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New Malic Enzyme 1 Inhibitor Design Using Fragmental-Based Virtual Screening and Molecular Dynamic Simulation

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Abstract

Cancer is a deadly disease with a high mortality rate and the most advanced oncology centers of the world are trying to find solutions for it. The most advanced method used for treating cancer is based on the change of metabolism in cancerous cells, namely, the rise of enzymes participating in metabolism and the supply of energy to the cell. Malic enzyme 1 regulates the redox equilibrium, cellular energy, and the synthesis of biomolecules by conversion of a TCA cycle intermediate, malic acid, into NADPH and pyruvic acid. Thus, Malic enzyme 1 seems like a capable asset in the treatment of cancer and its inhibitors can be used to hinder the growth of the cancerous cell. In the present study, we have been using Fragment-based method of drug discovery using molecular dynamic simulation and molecular docking in order to create an inhibitor for malic enzyme 1. After testing roughly ninety thousand pieces of interaction with the binding site of NAD (P) with regard to the type of interactions, binding energy, and the compound orientation on the site, finally, combination 1f was selected as the best inhibitor.

Introduction

Alterations of oncogenic genes such as transitions, spot mutations, and additions and losses of genes are spotted in malignant tumors, and in the treatment of cancer, the oncogenes and the related downstream signals is the focus [1]. Recently, it has been demonstrated that the cellular metabolism of cancer cells evolves to adapt to alterations in the microenvironment and the gene, as well as to survive and maintain the growth [2]. Thus, now researchers are considering the re-targeting of cellular metabolism for the treatment of cancer [3].

In a recent study by Pavlova and colleagues, six symptoms related to cancer were summarized. One of which was the use of intermediates of carboxylic acid (TCAs) and glycolysis for the biosynthesis of nicotinic amide adenine dinucleotide phosphate (NADPH) [4].

NADPH plays a key role in redox homeostasis and macromolecule biosynthesis in cells and acts as a reducer. It is created by enzymes of a metabolic nature, such as 6-phosphogluconate dehydrogenase (6PGD) and glucose 6-phosphate dehydrogenase (G6PD) from the malice enzymes (MEs), pentose phosphate route (PPP), enzymes of tetrahydrofolate oxidation pathways and isocytrate dehydrogenase (IDH). There have been studies on these NADPH-producing enzymes as they are believed to have the potential to be therapeutic targets for cancer [2,5,6].

Redux equilibrium, cellular energy, and the synthesis of biomolecules are regulated by MEs through conversion of malic acid, a TCA cycle intermediate, to NADPH and pyrovic acid. Three males enzyme isoforms have been documented in mammalian cells: a mitochondrial NAD+-dependent isoform (ME2); a cytosolic NADP+-dependent isoform (ME1); plus a mitochondrial NADP+-dependent isoform (ME3) [7,8]. ME1, which has a function different from mitochondrial ME2 and ME3, produces NADPH in cytoplasm. It has been reported that ME1 creates NADPH as much as the G6PD on the PPP Shunt, the primary source of NADP cells [9]. ME1 is used as a predictor marker and a prognostic indicator in cancer [10,11]. Thus, researches are regarding ME1 is a capable target for the treatment of cancer [12,13].

In a study by Wen et al. in 2012 [10], it was demonstrated that in hepatocellular carcinoma (HCC), the higher intensity of ME1 is largely related to a drop in progression-free survival and a decrease in overall survival. Through suppression of epithelial–mesenchymal transition processes in ROS-induced pathways [11], metastasis of HCC is reduced by inhibition of ME1 expression.

In a study by Chakrabarti in 2015 on lung cancer cell, is being shown that ME1 plays a key role in the growth of the cell. They proposed that in lung cancer, ME1 may be a possible therapeutic target and a predictive marker for radiotherapy [13].

In an article in Oncogenesis Journal by Murai and colleagues in 2017, they inhibited the ME1 gene using the siRNA. They demonstrated that inhibition of ME1 can disrupt the metabolism of the cell and thus inhibit the growth of cancer cell [14].

This study has been conducted with the aim of designing a ME1 inhibitor as a potential treatment for cancer. It is vital in the initial phases of drug discovery to find new enzyme inhibitors and new ligands for proteins. In fragment-based approaches, we identify fragments, weak molecules, in order to use them as inhibitors to create stronger inhibitors. This method has already resulted in several compounds that are currently in development in clinical trials or are pre-clinical phases [15-18]. This successful discovery of fragment-based precursors have resulted in wider usage in scientific and industrial organizations [19]. In the present study, we have been using this technique to design a ME1 inhibitor. Finally, the interactions and binding energy of the molecule obtained from docking were investigated with molecular dynamics.

Methods

Docking studies

In this study, two Maybridge fragment screening databases containing 90000 small molecules were employed. All sdf legends were converted to pdbqt.

We obtained the human NADP-dependent ME1 crystallographic structure of RCSB Protein Data Bank (PDBID: 2AW5, resolution: 2.5 Å; http://www.rcsb.org).

Aiming to prepare the protein structure in the AutoDockTools (ADT) program 1.5.6.[20], we acted according to the Docking protocol in our previous articles [21]; we removed all water molecules from the structure obtained from RCSB, then added the Polar hydrogens and assigned Kollman charges. We used the AutoDock 4.2 [22] software to dock small molecules into the malic enzyme protein. We used a 60 × 60 × 60 Å grid box (-0.562, 50.662 and-64.26) (x, y, and z) with 50 docking runs. We then selected the ligands with the most negative energy by comparing each ligand against the ME1 binding energy.

Molecular dynamic

We performed Molecular dynamics simulation by GROMACS 5.0.5 [23] using the OPLSAA force field in order to study the interaction energy of the best compound from docking with ME1 and ligands NADP. We placed the molecules in a cube box which contained TIP3P water molecule model. We replaced water molecules with Na^+ and Cl^- ions in order to create the Ionic conditions of 0.15 molar and neutralized the total system charge. We then executed an initial minimization of energy utilizing the steepest descent algorithm. Afterwards, we executed The NVT simulation for 50 ps and performed the final simulation using NPT ensemble for 30 nanoseconds. In order to maintain the 300° K temperature and 1 bar pressure, we used Nose-Hoover thermostat [24] and Brendsen barostat [25] respectively. We considered r=1.2 for Electrostatic and Vandalvlas and interactions.

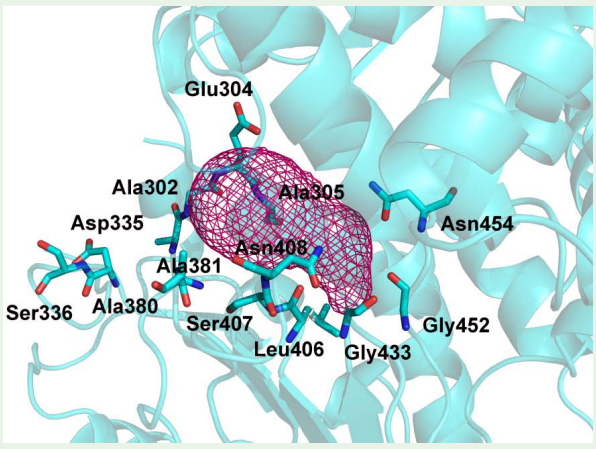

Figure 1: The NAD (P) binding site of ME1 and the important amino acids.

Results and Conclusion

Binding site and ligand conformation

We utilized the CAVAER 3.0.1 software [26] to precept the entrance cavity of NAD(P) binding site. We performed the present study by assigning a shell depth of 4´°A, a maximum probe radius of 0.9´°A, and a clustering threshold of 3.5´°A.

NADP-dependent malic enzyme (P48163) possesses a NAD/NADP-binding domain and an N-terminal domain. The NAD(P) binding site contains 6 strands of parallel beta-sheet and a core Rossmann-type fold. Most important amino acid residues in this domain are Ala302, Ala305, Ala380, Ala381, Asp335, Asn408, Asn454, Glu304, Gly303, Gly433, Gly452, Ser336, Ser407, and Leu406 (Figure 1).

Docking results

We selected 14 compounds Based on the forecasted binding energy obtained from the docking results (Figure 2).

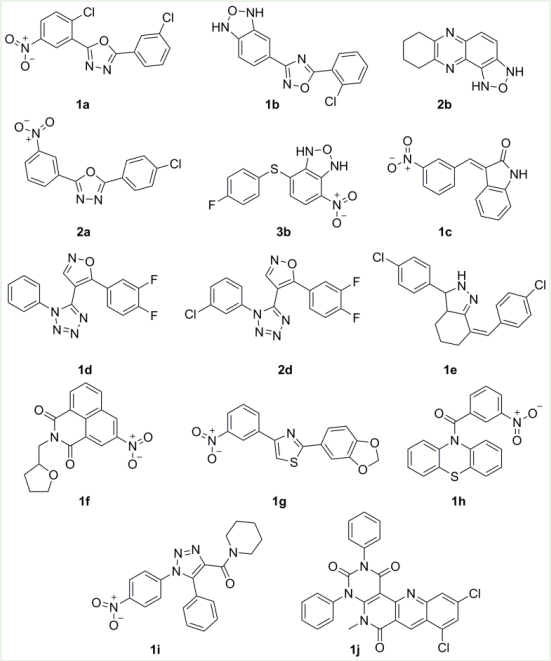

Figure 2: Chemical structures of 14 compounds chosen as the best ligands following the virtual screening result.

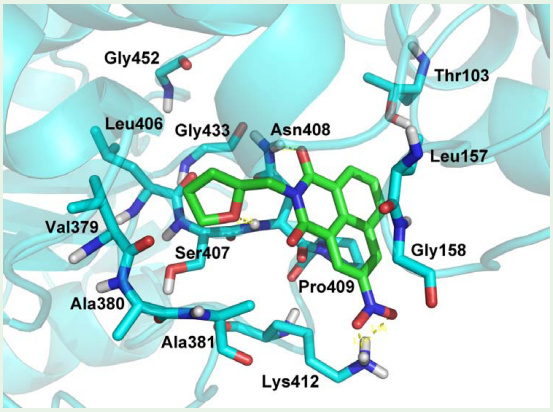

Figure 3: 1f compound's binding mode in the ME1 active site.

NADP interacts with the amino acids Asn408, Asn454, Ala380, Ser407, Leu406, Glu304 and Glu305 in the binding site. Ligands with stronger interactions than NADP connections are potentially able to inhibit NADP binding. We visually inspected the final hit compounds acquired using the VS approach to check for some important interactions with the ME1 NAD (P) binding site. These compounds, their binding energy, and their interaction in the ME1 NAD (P) binding site are depicted in Figure1S in supplementary file.

Based on binding energy acquired from docking results, interaction with main amino acid remains in the active site and alignment, we chose the Compound **1f** (5-nitro-2-((tetrahydrofuran-2-yl)methyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione) as the best-predicted compound.

As you can see in Figure 3, a hydrogen bond was formed by the carbonyl group at C7 isoquinoline with Asn408. Furthermore, another hydrogen bond was formed by the tetrahydrofuran ring's oxygen atom with Ser407. A hydrogen bond was formed by amino acid Lys412 with the 5-nitro phenyl substitution oxygen atom connected to isoquinoline. The amino acid remains Val379, Pro409 and Ala381 formed π-Alkyl interaction with tetrahydrofuran and phenyl rings. Also, a π-Sigma bond was formed in the NAD (P) binding pocket as a result of the interaction between the phenyl ring and Leu157. The Ser407 and Leu406 make van der Waals interaction with tetrahydrofuran ring.

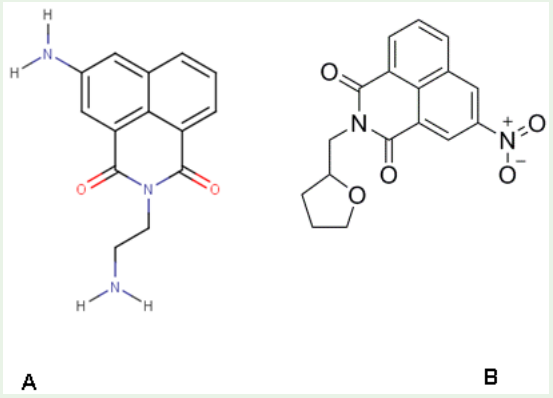

Figure 4: Chemical structures of Amonfide (A) and Ligand 1f (B).

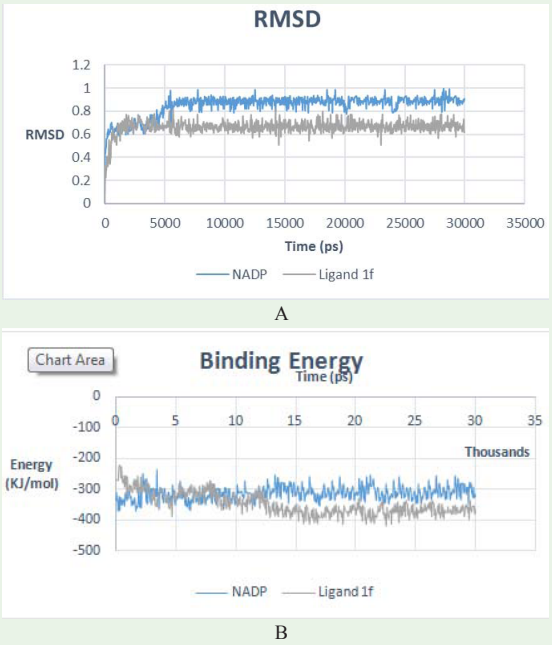

Figure 5: A) RMSD of the last protein structure from the initial structure for both systems contains ligand 1f (gray line) and NADP (blue line). B) The Binding energy of ligand 1f (gray line) and NADP (blue line) to the ME1.

The similarity of ligands with the drug library

We examined the similarity between roughly 95% of all 14 compounds with the drug structures of the Drug Bank. Compound 1f has a structurally similar formula (5-nitro-2 - ((tetrahydrofuran-2-yl) methyl) -1H-benzo [de] isoquinoline-1,3 (2H) -dione (Figure 4B) to Amonfide (Figure 4A).

Amonfide is a derivative of naphthalic acid. Amonfide suppresses topoisomerase II and that leads to the breaking of protein-related strands and disruption of DNA and RNA production. Thus, it can be used in cancer treatment. The anti-cancer effect of this compound with this material is also high due to its structural similarity of over 95%.

Binding Energy

We performed the molecular dynamics simulation to compare the binding energy of NADP and ligand 1f to the ME1. The RMSD graph demonstrated stability in the system (Figure 5A). Binding energy was calculated using g_mmpbsa package. Figure 5B depicts the binding

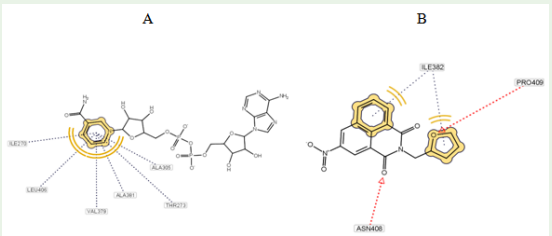

Figure 6: The sites of interaction between NADP (A) and ligand 1f (B) and ME1. The Red line and the black line show hydrogen bond and hydrophobic linkage respectively.

graphs of these two molecules. As you can see, the binding energy of the NADP to ME1 (-300 KJ/mol) is higher than that of 1f (-365 KJ/mol).

Interaction sites

After 30ns of molecular dynamics simulation, we investigated how each of the two ligand 1f molecules and NADP interacted with amino acids of the binding site (Figure 6). Only hydrophobic linkage was formed (black line) between NADP and ME1 (Figure 6a), while there are two hydrogen bonds between the ME1 and the ligand (red line) that resulted in the lower binding energy for ligand 1f.

Conclusion

ME1 converts malic acid to NADPH and acrylic acid and plays a role in regulating redox equilibrium, cellular energy, and biomolecule synthesis. Therefore, scientists consider ME1 as a potential target for the treatment of cancer. The inhibitors of this enzyme can be utilized to disrupt the growth of the cancerous cell.

In the present study, which aimed to design a ME1 inhibitor, fourteen compounds possessing binding energies ranging from (-5) to (-6) were chosen based on the results of VS.

Then, based how they reacted with the key linkage amino acids of Ala302, Ala305, Ala380, Ala381, Asp335, Asn408, Asn454, Glu304, Gly303, Gly433, Gly452, Ser336, Ser407, and Leu406, their binding site alignment and their resemblance to current drug combinations, we selected the 1f compound as the final compound that can potentially inhibit malic acid.

The molecular dynamics simulation demonstrated that the binding energy between ligand 1f and ME1 is lower than NADP and that it also forms stronger connections with the residues in the binding site. Thus, we expect that by binding to the site of NADP, ligand 1f can inhibit the binding and can hinder the ME1 function which leads to the collapse of the cancerous cell. As a result, we introduce compound 1f as a potential anti-cancer compound which is able to inhibit the metabolism of the cancerous cell.

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