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

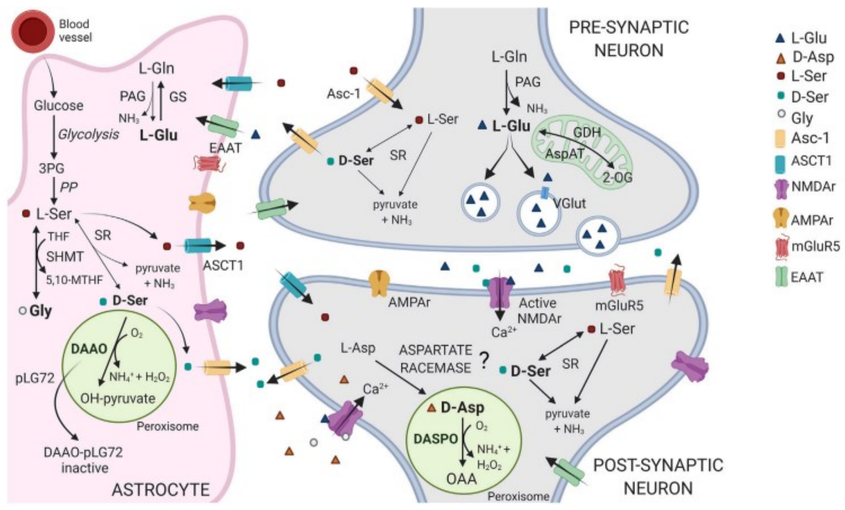
M. Cavinato*[1], A. Citarella*[2], V. Rabattoni*[3], H. Shehi*[1], D. Passarella[2], L. Pollegioni[3], M. Nardini[1]

[1]Department of Biosciences, University of Milan, Milan, Italy, [2]Department of Chemistry, University of Milan, Milan, Italy, [3]The Protein Factory 2.0 lab, DBSV, University of Insubria, Milan, Italy

1.INTRODUCTION

D-Aspartate (D-Asp) is a co-agonist of *N*-methyl D-Aspartate receptors stimulating neurotransmission in mammal brain. Notably, D-Asp levels are high in embryonal development and significantly decrease after birth due to the catabolic activity of hDASPO, indeed high expression levels of the enzyme are observed in rat models during post-natal development and DDO$^-$ individuals show higher levels of D-Asp due to gene knock-out[1].

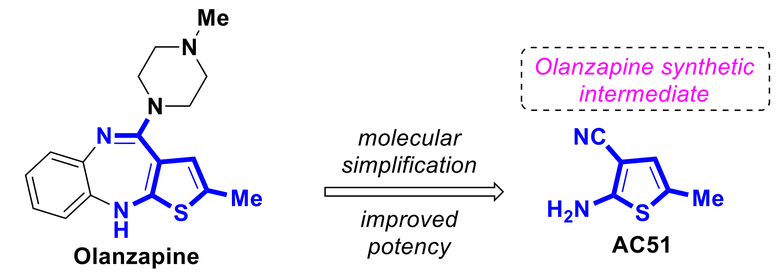
Depletion of D-Asp causes neurotransmission hypoactivation, that can contribute to schizphrenia symptoms. Severe reduction in D-Asp amount in *post-mortem* prefrontal cortex and striatum tissues samples was found in schizophrenia patients, while increased D-Asp levels are reported to be beneficial by improving neuronal plasticity[2]. Therefore, an innovative therapeutic approach is represented by the modulation of D-Asp in brain through hDASPO inhbition.

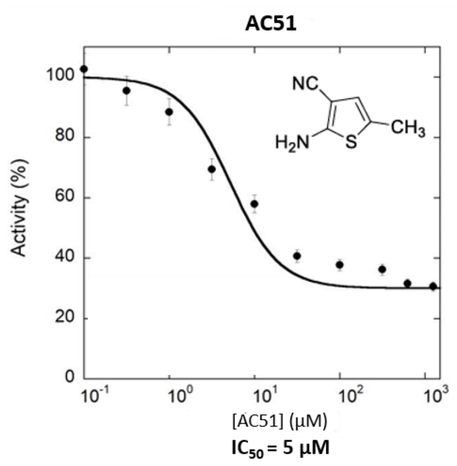


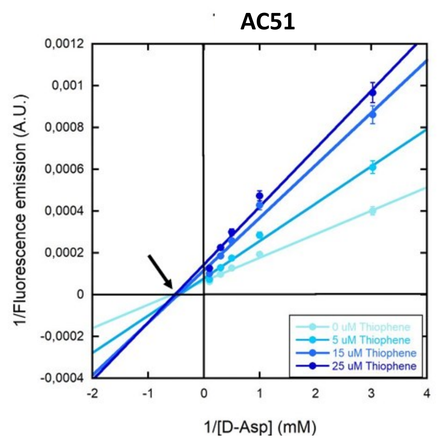
Pollegioni et al., Cell. Mol. Life Sci. (2010) 67:2387–2404

2. OLANZAPINE DERIVATIVES AS NEW hDASPO INHIBITORS

AC51 was extrapolated from olanzapine, a known hDASPO inhibitor, through molecular simplification approach. AC51 shows a better inhibitory activity (IC_{50} = 5 µM) than olanzapine (IC_{50} = 23.4 µM[3]) but the same non-competitive inhibition mechanism.

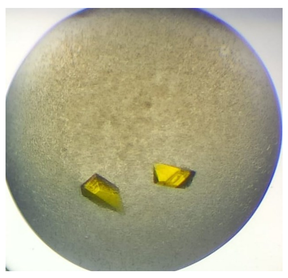






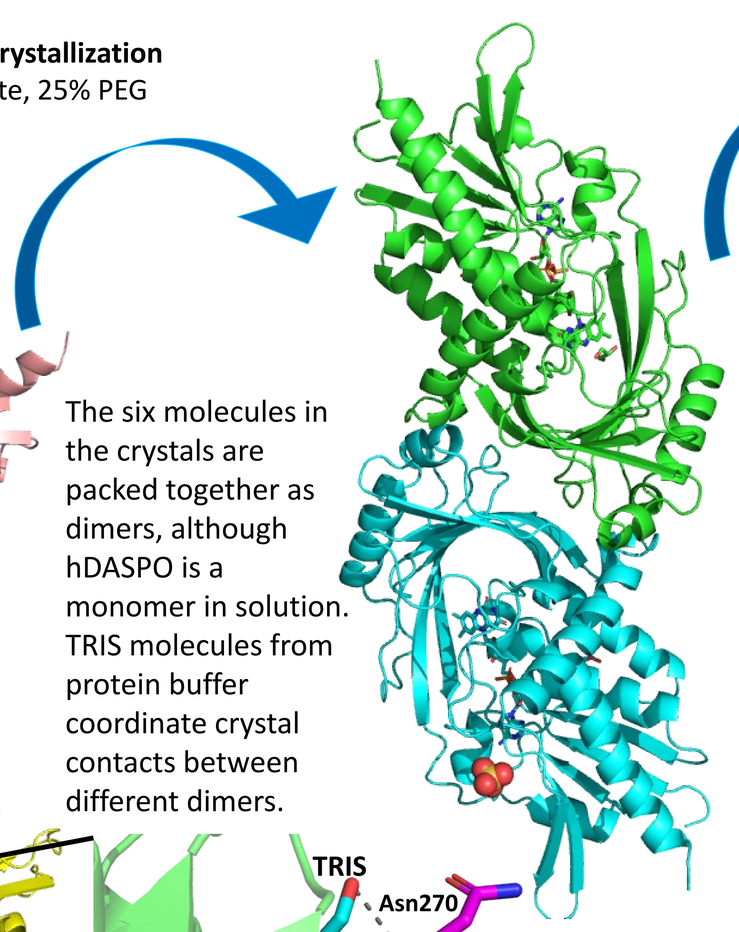
By increasing AC51 concentration only V_{MAX} changes indicating a **non-competitive mechanism** of inhibition

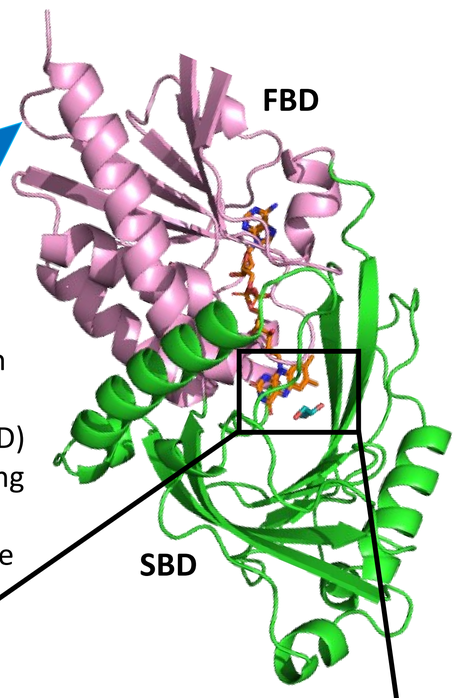
3. STRUCTURAL CHARACTERIZATION OF WILD TYPE hDASPO



WT hDASPO - AC51 co-crystallization
170 mM ammonium sulfate, 25% PEG 4000, 15% glycerol

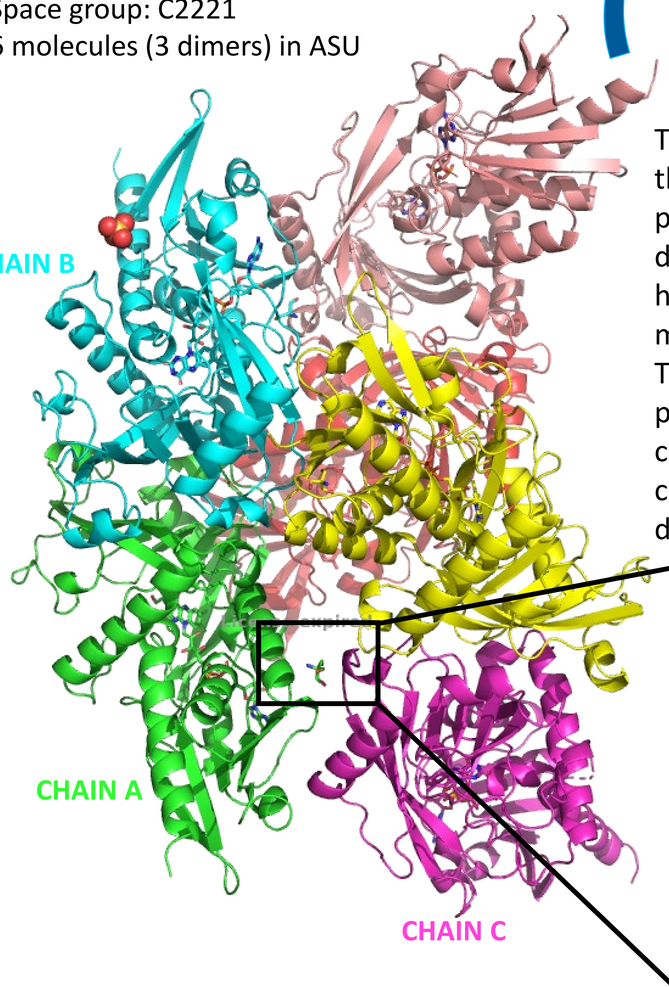
- Resolution: 2.5 Å
- Space group: C2221
- 6 molecules (3 dimers) in ASU



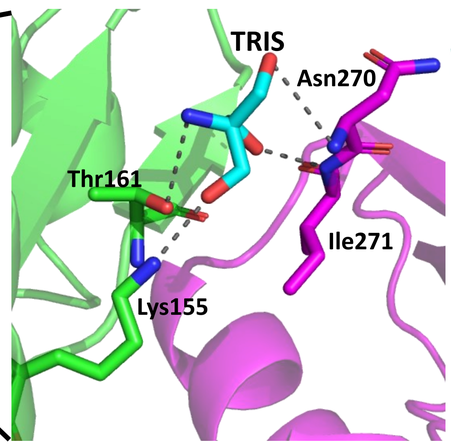


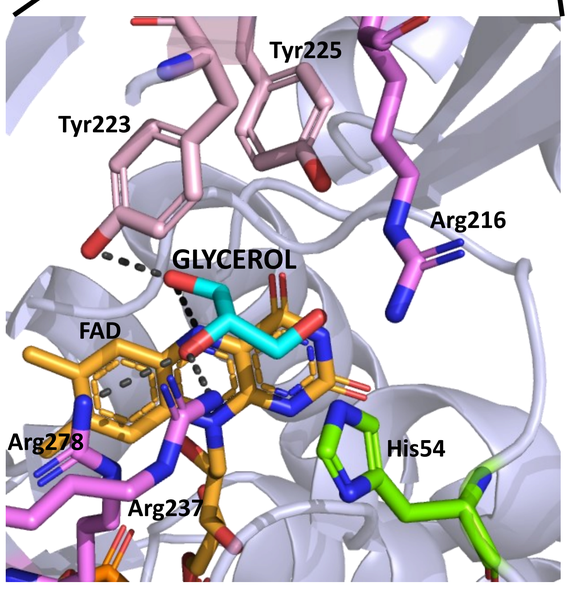
hDASPO monomer (40kDa) is divided in two domains: FAD binding domain (FBD) and substrate binding domain (SBD) containing the active site[4].

In the active site polar and positively charged residues (His54, Arg216, Tyr223, Tyr225, Arg237, Arg278, Ser308) can attract and accommodate the negatively charged (D-Asp) substrate. Hydrophobic residues (Ile52, Met50, Ala48) surround the pocket[4].



The six molecules in the crystals are packed together as dimers, although hDASPO is a monomer in solution. TRIS molecules from protein buffer coordinate crystal contacts between different dimers.





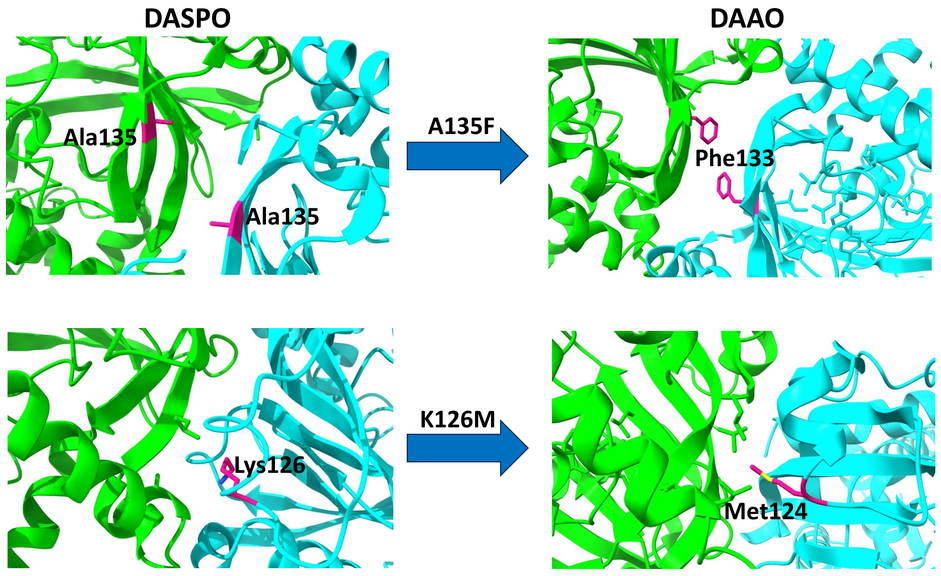
A glycerol molecule is present in the active site, stabilised by Tyr223, Arg237, Arg278 and FAD.

4. CONCLUSIONS AND FUTURE PERSPECTIVE

- AC51 displayed an IC_{50} = 5 µM overcoming the inhibitory activity of olanzapine (IC_{50} = 23.4 µM).
- AC51 inhibits hDASPO with a non-competitive mechanism.
- Wild type hDASPO structure was solved at 2.5 Å by molecular replacement exploiting 6RKF (*Molla et al., 2020*) as search model.
- Glycerol was modeled in the active site, mimicking the bound substrate.
- Although wild type hDASPO was co-crystallized with AC51, a corresponding electron density for the inhibitor was not clearly identified.

- AC51 and olanzapine represent new promising scaffolds to synthetise new inhibitors.
- Wild type hDASPO will be co-crystallized or soaked with high affinity inhibitors.
- Since a «dimeric» form is found in the crystal, mutations will be designed at the dimerization interface (based on the homologous D-aminoacid oxidase (DAAO)) to favour the dimeric form in solution with the aim and improve crystal quality.

Dimerization interface



Two of the proposed hDASPO mutants are designed to recover an hydrophobic environment present in DAAO by introducing Phe and Met at positions 135 and 126 of hDASPO, respectively.

REFERENCES

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3. Sacchi et al., (2017) Scientific Reports, 7
4. Molla et al., *FASEB Journal* (2020), *34*(1), 1182–1197

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