International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.7, No.1, pp 54-63, 2014-2015

Design of Cholera Toxin Antagonists by Molecular Docking from Polyphenolic Compounds of Tea.

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Abstract: Cholera is still a problem in third world countries and remains as a great threat to public health and safety. The effective and early treatment of cholera is to neutralise the toxin. So far there is no effective agent in the neutralisation of cholera toxin. 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 elucidation 3D structure of CT paved the way for the understanding of binding mechanism of CTB and thus researchers started to design inhibitors which can bind to the binding site of CTB. These inhibitors can be effective drugs in the prevention and treatment of cholera. Three polyphenols viz. disometin, quercetin and ternatin present in the tea are selected for the present study. Their derivatives were prepared *insilico* by ACD chemsketch software. These derivates were initially subjected to molecular docking in iGEMDOCK. Then the best derivates in terms of ADME properties and binding energy were further subjected to molecular docking by HEX version 8.0.0. From the present study it was found that the few derivatives of three natural polyphenols from tea viz. disometin, quercetin and ternatin have shown excellent binding energy values with good drug likeliness. These compounds are excellent drug candidates in the inhibition of cholera toxin and have the potential to be a treatment in the control of cholera.
Key words: Cholera toxin, polyphenols of tea, molecular docking.

Introduction

Cholera is still a problem in third world countries and remains as a great threat to public health and safety. It is an infection of small intestine that is caused by the bacterium *Vibrio cholera*[1]. Worldwide it affects 3-5 million people and causes 100,000-130,000 deaths[2]. Cholera toxin is the causative agent for the massive secretory diarrhoea[3]. Thus the treatment of cholera does not rely on antibiotic administration. The effective and early treatment of cholera is to neutralise the toxin. So far there is no effective agent in the neutralisation of cholera toxin.

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. Upon binding, the complex is taken into the cell *via* receptor-mediated endocytosis[4]. This is then followed by splitting of the A chain and Cys187 –Cys199 disulphide bond reduction that results in two fragments A1 and A2[5]. Then A1 gets translocated across the membrane to the cytosol. Gsu, the signalling protein needs ADP ribose moiety from NAD+ which it catalysed by A1. After this event, a series of events will lead to enormous loss of water from epithelial cells into the intestinal lumen, causing the characteristic watery diarrhoea of cholera.

Thus it is evident that for the action of CT the binding of CTB to the host cell is important. In early 1970, the GM1 ganglioside was identified as the receptor for CT[6]. One of the strategies to prevent adhesion of CTB to the cell surface involves design and synthesis of functional and structural mimics of GM1.

The efforts to solve a complete structure of cholera toxin by X-ray diffraction analysis were concluded during the 1990s[7, 8]. This paved the way for the understanding of binding mechanism of CTB and thus researchers started to design inhibitors which can bind to the binding site of CTB[9, 10]. These inhibitors can be effective drugs in the prevention and treatment of cholera.

Many researchers have found the presence of anti diarrhoeal activity of many medicinal plants[11, 12]. Few studies on tea extracts have shown that it has an activity against the infection of *Vibrio cholera*[13, 14]. The tea extract is a rich source of polyphenols. Thus an attempt was made in the present study to use the *insilico* method of molecular docking to screen the polyphenols of tea for its inhibitory properties and to prepare its derivatives to find an effective drug candidate in the prevention of cholera.

Materials And Methods

Protein preparation

The three dimensional crystal structure of cholera toxin was obtained from RCSB database (http://www.rcsb.org/pdb/explore/explore.do?structureId=1XTC)[8]. Its PDB code is 1XTC. Using Pymol software the water molecules were removed and the hydrogen atoms were added to the protein.

Generation and optimization of Ligand

Three polyphenols viz. disometin, quercetin and ternatin present in the tea are selected for the present study. Their structures (Figure 1) in SDF format was obtained from Pubchem database. The structures were converted to MDL format in Open Babel software (www.vcclab.org/lab/babel/start.html). The derivatives of the all three polyphenols were prepared in ACD chemsketch software[15]. ACD/ChemSketch is the powerful all-purpose chemical drawing and graphics package from ACD/Labs developed to draw the desired molecules and to store it in various desired formats. It also helps to generate IUPAC names and to calculate certain chemical properties of the chemicals. All the prepared compounds were saved in MDL format. Finally all the compounds were converted to PDB format by Open Babel software.

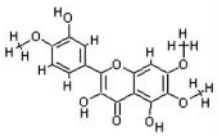
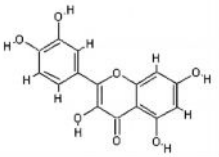
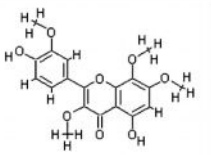
Polyphenol	Compound ID	IUPAC name	Structure
Disometin	CID: 5281612	5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chromen-4-one	
Quercetin	CID: 5280343	2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one	
Ternatin	CID: 5459184	5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-3,7,8-trimethoxychromen-4-one	

Figure 1: The name, compound ID, IUPAC name and structure of three polyphenol compounds.

Rapid virtual screenings of these compounds were performed in the docking tool iGEMDOCK v2.0[16]. A population size of 150 is set with 70 generation and one solution for quick docking. The ligands with low binding energy were selected for the further study. The selected ligands were then analyzed for drug- relevant properties based on "Lipinski's rule of five" and other drug like properties using ACD/iLab web portal.

Lipinski Rule of 5 (Ro5), also known as Lipinski Alert Index, is a filter that identifies compounds with low probability of useful oral activity because of poor absorption or permeation[17, 18].

In the discovery setting the Lipinski rule of 5 predicts that poor absorption or permeation is more likely when:

- there are more than 5 H-bond donors (nHDon)
- there are more than 10 H-bond acceptors (N + O)
- molecular weight (MW) is over 500
- Moriguchi's logP (MLogP) is over 4.15

Other important drug like properties that were checked was lead like scores and Ghose filter. Lead-like Scores (LLS) are defined as the ratio between the number of satisfied conditions over the total number of conditions.

Eight drug-like indices were proposed by Ghose-Viswanadhan-Wendoloski[19] in order to help to streamline the design of combinatorial chemistry libraries for drug design and develop guidelines for prioritizing large sets of compounds for biological testing. They are based on a consensus definition and have been derived from analysis of the distribution of some physicochemical properties (logP, molar refractivity, molecular weight, and number of atoms) and chemical constitutions of known drug molecules available in the Comprehensive Medicinal Chemistry (CMC) database and seven drug classes defined by disease state.

Protein – Ligand docking

The protein – ligand docking was performed by Hex version 8.0.0. Hex is an interactive molecular graphics program for calculating and displaying feasible docking modes of pairs of protein and DNA molecules. Hex can also calculate Protein-Ligand Docking, assuming the ligand is rigid, and it can superpose pairs of molecules using only knowledge of their 3D shapes[20]. It uses Spherical Polar Fourier (SPF) correlations to accelerate the calculations and its one of the few docking programs which has built in graphics to view the result[21].

The parameters used for the docking process were:

1. Correlation type – Shape + Electrostatics
2. FFT Mode - 3D
3. Post Processing- MM Energies
4. Grid Dimension - 0.6
5. Receptor range – 180
6. Ligand range – 180
7. Twist range – 360
8. Distance Range – 40

Results

Protein and ligand preparation

The 3D structure of cholera toxin is shown Figure 2. The subunit A contains 258 aminoacids. It is made up of 233 amino acids. The amino acids 19 to 212 represents A1 subunit and amino acids 213 to 258 represents A2 subunit. The amino acids 1 to 18 acts as signal peptide. The subunit B contains 124 amino acids. Alpha helices are coloured magenta, beta sheets are coloured yellow, turns are coloured pale blue, and all other residues are coloured white.

For each polyphenol compounds, 200 derivatives were prepared using ACD chemsketch software. It was converted to pdb format using OPEN BABEL software.

Figure 2: The 3D structure of cholera toxin viewed with Rasmol structure colour scheme

Screening and selection of ligands

On virtual rapid screening of disometin derivatives with iGEMDOCK software, three compounds were found to have good fit with a low binding energy. The Table 1 shows the energy values of the three compounds generated from disometin. From the table it is evident that all the three compounds have excellent total binding energy.

Table 1: The results of iGEMDOCK showing binding energies of the three disometin derivatives.

S.No.	Ligand	Total binding Energy (kcal/mol)	Vanderwaals force	H Bond
1.	3,5-dihydroxy-2-[(1*E*,3*Z*)-3-hydroxy-4-methoxyhexa-1,3,5-trien-1-yl]-6,7-dimethoxy-4*H*-chromen-4-one	-107.651	-104.66	-2.99057
2.	3,5,6-trihydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-methoxy-4*H*-chromen-4-one	-97.1809	-79.3493	-17.8316
3.	(3*Z*)-5-hydroxy-3-[(2*Z*)-1-hydroxy-2,3-dimethoxyprop-2-en-1-ylidene]-6-(3-hydroxy-4-methoxyphenyl)-2-methylidene-2,3-dihydro-4*H*-pyran-4-one	-99.3447	-78.2134	-21.1313

The Table 2 shows the Lipinski's rule of five and Table 3 shows other drug likeness properties of the selected three compounds. From the tables it is evident that all the three derivatives have good drug like properties.

Table 2: Lipinski's rule of five for disometin derivatives

S. No.	Ligand	Molecular weight	Xlog p	H bond donor	H bond acceptor
1.	3,5-dihydroxy-2-[(1*E*,3*Z*)-3-hydroxy-4-methoxyhexa-1,3,5-trien-1-yl]-6,7-dimethoxy-4*H*-chromen-4-one	364.35	1.77	0	8
2.	3,5,6-trihydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-methoxy-4*H*-chromen-4-one	346.29	0.9	1	8
3.	(3*Z*)-5-hydroxy-3-[(2*Z*)-1-hydroxy-2,3-dimethoxyprop-2-en-1-ylidene]-6-(3-hydroxy-4-methoxyphenyl)-2-methylidene-2,3-dihydro-4*H*-pyran-4-one	362.33	0.59	0	8

Table 3: Important drug like properties of disometin derivatives.

S. No.	Ligand	Bioavailability	Ghose filter	Lead likeness
1.	3,5-dihydroxy-2-[(1*E*,3*Z*)-3-hydroxy-4-methoxyhexa-1,3,5-trien-1-yl]-6,7-dimethoxy-4*H*-chromen-4-one	yes	yes	yes
2.	3,5,6-trihydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-methoxy-4*H*-chromen-4-one	yes	yes	yes
3.	(3*Z*)-5-hydroxy-3-[(2*Z*)-1-hydroxy-2,3-dimethoxyprop-2-en-1-ylidene]-6-(3-hydroxy-4-methoxyphenyl)-2-methylidene-2,3-dihydro-4*H*-pyran-4-one	yes	yes	yes

On virtual rapid screening of quercetin derivatives with iGEMDOCK software, four compounds were found to have good fit with a low binding energy. The Table 4 shows the energy values of the four compounds generated from quercetin. From the table it is seen that all the four compounds have excellent total binding energy.

Table 4: The results of iGEMDOCK showing binding energies of the four quercetin derivatives.

S.No.	Ligand	Total binding Energy (kcal/mol)	Vanderwaals force	H Bond
1.	2-(3,4-dihydroxyphenyl)-4*H*-chromene-3,5,7-triol	-84.1625	-69.3221	-14.8403
2.	2-[(2*Z*,4*Z*)-4,5-dihydroxyhexa-2,4-dien-1-yl]-3,5,7-trihydroxy-4*H*-chromen-4-one	-101.149	-83.3286	-17.8205
3.	(2*E*,5*Z*,7*E*,9*E*)-2-(3,4-dihydroxyphenyl)-3,6,8-trihydroxy-4*H*-oxecin-4-one	-96.7913	-75.0598	-21.7314
4.	2,3-dihydroxy-5-(3,5,7-trihydroxy-4-oxo-4*H*-chromen-2-yl)pyranium	-96.4629	-81.7793	-14.6835

The Table 5 shows the Lipinski's rule of five and Table 6 depicts other drug likeness properties of the selected four compounds. From the tables it is evident that all the four derivatives have good drug like properties.

Table 5: Lipinski's rule of five for quercetin derivatives

S. No.	Ligand	Molecular weight	Xlog p	H bond donor	H bond acceptor
1.	2-(3,4-dihydroxyphenyl)-4*H*-chromene-3,5,7-triol	288.25	1.71	0	6
2.	2-[(2*Z*,4*Z*)-4,5-dihydroxyhexa-2,4-dien-1-yl]-3,5,7-trihydroxy-4*H*-chromen-4-one	306.26	1.67	0	7
3.	(2*E*,5*Z*,7*E*,9*E*)-2-(3,4-dihydroxyphenyl)-3,6,8-trihydroxy-4*H*-oxecin-4-one	304.25	1.29	0	7
4/	2,3-dihydroxy-5-(3,5,7-trihydroxy-4-oxo-4*H*-chromen-2-yl)pyranium	305.21	1.75	0	8

Table 6: Important drug like properties of quercetin derivatives.

S. No.	Ligand	Bioavailability	Ghose filter	Lead likeness
1.	2-(3,4-dihydroxyphenyl)-4*H*-chromene-3,5,7-triol	yes	yes	yes
2.	2-[(2*Z*,4*Z*)-4,5-dihydroxyhexa-2,4-dien-1-yl]-3,5,7-trihydroxy-4*H*-chromen-4-one	yes	yes	yes
3.	(2*E*,5*Z*,7*E*,9*E*)-2-(3,4-dihydroxyphenyl)-3,6,8-trihydroxy-4*H*-oxecin-4-one	yes	yes	yes
4.	2,3-dihydroxy-5-(3,5,7-trihydroxy-4-oxo-4*H*-chromen-2-yl)pyranium	yes	yes	yes

On virtual rapid screening of ternatin derivatives with iGEMDOCK software, three compounds were found to have good fit with a low binding energy. The Table 7 shows the energy values of the three compounds generated from ternatin. From the table it is evident that all the three compounds have excellent total binding energy.

Table 7: The results of iGEMDOCK showing binding energies of the four ternatin derivatives.

S.No.	Ligand	Total binding Energy (kcal/mol)	Vanderwaals force	H Bond
1.	(2*Z*)-1-(2,6-dihydroxy-3-methoxyphenyl)-3-(4-hydroxy-3-methoxyphenyl)-2-methoxyprop-2-en-1-one	-98.3775	-80.4299	-17.9476
2.	5-hydroxy-2-[(2*Z*,4*E*)-4-hydroxyhexa-2,4-dien-1-yl]-3,7,8-trimethoxy-4*H*-chromen-4-one	-111.673	-107.595	-4.07783
3.	5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-3-methoxy-4*H*-chromen-4-one	-96.4543	-87.702	-8.75228

The Table 8 shows the Lipinski's rule of five and Table 9 shows other drug likeness properties of the selected three compounds. From the tables it is seen that all the three derivatives have good drug like properties.

Table 8: Lipinski's rule of five for ternatin derivatives.

S. No.	Ligand	Molecular weight	Xlog p	H bond donor	H bond acceptor
1.	(2*Z*)-1-(2,6-dihydroxy-3-methoxyphenyl)-3-(4-hydroxy-3-methoxyphenyl)-2-methoxyprop-2-en-1-one	346.33	3.56	3	7
2.	5-hydroxy-2-[(2*Z*,4*E*)-4-hydroxyhexa-2,4-dien-1-yl]-3,7,8-trimethoxy-4*H*-chromen-4-one	348.35	2.67	0	7
3.	5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-3-methoxy-4*H*-chromen-4-one	330.28	2.42	1	7

Table 9: Important drug like properties of ternatin derivatives.

S. No.	Ligand	Bioavailability	Ghose filter	Lead likeness
1.	(2*Z*)-1-(2,6-dihydroxy-3-methoxyphenyl)-3-(4-hydroxy-3-methoxyphenyl)-2-methoxyprop-2-en-1-one	yes	yes	yes
2.	5-hydroxy-2-[(2*Z*,4*E*)-4-hydroxyhexa-2,4-dien-1-yl]-3,7,8-trimethoxy-4*H*-chromen-4-one	yes	yes	yes
3.	5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-3-methoxy-4*H*-chromen-4-one	yes	yes	yes

Docking with Hex

All the selected ligands were then subjected to accurate docking with Hex version 8.0.0 to analyse its binding energies.

The Table 10 shows the Energy values of the disometin derivatives. From the table it is clear that all the derivatives show high energy values. The Figure 3 displays the docking pose of the three disometin derivatives.

Table 10: E-values of disometin derivatives obtained by docking in Hex.

S.No.	Ligand	E-value
1.	3,5-dihydroxy-2-[(1*E*,3*Z*)-3-hydroxy-4-methoxyhexa-1,3,5-trien-1-yl]-6,7-dimethoxy-4*H*-chromen-4-one	-329.64
2.	3,5,6-trihydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-methoxy-4*H*-chromen-4-one	-300.93
3.	(3*Z*)-5-hydroxy-3-[(2*Z*)-1-hydroxy-2,3-dimethoxyprop-2-en-1-ylidene]-6-(3-hydroxy-4-methoxyphenyl)-2-methylidene-2,3-dihydro-4*H*-pyran-4-one	-296.92

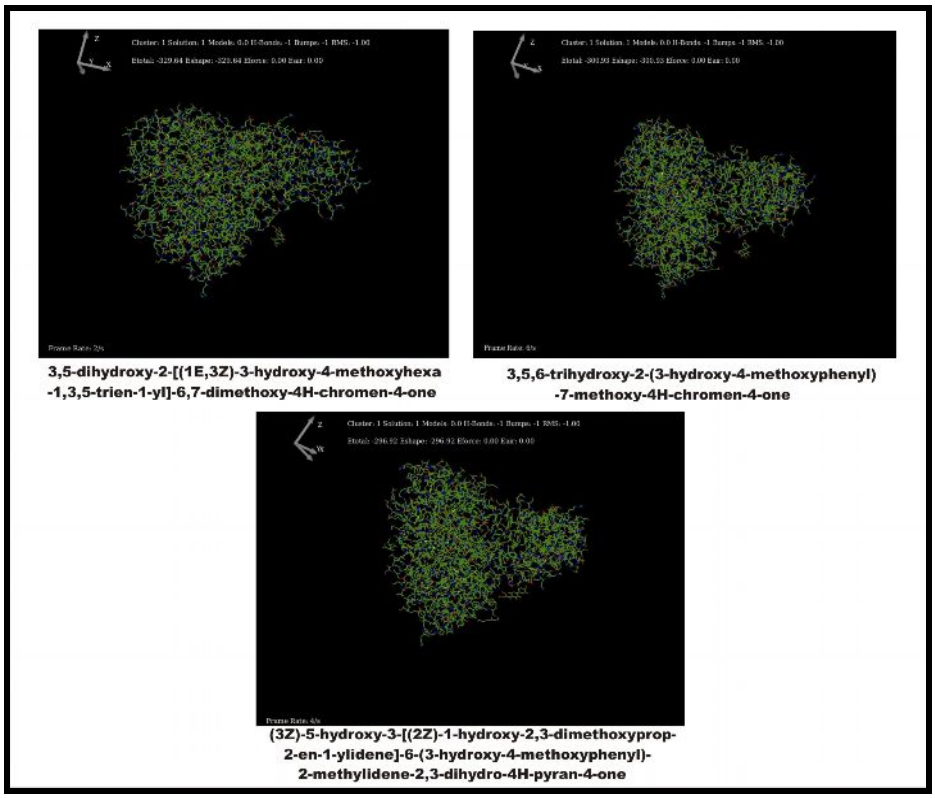


Figure 3: Docking pose of disometin derivatives in Hex.

The Table 11 depicts the Energy values of the quercetin derivatives. From the table it is evident that all the derivatives show high energy values. The Figure 4 shows the docking pose of the four quercetin derivatives.

Table 11: E-values of quercetin derivatives obtained by docking in Hex.

S.No.	Ligand	E-value
1.	2-(3,4-dihydroxyphenyl)-4*H*-chromene-3,5,7-triol	-265.83
2.	2-[(2*Z*,4*Z*)-4,5-dihydroxyhexa-2,4-dien-1-yl]-3,5,7-trihydroxy-4*H*-chromen-4-one	-275.99
3.	(2*E*,5*Z*,7*E*,9*E*)-2-(3,4-dihydroxyphenyl)-3,6,8-trihydroxy-4*H*-oxecin-4-one	-275.99
4.	2,3-dihydroxy-5-(3,5,7-trihydroxy-4-oxo-4*H*-chromen-2-yl)pyranium	-271.17

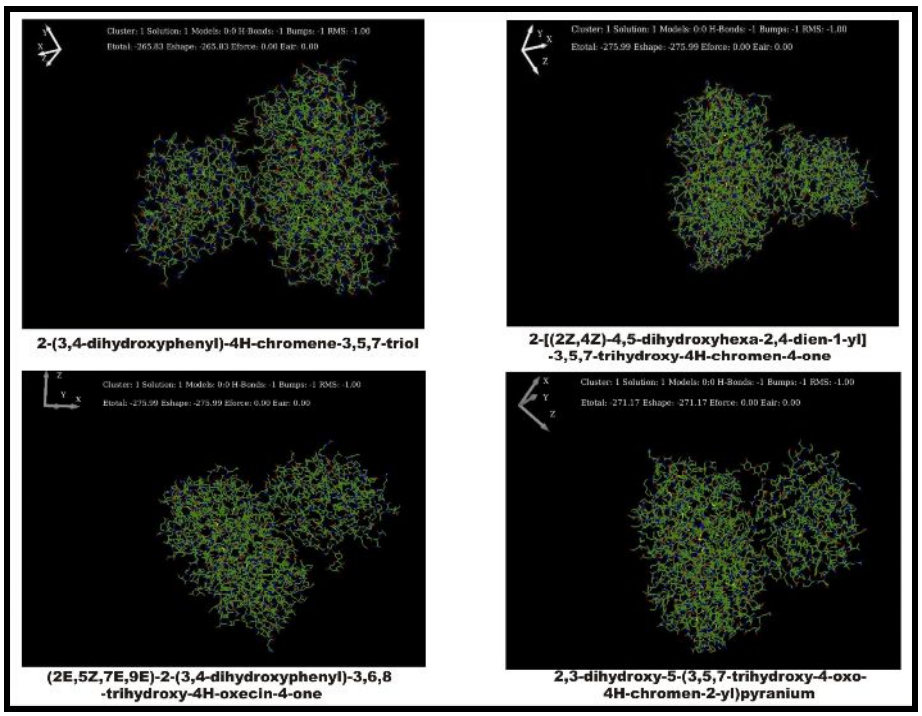


Figure 4: Docking pose of quercetin derivatives in Hex.

The Table 12 presents the Energy values of the ternatin derivatives. From the table it is evident that all the derivatives show high energy values. The Figure 4 depicts the docking pose of the three ternatin derivatives.

Table 11: E-values of ternatin derivatives obtained by docking in Hex.

S.No.	Ligand	E-value
1.	(2*Z*)-1-(2,6-dihydroxy-3-methoxyphenyl)-3-(4-hydroxy-3-methoxyphenyl)-2-methoxyprop-2-en-1-one	-323.32
2.	5-hydroxy-2-[(2*Z*,4*E*)-4-hydroxyhexa-2,4-dien-1-yl]-3,7,8-trimethoxy-4*H*-chromen-4-one	-311.15
3.	5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-3-methoxy-4*H*-chromen-4-one	-308.47

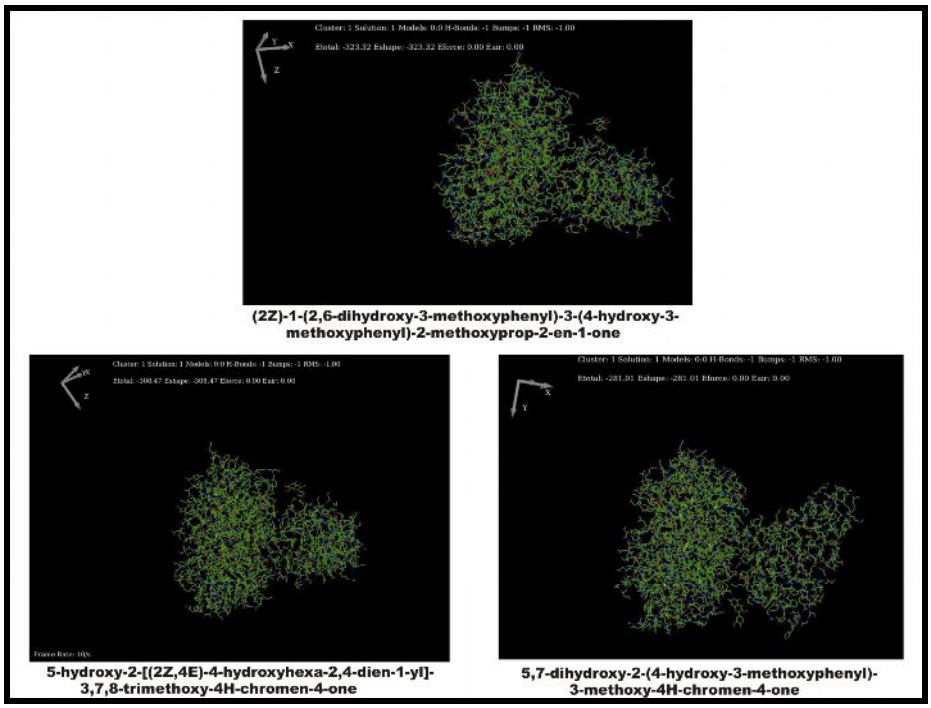


Figure 4: Docking pose of quercetin derivatives in Hex.

Discussion

The understanding of the mechanism of cholera and CT toxicity at the molecular level has allowed to identify natural products that can inhibit the CT. Such an important group of products are polyphenols from green and black tea. In the present study it has been shown that the derivatives of three natural polyphenols from tea viz. disometin, quercetin and ternatin has the property to bind the active site of cholera toxin and thereby can inhibit its activity. Many polyphenols from different plants have been shown to have anti-cholera activity. It has been shown that the polyphenols of apple has inhibitory effect on CT-catalyzed ADP-ribosylation of agmantine and it is due to the inhibition of the enzymatic activity of the A subunit of CT[22]. In another study, CTA inhibitory activity of proantho-cyanidines extracted from *Guazuma ulimfolia,* a medicinal plant used in Mexico for traditional treatment of diarrhoea has been proved[23]. In an another similar study, the bioactivity of rhubarb galloyl tannin (RG-tannin), a compound isolated from *Rhei rhizome,* against CT activities including ADP-ribosylation and fluid accumulation has been shown[24]. Based on these findings few docking studies were done with polyphenol structures to find its efficacy in inhibiting the cholera toxin[10, 25].

Conclusion

From the present study it was found that the selective derivatives of three natural polyphenols from tea viz. disometin, quercetin and ternatin have shown excellent binding energy values. These compounds are excellent drug candidates in the inhibition of cholera toxin and have the potential to be a treatment in the control of cholera.

Acknowledgements

This work was done under the ICMR-STS 2014 project sanctioned to Abiramavalli R. and the authors are grateful to Indian Council of Medical Research for the same.

References

1. Faruque S. M and Nair G. B., Vibrio cholerae: Genomics and Molecular Biology. Caister Academic Press, 2008, 24, 485 – 491.
2. Pierre-Hervé L. The Discovery of Cholera-Toxin as a Powerful Neuroanatomical Tool. J Mol Biol, 2001, 4, 3-23.
3. De Haan L and Hirst T. R., Cholera toxin: a paradigm for multifunctional engagement of cellular mechanisms. Mol. Membr. Biol, 2004, 21, 77–92.

4. Surewicz W. K., Leddy J. J. and Mantsch H. H., Structure, stability, and receptor interaction of cholera toxin as studied by Fourier-transform infrared spectroscopy. Biochemistry 1990, 29, 8106–8111.
5. Mekalanos J. J, Collier R. J and Romig W. R., Enzymic activity of cholera toxin. II. Relationships to proteolytic processing, disulfide bond reduction, and subunit composition. J Biol Chem. 1979, 254, 5855–5861.
6. Holmgren J., Lo¨nnroth I. and Svennerholm L., Fixation and inactivation of cholera toxin by GM1 ganglioside, Scand J Infect Dis., 1973, 5, 77–78.
7. Spangler B. D., Structure and function of cholera toxin and the related Escherichia coli heat-labile enterotoxin. Microbiol Rev 1992, 56, 622-647.
8. Zhang R. G., Scott D. L Westbrook M. L., Nance S., Spangler B. D., Shipley G. G. and Westbrook E. M., The three-dimensional crystal structure of cholera toxin. J Mol Biol 1995, 251, 563-573.
9. Hol W. G. J., Zhang Z. S., et al. Solution and crystallographic studies of branched multivalent ligands that inhibit the receptor-binding of cholera toxin. Journal of the American Chemical Society 2002, 124, 12991-12998.
10. Zhang G. T., Design and *in silico* screening of inhibitors of the cholera toxin. Expert Opinion on Drug Discovery 2009, 4, 923-938.
11. Chitme H. R., Chandra R. and Kaushik S., Studies on anti-diarrheal activity of *Calotropis gigantean* in experimental animals, J Pharmacol Pharm Sci 2004, 7,70-75.
12. Shoba F.G., Thomas M. Study of antidiarrhoeal activity of four medicinal plants in castor oil induced diarrhea, J Ethnopharmacol 2001, 76, 73-76.
13. Toda M., Okubo S., et al. The protective activity of tea against infection by Vibrio cholerae O1. J Appl Bacteriol 1991, 70, 109-112.
14. Toda M., Okubo S., et al. The Protective Activity of Tea Catechins against Experimental-Infection by Vibrio-Cholerae O1. Microbiol Immunol 1992, 36, 999-1001.
15. ACD/ChemSketch Freeware, version 10.00, Advanced Chemistry Development, Inc., Toronto, ON, Canada, 2012.
16. Yang J. M. and Chen C. C., GEMDOCK: A generic evolutionary method for molecular Docking. Proteins: Structure, Function, and Bioinformatics, 2004, 55, 288-304.
17. Lipinski C. A, Lombardo F., Dominy B. W. and Feeney P. J., Advanced Drug Delivery Reviews 1997, 23, 3-25.
18. Lipinski C. A., Lombardo F., Dominy B. W. and Feeney P. J., Advanced Drug Delivery Reviews 2001, 46, 3-26
19. Ghose A. K., Viswanadhan V. N., Wendolowski J. J. and Comb J., Chem. 1999, 1, 55-68.
20. Ritchie D. W., Evaluation of Protein Docking Predictions using Hex 3.1 in CAPRI rounds 1-2, Proteins, Structure, Fucntion and Genetics, Wiley-liss Inc.
21. Ritchie D. W. and Kemp G. J. L., Protein Docking Using Spherical Polar Fourier Correlations. PROTEINS: Struct. Funct. Genet. 2000, 39, 178-194.
22. Saito T., Miyake M., et al. Inhibition by apple polyphenols of ADPribosyltransferase activity of cholera toxin and toxin-induced fluid accumulation in mice. Microbiol Immunol 2002, 46, 249-255.
23. Hor M., Rimpler H., et al. Inhibition of intestinal chloride secretion by proanthocyanidins from Guazuma ulmifolia. Planta Med 1995, 61, 208-212.
24. Oi H., Matsuura D., et al. Identification in traditional herbal medications and confirmation by synthesis of factors that inhibit cholera toxin-induced fluid accumulation. Proc Natl Acad Sci USA 2002, 99, 3042-3046.
25. Podlipnik Č., Docking of Selected Natural Polyphenols to ARF Activated A1 Subunit of Cholera Toxin. Acta Chimica Slovenica 2009, 56, 156-165.

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