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Dammarane and Ergostane derivatives as prophylactic agents against SARS-CoV-2 host cell entry Inhibitors

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Abstract
A novel coronavirus designated as SARS-CoV-2 originated in Wuhan city in Hubei Province of China at the end of 2019.This been recently declared as Global Pandemic by WHO and termed as disease COVID-19. This believed to be Zoonotic in origin mainly from an Intermediate Horse shoe bat and suspected intermediate host of unknown origin. As of 11 May 2020, World had 4.1 Million confirmed cases in total, 1.4 Million recovered and deaths around 282000.There is a global emergency to identify potential drugs to treat the SARS-CoV-2. Currently, there is no specific treatment against the new virus. There is urgency to identifying potential antiviral agents or Vaccines to combat the disease. Many Repurposed been proposed, but most of them have toxicity and not specific to viral host cell inhibitors. Natural compounds proved many times as antiviral agents and have potential to act as host cell entry inhibitors. Dammarane and Ergostane terpenoid derivatives are such category of natural compounds selected to study as entry inhibitors of SARS-CoV-2. Dammarane compounds like Protopanaxidiols, Panaxatriol, Betulin, Stigmasterols like Betasitosterol and Ergostane compounds like Withanolide in comparison with AI predicted Thiocolchicoside and Prednisone were selected as ligands and docked against SARS-CoV-2 spike glycoprotein PDB ID 6VXX. Protopanaxidiols, Panaxatriol, Betulin, Arcapitin, Betasitosterol, prednisone, Thiocolchicoside, Withanolide compounds had shown strong binding with spike glycoprotein with ΔG -6.98, -6.5, -5.8,-6.02 -5.9, -5.95,-6.0, -6.75 kcal and Estimated Inhibition Constant, Ki values 100 uM, 70 uM, 43 uM, 18 uM, 25 uM, 42 uM, 54 uM and 23 uM. Betasitosterol, Panaxatriol and Betulin had shown cation pi interaction indicating strong binding efficacy. Amino acid side chain interaction of ligand with TYR, SER, ASN, THR, GLN, ARG, LEU of spike protein shown the selected molecules have good inhibitory effect against SARS-CoV-2. Thus we propose to study the cell entry inhibitory effect of Dammarane and Ergostane derivatives along with lactone skeleton in their structure via *In-vitro In-vivo* methods and to conduct proper clinical trials among COVID-19 patients.

Keywords: Dammarane, Ergostane, Protopanaxidiols, panaxatriol, prednisone, Betulin, Arcapitin, Betasitosterol, Withanolide, Thiocolchicoside, SARS-CoV-2, COVID-19

Introduction
Many human pathogeneses including some types of cancer are caused by different type of viruses in the history. Many viral associated hard to cure diseases and syndromes like Alzheimer's, type -1 diabetes and hepatocellular carcinoma have been associated with viral infections. Globalization and rapid urbanization raised the epidemic outbreaks caused by emerging and re-emerging viruses pose a critical threat to the public health. More threat occurs when preventive medicine is not available for such disease outbreaks. Examples include recent emergence of influenza virus, MERS, SARS, Ebola, dengue and Zika virus outbreaks. Till date no effective immunization programmes been successfully developed for the above viral outbreaks with few options of anti-viral drugs leftover. Hence, there is an urgency to discover novel antiviral drugs that are effective and economical to treat the viral outbreaks.

Traditional medicines and natural compounds provide rich sources of antiviral drug development. Identification of antiviral mechanism from these natural compounds should be taken as priority. Studies should be conducted on interaction of natural compounds with viral cell entry, replication, assembly and shredding.

Dammarane and Ergostane are the terpenoid derivatives as shown in image 1 and image 2 present in many tropical and subtropical plants including some herbs of temperate regions. These groups of compounds had shown antiviral, anticancer and anti-inflammatory properties. Dammarane compounds from Ginseng, Rheus chinensis, *Argyreia capitata* (Available in families of Convolvulaceae and Solanaceae), Tirucallane and Apotirucallane compounds from *Chisocheton paniculatus*, Xylocarpus granatum had shown inhibitory effect against HIV and Herpes virus.

Ergostane compounds from *Withania somnifera* had shown inhibitory effect against HIV virus.

Computer-aided drug discovery has recently had important successes: new biologically-active compounds have been predicted along with their receptor-bound structures and in several cases the achieved hit rates (ligands discovered per molecules tested) have been significantly greater than with HTS. The molecular docking approach can be used to model the interaction between a small molecule and a protein at the atomic level, which allow us to characterize the behaviour of small molecules in the binding site of target proteins as well as to elucidate fundamental biochemical processes. The docking process involves two basic steps: prediction of the ligand conformation as well as its position and orientation within these sites (usually referred to as pose) and assessment of the binding affinity.

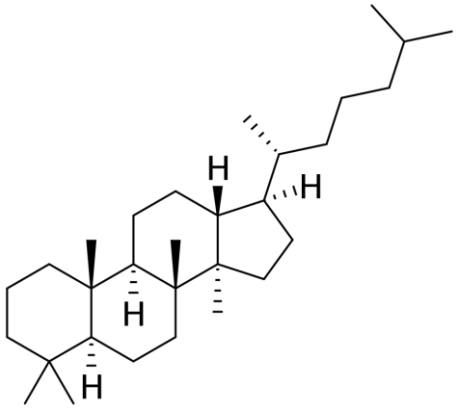


Image 1: Structure of Dammarane

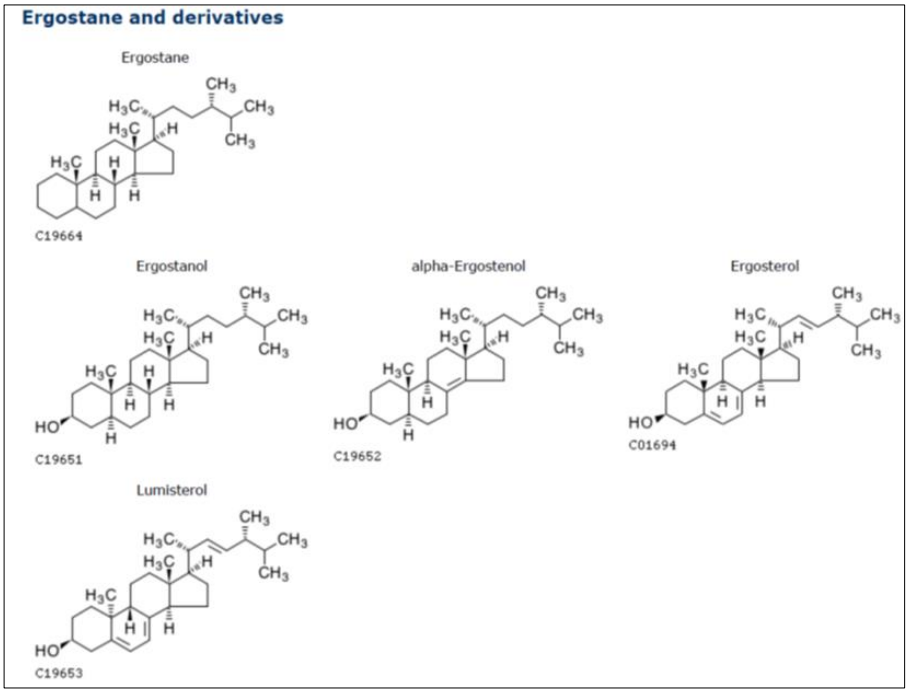


Image 2: Ergostane and derivatives

SARS-CoV-2 is a type of coronavirus emerged as a global pandemic from the city of Wuhan from China in December 2019. By May 2020 four million positive cases, two million recovery cases and 2, 90,000 deaths been registered globally because of COVID-19. SARS-CoV-2 virus contains single stranded positive RNA with nucleocapsid enveloped in lipid bilayer icosahedral capsule with spike glycoprotein, membrane protein and envelope protein on the surface. SARS-CoV-2 enters host cell through membrane receptors like ACE2, TMPRSS2 and other receptors like Furins, dectins, mucins etc. Spike glycoprotein binds to cell receptors like ACE2 and TMPRSS2 and enters host cell. A replication cycle begins in the cytoplasm of the host cell. Thus, by targeting spike glycoprotein viral host cell entry can be inhibited. In this brief report we summarized the antiviral activity mainly host cell entry from natural compounds like Dammarane compounds like Protopanaxidiols, Panaxatriol, Betulin, Stigmasterols like Betasitosterol and Ergostane compounds like Withanolide in comparison with AI predicted Thiocolchicoside and Prednisone were selected as ligands and docked against SARS-CoV-2 spike glycoprotein PDB ID 6VXX.

Methods

Crystal structures of the protein-ligand complexes used in this study obtained from Databank http://www.rcsb.org/pdb. Ligand site was carefully checked when asymmetric unit found different from biological unit. The biological unit used in this study was spike glycoprotein of SARS-CoV-2 with PDB code: 6VXX. From PDB Database Experimental protein-ligand binding affinities were taken. From recent publications protein ligand PDB core set was taken. The following criteria were taken for the structures to run docking: Crystallographic resolution lower than 3.2 Å non-covalent binding between protein and ligand. Structurally diverse ligands in complex with a heterogeneous collection of proteins

Derived from the complex crystal structure flexible docking of the ligand to rigid receptor carried out. Two different input methods were used to prepare the structures: (a), using Gasteiger charges for both the ligands and the proteins; (b) PM6 charges were calculated by MOPAC2009 for both the ligands and proteins. For the input structures Gasteiger charges were prepared as follows: Hydrogen atoms were added using AutoDock tools and ligand atoms and bond types were assigned. Gasteiger charges were used to calculate

Empirical charges. HEME and metal ions were kept for proteins and cofactors, and their atom types and bond types were assigned manually. Halogen, Sulphate atoms and water molecules were removed. Hydrogens were added in protein residues along with Gasteiger partial charges using AutoDock tools. Sulphate, halogens and water molecules were removed. Non-polar hydrogen atoms along with their charges were merged and Hydrogens are added in protein residues as well as Gasteiger partial charges using Auto Dock Tools. No further optimization of the protein structures was done.

PM6 method was used to assign semi-empirical modules by Mozyme function of MOPAC2009 program in Docking Server http://www,dockingserver.com. In the last step PM6 charges were calculated using MOPAC2009 software for the Ligand structures with semi-empirical charges and followed as above description. Halogens, Sulphate and water molecules were removed first for the Protein structures setup. AutoDock Tools were used to add Hydrogen atoms to the pdb structures. Mozyme functions of MOPAC2009 software was used to calculate the total charge of the protein and partial charges of the atoms. Further the calculated partial charges were applied.

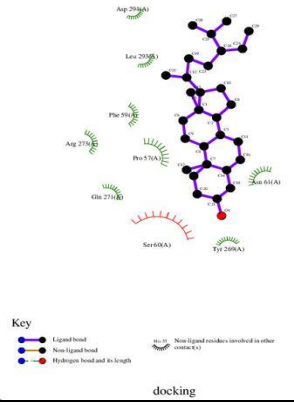
Dammarane compounds like Protopanaxidiols, Panaxatriol, Betulin, Stigmasterols like Betasitosterol and Ergostane compounds like Withanolide in comparison with AI predicted Thiocolchicoside and Prednisone are selected as ligands and Docking studies were performed using Docking Server http://www.dockingserver.com. In order to calculate partial charges while ligand setting Docking Server integrates Marvin http://www.chemaxon.com and MOPAC2009 at a given protonation state and for semi-empirical geometry optimization. For docking calculation, AutoDock 4 is integrated. PM6 method, QASP parameter modified (QASP = 0.00679) both Autogrid 4 and AutoDock 4 were used in case where protein and ligand partial charges were calculated.

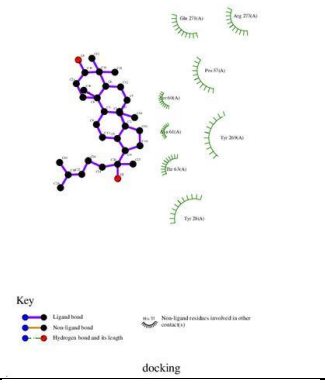
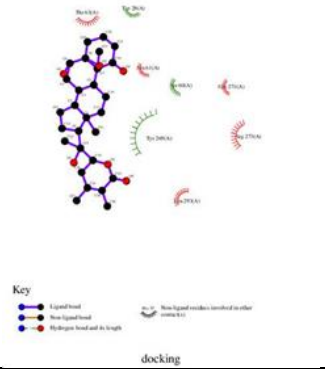
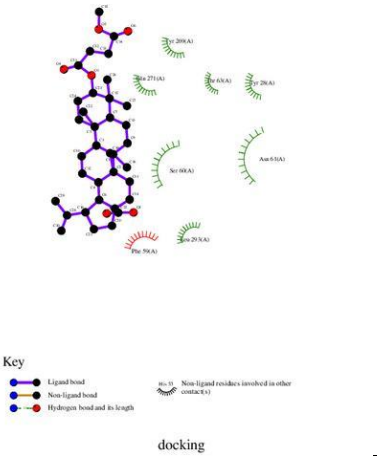
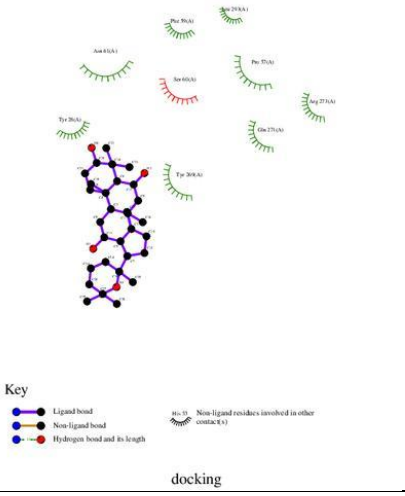
Following parameters are set in Docking Server: Grid parameters were built along with atom specific affinity maps were constructed using Autogrid 4. Using 6X60X60 grid points and spacing 0.375 Å, map files were generated, with maps centered on the experimentally fixed centre of the bound ligand. Lamarckian Genetic Algorithm was used to study Docking simulations. During docking the ligand parameters like orientation, torsion and Initial position were set randomly and all rotatable torsions were released while docking. 100 different runs which were set to terminate after maximum 2,500,000 energy evaluations for each docking with a population size of 250. RMSD between lowest energy, pose of ligand and complex crystal structure ligand pose was evaluated after each docking calculation.

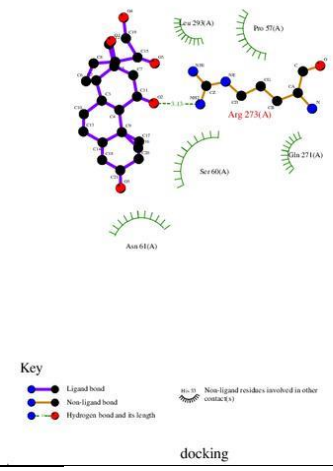
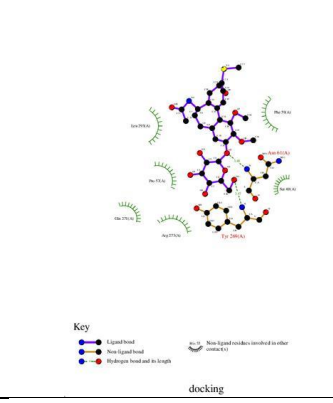
Results

In Table 1 structural and experimental data of the investigated protein-ligand complexes are summarized. The spike glycoprotein complex (PDBID: 6VXX) used in this study were all characterized by a resolution below 3.2 Å. The complex was chosen partly from the AutoDock 3.0 calibration set, from a recently published paper examining different docking software and from the core set of PDB Database. The chosen structures Dammarane compounds like Protopanaxidiols, panaxatriol, prednisone, Stigmasterols Betasitosterol and Ergostane compounds like Withanolide possess structurally diverse ligands shown in Table 1. It should be noted that for some structures with lower resolution (although chosen from the AutoDock 3.0 calibration set), an RMSD-based comparison of docked versus experimental structure might not always lead to a meaningful result as partial occupancies might occur that are not reflected by a single ligand structure. Using this dataset, ligand and protein structures were setup using two different methods, (i), calculating Gasteiger charges on both the ligands and the proteins using AutoDockTools and (ii), calculating PM6 charges on both the ligands and the proteins using MOPAC2009 on Docking Server (see Method section for details). Docking calculations were performed twice on the dataset (in case of both ligand and protein set up methods) and the results were then compared to the experimentally determined complex structures Protopanaxidiols, Panaxatriol, Betulin, Arcapitin, Betasitosterol, prednisone, Thiocolchicoside, Withanolide compounds had shown strong binding with spike glycoprotein with ΔG -6.98, -6.5, -5.8,-6.02, -5.9, -5.95,-6.0, -6.75 kcal and Estimated Inhibition Constant, Ki values 100 uM, 70 uM, 43 uM, 18 uM, 25 uM, 42 uM, 54 uM and 23 uM. Betasitosterol, Panaxatriol and Betulin had shown cation pi interaction indicating strong binding efficacy. Amino acid side chain interaction of ligand with TYR, SER, ASN, THR, GLN, ARG, LEU of spike protein shown the selected molecules have good inhibitory effect against SARS-CoV-2. Thus we propose use of Dammarane and Ergostane derivatives like Protopanaxidiols, Panaxatriol, Betulin, Arcapitin, Withanolide along with Betasitosterol, prednisone and Thiocolchicoside as prophylactic or primary treatment drugs along with palliative treatment protocols after conducting proper *In-vivo* and *In-vitro* studies

Table 1: Docking results for Dammarane and Ergostane derivatives showing prophylactic inhibition activity by binding spike protein of SARS-CoV-2

Compound Name	Estimated Ki	Free energy of Binding ΔG	2D Interactions
Betasitosterol	25 uM	-5.9 Kcal/mol	

Protopanaxidiols	100 uM	-6.98 Kcal/mol	
Withanolide A	23 uM	-6.75 Kcal/mol	
Betulin	43 uM	-5.8 Kcal/mol	
Panaxatriol	70 uM	-6.5 Kcal/mol	

Prednisone	42 uM	-5.95 Kcal/mol	
Thiocolchicoside	54 uM	-6.0 Kcal/mol	
Arcapitin	18 uM	-6.02 Kcal/mol	

Conclusion

Dammarane and Ergostane derivatives with lactone in their skeleton had shown promising results as antiviral agents earlier. They acted as inhibitors for both DNA and RNA viruses. In the present study Dammarane compounds like Protopanaxidiols, Panaxatriol, Betulin, Stigmasterols like Betasitosterol and Ergostane compounds like Withanolide in comparison with AI predicted Thiocolchicoside and Prednisone were selected as ligands and docked against SARS-CoV-2 spike glycoprotein PDB ID 6VXX. Protopanaxidiols, Panaxatriol, Betulin, Arcapitin, Betasitosterol, prednisone, Thiocolchicoside, Withanolide compounds had shown strong binding with spike glycoprotein with ΔG -6.98, -6.5, -5.8,-6.02 -5.9, -5.95,-6.0, -6.75 kcal and Estimated Inhibition Constant, Ki values 100 uM, 70 uM, 43 uM, 18 uM, 25 uM, 42 uM, 54 uM and 23 uM. Betasitosterol, Panaxatriol and Betulin had shown cation pi interaction indicating strong binding efficacy. Amino acid side chain interaction of ligand with TYR, SER, ASN, THR, GLN, ARG, LEU of spike protein shown the selected molecules have good inhibitory effect against SARS-CoV-2. Thus we propose to study the cell entry inhibitory effect of Dammarane and Ergostane derivatives along with lactone skeleton in their structure via *In-vitro In-vivo* methods and to conduct proper clinical trials among COVID-19 patients

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