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Design and synthesis of O-GlcNAcase inhibitors via 'click chemistry' and biological evaluations

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ABSTRACT

Protein O-GlcNAcylation has been shown to play an important role in a number of biological processes, including regulation of the cell cycle, DNA transcription and translation, signal transduction, and protein degradation. O-GlcNAcase (OGA) is responsible for the removal of O-linked β-*N*-acetylglucosamine (O-GlcNAc) from serine or threonine residues, and thus plays a key role in O-GlcNAc metabolism. Potent OGA inhibitors are useful tools for studying the cellular processes of O-GlcNAc, and may be developed as drugs for the treatment neurodegenerative diseases. In this study, Cu(I)-catalyzed 'Click' cycloaddition reactions between glycosyl azides and alkynes were exploited to generate inhibitory candidates of OGA. Enzymatic kinetic screening revealed that compound **7** was a potent competitive inhibitor of human O-GlcNAcase (K_i = 185.6 μM). Molecular docking simulations of compound **7** into *Cp*OGA (*Clostridium perfringens* OGA) suggested that strong π–π stacking interaction between the compound and W490 considerably contributed to improving the inhibitory activity.

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1. Introduction

The covalent attachment of a single *N*-acetylglucosamine moiety onto serine or threonine residues of proteins via a β-O-glycosidic linkage is termed the O-GlcNAc modification (O-GlcNAcylation). O-GlcNAcylation was discovered 26 years ago by Torres and Hart,[1] and it is one of the most common post-translational modifications related to cellular regulation and signal transduction.[1–3] So far, more than 1000 proteins involved in cellular processes (transporting, transcription, cell shaping, cell signaling and apoptosis[4–6]) have been identified to be O-GlcNAcylated. These proteins include nuclear pore proteins, cytoskeletal proteins, transcription factors, oncogenic proteins, tumor suppressor proteins and so on. It has been demonstrated that O-GlcNAcylation plays a significant role in many fundamental cellular processes, and its dysregulation contributes to the etiology of cardiovascular diseases, type-2 diabetes, cancer, and neurological disorders.[7–10] Especially, O-GlcNAcylation is closely related to Alzheimer's disease (AD). Many AD-associated proteins are modified by O-GlcNAc and phosphorylation, including tau, neurofilaments, and beta-amyloid precursor protein (APP).[11] Hyperphosphorylated tau accumulates into insoluble paired helical filaments, which is a major characteristic of AD. In the diseased brain, tau is hyperphosphorylated and underglycosylated at same Ser/Thr site as Thr231 and Ser396.[12]

The hydrolytic cleavage of O-GlcNAc from O-GlcNAcylated proteins is catalyzed by O-GlcNAcase (OGA). OGA contains two discrete domains: an N-terminal glycoside hydrolase domain and a C-terminal domain with histone acetyltransferase activity (Fig. 1).[13] According to primary sequence similarity, the N-terminal domain is classified into the glycoside hydrolase family 84 (http://www.cazy.org/),[14] OGA uses a catalytic mechanism involving substrate-assisted catalysis that relies on the involvement of the 2-acetamido group of the substrate to form a transient oxazoline intermediate (Fig. 2).[15–17] Asp^{174} and Asp^{175} have been identified as two key residues of human OGA involved in the de-GlcNAcylation.[16,18] In the first step, Asp^{174} directs and polarizes the 2-acetamido group to act as a nucleophile and form the oxazoline intermediate. Meanwhile, Asp^{175} acts as a general acid to facilitate departure of the aglycone leaving group. The following step is nearly the microscopic reverse of the first step. Asp^{174} facilitates departure of the 2-acetamido group, while Asp^{175} acts as a general base, promoting the attack of one molecule of water to yield the β-hemiacetal product.[16] Since O-GlcNAcylation is directly regulated by OGA, modulation of O-GlcNAc levels with small molecule inhibitors of OGA is a useful strategy to probe the role of this O-GlcNAcylation in a range of cellular processes. PUGNAc (Fig. 3) a nanomolar inhibitor of OGA (K_i = 46 nM), was recently reported by Vocadlo and co-workers.[15] However, PUGNAc lacked selectivity

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Figure 1. Sketch map of OGA.

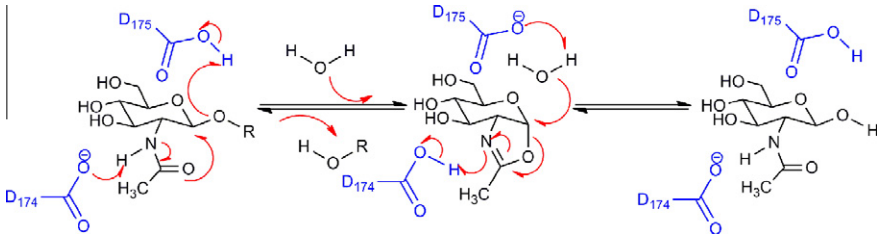


Figure 2. The proposed catalytic mechanism of OGA uses substrate-assisted catalysis involving a two-step double-displacement mechanism via forming a transient oxazoline intermediate.

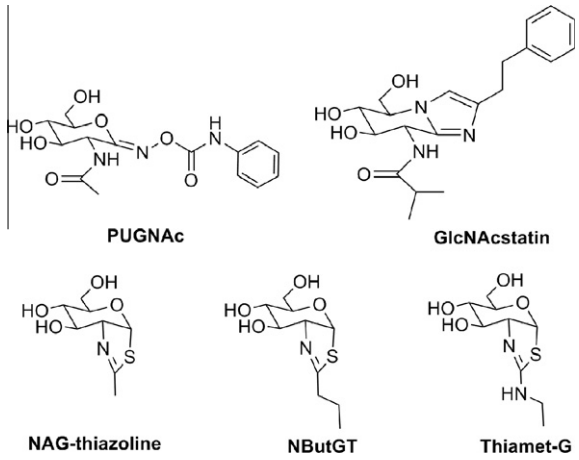


Figure 3. Some previously reported inhibitors of O-GlcNAcase.

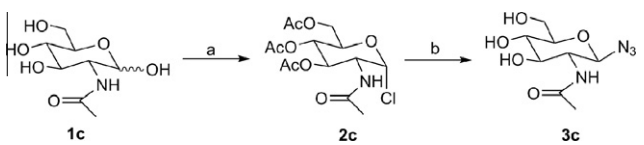
and did not cross the blood brain barrier.[15,19] GlcNAcstatin (Fig. 3) has been found to be an extremely potent inhibitor of a bacterial homolog of OGA from *Clostridium perfringens* (K_i = 4.6 pM).[20] Subsequently, Dorfmueller found that GlcNAcstatin showed a K_i value of 4.4 nM toward human OGA, and it also displayed 125-fold selectivity for human OGA over β-hexosaminidase A and β-hexosaminidase B (Hex A and B, K_i = 550 nM). In addition, GlcNAcstatin was shown to be a cell-permeant compound that modulated O-GlcNAcylation levels within the cells by inhibiting human OGA.[21] NAG-thiazoline (Fig. 3), which has an obvious geometric resemblance to the oxazoline intermediate, has been found to be a potent inhibitor of OGA (K_i = 70 nM) by virtue of its geometric mimicry of the transition state.[15,22] Unfortunately, NAG-thiazoline lacked selectivity and therefore perturbed multiple cellular processes. In order to improve the selectivity of NAG-thiazoline, NButGT (Fig. 3), which was generated by varying the bulk of the thiazoline substituent, displayed 800-fold selectivity for human OGA (K_i = 600 nM) over Hex A and B.[15,23] Although this inhibitor had reasonable potency and good selectivity, it had limited chemical stability in the solution over extended periods of a few days to weeks.[15,23] Thiamet-G (Fig. 3) has been identified as a potent inhibitor of OGA (K_i = 21 nM), which displayed exquisite 37,000-fold selectivity for OGA over Hex A and B.[23] Moreover, Thiamet-G was highly stable and effective in cell culture with an EC_{50} of 30 nM for increasing O-GlcNAc levels in PC12 rat pheochromocytoma cells.[23]

Huisgen 1,3-dipolar cycloadditions[24] are exergonic fusion processes that connect two unsaturated reactants and afford fast access to an enormous variety of five-membered heterocycles.[25] The Cu(I)-catalyzed [3+2] azide–alkyne cycloaddition reaction to generate triazole is the most useful member of this family. It provides an expedient method to connect azides and alkynes in high yields under mild conditions.[26,27] Moreover, the triazole moiety usually has favorable physicochemical properties, which are capable of interacting with the biological targets through hydrogen bonding, dipole–dipole, and π-stacking interactions. This reaction has become a powerful tool in generating combinatorial libraries,[28,29] and increasing the application of bioconjugation, discovery of the lead compound and optimization of the lead compound.[30,31] For example, recently, a click-based library of protein tyrosine phosphatase (PTP) inhibitor candidates was prepared via copper(I)-assisted 'click chemistry', in which a compound with an IC_{50} value of 4.7 μM against PTP1B was screened out.[32] Rossi and Basu prepared 1-glycosyl-4-phenyl triazoles via this reaction, some of which can inhibit the activity of certain glycosidases.[33] Poulsen and co-workers generated a novel class of carbonic anhydrase inhibitors that were glycoconjugate benzene sulfonamides prepared by 'click-tailing'.[34] Lee identified a potent and highly selective inhibitor of human α-1,3-fucosyltransferase by screening a GDP-triazole library synthesized via 'click chemistry'.[35] Herein, we described a rapid parallel synthesis of a small triazole-linked carbohydrate library via 'click chemistry'. Subsequent screening and evaluation revealed a potent inhibitor of human OGA.

2. Results and discussion

2.1. Synthesis of inhibitory candidates via 'click chemistry'

Glycosyl azide (Scheme 1, compound **3c**) was a key intermediate for the synthesis of glycosyl triazoles through the Cu(I)-catalyzed [3+2] azide–alkyne cycloaddition reaction. 2-Acetamido-2-deoxy-D-glucose (**1c**) was chosen as the starting material, which was reacted with acetyl chloride to afford 3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-glucopyranosyl chloride (**2c**) in a yield of 72%.[36] Subsequently, the desired glycosyl azide (**3c**) was prepared by nucleophilic substitution of compound **2c** with sodium



Scheme 1. Reagents and conditions: (a) AcCl (12 equiv), rt, 24 h, 72%; (b) Bu_4NHSO_4 (1 equiv), $NaHCO_3$ (satd), CH_2Cl_2, NaN_3 (3 equiv), rt, 1 h, 59%.

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