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In-silico Identification of Potential Inhibitors of Human Dihydrouridine Synthase 2 for Cancer Therapy

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The formation of dihydrouridine from uridine substrate is catalysed by the human tRNA-dihydrouridine synthase (hDus2) enzyme. The abundance of dihydrouridine, possibly accumulated due to the aberrant function of hDus2, is linked with carcinogenesis. In this study, we focused on hDus2 enzyme, in hopes of discovering novel molecule with affinity for its tRNA binding site. Using the computational method, we performed virtual screening of a natural compound library (NPACT) with Autodock Vina, followed by validation using Smina and Idock. The top hits ZINC08219592, ZINC44387960, and ZINC95098958 were further investigated for their ADME properties to assess their potential as drug candidates. Additionally, the electronic structure properties of the lead molecules were investigated using Density Functional Theory (DFT). Our findings suggest that the identified natural molecules may act as potential hDus2 binders, opening new possibilities for the development of targeted anticancer drugs. This study provides a foundation for further research and the potential advancement of cancer therapeutics targeting on hDus2.

Keywords: In-silico identification; Dihydrouridine; tRNA-dihydrouridine synthase (hDus2) enzyme; DFT

1 Introduction

A significant worldwide health issue with a high fatality rate is cancer. By 2025, it is anticipated that there will be 19.3 million new instances of cancer worldwide, mostly due to changing lifestyles and longer life expectancies[12,3]. At the molecular level, there are several factors that drive a cell toward uncontrolled proliferation. Deleterious lesions and modification in nucleic acidsare the major factors. Recently it has been brought to attention that the human tRNA-dihydrouridine synthase (hDus2) enzyme that catalyzes uridine into dihydrouridine is associated with the promotion of cancers[4–6]. We reasoned that disruption of hDus2 activity would strengthen the correlation between accumulated dihydrouridine levels and cancer progression. hDus2 provides a unique opportunity as a potential drug target and inhibitor of hDus2 can be developed as a potential anti-cancer drug.

In this study, we seek to develop highly specific candidate molecules that can interfere with the interactions between hDus2 and its substrates and open up new therapeutic options for cancer patients. We employed computational methods to screen a library of natural compounds (NPACT) using Autodock Vina[7] for virtual screening with an aim to identify molecules with a strong affinity for hDus2. To ensure the reliability of our findings, we rescreened the best hits with Smina[8] and Idock[9]. In addition to investigating binding affinities, we analyzed the ADME (absorption, distribution, metabolism, and excretion) properties of the top three hits. Understanding these properties is crucial in determining the potential of the identified inhibitors as candidate lead molecules that can be taken for further development. Additionally, we used Density Functional Theory (DFT) to calculate the HOMO-LUMO energy gap, providing insights into the electronic structure and activity of the lead molecules identified through virtual screening. Our findings suggest that identified best hits hold great promise as hDus2 inhibitors and may pave the way for development of novel cancer therapeutics.

2 Materials and Methods

2.1 Protein and Ligand Preparation

The protein data bank (PDB) of the Research Collaboratory for Structural Bioinformatics (RCSB) was used to get the crystal structure of hDus2 (4xp7)[4], at 1.90 Å resolution. Hetero atoms were removed as per protocol and structure was minimized using Chimera. For generating the protein file, polar

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hydrogen atoms were added, AD4 atom type was assigned and a Kollman charge was added using Autodock Tools. The ZINC[12] database was used to get the natural product library (NPACT) structures file for virtual screening. The receptor and drug molecule were finally saved in the PDBQT file for screening[11].

2.2 Virtual Screening

Virtual Screening (VS) is a computational method to analyze a large library of molecules[12,13]. VS was executed via the protein-ligand docking method of AutoDock Vina[7]. Using the previously prepared hDus2coordinates, the VS grid was constructed. Grid parameters were maintained as follows: Center_X = 3.79, Center_Y = - 3.563, and Center_Z = 0.1847 and Size_X = 31 Å, Size_Y = 24 Å, and Size_Z = 21 Å. The number of binding modes was restricted to nine, and the exhaustiveness was set at eight. Binding affinities of molecules with a higher affinity for the binding pocket of hDus2 were selected. To further validate the process the top selected compounds were screened using Smina[8] and Idock[9]. The results from AutodockVina were considered for the analysis. Further, the evaluation of the molecules for ADME properties and electronic properties were computed using SwissADME[14], a webserver-based tool and Argus Lab tool.

3 Results and Discussion

3.1 Virtual Screening

To identify inhibitors against hDus2, a structure-based virtual screening approach was used using Autodock Vina, Idock, and Smina tools. A collection of natural compounds (NPACT[15]) was obtained from the ZINC[12] database. Each molecule was evaluated based on its binding affinity. The top compounds with the highest binding scores were chosen based on their affinity towards hDus2, indicating their potential to inhibit the enzyme. The resulting compounds exhibited binding affinities ranging from -11.3 to -8.5 kcal/mol for Autodock Vina, -11.3 to -10 kcal/mol for Idock, and -11.2 to -8.4 kcal/mol for Smina (Table 1).

Table 1 — Binding Affinities of Natural Products Against hDus2 using Multiple Docking Tools (Vina, Smina and Idock)

Compounds	Vina	Smina	Idock
ZINC08219592	-10.4	-11.3	-10.4
ZINC08681672	-9.4	-9.3	-10.5
ZINC44387960	-11.3	-8.4	-11.2
ZINC95098943	-10.5	-10.7	-10.6
ZINC95098956	-11.0	-11.5	-10.0
ZINC95098958	-11.1	-11.7	-11.2
ZINC95099084	-10.1	-8.9	-10.4
ZINC95099365	-8.5	-9.0	-10.3
ZINC95099371	-8.9	-10.4	-10.5

Among them, ZINC08219592, ZINC44387960 and ZINC95098958 displayed the lowest energy with a binding affinity (Table 1). All selected compounds demonstrated considerable binding affinities towards hDus2, indicating their potential for inhibiting the enzyme (Table 1). Finally, the top three compounds were selected for further studies.

3.2 ADME Analysis

Considering the potential development of oral drugs ADME prediction is found to be effective. ZINC08219592 has the formula $C_{33}H_{54}O_8$ with a molecular weight of 578.78. The molecule has 3 rotatable bonds, 8 H-bond acceptors, and 4 H-bond donors. The MR (Molar Refractivity) value is 154.45, and the TPSA (Topological Polar Surface Area) is 117.84. The iLOGP is 3.94, XLOGP3 is 4.9, WLOGP is 3.62, and MLOGP is 2.56. The molecule has an ESOL Log S of -6.32, indicates low solubility. It does not inhibit the CYP1A2, CYP2C19, CYP2C9, CYP2D6, or CYP3A4 enzymes. The log Kp (partition coefficient) is -6.35 cm/s. ZINC08219592 violates 1 Lipinski rule and 0 violations in terms of Veber, Egan, and Muegge criteria. The Bioavailability Score is 0.55, indicating moderate bioavailability. ZINC44387960 has the formula $C_{39}H_{53}O_5$- with a molecular weight of 601.84. It has 5 rotatable bonds, 5 H-bond acceptors, and 1 H-bond donor. The MR value is 176.34, and the TPSA is 86.66. The iLOGP is 4.88, XLOGP3 is 9.65, WLOGP is 7.62, and MLOGP is 6.42. The molecule has an ESOL Log S of -9.42, indicating low solubility. The ESOL Solubility is 2.28E-07 mg/ml, and it is classified as insoluble. It does not inhibit the CYP1A2, CYP2C19, CYP2C9, CYP2D6, or CYP3A4 enzymes. The log Kp is -3.12 cm/s. It violates Lipinski rules and 0 violations in terms of Veber, Egan, and Muegge criteria. The Bioavailability Score is 0.56, indicating moderate bioavailability.

ZINC95098958 has the formula $C_{38}H_{51}O_6$- with a molecular weight of 603.81. It has 5 rotatable bonds, 6 H-bond acceptors, and 2 H-bond donors. The MR value is 172.7, and the TPSA is 106.89. The iLOGP is 4.52, XLOGP3 is 8.27, WLOGP is 6.34, and MLOGP is 5.43. The molecule has an ESOL Log S of -8.56, indicating low solubility. The ESOL Solubility is 1.65E-06 mg/ml. It does not inhibit the CYP1A2, CYP2C9, CYP2D6, or CYP3A4 enzymes. The log Kp is -4.11 cm/s. It violates Lipinski rules, and 0 violations in terms of Veber, Egan, and Muegge criteria. The Bioavailability Score is 0.56, indicating moderate bioavailability.

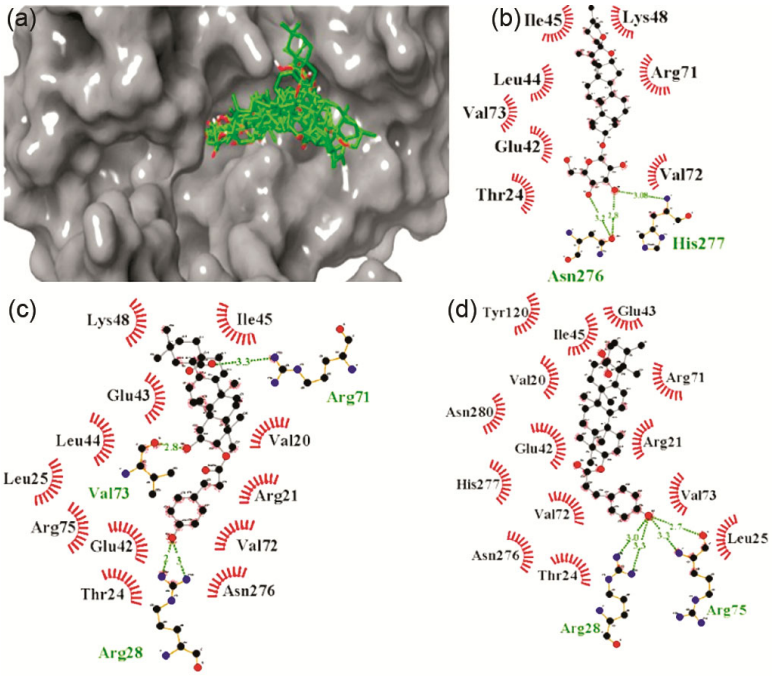

Fig. 1 — Surface and ligplot diagrams showing binding interactions of top three natural products with hDus2. (a) Surface diagram. Ligplot of compound (b) ZINC08219592, (c) ZINC95098958, (d) ZINC44387960. Residues showing hydrogen bonding are shown in green. Representation is drawn using Ligplot+[16].

3.3 Interaction of Compounds

All three natural products, namely Compound ZINC08219592, ZINC44387960, and ZINC95098958, were found to interact with hDus2at the tRNA binding site. ZINC08219592 interacted with Thr24, Glu42, Leu44, Ile45, Lys48, Arg71, Val32, Val73, Asn276, and His277, forming two hydrogen bonds with Asn276 and one hydrogen bond with His277. ZINC44387960 interacted with Val20, Arg21, Arg28, Thr24, Leu25, Glu42, Glu43, Ile45, Arg71, Val72, Val73, Arg75, Tyr120, Asn276, His277, and Asn280, with two hydrogen bonds with Arg28 and two hydrogen bonds with Arg75. ZINC95098958 interacted with Val20, Arg21, Thr24, Leu25, Arg28, Glu42, Glu43, Leu44, Ile45, Lys48, Arg71, Val72, Val73, Arg75, and Asn276, forming two hydrogen bonds with Arg28 and one hydrogen bond with Val73 (Figure 1). These interactions provide valuable insights into the binding mechanisms of these compounds with hDus2, offering potential avenues for further research and drug development targeting this protein.

3.4 Density Functional Theory

Using DFT analysis E_{HOMO}, E_{LUMO} and ΔE (energy gap) are calculated, which tells about the stability of individual molecules. The top three identified molecules have an energy gap in between the range of 0.563 to 1.1. The lowest energy was observed for the molecule ZINC95098958. ΔE for the other two top molecules ZINC08219592 and ZINC95098958 is 0.566 and 1.123 respectively.

4 Conclusion

In this study, an in-silicomethodology was used to identify potential compounds capable of inducing apoptosis by targeting hDus2, a key protein involved in cancer progression. Three natural compounds, namely ZINC08219592, ZINC44387960, and ZINC95098958, were discovered with a higher binding affinity ranging between − 11.1 and − 10.4 kcal/mol towards hDus2. However, further analysis revealed certain characteristics and limitations of these compounds based on ADME (absorption, distribution, metabolism, and excretion) analysis. ZINC08219592, ZINC44387960, and ZINC95098958 exhibited average solubility and showed some violations of Lipinski rule, indicating potential limitations in their pharmacokinetic properties. Overall, the present work identifies ZINC08219592, ZINC44387960, and ZINC95098958 as potential inhibitors of hDus2, presenting a promising avenue for the development of novel

anticancer therapeutics. However, further research, including modifications and in vitro validation, is imperative to enhance their suitability as cancer drug candidates. This study serves as a foundation for future investigations, ultimately aiming to advance targeted treatment strategies and improve the outcomes of cancer patients.

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