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Computational modelling of potential Zn-sensitive non- β -lactam inhibitors of imipenemase-1 (IMP-1)

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ABSTRACT

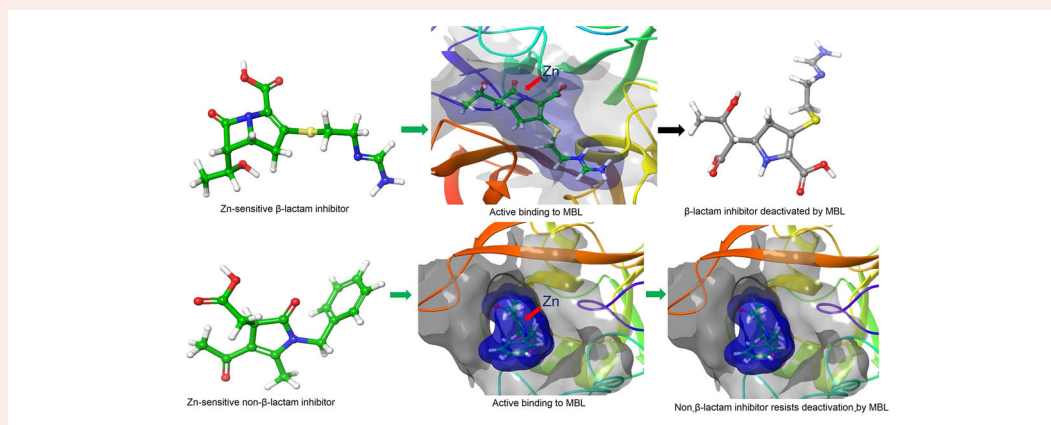
Antibiotic resistance (AR) remains one of the leading global health challenges, mostly implicated in disease-related deaths. The *Enterobacteriaceae*-producing metallo- β -lactamases (MBLs) are critically involved in AR pathogenesis through Zn-dependent catalytic destruction of β -lactam antibiotics, yet with limited successful clinical inhibitors. The efficacy of relevant broad-spectrum β -lactams including imipenem and meropenem are seriously challenged by their susceptibility to the Zn-dependent carbapenemase hydrolysis, as such, searching for alternatives remains imperative. In this study, computational molecular modelling and virtual screening methods were extensively applied to identify new putative Zn-sensitive broad-spectrum inhibitors of MBLs, specifically imipenemase-1 (IMP-1) from the IBScreen database. Three ligands, **STOCK35-30154**, **STOCK35-30418** and **STOCK35-30514** selectively displayed stronger binding interactions with the enzymes compared to reference inhibitors, **imipenem** and **meropenem**. For instance, the ligands showed molecular docking scores of -9.450 , -8.005 and -10.159 kcal/mol, and MM-GBSA values of -40.404 , -31.902 and -33.680 kcal/mol respectively against the IMP-1. Whereas, **imipenem** and **meropenem** showed docking scores of -9.038 and -10.875 kcal/mol, and MM-GBSA of -31.184 and -32.330 kcal/mol respectively against the enzyme. The ligands demonstrated good thermodynamic stability and compactness in complexes with IMP-1 throughout the 100 ns molecular dynamics (MD) trajectories. Interestingly, their binding affinities and stabilities were significantly affected in contacts with the remodelled Zn-deficient IMP-1, indicating sensitivity to the carbapenemase active Zn site, however, with non- β -lactam scaffolds, tenable to resist catalytic hydrolysis. They displayed ideal drug-like ADMET properties, thus, representing putative Zn-sensitive non- β -lactam inhibitors of IMP-1 amenable for further experimental studies.

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Zn-chelating inhibitors



Introduction

Antibiotic resistance (AR) of pathogenic germs (bacteria and fungi) occurs when the infective microorganisms develop defensive features against the drugs that are designed to

inhibit their growth. Although, AR occurs in non-pathogenic microorganisms, however, it is predominantly amplified in pathogenic concerns (Aarts & Margolles, 2014; Larsson & Flach, 2022). It remains one of the major public health

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challenges of the 21st century and is currently atop the leading causes of death globally (CDC, 2019; Murray et al., 2022). The current burden of AR is much greater than initially understood. For instance, in 2019, $\approx$ 4.95 million deaths were associated with antibiotic-resistant bacteria, with the highest rate (27.3 deaths/100,000) recorded in sub-Saharan Africa. The developed nations are also not exonerated from the menace. In the US in 2019, more than 2.8 million people were reportedly infected with AR-associated diseases annually while $>35,000$ of those cases resulted in mortality (CDC, 2019; Murray et al., 2022; Schaenzer & Wright, 2020). Although, the concerted efforts by the US healthcare system have reduced the death rate from AR generally by 18% as shown in CDC reports of 2013 and 2019 (CDC, 2019). However, the carbapenem-resistant *Enterobacteriaceae* and drug-resistant tuberculosis (TB) remain stable, a great concern. For instance, the rate of infection by the extended-spectrum beta-lactamases (ESBL)-producing *Enterobacteriaceae*, drug-resistant *Neisseria gonorrhoeae* and Erythromycin-resistant invasion group A strep has drastically increased by 50–315% within the time frame. The burden of the rapid global spread of AR has become critical and could result in more pathogenic organisms becoming much more lethal than the present ones if left unchecked (Murray et al., 2022). A recent scientific view also highlights the insufficiency in the explorations for identifying potent inhibitors of the metallo- β -lactamases (MBLs) beyond the serine-active β -lactams and expatiated the deserving urgent interventions to overcome the global health challenge. Thus, the expansion of therapeutic innovations to curb the infection menace, reduce the spread and treat the infected people remains critically required (CDC, 2019; Mojica et al., 2022).

Up to 18 pathogenic microorganisms have been identified with various degrees of threats ranging from urgent, and serious to concerning, among which the MBL-producing constitute the most alarming threats (CDC, 2019). Metallo- β -lactamases are renowned chromosomal enzymes produced by the carbapenem-resistant gram-negative bacteria with limited successful inhibitors in clinical conditions up to this moment to match their rapid global spread due to the incessant AR development (Kar et al., 2021; Tan et al., 2021). They are of great concern due to the prevalent implication in nosocomial infections caused by their producer superbugs, *Enterobacteriales* including *Acinetobacter baumannii*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, making the organisms among the leading causes of AR-attributed deaths (Murray et al., 2022). The imipenemase (IMP-1), Verona integron-encoded metallo- β -lactamase (VIM-1) and New Delhi metallo- β -lactamase (NDM-1) are among the most relevant MBLs since their respective first discoveries in 1988, 1997 and 2008. In their current variants of >1000 , they constitute an overwhelming challenge in AR science through the incessant epidemic evasion of almost all labelled β -lactam antibiotics even in combinations, except the monobactams (Boyd et al., 2020; Chen et al., 2017; Kar et al., 2021).

The β -lactam antibiotics remain the most frequently applied extended-spectrum β -lactam (ESBL) inhibitors in

clinical conditions for the treatment of MBL-associated infections including the severe and high-risk cases admitted into hospital intensive care units. Due to their broad-spectrum efficacy and relative tolerability, they are currently prescribed for treating patients of coronavirus 2019 with microbial complications (Iyer, 2022; Mojica et al., 2022). The drugs include carbapenem, ertapenem, imipenem and meropenem, all enriched with β -lactam ring (Figure 1a) essentially for effective inhibition of Ser-enzymes, transpeptidases by impairing their cell wall synthesis. However, their pharmacological efficacy is increasingly beleaguered by AR inclusively developed from drug misuse or overuse. The resultant effects especially on their lipophilic β -lactams include the activation of efflux pumps, alteration of targets, blockade of porin water-filled channels and most importantly, hydrolytic catalysis by the MBLs (Arjomandi et al., 2019; Ayipo, Osunniran, et al., 2021; Iyer, 2022; van Bambeke et al., 2017). The mechanisms of MBL-mediated AR occur through the covalent binding of the enzymes to β -lactam antibiotics at the Zn(II) coordination site, thereby inducing the catalytic hydrolysis and eventual cleavage of the β -lactam ring, the main pharmacophoric group (Ayipo, Osunniran, et al., 2022; Lima et al., 2020). Some of the enzymes such as the serine β -lactamases, KPC and OXA-48 subgroups are additionally involved in the deacetylation of the β -lactam antibiotics, thereby inducing the release of the hydrolysed/inactivated drug and protecting the bacterial cell wall against the antibiotic effects (Boyd et al., 2020; van den Akker & Bonomo, 2018). The coordination of mononuclear/binuclear Zn ions to the nucleophilic H₂O molecules resultantly induces cleavage, leading to their deactivation and eventual release of the drug (Figure 1b). However, potent Zn-sensitive inhibitors with non- β -lactam chemical scaffolds have been shown to resist deactivation by the MBLs, thereby amenable for preserving the antibiotic integrity against the enzymes (Figure 1c) (Arjomandi et al., 2019; Lisa et al., 2017). Thus, a continuous efforts for the development of new clinical inhibitors with broad-spectrum, multi-target mechanisms against the relevant MBLs and structural resistance to carbapenemase hydrolytic cleavage remains imperative (Yan et al., 2020).

Although, scientific efforts are continuously being channelled towards identifying effective candidates with strong inhibitory binding to the active sites of the MBLs and lesser susceptibility to the β -lactamase catalytic activity. However, no successful candidate is yet available for clinical applications, making a continuous search for effective candidates remains crucial.

Computer-aided drug design (CADD) method aids in faster, environmentally friendly and cost-effective identification of potential drug candidates from large compound libraries *via de novo* fragment- or structure-based designs. It represents a core element of the modern drug discovery phase, incorporating molecular and quantum modelling, bioinformatics and cheminformatics for the optimization of such potentials for the other two resource- and time-demanding phases of therapeutic development, i.e. experimental validation and registry. The technique has been extensively

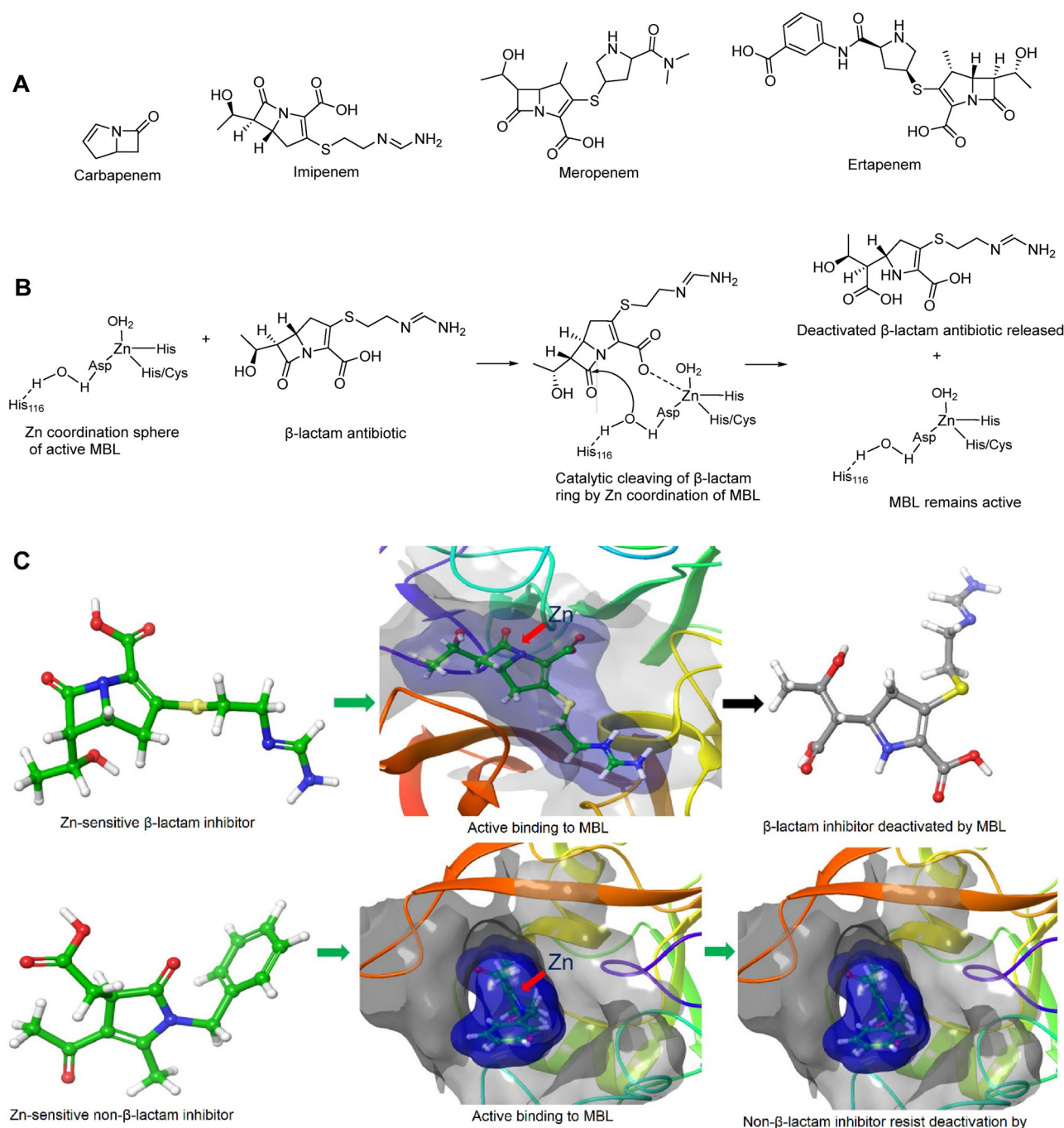


Figure 1. Chemical structures of some β-lactam inhibitors and catalysis by metallo-β-lactamases. (A) Some renowned β-lactam antibiotics with common β-lactam ring essentially for therapeutic actions (B) Illustration of the contribution of Zn-coordination sphere of metallo-β-lactamases to the deactivation of β-lactam antibiotics through the Zn-dependent cleavage of β-lactam rings. The drugs eventually become deactivated and released from the active site. (C) While β-lactam inhibitor is susceptible to deactivation, non-β-lactam, Zn-sensitive inhibitor actively binds to the MBL, sequesters Zn ion and resists deactivation.

applied to study molecular targets, mechanisms of biological events and structure-activity relationships (Prieto-Martínez et al., 2019; Sliwoski et al., 2014). The CADD of small molecules for drug discovery involves virtual screening, docking analysis, high throughput screening, molecular dynamics and Monte Carlo simulations with biological targets (Tomar et al., 2018). Recently, some potent inhibitors of the NDM-1 were identified from the ZINC database through virtual screening and molecular modelling (Wang et al., 2020). Similarly, a reported *de novo* fragment-based design also recognised potent small-molecule inhibitors targeting the broad-

spectrum MBLs through the interactions with Zn ion and other essential amino acid residues without susceptibility to carbapenemase deactivation (Cain et al., 2018). Some recent reports also demonstrated the successful identification of natural products as potential NDM-1 and VIM-1 inhibitors using virtual screening and *in silico* approaches (Kar et al., 2021; Salari-Jazi et al., 2021). However, only limited studies have been conducted on the discovery of non-β-lactam inhibitors of IMP-1 and no reports on the sensitivity toward the Zn site and broad-spectrum effects for resistance development. The IBS INTERBIOSCREEN (IBScreen, <https://www.>

[ibscreen.com/](https://www.ibscreen.com/)) represents one of the leading global databases of high-quality natural/synthetic chemicals for *de novo* screening and has been successfully explored for identifying some putative inhibitors (Ayipo, Ahmad, et al., 2022; Elekofehinti et al., 2021). As such, it is found worthy of further exploration in this *de novo* design for promising IMP-1 inhibitors.

Thus, this study is aimed at identifying potent Zn-chelating broad-spectrum inhibitors of the MBLs with structural resistance to the Zn-dependent catalytic deactivation. The IBScreen database was searched for potential ligands using a substructure of ML302F as a structural template. The retrieved ligands were virtually screened against the IMP-1, NDM-1 and VIM-1 using the SP module of Maestro 12.2 ligand-receptor flexible docking. The top-scored 100 ligands with the highest algorithm scoring functions of SP docking scores and targeted interactions were subjected to XP docking in the presence and absence of Zn ions at the active sites of the selected enzyme targets. The contributions of the Zn-coordination sphere to the catalytic functions of the enzymes and stability inducement by the ligands were further studied using MD simulations. The binding free energies of the selected ligands in complexes with IMP-1 were further calculated using molecular mechanics-generalized Born and surface area continuum (MM-GBSA). Finally, the retrieved ligands were assessed *in silico* for physicochemical, pharmacodynamics, pharmacokinetics and toxicological features as putative Zn-sensitive inhibitors of IMP-1 for further study.

Materials and method

Ligand selection and preparation

The open-access IBS INTERBIOSCREEN chemical library consisting of 555 093 screening compounds (<https://www.ibscreen.com/>) was searched for substructures of ML302F. The compound was chosen as a template due to its interesting structural resistivity to carbapenemase activity and potent Zn-sensitive and MBL-inhibiting profiles (González-Bello et al., 2020; Hinchliffe et al., 2016). The retrieved ML302F prototype ligands were downloaded in structure data file (SDF) format. The ligands together with the SDFs of the references, imipenem and meropenem obtained from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) were then imported into the workspace of the Maestro 12.12 suites for preparation using the LigPrep wizard (LigPrep, Schrodinger, LCC, New York, NY, 2019). The ligand preparation protocols such as the protonation state assignment, energy minimization, estimation of geometry and partial atomic charges using the OPLS-3e force field incorporated in Maestro 12.2 were adopted from relevant literature (Ayipo, Ahmad, et al., 2022; Harder et al., 2016). The minimized ligands in their ready-to-dock protonated states were saved in a LigPrep output folder.

Enzyme selection and preparation

Three MLB structures representing the IMP-1, NDM-1 and VIM were selected from the RCSB protein databank (PDB),

considering significance to the study, resolution and relevance in previous studies (Arjomandi et al., 2019; Kar et al., 2021; Moreira et al., 2021; Softley et al., 2020). These include a 1.8 Å X-ray-resolved structure of the IMP-1 MBL from *P. aeruginosa* in a complex with a plasmid-born inhibitor, biaryl succinic acid (BASA) (PDB 1JJT) (Toney et al., 2001); a hydrolysed imipenem-bound X-ray-resolved structure of NDM-1 with a resolution of 1.8 Å (PDB 5YPL) (Feng et al., 2017) and a 1.30 Å crystal structure of MBL VIM-1 in complex with an inhibitor, ML302F (Salimraj et al., 2019). The enzymes were downloaded in PDB format and imported into the workspace of the Maestro 12.2 software one after the other for preparation using the protein preparation wizard (Protein Preparation, Schrodinger, LCC, New York, NY, 2019). The preparation protocols for each receptor involved pre-processing, where the missing loops and chains were filled, and bond orders and protonation states were correctly assigned (Madhavi Sastry et al., 2013). The pre-process was followed by a review and modification of workspace content during which unwanted chains, water molecules and heteroatoms were deleted and the Epik state was regenerated at pH 7.0 ± 2.0 . Then refining the process consisting of the optimization step for assigning H-bonds, the prediction of ionizable groups (PROPKA) at pH 7.0 was run. Finally, the energy minimization was conducted at default settings by removing water molecules with less <3 H-bonds to non-water and convergence of heavy atoms to root mean square deviation (RMSD) 0.30 Å using the OPLS-3e force field (Harder et al., 2016). The enclosing grid boxes for active ligand dockings were separately generated by selecting the centroid of the workspace co-crystallized inhibitors. This allows for accurate active site detection since the reported potencies of the co-crystallized inhibitors are dependent on their binding positions. The x, y and z coordinates of the respective grid boxes were generated at default settings of the ligand docking length of 0–20 Å from the selected centroid of the co-crystallized ligands. The active site amino acid residues were identified and the pocket cavity (receptor grid box) of each prepared protein was saved in a gridbox.zip file. The protein preparation steps were repeated for each receptor, however, with the removal of Zn ions from the active sites of the enzymes to represent Zn-starved IMP-1, NDM-1 and VIM. The respective Zn-deficient remodelled receptors with similar grid boxes to the original active sites were also saved in gridbox.zip files accordingly (Ayipo, Osunniran, et al., 2022).

Molecular docking

The interactions of the prepared ligand and reference conformers with the respective enzymes were probed using the Standard precision (SP) and Extra precision (XP) molecular docking modules available in Maestro 12.2 (Ligand docking, Schrodinger, LCC, New York, NY, 2019). The redocking of co-crystallized ligands was used to validate the accuracy, precision and reproducibility of the docking procedure using the root mean square deviation (RMSD). Both complex structures of the PDB file of each enzyme as retrieved from the RCSB and respective docking pose were superimposed onto the

workspace of Maestro 12.2 (Structure alignment/superimposition, Schrodinger, LCC, New York, NY, 2019) and the RMSD value was computed for each superimposed pair. An RMSD value of less than 2.00 Å validates the docking protocol as accurately acceptable and reliable (Castro-Alvarez et al., 2017; Ramírez & Caballero, 2018). The SP module was applied to virtually screen the ligands and rank them in ascending order of docking scores and relevant interactions. 100 ligands with top-ranked binding affinities to the active sites of the receptors and interactions similar to the references were selected for the more discriminate, robust ligand sampling and refined torsion XP docking module (Parmar et al., 2021). The docking results were presented in the algorithms of the SP and XP docking scores in kcal/mol while the interactions of the ligands with active site residues were studied using the retrieved 2D binding pose views. Special attention was paid to the interactions between the ligands and the Zn ions at the active sites of the enzymes. The selected ligands were re-docked against the Zn-deficient receptors to evaluate the effects of the active site Zn ions to the binding affinities. The docking results were compared between the Zn-enriched and Zn-starved enzyme models to quantitatively study the sensitivity and specificity of the ligands towards the Zn site of the enzymes (Ayipo, Ahmad, et al., 2022).

Molecular dynamics

The stability of the selected promising ligands in complexes with IMP-1 of *P. aeruginosa* (PDB 1JJT) protein and their sensitivity toward the Zn site were evaluated using MD simulations on the OPLS-3e force field within the Desmond package (Desmond 2020-3, Schrodinger LLC) (Schrodinger Release 2020-3, 2021). Each ligand-protein complex was resolved in a solvent-soaked orthorhombic periodic box with a minimum distance of 10 Å between protein atoms and the box edges. The solvent was incorporated using a single-point charge water model while the system charges were neutralized with Na⁺ and Cl⁻ counter ions and a 0.15 M NaCl salt concentration was input to corresponding to the physiological system using the Desmond *System Builder* panel. The solvated system was then minimized and relaxed, utilizing OPLS-3e force field parameters as at the default Desmond protocols. The isothermal isobaric ensemble (Normal pressure and temperature/NPT) was set during the simulation, with a temperature and an atmospheric pressure of 300 K and 1.0315 bar respectively using the Nose-Hoover chain thermostat and Martyna-Tobias-Klein barostat methods (Jorgensen et al., 1996; Kalibaeva et al., 2003; Martyna, 1994). The simulation was carried out for a period of 100 ns, and trajectory snapshots were recorded at an interval of 100 ps. The MD trajectories were analyzed using Desmond's Simulation Interaction Diagram (SID) to predict the binding orientation of the ligand to the enzyme (Pawara et al., 2021) in the presence and absence of Zn sites. The classical MD simulation analysis was used to analyse the time-dependent interactive behaviour of IMP-1 protein-ligand complexes. The MD simulation analysis, in particular, gave precise insight into the fluctuation and structural or conformational changes of the

IMP-1 with/without Zn in complexes with selected ligands, as well as inter or intra-atomic interactions during a time range of 100 ns. The corresponding parameters observed from the MD trajectories include protein/ligand RMSD, protein C-α atoms RMS fluctuation (RMSF), the radius of gyration (Rg) and MM-GBSA binding free energy analysis.

Binding free energy calculations

The binding free energies of the selected ligands in complexes with Zn-enriched and Zn-deficient IMP-1 were calculated using the MM-GBSA approach. The Python script (*thermal_mmgsa.py*) was utilized to assess the Prime MM-GBSA binding free energy for 0–1000 frames, having a 10-steps sampling size in the simulation trajectory with the VSGB solvation model associated with the OPLS-3e force field. The overall ligand-protein complexes MM-GBSA-based binding free energy (ΔG_{Bind}) is calculated using the equation given below (Ayipo, Ahmad, et al., 2022).

$$\Delta G_{\text{bind}} = E_{\text{complex (minimized)}} - (E_{\text{ligand (minimized)}} + E_{\text{protein (minimized)}})$$

Physicochemical, pharmacokinetics and pharmacodynamics predictions

The physicochemical, pharmacokinetics, pharmacodynamics and toxicological properties which define the absorption, distribution, metabolism, excretion and toxicity (ADMET) profiles of the selected ligands were estimated *in silico* using the pkCSM-pharmacokinetics predictor (<http://biosig.unimelb.edu.au/pkcsml/>) by the input of SMILE files. The expression of a distance-based graph structural signature was employed to precisely calculate the fundamental physicochemical properties, atomic pharmacophore frequency counts and toxicophore fingerprints for the druggability of selected ligands adopting relevant protocols (Ayipo, Osunniran, et al., 2022; Pires et al., 2015). The predicted properties for defining the drug-like characteristics of the ligands include molecular weight (MW), number of rotatable bonds (for flexibility), number of hydrogen bond acceptor/donor (HBA and HBD), solubility (Log S), lipophilicity (log P) and topological polar surface area (TPSA). These inclusively indicate the potentials of the selected ligands for ADMET. Their druggability was also assessed through the propensities for crossing the blood-brain barrier (BBB), human gastrointestinal absorption, p-glycoprotein (pg) substrate and the inhibitory interactions with the metabolic cytochrome P450s. The benchmarks of the BDDC rules of 5 (RO5) and druggability, and Veber's influence of molecular properties on oral bioavailability were adopted (Benet et al., 2016; Veber et al., 2002).

Sequence alignment of selected bacterial MBLs with human and other eukaryotic MBLs, and possibilities of cross interactions

The MBLs are reportedly present in human beings contributing to diverse biological functions along disease/pharmacological pathways including cell detoxification, cell cycle progression,

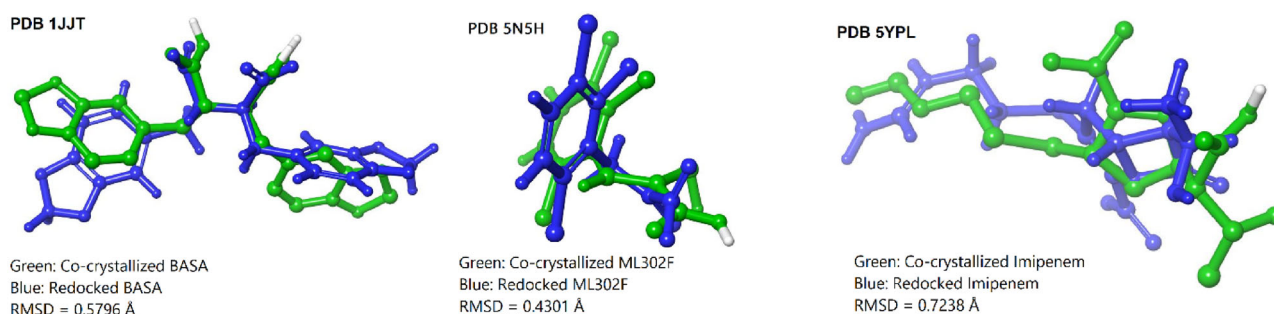


Figure 2. Superimposed structures of co-crystallized and re-docked reference ligands at the active pocket of the selected enzymes. The superimposed structures produced RMSD values in the range of 0.4301–0.7238 Å, indicating a good alignment and precise docking.

and conferment of cellular resistance to renowned antibiotics and anticancer drugs through digestion (Boyd et al., 2020; Diene et al., 2019; Pettinati et al., 2016). Similarities among the sequences of the bacterial MBLs under study, human MBL and other eukaryotic MBL were assessed, especially at the active catalytic sites for the possibilities of cross interactions with the selected ligands using the Maestro 12.2 (Multiple sequence alignment, Schrodinger, LCC, New York, NY, 2019) and the server-based TM-align (<https://zhanggroup.org/TM-align/>) (Zhang & Skolnick, 2005). The similarities are defined in order of alignment scores, RMSD values and TM-scores. An alignment score between 0 and 0.7 indicates a significant alignment with sufficient similarity, the smaller the better (http://gohom.win/ManualHom/Schrodinger/Schrodinger_2015-2_docs/maestro/help_Maestro/tools_menu/protein_structure_alignment.html). Similarly, TM-scores ranging from 0 to 1, indicate bad to good alignments (Zhang & Skolnick, 2005), whereas an RMSD of >3.00 Å is regarded as upper bound, although the RMSD benchmarks are associated with some drawbacks (Kufareva & Abagyan, 2012; Li, 2013). The possibility of cross interactions and binding affinities between the final hits and other MBLs present in humans and eukaryotes was assessed using molecular docking. The binding affinities of the ligands to the selected crystal structures, PDB ID 4V0H and PDB ID 1WW1 for human MBL domain-containing protein 1 (hMBLAC1) and tRNAse Z, an MBL from *Thermotoga maritima* respectively were defined by the algorithms docking analysis with the binding interactions observed through the binding poses.

Results and discussion

Ligand selection and preparation

The search for analogues of ML302F template inhibitor from the IBScreen chemical library returned 1012 substructures with similar pharmacophoric fingerprints to the template out of the 555 093 total screening compounds available in the database. The preparation of the retrieved 1012 IBScreen ligands and four reference inhibitors generated 2035 ready-to-dock conformers.

Molecular docking

The prepared receptor grid boxes consist of x, y, z coordinates of $57.81 \times 23.89 \times 34.06$; $-23.53 \times -13.96 \times -6.61$ and $-40.25 \times 75.87 \times 97.36$ for PDB 1JJT; PDB 5N5H and PDB

5YPL, respectively. The docking protocols were validated by redocking the co-crystallized ligands to the defined active ligand-binding pocket of each prepared receptor file. The superimposition of the co-crystallized and re-docked reference ligands, BASA, ML302F and imipenem produced poses with RMSD values of 0.5796 Å, 0.4301 Å and 0.7238 Å, respectively (Figure 2), thereby validating the protocols as accurate and reliable (Castro-Alvarez et al., 2017; Moreira et al., 2021; Ramírez & Caballero, 2018).

A derivative of succinic acid, BASA is a potent inhibitor co-crystallized in the active site of IMP-1 (PDB 1JJT), ML302F inhibitor in VIM-1 (PDB 5N5H) while imipenem is complexed within the active site of NDM-1 (PDB 5YPL) (Feng et al., 2017; Salimraj et al., 2019; Toney et al., 2001). From the XP docking scores (Table 1), BASA shows the highest binding affinity to the enzyme in the presence of Zn (column 1) as expected. The selected ligands mostly show competitive docking scores against the IMP-1 compared to the reference standard inhibitors, BASA, ML302F imipenem and meropenem. Selectively, IBScreen ligands, **STOCK3S-30514**, **-30154** and **-30418** showed consistently high binding affinities in the presence and absence of Zn. Similar trends could be observed in the docking scores against the VIM-1 and NDM-1 (columns 3 and 5, respectively), although **imipenem** and **meropenem** often-times displayed higher affinities against the enzymes in the presence of Zn ions. This is not unexpected since they are renowned inhibitors in clinical applications (Tan et al., 2021), however, challenged by AR development through the carbapenemase hydrolytic effects on their β -lactam ring (Lisa et al., 2017). Generally, the binding affinities represented by the docking scores are less than -7.00 kcal/mol in the presence of Zn, indicating strong binding. The close docking scores between the selected ligands and referenced standard inhibitors support similar binding affinities to the enzymes. More importantly, there exist significant differences between the docking scores of all the ligands in the presence of Zn (columns 1, 3, 5) and the absence of Zn (columns 2, 4, 6). Whereas, Zn plays important role in substrate coordination at the active sites of the enzymes, the process by which β -lactam antibiotics are deactivated (Lisa et al., 2017). The significant change in the binding affinities strongly buttresses the Zn-dependent substrate sensitivity and specificity of the enzymes as previously reported. Secondly, it supports the sensitivities of the ligands toward the Zn site and the scientific hypothesis of Zn-chelation by inhibitors as a plausible

Table 1. Docking scores of selected compounds against three MBLs in the presence and absence of Zn ions.

Title	XP Docking scores (kcal/mol)					
	IMP-1		VIM-1		NDM-1	
	PDB 1JJT	PDB 1JJT_NZ	PDB 5N5H	PDB 5N5H_NZ	PDB 5YPL	PDB 5YPL_NZ
STOCK3S-30514	−10.159	−8.912	−8.411	−6.639	−8.705	−6.388
STOCK3S-31000	−10.001	−5.322	−5.66	−4.532	−7.87	−5.994
STOCK3S-29912	−9.611	−3.639	−6.457	−4.616	−7.408	−4.835
STOCK3S-30154	−9.450	−6.042	−6.841	−6.102	−8.507	−4.739
STOCK3S-30148	−9.445	−2.291	−6.561	−2.561	−7.981	−1.725
STOCK3S-30503	−9.147	−3.880	−7.230	−4.980	−7.220	−5.985
STOCK3S-30418	−8.005	−5.572	−7.857	−5.128	−8.708	−5.872
STOCK3S-30509	−7.803	−5.333	−6.641	−4.456	−7.007	−3.265
Imipenem	−9.038	−7.872	−10.548	−8.713	−11.622	−6.574
Meropenem	−10.875	−7.567	−9.157	−6.508	−10.206	−6.504
BASA	−12.033	−8.643	−7.129	−6.683	−11.087	−8.587
ML302F	−8.915	−4.167	−5.374	−4.994	−7.123	−3.833

NZ = Zn-deficient. *See Supplementary file Tables S1–S3 for complete docking scores of 100 screened ligands.

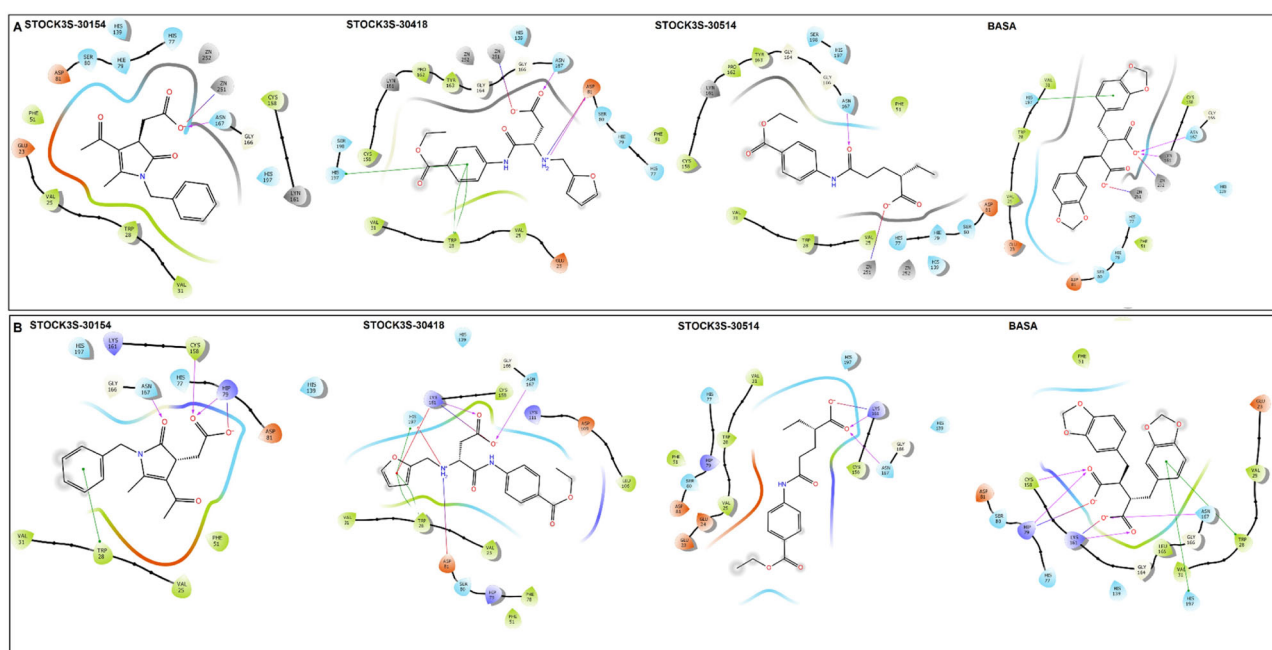


Figure 3. Binding poses of ligands with IMP-1 (PDB 1JJT) (A) With Zn ions at the active site (B) Without Zn ions at the active sites. Bonding interactions are shown as H-bonding (magenta arrow), π - cation (blue line), salt bridge (red-blue line) and π - π stacking (green line). The selected ligands show strong interaction with Zn and other amino acid residues implicated in the catalytic activity of IMP-1, potentiating Zn-chelation.

strategy for MBL deactivations (Ayipo, Osunniran, et al., 2022; Chen, 2020).

The binding poses showing the bonding and non-bonding interactions of the selected ligands and referenced co-crystallized inhibitors with the residues at the active site of IMP-1 are displayed in Figure 3. According to relevant previous studies, the active site components of the IMP-1 include the Zn1 and Zn2, Glu 23(59), Val 25(61), Trp 28(64), Phe 51(87), Asp 81(120), Lys 161(224) and Asn 167(233) (Arjomandi et al., 2019; Siemann et al., 2002; Yamaguchi et al., 2021; Yusof, 2015). The hydrophobic pocket contains Glu 23, Val 25 and Phe 51 within the proximity of the Zn(II) active site, while Trp 28 on the flexible loop 1 enhances the stabilization of the hydrophobic binding of the ligands in a closed form (Yamaguchi et al., 2021). The key amino acid residues for substrate sensitivity of the enzymes include Trp 28,

Lys 161 and Asn 167, and Zn ions for catalytic coordination (Arjomandi et al., 2019; Toney et al., 2001; Yamaguchi et al., 2021). The co-crystallized inhibitor, BASA interacted with Asn 167 through H-bonding to its succinate O[−] group in the presence of Zn (Figure 3A). It also formed salt bridges with Lys 161, Zn 251 and Zn 252 through its succinate O[−] ligands essential for Zn-dependent carbapenemase activity, and exhibited a hydrophobic π - π bonding with His 197 through benzyl moiety. Similar interactions with Asn 167 and Zn 251 could be observed in respect of the selected ligands, **STOCK3S-30154**, **STOCK3S-30418** and **STOCK3S-30514**. Additional H-bonding, salt bridge and π - π interactions were displayed by **STOCK3S-30418** towards Trp 28, Asp 81 and His 197. The similar inhibitory interactions between the selected database ligands and renowned co-crystallized inhibitors are indicative of potential similarities in

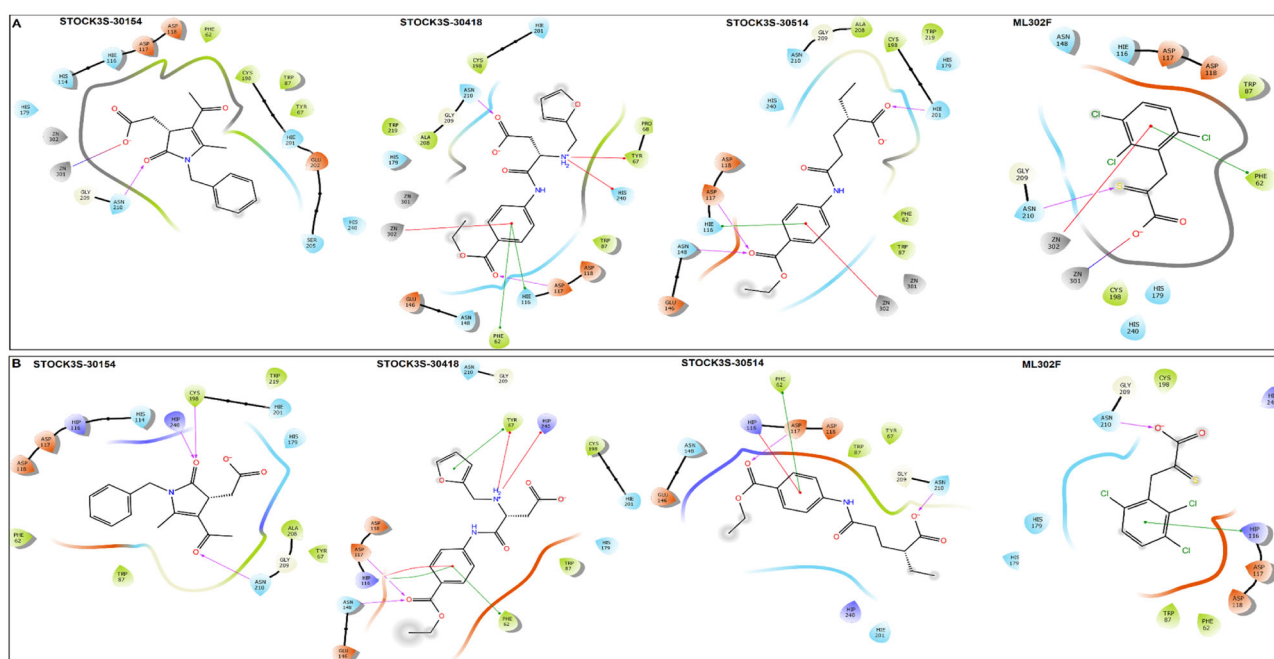


Figure 4. Binding poses of ligands with VIM-1 (PDB 5N5H) (A) In the presence of Zn ions at the active site (B) In the absence of Zn ions at the active sites. Bonding interactions are shown as H-bonding (magenta arrow), π -cation (blue line), salt bridge (red-blue line) and π - π stacking (green line). The selected ligands show strong interaction with Zn and other amino acid residues implicated in the catalytic activity of VIM-1, potentiating Zn-chelation.

pharmacological effects for the well-defined binding pocket similar structures (Naderi et al., 2019). The Zn ions represent essential pharmacophores at the host-pathogen interface (Bahr et al., 2022). All selected ligands commonly have electron-rich carboxylate groups configured towards the active pocket Zn ions, indicating a propensity for interactions. The ability of the ligands to electrostatically interact with Zn ions as shown by the ligands potentiates Zn-chelation effects consistently with the co-crystallized Zn-chelator, **BASA** (Toney et al., 2001). Whereas, such chelation effects as exhibited by Zn-binding motifs are strategic mechanisms of inducing Zn starvation on the enzymes for effective deactivation (Ayipo, Ahmad, et al., 2022; Yan et al., 2020). Relevantly, similar IMP-1-inhibitory interactions were reported for some ligands which were further confirmed as potent inhibitors in experimental studies (Kang et al., 2018), promoting the ligands as promising candidates. Moreover, all the selected ligands including the co-crystallized **BASA** exhibited significantly different bonding interactions with the active pocket residues in the absence of Zn (Figure 3B). Consistently, with the large variations in binding affinities as shown by the docking scores, these further indicate the invaluable contributions of Zn to the substrate sensitivity and specificity and enzyme integrity. Supportively, experimental evidence has shown that Zn-sequestering ligands could be essentially effective as selective inhibitors of the MBLs (Bush, 2018; Chen, 2020).

The bonding and nonbonding interactions of the selected and reference ligands at the active pocket of VIM-1 with and without Zn were displayed by 2D interaction diagrams (Figure 4). According to relevant previous studies (Kar et al., 2021; Tooke et al., 2019), Zn1 and Zn2 are common in binuclear MBLs while amino acid residues, Tyr 67, Trp 87, His 201, Glu 202, His 116, Asp 117, Asp 118, Ala 208, Asn 210 and His

240 inclusively constitute the substrate-binding catalytic pocket of the VIM-1. These residues are actively available in the defined substrate-binding pocket for the enzyme (Figure 4A). In the presence of Zn ion (Figure 4A), the co-crystallized reference inhibitor, **ML302F** displayed electrostatic interactions with Asn 210, Zn 301 and Zn 302 through H-bonding, salt bridge and π -cation formation, respectively. It formed an additional hydrophobic π - π bond with Phe 362 and non-bonding van der Waals interactions with other defined amino acid residues. Similar hydrophilic interactions to Asn 210 and Zn 301 were observed with the selected ligands under probe except for **STOCK3S-30514**. Interestingly, ligands **STOCK3S-30418** and **STOCK3S-30514** showed more bonding interactions, corroborating their higher binding scores than the reference **ML302F**. The ability of the ligands to accept H-bond from Asn 210 through the thiol group and form electrostatic bonds with the Zn moieties through the carboxylate O^- and aromatic π donor respectively indicate interactive ability with di-zinc B2 subclass of MBLs for Zn removal consistently with relevant previous studies (Hinchliffe et al., 2016). All selected ligands also display non-bonding interactions in similar patterns to **ML302F**. The template inhibitor, **ML302** has been proved experimentally to be highly effective against several clinical isolates of MBL-producing superbugs especially in combination with imipenem, through mixed uncompetitive/non-competitive Zn-inclined mechanisms (González-Bello et al., 2020). It was also reported to restore the antibiotic integrity of meropenem against VIM-1-producing bacterial strains through interactions similar to the aforementioned (Salimraj et al., 2019). The consistency between the binding patterns of the selected ligands and the template inhibitor, **ML302F** to the enzyme could potentiate similar activity (Joon S. Kang et al., 2018; Naderi et al., 2019), thereby supporting the ligands as putative inhibitors.

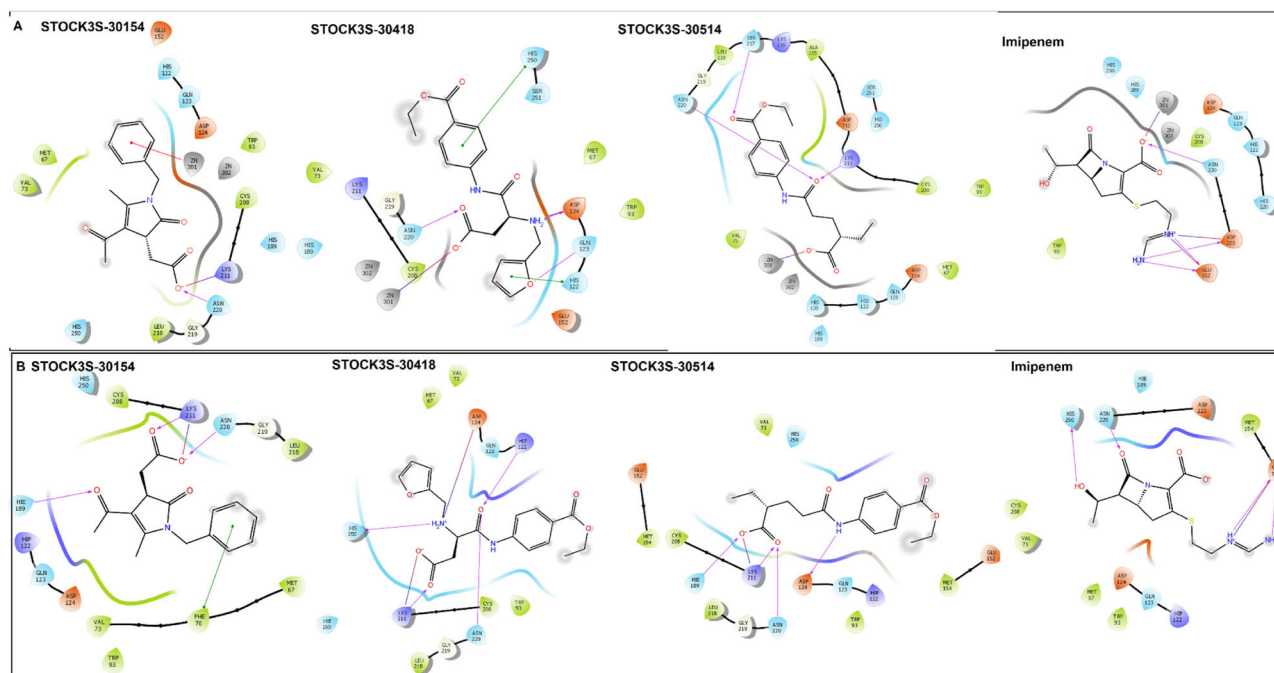


Figure 5. Binding poses of ligands with NDM-1 (PDB 5YPL) (A) In the presence of Zn ions at the active site (B) In the absence of Zn ions at the active sites. Bonding interactions are shown as H-bonding (magenta arrow), π - cation (blue line), salt bridge (red-blue line) and π - π stacking (green line). The selected ligands show strong interaction with Zn and other amino acid residues implicated in the catalytic activity of NDM-1, potentiating Zn-chelation.

Conversely, in the absence of the Zn site (Figure 4B), only H-bond interaction with Asn 210 was maintained by all the ligands including the co-crystallized **ML302F**. They all exhibited diverse bonding interactions, indicating a significant change in the sensitivity and substrate specificity of the enzyme due to the loss of Zn. This together with the noticeable changes in binding affinities to the enzyme Zn (Table 1) further support the ligands as Zn-sensitive and the idea of Zn starvation as a therapeutic pathway for enzyme deactivation.

The binding poses of interactions of the selected ligands with the active site residues of NDM-1 are illustrated in comparison with imipenem, a renowned NDM-1 inhibitor (Figure 5). Accordingly, the catalytic integrity of the enzyme strongly depends on the coordination of Zn ions to some key amino acid residues including His 120, 122, 189 and 250. Other residues such as Ile 35, Gln 123, Asp 124, Glu 152, Cys 208, Lys 211, Ser 217, Asn 220 and 223 are inclusively critical for the Zn-mediated activities (Kar et al., 2021; Salari-Jazi et al., 2021; Wang et al., 2020). In the presence of Zn (Figure 5A), **imipenem** showed H-bonding interaction with Glu 152, Asn 220 and Asp 223. It also formed salt bridges to Zn 301, Glu 152 and Asp 223, indicating strong electrostatic binding for potent inhibition. Similar interactions were exhibited by the selected database of non- β -lactam ligands. However, **STOCK3S-30418** engaged in additional H-bonding and salt bridge formation with Asp 124, and π - π interactions with His 122 and His 250. Whereas similar molecular interactions were reported with some experimentally proven Zn1-chelators in coordination with His 120, His 122 and His 189, as well as Zn2 surrounded by Asp 124, Cys 208 and His 250 with a water molecule, shared between the two metal ions during a substrate-binding (Chen et al., 2017). Residues Asp 124, Lys

211 and Gln 123 were also reported to participate in electrostatic interactions while His 122, Asp 124 and His 189 are involved in the cleavage of the β -lactam ring and Zn complexation. Also, Ser 217, Leu 218 and Asn 220 belong to loop L1 and contribute to the positioning of the β -lactam substrate within the active pocket. Interactions of these residues with the ligands, especially as shown by **STOCK3S-30418** could reduce the flexibility of the residues that contribute to antibiotic inactivation. (Moreira et al., 2021). The interactions could hypothetically distort the Zn-dependent activity of the enzyme (Chen et al., 2017). Additionally, ligand **STOCK3S-30514** exhibited nucleophilic H-bonding interaction involving its carbonyl group and serine residue at the active site, typical of renowned inhibitors reported with potent irreversible inhibition of the enzyme (Lima et al., 2020). Additionally, hydrophobic interactions of such active ligands with some of the residues such as Ile 35, His 189, Asp 124 and His 122 strongly stabilize their complexes with the enzyme. Overall, the interactions displayed by the selected database ligands are comparable to those recently reported for some potent inhibitors possibly due to close similarities in pharmacophores (Kar et al., 2021; Salari-Jazi et al., 2021; Wang et al., 2020), supporting the ligands as promising inhibitors. However, they showed diverse interactions in the absence of Zn (Figure 5B), indicating Zn-sensitivity and Zn-dependent behaviours. Taking together, the relevant bonding and nonbonding interactions exhibited by the selected ligands qualify them as promising Zn-sensitive inhibitors of NDM-1 for further rigorous assessment. The collective interesting results from the docking analysis support the three selected ligands, **STOCK3S-30154**, **STOCK3S-30418** and **STOCK3S-30514** worthy of further evaluation through MD simulation.

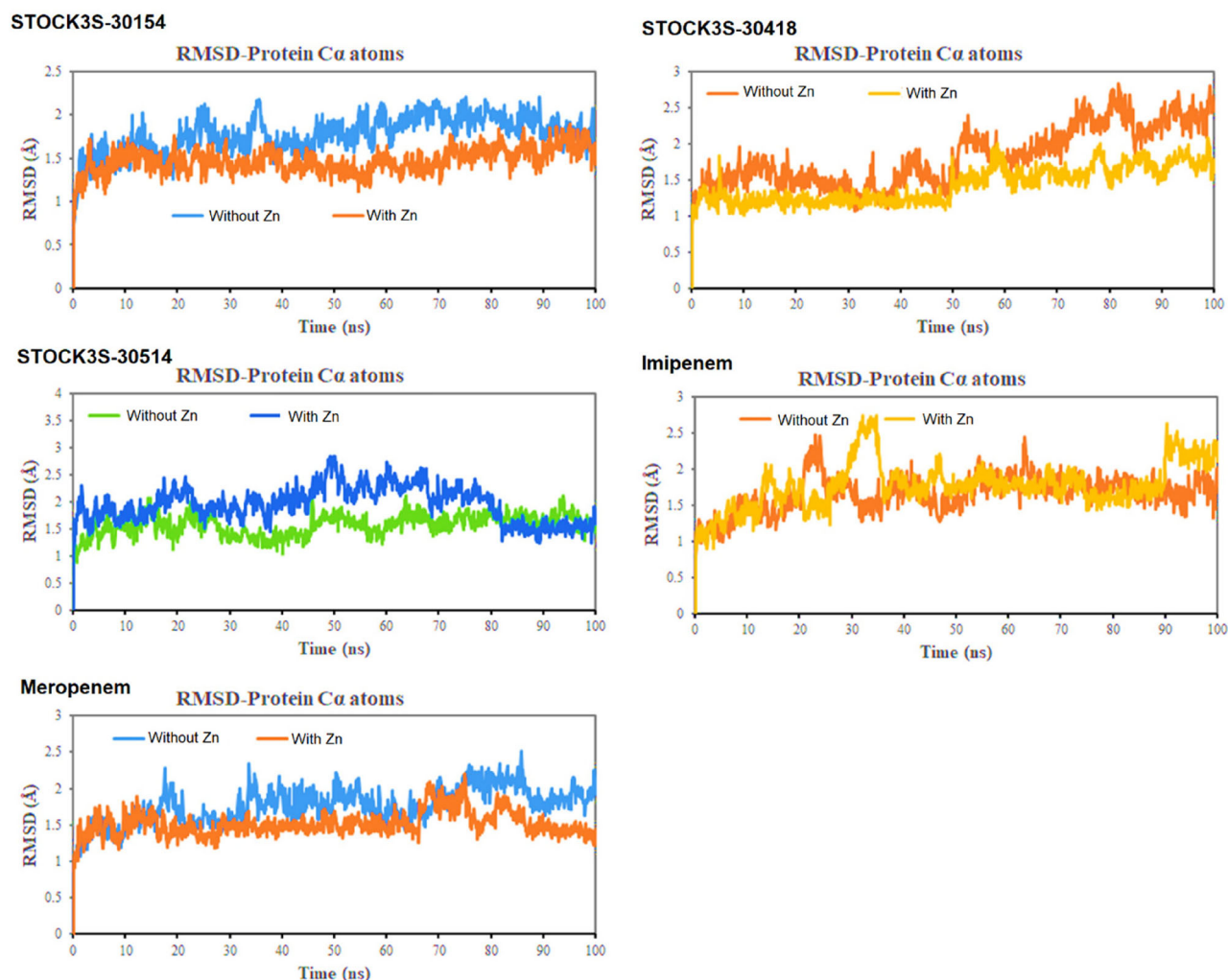


Figure 6. Time-dependent protein C α atoms RMSD plot bound with STOCK3S-30154, STOCK3S-30418, STOCK3S-30514, Imipenem, and Meropenem in the presence and absence of Zn. The presence of Zn ions confers more stability to the protein backbone than in the absence of Zn, indicating the relevance of Zn to enzyme integrity.

Molecular dynamics

Root mean square deviation

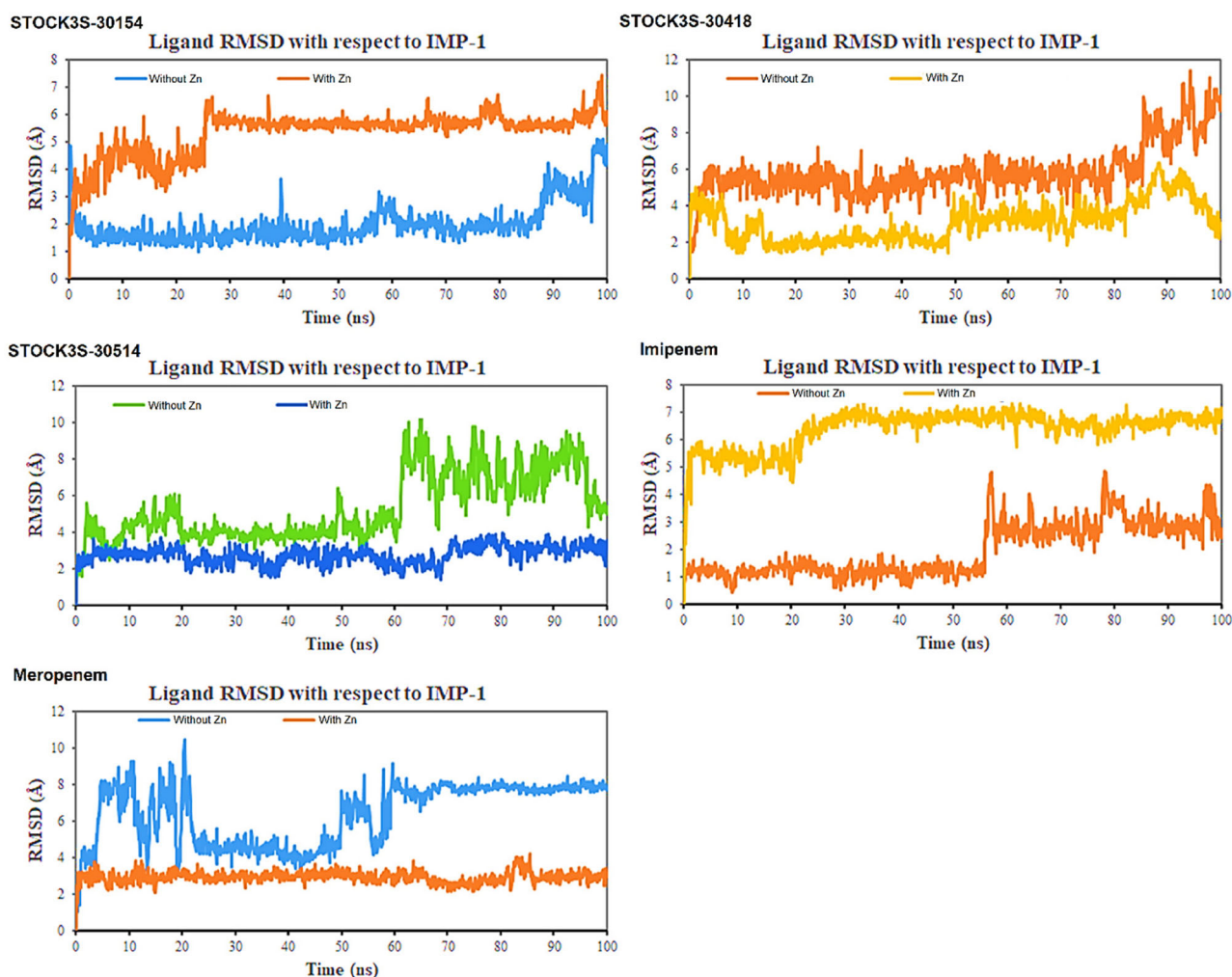
The RMSD values of protein C α atoms bound to each compound show how the protein backbone exists internally and changes structurally over time in comparison to the initial position. In terms of structural or conformational changes measured throughout simulations, RMSD values of less than 3 Å indicates a very close resemblance to the reference structure (Ayipo, Yahaya, et al., 2022; Pawara et al., 2021). The RMSD plots of the protein C α atoms of each complex with/without Zn were computed from the MD simulation trajectories as shown below (Figure 6) and supported by the average, maximum, and minimum RMSD values (Table 2). The RMSD plot in Figure 6 clearly showed that the average deviations of the protein C α atoms in complex with compounds **STOCK3S-30514**, **STOCK3S-30418**, **STOCK3S-30154**, **Imipenem**, and **Meropenem** were within the range of 0–2.7 Å, which is an acceptable range and suggests the stability of the system when bound to inhibitors. An in-depth examination of RMSD values indicated that all the selected ligands

including the reference inhibitors maintained good stability in complex with the enzyme in the presence and absence of Zn, except **STOCK3S-30418** which showed a slightly high RMSD in the absence of Zn towards the end of the simulation. The presence or absence of Zn ion in protein C α atoms associated with other ligands exhibited a similar type of fluctuation pattern with **imipenem** throughout the MD simulation, with values below 2.7 Å. Although a non-linear relationship exists between the RMSD and the presence of Zn for a few ns in the case of **STOCK3S-30514**, similarly with **imipenem**, however, the ligands thereafter maintained stability till the end of the simulation. Altogether, these demonstrated that the protein C α atoms were adequately stable with bound ligands as proposed IMP-1 inhibitors.

In parallel to the RMSD of the protein C α atoms, the RMSD of the ligands was calculated and found to be moderate for all ligands. For **STOCK3S-30418**, **STOCK3S-30514**, **STOCK3S-30154**, **Imipenem**, and **Meropenem**, the average ligand RMSD values in the presence and absence of Zn ion were determined to be 3.0817 and 5.8561, 2.7633 and 5.3196, 5.3164 and 1.9895, 6.3626 and 1.9655, 2.9319 and

Table 2. Minimum, Maximum and Average, RMSD, RMSF and Rg values of proposed inhibitors and control drugs.

	STOCK3S-30418		STOCK3S-30514		STOCK3S-30154		Imipenem		Meropenem	
	With Zn	Without Zn	With Zn	Without Zn	With Zn	Without Zn	With Zn	Without Zn	With Zn	Without Zn
Protein C-α atoms RMSD										
Min	0.924	0.886	1.0660	0.8840	0.8500	0.7340	0.8690	0.7930	0.9100	0.9110
Max	2.084	2.829	2.8410	2.1470	1.8900	2.2020	2.7410	2.4710	2.2010	2.5080
Average	1.4292	1.8168	1.9515	1.5712	1.4701	1.7710	1.7736	1.6600	1.5160	1.7673
Ligand RMSD to protein										
Min	1.3750	1.3420	1.3910	1.4650	1.5130	0.9660	2.1970	0.4500	1.5330	1.0090
Max	6.3460	11.4150	3.9710	10.3780	7.4330	5.1010	7.3780	4.8360	4.2090	10.4490
Average	3.0817	5.8561	2.7633	5.3196	5.3164	1.9895	6.3626	1.9655	2.9319	6.3690
Protein C-α atoms RMSF										
Min	0.3800	0.3500	0.3390	0.3470	0.3380	0.3290	0.3580	0.3250	0.3360	0.3900
Max	5.8450	6.1330	7.2650	4.3490	3.2200	6.8930	6.1050	4.8080	4.5880	6.9300
Average	1.0066	0.7795	0.8765	0.8137	0.7477	0.8424	0.8797	0.7749	0.7917	0.9166
Radius of gyration										
Min	4.1380	4.4150	4.8000	4.1910	3.2390	3.3800	3.3850	3.3390	4.0710	3.8480
Max	5.1560	5.2050	5.2110	5.0840	3.6150	3.6020	3.8930	3.9600	4.6130	4.2960
Average	4.6825	4.9010	5.0901	4.5532	3.4417	3.4951	3.6477	3.6470	4.2513	4.0235

**Figure 7.** Time-dependent ligand RMSD plot of STOCK3S-30154, STOCK3S-30418, STOCK3S-30514, Imipenem, and Meropenem to protein in the presence and absence of Zn. The ligands were relatively more stable throughout the trajectories of MD simulation in the presence of Zn, indicating their sensitivity towards Zn ions and the influence of Zn on the molecular functions of the enzyme.

6.3690 Å, respectively. The plots of RMSD versus the time of simulation of all ligands were depicted in Figure 7. The figures clearly show that the Zn ion has a significant effect on the ligand RMSD. The presence of Zn ion offers stability,

whereas its lack results in large changes in ligand RMSD concerning protein (Figure S8). It is clearly visible in **STOCK3S-30514**, where the absence of the Zn ion has significantly affected ligand stability as well as the conformation even

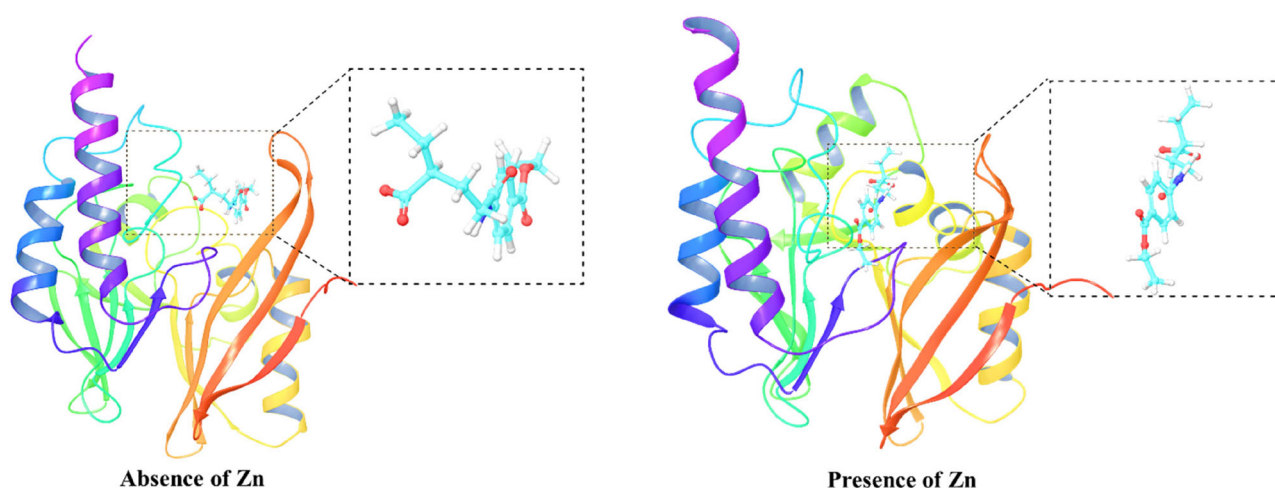


Figure 8. Conformational comparative analysis of the STOCK3S-30514 in contact with IMP-1 in the absence and presence of Zn ion. Although, the STOCK3S-30514 maintained strong contact with the enzyme, however, the absence of Zn significantly affects RMSD of STOCK3S-30514, indicating their sensitivities to the metal ion.

though the contact was maintained during the simulation. To examine the conformational changes that occur throughout the simulation, the comparative last frame of **STOCK3S-30514** confirmation in the absence and presence of a Zn ion is depicted in [Figure 8](#), indicating that the lack of a Zn ion also causes observable conformational changes in the ligand structure. These inclusively indicate the contribution of the active site zinc ions to the conformational stability of the enzymes and support the proposed zinc sequestration as an ideal mechanism for deactivating the catalytic integrity of the enzymes.

Collectively, the RMSD data suggest that the presence of the Zn ion significantly stabilises the protein-ligand complexes when compared to its absence. These supportively indicate the contribution of the active site zinc ions to the conformational stability of the enzymes, and support the proposed zinc sequestration as an ideal mechanism for deactivating the catalytic integrity of the enzymes (Chen, [2020](#); González-Bello et al., [2020](#); Moreira et al., [2021](#)).

Root mean square fluctuation

The RMSF analysis is also commonly applied to investigate the stability of protein-ligand complexes. It estimates the individual protein residue elasticity in terms of atomic fluctuations from their original positions during MD simulation (Ayipo, Yahaya, et al., [2021](#)). The RMSF values for the IMP-1 protein with and without Zn ion were calculated separately for the bound **STOCK3S-30418**, **STOCK3S-30514**, **STOCK3S-30154**, **Imipenem** and **Meropenem**, as illustrated in [Figure 9](#). The plots of the selected database ligands show a similar pattern with little variation in the fluctuation of amino residues for the IMP-1 protein, whether the Zn ion is present or not consistently with reference antibiotics. The amino acids present in the C and N terminal regions of the sequence fluctuated by around 5 Å when compared to other regions of the protein ([Figure 9](#)). The fluctuation for individual amino acids correlates with the pattern shown in the RMSD of complexes. In all complexes, major fluctuations are observed in

residues in the range of 26–28. Simulation of **STOCK3S-30418** reveals further fluctuation in the 52–57 amino acid region of IMP-1 protein. In the absence of Zn ion. [Table 3](#) shows the RMSF values of ligand-contacted residues during simulation. The average RMSFs of ligand-contacted residues are less than 3 Å, implying that the presence and absence of Zn ion in IMP-1 protein results in minimal fluctuation and relative secondary conformational stability upon binding of reported compounds. This suggestively poses the ligands as potential inhibitors of IMP-1 even after Zn sequestration, as such, they could act as stand-alone antibiotics upon further studies.

Radius of gyration (Rg)

The Rg analysis is an inclusive criterion for determining the root mean square distance of a group of atoms from their common centre of mass, which can reveal the system's overall compactness. The Rg of all ligand-IMP complexes was determined and the results are shown in [Figure 10](#). During the MD simulation, a steady fluctuation of Rg shows that the protein is stably folded, while the high variation of the Rg parameters reflects the unfolding of the protein (Ayipo, Ahmad, et al., [2021](#); Pawara et al., [2021](#)). In comparison, the selected ligands except **STOCK3S-30514** showed more compactness in complexes with the enzyme in the presence of Zn ion at the active site than in its absence throughout the simulation, consistently with the renowned inhibitors. The small variances in Rg values show that all of the systems nearly uniformly adopt compact protein structures, which may be attributable in part to significant contributions from the Zn ion in the protein structure. Overall, the selected database ligands showed sensitivities to the Zn site of the enzyme for compactness and could be ideally proposed as Zn-sensitive inhibitors with similar behaviour to imipenem and meropenem for further evaluation. Although, the variations regarding the Rg plots are diminutive in some cases, however, the consistency from other MD parameters including the RMSD, RMSF, ligand interaction plots and MMGB-SA

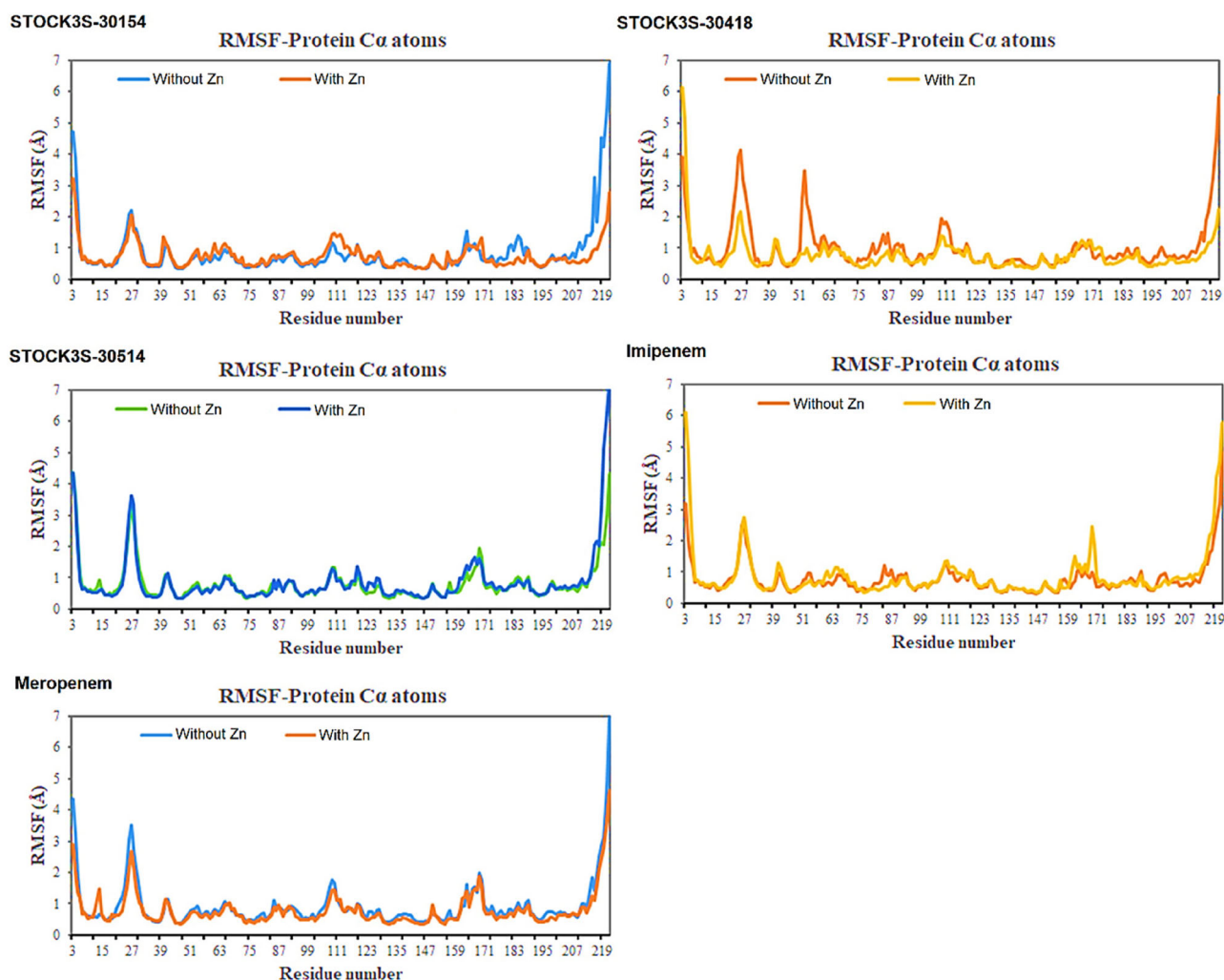


Figure 9. RMSF plot of protein Cα atoms bound with STOCK3S-30154, STOCK3S-30418, STOCK3S-30514, Imipenem, and Meropenem. Although, all the residues experienced averagely low fluctuations during the simulation, however, the residues were least fluctuated in the presence of Zn, indicating the underlying effect of Zn on the entire protein stability.

favours higher stability in the presence of Zn than in its absence. Moreover, a similar condition regarding the Rg simulation results was recently observed by Jahan and Nayeem when they studied the conformational stability of superoxide dismutase in osmolytes and this did not deter the ideal conclusion based on cumulative results from other dynamic studies (Jahan & Nayeem, 2020).

Ligand contacts with pharmacological residues

During the MD simulation, the selected IBScreen ligands made strong contacts with Zn ions and other amino acid residues critical to the inhibitory pathways of IMP-1. As shown in Figure 11 for instance, **STOCK3S-30418** has strong interactions with 26 amino acid residues in addition to the two Zn ions at the catalytic site of IMP-1 during the MD simulation, mostly through ionic and H-bonding in the presence and absence of Zn ions, respectively. The Zn ions critically represent a catalytic interface between the relevant pathogens and the host (Bahr et al., 2022). As such, the strong interactions of the ligands with the ions and their coordinated residues could significantly disrupt their chemistry,

thereby suppressing the catalytic integrity of the enzyme. Moreover, the prominent hydrophilic and non-covalent bonding of the ligands to the active site residues suggestively portrays an effective pathway for preserving and restoring the efficacy of antibiotics, especially the β -lactam class (Salari-Jazi et al., 2021). The contacts involve the relevant residues to the deactivating pathways of the enzyme (Toney et al., 2001; Yamaguchi et al., 2021), notably, Trp 28, Asp 81, Lys 161, Asn 167 and His 197 with the two Zn ions, consistently with the interactions shown by imipenem and other reference ligands (Supplementary File 1, Figures S1–S4).

Binding free energy

The binding affinity of ligands is mostly determined by non-bonded interactions at the binding site, such as hydrogen bonds, hydrophobic interactions and ionic interactions. The histogram of all complex binding contacts is provided in Supplementary File 1, Figure S1–S4 in the supplemental information, revealing that the ligands make major ionic interactions when the Zn ion is present, which improved their stability. The MM-GBSA approach estimates binding free energies (ΔG_{bind}) based on MD simulation trajectories using

Table 3. RMSF values of ligand-contacted residue with IMP-1 protein (C α atoms) after binding of lead compounds.

STOCK35-30418	STOCK35-30514				STOCK35-30154				Imipenem				Meropenem					
	With Zn		Without Zn		With Zn		Without Zn		With Zn		Without Zn		With Zn		Without Zn			
	Residue	RMSF	Residue	RMSF	Residue	RMSF	Residue	RMSF	Residue	RMSF	Residue	RMSF	Residue	RMSF	Residue	RMSF		
	Glu23	1.689	Asn26	3.042	Glu23	0.909	Val25	1.218	Val25	1.542	Glu24	1.169	Val25	1.76	Glu23	0.795	Glu23	1.22
	Val25	2.95	Trp28	3.324	Glu24	1.358	Trp28	1.535	Asn26	2.086	Val25	1.75	Asn26	2.507	Glu24	1.138	Val25	2.091
	Asn26	3.925	His77	0.415	Val25	1.899	Val31	0.792	Trp28	1.656	Asn26	2.366	Gly27	2.691	Val25	1.524	Asn26	3.058
	Gly27	4.128	His79	0.491	Asn26	2.641	Phe51	0.647	Val30	1.217	Gly27	2.746	Gly27	1.893	Trp28	2.173	Gly27	3.522
	Trp28	3.195	Asp81	0.502	Trp28	2.788	His77	0.415	Val31	1.077	Trp28	2.269	Val30	1.138	Val30	1.039	Trp28	2.733
	Val30	2.515	His139	0.542	Val30	1.273	His79	0.493	Pro32	0.666	Val31	0.806	Val31	0.861	Val31	0.918	Val30	1.463
	Val31	1.279	Cys158	0.516	Val31	0.998	Ser80	0.67	Phe51	0.644	Pro32	0.627	Pro32	0.687	Pro32	0.674	Val31	0.918
	Pro32	0.986	Ile160	0.661	Pro32	0.731	Asp81	0.599	His77	0.404	Phe51	0.612	Lys33	0.596	Phe51	0.718	Pro32	0.635
	Lys33	0.601	Lys161	1.001	Phe51	0.624	His139	0.479	His79	0.456	His77	0.391	Phe51	0.755	His77	0.381	Phe51	0.778
	Phe51	0.979	Pro162	0.959	His79	0.498	Cys158	0.469	Ser80	0.538	Phe78	0.465	His79	0.41	His79	0.438	His77	0.466
	His77	0.671	Tyr163	1.174	Ser80	0.564	Lys161	0.67	Asp81	0.547	His79	0.443	His77	0.503	Ser80	0.576	His79	0.595
	Phe78	0.641	Gly164	1.414	Asp81	0.553	Gly164	1.099	His139	0.607	Ser80	0.496	Ser80	0.631	Asp81	0.478	Ser80	0.664
	His79	0.7	Leu165	1.26	Trp124	0.517	Leu165	1.123	Asp81	0.611	Asp81	0.469	Asp81	0.641	Lys127	0.646	Asp81	0.696
	Ser80	0.888	Asn167	1.642	Lys127	0.718	Asp167	1.027	Lys161	0.586	Asp109	1.323	Ser93	0.961	His139	0.51	His139	0.66
	Asn128	1.144	Ser196	0.496	His139	0.532	Asp170	1.336	Tyr163	1.16	Lys127	0.706	His139	0.444	Cys158	0.538	Ile160	0.561
	Lys129	0.615	His197	0.563	Ile160	0.534	His197	0.545	Gly164	1.55	His139	0.461	Lys161	0.648	Lys161	0.721	Lys161	0.732
	His139	0.429	Ser198	0.775	Lys161	0.599	Lys161	0.912	Cys158	0.912	Cys158	0.489	Tyr163	1.046	Tyr163	1.207	Tyr163	0.91
	Cys158	0.635	Glu199	0.796	Tyr163	0.873	Leu166	1.076	Gly166	1.076	Lys161	1.164	Gly164	1.077	Gly164	1.388	Gly164	1.626
	Cys158	0.62	Gly164	1.192	Gly164	0.873	Asn167	1.13	Leu165	0.996	Leu165	0.996	Leu165	0.863	Leu165	0.873	Leu165	0.991
	Lys161	0.767	Leu165	0.917	Leu165	0.917	Gly169	0.919	Gly166	0.919	Gly166	1.253	Gly166	1.033	Gly166	1.355	Gly166	1.486
	Pro162	0.782	Leu165	0.949	Gly166	1.137	Asp167	0.619	Asp167	0.619	Asp167	0.996	Asn167	0.882	Asn167	1.489	Asn167	1.551
	Tyr163	0.735	Asn167	1.108	Asn167	1.345	His197	0.559	Leu168	0.559	Leu168	1.564	Asp170	0.835	Asp170	1.519	Ser196	0.69
	Leu165	1.089	Gly166	1.241	Ser196	0.492	Ser196	0.492	Gly169	0.492	Gly169	2.473	Ser196	0.732	Ser196	0.443	His197	0.751
	Gly166	1.241	Asn167	1.187	Asp170	1.052	His197	0.636	Asp170	5.475	Asp170	1.885	His197	0.904	His197	0.471	Ser198	0.742
	Asn167	1.187	Trp176	1.268	His197	0.621	Ser198	0.762	His197	0.621	His197	0.565	Ser198	0.925	Ser198	0.471	Ser198	0.742
	Asp170	1.268	His197	0.47	His197	0.716	Ser198	0.762	His197	0.716	His197	0.565	Ser198	0.925	Ser198	0.471	Ser198	0.742
	His197	0.47	Ser198	0.877	Ser198	0.716	Ser198	0.762	Ser198	0.716	Ser198	0.565	Ser198	0.925	Ser198	0.471	Ser198	0.742

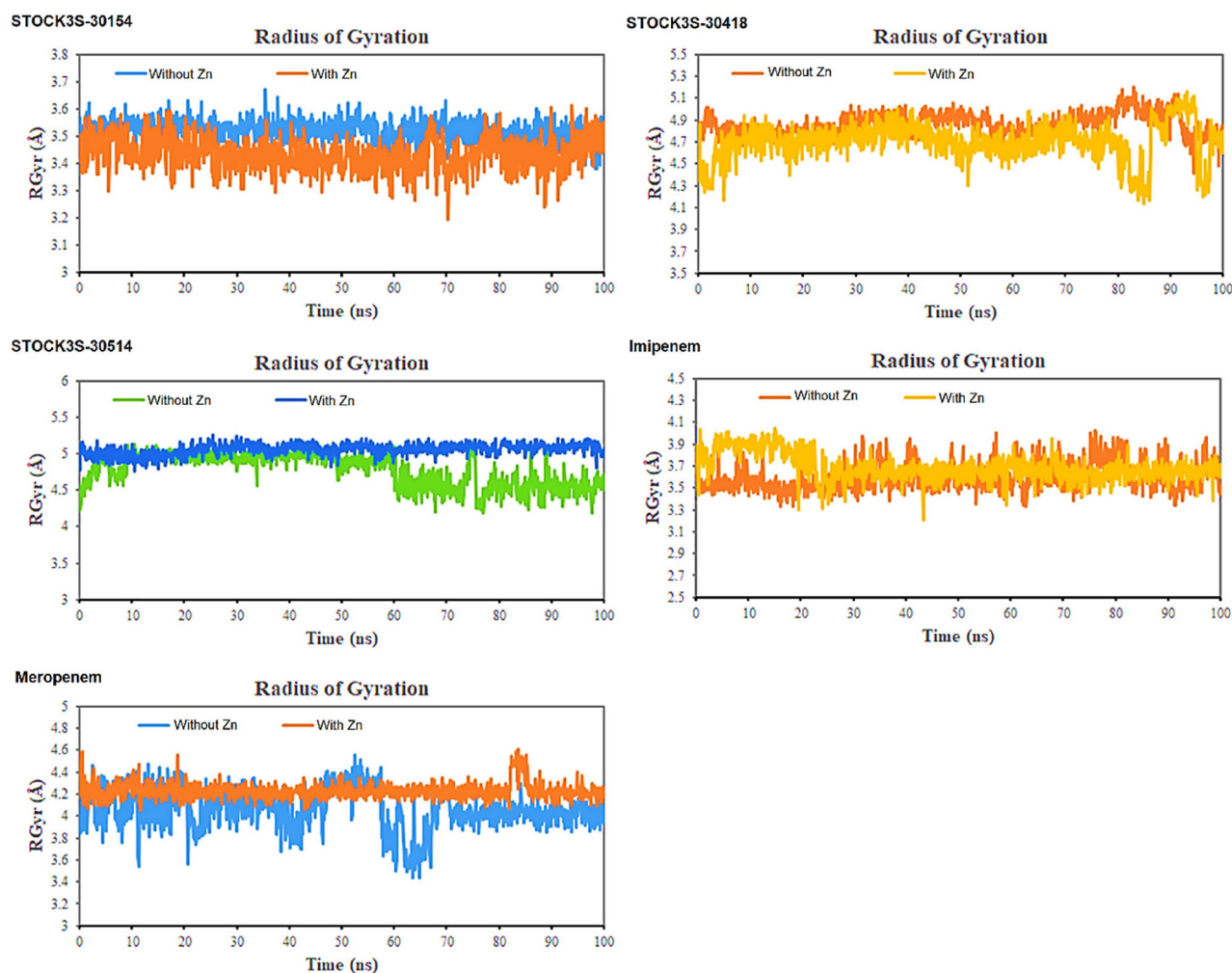


Figure 10. Radius of gyration plot representing the compactness of IMP-1 structure in complexes with selected ligands in the presence and absence of active pocket Zn ions. The ligands showed more compactness with the enzyme in the presence of Zn, supporting the influence of Zn on the catalytic interactions of the enzyme with the ligands and the sensitivity of the ligands to Zn ions.

the molecular mechanic method. The post-simulation MM-GBSA was computed from frame 0 to 1000 at every 10th frame, yielding a total of 100 structures of each protein-ligand complex, and the average binding energies are shown in Figure 12. A lower number indicates that the ligand binds to the target protein more strongly (Ayipo, Osunniran, et al., 2022; Pawara et al., 2021). In all compounds, the presence of the Zn complex has a higher ΔG_{bind} than the lack of the Zn in the complexes, which reveals that the presence of the Zn ion in protein provides for stability and binding affinity of proposed inhibitors. The significant variations in the binding affinities of the ligands to the Zn-enriched and Zn-deficient active sites of IMP-1 further support the sensitivities of the ligands to the Zn site, typical of Zn-chelating MBL inhibitors. Consistently, similar observations were reported for some emerging Zn-sensitive MBL inhibitors from experimental investigations (Ayipo, Osunniran, et al., 2022; Chen, 2020). The summative performances of the three ligands, **STOCK3S-30154**, **STOCK3S-30418** and **STOCK3S-30514** during MD simulations support their selection for further assessments such as drug-likeness characters through ADMET predictions.

Physicochemical, pharmacokinetics and pharmacodynamics predictions

The selected database ligands were evaluated for drug-like potentials using *in silico* predictors of the physicochemical, pharmacokinetics, pharmacodynamics and toxicological properties. The properties define their ADMET profiles as importantly for good delivery to pharmacological targets for metabolism, physiological effects and discharge from the system with diminutive toxicity. The server-based pk-CSM model applied was validated in agreement with experimental reports. It reportedly has good accuracy and precision for reliable predictions of new molecules for drugability probes (Pires et al., 2015). The procedure affords rapid, economical and less hazardous methods of screening promising drug-like molecules for further rigorous and time- and resource-demanding wet-lab analyses. The predicted properties for the selected database ligands, **STOCK3S-30154**, **STOCK3S-30418** and **STOCK3S-30514** are presented in comparison with clinical inhibitors, **imipenem** and **meropenem** (Table 4). All the ligands have MW of <500 g/mol, Log *P* values within −0.7 – +5.0, indicating high lipophilicity, and 6–13 rotatable

STOCK3S-30418

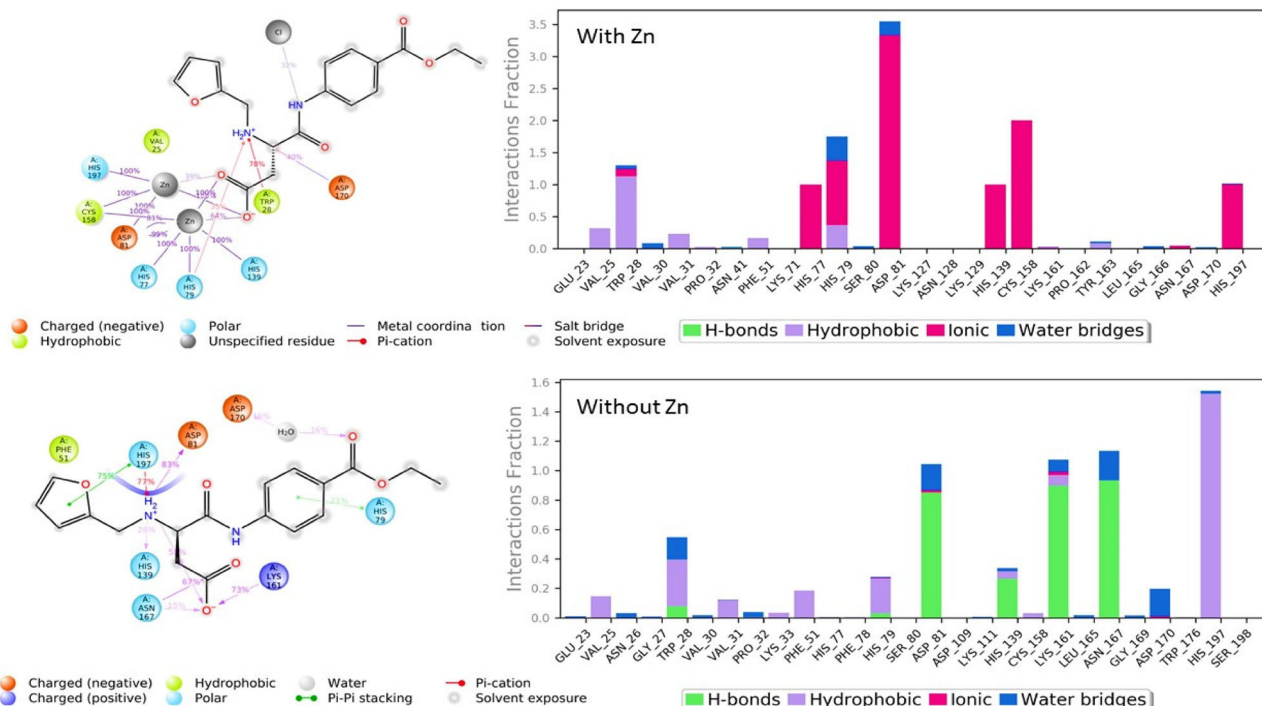


Figure 11. Binding interaction of compound STOCK3S-30418 with IMP-1 protein during 100 ns simulation period. The ligand exhibited strong contact with Zn and other amino acid residues essential for enzyme integrity, supporting the ligand as a putative Zn-chelating inhibitor.

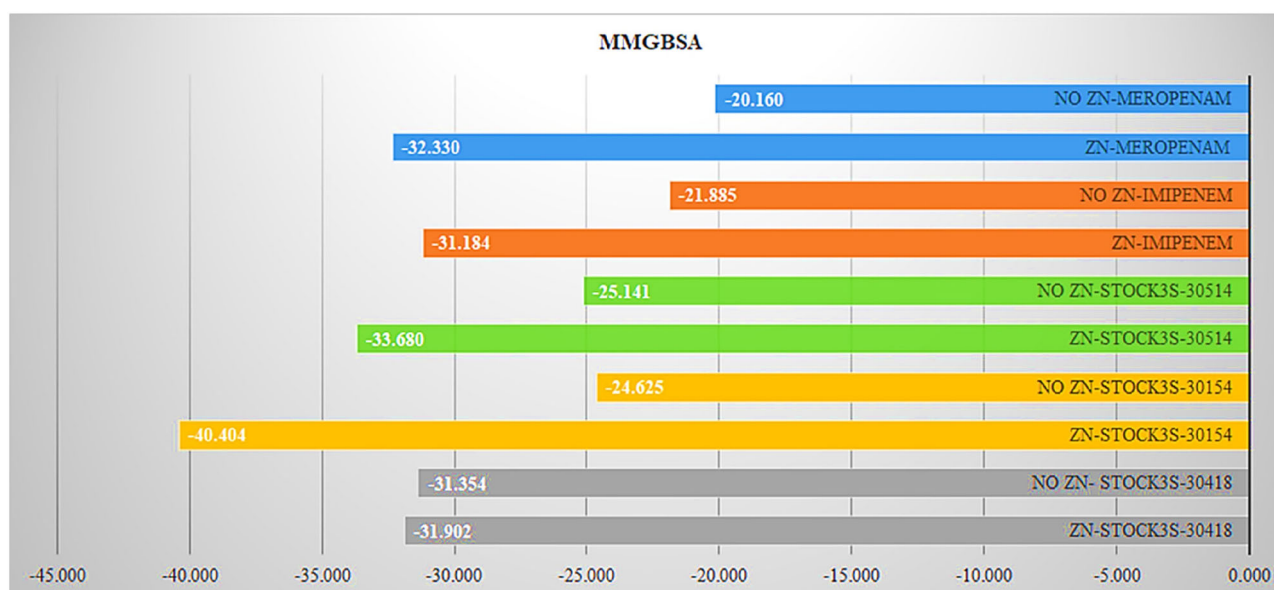


Figure 12. Post-simulation binding free energy analysis of selected ligands with IMP-1 through MM-GBSA method. The selected ligands most show a negatively higher binding free energy to IMP-1 than reference inhibitors either in the presence or absence of Zn, indicating stronger inhibitory interactions. All the ligands demonstrated significantly higher binding affinities to the enzyme in the presence of Zn, indicating the potential Zn-sensitivity, consistently with the reference Zn-sensitive inhibitors.

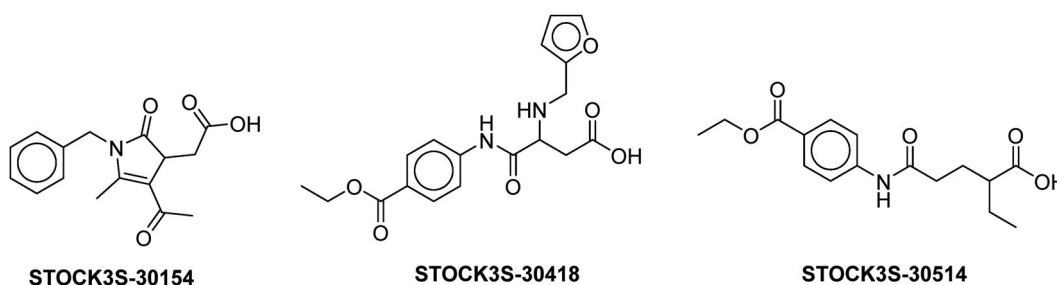
bonds for ideal flexibility except **STOCK3S-30154** and **meropenem**. They also possess HBA and HBD groups of less than 10 and 5, respectively, and TPSA between 20 and 130 Å² except for **STOCK3S-30418** and **meropenem**. According to Veber's hypothesis, the MW of <500 g/mol and rotatable bonds of ≈7 could influence more than 65% of fractions of the ligands for ≥20% oral bioavailability in a whole rat

system (Veber et al., 2002). These are consistent with the predicted absorption-related profiles such as water solubility scores (good), intestinal epithelial Caco2 permeability (low), human intestinal absorption (between 50 and 92% compared to reference drugs with 35–37%), skin permeability with good solubility score and low permeability across the human Caco-2 cell as mostly required for drug candidates. The

Table 4. Predicted ADMET properties for the selected ligands.

Property	Descriptor	30154	30418	30514	Imipenem	Meropenem
MW	(g/mol)	287.315	360.366	307.346	299.352	383.47
Log P	–	1.9827	2.0279	2.6928	–0.3857	–0.308
#Rot. bonds	–	5	9	8	6	5
#HBA	–	3	6	4	5	6
#HBD	–	1	3	2	3	3
TPSA	(Å) ²	122.424	149.694	129.023	120.082	156.137
Absorption	Water solubility (mol/L)	–2.451	–2.891	–2.914	–1.704	–2.568
Absorption	Caco2 permeability	1.009	0.007	0.225	–0.201	0.028
Absorption	Intestinal absorption (%) (human)	92.35	50.197	60.524	37.455	34.839
Absorption	Skin Permeability (Log Kp)	–2.913	–2.735	–2.732	–2.735	–2.735
Absorption	P-glycoprotein substrate	No	Yes	Yes	No	Yes
Absorption	P-glycoprotein I inhibitor	No	No	No	No	No
Absorption	P-glycoprotein II inhibitor	No	No	No	No	No
Distribution	VDss (human)	–0.681	–0.221	–0.727	–1.292	–1.191
Distribution	Fraction unbound (human)	0.243	0.563	0.295	0.668	0.766
Distribution	BBB permeability	–0.212	–1.142	–0.455	–1.145	–0.754
Distribution	CNS permeability	–2.643	–3.584	–2.795	–4.039	–3.705
Metabolism	CYP2D6 substrate	No	No	No	No	No
Metabolism	CYP3A4 substrate	Yes	No	No	No	No
Metabolism	CYP1A2 inhibitor	No	No	No	No	No
Metabolism	CYP2C19 inhibitor	No	No	No	No	No
Metabolism	CYP2C9 inhibitor	No	No	No	No	No
Metabolism	CYP2D6 inhibitor	No	No	No	No	No
Metabolism	CYP3A4 inhibitor	No	No	No	No	No
Excretion	Total Clearance (log ml/min/kg)	0.340	0.972	0.537	0.749	0.365
Excretion	Renal OCT2 substrate	No	No	No	No	No
Toxicity	AMES toxicity	No	No	No	No	No
Toxicity	Max. tolerated dose (human) (log mg/kg/day)	0.129	0.732	0.931	1.399	0.793
Toxicity	hERG I inhibitor	No	No	No	No	No
Toxicity	hERG II inhibitor	No	No	No	No	No
Toxicity	Oral Rat Acute Toxicity (LD50) (mol/kg)	2.315	2.366	2.240	1.785	1.815
Toxicity	Oral Rat Chronic Toxicity (LOAEL) (mg/kg/day)	1.233	1.607	1.441	1.167	2.170
Toxicity	Hepatotoxicity	Yes	Yes	No	Yes	Yes
Toxicity	Skin Sensitisation	No	No	No	No	No
Toxicity	<i>T. Pyriformis</i> toxicity (µg/L)	0.499	0.285	0.229	0.281	0.285
Toxicity	Minnow toxicity	0.932	1.263	0.187	3.263	3.695

Hint: Ligand annotations are preceded by STOCK3S-.

**Figure 13.** Chemical structures of new putative Zn-sensitive non-β-lactam inhibitors of IMP-1.**Table 5.** Sequence alignment results in similarities between the selected bacterial MBLs, and human and other eukaryotic MBL.

Similarity descriptor	Sequence similarities between bacterial, viral and human MBLs								
	IMP-1 VIM-1	IMP-1 NDM-1	VIM-1 NDM-1	IMP-1 hMBL	VIM-1 hMBL	NDM-1 hMBL	IMP-1 eMBL	VIM-1 eMBL	NDM-1 eMBL
Alignment score	0.103	0.122	0.146	0.425	0.522	0.682	0.574	0.644	0.629
RMSD (Å)	1.607	1.746	1.898	3.236	3.628	4.066	3.768	3.999	3.938
TM-score	0.92	0.90	0.90	0.65	0.70	0.68	0.48	0.47	0.48

Hint: hMBL = human MBL (PDB ID 4V0H); eMBL = MBL of another eukaryote, *Thermotoga maritima* (PDB ID 1WW1); Alignment score of ≤ 0.7 and RMSD ≤ 3.000 Å indicate ideal similarity, the smaller the better. TM-score of 0–1.0 indicates bad to good alignment.

displayed physicochemical parameters support ideal pharmacokinetics for lead-like candidates (Daina et al., 2017; Pires et al., 2015). In terms of distribution, the selected database ligands displayed a consistent volume of drug distribution,

fraction unbound, BBB and CNS permeability with the referenced clinical candidates. Interestingly, all three selected ligands display no expression for substrate/inhibitors of the metabolism-inclined cytochrome P450 isoenzymes family,

similarly to the standard drugs, except **STOCK3S-30154** against CYP3A4. This indicates their potential for fewer side effects and safety to normal cells as ideal pro-drugs. The predicted parameters for safe excretion from biosystems are also very impressive. For instance, they have a total clearance of 0.34–0.97 logs ml/min/kg and no signalling for the endogenous renal transporter (OCT2) substrate, consistently with renowned drug references. More importantly, they are predicted with almost zero potential for toxicity-defining parameters including the AMES, hERG I & II inhibition, skin sensitisation, hepatotoxicity, minnow toxicity and *Tetrahymena Pyriformis*, a model organism for evaluating toxicity in biomedical research. Cumulatively the selected ligands were predicted with ADMET properties that satisfy the BDDC rule of 5 and druggability, and other standards for drug candidates with insignificant violations (Benet et al., 2016; Daina et al., 2017; Pires et al., 2015; Veber et al., 2002).

Final hits

Sequel to the molecular modelling, virtual screening, molecular dynamics simulations and ADMET predictions, three promising ligands, **STOCK3S-30154**, **STOCK3S-30418** and **STOCK3S-30514** (Figure 13) were identified as promising inhibitors of some notable bacterial MBLs, IMP-1, VIM-1 and NDM-1. They demonstrate potential for Zn-sensitivity and the likelihood of broad-spectrum effects. They interacted with Zn ions and other essential amino acid residues through electron donor groups for Zn-chelation and strong binding affinities as pathways of inhibiting the enzymes, consistently with the relevant drugs. The selected ligands also displayed more stabilities and compactness in complexes with Zn-enriched IMP-1 compared to their Zn-starved counterpart. In similar previous studies, some inhibitors have been designed with mechanistic mimicry of the β -lactam substrates. They covalently form adducts with the catalytic serine through acylation as confirmed in pre-clinical experimental models. These interesting antibiotic-like effects are favoured by compounds with carbonyl, dicarboxylate, hydroxamate, sulphate and tetrazole ligands with which the active site Zn ions of the enzymes are sequestered through chelation (Arjomandi et al., 2019; Boyd et al., 2020; van den Akker & Bonomo, 2018). Among other promising candidates, thiosemicarbazone and their analogues such as N-(4-methylpiperazin-1-yl)-2-[(5Z)-4-oxo-2-thioxo-5-(2,3,6-trichlorobenzylidene)-1,3-thiazolidin-3-yl]acetamide (ML302) and its derivative, (2Z)-2-sulfanyl-3-(2,3,6-trichlorophenyl)prop-2-enoic acid (ML302F) have been reported with effective inhibitions inclusively through the Zn sequestration (González-Bello et al., 2020; Moreira et al., 2021). Interestingly, the identified ligands from this study also contain some of such activity-determining pharmacophores including carbonyl, carboxylate, hydroxamate, sulphate and azole moieties, supporting their potentials. They demonstrated ideal ADMET properties for pro-drug candidates and have a non- β -lactam molecular scaffold, supporting their insusceptibility for Zn-dependent deactivation by the enzymes.

Sequence alignment of selected bacterial MBLs with human and other eukaryotic MBLs, and possibilities of cross interactions

From the sequence alignment results, Table 5, the selected bacterial MBLs (PDB IDs 1JJT, 5YPL and 5N5H) show high sequence alignment and similarities among themselves with alignment scores of 0.103–0.146, RMSD of 1.607–1.898 Å and TM-score of 0.90–0.92. However, they display only fair sequence similarities to the hMBLAC1 and eukaryote tRNAse Z MBLs (PDB IDs 4V0H and 1WW1), indicated by alignment scores in the range of 0.425–0.682, high RMSD between 3.236 and 4.066 and average TM-scores between 0.47 and 0.70. Moreover, the selected bacterial MBLs (PDB IDs 1JJT, 5YPL and 5N5H) have some homologous sequence signatures in the active catalytic site residues to the human and other eukaryotic MBLs (PDB IDs 4V0H and 1WW1), especially at the conserved motifs II–V pivotal to their catalytic functions (Supplementary Figure S5) (Ishii et al., 2005; Pettinati et al., 2018). Similar sequence alignments were recently observed between some renowned MBLs (Salari-Jazi et al., 2021). These indicate possibilities for similar ligand-binding for pharmacological expressions. The extended docking analysis of the final hits, **STOCK3S-30154**, **–30418** and **–30514** against the human MBL (PDB ID 4V0H) showed very strong affinities with XP docking scores of –9.230, –12.230 and –8.181 kcal/mol, supporting potentials for inhibiting human MBL. Similarly, the ligands bound potently to the eukaryotic MBL, tRNAse Z from *T. maritima* (PDB ID 1WW1) with respective XP docking scores of –7.221, –7.723 and –5.467 kcal/mol, indicating a possibility for broad-spectrum interactions with MBLs of other pathogenic organisms. The binding poses (Supplementary Figure S6) illustrate the sensitivity of the ligands to the Fe site of the hMBLAC1 (PDB ID 4V0H) and Zn ion at the active site of the eukaryote (*Thermotoga maritima*) MBLAC1 (PDB ID 1WW1). The ligands interacted with essential amino acid residues for catalytic activities. For instance, in human MBL, the ligands bound to the active site His 117, His 119, Asp 120 and His 122, correspond to the His 116, His 118, Asp 120 and His 121 on the conserved motif II of the MBL superfamily including those under study. Also, His 172 (196), Asp 195 (221) and His 234 (263) on motifs III, IV and V respectively for catalytic activities (Pettinati et al., 2018). These support the potential of the selected ligands for inhibiting the human MBLs, some of which are known to degrade antibiotics such as penicillin (Diene et al., 2019). Similarly, the selected ligands interacted with and interacted with relevant amino acid residues at the conserved motifs II–V of the tRNAse Z MBLAC1 of the eukaryotic *T. maritima*. These include His 48 (116), His 50 (118), Asp 52 (120) and His 53 (121), commonly to the conserved motif II of the MBL superfamily. Others are His 134 (196), Asp 190 (221) and His 224 (263) of the conserved motifs III, IV and V, respectively. These correspond to the pharmacological pathways for inhibiting the correct processing of the pre-tRNA, a major function of the enzyme in eukaryotes (Ishii et al., 2005). Cumulatively, the ligands display potentials for broad-spectrum inhibition across various MBLs, amenable to designing stand-alone/adjuvant antibiotics.

Conclusion

The extensive application of computational molecular modeling and virtual screening herein has afforded the identification of putative inhibitors of MBLs, specifically IMP-1 from the IBScreen chemical library. Out of the 555 093 screening ligands available in the database, three hits, annotated as **STOCK3S-30154**, **STOCK3S-30418** and **STOCK3S-30514** were identified as promising Zn-sensitive, broad-spectrum inhibitors of the most relevant bacterial MBLs, especially, IMP-1.

Although, the study represents *de novo* and theoretical drug design, as such, requires rigorous *in vitro* and *in vivo* experimental validations, phase two of drug discovery for more robust conclusions. However, the study ideally reposes the required confidence for embarking on such resource-demanding explorations and models an economical, faster and eco-friendly approach involving structure- and ligand-based drug designs. Again, it represents a reliable discovery phase of therapeutic design. Thus, the three IBScreen database ligands, **STOCK3S-30154**, **STOCK3S-30418** and **STOCK3S-30514** are discovered as promising broad-spectrum, Zn-sensitive and non- β -lactam MBL inhibitors for further experimental studies.

Disclosure statement

The authors declare no conflict of interest.

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Author contributions

The manuscript was written with the contributions of all authors. All authors have approved the final version of the manuscript. Conceptualization, literature survey, data collection and writing by YOA, data collection and writing by AI, WAA and AL, research supervision and manuscript revision by MNM and HP.

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