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Research Article

NORTRACHELOGENIN -AN ACTIVE COMPONENT IN *ZINGIBER OFFICINALE* AS AN EFFICIENT THROMBOLYTIC AGENT – AN *IN SILICO* APPROACH

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ABSTRACT

Thrombosis is a very serious condition that is often treated with anti-coagulants or blood thinners. Due to the serious threat that it entails, it is necessary to identify efficient alternatives from natural sources to prevent thrombosis. This paper discusses one such attempt to study the efficiency of the phytoconstituents in *Zingiber officinale* for thrombolytic activity using molecular docking analysis. One of the bioactive compounds in *Zingiber officinale* namely Nortrachelogenin was assessed for its drug-likeliness using ADMETSAR server and it was found that the compound was in compliance with the Lipinski's rule of five. The binding affinity of Nortrachelogenin with the targets for thrombosis namely Protein Disulphide Isomerase, Plaminogen Activator Inhibitor-1, Plasmin, Thrombin Activated Fibrinolysis Inhibitor and thrombin was performed using Autodock following the standard procedure. Clopidogrel, the standard drug for thrombosis was used as a reference and it was found that Nortrachelogenin has more binding affinity and higher inhibition for PAL-I, Plasmin and TAFI than the standard drug Clopidogrel. This finding suggests further testing of Nortrachelogenin as potential thrombolytic agent that will be better than the existing drugs.

Keywords: Nortrachelogenin, Thrombosis, Thrombolysis, Docking, *In silico, Zingiber officinale*.

1. INTRODUCTION

Thrombosis is the clotting of blood in the blood vessel resulting in decreased rate of blood flow. Small clots dissolve easily and the bigger clots can lead to serious health problems including Myocardial infarction and thrombotic stroke when blood flow is blocked to heart and brain. Thrombolysis or fibrinolytic therapy is the treatment to dissolve the blood clots in the blood vessels, thereby improving the blood flow. There are many proteins that are involved in the mechanisms leading to thrombosis and some of them are Protein Disulphide Isomerase (PDI), Plasminogen Activator Inhibitor (PAI-1), Plasmin, TAFI and Thrombin.

Protein Disulphide Isomerase(PDI) belongs to PDI protein family which has at least one domain with a thioredoxin-like fold [1]. PDI is expressed in almost all mammalian cell but, Endoplasmic reticulum has it in higher abundance [2, 3]. It has three catalytic domain each for thiol redox reactions, disulphide exchange and Chaperone activity, all of which are necessary for the functioning of Endoplasmic reticulum [4]. It was found in laser induced arteriolar mouse models that PDI was essential for platelet thrombus formation, generation of fibrin and hemostasis [5].

Plasminogen Activator Inhibitor (PAI-1) is an inhibitor of Plasminogen activator, which belongs to the serpin superfamily [6]. Plasminogen activator is a serine protease which activates plasmin which is an important component of the fibrinolysis cascade. When PAI-1 inhibits Plasminogen activator, fibrinolysis is inhibited thus leading to thrombosis. PAI-1 can easily switch between active and inactive states [7]. In plasma, PAI-1 exists at very low concentrations of 5-20 ng/mL under normal conditions and has a dynamic regulation in response to a variety of stimuli.

The main fibrinolysin is the plasmin protein which is activated by serine proteases. Although plasmin is tightly regulated, it is important to design inhibitors to prevent excess unregulated plasmin [8]. Thrombin activated fibrinolysis inhibitor (TAFI) is a carboxypeptidase decreases the number of plasminogen binding sites through removal of C-terminal lysine and arginine thereby decreasing the generation of Plasmin [9, 10]. It belongs to non-serpin fibrinolysis inhibitor

which is activated by thrombomodulin-associated thrombin. Finally, thrombin is an important protein in hemeostasis. It activates platelets, procofactors FV and FVIII that are needed for its own generation. When 10nM of thrombin-antithrombin complex is formed, the propagation phase of clot formation starts.

Thrombosis can be prevented at various stages by inhibition of any of the proteins namely, PDI, PAI-1, Plasmin, TAFI of Thrombin. The current work is an aim to identify phytoconstituents in *Zingiber officinale* and test for its thrombolytic activity using molecular docking approach and compare its efficiency with the standard drug Clopidogrel.

2. MATERIAL AND METHODS

The chemical structures for the ligand molecules were created in Mol format using ACD Lab's Chemsketch. The ligands were assessed for compliance with Lipinski's rule using ADMETSAR Web server [11, 12]. Lipinski's rule of 5 is a rule to evaluate the drug-likeliness of chemical compounds [13]. The receptors for the docking were retrieved from Protein Data Bank [14]. Protein Disulphide Isomerase (PDB Id: 4EL1), Plaminogen Activator Inhibitor I (PDB Id:4AQH), Plasmin (PDB Id:1BML), Thrombin Activated Fibrinolysis Inhibitor(PDB Id:3D67), and Thrombin (PDB Id:3EQ0) were retrieved from PDB and used as receptors for the molecular docking studies. Molecular Docking of the identified receptor and synthesized ligands was performed using Autodock Tools (ADT) 1.5.6 [15] by employing the following method:

2.1. Preparation of Receptor and Ligand files

Autodock entails both the receptor and ligand in PDBQT format for assessing the binding affinity between them. PDBQT format restrains the atomic coordinates, partial charges and atom types. Initially, the receptor file in PDB format obtained from Protein Databank was accessed in Autodock Workspace. The water molecules in the receptor file were removed and implicit Hydrogen atoms were added. Finally, partial charges were added and the receptor file was saved in PDBQT format. Similarly, the ligand files in PDB format was retrieved by Autodock and saved in PDBQT format.

2.2. Preparation of Grid and Dock Parameter files

Autogrid 4.2 program in ADT was used to perform the grid computation. The grips maps with a dimension of 90X90X90 and spacing of 0.375 Å were centered along the ligand binding site. The receptor and ligand files in PDBQT format along with the grid maps were saved as the grid parameter file to execute the Autogrid program. After the autogrid calculation, Autodock parameter file was created with the receptor, ligand and selection of Autodock parameters.

2.3. Docking and Visualization

Docking was performed using Lamarckian Genetic Algorithm with Ten independent runs per ligand with an initial population of 150 randomly placed ligand on the receptor binding site. A maximum of $2.5X10^5$ evaluations on the energy will be carried out for $27X10^3$ generations with a mutation rate of 0.02 and a cross over rate of 0.80. The local-energy-minimization algorithm was limited to 100 steps for 6% of the population. To explore the conformational space of ligands, the overall translation steps was set to 0.2 Å, and the overall rotation and torsion rotation step were set to 5 in the docking studies. The Autodock 4.0 program in ADT was executed and the docking scores were reported using binding free energies in kcal/mol. The bound complex with the receptor and ligand was visualized using Pymol.

3. RESULTS AND DISCUSSION

The docking studies were performed with the targets Protein Disulphide Isomerase (PDB Id:4EL1), Plaminogen Activator Inhibitor I (PDB Id:4AQH), Plasmin (PDB Id:1BML), Thrombin Activated Fibrinolysis Inhibitor(PDB Id:3D67), and Thrombin (PDB Id:3EQ0) and the phytoconstituent in the *Zingiber officinale* namely Nortrachelogenin (Fig. 1). In addition, docking was done with the standard drug for thrombosis namely Clopidrogel (Fig. 2).

The assessment of the drug likeliness and ADME properties of Nortrachelogenin in comparison to the standard drug Clopidrogel (Table 1) reveals that the ligand Nortrachelogenin did not violate any of the Lipinski's rule of five. Nortrachelogenin has a mass of 358.35 Da, little higher than the standard Clopidrogel but well within the Lipinski range of 130 and 725 Daltons. The number of Hydrogen bond donors and acceptors in Nortrachelogenin are 7 and 3 respectively against 4 and 0 in Clopidrogel. The Log P value of 1.90 for Nortrachelogenin unravels its optimal hydrophobicity to be a drug. The number of rotatable bonds and good Human Intestinal adsorption value further validates its potential as a drug. Both the standard

Clopidrogel and the ligand Nortrachelogenin have negative Human Oral Bioavailability.

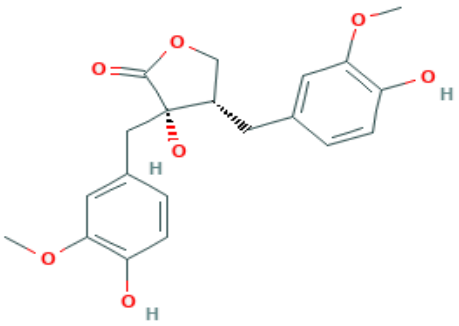


Fig. 1: Structure of Nortrachelogenin

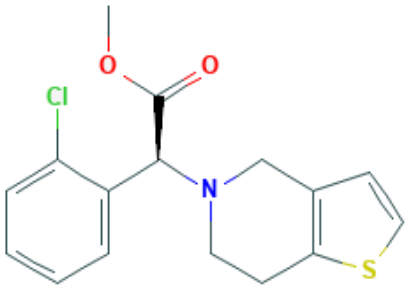


Fig. 2: Structure of Clopidrogel

Since, Nortrachelogenin follows all the rules for an ideal drug, molecular docking was performed with the selected target proteins to know its potential as a thrombolytic agent.

The propensity of Nortrachelogenin (Binding energy = -4.36 kcal/mol) to bind with PDI is much higher than that of the standard drug Clopidogrel as revealed by the higher binding energy. The binding energy per non-hydrogen atom of Nortrachelogenin called the ligand efficiency (-0.17 kcal/mol) is also higher than that of the standard drug Clopidogrel. The inhibitory constant which portrays the concentration at which half maximum inhibition happens for Nortrachelogenin (643.74 µM) is much higher than that of the standard drug Clopidogrel. The other internal energy parameters such as Intermolecular energy, Vanderwaals energy, electrostatic energy and torsional energy are also not better for Nortrachelogenin than the standard drug Clopidrogel. PDI has several roles in different disease states and its role in cardiovascular disease is detrimental. This is due to its role in thrombus formation by platelet accumulation [16]. Inhibition of PDI is one of the ways to prevent thrombosis in Coronary artery disease [17] and studies aim to identify such inhibitors for treating thrombosis. The molecular docking studies with PDI and Nortrachelogenin did not validate it as a better inhibitor than the available standard Clopidogrel.

Table 1: ADME properties of Nortrachelogenin and Clopidrogrel

Descriptors	Standard Values	Nortrachelogenin	Clopidogrel
Molecularweight (Da)	130.0 –725.0	358.35	321.83
Number of Hbond acceptors	2.0 - 20.0	7	4
Number of Hbond donors	0.0 / 6.0	3	0
QP log P foroctanol/ water	-2.0/6.5	1.90	3.67
Lipinski Rule of5 Violations	(maximu mis 4)	0	0
Number of rotatable bonds	>10 is poor oral availability	1	3
Human Intestinal Absorption	(<30% is poor)	Good	Good
Human Oral bioavailability	(>0 is positive)	Negative	Negative

The lower binding energy of Nortrachelogenin (-7.08 kcal/mol) as against -6.2 kcal/mol for the standard drug Clopidogrel with the target PAI-1 is a promising evidence for the use of Nortrachelogenin as a better alternative to Clopidogrel to treat thrombosis. To further validate it, the much lower inhibitory constant for Nortrachelogenin (6.46 µM) than Clopidogrel (28.57 µM) makes it a better alternative with lower dosage to treat thrombosis. All the other internal energy parameters such as Electrostatic energy, Vander waals energy and Torsional energy are better than Clopidogrel. Also, the number of hydrogen bonds formed by Nortrachelogenin with PAI-1 is equal to that of the number of hydrogen bonds formed by Clopidogrel (Table 4). In vitro studies have confirmed the reduced activity of plasminogen activation and a higher clot lysis time with higher levels of PAI-1 in plasma [18, 19]. Similar kind of evidence is seen in myocardial infarction where there are high levels of PAI-1 in plasma [20, 21]. These studies further

extrapolate the need to inhibit PAI-1 to treat thrombosis. *In silico* molecular docking studies has identified one such small molecule namely Nortrachelogenin, which has better affinity with PAL-1 than the standard Clopidogrel.

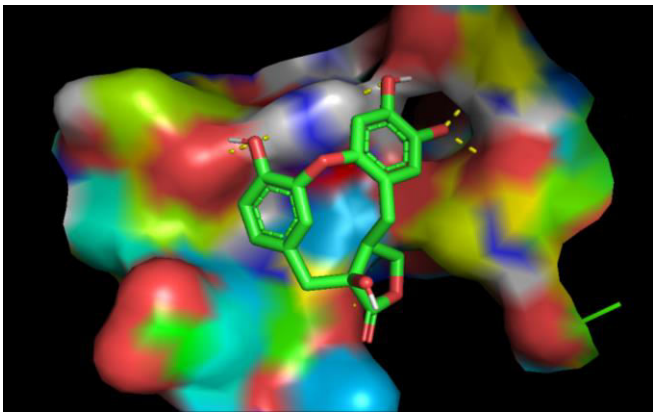


Fig. 3: Molecular Surface of PDI docked with (-)-Nortrachelogenin

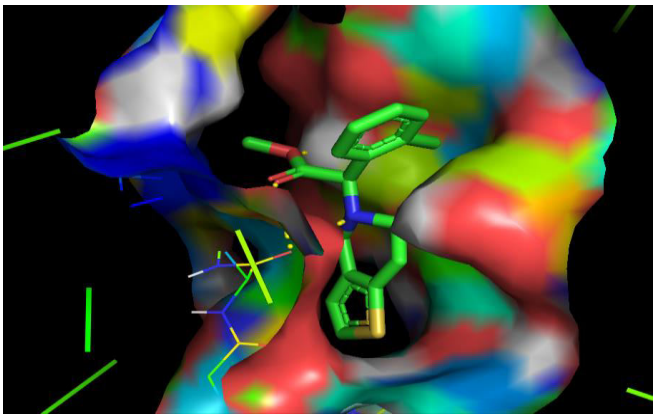


Fig. 4: Molecular Surface of PDI docked with Clopidogrel

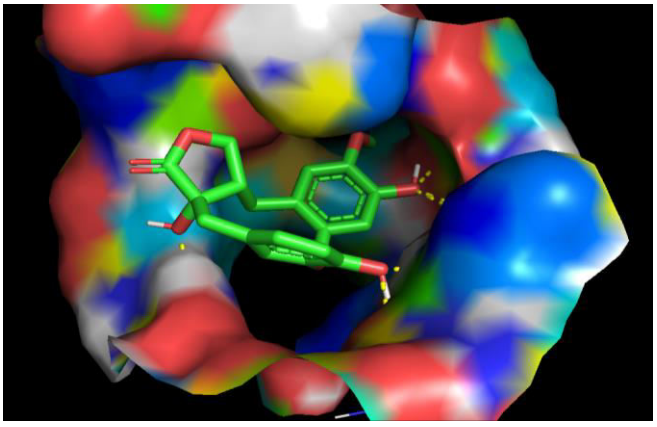


Fig. 5: Molecular Surface of PAI-1docked with (-)-Nortrachelogenin

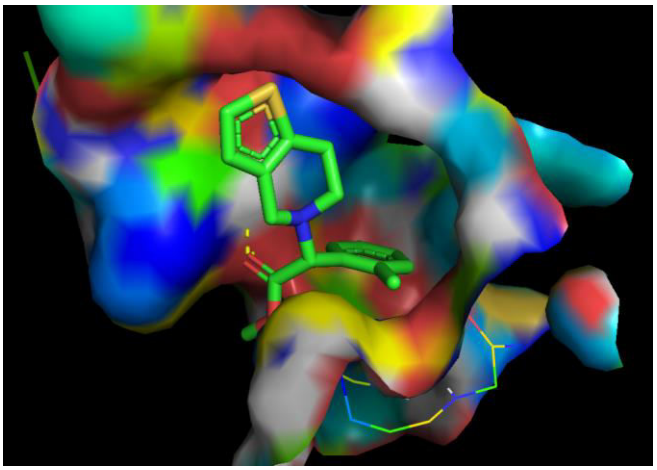


Fig. 6: Molecular Surface of PAI-1 docked with Clopidogrel

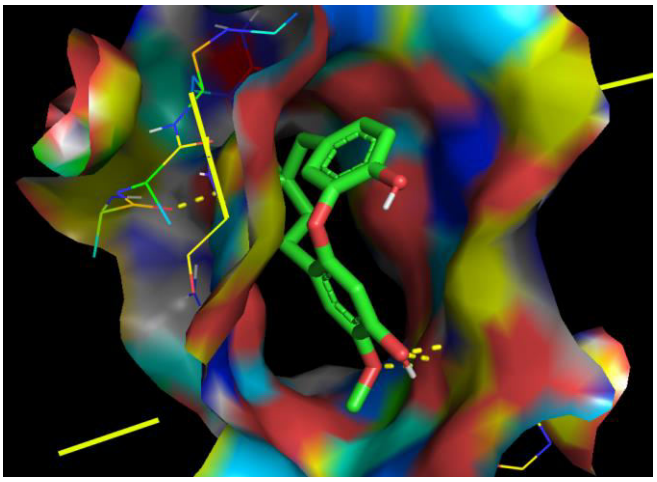


Fig. 7: Molecular Surface of Plasmin docked with (-)-Nortrachelogenin

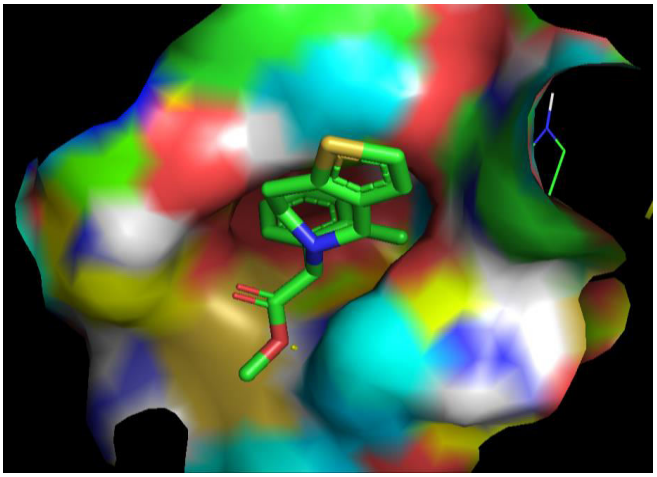


Fig. 8: Molecular Surface of Plasmin docked with Clopidogrel

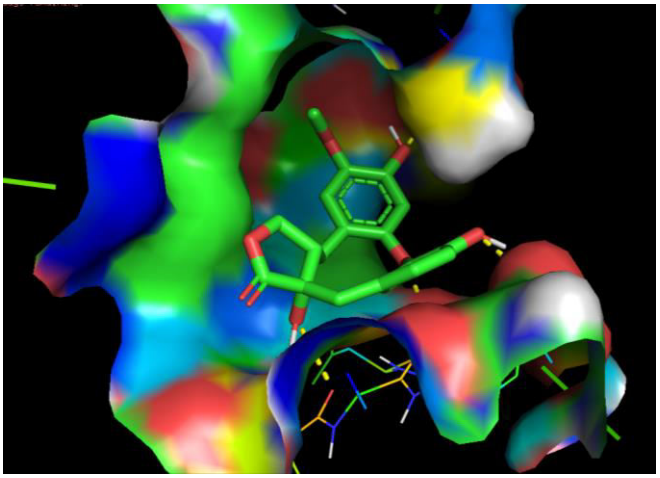


Fig. 9: Molecular Surface of TAFI docked with (-)-Nortrachelogenin

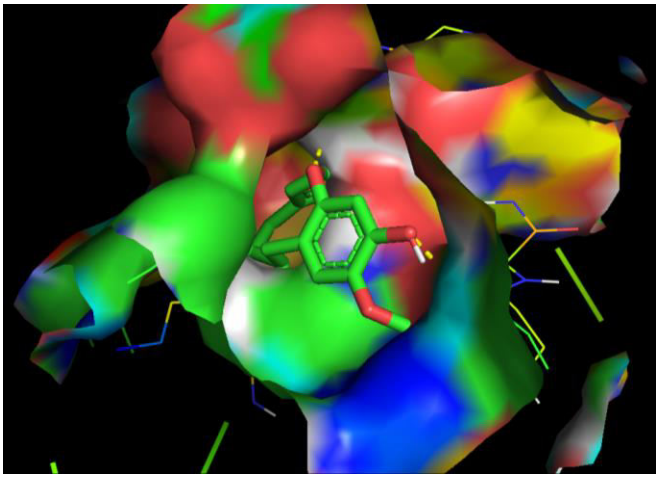


Fig. 11: Molecular Surface of Thrombin docked with(-)-Nortrachelogenin

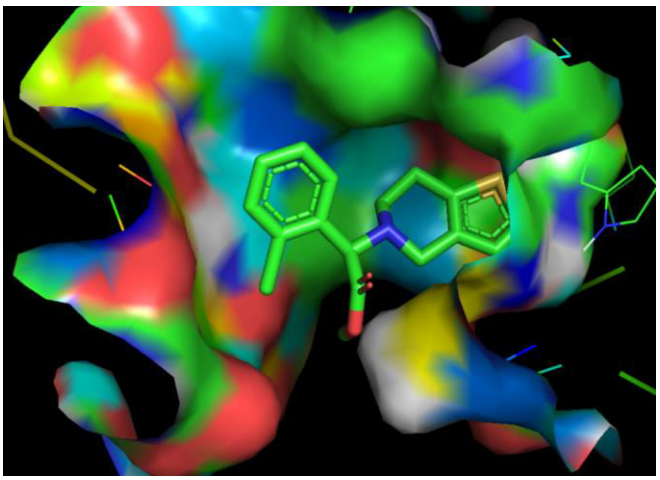


Fig.10: Molecular Surface of TAFI docked with Clopidogrel

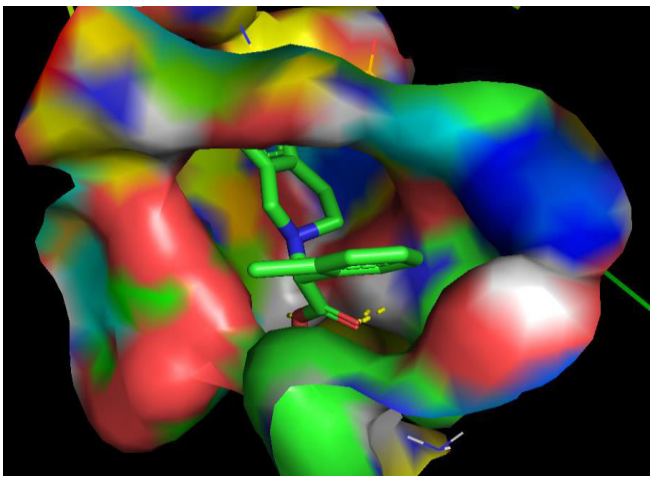
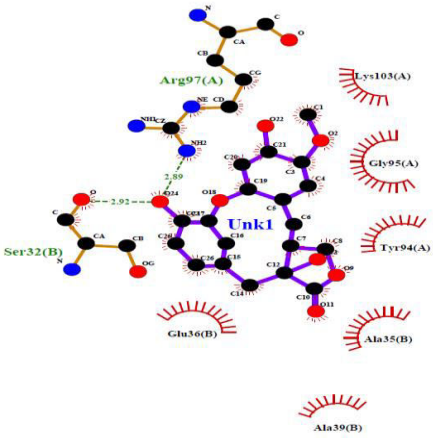
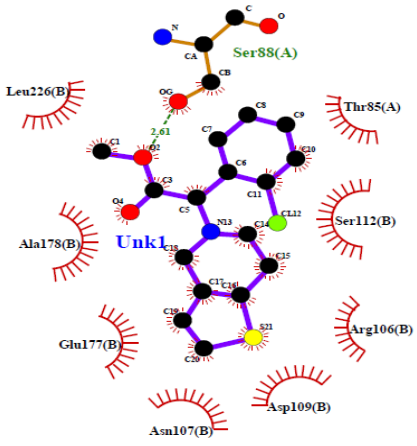


Fig. 12: Molecular Surface of Thrombin docked with Clopidogrel

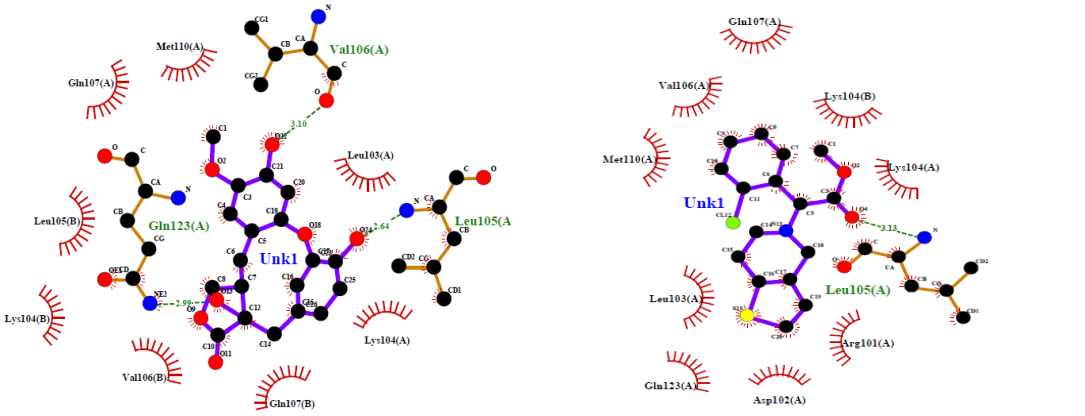


Nortrachelogenin



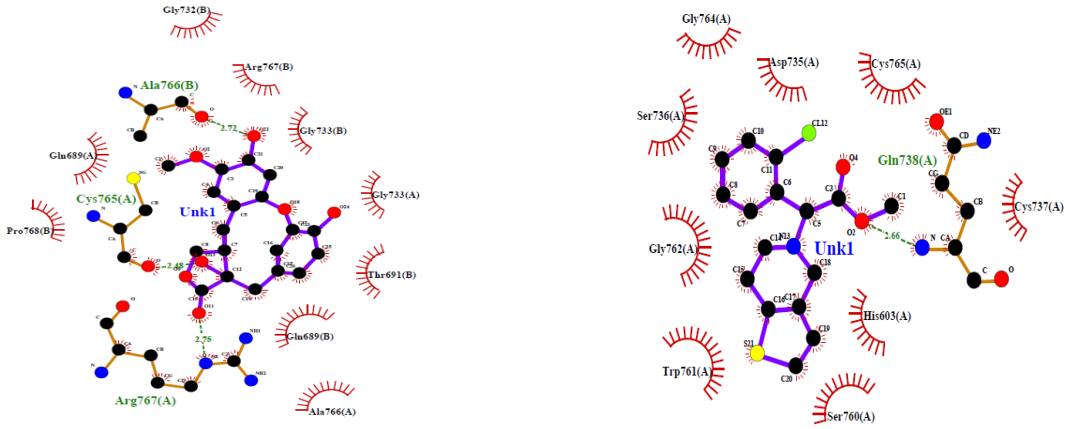
Clopidogrel

Fig. 13: Residues involved in interactions of PDI with ligands (4EL1)



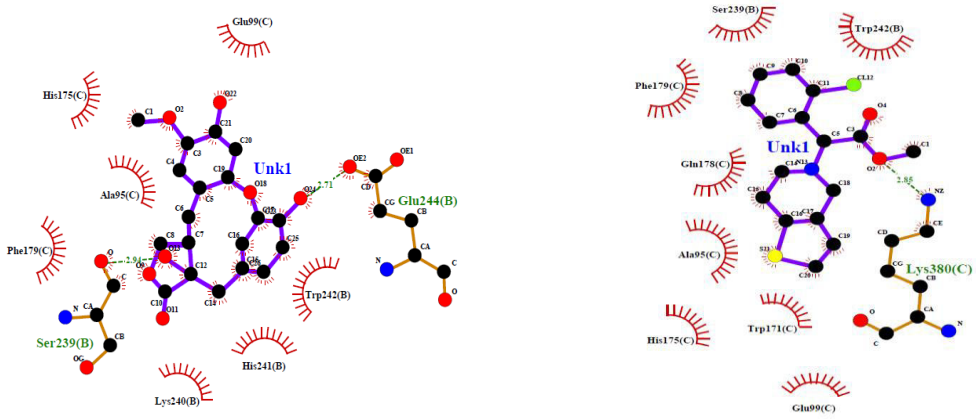
Nortrachelogenin **Clopidogrel**

Fig. 14: Residues involved in interactions of PAI-1 with ligands (4AQH)



Nortrachelogenin **Clopidogrel**

Fig 15: Residues involved in interactions of Plasmin with ligands (1BML)



Nortrachelogenin **Clopidogrel**

Fig 16: Residues involved in interactions of TAFI with ligands (3D67)

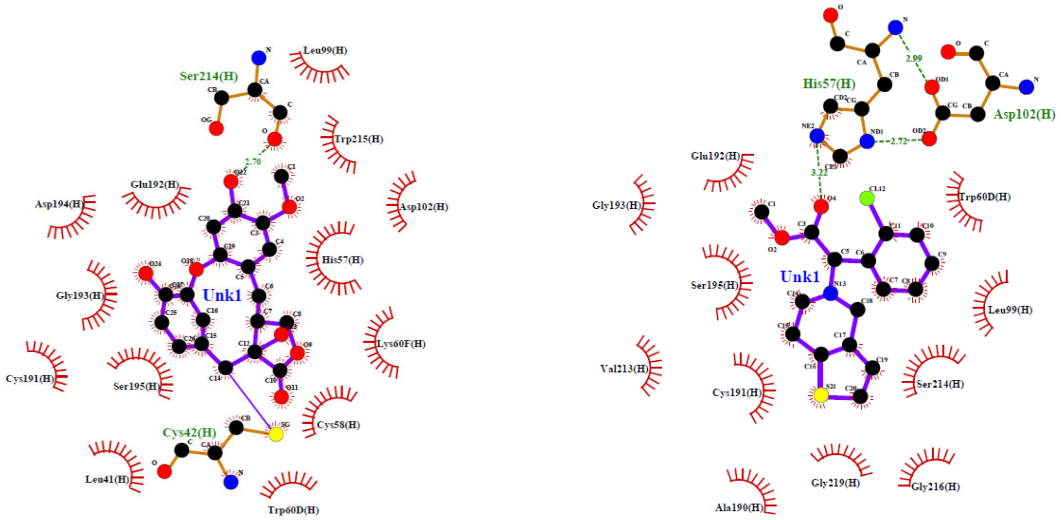


Fig 17: Residues involved in interactions of THROMBIN with ligands (3EQ0)

Table 2: Molecular Docking parameters for Nortrachelogenin and Clopidrogrel with PDI, PAI-1 and Plasmin

Parameters	PDI		PAL-1		PLASMIN	
	I	II	I	II	I	II
Binding Energy kcal/mol	-4.36	-6.51	-7.08	-6.2	-7.23	-6.82
Ligand Efficiency	-0.17	-0.31	-0.27	-0.3	-0.28	-0.32
Inhibitory Constant	643.74	16.8	6.46	28.57	4.99	9.94
Inhibitory Constant units	µM	µM	µM	µM	µM	µM
Intermol energy	-5.55	-7.71	-8.27	-7.39	-8.43	-8.02
Vdw_hb_desolv_energy	-5.39	-6.42	-8.16	-7.54	-8.17	-7.44
Electrostatic energy	-0.16	-1.28	-0.11	0.15	-0.25	-0.58
Total_Internal	-0.97	-1.24	-0.94	-1.27	-1.12	-0.87
Torsional_Energy	1.19	1.19	1.19	1.19	1.19	1.19
Unbound_Energy	-0.97	-1.24	-0.94	-1.27	-1.12	-0.87

I-Nortrachelogenin; II-Clopidogrel

Table 3: Molecular Docking parameters for Nortrachelogenin and Clopidrogrel with TAFI and Thrombin

Parameters	TAFI		Thrombin	
	I	II	I	II
Binding Energy kcal/mol	-6.88	-6.1	122.16	-8.57
Ligand Efficiency	-0.26	-0.29	4.7	-0.41
Inhibitory Constant	9.07	33.53	-	525.54
Inhibitory Constant units	µM	µM	-	nM
Intermol energy	-8.07	-7.3	120.97	-9.76
Vdw_hb_desolv_energy	-7.84	-7.19	121.31	-8.65
Electrostatic energy	-0.23	-0.11	-0.34	-1.11
Total_Internal	-0.81	-0.96	-1.08	-0.75
Torsional_Energy	1.19	1.19	1.19	1.19
Unbound_Energy	-0.81	-0.96	-1.08	-0.75

I-Nortrachelogenin; II-Clopidogrel

Table 4: H-bond Interactions of the Targets with the Ligands

Protein	Ligand	No. of Hydrogen bonds	Interacting Residues
PDI (4EL1)	Nortrachelogenin	2	A: Ser32, A: Arg97
	Clopidogrel	2	A: Ser88 (2)
PAL-1 (4AQH)	Nortrachelogenin	3	A: Leu103, A: Leu105, A: Gln123
	Clopidogrel	3	A: Gln107 A: Arg101, A: Leu103
PLASMIN (1BML)	Nortrachelogenin	2	B: Ala766 A: Arg767
	Clopidogrel	1	A: Gln738
TAFI (3D67)	Nortrachelogenin	2	B: Ser239, B: Glu244
	Clopidogrel	2	C: Gln178, C: Lys380
THROMBIN (3EQ0)	Nortrachelogenin	3	H: Lys60, H: Ser195 H: Lys60
	Clopidogrel	2	H: Gly193 H: Ser195

The lower binding energy of Nortrachelogenin (-7.23 kcal/mol) as against -6.82 kcal/mol for the standard drug clopidogrel with the target Plasmin is a promising evidence for the use of Nortrachelogenin as a better alternative to Clopidogrel to treat thrombosis. To further validate it, the much lower inhibitory constant for Nortrachelogenin (4.99 μM) than Clopidogrel (9.94 μM) makes it a better alternative with lower dosage to treat thrombosis. All the other internal energy parameters such as Electrostatic energy, Vanderwaals energy and Torsional energy are better than Clopidogrel. Also, the number of hydrogen bonds formed by Nortrachelogenin with Plasmin is more than that of the number of hydrogen bonds formed by Clopidogrel (Table 4). As Plasmin is produced from Plasminogen activator, inhibiting it would be measure to treat thrombosis. *In silico* molecular docking studies has identified one such small molecule namely Nortrachelogenin, which has better affinity with Plasmin than the standard Clopidogrel.

The propensity of Nortrachelogenin (Binding energy = -6.88 kcal/mol) to bind with TAFI is much lower than that of the standard drug Clopidrogel as revealed by the lower binding energy. The binding energy per non-hydrogen atom of Nortrachelogenin called the ligand efficiency (-0.26 kcal/mol) is higher than that of the standard drug Clopidrogel. The inhibitory constant which portrays the concentration at which half maximum inhibition happens for Nortrachelogenin (9.07 μM) is much lower than that of the standard drug Clopidrogel. The other internal energy parameters such as Intermolecular energy, Vanderwaals energy, electrostatic energy and torsional energy are also better for Nortrachelogenin than the standard drug Clopidogrel. Kraft *et al* [22] studied the role of elevated levels of TAFI with Thrombosis leading to Ischemic Stroke. The molecular docking studies with TAFI and Nortrachelogenin validated it as a better inhibitor for TAFI than Clopidrogrel.

Finally, Thrombin a key enzyme in hemostasis and thrombosis regulates pro- and anticoagulant reactions by interacting with other coagulation proteins and cellular receptors [23]. It could be well understood that there is no affinity between Nortrachelogenin with Thrombin as depicted by the positive binding free energy. The standard drug Clopidogrel binds with Thrombin with reasonable affinity because the binding energy is -8.57 kcal/mol.

4. CONCLUSION

Nortrachelogenin ,a bioactive molecule in *Zingiber officcinale* showed better affinity with PAL-1, Plasmin

and TAFI.From the *in silico* studies, it can be suggested that the ligand Nortrachelogenin can be used as a better alternative to Clopidogrel in case of PAL-1, Plasmin and TAFI as drug targets. *In silico* studies provides more evidence at the molecular level showing the interactions that take place between the targets and the chosen ligands. Hence, it can be concluded that Nortrachelogenin can be considered as an efficient thrombolytic agent.

5. ACKNOWLEDGEMENT

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Conflict of interest

The authors declare that they have no conflicts of interest.

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