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Research Article

In Silico Based Identification Of Novel Inhibitors For Selected MDR Protein From Shigella Species: A Validation Through Molecular Docking Analysis

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ABSTRACT

Gram-negative Enterobacteriaceae pathogenic strains of Shigella organisms cause bacillary dysentery and shigellosis. Three serogroups-Shigella dysenteriae, flexneri, boydii, and sonnei and one serotype-Sonnei, are groups A, B, C, and D, respectively. Shigella flexneri is the most common serogroup worldwide. Fecal-oral transmission is the main method, but sexual transmission has been recorded. These groups are among the most responsible for diarrheal diseases. In children, this species can lead to stunted growth due to severe and life-threatening illnesses. In the present day situation, managing this infection is still challenging as it can easily resist many antimicrobials, and overcoming this and finding potential inhibitors can help reduce this infection. As a result, this research aims to determine the potential inhibitors against the potential protein target through molecular docking analysis. According to the virulence factor, subcellular localisation, and role in infection, the aminoglycoside'-(9)O-adenyltransferase protein was the most potent target protein. The three-dimensional structure was designed and validated, it became known that designed model has a promising profile according to the Ramachandran plot analysis. Further, the molecular docking analysis targeted available ligands in ZINC databases and found 10 potential novel inhibitors. Among these, only 6 inhibitors were found to have the most potential as per toxicity analysis. These findings suggested that these selected inhibitors can be utilised to combat this infection; however, further experimental validation is required to confirm their efficacy.

Keywords— Diarrohea; Shigella; Molecular docking; Multidrug resistant; Novel inhibitors

I.INTRODUCTION

Enterobacteriaceae rod-shaped gram-negative, non-motile, non-spore-forming, facultative intracellular pathogens are Shigella. Bacillary dysentery (shigellosis) from Shigella is caused. They are classified as A, B, C, and D, with four species: Shigella dysenteriae, flexneri, boydii, and sonnei. A–C are physiologically identical, but biochemical metabolism studies identify S. sonnei (group D). The S. flexneri virulence plasmid encodes three virulence factors: T3SS, ipa proteins, and IcsA (used for cell-to-cell transmission). [1]. S. flexneri block early infection-induced inflammation. It uses OspI, an effector protein encoded by ORF169b on the Shigella large plasmid and secreted by Type III secretion. Reducing the TNF-α-Receptor-associated factor 6 (TRAF6) signalling pathway reduces the inflammatory response during bacterial invasion. [2]. Paracytophagy allows S. flexneri to travel straight from cell to cell via host cell actin. [3, 4]. The most genetically diverse Shigella is S.boydii [5]. Shigella bodyii has 18 serotypes [6]. Shigella survives stomach digestion and reaches intestinal mucosa epithelial cells. After multiplying intracellularly, the bacteria damage neighbouring epithelial cells. CDC shigellosis cases ranged from more than 17,000 from 1978 to 2003 to an all-time low of 14,000 in 2004 to nearly 20,000 in 2007 [7]. However, most cases go untreated or unreported [8]. The fecal-oral route transfers shigella species , and the majority of illnesses spread from person to person [7]. Inadequate

sanitation, malnutrition, low socioeconomic position, and lack of clean water contribute to disease spread [912]. A 10-100 organism infectious dosage and high person-toperson transmission efficiency make these agents highly contagious. In the United States, the FoodNet, a reporting system used by public health authorities that captures foodborne illness in over 13% of the population, records the incidence of foodborne illness. Shigellosis has historically been associated with epidemics in daycare centres, nursing homes, institutional settings (such as prisons), and cruise ships due to its relatively prevalent person-to-person transmission. Most shigella infections cause diarrhoea, fever, and stomach cramps. Mucuscontaining diarrhoea can range from mild to severe. Severe diarrhoea is 25%–50% bloody [11]. Rectal spasms, or "tenesmus," are common. S. flexneri infections can cause chronic consequences include joint pain, eye irritation, painful urination (Reiter's Syndrome), and stunted growth [13]. Antibiotics shorten illness and reduce contagiousness. Antibiotic overuse has caused Shigella antibiotic resistance, which threatens public health. Expanding antibiotic sensitivity in Shigella species is a global problem [14]. Multidrug-resistant strains originated mainly because bacteria easily acquire and pass on exogenous genes via mobile genetic components such transposons, plasmids, insertion sequences, genomic islands, and integrons [15, 16].

Biological warfare implementations require understanding toxins, basic virulence components, and resistance genes to antibiotics to identify potent inhibitors against emerging pathogens. The availability of bacterial MDR protein data has sparked the development of several novel approaches for determining potential targets against it. Conventional drug discovery methods have several limitations, including cost and time commitment; in silico-based technology has given researchers an enticing alternative for identifying inhibitors against MDR protein targets. The present study investigates the utility of computer-aided methods in studying the novel inhibitors for selected MDR protein from Shigella species. This research made use of an in silico approach, selected different MDR proteins from Shigella species, and identified novel inhibitors against them to generate knowledge about better therapeutic agents.

II. METHODOLOGY

A. Selection of the probable multi-drug resistance protein from Shigella spp.

A comprehensive literature review from scientific databases such as PubMed and Google Scholar and research articles from reputable journals of shigella were examined to establish the current state of knowledge regarding Shigella infections and MDR proteins.

B. Retrieval and Classification of MDR Proteins from the MDR Database

The MvirDB database retrieved critical information regarding protein toxins, virulence factors, multi-drug resistant proteins and genes associated with Shigella species. The collected MDR proteins were systematically classified based on their known drug resistance profiles and distinctive characteristics [17].

C. Online BLAST for Sequence Comparison

For sequence similarity analysis, the Basic Local Alignment Search Tool was used. It facilitated the identification of query protein sequences within Shigella species that exhibited significant similarity to known MDR proteins in other bacterial organisms, such as Klebsiella, Streptococcus, and Staphylococcus [18].

D. Transmembrane Protein Prediction (TMHMM) and Localisation (PSORTb)

Predictive tools, namely TransMembrane prediction (TMHMM) and Protein Localization Prediction (PSORTb) were utilised to evaluate protein properties. Transmembrane areas were predicted using TMHMM., while PSORTb recognised the localisation of proteins within cellular compartments [19, 20].

E. Selection of target protein

Based on the screening, the most significant potential target was selected, and their three-dimensional structure was modelled through the I-TASSER (https://zhanggroup.org/ITASSER/) software. Further, The selected target's primary sequence was utilised as a query input using the default parameter, and the 3D structure was modelled [21].

F. Binding Site Prediction with MetaPocket

MetaPocket, a bioinformatics tool, was harnessed for the predicted location of ligand binding areas on the chosen protein. It systematically identified potential binding pockets for ligands according to the protein's structural information [19, 20].

G. Molecular Docking Analysis

The study leveraged Molegro Virtual Docker, a specialised molecular docking software for molecular docking studies, used to assess the affinity for binding and interactions between selected ligands and the protein binding sites [22].

H. Toxicity Assessment

Toxicity prediction was performed using ToxPredict, a bioinformatics tool designed to evaluate the potential toxicity of chemical compounds. This comprehensive assessment encompassed various toxicity endpoints, including carcinogenicity and irritation, to ensure the safety of the compounds.

III. RESULT AND DISCUSSION

A. Mvir DB

Different multi-drug resistant proteins were collected using the MvirDB database. A total of 8404 multi-drug resistant protein sequences were retrieved. From the NCBI, a total of 217 shigella sequences were retrieved. In the multi-drug resistant result, some sequences do not give any hits found. So, after deleting those sequences from the shigella sequences, it gives 147 resistant targets.

B. Online BLAST

After that, we are performing an online blast including three different reference species, including Klebsiella (taxid: 570), Staphylococcus (taxid: 1279), and Streptococcus (taxid: 1301). It confirmed that those sequences have similarities for Klebsiella, Staphylococcus, and Streptococcus. Collect only those sequences which were present in Klebsiella, Staphylococcus, and Streptococcus. It gives 84 sequences in total.

C. PSORTb result

SeqID: gi|253400253|gb|ACT31400.1| aminoglycoside 3'-(9)O-adenyltransferase [Shigella flexneri]. Result analysis given in table 1.

D. TMHMM-based protein localization

Performing TMHMM gives the protein localisation where the protein is present. Out of 84 shigella sequences, there are 26 proteins which give a positive result for the TMHMM. If we know about protein localisation, then it is easy to identify where protein is present in the cell, and it is easy to locate the target protein. So, the target protein is useful in developing new drug molecules which bind to that target molecule and prevent particular diseases. Psort is also performed to find the localisation of the protein.

E. Selection of a Target Protein (Aminoglycoside'-(9)-O-Adenyltransferase)

Escherichia, Salmonella, Shigella, Enterobacter, Citrobacter, Acinetobacter, Proteus, Klebsiella, Serratia, Morganella, and Pseudomonas are all effectively treated with aminoglycoside antibiotics in vitro. Several Streptococci and Staphylococcus were detected. Streptococcus pneumonia and Bacteroides species have erratic in vitro activity.

The "-I aminoglycoside nucleotidyltransferase " Its hydroxyl group and position 9 can be modified to resist streptomycin and spectinomycin, respectively. Eight genes have been found for ANT(")-I. Gram-negative bacteria often contain ANT(")-I enzymes with 59% to 95% amino acid sequence identity. In streptomycin-resistant clinical isolates, over 90% had ANT(")I. Staphylococcus aureus and Corynebacterium glutamicum have transposons and plasmids containing the ANT(")-Ia gene. Species from Enterobacteriaceae, Pseudomonas aeruginosa, and Vibrio cholera clinical isolates typically feature ANT(")-I genes. The recent identification of the ANT(")-Ia gene among integrons on the large conjugative plasmid of an Enterococcus clinical isolate is concerning because streptomycin is used synergistically to treat serious Enterococcal infections after gentamicin resistance. Thus, the aminoglycoside'-(9)-Oadenyltransferase protein was chosen for further study, and its protein sequence was obtained from NCBI (https://www.ncbi.nlm.nih.gov/) entry ACT31400.

F. Three-dimensional structure prediction

The 3D structure were modelled using I-TASSER. Further, the I-TASSER software generated 5 models of input sequence based on different C-scores (Table 2).

Table 1: PSORTb result analysis report:

SeqID: NP_949347.1		
Analysis Report:		
CMSVM-	Unknown	[No details]
CytoSVM-	Unknown	[No details]
ECSVM-	Extracellular	[No details]
ModHMM-	Unknown	[No internal helices found]
Motif-	Unknown	[No motifs found]
OMPMotif-	Unknown	[No motifs found]
OMSVM-	OuterMembrane	[No details]

PPSVM-	Unknown	[No details]
Profile-	Unknown	[No matches to profiles found]
SCL-BLAST-	OuterMembrane, Extracellular	[matched **3646417**: Outer membrane (Autotransporter)]
SCL-BLASTe-	Unknown	[No matches against database]
Signal-	Non-cytoplasmic	[Signal peptide detected]
Localisation Scores:		
Cytoplasmic		0.00
CytoplasmicMembrane		0.00
Periplasm		0.00
OuterMembrane		5.87
Extracellular		4.13
Final Prediction:		
Unknown (This protein may have multiple localisation sites)		

Table 2 : Top 5 best model's c-score predicted by I -TASSER

Model no.	C-score
Model 1	-1.45
Model 2	-2.63
Model 3	-3.27
Model 4	-5.00
Model 5	-3.87

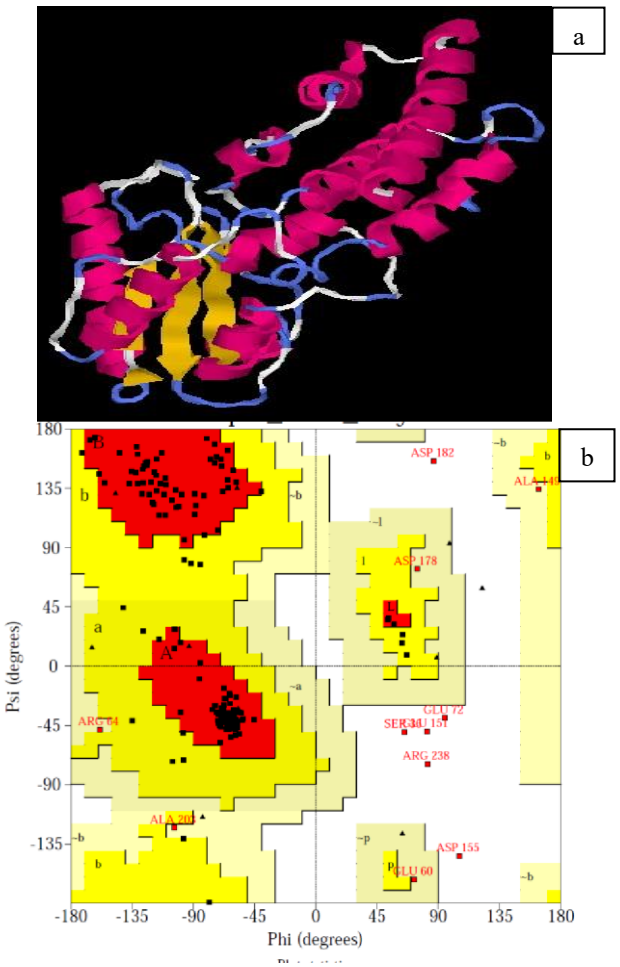


Fig. 1: a. Structure predicted by I-TASSER and b. Ramachandran plot.

C-score is I-TASSER's confidence score for anticipated model quality. It depends on threading template alignment significance and structure assembly simulation convergence factors. Model 1 has been selected for our further study because it has a lower c score. Models with high C-scores are confident, and vice versa. The TM-score and RMSD are good benchmarks for evaluating structural similarity and measuring structure modelling accuracy when the original structure is known. The TM-score is a measure of the query and template protein's global structural similarity. In cases where the native structure is not known, Predicting the quality of the modelling prediction, i.e. the distance between the predicted model and the native structures, becomes necessary. For this, the TM score is useful. The TM-score and RMSD of the predicted models relative to the native structures are based on the C-score. The c-score is highly correlated with TM-score and RMSD. A TM-score >0.5 indicates a proper topology model, and a TM-score <0.17 means a random similarity. In that case, the TM score is 0.54±0.15. Further, the modelled structure has 0.69 TM value and was validated, and it found that the designed model has a promising profile as 88.5% residue is in the most favoured region, 6.8% residue in the additional allowed region, 2.1% residues in generously allowed regions and only 2.6% residue are in the disallowed region. The three-dimensional structure of the target protein is as illustrated in Figure 1.

G. Binding Site Prediction with MetaPocket Protein structure assessment server ANOLEA. It calculates protein atomic energy. Non-local interactions between all heavy atoms of the twenty typical amino acids in the molecule are calculated. The server receives a PDB file with protein chains. This produces an energy profile with protein amino acid energy values (Figure 3).

Table 3: MVD Docking Score using zink database

ZINC id	pH range	Hbond Donor	H-bond Acceptor	Molecular Weight (gm/mol)	Rotatable bonds
ZINC1567 6026	pH 7	3	11	438.468	4
ZINC0879 0838	pH 7	2	7	420.47	5
ZINC1567 6264	pH 7	2	6	336.419	3
ZINC3542 4705	pH 7	4	8	386.5	6
ZINC4031 0115	pH 7	2	9	421.433	5
ZINC0213 0102	pH 7	2	8	389.391	6
ZINC1198 7865	pH 7	1	2	279.811	2

The distance-scaled finite ideal-gas reference state makes DFIRE an all-atom statistical potential. In the protein model, DFIRE often evaluates non-bonded atomic interactions.The model's pseudo energy indicates its quality and may be used to rate goal estimates. An energy-lower model is closer to the natural conformation. Fire energy score: -338.25

This protein's binding sites were found using MetaPocket 2.0. It predicts the protein's top three ligand binding sites.

A. Molecular Docking with Molegro Virtual Docker (MVD)

Docking utilises energy to investigate the binding mechanisms of two interaction molecules. Given a protein target's 3D shape, chemicals can fit in a "docking" cavity. Energy minimisation for the complex concludes the process. Docking builds ligand-protein binding models, clarifies key residues, and develops drugs from chemical databases. The ZINC database, which contains 1.5 lakh ligand molecules, was used for the docking. The top 10 molecular docking compounds were selected by score.

B. Toxicity Assessment of the chosen compounds

The toxicity evaluation of the final selected molecule was performed, and their different function are displayed in table 5. Toxicity is predicted for this ten-ligand molecule using ToxPredict. It gives pka values, persistence biodegradation, carcinogenicity, skin irritation, skin sensitisation and eye irritation. From that carcinogenicity is most dangerous for the body so those molecules which produce carcinogenicity then further study about those molecules were terminated. So, seven of the ten compounds are still available for research study. They were selected for further study for the carcinogenicity effect, and information was retrieved from the ZINC database. It is in the following table 4. The ZINC database gives all the information about the ligand, like hydrogen bond acceptor, donor, molecular weight, molecular formula etc., from which we can get the all physical representation of the ligand molecule. One molecule does not contain an amide group from that seven-ligand molecule. So, except for this molecule, six inhibitors are displayed in table 6.

Table 4: Details about the selected molecule

Ligand	MolDock Score	Rerank Score	HBond
ZINC16681947	-175.7	-145.9	-8.0
ZINC15676026	-175.1	-131.0	-8.3
ZINC08790838	-159.3	-114.5	-2.0
ZINC15676030	-156.2	-100.0	-7.5
ZINC15676264	-147.5	-110.1	-2.7
ZINC35424705	-147.0	-109.2	-10.8
ZINC40310115	-146.3	-107.4	-4.5
ZINC02130102	-146.0	-93.1	-5.6
ZINC70700903	-144.9	-106.0	-10.3
ZINC11987865	-143.5	-102.2	-2.5

The potential 3 ligand binding sites in your protein:

HEADER binding site ID: 1

RESI	GLY_9^40^	GLY_9^41^	LYS_9^43^	SER_9^36^	ASP_9^39^
RESI	PRO_9^204^	LEU_9^42^	ASP_9^206^	GLY_9^120^	ALA_9^203^
RESI	ILE_9^121^	ALA_9^119^	SER_9^46^	PRO_9^44^	HIS_9^45^
RESI	ASP_9^47^	GLY_9^35^	ILE_9^202^	VAL_9^38^	ASP_9^116^
RESI	SER_9^142^	TYR_9^34^	VAL_9^207^	LYS_9^205^	LEU_9^145^
RESI	HIS_9^141^	ALA_9^138^	ARG_9^100^	LEU_9^118^	ILE_9^48^
RESI	GLY_9^200^	ILE_9^117^	LYS_9^137^	LYS_9^201^	ASP_9^49^
RESI	ASN_9^115^	ALA_9^144^	TYR_9^195^	LEU_9^153^	GLU_9^140^
RESI	MET_9^1^	PHE_9^154^	VAL_9^146^	TYR_9^101^	ALA_9^208^
RESI	TRP_9^112^	THR_9^199^	GLN_9^113^	GLU_9^87^	ARG_9^114^
RESI	ASP_9^155^	PRO_9^102^	TRP_9^99^	ALA_9^103^	PRO_9^158^
RESI	VAL_9^157^	LYS_9^104^	SER_9^196^	GLU_9^111^	ASP_9^161^
RESI	GLU_9^159^	ARG_9^192^	LEU_9^162^	PRO_9^156^	ARG_9^63^
RESI	GLU_9^152^	ALA_9^85^	VAL_9^86^	GLY_9^78^	GLU_9^79^
RESI	PRO_9^77^	SER_9^80^			

HEADER binding site ID: 2

RESI	GLU_9^151^	GLU_9^159^	VAL_9^261^	LYS_9^263^	ALA_9^149^
RESI	PHE_9^163^	VAL_9^260^	PRO_9^158^	LEU_9^162^	PRO_9^148^
RESI	SER_9^196^	ALA_9^197^	THR_9^199^	PHE_9^154^	ASP_9^155^
RESI	PRO_9^156^	ARG_9^215^	LYS_9^259^	LYS_9^201^	GLU_9^214^
RESI	VAL_9^146^	GLY_9^147^	VAL_9^198^	ALA_9^212^	MET_9^213^
RESI	GLY_9^200^	LEU_9^145^	ALA_9^150^	TRP_9^194^	ILE_9^257^
RESI	GLU_9^256^	LEU_9^216^	PRO_9^217^	GLY_9^262^	ILE_9^202^

HEADER binding site ID: 3

RESI	PRO_9^98^	TRP_9^99^	PRO_9^102^	ASP_9^95^	GLU_9^106^
RESI	LEU_9^107^	LYS_9^104^	ARG_9^105^	ALA_9^103^	ARG_9^114^
RESI	LEU_9^118^	GLN_9^113^	ILE_9^117^	PHE_9^109^	THR_9^62^
RESI	GLY_9^110^	GLU_9^111^	ASP_9^161^	ARG_9^192^	VAL_9^157^
RESI	GLN_9^108^	TYR_9^34^	LEU_9^51^	ILE_9^96^	ARG_9^100^
RESI	THR_9^61^	ARG_9^63^	GLU_9^87^	ARG_9^64^	ASN_9^115^

Fig. 2: ANOLEA result

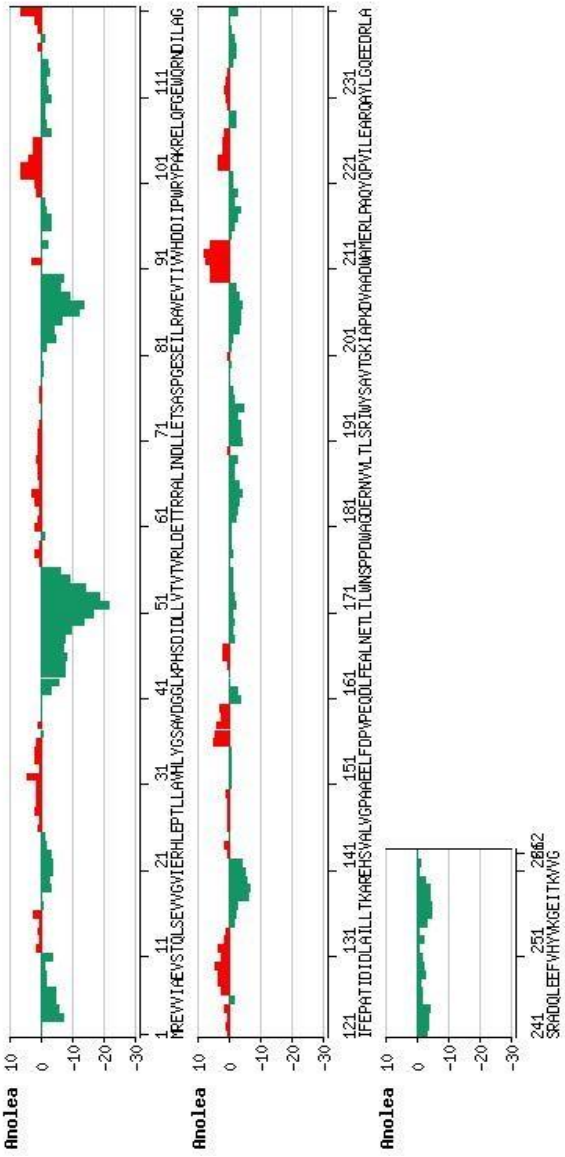
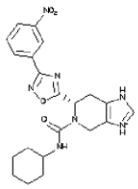
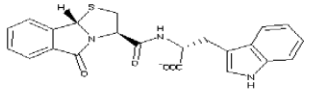
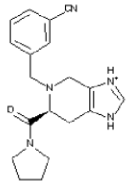
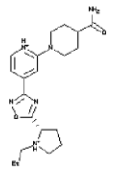
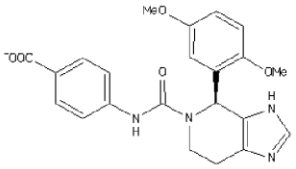
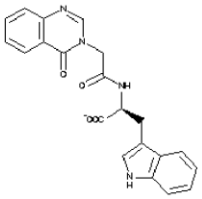


Fig. 3: Predicted binding site by MetaPocket

Table 5: Toxicity prediction for the top 10 molecules

ZINC ID	pKa	Persistence biodegradation	Carcinog -enicity	Skin Irritation/ corrosion	Skin sensitisation	Eye Irritation/ corrosion
ZINC16681947	7.76	class-2 (persistant chemical)	YES	not corrosive to skin	NO	Not skin corrosion R34 or R35
ZINC15676026	7.83	class-2 (persistant chemical)	NO	not corrosive to skin	YES	Not skin corrosion R34 or R36
ZINC08790838	4.03	class-2 (persistant chemical)	NO	unknown	NO	Not eye irritation R36
ZINC15676030	7.83	class-2 (persistant chemical)	YES	not corrosive to skin	NO	Not skin corrosion R34 or R36
ZINC15676264	7.76	class-2 (persistant chemical)	NO	unknown	YES	unknown
ZINC35424705	7.76	class-2 (persistant chemical)	NO	unknown	YES	unknown
ZINC40310115	2.83	class-2 (persistant chemical)	NO	not corrosive to skin	NO	Not skin corrosion R34 or R36
ZINC02130102	4.03	class-2 (persistant chemical)	NO	not corrosive to skin	NO	Not skin corrosion R34 or R36
ZINC70700903	7.13	class-2 (persistant chemical)	YES	unknown	NO	unknown
ZINC11987865	7.76	class-2 (persistant chemical)	NO	unknown	NO	unknown

Table 6 : Inhibitors reported in this study

Compound	Chemical structure
(6S)-N-cyclohexyl-6-[3-(3-nitrophenyl)-1,2,4-oxadiazol-5-yl]-1,4,6,7-tetrahydroimidazo[5,4-d]pyridine	
(2R)-3-(1H-indol-3-yl)-2-((3R)-5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-a]isoindole-3-carboxamido)propanoic acid	
3-[[(6S)-6-(pyrrolidine-1-carbonyl)-1,4,6,7-tetrahydroimidazo[5,4-d]pyridin-5-yl]methyl]benzonitrile	
1-[4-[5-[(2S)-1-propylpyrrolidin-2-yl]-1,2,4-oxadiazol-3-yl]-2-pyridyl]piperidine-4-carboxamide	
4-(4-(2,5-dimethoxyphenyl)-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridine-5-carboxamido)benzoic acid	
(S)-3-(1H-indol-3-yl)-2-(2-(4-oxoquinazolin-3(4H)-yl)acetamido)propanoic acid	

IV. CONCLUSION

In summary, our investigation has identified six unique inhibitors represented in table 6 are responsible for developing aminoglycoside resistance in Shigella strains. The identification of these inhibitors holds significant promise in the realm of pharmaceutical research addressing a broader spectrum of infections caused by shigella. This research underscores the significance of precision inhibition strategies to combat multidrug resistant pathogens and the potential for innovative therapeutic interventions in infectious diseases. Moreover, identifying and developing therapeutics against this infection will help address several sustainable development goals, such as no poverty, decreased inequality, and global health.

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