**Half maximal inhibitory concentrations (IC50) of selected compounds.**

To evaluate the ability of selected compounds to suppress the activities of the human PFK1 enzyme, the in vitro inhibitory capacities of selected compounds were determined on partially purified recombinant native and modified PFK-M and PFK-L enzymes [15]. Among the inhibitors that preferentially bind to the PFK-P/PFK-M type ATP site, two cmpds (No. 3 and 9) showed potent inhibition of the highly active modified sfPFK-M enzyme with half maximal inhibitory concentrations (IC50 values) of approximately 15 µM and 17 µM, respectively. Although inhibitor No. 3 also significantly reduced the activity of the native nPFK-M enzyme (IC50, 18 µM), not as strong inhibition of the native enzyme was observed by cmpds No. 9 (IC50, 41 µM) and cmpd 30 (IC50, 46 µM).

Compounds targeting PFK-L-type ATP-binding sites also successfully inhibited the recombinant sfPFK-L enzyme. Inhibitors No. 29 and 32 showed strong inhibition of the highly active sfPFK-L enzyme (IC50, 8 µM each). Similar half-maximal inhibitory concentrations were also observed with cmpds No. 30 and 31 (IC50, 9 µM each). The weakest inhibition was observed with cmpd No. 23 (IC50, 27 µM). However, all five inhibitors tested showed significantly weaker inhibitions of the native nPFK-L enzymes, ranging from IC50 values of 47 to 54 µM. It is interesting to note that compounds targeting the PFK-L type binding site inhibited the modifier sfPFK-L enzyme strongly concerning the native nPFK-L enzyme compared to the PFK-P/PFK-M type compounds (**Fig. 3**).

Consequently, the individual inhibitors might act differently by preventing lactate and/or SOX formation in different tumorigenic cell lines. To obtain more information on the efficiency of selected small molecule inhibitors for lactate/SOX suppression in cancer cells some additional preclinical tests were performed. Two compounds were selected for testing: cmpd No. 9 proved to be the most effective among compounds that preferentially dock to the ATP-binding type PFK-P/PFK-M, and cmpd No. 30 proved to be the most effective compound for lactate/SOX reduction among compounds that dock to the PFK-L isoenzyme.