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An Insilico Study of Quinic Acid Derivatives as Inhibitors of Com A, The Quorum Sensing Protein of Streptococcus Mutans Responsible for The Pathogenesis in Dental Caries

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Abstract

Dental caries is considered to be one of the important infectious diseases throughout the world. It was recognized that the Streptococcus mutans, plays an important role in cariogenesis. The oral streptococci in biofilms may communicate by a quorum-sensing CSP signalling system. The products of at least six genes, comAB, comX, and comCDE, are involved in CSP signalling. It has been proved that loss of Com A attenuates the formation of biofilm. In the present study quinic acid derivatives have been developed insilico as the inhibitors of Com A and a molecular docking study is performed to find its efficiency as inhibitors. The three-dimensional structure of Com A was retrieved from RCSB- PDB database. The possible binding sites of Com A were searched using binding site prediction 3DLIGANDSITE. The structure of quinic acid was obtained from ZINC database. A total of 100 ligands were generated with the help of software ACD chemsketch. Rapid virtual screenings of these compounds were performed in the docking tool iGEMDOCK v2.0. Based on the binding energy a total of five ligands were selected for the further study. The selected five ligands were then analyzed for drug- relevant properties based on "Lipinski's rule of five" and other drug like properties. The docking of five ligands was performed using AutoDock 4.0 software. From the present study, it has been found that (1R)-1, 3, 4-trihydroxy-5-methylcyclohexane carboxylic acid, which is a novel compound, a derivative of quinic acid, can act has an inhibitor for the Com A.

Keywords: *Streptococcus mutans, Dental Caries, Com A, Quinic Acid Derivatives and Molecular Docking*

1. Introduction

Dental caries is considered to be one of the important infectious diseases throughout the world. It forms an important cause for the tooth loss both in children and adults. Almost forty years ago it was recognized that the Streptococcus mutans, plays an important role in cariogenesis [1]. The ability of S. mutans to form dental caries relies on its successful colonisation in the oral cavity and to form biofilm [2].

The genetic transformation in nature has been reported for many oral streptococci in cultures. This occurs by induction of genetic competence through a competence stimulating peptide (CSP) signalling system [3]. The competence among the oral bacteria is a quorum sensing phenomenon. The products of at least six genes, comAB, comX, and comCDE, are involved in CSP signaling. CSP is encoded by comC. It is now clear that oral streptococci in biofilms may communicate by a quorum-sensing CSP signalling system [4]. In S. mutans, the loss of CSP results in biofilms with altered architectures [5].

ComA is an ABC transporter that, together with its accessory protein ComB, exports CSP following the removal of the leader peptide distal to a Gly-Gly motif (double- glycine leader) of the pre-CSP [6]. It has been proved that loss of Com A attenuates the formation of biofilm [7]. Thus inhibition of Com A by a structural analogue can in turn affect the formation of biofilm by S. mutans. Finding a structural analogue may possibly be a promising drug candidate for the control of dental caries.

Quinic acid is a cyclitol, a cyclic polyol. Its IUPAC name is (1R, 3R, 4R, 5R)-1,3,4,5-Tetrahydroxycyclohexanecarboxylic acid. It is a crystalline acid obtained from cinchona bark, tobacco leaves, carrot leaves, apples, peaches, coffee beans, and other plant products. It can also be made synthetically by hydrolysis of chlorogenic acid. The quinic acid is considered to be one of starting material for the synthesis of various new pharmaceuticals. Tamiflu, a drug used for the treatment of influenza A and B has been synthesised from quinic acid [8].

Thus, in the present study quinic acid derivatives have been developed insilico as the inhibitors of Com A and a molecular docking study is performed to find its efficiency as inhibitors.

2. Materials And Methods

Protein Preparation

The three-dimensional structure of Com A was retrieved from RCSB-PDB data base. Its PDB code is 3K8U.

Active Site Prediction

The possible binding sites of Com A were searched using binding site prediction 3 DLIGANDSITE, an online tool (http://www.ncbi.nlm.nih.gov/pubmed/2051 3649) [9]. The best flexible binding sites were selected for this study.

Generation and Optimization of Ligand

The structure of quinic acid was obtained from ZINC database. A total of 100 ligands in 2D format were generated with the help of software ACD chemsketch [10]. The ligands were saved in mol 2 format. The OPEN BABEL software (www.vcclab.org/lab/babel/start.html) was used to convert mol format to pdb format. Rapid virtual screenings of these compounds were performed in the docking tool iGEMDOCK v2.0 [11]. A population size of 150 is set with 70 generation and one solution for quick docking. Based on the binding energy a total of five ligands were selected for the further study. The selected five ligands were then analyzed for drug- relevant properties based on “Lipinski’s rule of five”. Other drug like properties were analysed using OSIRIS Property Explorer (http://www.organicchemistry.org/prog/peo/) and Mol soft: Drug-Likeness and molecular property explorer (http://www.molsoft.com/mprop/). On the basis of binding affinity and drug like properties, all these five ligands were taken for further molecular docking study.

Protein-Ligand Docking

The docking of five ligands was performed using AutoDock 4.0 software. Docking was performed to obtain a population of possible conformations and orientations for the ligands at the binding site and also its binding energy. Using the software, polar hydrogen atoms were added to the Com A and its non polar hydrogen atoms were merged. All bonds of ligands were set to be rotatable. All calculations for protein- ligand flexible docking were done using the Lamarckian Genetic Algorithm (LGA) method. The grid box with a dimension of 126 x 126 x 126 points was used so as to cover the entire enzyme binding site and accommodate ligands to move freely. The best conformation was chosen with the lowest docked energy, after the docking search was completed.

3. Results and Discussion

The 3D structure of Com A is shown Figure 1. It is made up of 760 amino acids. Peptidase family C39 domain lies between amino acids of 45 to 182. ATP-binding cassette, ABC transporter-type domain lies between amino acids of 578 to 760. The 3D structure is viewed as PDB file with Rasmol structure colour scheme. Alpha helices are coloured magenta, beta sheets are coloured yellow, turns are coloured pale blue, and all other residues are coloured white.

Its binding site was predicted using 3DLIGANDSITE. The binding site predicted are 14 THR, 15 ARG, 44 THR 45, ASN, 47 GLN, 48 GLY, 49 THR, 87 HIS, 94 LEU, 95 GLN, 96 HIS and 97 THR. The Figure 2 shows the 3D structure of Com A protein showing its binding sites.

A total of 100 ligands were derived from quinic acid using ACD chemsketch software. It was converted to pdb format using OPEN BABEL software. All the 100 ligands were then subjected to virtual rapid screening with iGEMDOCK software and five compounds were found to have good fit with a low binding energy. The structure and the IUPAC name of the five ligands were shown in the Figure 3. The selected five ligands were then studied for its drug relevant properties.

The Table 1 depicts the values related to the Lipinski’s rule of Five. From the table it is evident that all the five selected ligands obey the rule. The Table 2 shows the drug relevant properties of the five ligands. They all possess good drug score and drug likeness.

The Table 3 displays the results obtained in rapid virtual screening by iGEMDOCK of the five ligands. From the table it is clear that the five ligands have low total binding energies and thus were taken to further docking studies.

The five ligands were subjected to molecular docking using AutoDock tools. The binding sites 49 THR, 48 GLY, 47 GLN and 95 GLN of 3K8U were selected in autodock as flexible residues. The best confirmation of protein-ligand docking for the five ligands were selected based its total binding energy. The Table 4 depicts the results of the molecular docking. All the five ligands showed the low binding energy with the negative values. Among the five ligands, 1,3,4,5-tetra hydroxycyclohexa-2,4-diene-1-carboxylic acid and (1R)-1,3,4- trihydroxy-5-methylcyclohexane carboxylic acid (Ligand 1 and 2) are considered as the best inhibitors compared to others as it has the lowest energy values. The docking pose of the two ligands are given in Figure 4 and Figure 5.

Among these two ligands, (1R)-1, 3, 4- trihydroxy-5-methylcyclohexane carboxylic acid (Ligand 2) shows an excellent score of 1.6 and 0.9 for drug likeness and drug score respectively.

Figure 1: The 3D Structure of Com A Viewed with Rasmol Structure Colour Scheme

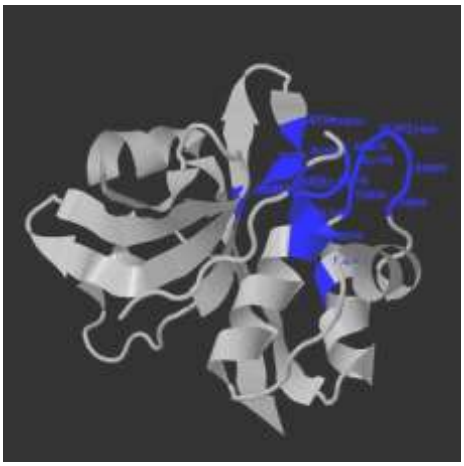

Figure 2: The 3D Structure of Com A Showing its Binding Site

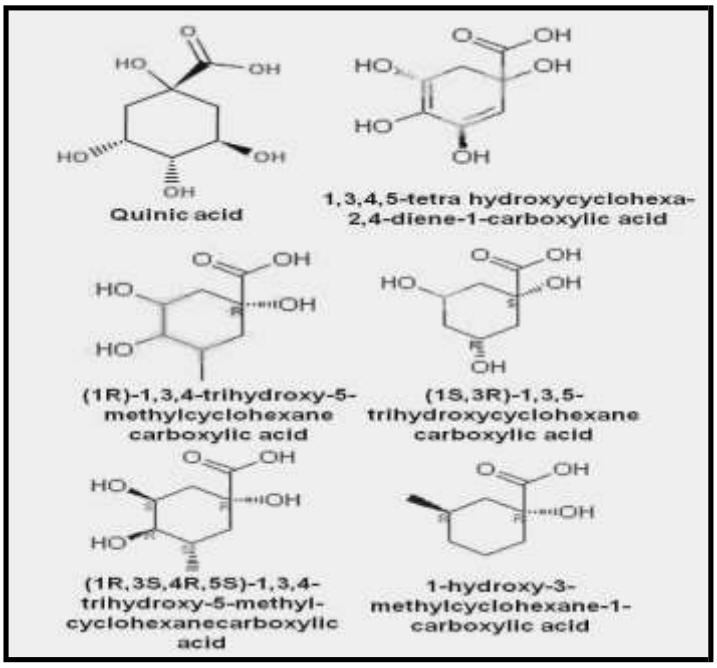

Figure 3: The Structure of Quinic Acid and the Five Ligands

TABLE 1: The Lipinski's Properties of the Selected Five Ligands

S. No.	Ligand	Molecular weight	Xlog p	H bond donor	H bond acceptor
1.	1,3,4,5-tetra hydroxycyclohexa-2,4-diene-1-carboxylic acid	188.135	-0.847	5	6
2.	(1R)-1,3,4-trihydroxy-5-methylcyclohexane carboxylic acid	186.163	0.034	4	5
3.	(1S,3R)-1,3,5-trihydroxycyclohexane carboxylic acid	170.164	0.368	3	4
4.	(1R,3S,4R,5S)-1,3,4-trihydroxy-5-methyl-cyclohexanecarboxylic acid	190.195	-1.157	4	5
5.	1-hydroxy-3-methylcyclohexane-1-carboxylic acid	158.197	0.909	2	3

TABLE 2: The Drug Relevant Properties of Selected Five Ligands

S. No.	Ligand	Drug likeness	Drug score	Mutagenic	Tumorigenic	Irritant
1.	1,3,4,5-tetra hydroxycyclohexa-2,4-diene-1-carboxylic acid	0.41	0.79	NO	NO	NO
2.	(1R)-1,3,4-trihydroxy-5-methylcyclohexane carboxylic acid	1.6	0.9	NO	NO	NO
3.	(1S,3R)-1,3,5-trihydroxycyclohexane carboxylic acid	0.51	0.8	NO	NO	NO
4.	(1R,3S,4R,5S)-1,3,4-trihydroxy-5-methyl-cyclohexanecarboxylic acid	1.6	0.9	NO	NO	NO
5.	1-hydroxy-3-methylcyclohexane-1-carboxylic acid	-2.47	0.52	NO	NO	NO

TABLE 3: The Results of iGEMDOCK Showing Binding Energies of Five Selected Ligands

S. No.	Ligand	Total binding Energy (kcal/mol)	Vanderwaals force	H Bond	Electrostatic bond
1.	1,3,4,5-tetra hydroxycyclohexa-2,4-diene-1-carboxylic acid	-72.83	-41.27	-33.17	1.61
2.	(1R)-1,3,4-trihydroxy-5-methylcyclohexane carboxylic acid	-70.45	-42.99	-27.46	0

3.	(1S,3R)-1,3,5-trihydroxycyclohexane carboxylic acid	-67.3	-46.78	-17.47	-3.05
4.	(1R,3S,4R,5S)-1,3,4-trihydroxy-5-methyl-cyclohexanecarboxylic acid	-50.33	-50.33	0	0
5.	1-hydroxy-3-methylcyclohexane-1-carboxylic acid	-50.55	-50.55	0	0

Table 4: The Results of AUTODOCK Showing Binding Energies of Five Selected Ligands

S. No.	Ligand	Total binding energy (kcal/mol)	Vanderwaals+ Hydrogen bond+ dissolvation energy	Electrostatic energy
1.	1,3,4,5-tetra hydroxycyclohexa-2,4-diene-1-carboxylic acid	-661000000	163000	-663000000
2.	(1R)-1,3,4-trihydroxy-5-methylcyclohexane carboxylic acid	-661000000	551000	-662000000
3.	(1S,3R)-1,3,5-trihydroxycyclohexane carboxylic acid	-553000000	751000	-554000000
4.	(1R,3S,4R,5S)-1,3,4-trihydroxy-5-methyl-cyclohexanecarboxylic acid	-590000000	544000	-591000000
5.	1-hydroxy-3-methylcyclohexane-1-carboxylic acid	933000	-1.66	-0.6

Figure 4: Docking Pose of Com A with 1, 3, 4, 5-tetra hydroxycyclohexa-2,4-diene-1-carboxylic acid

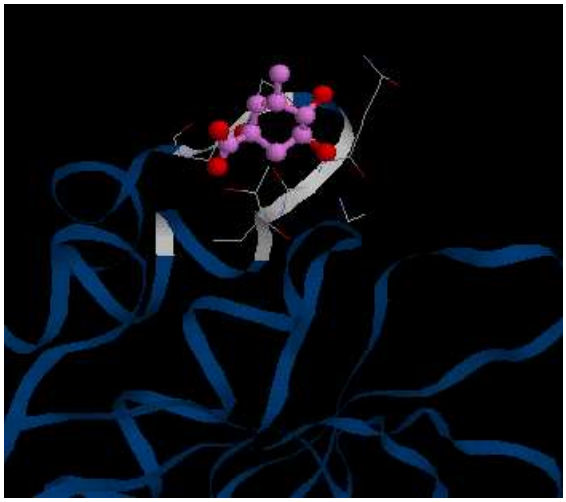

Figure 5: Docking Pose of Com A with (1R)-1,3,4-trihydroxy-5-methylcyclohexane carboxylic acid

4. Conclusion

The main prerequisite for the formation of dental caries is the ability of the S. mutans to form the biofilm. The important phenomenon in the formation of biofilm is the quorum sensing. Hence the inhibition of quorum sensing phenomenon will prevent the formation of dental caries. In the present study, with the help of molecular docking studies, a novel compound (1R)-1,3,4- trihydroxy-5-methylcyclohexane carboxylic acid, a derivative quinic acid is found to have the ability to inhibit Com A protein, an important protein in the phenomenon of quorum sensing. Thus it is concluded that this compound can act as an effective drug in the prevention of dental caries.

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