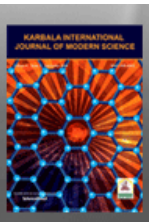

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Quercetin as an anticancer candidate for glioblastoma multiforme by targeting AKT1, MMP9, ABCB1, and VEGFA: An in silico study

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Abstract

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Keywords

Anticancer, Inhibitor, Medicine, Molecular dynamic, Quercetin

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RESEARCH PAPER

Quercetin as an Anticancer Candidate for Glioblastoma Multiforme by Targeting AKT1, MMP9, ABCB1, and VEGFA: An *in silico* Study

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Abstract

Quercetin, a natural compound present in various fruits and vegetables, shows promise as a potential inhibitor for glioblastoma multiforme (GBM) development. This study aims to examine the anti-GBM potential of Quercetin. The protein target of Quercetin is identified and analyzed using databases such as NCBI, SEA, CTD, and STRING. Protein−protein interaction (PPI) and functional annotation are carried out based on the obtained target proteins. Molecular docking and dynamics simulations are employed using AutoDock Vina and WebGro tools to analyze the interaction between Quercetin and its target proteins. The prediction of protein targets reveals that Quercetin directly targets four proteins associated with GBM. In conclusion, Quercetin demonstrates potential as an anti-GBM agent, specifically by targeting AKT1, MMP9, ABCB1, and VEGFA proteins.

Keywords: Anticancer, Inhibitor, Medicine, Molecular dynamic, Quercetin

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1. Introduction

Glioblastoma multiforme (GBM) is a grade IV histological malignancy tumor of the central nervous system, as defined by the World Health Organization (WHO). It encompasses a genetically and phenotypically heterogeneous group of primary brain neoplasms [1]. *De novo* or primary glioblastoma accounts for approximately 90% of GBM cases, originating from normal glial cells through multiple stages of tumorigenesis. Epidemiological data indicate an incidence of 2–3 GBM cases per 100,000 adults annually in Europe and North America. The occurrence of GBM is higher in Caucasians, particularly those residing in industrial areas [2]. Abnormal proliferation of GBM is influenced by molecules such as vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), and the absence of phosphotensin analog (PTEN). Additionally, downstream growth signaling pathways, including PI3K/AKT, may be activated [3]. Currently, various treatment options are available for GBM cases. Surgery is the initial choice to alleviate clinical symptoms, increase survival prospects, and reduce the need for steroids. However, GBMs typically recur, rendering surgery inadequate. Other treatments encompass radiation therapy (RT) and combined chemoradiotherapy with temozolomide, which is considered the gold standard therapy for this disease [4].

ATP binding cassette subfamily B member 1 (ABCB1) is a protein commonly overexpressed in multidrug-resistant human cancer cells. It functions as an ATP-binding cassette drug efflux transporter, implicated in drug resistance due to its ability to transport drug substrates out of cancer cells [5]. ABCB1 expression is observed in most brain tumors, including glioblastomas [6]. The involvement of ABCB1 in drug resistance is reinforced by a study demonstrating improved temozolomide response in a GBM cell model with the ABCB1 gene knocked out using CRISPR/Cas9 [7]. AKT1 is a downstream serine/threonine kinase within the RTK/PTEN/PI3K pathway, frequently mutated in GBM cases. A study also indicates that mice lacking AKT1 exhibit spontaneous apoptosis in the testes and thymus [8]. Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases. Among the MMP family members, MMP9 is implicated in the invasion and metastasis of head and neck squamous cell carcinoma. It is also known to be upregulated in higher-grade gliomas and can induce proliferation in glioblastoma. Additionally, glioblastoma tumor cells secrete VEGF, whose expression is elevated in glioblastoma tumors. Increased VEGFA expression activates the VEGFR pathway, promoting proliferation, migration, and survival of endothelial cells, consequently facilitating tumor angiogenesis and the formation of tumor blood vessels. This angiogenesis enables tumor cells to survive in a hypoxic and hostile environment [9].

Quercetin belongs to the flavonol subclass, one of the six subclasses of flavonoid compounds. It is characterized by its brilliant citron yellow needle crystal form and poor solubility in water while being relatively soluble in alcohol and lipids [14]. Similar to other flavonols, Quercetin possesses a 3-hydroxyflavone backbone, with hydroxyl (OH) groups attached to positions 3, 5, 7, 3′, and 4′ in its structure [15]. This active compound exhibits various biological actions, including anti-carcinogenic, anti-inflammatory, and antiviral activities [14]. Previous studies have reported that Quercetin induces apoptosis in the MCF7 breast cancer cell line [16]. Another *in vitro* study demonstrated that Quercetin inhibits lung cancer growth by targeting Aurora B kinase [17]. Furthermore, previous *in vivo* studies have shown that Quercetin inhibits colon carcinogenesis in C57BL/6J colorectal cancer mice [18], and induces cancer cell apoptosis in BALB/c mice as a breast cancer model [19]. Experimental research has also indicated that Quercetin inhibits glioblastoma cell proliferation *in vitro* [20]. These findings collectively highlight the substantial potential of Quercetin as an anticancer drug candidate. Therefore, the present research aims to investigate the ability of Quercetin as a potential anticancer candidate for GBM using *in silico* methods. The study will specifically focus on targeting ABCB1, AKT1, MMP9, and VEGFA proteins, which play significant roles in drug resistance, cell survival, proliferation, and angiogenesis associated with GBM.

2. Method

2.1. Target protein prediction and Protein–Protein Interaction (PPI) network generation

Target protein prediction involves predicting the direct and indirect targets of Quercetin. Direct targets were identified using STITCH (http://stitch.embl.de/) and SEA (http://sea.bkslab.org), which were then cross-referenced with glioblastoma multiforme-related proteins from the NCBI database (https://www.ncbi.nlm.nih.gov/). The selected target proteins were obtained from all four databases. Indirect targets were determined based on the direct target proteins using the STRING web server (https://string-db.org/). Cytoscape 3.7.2 software

was utilized to construct the Protein–Protein Interaction (PPI) networks.

2.2. Functional annotation analysis

Functional annotation analysis was performed to assess the roles of all target proteins in biological processes within cells. The Database for Annotation, Visualization, and Integrated Discovery (DAVID) web server (https://david.ncifcrf.gov/) was employed for this analysis. Further analysis utilized the KEGG databases (https://www.genome.jp/kegg/pathway.html) and Gene Ontology (http://geneontology.org/).

2.3. Molecular docking simulation

Molecular docking was conducted between Quercetin and the direct target proteins. The 3D structure of Quercetin was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Conformational energy optimization of the compounds was performed using the Open Babel plug-in integrated into PyRx 0.8 software [21]. The 3D structures of Akt1 (PDB ID 4ek1), MMP9 (PDB ID 1gkc), ABCB1 (PDB ID 6qex), and VEGFA (PDB ID 1flt) proteins were retrieved from the RCSB PDB database (https://www.rcsb.org/). These proteins were prepared by removing water molecules and other contaminants using Discovery Studio Visualizer 2019 software. Docking simulations were conducted on the active site of each protein using AutoDock Vina software integrated with PyRx [22]. The coordinates of the active site for each protein are provided in Table 1. Docking results were visualized using Discovery Studio Visualizer 2019 software.

2.4. Molecular dynamic simulation

The ligand topology for each complex was generated using the PRODRG2 server (http://davapc1.bioch.dundee.ac.uk/cgi-bin/prodrg) [23]. Molecular dynamics simulations were performed using the WebGro web server (https://simlab.uams.edu) with GROMOS96 43a1 as the force field. Environmental conditions were set to reflect physiological cell conditions, including 0.15M salt content, 310K temperature, and 1 bar pressure. The simulation duration was 20 ns, and the parameters assessed included RMSD (root mean square deviation) of the protein backbone, RMSD of ligand movement, and RMSF (root mean square fluctuation).

3. Result

3.1. Protein targets of quercetin associated with GBM

The target proteins of Quercetin were obtained from the SEA and STITCH databases, while the GBM-related proteins were retrieved from the NCBI database. Based on SEA analysis, 69 proteins were targeted by Quercetin, and 37 protein targets were identified from STITCH prediction. Additionally, there were 407 proteins related to GBM from the NCBI database. From these findings, four target proteins were selected from SEA and STITCH databases and linked to GBM: AKT1, MMP9, ABCB1, and VEGFA. These four proteins will be the focus of discussion in this study (Fig. 1A).

3.2. Protein–protein interaction (PPI) of quercetin-targeted proteins

The four selected target proteins of Quercetin interacted with other proteins associated with cancer development. MMP9 interacted with proteins involved in tumor cell invasions, such as MMP1 and MMP10. VEGFA interacted with proteins related to angiogenesis (FLT1, FLT4, and KDR) and cell proliferation (STAT3 and HIF1A). ABCB1 interacted with MAPK8 and TP53, which are implicated in tumor development. AKT1 interacted with proteins

Table 1. Active site and grid position of specific docking.

Proteins	PDB ID	Active site	Ref.	Grid coordinate (Å)	
				Center	Dimension
Akt1	6hhf	Trp80, Ile84, Glu85, Leu210, Leu264, Lys268, Val270, Tyr272, Arg273, Asp292	[10]	X: 5.0233, Y: 5.3081 Z: 14.1707	X: 17.6221 Y: 22.8262 Z: 25.5946
MMP9	1gkc	Glu353, Leu387, Met388, Met343, Leu346, Thr347, Arg394, Asp351, Leu428, Ile424, Met421, Leu525	[11]	X: 30.3707, Y: 0.4633 Z: 27.7685	X: 26.889 Y: 18.3735 Z: 20.6289
ABCB1	6qex	Ile306, Tyr307, Tyr310, Leu339, Phe343, Gln725, Phe728, Met949, Gly983	[12]	X: 30.3707, Y: 0.4633 Z: 27.7685	X: 30.3707, Y: 0.4633 Z: 27.7685
VEGFA	5t89	Asp34, Phe36, Pro40, Ile43, Ile46, Lys48, Ile83, Lys84, His86, Ile91	[13]	X: 30.3707, Y: 0.4633 Z: 27.7685	X: 30.3707, Y: 0.4633 Z: 27.7685

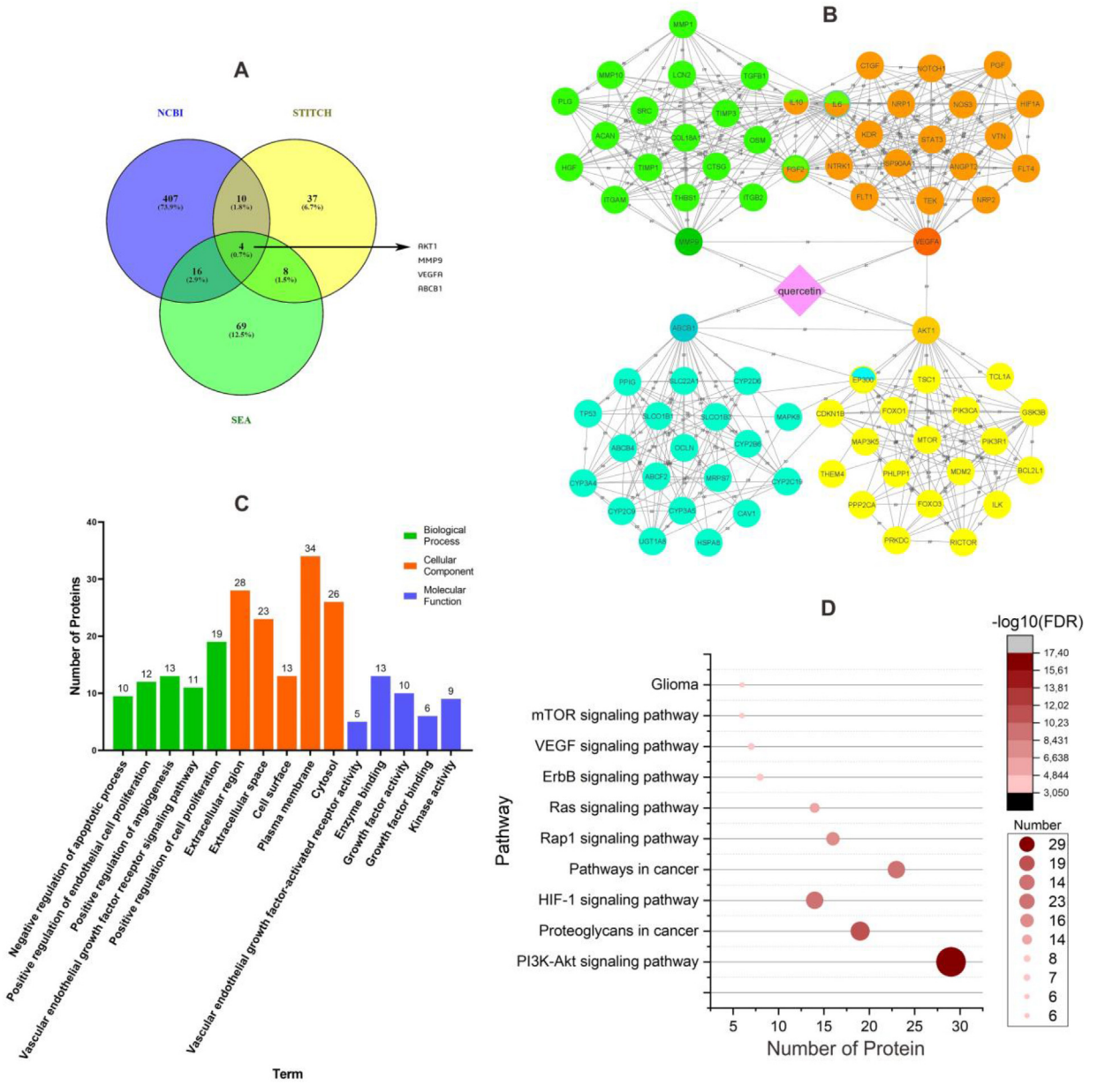

Fig. 1. Protein target prediction, PPI network, and functional annotation. A) Direct target of Quercetin based on three databases. B) Direct and indirect target of Quercetin in the form of PPI network. C) Functional annotation based on Gene Ontology database. D) Functional annotation based on KEGG pathway.

in the PI3K/AKT signaling pathway and the FOXO signaling pathway, both of which play a role in tumor cell growth. Additionally, AKT1 interacted with the antiapoptotic protein BCL2L1 (Figure 1B).

3.3. Functional annotation analysis

Functional annotation analysis was conducted using all proteins in the PPI network shown in Fig. 1B. Based on the Gene Ontology database, the target proteins of Quercetin were involved in biological processes related to tumor development, including "Positive regulation of cell proliferation," "VEGFR signaling pathway," "Positive regulation of angiogenesis," "Positive regulation of endothelial cell proliferation," and "Negative regulation of apoptotic processes" (Fig. 1C). The target proteins of Quercetin were also found in pathways associated with cancer development, such as the "PI3K-Akt signaling pathway" and "mTOR signaling pathway," according to the KEGG pathway analysis (Fig. 1D).

3.4. Molecular interaction of quercetin with the target proteins

Molecular docking results revealed that Quercetin interacted with the target proteins at the inhibitor-binding sites. The interaction between Quercetin and Akt1 exhibited a binding affinity score of −8.7 kcal/mol. Quercetin formed four hydrogen bonds and four hydrophobic interactions with Akt1, involving residues Gly159, Val164, and Lys179 (Fig. 2 and Table 2). The binding affinity score of the Quercetin-MMP9 complex (−9.1 kcal/mol) was more negative than that of the MMP9-inhibitor complex (−7.2 kcal/mol). Quercetin interacted with MMP9 at identical residues as the inhibitors, specifically Leu188, Val398, His401, and Tyr423 (Fig. 2B & Table 2). The interaction between Quercetin and ABCB1 had a low binding affinity (−8.3 kcal/mol) and involved the same chemical interactions as the inhibitors, namely Trp232, Phe343, Ser344, and Gln990 (Fig. 2C & Table 2). Quercetin formed complexes with VEGFA, exhibiting a binding affinity value of −7.2 kcal/mol, which was slightly similar to the VEGFA-inhibitor complex (−7.2 kcal/mol). The compound interacted with VEGFA at identical residues as the inhibitor, including Ser50, Gly59, and Asp63 (Fig. 2D & Table 2). The presence of similar interaction residues between Quercetin and the inhibitors suggests that Quercetin occupies a similar interaction position as the control inhibitors, indicating its potential as an inhibitor of the target proteins.

3.5. Stability of the interaction between quercetin and the target proteins

The stability of the ligand−protein complexes was assessed using molecular dynamics simulations, with the RMSD backbone protein representing the stability over a 20 ns simulation (Fig. 3). The structures of the Quercetin-bound Akt1, ABCB1, and VEGFA proteins remained relatively stable throughout the simulation (Fig. 3A, C, D). The structure of the Quercetin-bound MMP9 protein exhibited fluctuations until 12 ns but then stabilized for the remainder of the simulation (Fig. 3D). The stability of the protein structures was further assessed by examining the RMSF values of amino acid residues. Overall, the majority of complexes had RMSF values below 3 Å, indicating their tendency to remain stable during the simulation (Fig. 4). The stability of Quercetin when interacting with the proteins was reflected in the RMSD values of ligand movement. Quercetin exhibited a stable interaction with Akt1, MMP9, and VEGFA, as indicated by minimal fluctuations in the RMSD values of ligand movement (Fig. 5A and B, D). When interacting with ABCB1, Quercetin displayed fluctuations until 18 ns, after which it stabilized (Fig. 5C). The results of the molecular dynamics simulations suggest that the Quercetin-protein complexes tended to be stable throughout the simulation.

4. Discussion

Glioblastoma multiforme (GBM) is a cancer that occurs in the human Central Nervous System (CNS) and is caused by an uncontrolled division of glial cells [1]. These tumors commonly occur in the hemispheres and cerebellum, leading to increased intracranial pressure and hydrocephaly [2]. GBM cells exhibit varied morphologies, ranging from polygonal to spindle-shaped, with pleomorphic tumor cells and irregular nuclear membranes [24]. The transformation of glial cells into gliomas is driven by the accumulation of genetic alterations and abnormal regulation of growth factor signaling pathways, including Vascular Endothelial Growth Factor (VEGF), Epidermal Growth Factor (EGF), and proteins in the PI3K/Akt signaling pathway [3]. Synthetic drugs like temozolomide, bevacizumab, and lomustine have been developed to treat GBM [25]. However, research on bioactive compounds against GBM aims to explore alternatives to synthetic drugs with potential side effects [26]. This study introduces Quercetin as a potential anti-GBM compound.

Quercetin is naturally found in many fruits and vegetables [14]. It possesses five hydroxyl groups at positions 3, 5, 7, 3′, and 4′, some of which can undergo glycosylation to produce Quercetin derivatives [15]. The presence of multiple hydroxyl groups makes Quercetin a potent radical scavenger [27]. Quercetin also features a catechol structure (3′, 4′-dihydroxy groups in the C ring), which blocks substrate access to the catalytic site of the tyrosinase enzyme [28]. Previous research indicates that the hydroxyl group at C-3′ inhibits ATP interactions, resulting in protein kinase inactivation [29]. Moreover, Quercetin exhibits various pharmacological activities, including antioxidant, anti-inflammatory, immunosuppressant, antimicrobial, and anticancer properties [30]. *In vitro* and *in vivo* studies have demonstrated the toxicity of Quercetin against ovarian cancer cells [31]. Previous research also mentioned that Quercetin induced apoptosis and inhibit the breast cancer cell proliferation [32]. The mechanism of action of Quercetin involves inhibiting the activity of oncogenes such as Aurora B kinase, Cyclin D1, and Twist [16,17].

However, no studies have explored the benefits of Quercetin for GBM and its potential molecular mechanisms. Hence, this study aims to predict the mechanism of Quercetin as an anti-GBM agent through *in silico* analysis. Compared to conventional therapy such as nivolumab, Quercetin offers advantages in terms of lower toxicity [33]. A study by Simonelli et al. (2016) reported that nivolumab can cause hepatotoxicity, indicated by increased levels of aspartate aminotransferase and alanine transaminase

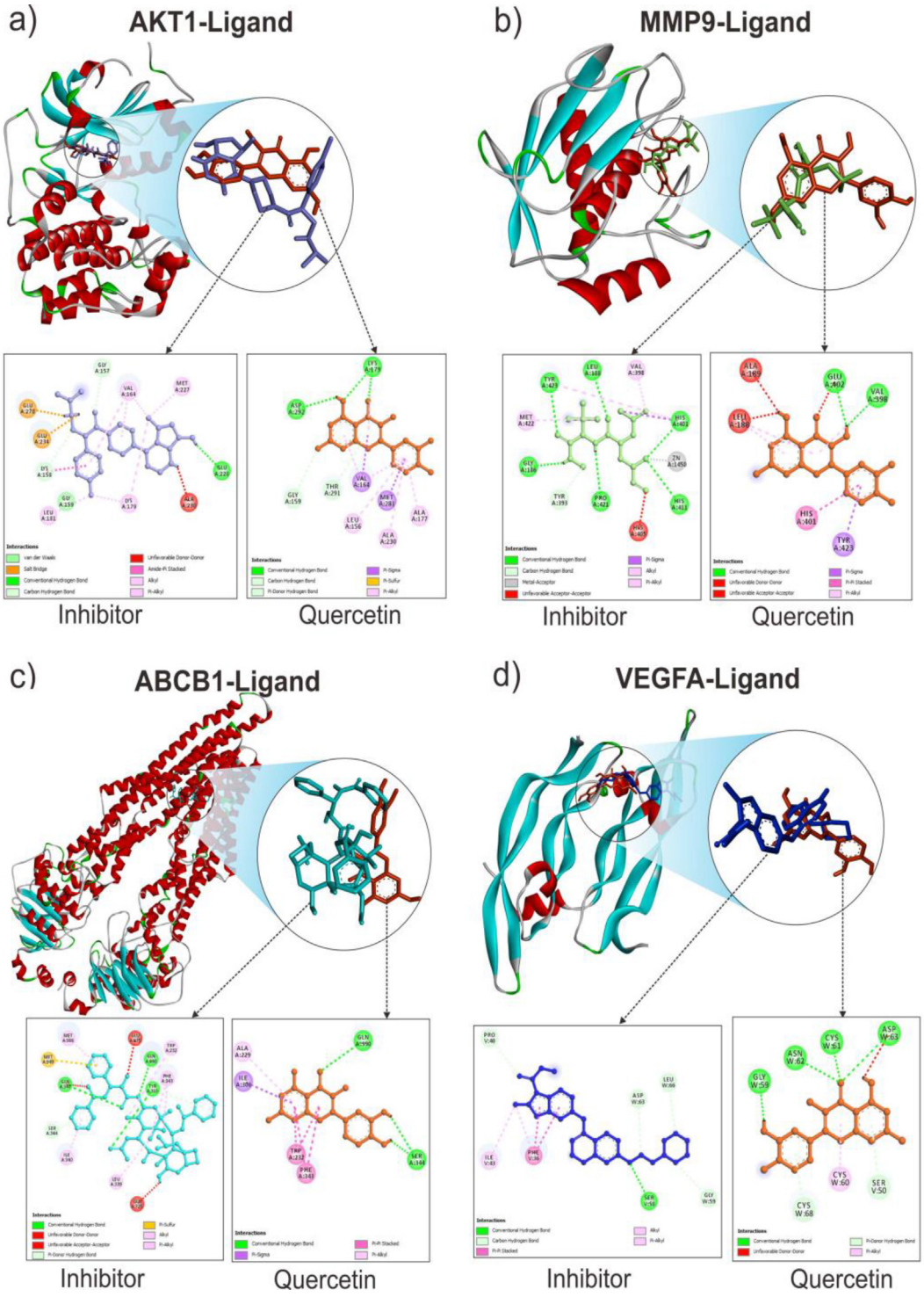

Fig. 2. Interaction between Quercetin and four direct target proteins. A) AKT1-Ligand, B) MMP9-Ligand, C) ABCB1-Ligand, D) VEGFA-Ligand.

Table 2. Interaction of Quercetin with the target proteins.

Protein	Ligand	Binding affinity (kcal/mol)	Position of chemical interaction	
			Hydrogen bond	Hydrophobic interaction
Akt1	Quercetin	−8.7	Gly159, Lys179, Thr291, Asp292	Leu156, Val164, Ala177, Ala230, Met281
	Inhibitor	−9.5	Gly157, Lys158, Gly159, Glu228	Val164, Lys179, Leu181, Met227
MMP9	Quercetin	−9.1	Val398, Glu402	Leu188, His401, Tyr423
	Inhibitor	−7.2	Gly186, Leu188, Tyr393, His401, His411, Pro421, Tyr423	Val398, Met422
ABCB1	Quercetin	−8.3	Ser344, Gln990	Ala229, Trp232, Ile306, Phe343
	Inhibitor	−9.8	Tyr310, Ser344, Gln347, Gln990	Trp232, Leu339, Ile340, Phe343, Met949, Met986
VEGFA	Quercetin	−7.2	Ser50, Gly59, Cys61, Asn62, Asp63, Cys68	Cys60
	Inhibitor	−7.3	Pro40, Ser50, Gly59, Asp63, Leu66	Phe36, Ile43

as markers of acute liver injury [34]. Conversely, Quercetin exhibits no liver toxicity and can even inhibit liver damage through its antioxidant activity [35].

This study identifies AKT1, MMP9, ABCB1, and VEGFA as the target proteins of Quercetin related to GBM. AKT1, also known as Protein Kinase B, is a central protein in the PI3K/Akt signaling pathway, regulating cell proliferation and survival [36]. Previous research has indicated that Akt1 activation promotes the survival of glioblastoma cells [37]. Another study has also suggested that inhibiting the PI3K/Akt signaling pathway can hinder GBM migration and proliferation [38]. MMP9, a matrix metalloproteinase, is implicated in tumor invasion and is overactive in GBM. Inhibition of MMP9 expression significantly reduces GBM invasion [39]. ABCB1, an ATP-binding cassette subfamily B member 1, is extensively studied in multi-drug resistance cases. Overexpression of ABCB1 in cancer cells confers resistance to chemotherapy drugs, while its inhibition enhances sensitivity to chemotherapy [40]. GBM exhibits upregulated ABCB1, rendering it prone to multi-drug resistance [41]. VEGF influences GBM angiogenesis, as continuous glioma cell division leads to oxygen deficiency and hypoxia. This hypoxic condition induces VEGF expression, promoting angiogenesis [42]. Inhibiting VEGF is a crucial strategy to impede GBM development [43]. Based on the target proteins, Quercetin exhibits potential anti-proliferative, anti-invasion, anti-angiogenesis, and anti-drug resistance activities in GBM.

The interaction between Quercetin and AKT1, MMP9, ABCB1, and VEGFA was investigated through molecular docking and dynamics simulations. Docking results indicate that Quercetin binds to the active sites of its target proteins, similar to the positive control inhibitor. The presence of more hydrogen bonds in the protein-ligand complex suggests stronger interaction stability [44]. Quercetin's interaction with its target proteins is characterized by a low binding affinity, indicating

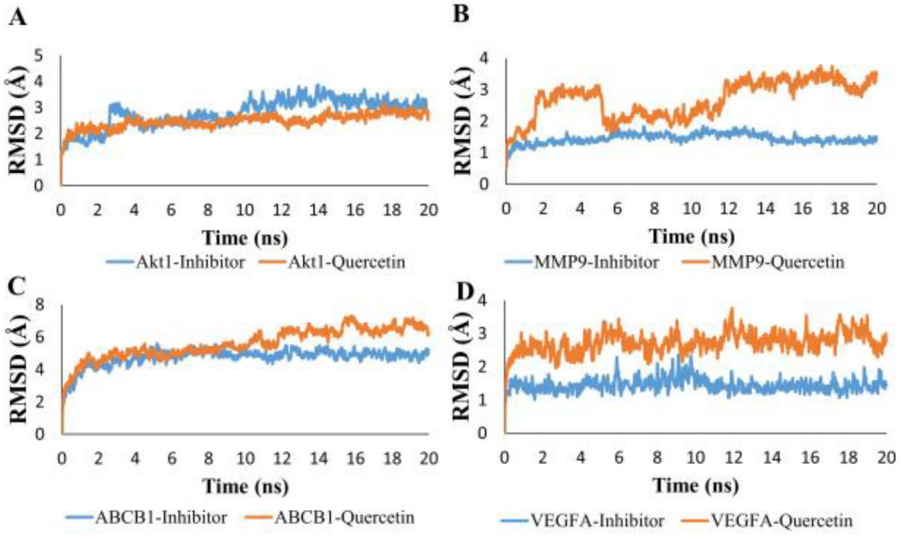

Fig. 3. RMSD backbone of the complex. A) AKT1-Ligand, B) MMP9-Ligand, C) ABCB1-Ligand, D) VEGFA-Ligand.

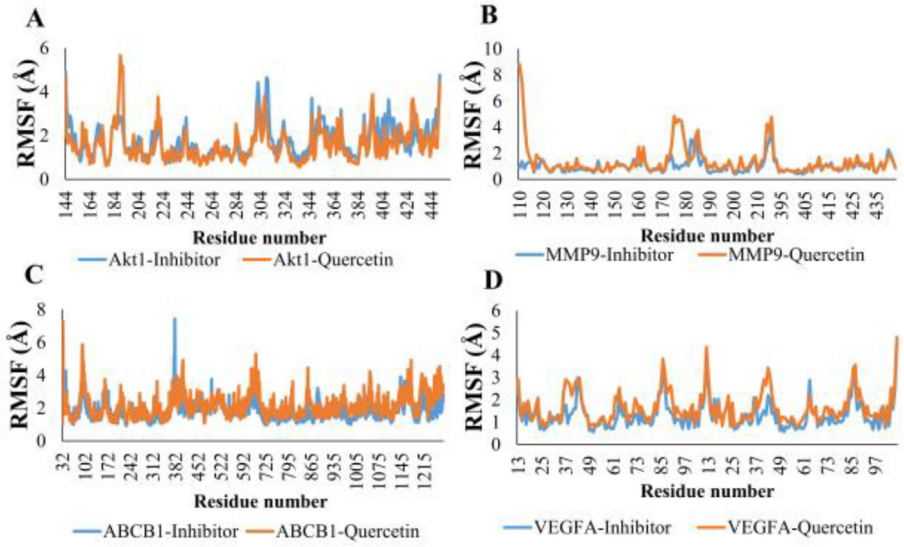

Fig. 4. RMSF of the complex. A) AKT1-Ligand, B) MMP9-Ligand, C) ABCB1-Ligand, D) VEGFA-Ligand.

robust interaction [45]. Molecular dynamics simulations confirm the docking results, as the protein structures remain stable with minimal fluctuations in RMSD values and RMSF values mostly below 3 Å [46,47]. As indicated by RMSD values, the ligand movement demonstrates stable interactions between Quercetin and AKT1, MMP9, ABCB1, and VEGFA, with minimal fluctuations observed [48]. Stable interactions indicate that Quercetin can act as an inhibitor of these overactive proteins in GBM. These findings align with previous studies demonstrating Quercetin's kinase inhibitor activity and its ability to inhibit the AKT/MM9 signaling pathway in breast cancer [49,50]. Additionally, other studies have reported Quercetin's inhibitory effects on angiogenesis, although the specific molecular mechanisms have not been elucidated [51]. This study predicts that Quercetin inhibits angiogenesis by targeting VEGFA protein activity. Furthermore, the study highlights Quercetin's potential to inhibit ABCB1, which is involved in drug resistance in cancer. These findings are consistent with previous research on Quercetin's inhibition of drug resistance [52,53]. The results of this study suggest that Quercetin has the potential to exhibit anti-proliferative, anti-invasion, anti-angiogenesis, and anti-drug resistance activities by targeting AKT1, MMP9, VEGFA, and ABCB1 proteins. However,

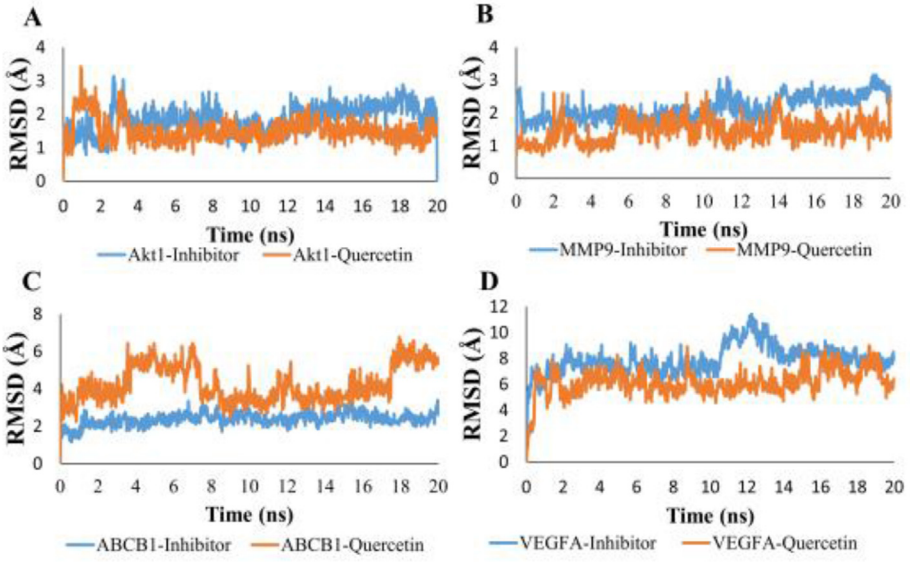

Fig. 5. RMSD ligand movement of the complex. A) AKT1-Ligand, B) MMP9-Ligand, C) ABCB1-Ligand, D) VEGFA-Ligand.

further research using cell lines and experimental animal models is necessary to validate these findings.

5. Conclusion

Quercetin has been predicted to act as an inhibitor for GBM-related proteins, including AKT1, MMP9, ABCB1, and VEGFA, by stably binding to their inhibitor sites. As a result, Quercetin is anticipated to inhibit GBM growth through apoptosis induction, proliferation inhibition, drug resistance blockade, and angiogenesis inhibition. However, further research utilizing animal models is required to confirm the findings of this study.

Conflicts of interest

The authors declare no conflicts of interest in the present study.

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