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Synthesis, *in-vitro* evaluation, molecular docking, and kinetic studies of pyridazine-triazole hybrid system as novel α-glucosidase inhibitors

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ABSTRACT

In this study, we reported the discovery of pyridazine based 1,2,3-triazole derivatives as inhibitors of α-glucosidase. All target compounds exhibited significant inhibitory activities against yeast and rat α-glucosidase enzymes compared to positive control, acarbose. The most potent compound **6j**, ethyl 3-(2-(1-(4-nitrobenzyl)-1*H*-1,2,3-triazol-4-yl)ethyl)-5,6-diphenylpyridazine-4-carboxylate exhibited IC_{50} values of 58, and 73 μM. Docking studies indicated the responsibility of hydrophobic and hydrogen bonding interactions in the ligand-enzyme complex stability. The *in-vitro* safety against the normal cell line was observed by toxicity evaluation of the selected compounds.

1. Introduction

A progressive metabolic disorder, Diabetes (diabetes mellitus), characterized by high glucose levels, affects millions of people's life worldwide. Assorted into type 1 and type 2, diabetes is resulted from the unevenness in the action or in the secretion of insulin [1,2]. The type 2 diabetes mellitus (T2DM) and its total societal costs have the sensible and tremendous impact on societies. Regarding the expectations about the growing rate and high risks associated with diabetic patients, the pharmacological treatment strategies along with the changes in lifestyle should be considered to improve the well-being of patients. Most of diabetes mellitus type-2 patients, a non-insulin dependent diabetes, are suffering from hyperglycemia which led to the serious health disorders including heart disease and stroke, renal failure, optic neuropathy, nerve damage, foot problems, and skin complications. Due to the importance of hyperglycemia and its serious long-term complications, many treatment approaches have been devised. α-Glucosidase inhibitors have been considered as the subject of several research studies during the last decades for the treatment of diabetes. α-Glucosidase is a catabolic enzyme of intestinal brush border which catalyzes the hydrolysis of α-1,4-glucoside bond in oligosaccharides to form absorbable monosaccharides [3,4]. The inhibition of this enzyme could reduce or slow the postprandial hyperglycemia by retarding the absorption of glucose [5–9]. Biguanides [10], sulfonylureas [11], and thiazolidindiones [12,13] were found effective in the treatment of diabetes. Moreover, miglitol, nojirimycin, voglibose, and acarbose [14–17] are therapeutically glucosidase inhibitors in the market, effectively manage the symptoms of this metabolic disorder by regulating the glucose level. The reported side effects involving hepatic disorders, gastrointestinal, and diarrhea [18,19] along with limited hyperglycemic activity have motivated medicinal chemists to design selective and potent non-glycosidic based compounds as potential therapeutic agents.

Click reaction is one of the most important chemical transformations, employed in academic and pharmaceutical researches to form triazole ring [20]. In particular, copper (I)-catalyzed azide-alkyne cycloaddition (CuAAC) is known for its distinct advantages, such as exquisite

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selectivity, broad scope, and mild reaction condition [21–23]. Triazoles are one of the most important toolkits in medicinal chemistry [24–29], extensively used to synthesize lead analogues by providing the good mimics of different functional groups involving amide bond, ester bond and various heterocycles. This valuable heterocyclic core have also found applications in functional materials [30–33]. Therefore, several hybrid molecules containing 1,4-disubstituted-1,2,3-triazoles with diverse range of bioactivities have been reported and found their way to the market [34].

Pyridazine is the ubiquitous nitrogen containing heterocyclic core found in a wide range of molecules with immense biological functionalities [35–37]. Some investigations reported that applications of pyridazinones as antihypertensive, antifungal, anti-inflammatory, anticancer, and antimicrobial agents [38–43]. Accordingly, tremendous effort has been devoted to the hybridization of this valuable core to afford multi-target novel bioactive compounds. To date, different classes of glucosidase inhibitors with different chemical scaffolds have been developed. Given the importance of triazines in the inhibition of glucosidase, we were keen to demonstrate the utility of pyridazine as its bioisostere. In addition, many triazole-containing glucosidase inhibitors have been found in the literature. Fig. 1, schematically presented the previously reported compounds bearing these prominent cores [44–50].

Previously, we have reported on the synthesis of glucosidase inhibitors [51,52], so, in pursuit of our goal of discovering novel bioactive agents [53–55], herein, we designed and synthesized a series of glucosidase inhibitors based on the pyridazine core according to the bioisosterism principle. The sixteen final compounds showed better activities compared to the activity of the positive control.

2. Experimental

2.1. General chemistry

All commercially available reagents and solvents, used without further purification, were purchased from Merck and Sigma-Aldrich companies. Bruker FT-500 MHz spectrometer was used to confirm the structure of the target compounds by ^{1}H- and ^{13}C NMR spectra in DMSO-d_6 as the solvent. Chemical shifts reported in δ parts per million (ppm) downfield from tetramethylsilane (TMS) as the internal standard. All reactions were monitored through thin-layer chromatography (TLC) on silica gel 250 mm, F254 plastic sheets. Elemental analysis was recorded by a Perkin Elmer 2400 (automatic elemental analyzer). Melting points were determined with a Kofler hot-plate microscope apparatus and are uncorrected. IR spectroscopy was performed using a Nicolet FT-IR Magna 550 spectrograph (KBr disks).

2.1.1. Synthesis of ethyl-2,3-dihydro-3-oxo-5,6-diphenylpyridazine-4-carboxylate (3)

A cooled flask was charged with sodium (0.05 mol) and ethanol (200 mL). Upon disappearance of sodium, compound **2** (0.05 mol) and diethyl malonate (0.075 mol) were added at 0 °C. The mixture was cooled to room temperature and was stirred at reflux temperature for 3 h. After this time, the mixture was evaporated under reduced pressure and the residue was acidified with HCl (1 N). The slight yellow solid was simply filtered and washed with cold water. mp. lit. 218–219 °C [56], mp. obtained; 217–219 °C.

2.1.2. Synthesis of ethyl-3-mercapto-5,6-diphenylpyridazine-4-carboxylate (4)

Compound **3** (20 mmol), toluene (150 mL), and Lawesson's reagent (10 mmol) were added to the flask and the mixture was refluxed for 18 h. After this time, the mixture was concentrated and the residue was recrystallized from petroleum ether/ethyl acetate, mp. lit. 230 °C [57], mp. obtained; 228–230 °C.

2.1.3. Synthesis of ethyl-5,6-diphenyl-3-(prop-2-ynylthio)pyridazine-4-carboxylate (5)

To the stirred solution of ethyl-3-mercapto-5,6-diphenylpyridazin-4-carboxylate (5 mmol), K_2CO_3 (5 mmol) in DMF (10 mL), propargyl bromide (6 mmol) was added dropwise and the mixture was stirred at 50 °C. After completion, checked by TLC, the reaction was quenched with ice/water solution and the resulted precipitate was filtered, washed with water and used without further purification.

2.1.3.1. Ethyl 5,6-diphenyl-3-(prop-2-yn-1-ylthio)pyridazine-4-carboxylate (5). Light-yellow solid; yield: 92%; mp: 79–81 °C; IR (KBr, cm^{-1}): 2173, 1734 (C═O), 1322, 1192; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.34 (t, J = 6.9 Hz, 3H, CH_3), 2.38 (t, J = 3.0 Hz, 1H), 4.00 (d, J = 2.9 Hz, 2H, S-CH_2), 4.33 (q, J = 6.9 Hz, 2H, CH_2-CH_3), 7.38–7.41 (m, 2H, Ar), 7.47–7.56 (m, 5H, Ar), 7.57–7.58 (m, 1H, Ar), 7.97 (d, J = 8.0 Hz, 2H, Ar); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 14.22 (CH_3), 18.66 (SCH_2), 60.68 (OCH_2), 72.36 (C≡CH), 79.23 (C≡CH), 127.06 (CH), 127.92 (CH), 128.45 (CH), 129.02 (CH), 129.85 (CH), 129.99 (CH), 130.37 (C), 134.87 (C), 135.95 (C), 138.35 (C), 155.72 (C═N), 159.20 (C═N), 166.88 (C═O); Anal. Calcd. For $C_{22}H_{18}N_2O_2S$: C, 70.57; H, 4.85; N, 7.48. Found: C, 70.16; H, 4.83; N, 7.52; ESI-MS m/z: 375.11 $[M + H]^+$.

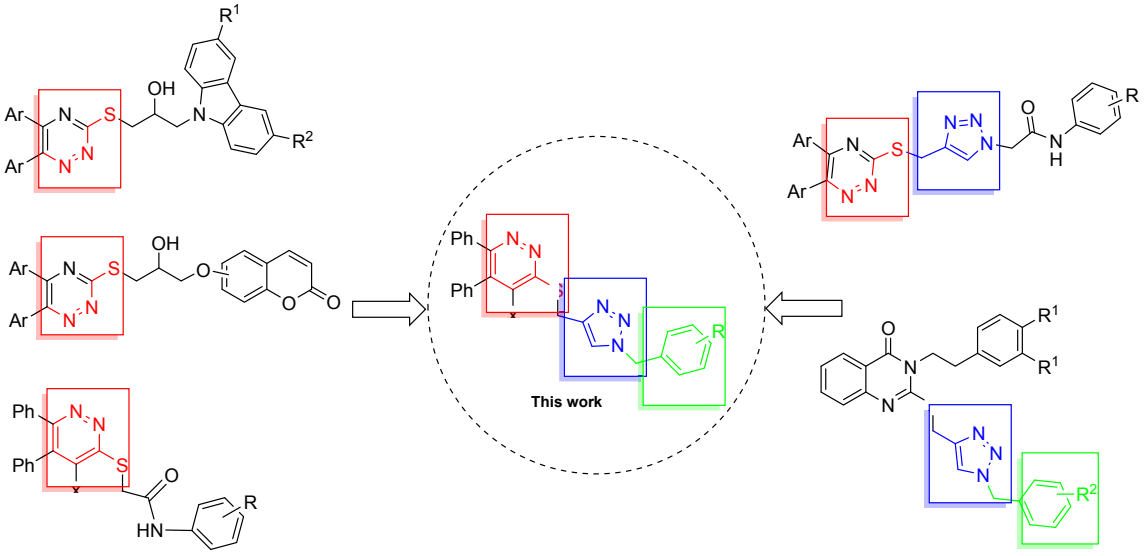


Fig. 1. Previously described triazole and triazine-containing analogues with alpha-glucosidase inhibitory activity.

2.1.4. General procedure for the preparation of compounds (6a-6 m)

A solution of benzyl bromide/chloride derivatives (1 mmol), sodium azide (1 mmol) were stirred in a H_2O/*tert*-buthanol (1:1, 10 mL) for 1 h. After this time, compound **5** (1 mmol), triethyl amine (1 mmol), sodium ascorbate (10 mol%) and copper (II) sulfate (10 mol%) were added to the reaction mixture and the reaction was stirred for 6–10 h. After this time, the ice/water solution was added to the mixture and the resultant solid was recrystallized from ethanol.

2.1.4.1. Ethyl 3-(((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)thio)-5,6-diphenylpyridazine-4-carboxylate (6a). White solid; yield: 83%; mp: 118–120 °C; IR (KBr, cm^{-1}): 1723 (C═O), 1639, 1458, 1319, 1194; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.84 (t, J = 7.2 Hz, 3H, CH_3), 4.02 (q, J = 7.2 Hz, 2H, CH_2-CH_3), 4.74 (s, 2H, S-CH_2), 5.58 (s, 2H, N-CH_2), 7.12 (d, J = 7.1 Hz, 2H, Ar), 7.27–7.35 (m, 13H, Ar), 8.16 (s, 1H, *N*-CH); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 13.78 (CH_3), 25.23 (SCH_2), 53.22 (NCH_2), 62.56 (OCH_2), 124.43, 124.47, 128.37, 128.57, 128.79, 129.10, 129.18, 129.31, 129.45, 129.47, 130.13, 130.90, 134.08, 135.64, 136.42, 136.49, 143.36 (S-CH_2-**C**), 155.88 (C═N), 157.81 (S-**C**═N), 164.51 (C═O); Anal. Calcd. For $C_{29}H_{25}N_5O_2S$. C, 68.62; H, 4.96; N, 13.80. Found: C, 68.47; H, 4.67; N, 13.96; ESI-MS *m/z*: 508.18 $[M + H]^+$.

2.1.4.2. Ethyl 3-(((1-(2-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)thio)-5,6-diphenylpyridazine-4-carboxylate (6b). Gray solid; yield: 63%; mp: 116–118 °C; IR (KBr, cm^{-1}): 1728 (C═O), 1640, 1456, 1322, 1184; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.87 (t, J = 7.1 Hz, 3H, CH_3), 4.04 (q, J = 7.1 Hz, 2H, CH_2-CH_3), 4.76 (s, 2H, S-CH_2), 5.66 (s, 2H, N-CH_2), 7.14 (d, J = 6.7 Hz, 2H, Ar), 7.19–7.35 (m, 11*H*, Ar), 7.40–7.42 (m, 1H, Ar), 8.14 (s, 1H, *N*-CH); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 13.79 (CH_3), 25.24 (SCH_2), 47.36 (NCH_2), 62.57 (OCH_2), 116.06 (d, $^2J_{C\text{-}F}$ = 20.0 Hz), 123.30 (NCH), 124.56, 125.28, 128.36 (2C), 128.79, 129.11 (2C), 129.32 (2C), 129.47 (2C), 130.14, 130.96, 131.15, 131.21, 134.10, 135.65, 136.45, 143.32 (S-CH_2-**C**), 155.90 (C═N), 157.84 (S-**C**═N), 160.54 (d, $^1J_{C\text{-}F}$ = 246.7 Hz), 164.51 (C═O); Anal. Calcd. For $C_{29}H_{24}FN_5O_2S$. C, 66.27; H, 4.60; N, 13.32; Found: C, 66.01; H, 4.82; N, 13.06; ESI-MS *m/z*: 526.17 $[M + H]^+$.

2.1.4.3. Ethyl 3-(((1-(3-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)thio)-5,6-diphenylpyridazine-4-carboxylate (6c). Gray solid; yield: 79%; mp: 125–127 °C; IR (KBr, cm^{-1}): 1727 (C═O), 1639, 1458, 1321, 1183; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.87 (t, J = 7.1 Hz, 3H, CH_3), 4.05 (q, J = 7.1 Hz, 2H, CH_2-CH_3), 4.77 (s, 2H, S-CH_2), 5.63 (s, 2H, N-CH_2), 7.21–7.23 (m, 5H, Ar), 7.29–7.35 (m, 8H, Ar), 7.40–7.44 (m, 1H, Ar), 8.21 (s, 1H, *N*-CH); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 13.79 (CH_3), 25.25 (SCH_2), 52.56 (NCH_2), 62.57 (OCH_2), 115.14, 115.34, 115.53, 124.46 (d, $^4J_{C\text{-}F}$ = 3.75 Hz), 124.61, 128.35 (2C), 128.79 (d, $^2J_{C\text{-}F}$ = 25.8 Hz), 129.21 (d, $^2J_{C\text{-}F}$ = 25.8 Hz), 129.47 (2C), 130.14 (2C), 130.95 (2C), 131.28 (d, $^3J_{C\text{-}F}$ = 8.5 Hz), 134.00, 135.65, 136.45, 139.18 (d, $^3J_{C\text{-}F}$ = 7.6 Hz), 143.49 (S-CH_2-**C**), 155.87 (C═N), 157.84 (S-**C**═N), 162.58 (d, $^1J_{C\text{-}F}$ = 244.3 Hz), 164.51 (C═O); Anal. Calcd. For $C_{29}H_{24}FN_5O_2S$. C, 66.27; H, 4.60; N, 13.32; Found: C, 66.52; H, 4.43; N, 13.51. ESI-MS *m/z*: 526.17 $[M + H]^+$.

2.1.4.4. Ethyl 3-(((1-(3-bromobenzyl)-1H-1,2,3-triazol-4-yl)methyl)thio)-5,6-diphenylpyridazine-4-carboxylate (6d). Off-white solid; yield: 76%; mp: 136–137 °C; IR (KBr, cm^{-1}): 1721 (C═O), 1631, 1453, 1319, 1149; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.88 (t, J = 7.1 Hz, 3H, CH_3), 4.05 (q, J = 7.1 Hz, 2H, CH_2-CH_3), 4.77 (s, 2H, S-CH_2), 5.61 (s, 2H, N-CH_2), 7.14–7.15 (m, 2H, Ar), 7.30–7.36 (m, 10*H*, Ar), 7.54–7.55 (m, 2H, Ar), 8.21 (s, 1H, *N*-CH); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 13.27 (CH_3), 33.94 (SCH_2), 52.03 (NCH_2), 58.64 (OCH_2), 112.44, 122.79 (C-Br), 122.93, 127.58 (2C), 127.96 (2C), 128.03, 128.27 (2C), 128.40, 128.44 (2C), 128.68, 129.89, 131.00, 132.25, 132.39, 132.45, 136.54, 137.96, 143.40 (S-CH_2-**C**), 146.76 (C═N), 153.87 (S-**C**═N), 164.32 (C═O); Anal. Calcd. For $C_{29}H_{24}BrN_5O_2S$. C, 59.39; H, 4.12; N, 11.94. Found: C, 59.28; H, 4.15; N, 12.02. ESI-MS *m/z*: 586.09 $[M + H]^+$.

2.1.4.5. Ethyl 3-(((1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)thio)-5,6-diphenylpyridazine-4-carboxylate (6e). White solid; yield: 85%; mp: 124–126 °C; IR (KBr, cm^{-1}): 1721 (C═O), 1639, 1456, 1309, 1214; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.84 (t, J = 7.1 Hz, 3H, CH_3), 4.02 (q, J = 7.1 Hz, 2H, CH_2-CH_3), 4.73 (s, 2H, S-CH_2), 5.57 (s, 2H, N-CH_2), 7.12 (dt, J = 6.8, 1.6 Hz, 2H, Ar), 7.16–7.20 (m, 2H, Ar), 7.29–7.39 (m, 10*H*, Ar), 8.15 (d, J = 2.4 Hz, 1H, *N*-CH); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 13.79 (CH_3), 25.26 (SCH_2), 50.16 (NCH_2), 52.40 (OCH_2), 115.24 (d, $^2J_{C\text{-}F}$ = 20.0 Hz), 122.02 (2C), 127.17, 128.09 (2C), 128.11, 129.07 (2C), 129.88 (2C), 129.99 (2C), 130.01, 130.30 (d, $^3J_{C\text{-}F}$ = 7.9 Hz, 2C), 133.09 (d, $^4J_{C\text{-}F}$ = 3.1 Hz), 134.97, 136.19, 138.45, 144.03 (S-CH_2-**C**), 155.67 (C═N), 158.66 (S-**C**═N), 163.13 (d, $^1J_{C\text{-}F}$ = 252.1 Hz), 164.49 (C═O); Anal. Calcd. For $C_{29}H_{24}FN_5O_2S$. C, 66.27; H, 4.60; N, 13.32. Found: C, 65.93; H, 4.84; N, 13.06; ESI-MS *m/z*: 526.17 $[M + H]^+$.

2.1.4.6. Ethyl 3-(((1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)thio)-5,6-diphenylpyridazine-4-carboxylate (6f). White solid; yield: 81%; mp: 161–162 °C; IR (KBr, cm^{-1}): 1724 (C═O), 1632, 1454, 1324, 1166; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.88 (t, J = 7.1 Hz, 3H, CH_3), 4.04 (q, J = 7.1 Hz, 2H, CH_2-CH_3), 4.78 (s, 2H, S-CH_2), 5.46 (s, 2H, N-CH_2), 7.28 (d, J = 8.5 Hz, 2H, Ar), 7.38–7.46 (m, 6H, Ar), 7.53–7.58 (m, 4H, Ar), 7.97 (d, J = 8.5 Hz, 2H, Ar), 8.11 (s, 1H, *N*-CH); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 13.82 (CH_3), 31.64 (SCH_2), 52.85 (NCH_2), 59.62 (OCH_2), 122.05, 127.23, 128.09 (2C), 128.12, 128.66 (2C), 129.07 (2C), 129.88 (2C), 129.91 (2C), 129.99 (2C), 130.13, 132.37, 134.97 (C-Cl), 135.05, 136.19, 138.45, 144.03 (S-CH_2-**C**), 154.62 (C═N), 156.63 (S-**C**═N), 164.90 (C═O); Anal. Calcd. For $C_{29}H_{24}ClN_5O_2S$: C, 64.26; H, 4.46; N, 12.92. Found: C, 64.41; H, 4.14; N, 12.67; ESI-MS *m/z*: 542.14 $[M + H]^+$.

2.1.4.7. Ethyl 3-(((1-(4-bromobenzyl)-1H-1,2,3-triazol-4-yl)methyl)thio)-5,6-diphenylpyridazine-4-carboxylate (6g). White solid; yield: 84%; mp: 129–131 °C; IR (KBr, cm^{-1}): 1723 (C═O), 1636, 1457, 1323, 1156; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.84 (t, J = 7.1 Hz, 3H, CH_3), 4.01 (q, J = 7.1 Hz, 2H, CH_2-CH_3), 4.72 (s, 2H, S-CH_2), 5.55 (s, 2H, N-CH_2), 7.11 (d, J = 7.3 Hz, 2H, Ar), 7.22–7.24 (m, 2H, Ar), 7.26–7.33 (m, 8H, Ar), 7.53 (d, J = 8.2 Hz, 2H, Ar), 8.14 (s, 1H, *N*-CH); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 13.74 (CH_3), 32.56 (SCH_2), 51.41 (NCH_2), 59.46 (OCH_2), 112.86, 121.06 (C-Br), 122.10, 127.55 (2C), 127.73 (2C), 128.06, 128.30 (2C), 128.48 (2C), 128.79, 131.14 (2C), 131.90 (2C), 132.39, 132.45, 136.10, 137.96, 143.40 (S-CH_2-**C**), 146.76 (C═N), 153.87 (S-**C**═N), 164.73 (C═O); Anal. Calcd. For $C_{29}H_{24}BrN_5O_2S$. C, 59.39; H, 4.12; N, 11.94. Found: C, 59.15; H, 4.39; N, 12.10; ESI-MS *m/z*: 586.09 $[M + H]^+$.

2.1.4.8. Ethyl 3-(((1-(2,6-difluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)thio)-5,6-diphenylpyridazine-4-carboxylate (6h). Off-white solid; yield: 66%; mp: 148–150 °C; IR (KBr, cm^{-1}): 1723 (C═O), 1636, 1457, 1323, 1156; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.87 (t, J = 7.0 Hz, 3H, CH_3), 4.04 (q, J = 7.1 Hz, 2H, CH_2-CH_3), 4.76 (s, 2H, S-CH_2), 5.67 (s, 2H, N-CH_2), 7.14–7.19 (m, 4H, Ar), 7.28–7.36 (m, 8H, Ar), 7.48–7.56 (m, 1H, Ar), 8.14 (s, 1H, *N*-CH); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 13.78 (CH_3), 25.18 (SCH_2), 41.32 (NCH_2), 62.56 (OCH_2), 112.30 (d, $^2J_{C\text{-}F}$ = 4.9 Hz), 112.46, 124.56, 128.37 (2C), 128.79 (2C), 129.11, 129.31, 129.47 (2C), 130.14 (2C), 130.97, 132.18 (d, $^3J_{C\text{-}F}$ = 10.4 Hz), 134.11, 135.66 (2C), 136.46, 143.22 (S-CH_2-**C**), 155.89 (C═N), 157.85 (S-**C**═N), 161.28 (d, $^1J_{C\text{-}F}$ = 249.1 Hz), 164.51 (C═O); Anal. Calcd. For $C_{29}H_{23}F_2N_5O_2S$: C, 64.08; H, 4.26; N, 12.88; Found: C, 63.88; H, 4.48; N, 12.92; ESI-MS *m/z*: 544.16 $[M + H]^+$.

2.1.4.9. Ethyl 3-(((1-(2,5-dichlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)thio)-5,6-diphenylpyridazine-4-carboxylate (6i). Off-white solid; yield: 64%; mp: 110–112 °C; IR (KBr, cm^{-1}): 1721 (C═O), 1632, 1449, 1300,

1187; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.87 (t, J = 7.1 Hz, 3H, CH_3), 4.04 (q, J = 7.1 Hz, 2H, CH_2-CH_3), 4.78 (s, 2H, S-CH_2), 5.70 (s, 2H, N-CH_2), 7.13–7.14 (m, 2H, Ar), 7.27–7.37 (m, 9H, Ar), 7.48 (dd, J = 8.6, 2.6 Hz, 2H), 8.18 (s, 1H, *N*-CH); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 13.79 (CH_3), 25.19 (SCH_2), 50.68 (NCH_2), 62.57 (OCH_2), 125.02, 128.36 (2C), 128.80 (2C), 129.12, 129.32, 129.46 (2C), 130.13 (2C), 130.51, 130.68, 130.98, 131.81, 131.97 (C-Cl), 132.53 (C-Cl), 134.10, 135.63, 135.79, 136.44, 143.38 (S-CH_2-**C**), 155.80 (C═N), 157.82 (S-**C**═N), 164.50 (C═O); Anal. Calcd. For $C_{29}H_{23}Cl_2N_5O_2S$: C, 60.42; H, 4.02; N, 12.15; Found: C, 60.09; H, 3.90; N, 12.28; ESI-MS *m/z*: 579.09 $[M + H]^+$.

2.1.4.10. Ethyl 3-(((1-(3-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methyl)thio)-5,6-diphenylpyridazine-4-carboxylate (6j). Gray solid; yield: 82%; mp: 137–139 °C; IR (KBr, cm^{-1}): 1728 (C═O), 1641, 1459, 1325, 1188; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.87 (t, J = 7.1 Hz, 3H, CH_3), 4.05 (q, J = 7.1 Hz, 2H, CH_2-CH_3), 4.77 (s, 2H, S-CH_2), 5.78 (s, 2H, N-CH_2), 7.14–7.15 (m, 2H, Ar), 7.29–7.37 (m, 8H, Ar, *N*-CH), 7.67–7.70 (m, 1H, Ar), 7.77 (d, J = 7.7 Hz, 1H, Ar), 8.20–8.22 (m, 2H), 8.27 (s, 1H, Ar); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 13.79 (CH_3), 25.19 (SCH_2), 52.20 (NCH_2), 62.57 (OCH_2), 122.02, 122.19, 123.45, 127.17, 128.12, 128.19 (2C), 129.12 (2C), 129.63, 129.88 (2C), 129.98 (2C), 130.38, 133.73, 134.97, 136.09, 137.49, 138.45, 144.03 (S-CH_2-**C**), 148.14 (C-NO_2), 155.83 (C═N), 157.85 (S-**C**═N), 164.50 (C═O); Anal. Calcd. For $C_{29}H_{24}N_6O_4S$: C, 63.03; H, 4.38; N, 15.21. Found: C, 62.90; H, 4.60; N, 15.40; ESI-MS *m/z*: 553.16 $[M + H]^+$.

2.1.4.11. Ethyl 3-(2-(1-(4-nitrobenzyl)-1H-1,2,3-triazol-4-yl)ethyl)-5,6-diphenylpyridazine-4-carboxylate (6k). Gray solid; yield: 89%; mp: 149–151 °C; IR (KBr, cm^{-1}): 1730 (C═O), 1642, 1459, 1325, 1187; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.85 (t, J = 7.0 Hz, 3H, CH_3), 4.01 (q, J = 7.1 Hz, 2H, CH_2-CH_3), 4.75 (s, 2H, S-CH_2), 5.76 (s, 2H, N-CH_2), 7.11 (d, J = 7.0 Hz, 2H, Ar), 7.26–7.34 (m, 8H, Ar), 7.50 (d, J = 8.3 Hz, 2H, Ar), 8.20 (d, J = 8.7 Hz , 2H, Ar), 8.23 (s, 1H, *N*-CH); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 14.24 (CH_3), 22.20 (SCH_2), 52.42 (NCH_2), 60.61 (OCH_2), 122.02, 123.76 (2C), 127.17, 128.12, 128.19 (2C), 129.12 (2C), 129.17 (2C), 129.88 (2C), 129.98 (2C), 130.38, 134.97, 136.09, 138.45, 140.28, 144.03 (S-CH_2-**C**), 147.03 (C-NO_2), 155.68 (C═N), 158.66 (S-**C**═N), 164.49 (C═O); Anal. Calcd. For $C_{29}H_{24}N_6O_4S$: C, 63.03; H, 4.38; N, 15.21. Found: C, 63.22; H, 4.51; N, 15.35; ESI-MS *m/z*: 553.16 $[M + H]^+$.

2.1.4.12. Ethyl 3-(((1-(3-methoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)thio)-5,6-diphenylpyridazine-4-carboxylate (6l). Gray solid; yield: 71%; mp: 154–156 °C; IR (KBr, cm^{-1}): 1730 (C═O), 1641, 1462, 1325, 1188; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.86 (t, J = 7.1 Hz, 3H, CH_3), 3.71 (s, 3H, OCH_3), 4.03 (q, J = 7.0 Hz, 2H, CH_2-CH_3), 4.74 (s, 2H, S-CH_2), 5.54 (s, 2H, N-CH_2), 6.84–6.90 (m, 3H, Ar), 7.12–7.15 (m, 3H, Ar), 7.25–7.36 (m, 8H, Ar), 8.15 (s, 1H, *N*-CH); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 14.24 (CH_3), 22.20 (SCH_2), 52.84 (NCH_2), 55.15 (OCH_3), 60.61 (OCH_2), 112.78, 113.00, 121.87, 122.02, 127.17, 128.12, 128.15 (2C), 129.12 (2C), 129.50, 129.88 (2C), 129.99 (2C), 130.13, 134.97, 136.19, 136.58, 138.45, 144.03 (S-CH_2-**C**), 155.67 (C═N), 158.66 (S-**C**═N), 159.53 (**C**-OMe), 167.00 (C═O); Anal. Calcd. For $C_{30}H_{27}N_5O_3S$: C, 67.02; H, 5.06; N, 13.03. Found: C, 66.92; H, 5.31; N, 13.22; ESI-MS *m/z*: 538.19 $[M + H]^+$.

2.1.4.13. Ethyl 3-(((1-(4-methoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)thio)-5,6-diphenylpyridazine-4-carboxylate (6m). Off-white solid; yield: 74%; mp: 123–125 °C; IR (KBr, cm^{-1}): 1722 (C═O), 1639, 1458, 1300, 1161; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.87 (t, J = 7.1 Hz, 3H, CH_3), 3.73 (s, 3H, OCH_3), 4.04 (q, J = 7.2 Hz, 2H, CH_2-CH_3), 4.74 (s, 2H, S-CH_2), 5.50 (s, 2H, N-CH_2), 6.92 (d, J = 8.7 Hz, 2H, Ar), 7.13 (d, J = 7.1 Hz, 2H, Ar), 7.28–7.37 (m, 10*H*, Ar), 8.10 (s, 1H, *N*-CH); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 14.25 (CH_3), 23.60 (SCH_2), 52.03 (NCH_2), 55.23 (OCH_3), 60.67 (OCH_2), 112.44, 113.80 (2C), 122.93, 127.72 (2C), 128.03, 128.10 (2C), 128.44 (2C), 128.50, 128.65, 125.68, 132.39, 135.55, 137.03, 138.27, 146.76 (S-CH_2-**C**), 147.59 (C═N), 154.94 (S-**C**═N), 159.16 (**C**-OMe), 164.27 (C═O); Anal. Calcd. For $C_{30}H_{27}N_5O_3S$: C, 67.02; H, 5.06; N, 13.03. Found: C, 67.22; H, 5.28; N, 13.21; ESI-MS *m/z*: 538.19 $[M + H]^+$.

2.1.5. General procedure for the preparation of compounds (7a-7c)

The appropriate compounds (**6a**, **6e**, **6 g**, 1 mmol) were dissolved in ethanol (5 mL) and the NaOH solution (1 M, 5 mL) was added and the mixture was refluxed. Upon completion (1–2 h), checked by TLC, the mixture was acidified to pH = 1 and the resultant solid was filtered and washed with water.

2.1.5.1. 3-(((1-Benzyl-1H-1,2,3-triazol-4-yl)methyl)thio)-5,6-diphenylpyridazine-4-carboxylic acid (7a). White solid; yield: 76%; mp: 192–194 °C; IR (KBr, cm^{-1}): 3056 (COOH), 1719 (C═O), 1642, 1463, 1326, 1200; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 4.74 (s, 2H, S-CH_2), 5.59 (s, 2H, N-CH_2), 7.15–7.17 (m, 2H, Ar), 7.28–7.38 (m, 13H, Ar), 8.16 (s, 1H, *N*-CH), 14.12 (s, 1H, OH); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 21.48 (SCH_2), 52.10 (NCH_2), 121.99, 128.03, 128.04 (2C), 128.07 (2C), 128.09, 128.42 , 129.08, 129.67, 129.85, 129.98, 130.02, 134.78, 136.21, 136.26, 138.36, 144.03 (S-CH_2-**C**), 157.65 (C═N), 158.37 (S-**C**═N), 166.88 (C═O); Anal. Calcd. For $C_{27}H_{21}N_5O_2S$: C, 67.62; H, 4.41; N, 14.60. Found: C, 67.43; H, 4.29; N, 14.75; ESI-MS *m/z*: 480.14 $[M + H]^+$.

2.1.5.2. 3-(((1-(4-Fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)thio)-5,6-diphenylpyridazine-4 carboxylic acid (7b). White solid; yield: 93%; mp: 212–213 °C; IR (KBr, cm^{-1}): 3051 (COOH), 1705 (C═O), 1636, 1468, 1301, 1166; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 4.74 (s, 2H, S-CH_2), 5.58 (s, 2H, N-CH_2), 7.16–7.21 (m, 4H, Ar), 7.28–7.33 (m, 8H, Ar), 7.37–7.40 (m, 2H, Ar), 8.17 (d, J = 2.5 Hz, 1H, *N*-CH), 13.02 (s, 1H, OH); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 22.20 (SCH_2), 52.17 (NCH_2), 115.24 (d, $^2J_{C\text{-}F}$ = 20 Hz, 2C), 122.02, 128.07, 128.09, 129.03, 129.67, 129.85, 130.03 (2C), 130.06, 130.30 (d, $^3J_{C\text{-}F}$ = 7.5 Hz, 133.09 (2C), 134.78, 136.21, 138.36, 144.03 (S-CH_2-**C**), 157.52 (C═N), 158.23 (S-**C**═N), 162.27 (d, $^1J_{C\text{-}F}$ = 252.1 Hz), 167.42 (C═O); Anal. Calcd. For $C_{27}H_{20}FN_5O_2S$: C, 65.18; H, 4.05; N, 14.08. Found: C, 65.36; H, 3.87; N, 14.22; ESI-MS *m/z*: 498.14 $[M + H]^+$.

2.1.5.3. 3-(((1-(4-Bromobenzyl)-1H-1,2,3-triazol-4-yl)methyl)thio)-5,6-diphenylpyridazine-4-carboxylic acid (7c). White solid; yield: 81%; mp: 211–213 °C; IR (KBr, cm^{-1}): 3105 (COOH), 1695 (C═O), 1649, 1451, 1309, 1217; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 4.74 (s, 2H, S-CH_2), 5.58 (s, 2H, N-CH_2), 7.14 (dd, J = 7.7, 1.9 Hz, 2H, Ar), 7.25 (d, J = 8.4 Hz, 2H, Ar), 7.28–7.34 (m, 8H, Ar), 7.56 (d, J = 8.4 Hz, 2H, Ar), 8.17 (s, 1H, *N*-CH), 12.99 (s, 1H, OH); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 23.29 (SCH_2), 53.79 (NCH_2), 121.87 (C-Br), 122.16, 128.09, 128.10, 129.09, 129.68, 129.88, 130.02, 130.03, 130.28, 131.57, 134.79, 135.31, 136.11, 138.36, 144.03 (S-CH_2-**C**), 157.49 (C═N), 158.23 (S-**C**═N), 167.51 (C═O); Anal. Calcd. For $C_{27}H_{20}BrN_5O_2S$: C, 58.07; H, 3.61; N, 12.54. Found: C, 57.81; H, 3.44; N, 12.71; ESI-MS *m/z*: 558.06 $[M + H]^+$.

2.2. Biological results

2.2.1. In-vitro α-glucosidase inhibition assay

α-Glucosidase (Saccharomyces cerevisiae, EC3.2.1.20, 20 U/mg) and substrate (*p*-nitrophenyl glucopyranoside) were purchased from Sigma-Aldrich. Desired concentrations of enzyme were prepared by potassium phosphate buffer (pH 6.8, 50 mM), and the target compounds were dissolved in DMSO (10% final concentration). The enzyme solution (20 μL), different concentrations of compounds (20 μL), and potassium phosphate buffer (135 μL) were added to the 96-well plate and incubated at 37 °C for 10 min. Then, *p*-nitrophenyl glucopyranoside as

substrate (25 μL, 4 mM) was added to each well and allowed to be incubated at 37 °C for 20 min. Finally, the change in the absorbance was measured at 405 nm by using spectrophotometer (Gen5, Power wave xs2, BioTek, America). DMSO and acarbose were used as the control and standard inhibitor, respectively. The percentage of inhibition for target compounds, control, and the standard inhibitor was calculated by using the following formula:

$$\%\text{Inhibition} = [(\text{Abs control} - \text{Abs sample})/\text{Abs control}] \times 100$$

IC_{50} values of tested compounds were obtained from the nonlinear regression curve using the Logit method.

2.2.2. Rat α- glucosidase assay

Rat small intestine α-glucosidase (EC 3.2.1.20) was prepared according to the method published by Lossow et al. (1964). Enzyme in vitro activity was determined by recording the release of 4-nitrophenol from Pnitrophenyl α-D glucopyranoside as previously described by Kim *et al.* [59,60]. Final volume of 200 μL of assay solution was prepared in a 96-well plate as follow: the enzyme solution (190 μL, 0.15 units/ml), different concentrations of compounds 1, 10, 20, 50, 100, 500 and 1000 μM (5 μL), and potassium phosphate buffer. Test compounds were dissolved in DMSO (not exceed than 5% of final volume). After 10 min. of pre-incubation at 37 °C, *p*-nitrophenyl glucopyranoside as substrate (5 μL, 3 mM), was added to the enzyme solution and let to be incubated for one hour at 37 °C. Finally, the change in the absorbance was followed at 405 nm using Cytation 3 hybrid microplate reader (BioTek, USA). DMSO and acarbose were used as the control and standard inhibitor, respectively. The extent of enzyme inhibition was calculated in the presence of different concentrations of compounds by using the following formula and data presented as percentage of inhibition.

$$\%\text{Inhibition} = [(\text{Abs control} - \text{Abs sample})/\text{Abs control}] \times 100$$

IC50 values of tested compounds were obtained from the nonlinear regression curve using GraphPadprism 6.0 (San Diego, California, USA).

2.2.3. Kinetic studies

The mode of inhibition of the most active compound **6j**, identified with the lowest IC_{50}, was investigated against α-glucosidase activity with different concentrations of *p*-nitrophenyl α-D-glucopyranoside (2–10 mM) as substrate in the absence and presence of sample **6j** at different concentrations (0, 45, 65, and 85 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 (K_i) value was constructed by secondary plots of the inhibitor concentration [I] versus K_m.

2.2.4. Cytotoxic studies

The cytotoxic studies of selected compounds were performed according to the previously reported literature [52].

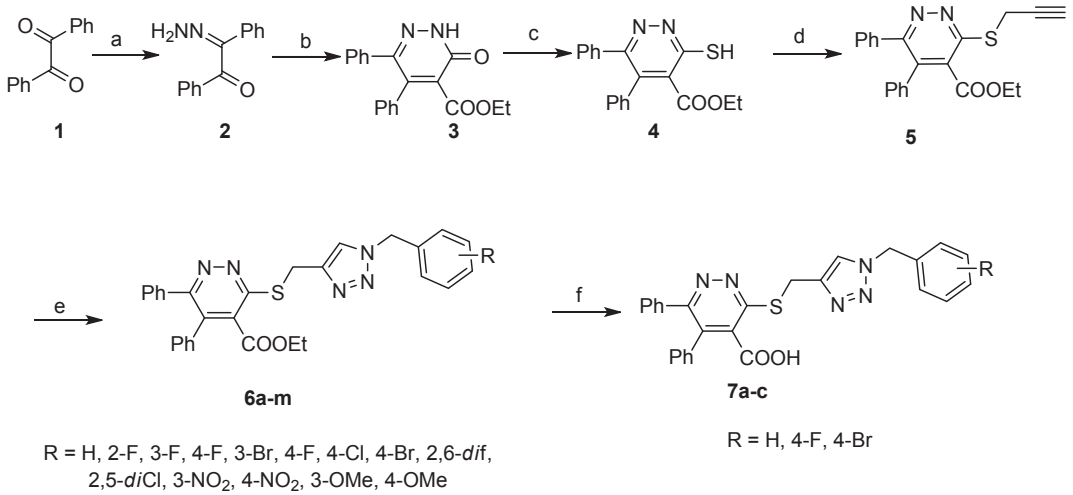
2.2.5. Docking studies

Docking study of compound **6j** was done using Autodock 4.2.1 software. The structure of *S. cerevisiae* isomaltase (PDB: 3A4A with 1.6 Å resolution) was taken from RCSB data bank and the docking procedure was carried out as previously reported [52]. The docking results were analyzed by Discovery Studio visualizer 4.5.

3. Results and discussion

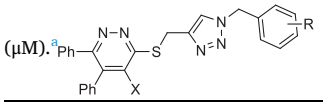
3.1. Chemistry

The synthesis of final compounds (**6a-m**) was achieved through the five step reaction. Scheme 1 showed the synthetic route used to prepare final compounds, starting from benzil. Benzil constituted a useful starting material for the synthesis of heterocyclic compounds. According to the related reports on the construction of pyridazine core, the reaction of benzil and hydrazine gave 2-hydrazonoe-1,2-diphenyl ethanone **2** which upon treatment with diethyl malonate by using sodium ethanolate afforded cyclized product **3**. Ethyl-2,3-dihydro-3-oxo-5,6-diphenylpyridazine-4-carboxylate was converted to compound **4** by the action of Lawesson's reagent. The propargylic unit at the side chain was installed by the treatment of compound **4** in *N,N*-dimethyl formamide (DMF) with propargyl bromide in the presence of potassium carbonate. The click reaction proceeded smoothly by employing 10 mol percent sodium ascorbate/copper sulfate and 1 equiv. of triethyl amine in water/*tert*-buthanol, affording 1,4-disubstituted triazole ring in good yields with complete regioselectivity. The corresponding acids could be easily obtained from the saponification reaction with ethanolic sodium hydroxide solution. The synthetic protocol was examined on a series of substituted benzyl bromides/chlorides and the data are collated in Table 1.



Scheme 1. Synthesis of target compounds. Reagents and conditions: a) hydrazine hydrate, methanol, reflux, 15 min., 79%, m.p. lit. 149–151 °C [58], m.p. obtained; 148–150 °C; b) Na, EtOH, diethyl malonate, reflux, 3 h, 47%; c) Lawesson's reagent, toluene, reflux, 18 h, 94%; d) propargyl bromide, DMF, K_2CO_3, 50 °C, 2 h, 92%; e) (i) benzyl bromide/chloride derivatives, NaN_3, H_2O/*tert*-buthanol, Et_3N, 1 h; (ii) sodium ascorbate, copper (sulfate, **5**, 6–10 h; f) NaOH (1 M), EtOH, reflux, 1–2 h.

Table 1
α-Glucosidase inhibitory activity of the target compounds, presented as IC_{50} (μM).[a]



Compound	X	R	IC_{50} (μM)[b]	IC_{50} (μM)[c]
6a	COOEt	H	307.4 ± 4.3	220.7 ± 52.6
6b	COOEt	2-F	198.0 ± 2.0	119.7 ± 12.9
6c	COOEt	3-F	321.5 ± 4.5	148.3 ± 16.3
6d	COOEt	3-Br	238.4 ± 3.7	278.5 ± 57.2
6e	COOEt	4-F	449.5 ± 6.6	166.2 ± 31.3
6f	COOEt	4-Cl	213.3 ± 3.3	284.5 ± 59.1
6g	COOEt	4-Br	190.7 ± 1.9	304.7 ± 46.9
6h	COOEt	2,6-*di*F	202.5 ± 3.1	89.1 ± 15.7
6i	COOEt	2,5-*di*Cl	107.7 ± 1.2	193.1 ± 42.6
6j	COOEt	3-NO_2	85.6 ± 0.5	73.7 ± 11.4
6k	COOEt	4-NO_2	414.2 ± 6.3	118.4 ± 21.2
6l	COOEt	3-OMe	220.6 ± 3.5	466.8 ± 39.1
6m	COOEt	4-OMe	634.0 ± 9.2	412.5 ± 45.4
7a	COOH	H	489.2 ± 7.0	294.6 ± 43.7
7b	COOH	4-F	453.0 ± 6.8	179.1 ± 33.2
7c	COOH	4-Br	249.4 ± 3.9	215.4 ± 20.8
	Acarbose	–	750.0 ± 10.0	318.2 ± 23.9

[a] Values are the means of three replicates ± standard deviation (SD).
[b] The activity against Saccharomyces cerevisiae.
[c] The activity against rat small intestine α-glucosidase.

3.2. α-Glucosidase inhibitory activity

All target compounds were evaluated for their abilities to inhibit α-glucosidase activity of the extracted enzyme from *Saccharomyces cerevisiae* and rat small intestine α-glucosidase. The activity against both enzymes showed same trend and the most active compound was **6j**. From the reported data, the preliminary structure–activity relationship against *Saccharomyces cerevisiae* can be drawn. All synthesized compounds had better inhibitory activities than acarbose, exhibiting IC_{50} values ranging from 85.6 to 634.0 μM. The most potent compound was 3-nitro containing derivative, **6j**. The influence of halogens involving fluorine, chlorine, and bromine at different positions was examined. Moving fluorine from *ortho* to *meta* led to the decreased potency. Substitution of the most electronegative atom, fluorine as the lipophilic group, at *para* position was not tolerated and led to the less potent compound **6e** with IC_{50} = 449.5 μM among halogen containing derivatives.

Replacing fluorine at *meta* position (**6c**, IC_{50} = 321.5 μM) with bromine resulted in increased potency, (**6d**, IC_{50} = 238.4 μM). The replacement of the fluorine at *para* position with chlorine and bromine yielded more potent compounds with IC_{50} values of 213.3, 190.7 μM, respectively. The introduction of second fluorine atom at *ortho* position, **6h**, did not lead to the significant enhancement in the activity compared to mono substituted derivative **6b**. 2,5-Dichloro-substituted compound **6i** *vs* **6h** exhibited 2-fold improvements in activity indicating that dichloro substitution was more favorable than difluoro substituted one. Interestingly, the introduction of nitro group at *meta* position produced the most potent compound **6j**, IC_{50} = 85.6 μM, with almost 10-fold more potency compared to acarbose. IC_{50} = 750.0 μM. While, moving this functional group to the *para* position led to the compound with 5-times less activity compared to **6j**. The presence of electron-donating group at *para* position, **6m**, dramatically decreased the enzyme inhibition compared to unsubstituted and *meta*-substituted analogue, resulted in the weakest compound with IC_{50} value of 634.0 μM.

We expanded the scope of our investigation by the replacement of ester with acidic group. Compared with ester containing compounds, no improvement in the inhibitory profile was observed. For compounds bearing hydrogen, chlorine and fluorine at *para* position, the presence of acidic group led to the decreased inhibitory effects, (**7a**, **7b**, and **7c**; IC_{50s} = 489.2, 453.0, 249.4 μM, respectively). Based on the evaluation against rat enzyme, all compounds except **6l** and **6 m** were more active compared to acarbose. The IC_{50} inhibition of acarbose against rat a-glucosidase was found to be 318.2 ± 23.9 μM. Comparing the presence of halogens at *para* position, the less electronegative atom, meaning bromine (**6 g**) exhibited the less inhibitory activity. The reverse trend was observed by replacing fluorine with bromine at *meta* position. The introduction of second fluorine enhanced the inhibitory activity (**6b** *vs* **6h**). The most active compounds was **6j.** The interesting point is moving this group to *para* position decreased the activity. The less active compounds contained methoxy group at *meta* and *para* positions (Table 1).

3.3. Kinetic studies

The mode of inhibition of the target derivatives was investigated by kinetic studies on compound **6j** (Fig. 2). The competitive mode of inhibition was determined for the most active compound. The unchanged V_{max} value and increased k_m value which were determined by Lineweaver-Burk plot, indicated this fact. Moreover, by drawing the plot of the K_m versus different concentration of inhibitor, K_i of 82 μM, was determined for compound **6j**.

3.4. Cytotoxic studies

In order to investigate the cytotoxicity of these compounds, the active ones, **6g**, **6j**, and **7c**, from both series were tested against the normal cell line. In case of this cell line, HDF, no toxicity was observed with either compound.

3.5. Docking study

According to the biological results, the most active compound **6j** was selected and docked into the active site of α-glucosidase enzyme to identify the binding modes and interactions. Molecular docking study was accomplished by Autodock 4.2.1 software package and the 3D and 2D structure of docking interactions were depicted by Discovery Studio visualizer 4.5. Crystal structure of *S. cerevisiae* α-glucosidase is not prepared yet, so the crystal structure of S. cerevisiae isomaltase (PDB: 3A4A) was used due to its high similarity to *S. cerevisiae* α-glucosidase.

The best score docking conformation of **6j** was shown in Fig. 3. Compound **6j** established two conventional hydrogen bonds and several hydrophobic interactions with the active pocket of enzyme which enhanced the stability of the ligand-enzyme complex. The triazole ring of **6j** formed a hydrogen bond with His 280 of distance 2.46 Å and the nitro group made the second hydrogen bond with Asn 415 of distance 2.87 Å. This ligand exhibited π-cation and π-anion interactions with Arg 442 and Asp 352, respectively. Moreover, the sulfide group of this compound made π-sulfur interaction with Phe 303. Other interactions including π-π stacked, π-π *T*-shaped, and van der Waals were observed between two phenyl moieties of this ligand and a number of residues.

4. Conclusion

In conclusion, for the first time, we have demonstrated the α-glucosidase inhibitory activity of pyridazine-containing compounds. By this method, sixteen compounds have been synthesized through the five-step approach and their *in-vitro* inhibitory activity against yeast and rat small intestine α-glucosidase enzymes were investigated. Structure-activity relationship investigation indicated compound **6j** with nitro group at *meta* position as the most active compound. The enhanced activity of final compounds suggested this series as the good candidates for further development. Our group is also engaged in expanding the library of pyridazine-containing compounds as glucosidase inhibitors.

Declaration of Competing Interest

The authors declare that they have no known competing financial

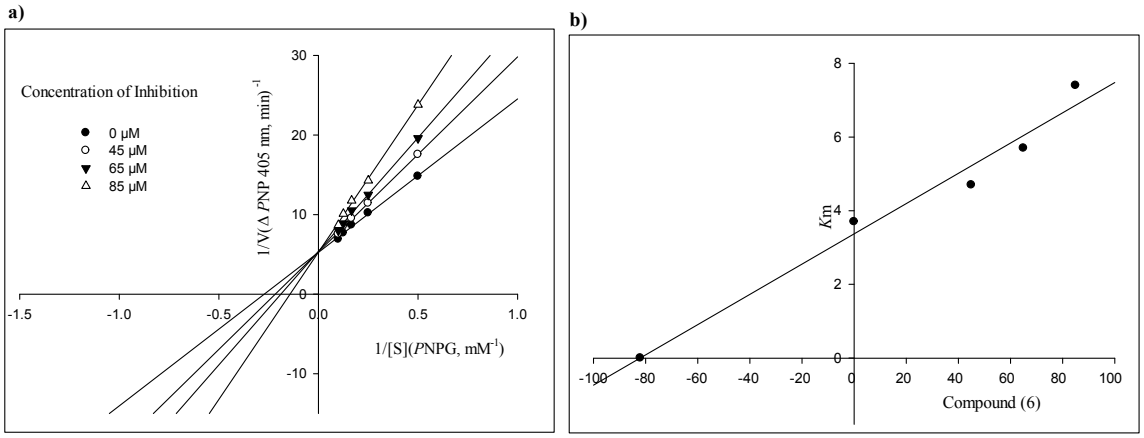


Fig. 2. Kinetics of α-gluosidase inhibition by **6j**. (a) The Lineweaver-Burk plot in the absence and presence of different concentrations of **6j** (μM); (b) the secondary plot between K_m and various concentrations of **6j**.

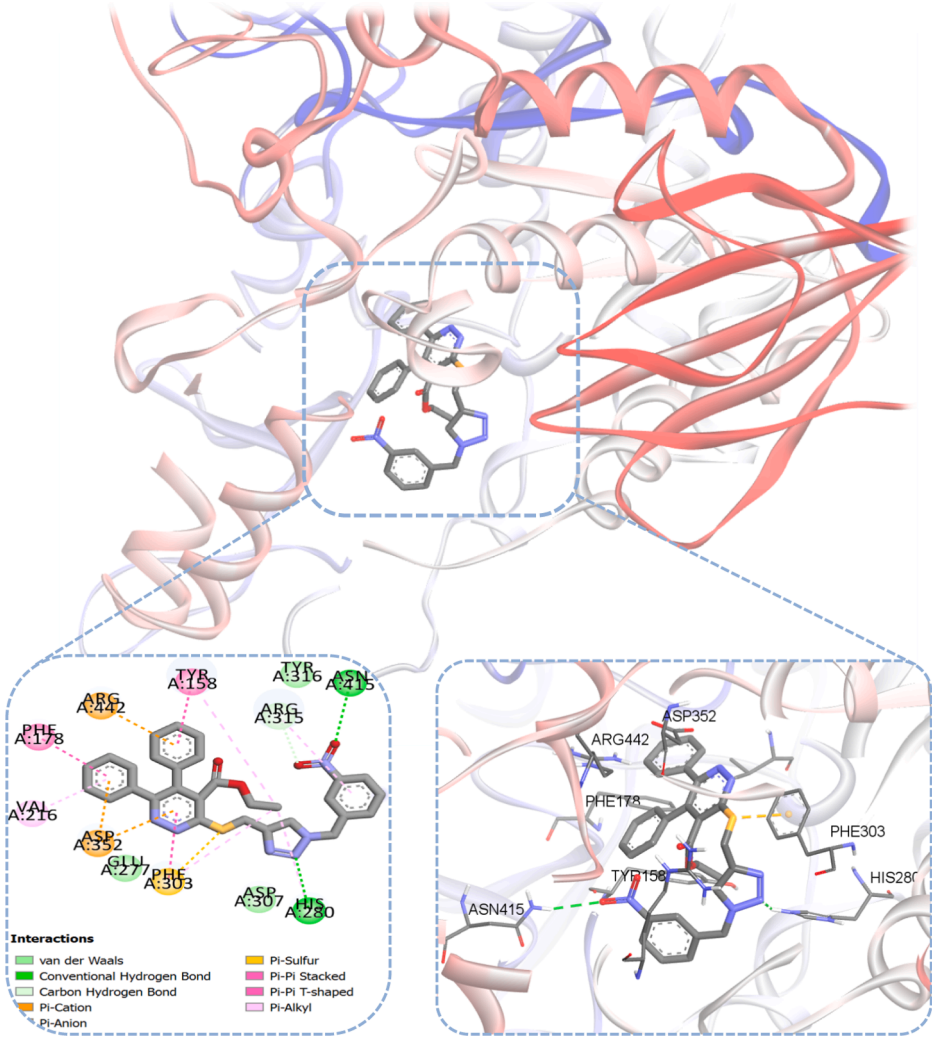


Fig. 3. The 3D binding conformation and 2D binding conformation of compound **6j**.

interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2021.104670.

References

[1] G.M. Reaven, Banting lecture 1988. Role of insulin resistance in human disease, Diabetes 1988 (37) (1988) 1595–1607.
[2] J.J. Marín-Peñalver, I. Martín-Timón, C. Sevillano-Collantes, F.J. Del Cañizo-Gómez, Update on the treatment of type 2 diabetes mellitus, World J. Diabetes 15 (2016) 354–395, https://doi.org/10.4239/wjd.v7.i17.354.
[3] Y. Moritoh, K. Takeuchi, M. Hazama, Voglibose, an α-glucosidase inhibitor, to increase active glucagon-like peptide-1 levels, Mol. Cell Pharmacol. 1 (2009) 188–192, https://doi.org/10.4255/mcpharmacol.09.22.
[4] S. Chiba, Molecular Mechanism in α-Glucosidase and Glucoamylase, Biotechnol. Biochem. 61 (1997) 1233–1239, https://doi.org/10.1271/bbb.61.1233.
[5] U. Saqib, M.I. Siddiqi, Int. J. Integr. Biol. 2 (2008) 115–121.
[6] F.A. van de Laar, Alpha-glucosidase inhibitors in the early treatment of type 2 diabetes, Health Risk Manag. 4 (2008) 1189–1195, https://doi.org/10.2147/vhrm.s3119.
[7] N.S. Moorthy, M.J. Ramos, P.A. Fernandes, Studies on α-glucosidase inhibitors development: magic molecules for the treatment of carbohydrate mediated diseases, Mini Rev. Med. Chem. 12 (2012) 713–720, https://doi.org/10.2174/138955712801264837.
[8] W. Benalla, S. Bellahcen, M. Bnouham, Antidiabetic medicinal plants as a source of alpha glucosidase inhibitors, Curr. Diabetes Rev. 6 (2010) 247–254, https://doi.org/10.2174/157339910791658826.
[9] R. Tundis, M.R. Loizzo, F. Menichini, Natural products as alpha-amylase and alpha-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: an update, Mini Rev. Med. Chem. 10 (2010) 315–331, https://doi.org/10.2174/138955710791331007.
[10] K. Cusi, R.A. DeFronzo, Metformin: A review of its metabolic effects, Diabetes Rev. 6 (1998) 89–131.
[11] H.E. Lebovitz, Insulin secretagogues: old and new, Diabetes Rev. 7 (1999) 139–153.
[12] M. Diamant, R.J. Heine, Thiazolidinediones in type 2 diabetes mellitus: current clinical evidence, Drugs 63 (2003) 1373–1406, https://doi.org/10.2165/00003495-200363130-00004.
[13] H.E. Lebovitz, Diabetes Rev. 6 (1998) 132–145.
[14] H.S. Yee, N.T. Fong, A Review of the Safety and Efficacy of Acarbose in Diabetes Mellitus, Pharmacotherapy 16 (1996) 792–805, https://doi.org/10.1002/j.1875-9114.1996.tb02997.x.
[15] C. Rosak, G. Mertes, Critical evaluation of the role of acarbose in the treatment of diabetes: patient considerations, Diabetes Metab. Syndr. Obes. 5 (2012) 357–367, https://doi.org/10.2147/DMSO.S28340.
[16] A.J. Scheen, Is there a role for alpha-glucosidase inhibitors in the prevention of type 2 diabetes mellitus? Drugs 63 (2003) 933–951, https://doi.org/10.2165/00003495-200363100-00002.
[17] A.J.J. Reuser, H.A. Wisselaar, An evaluation of the potential side-effects of alphaglucosidase inhibitors used for the management of diabetes mellitus, Eur. J. Clin. Invest. 24 (1994) 19–24, https://doi.org/10.1111/j.1365-2362.1994.tb02251.x.
[18] A. Murai, K. Iwamura, M. Takada, K. Ogawa, T. Usui, J. Okumura, Control, of post prantial hyperglycaemia by galactosylmaltobionolactone and its novel anti-amylase effect in mice, Life Sci. 71 (2002) 1405–1415, https://doi.org/10.1016/s0024-3205(02)01844-1.
[19] S.Y. Lee, A. Mediani, A.A.H. Nur, A.B.S. Azliana, F. Abas, Antioxidant and α-glucosidase inhibitory activities of the leaf and stem of selected traditional medicinal plants, Int. Food Res J. 21 (2014) 165–172.
[20] J.S.S. Neto, G. Zeni, A decade of advances in the reaction of nitrogen sources and alkynes for the synthesis of triazoles, Coord. Chem. Rev. 409 (2020), 213217, https://doi.org/10.1016/j.ccr.2020.213217.
[21] V.V. Rostovsev, L.G. Green, V.V. Fokin, K.B. Sharpless, A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective "Ligation" of Azides and Terminal Alkynes, Angew. Chem. Int. Ed. Engl. 41 (2002) 2596–2599, https://doi.org/10.1002/1521-3773(20020715)41:14<2596::AID-ANIE2596>3.0.CO;2-4.
[22] H.C. Kolb, M.G. Finn, K.B. Sharpless, Click Chemistry: Diverse Chemical Function from a Few Good Reactions, Angew. Chem. Int. Ed. 10.1002/1521-3773(20010601)40:11<2004::aid-anie2004>3.3.co;2-x.
[23] K. Bozorov, J. Zhao, H.A. Aisa, 1,2,3-Triazole-containing hybrids as leads in medicinal chemistry: A recent overview, Bioorg. Med. Chem. 27 (2019) 3511–3531, https://doi.org/10.1016/j.bmc.2019.07.005.
[24] E. Bonandi, M.S. Christodoulou, G. Fumagalli, D. Perdicchia, G. Rastelli, D. Passarella, The 1,2,3-triazole ring as a bioisostere in medicinal chemistry, Drug Discov. Today 22 (2017) 1572–1581, https://doi.org/10.1016/j.drudis.2017.05.014.
[25] O.V. Andreeva, B.F. Garifullin, R.R. Sharipova, I.Y. Strobykina, A.S. Sapunova, A. D. Voloshina, M.G. Belenok, A.B. Dobrynin, L.R. Khabibulina, V.E. Kataev, Glycosides and Glycoconjugates of the Diterpenoid Isosteviol with a 1,2,3-Triazolyl Moiety: Synthesis and Cytotoxicity Evaluation, J. Nat. Prod. 83 (2020) 2367–2380, https://doi.org/10.1021/acs.jnatprod.0c00134.
[26] Z. Xu, 1,2,3-Triazole-containing hybrids with potential antibacterial activity against methicillin-resistant Staphylococcus aureus (MRSA), Eur. J. Med. Chem. 206 (2020), 112686, https://doi.org/10.1016/j.ejmech.2020.112686.
[27] N. Rezki, M.A. Almehmadi, S. Ihmaid, A.M. Shehata, A.M. Omare, H.E.A. Ahmed, M.R. Aouad, Novel scaffold hopping of potent benzothiazole and isatin analogues linked to 1,2,3-triazole fragment that mimic quinazoline epidermal growth factor receptor inhibitors: Synthesis, antitumor and mechanistic analyses, Bioorg. Chem. 103 (2020), 104133, https://doi.org/10.1016/j.bioorg.2020.104133.
[28] M.R. Aouad, M.A. Almehmadi, N. Rezki, F.F. Al-blewi, M. Messali, I. Ali, Design, click synthesis, anticancer screening and docking studies of novel benzothiazole-1,2,3-triazoles appended with some bioactive benzofused heterocycles, J. Mol. Struct. 1188 (2019) 153–164, https://doi.org/10.1016/j.molstruc.2019.04.005.
[29] M.R. Aouad, M.A. Soliman, M.O. Alharbi, S.K. Bardaweel, P.K. Sahu, A.A. Ali, M. Messali, N. Rezki, Y.A. Al-Soud, Design, Synthesis and Anticancer Screening of Novel Benzothiazole-Piperazine-1,2,3-Triazole Hybrids, Molecules 23 (2018) 2788–2802, https://doi.org/10.3390/molecules23112788.
[30] M. Villiou, J.I. Paez, A. del Campo, Photodegradable Hydrogels for Cell Encapsulation and Tissue Adhesion, ACS Appl. Mater. Interfaces 12 (2020) 37862–37872, https://doi.org/10.1021/acsami.0c08568.
[31] J. Huo, H. Hu, M. Zhang, X. Hua, M. Chen, D. Chen, J. Liu, G. Xiao, Y. Wang, Z. Wen, A mini review of the synthesis of poly-1,2,3-triazole-based functional materials, RSC Adv. 7 (2017) 2281–2287, https://doi.org/10.1039/C6RA27012C.
[32] L.D. Rodrigues, D. Sunil, D. Chaithra, P. Bhagavath, 1,2,3/ 1,2,4-Triazole containing liquid crystalline materials: An up-to-date review of their synthetic design and mesomorphic behavior, J. Mol. Liq. 297 (2020), 111909, https://doi.org/10.1016/j.molliq.2019.111909.
[33] D. Brunel, F. Dumur, Recent advances in organic dyes and fluorophores comprising a 1,2,3-triazole moiety, New J. Chem. 44 (2020) 3546–3561, https://doi.org/10.1039/c9nj06330g.
[34] H.C. Kolb, K.B. Sharpless, The growing impact of click chemistry on drug discovery, Drug Discov. Today 8 (2003) 1128–1137, https://doi.org/10.1016/s1359-6446(03)02933-7.
[35] R. Bansal, S. Thota, Pyridazin-3(2*H*)-ones: the versatile pharmacophore of medicinal significance, Med. Chem. Res. 22 (2013) 2539–2552, https://doi.org/10.1007/s00044-012-0261-1.
[36] Y.M. Loksha, M.M. Abd-Alhasee, Synthesis and biological screening of some novel 6-substituted 2-alkylpyridazin-3(2*H*)-ones as anti-inflammatory and analgesic agents. Arch Pharm Chem, Life Sci. 1900295 (2020), https://doi.org/10.1002/ardp.201900295.
[37] B. Singh, R. Bhatia, B. Pani, D. Gupta, Synthesis, crystal structures and biological evaluation of new pyridazine derivatives, J. Mol. Struct. 1200 (2020), 127084, https://doi.org/10.1016/j.molstruc.2019.127084.
[38] R.R. Harris, L. Black, S. Surapaneni, T. Kolasa, S. Majest, M.T. Namovic, G. Grayson, V. Komater, D. Wilcox, L. King, K. Marsh, M.F. Jarvis, M. Nuss, H. L. Nellans, G.A. Pruesser, B. Reinhart, P. Cox, A. Jacobson, M. Stewart, C.G. Carter, R.L. Bell, ABT-963 [2-(3, 4-difluoro-phenyl)-4-(3-hydroxy-3-methyl-butoxy)-5-(4-methanesulfonyl-phenyl)-2*H*-pyridazin-3-one], a highly potent and selective disubstituted pyridazinone cyclooxgenase-2 inhibitor, J. Pharmacol. Exp. Ther. 311 (2004) 904–912, https://doi.org/10.1124/jpet.104.070052.
[39] D. Sharma, R. Bansal, Synthesis of 2-substituted-4-aryl-6-phenylpyridazin-3(2*H*)-ones as potential anti-inflammatory and analgesic agents with cardioprotective and ulcerogenic sparing effects, Med. Chem. Res. 25 (2016) 1574–1589, https://doi.org/10.1007/s00044-016-1588-9.
[40] E.M. Ahmed, M.S.A. Hassan, A.A. El-Malah, A.E. Kassab, New pyridazine derivatives as selective COX-2 inhibitors and potential antiinflammatory agents; design, synthesis and biological evaluation, Bioorg. Chem. 95 (2020), 103497, https://doi.org/10.1016/j.bioorg.2019.103497.
[41] P.G. Sergeev, V.G. Nenajdenko, Recent advances in the chemistry of pyridazine - An important representative of six-membered nitrogen heterocycles, Russ. Chem. Rev. 89 (2020) 393–429, https://doi.org/10.1070/RCR4922.
[42] G.S. Deora, C.X. Qin, E.A. Vecchio, A.J. Debono, D.L. Priebbenow, R.M. Brady, J. Beveridge, S.C. Teguh, M. Deo, L.T. May, G. Krippner, R.H. Ritchie, J.B. Baell, Substituted Pyridazin-3(2*H*)-ones as Highly Potent and Biased Formyl Peptide Receptor Agonists, J. Med. Chem. 62 (2019) 5242–5248, https://doi.org/10.1021/acs.jmedchem.8b01912.
[43] J. Krall, F. Bavo, C.B. Falk-Petersen, C.H. Jensen, J.O. Nielsen, Y. Tian, V. Anglani, K.T. Kongstad, L. Piilgaard, B. Nielsen, D.E. Gloriam, J. Kehler, A.A. Jensen, K. Harpsøe, P. Wellendorph, B. Frølund, Discovery of 2-(Imidazo[1,2-b]pyridazin-2-yl)acetic Acid as a New Class of Ligands Selective for the γ-Hydroxybutyric Acid (GHB) High-Affinity Binding Sites, J. Med. Chem. 62 (2019) 2798–2813, https://doi.org/10.1021/acs.jmedchem.9b00131.
[44] S. Imanparast, M.A. Faramarzi, F. Bandarian, E.N. Esfahani, M. Safavi, H. Rastegar, Design and synthesis of novel quinazolinone-1, 2,3-triazole hybrids as new antidiabetic agents: In vitro α-glucosidase inhibition, kinetic, and docking study, Bioorg. Chem. 83 (2019) 161–169, https://doi.org/10.1016/j.bioorg.2018.10.023.
[45] F.Z. Basha, New carbazole linked 1,2,3-triazoles as highly potent non-sugar α-glucosidase inhibitors, Bioorg. Chem. 74 (2017) 72–81, https://doi.org/10.1016/j.bioorg.2017.07.006.
[46] G. Wang, Z. Peng, J. Wang, X. Li, J. Li, Synthesis, in vitro evaluation and molecular docking studies of novel triazine-triazole derivatives as potential α-glucosidase inhibitors, Eur. J. Med. Chem. 125 (2017) 423–429, https://doi.org/10.1016/j.ejmech.2016.09.067.
[47] F. Rahim, K. Ullah, H. Ullah, A. Wadood, M. Taha, A.U. Rehman, I. Uddin, M. Ashraf, A. Shaukat, W. Rehman, S. Hussain, K.M. Khan, Triazinoindole analogs as potent inhibitors of α-glucosidase: Synthesis, biological evaluation and

molecular docking studies, Bioorg. Chem. 58 (2015) 81–87, https://doi.org/10.1016/j.b.ioorg.2014.12.001.
[48] G. Wang, J. Wang, D. He, X. Li, J. Li, Z. Peng, Synthesis and biological evaluation of novel 1,2,4-triazine derivatives bearing carbazole moiety as potent α-glucosidase inhibitors, Bioorg. Med. Chem. Lett. 26 (2016) 2806–2809, https://doi.org/10.1016/j.bmcl.2016.04.071.
[49] G. Wang, X. Li, J. Wang, Z. Xie, L. Li, M. Chen, S. Chen, Y. Peng, Synthesis, molecular docking and α-glucosidase inhibition of 2-((5,6-diphenyl-1,2,4-triazin-3-yl)thio)-N-arylacetamides, Bioorg. Med. Chem. Lett. 27 (2017) 1115–1118, https://doi.org/10.1016/j.bmcl.2017.01.094.
[50] Y. Chinthala, A.K. Domatti, A. Sarfaraz, S.P. Singh, N.K. Arigari, N. Gupta, S.K.V. N. Satya, J.K. Kumar, F. Khan, A.K. Tiwari, G. Paramjit, Synthesis, biological evaluation and molecular modeling studies of some novel thiazolidinediones with triazole ring, Eur. J. Med. Chem. 70 (2013) 308–314, https://doi.org/10.1016/j.ejmech.2013.10.005.
[51] S. Moghimi, M. Toolabi, S. Salarinejad, L. Firoozpour, S.E. Sadat Ebrahimi, F. Safari, S. Mojtabavi, M.A. Faramarzi, A. Foroumadi, Design and synthesis of novel pyridazine *N*-aryl acetamides: *In-vitro* evaluation of α-glucosidase inhibition, docking, and kinetic studies, Bioorg. Chem. 102 (2020) 14071, https://doi.org/10.1016/j.bioorg.2020.104071.
[52] F. Peytam, M. Adib, R. Shourgeshty, L. Firoozpour, Jahani M Rahmanian-Jaz-M, S. Moghimi, K. Divsalar, M.A. Faramarzi, S. Mojtabavi, F. Safari, M. Mahdavi, A. Foroumadi, An efficient and targeted synthetic approach towards new highly substituted 6-amino-pyrazolo[1,5-a]pyrimidines with α-glucosidase inhibitory activity, Sci. Rep. 10 (2020) 2595, https://doi.org/10.1038/s41598-020-59079-z.
[53] M. Toolabi, S. Moghimi, T. OghabiBakhshaiesh, S. Salarinejad, A. Aghcheli, Z. Hasanvand, E. Nazeri, A. Khalaj, R. Esmaeili, A. Foroumadi, 6-Cinnamoyl-4-arylaminothienopyrimidines as highly potent cytotoxic agents: Design, synthesis and structure-activity relationship studies, Eur. J. Med. Chem. 185 (2020), 111786, https://doi.org/10.1016/j.ejmech.2019.111786.
[54] Z. Mojallal-Tabatabaei, P. Foroumadi, M. Toolabi, F. Goli, S. Moghimi, S. Kaboudanian-Ardestani, A. Foroumadi, 2-(Bipiperidin-1-yl)-5-(nitroaryl)-1,3,4-thiadiazoles: Synthesis, evaluation of in vitro leishmanicidal activity, and mechanism of action, Bioorg. Med. Chem. 3682–3691 (2019), https://doi.org/10.1016/j.bmc.2019.07.009.
[55] L. Faraji, H. Nadri, A. Moradi, S.N.A. Bukhari, B. Pakseresht, F.H. Moghadam, S. Moghimi, M. Abdollahi, M. Khoobi, A. Foroumadi, Aminoalkyl-substituted flavonoids: synthesis, cholinesterase inhibition, β-amyloid aggregation, and neuroprotective study, Med. Chem. Res. 28 (2019) 974–983, https://doi.org/10.1007/s00044-019-02350-4.
[56] S. Radwan, E. Bakhite, Synthesis of Novel Thieno[2,3-c]pyridazinesand Related Heterocycles, Monatsh. Chem. 130 (1999) 1117–1128, https://doi.org/10.1007/PL00010290.
[57] P. Schmidt, J. Druey, Heilmittelchemische Studien in der heterocyclischen Reihe. 5. Mitteilung. Pyridazine II. Eine neue Pyridazinsynthese, Helv. Chim. Acta 15 (1954) 134–140, https://doi.org/10.1002/hlca.19540370116.
[58] M.S.A.Y. Al-kahraman, Yasinzai M Al-kahraman, G.S. Singh, Evaluation of some classical hydrazones of ketones and 1,2-diketones as antileishmanial, antibacterial and antifungal agents, Arch. Pharm. Res. 35 (2012) 1009–1013, https://doi.org/10.1007/s12272-012-0608-7.
[59] W.J. Lossow, R.H. Migliorini, N. Brot, I.L. Chaikoff, Effect of total exclusion of the exocrine pancreas in the rat upon in vitro esterification of C14 – labeled cholesterol by the intestine and upon lymphatic absorption of C14 – labeled cholesterol, J. Lipid Res. 5 (1964) 198–202, https://doi.org/10.1016/S0022-2275(20)40238-X.
[60] J.H. Kim, C.W. Cho, H.Y. Kim, K.T. Kim, G.-S. Choi, H.-H. Kim, I.S. Cho, S.J. Kwon, S.-K. Choi, J.-Y. Yoon, S.Y. Yang, J.S. Kang, Y.H. Kim, α-Glycosidase inhibition by prenylated and lavandulyl compounds from Sophora flavescens roots and in silico analysis, Int. J. Biol. Macromol. 102 (2017) 960–969, https://doi.org/10.1016/j.ijbiomac.2017.04.092.