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RESEARCH ARTICLE
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Evaluation Of Antibacterial Potential of Oxazole Derivative of Haemagglutinin of Porphyromonas Gingivalis Using in Silico Molecular Docking and Admet Prediction

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ABSTRACT

Introduction: Porphyromonas gingivalis is known to produce a lot of virulence factors that could pave their way into the gingivae and cause tissue damage either directly or indirectly, by induction of inflammation. Hemagglutinins are also considered important virulence factors, as they can be a pathway to acquire hemin, which is necessary for bacterial growth, from erythrocytes

Materials And Methods: The 2D structures (mol)of the oxazole compounds (PJ1-PJ8) were drawn using ChemDraw and analysed using Chem3D soNware. The homology modelling of the haemagglutinin protein was done using Swiss model.The graphical user interface program AutoDock Vina was used for ligand - protein docking simulations , the docking algorithm provided with AutoDock Vina was used to search for the best docked confirmation between ligand and protein. The SwissAdme and Protox online servers was used for estimating the absorption, distribution, metabolism and excretion.Statustical analysis : ANOVA($P<0.05$)

Results: The SwissADME prediction outcome showed that the isolated compounds satisfy the Lipinski's rule of five with zero violations having molecular weight less than 500 and lipophilicity values (iLogP) values less than 5.PJ2,3 and PJ5 were better compounds as they were non carcinogenic, mutagenic, cytotoxic and non immunotoxic

Conclusion: We were able to conclude from our study that the docking scores of ligands were better than the standard control drugs that were available and is found to be as a better alternative. Compounds 2,3 were found to be better drugs in exhibiting potential inhibitory action against the haemagglutinin of P.gingivalis.

Keywords: *Periodontitis, Porphyromonas gingivalis, In-silico analysis, virulence factor, heamaglutinin, innovation*

INTRODUCTION

Periodontal disease generally refers to inflammatory pathologic state of the gingiva and the supporting structures of the periodontium and is a serious gum infection which include gingival, alveolar bone, periodontal ligament, and cementum[1][2]. Globally periodontitis is becoming a more prevalent disease which is caused by the growth of favourable microorganisms such as Porphyromonas gingivalis.

Porphyromonas gingivalis is a Gram-negative oral anaerobe that plays an important role in the pathogenesis of periodontitis[3].Due to high prevalence of P. gingivalis in periodontal lesions, it is speculated that the virulence factors from P.gingivalis react with the host microbiota and creates a conducive environment for facilitating the growth of P.gingivalis leading to further progression of the disease.

P. gingivalis is known to produce a lot of virulence factors that could pave their way into the gingivae and cause tissue damage either directly or indirectly, by induction of inflammation.Virulence factors may be defined as the constituents or metabolites of an organism which are produced as they are essential for the survival of the micro organisms and can lead to host destruction .For further multiplication in a host, the unfavourable pathogen needs to overcome the host defence mechanisms in order to bring about their colonisation[4].The presence of virulence factors helps in colonisation such as lipopolysaccharides, proteases, and fimbriae , haemagglutinin which help in the outgrowth of the bacterial colony [5].

Haemagglutinin molecules which are encoded by many protease genes in P. gingivalis have the capacity to degrade a wide range of host proteins[6]. Haemagglutinins can function as adhesins and are required for virulence of several bacterial pathogens[7]. Hemagglutinins are also considered important virulence factors, as they can be a pathway to acquire hemin, which is necessary for bacterial growth, from erythrocytes[8]. In addition, an association between periodontal disease and cardiovascular disease has been found in many epidemiological studies .The accumulation of clinical and experimental evidence also suggests that periodontal infection may be a contributing risk factor for heart disease[9]. For example, the occurrence of P. gingivalis in subgingival sites correlates with the detection of P. gingivalis in coronary artery plaque samples .A correlation between high levels of antibody to P. gingivalis and prevalence of coronary heart disease has also been observed.

In this study we analyse the potential haemagglutinin inhibiting agents to prevent further spread and colonisation of P.gingivalis and reduce the incidence of periodontitis.

Our team has extensive knowledge and research experience that has translate into high quality publications [10]–[18]

MATERIALS AND METHODS

The 2D structures (mol)of the oxazole compounds (PJ1-PJ8) were drawn using ChemDraw and analysed using Chem3D software. The homology modelling of the haemagglutinin protein was done using Swiss model. The total lowest energy of the tile chemical was found by structural optimisation procedure. The 3D coordinates were found for each molecule using optimal structure.

The 3D structure of the protein Haemagglutinin was obtained from the protein data bank. Protein data bank was used to download the crystal structure of the protein haemagglutinin and was synthesised in accordance with the protocol followed universally. Co factors and water molecules were chosen for elimination.

Autodock Vina was used to dock the protein and the synthesised novel compounds onto the active sites of the protein. The chemical structure of the compounds was drawn using chemtool software with proper guidelines. The protein preparation was done using the Auto preparation of target protein file Auto Dock4.2. The grid box for the docking was selected and it provides capacity to concentrate the region of interest. The best docked conformation between the compounds synthesised and the protein was analysed using the docking algorithm provided by Auto Dock Vina.

The SwissADME and ProTox online servers was used to estimate the absorption, distribution, metabolism and elimination properties of the synthesised compounds. This properties helps us to characterise the novel compounds and compare them with the standard control drugs

that were earlier available. This prediction is based on Lipinski's rule of five. The SwissADME also provides insight into further properties such as polar surface area, rotatable bonds, hydrogen donors and acceptors. The toxicities and endpoints along with LD50 value of ligands was predicted using ProTox online servers. The Docking results were contrasted with the therapeutic compounds that have been clinically evaluated using statistical analysis ANOVA($P<0.05$).

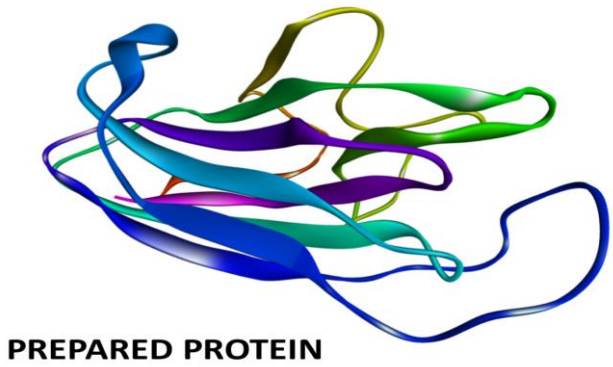
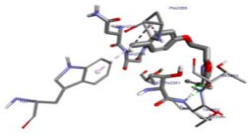
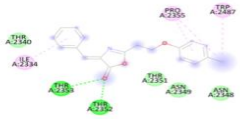
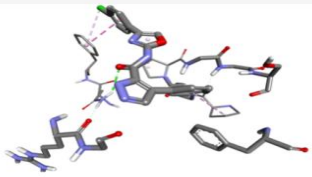
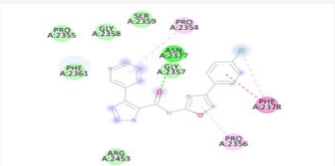
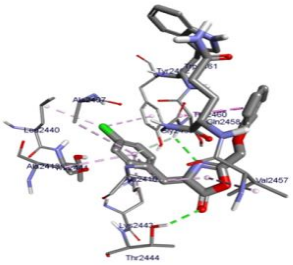
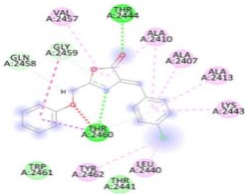
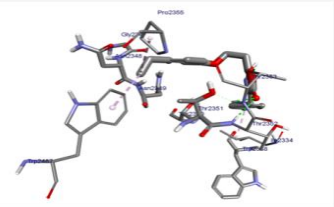
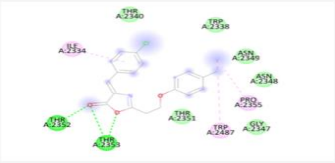
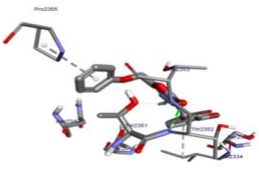
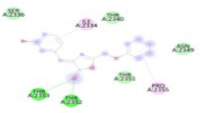


FIGURE 1: The above figure is the prepared protein

RESULTS AND DISCUSSION

TABLE 1: The above table shows the synthesised compounds and their molecular structure

LIGANDS		
PJ1		
PJ2		
PJ3		
PJ4		
PJ5		

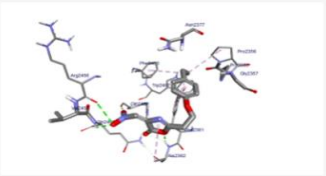
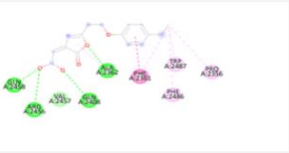
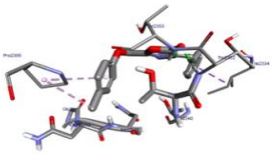
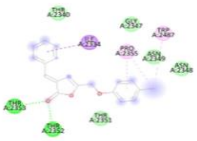
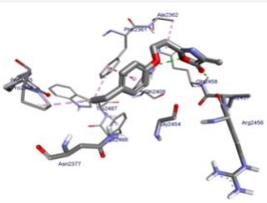
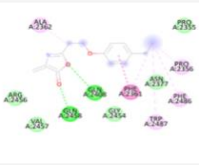
LIGANDS		
PJ6		
PJ7		
PJ8		

TABLE 2: The above table shows the ADME properties of the synthesised compounds

Compound	MW	iLogP	HBD (n_{OHNH})	HBA (n_{ON})	nrotb	MR	TPSA	Lipinski #violations	Bio availability score
Lipinski*	≤500	≤5	≤5	≤10	≤10	-	-		
Veber**	-	-	-	-	-	-	≤ 140		
PJ1	307.34	3.21	0	4	5	92.92	47.89	0	0.55
PJ2	364.79	2.38	2	4	5	98.74	83.81	0	0.55
PJ3	313.74	3.14	0	4	4	88.16	47.89	0	0.55
PJ4	355.81	3.93	0	4	6	102.74	47.89	0	0.55
PJ5	358.19	3.2	0	4	4	90.85	47.89	0	0.55
PJ6	290.27	2.18	0	6	6	80.55	93.71	0	0.55
PJ7	293.32	3.11	0	4	4	88.11	47.89	0	0.55
PJ8	245.27	2.89	0	4	5	72.45	47.89	0	0.55
Amoxicillin	365.4	1.46	4	6	5	94.59	158.26	0	0.55
Moxifloxacin	401.43	2.78	2	6	4	114.05	83.8	0	0.55
Sulfanilamide	172.2	0.61	2	3	1	41.84	94.56	0	0.55
Sulfamethoxazole	253.28	1.03	2	4	3	62.99	106.6	0	0.55

Compound	log Kp (cm/s)	GI absorption	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
PJ1	-5.43	High	Yes	No	Yes	Yes	Yes	No	No
PJ2	-5.65	High	No	No	Yes	Yes	Yes	Yes	Yes
PJ3	-5.21	High	Yes	No	Yes	Yes	Yes	No	No
PJ4	-4.97	High	Yes	No	Yes	Yes	Yes	No	No
PJ5	-5.44	High	Yes	No	Yes	Yes	Yes	No	No
PJ6	-6.44	High	No	No	Yes	No	No	No	No
PJ7	-5.27	High	Yes	No	Yes	Yes	Yes	No	No
PJ8	-5.8	High	Yes	No	Yes	Yes	No	No	No
Amoxicillin	-9.94	Low	No	No	No	No	No	No	No
Moxifloxacin	-8.32	High	No	Yes	No	No	No	Yes	No
Sulfanilamide	-7.79	High	No	No	No	No	No	No	No
Sulfamethoxazole	-7.21	High	No	No	No	No	No	No	No

TABLE 3: The above table shows the toxicity values of the synthesised compounds

Compound			Toxicity				
	[a]LD_{50} (mg/kg)	Class	HEPATOTOXICITY	CARCINOGENICITY	IMMUNOTOXICITY	MUTAGENICITY	CYTOTOXICITY
PJ1	2500	5	Inactive	Active	Inactive	Inactive	Inactive
PJ2	1000	4	Active	Inactive	Inactive	Inactive	Inactive
PJ3	925	4	Active	Inactive	Inactive	Inactive	Inactive
PJ4	1190	4	Active	Inactive	Active	Inactive	Inactive
PJ5	2500	5	Active	Inactive	Inactive	Inactive	Inactive
PJ6	1190	4	Active	Inactive	Active	Inactive	Inactive
PJ7	2500	5	Inactive	Active	Inactive	Inactive	Inactive
PJ8	2800	5	Inactive	Active	Inactive	Inactive	Inactive
Amoxicillin	15000	6	Inactive	Inactive	Inactive	Inactive	Inactive
Moxifloxacin	2000	4	Inactive	Inactive	Inactive	Active	Inactive
Sulfanilamide	3000	5	Inactive	Active	Inactive	Inactive	Inactive
Sulfamethoxazole	2300	5	Active	Active	Inactive	Inactive	Inactive

[a]LD_{50}: lethal dose parameter

TABLE 4: The above table shows the affinity of the ligand/ docking scores

Ligands	Docking scores/Affinity (kcal/mol)	H-bond	Amino Acid Residual interactions	
			Hydrophobic/Pi-Cation	Van dar Waals
PJ1	-6.8	THR:2353, THR:2352	ILE: 2334, PRO: 2355, TRP: 2487	THR: 2340, ASN: 2348, ASN: 2349, THR: 2351
PJ2	-7.3	ASN:2377	PRO: 2354, PHE: 2378, PRO: 2356	GLY:2357, ARG: 2453, SER:2359, GLY: 2358, PHE: 2361, PRO:2355
PJ3	-6.9	THR: 2444, THR: 2460	VAL: 2457, TYR: 2462, LEU: 2440, ALA: 2410, ALA: 2407, ALA: 2413, LYS: 2443	GLY: 2459, GLN: 2458, TRP: 2461, TRP: 2441
PJ4	-6.6	THR: 2353, THR: 2352	TRP: 2487, PRO:2355, ILE:2334	THR: 2340, TRP:2338, ASN: 2349, ASN:2348, GLY: 2347, THR: 2351
PJ5	-6.6	THR: 2353, THR: 2352	ILE: 2334, PRO: 2355	SER :2336, THR:2340, ASN: 2349, THR: 2351
PJ6	-6.1	GLN: 2458, ARG: 2456, GLN:2408, ALA: 2362	PHE :236, TRP:2487, PRO:2356, PHE: 2486	VAL: 2457
PJ7	-7	THR: 2353, THR: 2352	ILE:2334, PRO: 2355, TRP A:2487	THR:2351, THR: 2340, ASN:2349, ASN:2348, GLY:2347
PJ8	-5.5	GLN:2458, GLN:2408	PRO :2356, PHE: 2486, PHE :2361, TRP:2487, ALA: 2362	PRO :2355, ASN: 2377, GLY: 2454, VAL: 2457, ARG: 2456
Amoxicillin	-6.4	PRO :2355, TRP: 2487, ALA: 2362, GLN: 2408	PRO: 2356	GLN: 2458, GLY:2454, GLY: 2357, PHE: 2486, PHE: 2361, ASN: 2377, PRO:2354, SER: 2360

Moxiflaxcin	-6.4	TRP: 2338, ASN: 2349	ILE: 2334, PRO: 2355	THR: 2340, ASN: 2348, THR: 2351, THR: 2353, THR: 2352, SER: 2336, PRO: 2335
Sulfanilamide	-5.3	THR: 2441, ALA: 2407	THR: 2460, LYS: 2443, ALA: 2410, ALA: 2413	GLY: 2459, CYS: 2406, LEU: 2440, THR: 2444
Sulfamethoxazole	-6	SER: 2359, SER: 2360	ILE: 2452, PHE: 2361, PHE:2486, PHE: 2378	GLY: 2357, PRO: 2356, ARG: 2453, PRO: 2354

Oral cavity is a wide and favourable environment for the growth of various micro organisms that can cause infection. The oral flora requires defence mechanism in order to prevent the infection. The infection on further spread can damage the gums and cause transition of gingivitis to periodontitis. The red complex bacteria especially Porphyromonas gingivalis is the most common pathogen in causing periodontitis and many research work are now carried out to study the disease pathogenesis and strategies to inhibit the virulence mechanisms. Molecular modelling in computer aided drug design is one of the most advanced and virtual mechanism of studying the drug receptor interaction. Docking is the computational process where the appropriate ligand is identified that fits the proteins binding site and stops the virulence mechanism of the bacteria.

In our present study we have studied the molecular docking interaction between the synthesised oxazole compounds and haemagglutinin protein of P.gingivalis and compared them with clinically approve drugs such as Amoxicillin, Sulfanilamide, Sulfamethoxazole, Moxifloxacin.

In Silico Analysis

In Silico Drug Likeness And Toxicity Analysis

The ADME predictors are particularly important as they enable us to analyse the properties of absorption, distribution, metabolism and elimination of the synthesised novel compounds. The SwissADME prediction outcome showed that the isolated compounds satisfy the Lipinski's rule of five with zero violations having molecular weight less than 500 and lipophilicity values (iLogP) values less than 5[9], [19]. The Kp values of all molecules are within the range of (-5.21-6.44 cm/s) when compared to the standard drugs available which were within the range of (-7.21-9.44 cm/s) inferring low skin permeability. The Gastro intestinal absorption rate was higher in all the oxazole compounds synthesised and also had better blood brain barrier penetration than the control drugs used in the study[20]

Acute toxicity prediction analysis showed that toxicity class classification and LD 50 values of all the synthesised compounds were less than the control drugs. The compounds which were carcinogenic were eleminated such as PJ1, PJ7, PJ8.

The properties of hepatotoxicity , carcinogenecity, immunotoxicity , mutagenicity and cytotoxicity were compared within the newly synthesised compounds and standard drugs available (amoxicillin, moxifloxacin, sulfanilamide, Sulfamethaxazole) and found that PJ2,3 and PJ5 were better compounds as they were non carcinogenic, mutagenic, cytotoxic and non immunotoxic.

Molecular docking results

The H bond, vanderwaals, and hydrophobic bonds were analysed in the ligands and given a docking score which showed that PJ 2 and 3 had better docking score with value of -7.3 and -6.9 respectively, when compared to clinical drugs and was considered as a better alternative to inhibit the action of the bacteria P.gingivalis. More negative the docking score better was the binding of the ligand to the protein[21][22].

Inhibition of virulence factors could prevent or slow down the progression of periodontitis. The present study analyse the interaction between the virulence factors of Porphyromonas gingivalis and the ligands which were synthesised from the oxazole compounds. Docking studies with Autodock software revealed that ligands was able to bond with the selected protein of the bacteria indicating their affinity when compared to the standard control drugs. PJ2,3 compounds showed significant interaction with the virulence factor haemagglutinin and could play an important part in preventing the progression of periodontitis mediated by P.gingivalis.

CONCLUSION

In this study we have analysed the bond interaction between the newly synthesized oxazole compound and the protein. We were able to conclude from our study that the docking scores of ligands were better than the standard control drugs that were available and is found to be as a better alternative. Compounds 2,3 were found to be better drugs in exhibiting potential inhibitory action against the haemagglutinin of P.gingivalis. All synthesised compounds were exhibiting promising efficiency but compounds PJ 2,3 were the best among them.

CONFLICT OF INTEREST

The authors assert that there is no conflict of interest

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