BIOLOGICAL EVALUATION AND MOLECULAR MODELING OF PEPTIDOMIMETIC COMPOUNDS AS INHIBITORS FOR O-GLCNAC TRANSFERASE (OGT)

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Introduction

O-linked N-acetylglucosamine (O-GlcNAc) is a ubiquitous, single N-acetyl-glucosamine sugar that cycles on and off serine or threonine residues in nuclear, cytoplasmic, and mitochondrial proteins. O-GlcNAc is added to proteins by a single enzyme, O-GlcNAc transferase (OGT), while the enzyme O-GlcNAcase (OGA) removes the modification. The synthesis of UDP-GlcNAc, the substrate for OGT, occurs through the hexosamine biosynthetic pathway. Furthermore, OGT activity is sensitive to a wide range of UDP-GlcNAc concentrations. Due to the O-GlcNAcylation important in metabolic diseases as cancer and diabetes, the number of studies focused to unveil OGT catalysis increased (Santos et al., 2018). Studies on OGT's catalysis mechanism allowed the discovery of numerous enzymatic inhibitors, therefore, most of OGT inhibitors available until now are limited due to several factors such as lack of specificity, cytotoxicity, or solubility (Makwana et al., 2019). The present work focuses on the study of peptidomimetic compounds derived from isomannide and isosorbide as a novel class of competitive inhibitors of OGT.

Method

The derivative compounds were previously synthesized by our group (Muri, 2012; Oliveira et al., 2013). Biological assays were performed using the recombinant hOGT enzyme, followed by the protocol described by Albuquerque (2019). The molecular docking study was performed using GOLD 5.2.2 program (Jones et al., 1997) with Goldscore function. The X-ray crystal structure of hOGT (PDB:5NPS, 0.68Å) was used for the molecular docking experiment and the 3D structure isomannide and isosorbide derivatives were built up using Spartan v10 software (Dewar et al., 1985). The binding site was defined as a 10Å sphere for the nitrogen of the imidazole group from His901 (ND1His901) and the number of genetic algorithm run was set at 10. The molecular dynamics simulations (MDS) were performed to the best pose obtained by molecular docking study using the GROMACS 2019 program (Berendsen et al., 1995) with the Charmm36 force field (Huang et al., 2016).

Results / Discussion

Two isomannide derivatives (LQMed 269 and 330) showed activity against OGT enzyme with the half maximal inhibitory concentration (IC_{50}) of 11.7 and 159μM, respectively. The LQMed 330 exceeded the IC_{50} of the Alloxan (100μM) (Konrad et al., 2002) and the activity was comparable with two potent *in vitro* inhibitors found in the literature: UDP-5SGlcNAc (8μM) (Gloster et al., 2011), and OSMI-1 (2.7μM)

(Ortiz-Meoz et al., 2015). The molecular docking study of the LQMed 330 showed hydrogen bond interactions with N-Catalytic (Asn557 and His562) and C-Catalytic domain (Gln839, Lys898, Thr922, and Asp925), which form the catalytic binding site. The MDS was conducted for 80ns, which showed a great stability for the OGT α-carbon atoms of N-cat and C-cat domains, whereas the LQMed 330 had a slight movement into the biding site (2.86 ± 0.39Å). The analyses of the hydrogen bond in the OGT330 complexes over to 80ns of MDS showed that the most important interactions occurred with residues Gln839 and Lys898 with a lifetime greater than 70%, while interactions with residues Asn557 and Thr922 occurred with a lifetime less than 10% but dispersed throughout MDS. Therefore, it is possible to suggest that the maintenance of interactions by hydrogen bonding with residues at the N-cat domain could be one of the explanations for the high inhibition activity of LQMed 330.

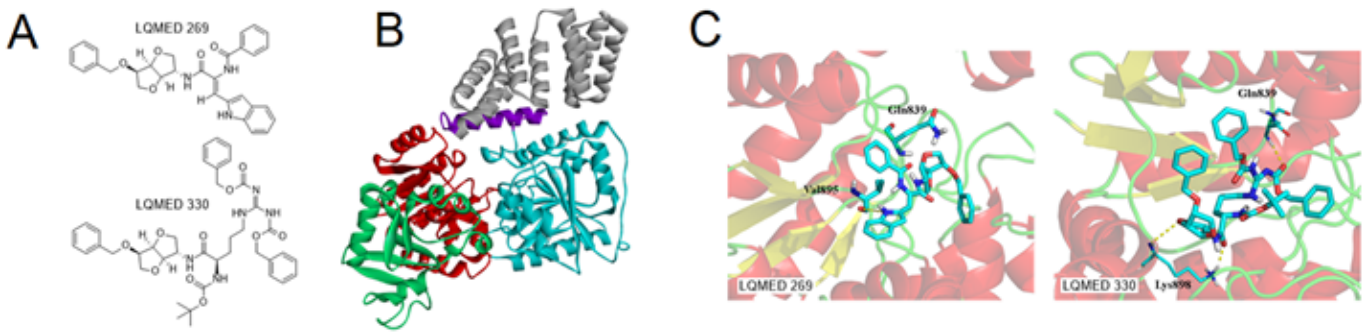

Figure 1 - A) Structures of active compounds. B) OGT structure. C) OGT-Compound interactions.

Conclusion

In conclusion, we report the discovery of new peptidomimetic-based hOGT inhibitors, LQMed 269 and LQMed 330, the latter presenting a lower IC_{50} value compared to the known Globin1 and Alloxan. We carried out molecular docking and MDS that allowed us to identify the main hydrogen bond interactions and dynamic behavior in the OGT-inhibitor complexes. The MDS, indicated that the hOGT binding site accepts bulky inhibitors without affecting the stability of the enzyme. The identification of LQMed 330 allows us to consider it as a new lead compound for future design of OGT inhibitors.

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