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Original Article

IN SILICO PREDICTION OF SOME INDOLE DERIVATIVES AGAINST VIRB8 FROM *BRUCELLA SUIS* AND CYANOBACTERIAL MEMBRANE-BOUND MANGANESE SUPEROXIDE DISMUTASE

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ABSTRACT

This study investigates green chemistry methods to design the indole derivatives with potential medicinal properties. When benzaldehyde compounds react with phenyl hydrazine and ortho-phosphoric acid in an acidic environment, synthesized the indole derivatives. There are two proteins chosen from the protein data bank: the crystal structure of VirB8 from *Brucella suis* (PDB ID: 2BHM) for evaluation of antibacterial potential and a type of manganese superoxide dismutase (MnSOD) from cyanobacteria (PDB ID: 1GV3) for antioxidant potential. The molecule 3g had the best docking score of -10.4 with the crystal structure of VirB8 from *Brucella suis* (PDB ID 2BHM), which means it has a strong ability to bind to the target receptor. The molecule 3j had the best docking score of -10.4 against manganese superoxide dismutase (MnSOD) in the membrane of cyanobacteria (PDB ID: 1GV3), showing that it strongly attaches to the target receptor. The result suggests that it has a strong affinity for binding to the target receptor. This result indicates that it has a strong affinity for the target receptor. The metronidazole is taken as a standard for comparison purposes and has a molecular docking score of -6.5 against the crystal structure of VirB8 from *Brucella suis*. Ascorbic acid is used as a standard. It has a molecular docking score of -5.7 against manganese superoxide dismutase (MnSOD), which is attached to the membrane of cyanobacteria. The remaining design molecules demonstrated excellent docking scores for both proteins when compared to the standard molecules, metronidazole and ascorbic acid. In this study, ProtTox II Software was used to predict the *in silico* toxicity of all the design molecules. It was found that molecules 3l and metronidazole were respiratory toxic, while compounds 3g, 3k, 3p, and 3r were neurotoxic. All designed molecules were screened for the drug-likeness property and obeyed the criteria of the Lipinski rule of five.

KEYWORDS: Indole, Virb8, Cyanobacterial, Antioxidant, Antimicrobial, CB DOCK-II.

1. Introduction

The pathogenic bacterium *Brucella suis* causes brucellosis, a zoonotic disease that puts humans and animals at significant risk [1]. Bacteria protect themselves in host species through the complicated VirB system for type IV secretion. This VirB system for type IV secretion is a good target for the development of new drugs because it is so important for *Brucella species* to move around inside host cells [2]. Nonetheless, the limited availability of medications and the potential for illness resistance complicate the development of effective therapies [3]. Cyanobacteria, known for their resilience to extreme environmental conditions, rely on the enzyme manganese superoxide dismutase (MnSOD) to manage oxidative stress [4]. Manganese superoxide dismutase (MnSOD) dismutates superoxide radicals, thereby shielding cells from oxidative damage. Because it is so important for keeping cells healthy, MnSOD has become a promising target for treating diseases linked to oxidative stress and microbial infections [5]. The medicinal potential of indole

derivatives has garnered considerable attention. This class of compounds is renowned for its extensive array of biological actions [6]. These compounds are promising prospects for therapeutic development due to their diverse pharmacological properties, including antibacterial, antioxidant, and anticancer actions. Indole derivatives can block the activity of cyanobacterial MnSOD and *Brucella suis* VirB8 because they can connect with various biological targets. Computer-based methods have changed how drugs are developed by allowing scientists to predict how chemicals will interact and find promising compounds before testing them in the lab. Researchers can use computer methods like molecular docking, molecular dynamics simulations, and virtual screening to study how well indole derivatives attach to specific target proteins, such as VirB8 and MnSOD. The identification of suitable candidates for further biological testing can be significantly expedited by these *in silico* predictions. This study aims to see how effective indole derivatives are as MnSOD inhibitors in cyanobacteria and VirB8 inhibitors in *Brucella suis* by using computer simulations along with actual experiments. Our aim is to understand how these compounds can be used to treat important microbial targets and help developed new treatment methods for brucellosis and diseases related to oxidative stress by looking at how their structure affects their activity. Indole derivatives are a major and intriguing group of heterocyclic drugs; they are well known in synthetic organic chemistry as well as medicinal chemistry [12]. Additionally, indole derivatives are involved in the synthesis of neurotransmitters such as serotonin, playing a crucial role in mood regulation and cognitive function. Indole derivatives are useful in medicinal chemistry because they have many biological activities, such as being antioxidants, antimicrobials, and anticancer agents [13]. Fig. 1 represents the basic nucleus of the indole ring.

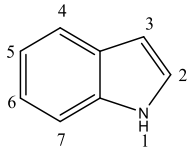

Fig. 1. Structure of indole

2. Methodology

2.1. Molecular Docking

2.1.1. The Protein Selection

There are two proteins selected for the molecular docking study. The first one is the crystal structure of VirB8 from *Brucella suis* (PDB ID: 2BHM), which is a bacterial protein selection [14]. Gram-negative bacteria commonly use Type IV secretion systems (T4SSs) as their secretion machinery. They infect human, animal, or plant cells and propagate antibiotic resistance. The second part is the structure of the antioxidant section of a manganese superoxide dismutase that is connected to the membrane of cyanobacteria (PDB ID: 1GV3). They are homodimer type 2 membrane proteins that protect this phototrophic organism against oxidative stress.

Metronidazole (Fig. 2) is an antibacterial drug that works well against illnesses caused by protozoa and anaerobic bacteria. A reference molecule is an important thing to think about when studying bacterial targets like *Brucella suis* VirB8. Because it interacts with bacterial proteins to damage DNA, it could be used as a standard to figure out how things will join. Metronidazole is easily taken, distributed, metabolized, and eliminated from the body. This makes it possible to compare how new indole derivatives work in the body and their docking scores. Ascorbic acid (Fig. 2) is a strong antioxidant that can be used to compare how well different chemicals work with the enzyme MnSOD, which helps protect cells from oxidative stress. Superoxide dismutase helps to get rid of harmful reactive oxygen species (ROS), which makes ascorbic acid an important molecule for understanding how antioxidants work. Ascorbic acid can help docking work better by making a clear pattern of binding and hydrogen bonding with enzyme sites that are involved in redox processes. Ascorbic acid works well with MnSOD's role to deal with oxidative stress, and metronidazole is good for the *Brucella* VirB8 protein because it helps fight germs. Both of the reference molecules can be used as starting points to compare the docking scores and interaction patterns of indole derivatives. By giving biological and pharmacological background, they help us understand how well the indole derivatives bind and how well they might work.

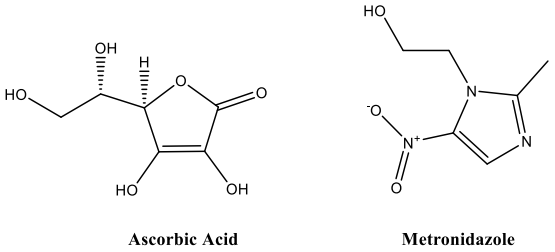

Fig. 2. Structure of molecules taken as standards

2.1.2. Ligand selection

An extensive literature review identified the recognized antimicrobial and antioxidant properties of the indole pharmacophore. This pharmacophore is notable for its versatility and significance in various applications. Because there are a lot of synthetic indole derivatives and compounds in the literature, this group was chosen for *in silico* studies [16, 17]. Furthermore, this research led to the development of additional novel molecules. All molecules were designed and drawn by using ChemBioDraw 14.0 software (Fig. 3).

2.1.3. Molecular docking

The molecular docking of designed indole compounds was done using CB-Dock II. The protein structures [PDB ID: 1GV3 and PDB ID: 2BHM] were taken from the Protein Data Bank, had unnecessary atoms and water taken out, and the spots where they could connect were found. The compounds structures were shown using ChemDraw software, the geometric configuration was optimized, and the structure was classified as a ligand. Ligand docking was performed by generating a receptor grid, and the resultant docking score was documented [18, 19].

2.2. *In silico* toxicity study

To develop safer and more environmentally sustainable products and chemicals, toxicity studies employ predictive toxicology. This technique reduces waste and exposure [20]. The organ toxicities of the ligands and their toxicological endpoints, including LD50 values, were

predicted utilizing the Protox-II program. Protox-II software can evaluate the hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity of compounds. The specified toxicity level was selected, the molecular structure drawn, and the results recorded [21, 22].

2.3. Drug Likeness/ ADME Properties

Optimizing the absorption, distribution, metabolism, and excretion variables is crucial in the setting of ADME. The body's metabolization of a drug influences not only its effectiveness but also its selectivity and effectiveness. The early stages of ADME predictions use a number of basic ADME or ADME-related metrics. These include intrinsic solubility (logS), partition coefficient (logP), and apparent partition coefficient (logD). Based on these characteristics, the ADME properties of a particular molecule are estimated using the Protox-II software. The Lipinski's rule of five is used to determine factors like molecular weight, logP, hydrogen bond donors, and acceptors [23, 24].

3. Results

When performing an *in silico* evaluations of antibacterial and antioxidant activities, indole ligands are usually chosen for this purpose. This selection process is based on a variety of established criteria and scientific facts. In order to conduct in vitro research, we chose indole ligands on the basis of their pharmacophoric qualities, proven biological activity, structural flexibility, and drug-likeness. When it comes to medicinal chemistry, indole is considered to be a favored scaffold, which indicates that it is frequently found as a substructure in a wide variety of biologically active compounds. Indole and its derivatives have been shown to possess a wide range of bioactivities, including antiviral, antibacterial, antioxidant, anticancer, and anti-inflammatory properties.

Additionally these molecules selected on the basis of their subsituent pattern as designed molecules contain additional aromatic ring like indomethacin molecule which is responsible for binding to the aromatic region at the receptor location.

The indole rings may be easily altered in a variety of locations, which enables designers to exercise their creativity while maintaining the important features of size, electrical conductivity, and fat-loving properties. Pharmaceuticals produced from indole have been the subject of substantial research both *in vitro* and *in vivo* conducted by researchers. *In vitro* studies include tests such as DPPH, ABTS, and FRAP to determine how well substances can fight against oxidation. Other tests include Minimum Inhibitory Concentration (MIC) to determine how effective they are against bacteria. Additionally, cytotoxicity tests (such as MTT) are performed on various types of cells, including animal cells and bacteria. The scope of *in vivo* research includes several different indole compounds have been shown to have protective effects in animal models of oxidative stress and infection. The prevalence of *in vivo* research continues to be lower than that of *in vitro* and *in silico* investigations. This drop is mostly due to the factors of increasing costs and regulatory restraints.

For the purpose of finding the possibility that it exhibits antibacterial properties, the crystal structure of VirB8 from *Brucella suis* (PDB ID: 2BHM) has been selected. The protein is chosen based on their classification, organism, expression system, and resolution. Each of these factors is taken into consideration. The bacterial protein known as VirB8 from *Brucella suis*, which has an expression mechanism that is similar to that of *Escherichia coli*, has a crystal structure that has been assigned the reference number 2BHM in the Protein Data Bank. Two and forty angstroms are the resolution of the protein. The structure of the catalytic region of a cyanobacterial membrane-bound manganese superoxide dismutase, which has a resolution of 2.0 Å and has the PDB ID of 1GV3, has been chosen for the purpose of evaluating the antioxidant capability using an *in silico* method. When selecting this protein, the same factors that were used to choose the antimicrobial protein were taken into consideration. The protein, which is a membrane-bound manganese superoxide dismutase that is found in cyanobacteria, belongs to the manganese superoxide dismutase class and originates from the organism *Nostoc sp.* PCC 7120 = FACHB-418. It is expressed using the *Escherichia coli* BL21 (DE3) expression system. There is a resolution of 2.00 Å for the protein here.

3.1. Molecular docking

Fig. 3 shows the indole-based molecules designed for molecular docking.

The designed indole analogues were analyzed, particularly focusing on their molecular docking score and interactions with a specific protein receptor. Molecule 3g, which is 2-(4'-bromo-[1,1'-biphenyl]-4-yl)-2-methylindoline, showed the highest docking score of -8.8 in the case of cyanobacterial membrane-bound manganese superoxide dismutase (MnSOD) (PDB ID: 1GV3), and the reference molecule taken for comparison is ascorbic acid, showing a molecular docking score of -5.7. This protein is taken for the evaluation of the antioxidant potential of all design molecules. The remaining molecules show the molecular docking score from the range -5.8 to -7.7 for the antioxidant potential. Table 1 represents the molecular docking score of designed indole molecules by CB Dock II software.

The molecule 3j, which is 2-(3-methoxyphenyl)-1H-indole against crystal structure of VirB8 from *Brucella suis* (PDB ID: 2BHM), shows the highest molecular docking score, which is -10.4; the reference molecule taken for comparison is metronidazole, showing a molecular docking score of -6.5. This protein is collected to evaluate the antimicrobial potential of all design molecules. The remaining molecules show a molecular docking score in the range of -6.3 to -9.9 for antimicrobial potential. Table 2 displays the binding sites of both proteins for their respective molecules. Table 2 shows the order of nearby amino acids for chosen design molecules with high docking scores and standard molecules used for molecular docking. The designed indole-based molecule 3g has a high docking score against cyanobacterial membrane-bound manganese superoxide dismutase (MnSOD) (PDB ID: 1GV3) and shows promise as an antioxidant compared to the standard molecule, ascorbic acid. Similarly, molecule 3j appears to be effective against bacteria when tested against the crystal structure of VirB8 from *Brucella suis* (PDB ID: 2BHM), much like how metronidazole is used as a standard treatment. Both molecules, 3g and 3j, have potential for further investigation on the basis of their primary *in silico*

investigation.

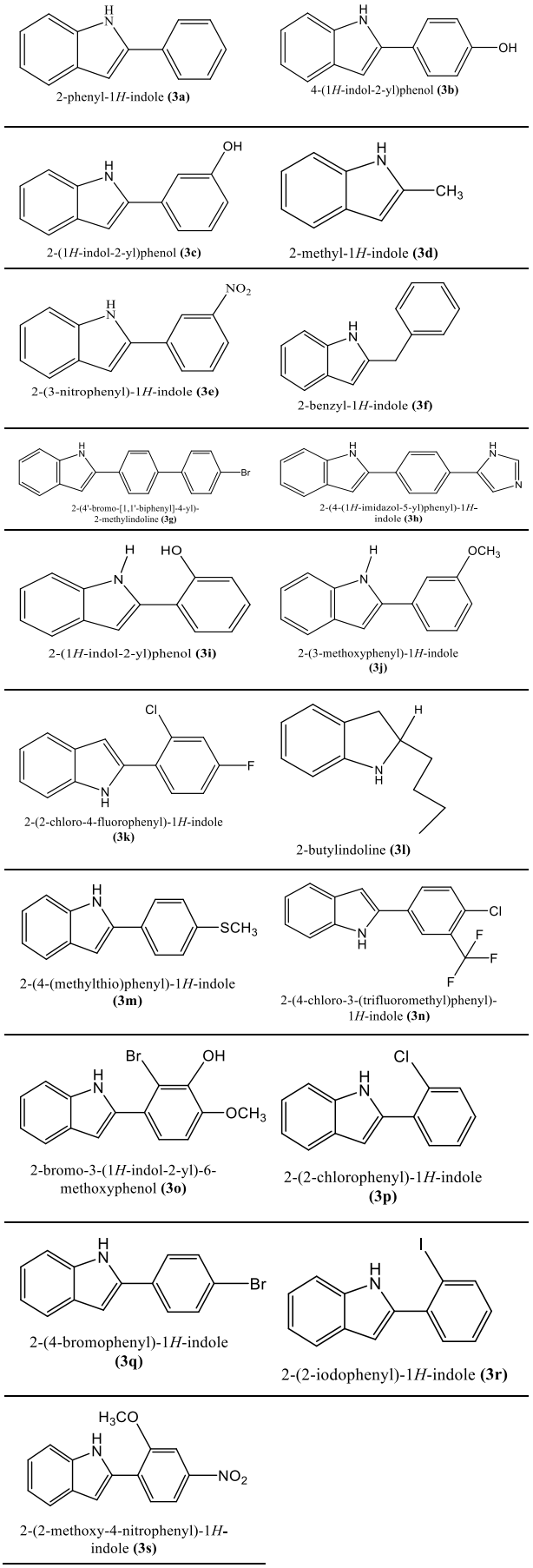

Fig. 3. Designed indole molecules for molecular docking.

Table 1. Molecular docking score of designed indole molecules by CB Dock II software

Sr. No	Molecule	Crystal structure of VirB8 from *Brucellasuis* (PDB ID: 2BHM)	Cyanobacterial membrane-bound manganese superoxide dismutase (MnSOD)(PDB ID:1GV3)
		Standard	
1.	Ascorbic acid	-	-5.7
2.	Metronidazole	-6.5	-
		Indole Derivatives	
1.	**3a**	-8.0	-6.1
2.	**3b**	-8.6	-6.7
3.	**3c**	-8.3	-6.8
4.	**3d**	-6.7	-5.8
5.	**3e**	-8.6	-7.4
6.	**3f**	-8.1	-6.9
7	**3g**	-9.6	**-8.8**
8.	**3h**	-9.3	-7.6
9.	**3i**	-8.4	-6.5
10.	**3j**	**-10.4**	-7.0
11.	**3k**	-7.6	-7.0
12.	**3l**	-6.3	-5.8
13.	**3m**	-8.2	-7.2
14.	**3n**	-9.5	-7.5
15.	**3o**	-7.6	-7.0
16.	**3p**	-8.6	-6.5
17.	**3q**	-8.3	-6.4
18.	**3r**	-7.4	-6.4
19.	**3s**	-9.9	-7.7
20.	**3t**	-8.1	-6.1

There are several reasons that might contribute to a low docking score for indole-based compounds. This score indicates that the expected binding strength is poor. These factors are linked to the target binding site's traits and the molecule's structure. There are several factors that lead to inadequate complementarity with the binding site, including steric conflicts, shape incompatibilities, and improper molecular orientation. It is possible for residues in the binding pocket to contact either the indole ring or its substituents. The planar shape of the indole ring may not fit well in the binding pocket. There is a possibility that the docking algorithm will not be able to come up with a position that allows for productive interactions. Interactions such as hydrogen bonding, ionic interactions, and hydrophobic mismatch are examples of some of the critical interactions that might occasionally be absent. Some scoring algorithms may not give sufficient weight to π-π stacking, which involves aromatic rings similar to those found in indole, as well as other minor interactions or interactions. There is a possibility that the posture prediction will not be accurate if sufficient consideration is not given to the metal ions or water molecules present at the spot.

Fig. 4a and Fig. 5a show the catalytic portion of cyanobacterial membrane-bound manganese superoxide dismutase (MnSOD) (PDB ID: 1GV3) proteins with standard ascorbic acid and designed indole molecule 3g in the hide receptor interaction represented in 3D using CB Dock software, respectively. Fig. 4b and Fig. 5b show the catalytic portion of cyanobacterial membrane-bound manganese superoxide dismutase (MnSOD) (PDB ID: 1GV3) proteins with standard ascorbic acid and designed indole molecule 3g in drug-receptor interaction represented in 3D using CB Dock software, respectively.

Fig. 6a and Fig. 7a show the crystal structure of VirB8 from *Brucella suis* (PDB ID: ID: 2BHM) represented in 3D with standard metronidazole and designed indole molecule 3j in drug-receptor interaction using CB Dock software, respectively. Fig. 6b and Fig. 7b show the crystal structure of VirB8 from *Brucella suis* (PDB ID: 2BHM) represented in 3D with standard metronidazole and designed indole molecule 3j in drug-receptor interaction using CB Dock software, respectively.

Table 2. Sequence of surrounding amino acids of selected design molecules with high docking scores and standard molecules taken for molecular docking

Structure name	PDB ID	Amino acids	Docking Score
Ascorbic Acid	1GV3	**Chain A:** LYS65 HIS66 ALA69 TYR70 ASN73 ASN110 TRP202 HIS204 **Chain B:** GLY158 GLY159 ASP160 ARG161 PHE162 GLY163 VAL201 GLU203 TYR206 GLN211 ASN212 ARG213 ARG214 PRO215	-5.7
Metronidazole	2BHM	**Chain A:** ARG114 GLU115 THR116 TYR117 TYR141 GLN144 PHE145 LEU151 TYR155 THR161 LYS182 THR184 TRP198 TYR229 ARG230 VAL231 ASP232 PRO233 **Chain D:** ARG114 GLU115 THR116 TYR117 GLN144 PHE145 LEU151 TYR155 THR161 LYS182 TRP198 TYR229 ARG230 VAL231 ASP232 PRO233	-6.5
3g	1GV3	**Chain A:** LYS152 ASN155 GLN156 GLY159 ASP160 ARG161 PHE162 GLY163 GLU203 TYR206 ASN212 ARG213 ARG214 PRO215 LEU218 **Chain B:** ASP64 LYS65 HIS66 ALA68 ALA69 TYR70 ASN72 ASN73 THR105 THR106 ASN109 ASN110 TRP202 HIS204	-8.8
3j	2BHM	**Chain A:** ARG114 GLU115 THR116 TYR117 GLN144 LEU151 TYR155 VAL159 THR161 LYS182 THR184 TRP198 TYR229 ARG230 VAL231 ASP232 PRO233 **Chain D:** ARG114 GLU115 THR116 TYR117 TYR141 GLN144 LEU151 TYR155 VAL159 THR161 LYS182 THR184 TRP198 TYR229 ARG230 VAL231 ASP232 PRO23	-10.4

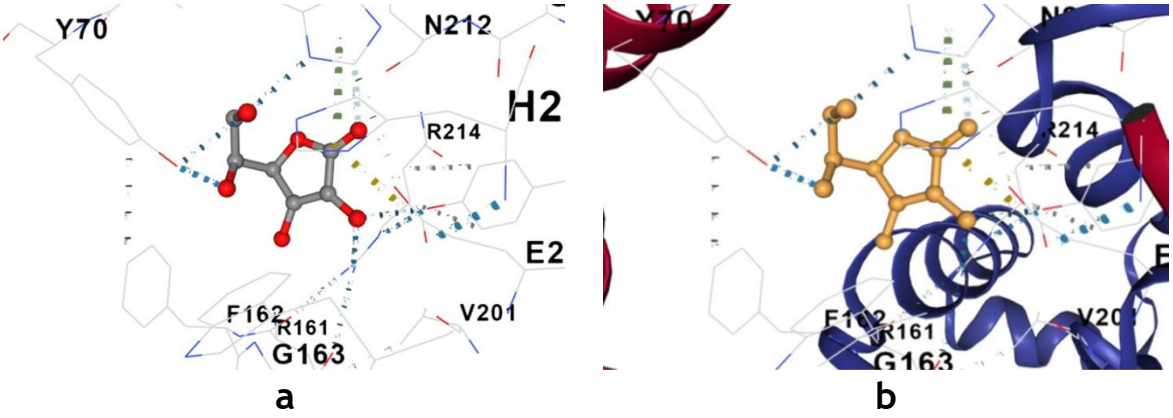

Fig. 4. a. The catalytic portion of cyanobacterial membrane-bound manganese superoxide dismutase (MnSOD) (PDB ID: 1GV3) proteins with standard ascorbic acid in the hide receptor interaction represented in 3D using CB Dock software. **b.** The catalytic portion of cyanobacterial membrane-bound manganese superoxide dismutase (MnSOD) (PDB ID: 1GV3) proteins with standard ascorbic acid in drug-receptor interaction represented in 3D using CB Dock software.

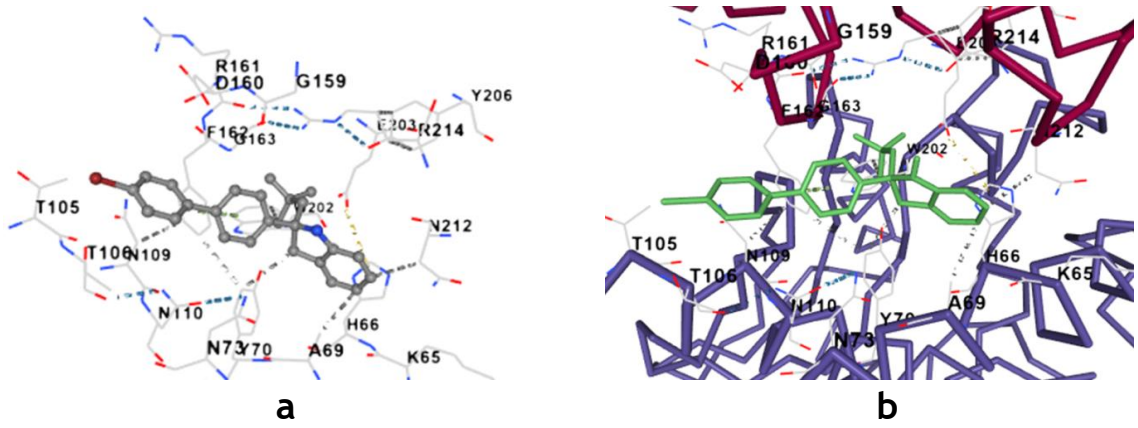

Fig. 5. a. The catalytic portion of cyanobacterial membrane-bound manganese superoxide dismutase (MnSOD) (PDB ID: 1GV3) proteins with the design molecule 3g in drug hide receptor interaction represented in 3D using CB Dock software. **b.** The catalytic portion of cyanobacterial membrane-bound manganese superoxide dismutase (MnSOD) (PDB ID: 1GV3) proteins with design molecule 3g in drug receptor interaction represented in 3D using CB Dock software.

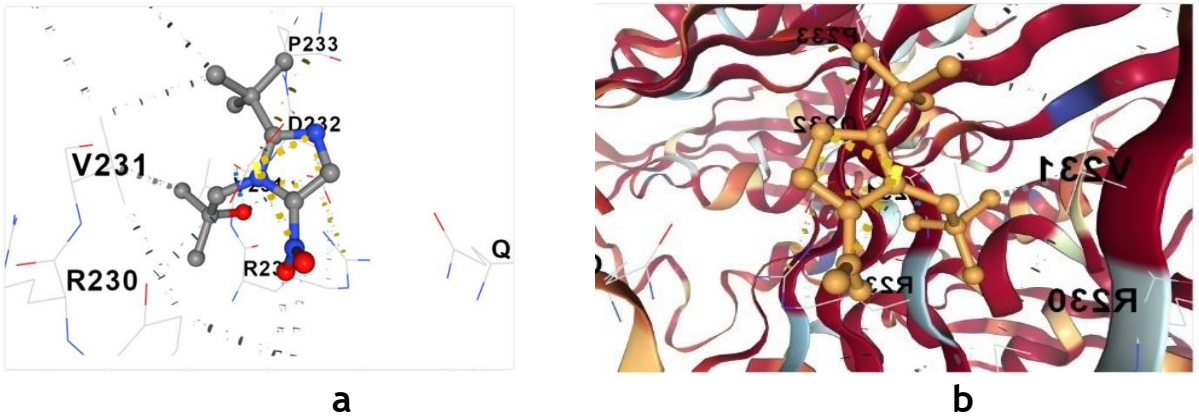

Fig. 6. a. The crystal structure of VirB8 from *Brucella suis* (PDB ID: ID: 2BHM) represented in 3D with standard metronidazole in drug-receptor interaction using CB Dock software. **b.** The crystal structure of VirB8 from *Brucella suis* (PDB ID: 2BHM) represented in 3D with standard metronidazole in drug-receptor interaction using CB Dock software.

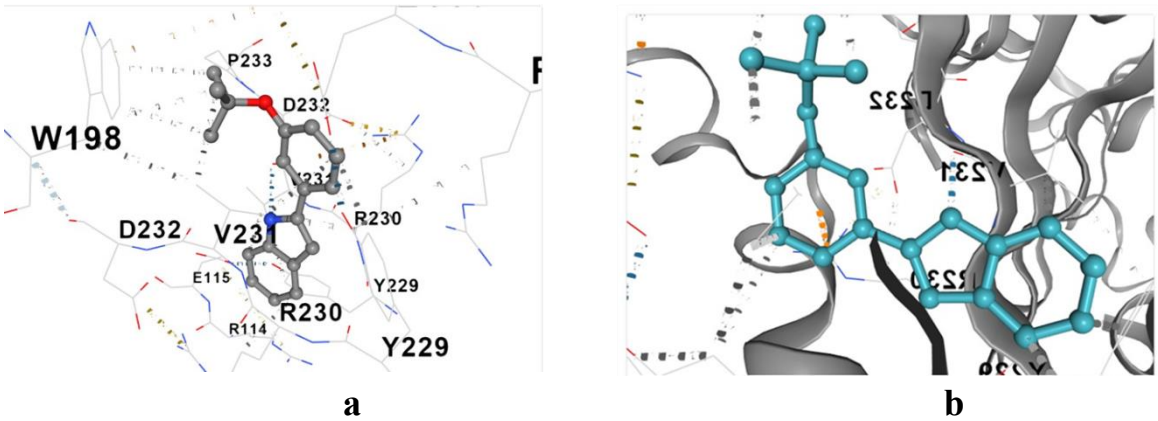

Fig. 7. a. The crystal structure of VirB8 from *Brucella suis* (PDB ID: 2BHM) designed indole molecule 3j in the drug-hiding receptor interaction represented in 3D using CB Dock software. **b.** The crystal structure of VirB8 from *Brucella suis* (PDB ID: 2BHM) with the designed indole molecule 3j in drug receptor interaction represented in 3D using CB Dock software.

3.2. *In silico* Toxicity Prediction

The in silico toxicity prediction was carried out by using ProTox II software, which is an online available tool. Assessing the combined harmful effects is a vital component of the process of developing drugs. In this study, it is found that designed molecules can harm the liver, brain, kidneys, lungs, and heart, and we checked how toxic the ligand is using the PROTOX-II server. We used ascorbic acid as a standard to check for antioxidant effects and found it to be safe; for testing antimicrobial effects, we used metronidazole, which was determined to be harmful to the respiratory system. These results were comparative to the previous literature survey. The molecule 3l was found to be respiratory toxic, while molecules 3g, 3k, 3p, and 3r were found to be neurotoxic. All other remaining molecules were found to be nontoxic. Here, the threshold of 0.70 was considered by the software for toxicity prediction. Table 3 represents In silico Toxicity Prediction by ProTox II Software for designed.

3.3. Drug Likeness Screening/ Lipinski's Rule of Five

The molecular weights of the designed compounds vary from 131.17 to 364.28; metronidazole and ascorbic acid possess molecular weights of 171.15 and 176.12, respectively. Ascorbic acid possesses log P values of 1.41, and metronidazole possesses log P values of 0.62, which is acceptable as per the Lipinski rule of five, while the designed compounds have log P values between 2.48 to 6.14. Ascorbic acid and metronidazole have values of 3 and 9 for hydrogen bond acceptors, respectively, while their donor values are 1 and 4. Moreover, the hydrogen bond donor and acceptor values of the proposed compounds are within an acceptable range below 5 for hydrogen bond donors and below 10 for hydrogen bond acceptors. All molecules will satisfy the conditions of Lipinski's Rule of Five, which serves as a fundamental principle for the development of novel compounds based on anticipated characteristics. Table 4 represents the Drug likeness screening for designed indole molecules.

Currently, there is no experimental evidence indicating that metronidazole binds to VirB8 from Brucellasuis. Metronidazole is primarily effective against anaerobic bacteria and certain protozoa, and its mechanism of action involves the reduction of its nitro group within anaerobic cells, leading to DNA damage. It is not known to target type IV secretion system (T4SS) components like VirB8. However, research has identified small-molecule inhibitors that bind to VirB8 and disrupt

Table 3. *In silico* Toxicity Prediction by ProTox II Software

Sr. No.	Designed Molecule	Hepatotoxicity with threshold value	Neurotoxicity with threshold value	Nephrotoxicity with threshold value	Respiratory toxicity with threshold value	Cardiotoxicity with threshold value
Standard						
1.	Metronidazole	0.87	0.70	0.52	0.75	0.74
2.	Ascorbic Acid	0.86	0.94	0.62	0.83	0.79
Indole derivative						
1	3a	0.52	0.58	0.85	0.89	0.93
2	3b	0.53	0.57	0.66	0.61	0.87
3	3c	0.52	0.55	0.61	0.52	0.83
4	3d	0.58	0.51	0.85	0.88	0.91
5	3e	0.58	0.57	0.72	0.66	0.56
6	3f	0.58	0.60	0.88	0.50	0.91
7	3g	0.54	0.70	0.87	0.65	0.85
8	3h	0.58	0.66	0.83	0.74	0.96
9	3i	0.52	0.55	0.61	0.52	0.83
10	3j	0.51	0.58	0.74	0.67	0.79
11	3k	0.53	0.72	0.82	0.77	0.89
12	3l	0.81	0.61	0.9	0.78	0.81
13	3m	0.60	0.56	0.82	0.82	0.90
14	3n	0.52	0.69	0.83	0.76	0.89
15	3o	0.64	0.51	0.54	0.57	0.71
16	3p	0.57	0.74	0.82	0.79	0.89
17	3q	0.56	0.65	0.82	0.87	0.89
18	3r	0.52	0.70	0.81	0.78	0.88
19	3s	0.61	0.67	0.59	0.61	0.59
20	3t	0.52	0.58	0.58	0.89	0.93

Colour coding represents:

Active Below Threshold	Inactive Below Threshold	Active Above Threshold	Inactive Below Threshold

Table 4: Drug likeness Screening/ Lipinski rule of five

Molecule	Compound	Mass	Log P	H Bond Acceptor	H Bond Donor
			Standard		
1	Metronidazole	171.15	0.62	3	1
2	Ascorbic Acid	176.12	1.41	6	4
			Indole Derivatives		
1	3a	193.24	3.83	0	1
2	3b	209.24	3.54	1	2
3	3c	209.24	3.54	1	2
4	3d	131.17	2.48	0	1
5	3e	238.24	4.27	1	1
6	3e	207.27	3.76	0	1
7	3f	364.28	6.14	1	1
8	3g	259.31	4.23	1	2
9	3h	209.24	3.54	1	2
10	3i	223.27	3.84	1	1
11	3j	245.68	4.63	0	1
12	3k	175.27	3.35	1	1
13	3l	239.34	4.56	1	1
14	3m	295.69	5.51	0	1
15	3n	318.17	4.31	2	2
16	3o	227.69	4.49	0	1
17	3p	272.14	4.6	0	1
18	3q	319.14	4.44	0	1
19	3r	268.27	3.61	2	1
20	3s	193.24	3.83	0	1

its function. Smith et al. (2012) reported that the inhibitor B8I-1 binds (to a conserved surface groove on VirB8, opposite its dimerization interface. This binding involves interactions with residues such as K182, Q144, R114, and W198, and effectively inhibits VirB8 dimerization, thereby impairing T4SS assembly and function [25]. Paschos *et al.* (2006) detailed the structural and functional analysis of VirB8, highlighting its interactions with other T4SS components like VirB4 and VirB10. Mutations at specific residues (e.g., M102, Y105, E214) were shown to affect VirB8's ability to self-associate and interact with its partners, underscoring the protein's critical role in T4SS assembly [3]. In the conclusion, while metronidazole does not bind to VirB8, targeted inhibitors like B8I-1 have been identified and characterized for their ability to disrupt VirB8 function, offering potential avenues for antimicrobial development against *Brucella suis*.

Similarly, there is no evidence from experiments that suggests that ascorbic acid directly binds to manganese superoxide dismutase (MnSOD) that is attached to the membrane of cyanobacteria. Researchers have studied the structure and function of manganese superoxide dismutases (MnSODs) in cyanobacteria like *Anabaena* PCC 7120 and *Leptolyngbya valderiana* BDU20041 a lot, but none of these studies have found any interactions with ascorbic acid. In the research done by Priya *et al.* (2010) and Atzenhofer *et al.* (2002), the structure of the part of MnSOD from *Anabaena* PCC 7120 that helps it work was studied in detail, with a clarity of 2.0 Å. The findings from this analysis gave a clear picture of how the enzyme works and its two-part structure, showing its role in protecting against oxidative stress. Similarly, studies on *Leptolyngbya valderiana* BDU20041 have focused on how the enzyme responds to oxidative stress and its role in handling reactive oxygen species [2, 26]. The current research does not suggest a direct binding interaction between ascorbic acid and cyanobacterial MnSODs. This is although ascorbic acid is a well-known antioxidant that has the ability to alter several pathways associated with oxidative stress.

4. Conclusions

This study successfully makes a group of new indole derivatives that have intriguing biological activities, like strong antimicrobial and antioxidant abilities. Two proteins from the Protein Data Bank are VirB8 from *Brucella suis* (PDB ID: 2BHM) and MnSOD from cyanobacteria (PDB ID: 1GV3). What they show is the crystal structure of VirB8. Through *in silico* docking studies, the compounds showed favorable interactions with the catalytic portion of both selected proteins for their antioxidant and antimicrobial potential. One of the molecules, 3g, had the best docking score of -10.4 against the crystal structure of VirB8 from *Brucella suis* (PDB ID 2BHM). This means that it binds strongly to the target receptor. Manganese superoxide dismutase (MnSOD),

associated with the cyanobacteria membrane (PDB ID 1GV3), had a good fit (-10.4 points) with the 3j molecule. This signal indicates a robust affinity for binding to the target receptor. The score indicates a robust affinity for the target receptor. Metronidazole is used as a standard because it has a molecular docking score of -6.5 when compared to the crystal structure of Brucellasuis VirB8. Ascorbic acid serves as a standard. Manganese superoxide dismutase (MnSOD), which connects to the membrane of cyanobacteria, requires -5.7 to dock. It was found that the residual design molecules docked better with both proteins than the reference compounds, metronidazole and ascorbic acid. Additionally, ProtTox II software was employed to forecast the *in silico* toxicity of all the designed compounds. Molecules 3l and metronidazole exhibited respiratory toxicity, whereas molecules 3g, 3k, 3p, and 3r showed neurotoxicity. All designed compounds were evaluated for drug-likeness and conformed to Lipinski's Rule of Five criterion.

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References:

1. Terradot, L.; Bayliss, R.; Oomen, C.; Leonard, G. A.; Baron, C.; Waksman, G. Structures of two core subunits of the bacterial type IV secretion system, VirB8 from *Brucella suis* and ComB10 from *Helicobacter pylori*. *Proc. Natl. Acad. Sci.* **2005**, *102* (12), 4596-4601. DOI: 10.1073/pnas.0408927102
2. Atzenhofer, W.; Regelsberger, G.; Jacob, U.; Peschek, G. A.; Furtmüller, P. G.; Huber, R.; Obinger, C. The 2.0 Å resolution structure of the catalytic portion of a cyanobacterial membrane-bound manganese superoxide dismutase. *J. Mol. Biol.* **2002**, *321* (3), 479-489. DOI: 10.1016/S0022-2836(02)00624-1
3. Paschos, A.; Patey, G.; Sivanesan, D.; Gao, C.; Bayliss, R.; Waksman, G.; O'callaghan, D.; Baron, C.; Dimerization and interactions of *Brucella suis* VirB8 with VirB4 and VirB10 are required for its biological activity. *Proc. Natl. Acad. Sci.* **2006**, *103* (19), 7252-7257. DOI: 10.1073/pnas.0600862103
4. Elkaeed, E.B.; Yousef, R.G.; Elkady, H.; Gobaara, I.M.; Alsfouk, A.A.; Husein, D.Z.; Ibrahim, I.M.; Metwaly, A.M.; Eissa, I.H. The assessment of anticancer and VEGFR-2 inhibitory activities of a new 1 H-indole derivative: in silico and in vitro approaches. *Processes* **2022,** 10(7), Art. No: 1391. DOI: 10.3390/pr10071391
5. Regelsberger, G.; Atzenhofer, W.; Ruker, F.; Peschek, G.A.; Jakopitsch, C.; Paumann, M.; Furtmüller, P.G.; Obinger, C. Biochemical characterization of a membrane-bound manganese-containing superoxide dismutase from the cyanobacterium *Anabaena* PCC 7120. *J. Biol. Chem.* **2002,** *277(46),* 43615-43622. DOI: 10.1074/jbc.M207691200
6. Surana, K.R.; Ahire, E.D.; Sonawane, V.N.; Talele, S.G.; Biomolecular and molecular docking: A modern tool in drug discovery and virtual screening of natural products. In *Applied Pharmaceutical Practice and Nutraceuticals*. Apple Academic Press **2021,** 209-223. DOI: 10.1201/9781003054894
7. Hoppner, C.; Carle, A.; Sivanesan, D.; Hoeppner, S.; Baron, C. The putative lytic transglycosylase VirB1 from Brucellasuis interacts with the type IV secretion system core components VirB8, VirB9 and VirB11. *Microbiol*ogy **2005**, *151(11),* 3469-3482. DOI: 10.1099/mic.0.28326-0
8. Priya, B.; Premanandh, J.; Dhanalakshmi, R.T.; Seethalakshmi, T.; Uma, L.; Prabaharan, D.; Subramanian, G.; Comparative analysis of cyanobacterial superoxide dismutases to discriminate canonical forms. *BMC Genomics*, **2007**, *8*, Art. No: 435. DOI: 10.1186/1471-2164-8-435
9. Andrieux, L.; Bourg, G.; Pirone, A.; O'Callaghan, D.; Patey, G. A single amino acid change in the transmembrane domain of the VirB8 protein affects dimerization, interaction with VirB10 and *Brucella suis* virulence. *FEBS Lett.* **2011,** 585(15), 2431-2436. DOI: 10.1016/j.febslet.2011.07.004
10. Shah, M.; Kumar, A.; Singh, A.K.; Singh, H.; Narasimhan, B.; Kumar, P.; In silico studies of Indole Derivatives as Antibacterial agents. *J. Pharmacopuncture* **2023,** 26(2), 147-157. DOI: 10.3831/KPI.2023.26.2.147
11. Parthiban, A.; Sivasankar, R.; Rajdev, B.; Asha, R.N.; Jeyakumar, T.C.; Periakaruppan, R.; Naidu, V.G.M. Synthesis, *in vitro, in silico* and DFT studies of indole curcumin derivatives as potential anticancer agents. *J. Mol. Struc.* **2022,** *1270*, Art. No: 133885. DOI: 10.1016/j.molstruc.2022.133885
12. Kumari, A.; Singh, R.K. Synthesis, molecular docking and ADME prediction of 1H-indole/5-substituted indole derivatives as potential antioxidant and anti-inflammatory agents. *Med. Chem.* **2023,** 19(2), 163-173. DOI: 10.2174/1573406418666220812152950
13. Dileep, K.V.; Remya, C.; Tintu, I.; Haridas, M.; Sadasivan, C. Interactions of selected indole derivatives with phospholipase A 2: in silico and in vitro analysis. *J. Mol. Model.* **2013**, *19*, 1811-1817. DOI: 10.1007/s00894-012-1741-4
14. Balupuri, A.; Gadhe, C.G.; Balasubramanian, P.K.; Kothandan, G.; Cho, S.J. In silico study on indole derivatives as anti HIV-1 agents: a combined docking, molecular dynamics and 3D-QSAR study. *Arch. Pharm. Res.* **2014,** *37*, 1001-1015. DOI: 10.1007/s12272-013-0313-1
15. Pingaew, R.; Mandi, P.; Prachayasittikul, V.; Thongnum, A.; Prachayasittikul, S.; Ruchirawat, S.; Prachayasittikul, V. Investigations on anticancer and

antimalarial activities of indole-sulfonamide derivatives and in silico studies. *ACS Omega* **2021,** *6(47),* 31854-31868. DOI: 10.1021/acsomega.1c04552

16. Rudrapal, M.; Celik, I.; Chinnam, S.; Çevik, U.A.; Tallei, T.E.; Nizam, A.; Joy, F.; Abdellattif, M.H.; Walode, S.G. Analgesic and anti-inflammatory potential of indole derivatives. *Polycyclic Aromatic Compounds* **2023**, *43(9),* 7732-7753. DOI: 10.1080/10406638.2022.2139733
17. Miller, A.F.; Superoxide dismutases: ancient enzymes and new insights. *FEBS Lett.* **2012,** *586(5),* 585-595. DOI: 10.1016/j.febslet.2011.10.048
18. Uma, L.; Prabaharan, D.; Priya, B.; Subramanian, G.; Role of cyanobacterial oxidases in bioremediation-an overview. *Al. Bio. Biotech.* **2009,** 251-261.
19. Venkataramanaa, C.H.S.; Sravania, K.R.; Singha, S.S.; Madhavanb, V.; In-silico ADME and toxcity studies of some novel indole derivatives. *J. Appl. Pharm. Sci.* **2011,** *01(10),* 159-162.
20. Surana, K.R.; Mahajan, S.K. In silico study of chromane ring compound rubranonoside from *Plumeria rubra* as anticancer potential. *Trend. Sci.* **2022**, *19(24),* 3305-3305. DOI: 10.48048/tis.2022.3305
21. Surana, K.R.; Jadhav, P.S.; Shewale, H.S.; Wagh, D.B.; Mahajan, S.K.; Musale, J.V. In silico and Biological Evaluation of Anti-Inflammatory Activity of synthesized Benzimidazoles Derivatives. *Biosci. Biotechnol. Res. Asia.* **2024,** *20(3),* 1241-1253. DOI: 10.13005/bbra/3300
22. Aher, S.N.; Sonawane, S.N.; Sonawane, P.R.; Surana, K.R.; Mahajan, S.K.; and Patil, D.M. In silico Drug Design, Synthesis and Evaluation of Anti-inflammatory Activity Pyrimidine Analogue. *Biosci. Biotechnol. Res. Asia.* **2024,** *21(2),* 741-753. DOI: 10.13005/bbra/3261
23. Balekundri, A.; Ahire, E. D. Bacterial network construction and molecular docking approach to study interaction of *Myristica fragrans* on Acne infections: Molecular docking approach to study interaction of *Myristica fragrans*. *Community Acquir. Infect.* **2023**,*10*. DOI: 10.54844/cai.2023.0326
24. Ahire, E. D.; Kshirsagar, S. J. In Silico Investigation of Surfactants as Potential Permeation Glycoprotein Inhibitors for Formulation Development. *Adv. Biores.* **2023**, *12*, 115-120. DOI: 10.15515/abr.0976-4585.14.4.115120.
25. Smith, M.A.; Coinçon, M.; Paschos, A.; Jolicoeur, B.; Lavallée, P.; Sygusch, J.; Baron, C. Identification of the binding site of *Brucella* VirB8 interaction inhibitors. *Chem. Biol.* **2012,** *19(8),* 1041-1048. DOI: 10.1016/j.chembiol.2012.07.007
26. Priya, B.; Sivaprasanth, R.K.; Jensi, V.D.; Uma, L.; Subramanian, G.; Prabaharan, D. Characterization of manganese superoxide dismutase from a marine cyanobacterium *Leptolyngbya valderiana* BDU20041. *Saline Syst.* **2010,** *6,* Art. No: 6. DOI: 10.1186/1746-1448-6-6