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RESEARCH ARTICLE
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Evaluation of antimicrobial potential of oxazole compounds against Mfa1 virulence factor of Porphyromonas gingivalis using In silico molecular docking and ADMET predictions

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ABSTRACT

Introduction: A chronic inflammatory condition called periodontitis affects around half of adult Indians. One of the main events in the beginning and progression of periodontal disease is the colonisation of the oral cavity by the Gram-negative bacterial pathogen Porphyromonas gingivalis. Throughout the progression of the illness, P. gingivalis interacts with host cells and other bacteria through adhesive surface features known as fimbriae (pili). A new method that includes the proteolytic digestion of lipidated precursor subunits and their subsequent polymerization on the bacterial surface is used to build the P. gingivalis fimbriae. The P. gingivalis fimbriae are promising targets for anti-infective treatments to prevent or cure periodontal disease because of their extracellular assembly process and key roles in pathogenesis.

Aim: To identify potiential inhibitors of Mfa1 a virulence factor of P. gingivalis

Materials and methods: The structure of Mfa1 was predicted by the SWISS-MODEL web server and the structure was evaluated by different web tools. The structure of virulence factor of Porphyromonas gingivalis was drawn using Chem3D ultra 11.0 software. The structure of important protein virulence factors of red complex bacteria of periodontitis was determined by the SWISS-MODEL web server. The interaction study between oxazole compound and virulence factors was carried out by molecular docking using Auto dock version 4.0 software and pyDock WEB server.

Results & Discussion: The selected ligands show better interactions with the model led protein within the binding sites. Ligands NV1-4 and NV6 obey Lipinski's rule of 5 with low toxicity profile and give better interaction score. These ligands can be validated and can be used as it has better absorption and no cytotoxicity

Conclusion: After comparing all the ligands with each other, we can conclude that NV6 could be a potential drug inhibitor against Mfa1 in P. gingivalis in periodontitis, owing to its high LD50, inactive carcinogenic, mutagenic and immunogenic effect. It also had high hydrogen bond forming capacity.

Keywords: *Periodontitis, Porphyromonas gingivalis, Molecular docking, In silico analysis, drug development*

INTRODUCTION

Periodontitis is chronic inflammatory condition affecting the supporting tissue of the teeth. It's high global prevalence has identified it as one of the main oral diseases that requires prompt identification and treatment. The primary etiologic factor for the initiation of periodontal disease is dental plaque of which the red complex bacteria *Prophyromonas gingivalis, Treponema denticola* and *Tanerella forsythia* have been designated as perio pathogens. These bacteria have an arsenal of virulence factors that play a crucial role in their survival, colonisation and multiplication to form a dysbiotic microbial milieu. (1)

The periodontal pathogen *Porphyromonas gingivalis* has gained much attention due to its significant role as a keystone pathogen in forming a dysbiotic environment. It has the ability for invasion of epithelial cells and dysregulation of host's immune response and activate inflammatory pathways. It expresses various virulence factors like lipopolysaccharides, fimbriae, gingipains, capsules. *P. gingivalis* fimbriae (pili) are proteinaceous, filamentous appendages that protrude from the bacterial cell surface. They play crucial roles in biofilm formation, auto-aggregation, co-aggregation with oral bacteria, adhesion to host molecules, and host cell invasion. Each fimbria is composed mainly of FimA (major fimbriae) and Mfa1 (minor fimbriae) protein polymers encoded by fimA and mfa1 in the fim and mfa gene clusters, respectively. (2) With the emergence of microbial resistance causing severe and refractory forms of periodontal disease, development and designing of newer drugs targeting such specific virulence factors are the need of the hour. (3)

Oxazole rings containing nitrogen and oxygen atoms are considered as prime scaffolds for the drug discovery. Recent studies have explored the pharmaceutical properties of oxazole compounds that have the ability to readily bind with a variety of enzymes and receptors in biological systems. They have displayed therapeutic potential with antibacterial, antifungal, antiviral, antitubercular, anticancer, anti-inflammatory properties. (4) (5)

Drug development is a branch of science that applies various in silico analysis techniques, molecular docking methods and online ADMET predictions to discover new potential drug targeting specific proteins. In this study, we have studied the potential antimicrobial effects of oxazole compounds against mfa1 protein of *Prophyromonas gingivalis.*

MATERIALS AND METHODS

Preparation Of Protein

The 3D structure of the protein Mfa1 was retrieved from protein data bank (5NF2). Protein Data Bank was used to download the crystal structures of the Mfa1 protein and was prepared in accordance with accepted protocol and practices around the world. Cofactors and water molecules were chosen for elimination. Prior to adding polar hydrogens to the protein, previously attached ligands were removed using Auto Preparation of target protein file Auto Dock 4.2.6. (MGL tools 1.5.6).

Preparation Of Ligands

The 2D structures (mol) of oxazole compounds (NV1-NV6) were made using ChemDraw and analyzed using Chem3D software. Using Chem-Draw 16.0, the 2D structures (mol) of the synthesized compounds (NV1–NV6) were drawn and individually examined. During the optimization procedure all the parameters were set in order to obtain a stable structure with minimum energy. The 3D coordinates (PDB) of each molecule was obtained through optimized structure. As per standard protocol, the protein and ligands were prepared for molecular docking. The graphical user interface program AutoDock

Vina was used for ligand protein docking simulations.

Auto Dock Vina Analysis

For the grid box for docking simulations, Auto Dock 4.2.6, a graphical user interface application, was used. We experimented with a variety of docking pockets and positions before creating the grid in accordance with the best outcomes. The optimal docked configuration between the ligand and protein was looked for using the docking algorithm offered by Auto Dock Vina. For each ligand, a maximum of nine conformers were produced. PyMOL and Discovery studio visualizer were used to analyze the interactions between the target protein and ligands by choosing the conformations with the most advantageous (least) free binding energy. The docking algorithm provided with AutoDock Vina was used to search for the best docked conformation between ligand and protein. (6)

Drug Likeness and Toxicity Testing

The SissADME and ProTox online servers were used for estimating the absorption, distribution, metabolism and excretion. This forecast points ushers in the direction of drug effectiveness and offers insights into whether or not the examined ligand has characteristics that are consistent with being an orally active medicine. This prediction is based on Lipinski's rule of five, a theory that has already been established by Lipinski et al. To estimate in-silico pharmacokinetic parameters, the chemical structures of the substances (1-6) were translated to their canonical simplified molecular input line entry system (SMILE). The SwissADME predictor offers details on a compound's total polar surface area, rotatable bonds, hydrogen donors, and hydrogen acceptors. Additionally, Lipinski et al. assessed the ligands using SwissADME and PreADMET predictors. Using Pro Tox II and OSIRIS Property Explorer, the ligands' organ toxicities, toxicological endpoints, and LD50 were predicted. The analysis of the compounds were contrasted with those of the reference medications such as amoxicillin, moxifloxacin, sulfonamide and sulfathiazole.

RESULTS

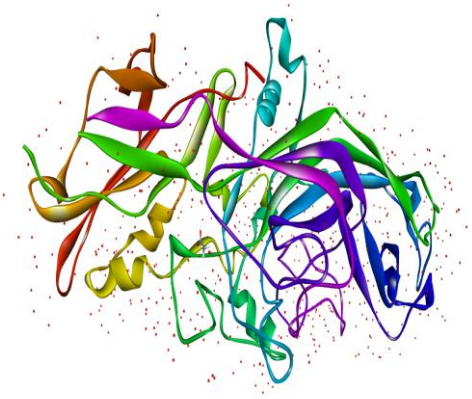


FIGURE 1: Protein preparation 5NF2

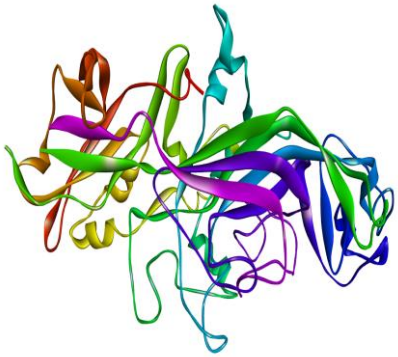


FIGURE 2: Prepared protein

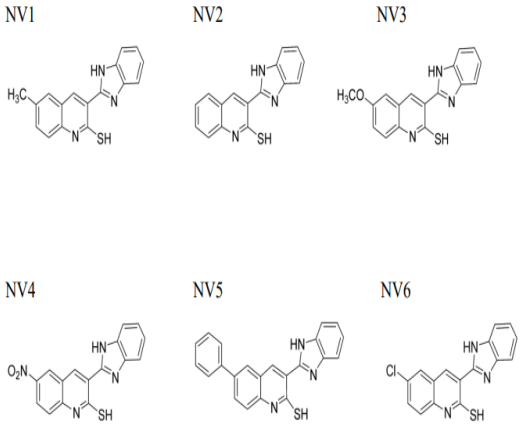
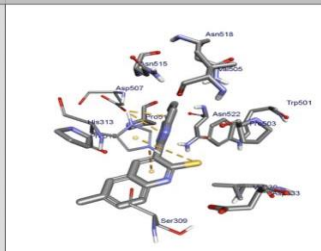
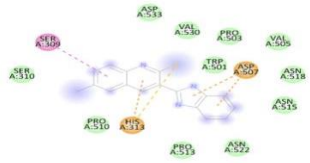
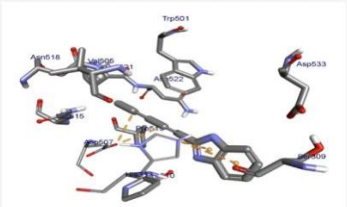
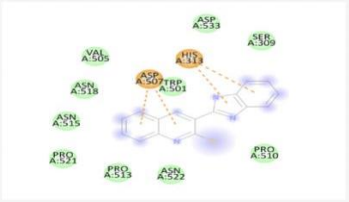
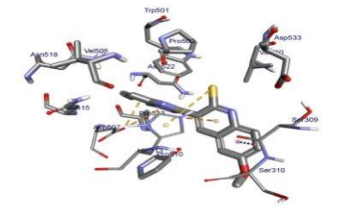
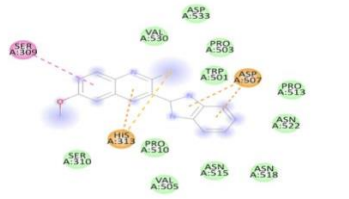
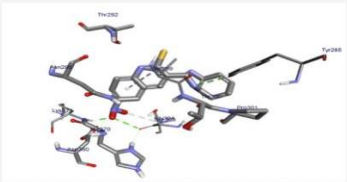
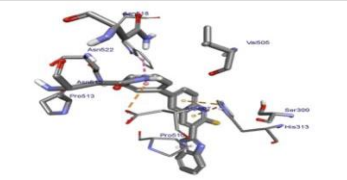
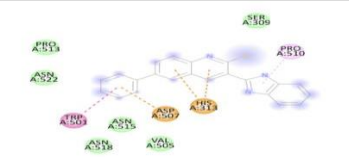
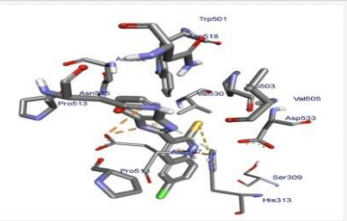
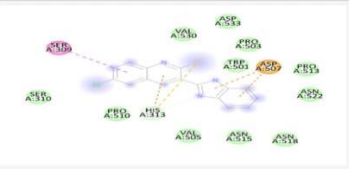
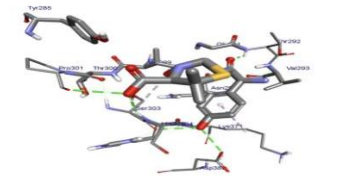
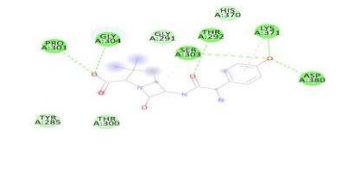
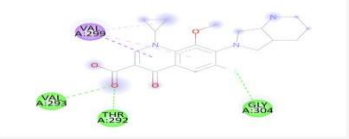


FIGURE 3: Ligand preparation

TABLE 1: Showing analysis and interaction of NV proteins and the control group

COMOUND	ANALYSIS	INTERACTIONS
NV1		
NV2		
NV3		
NV4		
NV5		
NV6		
AMOXICILLIN		
MOXIFLOXACIN		

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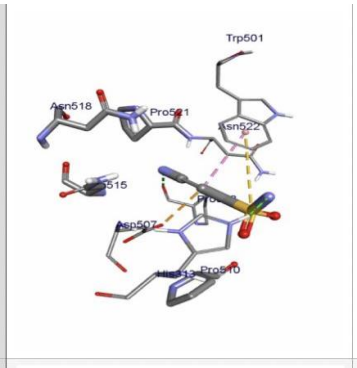
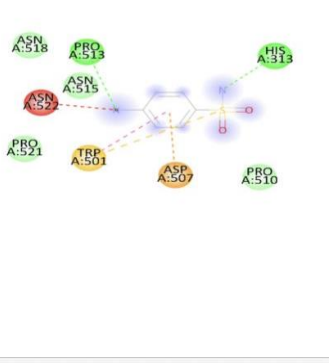
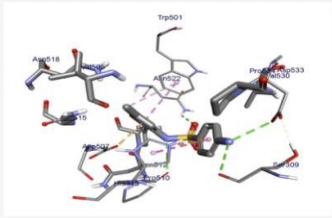
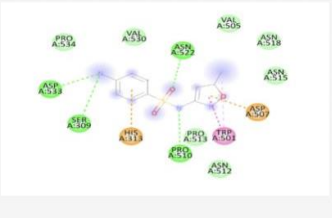
SULFANIMIDE		
SULFAMETHOXAZOLE		

TABLE 2: Molecular docking scores and residual amino acid interactions of Imidazole Quinoline compounds (NV1-NV6) against Minor fimbrium subunit Mfa1 of *Porphyromonas gingivalis* (PDB ID 5NF2).

Ligands	**Docking scores/Affinity (kcal/mol)**	**H-bond**	**Amino Acid Residual interactions**	
			Hydrophobic/Pi-Cation	**Van dar Waals**
NV1	-7.5		HIS-313, ASP-507, SER-309	ASP-533, VAL-530, PRO-503, VAL-505, ASN-518, ASN-515, TRP-501, ASN-522, PRO-513, PRO-510, SER-310
NV2	-8.1		HIS-313, ASP-507	ASP- 533, SER-309, PRO-510, ASN-522, PRO-513, PRO-521, ASN-515, ASN-518, VAL-505
NV3	-7.3		SER- 309, ASP- 507, HIS-313	VAL-530, ASP-533, PRO-503, TRP-501, PRO-513, ASN-522, ASN-518, ASN-515, PRO-510, VAL-505, SER-310
NV4	-6.2	LYS-371, SER-303, TYR-285	VAL-299	HIS-370, ASN-296, ASP-380, GLY-304
NV5	-7.7		TRP-501, PRO-510, HIS-313, ASP-507	PRO-513, ASN-522, SER-309, ASN-515, VAL-505, ASN-518

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NV6	-7.5	HIS- 313	ASP-507, SER-309	VAL-530, ASP-533, PRO-503, TRP-501, PRO-513, ASN-522, ASN-518, ASN-515, VAL-505, PRO-510, SER-310
Amoxicillin	-6.3	PRO-301, GLY-304, SER-303, THR-292, LYS-371, ASP-380		GLY-291, HIS-370, THR-300, TYR-285
Moxiflaxcin	-5.8	VAL- 293, THR-292, GLY-304	VAL-299	
Sulfanilamide	-5.7	PRO-513, HIS-313	ASN-522, TRP-501, ASP-507	ASN-518, ASN-515, PRO-521, PRO-510
Sulfamethoxazole	-6.8	ASP-533, SER-309, ASN-522, PRO-510	HIS-313, ASP-507, TRP-501	PRO-534, VAL-530, VAL-505, ASN-518, ASN-515, PRO-513, ASN-512

TABLE 3,4: Showing ADME predictions of isolated compounds, computed by SwissADME

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		
NV1	291.37	2.34	1	2	1	89.05	80.37	0	0.55
NV2	277.34	1.92	1	2	1	84.08	80.37	0	0.55
NV3	307.37	2.35	1	3	2	90.57	89.6	0	0.55
NV4	322.34	1.8	1	4	2	92.9	126.19	0	0.55
NV5	353.44	2.75	1	2	2	109.52	80.37	1	0.55
NV6	311.79	2.22	1	2	1	89.09	80.37	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**
NV1	-5.05	High	No	Yes	Yes	Yes	Yes	Yes	Yes
NV2	-5.22	High	No	Yes	Yes	Yes	No	Yes	Yes
NV3	-5.43	High	No	Yes	Yes	Yes	Yes	Yes	Yes
NV4	-5.62	High	No	No	Yes	Yes	Yes	Yes	Yes
NV5	-4.53	High	No	Yes	Yes	Yes	No	No	Yes
NV6	-4.99	High	No	Yes	Yes	Yes	Yes	No	Yes
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 5: Showing prediction of toxicity of synthesised compounds computed by Protox

Compound			Toxicity						
	[a]LD_{50} (mg/kg)	Class	HEPATOTOXICITY	CARCINOGENICITY	IMMUNOTOXICITY	MUTAGENICITY	CYTOTOXICITY		
NV1	1190	4	Active	Inactive	Active	Inactive	Inactive		
NV2	100	3	Active	Inactive	Inactive	Active	Inactive		
NV3	3000	5	Active	Active	Inactive	Active	Inactive		
NV4	73	3	Active	Active	Inactive	Active	Inactive		
NV5	100	3	Active	Inactive	Inactive	Active	Inactive		
NV6	3710	5	Active	Inactive	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

DISCUSSION

One of the most crucial virtual screening techniques to research drug-receptor interaction, molecular modelling is essential to computer-aided drug designing. Finding a ligand that is suitable and fits the protein's binding site energetically and geometrically is known as docking. In the current investigation, the relationship between the mfa1 protein of porphyromonas gingivalis and oxazole compounds were examined.

The compounds were docked into the binding region of the protein in order to analyse the interactions and binding affinities between the synthetic chemicals and bacterial proteins in a 3D environment. The proteins and compounds were docked into the active sites of the proteins using AutoDock Vina and our previously published technique. Using the Chem Office tool (ChemDraw) and the appropriate orientation, the chemical structures of the compounds were created. ChemBio3D was then used to minimise the energy of each molecule. The ligand molecules with the lowest energy were then sent into AutoDock Vina to complete the docking process. From the protein data library, the crystal structure of the receptor molecules for the oxazole binding protein 5NF2 was retrieved. The target protein was generated by leaving the related residue with protein utilising Auto preparation of target protein file Auto Dock 4.2 after the co-crystallized ligand was removed, water molecules were deleted, polar hydrogens, and cofactors were added. The grid box for the docking simulation was configured using the graphical user interface application. A grid was employed to encircle the macromolecule's region of interest. With the help of the docking method offered by Auto Dock Vina, the optimum docked configuration between the chemicals and the protein was investigated. The most favourable confirmations with the lowest free binding energy were chosen by Discovery Studio Visualizer to analyse the interactions between the target receptor and ligands. (7)

In the present study we have elucidated the molecular docking interaction between the synthesised oxazole compounds and virulence factor Mfa1 from P. ginigivalis. We compared theit affinity with the interaction noted with clinically available drugs. The synthesized compounds NV1-NV6 were found to have minimum binding energy ranging from -6.2 To -8.1 kcal/mol while the docking affinity of control drugs ranged from -5.7 to -6.8 kcal/mol (Table 2). The oxazole compound with highest docking affinity was NV4 (-6.2 kcal/mol), which was very similar to the docking affinity noted with Amoxicillin (-6.3 kcal/mol). The binding affinity, H-bond and residual interaction of synthesised compounds and clinical drugs are summarised in Table 2. On the comparison of the similarity of amino acid residual interactions between the compounds and the control drugs, maximal similarity was noted between NV 1, NV3, NV5, NV6 and Sulfamethoxazole and Sulfanilamide. (8)

The SwissADME prediction results indicate that all the compounds satisfy Lipinski’s rule of 5,

except NV5 with 1 violation. The Kp values of all molecules are within the ranged of -5.62 to -4.53 cm/s inferring low skin permeability. The SwissADME prediction parameters showed that all the compounds have high gastrointestinal absorption and none of them show blood brain barrier permeability. These predictions show that they can be active pharmacological agents. A range of cytochromes (CYP's) regulates the drug metabolism, particularly the biotransformation of drug molecules are regulated by CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4. The prediction results exhibits that all the compounds except the control drugs are found to be potential inhibitors of CYP1A2, CYP2C19 and CYP3A4.

The in-silico prediction results of absorption, distribution, metabolism and excretion (ADME) for isolated compounds and control drugs was done. The acute toxicity prediction results such as toxicity classification and LD50 values indicate that none of the compounds has shown acute toxicity. It gives us results of hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity and cytotoxicity. All the compounds were found to show hepatoxicity. Compounds 3 and 4 were found to be carcinogenic, compounnd 1 was found to be immunotoxic. All compounds except 1 and 6 were found to be mutagenic. Neither of the compounds exhibited cytotoxicity. Hence, based on ADMET prediction analysis, the compound 6 may be a potential drug candidate in the investigation.

Our team has extensive knowledge and research experience that has translate into high quality publications ((9–16))

Because the oral cavity is a good environment for a variety of bacteria to colonise, oral flora needs a wide range of defence mechanisms to avoid infection. Since red complex bacteria are significant periodontal pathogens, a great deal of research is being done to understand the disease's etiology and methods to reduce their virulence. Understanding the virulence factors and mechanisms of these periopathogens will enable us to map and develop efficient drugs to control them. Socransky, S. S., & Haffajee, A. D. (1994).

CONCLUSION

After comparing all the ligands with each other, we can conclude that NV6 could be a potential drug inhibitor against Mfa1 in P. gingivalis in periodontitis, owing to its high LD50, inactive carcinogenic, mutagenic and immunogenic effect. It also had high hydrogen bond forming capacity.

One of the key elements of the host's natural innate immunity is oxazole compounds. In this study, molecular docking revealed that oxazole compounds interacted with different periodontitis virulence factors, suggesting that it might be used as an adjunct or alternative to antibiotics for the treatment of periodontitis. Using a variety of web resources, it was discovered that oxazole compounds are non-toxic. It has a lot of potential use in the drug development process and might be a good option for the treatment of periodontitis.

Author Contributions

Dr. Parkavi: Review and Editing, Supervision, Validation, Methodology.
Navya Khanna: Original draft preparation, Data curation, Investigation.
Dr. Rajalakshmanan : Conceptualization, Software, Formal Analysis

CONFLICT OF INTEREST

The author assert that there is no conflict of interest

ACKNOWLEDGMENT

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Future Scope

The molecule has to be developed for future research. Compounds with functional groups similar to the lead molecules have to be explored.

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2. Saveetha Institute of Medical and Technical Sciences
3. Saveetha University
4. Deepak Bearing Industries, New Delhi

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