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Molecular Docking Analysis of Bacoside A with Selected Signalling Factors Involved in Glioblastoma

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Authors' contributions

This work was carried out in collaboration among all authors. Author PV designed the study, performed in-vitro assay and molecular docking study, evaluated the molecular docking data, analysed the data using statistical methods and wrote the manuscript. Author SS evaluated of molecular docking results and proof-read the manuscript. Author GSK helped in data analysis. The corresponding author VPV suggested the concept, helped in designing the study, supervised the whole research progress, reviewed, corrected and proof-read the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Glioblastoma is the malignant tumor affecting the central nervous system. Despite the advancement in treatment modalities, presence of blood-brain barrier, recurrence after surgical removal, resistance to radiotherapy and chemotherapy remain the major obstacles for long term survival of the patients. In this context, finding suitable therapeutics which have anticancer potential and are nontoxic might be useful to improve the overall survival of GBM. Plant products are safer, nontoxic and cheaper when compared to the chemotherapeutic drugs in trend which are expensive and highly toxic, owing to their systemic effects. Bacoside A (BA) is one such plant constituent isolated from *Bacopa monnieri* which offers neuroprotection and possesses anticancer potential. The present investigation elucidates the specific interaction of BA with various cell surface receptors, signal transduction proteins, effector proteins and transcription factors involved in glioblastoma signalling such as EGFR/Ras/Raf/MAPK pathway, Notch signalling and Wnt-beta

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catenin signalling through molecular docking studies. The interaction between BA and the target proteins of glioblastoma were analysed through the Glide module (Version 6.5) of Schrodinger Suite (2015) software. According to the results of molecular docking, jagged-1 ligands interact with BA with stronger affinity and Frizzled receptors interact with least affinity in terms of glide score. The results indicate that BA interacts well with the polar amino acids such as Asn, Trp, Arg, Ser, Thr, Tyr, Gln, Asp, Lys and Glu. The ligand also showed interactions with specific hydrophobic amino acids such as Val, Ala and Leu in all the protein targets studied. The in vitro cytotoxicity studies reveal the cytotoxic potential of BA on the U87MG glioblastoma cell line. This study warrants further investigation to elucidate modulations on cell signalling pathways resulting from the specific BA-target interactions in glioblastoma.

Keywords: Glioblastoma; Bacopa monnieri; Bacoside A; molecular docking; EGFR signaling; notch signaling; Wnt-beta catenin signaling.

1. INTRODUCTION

Glioblastoma (GBM) is one of the aggressive tumors of the central nervous system which accounts for 57.3% of all gliomas and 48.3% of all malignant brain tumors. Also, the prevalence of GBM increases with age and is normally diagnosed at a median age of 65 years [1,2]. Although the treatment modalities are significantly advanced, the prognosis of the affected individuals remains poor with a mean survival rate of less than 2 years [3,4]. The primary treatment option for glioblastoma includes surgery involving the complete removal of the tumor, followed by treatment with temozolomide (TMZ) and radiotherapy [5,6]. However, current treatment regimens are not satisfactory as they do not seem to improve patient's survival significantly. Further, the major concerns in the treatment of GBM include the presence of blood-brain barrier, recurrence after surgical removal, and resistance to radiotherapy and chemotherapy [7]. Hence, the current study involves identification of suitable anticancer phytochemicals that potentially improve the overall survival of GBM and help to overcome the afore-mentioned issues.

Molecular docking studies have been extensively used in drug designing as it helps to predict the ligand-receptor interaction and to categorize chosen pharmacological compounds on the basis of their binding energy or fitness score [8]. In the present study, important targets involved in GBM pathogenesis were identified by extensive literature study. Pathways such as EGFR/Ras/Raf/MAPK signalling [9], Notch signalling [10] and Wnt/β-catenin signalling [11] were found to be significant in the pathogenesis of GBM and proteins were selected from these pathways for docking. However, the exact mechanism of action of BA in glioblastoma has not been evaluated so far. Hence, in the present study, the interaction of the selected proteins with BA, was analysed through molecular docking. The various parameters considered for evaluating the interaction between the protein and the ligand include: the docking score, docking energy, the interacting amino acids and hydrogen bonding.

2. METHODOLOGY

2.1 Chemicals and Reagents

Bacoside A was purchased from Natural remedies Pvt Ltd, Bangalore, India. DMEM media, Fetal Bovine Serum (FBS), antibiotics and other fine chemicals were obtained from HiMedia Laboratories (Mumbai, India).

2.2 Animal Cell Culture Maintenance

Human GBM cancer cell line (U87MG) was procured from National Centre for Cell Science (NCCS), Pune, India. Cell line was maintained by culturing in DMEM media containing 10% FBS and Penicillin (100 Units/ml), Streptomycin (30 µg/ml) and Gentamicin (20 µg/ml).

2.3 Cell Proliferation Assay - Sulforhodamine B Assay (SRB Assay)

The cell density on the basis of protein content was assessed by SRB assay [12]. Microtitre plates having 96-wells were inoculated with 1 x 10^4 U87MG cells/ml. The media used was removed after 16 hours of attachment to the plate and different concentrations of BA (5, 10, 15, 20, and 25 µM) diluted in incomplete media was added and kept for 24 hours to determine "the effect of the compound on the cell viability" in a dose dependent manner. Once the treatment period was over, 10% (wt/vol) trichloroacetic acid was added to the plate and placed in 4^0C for 1 hr

to fix the cells. Further, the plate was washed well by immersing in tap water in a tray and dried at room temperature. Then, SRB staining solution prepared in 1 % acetic acid was added to the wells and kept at room temperature for 1hr. The SRB stain used was removed by washing the wells four times with 1% (vol/vol) acetic acid. The solubilization of the protein-bound dye was done by the addition of 10 mM Tris base solution followed by shaking the plate for 10 minutes. The absorbance (OD) was determined at 510 nm by using a microplate reader (BioTek, USA). The results were shown as percentage viability.

2.4 Preparation of Protein

The 15 proteins considered for the study were Epidermal growth factor receptor , EGFR (PDB ID: 1IVO), Ras (PDB ID:1CTQ), Raf (PDB ID:3IQJ), Vascular endothelial growth factor receptor, VEGFR (PDB ID:1YWN), Delta like ligand, DLL-1 (PDB ID:4XBM), Jagged-1(PDB ID:4X17), Notch-2 (PDB ID:2OO4), Gamma secretase (PDB ID:5A63), Hes (PDB ID:2MH3), Hey (PDB ID:2DB7), Beta Catenin (PDB ID: 2Z6H), Glycogen synthase kinase 3 beta, GSK 3β (PDB ID:1GNG), Frizzled Receptor (PDB ID:6BD4), Wnt-3 (PDB ID: 2YZQ), p53 (PDB ID:1YC5). The structure of the selected protein targets used for the molecular docking study were retrieved from the PDB (Protein Data Bank, hyperlink: http://www.rcsb.org/pdb/-home/home.do).

Protein structures were refined in order to solve the geometrical inconsistencies and hydrogen bonds were optimized using protein preparation wizard of Glide module Version 6.5) of Schrodinger suite. The selected chains of the target proteins have undergone corrections for missing hydrogen for assigning proper bond orders and overlap searches. Water molecules were removed within a distance of 5 Å which are not involved in ligand binding. Finally, the proteins were minimised to a value of 0.30 Root Mean Square Deviation (RMSD). Both RMSD and OLPS-2005 (optimized potential for liquid simulation) were used to perform the minimization [13].

2.5 Ligand Preparation

The structure of Bacoside A (CID_53398644) was retrieved from PubChem. (https://pubchem.ncbi.nlm.nih.gov/) (Fig. 1). The structure of the ligand was optimized by the LigPrep wizard Schrodinger Maestro 11.9 (Glide). Glide ligand docking jobs require a set of previously calculated receptor grids and one or more ligand structures. Preparation of the ligands before docking is strongly recommended. Corrected Lewis structure was generated for a ligand; it is skipped by the docking job. Glide also automatically skips ligands containing un parametrized elements, such as arsenic, or atom types not supported by the OPLS force fields, such as explicit lone pair “atoms”. Corrections such as 2D to 3D conversion, addition of hydrogen, stereochemistry, low energy state, corrections of bond lengths and bond angles, ring conformations along with minimization and optimizations were done using OPLS3 force field [14].

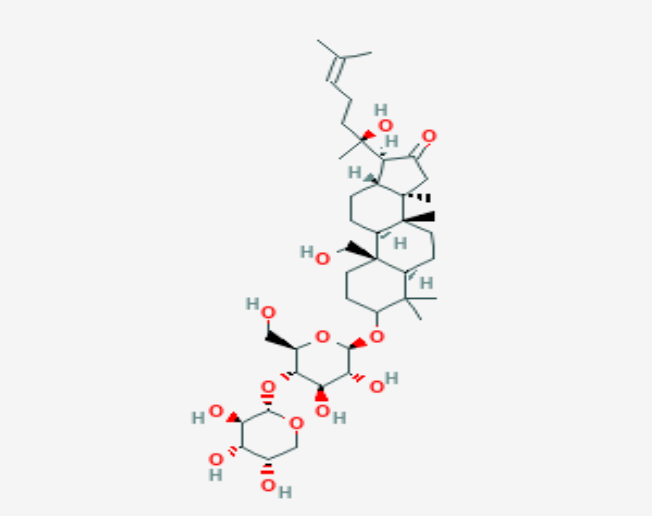


Fig. 1. Structure of Bacoside A retrieved from PubChem

2.6 Grid Generation

The receptor grid was prepared using the grid generation tool of Maestro 11.9 of the Schrödinger suite. The receptor grid can be set up and generated from the Receptor Grid Generation panel. The grid box is generated on the center of the macromolecule. The active site of the receptor is represented by a box at the center of the ligand of interest. Then using the default Glide settings, a grid box centered on the ligand was generated. Ligand docking jobs cannot be performed until the receptor grid has been generated. Receptor grid generation requires a "prepared" structure: an all-atom structure with appropriate bond orders and formal charges. The atoms were of size equal to Van der Waals radii of 1.0 (scaling factor) > while the partial atomic charge was less than 0.25 (van der Waals radius) defaults. The ligand is able to bind forming the achievable conformation using this receptor grid which also highlights the active site of the protein as with the co-crystallized ligand molecule. This co-crystallized ligand will then be removed from the active site to be occupied by our ligand of interest [14].

2.7 Molecular Docking

The Docking analysis and characterization of binding pocket was performed by using the accomplished using Maestro-GLIDE module of the Schrödinger suite [15-17]. The optimized ligand was then docked in a flexible manner to the active site of the protein drawn within the grid box using the extra precision (XP) feature of Glide module, version 5.6, 2010 [18]. It also helps in generating the 2D ligand interaction diagram corresponding to analysis of each protein-ligand interaction. A post docking energy minimization was applied in the analysis. The characterization of binding pockets of the ligand was done by generating Different molecular interactions represented by varying colour patterns and shapes.

2.8 Selection of the Best-Scored Pose

The best docking poses for the selected protein-ligand were primarily scrutinized by the docking scores but values of different energies, number of H bonds, and visual inspection of all docking poses in Maestro (Schrodinger, USA) were also taken into account. Interaction energy between protein and ligand can be related to binding affinities. Different criteria were laid down to select the best docked structure for each ligand. Then, rankings were derived by directly using the Glide G Score.

3. RESULTS AND DISCUSSION

3.1 Bacoside A Induced Cytotoxicity Glioblastoma U87MG Cell Lines

In order to ascertain the toxicity of BA in GBM cell line U87MG, cell viability was measured by SRB assay after exposure to BA at various concentrations for 24 hours of treatment. Treatment with BA inhibited GBM cell growth in a dose-dependent manner. The concentration required by the U87MG cells to cause 50% inhibition (IC_{50}) was found to be 22 µM (Fig. 2). Previous literature shows extensive works on the effects of *Bacopa monnieri* and its constituents against different cancer cell lines such as MCF-7 and MDA-MB 231 cell line [19] mammary carcinoma and oral cancer cell lines [20]. Studies suggest that BA inhibits the cell proliferation in Hep G2 cells with an IC_{50} value of 0.625 µg/ml [19]. BA induced Sub G0 arrest in the GBM cell line through the notch signalling pathway and they had found out the IC_{50} value of (83.01 µg/ml) [21].

3.2 Target Protein Selection

Previous literature documents the usefulness of several plants and their products as medhya rasayana (which improve mental health). Brahmi isolated from *Bacopa monnieri* which is traditionally known as "medhya rasayana," because it is found to enhance the cognitive properties of brain and hence is prevalent among the practitioners of Ayurveda, where it is utilised to treat multiple ailments like loss of memory, epilepsy, inflammation, fever, asthma, etc. There exists evidence for the wide spectrum of its activity in treating insomnia, insanity, depression, psychosis, epilepsy, Parkinson's disease, Alzheimer's disease and stress [22]. Several reports suggest the anti-inflammatory, analgesic, antipyretic, sedative, free radical scavenging and anti-lipid peroxidative activities of Brahmi [23-25]. The *in-vitro* and *in-vivo* studies in the recent past have unveiled the significant antitumor and cytotoxic activity of Bacopa extract [26,27] and Bacoside A in many human cancer cell lines, including hepatocellular carcinoma [28,29], sarcoma [30], mammary carcinoma [31], breast cancer [32]. Aithal and Rajeswari [33] reported BA induced cell cycle arrest and apoptosis *via*

notch signalling in glioblastoma. The majority of the pharmacological properties of Bacopa *monnieri* can be attributed to its major phyto constituent Bacoside A which was first reported by Chatterji et al. in 1965 [34].

Epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase which is overexpressed and its aberrant signalling has been observed in GBM. Though several mutations to EGFR are identified, EGFRvIII (EGFR type III, EGFRvIII, del2-7, ΔEGFR) is the most common type among them which is unable to bind to the ligand [35,36]. Hence, its signalling occurs in a constitutive manner. Usually, it is found in association with wild type EGFR [37]. EGFR can regulate various signalling pathways such as PI3K/AKT, RAS/MAPK, and JAK2/STAT. Therefore, EGFR functions as a hub for regulating various cellular processes [38,39]. Small molecule receptor tyrosine kinase inhibitors such as gefitinib, erlotinib, lapatinib, monoclonal antibodies like cetuximab, nimotuzumab, CAR-T Cells Targeting EGFRvIII are some of the treatment modalities targeting EGFR [40].

GBM tissues express high levels of VEGF, an important angiogenic protein with an upregulation of VEGFR2. VEGF binding to VEGFR leads to an increased vascular permeability [41,42]. VEGF binding to VEGFRs on tumour blood vessels also activates endothelial cell proliferation, survival, and migration [43].

Ras is a small GTP-binding protein, which is the common upstream activator molecule of several signaling pathways including Raf/MEK/ERK, PI3K/Akt and Ral/EGF/Ral [44]. Constitutive expression of Ras proteins is observed in 30% of cancers which arise as a result of either the amplification of *ras* proto-oncogenes or due to activation of mutations [45]. Experimental results done on transgenic mice model suggests that activated Ras alone is sufficient to transform normal astrocytes and neuronal precursor cells into malignant gliomas [46,47]. Nevertheless, it contributes an indispensable role in gliomagenesis, activated Ras-Raf signaling axis may also be crucial in the maintenance of malignant gliomas. Moreover, increased Ras-RAF-ERK activity is a common characteristic of malignant gliomas with deregulated EGFR and PDGFR pathways [48]. In contrast, the transcriptional down-regulation of Ras-RAF-ERK and the small molecular inhibitors reduce growth of glioma.

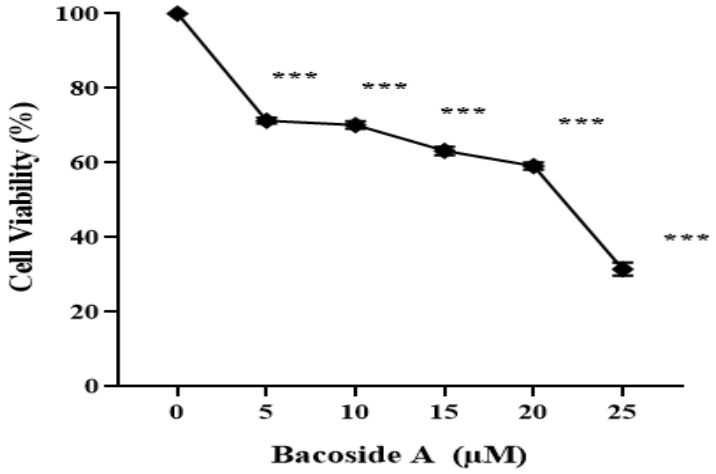


Fig. 2. To determine the cytotoxic effect of Bacoside A, U87MG cells were treated with different concentrations (1-25µM) for 24hrs. Cell viability of U87MG cells checked after the treatment schedule through SRB assay. The Bacoside A induced cytotoxic effects on U87MG cells in a dose depended manner (Fig. 1)

*Data were obtained & analyzed from experiments carried out in triplicates and expressed as Mean ± S.D. ***p<0.001 when compared to control (One-way ANOVA followed by Tukey's multiple comparison test). The IC25 & IC50 values were determined as 12 µM & 22 µM respectively*

Another vital component of Ras/Raf/MEK/ERK signaling pathway is Raf which can also be targeted for GBM treatment. The RAF family consists of three kinases namely A-, B-, and C-RAF/RAF-1. These serine/threonine kinases have a common structure with an N-terminal regulatory region and a C-terminal catalytic domain [49]. Levels of RAF-1, BRAF proteins and RAF kinase activity are increased in human GBM samples and constitutive activation of Raf-1 Induces Glioma Formation in mice [50,51]. Though Sorafenib, a Raf kinase inhibitor, has been tested in combination with Erlotinib (an EGFR tyrosine kinase inhibitor) and in a phase II trial for patients with recurrent GBM, their combinational therapy have not manifested any desired impact due to its failure to reach the goal of a 30% improved survival time. Seemingly, this owes to the pharmacokinetic interaction between the drugs that lessens their efficacy.

Notch signalling often gets triggered in human gliomas and assures the self-renewal ability of glioma stem cells [52]. It follows that the expression of Notch-1 predicts poor patient survival in proneural and classic glioblastomas [53]. Notch is a cytoplasmic receptor and is able to bind to two types of ligands Delta-like (Dll1-3 and -4) and Jagged (Jagged1 and -2) on itself (cis-activation) or a neighbouring cell (transactivation) [54]. In comparison with the normal brain cells, mRNA and protein levels of Notch1, Notch4, Dll1, Dll4, Jagged1, CBF1, Hey1, Hey2, and Hes1 would be higher in brain tumour cells which correlates with an elevated expression of VEGF and pAKT, and reduced levels of PTEN [55,56]. Similarly, differentiated cells within the tumour express higher levels of Dll1 compared to Glioblastoma Stem cells (GSCs), contributing to Notch signalling activation in GSCs [57]. Generally, the Notch activity is measured by the expression levels of its direct target genes. Hey is one of the main downstream effectors of notch signalling and there are reports linking the expression of Hes/Hey transcriptional repressors to cancer prognosis. Recurrently, Hey-1 expresses in GBM among other astrocytomas. Patients expressing higher levels of Hey-1 are related to two-fold shorter disease-free survival in comparison with the patients carrying Hey-1 negative tumours [56]. Notch also activates Hes-1, which is a transcriptional repressor that arrests cell cycle [56]. Receptor-ligand binding triggers sequential cleavage events by a disintegrin and metalloprotease (ADAM) and Gamma secretase that culminate in release of the Notch intracellular domain (NICD) and translocation to the nucleus. Interaction with the RBP-J (or CSL) transcription factor and recruitment of a transcriptional coactivator, mastermind-like family (MAML), then enact changes in gene expression [57]. At present, there is a growing interest in developing therapies targeting Notch signalling pathways at various cascade levels. Monoclonal antibodies, antisense or RNA interference, receptor, and glycosylation/protease inhibitor strategies have been developed targeting NOTCH receptors and ligands. Out of these, γ-secretase inhibitors (GSIs) targeting receptor activation have been examined in different preclinical models and numerous clinical trials as anticancer agents that inhibit active NICD's release from the receptor by the γ-secretase complex [58].

Wnt/*β*-catenin signalling plays a notable role in the proliferation of glioma tumour cells and tumour progression and also promotes growth and invasion through the maintenance of stem cell properties [59,60]. Binding of Wnt ligands to the cell surface receptors like Frizzled and LRP families triggers the signalling which subsequently causes the disassembly of the complex consisting of AXIN, adenomatous polyposis coli (APC), and GSK3*β*, thereby stabilizing *β*-catenin. By virtue of this, *β*-catenin is translocated from the cytoplasm into the nucleus where it forms a complex with T-cell factor/lymphoid enhancer factor (TCF/LEF) and thereby promoting transcription of multiple target genes including c-MYC and cyclin D1 [61]. *β*-catenin is a prognostic marker and its increased levels of mRNA and protein in GBM and astrocytomas of high grade indicates its role in malignant transformation [62]. In addition, constitutive activation of *β*-catenin increased the proliferation of mouse neural progenitor cells *in-vivo*, whereas deletion of *β*-catenin decreased their proliferation and cellular invasion in U87MG and LN229 GBM cells [63]. An increased expression of positive regulators of WNT signalling also found to regulate EMT-associated genes, such as ZEB1, SNAIL, TWIST, SLUG, and N-cadherin, indicating the role of WNT in EMT [64,65]. Hence, targeting Wnt signalling is a novel therapeutic method to kill GBM. For instance, inhibition of GSK3β activity also induces tumour cell differentiation and enhances apoptosis in glioblastoma [66]. Moreover, GSK3β inhibition elevated the level of tumour suppressors p53 and p21 in the cells carrying wild type *TP53* along with downregulation of cyclin-dependent kinase 6 (CDK6) and

decreased RB phosphorylation despite the cell genotype [67].

p53 is a known tumour suppressor which primarily mediates its effect by regulating genes in cell cycle arrest, apoptosis, stem-cell differentiation and cellular senescence. The mutational status of *TP53* is associated with GBM progression and p53 inactivation is correlated with a more invasive, less apoptotic, more proliferative and more stem-like phenotype [68]. Moreover, stress signals, such as DNA damage, hypoxia, heat shock and cold shock elicit a p53 response. The p53 protein further activates p21 that inhibits Cdk4/Cyclin D, Cdk2/Cyclin E complexes and cyclin B which helps to stop cell cycle progression. Due to the vitality of p53 in GBM pathogenesis, a gene therapy approach is also suggested to restore p53 expression [69].

3.2.1 Docking profile of Bacoside A with target proteins

Anticancer potential of phytochemicals or drugs was assessed by means of different *in-vitro*, *in-vivo*, and computational methods. However, molecular docking has been considered as an attractive method for drug designing in any disease [70,71].

Molecular interaction between protein and ligand predicts the binding conformation or pose of the ligand bound to the protein, which can be quantified, based on the shape and electrostatic interaction between the ligand and protein 1. The totality of interaction observed is approximated to be the docking score of the ligand into the binding pocket of the protein [72]. Docking score is expressed in the negative value of energy in Kcal/mol where the lower the negative total energy E, the stronger the interaction between the ligands and the protein 1 [73]. Docking approach predicts the best binding conformation of the compounds at the binding pocket of the protein.

Molecular docking studies were carried out for 15 target proteins from EGFR/Ras/Raf/MAPK pathway, Notch signalling and Wnt-beta catenin pathway with Bacoside A. The docked complexes were further subjected to post docking analysis i.e, energy comparison between current complex energy and minimised complex energy. Both glide score and Glide energy were computed for all 15 protein complexes (Table 1). Conformers are generated for Bacoside A and docked with all target proteins.

Energies were calculated as current energy and minimised energy because the entire protein complex has undergone a minimization process by the macromodel Module from Schrodinger. It was found that invariably the entire protein complex has minimized energy which confirms the stability of protein after its domain interaction. Out of the 15 proteins considered for the study, interaction of BA with jagged 1 showed the least Glide score and hence maximum interaction. The highest Glide score was found when the frizzled receptor was allowed to interact with BA. Schrodinger Glide calculated the total docking score between Jagged-1 and BA. Total docking score of the complex, bond energy and model energy depicts the efficacy of BA in the surface solvent area and the energy it takes to occupy is -82407.026 KJ/mol. Oxygen as a protein atom of Jagged-1 from CYS88 and SER300 were interacted with Hydrogen from ligand (BA) atoms with the bond distance of 2.028 Å, 2.231 Å respectively. The intermolecular interaction is perceived in –OH group as functional which is as side chains of CYS88 and SER300. Hydrogen as a protein atom of Jagged-1from ALA153 interacted with Oxygen from ligand (BA) atom with the bond distance of 2.144 Å. The intermolecular interaction is perceived in –OH group as functional which is as side chains of ALA153.

Out of the 15 proteins docked with BA from 3 different pathways, at least three of the components from each pathway have a glide score less than -8.0. They are EGFR, VEGFR, Raf, p53 from EGFR/Ras/Raf/MAPK pathway, Jagged-1, Gamma secretase and Notch-2 from notch signalling and Gsk-3 beta, wnt-3 and beta-catenin from Wnt-beta catenin signalling. The only receptor that is showing the least interaction out of the 15 protein ligand interaction I frizzled receptor and the glide score is -4.8 having glide energy of -9675.0586 KJ/mol. Several frizzled receptor inhibitors have been identified and their docking results were also published. The two most potent compounds, SRI35959 and SRI37892, were docked separately into the putative binding site of the Fzd7-TMD model. The docked models suggested that both hits SRI35959 and SRI37892 accommodates into the Fzd7 active site. However, SRI37892 bound relatively tighter than SRI35959 with a docking score (which mimics the binding affinity) of -12.0 kcal/mol as compared to -10.3 kcal/mol

of SRI35959, which is consistent with the observed experimental results that SRI37892 is a more potent inhibitor [74].

Hydrogen bonds play an essential role in stabilizing the protein-ligand interactions [75]. The transmembrane receptors EGFR and VEGFR have 7 hydrogen bonds with the ligand and the cytoplasmic receptor notch-2 has 6 hydrogen bonds (Fig. 3 and Table 2). However, the frizzled receptor interacts with BA via only two hydrogen bonds. From the amino acid interactions of all the target proteins with BA, it was clear that the interactions were stronger with the polar amino acids such as Asn, Trp, Arg, Ser, Thr, Tyr, Gln, Asp, Lys and Glu. The ligand also showed interactions with specific hydrophobic amino acids such as Val, Ala and Leu in all the protein targets studied.

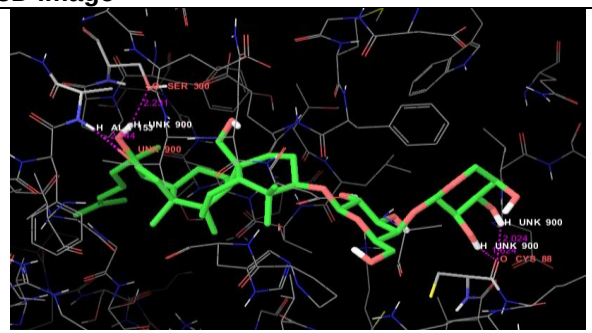
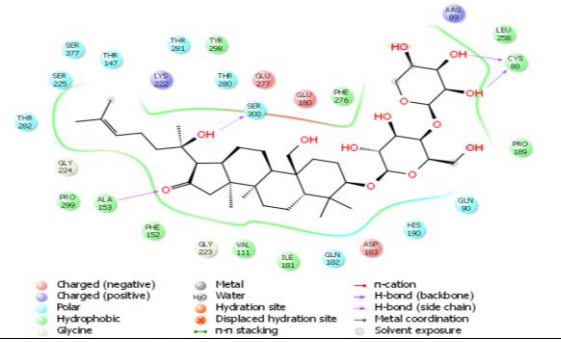
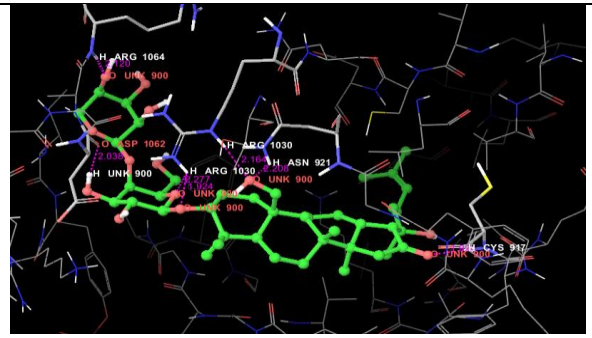
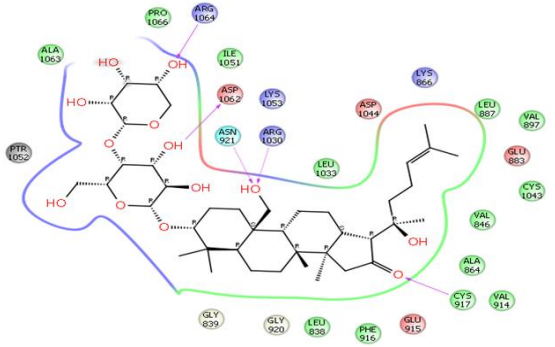
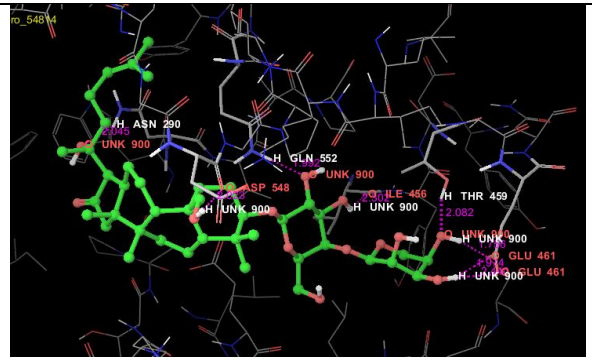
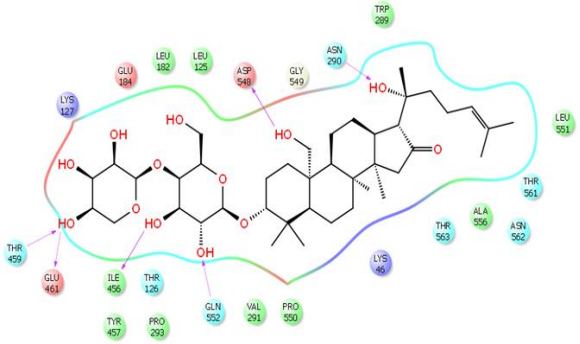
A recent study proved that bacoside A inhibits the expression of notch 1 in GBM cell line and is found to inhibit the GBM pathogenesis via notch signaling and induces Sub G0 arrest [21]. Similarly, Moskwa et al. [76] reported a study which evaluated the efficacy of combination of polish propolis and *Bacopa monnieri* extract in glioblastoma cell line and it was found that combination showed effective anticancer potential and the cell death increased substantially when used in combination. It could be due to the excessive amount of triterpene saponins in the compound. Anticancer activity of the extract of *B.monnieri* in several cancer cell lines such as colon, lung, cervix, and breast and also in Ehrlich ascites carcinoma (EAC)-treated mice were also reported. The same group of researchers performed an *in-silico* screening and reported that bacosides and curcubitacins were responsible for the anticancer activity exhibited by *B. monnieri* [77]. Another study reported that *Bacopa monnieri* extract and bacoside A could upregulate CaMK2A and stimulate its phosphorylation in glioblastoma cells via increased calcium release from the endoplasmic reticulum, thereby inducing cell death termed macropinocytosis [78]. Hence, the present study concluded that bacoside A can cause inhibition on GBM pathogenesis through any of the signalling pathways. However, it warrants future studies both *in-vitro* and *in-vivo*.

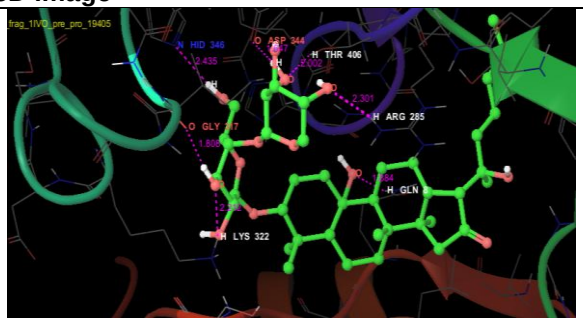
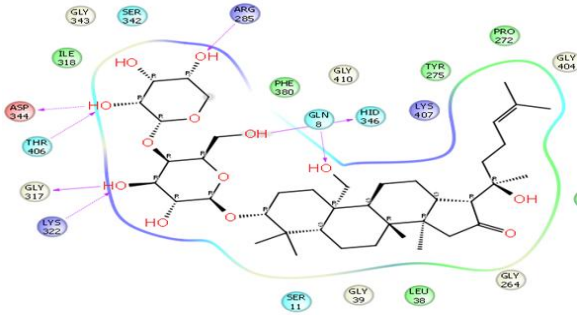
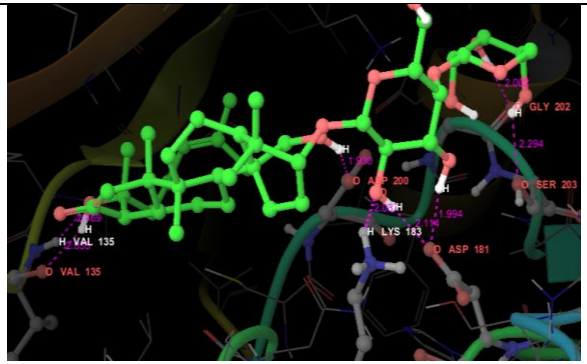
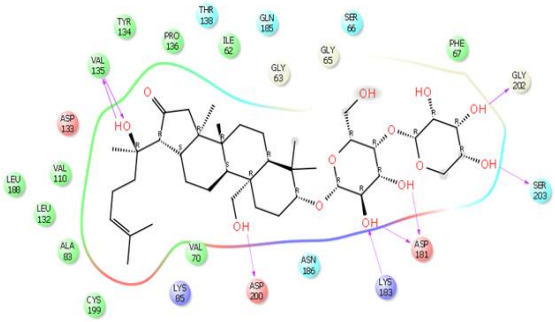
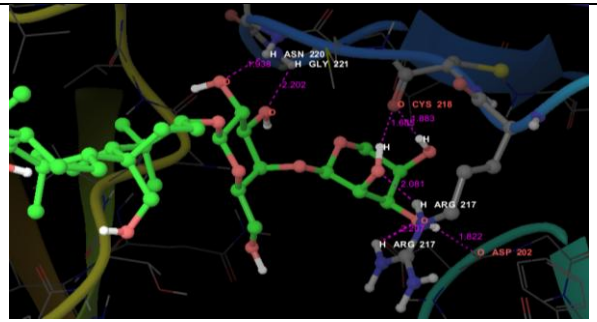
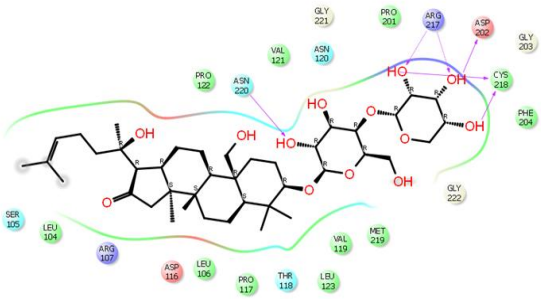
Table 1. Glide SP docking score of Bacoside A with the cancer target proteins

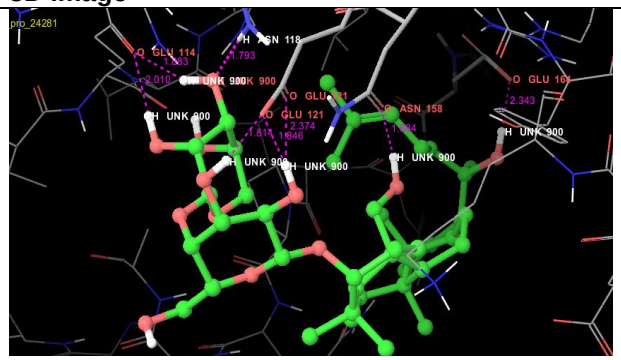
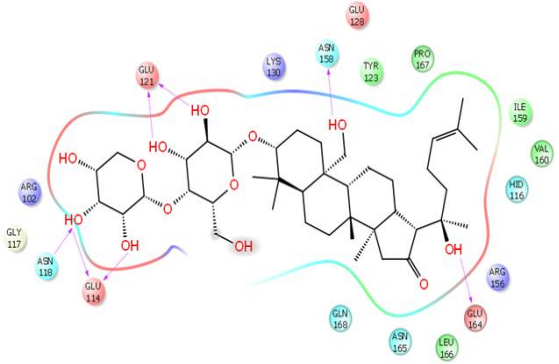
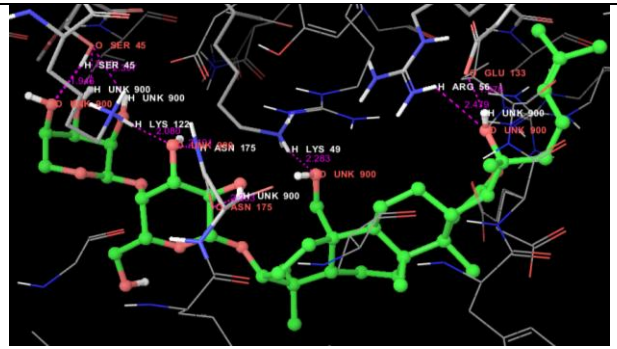
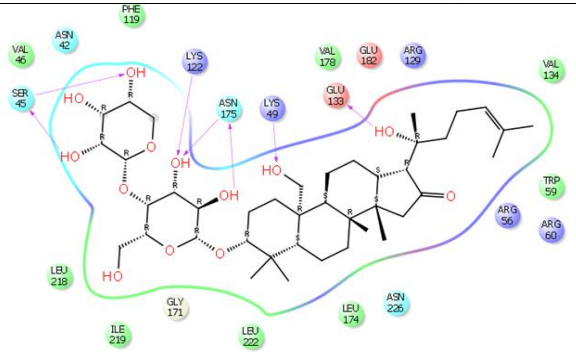
S. No	Target Proteins	Glide Score	Glide Energy (kJ/mol)
1	Jagged-1	-11.2	-82407.026
2	VEGFR	-10.4	-10933.9
3	Gamma secretase	-10	-181166.03
4	EGFR	-10	-45420.8789
5	GSK3- Beta	-9.9	-18164.5469
6	WNT-3	-9.2	-4406.6562
7	P53	-8.72	-48349.47
8	Raf	-8.3	-10627.63
9	Notch-2	-8.2	-7247.2
10	Beta catenin	-8.1	-25205.3046
11	Dll-1	-7.8	-19467.57
12	Hes	-7.3	-4328.17
13	Ras	-5.7	-9904.05
14	Hey	-5.6	0
15	Frizzled Receptor	-4.8	-9675.0586

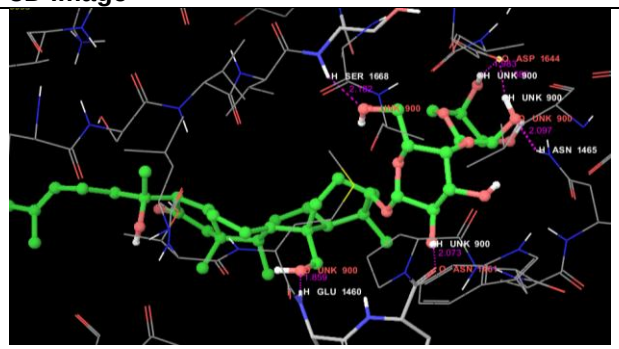
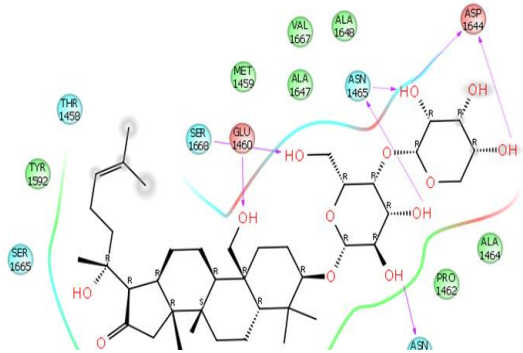
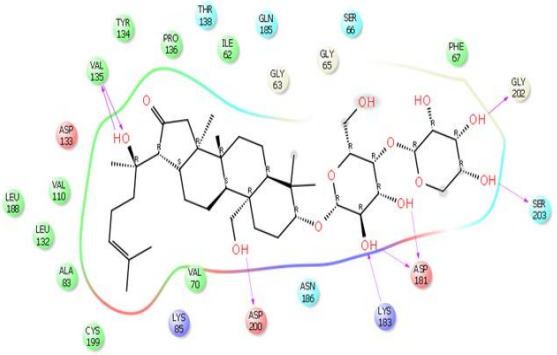
Table 2. Hydrogen bonding interaction between the amino acid residues of Bacoside A and target protein after molecular docking

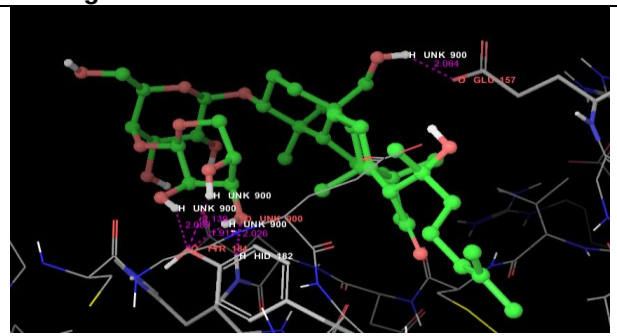
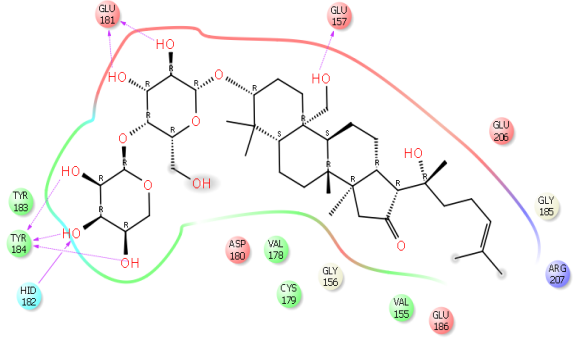
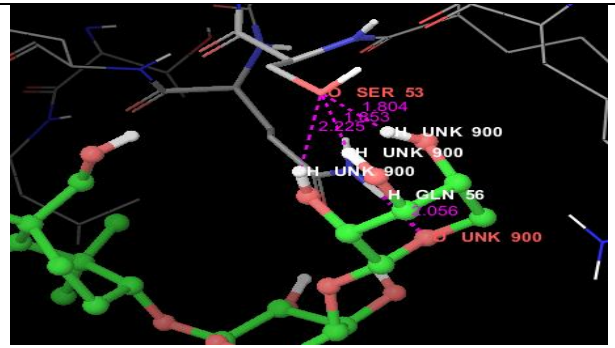
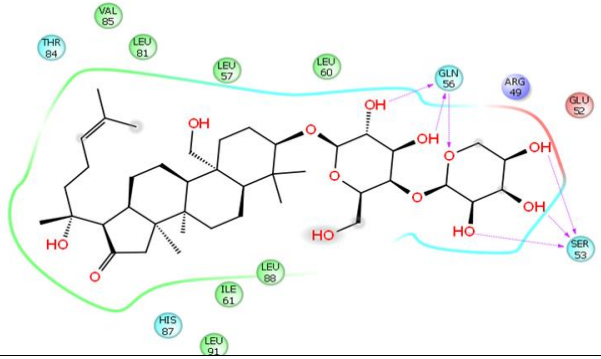
Ligand	Signalling	Protein name	PDB ID	No of hydrogen bonds	Interacting amino acid residues
Bacoside A	EGFR/Ras/Raf/MAPK pathway	EGFR	1IVO	7	GLN8,ARG285,THR406,LYS322, ASP344,GLY317, HIS346.
		Ras	1CTQ	7	ASP119, ASP30, VAL29, SER17, ASP33, CYS917
		Raf	3IQJ	9	SER45, GLU133, and ASN175, ARG56, LYS49, LYS122,
		VEGFR	1YWN	7	ARG1030, ARG1064, ASN921,CYS917, ASP1062.
		p53	1YC5	8	GLU121 ,GLU114 ,GLU164,ASN158 , ASN118
	Notch Signalling	DLL-1	4XBM	5	TYR184 ,GLU157, HIS182.
		Jagged-1	4X17	4	CYS88,SER300, ALA153
		Notch-2	2OO4	6	ASP1644,ASN1461, GLY1460, SER1668, ASN1465
		Gamma- secretase	5A63	8	GLU461, ASP548, ILE456, ASN290, GLN552,THR459.
		Hes	2MH3	4	SER53, GLN56
		Hey	2DB7	4	ASP13,ASN51, SER16
	Canonical Wnt- Beta catenin signaling	β - catenin	2Z6H	6	LYS354, CYS466, SER425, ASP390, SER351,VAL349
		GSK -3β	1GNG	7	ASP181, VAL135, ASP200 , SER203, GLY202,LYS182
		Frizzled Receptor	6BD4	2	GLU52,LEU41
		Wnt-3	2YZQ	5	ARG217, GLY221,ASN220, CYS218, ASP202, GLY221.

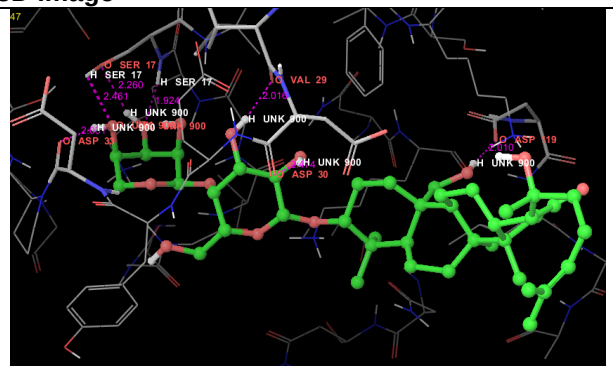
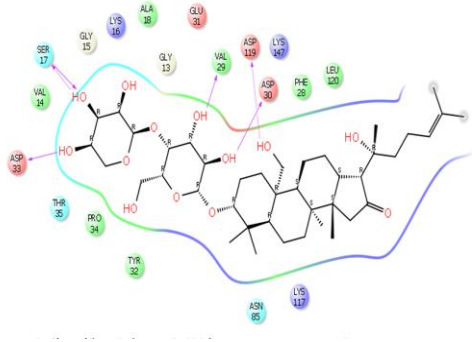
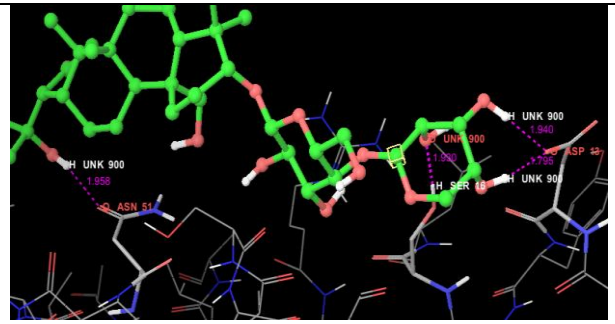
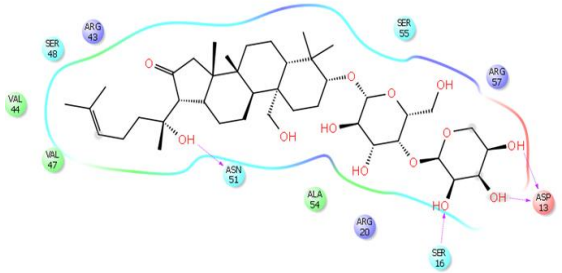
Protein Name	3D image	2D image
Jagged-1		
VEGFR		
Gamma secretase		

Protein Name	3D image	2D image
EGFR		
GSK 3 beta		
Wnt-3		

Protein Name	3D image	2D image
p53		
Raf-1		

Protein Name	3D image	2D image
Notch-2		
β-catenin		

Protein Name	3D image	2D image
Dll-1		
Hes		

Protein Name	3D image	2D image
Ras		
Hey		

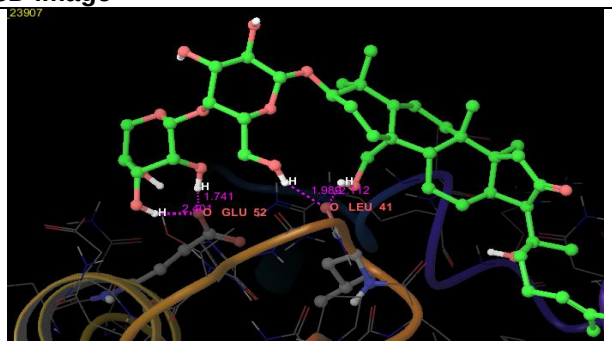
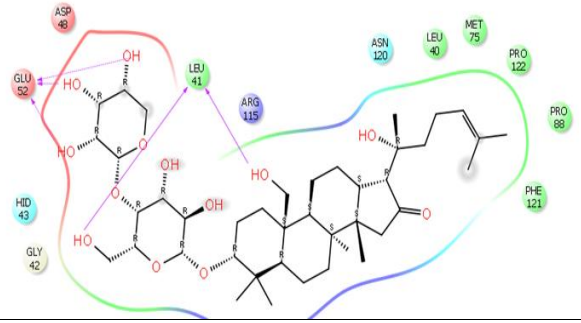
Protein Name	3D image	2D image
Frizzled Receptor		

Fig. 3. Molecular docking studies of Bacoside A with Jagged-1, VEGFR, Gamma secretase, EGFR, GSK-3 beta, Wnt-3,p53,Raf,Notch-2, Beta catenin, Dll-1,Hes,Ras, Hey, Frizzled receptor arranged according to the increasing order of their glide score. The figure on left side represents 3D image whereas second image represents 2D view of the docked complex in which hydrogen bonding has been shown

4. CONCLUSION

The present study evaluates the anti-glioma activity of BA through *in-silico* studies. The docking studies revealed that BA obtained interacts with the proteins EGFR, VEGFR, Ras, Raf, DLL-1, Notch-2, Jagged-1, Gamma secretase, Hey, HIF 1-α, p53, Wnt-3, Frizzled Receptor, Beta catenin, GSK3- Beta. Out of the 15 proteins which seem to interact with BA, its interaction with jagged 1(a ligand of Notch receptor) and TGF 1α protein have the best glide score while the frizzled receptor showed minimum glide score. The binding interaction of BA with target proteins is in the following order, Jagged-1> VEGFR> Gamma secretase> EGFR> GSK 3-Beta > Wnt-3 > p53> Raf> Notch-2> β-Catenin > Dll-1 > Hes > Ras> Hey>Frizzled receptor. The amino acid interactions of all the target proteins with BA showed that the interaction is comparatively stronger with the polar amino acids such as Asn, Trp, Arg, Ser, Thr, Tyr, Gln, Asp, Lys and Glu. BA also interacts with specific hydrophobic amino acids such as Val, Ala and Leu in all the protein targets studied. The results of these molecular docking studies indicate the therapeutic potential of BA against glioblastoma and suggest its potential for use as a molecular therapeutic. It warrants further validation of these results through *in-vitro* and *in-vivo* studies.

DISCLAIMER

Commonly used products in research in our area are exploited in this study. There is absolutely no conflict of interest between the authors and manufacturers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ostrom Q, Cioffi G, Gittleman H, Patil N, Waite K, Kruchko C. Barnholtz-Sloan JJN-o: CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2012–2016. Neuro-Oncol. 2019;21:v1–v100.
2. Ostrom QT, Gittleman H, Truitt G, Boscia A, Kruchko C, Barnholtz-Sloan JS. CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2011–2015. Neuro-Oncol. 2018; 20(suppl_4):iv1–iv86
3. Paszat L, Laperriere N, Groome P, Schulze K, Mackillop W, Holowaty E. A population-based study of glioblastoma multiforme. Int J Radiat Oncol Biol Phys. 2001;51(1):100–7.
4. Stupp R, Mason WP, Van Den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. New England Journal of Medicine. 2005; 352(10):987-96.
5. Krex D, Klink B, Hartmann C, von Deimling A, Pietsch T, Simon M, Sabel M, Steinbach JP, Heese O, Reifenberger G, Weller M, Schackert G. Long-term survival with glioblastoma multiforme. Brain. 2007;130: 2596–2606.
6. Burton EC, Lamborn KR, Feuerstein BG, Prados M, Scott J, Forsyth P, Passe S, Jenkins RB, Aldape KD. Genetic aberrations defined by comparative genomic hybridization distinguish long-term from typical survivors of glioblastoma. Cancer Res. 2002;62:6205–6210.
7. Saito N, Fu J, Zheng S, Yao J, Wang S, Liu DD, Yuan Y, Sulman EP, Lang FF, Colman H, et al. A high Notch pathway activation predicts response to γ secretase inhibitors in proneural subtype of glioma

tumor-initiating cells. Stem Cells. 2014;32: 301–312.
8. Kitchen DB, Decornez H, Furr JR, Bajorath J. Docking and scoring in virtual screening for drug discovery: methods and applications. Nature Reviews Drug Discovery. 2004;3(11):935-49.
9. Pearson JR, Regad T. Targeting cellular pathways in glioblastoma multiforme. Signal Transduction and Targeted Therapy. 2017;2(1):1.
10. Bazzoni R, Bentivegna A. Role of notch signaling pathway in glioblastoma pathogenesis. Cancers. 2019;11(3):292.
11. Nager M, Bhardwaj D, Cantí C, Medina L, Nogués P, Herreros J. β-Catenin signalling in glioblastoma multiforme and glioma-initiating cells. Chemotherapy Research and Practice; 2012.
12. Vichai V, Kirtikara K. Sulforhodamine B colorimetric assay for cytotoxicity screening. Nat Protoc. 2006;1(3):1112–1116.
13. Anonymous. Schrodinger release 2018-4: Protein Preparation Wizard; Epik, Schrödinger, LLC, New York. NY. Impact, Schrödinger, LLC, New York, NY, 2018; Prime, Schrödinger, LLC, New York, NY; 2018.
14. Anonymous. Schrödinger release. LigPrep, Schrödinger, LLC. New York, NY. 2018;4.
15. Artese A, Costa G, Ortuso F, Parrotta L, Alcaro S. Identification of new natural DNA G -quadruplex binders selected by a structure -based virtual screening approach. Molecules. 2013;18:12051-12070.
16. Friesner RA, Murphy RB, Repasky MP, Frye LL, Greenwood JR, Halgren TA, Sanschagrin PC, Mainz DT. Extra precision glide: Docking and scoring incorporating a model of hydrophobic enclosure for protein− ligand complexes. Journal of Medicinal Chemistry. 2006; 49(21):6177-96.
17. Schrodinger Inc. Schrodinger Release 2018 version 1 Maestro, Schrodinger, LLC, New York, NY; 2018.
18. Glide, Version 5.6, Schrodinger. LLC, New York, NY, USA; 2010.
19. Mallick MN, Akhtar MS, Najm MZ, Tamboli ET, Ahmad S, & Husain SA. Evaluation of anticancer potential of *Bacopa monnieri* L. against MCF-7 and MDA-MB 231 cell line. Journal of Pharmacy & Bioallied Sciences. 2015;7(4):325.
20. Nivesh Krishna R, Gayathri R, Priya V. Genotoxicity potential of Bacopa monnieri on oral cancer cell lines by DNA fragmentation. International Journal of Pharmaceutical Sciences Review and Research. 2016;39(1):240–242.
21. Aithal MG, Rajeswari N. Bacoside A induced sub-G0 arrest and early apoptosis in human glioblastoma cell line U-87 MG through notch signaling pathway. Brain Tumor Research and Treatment. 2019; 7(1):25-32.
22. Russo A, Borrelli F. Bacopa monniera, a reputed nootropic plant: An overview. Phytomedicine. 2005;12:305–17.
23. Kishore K, Singh M. Effect of bacosides, alcoholic extract of Bacopa monniera Linn. (brahmi), on experimental amnesia in mice. Indian J Exp Biol. 2005;43:640–2.
24. Anbarasi K, Vani G, Balakrishna K, Desai CS. Creatine kinase isoenzyme patterns upon chronic exposure to cigarette smoke: protective effect of Bacoside A. Vascul Pharmacol. 2005;42:57–61.
25. Vishnupriya P, Padma VV. A review on the antioxidant and therapeutic potential of *Bacopa monnieri*. React Oxygen Spec. 2017;3:111-120.
26. Kumar EP, Elshurafa AA, Elango K, Subburaju T, Suresh B. Cytotoxic and anti -tumour properties of ethanolic extract of *Bacopa monnieri* (L) penn. Anc Sci Life. 1998;17:228-34.
27. Peng L, Zhou Y, Kong de Y, Zhang WD. Antitumor activities of dammarane triterpene saponins from *Bacopa monniera*. Phytother Res. 2010;24:864-8.
28. Janani P, Sivakumari K, Geetha A, Yuvaraj S, Parthasarathy C. Bacoside A downregulates matrix metalloproteinases 2 and 9 in DEN-induced hepatocellular carcinoma. Cell Biochemistry and Function: Cellular biochemistry and its modulation by active agents or disease. 2010;28(2):164-169.
29. Kalachaveedu M, Adapala D, Punnoose AM, & Kuruvilla S. Brahmi saponins inhibit proliferation of Hep G2 cells by blocking cell cycle progression and inducing apoptosis. In XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014): V World 1125. 2014;173-180.
30. Rohini G, Devi CS. Bacopa monniera extract Induces apoptosis in murine sarcoma cells (S-180). Phytotherapy

Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2008;22(12):1595-1598.
31. Kalyani MI, Lingaraju SM, Salimath BP. A pro-apoptotic 15-kDa protein from Bacopa monnieri activates caspase-3 and downregulates Bcl-2 gene expression in mouse mammary carcinoma cells. J Nat Med. 2013;67:123-36.
32. Singh HK, Dhawan BN. Neuropsychopharmacological effects of the ayurvedic nootropic *Bacopa monnieri* Linn. Indian J Pharmacol. 1997;29:S359-65.
33. Ekstrand AJ, James CD, Cavenee WK, Seliger B, Pettersson RF, Collins VP. Genes for epidermal growth factor receptor, transforming growth factor α, and epidermal growth factor and their expression in human gliomas *in vivo*. Cancer Res. 1991;51:2164–2172.
34. Wong AJ, Ruppert JM, Bigner SH, Grzeschik CH, Humphrey PA, Bigner DS, Vogelstein B. Structural alterations of the epidermal growth factor receptor gene in human gliomas. Proc Natl Acad Sci USA. 1992;89:2965–2969.
35. Biernat W, Huang H, Yokoo H, Kleihues P, Ohgaki H. Predominant expression of mutant EGFR (EGFRvIII) is rare in primary glioblastomas. Brain Pathol. 2004;14:131–136.
36. Eskilsson E, Rosland GV, Solecki, Wang Q, Harter PN, Graziani G, Verhaak RGW, Winkler F, Bjerkvig R, Miletic H. EGFR heterogeneity and implications for therapeutic intervention in glioblastoma. Neuro Oncol. 2018;20:743–752.
37. An Z, Aksoy O, Zheng T, Fan QW, Weiss WA. Epidermal growth factor receptor and EGFRvIII in glioblastoma: Signaling pathways and targeted therapies. Oncogene. 2018;37:1561–1575.
38. Oprita, Alexandru, Stefania-Carina Baloi, Georgiana-Adeline Staicu, Oana Alexandru, Daniela E. Tache, Suzana Danoiu, Elena S. Micu, and Ani-Simona Sevastre. Updated Insights on EGFR Signaling Pathways in Glioma. International Journal of Molecular Sciences. 2021;22(2)587.
39. Steiner HH, Karcher S, Mueller MM, Nalbantis E, Kunze S, Herold-Mende C. Autocrine pathways of the vascular endothelial growth factor (VEGF) in glioblastoma multiforme: Clinical relevance of radiation-induced increase of VEGF levels. J Neurooncol. 2004;66:129–138.
40. Joensuu H, Puputti M, Sihto H, Tynninen O, Nupponen NN. Amplification of genes encoding KIT, PDGFRalpha and VEGFR2 receptor tyrosine kinases is frequent in glioblastoma multiforme. J Pathol. 2005; 207:224–231.
41. Abounader R, Laterra J. Scatter factor/ hepatocyte growth factor in brain tumor growth and angiogenesis. Neuro Oncol. 2005; 7: 436–451.
42. Peyssonnaux S, Provot MP, Felder-Schmittbuhl G, Calothy A. Eychéne. Induction of postmitotic neuroretina cell proliferation by distinct Ras downstream signaling pathways. Mol. Cell. Biol. 2000; 20:7068-7079.
43. Flotho C, Valcamonica S, Mach-Pascual S, Schmahl G, Corral L, Ritterbach J, Hasle H, Arico M, Biondi A, Niemeyer CM. RAS mutations and clonality analysis in children with juvenile myelomonocytic leukemia (JMML). Leukemia. 1999;13(1):32-7.
44. Stirewalt KJ, Kopecky S, Meshinchi FR, Appelbaum ML, Slovak CL, Willman JP, Radich. FLT3, RAS, and TP53 mutationsin elderly patients with acute myeloid leukemia, Blood. 2001;97:3589–3595.
45. Abel TW, Clark C, Bierie B, Chytil A, Aakre M, Gorska A, Moses HL. GFAP-Cre-mediated activation of oncogenic K-ras results in expansion of the subventricular zone and infiltrating glioma. Mol. Cancer Res. 2009;7:645–653.
46. Shannon P, Sabha N, Lau N, Kamnasaran D, Gutmann DH, Guha A. Pathological and molecular progression of astrocytomas in a GFAP: 12 V-Ha-Ras mouse astrocytoma model. Am. J. Pathol. 2005;167:859–867.
47. Lo HW, Cao X, Zhu H, Ali-Osman F. Constitutively activated STAT3 frequently coexpresses with epidermal growth factor receptor in high-grade gliomas and targeting STAT3 sensitizes them to Iressa and alkylators. Clin Cancer Res. 2008; 14(19):6042-54.
48. Ebisch A, Czernilofsky AP, Keri G, Smigelskaite J, Sill H, Troppmair J. Signaling through RAS-RAF-MEK-ERK: from basics to bedside. Curr Med Chem. 2007;14: 601–623.
49. Lyustikman Y, Momota H, Pao W, Holland EC. Constitutive activation of Raf-1 induces glioma formation in mice. Neoplasia. 2008;10(5):501-510.

50. Hu YY, Zheng MH, Cheng G, et al. Notch signaling contributes to the maintenance of both normal neural stem cells and patient-derived glioma stem cells. BMC Cancer. 2011;11:82.
51. Hai L, Zhang C, Li T, et al. Notch1 is a prognostic factor that is distinctly activated in the classical and proneural subtype of glioblastoma and that promotes glioma cell survival via the NF-κB(p65) pathway. Cell Death Dis. 2018;9:158.
52. Hori K, Sen A, Artavanis-Tsakonas S. Notch signaling at a glance. Pt 10*J.* Cell Sci. 2013;126:2135–2140.
53. Hulleman E, Quarto M, Vernell R, Masserdotti G, Colli E, Kros JM, Levi D, Gaetani P, Tunici P, Finocchiaro G, et al. A role for the transcription factor HEY1 in glioblastoma. J. Cell. Mol. Med. 2009;13: 136–146.
54. Zhang X, Chen T, Zhang J, Mao Q, Li S, Xiong W, Qiu Y, Xie Q, Ge J. Notch1 promotes glioma cell migration and invasion by stimulating β-catenin and NF-κB signaling via AKT activation. Cancer Sci. 2011;103:181–190.
55. Zhu TS, Costello MA, Talsma CE, Flack CG, Crowley JG, Hamm LL, He X, Hervey-Jumper SL, Heth JA, Muraszko KM, et al. Endothelial cells create a stem cell niche in glioblastoma by providing NOTCH ligands that nurture self-renewal of cancer stem-like cells. Cancer Res. 2011;71:6061–6072.
56. Sang L, Coller HA, Roberts JM. Control of the reversibility of cellular quiescence by the transcriptional repressor hes1. Science. 2008;321:1095-1100.
57. Kovall RA, Blacklow SC. Mechanistic insights into notch receptor signaling from structural and biochemical studies. Curr Top Dev Biol. 2010;92:31–71.
58. Ran Y, Hossain F, Pannuti A, Lessard CB, Ladd GZ, Jung JI. Golde TE. γ-Secretase inhibitors in cancer clinical trials are pharmacologically and functionally distinct. EMBO Molecular Medicine. 2017; 9(7):950-966.
59. Zhang K, Zhang J, Han L et al. Wnt/Beta-catenin signaling in glioma. J Neuroimmune Pharmacol. 2012;7:740–749.
60. Gong A and Huang S. FoxM1 and Wnt/β-catenin signaling in glioma stem cells. Cancer Res. 2012;72(22):5658-62.
61. Lee Y, Lee, JK, Ahn S, et al. WNT signaling in glioblastoma and therapeutic opportunities. Lab Invest. 2016;96:137–150.
62. Sareddy GR, Panigrahi M, Challa S, Mahadevan A, Babu PP. Activation of Wnt/beta-catenin/Tcf signaling pathway in human astrocytomas. Neurochem Int. 2009;55(5):307-17.
63. Yue X, Lan F, Yang W, et al. Interruption of beta-catenin suppresses the Egfr pathway by blocking multiple oncogenic targets in human glioma cells. Brain Res. 2010; 1366:27–37.
64. Han SP, Kim JH, Han ME et al. Snai1 is involved in the proliferation and migration of glioblastoma cells. Cell Mol Neurobiol. 2011;31:489–496.
65. Yang HW, Menon LG, Black PM et al. Snai2/Slug promotes growth and invasion in human gliomas. BMC Cancer. 2010; 10:301.
66. Kotliarova S, Pastorino S, Kovell LC, Kotliarov Y, Song H, Zhang W, Bailey R, Maric D, Zenklusen JC, Lee J, and Fine HA. Glycogen synthase kinase-3 inhibition induces glioma cell death through c-MYC, nuclear factor-kappaB, and glucose regulation. Cancer Res. 2008;68:6643–6651.
67. Miyashita K, Kawakami K, Nakada M, Mai W, Shakoori A, Fujisawa H, Hayashi Y, Hamada J, Minamoto T. Potential therapeutic effect of glycogen synthase kinase 3beta inhibition against human glioblastoma. Clin Cancer Res. 2009; 15(3):887–897.
68. Zhang, Y, Dube C, Gibert M, Cruickshanks N, Wang B, Coughlan M, Abounader R. The p53 pathway in glioblastoma. Cancers. 2018;10(9):297.
69. Pearson J, Regad T. Targeting cellular pathways in glioblastoma multiforme. Sig Transduct Target Ther. 2017;2:17040.
70. Tabassum S, Zaki M, Afzal M, Arjmand F. Synthesis and characterization of Cu (II)-based anticancer chemotherapeutic agent targeting topoisomerase Iα: *In vitro* DNA binding, pBR322 cleavage, molecular docking studies and cytotoxicity against human cancer cell lines. European Journal of Medicinal Chemistry. 2014;74: 509-23.
71. Zahra SN, Khattak NA, Mir A. Comparative modeling and docking studies of p16ink4/Cyclin D1/Rb pathway genes in lung cancer revealed functionally interactive residue of RB1 and its functional partner E2F1. Theoretical

Biology and Medical Modelling. 2013; 10(1):1-9.
72. Pagadala NS, Syed K, Tuszynski J. Software for molecular docking: A review. Biophysical reviews. 2017;9(2):91-102.
73. Ashwini S, Varkey SP, Shantaram M. In Silico docking of polyphenolic compounds against Caspase 3-HeLa cell line protein. Int J Drug Dev Res. 2017;9:28-32.
74. Zhang W, Lu W, Ananthan S, Suto MJ, & Li Y. Discovery of novel frizzled-7 inhibitors by targeting the receptor's transmembrane domain. Oncotarget. 2017;8(53):91459.
75. Chou KC. Review: Structural bioinformatics and its impact to biomedical science. Curr. Med. Chem. 2004;11:2105-2134.
76. Moskwa J, Naliwajko SK, Markiewicz-Żukowska R, Gromkowska-Kępka KJ, Nowakowski P, Strawa JW, Borawska MH, Tomczyk M, Socha K. Chemical composition of Polish propolis and its antiproliferative effect in combination with Bacopa monnieri on glioblastoma cell lines. Scientific Reports. 2020;10(1): 1-6.
77. Mallick MN, Khan W, Parveen R, Ahmad S. Exploring the cytotoxic potential of triterpenoids-enriched fraction of Bacopa monnieri by implementing *in vitro*, *in vivo*, and *in silico* approaches. Pharmacognosy Magazine. 2017;13(Suppl 3):S595
78. John S, Sivakumar KC, Mishra R. Bacoside A induces tumor cell death in human glioblastoma cell lines through catastrophic macropinocytosis. Frontiers in Molecular Neuroscience. 2017;15(10):171.

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