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ORIGINAL ARTICLE

Ganoderic acid targeting multiple receptors in cancer: in silico and in vitro study

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Abstract Receptor tyrosine kinases (RTKs) are transmembrane high-affinity surface receptors responsible for cell migration, adhesion, apoptosis, metabolism, and cell proliferation activities in various cancers. Minute aberration in the RTK signaling modulates the downstream signaling pathways that results in cancer. Ganoderic acid is a triterpene isolated from *Ganoderma lucidum*, which is renowned for its therapeutics effect, especially in cancer. The present study discusses receptor-based molecular docking of insulin receptor (IR), insulin-like growth factor receptor 1 (IGFR-1), vascular endothelial growth factor receptor-1 (VEGFR-1), vascular endothelial growth factor receptor-2 (VEGFR-2), and estrogen receptor (ER) with 50 isoforms of ganoderic acid along with natural inhibitors. These receptors were assessed for toxicity (ADMET) by using Maestro 9.6 (Schrödinger Inc). The calculated docking free energy yielded an excellent dock score for the ganoderic acid when docked with proteins IR, IGFR-1, VEGFR-1, VEGFR-2, and ER, suggesting its potential in combating cancer. Protein–ligand profile highlighted the binding interactions comprising lipophilic, hydrogen bonding, pi-pi stacking interactions, and noncovalent bonding which play a pivotal role in targeting cancer. In silico studies revealed structure of ganoderic acid A as best isoforms among 50 isoforms which exhibits biological activity in liver cancer cells. Ganoderic acids A significantly decrease the viability, proliferation, and oxidative stress in a dose-dependent manner in liver cancer cells.

Keywords Ganoderic acid · Cancer · Receptor tyrosine kinase · Molecular docking · Oxidative stress

Abbreviations

IR	Insulin receptor
IGFR-1	Insulin-like growth factor receptor 1
VEGFR-1	Vascular endothelial growth factor receptor-1
VEGFR-2	Vascular endothelial growth factor receptor-2
ER	Estrogen receptor
ROS	Reactive oxygen species
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
PBS	Phosphate-buffered saline
DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethyl sulfoxide
FBS	Fetal bovine serum

Introduction

Alteration in cellular genome results from progressive accumulation of mutations in the genes controlling growth and differentiation which results in cancer [1]. Chromosomal instability, epigenetic silencing, and change in the functioning of tumor suppressor genes further advances cancer [2]. Receptor tyrosine kinases (RTKs) are transmembrane high-affinity surface receptor regulating numerous functions of cell migration, adhesion, apoptosis, metabolism, and cell proliferation [3]. Different studies highlighted the role of RTKs in signaling

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network involved in various cancers such as prostate, breast, lung, and ovarian cancers [4, 5]. RTKs have different families comprising insulin receptor (IR), insulin-like growth factor receptor (IGFR), epidermal growth factor receptor (EGFR), vascular endothelial growth factor recepror-1 (VEGFR-1), vascular endothelial growth factor recepror-2 (VEGFR-2), and estrogen receptor (ER) [6]. The multiple molecular mechanisms alter the cellular metabolism by modulating the activity of cell receptors and adapter molecules embedded in the plasma membrane. In normal physiology, cell receptors are embedded in the plasma membrane which are in an inactive form which on receiving signal, get phosphorylated and activated. This in turn activates various enzymes, ion channels, and transporters in the cell when the specific receptor is linked to the specific adapter in a cellular biochemical pathway [7]. In complex diseases such as cancer, numerous gene networks and receptor get interlinked playing a significant role in such diseases [8]. Cross talk among different proteins and their adapter molecules makes it challenging to hold responsible any single pathway, as an aberration in more than one pathway may be causing the debilitating condition [9].

Numerous RTK inhibitors have been designed for active involvement in signal transduction mediated by RTKs [10, 11]. One such renowned natural product is *Ganoderma lucidum*, polypore family of mushrooms with myriad therapeutic indications [12, 13]. Among different bio-constituents in *G. lucidum*, ganoderic acid was explored for revealing mechanism in various cancer pathways [14]. Literature revealed that ganoderic acid DM inhibits prostate cancer cell growth and blocks osteoclastogenesis by inhibiting 5a-reductase and androgen receptor (AR)-binding activity [15]. Similarly, ganoderic acid A inhibits JAK-STAT3 signaling pathway by ganoderic acid A in the HepG2 cells [16]. Furthermore, ganoderic acid Me down-regulates matrix metalloproteinases 2/9 gene expression and inhibits tumor invasion [17]. These studies unable to expose the mechanistic binding of ganoderic acids, therefore, need to explore binding mechanism. In the present study, molecular docking of 50 isoforms of ganoderic acid was performed on different membrane receptors which initiate and modulate the downstream signaling in various diseases. The receptors were IR, IGFR-1, VEGFR-1, VEGFR-2, and ER. After docking, best isoform of ganoderic acid was checked for its biological activity in the liver cancer cell line.

Methodology

Preparation of ligands and protein molecule

The protein-crystallized structure was retrieved from PDB site of IR (PDB; 3ETA), IGFR (PDB; 1K3A), VEGFR-1 (PDB; 3HNG), VEGFR-2 (PDB; 2OH4), and ER (PDB; 3ERT). The processing of proteins includes removal of the water molecule in relation to the domain topology that usually interacts with hydrophobic regions. Furthermore, polar hydrogen was incorporated to complete the inappropriate valency of the protein atoms which increased the polarizability of bonds [14]. The increased polarizability improved the probability of ligand–protein interactions for the better modeling of the structure. Further processing was evaluated for the stereochemical quality by analyzing residue-by-residue geometry as well as overall structural geometry. The next crucial step was the preparation of different isoforms of ganoderic acid and IR, IGFR, VEGFR-1, VEGFR-2, and ER natural inhibitors structures by software ChemBioDraw Office [14] (licensed at Cambridge's soft). Different natural inhibitors of RTK includes such as quercetin, curcumin, withaferin A, betulinic acid, pristimerin, and ursolic acid [18–23]. Later, Ligprep was used for the ligand preparation using Maestro 9.3. Ligprep was corrected by addition and optimization of hydrogen bonds for correcting the valency, removal of bad contacts, creation of disulfide bonds, capping of protein terminals optimization of bond lengths, and fixing of missing residues in order to make protein ready for docking. The prepared structure was then minimized and optimized in the optimized potential for the liquid simulation (OPLS 2005) force field to acquire an energetically stable geometry [24–26].

Receptor grid formation

Grid pre-calculates grid maps of interaction energies for various atom types, such as aliphatic carbons, aromatic carbons, hydrogen bonding oxygen, and so on, with a macromolecule such as a protein, DNA, or RNA prior to docking [14]. These grid maps are then used by GLIDE docking calculations to determine the total interaction energy for a ligand with a macromolecule. Grid mapping, which calculates the crucial coordinates over the protein atomic data, assigned the coordinates of the IR, IGFIR, VEGFR-1, VEGFR-2, and ER for docking. Moreover, grid mapping affords an appropriate surface topology for the ligand atoms for interaction with the IR, IGFIR, VEGFR-1, VEGFR-2, and ER domains. Grid mapping is a pre-requisite to direct different isoforms of ganoderic acids to look for their region of the firm affinity of the IR, IGFR, EGFR, VEGFR-1, VEGFR-2, and ER domains. Grid was generated for the search of favorable interaction and best post during docking which represents conformation, position, and orientation about the receptors. Other parameters such as sites, constraints, rotatable groups, and excluded volume, which are the default setting of the Maestro 9.3 are used [27].

GLIDE molecular docking

Molecular docking procedures were carried out after preparing the ligand, protein, and the grid preparation on the active

site of the protein using Maestro 9.6 (Schrödinger Inc). GLIDE docking software predicts best computational simulation binding orientation to the protein target. GLIDE molecular docking output GScore (empirical scoring function) is a combination of various parameters vital for binding energy [14]. The GScore is calculated by analyzing the ligand–protein interaction energies, root mean square deviation (RMSD), hydrophobic interactions, hydrogen bonds, internal energy, π–π stacking interactions, and desolvation. GLIDE module of the XP visualizer analyzes the specific ligand–protein interactions. Ligands were docked with the X-ray crystal structure of IR (PDB; 3ETA), IGFR (PDB; 1K3A), VEGFR-1 (PDB; 3HNG), VEGFR-2 (PDB; 2OH4), and ER (PDB; 3ERT) using GLIDE. The best possible fit compound results after docking were analyzed for thermodynamic optimal energy value, the potential of bonding and conformations, types of interactions, residues involved during the interaction, and distance among different residues [28, 29].

ADME properties

Qikprop is an important tool that calculates properties of the valid descriptors and pharmaceutically relevant molecules by comparing their values with those of 95 % of already known pharmaceutical drugs. Absorption, distribution, metabolism, excretion, and toxicity (ADME/T) properties of the docked ligand molecules were subjected in QikProp tool. It analyzes and predicts different properties of drug about the use and aftermaths of drug intake, i.e., absorption, distribution, metabolism, and excretion. It gives the information about $QPlogP_{o/w}$, QPlogBB, overall CNS activity, Caco2, MDCK cell permeability, logKhsa for human serum albumin binding, and the percentage of human oral absorption [30].

Materials and methods

Ganoderic acid A (≥98 %) was purchased from Sigma-Aldrich and dissolved in dimethyl sulfoxide (DMSO) at a concentration of 50 mM, stored at −20 °C. Hepatocellular carcinoma cell line (Hep G2) used in the study was procured from the National Centre for Cell Sciences, Pune, India. The cell line was carefully maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % heat-inactivated fetal bovine serum (FBS), 1 % penicillin (units/mL), streptomycin (100 mg/mL). Cells were maintained at 37 °C in a humidified atmosphere with 5 % CO_2.

MTT cell proliferation assay

Effect of different treatments of ganoderic acid A on the growth of hepatocellular carcinoma cells (Hep G2) was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay in triplicates. Approximately, 10,000 cells were seeded in the 96-well plate in media containing 10 % FBS and incubated overnight at 37 °C followed by serum starvation for 24 h. Cells were exposed to the different concentrations of ganoderic acid A (5, 10, 20, 50, 80 μM/mL) in serum-free media which was incubated for 48 h. At the end of 48 h, the medium was removed and replaced with 100 μL of MTT (0.5 mg/mL in phosphate-buffered saline (PBS)) in 10 % FBS-containing media and incubated at 37 °C for 4 h in the dark. The supernatant was removed from the wells and the reduced MTT, formazan complex, was solubilized in 200 μL/well DMSO. Absorbance was measured at 570 nm using microplate reader.

Evaluation of cytotoxic/apoptotic effect

In another set of experiment, the rate of cell death in response to ganoderic acid A with treatment was assessed by the trypan blue exclusion test; 2×10^5 Hep G2 cells were seeded in six-well culture plates. The adhered cells were treated with ganoderic acid A (5, 10, 20, 50, 80 μM/mL) for 48 h. After treatment, both floating cells in the medium and adhered cells on the plate were collected and concentrated by centrifugation. Cell viability was estimated by staining with 0.4 % trypan blue for 15 min. Both live (unstained) and dead (stained) cells were counted in three replicates using Automated cell counter (Invitrogen). Percent data of dead cells was calculated and used as an indicator of the degree of cell death.

Effect of ganoderic acid A on ROS levels (H_2DCF-DA assay)

Intracellular reactive oxygen species (ROS) level was measured with 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCF-DA), which undergoes rapid oxidation into the highly fluorescent 2′,7′-dichlorofluorescein (DCF) in the presence of intracellular ROS. Hep G2 cells (2×10^5) were seeded in six-well culture plates and treated with ganoderic acid A (5, 10, 20, 50, 80 μM/mL) for 24 h. Cells were washed with PBS and incubated in PBS containing 10 μM H_2DCFDA for 30 min at 37 °C. The cells were washed with PBS to remove excess dye, and the fluorescence was measured at 485 and 530 nm wavelengths.

Nitroblue tetrazolium reduction assay

Hep G2 cells at 8×10^3 were seeded into the 96-well plates in DMEM containing 10 % FBS and 1 % penicillin/streptomycin and then incubated at 37 °C overnight followed by serum starvation for 24 h. After 24 h, the media were changed with fresh complete medium (200 μL) and exposed to different ganoderic acid A 5, 10, 20, 40, and 80 μM concentrations.

After 48 h, the medium were removed and incubated with 100 μL 0.1 % nitroblue tetrazolium reduction assay (NBT), incubated for 4 h. The reduced NBT was solubilized with 100 μL 2 M KOH and 100 μL DMSO for 30 min, and absorbance was measured at 570 nm using an ELISA plate reader.

Statistical analysis

Results were expressed as the mean ± standard deviation of experiments performed in triplicates. Data obtained was subjected to one-way analysis of variance (ANOVA), and significant differences of the mean were determined statistically using Tukey's test using SigmaPlot 11.

Results and discussion

IR (3EKK)

IGFR

VEGFR-1

VEGFR-1 (3HNG)

VEGFR-2 (1Y6A)

Estrogen receptor (3ERT)

In silico studies

The multicellular organism requires a complex system to communicate a variety of physiological functions. The normal functioning of cell is regulated by the switching on or off of the membrane receptor which on external stimulus gets phosphorylated and activates downstream signaling. Among different receptors forming an integral part of the plasma membrane, RTKs play a prominent role in the transmission of signals. Among RTKs, different families such as VEGFR-1, VEGFR-2, IR, ER, and IGFR perform different function crucial to the normal functioning of the cell. The current studies convey new perception into the biological and molecular mechanisms of VEGFR-1, VEGFR-2, IR, ER, and IGFR receptors with different isoforms of ganoderic acid involved in various abnormalities. Ganoderic acid DM inhibits prostate cancer cell growth and blocks osteoclastogenesis by inhibiting 5a-reductase inhibitory and AR-binding activity [15]. Similarly, ganoderic acid A inhibits JAK-STAT3 signaling pathway by ganoderic acid A in the HepG2 cells [16]. Furthermore, ganoderic acid Me down-regulates matrix metalloproteinases 2/9 gene expression and inhibits tumor invasion [17].

The receptor-based molecular docking was carried for these above receptors with 50 isoforms of ganoderic acid with their natural inhibitors. Molecular docking identifies the maximum energy, protein–ligand interactions involved, orientations, and conformations best suited for drug preparation. Molecular docking study predicts the preferred orientation to form a stable complex and finds the strength of association of binding affinity by different docking parameters.

Ganoderic acid (GAs) with 50 isoforms and triterpenes of *G. lucidum* were docked with X-ray crystal structure-retrieved IR (PDB, 3EKK), IGFR (PDB, IK3A), VEGFR-1 (PDB, 3HNG), VEGFR-2 (PDB, 1Y6A), estrogen receptor (3ERT) from Protein Data Bank (Tables 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10). In triterpene of *G. lucidum*, the ganoderic acid has lanosterol scaffold and variation in its isoforms varies from the functional group or in the side chain. The structure of receptor complexed with ganoderic acid highlighted the lipophilic, electrostatic, and hydrogen bonding interaction and considered to be the main contributor in protein–ligand interaction. In molecular docking process of IR, ganoderic acid alpha exhibits best docking parameters with GScore (−10.66), Lipophilic Evdw (−3.12), GLIDE emodel (32.12), H bond (−2.03), and hydrogen bonding forming the significant interaction which forms the stability of the complex. Different residues involved in hydrogen bonding were Arg 1136, Asn 1132, and Asn 1137 with 1.88, 3.83, and 3.62 Å bond lengths, respectively. Other residues forming the covalent and noncovalent bindings are shown in Fig. 1. Analysis of molecular docking showed that –COOH groups present in ganoderic acid were indulged in interaction with IR with polar amino acids. Among different natural inhibitors, quercetin exhibits best-docked docking parameters with Lipophilic Evdw (−2.45), GLIDE emodel

Table 1 Binding affinities, scores, and interaction of residues of IR with different isoforms of ganoderic acid along with natural inhibitors

S. no.	Ligand type	GScore (kcal/mol)	Lipophilic EvdW	GLIDE emodel	H bond	H bond length (Å)	Protein–ligand interaction
1	Ganoderic acid alpha	−10.66	−3.12	32.127	−2.03	1.88, 3.83, 3.62	Arg 1136, Asn 1132, Asn 1137
2	Quercetin	−9.36	−2.45	−56.042	−0.51	0.76, 0.63	Lys 1030, Glu 1077
3	Curcumin	−8.601	−2.24	−65.352	−2.32	2.63	Gln 1004
4	Ganoderic acid DF	−7.879	−3.85	31.103	−1.34	1.78, 1.83, 2.98	Asn 1132, Asn 1137, Arg 1136
5	Ganolucidic acid A	−7.663	−3.17	40.058	−2.04	1.53, 2.86	Asn 1137, Arg 1136
6	Ganoderiol A	−7.486	−2.74	29.236	−1.82	2.53	Asn 1137
7	Ganoderic acid J	−7.450	−3.11	44.687	−2.71	3.62, 2.83, 4.02	Asn 1132, Asn 1137, Arg 1136
8	Ganoderic acid C1	−7.351	−0.12	39.418	−1.67	3.62, 3.84, 0.79	Asn 1132, Asn 1137, Arg 1136
9	Ganoderic acid F	−7.027	−1.38	−23.408	−2.83	3.62, 1.73	Asn 1137, Arg 1136
10	Ganoderic acid beta	−6.958	−1.86	42.973	−1.11	1.56, 3.62, 2.61	Asn 1132, Asn 1137, Arg 1136
11	Ganolucidic acid B	−6.813	−3.77	47.821	−1.29	3.69, 2.83, 2.83	Asn 1132, Asn 1137, Arg 1136
12	Ganoderic acid Lm2	−6.699	−3.24	−24.577	−1.94	3.73, 3.62, 3.83	Asn 1132, Asn 1137, Arg 1136
13	Ganoderic acid theta	−6.568	−2.59	−22.106	−2.45	1.74, 3.28, 2.59	Asn 1132, Asn 1137, Arg 1136
14	Ganoderic acid T-Q	−6.488	−3.18	12.456	−0.71	3.33, 2.72, 2.93	Asn 1132, Asn 1137, Arg 1136
15	Ganoderic acid Me	−6.448	−2.67	−25.937	−2.14	2.83, 3.82, 0.45	Asn 1137, Arg 1136, Lys 1085
16	Ganoderic acid Mk	−6.406	−1.89	−18.853	−0.99	1.73, 2.83, 2.93	Asn 1132, Asn 1137, Arg 1136
17	Ganoderic acid TR	−6.315	−2.18	40.306	−1.64	3.64, 3.84, 3.76	Asn 1132, Asn 1137, Arg 1136
18	Ganoderic acid Sz	−6.236	−3.95	45.563	−1.81	2.11, 1.87, 1.98	Asn 1132, Asn 1137, Arg 1136
19	Ganodermatriol	−6.100	−2.89	35.959	−2.56	1.87	Asn 1137
20	Ganoderiol F	−6.050	−2.7	36.489	−0.64	2.85, 3.84, 3.65	Asn 1132, Asn 1137, Arg 1136
21	Ganodermanontriol	−6.005	−3.64	35.600	−2.02	2.53	Arg 1136
22	Ganoderic acid Am1	−5.997	−3.84	35.589	−1.79	3.75, 3.65, 3.81	Asn 1132, Asn 1137, Arg 1136
23	Withaferin A	−5.357	−1.98	55.183	−1.54	3.65	Asn 1137
24	Ganoderiol B	−5.258	−3.62	34.389	−2.26	3.65, 3.76	Asn 1137, Arg 1136
25	Ganoderol B	−5.041	−2.93	20.287	−1.22	3.85	Asn 1137
26	Ganoderic acid C2	−4.909	−3.81	44.915	−0.77	2.11	Asp 1083
27	Ganoderic acid A	−4.500	−3.01	43.492	−0.73	1.39	Asp 1083
28	Lucialdehyde B	−4.110	−2.8	48.167	−2.16	3.84, 2.93	Asn 1132, Arg 1136
29	Ganoderic acid T	−3.958	−2.55	44.001	−1.59	3.93, 3.03	Lys 1030, Met 1079
30	Ganoderic acid Y	−3.920	−2.77	25.225	−1.83	2.79	Asp 1083
31	Ganolucidic acid E	−3.917	−1.98	51.472	−1.58	2.44, 2.01	Asp 1083, Arg 1036
32	Ganoderic acid R	−3.816	−2.81	9.226	−1.37	3.99	Met 1079
33	Ganodermic acid S	−3.816	−2.81	9.226	−0.62	1.06	Met 1079
34	Ganosporeric acid A	−3.745	−2.89	−16.532	−1.73	2.22, 3.95, 3.86	Asn 1132, Asn 1137, Arg 1136
35	Ganoderic acid D	−3.743	−3.4	−30.775	−0.63	2.92	Lys 1085
36	Ganoderic acid B	−3.74	−2.65	−34.87	−1.63	3.56, 2.74	Lys 1030, Met 1079
37	Ganoderic acid F	−3.691	−2.67	−38.87	−1.44	2.19	Arg 1036
38	Ganoderic acid TR1	−3.686	−3.05	37.620	−0.57	1.78	Met 1079
39	Ganoderic acid B methyl ester	−3.607	−1.63	−32.723	−0.62	1.98	Met 1079
40	Lucialdehyde C	−3.144	−2.86	23.995	−1.02	2.21	Asn 1132
41	Ganoderic acid E	−2.874	−0.81	43.895	−1.29	3.54	Arg 1136
42	Methyl ganoderate D	−2.741	−3.83	−31.523	−0.83	3.56	Arg 1136
43	Ganoderic acid DM	−2.157	−3.12	54.989	−1.69	3.73, 2.63, 3.43	Asn 1132, Asn 1137, Arg 1136
44	Ganolucidic acid E	−2.012	−3.12	32.43	−0.68	3.32	Asn 1132
46	Ganoderic acid X	−1.911	−2.11	−44.94	−1.17	2.85, 2.98	Asn 1132, Arg 1136
47	Ganoderic acid TR1	−1.901	−2.95	−38.38	−1.72	2.98	Arg 1136
48	Betulinic acid	−1.868	−1.84	−22.85	−1.63	2.98	Arg 1136
49	Pristimerin	−1.859	−2.74	10.11	−1.66	3.43	Asn 1132
50	Ganoderic acid K	−1.644	−2.76	−55.98	−1.09	2.98	Arg 1136
51	Lucidenic acid P	−1.532	−2.85	10.11	−0.94	3.43	Asn 1132
52	Ganoderic acid C6	−1.522	−2.78	−28.73	−0.73	2.98	Arg 1136
53	Ganoderic acid H	−1.517	−1.98	23.95	−0.49	3.43	Asn 1132
54	Lucialdehyde B	−1.387	−2.31	10.78	−1.06	2.46	Arg 1136
55	Lucidenic acid A	−1.15	−2.52	22.57	−0.65	1.96	Arg 1136
56	Ursolic acid	−1.06	−1.66	18.66	−0.52	3.49	Arg 1136
57	Tirucallol	−0.95	−0.68	10.43	−1.28	2.58	Arg 1136

Table 2 Evaluation of drug-like properties of IR with different isoforms of ganoderic acid along with natural inhibitors by Qikprop

Molecule	MW	Dipole	QPlogPo/w (−2.0 to 6.5)	QPlogHERG (acceptable range, above −5.0)	QPP Caco (nm/s; 25—poor; 500—great)	QPlogBB (−3 to 1.2)	QPP MDCK (nm/s)	QPlogKp (−8.0 to −0.1)
Ganoderic acid alpha	574.71	5.979	2.604	−2.689	6.365	−2.714	2.661	−5.7
Curcumin	368.385	6.541	2.434	−4.85	118.734	−2.002	49.44	−3.369
Quercetin	304.256	7.315	0.163	−4.78	24.361	−2.22	8.924	−5.389
Ganoderic acid DF	516.673	7.96	2.832	−2.703	11.931	−2.372	5.247	−5.266
Ganolucidic acid A	500.674	4.293	3.574	−2.583	15.452	−2.151	6.939	−5.143
Ganoderic acid alpha	574.71	9.699	2.706	−2.816	6.325	−2.771	2.643	−5.705
Ganoderiol A	474.723	2.959	4.854	−4.341	555.461	−1.3	262.03	−2.885
Ganoderic acid J	514.658	1.777	2.722	−2.537	10.431	−2.311	4.537	−5.475
Ganoderic acid C1	514.658	5.424	2.775	−2.623	12.031	−2.278	5.295	−5.355
Ganoderic acid F	570.678	6.21	2.48	−2.306	17.558	−2.003	7.966	−5.036
Ganoderic acid beta	484.675	6.907	4.551	−2.717	31.499	−1.8	14.984	−4.614
Ganolucidic acid B	502.69	6.586	3.661	−2.579	18.507	−2.128	8.433	−4.895
Ganoderic acid Lm2	514.658	5.637	2.996	−2.503	22.26	−1.949	10.296	−4.805
Ganoderic acid theta	530.657	3.893	1.931	−2.521	7.863	−2.481	3.343	−5.597
Ganoderic acid T-Q	510.712	7.422	6.187	−2.903	50.764	−1.598	25.099	−4.117
Ganoderic acid Me	554.765	6.545	6.509	−2.053	74.462	−1.307	37.974	−3.756
Ganoderic acid Mk	556.738	5.681	5.855	−2.917	41.635	−1.816	20.258	−4.097
Ganoderic acid TR	468.675	5.57	5.525	−2.565	72.537	−1.347	36.914	−3.8
Ganoderic acid Sz	452.676	6.302	6.421	−2.485	115.123	−1.068	60.816	−3.502
Ganodermatriol	456.707	5.565	5.159	−4.355	643.84	−1.171	307.37	−2.828
Ganoderiol F	454.692	5.02	5.388	−4.642	540.743	−1.244	254.534	−3.078
Ganodermanontriol	472.707	4.741	4.761	−4.108	409.6	−1.327	188.52	−3.242
Ganoderic acid Am1	514.658	4.787	2.723	−2.515	10.744	−2.29	4.685	−5.45
Ganoderic acid Me	554.765	5.028	7.003	−2.403	130.587	−1.133	69.691	−3.26
Ganodermatriol	456.707	5.765	5.163	−4.431	581.648	−1.23	275.407	−2.918
Withaferin A	470.605	4.744	2.894	−4.473	232.281	−1.356	102.114	−3.928
Ganoderic acid DF	516.673	8.239	2.724	−2.084	14.273	−2.082	6.369	−5.114
Ganoderiol B	470.691	3.462	4.249	−4.559	220.439	−1.697	96.499	−3.755
Ganoderol B	440.708	4.125	6.375	−4.465	1534.173	−0.685	785.702	−2.285
Ganoderic acid C2	518.689	3.542	2.841	−2.478	14.355	−2.269	6.408	−5.014
Ganoderic acid A	516.673	3.013	2.741	−2.132	16.616	−2.033	7.505	−4.986
Lucialdehyde B	452.676	1.17	5.039	−4.408	452.709	−1.124	210.054	−3.617
Ganoderic acid T	612.802	4.34	6.526	−3.166	20.574	−2.245	9.455	−4.726
Ganoderic acid	454.692	4.969	6.31	−2.605	121.642	−1.13	64.546	−3.366
Ganolucidic acid E	484.675	3.963	4.497	−2.563	30.435	−1.765	14.437	−4.632
Ganoderic acid R	554.765	5.414	6.834	−3.254	32.261	−1.981	15.376	−4.41
Ganodermic acid S	554.765	5.414	6.834	−3.254	32.261	−1.981	15.376	−4.41
Ganosporeric acid A	526.625	7.798	1.691	−1.896	17.202	−1.828	7.792	−5.149
Ganoderic acid D	514.658	7.271	2.987	−2.518	26.1	−1.895	12.228	−4.701
Ganoderic acid TR1	468.675	3.28	5.317	−2.213	70.726	−1.286	35.919	−3.842
Ganoderic acid Y	454.692	2.714	6.377	−2.626	127.102	−1.115	67.683	−3.329
Ganoderic acid Sz	452.676	2.475	6.339	−2.429	115.722	−1.055	61.158	−3.497
Lucialdehyde C	468.718	1.104	5.652	−4.548	532.649	−1.184	250.418	−3.23
Ganoderic acid E	512.642	5.211	2.631	−2.35	13.746	−2.063	6.114	−5.338
Methyl ganoderate D	528.684	6.868	3.253	−4.773	218.703	−1.679	95.678	−4.066
GA-B methyl ester	528.684	6.508	3.065	−4.568	126.592	−1.927	52.985	−4.411
Ganoderic acid DM	468.675	6.078	5.473	−2.475	76.145	−1.278	38.902	−3.962
Ganolucidic acid E	484.675	6.258	4.52	−2.875	65.111	−1.685	26.65	−4.72
Ganoderic acid X	512.728	3.773	6.19	−2.67	56.843	−1.45	48.431	−4.34
Ganoderic acid TR1	468.675	3.78	5.63	−2.54	55.54	−1.63	42.69	−4.363
Betulinic acid	456.707	1.63	5.18	−2.74	159.8	−1.76	208.226	−3.52
Pristimerin	464.644	9.048	5.53	−4.309	102.53	−1.42	94.82	−4.44
Ganoderic acid K	574.71	5.159	3.39	−2.72	37.912	−1.28	23.21	−4.374
Lucidenic acid P	518.646	7.538	4.632	−2.06	23.792	−2.32	17.51	−4.66
Ganoderic acid C6	530.657	3.821	2.52	−3.43	25.74	−1.57	12.46	−3.73
Ganoderic acid H	572.694	7.663	3.36	−2.08	11.53	−1.32	13.98	−4.882
Lucialdehyde B	452.676	1.747	3.73	−2.73	161.1	−2.184	86.92	−3.89
Lucidenic acid A	458.594	2.395	4.74	−3.28	42.55	−2.63	28.19	−3.73
Ursolic acid	456.707	3.403	5.62	−2.74	311.8	−1.04	77.74	−3.73
Tirucallol	426.724	1.816	6.93	−4.85	1122.7	−0.082	999.92	−1.91

Table 3 Binding affinities, scores, and energies of IGFR with different isoforms of ganoderic acid along with natural inhibitors

S. no.	Ligand type	GScore (kcal/mol)	Lipophilic EvdW	GLIDE emodel	H bond	H bond length (Å)	Protein–ligand interaction
1	Ganoderic acid A	−9.672	−3.12	−73.686	−1.83	1.84, 2.17	Lys 1003, Leu 975
2	Quercetin	−8.724	−2.59	−62.315	−2.45	2.23, 2.63, 2.83	Lys 1003, Gly 1122, Leu 975
3	Curcumin	−6.406	−3.26	−45.651	−1.32	1.99, 2.01	Gly 1122, Ser 979
4	Withaferin A	−6.334	−2.04	−58.215	−1.38	3.1, 1.03	Met 1052, Glu 7
5	Ganodermanontriol	−6.230	−2.43	−58.137	−1.84	3.83, 3.11	Gln 799, Met 1052
6	Lucidenic acid C	−6.073	−1.67	−59.906	−2.1	2.93, 2.11, 0.88	Met 1052, Glu 7, Glu 1050
7	Ganoderic acid B methyl ester	−5.979	−1.57	−51.587	−0.742	0.91, 3.66	Met 1052, Gln 799
8	Methyl ganoderate D	−5.827	−2.52	−45.959	−1.65	2.82	Met 1052
9	Ganoderic acid D	−5.775	−3.05	−55.404	−1.63	2.89, 2.99	Glu 7, Lys 1003
10	Ganoderic acid G	−5.761	−2.37	−56.235	−1.83	2.18	Lys 1003
11	Ganoderic acid B	−5.671	−2.92	−40.081	−0.84	3.21	Ser 979
12	Ganoderic acid alpha	−5.466	−3.44	−47.548	−2.52	3.21	Ser 979
13	Ganoderic acid K	−5.427	−2.92	−51.842	−2.94	3.77	Ser 979
14	Lucidenic acid P	−5.425	−3.47	−56.234	−0.73	3.82, 3.04	Ser 979, Met 1052
15	Ganoderic acid theta	−5.410	−1.85	−48.914	−1.74	2.45	Met 1052
16	Ganoderic acid C6	−5.307	−1.92	−54.163	−1.45	3.63	Ser 979
17	Ganosporeric acid A	−5.275	−3.92	−48.537	−0.84	2.59	Lys 1003
18	Ganoderic acid C2	−5.185	−2.62	−49.896	−1.53	2.67, 3.13	Glu 7, Met 1052
19	Ganolucidic acid B	−5.177	−2.22	−41.901	−1.74	3.19	Lys 1003
20	Ganoderic acid H	−5.126	−2.33	−40.023	−1.94	3.98, 3.92	Lys 1058, Ser 979
21	Ganoderiol F	−5.090	−3.63	−51.399	−1.78	4.03	Lys 1003
22	Ganodermatriol	−5.022	−1.24	−50.193	−1.49	3.67, 3.33	Glu 7, Met 1052
23	Ganoderiol A	−5.011	−3.73	−43.838	−2.55	3.82	Met 1052
24	Ganoderiol B	−4.970	−2.47	−49.307	−1.99	3.83	Met 1052
25	Ganoderic acid Me	−4.952	−3.47	−36.219	−1.22	2.93	Lys 1003
25	Lucidenic acid A	−4.894	−3.42	−46.342	−0.67	2.93	Lys 1003
26	Ganoderic acid C1	−4.871	−2.66	−44.738	−0.93	3.49, 2.87, 3.92	Gly 1122, Glu 1050, Glu 7
27	Ganoderic acid Mk	−4.805	−3.45	−48.780	−1.11	2.93	Lys 1003
28	Ganoderic acid DF	−4.797	−2.92	−43.051	−1.02	2.93	Lys 1003
29	Ganoderic acid Am1	−4.760	−2.46	−49.529	−1.83	2.93	Lys 1003
30	Lucialdehyde C	−4.749	−2.55	−45.118	−0.18	3.66	Met 1052
31	Ganoderic acid T	−4.715	−2.83	−34.509	−0.73	3.72	Ser 979
32	Ganoderic acid R	−4.663	−3.33	−36.427	−1.71	3.12	Lys 1003
33	Ganodermic acid S	−4.579	−2.84	−46.918	−2.34	3.13, 3.02	Lys 1003, Glu 7
34	`Ganoderic acid F	−4.523	−3.42	−44.981	−1.93	3.74, 3.72	Met 1052, Leu 975
35	Ursolic acid	−4.416	−2.67	−37.127	−0.72	3.03, 1.93	Met 1052, Gln 977
36	Ganoderic acid beta	−4.403	−2.54	−29.530	−1.38	2.12, 2.56	Glu 1050, Glu 7
37	Tirucallol	−4.371	−2.37	−40.204	−1.49	3.83	Lys 1003
38	Lucialdehyde B	−4.354	−2.55	−36.713	−0.56	3.74	Met 1052
39	Ganoderic acid T-Q	−4.265	−2.03	−45.790	−2.72	3.76	Lys 1003
40	Ganolucidic acid A	−4.173	−2.73	−32.978	−2.06	3.78	Lys 1003
41	Ganoderic acid S	−4.080	−1.47	−45.398	−1.94	3.76	Lys 1003
42	Ganoderic acid Sz	−4.053	−3.47	−36.521	−1.26	3.86	Lys 1003
43	Ganoderic acid J	−3.960	−3.88	−11.512	−0.67	1.84	Lys 1003
44	Ganoderic acid X	−3.951	−2.61	−40.997	−0.93	4.09,2.61	Gly 1122, Glu 1050
45	Ganoderic acid TR1	−3.907	−3.56	−39.257	−0.99	3.76	Met 1052
46	Ganoderic acid Y	−3.866	−2.66	−42.910	−1.86	3.99	Lys 1003
47	Ganolucidic acid E	−3.755	−2.04	−35.149	−1.83	2.18	Lys 1003
48	Ganoderol B	−3.725	−2.09	−38.145	−1.73	2.56, 3.12	Lys 1003, Gly 1122
49	Ganoderic acid DF	−3.625	−1.23	−33.427	−0.48	2.82	Met 1052
50	Ganoderol B	−3.432	−2.76	−28.488	−0.47	3.93	Met 1052
51	Betulinic acid	−3.360	−2.77	−29.463	−0.33	3.87	Met 1052
52	Pristimerin	−3.158	−3.32	−32.549	−0.97	3.72	Lys 1003
53	Ganoderic acid Lm2	−3.157	−3.93	−34.302	−2.87	3.72	Lys 1003
54	Ganoderic acid E	−3.075	−2.93	−34.757	−1.64	2.98, 2.88	Lys 1003, Lys 1058
55	Celastrol	−2.808	−2.88	−20.603	−1.22	2.19	Arg 1109
56	Ganoderic acid TR	−1.957	−2.84	−17.039	−1.01	3.87	Met 1052

Table 4 Evaluation of drug-like properties of the different isoforms of ganoderic acid and natural inhibitors of IGFR by Qikprop

Molecule	MW	Dipole	QPlogPo/w (−2.0 to 6.5)	QPlogHERG (acceptable range, above −5.0)	QPP Caco (nm/s; 25—poor; 500—great)	QPlogBB (−3 to 1.2)	QPP MDCK (nm/s)	QPlogKp (−8.0 to −0.1)
Ganoderic acid A	516.673	3.269	4.42	−4.78	66.52	−2.651	33.73	−4.521
Quercetin	304.256	5.433	0.131	−4.612	25.453	−2.155	9.357	−5.377
Curcumin	368.385	5.908	2.967	−6.41	244.079	−2.021	107.732	−2.526
Withaferin A	470.605	7.92	2.928	−4.413	233.922	−1.337	102.894	−3.916
Ganodermanontriol	472.707	1.802	4.74	−4.07	434.406	−1.301	200.89	−3.217
Lucidenic acid C	476.609	5.169	1.895	−1.641	17.314	−1.813	7.847	−5.047
Ganoderic acid B methyl ester	528.684	1.658	3.184	−4.516	193.892	−1.712	84.001	−4.039
Methyl ganoderate D	528.684	4.893	2.625	−4.283	62.068	−2.107	24.524	−5.129
Ganoderic acid D	514.658	5.974	2.53	−2.307	8.222	−2.331	3.509	−5.676
Ganoderic acid G	532.673	2.956	1.988	−2.396	10.458	−2.379	4.55	−5.28
Ganoderic acid B	516.673	5.931	2.855	−2.432	14.621	−2.184	6.537	−5.094
Ganoderic acid alpha	574.71	3.301	2.712	−2.185	23.666	−1.959	11	−4.592
Ganoderic acid K	574.71	5.159	2.555	−2.565	7.232	−2.606	3.054	−5.592
Lucidenic acid P	518.646	7.538	2.594	−2.171	15.706	−2.009	7.063	−5.13
Ganoderic acid theta	530.657	9.125	1.701	−2.321	6.951	−2.461	2.926	−5.705
Ganoderic acid C6	530.657	3.821	1.671	−2.213	5.867	−2.502	2.436	−5.865
Ganosporeric acid A	526.625	6.595	1.412	−2.323	5.21	−2.475	2.142	−6.157
Methyl ganoderate D	528.684	3.234	2.321	−4.023	64.61	−2.005	25.611	−5.095
Ganoderic acid C2	518.689	11.055	2.89	−2.185	27.256	−1.899	12.815	−4.472
Ganolucidic acid B	502.69	6.236	3.737	−2.137	44.106	−1.623	21.56	−4.162
Ganoderic acid H	572.694	7.663	2.539	−2.755	7.627	−2.598	3.235	−5.643
Ganoderiol F	454.692	6.476	5.475	−4.655	642.062	−1.167	306.453	−2.929
Ganodermatriol	456.707	3.447	4.829	−3.799	897.892	−0.948	440.343	−2.564
Ganoderiol A	474.723	6.615	4.749	−4.321	438.736	−1.4	203.055	−3.085
Ganoderiol B	470.691	7.28	4.499	−4.304	471.884	−1.297	219.687	−3.093
Ganoderic acid Me	554.765	8.04	7.032	−2.574	149.555	−1.105	80.695	−3.141
Lucidenic acid A	458.594	2.395	2.774	−2.263	22.02	−1.772	10.176	−5.037
Ganoderic acid C1	514.658	9.563	2.887	−2.645	15.897	−2.159	7.155	−5.12
Ganoderic acid Mk	556.738	3.773	5.718	−2.374	34.664	−1.754	16.617	−4.274
Ganoderic acid DF	516.673	6.531	2.928	−2.26	21.532	−1.961	9.932	−4.767
Ganoderic acid Am1	514.658	3.824	2.436	−2.23	9.092	−2.261	3.911	−5.591
Lucialdehyde C	468.718	3.57	5.773	−4.396	588.124	−1.12	278.723	−3.189
Ganoderic acid T	612.802	5.481	6.831	−3.661	12.112	−2.692	5.333	−5.139
Ganoderic acid R	554.765	6.121	6.235	−2.466	34.058	−1.741	16.304	−4.423
Ganodermic acid S	554.765	6.121	6.235	−2.466	34.058	−1.741	16.304	−4.423
Ganoderic acid F	570.678	7.593	2.552	−2.569	10.124	−2.336	4.394	−5.5
Ursolic acid	456.707	3.403	6.062	−1.572	277.45	−0.406	157.375	−3.135
Ganoderic acid beta	484.675	4.954	4.589	−2.702	32.198	−1.777	15.344	−4.575
Tirucallol	426.724	1.816	7.466	−4.178	4458.807	−0.084	2489.307	−1.679
Lucialdehyde B	452.676	1.747	4.921	−4.069	464.605	−1.043	216.026	−3.588
Ganolucidic acid B	502.69	6.963	3.99	−1.844	96.176	−1.237	50.073	−3.504
Ganoderic acid T-Q	510.712	8.237	6.139	−2.639	51.963	−1.521	25.74	−4.108
Ganolucidic acid A	500.674	8.342	3.315	−2.068	17.635	−1.928	8.004	−5.032
Ganoderiol A	474.723	3.39	4.813	−4.315	513.686	−1.33	240.795	−2.955
Ganoderic acid S	452.676	3.932	6.574	−2.613	160.658	−0.948	87.189	−3.224
Ganoderic acid A	516.673	7.548	2.823	−2.434	18.64	−2.078	8.499	−4.889
Ganoderic acid Sz	452.676	3.253	6.495	−2.709	117.84	−1.105	62.368	−3.491
Ganoderic acid J	514.658	4.059	2.976	−2.494	26.148	−1.887	12.252	−4.7
Ganoderic acid X	512.728	3.773	6.19	−2.952	56.321	−1.617	28.081	−3.918
Ganoderic acid TR1	468.675	3.78	5.32	−2.169	128.453	−1.031	68.461	−3.347
Ganoderic acid Y	454.692	4.885	6.465	−2.625	133.613	−1.095	71.439	−3.296
Ganoderiol A	474.723	6.817	4.735	−4.05	534.444	−1.264	251.33	−2.919
Ganolucidic acid E	484.675	6.258	4.472	−2.508	35.111	−1.685	16.85	−4.509
Ganoderol B	440.708	4.486	6.019	−4.17	1657.168	−0.617	854.003	−2.219
Ganoderic acid DF	516.673	5.548	2.906	−2.427	20.618	−2.031	9.477	−4.804
Ganoderic acid D	514.658	4.441	2.737	−1.861	16.595	−1.892	7.495	−5.083
Ganoderol B	440.708	2.498	6.223	−4.036	1903.527	−0.545	992.019	−2.103
Betulinic acid	456.707	1.633	6.176	−1.739	359.488	−0.379	208.226	−2.786
Ganoderic acid R	554.765	7.925	6.738	−3.141	33.16	−1.922	15.839	−4.364
Ganodermic acid S	554.765	7.925	6.738	−3.141	33.16	−1.922	15.839	−4.364
Pristimerin	464.644	9.048	5.212	−4.309	482.397	−0.839	224.982	−3.64
Ganoderic acid Lm2	514.658	6.78	2.793	−2.415	15.553	−2.079	6.988	−5.111
Ganoderic acid E	512.642	3.812	2.248	−2.116	8.208	−2.204	3.502	−5.774
Celastrol	450.617	5.881	4.915	−1.993	72.431	−0.97	36.855	−4.134
Ganoderic acid Sz	452.676	4.808	6.495	−2.335	178.493	−0.857	97.697	−3.146

Table 4 (continued)

Molecule	MW	Dipole	QPlogPo/w (−2.0 to 6.5)	QPlogHERG (acceptable range, above −5.0)	QPP Caco (nm/s; 25—poor; 500—great)	QPlogBB (−3 to 1.2)	QPP MDCK (nm/s)	QPlogKp (−8.0 to −0.1)
Ganoderic acid Mk	556.738	8.7	5.633	−3.194	13.713	−2.431	6.099	−5.029
Ganoderic acid theta	530.657	3.315	1.824	−2.529	4.864	−2.697	1.989	−6.002
Ganoderic acid TR	468.675	2.232	5.559	−2.718	73.44	−1.384	37.41	−3.809
Pristimerin	464.644	10.739	5.088	−4.469	250.562	−1.154	110.828	−4.193
Ganoderic acid DM	468.675	6.133	5.572	−2.664	71.192	−1.351	36.174	−4.013

Table 5 Binding affinities, scores, and energies of different compounds with VEGFR-1 receptor

S. no.	Ligand type	GScore (kcal/mol)	Lipophilic EvdW	GLIDE emodel	H bond	H bond length (Å)	Protein–ligand interaction
1	Ganoderic acid C2	−9.906	−2.4	−50.790	−2.162	2.16, 3.57, 1.41, 3.58, 2.67	Arg 1021, Asp 807, Ile 1019, Lys 861, Asp 1040
2	Curcumin	−8.546	−3.3	−66.721	−0.480	2.12, 3.05	Il1 1019, Phe 1041
3	Ganoderic acid A	−9.906	−2.4	−50.437	−1.23	1.21, 2.75, 2.76	Arg 1021, Lys 873, Glu 878
4	Withaferin A	−7.553	−2.2	−28.294	−1.660	1.52, 2.67	Arg 1021, Asp 1040
5	Quercetin	−7.400	−2.8	−54.103	−2.719	1.88, 2.16	Arg 1021, Asp 1040
6	Lucialdehyde C	−7.382	−0.2	−37.665	−1.050	1.86, 3.26	Arg 1021, Asp 1040
7	Ganolucidic acid A	−6.588	−1.8	−52.363	−0.350	2.78, 4.67, 1.86	Arg 1021, Asp 1040, Ile 1019
8	Ganoderic acid C6	−6.537	−0.8	−62.557	−1.171	2.11, 3.76	Arg 1021, Asp 1040
9	Ganoderic acid H	−6.369	−1.7	22.217	−0.607	2.77, 1.09	Arg 1021, Lys 873
10	Ganolucidic acid B	−6.294	−1.8	−65.381	−1.360	3.13, 3.67, 0.19	Arg 1021, Asp 1040, Ile 1019
11	Ganoderic acid B	−6.259	−2.7	−41.421	−0.830	3.13, 1.69, 2.43, 3.76	Arg 1021, Asp 1040, Lys 861, Asp 807
12	Ganoderic acid C1	−6.257	−2.9	−38.616	−1.647	1.72, 3.45	Arg 1021, Asp 1040
13	Ganoderic acid F	−6.196	−2.5	10.022	−1.016	1.87	Arg 1021
14	Ganoderic acid G	−6.135	−2.7	−46.966	−1.689	2.83, 2.95	Arg 1021, Asp 1040
15	Ganoderic acid DF	−6.031	−0.7	−46.046	−2.430	1.83, 2.12, 3.77, 1.49	Arg 1021, Ile 1019, Glu 878, Lys 873
16	Ganoderic acid C6	−6.030	−3.6	−53.633	−0.830	1.53, 2.58	Arg 1021, Asp 1040
17	Ganoderic acid D	−5.882	−3.8	−42.800	−1.139	3.46, 1.58	Arg 1021, Asp 1040
18	Ganoderic acid alpha	−5.854	−1.8	41.318	−1.100	2.35	Arg 1021
19	Ganoderic acid J	−5.794	−2.6	−55.127	−1.485	3.71, 3.36	Arg 1021, Lys 873
20	Ganoderic acid K	−5.609	−3.4	45.682	−1.232	2.41, 3.14	Arg 1021, Ile 1019
21	Ganoderic acid Am1	−5.433	−0.3	−56.094	−0.900	1.85, 3.47, 2.92	Arg 1021, Asp 1040, Lys 861
22	Lucialdehyde B	−5.254	−2.8	−45.997	−1.901	3.88, 3.31	Arg 1021, Asp 1040
23	Tirucallol	−5.211	−2.3	56.542	−0.891	3.72	Asp 1040
24	Ganoderic acid Sz	−5.199	−3.1	−41.760	−1.743	3.72	Asp 1040
25	Ganoderic acid beta	−5.138	−1.5	−55.488	−1.786	3.72	Asp 1040
26	Ganoderic acid theta	−4.943	−1.3	−46.653	−2.296	1.94, 2.73	Arg 1021, Asp 807
27	Ganoderic acid Lm2	−4.904	−1.3	−44.129	−0.457	2.73, 4.83, 0.79	Arg 1021, Lys 873, Glu 878
28	Ganoderic acid TR	−4.879	−1.3	−41.387	−0.842	1.69, 2.53	Lys 873, Glu 878
29	Lucidenic acid C	−4.867	−1.5	−44.140	−1.823	2.78, 3.02	Arg 1021, Asp 807
30	Ganolucidic acid E	−4.741	−0.3	−19.096	−1.285	2.94, 1.8, 2.81	Arg 1021, Lys 873, Ala 874
31	Ganoderic acid DF	−3.635	−2.6	−52.343	−2.091	2.83, 2.84, 1.99, 0.83	Arg 1021, Lys 861, Asp 807, Asp 1040
32	Ganoderic acid B methyl ester	−3.621	−2.2	−51.587	−0.742	2.83	Arg 1021
33	Lucidenic acid P	−3.525	−1.6	−56.234	−0.731	2.99, 1.73	Lys 873, Glu 878
34	Ganoderic acid Am1	−3.411	−1.3	−37.532	−1.45	2.89, 3.61	Asp 1040, Lys 861
35	Ganoderic acid T-Q	−3.41	−1.3	−37.42	−2.76	3.82, 3.53	Asp 1040, Lys 861
36	Ganolucidic acid A	−3.293	−0.82	−43.71	−1.69	3.01	Lys 873
37	Ganoderic acid R	−3.183	−0.75	−33.18	−2.42	3.76	Lys 861
38	Ganoderic acid X	−3.127	−1.1	−22.18	−2.81	2.51	Arg 1021
39	Ganoderic acid TR1	−3.079	−2.7	−44.83	−1.63	1.88, 2.24	Asp 1040, Lys 861
40	Ganoderic acid Y	−2.96	−1.1	−39.59	−2.11	3.01	Asp 1040
41	Ganosporeric acid A	−2.92	−1.3	−42.63	−2.01	2.99	Glu 878
42	Ganoderol B	−2.852	−1.3	−62.73	−0.94	3.98	Arg 1021
43	Ursolic acid	−2.846	−0.7	−55.63	−0.99	3.51	Arg 1021
44	Ganoderic acid Mk	−2.791	−1.2	−22.62			Lys 861, Arg 1021
45	Ganoderic acid T	−2.79	−0.62	−11.73	−1.81	1.92	Lys 861
46	Ganodermic acid S	−2.748	−1.21	−22.71	−1.89	2.22	Arg 1021
47	Ganoderic acid beta	−2.715	−1.7	−43.76	−0.73	2.77	Asp 1040

Table 5 (continued)

S. no.	Ligand type	GScore (kcal/mol)	Lipophilic EvdW	GLIDE emodel	H bond	H bond length (Å)	Protein–ligand interaction
48	GA-S	−2.706	−1.6	−22.68	−1.63	3.61	Glu 878
49	Ganoderic acid T-Q	−2.606	−2.8	−43.896	−1.231	3.88	Arg 1021
50	Ganoderic acid Lm2	−2.556	−1.9	−54.991	−1.679	3.22, 2.43, 3.51	Arg 1021, Asp 1040, Lys 861
51	Ganoderic acid E	−2.082	−2.8	6.045	−1.03	2.01	Arg 1021
52	Celastrol	−2.082	−1.1	6.045	−105	3.78	Arg 1021
53	Pristimerin	−2.009	−3.32	−22.87	−1.21	2.89	Lys 861

Table 6 Evaluation of drug-like properties of the different isoforms of ganoderic acid and natural inhibitors of VEGFR-1 by Qikprop

Molecule	MW	Dipole	QPlogPo/w (−2.0 to 6.5)	QPlogHERG (acceptable range, above −5.0)	QPP Caco (nm/s; 25—poor; 500—great)	QPlogBB (−3 to 1.2)	QPP MDCK (nm/s)	QPlogKp (−8.0 to −0.1)
Ganoderic acid C2	518.689	2.882	3.175	−4.826	53.931	−2.386	12.62	−4.821
Curcumin	368.385	1.681	2.902	−6.281	220.101	−2.046	96.339	−2.643
Quercetin	304.256	6.942	0.174	−4.842	23.224	−2.259	8.475	−5.423
Withaferin A	470.605	6.119	3.061	−4.575	217.675	−1.408	95.192	−3.98
Ganolucidic acid B	502.69	4.545	3.628	−2.358	20.379	−2.015	9.359	−4.814
Withaferin A	470.605	9.743	3.079	−4.598	235.306	−1.377	103.552	−3.909
Lucialdehyde C	468.718	3.637	5.561	−4.195	701.938	−1.343	337.456	−3.011
Ganoderic acid F	570.678	6.665	2.209	−2.546	5.586	−2.594	2.31	−6.002
Ganoderic acid A	516.673	5.061	2.727	−2.255	10.499	−2.268	4.57	−5.374
Ganoderic acid D	514.658	2.434	2.668	−2.412	11.784	−2.212	5.177	−5.372
Ganoderic acid C1	514.658	3.626	2.619	−2.229	11.501	−2.16	5.043	−5.393
Ganoderic acid B	516.673	5.874	2.932	−2.288	17.192	−2.067	7.787	−4.957
Ganoderic acid alpha	574.71	2.281	2.352	−2.305	7.804	−2.474	3.316	−5.528
Ganoderic acid H	572.694	2.764	2.227	−2.29	8.409	−2.376	3.595	−5.561
Ganoderic acid G	532.673	3.603	2.024	−2.381	10.442	−2.375	4.543	−5.282
Ganoderic acid beta	484.675	5.691	4.529	−2.482	33.322	−1.702	15.923	−4.555
Lucialdehyde B	452.676	2.338	4.871	−3.944	542.017	−0.957	255.182	−3.467
Ganoderic acid E	512.642	5.162	2.525	−2.534	11.208	−2.218	4.904	−5.511
Lucidenic acid A	458.594	6.725	2.778	−2	21.374	−1.708	9.853	−5.062
Ganoderic acid Sz	452.676	3.088	6.547	−2.725	119.9	−1.098	63.548	−3.469
Ganoderic acid DM	468.675	4.817	5.562	−2.714	74.261	−1.341	37.863	−3.969
Ganoderic acid C6	530.657	5.423	1.952	−2.587	7.734	−2.524	3.284	−5.631
Ganoderic acid alpha	574.71	7.068	2.692	−2.602	10.966	−2.433	4.79	−5.241
Ganoderic acid DF	516.673	3.486	2.859	−2.625	12.762	−2.312	5.643	−5.209
Ganoderic acid Am1	514.658	6.058	2.629	−2.39	10.138	−2.27	4.4	−5.499
Ganoderic acid Lm2	514.658	7.188	2.761	−2.743	10.571	−2.368	4.604	−5.436
4091	129.164	5.487	0.684	−3.029	260.059	−1.017	115.375	−6.231
Ganospororic acid A	526.625	1.892	1.644	−2.399	8.06	−2.314	3.434	−5.789
Ganoderic acid C6	530.657	3.14	2.001	−2.409	11.835	−2.269	5.201	−5.273
Ganoderic acid J	514.658	5.171	2.75	−2.602	14.24	−2.194	6.352	−5.212
Methyl ganoderate D	528.684	7.354	3.007	−4.494	145.586	−1.789	61.629	−4.409
Ganolucidic acid A	500.674	5.155	3.448	−2.093	18.88	−1.907	8.617	−4.974
Ganolucidic acid A	500.674	5.75	3.61	−1.975	43.492	−1.529	21.236	−4.27
Ganolucidic acid E	484.675	7.956	4.437	−2.324	30.229	−1.709	14.332	−4.662
Ursolic acid	456.707	5.373	6.171	−1.763	261.273	−0.454	147.481	−3.198

Table 7 Binding affinities, scores, and energies of different compounds with VEGFR-2 receptor

S. no.	Ligand type	GScore (kcal/mol)	Lipophilic EvdW	Electro	GLIDE emodel	H bond	H bond length (Å)	Protein–ligand interaction
1	Ganoderic acid A	−9.9	−3.03	−0.48	−66.674	−0.24	1.11, 2.11	Lys 1041, Glu 848
2	Quercetin	−9.32	−4.0	−1.92	−53.422	−2.47	3.29, 0.23, 1.82	Ile 1042, Leu 1033, Pro 837
3	Curcumin	−9.21	−4.67	−1.61	−75.479	−0.92	2.22, 2.75, 2.99	Glu 883, Leu 1033, Glu 848
4	Ganoderic acid Mk	−6.46	−3.95	−1.6	8.136	−1.28	3.58, 0.74, 0.62	Lys 836, Pro 837, Ile 1042
5	Ganolucidic acid A	−5.89	−2.89	−1.11	−21.259	−1.9	4.12, 2.11	Lys 1041, Glu 848
6	Withaferin A	−5.88	−3.01	−1.92	−19.990	−0.25	2.48, 2.79	Lys 1041, Glu 848
7	Ganodermatriol	−5.87	−3.64	−2.02	6.006	−0.7	2.73, 3.11	Ile 1042, Asp 1044
8	Ganoderic acid T	−5.55	−3.84	−1.32	13.408	−0.86	3.22, 0.48	Lys 1041, Lys 836
9	Methyl ganoderate D	−5.54	−3.18	−0.7	−41.888	−0.23	1.22	Leu 1033
10	Ganoderiol F	−5.27	−3.62	−1.12	−36.845	−0.35	2.33	Leu 1033
11	Ganoderic acid G	−5.11	−2.67	−1.74	−23.878	−0.59	3.64, 3.89	Lys 1041, Leu 838
12	Ganoderic acid DF	−5.11	−2.7	−0.68	−18.944	−1.76	2.17, 1.99	Lys 1041, Leu 838
13	Ganoderic acid Lm2	−4.91	−3.01	−1.59	−37.164	−0.42	2.76, 1.43	Lys 1041, Leu 1033
14	Ganoderic acid J	−4.85	−2.8	−1.36	−45.202	−0.77	2.26, 1.88	Lys 1041, Glu 848
15	Ganodermanontriol	−4.65	−2.86	−1.95	−35.210	−0.37	1.42, 0.52	Leu 1033
16	Ganoderic acid alpha	−4.65	−0.81	−2.56	−20.275	−1.82	1.11, 1.57	Arg 1064, Arg 927
17	Lucidenic acid C	−4.52	−1.98	−1.71	−21.526	−0.53	3.65	Leu 838
18	Ganoderic acid S	−4.48	−2.81	−0.52	−13.685	−1.56	2.77	Lys 1041
19	Ganoderic acid D	−4.33	−2.81	−0.53	−36.220	−0.59	2.55	Lys 836
20	Lucialdehyde B	−4.25	−3.4	−0.5	−9.689	−0.15	2.82	Leu 1033
21	Ganoderic acid Am1	−4.25	−3.05	−0.34	−50.773	−0.47	2.95	Asn 921
22	Ganoderic acid K	−4.22	−1.29	−2.76	80.802	−0.54	2.06	Leu 838
23	Ganoderic acid E	−4.21	−3.1	−0.32	−44.017	−0.52	2.92, 0.83	Leu 1033, Asn 921
24	Ganoderiol B	−4.19	−2.87	−1.44	−23.009	−0.46	1.91, 0.73	Lys 1041, Ile 1042
25	Ganoderic acid DM	−4.18	−3.23	−0.92	−15.974	−0.45	2.03	Lys 1041
26	Ganoderic acid TR1	−4.09	−2.61	−1.35	−21.471	−0.44	3.31, 3.83	Lys 1041, Leu 1034
27	Ganoderic acid C1	−4.09	−2.93	−0.34	−43.140	−0.36	2.82, 3.93	Leu 1033, Asn 921
28	Ganoderic acid B methyl ester	−3.99	−3.62	−0.43	−49.955	−1.11	3.43	Asn 921
29	Ganolucidic acid B	−3.98	−2.96	−0.43	−41.936	−0.32	3.86	Leu 1033
30	Ganoderic acid C2	−3.94	−2.59	−0.62	−43.521	−0.25	3.32	Leu 1033
31	Ganoderic acid B	−3.91	−2.79	−0.3	−41.243	−0.42	3.93	Leu 1033
32	Ganolucidic acid E	−3.88	−2.26	−1.05	−38.717	−0.49	3.72	Leu 1033
33	Ganoderic acid Me	−3.73	−2.73	−0.7	−29.997	−0.35	2.36	Asn 921
34	Ganoderic acid R	−3.71	−3.47	−0.15	−26.589	−0.3	3.64	Asn 921
35	Ganodermic acid S	−3.71	−3.47	−0.15	−26.589	−0.3	3.64	Asn 921
36	Ganoderic acid T-Q	−3.68	−3.42	−0.17	−17.836	−0.27	3.64	Asn 921
37	Ganoderic acid H	−3.56	−3.05	−0.35	19.562	−0.42	2.99	Asn 921
38	Ganoderic acid Sz	−3.39	−3.37	0	−11.772	−0.03	2.83	Asp 1044
39	Ganoderic acid F	−3.38	−1.92	−1.05	−29.256	−0.29	3.63	Leu 1033
40	Ganoderic acid TR	−3.38	−2.46	−0.35	−33.703	−0.56	3.65	Leu 1033
41	Betulinic acid	−3.37	−2.99	−0.24	42.626	−0.19	3.9	Leu 1033
42	Lucidenic acid A	−3.32	−2.25	−0.35	−18.007	−0.61	3.4, 3.98	Leu 1033, Lys 836
43	Ganoderic acid beta	−2.73	−2.71	0	29.200	−0.36	2.76	Asn 921
44	Ganosporeric acid A	−2.71	−2.36	−0.35	−39.577	−0.48	3.65	Leu 1033
45	Ursolic acid	−1.61	−1.24	−0.7	−25.069	−0.28	3.43	Lys 836
46	Pristimerin	−1.21	−1.42	0	−31.564	−0.07	2.8	Leu 1034
47	Celastrol	−1.1	−0.84	−0.35	−29.905	−0.37	1.78	Asn 921
48	Ganoderic acid Y	−0.972	−0.76	−0.15	−41.243	−0.43	2.17	Asp 1044
49	Lucialdehyde C	−0.94	−2.78	−0.15	−27.82	−0.21	3.76	Leu 1033
50	Ganoderic acid X	−0.63	−2.73	−0.35	−35.75	−0.42	3.02	Leu 1033
51	Betulinic acid	−0.61	−1.76	−0.78	−32.53	−0.52	1.98	Asn 921
52	Lucidenic acid P	−0.53	−0.67	−0.27	−43.76	−0.11	2.78	Asp 1044
53	Ganoderic acid C6	−0.49	−1.11	−0.12	−39.89	−0.09	2.78	Asn 921

Table 8 Evaluation of drug-like properties of the different isoforms of ganoderic acid and natural inhibitors of VEGFR-2 by Qikprop

Molecule	MW	Dipole	QPlogPo/w (−2.0 to 6.5)	QPlogHERG (acceptable range, above −5.0)	QPP Caco (nm/s; 25—poor; 500—great)	QPlogBB (−3 to 1.2)	QPP MDCK (nm/s)	QPlogKp (−8.0 to −0.1)
Ganoderic acid A	516.673	3.269	4.562	−4.74	21.76	−2.059	24.83	−4.834
Quercetin	304.256	6.82	0.179	−4.9	23.586	−2.269	8.618	−5.4
Curcumin	368.385	6.82	2.93	−6.241	232.471	−2.012	102.21	−2.609
Ganoderic acid Mk	556.738	5.413	5.534	−3.218	12.718	−2.485	5.622	−5.106
Ganolucidic acid A	500.674	7.693	3.682	−2.547	18.127	−2.067	8.246	−5.009
Withaferin A	470.605	6.893	3.087	−4.425	276.463	−1.27	123.261	−3.782
Ganodermatriol	456.707	1.172	5.236	−4.296	593.023	−1.197	281.234	−2.903
Ganoderic acid T	612.802	6.202	6.498	−3.006	19.853	−2.203	9.098	−4.752
Methyl ganoderate D	528.684	10.348	2.752	−4.268	130.298	−1.774	54.664	−4.503
Ganoderiol F	454.692	4.29	5.609	−4.631	843.147	−1.038	411.396	−2.695
Ganoderic acid G	532.673	6.644	1.99	−2.334	8.503	−2.447	3.638	−5.456
Ganoderic acid DF	516.673	8.847	2.769	−2.215	12.637	−2.175	5.583	−5.217
Ganoderic acid Lm2	514.658	9.657	2.415	−2.422	6.925	−2.431	2.914	−5.784
Ganoderic acid J	514.658	2.97	2.619	−2.351	10.508	−2.241	4.574	−5.469
Ganodermanontriol	472.707	5.077	5.264	−4.962	483.195	−1.432	225.384	−3.101
Ganoderic acid alpha	574.71	6.909	2.572	−2.479	7.793	−2.54	3.311	−5.529
Lucidenic acid C	476.609	4.949	1.968	−2.108	14.423	−2.025	6.441	−5.202
Ganoderic acid D	514.658	5.272	2.723	−2.156	15.882	−1.999	7.148	−5.12
Ganoderic acid A	516.673	3.269	2.612	−2.192	11.561	−2.206	5.071	−5.292
Lucialdehyde B	452.676	4.472	4.982	−3.979	555.078	−0.952	261.834	−3.443
Ganoderic acid Am1	514.658	2.736	2.614	−2.353	11.677	−2.196	5.126	−5.38
Ganoderic acid K	574.71	9.365	2.818	−2.354	24.05	−2.002	11.194	−4.578
Ganoderic acid E	512.642	5.231	2.316	−1.951	11.52	−2.008	5.052	−5.487
Ganoderiol B	470.691	1.761	4.467	−4.479	294.484	−1.547	131.968	−3.509
Ganoderic acid DM	468.675	2.241	5.445	−2.439	70.039	−1.307	35.542	−4.035
Ganoderic acid TR1	468.675	4.056	5.234	−2.688	61.54	−1.45	30.904	−3.94
Ganoderic acid C1	514.658	7.632	2.865	−2.347	17.724	−2.012	8.048	−5.028
Ganoderic acid B methyl ester	528.684	1.817	2.931	−4.557	92.871	−2.063	37.91	−4.664
Ganolucidic acid B	502.69	1.453	3.551	−2.372	15.744	−2.132	7.081	−5.032
Ganoderic acid C2	518.689	2.882	2.644	−2.402	8.616	−2.467	3.691	−5.444
Ganoderic acid B	516.673	1.649	2.808	−2.009	17.049	−1.985	7.717	−4.964
Ganolucidic acid E	484.675	5.322	4.505	−2.388	29.889	−1.724	14.158	−4.651
Ganoderic acid Me	554.765	6.48	7.029	−3.137	54.652	−1.697	27.183	−3.982
Ganoderic acid R	554.765	1.483	7.121	−3.221	57.794	−1.694	28.876	−3.934
Ganodermic acid S	554.765	1.483	7.121	−3.221	57.794	−1.694	28.876	−3.934
Ganoderic acid T-Q	510.712	9.974	6.189	−2.841	63.485	−1.485	31.961	−3.945
Ganoderic acid H	572.694	3.797	2.822	−2.212	29.465	−1.814	13.941	−4.503
Ganoderic acid Sz	452.676	3.363	6.588	−2.744	125.57	−1.08	66.802	−3.425
Ganoderic acid F	570.678	5.653	2.104	−2.406	5.228	−2.567	2.151	−6.058
Ganoderic acid TR	468.675	4.683	5.534	−2.768	66.195	−1.437	33.438	−3.881
Betulinic acid	456.707	3.198	6.181	−1.864	338.575	−0.416	195.164	−2.835
Lucidenic acid A	458.594	5.936	2.569	−1.749	18.875	−1.688	8.615	−5.167
Ganoderic acid beta	484.675	2.701	4.534	−2.543	34.52	−1.703	16.543	−4.525
Ganosporeric acid A	526.625	4.871	1.671	−2.622	6.55	−2.493	2.744	−5.964
Ursolic acid	456.707	3.556	6.09	−1.622	259.188	−0.438	146.209	−3.192
Pristimerin	464.644	8.794	5.297	−4.244	561.924	−0.763	265.327	−3.511
Celastrol	450.617	6.106	4.943	−1.99	71.633	−0.973	36.417	−4.14
Ganolucidic acid B	502.69	6.914	3.71	−2.546	23.669	−2.008	11.002	−4.688

Table 9 Binding affinities, scores, and energies of different compounds along with natural inhibitors with ER receptor

S. no.	Ligand type	GScore (kcal/mol)	Lipophilic EvdW	GLIDE emodel	H bond	H bond length (Å)	Protein–ligand interaction
1	Ganoderic acid C6	−8.863	−3.26	−52.948	−1.48	2.72, 2.57	Tyr 739, Glu 893
2	Curcumin	−8.143	−3.62	−48.681	−0.96	3.52, 3.82, 2.91, 1.9	Arg 394, Glu 353, Asp351, Trp 383
3	Ganoderic acid J	−6.616	−1.59	−51.899	−1.1	2.72	Lys 905
4	Ganoderic acid C2	−5.565	−2.42	−42.239	−0.9	2.91, 2.87	Tyr 739, Glu 897
5	Ganoderiol B	−5.936	−2.79	−43.566	−1.86	1.78	Tyr 739
7	Methyl ganoderate D	−5.819	−2.24	−50.883	−0.54	2.11	Ile 898
8	Ganoderic acid Lm2	−5.652	−2.73	−36.946	−0.82	2.92	Tyr 739
9	Ganoderic acid C1	−5.588	−1.47	−42.434	−1.89	2.99, 3.38	Tyr 739, Ile 898
10	Ganoderic acid A	−5.576	−1.47	−40.971	−1.01	2.94	Tyr 739
11	Ganoderic acid G	−5.549	−3.05	−43.127	−1.14	1.83	Glu 897
12	Ganoderic acid Am1	−5.415	−1.37	−59.075	−0.17	2.16	Ile 898
13	Ganoderic acid B methyl ester	−5.375	−1.92	−45.873	−0.93	1.93	Glu 893
14	Ganoderic acid D	−5.325	−1.46	−33.773	−1.83	1.78, 3.86	Tyr 739, Ile 898
15	Ganoderic acid DF	−5.313	−2.99	−46.810	−0.66	3.44	Ile 898
16	Ganoderic acid E	−5.260	−3.17	−49.262	−1.77	2.72, 1.03	Lys 905, Gln 902
17	Withaferin A	−5.208	−2.85	−24.170	−2.04	2.94	Tyr 739
18	Ganoderic acid B	−5.207	−3.82	−50.692	−0.83	2.52, 2.78	Tyr 739, Glu 897
19	Ganoderic acid alpha	−4.932	−3.92	−38.817	−1.85	1.83, 1.92	Ser 900, Val 901
20	Ganoderic acid K	−4.881	−2.22	−46.583	−1.47	3.8, 2.09, 2.94	Lys 910, Val 903, Met 894
21	Ganodermatriol	−4.866	−2.16	−33.261	−2.05	2.66	Tyr 739
22	Lucidenic acid C	−4.603	−2.64	−47.632	−1.77	1.43	Glu 897
23	Ganoderic acid Mk	−4.601	−3.63	10.546	−0.83	1.73	Glu 897
24	Ganolucidic acid A	−4.593	−1.24	−41.663	−2.92	3.33, 3.29	Tyr 739, Ile 898
25	Quercetin	−4.547	−3.73	−38.864	−1.83	2.12, 1.56	Tyr 739, Val 901
26	Ganoderic acid TR1	−4.515	−2.47	−40.911	−2.01	2.69	Tyr 739
27	Ganoderic acid F	−4.356	−3.47	−38.124	−0.11	3.87	Met 894
28	Lucialdehyde B	−4.291	−2.42	−40.447	−0.05	2.74	Glu 897
29	Ganoderic acid DM	−4.266	−2.05	−42.363	−1.82	2.92	Tyr 739
30	Lucidenic acid P	−4.262	−3.37	−36.533	−2.11	2.67	Ile 898
31	Ganoderic acid T-Q	−4.172	−3.92	−29.502	−1.83	3.55, 0.86	Tyr 739, Met 894
32	Ganoderic acid H	−3.937	−2.01	−42.741	−1.93	2.93, 0.94	Met 894, Lys 910
33	Lucidenic acid A	−3.911	−2.83	−38.504	−0.99	1.44	Ile 898
34	Ganoderic acid TR	−3.696	−3.84	−36.219	−1.45	2.9, 2.68	Tyr 739, Met 894
35	Ganoderic acid Me	−3.525	−3.24	−39.338	−2.83	3.22	Glu 897
36	Ganolucidic acid B	−3.511	−3.45	−42.67	−1.66	3.79	Met 894
37	Ursolic acid	−3.449	−3.54	−22.744	−0.82	2.01	Lys 905
38	Ganosporeric acid A	−3.179	−3.37	−35.098	−1.73	2.64	Tyr 739
39	Methyl ganoderate D	−2.981	−2.11	−44.941	−2.63	4.23	Gln 902
40	Ganoderic acid X	−2.962	−2.65	−66.731	−1.72	2.93, 1.63	Lys 910, Glu 897
41	Ganolucidic acid E	−2.96	−2.85	43.661	−1.81	2.76	Ile 898
42	Betulinic acid	−2.733	−2.84	−38.504	−2.82	2.45, 1.77	Gln 902, Met 894
43	Ganoderic acid S	−2.699	−1.88	−33.485	−1.39	3.11	Ile 898
44	Ganoderic acid Sz	−2.629	−2.99	−22.869	−0.47	2.73	Gln 902
45	Celastrol	−2.61	−1.66	−29.51	−1.93	1.92	Gln 902
46	Ganodermic acid S	−2.595	−1.73	−12.865	−0.66	2.02	Glu 897
47	Ganoderic acid T	−2.281	−1.32	−23.683	−0.07	2.02	Glu 897
48	Ganoderic acid Y	−2.198	−2.63	−35.157	−0.52	2.45, 2.93	Gln 902, Lys 910
49	Ganoderic acid R	−1.899	−3.72	−54.378	−1.01	2.63	Ile 898
50	Lucialdehyde C	−1.821	−1.73	−27.685	−0.27	2.99	Lys 910
51	Ganoderic acid beta	−1.561	−2.73	−31.236	−1.71	3.61	Met 894
52	Pristimerin	0.365	−2.91	−30.352	−1.22	3.45	Lys 910
53	Ganoderic acid theta	0.588	−2.98	−34.430	−0.19	2.05, 3.9	Val 903, Lys 910

Table 10 Evaluation of drug-like properties of the different isoforms of ganoderic acid and natural inhibitors of ER by Qikprop

Molecule	MW	Dipole	QPlogPo/w (−2.0 to 6.5)	QPlogHERG (acceptable range: above-5.0)	QPP Caco (nm/s; 25—poor; 500—great)	QPlogBB (−3 to 1.2)	QPP MDCK (nm/s)	QPlogKp (−8.0 to −0.1)
Ganoderic acid C6	530.657	7.095	2.029	−2.73	9.226	−2.5	3.974	−5.482
Curcumin	368.385	8.338	2.693	−5.378	249.11	−1.793	110.137	−2.669
Ganoderic acid J	514.658	7.055	2.662	−1.918	19.492	−1.843	8.919	−4.947
Ganoderiol B	470.691	5.596	4.418	−4.658	249.191	−1.662	110.173	−3.637
Methyl ganoderate D	528.684	5.114	3.165	−5.037	91.495	−2.189	37.303	−4.801
Ganoderic acid A	516.673	4.474	2.782	−2.444	11.641	−2.289	5.109	−5.287
Ganoderic acid G	532.673	6.422	2.088	−2.656	9.707	−2.508	4.198	−5.344
Ganoderic acid Lm2	514.658	5.237	2.755	−2.704	9.012	−2.43	3.874	−5.579
Ganoderic acid C1	514.658	3.82	2.826	−2.627	13.665	−2.222	6.076	−5.247
Ganoderic acid C2	518.689	4.698	2.873	−2.639	12.364	−2.392	5.453	−5.14
Ganoderic acid Am1	514.658	5.918	2.883	−2.77	14.218	−2.255	6.342	−5.214
Ganoderic acid B methyl ester	528.684	2.559	3.191	−4.87	142.934	−1.952	60.416	−4.28
Ganoderic acid D	514.658	5.435	2.912	−2.65	15.458	−2.174	6.942	−5.143
Ganoderic acid DF	516.673	6.41	2.84	−2.357	18.03	−2.068	8.199	−4.917
Ganoderic acid Am1	514.658	6.555	2.839	−2.381	21.546	−1.938	9.939	−4.863
Ganoderic acid E	512.642	8.134	2.695	−2.364	22.134	−1.86	10.233	−4.936
Withaferin A	470.605	10.37	3.019	−4.581	221.117	−1.403	96.82	−3.97
Ganoderic acid B	516.673	4.03	2.892	−2.673	12.462	−2.341	5.5	−5.229
Ganoderic acid D	514.658	5.298	2.751	−2.514	12.196	−2.233	5.373	−5.343
Ganoderic acid alpha	574.71	7.818	2.572	−2.296	9.151	−2.402	3.939	−5.394
Ganoderic acid K	574.71	8.839	2.826	−3.117	9.647	−2.697	4.17	−5.349
Ganodermatriol	456.707	5.191	5.269	−4.454	515.519	−1.288	241.725	−3.022
Ganoderic acid DF	516.673	6.364	2.925	−2.249	24.852	−1.897	11.598	−4.646
Lucidenic acid C	476.609	7.571	1.948	−1.946	17.487	−1.896	7.932	−5.039
Ganoderic acid Mk	556.738	6.84	5.326	−2.397	36.335	−1.741	17.485	−4.237
Ganolucidic acid A	500.674	8.333	3.781	−2.679	23.502	−1.993	10.918	−4.79
Quercetin	304.256	8.091	0.191	−4.818	27.291	−2.18	10.089	−5.28
Ganoderic acid TR1	468.675	3.109	5.356	−2.774	66.491	−1.437	33.6	−3.877
Ganoderic acid F	570.678	5.307	2.355	−2.615	9.727	−2.372	4.208	−5.534
Lucialdehyde B	452.676	2.208	5.01	−4.256	462.967	−1.079	215.203	−3.583
Ganoderic acid DM	468.675	5.914	5.602	−2.639	74.31	−1.322	37.89	−3.965
Lucidenic acid P	518.646	5.905	2.667	−1.885	28.023	−1.686	13.205	−4.641
Ganoderic acid T-Q	510.712	4.315	6.248	−2.991	45.115	−1.683	22.094	−4.231
Ganoderic acid F	570.678	4.522	2.248	−2.533	8.638	−2.394	3.701	−5.634
Ganolucidic acid A	500.674	5.555	3.657	−2.362	27.557	−1.825	12.968	−4.655
Ganoderic acid H	572.694	7.057	2.439	−2.745	6.399	−2.675	2.676	−5.792
Lucidenic acid A	458.594	5.788	2.611	−2.006	16.548	−1.816	7.473	−5.278
Ganoderic acid K	574.71	5.45	2.643	−2.608	6.809	−2.65	2.861	−5.643
Ganoderic acid TR	468.675	4.408	5.583	−2.917	68.01	−1.458	34.43	−3.845
Ganoderic acid Me	554.765	5.43	7.236	−2.863	66.225	−1.537	33.454	−3.832
Ursolic acid	456.707	2.76	6.116	−1.69	313.444	−0.373	179.555	−3.038
Pristimerin	464.644	8.116	5.385	−4.552	481.585	−0.885	224.573	−3.635
Ganoderic acid theta	530.657	3.939	1.85	−2.548	6.824	−2.546	2.868	−5.7
Ganosporeric acid A	526.625	2.962	1.881	−3.08	6.915	−2.662	2.91	−5.918

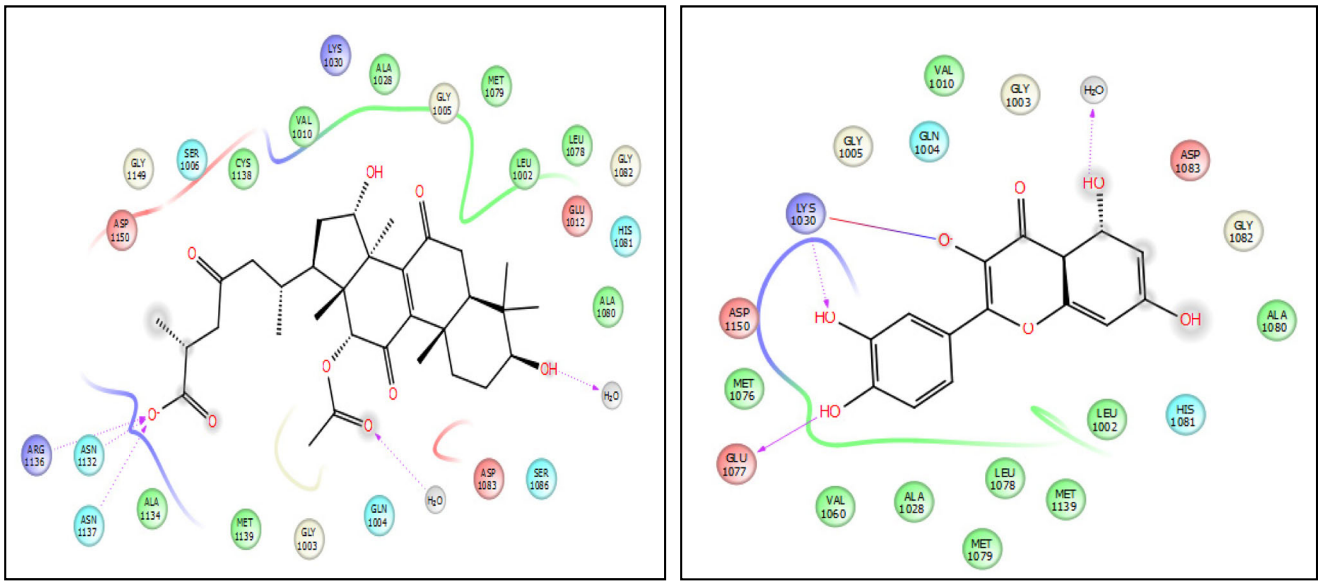

Fig. 1 Protein–ligand interaction profile of IR (PDB, 3EKK) with ganoderic acid and quercetin. Protein–ligand interaction profile revealed that residues involved in ganoderic-binding acid were Arg 1136, Asn 1132, and Asn 1137 whereas Lys 1030 and Glu 1077 were involved in quercetin

(56.042), GScore (−9.36), and Lys 1030 and Glu 1077 residues participating in hydrogen bonding (Fig. 1). Interaction in quercetin exhibits involvement of both phenyl moiety and chromone moiety during docking, which makes it crucial for designing effective target. Other isoforms and natural inhibitors with docking score and other parameters are represented in Table 1. In general, docking depicted the role of Arg 1136, Asn 1132, Asn 1137, Glu 1077, and Gln 1004 residues in the active sites which forms the basis of the stability of the complex.

During docking with IGFR (PDB, IK3A) with 50 isoforms of ganoderic acid, best-docked isoform was lanosterol struc-

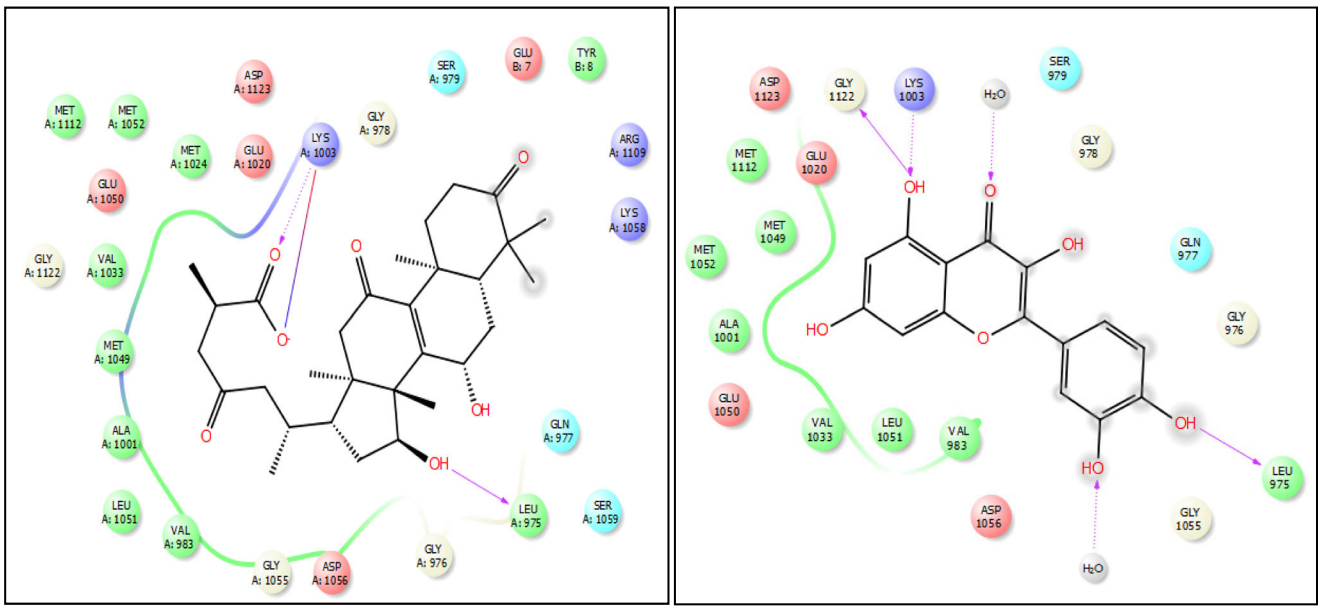

Fig. 2 Protein–ligand interaction profile of IGFR (PDB, IK3A) with ganoderic acid and 5280343. Protein–ligand interaction profile revealed that residues involved in ganoderic-binding acid were Lys 1003 and Leu 975 whereas Lys 1003, Gly 1122, and Leu 975 were involved in quercetin

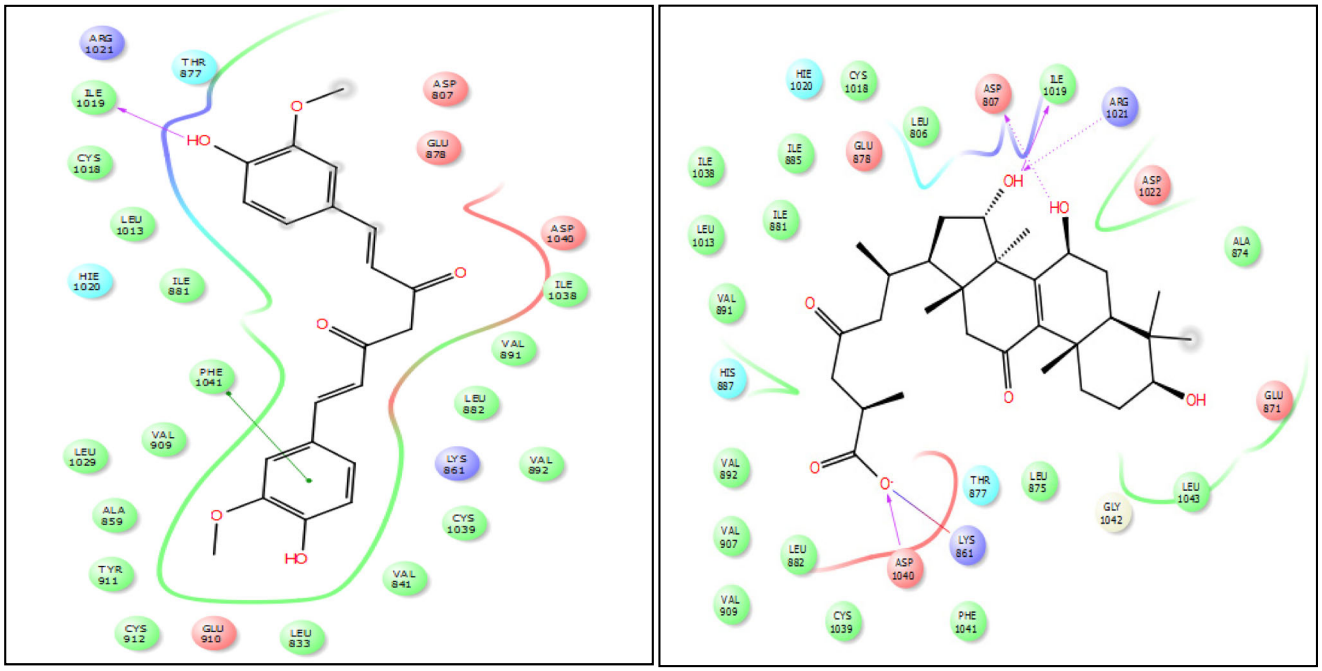

Fig. 3 Protein–ligand interaction profile of VEGFR-1 (PDB, 3HNG) with ganoderic acid C2 and curcumin. Protein–ligand interaction profile revealed that residues involved in ganoderic C2-binding acid were Arg 1021, Asp 807, Ile 1019, Lys 861, and Asp 1040 whereas Il1 1019 and Phe 1041 were involved in curcumin

ture ganoderic acid A. Ganoderic acid A during interaction displayed GScore (−9.672), Lipophilic Evdw (−3.12), GLIDE emodel (−.73.686), and participation of Lys 1003 and Leu 975 (Fig. 2) with 1.84 and 2.17 Å, respectively, hydrogen bond length in the hydrogen bonding. On the other hand, natural inhibitor quercetin have GScore (−8.724),

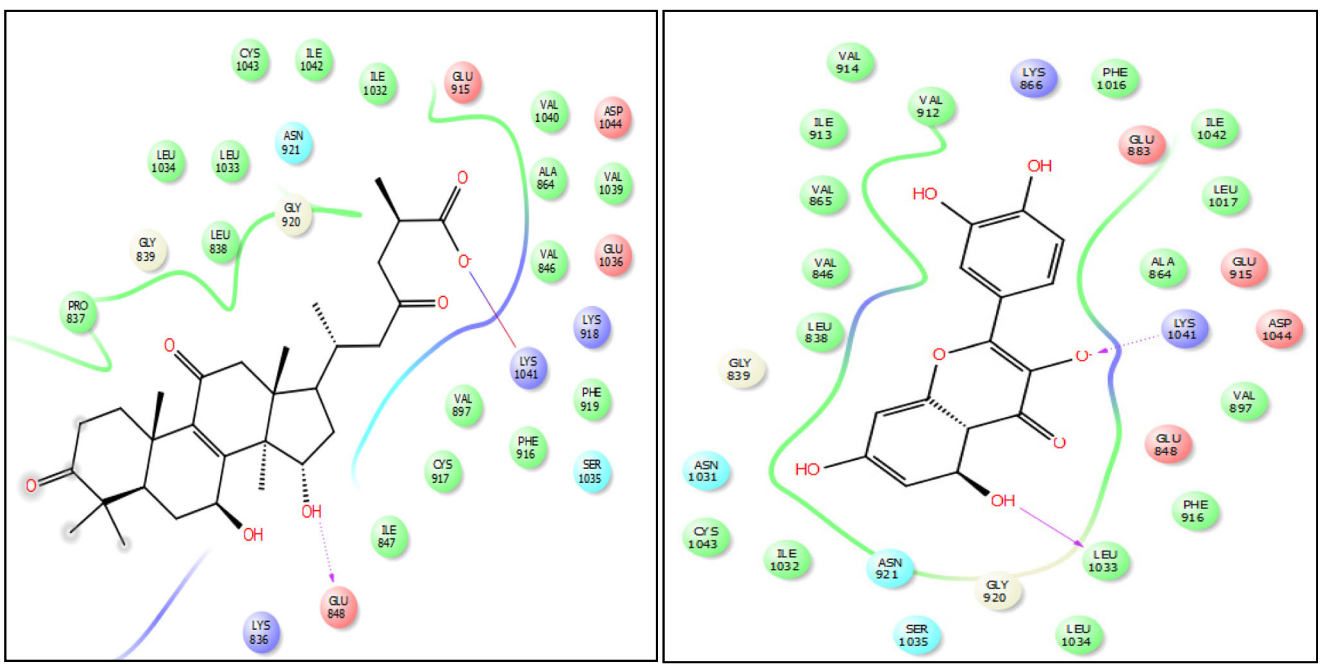

Fig. 4 Protein–ligand interaction profile of VEGFR-2 (PDB, IY6A) with ganoderic acid and quercetin. Protein–ligand interaction profile revealed that residues involved in ganoderic-binding acid were Lys 1041 and Glu 848, whereas Ile 1042, Leu 1033, and Pro 837 were involved in hydrogen bonding in the quercetin. Furthermore, other residues present around ganoderic acid and quercetin make the poses strong and stable

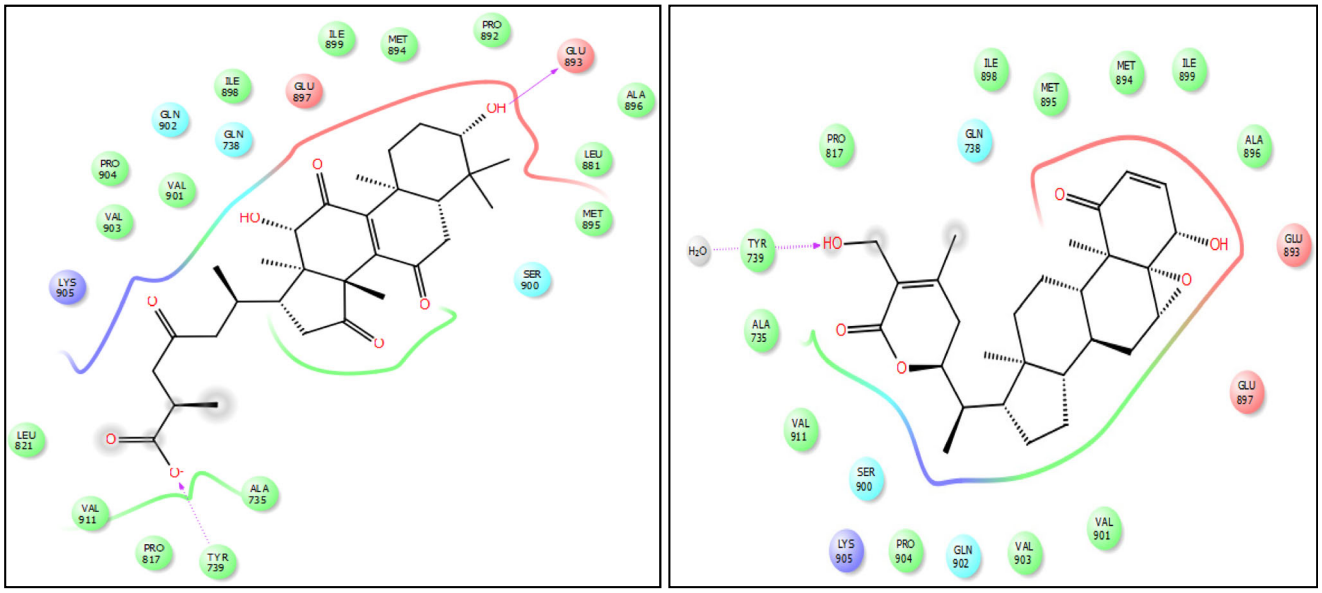

Fig. 5 Protein–ligand interaction profile of ER (PDB, 3ERT) with ganoderic acid C6 and withaferin A. Protein–ligand interaction profile revealed that residues involved in binding in GA-C6 were Tyr 739 and Glu 893 whereas residue Tyr 739 were involved in withaferin A

Lipophilic Evdw (−2.59), GLIDE emodel (−62.31) with Lys 1003, Gly 1122, and Leu 975 forming the hydrogen bonding with 2.23, 2.63, and 2.83 Å hydrogen bond lengths, respectively. In ganoderic acid, −COOH also shows interaction with IGF, thus it can be a target for modulating signaling process. Other isoforms and natural inhibitors with GScore, Lipophilic, Electro, GLIDE emodel, H bond length, and protein–ligand interaction are represented in Table 3.

During docking of VEGFR-1 (PDB, 3HNG), best-docked isoform was ganoderic acid C2 with GScore (−9.906), Lipophilic Evdw (−2.4), and GLIDE emodel (−50.437) which formed hydrogen bonding with Arg 1021, Asp 807, Ile 1019, Lys 861, and Asp 1040 (Fig. 3). In ganoderic acid C2, charged residue Asp and Lys were involved with the COOH group, whereas lanosterol moiety were actively participated with Arg, Asp, Ile, and Lys residues. On the other hand, curcumin was indulged with Ile 1019 and Phe 1041 residues with GScore (−8.546), Lipophilic Evdw (−3.3), and GLIDE emodel (−66.72) (Fig. 3). Other isoforms and natural inhibitors are represented in Table 5.

Molecular docking of different isoforms of ganoderic acid with VEGFR-2 (PDB, 1Y6A) revealed ganoderic acid A as the best-docked isoform with GScore (−9.906), Lipophilic Evdw (−3.03), and GLIDE emodel (−66.67) with Lys1041 and Glu 848 (Fig. 4). Among natural inhibitors, quercetin shows GScore (−9.32), Lipophilic Evdw (−4.0), and GLIDE emodel (−53.42) with participation of Ile 1042, Leu 1033, and Pro 837 residues during interaction (Fig. 4). The active participation of the chromone moiety in quercetin during docking process of VEGFR-2 highlighted its engagement in the signaling process. Other isoforms of ganoderic acid and their natural inhibitors are represented in Table 7.

Molecular docking with estrogen receptor (PDB, 3ERT) exposed ganoderic acid C6 as a best-docked isoform with GScore (−8.863), Lipophilic Evdw (−3.26), and GLIDE emodel (−52.948) forming hydrogen bonding with Tyr 739 and Glu 893 residues (Fig. 5). Among natural inhibitors, curcumin has GScore (−8.143), Lipophilic Evdw (−3.62), and GLIDE emodel (−48.681) forming bonding with Arg 394, Glu 353, Asp351, and Trp 383 (Fig. 5). Other isoforms and natural inhibitors of estrogen receptor are represented in Table 9.

After analyzing different parameters in receptor-based molecular docking studies of 50 isoforms of ganoderic acid in IR, IGFR, VEGFR-1, VEGFR-2, and ER, it concludes that different isoforms have a different affinity towards different

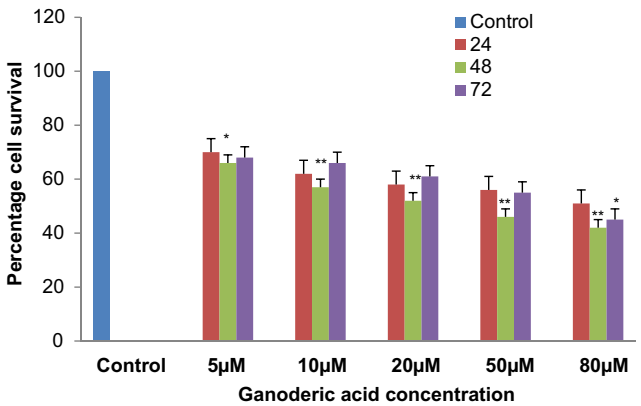

Fig. 6 Ganoderic acid A reduces the cell viability and growth of the Hep G2 cells in a dose-dependent manner determined by the MTT assay. *$P = 0.05$; **$P = 0.01$ versus control

receptors. Overall, it was concluded that triterpenoids of *Ganoderma* species comprise aldehyde, alcohols, and acidic derivatives. The structures have a tetracyclic ring with one double bond at a different position, and the branch ends with a carboxyl group while others with some modification. In molecular docking of ganoderic acid and its isoforms, −COOH group have a crucial role in different activities such as inhibitory effects whereas –C = O or –OH group also have a role in signaling through different receptors. Natural inhibitors in particular, quercetin, have a role both of phenyl moiety and chromone moiety during the interaction. After analyzing in silico studies, ganoderic acid A was analyzed for the biological activity in the liver cancer cell line.

ADME properties

Binding affinity prediction is useful for analyzing the pharmacokinetic and pharmacodynamic properties. Qikprop is an important tool that calculates properties of the valid descriptors and pharmaceutically relevant molecules by comparing their values with those of 95 % of already known pharmaceutical drugs. Ganoderic acid and its 50 isoforms were checked for ADME properties [31]. The most interesting aspect of these compounds are their admirable QPlogPo/w and QPlogHERGK channels and QPlogBB, QPlogKP, and QPlogKhsa values that satisfy the Lipinski's rule of five (Tables 2, 4, 6, 8, and 10). Some of the isomers of ganoderic acid in different receptors did not satisfy all aspect and needed some modifications in the basic lanosterol structure (Tables 2, 4, 6, 8, and 10). Predicted IC_{50} value for blockage of HERG K+ channels (acceptable range, above −5.0); QPP Caco predicted apparent Caco2 cell permeability in nanometers per second. Caco2 cells is a model for the gut–blood barrier (nm/s): <25—poor and >500—great QPlogBB predicted brain–blood partition coefficient QPP MDCK predicted apparent MDCK cell permeability in nanometers per second. MDCK cells are considered to be a good mimic for the blood–brain barrier (nm/s): <25—poor and >500—great QPlogKP predicted skin permeability and percentage of human oral absorption: <25 % is poor and >80 % is high [32].

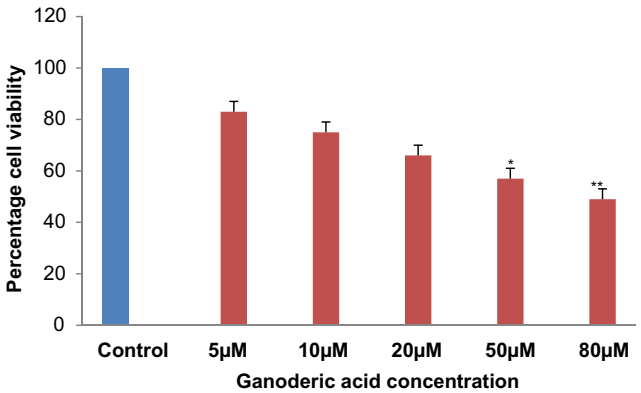

Fig. 7 Ganoderic acid A significantly reduces the cell number of Hep G2 cells in a dose-dependent manner determined by the trypan blue exclusion test of the cell. *$P = 0.05$; **$P = 0.01$ versus control

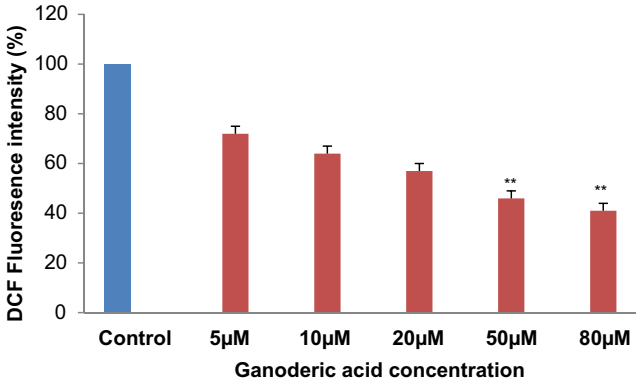

Fig. 8 DCF fluorescence intensity (%) in Hep G2 cells in a dose-dependent manner cells in response to ganoderic acid A. *$P = 0.05$; **$P = 0.01$ versus control

Effects of ganoderic acid on cell growth of cancer cells

To measure the cytotoxicity of ganoderic acid A in cancer cells, MTT assay were performed. Hepatocellular carcinoma cells Hep G2 were treated with different concentrations of ganoderic acid A (5, 10, 20, 50, 80 μM) at different time intervals (24, 48, and 72 h). Ganoderic acid A exhibited a remarkable reduction in cell proliferation in cancer cells in a concentration-dependent manner (Fig. 6).

Moreover, trypan blue exclusion test of cell viability was performed to determine the number of the treatments of ganoderic acid A. Trypan blue exclusion test determined the viable and dead cells. Ganoderic A acid exhibited a pronounced decrease in cell viability (Fig. 7). Overall, it can be inferred that ganoderic acid reduced cell viability in a concentration-dependent manner.

To determine the intracellular ROS-scavenging ability of the ganoderic acid A, H_2DCF-DA assay was carried out with Hep G2 cells. The data obtained from the assay highlighted the ganoderic acid A potential to scavenge the

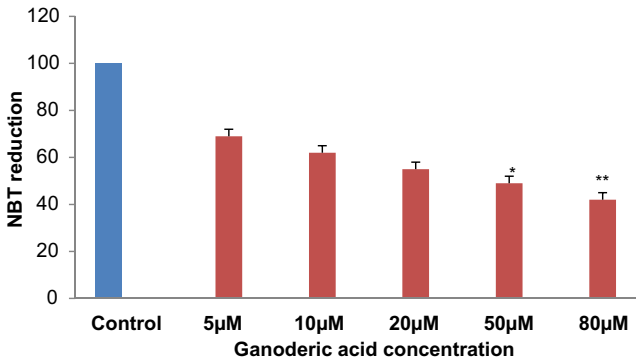

Fig. 9 Ganoderic acid A significantly reduces the cell number of Hep G2 cells in a dose-dependent manner determined by the NBT reduction assay. *$P = 0.05$; **$P = 0.01$ versus control

reactive oxygen species. It showed significant inhibition as compared with control in dose-dependent manner (Fig. 8). In Hep G2 cells, ganoderic acid A causes a reduction of ROS to 50 % in 50 μM and higher concentration in NBT reduction assay (Fig. 9). Thus, the study concludes that ganoderic acid A has a potential to inhibit proliferation, viability, and ROS in the Hep G2 cells.

Conclusion

Receptor tyrosine kinases play an important role in the functioning of cell proliferation, differentiation, and survivability. Minute irregularity in processing of RTK and its signaling network by mutation and chromosomonal instability may lead to cancer. Targeting RTKs provides the platform to hinder numerous adapter molecules important in the physiology of the cell. The present study of molecular docking of different receptors provides an in-depth understanding of protein–ligand interaction, which provides strength to the cell inhibitory activity as well as for ADMET studies. Natural products are accredited with different constituents having broad range of activity particularly in cancer. Ganoderic acid A significantly decreases proliferation, viability, and ROS in the Hep G2 cells and proves to be a better option for drug designing. The present working group is engaged in finding the anticancer property of *G. lucidum*.

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Compliance with ethical standards

Conflicts of interest None

References

1. Croce CM. Oncogenes and cancer. N Engl J Med. 2008;358(5):502–11.
2. Anand SS, Gill BS. Breakthroughs in epigenetics. Pharm Tutor. 2015;3(7):16–24.
3. Ullrich A, Schlessinger J. Signal transduction by receptors with tyrosine kinase activity. Cell. 1990;61(2):203–12.
4. Negi A, Gill B. Success stories of enolate form of drugs. Pharm Tutor. 2013;1(2):45–53.
5. Zwick E, Bange J, Ullrich A. Receptor tyrosine kinase signalling as a target for cancer intervention strategies. Endocr Relat Cancer. 2001;8(3):161–73.
6. Gill BS, Kumar S. Triterpenes in cancer: significance and their influence. Mol Biol Rep. 2016;43(9):881–96. doi:10.1007/s11033-016-4032-9.
7. Schlessinger J. Cell signaling by receptor tyrosine kinases. Cell. 2000;103(2):211–25.
8. Gill BS, Alex JM, Kumar S. Missing link between microRNA and prostate cancer. Tumor Biol. 2016;37(5):5683–704.
9. Vogelstein B, Kinzler KW. The multistep nature of cancer. Trends Genet. 1993;9(4):138–41.
10. Negi ABG, Anand SS. Tilling: versatile reverse genetic tool. Pharma Tutor. 2014;2(1):26–32.
11. Hollosy F, Keri G. Plant-derived protein tyrosine kinase inhibitors as anticancer agents. Curr Med Chem Anticancer Agents. 2004;4(2):173–97.
12. Gill BS, Sharma P, Kumar R, Kumar S. Misconstrued versatility of *Ganoderma lucidum*: a key player in multi-targeted cellular signaling. Tumor Biol. 2015;37(3):2789–804.
13. Yuen JW, Gohel MDI. Anticancer effects of *Ganoderma lucidum*: a review of scientific evidence. Nutr Cancer. 2005;53(1):11–7.
14. Gill BS, Kumar S. Differential algorithms-assisted molecular modeling-based identification of mechanistic binding of ganoderic acids. Med Chem Res. 2015;24(9):3483–93.
15. Liu J, Shiono J, Shimizu K, Kukita A, Kukita T, Kondo R. Ganoderic acid DM: anti-androgenic osteoclastogenesis inhibitor. Bioorg Med Chem Lett. 2009;19(8):2154–7.
16. Yao X, Li G, Xu H, Lü C. Inhibition of the JAK-STAT3 signaling pathway by ganoderic acid A enhances chemosensitivity of HepG2 cells to cisplatin. Planta Med. 2012;78(16):1740–8.
17. Chen N-H, Liu J-W, Zhong J-J. Ganoderic acid Me inhibits tumor invasion through down-regulating matrix metalloproteinases 2/9 gene expression. J Pharmacol Sci. 2008;108(2):212–6.
18. Hong R-L, Spohn WH, Hung M-C. Curcumin inhibits tyrosine kinase activity of p185neu and also depletes p185neu. Clin Cancer Res. 1999;5(7):1884–91.
19. Huang YT, Hwang JJ, Lee PP, Ke FC, Huang JH, Huang CJ, et al. Effects of luteolin and quercetin, inhibitors of tyrosine kinase, on cell growth and metastasis-associated properties in A431 cells overexpressing epidermal growth factor receptor. Br J Pharmacol. 1999;128(5):999–1010.
20. Kwon HJ, Shim JS, Kim JH, Cho HY, Yum YN, Kim SH, et al. Betulinic acid inhibits growth factor-induced in vitro angiogenesis via the modulation of mitochondrial function in endothelial cells. Cancer Sci. 2002;93(4):417–25.
21. Zhang X, Mukerji R, Samadi AK, Cohen MS. Down-regulation of estrogen receptor-alpha and rearranged during transfection tyrosine kinase is associated with withaferin a-induced apoptosis in MCF-7 breast cancer cells. BMC Complement Altern Med. 2011;11(1):84.
22. Zhang W, Hong D, Zhou Y, Zhang Y, Shen Q, Li J-Y, et al. Ursolic acid and its derivative inhibit protein tyrosine phosphatase 1B, enhancing insulin receptor phosphorylation and stimulating glucose uptake. BBA Gen Subjects. 2006;1760(10):1505–12.
23. Mu X, Shi W, Sun L, Li H, Jiang Z, Zhang L. Pristimerin, a triterpenoid, inhibits tumor angiogenesis by targeting VEGFR2 activation. Molecules. 2012;17(6):6854–68.
24. Jorgensen WL, Maxwell DS, Tirado-Rives J. Development and testing of the OPLS all-atom force field on conformational energetics and properties of organic liquids. J Am Chem Soc. 1996;118(45):11225–36.
25. Jorgensen WL, Tirado-Rives J. The OPLS [optimized potentials for liquid simulations] potential functions for proteins, energy minimizations for crystals of cyclic peptides and crambin. J Am Chem Soc. 1988;110(6):1657–66.
26. Shivakumar D, Williams J, Wu Y, Damm W, Shelley J, Sherman W. Prediction of absolute solvation free energies using molecular dynamics free energy perturbation and the OPLS force field. J Chem Theory Comput. 2010;6(5):1509–19.

27. Repasky MP, Shelley M, Friesner RA. Flexible ligand docking with GLIDE. Curr Protoc Bioinform. 2007;8.12.1–8.12.36
28. Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ, Mainz DT, et al. GLIDE: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. J Med Chem. 2004;47(7):1739–49.
29. Friesner RA, Murphy RB, Repasky MP, Frye LL, Greenwood JR, Halgren TA, et al. Extra precision glide: docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes. J Med Chem. 2006;49(21):6177–96.
30. Jorgensen WL, Duffy EM. Prediction of drug solubility from structure. Adv Drug Deliv Rev. 2002;54(3):355–66.
31. Singh P, Bast F. High-throughput virtual screening, identification and in vitro biological evaluation of novel inhibitors of signal transducer and activator of transcription 3. Med Chem Res. 2015;24(6):2694–708.
32. Gill BS, Navgeet, Kumar S. Ganoderic acid A targeting β-catenin in Wnt signaling pathway: in silico and in vitro study. Interdisciplinar Sci Comput Life Sci. 2016. doi:10.1007/s12539-016-0182-7.