International Journal of Life Sciences and Biotechnology
Önder, A., Davutlar, G., Ay, M., and Cömert Önder, F. Virtual Screening, Molecular Docking, and Molecular Dynamics Simulation Studies on Potential Phytochemicals as Sphingosine Kinase 1 Inhibitors for Cancer Therapy. Journal of Life Sciences and Biotechnology, 2024 7(2): p. 88-101.
https://doi.org/10.38001/ijlsb.1199393
Araştırma Makale/ Research article

Virtual Screening, Molecular Docking, and Molecular Dynamics Simulation Studies on Potential Phytochemicals as Sphingosine Kinase 1 Inhibitors for Cancer Therapy

Alper Önder[1], Gülce Davutlar[2], Mehmet Ay[1], Ferah Cömert Önder[3]*

ABSTRACT
Sphingosine kinases (SphKs) as lipid kinases catalyze the phosphorylation of sphingosine (Sph) to sphingosine-1-phosphate (S1P). Targeting the S1P signaling pathway is a significant strategy for many human diseases. Herein, we evaluated main prenylated bioactive components of a medicinal plant and performed a virtual screening study with flavonoid compounds and then, molecular docking and molecular dynamics (MD) simulation for the targeted cancer therapy. *In silico* ADMET and drug-likeness results were determined by BIOVIA Discovery Studio (DS). Molecular docking and molecular dynamics (MD) simulations were carried out by using Glide/SP and Desmond of Maestro with the filtered ligands. Glide/SP docking results showed higher binding affinity with xanthohumol (XN), 8-prenylnaringenin (8-PN), and neobavaisoflavone against SphK1. Three hits displayed strong hydrogen binding between the specific amino acid residues of targeting SphK1. There were no significant structural changes between SphK1-XN and SphK1-neobavaisoflavone complexes during 200 ns MD simulation analysis performed by GROMACS. Root-mean square deviation (RMSD) average values of XN- and neobavaisoflavone-protein complexes were compared to free SphK1 and were found as 0.2626 nm, 0.2589 nm, and 0.2508 nm, respectively. As a result, XN and 8-PN, and neobavaisoflavone have been determined as potential inhibitor candidates of SphK1 to examine for further *in vitro* and *in vivo* studies.

ARTICLE HISTORY
Received
06 March 2024
Accepted
31 May 2024

KEY WORDS
Sphingosine kinase,
virtual screening,
cancer,
molecular docking,
MD simulation

Introduction

Sphingolipids are lipid signaling molecules that contribute to the regulation of cellular processes [1]. Sphingomyelin metabolites are ceramide (Cer), sphingosine (Sph) and sphingosine-1-phosphate (S1P) and play an important role in the development of diseases such as cancer [2]. Ceramide and sphingosine act as pro-apoptotic molecules involved in cell cycle arrest and inducing apoptosis [3]. Moreover, increasing the level of ceramide or sphingosine with the change of this balance may provide a therapeutic intervention in cancer [4]. Sphingosine-1-phosphate (S1P), that interconvertible opposite effect with other metabolites creates sphingolipid rheostat, plays a role in cell proliferation, cell survival, apoptosis, angiogenesis, and inflammation. Inhibition of SphK1 activity or decreasing the level of S1P may be effective in human disease such as cancer [5]. According to previously reported studies, an upregulation of SphK1 has been reported in various cancer types such as lung, brain, breast, prostate etc.. [6-9]. SphK1 also possesses a role in human cancer cells to certain chemotherapeutic drugs [10]. It was reported that there was a collaboration between overexpression of SphK1 in malignant tissues [11]. Moreover, signaling of S1P/SphK1 is associated with a wide variety of metabolic and inflammatory diseases such as diabetes, pulmonary fibrosis, Alzheimer's disease, obesity, rheumatoid arthritis, and sepsis [11]. These findings indicate that SphK1 is an interesting drug target for the discovery of new molecules for SphK1-related diseases.

[1] Natural Products and Drug Research Laboratory, Department of Chemistry, Faculty of Science, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye
[2] Department of Medical System Biology, School of Graduate Students, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye
[3] Department of Medical Biology, Faculty of Medicine, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye
* Correspondence Author: Assoc. Prof. Ferah Cömert Önder, e-mail: ferahcomertonder@comu.edu.tr

Due to the remarkable role in cancer and other human diseases, the researchers have been interested in developing new therapeutics for SphK1 drug targets [12]. Up to date, although many therapeutics have been developed and investigated by *in vitro* and *in vivo* studies. There is still need to determine newly potential candidates due to the limited usage of synthetic compounds because of side effects or non-bioavailability. Natural compounds are used to discover specific inhibitors for drug targets with their no or lower side effects. Furthermore, many naturally occurring compounds isolated from plants have a broad-spectrum for their biological activities such as anticancer, anti-inflammatory etc.. Thus, it is thought that natural compounds are potential candidates to be used in further studies [13]. In recent years, scientists have tried to understand *in vitro* and *in vivo* activity of phytochemicals used in traditional medicine [14]. Since the effects on medicinal plants have been also reported, it is very important to further develop and research studies with these medicinal plants for the prevention or treatment of diseases [15]. These literature findings can provide the basis for the design of new drugs for the treatment of important diseases such as cancer, diabetes, and Alzheimer's disease [16].

Humulus lupulus L. (hops) plant has been used for medicinal purposes since ancient times [16,17]. Hop cones contain a lot of secondary metabolites and the main compounds including bitter acids, flavonoids, terpenes, and chalcones have been found in female inflorescences of hops [17-19]. It has been reported that the prenylated flavonoids from hop cones such as xanthohumol (XN), iso-xanthohumol (IXN), and 8-prenylnaringenin (8-PN) has various biological activities including cardiovascular, anticancer, antiinflammatory, antioxidant, and pro-apoptotic effects etc. [18-21]. Furthermore, it has been reported that bitter acids including isohumulones obtained from hop cones significantly reduce the level of cholesterol in the blood [22].

Herein, the aim of this study is to investigate the main prenylated components of hop by the computational studies containing molecular docking and molecular dynamics (MD) simulation and ADMET prediction as potential candidates against SphK1. Furthermore, a flavonoid compound library was evaluated by virtual screening, molecular docking, and MD simulation studies to determine their binding affinities for targeting protein. Molecular docking and MD simulation results show that for the first time XN and neobavaisoflavone are two hits that may be useful for future studies in inhibiting SphK1 especially in cancer treatments. The workflow for this study is shown in Figure 1.

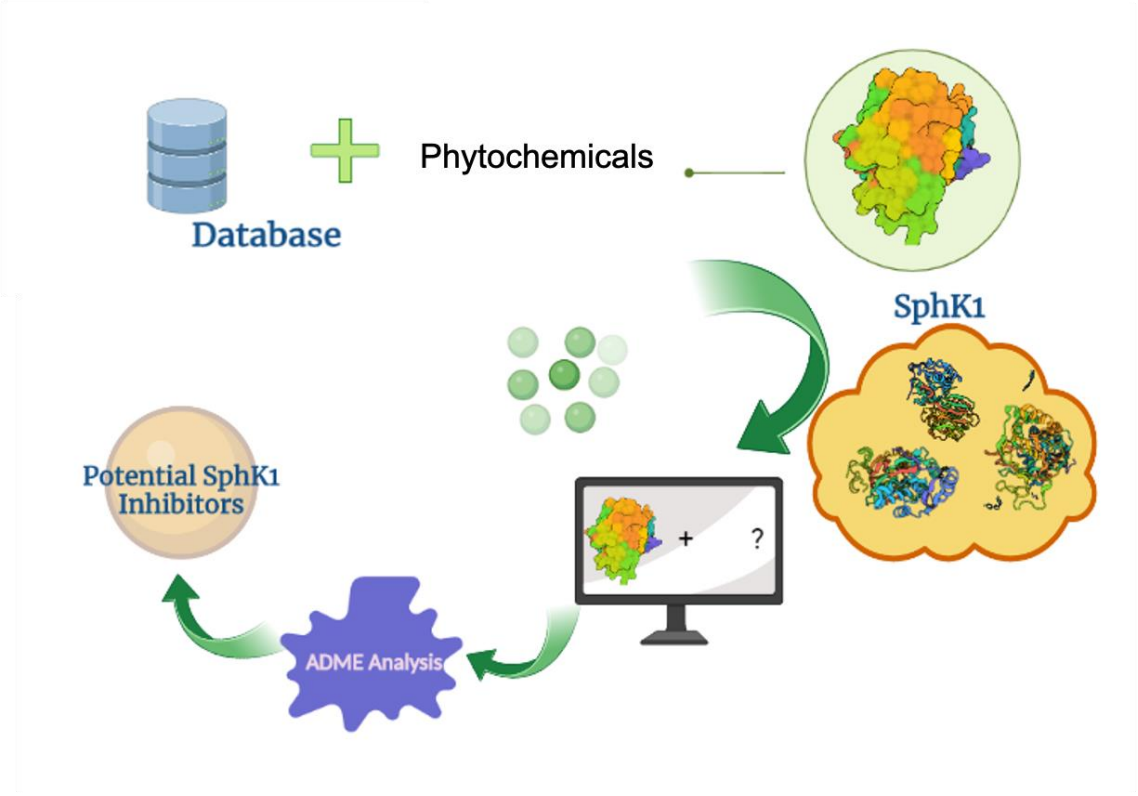

Fig 1 A representative workflow for this study.

Material and Methods

Protein and ligand preparation

The crystal structures of SphK1 were downloaded from the Protein Data Bank (PDB) (3VZB, 3VZC, 4V24) (https://www.rcsb.org/). According to our previously reported study, the calculation parameters were used [23]. Default settings of Maestro were used. Protein preparation wizard and Prime module of Maestro were used to prepare the target protein before docking (Schrödinger Release 2021-4, LLC, New York, NY). PROPKA was used to add hydrogen atoms [24]. Hop components were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/). Downloaded ligands were prepared by using the LipPrep module of Maestro. The ionization state was calculated at neutral pH by using Epik [25]. OPLS2005 force field was used and 0.3 Å RMSD was considered.

Molecular docking

Glide/SP (Standard Precision) method was used to determine the binding pose and molecular interactions between the protein-ligand complex [26]. Default settings of flexible ligand docking were kept as 5000 poses per ligand, and best 400 poses per ligand for energy minimization. The Grid box was formed by using co-crystal ligand [12, 23].

Structure-based virtual screening

A flavonoid compound library was downloaded from Selleckchem (https://www.selleckchem.com/). Firstly, ADMET descriptors and Filter by Lipinski and Veber Rule (Ro5) modules of BIOVIA Discovery Studio (DS) were used for the filtration of ligands, respectively [27]. Aqueous solubility [28], brain barrier penetration [29], CYP2D6 binding [30], hepatotoxicity [31], intestinal absorption [32], and plasma protein binding [33] were selected in ADMET Descriptors module of DS. Throughout the Filter by Lipinski and Veber Rules module of DS, hydrogen bond donors, acceptors, molecular weight, and AlogP values were 5, 10, 500, and 5, respectively. According to Veber rule, rotatable bonds, polar surface area, hydrogen bond donors and acceptors were 10, 140, and 12, respectively. Then, the resulting 136 compounds were prepared by using the LigPrep module of Maestro for molecular docking study and prepared candidates were docked to SphK1 target protein.

Molecular dynamics (MD) simulation

The top docking poses of SphK1-XN and SphK1-neobavaisoflavone obtained following docking were prepared by using Chimera software for MD simulation [34]. Molecular topology files were formed by accessing www.swissparam.ch [35] MD simulation analysis of protein and protein-ligand complexes were carried out by GROMACS 2023.1 using CHARMM force field parameters [36-38]. TIP3P water model was used for each protein [39]. The simulation box was neutralized by using Na+ and Cl- ions. The energy minimization was performed for each MD simulation system. The temperature of each system was gradually increased from 0 K to 300 K during the equilibration period at constant volume, pressure (1 atm), and temperature (300 K) under periodic boundary conditions. It was carried out for 200 ns at molecular mechanics level on free SphK1, SphK1-XN, and SphK1-neobavachin. The resulting trajectories were saved for further analysis using GROMACS' internal utilities. The output files (.xvg) were analyzed by XMGRACE software [40,41].

Results

Molecular docking study of hop flavonoids

To understand the binding pattern between phloroglucinol core structure, prenyl groups and target protein, a molecular docking study was performed with major bioactive prenylated components of *H. lupulus* L.. Hop prenylated bioactive components including XN, IXN, xanthogalenol, desmethylxanthohumol (DesXN), 6-prenylnaringenin (6-PN), 8-PN, and bitter acids such as humulone, lupulone, cohumulone, and colupulone were docked against target protein (3VZB). These results showed that XN, 8-PN, and IXN had the highest binding scores as -8.904 kcal/mol, -8.582 kcal/mol, and -8.148 kcal/mol, respectively. In addition, XN derivatives, DesXN and xanthogalenol displayed lower binding scores as -7.927 kcal/mol and -7.889 kcal/mol, respectively. Cohumulone is one of the alpha acids of hops and is calculated for its binding energy as -5.978 kcal/mol. The calculated binding scores of major prenylated components of hop cones were given in Table 1.

Furthermore, according to the interactions between ligand and target protein, these ligands showed the expected interactions such as H-binding and pi-alkyl with important amino acid residues such as Asp178, Phe288, Phe173, Met306, and Leu299 of Sph binding of SphK1. In general, the ligands interacted with the substrate-binding Asp178 residue of SphK1. Strong hydrogen bonding between hydroxyl groups of ligands

and Asp178 was observed. A detailed binding pattern of these compounds with SphK1 is illustrated in Figure 2 and 3. As a result, hop components XN and 8-PN could be potential SphK1 inhibitors.

Table 1 Glide/SP docking scores of major prenylated bioactive components of hop cones against SphK1

No	Ligand	Glide/SP (kcal/mol) (3VZB)	Ligand	Glide/SP (kcal/mol) (4V24)
1	XN	-8.904	8-PN	-9.507
2	8-PN	-8.582	XN	-8.906
3	IXN	-8.148	IXN	-8.366
4	DesXN	-7.889	Cohumulone	-7.750
5	Xanthogalenol	-7.331	Adhumulone	-7.690
6	Cohumulone	-5.978	DesXN	-7.545
7	6-PN	-5.406	6-PN	-7.290

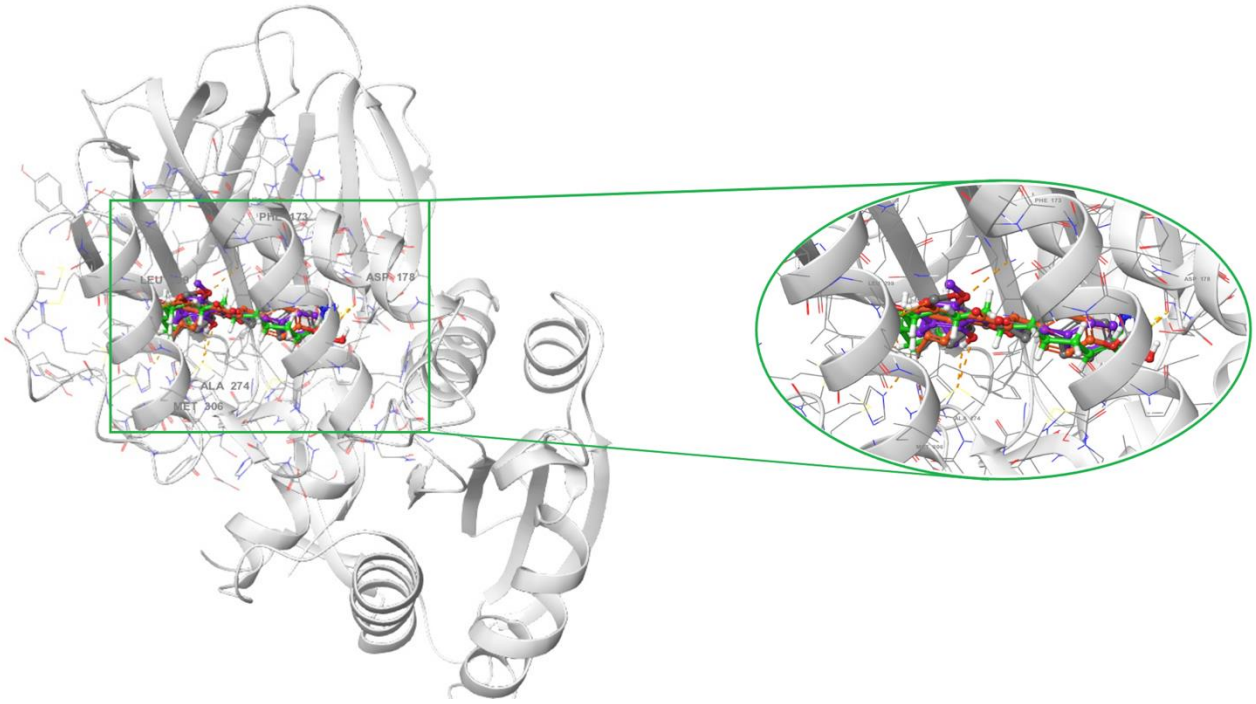

Fig 2 Binding pattern of XN, 8-PN, and IXN (pdb: 3VZB). Compounds were given as colored in magenda, orange, and grey, respectively. Key residues were labeled.

When hop prenylated components were docked against protein (pdb: 4V24), 8-PN and XN displayed the highest binding scores as -9.507 kcal/mol and -8.906 kcal/mol, respectively, followed by IXN (-8.366 kcal/mol) and cohumulone (-7.750 kcal/mol). Adhumulone (-7.690 kcal/mol) is another alpha acid that exhibits a similar score like cohumulone. In conclusion, among *H. lupulus* L. components, XN and 8-PN displayed the best binding scores against SphK1. Glide/SP docking scores were given in Table 1. Top docking poses of hit ligands are shown in the binding pocket of protein in Figure 4. Binding mode prediction of 8-PN and known inhibitor SKI-II is given in Figure 5.

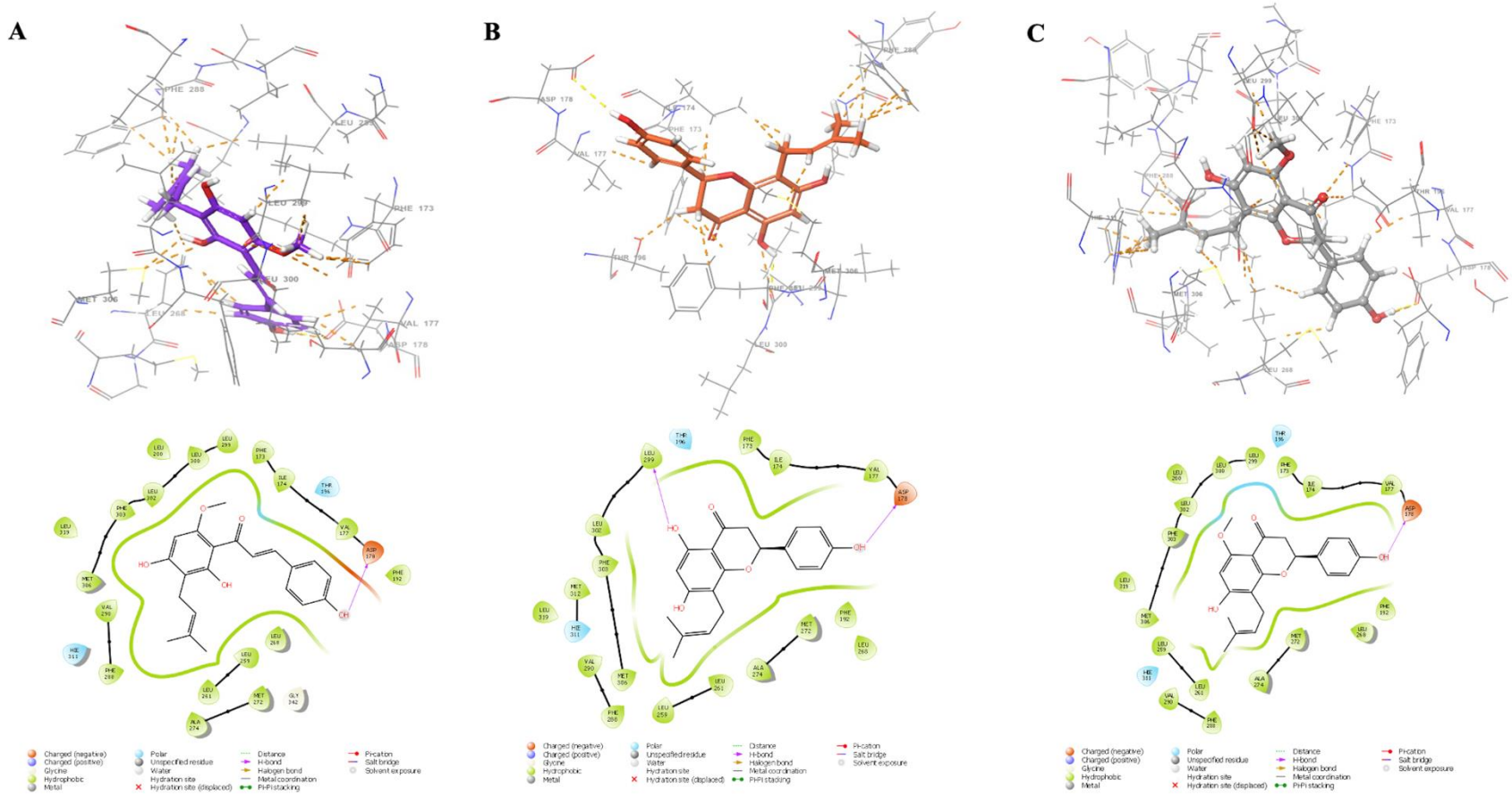

Fig 3 Binding pattern and 2D interaction diagram of XN (A), 8-PN (B), and IXN (C) (pdb: 3VZB).

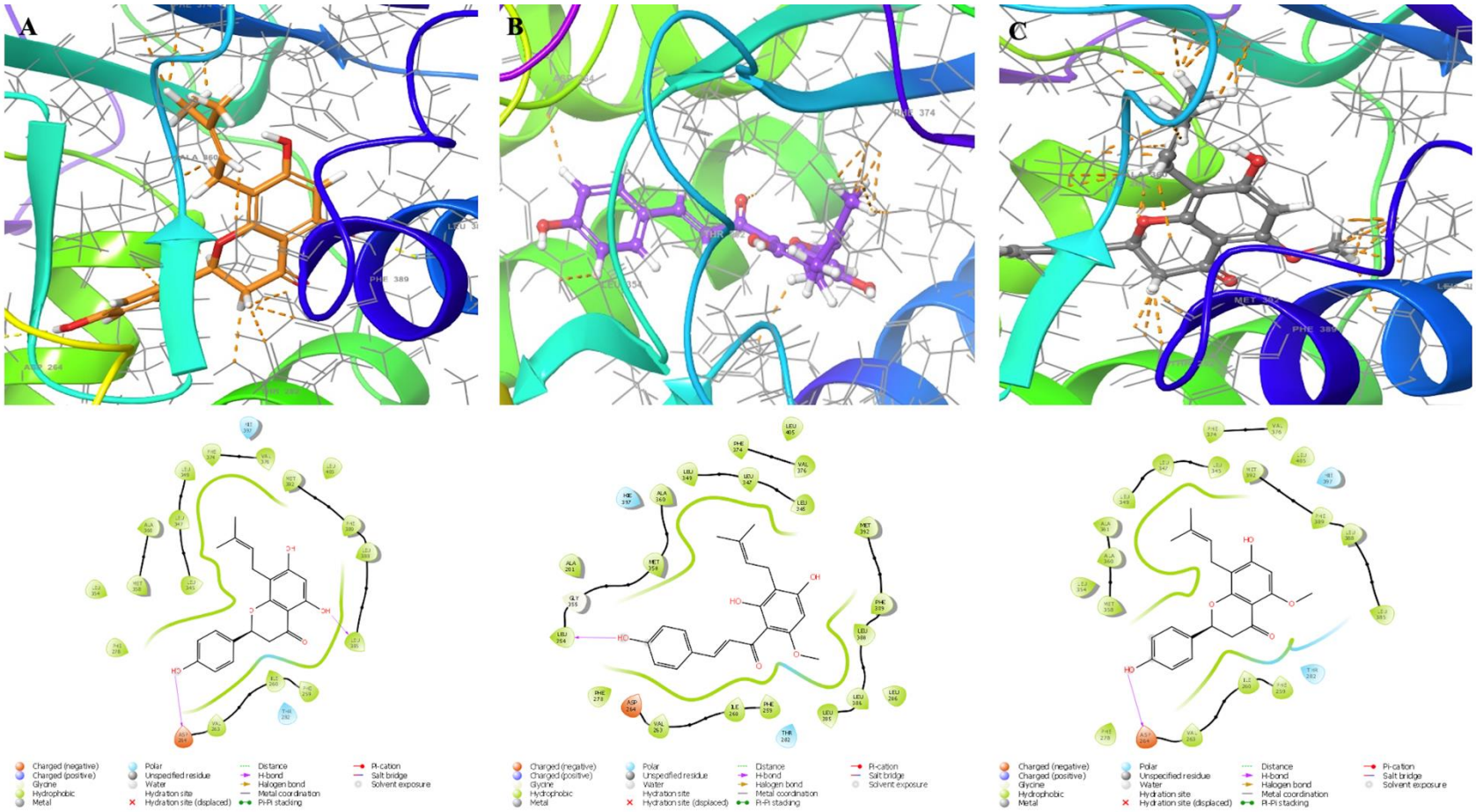

Fig 4 Binding pattern and 2D interaction diagram of 8-PN (A), XN (B), and IXN (C) (pdb: 4V24).

Structure based virtual screening

To understand the potential role of major prenylated flavonoids of *H. lupulus* L. against SphK1, we have thought to analyze flavonoid-based natural compounds. For this purpose, a structure-based virtual screening was performed by using a flavonoid compound library of Selleckchem. Following ADMET and Ro5 analysis in DS, filtered 136 compounds were docked against SphK1 by Glide/SP method. Throughout these flavonoid compounds, it is indicated that the selected hits with high binding scores were given in Table 2.

Table 2 Glide/SP docking scores of selected naturally occurring flavonoid compounds against SphK1 (pdb: 3VZB).

No	Ligand	Glide/SP (kcal/mol)	No	Ligand	Glide/SP (kcal/mol)
1	Neobavaisoflavone	-9.650	8	Genistein	-8.998
2	Licochalcone A	-9.609	9	Daidzein	-8.994
3	Corylin	-9.252	10	XN	-8.904
4	Tectorigenin	-9.161	11	Naringenin	-8.419
5	Equol	-9.108	12	Luteolin	-8.352
6	alpha-Naphthoflavone	-9.089	13	Taxifolin	-8.348
7	Glycitein	-9.019	14	Quercetin	-8.328

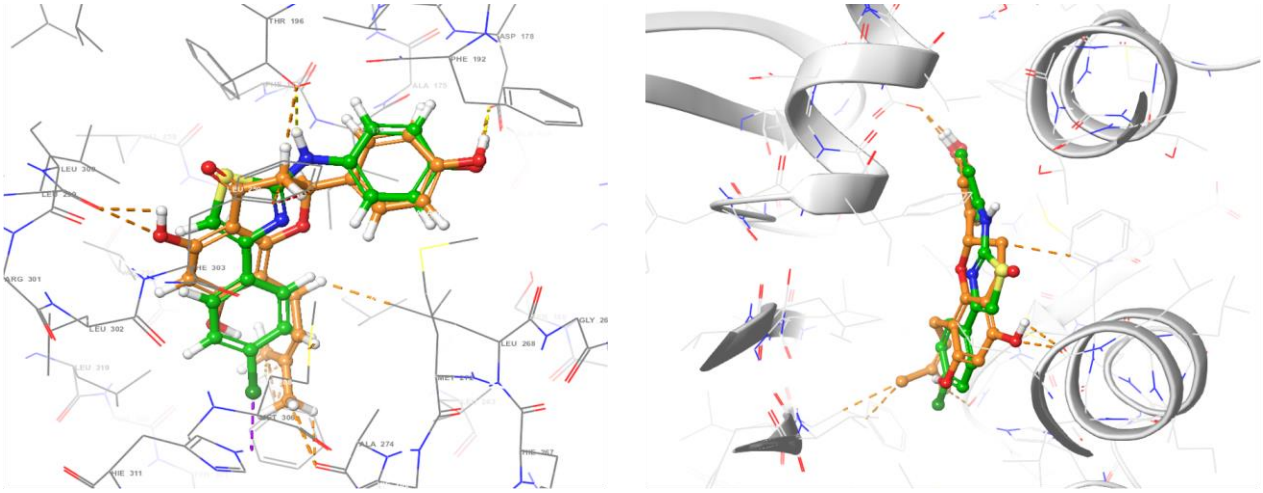

Fig 5 Binding pattern of 8-PN (orange) and SKI-II (green) (pdb: 3VZC).

Among a series of screened flavonoid compounds (pdb: 3VZC), the docking score was found for 2',3',4'-trihydroxy flavone (2D08) as -9.910 kcal/mol and also, isobavachin, which is a 8-prenylated flavone (-9.761 kcal/mol) exhibited higher binding score than quercetin. The interactions were observed between main residues such as Asp178, Phe303, Thr196, and Ile174. Other important naturally occurring compound genkwanin (especially known as apigenin derivative) is a O-methylated flavone has the highest docking score as -9.288 kcal/mol with H-binding between Asp178 and hydroxyl group and pi-pi stacking between Phe303 and phenyl ring. It was followed by butein is a tetrahydroxy chalcone compound (-9.288 kcal/mol) with the interactions between important residues such as Asp178 and 3,4-dihydroxy groups on phenyl ring. Fisetin is a flavonol, known as another dietary antioxidant, exhibits a good binding score (-9.074 kcal/mol) against SphK1 like quercetin and taxifolin (also called as dihydroquercetin) (-8.925 kcal/mol).

On the other hand, daidzein, taxifolin, luteolin, naringenin, and quercetin significantly placed in the substrate binding pocket of SphK1. The binding scores of neobavaisoflavone and licochalcone were calculated with high binding scores as -9.650 kcal/mol and -9.609 kcal/mol, respectively. The main interactions were observed at amino acid residues including Asp178 and Leu299 with hydroxyl groups of chromen-4-one core structure and phenyl ring, respectively. The interactions such as H-binding of licochalcone A with the high docking score have been identified between Asp178 and 4-hydroxy phenyl. Thus, both compounds displayed H-bond interaction with the substrate binding site of target protein. The binding pattern against target protein was given in Figure 6.

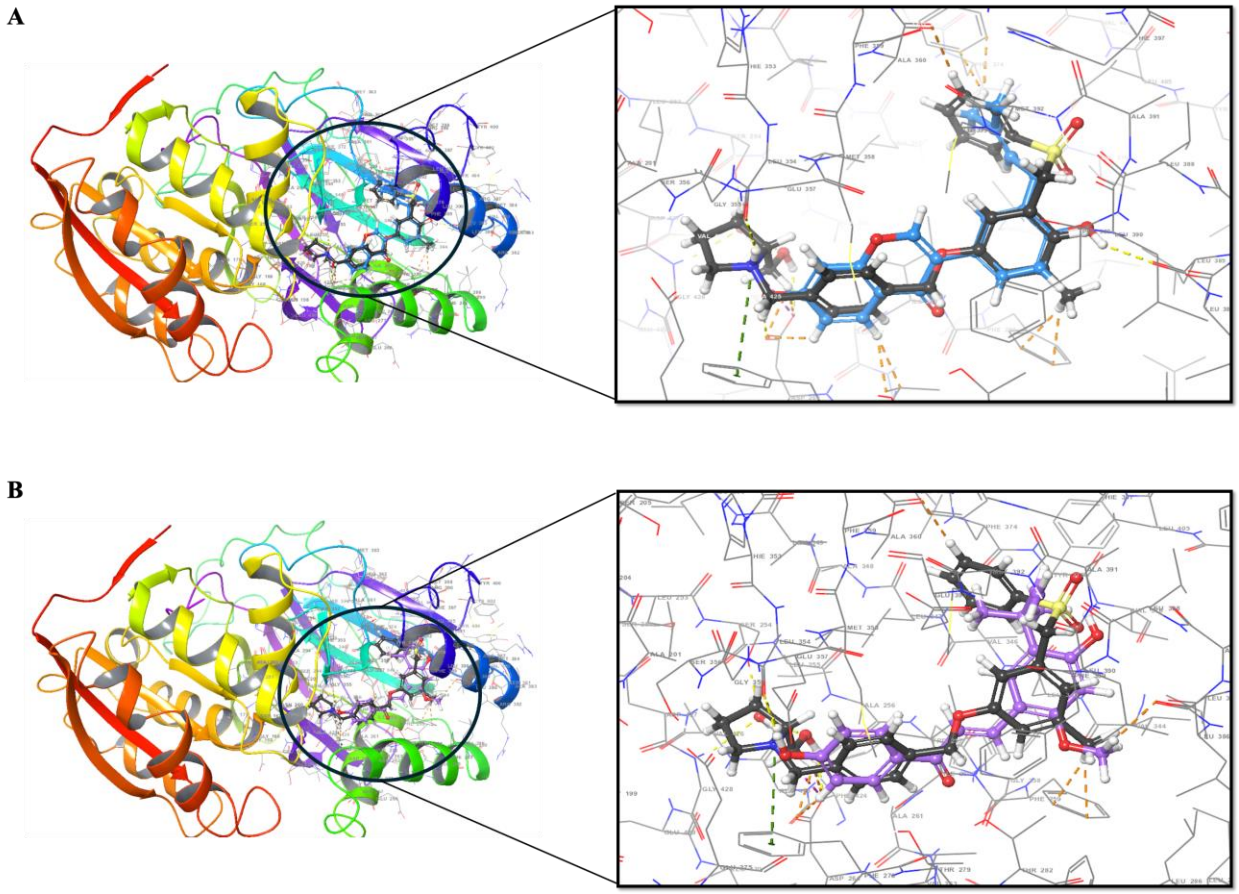

Fig 6 Binding pattern of neobavaisoflavone (blue) and PF543 (dark grey) (A) and licochalcone A (lilac) and PF543 (dark grey) (B).

Neobavaisoflavone exhibited strong H-bond interactions between amino acid residues Asp264 and Leu385 with 7-hydroxy group of chromen-4-one and 4-hydroxy substituted phenyl ring. Whereas 7-hydroxy group of substituted chromen-4-one core structure of daidzein interacted with Leu385. In addition, 4-hydroxyphenyl of licochalcone A showed strong H-bond with Leu354. These flavonoids and chalcone compounds may be revealed as potential SphK1 inhibitors in place of known inhibitor PF543. Besides, XN was found with the same binding pattern as known inhibitor PF543. Consequently, it is clear that hydroxyl groups of flavonoids contribute to the inhibitor activity with strong H-binding for targeted SphK1. In addition, van der Waals interactions have a role for binding the pattern of the flavonoids to the target protein with alkyl groups.

Molecular Dynamics (MD) Simulation

MD simulations were performed to understand protein-ligand complex. When XN and neobavaisoflavone were bond to the target protein, the structural behavior of SphK1 was evaluated with MD trajectories analysis. It appears that the structural conformation is better stabilized by XN. As is known, the structural deviation and conformational changes of a protein was determined with the calculations of root-mean square deviation (RMSD). Herein, RMSD average values of XN- and neobavaisoflavone-protein complex were compared to free SphK1 and were found as 0.2626 ± 0.026 nm, 0.2589 ± 0.029 nm, and 0.2508 ± 0.033 nm, respectively. Thus, it is though that there have been no significant changes in RMSD values (Figure 7a). Root mean square fluctuation (RMSF) shows that structural flexibility, the average fluctuation of each residue with the binding of ligand (Figure 7b). Radius of gyration (Rg) is given for the analyze the conformational changes and stability of the protein. The average Rg was found as approximately 2.035 ± 0.010 nm, 2.040 ± 0.012 nm, and 2.051 ± 0.009 nm for free SphK1 and SphK1-XN and SphK1-Neo, respectively. It shows that there is no significant structural changes during the analysis (Figure 7c). Solvent Accessible Surface Area (SASA) has displayed the average values 156.86 ± 3.08 nm^2 and 164.91 ± 2.72 nm^2, and 156.40 ± 2.44 nm^2 for free SphK1, SphK1-XN, and SphK1-Neo, respectively. Although neobavaisoflavone was no structural changes in SphK1, XN had a little change compared to free SphK1 (Figure 7d).

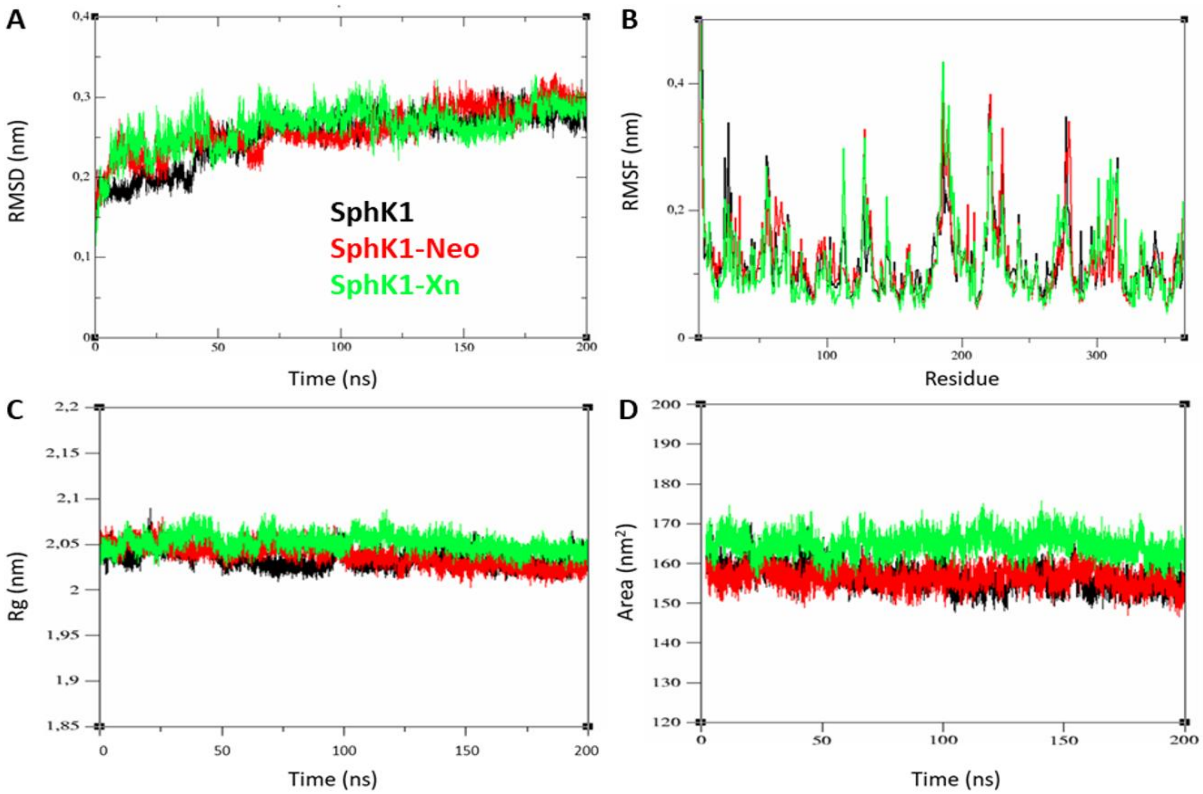

Fig 7 Structural dynamics of SphK1 before and after XN and Neobavaisoflavone complex. (A) RMSD plot of SphK1. (B) RMSF plot of SphK1. (C) Radius of gyration (Rg). (D) SASA plot of SphK1. The values were obtained from the 200 ns MD simulation. Black, red, and green represent values for free SphK1, SphK1-Neobavaisoflavone, and SphK1-XN complex, respectively.

ADMET, drug likeness and toxicity prediction

The molecular weight (MW) was filtered to a value less than 500 g/mol. *In silico* pharmacokinetic properties including ADMET descriptors, drug similarity and ADMET solubility, CYP2D6 binding, hepatotoxicity, blood brain barrier (BBB) penetration, plasma protein binding (PPB), intestinal absorption, MW, and number of H acceptors and donors are given in Table 3 and 4. According to these

results, ADMET solubility level was found in the range of 2 and 3. Thus, these values indicate that all compounds are soluble in water. The ligands have a good human intestinal absorption level between 0 and 1, indicating that all selected compounds will be well absorbed by the gut. The blood brain-barrier permeability of all compounds was evaluated, and it was observed that the compounds had values between 1-4. Predictive inhibitors of CYP450 enzymes (3A4, 2D6 and 2C9) are important for drug metabolism and do not implicate all compounds as inhibitors of CYP450 enzymes. It was found that all compounds did not show hepatotoxicity. The plasma protein binding level of all compounds ranged from 0.00 to 0.957.

Conclusion and Discussion

Absorption, distribution, metabolism, and excretion are investigated of ADME properties that are of great importance for drug development strategy to gain information about the potential of success of candidate therapeutic molecules [42]. Flavonoid compounds were analyzed with their ADMET properties including toxicity prediction and Ro5, and the resulting compounds were screened against SphK1 [43]. The number of hydrogen bond donors and acceptors [44] which is an important molecular identifier and serves to predict human oral bioavailability, and too many H-bond donors and acceptors negatively affect the permeability of compounds. H-bond acceptors and donors must be less than or equal to 10 and 5, respectively. All compound numbers of hydrogen bond acceptors and donors do not violate Ro5. In conclusion, this study contributed to the identification of the best flavonoids and bioactive components of *H. lupulus* L. regarding pharmacokinetic properties and the compounds could be used as potential drug targets. Throughout the reported studies, bioactive natural products were identified as potential SphK1 inhibitors. ZINC database library was filtered by using Lipinski's rule, ADMET, carcinogenicity, and PAINS. In a previously reported study, quercetin was investigated against SphK1 target by molecular docking study [41]. It was reported that its binding affinity was calculated as -8.2 kcal/mol. Thus, the interactions of quercetin with amino acid residues of target protein have been revealed as Asp178, Thr196, Ile174, Phe303, and Met306 [41]. As a result, a flavonoid quercetin was determined as a potent inhibitor of SphK1 due to the direct interaction with the substrate binding pocket in a reported study [41]. Consequently, the researchers suggested that dietary phytochemicals could be new therapeutics to develop SphK1 inhibitors [41]. Selected hits exhibited the high binding scores toward SphK1 in the range of -9.65 kcal/mol and -8.33 kcal/mol. However, the binding affinities of SphK1 inhibitors SKI-II and PF543 were calculated as -9.1 and -8.8 and 9.90 kcal/mol, respectively, in the previously reported studies [45,46]. According to each subtype of a flavonoid compound, has a hydroxyflavone core structure substituted with hydroxyl groups, binding scores show similarity with a flavonoid quercetin (-8.328 kcal/mol) with the interactions of specific amino acid residues such as Asp178, Thr196, Leu268, and Ile174. High binding affinities were determined in the range of -12.2 kcal/mol and -11.8 kcal/mol. Among these compounds, substituted naphthalene carbamate and substituted phenyl have been reported as potential inhibitor candidates [45]. In the other study, filtered by drug likeness and ADMET properties ligand-based virtual screening was applied to determine hit candidates by using various databases [46]. According to molecular docking study, hit compounds were determined with the highest binding energies compared to known inhibitor PF543. Hit1 compound was reported at the interactions with active site residues such as Phe259, Leu354, Asp254, and Ile260 by two conventional H-bonds compared to known inhibitors [46]. The interactions were observed between the 4-hydroxy group and phenyl ring bound to residues Asp178 and Phe303 of substrate binding pocket with H-bond and pi-pi stacking, respectively.

Table 3 ADMET Profiles of hop plant components against SphK1 (pdb: 3VZB).

No	Ligand	MW	ADMET solubility level[a]	BBB level[b]	Absorption level[c]	CYP2D6[d]	Hepatotoxicity[e]	PPB level[f]	H Acceptor	H Donor
1	XN	427.921	2	1	0	0	0	0	5	0
2	8-PN	270.28	3	2	0	0	0.001	0.859	4	1
3	IXN	338.397	2	1	0	0	0.004	0.157	4	2
4	DesXN	254.238	3	1	0	0.575	0.985	0.799	4	2
5	Xanthogalenol	284.263	3	2	0	0.008	0.738	0.957	5	2
6	Cohumulone	322.355	2	3	0	0.016	0.003	0.736	4	2
7	6-PN	254.238	3	1	0	0.041	0.048	0.298	4	2

[a]Aqueous-solubility level: 0 (extremely low); 1 (very low, but possible); 2 (low); 3 (good). [b]Blood Brain Barrier level: 0 (very high penetrant); 1 (high); 2 (medium); 3 (low); 4 (undefined). [e]Human-intestinal absorption level: 0 (good); 1 (moderate); 2 (poor); 3 (very poor). [d]Cytochrome P450 2D6 level: 0 (non-inhibitor); 1 (inhibitor). [e]Hepatotoxicity: 0 (nontoxic); 1 (toxic). [f]Plasma Protein Binding: 0 (absorbent weak); 1 (absorbent strong).

Table 4 ADMET Profiles of selected flavonoid compounds against SphK1 (pdb: 3VZB).

No	Ligand	MW	ADMET solubility level[a]	BBB level[b]	Absorption level[c]	CYP2D6[d]	Hepatotoxicity[e]	PPB level[f]	H Acceptor	H Donor
1	Neobavaisoflavone	322.36	2	1	0	0.016	0.003	0.736	4	2
2	Licochalcone A	338.40	2	1	0	0	0.004	0.157	4	2
3	Corylin	320.34	2	1	0	0.007	0.423	0.188	4	1
4	Tectorigenin	300.26	3	3	0	0.007	0.691	0.882	6	3
5	Equol	242.27	3	1	0	0.005	0.578	0.872	3	2
7	Glycitein	284.26	3	3	0	0.008	0.738	0.957	5	2
8	Genistein	270.24	3	3	0	0.666	0.989	0.805	4	3
9	Daidzein	254.24	3	2	0	0.575	0.985	0.799	5	2
10	XN	354.40	2	4	0	0	0.000	0.026	5	3
11	Taxifolin	304.25	3	4	1	0	0.014	0.098	7	5
12	Luteolin	286.24	3	4	0	0.618	0.526	0.324	6	4
13	Quercetin	302.24	3	4	1	0.431	0.964	0.611	7	5
14	Naringenin	272.25	3	3	0	0	0.034	0.685	5	3

Herein, flavonoids including luteolin, daidzein, naringenin, and kaempferol were identified as potential SphK1 inhibitors in place of known SKI-II that is a lipid substrate competitive inhibitor [47]. According to structural changes of SphK1-quercetin complex, RMSD values were reported as 0.37 nm and 0.38 nm for SphK1 free and SphK1-quercetin complex, respectively. It was reported that the structure of SphK1 was stabilized with the binding of quercetin. In addition, a little conformational change in SphK1 was observed at Rg and SASA values for free SphK1 and SphK1-quercetin were given as 1.96 nm and 2.03 nm, and 145.61 nm^2 and 153.09 nm^2, respectively [44]. One of the reported MD simulation analyses shows that identified by structure-based virtual screening two natural compounds from the ZINC database can include potential scaffolds for further studies. The results of RMSD, RMSF, Rg, and SASA obtained by using GROMACS for free SphK1 and two ligands reported between 0.30-0.28 nm, 0.13-0.11 nm, 1.96-2.00 nm, and 145.61-147.05 nm^2, respectively [45]. In another study, MD simulation analysis was performed by SphK1, SphK1-PF543, and hits. *In silico* analysis indicated that the identified compounds had strong inhibitor potential to be used in the discovery and development of new SphK1 inhibitor candidates [12]. As a result, inhibition of SphKs using small molecules is an important strategy for cancer treatments and others. Due to its remarkable role in cancer progression, metastasis, and other diseases, SphK1 has emerged as a new therapeutic target to combat these diseases and develop effective therapeutics.

Herein, we identified some potential prenylated flavonoid compounds against SphK1. XN and prenylnaringenin compounds are the main components of *H. lupulus* L. medicinal plant. These compounds are widely used in biological activity studies such as cancer and inflammation-related metabolic pathways [48-50]. This study also indicates that these prenylated bioactive compounds can be potential for targeted SphK1. These results were supported by the structure-based virtual screening study by using a flavonoid compound library. Our findings have shown that prenylated flavonoid compounds with hydroxyl groups may be potential inhibitor candidates to be used in SphK1-targeted drug discovery and development. *In vitro* studies are recommended.

Funding
This study was supported by Çanakkale Onsekiz Mart University, the Scientific Research Coordination Unit (Project number TSA-2021-3729). The website biorender.com was utilized for creating the workflow.

Data Availability statement
The authors confirm that the data supporting this study are cited in the article.

Compliance with ethical standards

Conflict of interest
The authors declare no conflict of interest.

Ethical standards
The study is proper with ethical standards.

Authors' contributions
Concept- A.O., M.A., F.C.O.; Design- A.O., M.A., F.C.O.; Data Collection-Analysis-Writing- A.O., G.D., M.A., F.C.O.

References

1. Hannun, Y.A., Obeid LM. Sphingolipids and their metabolism in physiology and disease. Nature Reviews Molecular Cell Biology, 2018. 19(3): p. 175-191. https://doi:10.1038/nrm.2017.107.
2. Quinville, B.M., Deschenes, N.M., Ryckman, A.E., Walia, J.S. A Comprehensive Review: Sphingolipid Metabolism and Implications of Disruption in Sphingolipid Homeostasis. International Journal of Molecular Science, 2021. 22(11): p. 5793. https://doi:10.3390/ijms22115793.
3. Haddadi, N. Dicing and Splicing Sphingosine Kinase and Relevance to Cancer. International Journal of Molecular Sciences, 2017. 18(9): p. 1891. https://doi:10.3390/ijms18091891.

4. Haass, N.K. Switching the Sphingolipid Rheostat in the Treatment of Diabetes and Cancer Comorbidity from a Problem to an Advantage. BioMed Research International 2015. p. 1-9. https://doi:10.1155/2015/165105.
5. Chalfant, C.E., Spiegel, S. Sphingosine 1-phosphate and ceramide 1-phosphate: expanding roles in cell signaling. Journal of Cell Science, 2005. 118(Pt 20): p. 4605-4612. https://doi:10.1242/jcs.02637.
6. Govindarajah, N., Clifford, R., Bowden, D., Sutton, P.A., Parsons, J.L., Vimalachandran, D. Sphingolipids and acid ceramidase as therapeutic targets in cancer therapy. Critical Reviews in Oncology/Hematology, 2019. 138: p. 104-111. https://doi:10.1016/j.critrevonc.2019.03.018.
7. Riboni, L. et al., Ceramide levels are inversely associated with malignant progression of human glial tumors. Glia, 2002. 39(2): p.105–113. https://doi:10.1002/glia.10087.
8. Nava, V.E., et al., Sphingosine enhances apoptosis of radiation-resistant prostate cancer cells. Cancer Research, 2000. 60(16): p. 4468-4474.
9. Alshaker, H., et al., Therapeutic potential of targeting SK1 in human cancers. Advances In Cancer Research, 2013. 117: p. 143-200. https://doi:10.1016/B978-0-12-394274-6.00006-6.
10. Shida, D., et al., Targeting SphK1 as a new strategy a gainst cancer. Current Drug Targets, 2008. 9(8): p. 662–673. https://doi:10.2174/138945008785132402.
11. Gault, C.R., Obeid, L.M. Still benched on its way to the bedside: sphingosine kinase 1 as an emerging target in cancer chemotherapy. Critical Reviews in Biochemistry and Molecular Biology, 2011. 46(4): p. 342–351. https://doi:10.3109/10409238.2011.597737.
12. Erdoğan, M., Comert Onder, F. Synthesis, anticancer activity and molecular modeling study of novel substituted triazole linked tetrafluoronaphthalene hybrid derivatives. Journal of Biomolecular Structure and Dynamics, 2023. p. 1-20. https://doi:10.1080/07391102.2023.2252914.
13. Atanasov, A.G., et al., Natural products in drug discovery: advances and opportunities. Nature Reviews Drug Discovery, 2021. 20(3): p. 200-216. https://doi:10.1038/s41573-020-00114-z.
14. Tanaka, N., Kashiwada, Y. Phytochemical studies on traditional herbal medicines based on the ethnopharmacological information obtained by field studies. Journal of Natural Medicines, 2021. 75(4): p. 762-783. https://doi:10.1007/s11418-021-01545-7.
15. Sofowora, A., et al., The role and place of medicinal plants in the strategies for disease prevention. African Journal of Traditional, Complementary and Alternative Medicines, 2013. 10(5): p. 210-29. https://doi:10.4314/ajtcam.v10i5.2.
16. Carbone, K., Gervasi, F. An Updated Review of the Genus Humulus: A Valuable Source of Bioactive Compounds for Health and Disease Prevention, Plants. 2022. 11(24): p. 3434. https://doi:10.3390/plants11243434.
17. Knez Hrnčič, M., et al., Hop Compounds: Extraction Techniques, Chemical Analyses, Antioxidative, Antimicrobial, and Anticarcinogenic Effects. Nutrients, 2019. 11: p. 257. https://doi:10.3390/nu11020257
18. Onder, F.C., Kalin, S., Sahin, N., Davutlar, G., Abusharkh, K.A.N., Maraba, O., Hal, R.S., Ay, M., Nahar, L. Sarker, S.D. Major Bioactive Prenylated Flavonoids from Humulus lupulus L., Their Applications in Human Diseases and Structure-Activity Relationships (SAR) - A Review. Pharmaceutical Sciences, 2023. 30(1): pp. 1-20. https://doi:10.34172/PS.2023.18
19. Lin, M., et al., Role of Characteristic Components of Humulus lupulus in Promoting Human Health. Journal of Agricultural and Food Chemistry, 2019. 67(30): p. 8291-8302. https://doi:10.1021/acs.jafc.9b03780.
20. Abiko, Y., et al., Hops components and oral health. Journal of Functional Foods, 2022. 92: p. 105035.
21. Dostálek, P., et al., Hop Phytochemicals and Their Potential Role in Metabolic Syndrome Prevention and Therapy. Molecules, 2017. 22(10): p. 1761. https://doi:10.3390/molecules22101761.
22. Miura, Y., et al., Dietary isohumulones, the bitter components of beer, raise plasma HDL-cholesterol levels and reduce liver cholesterol and triacylglycerol contents similar to PPARalpha activations in C57BL/6 mice. British Journal of Nutrition, 2005. 93(4): p. 559-67. https://doi:10.1079/bjn20041384.
23. Kalin, S, Comert Onder, F. Discovery of potential RSK1 inhibitors for cancer therapy using virtual screening, molecular docking, molecular dynamics simulation, and MM/GBSA calculations. Journal of Biomolecular Structure and Dynamics, 2023. p. 1-21. https://doi:10.1080/07391102.2023.2291830.
24. Protein Preparation Version. Epik Schrödinger, LLC, New York.
25. Shelley, J.C., et al., Epik: a software program for pK(a) prediction and protonation state generation for drug-like molecules. Journal of Computer-Aided Molecular Design, 2007(12); 12: p. 681-91. https://doi:10.1007/s10822-007-9133-z.
26. Friesner, R.A., et al., Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. Journal of Medicinal Chemistry, 2004; 47(7): p. 1739-49. https://doi:10.1021/jm0306430.
27. Rampogu, S., et al., Discovery of Lonafarnib-Like Compounds: Pharmacophore Modeling and Molecular Dynamics Studies. ACS Omega, 2020. 5(4): p. 1773-1781. https://doi:10.1021/acsomega.9b02263.
28. Cheng, A., et al., Prediction of aqueous solubility of a diverse set of compounds using quantitative structure-property relationships. Journal of Medicinal Chemistry, 2003. 46(17): p. 3572-3580. https://doi:10.1021/jm020266b.
29. Kelder, J., et al., Polar molecular surface as a dominating determinant for oral absorption and brain penetration of drugs. Pharmaceutical Research, 1999. 16(10): p. 1514-1519. https://doi:10.1023/a:1015040217741.
30. Oprea, T.I., et al., Is there a difference between leads and drugs? A historical perspective. Journal of Chemical Information and Modeling, 2001. 41(5): p. 1308-15. https://doi:10.1021/ci010366a.

31. Cheng, A., Dixon, S.L. *In silico* models for the prediction of dose-dependent human hepatotoxicity. Journal of Computer-Aided Molecular Design, 2003. 17(12): p. 811-823. https://doi:10.1023/b:jcam.0000021834.50768.c6 .
32. Egan, W.J., Prediction of Drug Absorption Using Multivariate Statistics. Journal of Medicinal Chemistry, 2000. 43(21): p. 3867-3877. https://doi:10.1021/jm000292e.
33. Colmenarejo, G., et al., Cheminformatic models to predict binding affinities to human serum albumin. Journal of Medicinal Chemistry, 2001. 44(25): p. 4370-4378. https://doi:10.1021/jm010960b.
34. Pettersen, E.F., et al., UCSF Chimera—A visualization system for exploratory research and analysis. Journal of Computational Chemistry, 2004. 25(13): p. 1605-12. https://doi:10.1002/jcc.20084.
35. Zoete, V., et al., SwissParam: a fast force field generation tool for small organic molecules. Journal of Computational Chemistry, 2011. 32(11): p. 2359–2368. https://doi:10.1002/jcc.21816.
36. MJ. Abraham, T. et al., GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputer. SoftwareX 2015. 1–2: p. 19–25. https://doi.org/10.1016/j.softx.2015.06.001 .
37. Abraham, M., et al. 2023. Zenodo. https://doi.org/10.5281/zenodo.7852189.
38. Brooks, B.R. et al., CHARMM: the biomolecular simulation program. Journal of Computational Chemistry, 2009. 30(10): p. 1545-614. https://doi:10.1002/jcc.21287.
39. Mark, P., Nilsson, L. Structure and Dynamics of the TIP3P, SPC, and SPC/E Water Models at 298 K. J. Phys. Chem. A, 2001. 105(43): p. 9954–9960. https://doi.org/10.1021/jp003020w.
40. Turner, P.J. (2005). XMGRACE, Version 5.1.22. Center for Coastal and Land-Margin Research, Oregon Graduate Institute of Science and Technology, Beaverton, OR.
41. Gupta, P., et al., Evaluation of binding and inhibition mechanism of dietary phytochemicals with sphingosine kinase 1: Towards targeted anticancer therapy. Scientific Reports, 2019. 9(1): p. 18727. https://doi.org/10.1038/s41598-019-55199-3.
42. Nagarajan, K., et al., Spectral analysis (FT-IR, FT-Raman, UV and NMR), molecular docking, ADMET properties and computational studies: 2-Hydroxy-5-nitrobenzaldehyde. Journal of the Indian Chemical Society, 2023. 100(3): p. 100927. https://doi.org/10.1016/j.jics.2023.100927.
43. Sharma, K., et al., Synthesis, spectroscopic characterization, electronic and docking studies on novel chalcone derivatives (3DPP and 5PPD) by experimental and DFT methods. Journal of Molecular Structure, 2022. 1256(132553): p. 132553. https://doi.org/10.1016/j.molstruc.2022.132553.
44. Veber, D.F., et al., Molecular properties that influence the oral bioavailability of drug candidates. Journal of Medicinal Chemistry, 2002. 45(12): p. 2615-23. https://doi.org/10.1021/jm020017n.
45. Jairajpuri, D.S., et al., Identification of Sphingosine Kinase-1 Inhibitors from Bioactive Natural Products Targeting Cancer Therapy. ACS Omega, 2020. 5(24): p. 14720-14729. https://doi:10.1021/acsomega.0c01511.
46. Khan, F.I., et al., Identifying novel sphingosine kinase 1 inhibitors as therapeutics against breast cancer. Journal of Enzyme Inhibition and Medicinal Chemistry, 2020. 35(1): p. 172-186. https://doi:10.1080/14756366.2019.1692828.
47. Wang, Z., et al., Molecular basis of sphingosine kinase 1 substrate recognition and catalysis. Structure, 2013. 21(5): p. 798-809. https://doi:10.1016/j.str.2013.02.025 .
48. Arshad, L., et al., Immunosuppressive Effects of Natural α,β-Unsaturated Carbonyl-Based Compounds, and Their Analogs and Derivatives, on Immune Cells: A Review. Frontiers in Pharmacology, 2017. 8: p. 1663-9812. https://doi:10.3389/fphar.2017.00022.
49. Lin, M., et al., Bioactive flavonoids in Moringa oleifera and their health-promoting properties. Journal of Functional Foods, 2018. 47(4): p. 469−479. https://doi:10.1016/j.jff.2018.06.011.
50. Yang, X., et al., Prenylated flavonoids, promising nutraceuticals with impressive biological activities. Trends in Food Science & Technology, 2015. 44(1): p. 93-104. https://doi.org/10.1016/j.tifs.2015.03.007.