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# Computer-aided discovery and biological characterization of human lactate dehydrogenase 5 inhibitors with anti-osteosarcoma activity

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ABSTRACT

Human lactate dehydrogenase 5 (*h*LDH5) is overexpressed in various tissues of human tumors, which could be a potential therapeutic target for cancer treatment. Herein, we describe the computer-aided discovery and biological characterizations of *h*LDH5 inhibitors with anti-osteosarcoma activity. Biochemical assay indicated that the identified compounds **3** and **9** strongly inhibited *h*LDH5 function with $EC_{50}$ values of 0.67 and 0.39 μM, respectively. The MTT assay revealed that most of the identified inhibitors had little effect on MG-63 cell proliferation at 4 μM, only **9** reduced the cancer cell proliferation at the same concentration, with an $IC_{50}$ value of 3.18 μM. Our data suggested that **9** could be a starting lead of developing potent *h*LDH5 inhibitor for the anti-osteosarcoma agents in cancer treatment.

Cancer cell features a switch in glucose metabolism from mitochondrial oxidative phosphorylation (OXPHOS) to cytoplasm-based glycolysis for energy production.[1] This altered glucose metabolic pathway satisfies the survival and proliferation for tumor progression.[2] So specifically targeting this glycolytic 'addiction' in tumors may offer opportunities for cancer treatment.[3] Some enzymes and transporters involved in the glycolysis pathways are thus considered as promising therapeutic targets for developing effective anticancer therapeutics.

Human lactate dehydrogenase 5 (*h*LDH5) plays a crucial role in glycolysis, which is responsible for the conversion of pyruvate to lactate at the end of the glycolytic process.[4] It has been reported that *h*LDH5 protein was highly overexpressed in a variety of glycolytic human cancers, such as osteosarcoma cancer,[5] melanoma cancer,[6] colorectal cancer,[7] pancreatic cancer,[8] prostate cancer,[9] lung cancer,[10] and endometrial cancer.[11] The overexpressed *h*LDH5 protein conferred the growth advantages of cancer cells by ensuring a sustainable energy supply, which correlated with the aggressive phenotypes and poor prognosis in several tumors.[12] In addition, recent studies suggested that the inhibition of *h*LDH5 reduced the invasive and metastatic potential of cancer cell by decreasing their proliferation ability.[13] In fact, the silencing of *h*LDH5 gene by short hairpin RNA (shRNA) markedly delayed the ability of tumor formation and growth in a renal cancer xenograft mouse mode.[14] Furthermore, the inhibition of *h*LDH5 by small molecules increased oxygen consumption and decreased lactate formation in cancer cells, which led to the increased production of mitochondrial reactive oxygen species (ROS) and oxidative stress, thus resulting in the cell death.[15] All these facts, together with the fact that individuals with *h*LDH5 deficiency do not demonstrate any serious pathology under normal conditions, making *h*LDH5 a potentially important and relatively safe therapeutic target to mitigate the highly activated glycolysis pathway in cancer cells.

To date, very limited *h*LDH5 inhibitors with cellular activity have been developed,[16] and none of them was used in real clinical trials. This is probably due to the reasons that the active site of *h*LDH5 located at a rather deep position and its accessibility is difficult. Meanwhile, the active site of *h*LDH5 is rich in cationic residues. To keep good interactions with the target, inhibitors usually armed with negatively charged moieties. However, inhibitors of such nature are usually poorly permeable across various cellular barriers and therefore lead to a low bioavailability. Until recently, Purkey et al. reported the structure-based design of trisubstituted hydroxylactams,[17] from which a cell active compound **1** was discovered (Fig. 1). Analysis of the 2.2-Å X-ray co-crystal of *h*LDH5-**1** complex (PDB code: 4ZVV) suggested that **1** bound to *h*LDH5 in the active site adjacent to NADH, forming hydrogen bonds with the active site amino acids Arg168, Asn137, and His192 (Fig. 4**A**). Based on the binding model of *h*LDH5-**1** complex, herein we report the

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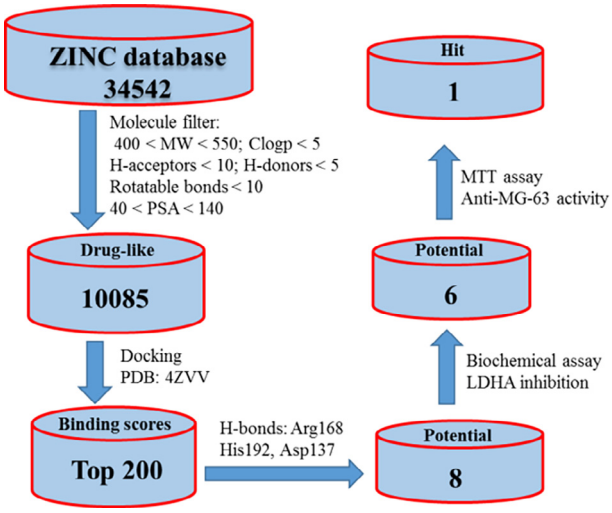


**Fig. 1.** Flow chart of the multistep virtual screening strategy.

computer-aid high-throughput virtual screening approach and biological evaluations to identify cellular active *h*LDH5 inhibitors.

Firstly, the co-crystal of *h*LDH5-**1** complex (PDB code: 4ZVV) was downloaded from the protein data bank (http://www.rcsb.org/) and optimized in Sybyl-X 2.0 software package. Then the ZINC database containing 34,542 compounds was filtered to rule out compounds that do not maintain the drug-likeness, the remaining compounds 10,085 were then optimized by the ligand structure preparation procedure in the Sybyl software. The optimized compounds in library was then docked into the binding pocket adjacent to NADH pocket in *h*LDH5 (Fig. 1). The top-ranked 200 compounds were chosen and potential compounds were finally selected if they formed hydrogen bond interactions with the amino acids of His192, Asn137, and Arg168 in *h*LDH5, which were involved in the interactions of compound **1** in *h*LDH5. Followed these procedures, 8 candidates (compounds **2–9**, shown in Fig. 2) were identified and purchased from local supplier, which were then evaluated for their biological roles in *h*LDH5 function.

Then, we tested the inhibitory effect of **2–9** on *h*LDH5 protein by measuring the NADH decrease during the conversion of pyruvate to lactate in *h*LDH5 catalytic pathways, **1** was used as positive control. As

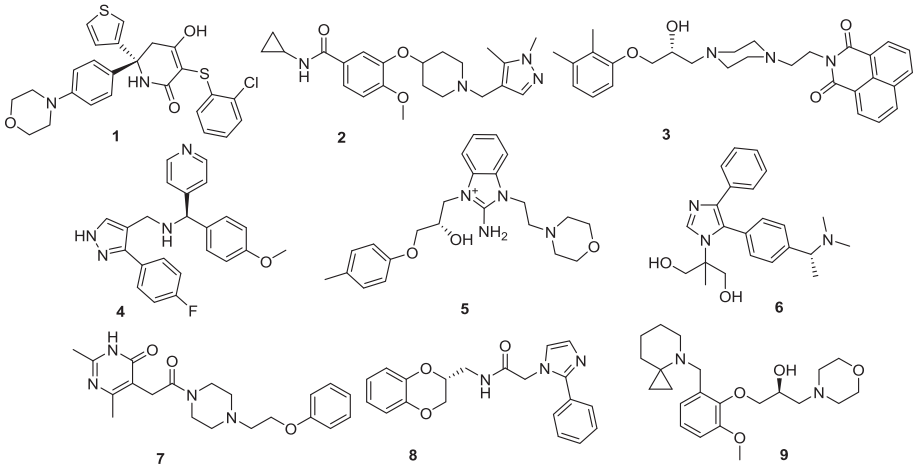


**Fig. 2.** Chemical structures of compound **1** and the identified potential *h*LDH5 inhibitors **2–9**.

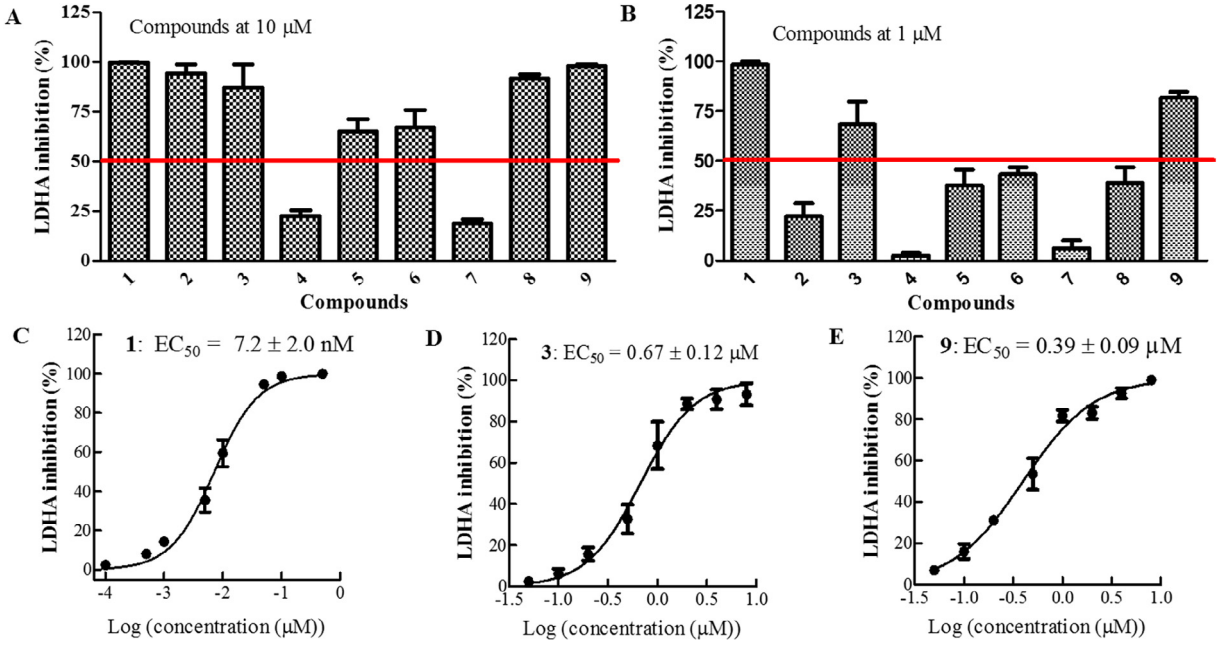


**Fig. 3.** Compound **1** and the identified compounds **2–9** inhibited *h*LDH5 activity. A/B: Compounds **1–9** at 10 or 1 μM reduced *h*LDH5 activity; C–E: $EC_{50}$ values of compounds **1**, **3** and **9** inhibited *h*LDH5 activity.