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# Interaction of ganoderic acid on HIV related target: molecular docking studies

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**Abstract:**
Finding the ultimate HIV cure remain a challenging tasks for decades. Various active compounds have been tested against various components of the virus in the effort to halt the virus development in infected host. The idea of finding cure from known pharmacologically active natural occurring compounds is intriguing and practical. Ganoderma lucidum (Ling-Zhi or Reishi) is one of the most productive and pharmacologically active compounds found in Asian countries. It has been used traditionally for many years throughout different cultures. More than a decade ago, el-Mekkawy and co-workers (1998) have tested several active compounds found in this plant. They have successfully identified several active compounds with reasonable inhibitory activity against HIV protease however; no further studies were done on these compounds. This study aimed to elucidate interactions for one of the active compounds of Ganoderma lucidum namely ganoderic acid with HIV-1 protease using molecular docking simulation. This study revealed four hydrogen bonds formed between model34 of ganoderic acid B and 1HVR. Hydrogen bonds in 1HVR-Model34 complex were formed through ILE50, ILE50′, ASP29 and ASP30 residues. Interestingly similar interactions were also observed in the native ligand in 1HVR. Furthermore, interactions involving ILE50 and ILE50′ residues have been previously identified to play central roles in HIV-1 protease-ligand interactions.These observed interactions not only suggested HIV-1 protease in general is a suitable target for ganoderic acid B, they also indicated a huge potential for HIV drug discovery based on this compound.

**Background:**
Ganoderma lucidum are filamentous fungi come from Basidiomycota phylum and Ganodermataceae family. It has been used extensively as home remedy in traditional Chinese medicine in many Asian countries for 2000 years. G. lucidum also known as Ling-Zhi in China and Korea and Reishi or Mannentake in Japan - has received wide of attention in recent year due to its extraordinarily pharmacological functions **[1].** The pharmacological effect of G. lucidum is based on their powerful immune-modulating activity and immune potential capability which support and enhance the immune system due to 200 active elements from fruiting body, mycelia, spores and anther. The active elements can be categorized into water soluble, organic soluble and volatile soluble compounds and include polysaccharide, polysaccharide-peptide complex, β-glucans, lectins, organic germanium, adenosine, triterpenoids and nucleosides **[2, 3].** Triterpenoids are common chemical constituent of G. lucidum. However, due to their unique properties, the importance of triterpenoids is established not only within the species but also extends to the chemotaxonomy of Ganoderma genus **[4].** Recently, more than 140 different triterpenoids have been isolated and characterized with ganoderic acids as one of the main triterpenoids present in G. lucidum **[2, 3].** Ganoderic acid is highly oxygenated lanostane-type triterpenoids and due to its valuable medicinal of pharmacological values, a lot of studies have been done on process development in

 

enhancing production of ganoderic acid **[1].** Most identified ganoderic acids are named using Latin or Greek alphabets along with numbering systems.

Biological effects of ganoderic acids as reported in the literature were antitumor, anti-HIV, antihypertensive, antihepatotoxic, anti-inflammation, inhibition of histamine release, hypocholesterolemic, anti-complement, antinociceptive, antioxidant, antiaging activities and platelet aggregation effects **[5, 6, 7, 8, 9, 10, 11, 12,].** More than a decade ago, El-Mekkawy and co-workers **[6]** have tested active compounds from Ganoderma lucidum against HIV proliferation and HIV protease. They have successfully identified thirteen compounds with inhibitory activities against HIV protease (HIV-PR) and proliferation of HIV-1 from G lucidum. Five of those compounds belong to ganoderic acid family namely ganoderic acid A, B, C1, H and α. Today, it is a common practice to utilize molecular modelling to complement conventional wet lab research especially in the field of drug discovery. Molecular modelling such as molecular docking and reverse docking has developed by leaps and bounds in the last decades in terms of technology and computing power. Emergence of this field has open new possibilities and ways to learn molecular interaction between molecules.Molecular docking can be described as the process of docking a small molecule (ligand, inhibitor and etc.) to a macromolecule with the objective of observing and studying interactions between these molecules. Reverse docking can be described as the process of searching a small molecule-protein target over a large database of potential protein targets. In contrast to conventional docking approach, reverse docking screens the molecule against specific database. The reverse docking terms rooted from the fact that this approach starts from the molecule of interest (usually ligands) whilst in conventional molecular docking usually starts with the potential targets (the proteins). From HIV drug development perspective, a combination of molecular modelling tools namely molecular dynamics and molecular docking has led to the discovery of the first HIV-integrase inhibitor. Previously, these methods have successfully pointed out a previously unidentified region in HIV-integrase and this region later on became a potent target for HIV-integrase inhibitor **[13].** This report aims to study molecular perspective of interaction for ganoderic acid compounds with HIV inhibitory activity as identified by el-Mekkawy and co-workers **[6]** using molecular docking simulation. Ganoderic acid compounds with HIV inhibitory activity were studied using two molecular docking approaches (reverse molecular docking and molecular docking). Outcomes from these docking studies are reported here.

**Methodology:**

Compound of interest were identified from previous work by el-Mekkawyand co-workers **[6].** Four compounds with IC50 ranging from 0.17 mM to 0.20 mM were chosen as the compound of interest.They are ganoderic acid α (0.19 mM), ganoderic acid B (0.17 mM), ganoderic acid C1 (0.18 mM) and ganoderic acid H (0.20 mM). Three dimensional structures of these compounds were obtained from PubChem database **[14].** All four structures were submitted to TarFisDock server **[15]** against the Potential Drug Target Database (PDTD) **[16]** for target identification by reverse docking. Viral infections were selected as the target criteria and default parameters defined in the server were used. Targets were selected and filtered based on their match with pharmacology activities as found in literatures, the crystal structure resolution and distribution in regards to compounds of interest. These selected protein targets were subsequently used in molecular docking studies where their three dimensional structure files were downloaded from the RCSB Protein Data Bank **[17].** All docking studies were performed using the Autodock 4 and ADT suite program **[18].** Hydrogen atoms, Gasteiger charges and torsion angles were computed and added to the ganoderic acid compounds (ganoderic acid α, B, C1, and H). For macromolecules, all water molecules and ligand were removed prior preparing the input files. Hydrogen atoms and Gasteiger charges were computed and added to the macromolecules. Active site of the target was highlighted and 50x60x60 grid box with 0.375Å grid spacing was drawn to cover the active site of the target. Each docking trial was initiated with 100 runs. Population size, energy evaluations, mutation and crossover rates and local search probability were kept as default. Molecular docking results were analyzed by cluster number, and hydrogen bonding comparison with the target's native ligand.

**Discussion:**

***Reverse Docking***

Based on the results by TarFisDock, two types of HIV target proteins were identified forboth ganoderic acid α (HIV-1 Reverse Transcriptase, HIV Protease) and ganoderic acid C1 (HIV Protease, HIV-1 Integrase). On the other hand, only HIV Protease was identified for both ganoderic acid B and ganoderic acid H. This is in good agreement with studies carried out by el-Mekkawy and co-workers **[6]** where they also demonstrated inhibition of HIV Protease by ganoderic acid α, B, C1 and H. Based on these potential targets resolution, their distribution with regard to compounds of interest and by correlation to el-Mekkawy and co-workers' findings, 1DIF and 1HVR (both are HIV-1 Protease) were selected as target for further molecular docking analysis.

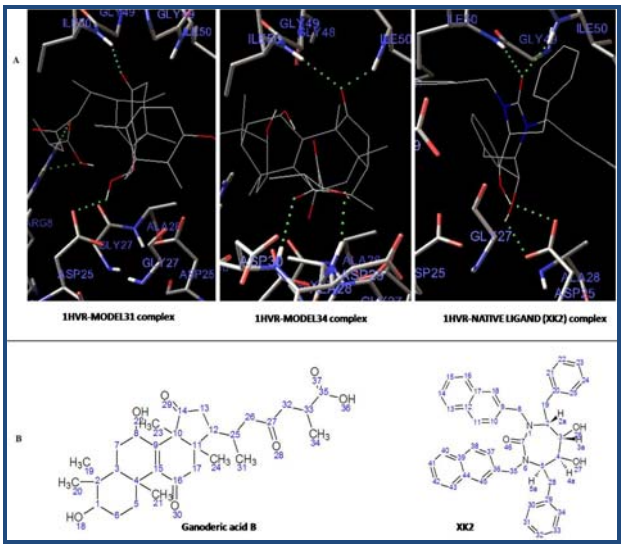


**Figure 1: (A)** Hydrogen bond networks as shown in 1HVR-Model 31 complex, 1HVR-Model 34 com-plex, 1HVR-native

ligand (XK2) complex**. (B)** Schematic representation of ganoderic acid B and XK

*Molecular Docking*

Summary of molecular docking results are shown in **Table 1. (See supplementary material)** Ganoderic acid B scored the lowest cluster number in both docking studies of 1HVR and 1DIF. Interestingly, this compound also scored the lowest mean estimated ΔG (-8.83 Kcal/mol) and with 78% of cluster dominance in the molecular docking study of 1HVR. Furthermore this results was also consistent to the previous data from el-Mekkawy and co-workers **[6]** where the lowest IC50 (0.17 mM) were obtained from ganoderic acid B. Cluster number, mean estimated ΔG and percentage of dominant cluster can be employed as comparative tools to asses molecular docking results. Cluster number represents variance of the docked structure, mean estimated ΔG represents energy state of each structure and percentage of dominant cluster represents the simulated structure dominance. Low cluster number, mean estimated ΔG and high percentage of dominant cluster are desirable in molecular docking simulation. Molecular docking of ganoderic acid B and 1HVR returned better results as compared to 1DIF, both in cluster number and mean estimated ΔG as compared to other docking studies. This makes ganoderic acid B an ideal candidate for further molecular interactions studies. Molecular interactions were studied in the best two conformations of molecular dockingstudies of 1HVR-ganoderic acid B. The first conformation was model 31 and the second conformation was model 34. Model 31 was the lowest scoring ΔG of all 1HVR-ganoderic acid B docking runs. On the other hand model 34 was the lowest scoring ΔG in cluster 2 (the most dominant cluster) of 1HVR-ganoderic acid B docking.

*1HVR-Ganoderic acid B molecular interaction*

Summary of these hydrogen bond interactions is shown in **Figure 1.** Four hydrogen bonds were observed in 1HVR-Model 34 complex.Amide hydrogen at ILE50 and ILE50′ formed two hydrogen bonds with oxygen atom at position 29 of ganoderic acid B. Another hydrogen bond was observed between amide of ASP30 and oxygen atom at position 37. The last hydrogen bond was between amide hydrogen of ASP29 and hydroxyl at position 36. Four hydrogen bonds were also observed in the 1HVR-Model 31 complex. Amide hydrogen at ILE50 formed hydrogen bond with oxygen atom at position 30. Amide hydrogen of ARG8 formed two hydrogen bonds with oxygen atom at position 37. The last hydrogen bond was between ASP25 and hydroxyl group at position 22.

Interestingly similar hydrogen bonding patterns were also observed in the 1HVR native ligand (XK2) complex in the 1HVR crystal structure. Two hydrogen bonds were observed between amide hydrogen of ILE50 and ILE50′ andoxygen atom position 7. Another two hydrogen bonds were from hydroxyl atoms position 26 and 27 with ASP25. Interaction of amide hydrogen for ILE50 and ILE50′, structural water molecule and the ligand or inhibitor through hydrogen bond is a common and a very important feature found in HIV-1 protease **[19].** To further highlight the importance of this ILE50-ILE50′-ligand interactions, Lam and co-workers had designed their HIV-1 protease inhibitor solely based on this feature **[20].** The lack of structural water molecule in our docking studies is due to the limitation of the method where molecular docking wasperformed on simplified environment by removing all water molecules from the macromolecule, a common practice in molecular docking routine. Nevertheless, the strong interaction between ILE50, ILE50′ and ganoderic acid B can still be established and observed in this study. This finding illuminates the significance of ganoderic acid B might be a potential inhibitor for HIV-1 protease.

**Conclusions:**

Ganoderic acid B produced better molecular docking results compared to other ganoderic acid compounds. This is in good agreement with previous report where ganoderic acid B was reported to have the lowest IC50 **[6].** Molecular interactions study revealed ganoderic acid B interactions with important residues of 1HVR, thus making 1HVR and HIV-1 protease in general suitable targets for this compound. The fact that ganoderic acid B is a naturally occurring compound and was found to interact with one of the most important feature of HIV-1 protease indicated a huge potential for HIV cure discovery based on this compound.More rigorous binding free energy calculation such as thermodynamics integration, free energy perturbation or molecular dynamic simulation as well as different vantage points such protonation state of the interacting molecules would be implemented in upcoming worksto obtain more in-depth information.

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## Supplementary material:

**Table 1:** Summary of 1HVR and 1DIF molecular docking:

| Compound | 1HVR molecular docking | | | | 1DIF molecular docking | | | | $IC_{50}$ (mM) [6] |
|---|---|---|---|---|---|---|---|---|---|
| Ganoderic acid α | **Mean estimated Δ*G* (Kcal mol$^{-1}$)** | **Lowest estimated Δ*G* (Kcal mol$^{-1}$)** | **Number of cluster** | **% dominant cluster** | **Mean estimated Δ*G* (Kcal mol$^{-1}$)** | **Lowest estimated Δ*G* (Kcal mol$^{-1}$)** | **Number of cluster** | **% dominant cluster** | |
| Ganoderic acid B | -8.43 | -9.71 | 12 | 42 | -7.53 | -8.63 | 15 | 45 | 0.19 |
| Ganoderic acid C1 | -8.83 | -9.67 | 5 | 78 | -6.99 | -7.97 | 6 | 40 | 0.17 |
| Ganoderic acid H | -8.63 | -10.10 | 8 | 26 | -8.13 | -8.53 | 9 | 28 | 0.18 |
| Ganoderic acid α | -7.02 | -8.19 | 19 | 25 | -6.59 | -7.57 | 12 | 53 | 0.20 |