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Research Article

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Kaempferol derivatives as a potent inhibitor of LYS-Gingipain KGP which is the predominant factor in the development of periodontitis

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ABSTRACT

Porphyromonas gingivalis is recognised as one of the important pathogens causing periodontal disease. Its ability to attach to other cells and matrix, its toxic products, ability to evade and disturb host defenses and trigger inflammation contributes to its virulence. Extracellular proteinases (gingipains) are considered important virulence factors. Among the gingipains, the most important method of invasion of Porphyromonas gingivalis is by adhesion, colonization and the hemolysis of erythrocytes. Upon engagement to their ligands they can cause the hemolysis of erythrocytes. Lys-gingipain KGP is the most potent fibrinogen/fibrin degrading enzyme and is involved in the bleeding tendency at the diseased gingiva. Further it has been suggested that the development of a strategy to block the Lys-gingipain KGP can potentially help in the prevention and treatment of periodontitis. The three dimensional structure of Lys-gingipain KGP 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 AutoDock 4.0 software. In the present study, the possible drug candidates ie., 2-(4-hydroxyphenyl)-4-imino-1,4-dihydroquinoline-3,5,7-triol, 3,7-dihydroxy-6-(4-methylphenyl) quinolin-8(5H)-one, 5-hydroxy-2-(4-hydroxy phenyl)-3-methyl quinolin-4(1H)-one and 3-(4-fluoro phenyl)-5,8-dimethyl-1,4-dihydro naphthalene-2,6-diol have been designed that can be used in control and treatment of periodontitis caused by P. gingivalis

Keywords: Lys-gingipain KGP, periodontitis, kaempferol derivatives, molecular docking, autodock.

INTRODUCTION

Porphyromonas gingivalis (Pg) is suspected to be one of the important causative agents of chronic and aggressive forms of periodontal disease.[1] These organisms produce several virulence factors including fimbriae, and its adhesions, membrane bound vesicles, lipopolysaccarides, hemolysins and proteases. [2,3]Arg and Lys gingipains are the main extracellular and cell boundendopeptidases produced by Pg. [4] Two genes code for Arggingipain (RgpA and RgpB) and one code for Lysine (Kgp)[4,5]RgpA and Kgp have a catalytic domain and a haemagglutination / adhesion domain while RgpB does not have the latter[5]

The trypsin like activity of the organism Pg results in down regulation of PMN's, degrade extracellular proteins and bioactive peptides such as C5, prekallikrinin, kininogen.[6]Kgp plays an important role in Fe acquisition by binding to haemoglobulin in erythrocytes and also cleaves proteins after lysine residues[6]and also have a potent degrading

enzyme for fibrinogen and involved in the bleeding tendency in diseased gingiva.[7] On the bacterial membrane surface RgpA and Kgp form large complexes that causes proteolysis, haemacquisition, platelet activation, red blood cell agglutination, haemolysis, and adhesion to extracellular matrix.[8] Reynolds et al[9] implicated Kgp and RgpB as the primary virulence factor of Pg in murine models of alveolar bone destruction. It has been advocated that gingipains are the main virulence factors of Pg and should be targeted for the treatment and prevention of periodontitis.[9]

It has been suggested that gingipain inhibition by vaccination and gingipains specific inhibitors would be a useful therapy for periodontitis caused by Pg. Grenier and La[10] published a review of proteases of Pg as potential targets for plant derived products. Polyphenols isolated from Cranberry and green tea were found to inhibit several proteases produced by Pg. Kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one) is a flavonoid found in many edible plants (e.g. tea, broccoli, cabbage, kale, beans, endive, leek, tomato, strawberries and grapes) and in plants or botanical products commonly used in traditional medicine. Numerous preclinical studies have shown that kaempferol and some glycosides of kaempferol have a wide range of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, anticancer, cardioprotective, neuroprotective, antidiabetic, anti-osteoporotic, estrogenic/antiestrogenic, anxiolytic, analgesic and antiallergic activities.[11] Many studies have found that green tea derived kaempferol and its related compounds have been found to be effective in the treatment of Pg and its related periodontal disease.[12,13,14]Hence an attempt is made in this study to evaluate kaempferol and its derivatives as the inhibitor of Lysine gingipain KGP.

EXPERIMENTAL SECTION

This work was performed in the medical informatics facility of Department of Microbiology, Tagore Medical College and Hospital with prior permission.

PROTEIN PREPARATION

The three dimensional structure of Lysine gingipain KGP was retrieved from RCSB database (http://www.rcsb.org/pdb/explore/explore.do?structureId=3km5). Its RCSB code is 3KM5.

ACTIVE SITE PREDICTION

The possible binding sites of gingipains were searched using binding site prediction 3DLIGANDSITE, an online tool (http://www.ncbi.nlm.nih.gov/pubmed/20513649)[20]. The binding site thus obtained was selected for this study.

GENERATION AND OPTIMIZATION OF LIGAND

The structure of kaempferol (Figure 1) was obtained from pubchem database. Its compound ID is5280863. 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[15]. 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[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 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 Lys-gingipain (Kgp) 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.

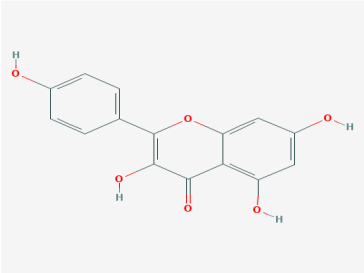

Figure 1: Structure of kaempferol

RESULTS AND DISCUSSION

The 3D structure of Lys-gingipain KGP 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.

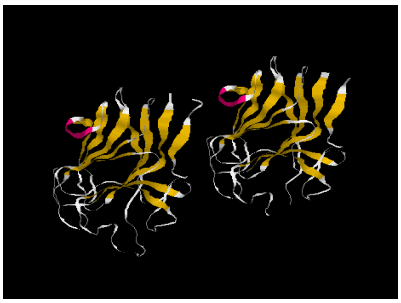

Figure 2: The 3D structure of Lys-gingipain KGP viewed with Rasmol structure colour scheme

A total of 500 ligands were derived from kaempferol using ACD chemsketch software. It was converted to pdb format using OPEN BABEL software. On virtual rapid screening with iGEMDOCK software, four compounds were found to have good fit with a low binding energy.

The 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. The structure and the IUPAC name of the four ligands were shown in the Figure 4.

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

S.no	Ligand	Total Energy	VanderWaals Forces	HBond	Elec
1	2-(4-hydroxyphenyl)-4-imino-1,4-dihydroquinoline-3,5,7-triol	-90.7854	-77.9373	-12.8481	0
2	3,7-dihydroxy-6-(4-methylphenyl)quinolin-8(5*H*)-one	-86.8828	-78.1666	-8.71621	0
3	5-hydroxy-2-(4-hydroxyphenyl)-3-methylquinolin-4(1*H*)-one	-85.3846	-74.3527	-11.0319	0
4	3-(4-fluorophenyl)-5,8-dimethyl-1,4-dihydronaphthalene-2,6-diol	-85.6023	-81.0006	-4.60169	0

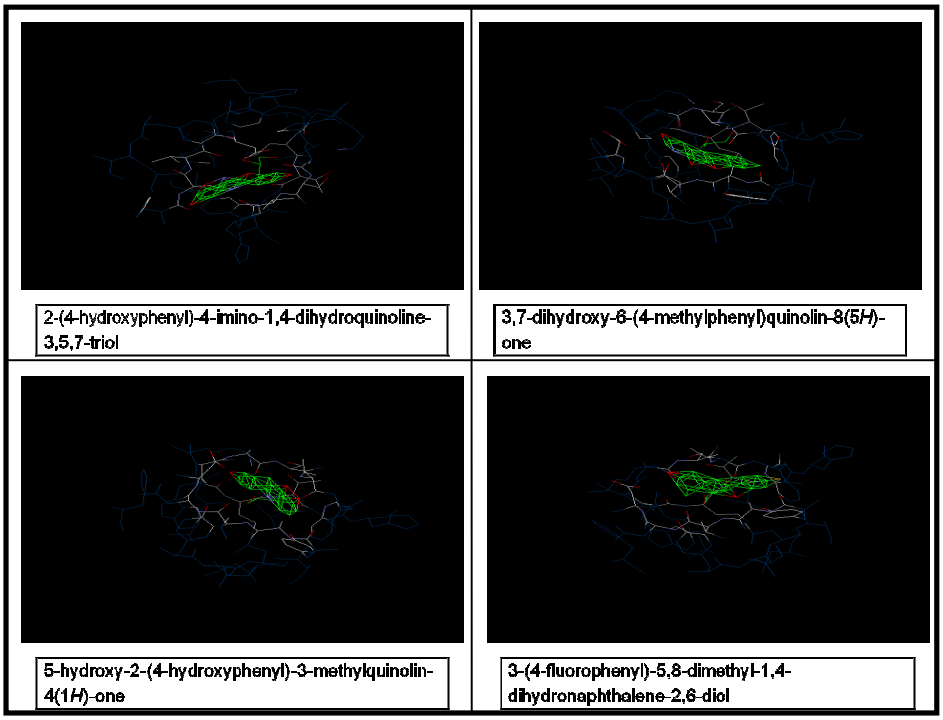

Figure 3: Docking pose of the four ligands with Lys-gingipain KGP

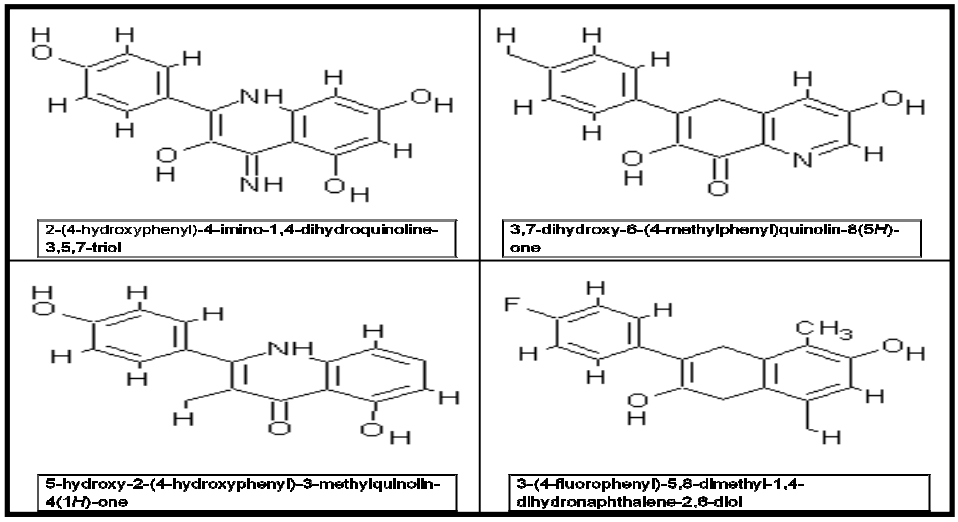

Figure 4: The structure and the IUPAC name of the four ligands

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

S. No.	Ligand	Molecular weight	Xlog p	H bond donor	H bond 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

The four ligands were subjected for its ADMET properties. The 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. The Table 3 shows the drug relevant properties of the four ligands. They all possess good drug score and drug likeness.

Table 3: The drug relevant properties of selected four ligands

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

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. The Table 4 depicts the results of the molecular docking. All the four ligands showed the low binding energy with the negative values. Its best docking pose is shown in Figure 6. From the table is it is evident that all four ligands show good binding properties

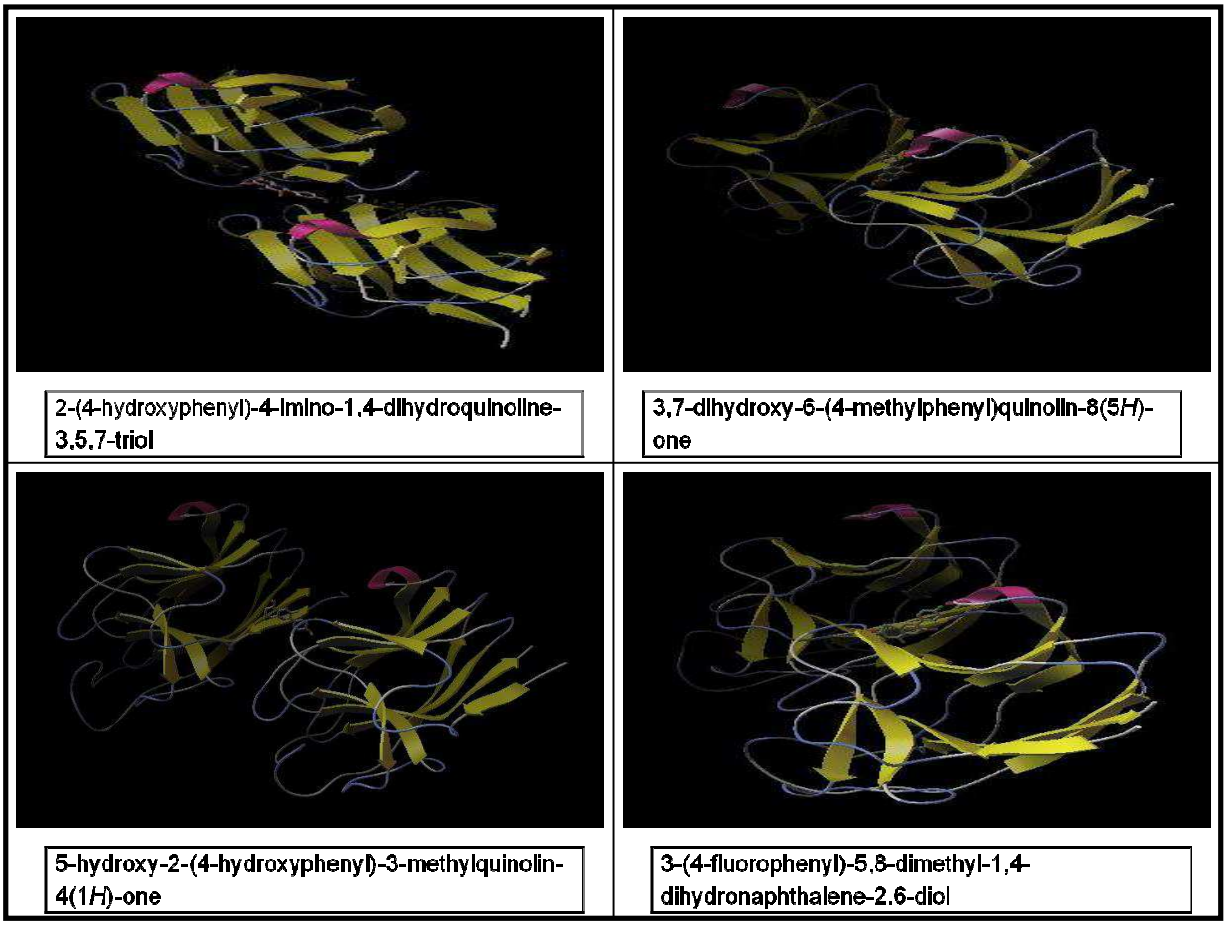

Figure 6: Docking pose of the four ligands with Lys-gingipain KGP

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

S.no	Ligand	Total binding energy (kcal/mol)
1	2-(4-hydroxyphenyl)-4-imino-1,4-dihydroquinoline-3,5,7-triol	Binding Energy: -8.1
2	3,7-dihydroxy-6-(4-methylphenyl)quinolin-8(5*H*)-one	Binding Energy: -8.26
3	5-hydroxy-2-(4-hydroxyphenyl)-3-methylquinolin-4(1*H*)-one	Binding Energy: -8.59
4	3-(4-fluorophenyl)-5,8-dimethyl-1,4-dihydronaphthalene-2,6-diol	Binding Energy: -8.33

The results confirm that the four ligands hve the ability to bind to gingipains and can act as antagonist. Among the four ligands, 5-hydroxy-2-(4-hydroxyphenyl)-3-methylquinolin-4(1*H*)-one (Ligand 3) have excellent binding energy coupled with good ADMET properties.

CONCLUSION

Kgp induces kallekrinin activation, activate blood coagulation, bone loss, fibrin degradation, hemolysis and also adhesion to cells and extracellular matrix. Hence, Lys-gingipain KGP is one of the important factors involved in the haemolysis of erythrocytes and also in the adhesion and colonization of P.gingivalis. Hence blocking Lys-gingipain KGP will prevent these and thereby helping 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 that the drug candidates2-(4-hydroxyphenyl)-4-imino-1,4-dihydroquinoline-3,5,7-triol, 3,7-dihydroxy-6-(4-methylphenyl)quinolin-8(5H)-one, 5-hydroxy-2-(4-hydroxyphenyl)-3-methylquinolin-4(1H)-oneare excellent in the control of periodontitis.

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