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Synthesis, in vitro and computational studies of 1,4-disubstituted 1,2,3-triazoles as potential α-glucosidase inhibitors

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ABSTRACT

1,4-Disubstituted-1,2,3-triazoles were synthesized by Cu(I) catalyzed click reaction, where the azides, with electron donating and electron withdrawing groups acted as 1,3-dipoles and 1-ethynyl-1-cyclohexanol served as the terminal alkyne. These synthesized triazoles were subjected to enzymatic assay which showed promising activity against α-glucosidase; 1-(2-cyano-4-nitrophenyl)-4-(1-hydroxycyclohexyl)-1*H*-1,2,3-triazole **3m** being the most active members of the library. Molecular docking studies of these triazoles with the homology-modeled α-glucosidase protein were also performed to delineate ligand–protein interactions at molecular level which suggested that Phe157, Arg312 and His279 are the major interacting residues in the biding site of the protein and may have a significant role in the inhibition of enzyme's function.

Glucosidases are responsible for the hydrolytic cleavage of glycosidic bonds of oligosaccharides. The catalytic specificity of glucosidases depends on the number of monosaccharides, the position of cleavage site, and the configuration of the hydroxyl groups in the substrate.[1] The most extensively studies among the glucosidases are α- and β-glucosidases which differ in the orientation of carboxylic acid side chains during catalysis.[2] α-Glucosidase (EC 3.2.1.20) has drawn a special interest to the pharmaceutical research community because of its catalytic activity resulted in the retardation of glucose absorption and the decrease in postprandial blood glucose level.[3–5] Diabetes mellitus is one of the most widespread chronic metabolic disorder and the frequent prevalence and drastic incidence of hyper- and hypo-glycemia affects the quality of life of people globally. It is, therefore, imperative to develop safe and efficacious medicines for treating type 2 diabetes mellitus (T2DM). The α-glucosidase inhibitors act by a reversible inhibition of α-glucosidase by interacting with the active center of α-glucosidase.[6]

α-Glucosidase has also been well appreciated as a therapeutic target for other carbohydrate mediated diseases including cancer,[7] viral infections,[7,8] hepatitis and Alzheimer's disease.[9] These include newly identified synthetic compounds,[10–22] transition state analogs,[12] and natural products isolated from various species.[23–25]

The importance of triazoles in medicinal chemistry is undeniable. Particularly, 1,2,3-triazoles have grabbed attention in the drug-discovery field since the inception of the 'click' chemistry concept by Sharpless.[26–28] Rossi et al. and Basu et al.[29] reported the synthesis of 1-glycosyl-4-phenyltriazoles linked to the anomeric carbon atom of a sugar unit, exhibiting a strong Ki for β-galactosidase of *Escherichia coli*. In another work, Perion et al.[30] reported the synthesis of 6-deoxyglucosyltriazoles (linked via **C4** of the sugar unit) with a 2-fold higher inhibitory activity against yeast *R*-glucosidase (K_i = 73 μM) than acarbose.[31]

Huisgen's 1,3-dipolar [3+2] cycloaddition[32] of alkynes and azides is one of the most common synthetic strategies to approach 1,2,3-triazoles. However, this cycloaddition leads to a regioisomeric mixture of 1,4- and 1,5-disubstituted-1,2,3-triazoles. A major breakthrough in the area of [3+2] cycloaddition, by Sharpless in 2002,[33] has made it highly efficient process even in complex chemical and biological environments.[34]

It was, therefore, envisaged to design and synthesize 1,4-disubstituted-1,2,3-triazoles. Copper azide alkyne cycloaddition (CuAAC) click strategy is employed for the synthesis of

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1,4-disubstituted-1,2,3-triazoles for its advantages of being efficient, easy-to-use, regioselective, hazard-free and excellent yielding.[35,36]

Herein, we synthesized a library of 1-aryl-4-(1-hydroxycyclohexyl)-1,2,3-triazoles by a copper-catalyzed [3+2] cycloaddition of aryl azides with 1-ethynyl-1-cyclohexanol by following click chemistry.

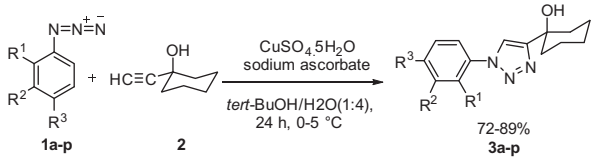
Most of the α-glucosidase inhibitors reported in the literature stem either from the isolation of new scaffolds by high throughput screening (HTS) or the generation of the improved derivatives of pre-existing inhibitors. Three-dimensional (3D) structures of proteins provide valuable insights into the molecular basis of protein function. However, the lack of 3D structure of human α-glucosidases limits the mechanistic and pharmacological studies of this enzyme. Indeed, the structural information of α-glucosidases has been reported merely for a few bacterial and fungal strains only in ligand-free forms.[37,38] This limitation has made it a difficult task to discover good lead compounds against α-glucosidases. Comparative modeling is the only method among all current theoretical approaches that can reliably generate a 3D model of a protein (drug target) from its amino acid sequence in an affordable time frame. Protein folding simulations by ab initio methods is another established method; however, it is not very practical due to high time and computation demands.[39,40]

In this study, we have identified a novel class of α-glucosidase inhibitors in a systematic manner starting with the synthesis of non-glycosidic triazoles, in vitro enzyme assay and homology modeling of the receptor (α-glucosidase) followed by the molecular docking to investigate ligand, enzyme interactions and to rationalize in vitro results obtained in the wet lab. We applied molecular docking studies with the aim to predict new α-glucosidase inhibitors and putative ligands that can reduce the prostglandal blood glucose level in vivo, thereby helping in addressing diabetic related issues.

Various aryl, heteroaryl and aliphatic azides (**1a**–**s**) were prepared by following previous reported methods.[41,42] The synthesized azides (**1a**–**p**) treated with 1-ethynyl-1-cyclohexanol in presence Cu(I) catalyst which was generated in situ by the reduction of $CuSO_4{\cdot}5H_2O$ with sodium ascorbate to afford 1-aryl-(1-hydroxycyclohexyl)-1,2,3-triazoles (**3a**–**p**) (Scheme 1).[43] Similarly compounds **3q**–**s** from azides **1q**–**s** and 1-ethynylcyclohexanol.[44] Besides using 1-ethynylcyclohexanol, phenyl acetylene was also used for the synthesis of **3t** and **5a**–**l**[45–53] as shown in Schemes 2 and 3, respectively.

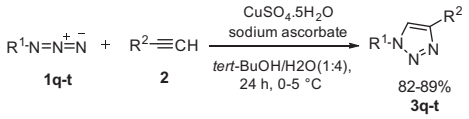
Another Set of triazoles **6a**–**f** with the aliphatic chain at C-4 was synthesized by one pot protocol using an alkyl halide and NaN_3 as given in Scheme 4.[41] All the synthesized compounds were characterized by spectroscopic techniques.

In brief, α-glucosidase (Sigma, type III, from yeast) was dissolved in buffer A (0.1 mol/L potassium phosphate, 3.2 mmol/



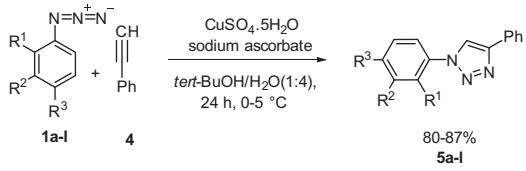
3a: R^1 = Cl; R^2 = H; R^3 = H
3b: R^1 = H; R^2 = Cl; R^3 = H
3c: R^1 = H; R^2 = H; R^3 = Cl
3d: R^1 = H; R^2 = H; R^3 = Br
3e: R^1 = H; R^2 = OCH_3; R^3 = H
3f: R^1 = H; R^2 = H; R^3 = OCH_3
3g: R^1 = H; R^2 = H; R^3 = CH_3
3h: R^1 = Cl; R^2 = H; R^3 = CH_3
3i: R^1 = F; R^2 = H; R^3 = CH_3
3j: R^1 = NO_2; R^2 = H; R^3 = H
3k: R^1 = H; R^2 = NO_2; R^3 = H
3l: R^1 = H; R^2 = H; R^3 = NO_2
3m: R^1 = CN; R^2 = H; R^3 = NO_2
3n: R^1 = $COCH_3$; R^2 = H; R^3 = H
3o: R^1 = H; R^2 = $COCH_3$; R^3 = H
3p: R^1 = H; R^2 = H; R^3 = $COCH_3$

Scheme 1. Synthesis of -triazoles **3a**–**p**.



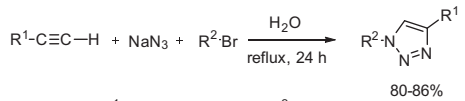
3q: R^1 = 2-naphthyl; R^2 = 1-cyclohexyl-1-ol
3r: R^1 = 4-pyridyl; R^2 = 1-cyclohexyl-1-ol
3s: R^1 = $-CH_2CO_2CH_3$; R^2 = 1-cyclohexyl-1-ol
3t: R^1 = $-CH_2CO_2CH_3$; R^2 = phenyl

Scheme 2. Synthesis of -triazoles **3q**–**t**.



5a: R^1 = Cl; R^2 = H; R^3 = H
5b: R^1 = H; R^2 = Cl; R^3 = H
5c: R^1 = H; R^2 = H; R^3 = Cl
5d: R^1 = H; R^2 = H; R^3 = Br
5e: R^1 = H; R^2 = OCH_3; R^3 = H
5f: R^1 = NO_2; R^2 = H; R^3 = H
5g: R^1 = H; R^2 = NO_2; R^3 = H
5h: R^1 = H; R^2 = H; R^3 = NO_2
5i: R^1 = CN; R^2 = H; R^3 = NO_2
5j: R^1 = $COCH_3$; R^2 = H; R^3 = H
5k: R^1 = H; R^2 = $COCH_3$; R^3 = NO_2
5l: R^1 = H; R^2 = H; R^3 = $COCH_3$

Scheme 3. Synthesis of triazoles **5a**–**l**.



6a: R^1 = 1-cyclohexyl-1-ol; R^2 = CH_2Ph
6b: R^1 = 1-cyclohexyl-1-ol; R^2 = C_4H_9
6c: R^1 = 1-cyclohexyl-1-ol; R^2 = C_6H_{13}
6d: R^1 = 1-cyclohexyl-1-ol; R^2 = C_8H_{17}
6e: R^1 = 1-cyclohexyl-1-ol; R^2 = $C_{16}H_{33}$
6f: R^1 = phenyl; R^2 = $C_{16}H_{33}$

Scheme 4. Synthesis of triazoles **6a**–**f**.

L-$MgCl_2$, pH 6.8) (0.1 units/ml), *p*-nitrophenyl-α-D-glucopyranoside dissolved in buffer A at 6 mmol/L was used as substrate. 102 μL buffer B (0.5 mol/L potassium phosphate, 16 mmol/L-$MgCl_2$, pH 6.8), 120 μL sample solution (0.6 mg/ml in DMSO), 282 μL water and 200 μL substrate were mixed. This mixture was incubated in a water bath at 37 °C for 5 min and then 200 μL enzyme solution was added and mixed. The enzyme reaction was carried out at 37 °C for 30 min followed by the addition of 1.2 ml 0.4 mol/L glycine buffer (pH, 10) to terminate the reaction. Enzyme activity was quantified by measuring the absorbance at 410 nm. Deoxynojirimycin hydrochloride (DNJ) was used as standard inhibitor.[54]

The synthesized library of triazoles was subjected to α-glucosidase inhibition assay. The IC_{50} values and percent inhibition of these compounds are given in Table 2. Compounds **3m** and **5a** proved themselves as potent inhibitors while **3a**, **3e**, **3l**, **3o** and **5k** inhibited α-glucosidase inhibitors from good to moderate extent. These results were corroborated by molecular docking studies with their α-glucosidase inhibitory potential.

The protein data bank (pdb) contains a number of entries for α-glucosidase enzymes; however, all of them belong to strains other than yeast. Therefore, it is more realistic to model a 3D structure of yeast α-glucosidase directly from its amino acid sequence to have a direct comparison with the results obtained from in vitro assays. The BLAST program[55] was used that identified the pdb entry 3A47[56] as a top hit with the maximum sequence identity of over 72% with the target protein. In the earlier studies 1UOK.pdb was used as a template which has only 38.5% of sequence identity with