Discovery of new human DHODH inhibitors using a Structure-Based and a Ligand-Based Pharmacophore approaches

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Dihydroorotate Dehydrogenase (DHODH) is a mitochondrial protein involved in *de novo* pyrimidine biosynthesis, catalyzing the ubiquinone-mediated oxidation of dihydroorotate (DHO) to orotate. DHODH is considered to be a validated target for the development of immune-modulating agents[1]. In this work two different approaches were used to create a pharmacophore model, which was subsequently used in a screening against a large compound library with the aim to identify new DHODH inhibitors.

In the structure-based approach we used a crystal structure of DHODH in complex with a ligand, N-(alkylcarbonyl)anthranilic acid derivative (PDB 2WV8).[2] By using *PHASE*[3], a pharmacophore model was created. To improve the selectivity of the pharmacophore hypotheses, a receptor-based approach[4] was used and adding exclusion volumes optimized the model. The selectivity of the model was then proved using a test set of 37 molecules (26 active and 11 inactive compounds). In the following, a library of 6786 compounds downloaded from *ZINC*[5] website was screened. 900 molecules were identified as hits. All these molecules were then docked into DHODH (PDB 1D3G) using *GLIDE*[6-7] in *Standard Precision* mode. The 322 hits resulting from this round were re-docked into the structure with *Extra-Precision* modality, leading to 247 potential DHODH inhibitor compounds.

The 26 active molecules from the test set used in the structure-based approach were used to create a ligand-based pharmacophore model with the program *Ligand Scout*[8]. This approach allows the comparison of all the common structural parts of the active molecules. Several hypotheses were then analyzed and refined, manually adding exclusion volumes and increasing selectivity and capability to distinguish active and inactive compounds. In the following the above-mentioned *ZINC* library was screened leading to 137 hits. Those hits were, as before, docked in both *Standard* and *Extra-Precision* modes, using the program *GLIDE.* Eventually, only 115 compounds were well docked into the 1D3G structure. Several of those were identical to the compounds found using the structure-based approach (e.g *ZINC08438775,* ZINC02165050).

The results show how both methodologies lead to very similar results, in terms of scaffold similarity and Docking Score. 19 different molecules with Docking Score values up to -14.04 were bought for the future activity evaluation.

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