Molecular docking analysis of two bioactive molecules KLUF10 and KLUF13 isolated from the marine bacteria Micrococcus sp. OUS9 with TNF alpha

Shanthi Kumari1,3, Pabba Shivakrishna2,* & K. Sreenivasulu3

1Osmania University, Department of microbiology, Hyderabad, India; 2Lavin laboratories, Hyderabad, India; 3KLEF University, Andhra Pradesh, India; *Corresponding author: Dr. Pabba Shivakrishna: E-mail: Shiva-krishnapabba@yahoo.com

Received March 15, 2021; Revised April 21, 2021; Accepted April 30, 2021, Published May 31, 2021

Declaration on official E-mail:
The corresponding author declares that official e-mail from their institution is not available for all authors

Declaration on Publication Ethics:
The authors state that they adhere with COPE guidelines on publishing ethics as described elsewhere at https://publicationethics.org/. The authors also undertake that they are not associated with any other third party (governmental or non-governmental agencies) linking with any form of unethical issues connecting to this publication. The authors also declare that they are not withholding any information that is misleading to the publisher in regard to this article

Abstract:
Tumor necrosis factor-alpha (TNF-α) is known to be linked with tumor. Therefore, it is of interest to document the Molecular docking analysis of two bioactive molecules KLUF10 and KLUF13 isolated from the marine bacteria Micrococcus sp. OUS9 with TNF alpha. We report the molecular interactions of KLUF10 and KLUF13 with TNF alpha.

Kew Words: TNF-α, KLUF10, KLUF13, PDB ID 2AZ5, Docking.

Background:
Oceans occupy about 70% of the Earth’s surface area, and many aquatic species have novel components that are not found on land, as well as specific biological properties of high activity and efficacy. Since the National Cancer Institute (NCI) began screening marine tools for anti-cancer activity in 1968, research on marine drugs has evolved into a distinct field [1]. Docking is a process that predicts the preferred orientation of one molecule to another when they are bound together to form a stable complex [2] and it plays an important role in drug rational design. The in-silico method is a low-cost and fast approach for identifying protein targets of natural based ingredients [3] By analyzing the interactions between small molecule ligands and receptor biomacromolecules, this method could predict the binding mode and affinity strength, and then realize structure-based drug design, which is of great significance to the molecular mechanisms of pharmacological activities, structure prediction of protein-ligand complexes, and targeted drug screening [4,5,6] A number of molecular modeling and docking studies have been done for predicting molecular targets and molecular mechanism of ginsenosides [7, 8, 9,10] The most widely used computational technique for characterization of protein-ligand binding sites is molecular docking. A variety of molecular simulation and docking experiments have been conducted in order to predict ginsenoside molecular targets and
molecular mechanisms [7,8]. Therefore, it is of interest to document the Molecular docking analysis of two bioactive molecules KLUF10 and KLUF13 isolated from the marine bacteria Micrococcus sp. OUS9 with TNF alpha.

Table 1: Molecular docking energies for compounds KLUF10 & KLUF13

| Ligand name | Electrostatic energy (kJ/mol) | Van der Waals Energy (kJ/mol) | Binding energy (kJ/mol) | C1 |
|-------------|-------------------------------|------------------------------|------------------------|----|
| KLUF10 (Zeaxanthin) | -9.817 | 4.521 | -95.449 | 31.377 | -41.212 |
| C1 | 7.427 | -1.446 | 7.235 | 23.759 | -36.84683 |

C1 = KLUF13 (1-(1-(4-methoxyphenyl)-2-(methylamino) ethyl) cyclohexanol)

Table 2: Hydrogen bonding residues, interacting atoms and H-distance between protein and novel compounds

| Ligand name | Interacting amino acids | Interacting atoms | H-distance |
|-------------|-------------------------|-------------------|------------|
| KLUF10 (Zeaxanthin) | Tyr119, Leu120, Gly121, Gly122, Ser60, Leu57, Tyr151 | No Hydrogen bond | - |
| C1 | Tyr119, Leu120, Gly121, Gly122, Ser60, Leu57, Tyr151 | C4:H30 - A: LEU120:O | 2.022 |

C1 = KLUF13 (1-(1-(4-methoxyphenyl)-2-(methylamino) ethyl) cyclohexanol)

Materials and methods:

Sampling site and sample collection
Seawater and soil samples were collected from coastal locations of Nellore, Visakha Patnam and Bapatla, under aseptic conditions and were processed within 1-2 hours after collection [11,12]. The isolated bacteria were screened and identified by molecular characterization (using 16s rRNA sequencing), as per our previous study [13].

Extraction of crude extracts
The shake-flask fermentation was performed using 250ml capacity Erlenmeyer flasks for the selected active bacterial strains, containing 100ml of Zobell broth medium. The pure selected bacteria strain was inoculated with 1ml culture suspension for the sterilized fermented broth. Fermentation was conducted on a rotary shaking incubator by incubating inoculated flasks at 28 °C, 250 rpm for five days. The fermentation broth was centrifuged for crude extract preparation at 10,000rpm for 20 min after incubation.

Purification of the active compounds
The column chromatography had chosen with silica gel of L00-200 μm particle size. The gel was suspended for the packing of the column with petroleum ether. The column was formed by a corning glass tube 40 cm long with a glass stopper at the bottom and an internal diameter of 2.5 cm. The column’s final size was 25X2.5 cm. The column had methanol balanced. The sample was not exceeding 5 ml, and the flow rate was kept to 0.2 ml/min, with the chloroform gradient water system methanol (9:1, 7:3, 1:1). Then the column was washed with hexaneand methanol. 10ml fractions were gathered and all the different fractions were analyzed with each solvent scheme. All pooled fractions have been tested with antimicrobial agents. The active fraction was analyzed and characterized by NMR spectroscopy and Mass spectroscopy.

Docking study

Protein preparation
TNF-α (PDB ID 2AZ5) protein X-ray crystallographic structure (resolution 3.0) was obtained from the Protein Data Bank. Water molecules, and other heteroatoms ligands, with chains B, C, and D, were excluded from the protein molecule. The CHARMm force field was used to bind hydrogen atoms to the protein. Using the Accelyrs Discovery studio (version 2.1), energy was minimized using the conjugate gradient procedure with an RMS gradient of 0.01kcal/mol.

Ligand preparation
The ligand molecules (KLUF10-zeaxanthin and KLUF13- novel compound) structure were drawn in Hyperchem molecular modeling and visualization tool (version 7.5) and the energy was minimized using Accelyrs Discovery studio client (version 2.1) software. The minimized protein and ligands were saved in PDB and mol-2 format, respectively for further analysis as shown in the Figure 1 and the energy values obtained.

Docking
The protein and ligand docking studies were performed by using (using a grid-based MD docking algorithm, CDOCKER (CHARMm-based MD docking tool) software (Wu et al. 2003). The CDOCKER interaction energy as an estimation of molecular complex binding affinity was used in this study.
Figure 1: Molecular Docking simulation results: (A) Secondary structure of TNF-α with a small molecule inhibitor (2AZ5), (B) three-dimensional structure of 2AZ5_A chain represented in Wireframe model (Black -Carbon, Red-Oxygen, Blue -nitrogen, Yellow-Sulphur), (C) Prepared protein, (D) Shows identification of active site pocket.
Results and Discussion:

Marine organisms have developed biochemical and physiological processes including the production of bioactive metabolites for reasons such as reproduction, communication, and predation, infection, and competition protection (Halvorson, 1998). Virtually, every kind of marine organism displays different molecules with distinctive structural characteristics, due to their physical and chemical circumstances in the marine environment. In the present investigation, the total 29 different bacterial cultures were isolated from different locations of Nellore district regions the bacterial colonies present on agar plates with morphologically different pigment producing has been identified. The chosen colonies were screened by well diffusion for antagonistic activity and identified by 16s molecular identification method. The crude extract form Micrococcus sp. OUS9 was separated using column chromatography and from the different fractions obtained from the column the KLUF10 and KLUF13 were shown bioactive nature compare to other fractions. Hence, fraction KLUF10 and KLUF13 was subjected to further analysis to identify the bioactive compound NMR studies. The two fractions were revealed as KLUF10 (Zeaxanthin) KLUF13(1-(1-(4-methoxyphenyl)-2-(methylamino) ethyl) cyclohexanol).

Docking studies

Two bioactive compounds isolated from marine bacteria Micrococcus sp. OUS9 KLUF10 and KLUF13substances were selected as possible lead structures and optimized. The inhibitory potential of the obtained structures to PDB ID 2AZ5 were evaluated by means of molecular docking using a grid-based MD docking algorithm, CDOCKER (CHARMm-based DOCKER), which offers all the advantages of full ligand flexibility (including bonds, angles, dihedrals). From the docking studies KLUF10 shows the negative CDOCKER energy score (-95.449 K.cal/mol) with binding energy of -41.212 K.cal/mol (Table 1 and Table 2) and forms non-bonded interactions with active site residues Tyr119, Leu120, Gly121, Gly122, Ser60, Leu57, Tyr151 (Figure 2). Whereas another compound KLUF13 shows CDOCKER energy score (7.235K.cal/mol) with binding energy of -36.84683K.cal/mol with one Hydrogen bonding interaction (Figure 3). Hydrogen bond is formed between the oxygen atom of LEU120 interacting with NH atom of the KLUF10 (C4:H30 - A: LEU120: O) with a hydrogen bond distance of 2.022Å.

Conclusion:

We report the optimal molecular interactions of KLUF10 and KLUF13 with TNF alpha for further considerations.

Acknowledgment:

The authors are thankful to the management of KLEF, Guntur, Osmania University, Hyderabad, Lavin laboratories, Hyderabad.
and Averin Biotech, Hyderabad for providing the lab facilities needed to carry out this present study.

Conflict of interest:
We declare that we have no conflict of interest

References:
[1] Ruggieri GD Science 1976 194:491.
[2] Lengauer T et al. Curr. Opin. Struct. Biol. 1996 6:402.
[3] Chen X et al. Nat Prod Rep. 2003 20:432.
[4] Villoutreix BO et al. Curr. Pharm. Biotechnol. 2008 9:103.
[5] Vakser IA Biophys. J. 2014 107:1785.
[6] Xue LC et al. FEBS Lett. 2015 589:3516.
[7] Chen RJ et al. Sin. 2009 30:61.
[8] Qu C et al. Spectrochim Acta A Mol Biomol Spectrosc. 2010 78:676.
[9] Sathishkumar et al. J. Enzyme. Inhib. Med. Chem. 2011 27:685.
[10] Sathishkumar et al. Comput. Biol. Med. 2013 43:786.
[11] Jayaprakashvel M et al. Adv. Biotech. 910:39.
[12] Krishna PS et al. Indian J. Microbiol. 2015; 55:292.
[13] Kumari KS et al. Saudi Journal of Biological Sciences, 2020 27:2398.
