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New thiosemicarbazide-1,2,3-triazole hybrids as potent α-glucosidase inhibitors: Design, synthesis, and biological evaluation

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ABSTRACT

A new series of thiosemicarbazide-1,2,3-triazole hybrids **10a-o** has been synthesized, characterized by ^{1}H NMR, ^{13}C NMR, and screened for their *in vitro* α-glucosidase inhibitory activity. All of the synthesized compounds displayed excellent α-glucosidase inhibitory activity with IC_{50} values in the range of 75.0 ± 0.5 to 253.0 ± 0.5 μM, as compared to the standard drug acarbose ($IC_{50} = 750.0 \pm 1.5$ μM). Among the synthesized compounds, compound **10h** ($IC_{50} = 75.0 \pm 0.5$) with 4-methoxy group at phenyl part of thiosemicarbazide moiety and 2,6-dichloro substituents at benzyl moiety was found to be the most potent compound. Kinetic analysis revealed that compound **10h** is a competitive inhibitor for α-glucosidase. Docking study of compound **10h** in the active site of α-glucosidase showed that this compound interacted with residues His239, His279, Glu304, Gly306, and Arg312.

1. Introduction

α-Glucosidase is a digestive enzyme, which plays a vital role in the breakdown of disaccharide and polysaccharides in the intestine [1]. α-Glucosidase inhibitors could diminish postprandial plasma glucose levels and, as a result, they decreased postprandial hyperglycemia. Therefore, α-glucosidase is an essential target for the treatment of type 2 diabetes [2]. Furthermore, α-glucosidase also has been introduced as an attractive therapeutic target for other carbohydrate-mediated diseases including HIV, cancer, and hepatitis [3–5]. Acarbose, miglitol, and voglibose are α-glucosidase inhibitors that have been approved for the clinical use. These drugs have side effects such as flatulence, pain, bloating, diarrhea, and abdominal discomfort [6]. Hence, the search for new agents with high α-glucosidase inhibitory activity and low side effects is still in progress [7–9].

Thiosemicarbazide and thiourea are two sulfur-containing scaffolds, which are found in the many biologically active compounds with antitumor, antibacterial, antifungal, anticonvulsant, and antioxidant activities [10–19]. Furthermore, derivatives of these scaffolds such as thiosemicarbazide derivatives **A** and thiourea derivatives **B** have been reported to show high inhibitory activity against α-glucosidase (Fig. 1) [20,21]. On the other hand, recently, several groups of α-glucosidase inhibitors containing Schiff-base such as compounds **C** have been synthesized (Fig. 1) [22].

1,2,3-Triazole is a five-membered ring with three nitrogen atoms and applied as an important building block in many biologically active compounds with various pharmacological activities [23–25]. One of the reported pharmacological effects of 1,2,3-triazole derivatives is α-glucosidase inhibition [26–29]. In this respect, newly, our research group by using molecular hybridization reported a new series of 1,2,3-triazole-quinazolinone hybrids **D**

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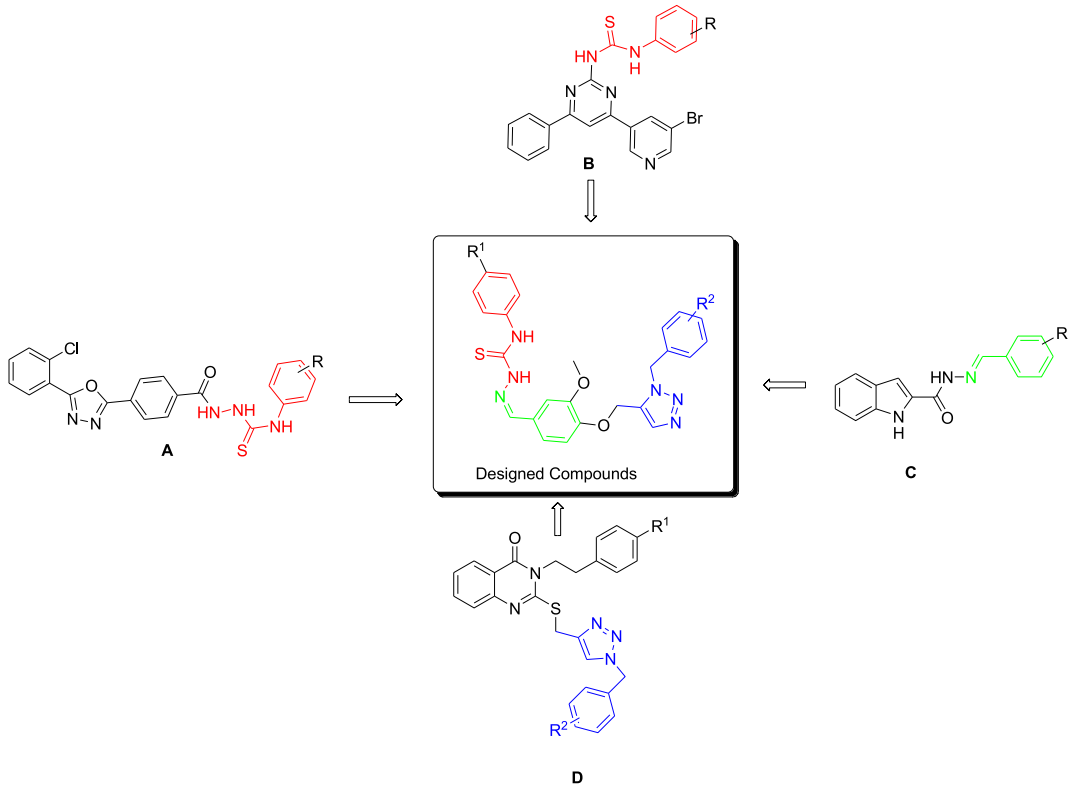


Fig. 1. Rational design of new thiosemicarbazide-1,2,3-triazole hybrids as potent α-glucosidase inhibitors based on molecular hybridization.

as potent α-glucosidase inhibitors [30]. Therefore, herein, with focusing on structures **A**, **B**, **C**, and **D** as potent α-glucosidase inhibitors, a new series of thiosemicarbazide-1,2,3-triazole hybrids **10a-o** are designed as the follow up efforts in the development of new α-glucosidase inhibitors by molecular hybridization [31–33]. Designed compounds were synthesized and evaluated against α-glucosidase. Kinetic and docking studies were also performed to assay the interaction of these compounds with α-glucosidase.

2. Method and material

Melting points of the target compounds **10a-o** was measured on a Kofler hot stage apparatus. ^{1}H and ^{13}C NMR spectra of title compounds were determined on a Bruker FT-500 *via* TMS (internal standard). IR spectra re-coded using KBr disks on a Nicolet Magna FTIR 550 spectrophotometer. Mass spectra for selected compounds **10a**, **10d**, and **10l** were obtained with an Agilent Technology (HP) mass spectrometer operating at an ionization potential of 70 eV. Elemental analysis was performed by an Elementar Analysen system GmbH VarioEL CHN mode.

2.1. General procedure for the synthesis of 4-phenylthiosemicarbazides 3

A solution of isothiocyanate derivatives **1** (1 mmol) and hydrazine **2** (1 mmol) in Et_2O (15 mL) was stirred at room temperature for 6 h. Later, the reaction mixture was filtered off and precipitated product was washed with Et_2O to obtain pure 4-phenylthiosemicarbazides **3.**

2.2. General procedure for the synthesis of 3-methoxy-4-(prop-2-ynyloxy)benzaldehyde 6

A suspension of 4-hydroxy-3-methoxybenzaldehyde **4** (1 mmol) and K_2CO_3 (1 mmol) in DMF (5 ml) was stirred at room temperature for 1 h. At that point, it was added to a solution of propargyl bromide **5** (1.2 mmol) in DMF (10 ml) in a dropwise manner, and the reaction mixture was stirred at room temperature for 2 h. Once the reaction was completed (checked by TLC), it was poured into crushed ice, and the obtained mixture was filtered off and residue was recrystallized in ethanol to obtain 3-methoxy-4-(prop-2-ynyloxy) benzaldehyde **6**.

2.3. General procedure for the synthesis of 1-(3-methoxy-4-(prop-2-ynyloxy)benzylidene)-4-phenylthiosemicarbazides 7

A mixture of 4-phenylthiosemicarbazides **3** (1 mmol) and 3-methoxy-4-(prop-2-ynyloxy) benzaldehyde **6** (1 mmol) at ethanol in the presence of PTSA (para-toluene sulfonic acid) was refluxed for 6 h. Then, the obtained mixture was filtered off and residue was recrystallized in the ethyl acetate/n-hexane to gain pure 1-(3-methoxy-4-(prop-2-ynyloxy) benzylidene) -4-phenylthiosemicarbazides **7.**

*2.4. General procedure for the synthesis of thiosemicarbazide-1,2,3-triazole derivatives **10a-o***

At first, azide derivatives **9** were prepared in situ. For this purpose, a solution of benzyl halides **8** (1.1 mmol), sodium azide (0.9 mmol), and Et_3N (1.3 mmol) in the mixture of water plus t-BuOH (10 mL, 1:1) was stirred at room temperature for 1 h. Then and there, a mixture of 1-(3-methoxy-4-(prop-2-ynyloxy)benzylidene)-4-phenylthiosemicarbazides **7** (1 mmol), sodium ascorbate, and $CuSO_4 \cdot 5H_2O$ (7 mol %) was added to the prepared benzyl azide derivative **9,** and obtained mixture was stirred at room temperature for 24–48 h. Upon completion of the reaction (monitored by TLC), reaction mixture was diluted with cold water and poured into crushed ice. Then, precipitated products **10a-o** were filtered off, washed with water, and purified by recrystallization in ethanol.

*2.4.1. (E)-1-(4-((3-(2,4-dichlorobenzyl)-3H-1,2,3-triazol-4-yl) methoxy)-3-methoxybenzylidene)-4-phenylthiosemicarbazide (**10a**)*

White Powder; Mp: 157–159 °C; Yield: 72%; ^{1}H NMR (500 MHz, $CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*): 3.91 (s, 3H; OCH_3), 5.34 (s, 2H; CH_2N), 5.64 (s, 2H; CH_2O), 7.11 (d, $J = 8.3$ Hz, 1H; H_5), 7.15–7.18 (m, 2H; $H_{4'',6}$), 7.25 (s, 1H; H_2), 7.27–7.29 (m, 2H; $H_{5',6'}$), 7.43 (d, $J = 7.9$ Hz, 2H; $H_{2'',6''}$), 7.71 (s, 1H; $CH_{triazole}$), 7.91 (s, 1H; H-C=N), 9.16 (s, 1H; NH-Ph), 10.15 (s, 1H; NH-C=S); ^{13}C NMR ($CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*):, 51.4, 56.0, 62.9, 109.4, 113.8, 122.3, 123.3, 124.6, 126.2, 126.7, 128.0,128.9, 129.9130.9, 131.3, 134.3, 135.7, 137.9, 143.1, 144.2, 150.0, 150.1, 175.8; IR (KBr, cm^{-1}): 3429, 3302 (N–H stretching), 3144 (Aromatic C–H stretching), 1546 (C=N), 1506 (C=C), 1265 (C-N), 1201 (C=S), 1137 (C-O); MS (70 eV): $m/z = 540$ [M^+]; Anal. Calcd. For $C_{25}H_{22}Cl_2N_6O_2S$: C, 55.46; H, 4.10; N, 15.52; Found: C, 55.21; H, 4.32; N, 15.71.

*2.4.2. (E)-1-(4-((3-(2,6-dichlorobenzyl)-3H-1,2,3-triazol-4-yl) methoxy)-3-methoxybenzylidene)-4-phenylthiosemicarbazide(**10b**)*

Cream to white Powder; Mp: 220–222 °C; Yield: 88%; ^{1}H NMR (500 MHz, $CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*): 3.91 (s, 3H; OCH_3), 5.32 (s, 2H; CH_2N), 5.88 (s, 2H; CH_2O), 7.13–7.18 (m, 2H; $H_{5,6}$), 7.24–7.28 (m, 2H; $H_{2,4'}$),7.32 (t, $J = 7.7$ Hz, 1H; H_4), 7.41–7.45 (m, 4H; $H_{3'',5'',2'',6''}$), 7.67–7.68 (m, 3H; $H_{3',5',\ triazole}$), 7.86 (s, 1H; H-C=N), 9.15 (s, 1H; NH-Ph), 9.82 (s, 1H; NH-C=S); ^{13}C NMR ($CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*): 49.0, 56.5, 62.9, 109.1, 114.1, 122.2, 123.0, 124.6, 126.2, 126.7, 128.8, 128.9, 130.0, 131.1, 133.8, 136.8, 137.9, 139.9, 143.0, 143.5, 149.9, 150.2, 175.6; IR (KBr, cm^{-1}): 3438, 3294 (N–H stretching), 3150 (Aromatic C–H stretching), 1595 (C=N), 1551 (C=C), 1267 (C-N), 1199 (C=S), 1138 (C-O); Anal. Calcd. for $C_{25}H_{22}Cl_2N_6O_2S$: C, 55.46; H, 4.10; N, 15.52; Found C, 55.61; H, 4.27; N, 15.39.

*2.4.3. (E)-1-(4-((3-(3,4-dichlorobenzyl)-3H-1,2,3-triazol-4-yl) methoxy)-3-methoxybenzylidene)-4-phenylthiosemicarbazide (**10c**)*

White Powder; Mp: 178–180 °C; Yield: 88%; ^{1}H NMR (500 MHz, $CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*): 3.92 (s, 3H; OCH_3), 5.34 (s, 2H; CH_2N), 5.50 (s, 2H; CH_2O), 7.11 (t, $J = 8.2$ Hz, 2H; $H_{3'',5''}$), 7.18 (d, $J = 8.3$, 1H; H_5), 7.25–7.28 (t, $J = 7.7$ Hz, 2H; $H_{4'',2}$), 7.37 (s, 1H, $H_{2'}$), 7.42–7.47 (m, 3H; $H_{6,5',6'}$), 7.63 (s, 1H, $H_{triazole}$), 7.67 (d, $J = 7.5$ Hz, 2H, $H_{2'',6''}$), 7.87 (s, 1H; H-C=N), 9.15 (s, 1H; NH-Ph), 9.85 (s, 1H; NH-C=S); ^{13}C NMR ($CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*): 53.4, 56.1, 62.9, 109.1, 109.2, 113.6, 121, 122.2, 123, 124.6, 126.7, 127.3, 128.8, 130.0, 134.4, 137.9, 143.0, 144.4, 146.0, 150.0, 175.8; IR (KBr, cm^{-1}): 3387, 3302 (N–H stretching), 3139 (Aromatic C–H stretching), 1595 (C=N), 1544 (C=C), 1266 (C-N), 1198 (C=S), 1135 (C-O); Anal. Calcd. for $C_{25}H_{22}Cl_2N_6O_2S$: C, 55.46; H, 4.10; N, 15.52; Found C, 55.24; H, 4.02; N, 15.72.

*2.4.4. (E)-1-(4-((3-(2-methylbenzyl)-3H-1,2,3-triazol-4-yl) methoxy)-3-methoxybenzylidene)-4-(4-methoxyphenyl) thiosemicarbazide (**10d**)*

Cream Powder; Mp: 94–96 °C; Yield: 79%; ^{1}H NMR (500 MHz, $CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*): 3.84 (s, 3H, OCH3), 3.88 (s, 3H; OCH_3), 5.31 (s, 2H; CH_2N), 5.55 (s, 2H; CH_2O), 6.94 (d, $J = 8.1$ Hz, 2H; $H_{3'',5''}$), 7.10 (d, $J = 8.4$, 1H; H_5), 7.16 (d, $J = 7.2$, 2H; $H_{2'',6''}$), 7.21–7.24 (m, 3H, $H_{2,\ 4',\ 5'}$), 7.29 (d, $J = 9.4$ Hz, 1H; $H_{3'}$), 7.48–7.50 (m, 3H; $H_{6,6',triazole}$), 7.90 (s, 1H; H-C=N), 9.00 (s, 1H; NH-Ph), 10.16 (s, 1H; NH-C=S); ^{13}C NMR ($CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*): 19.2, 52.3, 55.5, 56.3, 63.1, 109.4, 113.8, 114.1, 122.2, 122.8, 126.6, 126.7, 127.0, 129.2, 129.5, 131.1, 132.3, 134.6, 136.9, 143.1, 143.8, 149.0, 150.0, 158.0, 176.4; IR (KBr, cm^{-1}): 3434, 3302 (N–H stretching), 3147 (Aromatic C–H stretching), 1596 (C=N), 1510 (C=C), 1262 (C-N), 1199 (C=S), 1136 (C-O); MS (70 eV): $m/z = 516$ [M^+]; Anal. Calcd. for $C_{27}H_{28}N_6O_3S$: C, 62.77; H, 5.46; N, 16.27 Found C, 63.10; H, 5.33; N, 15.97.

*2.4.5. (E)-1-(4-((3-(3-methoxybenzyl)-3H-1,2,3-triazol-4-yl) methoxy)-3-methoxybenzylidene)-4-(4-methoxyphenyl) thiosemicarbazide (**10e**)*

White to cream Powder; Mp: 125–127 °C; Yield: 90%; ^{1}H NMR (500 MHz, $CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*): 3.78 (s, 3H, OCH3), 3.84 (s, 3H; OCH_3), 3.89 (s, 3H; OCH_3), 5.32 (s, 2H; CH_2N), 5.51 (s, 2H; CH_2O), 6.80 (m, 1H; $H_{2'}$), 6.86 (d, $J = 7.9$ Hz, 1H; $H_{4'}$), 6.90 (d, $J = 7.9$ Hz, 1H; $H_{6'}$), 6.95 (d, $J = 8.1$ Hz, 2H, $H_{3'',5''}$), 7.09 (d, $J = 8.3$ Hz, 1H, H_5), 7.16 (d, $J = 8.3$ Hz, 1H, H_6), 7.23 (s, 1H, H_2), 7.28–7.29 (m, 1H, $H_{5'}$), 7.49 (d, $J = 8.1$ Hz, 2H; $H_{2'',6''}$), 7.60 (s, 1H; $H_{triazole}$), 7.89 (s, 1H; H-C=N), 9.00 (s, 1H; NH-Ph), 10.17 (s, 1H; NH-C=S); ^{13}C NMR ($CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*): 54.2, 55.3, 55.5, 56.1, 63.1, 109.2, 113.7, 113.8, 114.1, 114.3, 120.3, 122.1, 123.0, 126.8, 127.1, 130.2, 130.8, 135.8, 143.1, 144.0, 149.9, 150.1, 158.1, 160.2, 175.8; IR (KBr, cm^{-1}): 3441, 3261 (N–H stretching), 3133 (Aromatic C–H stretching), 1595 (C=N), 1511 (C=C), 1265 (C-N), 1201 (C=S), 1139 (C-O); Anal. Calcd. for $C_{27}H_{28}N_6O_4S$: C, 60.89; H, 5.30; N, 15.78 Found C, 60.53; H, 5.21; N, 16.31.

*2.4.6. (E)-1-(4-((3-(2-chlorobenzyl)-3H-1,2,3-triazol-4-yl) methoxy)-3-methoxybenzylidene)-4-(4-methoxyphenyl) thiosemicarbazide (**10f**)*

White to cream Powder; Mp: 133–135 °C; Yield: 82%; ^{1}H NMR (500 MHz, $CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*): 3.84 (s, 3H, OCH3), 3.90 (s, 3H; OCH_3), 5.33 (s, 2H; CH_2N), 5.68 (s, 2H; CH_2O), 6.90 (d, $J = 8.7$ Hz, 2H; $H_{3'',5''}$), 7.10 (d, $J = 8.4$ Hz, 1H; H_5), 7.16 (d, $J = 8.4$ Hz, 1H; H_6), 7.21 (d, $J = 7.5$ Hz, 1H, $H_{6'}$), 7.24 (s, 1H, H_2), 7.26–7.28 (m, 1H, $H_{4'}$), 7.33 (t, $J = 7.7$ Hz, 1H, $H_{5'}$), 7.45 (d, $J = 7.7$ Hz, 1H, $H_{3'}$), 7.50 (d, $J = 8.7$ Hz, 2H; $H_{2'',6''}$), 7.69 (s, 1H; $H_{triazole}$), 7.88 (s, 1H; H-C=N), 9.00 (s, 1H; NH-Ph), 10.03 (s, 1H; NH-C=S); ^{13}C NMR ($CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*): 51.6, 51.6, 55.3, 56.3, 63.3, 109.2, 113.8, 114.1, 122.1, 123.3, 126.8, 127.0, 127.6, 129.9, 130.3, 130.5, 130.8, 132.2, 133.6, 143.1, 144.0, 149.9, 150.1, 158.1, 176.4; IR (KBr, cm^{-1}): 3436, 3294 (N–H stretching), 3149 (Aromatic C–H stretching), 1595 (C=N), 1504 (C=C), 1267 (C-N), 1199 (C=S), 1138 (C-O); Anal. Calcd. for $C_{26}H_{25}ClN_6O_3S$: C, 58.15; H, 4.69; N, 15.65 Found C, 57.95; H, 4.83; N, 15.48.

*2.4.7. (E)-1-(4-((3-(2,4-dichlorobenzyl)-3H-1,2,3-triazol-4-yl) methoxy)-3-methoxybenzylidene)-4-(4-methoxyphenyl) thiosemicarbazide (**10g**)*

White Powder; Mp: 164–166 °C; Yield: 73%; ^{1}H NMR (500 MHz, $CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*): 3.84 (s, 3H, OCH3), 3.90 (s, 3H; OCH_3), 5.33 (s, 2H; CH_2N), 5.64 (s, 2H; CH_2O), 6.95 (d, $J = 8.9$ Hz, 2H; $H_{3'',5''}$), 7.09 (d, $J = 8.3$ Hz, 1H; H_5), 7.14–7.17 (m, 2H; $H_{2,6}$), 7.24–7.27 (m, 1H, $H_{5',6'}$), 7.46 (s, 1H, $H_{3'}$), 7.49 (d, $J = 8.9$ Hz, 2H, $H_{2'',6''}$), 7.70 (s, 1H; $H_{triazole}$), 7.90 (s, 1H; H-C=N), 9.00 (s, 1H; NH-Ph), 10.18 (s, 1H; NH-C=S); ^{13}C NMR ($CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*): 50.6, 55.6, 56.3, 63.1, 109.2, 113.3, 114.1, 121.7, 123.3, 126.9, 127.0, 127.8, 129.8, 130.8, 131.4, 134.4, 136.0, 137.9, 142.7, 144.3, 150.1, 158.3, 176.6; IR (KBr, cm^{-1}): 3437, 3292 (N–H stretching), 3142 (Aromatic C–H stretching), 1595 (C=N), 1509 (C=C), 1261 (C-N), 1194 (C=S), 1137 (C-O); Anal. Calcd. for $C_{26}H_{24}Cl_2N_6O_3S$: C, 54.64; H, 4.23; N, 14.71 Found C, 54.89; H, 4.37; N, 14.43.

*2.4.8. (E)-1-(4-((3-(2,6-dichlorobenzyl)-3H-1,2,3-triazol-4-yl) methoxy)-3-methoxybenzylidene)-4-(4-methoxyphenyl) thiosemicarbazid (**10h**)*

White Powder; Mp: 184–186 °C; Yield: 74%; ^{1}H NMR (500 MHz, $CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*): 3.85 (s, 3H, OCH3), 3.91 (s, 3H; OCH_3), 5.31 (s, 2H; CH_2N), 5.87 (s, 2H; CH_2O), 6.96 (d, $J = 8.4$ Hz, 2H; $H_{3'',5''}$), 7.12–7.17 (m, 2H; $H_{5,6}$), 7.24 (s, 1H; H_2), 7.32 (t, $J = 8.4$ Hz, 1H,

$H_{4'}$), 7.42 (d, $J = 8.4$ Hz, 2H, $H_{2'',6''}$), 7.50 (d, $J = 8.4$ Hz, 2H, $H_{3',5'}$), 7.68 (s, 1H; $H_{triazole}$), 7.85 (s, 1H; H-C=N), 9.00 (s, 1H; NH-Ph), 9.83 (s, 1H; NH-C=S); ^{13}C NMR ($CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*): 49.2, 55.5, 56.6, 62.9, 109.0, 113.8, 114.1, 122.2, 123, 124.6, 126.7, 126.9, 128.9, 130.7, 131.1, 134.2, 136.9, 139.4, 143.0, 143.5, 149.6, 150.1, 158.1, 173.5; IR (KBr, cm^{-1}): 3425, 3304 (N–H stretching), 3147 (Aromatic C–H stretching), 1594 (C=N), 1511 (C=C), 1262 (C-N), 1198 (C=S), 1138 (C-O); Anal. Calcd. for $C_{26}H_{24}Cl_2N_6O_3S$: C, 54.64; H, 4.23; N, 14.71 Found C, 54.43; H, 4.37; N, 14.93.

2.4.9. (E)-1-(4-((3-(3,4-dichlorobenzyl)-3H-1,2,3-triazol-4-yl) methoxy)-3-methoxybenzylidene)-4-(4-methoxyphenyl) thiosemicarbazide (**10i**)

White to cream Powder; Mp: 107–108 °C; Yield: 72%; ^{1}H NMR (500 MHz, $CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*): 3.84 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 5.33 (s, 2H; CH_2O), 5.50 (s, 2H; CH_2O), 6.95 (d, $J = 8.8$ Hz, 2H; $H_{3'',5''}$), 7.08 (d, $J = 8.3$ Hz, 1H, H_5), 7.12 (d, $J = 9.0$, 1H; $H_{6'}$), 7.16 (d, $J = 9.0$ Hz, 1H; $H_{5'}$), 7.24 (s, 1H, H_2), 7.37 (s, 1H, $H_{2'}$), 7.44–7.49 (m, 3H, $H_{6,2'',6''}$), 7.64 (s, 1H; $H_{triazole}$), 7.90 (s, 1H; H-C=N), 9.00 (s, 1H; NH-Ph), 10.21 (s, 1H; NH-C=S); ^{13}C NMR ($CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*): 55.9, 55.5, 56.1, 63.0, 109.2, 113.7, 114.1, 122.1, 123.0, 126.9, 127.0, 127.1, 127.3, 130.0, 130.8, 131.1, 133.2, 134.5, 143.1, 144.4, 149.9, 158.1, 176.4; IR (KBr, cm^{-1}): 3309 (N–H stretching), 3142 (Aromatic C–H stretching), 1594 (C=N), 1511 (C=C), 1261 (C-N), 1200 (C=S), 1133 (C-O); Anal. Calcd. for $C_{26}H_{24}Cl_2N_6O_3S$: C, 54.64; H, 12.41; N, 14.71 Found C, 54.81; H, 12.57; N, 14.63.

2.4.10. (E)-1-(4-((3-(4-bromobenzyl)-3H-1,2,3-triazol-4-yl) methoxy)-3-methoxybenzylidene)-4-(4-methoxyphenyl) thiosemicarbazide (**10j**)

Cream Powder; Mp: 116–118 °C; Yield: 79%; ^{1}H NMR (500 MHz, $CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*): 3.84 (s, 3H, OCH3), 3.90 (s, 3H; OCH_3), 5.32 (s, 2H; CH_2N), 5.49 (s, 2H; CH_2O), 6.94 (d, $J = 7.0$ Hz, 2H; $H_{3'',5''}$), 7.15 (d, $J = 7.0$ Hz, 2H, $H_{2'',6''}$), 7.24 (s, 1H; H_2), 7.35 (d, H = 8.3 Hz, 1H; H_5), 7.46–7.52 (m, 5H, $H_{6,\ 2',3',5',6'}$), 7.14 (d, $J = 8.3$, 1H, H_6), 7.23 (s, 1H, H_2), 7.28–7.31 (m, 1H, $H_{5'}$), 7.39 (d, $J = 8.7$ Hz, 2H; $H_{2'',6''}$), 7.60 (s, 1H; $H_{triazole}$), 7.90 (s, 1H; H-C=N), 9.00 (s, 1H; NH-Ph), 10.20 (s, 1H; NH-C=S); ^{13}C NMR ($CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*):53.4, 55.6, 56.3, 62.9, 109.1, 113.7, 113.9, 114.1, 121.7, 122.3, 123.0, 127.0, 129.6, 130.7, 132.2, 132.4, 132.6, 133.4, 143.0, 145.4, 149.4, 150.1, 158.0, 176.4; IR (KBr, cm^{-1}): 3435, 3325 (N–H stretching), 3144 (Aromatic C–H stretching), 1597 (C=N), 1513 (C=C), 1260 (C-N), 1196 (C=S), 1137 (C-O); Anal. Calcd. for $C_{26}H_{25}BrN_6O_3S$: C, 53.70; H, 4.33; N, 14.45 Found C, 53.93; H, 4.49; N, 14.29.

2.4.11. (E)-1-(4-((3-(3-methoxybenzyl)-3H-1,2,3-triazol-4-yl) methoxy)-3-methoxybenzylidene)-4-(4-(dimethylamino)phenyl) thiosemicarbazide (**10k**)

Cream to white Powder; Mp: 143–145 °C; Yield: 82%; ^{1}H NMR (500 MHz, $CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*): 2.98 (s, 6H, NCH3), 3.78 (s, 3H; OCH_3), 3.89 (s, 3H; OCH_3), 5.31 (s, 2H; CH_2N), 5.50 (s, 2H; CH_2O), 6.76 (d, $J = 8.8$ Hz, 2H; $H_{3'',5''}$), 6.79 (s, 1H, $H_{2'}$), 6.86 (d, $J = 7.0$ Hz, 1H; $H_{4'}$), 6.90 (d, $J = 7.0$ Hz, 1H; $H_{6'}$), 7.08 (d, $J = 8.3$ Hz, 1H, H_5), 7.14 (d, $J = 8.3$, 1H, H_6), 7.23 (s, 1H, H_2), 7.28–7.31 (m, 1H, $H_{5'}$), 7.39 (d, $J = 8.7$ Hz, 2H; $H_{2'',6''}$), 7.59 (s, 1H; $H_{triazole}$), 7.89 (s, 1H; H-C=N), 8.95 (s, 1H; NH-Ph), 10.23 (s, 1H; NH-C=S); ^{13}C NMR ($CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*): 40.5, 54.2, 55.3, 56.0, 63.1, 109.2, 112.4, 112.8, 113.7, 113.7, 114.3, 120.3, 122.0, 123.0, 126.8, 127.0, 130.2, 135.8, 137.7, 142.8, 144.1, 149.3, 150.0, 160.1, 176.6; IR (KBr, cm^{-1}): 3440, 3259 (N–H stretching), 3127 (Aromatic C–H stretching), 1591 (C=N), 1511 (C=C), 1263 (C-N), 1201 (C=S), 1140 (C-O); Anal. Calcd. for $C_{28}H_{31}N_7O_3S$: C, 61.63; H, 5.73; N, 17.97 Found C, 61.51; H, 5.84; N, 17.73.

2.4.12. (E)-1-(4-((3-(2-chlorobenzyl)-3H-1,2,3-triazol-4-yl) methoxy)-3-methoxybenzylidene)-4-(4-(dimethylamino)phenyl) thiosemicarbazide(**10l**)

Light green Powder; Mp: 175–177 °C; Yield: 85%; ^{1}H NMR (500 MHz, $CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*): 2.98 (s, 6H, NCH3), 3.88 (s, 3H; OCH_3), 5.33 (s, 2H; CH_2N), 5.67 (s, 2H; CH_2O), 6.76 (d, $J = 8.8$ Hz, 2H; $H_{3'',5''}$), 7.08 (d, $J = 8.3$ Hz, 1H, H_5), 7.15 (d, $J = 8.3$ Hz, 1H; H_6), 7.20 (d, $J = 7.5$ Hz, 1H; $H_{6'}$), 7.24–7.27 (m, 2H, $H_{2,4'}$), 7.32 (t, $J = 7.5$, 1H, $H_{5'}$), 7.39 (d, $J = 8.7$ Hz, 2H; $H_{2'',6''}$), 7.69 (d, $J = 7.5$ Hz, 1H, $H_{3'}$), 7.69 (s, 1H; $H_{triazole}$), 7.93 (s, 1H; H-C=N), 8.96 (s, 1H; NH-Ph), 10.48 (s, 1H; NH-C=S); ^{13}C NMR ($CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*): 40.8, 51.6, 56.3, 62.9, 107.8, 109.2, 110.2, 112.4, 113.8, 122.0, 123.3, 126.9, 127.6, 127.7, 129.9, 130.3, 130.4, 132.2, 136.0, 142.9, 144.1, 149.4, 149.8, 176.4; IR (KBr, cm^{-1}): 3440, 3259 (N–H stretching), 3127 (Aromatic C–H stretching), 1591 (C=N), 1511 (C=C), 1263 (C-N), 1201 (C=S), 1140 (C-O); MS (70 eV): $m/z = 550$ [M^+]; Anal. Calcd. for $C_{28}H_{31}N_7O_3S$: C, 61.63; H, 5.73; N, 17.97 Found C, 61.51; H, 5.84; N, 17.73.

2.4.13. (E)-1-(4-((3-(2,4-dichlorobenzyl)-3H-1,2,3-triazol-4-yl) methoxy)-3-methoxybenzylidene)-4-(4-(dimethylamino)phenyl) thiosemicarbazide (**10m**)

Light green Powder; Mp: 189–191 °C; Yield: 81%; ^{1}H NMR (500 MHz, $CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*): 2.99 (s, 6H, NCH3), 3.91 (s, 3H; OCH_3), 5.33 (s, 2H; CH_2N), 5.64 (s, 2H; CH_2O), 6.76 (d, $J = 8.8$ Hz, 2H; $H_{3'',5''}$), 7.09 (d, $J = 8.3$ Hz, 1H; H_5), 7.11–7.16 (m, 2H; $H_{5',6'}$), 7.25–7.28 (m, 2H, $H_{2,6}$), 7.39 (d, $J = 8.8$ Hz, 2H, $H_{2'',6''}$), 7.46 (s, 1H, $H_{3'}$), 7.70 (s, 1H; $H_{triazole}$), 7.89 (s, 1H; H-C=N), 8.95 (s, 1H; NH-Ph), 10.19 (s, 1H; NH-C=S); ^{13}C NMR ($CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*): 40.6, 50.7, 56.4, 62.9, 109.1, 112.4, 113.7, 122.1, 123.3, 126.8, 126.9, 127.0, 128.0, 129.8, 130.9, 131.2, 134.2, 135.7, 142.8, 144.2, 149.4, 149.8, 149.9, 176.5; IR (KBr, cm^{-1}): 3306 (N–H stretching), 3144 (Aromatic C–H stretching), 1591 (C=N), 1514 (C=C), 1267 (C-N), 1200 (C=S), 1134 (C-O); Anal. Calcd. for $C_{27}H_{27}Cl_2N_7O_2S$: C, 55.48; H, 4.66; N, 16.77 Found C, 55.79; H, 4.49; N, 16.61.

2.4.14. (E)-1-(4-((3-(3,4-dichlorobenzyl)-3H-1,2,3-triazol-4-yl) methoxy)-3-methoxybenzylidene)-4-(4-(dimethylamino)phenyl) thiosemicarbazide (**10n**)

Light green Powder; Mp: 135–137 °C; Yield: 85%; ^{1}H NMR (500 MHz, $CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*): 2.99 (s, 6H, NCH3), 3.90 (s, 3H; OCH_3), 5.33 (s, 2H; CH_2N), 5.49 (s, 2H; CH_2O), 6.76 (d, $J = 8.7$ Hz, 2H; $H_{3'',5''}$), 7.07 (d, $J = 8.2$ Hz, 1H; H_5), 7.11–7.15 (m, 2H; $H_{5',6'}$), 7.24 (s, 1H, H_2), 7.38–7.40 (m, 3H, $H_{\ 2',2'',6''}$), 7.45 (d, $J = 8.2$ Hz, 1H, H_6), 7.63 (s, 1H; $H_{triazole}$), 7.88 (s, 1H; H-C=N), 8.95 (s, 1H; NH-Ph), 10.13 (s, 1H; NH-C=S); ^{13}C NMR ($CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*): 40.8, 52.9, 56.1, 62.9, 109.0, 109.1, 112.4, 113.7, 122.0, 123.0, 126.8, 126.9, 127.1, 127.3, 130.0, 131.1, 133.2, 134.6, 142.8, 144.5, 149.4, 149.7, 150.0, 176.4.; IR (KBr, cm^{-1}): 3434, 3316 (N–H stretching), 3141 (Aromatic C–H stretching), 1595 (C=N), 1520 (C=C), 1267 (C-N), 1198 (C=S), 1134 (C-O); Anal. Calcd. for $C_{27}H_{27}Cl_2N_7O_2S$: C, 55.48; H, 12.13; N, 16.77 Found C, 55.69; H, 12.21; N, 16.61.

2.4.15. (E)-1-(4-((3-(4-bromobenzyl)-3H-1,2,3-triazol-4-yl) methoxy)-3-methoxybenzylidene)-4-(4-(dimethylamino)phenyl) thiosemicarbazide (**10o**)

White Powder; Mp: 120–122 °C; Yield: 80%; ^{1}H NMR (500 MHz, $CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*): 3.00 (s, 6H, NCH_3), 4.00 (s, 3H; OCH_3), 5.33 (s, 2H; CH_2N), 5.50 (s, 2H; CH_2O), 6.77 (d, $J = 8.8$ Hz, 2H; $H_{3'',5''}$), 7.09 (d, $J = 8.3$, 1H; H_5), 7.14–7.17 (m, 3H; $H_{2'',6'',6}$), 7.25 (s, 1H, H_2), 7.40 (d, $J = 8.6$ Hz, 2H; $H_{2',6'}$), 7.52 (d, $J = 8.3$ Hz, 2H; $H_{3',5'}$), 7.59 (s, 1H, $H_{triazole}$), 7.84 (s, 1H; H-C=N), 8.93 (s, 1H; NH-Ph), 9.85 (s, 1H; NH-C=S); ^{13}C NMR ($CHCl_3$-d_6, 25 °C, TMS) (*δ*, *ppm*): 40.5, 53.7, 56.2, 63.0, 106.8, 109.1, 109.3, 112.5, 113.7, 122.1, 122.9, 123.0, 126.7, 126.9, 129.7, 132.1, 132.4, 142.5, 143.8, 144.3, 144.9, 149.3, 149.9, 176.6; IR

(KBr, cm^{-1}): 3415, 3293 (N–H stretching), 3142 (Aromatic C–H stretching), 1594 (C=N), 1543 (C=C), 1266 (C-N), 1196 (C=S), 1135 (C-O); Anal. Calcd. for $C_{27}H_{28}BrN_7O_2S$: C, 54.55; H, 4.75; N, 16.49 Found C, 54.13; H, 4.91; N, 16.82.

2.5. α-Glucosidase inhibition assay

The α-glucosidase inhibitory activities of thiosemicarbazide-1,2,3-triazole derivatives **10a-o** were determined according to the literature protocol [31]. The *Saccharomyces cerevisiae* form of the α-glucosidase enzyme (EC 3.2.1.20) and p-nitrophenyl glucopyranoside (pNPG) as substrate were prepared in potassium phosphate buffer (pH 6.8, 50 mM). The enzymatic reaction mixture composed of enzyme buffer (20 μL, 1 U/mL), the substrate (25 μL, 0.5 mM), the test compound (20 μL), and potassium phosphate buffer (135 μL) was incubated at 37 °C for 30 min. The enzymatic activity of α-glucosidase was evaluated by spectrophotometer (Gen5, Power wave xs2, BioTek, America) at a wavelength of 405 nm.

2.6. Kinetic study

Compound **10h** (0, 35, 55 and 75 μM, 20 μL) was added to the enzyme solution (1 U/Ml, 20 μL) at 30 °C and incubated for 15 min. The substrate (pNGP) at different concentrations (1–4 mM) was then added to initiate the enzyme reaction and change in absorbance was measured for 20 min at 405 nm by using spectrophotometer (Gen5, Power wave xs2, BioTek, America) [31].

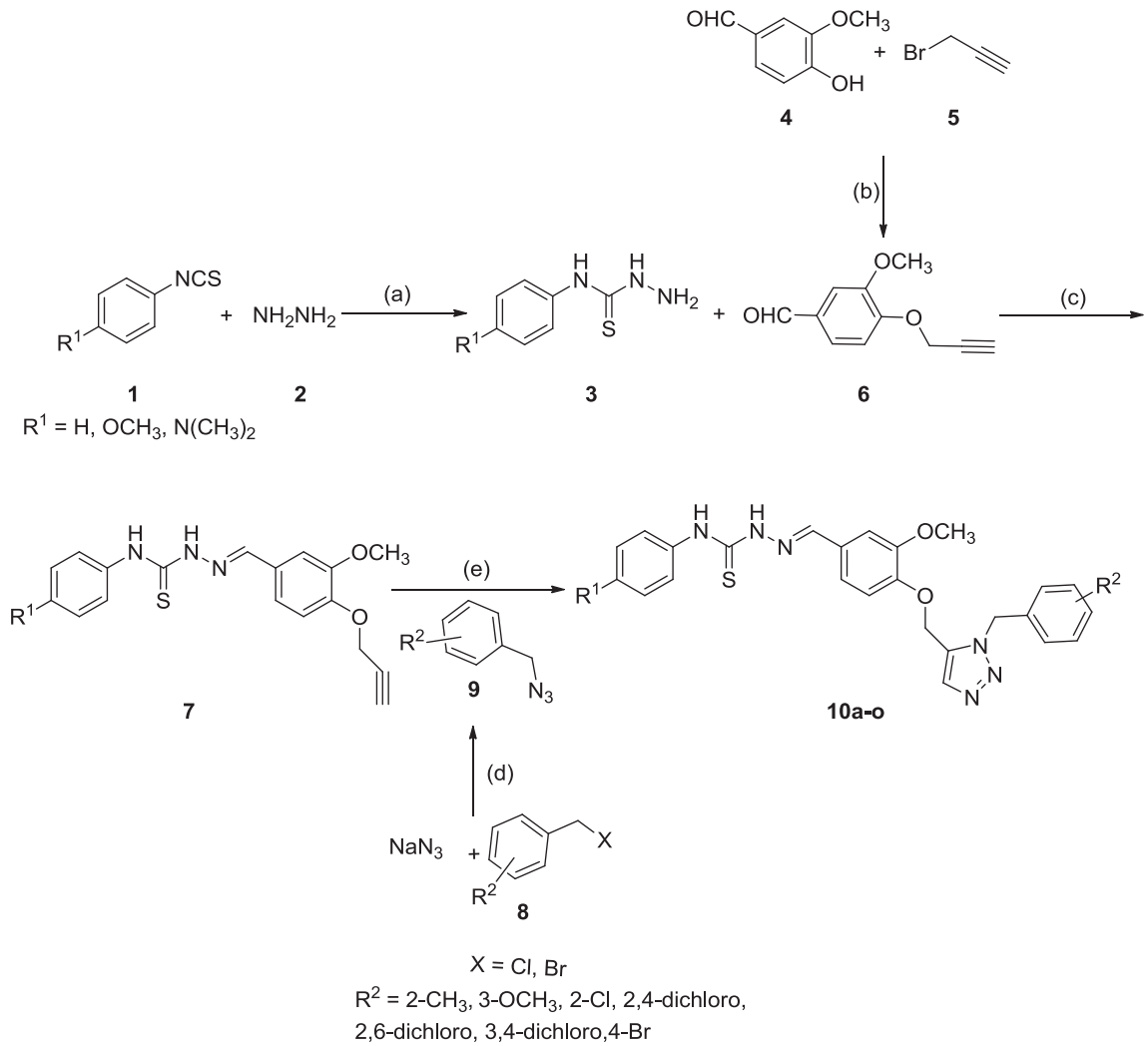
2.7. Molecular docking study

Docking study of the selected compounds **10b**, **10h**, and **10m** was carried out according to the literature protocol [33].

3. Results and discussion

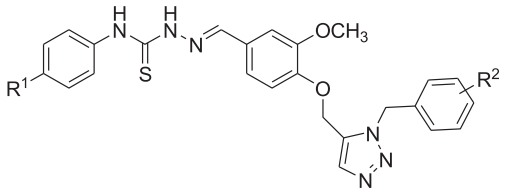
3.1. Chemistry

The synthetic route for the synthesis of thiosemicarbazide-1,2,3-triazole hybrids **10a-o** has been depicted in Scheme 1. Firstly, a mixture of isothiocyanat derivatives **1** and hydrazine **2** in diethylether (Et_2O) was stirred at room temperature for 6 h, to afford 4-phenylthiosemicarbazides **3**. On the other hand, 3-methoxy-4-(prop-2-ynyloxy)benzaldehyde **6** was produced by a reaction between 4-hydroxy-3-methoxybenzaldehyde **4** and propargyl bromide **5** in presence of K_2CO_3 in DMF at room temperature. In the next step, the 4-phenylthiosemicarbazides **3** reacted with 3-



Scheme 1. Reagents and conditions for the synthesis of compounds **10a-o**: (a) Et_2O, room temperature, 6 h; (b) K_2CO_3, DMF, room temperature, 3 h; (c) ethanol, PTSA, 50 °C, 6 h; (d) NaN_3, NEt_3, H_2O/t-BuOH, 1 h (e) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, room temperature, 24–48 h.

Table 1
In vitro α-glucosidase inhibitory activity of thiosemicarbazide-1,2,3-triazole hybrids **10a-o**.



Compound	R^1	R^2	IC_{50} (μM)[a]
10a	H	2,4-Dichloro	208.3 ± 0.9
10b	H	2,6-Dichloro	99.5 ± 0.6
10c	H	3,4-Dichloro	253.0 ± 0.5
10d	OCH_3	2-CH_3	143.7 ± 0.6
10e	OCH_3	3-OCH_3	104.4 ± 1.2
10f	OCH_3	2-Cl	90.0 ± 0.4
10g	OCH_3	2,4-Dichloro	125.2 ± 1.3
10h	OCH_3	2,6-Dichloro	75.0 ± 0.5
10i	OCH_3	3,4-Dichloro	135.6 ± 0.8
10j	OCH_3	4-Br	96.3 ± 0.9
10k	$N(CH_3)_2$	3-OCH_3	155.0 ± 0.5
10l	$N(CH_3)_2$	2-Cl	217.4 ± 0.8
10m	$N(CH_3)_2$	2,4-Dichloro	124.5 ± 1.0
10n	$N(CH_3)_2$	3,4-Dichloro	194.0 ± 0.7
10o	$N(CH_3)_2$	4-Br	231.4 ± 1.0
Acarbose	–	–	750.0 ± 1.5

[a] Values are the mean ± SD. All experiments were performed at least three times.

methoxy-4-(prop-2-ynyloxy)benzaldehyde **6** in ethanol at room temperature, to give 1-(3-methoxy-4-(prop-2-ynyloxy)benzylidene)-4-phenylthiosemicarbazides **7**. The latter compounds were prone to be participated in click reaction. Accordingly, various benzyl halide derivatives **8** and sodium azide reacted in the mixture of H_2O and t-BuOH (1:1) in the presence of Et_3N at room temperature. Then, a mixture of 1-(3-methoxy-4-(prop-2-ynyloxy)benzylidene)-4-phenylthiosemicarbazides **7**, sodium ascorbate, and CuSO4·5H2O was added to the freshly prepared benzyl azide derivatives **9** and the reaction was continued at room temperature to afford the desired compounds **10a-o**.

The structures of the synthesized compounds **10a-o** were determined by ^1H and ^{13}C NMR, DEPT, IR, and elemental analysis. The IR spectra showed the expected absorption of stretching bands for NH at 3261-3441 cm^{-1}. Furthermore, these compounds displayed characteristic stretches of C=N and bonds around 1546-1597 cm^{-1} and 1194-1201 cm^{-1}, respectively. ^1H NMR spectra of the synthesized compounds showed signals at 6.75–7.68 ppm corresponding to protons related to phenyl groups, besides a singlet in the region of 7.49–7.71 ppm attributed to triazole proton. In addition, the two singlets appeared at 5.31–5.34 ppm and 5.49–5.88 ppm, related to benzyl protons and -OCH_2- group connected to triazole ring, respectively. The signals of protons belonging to the NH connected to the phenyl ring and -C=S group appeared around 8.95–9.16 ppm and 9.84–10.48 ppm, respectively. Moreover, the singlet in the region of 7.85–7.92 ppm related to imine proton (H-C=N) confirms the success of the reaction between thiosemicarbazide and vaniline carbaldehyde. Similarly in ^{13}CNMR spectra, the azomethane group (H-C=N) occurred at 142.5–143.8 ppm as well as the C=S group appeared at 173.5–176.6 ppm.

All title compounds **10a-o** could exist in either the E or Z isomeric form of the imono bond (–CH=N-). In this regard, compound **10f** was selected for elucidation of isomeric structure of the synthesized compounds by 2D NMR. In 2D NMR NOESY spectrum, the special correlation between hydrogen of –NH-C=S at 10.03 ppm and azomethine (H-C=N-) at 7.88, as well as no spatial interaction between the hydrogen in position 2 of the aromatic ring (-CH=C-OCH3) at 7.24 ppm and the hydrogen azomethine (H-C=N-) at 7.88, indicated the presence of a E isomeric form, which corroborates the NOESY spectrum of E-thiosemicarbazone presented in literature [34].

3.2. α-Glucosidase inhibitory activity

Synthesized compounds **10a-o** were evaluated against α-glucosidase to check their *in vitro* inhibitory activity. As can be seen in Table 1, thiosemicarbazide-1,2,3-triazole derivatives **10a-o** can be divided into three groups; (i) phenylthiosemicarbazides **10a-c**, (ii) 4-methoxyphenylthiosemicarbazides **10d-j**, and (iii) 4-dimethylaminophenylthiosemicarbazides **10l-o**. In each group, the substituents on the phenyl ring of benzyl moiety were altered to optimize activity against α-glucosidase.

Achieved results showed that all the synthesized compounds have excellent inhibitory activity in the range of $IC_{50} = 75.0 \pm 0.5$–253.0 ± 0.5 μM, when compared to the standard drug acarbose ($IC_{50} = 750.0 \pm 1.5$ μM) (Table 1). 4-Methoxyphenylthiosemicarbazides **10h**, **10f** and **10j** displayed the highest activity (75.0 ± 0.5, 90.0 ± 0.4, and 96.3 ± 0.9 μM, respectively).

The inhibitory activity of phenylthiosemicarbazide derivatives **10a-c** against α-glucosidase demonstrated that 2,6-dichloro derivative **10b** ($IC_{50} = 99.5 \pm 0.6$ μM) have the most potent activity, while 3,4-dichloro derivative **10c** ($IC_{50} = 253.0 \pm 0.5$ μM) were less active compounds amongst all the synthesized compounds. Changing the second chloro group position of compound **10b** from C-6 to C-4, producing compound **10a** ($IC_{50} = 208.3 \pm 0.9$ μM) diminished the activity drastically.

In the 4-methoxyphenylthiosemicarbazide series (compounds **10d-j**), 2,6-dichloro, 2-Cl, and 4-Br substituted compounds **10h**, **10f**, and **10j** showed the highest activity in this series and among all the synthesized compounds. 3-Methoxy derivative **10e** showed good inhibitory activity in the second series. Moreover, the inhibitory activity of remaining substituted compounds of this series based on the substituent type is in the order of 2,4-dichloro (**10g**) > 3,4-dichloro (**10i**) > 2-CH_3 (**10d**).

In the third group, 3-methoxy derivative **10k** showed good inhibitory activity against α-glucosidase. Compound **10l** ($IC_{50} = 217.4 \pm 0.8$ μM) with chloro substituent on 2-position of benzyl moiety showed moderate activity in comparison to other synthesized compounds. Adding the second chlorine atom to benzyl moiety of compound **10l** led to an inhibitory activity improvement as observed in the compounds **10m** and **10n.** For that matter, the most potent compound in 4-dimethylaminophenylthiosemicarbazide series is 2,4-dichloro derivative **10m** with IC_{50} value 124.5 ± 1.0 μM. The less active compound also in this series was 4-bromo derivative **10o** ($IC_{50} = 231.4 \pm 1.0 \pm 0.8$ μM).

The results revealed phenylthiosemicarbazides were less active than their analogs of the second and third groups. Moreover, 4-methoxyphenylthiosemicarbazide derivatives except for compound **10g** were more potent than their 4-dimethylaminophenylthiosemicarbazide analogs (compounds **10f**, **10i**, and **10j** vs. compounds **10l**, **10n**, and **10o**, respectively). Inhibitory activities in 2, 4-dichloro derivatives **10g** of the second series and **10m** of the third series were approximately the same.

3.3. Enzyme kinetic study

A kinetic study was carried out on the most potent compound **10h** to evaluate the inhibition mode of synthesized compounds on α-glucosidase. For this purpose, the reaction rates were measured

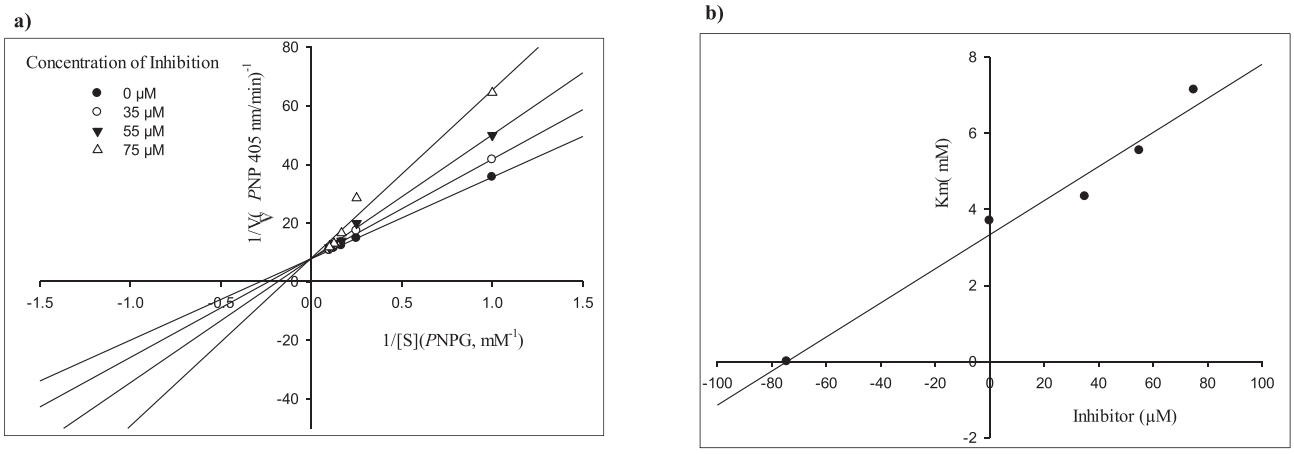


Fig. 2. Kinetics of α-glucosidase inhibition by compound **10h** (inhibitor). (a) The Lineweaver– Burk plot in the absence and presence of different concentrations of compound **10h**; (b) The secondary plot between K_m and various concentrations of compound **10h**.

in the absence and presence of different inhibitor concentrations (compound **10h**) with various concentrations of substrate (p-nitrophenyl glucopyranoside), and then graphs were drawn by the Lineweaver–Burk plot (Fig. 1a). As the concentrations of compound **10h** increased, V_{max} values did not change; however, K_m value increased, thereby indicating that compound **10h** was a competitive inhibitor for α-glucosidase (Fig. 2a). The K_i value was calculated directly by secondary re-plotting of Lineweaver–Burk plots against the different concentrations of compound **10h** (Fig. 2b). The results proved that compound **10h** was a competitive inhibitor against α-glucosidase with a K_i of 74.5 μM. Furthermore, according to plots 2a and 2b, the concentrations of substrate and inhibitor are variable and the rate of reaction is dependent on the reactant concentrations, therefore the reaction order is considered as first order kinetics.

3.4. Docking study

Docking simulation was employed to investigate the interaction pose of the synthesized compounds in the active site of α-glucosidase. For this propose, the most potent compound among all of the synthesized compound (**10h** of the second series) and the most potent compounds of the first and third series (compounds **10b** and **10m**, respectively) were selected. Due to the lack of a crystallographic structure for *Saccharomyces cerevisiae* α-glucosidase (used in the *in vitro* evaluation), a homology model of this enzyme was constructed using SWISS-MODEL Repository [33]. The superposed structure of the standard inhibitor acarbose and the selected compounds **10b**, **10h**, and **10m** in the active site of the modeled α-glucosidase is shown in Fig. 3a. The detailed interaction mode of acarbose revealed that this drug established interactions with residues Asn241, Thr307, His279, Glu304, Pro309, Ser308, Arg312, Thr301, and Gln322 in the active site of α-glucosidase (Fig. 3b).

As depicted in Fig. 4a, in the most potent compound **10h**, the 4-methoxy substituent on phenyl group formed a hydrogen bond with residue Gly306. In this compound, an NH unit of thiosemicarbazide moiety interacted with residue Glu304 *via* electrostatic interaction. 3-Methoxybenzylidene moiety of the compound **10h** created two π-cation interactions with His279 and His239 as well as a π-π interaction with His279. The latter residue also formed a hydrogen bond with the nitrogen atom of 1,2,3-triazole ring. Furthermore, an interaction between the 2-chloro substituent of 2, 6-dichlorobenzyl group and Arg312 was also observed.

NH units of thiosemicarbazide moiety of the compound **10b** formed three hydrogen bonds with Thr307 and Glu304. The latter residue also showed an electrostatic interaction with one of the NH units of thiosemicarbazide moiety. Phenyl ring of 3-methoxybenzylidene moiety of compound **10b** interacted with His279 *via* a π-cation interaction while this moiety in the compound **10h** formed three interactions with His279 and His239. 1,2,3-triazole ring of compound **10b** formed π-π and π-cation interactions with His 239 while this ring in the compound **10h** created a hydrogen bond with His279. The 2-chloro substituent of the 2,6-dichlorobenzyl group in compound **10b**, similar to compound **10h**, interacted with Arg312.

As can be seen in Fig. 4c, thiosemicarbazide moiety of the compound **10m** established a hydrogen bonds as well as electrostatic interaction with residues Thr307 and Glu304, respectively. 3-methoxybenzylidene moiety of this compound, unlike compounds **10h** and **10b**, cannot interact with active site. 1,2,3-Triazole ring of

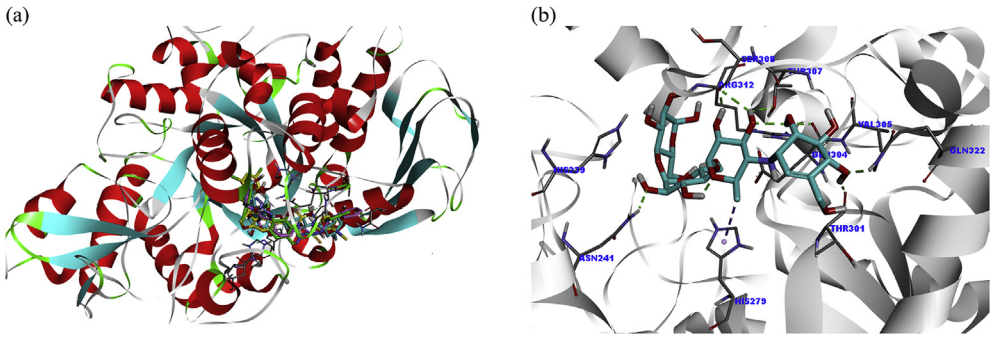


Fig. 3. (a) Superimposition structure of acarbose (cyan) and the most potent compounds **10h** (green), **10b** (pink), **10m** (yellow); (b) interaction mode of acarbose in the active site of α-glucosidase.

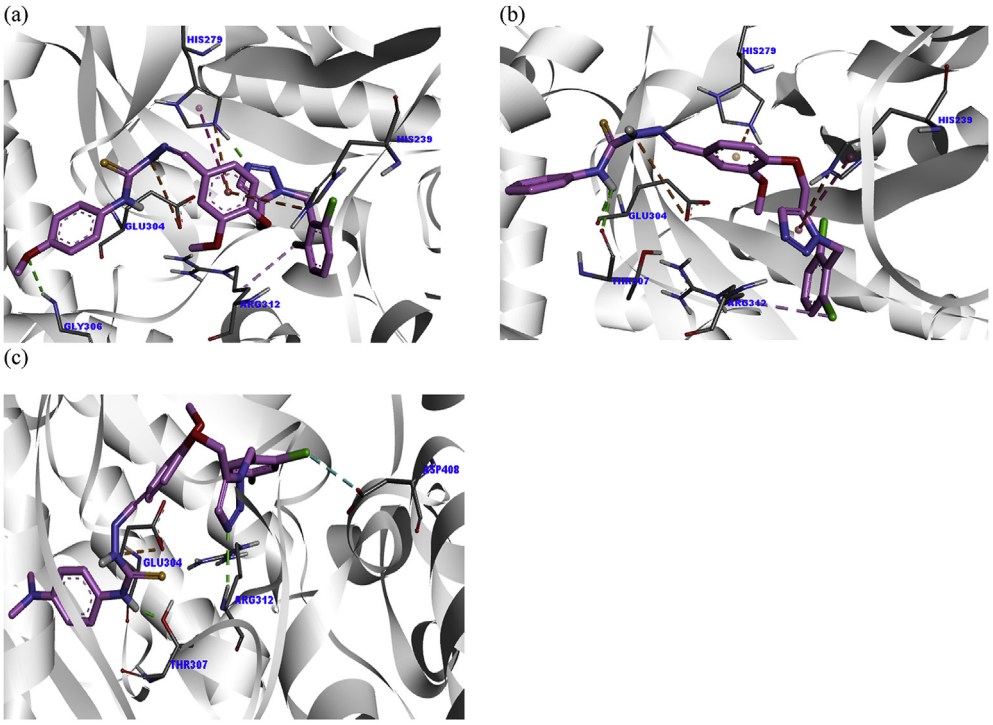


Fig. 4. The interaction modes of the most potent compounds **10h** (a), **10b** (b), and **10m** (c) in the active site of α-glucosidase.

compound **10m** interacted with residue Arg312 through a hydrogen bond. The 2-chloro substituent of the 2,4-dichlorobenzyl group in compound **10m** interacted with Asp408.

4. Conclusion

In conclusion, we designed and synthesized a novel series of thiosemicarbazide-1,2,3-triazole hybrids **10a-o**. Further, α-glucosidase inhibitory activity of the synthesized compounds **10a-o** was evaluated. The obtained results showed that all the synthesized thiosemicarbazide-1,2,3-triazole hybrids are more potent than standard drug acarbose. Kinetic study of the most potent compound **10h** indicated that it competitively inhibited α-glucosidase. Furthermore, docking study showed that selected compounds **10b**, **10h**, and **10m** interacted with important residues in the active site of α-glucosidase. In summary, the results have shown that these thiosemicarbazide-1,2,3-triazole hybrids are a new class of α-glucosidase inhibitors.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molstruc.2019.04.082.

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