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In silico drug designing approach to treat infectious disease using mangrove through docking analysis

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Abstract

The term "*In - silico*" has been established since 1989 meaning "any biological experiment on or in the computer". *In - silico* software has been used in discovery and optimization of bioactive compounds with affinity to a particular target and clarification of absorption, distribution, metabolism, excretion and toxicity properties (ADME/Tox). Urinary Tract Infection (UTI) is an extensive economic burden on the society. *Escherichia coli* is the most common cause of all types of UTI. Antimicrobial properties of plants have been increasingly reported from different parts of the world. Mangrove plants have been used for centuries to treat several disorders. *Avicennia marina* contains active antimicrobial compounds. Hence, few compounds of *A. marina* (Lupeol, phytol, avicequinone C, beta-amyrin and beta-sitosterol) were selected in the present study. Docking was performed between the ligands and virulent proteins of *E. coli.* LIBDOCK score and Lipinski's rule of 5 was calculated followed by a drug like properties of the ligands by ADME calculations. From the result out of 5 compounds, phytol was confirmed as the most promising compound against pathogen. Therefore, the present study played a guiding role in developing new inhibitors with better binding affinities towards the proteins, followed by invention of drug to treat UTI.

Key words

Avicennia marina, Drug discovery, Mangrove plant, Molecular docking, Urinary tract infection

Introduction

Plants are very important source of medicine for human beings. The active constituents of plants are being used as precursor for synthesis of many drugs (Dhankhar *et al*., 2011). Plant-derived drugs has been a part of the evolution of human, healthcare for thousands of years. Plant-based drugs are commonly used in India and China (Duraipandiyan and Ignacimuthu, 2007). Mangrove forest is economically and ecologically important. They are edible plants and have rich medicinal value (Nuntavan Bunyapraphatsara *et al*., 2003). Mangroves are biochemically unique, producing a wide array of novel natural products. The extract of mangrove has been used for centuries as a popular method for treating several health disorders (Bobbarala *et al*., 2009). The mature leaves of *A. marina* have potential medicinal importance (Abeysinghe *et al*., 2006).

Urinary tract is the most widespread site of bacterial infection in humans. *E. coli* is the far most frequent species infecting this site and accounting for more than 80% of community-acquired infections (Kunin, 1987). The infectious diseases remain one of the greatest challenges to global health. Urinary tract infection (UTI) is the second most common clinical disease and possess a significant healthcare burden (Foxman, 2010). UTI usually begin as a bladder infection but often ascends to affect the kidneys, and

can ultimately result in renal failure or dissemination to the blood (Foxman, 2002). Uropathogenic *E. coli* makes its eradication more difficult, and its incidences are also increasing steadily (Nandalal and Somashekar, 2007).

Cytotoxic necrotizing factor 1 (CNF1) are toxins produced by uropathogenic *E. coli* (UPEC) (Tamako *et al*., 2015). Outer membrane protein (ompT) is one of the virulent gene thought to be important in *E. coli* mediated UTI (Carl Marrs *et al*., 2002). Extended-spectrum beta-lactamase (ESBL) producing *E. coli* are emerging worldwide. The incidence of UTI has increased due to ESBL-producing *E. coli* (Picozzi *et al*., 2013). Keeping rational drug designing in mind, an effort was made to design a natural drug for UTI, caused by *E. coli*, using *A. marina* compounds by docking against virulent proteins.

Bioinformatics is an emerging field with a potential to significantly improve how drugs are found, brought for clinical trials and finally released in marketplace. Bioinformatics can be thought of as a central hub that unites several disciplines and methodologies. Methods developed to facilitate and accelerate the drug designing process are Rational Drug Design (RDD). These processes are used in the biopharmaceutical industry to discover and develop new drugs. RDD uses a variety of computational methods to identify novel compounds. One of the methods is docking of drug molecules with receptors. The site of drug action, which is ultimately responsible for the pharmaceutical effect is a receptor. Docking allows the scientist to virtually screen a database of compounds and predict the strongest binders based on various scoring functions. It explores ways in which two molecules, such as drugs and enzyme receptor fit together and dock well to each other. The molecules binding to a receptor inhibit their function and thus, act as a drug (Daisy *et al*., 2008). The *in silico* molecular docking is one of the most powerful techniques to discover novel ligands for receptors of known structure and thus, play a key role in structure-based drug design (Brooijmans *et al*., 2003). The objective of the present study is to find out ligands and level of its inhibitory effect on pathogens.

Materials and Methods

Selection of protein and preparation of its structure : Rho-activating domain of *E. coli* CNF1, the crystal structure of ompT from *E. coli* and crystal structure of native CTX-M-15 of ESBL of *E. coli* were selected for the present study. The protein structures were downloaded from protein data bank (PDB) (http://www.rcsb.org/pdb/) established by Brookhaven National Laboratories (BNL) in 1971. The PDB ID of these proteins were 1HQ0, 1I78 and 4HBT, respectively. The proteins were prepared for docking by removing water molecules and heteroatom from the downloaded protein structures by Chemistry at HARvard Macromolecular Mechanics (CHARMM) 36 force fields, version - 3.0.1.

Active site prediction for selected proteins : Based on the score value, the best active sites for all the proteins were chosen from various sites predicted by the software, where the ligand could bind and interact after energy minimization.

Selection of ligand : Lupeol (L1), phytol (L2), avicequinone c (L3), beta-amyrin (L4) and beta-sitosterol (L5) present in *A.marina* were selected for the study. The ligand structures were downloaded from PubChem database. The PubChem ID (CID) of these ligands was 259846, 145386, 10563004, 73145 and 222284, respectively.

Interaction studies of binding and calculation of ADME / TOX : The exact fit of the ligand to a receptor was studied using LibDock module in the Discovery Studio Accelrys® software corporation, San Diego, USA. The interactions of the ligands (drugs) with the target proteins were analyzed using the receptor-ligand interaction protocol of the software. The receptor cavities were explored and the active site residues selected were used for interaction with the drugs. Scoring functions implemented in docking programs made various assumptions and simplifications in the evaluation of modeled complexes, which includes terms of hydrogen bonds employed to rank the docked bases and to assess the binding site and the number of rotatable bonds present. With the intention of finding out drug-like properties for the ligands, ADME calculation and Lipinski's rule of five were calculated by using Accord for Excel, version - 6.1. The structure of the ligands were directly introduced into the software by using the edit chemistry module. Using function module from this software, blood-brain penetration level, aqueous solubility, cytochrome 450 binding, hepatotoxicity and plasma protein level were calculated.

Results and Discussion

According to the results of docking, out of 5 compounds, only 4 had docked with Rho-activating domain of *E. coli* CNF1; and 5 had docked with ompT and CTX-M-15 ESBL of *E. coli* (Table 1). The compound phytol had a high Libdock score and low absolute energy with Rho-activating domain of *E. coli* CNF1, ompT and CTX-M-15 ESBL of *E. coli*. The docked pose of these three are shown in Fig. 1, 2 and 3, respectively. In the figure, green lines denoted hydrogen bonds. All the amino acid residues involved in the molecular interactions are also shown in green color, and the ligands in grey color.

The compound beta-sitosterol had a high score with Rho-activating domain of *E. coli* CNF1 and ompT, but

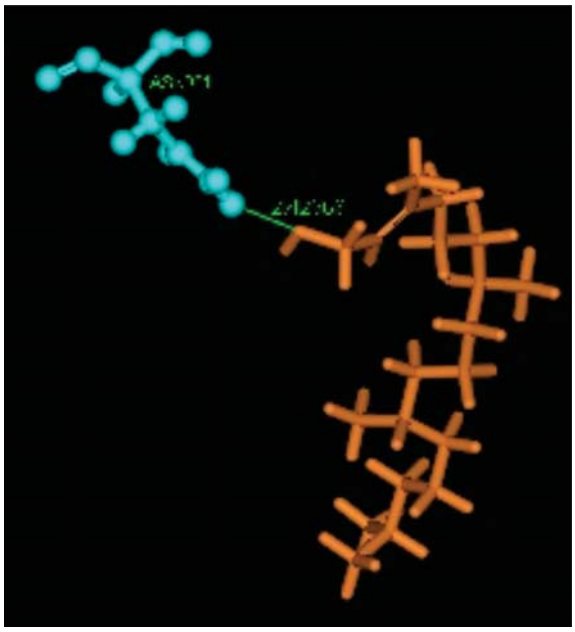

Fig. 1: Drug-Receptor interaction. The docked complex of phytol (brown colour) with Rho-activating domain of *E. coli* CNF1 (blue colour). Hydrogen bond and aminoacid exist in green colour

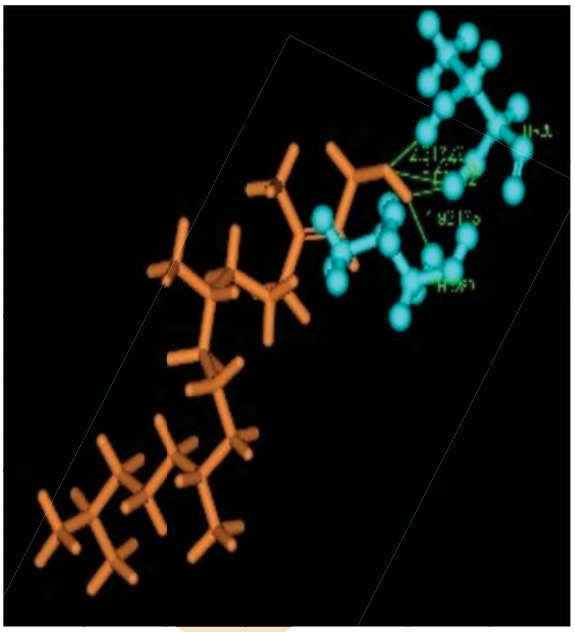

Fig. 2 : Drug-Receptor interaction. The docked complex of phytol (brown colour) with crystal structure of ompT from *E. coli* (blue colour). Hydrogen bonds and aminoacids exist in green colour

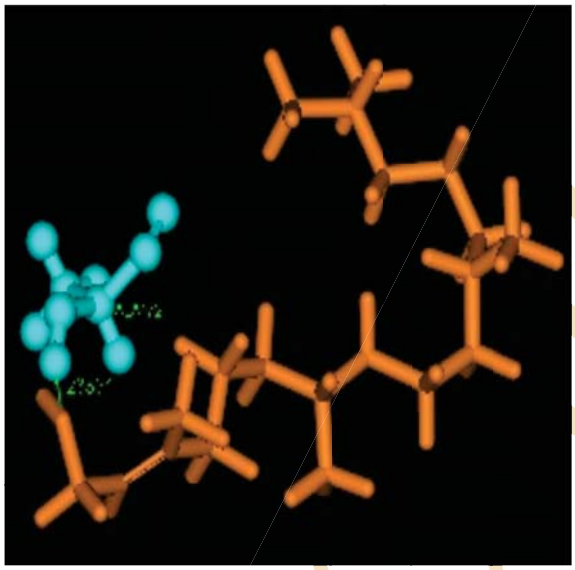

Fig. 3 : Drug-Receptor interaction. The docked complex of phytol (brown colour) with crystal structure of native CTX-M-15 of ESBL of *E. coli* (blue colour). Hydrogen bond and aminoacid exist in green colour

compound avicequinone C had a high score with CTX-M-15 ESBL of *E. coli*, followed by beta-sitosterol. ADME prediction is the most crucial step in drug discovery (Lipinski *et al*., 2001). All the calculated ADME parameters of the ligands (Phyto compounds) are tabulated in Table 2.

Oral administration is the most common route of drug administration. Most drugs in market are administered via oral and it is one of the convenient and cost effective routes of administration (Cheng and Merz, 2003). Low and good solubility is detrimental to good and complete oral absorption, and so early measurement of this property is of great importance in drug discovery (Lipinski, 2000). Aqueous solubility was used to predict the solubility of a compound in water at 25°C and it had eight different levels from 0-6. Aqueous solubility levels ranging from 0-2 indicates low solubility, level 3 indicates good solubility, level 4 indicates optimal solubility and level 5 indicates high solubility (Bevan and Lloyd, 2000). Compound lupeol showed optimal solubility, phytol and beta-sitosterol showed optimal solubility, while avicequinone C and beta-amyrin showed low solubility.

Blood-brain barrier (BBB) is a complex cellular system that helps in maintaining the homeostasis of central nervous system (CNS) by separating the brain from systemic blood circulation. Drugs acting at CNS should have the capacity to cross these barriers whereas other drugs crossing these barriers would cause unwanted side effects. The level 0 and 1 showed high penetration, level 2 showed medium penetration, level 3 showed low penetrations and level 4 showed undefined penetration level. BBB penetration levels

Table 1 : Summary of docking results of virulent proteins with ligands

Protein-Pubchem ID	Ligand	Libdock score	Absolute energy	No of hydrogen bond	Interacting molecule (Amino acid)	Bond length (A°)
1HQ0	L2	114.056	24.599	1	ASN921	2.42568
	L4	55.231	55.52	1	THR 892	2.27819
	L5	106.916	47.441	2	GLY 890	2.1133
					LYS 945	1.8205
1I78	L1	85.098	110.11	1	ASN 254	2.18545
	L2	112.962	27.239	4	THR 20	1.61221
						2.28493
						2.21329
					THR 289	1.92183
	L3	91.96	73.827	4	TRP 71	2.01771
					ASN 286	2.4801
					ASN 254	2.40404
					GLN 135	2.33116
	L5	102.42	51.268	1	ASN 57	2.32084
4HBT	L2	123.511	28.347	1	ALA 172	1.23354
	L3	110.292	73.907	1	THR 264	2.08098
	L4	85.546	64.844	1	SER 130	2.33713
	L5	101.75	54.927	2	SER 70	1.5694
					SER 130	2.02179

Table 2 : Summary of ADME results of the ligands

Ligands	Aqueous solubility logarithmic level	Aqueous solubility level	Blood brain barrier penetration level	CYP456	Hepatatoxicity	Plasma protein binding level
L1	-1.933479	4	2	0	0	1
L2	-3.127553	3	1	0	0	2
L3	-8.802103	0	4	0	1	2
L4	-8.756904	0	4	0	0	2
L5	-3.657237	3	2	0	1	2

of phyto compounds were predicted in order to find out whether these compounds acted on CNS (David Clark, 1999). Compound phytol showed high penetration.

Lupeol and beta-sitosterol showed medium penetration. Hence, these drugs showed possibility of crossing the BBB. Other compounds such as avicequinone C and beta-amyrin had undefined penetration level to cross blood–brain barrier. Hence, for these compound, the chances of CNS side effect were low or absent.

Cytochrome 450 (CYP450) is an enzyme that catalyzes oxidation of organic substance. These are major enzymes involved in drug metabolism (Guengerich, 2008). Most of the drugs undergo metabolism *via* cytochrome P450 enzymes (Williams *et al*., 2002). Cytochrome 450 (CYP450) predicts CYP2D6 enzyme inhibition using 2D chemical structure, and has 2 level namely 0 for non-inhibitor and 1 for inhibitor (Susnow and Dixon, 2003). CYPs often have distinct role in xenobiotic metabolism with active sites that enable broad and overlapping substrate specificity (Ekins and Rose, 2002). All the phytocompounds fell in level 0, and these phytocompounds were non-inhibitor and unfavourable to inhibit the CYP2D6 enzyme.

Liver synthesizes, concentrates and secretes bile acids and excretes other toxicants, such as bilirubin. Drug-induced injury to hepatocytes and bile duct cells can lead to cholestasis. Cholestasis, in turn, causes intrahepatic accumulation of toxic bile acids and excretory products, which promotes further hepatic injury (Hartmut Jaeschke *et al*., 2002). Hepatotoxicity predicts potential organ toxicity for a wide range of structurally diverse compounds and has 2 levels, namely, 0 for non-toxic and 1 for toxic (Dixon and Villar, 1999). Phytocompounds lupeol, phytol and beta-amyrin have non-toxic effect and do not cause liver injury. Other compounds such as avicequinone C and beta-sitosterol have toxic effect and cause dose-dependent liver injuries.

Plasma binding protein helps in identifying the binding of inhibitors to carrier protein in the blood. It is generally assumed that only free drug can cross membrane

and bind to the intended molecular target, therefore, it is important to find the binding of plasma protein (Han van de Waterbeemd and Eric Gifford, 2003; Dixon and Merz, 2001). Plasma protein has three levels of binding capacity, namely level 0 has <90%, level 1 has ≥90% and level 2 has ≥95%. Phytocompounds such as phytol, avicequinone C, beta-amyrin and beta-sitosterol have a binding capacity of ≥95% to cross the membrane and are bound to plasma protein and another lupeol has a binding capacity of ≤90% to cross the membrane and bound to the plasma protein.

Out of five, most of the compounds satisfied ADME properties and Lipinski's rule of 5. Based on the *in silico* drug designing analysis through molecular docking results, phytol *A.marina* possessed antibacterial activity by inhibiting the activity of all the three virulent proteins of *E. coli*, and forming a strong atomic interaction with the active site residues. Hence, the compound was considered as a drug to treat infectious diseases.

The compound phytol is an acyclic diterpene alcohol. It is used as a precursor for the manufacture of synthetic forms of vitamin E and vitamin K1 (Daines *et al*., 2003). It is also used in industries to make fragrances, cosmetics, shampoos, toilet soaps, household cleaners and detergents (McGinty *et al*., 2010). It has antischistosomal activities and provides a basis for subsequent experimental and clinical trials.

The low toxicity, high bioactivity and tolerance by mammals support the potential of phytol as a new lead compound for human schistosomiasis (Josué de Moraes *et al*., 2014). Interestingly, the compound has been found to be a naturally occurring antimicobacterial agent, having a minimum inhibitory concentration (MIC) of 2 $\mu g\ ml^{-1}$ against *Mycobacterium tuberculosis* $H_{37}Rv$ strain, radiorespirometrically (Rajab *et al*., 1998).

Various therapeutic activities of phytol have been reported in previous studies, including its activity against mycobacteria (Saikia *et al*., 2010) anticonvulsant (Costa *et al*., 2012), antispasmodic (Pongprayoon *et al*., 1992) and anticancer activities (Lee *et al*., 1999). Also, it exerts an anti-inflammatory action and inhibited polymorphonuclear cell migration, probably by decreasing tumor necrosis factors- α and Interleukin- β levels (Jand Venes Medeiros *et al*., 2013) the finally, the present study may provide a hope to overcome failures of drug resistance profiles.

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