Research Article**Molecular docking studies of interaction between protease activated inhibitor receptor as the crucial protein and hydroxyl cinnamic acid as ligands****Muttevi Hyagreva Kumar¹, Prabhu^{2*}, C. Sumathi Jones³, Aparna Ravi⁴**^{1,2}Sree Balaji Medical College & Hospital (SBMCH), Bharath University, BIHER, Chennai, India³Asan Memorial Dental College and Hospital (AMDECH), Chengalpattu, India⁴Azzazia institute of medical science and research, Kollam, India

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Abstract

Background: Cinnamic acid is a key intermediate in shikimate and phenylpropanoid pathway which is a precursor of many aromatic amino acid, alkaloids and indole derivatives. The biological activities of cinnamic acid derivatives include anti TB, antidiabetic, antioxidant, antimicrobial, as a flavoring agent, hepatoprotective, CNS depressant, anticholesteremic, antifungal, antihyperglycemic, antimalarial, antiviral, anxiolytic, cytotoxic, anti-inflammatory agents. **Objective:** The interaction between the protein and the ligands are well established by docking studies. **Material and methods:** The present study involves the interaction between protease activated receptor inhibitor as the crucial protein and hydroxyl Cinnamic acid as ligands. Molecular properties, toxicity and ADMET properties of these compounds are calculated using molinspiration and PreADMET calculator. Docking studies were performed using autodock 4.0. the molecular properties and toxicity were analyzed and energy obtained for hydroxyl cinnamic acid and protease activated receptor indicate that these ligands interact with specific residues in the active site regions. **Results and conclusion:** The molecular formula and molecular weight were $C_9H_8O_2$ and 148.05, and the number of hydrogen bond and polar surface area was found to be 2 and $28.62 \text{ \AA}^2$. The molecular volume is $153.60 \text{ \AA}^3$ and the stereo centers were zero obtained as the drug like lines score was found to be -1.13. The value of solubility, molecular weight, drug likeliness, drug score, mutagenic and anti-tumor activities and implies that cinnamic acid is devoid of toxic and mutagenic effects. The results from the study illustrates the possibility of utilizing the ligand cinnamic acid as an antagonist / inhibitor that can modulate the activity of protease activated receptor. However, further experimental studies may be required to specify accurately the binding affinity and duration of inhibition of the ligand.

Keywords: Cinnamic acid, protease activated receptor, ligand, docking**Introduction**

p-methoxy cinnamic acid have been identified as main bioactive molecules in many herbs. Cinnamic acid and its derivatives are naturally occurring substances found in fruit, vegetables, and flowers are consumed as dietary phenolic compounds. Cinnamic acid commonly is found in free form and ester form in essential oils, resins and balsams, oil of cinnamon, balsam of peru and balsam of tolu etc. has very high therapeutic applications that include antioxidant, hepatoprotective, anti TB,

anxiolytic, insect repellent, antidiabetic, anticholesteremic, antimicrobial, as a fragrance material, antimalarial, anti-inflammatory agents. Cardiogenic and antiangiogenic activities of cinnamic acid is due to the presence of Phytosterols. Cinnamic acid is a key intermediate, in shikimate and phenylpropanoid pathways. Shikimic acid is a precursor of many alkaloids aromatic amino acids and indole derivatives (Bothara et al., 1998; Rahman et al., 2013; Lone et al., 2014) It is also a U-V ray absorbent (Sairam, 1998) The derivatives of Cinnamic acid are reported to have strong antioxidant effects. (Sharma, 2011). The p-hydroxy and methoxy groups in cinnamic acid derivatives also showed good insulin releasing activity (Kim et al., 2004). Due to the presence of Esters, amides and substituted derivatives of cinnamic acid it also shows anti-

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microbial activity (Sun et al., 2007). The role of hepatoprotective property and CNS depressant activity is due to the presence of Hydroxy cinnamic acid and halogenated cinnamic acid and 3- phenyl propionyl moiety will result in anti malarial activity (Barontini et al., 2014). The lipid lowering efficacy by the derivatives of cinnamic acid showed anticholesterolemic activity (Vu et al., 1991; Adisakwattanna et al., 2008). The derivatives of cinnamic acid also possess to have antiviral and antifungal property. cinnamic acid also showed the anti-inflammatory activity of on E- selectin mediated cell-cell adhesion and the derivative of cinnamic acid also shows anti-anxiety (Turm et al., 2010).

Enhanced expression of PAR1 was observed in invasive and metastatic tumours and, interestingly, the expression levels directly correlated with the degree of invasiveness of the cancer. It belongs to the G-protein-coupled receptors (GPCRs), which mediate cellular response to specific proteases (Gieseler et al., 2013).

Computational methods have become a routine method during the drug discovery process, in which molecular docking is one of the important techniques for accelerating the process. Making a prediction of the ligand-receptor complex structure is the main role of computational methods that use molecular docking. Docking can be achieved through two important steps: first by sampling conformations of the ligand in the binding pocket of the protein; then ranking these conformations via a scoring function (Turm et al., 2010). In this study three dimensional study of cinnamic acid was mined from the Pubchem compound library 3- dimensional entity of cinnamic acid and was imported as structure data file and it was converted into protein data file using molecule file convertor and docked with protease activator to analyse the toxicity and clotting effects of cinnamic acid.

Materials and methods

Molecular properties and bioactivity by molinspiration

In this study the data was retrieved from biological databases like protein data bank and carried out with on line tools like Molinspiration and ADME prediction and auto dock. Protein data bank is a repository for the 3-D structural data of large biomolecules such as protein, DNA and RNA. NCBI Pubchem is a chemical compound database that provides information on biological activities of small molecules. The structural data base calculates drug related properties like mutagenic, tumorigenic, irritant, reproductive effective, clp, solubility, Mol weight, drug likeness. Molecular properties such as drug likeness, Milog P and TPSA. MilogP were predicted by web based software molinspiration as a sum of fragment based contribution and correction factors. ADME is used in pharmacokinetics and pharmacology for absorption, distribution, metabolism and

elimination and autoDock uses genetic Algorithm for molecular docking (Ernawati et al., 2017)

Preparation of protein

The crystal structure of protease inhibiting factor used in this study was retrieved from RCSB protein Data Bank. Hydrogen was added and the protein was prepared as PDBT in Autodock and was used further for docking studies. The ligands are h- cinnamic acid and SDF files were retrieved from NCBI Pubchem and prepared for docking using SPDBV. Optimization of the ligands was carried out in Auto dock 4.0.

Toxicity studies

The toxicity analysis of ligands were carried out by Molinspiration and Pre ADMET and were selected to perform docking studies using autodock softwares. The permeability across the cell membrane which is predicted by Milog P parameter, the hydrogen bonding potential of compound which is predicted by total polar surface, total volume and rotatable bonds which predicts the molecular flexibility and above interprets the absorption index and bioavailability of drugs (Gratio et al., 2010).

Computational methods

Docking calculations using docking server and the docking parameters were set default before the docking the ligand actions, the gasteiger partial charges were added and rotatable bonds were selected and further hydrogen atom, non polar hydrogen bonds were merged and salvation parameters were implemented for molecular docking using auto dock tools.

Docking calculations were performed at a eugenol with metatrophic glutamate receptor affinity grid map of xxa grid point and 0.375 a spaces were generated using the auto grid program (Gehlhaar et al., 1995).

Result and Discussion

Docking study

Docking was performed between protease activator receptor and cinnamic acid using auto dock and binding energy values are indicated in table 1 and interaction are indicated in figures 1 and 2.

The values of ligands of p- cinnamic acid are 3396, 357.50 area and transformation occurs from -2.88 to 57.34 respectively. The molecular formula and molecular weight were $C_9H_8O_2$ and 148.05, and the number of hydrogen bond and polar surface area was found to be 2 and $28.62 \text{ \AA}^2$. The molecular volume is $153.60 \text{ \AA}^3$ and the stereo centers were zero obtained as the drug like lines score was found to be 1.13.

Table 1a. Pharmacokinetic profile of the ligand cinnamic acid

Absorption Human intestinal absorption (HIA%)	In vitro Ca Co-2 cell permeability(nm/sec)	In vitro MDCK cell permeability (Nm/sec)	IN vitro skin permeability (logkp,cm/hour)	In vitro blood brain penetration (c. brain / C. blood)	Distribution In vitro plasma protein binding (%)
98.059	21.5421	225.843	-1.7416	1.93217	73.964

Table 1b. Toxicity evaluation of cinnamic acid using property calculator

Drug likeness score	Mutagenic Carcino-Mouse	Tumoric
-1.13	negative	negative

Table 1c. Molinspiration property values of cinnamic acid

Millogp	TPSA	Mol.wt	No.N	N.ohnh	violations	Nrotb
2.33	28.62	148.05	3	2	0	2

Bioavailability and Toxicity studies

To recognize as drug bioactivity is very essential and the parameters such as G- protein coupled receptor (GPCR), ion channel modulator, kinase inhibitor, nuclear receptor and protease inhibitor and enzyme inhibitor are predicted as 0.15, -0.07-0.26, 0, -0.38 and 0.28. The docking cycles and other parameters were used according to the Murris method and the docking score profile were presented as shown in the figure 1.

Furthermore, the figures 3a and 3b indicate the value of solubility, molecular weight, drug likeness, drug score, mutagenic and anti-tumor activities and implies that cinnamic acid is devoid of toxic and mutagenic effects. By using Pre ADMET caco-2 cell permeability, plasma protein binding, blood brain barrier, skin permeability, human intestinal penetration and the properties of the compounds were calculated. The compounds have medium cell permeability and have strong plasma protein binding and the compounds were assessed for inhibiting protease activator by molecular docking studies and binding interactions (Table 1a, 1b and 1c).

The docking results suggest that the compound 3d has good orientation shape with active site by hydrogen bonds (Figure 2), hydrophobicity (figure 3d) and electrostatic interaction indicating that the molecule has a good interaction with the receptor. High Molinspiration values observed in protease indicates cinnamic acid to possess antagonistic activity (Figure 3c).

A molecule having bioactivity score more than 0.00 is most likely to exhibit considerable biological activities, while values -0.50 to 0.00 are expected to be moderately active and if score is less than

0.50 it is presumed to be inactive (Figure 3 c).

Figure 1. Ribbon diagram of the Docking of Activated protease inhibitor with Cinnamic acid **Figure 2.** Binding mode of protease activated inhibitor receptor and hydroxyl cinnamic acid as ligands

Figure 3a. Molecular properties and Drug likeness

Figure 3b. Model score of drug likeness

Figure 3c. Molinspiration bioactivity score of ligands

Figure 3d. Hydrophobicity of activated protease inhibitor

PARs are normally activated by the proteolytic exposure of an occult tethered ligand. Proteinase activated receptors are a sub family of G- protein coupled receptors and plays a major role in hemostasis, thrombosis, wound healing, inflammation, cancer progression and embryonic development and they are main target for the coagulation enzyme thrombin (Ooi et al., 2006). The unmasked amino termino function as tethered ligand were selected as drug target and cinnamic acid binds to structures on the cellular membrane to trigger conformational changes and signals to exhibit a mechanism of hemostatic activation. Many traditional plants and herbs have been identified to have hemostatic property and cinnamic acid have been identified as bio active molecules to have hemostatic property. The bioactivities were also screened through insilico analysis and bioactivities tools calculates the drug solubility, absorption digestive capacity and metabolic rate at receptor level and monslinspiration tool were used to predict the molecular properties and molsoft drug likeliness score of cinnamic acid was predicted and bioactivity parameters indicates that cinnamic acid as potent target for hemostatic properties (Schulz-Gasch and Stahl, 2004).

In general, drugs in the protease and GPCR-peptidic families are characterized by significantly higher average molecular weight,

while those in the ion channel family have lower average molecular weight. The ligand cinnamic acid have good biological activity and enzyme inhibitor score (0.15 and 0.23) respectively. Topological polar surface area is very much correlated with the hydrogen bonding of a molecule and is a very good as topological polar surface area is very much correlated with the hydrogen bonding (Han et al., 2011; Asif Husain et al., 2016).

Conclusion

Protease activated receptor inhibition is a unique mechanism and promising tool as a novel therapeutic target for cessation of the progression of invasive and metastatic cancers. The results from the study illustrates that possibility of utilizing, the ligand cinnamic acid as an antagonist /inhibitor that can modulate the activity of protease activated receptor. However, further experimental studies may be required to specify accurately the binding affinity and duration of inhibition of the ligand.

Authors Contribution

Muttevi Hyagreva Kumar has obtained cinnamic acid and docking studies were carried out as part of his thesis Dr prabhu supervised the research and contributed in the

writing, design, synthesis, biological evaluation and analysis of the data. Dr. C. Sumathi have been involved in drug likeliness and toxicity analysis, interpretation of results, manuscript writing and communicating to journal. Aparna Ravi was assisting in overall study. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that there exists no conflict of interest among the authors.

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