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Research paper

Design, synthesis and biological evaluation of small molecules as potent glucosidase inhibitors

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ABSTRACT

Herein we have reported design, synthesis and *in vitro* biological evaluation of a library of bicyclic lactams that led to the discovery of compounds **6** and **7** as a novel class of α-glucosidase inhibitors. They inhibited α−glucosidase (yeast origin) in a mixed type of inhibition with an IC_{50} of ~150 nM. Molecular docking studies further substantiated screening results. Interestingly phenotypic screening of this library against the human malaria parasite revealed **7** as a potent antiplasmodial agent.

1. Introduction

Diabetes mellitus is one of the most common non-communicable diseases of the globe [1,2]. The prevalence of diabetes is estimated to double by 2030 with 69% increase in developing countries and 20% increase among adults in developed countries. Despite phenomenal progress in medical science, diabetes continues to be a major killer [3].

Diabetes is a disease characterized by chronic hyperglycemia that leads to development of macro and micro vascular complication [4]. Hence, one of the therapeutic approaches in type 2 diabetes is to reduce the demand for insulin by lowering the corresponding postprandial hyperglycemic levels *via* inhibition of enzymes in the digestive organs such as the α-glucosidases [5]. Research has shown that inhibition of α-glucosidase enzyme located at the intestinal brush border of the intestine may play a role in the lowering of postprandial hyperglycemia [5].

To date, the only reported inhibitors of glycosidase enzymes are either complex natural products, like Validoxylamine A, MDL 25637 (α-homonojirimycin-7-*O*-β-D-glucopyranoside), trehazolin, acarbose, nojirimycin, castanospermineetc., or synthetic analogues like DNJ, N-butyl-DNJ, DANA and others [6]. Iminosugars and thiosugar derivatives are also one of the most promising glycosidase inhibitors [7]. However there are not very many non-sugar synthetic compounds as potent glycosidase inhibitors [8–15]. Hence, discovery of such molecules will have a niche appeal and applicability in the pharmaceutical world.

Herein we report our investigation involving identification of a small molecule inhibitor of α-glucosidase *via* routine glucosidase profiling of small molecule libraries unbiased towards any specific targets. This exercise resulted in generating a potential hit compound **1** possessing bicyclic lactam motif. Design and synthesis of a focused library of bicyclic lactams around this motif with diverse functionalities followed by *in vitro* screening against yeast α-glucosidase enzyme generated **6** and **7** as most potent compounds with IC_{50} ~150 nM. Reaction kinetics suggested that they are mixed inhibitors.

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In addition phenotypic screening against *3D7* strain of Plasmodium falciparum revealed decent antiplasmodial activity of **7**.

2. Results and discussion

2.1. Initial screening

To begin with we subjected our in-house compound collection, unbiased towards any specific targets in a routine glycosidase panel profiling. The exercise resulted in generating **1** as an interesting hit molecule with IC_{50} of 2 μM against yeast α-glucosidase. **1** was obtained by the dihydroxylation of the previously reported cyclic enelactam **2** [16].

2.2. Library design and synthesis

In a bid to generate the library from **1** we envisioned **8,** the diastereomer of **1,** as a library member followed by 6-membered bicyclic lactams **9** and **10** as the higher homologs of **1**. Additionally, we choose **3** and **5** where oxygen is located differently than in **1**. Also included in the library are the dehydroxylated analogue **6** and the unsaturated analogues **7** and **2** (the immediate precursor of **1**). We further envisioned that epoxide analogues **4** and **11** will streamline the SAR, hence they were synthesized too (Fig. 4). In a bid to diversify the aromatic moiety of the bicyclic lactams, benzyl and indoline substituted bicyclic lactams **12** and **13** were incorporated. Finally we envisioned a set of dimeric bicyclic lactams **14**–**17** in our library. By virtue of possessing diverse biological activity the dimeric compounds have lately gained lot of importance in the pharmaceutical world and that prompted their inclusion in this focused library (Fig. 1)

Synthesis of compounds **3**, **4**, **5** and **10** were accomplished following literature procedures [17–19]. Compound **2** was obtained from bicyclic lactam **18**, by phenyl selenium bromide mediated addition-reduction followed by oxidation of the arylselenium intermediate with hydrogen peroxide. **18** in turn was obtained by condensing *S*-pyroglutaminol and benzaldehyde with catalytic *p*-toluenesulfonic acid (PTSA) in refluxing toluene. Dihydroxylation of **2** in presence of *N*-methylmorpholine-N-Oxide and osmium tetraoxide in 1:1 acetone: water afforded **1**. In a different reaction, epoxidation of **2** with meta-perbenzoic acid (mCPBA) resulted in **4**. In a similar effort compound **6** (synthesized from *R*-pyroglutaminol and benzaldehyde) was converted to **7**. And a similar dihydroxylation of **7** yielded **8**. Epoxidation of **7** provided **11**. Simple condensation of *S*-phenylalaninol and *S*-Tryptophanol with 2-(2-nitrophenyl)-4-oxopentanoic acid in toluene in catalytic *p*-toluene sulfonic acid furnished **12** and **13**. Finally oxidative homoenolate coupling of bicyclic lactams **5**, **18**, **19** and **20** in presence of lithium bis(trimethylsilyl)amide (2.1 eq.) and 2.1 eq. of the oxidant iodine (I_2) afforded **14**–**17**. The synthesis of the library was designed in a way where each member of the library can be obtained in minimum number of steps and with high steps/molecule ratio (~1.7), thereby making the library generation more facile (Fig. 2).

2.3. In vitro assay against α-glucosidase (yeast origin)

We screened all the compounds against α-glucosidase enzyme of yeast origin along with acarbose, a known α-glucosidase inhibitor, as the positive control. The percentage inhibition on the α-glucosidase was measured at a concentration of 10 μM–50 μM (refer *SI* for experimental details). To assess the potency of our compounds, the inhibitory activities were plotted against the concentrations using non-linear regression approach (sigmoid curves) (cf. *SI*) from which their IC_{50} values were computed (Table 1). Among the compounds screened, **6** and **7** exhibited strong inhibition against α-glucosidase with IC_{50}s of 0.17 and 0.15 μM respectively, faring much better than acarbose control (900 nM). Compounds **2** and **8** also bind effectively with IC_{50} ranging between 0.19 & 0.21 μM. To further probe the inhibitory effect of our compounds, we obtained their dose responses against α-glucosidase. The results showed that the activity of α-glucosidase was reduced by majority of our compounds (**2**, **6**, **7** and **8**) in a dose-reliant fashion thereby indicating their strong affinity towards the enzyme (Fig. 3).

2.4. Molecular docking and modelling

With the IC_{50} values in hand, we wanted to understand the binding interactions of the initial compound **1** and most active compounds **6** and **7** with α–glucosidase. This would enable us to design more active analogues of **1**. Even though the X-ray crystallographic structure of α-glucosidase has been reported for some bacteria, the three dimensional structural information is not available for the yeast α-glucosidase enzyme. Hence we constructed the 3D structure of α-glucosidase by homology modelling. The sequence of α-glucosidase was downloaded from Uniprot (ID: P10253). BLASTP® against protein data bank was performed in order to identify the template for sequence alignment. Human Maltase-Glucoamylase (PDB ID: 2QLY, 3L4T) were showing good similarity to our query sequence [20]. Residues starting from 89 are aligning to these PDB and showing 44% identity. These two protein structures were therefore selected to model α-glucosidase using homology modelling. The homology model of α-glucosidase was built using Modeller v9.14. Three models were generated using Modeller v9.14 and the model having best DOPE score is selected for further studies [21]. The energy of the model was minimized using MOE energy minimization algorithm using Force Field MMFF94x. The stereochemical quality of this model was validated by the Ramachandran plot using the PROCHECK program. 98.1% residues are in the favoured and allowed regions, only 1.9% are outliers and no active site residue lie in this outlier region. Further the model is also validated using verify 3D program (Fig. 3).

Molecular modelling of **1** revealed that it was bound deeply into the binding cavity of α-glucosidase and showed interaction with the residues Lys 609, Asp 724, Leu 613, Gln 688, Ile 726, Ala 610, Thr 725, Gln 575, Phe 579, Val 728 & Asp 686 residues. **1** was found to form a hydrogen bond with the δ-amino moiety of Lys 609 and carbonyl group of amide moiety of Asp 724 (see Fig. 5).

Next both compounds **6** and **7** were docked against the protein (Figs. 6 and 7). **6** and **7** bind to the active site residues of modelled α-glucosidase protein by forming one hydrogen bond and few other non-bonded interactions. For **6,** the hydrogen bond is formed with Asp 686 residue while His 729, Asn 727, Val 728, Thr 725, Pro 722, Ile 726, Gln 688, Leu 719, Pro 720 residues shared non-bonded interactions. Similarly **7** binds to the active site residues of modelled α-glucosidase protein by the hydrogen bond formed with Asp 686 residue while Thr 725, Gln 688, His 729, Pro 722, Ile 726, Val 728, Asn 727, Leu 719, Pro 720 residues shared non-bonded interactions. **6** and **7** showed similar binding with the receptor but the energy of the docked model is different. **1** showed stronger binding as compared to **6** and **7** but the stability of docked model is better in case of **6** and **7**. This may be the reason why **6** and **7** demonstrated better activity than **1** (Fig. 6a and b).

2.5. Reaction kinetics study

Enzyme kinetic assays were conducted with the most active compounds *viz.* **6** and **7** to understand their mode of inhibition to α-glucosidase. This was determined by employing primary