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Preliminary Identification of Lactate Dehydrogenase Inhibitors towards Anticancer Drug Development

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Abstract

In tumor cells undergoing rapid cellular division, significantly increased quantities of lactic acid are produced in an anaerobic respiration via the Cori cycle. While this produces a lower of the pH in the local environment, the hepatic conversion of the generated lactate into glucose places a huge energy demand on the body which makes the patient weaker and results in cancer cachexia. Thus, instead of producing acetyl CoA, the dividing tumorous cells synthesize lactic acid catalyzed by the enzyme lactate dehydrogenase (LDH). LDH's involvement in tumor initiation and metabolism primarily involves a state of fermentative glycolysis catalyzed by the A form of the enzyme which allows tumorous cells convert the majority of their glucose stores into lactate even under anaerobic conditions which invariably shifts the utilization of glucose metabolites from simple energy production to an active promotion of accelerated cell growth and replication. This makes LDH a vital target for drug development. And in the present work a combination of virtual screening, database scouting and biophysical analysis of binding site properties have been employed in analysis the interaction of about 30,000 compounds with LDH. Using a synthetic NADH inhibitor, as a reference, only four compounds were found to demonstrate stronger binding features than the inhibitor. This preliminary in silicon screening represents the foundational effort in a cancer drug discovery project aimed at generating specific inhibitors of LDH for use in cancer therapeutics.

Keywords: Inhibitors; Anticancer therapeutics; Drug development; Drug discovery

Introduction

Rapidly growing tumor cells produce increased quantities of lactic acid (because of their anaerobic form of respiration through the Cori cycle), causing high acidity in the local environment. When this lactate is used for gluconeogenesis to produce glucose by the liver; energy consumption increases, thus making the patient weaker [1]. This is one of the reasons for cancer cachexia (rapid loss of weight). Thus, instead of producing Acetyl CoA, they produce lactic acid (with the aid of an enzyme called Lactate dehydrogenase) which is undesired for by the body tissues especially by the heart and muscles because it causes myocardial infarction and muscle cramps respectively.

LDH is involved in tumor initiation and metabolism. Cancer cells rely on anaerobic respiration for the conversion of glucose to lactate even under oxygen-sufficient conditions, a process known as the Warburg effect [2]. This state of fermentative glycolysis is catalyzed by the A form of LDH allowing tumorous cells convert the majority of their glucose stores into lactate regardless of oxygen availability, and shifting the use of glucose metabolites from simple energy production to the promotion of accelerated cell growth and replication [3]. For this reason, LDH A and the possibility of inhibiting its activity has been identified as a promising target in cancer treatments focused on preventing carcinogenic cells from proliferating.

Chemical inhibition of LDH A has demonstrated marked changes in metabolic processes and overall survival carcinoma cells. Oxalate is a cytosolic inhibitor of LDH A that significantly decreases ATP production in tumorous cells as well as increasing production of reactive oxygen species (ROS). These ROS drive cancer cell proliferation by activating kinases that drive cell cycle progression growth factors at low concentrations, [4] but can damage DNA through oxidative stress at higher concentrations. Secondary lipid oxidation products can also inactivate LDH and impact its ability to regenerate NADH, [3] directly disrupting the enzymes ability to convert lactate to pyruvate [5].

LDH test is also performed in various circumstances as an index for cellular or tissue damage, especially the heart, liver, kidney, muscles, brain, blood cells, and lungs. A higher than normal level (105-133IU/L) could be an indicator for tumor genesis in the lungs, etc. [6].

$$\text{Pyruvate} + \text{NADh} + H^+ \rightleftharpoons \text{Lactate} + NAD^+$$

The equilibrium of the reaction strongly favors lactate formation.

The traditional method of discovering new drugs involves the *in vitro* screening of large compound libraries (often in millions) against the miniaturized model of the disease-isolated tissues or organ systems. A combination of a high attrition rate, prohibitively exorbitant cost of development, and a steep regulatory qualifying criteria demanded by regulatory bodies have pressured pharmaceutical companies and research bodies to evolve different techniques that afford a greater deal of control on the discovery process. One of such methods, sometimes referred to as rational drug design, utilizes receptor information as it were in carving out or identifying small molecules that are capable of forming an energetically favorable interaction within the binding site. Such approach decomposes the basic mechanisms of drug-induced transduction and transduction inhibition into sub molecular and atomic contributions. Computer-aided drug design was a natural offshoot of this line of research. The continually improving computational capabilities are increasingly being explored in computing interaction

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indices between ligands and protein receptors which can be in the form of static observables such as binding free energy involving single frames, or dynamic properties like downstream conformational processes following ligand binding. Improvements in ligand docking and force field developments have seen simulated interactions approach traditional *in vitro* screening methods in modeling Biomolecular processes. While the *in vitro* screening tests must still be performed for the system under study, the use of in silicon methods allows the researcher to start viewing the research question from a molecular and atomistic perspective right from the commencement of the investigation. And with virtual screening, the researcher is not immediately constrained by the chemical space available to synthesis: This allows him accessing much wider chemical space that can be screened against the binding site (orthostatic or allosteric) of interest. In the present work, we have exploited this aspect of virtual screening to search a fairly wide chemical space for agents capable of specific interaction with LDH binding site [7-11].

Method

From the protein database available at http://www.rcsb.org/pdb/home/home.do we downloaded the X-ray crystallographic structure of lactate dehydrogenase complexes with the synthetic inhibitor 1, 4-dihydronicotinamide adenine dinucleotide (here after referred to as the inhibitor) and with PDB code 4RLS. The amino acids constituting the binding site of the specific binding inhibitor were identified and utilized in binding an interaction grid. We assembled a virtual library of small molecules containing about 30,000 compounds and including both existing drugs (FDA-approved) and secondary metabolites present in African plants. A combination of Igemdock [12] , PyRx 1.91 [13] virtual screening software, Open Babel 2.3.2 [14] for file conversion, Auto Dock Tools [15] for prediction validation, and VMD [16] for interaction analysis and validation was employed in screening and analyzing each of the 30,000 compounds against LDH binding site.

Result

The virtual screening was performed in batches using PyRx and beginning with exhaustiveness factor of 1 and gradually increasing it to 8. At each stage, all compounds featuring better binding energy than the inhibitor were retained and progressed to the next screening. After exhaustiveness factor 8, the remaining qualifying compounds were rescreened using Auto Dock vine and their atomic details of their interactions with the binding site residues investigated.

At the conclusion of the procedure, we were able to identify four (4) compounds that display stronger binding features than the synthetic inhibitor. The figures below show how these four compounds and the inhibitor interact within the binding site of lactate dehydrogenase. The interesting aspect of this outcome is the fact that some of the five compounds are existing drug molecules, in which case the concept of drug repurposing may be employed. The advantage of this is that it circumvents a discouragingly lengthy drug development process since most of the data required for regulatory approval are already available in public domain [17-19].

Having identified four compounds that are able to display binding properties superior to an existing synthetic inhibitor, the next phase of the cancer drug discovery project will involve the procurement of cancer models which will be assembled into a panel against which we will test the five drugs for their therapeutic properties in altering the disease course.

In Figure 1, a more detailed view of the interaction between the synthetic inhibitor and the binding site residues of lactate dehydrogenase has been presented. The binding involves extensive hydrogen bonding networks between the polar chemical groups in the inhibitor and polar side-chains of the binding site residues. The identified four compounds were able to form much stronger interactions with the binding site residues than those formed by the inhibitor.

Upon stringent analysis, it was carefully observed that not all models of a particular drug compound bound to the binding site; however, they still gave results which were not out of boundary. Thus, it became our duty to carefully screen and analyze docking result of each compound to extract the models which were well bound to the binding site.

After docking about 30,000 compounds, the compounds which binding to the binding and which gave the best binding energy were sorted out and selected. After each evaluation process, the drugs were analyzed and it was found that even in the same compound, there are different structural conformations called models which gave various results. The models which bound well to the real binding site on the receptor molecule were extracted. The following compounds were chosen upon critical analysis of their binding affinity and charges at the receptor binding site, Compound A, Compound B, Compound C, Compound D and Compound E, with compound A being the normal inhibitor after X-ray crystallographic techniques were employed (Tables 1-3).

The following 3D images obtained in the after the virtual screening give better understanding of the drug-receptor interaction in the molecular level (Figures 2-6).

Discussion

We have employed a stepwise approach, with increasingly stringent docking requirements, to identify a number of compounds that are capable of forming strong binding interaction with the orthostatic binding site of the enzyme lactate dehydrogenase. The pattern observed in the docking results indicates that at varying exhaustiveness slight but significant differences can occur in the obtained docking results. Even when the same ligand docking software has been employed we observed that the scoring function displays a general consistency in binding energies ranking; however, with respect to the selection of top ranking compounds, the observed variability in binding score is sufficiently significant to discriminate against the selection of some compounds. To reduce the effect of such variation, we have employed two docking protocols, PyRx 1.91 for virtual screening at different exhaustiveness followed by Auto Dock Vine for validation.

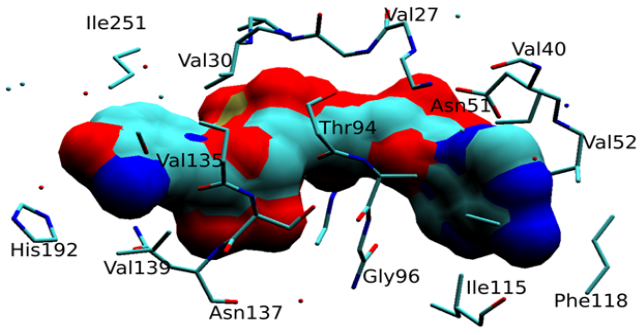

Figure1: 1,4-dihydronicotinamide adenine dinucleotide (surface representation) interacting with the amino acids in the binding site of LDH shown in stick representation. Red patches represent oxygen atoms, blue patches stand for nitrogen atoms while cyan coloration denotes carbon atoms.

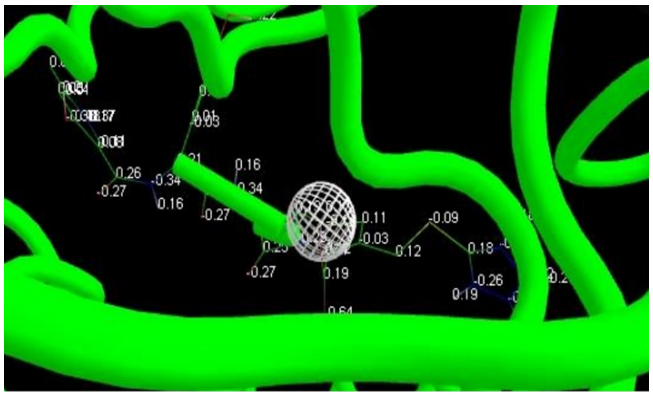

Figure 2: Pictorial representation of the molecular interaction at the binding site of the receptor between the inhibitor (Compound A) and the receptor showing the spatial distribution of charges (represented by the charges, negative and positive) at each carbon with respect to the various molecules and amino acid present at the binding site as illustrated in figure 1. Also note the center of the inhibitor compound displayed by the wire sphere. Picture generated from Vega ZZ [19] .

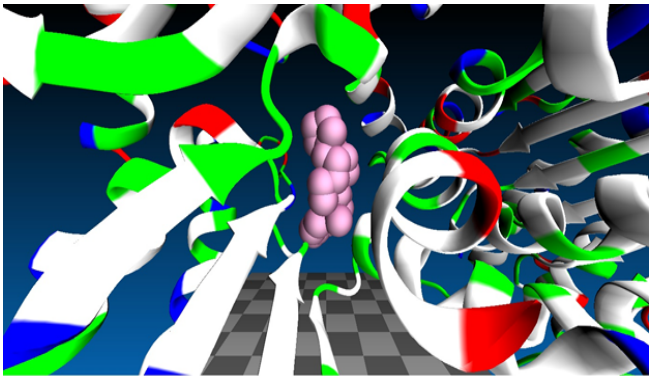

Figure 3: A pictorial representation of the Drug-receptor interaction focusing on the binding site. Notice Compound B fitting in to the binding site of the receptor molecule. Compound B is illustrated as the Brush metal color focused in the binding site of the receptor which is the coil leaf-like structure. Image generated using the Visual Molecular Dynamics (VMD) 1.92 tool.

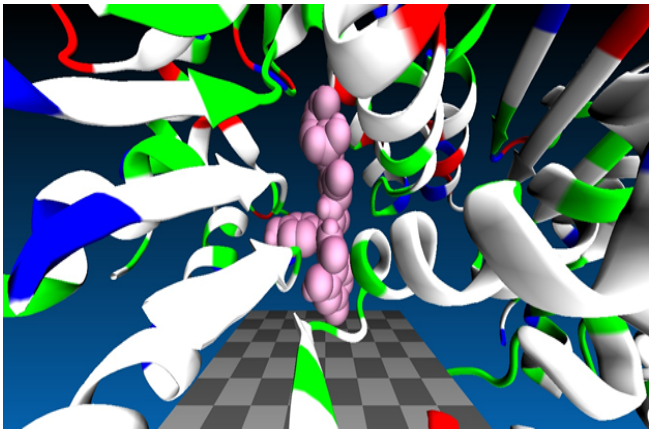

Figure 4: A pictorial representation of the Drug-receptor interaction focusing on the binding site. Notice Compound C with a different structural configuration from compound B fitting in to the binding site of the receptor molecule. Compound C is illustrated as the Brush metal color focused in the binding site of the receptor which is the coil leaf-like structure. Image generated using the Visual Molecular Dynamics (VMD) 1.92 tool.

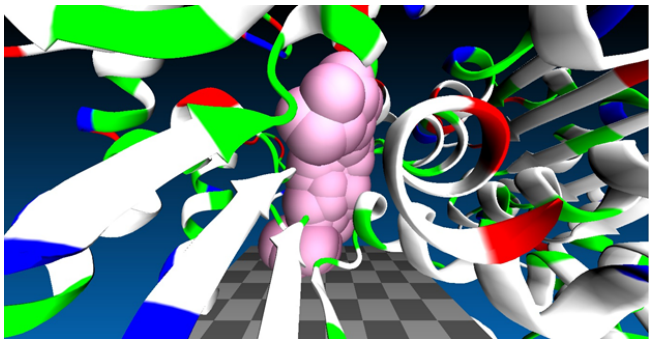

Figure 5: A pictorial representation of the Drug-receptor interaction focusing on the binding site. Notice Compound D fitting in to the binding site of the receptor molecule. Compound D is illustrated as the Brush metal color focused in the binding site of the receptor which is the coil leaf-like structure. Image generated using the Visual Molecular Dynamics (VMD) 1.92 tool.

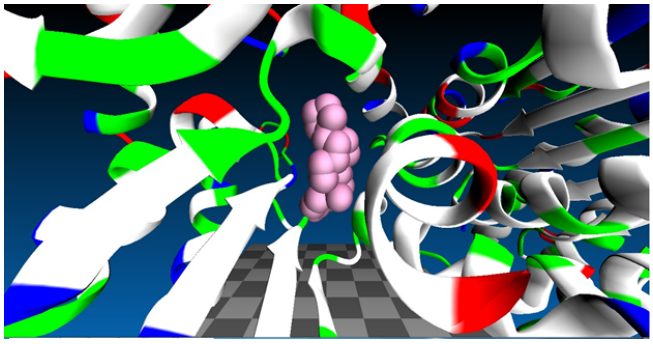

Figure 6: A pictorial representation of the Drug-receptor interaction focusing on the binding site. Notice Compound E fitting in to the binding site of the receptor molecule. Compound E is illustrated as the Brush metal color focused in the binding site of the receptor which is the coil leaf-like structure. Image generated using the Visual Molecular Dynamics (VMD) 1.92 tool.

The orthostatic binding site of NADH in LDH is made up of an amphipathic cavity delicately located at the intersection between seven helices and a ladder of parallel beta sheet structures. At the intersection of the different secondary structures, five loop structures can be identified which form the orthostatic binding site. It is the authors' opinion that specific interactions with the amino acids of the five loops form the basis of LDH activity modulation. And by interacting with loop residues Gly26 to Ala29 (loop 1), Asp51 to Leu53 (loop 2), Thr94 to Arg98 (loop 3), Val135 to Asn137 (loop 4) and His192 (loop 5), Compound A can specifically modulate LDH's activity. For a compound to specifically inhibit the enzymatic activities of LDH, such compound will be capable of binding and interacting with the identified loop residues within the orthostatic binding cavity and in so doing prevent the binding of the natural substrate. The thermodynamic attribute of drug binding to receptors makes it possible to employ the energetics of the ligand-receptor association in distinguishing ligands that are capable of spontaneous interaction (negative binding free energy) from those that are not (positive binding free energy). The availability of a crystal structure with the natural substrate co-crystallized with LDH further improved the amenability of our research question to an in silicon protocol. Thus in identifying inhibitors of LDH, the compounds were not only required to exhibit a negative binding energy at the binding site, their binding energies were also required to be more negative than the value obtained for Compound A.

At the conclusion of the virtual screening of about 30,000 compounds

S/N	Ligand	Binding energy (kcal/mol)
1	Inhibitor (Compound A)	-8.1
2	Compound B	-9.2
3	Compound C	-9.3
4	Compound D	-6.6
5	Compound E	-8.7

Table 1: At an exhaustiveness of 2, the following results were obtained.

S/N	Ligand	Binding energy (kcal/mol)
1	Inhibitor (Compound A)	-8.7
2	Compound B	-9.2
3	Compound C	-9.1
4	Compound D	-8.3
5	Compound E	-8.7

Table 2: At an exhaustiveness of 4, the following results were obtained.

S/N	Ligand	Binding energy (kcal/mol)
1	Inhibitor (Compound A)	-8.6
2	Compound B	-9.2
3	Compound C	-9.1
4	Compound D	-8.7
5	Compound E	-8.7

Table 3: At an exhaustiveness of 8, the following results were obtained.

and post docking validation of the top scoring compounds, only four compounds displaying stronger binding features than Compound A were identified. This suggests a high level of selectivity on the part of LDH for interacting ligands. This, we think, emanates partly from the presence of ionizable amino acids such as Asp51, Arg98, His192 and Asn 137 within the binding site that constitute an electrostatic filter in the selection of ligands. All four identified compounds were able to interact with the binding site residues employed by Compound A, even to a much greater extent as revealed in binding free energies lower than -8.1 kcal/mol (as the baseline exhaustiveness) obtained for Compound A.

Interestingly some of the four compounds are approved drugs. This implies that the respective drugs are being repurposed for a different therapeutic use with the advantage that the typically lengthy development process can be considerably shortened mostly because the data required for regulatory approval are already available in public domain.

Conclusion

Analysis of the interaction between the four compounds and LDH suggests the formation of extensive hydrogen bonding networks between the polar chemical groups in the inhibitor and polar side-chains of the binding site residues. Compounds B through E displayed better binding energetics mostly because they are able to recruit binding site substructure not employed by compound A involving other nonpolar residues. It is however not clear in which precise fashion these sub molecular interaction elements will translate into observable efficacy in both *in vitro* and *in vivo* experiments. But since most therapeutic efficacies involve receptor occupation as the first and most critical step in receptor activation or deactivation, we are confident that our in silicon search have generated good candidates with good potentials to be developed into LDH-inhibiting anticancer agents.

Having undergone various processes in the course of this project, it can hence be stated that there are available compounds which can inhibit the more deleterious Cori cycle in the body and thereby avert its consequences such as cancer cachexia, muscular dystrophy, and fatigue and lowering chances of death in patients suffering from cancer. Thus, inhibiting the enzymatic actions of this molecule in the conversion of pyruvate to lactate will go a long way in averting the outlined consequences of its actions. Theoretically speaking, the use of negative modulators of lactic acid dehydrogenase be used in the treatment of cancer will lead to fierier battle against cancer. This will cause histo toxicity and necrosis for the cancer cells with no significant damage to the normal body cells since lactate is not a very important metabolite in most biochemical pathways. Also, because cancer tissues seriously weaken patients by sapping off relatively large amount energy through the conversion of bio-hazardous lactate (when accumulated) back to glucose.

References

1. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: The next generation. Cell 144: 646-674.
2. Deberardinis RJ, Sayed N, Ditsworth D, Thompson CB (2008) Brick by brick: metabolism and tumor cell growth. Curr Opin Genet Dev 18: 54-61.
3. Vander Heiden MG, Cantley LC, Thompson CB (2009) Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 324: 1029-1033.
4. Knight JR, Milner J (2012) Oncogenic alterations of metabolism. Curr Opin Oncol 24: 68-75.
5. Dang CV, Semenza GL (1999) Oncogenic alterations of metabolism. Trends Biochem Sci 24: 68-72.
6. Vousden KH, Ryan KM (2009) P53 and metabolism. Nat Rev Cancer 9: 691-700.
7. Warburg O (1956) On the origin of cancer cells. Science 123: 309-314.
8. Irani K, Xia Y, Zweier JL, Sollott SJ, Der CJ, et al. (1997) Mitogenic signaling mediated by oxidants in Ras-transformed fibroblasts. Science 275: 1649-1652.
9. Ramanathan R, Mancini RA, Suman SP, Beach CM (2014) Covalent Binding of 4-Hydroxy-2-nonenal to Lactate Dehydrogenase Decreases NADH Formation and Metmyoglobin Reducing Activity. J Agric Food Chem 62: 2112-2117.
10. Yang JM, Chen CC (2004) GEMDOCK: a generic evolutionary method for molecular docking. Proteins 55: 288-304.
11. PyRx - Virtual Screening Tool.
12. Noel MO, Banck M, James CA, Morley C, Vandermeersch T, et al. (2011) Open Babel: An open chemical toolbox. Cheminformatics 3:33.
13. ADT/AutoDockTools
14. Software Downloads.Theoretical and Computational Biophysics Group. University of Illinois at Urbana-Champaign.
15. Trott O, Olson AJ (2010) Auto Dock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. Journal of Computational Chemistry 31: 455-461.
16. Markus Heller, Horst Kessler (2001) NMR spectroscopy in drug design. Pure Appl Chem 73: 1429-1436.
17. Connolly ML (1996) Molecular Surfaces: A Review. Network Science
18. Allison SJ, KnightJRP, Granchi C, Rani R, Minutolo F, et al. (2014) Identification of LDH-A as a therapeutic target for cancer cell killing via (i) p53/NAD(H)-dependent and (ii) p53-independent pathways. Oncogenesis 3: e102.
19. Software Projects. Drug Design Laboratory.