Design, synthesis, biological evaluation, and docking study of new acridine-9-carboxamide linked to 1,2,3-triazole derivatives as antidiabetic agents targeting α-glucosidase

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A new series of acridine-9-carboxamide-1,2,3-triazole derivatives **7a-m** were designed, synthesized, and evaluated as novel α-glucosidase inhibitors. Acridine-9-carboxamide-1,2,3-triazole scaffold has been designed by combination of effective moieties from potent α-glucosidase inhibitors. Most of the synthesized compounds were more potent than standard inhibitor acarbose. Among the title compounds, the most potent compounds were compounds **7j**,

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7k, and **7a** with IC_{50} values of 120.2 ± 1.0, 151.1 ± 1.4, and 157.6 ± 1.6 μM, respectively (IC_{50} value of acarbose = 750.0 ± 10.0 μM). Docking study of the most potent compounds demonstrated that these compounds formed stable complexes with α-glucosidase active site. Anti-α-amylase assay of compounds **7j**, **7k**, and **7a** was performed and no activity was observed. *In vitro* cytotoxicity assay of the latter compounds revealed that these compounds were not cytotoxic toward human normal (HDF) and cancer (MCF-7) cell lines. ADME and toxicity prediction of compounds **7j**, **7k**, and **7a** were also performed.

Keyword: Acridine-9-carboxamide; 1,2,3-Triazole; α-Glucosidase; Docking; ADME

INTRODUCTION

Diabetes mellitus is a prevalent metabolic disorder which led to defective homeostasis of glucose in the blood due to inadequate insulin secretion (Type 1) and/or defects in insulin action (Type 2) [1]. Based on the type of diabetes, various medications are used for regulation of blood glucose; commonly, insulin is used for type 1 diabetes and biguanidus, sulfonylureas, thiazolidinedones, and α-glucosidase inhibitors are used for type 2 diabetes [2-4]. α-Glucosidase is an intestinal enzyme that plays a major role in the degradation of carbohydrates to glucose so inhibiting it can reduce the release of glucose into the bloodstream [5]. α-Glucosidase inhibitors like acarbose, miglitol, and voglibose are used effectively for treatment of type 2 diabetes [6]. However these drugs created side effects such as hepatic disorders, flatulence, gastrointestinal diseases, improper absorption, and diarrhea [7, 8]. Therefore, during the last two decades, pharmaceutical chemists reported various α-glucosidase inhibitors with different scaffolds [9, 10]. Furthermore, there are evidences that shows α-glucosidase inhibitors can be useful in the treatment of other carbohydrate related diseases [11].

Aromatic tricyclic heterocycles such as acridine, xanthone, and carbazole exhibited various pharmacological properties such as anti-cancer, anticonvulsant, anti-HIV, antioxidant, anti-inflammatory, and anticholinesterase activities [12-14]. These scaffolds are also found in the potent α-glucosidase inhibitors such as compounds **A-C** (Fig. 1) [15-17]. Another important pharmacophore in the design of α-glucosidase inhibitors is 1,2,3-triazole ring. In addition to compounds **B** and **C**, this ring is also found in several strong α-glucosidase inhibitors such as compounds **D** [18]. Thus, in continuation of our attempts for design and synthesis of new heterocyclic compounds as potent α-glucosidase inhibitors, herein, we designed a new series of acridine-9-carboxamide-1,2,3-triazoles **7a-m** and synthesized them with simple reactions. Then, these compounds evaluated against yeast α-glucosidase by *in vitro* and *in silico* assays [19-21]. Since acridine is found in numerous anti-tumor agents, cytotoxic effects of title compounds were evaluated against cancer and normal human cells by MTT assay [22].

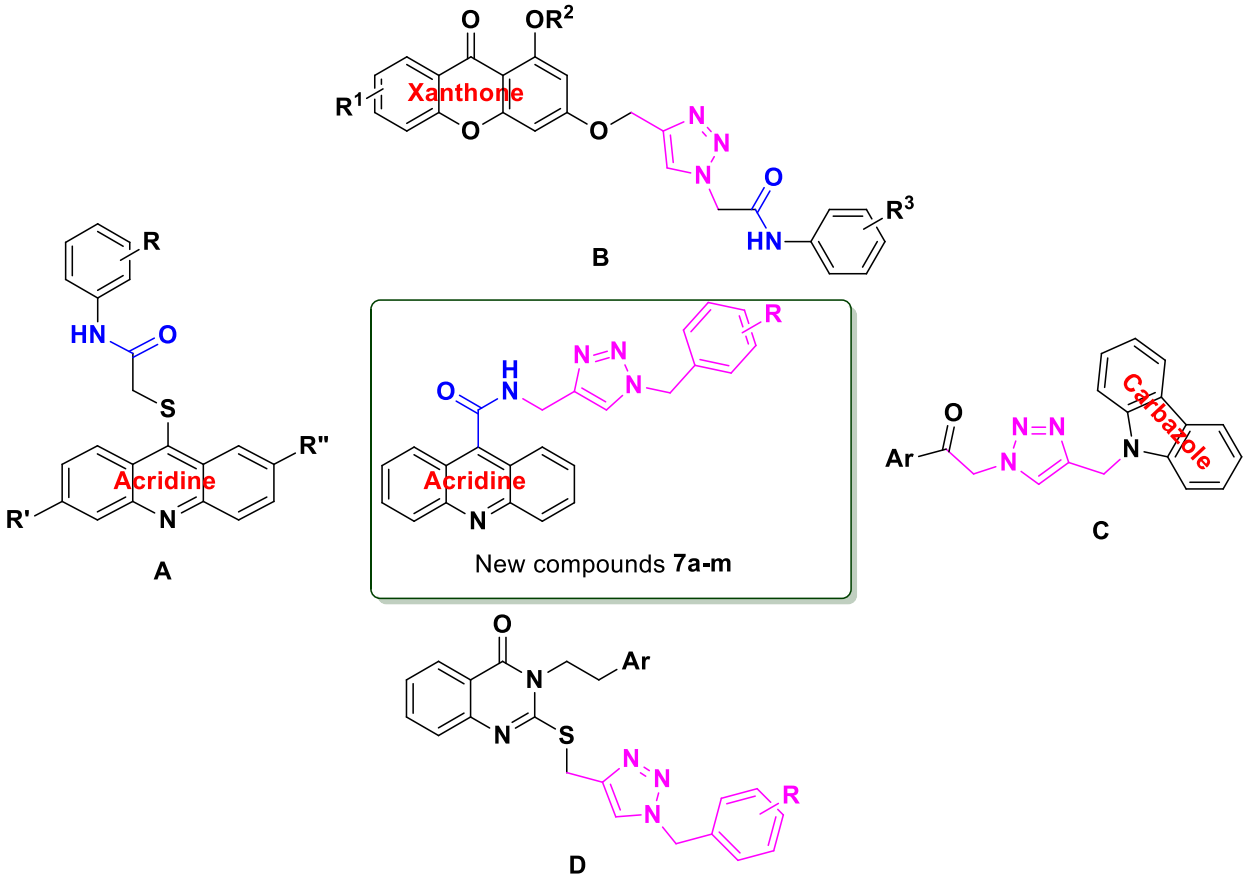
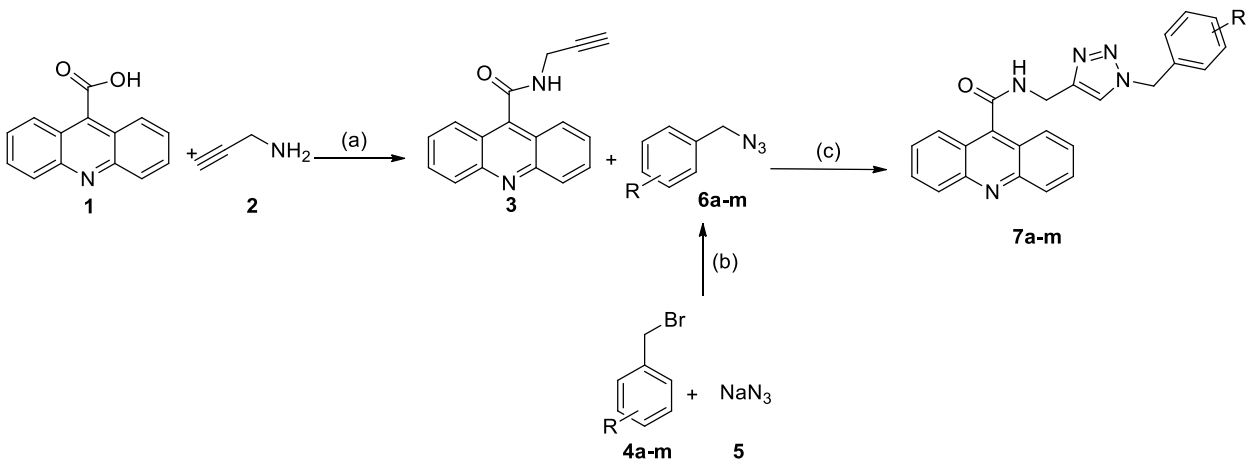


Figure 1. Design strategy for new α-glucosidase inhibitors **7a-m** with acridine-9-carboxamide 1,2,3-triazole scaffold.

RESULTS AND DISCUSSION

Chemistry. Synthetic procedure for the preparation of the acridine-9-carboxamide-1,2,3-triazole derivatives **7a-m** is depicted in Scheme 1. This procedure was started with reaction between acridine-9-carboxylic acid **1** and propargylamine **2** in the presence of HOBt/EDCI in dry acetonitrile for produce *N*-(prop-2-yn-1-yl)acridine-9-carboxamide **3**. On the other hand, azide derivatives **6a-m** were prepared according to our previous work [18]. In the final step, *N*-(prop-2-yn-1-yl)acridine-9-carboxamide **3**, catalytic amount of sodium ascorbate, and $CuSO_4$ were added to the freshly prepared azides **6a-m** to provide desired compounds **7a-m**.



Scheme 1. Synthesis of compounds **7a-m**. Reagents and conditions: (a) HOBT/EDCI, dry CH_3CN, R.T., 24 h; (b) NEt_3, H_2O/t-BuOH, R.T., 1 h; (c) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, R.T., 16–24 h.

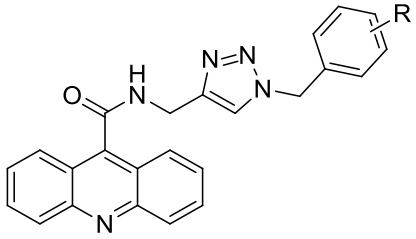
***In vitro* α-glucosidase inhibition assay.** The *in vitro* α-glucosidase inhibition of the synthesized compounds **7a-m** was evaluated against yeast form of this enzyme (*Saccharomyces cerevisiae*). The IC_{50} values of compounds **7a-m** in comparison to acarbose as standard drug are presented in Table 1. All the synthesized compounds (IC_{50} = 120.2 ± 1.0-624.4 ± 9.3 µM), with the exception of **7d** and **7f** (IC_{50} > 750 µM), exhibited higher inhibitory activity respect to acarbose (IC_{50} = 120.2 ± 1.0-624.4 ± 9.3 µM). Among the synthesized compounds, compounds **7j**, **7k**, and **7a** with inhibitory activities 6-4 folds more than standard inhibitor were the most potent compounds.

As can be seen in Table 1, compound **7a** with un-substituted pendant phenyl ring was the third most potent compound (IC_{50} = 157.6 ± 1.6 µM). The introduction of electron-donating substituents 2-metyl or 3-methoxy on pendant phenyl ring led to a significant decrease in inhibitory activity as observed in compounds **7b** (IC_{50} = 624.4 ± 9.3 µM) and **7c** (IC_{50} = 616.3 ± 9.0 µM), while inhibitory activity of remaining derivatives with electron withdrawing

substituents depend on the type and position of the substituent. In this regards, 2-fluoro derivative **7d** and 4-fluoro derivative **7f** were inactive against α-glucosidase while their chloro analogs (2-chloro derivative **7g** and 4-chloro derivative **7h**) exhibited high anti-α-glucosidase activity in comparison to standard inhibitor. Moreover, 3-fluoro derivative **7e** was a moderate inhibitor for α-glucosidase. Among the synthesized compounds, 3-bromo derivative **7j** (IC_{50} = 120.2 ± 1.0 µM) showed the most potent activity. Changing the position of bromo substituent in the pendant phenyl ring from C-3 to C-4, as in compound **7k** (second potent compound; IC_{50} = 151.1 ± 1.4 µM) slightly diminished the activity, while this changing from C-3 to C-2 dramatically decreased inhibitory activity as observed in compound **7i** (IC_{50} = 303.7 ± 4.3 µM). Similar to fluoro and bromo substituents, nitro substituent showed a better effect when placed in 3-position (compound **7l** vs **7m**).

Table 1

α-Glucosidase inhibitory activities of the synthesized compounds **7a-m**.



Compound	R	IC_{50} (µM)[a]	Compound	R	IC_{50} (µM)[a]
7a	H	157.6 ± 1.6	**7h**	4-Cl	165.2 ± 1.7
7b	2-CH_3	624.4 ± 9.3	**7i**	2-Br	303.7 ± 4.3
7c	3-OCH_3	616.3 ± 9.0	**7j**	3-Br	120.2 ± 1.0
7d	2-F	> 750	**7k**	4-Br	151.1 ± 1.4

7e	3-F	580.6 ± 7.7	**7l**	3-NO_2	229.7 ± 2.6
7f	4-F	> 750	**7m**	4-NO_2	404.2 ± 5.6
7g	2-Cl	216.6 ± 2.5	Acarbose		750.0 ± 10.0

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

Kinetic study. To give the inhibition mode of the synthesized compounds into α-glucosidase, an enzyme kinetic assay was performed on the most potent compound **7j**. As can be seen in Fig. 2a, with increase of concentration of inhibitor **7j**, V_m did not change while K_m increased. So, compound **7j** is a competitive inhibitor for α-glucosidase. The value of K_i for compound **7j** was 116 µM (Fig. 2b).

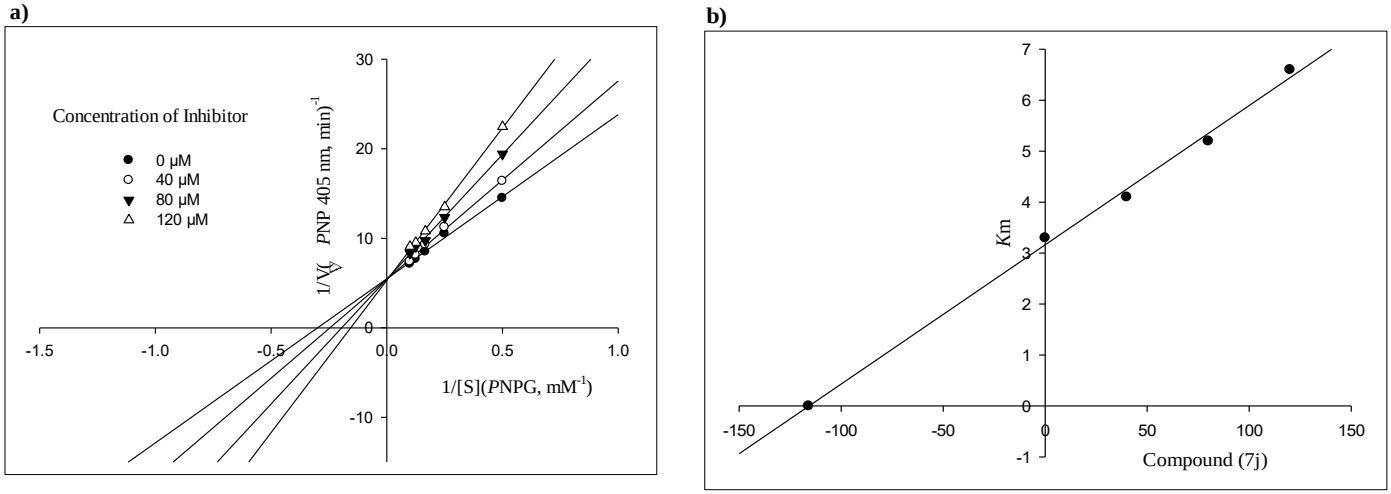


Figure 2. Kinetics of α-glucosidase inhibition by compound **7j**. (a) The Lineweaver– Burk plot in the absence and presence of different concentrations of compound **7j**; (b) The secondary plot between K_m and various concentrations of compound **7j**.

Docking study in α-glucosidase active site. Interaction poses of acarbose as standard inhibitor and the most potent compounds **7j**, **7k**, and **7a** were screened in the active site of modeled α-

glucosidase by molecular docking study [19]. Acarbose in α-glucosidase active site was showed in Fig. 3a and details of acarbose interaction in the active site was exhibited in 2-dimensional form in Fig. 3b. As can be seen in the latter Fig., acarbose established hydrogen bonds with active site residues Arg312, Thr307, Gln322, Thr301, Glu304, Asn241, and Ser308 and a hydrophobic interaction with His279. Furthermore, acarbose formed weak carbon hydrogen bonds with Phe157, Val305, Glu304, and His239 and two unfavorable interactions with Thr307 and Arg312. The binding energy of this drug in the active site of modeled α-glucosidase was -4.4 kcal/mol.

(a) (b)

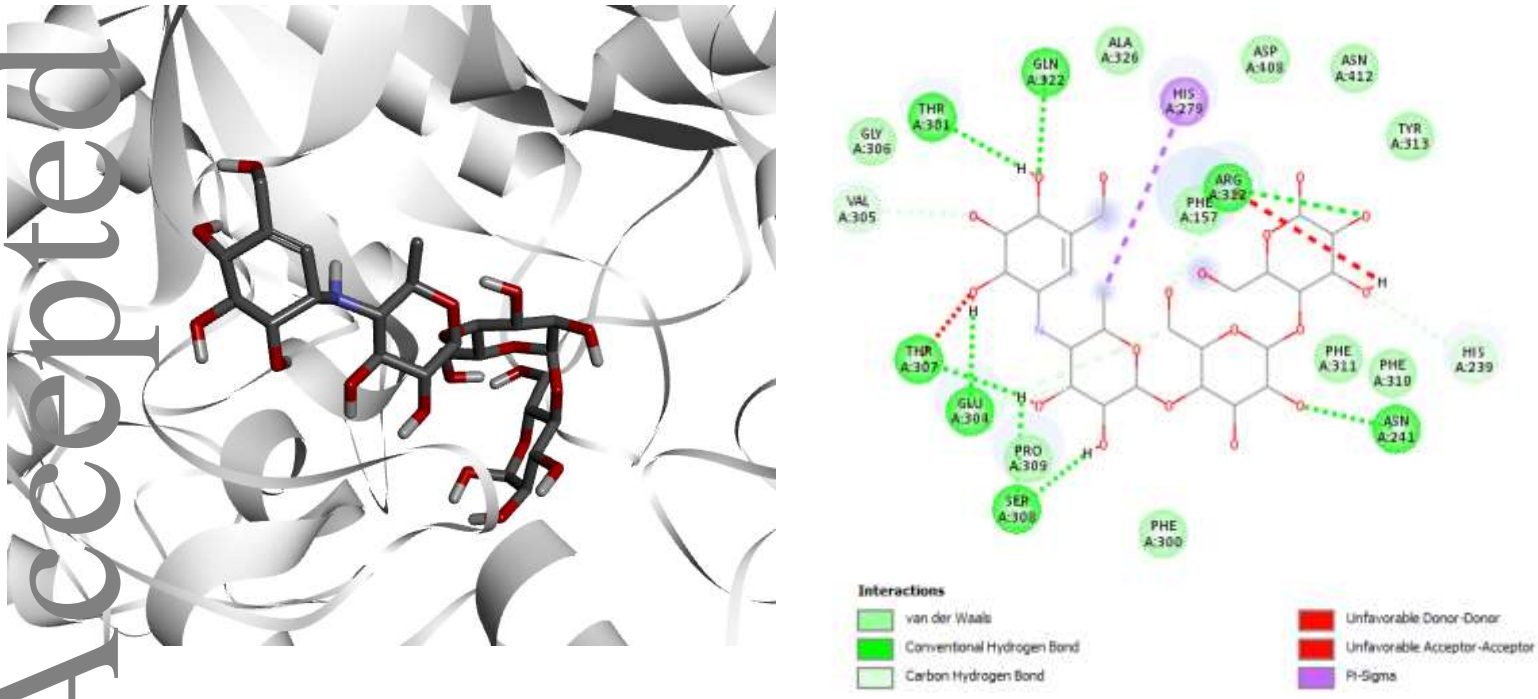


Figure 3. (a) Acarbose and (b) it interaction pose in the active site of modeled α-glucosidase.

1,2,3-Triazole ring of the most potent compound **7j** formed π-π and π-cation interactions with residues His239 and His279, respectively (Fig. 4a). This compound also established a π-anion

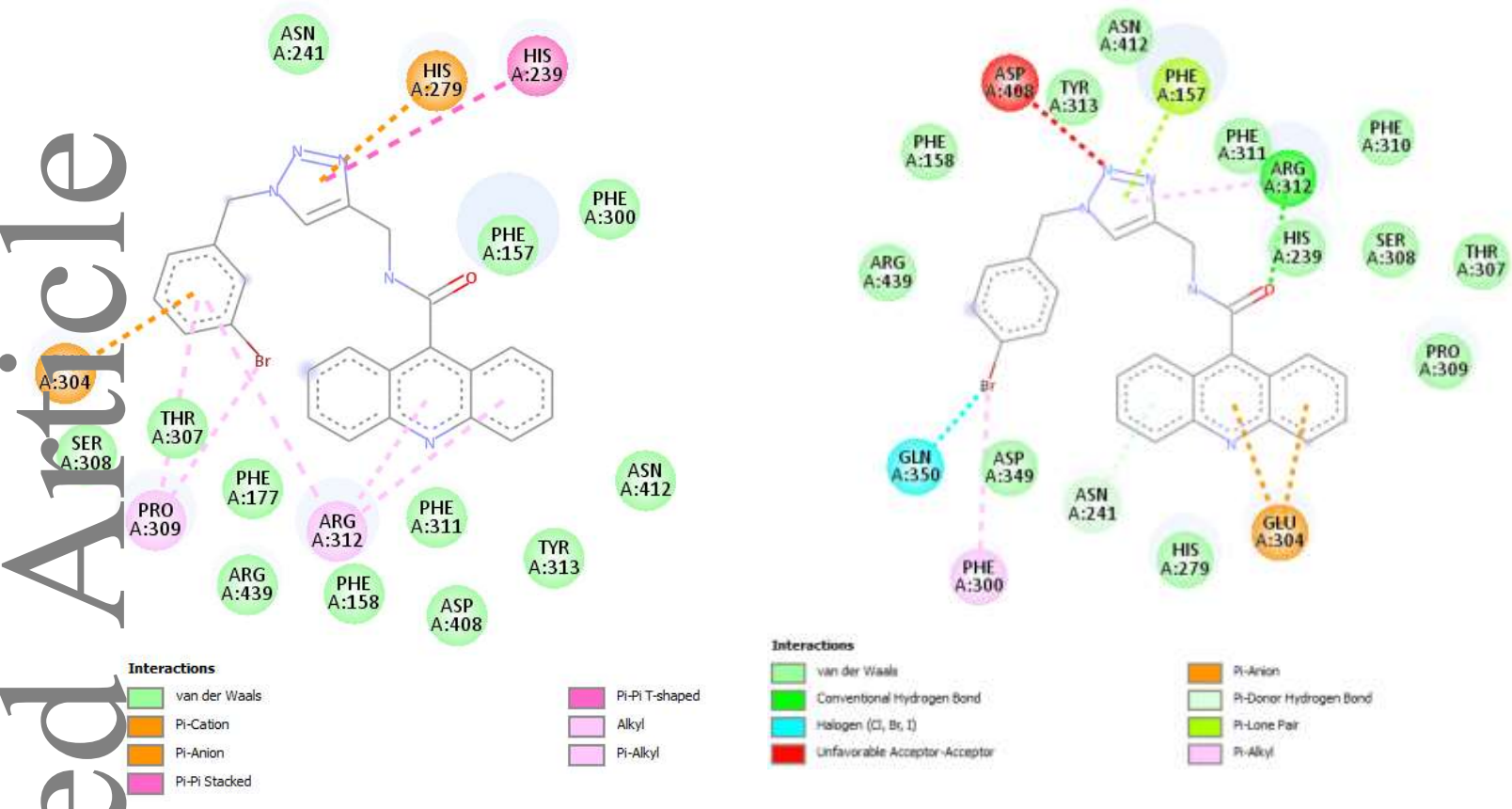
interaction with Glu304 *via* pendant 3-bromophenyl group. The latter group also formed a hydrophobic interaction with Pro309 *via* bromo substituent and two hydrophobic interactions with Pro309 and Arg312 *via* phenyl ring. Furthermore, acridine ring established two hydrophobic interactions with Arg312. The binding energy value for this compound was calculated to be -9.36 kcal/mol, which was lower from acarbose (−4.04 kcal/mol) and hence, compound **7j** can bind to α-glucosidase active site more easily than acarbose.

4-Bromo substituent of the second potent compound **7k** formed two hydrophobic interactions with Phe300 and Gln350. 1,2,3-triazole ring of this compound in addition two interactions with Arg312 (hydrophobic interaction) and Phe157 (π-Lone Pair), formed an unfavorable interaction with Asp408. Compound **7k** also established a hydrogen bond with Arg312 *via* carbonyl unit. Acridine ring of this compound formed π-anion interactions with Glu304 and a carbon hydrogen bond with Asn241.

As can be seen in Fig. 4., the interaction modes of the most potent compound **7j** and the third most potent compound **7a** are similar and have only two differences: 1) acridine ring of compound **7a** formed an additional π-π interaction with Phe158 and 2) un-substituted phenyl ring of compound **7a**, unlike compound **7j**, only formed a hydrophobic interaction with Pro309.

The comparison of binding energies of the most active compound **7j** (-9.36 kcal/mol) with the second potent compound **7k** (−9.09 kcal/mol) and third potent compound **7a** (-8.85 kcal/mol) demonstrated that theoretically the compound **7j** bound easily than compounds **7k** and **7a** to active site of α-glucosidase. This finding is in agreement to biological data (Table 1).

(a) (b)



(c)

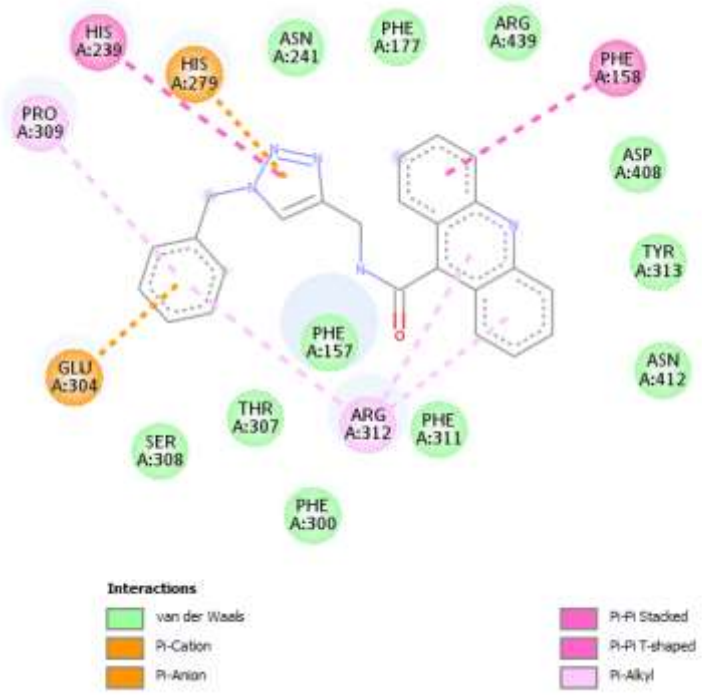


Figure 4. Interaction modes of the most active compounds **7j** (a), **7k** (b), and **7a** (C) in the active site pocket.

***In vitro* α-amylase inhibitory activity assay**. α-Amylase is a pancreatic carbohydrate-hydrolyzing enzyme that polysaccharides converted to oligo- and disaccharides [23]. Although α-amylase inhibition can play a positive role in lowering blood glucose level, it raises the amount of undigested polysaccharide in the intestine and causes gastrointestinal side effects [24]. Therefore, α-amylase inhibitory activity of the most potent compounds **7j**, **7k**, and **7a** were evaluated against porcine pancreatic α-amylase [20]. As can be seen Table 2, *In vitro* inhibition assay of the compounds **7j**, **7k**, and **7a** (IC_{50}s > 200) showed that these compounds are inactive against α-amylase in comparison to acarbose (IC_{50} = 108 ± 0.71 µM).

Table 2

Anti-α-amylase activity and cytotoxicity of compounds **7j**, **7k**, and **7a**.

Compound	Enzyme/Cell line		
	α-Amylase	MCF-7	HDF
7j	> 200	> 200	> 200
7k	> 200	> 200	> 200
7a	> 200	> 200	> 200
Acarbose	108 ± 0.71	-	-
Etoposide	-	22.08 ± 0.39	92.7 ± 1.2

***In vitro* cytotoxicity.** *In vitro* cytotoxicity of the compounds **7j**, **7k**, and **7a** was evaluated against human normal cell line HDF and human cancer cell line MCF-7 by MTT assay [15, 18]. Obtained results revealed that at 200 µM, studied compounds were non-cytotoxic against two cell line MCF-7 and HDF when compared with etoposide as an anti-tumor agent (Table 2).

ADME and Toxicity studies. ADME/T properties of the compounds **7j**, **7k**, and **7a** as most potent α-glucosidase inhibitors among the synthesized compounds and standard inhibitor acarbose were predicted using online software PreADMET (Table 3) [25]. As can be seen in

Table 3, new synthesized compounds and acarbose have poor penetration into Caco-2 cell, blood brain barrier (BBB), and skin. Moreover, compounds **7j**, **7k**, and **7a** have high human oral absorption (HIA) while acarbose does not show HIA. In term of toxicity, all studied compounds with the exception of the most potent compound **7j**, were mutagenic. *In silico* toxicity assay also demonstrated that cardiotoxicity acarbose is ambiguous while compounds **7j**, **7k**, and **7a** exhibited medium risk in term of cardiotoxicity (hERG inhibition). Furthermore, the latter new compounds, unlike acarbose, have not carcinogenic effect on mouse. On the other hand, only compound **7a**, like acarbose have not carcinogenic effect on rat.

Table 3. ADME/T Profile of the most potent compounds **7j**, **7k**, and **7a**.

ADME/T [a]	Compound			
	7j	**7k**	**7a**	**Acarbose**
Caco2	24.4555	24.4254	21.2183	9.44448
HIA	97.623813	97.623813	97.090144	0.000000
BBB	0.0296523	0.0282977	0.0218381	0.0271005
Skin Permeability	-2.76734	-2.76843	-2.87797	-5.17615
Ames test	Non-mutagen	Mutagen	Mutagen	Mutagen
hERG inhibition	Medium risk	Medium risk	Medium risk	Ambiguous
Carcino Mouse	Negative	Negative	Negative	Positive
Carcin Rat	Positive	Positive	Negative	Negative

[a] The recommended ranges for Caco2: <25 poor, >500 great, HIA: >80% is high <25% is poor, BBB = –3.0 – 1.2, and Skin_Permeability = –8.0 – –1.0.

CONCLUSION

In conclusion, new acridine-9-carboxamide-1,2,3-triazole derivatives were designed, synthesized, and evaluated as potent α-glucosidase inhibitors. Most of the synthesized compounds were more potent than acarbose and among them, the most potent compounds were 3-bromo derivative **7j**, 4-bromo derivative **7k**, and un-substituted derivative **7a** with inhibitory activity around 6-4 fold more than acarbose. Docking study was performed to elucidate the observed anti-α-glucosidase activity on the most potent compounds using Autodock tools. α-Amylase inhibitory activity and cytotoxicity of the most potent compounds in order to assess the possible side effects were screened and no anti-α-amylase activity and no cytotoxicity were observed. ADME/Toxicity prediction of the most potent compounds and acarbose were also performed.

EXPERIMENTAL

General chemistry. Melting points of acridine-9-carboxamide-1,2,3-triazoles **7a-m** were measured on a Kofler hot stage apparatus. The NMR (^{1}H and ^{13}C) and IR spectra of these compounds 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 **6a-m** were obtained according to our previous work [18].

Synthesis of *N*-(prop-2-yn-1-yl)acridine-9-carboxamide 3. A mixture of acridine-9-carboxylic acid **1** (1 mmol), hydroxybenzotriazole (HOBt, 0.13 g, 1 mmol), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI, 0.17 g, 1.1 mmol) was stirred in dry acetonitrile (10 mL) at room temperature for 30 min. After that, propargylamine **2** (0.05 g, 1 mmol) was added to the latter mixture and the reaction was continued at room temperature. After 24 hours,

acetonitrile was evaporated under vacuum and the obtained residue was dissolved in dichloromethane and washed with sodium carbonate 10%. Dichloromethane solution was dried by anhydrous sodium sulfate (Na_2SO4) and dichloromethane was evaporated to give pure compound **3**.

General procedure for the synthesis of acridine-9-carboxamide-1,2,3-triazole derivatives 7a–m. A mixture of compound **3** (1 mmol), CuSO4 (7 mol%), and sodium ascorbate was added to freshly prepared azides **6a-m** in H_2O and t-BuOH (10 mL, 1:1) and obtained mixture was stirred at room temperature for 16-24 h. Then, the reaction mixture was diluted with cold water, precipitated products **7a-m** 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)acridine-9-carboxamide 7a.** White solid; isolated yield: 89%; mp 166-168 °C; IR (KBr, υ): 3295, 3051, 2926, 1638 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.54 (s, 1H, NH-amide), 8.22 (s, 1H, H-triazole), 8.19 (d, *J* = 8.7 Hz, 2H, Ar), 7.97 (d, *J* = 8.7 Hz, 2H, Ar), 7.88 (ddd, *J* = 8.0, 6.6, 1.3 Hz, 2H, Ar), 7.63 – 7.58 (m, 2H, Ar), 7.41 – 7.34 (m, 5H, Ar), 5.66 (s, 2H, CH2-Ph), 4.75 (s, 2H, CH_2-NH); ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.53, 148.60, 145.07, 142.34, 136.69, 131.06, 129.70, 129.23, 128.60, 128.40, 127.10, 126.06, 123.70, 122.26, 53.27, 35.16; MS (70 eV, m/z [M^+]) = 393; Anal Calcd for $C_{24}H_{19}N_5O$, C, 73.27; H, 4.87; N, 17.80 found: C, 73.28; H, 4.83; N, 17.84.

***N*-((1-(2-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)acridine-9-carboxamide 7b.** White solid; isolated yield: 83%; mp 162-164 °C; IR (KBr, υ): 3289, 3054, 2922, 1630 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.54 (s, 1H, NH-amide), 8.19 (d, *J* = 8.7 Hz, 2H, Ar), 8.09 (s, 1H, H-triazole), 7.97 (d, *J* = 8.6 Hz, 2H, Ar), 7.93 – 7.81 (m, 2H, Ar), 7.66 – 7.51 (m, 2H, Ar), 7.33 – 7.07 (m, 4H, Ar), 5.67 (s, 2H, CH2-Ph), 4.77 (s, 2H, CH_2-NH), 2.34 (s, 3H, Me); ^{13}C NMR (126

MHz, DMSO-d_6) δ 166.51, 148.61, 144.97, 142.34, 136.83, 134.72, 131.06, 130.92, 129.72, 129.16, 128.80, 127.10, 126.72, 126.03, 123.69, 122.26, 51.48, 35.18, 19.11; MS (70 eV, m/z [M^+]) = 407; Anal Calcd for $C_{25}H_{21}N_5O$, C, 73.69; H, 5.19; N, 17.19 found: C, 73.74; H, 5.13; N, 17.14.

***N*-((1-(3-methoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)acridine-9-carboxamide 7c.** White solid; isolated yield: 88%; mp 169-171 °C; IR (KBr, υ): 3289, 3053, 2929, 1632 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.55 (t, *J* = 5.7 Hz, 1H, NH-amide), 8.22 (s, 1H, H-triazole), 8.19 (d, *J* = 8.8 Hz, 2H, Ar), 7.98 (d, *J* = 8.6 Hz, 2H, Ar), 7.90 – 7.86 (m, 2H, Ar), 7.63 – 7.58 (m, 2H, Ar), 7.32 (t, *J* = 7.9 Hz, 1H, Ar), 6.96 – 6.91 (m, 3H, Ar), 5.63 (s, 2H, CH2-Ph), 4.77 (d, *J* = 5.7 Hz, 2H, CH_2-NH), 3.74 (s, 3H, OCH_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.54, 159.93, 148.60, 145.09, 142.35, 138.12, 131.05, 130.39, 129.71, 127.10, 126.06, 123.71, 122.27, 120.48, 114.30, 113.81, 55.56, 53.19, 35.16; MS (70 eV, m/z [M^+]) = 423; Anal Calcd for $C_{25}H_{21}N_5O_2$, C, 70.91; H, 5.00; N, 16.54 found: C, 70.92; H, 5.10; N, 16.48.

***N*-((1-(2-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)acridine-9-carboxamide 7d.** White solid; isolated yield: 87%; mp 180-182 °C; IR (KBr, υ): 3289, 3051, 2924, 1635 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.54 (s, 1H, NH-amide), 8.26 – 8.16 (m, 3H, H-triazole& Ar), 7.98 (d, *J* = 8.6 Hz, 2H, Ar), 7.91 – 7.84 (m, 2H, Ar), 7.65 – 7.58 (m, 2H, Ar), 7.43 (dt, *J* = 19.7, 7.4 Hz, 2H, Ar), 7.32 – 7.22 (m, 2H, Ar), 5.73 (s, 2H, CH2-Ph), 4.76 (s, 2H, CH_2-NH); ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.54, 148.60, 145.03, 142.33, 131.27, 131.20, 131.07, 129.70, 127.11, 126.05, 125.33, 125.31, 123.78, 122.26, 116.20, 116.04, 49.06, 35.14; MS (70 eV, m/z [M^+]) = 411; Anal Calcd for $C_{24}H_{18}FN_5O$, C, 70.06; H, 4.41; N, 17.02 found: C, 70.09; H, 4.36; N, 17.01.

***N*-((1-(3-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)acridine-9-carboxamide 7e.** White solid; isolated yield: 85%; mp 218-220 °C; IR (KBr, υ): 3297, 3044, 2918, 1638 cm^{-1}; ^{1}H NMR (500

MHz, DMSO-d_6) δ 9.65 – 9.44 (m, 1H, NH-amide), 8.27 (s, 1H, H-triazole), 8.19 (d, *J* = 8.7 Hz, 2H, Ar), 7.99 (d, *J* = 9.2 Hz, 2H, Ar), 7.91 – 7.82 (m, 2H, Ar), 7.64 – 7.57 (m, 2H, Ar), 7.49 – 7.41 (m, 1H, Ar), 7.20 (t, *J* = 8.8 Hz, 3H, Ar), 5.70 (s, 2H, CH2-Ph), 4.77 (s, 2H, CH_2-NH); ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.54, 148.61, 145.15, 142.37, 139.42, 139.36, 131.36, 131.29, 131.05, 129.71, 127.08, 126.06, 124.49, 124.47, 123.89, 122.27, 115.54, 115.38, 115.30, 115.13, 52.58, 49.06, 35.16; Anal Calcd for $C_{24}H_{18}FN_5O$, C, 70.06; H, 4.41; N, 17.02 found: C, 70.14; H, 4.32; N, 17.03.

***N*-((1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)acridine-9-carboxamide 7f.** White solid; isolated yield: 83%; mp 173-175 °C; IR (KBr, υ): 3288, 3047, 2928, 1639 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.53 (t, *J* = 5.4, 4.9 Hz, 1H, NH-amide), 8.22 (s, 1H, H-triazole), 8.19 (d, *J* = 8.8 Hz, 2H, Ar), 7.98 (d, *J* = 8.7 Hz, 2H, Ar), 7.88 (t, *J* = 7.2 Hz, 2H, Ar), 7.65 – 7.56 (m, 2H, Ar), 7.48 – 7.39 (m, 2H, Ar), 7.24 (t, *J* = 8.8 Hz, 2H, Ar), 5.65 (s, 2H, CH2-Ph), 4.75 (d, *J* = 5.7 Hz, 2H, CH_2-NH); ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.53, 148.60, 145.11, 142.34, 132.93, 131.06, 130.80, 130.73, 129.71, 127.10, 126.07, 123.60, 122.26, 116.15, 115.98, 52.47, 35.16; Anal Calcd for $C_{24}H_{18}FN_5O$, C, 70.06; H, 4.41; N, 17.02 found: C, 70.01; H, 4.47; N, 17.06.

***N*-((1-(2-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)acridine-9-carboxamide 7g.** White solid; isolated yield: 81%; mp 214-216 °C; IR (KBr, υ): 3291, 3056, 2927, 1638 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.57 (d, *J* = 5.8 Hz, 1H, NH-amide), 8.20 (s, 1H, H-triazole), 8.18 (d, *J* = 2.9 Hz, 2H, Ar), 7.99 (d, *J* = 8.6 Hz, 2H, Ar), 7.91 – 7.86 (m, 2H, Ar), 7.65 – 7.60 (m, 2H, Ar), 7.56 (dd, *J* = 7.7, 1.4 Hz, 1H, Ar), 7.44 – 7.37 (m, 2H, Ar), 7.31 (dd, *J* = 7.6, 1.6 Hz, 1H, Ar), 5.78 (s, 2H, CH2-Ph), 4.78 (d, *J* = 5.6 Hz, 2H, CH_2-NH); ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.55, 148.61, 144.98, 142.33, 133.86, 133.16, 131.07, 131.03, 130.76, 130.14, 129.71, 128.20,

127.12, 126.05, 124.01, 122.27, 51.12, 35.16; Anal Calcd for $C_{24}H_{18}ClN_5O$, C, 67.37; H, 4.24; N, 16.37 found: C, 67.41; H, 4.23; N, 16.40.

***N*-((1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)acridine-9-carboxamide 7h.** White solid; isolated yield: 84%; mp 190-192 °C; IR (KBr, υ): 3293, 3052, 2921, 1637 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 8.69 (t, *J* = 5.7 Hz, 1H, NH-amide), 7.38 (s, 1H, H-triazole), 7.34 (d, *J* = 8.7 Hz, 2H, Ar), 7.14 (d, *J* = 8.7 Hz, 2H, Ar), 7.03 (ddd, *J* = 8.1, 6.6, 1.3 Hz, 2H, Ar), 6.79 – 6.71 (m, 2H, Ar), 6.62 (d, *J* = 8.5 Hz, 2H, Ar), 6.55 (d, *J* = 8.6 Hz, 2H, Ar), 4.82 (s, 2H, CH_2-Ph), 3.92 (d, *J* = 6.1, 5.6 Hz, 2H, CH_2-NH); ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.55, 148.61, 145.14, 142.35, 135.68, 133.36, 131.06, 130.41, 129.72, 129.24, 127.10, 126.07, 123.73, 122.27, 52.48, 35.18; Anal Calcd for $C_{24}H_{18}ClN_5O$, C, 67.37; H, 4.24; N, 16.37 found: C, 67.31; H, 4.28; N, 16.29.

***N*-((1-(2-bromobenzyl)-1H-1,2,3-triazol-4-yl)methyl)acridine-9-carboxamide 7i.** White solid; isolated yield: 81%; mp 157-159 °C; IR (KBr, υ): 3285, 3054, 2920, 1639 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 9.65 – 9.47 (m, 1H, NH-amide), 8.19 (d, *J* = 8.7 Hz, 3H, H-triazole& Ar), 8.00 (d, *J* = 8.6 Hz, 2H, Ar), 7.89 (t, *J* = 7.6 Hz, 2H, Ar), 7.73 (d, *J* = 7.9 Hz, 1H, Ar), 7.63 (t, *J* = 7.5 Hz, 2H, Ar), 7.43 (t, *J* = 7.4 Hz, 1H, Ar), 7.33 (t, *J* = 7.6 Hz, 1H, Ar), 7.25 (d, *J* = 7.5 Hz, 1H, Ar), 5.76 (s, 2H, CH2-Ph), 4.79 (d, *J* = 5.8, 4.6 Hz, 2H, CH_2-NH); ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.55, 148.60, 145.00, 142.32, 135.46, 133.41, 131.07, 130.96, 130.92, 129.71, 128.75, 127.13, 126.05, 124.01, 123.41, 122.27, 53.44, 35.17; Anal Calcd for $C_{24}H_{18}BrN_5O$, C, 61.03; H, 3.84; N, 14.83 found: C, 61.06; H, 3.78; N, 14.79.

***N*-((1-(3-bromobenzyl)-1H-1,2,3-triazol-4-yl)methyl)acridine-9-carboxamide 7j.** White solid; isolated yield: 83%; mp 193-195 °C; IR (KBr, υ): 3288, 3051, 2917, 1622 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 9.55 (t, *J* = 5.6 Hz, 1H, NH-amide), 8.27 (s, 1H, H-triazole), 8.19 (d, *J* = 8.7

Hz, 2H, Ar), 7.99 (d, *J* = 8.3 Hz, 2H, Ar), 7.90 – 7.85 (m, 2H, Ar), 7.63 – 7.59 (m, 3H, Ar), 7.58 – 7.55 (m, 1H, Ar), 7.37 (d, *J* = 5.0 Hz, 2H, Ar), 5.68 (s, 2H, CH2-Ph), 4.77 (d, *J* = 5.6 Hz, 2H, CH_2-NH); ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.54, 148.60, 145.17, 142.34, 139.30, 131.51, 131.45, 131.24, 131.06, 129.71, 127.56, 127.12, 126.06, 123.85, 122.34, 122.27, 52.44, 35.16; Anal Calcd for $C_{24}H_{18}BrN_5O$, C, 61.03; H, 3.84; N, 14.83 found: C, 61.07; H, 3.86; N, 14.82.

***N*-((1-(4-bromobenzyl)-1H-1,2,3-triazol-4-yl)methyl)acridine-9-carboxamide 7k.** White solid; isolated yield: 85%; mp 176-178 °C; IR (KBr, υ): 3292, 3057, 2920, 1639 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.55 (s, 1H, NH-amide), 8.27 – 8.10 (m, 3H, H-triazole& Ar), 7.98 (d, *J* = 8.5 Hz, 2H, Ar), 7.89 (t, *J* = 7.3 Hz, 2H, Ar), 7.62 (d, *J* = 8.0 Hz, 4H, Ar), 7.34 (d, *J* = 7.9 Hz, 2H, Ar), 5.66 (s, 2H, CH2-Ph), 4.76 (s, 2H, CH2-NH); ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.52, 148.61, 145.14, 142.33, 136.07, 132.15, 131.02, 130.70, 129.71, 127.08, 126.05, 123.71, 122.27, 121.90, 52.54, 35.17; Anal Calcd for $C_{24}H_{18}BrN_5O$, C, 61.03; H, 3.84; N, 14.83 found: C, 61.11; H, 3.82; N, 14.88.

***N*-((1-(3-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methyl)acridine-9-carboxamide 7l.** White solid; isolated yield: 81%; mp 222-224 °C; IR (KBr, υ): 3293, 3056, 2911, 1639 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.56 (t, *J* = 5.5 Hz, 1H, NH-amide), 8.34 (s, 1H, H-triazole), 8.30 (s, 1H, Ar), 8.25 – 8.16 (m, 3H, Ar), 8.00 (d, *J* = 8.6 Hz, 2H, Ar), 7.90 – 7.85 (m, 2H, Ar), 7.82 (d, *J* = 7.7 Hz, 1H, Ar), 7.71 (t, *J* = 7.9 Hz, 1H, Ar), 7.63 – 7.57 (m, 2H, Ar), 5.85 (s, 2H, CH2-Ph), 4.79 (d, *J* = 5.6 Hz, 2H, CH_2-NH); ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.57, 148.61, 148.36, 145.23, 142.34, 138.79, 135.19, 131.05, 130.89, 129.71, 127.10, 126.08, 124.02, 123.61, 123.30, 122.28, 52.23, 35.17; Anal Calcd for $C_{24}H_{18}N_6O_3$, C, 65.75; H, 4.14; N, 19.17 found: C, 65.72; H, 4.18; N, 19.15.

***N*-((1-(4-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methyl)acridine-9-carboxamide 7m.** White solid; isolated yield: 83%; mp 194-196 °C; IR (KBr, υ): 3298, 3050, 2926, 1631 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 9.61 – 9.47 (m, 1H, NH-amide), 8.31 (d, *J* = 1.6 Hz, 1H, H-triazole), 8.28 – 8.23 (m, 2H, Ar), 8.19 (d, *J* = 9.6 Hz, 2H, Ar), 8.01 (d, *J* = 8.7 Hz, 2H, Ar), 7.91 – 7.85 (m, 2H, Ar), 7.65 – 7.56 (m, 4H, Ar), 5.85 (s, 2H, CH2-Ph), 4.79 (d, *J* = 5.4 Hz, 2H, CH_2-NH); ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.57, 148.61, 147.72, 145.21, 144.09, 142.33, 131.06, 129.72, 129.51, 127.13, 126.09, 124.38, 124.17, 122.28, 52.38, 35.19; MS (70 eV, m/z [M^+]) = 438; Anal Calcd for $C_{24}H_{18}N_6O_3$, C, 65.75; H, 4.14; N, 19.17 found: C, 65.68; H, 4.17; N, 19.09.

***In vitro* α-glucosidase inhibition assay and kinetic study.** α-Glucosidase inhibition assay of acridine-9-carboxamide-1,2,3-triazoles **7a-m** and kinetic study of most potent compound **7j** were performed on yeast α-glucosidase (*Saccharomyces cerevisiae*, EC3.2.1.20, 20 U/mg) according to previous works [18-21].

Docking study. For docking study, since *Saccharomyces cerevisiae* form of α-glucosidase has not any crystallographic structure in protein data bank (PDB), homology model of it was constructed by using SWISS-MODEL Repository according to our previous paper [19].

***In vitro* α-amylase inhibition assay.** The α-amylase inhibitory activity of most potent compounds was screened against porcine pancreatic α-amylase by a reported colorimetric method and acarbose was used as reference drug [20].

***In vitro* cytotoxicity assay.** *In vitro* cytotoxicity assay of most potent compounds was determined by MTT assay according to literature [26]. For this study, two cell lines HDF and MCF-7 as normal and cancer cells and etoposide as positive control were used.

***In silico* ADME/T study.** *In silico* ADME/Tox studies of most potent compounds and standard drug acarbose were performed using the preADMET online server (http://preadmet.bmdrc.org/) [25].

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