



www.bioinformation.net
Volume 19(1)



Research Article

Received January 1, 2023; Revised January 30, 2023; Accepted January 31, 2023, Published January 31, 2023

DOI: 10.6026/97320630019057

Declaration on Publication Ethics:

The author's state that they adhere with COPE guidelines on publishing ethics as described elsewhere at <https://publicationethics.org/>. The authors also undertake that they are not associated with any other third party (governmental or non-governmental agencies) linking with any form of unethical issues connecting to this publication. The authors also declare that they are not withholding any information that is misleading to the publisher in regard to this article.

Declaration on official E-mail:

The corresponding author declares that lifetime official e-mail from their institution is not available for all authors

License statement:

This is an Open Access article which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited. This is distributed under the terms of the Creative Commons Attribution License

Comments from readers:

Articles published in BIOINFORMATION are open for relevant post publication comments and criticisms, which will be published immediately linking to the original article without open access charges. Comments should be concise, coherent and critical in less than 1000 words.

Edited by P Kanguane

Citation: Parthiban *et al.* Bioinformation 19(1): 57-62 (2023)

Molecular docking analysis of a virulence factor protein dentilisin from *Treponema denticola* with oxazole piperazine derivatives

Kandeeban Parthiban¹, Vishnu Priya Veeraraghavan*¹ Surya Sekaran², Kavitha Sankaran, Gayathri Rengasamy¹ & Rajalakshmanan Eswaramoorthy*²

¹Department of Biochemistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600077; ²Department of Biomaterials (Green lab), Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600077; *Corresponding author

Author contacts:

Kandeeban Parthiban - E-mail: 152101039.sdc@saveetha.com

Vishnu Priya Veeraraghavan - E-mail: vishnupriya@saveetha.com

Surya Sekaran - E-mail: suryas.sdc@saveetha.com

Kavitha Sankaran - E-mail: kavithas.sdc@saveetha.com

Gayathri Rengasamy - E-mail: gayathri.sdc@saveetha.com

Rajalakshmanan Eswaramoorthy - E-mail: rajalakshmanane.sdc@saveetha.com

Abstract:

Dentilisin is a surface protease synthesized by the cell wall of *Treponema denticola*. This protein aids in the invasion of the periodontal tissue by causing infection. To identify drug molecules that have better results, homology modeling of the dentilisin protein was constructed, and molecular docking was performed with the oxazole compounds (1-6) taken from previous studies that are not yet clinically used. Data shows that compounds 1, 2, 3 show better inhibiting properties.

Key words: antimicrobial agents, oxazole piperazine derivatives, *Treponema denticola*, Homology modeling, dentilisin

Background:

Treponema denticola is a helical-shaped, oral spirochete, periodontal pathogen that inflicts damage on host tissue in conjunction with other members of an intricate polymicrobial oral biofilm. These members primarily reside in the subgingival and periodontal pockets, which increases gingival apoptotic cell death and results in hemolysis of human RBC [1, 2]. In animal models, it can induce soft tissue degradation and loss of alveolar bone, and its levels rise in the subgingival microbiome of individuals with periodontal disease when compared to healthy people. An operon comprising the genes *prcB*, *prcA*, and *prtP* encodes dentilisin [3, 4, and 5]. Among these, *prtP* encodes for a 72 kDa protease domain that disintegrates extracellular matrix elements like fibronectin, activates C3, and contributes in fibrinogen attachment [6, 7]. Additionally, dentilisin functions in immunomodulation, adhesion to fibronectin, as well as other processes. An active protease complex is synthesized on the surface of *T. denticola* by post-translational modification and interactions between both the expressed proteins [3, 8]. Dentilisin, also known as acylated chymotrypsin-like protease complex (CTLP), is a substantial virulence factor that triggers a spectrum of cytopathic effects that are consistent with the pathophysiology of periodontal disease [9, 10]. An oxygen-containing heterocyclic molecule called oxazole is crucial in the development of several biologically efficient drug molecule, [12,13] including medications that are anti-inflammatory, anti-cancer, anti-depressant, anti-microbial, anti-obesity, anti-diabetic, and analgesic. Similarly, for several years, studies into the anti-microbial characteristics of pyrrole derivatives have driven the synthesis and analysis of a variety of novel compounds, including monodeoxyputeroin and derivatives of 2-(2'-hydroxybenzoyl) pyrrole bromine [14, 15]. Therefore, it is of interest to document the molecular docking analysis data of a virulence factor protein dentilisin from *Treponema denticola* with oxazole piperazine derivatives for consideration in drug discovery.

Materials and methods:**Preparation of ligands:**

The 2D mol structures of the selected oxazole piperazine derivatives (1-6) were drawn, and energy minimized using ChemDraw (Figure 1) and Chem3D software respectively (ChemOffice suite 16.0). During the optimization method, all parameters were selected in order to achieve a stable structure with the least amount of energy. Each molecule's 3D coordinates (PDB) were determined using optimized structure [16].

Homology modeling of the dentilisin protein:

The fasta sequence of the protein dentilisin was derived from the Uniprot database (Uniprot ID: P96091). The query sequence is then

submitted to the Swiss Model online server and the structure is built using the template with maximum sequence identity. Based on the structure assessment and Ramachandran favored region, the built model was further analyzed for protein preparation [17, 18, 19].

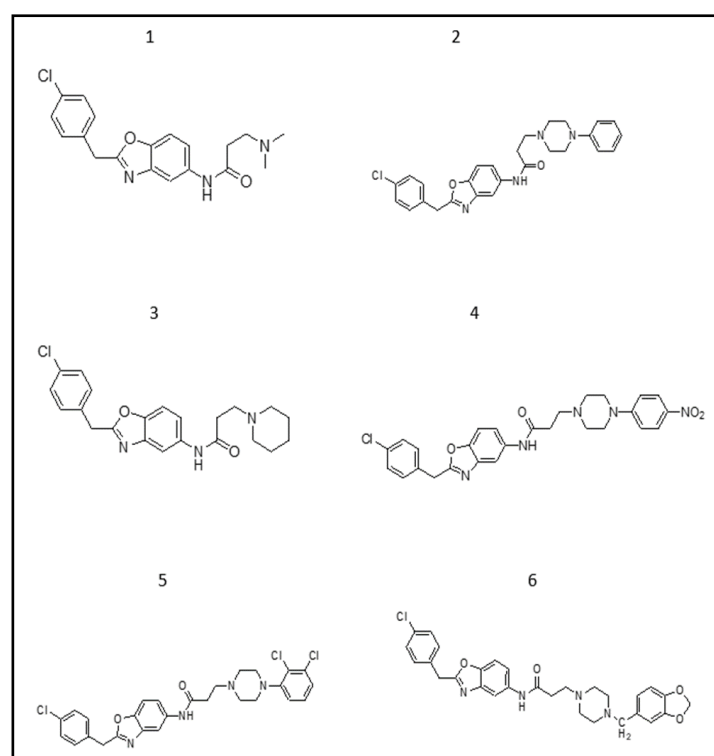


Figure 1: 2D structures of the oxazole piperazine compounds (1-6)



Figure 2: Homology modeled and prepared Dentilisin protein of *Treponema Denticola*

Preparation of protein macromolecule:

The homology modeled structure (**Figure 2**) of the protein dentilisin of *Treponema denticola*, was prepared as per standard protocol practiced globally using the software Biovia Discovery studio. Water molecules, other hetero atoms were chosen for elimination. The previously connected ligands were removed, and the protein was prepared by adding polar hydrogens and kollman charges with Auto Prep.

Auto dock Vina analysis: The graphical user interface Auto Dock vina was used for Ligand-Protein docking interactions (**Figure 3 & Figure 4**). Auto Dock Tools (ADT), a free visual user interface (GUI) for the AutoDock Vina software, was used for the molecular docking research. For each ligand, nine alternative conformations were created and ranked based on their binding energies utilizing Auto Dock Vina algorithms.

Drug-likeness and toxicity predictions: SwissADME and PROTOX-II online servers were used. This prediction points users in the direction of drug efficiency, gives information on whether or not the examined ligands have features consistent with becoming an orally active medication. This prediction is based on Lipinski et al's previously established idea known as Lipinski's rule of five [20].

Statistical Analysis: One way ANOVA was used for statistical analysis. The clinically proven drugs are used as control and the results are compared. The significance of the results was found to be $p < 0.05$.

Results:**Molecular docking interaction against protein dentilisin:**

All compounds with the protein have binding energy in the range of -6.9 to -8.5 Kcal/mol (**Table 1**). The compounds show an H binding interaction similar to that of sulfamethoxazole (-5.6). Clinically proven drugs shown in lead binds to the binding site of protein. Azithromycin binds to the Gln-145, Glu-441, Thr-442, Lys-

430 binding site. Sulfanilamide binds to the Asp-79, Asp-76, Thr-74 binding sites. Sulfamethoxazole binds to the Ser-291, Arg-299 binding site of the protein. All compounds show similar binding affinity as the lead molecules within the binding site.

ADME & Lipinski rule of five:

The chosen compounds show a logKp value within the range of -6 to -5.12 cm/surface (**Table 2**). It is inferred that, more the negative value, the higher will be the permeation of skin. All the compounds show lesser negative values of log Kp than the lead compounds. The GI absorption of the compounds is high and there will be no involvement of carrier molecules to perform their function. This is similar to the control compound Sulfamethoxazole and Sulfanilamide. The compounds 4, 5, 6 and the control group do not show any blood brain barrier permeability. However, compounds 1, 2, and 3 shows blood brain barrier permeation. The compound 4, 5, 6 and the control group taken doses not inhibit the CYP. Nonetheless, compounds 1, 2, and 3 are the inhibitors for CYP. Therefore, the compounds 1, 2, and 3 can be used as potentially lead drug molecules. The compounds 1, 2, and 3 obey the Lipinski rule of five and are similar to the control groups Sulfamethoxazole and Sulfanilamide (**Table 3**). Additionally, the control group molecule Azithromycin and compounds 4, 5, and 6 shows violation in the molecular weight (748.9, 519.98, 543.87, 533.02 respectively). Also, Azithromycin and compound 4 violates TPSA score (180.08, 107.43). In spite of these exceptions, this can still be used as a drug as these violations don't directly affect its function. Compared to this, the oxazole compounds don't have any violations and therefore can be used as lead molecules.

Toxicity profile:

The compounds show class 4 toxicity and have inactive Immunotoxicity, cytotoxicity and hepatotoxicity (**Table 4**). The lethal dose parameters of the chosen molecules are lesser than the control group and thus can be used as drugs.

Table 1: Molecular docking scores and residual amino acid interactions of oxazole compounds (1-6) along with control groups against Dentilisin of *Treponema denticola* (Homology modeled).

Ligands	Docking scores/Affinity (kcal/mol)	H-bond	Amino Acid Residual interactions	
			Hydrophobic/Pi-Cation	Van dar Waals
1	-6.9	Asp-79	Leu-96, Trp-104	Ser-95, Ile-94, Asn-93, Lys-80, Thr-81, Val-90, Thr-74, Asp-76
2	-7.9	Glu-437	Trp-253, Lys-209, Ile-274, Tyr-440	Ile-249, Asn-207, Asp-438, Lys-211, Gly-275
3	-7.5	Ser-95	Trp-104, Thr-97, Leu-96, Leu-310	Lys-80, Asp-79, Thr-74, Asp-76, Ile-94, Asn-93, Arg-324, Lys-315, Asn-242
4	-7.8	Val-326, Arg324	Lys-319, Gln-342, Lys-195	Ser-325, Ile-316, Leu-317, Gln-345, Ile-343, Asn-465
5	-8.5	Asp-438	Trp-253, Lys-209, Glu-437, Try-440, Ile-274	Ile-249, Asn-207, Lys-211, Gly-275, Glu-170, Pro-169
6	-8.4	Try-237, His-130	Arg-299, Lys-113, Tyr-132, Lys-133	Ser-297, Gly-298, Gly-235, Thr-236, Gly-68, Thr-69, Phe-70, Asp-131
Azithromycin	-6.7	Gln-145, Glu-441, Thr-442, Lys-430	Pro-169, Asn-144	Ser-153, Pro-152, Asp-151, Thr-440, Asn-173
Sulfanilamide	-5.6	Asp-79, Asp-76, Thr-74	Trp-104	Thr-102, Ile-94, Tyr-103, Asn-93
Sulfamethoxazole	-6.2	Ser-291, Arg-299	Ser-302, Tyr-237	Ile-238, Tyr-224, Trp-301, Leu-223, Gly-293, Gly-298, Ser-297, Glu-295, Lys-133,

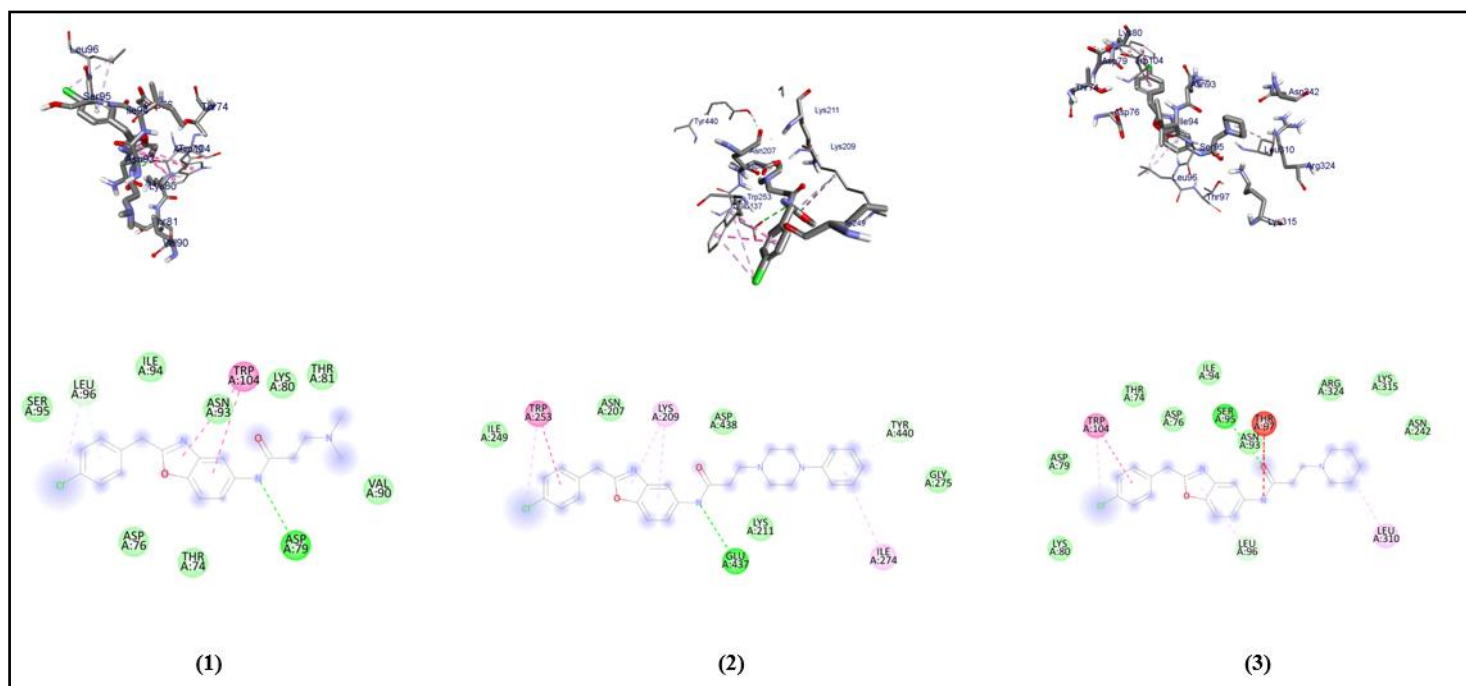


Figure 3: Molecular docking interaction analysis of compounds 1, 2, and 3 against the protein denilisin

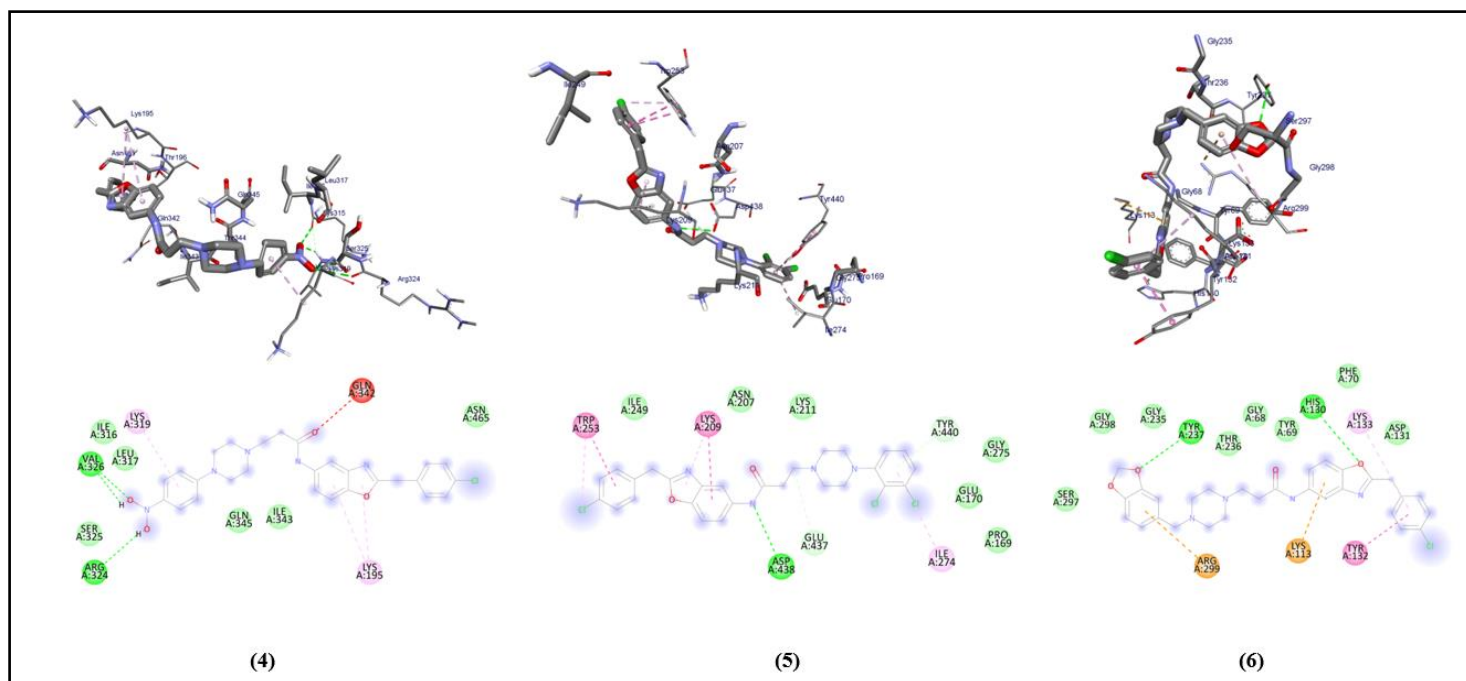


Figure 4: Molecular docking interaction analysis of compounds 4, 5, and 6 against the protein denilisin

Table 2: ADMET and SwissADME properties of oxazole compounds (1-6)

Compound	log Kp (cm/s)	GI absorption	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
1	-6	High	Yes	No	Yes	Yes	Yes	Yes	Yes
2	-5.58	High	Yes	No	Yes	Yes	Yes	Yes	Yes
3	-5.65	High	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	-5.98	High	No	Yes	No	Yes	Yes	Yes	Yes
5	-5.12	High	No	Yes	No	Yes	Yes	Yes	Yes

6	-6.28	High	No	Yes	No	Yes	Yes	Yes	Yes
Azithromycin	-8.01	Low	No	Yes	No	No	No	No	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: Lipinski & Veber rule.

Compound	MW	iLogP	HBD (noHNNH)	HBA (noN)	nrotb	MR	TPSA	Lipinski #violations	Bio availability score
Lipinski*	≤500	≤5	≤5	≤10	≤10	-	-		
Veber**	-	-	-	-	-	-	≤ 140		
1	357.83	3.46	1	4	7	100.1	58.37	0	0.55
2	474.98	4.31	1	4	8	143.37	61.61	0	0.55
3	397.9	3.97	1	4	7	116.32	58.37	0	0.55
4	519.98	3.32	1	6	9	152.19	107.43	1	0.55
5	543.87	4.42	1	4	8	153.39	61.61	2	0.17
6	533.02	4.93	1	7	9	153.68	80.07	1	0.55
Azithromycin	748.98	4.76	5	14	7	200.78	180.08	2	0.17
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

Table 4: Toxicity profile of the oxazole compounds (1-6)

Compound	Toxicity							
	^a LD ₅₀ (mg/kg)	Class	HEPATOTOXICITY	CARCINOGENICITY	IMMUNOTOXICITY	MUTAGENICITY	CYTOTOXICITY	
1	1000mg/kg	4	Inactive	Inactive	Inactive	Inactive	Inactive	
2	1600mg/kg	4	Inactive	Inactive	Inactive	Inactive	Inactive	
3	1500mg/kg	4	Inactive	Inactive	Inactive	Inactive	Inactive	
4	1420mg/kg	4	Inactive	Active	Inactive	Active	Inactive	
5	1500mg/kg	4	Inactive	Inactive	Inactive	Inactive	Inactive	
6	1000mg/kg	4	Inactive	Active	Inactive	Active	Inactive	
Azithromycin	2000mg/kg	4	Inactive	Inactive	Active	Inactive	Inactive	
Sulfanilamide	3000mg/kg	5	Inactive	Active	Inactive	Inactive	Inactive	
Sulfamethoxazole	2300mg/kg	5	Active	Active	Inactive	Inactive	Inactive	

^aLD₅₀: lethal dose parameter**Discussion:**

The selected compounds (Oxazole piperazine derivatives) 5 (-8.5 kcal/mol) and 6 (-8.4 kcal/mol) show better interaction compared to the clinically proven drugs (-6.9 kcal/mol & -7.9 kcal/mol). The compounds 1, 2, 3 obey Lipinski's rule of 5 similar to sulfanilamide and Sulfamethoxazole. Compounds 4, 5, 6 do not obey Lipinski's rule of 5 which is similar to Azithromycin. The selected compounds show better skin permeability (log K_p -6.28 to -5.12 (cm/s)). Compounds 1, 2, 3 & 5 have no toxicity. The reported compounds have stable H bonding, weak hydrophobic and van der Waals interactions within the binding pocket in targeted protein. The SwissADME prediction parameters showed that all the compounds (1- 6) have high gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeation and all compounds are substrates of permeability glycoprotein (P-gp) except 1. The CYP's interaction result showed that all the compounds are inhibitors for CYP2C19, CYP2C9, and CYP2D6. Compounds 4, 5 and 6 were found to be potential inhibitors for CYP1A2. For CYP3A4, compound Azithromycin, sulfanilamide and sulfamethoxazole were found to be non-inhibitors whereas compounds 1-6 were found to be potential inhibitors [21].

Conclusion:

The selected ligands (1-6) show optimal interactions with modeled protein within the binding sites. Ligands 1, 2, and 3 obeys Lipinski's rule of 5 with low toxicity profile and have better interaction score (-6.9, -7.9, and -7.5 kcal/mol) than Sulfanilamide and Sulfamethoxazole (-5.6 and -6.2 kcal/mol). Therefore, compounds

1, 2 & 3 are inferred to be the potential inhibitors for the virulence factor dentilisin.

Acknowledgement:

The author would like to express their gratitude to Saveetha University for providing them with the computer work station and software's to conduct the study and Kamala dental speciality hospital.

References:

- [1] Markov A. *et al. Stem cell research & therapy* 2021 **12**:192. [PMID: 33736695]
- [2] Zeng H *et al. Microbiology spectrum* 2021 **9**:e0070121. [PMID: 34585987]
- [3] Ishihara K. *et al. Infection and immunity* 1996 **64**:5178. [PMID: 8945563]
- [4] Lee SY *et al. Journal of bacteriology* 2002 **184**:3864. [PMID: 12081957]
- [5] Godovikova V *et al. Journal of bacteriology* 2010 **192**:3337. [PMID: 20435733]
- [6] Felicita AS & Sumathi Felicita A, *The Saudi Dental Journal* 2017 p185.
- [7] Yamazaki T *et al. Microbes and infection* 2006 **8**:1758. [PMID: 16815066]
- [8] Ganther S *et al. PLoS pathogens* 2021 **17**:e1009311. [PMID: 34255809]
- [9] Grenier D *et al. Infection and immunity* 1990 **58**:347. [PMID: 2404867]

- [10] Chellapa LR *et al.* *Bioinspired Biomimetic and Nanobiomaterials* 2020 184.
- [11] Muthukrishnan L, *Chemico-biological interactions* 2021 **337**:109397. [PMID: 33508305]
- [12] Mohamed MS *et al.* *Acta pharmaceutica* 2009 **59**:145. [PMID: 19564140]
- [13] Aatif M *et al.* *Antibiotics (Basel Switzerland)* 2022 **11**. [PMID: 36551407]
- [14] Aliye M *et al.* *Scientific reports* 2021 **11**:10101. [PMID: 33980935]
- [15] Mohanavel V *et al.* *Journal of Materials Research and Technology* 2020 p14662.
- [16] Zeleke D *et al.* *Journal of chemistry and chemical engineering* 2020
- [17] Bitew M *et al.* *PloS one* 2021 **16**:e0260853. [PMID: 34890431]
- [18] Anza M *et al.* *Adv appl bioinform chem* 2021 **14**:117. [PMID: 34447254]
- [19] Eswaramoorthy R *et al.* *Adv appl bioinform chem* 2021 **14**:13. [PMID: 33584098]
- [20] Ganapathy D *et al.* *Oral oncology* 2022 **125**:105725. [PMID: 35051852]
- [21] Kumar KRR *et al.* *American Journal of Orthodontics and Dentofacial Orthopedics* 2011 **479**.
-