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Multiform structure-based identification of natural phytochemical inhibitors targeting RAMP1 for migraine therapy

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Abstract

Migraine is a prevalent neurological disorder primarily driven by activation of calcitonin gene-related peptide (CGRP) receptor system, in which receptor activity modifying protein 1 (RAMP1) plays a critical structural and functional role in stabilizing CGRP receptor complex. In this analysis, an *in-silico* drug designing approach was utilized for the identification of naturally existing phytochemicals that have strong inhibitory activity against RAMP-1. In present study, an *in-silico* drug discovery approach was employed to identify naturally occurring phytochemicals with potential inhibitory activity against RAMP1. The three-dimensional structure of RAMP1 was retrieved and prepared for molecular docking analysis, while twenty-five phytochemicals with reported anti-inflammatory properties were selected as candidate ligands. Molecular docking was performed to evaluate the binding affinity and protein-ligand interactions. Ursonic acid exhibited highest binding affinity (-7.8kcal/mol), followed by rutin (-7.1 kcal/mol) and β-sitosterol (-7.0 kcal/mol). Interaction analysis of the docked complexes identified 36 unique amino acid residues involved in ligand binding with Tyr-66, Trp-84, Pro85, Val-89, Asp-90, Phe-93 and His-97. ADMET analysis revealed that 23 of the 25 compounds compiled with Lipinski's Rule of Five. By integrating molecular docking with pharmacokinetics and toxicity predictions, ursonic acid and β-sitosterol were identified as the most significant phytochemical candidates for further analysis as potential RAMP1 target as anti-migraine agent.

Introduction

Migraine is a persistent neurological disorder characterized by recurrent attacks of moderate to severe headaches, usually accompanied by nausea, aura, vomiting, phonophobia, and photophobia. It affects approximately 14% of the global population and is particularly common in women aged 15-49 years, who experience attacks that are 2-3 times more frequent, longer-lasting, and more severe than those in men [1]. Chronic migraine has a significant impact, affecting approximately 1-2% of the general population and nearly 8% of individuals suffering from migraine. It typically evolves from episodic migraine, with approximately 3% of cases progressing to chronic migraine each year. The condition can be improved and, in some cases, be reversed; nearly 26% of patients with chronic migraine achieve recovery within two years [2]. Various risk factors for migraine have been identified, including aging, head trauma, overuse of caffeine or medication, low socioeconomic status, stress, irregular sleep patterns, obesity, and depression [3].

The etiology of migraine is considered multifactorial and is believed to begin with the overactivation of the trigeminovascular system. This system regulates cerebral blood flow and pain signaling in the head, and its activation results in the release of several neuropeptides into the meninges. These neuropeptides include calcitonin gene-related peptide (CGRP), neurokinin A, calcitonin, and substance P. The release of these chemicals causes neurogenic inflammation, which stimulates and sensitizes nerve endings, thereby initiating a headache [4]. The pathogen of migraine is highly complex. Oxidative stress, neuronal injury, and inflammatory pathways interact to form an intricate network that contributes to both initiation and persistence of migraine [5]. Genetic susceptibility and environmental factors, such as stress, hormonal fluctuations, dietary habits, and sensory stimuli, also contribute to migraine development by influencing neural excitability and inflammatory responses [6].

The CGRP receptor is a heterodimer complex of the calcitonin receptor-like receptor (CLR) and receptor-modifying protein 1 (RAMP1). RAMP1 is a single-pass transmembrane protein characterized by a large extracellular N-terminus domain and consists of 148 amino acids. It is an essential accessory protein responsible for the stabilization of the CGPR receptor complex, as it associates with CLR to form a functional receptor complex of approximately 66kDa [7]. During migraine development, activation of the trigeminovascular system contributes to the release of CGRP from the terminals of the trigeminal nerve. CGPR exerts its effects by binding to its functional receptors, leading to activation of G-protein-mediated signaling, increased intracellular cAMP levels, and initiation of downstream signaling pathways such as protein kinase A [8]. These events promote vasodilation, neurogenic inflammation, and mast cell activation, all of which contribute to migraine pain [9]. Furthermore, epigenetic modifications such as DNA methylation of the RAMP1 promoter region may influence the regulation of this receptor and contribute to migraine susceptibility [10].

Migraine is a widespread neurological disorder; however, current therapeutic approaches remain limited by poor efficacy, adverse effects, and contraindications. Although CGRP-targeting therapies have significantly improved management, incomplete therapeutic responses remain a major challenge. RAMP1 plays a significant role in the pathophysiology of migraine; however, its full therapeutic potential remains largely unexplored [11]. Therefore, the identification of novel naturally occurring bioactive compounds targeting RAMP1 may facilitate the development of safer and more effective anti-migraine therapies [12]. Computational drug design provides a rapid and cost-effective approach to overcome the limitations of conventional migraine drug discovery [13]. Using bioinformatics tools, the 3D structure of RAMP1 can be analyzed, and various phytochemicals can be screened through molecular docking for the identification of potential inhibitors. Furthermore, the evaluation of protein-ligand interactions helps to identify key binding residues and prioritize promising compounds based on their binding affinities [14].

Materials and Methods

Retrieval and preparation of the target protein

The three-dimensional structure of the target protein was retrieved from Universal Protein Resource Knowledgebase (Uniprot: Q16602) [15]. This protein consists of 461 amino acids distributed in chains A and B. UniprotKB is a comprehensive, freely accessible repository of protein sequences and functional information maintained jointly by Swiss

Institute of Bioinformatics (SIB) and (EMBL-EBI) [16]. The FASTA sequence was retrieved, and the 3D structure was obtained from the Protein Data Bank (PDB). The protein structure was subsequently processed using SWISS-PDB Viewer [17] to remove unnecessary molecules, and the cleaned protein structure was saved for molecular docking analysis.

Retrieval of ligands

The phytochemical compounds used as ligands in this study were collected from Dr. Duke's Phytochemical and Ethnobotanical Database [18]. This is extensive, publicly available database developed by James A. Duke at the United States Department of Agriculture. The compounds were selected based on their reported biological activity and association with anti-inflammatory properties. The chemical structures of the selected phytochemicals were downloaded from PubChem [19]. The ligands were initially retrieved in SDF format and converted into PDB form after energy minimization using ChemDraw [20]. Each ligand structure was subsequently imported into the AutoDock Vina [14].

Molecular docking analysis

Molecular docking was carried out using AutoDock Vina. The prepared RAMP1 structure was used as the receptor, while the selected phytochemical compounds were used as ligands. Docking analysis was performed to evaluate the binding affinity of each compound with the target protein. The best docking pose for each ligand was selected based on the lowest binding energy score (kcal/mol), where lower binding energy values indicate stronger interactions between the ligand and target protein. A grid box encompasses the entire protein surface was defined to enable unbiased identification of potential binding regions. A grid box with dimensions of 30 × 30 × 30 Å and a grid spacing of 1 Å was used to cover the complete protein surface. The exhaustiveness parameter, which controls the computational search during docking, was set to set to default value of 8.

Visualization and interaction analysis

Protein-ligand interactions were analyzed using BIOVIA Discovery Studio [21]. The interaction evaluated included hydrogen bonds, hydrophobic interactions, van der Waals interactions, Pi-Pi stacking and carbon-hydrogen bonds. Hydrogen bonds were considered essential for determining binding specificity and stabilizing the ligand within the active site, whereas hydrophobic interactions and van der Waals contacts significantly to binding affinity through favorable non-covalent interactions. Aromatic interaction, including π–π stacking and π–alkyl interactions, further enhanced complex stability by promoting interactions. The most stable binding conformations were determined by comparing the quantity, kind and geometric arrangement of these interactions among the top-ranked docking poses. Residues that repeatedly interacted with multiple high-affinity ligand complexes were identified as candidate hotspot residues within the RAMP1 binding pocket.

ADMET analysis

The absorption, distribution, metabolism, excretion and toxicity (ADMET) properties of the selected compounds were evaluated using ADMETlab 3.0 web server [22]. The SMILES strings of the selected compounds were retrieved from the PubChem database and submitted to the server for property prediction. The predicted physiochemical parameters included molecular weight (MW), number of hydrogen bond acceptors [22], number of hydrogen bond donors (nHD), topological polar surface area (TPSA), number of rotatable bonds (nRot), lipophilicity (logP), aqueous solubility (logS) and follow the Lipinski's Rule of Five (RO5). These descriptors were used to assess the physicochemical properties of the compound and its potential oral bioavailability. The evaluated absorption and toxicity parameters included Caco-2 cell permeability, carcinogenicity, mutagenicity, AMES toxicity and rat oral acute toxicity probability. The utilized methodology (**Fig. 1**) has been extensively reported in numerous studies [23]. The CADD pipeline, including the steps of target identification, protein structure prediction, virtual screening, molecular docking, molecular dynamics simulation, binding free energy estimation, and pharmacokinetic evaluation, has been well reported[24]and validated in many peer-reviewed studies and therefore is a reliable and reproducible process for the identification and optimization of new therapeutic compounds before experimental testing [13].

Results

Three-dimensional protein structure

3D structure of RAMP-1 was retrieved and visualized

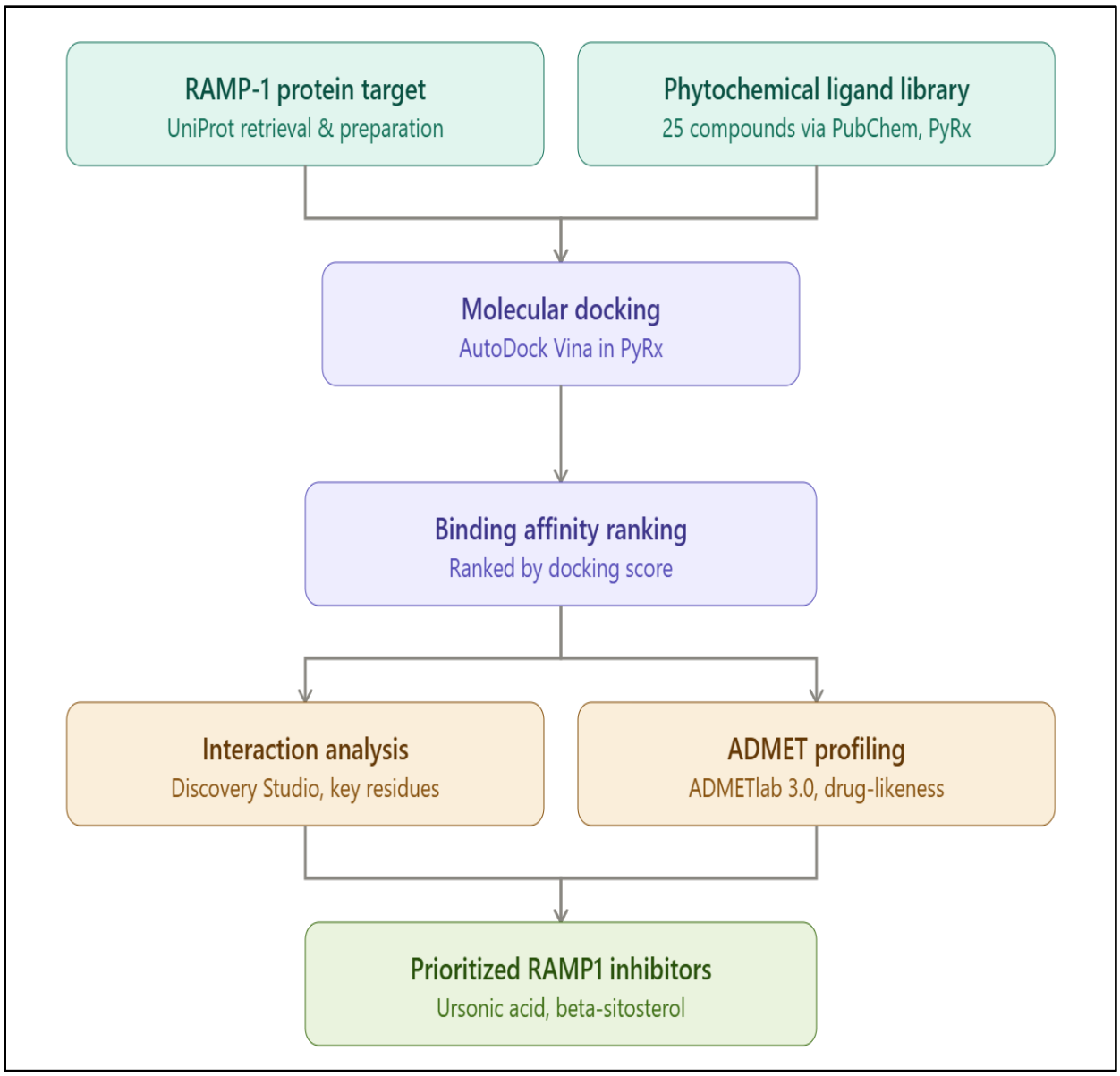

Fig. 1: Schematic workflow of the computational drug design pipeline used to identify phytochemical inhibitors of RAMP1

(**Fig. 2**). The cleaned protein structure was subsequently used as the receptor molecule for evaluating the binding affinity of the selected antiviral compounds through molecular docking analysis.

Structures of ligands

A total of twenty-five ligands were selected for molecular docking analysis against the RAMP1 protein. The optimized ligand structures (**Fig. 3**) were used for molecular docking to evaluate their binding affinity. The molecular docking analysis revealed that several ligands exhibited favorable interactions with the target protein and showed strong binding affinity.

Molecular docking of phytochemicals with RAMP-1 protein

The selected compounds and the target protein were subjected to molecular docking. The resulting protein-ligand complexes exhibited different binding affinities which were ranked from the strongest to weakest binding (**Table 1**).

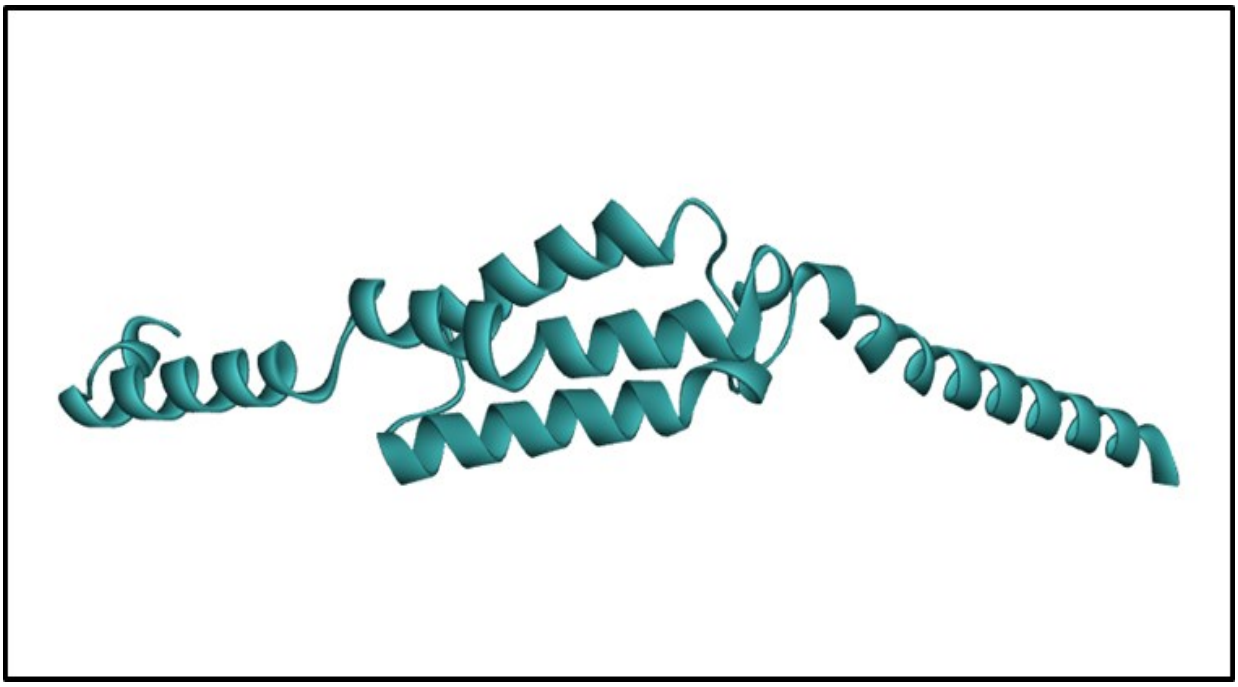

Fig. 2: Three-dimensional structure of receptor activity-modifying Protein-1

Table 1: Binding affinities of phytochemical compounds docked against RAMP1

Rank	Compound	Binding energy (kcal/mol)	Classification
1	Ursonic acid	−7.8	Strong binder
2	Rutin	−7.1	Strong binder
3	Beta-sitosterol	−7.0	Strong binder
4	Quercetin	−6.7	Moderate binder
5	Luteolin	−6.6	Moderate binder
6	Curcumin	−6.5	Moderate binder
7	Kaempferol	−6.4	Moderate binder
8	Beta-carotene	−6.3	Moderate binder
9	Rosmarinic acid	−5.6	Moderate binder
10	Chlorogenic acid	−5.6	Moderate binder
11	Coumarin	−5.4	Moderate binder
12	Linalool	−5.0	Moderate binder
13	Thymol	−5.0	Moderate binder
14	Caffeic acid	−4.9	Weak binder
15	Pectin	−4.8	Weak binder
16	Camphor	−4.8	Weak binder
17	Eugenol	−4.7	Weak binder
18	Limonene	−4.7	Weak binder
19	Protocatechuic acid	−4.7	Weak binder
20	Citral	−4.6	Weak binder
21	1,8-Cineole	−4.6	Weak binder
22	Ascorbic acid	−4.5	Weak binder
23	Geraniol	−4.4	Weak binder
24	Linoleic acid	−4.2	Weak binder
25	Menthol	−3.6	Weak binder

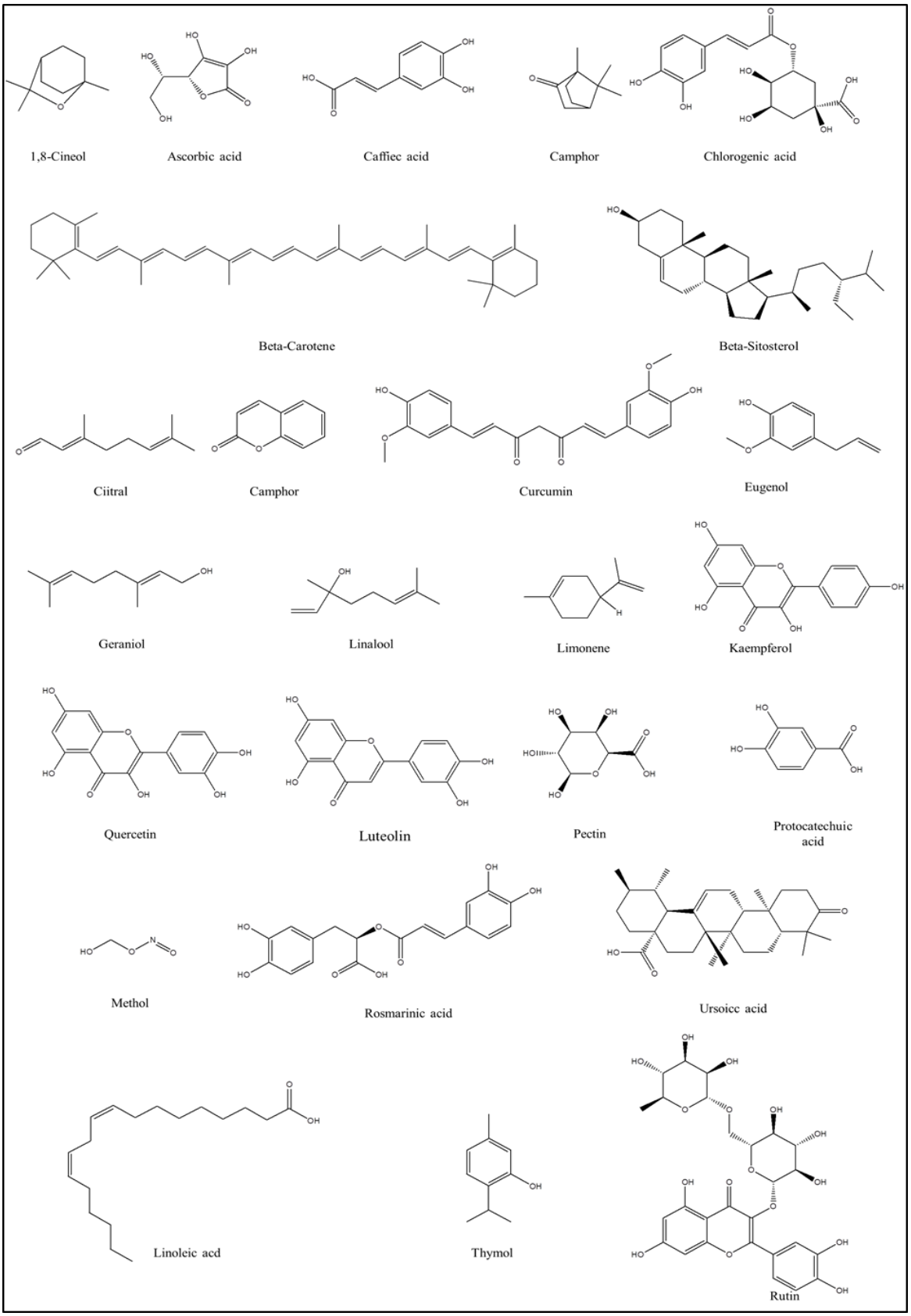

Fig. 3: Two-dimensional chemical structures of the twenty-five phytochemical ligands

Protein-ligand interaction analysis

The docked complexes of all 25 phytochemicals-RAMP1 complexes were analyzed, and promising results were observed. Visualization of each complex in both 3D and 2D interaction mode revealed the specific amino acid residues of RAMP1 involved in hydrogen bonding, hydrophobic interactions, pi–pi stacking, and van der Waals interactions with each ligand (**Table 2**). Across all 25 complexes, a total of 36 unique RAMP1 residues were identified as participating in ligand interactions, spanning the region from residue 14 to residue 109.

In silico ADMET profiling

Evaluation of physicochemical properties revealed that 23 of the 25 compounds complied with Lipinski's Rule of Five, indicating favorable drug-likeness and a high probability of acceptable oral bioavailability (Table 3). Only β-carotene and rutin did not follow the RO5 rule. β-carotene exceeded the acceptable limits due to its high molecular weight (536.44 Da) and high lipophilicity (logP = 7.713), whereas rutin failed because of its large molecular weight (610.15 Da). The topological polar surface area varied considerably among the compounds, ranging from 0 $Å^2$ to 269.43 $Å^2$. This variation was generally associated with differences in the predicted aqueous solubility (logS) and membrane permeability (**Table 3**).

Table 2: Interacting RAMP1 residues for all 25 phytochemical–RAMP1 complexes

Sr #	Compound	Interacting residues
1	1,8-Cineole	Tyr-66, Trp-84, Pro-85, Val-89, Asp-90, Phe-93, His-97
2	Ascorbic acid	Met-23, Thr-24, Cys-27, Glu-29, Cys-82, Phe-83, Asn-86, Aal-87
3	Beta-carotene	Trp-14, Leu-15, Leu-17, Ala-18, Leu-21, Phe-22, Thr-25, Gln-28, Glu-29, Ala-30
4	Beta-sitosterol	Trp-59, Ile-63, Tyr-66, Arg-67, Ala-70, Trp-84, Pro-85, Val-89, Asp-90, Phe-93, Leu-94, His-97
5	Caffiec-acid	Gln-45, Glu-49, Gly-52, Glu-53, Trp-56, Arg-99 Tyr-100
6	Camphor	Tyr-66, Ala-70, Trp-84, Pro-85, Val-89, Asp-90, Phe-93, His-97
7	Chlorogenic-acid	Met-23, Thr-24, Ala-26, Cys-27, Glu-29, Gly-81 Cys-82, Phe-83, Trp-84, Pro-85, Asn-86, Ala-87, Glu-88
8	Citral	Trp-59, His-97, Gly-98, Phe-101, Arg-102, Cys-104, Pro-105, Ile-106, Arg-109
9	Coumarin	Tyr-66, Trp-84, Pro-85, Val-89, Asp-90, Phe-93, His-97
10	Curcumin	Trp-59, Tyr-66, ALA-70, Trp-84, Pro-85, Val-89, Asp-90,Phe-93, His-97, Phe-101, Pro-105, Ile-106, Arg-109
11	Eugenol	Trp-59, Tyr-66, His-97, Phe-101, Cys-104, Ile-106, Arg-109
12	Geraniol	ARG-37, Leu-41, Gln-45, Glu-88, Phe-92, Ala-95, Val-96, Arg-99, Tyr-100
13	Kampferol	Trp-59, Tyr-66, His-97, Phe-101, Cys-104, Pro-105, Ile-106, Arg-109
14	Limonene	Tyr-66, Ala-70, Trp-84, Val-89, Asp-90, Phe-93, Leu-94
15	Linalool	Trp-59, Tyr-66, Ala-70, Trp-84, Pro-85, Val-89, Asp-90, Phe-93, His-97, Phe-101, Cys-104, Arg-109
16	linoleic acid	Trp-59, Ile-63, Tyr-66, Arg-67, Ala-70, Trp-84, Pro-85, Val-89, Phe-93
17	Luteolin	Trp-59, Thr-62, His-97, Gly-98, Phe-101, Arg-102, Cys-104, Ile-106, Arg-109
18	Menthol	Trp-56, Cys-57, Asp-58, Trp-59, Thr-62, Phe-101, Cys-104, Arg-109
19	Protocatechuic acid	Met-23, Thr-24, Cys-27, Gln-28, Glu-29, Gly-81, Cys-82, Phe-83, Asn-86
20	Pectin	Glu-53, Trp-56, Glc-98, Arg-99, Tyr-100, Phe-101, Arg-102, Ser-103
21	Quercetin	Trp-59, Leu-94, His-97, Gly-98, Phe-101, Arg-102, Cys-104, Pro-105, Ile-106, Arg-109
22	Rosmarinic acid	Trp-59, Tyr-66, Phe-93, Leu-94, His-97, Phe-101, Arg-102, Cys-104, Pro-105, Ile-106, Arg-109
23	Rutin	Tyr-66, Val-89, Asp-90, Phe-93, Leu-94, His-97, Gly-98, Phe-101, Arg-102, Ser-103, Cys-104, Pro-105, Ile-106, Arg-109
24	Thymol	Tyr-66, Ala-70, Trp-84, Pro-85, Val-89, Asp-90, Phe-93, Leu-94, His-97
25	Ursonic acid	Trp-59, Ile-63, Try-66, Arg-67, Ala-70, Trp-84, Pro-85, Val-89, Asp-90, Phe-93

Table 3: Physicochemical and drug-likeness properties of the selected phytochemical compounds

Compound	Molecular Weight (MW)	nHA	nHD	nRot	TPSA	logS	logP
1,8-Cineole	154.14	1.0	0.0	0.0	9.23	-1.383	2.168
Ascorbic acid	176.03	6.0	4.0	2.0	107.22	0.232	-1.707
Beta-carotene	536.44	0.0	0.0	10.0	0.0	-7.571	7.713
Beta-Sitesterol	414.39	1.0	1.0	6.0	20.23	-7.221	8.004
Caffeic acid	180.04	4.0	3.0	2.0	77.76	-1.837	1.19
Camphor	330.15	5.0	3.0	4.0	94.83	-4.032	2.705
Chlorogenic acid	354.1	9.0	6.0	5.0	164.75	-2.958	1.036
Citral	152.12	1.0	0.0	4.0	17.07	-2.968	3.284
Coumarin	146.04		0.0	0.0	30.21	-1.997	1.472
Curcumin	368.13	6.0	2.0	8.0	93.06	-3.62	2.147
Eugenol	164.08	2.0	1.0	3.0	29.46	-1.891	2.321
Geraniol	154.14	1.0	1.0	4.0	20.23	-2.954	3.428
Kampferol	286.05	6.0	4.0	1.0	111.13	-3.648	1.965
Limonene	136.13	0.0	0.0	1.0	0.0	-4.123	4.541
Linalool	154.14	1.0	1.0	4.0	20.23	-1.806	2.512
Linoleic acid	280.24	2.0	1.0	14.0	37.3	-5.426	6.953
Luteolin	286.05	6.0	4.0	1.0	111.13	-4.017	2.247
Menthol	77.01	4.0	1.0	2.0	58.89	0.722	-0.175
Pectin	194.04	7.0	5.0	1.0	127.45	0.559	-2.004
Protocatechuic acid	154.03	4.0	3.0	1.0	77.76	-1.697	1.001
Quercetin	302.04	7.0	5.0	1.0	131.36	-3.722	1.448
Rosmarinic acid	360.08	8.0	5.0	7.0	144.52	-3.033	2.005
Rutin	610.15	16.0	10.0	6.0	269.43	-2.397	0.986
Thymol	150.1	1.0	1.0	1.0	20.23	-2.067	3.161
Ursonic acid	454.34	3.0	1.0	1.0	54.37	-4.767	4.547

The predicted Caco-2 permeability values indicated that phenolic acid and terpenoids generally possessed favorable characteristics for intestinal absorption, with values ranging between -4.11 and -5.50. Compounds including coumarin, limonene, citral, and geraniol. Thymol and eugenol illustrated comparatively good permeability, which showed efficient intestinal absorption. Among them, pectin exhibited an exceptionally high positive Caco-2 value, that reflecting that it is a polysaccharide structure and signifies passing through the lining of the intestine. However, compounds such as rosmarinic acid, citral, menthol, and chlorogenic acid urge caution due to marked carcinogenicity and mutagenicity. These findings should be considered when prioritizing candidates for further drug design procedures (**Table 4**).

Discussion

Bioinformatics is an interdisciplinary science that integrates biology, computer science, mathematics and statistics to capture, organize, analyze, and realize complex biological information and analyze biological data [25]. It has become an indispensable tool for the analysis of genomic, transcriptomic, proteomic, metabolomic and structural biological data in modern biological and biomedical research [26-28]. One of the most versatile computational techniques, computer-aided drug design (CADD) has become one of the most powerful methods used to accelerate the drug discovery process [29]. CADD can significantly redue the time, cost, and experimentation needed to identify viable lead compounds [30]. The efficacy of CADD in contemporary drug discovery has been evidenced in numerous computational studies [29] identifying novel therapeutic candidates against a variety of diseases including COVID-19 [31, 32], neurological disorders [24], and cancer [33, 34]. Additionally, these computational methods have been extensively used[34] to find inhibitors of bacterial, viral, fungal, and parasitic pathogens, as well as in vaccine design and precision medicine [35]. The present study aimed to identify naturally derived RAMP1 inhibitors as an alternative approach for anti-migraine drug development, and the molecular docking results support this rationale [23]. Ursonic acid, rutin, and beta-sitosterol formed the strongest-binding complexes, each falling in the range that is considered significant stable, favorable protein-ligand association. Particularly, the residues that most regularly repeat in these high-affinity interactions include Tyr-66, Trp-84, Pro-85, Val-89, Asp-90, Phe-93, and His-97. This assembly suggests that a discrete

Table 4: Predicted ADMET profile of the selected twenty-five phytochemicals

Compound	Caco-2 Permeability	Carcinogenicity	Mutagenicity	AMES Toxicity	Rat Oral Acute Toxicity	Lipinski Rule
1,8-Cineole	-4.58	0	0	0.499	0.28	Accepted
Ascorbic acid	-6.009	0	1	0.45	0.084	Accepted
Beta-carotene	-4.888	0	0	0.456	0.624	Rejected
Beta-Sitesterol	-5.122	0	0	0.139	0.111	Accepted
Caffeic acid	-4.94	1	0	0.274	0.079	Accepted
Camphor	-4.817	1	0	0.175	0.077	Accepted
Chlorogenic acid	-6.426	1	1	0.386	0.054	Accepted
Citral	-4.486	1	1	0.43	0.262	Accepted
Coumarin	-4.11	0	1	0.593	0.478	Accepted
Curcumin	-5.471	1	1	0.293	0.092	Accepted
Eugenol	-4.57	0	0	0.468	0.346	Accepted
Geraniol	-4.426	0	1	0.228	0.086	Accepted
Kampferol	-5.969	0	0	0.546	0.488	Accepted
Limonene	-4.581	0	0	0.192	0.207	Accepted
Linalool	-4.721	0	0	0.369	0.192	Accepted
Linoleic acid	-5.051	0	0	0.107	0.033	Accepted
Luteolin	-5.192	0	0	0.65	0.51	Accepted
Menthol	-4.449	1	3	0.973	0.702	Accepted
Pectin	6.424	0	0	0.492	0.048	Accepted
Protocatechuic acid	-5.304	0	0	0.371	0.255	Accepted
Quercetin	-6.177	0	0	0.586	0.48	Accepted
Rosmarinic acid	-6.513	1	1	0.549	0.312	Accepted
Rutin	-6.547	0	0	0.756	0.044	Rejected
Thymol	-4.48	0	0	0.305	0.311	Accepted
Ursonic acid	-5.534	0	0	0.229	0.407	Accepted

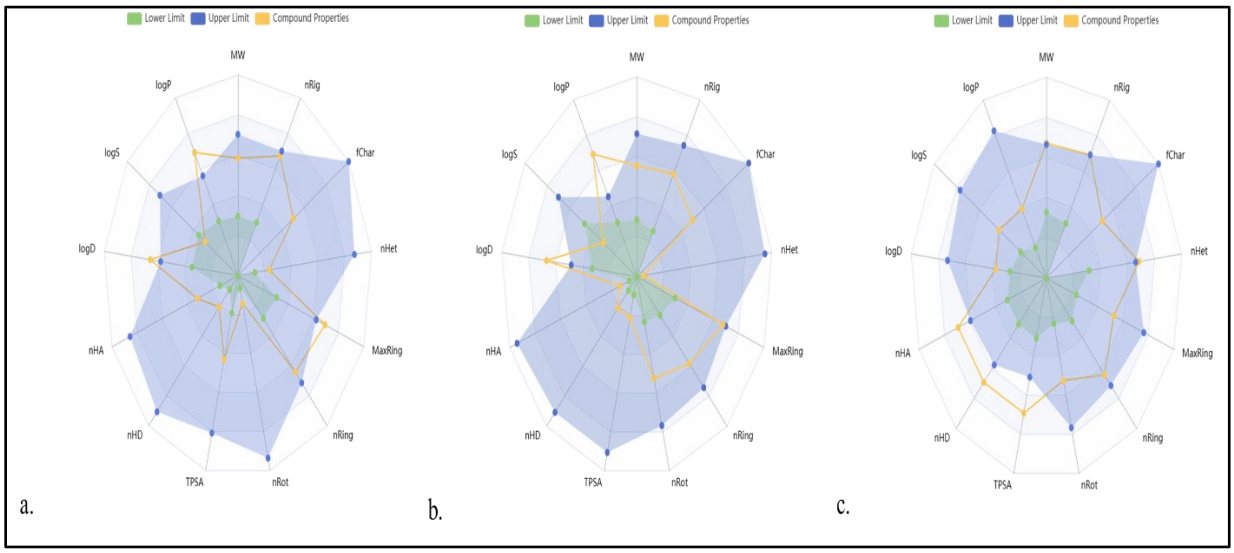

Fig. 4: Radar graph shows the ADMET properties of the top three scrutinized compounds (a) Ursonic acid (b) Beta-Sitosterol and (c) Rutin.

hydrophobic and aromatic-rich sub-pocket within the RAMP1 extracellular domain may function as a drugable hotspot, a finding that could help direct future structure-based design or fragment optimization efforts targeting this region rather than the full protein surface [36].

The binding affinity provided an initial basis for ranking candidates, the ADMET analysis proved to distinguish possible drug leads from computationally strong, while pharmacologically unfavorable hits [37, 38]. Although rutin exhibited the second highest docking affinity, it did not follow Lipinski's Rule of Five as its molecular weight is 610.15 Da, and strong

hydrogen bonding capacity and characteristics that are commonly associated with reducing oral bioavailability and confined membrane permeability. β-carotene was similarly excluded due to excessive lipophilicity (logP = 7.713). Conversely, ursonic acid combined a strong binding affinity with an acceptable physicochemical profile, reinforcing its position as the most promising candidate to emerge from this screen. Similarly, beta-sitosterol retained a favorable balance between affinity and drug-likeness, where its comparatively higher molecular weight was considered a secondary lead.

The toxicity predictions add a further layer of refinement to candidate prioritization. Several phenolic compounds, including rosmarinic acid, chlorogenic acid, citral, and menthol, were returned cautions for carcinogenicity and mutagenicity potential despite reasonable binding scores, underscoring that binding affinity alone is an insufficient criterion for lead compounds selection. These conclusions highlighted the importance of ADMET evaluation along with molecular docking earlier in the pipeline that followed in drug discovery, instead of considering pharmacokinetics evaluation only at the final stages [24] .

Despite these optimistic findings, the results should be interpreted under the limitations associated with the *in silico* approach. The molecular docking analysis depends on a single rigid receptor conformation [39]. That does not justify the conformational flexibility of RAMP-1 or strongly induced-fit interactions during the binding of ligand. The ADMET analysis represents computational predictions that are based on statistical models and do not replace experimentally verified pharmacokinetic and toxicological data. Hence, the lead compounds, especially ursonic acid and beta-sitosterol, should be taken as a favorable candidate for further analysis.

Conclusion

This study applied a computational drug discovery approach to identify potential phytochemical inhibitors for RAMP1. Molecular docking identified ursonic acid, rutin, and β-sitosterol as the most significant ligands, exhibiting the highest binding affinities and interacting with several conserved residues that form a common binding hotspot. ADMET analysis prioritizes ursonic acid and β-sitosterol, having favorable drug-likeness, safety profiles, and pharmacokinetic properties. These findings highlight the importance of integrating molecular docking and ADMET evaluation for a lead compound.

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Conflict of interest

The authors declare no conflict of interest.

References

[1] Edvinsson L, Holm A. Understanding RAMP1's role in hormonal migraine may improve CGRP-targeted therapies. Taylor & Francis; 2026.
[2] May A, Schulte LH. Chronic migraine: risk factors, mechanisms and treatment. Nature Reviews Neurology. 2016;12:455-64.
[3] Amiri P, Kazeminasab S, Nejadghaderi SA, Mohammadinasab R, Pourfathi H, Araj-Khodaei M, et al. Migraine: A Review on Its History, Global Epidemiology, Risk Factors, and Comorbidities. Frontiers in Neurology. 2022; Volume 12 - 2021.
[4] Aksoydan B, Durdagi S. Molecular simulations reveal the impact of RAMP1 on ligand binding and dynamics of calcitonin gene-related peptide receptor (CGRPR) heterodimer. Computers in Biology and Medicine. 2022;141:105130.
[5] Zhao L, Huang H, Zhu J, Liu X, Liu H, Wu X, et al. Mechanistic Insights into Toutongping Granule for Migraine: A Multi-Pathway Intervention Study in Rats. Natural Product Communications. 2026;21:1934578X261433853.
[6] Lou G, Sophie L, Marta W-P, Dilara O, Karol M, Wietse W, et al. Genetics of migraine: where are we now? Journal of Headache and Pain (Web). 2023;24:1-23.
[7] Szymanowicz O, Słowikowski B, Poszwa J, Goutor U, Wiszniewska M, Jagodziński PP, et al. CACNA1A Genetic Variants and Their Potential Involvement in Migraine Pathogenesis. International Journal of Molecular Sciences. 2025;26:8083.
[8] Kowalska M, Prendecki M, Kozubski W, Lianeri M, Dorszewska J. Molecular factors in migraine. Oncotarget. 2016;7:50708.
[9] Zhang H, Jiang L, Xian Y, Yang S. Familial hemiplegic migraine type 2: a case report of an adolescent with ATP1A2 mutation. Frontiers in Neurology. 2024;Volume 15 - 2024.
[10] Domitrz I, Kosiorek M, Żekanowski C, Kamińska A. Genetic studies of Polish migraine patients: screening for causative mutations in four migraine-associated genes. Human Genomics. 2016;10:3.

[11] Edvinsson L, Haanes KA, Warfvinge K, Krause DN. CGRP as the target of new migraine therapies—successful translation from bench to clinic. Nature Reviews Neurology. 2018;14:338-50.
[12] Salaria D, Rolta R, Mehta J, Awofisayo O, Fadare OA, Kaur B, et al. Phytoconstituents of traditional Himalayan Herbs as potential inhibitors of Human Papillomavirus (HPV-18) for cervical cancer treatment: An In silico Approach. PLoS One. 2022;17:e0265420.
[13] Sehgal SA, Tahir RA, Waqas M. Quick Guideline for Computational Drug Design (Revised Edition): Bentham Science Publishers; 2021.
[14] Dallakyan S, Olson AJ. Small-molecule library screening by docking with PyRx. Chemical biology: methods and protocols: Springer; 2014. p. 243-50.
[15] Consortium U. UniProt: a worldwide hub of protein knowledge. Nucleic acids research. 2019;47:D506-D15.
[16] Pundir S, Martin MJ, O'Donovan C. UniProt protein knowledgebase. Protein Bioinformatics: From Protein Modifications and Networks to Proteomics: Springer; 2017. p. 41-55.
[17] Guex N, Peitsch MC. SWISS-MODEL and the Swiss-Pdb Viewer: an environment for comparative protein modeling. electrophoresis. 1997;18:2714-23.
[18] Ben W. Dr. Duke's phytochemical and ethnobotanical databases. Choice Rev Online. 2001;38:38-3317.
[19] Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, et al. PubChem substance and compound databases. Nucleic acids research. 2016;44:D1202-D13.
[20] Mills N. ChemDraw Ultra 10.0 CambridgeSoft, 100 CambridgePark Drive, Cambridge, MA 02140. www. cambridgesoft. com. Commercial Price: 1910fordownload, 2150 for CD-ROM; Academic Price: 710fordownload, 800 for CD-ROM. ACS Public; 2006.
[21] Baroroh U, Biotek M, Muscifa ZS, Destiarani W, Rohmatullah FG, Yusuf M. Molecular interaction analysis and visualization of protein-ligand docking using Biovia Discovery Studio Visualizer. Indonesian Journal of Computational Biology (IJCB). 2023;2:22-30.
[22] Fu L, Shi S, Yi J, Wang N, He Y, Wu Z, et al. ADMETlab 3.0: an updated comprehensive online ADMET prediction platform enhanced with broader coverage, improved performance, API functionality and decision support. Nucleic acids research. 2024;52:W422-W31.
[23] Sehgal SA, Hassan M, Rashid S. Pharmacoinformatics elucidation of potential drug targets against migraine to target ion channel protein KCNK18. Drug design, development and therapy. 2014:571-81.
[24] Tahir RA, Sehgal SA. Pharmacoinformatics and molecular docking studies reveal potential novel compounds against schizophrenia by target SYN II. Combinatorial chemistry & high throughput screening. 2018;21:175-81.
[25] Sehgal SA. Pharmacoinformatics, adaptive evolution, and elucidation of six novel compounds for schizophrenia treatment by targeting DAOA (G72) isoforms. BioMed research international. 2017;2017:5925714.
[26] Wu J, Khaliq H, Ke Y, Ullah Q, Sehgal SA, Yi X. Network pharmacology and integrative bioinformatics analyses identify PDE1A as a key target of pirfenidone in idiopathic pulmonary fibrosis. Plos one. 2026;21:e0342991.
[27] Azmi MB, Khan F, Asif U, Hoda F, Sehgal SA, Qureshi SA, et al. Computational exploration of Withania coagulans-derived natural inhibitors targeting HMG-CoA reductase: A pharmacokinetic, docking and molecular dynamics study for cholesterol regulation in Mus musculus. Pak J Pharm Sci. 2026;39:999-1020.
[28] Azmi MB, Sehgal SA, Asif U, Musani S, Abedin MFE, Suri A, et al. Genetic insights into obesity: in silico identification of pathogenic SNPs in MBOAT4 gene and their structural molecular dynamics consequences. Journal of Biomolecular Structure and Dynamics. 2024;42:13074-90.
[29] Waseem HB, Shakeel M, Hassan F-u, Yaqoob A, Iqbal A, Khalid A, et al. In silico Identification and Computational Screening of Potential AFP Inhibitors Against Liver Cancer. Medicinal Chemistry. 2025;21:554-65.
[30] Jin Y, Sehgal SA, Hassan F, Liu G. In Silico Identification of Novel Compounds as Anthelmintics Against Haemonchus contortus Through Inhibiting β-Tubulin Isotype 1 and Glutathione S-Transferase. Animals. 2025;15:1846.
[31] Sajid M, Marriam S, Mukhtar H, Sohail S, Sajid M, Sehgal SA. Epitope-based peptide vaccine design and elucidation of novel compounds against 3C like protein of SARS-CoV-2. PloS one. 2022;17:e0264700.
[32] Marriam S, Afghan MS, Nadeem M, Sajid M, Ahsan M, Basit A, et al. Elucidation of novel compounds and epitope-based peptide vaccine design against C30 endopeptidase regions of SARS-CoV-2 using immunoinformatics approaches. Frontiers in Cellular and Infection Microbiology. 2023;13:1134802.
[33] Tur Razia I, Kanwal A, Riaz HF, Malik A, Ahsan M, Saleem Khan M, et al. Recent trends in computer-aided drug design for anti-cancer drug discovery. Current Topics in Medicinal Chemistry. 2023;23:2844-62.
[34] Sehgal SA, Kanwal S, Tahir RA, Khalid Z, Hammad MA. In silico elucidation of potential drug target sites of the Thumb Index Fold Protein, Wnt-8b. Tropical Journal of Pharmaceutical Research. 2018;17:491.
[35] Al Hashmi S, Tahir RA, Sehgal SA. Network pharmacology-based identification of potential drug targets and bioactive compounds in Lycii Fructus (Gouqizi) for the therapeutics of Parkinson's disease. Frontiers in Pharmacology. 2025;16:1714071.
[36] Moore EL, Salvatore CA. Targeting a family B GPCR/RAMP receptor complex: CGRP receptor antagonists and migraine. British journal of pharmacology. 2012;166:66-78.
[37] Sehgal SA, Hammad MA, Tahir RA, Akram HN, Ahmad F. Current therapeutic molecules and targets in neurodegenerative diseases based on in silico drug design. Current neuropharmacology. 2018;16:649-63.
[38] Kanwal S, Jamil F, Ali A, Sehgal SA. Comparative modeling, molecular docking, and revealing of potential binding pockets of RASSF2; a candidate cancer gene. Interdisciplinary Sciences: Computational Life Sciences. 2017;9:214-23.
[39] Sehgal SA, Khattak NA, Mir A. Structural, phylogenetic and docking studies of D-amino acid oxidase activator (DAOA), a candidate schizophrenia gene. Theoretical Biology and Medical Modelling. 2013;10:3.