UNIVERSITI PUTRA MALAYSIA

DESIGN AND CHARACTERIZATION OF INHIBITORY PEPTIDES AGAINST BLEG1_2478, B3 SUBCLASS METALLO-β-LACTAMASE

GAYATHRI A/P SELVARAJU

FBSB 2021 36

DESIGN AND CHARACTERIZATION OF INHIBITORY PEPTIDES AGAINST BLEG1_2478, B3 SUBCLASS METALLO-β-LACTAMASE

By

GAYATHRI A/P SELVARAJU

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

April 2021

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

DESIGN AND CHARACTERIZATION OF INHIBITORY PEPTIDES AGAINST BLEG1_2478, B3 SUBCLASS METALLO-β-LACTAMASE

By

GAYATHRI A/P SELVARAJU

April 2021

Chairman : Associate Professor Normi binti Mohd Yahaya, PhD
Faculty : Biotechnology and Biomolecular Sciences

Metallo-β-lactamase (MBL) is an enzyme which hydrolyses β-lactam antibiotics. Their production by bacteria, particularly bacterial pathogens, is one of the mechanisms used to resist the action of the antibiotics. MBL requires zinc ions for this particular function. There are four classes of MBLs, B1, B2, B3 and B4 MBLs. Among them B3 MBLs do not have available clinical inhibitors and they have the widest substrate degradation spectrum. Previously, a hypothetical protein (HP) termed Bleg1_2478, which has a 3-D predicted structure and tested activity spectrum similar to B3 class MBL was discovered from *Bacillus lehensis* G1 alkaliphile. However, phylogenetic analysis showed that it is not related to any currently circulating B3 MBLs. As clinical inhibitors for B3 MBL are absent and that Bleg1_2478 is not related to any currently circulating B3 MBLs, there is a need to develop inhibitors specifically for Bleg1_2478. Therefore, this study aimed to design and characterise peptides as inhibitors against Bleg1_2478. Inhibitory peptides were designed by retrieving peptides from $CAMP_{R3}$ database and subsequently derivated based on functional residues around Bleg1_2478 active site which contains the zinc binding site. The binding energies of the peptides were determined via fixed protein-ligand docking using YASARA and AutoDock Vina software and compared with those of the preferred substrate, ampicillin. As a result, nine peptides with higher binding energies (>8.52 kcal/mol) towards the enzyme Bleg1_2478 were successfully designed. These peptides were then used for global protein-ligand docking to investigate other possible binding sites on the protein other than its active site. Inhibitory assay of these peptides on purified Bleg1_2478 recombinant protein was performed at 1, 10 and 20 μM respectively. The inhibitory peptides, RSWPWH and SSWWDR, depicted approximately 50% of inhibition of Bleg1_2478 at concentrations as low as 0.90 μM and 0.50 μM respectively. Analysis of the peptide-protein interaction via isothermal titration calorimetry (ITC) showed a 1.5 and 3 fold increase in the binding affinity of RSWPWH and SSWWDR respectively towards

Bleg1_2478; as compared to ampicillin. More significant is the binding strength of these peptides whereby they exhibited a respective 34 to 68-fold increase compared to ampicillin. Similar to ampicillin, both of the inhibitory peptides bind to Bleg1_2478 at one binding site, as can be observed from their stoichiometric value. Physicochemical computation of both peptides revealed, the basic or cationic nature of RSWPWH and its predicted binding site near the vicinity of the active site of Bleg1_2478 may have contributed for to its ease of interaction with Bleg1_2478, hence, giving forth free energy (G) and enthalpy factor values that are more favourable and spontaneous. The dissociation constant, K_d, revealed that RSWPWH is more susceptible to dissociate from the protein due to the location of its binding site which expose it to pH changes caused by the cellular environment. On the other hand, SSWWDR inhibitory peptide is less prone to dissociate from the protein as it has zero net charge and it binds to the narrow groove of the Bleg1_2478 active site, an area that is less accessible and less susceptible to changes in the cellular environment. In conclusion, both peptides obtained can be used as a potential inhibitor against Bleg1_2478 and possibly other B3 MBLs.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

REKAAN DAN PENCIRIAN PEPTIDA PERENCATAN TERHADAP BLEG1_2478, METALLO-β-LACTAMASE SUBKELAS B3

Oleh

GAYATHRI A/P SELVARAJU

April 2021

Pengerusi : Profesor Madya Normi binti Mohd Yahaya, PhD
Fakulti : Bioteknologi dan Sains Biomolekul

Metalo-β-laktamase (MBL) ialah enzim yang menghidrolisis antibiotik β-laktam. Penghasilannya oleh bakteria, terutama bakteria patogenik merupakan salah satu mekanisme yang digunakan untuk merintangi tindakan antibiotik. MBL memerlukan ion zink bagi fungsi ini. Terdapat 4 kelas MBL iaitu B1, B2, B3 and B4 MBL. Di kalangannya subkelas B3 MBL tidak mempunyai sebarang perencat klinikal dan ia mempunyai spektrum degradasi substrat yang paling luas. Sebelumnya, suatu protein hipotetikal (HP) yang dkenali sebagai Bleg1_2478, yang mempunyai struktur ramalan 3D dan spektrum aktiviti yang diuji yang sama seperti subkelas B3 MBL telah ditemui di dalam *Bacillus lehensis* G1 alkalifil. Namun, analisis filogenetik menunjukkan bahawa ia tidak mempunyai perkaitan dengan sebarang B3 MBL yang sedang beredaran. Oleh kerana perencat klinikal untuk B3 MBL masih tiada dan Bleg1_2478 tidak berkaitan dengan sebarang B3 MBL yang sedang beredaran, terdapat keperluan untuk membangunkan perencat khususnya untuk Bleg1_2478. Maka, kajian ini bertujuan untuk mereka cipta dan mencirikan peptida sebagai perencat terhadap Bleg1_2478. Peptida perencat telah direka dengan memuat turun peptida dari pangkalan data CAMPR3 dan kemudiannya diterbitkan berdasarkan residu-residu fungsian di sekitar tapak aktif Bleg1_2478 yang mengandungi tapak pengikatan zink. Tenaga pengikatan peptida ditentukan melalui pendokan tetap protein-ligan dengan menggunakan perisian YASARA dan AutoDock Vina dan dibandingkan dengan substrat terpilih iaitu ampisilin. Hasilnya, sembilan peptida dengan tenaga pengikatan yang lebih tinggi (>8.52 kcal/mol) terhadap enzim Bleg1_2478 berjaya direka cipta. Peptida-peptida ini kemudian digunakan untuk pendokan global protein-ligan untuk menyiasat tapak pengikatan yang berkemungkinan ada pada protein tersebut selain daripada tapak aktifnya. Ujian perencatan peptida ini pada protein rekombinan Bleg1_2478 yang telah ditulenkan dilakukan pada 1, 10 dan 20 μM masing-masing. Peptida perencat, RSWPWH dan SSWWDR, menunjukkan anggaran 50% perencatan pada Bleg1_2478 pada kepekatan serendah 0.90 μM dan

0.50 μM masing-masing. Analisis interaksi peptida-protein melalui isotermal kalorimetri penittratan (ITC) menunjukkan peningkatan sebanyak 1.5 dan 3 kali ganda dalam afiniti pengikatan RSWPWH dan SSWWDR masing-masing terhadap Bleg1_2478; berbanding dengan ampisilin. Lebih signifikan ialah kekuatan pengikatan peptida ini di mana kedua-duanya menunjukkan peningkatan 34 hingga 68 kali ganda berbanding ampisilin. Sama seperti ampisilin, kedua-dua peptida perencat mengikat pada Bleg1_2478 pada satu tapak pengikat, seperti yang boleh diperhatikan daripada nilai stoikiometrinya. Pengiraan fizikokimia kedua-dua peptida mendedahkan bahawa sifat bes atau kationik RSWPWH dan tapak pengikatannya yang diramalkan berhampiran kawasan aktif Bleg1_2478 mungkin telah menyumbang kepada kemudahannya berinteraksi dengan Bleg1_2478, maka, memberikan tenaga bebas (G) dan nilai faktor entalpi yang lebih digemari dan spontan. Pemalar penceraian, K_d, mendedahkan bahawa RSWPWH lebih cenderung untuk berpisah dari protein tersebut kerana lokasi tapak pengikatannya yang mendedahkannya kepada perubahan pH yang disebabkan oleh persekitaran sel. Sebaliknya, peptida perencat SSWWDR tidak cenderung untuk berpisah daripada protein tersebut kerana ia mempunyai cas bersih sifar dan ia mengikat pada alur sempit tapak aktif Bleg1_2478, iaitu suatu kawasan yang sukar diakses dan kurang cenderung terhadap perubahan persekitaran sel. Kesimpulannya, kedua-dua peptida yang diperoleh boleh digunakan sebagai perencat yang berpotensi terhadap Bleg1_2478 dan kemungkinan juga B3 MBL yang lain.

ACKNOWLEDGEMENTS

Foremost, I would like to express gratitude to my supervisor, Associate Professor Dr. Normi Mohd Yahaya, for giving me the opportunity to work in this project. I could not find words to express my love and respect for her. She has been a great supervisor who always motivates me, and she is honest, strict, and patient. Her guidance helped me a lot in research and writing this thesis, and it is impossible to complete this project without her support. Next, I would like to thank my supervisory committee, Professor Dato' Dr. Abu Bakar Bin Salleh, and Associate Professor Dr. Adam Leow Thean Chor, for their guidance and insights on this research area. Moreover, my sincere appreciation to all the lecturers of the Enzyme and Microbial Technology (EMTech) research group.

My sincere gratitude to Ms. Azyyati Binti Mohd. Padzil, Malaysian Genome Institute (MGI), for guiding me in Isothermal Titration Calorimetry (ITC). My sincere thanks extended to all the postgraduate students of EMTech and Protein Engineering Laboratory, who shared their knowledge and also supported me. My gratitude extended to the staff of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia (UPM), for their help and cooperation. I am so grateful to *L'Oréal-Unesco Fellowship for Women in Science* grant (Grant No.: 6300833), Putra Research Grant UPM (Grant No.:9502100), and Graduate Research Fellowship (GRF) for providing financial support to carry out this research.

I am very much indebted to my parents, Mr. Selvaraju Gopal and Mrs. Tulasiamah Angamuthu, and all my family members for their love and moral support. Last but not least, my friends Ashwaani Mangavelu, Revathi Kavirajan, Durgadevi Mohan, and Fatin Lyana Azman Shah, who stood with me in my ups and downs. Finally, my sincere appreciation extended to the people who indirectly contributed to this research. Thank you.

This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Normi binti Mohd Yahaya, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Abu Bakar bin Salleh, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Adam Leow Thean Chor, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 09 September 2021

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software

Signature: _____ Date: _____

Name and Matric No: Gayathri a/p Selvaraju

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) were adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: Associate Professor Dr. Normi binti Mohd Yahaya

Signature: _____
Name of Member of Supervisory Committee: Professor Dr. Abu Bakar bin Salleh

Signature: _____
Name of Member of Supervisory Committee: Associate Professor Dr. Adam Leow Thean Chor

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
µg/mL	Microgram per milliliter
µL	Microliter
µM	Micro molar
3D	Three Dimensional
Å	Angstrom
A_{600nm}	Optical density at wavelength 600 nanometer
Ala	Alanine
AMP	Antimicrobial peptide
AMR	Antimicrobial resistance
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
BSA	Bovine serum albumin
CA	Community-associated
Cation-π	Cation-pi
Cys	Cysteine
DNA	Deoxyribonucleic acid
EDTA	Ethylene-diamine-tetraacetic acid
g	Gram
Gln	Glutamine
Glu	Glutamic acid
Gly	Glycine
HA	Hospital-associated

H-Bond	Hydrogen Bond
His	Histidine
HIV	Human immunodeficiency virus
Ile	Isoleucine
IPTG	Isopropyl-Beta-D-Thiogalactoside
Kcal/ mol	kilocalorie per mole
k Da	Kilo Dalton
kJ/ mol	kilo Joule per mole
L	Litre
LB	Luria-Bertani
Leu	Leucine
M	Molar
MBL	Metallo-β-lactamase
Met	Methionine
$MgSO_4$	Magnesium Sulphate
mL	Milli Liter
mM	Millimolar
MRSA	Methycillin-resistant staphylococcus aureus
NaCl	Sodium chloride
$NaPO_4$	Sodium phosphate
Ni	Nickel
nm	Nano meter
ns	Non-susceptible
OD	Optical Density
PBP	Penicillin-binding protein
Phe	Phenylalanine

Pro	Proline
s	Seconds
SDS - PAGE	Sodium deodecyl sulphate Polyacrylamide gel electrophoresis
Ser	Serine
Thr	Threonine (T)
Tyr	Tyrosine
w/v	Weight per volume
WHO	World Health Organization
Zn	Zinc
$ZnSO_4$	Zinc Sulfate
α	Alpha
β	Beta
π-π	pi-pi

CHAPTER 1

INTRODUCTION

β-lactam antibiotics have been used widely as frontline therapeutics in treating bacteria related infections and diseases (Huttner et al., 2015). These molecules specifically target bacterial pathogens by interfering bacterial cell wall synthesis, which will eventually cause cell lysis. However, the emergence of antimicrobial resistance (AMR) among bacterial pathogens had raised major concerns in global public health.

AMR can render the commonly used antibiotics and antimicrobial therapy ineffective, prolong hospital stay and increase medical expenses. Severe cases of AMR can lead to more complicated medical procedures such as surgery to remove the focal point of infection and even untimely deaths. AMR will exert a huge impact on the world economy in the future if the current situation is not tackled. According *Antimicrobial Resistance:Tackling a crisis for the health and wealth of nations* (2014), the forecasted death toll by the year 2050 due to AMR is reported to be 300 million people with declination of world's GDP around 2 to 3.5% (O'Nei, 2014).

AMR among bacteria can be acquired through various means i.e. through: (1) hydrolysis or inactivation of antibiotics by synthesizing enzymes (Bassetti et al., 2013); (2) redox process i.e. exploiting the oxidation or reduction process of the antibiotics (Yang et al., 2004);(3) modification of antibiotics by chemical substitution (Schwarz et al., 2005); (4) target modification (modification of the target site) (Spratt, 1994); (5) mutations on genes that encode the target or efflux pump that effect antibiotics uptake (Ruiz, 2003); (6) horizontal gene transfer where resistant genes are transferred from one pathogen to another via transduction, conjugation or transformation (Abushaheen et al., 2020).

One of the well-studied AMR mechanisms involves β-lactamase enzymes. β-lactamases deactivates β-lactam antibiotics before they reach their target. They exert their function by hydrolysing the β-lactam ring of the β-lactam antibiotics. Metallo-β-lactamases (MBLs) are β-lactamases that specifically require Zn^{2+} metal ions as cofactor for their catalysis and are among the most studied group of β-lactamases due to their broad substrate spectrum. Among MBLs, the B3 subclass in particular has garnered attention in the recent decade due to their inability to be inhibited by commonly used clinical inhibitors or drug combinations (Rojas *et al*., 2017; Gangadharappa et al., 2019).

As a result, the growth of the antibiotics industry in search of new molecules experienced tremendous growth. The *Antibiotics Market Size and Share: Industry Trends Report, 2019-2026* (2019) highlighted that the global antibiotics market for the year 2018 was USD 45.31 billion and was forecasted to reach USD 62.06 billion by the year 2025 with the compound annual growth rate (CAGR) of 4.0% per year. One of the main reasons for the increment in growth of the market size is due to the imbalance of supply and demand.

Previously, a hypothetical protein termed Bleg1_2437 (currently renamed as Bleg1_2478) which has comparable sequence identity to MBL in the range of 43-65% was discovered from the pool of hypothetical proteins of *Bacillus lehensis* G1 alkaliphile. Its predicted *in silico* structure revealed that Bleg1_2478 contained the αββα fold and global topology similar to MBLs. Analysis on its active site and metal-binding ligands revealed similarity to B3 MBLs. Biochemical analysis of purified recombinant Bleg1_2478 protein showed β-lactam hydrolysis with ampicillin as the preferred substrate (Tan et al., 2017). However, based on evolutionary relationship, it did not exhibit relatedness to other currently circulating B3 MBLs (Tan et al., 2017).

With the lack of inhibitors against B3 MBLs and the fact that Bleg1_2478 is not related to any currently circulating B3 MBLs, and yet has the ability to hydrolyse β-lactam antibiotics, this is of concern to local public health related to AMR. Hence, the general aim of this study is to design inhibitory peptides against Bleg1_2478 B3 subclass MBL and characterize their inhibitory potential and properties. The specific objectives are:

1. To design inhibitory peptides against Bleg1_2478 via *in silico* approach
2. To evaluate their binding properties and effect on the structure of Bleg1_2478 B3 MBL via docking analysis
3. To biochemically and biophysically characterize the inhibitory activities and properties of the peptides via *in vitro* assays with Bleg1_2478 purified enzyme.

REFERENCES

Abushaheen, M. A., Fatani, A. J., Alosaimi, M., Mansy, W., George, M., Acharya, S., Rathod, S., Divakar, D.D., Jhugroo, C., Vellappally, S., and Khan, A. A. (2020). Antimicrobial resistance, mechanisms and its clinical significance. *Disease-a-Month*, *66*(6), 100971.

Ambler, R. P. (1980). The structure of β-lactamases. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, *289*(1036): 321-331.

Antimicrobial Resistance:Tackling a crisis for the health and wealth of nations (2014). *Review on Antimicrobial Resistance*. Retrieved on 25th May, 2021 from https://amr-review.org/sites/default/files/AMR%20Review%20Paper%20-%20Tackling%20a%20crisis%20for%20the%20health%20and%20wealth%20of%20nations_1.pdf

Antibiotics Market Size & Share: Industry Trends Report, 2019-2026. (2019).Retrieved on 15th June, 2020 from https:// www. grandviewresearch. com/industry-analysis/antibiotic-market

Baier, F., and Tokuriki, N. (2014). Connectivity between catalytic landscapes of the metallo-β-lactamase superfamily. *Journal of Molecular Biology*, *426*(13), 2442-2456.

Balsalobre, L., Blanco, A., and Alarcón, T. (2019). Beta-Lactams. In J. Capelo-Martínez and G. Igrejas (Eds), *Antibiotic Drug Resistance*. (pp. 57-72). John Wiley & Sons, Inc.

Bassetti, M., Merelli, M., Temperoni, C., and Astilean, A. (2013). New antibiotics for bad bugs: where are we?. *Annals of Clinical Microbiology and Antimicrobials*, *12*(1), 22.

Bebrone, C. (2007). Metallo-β-lactamases (classification, activity, genetic organization, structure, zinc coordination) and their superfamily. *Biochemical Pharmacology*, *74*(12), 1686-1701.

Bebrone, C., Lassaux, P., Vercheval, L., Sohier, J. S., Jehaes, A., Sauvage, E., and Galleni, M. (2010). Current challenges in antimicrobial chemotherapy. *Drugs*, *70*(6), 651-679.

Bennett, P. M. (2008). Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria. *British Journal of Pharmacology*, *153*(S1), S347-S357.

Boeckel, T. P., Gandra, S., Ashok, A., Caudron, Q., Grenfell, B. T., Levin, S. A., and Laxminarayan, R. (2014). Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. *The Lancet Infectious Diseases, 14*(8), 742-750.

Boschi, L., Mercuri, P. S., Riccio, M. L., Amicosante, G., Galleni, M., Frère, J. M., andRossolini, G. M. (2000). The *Legionella (Fluoribacter) gormanii* metallo-β-lactamase: a new member of the highly divergent lineage of molecular subclass B3 β-lactamases. *Antimicrobial Agents and Chemotherapy*, *44*(6), 1538-1543.

Beta-lactam and Beta-lactamase Inhibitors Market 92019). Allied Market Research. Retrieved on 14th January, 2020 from http://www. alliedmarketresearch.com/beta-lactam-and-beta-lactamase-inhibitor-market.

Brem, J., Van Berkel, S. S., Aik, W., Rydzik, A. M., Avison, M. B., Pettinati, Umland, K.D., Kawamura A., Spencer, J., Claridge, T.D.W., McDonough, M. A. and Schofield, C.J. (2014). Rhodanine hydrolysis leads to potent thioenolate mediated metallo-β-lactamase inhibition. *Nature Chemistry*, *6*(12), 1084.

Bush, K., and Bradford, P. A. (2016). β-Lactams and β-lactamase inhibitors: an overview. *Cold Spring Harbour Perspectives in Medicine*, *6*(8): a025247.

Bush, K, Jacoby, G.A., and Medeiros, A.A. (1995). A functional classification scheme for beta-lactamases and its correlation with molecular structure. *Antimicrobial Agents and Chemotherapy*, *39*(6), 1211-1233.

Butler, M. S., Blaskovich, M. A., and Cooper, M. A. (2017). Antibiotics in the clinical pipeline at the end of 2015. *The Journal of Antibiotics*, *70*(1), 3-24.

Buynak, J. D., Chen, H., Vogeti, L., Gadhachanda, V. R., Buchanan, C. A., Palzkill, T., Shaw, R. W., Spencer, J. and Walsh, T. R. (2004). Penicillin-derived inhibitors that simultaneously target both metallo-and serine-β-lactamases. *Bioorganic & Medicinal Chemistry Letters*, *14*(5), 1299-1304.

CDC (2013). Antibiotic. threats in the United States. Centers for Diseases Control (CDC) Georgia

Chen, D. E., Willick, D. L., Ruckel, J. B., & Floriano, W. B. (2015). Principal component analysis of binding energies for single-point mutants of hT2R16 bound to an agonist correlate with experimental mutant cell response. *Journal of Computational Biology*, *22*(1), 37-53.

Cho, H., Uehara, T., and Bernhardt, T. G. (2014). Beta-lactam antibiotics induce a lethal malfunctioning of the bacterial cell wall synthesis machinery. *Cell*, *159*(6), 1300-1311.

Coan, K. E., Maltby, D. A., Burlingame, A. L., & Shoichet, B. K. (2009). Promiscuous aggregate-based inhibitors promote enzyme unfolding. *Journal of Medicinal Chemistry*, *52*(7), 2067-2075.

Cornaglia, G., Giamarellou, H., and Rossolini, G. M. (2011). Metallo-β-lactamases: a last frontier for β-lactams?. *The Lancet Infectious Diseases*, *11*(5), 381-393.

Crowder, M. W., Spencer, J., and Vila, A. J. (2006). Metallo-β-lactamases: novel weaponry for antibiotic resistance in bacteria. *Accounts of Chemical Research*, *39*(10), 721-728.

Damian, L. 2013. Isothermal Titration Calorimetry for Studying Protein–Ligand Interactions. In *Protein-Ligand Interactions. Methods in Molecular Biology (Methods and Protocols)*, eds. M. A. Williams and T. Daviter, pp. 103-118. Totowa, NJ: Humana Press.

Drawz, S. M., and Bonomo, R. A. (2010). Three Decades of Beta-Lactamase. *Clinical Microbiology Review, 23*(1), 160-201.

Duff, M.R., Grubbs, J. and Howell, E.E. 2011. Isothermal titration calorimetry for measuring macromolecule-ligand affinity. *Journal of Visualized Experiments* 55: 2796.

Faridoon, M., Hussein, W. M., Vella, P., Ul Islam, N., Ollis, D., Schenk, G., and McGeary, R. P. (2012). 3-Mercapto-1, 2, 4-triazoles and N-acylated thiosemicarbazides as metallo-β-lactamase inhibitors. *Bioorganic & Medicinal Chemistry Letters*, *22*(1), 380-386.

Felici, A., Amicosante, G., Oratore, A., Strom, R., Ledent, P., Joris, B., Fanuel, L., and Frère, J. M. (1993). An overview of the kinetic parameters of class B β-lactamases. *Biochemical Journal*, *291*(1), 151-155.

Feng, L., Yang, K. W., Zhou, L. S., Xiao, J. M., Yang, X., Zhai, L., Zhang, Y.L. and Crowder, M. W. (2012). N-heterocyclic dicarboxylic acids: broad-spectrum inhibitors of metallo-β-lactamases with co-antibacterial effect against antibiotic-resistant bacteria. *Bioorganic & Medicinal Chemistry Letters*, *22*(16), 5185-5189.

Ford, C. B., Shah, R. R., Maeda, M. K., Gagneux, S., Murray, M. B., Cohen, T., Johnston, J.C., Gardy, J., Lipsitch, M. and Fortune, S. M. (2013). Mycobacterium tuberculosis mutation rate estimates from different lineages predict substantial differences in the emergence of drug-resistant tuberculosis. *Nature Genetics*, *45*(7), 784-790.

Galleni, M., and Frère, J. M. (2007). Kinetics of β-lactamases and penicillin binding proteins. In R.A.Bonomo and M.Tolmasky (Eds), *Enzyme-Mediated Resistance to Antibiotics* (pp. 195-213). *American Society of Microbiology*.

Gallivan, J. P., and Dougherty, D. A. (1999). Cation-π interactions in structural biology. *Proceedings of the National Academy of Sciences*, *96*(17), 9459-9464.

Gan, M., Liu, Y., Bai, Y., Guan, Y., Li, L., Gao, R., He, W., You, Xuefu, Li, Y., Yu, L., and Xiao, C. (2013). Polyketides with New Delhi metallo-β-lactamase 1 inhibitory activity from *Penicillium* sp. *Journal of Natural Products*, 76(9), 1535-1540.

Gangadharappa, B. S., Sharath, R., Revanasiddappa, P. D., Chandramohan, V., Balasubramaniam, M., and Vardhineni, T. P. (2019). Structural insights of metallo-beta-lactamase revealed an effective way of inhibition of enzyme by natural inhibitors. *Journal of Biomolecular Structure and Dynamics*, *38*(13), 3757-3771.

Garau, G., Bebrone, C., Anne, C., Galleni, M., Frère, J. M, and Dideberg, O. (2005). A metallo-β-lactamase enzyme in action: crystal structures of the monozinc carbapenemase CphA and its complex with biapenem. *Journal of Molecular Biology 345*(4), 785-795.

Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S., Wilkins, M. R., Appel, R. D., and Bairoch, A. (2005). Protein Identification and Analysis Tools on the ExPASy Server. *The Proteomics Protocols Handbook,* 571-607.

Gomes, C. M., Frazão, C., Xavier, A. V., Legall, J., and Teixeira, M. (2002). Functional control of the binuclear metal site in the metallo-β-lactamase-like fold by subtle amino acid replacements. *Protein Science*, *11*(3), 707-712.

González, M. M., Kosmopoulou, M., Mojica, M. F., Castillo, V., Hinchliffe, P., Pettinati, I., Brem, J., Schofield, C.J., Mahler, G., Bonomo, R.A., Llarrull, L.I., Spencer, J., and Vila, A.J. (2015). Bisthiazolidines: a substrate-mimicking scaffold as an inhibitor of the NDM-1 carbapenemase. *ACS Infectious Diseases*, *1*(11), 544-554.

González, M. M., and Vila, A. J. (2016). An elusive task: a clinically useful inhibitor of metallo-β-lactamases. In Supuran C., Capasso C. (Eds) Zinc Enzyme Inhibitors (pp. 1-34). Springer, Cham.

Gudeta, D. D., Pollini, S., Docquier, J. D., Bortolaia, V., Rossolini, G. M., and Guardabassi, L. (2016). Biochemical characterization of CPS-1, a subclass B3 metallo-β-lactamase from a *Chryseobacterium piscium* soil isolate. *Antimicrobial Agents and Chemotherapy*, *60*(3), 1869-1873.

Hall, B. G., Salipante, S. J., and Barlow, M. (2004). Independent Origins of Subgroup Bl+ B2 and Subgroup B3Metallo-β-Lactamases. *Journal of Molecular Evolution*, *59*(1), 133-141.

Hammond, G. G., Huber, J. L., Greenlee, M. L., Laub, J. B., Young, K., Silver, L. L., Balkovec, J.M., Pryor, K.D., Wu, J.K., Leiting, B., Pompliano, D. L. and Toney, J.H. (1999). Inhibition of IMP-1 metallo-β-lactamase and sensitization of IMP-1-producing bacteria by thioester derivatives. *FEMS Microbiology Letters*, *179*(2), 289-296.

Hiraiwa, Y., Morinaka, A., Fukushima, T., and Kudo, T. (2009). Metallo-β-lactamase inhibitory activity of phthalic acid derivatives. *Bioorganic & Medicinal Chemistry Letters*, 19(17), 5162-5165.

Holmes, A. H., Moore, L. S., Sundsfjord, A., Steinbakk, M., Regmi, S., Karkey, A., Guerin, P., and Piddock, L. J. (2016). Understanding the mechanisms and drivers of antimicrobial resistance. *The Lancet*, 387(10014), 176-187.

Holten, K. B., and Onusko, E. M. (2000). Appropriate prescribing of oral beta-lactam antibiotics. *American Family Physician*, *62*(3), 611-620.

Hussein, W. M., Fatahala, S. S., Mohamed, Z. M., McGeary, R. P., Schenk, G., Ollis, D. L., and Mohamed, M. S. (2012). Synthesis and Kinetic Testing of Tetrahydropyrimidine-2-thione and Pyrrole Derivatives as Inhibitors of the Metallo-β-lactamase from *Klebsiella pneumonia* and *Pseudomonas Aeruginosa*. *Chemical Biology & Drug Design*, 80(4), 500-515.

Huttner, A., Harbarth, S., Hope, W. W., Lipman, J., and Roberts, J. A. (2015). Therapeutic drug monitoring of the β-lactam antibiotics: what is the evidence and which patients should we be using it for?.*Journal of Antimicrobial Chemotherapy*, 70(12), 3178-3183.

Ishii, Y., Eto, M., Mano, Y., Tateda, K., and Yamaguchi, K. (2010). In vitro potentiation of carbapenems with ME1071, a novel metallo-β-lactamase inhibitor, against metallo-β-lactamase-producing *Pseudomonas aeruginosa* clinical isolates. *Antimicrobial Agents and Chemotherapy*, 54(9), 3625-3629.

Ju, L. C., Cheng, Z., Fast, W., Bonomo, R. A., and Crowder, M. W. (2018). The continuing challenge of metallo-β-lactamase inhibition: mechanism matters. *Trends in Pharmacological Sciences*, *39*(7), 635-647.

Kapoor, G., Saigal, S., and Elongavan, A. (2017). Action and resistance mechanisms of antibiotics: A guide for clinicians. *Journal of Anaesthesiology, Clinical Pharmacology*, *33*(3), 300–305.

Kim, Y., Maltseva, N., Wilamowski, M., Tesar, C., Endres, M., and Joachimiak, A. (2020). Structural and biochemical analysis of the metallo-β-lactamase L1 from emerging pathogen Stenotrophomonas maltophilia revealed the subtle but distinct di-metal scaffold for catalytic activity. *Protein Science*, *29*(3), 723-743.

King, A. M., Reid-Yu, S. A., Wang, W., King, D. T., De Pascale, G., Strynadka, N. C., Walsh, T.R.W., Coombes, B.K. and Wright, G. D. (2014). Aspergillomarasmine A overcomes metallo-β-lactamase antibiotic resistance. *Nature*, *510*(7506), 503-506.

Klingler, F. M., Wichelhaus, T. A., Frank, D., Cuesta-Bernal, J., El-Delik, J., Müller, H. F., Sjuts, H., Gottig, S., Koenigs, A., Proschak, E., Pos, K.E. and Pogoryelov, D. (2015). Approved drugs containing thiols as inhibitors of metallo-β-lactamases: strategy to combat multidrug-resistant bacteria. *Journal of Medicinal Chemistry*, *58*(8), 3626-3630.

Krieger, E., Koraimann, G., and Vriend, G. (2002). Increasing the precision of comparative models with YASARA NOVA - A self-parameterizing force field. *Proteins: Structure, Function and Genetics*, *47*(3), 393–402.

Kurosaki, H., Yamaguchi, Y., Higashi, T., Soga, K., Matsueda, S., Yumoto, H., Misumi, S. and Goto, M. (2005). Irreversible Inhibition of Metallo-β-lactamase (IMP-1) by 3- (3-Mercaptopropionylsulfanyl) propionic Acid Pentafluorophenyl Ester. *Angewandte Chemie International Edition*, *44*(25), 3861-3864.

Laemmli, U. K. (1970). Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. *Nature*, *227*(5259), 680–685.

Lai, C. C., Lee, K., Xiao, Y., Ahmad, N., Veeraraghavan, B., Thamlikitkul, V.,Tambyah, P.A., Nelwan, R.H.H, Shibl, A.M., Wu, J.J, and Seto, W. H. (2014). High burden of antimicrobial drug resistance in Asia. *Journal of Global Antimicrobial Resistance*, *2*(3), 141-147.

Lakshmi, R., Nusrin, K. S., Ann, G. S., and Sreelakshmi, K. S. (2014). Role of beta lactamases in antibiotic resistance: A review. *International Research Journal of Pharmacy*, *5*(2), 37-40.

Lee, N., Yuen, K. Y., and Kumana, C. R. (2003). Clinical role of β-lactam/β-lactamase inhibitor combinations. *Drugs*, *63*(14), 1511-1524.

Lienard, B. M., Garau, G., Horsfall, L., Karsisiotis, A. I., Damblon, C., Lassaux, P., Papamicael, C., Roberts, G.C., Galleni, M., Dideberg, O., Frère, J.M., and Schofield, C.J. (2008). Structural basis for the broad-spectrum inhibition of metallo-β-lactamases by thiols. *Organic & Biomolecular Chemistry*, *6*(13), 2282-2294.

Lienard, B. M., Horsfall, L. E., Galleni, M., Frère, J. M., and Schofield, C. J. (2007). Inhibitors of the FEZ-1 metallo-β-lactamase. *Bioorganic & Medicinal Chemistry Letters*, *17*(4), 964-968.

Liu, X. L., Shi, Y., Kang, J. S., Oelschlaeger, P., and Yang, K. W. (2015). Amino acid thioester derivatives: a highly promising scaffold for the development of metallo-β-lactamase L1 inhibitors. *ACS Medicinal Chemistry Letters*, *6*(6), 660-664.

Lonsdale, R., and Ward, R. A. (2018). Structure-based design of targeted covalent inhibitors. *Chemical Society Reviews*, *47*(11), 3816-3830.

Marston, H. D., Dixon, D. M., Knisely, J. M., Palmore, T. N., and Fauci, A. S. (2016). Antimicrobial resistance. *Jama*, *316*(11), 1193-1204.

Minond, D., Saldanha, S. A., Subramaniam, P., Spaargaren, M., Spicer, T., Fotsing, J. R., Weide, T., Fokin, V.V., Sharpless k.B., Galleni, M., Bebrone, C., Lassaux, P., and Hodder, P. (2009). Inhibitors of VIM-2 by screening pharmacologically active and click-chemistry compound libraries. *Bioorganic & Medicinal Chemistry*, *17*(14), 5027-5037.

Mohamed, M. S., Hussein, W. M., McGeary, R. P., Vella, P., Schenk, G., and El-hameed, R. H. A. (2011). Synthesis and kinetic testing of new inhibitors for a metallo-β-lactamase from *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. *European Journal of Medicinal Chemistry*, *46*(12), 6075-6082.

Moloughney, J. G., D. Thomas, J., and Toney, J. H. (2005). Novel IMP-1 metallo-β-lactamase inhibitors can reverse meropenem resistance in Escherichia coli expressing IMP-1. *FEMS Microbiology Letters*, *243*(1), 65-71.

Montagner, C., Nigen, M., Jacquin, O., Willet, N., Dumoulin, M., Karsisiotis, A. I., and Matagne, A. (2016). The role of active site flexible loops in catalysis and of zinc in conformational stability of Bacillus cereus 569/H/9 β-lactamase. *Journal of Biological Chemistry*, *291*(31), 16124-16137.

Muley, L., Baum, B., Smolinski, M., Freindorf, M., Heine, A., Klebe, G., and Hangauer, D. G. (2010). Enhancement of hydrophobic interactions and hydrogen bond strength by cooperativity: synthesis, modeling, and molecular dynamics simulations of a congeneric series of thrombin inhibitors. *Journal of Medicinal Chemistry*, *53*(5), 2126-2135.

Nagano, R., Adachi, Y., Hashizume, T., and Morishima, H. (2000). In vitro antibacterial activity and mechanism of action of J-111,225, a novel 1 β-methylcarbapenem, against transferable IMP-1 metallo-β-lactamase producers. *Journal of Antimicrobial Chemotherapy*, *45*(3), 271-276.

Olsen, L., Jost, S., Adolph, H. W., Pettersson, I., Hemmingsen, L., and Jorgensen, F. S. (2006). New leads of metallo-β-lactamase inhibitors from structure-based pharmacophore design. *Bioorganic & Medicinal Chemistry*, *14*(8), 2627-2635.

Palzkill, T. (2013). Metallo-β-lactamase structure and function. *Annals of the New York Academy of Sciences*: *1277*, 91–104.

Payne, D. J., Hueso-Rodríguez, J. A., Boyd, H., Concha, N. O., Janson, C.A., Gilpin,M., Bateson, Cheever, C., Niconovich, N.L., Pearson, S., Rittenhouse, S., Tew, D., Diez, E., Perez, P., de la Fuente, J. Rees, M. and Rivera-Sagredo, A. (2002). Identification of a series of tricyclic natural products as potent broad-spectrum inhibitors of metallo-β-lactamases. *Antimicrobial Agents and Chemotherapy*, *46*(6), 1880-1886.

Phelan, E. K., Miraula, M., Selleck, C., Ollis, D. L., Schenk, G., and Mitić, N. (2014). Metallo-β-Lactamases: A Major Threat to Human Health. *American Journal of Molecular Biology AJMB*, *04*(03), 89-104.

Pratt, R. F., and McLeish, M. J. (2010). Structural relationship between the active sites of β-lactam-recognizing and amidase signature enzymes: convergent evolution?. *Biochemistry*, *49*(45), 9688-9697.

Qian, S. B., Waldron, L., Choudhary, N., Klevit, R. E., Chazin, W. J., and Patterson, C. (2009). Engineering a ubiquitin ligase reveals conformational flexibility required for ubiquitin transfer. *Journal of Biological Chemistry*, *284*(39), 26797-26802.

Rojas, L. J., Hujer, A. M., Rudin, S. D., Wright, M. S., Domitrovic, T. N., Marshall, S. H., Hujer, K.M., Richter, S.S., Cober, E., Perez, F., Adams, M.D., D, D., and Bonomp, R.A. (2017). NDM-5 and OXA-181 beta-lactamases, a significant threat continues to spread in the Americas. *Antimicrobial Agents and Chemotherapy*, *61*(7).

Rotondo, C. M., and Wright, G. D. (2017). Inhibitors of metallo-β-lactamases. *Current Opinion in Microbiology*, 39, 96-105.

Rotondo, C. M., Marrone, L., Goodfellow, V. J., Ghavami, A., Labbé, G., Spencer, J., Dmitrienko, G.I., & Siemann, S. (2015). Arginine-containing peptides as potent inhibitors of VIM-2 metallo-β-lactamase. *Biochimica et Biophysica Acta (BBA)-General Subjects*, *1850*(11), 2228-2238.

Rozwandowicz, M., Brouwer, M. S. M., Fischer, J., Wagenaar, J. A., Gonzalez-Zorn, B., Guerra, B., Mevius, D.J., and Hordijk, J. (2018).Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae. *Journal of Antimicrobial Chemotherapy*, *73*(5), 1121-1137.

Ruiz, J. (2003). Mechanisms of resistance to quinolones: target alterations, decreased accumulation and DNA gyrase protection. *Journal of Antimicrobial Chemotherapy*, *51*(5), 1109-1117.

Sacha, P., Wieczorek, P., Hauschild, T., Zórawski, M., Olszańska, D., and Tryniszewska, E. (2008). Metallo-beta-lactamases of Pseudomonas aeruginosa--a novel mechanism resistance to beta-lactam antibiotics. *Folia Histochemica et cytobiologica*, *46*(2), 137-142.

Sawa, T., Kooguchi, K., and Moriyama, K. (2020). Molecular diversity of extended-spectrum β-lactamases and carbapenemases, and antimicrobial resistance. *Journal of Intensive Care*, *8*(1), 13.

Schwarz, S., Cloeckaert, A., and Roberts, M. C. (2005). Mechanisms and spread of bacterial resistance to antimicrobial agents. In F.M. Aerustrup (Ed), *Antimicrobial resistance in bacteria of animal origin* (pp.73-98). ASM Press.

Siemann, S., Evanoff, D. P., Marrone, L., Clarke, A. J., Viswanatha, T., and Dmitrienko, G. I. (2002). N-arylsulfonyl hydrazones as inhibitors of IMP-1 metallo-β-lactamase. *Antimicrobial Agents and Chemotherapy*, *46*(8), 2450-2457.

Somboro, A. M., Sekyere, J. O., Amoako, D. G., Essack, S. Y., and Bester, L. A. (2018). Diversity and proliferation of metallo-β-lactamases, a clarion call for clinically effective metallo-β-lactamase inhibitors. *Applied and Environmental Microbiology*, *84*(18), e00698-18.

Spratt, B. G. (1994). Resistance to antibiotics mediated by target alterations. *Science*, *264*(5157), 388-393.

Stachyra, T., Péchereau, M. C., Bruneau, J. M., Claudon, M., Frère, J. M., Miossec, C., Coleman, K., and Black, M. T. (2010). Mechanistic studies of the inactivation of TEM-1 and P99 by NXL104, a novel non-β-lactam β-lactamase inhibitor. *Antimicrobial Agents and Chemotherapy*, *54*(12), 5132-5138.

Sun, Q., Law, A., Crowder, M. W., and Geysen, H. M. (2006). Homo-cysteinyl peptide inhibitors of the L1 metallo-β-lactamase, and SAR as determined by combinatorial library synthesis. *Bioorganic & Medicinal Chemistry Letters*, *16*(19), 5169-5175.

Tan, S. H., Normi, Y. M., Leow, A. T. C., Salleh, A. B., Murad, A. M. A., Mahadi, N. M., and Rahman, M. B. A. (2017). Danger lurking in the "unknowns": structure-to-function studies of hypothetical protein Bleg1_2437 from Bacillus lehensis G1 alkaliphile revealed an evolutionary divergent B3 metallo-beta-lactamase. *The Journal of Biochemistry*, *161*(2), 167-186.

Thomas, P. W., Cammarata, M., Brodbelt, J. S., and Fast, W. (2014). Covalent Inhibition of New Delhi Metallo-β-Lactamase-1 (NDM-1) by cefaclor. *ChemBioChem*, *15*(17), 2541-2548.

Toney, J. H., Fitzgerald, P. M., Grover-Sharma, N., Olson, S. H., May, W. J.,Sundelof, J. G., Vanderwall, D.E., Clearly, K.A., Grant, S.K. Wu, J.K., Kozarich, J.W., and Pompliano, D.L. (1998). Antibiotic sensitization using biphenyl tetrazoles as potent inhibitors of *Bacteroides fragilis* metallo-β-lactamase. *Chemistry & Biology*, *5*(4), 185-196.

Toney, J. H., Hammond, G. G., Fitzgerald, P. M., Sharma, N., Balkovec, J. M., Rouen, G. P., Olson, S.H., Hammond, M.L., Greenlee, M.L., and Gao, Y. D. (2001). Succinic acids as potent inhibitors of plasmid-borne IMP-1 metallo-β-lactamase. *Journal of Biological Chemistry*, *276*(34), 31913-31918.

Toussaint, K. A., and Gallagher, J. C. (2015). β-Lactam/ β-lactamase inhibitor combinations: from then to now. *Annals of Pharmacotherapy*, *49*(1), 86-98.

Trott, O., and Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, *31*(2), 455–461.

Van Boeckel, T. P., Brower, C., Gilbert, M., Grenfell, B. T., Levin, S. A., Robinson, T. P., Teilant, A., and Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences*, *112*(18), 5649-5654.

Vella, P., Miraula, M., Phelan, E., Leung, E. W., Ely, F., Ollis, D. L., McGeary, R.P., Schenk, G., and Mitić, N. (2013). Identification and characterization of an unusual metallo-β-lactamase from Serratia proteamaculans. *JBIC Journal of Biological Inorganic Chemistry*, *18*(7), 855-863.

Ventola, C. L. (2015). The antibiotic resistance crisis: part 1: causes and threats. *Pharmacy and Therapeutics*, *40*(4), 277–283.

Wachino, J. I., Yamaguchi, Y., Mori, S., Jin, W., Kimura, K., Kurosaki, H., and Arakawa, Y. (2016). Structural insights into recognition of hydrolyzed carbapenems and inhibitors by subclass B3 metallo-β-lactamase SMB-1. *Antimicrobial Agents and Chemotherapy*, *60*(7), 4274-4282.

Waghu, F. H., Barai, R. S., Gurung, P., and Idicula-Thomas, S. (2016). CAMPR3: a database on sequences, structures and signatures of antimicrobial peptides. *Nucleic Acids Research*, *44*(D1), D1094-D1097.

Walsh, T. R., Toleman, M. A., Poirel, L., and Nordmann, P. (2005). Metallo-β-lactamases: the quiet before the storm?. *Clinical Microbiology Reviews*, *18*(2), 306-325.

Wang, G., Li, X., and Wang, Z. (2016). APD3: the antimicrobial peptide database as a tool for research and education. *Nucleic Acids Research*, *44*(D1), D1087-D1093.

Wang, X., Lu, M., Shi, Y., Ou, Y., and Cheng, X. (2015). Discovery of novel New Delhi metallo-β-lactamases-1 inhibitors by multistep virtual screening. *PloS One, 10*(3), e0118290.

Weide, T., Saldanha, S. A., Minond, D., Spicer, T. P., Fotsing, J. R., Spaargaren, M., Frere, J.M., Sharpless, K.B., Hodder, P.S., and Fokin, V. V. (2010). NH-1, 2, 3-triazole inhibitors of the VIM-2 metallo-β-lactamase. *ACS Medicinal Chemistry Letters*, *1*(4), 150-154.

WHO, (2014). Antimicrobial resistance global report on surveillance. 2014. World Health Organization (WHO). WHO. Geneva.

WHO. (2018). Global antimicrobial resistance surveillance system (GLASS) report: early implementation 2017–2018 (2018). World Health Organization (WHO). Geneva.

Wilke, M. S., Lovering, A. L., and Strynadka, N. C. (2005). β-Lactam antibiotic resistance: a current structural perspective. *Current Opinion in Microbiology*, *8*(5), 525-533.

Wishart, D. S., Knox, C., Guo, A. C., Shrivastava, S., Hassanali, M., Stothard, P., Chang, Z. and Woolsey, J. (2006). DrugBank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Research*, *34*(suppl_1), D668-D672.

Xiang, Y., Chang, Y. N., Ge, Y., Kang, J. S., Zhang, Y. L., Liu, X. L., Oelschlaeger, P., & Yang, K. W. (2017). Azolylthioacetamides as a potent scaffold for the development of metallo-β-lactamase inhibitors. *Bioorganic & Medicinal Chemistry Letters*, *27*(23), 5225-5229.

Yang, W., Moore, I. F., Koteva, K. P., Bareich, D. C., Hughes, D. W., and Wright, G. D. (2004). TetX is a flavin-dependent monooxygenase conferring resistance to tetracycline antibiotics. *Journal of Biological Chemistry*, *279*(50), 52346-52352.

Zhang, Y. L., Yang, K. W., Zhou, Y. J., LaCuran, A. E., Oelschlaeger, P., and Crowder, M. W. (2014). Diaryl-Substituted Azolylthioacetamides: Inhibitor Discovery of New Delhi Metallo-β-Lactamase-1 (NDM-1). *ChemMedChem*, *9*(11), 2445-2448.