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Tetraprenyltoluquinone Inhibits the Tumor Marker Aldo-Keto Reductase: An in Silico Study

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***Abstract*—Cancer is one of the most common causes of death in the globe. The development of new cancer medicines and the identification of new therapeutic targets is still a pressing necessity. The protein AKR1B10 was discovered to be a valuable biomarker for the diagnosis and prognosis of some malignancies. Over expression of the AKR1B10 gene is found in lung cancer, oral squamous cell carcinoma, breast cancer, cholangiocarcinoma, pancreatic cancer and liver cancer. AKR1B10 is implicated in detoxification, retinoic acid metabolism, and lipid synthesis, among other pathological actions. AKR1B10 is known to be carcinogenic and can be utilized as a tumor marker, according to research. The tetraprenyltoluquinone compound is an isolate from the bark of kandis (*Garcinia cowa*, Roxb) which has been reported to have anticancer activity in vivo and in vitro and has the potential to be developed as an anticancer drug derived from natural ingredients. This study aims to determine the activity of the tetraprenyltoluquinone compound in silico with the target of the AKR1B10 protein. The method used is molecular docking using PLANTS (Protein Ligand ANT System) for protein visualization and preparation and Ligplus program for visualizing amino acids. The docking score results showed that the AKR1B10 protein interaction with the test ligand tetraprenyltoluquinone is lower than the native ligand, which means the binding energy of tetraprenyltoluquinone to the AKR1B10 (PDB ID: 1ZUA) protein was higher than the native ligand tolrestat. These results indicate that tetraprenyltoluquinone is a potential inhibitor of the AKR1B10 protein in the pathway of cancer.**

***Keywords*— AKR1B10; cancer; in silico; molecular docking; tetraprenyltoluquinone.**

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I. INTRODUCTION

One of the aldehyde-ketone reductase family members, aldo-keto reductase family 1 member B10 (AKR1B10), is an oncogene that leads to human cancers such as liver, lung, and breast cancer [1]–[3]. The main metabolic changes linked to cancer include aberrant glucose and amino acid absorption, as well as the preferred use of metabolic pathways for biomass and nicotinamide adenine dinucleotide phosphate synthesis (NADPH) [4]–[6]. For conjugation reactions to complete, human aldo-keto reductases (AKRs) catalyze the NADPH-dependent reduction of carbonyl groups to alcohols [7]–[9]. In recent years, scholars have been paying more and more attention to it.

The AKR1B10 gene is found on chromosome 7q33, and the AKR1B10 protein is 316 amino acids long [10], [11]. AKR1B10 is implicated in detoxification, retinoic acid metabolism, and lipid synthesis, among other pathological actions. AKR1B10 is known to be carcinogenic and can be utilized as a tumor marker, according to research. [12], [13].

Tetraprenyltoluquinone is a compound isolated from the hexane fraction of kandis acid bark (*Garcinia cowa*, Roxb) [14], which has been reported to have anticancer activity in vivo tested using mice. Administration of tetraprenyltoluquinone compound at a dose of 800 mg/Kg for 14 days in mice with H460 cells implanted subcutaneously in the right pelvis could slow tumor growth within five days of administration of the compound [15].

In vitro has also been tested against cell line H460 [16]. This compound exhibits anticancer activity by inducing cell cycle arrest. Cell cycle analysis showed that the compound caused arrest in the Go/G1 phase. In addition, this compound was selective against H460 lung cells, while MCF-7 breast cancer cells and DU-145 prostate cancer cells showed no activity [17]. This gives the possibility that this compound could be a potential new chemotherapy for cancer.

An in Silico approach with the molecular docking method is used by utilizing information from the target protein's structure and physicochemical properties to predict the protein's active site that can cause biological activity [18], [19]. This study aims to use the docking approach to

investigate the influence of tetraprenyltoluquinone on the activity of the AKR1B10 protein. This study can predict the effect of tetraprenyltoluquinone against cancer cells with specific and selective target proteins for cancer therapy which encourages the discovery and optimization of bioactive compounds in the cancer drug development process. The interaction of tetraprenyltoluquinone and the Native ligand (TOL) with the AKR1B10 protein was investigated in this study.

II. Material and Method

A. Materials

The Marvin Sketch 5.2.5.1 application was used to construct two-dimensional (2D) structures of the tetraprenyltoluquinone molecule. The hydrogen atom was used to turn the two-dimensional structures into three-dimensional ones, and then conformation optimization was performed. Figure 1 shows the structure of the target protein AKR1B10, which was retrieved from the Protein Data Bank (PDB) (http://www.rcsb.org, PDB ID: 1ZUA).

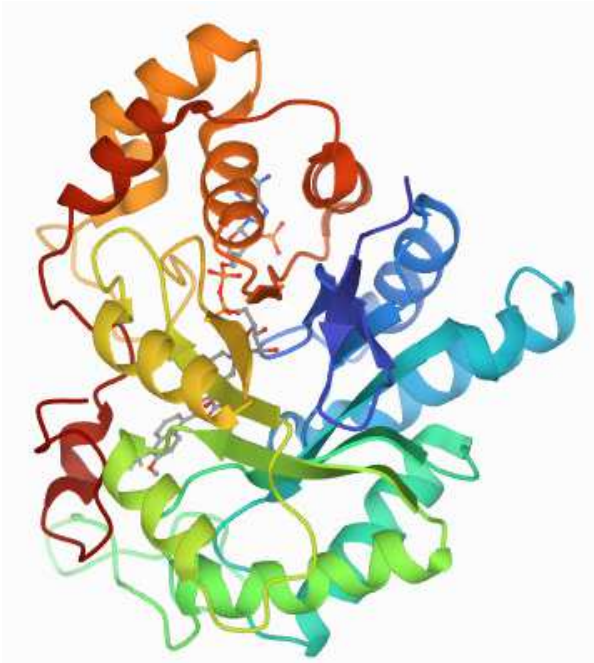


Fig. 1 The structure of tetraprenyltoluquinone's target protein and its native ligand

B. Tools

A set of computers with Windows 10 Pro, 64-bit operating system, Intel(R) Core (TM) i3-5005U CPU @ 2.00GHz 2.00 GHz, RAM 12 GB, and HDD 1 TB were employed for this study. Molecular docking software PLANTS (Protein-Ligand ANT-System), protein visualization and preparation software YASARA version 10.1.8 (C) 1993-2010 by Elmar Kreiger (licensed to Hari Purnomo Universitas Gadjah Mada), and the Ligplus tool for viewing amino acids.

C. Method

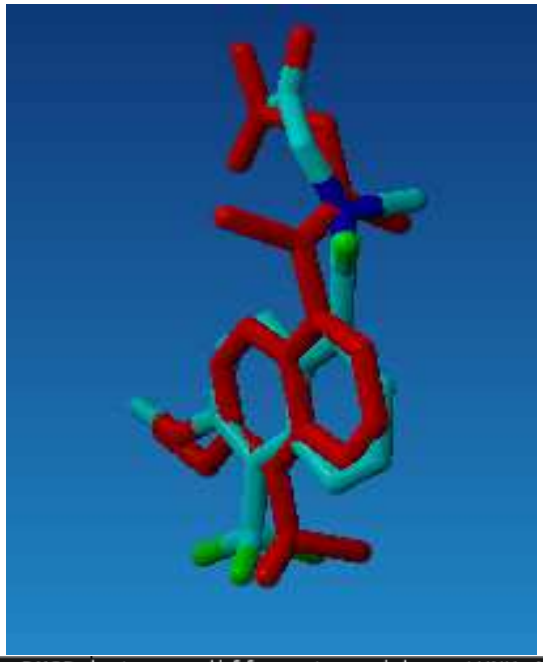
The active version of the protein that binds to the natural ligand was chosen. The YASARA program then deleted the native ligand to make a cavity. This area will be utilized to investigate how the ligand (tetraprenyltoluquinone) interacts with the target protein. Binding was validated using ligand copy and the computation of Root Mean Square Distances (RMSD) of native ligand. Using the PLANTS software, the target protein was separated from the native ligand. The protein is then coupled with its native ligand. The RMSD of a natural ligand with heavy atoms is investigated. The protocol has been received if the RMSD value is less than 2.0 angstroms, and the test chemical can be docked to the target protein. A protein with a different code was utilized if the RMSD value was greater than 2.0 angstroms. Tetraprenyltoluquinone molecule attached to the protein after the native ligand was removed with the PLANTS software. The analysis will reveal which compound has the lowest conformation and is most likely to bind to the target protein. To visualize amino acid residues that interact with the ligand, the Ligplus tool is utilized.

III. Result and Discussion

The crystal structure of human AKR1B10 in complex tolrestat was employed as a starting point. Although over 100 crystal structures of AKR1B10 and its inhibitor complexes have been identified, there is only one structure of AKR1B10 in complex with the strong AKR1B10 inhibitor tolrestat (PDB ID: 1ZUA). Two new structures of the AKR1B10 V301L mutant in association with the inhibitors fidalrestat and sorbinil (PDB ID: 4GAB and 4GA8, respectively) have been reported. These structures reveal almost similar inhibitor binding patterns to the corresponding AKR1B1-inhibitor complex structures [20].

An enzyme's ligand binding mechanisms or substrate coordination can be explored and predicted using molecular docking simulations. For simple to advanced molecular modelling applications, YASARA is a computer program with no specific system requirements [21], [22]. YASARA has all of the tools needed to examine macromolecular structures interactively. The ligand is commonly docked from a random place outside the enzyme, allowing both the ligand and the enzyme to be flexible. The ligand's binding mechanisms in the enzyme are investigated, and the binding energy is computed as a result [23].

The docking technique was confirmed by the RMSD score of the target protein PDB with its native ligand. The RMSD between the target protein PDB and the native ligand was less than 2 angstroms in this investigation (Fig 2 and Table I). Therefore, tetraprenyltoluquinone can be docked to the target protein. When performing protein-ligand docking simulations, its standard to start by seeing whether the computational method can replicate an experimental 3D structure for a complex including at least one ligand. If such a structure exists, we use it to see if a particular molecular docking strategy can predict the crystallographic position of the ligand in the protein structure, a process known as redocking. The root-mean-square deviation (RMSD) between the ligand's crystallographic position and the pose is the most commonly used criterion to assess redocking success (generated by the computer simulation). Compared to crystallographic structures, we predict the best docking simulations to have RMSD values of less than 2.0 A. [24].



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WARNING - Calculating RMSD between different residues 'UNK A 1' and 'TOL A 1320'
WARNING - Calculating RMSD between different residues 'UNK A 1' and 'TOL A 1320'
WARNING - Calculating RMSD between different residues 'UNK A 1' and 'TOL A 1320'
Molecule A and Molecule A have 1.2419 A RMSD
Molecule A and Molecule A have 0.0000 A RMSD
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Fig. 2 Target protein and native ligand RMSD score

TABLE I
RMSD SCORE

Protein	PDB ID	*Native* ligan	RMSD (Angstrom)
AKR1B10	1ZUA	TOL	1.2419

The docking score of interaction between tetraprenyltoluquinone compound and native ligand with protein AKR1B10 is presented in Table II. In Figure 3, the interaction of tetraprenyltoluquinone with the protein AKR1B10 is seen.

TABLE II
DOCKING SCORE COMPOUND WITH AKR1B10

Compound	Docking score (kcal/mol)
tetraprenyltoluquinone	-102.65
Native ligand (TOL)	-93.92

The interaction tetraprenyltoluquinone as a ligand with the target protein was visualized using the Ligplus program. According to the findings, the amino acids involved in the interaction of tetraprenyltoluquinone with target protein and TOL with target protein were shown to be equivalent (Table III.).

By measuring the immunohistochemistry levels of AKR1B10 in tissue, the protein AKR1B10 has the potential to be used as a prognostic biomarker for cancer [13]. It is now thought to be a valuable biomarker for cancer diagnosis and prognosis in some cases. Lung cancer (including smoking-related lung adenocarcinoma and lung squamous cell carcinoma), oral squamous cell carcinoma, breast cancer cholangiocarcinoma, pancreatic cancer and liver cancer have all been linked to overexpression of the AKR1B10 [20].

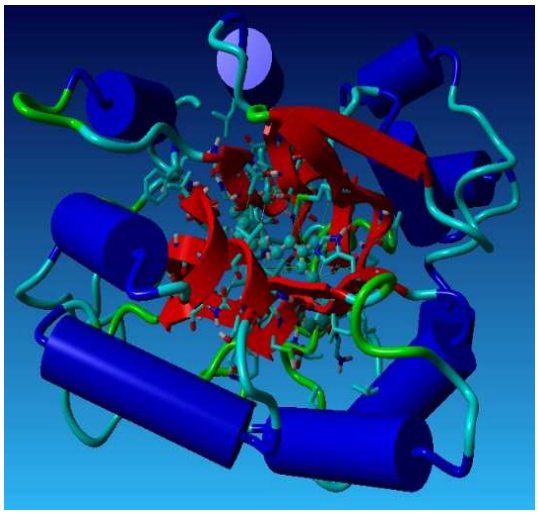
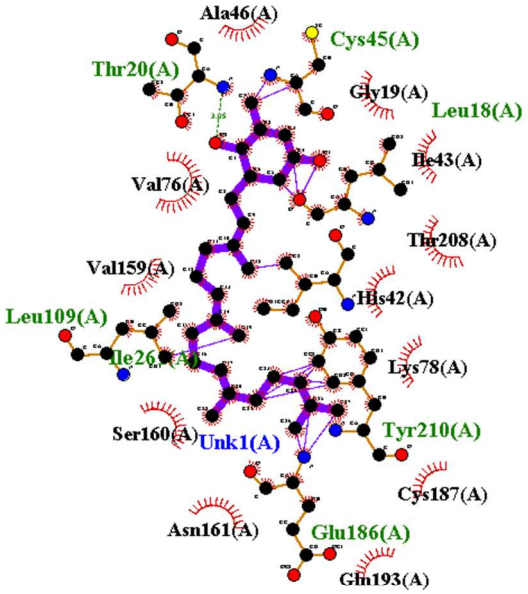
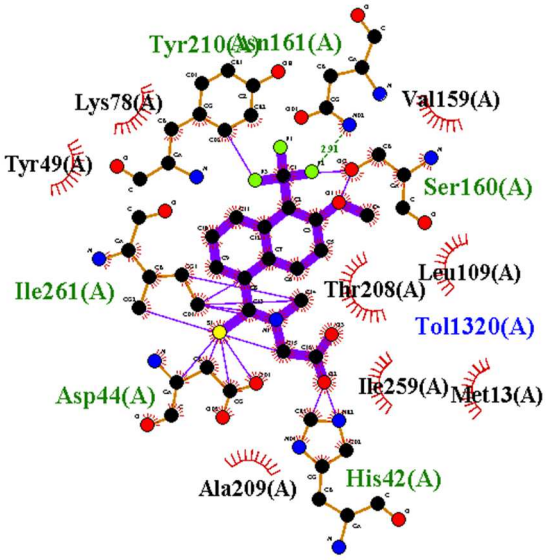


Fig. 3 The visualization of interaction between tetraprenyltoluquinone with protein AKR1B10.

The target protein used was AKR1B10 with PDB code 1ZUA which was studied in silico with molecular docking. Molecular docking studies are a technique that can be used for new drug discovery [23], [24]. To obtain the RMSD score of the PDB target protein with its native ligand, the docking approach must be confirmed by redocking. The RMSD score obtained in this investigation is less than 2 Angstroms, as indicated in Table I and Fig. 2. Because the validation requirements are met, the data indicate that the test compound's docking method can proceed [25].

TABLE III
INTERACTION OF LIGAND

Ligand	Interaction	Amino acid interaction/similar
tetraprenyltoluquinone		Ala46, Thr20, Val76, **Val159**, **Leu109**, **Ile261**, **Ser160**, **Asn161**, Glu186, Gln193, Cys187, **Tyr210**, **Lys78**, **His42**, **Thr208**, Ile43, Leu18, Gly19, Cys45
Native ligand		**Tyr210**, **Lys78**, Tyr49, **Ile261**, Asp44, Ala209, **His42**, Ile259, Met13, **Thr208**, **Leu109**, **Ser160**, **Val159**, **Asn161**

The goal of validation is to calibrate the docking method's accuracy. The native ligand location is compared to the protein that has been tested experimentally at the ligand binding site using RMSD during the validation procedure. Predictions with an RMSD score of 2 Angstrom are generally regarded as successful, but a value greater than 3 indicates a failed docking [26].

The value of the docking score for the interaction between tetraprenyltoluquinone and native ligand against the target protein is shown in Table II and the display of the interaction can be seen in Fig. 3. From the results obtained, it can be seen that the docking score of the interaction between the AKR1B10 protein and the test ligand tetraprenyltoluquinone is smaller than the native ligand. The native ligand used in this protein, tolrestat, was revealed as a potent inhibitor of AKR1B10 *n vitro* and *in vivo* [27], [28]. The Gibbs free energy (G), which represents the strength of the binding between the test ligand and the protein, is used to calculate the docking score. A low G value means the ligand and protein bindings are strong, and the conformation is stable. If G is higher, on the other hand, the connection is weak, and the conformation is unstable. Better ligand conformation results from stable ligand conformation [29], [30]

The interaction displays of tetraprenyltoluquinone and native ligands to the target protein can be seen in Table III using the Ligplus program. The results found that several amino acid equations are involved in the interaction between tetraprenyltoluquinone and protein and between native ligands and protein. A total of 9 same amino acid residues were involved in the interaction, namely Tyr210, Lys78, Ile261, His42, Thr208, Leu109, Ser160, Val159, Asn161.

This study shows that the tetraprenyltoluquinone compound binds to a stronger and more stable ligand than the AKR1B10 inhibitor. The inhibitor used has been experimentally proven to bind to the target protein. These

results indicate that tetraprenyltoluquinone compounds are predicted to be very potential as AKR1B10 inhibitors.

IV. Conclusion

The docking score of the AKR1B10 protein interaction with the test ligand tetraprenyltoluquinone is lower than the native ligand which is a AKR1B10 inhibitor which indicates that the tetraprenyltoluquinone compound is more stable and has strong potential as an inhibitor of the AKR1B10 protein in the cancer.

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