

Overexpression of human Kynurenine Aminotransferase-I for Inhibitor Studies

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Schizophrenia is a devastating mental disorder, affecting ~1% of the population, across all races and cultures. High levels of kynurenic acid often accompanies the occurrence of several neurological disorders including schizophrenia. Overactivity in the enzyme Kynurenine Aminotransferase-I (KAT-I) which produces this metabolite, is believed to be an important link in the pathophysiology and makes the kynurenine pathway a valuable target for the treatment of these diseases. Computational biochemistry and other bioinformatics tools have been used to generate potential inhibitors for this enzyme. The aim of this project is to express large amounts of purified human KAT-I (hKAT-I) to test the potency of lead compounds, obtain co-crystal structures of enzyme-inhibitor complexes and subsequent lead optimisation to guide drug discovery. In preliminary assays 8 out of 11 candidate compounds chosen from a virtual screen were found to inhibit KAT-1, with the strongest possessing an apparent K_i of 2.8 $\mu\text{mol/L}$. These assays were performed with semi-purified recombinant hKAT-1 and further compounds await testing.

The gene for hKAT-I has been expressed using the Baculovirus-insect cell system involving the virus AcMNPV and the Sf9 cell line. We pursued two lytic strategies; the Bac-N-Blue and BaculoDirect expression systems. The Bac-N-Blue strategy involves plaque assays for recombinant virus selection based on concomitant β -galactosidase expression, and a three-step purification procedure. Our BaculoDirect approach, which obviates the need for plaque assays, incorporated a histidine tag with V5 epitope to institute one-step purification. Our restriction enzyme digests and PCR analyses are consistent with correct gene incorporation into the transfer vector and entry clone. Using Bac-N-Blue we detected specific cell - derived KAT-I enzyme activity. Activity was determined by incubating with the substrate kynurenine and cofactors, and quantifying the kynurenic acid produced using HPLC with fluorescence detection. Both the Bac-N-Blue and BaculoDirect systems appear suitable for large-scale recombinant KAT-I expression. Unfortunately the C-terminal 6xHis tagged hKAT-1 showed no activity. Purification has proceeded with the untagged hKAT-1, to provide protein for the structure guided approach to inhibitor design.