IJPSR (2023), Volume 14, Issue 5 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 07 September 2022; received in revised form, 20 October 2022; accepted 17 November 2022; published 01 May 2023

MOLECULAR PROPERTY AND TOXICITY PREDICTIONS AND EVALUATION OF ANTI-ARTHRITIC AND ANALGESIC ACTIVITIES OF L-ASCORBIC ACID DERIVATIVES USING *IN-SILICO* MODELS

M. S. Priya [1], N. Deepak Venkataraman [*2], I. Sheik Nasar [2], R. Velmurugan [1] and T. Purushoth Prabhu [3]

Department of Pharmaceutical Chemistry [1], Saveetha College of Pharmacy, Saveetha Institute of Medical and Technical Sciences, Chennai - 602015, Tamil Nadu, India.
Department of Pharmacology [2], East Point College of Pharmacy, East Point Group of Institutions, Bangalore - 560049, Karnataka, India.
Department of Pharmacognosy [3], C. L. Baid Metha College of Pharmacy, Chennai - 600097, Tamil Nadu, India.

Keywords:

Docking, Rheumatoid arthritis, Molecular property prediction, Toxicity prediction, L-Ascorbic acid derivatives, *In-silico* models

Correspondence to Author:
N. Deepak Venkataraman

Associate Professor,
Department of Pharmacology,
East Point College of Pharmacy, East Point Group of Institutions, Bangalore - 560049, Karnataka, India.

E-mail: deepakvenkataraman.n@gmail.com

ABSTRACT: Docking has become an indispensable method for predicting a molecule's efficacy and toxicity. This technique is of great value in reducing the pain and suffering caused due to toxic drugs when tested in animals. Rheumatoid arthritis and the pain associated with the condition is a curse to mankind. In this research, the molecular property and toxicity predictions and evaluation of anti-arthritic and analgesic activities of L-Ascorbic acid derivatives were carried out using *in-silico* models. The molecular property and toxicity were predicted using the software called as OSIRIS. Molecular properties of compounds like ClogP, solubility, molecular weight and drug-likeness were evaluated. Toxicity parameters like mutagenicity, tumorigenicity, irritancy and reproductive effect were also examined. The anti-arthritic and analgesic activities were analyzed using CDOCKER. All the designed 10 molecules were docked with Hase enzyme for evaluation of anti-arthritic activity and opioid receptor for evaluation of analgesic activity. The results showed favourable ClogP, solubility, molecular weight, and drug-likeness values and excellent dock scores with toxicity profiles except for C4 and C6. Compound C10 showed exceptional results with respect to anti-arthritic and analgesic activity. Further L- Ascorbic acid derivatives can be synthesized and *in-vitro* and pre-clinical studies can be carried out to establish their efficacy in Rheumatoid arthritis and pain.

INTRODUCTION: Molecular docking is defined as an optimization problem, which would define the "best-fit" orientation of a ligand that actually binds to a particular protein. During the whole process, ligand and protein tend to adjust their conformation in order to achieve a complete "best-fit".

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DOI: 10.13040/IJPSR.0975-8232.14(5).2547-54

This article can be accessed online on www.ijpsr.com

DOI link: http://doi.org/10.13040/IJPSR.0975-8232.14(5).2547-54

This sort of adjustment of conformation, which results in the wholesome binding, is called "induced fit" [1].

Aim of Docking [2]: To accomplish an optimized orientation for the protein and the ligand.

To attain relative orientation among the protein and the ligand so that free energy concerned with the complete system is minimized to a maximum.

Docking Approaches:

- Shape complementarity
- Simulation

Shape Complementarity [2]: Shape complementarity/ Geometric matching method designates the protein and ligand like a set of structures (molecular surface/ complementarity surface) that will make them easily dockable. These descriptions may incorporate the molecular surface/ descriptors of complementary surface. In this scenario, the molecular surface of receptor is explained in terms of the solvent-accessible surface area of it, and the molecular surface of ligand is explained in the aspect of the matching surface description. The complementarity among the surfaces of the two leads to the shape matching description which may help to find the complementary position for docking the target and the ligand molecule.

Simulation [2]: In this approach, protein and ligand are divided by some substantial distance. After certain moves, ligand discovers its position inside the protein active site and increases the energy of the system. After every move, the whole energy of the structure is calculated.

Mechanics of Docking [2]: To perform a docking, the first requirement represents the structure of the protein of interest. The structure has been determined using biophysical techniques like

- ✓ X-ray crystallography
- ✓ NMR spectroscopy

Scoring Function [2]: The scoring function takes a posture as input and provides a number specifying the probability that the posture represents an advantageous binding interaction. Most scoring functions are physics-based molecular mechanics force fields that estimate the energy of the pose; a limited (negative) energy indicates a stable system and, thus a likely binding interaction.

Another approach is to develop a statistical model for interactions from an extensive databank of protein-ligand complexes, such as the Protein database, and scrutinise the best fit of the posture as per the inferred potential.

There are a tremendous number of structures from X-ray crystallography for complexes between proteins and high-affinity ligands. Still, comparatively fewer for low-affinity ligands as the later complexes tend to be less stable and, therefore, more difficult to crystallize.

Applications [2]:

Hit Determination: Docking combined with a score function that can be utilised to screen large data of potential entities rapidly. *In-silico* to determine molecules that are most probably to bind to the target of interest (a protein molecule).

Lead Optimization: Docking could be utilized to ascertain where and which relative orientation of a ligand binds to the concerned protein under study. This information may, in turn, be utilized to plan more potent and choosy analogues.

Computational Protein-ligand Techniques [2]: Docking of a ligand inside a receptor is a geometric search program. Most docking approaches keep the receptor rigid and the ligand flexible during docking. Some of the available protein-ligand docking software for structural-based virtual screening are

Glide, Accelrys Discovery Studio, AutoDock, Flex, Fred, Ligand Fit, and Gold

Discovery Studio Overview [2]: Discovery Studio is a software suite of life science molecular design solutions for computational chemists and computational biologists to help reduce the time and cost involved in the discovery and advancement of innovative, fresh drugs, therapies, and other biologically active substances. This will comprise a wide range of informatics capabilities and modelling tools to explore and characterize targets (protein molecule) and design new drugs. It is a powerful research platform for the life science disciplines that integrates a comprehensive suite of informatics, modelling, and simulation solutions and provides access to them on standard desktop personal computers. These tools address specific research problems encountered by scientists working across the whole discovery process.

Discovery Studio Applications [2]:

DS Gene: A streamlined desktop tool for accessing, manipulating, and analyzing DNA and sequences of protein. DS Gene provides an intuitive and flexible interface for examining various types of data, including DNA, protein, and 3D molecular representations.

DS Viewer Pro: An easy-to-use molecular visualization application that allows researchers to

develop and interact with high-quality 3D models of chemical structures on standard Windows PCs. DS Viewer Pro renders sophisticated images that previously were only available through expensive Unix workstations.

DS MedChem Explorer: Enable chemists to design active compounds for prescreening synthesis candidates faster using an *in-silico* approach. It provides enterprise-wide access to pharmacophore modelling, conformation analysis, property calculation, ADME prediction, and more.

DS Project KM: The backbone of Discovery Studio's knowledge-centric system, DS Project KM is a groupware infrastructure that integrates applications and enriches collaboration between research team members from biology to late-stage lead optimization.

Docking Procedure using Accelrys Discovery Studio [3, 4]:

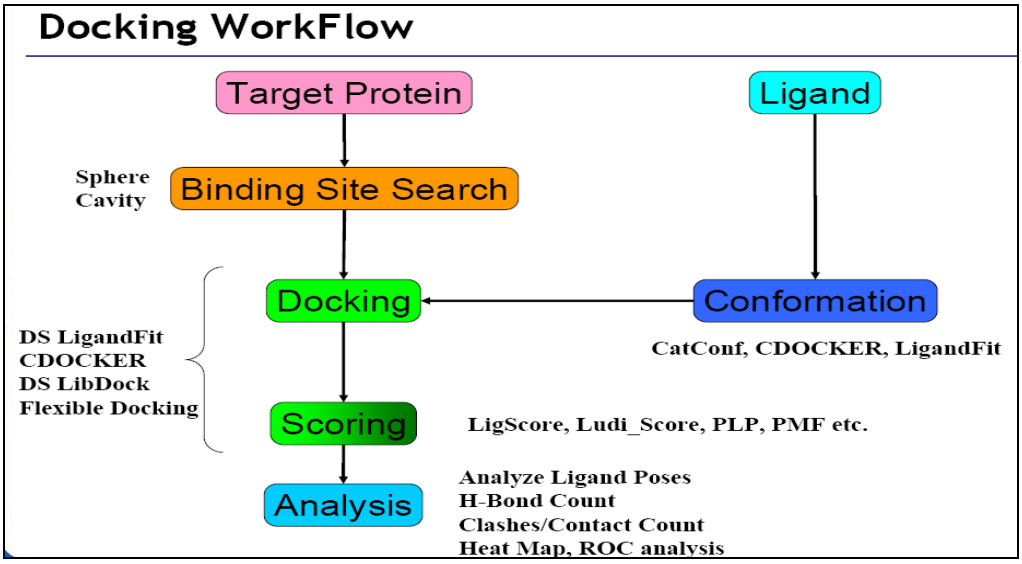


FIG. 1: STEPS INVOLVED IN DOCKING

OSIRIS - Molecular Property Prediction: The OSIRIS Property Explorer is used to draw chemical entities and determines on the fly several drug-relevant characteristics (solubility, CLogP, Molecular Weight, Overall Drug Score, Assessment of toxicity risk *etc*.) when a structure is effective. Prediction interpretations are respected and colour-coded. Properties with high risks of undesired effects like mutagen city or poor intestinal absorption, are shown in red. Whereas a green colour indicates drug-conform behaviour.

Mutagenicity: The information related to genetics should be similar in almost all cells of living things. But, mutation may occur in genetic data affecting a cell or living being to be dissimilar from others. Mutagenicity may lead to abnormalities of the future generations.

Teratogenicity: Teratogenicity can cause birth-death, abnormalities, death or developmental delays.

Reproductive Effect: Reproductive toxins could cause sterility, reduction in fertility or further deleterious reproductive effects.

Tumorigenicity: Carcinogens are recognized by their capacity to cause cancer in exposed labors, other people or assessment animals. Numerous occupational cancers have a extensive latency period, implying that cancer may mature in 10-20 yrs. / longer afterwards explosive to the carcinogen. Primary irritant dermatitis is produced by chemicals that directly cause skin irritation.

Toxicity Risk: Toxicity risk signals are a sign that the structure which is drawn may be harmful in connection with the risk category mentioned. However, risk signals are not meant to be a fully trustable toxicity prediction, nor must be decided from the lack of risk signals that a specific substance is absolutely free of any harmful effect.

Hydrophilicity: The log P of a chemical compound that is the logarithm of its partition coefficient among octanol and water Log ($C_{octanol}$ / C_{water}) is a successfully-established measurement of the hydrophilicity of the compound. Low hydrophilicity and elevated Log P values will lead to poor absorption or permeation. It has been observed that compounds to possess a rational probability of being well absorbed based on the log P values which should not be more than 5.0.

Solubility: Aqueous medium's soluble compounds considerably affect their absorption and distribution properties. Characteristically, little solubility goes beside a bad absorption property, and hence the broad aim is to escape poorly soluble compounds.

Molecular Weights: Optimizing compounds for elevated activity on biological targets often nearly goes beyond enhanced molecular weights. Yet, compounds with increased weights are less expected to be absorbed and hence to always attain the place of work.

Drug Likeness: *In-silico* prediction of drug-like properties is a boon for pharmaceutical industries to invest in billion blockbuster drugs it serves to identify compounds suitable for drug development and production capacity.

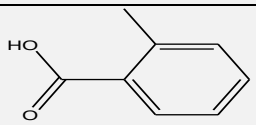
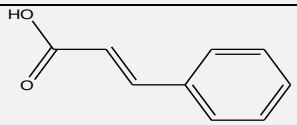
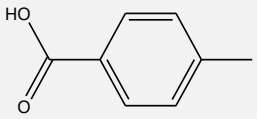
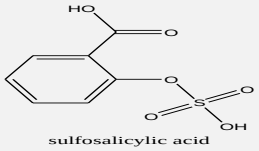
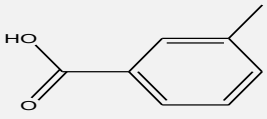
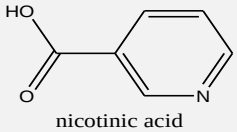
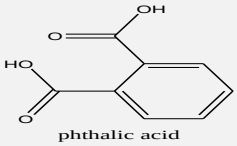
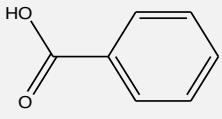
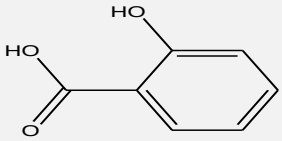
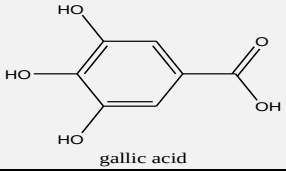
The computer programmer OSIRIS is used to predict mutagenicity, irritancy, and reproductive effect. This programmer gives overall drug score values to consider the compound as a drug. It is used to screen the virtual compound library to select compounds most likely to have high binding affinities represented as drug scores. Drug likeness is partly built on topological descriptors, fingerprints of MDL configuration keys or additional properties like molecular weights and C log P.

Drug Score: The drug score pools drug-likeness, C log P, molecular weight, log S, and toxicity risks in one convenient value rather than might be utilized to determine the compound's total potential to succeed as a drug. A drug score of 1.0, 0.8 and 0.6 indicates no, medium, and high risk, respectively.

Software Programmes: Several software programs are present for character explorer, including INSILCOFIRST, TOXTREE, and TOPKAT.

MATERIALS AND METHODS:

TABLE 1: COMPOUNDS DESIGNED AND THEIR CHEMICAL STRUCTURES

Compound code	Chemical structure	Compound code	Chemical structure
C1	 o-toluic acid	C6	 cinnamic acid
C2	 p-toluic acid	C7	 sulfosalicylic acid
C3	 m-toluic acid	C8	 nicotinic acid
C4	 phthalic acid	C9	 benzoic acid
C5	 salicylic acid	C10	 gallic acid

Docking using CDOCKER [5]: CDOCKER is an application of a CHAR Mm inspired docking tool utilizing a rigid receptor. The following steps are included in the CDOCKER protocol **Fig. 1.**

Step 1: A set of ligand conformations are generated using high-temperature molecular dynamics with numerous random seeds. The stage can only be skipped to dock the input conformation(s).

Step 2: Arbitrary orientations of the conformations are produced by translating the center of the ligand to a specified location inside the receptor active site, and performing a series of random rotations. Softened energy is calculated and the orientation is kept if the energy is less than a specified threshold. This process continues until either the desired number of low-energy orientations is found or the maximum number of bad orientations has been tried. This stage can be skipped to use the input orientation only.

Step 3: Every orientation is exposed to simulated annealing molecular dynamics. The temperature is ignited up to a high temperature, after which cooled to target temperature.

Step 4: A ligand is minimized to a maximum extent in the rigid receptor by using non-softened potential is done.

Step 5: For every final posture, the CHARMm energy (ligand strain plus interaction energy) and the energy of interaction are only calculated. The postures are arranged by CHARMm energy, and top-scoring (most negative, thus favourable to binding) poses are kept unchanged.

C-Docker Steps: Define the receptor and search for binding sites; prepare and run the dock ligands (c-docker) protocol.
Procedure: Open the receptor protein and apply the CHARMM force field. Define the selected molecule as a receptor, then select the ligand to define the sphere from the selection. Open the C-docker protocol and set the parameters; run the protocol.

DockScore: Candidate ligand postures are assessed and prioritized per the Dock Score function. There are two varieties of DockScore. One is founded on a force field estimate, and the other is based on the PLP, which is Piecewise Linear Potential function.

Equation 1:

DockScore (force field) = - (ligant / receptor interaction energy + ligant internal energy)

Equation 2:

DockScore (PLP) = - (PLP potential)

There are two energy terms in the force field version of DockScore, the interaction energy and internal energy of the ligand with the receptor. The interaction energy is considered as the totality of the electrostatic energy and the van der Waals energy. The calculation of the energy of interaction can be time-consuming. A grid-based approximation of the ligand/receptor energy of interaction is engaged to decrease the time required for this computation. The energy of the ligand's internal environment is calculated using the force field version of DockScore. The reason for including the energy of the internal environment is to evade ligand conformation with worse interior non-bond clashes. The PLP form of DockScore utilises the PLP1 role due to the functional form of PLP1 permits it to be easily denoted with a grid-based method.

Molecular Property and Toxicity Prediction [4]:
Prediction of Molecular Properties:
Step 1: The prediction process relies on a precompiled structure fragment that raises toxicity alerts in case they are encountered in the structure currently drawn.

Step 2: This section (fragment) lists were formed by thoroughly shredding all the compounds in the databank recognized to be active in a definite toxicity class. Shredding any molecule as the very first cut at each and every rotatable bond results in a set of central fragments.

Step 3: These, in turn, were utilized to rebuild all possible larger fragments being a substructure of the original molecule.

Step 4: Later, a substructure exploration procedure determined the incidence frequency of any fragment inside all chemical compounds of the concerned toxicity class.

RESULTS AND DISCUSSION:

Docking Studies of Anti-arthritic Activity with Hyaluronidase Enzyme:

TABLE 2: DOCKING SCORES OF ANTI-ARTHRITIC ACTIVITY

Sl. no.	Protein Code – 1 loh (Hyaluronidase)		
	Compound code	Dock score	Ligand internal energy
1	C1	-19.768	45.63
2	C2	-26.5804	53.50
3	C3	-29.304	46.66
4	C4	-30.813	56.15
5	C5	-24.144	47.30
6	C6	-9.991	42.46
7	C7	-33.9841	59.26
8	C8	-17.912	41.56
9	C9	-24.397	44.80
10	C10	-47.146	57.24

TABLE 3: MOST/LEAST ACTIVE INHIBITOR MOLECULES HYDROGEN BOND AND ACTIVE SITE AMINO ACIDS

Active	Compound code	Hydrogen bond distance	Active site amino acids
Most	C10	1	TRP 292
		2	ASN 580
		3	ARG 300
		4	ASP 293
		5	ASN 290
		6	ARG 243
		7	HIS 399
Least	C6	1	ARG 300

Docking Studies of Analgesic Activity with mu Receptor

TABLE 4: DOCKING SCORES OF ANALGESIC ACTIVITY

Sl. no.	PROTEIN CODE – 4DKL (MU Receptor)		
	Compound code	Dock score	Ligand internal energy
1	C1	-21.39	49.27
2	C2	-29.91	47.18
3	C3	-29.40	44.87
4	C4	-29.13	57.97
5	C5	-22.75	44.44
6	C6	-17.384	42.68
7	C7	-28.59	49.34
8	C8	-22.41	47.28
9	C9	-24.79	46.98
10	C10	-46.12	55.45

TABLE 5: MOST/LEAST ACTIVE INHIBITOR MOLECULES

Active	Compound Code
Most	C10
Least	C6

TABLE 6: MOLECULAR PROPERTIES OF COMPOUNDS BY OSIRIS

Compd.	ClogP	Solubility	Molecular weight (gm)	Drug Likeness	Drug score
C1	3.24	-4.45	440	0.54	0.47
C2	3.24	-4.45	440	0.26	0.5
C3	3.24	-4.45	440	-1.15	0.39
C4	1.65	-3.79	500	-6.61	0.12
C5	2.01	-3.71	444	0.44	0.61
C6	2.86	-4.51	464	-2.86	0.2
C7	-2.8	-0.39	604	-2.03	0.34
C8	0.45	-2.18	414	2.65	0.81
C9	2.61	-3.77	412	1.2	0.65
C10	0.81	-1.99	508	1.93	0.67

TABLE 7: TOXICITY PREDICTION BY OSIRIS

Compd.	Mutagenicity	Tumorigenicity	Irritancy	Reproductive effect
C1	Green	Green	Green	Green
C2	Green	Green	Green	Green
C3	Green	Green	Green	Green
C4	Green	Green	Red	Red
C5	Green	Green	Green	Green
C6	Green	Green	Red	Green
C7	Green	Green	Green	Green
C8	Green	Green	Green	Green
C9	Green	Green	Green	Green
C10	Green	Green	Green	Green

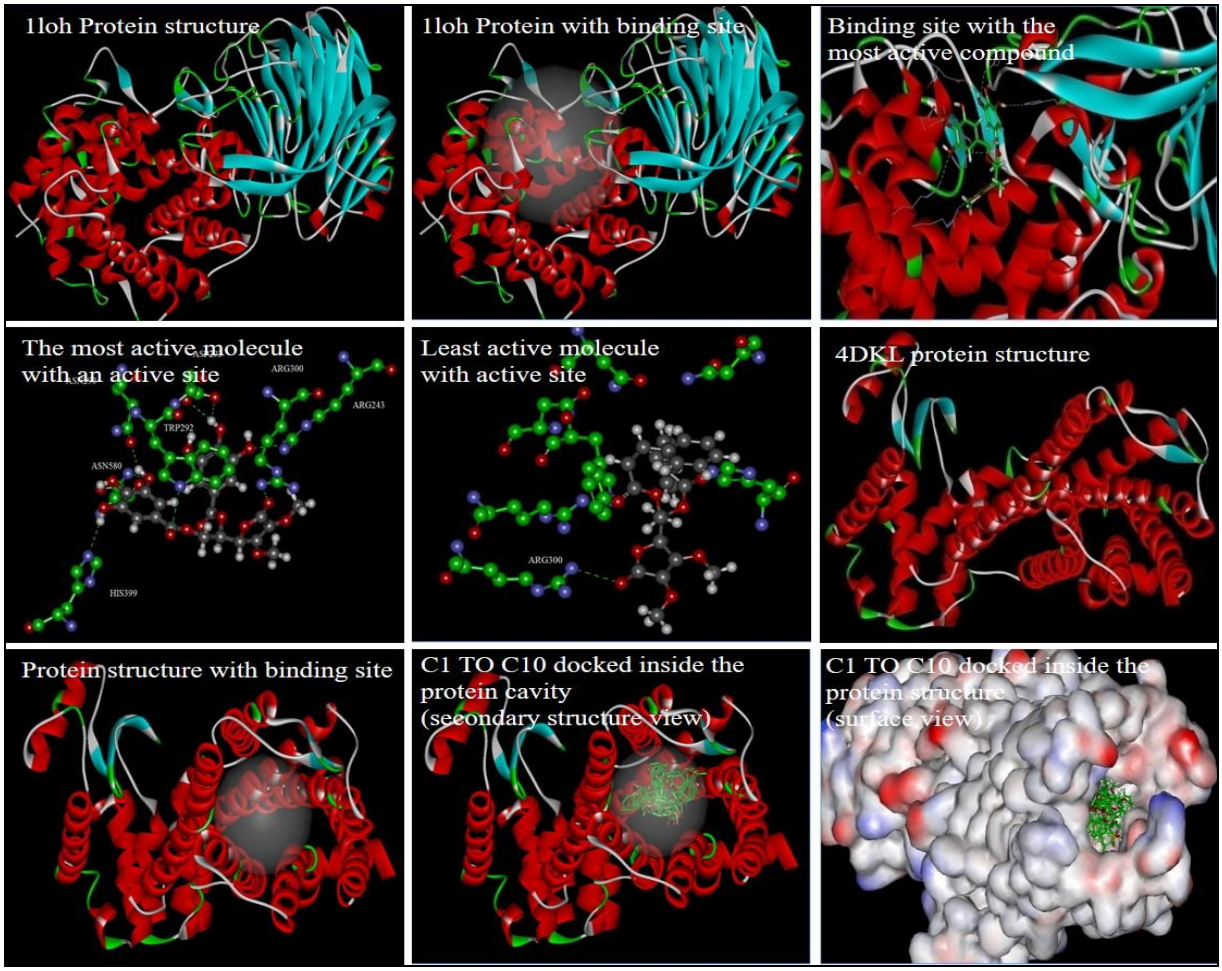


FIG. 2: DOCKING ANALYSIS

The compounds (C1-C10) in **Table 1** were subjected to virtual screening analysis, including drug-likeness screening and molecular docking approaches (**Fig. 2.**

All 10 molecules were docked as per CDOCKER docking method.

The 2 different proteins used are as follows,

- ✓ Hase enzyme for anti-arthritic activity.
- ✓ Opioid receptor for analgesic activity.

Hyaluronidase Enzyme: All the test molecules are successfully docked with this protein for antiarthritic activity by CDOCKER. The molecule C10, which is substituted with gallic acid in the 5th position and 6th position of the ring, showed a low dock score i.e. -47.146. The lowest dock score indicates the highest activity. So, it was concluded that C10 showed the highest anti-arthritic activity **Table 2**. The ascending order of the dock score of all the molecules is as follows,

C10>C7>C4>C3>C2>C9>C5>C1>C8>C6

The most active molecule C10 containing 7 hydrogen bonds with 7 amino acids present in the active site was compared to the least effective molecule C6 containing only 1 hydrogen bond. It indicates the binding capability of the ligand molecule is better when more hydrogen bonds with active site amino acids are present in **Table 3.**

Opioid Receptor: All the test molecules are successfully docked with this protein for analgesic activity by CDOCKER. The molecule C10 showed the lowest dock score *i.e.*, -46.124 compared to other test molecules. The lowest dock score indicates the highest analgesic activity, so it was concluded that C10 showed the highest analgesic activity **Table 4**. The ascending order of dock scores of all the molecules is as follows,

C10>C2>C3>C4>C7>C9>C5>C8>C1>C6

In general, analgesic activity is increased in the presence of an OH group. The compound C10 is having 6 OH groups and hence C10 shows a better docking score with the specific binding site **Table 5.** The molecular properties of the compounds (C1-C10) were within normal limits specified **Table 6.** All the compounds were free from toxicity risk (exhibited green colour) except C4 and C6, which showed toxicity (exhibited red colour) concerning irritancy and reproductive effect, respectively, which was confirmed by drug-likeness behaviour **Table 7.**

CONCLUSION: The designed molecules were found to show good drug-likeness, good log Pa value, and excellent dock scores with on toxicity profile except for C4 and C6 before the wet lab. As already stated *in-silico* studies cannot be entirely relied upon for toxicity or lack of toxicity properties but help to identify potentially poisonous drugs before carrying out pre-clinical studies.

The *in-silico* studies also showed promising analgesic and anti-arthritic properties of L-Ascorbic acid derivatives. Further L- Ascorbic acid derivatives can be synthesized, and *in-vitro* and pre-clinical studies can be carried out to establish their efficacy in Rheumatoid arthritis and pain.

ACKNOWLEDGEMENT: I would like to register my sincere gratitude to my Principal and Head of the Department of Pharmaceutical chemistry at Saveetha College of Pharmacy, Chennai- 600097.

CONFLICTS OF INTEREST: Nil

REFERENCES:

1. Ahmad Farouk Eweas, Amr Ali Alhossary and Ahmed S: Abdel-Moneim: Molecular Docking Reveals Ivermectin and Remdesivir as Potential Repurposed Drugs Against SARS-CoV-2. Front Microbiol 2021; 11: 592908.
2. Fons N: Focus: Drug Development: Textbook of Drug Design and Discovery, CRC Press (Taylor & Francis Group). Fifth Edition 2017.
3. Bender BJ, Gahbauer S and Luttens A: A practical guide to large-scale docking. Nat Protoc 2021; 16: 4799–4832.
4. Pedro HM, Torres, Ana CR, Sodero, Paula Jofily and Floriano P: Silva-J, Key Topics in Molecular Docking for Drug Design. Int J Mol Sci 2019; 20 (18): 4574.
5. Shuai Wang, Jun-Hao Jiang, Ruo-Yu Li and Ping Deng: Docking-based virtual screening of TβR1 inhibitors: evaluation of pose prediction and scoring functions. BMC Chem 2020; 14(1): 52.

How to cite this article:
Priya MS, Venkataraman ND, Nasar IS, Velmurugan R and Prabhu TP: Molecular property and toxicity predictions and evaluation of anti-arthritic and analgesic activities of l-ascorbic acid derivatives using *in-silico* models. Int J Pharm Sci & Res 2023; 14(5): 2547-54. doi: 10.13040/IJPSR.0975-8232.14(5).2547-54.