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Virtual screening using the ligand ZINC database for novel lipoxygenase-3 inhibitors

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Abstract:
The leukotrienes constitute a group of arachidonic acid-derived compounds with biologic activities suggesting important roles in inflammation and immediate hypersensitivity. Epidermis-type lipoxygenase-3 (ALOXE3), a distinct subclass within the multigene family of mammalian lipoxygenases, is a novel isoenzyme involved in the metabolism of leukotrienes and plays a very important role in skin barrier functions. Lipoxygenase selective inhibitors such as azelastine and zileuton are currently used to reduce inflammatory response. Nausea, pharyngolaryngeal pain, headache, nasal burning and somnolence are the most frequently reported adverse effects of these drugs. Therefore, there is still a need to develop more potent lipoxygenase inhibitors. In this paper, we report the screening of various compounds from the ZINC database (contains over 21 million compounds) using the Molegro Virtual Docker software against the ALOXE3 protein. Screening was performed using molecular constraints tool to filter compounds with physico-chemical properties similar to the 1N8Q bound ligand protocatechuic acid. The analysis resulted in 4319 Lipinski compliant hits which are docked and scored to identify structurally novel ligands that make similar interactions to those of known ligands or may have different interactions with other parts of the binding site. Our screening approach identified four molecules ZINC84299674; ZINC76643455; ZINC84299122 & ZINC75626957 with MolDock score of -128.901, -120.22, -116.873 & -102.116 kcal/mol, respectively. Their energy scores were better than the 1N8Q bound co-crystallized ligand protocatechuic acid (with MolDock score of -77.225 kcal/mol). All the ligands were docked within the binding pocket forming interactions with amino acid residues.

Keywords: lipoxygenase, ZINC database, virtual screening, Molegro Virtual Docker.

Background:
Lipoxygenases are a class of enzymes that convert arachidonic, linoleic, and other polyunsaturated fatty acid into biologically active metabolites involved in the inflammatory and immune responses **[1].** There are five active LOXs found in human beings: 5-LOX, 12*S*-LOX, 12*R*-LOX, 15-LOX-1, and 15-LOX-2. A sixth gene family member, epidermal LOX type 3 (eLOX3, gene symbol *ALOXE3*) was described first in the mouse **[2],** and in humans in 2001 **[3].** Widely expressed in animals and plants, and also in some bacteria and fungi, LOX enzymes participate in diverse physiological and pathological processes. Mammalian 12*R*-LOX and epidermal lipoxygenase-3 (eLOX3), for example, play an indispensable role in skin barrier formation **[4, 5].** A distinctive characteristic of LOX enzymes in general is that the resting enzyme requires activation before catalytic reactions with fatty acids and molecular oxygen can begin **[6].** eLOX3 is an enzyme that in humans is encoded by the *ALOXE3* gene. Unlike other lipoxygenases, the eLOX3 enzyme does not act directly on fatty acids. Instead, it processes the product of another lipoxygenase reaction, a fatty acid hydroperoxide. The substance produced is later converted to a signaling molecule that is involved in the growth and division (proliferation) and specialization (differentiation) of skin cells. The eLOX3 enzyme is thought to play a role in the

formation and maintenance of the fat (lipid) membrane of the cells that make up the outermost layer of the skin (the epidermis). The epidermis helps prevent water loss, regulates body temperature, and protects against infection. At least 10 mutations in the *ALOXE3* gene have been found to cause nonbullous congenital ichthyosiform erythroderma (NBCIE). Most of these mutations change single protein building blocks (amino acids) in the eLOX3 enzyme. Many *ALOXE3* gene mutations lead to the production of a nonfunctional eLOX3 enzyme, which impairs the formation of the lipid membrane of cells within the epidermis. Problems with this protective barrier underlie the skin abnormalities and other features of NBCIE **[7].**

Several classes of compounds having selective lipoxygenase inhibitory activity have been reported in the literature. Many 5-LOX inhibitors have subsequently been developed, and several have displayed efficacy in asthma models, such as allergen-induced bronchoconstriction. For example, 1, 5-diarylpyrazoles, indolizines and indoles **[8].** However, evidence suggests that adverse reactions such as neuropsychiatric events, including sleep disorders, behavioral changes, headache, nasal burning and somnolence are associated with prolonged use of lipoxygenase selective inhibitors like zileuton and azelastine **[9, 10].** Thus, there is still a need for novel, selective, and potent inhibitors of active LOXs with an improved profile compared to current lipoxygenase inhibitors. Traditional synthesis of a series of new compounds utilizing combinatorial chemistry and high-throughput screening can be carried out at high cost and also are time consuming; whereas on the other hand, screening small molecule databases for novel compounds represents an alternative process. Docking various ligands to the protein of interest followed by scoring to determine the affinity of binding and to reveal the strength of interaction has become increasingly important in the context of drug discovery. Screening large databases of compounds can provide a feasible, alternative technique against highthroughput screening, but depends on the fast and accuracy of the docking algorithm. Hence, in this paper we report screening a library of compounds from ZINC database **[11]** against eLOX3 protein, 1N8Q (PDB ID) with bound ligand protocatechuic acid extracted from protein data bank, by utilizing a fast, exhaustive docking software Molegro Virtual Docker **[12].**

In recent years, virtual screening has reached a status of a dynamic and lucrative technology in probing for novel drug like compounds or so called hits in the pharmaceutical industry **[13].** The technique employed is based on the prediction of binding modes and binding affinities of each compound in the dataset by means of docking to an X-ray crystallographic structure. Virtual screening utilizes docking and scoring of each compound from a dataset. It is important to visualize the docked poses of high-scoring compounds because many ligands are docked in different orientations and may often miss interactions that are known to be important for the target receptor. This sort of study becomes more difficult as the size of the dataset increases. Therefore, an alternative approach is to eliminate unpromising compounds before docking by restricting the dataset to drug like compounds, by filtering the dataset based on appropriate property and sub-structural features and by performing diversity analysis. These approaches can be highly effective in reducing the dataset to be docked to the order of 10^3 to 10^4 compounds **[14].**

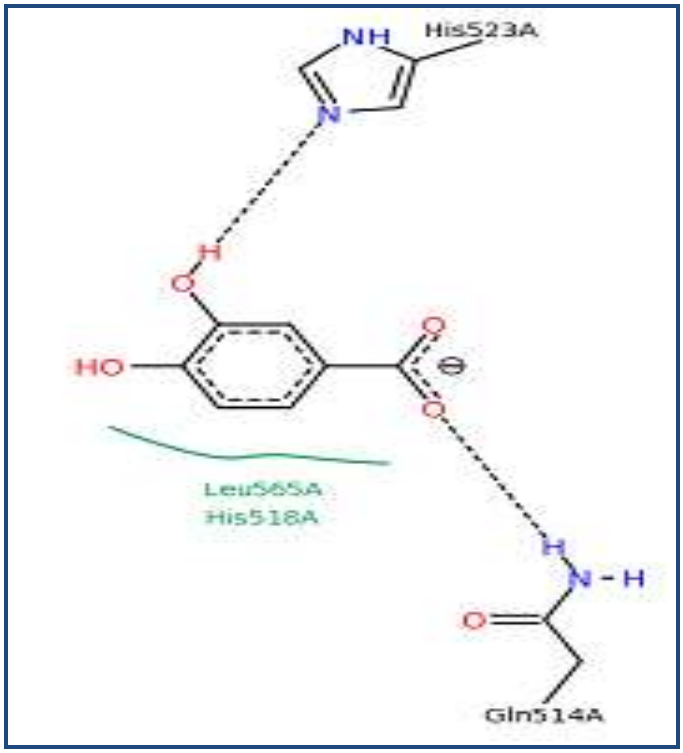

Figure 1: Diagram illustrating the interactions between 1N8Q protein and protocatechuic acid. Legend: black dashed lines - hydrogen bonds **[15].**

Methodology:

Receptor X-ray structure

The 3D coordinates of the crystal structure of eLOX3 in complex with protocatechuic acid (PDB code: 1N8Q) was selected as the receptor model in virtual screening program. We used the chemical compound library, ZINC database and the docking program Molegro Virtual Docker for the study. **Figure 1**, illustrating the hydrogen bond interactions between protocatechuic acid and eLOX3 protein (PDB code: 1N8Q).

Ligand ZINC database

ZINC, an acronym for 'ZINC is not commercial, a free database for virtual screening' contains over 21 million compounds in ready-to-dock, 3D formats, available at the URL http://zinc.docking.org. Molecules in ZINC are annotated by molecular property that include molecular weight, number of rotatable bonds, calculated log P, number of hydrogen-bond donors, hydrogen-bond acceptors, chiral centers, chiral double bonds (E/Z isomerism), polar and apolar desolvation energy (in kcal/mol), net charge and rigid fragments. The database contains 'lead-like' molecules, having molecular weight in the range 150 to 350 with calculated log P < 4, number of hydrogen bond donors ≤ 3 three, and number of hydrogen-bond acceptors ≤ 6. ZINC provides several search criteria such as molecular property constraint, ZINC codes, vendor based, and molecular substructure search **[11].**

Molegro Virtual Docker

Molegro is a Danish company, developing novel high-quality drug discovery and data mining software, founded in 2005 by

Rene Thomsen and Mikael Hvidtfeldt Christensen **[12].** We used the template docking available in Molegro virtual docker and evaluated MolDock, rerank and protein-ligand interaction scores from MolDock and MolDock [GRID] options. Template docking is based on extracting the chemical properties like the pharmacophore elements of a ligand bound in the active site and using that information for docking structurally similar analogs. We used the default settings, including a grid resolution of 0.30 Å for grid generation and a 15 Å radius from the template as the binding site. We used the MolDock optimizer as a search algorithm, and the number of runs was set to 10. A population size of 50, maximum iteration of 2000, scaling factor of 0.50, crossover rate of 0.90 and a variation-based termination scheme for parameter settings were used. The maximum number of poses was set to a default value of 5.

Screening

Before screening ZINC database, the docking protocol was validated. 1N8Q protein bound ligand protocatechuic acid was docked into the binding pocket to obtain the docked pose and the RMSD (Root Mean Square Deviation) of all atoms between these two conformations indicating that the parameters for docking simulation were good in reproducing the X-ray crystal structure. Therefore, the ZINC database was screened for compounds similar to protocatechuic acid structural features (structure based search) and by providing molecular constraints (property based search). ZINC database was also screened for compounds having properties similar to the known inhibitors of LOXs. The physicochemical properties such as log P value, H-bond donors, Hbond acceptors, molecular weight and rotational bonds, for protocatechuic acid ligand and known inhibitors of LOXs were calculated using the molinspiration server **[16].**

Discussion:

Lipinski's "Rule of 5" states, that most "drug-like" molecules have log P < or = 5, number of hydrogen bond acceptors < or = 10, number of hydrogen bond donors < or = 5 and molecular weight < or = 500 g/mol. Molecules violating more than one of these rules may have problems with bioavailability. The rule is called "Rule of 5", because the border values are 5, 500, 2*5, and 5 **[17].** Number of rotatable bonds is a simple topological parameter which is a measure of molecular flexibility. It has been shown to be a very good descriptor of oral bioavailability of drugs **[18].** Rotatable bond is defined as any single non-ring bond, bounded to nonterminal heavy (i.e., non-hydrogen) atom. Amide C-N bonds are not considered because of their high rotational energy barrier. The physico-chemical properties (log P value, H-bond acceptors, H-bond donors, molecular weight and rotational bonds) for protocatechuic acid ligand and known inhibitors of LOXs (aromadedrin, azelastin, robinetin, zileuton, caffeic and eriodictyol) were calculated using the molinspiration server. For these compounds range of calculated log P values was from 0.88 to 4.222, number of H-bond acceptors ranged from 4 to 7, number of H-bond donors ranged from 1 to 5, molecular weight ranged from 154.121 g/mol to 367.88 g/mol and number of rotational bonds ranged from 1 to 3.

We searched the ZINC database based on range values of properties of protocatechuic acid and known inhibitors of LOXs and identified 4319 hits that were Lipinski compliants. We docked these 4319 compounds using Molegro virtual docker and evaluated binding compatibility with the receptor based on docked energy (in kcal/mol). The docking tool generated 50 conformers for each docked molecule in about 1-2 minutes of CPU time. The virtual screening technique employed in this study identified diverse, yet specific ligands that bind in a comparable manner similar to protocatechuic acid binding for eLOX3. This approach identified four compounds ZINC84299674 ([5-(5,7-dimethyl-1H-indol-2-yl)-1H-pyrrol-3-yl] methanamine); ZINC76643455 (4-(4-methyl-1H-indol-2-yl)-1H-pyrrol-3-amine); ZINC84299122 ([5-(1H-indol-2-yl)-1H-pyrrol-3-yl]methanamine) & ZINC75626957 (4-[(E)-2-(2- thienyl)vinyl]benzene-1,2-diamine) from the ZINC database for compatible binding with eLOX3 **Table 1 (see supplementary material).**

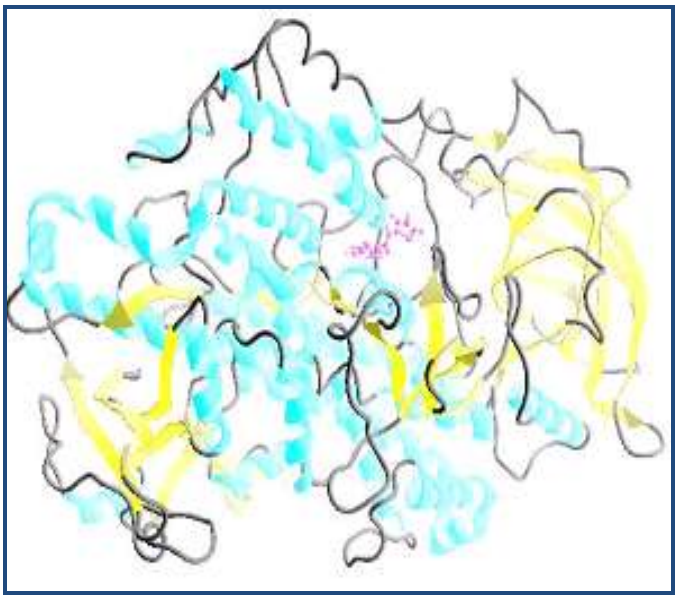

Figure 2: Schematic diagram illustrating the docking of ZINC84299674 (pink) (ligand with best MolDock score) to eLOX3 protein (pdb id 1N8Q) to form a protein-ligand complex.

eLOX3 co-crystallized protocatechuic acid ligand resulted in MolDock score of -77.225 kcal/mol. Therefore, any molecule from the dataset which shows a score lower than-77.225 kcal/mol would be regarded as ligands with higher binding affinity. In other words, these four zinc ligands would act as inhibitors against eLOX3 protein and such screening analysis forms the basis when millions of ligands are available in compound libraries such as the ZINC database. The docked energies of the four zinc ligands are given in (**Table 1).** Active site of eLOX3 offers many different binding modes for these compounds as they are strongly dependent on the attached substituent. All the ligands were docked deeply within the binding pocket region forming interactions with Thr337, Ser326, Lys332 and Glu333. In **(Table 1)** the binding affinities and the possible number of interactions based on chemical property identifiers are reported. Our screening approach identified four molecules ZINC84299674 (MolDock score of -128.901 kcal/mol); ZINC76643455 (MolDock score of -120.22 kcal/mol); ZINC84299122 (MolDock score of -116.873 kcal/mol) and ZINC75626957 (MolDock score of -102.116 kcal/mol) from the ZINC database. Their energy scores were better than the 1N8Q bound co-crystallized ligand

protocatechuic acid with MolDock score of - 77.225 kcal/mol. **Figure 2**, illustrating the protein-ligand complex between ZINC84299674 and eLOX3 protein (PDB code: 1N8Q).

Conclusion:

Finding novel compounds as starting points for lead optimization is a major challenge in drug discovery. Virtual screening is a powerful technique for identifying hit molecules as starting points for medicinal chemistry. The number of methods and software which use the ligand and target-based virtual screening approaches is increasing at a rapid pace. It has been clearly demonstrated that the approach utilized in this study is successful in finding four novel eLOX3 inhibitors from the ZINC database. ZINC84299674, in particular, showed high binding affinity with MolDock score of -128.901 kcal/mol against 1N8Q. The ligand was docked deeply within the binding pocket region forming hydrogen bond interaction with Thr337. Therefore, this study states the importance of small molecule libraries and their use to enhance drug discovery process prior synthesis. This approach to screen novel compounds as eLOX3 inhibitors from ZINC database depends on various parameters such as Lipinski's rule of 5, pharmacophoric groups attached on the ligand, size of the dataset and compound libraries among others. Further, work can be extended to study the receptor ligand interactions experimentally and evaluation of their biological activity would help in designing compounds based on virtual screening techniques.

Acknowledgment:

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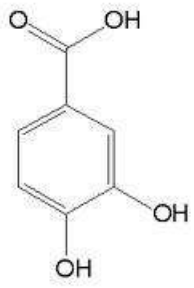
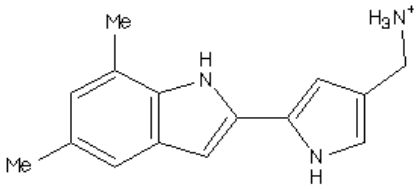
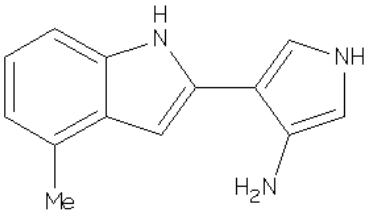
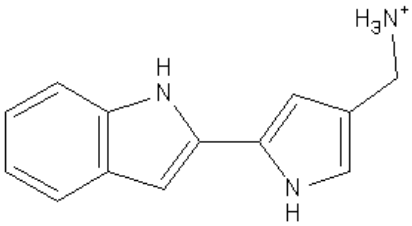
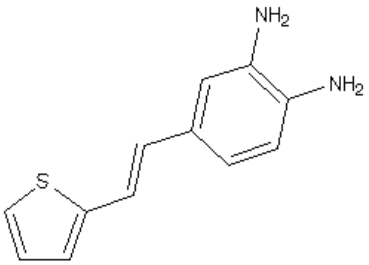
Supplementary material:

Table 1: Interaction parameters of eLOX3 with the predicted ZINC ligands and protocatechuic acid.

S. No.	Compound	Structure	MolDock Score (kcal/mol)	Rerank Score	HBond	Interacting residues
1	protocatechuic acid		-77.225	-62.511	-6.13446	His523, Gln514
2	ZINC84299674		-128.901	-100.667	-4.56216	Thr337
3	ZINC76643455		-120.22	-83.6125	-6.78915	Ser326, Thr337
4	ZINC84299122		-116.873	-55.4161	-5.05526	Lys332, Glu333
5	ZINC75626957		-102.116	-85.4102	-1.0764	Glu333