WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 2, 28-35. Research Article ISSN 2277– 7105

ANTIHYPERLIPIDEMIC DOCKING STUDY OF MONASCIN AND ANKAFLAVIN COMPOUNDS FROM *MONASCUS PURPUREUS* WITH SOME TARGETS RELATED WITH HYPERLIPIDEMIA

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Article Received on
25 November 2020,

Revised on 15 Dec. 2020,
Accepted on 05 Jan. 2021

DOI: https://doi.org/10.17605/OSF.IO/PDS8R

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ABSTRACT

Objective: Virtual screening methods are promising and useful to find natural active compounds that have activity and effectiveness against a specific receptor. This study is to determine the mechanism of interaction between monascin and ankaflavin compounds from Red Yeast Rice (*Monascus purpureus*) against receptors associated with antihyperlipidemic activity. This study used 3 target receptors associated with lipid metabolism: Niemann Pick C1 Like1 Protein (NPC1L1), Farnesiod X-Receptor (FXR), and Lanosterol 14α-Demethylase (LDM). The molecular interactions of monascin and ankaflavin are compared with the native ligands at the active site of the receptor. **Materials and Methods:** Ligand files and target receptors are obtained by downloading at https://pubchem.ncbi.nlm.nih.gov/ and https://www.rcsb.org/. The docking process begins with ligand and receptor preparations using Pyrx software, MgTool, then molecular docking processes and visualization of interactions with AutoDock Vina and Discovery Studio Visualizer. **Results:** The monascin and ankaflavin ligand binding activity score with FXR is -8.9 kcal/mol; -10.2 kcal/mol, with LDM -9.3 kcal/mol; -9.0 kcal/mol, and with NPC1L1 -4.5 kcal/mol; -3.9 kcal/mol. **Conclusions**: The binding activity data from molecular docking concluded that monascin and ankaflavin compounds had strong antihyperlipidemic activity through inhibition mechanisms at FXR and LDM receptors.

KEYWORDS: Molecular docking, antihyperlipidemic, monascin, ankaflavin, FXR, LDM, NPCL1.

INTRODUCTION

Hyperlipidemia is a medical condition related to lipid metabolism disorders characterized by an increase in plasma concentrations of various lipid and lipoprotein fractions and can lead to heart disease. Increased serum TC, VLDL, LDL, HDL, and TG that contribute to complications: coronary heart attack, coronary artery syndrome, stroke, atherosclerosis, myocardial and pancreatic infarction.[1,2] Medicinal plants are now more profitable than modern drugs, taking into account aspects of better patient tolerance, low toxicity effects, and relatively avoidable side effects and resistance.[3]

Monascus purpureus or Red Yeast Rice (RYR) has been known since the Ming Dynasty (1368-1644) as a food, preservative, food coloring, and also for medicinal purposes.[4] The resulting color pigments have been widely used to improve food quality. Meanwhile, the bioactive compounds from secondary metabolites have been scientifically proven as anti-fatigue, antihypertensive, anti-inflammatory, antioxidant, anti-tumor, antihyperlipidemic, anti-tumor, etc. *Monascus sp* is also known to have no adverse side effects.[4,5,6] Other studies have shown that monascin and ankaflavin compounds show a significant effect on reducing cholesterol levels, serum TG TC, and LDL-C.[7]

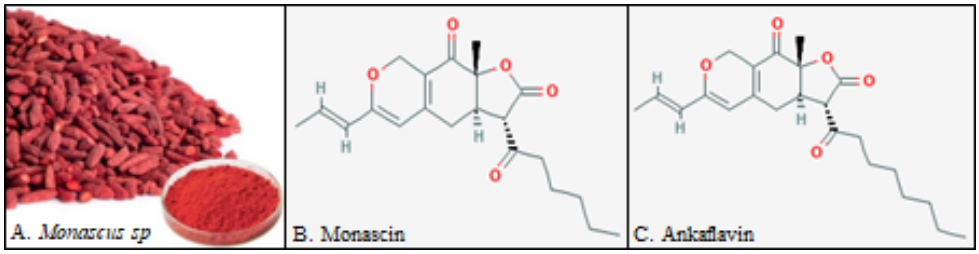

Figure 1: (A). *Monascus sp*, (B). Monascin, (C). Ankaflavin.

The computational method approach, molecular docking, helps by screening molecules based on their free binding energy and provides hypotheses how these molecules can exert activity in inhibiting target receptors.[8] This approach can provide data from new molecular pledge for direct and rational drug discovery, with the advantages of economical cost and shorter research time and high efficiency.[9] The active compounds monascin and ankaflavin will be used as hyperlipidemic agents against 3 target receptors related to lipid metabolism: Niemann Pick C1 Like1 Protein (NPC1L1), Farnesiod X-Receptor (FXR), and Lanosterol 14α-Demethylase (LDM). The resulting binding affinity value will then be compared with the native ligand that binds to the receptor.

NPC1L1 is a receptor target that is related to the direct intestinal sterol transfer mechanism. This receptor (PDB code ID: 3QNT) is a molecular target for the cholesterol-lowering drug ezetimibe.[10,11] FXR as a target receptor (PDB code ID: 1OSH) associated with bile acid (BA) sensors, plays a role in maintaining balance in cholesterol metabolism, and plays a role in the function of lipid homeostasis and absorption of fats and vitamins. FXR is also known to play a role in lowering plasma triglyceride levels.[12,13] The target of the LDM receptor (PDB ID code: 3LD6) has an important role in the catalysis of cholesterol biosynthesis, by reducing the l4α group-methyl group of lanosterol. LDM can also act as a promising therapeutic agent in the treatment of hypercholesterolemia.[14,15]

MATERIALS AND METHODS

Software and Tools

Protein Data Bank (http://www.rcsb.org/pdb), PubChem (https://pubchem.ncbi.nlm.nih.gov), ChemDraw Ultra 12.0, AutoDock Vina 1.1.2, MGL tools, Pyrx, Discovery Studio Visualizer.

Ligand Preparation

Monascin and ankaflavin and various ligands (positive control) were used as ligands for docking studies were listed in Table 1.

Table 1: Ligands used in the study.

No	Ligand	Molecular Formula	References
1	Monascin	$C_{21}H_{26}O_5$	[4]
2	Ankaflavin	$C_{23}H_{30}O_5$	[5]
3	Fexaramine	$C_{32}H_{36}N_2O_3$	[12]
4	Ketoconazole	$C_{26}H_{28}Cl_2N_4O_4$	[15]
5	Ezetimibe	$C_{24}H_{21}F_2NO_3$	[10]

Protein Preparation

All protein target files can be obtained in the protein data bank (http://www.rcsb.org). The target receptor file was then carried out with protein preparation and converted into a file in the PDBQT format using Discovery Studio and PyRx software.

Docking Studies Using AutoDock Vina

The structure of monascin, ankaflavin, and other ligands was minimized energy first by using AutoDock Vina 1.1.2. The prepared receptor files and ligands must be in PDBQT format files. The molecular docking process uses a closed receptor in a box with a distance of 1 Å.

This is so that the receptors are rigid and the ligands are flexible molecules. The energy of the interaction between the ligand and the receptor is calculated and compared (kcal/mol).

Protein–Ligand Interactions

The molecular interactions that form between the ligand and the target receptor in the active bond bag can be visualized in 2D with the Discovery studio visualizer program. This 2D visualization can explain molecular interactions that occur such as hydrogen bonds, hydropbobicity, bond distances and also the amino acids that interact through these bonds.

RESULTS AND DISCUSSION

Ligand Preparation

The molecular structure of the ligands can be downloaded at the link https://pubchem.ncbi.nlm.nih.gov/. Then the structure is minimized and saved in the PDBQT file format with the PyRx program. The physicochemical properties of all the ligands are shown in Table 2.

Table 2: Physiochemical parameters of ligand.

No	Ligand	Molecular Weight (Da)	Hydrogen Bond Donor	Hydrogen Bond Acceptor	Log P	Minimize Energy
1	Monascin	358.4	0	5	3.443	430.82
2	Ankaflavin	386.5	0	5	4.224	605.52
3	Fexaramine	496.6	0	4	6.719	588.08
4	Ketoconazole	531.4	0	7	4.206	547.92
5	Ezetimibe	409.3	2	3	4.888	936.66

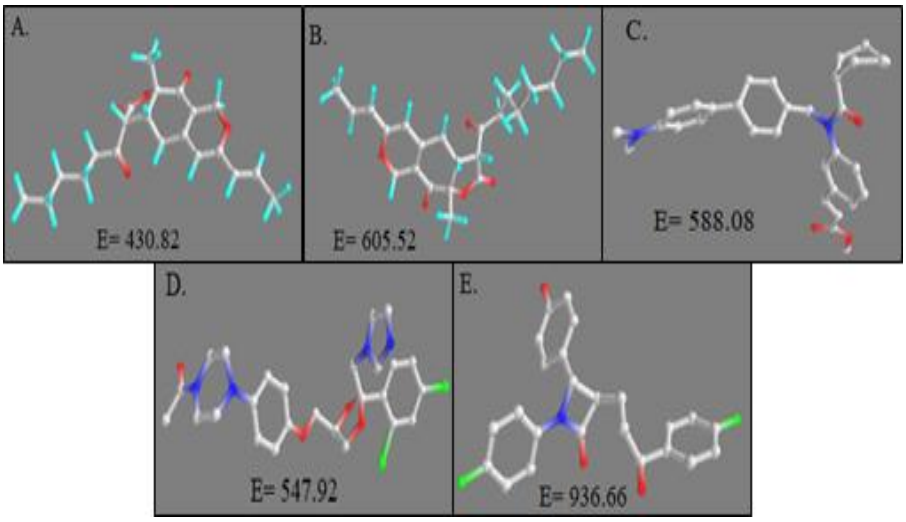

Figure 2: 3D ligand structure and energy minimized results. (A). Monascin, (B). Ankaflavin, (C). Fexaramine, (D). Ketoconazole, (D). Ezetemibe.

Protein Preparation

Receptor targets for antihyperlipidemia are obtained through the Protein Data Bank link (http://www.rcsb.org/pdb). The file is then converted into PDBQT format using the AutoDock Tool (figure 3). The target receptors are readily available for ligand docking.

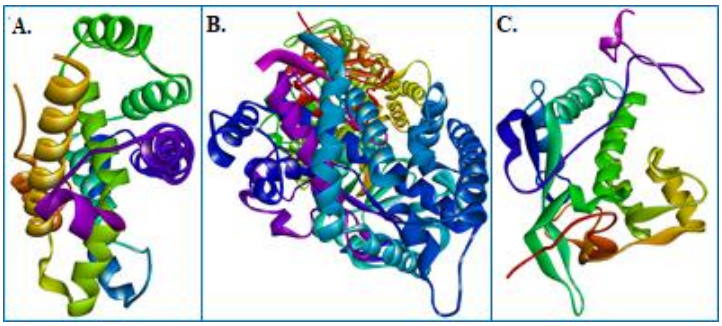

Figure 3: 3D structure of receptor, (A) FXR; (B) LDM; (C) NPC1L1.

Docking Studies Using AutoDock Vina

Docking of Monascin and Ankaflavin into PDB structure of Farnesiod X-Receptor (PDB ID: 1OSH)

The docking molecule results between Fexaramine, and ankaflavin against the FXR receptor do not have the same amino acid that forms a bond. The interaction between monascin and the FXR receptor has hydrogen bonds to the amino acids Tyr 365, Tyr 373, and Ser 336. Whereas compared to the interaction of ankaflavin and fexaramine on the FXR receptor, it has similarities in its amino acid binding: Met 487, Leu 134, Met 381, Ile 379, Met 378, His 489. Ankaflavin has hydrogen bonds which are interactions with the amino acids His 489, His 236. Docking analysis was performed by evaluating the binding affinity score indicating that the ligands were fexaramine (-10.9 kcal/mol), monascin (-8.9 kcal/mol), and ankaflavin (-10.2 kcal/mol). This provides information that both monascin and ankaflavin have antihyperlipidemic activity through inhibitory mechanisms at the FXR receptors, where ankaflavin shows a more dominant activity than monascin.

Docking of Monascin and Ankaflavin into PDB structure of Lanosterol 14α-Demethylase Receptor (PDB ID: 3LD6)

The molecular interactions between monascin and ketoconazole ligand against the LDM receptor have similarities only to the binding of the amino acid Phe 105, while the ankaflavin and ketoconazole ligand against the LDM receptor occur at amino acids Phe 205, Asn 211, Gly 212, Thr 51, Met 50, Leu 52, Pro 215, Ser 53, Ser 102, Ile 105, and Leu 213. Hydrogen

bonds are only formed on the interaction of the ankaflavin ligand and the LDM receptor on the amino acids Leu 52, and Ser 53. The binding activity score shows that ankaflavin has a better bond affinity (-9.3 kcal/mol) versus monascin (-10.0 kcal/mol). These values indicate that monascin and ankaflavin have antihyperlipidemic activity via LDM receptor inhibition mechanisms.

Docking of Monascin and Ankaflavin into PDB structure of Niemann-Pick C1 Like-1 Receptor (PDB ID: 1EZF)

The molecular docking results between the monascin and ezetimibe ligands on the NPC1L1 receptor showed no similarity in the amino acids in the bonds. Meanwhile, the ligands ankaflavin and ezetimibe against the NPC1L1 receptor have the same bonding to the amino acid Ile 288 and have hydrogen bonds to the amino acids Tyr 233, Ser 224, Ile 288, and Pro 209. The binding affinity score of ankaflavin (-3.9 kcal / mol) , and monascin (-4.5 kcal / mol) showed that these two ligands did not have antihyperlipidemic activity through inhibition of the NPC1L1 receptor mechanism.

Table 4: Comparative binding affinity of different ligands with receptors.

No.	Receptor	Ligand	Binding Affinity (kcal/mol)
1	Farnesiod X-Receptor	Fexaramine Monascin Ankaflavin	-10.9 -10.2 -8.9
2	Lanosterol 14α-Demethylase (LDM)	Ketoconazole Monascin Ankaflavin	-10.5 -9.0 -9.3
3	Niemann-Pick C1 Like-1 (NPC1L1)	Ezetimibe Monascin Ankaflavin	-7.0 -4.5 -3.9

The results of the visualization of ligand and receptor interactions with The Discovery studio visualizer program can be seen in Figure 4.

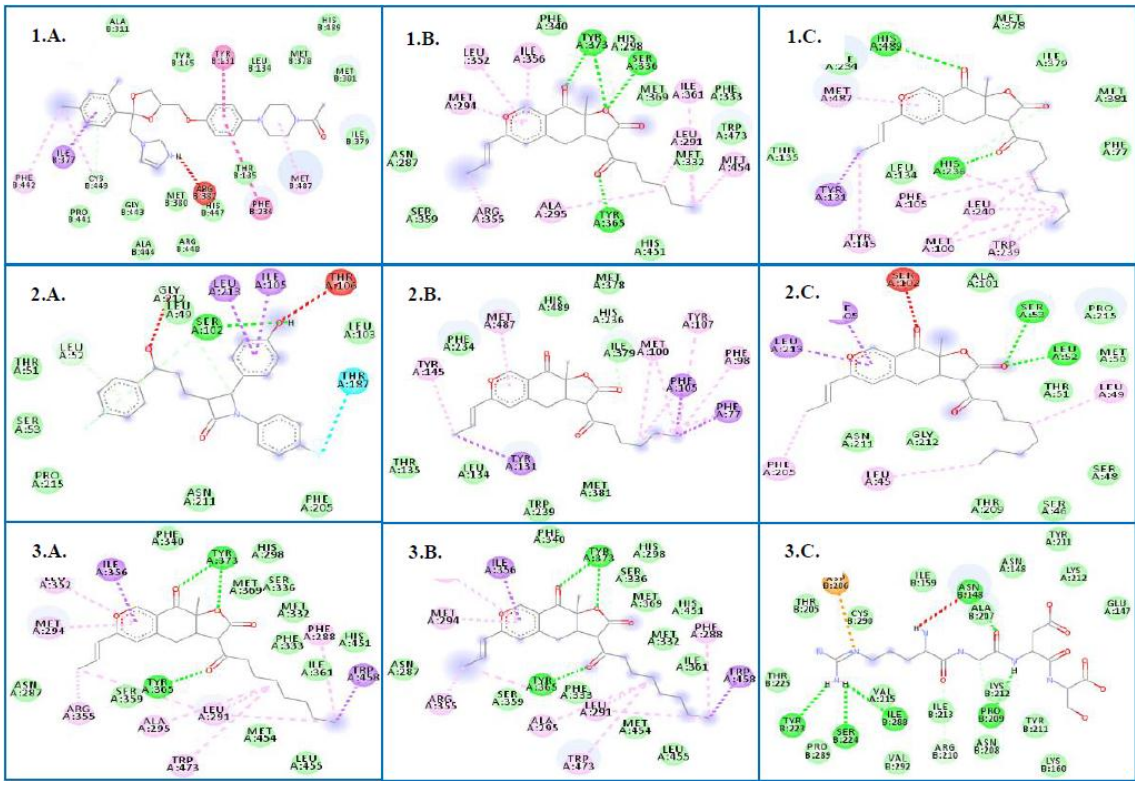

Figure 4: Interaction of ligands and target receptors. (1.A) Fexaramine bound to FXR, (1.B) Monascin bound to FXR, (1.C) Ankaflavin bound to FXR, (2.A) Ketoconazole bound to LDM, (2.B) Monascin bound to LDM, (2.C) Ankaflavin bound to LDM, (3.A) Ezetimibe bound to NPC1L1, (3.B) Monascin bound to NPC1L1, (3.C) Ankaflavin bound to NPC1L1.

CONCLUSIONS

Molecular docking studies of monascin and ankaflavin compounds from the *Monascus purpureus* have potential antihyperlipidemic activity through the mechanism of inhibition of FXR and LDM receptors.

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