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Identification of a potent inhibitor targeting human lactate dehydrogenase A and its metabolic modulation for cancer cell line

Chun-Ye Chen [a,*,†], Yan Feng [b,†], Jing-Yu Chen [c], Hao Deng [d]

[a] Key Laboratory of Biorheological Science and Technology, Ministry of Education, Bioengineering College, Chongqing University, Chongqing 400030, PR China
[b] Cancer Center, Daping Hospital and Research Institute of Surgery, Third Military Medical University, Chongqing 400042, PR China
[c] Department of Neurosurgery, Southwest Hospital, Third Military Medical University, Chongqing 400038, PR China
[d] Institute of Hepatobiliary Surgery, Southwest Hospital, Third Military Medical University, Chongqing 400038, PR China

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ABSTRACT

Targeting LDHA represents a promising strategy for the development of new anti-cancer agents. We report herein the identification of a potent compound as a direct LDHA inhibitor. The in vitro enzymatic assay revealed that the **VS-2** had good inhibitory potency (IC_{50} = 0.25 μM) to LDHA. Cytotoxic assay suggested that the **VS-2** could inhibit MCF-7 cancer cell growth, with the IC_{50} value low to 1.54 μM. The seahorse XF24 experiment validated that the **VS-2** served as a modulator to reprogram MCF-7 cancer cell metabolism from glycolysis to mitochondrial respiration.

Carbohydrate/glucose metabolism is the most important pathway to provide adenosine triphosphate (ATP) in human body. After glucose is transported into cells, it is metabolized to pyruvate by glycolysis. In normal cells, pyruvate was completely metabolized via the tricarboxylic acid cycle (TCA) in mitochondria.[1] Contrarily, in cancer cells the majority of pyruvate was transformed to lactate even in the presence of adequate oxygen (Fig. 1).[2] Given the unique glycolytic metabolism of most cancer cells, inhibition of aerobic glycolysis in cancer cells to impede tumor growth and proliferation could be exploited for the anticancer therapies.[3]

The final step in aerobic glycolysis is catalyzed by lactate dehydrogenase A (LDHA, known as LDH-5), which uses NADH as a co-factor to transfer a hydride to the pyruvate ketone moiety, conversion pyruvate to lactate in the cell cytosol.[4–7] While another isoform lactate dehydrogenase B (LDHB, known as LDH-1) preferentially catalyzes the reverse reaction in which lactate is converted to pyruvate.[8,9] It has been widely accepted that LDHA expression was constantly up-regulated in tumors, and closely correlated with tumor size and poor prognosis.[10] In addition, silencing LDHA expression by small hairpin RNA in tumor cells could induce decrease in cell proliferation, migration and in vivo tumorigenesis.[11] A further interesting evidence is that inhibition of LDHA was found to overcome the trastuzumab resistance in breast cancer cells.[12] Therefore, developing anticancer agents that target glycolytic enzymes, such as LDHA, may be a promising approach for cancer therapy.[13]

Although the crystal structure of LDHA is well characterized, only few successful attempts to inhibit LDHA by small compounds have been reported. As shown in Figure 2, oxamate is a LDHA inhibitor, which competes with pyruvate substrate, but it is nonselective with very poor potency (K_i = 138 μM).[14] Another LDHA inhibitor is gossypol, a natural polyphenol dialdehyde, the cytotoxicity to normal cells appears to limit its further clinical application.[15] FX11 is an efficient LDHA inhibitor, with the value of K_i low to 8 μM, which was also proved to inhibit the tumor progression both in vitro and in vivo.[16] The *N*-hydroxyindole **1** was evaluated as a promising LDHA inhibitor, however, its potency is far from satisfactory. Inhibitors **2**–**4** were also reported to show inhibitory activities to LDHA at low micromolar and nanomolar ranges, but the authors reported limited cellular activities or the inhibitors were not suitable for studying the pharmacokinetics.[17,5,9] So discovery potent LDHA inhibitors with cellular activities is really imperative.

Herein we report a computational-aided method that combines with in vitro enzymatic assay, cytotoxic assay, and bioenergetics profile experiments to identify a LDHA inhibitor as biologically active and potentially therapeutically useful agent. Firstly, the crystal structure of LDHA–ligand complex (PBD entry: 4qsm) was selected from the Protein Data Bank in view of its clear electron density for structure-based drug design,[16] then the crystal

* Corresponding author. Tel./fax: +86 23 65102507.
E-mail address: chunyechen713@gmail.com (C.-Y. Chen).
† These authors contributed equally to this study.

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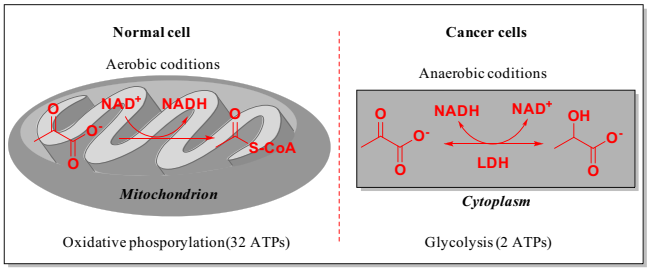

Figure 1. Schematic diagram of pyruvate metabolic pathways in normal cells and tumor ones. In normal cells, pyruvate is transformed to acetyl-CoA by pyruvate dehydrogenase complex. However, in proliferating cancer cells, 85% pyruvate is fermented into lactate and only 5% pyruvate enters into TCA cycle, generating only 2 mol of ATPs per glucose oxidized completely.

structure was subjected to series of optimization such as repair side-chain, correct atoms type and hydrogens addition. Secondly, a commercial available database from ChemBridge with totally 200,852 molecules was first filtered in order to discard some compounds that do not follow the drug-like criteria (300 < molecular weight < 500, $-3.5 < C\log p < 6$, 0 < number of rings < 4, 0 < rotational bonds < 10, 0 < donor < 5, 0 < acceptor < 8). Next, the remaining 100,342 compounds were subjected by removing some undesirable chemical groups (e.g., Michael acceptors, crown-ethers and their analogs, disulfides, epoxides, salts, etc.). Thirdly, the compounds were filtered by structural clustering analysis using Discovery Studio (version 2.5, Accelrys, Inc.). Finally, the remaining 5688 compounds were docked to the binding pocket of NADH in LDHA using Surflex-Dock software (Sybyl-X 2.0). The compounds were selected if satisfying the following criteria: one is the binding scores should higher than 4.90, which was calculated from the interaction between the compound **2** (Fig. 2) and LDHA. The other is that compounds should contact with residues Arg 99 and Asp 52 of LDHA to form hydrogen bonds interaction. Following these rules, we successfully discovered 4 candidates (Fig. 3) that proceeded for experimental validation.

Then we started our experiments by measuring the inhibitory activity of the identified candidates and the reference compounds **2** to human LDHA purified isoform. The enzymatic activities were determined by the measurement of the disappearance of NADH during the conversion of pyruvate to lactate. As shown in Table 1, all of the identified compounds exhibited a satisfactory inhibition to LDHA, with the IC_{50} values ranging from 0.25 to 7.8 μM. Compared to the reference compound **2**, all of the identified compounds showed better inhibitions against LDHA except VS-3. This result was also in accordance with the evidence obtained from our

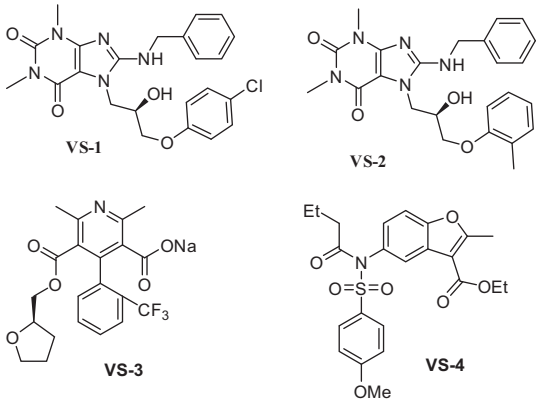

Figure 3. Chemicals identified from virtual screening.

Table 1
The identified compounds were tested on purified human LDHA and MCF-7 cancer cell line

Compound ID	Binding scores[a]	LDHA inhibition IC_{50}[b] (μM)	Cell growth inhibition IC_{50}[c] (μM)	*Clogp*
VS-1	7.60	2.3 ± 0.50	3.5 ± 0.21	3.72
VS-2	8.58	0.25 ± 0.05	1.54 ± 0.12	3.36
VS-3	5.87	7.8 ± 1.02	10.8 ± 0.95	4.16
VS-4	7.40	3.5 ± 0.47	>30	5.99
2	4.90	4.8 ± 0.54	>30	3.99

[a] Binding scores were obtained by docking experiments.
[b] LDHA inhibition data were reported as mean mean ± SD from two separate runs.
[c] IC_{50} is 50% inhibitory concentration; values are the mean ± SD from at least triplicate cytotoxicity experiments.

docking experiment (binding scores shown in the Table 1). In the Figure 4, we could see that the Arg 99 and Asp 52 contact with compound **2** to form two hydrogen bonds, the binding scores was 4.90, suggesting a moderate inhibitory activity towards LDHA. Interestingly, our identified compounds successfully picked up some other interactions apart from the two hydrogen bonds interaction. The **VS-1** and **VS-2** contacted with the residue of Tyr 83 to form additional two hydrogen bonds. And the **VS-3** and **VS-4** interacted directly with Arg 112 and Gly 97 to form two hydrogen bonds, respectively. All of these interactions contribute a lot to the improvement of the binding scores.

Next we tested the compounds' capability of inhibiting cancer cell Michigan Cancer Foundation-7 (MCF-7) growth by standard

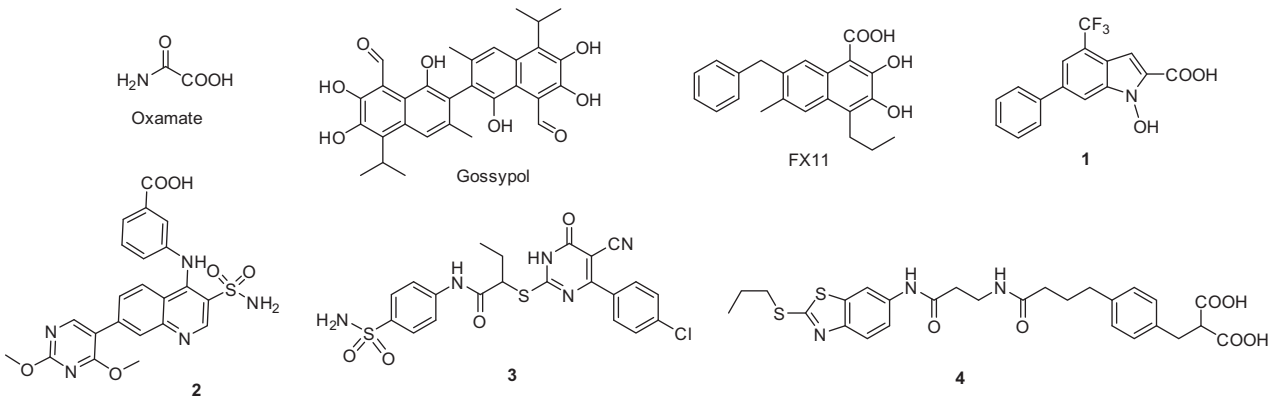

Figure 2. Structures of reported LDHA inhibitors.