Evaluation of Antibacterial Potential of Oxadiazole Derivative Compounds Against Peptidyl Arginine Deiminases of P.Gingivalis Using in Silico Molecular Docking and Admet Prediction

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Abstract

Background:Periodontitis is a widespread chronic polymicrobial inflammatory condition which is associated with multifactorial causation. Red complex bacteria have a significant role in the pathophysiology of periodontitis. Porphyromonas gingivalis has been termed as keystone pathogen since they play a crucial role for the development of symbiosis by creating a community-wide impact with low-abundance bacteria. Various virulence factors are found in P.gingivalis which includes lipopolysaccharide (LPS), gingipains, fimbriae, pili, lectins, capsule, peptidyl arginine deiminases, collagenases, superoxide dismutases and proteases. The virulence factor under emphasis here in our study is the enzyme Porphyromonas gingivalis peptide arginine deiminases (PPAD).Aim: Our study aimed to identify the potential inhibitors of peptidyl arginine deiminases of P.gingivalis with oxadiazole compounds. Materials and methods: The computerised crystal structure of the receptor molecules and the P.gingivalis peptidyl arginine deiminases (PDB ID: 5AK7) were taken from the protein data bank, and protein processing was carried out in accordance with the accepted procedures and practises around the world. A maximum of six conformers were developed for each of the ligands in order to explore the best-docked conformation between the ligand and protein using the docking technique offered by Auto Dock Vina. To estimate in-silico pharmacokinetic parameters, the Swiss ADME tool was used with the derived chemical structures of the synthesised compounds (1-6) to create their canonical simplified molecular input line entry system (SMILE). Using ProTox II and OSIRIS Property Explorer, the ligands' organ toxicities, toxicological endpoints, and LD50 were deciphered. Amoxicillin, Moxifloxacin, Sulfanilamide, and Sulfamethoxazole, four common medications, were compared to the analyses of the synthesised compounds. Results and discussion: The produced compounds (1-6) were discovered to have minimal binding energies ranging from -6.3 to -8.3 kcal/mol, with compound 2 (-8.3 kcal/mol) producing the best results. This outcome validated the findings of the experimental study that indicated a potential antibacterial in-vitro. The produced compounds may be a viable antibacterial agent against the P.gingivalis strain, according to the overall docking results. The SwissADME prediction findings show that the compounds (2,5,6) completely adhere to Lipinski's rule of five. Conclusion: Results show that all selected ligands (1-6) exhibit better interactions with the target protein within the binding sites. Ligands 2,5, and 6 obey Lipinski's rule of 5 with low toxicity profile and provide a better interaction score. Compounds with similar functional groups and its interactions can be explored for further studies. The molecule could be further developed and in the future, these drugs could be a better alternative to standard drugs.

Keywords: Insilico, Periodontitis, Peptidyl arginine deiminases, Porphyromonas gingivalis, red complex bacteria

1. Introduction

Periodontitis is a widespread chronic polymicrobial inflammatory condition which is associated with multifactorial causation (1). It is nothing but the destruction of supporting structures of the teeth such as gingiva, cementum, periodontal ligament, and alveolar bone and ultimately leads to the loosening of teeth (2). Periodontal pathogens were grouped by Dr. Sigmund Socransky in 1998 into the red complex, orange complex, green complex, orange-associated complex, and an Aa complex. (3). Bacteria established in the green and orange-associated clusters are early colonizers and the most pathogenic red-complex bacteria are the final bacteria that colonize and lead to the destruction of the periodontium. The pathogenesis of periodontitis is significantly influenced by red complex bacteria. The red complex group of bacteria, which includes

doi.org/10.31838/hiv23.03.47 Received: 26.10.22, Revised: 22.11.22, Accepted: 13.12.22.

Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola, is often not organized separately but collectively in the periodontal pockets, suggesting that these bacteria cooperate to cause the deterioration of periodontal tissues (4).

A new model of periodontal pathogenesis that postulates that periodontal disease is caused by a synergistic and dysbiotic microbial population rather than by a small number of bacteria usually classified as "perio pathogens" is consistent with modern metagenomic and mechanistic research (5). Keystone pathogens are low-abundance bacteria that have a significant impact on the community and are essential for the emergence of dysbiosis; the best-documented example of such a pathogen is Porphyromonas gingivalis (6). Recent findings show that P. gingivalis' pathogenicity is largely dependent on its capacity to establish residence in the subgingival environment and to compromise innate immunity in a way that decouples the inflammatory response—which is nutritionally advantageous for the bacteria—from the antimicrobial pathways. While interactions with early colonising bacteria are necessary for P. gingivalis to establish itself, coexisting species gain from P. gingivalis' immune subversion approaches (7).

Lipopolysaccharide (LPS), gingipains, fimbriae/pili, collagenase, lectins, capsule, protease, and superoxide dismutase are only a few of the virulence factors produced by P. gingivalis. The peptidyl-arginine deiminase enzyme from P. gingivalis is the main virulence factor in this study (PPAD) (8). By deiminating arginine residues in proteins and peptides and changing them to citrulline, this enzyme modifies both bacterial and host proteins. PPAD is anchored into the outer membrane and is present on the bacterial surface (9). PPAD is believed to be involved in interactions with eukaryotic cells, such as neutrophils, macrophages, and epithelial cells, as a key virulence factor.(10). Additionally, P.gingivalis citrullinated the proteins that constitute its cell envelope, producing in a PPAD-dependent manner a pool of potent antigenic epitopes that can overcome the tolerance to particular citrullinated host peptides (11). Auto-antibodies against citrullinated proteins can be produced as a byproduct of loss of tolerance (ACPAs) (12). Patients with severe periodontitis had higher levels of ACPAs (13). The study of the virulence factors of red complex bacteria and how they can be inhibited is a crucial field of study that could lead to new treatments for periodontitis. Our team has extensive knowledge and research experience that has translate into high quality publications (14–23)

The objective of the study includes the formulation of drug inhibitors, preparation of proteins and ligands, Molecular docking (AutoDock Vina), insilico evaluation of ADMET properties. With this background, our study aimed to identify the potential inhibitors of peptidyl arginine deiminases of P.gingivalis with oxadiazole compounds

2. Materials and methods

Data on the physical and spectral properties of newly synthesised oxadiazole compounds

SJ1)
O=S(NC1=NC(OC)N=C(OC)C1)(C2=CC=C(/N=N/C3=CC=C(N(CCO)CCO)C=C3)C=C2)=O
SJ2)
O=S(NC1=CC=CC=C1)(C2=CC=C(/N=N/C3=CC=C(O)C(C(O)=O)=C3)C=C2)=O
SJ3)
O=S(NC1=NC(OC)N=C(OC)C1)(C2=CC=C(/N=N/C3=CC=C(N(CCO)CC)C=C3)C=C2)=O
SJ4)
O=S(NC1=NC(OC)N=C(OC)C1)(C2=CC=C(/N=N/C3=CC=C(N(CCO)C)C=C3)C=C2)=O
SJ5)
O=S(NC1=NC(OC)N=C(OC)C1)(C2=CC=C(/N=N/C3=CC=C(O)C=C3)C=C2)=O
SJ6)
NC1=CC=C(S(=O)(N(C2=NOC(C)C=C2)[H])=O)C=C1

In-silico molecular docking methodology

Preparation of ligands

Using Chem-Draw 16.0, the 2D structures (mol) of the synthesised oxadiazole compounds (SJ1–SJ6) were drawn and each structure was analysed. By using the DFT approach and the Gaussian 09 programme suite at the Becke-3-Lee-YangPar (B3LYP) level combined with the common 6-31G (d,p) basis set, specific molecules were addressed quantum mechanically. During the optimisation process, every parameter was determined to produce a stable structure with the least amount of energy. The title compound's global minimum energy was deduced via the structure optimization process. Following that, an optimised structure was used to obtain the 3D coordinates (PDB) of each of the molecules.

Preparation of protein

The computed crystal structure of the receptor molecules from P. gingivalis (PDB ID: 5AK7) was taken from the protein data bank, and the protein was prepared in accordance with global best practises. Cofactors and water molecules were removed. To prepare the protein, polar hydrogens were added using Auto Preparation of target protein file Auto Dock 4.2.6 after the previously connected ligands were separated (MGL tools 1.5.6).

Auto dock Vina analysis

The grid box for docking simulations was created using the AutoDock 4.2.6 graphical user interface application. We tried a variety of different docking pockets and positions before developing the grid based on the best results. A maximum of six conformers were developed for each of the ligands in order to explore the best-docked conformation between the ligand and protein using the docking technique offered by Auto Dock Vina. The target protein's interactions with its ligands were examined using PyMOL and Discovery Studio Visualizer, and the conformations with the most advantageous (least) free binding energy were selected.

The Auto Dock Tools (ADT), a free graphic user interface (GUI) for the AutoDock Vina programme, was used to carry out the molecular docking research. The grid box was created using a grid point spacing of 0.375 A and the

coordinates 20 20 20, which stand for the x, y, and z directions, respectively. the central grid boxes of 2XCT's 623062A and 1DNU's 654065A. According to their binding energies, six distinct conformations were created for each ligand designated to obtain Auto Dock Vina functions. Utilizing various colours, sticks, ribbons, and lines, binding pockets, H-bonds, and other hydrophobic and electrostatic interactions are represented.

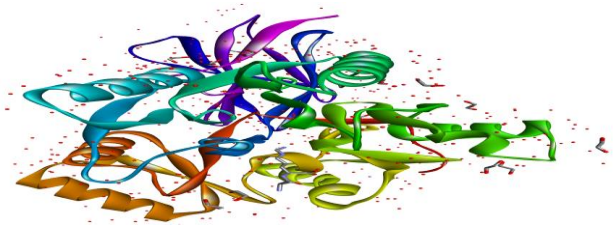
In-silico drug-likeness and toxicity predictions

This prediction informs users of the route to drug effectiveness and provides information on whether the investigated ligand exhibits characteristics consistent with existing in an orally active medication or not. This type of prediction is based on Lipinski's rule of five, a theory that was previously established by Lipinski et al. To estimate in-silico pharmacokinetic parameters, the SwissADME tool was used with the derived chemical structures of the synthesised compounds (1-6) to create their canonical simplified molecular input line entry system (SMILE). This SwissADME predictor collects information on a compound's total polar surface area, rotatable bonds, hydrogen donors, and hydrogen acceptors. Similar Lipinski et al. screening was done on the ligands utilising SwissADME and PreADMET predictors. Using ProTox II and OSIRIS Property Explorer, the ligands' organ toxicities, toxicological endpoints, and LD50 were deciphered. Amoxicillin, Moxifloxacin, Sulfanilamide, and Sulfamethoxazole, four common medications, were compared to the analyses of the synthesised compounds.

3. Results and Discussion

Protein preparation

5AK7 - peptidyl arginine deiminases



Prepared protein

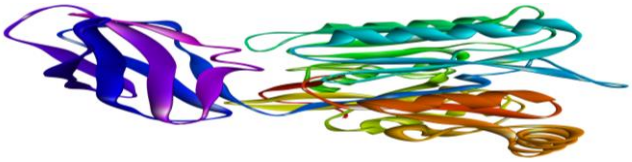


Table 1: Interaction of the synthesized compound and standard drugs

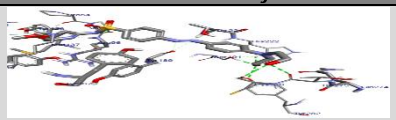
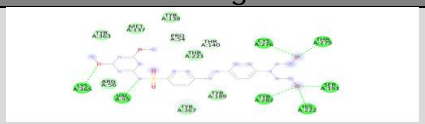
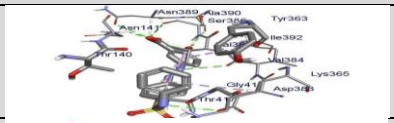
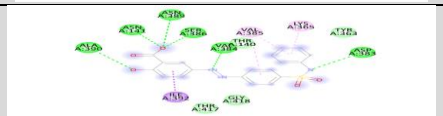
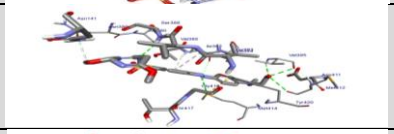
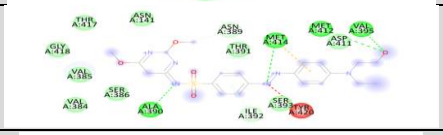
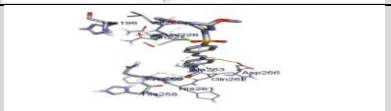
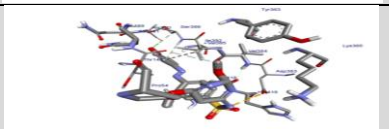
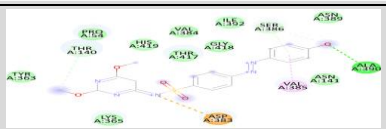
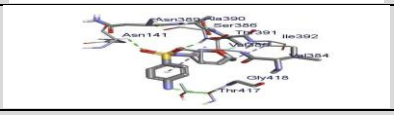
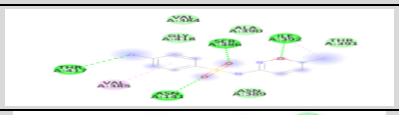
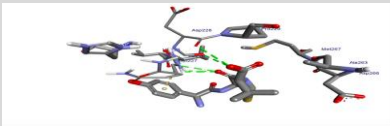
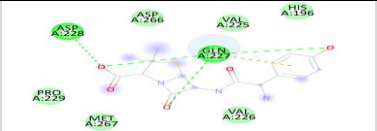
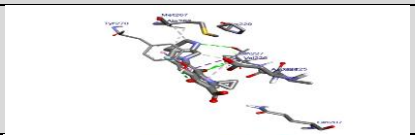
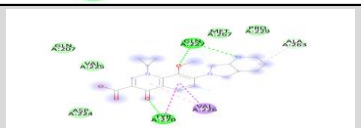
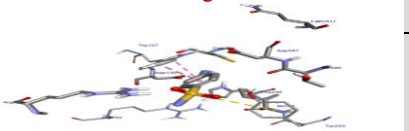
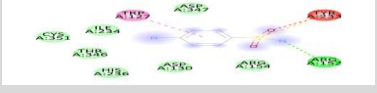
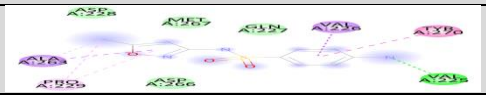
SJ1		
SJ2		
SJ3		
SJ4		
SJ5		
SJ6		
Amoxicillin		
Moxifloxacin		
Sulfanilamide		
Sulfamethoxazole		

TABLE-2 Molecular docking scores and residual amino acid interactions of Oxadiazole compounds against peptidylarginine deiminase of Porphyromonas gingivalis				
Ligands	Docking scores/Affinity (kcal/mol)	H-bond	Amino Acid Residual interactions	
			Hydrophobic/Pi-Cation	Van dar Waals
SJ1	-7.2	LYS-365, VAL-55, CYS-276, THR-275, SER-191, HIS-222, TYR-282, THR-140	PRO-54, THR-140	TYR-363, MET-137, TYR-138, THR-221, TYR-189, TYR-367
SJ2	-8.3	ALA-390, ASN-141, ASN-389, SER-386, VAL-384, ASP-383	ILE-392, VAL-385, LYS-365	THR-140, TYR-363, GLY-418, THR-417
SJ3	-7.2	ALA-390, MET-414, MET-412, VAL-395	TYR-420, ILE-392, ASN-389	ASN-141, THR-417, GLY-418, VAL-385, SER-386, VAL-384, THR-391, SER-393, ASP-411
SJ4	-7	GLN-227, GLN-262, HIS-261, PRO-259	VAL-226, VAL-225, ASP-266, ALA-263,	HIS-196, HIS-258, GLN-260
SJ5	-7.5	GLN-227, GLN-262, PRO-259, HIS-261	VAL-226, VAL-225, ASP-266, ALA-263,	HIS-258, GLN-260, HIS-196
SJ6	-6.3	THR-417, ASN-141, SER-386, ILE-392	VAL-385	VAL-384, GLY-418, ALA-390, THR-391, ASN-389
Amoxicillin	-6.6	ASP-228, GLN-227	-	ASP-266, VAL-225, HIS-196, VAL-226, MET-267, PRO-229
Moxifloxacin	-7.5	TYR-270, GLN-227	VAL-226, ALA-263	GLN-207, VAL-225, ASP-224, MET- 267, PRO-229
Sulfanilamide	-5.4	ARG-152	TRP-127, TYR-233	ASP-347, ILE-234, CYS-351, THR-346, HIS-236, ASP-130, ARG-154
Sulfamethoxazole	-6	VAL-225	VAL-226, TYR-270, ALA-263, PRO-229	ASP-228, MET-267, GLN-227, ASP-266

Table- 3, 4 Lipinski's Analysis									
Compound	MW	iLogP	HBD (nOHNH)	HBA (nON)	nrotb	MR	TPSA	Lipinski #violations	Bio availability score
Lipinski*	≤500	≤5	≤5	≤10	≤10	-	-		
Veber**	-	-	-	-	-	-	≤ 140		
SJ1	504.56	3.42	3	10	12	137.21	166.15	2	0.17
SJ2	397.4	1.71	3	7	6	103.16	136.8	0	0.56
SJ3	488.56	3.29	2	9	11	136.05	145.92	1	0.55
SJ4	474.53	3.03	2	9	10	131.24	145.92	1	0.55
SJ5	417.44	1.93	2	9	7	113.09	142.68	0	0.55
SJ6	267.3	0.98	2	4	3	71.48	102.16	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 permeability	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor
SJ1	-8.63	Low	No	Yes	No	No	No	No
SJ2	-5.93	Low	No	No	No	No	Yes	No
SJ3	-7.78	Low	No	Yes	Yes	No	Yes	No
SJ4	-7.95	Low	No	Yes	No	No	No	No
SJ5	-7.46	Low	No	Yes	No	No	No	No
SJ6	-7.31	High	No	No	No	No	No	No
Amoxicillin	-9.94	Low	No	No	No	No	No	No
Moxifloxacin	-8.32	High	No	Yes	No	No	No	Yes
Sulfanilamide	-7.79	High	No	No	No	No	No	No
Sulfamethoxazole	-7.21	High	No	No	No	No	No	No

Table- 5 toxicity Analysis							
Compound			Toxicity				
	Ald50 (Mg/Kg)	Class	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
SJ1	1000	4	Inactive	Active	Inactive	Inactive	Inactive
SJ2	7400	6	Active	Active	Inactive	Inactive	Inactive
SJ3	1000	4	Inactive	Active	Inactive	Inactive	Inactive
SJ4	1000	4	Inactive	Active	Inactive	Inactive	Inactive
SJ5	4000	5	Inactive	Active	Inactive	Inactive	Inactive
SJ6	8900	6	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							

Numerous studies are being conducted to understand the aetiology of the disease and to find ways to block the virulence mechanisms of red-complex bacteria, which are essential periodontal pathogens. Since the inhibition of virulence factors could prevent or halt the progression of periodontitis, several inhibitors from both natural and synthetic sources are also being developed (24). Among these technologies, molecular modelling is crucial to computer-aided drug design and one of the most important virtual screening strategies to investigate drug-receptor interaction. (25). Docking is a computational method for expressing an appropriate ligand that energetically and geometrically matches the protein's binding site. As a result, the current study evaluated how oxadiazole chemicals interact with the peptide arginine deaminases pathogenicity factors of red complex bacteria that cause periodontitis. Azoles are a large and promising class of five-membered heterocyclic compounds that can contain sulphur or oxygen atoms as well as one to five nitrogen atoms. Numerous azole compounds have also discovered a number of additional potential biological features, in addition to their widespread use as powerful antifungal drugs (26).

Table 1 lists the 3D interactions and binding affinities between synthetic chemicals and bacterial virulence factors that were docked into the protein's binding region. Using our recently published procedure, AutoDock Vina was used to dock the protein (PDB ID: 5AK7) and the produced chemicals (1-6) into the active site of proteins. Chemical structures for the compounds were shown using the Chem Office application (ChemDraw 16.0). Following each molecule's energy minimization, structures with the correct orientation were assigned using ChemBio3D. The energy-minimized ligand molecules were also used as input in AutoDock Vina to complete the docking simulation. The Porphyromonas gingivalis peptide arginine deaminase crystal structure (PDB ID: 5AK7) was retrieved from the protein data bank database, along with the crystal structure of the receptor molecules. The target protein file was organised by leaving the associated residue with protein using Auto preparation of target protein file Auto Dock 4.2 (MGL tools 1.5.7) in accordance with the reported standard protocol. The protein was further prepared by removing the co-crystallized ligand, eliminating water molecules, adding polar hydrogens, and cofactors. The region of interest in the macromolecules was encircled by a grid box that was prepared for docking simulations using a graphical user interface tool. Using the docking technique offered by AutoDock Vina, the best-docked configuration between the chemicals and the protein was investigated. For each ligand, a maximum of six conformers were examined throughout the docking procedure. The Discovery studio visualizer calculated the conformations with the most acceptable (least) free binding energy for the subsequent computation of the interactions between the target receptor and ligands. H-bonds (distance range 2-3.5 A) and the interfacing residues are shown as a ball and stick model depiction, while the ligands were shown in various colorations.

According to Table 2, the synthesized compounds (1-6) had docking affinity from -6.3 to -8.3 kcal/mol, with compound 2 (-8.3 kcal/mol) producing the best results. Table 2 also summarises the residual interaction, H-bond, and binding affinity of synthetic chemicals and clinical medicines. The manufactured compounds (1-6) displayed a similar residual interaction profile with amino acid residues compared to the conventional medicines. The in-silico investigation demonstrated improved activity in the compounds 2 (-8.3 kcal/mol) and 5 (-7.5 kcal/mol). When compounds 2 and 5 were subjected to the results of the in silico molecular docking analysis, they had greater residual interactions and docking scores than those of the common medications Amoxicillin, Moxifloxacin, Sulfanilamide, and Sulfamethoxazole. This outcome validated the findings of the experimental study that indicated a potential antibacterial in-vitro. The produced compounds may be a viable antibacterial agent against the P.gingivalis strain, according to the docking results overall.

The SwissADME prediction findings are summarised in Table 3, which shows that the compounds (2,5,6) satisfy Lipinski's rule of five with no violations. The anticipated logP values shown in Table 3 indicate that they have the best lipophilicity (ranging from 0.98 to 3.29). According to Table 4, all of the

synthesised compounds have Kp values between (-5.93 and -8.63 cm/s), which is lower than the range for typical antibiotics (-7.21 to -9.94 cm/s) and indicates low skin permeability. With the exception of compound 6, all of the compounds exhibit low gastrointestinal (GI) absorption, according to the SwissADME prediction parameters. Furthermore, neither the synthetic substance nor the reference material passes through the blood-brain barrier (BBB). Additionally compounds 2, 6, are not permeability glycoprotein substrates (P-gp). These results support the hypothesis that the produced compounds 2,5,6 may function as potent pharmacological agents. Several cytochromes (CYPs) control how drugs are metabolised, but CYP1A2, CYP2C19, CYP2C9, and CYP2D6 specifically control how drug molecules are biotransformed. The prediction outcome shows that compound 3 has been identified as a potential CYP1A2 inhibitor. The usual medications and none of the synthetic substances are CYP2C19 inhibitors. The substances 2-3 have the potential to inhibit CYP2C9. All of the other substances are not CYP2D6 inhibitors, with the exception of the common medication moxifloxacin. The Absorption, Distribution, Metabolism, and Excretion (ADME) in silico prediction findings for isolated chemicals and prescription medications are assessed using ProTox and are shown in Table 4. Compound 6 has demonstrated acute toxicity, according to data from acute toxicity prediction studies including toxicity class categorization and LD50 values. Results for hepatotoxicity, carcinogenicity, mutagenicity, and cytotoxicity are provided by the toxicological prediction. It was anticipated that none of the usual medications or isolated chemicals would be cytotoxic. All synthetic substances, with the exception of moxifloxacin and amoxicillin, are carcinogenic. The hepatotoxicity of compound 2 is comparable to that of sulfamethoxazole. Thus, the compounds 1, 3, and 4 may be a promising drug contender for the research of novel medications against P.gingivalis based on ADMET prediction analysis.

4. Conclusion

The emergence of drug-resistant pathogens is a major global threat to treating any disease. Search for a novel drug that is effective and less toxic has been a major goal in research. Periodontitis is a widespread polymicrobial chronic inflammatory condition which is attributed with multifactorial causation. *Porphyromonas gingivalis* is the red complex bacteria mainly associated with periodontitis. Our study targeted the virulence factor peptidyl arginine deaminases of *P.gingivalis* with molecular docking using oxadiazole compounds. Results show that all selected ligands (1-6) exhibit better interactions with the target protein within the binding sites. Ligands 2,5, and 6 obey Lipinski's rule of 5 with low toxicity profile and provide a better interaction score. Compounds with similar functional groups and its interactions can be explored for further studies. The molecule could be further developed and in the future, these drugs could be a better alternative to standard drugs.

5. Acknowledgement

I would like to thank the Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai for their valuable inputs for the study.

Conflict of interest

The authors declare no conflict of interest.

Funding:

The present project is supported by
Saveetha Institute of Medical and Technical Sciences, Chennai
Saveetha Dental College and Hospitals, Saveetha University,Chennai
Kanagaraj stores, Chennai (KAT/2025)

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