COMBINING IN VINTRO AND IN SILICO TECHNIQUES TO DISCOVER NEW HITS AGAINST CYSTATHIONINE GAMMA-LYASE (CSE) ENZYME

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Hydrogen sulfide, H_2S, is a highly diffusible gasotransmitter that has emerged as an important gaseous mediator in cellular physiology and pathology. Cystathionine gamma-lyase (CSE) is one major H_2S-producing enzyme primarily expressed in peripheral tissues. It consists of four identical monomers with a covalently bound pyridoxal 5'-phosphate (PLP) cofactor, which is crucial for maximal CSE enzymatic activity bound via the active site Lys212 residue. CSE expression and activity as well as the produced H_2S, play a key role in both health and diseases, making CSE an interesting pharmacological target.

This work focuses in combining in vitro and in silico techniques in order to discover new hits targeting CSE enzyme. Five hundred compounds of the in-house Pharmalab library were tested against CSE using the Methylene blue chromatometric method. Bacterial expression and purification of the enzyme CSE tag was carried out. Two molecules showed alteration of the enzymatic activity, a dibenzofuran derivative reducing H_2S production, while a 1,4,6-trimethoxyphenyl derivative increasing it. To establish Structure Activity Relationships prior to any further synthetic efforts, similar molecules were identified and purchased from the NCI chemical library (260.000 compounds) using a consensus virtual screening protocol. To this end the two compounds were used as queries to perform two-dimension (2D) and three-dimension (3D) similarity tests against the NCI library and the results were combined through docking-scoring calculations (Glide-Schrödinger Maestro). The top ranked compounds were further filtered based on visual inspection and 40 entries were selected for further investigation. Activity results enabled the SAR formulation suggesting bulky groups in the aliphatic chain of the 1,4,6-thrimethoxyphenyl derivative are likely to decrease the activity whereas the trimethoxy groups, are likely to play an important positive role. On the other hand the dibenzofuran ring seems to play an important role in decreasing the enzyme activity, whereas the side chain of the aromatic system could be altered. Future studies involve the synthesis of a series of compounds based on these first SAR.