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RESEARCH ARTICLE
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Evaluation of antibacterial potential of oxazole derivative compounds against Mirolysin toxin of Tannerella forsythia using In silico molecular docking and Admet prediction

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ABSTRACT

Introduction: mirolysin is a metalloproteinase secreted by Tannerella forsythia which is associated with periodontitis. Mirolysin inhibits the classical and lectin complement pathways. contribute to excessive and sustained inflammation at the site of infection. In this study we are analyzing the antimicrobial potential of oxazole compounds against the Mirolysin toxin of T. forsythia via insilico targeting.

Materials and Methods: 7 oxazole ligands were fabricated using Chem-Draw and Chem-3D software. The structure of the receptor molecule Mirolysin was downloaded from the protein databank. The preparation of the Mirolysin protein of T. forsythia was done using Biovia discovery studio. The ligand-protein interaction was assessed via Auto-Doc Vina. The data was the input into SwissADME and PROTOX softwares to assess their efficiency, potential side effects and toxicity.

Results: The docking score of all 7 prepared drugs shows better affinity than the control groups indicating increased efficacy of the drugs. VD2, VD4, VD5, VD6, VD7 show good GI absorption. The toxicity class of all drugs were 4 and based on the hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity and cytotoxicity, it can be seen that VD2, VD5 and VD7 are relatively safer groups of drugs.

Conclusion: Based on the toxicity levels and properties of the drugs VD2, VD5 and VD7 are potential drug candidates for further development. The prepared drugs showed better properties when compared to the clinically available compounds. Thus, further development of the lead molecules will aid in better treatment regimen.

Keywords: *In silico analysis, Molecular Docking, Periodontitis, Drug designing, Mirolysin, Tanerella Forsythia*

INTRODUCTION

Drug designing and development is a fundamental part of the biomedical and pharmaceutical industry. Potential target identification for a known bioactive compound plays a major role in the process(1). The main cause for failure of drugs to obtain approval for entering the market and clinical practices is due to the severe side effects and cross reactivity with other medications that are usually observed in the later stages of clinical trials(2). Approaches based on mRNA expression and protein affinity isolation and mass spectrometric analysis are some conventional methods usually followed for identifying the potential targets for the drug. However it is not cost and time effective and requires a lot of resources. Thus, Insilico targeting is considered a cheaper and more accessible alternative for target identification and for studying the effects of the potential drug(3). It aids in the identification of the mechanism of action of the drug or bioactive molecule by prediction of the potential interaction between the drug and target. It also helps in the assessment of the adverse effects, evaluation of any secondary reactions and in cases where there is beneficial secondary reaction, can be used for drug repurposing applications(4). The pharmacological parameters generally noted include drug-likeness, toxicity, absorption, distribution, metabolism and excretion(5). There are 2 methods of targeting, namely, receptor based and ligand based methods. Ligand based techniques are used when the bioactive compound shows similarity to an existing compound or molecule.

It has more flexibility and lesser computational requirements. Receptor based methods can aid in predicting the effects of undiscovered compounds. Generally ligand based approach is combined with receptor based approach for identification of new targets and biological activities for the query compounds(6). In this way, molecular docking analysis and online ADMET assessments including Pro-Tox II, SwissADME and OSIRIS property explorer give a more cost effective and accessible direction to researchers worldwide(7). DFT analysis helps in optimizing the geometry and aids in identification of the role of charge distribution in the development of potential drug candidates(8).

Periodontitis is the chronic inflammation of the periodontium surrounding the tooth that involves the association between periodontal tissues, bacterial populations, inflammatory mediators and immune response of the host. The pathway and pathogenesis of periodontitis is associated with a dysregulated host inflammatory-immune response to intra-oral plaque bacteria(9). The periopathogenic bacteria express various virulence factors and cellular components that initiate the host response. The most pathogenic and virulent bacteria involved in the later stages of periodontitis are the red-complex bacteria which include Treponema denticola, Porphyromonas gingivalis and Tannerella forsythia. These bacteria also produce endotoxins that can lead to systemic conditions such as respiratory tract disorders, vascular diseases, adverse pregnancy outcomes, diabetic complications.

Tannerella forsythia of the family Cytophaga-Bacteroides is an gram negative bacteria which is usually found in the subgingival region in the oral cavity(10). T. forsythia can be said to be a major contributor of periodontitis due to its unique glycosylated S-layer that tends to promote the adherence of the bacteria to the gingival cell surfaces of the host(11). The S-layer also causes attenuation of the host immune response thus leading to increased severity and destruction of bone(12). However, even though there has been many evidences supporting the association of the bacterium with periodontitis, there has not been much studies done in this field. This is majorly due to the difficult and fastidious requirements for growth and culture of the bacterium. Genetic manipulations are also found to be difficult to perform and no gene complementation systems are currently available for T.forsythia(13).

Mirolysin, a metalloprotease released by T. forsythia, is a member of the M43 family and of the subfamily M43B. It was later renamed to LysargiNase due to its specificity(14). Mirolysin has shown to contribute to excessive and sustained inflammation at the site of infection(15). It has also shown inhibitory activity against LL-37 which is a cathelicidin-derived antimicrobial peptide found in humans. LL-37 is found to play an important role in the maintenance of homeostasis in the periodontium by preventing the activation of Factor C by lipopolysaccharides, a component of the outer membrane of Gram-negative bacteria(16). Factor C is an endotoxin-responsive, intracellular serine protease zymogen that initiates the coagulation cascade system . In a study by Koneru et al., it was observed that mirolysin cleaved LL-37 and abolished the ability of LL-37 to neutralize the lipopolysaccharide(17). Thus, mirolysin could interfere with clotting at the site of the infection that is likely to cause the abundant proteins in the gingival crevicular fluid which generates a pool of peptides that is required for the growth of saccharolytic bacteria.

Since LL-37 possesses immunoregulatory properties that are regulated by binding and neutralizing the proinflammatory property of lipopolysaccharides or endotoxins, its degradation by mirolysin can result in prolonged inflammation at the site of infection resulting in aggravation of the disease(17,18).

Oxazoles are a doubly unsaturated 5-membered ring having one oxygen atom at position 1 and a nitrogen at position 3 separated by a carbon in-between(19). Many researches have been conducted on the antimicrobial activities of oxazole compounds. Oxazoles and its derivatives are a part of number of medicinal compounds which includes aleglitazar (antidiabetic), ditazole (platelets aggregation inhibitor), mubritinib (tyrosine kinase inhibitor), oxaprozin (COX-2 inhibitor) and so on(20). In this study we are analyzing the antimicrobial potential of oxazole compounds against the Mirolysin toxin of T. forsythia via insilico targeting. Our team has extensive knowledge and research experience that has translate into high quality publications (21–30)

MATERIALS AND METHOD

In-silico molecular docking methodology

Ligand preparation

Chen Draw 16.0 was used to draw and analyse the 2D structures of the synthesised compounds VD 1- VD 7. Optimisation procedure was done with parameters set in order to obtain a stable and minimal energy structure. The structure optimisation procedure was used to determine the minimum energy of the compound and the 3D coordinates were obtained.

Preparation of protein and Auto Dock Vina analysis

The structure of the receptor molecule Mirolysin was downloaded from the protein databank. Auto preparation of target protein file Auto Dock 4.2.6 was used to detach previously attached ligands and polar hydrogens were added to prepare the protein. Docking simulations were done using the interface by setting up grid boxes. Auto Dock Vina provided the docking algorithm to search for the best conformation between protein and ligand. Discovery studio visualiser was then used to analyse the interactions between target protein and ligands.

In-silico drug-likeness and toxicity predictions

The efficacy and likeliness of the prepared ligands were assessed against 4 antimicrobial drugs available in the market- amoxicillin, moxifloxacin, sulfanilamide and sulfamethoxazole. This is based on the lipinski's rule of 5 by Lipinksy et al., that states that the molecular weight should be ≥500g/mol, the iLogP value (lipophilicity) value should be ≥5, the hydrogen bond donors should be ≥5, hydrogen bond acceptors should be ≥10 and nrotb value should be ≥10. The ligands were transformed to their simplified molecular formula using the simplified molecular input line entry system or SMILE and the insilico pharmacokinetic parameters were estimated using the SwissADME software. The toxicity levels and LD50 were predicted using PROTOX II software.

Physical and simplified molecular data of synthesised oxazole compounds:

VD1-
FC1=CC=CC=C1C(O4)=NC(C5=CC=C(OC)C(OC)=C5OC)=C4SC3=NC2=CC=CC=C2S3

VD2-
BrC1=CC(C2=C(C4=CC=C(OCO5)C5=C4)N=C(C3CCN(C(C)=O)CC3)O2)=CC=C1

VD3 -
COC1=CC=C(C2=C(SC3=NN=C(C)S3)OC(C4=CC=CC=C4F)=N2)C(OC)=C1OC

VD4 -
COC1=CC=C(C2=COC(N3N=C(C5=CC=CC=C5)CC3C4=CC=CC=C4)=N2)C=C1

VD5 -
ClC1=CC(C2=C(C4=CC=C(OCO5)C5=C4)N=C(C3CCN(C(C)=O)CC3)O2)=CC=C1

VD6 -
COC1=CC=C(C2=C(SC3=NC=CC=N3)OC(C4=CC=CN=C4)=N2)C(OC)=C1OC

VD7 -
CC1=CC(C2=C(C4=CC=C(OCO5)C5=C4)N=C(C3CCN(C(C)=O)CC3)O2)=CC=C1

RESULTS

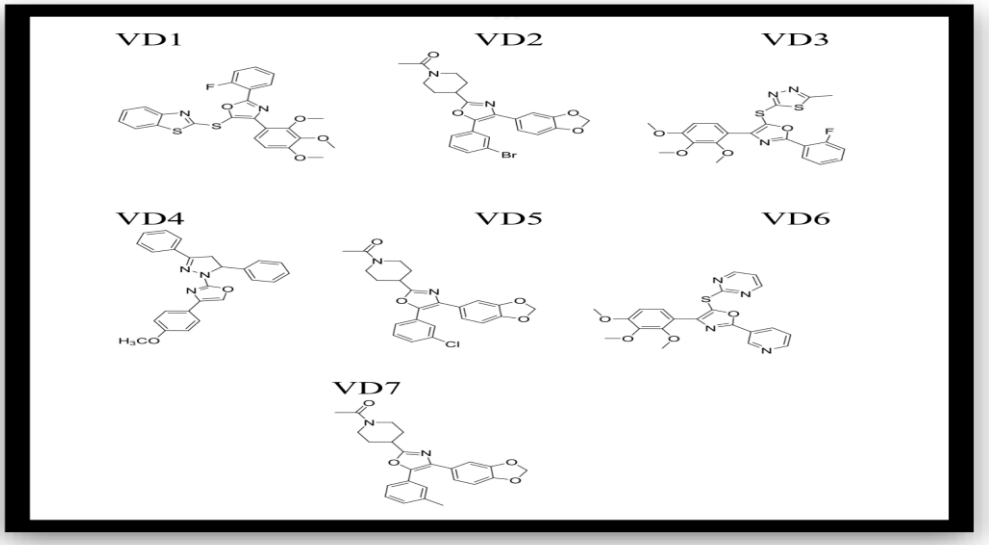


FIGURE 1: Fabricated oxazole compounds

TABLE 1: molecular docking scores and residual amino acid interactions of oxazole compounds vs Mirolysin protein

Table 3. Molecular docking scores and residual amino acid interactions of Oxazole compounds (VD1-VD7) against Mirolysin protein of Tenneralla forsythia (PDB ID 7OD0).

Ligands	Docking scores/Affinity (kcal/mol)	H-bond	Amino Acid Residual interactions	
			Hydrophobic/Pi-Cation	Van dar Waals
VD1	-8.9	TYR-258	HIS-234, GLU-225, TYR-286, HIS-224, MET-292, LEU-181	GLU-260, TYR-216, LEU-180, GLY-182, THR-221, ARG-220, THR-287
VD2	-10.7	-	TYR-286, TYR-258, LEU-181	ASP-289, ARG-220, MET-292, THR-287, THR-221, GLY-182, ALA-184, HIS-234
VD3	-8.4	-	GLU-260, TYR-286, LEU-181	TYR-258, THR-287, ASP-289, MET-292, ARG-220, THR-221, GLU-225, GLY-182
VD4	-8.7	ALA-184, TYR-286	TYR-258, LEU-180, PHE-186, TYR-183, HIS-228, LEU-181	GLU-260, TYR-216, MET-147, THR-221, GLU-225, HIS-224
VD5	-10.6	-	LEU-181, TYR-258, TYR-286	ASP-289, ARG-220, THR-221, GLU-225, ALA-184, HIS-234, GLY-182, THR-287, TYR-216, ASP-179
VD6	-8.3	-	HIS-228, TYR-286, LEU-181, TYR-216, HIS-224, ASP-289, ASP-285	TYR-183, ALA-184, MET-147, LEU-180, GLY-182, HIS-234, MET-292
VD7	-10.5	-	LEU-181, TYR-286, TYR-258	-

TABLE 2: molecular docking scores and residual amino acid interactions of control drugs vs Mirolysin protein

Amoxicillin	-8.1	LEU-181, GLU-225, ASP-289, THR-287	HIS-225	TYR-258, GLU-260, GLY-182, TYR-216, MET-292, THR-224
Moxiflaxcin	-8.3	GLU-260, GLY-182, HIS-224, HIS-234, HIS-228	TYR-183, TYR-286, LEU-181	ALA-184, GLU-225, THR-221, TYR-216
Sulfanilamide	-6.2	ASP-289, THR-287, HIS-224, GLU-225, GLY-182	LEU-181	MET-292, THR-221, HIS-234
Sulfamethoxazole	-7.8	GLY-182, HIS-228, HIS-234, TYR-286, LEU-181, THR-287	GLU-225, TYR-216	LEU-180

TABLE 3: Drug-likeness 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		
VD1	494.56	4.83	0	7	7	130.55	120.15	0	0.55
VD2	469.33	4.22	0	5	4	119.84	64.8	0	0.55
VD3	459.51	4.08	0	8	7	115.81	133.04	0	0.55
VD4	395.45	3.96	0	4	5	124.25	50.86	0	0.55
VD5	424.88	4.09	0	5	4	117.15	64.8	0	0.55
VD6	422.46	3.61	0	8	7	110.8	117.69	0	0.55
VD7	404.46	4.19	0	5	4	117.11	64.8	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
VD1	-4.44	Low	No	Yes	No	No	Yes	Yes	No
VD2	-6.15	High	Yes	Yes	Yes	Yes	Yes	No	Yes
VD3	-5.37	Low	No	No	Yes	No	Yes	Yes	Yes
VD4	-4.88	High	Yes	Yes	Yes	Yes	Yes	No	Yes
VD5	-5.92	High	Yes	Yes	Yes	Yes	Yes	No	Yes
VD6	-6.38	High	No	No	Yes	No	Yes	Yes	Yes
VD7	-5.99	High	Yes	Yes	Yes	Yes	Yes	Yes	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 4: Predication of toxicity of synthesized compound computed by Pro-Tox software.

Compound	Toxicity						
	[a]LD_{50} (mg/kg)	Class	HEPATOTOXICITY	CARCINOGENICITY	IMMUNOTOXICITY	MUTAGENICITY	CYTOTOXICITY
VD1	800	4	Active	Inactive	Active	Inactive	Inactive
VD2	1600	4	Inactive	Inactive	Active	Inactive	Inactive
VD3	1000	4	Active	Inactive	Active	Inactive	Inactive
VD4	1000	4	Active	Active	Inactive	Active	Inactive
VD5	1600	4	Inactive	Inactive	Active	Inactive	Inactive
VD6	800	4	Active	Active	Active	Inactive	Inactive
VD7	1600	4	Inactive	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

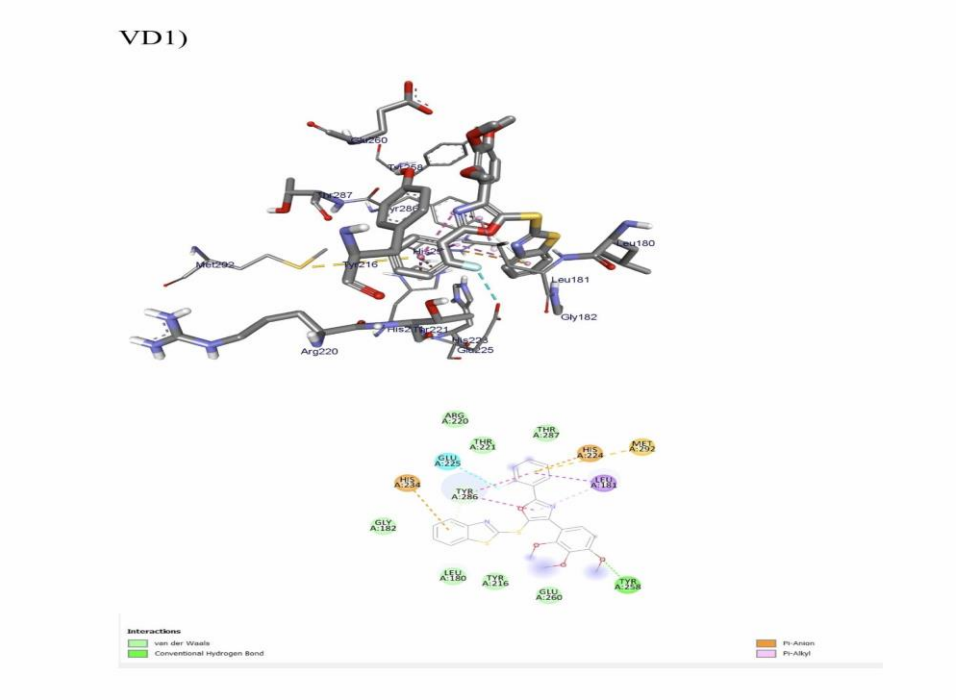
DISCUSSION

In our study, 7 oxazole ligands were fabricated using Chem-Draw and Chem-3D software (figure 1). The preparation of the Mirolysin protein of T. forsythia was done using Biovia discovery studio. The ligand-protein interaction was assessed via Auto-Doc Vina. The data was then input into SwissADME and PROTOX softwares to assess their efficiency, potential side effects and toxicity.

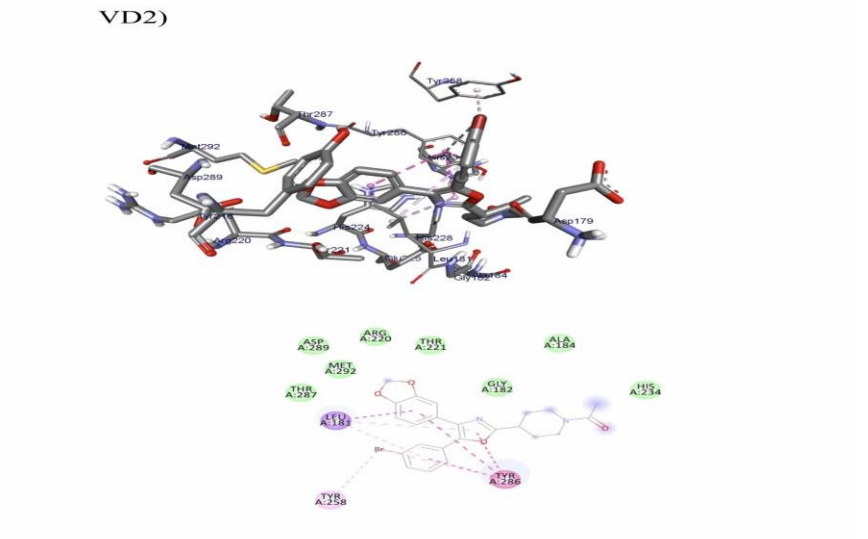
In silico molecular docking scores showed the high affinity of the synthesised drugs (VD1 to VD7) to the protein Mirolysin when compared to the commercially available controls amoxicillin, moxifloxacin, sulfanilamide and sulfamethoxazole.. The binding affinity of the synthesised drugs ranged between -10.07 to-8.3 kcal/mol with highest docking affinity scores expressed by VD2(-10.7kcal/mol), VD5 (-10.6 kcal/mol) and VD7 (-10.5kcal/mol).

On assessment of the ligands using the SwissADME software, they were found to fulfil all the Lipinsky's rules and no violation was to be found (Table 3). The logKp values which indicate the skin permeability of the drug were in the range of –4.44 to –6.38 cm/s showing high permeability levels. IlogP value denotes the lipophilicity of a drug. The synthesised drug ligands show high lipophilicity in the range of 3.61 to 4.83. SwissADME analysis shows that VD2, VD4, VD5, VD6, VD7 exhibit good GI absorption and VD2, VD4, VD5 and VD7 show blood brain barrier permeability which may or may not be preferable in certain cases.

The metabolism and bio transformation of the drug is regulated by cytochromes. Based on the results it can be seen that CYP1A2 and CYP3A4 are inhibited by all synthesised compounds except VD1, CYP2CI9 is inhibited by VD2, VD4, VD5 and VD7, CYP2C9 is inhibited by all compounds and CYP2D6 is inhibited by VD1, VD3, VD6 and VD7. The toxicity prediction places all the synthesised drugs in class 4 level and the LD50 levels are lower than the commercially available drugs (Table 4). Based on the organ toxicities it can be seen that VD2, VD5 and VD7 are relatively safer groups of drugs and may be potential drug candidates for further investigation.

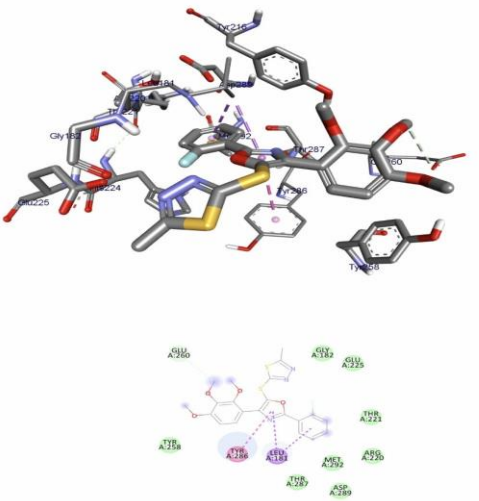


Compound 1



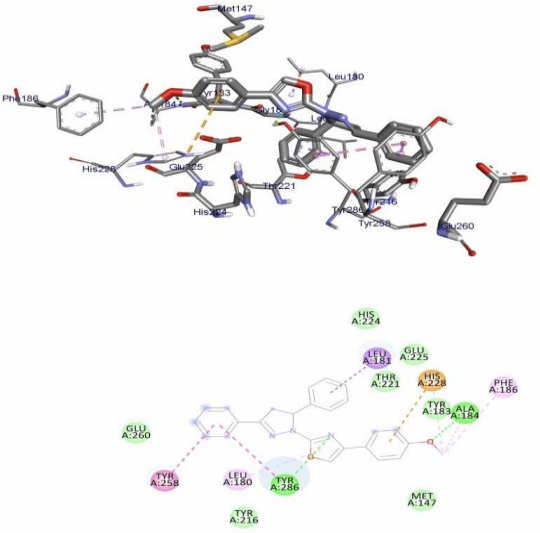
Compound 2

VD3)



Compound 3

VD4)



Compound 4

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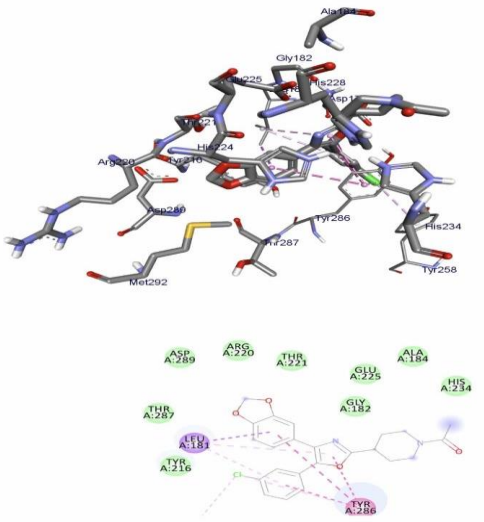
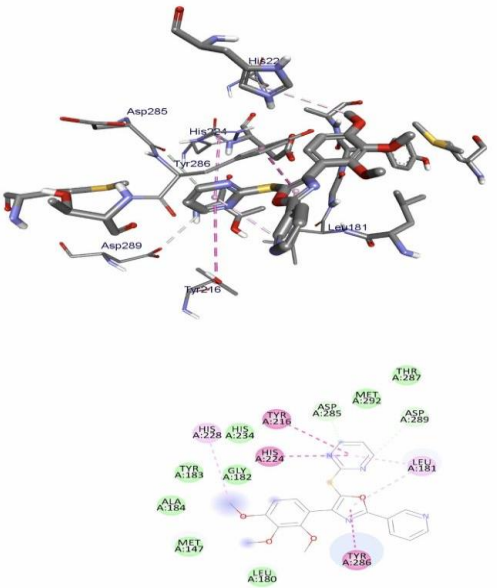
VD5)

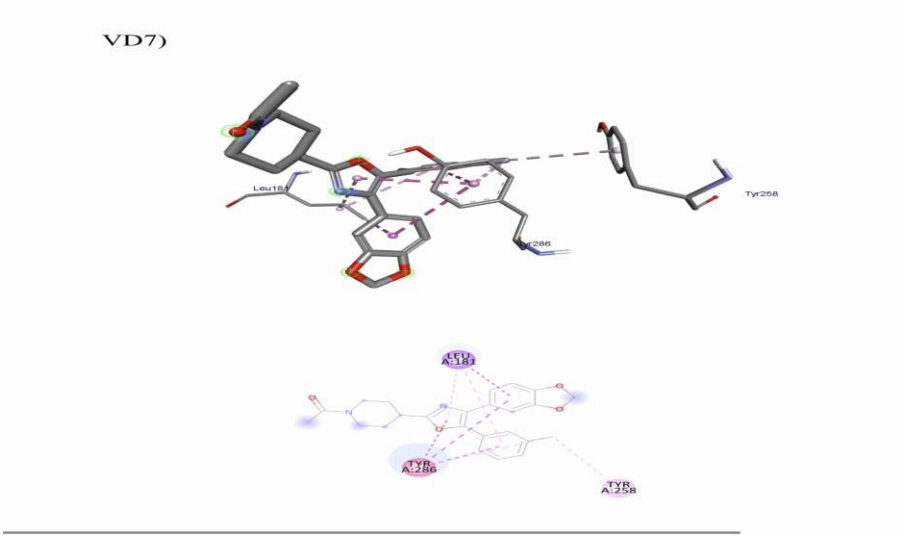


Compound 5

VD6)



Compound 6



Compound 7

CONCLUSION

The prepared drugs showed better binding affinity with the protein in the in silico approach when compared to the clinically available compounds. All synthesized drugs exhibited promising docking efficiency with Mirolysin with VD2, VD5 and VD5 as the best among them. Based on the toxicity levels and properties of the drugs, VD1, VD2, VD5 and VD7 are potential antimicrobial drug candidates for further development. The limitations of this research are that it is only a computational analysis and further invivo and invitro studies have to be performed inorder to get more accurate results. Thus, further development of the lead molecules will aid in better treatment regimen.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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