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RESEARCH ARTICLE

Study on the interaction of triaryl-dihydro-1,2,4-oxadiazoles with α-glucosidase

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Abstract

Purpose One of the therapeutic approaches in the management of Type 2 diabetes is delaying the absorption of glucose through α-glucosidase enzymes inhibition, which can reduce the incidence of postprandial hyperglycemia. The existence of chronic postprandial hyperglycemia impaired the endogenous antioxidant defense due to inducing oxidative stress induced pancreatic β-cell destruction through uncontrolled free radicals generation such as ROS, which in turn, leads to various macrovascular and microvascular complications. This study aimed to synthesize 2-aryl-4,6-diarylpyrimidine derivatives, screen their α-glucosidase inhibitory activity, perform kinetic and molecular docking studies.

Methods A series of 3,4,5-triphenyl-4,5-dihydro-1,2,4-oxadiazole derivatives were synthesized and their α-glucosidase inhibitory activity was screened in vitro. Compounds 6a-k were synthesized via a two-step reaction with a yield between 65 and 88%. The structural elucidation of the synthesized derivatives was performed by different spectroscopic techniques. α-Glucosidase inhibitory activity of the oxadiazole derivatives 6a-k was evaluated against *Saccharomyces cerevisiae* α-glucosidase.

Results Most of the synthesized compounds demonstrated α-glucosidase inhibitory action. Particularly compounds **6c, 6d** and **6 k** were the most active compounds with IC_{50} values 215 ± 3, 256 ± 3, and 295 ± 4 μM respectively. A kinetic study performed for compound **6c** revealed that the compound is a competitive inhibitor of *Saccharomyces cerevisiae* α-glucosidase with K_i of 122 μM. The docking study also revealed that the two compounds, **6c** and **6 k**, have important binding interactions with the enzyme active site.

Conclusion The overall results of our study reveal that the synthesized compounds could be a potential candidate in the search for novel α-glucosidase inhibitors to manage the postprandial hyperglycemia incidence.

Keywords 1,2,4-oxadiazoles · α-Glucosidase activity · Docking · Kinetic study

Background

A serious public health issue globally with multiple etiologies is Diabetes mellitus (DM) which is characterized by a failure of glucose homeostasis. The important result of the metabolic disturbances of carbohydrate, fat, and protein due to absolute or relative defects in insulin secretion and/or insulin action has been recognized as DM [1]. Type 2 diabetes mellitus (T2DM) accounts approximately 90% of all diabetic cases, which indicates a strict increase in its prevalence partly due to ageing population, overweight, and obesity [2]. Even though T2DM can be managed with physical exercise, diet, and modern drugs, there are several cases need to drug therapy. The currently available oral hypoglycemic drugs exhibit adverse side effects on an extended use including edema (Thiazolidinediones), hypoglycemia (Sulphonylureas),

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weight gain (Sulphonylureas and Thiazolidinediones), folate and B12 malabsorption and lactic acidosis (Metformin), and gastrointestinal symptom (Acarbose) [3]. The currently available α-glucosidase inhibitors, such as acarbose has showed several side effects including hypoglycemia at higher doses, liver problems, diarrhea, and lactic acidosis. Therefore, there is an urgent need to discover and develop potential α-glucosidase inhibitors in recent years.

In the management of DM, the inhibition of carbohydrate-hydrolyzing enzymes and regulating those constituents that cause the glucose uptake from the bloodstream is important [4]. In T2DM when the insulin secretion becomes insufficient or the cells fail to recognize the secreted insulin, cells glucose absorption increases which results in elevated glucose level that leads to postprandial hyperglycemia [5]. On the other hand, long term high plasma level of glucose causes to impair the endogenous antioxidant agents such as glutathione, coenzyme-Q and α-lipoic acid. Decreasing the level of this antioxidant increases the level of reactive oxygen species (ROS), hydroxyl and nitric oxide (NO) radicals that induces pancreatic β-cell destruction. Furthermore, uncontrolled free radicals generation leads to various microvascular, macrovascular complications, retinopathy, cardiovascular diseases, and nephropathy [6–9]. Thus in T2DM patients, delaying the absorption of digestible dietary carbohydrates is an important therapeutic approach to control the postprandial hyperglycemia to reduce the impact of T2DM through α-glucosidase enzyme inhibition [10, 11]. α-Glucosidase is the important catalyzing enzyme that involves in the process of the hydrolysis of 1,4-α-glycosidic linkages of carbohydrates and converts into monosaccharides (α-glucose), which can be absorbed into the blood [12]. The inhibition of this enzyme is the most valuable therapeutic approaches to delay the absorption of glucose and dropping the postprandial blood glucose level, which can potentially reduce the impact of T2DM [11]. On these bases, various small molecules both synthetic and isolates of natural product extracts have been reported with potential α-glucosidase inhibitory activities [13, 14].

On the other side, there are some reports on α-glucosidase inhibitory activity of 1,2-diaryl heterocycles. For example a series of 5,6-diphenyl-1,2,4-triazine derivatives (Fig. 1a) showed good activity for inhibition of α-glucosidase enzyme [15]. Other heterocyclic rings such as imidazole and pyrrole as central core contains 1,2-diaryl substitution (Fig. 1b, c) have been reported as anti-hyperglycemic agents [16, 17].

With respect to the above background, in the current research of new, simple and easily obtained compounds that could be active against α-glucosidase, herein, we have reported synthesis and evaluation of glucosidase inhibitory activity of new 4,5-dihydro-3,4,5-triary-1,2,4-oxadiazole derivatives.

Materials and methods

Materials

All chemicals compounds and reagents were purchased from Merck (Darmstadt, Germany). Melting points were determined with a Kofler hot stage apparatus (Reichert, Vienna, Austria) and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded with Bruker FT-500 and Bruker FT-125 spectrometer (Bruker, Darmstadt, Germany), respectively, using TMS as the internal standard and $CdCl_3$ as a solvent. The chemical shifts (δ) values were expressed in parts per million (ppm), the coupling constants (J) in hertz and spin multiplicities are given as s (singlet), d (doublet), t (triplet), and m (multiplet).

Chemistry

General pSrocedure for synthesis of 3,4,5-triaryl-4,5-dihydro-1,2,4-oxadiazole

To a solution of N-benzylideneaniline derivative (1 eq) in ether was added N-hydroxybenzimidoyl chloride (1 eq) and triethylamine (1.2 eq) successively. The reaction mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure. The residue was crystallized in acetic acid-water to yield 3,4,5-triaryl-4,5-dihydro-1,2,4-oxadiazoles. Compound **6a** was prepared as the published procedures [18] and the structure was confirmed with reported spectroscopic data.

3-(4-chlorophenyl)-4,5-diphenyl-4,5-dihydro-1,2,4-oxadiazole (6b)

Yield: 73%. Mp: 133-135 °C. ^{1}HNMR ($CdCl_3$) δ; 6.53(s, 1H, H5-oxadiazole), 6.81(d, J = 7.5 Hz, 2H), 7.10–7.14(m, 1H), 7.19–7.22(m, 2H), 7.32(d, J = 8 Hz, 2H), 7.45(bs, 3H), 7.55(d, J = 8 Hz, 2H), 7.59(bs, 2H). ^{13}CNMR ($CdCl_3$) δ; 154.4, 141.0, 138.8, 136.6, 129.9, 129.7, 129.3, 129.0, 128.9, 127.2, 125.9, 124.3, 123.9, 100.6. Mass (M/z, (%)); 334 (45), 257(5), 228 (100), 180 (47), 105 (21), 77 (49), 57 (38). Anal. Calcd. For $C_{20}H_{15}ClN_2O$: C, 71.75; H, 4.52; N, 10.59. Found: C, 71.54; H, 4.63; N, 10.47.

3,4-bis(4-chlorophenyl) -5-phenyl-4,5-dihydro-1,2,4-oxadiazole (6c)

Yield: 88%. Mp: 143-144 °C. ^{1}HNMR ($CdCl_3$) δ; 6.49(s, 1H, H5-oxadiazole), 6.73(d, J = 7.4 Hz, 2H), 7.16(d, J = 7.5 Hz, 2H), 7.34(d, J = 7.4 Hz, 2H), 7.46(m, 3H), 7.53(d, J = 7.5 Hz, 2H), 7.57(m, 2H). ^{13}CNMR ($CdCl_3$) δ; 154.1, 139.4, 138.4, 136.8, 131.5, 130.1, 129.5, 129.2, 129.2, 129.0, 127.2, 125.5, 123.5, 100.5. Mass (M/z, (%)); 368 (37), 352 (10), 246(50), 213 (11), 185 (36), 129 (44), 111 (40), 83 (77), 77 (40), 55

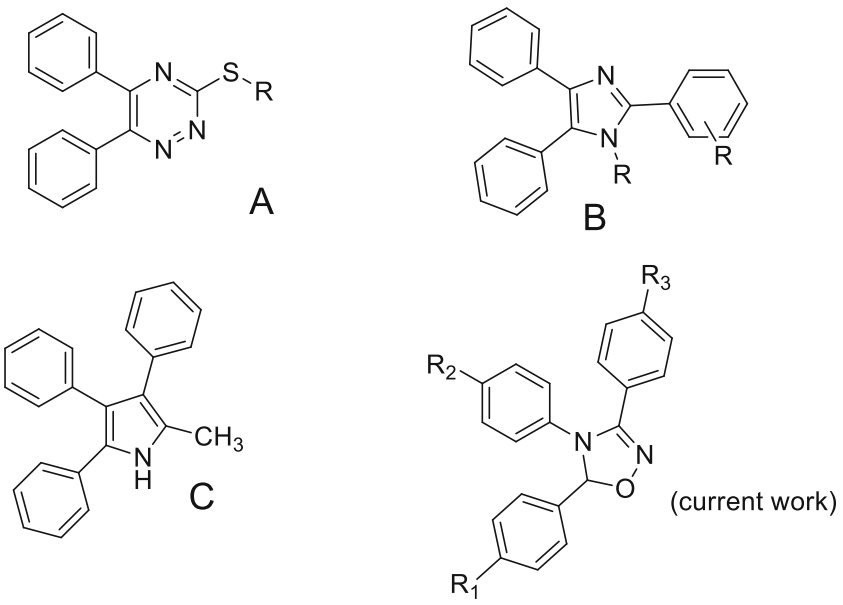


Fig. 1 Chemical structures of some compounds with α-glucosidase inhibitory and anti-hyperglycemia activity

(100). Anal. Calcd. For $C_{20}H_{14}Cl_2N_2O$: C, 65.06; H, 3.82; N, 7.59. Found: C, 65.26; H, 3.61; N, 7.44.

3-(4-chlorophenyl)-4-(4-fluorophenyl) -5-phenyl-4,5-dihydro-1,2,4-oxadiazole (6d)

Yield: 78%. Mp: 143-145 °C. ^{1}HNMR ($CdCl_3$) δ; 6.45(s, 1H, H5-oxadiazole), 6.78–6.82(m, 2H), 6.88–6.92(t, 2H, J = 9.0), 7.32(d, J = 8.5 Hz, 2H), 7.45–7.48(m, 3H), 7.52(d, J = 8.5 Hz, 2H), 7.55–7.60(m, 2H). ^{13}CNMR ($CdCl_3$) δ; 161.7, 154.6, 138.3, 137.0, 136.7, 130.00, 129.6, 129.2, 129.0, 128.9, 127.3, 126.8, 126.8, 123.6, 116.4, 116.3, 101.0. Mass (M/z, (%)); 352 (25), 289 (19), 246 (100), 198 (27), 153 (21). Anal. Calcd. For $C_{20}H_{14}ClFN_2O$: C, 68.09; H, 4.00; N, 7.94. Found: C, 68.31; H, 3.91; N, 7.67.

3,4-bis(4-chlorophenyl)-5-(p-tolyl) -4,5-dihydro-1,2,4-oxadiazole (6e)

Yield: 86%. Mp: 120-122 °C. ^{1}HNMR ($CdCl_3$) δ; 2.41(s, 3H, CH_3), 6.46(s, 1H, H5-oxadiazole), 6.72(d, J = 8.5 Hz, 2H), 7.16(d, J = 8.5 Hz, 2H), 7.26(d, J = 8.0 Hz, 2H), 7.34(d, J = 8.0 Hz, 2H), 7.45(d, J = 8.0 Hz, 2H), 7.52(d, J = 8.0 Hz, 2H). ^{13}CNMR ($CdCl_3$) δ; 154.1, 140.1, 139.4, 136.8, 135.3, 131.4, 129.6, 129.4, 129.2, 129.1, 127.1, 125.5, 123.6, 100.5, 21.4. Mass (M/z, (%)); 382 (33), 262(100), 229 (34), 192 (11), 125 (17), 91 (18). Anal. Calcd. For $C_{21}H_{16}Cl_2N_2O$: C, 65.81; H, 4.21; N, 7.31. Found: C, 65.66; H, 4.09; N, 7.25.

4-(4-chlorophenyl)-3-(4-fluorophenyl)-5-(p-tolyl) -4,5-dihydro-1,2,4-oxadiazole (6f)

Yield: 86%. Mp: 80-82 °C. ^{1}HNMR ($CdCl_3$) δ; 2.43(s, 3H, CH_3), 6.46(s, 1H, H5-oxadiazole), 6.72(d, J = 8.5 Hz, 2H), 7.05(t, 2H, J = 8.0 Hz), 7.15(d, J = 8.5 Hz, 2H), 7.27(d, J = 8.5 Hz, 2H), 7.46(d, J = 8.0 Hz, 2H), 7.56–7.60(m, 2H). ^{13}CNMR ($CdCl_3$) δ: 165.0, 163.0, 154.1, 139.5, 138.3, 131.4, 130.1, 130.0, 130.0, 129.4, 128.9, 127.1, 125.5, 121.2, 116.2, 116.0, 100.4, 21.4. Mass (M/z, (%)); 366 (25), 246(100, 212 (29), 95 (18), 69 (35). Anal. Calcd. For $C_{21}H_{16}ClFN_2O$: C, 68.76; H, 4.40; N, 7.64. Found: C, 68.39; H, 3.99; N, 7.78.

3-(4-chlorophenyl)-4-(4-fluorophenyl)-5-(p-tolyl) -4,5-dihydro-1,2,4-oxadiazole (6 g)

Yield: 65%. Mp: 125-128 °C. ^{1}HNMR ($CdCl_3$) δ; 2.38(s, 3H, CH_3), 6.42(s, 1H, H5-oxadiazole), 6.72–6.808(m, 2H), 6.89(t, J = 7.1 Hz), 2H), 7.26(d, J = 8.0 Hz, 2H), 7.33(d, J = 8.5 Hz, 2H), 7.45(d, J = 8.0 Hz, 2H), 7.51(d, J = 8.5 Hz, 2H).^{13}CNMR ($CdCl_3$) δ; 161.6, 159.7, 154.6, 140.1, 136.9, 136.6, 135.3, 129.6, 129.2, 129.0, 127.3, 126.8, 126.7, 123.7, 116.4, 116.2, 101.0, 21.4. Mass (M/z, (%)); 366 (18), 246(100), 213 (32), 95 (27), 69 (34). Anal. Calcd. For $C_{21}H_{16}ClFN_2O$: C, 68.76; H, 4.40; N, 7.64. Found: C, 68.46; H, 4.19; N, 7.39.

3-(4-chlorophenyl)-5-(4-methoxyphenyl) -4-phenyl-4,5-dihydro-1,2,4-oxadiazole (6 h)

Yield: 72%. Mp: 119-122 °C. ^{1}HNMR ($CdCl_3$) δ; 3.85(s, 3H, OCH_3), 6.49(s, 1H, H5-oxadiazole), 6.79(d, J = 7.5 Hz, 2H), 6.96 (d, J = 8.0 Hz, 2H), 7.13(m, 1H), 7.19(t, J = 7.5 Hz, 2H), 7.31(d, J = 8.0 Hz, 2H), 7.50–7.56(m, 4H). ^{13}CNMR ($CdCl_3$) δ; 160.83, 154.36, 140.86, 136.49, 130.80, 129.30, 129.23, 128.97, 128.70, 125.86, 124.44, 124.06, 114.21, 100.52, 55.35. Mass (M/z, (%)); 364 (36), 228 (100), 216 (41), 1135 (29), 77 (39). Anal. Calcd. For $C_{21}H_{17}ClN_2O_2$: C, 69.14; H, 4.70; N, 7.68. Found: C, 69.26; H, 4.55; N, 7.54.

3-(4-chlorophenyl)-4-(4-fluorophenyl)-5-(4-methoxyphenyl) -4,5-dihydro-1,2,4-oxadiazole (6i)

Yield: 76%. Mp: 98-102 °C. ^{1}HNMR ($CdCl_3$) δ; 3.88(s, 3H, OCH_3), 6.40(s, 1H, H5-oxadiazole), 6.76–6.79(m, 2H), 6.89 (t, J = 9.0 Hz, 2H), 6.97(d, J = 8.5 Hz, 2H), 7.32(d, J = 8.5 Hz, 2H), 7.49(d, J = 8.0 Hz, 4H). ^{13}CNMR ($CdCl_3$) δ; 161.7, 160.9, 159.7, 154.6, 136.8, 136.4, 130.2, 129.2, 129.0, 128.9, 126.9, 126.9, 123.7, 116.4, 116.2, 114.2, 100.9, 55.4. Mass (M/z, (%)); 382 (13), 258(50), 246 (88), 138 (54), 123 (100), 95 (52), 69 (41). Anal. Calcd. For $C_{21}H_{16}ClFN_2O2$: C, 65.89; H, 4.21; N, 7.32. Found: C, 65.53; H, 4.11; N, 7.46.

4-(4-chlorophenyl)-3-(4-fluorophenyl)-5-(4-methoxyphenyl) -4,5-dihydro-1,2,4-oxadiazole (6j)

Yield: 71%. Mp: 80-83 °C. ^{1}HNMR ($CdCl_3$) δ; 3.88(s, 3H, OCH_3), 6.45(s, 1H, H5-oxadiazole), 6.71(d, J = 8.5 Hz, 2H), 6.97 (d, J = 8.5 Hz, 2H), 7.05(t, J = 9.0 Hz, 2H), 7.17(d, J = 8.5 Hz, 2H), 7.49(d, J = 9.0 Hz, 2H), 7.55(m, 2H). ^{13}CNMR ($CdCl_3$) δ; 165.0, 163.0, 154.0, 140.1, 139.5, 135.4, 131.3, 130.1, 130.0, 129.6, 129.4, 127.2, 125.5, 121.4, 116.1, 116.0, 100.4, 54.9. Mass (M/z, (%)); 382 (21), 246 (100), 233 (19), 135 (13). Anal. Calcd. For $C_{21}H_{16}ClFN_2O_2$: C, 65.89; H, 4.21; N, 7.32. Found: C, 65.73; H, 3.98; N, 7.40.

3,4-bis(4-chlorophenyl)-5-(4-methoxyphenyl) -4,5-dihydro-1,2,4-oxadiazole (6 k)

Yield: 66%. Mp: 79-81 °C. ^{1}HNMR ($CdCl_3$) δ; 3.85(s, 3H, OCH_3), 6.45(s, 1H, H5-oxadiazole), 6.71(d, J = 9.0 Hz, 2H), 6.96(d, J = 9.0 Hz, 2H), 7.15(d, J = 8.5 Hz, 2H), 7.34(d, J = 8.5 Hz, 2H), 7.48–7.51(m, 4H). ^{13}CNMR ($CdCl_3$) δ; 160.4, 159.1, 139.3, 136.7, 131.5, 130.2, 129.4, 129.2, 129.1, 128.7, 125.7, 123.7, 114.3, 100.5, 55.4. Mass (M/z, (%)); 398 (20), 262(100), 246 (230), 135 (19), 111 (17). Anal. Calcd. For $C_{21}H_{16}Cl_2N_2O_2$: C, 63.17; H, 4.04; N, 7.02. Found: C, 63.36; H, 3.91; N, 7.29.

Biological evaluation

In vitro α-glucosidase inhibition activity

The α-glucosidase inhibitory activity of the synthesized compounds was evaluated using p-nitrophenyl-α-D-glucopyranoside (pNPG) as a substrate based on previously reported method (Nikookar et al., 2018). The α-glucosidase enzyme (EC3.2.1.20, *Saccharomyces cerevisiae*, 20 U/mg) and substrate (pNPG) were purchased from Sigma-Aldrich. The desired concentrations of enzyme were prepared in potassium phosphate buffer (pH 6.8, 50 mM), and the target compounds 6a-k were dissolved in DMSO (10% final concentration). A volume of 20 μL of α-glucosidase solution, different concentrations of the target compounds 6a-k (20 μL), and 135 μL of potassium phosphate buffer were added to the 96-well plate and incubated at 37 °C for 10 min. Then, 25 μL of 4 mM p-nitrophenyl-α-D-glucopyranoside solution in 0.05 M phosphate buffer (pH = 6.8) was added to each well and the reaction mixture was allowed to incubate for 20 min at 37 °C. Finally, the reaction was terminated by adding 50 μL of 0.2 M sodium carbonate solution and then, the change in absorbance was measured at 405 nm with a spectrophotometer (Gen5, Power wave xs2, BioTek, America). DMSO and acarbose were used as the control and standard inhibitor, respectively. The α-glucosidase inhibitory activity was expressed as the percentage of enzyme inhibition for each synthesized compound and calculated by the following formula [19, 20]:

% Inhibition = (Control Absorption-Sample Absorption)/(Control Absorption) × 100.

The concentration of the target compounds required to inhibit 50% of the enzyme activity (IC_{50}) values was obtained from non-linear regression curve using the Logit method.

Enzyme kinetic studies

The mode of inhibition of the most active compound **(6c)**, identified with the lowest IC_{50}, was investigated against alpha-glucosidase activity with different concentrations of p-nitrophenyl α -D-glucopyranoside (2–10 mM) as substrate in the absence and presence of 6c at different concentrations (0, 105, 160 and 215 μM). A Lineweaver–Burk plot was generated to identify the type of inhibition and the Michaelis–Menten constant (Km) value was determined from plot between reciprocal of the substrate concentration (1/[S]) and reciprocal of enzyme rate (1/V) over various inhibitor concentrations. Experimental inhibitor constant (Ki) value was constructed by secondary plots of the inhibitor concentration [I] versus Km.

Molecular docking

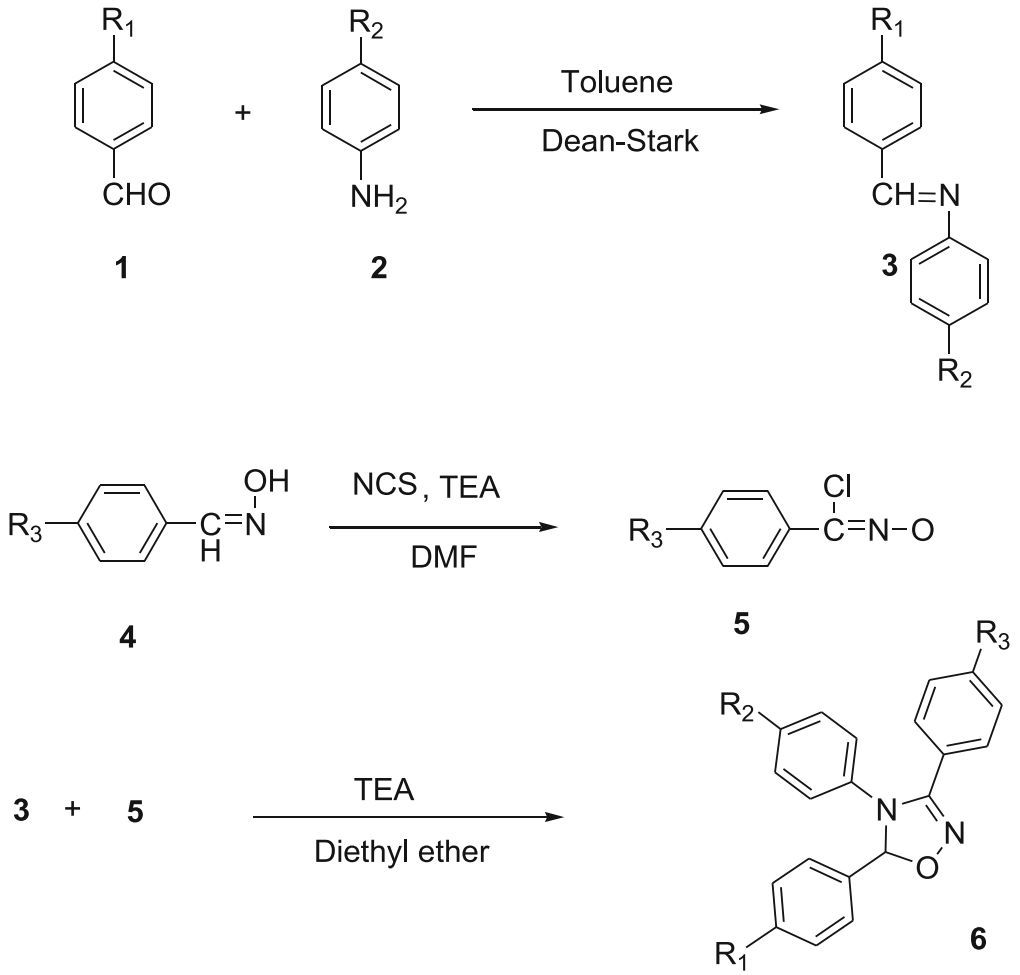
To develop more understanding in the mode of binding of the synthesized compounds, molecular docking studies were carried out. AutoDock version 4.2.6, AutoDock Tools (ADT) version 1.5.6 and Discovery studio v16.1.0.15350 were used to conduct the docking studies. The most potent compounds from the series was generated as ligand with their 3D structure, 6c and 6d using chemDraw Ultra 12.0.2 version of Cambridge University. Since there is no report yet about the crystal structure of α-glucosidase from *Saccharomyces cerevisiae* for preparing the protein for docking, we retrieved the crystal structure of isomaltase from *Saccharomyces cerevisiae* (PDB ID: 3A4A; Resolution 1.6 Å) from protein Data Bank (www.rcsb.org/pdb/). Then, the protein structure was prepared by removing the water molecules and original inhibitors. The ligand and protein pdbqt files were prepared and grid box formation was accomplished using AutoDock

Tools. AutoGrid was used in order to prepare the grid map using a grid box. The dimension of the grid size was set to 60 × 60 × 60 with grid spacing of 0.375 Å. The center of grid box was designated in coordinates x = 21.277, y = −0.773, and z = 18.648. Fifty runs of the AUTODOCK search by the Lamarckian genetic algorithm were performed for each docked system. So as to analyze the interactions between the target enzyme and the inhibitor, the best pose of each ligand was designated and the result was visualized with Discovery Studio Visualizer v16.1.0.15350 (Accelrys, San Diego, USA).

Results and discussion

Chemistry

The procedure for preparation of compounds **6** has been illustrated In Scheme 1. One of the reported synthesis route for preparation of 1,2,4-oxadiazoles is involved of the 1,3-dipolar cycloaddition of benzonitrile oxide and imines [18]. There are some reports that describe the dimerization of benzonitrile oxide within a few minutes to several days related to nature of substituted group in aryl ring [21]. As illustrated in scheme 1, an easy method for preparation of the final compounds **(6)** from condensation of 4-substituted benzohydroxyiminoyl chlorides **(5)** and imines **(3)** in diethyl ether was used. 4-Substituted benzohydroxyiminoyl chlorides were prepared from arylaldehyde oximes and N-chlorosuccinimide in DMF as reported procedure [22]. The compounds were characterized by ^{1}H and ^{13}C nuclear magnetic resonance, Mass spectroscopy and CHN analysis. In ^{1}HNMR spectra of the derivatives **(6)** a typical singlet signal at 6.45–6.52 ppm range is attributed to the H_5-oxadiazole ring. Other marker peaks in ^{13}CNMR spectra of compound 6 are the signals at 123 and 100 ppm that has been referred to C_3 and C_5 of oxadiazole ring, respectively. General procedure for preparation of **6a-k** was reported.



6a:R_1, R_2, R_3= H; **6b**: R_1, R_2=H, R_3=Cl; **6c**; R_1=H, R_2, R_3=Cl; **6d**: R_1=H, R_2=F, R_3=Cl; **6e**:R_1=Me, R_2, R_3=Cl; **6f**:R_1=Me, R_2=Cl, R_3=F; **6g**:R_1=Me, R_2=F, R_3=Cl; **6h**:R_1=OMe, R_2=H, R_3=Cl; **6i**: R_1=OMe, R_2=F, R_3=Cl; **6j**: R_1=OMe, R_2=Cl, R_3=F; **6K**:R_1=OMe, R_2, R_3=Cl

Scheme 1 Synthesis pathway for preparation of 6a-k

In vitro α-glucosidase inhibition activity

The activity of the target compounds **6a-k** was assessed in vitro against yeast α-glucosidase (EC 3.2.1.20) inhibition. The usage of this source of enzyme has been reported for inhibitory properties of α-glucosidase in numerous papers [15, 16, 19, 23–25, and]. A slightly different classification of α-glucosidases that is based on recognition of homologous sites of the enzymes' amino acid sequences has been reported [26]. 3D structures have also been obtained for the enzymes from A. oryzae, pig pancreas, *A. niger*, barley, and *Pseudomonas stutzeri*. All these structures have A similar topological features has been found in all these structres. In all these enzymes, the active site is a slit formed at the C-terminus of the β-sheet of the (β/α) 8 barrel [27]. Furthermore, a closeness results has been reported for assessment of α-glucosidase inhibitory activity using two different kind of enzyme (α-glucosidase EC 3.2.1.20; Sigma and rat intestinal α-glucosidase source). For kaempferol, a natural product separated from herb, IC_{50} were 61 μM and 59 μM respectively. The percentage of enzyme inhibition at concentration of 0.83 μM was reported as 68% and 55% respectively [28]. In this study, yeast α-glucosidase was used as a eukaryotic enzyme. Compound **6c** was bound to active site on the enzyme and competed with the substrate for binding to the active site, so the type of inhibition was competitive inhibition. The results were compared with acarbose, the marketed α-glucosidase inhibitor, as a standard drug. The activity of acarbose has been reported in a range of 600–800 μM related to source of the enzyme. In our experiments a 750 ± 12 μM was found for IC_{50} of acarbose in triplicated tests. The in vitro α-glucosidase inhibitory activity was carried out based on the previously reported method [23]. The results were described as the concentration of the target compounds required to inhibit 50% of the α-glucosidase activity (IC_{50} values) [29] and are summarized in Table 1.

The biological activity of the target compounds **6a-k** was illustrated in Table 1 that shows the better inhibitory activity for **6a-k** than the standard drug, acarbose. Optimization of the α-glucosidase inhibitory activity of 3,4,5-triaryl 1,2,4-oxadiazoles derivatives was performed using different substitution on 4 position of the phenyl rings. The differences in the pattern of inhibition of the synthesized compounds are mainly due to the nature of the substituents on the phenyl moiety.

The most potent compounds were **6c**, **6d**, and **6 k** with IC_{50} values 215 ± 3, 256 ± 3 and, 295 ± 4 μM, respectively while the IC_{50} value of acarbose was 750 ± 8.7 μM (Table 1). The other target compounds showed a moderate inhibitory activity around 1.64–2.45 folds more than acarbose with the IC_{50} values in the range of 305 ± 4 to 455 ± 6 μM. The comparison between activities of synthesized compounds shows that the substitution on the aryl ring is critical for inhibition of α-glucosidase as compound 6a was completely inactive in 2 mM. Substitution on R_3 especially chlorine atom increases the activity. The best group in R_1 is hydrogen if chlorobenzene has been substituted as the aryl number 3. In this case, both of

Table 1 α-Glucosidase inhibition assay of **6a-k**

Compounds	R_1	R_2	R_3	IC_{50}(μM)
Acarbose				750 ± 12
6a	H	H	H	inactive
6b	H	H	Cl	346 ± 5
6c	H	Cl	Cl	**215 ± 3**
6d	H	F	Cl	**256 ± 3**
6e	Me	Cl	Cl	455 ± 6
6f	Me	Cl	F	318 ± 4
6 g	Me	F	Cl	362 ± 5
6 h	OCH_3	H	Cl	305 ± 4
6i	OCH_3	F	Cl	444 ± 6
6j	OCH_3	Cl	F	314 ± 4
6 k	OCH_3	Cl	Cl	**295 ± 4**

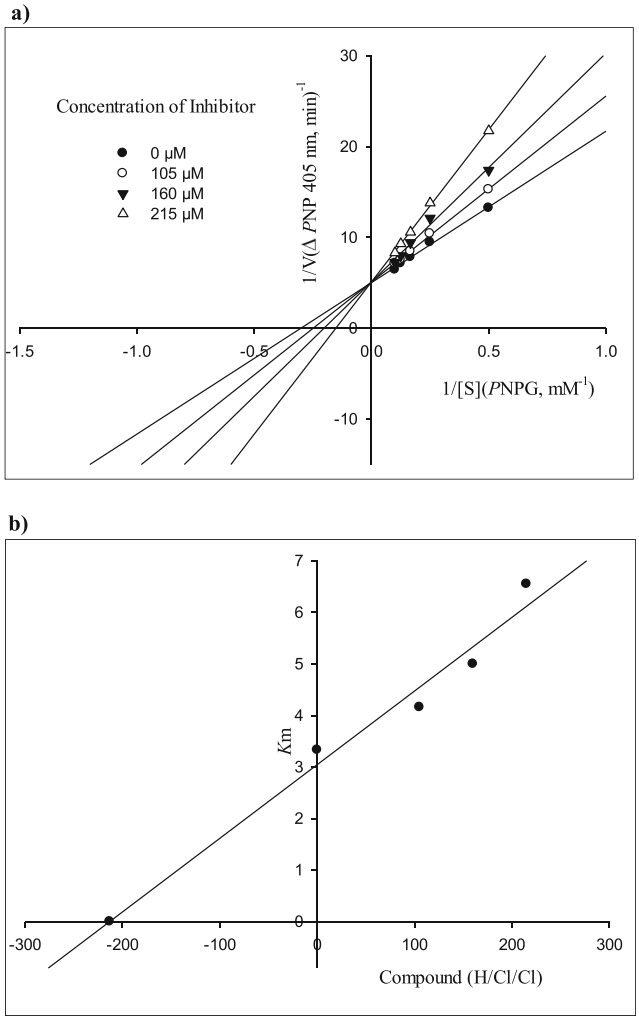


Fig. 2 Kinetics of alpha-glucosidase inhibition by 6c. (**a**) The Lineweaver-Burk plot in the absence and presence of different concentrations of 6c; (**b**) The secondary plot between Km and various concentrations of 6c

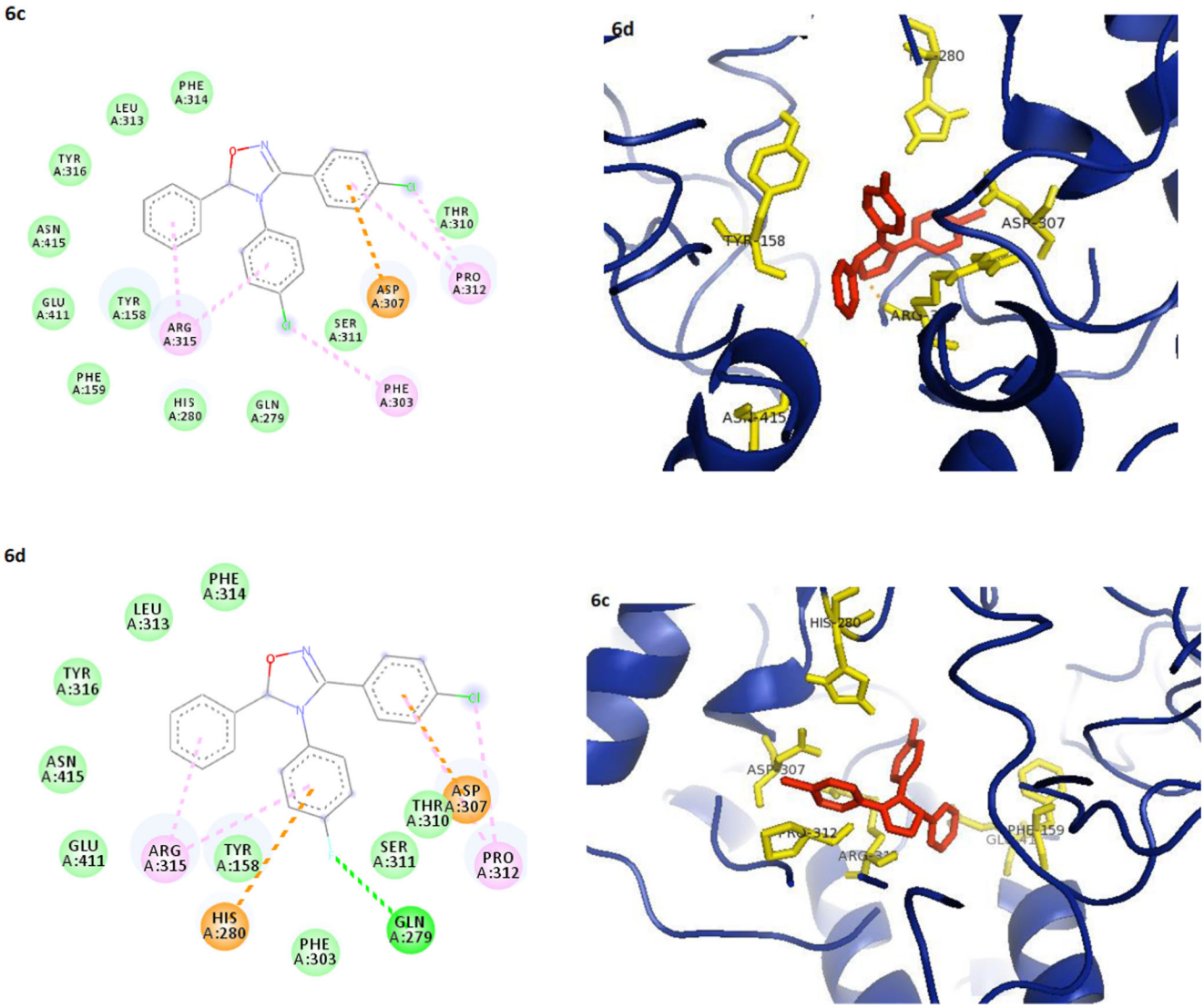


Fig. 3 Possible mode of interaction between the most potent compounds, 6c and 6d, and the enzyme active site

chlorine and fluorine atom has been tolerated as R_2 position and show better activity in comparison of hydrogen.

Enzyme kinetic studies

According to Fig. 2a, the Lineweaver-Burk plot showed that the K_m gradually increased and V_{max} remained unchanged with increasing inhibitor concentration indicating a competitive inhibition. The results show **6c** bind to active site on the enzyme and compete with the substrate for binding to the active site. Furthermore, plot of the K_m versus different concentration of inhibitor gave an estimate of the inhibition constant, K_i of 213 μM (Fig. 2b).

Docking study

In the modern area of drug discovery, molecular docking techniques have been widely applied in order to understand the interaction of the drugs with the receptors. In particular molecular docking techniques are used to assess the binding affinity and orientation of drug molecules in the possible target's site. Basically, the goal of molecular docking is accurate structural modeling and correct prediction of activity. Therefore, molecular docking studies have shaped the approach of drug design to a situation where the structure of the drug molecule will be designed based on the information obtained from the 3D binding fit on the receptor site [30].

After biological investigations, the possible mode of interaction between the most potent compounds, **6c** and **6d** with the enzyme active site were check by docking study. In the case of compound **6c**, PHE303, PRO312 and ARG315 residues of the active site had Π-alkyl interaction with the **6c**. ASP307 residue has also showed the Π-anion interaction with the **6c**. There are also some other van der waals interaction between **6c** and other residues in the active site. For compound **6d**, PRO312 and ARG315 residues showed Π-alkyl interaction with the **6d**. HIS280 and ASP307 residues had also showed the Π-anion

interaction with the **6d**. There is also hydrogen bond interaction between GLN279 and **6d**. Some other interactions are also detectable between **6d** and reside in the active site of the enzyme such as lipophilic interaction (Fig. 3).

Conclusion

In summary, a series of triaryl-dihydro-1,2,4-oxadiazoles derivatives were synthesized via condensation of N-benzylideneaniline derivative and N-hydroxybenzimidoyl chloride in diethyl ether in triethylamine. The reported derivatives exhibited α-glucosidase inhibition activity, among of them were better than reference compound (acarbose). Compounds **6c**, **6d**, and **6 k** showed potent activity among the series. Compound **6c** bearing chlorine substituent on R_2 and R_3 exhibited the most potent α-glucosidase inhibitory activity with an IC_{50} value of 215 ± 3 μM. The kinetic study showed that **6c** bind to active site on the enzyme and competed with the substrate for binding to the active site. Furthermore, plot of the K_m versus different concentration of inhibitor gave an estimate of the inhibition constant, K_i of 213 μM. Similarly, the molecular docking study also revealed that the two compounds have important binding interactions with the enzyme active site and can bind to the enzyme freely than the reference standard acarbose. Possible mode of interaction between the most potent compounds, **6c** and **6d**, with the active site of enzyme was studied. The Π-anion and Π-alkyl interaction were distinguished between **6c** and **6d** with the residue in the active site. The outcomes from the molecular docking studies of compounds **6c** and **6d** supported the results obtained from the in vitro assay. In conclusion, the synthesized compounds of this class of heterocyclic motifs have shown promising results for further development of potent, selective and efficacious inhibitors against the α-glucosidase enzyme that could be a potential candidate for design anti-diabetes new agents.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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