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IN SILICO SCREENING OF SOME COMMERCIALLY AVAILABLE ALKALOIDS AGAINST ANGIOTENSIN CONVERTING ENZYME USING LAMARCKIAN GENETIC ALGORITHM

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Abstract: Alkaloids are the dynamic components that may show an essential part in preventing the hypertension. Captopril, a known angiotensin converting enzyme inhibitor was considered as the standard. An *in silico* screening study was performed to detect the inhibitory potential of the alkaloids against angiotensin converting enzyme by using the Autodock 4.2 software. The alkaloids ajmaline, atropine, caffeine and emetine were selected for the present study. The results revealed that all the selected alkaloids exhibited binding energy ranging between -9.75 kcal/mol to -4.98 kcal/mol when compared with the standard (-5.38 kcal/mol). Inhibition constant (77.38 nM to 225.41 µM) and Intermolecular energy (-11.84 kcal/mol to -6.17 kcal/mol) of the alkaloids also concur with the binding energy. Hence, these alkaloids can be further scrutinized for *in vivo* studies and thereby it may act as potential chemical entities for the prevention and treatment of hypertension.

Keywords: Alkaloids; Angiotensin converting enzyme; Binding energy; Inhibition constant; Intermolecular Energy

I. Introduction

Hypertension is defined as a chronic condition in the medical field in which the arteries blood pressure is raised above the normal. Primary hypertension may produce as an outcome of an environmental or genetic sources and secondary hypertension has multiple pathophysiology which include vascular, renal and endocrine complications (Wong and Mitchell, 2007; Singer and Kite, 2008). Statistically primary hypertension occurs in 90 to 95% of adults where secondary hypertension showed in 2 to 10% of adults. Hypertension is the primary root for cardiovascular impermanence was stated by the World Health Organization (Sever and Messerli, 2011). Globally majority of the hypertensive people are ignorant of their illness which was predicted by the World Hypertension League (Folkow, 1982).

Angiotensin converting enzyme (ACE) ramblingly elevates the blood pressure by influencing the blood vessels. The constriction of blood vessels occurs due to the conversion of angiotensin I to angiotensin II. ACE is a potent vasoconstrictor which catalyses angiotensin I into angiotensin II and decreases bradykinin which is a dominant vasodilator (Zhang et al. 2000). ACE inhibitor is primarily used in the management of hypertension and other cardiovascular disorders (Imig, 2004). This class of drugs results in the blood vessels relaxation and reduced blood volume, which flinches to decrease blood pressure (Lazartigues et al. 2007).

Alkaloid, a chemical stuff of plant source encompassed of carbon, hydrogen, nitrogen, and oxygen. Alkaloids occur predominantly in altered genera of seed plants, such as the tobacco and

opium poppy plants (Davies and Shepherd, 1955). Alkaloids were present in almost all parts of these plants, including the seeds, bark, leaves, and roots (John, 2000). Most of the alkaloids have therapeutic effects on the nervous system of humans and animals. Active constituents of alkaloids were reported for various pharmacological activities like, sedatives, anaesthetics, relaxants, stimulants, and tranquilizers (Cushnie et al. 2014). In the management of malaria quinine is used and it may induce dizziness in large doses. Cocaine and morphine are the competent drugs for releasing pain temporarily without inducing loss of consciousness (Kittakoop et al. 2014).

Docking is a method of screening the compounds from the database and predicting the stoutest binders based on diverse scoring functions (Gschwend et al. 1996). The binding geometry of two interacting fragments is studied in molecular docking (Irwin et al. 2005). It estimates the ideal orientation of receptor and ligand to elucidate a stable complex. The various computational software like AutoDock 4.2, GOLD, SLIDE and GLIDE is used to screen the binding of small molecules to a known target receptors (Cosconati et al. 2010; Morris et al. 2009). Hence, the objective of the present study was to screen the some commercially available alkaloids against ACE using *in silico* docking studies.

II. **Experimental Section**

II. 1. Softwares required

Python 2.7 - language was downloaded from www.python.com, Cygwin was downloaded from www.cygwin.com, Molecular graphics laboratory (MGL) tools and AutoDock4.2 was downloaded from www.scripps.edu, ChemSketch was downloaded from www.acdlabs.com, Discovery studio visualizer 2.5.5 was downloaded from www.accelrys.com. Online SMILES translation was performed using cactus.nci.nih.gov/translate/.

2. Docking Evaluation Methodology

Research Collaboratory for Structural Bioinformatics (RCSB) is the global library for 3D structure of enzyme targets and their multiplexes, as retrieved by NMR spectroscopy, X-ray crystallography, and cryoelectron microscopy (Thomsen et al. 2006; Goodsell et al. 1996). The crystal structure of human angiotensin-converting enzyme in multifaceted with angiotensin-II was downloaded from Research Collaboratory for Structural Bioinformatics (RCSB) Protein Bank (PDB ID: 4APH). The PDB file of the target enzyme was prepared and the hetero atoms were removed in the molecule with the help of Accelrys Discovery Studio viewer (Fig. 1).

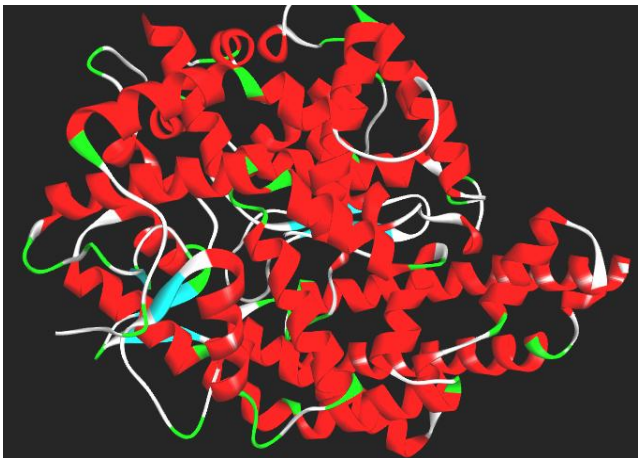

Fig. 1 *crystal structure of human angiotensin-converting enzyme in multifaceted with angiotensin-II*

In figure 2, the selected alkaloids such as ajmaline, atropine, caffeine, emetine and standard captopril were built using Chemsketch and then optimized using “Prepare Ligands” module in AutoDock 4.2. The optimized ligand molecules were docked into the refined target enzyme (4APH) in the AutoDock 4.2.

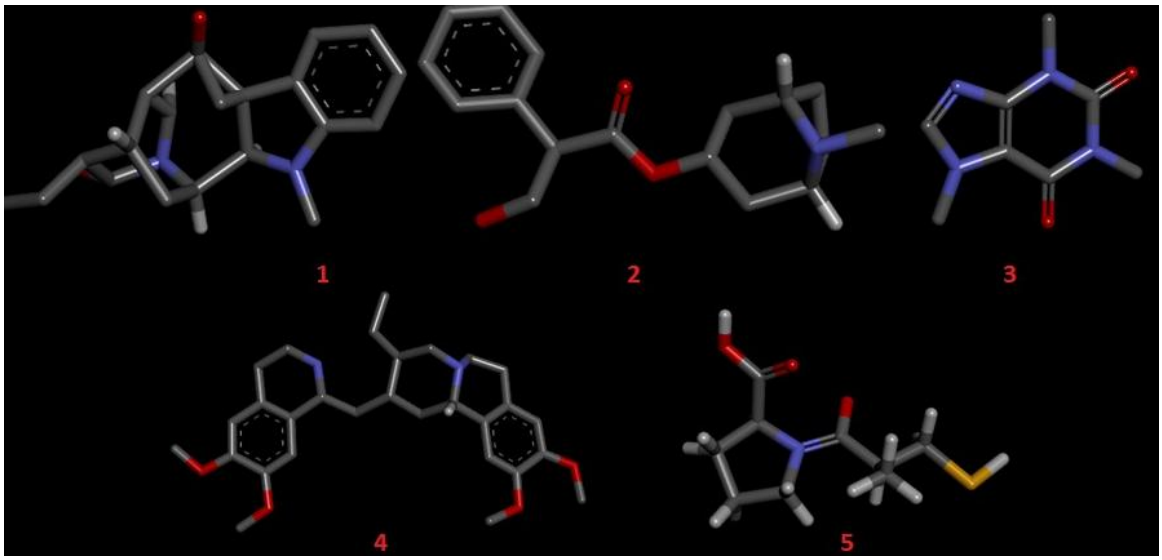

Fig. 2 *Optimized ligand molecules (1 ajmaline, 2 atropine, 3 caffeine, 4 emetine and 5 captopril)*

Lamarckian genetic algorithm principle used in the AutoDock 4.2 recognizes the conformational space of the alkaloids. Nonpolar hydrogen atoms were removed from the target enzyme file and inclusion of their partial charges were carried out. The computational program converts 'Mol' form to 'pdbqt' form for to screen the alkaloids screening against target file. Gasteiger charges were included for the target enzyme instead of Kollman charges which used in the older versions of AutoDock 4.2 (Madeswaran et al. 2013).

Target enzyme was surrounded on a three dimensional grid point in the AutoGrid module. The interaction energy of each atom in the alkaloids was determined against the target enzyme. Population size of 150 individuals, maximum of 27000 generations, 2.5 million energy evaluations, and number of top entities to mechanically endure to next generation of 1, crossover rate of 0.8 and mutation rate of 0.02 was selected as important docking parameters in the docking module (Madeswaran et al. 2012). For each enzyme ligand complex, 10 docking simulations were made with default restrictions. AutoDock 4.2 was run multiple times to acquire various docked simulations, and which was further used to estimate the predicted docking parameters (Madeswaran and Asokkumar, 2015).

III. Results and Discussion

Table 1*. Lipinski's rule of five for the selected alkaloids*

Compounds	Molecular Weight	log P	HBDH	T_PSA	Rule of 5
Ajmaline	326.441	2.857	2.000	46.940	0.000
Atropine	289.377	1.801	1.000	49.770	0.000
Caffeine	194.194	0.082	0.000	61.820	0.000
Emetine	480.652	3.311	1.000	52.190	0.000
Captopril	218.296	-1.981	2.000	60.540	0.000

Lipinski's rule of five evaluates certain pharmacological, biological and absorption, distribution, metabolism and excretion (ADME) properties of the lead molecule. The selected lead molecule that exceeds molecular weight (Mw) more than 500 Da, calculated log *P* value greater than 5, hydrogen-bond donors more than 5, hydrogen-bond acceptors more than 10 is unlikely to be further chased as a probable drug candidate, because it may possess nonexistence properties which is essential for its

pharmacokinetic properties (Wolber and Langer, 2005). The drug candidature scores of the chosen alkaloids were assessed with the help of Lipinski's rule of five with the help of MedChem Designer. All the selected alkaloids exhibited tremendous score which provide that these compounds may look for its pharmacological activity (Table 1).

Three dimensional quantitative structural activity relationship evaluations exhibited the residues of Glu 384 and His 387 in the energetic site surrounded in the lipophilic groups along with the sulfonyl portion of lisinopril and enalapril inhibitors. Evaluation of dipeptides by *in silico* docking studies and evaluation of intestinal stability anticipated a number of non-phosphorylated and phosphorylated dipeptides to have Angiotensin Converting Enzyme inhibitory activity. The inhibitory concentration (IC_{50}) values from *in vitro* pharmacological studies revealed that the phospho dipeptides were not remarkable inhibitors of the target enzyme. The N-terminal amino acid residues like Asp, Gly, and Pro and the C-terminal residues Pro, Ser, Thr, and Asp were stabilizing dipeptides toward luminal enzymatic peptide hydrolysis. QSAR modelling did not disclose significant association for intestinal permeability. Two dimensional fingerprint representations were recognized for its dipeptides ACE inhibitory activity (Norris et al. 2012). The binding orientation of the compounds within the dynamic site of the macromolecule was investigated using Accelrys discovery studio viewer. It yields a three dimensional visualization of the drug-target interaction (Mitrasinovic, 2010).

Novel series of acridine-coumarin hybrids were synthesized and biologically evaluated their inhibitory potential of AChE and BuChE. The newly synthesized derivatives 9a-d have shown higher activity against human AChE compared with 7-MEOTA as the standard drug. Among them derivative 9b exhibited the most potent AChE inhibitory activity, with an IC_{50} value of 5.85μM compared with 7-MEOTA (IC_{50}=15μM). Molecular modelling studies were performed to predict the binding modes of compounds 9b, 9c and 9f with hAChE/hBuChE (Hamulakova et al. 2017).

Two-dimensional similarity search were performed with selected inhibitor, keeping in view the physiochemical properties of the inhibitor. Docking studies revealed that Glu-53, Thr-54, Lys-58, Val-85, Ser-86, Tyr-87, Leu-88, Glu-90, Leu-95, Val-98, Ser-100, Glu-112, Tyr-116, Lys-120, Asp-121, and Arg-122 were critical residues for receptor-ligand interaction. The C-terminal of selected isoforms is conserved, and binding was observed on the conserved region of isoforms. Further analysis of this inhibitor through site-directed mutagenesis could be helpful for exploring the details of ligand-binding pockets (Sehgal et al. 2015).

Cholinesterase inhibitory potential of withanolide A was elucidated and its associated binding mechanism. Binding interactions of the ligand to the receptor were predicted with the help of computational docking studies. Ligand interaction with the human AChE residues was found to be Thr78, Trp81, Ser120 and His442 as active sites, it could be responsible for its inhibitory activity. The study further proved the evidence for consideration of withanolide A as a potential molecule in prevention and management of AD (Grover et al. 2012).

In Fig. 3, the probable binding orientations of the emetine alkaloid was found that, Tyr 287, Ser 298, Met 299, Asp 300, Thr 301, Thr 302 and Leu 375. Similarly the favoured binding sites of the standard captopril was identified that, Tyr 287, Ser 298, Met 299, Thr 301, Leu 375, Phe 446 and Lys 449. This evidences that the effective binding orientations were present in the emetine alkaloid when matched with standard. These amino acid fragments were actively participated in hydrophilic and hydrophobic connections and also play major role in the inhibition of target enzyme. The similar binding orientations were shown in both emetine and the standard such as Tyr 287, Ser 298, Met 299, Asp 300, Thr 301, Thr 302, and Leu 375. This further demonstrates the role of emetine in the ACE inhibition.

In silico docking studies revealed that, the selected alkaloids have considerable docking scores against the angiotensin converting enzyme. The binding energies of the selected alkaloids were exhibited in the ranging between -9.75 kcal/mol to -5.68 kcal/mol (Table 2). All the selected alkaloids had presented admirable binding energy when compared to the standard captopril (-5.38 kcal/mol).

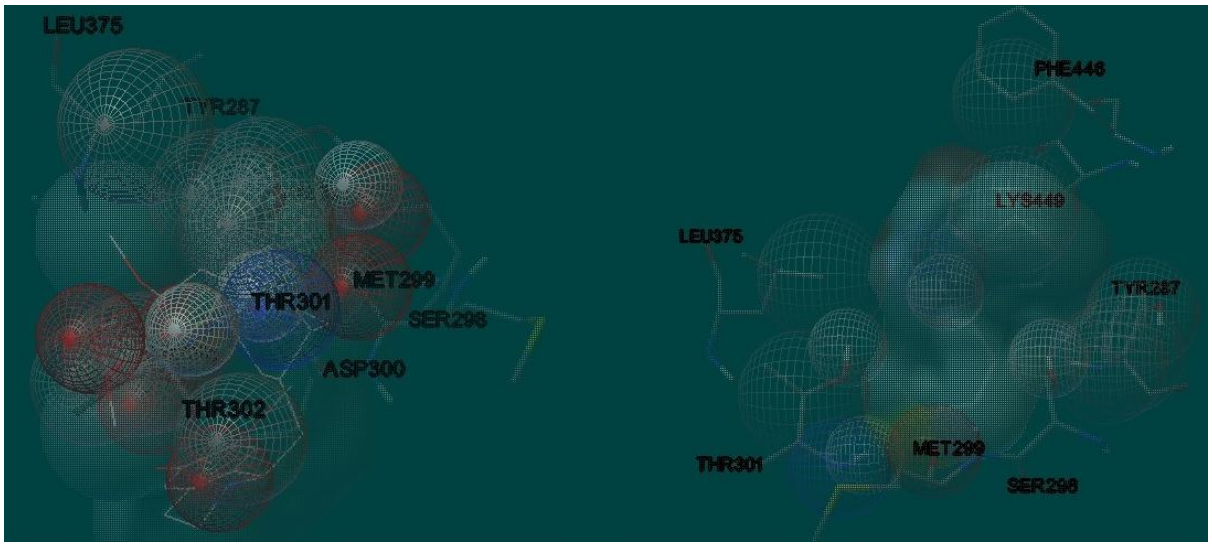

Fig. 3. *Docked poses of emetine and captopril against target enzyme*

Table 2*. Binding energies of the alkaloids based on their rank*

Compounds	Binding energies of the compounds (kcal/mol)									
Ajmaline	-5.68	-5.49	-5.30	-5.21	-5.18	-4.52	-4.36	-4.34	-4.15	-3.74
Atropine	-8.01	-7.36	-6.99	-6.72	-6.57	-6.26	-5.9	-6.21	-6.04	-5.30
Caffeine	-5.78	-5.65	-5.64	-4.78	-4.77	-4.7	-4.64	-4.36	-4.33	-4.08
Emetine	-9.75	-9.46	-8.65	-8.53	-8.5	-8.05	-7.87	-7.25	-6.82	-6.50
Captopril	-5.38	-4.85	-4.06	-5.19	-5.01	-4.83	-4.64	-4.4	-4.39	-3.75

In addition to the binding energy, two other docking parameters like inhibition constant (K_i) and intermolecular energy were evaluated. As shown in table 3, the selected alkaloids exhibited the inhibition constant ranging from 71.38 nM to 164.41 µM. All the selected compounds had decreased inhibition constant values when compared to the standard (113.13 nM). Inhibition constant is directly related to the binding energy. Thus, the ACE inhibitory activity of the alkaloids was compared with the Captopril.

Table 3*. Inhibition Constant of the alkaloids based on their rank*

Compounds	Inhibition Constant of the compounds based on their rank (*nM, **mM)									
Ajmaline	164.41	178.72	182.46	222.16	439.97	485.01	635.45	663.46	907.34	1.82**
Atropine	1.34	4.03	7.47	11.81	15.34	25.72	47.73	28.21	37.26	130.02
Caffeine	57.91	72.75	73.16	314.62	317.12	360.68	394.99	640.13	664.4	1.02**
Emetine	71.38*	116.9*	456.83*	556.07*	586.98*	1.27	1.7	4.88	9.94	17.13
Captopril	113.13	278.27	1.05**	156.16	213.36	289.28	397.77	598.2	602.55	1.77**

As shown in table 4, the compounds showed intermolecular energy ranging from -11.84 kcal/mol to -6.96 kcal/mol and which was lesser when compared to the standard (-6.88 kcal/mol). Intermolecular energy is also directly proportionate to the binding energy. In the present study we noticed that reduce in intermolecular energy of all the selected alkaloids with a concurrent decrease in the binding energy. Further these results enhance the ACE inhibitory activity of all the selected alkaloids.

Table 4. *Intermolecular energies of the alkaloids based on their rank*

Compounds	Intermolecular energies of the compounds based on their rank (kcal/mol)									
Ajmaline	-6.96	-6.74	-6.45	-5.85	-5.77	-5.71	-5.55	-5.53	-5.34	-4.93
Atropine	-9.50	-8.85	-8.49	-8.21	-8.06	-7.75	-7.39	-7.70	-7.53	-6.79
Caffeine	-5.78	-5.65	-5.64	-4.78	-4.77	-4.7	-4.64	-4.36	-4.33	-4.08
Emetine	-11.84	-11.55	-10.74	-10.62	-10.59	-10.13	-9.96	-9.33	-8.91	-8.59
Captopril	-6.88	-6.34	-5.56	-6.68	-6.50	-6.32	-6.13	-5.89	-5.88	-5.25

Alkaloids are able to prevent the onset of various degenerative diseases by free radical scavenging or binding with the oxidative reaction catalyst. Several studies have been done in evaluation of alkaloids from various plants for its wide range of pharmaceutical activities. Based on the *in silico* docking studies through AutoDock 4.2, angiotensin converting enzyme inhibitory activity of the selected alkaloids was found to be decreased in the order of emetine, atropine, caffeine, and ajmaline respectively. Alkaloids are the important secondary metabolites that are known to possess therapeutic properties due to the heterocyclic ring system and its structural collaborations. From the results it can be derived that emetine has a better chance to act as an angiotensin converting enzyme inhibitor candidate.

IV. Conclusion

Molecular docking study is one of the novel techniques to predict the pharmacological activity of the small molecules against known target enzyme. In the present study, it can be concluded that the selected alkaloids showed gratified binding interactions against angiotensin converting enzyme. The alkaloid emetine exhibited remarkable binding interactions and orientations against the active site of ACE. Further *in vitro* and *in vivo* estimations are necessary to design potent drug candidate to prevent and manage the treatment of hypertension.

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