In search of inhibitors of hDASPO as new target for schizophrenia and autism spectrum disorders

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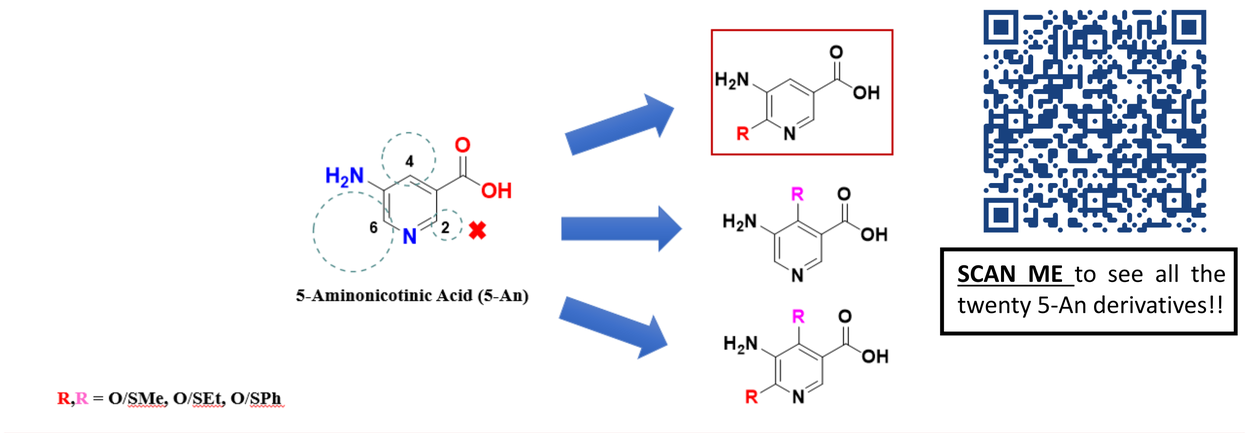
1. INTRODUCTION

D-aspartate is present in brain as N-methyl D-aspartate receptor (NMDAR) co-agonists for neurotransmission. Great interest is focussed on D-Asp metabolism in brain as disequilibrium, due to incorrect expression of catabolic enzyme human D-Aspartate oxidase (hDASPO), leads to hypo- or hyperactivation of NMDAR neurotransmission contributing to psychiatric and neurodevelopmental disorders like schizophrenia (SCZ) or autism spectrum disorders (ASD) respectively[1]. The 3D structure of hDASPO allowed to find potential inhibitors for the development of new therapies against SCZ. 5-animonicotinic acid (5-An) and olanzapine were indicated as inhibitors in the micromolar range[2],[3] , thus we started exploring 5-An functionalization in parallel with the synthesis of olanzapine reaction intermediates and evaluate their inhibitory activity. In addition, data obtained from 3D structures can elucidate interactions in enzyme-inhibitor complexes and guide future analysis as well as alternative experimental strategies.

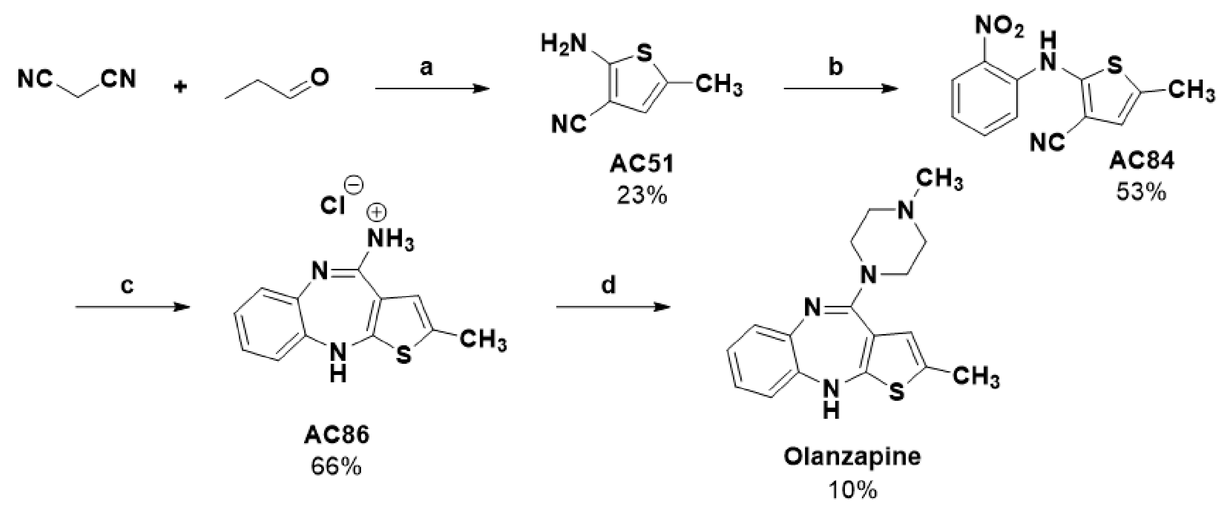
[1] Pollegioni et al., (2021) *Frontiers in Molecular Biosciences*, *8.* [2] Katane et al., (2015) *Journal of Medicinal Chemistry*, *58*(18), 7328–7340. [3] Sacchi et al., (2017) *Scientific Reports*, *7*.

2. SYNTHESIS OF 5-An DERIVATIVES



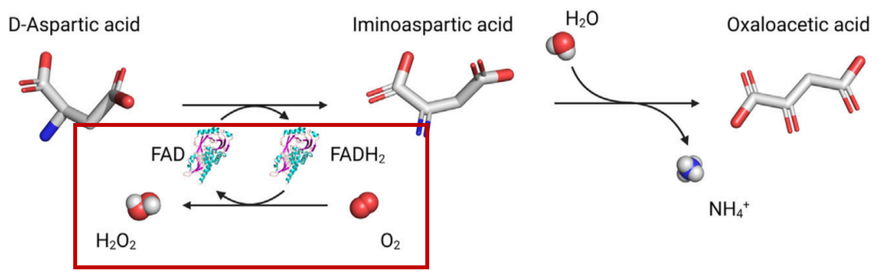
5-An was modified, following guidelines from literature and docking models, starting from the 6-derivative class (red square) and a total of twenty derivatives was obtained (see QC above). A three-step reaction was set up: (1) a nucleophilic substitution to insert the desired OR or SR group at position 6. (2) The reduction of NO_2 group into NH_2 group. (3) An hydrolysis to obtain the COOH group at position 3.
Fukumoto et al., (2012) *Tetrahedron Letters*, *53*(5), 535–538.

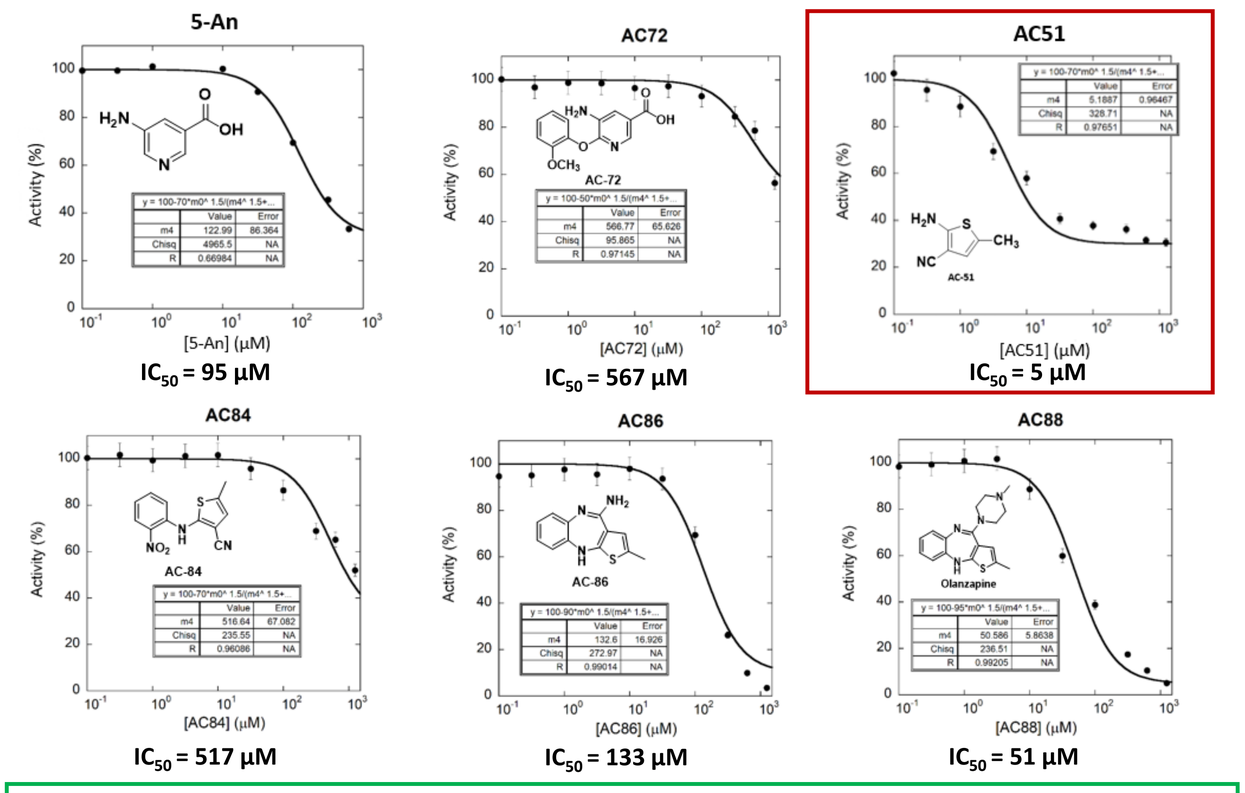
3. SYNTHESIS OF OLANZAPINE



Synthesis of olanzapine was obtained following literature, then olanzapine and all intermediates AC51, AC84, and AC86 were collected.
Reagents and Conditions: (a) Sulphur, DMF, TEA, rt; (b) 2-fluoronitrobenzene, NaH, 0 °C to RT; (c) $SnCl_2$, HCl 6M, EtOH, refl;
(d) *N*-methyl piperazine, DMSO/Toluene 1:1, refl, 20 h.
Gao et al., (2013) *Bioorganic and Medicinal Chemistry Letters*, *23*(7), 1953–1956.

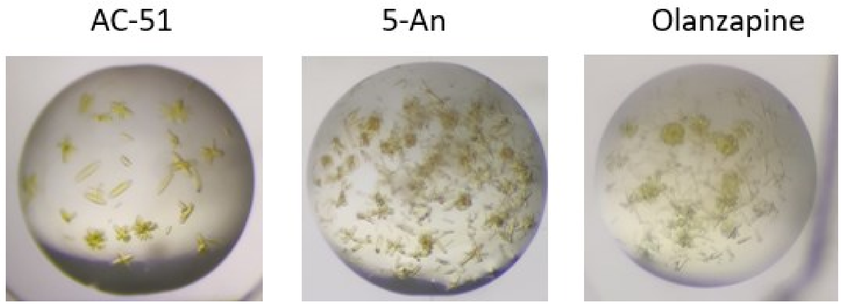
4. INHIBITION ASSAY



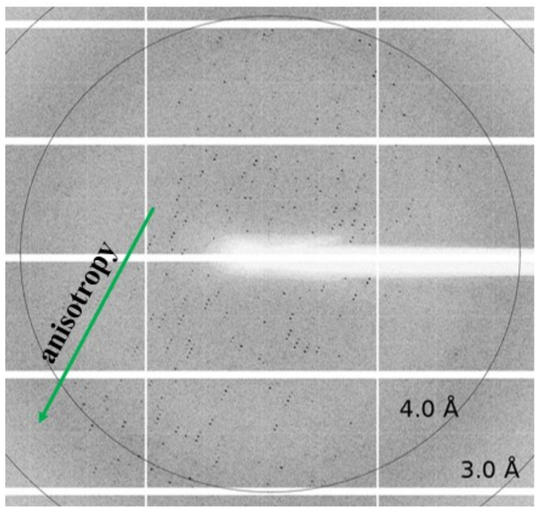


In the Amplex Ultrared inhibition assay the fluorescence from the oxidation of H_2O_2 , derived from the enzymatic reaction, by the Ultrared reagent is measured at the excitation and emission wavelengths of 530 and 590 nm, respectively. Inhibition is represented by a reduction in fluorescence. The inhibition assay was performed at the University of Insubria (Varese) by collaborators from the Loredano Pollegioni's group. AC51 is the best inhibitor (IC_{50} = 5 µM).

5. CO-CRYSTALLIZATION TRIALS



JSCG (Molecular dimensions)
E11 -> 160 mM $CH3COOCa_2$, 80 mM sodium cacodylate pH 6.5, 14,4% PEG 8K, 20% glycerol.



hDASPO (14 mg/mL) and inhibitors (5A-n, AC51 and olanzapine, 6 mM) were incubated for one hour at room temperature. Then, JCSG and SG1 (Molecular Dimensions) commercial screenings were selected, and Oryx4 Crystallization Robot (Douglas Instruments) was used to fill the plates, which were stored at 21 °C.
Data collection was performed at the European Synchrotron Radiation Facility (ESRF) in Grenoble, France. Crystals containing hDASPO-5An complex showed diffraction, however data processing failed due to poor resolution, twinning, and low number of spots.

6. CONCLUSIONS AND PERSPECTIVES

These results confirm 5-An and olanzapine as hDASPO weak inhibitors, and identify AC51 (IC_{50} = 5 µM) as best inhibitor known up to now. AC51 and AC86 will be functionalized to enhance their inhibitory activity, that will be then evaluated in the Amplex Ultrared inhibition assay. In addition, inhibitors binding activity will be tested by Isothermal Titration Calorimetry (ITC) and Microscale Thermophoresis (MST) to measure their affinity with hDASPO.
Data collection on co-crystals obtained from E11 condition (JCSG, Molecular Dimensions) was not successful due to scarce diffraction, anisotropy and twinning, therefore seeding tecnique as well as further optimization will be attempted to improve crystal reproducibility and diffraction.

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