

Identification of natural inhibitors against angiotensin I converting enzyme for cardiac safety using induced fit docking and MM-GBSA studies

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ABSTRACT

Background: Cleistanthins A and B are isolated compounds from the leaves of *Cleistanthus collinus* Roxb (Euphorbiaceae). This plant is poisonous in nature which causes cardiovascular abnormalities such as hypotension, nonspecific ST-T changes and QTc prolongation. The biological activity predictions spectra of the compounds show the presence of antihypertensive, diuretic and antitumor activities. **Objective:** Objective of the present study was to determine the *in silico* molecular interaction of cleistanthins A and B with Angiotensin I- Converting Enzyme (ACE-I) using Induced Fit Docking (IFD) protocols. **Materials and Methods:** All the molecular modeling calculations like IFD docking, binding free energy calculation and ADME/Tox were carried out using Glide software (Schrödinger LLC 2009, USA) in CentOS EL-5 workstation. **Results:** The IFD complexes showed favorable docking score, glide energy, glide emodel, hydrogen bond and hydrophobic interactions between the active site residues of ACE-I and the compounds. Binding free energy was calculated for the IFD complexes using Prime MM-GBSA method. The conformational changes induced by the inhibitor at the active site of ACE-I were observed based on changes of the back bone C α atoms and side-chain chi (x) angles. The various physicochemical properties were calculated for these compounds. Both cleistanthins A and B showed better docking score, glide energy and glide emodel when compared to captopril inhibitor. **Conclusion:** These compounds have successively satisfied all the *in silico* parameters and seem to be potent inhibitors of ACE-I and potential candidates for hypertension.

Key words: ADME/Tox, Chi angles, Cleistanthin A, Cleistanthin B, Induced fit docking, Prime MM-GBSA

INTRODUCTION

Plants are found to be the treasure chest of biological substances/drugs such as anti-cholinergic agents, skeletal muscle relaxants and salicylates. In some cases, compounds that exhibit medicinal properties have been obtained from plants which are considered to be toxic. Atropine, D-tubocurarine, hyoscine, digoxin and strychnine were isolated from toxic plants. *Cleistanthus collinus* Roxb., (Euphorbiaceae) is one such toxic plant, which exerts significant toxicity on cardiovascular, renal and respiratory system. The toxic

effect also induces metabolic acidosis and alters liver and kidney functions. From the leaves of *Cleistanthus collinus*, more than 25 compounds were identified in which some compounds including cleistanthin A and cleistanthin B were reported as toxic.^[1] Both cleistanthins A and B are glycosides commonly present in Euphorbiaceae family plants.^[2]

Cleistanthin A is also present in the leaves of *Phyllanthus taxodiifolius* Beille., (Euphorbiaceae) and this plant is commonly used as a traditional diuretic agent among Thai people.^[3] The predicted biological activity spectra of cleistanthins A and B showed the presence of hypotensive effect, diuretic and antitumor activities. Both compounds had significant antineoplastic and hypotensive effects in rodents and its cell lines.^[4,5] The *in vitro* studies of cleistanthins A and B compounds showed a significant diuretic effect but the effect was not comparable with

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standard diuretic agents.^[4] Hence; the present study aims to determine the possible interactions and binding free energy of cleistanthins A and B with target of ACE-I using Induced Fit Docking and Prime Molecular Mechanics Generalized Born Surface Area (MM-GBSA) analysis.

MATERIALS AND METHODS

Ligand preparation and biological activity prediction

The natural compounds of cleistanthins A and B were isolated and purified from the leaves of *Cleistanthus collinus* using column chromatographic method and the structures were determined.^[4] These structures [Figure 1] were built using builder panel in Maestro and ligand preparation was carried out for these compounds by Ligprep 2.3 module (Schrödinger, USA, 2009). Ligprep performs addition of hydrogens, 2D to 3D conversion, realistic bond lengths and bond angles, low energy structure with correct chiralities, ionization states, tautomers, stereochemistries and ring conformations. The energy minimized compounds were subjected to biological activity prediction based on their structural orientation using PASS (Prediction of Activity Spectra for Substances) tool.^[6]

Protein preparation

The PASS prediction results also showed that these compounds have anti-pulmonary hypertension property. Based on the results of *in vitro*, *in vivo* and *in silico* PASS studies,^[1,3] the x-ray crystal structure of human testicular Angiotensin I-Converting Enzyme (tACE-I) with captopril complex was recovered from Protein Data Bank (1UZF). The ACE is a zinc metallopeptidase that plays an important role of catalyzing the proteolysis of angiotensin I to the vasopressor angiotensin II. ACE, angiotensin I and II are part of renin-angiotensin system which regulates the blood

pressure, volume of fluids in the body. ACE catalyses the conversion of angiotensin I to II leading to vasoconstriction. ACE inhibitors block the conversion of angiotensin I to II thereby reducing the cardiac index and increasing natriuresis.^[7] Selection of potent inhibitors to this enzyme, may lead to development of new drugs for the treatment of cardiovascular diseases. Captopril is the first approved drug as an orally active ACE inhibitor for treatment of human hypertension, which was accomplished in 1981 by Cushman and Ondetti.^[8]

Induced fit docking

In the standard (rigid) mode of docking, as the protein is held rigid and the ligand is free to rotate, the simulation may provide misleading results. Also, many proteins undergo side-chain (χ angles) or backbone ($C\alpha$) conformational changes or both, while ligand binds at the active site of the target. These conformational changes allow the protein to generate close conformations to the shape of the ligand and lead to good binding affinity complex. In this study, the IFD (flexible docking) was carried out using Glide software (Schrödinger LLC 2009, USA) to predict accurate concomitant structural movements when ligand binds at the active site of the protein.^[9] The energy minimized human tACE-I-captopril complex was subjected for IFD studies. At the active site of tACE-I complex, the captopril binding was used to generate the grid for IFD calculation. While docking, the default van der Waals radii (0.50) was used for non-polar atoms of the receptor and ligands (captopril, cleistanthin A and cleistanthin B). In IFD study, the active site residues of tACE-I conform more closely to the shape and binding mode of the compounds. The energetically favorable IFD complexes were obtained for all compounds and the best poses were selected by docking score, glide energy

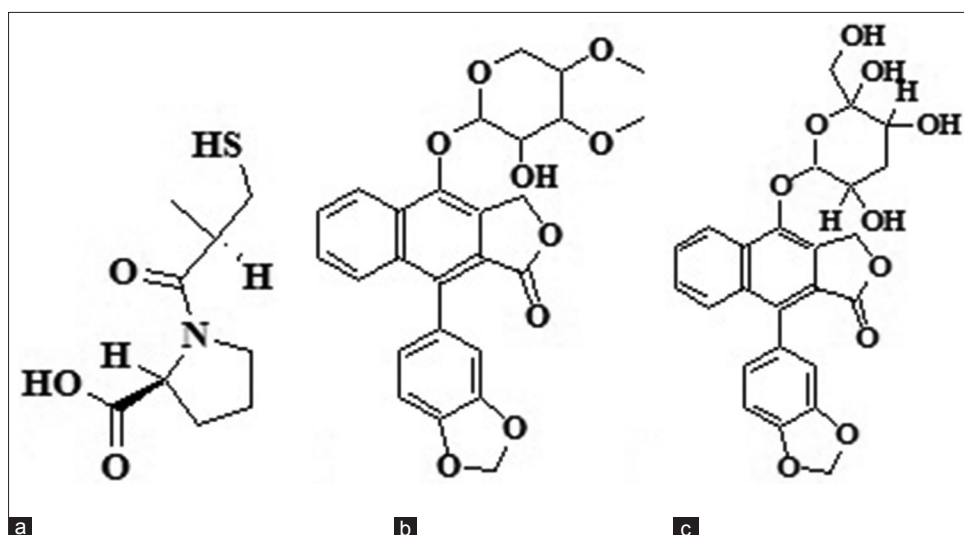


Figure 1: Chemical diagrams of (a) Captopril, (b) cleistanthin A and (c) cleistanthin B inhibitors used in the *in silico* study

and glide emodel for further calculations. *Ligplot^{A10}* and *PyMol^{A11}* were used for analyzing the hydrogen bond and hydrophobic interactions.

Prime MM-GBSA

The ligand strain energies and ligand binding energies were calculated for the natural and captopril compounds using Prime MM-GBSA modules (Schrödinger LLC 2009, USA). The best poses of IFD complexes were chosen to obtain the binding free energy calculation. Prime MM-GBSA is a method that combines Optimized Potential for Liquid Simulations-All Atoms (OPLSAA) force field, molecular mechanics energies (EMM), an SGB solvation model for polar solvation (GSGB), and a non-polar solvation term (GNP) composed of the non-polar solvent accessible surface area and van der Waals interactions.

The total binding free energy:

$$\Delta G_{\text{bind}} = G_{\text{complex}} - (G_{\text{protein}} + G_{\text{ligand}})$$

C α and χ angles conformational changes

The best IFD complexes and the crystal structure of tACE-I were taken for analyzing the conformational changes of C α atoms and χ angles at the active site. The IFD complexes were superimposed with the crystal structure of tACE-I for the structure based alignment matching using *Chimera*.^[12] Needleman-Wunsch algorithm and BLOSUM-62 matrix were used in *chimera* for aligning the amino acid residues based on the alignment of the helix and strand (C α atom) of the IFD complexes with the respective helix and strand (C α atom) of the crystal structure. Then, the side-chain χ angles (χ_1 (N-C α -C β -C γ), χ_2 (C α -C β -C γ -C δ), χ_3 (C β -C γ -C δ -C ϵ), and χ_4 (C γ -C δ -C ϵ -C ζ)) were analyzed to understand the conformational changes at the active site of tACE-I in the IFD complexes. These side-chain χ angles were calculated and analyzed using *MolProbity*^[13] and *Chimera* software.

Absorption, Distribution, Metabolism and excretion (ADME)/ Tox

The energy minimized natural compounds were subjected into ADME/Tox calculations using Qikprop 3.2 (Schrödinger 2009). Qikprop is quick and accurate; it predicts structurally significant descriptors and pharmaceutically relevant properties of organic molecules. QikProp provides ranges for comparing a particular molecule's physicochemical properties with those of 95% of known drugs.

RESULTS AND DISCUSSION

Docked complex analyses

Captopril was redocked at the active site of tACE-I [Figure 2a]. The docked complex has the docking score = -4.09, Glide energy = -32.19 Kcal/mol and Glide emodel = -45.66 Kcal/mol [Table 1]. The redocked complex was superimposed with the crystal structure of tACE-I and the RMSD was 0.237Å. Captopril has maintained four hydrogen bond interactions with the active site residues. In this compound, the O2 atom has trifurcated hydrogen bond acceptor interactions with Gln 281 (NE2), Lys 511 (NZ) and Tyr 520 (OH) and O3 atom has interacted with His 513 (NE2) residue. Zn (II) ion forms tetrahedral coordination geometry with Glu 384 (OE2), Glu 411 (OE1), Glu 411 (OE2) residues and sulfur atom in the ligand. This complex has the binding free energy of -16.41 Kcal/mol.

Seven hydrogen bond interactions were observed in the tACE-I-cleistanthin A complex and trifurcated hydrogen bond donor interactions were observed between ligand O9 atom and the active site residues of Glu 384 (OE2), Glu 384 (OE1) and Glu 411 (OE2). The residue Tyr 523 (OH) has maintained two hydrogen bond interactions with ligand atoms of O5 and O8 with distances 3.14 Å and 2.63 Å,

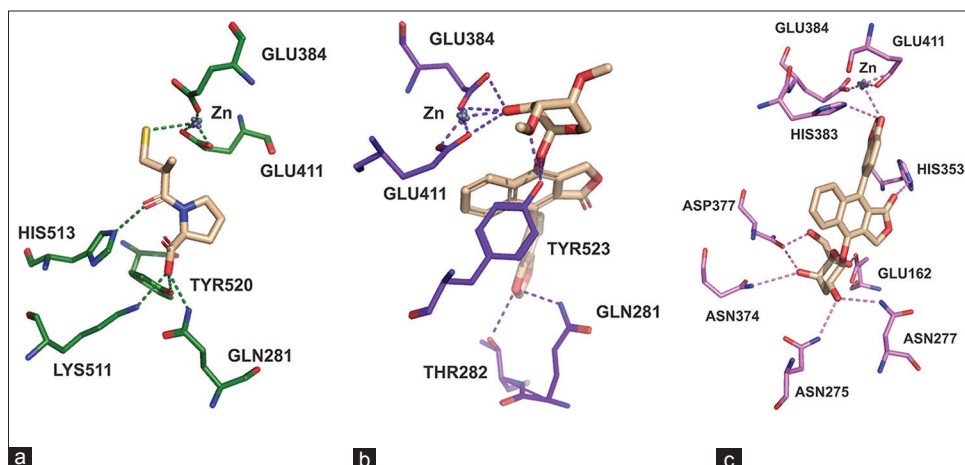


Figure 2: Hydrogen bond interactions (dotted lines) of (a) captopril, (b) cleistanthin A and (c) cleistanthin B with active site residues of tACE

respectively [Table 1 and Figure 2b]. Zn (II) ion has formed a tetrahedral coordination geometry which has three interactions with the active site residues (Glu 384 (OE2), Glu 411 (OE1) and Glu 411 (OE2)) and one interaction with O9 atom in the ligand. The ligand showed the docking score = -5.83, Glide energy = -49.19 Kcal/mol and Glide emodel = -94.06 Kcal/mol at the active site of tACE-I [Table 1]. This complex had binding free energy = -47.82 Kcal/mol.

Docked orientation of the cleistanthin B with the active site residues of tACE-I showed that the docking score = -6.68, Glide energy = -60.20 Kcal/mol and Glide emodel = -120.77 Kcal/mol. The ligand has eight hydrogen bond interactions with the active site residues. The atom O7 has two hydrogen bond interactions with Asn 227 (ND2) and Asn 285 (ND2). The residues Asn 374 (ND2) and Asp 377 (OD1) interact with O8 atom of the ligand. Moreover, the O9 atom also has two hydrogen bond interactions with His 353 (NE2) and Glu 162 (OE2) residues. O3 and O10 atoms have one interaction with His 383 (NE2) and Asp 377 (OD2), respectively. The Zn (II) ion

has tetrahedral coordination geometry with Glu 384 (OE2), Glu 411 (OE1), Glu 411 (OE2) residues and O3 atom of the ligand [Table 1 and Figure 2c]. This complex shows the binding free energy of -49.32 Kcal/mol.

Conformation changes of C α and side-chain X angles

The C α conformational changes of Ches A and B complexes were compared with crystal structure of tACE-I and the RMSD values are 0.24 Å and 0.23 Å for Ches A and B compounds, respectively. The side-chain conformational changes were analyzed by measuring chi angles at the active site residues for all the complexes. Glu 162, Gln 281, Thr 282, His 353, Asp 377, His 383, Glu 384, His 387, Glu 411, Phe 512 and Tyr 523 residues showed side-chain conformational changes at the active site of the tACE-I. These results show that all the compounds induced conformational changes at the active site residues [Figure 3 and Table 2].

Analysis of physicochemical properties

The natural compounds were taken for physicochemical properties analyzed using Qikprop simulation. The

Table 1: Docking score, glide energy, glide emodel, hydrogen bond interactions and binding free energy of Induced fit docked complexes

Inhibitor	Inhibitor atom	H-bond interaction	Residues	Distance of D...A (Å)	Docking score	Glide energy (Kcal/mol)	Glide emodel (Kcal/mol)	ΔG bind (Kcal/mol)					
Captopril	O2	O...H-N	GLN 281 (NE2)	2.85	-4.09	-32.19	-45.66	-16.41					
	O3	O...H-N	HIS 513 (NE2)	2.87									
	O2	O...H-N	LYS 511 (NZ)	2.99									
	O2	O...H-O	TYR 520 (OH)	2.91									
	Zn	Zn...O	GLU 384 (OE2)	1.90									
	Zn	Zn...O	GLU 411 (OE1)	1.94									
	Zn	Zn...O	GLU 411 (OE2)	2.01									
	Zn	Zn...S	Captopril	3.15									
Ches-A	O2	O...H-N	GLN 281 (NE2)	2.81	-5.83	-49.19	-94.06	-47.82					
	O2	O...H-O	THR 282 (OG1)	3.11									
	O5	O...H-O	TYR 523 (OH)	3.14									
	O8	O...H-O	TYR 523 (OH)	2.63									
	O9	O-H...O	GLU 384 (OE1)	2.66									
	O9	O-H...O	GLU 384 (OE2)	2.76									
	O9	O-H...O	GLU 411 (OE2)	3.07									
	Zn	Zn...O	GLU 411 (OE1)	1.94									
	Zn	Zn...O	GLU 411 (OE2)	1.96									
	Zn	Zn...O	GLU 384 (OE2)	1.93									
	Zn	Zn...O	Ches-A	2.22									
	Ches-B	O9	O-H...O	GLU 162 (OE2)					2.92	-6.68	-60.20	-120.77	-49.32
		O7	O...H-N	ASN 277 (ND2)					3.01				
O7		O...H-N	ASN 285 (ND2)	3.11									
O4		O...H-N	HIS 353 (NE2)	2.82									
O8		O...H-N	ASN 374 (ND2)	3.54									
O8		O-H...O	ASP 377 (OD1)	2.72									
O10		O-H...O	ASP 377 (OD1)	2.48									
O3		O...H-N	HIS 383 (NE2)	3.17									
Zn		Zn...O	GLU 384 (OE2)	1.92									
Zn		Zn...O	GLU 411 (OE1)	1.96									
Zn		Zn...O	GLU 411 (OE2)	2.05									
Zn		Zn...O	Ches-B	2.75									

molecular weight of the compounds is less than 500Da and QPlogPo/w (octanol/water partition coefficient) for all the inhibitors is less than five. These compounds satisfy the partition coefficient of octanol/gas (QPlogPoct), water/gas (QPlogPw) and brain/blood (QPlogBB) values as per the rules. The aqueous solubility (QPlogS) and the skin permeability (QPlogKp) were predicted for the compounds. Total solvent accessible surface area (SASA), hydrophobic component of the SASA (FOSA) and hydrophilic component of the SASA (FISA) values abide the ranges specified in the Qikprop. The natural

compounds have favorable oral absorption score of 3, when qualitative human oral absorption was predicted. Polar nitrogen and oxygen van der Waals surface area (PSA) of compounds satisfy the range in physicochemical calculation [Tables 3a and b].

CONCLUSION

The natural compounds and captopril were subjected for IFD and Prime MM-GBSA studies to observe docking score, glide energy, glide emodel, hydrogen bond and hydrophobic interactions and binding free energy between these ligands and the active site residues of tACE-I. The values of these for natural compounds were compared with redocked captopril-tACE-I complex. The comparison shows that cleistanthins A and B have better docking score, glide energy, glide emodel and binding free energy compared to captopril. Moreover, hydrogen bond and hydrophobic interactions of the natural compounds have shown favorable interactions with active site residues.

The natural compounds have favorable results in physicochemical properties of partition coefficient of QPlogPoct, QplogPw and QplogBB and surface area calculations of SASA, FOSA, FISA, and PSA. According to molecular weight calculation and the human oral absorption properties, both the compounds are below 500 Da with high oral absorption value. Both compounds satisfy all the *in silico* parameters like docking score, glide

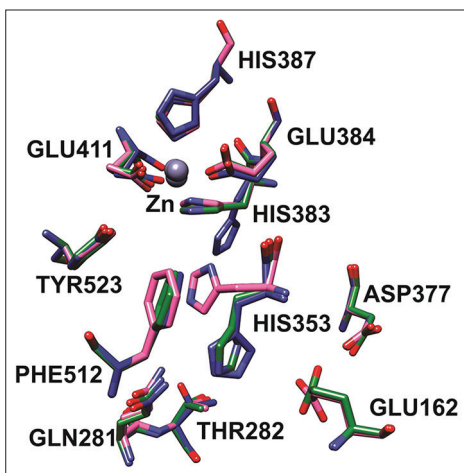


Figure 3: Side-chain conformational changes in the tACE complexes captopril, cleistanthin A and B bound complexes are colored in green, blue and pink, respectively

Table 2: Conformational changes of the side-chain Chi angles (χ_1 , χ_2 , χ_3 , and χ_4) of the docked complexes

Residues	Captopril				SBDD2				SBDD3			
	χ_1	χ_2	χ_3	χ_4	χ_1	χ_2	χ_3	χ_4	χ_1	χ_2	χ_3	χ_4
Glu 162	296.8	289.9	95.1	-	296.8	289.9	95.1	-	297.5	290.5	39.6	-
Gln 281	72.8	184.2	34	-	73.6	196	28	-	74.1	178.9	46.4	-
Thr 282	178.5	-	-	-	289.2	-	-	-	174.5	-	-	-
His 353	285.6	299.3	-	-	274.8	167.3	-	-	194.3	281.6	-	-
Asp 377	281.2	57.5	-	-	275.5	33.9	-	-	275.1	33	-	-
His 383	177.2	78	-	-	294.8	188	-	-	178.3	82	-	-
Glu 384	291.9	299	112.7	-	300.5	289.4	105.2	-	292.2	298	107.1	-
His 387	281.3	265.8	-	-	274.3	274.9	-	-	285.3	263.5	-	-
Glu 411	288.7	295.4	163.5	-	287.4	295.6	172.7	-	284.3	292.5	163.2	-
Phe 512	174.4	51.3	-	-	179	53.5	-	-	174.3	76.8	-	-
Tyr 523	307.7	113.4	-	-	308.2	117.8	-	-	305.4	115	-	-

Table 3a: Calculation of Lipinski's rule of five, partition coefficient and skin permeability

Inhibitor (range)	MW (130.0-725.0)	Volume (500.0-2000.0)	donorHB (†) (0.0-6.0)	acceptHB (†) (2.0-20.0)	QPlogPo/w (-2.0-6.5)	Rule of five (\$) (0-5)	QPlogPoct (8.0-35.0)	QPlogPw (4.0-45.0)	QPlogS (-6.5-0.5)	QPlogBB (-3.0-1.2)	QPlogKp (-8.0-1.0)
Ches-A	480.4	1283.9	1	9.0	2.0	0	22.8	15.5	-3.0	-0.4	-1.4
Ches-B	482.4	1249.2	4	10.0	1.0	0	27.3	19.9	-3.0	-1.5	-3.3

Estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution. Values are averages taken over a number of configurations, so they can be non-integer. †Number of Lipinski's rule of five violations. QPlogPoct, QPlogPw, QPlogS and QPlogBB were predicted partition coefficient of octanol/gas, water/gas, aqueous solubility and brain/blood, respectively. QPlogKp was predicted skin permeability (log kp)

Table 3b: Surface area calculation and human oral absorption

Inhibitor (range)	SASA (300.0-1000.0)	FOSA (0.0-750.0)	FISA (7.0-330.0)	Human oral absorption	PSA (7.0-200.0)
Ches-A	671.0	344.7	74.8	3	107.7
Ches-B	650.8	229.0	186.5	3	151.2

SASA: Total solvent accessible surface area; FOSA: Hydrophobic component of the SASA (saturated carbon and attached hydrogen); FISA: Hydrophilic component of the SASA (SASA on N, O, and H on heteroatoms); PSA: Van der waals surface area of polar nitrogen and oxygen atoms

energy, glide emodel, binding free energy, ADME/Tox and non-bonded interactions. Hence, cleistanthins A and B could exert potent inhibition of the tACE-I and they have favorable physicochemical properties. The inhibition of ACE by cleistanthins A and B could be revealed by *in vivo* and *in vitro* studies and if so, these compounds may be attempted to be used against cardiovascular diseases.

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