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In Silico Study of Anti-Cholera Activity of Some Tetracycline Molecules

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ABSTRACT

Cholera is a major health challenge most especially in the developing countries of the world. Cholera toxin (CTX or CT) is a protein complex which is secreted by the pathogenic organism, *Vibrio cholerae*. Several attempts have been made to eliminate the cholera toxin which is an oligomeric complex containing six protein subunits (AB_5) connected by a disulfide bond. Antibiotics have shown high level of potency in inhibiting the activity of the pathogen. World Health Organization (WHO) has recommended tetracycline as the most preferred choice for cholera control. Herein, the inhibitory effect of some tetracycline molecules against cholera causing bacteria, *Vibrio cholera* was investigated by molecular docking. The protein responsible for the bacterial disease was retrieved from protein data bank and docked against some teracycline compounds. The combination of Density Functional Theory (DFT), and molecular docking methods were used to investigate the inhibitory activity of five selected tetracycline molecules as an anticholera agent. The calculated molecular descriptors from quantum chemical method (DFT) were used. Molecular descriptors computed are log P, solvation energy and average electronic charges on all heteroatoms. Inhibition result was in the order: Tetracycline>Methacycline>Androtetracycline>Oxytetracycline/Chlorocycline.

Keywords: Cholera, Tetracycline molecules, Density Functional Theory (DFT), Molecular docking, Binding Affinity, Amino acid residue, Hydrogen bond.

INTRODUCTION

Cholera is a life- threatening disease most especially in the third world nations. It is caused by improper disposal of sewage and drinking of unpurified water and consumption of contaminated food [1]. Cholera has been reported to cause 100,000- 130,000 deaths of the World's population in the year 2010 [2].

The major cause of cholera is the bacteria known as *Vibrio cholerae. V.* cholera has about 209 serogroups depending on the O antigenic structures among which *V. cholerae* O1 and O139 have been found to cause widespread cholera epidemics. *V. cholerae* O139 serogroup strain was

first discovered in Bangladesh and India in 1992 [3]. Research has revealed that the two major virulence gene clusters responsible for the pathogenicity of V. cholerae O1 and O139 are CTXΦ prophage [4] which carries the CTXA and CTXB genes (the gene that encode cholera toxin (CT) which is responsible for severe diarrhea and toxin coregulatedpilus (TCP), which is responsible for the transportation of genes for the biosynthesis of the TCP needed for colonization of the small intestinal epithelium [5]. CTXΦ is a filamentous, hysogenic bacteriophage whose genome encodes cholera toxin [6].

Cholera toxin produced by the bacterium *Vibrio cholera* is made up of six protein subunits, a subunit with enzymatic activity and five B subunits with receptor binding. The active site of cholera toxin is in the single B subunit and a single solvent mediated hydrogen bond from the amino-acid residue Gly 33 to the neighboring subunit [7]. Cholera toxin sticks to the plasma membrane of intestinal epithelial cells releasing an enzymatically active sub unit which causes an increase in the production of adenosine 5-monophosphate (cAMP). The high level of cAMP in the cell leads to enormous secretion of electrolytes and water into the intestinal lumen [8]. *V. cholera s*trains possess a great public health treat among other pathogens. The earlier symptoms of cholera are diarrhea and vomiting resulting to dehydration and electrolyte imbalance.

The potency of some antibiotics for combating *Vibrio cholerea,* O395 cannot be over-emphasized. However, one of the strains most prevalent in India, *V. cholera* O139 has been established to defy fluoroquinolones [9]. Polyphenolic compounds of Tea have been found to be an excellent drug inhibitor of cholera toxin and a series of possible 1,2,3-triazole- bearing benzensulfonamides have been optimized as new antibiotic agent [10]. While vaccines against cholera exist, it is believed that oral rehydration therapy remains an effective treatment method, good sanitation and access to portable clean water will help in the long term solution to the spread of cholera [11].

The inhibitory activity of D-Galactose- based divalent neoglycopeptides for cholera toxin has been investigated [12]. Recent discovery of the antibiotic resistance in *V. cholera* drew attention to the production of novel therapeutics that counteract virulence rather than the viability of the pathogen which to the exploration of rare marine actinobacterium (RMA) being inhibitive to the formation of V. cholera biofilm [13]. Antibiotic therapy was reported to be crucial for the treatment of cholera, as it stops diarrhea [14]. The tetracycline is an important broad spectrum antibiotics used for the prophylaxis and treatment of variety of bacterial infections. This is the

preferred choice for the control of cholera as recommended by the World Health Organization (WHO) [15].

Therefore, this research would help reveal the annotation of one of the most devastating human pathogens by exploring the use of some cycline molecules by computational study.

METHODOLOGY

A personal laptop computer with the following specifications; 250 gigabyte hard drive, 8 gigabyte ram, 7th generation with intel core i5 processor was employed for the computational calculations in this research work. The tetracyclne compounds were optimized using Spartan,14 v 1.1.4 and the descriptors that described the anti-cholera main protease activities were obtained. The optimized compounds were docked into the active pocket of the main protease (PDB code: 6w637 using AutoDock Tool 1.5.6.17. The grid box centre was (X = -40.481, Y =-5.881, Z = 47.26) and box size (X = 40, Y = 40, Z = 40). The spacing was set to be 1.00Å. The binding affinities and the molecular interaction for each complex were observed.

RESULTS AND DISCUSSION

Molecular Descriptors

The calculated molecular descriptors for the five tetracycline molecules, Anhydrotetracycline (T1), Tetracycline (T2), Oxytetracycline (T3), Methacycline (T4) and Chlorotetracycline (T5) are shown in Table 1. The HOMO and the LUMO are vital descriptors that offer realistic qualitative facts about the excitation properties of molecules [16-18]. The calculated electronic descriptors band gaps which is essentially the difference between LUMO and HOMO were 3.45eV, 4.01eV, 3.24eV, 3.34eVand 3.39eV for T1, T2, T3, T4 and T5 respectively. Therefore, the band gaps are in the order T2 > T1 > T5 > T4 > T3. The lower the band gap the easier the excitation and the better the ability of a molecule to donate an electron (s) to the surrounding. The log P values are less than 5 indicating ability to dissolve in lipophilic solvents. The calculated log P are: 3.65, 4.06, 3.96, 3.79, and 3.14 for T1, T2, T3, T4 and T5 respectively. Therefore, compounds T1 to T5 are effective in term of lipophilicity. The calculated values for ovality which is the degree of deviation from perfect circularity of the cross section of the core or cladding of fiber [19] are 1.53, 1.52, 1.52, 1.54 and 1.52 for T1, T2, T3, T4 and T5 respectively. Also, the dipole moment which is the product of the magnitude of the charge and the distance of separation between the charges [20] was11.1 debye, 13.13 debye, 9.72 debye, 9.93 debye and

10.24 debye for T1, T2, T3, T4 and T5 respectively. However, large value of dipole moment has been attributed to the anomalous property of individual molecule [21].

Docking and Scoring

The docking simulation and solvent environment of each tetracycline compound (ligand) produced nine conformations and the best conformation is assumed to be the conformation with highest energy of binding (i.e. more negative value) in each docking. The binding affinities for compounds T1-T5 are displayed in Table 2. Interaction between the cholera toxin and tetracycline molecules is basically due to *Van derWaal*, hydrogen bond interactions. Androtetracycline showed two hydrogen bonds with GLN A:56 and GLN A: 49 protein residue,

Tetracycline basically bonded to the cholera protein residue via three hydrogen bonds with PHEA:25, ILEA:24 and SERA:26. While, Oxytetracycline was bonded to the protein residue via one unfavourable acceptor-acceptor bond with ALA A:92 and four hydrogen bonds with TVR A:12 and GNL A:61. Methacycline binds to the protein residue by four hydrogen bonds with TYR A:12, HIS A:13, ASN A :14 and THR A:15 and one unfavourable donor-donor bond with ASN A:14 and one *Van der Waal* force with THR A:15. Chlorocycline showed six hydrogen bonds with VAL A:52, GLN A:56, GLN A: 49. ARG A:35, LYS A:34 and SER A:55.

Molecular docking reveals the binding geometries of a ligand with a target. The three major reasons for docking includes characterization of binding sites, positioning of the ligand into the binding site and the evaluation of the strength of interaction [22] and for a ligand to be bioavailable and possibly potential drug, it's Hydrogen bond acceptor < 10, molecular mass < 500 Daltons, log p < 5 and it should have a strong inhibition value of 7100 [23].

Table1: The calculated molecular descriptors from the compounds T1-T5 for anti –cholera

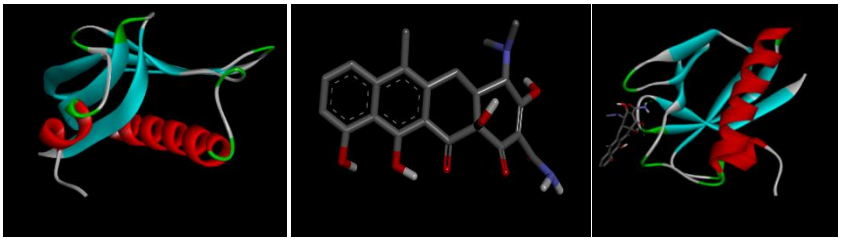
M	HOMO (eV)	LUMO (eV)	BG	LOG P	DM (Debye)	PSA (amu)	MW	HBD	HBA	A (A^2)	V (A^3)	OVALITY (A2)
T1	-5.49	-2.04	3.45	-3.65	11.40	145.62	444.44	6	10	407.11	407.40	1.53
T2	-6.05	-2.04	4.01	-4.06	13.13	157.50	460.44	7	11	411.03	413.23	1.52
T3	-5.29	-2.05	3.24	-3.96	9.72	139.63	442.42	6	10	401.12	402.99	1.52
T4	-5.50	-2.16	3.34	-3.79	9.93	144.02	478.89	6	10	419.30	420.60	1.54
T5	-5.44	-2.05	3.39	-3.14	10.24	127.5	426.43	5	9	395.18	394.97	1.52

BG- Band gap, DM- Dipole moment, PSA- Polar surface area, MW- Molecular weight, HBD- Hydrogen bond donor, HBA- Hydrogen bond acceptor, A-Area, V- Volume

Table2: Docking Scores of Conformations of the studied Tetracyline Molecules

Molecule	Binding Affinity (kcal mol^{-1})
T1	-7.0
T2	-7.6
T3	-6.9
T4	-7.2
T5	-6.9

Binding Interactions between Ligands and protein receptor for T1,T2,T3,T4 and T5



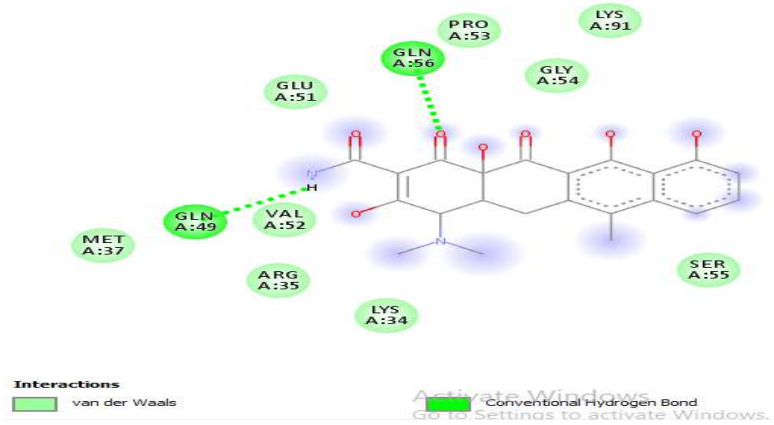


Figure1: Molecular Docking of Anhydrotetracycline

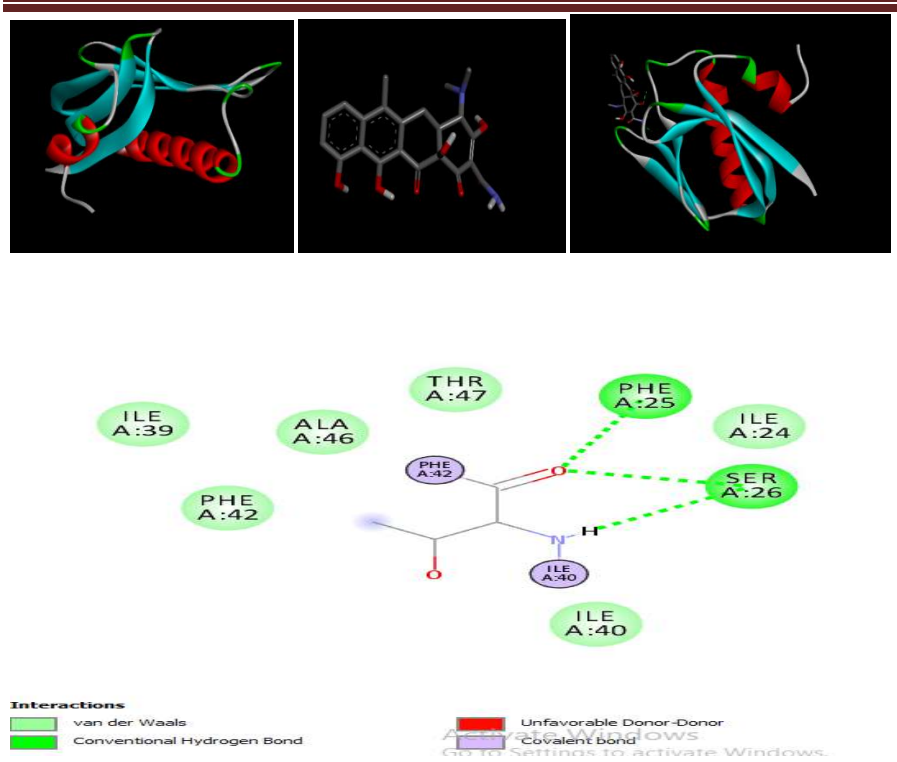
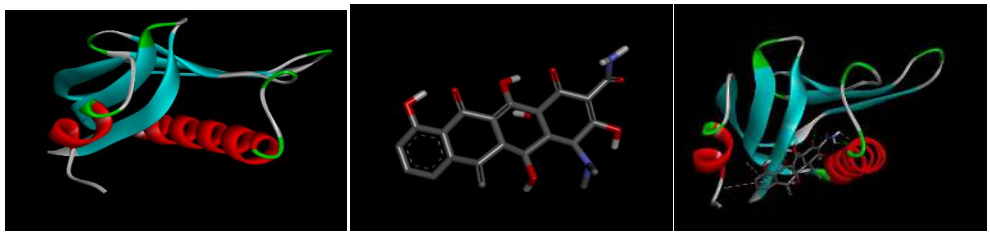


Figure 2: Molecular Docking of Tetracycline



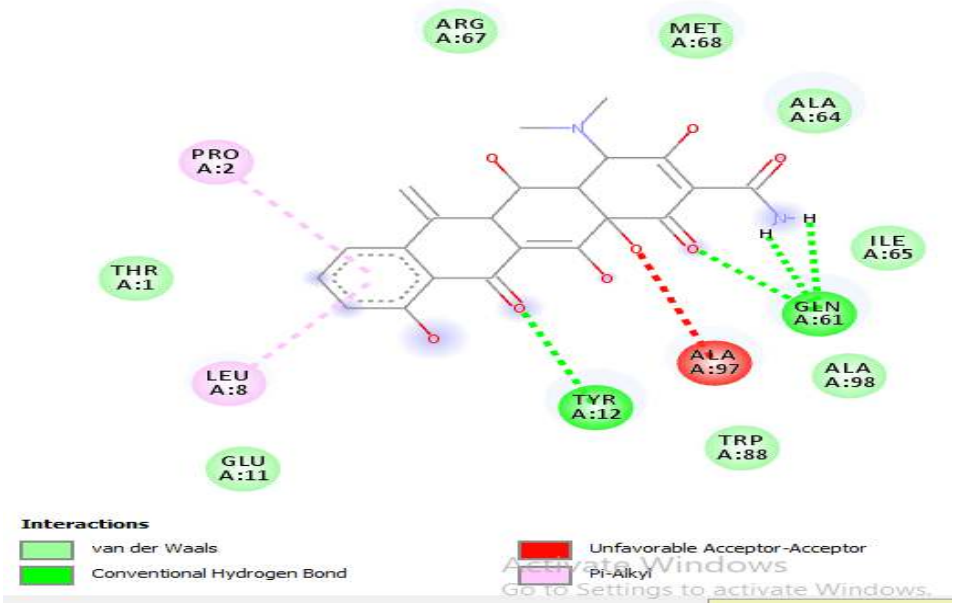
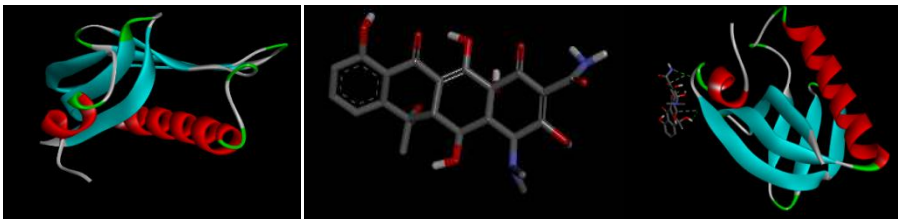


Figure 3: Molecular Docking of Oxytetracycline



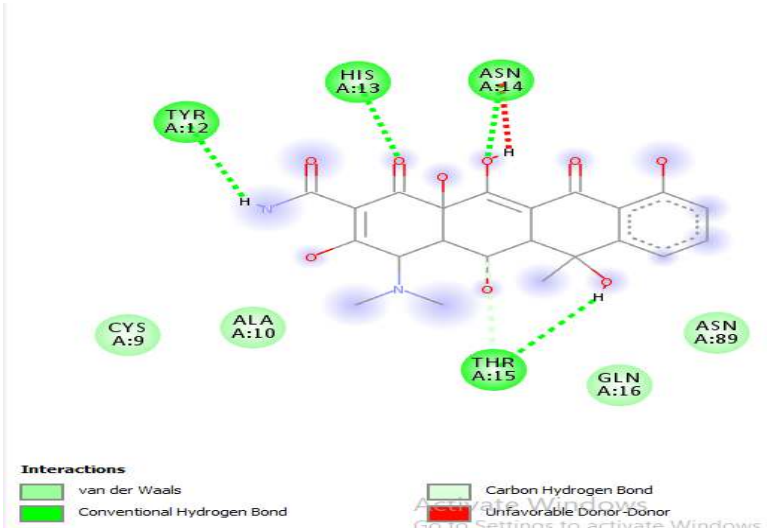


Figure 4: Molecular Docking of Methacycline

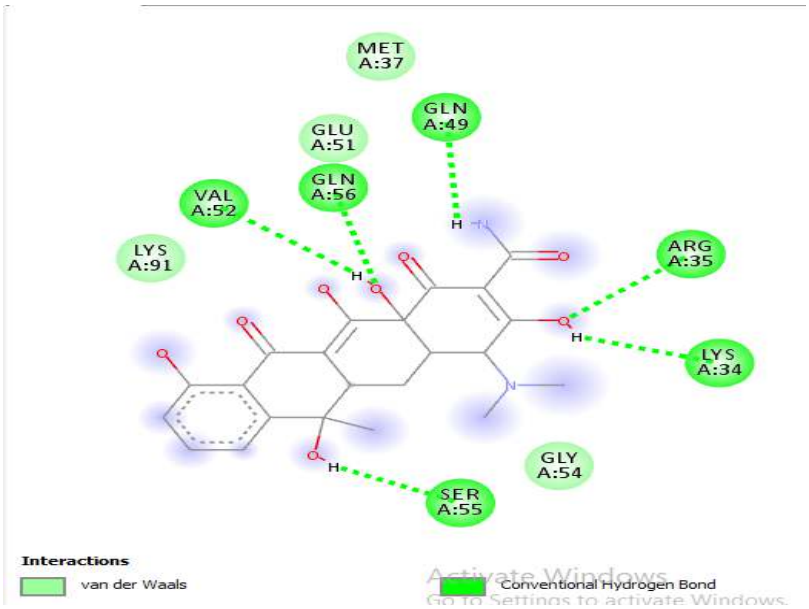


Figure 5: Molecular Docking of Chlorocycline

CONCLUSION

Antibiotics have been reported to be very effective for curing cholera. Inhibition is in the order: Tetracycline >Methacycline>Anhydrotetracycline>Oxytetracycline/Chlorocycline. Therefore, Tetracycline is the most potent antibiotic studied for curing cholera as Oxytetracycline and Chlorocycline have equal potency.

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