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Quinic Acid Derivatives as Inhibitors of Glucosyltransferase Si, A Virulence Factor of *Streptococcus Mutans* in The Pathogenesis of Dental Caries

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Abstract

The important prerequisite for the formation of dental caries is the ability of Streptococcus mutans to form the biofilm. It has been proved that the formation of biofilm is mediated mainly by the enzymatic action of the Glucosyltransferase (GTF) enzymes. These enzymes are considered fundamental for the virulence of S. mutans in the causation of dental caries. In the present study quinic acid derivatives have been developed insilico as the inhibitors of GTF-SI enzyme and a molecular docking study is preformed to find its efficiency as inhibitors. The three-dimensional structure of GTF-SI was retrieved from MMDB database. The possible binding sites of GTF-SI 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 GTF-SI.

Keywords: *Streptococcus mutans, Dental caries, Glucosyltransferase-SI, Quinic acid derivatives and Molecular docking*

1. Introduction

Dental caries is one of the important diseases worldwide that affects many school going children that leads to inconvenience due to pain and even the loss of tooth. The oral microflora of human being is highly complex[1]. However various studies conducted have proved conclusively that the mutans streptococci in particular *Streptococcus mutans* as the principal cariogenic bacteria[2]. Further, the research in the etiology of dental caries had established the central role of glucan in sucrose dependent adhesion and the correlation between sucrose consumption and increased caries rates[3]. One of the major characteristics of *S. mutans* in causing dental caries is its ability to adhere firmly to the tooth surface in the presence of sucrose[4]. It has been proved intelligibly that this adherence is mediated mainly by the enzymatic action of the Glucosyltransferase (GTF) enzymes[5]. These enzymes are considered fundamental for the virulence of *S. mutans* in the causation of dental caries[6].

Most strains of *S. mutans* contain three distinct gtf genes: gtfB coding for the GTF-I enzyme, gtfC expressing a similar GTF-SI, and gtfD coding for the GTF-S enzyme. The first two enzymes synthesize primarily water-insoluble glucans, whereas the latter produces water-soluble glucans. The GTFs (E.C. 2.4.1.5) are isoenzymes. The molecular weight of GTFs ranges from 150 to 180 kDa. These enzymes catalyze the transfer and addition of a glucosyl moiety to the terminal site of a primer or elongating glucan according to the reaction scheme[7]:

Sucrose + (1,6-a-D-glucosyl) *n* àD-fructose+(1,6-a- D-glucosyl) *n*+1

The glucans consist of an α-(1-6)-linked glucose polymer with α-(1-3) branch linkages[8]. The glucan is sticky in nature and facilitates the adherence of the bacteria to the tooth to form a firm biofilm[9]. A significant constituent of the plaque biofilm is water insoluble glucans, which are formed primarily

through the activity of gtfB and gtfC[10]. The gtfC expressing GTF-SI enzyme has been proved to have an essential role in the sucrose dependent attachment to smooth dental surface[11].

This enzyme is involved in the synthesis of both soluble and insoluble glucans[12]. It is also further proved that the absence of this enzyme has drastically reduced the ability of adherence of *S. mutans*[13]. Hence inhibition of GTF-SI by the structural analogue can be a strategy to control adherence of *S.mutans* and thus can prevent the dental caries.

Quinic acid is a cyclitol, a cyclic polyol. Its IUPAC name is (1*R*,3*R*,4*R*,5*R*)-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[14].

Thus, in the present study quinic acid derivatives have been developed *insilico* as the inhibitors of GTF-SI enzyme and a molecular docking study is preformed to find its efficiency as inhibitors.

2. Materials And Methods

Protein preparation

The three-dimensional structure of GTF-SI was retrieved from MMDB database

(http://www.ncbi.nlm.nih.gov/Structure/mmdb/)[15]. Its MMDB code is 89340 and PDB code is 3AIB.

Active site prediction

The possible binding sites of GTF-SI were searched using binding site prediction 3DLIGANDSITE, an online tool (http://www.ncbi.nlm.nih.gov/pubmed/20513649)[16]. The binding site thus obtained was 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[17]. 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 v2.018. 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" and other drug like properties using OSIRIS Property Explorer

http://www.organicchemistry.org/prog/peo/), 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 GTF-SI and its non-polar hydrogen atoms were merged. All bonds of ligands were set to be rotatable. All calculations for protein- ligand flexible docking was 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 GTF-SI is shown Figure 1. It is made up of 1455 amino acids. Its catalytic activity is found to be present from the amino acid of 250 to 1050. 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.

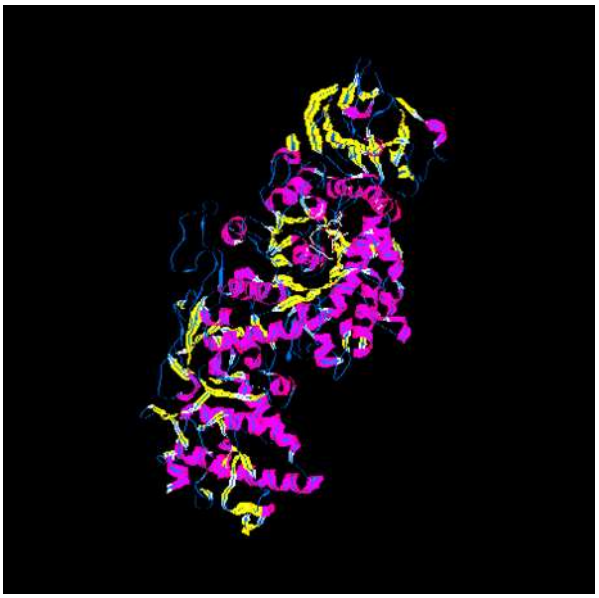

Figure 1: The 3D structure of GTF-SI viewed with Rasmol structure colour scheme

Its binding site was predicted using 3DLIGANDSITE. The binding site predicted are 381 HIS, 383 GLN, 434 LEU, 592 GLN, 906 SER, 907 PHE, 909 ASP, 962 TYR and 1052 ASN. The figure 2 shows the 3D structure of GTF-SI protein showing its binding sites.

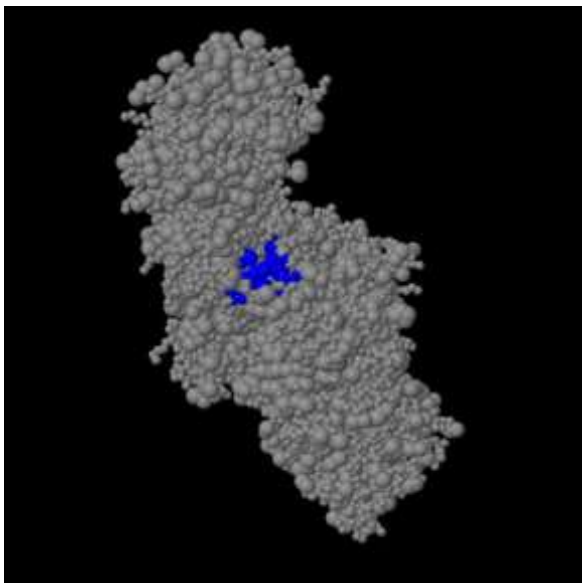

Figure 2: The 3D structure of GTF-SI showing its binding site

A total of 100 ligands were derived from quinic acid using ACD chemsketch software. It was converted to pdb format using OPEN BABEL software. The drug relevant properties of all these 100 ligands were studied. On virtual rapid screening with iGEMDOCK software, five compounds were found to have good fit with a low binding energy. The structure and the IUPAC name of the five ligands

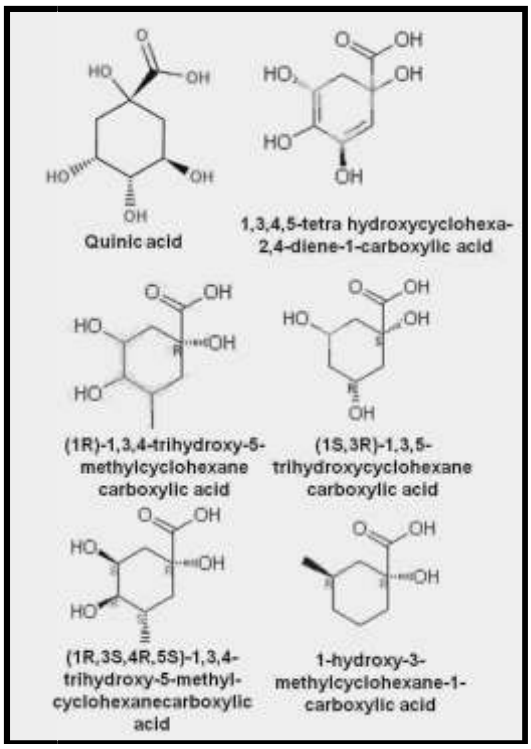

Figure 3: The structure of quinic acid and the five ligands

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.

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

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.

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	-83.322	-47.6027	-34.3729	-1.34642
2.	(1R)-1,3,4-trihydroxy-5-methylcyclohexane carboxylic acid	-83.9704	-54.8839	-28.8004	-0.28607
3.	(1S,3R)-1,3,5-trihydroxycyclohexane carboxylic acid	- 80.6225	-43.8194	-33.5911	-3.21202
4.	(1R,3S,4R,5S)-1,3,4-trihydroxy-5-methyl-cyclohexanecarboxylic acid	-58.0483	-58.0483	0	0
5.	1-hydroxy-3- methylcyclohexane-1-carboxylic acid	-50.4658	-50.4658	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	-672000000	9.16	-685000000
2.	(1R)-1,3,4-trihydroxy-5-methylcyclohexane carboxylic acid	-154000000	-1.19	-168000000
3.	(1S,3R)-1,3,5-trihydroxycyclohexane carboxylic acid	-155000000	30.7	-168000000
4.	(1R,3S,4R,5S)-1,3,4-trihydroxy-5-methyl-cyclohexanecarboxylic acid	-136000000	-0.62	-15000000
5.	1-hydroxy-3-methylcyclohexane-1-carboxylic acid	-601000000	34.89	-615000000

The five ligands were subjected to molecular docking using AutoDock tools. The best confirmation of protein-ligand docking for the five ligands were selected based its total binding energy (Figure 4). The Table 4 depicts the results of the molecular docking. All the five ligands showed the low binding energy with the negative values. However, basedon Vanderwaals force and hydrogen bond energy, (1R)-1,3,4-trihydroxy-5- methylcyclohexane carboxylic acid (Ligand 2) is considered as the best inhibitor compared to others as it has the lowest energy of -1.19. Further from Table 2 it is evident that it has very good drug likeness and drug score i.e., 1.6 and 0.9 respectively.

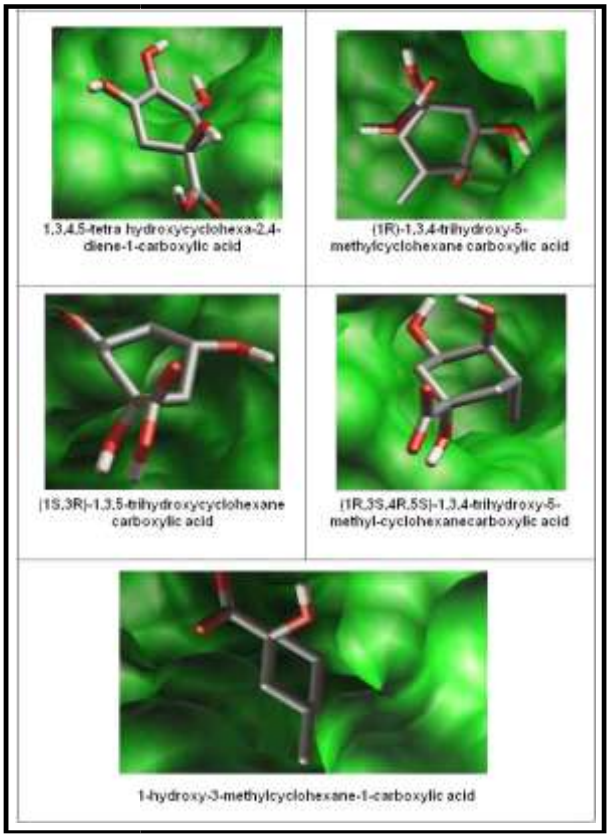

Figure 4: Docking pose of the five ligands with GTF-SI

4. Conclusion

The main virulence factor for S. mutans for its cariogenic property is GTF-SI. 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 a structural analogue for the GTF-SI. Thus, it can act as the inhibitor of the enzyme and thus can prevent the formation of dental biofilm (plaque), an important prerequisite for the formation of dental caries. Hence this compound has the potential of being a drug for the prevention of dental caries.

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