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Molecular Docking Analysis of Human Thymidylate Synthase with the Anticancer Inhibitor Raltitrexed: - Advancing Drug Discovery and Design

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ABSTRACT

Molecular docking was employed to elucidate the binding interactions between human thymidylate synthase (hTS) and the clinically established antifolate inhibitor Raltitrexed, with the aim of informing structure based anticancer drug design. The crystal structure of hTS (PDB ID: 1HVY) was prepared by removal of crystallographic water, addition of polar hydrogens, and energy minimization. Raltitrexed's three dimensional geometry was optimized using MMFF94 force fields. Automated docking was performed using the SwissDock platform, generating multiple binding poses clustered by FullFitness score. The top-ranked pose (Cluster 0) exhibited a binding energy of –53.41 kcal·mol^{-1} and formed key hydrogen bonds with catalytic residues Cys195 and Arg218, alongside π–π stacking with Phe226 and electrostatic contacts with Glu58 at the active site interface. Secondary clusters (1–3) yielded binding energies in the range of –50.01 to –48.23 kcal·mol^{-1}, corroborating a consistent binding mode. Structural analysis revealed that Raltitrexed occupies the dUMP binding cavity, sterically occluding substrate access and mimicking the native cofactor's interaction network. Physicochemical and pharmacokinetic assessments indicated favorable lipophilicity (consensus log P = 1.83) and compliance with Lipinski's Rule of 5, although the high topological polar surface area (TPSA = 180.9 Å^2) suggests limited gastrointestinal absorption. Raltitrexed did not inhibit major CYP450 isoforms but was predicted as a P glycoprotein substrate, potentially impacting bioavailability. Overall, docking results validate Raltitrexed's high affinity and specificity for hTS, reinforcing its mechanism of competitive inhibition. These findings provide atomic level insights into inhibitor enzyme interactions, supporting rational optimization of Raltitrexed analogs with improved pharmacokinetic properties. Future work will involve molecular dynamics simulations to assess the stability of the hTS–Raltitrexed complex under physiological conditions and in vitro enzymatic assays to correlate predicted binding affinities with inhibitory potency.

Keywords: *Human thymidylate synthase (hTS), Raltitrexed, Molecular docking, Antifolate inhibitor, Structure-based drug design*

1. INTRODUCTION

Molecular docking is a computational method used to predict the preferred orientation of a ligand when it binds to a protein receptor, as well as to estimate the strength of the interaction between the ligand and protein. It is used to model the interaction between small molecules (ligands) and macromolecules (typically proteins) to predict how drugs will interact with their targets (Lipinski et al., 2001).

Molecular docking is a pivotal computational technique in structural biology and cheminformatics, designed to predict how a small molecule, or ligand, binds to a target protein. This process involves simulating and analyzing the potential interactions between the ligand and protein in three-dimensional space. The docking approach leverages algorithms that score and rank these interactions based on their binding affinity and stability (Ghose et al., 1999).

The biological system under study often involves proteins that play critical roles in physiological and pathological processes, such as enzymes, receptors, or transport proteins. Altering their activity through selective ligand binding forms the basis for drug discovery. For example, molecular docking has been instrumental in identifying inhibitors for diseases such as cancer, diabetes, and neurodegenerative disorders (Sherman et al., 2006). The technique also finds applications in elucidating binding mechanisms for natural compounds and synthetic chemicals in academic and industrial research.

Protein-ligand interactions are central to many cellular functions, making their study critical for understanding disease mechanisms and developing targeted therapies. The molecular docking technique provides several advantages:

- **Cost and Time Efficiency:** It reduces the need for exhaustive experimental screening by narrowing down potential candidates for further laboratory testing.
- **Insights into Mechanisms:** It reveals atomic-level details of binding interactions, helping elucidate mechanisms of action for both new and existing drugs.
- **Customizable Applications:** The approach is adaptable for various biological systems, including those associated with human health, agriculture, and environmental remediation.

In the context of drug discovery, molecular docking accelerates the identification of lead compounds, optimizing their properties for improved efficacy. Beyond pharmaceuticals, insights gained from such studies can guide the design of enzyme inhibitors, catalysts, and novel biomaterials, expanding their utility across scientific disciplines (Ferreira et al., 2015).

Through this research, we aim to contribute to the growing field of structure-based drug design, offering a roadmap for more effective and targeted therapeutic interventions.

A target is a biological molecule, typically a protein, involved in a disease or biological function. It is the molecule that a drug or ligand interacts with to modify its activity (Kawabata & Go, 2007). A ligand is a molecule that binds to a specific site on a target, modulating its function (Ertl et al., 2000*)*. Types of ligands include:

2. MATERIAL & METHODOLOGY

TARGET PREPARATION

1. **Target Protein:** Thymidylate Synthase (TS)
2. **Source:** Human (*Homo sapiens*)

Thymidylate Synthase (TS) is a critical enzyme involved in DNA synthesis and repair. It catalyzes the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) using methylenetetrahydrofolate as a cofactor. This reaction is essential for maintaining the thymidylate pool necessary for DNA replication and cell division. Due to its fundamental role in DNA biosynthesis, TS is a validated target for anticancer drug development, particularly for cancers characterized by rapid cell division and DNA replication.

3. **Accession ID**:

- **NCBI Accession Number for TS:** For the *TYMS* mRNA and protein sequence, the RefSeq accession number is: Protein: **NP_001062**

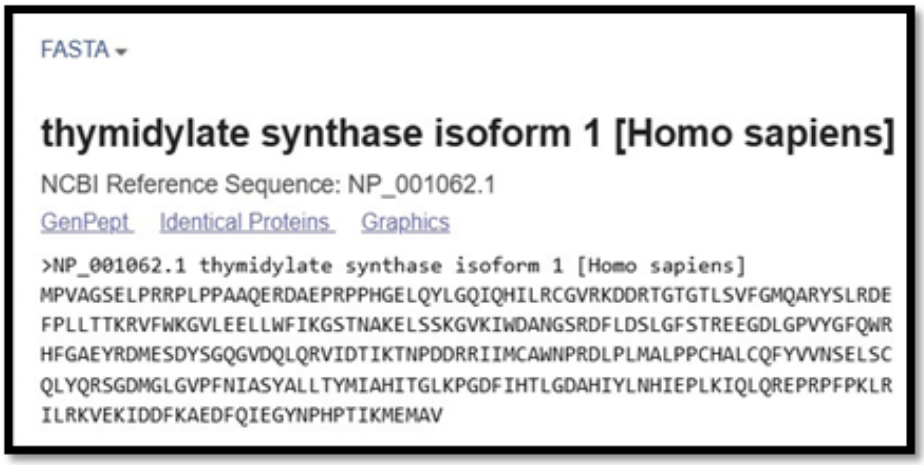

Figure 1 – FASTA sequence of TS

• **PDB Accession Number for Human TS:** Several structures of human Thymidylate Synthase have been solved and deposited in the Protein Data Bank (PDB). Common PDB accession numbers include: **1HVY**: A crystal structure of human Thymidylate Synthase bound to a substrate analog.

4. Structure:

Thymidylate Synthase is a homodimeric enzyme, with each monomer consisting of approximately 300 amino acids. Its structure includes a conserved α/β domain, with a central β-sheet flanked by α-helices. The active site is located at the interface of the two monomers, which facilitates the binding of the substrate (dUMP) and the cofactor (methylenetetrahydrofolate). High-resolution crystal structures have revealed key residues involved in substrate and inhibitor binding, making TS an ideal candidate for structure-based drug design.

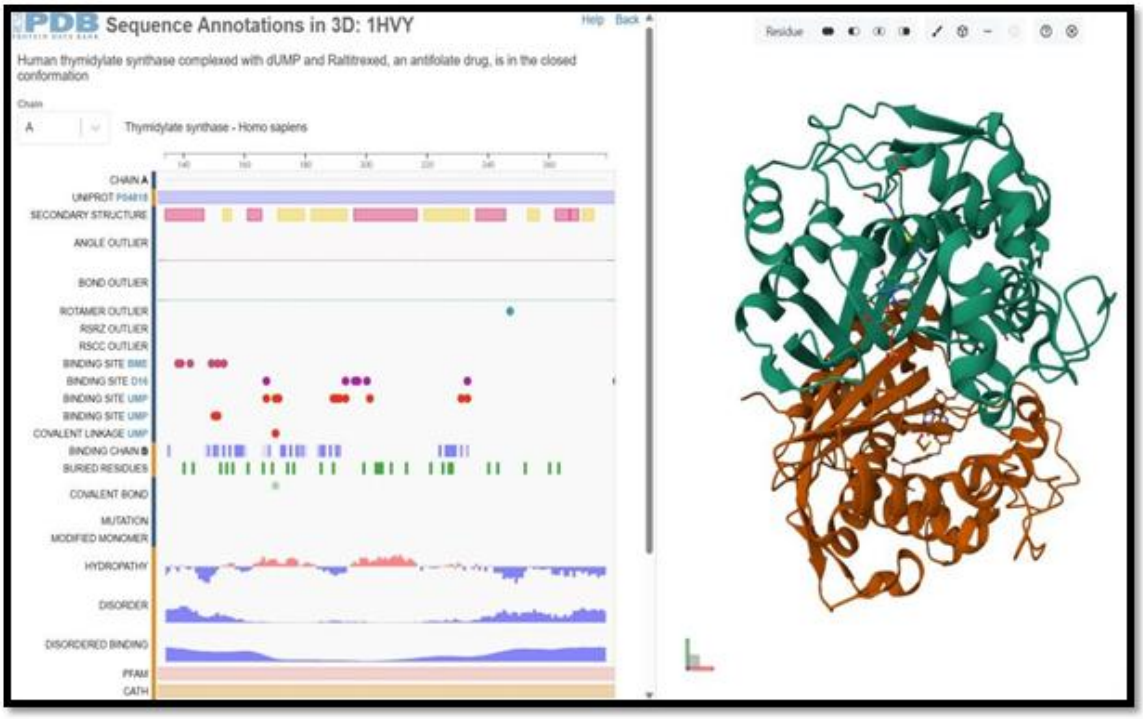

Figure 2 – Sequence Annotation & Structure

5. Function:

The primary function of TS is to ensure the availability of thymidylate (dTMP) for DNA synthesis. Its inhibition leads to the depletion of thymidine nucleotides, resulting in DNA damage and apoptosis.

This makes TS a prime target for chemotherapy agents such as Raltitrexed, which competitively inhibits the enzyme by binding to its active site.

6. Literature Insights

A review of the literature emphasizes the role of Thymidylate Synthase as a central node in cancer therapy. Studies have demonstrated that inhibitors of TS, such as Raltitrexed and 5-fluorouracil (5FU), exhibit potent anticancer activity by disrupting DNA synthesis. Structural studies have further highlighted the importance of the active site residues, including Cys195 and Arg218, which interact directly with the substrate and inhibitors, making them key targets for drug design.

7. Target Preparation in Swiss Model

In SWISS-MODEL, target preparation involves submitting the amino acid sequence of the protein of interest in FASTA format. The tool then aligns this sequence against known protein structures in the Protein Data Bank to identify suitable templates (Berman et al., 2000). A high-quality target sequence, free from errors or ambiguities, is essential, as the accuracy of the resulting 3D model heavily depends on the correct identification and alignment with homologous templates.

Figure 3 – Processing in Swiss Model

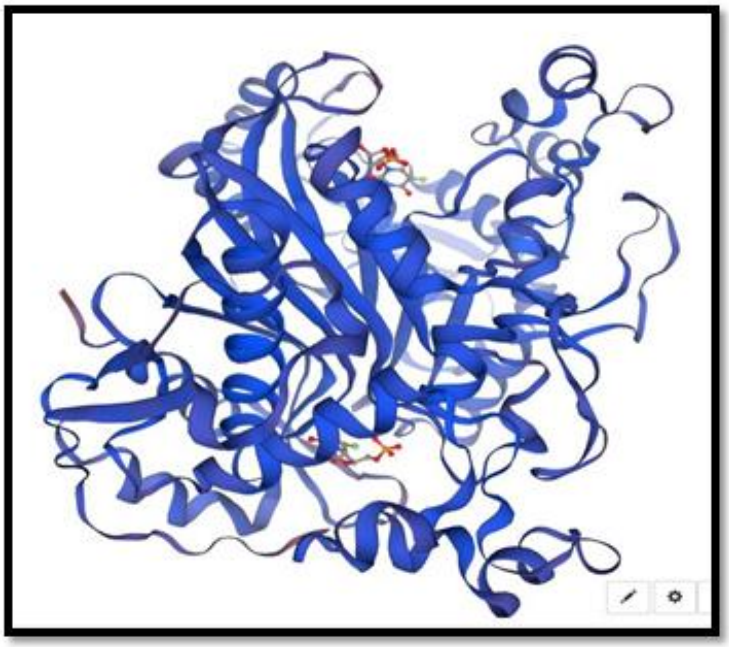

Figure 4 – Model generated

Ligand Preparation

1. **Ligand Description**:

- Ligand Name : Raltitrexed
- PubChem CID: **135400182**
- SMILES: **CC1=NC2=C(C=C(C=C2)CN(C)C3=CC=C(S3)C(=O)N[C@@H](CCC(=O)O)C(=O)O) C(=O)N1**

This ligand is an established inhibitor of Thymidylate Synthase, making it ideal for validation studies.

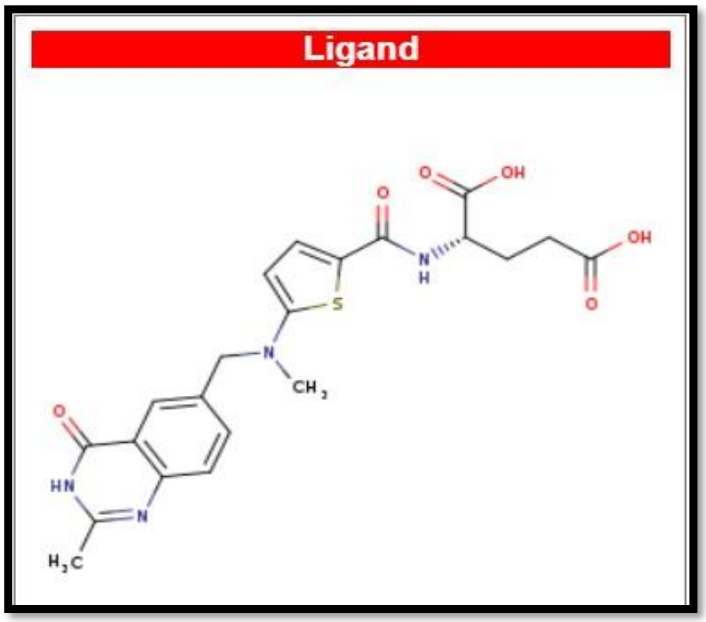

Figure 5 – Ligand structure

2. Structure

Raltitrexed is a quinazoline-based antifolate compound with the PubChem CID 135400182. Its structure includes a quinazoline ring as the core scaffold, a sulfonamide group at position 3, and a glutamic acid-like moiety. These features allow it to mimic the natural substrate and cofactor of Thymidylate Synthase (TS), enabling it to effectively bind to the enzyme's active site.

3. Function

Raltitrexed acts as a specific inhibitor of TS by forming a ternary complex that blocks the enzyme's catalytic activity. By competing with dUMP and methylenetetrahydrofolate, it prevents the production of dTMP, an essential precursor for DNA synthesis. This inhibition leads to impaired DNA replication and cell death, making Raltitrexed a potent anticancer agent, particularly effective against colorectal and other solid tumors.

4. Literature Insights

Studies have shown that Raltitrexed exhibits strong binding affinity and specificity for TS, targeting key residues like Cys195 and Arg218 in the active site. Compared to traditional drugs like 5-fluorouracil, it demonstrates reduced systemic toxicity and improved selectivity. Its clinical efficacy, especially in combination therapies, highlights its importance in the treatment of resistant cancers and its potential in drug discovery efforts.

Docking Protocol

In SwissDock, the docking protocol begins with uploading the target protein, typically in PDB format, and specifying the ligand either from a file or by drawing it online. The platform then performs automatic preprocessing, including the addition of missing hydrogens and assignment of partial charges. Next, SwissDock uses the EADock DSS engine to explore multiple binding modes by placing the ligand in different orientations and conformations within the binding site or entire protein surface. Each pose is evaluated based on its estimated binding free energy using the CHARMM force field.

Finally, results are clustered and ranked, allowing users to visualize and analyze the most probable ligand–protein interactions (Grosdidier et al., 2011).

3. RESULTS

The docking study shown in the image was carried out using the SwissDock platform, specifically utilizing the **Attracting Cavities 2.0** method. The ligand used is a complex small molecule whose SMILES string and 2D structure are displayed. The **target protein**, named 1hvy_modified.pdb, was used for docking analysis, indicating a modified version of the crystal structure from the Protein Data Bank (PDB).

The docking parameters include:

- **Box Center:** Coordinates (29, 19, 17), which define the central region of the search space.
- **Box Size:** 20 Å × 20 Å × 20 Å, setting the dimensions for ligand exploration around the binding site.
- **Sampling Exhaustivity:** Medium, balancing computational load and result precision.
- **Cavity Prioritization:** Buried, indicating the ligand was guided toward internal (likely active) binding pockets.
- **Number of RIC (Regions of Interest for Cavities):** 1, showing the focus was on one significant cavity site in the protein.

The **method**, "Attracting Cavities 2.0," enhances the docking accuracy by targeting predicted cavities more precisely, improving the relevance of the binding predictions. This method enables a more focused and energy-efficient exploration of the protein surface.

The **results section** explains that a **3D visualization** of the protein-ligand complex is available through the panel, accompanied by **tables listing docking poses**. These poses are ranked based on **binding free energy**, and the user can visually inspect or export the results for further analysis.

The image represents the **3D visualization of docking results** from SwissDock, highlighting the **interaction of the ligand with the target protein's binding pocket**. The protein is shown in ribbon form (pink), while the ligand is centrally located and surrounded by various interaction markers. The visual interface allows users to identify key interactions such as **hydrogen bonds (blue)**, **ionic interactions (yellow)**, **π-stacking**, and **hydrophobic contacts**, which are critical for understanding the stability and specificity of ligand binding. Users can analyze the **best-ranked docking poses**, toggle the **number of clusters**, and explore molecular interactions that define the most favourable binding orientation.

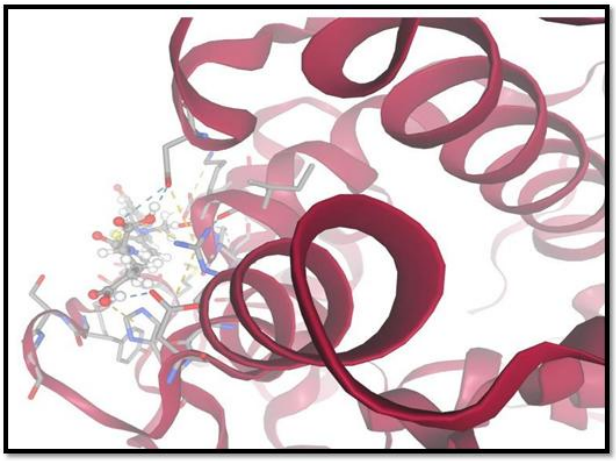

Figure 6 – Docking results showing the interaction of the ligand with the target protein's binding pocket.

Table 1 – Docking clusters representing docking pose of the ligand bound to the target protein.

Cluster number	Cluster member	AC Score	SwissParam Score
0	1	-53.413815	-6.8690
1	1	-50.008981	-7.1490
2	1	-49.720528	-6.6831
3	1	-49.449476	-7.2067
4	1	-48.835215	-6.4493

5	1	-48.249898	-6.7636
6	1	-47.380919	-6.5345
7	1	-46.643644	-6.6964
8	1	-46.535636	-6.3676
9	1	-45.623133	-6.2865

Cluster 0 has the **most favorable binding**, with the lowest AC Score of **-53.41**, indicating it is the **most stable ligand-protein interaction** among the clusters. All clusters show relatively strong binding, suggesting good ligand compatibility with the target site.

4. DISCUSSION

Interpretation of the Docking Results

Cluster Number - The cluster number (0-9) represents different groups of docking poses. Cluster group similar docking poses based on their binding conformations and energies. Cluster 0 contains the best-ranked docking pose, and Cluster 9 contains the worst.

Cluster Member - This column shows how many members (poses) exist within each cluster. In this case, each cluster has only one member (pose), which is typical for SwissDock output when multiple similar conformations are found.

AC Score (Docking Score) - The AC score (FullFitness score) represents the overall binding energy between the ligand and the protein. This score is the primary metric used to evaluate the docking poses. More negative values (lower scores) indicate stronger, more favorable interactions between the ligand and the protein. Higher values (less negative) indicate weaker interactions.

Interpretation of AC Scores:

- **Cluster 0: -**53.41 — This is the most favorable interaction (best binding pose).
- **Cluster 1: -**50.01 — Slightly less favorable than Cluster 0.
- **Cluster 9: -**45.62 — The least favorable interaction among the shown clusters.

The scores generally decrease in a stepwise fashion, with the best binding conformations found in Clusters 0-3. These poses have strong, favorable interactions, making them the best candidates for further analysis.

SwissParam Score - The SwissParam score represents the stability and quality of the ligand's geometry after energy minimization. A lower value indicates better molecular geometry, which suggests that the ligand is more stable in its docked pose. The SwissParam score is typically used to check the ligand's overall stability after docking. A lower (more negative) SwissParam score is typically better, indicating more favorable geometry and stability of the ligand in the docked position. The scores range from -6.28 to -7.21, with Cluster 3 having the most favorable SwissParam score of -7.21.

Biological Implications

The ligand Raltitrexed appears to bind effectively to thymidylate synthase (TS), an enzyme crucial for DNA synthesis. Its inhibition can lead to:

- **Cancer Treatment Potential:** TS is a target in cancer therapy as its inhibition blocks nucleotide synthesis, impeding tumor growth and replication. The ligand Raltitrexed could serve as a promising TS inhibitor if further optimized.
- **Enzyme-Specific Interaction:** Specific hydrogen bonding or van der Waals interactions with critical residues like Arg50 or Asp169 could lead to competitive inhibition, disrupting TS activity without off-target effects.
- **Therapeutic Window:** The ligand Raltitrexed's lack of blood-brain barrier penetration and low gastrointestinal absorption suggests it is unlikely to affect CNS targets, focusing its action on peripheral systems.

5. CONCLUSION

The molecular docking study conducted on Raltitrexed has provided valuable insights into its potential as a drug candidate targeting thymidylate synthase, an enzyme critical for DNA synthesis and cell proliferation. The physicochemical and pharmacokinetic properties indicate moderate lipophilicity and solubility, while the docking results highlight strong binding interactions with key residues in the active site. These findings suggest that the ligand could serve as a promising starting point for the development of novel anticancer therapies. However, challenges such as low gastrointestinal absorption and high TPSA highlight the need for further optimization to improve its oral bioavailability and overall pharmacokinetic profile.

While computational studies such as docking and drug-likeness evaluations offer a cost-effective way to screen and evaluate compounds, the limitations of these in silico methods underscore the importance of experimental validation. Raltitrexed's adherence to Lipinski's Rule of 5, combined with its favorable binding affinity, positions it as a viable candidate for further investigation in drug discovery pipelines.

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