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Computational Docking Study of Alpha-Fetoprotein Binding Affinity with Isoindole Using AutoDock

Abstract – Alpha-fetoprotein (AFP) is a well-established biomarker for monitoring hepatocellular carcinoma (HCC). Targeting of AFP remains as a clinical challenge with limit survival benefits with current drugs. Recent studies suggested anti-proliferative activities of isoindole in cancer. To date, the interaction between AFP and isoindole is yet to be determined. Hence, the study aimed to determine the binding interaction between isoindole and the AFP. Molecular docking simulations were performed using AutoDock 4.2.6 with the crystal structure of AFP (PDB ID: 8X1N). Binding poses were clustered and evaluated based on binding energy and RMSD. The top docking pose exhibited a binding affinity of –6.04 kcal/mol and localized isoindole within a hydrophobic surface pocket of AFP. Clustering analysis demonstrated consistent binding conformations, with 41 out of 100 poses forming a dominant low-RMSD cluster (Table 1). The isoindole binding pocket was predominantly composed of hydrophobic and neutral residues, including Leu132, Ala134, Phe170, and Tyr202, forming van der Waals interactions with the ligand. No strong hydrogen bonding was observed in the top pose, consistent with the hydrophobic nature of isoindole. The spatial orientation also suggests potential accessibility from the solvent side, supporting the possibility of AFP-ligand interaction occurring at or near the plasma membrane or extracellular domain. These residues are probably crucial for the binding and recognition of isoindole. The binding is stabilized by hydrophobic interactions (Leu, Phe). Isoindole may be able to prevent AFP from playing a part in the development of cancer if the binding pocket is close to the functional domain of AFP. By interacting with aromatic rings, aromatic residues like Tyr and Phe increase affinity. These interactions demonstrate that isoindole fits into the binding site of AFP nicely and can function as a possible modulator or inhibitor. The finding indicated a stable interaction between isoindole and AFP, with potential relevance to its uptake by HCC cells. AFP may play a role in modulating isoindole transport or bioavailability. This warrants further validation by *in vitro* uptake assays and mechanistic studies.

Keywords – Isoindole, hepatocellular carcinoma, in silico

1 INTRODUCTION

Alpha-fetoprotein (AFP) is a well-established biomarker for monitoring hepatocellular carcinoma (HCC). Targeting of AFP remains as a clinical challenge with limited survival benefits with current drugs. Recent studies suggested anti-proliferative activities of isoindole in cancer. To date, the interaction between AFP and isoindole is yet to be determined. Hence, the study aimed to determine the binding interaction between isoindole and the AFP.

2 MATERIALS&METHODS

2.1 Retrieval of Structures

The crystal structure of alpha-fetoprotein (AFP) was retrieved from the Protein Data Bank (PDB ID: 8X1N) and saved in PDB format. Isoindole was obtained from publicly available chemical databases (e.g., ChemSpider) and converted to compatible formats (MOL/SDF) for docking preparation.

2.2 Receptor Preparation

The AFP structure was opened in AutoDockTools (ADT). All water molecules, crystallographic ligands, and non-essential atoms were removed. Polar hydrogens were added, followed by merging of non-polar hydrogens. Kollman charges were assigned to the receptor, and the final structure was saved in PDBQT format using AutoDockTools.

2.3 Ligand Preparation

The isoindole structure was converted to PDB format using Open Babel.Hydrogen were added and geometry was checked to ensure proper protonation. Torsional bonds were assigned to

generate the **torsion tree**, and Gasteiger charges were applied. The final ligand was saved as ligand.pdbqt.

2.4 Workspace Setup

AutoDock 4.2.6 and AutoGrid executables were placed inside the working directory to ensure direct access during docking simulations.The working directory was set as the default startup directory in ADT to streamline file generation.

2.5 Grid Box Configuration

The binding search area was defined by generating a grid box around the predicted binding pocket of AFP. The grid center and dimensions were adjusted to fully cover the target region. Grid spacing was set according to AutoDock parameters (default 0.375 Å unless otherwise modified). A Grid Parameter File (GPF) was generated for AutoGrid calculations.

2.6 Docking Simulation

Docking was performed using the Lamarckian Genetic Algorithm (LGA) in AutoDock 4.2.6. Key parameters such as the number of GA runs, population size, evaluations, and generations were set according to standard AutoDock protocols (with specific values reported in the Results section). A Docking Parameter File (DPF) was generated and used to execute docking with AutoDock 4. AutoGrid was run prior to docking to compute interaction energy maps for all atom types in the ligand.

2.7 Analysis of Docking Results

Docking log files (DLG) were loaded into ADT for analysis. All predicted binding poses were clustered based on root-mean-square deviation (RMSD) with the appropriate clustering threshold. The best binding pose was identified based on: lowest binding energy, cluster size (dominant conformational group), and orientation within the binding pocket. Interacting residues were identified based on proximity, hydrophobic contacts, π–π interactions, and hydrogen bonding when present. Visualizations of receptor–ligand interactions were generated for reporting.

3 CONCLUSION

The finding indicated a stable interaction between isoindole and AFP, with potential relevance to its uptake by HCC cells. AFP may play a role in modulating isoindole transport or bioavailability. This warrants further validation by *in vitro* uptake assays and mechanistic studies.

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Table 1. Clustering of Docked Isoindole–AFP Poses Based on RMSD and Binding Energy

Cluster	Number of Poses	RMSD Range (Å)	Binding Energy Range (kcal/mol)	Description
Cluster 1	41	≤ 2.0	–6.04 to –5.7	Dominant cluster; stable and consistent binding
Cluster 2	22	2.5 – 3.5	–5.6 to –5.2	Alternate pose; less favored conformation
Cluster 3+	≤ 10 per cluster	> 4.0	–5.4 to –4.6	Structurally diverse; lower relevance