DISCOVERY OF PIPERAZINE MOLECULES WITH INHIBITORY ACTIVITY AGAINST N-ACETYLTRANSFERASE D

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N-terminal acetylation, catalyzed by N-terminal acetyltransferases (NATs), constitutes a frequent protein modification associated with various biological responses. N-acetyltransferase D (NatD or NAA40) is one of the most selective NATs that catalyzes solely the acetylation of the N-terminus of histones H4 and H2A. Dysregulation of this enzyme has been correlated with multiple cases of carcinogenesis. At present, there is only one peptide molecule with inhibitory activity against NatD, a reality that signifies the need for the development of novel drug-like inhibitors.

In this study, the search for small molecules exhibiting inhibitory activity against NatD will be presented. Initially, a virtual screening of the in-house chemical repository (Pharmalab ~2500 compounds) was performed, utilizing both 3D-similarity methodologies and docking calculations. The oligopeptide SGRGK corresponding to the amino acid sequence recognized by NatD, was divided into smaller segments and 5 queries were created based on crystallographic data (PDB IDs: 4U9W, 4U9V). Preparation of Pharmalab was carried out, using both ConfGen (Schrodinger software) and Omega (OpenEye software) and two library versions were created. 3D-similarity screening was performed by ROCS (OpenEye software) and two lists of structurally and chemically similar compounds were calculated for every query. The results were combined for both versions of the library with the assistance of an R script, and two rankings were produced. Subsequently, Pharmalab was further explored with docking simulations using GLIDE (Schrödinger software). Docking and 3D similarity screening results were merged into a final list of potentially active molecules against NatD, using a consensus methodology. Top ranked molecules containing a piperazine group were selected and evaluated in vitro by two orthogonal assays, namely Differential Scanning Fluorimetry (DSF) and a Fluorescence based enzymatic assay. Four molecules with inhibitory activity were discovered with IC50 of ~200 μM. Further evaluation of the selected ligands will be undertaken.