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Gedunin Modification for Anticancer Properties Improvement against the Human NAD^+ Kinase

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Abstract: Background: Cancer, also called malignancy, is an abnormal growth of cells. There are more than 100 types of cancer, including breast cancer, skin cancer, lung cancer, colon cancer, prostate cancer, and lymphoma. Symptoms vary depending on the type. Cancer treatment may include chemotherapy, radiation, and/or surgery. NAD^+ kinase (NADK) catalyzes the phosphorylation of nicotinamide adenine dinucleotide (NAD^+) to nicotinamide adenine dinucleotide phosphate ($NADP^+$) using ATP as the phosphate donor. $NADP^+$ is then reduced to NADPH by dehydrogenases, in particular glucose-6-phosphate dehydrogenase and the malic enzymes. NADPH functions as an important cofactor in a variety of metabolic and biosynthetic pathways. The demand for NADPH is particularly high in proliferating cancer cells, where it acts as a cofactor for the synthesis of nucleotides, proteins, and fatty acids.

Materials and Methods: The Modification made to gedunin was done by substituting a CH_3 group from the compound with NH_2 using the marvin sketch software. The designed structural modifications were saved as mrv files which were then converted into SMILES strings for docking using the OpenBabel software. Molecular docking to predict the binding energy of gedunin and the modified analogue carried out using the AutoDock Vina software while pharmacokinetics parameters for each compound was predicted using the SwissADME server.

Result: Following the modifications effected on gedunin, a positive result was observed as regarding the binding energies of the compounds. The NH_2 analogue of gedunin exhibited a higher binding energy for the inhibition of the human NAD^+ kinase enzyme. Both compounds are predicted safe for administration as they both cannot permeate the blood brain barrier.

Conclusion: Results from the molecular docking study showed that the NH_2 analogue of gedunin might be a better anticancer agent. Laboratory synthesis and preclinical studies on the compound is therefore recommended.

Keywords: Cancer; Gedunin; NAD^+ kinase; Phosphorylation; Modification.

Introduction

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body [1]. These contrast with benign tumors, which do not spread to other parts of the body [2]. Possible signs and symptoms include a lump, abnormal bleeding, prolonged cough, unexplained weight loss and a change in bowel movements.[1] While these symptoms may indicate cancer, they may have other causes [3]. Over 100 types of cancers affect humans [2].

When cancer begins, it produces no symptoms. Signs and symptoms appear as the mass grows or ulcerates. The findings that result depend on the cancer's type and location. Few symptoms are specific. Many frequently occur in individuals who have other conditions. Cancer is a "great imitator". Thus, it is common for people diagnosed with cancer to have been treated for other diseases, which were hypothesized to be causing their symptoms [4].

NAD^+ kinase is an enzyme that converts nicotinamide adenine dinucleotide (NAD^+) into $NADP^+$ through phosphorylating the NAD^+ coenzyme [5]. $NADP^+$ is an essential coenzyme that is reduced to NADPH primarily by the pentose phosphate pathway to provide reducing power in biosynthetic processes such as fatty acid biosynthesis and nucleotide synthesis [6]. The structure of the NADK from the archaean *Archaeoglobus fulgidus* has been determined [7]. In humans, the genes *NADK* and *MNADK* encode NAD^+ kinases localized in cytosol and mitochondria, respectively [8, 9]. Similarly, yeast have both cytosolic and mitochondrial isoforms, and the yeast mitochondrial isoform accepts both NAD^+ and NADH as substrates for phosphorylation [10].

Highly proliferating cancer cells require sufficient amounts of NADH and NADPH to act as reducing agents for reductive synthesis of nucleic acids, protein, and lipid biosynthesis [11]. A lack of these precursors

can lead to a halt in cell growth and eventual cell death. NADPH is also key to the maintenance of a healthy redox status in cells, as it is involved in neutralizing the reactive oxygen species (ROS) associated with rapid growth [12]. To achieve the metabolic and ROS-mediating requirements associated with rapid proliferation, cancer cells can alter the expression and regulation of metabolic genes. Indeed, several of these genes are under the regulation of oncogenes and tumor suppressor proteins [13]. For example, the tumor suppressor p53 exerts control of NADPH levels by inhibiting glucose-6-phosphate dehydrogenase (G6PD) activity and by repressing activity of the malate dehydrogenase enzymes M1 and M2, both of which contribute to the cellular NADPH pool [14]. When p53 is mutated or rendered nonfunctional through interaction with inhibitory proteins, this control is lost; as a result, cancer cells generate more NADPH for protection from ROS and macromolecular synthesis to allow rapid proliferation. Another example is the M2 splice variant of pyruvate kinase (PKM2), which is subjected to complex regulation by both oncogenes and tumor suppressors; overexpression of PKM2 leads to the increased production of NADPH by diverting glucose metabolism into the pentose phosphate pathway [15].

The aim of this study is to improve on the potency of gedunin against the human NAD^+ kinase through molecular docking procedures and the singular substitution of the CH_3 attachment of gedunin with NH_2.

Materials and Methods

Protein preparation

The crystal structure of the human NAD^+ kinase was obtained from the Protein Data Bank, PDB 3PFN (Figure 1). The protein structure was subjected to a refinement protocol using the Pymol viewer [16].

Designing of the Gedunin structural analogue

The 2D structure of gedunin (Figure 2) was drawn with the aid of the Marvin Sketch software [17]. The structural analogue of gedunin (Figure 3) was developed with a structural modification and a different substituent [18]. The CH_3 substituent of gedunin was replaced with an NH_2 group. The structure was built with the Marvin Sketch software and minimized using the Chimera software [19, 20].

Molecular docking

Molecular docking (Figure 4 and 5) was performed using the AutoDock Vina Software [21]. Physicochemical, lipophilicity, solubility, pharmacokinetics and Lipinski druglikeness of gedunin and its NH_2 analogue was determined using the SwissADME Server [22].

Physiochemical Characteristics

The Physiochemical characteristics of the human NAD^+ kinase was predicted using the ExPASy ProtParam server [23].

Result and Discussion

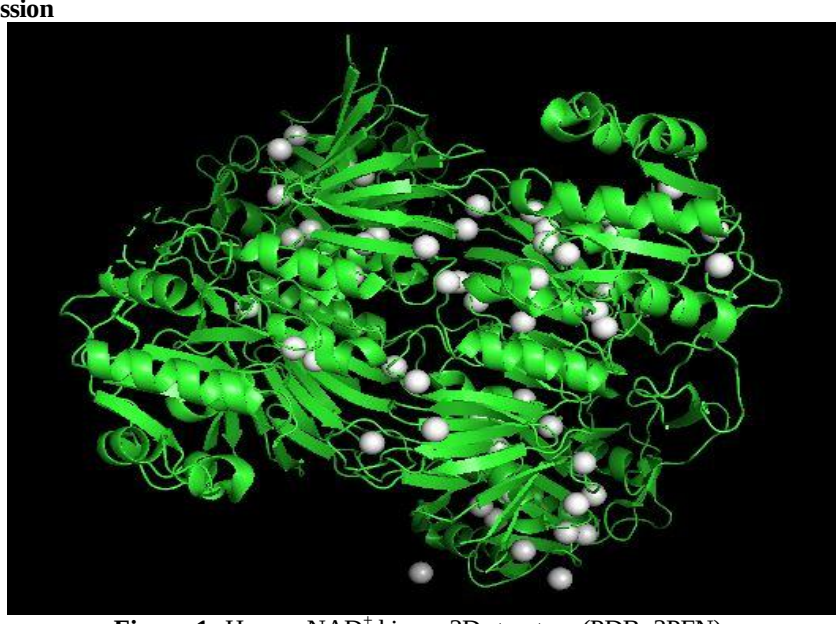

Figure 1: Human NAD^+ kinase 3D structure (PDB: 3PFN)

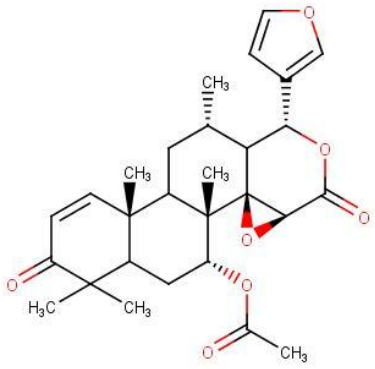

Figure 2: Gedunin 2D Structure

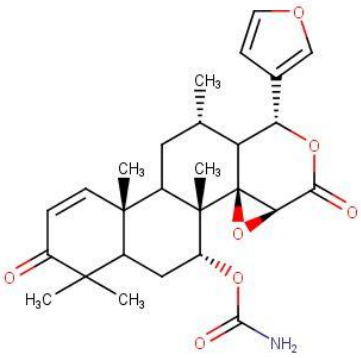

Figure 3: NH_2 Analogue

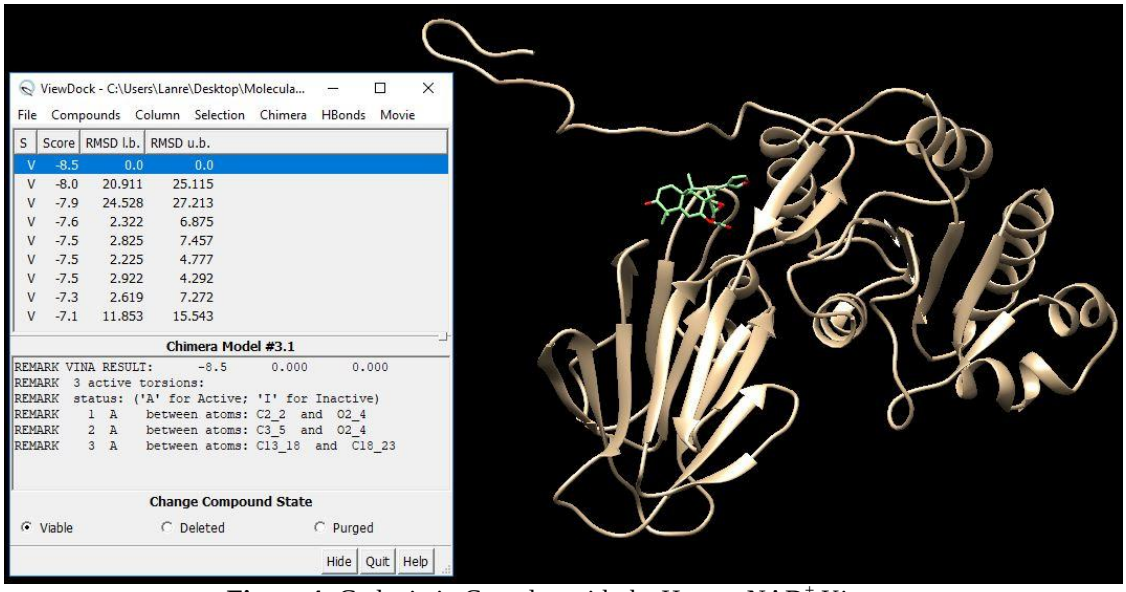

Figure 4: Gedunin in Complex with the Human NAD^+ Kinase

Figure 5: NH_2 Analogue of Gedunin in Complex with the Human NAD^+ Kinase

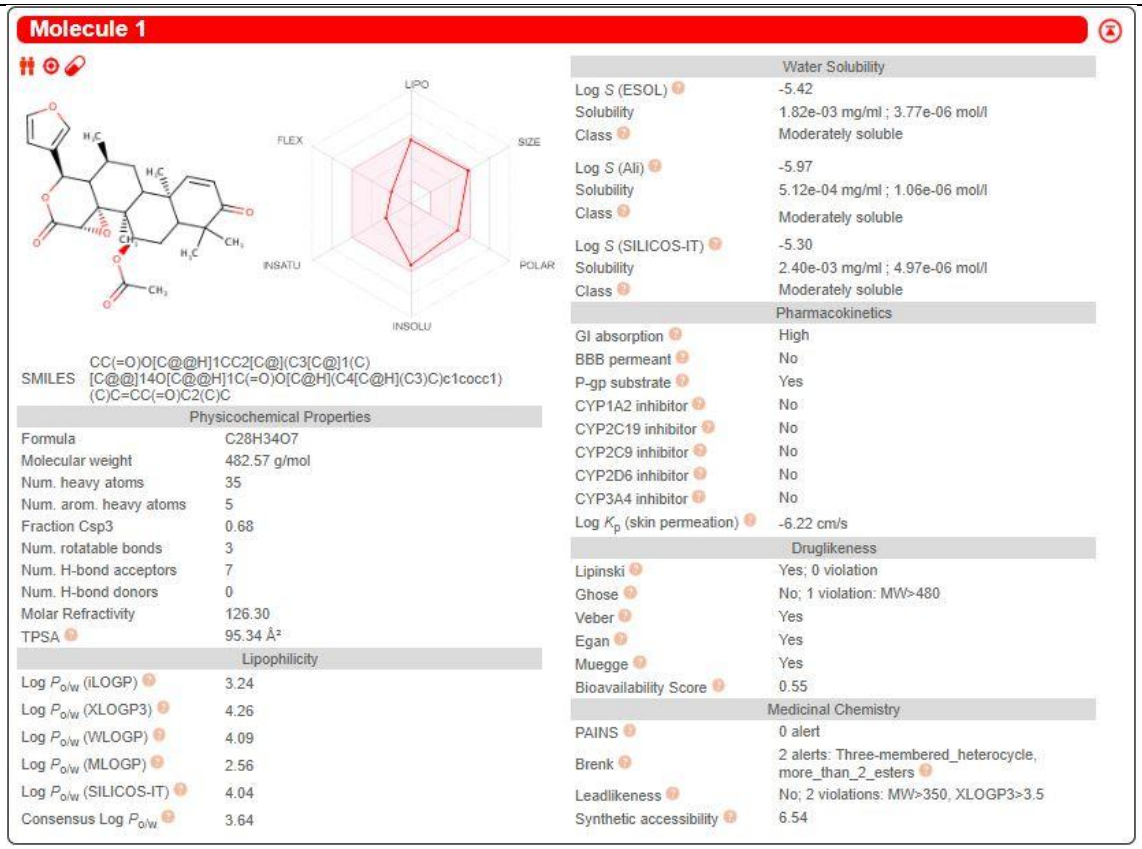

Figure 6: Druglikeness Prediction of Gedunin

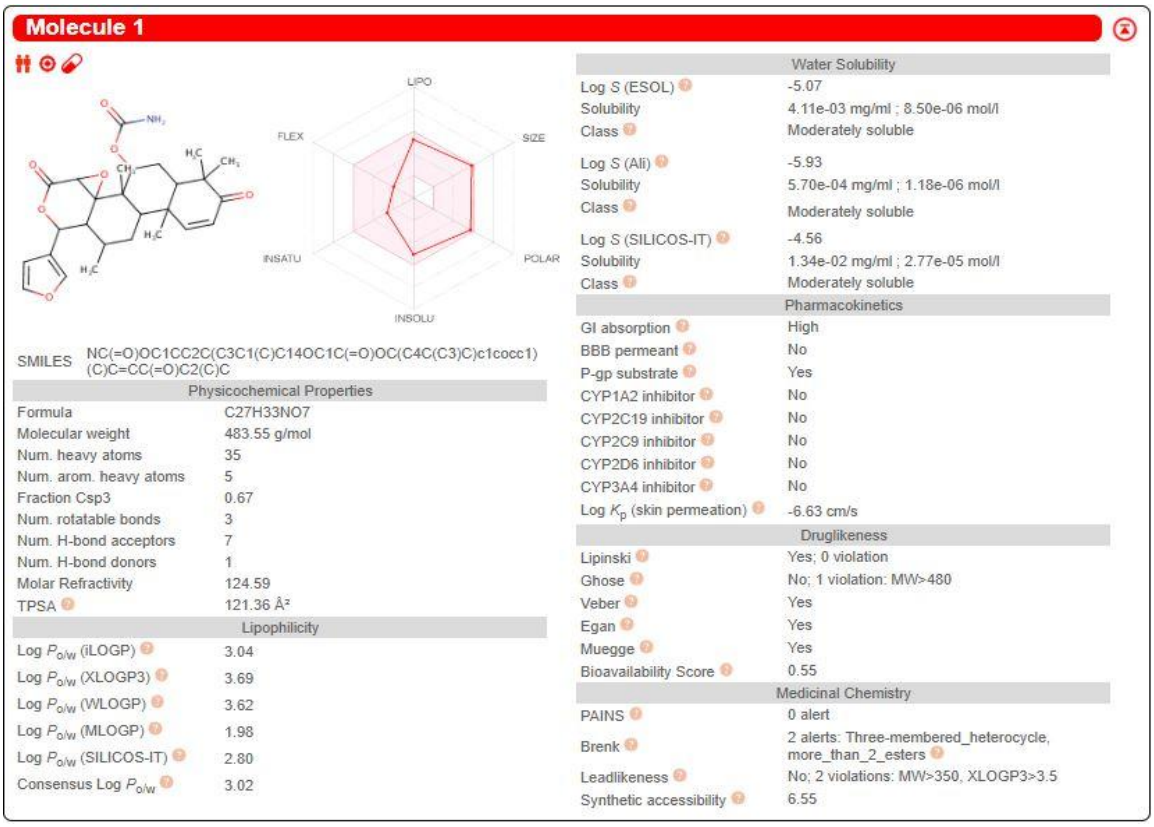

Figure 7: Druglikeness Prediction of the Gedunin NH_2 Analogue

The theoretical *pI* of the human NAD^+ kinase predicted through the biochemical characterization analysis has predicted the protein to be slightly acidic with a value of 6.70 [24]. The hydrophobicity scale produced values that define relative hydrophobicity of amino acid residues. The more positive the value, the more hydrophobic the amino acids located in that region of the protein [25]. The GRAVY calculator used in predicting the hydrophobicity assigned to the protein a value of -0.025. This result implies that the human NAD^+ kinase exhibit a hydrophobic character.

The instability index is a pointer to the stability of a protein in a test tube. A protein whose instability index is greater than 40 is predicted as unstable and a value below 40 predicts the protein may be stable [25]. The human NAD^+ kinase is therefore an unstable protein with an instability index of 45.61. This makes it an ideal therapeutic target for drug-like compounds

Lipinski's rule of five also known as the Pfizer's rule of five or simply the rule of five (RO5) is a rule of thumb to evaluate druglikeness or determine if a chemical compound with a certain pharmacological or biological activity has chemical properties and physical properties that would make it a likely orally active drug in humans [19 26]. Lipinski's rule states that, in general, an orally active drug has no more than one violation of the following criteria: No more than 5 hydrogen bond donors (the total number of nitrogen–hydrogen and oxygen–hydrogen bonds), no more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms), a molecular mass less than 500 daltons, an octanol-water partition coefficient log *P* not greater than 5 [27]. The NH_2 analogue of gedunin can as such be regarded as druglike because it has violated none of the lipinski's rule.

The polar surface area (PSA) or topological polar surface area (TPSA) of a molecule is defined as the surface sum over all polar atoms, primarily oxygen and nitrogen, also including their attached hydrogen atoms. Molecules with a polar surface area of greater than 140 angstroms squared tend to be poor at permeating cell membranes [28]. For molecules to penetrate the blood–brain barrier (and thus act on receptors in the central nervous system), a PSA less than 90 angstroms squared is usually needed [29]. The TPSA value of the NH_2 analogue of gedunin is 121.36 angstroms (Fig 7). This means that the compound lacks the blood brain barrier permeation ability hence safe for oral administration

The P-glycoprotein is involved in limiting the harmful exposure of toxins, drugs, and xenobiotics to the body by extruding them out of cells. It is increasingly recognized to play an important modulating role in the pharmacokinetic properties of many clinically important therapeutic agents and because of its importance in pharmacokinetics, its screening has to be incorporated into the drug discovery process [29]. The presence of the P-glycoprotein is the reason for the multidrug resistance attribute exhibited by cancer cells and the pharmacokinetics result on the NH_2 analogue of gedunin showed that it is a P-glycoprotein substrate.

A method to estimate ease of synthesis (synthetic accessibility) of drug-like molecules is needed in many areas of the drug discovery process. The assessment of synthetic accessibility (SA) of a lead candidate is a task which plays a role in lead discovery regardless of the method the lead candidate has been identified with. After normalization, the SA Score ranges from 1 (very easy) to 10 (very difficult) [30]. The synthesis of the NH_2 analogue of gedunin has a 6.55 synthetic accessibility value which makes the compound slightly difficult to synthesize.

The increased application of molecular docking methods in the pharmaceutical industry and academia is a direct result of increase in computer speed, and the reliability of simulation theories and docking software [31]. The NH_2 substituted analogue of gedunin bound tighter to the human NAD^+ kinase than gedunin with a binding score of -8.8 and -8.5Kcal/mol respectively.

Conclusion

Results from this study suggested that the modification of natural compounds might positively affect their efficacy due to observations from the molecular docking results. Modifying natural compounds has also been suggested to improve the safety of drugs for oral administration as observed from the in silico pharmacokinetics study. The binding affinity of the NH_2 analogue of gedunin to the human NAD^+ kinase suggested that it might be a better anticancer agent than gedunin which is occurring in its natural form. It is therefore suggested that the laboratory synthesis and preclinical studies on this compound be carried out to confirm its activity as a better anticancer agent. We also recommend further modifications on geunin for it t become a non substrate to the P-glycoprotein as this will increase its anticancer activity.

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