Discovering new promising inhibitors of human D-aspartate oxidase for D-aspartate modulation in schizophrenia

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D-aspartate (D-asp) contributes, with D-serine, to the neurotransmission of N-methyl-D-aspartate receptors (NMDAR) in mammalian brain. Neurotransmission impairment is typical of some psychiatric and neurodevelopmental pathologies like schizophrenia, where depletion of D-asp is caused by overexpression of its catabolic enzyme human D-aspartate oxidase (hDASPO). In this context, hDASPO is considered an interesting target for the development of new inhibitors in attempt to modulate excessive D-asp depletion and improve neurotransmission in patients. Olanzapine is a weak inhibitor of hDASPO and was chosen as scaffold to rationally synthetize new derivatives with better inhibitory activity. The first intermediate of olanzapine synthesis, 2-amino-5-methylthiophene-3-carbonitrile (AC-51), represents new promising inhibitor (IC_{50} = 5 μM). Understanding the molecular interactions between hDASPO and this new inhibitor is crucial to guide further rational design and achieve better potency. To this aim, the wild type hDASPO was co-crystallized with AC-51 and molecular replacement was applied to diffraction data exploiting the available crystallographic structure (PDB: 6RKF) as a search model (previously published in Molla G et al. (2020) The FASEB Journal. 2020; 34: 1182–1197). The best crystal diffracted at 2.5 Å resolution, showing a C2221 symmetry, with six molecules in the asymmetric unit. However only four out of six protein molecules display electron density in the active site and further evaluations are needed to understand the nature of the bound ligands. Another issue is the poor reproducibility of the crystals. In the hDASPO crystals, protein molecules form dimers thanks to the presence of a phosphate molecule at the protein-protein interface. Thus, we propose to insert site-specific mutations that could stabilize the proteins towards a dimeric form with the aim of favouring crystallization and improving the order and stability of the crystal lattice.

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