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Discovery of 2-((3-cyanopyridin-2-yl)thio)acetamides as human lactate dehydrogenase A inhibitors to reduce the growth of MG-63 osteosarcoma cells: Virtual screening and biological validation ☆

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ABSTRACT

Lactate dehydrogenase A (LDHA) has emerged as an attractive target in the oncology field. In this paper, we present the identification of 2-((3-cyanopyridin-2-yl)thio)acetamide-containing compounds as LDHA inhibitors. The in vitro enzymatic assay suggested that inhibitor **9** had good inhibitory potency against LDHA with IC_{50} value as 1.24 μM. Cytotoxicity assay showed that inhibitor **9** strongly inhibited the proliferation of cancer cell MG-63 (EC_{50} = 0.98 μM). These findings indicated that inhibitor **9** could be employed as a lead for developing more potent LDHA inhibitor with anti-proliferative potency.

Many cancer cells preferentially convert glucose into lactate through glycolysis even under normal oxygen conditions, a phenomenon known as Warburg effect.[1] Despite the low efficiency of producing adenosine triphosphate (ATP) by glycolysis rather than oxidative phosphorylation in mitochondrion, the increased rate of glycolysis in cancer cells is believed to ensure rapid generation of energy and biomass needed for cellular proliferation.[2] This metabolic switch categorized as an emerging hallmark of cancer,[3] and thus, inhibition of the altered metabolic processes has been considered as a promising approach in the search for new cancer therapies.

The final step of glycolysis is catalyzed by LDHA, also known as LDH-5, which is a key glycolytic enzyme that catalyzes the lactate formation in cytosol,[4] while another isoform lactate dehydrogenase B (LDHB, known as LDH-1) favoring the backward reaction in which lactate is converted to pyruvate.[5] It has been widely accepted that LDHA is over-expressed in human tumor tissues and found to correlate with tumor size and poor prognosis,[6] and silencing LDHA expression by small hairpin RNA in tumor cells could induce decrease cell proliferation, migration and in vivo tumorigenesis.[7] These observations make LDHA a promising target for the development of novel anticancer agents.

To date, several examples of human LDHA inhibitors have been reported in scientific and patent literature. As shown in Figure 1, Gossypol is a known LDHA inhibitor, a natural polyphenol dialdehyde extracted from cotton seeds, which is also highly cytotoxic and promiscuous.[8] Another LDHA inhibitor is FX11, with a K_i value as 8 μM and a >10-fold selectivity over LDHB, which has been recently proved to inhibit the tumor progression in vitro and in vivo.[9] *N*-Hydroxyindole **1** is a promising LDHA selective inhibitors, which also shows inhibitory activity toward a wide range of tumor cells, with IC_{50} value low to 10.8 μM.[10] Inhibitors **2–4** were reported to show inhibitory potencies against LDHA at micromolar or nanomolar ranges, but have limited cellular activities or the inhibitors were not suitable for studying the pharmacokinetics.[11,12] So it is very urgent to discover potent LDHA inhibitors with anti-proliferative activity. Herein we present a computational-aided method that allow us to identify new LDHA inhibitors with interesting enzymatic potency in vitro. In addition, the lowest enzymatic potency inhibitor **9** could reduce the growth of MG-63 cancer cell with EC_{50} value as 0.98 μM.

Firstly, the crystal structure of LDHA-compound **2** complex (PDB code: 4QSM) was picked from the protein data bank (http://www.rcsb.org/pdb/) as template for molecular docking. The protein structure was subjected to series of optimization such

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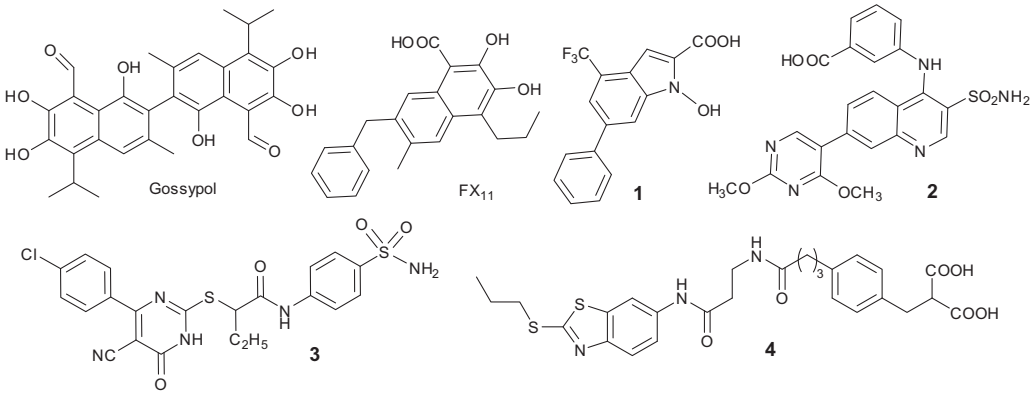

Figure 1. Chemical structures of the reported LDHA inhibitors.

as hydrogens addition and energy minimization in Protein Preparation Workflow of Sybyl-X 2.0 software package. Secondly, a commercially available database with 5236 compounds was downloaded from ZINC database (http://zinc.docking.org), then compounds were prepared by the Ligand Structure Preparation procedure in the software. Finally, the optimized compounds were docked into the binding pocket of compound **2** in LDHA using Surflex-Dock software, the top-ranked 150 compounds with the highest total binding scores were selected. The potential compounds were chosen if satisfying two criteria: one is the total binding score higher than 5.43, which is calculated from the interaction between compound **2** and LDHA. The other one is that compound should form no less than three hydrogen bonds with residues of Arg 99, Asp 52, Arg 112 or Gly 97 of LDHA. Following these two rules, 18 candidates were identified, but compounds **6**, **7**, and **9** sharing the 2-((3-cyanopyridin-2-yl)thio)acetamide motif attracted our interests (see Fig. 2). Moreover, we found that compounds **5** and **8** also had similar framework, but failed to pick up some interactions with residues Asp 52, Arg 99, Arg 112 or Gly 97. So the 5 compounds were purchased from local supplier for further biological validation, with the aim of gaining some information to further modify this series of compounds.

With the identified inhibitors (**5–9**) and reference compound **2** in our hand, we started our exploratory study by testing the inhibitory activity of the compounds to human LDHA isoform. The enzymatic assay were determined by measuring of the disappearance of NADH at 340 nm during the conversion of pyruvate to lactate. As shown in Table 1, most of the selected inhibitors displayed an exciting inhibitory activity, with IC_{50} values ranging from 1.24 to 22.47 μM. In contrast, the inhibitory potency for compound **5** to LDHA was undetectable (IC_{50} > 100 μM) under the same condition, this could be explained by the disappearance of hydrogen bonds interaction with Gly 97 and Arg 99 in LDHA. Compared to the reference compound **2** (IC_{50} = 7.40 μM), most of the identified compounds showed better inhibition potencies. The increased inhibitory activity against LDHA could be partially explained by the evidences obtained from our docking experiment (Fig. 3). Similar to compound **2**, the selected inhibitors **6**, **7**, and **9** retained the hydrogen bonds interaction with residues Asp 52, Gly 97, and Arg 99, additionally, they successfully picked up hydrogen bond interaction with residue Tyr 83, which may contribute the enhanced inhibitory potency. From the data we could conclude that substituents on A ring play a decisive role for exerting inhibitory potency, and the amide, not the ester linkage is necessary for compound to retain inhibitory activity.

Next we have interest to know whether the identified inhibitors could reduce the growth of MG-63 cancer cell. So we measured the anticancer activity of the identified compounds against MG-63 cancer cell by using MTT assay. As shown in Table 1, most of the compounds, with EC_{50} values as 6.72, 5.78, and 0.98 μM, respectively, strongly blocked the MG-63 cancer cell proliferation at very low concentration. As expected, compound **5** did not show any

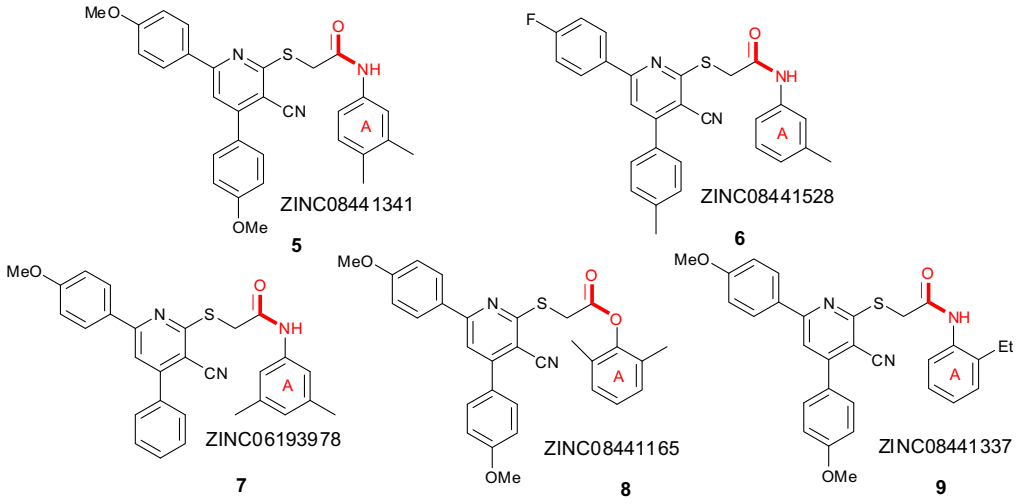

Figure 2. Chemical structures of the potential LDHA inhibitors identified by the virtual screening method.