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Synthesis, *in vitro* evaluation, and molecular docking studies of novel hydrazineylideneindolinone linked to phenoxymethyl-1,2,3-triazole derivatives as potential α-glucosidase inhibitors

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ABSTRACT

In this work, a novel series of hydrazineylideneindolinone linked to phenoxymethyl-1,2,3-triazole derivatives were designed, synthesized, and evaluated for their anti-α-glucosidase activity due to an urgent need to develop effective anti-diabetic agents. Among tested 15 compounds, 8 derivatives (**9a**, **9b**, **9c**, **9d, 9e, 9f, 9h,** and **9o)** demonstrated superior potency compared to that of positive control, acarbose. Particularly, compound **9d** possessed the best anti-α-glucosidase activity with around a 46-fold improvement in the inhibitory activity. Additionally, **9d** showed a competitive type of inhibition in the kinetic study and the molecular docking study demonstrated that it well occupied the binding pocket of the catalytic center through desired interactions with residues, correlating to the experimental results.

1. Introduction

Diabetes mellitus (DM) is one of the most health problems of the present era, affecting millions of people worldwide [1]. According to the World Health Organization (WHO), more than 420 million people suffer from diabetes all around the world and the number would be expected to increase to 642 million in 2040 [2]. DM commonly referred as diabetes, is a group of metabolic disorders of carbohydrate metabolism characterized by the uncontrolled and inappropriately high blood sugar (hyperglycemia) [3]. Diabetes is classified as type I (insulin-dependent), type II (noninsulin-dependent), gestational, and other uncommon diabete types based on its pathogenesis [4,5]; however type II is the most common form of DM, accounting for around 90%- 95% of all diabetes cases [6]. Current therapeutic approaches to treat type II DM include α-glucosidase inhibitors, glucagon-like peptide-1 agonists, and dipeptidyl peptidase-IV (DPP-IV) inhibitors [7]. α-Glucosidase (EC 3.2.1.20) is the enzyme, located in the brush border of the small intestine and cleave α(1 → 4) bonds from the non-reducing side in carbohydrates. This process produces absorbable monosaccharides (single α-glucose) which increase plasma glucose levels and postprandial hyperglycemia [8]. Evidence has shown that α-glucosidase enzyme is a promising therapeutic target against type II DM and its complications especially obesity, neuropathy, and vascular diseases [9] due to its high efficacy in decreasing fasting and postprandial insulin levels with limiting the systemic side effects.

In the past few decades, various natural, semisynthetic, and synthetic α-glucosidase inhibitors have been reported with sugar mimic [10], chalcone [11,12], alkaloid [13,14], xanthone[15], azole [16], steroid [17,18], and peptide structure [7]. Currently, acarbose, miglitol, voglibose, and emiglitate are four α-glucosidase inhibitors prescribed as drugs [15]. Acarbose, an oral anti-diabetic drug launched in 1990 is currently used in the treatment of type II DM [19]. However, various side effects including flatulence and abdominal pain are related to acarbose. With the drastic increase in the incidence and prevalence of DM without significant progress in the field of anti-diabetic drug developments, there is an urgent need to discover new efficient and potent

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α-glucosidase inhibitors.

Isatin is one of the most important heterocycles having various biological and pharmacological activities including antimicrobial [20], antiviral [21], anticonvulsant [22], anticancer [23,24], and anti-diabetic [25,26] properties. Isatin has attracted much attention as a core structure in targeting α-glucosidase [25,27]. Various types of synthetic isatin-based α-glucosidase inhibitors have been recently introduced [25,28–30]. 1,2,3-triazole ring has also been fully developed in the field of medicinal chemistry and drug development by the introduction of click reaction. Specifically, promising anti-α-glucosidase potency has reported for derivatives bearing 1,2,3-triazole moiety [31–34]. On the other hand, the 1,2,3-triazole ring is constructed *via* multicomponent azide-alkyne click reaction which permits the synthesis of adducts in a time and cost-effective manner.

In continuation of our ongoing research on designing novel compounds with bioactivity against α-glucosidase [8,35]; herein, we report the design and synthesis of new hydrazineylideneindolinone linked to different phenoxymethyl-1,2,3-triazole derivatives. In the hybrid series **9a-o**, various electronic and lipophilic environments were introduced to study their impact on the *in vitro* inhibitory activity. In addition, kinetic evaluation as well as *in silico* α-glucosidase inhibitory potentials were performed.

2. Results and discussion

2.1. Designing

Isatin (indole-2,3-dione) is known as an important biological and pharmacological heterocyclic system with two cyclic rings. The six-membered ring has an aromatic character, whereas the five-membered one possesses an anti-aromatic character [36]. Recent studies showed that isatin has attracted much attention as a core structure in targeting α-glucosidase [25]. Various types of synthetic isatin-based α-glucosidase inhibitors were introduced [25,28,29]. Rahim et al. reported isatin schiff base derivatives (compound **A**, Fig. 1.) as a strong α-glucosidase inhibitor with IC_{50} = 2.2 ± 0.25 μM for the most active analog among the series. From the docking viewpoint, it can be seen that compound **A** fits well into the catalytic active site making interactions with the Arg212, His239, Glu276 and Thr307 [27]. Recently, chromone-isatin derivative as a promising α-glucosidase inhibitor was synthesized. Based on SAR evaluations, compound **B** (Fig. 1) was regarded as the most potent compound with an IC_{50} value of 3.18 ± 0.12 μM due to the presence of 4-bromobenzyl pendant group [37].

The recent findings on α-glucosidase inhibitors showed that phenoxy linked to 1,2,3-triazole could effectively inhibit the enzyme [32,38]. In particular, the modified phenoxy-1,2,3-triazole series reversibly inhibited the α-glucosidase enzyme in a competitive manner without toxicity towards normal fibroblast cells. The IC_{50} values were ranging from 13.0 to 75.5 ± 7.0 μM (compound **C** as the most potent compound was depicted in Fig. 1) [39]. Compound **D** (Fig. 1) as the hybrid derivatives of phenoxy-benzimidazole and aryl-1,2,3-triazole exhibited much higher inhibitory activity than its parental compounds with outstanding potential [31].

From the synthetic point of view, isatine was used as an initiator with excellent α-glucosidase inhibitory potency. Based on the previous study, we also examined the introduction of the aromatic bromobenzyl group into the X position of the isatin ring to evaluate the inhibitory activities. On the other hand, different Y-substituted phenoxymethyl-1,2,3-triazole was applied in the central core of the molecule as a suitable electron-rich moiety with the potential to participate in the interactions with the active site. Derivatization of the hybridized backbone was performed at the Z position through a multi-component click reaction. Therefore, it seems that hybridization of these three scaffolds in a single molecule can improve pharmacological activity. In this direction, a novel series of hydrazineylideneindolinone linked to phenoxymethyl-1,2,3-triazoles were rationally designed, synthesized, and evaluated as α-glucosidase inhibitors. Kinetic study as well as *in silico* evaluations were performed on the synthesized hybrids.

2.2. Chemistry

The synthesis of compounds **9** has been schematically shown in Scheme 1.

Isatin or it 4-bromobenzyl derivative **1** reacted with hydrazine hydrate **2** in methanol under reflux conditions to obtain hydrazineylideneindolinone derivative **3**. Then, compound **3** reacted with aldehyde **4** in the presence of catalytic amounts of SiO_2-H_2SO_4 or acetic acid (HOAc) under microwave irradiation (700 W) for 10–12 min to prepare prop-2-yn-1-yloxybenzylidene-hydrazineylideneindolinone derivatives **5**. Finally, click reaction of compound **5** and azides **8** (prepared by the reaction of benzyl chloride/bromide derivative **6** and sodium azide **7**) led to the formation of products **9**. The structure of all compounds was confirmed using NMR and IR spectroscopy as well as elemental analysis. NMR characterization indicated the presence of two conformers *anti* and *syn* due to hydrazone moiety.

2.3. In vitro α-glucosidase enzymatic assay

The results of the α-glucosidase inhibitory assay were shown in Table 1. In this series, compound **9d** bearing *ortho*-fluorobenzyl showed exceptionally high potency against α-glucosidase with an IC_{50} value of 16.43 μM which is significantly lower than that of acarbose as the positive control (IC_{50} = 750.0 ± 10.0 μM). Based on biological results, the structure–activity relationship (SAR) was constructed for the designed

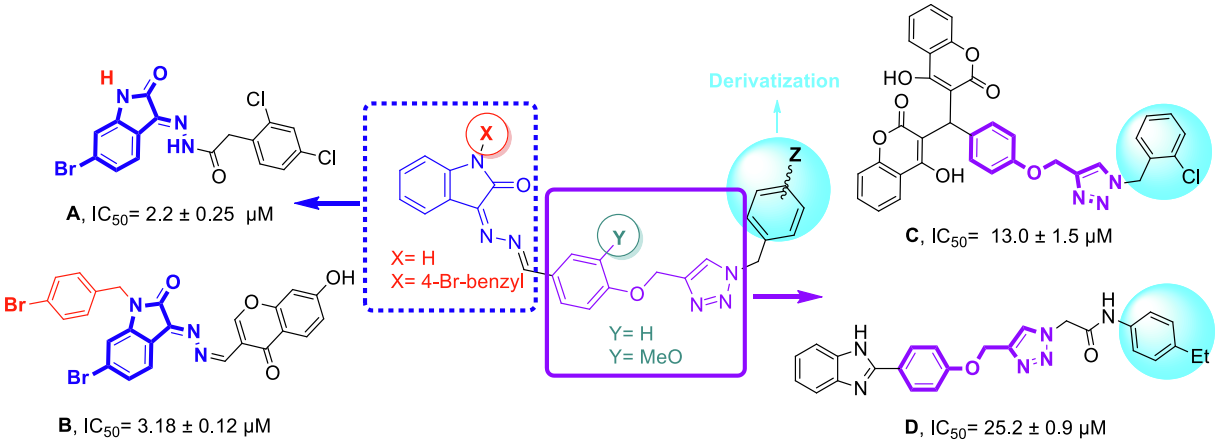
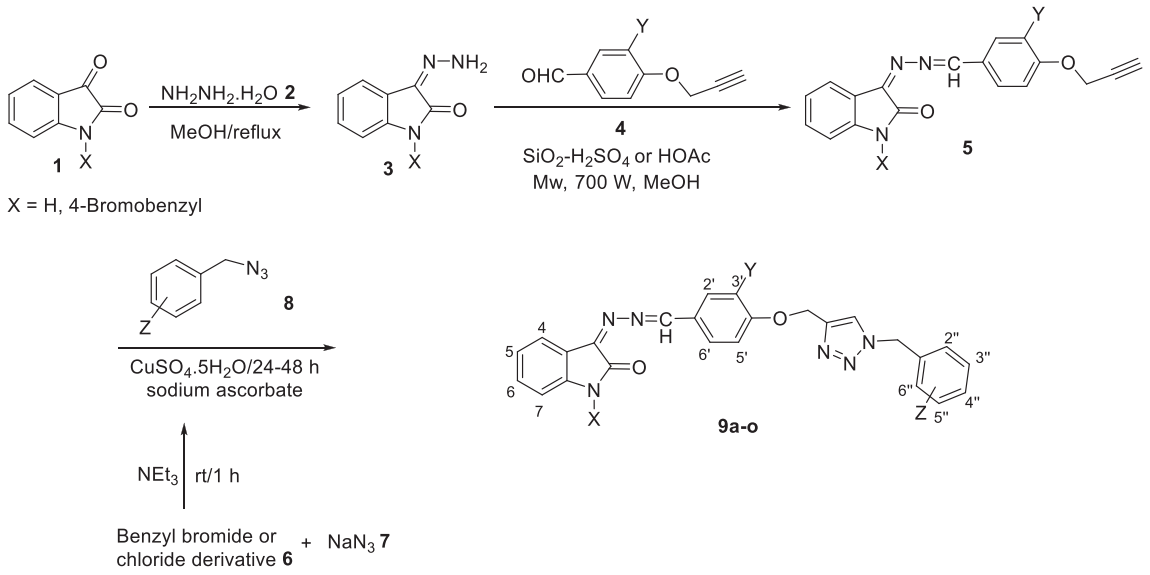
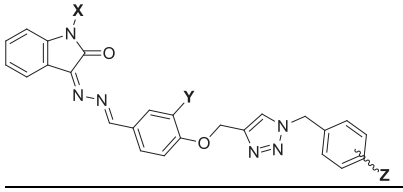


Fig. 1. Novel hydrazineylideneindolinone linked to phenoxymethyl-1,2,3-triazoles by hybridization of isatine and phenoxymethyl-1,2,3-triazole.



Scheme 1. Synthesis of compounds **9a-o**.

Table 1
α-Glucosidase inhibitory activity of compounds **9a-o.**



Entry	Compound	X	Y	Z	IC_{50} (μM) [a]
1	**9a**	H	H	H	100.47 ± 0.22
2	**9b**	H	H	2-Me	96.03 ± 0.06
3	**9c**	H	H	4-Me	43.51 ± 0.15
4	**9d**	H	H	2-F	16.43 ± 0.01
5	**9e**	H	H	4-F	86.8 ± 0.12
6	**9f**	H	OMe	H	215.42 ± 0.08
7	**9 g**	H	OMe	2-Me	>750
8	**9 h**	H	OMe	4-Me	110.59 ± 0.44
9	**9i**	H	OMe	2-F	>750
10	**9j**	H	OMe	4-F	>750
11	**9 k**	4-Br-C_6H_4-CH_2	H	H	>750
12	**9 l**	4-Br-C_6H_4-CH_2	H	2-Me	>750
13	**9 m**	4-Br-C_6H_4-CH_2	H	4-Me	>750
14	**9n**	4-Br-C_6H_4-CH_2	H	2-F	>750
15	**9o**	4-Br-C_6H_4-CH_2	H	4-F	96.89 ± 0.26
16	Acarbose				750.0 ± 10.0

[a] Data represented in terms of mean ± SD.

scaffold. In order to better understand the SAR, synthetic compounds were divided into three groups.

3. Evaluating the effect of Z moiety on hydrazineylideneindolinone derivatives (X = H and Y = H)

In cases of **9a-e**, it can be seen that the unsubstituted compound (**9a**) demonstrated an IC_{50} value of 100.47 μM with around 7 times improvement in potency compared to that of acarbose as a positive control. As can be seen in compounds **9b** (Z = 2-Me) and **9c** (Z = 4-Me), the introduction of the methyl group as electron-donating moiety had a positive effect on α-glucosidase inhibitory activity compared with the unsubstituted one. Substitution of small electron-withdrawing groups such as fluoro at Z played a significant role in α-glucosidase inhibition. In detail, **9d** as the top potent α-glucosidase inhibitor generated *via* introducing 2-F into the benzyl moiety at Z position showed an IC_{50} value of 16.43 μM followed by **9e** (Z = 4-F) with an IC_{50} value of 86.8 ± 0.12 μM.

4. Evaluating the effect of Z moiety on hydrazineylideneindolinone derivatives (X = H and Y = OMe)

From the screening data of **9f-j**, it was revealed that **9f** as unsubstituted derivative in this set might be considered as the moderate α-glucosidase inhibitor with IC_{50} = 215.42 ± 0.08 μM. Although the presence of *para* methyl as an electron-donating group (**9 h,** IC_{50} = 110.59 ± 0.44 μM) led to around two-fold improvement of α-glucosidase inhibitory activity comparing with un-substituted one (**9f**), the inhibitory potency was not improved in **9g** bearing *ortho* methyl group (IC_{50} > 750 μM). Unexpectedly, there was a dramatic reduction in α-glucosidase inhibitory activity *via* introducing fluorobenzyl moiety as an electron-withdrawing group at Z (**9i,** Z = 2-F with IC_{50} > 750 and **9j,** Z = 4-F with IC_{50} > 750).

5. Evaluating the effect of Z moiety on 4-bromobenzyl-hydrazineylideneindolinone derivatives (X = 4-bromobenzyl and Y = H)

To further improve anti-α-glucosidase activity, the impact of 4-bromobenzyl substitution on the indolin-2-one at X position was explored (**9 k-o**). Disappointingly, **9k** as the un-substituted derivative in this set did not improve potency compared to **9a** and **9f** or even the positive control. Also, methyl substitutions on the benzyl ring (**9l** Z = 2-Me with IC_{50} > 750 and **9m** Z = 4-Me with IC_{50} > 750) displayed no α-glucosidase inhibitory property in the whole range of concentrations studied. Investigation of the fluorinated derivatives showed that unlike the other sets, in this category 4-F (**9o,** Z = 4-F with IC_{50} = 96.89 ± 0.26 μM) comparing with that of 2-F (**9n**, Z = 2-F with IC_{50} > 750 μM) at Z position led to an improvement of α-glucosidase inhibitory activity.

6. SAR assessments regarding the potential of all sets it can be understood that

- In most cases, the target hydrazonoindolin-2-one derivatives had superior inhibitory activity toward α-glucosidase compared to 4-bromobenzyl-hydrazonoindolin-2-one counterpart. This may be due to the bulkiness of the bromobenzyl on hydrazonoindolin-2-one ring which cannot properly enter the active site and hinders the interactions with the residues of the mentioned area.

- The substitution of MeO as a bulky electron donating group at Y position led to decrease in the potency comparing with the unsubstituted counterpart. This trend can be seen in compound **9f** (IC_{50} = 215.42 ± 0.08 μM) and **9a** (IC_{50} = 100.47 ± 0.22 μM) or **9j** (IC_{50} > 750) and **9d** (IC_{50} = 215.42 ± 0.08 μM).
- Mostly, introduction of *para* methyl group into the benzyl pendant at Z position compared to *ortho* methyl had a positive effect on anti-α-glucosidase activity.
- Overall, it seems that compounds possessing fluoride at Z induced better α-glucosidase inhibitory activity compared to other derivatives. In another study done by Xie et al, the same trend was observed so that among different derivatives, the derivative possessing 2-fluorobenzyl substituent displayed the highest inhibitory activity [40].

The summary of the SAR to improved α-glucosidase inhibitory activity was depicted in Fig. 2.

6.1. Enzyme kinetic studies

According to results obtained from the kinetic study of compound **9d** and the related Lineweaver-Burk plot (Fig. 3), the K_m gradually increased and V_{max} remained unchanged with increasing inhibitor concentration which indicated a competitive inhibition. The results showed that compound **9d** bound to the active site of the enzyme and competed with the substrate for binding to the active site. Furthermore, the plot of the K_m versus different concentrations of inhibitor gave an estimate of the inhibition constant, K_i of 16.33 μM (Fig. 3).

6.2. Docking analyses

The docking analysis of **9d** as the most active and **9n** as the least potent compounds against the α-glucosidase enzyme has been achieved using Gold software with ChemScore fitness function. To evaluate the accuracy of docking, the redocking process of acarbose (as a crystallographic ligand) with human lysosomal acid-alpha-glucosidase (PDB ID: 5NN8) was performed. Alignment of the best pose of acarbose in the active site of α-glucosidase and crystallographic ligand was shown in Fig. 4. Both shared a common binding mode which confirming the reliability and accuracy of the used method for *in silico* studies. Next, the docking assessments of the two mentioned compounds were done based on the same protocol performed on the standard drug. The ChemScore fitness value of **9d** and **9n** plus their interactions with residues in the α-glucosidase active site were documented in Table 2.

Fig. 5 showed the docking interactions of the most potent compound **9d** (IC_{50} = 16.43 ± 0.01 μM). Docking evaluation depicted two pi-pi, two pi-alkyl, and one pi-sulfur interaction with the indolinone ring as well as two hydrogen bound interactions between Asp518 and Met519 with NH of indolinone. Another hydrogen bonding interaction was generated between C═O of the indolinone ring and Arg600. On the other side of the molecule, 2-fluorobenzyl made a pi-pi stacking with Phe525 amino acid. The 1,2,3-triazole ring also constructed two pi-alkyl interactions with leu283 and Ala555 plus pi-sulfur interaction with Asp282. Another hydrogen bonding and pi-alkyl interactions were generated between phenoxy moiety and Ala284.

3D interaction pattern of compound **9n** (Fig. 6) showed two pi-alkyl interactions between Leu766 and 4-bromobenzyl group. The mentioned ring also participated in pi-pi interactions with Trp376. The indolinone fused ring made two pi-pi, two pi-sulfur, and one pi-anion interactions with Trp481, Met519, and Asp616, respectively. Terminal 2-fluorobenzyl participated in pi-pi interactions with Arg527 and Ala555. Also, the phenoxy linker was fixed through pi-anion interaction with Asp282. Although this compound showed interactions within the α-glucosidase enzyme, low ChemScore value and no hydrogen bond interactions between **9n** and enzyme could justify its low potency.

7. Conclusion

Following our expertise in the rational design of α-glucosidase inhibitors [35,41]; herein, we designed, synthesized, and evaluated highly potent α-glucosidase inhibitors from the hydrazineylideneindolinone linked to phenoxymethyl-1,2,3-triazoles. In this regard, 15 derivatives were designed and synthesized. The SAR data obtained by the anti-α-glucosidase screening of synthesized compounds showed a promising inhibitory effect of derivatives containing hydrazineylideneindolinone moiety comparing with 4-bromobenzyl-hydrazineylideneindolinone counterparts. The most active compound **9d** bearing 2-fluorobenzyl group (IC_{50} = 16.43 ± 0.01 μM) showed 47 times better inhibitory effect than acarbose. The gold score determined *via in silico* study for the designed compounds were strongly correlated with the experimentally α-glucosidase inhibitory activity. Molecular docking studies of **9d** into α-glucosidase active site revealed that this molecule completely fit into the α-glucosidase pockets and formed hydrogen bond, pi-pi, pi-aryl, pi-anion, and pi-sulfur interactions. Thus, the tested hybrid appears as a good candidate for initiating lead drug discovery program.

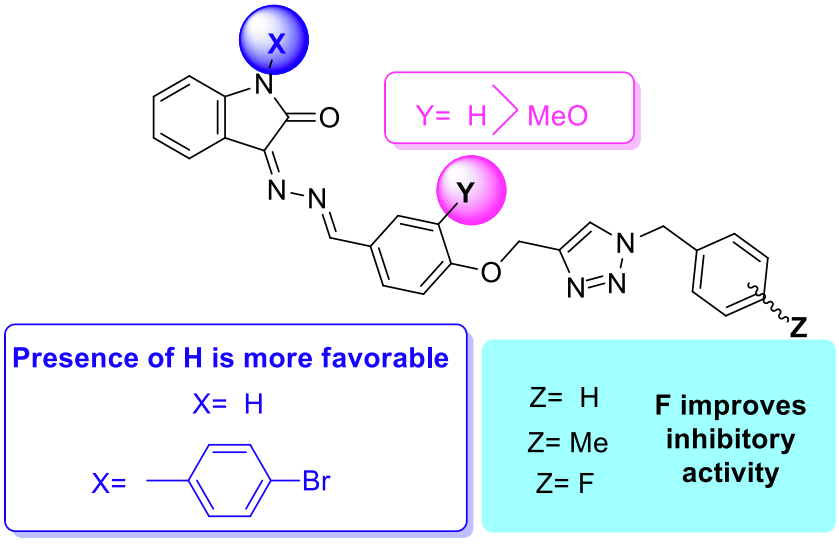


Fig. 2. Summary of the SAR for the indolinone substituted phenoxy-methyltriazole derivatives.

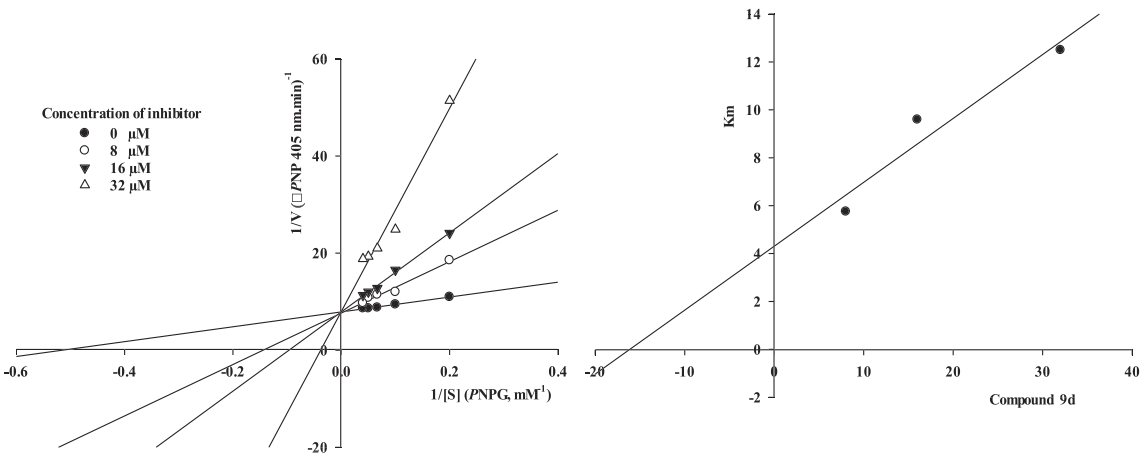


Fig. 3. Kinetic study of inhibitor **9d** against α-glucosidase. Lineweaver-Burk plot (left) and double reciprocal Lineweaver-Burk plot (right) are shown.

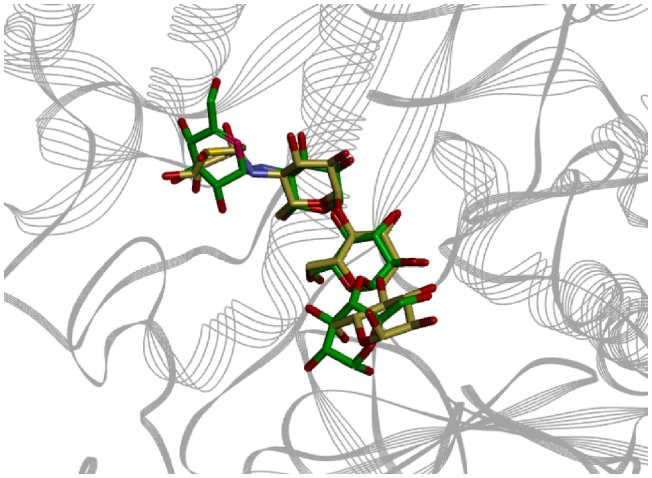


Fig. 4. Depiction of crystallographic ligand (yellow) and docked acarbose (green) in the active site of α-glucosidase enzyme. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Docking scores and interactions of compounds against the α-glucosidase (PDB ID: 5NN8

Compound	ChemScore	Type of Interactions	Amino acid
9d	71.46	Hydrogen bond	Ala284
		Hydrogen bond	Arg600
		Hydrogen bond	Met519
		Hydrogen bond	Asp518
		pi-pi	Phe525
		pi-pi	Trp481
		pi-pi	Trp481
		pi-alkyl	Ala555
		pi-alkyl	Leu283
		pi-alkyl	Ala284
		pi-alkyl	Met519
		pi-alkyl	Leu405
		pi-anion	Asp282
		pi-sulfur	Met519
9*n*	55.20	pi-pi	Trp481
		pi-pi	Trp481
		pi-pi	Trp376
		pi-alkyl	Ala555
		pi-alkyl	Arg527
		pi-alkyl	Leu677
		pi-alky	Trp376
		pi-anion	Asp282
		pi-anion	Asp616
		pi-anion	Met519

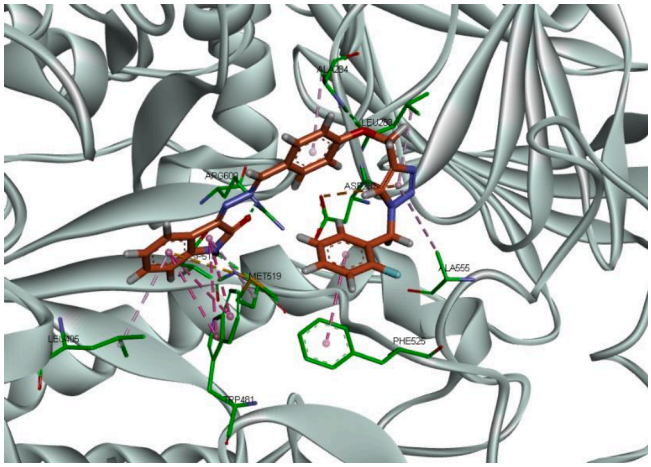


Fig. 5. 3D interaction pattern of compounds **9d** in the α-glucosidase active site.

8. Experimental

8.1. Chemistry

8.1.1. Synthesis of hydrazineylideneindolinone derivatives *3*

A mixture of isatine or its derivative **1** (1 mmol) and hydrazine hydrate **2** (1 mmol) in methanol (10 mL) was refluxed for 4 h. After completion of reaction (checked by TLC), the precipitated product was filtered off, washed with cold methanol, and used for further reaction.

8.1.2. Synthesis of prop-2-yn-1-yloxybenzylidene-hydrazineylideneindolinone derivatives *5*

A mixture of compound **3** (1 mmol) and aldehyde derivative **4** [42] (1 mmol) in methanol (10 mL), in the presence of catalytic amounts of SiO_2-H_2SO_4 [43] was reacted under microwave irradiation at 700 W for 10–12 min (1 min interval). After completion of the reaction (checked by TLC), the catalyst was filtered off, the filtrate let to be cooled down, and the precipitates were filtered off to afford compound **5**. It should be noted that the reaction could be achieved in the presence of a few drops of acetic acid.

*Synthesis of compounds **9***: Synthesis of the title compounds **9** was achieved by the click reaction of compound **5** and *in situ* prepared azides **8**. The reaction was initiated by stirring the mixture of benzyl chloride/

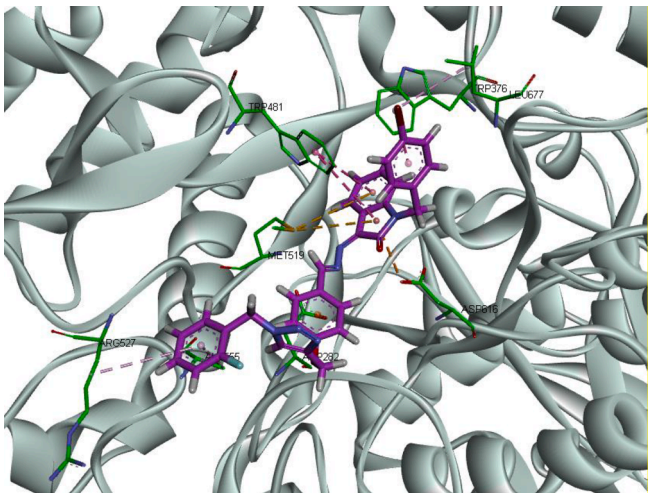


Fig. 6. 3D interaction pattern of compound **9n** in the active site of the α-glucosidase enzyme.

bromide derivative **6** (1.1 mmol) and sodium azide **7** (0.06 g, 0.9 mmol) in the presence of triethylamine (0.13 g, 1.3 mmol) in water (4 mL) and *tert*-butyl alcohol (4 mL) at room temperature for 30 min. Next, compound **5** (0.5 mmol) and $CuSO_4{\cdot}5H_2O$ (7 mol%) were added to the reaction mixture and it was continued for 24–48 h. After completion of the reaction as checked by TLC, the mixture was poured on crushed ice, the precipitates were filtered off and washed with water. In the case of some compounds, they were purified using plate chromatography eluting with ethyl acetate and petroleum ether.

8.1.3. 3-((4-((1-Benzyl-1H-1,2,3-triazol-4-yl)methoxy)benzylidene) hydrazineylidene)indolin-2-one ***9a***

Yield: 55%, mp = 153–155 °C. IR (KBr): 3394, 1732, 2825, 1606, 1537, 1508 cm^{-1}. ^{1}HNMR (500 MHz, DMSO-d_6) (two isomers): 10.83 (s, 1H, NH), 8.64 (s, 1H, CH), 8.34 (s, 1H, triazole), 8.04 (d, J = 7.5 Hz, 1H, H4), 7.96 (d, J = 9.0 Hz, 2H, H2′, H6′), 7.38–7.33 (m, 6H, H6, H2″, H3″, H4″, H5″, H6″), 7.23 (d, J = 9.0 Hz, 2H, H3′, H5′), 7.04 (t, J = 7.5 Hz, 1H, H5), 6.90 (d, J = 7.5 Hz, 1H, H7), 5.63 (s, 2H, CH_2), 5.27 (s, 2H, CH_2), 5.22 (s, 2H, CH_2) ppm. ^{13}CNMR (125 MHz, DMSO-d_6) (two isomers): 165.2, 162.4, 161.2, 151.2, 145.3, 143.0, 136.4, 134.0, 131.5, 130.4, 129.4, 129.2, 128.6, 128.4, 126.8, 125.4, 122.8, 117.1, 116.5, 116.0, 111.2, 61.8, 53.4 ppm. Anal. calcd. for $C_{25}H_{20}N_6O_2$: C, 68.80; H, 4.62; N, 19.25. Found: C, 68.58; H, 4.44; N, 19.50.

8.1.4. 3-((4-((1-(2-Methylbenzyl)-1H-1,2,3-triazol-4-yl)methoxy) benzylidene)hydrazineylidene)indolin-2-one ***9b***

Yield: 75%, mp = 135–138 °C. IR (KBr): 3419, 2827, 1739, 1603, 1432 cm^{-1}. ^{1}HNMR (500 MHz, DMSO-d_6) (two isomers): 10.84 (s, 1H, NH), 10.66 (s, 1H, NH), 8.64 (s, 1H, CH), 8.24 (s, 1H, triazole), 8.01–7.80 (m, 3H, H4, H2′, H6′), 7.40–6.91 (m, 9H, H5, H6, H7, H3′, H5′, H3″, H4″, H5″, H6″), 5.63 (s, 2H, CH_2), 5.27 (s, 2H, CH_2), 5.23 (s, 2H, CH_2), 2.30 (s, 3H, CH_3) ppm. ^{13}CNMR (125 MHz, DMSO-d_6): 165.2, 162.0, 161.1, 151.7, 145.6, 145.3, 142.2, 136.7, 134.5, 134.0, 132.2, 131.5, 130.9, 130.4, 129.1, 128.8, 126.7, 125.6, 116.0, 115.6, 115.6, 61.8, 51.5, 19.1 ppm. Anal. calcd. for $C_{26}H_{22}N_6O_2$: C, 69.32; H, 4.92; N, 18.66. Found: C, 69.51; H, 5.18; N, 18.80.

8.1.5. 3-((4-((1-(4-Methylbenzyl)-1H-1,2,3-triazol-4-yl)methoxy) benzylidene)hydrazono)indolin-2-one ***9c***

Yield: 50%, mp = 173–176 °C. IR (KBr): 3442, 2924, 1730, 1606, 1544, 1509 cm^{-1}. ^{1}HNMR (500 MHz, DMSO-d_6) (two isomers): 10.84 (s, 1H, NH), 8.64 (s, 1H, CH), 8.61 (s, 1H, CH), 8.31 (s, 1H, triazole), 8.29 (s, 1H, triazole), 8.03 (d, J = 8.0 Hz, 1H, H4), 7.94 (d, J = 9.0 Hz, 2H, H2′, H6′), 7.80 (d, J = 8.0 Hz, 1H, H4), 7.38–7.03 (m, 8H, H5, H6, H3′, H5′, H2″, H3″, H5″, H6″), 6.89 (d, J = 8.0 Hz, 1H, H7), 5.57 (s, 2H, CH_2), 5.26 (s, 2H, CH_2), 5.21 (s, 2H, CH_2), 2.25 (s, 3H, CH_3) ppm. ^{13}CNMR (125 MHz, DMSO-d_6) (two isomers): 165.3, 162.5, 161.8, 161.0, 160.8, 151.3, 145.3, 138.0, 134.0, 133.3, 131.5, 130.4, 129.8, 129.4, 128.5, 126.8, 126.7, 125.4, 122.8, 117.1, 115.9, 115.6, 111.2, 61.8, 53.2, 21.1 ppm. Anal. calcd. for $C_{26}H_{22}N_6O_2$: C, 69.32; H, 4.92; N, 18.66. Found: C, 69.20; H, 5.20; N, 18.52.

8.1.6. 3-((4-((1-(2-Fluorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy) benzylidene)hydrazineylidene)indolin-2-one ***9d***

Yield: 46%, mp = 157–160 °C. IR (KBr): 3231, 2850, 1735, 1609, 1539, 1511 cm^{-1}. ^{1}HNMR (500 MHz, DMSO-d_6) (two isomers): 10.82 (s, 1H, NH), 8.63 (s, 1H, CH), 8.31 (s, 1H, triazole), 8.29 (s, 1H, triazole), 8.03 (d, J = 7.9 Hz, 1H, H4), 7.95 (d, J = 8.6 Hz, 2H, H2′, H6′), 7.80 (d, J = 9.0 Hz, 1H, H4), 7.42–7.25 (m, 7H, H6, H3′, H5′, H3″, H4″, H5″, H6″), 7.14 (d, J = 9.0 Hz, 1H, H7), 7.03 (t, J = 7.9 Hz, 1H, H5), 6.89 (d, J = 7.9 Hz, 1H, H7), 5.68 (s, 2H, CH_2), 5.26 (s, 2H, CH_2), 5.21 (s, 2H, CH_2). ^{13}CNMR (125 MHz, DMSO-d_6) (two isomers): 165.2, 162.4, 161.8, 160.9, 160.6 (d, $J_{C\text{-}F}$ = 243.0 Hz), 151.2, 145.3, 143.1, 133.9, 131.3, 131.2 (d, $J_{C\text{-}F}$ = 30.7 Hz), 130.4, 129.3, 126.8, 125.5, 125.3 (d, $J_{C\text{-}F}$ = 16.7 Hz), 123.2 (d, $J_{C\text{-}F}$ = 14.5 Hz), 122.8, 117.1, 115.9 (d, $J_{C\text{-}F}$ = 20.7 Hz), 115.9, 115.6, 111.2, 61.7, 47.4 ppm. Anal. calcd. for $C_{25}H_{19}FN_6O_2$: C, 66.07; H, 4.21; N, 18.49. Found: C, 65.86; H, 4.40; N, 18.22.

8.1.7. 3-((4-((1-(4-Fluorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy) benzylidene)hydrazineylidene)indolin-2-one ***9e***

Yield: 66%, mp = 164–167 °C. IR (KBr): 3415, 2815, 1730, 1606, 1541, 1509 cm^{-1}. ^{1}HNMR (500 MHz, DMSO-d_6) (two isomers): 10.80 (s, 1H, NH), 8.62 (s, 1H, CH), 8.31 (s, 1H, triazole), 8.28 (s, 1H, triazole), 8.04 (d, J = 8.0 Hz, 1H, H4), 7.94 (d, J = 8.5 Hz, 2H, H2′, H6′), 7.40–7.17 (m, 7H, H6, H3′, H5′, H2″, H3″, H5″, H6″), 7.03 (t, J = 8.0 Hz, 1H, H5), 6.89 (d, J = 8.0 Hz, 1H, H7), 5.61 (s, 2H, CH_2), 5.25 (s, 2H, CH_2), 5.21 (s, 2H, CH_2) ppm. ^{13}CNMR (125 MHz, DMSO-d_6): 165.2, 162.4, 162.1, 160.8 (d, $J_{C\text{-}F}$ = 250.0 Hz), 151.3, 145.4, 138.2, 133.8, 131.4, 130.7, 129.9, 129.8, 129.3, 126.8, 125.8, 125.2, 117.1, 116.0 (d, $J_{C\text{-}F}$ = 25.7 Hz), 110.8, 61.7, 52.6 ppm. Anal. calcd. for $C_{25}H_{19}FN_6O_2$: C, 66.07; H, 4.21; N, 18.49. Found: C, 66.30; H, 4.35; N, 18.66.

8.1.8. 3-((4-((1-Benzyl-1H-1,2,3-triazol-4-yl)methoxy)-3-methoxybenzylidene)hydrazineylidene)indolin-2-one ***9f***

Yield: 30%, mp = 198–200 °C. IR (KBr); 3403, 2923, 2853, 1727, 1616, 1540, 1504 cm^{-1}. ^{1}HNMR (500 MHz, DMSO-d_6) (two isomers): 10.84 (s, 1H, NH), 8.63 (s, 1H, CH), 8.56 (s, 1H, CH), 8.32 (s, 1H, triazole), 8.00 (d, J = 7.8 Hz, 1H, H4), 7.56–7.33 (m, 8H, H6, H2′, H6′, H2″, H3″, H4″, H5″, H6″), 7.04 (t, J = 7.8 Hz, 1H, H5), 6.90–6.89 (m, 2H, H7, H5′), 5.62 (s, 2H, CH_2), 5.24 (s, 2H, CH_2), 5.20 (s, 2H, CH_2), 3.83 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3). ^{13}CNMR (125 MHz, DMSO-d_6) (two isomers): 165.2, 161.8, 161.2, 153.3, 151.5, 151.0, 149.8, 149.7, 145.3, 143.0, 136.4, 133.9, 129.2, 128.6, 128.4, 127.0, 126.1, 125.5, 124.1, 123.7, 122.8, 117.0, 113.8, 113.5, 113.2, 111.3, 111.2, 110.3, 62.1, 55.9, 53.3 ppm. Anal. calcd. for $C_{26}H_{22}N_6O_3$: C, 66.94; H, 4.75; N, 18.02. Found: C, 67.22; H, 4.59; N, 17.85.

8.1.9. 3-((3-Methoxy-4-((1-(2-methylbenzyl)-1H-1,2,3-triazol-4-yl) methoxy)benzylidene)hydrazineylidene)indolin-2-one ***9 g***

Yield:24%, mp = 140–143 °C. IR (KBr): 3431, 2925, 2854, 1724, 1620, 1539, 1506 cm^{-1}. ^{1}HNMR (500 MHz, DMSO-d_6) (two isomers): 10.96 (s, 1H, NH), 8.64 (s, 1H, CH), 8.58 (s, 1H, CH), 8.23 (s, 1H, triazole), 8.01 (d, J = 7.5 Hz, 1H, H4), 7.57–7.19 (m, 7H, H6, H2′, H6′, H3″, H4″, H5″, H6″), 7.09 (d, J = 7.5 Hz, 1H, H5′), 7.04 (t, J = 7.5 Hz, 1H, H5), 6.91 (d, J = 7.5 Hz, 1H, H7),

5.63 (s, 2H, CH_2), 5.45 (s, 2H, CH_2), 5.25 (s, 2H, CH_2), 5.20 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3). ^{13}CNMR (125 MHz, DMSO-d_6): 164.7, 161.2, 151.0, 150.5, 150.2, 149.3 144.9, 142.4, 136.3, 134.1, 133.5, 130.4, 128.8, 128.7, 128.3, 126.6, 126.2, 125.1, 122.3, 117.0, 116.6, 113.5, 110.8, 61.7, 55.5, 51.0, 19.2 ppm. Anal.

calcd. for $C_{27}H_{24}N_6O_3$: C, 67.49; H, 5.03; N, 17.49. Found: C, 67.66; H, 5.21; N, 17.20.

8.1.10. 3-((3-Methoxy-4-((1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl) methoxy)benzylidene)hydrazineylidene)indolin-2-one **9 h**

Yield: 27%, mp = 13–136 °C. IR (KBr); 3449, 2923, 2853, 1727, 1616, 1541, 1504 cm^{-1}. ^{1}HNMR (500 MHz, DMSO-d_6) (two isomers): 10.91 (s, 1H, NH), 8.57 (s, 1H, CH), 8.56 (s, 1H, CH), 8.28 (s, 1H, triazole), 7.98 (d, J = 7.5 Hz, 1H, H4), 7.55–6.92 (m, 7H, H5, H6, H7, H2′, H5′, H6′, H2″, H3″, H5″, H6″), 5.55 (s, 2H, CH_2), 5.22 (s, 2H, CH_2), 5.19 (s, 2H, CH_2), 3.83 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3). ^{13}CNMR (125 MHz, DMSO-d_6) (two isomers): 161.1, 160.9, 150.6, 149.2, 145.1, 143.8, 142.5, 140.2, 137.4, 133.4, 132.9, 129.3, 128.7, 128.0, 126.5, 124.9, 123.6, 122.2, 116.9, 113.4, 113.1, 110.9, 110.7, 61.7, 55.5, 52.6, 20.7 ppm. Anal. calcd. for $C_{27}H_{24}N_6O_3$: C, 67.49; H, 5.03; N, 17.49. Found: C, 67.20; H, 4.81; N, 17.70.

8.1.11. 3-((4-((1-(2-Fluorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-methoxybenzylidene)hydrazineylidene)indolin-2-one **9i**

Yield: 28%, mp = 193–196 °C. IR (KBr); 3483, 2923, 2853, 1732, 1618, 1594, 1538, 1505 cm^{-1}. ^{1}HNMR (500 MHz, DMSO-d_6) (two isomers): 10.95 (s, 1H, NH), 8.64 (s, 1H, CH), 8.58 (s, 1H, CH), 8.32 (s, 1H, triazole), 8.01 (d, J = 7.5 Hz, 1H, H4), 7.57–7.23 (m, 8H, H6, H2′, H5′, H6′, H3″, H4″, H5″, H6″), 7.04 (t, J = 7.5 Hz, 1H, H5), 6.91 (d, J = 7.5 Hz, 1H, H7), 5.70 (s, 2H, CH_2), 5.25 (s, 2H, CH_2), 5.20 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3). ^{13}CNMR (125 MHz, DMSO-d_6): 164.7, 164.0, 161.2, 160.1 (d, $J_{C\text{-}F}$ = 235.0 Hz), 151.0, 150.6, 149.4, 142.5, 133.5, 130.8, 128.8, 126.6, 125.2, 124.9, 123.6, 122.8 (d, $J_{C\text{-}F}$ = 15.6 Hz), 122.3, 120.5, 116.6, 115.6 (d, $J_{C\text{-}F}$ = 20.8 Hz), 113.4, 113.3, 110.8, 61.6, 54.9, 46.9 ppm. Anal. calcd. for $C_{26}H_{21}FN_6O_3$: C, 64.46; H, 4.37; N, 17.35. Found: C, 64.22; H, 4.18; N, 17.22.

8.1.12. 3-((4-((1-(4-Fluorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-methoxybenzylidene)hydrazineylidene)indolin-2-one **9j**

Yield: 25%, mp = 158–161 °C. IR (KBr): 3385, 2925, 2854, 1732, 1620, 1539, 1508 cm^{-1}. ^{1}HNMR (500 MHz, DMSO-d_6) (two isomers): 10.93 (s, 1H, NH), 8.64 (s, 1H, CH), 8.58 (s, 1H, CH), 8.34 (s, 1H, triazole), 8.31 (s, 1H, triazole), 8.00 (d, J = 7.5 Hz, 1H, H4), 7.57–7.21 (m, 8H, H6, H2′, H5′, H6′, H2″, H3″, H5″, H6″), 7.03 (t, J = 7.5 Hz, 1H, H5), 6.90 (d, J = 7.5 Hz, 1H, H7), 5.62 (s, 2H, CH_2), 5.43 (s, 2H, CH_2), 5.24 (s, 2H, CH_2), 5.19 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3). ^{13}CNMR (125 MHz, DMSO-d_6) (two isomers): 164.7, 162.9, 162.3, 161.2, 160.6 (d, $J_{C\text{-}F}$ = 224.6 Hz), 151.1, 150.6, 150.2, 149.3, 149.2, 148.0, 144.9, 144.0, 142.7, 133.4, 132.2, 131.6, 131.1, 130.3 (d, $J_{C\text{-}F}$ = 8.6 Hz), 128.8, 127.2, 126.6, 124.9, 123.6, 123.0, 122.3, 116.6, 115.6 (d, $J_{C\text{-}F}$ = 21.4 Hz), 113.4, 113.2, 110.8, 63.0, 61.2, 55.9, 52.4 ppm. Anal. calcd. for $C_{26}H_{21}FN_6O_3$: C, 64.46; H, 4.37; N, 17.35. Found: C, 64.60; H, 4.51; N, 17.15.

8.1.13. 3-((4-((1-Benzyl-1H-1,2,3-triazol-4-yl)methoxy)benzylidene) hydrazono)-1-(4-bromobenzyl)indolin-2-one **9 k**

Yield: 50%, mp = 182–185 °C. IR (KBr): 3350, 2923, 2852, 1726, 1604, 1532, 1509, 1463 cm^{-1}. ^{1}HNMR (500 MHz, DMSO-d_6) (two isomers): 8.70 (s, 1H, CH), 8.63 (s, 1H, CH), 8.34 (s, 1H, triazole), 8.32 (s, 1H, triazole), 8.13 (d, J = 8.0 Hz, 1H, H4), 7.98 (d, J = 8.8 Hz, 2H, H2′, H6′), 7.54 (d, J = 8.4 Hz, 2H, 4-bromobenzyl), 7.42–7.32 (m, 8H, H6, H2″, H3″, H4″, H5″, H6″, 4-bromobenzyl), 7.24 (d, J = 8.8 Hz, 2H, H3′, H5′), 7.11 (t, J = 8.0 Hz, 1H, H5), 7.01 (d, J = 8.0 Hz, 1H, H7), 5.63 (s, 2H, CH_2), 5.27 (s, 2H, CH_2), 5.22 (s, 2H, CH_2), 4.96 (s, 2H, CH_2) ppm. ^{13}CNMR (125 MHz, DMSO-d_6) (two isomers): 164.2, 163.1, 162.2, 160.3, 150.3, 146.4, 145.3, 143.1, 136.5, 136.1, 133.8, 132.1, 131.6, 130.0, 129.2, 128.4, 127.2, 126.5, 126.4, 125.7, 124.6, 123.7, 123.6, 121.3, 116.6, 116.0, 106.9, 62.0, 53.5 ppm. Anal. calcd. for $C_{32}H_{25}BrN_6O_2$: C, 63.48; H, 4.16; N, 13.88. Found: C, 63.21; H, 4.33; N, 13.60.

8.1.14. 1-(4-Bromobenzyl)-3-((4-((1-(2-methylbenzyl)-1H-1,2,3-triazol-4-yl)methoxy)benzylidene)hydrazono)indolin-2-one **9 l**

Yield: 30%, mp = 172–177 °C. IR (KBr): 3350, 2922, 2852, 1729, 1603, 1531, 1510 cm^{-1}. ^{1}HNMR (500 MHz, DMSO-d_6) (two isomers): 8.70 (s, 1H, CH), 8.63 (s, 1H, CH), 8.25 (s, 1H, triazole), 8.21 (s, 1H, triazole), 8.13 (d, J = 7.8 Hz, 1H, H4), 7.98 (d, J = 8.7 Hz, 2H, H2′, H6′), 7.54 (d, J = 8.5 Hz, 2H, 4-bromobenzyl), 7.41 (t, J = 7.8 Hz, 1H, H6), 7.34 (d, J = 8.5 Hz, 2H, 4-bromobenzyl), 7.25–7.08 (m, 5H, H5, H3″, H4″, H5″, H6″), 7.01 (d, J = 7.8 Hz, 1H, H7), 5.63 (s, 2H, CH_2), 5.27 (s, 2H, CH_2), 5.22 (s, 2H, CH_2), 4.96 (s, 2H, CH_2), 2.31 (s, 3H, CH_3) ppm. ^{13}CNMR (125 MHz, DMSO-d_6): 164.1, 163.1, 162.5, 162.0, 150.2, 145.3, 142.8, 136.8, 135.8, 134.6, 133.8, 132.1, 131.6, 130.8, 130.0, 129.4, 129.2, 129.0, 126.7, 125.5, 123.7, 121.2, 116.7, 116.0, 110.4, 62.4, 51.4, 19.1 ppm. Anal. calcd. for $C_{33}H_{27}BrN_6O_2$: C, 63.98; H, 4.39; N, 13.57. Found: C, 64.18; H, 4.60; N, 13.33.

8.1.15. 1-(4-Bromobenzyl)-3-((4-((1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methoxy)benzylidene)hydrazono)indolin-2-one **9 m**

Yield: 30%, mp = 185–188 °C. IR (KBr): 3355, 2922, 2853, 1711, 1603, 1539, 1510 cm^{-1}. ^{1}HNMR (500 MHz, DMSO-d_6) (two isomers): 8.70 (s, 1H, CH), 8.62 (s, 1H, CH), 8.30 (s, 1H, triazole), 8.13 (d, J = 8.0 Hz, 1H, H4), 7.98 (d, J = 8.5 Hz, 2H, H2′, H6′), 7.54 (d, J = 8.5 Hz, 2H, 4-bromobenzyl), 7.41 (t, J = 8.0 Hz, 1H, H6), 7.33 (d, J = 8.5 Hz, 2H, 4-bromobenzyl), 7.24–7.21 (m, 4H, H2″, H3″, H5″, H6″), 7.17 (d, J = 8.5 Hz, 2H, H3′, H5′), 7.11 (t, J = 8.0 Hz, 1H, H5), 7.00 (d, J = 8.0 Hz, 1H, H7), 5.56 (s, 2H, CH_2), 5.26 (s, 2H, CH_2), 5.21 (s, 2H, CH_2), 4.96 (s, 2H, CH_2), 2.27 (s, 3H, CH_3) ppm. ^{13}CNMR (125 MHz, DMSO-d_6): 164.1, 163.2, 161.9, 150.3, 147.7, 147.5, 145.1, 143.0, 137.9, 136.1, 133.8, 133.5, 132.1, 131.6, 130.0, 129.7, 128.5, 126.7, 125.4, 121.2, 116.7, 116.1, 115.1, 62.7, 53.1, 21.1 ppm. Anal. calcd. for $C_{33}H_{27}BrN_6O_2$: C, 63.98; H, 4.39; N, 13.57. Found: C, 64.25H, 4.15; N, 13.70.

8.1.16. 1-(4-Bromobenzyl)-3-((4-((1-(2-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)benzylidene)hydrazono)indolin-2-one **9n**

Yield: 17%, mp = 158–161 °C. IR (KBr): 3350, 2922, 2852, 1727, 1602, 1462 cm^{-1}. ^{1}HNMR (500 MHz, DMSO-d_6) (two isomers): 8.70 (s, 1H, CH), 8.63 (s, 1H, CH), 8.33 (s, 1H, triazole), 8.13 (d, J = 8.0 Hz, 1H, H4), 7.98 (d, J = 8.6 Hz, 2H, H2′, H6′), 7.54 (d, J = 8.5 Hz, 2H, 4-bromobenzyl), 7.43–7.23 (m, 6H, H6, H3′, H5′, H3″, H4″, H5″, H6″), 7.12 (t, J = 8.0 Hz, 1H, H5), 7.01 (d, J = 8.0 Hz, 1H, H7), 5.69 (s, 2H, CH_2), 5.27 (s, 2H, CH_2), 5.22 (s, 2H, CH_2), 4.96 (s, 2H, CH_2) ppm. ^{13}CNMR (125 MHz, DMSO-d_6) (two isomers): 164.0, 162.1 (d, $J_{C\text{-}F}$ = 255.9 Hz), 162.0, 161.7, 161.6, 150.3, 149.6, 148.2, 147.3, 145.1, 142.8, 136.0, 133.7, 132.1, 131.7, 131.3, 130.0, 129.2, 126.9, 125.7, 125.3, 123.4, 121.1, 121.0, 116.9, 116.1, 115.9 (d, $J_{C\text{-}F}$ = 15.5 Hz), 110.4, 62.0, 47.9 ppm. Anal. calcd. for $C_{32}H_{24}BrFN_6O_2$: C, 61.65; H, 3.88; N, 13.48. Found: C, 61.84; H, 3.60; N, 13.20.

8.1.17. 1-(4-Bromobenzyl)-3-((4-((1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)benzylidene)hydrazono)indolin-2-one **9o**

Yield: 18%, mp = 174–178 °C. IR (KBr): 3355, 2922, 2852, 1715, 1604, 1543, 1504, 1463 cm^{-1}. ^{1}HNMR (500 MHz, DMSO-d_6) (two isomers): 8.70 (s, 1H, CH), 8.63 (s, 1H, CH), 8.35 (s, 1H, triazole), 8.13 (d, J = 7.8 Hz, 1H, H4), 7.98 (d, J = 8.8 Hz, 2H, H2′, H6′), 7.54 (d, J = 8.5 Hz, 2H, 4-bromobenzyl), 7.42–7.40 (m, 3H, H6, H2″, H6″), 7.33 (d, J = 8.5 Hz, 2H, 4-bromobenzyl), 7.25–7.19 (m, 4H, H3′, H5′, H3″, H5″), 7.11 (t, J = 7.8 Hz, 1H, H5), 7.01 (d, J = 7.8 Hz, 1H, H7), 5.62 (s, 2H, CH_2), 5.27 (s, 2H, CH_2), 5.21 (s, 2H, CH_2), 4.96 (s, 2H, CH_2). ^{13}CNMR (125 MHz, DMSO-d_6) (two isomers): 164.0, 162.6, 162.0 (d, $J_{C\text{-}F}$ = 250.0 Hz), 162.2, 162.0, 150.4, 145.4, 143.1, 136.0, 133.9, 133.8, 132.8, 132.7, 132.1, 131.7, 131.6, 130.9, 130.8, 130.0, 126.7, 125.3, 123.6, 121.0, 116.7, 116.0, 115.7 (d, $J_{C\text{-}F}$ = 15.5 Hz), 111.8, 62.0, 52.5 ppm. Anal. calcd. for $C_{32}H_{24}BrFN_6O_2$: C, 61.65; H, 3.88; N, 13.48. Found: C, 61.38; H, 3.71; N, 13.60.

8.2. In vitro α-glucosidase inhibition assay

The assay was performed exactly according to our previous report [44].

8.3. Enzyme kinetic studies

The mode of inhibition of the most active compound **9d**, identified with the lowest IC_{50}, was investigated against an α-glucosidase activity with different concentrations of *p*-nitrophenyl *α*-D-glucopyranoside (2–10 mM) as the substrate in the absence and presence of compound **9d** at different concentrations (0, 8, 16, and 33 μM). A Lineweaver–Burk plot was generated to identify the type of inhibition and the Michaelis–Menten constant (K_m) value was determined from the plot between reciprocal of the substrate concentration (1/[S]) and reciprocal of enzyme rate (1/V) over various inhibitor concentrations. Experimental inhibitor constant (K_i) value was constructed by the secondary plots of the inhibitor concentration [I] versus K_m.

8.4. Docking analysis

X-ray crystal structure of α-glucosidase with PDB code of 5NN8 was downloaded from PDB website (https://www.rcsb.org/). The protein structure was prepared using the Discovery Studio Client so that ligands were removed from 5NN8 and all hydrogens were added. The binding site of the enzyme for the docking process was defined using the native ligand acarbose. GOLD docking program with ChemScore function was used for docking analyses and re-dock acarbose inside the 5NN8 was first applied. All other options were set as default. Next, compounds **9d** and **9n** were sketched using hyperchem software and energy minimization was conducted using the MM1 force field. Similarly, the GOLD program was applied for doing docking analyses for the mentioned compounds with ChemScore function in GOLD. The top-score binding poses were used for further analysis. Protein-ligand interactions were analyzed with Discovery Studio Visualizer.

Declaration of Competing Interest

The authors declare that they have no known competing financial 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.2021.104869.

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