DIAMINOCYCLOPENTANE GLYCOMIMETICS AS SELECTIVE *O*-GlcNAcASE INHIBITORS: NEW APPROACH TO ALZHEIMER DISEASE TREATMENT

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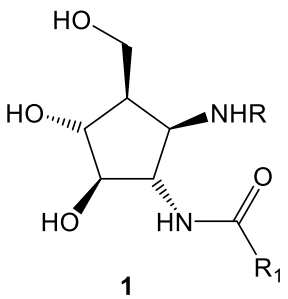
Alzheimer's disease (AD) is the most prevalent form of senile dementia, accounting for 60-80% of cases in people over the age of 65. Amidst the intricate tapestry of AD, two cardinal pathological features predominate: the formation of neurotoxic amyloid β-plaques and the emergence of neurofibrillary tangles. The latter arises from the **abnormal hyperphosphorylation of tau protein**. This **pathological phosphorylation can be prevented by selective inhibition of *O*-GlcNAcase** (*O*-*N*-acetyl-β-D-glucosaminidase, GH84), which already advanced to clinical trials. **A new class of compounds**, namely highly **substituted diaminocyclopentanes** including L-lysine adducts (**1**), have been discovered as potent inhibitors of human *O*-GlcNAcase, an enzyme crucial for protein de-*O*-glycosylation [1-3]. These inhibitors with K_i **at low nanomolar concentrations** exhibit exceptional selectivity and reversibility and are the first example of human *O*-GlcNAcase inhibitors that are structurally related to the transition state (coined as “extended substrate mimetics”) of the rate-limiting step with the “aglycone” still in bond-length proximity. The new inhibitors proved a high selectivity over human β-hexosaminidase B (100 000 ×), negligent toxicity up to 1 mM (HepG2; Balb/3T3 cells), and **increased protein *O*-GlcNAcylation in murine neuronal astrocyte cell** culture. Selected compounds were shown to pass the blood-brain-barrier model of brain microvascular cells (hCMEC/D3) and human astrocytes (P10251-IM-HA). Tests on **human brain organoids** (over)producing human β-amyloid protein bearing trisomy at chromosome 21 clearly proved increasing GlcNAc-ylation and a decrease in phosphorylation of the tau protein. Pilot experiments with the **transgenic mouse model P301S** (PS19), which carries the T34 isoform of the microtubule-associated protein tau, showed a decrease in tau protein levels in the brain after intracranial administration of the compound.



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References:

1. Weber, P., Bojarová, P., Křen, V., Stütz, A. E., Wrodnigg, T. M., *et al. Bioorg. Chem.* **2024**, *148*, 107452.
2. Weber, P., Bojarová, P., Křen, V., Stütz, A. E., Wrodnigg, T. M., *et al.* Bioorg. Chem. **2023**, *59*, 10404.
3. Weber, P., Mitrečić, D. Bojarová, P., Slámová, K., Křen, V., Stütz A. E., *et al.*. Chem. Comm. **2022**, *58*, 8838.