Inhibitors and PROTACs: two alternative strategies to modulate endogenous D-Aspartate targeting human D-Aspartate Oxidase

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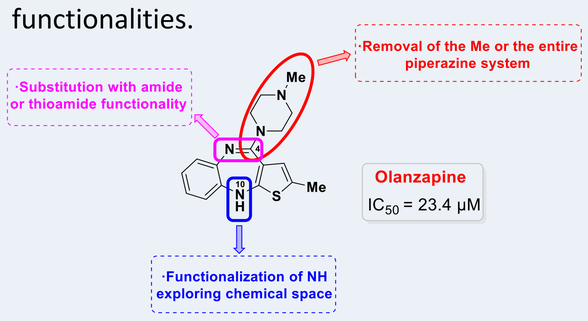
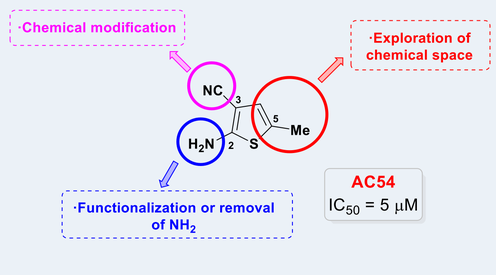
D-Aspartate and DASPO in schizophrenia

D-Aspartate (D-Asp) plays a role as co-agonist in stimulating N-methyl D-Aspartate receptors (NMDARs) in mammalian brain. Neurotransmission hypo-stimulation in schizophrenia is associated with reduced levels of D-Asp, due to the overexpression of the catabolic flavoenzyme D-Aspartate oxidase (DASPO), while a supplement of D-Asp is reported to be beneficial by improving neuronal plasticity in patients[1]. Therefore, targeting DASPO to modulate endogenous D-Asp levels represents an innovative strategy to counteract schizophrenia symptoms. Two strategies are proposed: the rational design of DASPO inhibitors and the activation of DASPO degradation through proteolysis targeting chimeras (PROTACs).

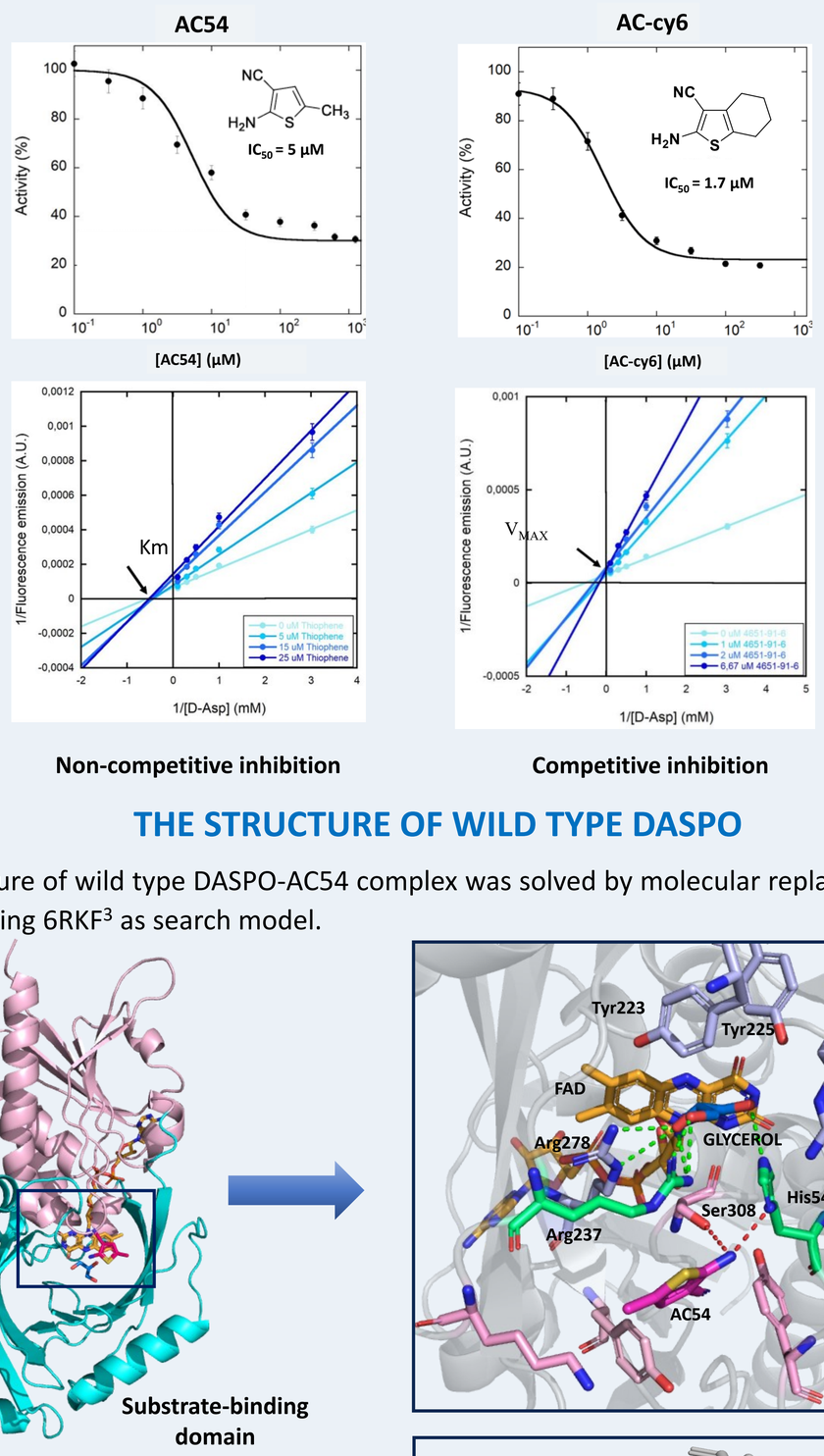
DESIGN AND TESTING NEW DASPO INHIBITORS

Olanzapine (IC_{50} = 23.4 µM[2]) and its first synthesis intermediate 2-amino-5-methylthiophene-3-carbonitrile (AC54) (IC_{50} = 5 µM) are promising DASPO inhibitors, thus a vast library of derivatives was synthetized by adding new chemical functionalities.





In Amplex UltraRed assay, AC-cy6 showed improved activity (IC_{50} = 1.7 µM) respect to AC54. In addition, AC-cy6 showed a competitive type of inhibition, while AC54 showed a non-competitive mechanism.



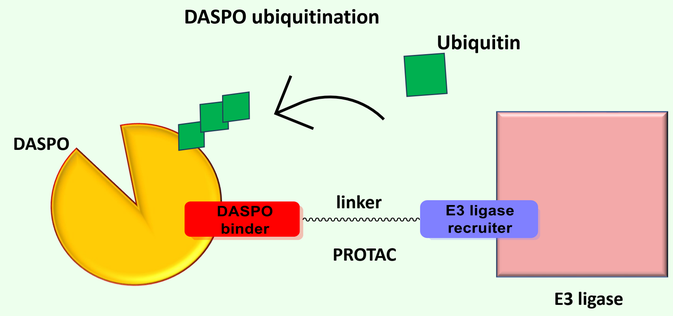
THE STRUCTURE OF WILD TYPE DASPO

The structure of wild type DASPO-AC54 complex was solved by molecular replacement at 2.5 Å using 6RKF[3] as search model.

A glycerol molecule was modeled in the active site of DASPO molecules, interacting with His54, Arg237, Arg278.
AC54 (magenta) was modeled in a density right in front of the active site stabilising the closed conformation of His54 and contacting Ser308.

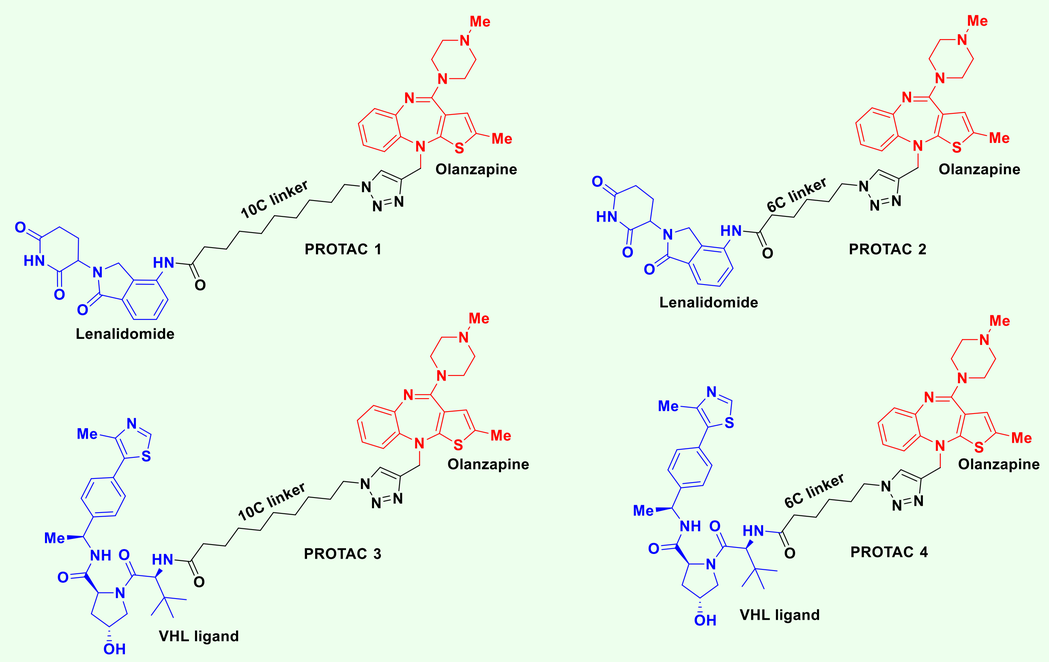
THE PROTAC TECHNOLOGY

PROTACs are dual compounds consisting of a binder for the protein of interest (DASPO) and a E3 ligase recruiter bound together by a linker[4]. Olanzapine was chosen as DASPO binder, while lenalidomide and VHL were selected as E3 ligase recruiters. The two partners were linked together by aliphatic chains of different length. In this way olanzapine should bind to DASPO and the E3 ligase recruiter should attract E3 ligase to promote ubiquitination for subsequent degradation of DASPO.



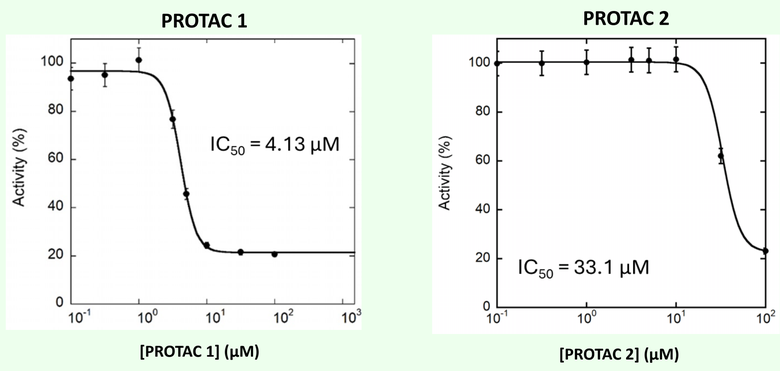
PROTAC SYNTHESIS

Click chemistry[5] was exploited to link olanzapine, carrying an alkyne moiety, to lenalidomide or VHL ligand bearing the azide group at the end of the linker. Four PROTACs were synthesized.



BIOLOGICAL EVALUATION

The well established Amplex UltraRed assay protocol was used to evaluate the binding of PROTACs to DASPO by extrapolating IC_{50} values. PROTACs bearing lenalidomide as E3 ligase recruiter showed binding and interesting activity.



CONCLUSIONS AND FUTURE PERSPECTIVES

- ✓ AC-cy6 showed the best activity (IC_{50} = 1.7 µM) against DASPO and will be evaluated in cell lines as well as AC54 and olanzapine.
- ✓ Inhibition mechanism studies showed non-competitive inhibition for AC54, while AC-cy6 showed competitive inhibition.
- ✓ The structure of wild type DASPO-AC54 complex was obtained at 2.5 Å and DASPO will be co-crystallized or soaked with high affinity inhibitors.
- ✓ Four PROTACs were successfully synthesized, and preliminary assays show that PROTAC 1 can bind better to DASPO (IC_{50} = 4.3 µM). Binding constants will be better evaluated with appropriate methods (MST, ITC).
- ✓ In future, the formation of the ternary complex and the ability of PROTACs to promote DASPO degradation in cells will be evaluated .

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1. Errico et al., Journal of Psychiatric Research (2013); 2. Sacchi et al., Scientific Reports (2017) ; 3. Molla et al., FASEB Journal (2020), 4. Chirnomas et al., Nat. Rev. Clinical Oncology (2023) 5. Li et al., JMedChem (2023)