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Research Article

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Kaempferol derivatives as a potent inhibitor of Karilysin, an important virulence factor of *Tanerella forsythia*

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ABSTRACT

One of the virulent gram-negative periodontopathogenic bacterium causing periodontitis is Tanerella forsythia, which is also a part of the red complex. Karilysin, recently identified potent virulent factor of T.forsythia acts by deactivation of complement system and also development of resistance against the antimicrobial peptide LL-37, thereby making T. forsythia viable in the sub-gingival plaque. So based on these, it has been aimed that the development of a strategy to block the Karilysin by a novel compound can potentially help in the prevention and treatment of periodontitis. The three dimensional structure of Karilysin was retrieved from RCSB database. A total of 500 ligands in 2D format were generated from the basic structure of kaempferol with the help of software ACD chemsketch. Rapid virtual screenings of these compounds were performed in the docking tool iGEMDOCK v2.0. The molecular docking of ligands was performed using Auto Dock 4.0 software. In the present study, 5-hydroxy-2-(4-hydroxyphenyl)-3-methylquinolin-4(1H)-one has been found to have very good inhibitory property based on molecular docking study. Further the compound shows a good ADMET properties based on studies in OSIRIS. Hence it is concluded that 5-hydroxy-2-(4-hydroxyphenyl)-3-methylquinolin-4(1H)-one is an excellent drug candidate in the prevention and treatment of periodontitis.

Keywords: Karilysin, periodontitis, kaempferol derivatives, molecular docking, autodock.

INTRODUCTION

Arguably periodontitis is the most prevalent bacterial- driven chronic inflammatory disease of humans. [1]. The incidence of periodontal disease has been reported to range from 30% of the population in developed countries to over 70% of the population in developing countries, with severe disease inflicting 7–15% of the human population world-wide[2]. Periodontitis is a major cause of tooth loss, and is also implicated in the onset, development and/or progression of systemic diseases such as cardiovascular diseases, rheumatoid arthritis and Alzheimer disease.

The involvement of bacteria in the etiology of periodontitis is unquestionable, but the composition of the causative species is still being debated. Presently, over 500 bacterial species have been detected in periodontal plaque but only a handful, including *Tanerella forsythia*, gram-negative bacterium, have been implicated as the major etiological pathogens of chronic periodontitis, the most prevailing form of periodontal disease. *Tanerella forsythia* produces a variety of virulence factors, including bioactive metabolic products, fimbriae and an array of proteolytic enzymes. Metallopeptidases (MPs) are among the virulence factors secreted by this pathogenic bacteria at the site of infection. The identified peptidases in *T. forsythia* are cysteine proteinase, PrtH and the MP karilysin[3]. The latter is a 472-residue secretory protein comprising a 20-residue signal sequence, a 14-residue pro-peptide, an 18-kDa catalytic peptidase domain (CD), and a 30- kDa C-terminal domain of unknown function. The enzyme requires calcium for

activity and thermal stability and it cleaves the bonds preferentially N-terminal of bulky hydrophobic residues. It efficiently degraded elastin, fibrinogen, and fibronectin, thus pointing to possible roles in periodontitis progression[4]. Karilysin's bactericidal activity is attributed to its inactivation of LL-37 in a time and concentration dependent manner through limited proteolysis.[4] Proteolytic inactivation of LL-37 bactericidal activity by karilysin may protect LL-37-sensitive species in the sub-gingival plaque and maintain the local inflammatory reaction driven by LPS from gram-negative bacteria. Another protective effect of karilysin against serum bactericidal activity was attributable to its ability to inhibit complement system at several stages[5]. Consequently, the karilysin protease may directly contribute to periodontal tissue damage and the development and/or progression of chronic periodontitis. Thereby, karilysin presents as a potential drug target to fight against *Tanerella forsythia*.

Polyphenols are derived from plant metabolites and comprise the component of human dietary antioxidants[6]. Flavonols beong to the subclass of polyphenols[7], of which Kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one) is a low molecular weight flavonol (286.2 g/moL), that has been identified in many plants used in traditional medicine, including *Equisetum* species, *Sophora japonica*, and *Ginkgo biloba*, and edible plants, including beans, broccoli, cabbage, gooseberries, grapes, kale, strawberries, tea, and tomatoes[8,9]. Kaempferol intake has shown reduced risk of few varieties of cancer and cardiovascular diseases[8]. It has been documented that kaempferol has antioxidative, antimicrobial, anti-inflammatory, lipolytic[10], and anticancer properties[11,12]. Hence this study was done to identify the Kaempferol derivatives efficacy against Karilysin.

EXPERIMENTAL SECTION

PROTEIN PREPARATION

The three dimensional structure of Karilysin was retrieved from RCSB database (http://www.rcsb.org/pdb/explore/explore.do?structureId=2xs3). Its RCSB code is 2XS3

GENERATION AND OPTIMIZATION OF LIGAND

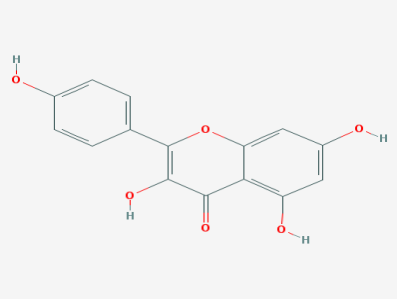

Figure 1. Structure of Kaempferol

The structure of kaempferol (Figure 1) was obtained from pubchem database. Its compound ID is 5280863. Its IUPAC name is *3, 5, 7-trihydroxy-2-(4-hydroxyphenyl)chromen-4-one*. The kaempferol has a molecular weight of 286.2363 [g/mol] and its XlogP3 value is 1.9. A total of 500 ligands in 2D format were generated from the basic structure of kaempferol with the help of software ACD chemsketch[13]. 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[14]. 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"[15] 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 ligands were taken for further molecular docking study.

PROTEIN – LIGAND DOCKING

The docking of 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 Karilysin 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.

RESULTS AND DISCUSSION

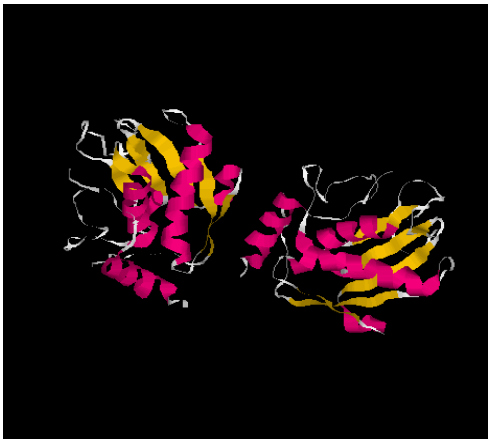

Figure 2. The 3D structure of Karilysin viewed with Rasmol structure colour scheme

The 3D structure of Karilysin is shown Figure 2. It is made up of 2,164 amino acids. Alpha helices are coloured magenta, beta sheets are coloured yellow, turns are coloured pale blue, and all other residues are coloured white. On virtual rapid screening with iGEMDOCK software, four compounds were found to have good fit with a low binding energy.

Ligand	Total Energy	VanderWaals Forces	HBond	Elec
2-(4-hydroxyphenyl)-4-imino-1,4-dihydroquinoline-3,5,7-triol(kaem 1)	-97.0438	-85.1625	-11.8813	0
3,7-dihydroxy-6-(4-methylphenyl)quinolin-8(5*H*)-one(kaem 2)	-92.4644	-83.5886	-8.87577	0
5-hydroxy-2-(4-hydroxyphenyl)-3-methylquinolin-4(1*H*)-one(kaem 3)	-97.1604	-80.9097	-16.2507	0
3-(4-fluorophenyl)-5,8-dimethyl-1,4- dihydronaphthalene-2,6-diol(kaem 4)	-93.3095	-87.0601	-6.24948	0

Table 1: The results of iGEMDOCK showing binding energies of four selected ligands

Table 1 displays the results obtained in rapid virtual screening by iGEMDOCK of the four ligands. From the table it is clear that the four ligands have low total binding energies and thus were taken to further docking studies. Their docking pose is shown in Figure 3.

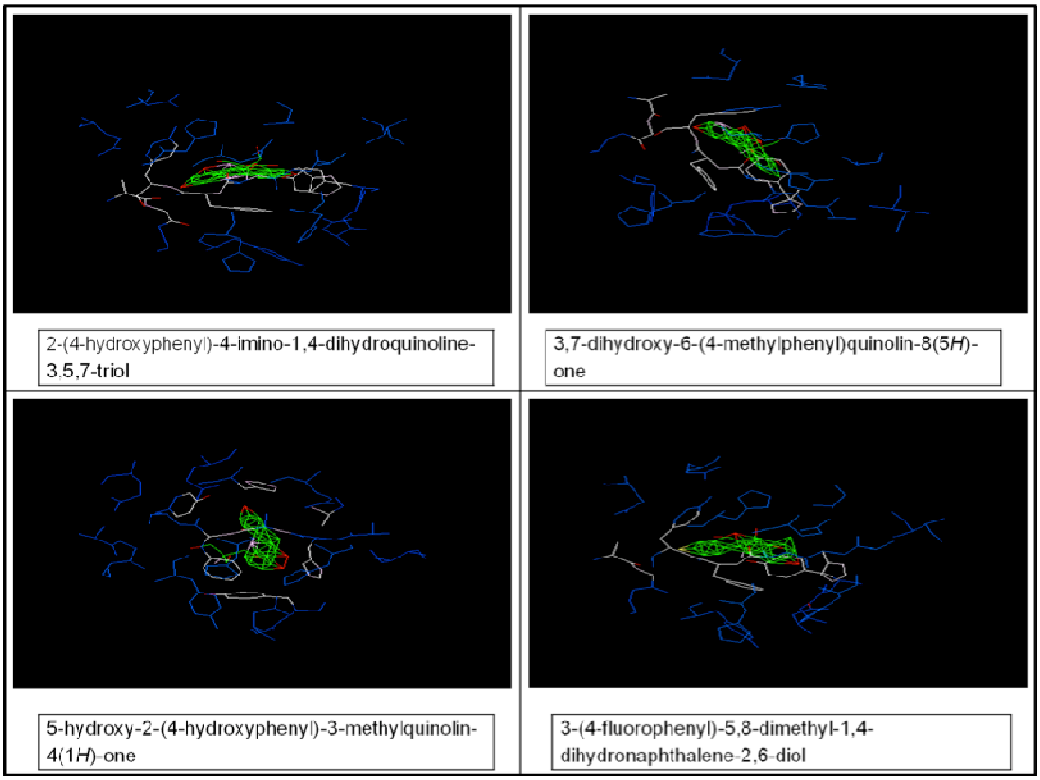

Figure 3: Docking pose of the four ligands with Karilysin in iGEMDock

The structure and the IUPAC name of the four ligands are shown in Figure 4. The four ligands were subjected for its ADMET properties.

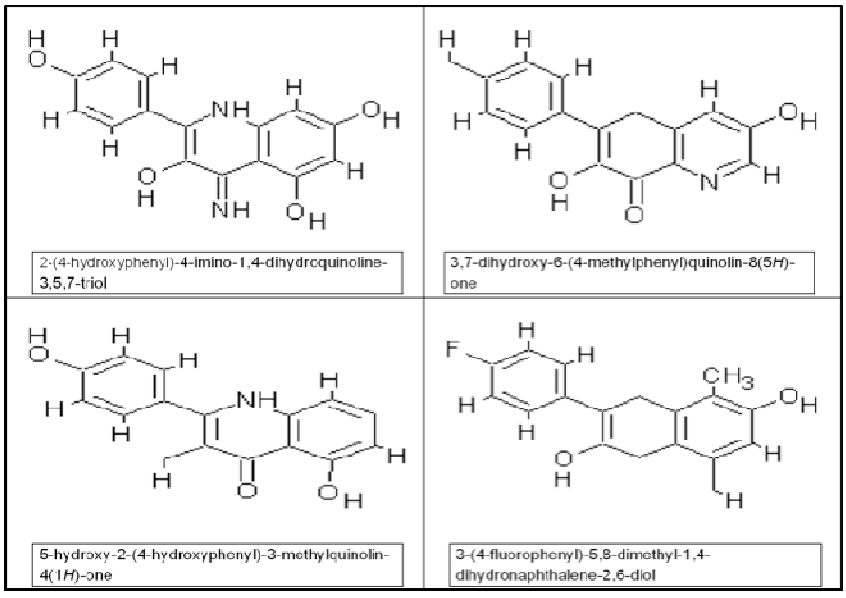

Figure 4:The four ligands derived from kaempferol using ChemSketch

Table 2 depicts the values related to the Lipinski's rule of Five. From the table it is evident that all the four selected ligands obey the rule

S. No.	Ligand	Molecular weight	Xlog p	Hbond donor	Hbond acceptor
1	2-(4-hydroxyphenyl)-4-imino-1,4-dihydroquinoline-3,5,7-triol	282.06	2.002	4	6
2	3,7-dihydroxy-6-(4-methylphenyl)quinolin-8(5*H*)-one	263.06	2.178	2	4
3	5-hydroxy-2-(4-hydroxyphenyl)-3-methylquinolin-4(1*H*)-one	263.06	1.51	2	4
4	3-(4-fluorophenyl)-5,8-dimethyl-1,4-dihydronaphthalene-2,6-diol	275.05	1.677	2	2

Table 2: The Lipinski's properties of the selected four ligands

Table 3 shows the drug relevant properties of the four ligands. They all possess good drug score and drug likeness.

S.NO	Ligand	Drug Likeness	Drug score	Mutagenic	Tumorigenic	Irritant
1	2-(4-hydroxyphenyl)-4-imino-1,4-dihydroquinoline-3,5,7-triol	1.33	0.84	No	No	No
2	3,7-dihydroxy-6-(4-methylphenyl)quinolin-8(5*H*)-one	0.47	0.73	No	No	No
3	5-hydroxy-2-(4-hydroxyphenyl)-3-methylquinolin-4(1*H*)-one	1.67	0.79	No	No	No
4	3-(4-fluorophenyl)-5,8-dimethyl-1,4-dihydronaphthalene-2,6-diol	1.59	0.67	No	No	No

Table 3: The drug relevant properties of selected four ligands

The four ligands were subjected to molecular docking using AutoDock tools. The best confirmation of protein-ligand docking for the four ligands were selected based its total binding energy hydrogen bonding.

Table 4: The results of AUTODOCK showing binding energies of four selected ligands

Table 4 depicts the results of the molecular docking. All the four ligands showed the low binding energy with the negative values.

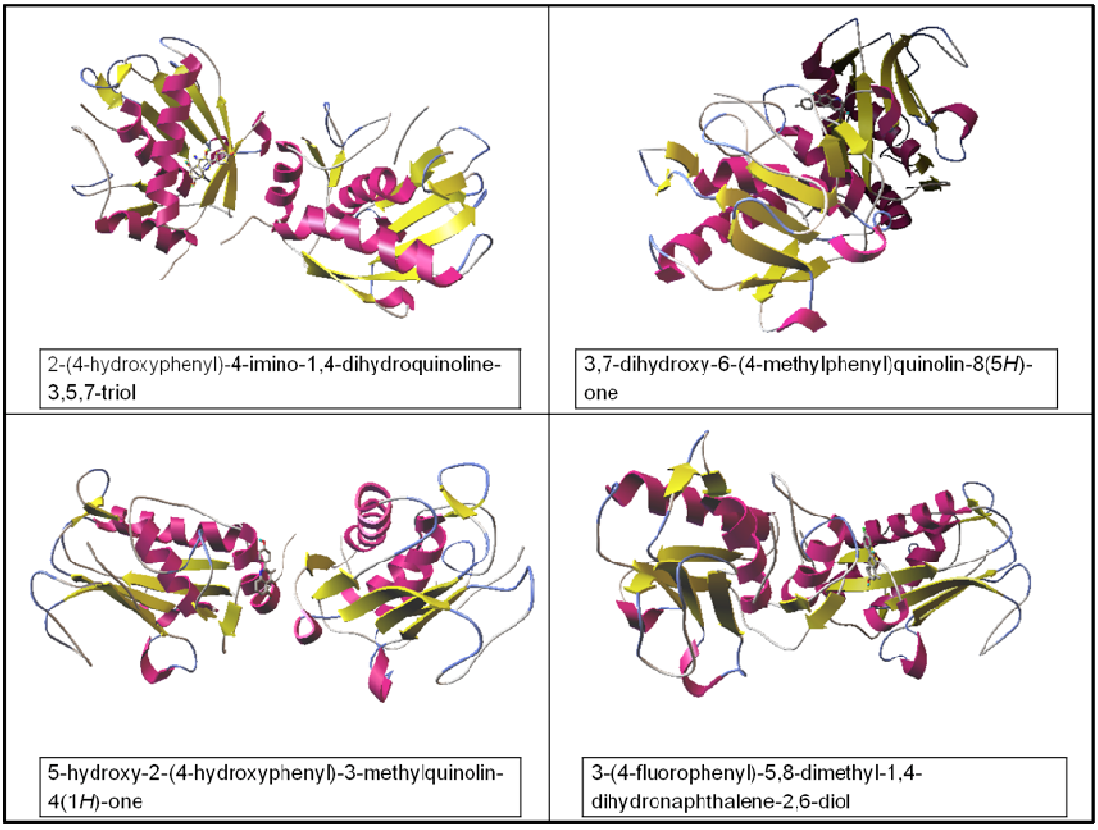

Figure 5: Docking pose of the four ligands with Karilysin in AUTODOCK

Its best docking pose is shown in Figure 5. From Table 4 it is evident that all four ligands show good binding properties. Among the four ligands, 5-hydroxy-2-(4-hydroxyphenyl)-3-methylquinolin-4(1*H*)-one (Ligand 3) has excellent binding energy coupled with good ADMET properties.

ACD/ChemSketch is a drawing package that allows us to draw chemical structures including organics, organometallics, polymers, and Markush structures. It also has several features such as calculation of molecular properties (e.g., molecular weight, density, molar refractivity etc.), 2D and 3D structure cleaning and viewing, functionality for naming structures (fewer than 50 atoms and 3 rings), and prediction of log*P*. Molecular docking softwares finds its major usage in drug research industry. The most important application of docking software is virtual screening (VS). Many tools like GEMDOCK, DOCK, AutoDock, and GOLD have been developed for virtual screening.

Lipinski's Rule of Five (Ro5)[15] states that a compound is more likely to exhibit poor absorption or permeation when two or more of the following physicochemical criteria are fulfilled: the molecular weight (MW) is greater than 500Da; the calculated logP (ClogP) is greater than 5; there are more than 5 hydrogen- bond donors or the number of hydrogen-bond acceptors (nitrogen and oxygen atoms) is greater than 10.

Various docking studies have been conducted in the past years and still today to create a viable drug compound against many diseases which does not have a definitive targeted cure. Targeting a specific protein or an enzyme specific of an organism or a disease is highly effective in preventing or terminating the progression of the disease, thereby increasing the chances of a good prognosis and effectively decreasing the morbidity of the patient.

The compound kaempferol, a natural flavonol occurring in various plants and plant derived foods has a wide variety of medicinal properties like anticancer, antibacterial, anti-diabetes, antiviral and it presents as an excellent antioxidant. There are several documented evidence regarding these properties.

Very few studies have been done against periodontitis, particularly against a different periodonto pathogenic organism but not against this organism. It is well known that T. forsythia is a significant organism in causing chronic periodontitis and it is part of the red complex. So inhibition of T. forsythia can be highly effective in controlling periodontitis. Karilysin, a supremo virulence enzyme of T. forsythia presented as a perfect drug target. So planning and execution of kaempferol's derivatives against karilysin has yielded excellent results, thereby proving that the derivatives have an extremely good amount of medicinal properties against periodontitis.

CONCLUSION

Karilysin is one of the important virulent factors involved in inhibiting the complement system in various stages and also making T. forsythia resistant against disruption by the antimicrobial peptide, LL-37. Hence blocking Karilysin will prevent these and thereby help in the prevention and treatment of periodontitis. In the present study, 5-hydroxy-2-(4-hydroxyphenyl)-3-methylquinolin-4(1*H*)-one has found to have very good inhibitory property based on molecular docking study. Further the compound shows a good ADMET properties based on studies in OSIRIS. Hence it is concluded 5-hydroxy-2-(4-hydroxyphenyl)-3-methylquinolin-4(1*H*)-one is an excellent drug candidate in the control of periodontitis.

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