Ali et al | Article 9110
J Pure Appl Microbiol. 2024;18(2):1167-1176. doi: 10.22207/JPAM.18.2.35
Received: 06 November 2023 | Accepted: 23 January 2024
Published Online: 27 May 2024

P-ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

Novel Drug Repurposing Strategy as an Alternative Therapeutic Concept for Scrub Typhus Using Computational Studies

S. Mohamed Akram Ali[1], N. Helina[2], S. Vinoth Kumar[2], E. Varshini[3], K.MF. Thawfeeq Ahmad[3] and H. Rajamohamed[4]*

[1]Department of Pharmacy Practice, Sri Shanmugha College of Pharmacy, Sankari, Tamil Nadu, India.
[2]Department of Pharmaceutical Chemistry, Thanthai Roever College of Pharmacy, Perambalur, Tamil Nadu, India.
[3]Department of Pharmacy Practice, Thanthai Roever College of Pharmacy, Perambalur, Tamil Nadu, India.
[4]Department of Pharmacy Practice, JSS College of Pharmacy, JSS Academy of Higher Education and Research, Nilgiris, Tamil Nadu, India.

Abstract

Scrub typhus is one of the most underdiagnosed and unreported febrile illnesses caused by an obligate intracellular bacterium named *Orientia tsutsugamushi* and the antibiotics were the commonly prescribed drugs to treat the condition. Due to the widespread development of antimicrobial resistance to the standard drugs, the new therapeutic approach is warranted. The drug repurposing approach plays a novel concept in identifying alternative therapies to fight against pathogens. To investigate the anti-scrub typhus activity of nine newly FDA-approved antibiotics from 2018-2019 against *Orientia tsutsugamushi* deubiquitylase (OtDUB) compared with standard drugs. The structure of ligands was retrieved from the PubChem database and the crystal structure of target OtDUB (PDB ID: 6UPU) with a resolution of 2.2 A° was retrieved from the Protein data bank. Molecular docking studies were performed using PyRx version 0.8 and the amino acid interactions were visualized using BIOVIA Discovery studio and the pharmacokinetic properties of the drugs were analysed by SWISS ADME software. The binding affinity of the drugs to deubiquitylase and amino acids was determined using the *In silico* approach, the drug Omadacycline shows superior activity when compared with other drugs. Based on our preliminary *in-silico* docking studies, we conclude that Omadacycline may be repurposed for the treatment of scrub typhus as it shows a higher binding affinity of -8.6 kcal/mol when compared to the standard drugs. For the further advancement of the study, *in vitro* and *in vivo* studies should be performed.

Keywords: Antibiotics, Drug Repurposing, Omadacycline, *Orientia tsutsugamushi* Deubiquitylase, Scrub typhus

***Correspondence:** rajahaithar@jssuni.edu.in

Abbreviations: FDA, Food and Drug Administration; OtDU, *Orientia tsutsugamushi* deubiquitylase, ARDS, Acute Respiratory Distress Syndrome; CSF, Colony-Stimulating Factor

Citation: Ali SMA, Helina N, Kumar SV, Varshini E, Ahmad KMFT, Rajamohamed H. Novel Drug Repurposing Strategy as an Alternative Therapeutic Concept for Scrub Typhus Using Computational Studies. *J Pure Appl Microbiol.* 2024;18(2):1167-1176. doi: 10.22207/JPAM.18.2.35

INTRODUCTION

In recent years, Rickettsia disease is re-emerging infection found to be the second most common origin of non-malarial febrile illness mainly in the South East Asia after dengue. Recently, there has been a high risk of morbidity and mortality due to infection in different parts of India and neighbouring countries.[1]

Scrub typhus is caused by the gram-negative obligate bacterium *Orientia tsutsugamushi* in the order Rickettsia which is transmitted by the larval stage of the infected vector, a Leptotrombidium mite in the family Trombiculidae. Scrub typhus is usually underdiagnosed because of the non-specific clinical manifestations, lack of access to specific and sensitive diagnostic tests and low suspicion among clinicians. However early treatment helps in shortening the disease course and minimises fatalities. Many states in India have reported a high number of cases of this disease. According to the current estimates, the mortality of Scrub typhus in the present day is in the range of 40% to 45% for cases not treated with antibiotics.[2] Scrub typhus is an endemic disease that affects the Tsutsugamushi Triangle extending from South and East Asia to the Asian Pacific to Northern Australia, where the Tsutsugamushi Triangle is a region of around 13,000,000 Km^2 area of Asia.[3]

The pathogenesis of scrub typhus includes where the host gets infected by *Orientia tsutsugamushi*, then it takes 10-12 days for incubation in the host and develops an eschar-like lesion at the site of the larval bite. The onset of the disease is identified by an unknown fever origin, headache, cough, myalgia and gastrointestinal symptoms.[4] *Orientia tsutsugamushi* enters endothelial cells to cause perivascular inflammatory lesions and disseminated vasculitis that leads to increased vascular permeability and end-organ injury to organs such as pulmonary, heart, and kidney and in some cases, it may cause grievous complications like renal failure, hepatitis, acute respiratory distress syndrome (ARDS) and meningoencephalitis and myocarditis.[5] There were numerous cytokines formed such as granulocyte colony-stimulating factor (CSF), macrophages – CSF, tumour necrosis factor – α and interferon γ, where NK T-cells and the cytotoxic T-lymphocytes serve a major aspect in attacking the defiled host cells. The defence mechanism of the host was downregulated by the organism, where GP-96 is downregulating on the endothelial cells and the macrophages, which play a major part in antigen presentation, antibody production and dendritic cell functioning.[6]

The most frequently observed target cells for this bacterium include endothelial cells, fibroblasts, macrophages, neutrophils and lymphocytes, where endothelial cells are one of the major cellular targets of *O. tsutsugamushi* during systemic infection.[7,8]

Bacterial pathogens often manipulate the host cell membrane-trafficking pathways for the benefit of pathogens to enter the cell, to humiliate immune responses or to gain nutrients.[9] One such protein OtDUB reserved from *Orientia tsutsugamushi* contains a deubiquitylase (DUB) and additional domains, where the expression of OtDUB disturbs the trafficking of membranes through various mechanisms. The presence of protein plays a major role in infection because the ubiquitin-proteasome system performs an important role in host cell defence.[10,11] OtDUB contains a Ubiquitin binding domain ($OtDUB_{UBD}$), a guanine nucleotide exchange factor domain ($OtDUB_{GEF}$) Phosphatidyl serine- binding domain and a domain attached to clathrin adaptor protein complexes 1 & 2 (AP-1, AP-2) without intermediary. During infection, OtDUB affiliates with the endosomal system through its preferential binding with AP-2 complexes.[12] Thus, ubiquitination and DUBs play a significant role in the host immune response during infections, so these could be chosen as potential targets in therapeutics and effective treatments.

Because of non-specific symptoms and signs of scrub typhus, there may be overlap with other tropical infections such as dengue, typhoid, malaria and leptospirosis.[13] Most commonly antimicrobials such as beta-lactam antibiotics are recommended for the management of common infections including typhoid fever.[14] Clinically Scrub typhus has been treated with chloramphenicol or doxycycline. In recent decades, chloramphenicol usage has been minimized because of its toxicity problem, hence making azithromycin, a preferential drug of choice for the initial stage of scrub typhus.[15]

Due to the development of antimicrobial resistance leading to minimal utilization of these agents,[16] the drug repurposing approach using computational tools provides an opportunity to find a potential drug to combat resistance development in serious infectious diseases.[17] This approach provides several merits compared with the development of new drugs, due to its faster and low-cost process and while the drugs were tested in clinical trials for safety and toxicity studies before introducing the drug into the market.[18,19] The current work focuses on the drug repurposing approach using newly FDA-approved antibiotics for their anti-scrub typhus activity targeting *Orientia tsutsugamushi* deubiquitylase (OtDUB) through molecular docking studies. The outcome of the study will help clinicians and pharmaceutical experts to investigate and further validate these drugs as potent anti-scrub typhus against *Orientia tsutsugamushi* infection.

MATERIALS AND METHODS

In silico docking studies

Protein preparation

The crystal structure of the deubiquitylase (*Orientia tsutsugamushi* OtDUB in complex with three molecules of ubiquitin) of PDB ID-6UPU with a resolution of 2.2 A° is used as a target and retrieved through Protein Data Bank. The Protein was prepared using the Biovia Discovery Studio tool, where the water molecules, heteroatoms and the chains B, C, D, F, and G were removed from the macromolecule. Finally, polar hydrogen was added to the target protein and saved in '.pdb' format. The prepared protein is shown in Figure 1.

Ligand preparation

Nine new FDA-approved antibiotic drugs from 2018-2019 and standard drugs for scrub typhus were chosen for this study. Chemical structures were retrieved from the PubChem Database and shown in Table 1. By using Biovia Discovery Studio, the three-dimensional geometry of the compounds was generated and saved in '.pdb' format for further interaction with the target.

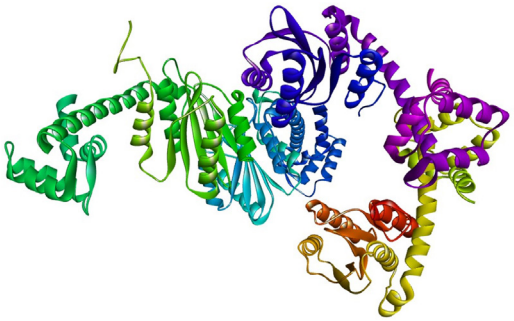


Figure 1. Prepared protein of *Orientia tsutsugamushi* Deubiquitylase (OtDUB)

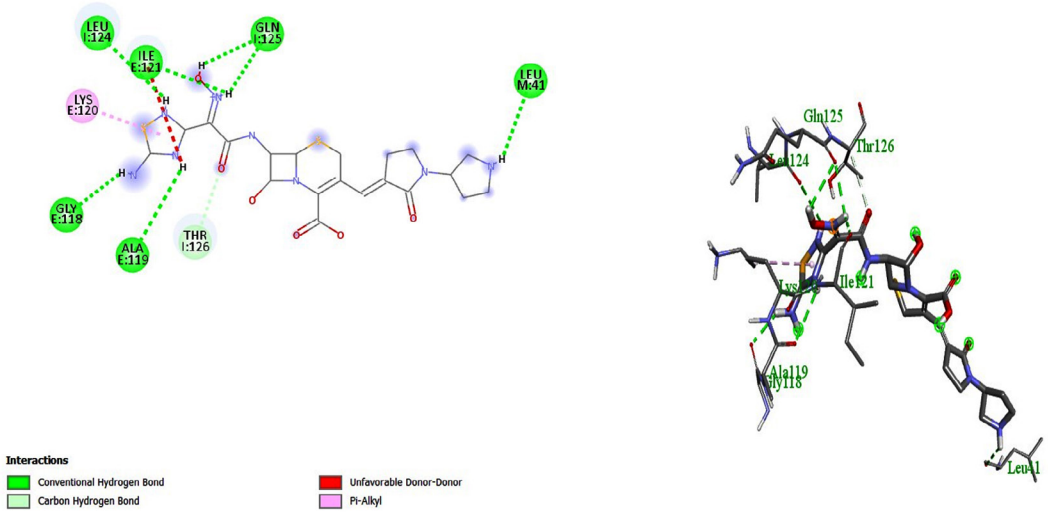
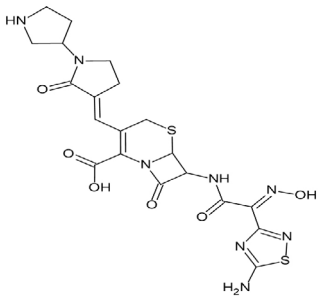
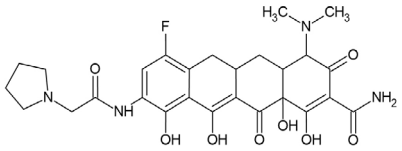
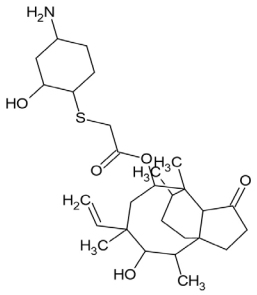
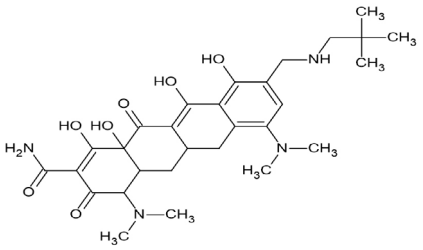
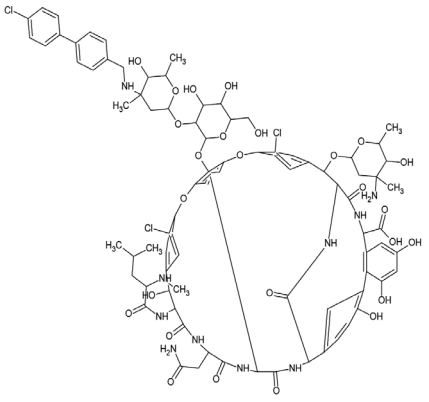
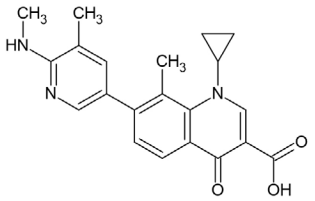


Figure 2. 2D and 3D interactions of Ceftobiprole with *Orientia tsutsugamushi* Deubiquitylase (OtDUB)

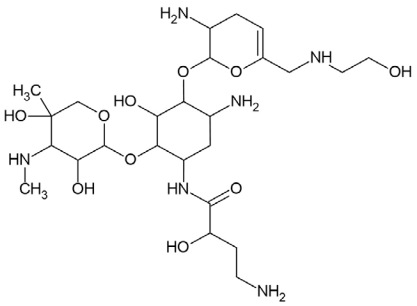
Table 1. Chemical structures of the FDA-approved new drugs

No	FDA-Approved Drugs	Chemical structure
1.	Ceftobiprole	
2.	Eravacycline	
3.	Lefamulin	
4.	Omadacycline	
5.	Oritavancin	

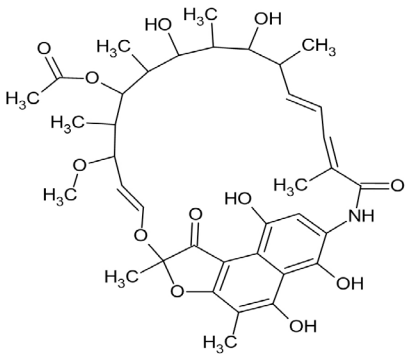
6. Ozenoxacin



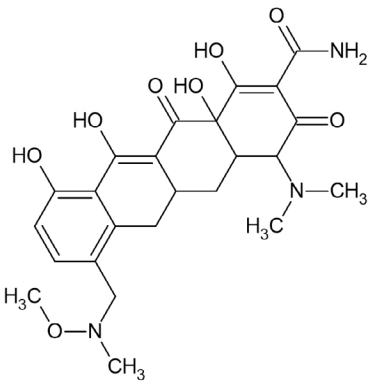
7. Plazomicin



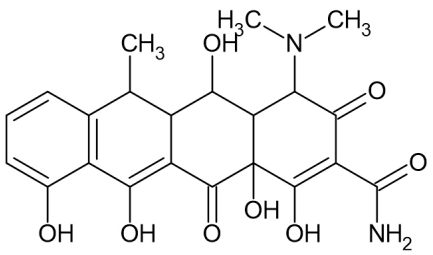
8. Rifamycin



9. Sarecycline



10. Doxycycline



Molecular docking studies

The ligands were docked against deubiquitylase (DUB) and were performed by using the Auto dock tool complied in Pyrx software which is an open-source virtual screening tool. The prepared target has been opened in PyRx software and the macromolecule is converted to PDBQT and then the prepared ligand is imported and minimized, finally converted to PDBQT format. After the ligand and target are ready for docking, click vina wizard, then select the target and molecules for docking, and click forward results in the auto grid generated were predetermined around the active site of the protein. When the docking process is completed, the results are saved as a '.csv' file for future purposes and the docked ligand is analysed for interactions.

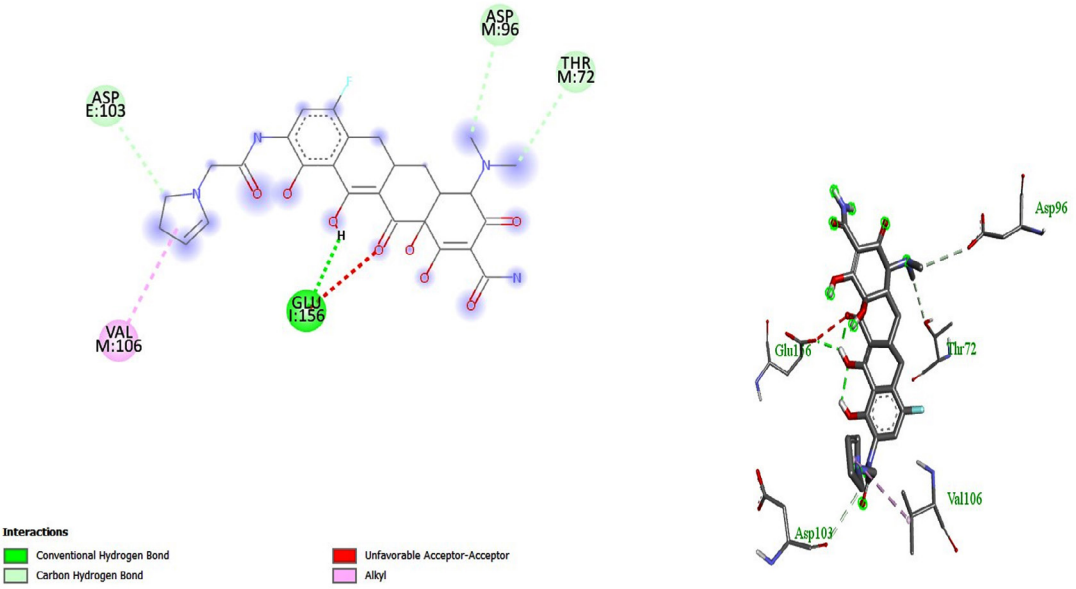


Figure 3. 2D and 3D interactions of Eravacycline with *Orientia tsutsugamushi* Deubiquitylase (OtDUB)

Table 2. Binding affinity for New FDA approved antibiotics and Standard drug

No.	Compound	Binding affinity (kcal/mol)	Hydrogen bond interactions with amino acids	Other interactions
1.	Ceftobiprole	-8.3	Leu 124, Ile 121, Gln 125, Gly 118, Ala 119, Leu 41	Lys 120, Thr 126
2.	Eravacycline	-8.5	Glu 156	Val 106, Asp 103, Asp 96, Thr 72
3.	Lefamulin	-7.9	Arg 117, Tyr 12, Lys 192,	Gln 5
4.	Omadacycline	-8.6	Asn 122, Met 98, Ser 101, Gly 99	Gln 125, Ile 152, Leu 124, Val 100, Pro 151
5.	Oritavancin	-7.2	Lys 113, Thr 155	Ile 109, Val 106, Val 106
6.	Ozenoxacin	-7.3	Ser 93	Gln 150, Met 146, Ile 152, Pro 151
7.	Plazomicin	-6.2	Lys 192, Arg 177, Gln 5	Gln 21
8.	Rifamycin	-8.5	Pro 117, Ile 121, Asn 42, Leu 41, Thr 107	-
9.	Sarecycline	-7.8	Arg 242, Asn 176, Gln 21, His 25	Glu 238, Lys 24
10.	Doxycycline	-7.8	Ile 152, Thr 126, Leu 124	Ile 121

Visualization and analysis of ligand interactions

The protein–ligand interactions were analysed by using Discovery studio visualizer 4.0 a free viewer software. The protein molecule in the form of '.pdbqt' form was imported on the graphical interface of the molecule window followed by the '.pdbqt' file output of ligand. The 2D and 3D structures of compounds with the highest affinity were analysed and visualized.

Physiochemical and pharmacokinetic properties

The screened ligand molecules were further evaluated for their physicochemical and pharmacokinetic properties using the SWISS ADME[20] and PREADMET server. The SMILE format of the ligand molecules was used as input data and uploaded to the server.

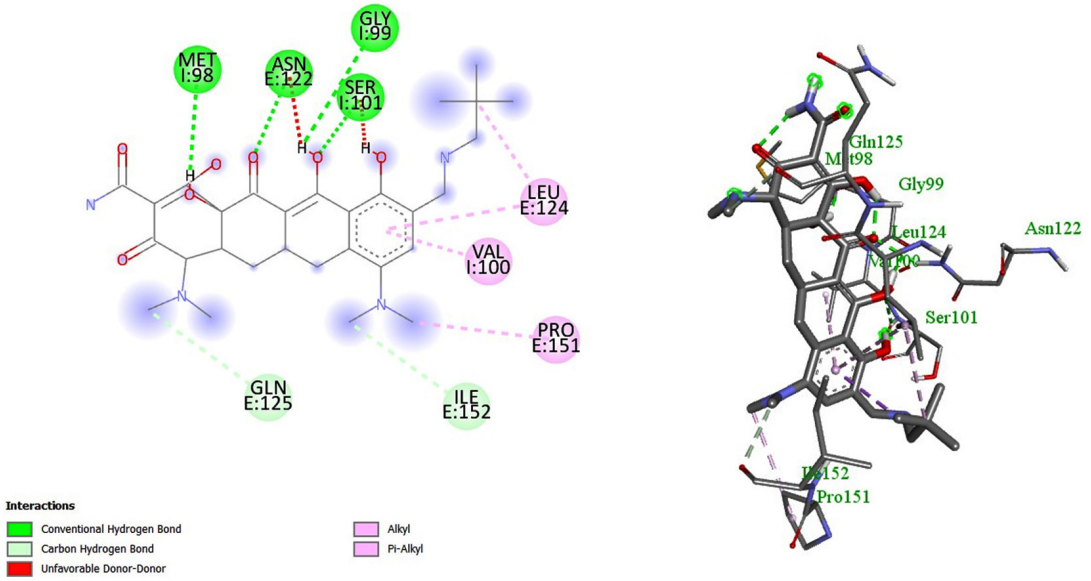


Figure 4. 2D and 3D interactions of Omadacycline with *Orientia tsutsugamushi* Deubiquitylase (OtDUB)

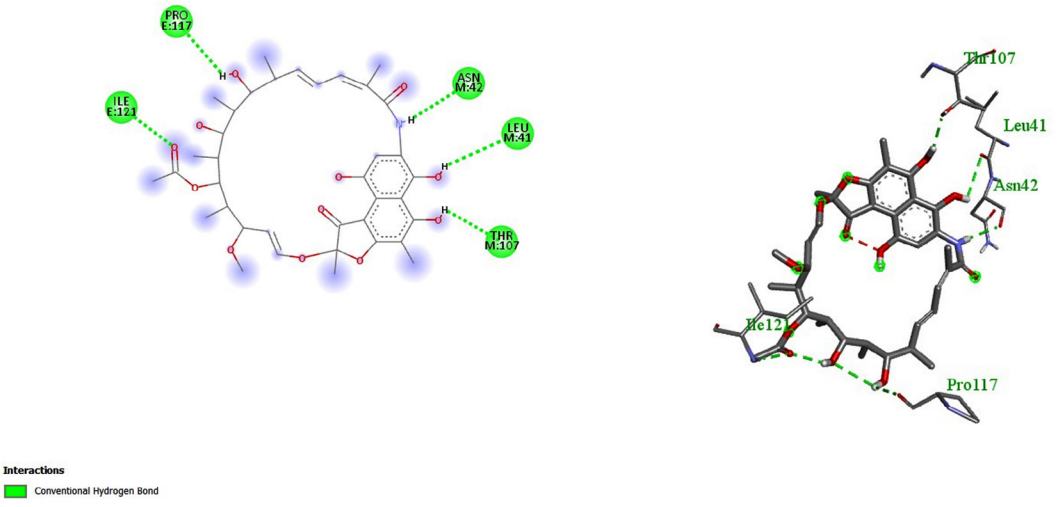


Figure 5. 2D and 3D interactions of Rifamycin with *Orientia tsutsugamushi* Deubiquitylase (OtDUB)

Table 3. Pharmacokinetic Properties of the FDA-Approved New Drugs and Standard Antibiotics for Scrub Typhus

No	DRUG	MF	MW	HBA	HBD	TPSA	GI ABS	BBB	PGP SUBS	Bioavailability score	Synthetic accessibility	Lipinski rule
1.	Ceftobiprole	$C_{20}H_{22}N_8O_6S_2$	534.57	10	5	256.98 Å^2	Low	No	No	0.17	5.61	No
2.	Eravacycline	$C_{27}H_{31}FN_4O_8$	558.56	11	6	193.73 Å^2	Low	No	Yes	0.11	5.56	No
3.	Lefamulin	$C_{28}H_{45}NO_5S$	507.73	6	3	135.15 Å^2	Low	No	Yes	0.55	6.95	Yes
4.	Omadacycline	$C_{29}H_{40}N_4O_7$	556.65	9	6	176.66 Å^2	Low	No	Yes	0.11	5.93	No
5.	Oritavancin	$C_{86}H_{97}Cl_3N_{10}O_{26}$	1793.1	29	20	561	-	-	-	-	-	No
6.	Ozenoxacin	$C_{21}H_{21}N_3O_3$	363.41	4	2	84.22	High	No	No	0.56	2.90	Yes
7.	Plazomicin	$C_{25}H_{48}N_6O_{10}$	592.68	15	11	269.29	Low	No	Yes	0.17	7.15	No
8.	Rifamycin	$C_{37}H_{47}NO_{12}$	697.77	12	6	201.31	Low	No	Yes	0.17	8.26	No
9.	Sarecycline	$C_{24}H_{29}N_3O_8$	487.50	10	5	173.86	Low	No	Yes	0.11	5.34	Yes
10.	Doxycycline	$C_{22}H_{24}N_2O_8$	444.43	9	6	181.62	Low	No	Yes	0.11	5.25	Yes

Abbreviations: MF, Molecular Formula; MW, Molecular Weight; HBA, Hydrogen Bond Acceptors; HBD, Hydrogen Bond Donors; TPSA, Topological Polar Surface Area; GI ABS, Gastrointestinal Absorption; BBB, Blood-Brain Barrier

RESULTS AND DISCUSSION

Docking studies

Pyrx software is a virtual screening tool used for docking between ligands and receptors. Molecular docking studies were performed to study the anti-scrub typhus activity for Nine new FDA-approved antibiotics against *Orientia tsutsugamushi* deubiquitylase (PDB ID-6UPU). All the ligands exhibit various binding affinities (ΔG kcal/mol) ranging from -8.6 to -5.9 kcal/mol for *Orientia tsutsugamushi* Deubiquitylase (OtDUB). From the results, Ceftobiprole (-8.3 kcal/mol), Eravacycline (-8.5 kcal/mol), Omadacycline (-8.6 kcal/mol) and Rifamycin (-8.5 kcal/mol) showed a greater affinity o and compared to that of standard drug Doxycycline of -7.8 kcal/mol.

The drug Ceftobiprole forms six hydrogen bonding interactions with leu 124, Ile 121, Gln 125, Gly 118, Ala 119, and Leu 41 of the active site of the target, where these amino acids form hydrogen bonding with nitrogen atoms of the ligand, pi-alkyl interactions with Lys 120 and carbon-hydrogen bond with Thr 126 and shown in Figure 2. Eravacycline forms one hydrogen bonding interaction between the oxygen atom and with the hydrogen atom of Glu 156 an unfavourable acceptor-acceptor bond with Glu 156, alkyl interaction with Val 106 and carbon–hydrogen bond with Asp 103, Asp 96, Thr 72 as shown in Figure 3. Omadacycline forms four hydrogen bonding interactions between the oxygen atom and with the hydrogen atom of Asn 122, Met 98, Ser 101, Gly 99, carbon-hydrogen bond with Gln 125, Ile 152 and pi-alkyl interaction with Leu 124, Val 100, Pro 151 were depicted in Figure 4. Rifamycin forms one hydrogen bonding interaction between the oxygen atom and with hydrogen atom of Gln 125 as shown in Figure 5, where the standard drug doxycycline showed three hydrogen bonding interactions with Ile 152, Thr 126, Leu 124 and pi-alkyl interaction with Ile 121 of the target site as shown in Figure 6. The binding affinity for the New FDA drugs and standard drugs are shown in Table 2.

Physiochemical and pharmacokinetic analysis

To assess the bioavailability of orally administered drugs, it should obey the Lipinski rule of five, which states that the molecular

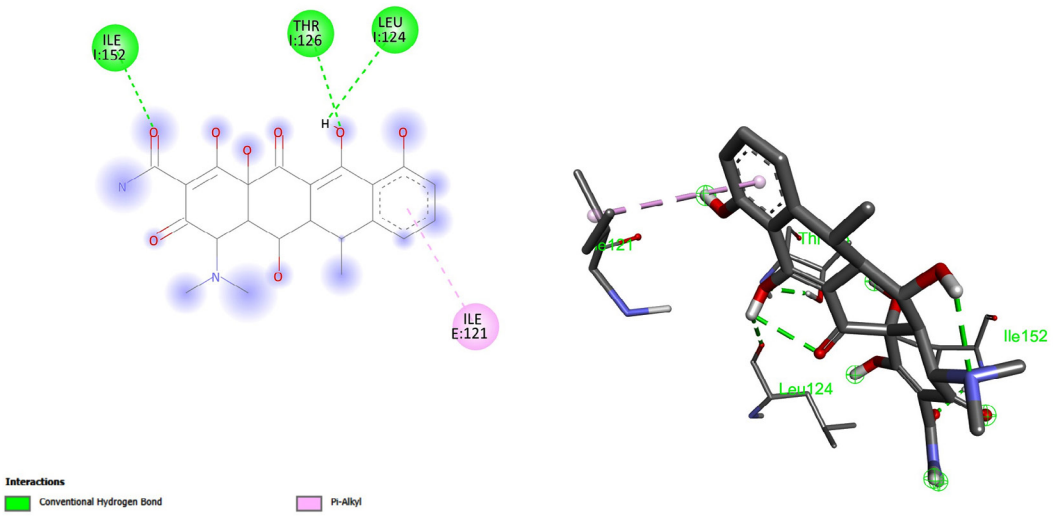


Figure 6. 2D and 3D interactions of Doxycycline with *Orientia tsutsugamushi* Deubiquitylase (OtDUB)

weight < 500, hydrogen bond donor < 5, hydrogen bond acceptor <10 and log p <5.[21] Early analysis of ADME in the drug discovery phases minimize the pharmacokinetic-related problem during the clinical phase.[22] Based on the criteria, all the ligand molecules do not obey the Lipinski rule, except Lefamulin, Ozenoxacin, Sarecycline, and Doxycycline, but they still fit into the FDA-approved drugs. Except for ozenoxacin, most of the drugs showed poor gastrointestinal absorption and all the drugs did not penetrate the blood-brain barrier; the results are shown in Table 3.

CONCLUSION

In recent decades, Scrub typhus has remained an important health issue in endemic places but antibiotic resistance for scrub typhus is still existing. So, drug repurposing is the new concept to discover alternative therapy for Scrub typhus. In this study, we conclude that Omadacycline may be repurposed for the treatment of scrub typhus as it shows a higher binding affinity of -8.6 kcal/mol to OtDU and other amino acids when compared to the standard drug and other new FDA-approved drugs. For the further advancement of the study, *in vitro* and *in vivo* studies should be performed.

ACKNOWLEDGMENTS

None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

SVK designed the study. NH, KMFTA and EV performed the docking study and interpreted the data. SMAA and HR wrote, reviewed and edited the manuscript. All authors read and approved the final manuscript for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

REFERENCES

1. Sarangi R, Pradhan S, ch Debata N and Mahapatra S. Clinical profile of scrub typhus in children treated in a tertiary care hospital in eastern India. *Pediatria Polska.* 2016;91(4):308-311. doi: 10.1016/j.pepo.2016.04.005
2. Kore VB, Mahajan SM. Recent threat of scrub typhus in India: a narrative review. *Cureus.* 2022;14(10).

doi: 10.7759/cureus.30092
3. Kumar D, Raina DJ, Gupta S, Angurana A. Epidemiology of scrub typhus. *JK Science*. 2010;12(2):60.
4. Rajapakse S, Rodrigo C, Fernando D. Scrub typhus: pathophysiology, clinical manifestations and prognosis. *Asian Pac J Trop Med.* 2012;5(4):261-264. doi: 10.1016/S1995-7645(12)60036-4
5. Trent B, Fisher J, Soong L. Scrub typhus pathogenesis: innate immune response and lung injury during *Orientia tsutsugamushi* infection. *Front Microbiol.* 2019;10:2065. doi: 10.3389/fmicb.2019.02065
6. Rajapakse S, Weeratunga P, Sivayoganathan S and Fernando SD. Clinical manifestations of scrub typhus. *Trans R Soc Trop Med Hyg.* 2017;111(2):43-54. doi: 10.1093/trstmh/trx017
7. Salje J. Cells within cells: Rickettsiales and the obligate intracellular bacterial lifestyle. *Nat Rev Microbiol.* 2021;19(6):375-390. doi: 10.1038/s41579-020-00507-2
8. Ge Y, Rikihisa Y. Subversion of host cell signaling by *Orientia tsutsugamushi*. *Microbes Infect.* 2011;13(7):638-648. doi: 10.1016/j.micinf.2011.03.003
9. Schertzer JW, Whiteley M. Bacterial outer membrane vesicles in trafficking, communication and the host-pathogen interaction. *J Mol Microbiol Biotechnol.* 2013;23(1-2):118-130. doi: 10.1159/000346770
10. Berk JM, Lim C, Ronau JA, et al. A deubiquitylase with an unusually high-affinity ubiquitin-binding domain from the scrub typhus pathogen *Orientia tsutsugamushi. Nat Commun.* 2020;11:2343. doi: 10.1038/s41467-020-15985-4
11. Adcox HE, Berk JM, Hochstrasser M, Carlyon JA. *Orientia tsutsugamushi* OtDUB Is Expressed and Interacts with Adaptor Protein Complexes during Infection. *Infect Immun.* 2022;90(12):e0046922. doi: 10.1128/iai.00469-22
12. Berk JM, Lee MJ, Zhang M, Lim C, Hochstrasser M. OtDUB from the human pathogen Orientia tsutsugamushi modulates host membrane trafficking by multiple mechanisms. *Mol Cell Biol.* 2022;42(7):e0007122. doi: 10.1128/mcb.00071-22
13. Rebecca J, Varghese GM. Scrub typhus: a reemerging infection. *Curr Opin Infect Dis.* 2020;33(5):365-371. doi: 10.1097/QCO.0000000000000664
14. El Sayed I, Liu Q, Wee I, Hine P. Antibiotics for treating scrub typhus. *Cochrane Database of Systematic Reviews.* 2018;9(9):CD0012150. doi: 10.1002/14651858.CD002150.pub2
15. Varghese GM, Dayanand D, Gunasekaran K, et al. Intravenous Doxycycline, Azithromycin, or Both for Severe Scrub Typhus. *N Engl J Med.* 2023;388(9):792-803. doi: 10.1056/NEJMoa2208449
16. Kelly DJ, Fuerst PA, Richards AL. The historical case for and the future study of antibiotic-resistant scrub typhus. *Trop Med Infect Dis.* 2017;2(4):63. doi: 10.3390/tropicalmed2040063
17. Park K. A review of computational drug repurposing. *Trans Clin Pharmacol.* 2019;27(2):59-63. doi: 10.12793/tcp.2019.27.2.59
18. Rangel-Vega A, Bernstein LR, Mandujano-Tinoco EA. Garcia-Contreras SJ, Garcla-Contreras R. Drug repurposing as an alternative for the treatment of recalcitrant bacterial infections. *Front Microbiol.* 2015;6:82. doi: 10.3389/fmicb.2015.00282
19. Liu Y, Tong Z, Shi J, Li R, Upton M, Wang Z. Drug repurposing for next-generation combination therapies against multidrug-resistant bacteria. *Theranostics.* 2021;11(10):4910-4928. doi: 10.7150/thno.56205
20. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep.* 2017;7:42717. doi: 10.1038/srep42717
21. Lipinski CA. Lead- and drug-like compounds: the rule-of-five revolution. *Drug Discov Today Technol.* 2004;1(4):337-341. doi: 10.1016/j.ddtec.2004.11.007
22. Hay M, Thomas DW, Craighead JL, Economides C, Rosenthal J. Clinical development success rates for investigational drugs. *Nat Biotechnol.* 2014; 32:40-51. doi: 10.1038/nbt.2786