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Comparative Screening analysis of Natural metabolites against a cholera toxin

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ABSTRACT

Cholera is one of the earliest infections to be studied by epidemiological methods. Worldwide it affects 3-5 million people and causes 100,000-130,000 deaths a year as of 2010. Cholera is an infection of the small intestine that causes a large amount of watery diarrhea; it is caused by the bacterium *vibrio cholerae*. The cholera toxin (CTX or CT) is an oligomeric complex made up of six protein subunits a copy of A subunit and the five copies of B subunit, connected by a disulfide bond. This study is based on targeting the CTX using computational tools, a natural metabolite mangiferin was taken and its chemical features are generated using common pharmacophore analysis, using minmaybridge database and taken lead compound were optimized and the Lipinski's Rule of 5 properties were analyzed. Here, the binding affinity of protein – ligand interaction studied and displayed using ligand fit.

INTRODUCTION

Cholera is an infection of the small intestine that is caused by the bacterium *vibrio cholerae* (Farques, 2008). The main symptoms are profuse watery diarrhea and vomiting. Transmission is primarily through consuming contaminated drinking water or food. The severity of the diarrhea and vomiting can lead to rapid dehydration and electrolyte imbalance. Primary treatment is with oral rehydration solution and if these are not tolerated intravenous fluids. Antibiotics are beneficial in those with severe disease. Worldwide it affects 3-5 million people and causes 100,000-130,000 deaths a year as of 2010 (Rosenberg, 1987). Cholera was one of the earliest infections to be studied by epidemiological methods (Pierre, 2001).

Signs and Symptoms

The primary symptoms of cholera are profuse painless diarrhea and vomiting of clear fluid. These symptoms usually start suddenly, one to five days after ingestion of the bacteria. The diarrhea is frequently described as "rice water" in nature and may have a fishy odor. An untreated person with cholera may produce 10-20 liters of diarrhea a day with fatal results. For every symptomatic person there are 3 to 100 people who get the infection but remain asymptomatic. If the severe diarrhea and vomiting are not aggressively treated it can, within hours, result in life-threatening dehydration and electrolyte imbalances. The typical symptoms of dehydration include low blood pressure, poor skin turgor (wrinkled hands), sunken eyes, and a rapid pulse (Byrne, 2008).

Mechanism of Action

Most bacteria, when consumed, do not survive the acidic conditions of the human stomach. The few bacteria that do survive conserve their energy and stored nutrients during the passage through the stomach by shutting down much protein production. When the surviving bacteria exit the stomach and reach the small intestine, they need to propel themselves through the thick mucus that lines the small intestine to get to the intestinal walls, where they can thrive. *v. cholerae* bacteria start up production of the hollow cylindrical protein flagellin to make flagella, the cork-screw helical fibers they rotate to propel themselves through the mucus of the small intestine (Merrel, 2002). Once the cholera bacteria reach the intestinal wall, they no longer need the flagella propellers to move (Burnshaw *et al.*, 2000). The bacteria stop producing the protein flagellin, thus again conserving energy and nutrients by changing the mix of proteins which they manufacture in response to

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the changed chemical surroundings. On reaching the intestinal wall, *v. cholerae* start producing the toxic proteins that give the infected person a watery diarrhea. This carries the multiplying new generations of *v. cholerae* bacteria out into the drinking water of the next host if proper sanitation measures are not in place (Haynes, 1997).

The cholera toxin (CTX or CT) is an oligomeric complex made up of six protein subunits: the A subunit (part A), and five copies of the B subunit (part B), connected by a disulfide bond. The five B subunits form a five-membered ring that binds to GM1 gangliosides on the surface of the intestinal epithelium cells. The A1 portion of the A subunit is an enzyme that ADP-ribosylates G proteins, while the A2 chain fits into the central pore of the B subunit ring. Upon binding, the complex is taken into the cell *via* receptor-mediated endocytosis. Once inside the cell, the disulfide bond is reduced, and the A1 subunit is freed to bind with a human partner protein called ADP-ribosylation factor 6 (Arf6) (O'Neal, 2005).

Binding exposes its active site, allowing it to permanently ribosylate the Gs alpha subunit of the heterotrimeric G protein (De Haan, 2004). This results in constitutive cAMP production, which in turn leads to secretion of H_2O, Na^+, K^+, Cl^-, and HCO_3^- into the lumen of the small intestine and rapid dehydration. The gene encoding the cholera toxin is introduced into *v. cholerae* by horizontal gene transfer. Virulent strains of *v. cholerae* carry a variant of temperate bacteriophage called CTXf or CTXφ (Davis, 2003).

Existing Antibiotics

Antibiotic treatments for one to three days shorten the course of the disease and reduce the severity of the symptoms. People will recover without them, however, if sufficient hydration is maintained (Sehdev, 2002).

Doxycycline is typically used first line, although some strains of *v. cholerae* have shown resistance. Testing for resistance during an outbreak can help determine appropriate future choices. Other antibiotics that have been proven effective include cotrimoxazole, erythromycin, tetracycline, chloramphenicol, and furazolidone. Fluoroquinolones, such as norfloxacin, also may be used, but resistance has been reported (Smith, 2006).

MATERIALS AND METHODS

The co-ordinates for all the proteins obtained from Research Colloboration for Structural Bioinformatics (RCSB, www.rcsb.org) (PDB ID 2A5G). The structure was derived from RCSB in which all the units contain same binding site for the ligand (Zhang R *et al.*, 1995). In the present study multiple ligand was targeted. The complex was prepared by the module protein preparation wizard, where hydrogens were added automatically and refinement was done shown in Fig. 1. Water molecules were removed for the protein-ligand interactions and bond orders were re-assigned. The structure was minimized to a Root mean Square deviation of 0.30Å.

Pharmacophore Generation

The Common feature Pharmacophore Generation protocol generates pharmacophore that are common to a set of active ligands. Optionally, it can excluded volumes to the pharmacophore based on the information from a set of inactive.

MANGIFERIN

Mangiferin is a xanthonoid, a chemical compound found in mangoes and in Anemarrhena asphodeloides rhizomes. This molecule is a polyphenol and shows antimicrobial and antioxidant activities. It shows inhibitory effects on type II 5α-reductase in vitro. It shows gastro protective and antidiabetic effects in rodents. Mangiferin, 1,3,6,7-tetra hydroxyl xanthone-C2-beta-D-glucoside, is one of xanthone derivatives and C-glucosylxanthones (Blake, 2003).

Fig. 1: Structure of Mangiferin.

Toxicity Prediction

ADMET provides protocols and tools that allow you to compute the adsorption, Distribution, Metabolism, Excretion and Toxicity properties of small molecules. The tool TOPKAT method for assessing toxicity prediction for organic compounds also it helps to assess an environmental fate, ecotoxicity, toxicity, mutagenicity, and reproductive/developmental toxicity of chemicals.

Common feature pharmacophore for the ligand

The common chemical structures of the mangiferin is generated using Hip-Hop catalyst-Cerisus 2 is shown below. HipHop1 is taken for further study to find the leads fits to this Hiphop1 using Minimaybridge database which is in-built in Accelrys Discovery Studio 2.5.

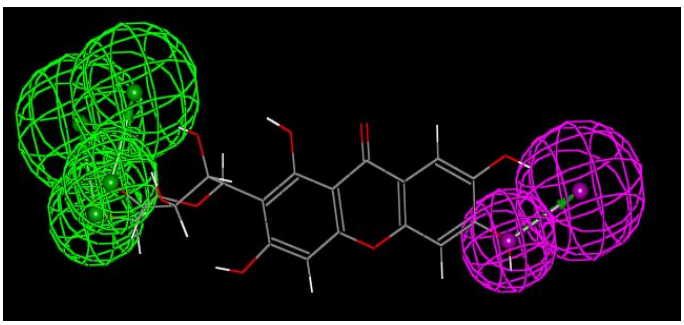

Fig. 2: represents the pharmacophoric relationship of the protein

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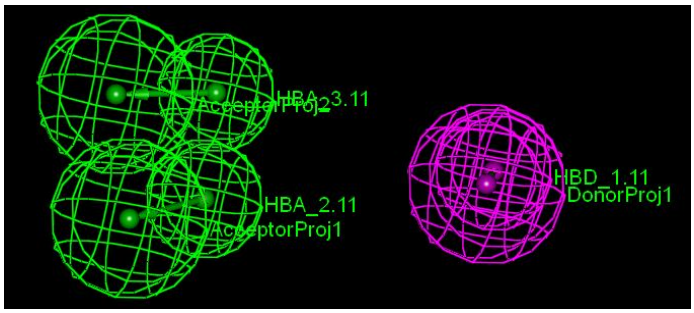

Fig.. 3: represents the pharmacophoric relationships with indications.

RESULTS AND DISCUSSION

Receptor Ligand Interaction

The interaction between the receptor and the ligand are fundamental to drug discovery. The tool Discovery Studio provides a set of methods for predicting and analyzing the interactions between protein receptors and ligands. These methods allow us to carry out the structure based design or even to examine the possible interactions with theoretical structures such as homology models. A common technique pertaining to the receptor ligand interaction is docking. The Table 1 shows the docking of ligand to active site amino acids of Cholera toxin A.

Table. 1: rep. about the energy value and dock score of both the similar drug molecules.

Name of the compound	Dock Score	Energy Value
DSHS 00507	23.876	5.316
NRB 03988	39.099	0.322

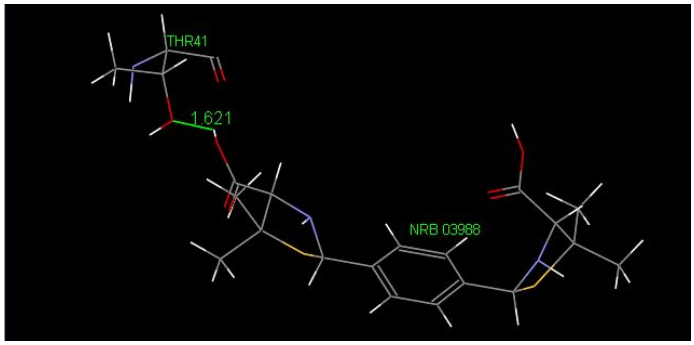

Fig. 4a: Interaction of cholera toxin A with NRB 03988

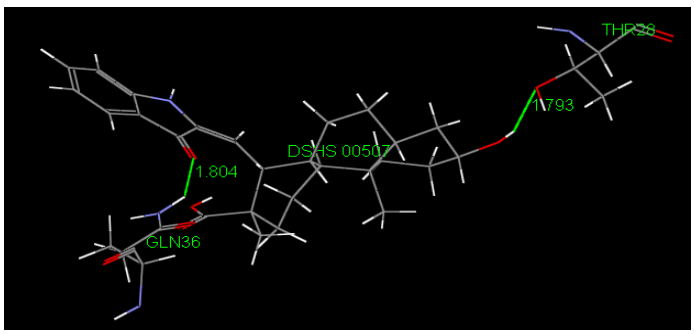

Fig. 4b: Interaction of cholera toxin A with DSHS00507.

Calculation of Molecular Properties

The molecular property of the ligand is calculated using the Lipinski's rule of five. This rule is important for drug development where a pharmacologically active lead structure is optimized step-wise for increased activity and selectivity, as well as drug-like properties as described by Lipinski's rule.

- Molecular weight (M.Wt) ≤ 500,
- Lipophilicity Clog P ≤ 5
- H-bond donors ≤ 5 (sum of OH and NH)
- H-bond acceptors ≤ 10 (sum of O and N atoms)

Here, the 38 molecules that were calculated for molecular properties. Out of that 31 molecules were taken for further studies,7 molecules are ruled out using Lipinski's Rule of 5.

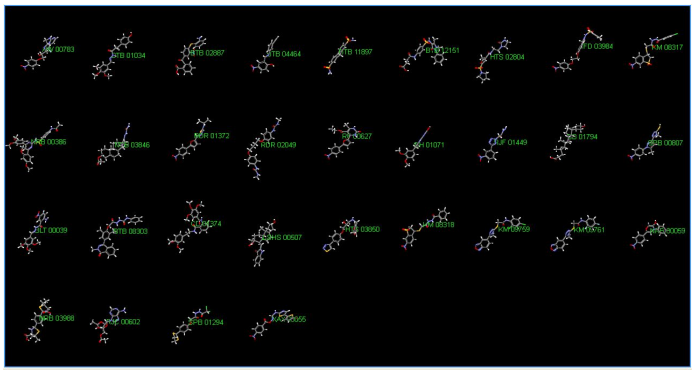

Fig. 5: shows that the structure taken to study for Lipinski's rule of 5.

ADMET Properties

The 31 ligands were identified and observed for ADMET properties. Among those only four compounds namely **RDR 01732, SB0174, NRB 03988 and DSHS 00507** satisfied the ADMET criteria and shown in Table 2.

Table. 2: describes about the ADME values of selected drug- like molecule.

S. No	Name	Blood Brain Barrier	Absorbtion	Solubility	Hepatoxicity	Cyp2d6
1	RDR 01372	2	0	2	0	0
2	SB 01794	2	0	2	0	0
3	DSHS 00507	4	0	2	0	0
4	NRB 03988	3	0	2	0	0

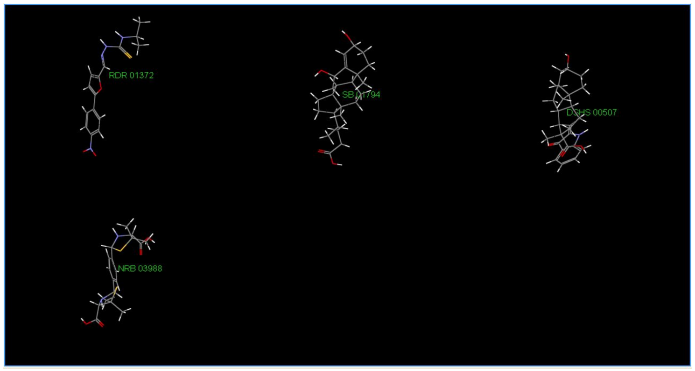

Fig. 6: indicates the compounds satisfied by ADME properties.

TOXICITY PREDICTION

TOPKAT tool is for assessing toxicity prediction of organic compounds. It can be used to assess environmental fate, ecotoxicity, toxicity, mutagenicity, and reproductive/developmental toxicity of chemicals. Here, the compounds NRB03988 and DHS00507 passed the TOPKAT analysis. Hence, the above mentioned compounds were considered for an active compound towards colera toxin.

CONCLUSION

The study is based on identifying the binding affinity between the protein and ligands namely, mangiferin and similar compounds NRB03988 and DHS00507 to analyze for cholera disease also evaluated using various tools. The drug molecule, which is perfectly, binds with the protein in the regions of Thr 28 and Gln 36. So, this analysis suggests that the studied of small molecule is suitable for drug improvement of cholera targets. Further, studies to be verified on *in vivo* analysis and to be investigated towards toxicity.

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