



Received on 09 June, 2017; received in revised form, 09 August, 2017; accepted, 17 August, 2017; published 01 March, 2018

DOCKING STUDIES OF *GANODERMA LUCIDUM*

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Keywords:

Ganoderma lucidum, Molecular docking, Arguslab, DOCK 6.3

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ABSTRACT: Ganoderma is a miracle edible mushroom with unique composition and miraculous health benefits. The investigation aimed to elucidate interactions for the active compounds of *Ganoderma lucidum* namely Ganoderol A, Ganoderol B, Ganoderol C and Ganoderic Acid Y with Lanosterol 14 alpha demethylase enzyme using molecular docking software Arguslab. The effect of ganomycin1 and 2 as an inhibitor against HIV 1 protease and Tyrosinase were carried out using molecular docking software DOCK 6.3. The *in-silico* docking analysis of *Ganoderma lucidum* triterpenes was found to be effective as inhibitors of Lanosterol 14 alpha demethylase. The grid score and best scored conformations of Ganomycin 1 and 2 suggest that both have a significant inhibitory effect on these enzymes, comparable to the known inhibitors. The docking results showed significant inhibitory action of these compounds indicating the possibility that they can be used as potent drugs in the future. Thus these natural compounds have diverse structural and biological properties which can serve them to be utilized as a valuable lead molecule for the treatment of various diseases.

INTRODUCTION: *Ganoderma* is a genus of polypore mushroom. It is a valuable herb due to its biological activity such as anti-tumor, antioxidant, antidiabetic, anti-inflammatory, antimicrobial, immune-modulatory and hypo-lipidemic effect ¹. The pharmacological active compounds in *Ganoderma lucidum* are the polysaccharides, triterpenoids, alkaloids, organic germanium, ergosterols include farnesyl hydroquinones named ganomycin 1 and ganomycin 2 ^{2,3}. High cholesterol levels are deemed as a risk factor in heart disease. In cholesterol synthesis, HMG CoA reductase is the rate limiting enzyme and is inhibited by various drugs.

The triterpenoids in *Ganoderma lucidum* are potent inhibitors of the lanosterol 14 alpha demethylase, an enzyme of the cholesterol biosynthetic pathway that acts later in the pathway ultimately inhibiting the production of cholesterol. Lanosterol 14 alpha demethylase (or CYP_{51A1}, P450_{14DM}) is a cytochrome P450 (family 51, subfamily A, polypeptide 1) enzyme that is involved in the demethylation of lanosterol 2 thereby converting lanosterol to cholesterol. Inhibitors of P450_{14DM} are potential therapeutic agents for the treatment of hypercholesterolemia.

Several drugs, such as lovastatin and cholesteryl amine, are currently used to lower the serum cholesterol; they are expensive and are associated with adverse side effects. This leads to increasing demand for natural products with anti-lipidemic activity with fewer side effects. There are 130 triterpenoids extracted from the non-polar fractions of *G. lucidum* extracts.

QUICK RESPONSE CODE 	DOI: 10.13040/IJPSR.0975-8232.9(3).1100-05
	Article can be accessed online on: www.ijpsr.com
DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(3).1100-05	

All of them are highly oxygenated lanosterol derivatives with pharmacological activity, known as ganoderic acids, ganoderols, ganolucidic acids, lucidones and lucidenic acids.

HIV 1 protease is a retroviral aspartyl protease, which plays a critical role in viral replication. The inhibition of virus specific aspartyl protease would result in the production of immature and non-infectious virions. The known protease inhibitors have been known to cause hepatotoxicity, hyperglycemia, hyperlipidemia and lipodystrophy⁴. This necessitates the discovery of protease inhibitors from natural sources. Tyrosinase is a copper containing enzyme. It is linked with skin pigmentation, Parkinson's and other neurodegenerative diseases oxidizing excess dopamine to

produce DOPA quinones, highly reactive compounds that induce neuronal damage and cell death. Hydroquinone is a strong inhibitor of tyrosinase and Ganomycin 1 and Ganomycin 2 are active compounds of *G. lucidum* which are derivatives of Hydroquinone.

Computational study such as molecular docking is the best bioinformatics tool to evaluate whether the active compounds are a good ligand for the target receptor enzyme⁵. It helps to obtain the best geometry of ligand- receptor complex and calculate the energy of interactions for different ligands to design more effective ligands⁶. Elucidation of ligand binding mechanisms is required to obtain more selective and potent drugs⁷.

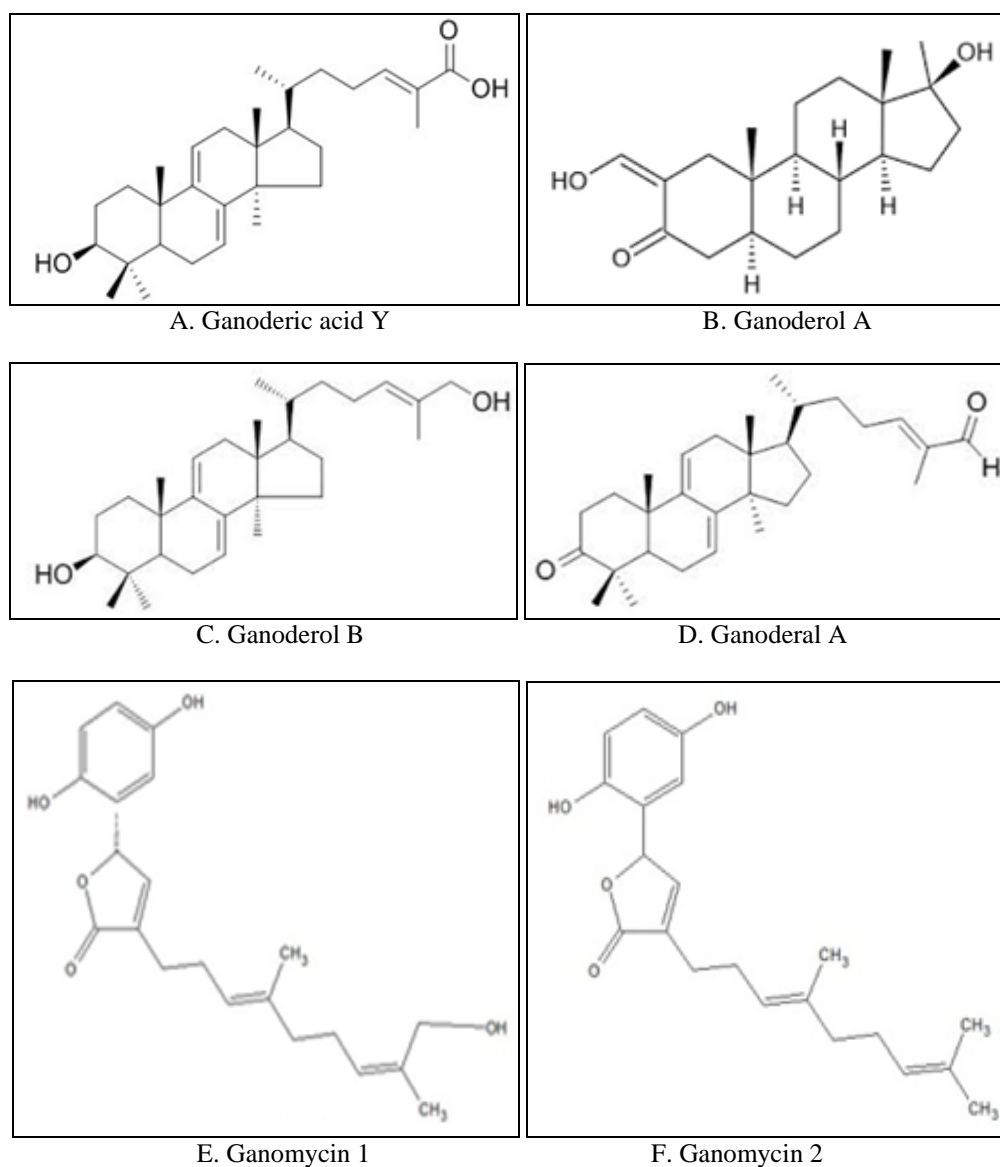


FIG. 1: CHEMICAL STRUCTURES OF THE LIGANDS

Preparation of receptor: The receptor structures were downloaded from the protein data base (PDB) of National Center of Biotechnology Information (NCBI) (<http://www.rcsb.org/>) with PDB code of (Lanosterol 14 alpha demethylase) 3JUS, (Tyrosinase) 1WX2.PDB, (HIV-1 Protease) 1EBZ.PDB respectively. All the bound water molecules, ligands and cofactors were removed from the original structure file which was taken in PDB format. The protein was characterized using *in-silico* methods where the hydrophobic or cavity region in the co-crystallized protein - drug complex was identified. The ligand associated within the complex was identified and removed by using Chimera. Using Chimera, water molecules were removed from the receptor structure and Gasteiger charges and hydrogen atoms were added to the receptor structure and the structure of the receptor were saved for docking.

Preparation of Ligands: The molecular structures of the ligands Ganoderol A, Ganoderol B, Ganoderol A and Ganoderic Acid Y, Ganomycin 1 and 2 were drawn using ChemsSketch. The ligands were further processed in Chimera where nominal gasteiger charges and hydrogen atoms were added to the structure to prepare the ligands for docking. Lowest energy conformation was selected and used for docking.

Docking Methodology using Argus Lab 4.0.1: After the preparation of the protein and ligand, molecular docking studies were performed by ArgusLab software to evaluate the interactions. ArgusLab 4.0.1 (Mark A. Thompson, Planaria Software LLC, Seattle, WA, USA, <http://www.arguslab.com>) is the electronic structure program that is based on the quantum mechanics. It

predicts the molecular structures, geometry optimization, potential energies, and vibration frequencies of coordinates of atoms, bond length, and bond angle.^{8,9} The docking of protein ligand complex was performed by selecting Argus Dock as the docking engine. The best docking model was selected according to the lowest AScore calculated by ArgusLab, as the lowest energy poses indicate the highest binding affinity whereas high energy produces the unstable conformation.

Docking Methodology using DOCK 6.3: DOCK is a Unix based scientific software. It is used for computational structure prediction of ligand-protein complexes, search databases of ligands that inhibit enzyme activity or bind a particular protein or nucleic acid target, examine possible orientations for protein-protein and protein DNA complexes. DOCK 6.3 includes two programs SPHGEN, which identifies the active site and generates the sphere centers that fill the active site and GRID, which generates the scoring grids¹⁰. The grid-box is generated using SHOWBOX program. Within the docking suite of the programs, the program DOCK matches spheres with ligand atoms and uses scoring grids to evaluate ligand orientations. The DOCK program identifies the best conformation with the lowest binding energy.

RESULTS AND DISCUSSION: The docking studies of Ganoderol A, Ganoderol B, Ganoderic acid Y and Ganoderol A with Lanosterol 14 alpha demethylase with PDB code 3JUS was carried out by Argus Lab 4.0.1. The least binding energy exhibits the highest activity which has been observed by the ranking poses generated by scoring function of Argus Lab and are given in **Table 1**.

TABLE 1: DOCKING SCORE AND MOLECULAR PROPERTIES OF LIGAND

Compound	Energy value ΔG (Kcal/mol)	MW (g/mol)	Hydrogen donor	Hydrogen acceptor	Log P	TPSA
Ganoderic acid Y	-17.70	454.695	2	3	7.3	57.5
Ganoderol A	-16.67	438.696	1	2	7.1	37.3
Ganoderol B	-14.04	440.712	2	2	7.4	40.5
Ganoderol A	-12.99	436.68	0	2	6.9	34.1

Keong reported the hypocholesterolemic effect of the mushroom fruiting bodies of some putative active compounds extracted from *G. lucidum*.¹¹ The Lipinski's rule of five for an ideal drug should have Molecular Weight value of ≤ 500 , Hydrogen

bond Donor ≤ 5 , Hydrogen bond Acceptor ≤ 10 and Partition coefficient (Log P) value ≤ 5 .^{6,12} Out of the four ligands, Ganoderol A was found to possess the lowest binding energies with -12.99 Kcal/mol and is likely to obey the Lipinski's rule of five with

the partition coefficient (Log P) which was more than 5 indicating the hydrophilic nature of Ganoderal A. The topological polar surface area

(TPSA) is an indicator of ligand hydrophilicity. It was found to be less than 140 Å and hence has good cell permeability.

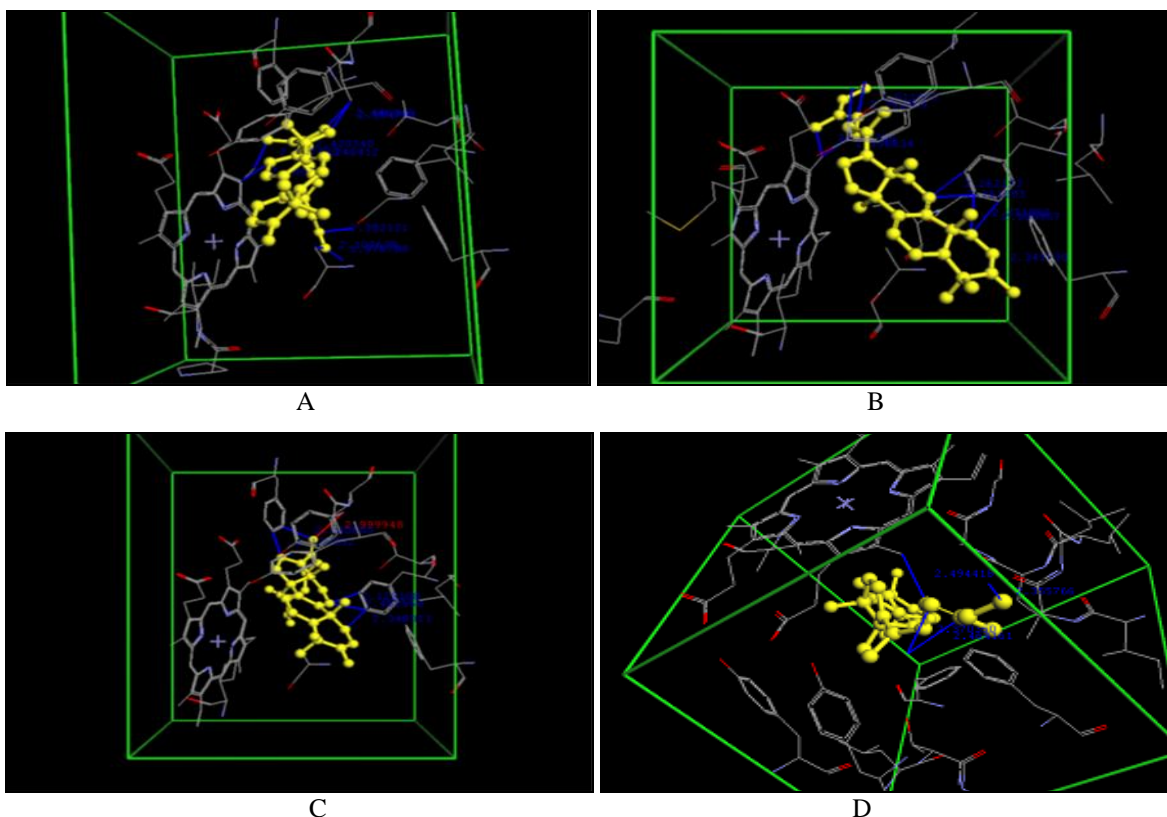


FIG. 2: DOCKING INTERACTIONS OF RECEPTOR LANOSTEROL 14 ALPHA DEMETHYLASE (YELLOW) WITH THE LIGANDS [A] GANODERIC ACID Y AND [B] GANODEROL A [C] GANODEROL B [D] GANODERAL A

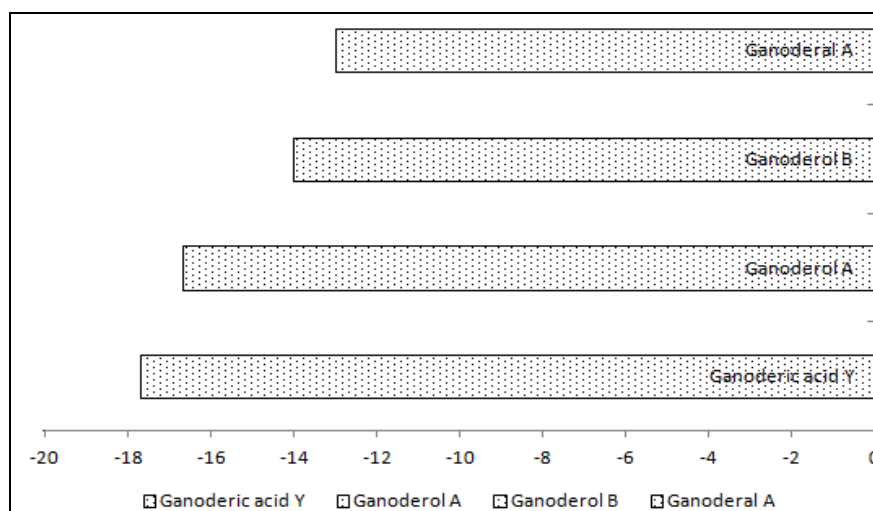


FIG. 2: COMPARISON OF BINDING FREE ENERGIES OF VARIOUS LIGANDS WITH LANOSTEROL 14 ALPHA DEMETHYLASE BY ARGUS LAB

TABLE 2: DOCKING SCORE AND MOLECULAR PROPERTIES OF LIGAND GANOMYCIN 1 AND 2 WITH RECEPTOR HIV PROTEASE* AND TYROSINASE **

Ligand	Van der Walls energy	Electrostatic energy	Total Grid score
Ganomycin 1*	-65.103279	0.00000	-65.103279
Ganomycin 2*	-63.544552	0.00000	-63.544552
Ganomycin 1**	-54.654087	0.00000	-54.654087
Ganomycin 2**	-56.174919	0.00000	-56.174919

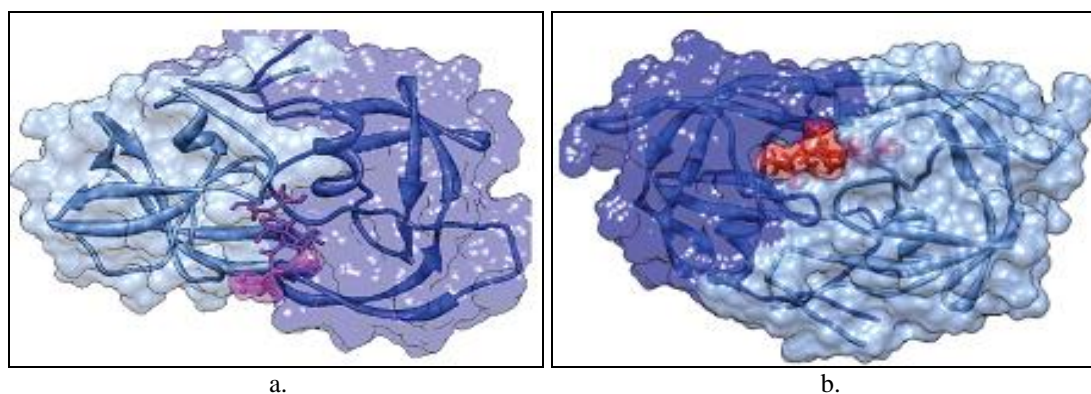


FIG. 3: DOCKING INTERACTIONS OF RECEPTOR HIV 1 PROTEASE a. WITH GANOMYCIN 1 b. WITH GANOMYCIN 2

The best fit for Ganomycin 1 and Ganomycin 2 is shown to be located in the cleft between the active site dimer interface and the residues 41-49 of both the monomers of the protease that comprise the β -strands a', that acts as a flap. This cleft is enclosed on each side by the residues 79 - 82 of chain A and chain B respectively. The total grid score of all the ligands seem to have been contributed solely by van der waals interaction. Ganomycin are highly flexible structures, with the presence of 14 rotatable angles. The presence of these angles allowed the flexible docking of DOCK to predict the ligands tightly wrapped orientation within the cleft. The docking results of Ganomycin 1 and Ganomycin 2 with HIV 1 protease show significant inhibitory action of these compounds, indicating the

possibility that they can be used in the anti-retroviral therapy as potent drugs. A triterpenoid isolated from the stem of *Ganoderma sinense* showed higher affinity towards HIV-1 protease with binding energy of -11.4kcal/mol using AutoDock 4.2 software¹³. Similar effect of ganoderic acid B was studied by Akbar and Yam on HIV 1 protease and indicated a huge potential for HIV treatment¹⁴. Hattori *et al.* also studied ganomycin 2 as an anti-HIV agent^{7, 15}. Majority of anti-viral in-vitro investigations on *Ganoderma sp.* from fruiting bodies against the protease enzyme of HIV virus were reported by Basnet *et al.*, 2017¹⁶. Zhu *et al.*, also suggested that lanostane triterpenoids from *G. lucidum* to possess anti-influenza potential¹⁷.

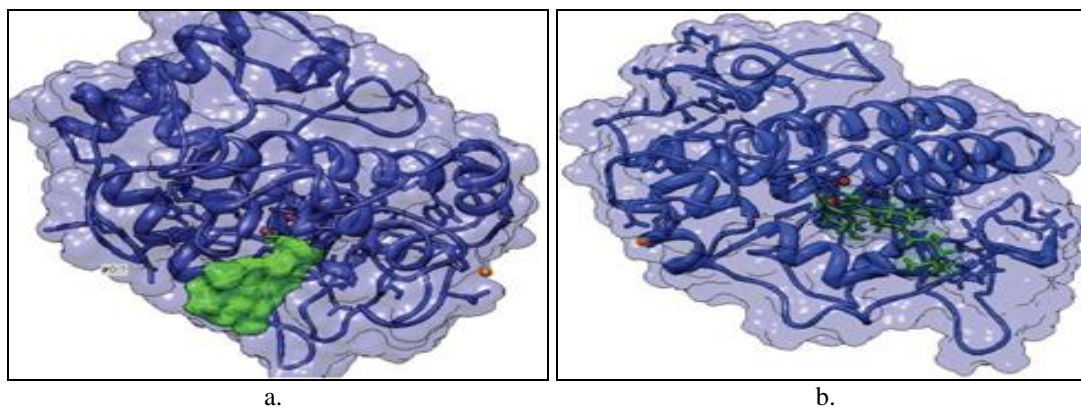


FIG. 4: DOCKING INTERACTIONS OF RECEPTOR TYROSINASE (BLUE) AND THE COPPER ATOMS OF THE STRUCTURE (RED) a. WITH GANOMYCIN 1 (LIGHT GREEN) b. WITH GANOMYCIN 2 (GREEN)

The docking simulation of binding between Ganomycin 1 and 2 with tyrosinase was successful in producing a significant score (binding energy was -54.654Kcal/mol for ganomycin1 and -56.174Kcal/mol for ganomycin 2). Ganomycin 1 binds in close proximity to the copper ions of the active site of tyrosinase whereas, ganomycin 2 binds with copper ions and also forms an H-bond with the Arg

54 of the tyrosinase structure. The docking simulations provided informative data for ganomycin as a tyrosinase inhibitor identifying inducing residues near the active site pocket, which might directly affect the substrate docking and catalysis by inducing structural modifications. Yin and his coworkers carried out combination of inhibition kinetics and computational modeling of

phthalic acid on tyrosinase inhibition¹⁸. Kim et al., investigated the *in-vitro* whitening effect of ganodermanondiol as a tyrosinase inhibitor present in *G. lucidum*¹⁹.

CONCLUSION: The molecular docking interactions revealed Ganoderal A with lowest binding energies using Arguslab software. It would be a promising drug as it acts as an inhibitor against Lanosterol 14 alpha demethylase. Ganomycin 1 is a potential inhibitor for HIV 1 protease and tyrosinase enzyme with the lowest energies. These natural active compounds from *G. lucidum* can be area for further investigational studies and may prove useful for the screening of potential enzyme inhibitors.

ACKNOWLEDGEMENT: The authors are thankful to Dr. Selvaa Kumar C., Associate Professor and Mr. Pramod Gupta, Assistant Professor at School of Biotechnology and Bioinformatics, D. Y. Patil University, Navi Mumbai for their technical support to our research work.

CONFLICT OF INTEREST: The authors declare no conflict of interest.

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How to cite this article:

Gupte A, Palande A, Venkata S and Pol R: Docking studies of *Ganoderma lucidum*. Int J Pharm Sci Res 2018; 9(3): 1100-05. doi: 10.13040/IJPSR.0975-8232.9(3).1100-05.