Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2015; 7(4); 781-784

ISSN: 0975-4873

Research Article

CXCR4 Inhibitory Activity Analysis of Linoleic Acid Isolated from Ethanolic Extract of *Cayratia trifolia* (L.): An Molecular Docking Simulation

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Available Online: 21st July, 2015

ABSTRACT
Chemokine Receptor type 4 (CXCR4) is the increasing interest as a drug target, which is involved in many disease states including more than 23 types of cancer and several immunodeficiency disorders. On the other hand, the chemical constituents of medicinal plant are helpful in the discovery of therapeutic agents. Therefore the main aim of the study was to analyze the inhibitory activity of linoleic acid against CXCR4. Previous studies the natural compound of linoleic acid was isolated and identified from ethanolic extract of *Cayratia trifolia.* The molecular docking analysis was carried out to find the CXCR4 inhibitory activity of the isolated compound. Results, the isolated compound of linoleic acid possess comparable good Glide score and Glide energy when compared with FDA approved drug. Based on the results, it can be concluded that, the isolated compound of linoleic acid may act as novel inhibitor aginst CXCR4 and further it can be lead to development of therapeutic agent for variety of cancers and other disorders.

Keywords: *Cayratia trifolia* (L.); Linoleic acid; CXCR4; Molecular docking analysis.

INTRODUCTION

The chemokine gradients are playing significant role movement of cells in a variety of normal and pathologic processes. Cancers have a complex chemokine network that may influence the leucocyte infiltrate and angiogenesis[1]. Malignant cells can also state the chemokine receptors and respond to chemokine gradients and this may be associated with the development and spread of cancer. Different cancers express different CC and CXC chemokine receptors and the corresponding ligands are sometimes expressed at sites of tumour spread[2,3]. There is one chemokine receptor, however, Chemokine Receptor type 4 (CXCR4) is a G-protein-coupled membrane receptor which is present in various cell types. It is increasing interest as a drug target and it can be involved in many disease states including more than 23 types of cancer and several immunodeficiency disorders[4]. CXCR4 has been shown to play a critical role in (breast) cancer progression and metastatic spread. It has been reported that 69% of ductal carcinoma in situ (DCIS) lesions are CXCR4-positive. Over-expression of CXCR4 has also been suggested to be of value for imaging applications[5]. As a number of reviews have recently been published highlighting CXCR4 as a target in HIV and its role in cancer metastasis[6].

Consideration of the bioactive compounds from the medicinal plant is helpful to discovery of therapeutic agents as well as new sources of economic materials like oil and gums[7]. Secondary metabolites from medicinal plants have demonstrated to be an excellent reservoir of new medical compounds[8]. Numbers of bioactive compounds are present in medicinal plants which are widely used against variety of diseases[9]. *Cayratia trifolia* (L.) is the medicinal plant belongs to the family of Vitaceae, commonly known as Fox grape in English is native to India, Asia and Australia. It is a perennial climber having trifoliated leaves with (2-3 cm), long petioles and ovate to oblong-ovate leaflets. Flowers are small greenish white brown in color. Fruits are fleshy, juicy, dark purple or black, nearly spherical, about 1 cm in diameter[10]. The whole plant is used as anti diuretic, in tumors, neuralgia and splenopathy. It has been reported to contain huge amount of bioactive compounds such as yellow waxy oil, steroids, terpenoids, flavonoids and tannins[11]. The bark extract has been reported to have antiviral, antibacterial, antiprotozoal, hypoglycemic, anticancer and diuretic activities in animal models[12]. Therefore, the aim of the present research work is to analyze the inhibitory activity of linoleic acid (Isolated

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Table 1: GlideScores and GlidEnergies of various ligands with CXCR4 (the natural compounds are identified from ethanolic extract of *Cayratia trifolia* (L.) and carboplatin is a FDA approved drug) complexes are given as calculated from the molecular docking studies.

S. No	Compounds	Glide Score	Glide Energy
1	Linoleic acid	-4.585	-32.114
2	Carboplatin (FDA drug)	-6.024	-25.039

Table 2: ADME properties of selected compounds as predicted by using QikProp module of Schrodinger suite are listed.

S. No	Ligands	Molecular Weight (g/mol)	H-Bond donor	H-Bond acceptor	Log *P* O/W
1	Linoleic acid	280.44	1	2	5.84
2	Carboplatin FDA	144.12	0	2	1.35

from ethanolic extract of *Cayratia trifolia*) against CXCR4 computational molecular analysis.

MATERIALS AND METHODS

Computational molecular analysis

Ligand selection preparation

Based on the previous studies, the isolated compound of linoleic acid[13] and FDA approved drug of Carboplatin (standard drug for comparison) were selected and prepared using the LigPrep 2.4 module from Schrodinger suit[14] for molecular docking analysis. The structure of each ligands were optimized by means of the OPLS 2005 force field using a default setting.

Preparation of protein structure

The high resolution crystal structure of (3D structure) CXCR4 was retrieved from the Protein Data Bank (PDB ID: 3OE6) and it was prepared by protein preparation wizards (standard methods) that are available in grid-based ligand docking with energetics[15]. Protein was optimized using sample water orientation and minimized by using RMSD 0.30 Å and OPLS (2005) force field.

Active site prediction

The binding pockets (active site) and functional residues in CXCR4 were identified and characterized by Site- Map 5.5 module from Schrodinger suit[16]. SiteMap calculation begins with an initial search step that identifies or characterizes- through the use of grid points- one or more regions on the protein surface that may be suitable for binding ligands to the receptor. Contour maps were then generated, hydrogen binding possibilities, hydrophilic maps, produced hydrophobic are may guide the protein-ligand docking analysis[17].

Molecular docking analysis

The docking analysis was performed by using the standard precision (SP) which is Standard mode of Glide 5.6 (Gridbased Ligand Docking with Energetic) module from Schrodinger suit[18]. The selected natural compound of linoleic acid and FDA approved drug were docked in to the binding site of CXCR4 using Glide modiule. The scaling Vander Waals radii were 1.0 in the receptor grid generation. Grid was prepared with the bounding box set on 20Aº. The co-ordinates of this enclosing box with the help of the active site residues to be set default. The force field is using for the docking protocol is OPLS_2005. The docked lowest-energy complexes were found in the majority of similar docking conformations[19].

ADME properties prediction

The CXCR4 ligands of linoleic acid and FDA drug were checked for their ADME properties using QikProp 2.3 module[20]. It helps to analyze the pharmacokinetics and pharmacodynamics of the ligands by accessing the drug like properties. The significant ADME properties such as Molecular weight (MW), H-Bond donor, H Bond acceptor and log P (O/W) were predicted.

RESULTS AND DISCUSSION

In India, great number of plant species had been screened for their pharmacological properties but still vast wealth of rare species is unexplored[21]. Medicinal plants are at interest to the field of novel drug development, as most of the drug industries depend on medicinal plants for the production of novel bioactive compounds[22]. The isolated bioactive compound of linoleic acid posses many biological activities such as anti-inflammatory, anti-microbial and anti-diabetic activities[23]. The best active site (binding pocket/site) was preferred based on the site score and hydrophobic/hydrophilic areas, which holds better binding cavity[24]. The binding site residues of CXCR4 were predicted and it may involve in the binding of substrate and small molecule. Thus, all these residues were confirmed as CXCR4 active site residues and picked to generate grid in the centroid of these residues for molecular docking approach.

Normally the molecular docking study is used to predict the binding orientation of small molecule drug candidate to their protein targets in order to predict the affinity and activity of the small molecule. In the docking results of linoleic acid and FDA approved drug of Carboplatin were complexes with CXCR4 protein shown in Table 1. Among that the natural compound of linoleic acid has good binding affinity when compared with FDA drug.

In the CXCR4-linoleic acid complex (Figure 1) possess Glide score of -4.585 and Glide energy of -32.114 kcal/mol, when compared with CXCR4/carboplatin complex which has Glide score of -6.02 and Glide energy of -25.03 kcal/mol. The linoleic acid strongly binds in hydrophobic region of CXCR4 at LYS239 residue. In the same way CXCR4/carboplatin complex also possess good affinity (Figure 2).

Chemokines and chemokine receptors regulate the physiological movement of immune cells in the body[25]. Among the family of chemokine and chemokine receptors mediating tumor cell invasion and metastasis, CXCL12/CXCR4 has gained a central role in different types of tumors in mediating tumor growth, angiogenesis

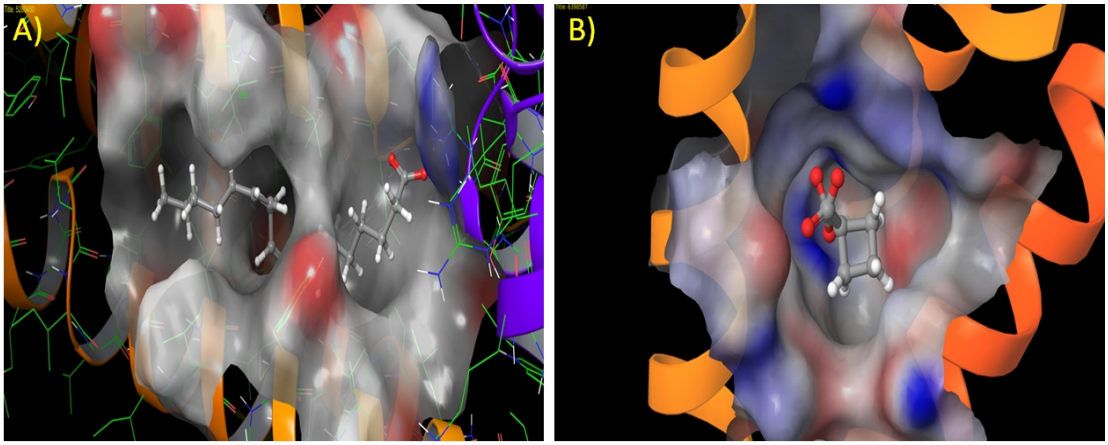

Figure 1: Docking complex of CXCR4 with A) Linoleic acid and B) FDA approved drug generated by using Glide-SP module of Schrodinger suite are shown in this figure. The proteins, ligands and binding pockets are represented in ribbon, sticks and surface models, respectively.

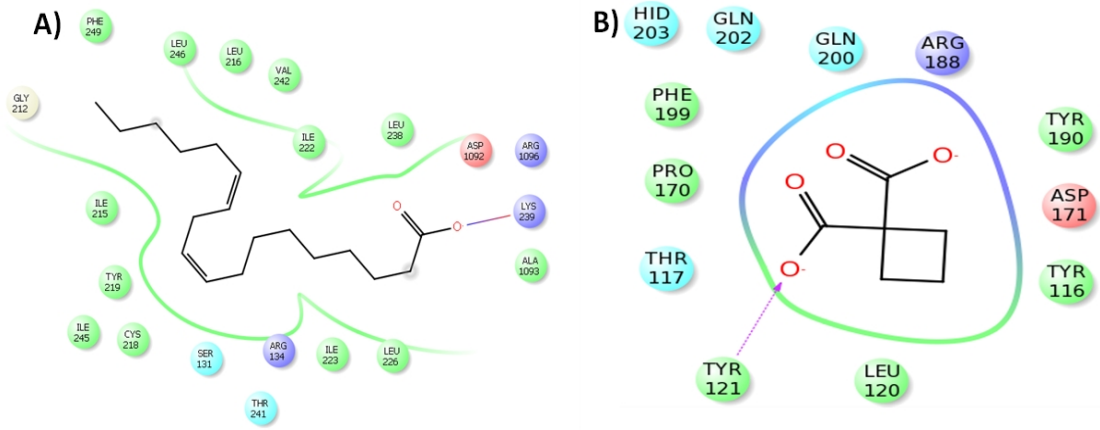

Figure 2: Residues of the CXCR4 that are within 4 Å proximities to A) Linoleic acid and B) FDA approved drug are illustrated in 2D graphics. Dotted arrow lines denote 'Hydrogen bonds' between the corresponding atoms.

and metastasis. In prostate cancer cells, CXCL12 and CXCR4 play a key role in invasion and metastasis, leading to development and expansion of osseous metastasis[26,27]. Thus, targeting CXCR4 can have dual effects on inhibiting primary tumor growth and metastasis or mono effect on inhibiting either tumor growth or metastasis[28]. In the molecular docking analysis the highest negative value of glide score and glide energy indicated that, these complexes may have good affinity[29] and this compound may act as good CXCR4 inhibitor.

The ADME properties prediction of linoleic acid was under acceptable range. The limitations of ADME properties are: not more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptor, molecular mass less than 500 daltons, an octanol- water partition coefficient log P not greater than 5 (Table 2).

CONCLUSION

In the present, the isolated and identified natural compound of linoleic acid was analyzed for their inhibitory activity against CXCR4 using molecular docking analysis and the drug like properties also were predicted. In the results, linoleic acid was strongly binds with CXCR4 when compared with FDA drug. The ADME properties of these compounds were acceptable range. Therefore, based on the results, it can be concluded that, the isolated bioactive compound of linoleic acid may work as novel inhibitor against CXCR4 and it may leads to development of drug agents for variety of cancers and other disorders.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interests regarding the publication of this paper.

ACKNOWLEDGEMENT

The authors are thankful to our Chancellor, Chief Executive Officer, Vice-Chancellor and Registrar of Karpagam University for providing facilities and encouragement. Our grateful thanks to Indian Council of Medical Research (ICMR), New Delhi for providing the financial support (File No: BIC/11(19)/2013) to this research work. We extend our deepest thanks to Dr. D. Jeya Sundra Sharmila, Karunya University for providing us an opportunity to use Schrodinger Suite (in silico analysis).

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