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Pyrimidine Derivatives as A-Glucosidase Inhibitors: Synthesis, Biological Activity Evaluation, Kinetic Analysis and Docking Study

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ABSTRACT

A new series of 2,4,6-triaryl pyrimidine derivatives were synthesized in order to investigate their α-glucosidase inhibitory activity in vitro. The designed pyrimidine derivatives 4a-k was synthesized in good yield (55-86%) via a two-step reaction. The structure of the synthesized compounds was confirmed by different spectroscopic techniques (IR, NMR, and Mass spectroscopy). The in vitro α-glucosidase inhibition activities of the synthesized compounds 4a–k was also evaluated against Saccharomyces cerevisiae α-glucosidase. All the synthesized compounds showed α-glucosidase inhibitory activities except compounds 4e, 4i and 4j. Compounds 4d and 4f were the most active with IC50 values of 168.9 ± 6.7 and 228.4 ± 8.4 µM respectively. The kinetic study also revealed that compound 4d was a competitive inhibitor with Ki of 166 µM. Molecular docking study was performed for the two most active compounds.

Keywords: α-Glucosidase; 2,4,6-Triaryl pyrimidine; Synthesis; Docking

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INTRODUCTION

The α-glucosidase, a key enzyme in the digestion of carbohydrate is a membrane-bound enzyme found in the intestinal epithelium of the small intestine. It acts on the non-reducing end of the sugar and releases α-D-glucose *via* hydrolyzing the α-glucopyranoside bond [1]. Hence, inhibiting the α-glucosidase enzyme is beneficial in the control of glucose absorption and reducing postprandial serum glucose level. Therefore, inhibition of α–glucosidase is an important strategy for the treatment of type-2 diabetes [2]. Studies indicated that α-glucosidase inhibitors had a beneficial effect on the long-term glycemic control as expressed by a decline in glycated hemoglobin A1c (HbA1c) in type-2 diabetes patients and delayed the onset of type-2 diabetes in individuals with impaired glucose tolerance [1,3]. Currently, three drugs are therapeutically used as anti-glucosidases [4]. Amongst the α-glucosidase inhibitors, acarbose (1), miglitol (2), and voglibose (3) (Figure 1) are being used for the treatment of type-2 diabetes [5]. However, management of diabetes without any side effect is still a challenge to the medical community [6].

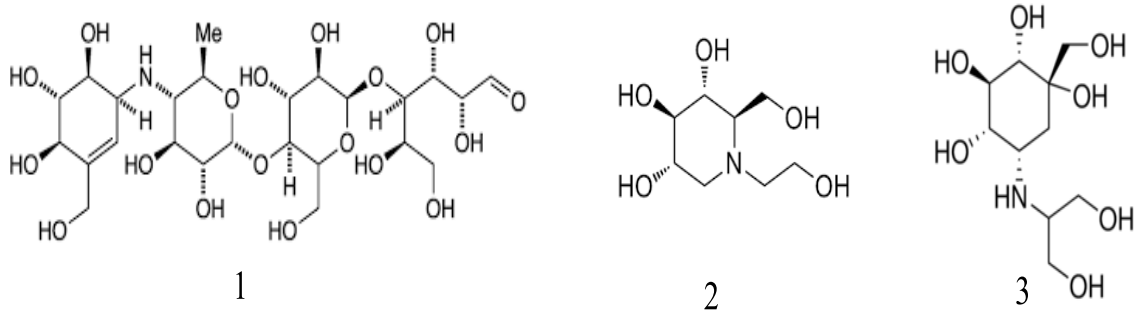


Figure 1. α-Glucosidase inhibitor antidiabetic drugs; acarbose (1), miglitol (2), and voglibose (3)

Pyrimidines are versatile compounds that exhibit a broad spectrum of pharmacological activities, including antibacterial, antiviral, anti-inflammatory, antihypertensive, antifungal, antidiabetic, anticonvulsant and anticancer activities [7]. Moreover, the pyrimidine moiety is widely found in natural products such as vitamin B1 (thiamine) and other therapeutic agents including sulfadiazine, flucytosine, trimethoprim and lamivudine [8]. Especially, some pyrimidines and pyrimidine fused ring containing compounds have been reported to have potent α-glucosidase inhibitory activities. Adib M et al., [9] reported in their study that all of the 6-amino-pyrido[2,3-d]pyrimidine-2,4-dione compounds tested in vitro against α-glucosidase were more active than the reference standard. Similarly, Barakat et al. [10] reported that the dihydropyrimidines derivatives synthesized in the study actively inhibited the α-glucosidase, where those with halogen groups in the ortho and Meta positions demonstrated a better biological activity and better drug-receptor interactions in comparison to their para counterparts. Yousefi et al. [11] and Panahi et al. [12] have also reported fused pyrimidine compounds that displayed antidiabetic activity *via* inhibiting α-glucosidase. Moreover, Shahidpour et al. [13] have reported that the pyrimidine derivatives in their study were active against mouse and yeast α-glucosidase. Therefore, this study is also focused on designing and synthesizing novel scaffolds containing tri substituted aryl-pyrimidines, which were tested for their α-glucosidase inhibitory activity. In addition, kinetic and molecular docking studies were done for the most active α-glucosidase inhibiting compounds.

EXPERIMENTAL SECTION

Chemistry

The chemicals used in this study were all obtained from the same supplier, Merck (Germany), and they were utilized without further purification. Melting points were determined by an Electro thermal 9100 apparatus and all the values are uncorrected. Shimadzu IR-460 spectrometer was used to record the IR spectra. ^{1}H and ^{13}C NMR ($CDCl_3$ solution) spectra of all the compounds were determined by using Bruker DRX-500 AVANCE (at 500.1 and 125.8 MHz) with TMS as an internal standard. MS were recorded with an Agilent Technology (HP) mass spectrometer operating (Ionization potential: 70 eV). The elemental analysis was obtained with an Elemetal Analyzer system GmbH VarioEL CHNS mode.

General Procedure for the Synthesis of Substituted 1,3-Diaryl Propenone Derivatives (3a-k)

A substituted methyl ketone (1 mmol) and a substituted aldehyde (1 mmol) were dissolved in a minimum amount of methanol (normally 5 ml) with stirring. NaOH pellets (300 mg) were added to it. In most cases, white to yellow solids were formed within a few minutes to 3 hr. The resulting solid material was filtered and washed three times with cold water. The products (3a-k) were recrystallized from ethanol [14].

General Procedure for the Synthesis of 2,4,6-Triaryl Pyrimidine Derivatives (4a-k)

To a 1,3-diaryl propenone derivative (3a-k) of 1 mmol in 10 ml isopropyl alcohol was added an equivalent amount of (1 mmol) of benzamidine hydrochloride. KOH pellets (300 mg) were added into the solution during 15 min. Dry air was bubbled through the reaction mixture. The reaction mixture was refluxed for 24 hr. Upon completion, the reaction mixture was poured into cold water. The resulting solid was filtered and washed with water. The products (**4a-k**) were recrystallized from ethanol [15].

4-(4-methoxyphenyl)-2-phenyl-6-(p-tolyl)pyrimidine (4a): Straw color solid; Yield (68%); mp 171-173°C; IR (KBr, cm^{-1}) 3028, 1587, 1514, 1365, 1257, 1174, 1022, 814, 756, 698; ^{1}H NMR (500 MHz, CDCl3, ppm): **δ** 8.72-8.70 (m, 2H), 8.24 (d, *J*=10.0 Hz, 2H), 8.16 (d, *J*=10.0 Hz, 2H), 7.88 (s, 1H, H_5-pyrimidine), 7.54-7.48 (m, 3H), 7.33 (dd, *J*=10.0 Hz, 2H, H_3, and H_5-2-phenyl), 7.05-7.02 (d, ***J***=15.0 Hz, 2H), 3.87 (s, 3H, -OCH_3), 2.43 (s, 3H, -CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): **δ 191.75,** 164.32, 164.20, 163.99, 161.80, 140.94, 138.37, 134.82, 130.43, 129.56, 128.72, 128.40, 128.36, 127.10 114.16, 108.99, 96.10, 77.33, 77.21, 77.01, 76.69, 55.39, 21.46; MS **(**m/**z, %)**: 352.2 (M+, 100), 337.1 (8), 321.2 (3), 234.1 (39), 132.1 (15), 115.1 (22), 89.1 (11), 77.0 (3).

2,4-diphenyl-6-(p-tolyl)pyrimidine (4b): White powder; Yield (59%); mp 150-151°C; IR (KBr, cm^{-1}) 3041, 1655, 1569, 1531, 1365, 837, 750; ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.73 (d, *J*=7.5 Hz, 2H, H_2 and H_6-2-phenyl), 8.29 (d, *J*=7.0 Hz, 2H), 8.20 (d, *J*=8.0 Hz, 2H), 7.98 (s, 1H, H_5-pyrimidine), 7.56-7.53 (m, 6H), 7.36 (d, *J*=8.0 Hz, 2H, H_3 and H_5-p-tolyl), 2.46 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 164.6, 164.5, 164.3, 141.1, 138.2, 137.6, 134.6, 130.7, 130.5, 129.6, 128.9, 128.4, 127.1, 109.9, 21.5; MS (m/z, %): 322.2 (M+, 100), 219.1 (48), 102.1 (48), 115.1 (48), 77.1 (14).

4-(4-chlorophenyl)-6-(4-methoxyphenyl)-2-phenylpyrimidine (4c): White solid; Yield (79%); mp 148-151°C; IR (KBr, cm^{-1}) 2843, 1585, 1524, 1362, 1255, 1171, 1088, 1036, 825, 754; ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.68 (d, *J*=7.5 Hz, 2H), 8.25 (d, *J*=9.0 Hz, 2H), 8.22 (d, *J*=8.5 Hz, 2H), 7.88 (s, 1H, H_5-pyrimidine), 7.53-7.50 (m, 6H), 7.05 (d, J=9.0 Hz, 2H), 3.9 (s, 3H,OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 164.3, 163.1, 161.9, 138.0, 136.8,

136.0, 130.6, 129.7, 129.0, 128.7, 128.5, 128.4, 128.3, 114.2, 109.0, 55.4; MS (m/z, %): 372.2 (M+, 100), 337.2 (5), 254.1 (11), 234.1 (18), 132.1(18), 89.0 (10).

4-(6-(4-chlorophenyl)-2-phenylpyrimidin-4-yl)-N,N-dimethylaniline (4d): Yellow solid; Yield (56%); mp 160-163°C; IR (KBr, cm^{-1}) 2910, 1572, 1510, 1371, 1203, 1095, 1036, 825, 754; ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 7.80-7.52 (dd, *J*=9.0 Hz, 4H), 6.98 (d, *J*=9.0 Hz, 2H), 6.65 (s, 1H, H_5-pyrimidine), 3.86 (s, 6H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 170.1, 162.51, 161.05, 160.9, 128.15, 127.4, 121.8, 120.4, 114.4, 114.3, 95.9, 77.24, 76.9, 76.7, 55.4, 55.3; MS (m/z, %): 385.2 (M+, 100), 145.1 (11), 105.1 (19), 71.1 (21).

4-(4-chlorophenyl)-6-(2,4-dichlorophenyl)-2-phenylpyrimidine (4e): White solid; Yield (62%); mp 186-188°C; IR (KBr, cm^{-1}) 2937, 1572, 1524, 1475, 1373, 1097, 1024, 831, 756; ^{1}H NMR (500 MHz, $CDCl_3$, ppm): *δ* 8.64-8.62 (m, 2H), 8.22-8.20 (d, J=9.0 Hz, 2H), 7.96 (s, 1H, H_5-pyrimidine), 7.82 (d, *J*=8.5 Hz, 1H), 7.56 (d, *J*=8.0 Hz, 1H, H_3-dichlorophenyl), 7.53-7.52 (m, 4H), 7.45-7.43 (dd, J=2.0 Hz, 1H) ; ^{13}C NMR (1125 MHz, $CDCl_3$, ppm): δ 164.7, 163.7, 162.7, 137.5, 137.2, 136.1, 135.7, 135.4, 133.0, 132.6, 130.9, 130.2, 129.1, 128.6, 128.5, 128.4, 127.7, 114.6. MS (m/z, %): 410.1 (M+, 77), 375.1 (22), 309.0 (6), 272.0 (50), 170.0 (36), 136.0 (63), 105.1 (33), 77.1 (59).

2-phenyl-4,6-di-p-tolylpyrimidine (4f): White solid; Yield (86.3%); mp 171-173°C; IR (KBr, cm^{-1}) 1571, 1514, 1356, 1182, 1016, 816, 750, 685; ^{1}H NMR (500 MHz, CDCl3, ppm): δ 8.72 (dd, *J*=7.3 Hz, *J*=5.0 Hz, 2H), 8.19 (m, 4H), 7.95 (s, 1H, H_5-pyrimidine), 7.53-7.50 (m, 3H), 7.35 (m, 4H), 2.45 (s, 6H, -CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 164.51, 141.01, 134.85, 130.49, 129.60, 128.42, 127.15, 109.56, 21.48; MS (m/z, %): 335.2 (M+, 100), 322.1 (4.4), 218.1 (35.5), 168.1 (4), 115.1 (57), 89.0 (7.4).

4,6-bis(4-methoxyphenyl)-2-phenylpyrimidine (4g): White solid; Yield (76.3%); mp 171-173°C; IR (KBr, cm^{-1}) 2971, 2842, 1606, 1571, 1508, 1367, 1172, 1026, 831; ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ=8.70 (d, *J*=7.0 Hz, 2 H, H_2 and H_6-2-phenyl), 8.26 (d, *J*=8.5 Hz, 4 H), 7.89 (s, 1H, H_5-pyrimidine), 7.53-7.52 (m, 3 H), 7.66 (d, *J*=8.5 Hz, 4H, H_3 and H_5-4,6-bis(4-methoxyphenyl)), 3.90 (s, 6 H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ=164.1, 163.83, 161.75, 138.39, 130.34, 130.04, 128.65, 128.33, 128.29, 114.12, 108.42, 55.34 ppm. MS (m/z, %): 368.3 (M+, 100), 265.1 (9.4), 250.1 (20), 132.1 (25), 117.1 (12), 89.1 (12).

2,4,6-triphenylpyrimidine (4h): White powder; Yield (63.2 %); mp>250°C. IR (KBr, cm^{-1}): 2945, 1644, 1527, 1394, 754, 700; ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.74-8.72 (d, *J*=7.0 Hz, 2H), 8.30-8.29 (d, *J*=7.0, 4H), 8.01 (s, 1H, H_5-pyrimidine), 7.58-7.52 (m, 9H); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 164.7, 163.9, 138.5, 137.8, 130.9, 130.6, 128.9, 128.7, 128.5, 127.5, 110.1; MS (m/z, %): 308.3 (M+, 100), 205.1 (83), 154.1 (8), 102.13 (91), 77.1 (24).

4,6-bis(4-chlorophenyl)-2-phenylpyrimidine (4i): White solid; Yield (64.5%); mp 202-204°C; IR (KBr, cm^{-1}): 2935, 1566, 1525, 1491, 1363, 1093, 1014, 827, 756; ^{1}H NMR (500 MHz, $CDCl_3$, ppm): *δ* 8.68-8.66 (m, 2H), 8.22 (d, *J*=8.5 Hz, 4H), 7.91 (s, 1H, H_5-pyrimidine), 7.53-7.51 (m, 7H); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 164.6, 163.6, 137.7, 137.1, 135.6, 130.9, 129.1, 128.5, 128.4, 109.6; MS (m/z, %): 376.2 (M+, 100), 341.2 (13), 273.1 (8), 238.1 (72), 136.0 (80), 101.0 (20), 75.0 (17).

4,6-bis(2,4-dichlorophenyl)-2-phenylpyrimidine (4j): White Solid; Yield (83.9%); mp 262-264°C; IR (KBr, cm^{-1}): 2918, 1591, 1525, 1473, 1375, 1105, 850, 806, 766; ^{1}H NMR (500 MHz, $CDCl_3$, ppm): *δ* 8.59-8.58 (m, 2H), 7.99 (s, 1H, H_5-pyrimidine), 7.84 (d, *J*=8.0 Hz, 2H), 7.57 (s, Hz, 2H, H_3 2,4-dichlorophenyl), 7.52-7.51 (m, Hz, 3H), 7.46

(m, 2H, H_3 and H_5-phenyl); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 162.8, 138.7, 137.4, 136.4, 135.6, 133.3, 132.7, 131.0, 130.4, 129.2, 128.6, 128.4, 127.7, 119.4. MS (m/z, %): 446.1 (M+, 65), 411.1 (18), 342.0 (6), 306.0 (9), 271.1 (9), 170.0 (100%), 77.1 (19).

4,6-bis(4-bromophenyl)-2-phenylpyrimidine (4k): White solid; Yield (71.8%); mp 207-208°C; IR (KBr, cm^{-1}): 3053, 1572, 1524, 1489, 1360, 1076, 1011, 820, 784; ^{1}H NMR (500 MHz, $CDCl_3$, ppm): *δ* 8.65-8.63 (m, 2H), 8.10 (d, *J*=8.5 Hz, 4H), 7.85 (s, 1H, H_5-pyrimidine), 7.65 (d, *J*=8.0 Hz, 4H), 7.53-7.52 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 164.5, 163.7, 137.6, 136.1, 132.2, 130.9, 128.9, 128.6, 128.5, 128.3, 128.2, 125.5, 109.5. MS (m/z, %): 466.1 (M+, 100), 385.2 (15), 282.1 (59), 203.1 (35), 182.0 (44), 153.0 (7), 101.1 (50), 75.1 (26).

***In vitro* α-Glucosidase Inhibition Assay**

The α-glucosidase inhibitory activity of the synthesized compounds was evaluated using *p*-Nitrophenyl-α-D-Glucopyranoside (pNPG) as a substrate based on the previously reported methods [16]. The α-glucosidase enzyme (EC3.2.1.20, *Saccharomyces cerevisiae*, 20 U/mg) and the substrate (pNPG) were purchased from Sigma-Aldrich. The enzyme was prepared in potassium phosphate buffer (pH 6.8, 50 mM), and 2,4,6-triaryl pyrimidine derivatives **4a-k** were dissolved in DMSO (10% of the final concentration). A volume of 20 µL of various concentration of the synthesized compounds **4a-k** and 135 µL of phosphate buffer containing 20 µL of the α-glucosidase solution was added to the 96-well plate and incubated for 10 min at 37°C. After pre-incubation, 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. 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 a control and reference drug, respectively. The α-glucosidase inhibitory activity was expressed as the percentage of enzyme inhibition for each synthesized compound and calculated by the following formula [16,17]:

$$\% \text{ Inhibition} = \frac{(\text{Control Absorption} - \text{Sample Absorption})}{Control\ Absorption} * 100$$

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

Enzyme Kinetic Studies

The mode of inhibition of the most active compound **4d**, identified with the lowest IC_{50}, was investigated against α-glucosidase activity with different concentrations of *p*-nitrophenyl *α*-D-glucopyranoside (1-10 mM) as a substrate in the absence and presence of sample **4d** at different concentrations (0, 80, 125 and 170 µM). A Lineweaver–Burk plot was generated to identify the type of inhibition and the Michaelis-Menten constant (K_m) 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 (K_i) value was constructed by secondary plots of the inhibitor concentration [I] *versus* K_m.

Molecular Docking Study

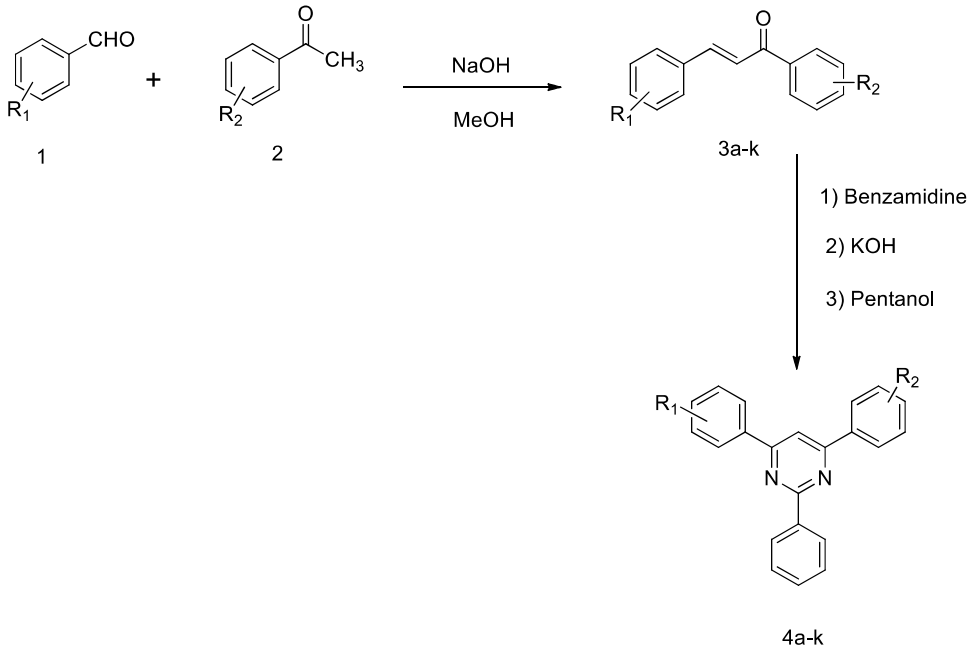
Homology modeling for *S. cerevisiae* α-glucosidase was done according to the method described by Imran et al. In order to identify the receptor with high sequence similarity to the *S. cerevisiae* α-glucosidase of the Protein Data Bank (PDB) was searched using SWISS-MODEL. Isomaltase from *S. cerevisiae* (PDB code 3A4A) was identified

to be 72% identical and found to have 85% similarity in sequence with the *S. cerevisiae* α-glucosidase [18,19]. Then subjected through sequence alignment and homology model using automated homology modeling pipeline SWISS-MODEL (managed by Swiss Institute of Bioinformatics) [20]. The quality check for the homology model obtained was done using PROCHECK. The structure of the most active compounds **4d** and **4f** was built and transformed to a 3D structure using MarvineSketch 19.1, 2018, ChemAxon [21] and then converted to pdbqt coordinate using Auto dock Tools [22]. Similarly, the pdbqt coordinate for the enzyme was produced by the Auto dock Tools after removing the water molecules and the inhibitors along with adding the polar hydrogens and assigning the Koullman charges. Then in the AUTOGRID for each atom type in the ligand, maps were calculated with 0.375 Å spacing between grid points, and the center of the grid box was placed at 60 60 60 with a spacing value of 0.375 Å. Flexible ligand docking was performed for the two most active compounds and the reference standard. For each docked system 50 runs were carried out by the AUTODOCK search using the Lamarckian genetic algorithm. The best pose of the runs was considered in analyzing the interaction between the enzyme and the docked compounds. The final results were visualized using Discovery Studio 2017 R2 Client and PyMol [23,24].

RESULTS AND DISCUSSION

Chemistry

The pyrimidine derivatives **4a-k** were synthesized in good yield (55-86%) as shown in **Scheme 1**. The target compounds were prepared *via* a two-step reaction. The first step includes the reaction of acetophenone and benzaldehyde derivatives in the presence of NaOH in methanol at room temperature to produce the corresponding chalcone. The second step involves the reaction of the already produced chalcone derivatives with benzamidine in amyl alcohol and KOH at 120°C. The structure of the target compounds was confirmed by spectral data from IR, ^{1}HNMR, ^{13}CNMR, and Mass spectroscopy.



Scheme 1. Synthesis of pyrimidine derivatives

***In vitro* α-Glucosidase Inhibitory Activity**

The *in vitro* α-glucosidase (*Saccharomyces cerevisiae*) inhibitory activity of the synthesized compounds was performed and the results were compared with the standard drug acarbose (IC_{50}=750.0 ± 5.0 µM). According to the IC_{50} values of the tested pyrimidine derivatives (4a-k), most of the compounds showed significant inhibition. The IC_{50} values for the test compounds were less than the reference standard, acarbose. The most active compounds were compound 4d and 4f with IC_{50} values 168.9 ± 6.7 and 228.4 ± 8.4 µM, respectively.

In order to study the trends in the structure-activity relationship and optimize the α-glucosidase inhibitory activity, different substituted benzaldehyde and acetophenone derivatives were utilized in the synthesis of the pyrimidine derivatives 4a-k, Table 1. Both electron donating and electron withdrawing groups such as such as methyl, methoxy, bromo and chloro have been used in the synthesis of the target compounds. In addition, some of the derivatives were symmetrical compounds 4h-4k, where the substitution on both sides is similar and useful to compare the structure-activity relationships (Figure 2).

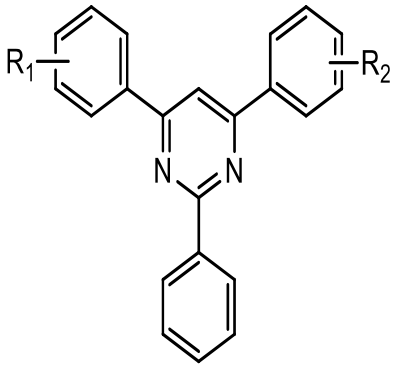


Figure 2. Structure of Pentanol

Table 1. *In vitro* α-Glucosidase inhibition activity of compounds 4a-k.

Compounds	R_1	R_2	IC_{50}(µM)
4a	4-Me	4-OMe	350.9 ± 10.1
4b	4-Me	H	277.9 ±7.5
4c	4-Cl	4-OMe	593.1 ± 11.2
4d	4-Cl	4-$N(Me)_2$	168.9 ± 6.7
4e	4-Cl	2,4-*di*-Cl	>750
4f	4-Me	4-Me	228.4 ± 8.4
4g	4-OMe	4-OMe	430.3 ± 10.3
4h	H	H	376.0 ±9.1
4i	4-Cl	4-Cl	>750
4j	2,4-*di*-Cl	2,4-*di*-Cl	>750
4k	4-Br	4-Br	457.3 ± 10.3
Acarbose			750.0 ± 5.0

Enzyme Kinetic Study

To evaluate the inhibition mechanism of the synthesized compounds, a kinetic study was performed on the most potent compound *4d*. The type of inhibition and the value of *Ki* were determined by Lineweaver-Burk and secondary re-plot of Lineweaver-Burk plots. According to Figure 3A, 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 sample **4d** bind to an active site on the enzyme and compete with the substrate for binding to the active site. Furthermore, the plot of the K_m versus different concentration of inhibitor gave an estimate of the inhibition constant, K_i of 166 µM (Figure 3B).

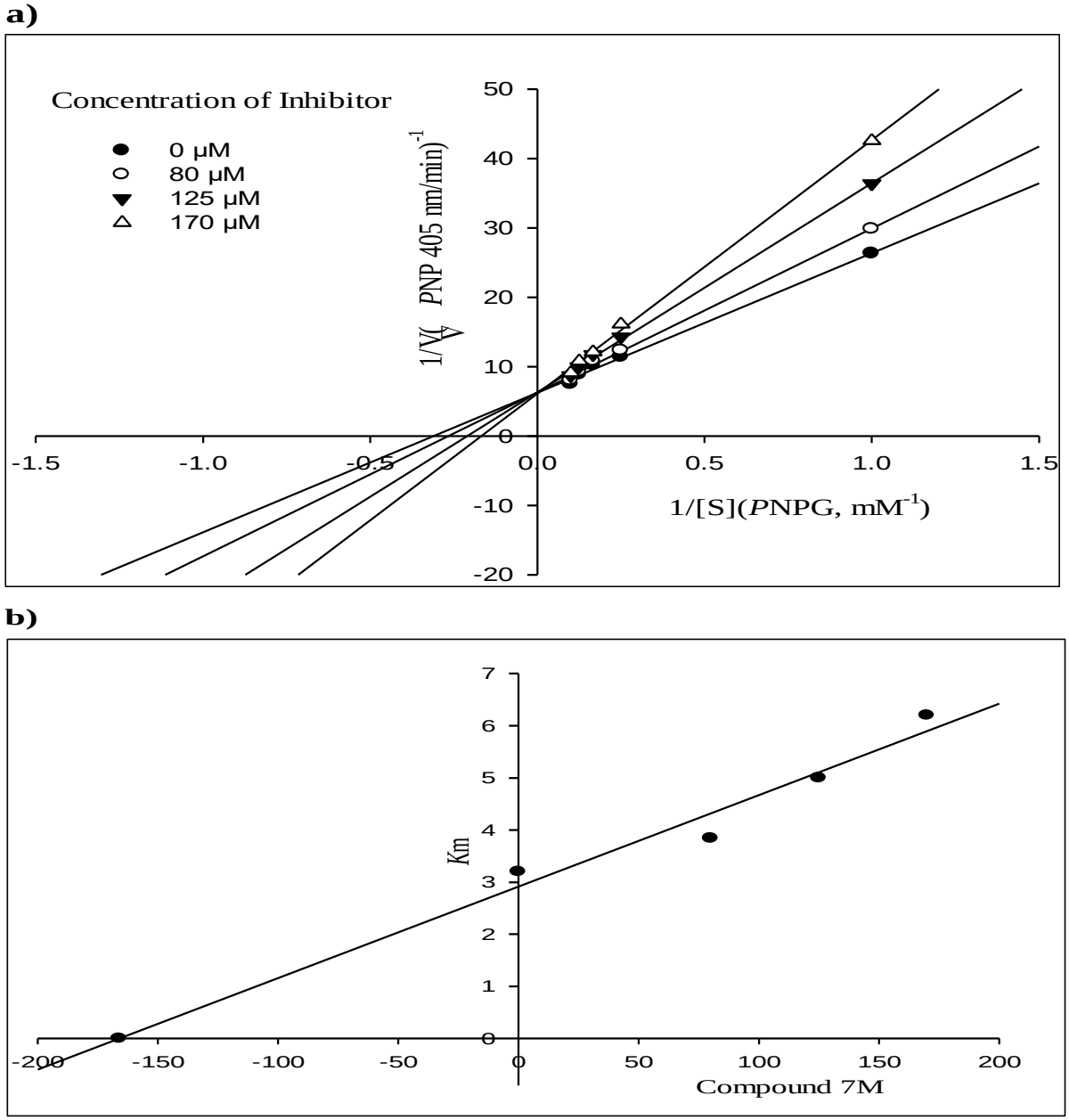


Figure 3. Kinetics of alpha-glucosidase inhibition by sample 4d. (A) The Lineweaver-Burk plot in the absence and presence of different concentrations of sample 4d; (B) The secondary plot between K_m and various concentrations of sample 4d

Docking Study

The docking study was performed using Auto Dock Tools (version 1.5.6) in order to determine the binding mode of the most active compounds **4d** and **4f** in the active site of *α-glucosidase*. The homology model of the *α*-glucosidase enzyme was utilized for the molecular docking since the crystallographic structure of the *Saccharomyces cerevisiae* *α*-glucosidase was not available in the RCSB protein data bank [18,25]. The superimposed structure of the most active compound (**4d**) and the reference standard acarbose and the superimposed structure of the two most active derivatives **4d** and **4f** the active site of homology model *α-glucosidase* is shown in Figure 4. As can be seen in Figure 3, acarbose interacted with Glu411, His351, Glu277, Arg442, His280, Ser311, Asp242, Phe303, Asp352, Pro342 and Gln279 residues in the active site of α-glucosidase.

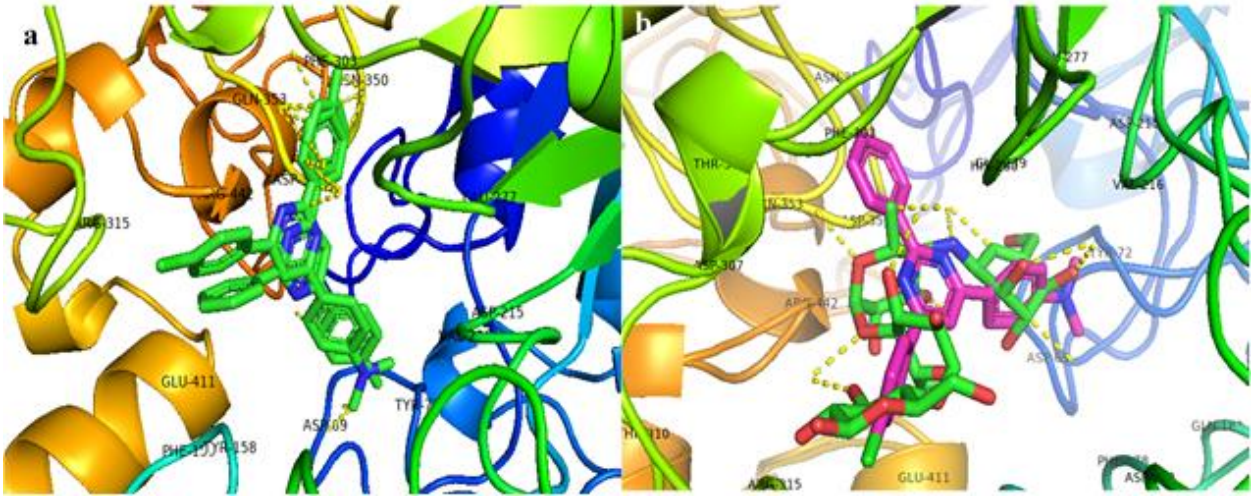


Figure 4. The superimposed binding mode of the most active compounds (compound 4d and 4f) (a) and superimposed binding mode of compound 4d and acarbose (b) in the active site of α-glucosidase

The mode of interaction of the most active derivative (**4d**) in the α-*glucosidase* enzyme active site *(*Figure 4*)* demonstrated that the various functional groups in the pyrimidine structure of the compound **4d** resulted in the hydrogen bond of the carbonyl function with Gln182, Asp215 and Asp69. The 2-aryl group and the pyrimidine ring of the compound ***4d*** formed **p-p** Phe303. In addition, the 2-aryl group interacted with Gln353 via pi-lone pairs. A **p**-anion interactions with Asp307 and Asp 352 was also formed with the substituted aryl functions. Hydrophobic interaction of the substituted aryl moieties resulted from interaction with Val216 and Arg315. Furthermore, the Cl in p-Cl-aryl ring interacted with Arg313 via pi-alkyl interactions. Essentially these are the interactions that played a major role in the α-glucosidase inhibitory activity of the compound 4d. Moreover, the interaction within the active site for the second most active compound **4f** is shown in Figure 5. Accordingly, compound **4f** has formed interactions with residues such as Phe178, Arg442, Asp352, Arg315, Tyr72, His216, Val112, Phe303, Gln353, and Tyr347.

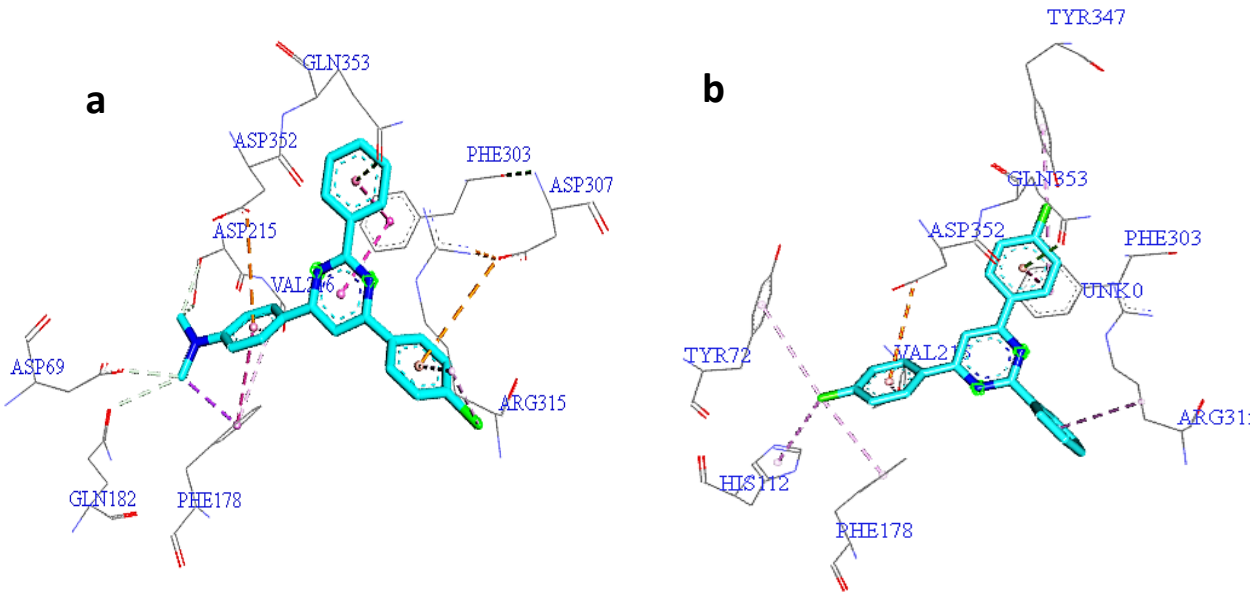


Figure 5. Binding mode of the most active compounds; (a) compound 4d and (b) compound 4f in the active site of α-glucosidase

CONCLUSION

In summary, the novel pyrimidine derivatives **4a-k** synthesized in this study are mostly active inhibitors of the α-glucosidase enzyme as compared to the standard drug acarbose. The better activity of compounds **4d** and **4f** suggests that the presence of a methyl group is important for the α-glucosidase enzyme inhibitory activity of the derivatives. Compound **4d** with dimethylamino substitution recorded the highest inhibition among the tested compounds with an IC_{50} value of 168.9 ± 6.7 µM. The enzyme kinetic study also revealed that the most active compound **4d** competitively inhibits the α-glucosidase enzyme. Furthermore, the docking study also demonstrated that the most active compounds **4d** and **4f** have various interactions with the residues in the receptors active site. The interactions that played a major role in the binding includes hydrogen bonds, p-anion interactions and hydrophobic interaction.

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