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ORIGINAL RESEARCH ARTICLE

Ganoderic Acid A Targeting β-Catenin in Wnt Signaling Pathway: In Silico and In Vitro Study

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Abstract Wnt signaling pathways are the group of signaling transduction controlling the embryonic development, cell proliferation, cell migration, cell fate specification, and body axis pattern. Nuclear accumulation of β-catenin in Wnt signaling is a widely recognized marker of poor cancer prognosis which regulates fat and glucose metabolism. Ganoderic acid is a triterpene isolated from fungus *Ganoderma lucidum* renowned for its pharmacological effects. The present study revealed the mechanistic study of β-catenin with 50 isoforms of ganoderic acid by molecular docking using Maestro 9.6 (Schrödinger Inc) in Wnt signaling pathway. Molecular docking reveals the binding interaction of β-catenin and ganoderic acid A with GScore (−9.44), kcal/mol, lipophilic EvdW (−2.86), electro (−0.72), Glide emodel (−50.401), MM-GBSA (−87.441), H bond (−1.91) with Lys 180 and Asn 220 residues involved in hydrogen bonding. Qikprop analyzed the absorption, distribution, metabolism, excretion, and toxicity and confirmed that most of the isoforms satisfies Lipinski rule but needs little modifications in their structure. The ganoderic acid A is the best-docked isoforms which inhibits the proliferation, viability, and intracellular ROS of pancreatic cancer RIN-5F cells in a dose-dependent manner.

Keywords Wnt signaling · β-catenin · Ganoderic acid · Diabetes II · Molecular docking

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Abbreviations

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

1 Introduction

Wnt signaling pathways are the group of signaling transduction pathway that passes the signal from extracellular to the intercellular. Wnt signaling role is a global regulator of embryonic development and controls cell proliferation, cell migration, cell fate specification, and pattern of the body axis [1]. The canonical Wnt signaling pathway is activated by binding Wnt protein to frizzled family receptor, which results in downstream signaling in the cell and regulates gene transcription. Upon activation of frizzled receptors, phosphoprotein Dishevelled (Dsh) in the cytoplasm gets a signal and transmitted to frizzled family receptor and Dsh in the cytoplasm [2]. In canonical Wnt pathway, β-catenin gets accumulated in the cytoplasm and further translocated into the nucleus and assist in activation of gene transcription (Fig. 1). Without Wnt signaling, there is no destruction of β-catenin in the cytoplasm by destruction complex of Axin, adenomatosis, polyposis coli (APC), casein kinase 1α (CK1α), glycogen synthase kinase 3 (GSK3), and protein phosphatase 2A (PP2A) [3]. Degradation of complex results in no accumulation of β-catenin in the cytoplasm as in non-canonical Wnt pathway. Nuclear accumulation of β-catenin is a widely recognized marker of poor cancer prognosis, makes cancer cell proliferation and regulates fat

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and glucose metabolism [4]. Beta-catenin/Wnt signaling involves in pancreas development, islet function, insulin secretion, production during diabetes [5]. Mutation in Wnt signaling pathway leads to numerous diseases such as breast and prostate cancer, type II diabetes, and glioblastoma [6, 7]. β-catenin is the core target of Wnt signaling by engaging in crucial protein interactions by behaving as both negative and positive effectors in the pathway. Direct interference of these interactions in β-catenin toward the modulation of their expression makes it hot spot target in drug designing [8].

Numerous discoveries and drug developments make the diagnosis of diseases facile [9], but the effective treatment strategy remains a mystery [10]. Natural products have pharmacological or biological activity and which modulates the signaling pathway in the cancer [11, 12]. One such renowned natural product is *Ganoderma lucidum*, polypore family of mushrooms with myriad therapeutic indications [13, 14]. Among different bio-constituents in *G. lucidum*, ganoderic acid was explored for revealing mechanism in different cancer pathways [15, 16]. In the present study, molecular docking was performed on β-catenin with 50 isoforms of ganoderic acid along with natural inhibitors to reveal the mechanistic binding of β-catenin in the Wnt canonical pathway.

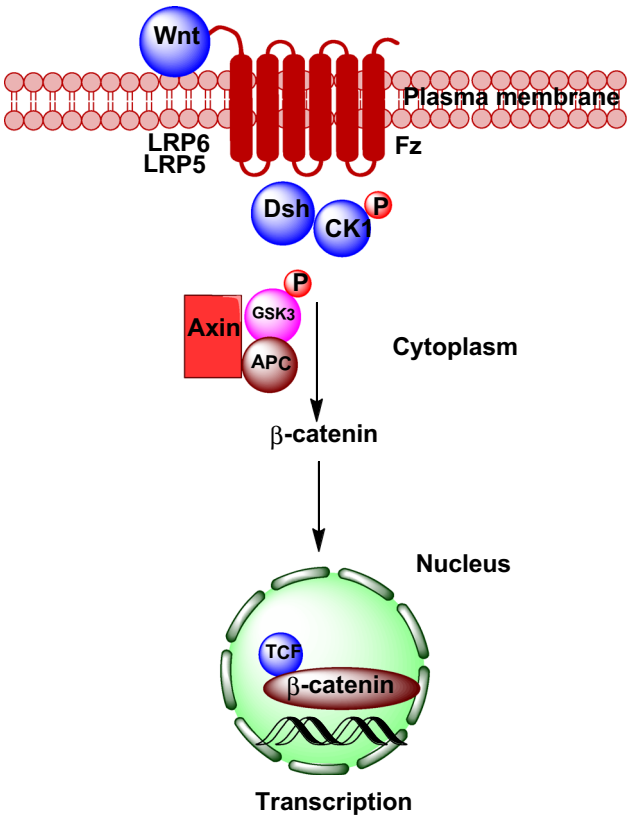

Fig. 1 Activation of canonical Wnt signaling pathway. Wnt protein binds and activates Frizzled (Fz) family receptor along with co-receptor. Upon activation, it activates phosphoprotein Dishevelled (Dsh) with the removal of axin that result in the destruction of the complex. Destruction complex comprises of axin, adenomatosis, polyposis coli (APC), casein kinase 1α (CK1α), glycogen synthase kinase 3 (GSK3), and protein phosphatase 2A (PP2A). Destruction of the complex results in the accumulation of β-catenin in the cytoplasm which later translocates to the nucleus and induces cellular response via TCF/LEF (T-cell factor/lymphoid enhancing factors)

2 Methodology

2.1 Preparation of Ligands and Protein Molecule

The protein crystallized structure was retrieved from Protein Data Bank site with β-catenin (PDB; 4DJS) [8]. Later, modification in protein's structure includes removal of water molecules in concern to the domain topology that usually interacts with hydrophobic regions. Subsequently, polar hydrogen is incorporated to fill the inappropriate valency of the protein atoms, ensuing in increased polarizability of bonds. The increased polarizability emphasized better modeling of the structure, for the computational work being carried out, which ultimately enhanced the probability of ligand–protein interactions. This processed protein structure was further 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 β-catenin inhibitor structures achieved using the software ChemBioDraw Office [16] (licensed @ Cambridge's soft). Natural inhibitors of β-catenin in Wnt signaling include quercetin, ursolic acid, curcumin, and others [17–19]. These compounds were subjected to the Ligprep for the ligand preparation by 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 terminal optimization of bond lengths, and fixing of missing residues in order make protein ready for docking. The prepared structure was then minimized and optimized in the optimized potential for the liquid simulations (OPLS 2005) force field to acquire an energetically stable geometry [20–23].

2.2 Receptor Grid Formation

Grid pre-calculates grid maps of interaction energies for various atom types, such as aliphatic carbons, aromatic carbons, and hydrogen bonding oxygen, with a macromolecule such as a protein, DNA or RNA before docking [16]. These grid maps are then used by GLIDE docking calculations to determine the total interaction energy for a ligand with a macromolecule. Grid mapping calculates the crucial coordinates over the protein atomic data and assigns

the coordinates of the β-catenin for docking. In addition, grid mapping affords an appropriate surface topology for the ligand atoms for interaction with the β-catenin domain. Grid mapping is a prerequisite to direct different isoforms of ganoderic acids to look for their region of the strong affinity of the β-catenin domain. Grid was generated for the search of favorable interaction and best post during docking which represent conformation, position, orientation about the receptors. Other parameters such as sites, constraints, rotatable groups, and excluded volume, which are the default setting of the Maestro 9.6, are used [24].

2.3 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. GLIDE docking tools predict the systematic and computational simulation method for predicting best binding orientation to the protein target. GLIDE molecular docking output GScore (empirical scoring function) is a combination of various parameters essential for binding energy. The GScore is calculated by calculating ligand–protein interaction energies, root mean square deviation (RMSD), hydrogen bonds, hydrophobic interactions, internal energy, π–π stacking interactions, and desolvation [16]. GLIDE module of the XP visualizer analyzes the specific ligand–protein interactions. Ligands were docked with the X-ray crystal structure of β-catenin (PDB; 4DJS), 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 [25, 26].

2.4 Prime/MM-GBSA Simulation (Free Energy Calculation)

The proper solvation facilitates the process of molecular recognition. The solvation effect estimates the binding affinities by MM-GBSA scoring, where MM means molecular mechanics, GB generalized born, SA solvent accessible surface area. In MM-GBSA simulation, electrostatics of ligand–receptor complex and solvation were analyzed which has an edge over prior methods of Coulomb-based terms. The Prime/MM-GBSA approach was used to calculate free energy and calculates the binding affinity of the receptor and the ligands [27]. The simulation was based on the structure obtained by receptor-based ligand molecular docking. The ligand poses were minimized by Prime, whereas energy of the complex was obtained by OPLS-2005 force field with VSGB2.0 solvent model in Generalized-Born/Surface Area continuum solvent model [28].

$$\Delta G_{\text{bind}} = \Delta E + \Delta G_{\text{solv}} + \Delta G_{\text{SA}}$$

$$\Delta E = E_{\text{complex}} - E_{\text{protein}} - E_{\text{ligand}}$$

where ΔE is the minimized energy of complex, protein and ligand

$$\Delta G_{\text{solv}} = G_{\text{solv(complex)}} - G_{\text{solv(protein)}} - G_{\text{solv(ligand)}}$$

where ΔG is the salvation free energy of the complex protein and ligand

$$\Delta G_{\text{SA}} = G_{\text{SA(complex)}} - G_{\text{SA(protein)}} - G_{\text{SA(ligand)}}$$

where ΔG_{SA} is surface area energy of the complex protein and ligand.

2.5 ADME Properties

Qikprop is an important tool that calculates properties of the significant 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 to 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 QP log Po/w, QP log BB, overall CNS activity, Caco-2, MDCK cell permeability, logKhsa for human serum albumin binding, the percentage of human oral absorption [29].

3 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. Pancreatic cancer cell line (RIN-5F) used in the study was procured from National Centre for Cell Sciences, Pune, India. The cell line was carefully maintained in RPMI media 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.

3.1 MTT Cell Proliferation Assay

Effect of different treatments of ganoderic acid A on the growth of pancreatic cancer cells (RIN-5F) was assessed by using the MTT assay in triplicates. Approximately 10,000 cells were seeded in the 96-well plate in RPMI 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 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 dimethyl sulfoxide (DMSO). Absorbance was measured at 570 nm using microplate reader.

3.2 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 RIN-5F 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 after 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 were calculated and used as an indicator of the degree of cell death.

3.3 Effect of Ganoderic Acid A ROS Levels (H_2DCF-DA Assay)

Intracellular 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. RIN-5F cells (2×10^5) were seeded in six-well culture plates and treated with ganoderic acid (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.

3.4 Statistical Analysis

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

4 Results and Discussion

The Wnt signaling pathway is an evolutionarily conserved pathway regulating cell fate determination, cell polarity, cell migration, neural patterning, and organogenesis during embryonic development. Aberrant activation of β-catenin in the Wnt signaling pathway is strongly intricated in the onset and progression of cancer and diabetes [8]. Present receptor-based molecular docking of β-catenin against 50 isoforms of ganoderic acid was carried out using Maestro 9.3 (Schrodinger 2012). Ganoderic acid (GA) with 50

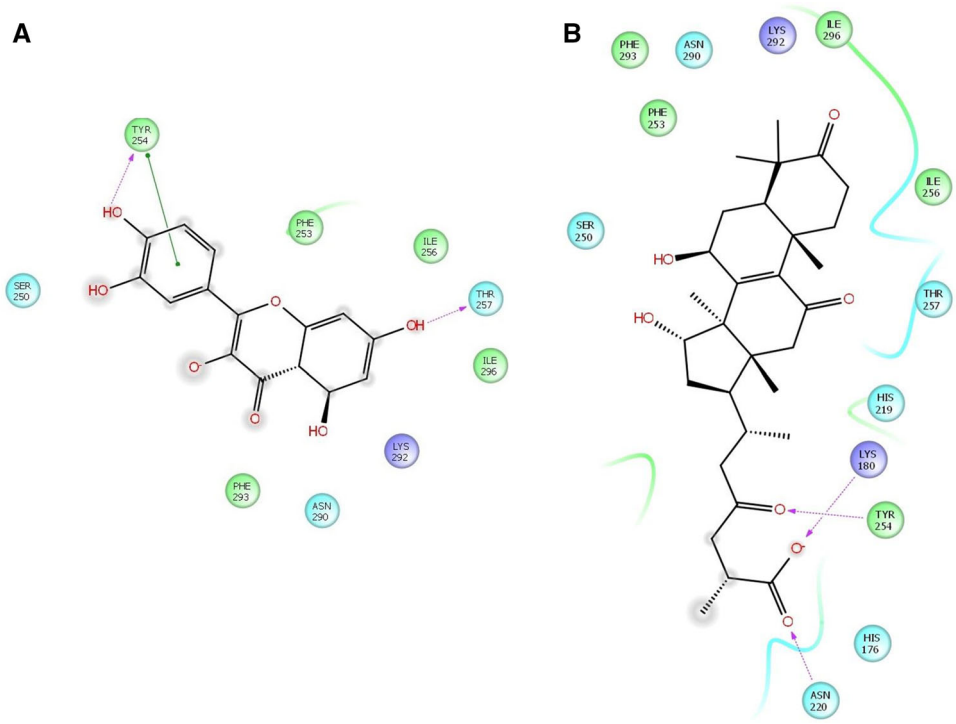

Fig. 2 Protein–ligand interaction profile of β-catenin (PDB: 4DJS) with ganoderic acid A and quercetin. Protein–ligand interaction profile revealed that residues involved in ganoderic acid binding acid were Arg 1136, Asn 1132, Asn 1137, whereas Lys 1030 and Glu 1077 were involved in quercetin

Table 1 Binding affinities, scores, and interaction of β-catenin with different isoforms of ganoderic acid along with natural inhibitors

S. no.	Ligand type	GScore (kcal/mol)	Lipophilic EvdW	Electro	Glide emodel	H bond	H bond length	MM-GBSA (kcal/mol)	Protein–ligand interaction
1	Ganoderic acid A	−9.44	−2.86	−0.72	−50.401	−1.91	1.34	−87.441	Lys 180,
							2.11		Asn 220
2	Ganodermanontriol	−8.088	−2.99	−0.61	−47.647	−2.7	2.51	−82.376	Asn 220
3	Ganoderic acid Lm2	−7.78	−2.25	−0.84	−41.949	−2.7	3.29	−72.267	Arg 212,
							1.78		Lys 180,
							1.12		Tyr 254
4	Ganoderic acid theta	−6.78	−2.6	−0.81	−45.361	−2.67	2.76	−79.261	Arg 212,
							1.78		Lys 180,
							3.02		Tyr 254
5	Ganoderiol A	−6.71	−2.67	−0.89	−43.065	−2.56	2.88	−78.543	Asn 220
6	Quercetin	−6.59	−2.01	−0.59	−33.771	−2.54	2.22	−81.466	Tyr 254,
							1.98		Thr 257
7	Ganoderic acid alpha	−5.52	−2.33	−0.99	−29.967	−2.33	2.51	−66.731	Lys 180,
							2.77		Asn 220,
							2.83		Tyr 254
8	Ganoderic acid G	−5.48	−2.8	−0.73	−37.546	−2.17	2.14	−72.565	Lys 180,
							3.74		Asn 220,
							1.41		Tyr 254
9	Ganolucidic acid B	−5.28	−1.5	−2.46	−38.191	−1.95	2.94	−72.451	Tyr 333,
							0.64		Lys 335,
							2.22		Arg 376
10	Ganoderic acid Me	−5.26	−1.69	−2.18	−31.777	−1.92	2.84	−72.229	Lys 335,
							2.75		Arg 376,
							1.98		Lys 292
11	Ganoderic acid D	−5.22	−1.83	−0.87	−37.253	−2.52	2.56	−66.989	Lys 180,
							3.67		Asn 220,
							1.04		Tyr 254,
							2.94		Thr 257
12	Ganoderic acid DF	−5.17	−2.51	−0.96	−46.090	−1.68	2.86	−71.573	Lys 180,
							2.73		Asn 220,
							2.84		Tyr 254
13	Ganoderic acid J	−5.16	−2.38	−0.96	−43.727	−1.68	2.99	−45.231	Lys 180
14	Ganoderic acid T-Q	−5.04	−2.96	−0.51	−43.530	−0.57	2.01	−69.349	Asn 220,
							2.90		Tyr 254
15	Ganoderic acid Mk	−5.04	−1.34	−2.44	−44.221	−1.96	2.72	−70.088	Tyr 333,
							1.69		Lys 335,
							3.02		Arg 376
16	Ganoderic acid C2	−4.92	−2.69	−0.69	−30.523	−1.93	3.82	−67.708	Lys 180,
							2.83		Asn 220,
							3.92		Tyr 254
17	Ganoderic acid K	−4.91	−1.35	−1.1	−41.549	−2.76	2.19	−62.221	Lys 180,
							2.92		Tyr 254,
							0.63		Arg 212,
							4.22		Thr 257
18	Ganoderiol B	−4.89	−3.06	−0.79	−38.073	−1.14	2.52	−44.635	Lys 180,
							3.29		Arg 212,
							4.32		His 176

Table 1 continued

S. no.	Ligand type	GScore (kcal/mol)	Lipophilic EvdW	Electro	Glide emodel	H bond	H bond length	MM-GBSA (kcal/mol)	Protein–ligand interaction
19	Ganoderic acid C1	−4.82	−2.11	−0.71	−33.849	−2.02	3.88	−57.342	Lys 180,
							2.91		Tyr 254
20	Ganolucidic acid A	−4.77	−1.77	−0.74	−27.877	−2.17	2.92	−48.226	Lys 180,
							3.92		Tyr 254
21	Withaferin A	−4.77	−2.42	−0.39	−37.183	−1.91	2.54	−58.188	Lys 180,
							1.37		Tyr 254,
							2.91		Asn 290
22	Ganoderiol F	−4.58	−2.86	−0.39	−40.862	−1.48	1.98	−41.555	His 176
23	Ganoderic acid TR	−4.58	−2.47	−0.48	−37.677	−0.85	2.22	−42.122	Lys 180,
							3.92		Asn 220
24	Ganoderic acid C6	−4.56	−2.32	−0.69	−46.860	−1.28	2.93	−47.078	Asn 290
25	Ganoderic acid T	−4.5	−2.59	−0.82	−26.811	−1.48	2.57	−45.908	Tyr 254,
							2.82		Asn 290,
							3.92		Lys 292,
							2.12		Lys 335
26	Ganosporeric acid A	−4.5	−2.36	−0.84	−47.864	−1.14	2.74	−44.732	Lys 180,
							2.94		Asn 220,
							1.98		Tyr 254
27	Ganoderic acid Am1	−4.47	−2.21	−0.8	−33.001	−1.72	2.83	−47.837	Lys 180,
							3.22		Asn 220,
							2.22		Tyr 254
28	Ganoderic acid Sz	−4.47	−3.06	−0.6	−39.824	−0.97	3.82	−43.668	Lys 180,
							2.62		Asn 220
29	Ganoderic acid X	−4.44	−2.15	−0.66	−40.375	−1.81	2.72	−45.827	Asn 290,
							4.33		Lys 292
30	Ganoderic acid S	−4.4	−2.99	−0.4	−40.739	−0.35	2.92	−40.463	Lys 180,
							1.52		Asn 220,
							2.11		Tyr 254
31	Methyl ganoderate D	−4.2	−2.36	−0.46	−41.273	−1.51	2.76	−39.537	Tyr 254,
							2.72		Arg 212
32	Curcumin	−4.07	−3.1	−0.2	−52.277	−0.96	2.92	−51.968	Asn 290,
							2.94		Lys 335,
							3.82		Asp 299
33	Ganoderic acid E	−3.99	−2.79	−0.57	−47.309	−0.38	2.92	−34.293	Arg 212,
							3.23		Asn 290
34	Ganoderic acid TR1	−3.98	−2.41	−0.61	−39.206	−1.04	2.82	−36.643	Lys 180,
							2.72		Asn 220
35	Ganodermatriol	−3.94	−2.55	−0.65	−38.619	−1.31	1.72	−37.836	Lys 180,
							1.92		Asn 220
36	Lucidenic acid P	−3.94	−1.79	−0.76	−32.108	−1.92	3.22	−44.551	Lys 292,
							1.83		Thr 257
37	Ganoderic acid R	−3.84	−1.89	−0.67	−34.543	−1.78	3.42	−33.748	Lys 292,
							2.81		Asn 290,
							2.82		Arg 212
38	Ganodermic acid S	−3.85	−1.89	−0.67	−34.543	−1.78	2.02	−35.675	Lys 292,
							3.82		Asn 290,
							2.91		Arg 212
39	Ganoderic acid B	−3.81	−2.81	−0.49	−41.891	−0.31	2.01	−30.564	Asn 290

Table 1 continued

S. no.	Ligand type	GScore (kcal/mol)	Lipophilic EvdW	Electro	Glide emodel	H bond	H bond length	MM-GBSA (kcal/mol)	Protein–ligand interaction
40	Pristimerin	−3.8	−1.81	−0.44	−31.900	−1.63	2.92	−41.667	Asn 220,
							2.99		Tyr 254
41	Ganoderic acid Y	−3.74	−2.06	−0.52	−32.911	−1.6	2.03	−31.763	Lys 292
42	Lucidenic acid A	−3.7	−2.14	−0.6	−37.102	−1.05	1.55	−44.867	Tyr 254,
							0.79		Asn 290
43	Ganoderic acid H	−3.6	−2.31	−0.43	−40.334	−0.79	2.18	−29.692	Tyr 254,
							2.92		Lys 292,
							1.99		Tyr 333
44	Lucidenic acid C	−3.53	−2.24	−0.58	−36.487	−0.88	2.92	−39.672	Asn 220,
							1.52		Tyr 254
45	Ganoderic acid B methyl ester	−3.5	−1.61	−0.44	−31.331	−1.83	2.81	−29.299	Arg 212,
							2.93		Ser 250,
							1.88		His 219
46	Ganoderic acid beta	−3.49	−2.68	−0.52	−38.517	−0.61	2.56	−29.287	Lys 180,
							1.91		Asn 220
47	Ursolic acid	−3.42	−1.82	−0.59	−28.147	−1.19	1.92	−33.562	Lys 292,
							2.22		Asn 261
48	Betulinic acid	−3.4	−2.53	−0.24	−36.705	−0.7	2.82	−35.631	Asn 261
49	Ganoderic acid F	−3.34	−2.14	−0.48	−37.505	−0.66	2.91	−27.565	Tyr 254,
							0.79		Lys 292,
							2.88		Thr 257
50	Ganolucidic acid E	−3.21	−2.06	−0.52	−33.554	−0.84	2.83	−27.735	Lys 180,
							3.32		Asn 220
51	Lucialdehyde B	−3.19	−2.66	−0.22	−37.555	−0.35	3.76	−22.576	Lys 180
52	Lucialdehyde C	−3.17	−2.37	−0.32	−31.189	−0.92	2.93	−27.455	Asn 290,
							2.78		Arg 212
53	Tirucallol	−2.96	−2.34	−0.17	−18.012	−0.56	2.73	−31.886	Tyr 254
54	Ganoderic acid DM	−2.85	−2.07	−0.51	−38.115	−1.8	3.22	−24.451	Lys 180,
							2.74		Arg 212
55	Ganoderol B	−2.83	−1.74	−0.62	−6.188	−1.05	2.93	−25.766	His 235,
							2.66		Lys 335
56	Celastrol	−2.21	−1.85	−0.21	−16.577	−0.48	3.82	−31.781	Asn 290

Predicted IC50 value for blockage of HERG K+ channels; (acceptable range: above −5.0); QPP Caco, predicted apparent Caco-2 cell permeability in nm/s. Caco-2 cells are a model for the gut–blood barrier; (nm/s) <25-poor >500-great; QPlogBB, predicted brain/blood partition coefficient; QPP MDCK, predicted apparent MDCK cell permeability in nm/s. MDCK cells are considered to be a good mimic for the blood–brain barrier; (nm/s) <25-poor >500-great; QP log KP, predicted skin permeability; percentage of human oral absorption; (<25 % is poor and >80 % is high)

isoforms, triterpenes of *G. lucidum,* was docked with X-ray crystal structures of β-catenin retrieved from Protein Data Bank. The crystal structure of beta-catenin in complex with a stapled peptide inhibitor was determined with 3.03 Å resolutions. Triterpene of *G. lucidum*, ganoderic acid, has lanosterol scaffold, and variation in its isoforms varies to the functional group or in the side chain. The structure of receptor complexes with ganoderic acid highlighted the lipophilic, electrostatic, and hydrogen bonding interaction and considered to be the main contributor in protein–ligand interaction. Molecular docking identifies thermodynamic optimal energy value, the potential of bonding, nature of interactions, conformations or poses against receptor protein molecules.

Ganoderic acid A with β-catenin exhibits docking parameters such as GScore (−9.44), lipophilic Evdw (−2.86), Glide emodel (−50.401), H Bond (−1.91), and electro (−0.72). Lys 180 and Asn 220 form the hydrophobic interaction in the stability of the complex. Lys 180 and Asn 220 donate electrons to ganoderic acid forming hydrogen bonding, whereas other covalent and non-covalent were also contributing to the stability

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

Molecule	M.W	Dipole	Q P log Po/w (−2.0 to 6.5)	Q P log HERG (acceptable range: above −5.0)	QPP Caco (nm/s) <25-poor >500-great	Q P log BB (−3 to 1.2)	QPP MDCK (nm/s)	Q Plog Kp (−8.0 to −0.1)
Ganoderic acid A	516.673	8.844	2.767	−2.26	114.664	−2.126	116.557	−5.092
Ganodermanontriol	472.707	3.404	4.842	−4.409	354.54	−1.452	161.284	−3.362
Ganoderic acid Lm2	514.658	3.39	2.793	−2.524	11.115	−2.261	24.86	−5.387
Ganoderic acid theta	530.657	1.877	1.939	−2.627	6.935	−2.577	2.919	−5.701
Ganoderiol A	474.723	5.159	4.677	−4.187	337.672	−1.485	153.006	−3.306
Quercetin	304.256	6.3	0.197	−4.868	24.218	−2.247	8.867	−5.379
Ganoderic acid alpha	574.71	7.377	2.635	−2.304	110.911	−2.329	41.76	−5.245
Ganoderic acid G	532.673	6.607	1.935	−2.307	110.073	−2.364	44.37	−5.312
Ganolucidic acid B	502.69	4.604	3.941	−2.503	40.341	−1.757	19.578	−4.238
Ganoderic acid Me	554.765	6.321	6.925	−3.145	56.25	−1.678	28.043	−3.936
Ganoderic acid D	514.658	6.938	2.802	−2.437	14.133	−2.141	46.301	−5.219
Ganoderic acid DF	516.673	9.224	3.001	−2.464	18.581	−2.089	78.469	−4.892
Ganoderic acid J	514.658	4.45	2.89	−2.548	15.25	−2.145	6.841	−5.155
Ganoderic acid T-Q	510.712	3.298	6.074	−2.219	137.032	−1.01	73.417	−3.279
Ganoderic acid Mk	556.738	9.803	5.827	−3.353	24.417	−2.202	11.378	−4.526
Ganoderic acid C2	518.689	4.712	2.856	−2.46	17.226	−2.181	7.804	−4.859
Ganoderic acid K	574.71	3.633	2.647	−2.364	12.637	−2.286	5.583	−5.121
Ganoderiol B	470.691	2.364	4.379	−4.499	274.921	−1.573	122.518	−3.539
Ganoderic acid C1	514.658	7.231	2.844	−2.383	18.525	−2.004	8.442	−4.99
Ganolucidic acid A	500.674	12.406	3.793	−2.508	26.706	−1.882	12.535	−4.682
Withaferin A	470.605	6.239	3.049	−4.49	240.817	−1.345	106.176	−3.9
Ganoderiol F	454.692	2.938	5.504	−4.506	799.286	−1.037	388.314	−2.724
Ganoderic acid TR	468.675	3.63	5.434	−2.693	74.026	−1.371	37.734	−3.793
Ganoderic acid C6	530.657	6.721	2.098	−2.383	18.009	−2.077	8.188	−4.918
Ganoderic acid T	612.802	11.873	6.495	−3.337	11.629	−2.595	5.104	−5.214
Ganosporeric acid A	526.625	9.711	1.758	−2.49	10.944	−2.212	4.779	−5.531
Ganoderic acid Am1	514.658	7.354	2.714	−2.229	15.535	−2.032	6.979	−5.139
Ganoderic acid Sz	452.676	4.855	6.425	−2.531	123.917	−1.048	65.852	−3.449
Ganoderic acid X	512.728	9.701	5.754	−2.894	33.831	−1.854	16.187	−4.393
Ganoderic acid S	452.676	5.487	6.536	−2.525	186.23	−0.869	102.282	−3.104
Ganoderic acid Mk	556.738	3.58	5.725	−3.017	42.372	−1.831	20.645	−4.069
Ganoderic acid C6	530.657	9.842	2.045	−2.391	13.827	−2.195	6.154	−5.141
Methyl ganoderate D	528.684	4.773	2.442	−3.892	95.95	−1.799	39.27	−4.761
Curcumin	368.385	3.998	2.901	−6.307	231.398	−2.028	101.695	−2.599
Ganoderic acid E	512.642	5.666	2.695	−2.774	11.335	−2.301	4.964	−5.501
Ganoderic acid TR1	468.675	6.287	5.335	−2.575	63.073	−1.415	31.737	−3.932
Ganodermatriol	456.707	2.241	5.163	−4.371	525.248	−1.263	246.659	−3
Lucidenic acid P	518.646	7.271	2.758	−2.218	26.042	−1.807	12.199	−4.703
Ganoderic acid R	554.765	9.832	6.755	−3.263	19.997	−2.233	9.169	−4.842
Ganodermic acid S	554.765	9.832	6.755	−3.263	19.997	−2.233	9.169	−4.842
Ganoderic acid B	516.673	6.39	2.91	−1.585	32.442	−1.614	15.469	−4.421
Pristimerin	464.644	11.962	5.043	−4.11	392.137	−0.888	179.848	−3.815
Ganoderic acid Y	454.692	4.314	6.146	−2.037	215.161	−0.793	119.56	−2.885
Lucidenic acid A	458.594	6.667	2.693	−2.057	18.689	−1.78	8.523	−5.175
Ganoderic acid H	572.694	2.096	2.85	−3.28	8.177	−2.791	3.488	−5.585
Lucidenic acid C	476.609	2.202	1.991	−1.976	16.329	−1.933	7.366	−5.097

Table 2 continued

Molecule	M.W	Dipole	Q P log Po/w (−2.0 to 6.5)	Q P log HERG (acceptable range: above −5.0)	QPP Caco (nm/s) <25-poor >500-great	Q P log BB (−3 to 1.2)	QPP MDCK (nm/s)	Q Plog Kp (−8.0 to −0.1)
Ganoderic acid B methyl ester	528.684	3.828	2.944	−4.611	106.881	−2.016	44.127	−4.548
Ganoderic acid beta	484.675	2.429	4.48	−2.4	36.23	−1.646	17.43	−4.494
Ursolic acid	456.707	1.837	6.101	−1.71	305.136	−0.386	174.416	−3.061
Betulinic acid	456.707	3.2	5.903	−1.955	351.066	−0.415	202.958	−2.827
Ganoderic acid F	570.678	5.721	2.197	−2.553	4.623	−2.682	1.883	−6.162
Ganolucidic acid E	484.675	5.16	4.517	−2.456	35.63	−1.675	17.119	−4.525
Lucialdehyde B	452.676	4.505	5.097	−4.4	459.091	−1.112	213.256	−3.594
Lucialdehyde C	468.718	3.082	5.467	−4.014	629.238	−1.015	299.842	−3.099
Tirucallol	426.724	1.698	7.373	−4.036	4408.951	−0.081	2459.236	−1.679
Ganoderic acid DM	468.675	7.759	5.617	−2.641	76.055	−1.317	38.852	−3.96
Ganoderol B	440.708	2.609	6.436	−4.652	1464.896	−0.729	747.425	−2.325
Ganoderic acid theta	530.657	7.372	1.615	−2.375	4.921	−2.637	2.014	−6.006
Celastrol	450.617	5.732	4.899	−1.893	72.535	−0.949	36.912	−4.131

represented (Fig. 2). Molecular docking revealed the interaction during binding of other best isoforms with Gscore such as ganodermanontriol (−8.088) ganoderic Acid Lm2 (−7.78) with the involvement of Asn 220, Arg 212, Lys 180, and Tyr 254 residues during the interaction. Molecular docking of β-catenin against the natural compounds revealed that quercetin has best docking parameters with GScore (−6.59), lipophilic Evdw (−2.01), Glide emodel (−33.771), H Bond (−2.54), and electro (−0.59). Residues Tyr 254 and Thr 257 exhibits hydrogen bonding and π–π stacking, which contributes to the stability of complex (Fig. 2). Other natural inhibitors with Gscore Withaferin A (−4.77) and curcumin (−4.07) involves Lys 180, Tyr 254, Asn 290, Asn 290, Lys 335, Asp 299 residues during the interaction. Molecular interaction revealed that Lys 180, Asn 220, Tyr 254, Asn 220, Thr 257, Tyr 333, Lys 335, Arg 376, Arg 212 residues were indulged in the active site during docking process. Different isomers of ganoderic acid along with natural inhibitors exhibit different residues and are presented in (Table 1). Interaction highlighted the involvement of hydrogen, electrostatic, pi–pi stacking, covalent, and non-covalent bonding.

4.1 ADME Properties

Binding affinity prediction is useful for analyzing the pharmacokinetic and pharmacodynamic properties. Qikprop is an important tool that calculates the properties of the important 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. Most interesting aspect of these compounds is their admirable Q P log Po/w, Q P log HERG K channels, Q P log BB, Q P log KP, and Q P log Khsa values that satisfy the Lipinski's rule of five (Table 2). Some of the isomers in different receptors did not satisfy all aspect and needed some modifications in the basic lanosterol structure. The pharmacokinetic and pharmacodynamic parameters of ganoderic acid were found to be satisfactory as in drug-like molecules. Q P log P, Q P log HERG, QPP Caco, Q P log BB, QPP MDCK, Q P log Kp, Q P log Khsa, and percent human oral absorption of ganoderic acid were found to be 2.767, −2.26, 114.664, −2.126, 116.55, −5.092, respectively, and are satisfied by "Lipinski's Rule of five" as in drug-like molecules.

4.2 Effects of Ganoderic Acid A on Cell Growth of Cancer Cells

To measure the cytotoxicity of ganoderic acid A in cancer cells, MTT assay was performed in pancreatic cancer cells (RIN-5F), and the cells 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. 3).

Ganoderic acid A showed up to 50 % of inhibition at 50 mg/mL and above concentration and best marked in 48 h. Moreover, trypan blue exclusion test of cell viability was performed to determine the number of the treatments of ganoderic acid. The viable and dead cells were determined by trypan blue exclusion test.

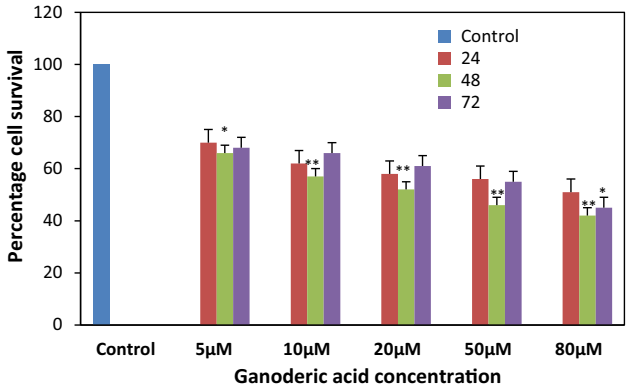

Fig. 3 Ganoderic acid A reduces the cell viability and growth of the RIN-5F cells in a dose-dependent manner determined by the MTT assay. $*P = 0.05$ and $**P = 0.01$ versus control

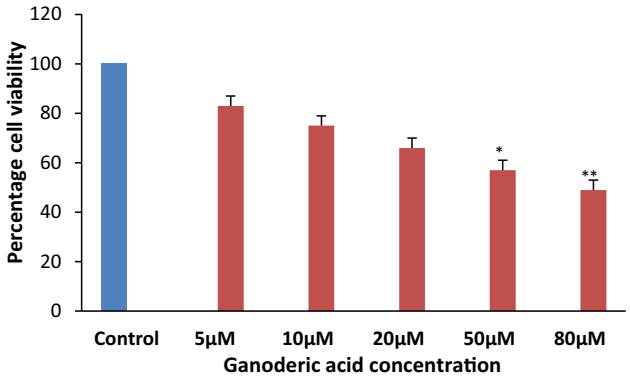

Fig. 4 Ganoderic acid A significantly reduces the cell number of RIN-5F cells in a dose-dependent manner determined by the trypan blue exclusion test of the cell. $*P = 0.05$ and $**P = 0.01$ versus control

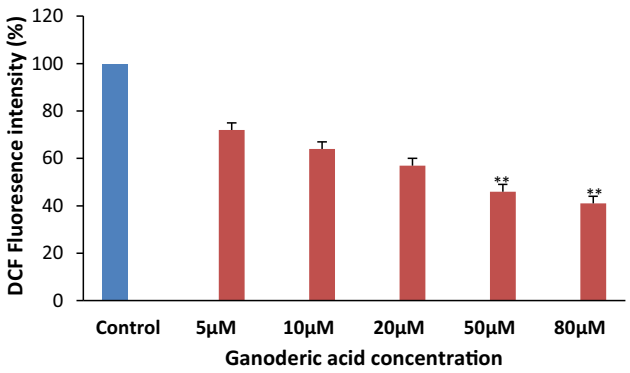

Fig. 5 DCF fluorescence intensity (%) in RIN-5F cells in a dose-dependent manner in response to ganoderic A acid. $*P = 0.05$ and $**P = 0.01$ versus control

Treatment of RIN-5F cells with (5, 10, 20, 50, 80 μM) of ganoderic acid A for the duration of 48 h exhibited a pronounced decrease in cell viability to exactly 17, 25, 34, 43, 51 %, respectively (Fig. 3). Overall, it can be inferred that ganoderic acid A reduced cell viability in a concentration-dependent manner (Fig. 4).

To determine the intracellular ROS scavenging ability of the ganoderic acid A, H_2DCF-DA assay was carried out with RIN-5F cells. The data obtained from the assay highlighted that ganoderic acid A potentially scavenges the reactive oxygen species. It showed 50 % inhibition significantly as compared to control (Fig. 5). It concludes that ganoderic acid A has potential to inhibit RIN-5F cell line.

5 Conclusion

Wnt signaling is well recognized in the development process intriguing embryonic induction, the generation of cell polarity, and the specification of cell fate. Deregulation in the expression of β-catenin in signaling results in various anomalies. The present study provides an in-depth understanding of protein–ligand interaction which provides strength to the cell inhibitory activity as well as for ADMET studies. Ganoderic acid A inhibited cell proliferation and cell viability, and scavenged the reactive oxygen species effectively in pancreatic cells. Ganoderic acid A targets β-catenin in the Wnt signaling pathways and effectively regulates its functioning and formulating drugs. Ganoderic acid A has Lys 180 and Asn 220 active residues actively forming hydrogen bonding and forms the stability of the complex during the interaction. Furthermore, in ADMET studies, some isoforms need some modification in the basic lanosterol scaffold, so it fulfills Lipinski rule. This study throws light on active residues present in a complex of signaling cascade of Wnt signaling.

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

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