Targeting the TrxR protein-protein interface by knowledge based virtual screening

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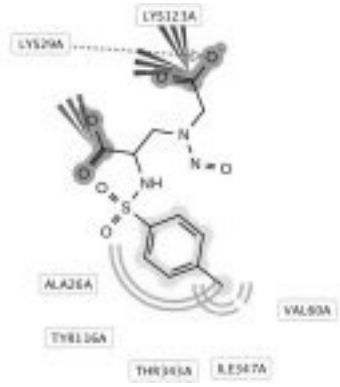
Thioredoxin reductase (TrxR) is a selenoprotein involved in various processes regulating the cell's redox state. It represents a promising target enzyme for cancer therapy and the treatment of arthritic diseases. Therapeutic organometals form a complex with selenocysteine, a C-terminal residue decisive for the catalytic activity. Further catalytic cysteine residues and the co-factor binding site are more centrally located in the 499 amino acid protein. Crystal structures reveal that two TrxR proteins dimerize in a head-to-tail arrangement. The substrate binding site is formed by the groove between the two subunits and both of them are involved in the catalytic mechanism [1]. The first crystal structure containing selenium (PDB codes 3EAN and 3EAO) and mutation experiments revealed new insights on the position of the C termination within the voluminous groove [2].

We intend to identify metal-free inhibitors of TrxR targeting the catalytic site of the homodimer. Since the binding mode of the substrate is unknown, we focused on the crucial contact between the selenocysteine with the second subunit.

We examined the protein-protein interface with a probe based method [3]. Within the potential binding site we performed de-novo design for hypothetical ligands serving as templates for virtual screening. The most relevant chemical features for the putative interactions between the protein and the virtual ligands were represented by a 3D pharmacophore model [4].

Biological testing of 18 compounds selected from the screening results identified four compounds with micromolar activity.



Binding poses suggested by docking experiments encourage our hypothesis of a binding mode, which blocks the positioning of the selenocysteine. The most active compound is placed in a small cavity next to the catalytic disulfid bridge. Especially ionic interactions with lysine residues resemble those of the selenocysteine motive in the C termination of the second subunit.

This new inhibitors of TrxR are not only relevant due to their biological activity, but might be first leads for the structure guided development of TrxR inhibitors.

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[2] Q. Cheng, T. Sandalova, Y. Lindqvist, E.S. Arnér, *J Biol Chem*, **2009**, *284*, 3998-08.
[3] P.J. Goodford, *J Med Chem*, **1985**, *28*, 849-857.
[4] G. Wolber, T. Langer, *J Chem Inf Model*, **2005**, *45*, 160-169.