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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

New phthalimide-benzamide-1,2,3-triazole hybrids; design, synthesis, α-glucosidase inhibition assay, and docking study

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Abstract

A new series of phthalimide-benzamide-1,2,3-triazole hybrids **8a–k** as α-glucosidase inhibitors was designed and synthesized. The biological evaluation of compounds **8a–k** against yeast α-glucosidase demonstrated that all they have excellent inhibitory activity in comparison with standard inhibitor acarbose. Among them, the most potent compound was compound **8d** with inhibitory activity 18.5-fold more than acarbose. Kinetic study revealed that α-glucosidase inhibition of compound **8d** was the competitive type. Furthermore, docking study suggested that compound **8d** is more stable than acarbose in the active site of α-glucosidase.

Keywords Phthalimide · Benzamide · 1,2,3-Triazole · Hybrid · α-Glucosidase

Introduction

α-Glucosidase is a hydrolyzing enzyme that found in the brush border surface of intestine. The main function of this enzyme is conversion of carbohydrates (polysaccharides and disaccharides) into absorbable monosaccharide glucose (Chiba 1997). Therefore, this enzyme is involved in carbohydrate-related diseases such as diabetes, cancer, viral infections, and pompe disease (Toeller 1994; Pili et al. 1995; Howe et al. 2013; Shimada et al. 2014). In terms of prevalence, the most important carbohydrate-related disease is type 2 diabetes and some of sugar-containing α-glucosidase inhibitors, such as acarbose, miglitol, and voglibose are successfully used in the treatment of this disease (Fig. 1) (Van De Laar et al. 2005). However, these inhibitors were prepared by tedious multistep reactions and their clinical use is associated with gastrointestinal side effects (Hollander 1992). Thus, the discovery of effective small molecules with convenient synthesis, high inhibitory activity against α-glucosidase, and low side effects is an attractive target for pharmaceutical scientists (Taha et al. 2020; Rafique et al. 2019; Rahim et al 2020; Taha et al. 2015; Khan et al. 2014).

Phthalimide is a fused heterocyclic structure that formed by the merger of benzene and pyrrole-2,5-dione. This scaffold was used as an important block in the design biological active compounds with properties such as anti-inflammatory,

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anticonvulsant, hypolipidemic, analgesic, and immunomodulatory activities (Lima et al. 2002; Bailleux et al. 1994; Chapman et al. 1979; Antunes et al. 2003; de Oliveira Cardoso et al. 2015). Furthermore, phthalimide derivative **A** is a known inhibitor for α-glucosidase (Dodo et al. 2008) (Fig. 2). Like phthalimide, 1,2,3-triazole ring widely used in design new molecules with various biological activities (Lauria et al. 2014). Recently, our research group introduced 1,2,3-triazole derivatives **B** with significant inhibitory effects against α-glucosidase (Fig. 2) (Saeedi et al. 2019). On the other hand, previously, structures **C** containing benzamide moiety was reported as potent α-glucosidase inhibitors (Khan et al. 2016) (Fig. 2). Therefore, in this study, in continuation of our attempts to introduce novel α-glucosidase inhibitors using molecular hybridization, for the first time, effective pharmacophore phthalimide and 1,2,3-triazole were attached together by a benzamide moiety in order to achieve phthalimide-benzamide-1,2,3-triazole hybrids **8a–k** as new α-glucosidase inhibitors (Saeedi et al. 2019). Eleven derivatives of the latter scaffold were synthesized and their in vitro α-glucosidase inhibition and docking study were also performed.

Material and methods

General chemistry

Melting points of phthalimide-benzamide-1,2,3-triazoles **8a–k** were measured on a Kofler hot stage apparatus. The NMR (^{1}H and ^{13}C) and IR spectra were obtained by using a Bruker FT-500 and Nicolet Magna FTIR 550 spectrophotometer on KBr disks, respectively. Mass spectrum was performed by an Agilent Technology (HP) mass spectrometer (ionization potential = 70 eV). Elemental analysis was measured by an Elementar Analysensystem GmbH VarioEL CHN mode. Compounds **7a–k** were obtained according to our previous work (Saeedi et al. 2019).

Synthesis of 3-(1,3-dioxoisoindolin-2-yl)benzoic acid 3

A mixture of phthalic anhydride **1** (10 mmol) and 3-aminobenzoic acid **2** (10 mmol) in acetic acid glycial (30 mL) was refluxed for 3 h. Then, solvent was decreased under reduced pressure and water was added to the obtained mixture. The pure precipitated product **3** was filtered off.

Synthesis of 3-(1,3-dioxoisoindolin-2-yl)-*N*-(prop-2-yn-1-yl)benzamide 5

A mixture of 3-(1,3-dioxoisoindolin-2-yl)benzoic acid **3** (4 mmol), propargylamine **4** (4 mmol), HOBT (4.8 mmol), EDCI (4.8 mmol), and NEt_3 (4.4 mmol) in anhydrous CH_2Cl_2 (20 mL) were stirred at room temperature for 30 min, and then were poured into water and the obtained mixture was extracted with CH_2Cl (3 × 15 ml) and organic layer dried over Na_2SO_4. Finally, CH_2Cl_2 was evaporated under reduced pressure and the residue used for the next reaction with no purification.

General procedure for the produce of phthalimide-benzamide-1,2,3-triazoles 8a–k

In order to do a click reaction in the last step, 3-(1,3-dioxoisoindolin-2-yl)-*N*-(prop-2-yn-1-yl)benzamide **5** (1 mmol), sodium ascorbate, and $CuSO_4.5H_2O$ (7 mol%) were added to the freshly prepared benzyl azide derivatives **7a–k**, and obtained mixture was stirred at room temperature for 12–14 h. After that, reaction mixture was poured into crushed ice and precipitated products **8a–k** were filtered off, washed with cold water, and purified by recrystallization in ethyl acetate.

N-((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)-3-(1,3-dioxoisoindolin-2-yl)benzamide (8a)

Off white solid; yield: 61%, mp = 172–174 °C; IR (KBr): 3351, 1709, 1650, 1583, 1385, and 1115 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): 4.30 (brs, 2H), 5.47 (*s*, 2H), 7.22–7.24 (m,

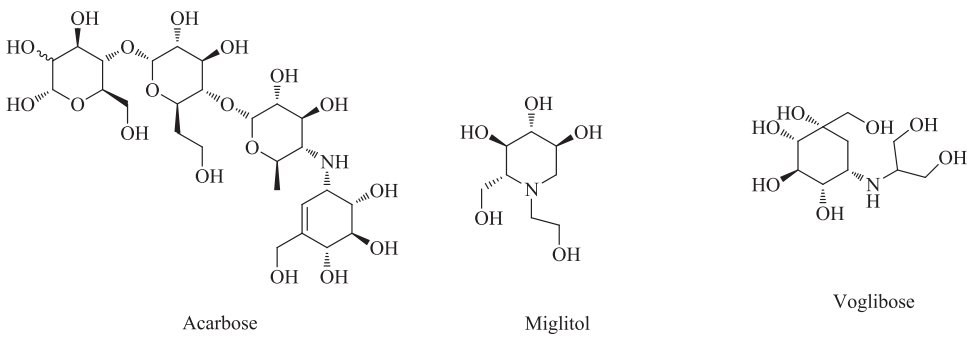


Fig. 1 Structures of well-known α-glucosidase inhibitors

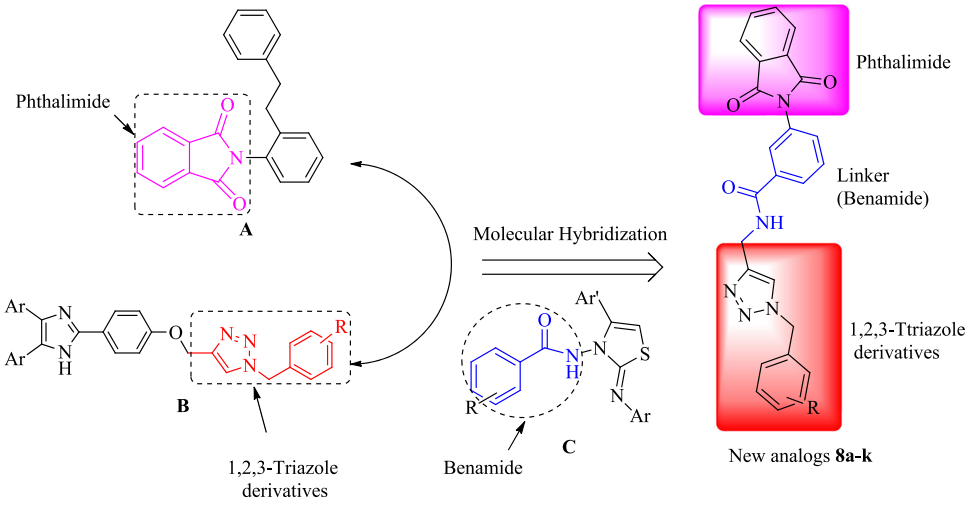


Fig. 2 Rational design of phthalimide-benzamide-1,2,3-triazole hybrids **8a–k** as potential anti-α-glucosidase agents

3H), 7.30–7.35 (m, 3H), 7.53–7.57 (m, 2H), 7.77–7.79 (m, 3H), and 7.90–7.94 (m, 4H).^{13}C NMR (125 MHz, $CDCl_3$): 35.4, 53.1, 123.9, 125.6, 127.0, 128.4, 129.0, 129.2, 129.5, 129.7, 131.6, 132.1, 134.3, 134.7, 135.2, 136.1, 166.5, 166.7, and 170.3. MS (70 eV): m/z = 437.1 [M+]. Anal. Calcd for $C_{25}H_{19}N_5O_3$: C, 68.64; H, 4.38; N, 16.01. Found: C, 68.75; H, 4.51; N, 16.12.

3-(1,3-dioxoisoindolin-2-yl)-*N*-((1-(4-methoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)benzamide (8b)

Off white solid; yield: 75%; mp = 95–97 °C; IR (KBr): 3336, 1726, 1642, 1382, and 1222 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): 3.79 (s, 3H), 4.70 (brs, 2H), 5.42 (*s*, 2H), 6.88 (*d*, *J* = 8.6 Hz, 2H), 7.10 (brs, 1H), 7.22 (d, *J* = 8.6 Hz, 2H), 7.55–7.59 (m, 2H), 7.79–7.81 (m, 4H), 7.87 (*s*, 1H), and 7.94–7.96 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): 33.5, 53.6, 54.8, 103.88, 104.33, 114.5, 118.4, 120.4, 120.7, 123.9, 125.2, 126.3, 126.7, 129.4, 129.6, 129.8, 131.6, 132.1, 132.9, 134.6, 135.6, 166.9, 179.5, and 190.1. Anal. Calcd for $C_{26}H_{21}N_5O_4$: C, 66.80; H, 4.53; N, 14.98. Found: C, 66.63; H, 4.9442; N, 15.06.

3-(1,3-dioxoisoindolin-2-yl)-*N*-((1-(2-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)benzamide (8c)

Off white solid; yield: 63%; mp = 169–171 °C; IR (KBr): 3334, 1722, 1657, 1586, 1437, 1381, and 1221 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): 4.80 (brs, 2H), 5.59 (*s*, 2H), 7.14–7.26 (m, 3H), 7.33–7.36 (m, 3H), 7.51–7.54 (m, 3H), and 7.70–7.79 (m, 5H). ^{13}C NMR (125 MHz, $CDCl_3$): 32.4, 53.1, 116.0, 116.2, 121.4, 124.0, 125.1, 125.7, 127.0, 129.5, 129.7, 131.4, 131.6, 132.1, 134.7, 160.7, 161.6, and 167.1. Anal. Calcd for $C_{25}H_{18}FN_5O_3$: C, 65.93; H, 3.98; N, 15.38. Found: C, 66.08; H, 3.84; N, 15.49.

3-(1,3-dioxoisoindolin-2-yl)-*N*-((1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)benzamide (8d)

Off white solid; yield: 81%; mp = 184–186 °C; IR (KBr): 3368, 1722, 1650, 1585, 1485, 1380, and 1223 cm^{-1}. 1H NMR (500 MHz, CDCl3): 4.62 (brs, 2H), 5.60 (*s*, 2H), 6.97–6.99 (m, 3H), 7.19–7.21 (m, 2H), 7.50–7.52 (m, 4H), 7.77–7.86 (m, 4H), and 8.02–8.04 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): 33.2, 52.1, 115.85, 116.0, 123.7, 126.1, 127.3, 129.2, 129.6, 130.6, 131.3, 131.8, 134.4, 161.8, 163.9, and 166.8. MS (70 eV): m/z = 455.3 [M +]. Anal. Calcd for $C_{25}H_{18}FN_5O_3$: C, 65.93; H, 3.98; N, 15.38. Found: C, 65.86; H, 3.78; N, 13.23.

N-((1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-3-(1,3-dioxoisoindolin-2-yl)benzamide (8e)

Off white solid; yield: 82%; mp = 190–192 °C; IR (KBr): 3321, 1709, 1652, 1584, 1483, 1389, and 1115 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): 4.52 (brs, 2H), 5.45 (*s*, 2H), 7.19 (*d*, *J* = 7.5 Hz, 2H), 7.32 (*d*, *J* = 7.5 Hz, 2H), 7.53–7.58 (m, 3H), 7.78–7.79 (m, 4H), 7.89 (*s*, 1H), and 7.93–7.94 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): 35.0, 53.0, 117.3, 123.9, 125.3, 126.7, 129.3, 129.4, 129.6, 131.6, 132.1, 132.8, 134.2, 134.6, 135.1, 139.5, 162.1, 165.3, and 166.9. Anal. Calcd for $C_{25}H_{18}ClN_5O_3$: C, 63.63; H, 3.84; N, 14.84. Found: C, 63.81; H, 3.97; N, 14.72.

N-((1-(2,6-dichlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-3-(1,3-dioxoisoindolin-2-yl)benzamide (8f)

Off white solid; yield: 85%; mp = 201–203 °C; IR (KBr): 3322, 1727, 1647, 1540, 1436, and 1222 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): 4.69 (brs, 2H), 5.80 (*s*, 2H), 7.24–7.28 (m, 1H), 7.49–7.56 (m, 2H), 7.77–7.83 (m, 2H), 7.82 (*d*, *J* =

7.6 Hz, 1H), 7.88 (brs, 1H), and 7.91–7.93 (m, 2H).^{13}C NMR (125 MHz, $CDCl_3$): 36.4, 53.1, 123.9, 125.5, 126.9, 129.0 (2C), 129.4, 129.6, 129.9, 131.2, 131.6, 132.0, 133.4, 134.7, 135.3, 136.9, 166.4, and 167.0. Anal. Calcd for $C_{25}H_{17}Cl_2N_5O_3$: C, 59.30; H, 3.38; N, 13.83. Found: C, 59.44; H, 3.51; N, 13.69.

N-((1-(3-bromobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-3-(1,3-dioxoisoindolin-2-yl)benzamide (8g)

Off white solid; yield: 79%; mp = 146–148 °C; IR (KBr): 3321, 1722, 1639, 1585, 1483, 1389, and 1115 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): 4.67 (*s*, 2H), 5.42 (*s*, 2H), 7.14 (*d*, *J* = 7.5 Hz, 1H), 7.20 (t, *J* = 7.7 Hz, 1H), 7.39 (*s*, 1H), 7.43–7.44 (m, 2H), 7.51 (t, *J* = 7.7 Hz, 1H), 7.55 (*d*, *J* = 7.7 Hz, 1H), 7.61–7.63 (m, 1H), 7.77–7.78 (m, 2H), 7.81 (*d*, *J* = 7.2 Hz, 1H), 7.88 (*s*, 1H), and 7.90–7.92 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): 35.5, 53.5, 123.1, 123.9, 124.0, 125.4, 126.7, 126.9, 129.5, 129.7, 130,8, 131.2, 131.6, 132.02, 132.08, 134.7, 134.8, 135.2, 136.7, 166.5, and 167.0. MS (70 eV): m/z = 515.1 [M+]. Anal. Calcd for $C_{25}H_{18}BrN_5O_3$: C, 58.15; H, 3.51; N, 13.56. Found: C, 58.26; H, 3.62; N, 13.41.

N-((1-(4-bromobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-3-(1,3-dioxoisoindolin-2-yl)benzamide (8h)

Off white solid; yield: 78%; mp = 196–198 °C; IR (KBr): 3315, 1709, 1651, 1585, 1388, and 1115 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): 4.39 (brs, 2H), 5.44 (*s*, 2H), 7.12 (d, *J* = 7.8 Hz, 2H), 7.48 (d, *J* = 7.8 Hz, 2H), 7.55–7.59 (m, 2H), 7.79–7.80 (m, 3H), 7.80–7.82 (m, 2H), 7.88 (s, 1H), and 7.93–7.95 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): 33.9, 52.6, 123.0, 123.9, 125.3, 126.7, 129.4, 129.6, 129.8, 131.6, 132.1, 132.3, 134.5, 164.7, and 166.95 (2C). Anal. Calcd for $C_{25}H_{18}BrN_5O_3$: C, 58.15; H, 3.51; N, 13.56. Found: C, 58.27; H, 3.43; N, 13.68.

3-(1,3-dioxoisoindolin-2-yl)-*N*-((1-(2-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methyl)benzamide (8i)

Off white solid; yield: 62%; mp = 177–179 °C; IR (KBr): 3311, 1662, 1585, 1388, and 1112, 720 cm^{-1}.^{1}H NMR (500 MHz, $CDCl_3$): 4.74 (brs, 2H), 5.93 (*s*, 2H), 7.04 (brs, 1H), 7.50–7.55 (m, 4H), 7.77–7.90 (m, 6H), 8.11 (d, *J* = 7.3 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): 35.5, 51.6, 124.2, 125.8, 125.9 (2C), 127.1, 129.7, 130.0 (2C), 130.1, 130.5, 130.7, 131.9, 132.3, 134.8, 135.0, 135.3, 147.8, 166.7, and 167.3. Anal. Calcd for $C_{25}H_{18}N_6O_5$: C, 62.24; H, 3.76; N, 17.42. Found: C, 62.38; H, 3.59; N, 17.69.

3-(1,3-dioxoisoindolin-2-yl)-*N*-((1-(3-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methyl)benzamide (8j)

Off white solid; yield: 58%; mp = 110–112 °C; IR (KBr): 3342, 1721, 1649, 1585, 1382, 719 cm^{-1}.1H NMR (500 MHz, CDCl3): 4.72 (brs, 2H), 5.62 (*s*, 2H), 7.50–7.51 (m, 2H), 7.54–7.58 (m, 4H), 7.79–7.94 (m, 6H), and 8.14–8.19 (m, 2H). 13C NMR (125 MHz, CDCl3): 35.1, 51.4, 123.2, 123.9, 124.0, 125.4, 126.9 (2C), 129.6, 129.8, 130.4, 131.6 (2C), 132.1, 134.1, 134.8, 135.1, 136.5, 148.6, 166.5, and 167.1. Anal. Calcd for $C_{25}H_{18}N_6O_5$: C, 62.24; H, 3.76; N, 17.42. Found: C, 62.13; H, 3.89; N, 17.33.

3-(1,3-dioxoisoindolin-2-yl)-*N*-((1-(4-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methyl)benzamide (8k)

Off white solid; yield: 83%; mp = 199–201 °C; IR (KBr): 3309, 1711, 1648, 1594, 1520, 1386, 1114, and 717 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): 4.73 (brs, 2H), 5.62 (*s*, 2H), 7.16–7.18 (m, 1H), 7.40 (*d*, *J* = 7 Hz, 2H), 7.55–7.59 (m, 2H), 7.81–7.86 (m, 5H), 7.93–7.95 (m, 2H), and 8.21 (*d*, *J* = 7.0 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): 35.7, 53.9, 124.3, 124.7, 125.6, 127.0, 129.1, 129.9, 130.0, 131.9, 132.5, 135.0, 135.3, 141.7, 148.5 (2C), 166.8, 167.4, and 170.2. MS (70 eV): m/z = 482.6 [M+]. Anal. Calcd for $C_{25}H_{18}N_6O_5$: C, 62.24; H, 3.76; N, 17.42. Found: C, 62.11; H, 3.87; N, 17.57.

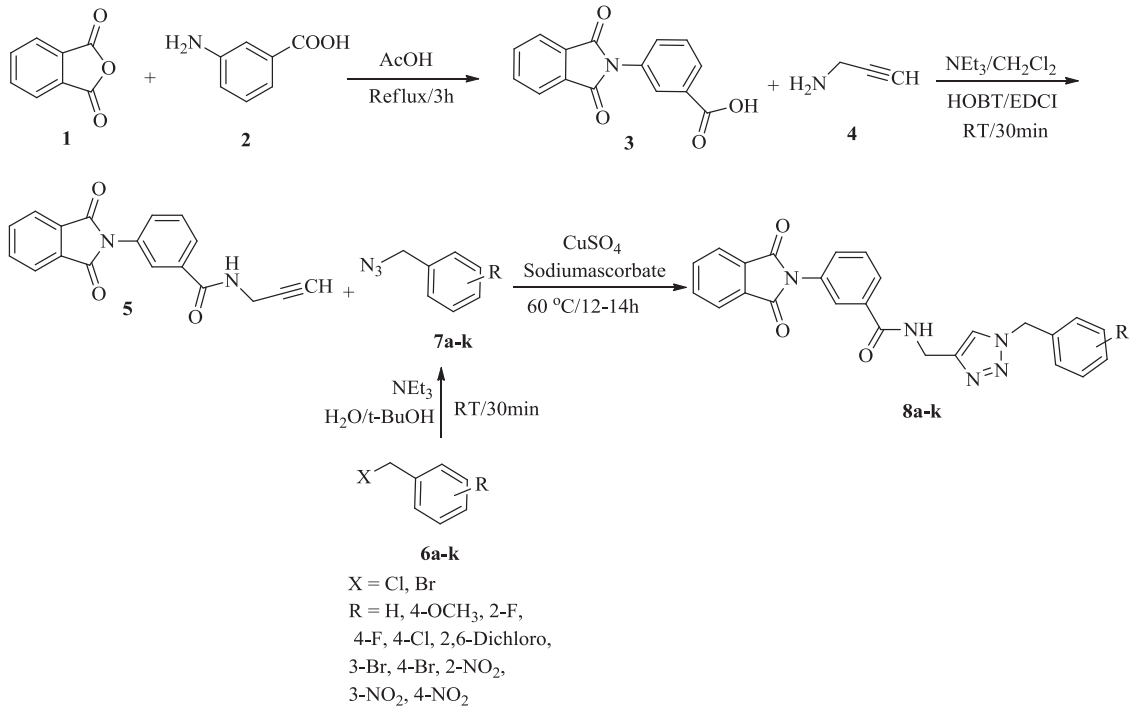
Biological evaluations

In vitro α-glucosidase inhibition of phthalimide-benzamide-1,2,3-triazoles **8a–k**, kinetic analysis of the most potent compound **8d**, and docking study of the standard drug and the most potent compounds **8d** and **8c** in the active site of α-glucosidase were performed exactly according to our previous work (Adib et al. 2018).

Results and discussion

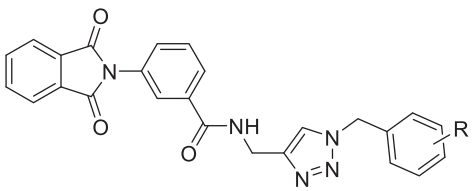
Chemistry

The synthesis route for the production of phthalimide-benzamide-1,2,3-triazoles **8a–k** has been depicted in Scheme 1. At first, phthalic anhydride **1** and 3-aminobenzoic acid **2** in acetic acid glycial were refluxed for 3 h to afford 3-(1,3-dioxoisoindolin-2-yl)benzoic acid **3**. The latter compound was converted to 3-(1,3-dioxoisoindolin-2-yl)-*N*-(prop-2-yn-1-yl) benzamide **5** in the presence of propargylamine **4**, HOBT/EDCI, and NEt_3 in anhydrous CH_2Cl_2 at room temperature.



Scheme 1 Synthesis of phthalimide-benzamide-1,2,3-triazoles **8a–k**

Table 1 In vitro α-glucosidase inhibitory activities of phthalimide-benzamide-1,2,3-triazoles **8a–k**



Compound	R	IC_{50} (μM)	Compound	R	IC_{50} (μM)[a]
8a	H	92.7 ± 1.6	**8g**	3-Br	89.5 ± 1.4
8b	4-OCH_3	131.3 ± 2.2	**8h**	4-Br	116.5 ± 2.0
8c	2-F	48.7 ± 0.8	**8i**	2-NO_2	54.1 ± 0.9
8d	4-F	40.5 ± 0.6	**8j**	3-NO_2	68.3 ± 1.0
8e	4-Cl	83.7 ± 1.2	**8k**	4-NO_2	77.5 ± 1.1
8f	2,6-Dichloro	109.6 ± 1.9	Acarbose	–	750.0 ± 10.0

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

Compound **5** was an appropriate prone to be participated in click reaction in order to construct 1,2,3-triazole ring (Kolb et al. 2001). For this purpose, different benzyl chloride/bromide derivatives **6a–k** and sodium azide reacted in the presence NEt_3 in the mixture of H_2O and t-BuOH (1:1) at room temperature to afford the fresh azide derivatives **7a–k**. Finally, compound **5**, $CuSO_4.5H_2O$, and sodium ascorbate were added to the azide derivatives **7a–k** at room temperature for 12–14 h at 60 °C to give the corresponding products **8a–k**.

Pharmacology

In vitro α-glucosidase inhibitory activity

Anti-α-glucosidase activity of phthalimide-benzamide-1,2,3-triazoles **8a–k** were screened by using α-glucosidase isolated from *Saccharomyces cerevisiae* (yeast). The obtained results demonstrated that all the title compounds exhibited excellent activity against α-glucosidase. These compounds were

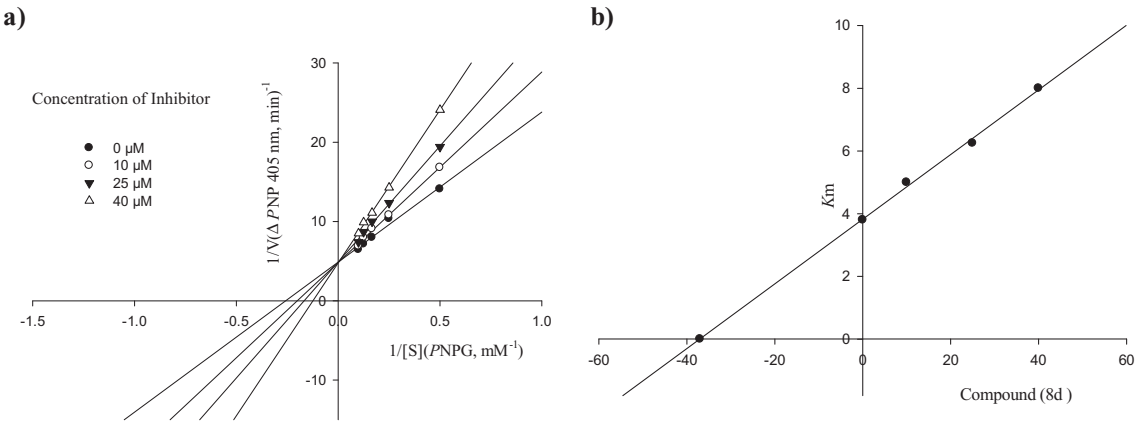


Fig. 3 **a** Lineweaver–Burk plots for the inhibition of α-glucosidase by compound **8d** and (**b**) the secondary plot between K_m and various concentrations of compound **8d**

around 18.5–5.7 (range of IC_{50} values = 40.5 ± 0.6–131.3 ± 2.2 μM) folds more potent than standard drug acarbose (IC_{50} = 750.0 ± 10.0 μM). As can be seen the chemistry section, in order to achieve optimal α-glucosidase inhibitor, we altered the substituents on the pendant phenyl ring.

As can be seen in the Table 1, among the synthesized compounds, the most potent compound was 4-fluoro derivative **8d** (IC_{50} = 40.5 ± 0.6 μM). Changing the position of the fluorine atom in the phenyl ring from 4-position to 2-position, as in compound **8c** (the second most potent compound with IC_{50} = 48.7 ± 0.8 μM), slightly diminished the inhibitory activity. Moreover, inhibitory activities of 4-substituted derivatives and un-substituted compound demonstrated that 4-fluoro derivative **8d** (IC_{50} = 40.5 ± 0.6 μM), 4-nitro derivative **8k** (IC_{50} = 77.5 ± 1.1 μM), and 4-chloro derivative **8e** (IC_{50} = 83.7 ± 1.2 μM) with strong electron withdrawing substituents have more inhibitory activity in comparison with compound **8a** (IC_{50} = 92.7 ± 1.6 μM) with un-substituted phenyl ring, compound **8 h** (IC_{50} = 116.5 ± 2.0 μM) with weak electron withdrawing substituent Br, and compound **8b** (IC_{50} = 131.3 ± 2.2 μM) with strong electron donating substituent methoxy (order of activity: F > NO_2 > Cl > H > Br > OCH_3). 4-Methoxy derivative **8b** also was the less active compound among the synthesized compounds. The tired most potent compound was 2-NO_2 derivative **8i** (IC_{50} = 54.1 ± 0.9 μM). Movement of NO_2 group of 2-position to 3 or 4-position led to decrease in inhibitory activities as observed in the compounds **8j** (IC_{50} = 68.3 ± 1.0 μM) and **8k** (IC_{50} = 77.5 ± 1.1 μM), respectively. It is worthy to note that in the cases of 3-nitro derivative **8j** (IC_{50} = 68.3 ± 1.0 μM) and 3-bromo derivative **8g** (IC_{50} = 89.5 ± 1.4 μM), compound **8j** with stronger electron withdrawing substituent has more inhibitory effect than compound with weaker electron withdrawing substituent (compound **8j** vs. compound **8g**). This pattern was also observed in the 4-substituted derivatives. The less active compounds among the halogenated derivatives were 2,6-dichloro derivative **8f** (IC_{50} = 109.6 ± 1.9 μM) and 4-bromo derivative **8h** (IC_{50} = 116.5 ± 2.0 μM).

Kinetic study

The kinetic analysis of α-glucosidase inhibition by the most potent compound **8d** was performed in order to determine the mechanism of inhibition and K_i value of this compound. As can be seen in Fig. 3a, with increasing the concentration of inhibitor (compound **8d**), the value of V_{max} remained constant and the value of K_m increased. This finding indicated that compound **8d** acted as competitive inhibitor of α-glucosidase. Moreover, the plot of K_m versus different concentrations of inhibitor gave an estimate of K_i (Inhibition constant) value of 37 μM for compound **8d** (Fig. 3b).

Docking study

In order to gain further insight into the inhibitory activities of the synthesized compounds against α-glucosidase, we investigated the binding modes of the standard drug acarbose and the most potent compounds **8d** and **8c** in the active site of target enzyme by using docking calculations (Adib et al. 2018). Figure 4 shows the binding mode between acarbose and α-glucosidase. This drug interacted with the residues Ser308, Glu304, Thr307, Val305, Thr301, Gln322, Arg312, His279, His239, and Asn241.

4-Fluoro and 3-Fluoro derivatives **8d** and **8c** were found to be more active than other synthesized inhibitors. The superposed structure of acarbose and the most potent compound **8d** revealed that the latter compound as well fitted in the active site of α-glucosidase (Fig. 5).

As can be seen in Fig. 6a, pendant 4-fluorophenyl moiety of compound **8d** interacted with Arg312,

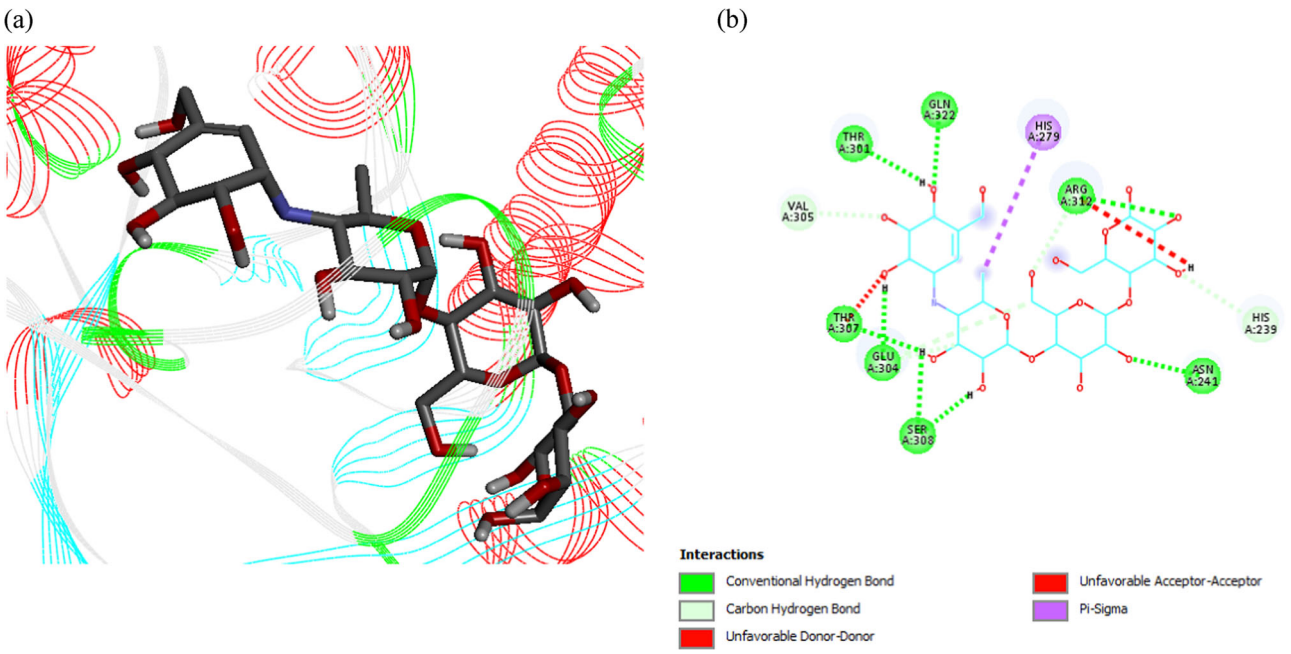


Fig. 4 (**a**) The 3D and (**b**) 2D predicted binding modes of acarbose in the active site pocket

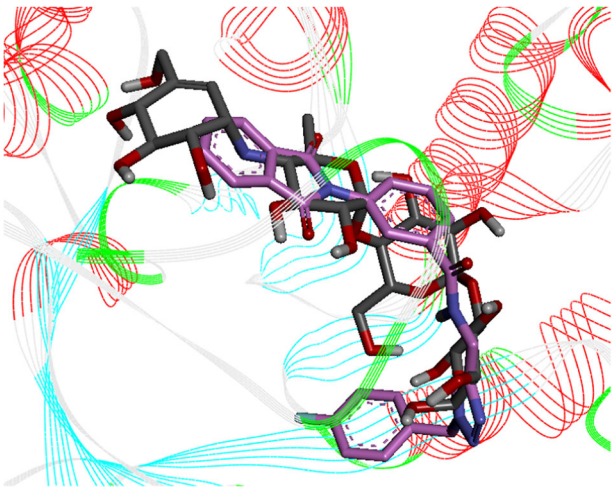


Fig. 5 Acarbose (gray) and the most potent compound **8d** (pink) superimposed in the active site pocket

Asp349, and Gln350 through fluoro substituent and Arg439 through phenyl ring. Residue Arg312 also formed a hydrophobic interaction with 1,2,3-triazole ring of compound **8d**. The latter ring also established a π–lone pair interaction with Phe157. Carbonyl unit of benzamide moiety formed a hydrogen bond with His239. Furthermore, phthalimide moiety of compound **8d** created the following interactions with active site: a π–anion interaction with Glu304 and two hydrophobic interactions with Pro309.

2-Fluoro substituent of pendant phenyl moiety in the second most potent **8c** interacted with Phe157, Asp408, and Asn412 and phenyl ring of this moiety formed a hydrophobic interaction with Arg312 (Fig. 6b). The latter amino acid also interacted with 1,2,3-triazole ring via a hydrogen bond and a hydrophobic interaction. Phenyl ring of benzamide moiety of compound **8c** established a π–π interaction with His279 and a π–anion interaction with Glu304. Furthermore, Glu304 also formed a π–anion interaction with phthalimide ring.

Calculation of binding energies of acarbose and the most active compounds **8d** and **8c** predicted that our new compounds with binding energies −9.17 and −9.12 can be attached to active site of α-glucosidase easier than standard drug acarbase with binding energies = −4.04 kcal/mol. These results are in agreement with in vitro α-glucosidase inhibition evaluation (Table 1).

Conclusion

In this paper, we combined phthalimide and 1,2,3-triazole derivatives by benzamide linker to design new potent α-glucosidase inhibitors. Designed phthalimide-benzamide-1,2,3-triazole hybrids **8a–k** were synthesized by click reaction and evaluated again yeast α-glucosidase. Obtained results demonstrated that all the title compounds were more potent than standard inhibitor and the most potent compound **8d** was a competitive inhibitor into α-glucosidase. Furthermore, docking study was performed

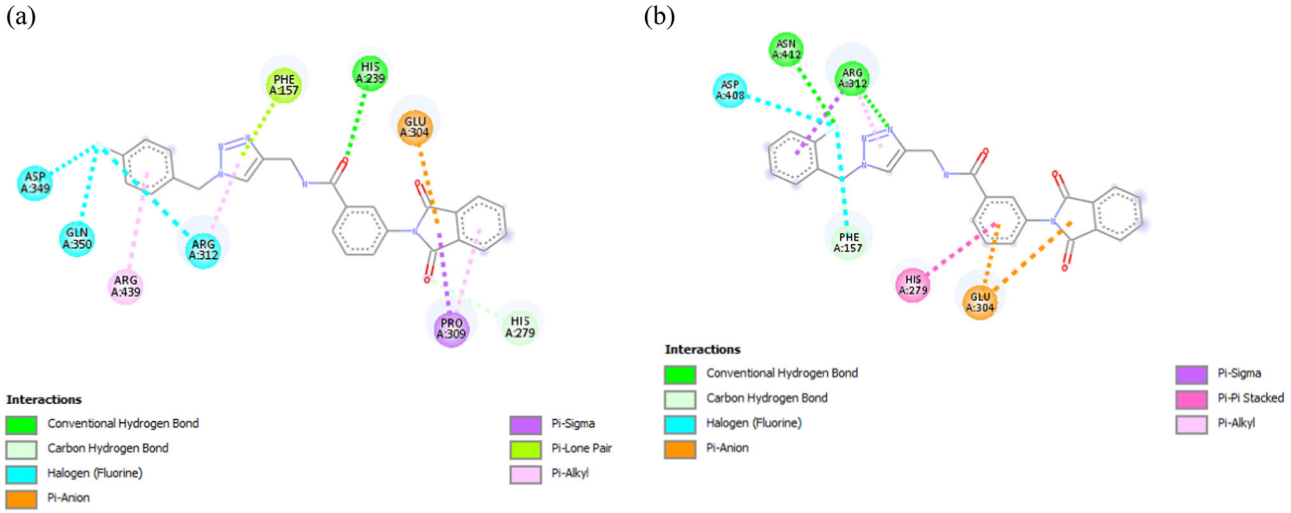


Fig. 6 Predicted binding modes of compounds (**a**) **8d** and (**b**) **8c** in the active site pocket

in order to predict possible interaction modes and binding energies of the standard drug and the most potent compounds in the active site of α-glucosidase.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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