"Computational methods for *in-silico* design of Ursolic acid against biological targets"

Kaniga P[1], Kavitha K[2*], Binoy Cheriyan[1], Kalaiselvi C[3], Rithikha S[1], Yokesh S[1], Kadir A[1]

[1]Department of Pharmaceutical Chemistry, Saveetha College of Pharmacy, SIMATS,

Chennai - 602 105, Tamil Nadu, India.

[2]Department of Pharmaceutical Chemistry, Prime College of Pharmacy, Prime Nagar, Errattyal,

Palakkad – 678 551, Kerala, India.

[3]Department of Pharmaceutics, SS Institute of Pharmacy, NH-544, Kuppanur, Sankari,

Salem – 637 301, Tamil Nadu, India.

*Corresponding author

Orcid ID: https://orcid.org/0000-0002-4303-6164

Correspondence:

Dr. Kavitha K
Professor,
Prime College of Pharmacy,
Prime Nagar, Erattyal,
Palakkad-- 678 551, Kerala, India.

Abstract:

In 1920, apple epicuticular waxes were the source of ursolicic acid, a pentacyclic triterpenoid also referred to as urson, prunol, malol, or 3β-hydroxyurs-12-en-28-oic acid. It is generally available for use in medicine since it is found in a wide range of plant species, including fruits, vegetables, and herbs. It is also commonly found in herbs and spices like thyme and rosemary. This study's main goal was to use in silico docking studies to look into the pharmacological potential of ursolic acid.ChemDraw ultra 8.0, AutoDock vina, and the Molecular Graphics Laboratory (MGL) tools were downloaded from Pyrx Virtual Screening Tools. Downloaded the Biovia Discovery studio visualizer at https://www.3dsbiovia.com/biovia-discovery.Swiss ADME was used to translate proteins into PDB format, whereas Chem 3D Pro 8.0 was used to translate ligand Mol files into PDB format. The chosen compound (UA) exhibits a good binding energy of -8.4 kcal/mol against anti-thrombotic activity (PDB ID: 1NFY). Additionally, the chosen compound (UA) has a better binding affinity of -9.5 kcal/mol against anti-inflammatory activity (PDB ID: 1CXY) and -9.0 kcal/mol against anti-cancer activity (PDB ID: 6GUE). However, the both the compounds produced better binding energy against targeted macro molecule with good ADMET Properties. All things considered, UA shows promise as a useful therapeutic agent for the prevention and treatment of a number of ailments, providing a potentially safer and more cost-effective substitute for traditional pharmaceuticals.

Keywords: Ursolic acid, Docking, Anti-Thrombolytic activity, Anti-Cancer activity, Anti-Inflammatory activity

Introduction

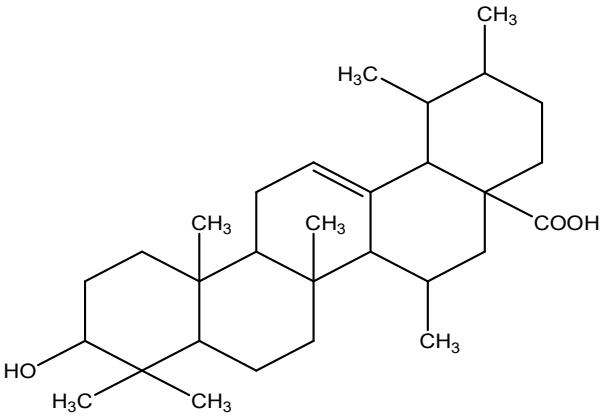
Inflammation is the complex biological response of vascular tissues to harmful stimuli, such as infections, injured cells, or irritants. It is the organism's protective attempt to eliminate the damaging stimuli and initiate the healing process of the tissue[1]. It does not mean to imply infection. It is erroneous to use the terms as synonyms, even in situations when infection causes inflammation: infection results from an external pathogen, whereas inflammation is the organism's reaction to the pathogen[2] . Chronic inflammation can develop if the underlying cause of the inflammation does not go away or if some of the control mechanisms meant to halt the process malfunction [3] . Prolonged inflammatory reactions can result in cell mutation and proliferation, which often creates an environment that is conducive to the development of cancer [4]. A group of diseases collectively referred to as cancers are typified by abnormal cell growth that can invade or spread to other body parts. Not all tumours are malignant; benign tumours do not spread to other parts of the body [5] .Symptoms may include a lump, abnormal bleeding, a long-lasting cough, unexplained weight loss, and altered bowel movements. These symptoms might be caused by cancer, but there might be other reasons. More than 100 distinct forms of cancer can affect people [6]. Tobacco use is linked to about 22% of cancer-related fatalities. An additional 10% can be attributed to obesity, inadequate nutrition, inactivity, and binge drinking. Some diseases, ionising radiation exposure, and exposure to contaminants in the environment are other concerns. Hepatitis B, hepatitis C, and human papilloma virus infections account for around 20% of cancer cases in underdeveloped nations. These elements work, at least in part, by altering a cell's genetic makeup. Usually, a great deal of genetic alteration is needed prior to cancer development. The Food and Drug Administration (FDA) has approved the combination of two targeted drugs, tucatinib (Tukysa) and trastuzumab (Herceptin), for patients with advanced colorectal cancer who overproduce the protein HER2. However, the medication has certain side effects, such as fatigue, diarrhoea, elevated liver enzymes, nausea, and vomiting. A liver issue is one of the more serious adverse effects. Inflammation of the vessel wall may also initiate thrombosis on an intact vein. Thrombolytics, also known as clot-busting drugs, are medications

used to dissolve blood clots in the blood vessels [7] . They work by activating a substance in the blood called plasmin, which helps break down the fibrin strands that hold the clot together. This process is crucial in restoring blood flow to organs and tissues affected by the clot [8] .There are several thrombolytic agents used in clinical practice, including alteplase (tPA), tenecteplase, and streptokinase. Each has its own characteristics, such as onset of action, duration of activity, and risk of side effects [9]. .Thrombolytic therapy is often accompanied by other treatments, such as antiplatelet drugs (e.g., aspirin) and anticoagulants, to prevent further clot formation and recurrence. Traditional plants are often perceived as better than synthetic drugs by some due to their perceived natural origin and historical use. They are believed to have fewer side effects and be gentler on the body. In 1920, apple epicuticular waxes were the source of ursolicic acid, a pentacyclic triterpenoid also referred to as urson, prunol, malol, or 3β-hydroxyurs-12-en-28-oic acid [10,11] . It is widely present in fruit peels, as well as in herbs and spices like thyme and rosemary, in a variety of plant species, including fruits, vegetables, and herbs, making it readily available for use in medicine [12,13]. In the end, the term CADD—computer-aided drug design—was approved to represent the use of computers in drug creation.10, 11 Advanced computational applications are considered to be effective tools. Prominent conquering is the use of these modules in conjunction with a specialized restraint that allows various computational programs to be worn to imitate drug-receptor exchanges in order to create fasting affinity. However, the methods' rejection, limited to the study of chemical connections, also necessitates findings. Due to its many uses, which include controlling digital compound libraries and designing compounds with specific physiochemical features. ADT is a programme set of automated docking tools that is intended to forecast the manner in which small molecules will attach to a target protein with a known three-dimensional structure [14,15]. AutoDock vina was used to ascertain the binding modes of those recently produced compounds in order to calculate their binding energies in the active sites [16] . With the Discovery Studio visualiser, the ligand's location within the enzyme binding site may also be seen. It might be helpful to understand the binding nature as well as to develop possible medication candidates [17].

Materials and Methods

The designed compound Ursolic Acid were afforded for prediction of anti-thrombotic, anti inflammatory and anti cancer activity of colon on crystal structure of PDB ID: INFY,1CX2 and

6GUE by molecular docking study [18]. This study extensively explored the in-silico activity of Ursolic acid.



LIGAND: UA

PDB : 1NFY **PDB : 6GUE** **PDB : 1CX2**

Figure 1: 3D crystal structure of macromolecules and the general structure of designed compounds.

Softwares required:

ChemDraw ultra 8.0, AutoDock vina, and the Molecular Graphics Laboratory (MGL) tools were downloaded from Pyrx Virtual Screening Tools. It was possible to download the Biovia Discovery studio visualizer at https://www.3dsbiovia.com/biovia-discovery. Swiss ADME was

used to translate proteins into PDB format, whereas Chem 3D Pro 8.0 was used to translate ligand Mol files into PDB format [19].

Methodology

One tool that is essential for comprehending the structure-activity relationship, binding energy, ligand-protein interaction, binding affinity, etc. is computer-aided drug design [20]. In order to assess the binding studies of our created chemical on the targeted enzyme, Auto dock was extensively utilised on this programme. The binding energy of the chosen molecule (UA) on the crystal structure of PDB IDs: INFY (Fig. 2), 1CX2 (Fig. 3), and 6GUE (Fig. 4) was obtained in 1971 at Brookhaven National Laboratory's Protein Data Bank (http://www.rcsb.org/pdp)[21,22,23].

Molecular Docking Studies:

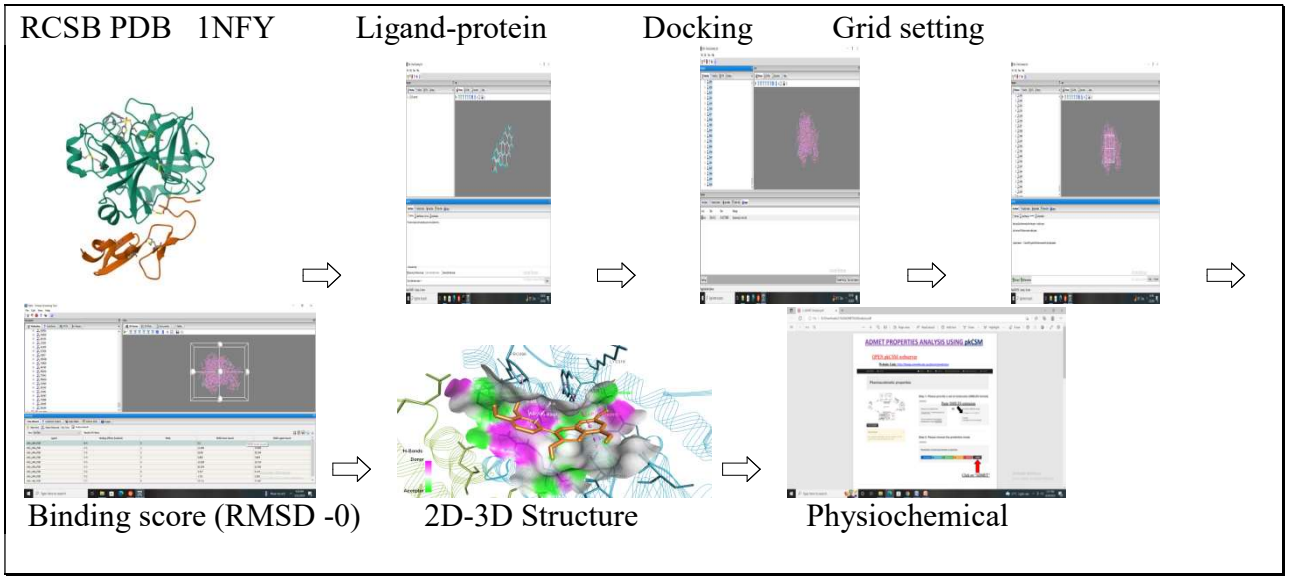


Fig.2: Protein Preparation & Docking method.

Preparation of Protein

The water molecules were taken out of the corresponding proteins prior to the molecular docking process beginning. The Swiss PDB viewer was used to further refine the chosen enzymes from the Protein Data Bank [24].

Preparation of Ligand

Chem draw (Cambridge, MA, USA) was used to sketch the ligand structures. Hydrogen atoms were added and water molecules were subtracted from the protein file (pdb) in order to further optimise it. After completing all of these steps, the ligand and protein were ready for docking [25].

Receptor Grid Generation

Receptor grid manufacture requires a "prepared" structure, which is an all-atom structure with the correct bond ordering and formal charges. AutoDock looks for advantageous contacts between a receptor molecule—typically a protein—and one or more ligand molecules. Multiple sets of fields are used to represent the receptor's shape and characteristics on a grid, allowing for increasingly precise scoring of the ligand poses. Selecting the location and size of the active site as it will be represented by receptor grids, setting up AutoDock limitations, and defining the receptor structure by excluding any potential co-crystallized ligand are all achievable with the tabs on the Receptor Grid Generation screen. Around the receptor's binding location, a grid region was created [26].

Docking validation

The ligand and active site crystal structures of a variety of enzymes, including the crystal structures of [PDB ID: INFY (Fig. 5), 1CX2 (Fig. 6), and 6GUE (Fig. 7)] underwent Auto Dock Vina testing in order to determine the binding energies. For the investigations, the genetic algorithm (GA) was used with its default parameters, and the docking grid box was adjusted to be about above 90. The number of runs and the other options in the search parameter were left at their default values [27]. The output displayed the docking calculations' results in word format. By choosing the ligand conformation that displayed the lowest binding energy (optimal pose), the conformation of the ligand docking data was determined. Using AutoDock techniques, the location and orientation of ligands within protein receptors as well as their interactions with amino acids that bind to the ligand were examined and depicted. Following the energy minimization, the top 10 conformations for each compound were simulated during the docking procedure [28].

Predicting bioactivity, drug likeness, and ADMET properties

Highly developed chemistry software, such as ChemDraw Ultra 8.0, ACD/ChemSketch version 12.0, and SMILES (simplified molecular-key procession access system), was used to drain the molecular configuration. Notation records were created and fed into intensively scheduled software to estimate the constraint. In silico research has proven to be a crucial tool for both lead optimization and drug innovation, helping to resolve the composite's action inside the host.. The cheminformatics server (http://www.molinspiration.com) was used to obtain the molecular descriptors, drug similarity features, and sulphonamides of quinazolin-4-one. The service provided the results of Lipinski's Rule 5 (RO-5). The pharmacokinetic property computation of ligands, or ADMET, is required to enable, scrutinize, and launch the efficacy in the host system. Additionally, the legacy of the ligand must be purposeful, with the formation of AdmetSAR. (http://lmmd.ecust.edu.cn/admetsar2/.[29]

Results

The order in which the molecules were created was determined by the results reported in the literature regarding the energetic binding locations of particular macromolecules. This work aims to investigate the structural characteristics that contribute to an efficient receptor-parameter interface. The well-known characteristics of H-bonding and π-stacking exchanges that have been documented in the literature are vital and expert in the binding of inhibitors to the active site of specific enzyme inhibitors. . With a few exceptions, all of the compounds exhibit hydrogen bond contact between the amino acid residues engaging within the newly created quinazolin-4-one derivatives, with many of them exhibiting Vanderwall attraction among odd amino acid residues into the necessary area. All of the chosen quinazolinones were found to exhibit outstanding fastening properties, such as an increased H-bond and vanderwalls' affinity for an essential amino acid. While pialkyl and pi-sigma interactions were analyzed, the majority of the active moiety was that which displayed hydrogen bond interactions over the enzyme. (UA) listed in Fig 1. The docking poses were identified based on their corresponding binding pockets and docking properties. Understanding the binding interactions with the targeted enzyme should be aided by these studies. .

Docking Analysis

In table.1, the docking score and interactions of synthesized compound were mentioned respectively on [PDB ID : INFY,1CX2 and 6GUE] . The results afforded excellent binding score were mentioned in the table.1.

One of the most widely used docking software tools, auto dock vina, was used to anticipate each ligand's binding energy against the intended macromolecule. This resulted in eight different binding poses. The ligand's binding affinity was then determined by taking the binding position with the highest binding energy that yielded an RMSD value of zero.

The selected compound (UA) against anti thrombotic activity (PDB ID:1NFY) (-8.4 kcal/mol) exist the good binding energy and also the selected compounds(UA) against anti inflammatory activity (PDB ID: 1CXY) possess better binding affinity of -9.5kcal/mol as well as against anti cancer activity (PDB ID: 6GUE) exist the binding affinity -9.0kcal/mol. however, the both the compounds produced better binding energy against targeted macro molecule.

According to the amino acid residue interaction, all compounds showed hydrogen bond interactions, and some of them also showed van der Waals attraction in different amino acid residues at the binding site.Because there are more hydrogen bonds and vanderwalls at the amino acid binding site, universal compounds were shown to have nearly greater binding affinity and interaction. Despite pialkyl and pi-sigma interactions being examined, hydrogen bond interactions dominate the most active molecule's interactions with the enzyme.

Molecular Interaction Studies

Drug likeliness, Bio activity and ADMET evaluation

In this case, the development of pharmaceuticals and ADME (Absorption, Distribution, Metabolism, Elimination) characteristics play a crucial role in the outcome, as they might eliminate potential competitors. Diminished characteristics could potentially lead to the release of molecules into targets, and toxicity is another crucial factor that frequently overshadows ADME assessments. In addition to recently elected candidates' calculations of the drug's molecular properties and bioactivity using cheminformatics software and the admetSAR databases' prophecy of ADMET belongings, Lipinski's rule is useful in evaluating the bioavailability of oral medications.

The selected compounds meet Veber's criteria by having TPSA of no more than 14 and rotatable bonds of fewer than 10. Additionally, it suggests that some chemicals might absorb well through the mouth. All of the novel quinazolin-4-one drug similarity features are shown in Table 1. Good intestinal absorption is indicated by a human intestinal absorption value of 0.9 or above. The

purpose of the AMES toxicity test evaluation was to determine whether or not the drugs are mutagenic. All of the designed compounds (UA) that were influenced by negative values did not exhibit mutagenicity or carcinogenicity, and they also showed lower oral acute toxicity (LD50), which is the dose-to-origin of 50% of the trial population.. Additionally, it was discovered to have a somewhat higher range, be deemed safe, and match the LD50 series shown in Table 3.Table 4 presents an analysis of the bioactivity value of the developed quinazolin-4-one derivatives, taking into account their roles as GPCR (G-protein coupled receptor), nuclear receptor ligand, ion-channel modulator, kinase inhibitor, protease inhibitor, and enzyme inhibitor. It is generally believed that a moiety exhibiting a bioactivity rating greater than 0.00 would demonstrate strong biological activity. When considering enzyme embarrassment in relation to additional pathways, the bioactivity assessment is 0.0.32 molecules have an enzyme inhibition bioactivity rating of more than 0.00, meaning that the appropriate system can measure their significant biological activity. The bioactivity of the newly created compounds ranges from 0.03 to 0.51. These outcomes support the rationale of the designed compounds against targets.

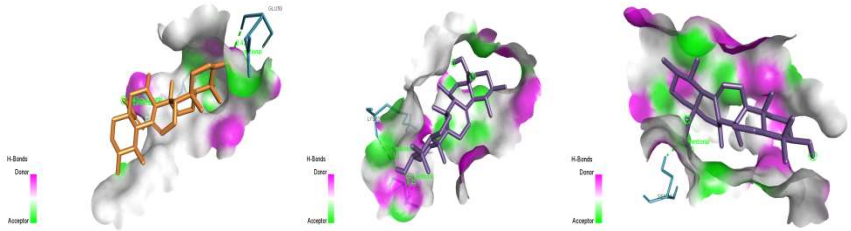
Table: 1.Properties and toxicity prediction of Ligands

Comp. Code UA	**Physiochemical properties**	MW	Logp	HBA	HBD	TPSA	nRB	No of violation
		456.71	6.95	2	2	57.53	1	1
Comp. Code UA	**ADMET Properties**	HIA	B.B.B	AMES toxicity	Carcino-genicity	LD 50-in rat (mol/kg)	-	-
		0.9972	0.7500	Non – toxic	Non-carcinogenic	3.348	-	-
Comp. Code UA	**Bioactivity Prediction**	GPCR Ligand	Ion Channel Modulator	Kinase Inhibitor	Nuclear Receptor Ligand	Protease Inhibitor	Enzyme Inhibitor	-
		0.23	0.03	-0.41	0.63	0.13	0.52	-
Comp. Code UA	**Binding affinity**	1NFY	1CX2	6GUE				
		-8.4 (kcal/mol)	-9.5 (kcal/mol)	-9.0 (kcal/mol)				

Table 2 Docking result and various interaction of tested compounds on targeted proteins

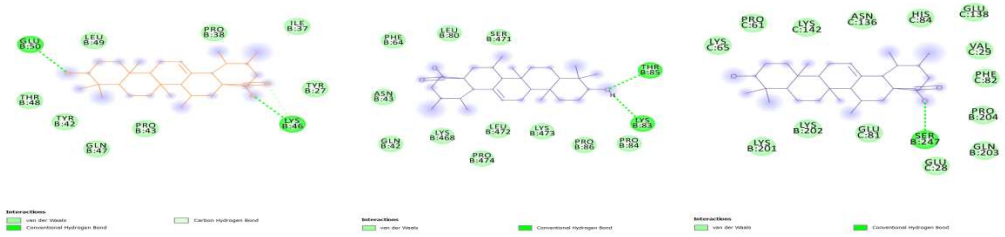
Comp code		Vander walls	H.bond
UA	1NFY	PRO B:38,43 TYR B:27,42 GLN B:47 THR B:48 ILE B:37 LEU B:49	GLU B:50 LYS B:46
	1CX2	PRO B:84,86,474 LEU B:80,472 LYS B:473,468 PHE B:64,SER B:471,GLN B:42,ASN B:43	LYS B:83 THR B:85
	6GUE	PHE B:64,LEU B:80,472,SER B:471,ASN B:43,GLN B:42,LYS B:468,473,PRO B:86,84,474	THR B:85,83

3D Structure of the binding poses ligand molecular intention with Macromolecules



PDB: 1NFY **PDB: 1CX2** **PDB: 6GUE**

2D Structure of the selected ligand molecular intention with Macromolecules



PDB: 1NFY **PDB: 1CX2** **PDB: 6GUE**

Discussion

In Docking studies, the best binding energy ranges from (-6.57 to -11.11 kcal/mol), average binding energy ranges from (-3.84 to – 7.41 kcal/mol) and poor binding energy ranges from (0.19 to 4.98 kcal/mol). So, based on this, Ursolic acid exhibits good binding energy (-8.4, -9.00 & -9.4 kcal/mol).

The selected compound (UA) against anti thrombotic activity (PDB ID:1NFY) (-8.4 kcal/mol) exist the good binding energy and also the selected compounds(UA) against anti inflammatory activity (PDB ID: 1CXY) possess better binding affinity of -9.5kcal/mol as well as against anti cancer activity (PDB ID: 6GUE) exist the binding affinity -9.0kcal/mol. however, the both the compounds produced better binding energy against targeted macro molecule. the selected ligands no violation with good drug likeness features also furthermore between entire chosen quinazolin-4(3H)-one candidates gone more the molecules have creditable HIA-(human intestinal absorption) and B.B.B-(blood brain barrier) among no carcinogenicity and AMES negative, as well as good bioavailability.

According to the amino acid residue interaction, all compounds showed hydrogen bond interactions, and some of them also showed van der Waals attraction in different amino acid residues at the binding site.Because there are more hydrogen bonds and vander walls at the amino acid binding site, universal compounds were shown to have nearly greater binding affinity and interaction. Despite pialkyl and pi-sigma interactions being examined, hydrogen bond interactions dominate the most active molecule's interactions with the enzyme present in table 2 Ursolic acid exhibits good binding energy, so the compound can compete better against other molecules to bind with its target. Higher negative binding energy means better stability of the complex.

Conclusion

Ursolic acid represents a promising natural compound with diverse pharmacological properties and therapeutic potential. Continued research efforts aimed at elucidating its molecular mechanisms of action and exploring its efficacy in various disease models are warranted. Moreover, further clinical studies are needed to validate its therapeutic benefits and establish its safety profile in humans.the selected drug candidates and ADMET characteristics complied with the "Lipinski rule of five." Overall, UA holds promise as a valuable therapeutic agent for the prevention

and treatment of various diseases, offering a potentially safer and more affordable alternative to conventional medications.

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Conflict of Interests

The authors declare that they have no conflict of interest.

ABBREVIATIONS:

ADMET: Absorption, Distribution, Metabolism, Toxicity; PDB: Protein data bank; HBA:hydrogen bond acceptor; HBD: hydrogen bond donor; HIA: human intestinal absorption; GPCR: G-Protein coupled receptor; B.B.B: Blood brain barrier; TPSA:Topical polar surface area; 3D:Three dimensional; RMSD: Root Mean Square Deviation.,UA-Urosolic acid.

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