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Original Article

In Silico Discovery of Natural Inhibitors against New Delhi Metallo-*β*-Lactamase-1: A Step towards Combating Superbug Resistance

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ABSTRACT

The emergence of New Delhi metallo-*β*-lactamase-1 (NDM-1) leads to a significant global health threat due to its ability to hydrolyze a broad range of *β*-lactam antibiotics, including carbapenems, leaving few alternatives for therapy. Therefore, identifying non-*β*-lactam inhibitors is crucial for combating NDM-1-mediated resistance. In this study, a multi-tiered virtual screening approach was employed against a library of 57,423 natural compounds from the Traditional Chinese Medicine Database@Taiwan. Structure-based virtual screening, including High Throughput Virtual Screening (HTVS), Standard Precision (SP), and extra-precision (XP) docking protocols, was performed using the Schrödinger suite. Drug-likeness was evaluated using Lipinski's Rule of Five and Jorgensen's Rule of Three, leading to the identification of ten promising hits. Among them, ZINC95909696 demonstrated a more favorable binding affinity of -10.041 kcal/mol, outperforming the co-crystallized *β*-lactam antibiotic ampicillin (-7.087 kcal/mol). Binding interaction analysis reveals hydrogen bonding with Asn220 and Lys211, along with coordination with the catalytically essential Zn^2 ion (Zn302), highlighting its potential as a non-*β*-lactam-based NDM-1 inhibitor. A 100 ns molecular dynamics simulation further confirmed the stability of the ZINC95909696–NDM-1 complex, as reflected by minimal fluctuations in RMSD and RMSF profiles. These results highlight ZINC95909696 as a compelling lead candidate for developing non-*β*-lactam therapeutics targeting NDM-1 *β*-lactamase.

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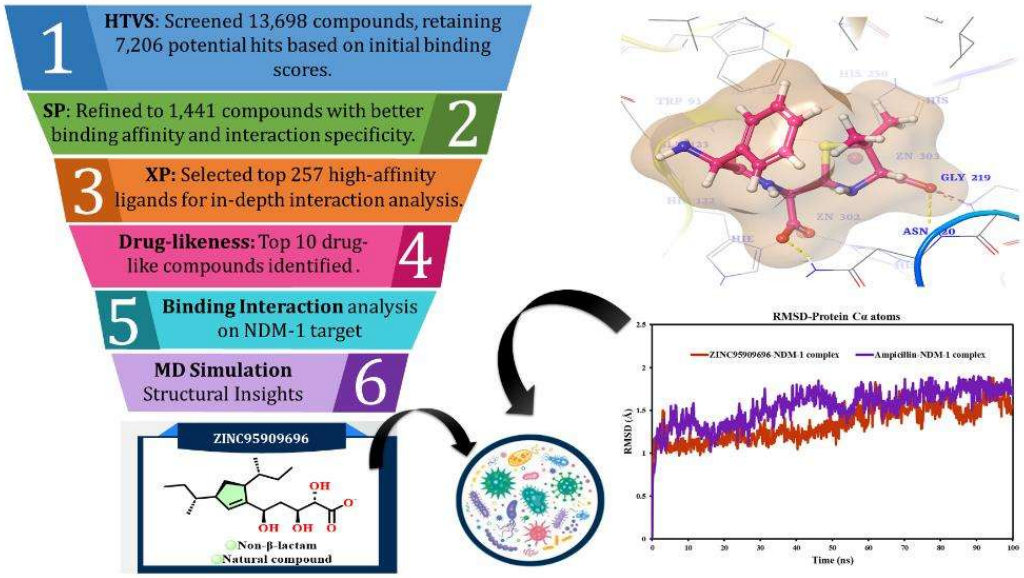
GRAPHICAL ABSTRACT



Introduction

The therapeutic use of antibiotics has transformed contemporary medicine by efficiently addressing bacterial illnesses, decreasing death rates, and markedly improving life expectancy. However, the rapid and widespread emergence of antimicrobial resistance (AMR) among bacterial pathogens has severely compromised the efficacy of many conventional antibiotics [1-4]. Antimicrobial resistance has become a major challenge in drug discovery, as it limits the efficacy of existing drug classes and complicates the development of new antibiotics with sustainable therapeutic value [5,6]. This growing resistance has not only increased the burden on healthcare systems globally, but has also emerged as a major hurdle in current drug discovery and development efforts [7]. Traditional antibiotic pipelines are drying up, and pharmaceutical innovations are often outpaced by the rapid evolution of resistant strains, making it increasingly difficult to develop drugs that remain effective over time [8]. One of the most alarming threats in this context is the bacterial ability to neutralize *β*-lactam antibiotics—the most extent used class—primarily through the production of *β*-lactamase enzymes [9]. These enzymes hydrolyze the *β*-lactam ring, an essential structural element of *β*-lactam antibiotics, therefore leaving them therapeutically ineffective [9-11]. New Delhi Metallo-*β*-lactamase-1 (NDM-1) has attracted considerable international interest among the several forms of *β*-lactamases due to its extensive hydrolytic activity [12,13]. First reported in 2008 from a Swedish patient previously hospitalized in India with a *Klebsiella pneumoniae* infection, NDM-1 has since been identified in multiple Gram-negative bacterial species, including *Escherichia coli*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Enterobacter cloacae* [14,15]. Its capability to confer resistance to nearly all *β*-lactam antibiotics, including penicillins, cephalosporins, and carbapenems, last line of defense against multidrug-resistant infections—positions NDM-1 as a critical target in the global fight against superbugs [16,17].

The clinical challenge is further intensified by the limited success in developing effective *β*-lactamase inhibitors. Although various compounds, such as captopril derivatives, thiols, boric acid analogs, sulfinamides, and natural products, have demonstrated *in vitro* inhibitory activity against NDM-1, none have yet received clinical approval [16-18]. This underscores the urgent need for the development of novel and potent NDM-1 inhibitors. Notably, non-*β*-lactam-based inhibitors offer a strategic advantage, as they are not easily hydrolyzed by *β*-lactamases and are less susceptible to bacterial resistance mechanisms such as efflux pumps and reduced membrane permeability [19,20].

The use of computational methods, such as molecular docking and molecular dynamics simulations, has emerged as a powerful tool in the search for potential inhibitors. In this context, natural compounds represent a rich source of structurally diverse and biologically active molecules. In the present study, a multilayer docking screening method was used to find possible natural inhibitors of NDM-1. from a library of 57,423 compounds available in the Traditional Chinese Medicine Database@Taiwan, using the Glide software suite. Among the screened compounds, ZINC95909696 was identified as a promising inhibitor of NDM-1 based on extra-precision (XP) docking, molecular dynamics (MD) simulation, and interaction analysis. These findings offer a potential new strategy for combating NDM-1-mediated resistance and may facilitate the future planning and growth of effective antibacterial agents. This compound's potential efficacy against NDM-1 could pave the way for new therapeutic strategies to combat antibiotic resistance. Further investigation into the mechanism of action and optimization of ZINC95909696 could lead to the development of clinically viable NDM-1 inhibitors.

Experimental

Selection and Preparation of NDM-1 Target Structure

The three-dimensional structure of New Delhi metallo-*β*-lactamase-1 (NDM-1) complexed with hydrolyzed ampicillin was obtained from the Protein Data Bank (PDB ID: 5ZGE) at a high resolution of 1.00 Å [21]. The structure comprises two chains, from which chain B was excluded, and only chain A was retained for further analysis. The zinc ion, crucial for the enzymatic activity of NDM-1, was maintained in its original coordination state. The Protein Preparation Wizard was used to prepare the protein for computational research. The refinement process encompassed several steps, including the removal of crystallographic water molecules situated more than 5 Å from the ligand-binding site, the addition of missing hydrogen atoms, the assignment of correct bond orders, and the optimization of the protonation states of titratable residues to reflect physiological pH conditions [22,23]. Finally, the structure underwent energy minimization to resolve any steric hindrance and ensure stability for molecular docking simulations.

Optimization of Natural Compound Dataset

A library comprising 57,423 natural compounds was obtained from the Traditional Chinese Medicine Database@Taiwan. The molecular structures were initially processed using the LigPrep module to convert 2D representations into accurate 3D conformations. To simulate physiological conditions, Epik was employed to generate all relevant ionization states at pH 7.4, along with biologically plausible tautomers and stereoisomers. This step ensured comprehensive coverage of each compound's structural diversity. Subsequently, all structures were energy-minimized using the OPLS3e force field, enabling correction of geometrical distortions and elimination of any steric strain, thus preparing the dataset for reliable molecular docking analyses [24-25].

Structure-based Virtual Screening and Molecular Docking Workflow

Structure-based virtual screening was carried out using the Schrödinger Virtual Screening Workflow, which integrates multiple precision levels to systematically narrow down potential inhibitors [26]. The prepared natural compound library was initially screened using High Throughput Virtual Screening (HTVS) mode to rapidly evaluate binding potential. Compounds ranking within the top 20% based on docking Score were then subjected to Standard Precision (SP) docking for more refined interaction analysis. Subsequently, the top 20% of SP-ranked hits were further processed using Extra-precision (XP) docking to obtain high-confidence binding poses and scores. This tiered approach enabled the efficient prioritization of candidate molecules

with strong binding affinity toward the NDM-1 active site.

Molecular Dynamics Simulation for Stability Assessment

MD simulations employing the Desmond simulation software were conducted to assess the dynamic stability of the protein-ligand complex. The system was solubilized in an SPC (Simple Point Charge) water model inside an orthorhombic box, maintaining a 10 *Å* buffer distance to the complex. Suitable counter ions were introduced to equilibrate the system. Before the simulation execution, energy reduction was performed to resolve steric conflicts and stabilize the system [27-30]. The simulation was performed under NPT ensemble conditions, characterized by a constant number of particles, pressure, and temperature. The system was maintained at a temperature of 300 *K* and a pressure of 1.0 bar, utilizing the Nose–Hoover thermostat and the Martyna–Tobias–Klein barostat, respectively [31-32]. The OPLS3e force field was used consistently to guarantee precise depiction of molecular interactions. A manufacturing run lasting 100 ns was conducted to assess the structural stability and binding characteristics of the ligand inside the active site of NDM-1.

Results and Discussion

High-Throughput Virtual Screening and Top-Hit Selection

Structure-based virtual screening of 13,698 natural compounds was conducted using a three-tier docking approach in the Schrödinger Virtual Screening Workflow, incorporating HTVS, SP, and XP docking modes. Each stage played a crucial role in balancing speed, accuracy, and computational efficiency. The combination of these three docking tiers allowed a reliable and systematic narrowing of the compound library, ultimately identifying strong candidate molecules for further evaluation using molecular dynamics simulations and binding free energy calculations. This approach enhances confidence in the selection of potential NDM-1 inhibitors and supports rational drug discovery efforts against resistant bacterial pathogens. The initial High Throughput Virtual Screening (HTVS) phase served as a rapid filtering step, efficiently eliminating compounds with weak binding potential (Figure 1). This allowed the prioritization of 7,206 molecules with Glide Docking Score values ranging from -5.708 to -9.253 kcal/mol, suitable for more refined docking. In the second tier, Standard Precision (SP) docking was applied to improve the accuracy of binding predictions. This method accounts for more detailed scoring functions and conformational flexibility, yielding 1,441 compounds with improved docking scores between -7.055 and -9.866 kcal/mol. Finally, the top 20% of SP hits were subjected to Extra-precision (XP) docking. XP docking applies more stringent scoring penalties and enhanced sampling, making it ideal for selecting the most promising ligands with high binding affinity and specificity. This stage identified 257 top-ranking compounds, with docking scores ranging from -9.866 to -13.865 kcal/mol. This systematic docking-based screening method was utilized to ascertain the most promising inhibitors of NDM-1, capable of binding effectively within its active site and overcoming its resistance mechanisms. The identified top hits were then subjected to further evaluation through Drug-Likeness Evaluation, molecular dynamics simulations and binding free energy analysis to assess their stability and interaction behavior in a dynamic biological environment.

Drug-Likeness Evaluation of Selected Hits

To evaluate the drug-likeness and oral bioavailability potential of the top hits identified through docking, an *in silico* Drug-Likeness was conducted using Lipinski's Rule of Five and Jorgensen's Rule of Three. These established filters are often used in early-stage drug research to forecast oral medication viability. This rule indicates that a molecule is more likely to exhibit oral bioactivity if its molecular weight is 500 *Da*

or less, it has no more than five hydrogen bond donors (HBD), no more than ten hydrogen bond acceptors (HBA), and an estimated octanol-water partition coefficient (logP) of five or below. Compounds violating more than one of these criteria are generally considered with poor permeability or absorption. A total of ten best compounds with favorable docking scores and drug-likeness parameters are presented in Table 1. Among them, five compounds (ZINC39029165, ZINC85488256, ZINC85488192, ZINC36422663, and ZINC95909696) fully complied with Lipinski's Rule of Five, suggesting strong oral drug potential. The remaining five compounds showed one violation each, primarily due to a higher number of hydrogen bond donors (HBD > 5), which may still be acceptable depending on the overall pharmacokinetic context. All selected compounds also satisfied Jorgensen's Rule of Three, indicating acceptable solubility (QPlogS < -5.7), good permeability (QPPCaco > 22 nm/s), and low metabolic liability (#metab < 7). Notably, ZINC95909696 displayed superior permeability (QPPCaco = 32.214), zero primary metabolites, and a predicted 69.87% human oral absorption, making it a strong candidate for further optimization.

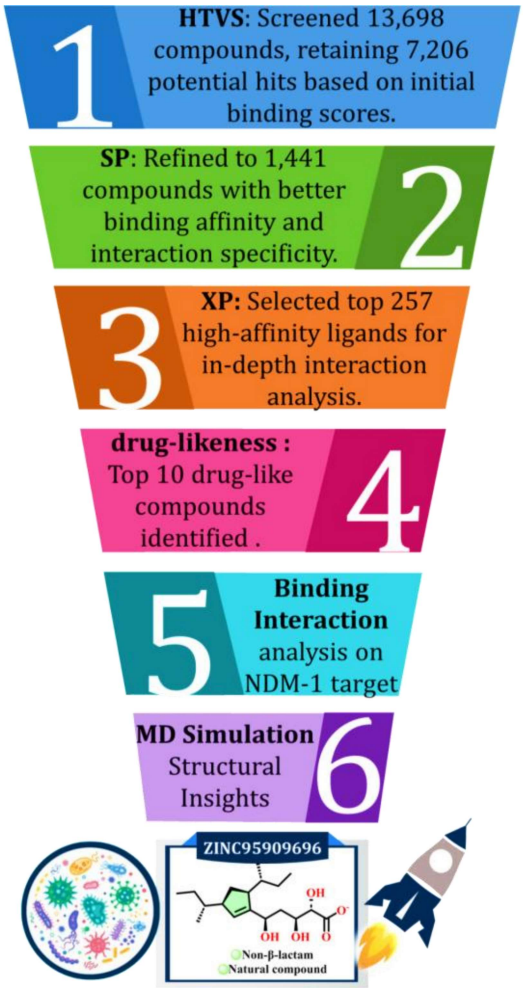


Figure 1: The workflow of the study to identify NDM-1 inhibitors

Table 1: Lipinski's rule of five, Jorgensen Rule of Five (Drug likeliness) prediction Compounds from TCM Database @ Taiwan

Name of Compounds	Docking Score	Lipinski's Rule of Five					Jorgensen Rule of Three				% Human Oral Absorption
		mol MW	Donor HB	Accpt HB	QPlog Po/w	Rule of Five	QPlogS	#metab	QPPCaco	Rule of Three	
ZINC39029165	-10.967	352.34	4	7.9	0.899	0	-2.839	3.093	5	1	40.982
ZINC85488256	-10.261	387.515	4	9.6	-0.335	0	-3.179	1.907	5	1	30.001
ZINC85488192	-10.154	421.579	3	7.9	1.409	0	-5.363	6.322	3	1	49.531
ZINC36422663	-10.143	324.376	3	7.7	-0.838	0	-2.161	13.2	4	1	42.094
ZINC95909696	-10.041	328.448	3	6.1	2.722	0	-3.425	32.214	5	0	69.872
ZINC06524388	-10.837	344.274	7	10.4	-1.403	1	-1.91	0.728	6	1	53.305
ZINC01529781	-10.715	354.313	6	9.65	-0.295	1	-2.46	1.658	5	1	46.193
ZINC13783510	-10.617	354.313	6	9.65	-0.463	1	-2.231	1.45	5	1	74.163
ZINC33830037	-10.586	354.313	6	9.65	-0.325	1	-2.25	1.654	5	1	46.54
ZINC85645585	-10.423	354.313	6	9.65	-0.283	1	-2.148	2.536	5	1	69.563
Co-crystal ligand	-7.087	367.419	4.25	7.75	-1.761	1	0.038	0.107	6	1	86.651

Lipinski's rule of five. The rules are: MW < 500, logPo/w<5, donor HB ≤ 5, and accept HB ≤ 10.
Jorgensen's rule of three. The three rules are: logS < -5.7, PCaco > 22 nm/s, and # Primary Metabolites < 7

Binding Interaction Analysis

According to the outcomes of the multilayered virtual screening and drug-likeness assessment, ZINC95909696 has been identified as a viable candidate for future exploration. This compound demonstrated a notable docking score of -10.041 kcal/mol, significantly better than the co-crystallized broad-spectrum *β*-lactam antibiotic Ampicillin, which served as the control and exhibited a docking score of -7.087 kcal/mol. Molecular docking analysis reveals that ZINC95909696 binds within the active site of NDM-1, overlapping with the binding region of the native substrate, suggesting its potential to competitively inhibit the enzyme. The interaction profile exhibited that ZINC95909696 forms hydrogen bonds with Asn220 and Lys211, which are crucial for stabilizing the ligand within the active site. Importantly, it also showed ionic interaction with Lys211 and ionic interaction with the catalytic Zn^{2+} ion (Zn302), a key feature for potent inhibition. Comparatively, the co-crystal ligand Ampicillin also interacts with Asn220 via hydrogen bonding, along with metal coordination involving both Zn302 and Zn303, and ionic contact with Lys211. The presence of similar binding interactions between ZINC95909696 and Ampicillin, particularly with Asn220, Lys211, and the zinc ion, highlights the structural compatibility of ZINC95909696 within the active site. However, a significant advantage of ZINC95909696 is that it lacks the *β*-lactam ring, making it structurally distinct and potentially less vulnerable to *β* lactamase mediated hydrolysis. The role of zinc ions in the NDM-1 active site is critical; it promote the hydrolysis of *β* lactam antibiotics by coordinating the water molecule responsible for nucleophilic attack. Inhibitors that can effectively chelate or interact with these zinc ions can disrupt this mechanism and block enzymatic activity. The observed metal coordination between ZINC95909696 and Zn302 underscores its potential as a non *β* lactam based inhibitor capable of effectively neutralizing NDM-1

function. The 2D and 3D visualizations of docking interactions are displayed in Figure 2, which clearly depicts the favorable orientation and critical contacts of ZINC95909696 within the NDM-1 active site. Overall, these findings support the potential of ZINC95909696 as a novel and effective NDM-1 inhibitor, meriting further stability analysis.

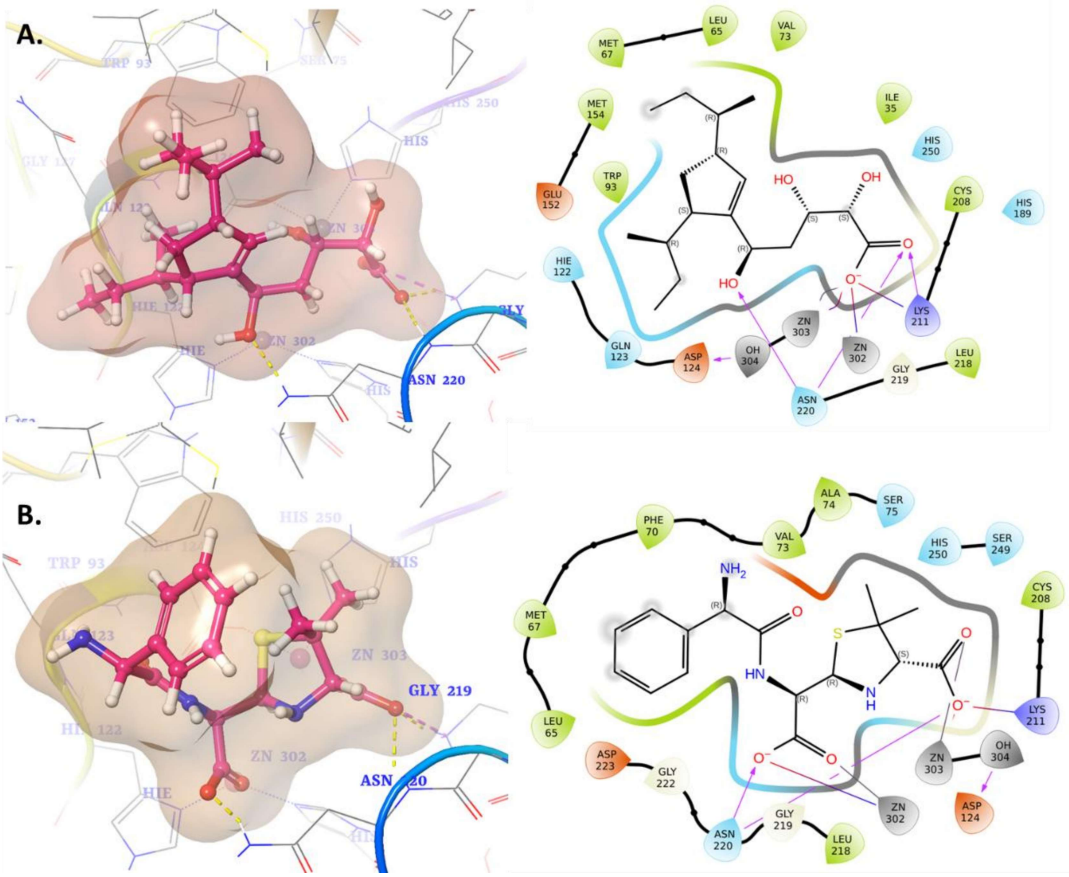


Figure 2: The binding interactions of identified compounds ZINC95909696 and co-crystallized ligand (Ampicillin) in the active site of NDM-1 (PDB ID: 5ZGE) protein

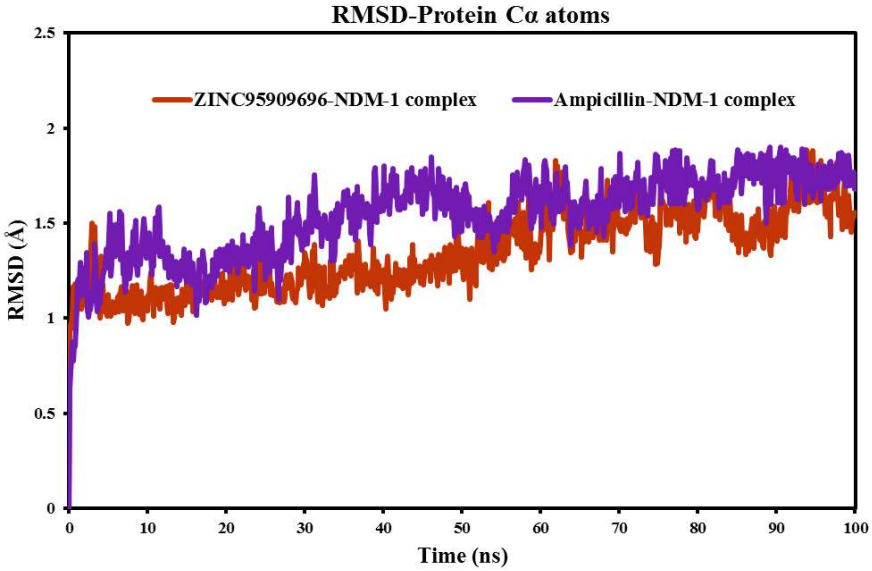


Figure 3: Time-dependent RMSD plot for the Cα atoms of the NDM-1 protein in complex with ZINC95909696 and Ampicillin the 100 ns MD simulations

Structural Insights from Molecular Dynamics Simulation

To explore the binding stability and dynamic behavior of ZINC95909696 within the active site of NDM-1, a 100 ns molecular dynamics (MD) simulation was conducted. This simulation provided valuable insights into the temporal stability of the protein–ligand complex under near-physiological conditions. Key MD parameters—including Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), and hydrogen bond analysis—were evaluated to assess conformational stability and residue-level flexibility throughout the simulation period.

RMSD tracks the average deviation of atomic positions from the initial structure over time and is a key indicator of the overall stability of a protein–ligand complex during simulation. A low and stable RMSD indicates minimal structural deviations and sustained ligand binding. The RMSD plot showed the initial fluctuations due to system equilibration, followed by minor deviations for the remainder of the simulation [33,34]. The average RMSD values for the ZINC95909696–NDM-1 and Ampicillin–NDM-1 complexes were 1.35 *Å* and 1.54 *Å*, respectively (Figure 3). Notably, no frames exceeded 2 Å RMSD, confirming the structural stability of both complexes throughout the 100 ns trajectory.

RMSF provides insights into the flexibility of individual amino acid residues in the protein during the simulation. Lower RMSF values denote rigid and stable regions, often crucial for ligand binding [34,35]. Both ZINC95909696–NDM-1 and Ampicillin–NDM-1 complexes showed an average RMSF of 0.72 Å. Residues such as Glu30, Ala172, Gly222, Thr41, Pro171, and Gly42 exhibited higher fluctuations, likely due to their location in flexible loop regions (Figure 4). Importantly, residues interacting with ZINC95909696—such as Phe70, Asn220, Met67, Leu218, Glu152, Asp223, Ile35, Leu65, Lys211, Trp93, Ala74, His189, His250, Gln123, Cys208, Asp124, His120, and His122—displayed minimal RMSF values, suggesting stable interactions with the ligand and limited mobility at the binding site.

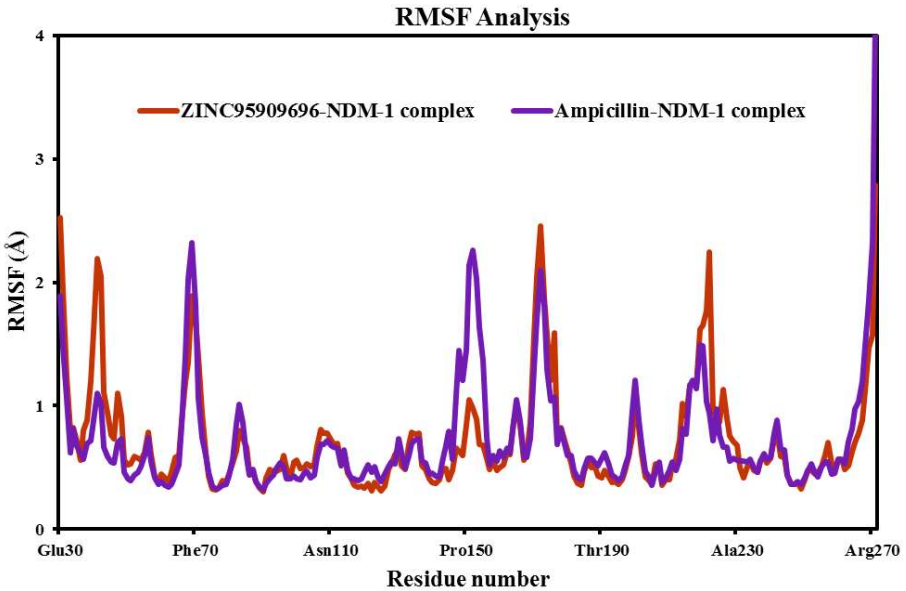


Figure 4: RMSF of individual amino acids Cα atoms of the NDM-1 protein in complex with ZINC95909696 and Ampicillin the 100 ns MD simulations

Hydrogen bonding analysis evaluates the frequency and stability of interactions between the ligand and protein during simulation. Stable hydrogen bonds are critical for strong and specific ligand binding [34,36]. The ZINC95909696–NDM-1 complex exhibited a maximum of four hydrogen bonds, with an average of 1.35 over the simulation time. In contrast, the Ampicillin–NDM-1 complex formed a maximum of three hydrogen bonds, with a

significantly lower average of 0.54 (Figure 5). The greater number and consistency of hydrogen bonds in the ZINC95909696 complex highlight its potential as a more stable and effective non-β-lactam inhibitor against NDM-1.

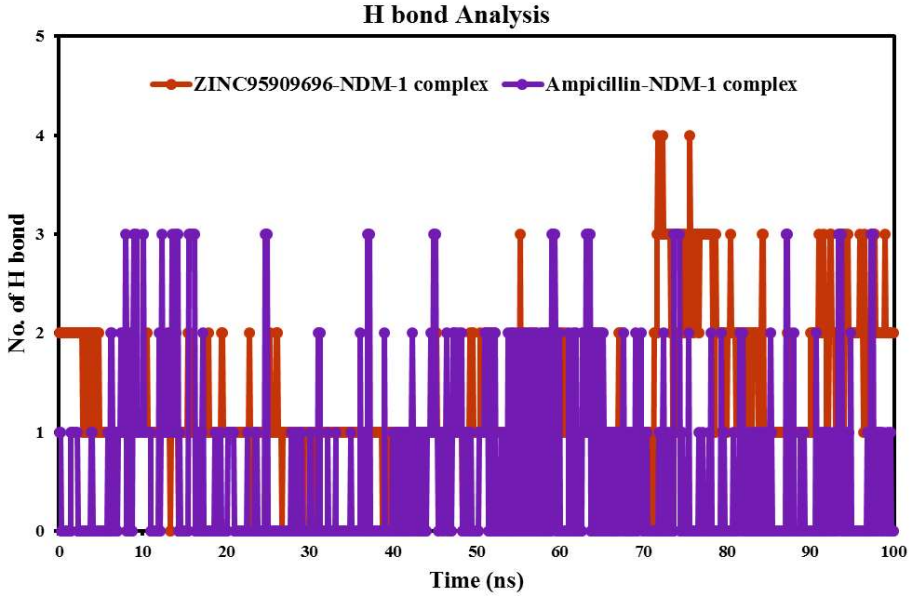


Figure 5: Time-dependent hydrogen analysis of ZINC95909696 and Ampicillin in complex with NDM-1 protein during 100 ns MD simulations

Binding Free Energy Analysis

Table 2: The MMGBSA binding free energy values (in kcal/mol) and their contributing components for ZINC95909696 and Ampicillin in complex with the NDM-1

MMGBSA Parameter	ZINC95909696- NDM-1 Complex	Ampicillin - NDM-1 Complex
ΔG_{Bind}	-17.83 ±3.74	-16.37 ±4.33
$\Delta G_{BindCoul}$	-48.02 ±14.34	-28.12 ±13.44
$\Delta G_{BindHbond}$	-1.02 ±0.44	-0.72 ±0.03
$\Delta G_{BindLip}$	-15.00 ±3.54	-2.18 ±0.59
$\Delta G_{Bindsol\_GB}$	80.39 ±21.67	91.16 ±13.65
$\Delta G_{Bindvdw}$	-31.11 ±2.87	-5.19 ±2.66

Molecular Mechanics Generalized Born Surface Area (MM-GBSA) calculations were performed to further validate the binding affinity of ZINC95909696 within the NDM-1 active site. ΔG_{Bind} Represents the total binding free energy between the ligand and protein. A more negative value indicates stronger binding affinity. The binding free energy (ΔG_{Bind}) for the ZINC95909696-NDM-1 complex was found to be -17.83 ± 3.74 kcal/mol, slightly more favorable than the reference Ampicillin-NDM-1 complex, which showed a ΔG_{Bind} of -16.37 ± 4.33 kcal/mol (Table 2). The binding energy decomposition reveals that ZINC95909696 benefits from strong van der Waals interactions ($\Delta G_{Bindvdw}$ = -31.11 ± 2.87 kcal/mol) and lipophilic contributions ($\Delta G_{BindLip}$ = -15.00 ± 3.54 kcal/mol), both significantly higher than those for Ampicillin. Additionally, the electrostatic (Coulombic) energy component ($\Delta G_{BindCoul}$) for ZINC95909696 was notably higher (-48.02 ± 14.34 kcal/mol) compared to Ampicillin (-28.12 ± 13.44

kcal/mol), further supporting strong binding interactions. While the solvation penalty ($\Delta G_{Bindsol\_GB}$) was higher for ZINC95909696 (80.39 ± 21.67 kcal/mol), the overall energy balance remained favorable. Collectively, these results affirm the superior binding affinity of ZINC95909696, highlighting it as a promising non-*β*-lactam inhibitor against NDM-1.

Conclusion

The emergence and global spread of NDM-1 represent a critical public health threat due to its potent ability to hydrolyze nearly all *β*-lactam antibiotics, including penicillins, cephalosporins, and carbapenems—the last line of defense against multidrug-resistant bacterial infections. The broad-spectrum resistance mechanism of this enzyme has rendered many conventional antibiotics ineffective, leading to frequent treatment failures and limited therapeutic options. Given the urgent need for alternative strategies, the discovery of novel non-*β*-lactam NDM-1 inhibitors is of paramount importance. In this study, a multi-tiered structure-based virtual screening approach was employed by library of 57,423 natural compounds from the Traditional Chinese Medicine Database@Taiwan using the Schrödinger suite. HTVS filtered the initial dataset to 7,206 candidates, which were subsequently refined to 1,441 through SP docking. Further narrowing using XP docking yielded 257 top-ranked ligands with high predicted binding affinity. ADME and drug-likeness profiling *via* Lipinski's Rule of Five and Jorgensen's Rule of Three identified ten compounds with favorable pharmacokinetic properties, among which ZINC95909696 emerged as the most promising hit, displaying a superior docking score of −10.041 kcal/mol compared to the co-crystal ligand ampicillin (-7.087 kcal/mol). Binding interaction analysis reveals that ZINC95909696 formed stable hydrogen bonds with Asn220 and Lys211 and coordinated with the catalytically essential Zn^{2+} ion (Zn302), crucial for NDM-1 activity. Notably, this compound lacks a β-lactam core, highlighting its potential to evade traditional resistance mechanisms. A 100 ns molecular dynamics simulation confirmed the structural stability of the ZINC95909696–NDM-1 complex, with consistent RMSD and RMSF profiles. Furthermore, MM-GBSA binding free energy calculations showed favorable ΔGBind values (-17.83 ± 3.74 kcal/mol), comparable to ampicillin (-16.37 ± 4.33 kcal/mol), with strong contributions from van der Waals, electrostatic, and hydrophobic interactions. These findings position ZINC95909696 as a strong lead candidate for developing next-generation, non-*β*-lactam NDM-1 inhibitors, addressing a critical gap in antimicrobial therapy.

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