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Evaluation of In-silico Pharmacokinetics, Cytotoxicity prediction and Molecular Docking Studies of Ganomestenol

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Abstract:
The present study finds out the *in-silico* pharmacokinetics, cytotoxicity prediction and molecular docking studies of Ganomestenol. The ganomestenol were subjected for *in silico* pharmacokinetics such as gastro intestinal absorption, P-glycoprotein, blood brain barrier, lipinski's rule etc., and toxicity prediction parameters such as hepatotoxicity, carcinogenicity, immunogenicity, mutagenicity and cell line cytotoxicity. The results showed that the compound may be drug candidate for various pharmacological studies. To support for the antimicrobial activity, *in-silico* drug Ganomestenol interaction with bacterial (PDB ID: 1ZKN) and fungal (PDB ID: 1EA1) target proteins were studied by using autodock4 tool showed good binding energy with target proteins.
Key words: Pharmacokinetics, Molecular Docking, antimicrobial activity, Ganomestenol etc.

1. Introduction

Ganoderma is a fungus which belongs to a Ganodermataceae, known as Polyporales. Several Ganoderma species are economically important for their medicinal properties and phytopathogenicity (Dai et al. 2007; 2009). *G. boninense* causes basal stem rot disease in palm and root-rot disease in Acacia trees caused by *G. steyaertanum, G. mastoporum*, and *G. philippii* (Susanto et al., 2005; Glen et al., 2009). *Ganoderma* is one of the valuable Asian medicines used for millennia (Bishop et al., 2000). Several biologically active triterpenes and sterols have been isolated from this mushroom and proved effective as cytotoxic, antiviral and anti-inflammatory agents. *Ganoderma* nutriceuticals are used as a remedy to treat more than 20 different illnesses, which include Migraine and headache, hypertension, arthritis, bronchitis (asthma), asthma, anorexia, gastritis, haemorrhoids, hypercholesterolaemia, nephritis, dysmenorrhoea, costipation, lupus esythematosis, hepatitis, leucopenia, cardiovascular problems and cancer including leukaemia (Sivakumar et al., 2006). Ganoderma was acclaimed as a divine herb that could use for good health and well being. This might be the case when certain mushrooms were treated as objects of worship on as objects of mysteries describing them as celestial herbs possessing panaceal properties. Now-a-days, modern research has revealed its active ingredients, which include polysaccharides, organic germanium, triterpenoids, adenosine, LZ-8 and an array of amino acids besides numerous mineral types. In additions showed significant effect for type-C hepatitis, type-B hepatitis, diabetes, gastric and duodenal ulcers etc. A significant effect against functional sterility was observed. And a certain degree of effectiveness against female illnesses such as climateric, dielectric disturbances, liver cirrhosis, chronic nephritis, hypertension, heart disease, atomic skin inflammation, such allergic diseases as bronchial asthma (Russel and Paterson, 2006; Shittu et al., 2006; El-

Mekkawy et al., 2000; Iwahokun et al., 2007).

The traditional Chinese medicines (TCM) and other oriental countries are huge treasure of world herbal medicinal knowledge. In comparison to Western medicine, they are regarded as the mainstay of all styles of traditional medicines, its international position increased rapidly in recent decades. TCM has come to be a very important component of the Chinese and Indian treasure of culture. It has abundant record in Materia Medica as unique sources of raw material of medicine. The inherited classes lay the foundation of its independent theories. TCM is the only exception which is esteemed as the world treasure of culture (Nasreen et al., 2005; Jonathan et al., 2007). Many health reports of Ganoderma are available for the immune system regulation. This is due the presence of polysaccharides and antioxidants in crude extracts of Ganoderma. The extract and its constituents are used to relive health disorders like influenza, cold, hepatitis and canker sores (Wachtel-Galor, 2011). Many health practitioners claim that Ganoderma species has effect on the immune system improvement because of presence of polysaccharides, secondary metabolites and antioxidants in *Ganoderma* extracts.

The computer-based (*in-silico*) predictions greatly reduced the risky, lengthy, and resource-intensive process. In-silico based methods predicts absorption, distribution, metabolism, excretion, and toxicity (ADMET), which could aid in the drug discovery and development (Ekins et al. 2007; Waring et al. 2015). ProTox-II assess toxicity prediction of compounds and provides valuable information about Hepatotoxicity, LD50, Cytotoxicity, Carcinogenicity, Mutagenicity and Toxicity class (Halder et al. 2019). The present study, Ganomestenol isolated from *Ganoderma* species were subjected for molecular docking against target proteins, ADMET and cytoxicity analysis for drug candidate in pharmacological studies. Previous study reported the isolation and characterization of Ganomestenol from *Ganoderma* species (Naveen Kumar KJ and Venkatesh, 2020). The present study focused on *in-silico* molecular interaction studies of Ganomestenol with target proteins, ADMET studies and cytoxicity analysis.

2. Materials and Methods

The Ganomestenol has been characterized based on the spectral information (Figure 1) and also showed good antimicrobial property against *E. coli, Pseudomonas solanacearum, Clavibacter michiganensis, Fusarium oxysporum, Alternaria solani, Curvularia lunata* and *Helminthosporium oryzae* (Naveen Kumar KJ and Venkatesh, 2020).

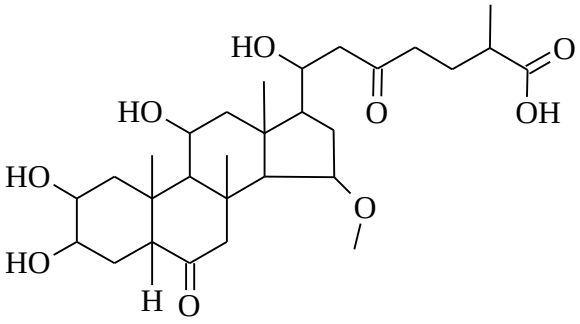

Figure 1: Structure of Ganomestenol

2.1 Molecular docking studies

Autodock4 was used to study the interaction of inhibitor or ligand or drug bound to the active site of biological receptors. The structure of ligand molecules Ganomestenol and Standard drugs were designed and 3-D coordinates were prepared using chemsketch 8.0. The protein crystal structure of antibacterial and antifungal target proteins, E.coli 24 kDa domain (PDB ID: 1KZN**)** (Lafitte et al., 2002) retrieved from Protein Data Bank (www.rcsb.org/pdb) and edited by removing the heteroatoms, adding C- terminal oxygen. Both ligands and protein molecule are saved in PDBQT. During docking, Gasteigere– Marsili partial charges (Gasteiger & Marsili, 1980) were assigned to the ligands and non-polar hydrogen atoms were merged. All torsions were allowed to rotate during docking. The grid map of protein *E.coli* 24kDa domain (PDB ID: 1ZKN) was centered at the following residues of the protein namely GLU58, ILE60, GLN72, ASP73, GLU131, VAL133,

GLN135, LYS162, THR163, GLY164, THR165, MET166 and grid map of protein cytochrome P450 14α-sterol demethylase (PDB ID:1EA1) at residues of TYR76, PHE78, MET79, PHE83, ARG96, MET99, LEU100, SER252, PHE255, ALA256, HIS259, THR260, LEU321, LEU324, CYS394, MET433 were predicted from the CASTp [Tian et al., 2009] and were generated with help of AutoGrid (Morris et al., 2009). The Lamarckian genetic algorithm was applied for minimization, using default parameters.

2.2 ADME, drug-likeness and toxicity evaluation

The MOL file and 'SMILES' of Ganomestenol were generated from chemsketch 8.0 and Swiss ADME tool. The compound Ganomestenol were subjected for Swiss ADME analysis for parameters such as molecular weight, LogP, number of hydrogen bond, number of hydrogen bond acceptor, number of hydrogen bond donor, gastro intestinal absorption, P-glycoprotein and Blood brain barrier etc., In addition, provides prediction of inhibitor of CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4 (Daina et al. 2017). To determine the Lipinski's rule five which likely orally active drug for chemical compound by same tool. More than two violations predict a compound as a non-orally available drug (Lipinski et al. 2004).

2.3 ProTox-II and Cytotoxicity Prediction by CLC Pred

The compound toxicity class and LD50 prediction were carried out by using ProTox-II (Drwal et al. 2014; Banerjee et al. 2018). The ProTox-II provides information of organ toxicity and carcinogenicity, immunotoxicity, mutagenicity and cytotoxicity of Ganomestenol. Cell Line Cytotoxicity Predictor (CLC PRED) tool were used for in silico prediction of cytotoxic effects of compound. The tool CLC PRED provides both normal and cancers cell lines based on structural formula (Lagunin, 2018).

2.4 Statistical Analysis

The ezANOVA (version 0.98) software was used to determine the mean and standard error.

3. Results and Discussion

The antimicrobial activity of the Ganomestenol against pathogen microorganisms were analyzed for the presence or absence of inhibition zone. The Ciprofloxacin (antibacterial) and Fluconazole (antifungal) were used as a standard drug for antimicrobial activity. In previous studies, Ganomestenol showed zone of inhibition against *Escherichia coli* (08±0.2), *Pseudomonas solanacearum* (06±0.2) and *Clavibacter michiganensis* (07±0.5). Early studies reported that the Ganomestenol showed the zone of inhbition against *Fusarium oxysporum* (08±0.4), *Curvularia lunata* (06±0.2), *Alternaria solani* (07±0.2) and *Helminthosporum oryzae* (06 ± 0.1) (Naveen Kumar KJ and Venkatesh, 2020).

In-silico analysis depict that molecular docking of Ganomestenol with *E.coli* 24kDa domain (PDB ID: 1ZKN) showed good binding energy -5.6 kcal/mol with two hydrogen bond compared to the Ciprofloxacin (-4.54 kcal/mol) (Table 1 and Figure 2). The docking of Ganomestenol with cytochrome P450 14α-sterol demethylase (PDB ID: 1EA1) showed significant binding energy -6.66 kcal/mol than Fluconazole (-4.1 kcal/mol) (Table 2 and Figure 3).

Table-1: *In-silico molecular docking* of Ganomestenol **with** *E.coli* 24kDa domain (PDB ID: 1ZKN)**.**

Compounds	**Binding energy kcal/mol**	**Ligand efficiency**	**Intermolecular energy kcal/mol**	**Electrostatic energy kJ/mol**	**H-bond**	**H-Bond with**
Ganomestenol	-5.6	-0.17	-7.84	-0.38	02	GLN72, GLU58
Ciprofloxacin	-4.54	-0.19	-5.18	0.18	01	GLN135

Table-2: *In-silico molecular docking* of Ganomestenol with cytochrome P450 14α-sterol demethylase (PDB ID: 1EA1).

Compounds	Binding energy kcal/mol	Ligand efficiency	Intermolecular energy kcal/mol	Electrostatic energy kJ/mol	H-bond	H-Bond with
Ganomestenol	-6.66	-0.2	-9.06	-0.57	01	GLN72
Fluconazole	-4.1	-0.19	-4.94	0.03	01	THR80

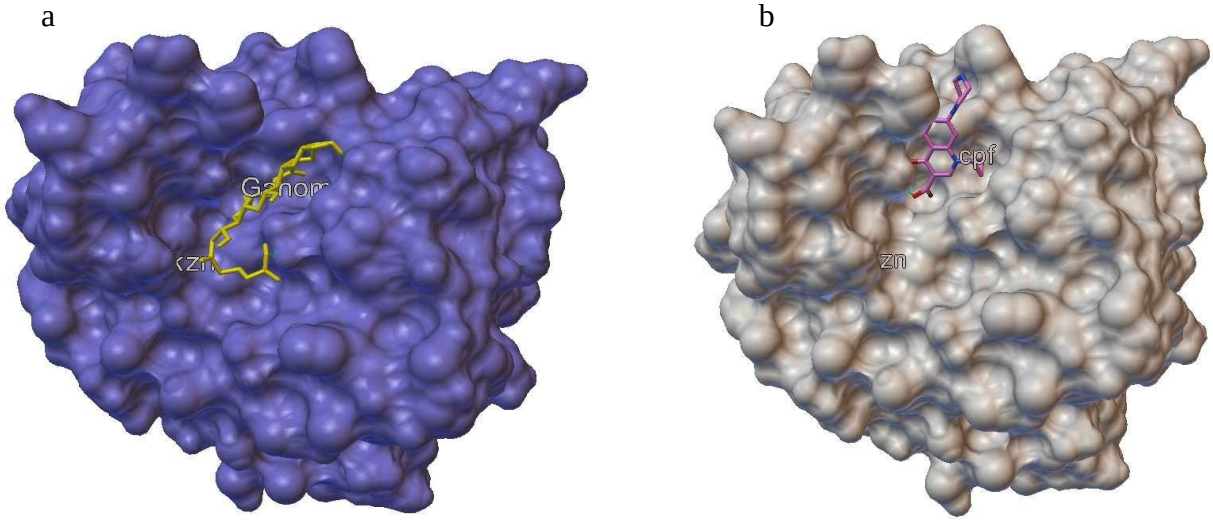

Figure 2: a) Molecular docking of Ganomestenol with E.coli 24kDa domain (PDB ID: 1ZKN) showed in Molecular Surface and b) Molecular docking of Ciprofloxacin with E.coli 24kDa domain (PDB ID: 1ZKN) showed in Molecular Surface.

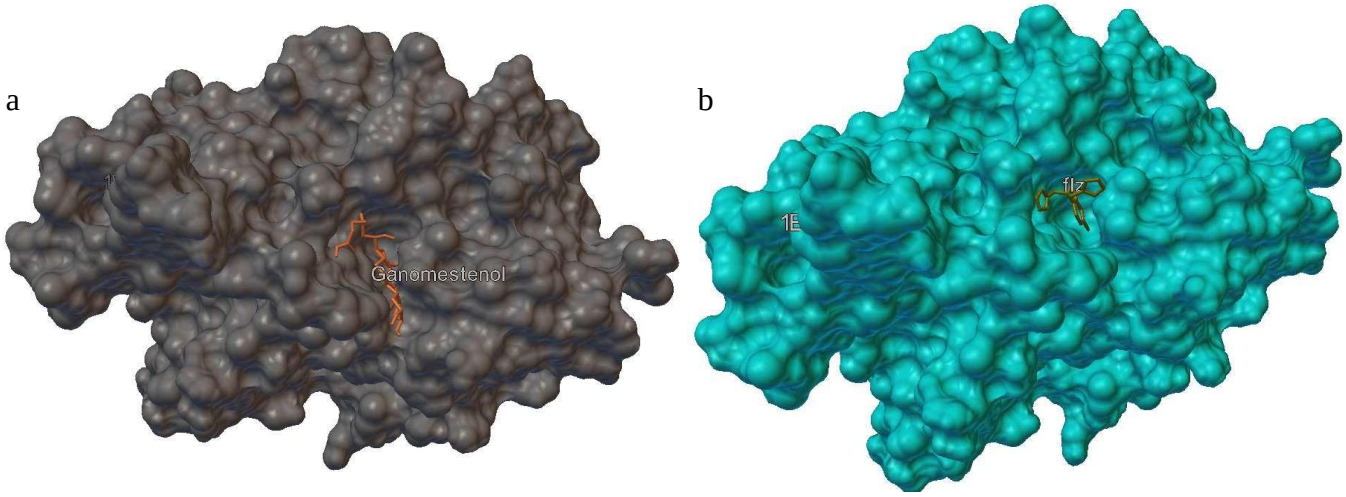

Figure 3: a) Molecular docking of Ganomestenol with cytochrome P450 14α-sterol demethylase (PDB ID: 1EA1) showed in Molecular Surface and b) Molecular docking of Ciprofloxacin with cytochrome P450 14α-sterol demethylase (PDB ID: 1EA1) showed in Molecular Surface.

In-silico pharmacokinetics and cytotoxicity predictions. The results showed that the SwissADME predicts the Ganomestenol have low gastrointestinal (GI) absorption, no blood–brain barrier (BBB) permeation and no substrates of permeability glycoprotein (P-gp). The CYP's interaction result showed that Ganomestenol are CYP3A4 Inhibitors. The

ADME and toxicity was shown in Table 3.

The toxicity prediction results showed that Ganomestenol are class classification 5 (harmful if swallowed). The toxicological prediction studies showed Ganomestenol are inactive in toxicity parameters such as hepatotoxicity, carcinogenicity, immunogenicity, cytotoxicity and mutagenicity. *In silico* cytotoxicity prediction of compound Ganomestenol showed that maximum Pa (Probability "to be active") value with DMS-114 cell line of lung carcinoma. The Pa value of other cell line was presented in table 4.

Table-3: ADME, Drug-likeness and Toxicity predictions of compound Ganomestenol by using SwissADME, Pro-Tox II and OSIRIS Property Explorer (http://www.organic-chemistry.org/prog/peo/).

Parameters		**Ganomestenol**
Formula		C29H46O9
Mol. Wt. (g/mol)		538.67 g/mol
NHD		5
NHA		9
NRB		8
TPSA(A^{o2})		161.59
LogP (cLogP)		1.67
Lipinski's Rule of Five Violation		One rule violated
log Kp cm/s		-9.12
GI Absorption		Low
BBB Permeability		No
Inhibitor Interaction	P-gp Substrate	No
	CYP1A2 Inhibitor	No
	CYP2C19 Inhibitor	No
	CYP2C9 Inhibitor	No
	CYP2D6 Inhibitor	No
	CYP3A4 Inhibitor	Yes
LD 50 (mg/kg)		5000
Toxicity Class		5
Organ Toxicity	Hepatotoxicity	Inactive
	Carcinogenicity	Inactive
	Immunotoxicity	Inactive
	Mutagenicity	Inactive
	Cytotoxicity	Inactive
	Irritant	Yes

***** NHD-number of hydrogen donor; NHA-number of hydrogen acceptor; NRB- number of rotatable bonds; TPSA-total polar surface area, Log Kp-skin permeation value; GI-gastro-intestinal; BBB-blood–brain barrier; P-gp-P-glycoprotein; CYP- cytochrome-P

Table 4: Cytotoxicity prediction of compound Ganomestenol by using CLC Pred.

Effect of Ganomestenol on various Cancer cell lines					
Pa	**Pi**	**Cell-line**	**Cell-line name**	**Tissue/organ**	**Tumor type**
0.355	0.005	SGC-7901	Gastric carcinoma	Stomach	Carcinoma
0.354	0.026	8505C	Thyroid gland undifferentiated (anaplastic) carcinoma	Thyroid	Carcinoma
0.335	0.017	Bel-7402	Hepatoma	Liver	Hepatoma
0.289	0.006	SW1573	Lung carcinoma	Lung	Carcinoma
0.405	0.127	DMS-114	Lung carcinoma	Lung	Carcinoma
0.283	0.063	MKN-7	Gastric carcinoma	Stomach	Carcinoma
0.324	0.168	NCI-H187	Small cell lung carcinoma	Lung	Carcinoma
0.208	0.069	St-4	Stomach carcinoma	Stomach	Carcinoma
0.291	0.167	A2058	Melanoma	Skin	Melanoma
0.185	0.063	A2780	Ovarian carcinoma	Ovarium	Carcinoma
0.233	0.143	SK-MEL-2	Melanoma	Skin	Melanoma
0.101	0.028	1A9	Ovarian adenocarcinoma	Ovarium	Adenocarcinoma
0.204	0.136	HL-60	Promyeloblast leukemia	Haematopoietic and lymphoid tissue	Leukemia
0.198	0.139	M19-MEL	Melanoma	Skin	Melanoma
0.185	0.126	HT-1080	Fibrosarcoma	Soft tissue	Sarcoma
0.115	0.059	SW480	Colon adenocarcinoma	Colon	Adenocarcinoma
0.203	0.151	EKVX	Non-small cell lung carcinoma	Lung	Carcinoma
0.146	0.095	ADR5000	Childhood T acute lymphoblastic leukemia	Blood	Leukemia
0.219	0.192	H9	T-lymphoid	Haematopoietic and lymphoid tissue	Leukemia
0.305	0.278	SK-MEL-1	Metastatic melanoma	Skin	Melanoma
0.214	0.188	UO-31	Renal carcinoma	Kidney	Carcinoma
0.120	0.100	DLD-1	Colon adenocarcinoma	Colon	Adenocarcinoma
0.085	0.074	Lu1	Lung carcinoma	Lung	Carcinoma
0.115	0.106	BGC-823	Stomach adenocarcinoma	Stomach	Adenocarcinoma
0.287	0.285	NCI-H838	Non-small cell lung cancer	Lung	Carcinoma

In CLC-Pred, Pa (Probability of Activity) and Pi (Probability of Inactivity) stand for the probability of a compound being active (cytotoxic) and inactive. The Pa value of 0.5 or higher suggests that a compound will exhibit strong likelihood of cytotoxicity and Pi Pa value of 0.5 or higher suggests low likelihood of cytotoxicity.

4. Conclusion

In-silico studies showed, molecule Ganomestenol have good binding efficiency against targeted proteins and support the *in-vitro* antimicrobial properties. The compound ganomestenol shows less toxicity value and lesser effect. Cytotoxicity prediction shows active in DMS-114 cell line of lung carcinoma. The compound ganomestenol were subjected *in-vitro* cytotoxicity studies further.

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