), 1600 – 1700 (-CO stretch), 3500 (bonded –OH stretch alcohol), 1200 – 1275 (ether –CO stretch). MS (ESI), *m/z*: 244.194, 169.059, 150.225, 134.969, 189.041; ^{1}H NMR spectrum in DMSO (δ, ppm): 7.38 (t, Ar-H),7.34 – 7.50 (m, 5H, Ar-H), 3.79 (s, 3H), 2.48 – 2.51 (s, Ar-C-H).

N-(3-Chlorophenyl)-4-hydroxy-3-methoxybenzamide (3e): Molecular formula-:$C_{14}H_{12}O_3Cl$; Yield: 2.09 g (85%); Color: brown amorphous powder; Solubility: soluble in acetone, water; insoluble in methanol, butanol, chloroform, carbon tetrachloride; M. P.: 295 – 298°C; IR spectrum (ν_{max}, cm^{-1}): 1550 – 1510 (CO-NH), 1550 (N-O stretch), 1600 – 1700 (-CO stretch), 3500 (bonded –OH stretch alcohol), 1200 – 1275 (ether –CO stretch), 680 (m-substituted benzene). MS (ESI), *m/z*: 278.150, 169.127, 131.12, 127.813. ^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 7.05 – 7.1 (m, Ar-H, 5H), 6.54 – 6.59 (M, 5H), 3.87 – 3.88 (d, H), 3.67 (s, H).

4-Hydroxy-3-methoxy-N-(3-nitrophenyl)benzamide (3f). Molecular formula; $C_{14}H_{12}O_5N_2$; Yield: 2.03 g (79%); Color: dark yellowish powder; Solubility: soluble in acetone, methanol and butanol; insoluble in water, chloroform, carbon tetrachloride; M. P.: 278 – 279°C; IR spectrum (ν_{max}, cm^{-1}): 1550 – 1510 (CO-NH), 1550 (N-O stretch), 1600 – 1700 (-CO stretch), 3500 (bonded –OH stretch alcohol), 1200 – 1275 (ether –CO stretch), 680 (m-substituted benzene). MS (ESI), *m/z*: 282.336, 262.691, 230.086, 207.201, 121.062, 169.262, 139.086. ^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 8.13 (dd, Ar-H), 8.1 – 8.13 (dd, o/m), 7.72 (d, Ar-H), 7.69 (d, Ar-H), 7.58 – 7.59 (dd, o/m), 7.33 – 7.38 (dt, di o/m), 7.38 (t, Ar-H), 7.261 ($CDCl_3$), 6.98 (s), 6.95 (s), 6.68 – 6.79 (dt, Ar-H), 6.81 – 6.82 (dd, Ar-H), 6.04 (N-H), 3.96 (s, OCH_3), 1.19 – 1.33 (monosubstituted benzene ring).

N-(4-Bromophenyl)-4-hydroxy-3-methoxybenzamide (3g). Molecular formula: $C_{14}H_{12}O_3NBr$; Yield: 2.48 g (87%); Color: green yellowish powder; Solubility: soluble in acetone, methanol, butanol. Insoluble in water, chloroform and carbon tetrachloride; M. P.: 284 – 285°C; IR spectrum (ν_{max}, cm^{-1}): 1550 – 1510 (-CO-NH), 1550 (N-O stretch), 1600 – 1700 (-CO stretch), 3500 (bonded –OH stretch alcohol), 1200 – 1275 (ether –CO stretch), 650 – 800 (p-disubstituted), 1600(aromatic C-C ring stretch). MS (ESI), *m/z*: 322.097,229.140, 215.099, 128.015, 169.127, 175.068 &173.920; ^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 7.72 (dd, H),, 6.66 – 7.72 (Ar-H), 7.69 (d, H), 7.58 – 7.59 (dd, o/m), 7.33 – 7.34 (dt, di o/m), 7.26 ($CDCl_3$), 6.98 (s), 6.95 (s), 6.79 (dd, Ar-H), 3.96 (s, OCH_3), 2.12 – 2.74 (Ar-C-H), 1.25 – 1.33 (monosubstituted benzene ring).

4-Hydroxy-3-methoxy-N-(2-nitrophenyl)benzamide (3h). Molecular formula: $C_{14}H_{12}O_5N_2$; Yield: 2.13 g (83%); Color: orange amorphous powder; Solubility: soluble in water, acetone. insoluble in chloroform, carbon tetrachloride methanol, butanol; M. P.: 279 – 281°C; IR spectrum (ν_{max}, cm^{-1}): 1550 – 1510 (-CO-NH), 1550 (N-O stretch),

1600 – 1700 (-CO stretch), 3500 (bonded –OH stretch alcohol), 1200 – 1275 (ether –CO stretch), 650 – 800 (p-disubstituted), 1600 (aromatic C-C ring stretch). MS (ESI), *m/z*: 282.336, 262.691, 230.086, 207.201, 121.062, 169.262, 139.086, 180.131. ^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 8.08 – 8.11 (dd, o/m), 7.34 – 7.37 (dt, di o/m), 7.26 ($CDCl_3$), 6.8 – 6.83 (dd, Ar-H), 6.68 – 6.79 (dt, Ar-H), 3.94 (s, OCH_3).

N-(2-Chlorophenyl)-4-hydroxy-3-methoxybenzamide (3i). Molecular formula: $C_{14}H_{12}O_3NCl$; Yield: 2.02 g (82%); Color: green yellowish powder; Solubility: soluble in water, chloroform, carbon tetrachloride, methanol; insoluble in acetone, butanol; M. P.: 302 – 304°C; IR spectrum (ν_{max}, cm^{-1}): 1550 – 1510 (-CO-NH), 1550 (N-O stretch), 1600 – 1700 (-CO stretch), 3500 (bonded –OH stretch alcohol), 1200 – 1275 (ether –CO stretch), 735 – 770 (o-disubstituted), 1600 (aromatic C-C ring stretch). MS (ESI), *m/z*: 278.150, 169.127, 131.121, 127.813,183.01. ^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 7.33 – 7.38 (dt, di o/m), 7.26 ($CDCl_3$), 6.68 – 6.79 (dt, Ar-H), 6.81 – 6.82 (dd, Ar-H),3.95 (s, OCH_3), 2.12 – 2.74 (Ar-C-H), 1.17 – 1.77 (monosubstituted benzene ring), 0.79 – 0.9 (shielded H).

N-(2-Aminophenyl)-4-hydroxy-3-methoxybenzamide (3j). Molecular formula: $C_{14}H_{14}O_3N_2$; Yield: 1.79 g (78%); Color: black amorphous powder; Solubility: soluble in water, acetone; insoluble in chloroform, carbon tetrachloride methanol, butanol; M. P.: 293 – 295°C; IR spectrum (ν_{max}, cm^{-1}): 1500 – 1510 (-CO-NH), 1550 (N-O stretch), 1600 – 1700 (-CO stretch), 3500 (bonded –OH stretch alcohol), 1200 – 1275 (ether –CO stretch),1600 (aromatic C-C ring stretch), 800 – 1000 (N-H wagging), 1620 (N-H bending), 1175 (C-N aromatic stretch). MS (ESI), *m/z*: 241.157, 211.116, 154.951, 119.104 ^{1}H NMR spectrum in DMSO (δ, ppm): 8.0 (dd, o/m), 7.7 (dd, 2H), 7.63 – 7.68 (d, H), 3.9 (s, OCH_3), 2.4 – 2.5 (Ar-H).

4-Hydroxy-3-methoxy-N-(3-methoxyphenyl)benzamide (3k). Molecular formula: $C_{15}H_{15}O_4N$; Yield: 1.97 g (81%); Color: black amorphous powder; Solubility: soluble in water, acetone. insoluble in chloroform, carbon tetrachloride methanol, butanol; M. P.: 280 – 281°C; IR spectrum (ν_{max}, cm^{-1}): 1500 – 1510 (-CO-NH), 1550 (N-O stretch), 1600 – 1700 (-CO stretch), 3500 (bonded –OH stretch alcohol), 1200 – 1275 (ether –CO stretch),1600 (aromatic C-C ring stretch), 800 – 1000 (N-H wagging), 1620 (N-H bending), 1175((C-N aromatic stretch), 680 (m-substituted benzene). MS (ESI), *m/z*: 274.167, 253.038, 244.059, 207.876, 179.118, 172.165, 154.951, 130.108, 128.015. ^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 7.72 (d, H), 7.69 (d, H), 7.58 (dd, o/m), 7.38(t, H), 7.31 (dt),7.26 ($CDCl_3$),6.95 (s), 6.87 – 6.88 (dd, Ar-H), 3.97 (s, OCH_3), 1.25 – 1.28 (monosubstituted benzene), 0.82 – 0.85 (shielded H).

3. EXPERIMENTAL PHARMACOLOGICAL PART

3.1. In Vitro Antidiabetic Activity

Alpha-amylase inhibitory activity of synthesized derivatives (3f-3k). The α-amylase inhibitory activity of synthesized derivatives was performed by Sigma – Aldrich kit with slight modification [9]. The inhibitory activity of different concentrations of synthesized derivative (**3a**) (10 – 100 μg/ml) was determined against α-amylase. The enzyme (0.5 %) was prepared in phosphate buffer (pH 6.8). Briefly, 500 μL of different concentrations of synthesized derivatives and 500 μL of 0.1M phosphate buffer (pH 6.8) containing α-amylase were incubated at 25°C for 10 min. After pre-incubation, 500 μL of 1% starch solution in 0.1 M phosphate buffer (pH 6.8) was added to each tube and further incubated at 25°C for 10 min. The reaction was stopped by addition of 1 mL of dinitrosalicylic acid reagent. The same was performed for control where drug solution was replaced with buffer. The test tubes were placed in a boiling water bath for 10 minutes and cooled. To each tube, 10 mL of distilled water was added and the absorbance was measured spectrophotometrically at 540 nm. The percentage (%) inhibition of enzyme activity was calculated using the following formula:

$$\text{Inhibition of } \alpha\text{-amylase activity (\%)} = [A_{540}\text{Control} - A_{540}\text{ Drug}/A_{540}\text{Control}] \times 100\%.$$

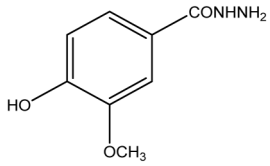
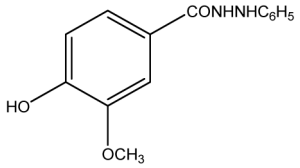
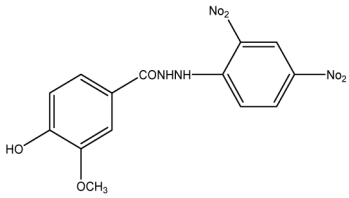
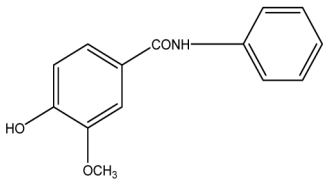
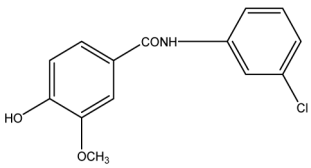
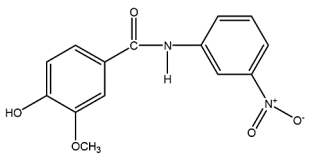
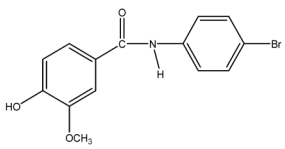
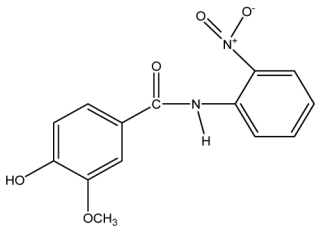
The IC_{50} value was determined from a plot of the percentage inhibition versus sample concentration. Acarbose was used as the standard control and the experiment was performed in duplicate.

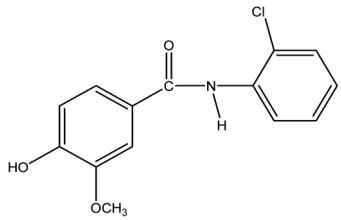
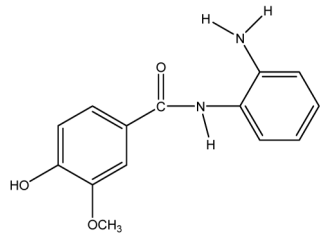
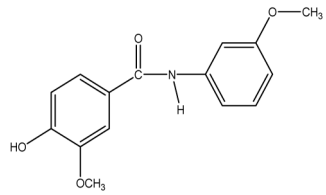
Alpha-glucosidase Inhibitory Activity of synthesized derivatives (3a-3k). The α-glucosidase inhibition assay was performed according to the standard procedure with slight modification [10]. α-Glucosidase (2 U/mL) was premixed with 20 μL of test sample at various concentration and incubated for 5 min at 37°C. 1 Mm para-nitrophenylglucopyranoside (20 μL in 50mM of phosphate buffer (pH 6.8) was added to initiate the reaction. The mixture was further incubated at 37°C for 20 min. The reaction was terminated by addition of 50 μL of 1 M sodium carbonate and the final volume was made up to150 μL. The α-glucosidase activity was determined spectrophotometrically at 405 nm by measuring the amount of para-nitrophenol released from pNPG. The assay was performed in triplicate. The concentration of test sample required to inhibit 50% of alpha glucosidase activity under the conditions was defined as the IC_{50}value. The experiment was repeated thrice with same protocol. The results are presented in Table 1.

3.2. Molecular Docking Studies

In this study, all the designed compounds were subjected to molecular docking to as determine their binding mode at human pancreatic alpha amylase. The biological target mole-

TABLE 1. Inhibitory Effects of Vanillic Acid Derivatives against α-Amylase and α- Glucosidase

Analog	Structure	IC_{50} mmol/mL α-amylase	IC_{50} mmol/mL α- glucosidase	Binding energy (kcal/mol)
3a		3.73 ± 0.01	4.54 ± 0.021	–6.4
3b		5.81 ± 0.002	6.31 ± 0.0184	–4.02
3c		5.82 ± 0.002	6.21 ± 0.0028	–3.58
3d		5.56 ± 0.021	6.89 ± 0.0008	–5.36
3e		5.14 ± 0.001	5.52 ± 0.013	–4.9
3f		5.78 ± 0.019	5.99 ± 0.002	–1.33
3g		4.92 ± 0.012	5.35 ± 0.006	–4.75
3h		5.85 ± 0.028	5.51 ± 0.0001	–2.03

Analog	Structure	IC_{50} mmol/mL α-amylase	IC_{50} mmol/mL α- glucosidase	Binding energy (kcal/mol)
3i		6.60 ± 0.001	5.42 ± 0.002	–1.18
3j		5.74 ± 0.002	5.14 ± 0.011	–4.02
3k		5.07 ± 0.002	7.00 ± 0.003	–5.44
Acarbose		7.36	6.9	**–6.8**

cule was downloaded from the protein data bank, PDB ID: 3IJ8 (Fig. 5) at a resolution of 1.43Å [11].The target was prepared for docking by removing all heteroatoms, nonreceptor atoms, water and other ions, etc. Molecular docking was performed for designed compounds **3a–3k** as potential α-amylase inhibitors. Compounds **3a–3k** were simulated using ChemDraw Ultra 8.0 software and converted into suitable model structures. The energy minimized structures are required for molecular docking and for the preparation of corresponding PDB files. Docking studies were performed on prepared ligands to predict the binding energy to find out the most acceptable docked pose. Confirmation of docking was done using Mcule platform.

4. RESULTS AND DISCUSSION

4.1. Chemistry

Initially, Carboxylic group of vanillic acid react with thionyl chloride to produce 4-hydroxy-3-methoxybenzoyl-chloride (**2**) according to the literature procedure [8]. During this reaction, the hydroxyl group of carboxylic acid is converted to a chlorosulfite intermediate making it a better leaving group. The chloride anion produced during the reaction acts a nucleophile. 4-Hydroxy-3-methoxybenzoylchloride **2** was reacted with potassium hydroxide and substituted amines to prepare the desired final derivatives **3a-3k** depicted in Fig. 4. All compounds were purified and the proposed structures were confirmed by ^{1}H NMR, melting point (M.P.) values, and mass spectrometry data.

4.2. In Vitro Anti-Diabetic Assay of Vanillic Acid Analogs

In this experiment, α-amylase and α-glucosidase has been selected to determine the anti-diabetic activity of all tested derivatives of vanillic acid with a slight modification. Acarbose was selected as positive control in the experiment. The group of chief intestinal enzymes like α-amylase and α-glucosidase were involved in the digestion of carbohydrates. Acarbose competitively inhibit these enzymes. Oral hypoglycemic drugs act mainly by inhibiting saccharide-hydrolyzing enzymes (alpha-amylase and alpha-glucosidase) in type-II diabetes. Digestion of carbohydrates can be prolonged through the inhibitions of these enzymes, resulting in

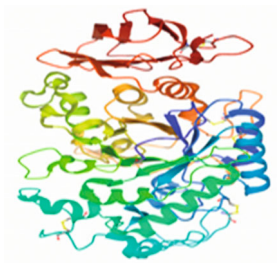


Fig. 5. 3D structure of pancreatic α-amylase PDB ID: 31J8.

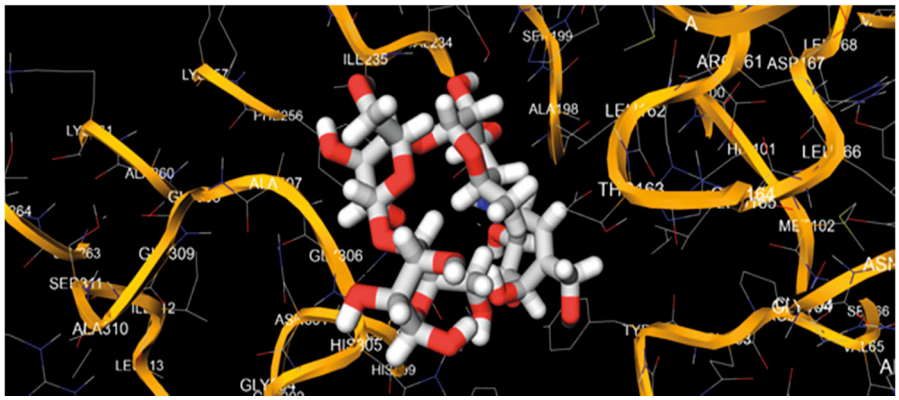


Fig. 6. Binding mode of acarbose docked with α-amylase.

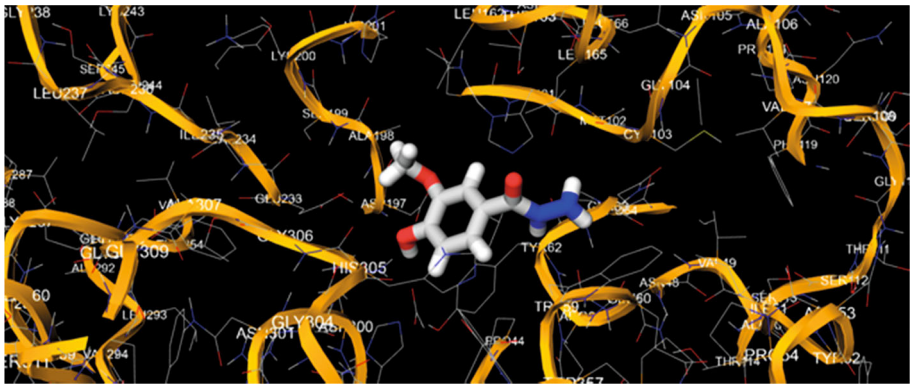


Fig. 7. Interaction and superimposed structure of 4-hydroxy-3-methoxybenzohydrazide (**3a**) with α-amylase amino acid residue.

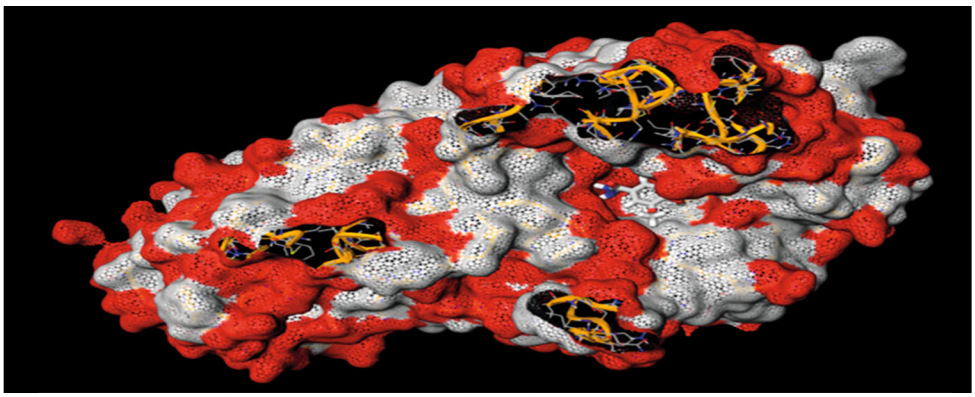


Fig. 8. Interaction and superimposed structure of 4-hydroxy-3-methoxybenzohydrazide (**3a**) with α-amylase surface.

the reduction of glucose absorption rate and postprandial plasma glucose level.

Different concentrations of vanillic acid derivatives were subjected to alpha-amylase and alpha-glucosidase inhibitory assay. The results are presented in Table 1. These data suggested that most of the derivatives exhibited significant enzymatic inhibitory activity, especially analog **3a** with the IC_{50} values of 3.71 and 4.56 mmol/mL and compound **3g** with the IC_{50} values of 4.92 and 5.35 mmol/mL against alpha-amylase and alpha-glucosidase, respectively. These values are lower than those for other analogs and acarbose as the positive control.

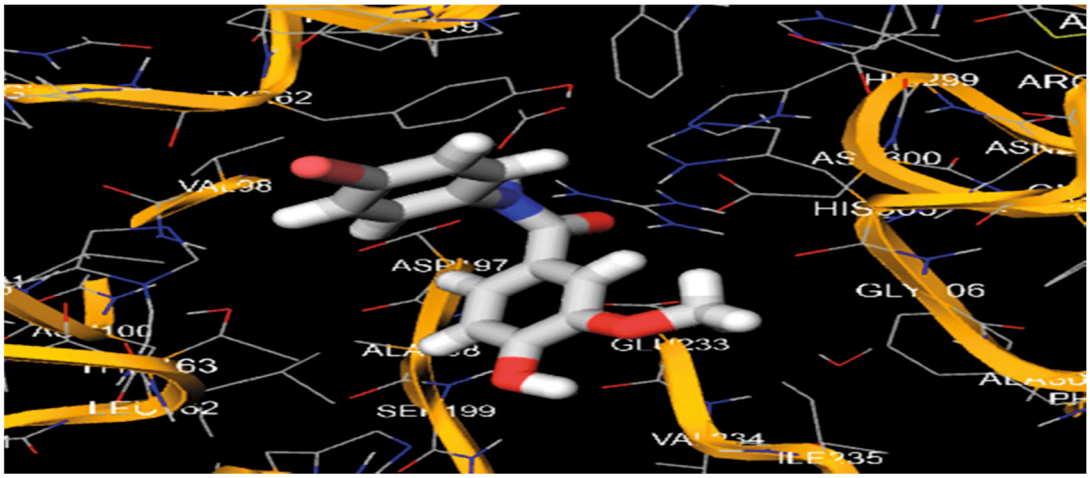


Fig. 9. Interaction and superimposed structure of N-(4-bromophenyl)-4-hydroxy-3-methoxybenzamide (**3g**) with α-amylase amino acid residue.

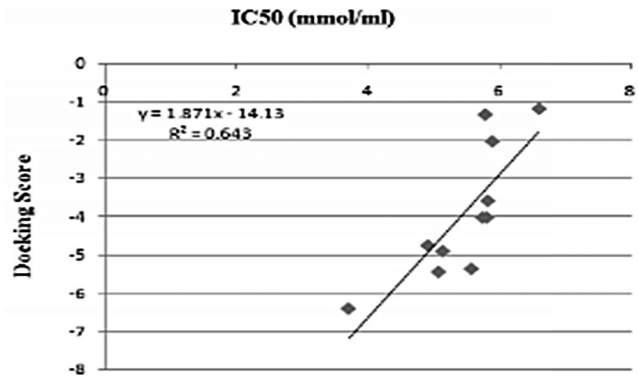


Fig. 10. Correlation plots of IC_{50} and docking score of vanillic acid derivatives vs. α-amylase.

4.3. Molecular Docking Studies

In order to study the binding interactions between alpha amylase with the ligands, all the compounds were docked and the binding interactions were calculated. To determine the complete intermolecular interactions between the ligand and target protein (31J8) (Fig. 5), an automated and virtual screening program AutoDock was used. Docking is a combination of algorithms and scoring functions. It performs grid-based ligand docking with energetics and finds for favorable interactions between one or more typically small ligand molecules and a typically larger receptor molecule, usually a protein. Three dimensional structural information of the target was taken from the Protein Data Bank (PDB). Compound structures were built with Chem Draw Ultra 10.0 [12]. The receptor preparation was done to delete water molecules not related to active sites and also to regenerate the native status, and also for the addition of hydrogen atoms.

As shown in Figs. 6, 7, 8, and 9, acarbose and synthesized compounds **3a** and **3g** are involved in similar binding interactions with the target protein molecule. Figure 6 shows that acarbose can form hydrogen bonds with ALA198, ASP197, TYR163, ILE235 and HIS305residues in the active site. While **3a** can interact with ALA198, ASP197, HIS305, TYR62 and GLN63 (Fig. 7) residues, 3g interacts with VAL198, ASP197, ALA198, GLU233, SER199 (Fig. 9). The binding free energy of acarbose was –6.8 kcal/mol and those for analogs **3a** and **3g** were –6.4 and –4.75 kcal/mol, respectively [13].

The experimentally most active compounds also exhibited a good correlation coefficient ($r^2 = 0.643$) between the docking score and IC_{50} values of the ligands (Fig. 10). Therefore, analog **3a** has significant inhibitory activity against α-amylase, which is also confirmed by *in vitro* enzymatic model.

Thus it can be postulated that the compounds **3a** and **3g** can be explored for *in vivo* activity in the near future. These studies will be helpful for further lead optimization and designing of new amylolytic inhibitors for the treatment of diabetes.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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