Indole alkaloids from Murraya koenigii as natural NPC1L1 inhibitors: An Insilico Approach

Deepesh Dileep

Department of Chemistry, S. D. College, Sanathanapuram P.O., Alappuzha, Kerala, India.
Email – deepesh.2552@gmail.com

Abstract: *It is assessed that several thousands of human lives are lost every year because of cardiovascular ailments as a result of dyslipidemic state of people. Individuals are generally considered at high danger of atherosclerosis due to food habits and genetic factors. Existing lipid lowering medications are costly and have side effects when used for prolonged therapy. Plant based research has again demonstrated its significance over the conventional statin drugs and its use has been reported widely. Cholesterol absorption within enterocytes is mediated by NPC1L1. Decrease or hindrance of NPC1L1 lowers intestinal cholesterol absorption by, 70% emphasizing its key role in dietary cholesterol absorption. This study was aimed to identify indole alkaloids with anti-dyslipidemic property from Murraya koenigii and analyze them for their efficiency in binding with NPC1L1. The docking studies indicate Mahanimbinol as the best compound that can inhibit NPC1L1 similar to Ezetimibe as it had strong binding affinity and interactions with the protein and had drug likeness based on the star value. The outcomes will be a decent beginning stage for future test and clinical investigations and might rise as a characteristic option.*

Key Words: *NPC1L1, Murraya koenigii, Docking, 3QNT*

1. INTRODUCTION:

As the expectations for everyday comforts and way of life changed over time, the pervasiveness of metabolic disorders won and expanded at speedier pace. Specifically, instances of dyslipidemia expanded drastically and are treated with statins. Cholesterol is a fundamental component of cell membranes, required for proper permeability and structural integrity. The correct control of cholesterol levels is basic for human wellbeing and people with raised cholesterol levels have expanded dangers for coronary illness.

The cholesterol in the diet is absorbed mainly in the intestine. After digestion in lumen and hydrolysis of dietary lipids, cholesterol is solubilized in mixed micelles containing bile acid and phospholipids. This solubilization promotes the movement of cholesterol from the massive lumen phase to the surface of the enterocyte. The absorption of cholesterol in the enterocytes is intervened by Niemann-Pick C1 Like 1 (NPC1L1), a protein expressed mainly in the intestine [1] and the target of the cholesterol absorption inhibitor Ezetimibe [2]. The reduction or inhibition of NPC1L1 reduces the absorption of intestinal cholesterol by 70%, which emphasizes its key role in the absorption of cholesterol in the diet. In transgenic mice, the effects of overexpression of NPC1L1 in the liver can be changed by hinderance with Ezetimibe, lowering plasma cholesterol levels to typical levels. In this way, NPC1L1 is a proven target to reduce cholesterol from dietary assimilation and liver reabsorption [3].

Murraya koenigii, ordinarily known as “Curry Patta”, is traditionally used in India as a spice for its characteristic flavor and aroma [4]. The aromatic leaves are considered tonic, antihelmintic, analgesic, digestive and aperitive, and are widely used in Indian cuisine to flavor food. The green leaves have been used for the treatment of itching, inflammation, fresh cuts, burses, dysentery, piles, vomiting and dropsy. Aqueous extracts of Murraya koenigii have a strong hypolipidemic activity and have been indicated for the treatment of mild form of diabetes [5]. The aqueous extract of M. koenigii leaves contains a range of active pharmacological agents, including alkaloids, flavonoids and tannins [6,7].

The point of molecular docking is to assess the feasible binding geometries of a ligand with a target whose target site is known. The binding poses include the position of the ligand relative to the receptor and conformational state of both. The present study has been carried out to compare the efficiency of the indole alkaloids of the plant M. koenigii and Ezetimibe against NPC1L1 using MOLECULAR DOCKING STUDIES.

2. MATERIALS AND METHODS:

The three-dimensional structure of the NPC1L1 target (PDBID: 3QNT) recovered from Protein Data Bank with a resolution of 2.83Å. 14 indole alkaloid structures and ezetimibe were searched against PubChem and stored in sdf format. Ligands and target proteins were imported into the workspace and prepared. Molegro Virtual Docker has been used to detect active sites. During docking, the first molecules are prepared and assigned links, bond orders, explicit hydrogens, charges, flexible twists, if missing in the Molegro Virtual Docker program [8] for both the protein and ligands. The scoring function used is the MolDock score. Atoms far from the binding site have been ignored.

Decrease the general calculation time. The search algorithm was taken as Moldock SE and no. of executions are taken 10 and the maximum iterations are 1500 with the population size 50 and with an energy threshold of 100.

After docking, the poses were organized according to the rerank score. The rerank score incorporates steric terms (LJ12-6) are approximations of Lennard-Jones, steric energy; MolDock score using a linear potential for the pieces for the approximation of steric energy [8]. The effectiveness of the ligand is generally defined as the ratio of free energy binding to the number of heavy atoms in a molecule [9]. Molegro Virtual Docker and was used to find Ligand Efficiency One (LE 1) as Moldock score divided by heavy Atoms count and Ligand Efficiency Two (LE 2) as rerank divided by the heavy atom no. The coefficients for the binding affinity terms were derived using multiple linear regression. The model was calibrated using a dataset of more than 200 complex structures of various PDB databases with known binding affinities (expressed in kJ / mol) [10]. ADMET properties (absorption, distribution, metabolism, excretion and toxicity) were estimated using Qikprop (Schrödinger Press 2015-1: QikProp, Schrödinger, LLC, New York, NY, 2015) Schrödinger Software Function to determine if phytochemicals has an undesirable effect.

3. RESULTS AND DISCUSSION:

A total of five cavities (Figure 1) were able to detected in NPC1L1 (PDB ID: 3QNT) by using Molegro Virtual Docker and were named cav1, cav2, cav3, cav4 and cav5, the volume and surface area details were given as Table 1. The volumes and surface areas of cav3, 4, and 5 were too small when compared to cav1 and 2 so that they were not considered for docking.

Figure 1: Five cavities detected in NPC1L1 (PDB ID: 3QNT)

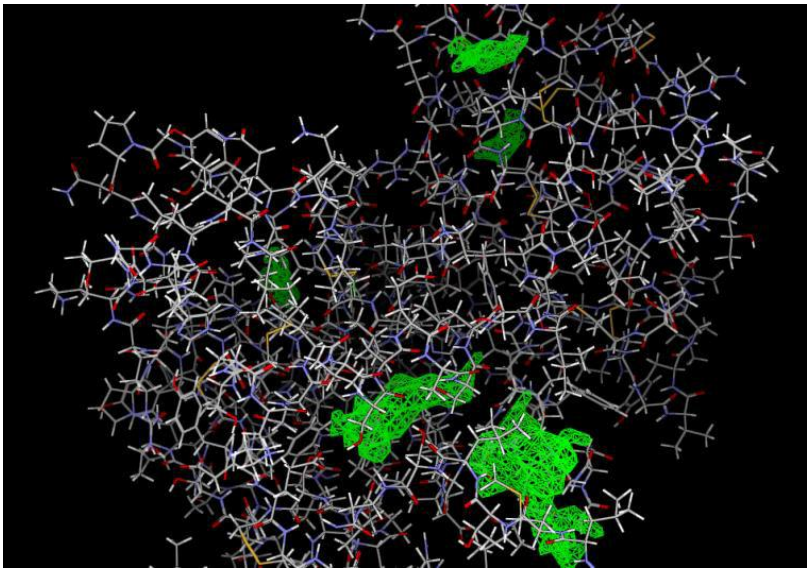


Table 1: Cavity information of NPC1L1

Cavity Name	Volume(Å^3)	Surface Area(Å^2)
Cav 1	72.192	218.88
Cav 2	71.68	194.56
Cav 3	18.944	80.64
Cav 4	16.896	74.24
Cav 5	12.8	57.6

All the 15 phytochemicals were used as ligand at mentioned 2 cavities of NPC1L1 and the results of the top ligands whose rerank score > -75 were selected and which were given in Table 2 and Table 3 of the context along with the hydrogen bond interaction values and other electrostatic interaction values.

Table 2: Docking result at Cavity one

Pubchem CID	Ligand	MolDock Score	Rerank Score	HBond	LE1	LE2	Affinity	Stars

131753005	Mahanimbinol	-129.1300	-87.1399	-4.8615	-5.1652	-3.4856	-24.8862	3
375142	Mukoeic acid	-102.2540	-82.1909	-2.5797	-5.6808	-4.5662	-25.7982	0
5318827	Koenine	-108.0230	-80.9226	-5.5180	-5.1439	-3.8535	-26.1299	1
150311	Ezetimibe	-141.3940	-75.1792	-4.8722	-4.7131	-2.5060	-28.3881	1

Table 3: Docking result at Cavity Two

Pubchem CID	Ligand	MolDock Score	Rerank Score	HBond	LE1	LE2	Affinity	Stars
150311	Ezetimibe	-149.1620	-93.8088	-2.9854	-4.9721	-3.1270	-27.7772	1
5318827	Koenine	-108.0210	-80.9185	-5.5202	-5.1439	-3.8533	-26.1310	1
375142	Mukoeic acid	-97.4204	-80.8671	-3.0845	-5.4123	-4.4926	-26.1518	0
131753005	Mahanimbinol	-138.2740	-75.6183	-2.5000	-5.5310	-3.0247	-20.2351	3

Figure 2: 3D and 2D interactions of Mahanimbinol with residues at cavity one

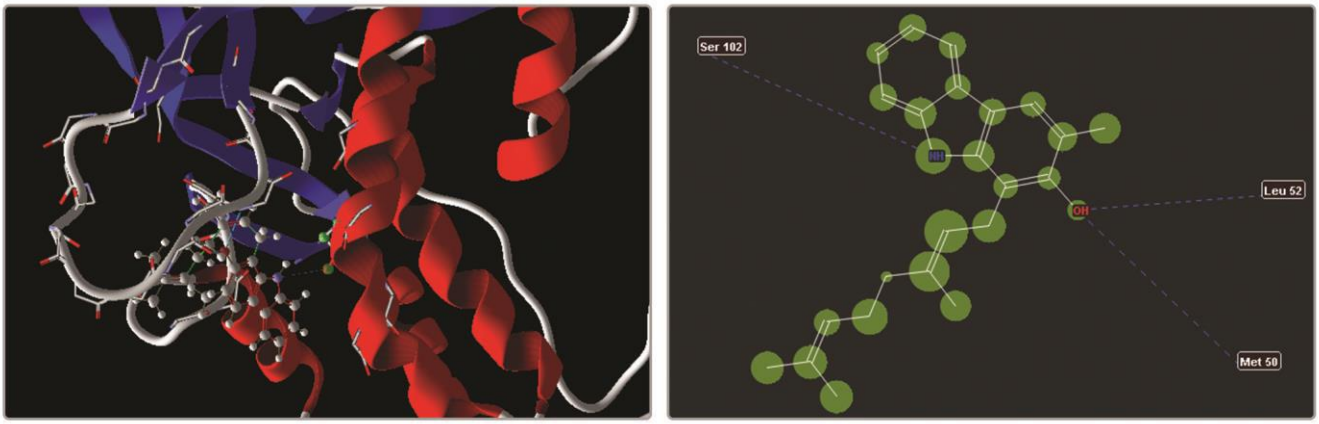
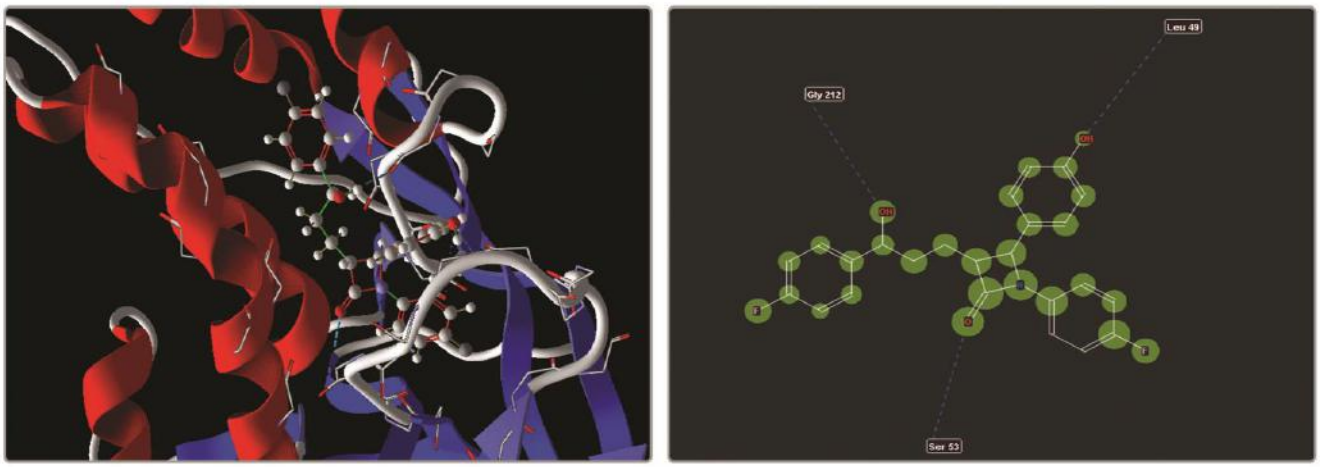


Figure 3: 3D and 2D interactions of Ezetimibe with residues at cavity two



From the results obtained, it has been observed that there exist excellent binding between selected phytochemicals of M. koenigii and NPC1L1. At cavity one, the Mahanimbinol showed a rerank score of -87.1399 and has 3 hydrogen bonds with the residues SER 102, LEU 52, MET 50. The H bond energy was found to be -4.8615 kJ/mol. On comparison with Ezetimibe, the drug which is recommended as a second line therapy for those intolerant of statins or unable to achieve target LDL cholesterol levels on statins alone had only a rerank score of -75.6183. This suggest that Mahanimbinol can be a better drug at the cavity one.

At the cavity two, the results were in the exact reverse order as observed at cavity one. Here Ezetimibe had the highest rerank score of -93.8088 and has 3 hydrogen bonds with GLY 212, LEU 49, SER 53. The H bond energy was

-2.9854 KJ/mol. This further emphasizes that Ezetimibe is a better drug than any other indole alkaloids at the cavity two.

4. CONCLUSION :

A thorough study was carried out over 14 phytochemicals of M. koenigii with the goal of identifying natural inhibitors relying on computational docking with MVD. Three indole alkaloids showed excellent rerank score. On comparison with the Second line drug Ezetimibe, the Mahanimbinol had a decent rerank score suggesting that it can emerge as a natural inhibitor of NPC1L1. Thus, it is hoped that the phytochemicals identified in this study if synthesized and tested in animal models would hold promising results for new drug discovery.

REFERENCES:

1. Altmann SW, Davis HR, Jr., Zhu LJ, Yao X, Hoos LM, et al. (2004) Niemann-Pick C1 Like 1 protein is critical for intestinal cholesterol absorption. Science303: 1201–1204.
2. Garcia-Calvo M, Lisnock J, Bull HG, Hawes BE, Burnett DA, et al. (2005) The target of ezetimibe is Niemann-Pick C1-Like 1 (NPC1L1). Proc Natl Acad Sci U S A 102: 8132–8137.
3. Kwon HJ, Palnitkar M, Deisenhofer J (2011) The Structure of the NPC1L1 N-Terminal Domain in a Closed Conformation. PLoS ONE 6(4): e18722. doi:10.1371/journal.pone.0018722
4. Chopra RN, Nayar SL, Chopra IC. (1996) Glossary of Indian Medicinal Plants. CSIR, New Delhi, p. 470.
5. Kesari AN, Kesari S, Singh SK, Gupta RK, Watal G. (2007) Studies on the glycemic and lipidemic effect of Murraya koenigii in experimental animals. Journal of Ethnopharmacology, 112, 305-311.
6. Chakrabarty M, Nath A, Khasnobis S, Chakrabarty M, Konda Y, Harigaya Y, Komiyama K. (1997) Carbazole alkaloids from Murraya koenigii. Phytochemistry, 46, 751-755.
7. (a) Wang YS, He HP, Shen YM, Hong X, Hao XJ. (2003) Two new carbazole alkaloids from Murraya koenigii. Journal of Natural Products, 66, 416-418; (b) Mandal S, Nayak A, Banerjee SK, Banerji J, Banerji A. (2008) A new carbazole alkaloid from Murraya koenigii Spreng (Rutaceae). Natural Product Communications, 3, 1679-1682; (c) Chakraborty M, Saha S, Mukhapadhyay S. (2009) Murrayakoeninol- a new carbazole alkaloid from Murraya koenigii (Linn) Spreng. Natural Product Communications, 4, 355-358.
8. R Thomsen & MH Christensen, J Med Chem. (2006) 49: 3315 [PMID: 16722650]
9. C Abad-Zapatero & JT Metz, Drug Discov Today. (2005) 10: 464 [PMID: 15809192]
10. R Wang et al. J Med Chem. (2004) 47: 2977 [PMID: 15163179].