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GENETICS

Evaluation of Interactions of Triterpenes in *M. charantia* with Proteins Involved in Vascularization in *In Silico*

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Abstract—Angiogenesis is an important process that plays an active role in tumorigenesis. VEGFRs, a member of the tyrosine kinase receptor family involved in this process, is known as the receptor for VEGF ligands in tumor cells. c-Src is an adapter protein located downstream of VEGFRs and plays a role in angiogenic signaling. SPARC protein has recently been shown to play a role in metastasis in various types of cancer. In this study, inhibition of angiogenesis via extracellular matrix and VEGF/VEGFRs is aimed. *Momordica charantia*; is a valuable plant used quite often in traditional medicine. Triterpenes from various regions of plant appear to be promising in in vitro cancer-related studies. In our study; literature was searched to identify possible triterpenes in this plant; triterpenes in fruit and seed were selected. The 2D and 3D structure files of these triterpenes were obtained from PubChem. The structure files of the ligands were prepared with various programs and converted to the appropriate file format. X-ray diffraction structures of proteins were obtained from RCSB PDB. These structure files were made suitable for molecular docking studies. Docking and scoring were performed with the Vina program to select the appropriate poses. According to the *in silico* analysis; It has been found that various triterpenes that can be obtained from *M. charantia* plant may inhibit VEGFRs, SPARC, and c-Src proteins. These results show that these triterpenes are promising in terms of new natural therapeutic routes and drug candidates for aggressive cancer therapy.

Keywords: angiogenesis, VEGFRs, SPARC, c-Src, molecular docking, protein inhibitors

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INTRODUCTION

Cancer is a cumulative disease that occurs with the disruption of molecular dynamics in the normal behavior mechanisms of cells (Cooper, 2018; Pecorino, 2012). Cancer is a second major health problem that leading cause of death worldwide (Pecorino, 2012; Roser and Ritchie, 2022; Siegel, 2019). In 2019, 1762450 new cases were diagnosed and 606880 deaths occurred in the United States. Besides, it has been observed that about 30% of new cases and about 15% of deaths result from aggressive cancers, such as breast cancer, melanomas, prostate cancer, and brain-related tumors (Siegel et al., 2019). According to these data, it is crucial to investigate possible drug-like compounds to be developed for the treatment of these aggressive cancers.

Aggressive cancers, such as breast, melanomas, and gliomas, have a complicated mechanism for tumor development and spread. One of these mechanisms, angiogenesis, is necessary for the development, growth, and spreading of solid tumors beyond 1–2 mm (Shahneh et al., 2013). This process enables tumor cells to spread to distant sites by bloodstream (Shahneh et al., 2013). In tumor angiogenesis, Vascular Endothelial Growth Factors (VEGFs) and their receptors (VEGFR1, VEGFR2, and VEGFR3) play a key role in developing new capillary vessels (Shahneh et al., 2013; Eilken and Adams, 2010; Ferrera, 2002; Adams and Alitalo, 2007). Especially vascular endothelial growth factor A (VEGFA) is essential and potent for the angiogenesis process in endothelial cells (Abhinand et al., 2016). Vascular endothelial growth factor receptor-1 (VEGFR1) is a protein that belongs to the vascular endothelial growth factor receptor (VEGFR) family, encoded by the FLT1 gene located on chromosome 13q12.3 (Markovic-Mueller et al., 2017; The Human Protein Atlas, 2022). VEGFR2 protein-coding gene that named FLK1 is located on chromosome 4q12 (Koch and Claesson-Welsh, 2011; GeneCards, 2022). VEGFR1 is a high-affinity ligand for VEGFA. VEGFA also has a ligand role for VEGFR2. But rather than VEGFA/VEGFR1 interaction, VEGFA/VEGFR2 interaction induces mitogenic signals in endothelial cells more strongly (Abhinand et al., 2016; Koch and Claesson-Welsh, 2011; Ho and Fong, 2015).

The Src (short for sarcoma) tyrosine kinase family (SFKs) is known as the non-receptor protein tyrosine kinases. SFKs have a key role for initiating various diseases, especially human malignancies (include solid and hematological). The function of Src protein in normal cells; to initiate downstream signal transduction by binding to various transmembrane proteins (such as VEGFR, EGFR, PDGFR, and FGFR) with its SH2 domain. Hyperactivation or overexpression of this protein is as known related to poor prognosis and tumor grade (Schenone et al., 2007).

In tumor angiogenesis, although the main elements are vascular endothelial growth factors and their receptors, recent studies show us that soluble factors in the tumor microenvironment result in angiogenesis. Like growth factors, such as VEGFA or PIGF, cytokines, chemokines, and various proteins play a key role in the angiogenesis signaling pathway (Eilken and Adams, 2010; Chang et al., 2017). Secreted Protein Acidic and Rich in Cysteine (SPARC) is a matricellular glycoprotein that has been associated with extensive tissue remodeling and tumorigenesis (Said, 2016). SPARC is a 303 amino acid protein and encoded by a single gene in human chromosome 5q31.1 (Said, 2016; Bradshaw and Sage, 2001; Lane and Sage, 1994). Mature SPARC protein has 286 amino acids (first 17 amino acids are signal peptide) with three major functional domains including an N-terminus acidic domain (NT), the follastatin-like domain (FS), and C-terminus domain (EC) (Said, 2016; Bradshaw and Sage, 2001; Lane and Sage, 1994). The NT domain comprised of amino acids 3–51, the FS domain comprised of amino acids 53–137, and the EC domain comprised of amino acids 138–286 (Said, 2016; Bradshaw and Sage, 2001; Lane and Sage, 1994; Pavanelli et al., 2017). In the follastatin-like region, there are also two Cu^{+2} binding sites within this domain and a (K)GHK sequence at amino acids 113–130 that encodes an angiogenic peptide when released (Bradshaw and Sage, 2001; Lane and Sage, 1994). In contrast, SPARC also shows an anti-tumor role in anti-angiogenesis (Chang et al., 2017). Due to this multifunctional structure of the protein, the role of SPARC in cancer development is still complicated.

Natural products have been used to treat various diseases since ancient times (Dutta et al., 2019). *Momordica charantia* (also known as kugua, bitter melon, bitter gourd, etc.) is a fruit that belongs to the Cucurbitaceae family and it is widely cultivated in the tropical and subtropical regions of the world (Jia et al., 2017). *M. charantia* contains several bioactive natural products such as triterpenoids, saponins, flavonoids, and alkaloids (Jia et al., 2017; Farooqi et al., 2018). *M. charantia* extracts and its monomer components have shown the strong anti-cancer effect on various tumors such as breast cancer, leukemia, prostate cancer, skin cancer and melanomas (Jia et al., 2017).

Triterpenoids, one of these natural compounds which in *M. charantia*, are metabolites of isopentenyl pyrophosphate oligomers. Triterpenes are a large family that contains various components such as triterpenic glycosides (saponins), phytosterols, and their precursors, and these components are potential therapeutics for the prevention and treatment various diseases including several cancers. Various studies have shown that triterpenoids inhibit angiogenesis by suppressing VEGFR2 activation (Jagan and Chinthalapally, 2012). It has also been stated that various triterpenes inhibit Src phosphorylation (Schenone et al., 2007; Jia et al., 2017; Jagan and Chinthalapally, 2012).

In this study, whether the inhibition of the angiogenesis-related regions of these proteins occurs with the triterpenes determined within the scope of the study's hypothesis is investigated *in silico* analysis. In recent years, in a study conducted on the glioblastoma multiforme cell line, it has been proven by in vitro experiments that *M. charantia* extract causes a decrease in SPARC and Src proteins, and it has been suggested that Sparc and Src proteins may play an effective role in the metastasis processes of this metastatic cancer (Erdogan and Eroglu, 2022). Also known that the SPARC protein temporarily regresses immediately after the injury state, and this condition promotes the VEGFA signal by temporarily inhibiting the tyrosine kinase activity of VEGFR. And this increases the disadvantages of proliferation at the angiogenesis stage through VEGFR1 stimulation. In line with all these preliminary data, it is aimed in this study to establish a hypothesis that the triterpene structures in *M. charantia* may inhibit these proteins and to test it with *in silico* bioinformatic analyses. Three-dimensional crystallography structure files of VEGFR receptors (1 and 2), SPARC and c-Src proteins were also obtained from RCSB PDB and used in molecular docking studies. Following *in silico* molecular docking analysis of these proteins and related triterpene structures, triterpene structures that were predicted to inhibit angiogenesis-related target proteins were predicted. With the estimated results of this study, we suggest that these triterpene structures may be angiogenesis inhibitors for use in in vitro and in vivo experiments.

MATERIALS AND METHODS

Systems, Softwares and Web Servers

The computer systems and computational programs used in this study are as follows; Computers ("MacBook Air, 1.8 GHz Dual-Core Intel Core i5 with macOS Catalina" and "HP Pavilion Laptop, Intel Core 15-7200U CPU, 2.50 GHz with Windows 10 Home"), MarvinSketch (ChemAxon, 2022), AutoDock Tools (Morris et al., 2009), Vina (Trott and Olson, 2010), Discovery Studio Visualizer, Molecular Operating Environment, SwissADME (Daina et al., 2017).

Table 1. Compliance values of the compounds used in the study according to Lipinski's 5 rule

Compound name	Molecular weight	MLogP	H-bond acceptors	H-bond donors
Cucurbitacin A	184.23 g/mol	1.50	3	0
Cucurbitacin B	378.50 g/mol	1.61	5	3
Cucurbitacin C	560.72 g/mol	1.85	8	4
Cucurbitacin E	556.69 g/mol	1.68	8	3
Cucurbitacin H	534.68 g/mol	0.74	8	5
Cucurbitacin I	514.65 g/mol	1.36	7	4
Cucurbitacin J	532.67 g/mol	0.66	8	5
Cucurbitacin O	518.68 g/mol	1.53	7	5
Cucurbitacin P	520.70 g/mol	1.62	7	5
Cucurbitacin R	518.68 g/mol	1.53	7	4
Cucurbitacin U	504.70 g/mol	2.42	6	4
Cucurbitacin Q	560.72 g/mol	1.85	8	4
Erythrodiol	442.72 g/mol	6.00	2	2
Euferol	426.72 g/mol	6.82	1	1
Goyaglycoside A	648.87 g/mol	2.25	9	5
Goyaglycoside B	648.87 g/mol	2.25	9	5
Goyaglycoside C	662.89 g/mol	2.42	9	4
Goyaglycoside D	662.89 g/mol	2.42	9	4
Karavilagenin A	486.77 g/mol	5.39	3	1
Karavilagenin B	472.74 g/mol	5.20	3	2
Karavilagenin C	472.74 g/mol	5.20	3	2
Karavilagenin E	456.70 g/mol	5.01	3	2
Karounodiol	440.70 g/mol	5.89	2	2
Kuguacin J	454.68 g/mol	4.82	3	2
Momordicin	479.73 g/mol	5.20	3	1
Momordicin I	472.70 g/mol	4.06	4	3
Momordicoside F1	632.87 g/mol	2.62	8	4
Momordicoside F2	618.84 g/mol	2.44	8	5
Momordicoside G	632.87 g/mol	2.62	8	4
Momordicoside I	618.84 g/mol	2.44	8	5
Momordol	440.66 g/mol	2.54	5	4
Octanorcucurbitacin A	342.47 g/mol	3.20	3	0
Octanorcucurbitacin B	342.47 g/mol	3.20	3	0
Octanorcucurbitacin D	388.50 g/mol	2.33	5	0
Taraxerol	426.72 g/mol	6.92	1	1
β-Amyrin	426.72 g/mol	6.92	1	1

Ligand Preparation for Molecular Docking Analysis

2D and 3D structures of chemicals are downloaded as ".sdf" file format from PubChem Data Bank (https://pubchem.ncbi.nlm.nih.gov). ADMET properties are calculated on SwissADME (Daina et al., 2017) webserver for all chemical structures. The ADMET properties of all compounds were evaluated based on Lipinski's rules and are shown in Table 1. 2D structure files are turned 3D structure file using MarvinSketch (ChemAxon, 2022) software. Energy minimization and partial charge analyzes of the ligand files prepared with MarvinSketch software were performed in MOE software, and they were structurally prepared to be recorded in the appropriate file format. Using AutoDock Tools (Morris et al., 2009), the root of the molecules and the number of torsions in ligand files were determined and saved as "pdbqt" file format for using AutoDock Vina.

Preparation of Receptor for Molecular Docking Analysis

As SPARC protein structure, the X-ray diffraction structure with PDB: 1BMO (Hohenester et al., 1997) from the RCSB PDB site (https://www.rcsb.org), as well as the X-ray diffraction structure with the PDB: 5T89 (Markovic-Mueller et al., 2017) for the VEGFR1 protein structure, X-ray diffraction structure of c-Src with PDB: 6WIW (Fang et al., 2020) and X-ray diffraction structure of VEGFR2 with PDB:3V2A (Brozzo et al., 2012) were obtained. Each PDB file is imported one by one into Discovery Studio Visualizer. For each PDB file; additional water molecules, ion structures, and hetatm structures in the PDB files have been removed. Missing side chains and hydrogen atoms are optimized using the AutoDock Tools software (Morris et al., 2009). When selecting all PDB files, the highest resolution is preferred for further analysis. Care has been taken to ensure that the PDB file is up to date.

Molecular Docking Procedure

Using AutoDock Tools (Morris et al., 2009), ligand files and protein files are saved as "pdbqt" file format so that they can be run in the Vina software (Trott and Olson, 2010). By creating a configuration file for the Vina software, docking was performed between proteins and ligands. The binding affinities of compounds capable of target site-specific interactions were determined for each protein individually. Outputs of Vina software analysis were visualized and checked with Discovery Studio Visualizer software and bond structures and lengths were determined for each compound. In this step, information on amino acids, bonds formed and their lengths in the regions where these compounds interact was also evaluated as a figure. In this regard, Fig. 1 for the binding of VEGFR1 protein to compounds, Fig. 2 for VEGFR2 protein and its interactions, Fig. 3 for c-Src protein and its interactions, and Fig. 4 to show the interaction of SPARC protein with compounds.

RESULTS

Evaluation of ADMET Properties of Triterpenic Phytochemicals Contained in M. charantia In Silico

In computer-aided drug design, the pharmacological drug-likeness of the compounds to be analyzed as drug candidates is important for future in vitro and in vivo experiments. ADMET (Absorption, Distribution, Metabolism, Elimination, and Toxicity) features are seen as a critical step in drug discovery studies (Thermo Fisher Scentific, 2022). Our analysis with SwissADME were based on the "5 rules of Lipinski" which are the rules used by SwissADME and Pfizer in finding drug-like ratios of the compounds to be investigated (Daina et al., 2017; Lipinski et al., 2001; Swiss Institute of Biotechnology, 2022) (Table 1). It was predicted that 18 of 28 compounds had high molecular weight, which could cause absorption and distribution problems in the body (Table 1). Also, it were predicted that 7 of these compounds did not comply with Lipinski's drug-likeness rules in terms of the LogP value used to determine the fat solubility. However, it were determined by SwissADME that these compounds have drug-likeness according to Lipinski's rules.

Molecular Docking Analysis with Triterpene Structures and Angiogenesis Receptors (VEGFR1 and VEGFR2) and Adapter Protein c-Src

As a result of our molecular docking studies for VEGFR1, 14 of 36 predicted drug-like triterpene structures (Karounodiol, Cucurbitacin A–B–C–E–I–P, Momordol, Karavilagenin B, Kuguacin J, Octanorcucurbitacin A–B–D) appear to bind to the binding site on the VEGFR1 receptor. It has been demonstrated by *in silico* predictions that these drug-like structures can form hydrogen bonds with amino acids, as well as carbon-hydrogen and various alkyl bonds in the target region. It can be speculated that the bonds formed may have the potential to inhibit stimuli for the angiogenesis process by creating protein-ligand interactions with high binding affinity with the amino acids in the binding site of VEGFA. However, when compared to Sunitinib (Sanchez-Leon et al., 2022) which is the strongest inhibitor of VEGFR1 proven in the literature by in vitro and in vivo experiments, it can be seen that only 7 of these 14 triterpene structures can be strong inhibitor candidates. As a result of this *in silico* molecular docking study, it can be predicted that the only triterpenes with a binding affinity of –6.2 kcal/mol that Sunitinib has is Karounodiol, Cucurbitacin A–C–E–I–P and Kuguacin J (Fig. 1). The results of this inhibitor candidate for VEGFR1 were obtained as an estimate by calculating them *in silico* with AutoDock Tools Vina, a computational biology tool.

According to the *in silico* ligand-binding results for VEGFR2, it is seen that only 6 of the 36 triterpene structures (Goyaglycoside A–C–D, Momordicoside F1–F2–I) interacting with the target site (Fig. 2). The ligand-binding affinities observed for these interactions are relatively high. At the same time, *in silico* results shown that the ligands often may make alkyl bonds. Besides, results of *in silico* molecular docking analysis shown that Momordicoside F2 may made a pi-sigma bond (Fig. 2). Generally, pi-interactions are bond types that are not affected by solvent/desolvation conditions (Bernaldez et al., 2018). Therefore, their presence in ligand-protein interactions may means that this interaction is strong. Sunitinib is a pharmaceutical that can be used for VEGFR1 as well as VEGFR2 inhibition, and therefore the binding affinity of sunitinib was used as a basis to compare the binding affinities of triterpenes for the VEGFR2 receptor. As a result of *in silico* molecular docking studies performed in this study, the binding affinity of sunitinib to

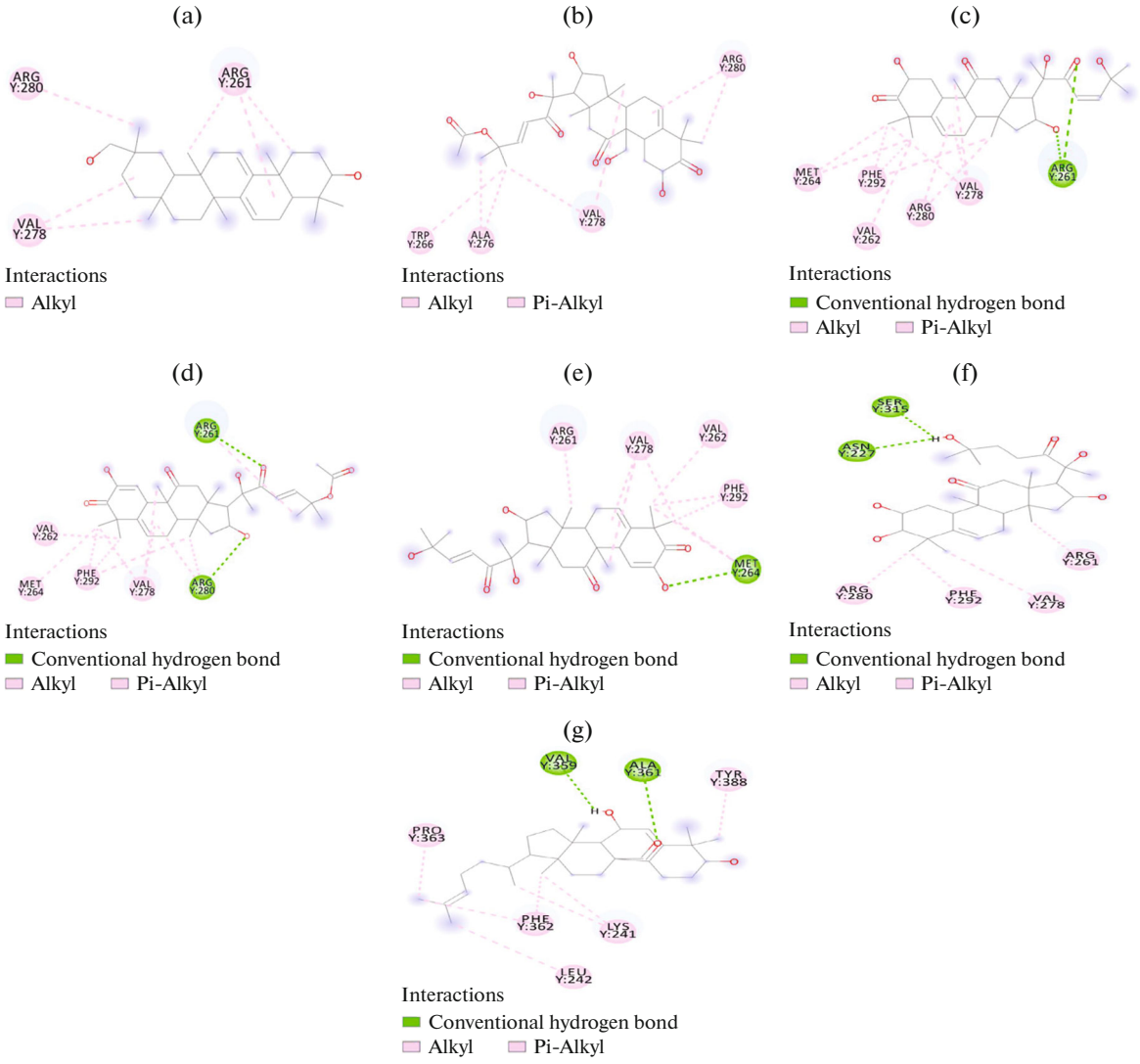

Fig. 1. Interactions of triterpenoid structures with VEGFR1 (a) Karounodiol (binding affinity: –6.2 kcal/mol), (b) Cucurbitacin A (binding affinity: –6.2 kcal/mol), (c) Cucurbitacin C (binding affinity: –6.4 kcal/mol), (d) Cucurbitacin E (binding affinity: –6.3 kcal/mol), (e) Cucurbitacın I (binding affinity: –6.2 kcal/mol), (f) Cucurbitacin P (binding affinity: –6.3 kcal/mol), (g) Kuguacin J (binding affinity: –6.3 kcal/mol).

VEGFR2 were estimated as –5.7 kcal/mol. *In silico* molecular docking studies conducted with triterpenes on the VEGFR2 receptor indicate that the binding affinities of 6 triterpenes (Goyaglycoside A–C–D, Momordicoside F1–F2–I) that were predicted to be effective may be much higher than sunitinib.

In the results that obtained for Src, 22 of 36 triterpene structures were found to bind to the target site in the best way. We found that the triterpene structures we studied showed a higher affinity for Src. Also, it has been observed that these compounds frequently make alkyl bonds as well as hydrogen bonds. It is observed that compounds with high binding affinity make more alkyl-type bonds, and hydrogen bonds and carbon-hydrogen bonds increase as the bonding affinity decreases. Compared with Dasatinib, which is known as an inhibitor of Src (Zhang al., 2020), it is seen that triterpene structures we turn to can also show binding affinities close to Dasatinib. While dasatinib showed a binding affinity of –8.9 kcal/mol, it was predicted as a result of molecular docking analysis that there may be two triterpene structures showing the same affinity in the same region. Therefore, 20 triterpenes with much lower affinity can be eliminated at this stage due to their binding affinity. In this way, β-amyrin and Momordicin (Fig. 3), which are predicted to show close affinity to Dasatinib in c-Src inhibition, are predicted as candidate inhibitors as a result of these *in silico* molecular docking analyses.

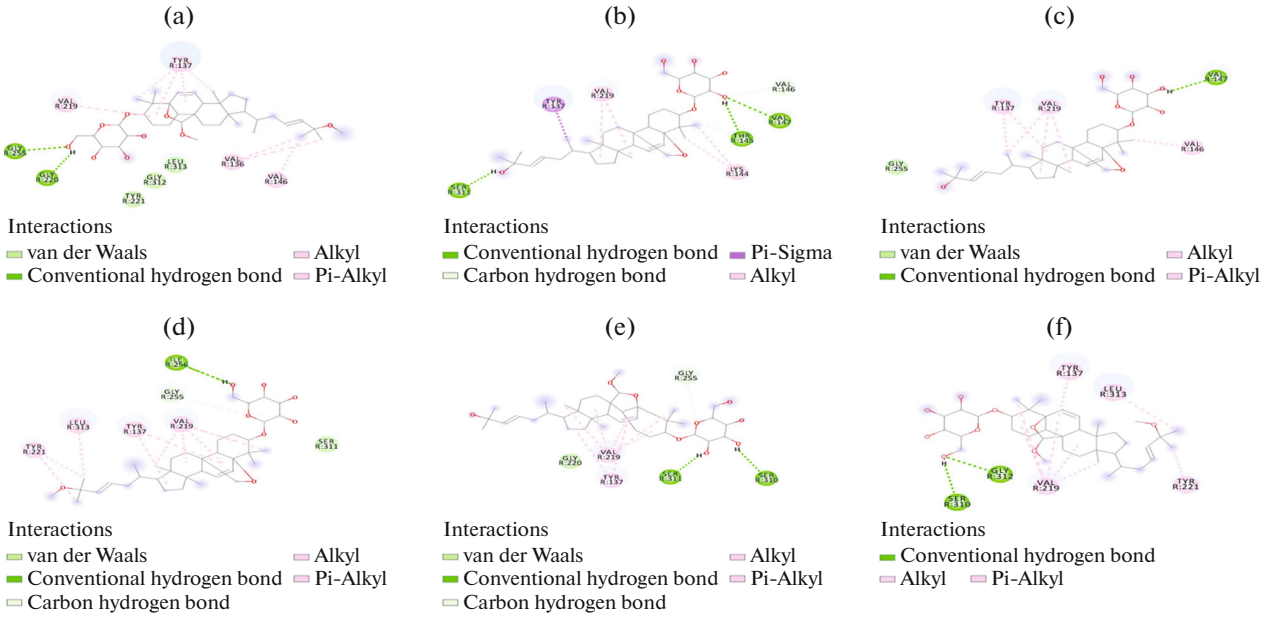

Fig. 2. Interactions of triterpenoid structures with VEGFR2 (a) Goyaglycoside D (binding affinity: –6.6 kcal/mol), (b) Momordicoside F2 (binding affinity: –6.5 kcal/mol), (c) Momordicoside I (binding affinity: –7.0 kcal/mol), (d) Momordicoside F1 (binding affinity: –6.5 kcal/mol), (e) Goyaglycoside A (binding affinity: –6.3 kcal/mol), (f) Goyaglycoside C (binding affinity: –7.1 kcal/mol).

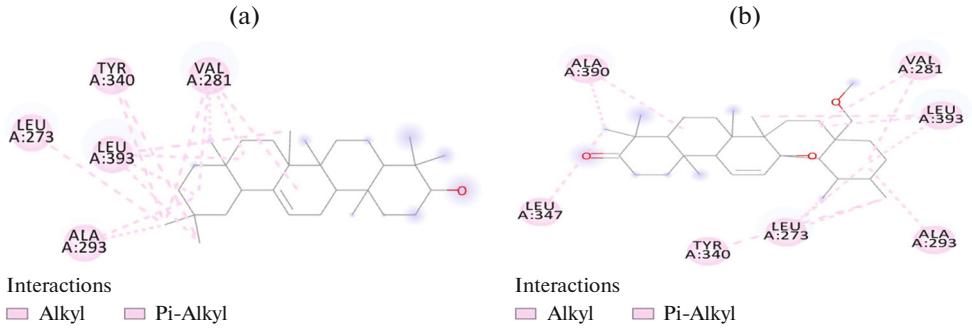

Fig. 3. Interactions of triterpenoid structures with c-Src (a) β-amyrin (binding affinity: –8.9 kcal/mol), (b) Momordicoside F2 (binding affinity: –8.9 kcal/mol).

Inhibition of the Angiogenic Effects of SPARC in the Extracellular Matrix

As a result of the molecular docking analysis with the SPARC protein and drug-like triterpenes, it was estimated that there was a total of 21 drug-like triterpene structures that were likely to bind to the angiogenesis-related amino acid sequences of this protein and were predicted to inhibit the angiogenesis relationship of the protein. These structures according to their best binding affinities are Taraxerol, Erythrodiol, Cucurbitacin A–B–C–E–I–O–U–Q, Goyaglycoside A–B–C–D, Momordicoside F1–F2–G–I, Karavilagenin C, Kuguacin J and Octanorcucurbitacin A (Fig 4). Also, as a result of *in silico* molecular docking analysis, it was predicted that the binding affinities of these 21 triterpene structures, which seem to have an effect on the angiogenesis-related region of SPARC protein, to this protein would be between –6.1 and –7.2 kcal/mol.

DISCUSSION

Momordica charantia; it is a plant that grows in subtropical regions, has a very high therapeutic value, and is used in traditional medicine. Demonstrating that *M. charantia* is effective as anti-inflammatory and anti-oxidant in various studies has also provided an important data source for the study of anti-cancer effects (Cao et al., 2018; Bortolotti et al., 2019). The anti-tumor, anti-inflammatory, immune modulator and anti-diabetic effects of most phytochemicals contained in *M. charantia* have been proven by several

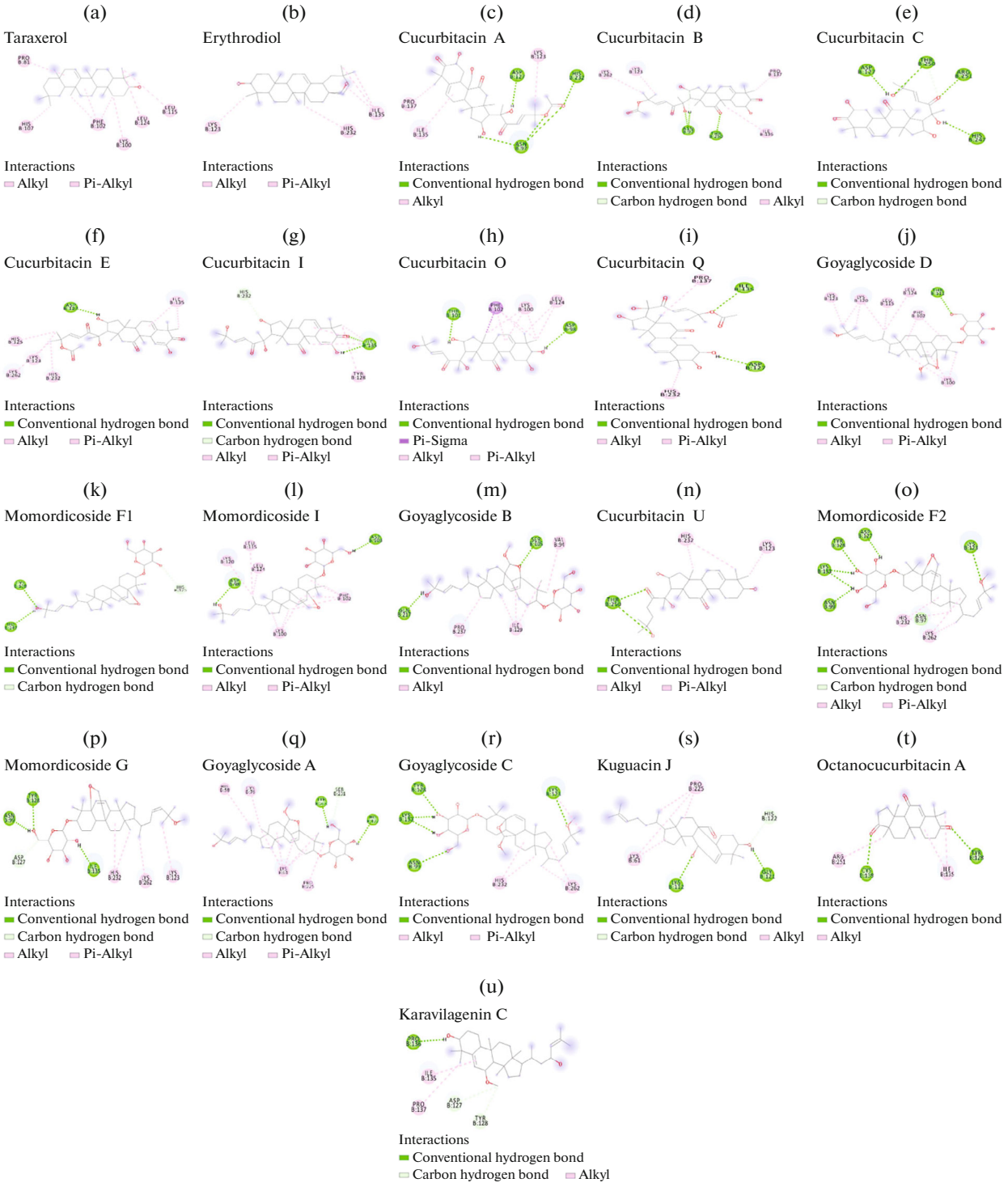

Fig. 4. Interactions of triterpenoid structures with SPARC (a) Taraxerol (binding affinity: –7.1 kcal/mol), (b) Erythrodiol (binding affinity: –6.8 kcal/mol), (c) Cucurbitacin A (binding affinity: –6.8 kcal/mol), (d) Cucurbitacin B (binding affinity: –6.8 kcal/mol), (e) Cucurbitacin C (binding affinity: –7.1 kcal/mol), (f) Cucurbitacin E (binding affinity: –6.2 kcal/mol), (g) Cucurbitacin I (binding affinity: –6.9 kcal/mol), (h) Cucurbitacin O (binding affinity: –6.1 kcal/mol), (i) Cucurbitacin Q (binding affinity: –6.1 kcal/mol), (j) Goyaglycoside D (binding affinity: –6.2 kcal/mol), (k) Momordicoside F1 (binding affinity: –6.9 kcal/mol), (l) Momordicoside I (binding affinity: –6.7 kcal/mol), (m) Goyaglycoside B (binding affinity: –7.0 kcal/mol), (n) Cucurbitacin U (binding affinity: –6.6 kcal/mol), (o) Momordicoside F2 (binding affinity: –7.2 kcal/mol), (p) Momordicoside G (binding affinity: –7.0 kcal/mol), (q) Goyaglycoside A (binding affinity: –7.0 kcal/mol), (r) Goyaglycoside C (binding affinity: –6.9 kcal/mol), (s) Kuguacin J (binding affinity: –6.8 kcal/mol), (t) Octanocucurbitacin A (binding affinity: –7.1 kcal/mol), (u) Karavilagenin C (binding affinity: –6.6 kcal/mol).

studies (Cao et al., 2018; Bortolotti et al., 2019; Wang et al., 2012). Among these phytochemicals; triterpene structures, whose biological activities have been emphasized recently, are being investigated in terms of anti-cancer properties.

VEGFA/VEGFR1 and VEGFA/VEGFR2 signaling play an important role in solid tumors in terms of the emergence of angiogenesis and increased proliferation. Studies have shown that these two receptors trigger angiogenesis. Although VEGFA is the ligand of both receptors, its binding affinity for VEGFR1 is higher. However, compared to VEGFR1, VEGFR2 plays a primary role in triggering angiogenesis. While VEGFR2 is so important, various studies have shown that VEGFR1 is also effective in triggering angiogenesis. It was determined that the PI3K/Akt signaling pathway was triggered by the activation of both receptors with VEGFA and stimulation of Src protein as a result of autophosphorylation and thus proliferation and migration were also stimulated (Weddell et al., 2017). Therefore, in this study, it is aimed to predict *in silico* computational analyses a candidate compound for inhibition of angiogenesis related receptors and adapter protein c-Src. At the same time, for SPARC protein, whose angiogenic effect has been frequently studied recently; to prevent angiogenic stimuli from the extracellular matrix, an attempt was made to predict the inhibitor candidate.

Regulation of the angiogenesis process in tumor cells has been associated with increased expression of SPARC protein (Pavanelli et al., 2017). In tumor structures with high metastatic character (such as gliomas, melanomas, breast, and prostate cancers), SPARC protein is highly overexpressed (Jianguo and Liling, 2014; Erdogan and Eroglu, 2022). The fact that it can stimulate angiogenesis in its metastatic tumor structures with its high expression and multifunctional structure makes it one of the potential targets for angiogenesis inhibition.

All analyses performed in this study were *in silico* analysis and based entirely on the estimated results given by algorithms created with various computational models. There were no in vitro or in vivo experiments have been performed for this compunds in scope of this study. During the *in silico* analyzes performed in this study, results were shown that none of the triterpene structures used as ligands had the potential to simultaneously inhibit VEFGR1 and VEGFR2, which play a role in the angiogenesis process. In addition, triterpene structures that may have the potential to simultaneously inhibit the angiogenesis-related region of the SPARC protein and the VEGFR1 receptor were also predicted as a result of *in silico* analyses. Accordingly, it was estimated that 8 of all drug-like triterpene structures examined in this study may provide co-inhibition at further in vitro or in vivo experiments. These compounds were; Cucurbitacin A–B–C–E–I, Kuguacin J, and Octanorcucurbitacin A. Similarly, compounds with the potential to jointly inhibit VEGFR2 and the angiogenesis-associated region of the SPARC protein were predicted *in silico* results; Momordicoside F1–F2–I and Goyaglycoside A–C–D. When an *in silico* molecular docking study was carried out for a structure that could simultaneously inhibit VEGFR1 and c-Src proteins, which play a downstream role in the angiogenic process in cancer cells, it was predicted that there were compounds that had the potential to inhibit VEGFR1 and c-Src proteins together, and these compounds were; Euferol, Octanorcucurbitacin A–B, Cucurbitacin E–P, Momordol and Kuguacin J. Although there were predicted common inhibitors of VEGFR2 and c-Src proteins in this pathway (Momordicoside F1–I and Goyaglycoside C–D), results were shown that there were no predicted compounds that may inhibit all three proteins VEGFR1, VEGFR2, and c-Src simultaneously. Provided that all these estimated *in silico* results can be supported by in vitro and in vivo experiments, it was envisaged that the combination of compounds can be tried for the inhibition of the angiogenesis system as an alternative way in cancer therapy, and as a result of various pharmacological studies, these natural compounds can be turned into valuable pharmaceuticals that can inhibit the angiogenic system. It was envisaged that the components with predicted inhibitory effects in this study can be developed for pharmaceutical purposes and will contribute to the development of alternative therapies for cancer patients within the scope of 'personalized therapy'.

Cucurbitacin-type triterpene structures are the compounds whose anti-cancer effects have been studied the most. In this context, when looking at the studies in the literature, it has been revealed that Cucurbitacin A can inhibit the mTOR/PI3K/Akt signaling pathway in ovarian cancer in vitro, and at the same time it can be a JAK2 protein inhibitor alone (Liu et al., 2018; Kaushik et al., 2015; Lee et al., 2010; Wang et al., 2017). When studies with Cucurbitacin B are examined; It has been found to reduce cell viability by inducing apoptosis in various types of cancer, downregulate protein expression by causing heavy methylation in various oncogenes (c-Myc, cyclin D1, and survivin) and inhibit oxidative stress (Dittharot et al., 2019; Sallam et al., 2018; Klungsaeng et al., 2019). Also, it has been found in studies on breast cancer that Cucurbitacin B partially downregulates the VEGF/FAK/MMP9 signal pathway, which leads to a decrease in metastasis and angiogenesis (Sinha et al., 2016).

Studies with Cucurbitacin C are very limited in the literature. The one article on anti-cancer properties was published in 2019 and according to this study, Cucurbitacin C triterpene; When low-dose therapy was applied in in vitro studies, it stopped the cell cycle of cancer cells in the G1 or G2/M stage, and when the high dose was administered, it directed the cells to apoptosis. In vivo studies, however, have been shown

to significantly reduce tumor growth in xenograft mice (Wu et al., 2019). In the literature researches about Cucurbitacin E; It has been found to stimulate apoptosis, suppresses cell viability, and causes cytotoxicity on various cancers (He et al., 2017; Song et al., 2018). Cucurbitacin E is the substrate of P-gp and BCRP proteins that cause multiple drug resistance and has been identified as an appropriate candidate to treat multiple drug resistance (Saeed et al., 2019).

While there is no study on Cucurbitacin H in the literature reviews, studies on Cucurbitacin I, have been shown that the triterpene structure significantly reduces the viability of various cancer cells at low concentrations, as well as a powerful inhibitor of the JAK2/STAT3 pathway, one of the important oncogenic pathways (Turner et al., 2020; Blaskovich et al., 2003; Dandawate et al., 2020). When we examine other cucurbitacin analogs, we see that it can only act as a selective STAT3 inhibitor concerning cucurbitacin Q (Sun et al., 2005; Ul Haq et al., 2019). However, it does not require inhibition of the Src protein in inducing apoptosis, supporting our results. The limited studies on Cucurbitacin O show that it only creates non-specific cytotoxicity (Meng et al., 2008). On the other hand, Cucurbitacin R stands out with its anti-inflammatory and immune-suppressive properties, as in most other analogs (Escandell et al., 2010a, 2010b). Apart from this, there are no anti-cancer studies on other cucurbitacin analogs (Cucurbitacin J, and P) that we examined.

As a result of our analysis on Cucurbitacin type triterpenes; we predicted that almost all analogs may have inhibitory effects, but their target-specific orientations are low. However, our *in silico* analysis were predict that Cucurbitacin C may have a target-specific orientation on SPARC and VEGFR1 proteins. Considering that SPARC protein has a single angiogenic region and this region is targeted in the study, we suggest that Cucurbitacin C may be an important natural inhibitor candidate for SPARC. Looking at the ADMET properties of Cucurbitacin C, which is the compound with the highest binding affinity for VEGFR1 protein and has a relatively high binding affinity for SPARC, it appears that the only disadvantage of being a natural drug candidate is the relatively high molecular weight of the compound. Other analogs appear to may be effective on the VEGFR1 protein with a progressively decreasing binding affinity. Similarly, results of *in silico* molecular docking analysis predict that they can act with increasingly decreasing binding affinities for SPARC protein. When looking at the general ADMET properties of Cucurbitacin type triterpene analogs, it is seen that the only disadvantage in front of them being natural drugs is the relatively high molecular weight of the compounds. Cucurbitacin type triterpenes, it was may predicted that they theoretically approach the Lipinski's 5 rule (Ro5), which is the criteria used for high oral bioavailability of a drug (Lipinski et al., 2001).

It has been observed in past studies that the compound Erythrodiol has anti-cancer and pro-apoptotic effects (Juan et al., 2008). For Euferol, studies on these effects are insufficient. Studies for the β-amyrin compound have defined its anti-inflammatory, anti-ulcerogenic, analgesic, and apoptotic properties (Dzubak et al., 2006; Cuesta-Rubio et al., 2017). It has been determined that the Taraxerol compound inhibits the apoptotic process through the mitochondrial pathway and at the same time plays a role as a tyrosinase inhibitor in the melanin production pathway in the case of hyperpigmentation (Yao et al., 2017; Chunhakant and Chaicharoenpong, 2019).

When compared with the findings of our molecular docking study, it was seen that Taraxerol could only be suitable as an angiogenesis inhibitor for SPARC. In the *in silico* analysis that made for Euferol, although it is seen that it may be an inhibitor candidate for VEGFR1 and Src proteins; results may say that the specificity for these proteins will be quite low since their water solubility is poor and their binding affinities are relatively low. For β-amyrin; our results conclude that, despite its poor water solubility, it may be a potent Src inhibitor. According to these data we have obtained, it is thought that β-amyrin may work as a potential natural angiogenesis inhibitor in further studies. Considering the estimated binding affinities obtained from *in silico* analyses, Erythrodiol can be seen as a less specific inhibitor candidate for c-Src protein than β-amyrin, but more than Euferol. Besides, Erythrodiol is a predicted inhibitor candidate according to molecular docking analysis for SPARC protein. However, considering the properties of ADMET, it may be evaluated as a natural inhibitor candidate in second place in further studies due to its very poor water solubility. Also, the relatively low binding affinities for these two proteins may weakens the inhibitor candidacy since there is no target-specific binding.

Studies on the effects of Octanorcucurbitacin on cancer have not been found in the literature reviews. Considering the results of our study, it was determined that ADME properties of these compound structures are very close to being drug candidates theoretically according to Lipinski rules. In addition, when we look at their interactions and binding affinities with VEGFR1, SPARC and Src proteins, they are thought to be weak natural inhibitor candidates for these proteins. It is clear that further studies need to be done.

Besides, studies with Kuguacin J found that this triterpene has antineoplastic and anti-cancer properties (Pitchakarn et al., 2011). Previous studies have revealed that Karounidiol can be chemopreventive used in renal cancers (Akihisa et al., 2001). Our findings show that Karounidiol is unlikely to be used as an angiogenesis inhibitor. Of the VEGFR1, VEGFR2, and c-Src proteins that stimulate angiogenesis, it has been found that it can only interact with VEGFR1 and

its binding affinity is relatively low. This compound, which is not specific for the angiogenesis system, seems more likely to be used as a chemopreventive. Studies on goyaglycosides are limited, especially in terms of cancer studies. There is limited evidence that some of the goyaglycosides (D and B) may have cytotoxicity (Zhang et al., 2020). In our analysis, it was seen that Goyaglycosides were effective on VEGFR2, c-Src, and SPARC proteins, and on the contrary, they were not specific to the target site on VEGFR1. We see that Goyaglycoside C and B show high specificity for VEGFR2 and c-Src proteins, respectively. Especially, the effect of Goyaglycoside C on VEGFR2 appears to have a higher binding affinity compared to the approved drug Sunitinib. On the other hand, when the ADME properties of goyaglycosides are examined, it is seen that the compliance with the Lipinski rules is theoretically impaired only due to their molecular weight. However, due to their high affinity, they may be considered as natural angiogenesis inhibitor candidates for further analysis.

When we look at the other studies on Karavilagenin analogs, it was seen that D and C analogs show cytotoxic effects (Ramalhete et al., 2018; Zhang et al., 2012). In particular, it has been observed that Karavilagenin C acts against multi-drug resistance at low concentrations by reversing resistance via P-gp protein (Ramalhete et al., 2018). As a result of the *in silico* analysis that made on Karavilagenins, it was seen that it may be considered as a natural inhibitor candidate due to its high binding affinity especially for c-Src. However, the binding affinity remains low compared to the Dasatinib molecule used as an inhibitor for c-Src. Considering the harmony of the analogs of Karavilagene with the Lipinski rules, it is noteworthy that they are theoretically close to the rules, but their water solubility values are inconvenient. The need for further analysis (*in silico*/in vitro/in vivo) is clearly seen here.

Studies investigating the relationship between Momordicoside analogs and cancer have shown that especially F1, F2, and I analogs have antiproliferative effects, while Momordicoside G triggers the shrinkage of inflammation-associated lung cancer lesions and healing of damage through stimulation of macrophages (Du et al., 2019; Hsiao et al., 2013). As a result of our analysis, we see that Momordicoside analogs may have anti-angiogenic properties besides these properties. As a result of the examination of ADME properties, it was seen that the only disadvantage of Momordicoside analogs according to the evaluations made according to Lipinski rules is their high molecular weight. In particular, the relatively high binding affinities in the VEGFR2, SPARC, and c-Src proteins to target sites make them considered natural inhibitor candidates. The presence of high binding affinity on the SPARC protein holds promise especially for the inhibition of the angiogenic region in this protein. Notable studies have been identified on momordicins and momordol. When the target-specific interaction of Momordicin compound with c-Src protein was examined, it was seen that it had the same affinity with Dasatinib used as an inhibitor.

As a result of our work; It is thought that triterpenes that can be obtained from the *Momordica charantia* plant can be evaluated as natural angiogenesis inhibitors. It is also clear that further analysis (*in silico*/ in vitro and in vivo) is required for these predictive values we have obtained. Although this study has deficiencies especially in the experimental sense, it also constitutes preliminary data for further *in silico*, in vitro or in vivo studies. The fact that the side effects of natural compounds are almost zero compared to synthetic or semi-synthetic compounds makes it logical to choose natural components in the treatment. For this reason, we suggest that the findings of our study are valuable due to the importance of research on phytochemicals. We recommend the use of the compound structures we have predicted as a preventive agent or to investigate combined therapies with further in vitro and in vivo experiments.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This article does not contain any in vitro or in vivo involving with animals or human participants performed by any of the authors.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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