MS8 – O1: Amino-thiadiazole as innovative inhibitors of human glutaminyl cyclase for the treatment of Alzheimer disease

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Glutaminyl cyclase is a zinc-dependent metallo-enzyme that catalyzes the formation of pyroglutamic acid (pGlu) from the N-terminal glutaminyl and glutamyl precursors of several bioactive peptides and proteins. Owing to the involvement in the formation of pGlu-modified amyloid peptides, human glutaminyl cyclase (hQC) represents a validated target for the treatment of neurodegenerative pathologies like Alzheimer disease (AD) and various forms of dementia including familial British and Danish dementias [1]. Amyloid β (Aβ) peptide, bearing a N-terminal pyroglutamate in position 3 [AβN3(pE)], has been reported as major N-truncated, modified constituent of intracellular, extracellular and vascular Aβ deposits in AD brain tissue [2]. AβN3(pE) forms soluble oligomers that favor the aggregation processes, also promoting the aggregation of nonmodified peptides [2].
Various attempts to express hQC in *Escherichia coli* resulted in serious drawbacks in terms of ease of production and very low yield of purified protein. To overcome these problems, we generated a hQC variant in which two surface residues implicated in protein aggregation were mutated into glutamic acids resulting in a soluble protein variant that provided very high protein yields in the bacterial expression system (~ 80 mg L^{-1} bacterial culture) [3]. Kinetic and structural analysis on this hQC variant revealed that it has conserved properties and structure compared to those of the native enzyme. Here, we are reporting the X-ray crystallographic screening of a library of amino-thiadiazole derivatives formerly developed towards a parasite enzyme target belonging to the folate pathway [4]. Some of these molecules have shown good activities when tested in kinetic inhibition assays toward our engineered variant. More than twenty structures of enzyme-inhibitor complexes have been determined providing key information about the interactions guiding the recognition of these innovative molecules in the hQC active site. Furthermore, the binding mode of these compounds, driven by the coordination of the amino-thiadiazole molecular core to the zinc(II) ion in the catalytic cavity, is quite peculiar and it does not resemble any inhibitor reported to date in the literature. The structural information achieved during this X-ray crystallographic screening will drive us in the development of more potent hQC inhibitors as new molecular tools for the treatment of neurodegenerative pathologies like Alzheimer disease.

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