Repurposing of Oxicam Derivatives to Inhibit NDM-1: Molecular Docking and Molecular Dynamic Simulation Studies

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Abstract:

The New Delhi Metallo-β-lactamase-1 (NDM-1) causes hydrolysis of broad spectrum β-lactam antibiotics, such as carbapenems, resulting in the development of antimicrobial resistance. Still, there are not any approved NDM-1 inhibitors, globally. Therefore, repositioning approved medicines as NDM-1 inhibitors to combine with carbapenems may be a crucial strategy to combat resistant pathogens. This study repurposes. Oxicam derivatives as inhibitors of bacterial NDM-1. The two-dimensional structures were obtained from the PubChem database. Twenty derivatives of oxicam were assessed computationally to realize their NDM-1 inhibition capability. To identify potential inhibitors of the NDM-1 target protein, a molecular docking protocol was used. In addition, drug-likeness and pharmacokinetic properties were predicted for the designed molecules. Three compounds with the most negative $\Delta G_{binding}$ results were chosen for additional study using molecular dynamic (MD) simulations. The compounds M010, M013, and M016 possessed a significantly more negative binding free energy than the positive control and other designed molecules, had stable MD simulations (Root-mean-square deviation < 0.5 Å), passed Lipinski's rule of five, and possessed favourable physicochemical and pharmacokinetic properties. The findings can inform *In vitro* studies of the promising compounds.

Keywords: Antibiotic resistance; drug repurposing; molecular docking study; molecular dynamics simulation; oxicam derivatives.

إعادة استخدام مشتقات أوكسيكام لمنع NDM-1: دراسات الالتحام الجزيئي والمحاكاة الديناميكية الجزيئية

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الخلاصة:

يسبب New Delhi Metallo-β-lactamase-1 (NDM-1) التحلل المائي للمضادات الحيوية واسعة النطاق من نوع β-lactam، مثل الكاربابينيمات، مما يؤدي إلى تطور مقاومة مضادات الميكروبات. ومع ذلك، لا توجد أي مثبطات NDM-1 معتمدة على مستوى العالم. ولذلك، فإن إعادة وضع الأدوية المعتمدة كمثبطات NDM-1 لتتحد مع الكاربابينيمات قد تكون استراتيجية حاسمة لمكافحة مسببات الأمراض المقاومة. هذه الدراسة تعيد الأغراض.

مشتقات الأوكسيكام كمثبطات للبكتيريا NDM-1. تم الحصول على الهياكل ثنائية الأبعاد من قاعدة بيانات PubChem. تم تقييم عشرين من مشتقات الأوكسيكام حسابيًا لتحقيق قدرتها على تثبيط NDM-1. لتحديد المثبطات المحتملة للبروتين المستهدف NDM-1، تم استخدام بروتوكول الالتحام الجزيئي. بالإضافة إلى ذلك، تم توقع التشابه الدوائي والخصائص

الدوائية للجزيئات المصممة. تم اختيار ثلاثة مركبات ذات نتائج ΔGbinding الأكثر سلبية لإجراء دراسة إضافية باستخدام عمليات المحاكاة الديناميكية الجزيئية (MD). تمتلك المركبات M010 وM013 وM016 طاقة ارتباط حرة سلبية أكثر بكثير من التحكم الإيجابي والجزيئات المصممة الأخرى، ولها محاكاة MD مستقرة (انحراف الجذر المتوسط <0.5 Å)، واجتازت قاعدة ليبينسكي الخمسة، وامتلكت مواتية. الخصائص الفيزيائية والكيميائية والحركية الدوائية. يمكن أن تفيد النتائج الدراسات المختبرية للمركبات الواعدة.

الكلمات المفتاحية: مقاومة المضادات الحيوية؛ إعادة استخدام المخدرات؛ دراسة الالتحام الجزيئي؛ محاكاة الديناميات الجزيئية. مشتقات أوكسيكام.

Introduction

The class of antimicrobial drugs known as β-lactam antibiotics is extensively prescribed globally (1). This category of antimicrobial agents have therapeutic advantages and known as a preferred antimicrobial medications because of ease of access, distinctive mode of action against bacterial cellular structures, and fewer side-effects (2). The significant growth of β-lactam resistance is due to the increased consumption, unnecessary prescriptions, and misuse of β-lactams during minor infections (3). The growth of resistance can be caused by Gram-negative bacteria, which are primarily responsible for this phenomenon. Carbapenems as a class of β-lactam antibiotics are commonly recognized as the ultimate resort in the management of bacterial infections (4). Development of novel antibiotics is an urgent global requirement because certain bacteria are resistance against not only available antibiotics but also empirical therapies, such as β-lactams, β-lactamase–β-lactams combinations, fluoroquinolone, lipopeptides, macrolides, and tetracyclines (5). The widespread distribution of resistance caused by *Enterobacteriaceae* to carbapenem, and the production of Metallo-β-lactamase (MBL) is a significant issue for public health. Over the span of years, the New Delhi Metallo-β-lactamase-1 (NDM-1) has appeared as a main contributor to the failure of carbapenem therapy (6). MBL(s) are categorized based on sequence alignment shown by common structural features into the following classes: B1, B2, and B3 (7). NDM-1 is belonged to B1 subclass and composed of 270 amino acid residues as a single chain of polypeptides, with 28 amino acids at N-terminus. Hydrolysis of β-lactam antibiotics can be performed by translocating the enzyme to the outer membrane of Gram-negative bacteria (8). The monomer of NDM-1 protein consists of five α helices and twelve β sheets. The initial pair of sheets are connected by an α helix, while the following pair are bordered by a fifth α helix, resulting in a structure that resembles a sandwich composed of αβ /βα elements (9) (Fig.1). The active site of NDM-1 is primarily consisted of L3, L7, and L10 loops, which are amino acid chains Leu65–Val73, Thr119–Met126, and Cys208–Leu221, respectively. Additionally, hydrolysis of the β-lactam rings is facilitated by two zinc ions (Zn1 and Zn2) at the bottom of the active site (10,11). Zn1 can generate a tetrahedral configuration (histidine site) through the formation of strong bonds with His120, 122, 189, and a water/hydroxyl group, resulting in the tightly bound zinc. The zinc ion Zn2 is characterized by a relatively low degree of coordination and is capable of binding to Aspa124, Cys208, and His250 amino acid residues, thereby forming a triangular conical structure (cysteine site). The interaction between Zn1 and Zn2 occurs via side chain of Asp124 (12,13). The sequence of NDM-1 exhibits remarkable differences from other MBLs, displaying the highest sequence homology with VIM-4 (38%), VIM-2 (32%), and IMP-1. The NDM-1 variant exhibits a significantly elongated N-terminus and a more hydrophobicity in L3, thereby promoting a stronger affinity towards inhibitor molecules (12,14) (Fig.2).

Hydrolysis of β-lactam antibiotics through β-lactamase is a crucial mechanism employed by bacteria to safeguard themselves against antibiotics and impede the antibiotics' access to the penicillin-binding protein (PBP) target site. The classification of these enzymes is based on their structural homology, which has resulted in the formation of four distinct classes, namely A, B, C, and D. The enzymes classified as A, C, and D are commonly referred to as serine β-lactamases (SBLs) due to their ability to utilize serine as a nucleophilic agent in the hydrolysis of the β-lactam bond. In contrast, class B enzymes are referred to as Metallo-β-lactamases (MBLs) because they are capable to cleave the β-lactam amide bond in antibiotics through the facilitation of Zn^{+2}, which activates the Zn^{+2}-bound water/hydroxide necessary for nucleophilic substitution (15) (Fig.3).

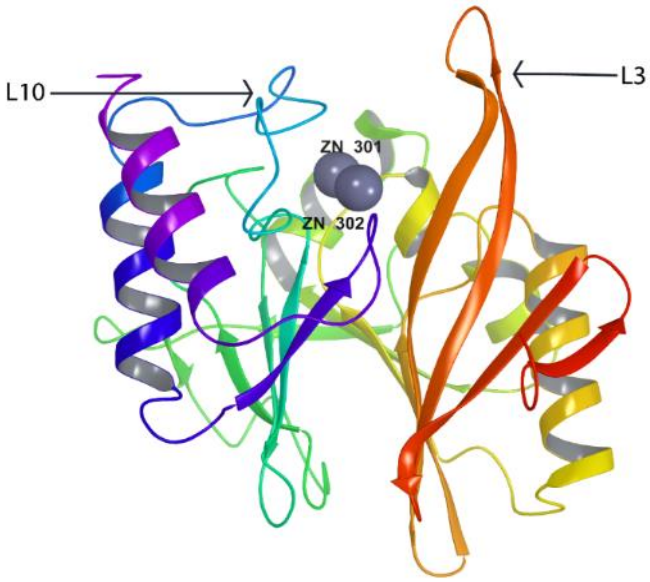

Figure (1) The 3D crystal structure of NDM-1 to show alpha helices, β sheets, zinc ions and loops.

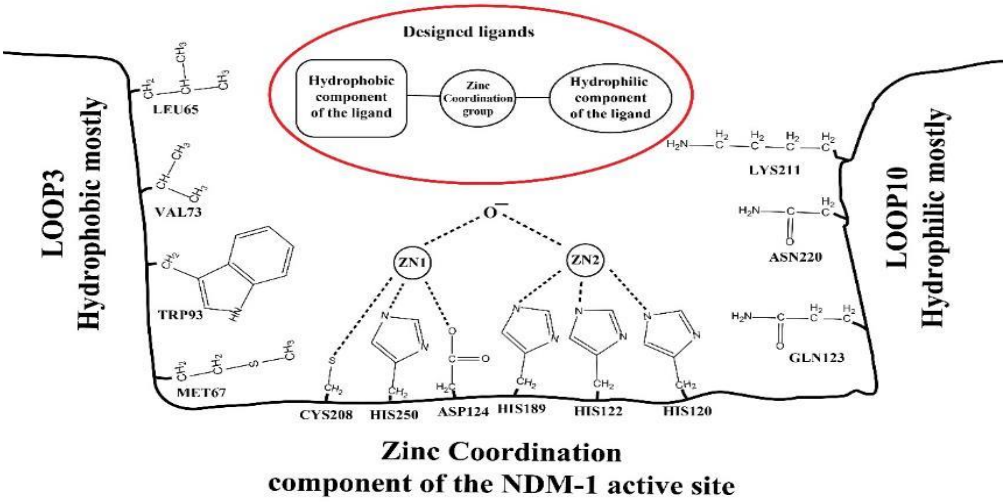

Figure (2) The active site of NDM-1 and the substrate scaffolds

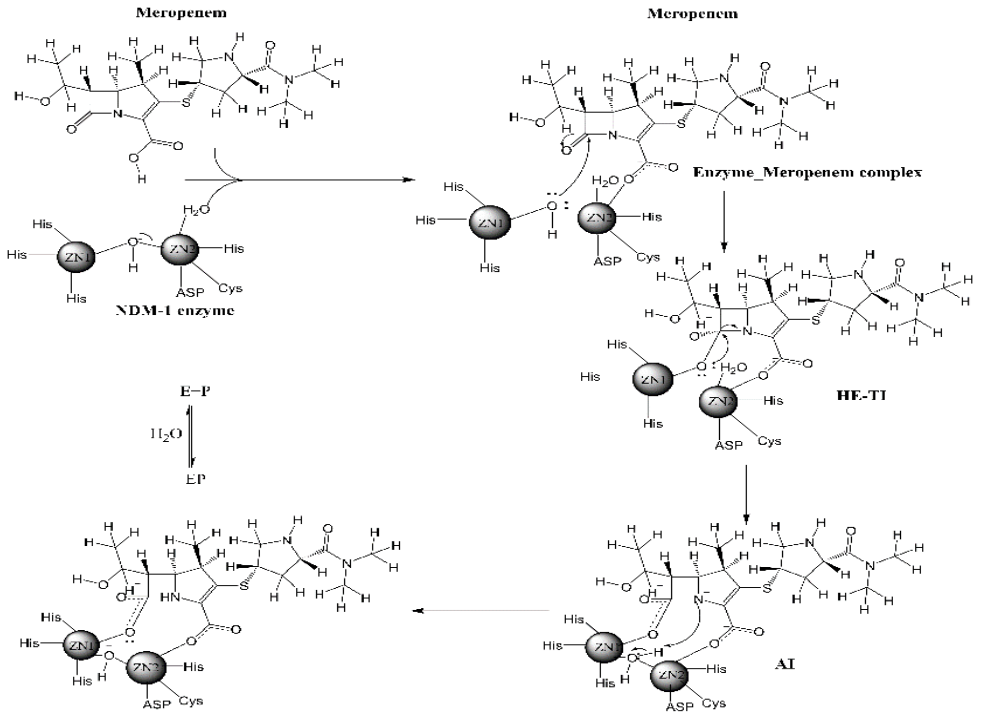

Figure (3) Reaction mechanism of NDM-1 with meropenem.; Enzyme, E; Product, P; High-Energy Tetrahedral Intermediate, HE-TI; Anionic Intermediate, AI; Enzyme:Product Complex, EP; and Tetrahedral Intermediate, TI; (37).

Administration of antibiotics with inhibitors is one of the remarkable approaches to address β-lactam resistance caused by β-lactamases (16). Inhibiting MBL could target different components of the enzyme and challengeable approach. NDM-1 inhibitors can be classified into three distinct types. The first category exerts its effect directly on Zn^{+2} located within the pocket. The second inhibitors category prevents the NDM-1–substrate contact through interacting with the amino acid residues of NDM-1. The third category of inhibitors has the capability to block NDM-1 activity through targeting the zinc ions and catalytic amino acid residues located in the NDM-1 active site (17). The inhibitors is predominantly encompassed zinc coordinating agents, including mercaptoaliphatic acid, thiosemicarbazones, 2-mercapto-azoles, dipicolinic acids, and boronic acid derivatives and chelating agents, such as EDTA, AMA, and NOTA (18). The inherent ability of heteroatoms to coordinate zinc makes heterocyclic compounds like pyrrole, pyridine, triazole, thiazole, quinoline, imidazolidine, and sulfamoyl a promising option for the development of MBL inhibitory compounds (19–22). Boronic acid derivatives have also shown potential as inhibitors due to their ability to interact with the catalytic hydroxide anion and form enzyme-inhibitor complexes (23,24). Furthermore, it has been observed that the inhibitors could selectively interact with the loops surrounding the cavity of the active site (18,25) . In addition to the numerous research on this field and reporting more than 500 potential inhibitors, none of the MBL inhibitor molecules have been approved to use as a drug in the market (18,26). Limitation to develop an effective inhibitor is related to the structural diversity of β-lactamases. Drug repurposing, which is also known as repositioning, redirecting, or reprofiling is developed as a convincing and financially feasible approach to identify new indications for the approved drugs

because of the significant risk, and protracted timeline associated with the development of novel drugs. Significant achievements have been observed in the recent years (27–30). Diverse computational approaches have been applied in the process of drug repositioning to enhance the efficacy and success rates of this strategy (28). Recent studies have illustrated drug repurposing to identify novel therapies for a range of medical conditions, including stroke, infectious diseases, neglected tropical diseases, cancer, metabolic diseases, and neurological disorders (31–36).

In 1972, the Pfizer drug company recognized a series of heterocyclic exocyclic dione/enols, which were also categorized as cyclic sulfonamides. The first FDA approved molecule was piroxicam. Then, a series of less toxic 'oxicams' were introduced during 1976-1982, such as tenoxicam, isoxicam, lornoxicam, meloxicam, and droxicam. Some of them were withdrawn because of their side effects, but meloxicam still available in the market (37). Non-steroidal anti-inflammatory drugs (NSAIDs) have a various biological activity due to the oxicam ring system. Many studies demonstrated antibacterial activity of the selected NSAIDs against *Staphylococcus aureus* and *E. coli* (38). Among the selected NSAIDs, piroxicam is able to inhibit bacterial biofilm formation (39–41). One of the significant features of oxicams is metal chelation (42). Previous studies confirmed the potential biological applications of oxicam compounds, like piroxicam and meloxicam, as well as their chelating ability with metal ions, such as Co(II), Cu(II), Fe(II), Fe(III), Ni(II), and Zn(II). With several transition metals, benzothiazine derivatives can form metal complexes in which they primarily function as bidentate or multidentate ligands, donating lone electron pairs into partially or entirely unoccupied orbitals of metals to produce coordinate complexes (40,42–44) .

Computer-aided drug design (CADD) has played a significant role in the initial stages of drug discovery through assisting the design and advancement of novel compounds. The application of *in silico* approaches in the development of MBL inhibitors targeting the NDM-1 protein has the potential to enhance project efficacy and reduce expenses associated with the development of new lead compounds (45). Molecular docking and molecular dynamic (MD) simulations study are the main approaches of CADD. Molecular docking studies realize the binding affinity and binding interactions between small molecules and receptor. through prediction of the favored pose of compound within the substrate binding site of receptor and the subsequent calculation of the score. However, MD simulation can estimate physical motion of the complex systems, evaluate the ligand's conformational changes within the protein binding site, and study the time-dependent dynamics of molecular systems (46). *In silico* approaches can also be applied to estimate physicochemical properties of lead molecules, such as size, polarity, and lipophilicity. These properties have a significant impact on the compound's binding to receptors (47,48). For example, increasing size and lipophilicity of the molecules lead to decrease the selectivity and accumulation of the drugs in a particular organ (49). Lipinski and colleagues (1997) formulated a metric, commonly referred to as Lipinski's or Pfizer's rule of 5, aimed to predict the pharmacokinetic characteristics of the lead molecules (50).

Designing MBL inhibitors are more difficult compared to the SBL inhibitors because MBL has the complex shape of Zn^{+2} and the d orbitals causes more obstacles. Furthermore, Zn^{+2} exhibits various coordination numbers and geometries (51). This study is based on the previous antibacterial activities of oxicams, aimed at designing NDM-1 inhibitors and

improving carbapenem efficacy against multidrug-resistant bacteria.

Methods

The present investigation involved a computational evaluation of the potential of repurposing non-antibiotic drugs, specifically oxicams as novel antibiotic adjuvants. The selection of oxicam was based on their structural similarity with chemical classes that have been extensively reviewed in the literature, as well as their compatibility with the NDM-1 active site. Oxicams are composed of zinc-coordinating groups, specifically thiazole and pyridine, as well as hydrophobic functional groups that are crucial for their interaction with hydrophobic amino acid residues located in the active site (Fig.2).

Ligand and protein preparation

The 3D structure of the ligands with explicit hydrogen atoms were generated from 2D structures (retrieved from PubChem) (Fig. 4) using mmff94 forcefield to minimize energy from Marvin software, v22.19 (https://chemaxon.com/products/marvin).
Then, pdb file for the compounds were generated (52). The crystal structure of NDM-1–NO9 complex was received from pdb platform (https://www.rcsb.org; access code 6KXI) (53). After that, Notepad++ software, v8.4.6, developed by Don Ho (https://notepad-plus-plus.org) was used to remove of the ligand, water molecules, and chain A from a protein dimer structure (54). Finally, chain B was preserved as a pdb file.

Molecular docking protocol

Binding free energy (ΔG) was estimated by docking NDM-1 with all the repurposed molecules using Assisted Molecular Docking (AMDock) program. This program was applied AutoDock4Zn forcefield. However, various external programs were also used to prepare the input structure files and establish the search space, such as ADT scripts, AutoLigand, Open Babel, and PDB2PQR (55). The Lamarckian genetic algorithm was used to generate docking poses. Polar hydrogens and Kollman charges were assigned to the target protein, while Gasteiger partial charges and non-polar hydrogens were assigned to the ligands. Torsion angles of the ligands were unconstrained during the docking experiments. This study applied empirical free energy in conjunction with the Lamarckian genetic algorithm (56,57). The search space's magnitude was determined using a grid center with coordinates X: 25, Y: 30, and Z: 15, and dimensions X:20, Y:20, and Z:20 Å for each magnitude. The ΔG values of all the docked complexes were evaluated. The most highly binding free energy was chosen for subsequent evaluation.

MD simulations protocol

After optimizing the ligands using AutoDock Tools, the protein-ligand complexes were placed in a dodecahedron-shaped water box containing TIP3P water molecules and additional sodium and chloride ions to simulate *in vivo* conditions. The systems were subjected to a 200 ps energy-efficient steepest descent before undergoing thermodynamic equilibrium using the CHARMM36 force field and GROMACS. A 100 ns production MD simulation was then performed, with a long-range Van der Waals cut-off rvdm selected at the energy minimization stage (58). The gen_vel option was activated with a gen_temp of 300 and -1 gen_seed. For the final production MD step, tau_p was set to 2.0 and ref_p was 1.0 using the Parrinello-Rahman method for pressure coupling (59). Python, programming languages, along with gmx rmsd, gmx rmsf, gmx area, and gmx code and gyrate tools, were used for MD analysis and evaluations.

Visualization and plotting software

The UCSF Chimera (1.16 package) platform (60), the PyMOL molecular graphics system (61), and the Biovia discovery studio visualizer (62) were used

to analyse interactions between the oxicam derivatives and the target protein. PyMOL was also used with AMDock to aid in defining the box of search space. In addition, Biovia discovery studio visualizer software (v21.1.0.20298) (62) was utilized to create and plot 2D representations of protein-ligand interactions, including hydrophobic and H-bonds.

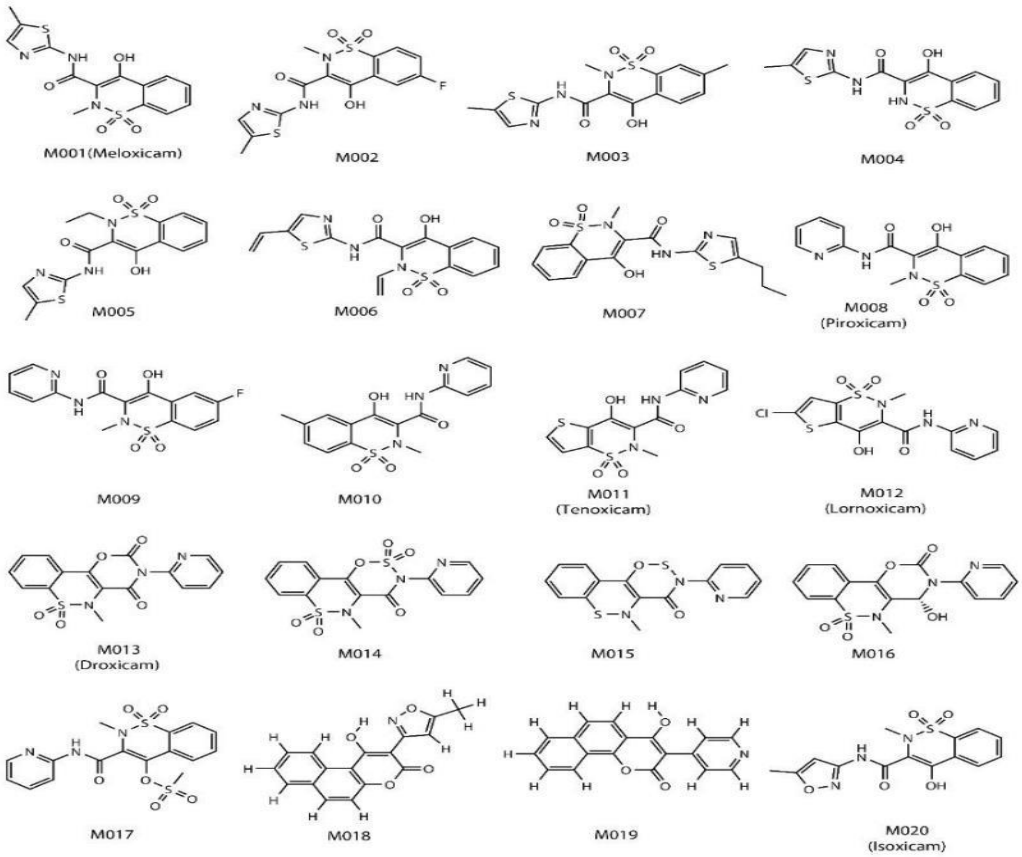

Figure (4) The 2D chemical structure of oxicam derivatives as NDM-1 inhibitors.

Results and Discussion

Docking of the oxicam derivatives

The objective of this experiment (63) was to determine binding interactions and binding affinity between the oxicam derivatives and NDM-1. During the docking procedure, the highest binding free energies of the complex systems were selected. As shown in table 1, the ΔG results of twenty compounds and the positive control (meropenem) were predicted. M018 had the highest ΔG (-11.45 kcal/mol), then, M013 (-10.61 kcal/mol) and M010 (-9.83 kcal/mol), respectively. The lowest ΔG value was referred to M007, which was -7.46 kcal/mol, but it still had higher ΔG than the positive control (-7.39 kcal/mol). RMSD was used for structural comparison and fitness between Meropenem and oxicam derivatives at receptor pocket (see table2.

Table (1) Chemical formula, ΔG, consensus LogP, compliance with rule of five metrics, and molar mass of the repositioned oxicam derivatives and meropenem.

Compound	Chemical formula	ΔG (kcal/mol)	Consensus LogP	Rule of 5 criteria	Molar mass (g/mol)
M001 (Meloxicam)	$C_{14}H_{13}N_3O_4S_2$	-8.08	1.73	Yes	351.03
M002	$C_{14}H_{12}FN_3O_4S_2$	-9.13	2.03	Yes	369.39
M003	$C_{15}H_{15}N_3O_4S_2$	-8.44	2.07	Yes	365.43
M004	$C_{13}H_{11}N_3O_4S_2$	-8.56	1.49	Yes	337.37
M005	$C_{15}H_{15}N_3O_4S_2$	-9.08	1.96	Yes	365.43
M006	$C_{16}H_{13}N_3O_4S_2$	-8.13	2.36	Yes	375.42
M007	$C_{16}H_{17}N_3O_4S_2$	-7.46	2.32	Yes	379.45
M008 (Piroxicam)	$C_{15}H_{13}N_3O_4S$	-8.88	1.38	Yes	331.35
M009	$C_{15}H_{12}FN_3O_4S$	-8.05	1.8	Yes	349.34
M010	$C_{16}H_{15}N_3O_4S$	-9.83	1.76	Yes	345.37
M011 (Tenoxicam)	$C_{13}H_{11}N_3O_4S2$	-8.55	1.06	Yes	337.37
M012 (Lornoxicam)	$C_{13}H_{10}ClN_3O_4S_2$	-9.66	1.59	Yes	371.82
M013 (Droxicam)	$C_{16}H_{11}N_3O_5S$	-10.61	1.3	Yes	357.34
M014	$C_{15}H_{11}N_3O_6S_2$	-8.56	0.76	Yes	393.39
M015	$C_{15}H_{11}N_3O_2S_2$	-9.11	2.8	Yes	329.40
M016	$C_{16}H_{13}N_3O_5S$	-9.28	0.88	Yes	359.36
M017	$C_{16}H_{15}N_3O_6S_2$	-8.31	1.30	Yes	409.44
M018	$C_{17}H_{11}NO_4$	-11.45	2.99	Yes	293.27
M019	$C_{18}H_{11}NO_3$	-9.43	3.07	Yes	289.28
M020 (Isoxicam)	$C_{14}H_{13}N_3O_5S$	-9.5	1.27	Yes	335.34
Meropenem	$C_{17}H_{25}N_3O_5S$	-7.39	-0.34	Yes	383.46

ClogP and drug-likeness properties

In drug discovery projects, it is crucial to make an initial prediction of the physicochemical properties of the lead molecule to identify a suitable candidate for further development (64). The LogP parameter is an essential metric to assess the physical and pharmacokinetic characteristics of a lead compound (65,66) . The present investigation employed SwissADME to assess the rule of five criteria and ClogP for the oxicam derivatives and the positive control compound (67). As shown in table 1 and table 2, most of the compound's ClogP were similar to the majority of antibacterial drugs available in the market, ranging from 1.9 to 5.19 (68). The most lipophilic molecules were M019 (3.07) and M018 (2.99), which they considered as a moderate lipophilic molecule. On the other hand, the most hydrophilic compounds were M016 (0.88) and M014 (0.76). It is remarkable that none of the repositioned molecules surpassed the hydrophilicity of meropenem. In addition to the importance of keeping the compounds ClogP in the range of oral bioavailability, maintaining the hydrophobic-hydrophilic balance of the compounds was also essential for their interactions with the receptor because of the availability hydrophobic residue and zinc ions in the NDM-1 pocket. Hydrophobic residues in the active site of NDM-1can be

interacted with the hydrophobic functional groups of the ligands. Furthermore, it should be noted that residue positions Cys208, Lys211, and Asn220 are encompassed within the L10 region of NDM-1. The coordination between Cys208 and Zn2 is observed, while the remaining residues establish interactions with the polar groups of the substrate (Fig. 2) (12).

Table (2) Lipinski's rule of five criteria of the repurposed ligands

Compound	Rotatable bonds	HB Donar	HB Acceptor	TPSA	RMSD (A°) compared to MER (Strict)	RMSD (A°) compared to MER (Flexible)
M001 (Meloxicam)	2	2	6	99.6	5.85	5.41
M002	3	2	6	136.22	5.70	5.43
M003	3	2	5	136.22	6.25	4.15
M004	3	3	5	145	6.03	5.43
M005	4	2	5	136	---	---
M006	5	2	5	136.22	6.14	5.89
M007	5	2	5	136.22	6.14	5.89
M008 (Piroxicam)	3	2	5	107.98	5.26	2.31
M009	3	2	6	107.98	5.99	5.25
M010	3	2	5	107.98	2.78	2.78
M011 (Tenoxicam)	3	2	5	136.22	4.99	4.26
M012 (Lornoxicam)	3	2	5	136.22	6.46	2.67
M013 (Droxicam)	1	0	6	110.86	---	0.79
M014	1	0	7	130.71	0.94	0.94
M015	1	0	3	109.44	---	0.98
M016	1	1	6	198.42	---	0.77
M017	5	1	7	139.50	5.44	2.89
M018	1	1	5	76.47	3.84	1.62
M019	1	1	4	63.33	---	1.63
M020	3	2	6	121.12	4.99	4.95
Meropenem	5	3	6	135.48	0	0

In conclusion, the effective inhibition of the NDM-1 enzyme needs such ligands moderately hydrophilic and highly lipophilic. Small molecules are the preferred choice for the design of novel drugs due to their favourable pharmacokinetic features (69). Drug-likeness properties were predicted to determine orally bioavailable medications of the tested molecules (70). The measurement of these properties was achieved through using Lipinski's rule of five criteria (71). All the compounds were followed rule of five and had MW < 500 Da. The highest MW as M017 (409.44 g/mol) (table 1). Nevertheless, strict adherence to the MW cut-off as a determinant of oral bioavailability may not be accurate because certain antibacterial agents have molecular weights of up to 900 Da and exhibit oral bioavailability, whereas other antibacterial agents with a MW < 500 Da, could not be administered orally. For example, meropenem's small MW and it's adherence to rule of five could not keep it taken orally because of its hydrophilic nature and inadequate absorption in the gastrointestinal tract (72).

Analysis of binding interactions

Numerous investigations have reported that ligand molecules containing functional groups such as pyridine, isoxazole, thiazole, sulfone, triazole, quinoline, and imidazolidine, along with hydrophobic part of the molecules, demonstrate efficacy and capability to inhibit MBLs (particularly NDM-1) by making zinc coordination and hydrophobic interactions in the active site (19–22,73–75). The repurposed molecules were found to possess hydrophobic components and could establish nonpolar interactions with the active site due to the presence of hydrophobic residues in proximity to the zinc center of the NDM-1 pocket (19). Molecular interactions of the crystal structure and three complexes (M010, M013 and M018) were investigated. The primary functional groups in M010 and M013 were pyridine and 1,2-Benzothiazine 1,1-Dioxide moieties, while isoxazole and benzo[h]chromen-2-one moieties in M018 (Fig. 4).

Meropenem binding interactions with NDM-1

Binding interactions between both hydrolyzed (crystal structure) and docked meropenem against NDM-1 were analyzed. As shown in figure 5A and 5B, meropenem of the crystal structure and the docked result were totally buried within the pocket of the target protein. Zinc ions of the NDM-1 active site is responsible on the metal coordination bond and β-lactam ring hydrolysis of the ligands (76,77). However, various binding interactions
were realized between the protein active site and the ligand. Hydrolysed meropenem was involved in zinc coordination interactions with the target. The amino acid residue interacted with the meropenem were found to be similar in the crystal and redocked structures. Although, redocked meropenem was created additional H-bonds with side chains of Gln123 and Asp124 of the receptor. The ligand conformations were changed when the crystal structure compared with the docked result because of the various interactions between meropenem and NDM-1 residues (see table3).

The NDM-1 binding interactions with M010, M013 and M018

The compounds M018, M013, and M010 had the highest binding free energies, respectively. Moreover, they passed Lipinski's rule of five and had optimal ClogP values (table 1). The docking results shown that the ligands exhibited numerous interactions with the receptor.
All three ligands showed essential interactions, such as zinc coordination between the pyridyl and isoxazole moieties of the ligands and the zinc ions present in the active site, as well as hydrophobic interactions was one of the significant interactions Fig. 6 and table 3. Compound M018 was found to participate in pi-pi stacking interactions with the imidazole side chain of His250 and naphthalene ring. A secondary pi-pi interaction was generated between Val73 and Lys211 and the naphthalene ring of M018 in the form of a pi-alkyl interaction. The observation of a pi-sigma interaction between the ligand's methyl group and the target's His122 was also noted. Hydrophobic naphthalene group of M018 could significantly increase ΔG because it was contacted with the receptor's hydrophobic residues (Val73 and His250). A H-bond was also formed between the carboxylate group of the ligand and Lys211 of the target (Fig. 6A). As shown in figure 6B, the primary hydrophobic interaction involved a pi-alkyl bond between the pyridyl group of M013 and the imidazole side chain of His122, as well as between Lys211 and the Benzothiazine 1,1-Dioxide moiety. A single H-bond was produced between Lys211 and the carboxylate group of the ligand. Furthermore, one of the interactions between M010 with NDM-1 was pi-alkyl hydrophobic interaction through His122 and Met154 of the protein and the methyl

group of benzothiazine 1,1-dioxide moiety of the ligand. A pi-sulphur hydrophobic interaction was obtained between Trp93 residue of the protein and the sulphur atom of benzothiazine 1,1-dioxide ring (Fig. 6C).

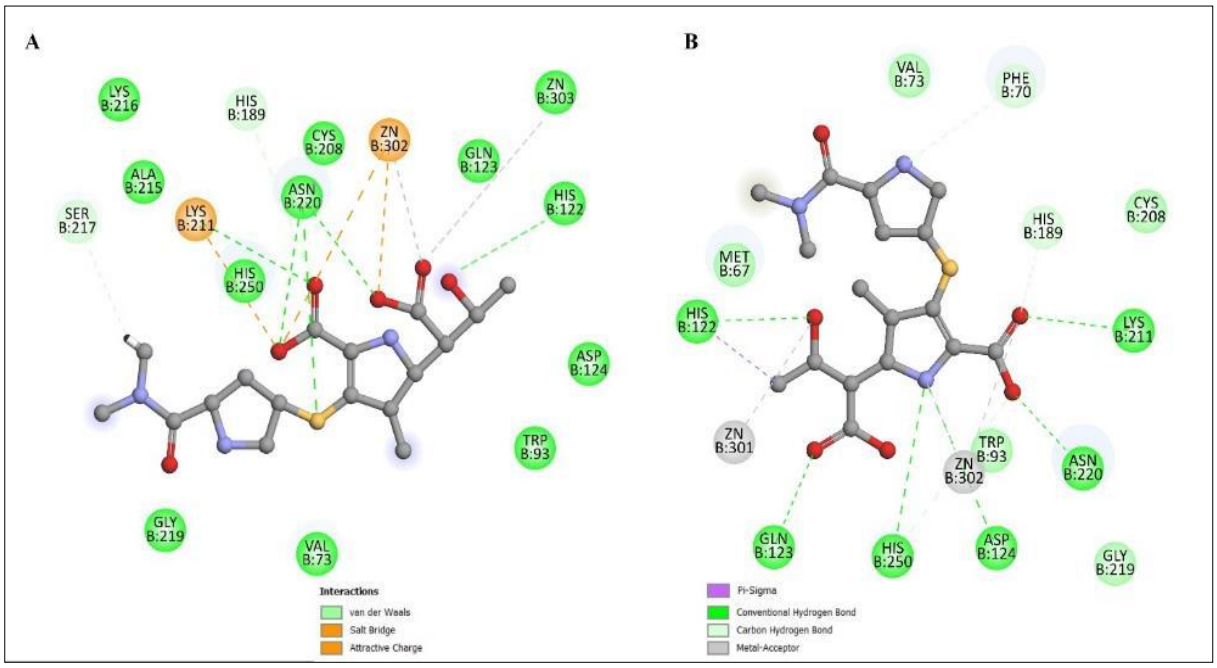

Figure (5) Estimated binding interactions between meropenem and NDM-1 in A) crystal structure, and B) redocked complex.

Table (3) Hydrogen bonding and hydrophobic interactions of the complexes between ligands and protein

Complexes	Amino acid residues involved in hydrogen bonding	Amino acid residues involved in hydrophobic interactions
M010	--------	Trp93, His122, Met154
M013	Lys211	His122, Lys211,
M018	Lys211	Val73, His250, Asn220, His122, Lys211
Hydrolyzed meropenem	His122, His250, Asp124, Asn220, Lys211	Lys211, Phe 70,

MD Simulation analysis of selected compounds

MD simulations can be identified as an effective tool in accelerating the initial stages of modern drug discovery and advancement (78). This approach will develop continuously and increase their application due to the advancements of theoretical and technological aspects of the field (78). MD simulations was applied to investigate the three ligand-receptor complex systems stability and dynamics under physiological conditions. The ligands were M010, M013, and M018 (77). The ligands were chosen based on their superior ΔG binding rank among the ligands that were selected. The root-mean-square deviation (RMSD) is a metric used to quantify the extent to which the protein backbone deviates from its original structural conformation to its basic conformation. The stability of a protein with respect to its structural conformation is determined by the deviations, which arise during the simulation (79). According to RMSD plot, the protein-ligand complexes

exhibited fluctuations ranging from approximately 1.0-3.0 Å across all the selected ligands, thereby indicating the stabilization of all three complex structures. The aforementioned oscillations are a typical characteristic of globular proteins and demonstrated a marked deviation from the maximum

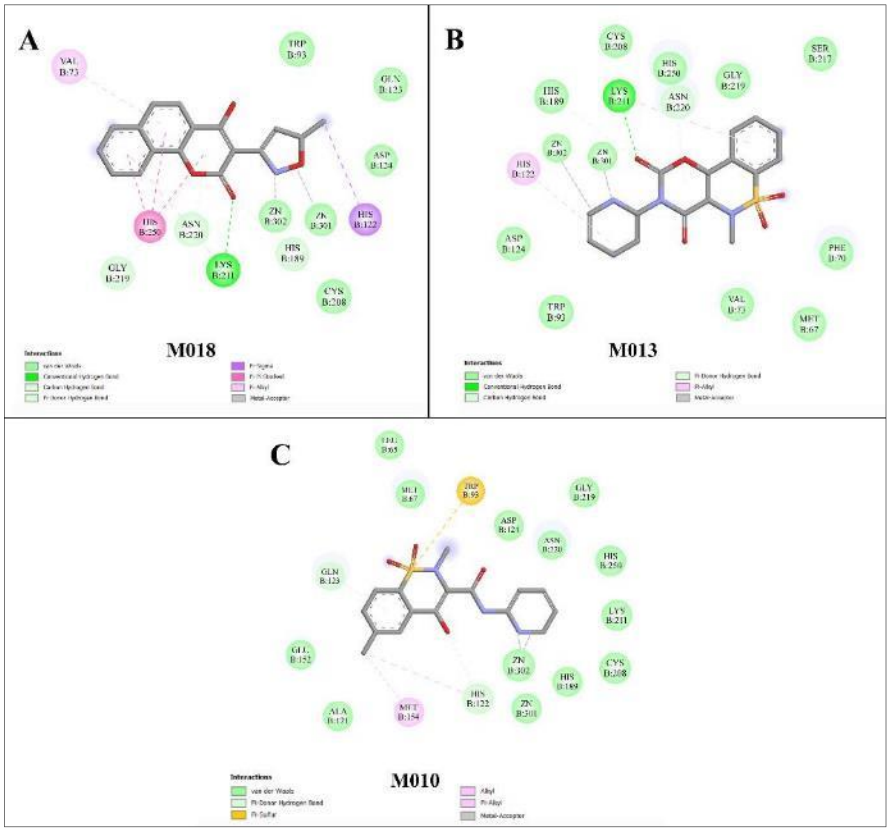

Figure (6) NDM-1 binding interactions with A) M018, B) M013, and C) M010.

threshold (2.0 Å) (77). The findings indicated that all three compounds showed robust interactions with the residues of NDM-1 pocket and formation of stable complexes (Fig. 7A). The next analysis of the complex systems was through quantifying the degree of variation of a given dataset from its mean value, which is known as Root Mean Square Fluctuation (RMSF). It characterizes the dynamic and flexible regions of protein-ligand complexes. The study of proteins reveals that regions with a high degree of structural flexibility, such as loops, turns, and coils, exhibit elevated root-mean-square fluctuation (RMSF), whereas regions with well-defined structures, such as α-helices and β-sheets, demonstrate lower RMSF (79). This study conducted an analysis of RMSF for three complex systems using the C-Alpha atoms of NDM-1. As shown in figure 7B, the results identified elevated fluctuations in certain residues due to the protein's loop. The RMSF plots facilitated the assessment of protein structure stability and the identification of flexible regions necessary for optimal conformational acquisition. The GROMACS algorithm was applied to calculate the radius of gyration for the chosen complex systems based on C-alpha atoms. This calculation could reveal the compactness of molecules.

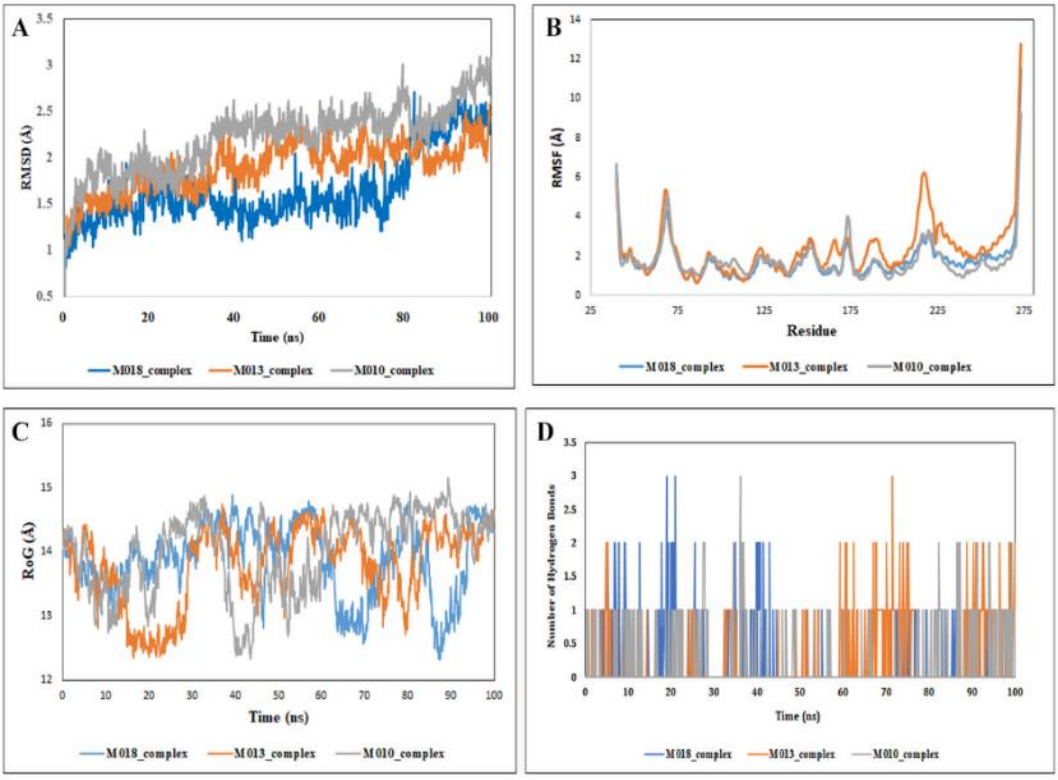

Figure (7) MD simulation between NDM-1 and three ligands (M010, M013, and M018). A) RMSD, B) RMSF, C) Radius of gyration, and D) ligand-receptor H-bonds.

The results obtained from the MD simulations indicated that the N and C terminal domains underwent frequent opening and closing movements. The complex systems exhibited a radius of gyration value ranging from 12.3-15.0 Å (Fig. 7C) (80). Investigations of H-bond interactions between a protein and ligand during MD simulations is crucial to determine the stability of a ligand- receptor complex (79). As illustrated in figure 7D, the established H-bonds during 100 ns MD simulation, exhibited a consistent level of interaction. On average, each complex created one H-bond, while the M013 and M018-NDM-1 complexes were able to form two H-bonds.

Conclusions

Repurposing approved drugs is an attractive and highly recommended approach because it could not only save time and cost but also decrease the failure rate. NDM-1 enzyme can hydrolyze carbapenems and all the β-lactam antibiotics. Therefore, the development of potent NDM-1 inhibitors has the potential to restore the susceptibility of multidrug-resistant bacteria to carbapenem antibiotics. In this study, twenty oxicam derivatives are repositioned as NDM-1 inhibitors. The selected compounds were evaluated computationally as NDM-1 inhibitor. Three compounds (M018, M013, and M010) were identified as the highest binding free energies and they had drug-likeness properties, particularly M018. Such compounds interacted with the enzyme's active region through metal coordination and hydrophobic bonds. These interactions prevent the enzyme from performing normal function. The findings suggested that the selected compounds may protect efficacy of carbapenems through binding with NDM-1 competitively and preventing carbapenems hydrolysis.

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Declarations
Conflict of interest the authors declare no conflicts of interest.

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