ISSN: 0975-766X
CODEN: IJPTFI
Available Online through *Research Article*
www.ijptonline.com

MOLECULAR DOCKING STUDIES OF NATURAL COMPOUND AS A NOVEL DRUG AGAINST SELECTED TARGETS OF UROPATHOGENS: *INSILICO STUDY*

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Received on 01-02-2015 *Accepted on 02-03-2015*

Abstract:

Urinary tract infections are the widest spread illness in India. Poor understanding and limited diagnosis is the major cause of the infection. This can be divided in two sub categories, such as complicated and uncomplicated infections. Most of this are bacterial and fungal, their cause is due to easy access to soft tissues. If a person is immunocompromised and hospitalised the chances of this infection is more than 70%. Due to lack of knowledge and less focus there is limited therapeutics available. High dose of broad spectrum antibiotics also now a day's getting resistance. Targeted therapy and natural drug selection is need of a hour. To achieve and develop new arena this pilot study was designed. Specific targets were selected from designated common occurring uropathogens namely *Pseudomonas aeruginosa, Klebsiella Pneumoniae and Escherichia coli.* Various active compounds were selected from plant leaves of *C.papaya* exibiting antimicrobial activity. Molecular docking studies were performed using Autodock 4.0 bioinformatics tool. All the compounds were screened for drug likeness activity as well as ADME analysis using bioinformatics software's. All the ccompounds have been screened and docked for binding energy analysis, the potential docked structures are then recommended for further studies.

Keywords: UTI, docking studies, ADMET, *Carica papaya*

Introduction

Infections of the soft tissues are the major and common problem of hospital acquired infections. Now a day's some diseases like tuberculosis, malaria are called as "Diseases of developing countries". Urinary tract infection associated

with hospitals are also comes under same category. India like developing country still suffering from the Nosocomial infection. What can be the reason? How it affects national health? Answers are less and no priority. Due to unawareness various factors are responsible, including educational factors, hygiene, funding, no importance, focus etc.

Bacterial infections are at high risk and threat to human beings indirectly. There are less symptoms, poor diagnosis and no targeted antibiotics.(1,2) Resistance in antibiotics is increasing day by day.

Even though the full genomes of the bacterial pathogens were discovered we are unable to catch the nerve. Indian scenario is very well described in various research articles in last decades, but there is no actions were taken. Insanitary conditions and poor knowledge about symptoms in population playing major role in spreading such infections. (3,4,5) If suspected, indiscriminate use of antibiotics occurs which ultimately results into resistance.

Herbal drug development is the one of the most possible and promising area where this issue can be overcome easily and safely. High interest in discovering natural antimicrobial leads to develop new drugs from ancient medicinal plants. *C. papaya* is the common plant available in India throughout all the seasons. Green leaves of this plant having some potential antimicrobial active agents reported by various authors.(6,7,8) We hypothesised to develop a new herbal antimicrobial drug against MDR uropathogens. Microbiological studies were performed previously and it is found that, there are certain bioactive compound which having MDR antibacterial activity, to understand more. All the compounds were screened using computational analysis using bioinformatics software's.

Methods:

Preparation of protein structure: Computational biology is vast and reliable method of drug discovery process. We have selected 3 protein targets from *Pseudomonas aeruginosa, Klebsiella*

pneumoniae and Escherichia coli were downloaded from database Protein Data Bank (PDB). (PDB: http://www.rcsb.org/ pdb/home/home. do) is (2VES, 1F0K, 4H0D) PDB id of the target proteins. All water molecules were removed and on final stage hydrogen atoms were added to receptor molecule. Table1.

Preparation of ligand structure:

Total 8 important natural compounds available in the plant extract of *C. papaya* were selected on the basis of their biological activity reported and molinspiron drawing tool used to draw the 3D structures.

Protein ligand interaction

All the three proteins and 8 ligands were subjected to docking studies individually using AUTODOCK 4.0 docking server, is based on the quantum mechanics, it predicts the molecular structures, active energies, geometry of structure, coordinates of atoms, bond length and bond angle present in their pocket.(9)

Drug likeness Analysis

Druglikeness activity prediction were performed using Molinspiron software to screen all the eight selected antibacterial compounds to designate as drug

Absorption, Distribution, Metabolism, and Excretion (ADME):

Were screened to analyse the drug like properties of the phytocompounds, calculation was performed by using the admet-SAR tool. The different parameters were calculated such as blood brain barrier, Aqueous solubility, metabolism, and carcinogenicity(10)

Computational Methods

Docking of the selected compounds and target selected were carried out using Autodock Server (11). Gasteiger partial charges were added to the ligand. Cleaning of the targets and ligand were done using Accelrys Discovery studio visualizer 4.0. Essential hydrogen atoms, Kollman united atom type charges, and salvation parameters were added with the aid of Auto Dock tools maps of 20 Å grid points and 0.375 Å spacing were generated using the Auto grid program automatically. Auto Dock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively.(12)

Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method. Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 10 different runs that were set to terminate after a maximum of 250,000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied. Also, due to high data output only the best score were selected and bonds were measured.

Results

The virulence factors are nothing but the proteins retrieved from the PDB database which enhances the infection by helping organism in their duplication. Structures of all the three different proteins of uropathogens downloaded from PDB shown in table1. The output of our previous work analysis shows active compounds tested microbiologically against bacterial strains (data not shown) which were selected for the docking purpose. Molecular docking was carried out using Lamarckian genetic algorithm (LGA), with Autodock tools, free energy of bindings were recorded for all the 8 compounds with 3 targets. Only remarkable docking scores were shown and discussed. 1H-Indole,3-[4-(1H-indol-3-yl)-2-thiazolyl found as the most suitable compound for all the targets with high dock score and various hydrogen bond formation i.e. -7.45kcal/mol. 2-Methoxy-4-vinylphenol also shows good score by forming strong hydrogen bonding with respective interacting amino acid chains. Table 3A,B and C.

Drug likeness activity were performed and all the compounds obeys Lipinski rule 5 and in the range of drug parameters, Out of three compounds 2-Methoxy-4-vinylphenol shows better score and definitely act as drug. Table 4

ADMET analysis shows range of results includes aqueous solubility among the all compounds, Blood brain barrier analysis generally used for penetration studies, level of 0-1 shows high penetration, in this study all the compounds shows high penetration levels hence considered to cross the barrier and act on Central nervous system. CYP450 are the important catalysers for drug metabolism, levels are from 0 and 1. In this study N-Aminomorpholine proved as inhibitors level. Data shown in table 5.

Table 1: Protein Retrieved from PDB.

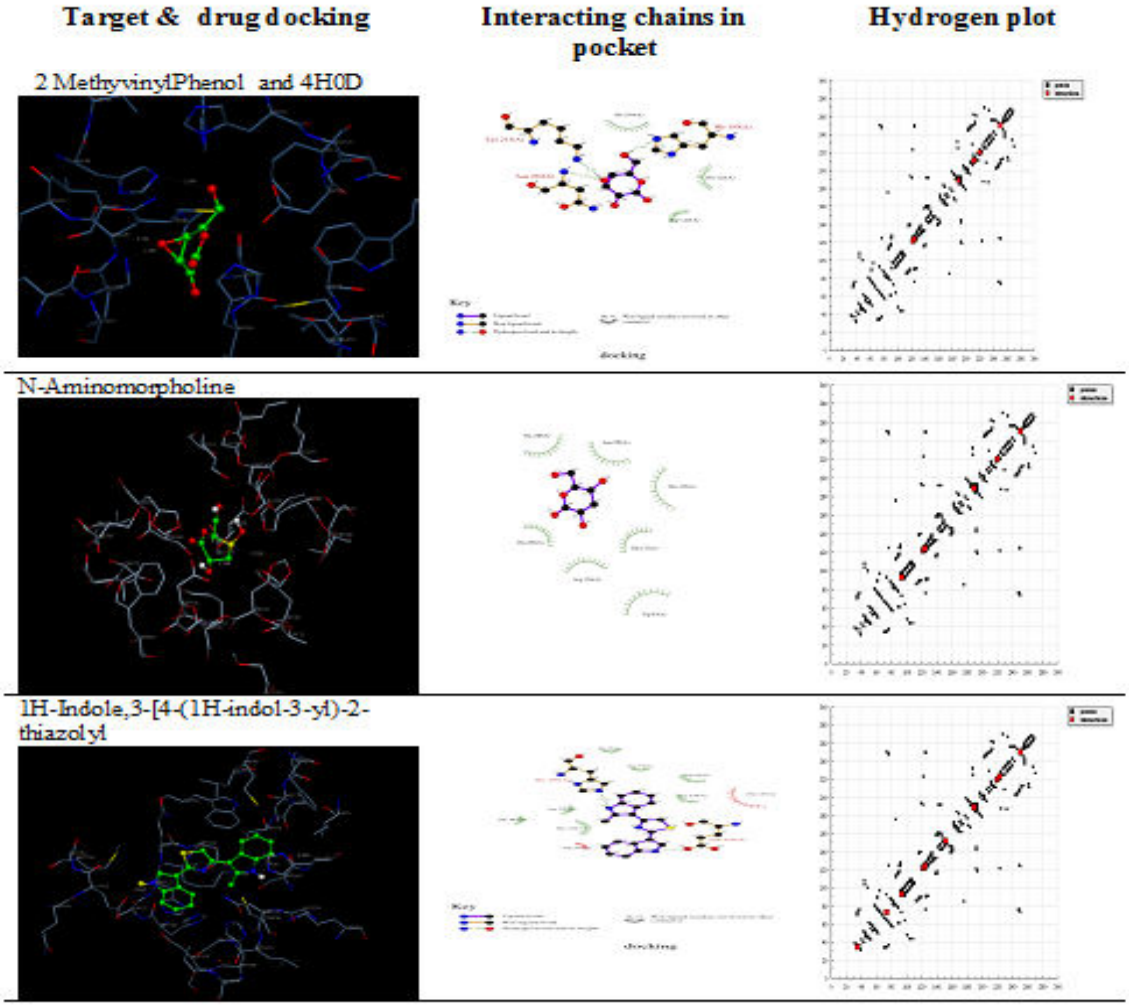
Sr No	Name of Protein	PDB Id
1	UDP-3-O-(R-3-hydroxymyristoyl)-N-acetylglucosamin deacetylase (LpxC) in Pseudomonas	2VES
2	MurG, membrane-associated glycosyltransferase	1F0K
3	Metallo beta-lactamase hydrolase	4H0D

Table 2: Protein Ligand Interaction Docking Scores.

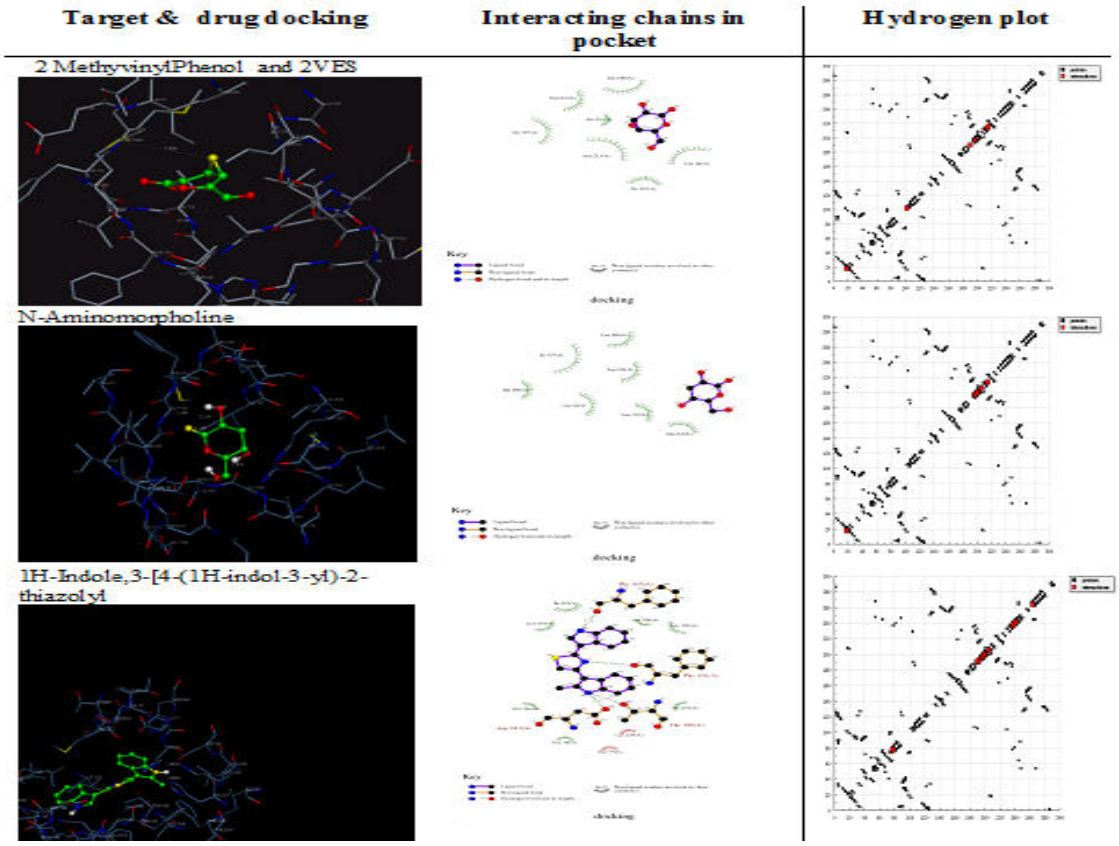
Ligands /Protein	2VES	1F0K	4H0D
N-Aminomorpholine	-4.85 kcal/mol	-4.38 kcal/mol	-3.78 kcal/mol
2-Methoxy-4-vinylphenol	-3.22 kcal/mol	-4.87 kcal/mol	-3.95 kcal/mol
1H-Indole,3-[4-(1H-indol-3-yl)-2-thiazolyl	-7.45 kcal/mol	-7.57 kcal/mol	-5.91 kcal/mol

Table 3 Docking interactions of receptor and ligands.

A) Best 3 Docking results of *Klebsiella pneumonia* target PDB ID 4H0D with hytocompounds.



B) Best docking view of *Pseudomonas aeruginosa* target PDBID 2VES with selected Phytocompounds.



C) Best three docking view of *E. coli* target PDBID 1F0K with selected Phytocompounds.

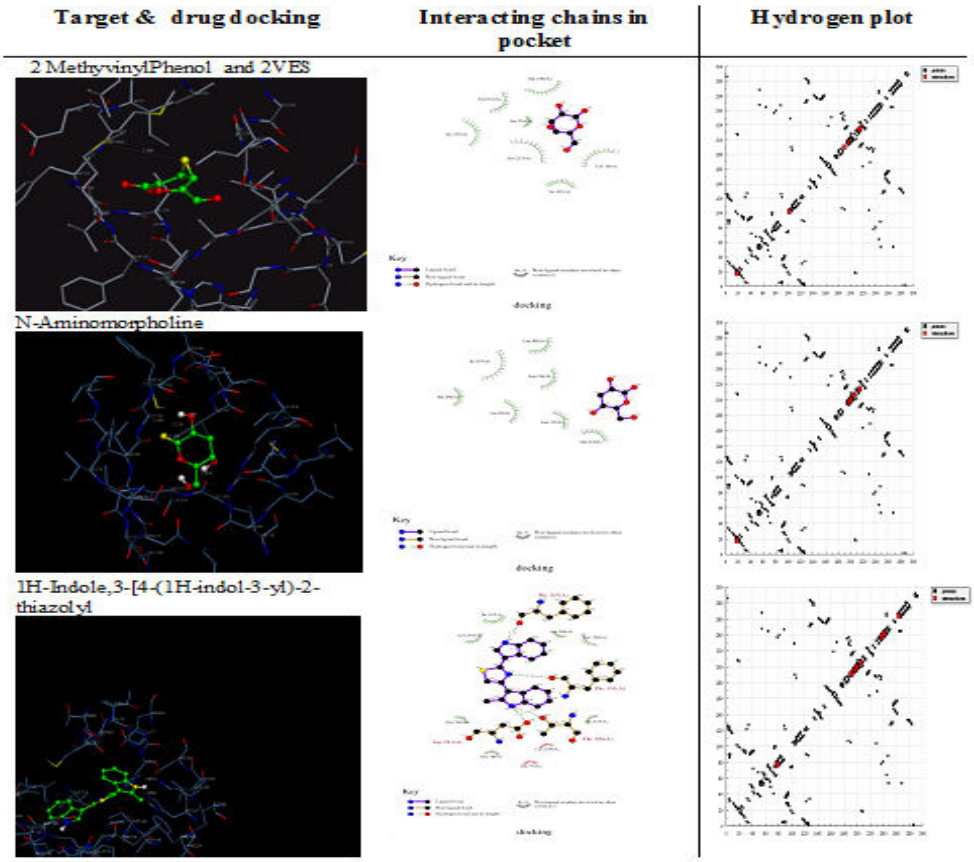


Table-4: Drug likeness activity of the selected ligands.

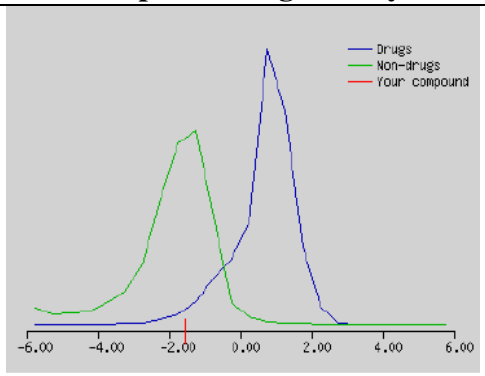
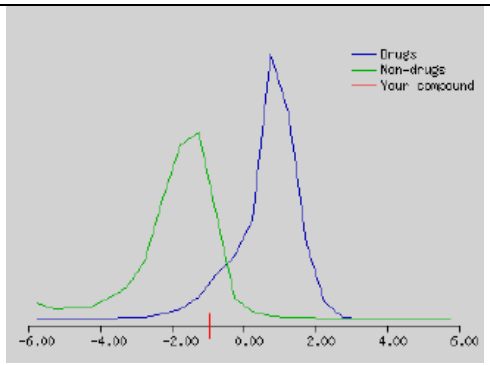
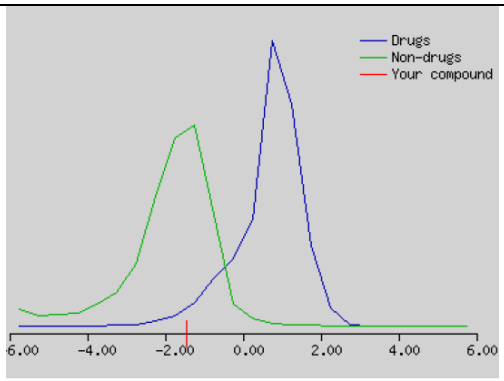
Name of the compounds	Graph showing activity	Drug likeness score
N-Aminomorpholine		**-1.54**
2-Methoxy-4-vinylphenol		**-0.96**
1H-Indole,3-[4-(1H-indol-3-yl)-2-thiazolyl		**-1.46**

Table 5: ADME analysis values of Phytocompounds

Ligands/ Test	Aqueous Solubility	BBB+	CYP450	AMES toxicity	Carcinogens
N-Aminomorpholine	-0.6090	0.9873	0.9148	0.5348-	0.8709 -
2-Methoxy-4-vinylphenol	-1.9439	0.8480	0.7598	0.9132-	0.8519-
1H-Indole,3-[4-(1H-indol-3-yl)-2-thiazolyl	-3.7499	0.8941	0.7353	0.5764	0.5548

Discussion

Antibacterial studies of the *C. papaya* plant leaves, stem, fruit etc were already studied and also mentioned in ancient literature. It is containing baskets full of health benfits for human health. Cancer drug derived from this plant is also pending by FDA. Various biological activities like mucolytic, wormicidal, antifungal available in phytoconstituents of leaves of papaya plant. We derived some of the compounds and checked for their antibacterial activity *invitro. Insilico* studies were used to confirm the results.

Pseudomonas aeruginosa is the gram negative bacilli available 99% in septicaemia, pus causing infections. It is having multifactorial stages of pathogenesis which means attachments, or adhesion, localization, and blood stream infection. This can also found in soft tissue of urinary tract of ill hospitalised patients. In Virulence there are more than dozen factors involves but currently LpXc is the more attracting target used for antibacterial drug development. LpxC [UDP-(3-*O*-acyl)-N-acetyl glucosamine deacetylase] is a metalloenzyme that catalyzes the first step in the lipid A biosynthesis in bacteria (13). Gram negative bacterial cell wall is full of LPS i.e. LpxC UDP-(3-*O*-acyl)-N-acetyl glucosaminedeacetylase which prevents entry of hydrophobic agents ultimately refluxes of antibiotics.(14) Hence we used phytocompounds to check its biological activity. We found that, compounds with amino group and indole group are more active and able to bind easily to this target.

Similarly, *Escherichia coli* also have the same mode of infection, it is gram negative walled organism. glycosyltransferase involved in peptidoglycan biosynthesis in gram negative bacteria, since last decade this MurG enzyme is extensively used for targeting. It is having alpha and beta domains.(15) Structural domains available in the cleft of this terminal of protein which act as donor and acceptable in bond formation. Due to this property, Target was selected and docked resulting the good scores of binding energies. *Klebsiella Pneumonia* is opportunistic pathogen and multidrug resistance. Selected target is beta lactamases available in Indian strains isolated in 2014 by Kim, Y Tesar, C. et al. It can be classified as hydrolases.(16) Generally they breaks beta lactum ring in antibiotics and alter their structures so ultimately it loses its antibacterial activity. Our docked compound such as indole and phenolic groups directly binds with the enzymes with good free energy of binding.

Conclusion

Therefore, in this alarming circumstances of developing resistance in uropathogens. Urgently alternate source of antimicrobial need to develop for better disease management. Aim of this study is to contribute in herbal drug development against multidrug resistant bacteria. We hope that *invitro* and *insilico* results will opens doors for further clinical studies.

Conflict of Interest:

The authors declare that there is no conflict of interests regarding the publication of this paper.

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