**Compound selection and preparation**

In the present study, we show that small molecules discovered to inhibit modified cancer-specific PFK1 iso-enzymes are a suitable means to reduce dysregulated glycolytic flux in cancer cells. A metabolic flux reduced to the level of normal cells avoids the formation of excess NADH and at the same time prevents the need for lactate and ROS formation in cancer cells (**Figure** 1).

To find the drugs inhibiting highly active, cancer-specific PFK1s, the atomic model of the human PFK-P iso-enzyme was designed based on the crystal structure of the human PFK-P tetramer (UniProt Q01813) in combination with ATP–Mg2+ at a resolution of 3.1 Å (Protein Data Bank accession number 4XYJ) [34]. In collaboration with the Laboratory for Molecular Modelling at the National Institute of Chemistry, Ljubljana, the ZINC Drug NOW database was first filtered to exclude expected aggregators and poorly soluble compounds. By using a supercomputer (CROW 16, National Institute of Chemistry, Ljubljana, Slovenia) with approximately 3,000 processor cores, large-scale virtual screening was performed by docking with ProBiS-Dock algorithm [35], [36] to the catalytic ATP binding site of PFK-P/PFK-M and PFK-L isoenzymes.

Initially, 38 compounds were selected to dock to the ATP binding site of human PFK-P isoenzyme of which 18 were commercially available and purchased from Enamine (Kyiv, Ukraine) (Inh, No, 1-8), ChemBridge (San Diego, USA) (Inh. No. 9-16) and ChemDiv (San Diego, CA) (Inh. No. 17-18). Because the human PFK-M (UniProt P08237-1) isoform has identical ATP-binding sites to that of the PFK-P isoform, the same compounds might equally inhibit both enzymes.

However, human PFK-L’s (UniProt P17858) ATP binding site differs from human PFK-P and human PFK-M isoenzymes in one amino acid residue. Distances among specific moieties on amino acid residues and ATP molecules at both ATP binding sites correspond to the interactions between the protein and ATP molecule (Figure 2a, b). Accordingly, an additional 15 potential PFK-L inhibitors were selected and purchased from Enamine (Kyiv, Ukraine) (Inh. No. 21-28) and ChemBridge (San Diego, CA) (Inh. No. 29-32, 34-36). The Inh. No. 33 was commercially unavailable.

All purchased compounds were supplied as 5mg lyophilized powder and dissolved in 100% dimethyl-sulfoxide (DMSO). According to the Cmpd’s molecular weight (MW), the amount of discontent was calculated to get 10 mM concentration. Finally, the volume needed to reach 100 µM/mL concentration in the medium by individual Compounds was determined.