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Research Article

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Molecular Docking and Pharmacokinetic Study of Gedunin against the NAD^+ Kinase of *Mycobacterium tuberculosis*

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Abstract *Background:* Tuberculosis (TB) is a potentially serious infectious disease that mainly affects the lungs. The bacteria that cause tuberculosis are spread from one person to another through tiny droplets released into the air via coughs and sneezes. Once rare in developed countries, tuberculosis infections began increasing in 1985, partly because of the emergence of HIV, the virus that causes AIDS. HIV weakens a person's immune system so it can't fight the TB germs. In the United States, because of stronger control programs, tuberculosis began to decrease again in 1993, but remains a concern. Many strains of tuberculosis resist the drugs most used to treat the disease. People with active tuberculosis must take several types of medications for many months to eradicate the infection and prevent development of antibiotic resistance.

Materials and Methods: The chemical structure and formular of gedunin was obtained from the PubMed database. This structure was redrawn using the ChemAxon software and converted into isomeric SMILES for the running of pharmacokinetic parameters using the SwissADME software. Molecular docking study was carried out using the Autodock Vina software while the 3D structure of *Mycobacterium tuberculosis* NAD^+ kinase was downloaded from the Protein Data Bank.

Results: The molecular docking of gedunin against the *Mycobacterium tuberculosis* NAD^+ kinase showed a high binding score of -8.4Kcal/mol while parameters obtained from the in silico pharmacokinetics studies showed that gedunin violated none of the lipinski's rule.

Discussion: Results from the molecular docking study showed that gedunin might be a potent antitubacular agent owing to the high binding energy observed against the *Mycobacterium tuberculosis* NAD^+ kinase. Gedinin has also been shown to be safe for oral administration based on results obtained from pharmacokinetic studies.

Keyword: Tuberculosis; Gedunin; Pharmacokinetics; NAD^+ kinase; Bacteria

Introduction

Tuberculosis (TB) is an infectious disease usually caused by the bacterium *Mycobacterium tuberculosis* (MTB) [1]. Tuberculosis generally affects the lungs, but can also affect other parts of the body. There are two types of TB conditions: latent TB infection and TB disease. In most people who breathe in TB bacteria and become infected, the body is able to fight the bacteria to stop them from growing. People with latent TB infection do not feel sick, do not have any symptoms, and cannot spread TB bacteria to others. If TB bacteria becomes active in the body and multiply, the person will go from having latent TB infection to being sick with TB disease. For this reason, people

with latent TB infection are often prescribed treatment to prevent them from developing TB disease.People with TB disease usually have symptoms and may spread TB bacteria to others [2].

Most infections do not have symptoms, in which case it is known as latent tuberculosis [2]. About 10% of latent infections progress to active disease which, if left untreated, kills about half of those infected [3]. The classic symptoms of active TB are a chronic cough with blood-containing sputum, fever, night sweats, and weight loss. It was historically called "consumption" due to the weight loss [4]. Infection of other organs can cause a wide range of symptoms [5]. Tuberculosis is spread through the air when people who have active TB in their lungs cough, spit, speak, or sneeze [6]. People with latent TB do not spread the disease.[1] Active infection occurs more often in people with HIV/AIDS and in those who smoke. Diagnosis of active TB is based on chest X-rays, as well as microscopic examination and culture of body fluids. Diagnosis of latent TB relies on the tuberculin skin test (TST) or blood tests [7].

Mycobacterium tuberculosis is a species of pathogenic bacteria in the family Mycobacteriaceae and the causative agent of tuberculosis [8]. First discovered in 1882 by Robert Koch, *M. tuberculosis* has an unusual, waxy coating on its cell surface primarily due to the presence of mycolic acid [9]. This coating makes the cells impervious to Gram staining, and as a result, *M. tuberculosis* can appear either Gram-negative or Gram-positive [10]. Acid-fast stains such as Ziehl-Neelsen, or fluorescent stains such as auramine are used instead to identify *M. tuberculosis* with a microscope. The physiology of *M. tuberculosis* is highly aerobic and requires high levels of oxygen. Primarily a pathogen of the mammalian respiratory system, it infects the lungs. The most frequently used diagnostic methods for tuberculosis are the tuberculin skin test, acid-fast stain, culture, and polymerase chain reaction [9]. *M. tuberculosis* is part of a complex that has at least 9 : *M. tuberculosis sensu stricto*, *M. africanum*, *M. canetti*, *M. bovis*, *M. caprae*, *M. microti*, *M. pinnipedii*, *M. mungi*, and *M. orygis* [11]. It requires oxygen to grow, does not produce spores, and is nonmotile [12, 13]. *M. tuberculosis* divides every 15–20 hours. This is extremely slow compared with other bacteria, which tend to have division times measured in minutes (*Escherichia coli* can divide roughly every 20 minutes). It is a small bacillus that can withstand weak disinfectants and can survive in a dry state for weeks. Its unusual cell wall is rich in lipids such as mycolic acid and is likely responsible for its resistance to desiccation and is a key virulence factor [14].

The plant product or natural product (gedunin) show an important role in diseases prevention and treatment through the enhancement of antioxidant activity, inhibition of bacterial growth, and modulation of genetic pathways [15]. The therapeutics role of number of plants in diseases management is still being enthusiastically researched due to their less side effect and affordable properties. It has been accepted that drugs based on allopathy are expensive and also exhibit some toxic effect on normal tissues and on various biological activities. It is a largely accepted fact that numerous pharmacologically active drugs are derived from natural resources including medicinal plants [16]. *Azadirachta indica* has complex of various constituents including nimbin, nimbidin, nimbolide, and limonoids and such types of ingredients play vital roles in diseases management through modulation of various genetic pathways and other activities. Numerous biological and pharmacological activities have been reported including antibacterial [17], antifungal [18], and anti-inflammatory. Earlier investigators have confirmed their role as anti-inflammatory, antiarthritic, antipyretic, hypoglycemic, antigastric ulcer, antifungal, antibacterial, and antitumour activities [19–22].

The aim of this research is to confirm the antibactarial activity of gedunin by targeting the *Mycobacterium tuberculosis* NAD^+ kinase using the molecular docking method.

Materials and Methods

Protein 3D Structure

The crystallized 3D structure of *Mycobacterium tuberculosis* NAD^+ kinase was downloaded from the Protein Data Bank repository with a PDB code of 1UOT. The Protein Data Bank (PDB) is a database for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids. The data, typically obtained by X-ray crystallography, NMR spectroscopy, or, increasingly, cryo-electron microscopy, and submitted by biologists and biochemists from around the world [23].

Gedunin 2D Structure Design

The 2D structure of gedunin was viewed from the PubMed database which is a database of chemical molecules and their activities against biological assays [24] and designed using the ChemAxon software [25].

Physiochemical Characteristics

The physiochemical characteristics of the *Mycobacterium tuberculosis* NAD^+ kinase was computed using the ExPASy ProtParam online server which computes various parameters such the molecular weight, amino acid composition, extinction coefficient, estimated half-life, theoretical pI, grand average of hydropathicity (GRAVY), aliphatic index and instability index [26].

File Conversion

The mrv file download of the MarvinSketch designed gedunin was converted into SMILES strings using the OpenBabel Graphics User Interface which is a chemical toolbox designed to speak the many languages of chemical data [27].

Secondary Structure Prediction

The *Mycobacterium tuberculosis* NAD^+ kinase secondary structure was predicted using the CFSSP online server. CFSSP (Chou & Fasman Secondary Structure Prediction Server) is an online protein secondary structure prediction server which predicts regions of secondary structure from the protein sequence such as alpha helix, beta sheet, and turns from the amino acid sequence [28].

Druglikeness Prediction

The druglikeness attributes of gedunin was predicted using the SwissADME server which is web tool that gives access to a pool of fast yet robust predictive models for physicochemical properties, pharmacokinetics, drug-likeness and medicinal chemistry friendliness, among which in-house proficient methods such as the BOILED-Egg, iLOGP and Bioavailability Radar [29].

Molecular Docking

This process was used to predict the binding energy between the *Mycobacterium tuberculosis* NAD^+ kinase and gedunin. Running of the docking process was done using the AutoDock vina software [30].

Results and Discussion

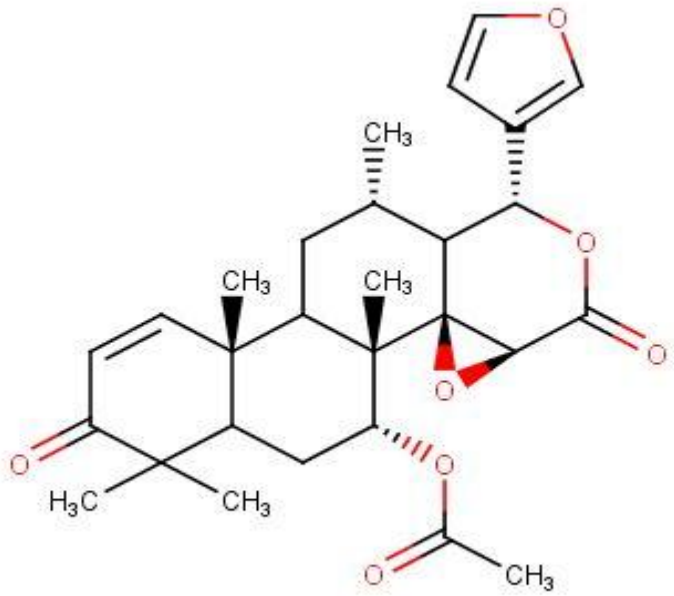


Figure 1: Gedunin 2D Structure

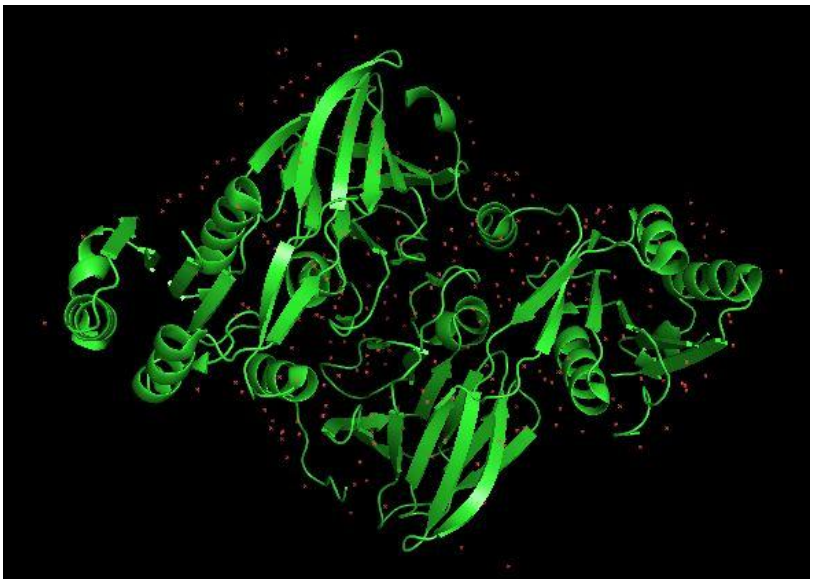
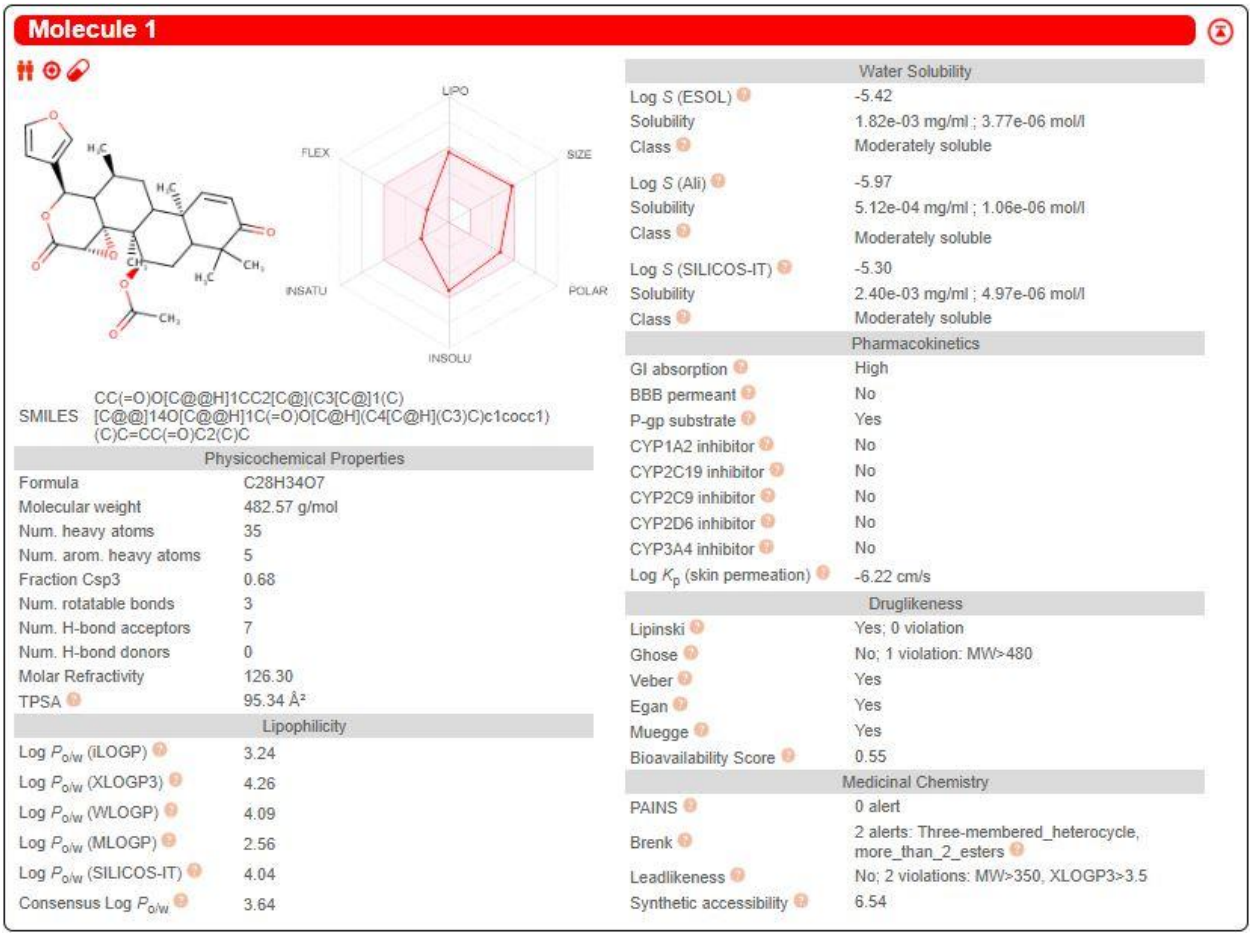


Figure 2: Mycobacterium tuberculosis NAD$^+$ kinase 3D Structure

Secondary Structure:

```
                  -           -           -           -           -           -
Query   1   MTAHRSVLLVVHTGRDEATETARRVEKVLGDNKIALRVLSAEAVDRGSLHLAPDDFRAHGVEIEVVDADQ  70
Helix   1       HHHHHHH      HHHHHHHHHHHHHHHHHHHHHHHHH      HHHHHHHHHHHHHHHHHHHHH  70
Sheet   1       EEEEEEEE              EEEE     EEEEE                EEEEEEEEEE       70
Turns   1                TT                TT          TT          T                 70
Struc   1   CCCCEEEEEEEECCTTHHHHHHHHHHEEHHTHHHEEEEEHHHHHHCTTCHHHHHTHHHHHEHHEHEHHHHH  70

                  -           -           -           -           -           -
Query  71   HAADGCELVLVLGGDGTFLRAAELARNASIPVLGVNLGRIGFLAEAEAEAIDAVLEHVVAQDYRVEDRLT 140
Helix  71   HHHHHHHHHH       HHHHHHHHHHHHHHHHHH    HHHHHHHHHHHHHHHHHHHHH      HHHH 140
Sheet  71         EEEEEE     EEE             EEEEEEEEEEEEEE          EEEEEEEEEEEEEEEEEEEE 140
Turns  71                TTT          T                                   T     T      140
Struc  71   HHHHHEHEEEECCTTTEHHHHHHHHTHHHEEEEEEEEEEEEEHHHHHHHHHEHHHHECEHHTEEEEDHEEE 140

                  -           -           -           -           -           -
Query 141   LDVVVRQGGRIVNRGHALNEVSLEKGPRLGVLGVVVEIDGRPVSAFGCDGVLVSTPTGSTAYAFSAGGPV 210
Helix 141   HHHHHH       HHHHHHHHHH       HHHHHHHHH                  HH          H 210
Sheet 141   EEEEEEEEEEEEEEEEEEEE          EEEEEEEEE        EEEEEEEEEEEEEEEEEEEE    E 210
Turns 141      T T      T          T T          TT                              T     210
Struc 141   EEEEEETEEEEEETHEHHHHHHHTCTCEEEEEEEHHHCTTCEEEEEEEEEEEEEEEEEEHEECCCCTCE 210

                  -           -           -           -           -           -
Query 211   LWPDLEAILVVPNNAHALFGRPIVTSPEATIAIEIEADGHDALVFCDGRREHLIPAGSRLEVTRCVTSVK 280
Helix 211   HHHHHHHHHH     HH          HHHHHHHHHHHHHHHHHHHHH     HH          HHHHHHHHH 280
Sheet 211   EEEEEEEEEE        EEEEEEE      EEEEE          EEE      EE        EEEEEEEEEE 280
Turns 211                T          T          T                 T T                 280
Struc 211   HHHHHHEEEEECTCHHCEEEEEEEHHHHHEHHHHHHHHHHHEEHHCTCTHEECCCCTCEEEEEEEEHH 280

                  -           -           -           -           -           -
Query 281   WARLDSAPFTDRLVRKFRLPVTGWRGKTPDIDCHAINSEQENCEHTAHRSVLLVVHTGRDEATETARRVE 350
Helix 281   HHHH          HHHHHHHH            HHHHHHHHHHHHHHHHHHHHHHHH          HHHHHHHHH 350
Sheet 281   E        EEEEEEEEEEEEEEE     EEEEEEEE EEEEEEEEE   EEEEEEEE          EE 350
Turns 281              T                T   T          T   T                TT          350
Struc 281   HHHHCCCCEEEEEEEHHEEEEEEEECCTCETEEHEEEHHHHHHHHHHHHHEEEEEEEECCTTHHHHHHHHH 350

Query 351   KVLGDNKIALRVLSAEAVDRGSLHLAPDDHRAHGVEIEVVDADQHAADGCELVLVLGGDGTFLRAAELAR 420
Helix 351   HHHHHHHHHHHHHHHHH      HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH      HHHHHHHHH 420
Sheet 351   EE       EEEEE                EEEEEEEEEE              EEEEEE    EEE       420
Turns 351      TT                TT          T                             TTT        T 420
Struc 351   EEHHTHHHEEEEEHHHHHCTTCHHHHHTHHHHHEHHEHEHHHHHHHHHHEHEEEECCTTTEHHHHHHHHT 420

                  -           -           -           -           -           -
Query 421   NASIPVLGVHLGRIGFLAEAEAEAIDAVLEHVVAQDYRVEDRLTLDVVVRQGGRIVNRGHALNEVSLEKG 490
Helix 421   HHHHHHHHHH    HHHHHHHHHHHHHHHHHHHHH      HHHHHHHHH      HHHHHHHHHHHH    490
Sheet 421      EEEEEEEEEEEEE            EEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE      490
Turns 421                                T          T          T T     T          T     490
Struc 421   HHHEEEEEEEEEEEHHHHHHHHHHEHHHHEEEHHTEEEEEHEEEEEEEEETEEEEEETHEHHHHHHHHTC 490

                  -           -           -           -           -           -
Query 491   PRLGVLGVVVEIDGRPVSAFGCDGVLVSTPTGSTAYAFSAGGPVLWPDLEAILVVPNNAHALFGRPIVTS 560
Helix 491     HHHHHHHHHH                                HH          HHHHHHHHHH     HH          HH 560
Sheet 491     EEEEEEEEE       EEEEEEEEEEEEEEEEEEEEE       EEEEEEEEEEEE       EEEEEEE    560
Turns 491 T                TT                          T                T          T     560
Struc 491   TCEEEEEEEHHHCTTCEEEEEEEEEEEEEEEEEEEHEECCCCTCEHHHHHHEEEEECTCHHCEEEEEEEHH 560

                  -           -           -           -           -           -
Query 561   PEATIAIEIEADGHDALVFCDGRREHLIPAGSRLEVTRCVTSVKLWARLDSAPFTDRLVRKFRLPVTGWRG 630
Helix 561   HHHHHHHHHHHHHHHHHHH     HH          HHHHHHHHHHHH          HHHHHHH      630
Sheet 561     EEEEE          EEE        EE       EEEEEEEEEEEE          EEEEEEEEEEEEEEE    630
Turns 561 T                      T T          T                T                     630
Struc 561   HHHEHHHHHHHHHHHHEEEEHCTCTHEECCCCTCEEEEEEEEEEHHHHHHCCCCEEEEEEHHEEEEEEECCC 630


Query 631 K 631
Helix 631   631
Sheet 631   631
Turns 631 T 631
Struc 631 T 631

Total Residues: H: 420   E: 370   T: 61
        Percent: H: 66.6  E: 58.6  T: 9.7
```

Figure 3: Mycobacterium tuberculosis NAD$^+$ kinase Secondary Structure



Molecule 1

SMILES: CC(=O)O[C@@H]1CC2[C@](C3[C@]1(C)[C@@]14O[C@@H]1C(=O)O[C@H](C4[C@H](C3)C)c1cocc1)(C)C=CC(=O)C2(C)C

Physicochemical Properties	
Formula	C28H34O7
Molecular weight	482.57 g/mol
Num. heavy atoms	35
Num. arom. heavy atoms	5
Fraction Csp3	0.68
Num. rotatable bonds	3
Num. H-bond acceptors	7
Num. H-bond donors	0
Molar Refractivity	126.30
TPSA	95.34 Å²

Lipophilicity	
Log $P_{o/w}$ (iLOGP)	3.24
Log $P_{o/w}$ (XLOGP3)	4.26
Log $P_{o/w}$ (WLOGP)	4.09
Log $P_{o/w}$ (MLOGP)	2.56
Log $P_{o/w}$ (SILICOS-IT)	4.04
Consensus Log $P_{o/w}$	3.64

Water Solubility	
Log *S* (ESOL)	-5.42
Solubility	1.82e-03 mg/ml ; 3.77e-06 mol/l
Class	Moderately soluble
Log *S* (Ali)	-5.97
Solubility	5.12e-04 mg/ml ; 1.06e-06 mol/l
Class	Moderately soluble
Log *S* (SILICOS-IT)	-5.30
Solubility	2.40e-03 mg/ml ; 4.97e-06 mol/l
Class	Moderately soluble

Pharmacokinetics	
GI absorption	High
BBB permeant	No
P-gp substrate	Yes
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-6.22 cm/s

Druglikeness	
Lipinski	Yes; 0 violation
Ghose	No; 1 violation: MW>480
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability Score	0.55

Medicinal Chemistry	
PAINS	0 alert
Brenk	2 alerts: Three-membered_heterocycle, more_than_2_esters
Leadlikeness	No; 2 violations: MW>350, XLOGP3>3.5
Synthetic accessibility	6.54

Figure 4: Druglikeness Prediction of Gedunin

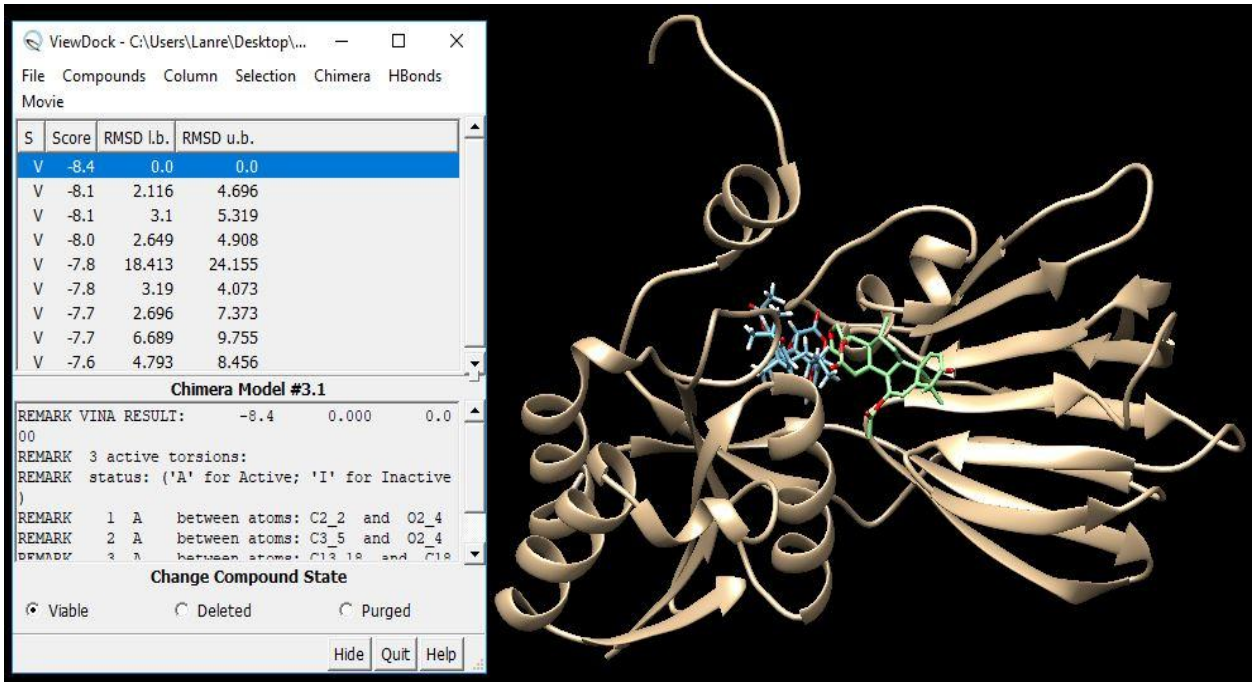


Figure 5: Gedunin in Complex with the Mycobacterium tuberculosis NAD^+ kinase

Mycobacterium tuberculosis NAD^+ kinase contains 635 amino acid residues. The docking pose of gedunin showed that it bound tightly to the active site of *Mycobacterium tuberculosis* NAD^+ kinase, as is evident from the superposition of gedunin in Figures 5. The interaction between gedunin and *Mycobacterium tuberculosis* NAD^+ kinase shows steric interaction with the amino acid residues. The calculated free energy of binding of gedunin was -8.4Kcal/mol (Figure 5). This confirms that the binding energy observed in this study is significantly related to the compound activity [31, 32]. Also, this proved the reliability of the docking results [33].

The solubility of a compound in water could improve its biotransformation and elimination as a drug [34]. Gedunin was soluble in water (figure 4). The molecular weight of gedunin was also less than 500g/mol, showing that it can be considered as a drug [35]. A compound can also be considered drug-like if it is characterized by high lipophilicity (less than 5) [36]. This is expressed as Log Po/w. The lipophilicity value of gedunin is less than 5 which means it is most likely to be a drug.

Lipinski's rule of 5 [37] helps in distinguishing between drug-like and non drug-like molecules. It predicts high probability of success or failure due to drug likeness for molecules complying with 2 or more of the following rules: Molecular mass less than 500g/mol; High lipophilicity (expressed as Log Po/w less than 5); Less than 5 hydrogen bond donors; Less than 10 hydrogen bond acceptors; Molar refractivity should be between 40-130. These filters help in early preclinical development and could help avoid costly late-stage preclinical and clinical failures [34]. Gedunin violated none of the Lipinski's rule, having a molecular weight of 482.57g/mol, 7 hydrogen bond acceptors, no hydrogen bond donor, consensus lipophilicity of 3.64 and a molar refractivity of 126.30 and therefore is likely to be a drug (figure 4).

High penetration is needed for most of the drugs targeting the central nervous system (CNS), whereas blood brain barrier (BBB) penetration should be minimized for non-CNS drugs to avoid undesired side-effects [38]. Pharmacokinetically, the gastrointestinal drug absorption of gedunin was high. Gedunin could also not cross the blood brain barrier (BBB) and this shows that it cannot cause any problem to the brain.

For synthetic accessibility, values of 5 to10 means that the drug could be synthesized [34]. This shows that the synthesis of gedunin might be slightly difficult haven shown a synthetic accessibility value of 6.54.

Secondary structure elements typically spontaneously form as an intermediate before the protein folds into its three dimensional tertiary structure [39]. It has been shown that α-helices are more stable, robust to mutations and designable than β-strands in natural proteins [40], thus designing functional all-α proteins is likely to be easier that designing proteins with both helices and strands; this has been recently confirmed experimentally [41]. The percentage helix according to the secondary structure prediction in figure 3 is 66.6. The high percentage helix is an indicator that the *Mycobacterium tuberculosis* NAD^+ kinase might be a stable enzyme.

Conclusion

The secondary structure prediction of the *Mycobacterium tuberculosis* NAD^+ kinase showed it is a stable protein owing to the percentage helix composition of the enzyme. This invariably requires a potent antibacterial agent hence, the need for gedunin. Gedunin has been shown to be safe for oral administration haven violated none of the lipinski's rule of five and with the predicted binding energy against the *Mycobacterium tuberculosis* NAD^+ kinase, we recommend the laboratory synthesis of gedunin and the direct targeting of the *Mycobacterium tuberculosis* NAD^+ kinase active site for the exhibition of its antimicrobial activity.

We also recommend the synthetic modification and molecular docking of gedunin against the *Mycobacterium tuberculosis* NAD^+ kinase for a probable increase in antimicrobial activity.

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