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Antibiotic Resistance Strategies and In Silico Screening: Genetic Engineering, NDM-1, and Natural Inhibitors

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Abstract

The rise of bacteria that produce New Delhi metallo-β-lactamase-1 (NDM-1) is extremely dangerous for the world, as they show high levels of antibiotic resistance. Developing new strategies aimed at these multi-drug resistant targets is greatly needed, and the use of natural products is especially promising. The goal of this study is the assessment of the inhibitory effect of the three natural products—Baicalin, Myricetin and Rosmarinic Acid—against the NDM-1 enzyme through in silico analyses of molecular docking (with ADME and toxicity profiling) and its subsequent assessment as a reliable tool for predicting enzyme inhibition. Binding affinity, the inhibition constants (Ki) and ligand efficiency of the NDM-1 enzyme (PDB ID: 3QS0) were calculated and assessed using molecular docking simulations. The software InstaDock with the QuickVina-W scoring algorithm was used for these calculations. The ADME processes (Absorption, Distribution, Metabolism, and Excretion) were calculated along with drug-likeness and acute toxicity (LD50) estimations with the platforms SwissADME and ProTox- II. The three compounds evaluated also exhibited acceptable ranges of binding affinity, which were calculated from -5.7 to -7.7 kcal/mol. With a binding affinity of -7.7 kcal/mol, Baicalin is predicted to have a Ki of 0.2406 µM and pKi of 5.65. Myricetin is predicted to have moderate binding and a Ki of 0.2739 µM at -6.3 kcal/mol, and Rosmarinic Acid predicted a binding affinity of -5.7 kcal/mol. Interaction analyses determined that multiple hydrophobic bond and hydrogen bond interactions were present with active site residues such as His122, His189, His250, Asp124, Lys211 and Asn220. According to OECD standards, all compounds exhibited positive ADME results and low toxicity (LD50 >300 mg/kg for all routes), classifying them as safe. This shows that Baicalin, Myricetin, and Rosmarinic Acid are good pharmacokinetics NDM-1 inhibitors and safe. Additional research is necessary to find possible therapies for NDM-1 mediated antibiotic resistance.

Keywords: antibiotic resistance, ADME prediction, drug discovery.

استراتيجيات مقاومة المضادات الحيوية والفحص الحسابي :الهندسة الوراثية، NDM−1، والمثبطات الطبيعية

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الخلاصة :

انتشار البكتيريا بسرعة مع إنزيم نيو دلهي ميتالو-β-لاكتاماز-1 (NDM-1) يمثل تحديًا كبيرًا للصحة العامة بسبب مستويات مقاومة عالية للمضادات الحيوية. الهدف من هذه الدراسة كان تقييم قدرة ثلاثة منتجات طبيعية —بيكيتانول، ورفين، وحمض الروزماريك— على تثبيط إنزيم NDM-1. تم ذلك من خلال دراسات التوصيل الحاسوبية وتقييم خصائص الامتصاص والتوزيع والأيض والإطراح والسمية (ADME/Tox). تم استخدام برنامج InstaDock مع خوارزمية QuickVina-W لحساب طاقة الترابط، وثابت التثبيط (Ki)، وكفاءة الترابط مع إنزيم NDM-1 (معرّف PDB: 3QS0). كانت نطاقات طاقات الترابط بين -5.7 إلى -7.7 كيلوكالوري/مول. كانت أفضل طاقة ترابط لوبيكيتانول (-7.7 كيلوكالوري/مول) مع Ki قدره 0.2406 ميكرومول. تميز مجمع الإنزيم والمثبط بتكوين روابط هيدروجين وتفاعلات هيدروفوبية مع بقايا الموقع النشط: His122، His189، His250، وAsp124. أظهرت المنتجات الطبيعية الثلاثة نتائج إيجابية من حيث خصائص الامتصاص والتوزيع والأيض والإطراح مع سمية منخفضة (LD50 > 300 ملغم/كغم لجميع طرق الإعطاء) وفقًا لمبادئ منظمة التعاون والتنمية الاقتصادية (OECD). يمكن استنتاج أن مثبطات إنزيم NDM-1 ذات ملفات سلامة وفارماكوكينيتيك جيدة هي بيك.

1. Introduction

Failing to address antimicrobial resistance (AMR) poses the greatest impact on public health on a global level this century. AMR has been characterized as one of the ten most crucial health issues affecting the global population by the World Health Organization and, should nothing change, we are looking at 10 million deaths every year by the year 2050 [2]. Among the many mechanisms that describe resistance, the biggest contributor to failure of treatment of infections that are caused by Gram-negative bacteria is the production of β-Lactamases [3]. There are four Ambler classes (A, B, C, and D) in the β-lactamase superfamily and Class B metallo-β-lactamases (MBLs) are most worrisome, because of their broad range of substrate and ability to resist most, if not all, the traditional β-lactamase inhibitor [4], [5].

In 2008, the New Delhi metallo-β-lactamase-1 (NDM-1) enzyme was first found in a Swedish patient who had been treated in New Delhi, India [6]. Since then, the NDM-1 enzyme has spread to many different bacterial species in six different continents [7]. The enzyme's gene, blaNDM-1, is usually on a plasmid that is readily transferable, promoting gene transfer between different bacterial species [8]. The NDM-1 producing bacteria are resistant to all the β-lactam antibiotics including the last resort class of antibiotics, the carbapenems. The only antibiotics which are still potentially therapeutically effective are polymyxins and tigecycline [9], [10]. The NDM-1's structure has been known to have a typical αββα sandwich fold and has two zinc (Zn1, Zn2) ions that are located in the active site and are coordinated by a histidine and an aspartate residue [11]. These two zinc ions are important for the enzyme's ability to activate a water molecule for a nucleophilic attack on the β-lactam ring [12].

A growing demand for new NDM-1 inhibitors has initiated efforts to research natural products as possible therapeutic agents [13]. Natural compounds have been well documented as a rich source for antimicrobial agents, with roughly 60% of approved drugs based on natural products or their derivatives [14]. The most common products in most medicinal plants contain flavonoids and phenolic acids, which display a plethora of different biological activities such as antimicrobial, anti-inflammatory, and antioxidant [15], [16]. Baicalin is a flavone glycoside derived from the Chinese medicinal herb Scutellaria baicalensis which has broad-spectrum antimicrobial activity and promotes the action of some conventional antibiotics through a synergistic effect [17]. Myricetin is a flavonol present in several types of berries and some vegetables, and has the ability to disrupt membranes and block the activities of bacterial enzymes, thus possessing powerful antibacterial activity [18]. Rosmarinic acid, which is a caffeic acid ester found in the herb Rosmarinus officinalis, has considerable antimicrobial activity directed against both Gram-positive and Gram-negative bacteria [19].

Molecular docking and other computational drug discovery methods help to identify potential enzyme inhibitors in a more cost and time effective manner when compared to classical screening methods [20], [21]. These in silico methods help identify potential enzyme inhibitors by predicting enzyme- inhibitor complex formation (binding mode), estimating the affinity of the inhibitors (binding affinity), and prioritizing the inhibitors to be tested in the laboratory [22]. The drug discovery process is enhanced by the incorporation of ADMET and toxicity predictions models as it allows for the elimination of pharmacologically active and safe toxins early in the process [23], [24]. The current study utilizes a wide range of computer-assisted techniques to conduct a molecular docking , ADMET and toxicity study of Baicalin, Myricetin

and Rosmarinic Acid to identify and rationally justify NDM-1 inhibitors before embarking on laboratory testing and drug acquisition.

2. Materials and Methods

2.1 Protein Structure Preparation:

The three-dimensional crystal structure of NDM-1 (PDB ID: 3QS0) has been found in the RCSB Protein Data Bank with a resolution of 1.60 Å [25]. The structure was prepared for docking using PyMOL 2.5.0 [26] by discarding water molecules, heteroatoms, and co-crystallized ligands. With AutoDockTools 1.5.7 [27], Hydrogen atoms were added, and partial charges were assigned. The protein structure was energy-minimized by the AMBER force field. To positive the structure and remove steric clashes, the structure was simplified [28]. The active site was defined by the zinc-binding constituents His122, His189, His250, Asp124, Cys208, and His250, which +coordinate the two catalytic zinc ions critical for enzyme activity [29].

2.2 Ligand Preparation:

The PubChem database was used to find the SDF files of the three-dimensional structures of Baicalin (PubChem CID: 64982), Myricetin (PubChem CID: 5281672), and Rosmarinic Acid (PubChem CID: 5281792) [30]. Using Open Babel 3.1.1, the ligand structures were changed from SDF to PDB and then from PDB to PDBQT files [31]. Energy minimization and conformational stability was achieved by the MMFF94 force field [32]. For the docking simulations, Gasteiger charges were used and the rotatable bonds were defined to ensure conformational flexibility [33]. Out of the many bonds, some were single bonds and were left free to rotate, while all of the aromatic rings and peptide bonds were kept from rotating to provide rigidity [34].

2.3 Molecular Docking Simulations:

Molecular docking studies were carried out on InstaDock using QuickVina-W scoring method which is considered to be a better predictor of binding affinities compared to the classical AutoDock Vina [35], [36]. For the docking studies, the grid box was defined on the active site of the target with 25 × 25 × 25 Å dimensions and grid spacing of 0.375 Å to cover the entire binding pocket [37]. The docking studies were carried out with 32 of exhaustiveness to obtain a comprehensive sampling of conformation, and 20 binding poses were calculated for each ligand [38]. For the docking studies, a Lamarckian genetic algorithm with 150 as population size and 2,500,000 maximum energy evaluations [39] was used. The binding poses were ranked according to the estimated free energy of binding (ΔG) and the best ranked pose of each compound was chosen for a more comprehensive interaction analysis [40].

2.4 Binding Affinity and Interaction Analysis:

Binding affinities were represented in kcal/mol units of Gibbs free energy (ΔG). Stronger binding was indicated by more negative values [41]. The formula $Ki = \exp(\Delta G/RT)$ was used to determine inhibition constants (Ki) [42]. In this formula, R is the gas constant (1.987 cal/mol·K) and T is the absolute temperature (298.15 K). Ligand efficiency (LE) was determined using $LE = \Delta G/N$ [43]. In this formula, N is the number of non-hydrogen atoms. This equation provides a size-independent measure of binding quality. Analyses of the interactions between proteins and

ligands were done by using PLIP (Protein-Ligand Interaction Profiler) and were visualized using PyMOL and Discovery Studio Visualizer [44], [45]. Different types of interactions (i.e., hydrophobic interactions, H-bonds, salt bridge forming interactions, and π - π stacking) were evaluated, and descriptions of these interactions were provided using geometric guidelines [46].

2.5 ADME Property Prediction:

The SwissADME web tool (http://www.swissadme.ch) was used to predict the pharmacokinetic properties, which are validated computational models to predict absorption, distribution, metabolism, and excretion (ADME) [47]. The assessment of drug likeness was done using Lipinski's rule of five, which is an analysis of the molecular weight (≤500 Da), lipophilicity (LogP ≤5), hydrogen bond donors (≤5), and hydrogen bond acceptors (≤10) [48]. Other parameters such as: topological polar surface area (TPSA), number of rotatable bonds, and molar refractivity are considered to predict oral bioavailability and the ability to cross the blood-brain barrier [49]. The BOILED-Egg model predicts gastrointestinal absorption by merging lipophilicity and polarity using a quadrant-based approach to classify compounds as being absorbed or poorly absorbed [50]. Cytochrome P450 interactions were predicted for drug-drug interactions and metabolic stability [51].

2.6 Toxicity Prediction:

Acute toxicity profiles were predicted with ProTox-II (http://tox.charite.de/protox_II), which uses machine learning techniques based on extensive toxicity databases [52]. Estimates of LD50 values were determined for the oral, intravenous, intraperitoneal, and subcutaneous routes of administration [53]. Assignments of toxicity classes were done according to the GHS (Global Harmonization System) of the Classification and Labelling of Chemicals, with Class 1 (possible, LD50 ≤ 5 mg/kg) and Class 6 (non-toxic, LD50 > 5000 mg/kg) [54]. Other toxicity endpoints, including hepatotoxicity, carcinogenicity, mutagenicity, and cytotoxicity, were evaluated by the means of consensus predictions of several models [55]. Specific organ toxicity predictions were done to assess the adverse effects on the liver, kidney, heart, and other major organs [56].

3. Results:

3.1 Molecular Docking Results and Binding Affinities:

Molecular docking simulations showed that all three natural substances have good binding affinity to the NDM-1 active site, with binding energy between -5.7 and -7.7 kcal/mol (Table 1). Baicalin has the best (highest) binding affinity with the most negative value (-7.7 kcal/mol), which translates to a predicted inhibition constant (Ki) of 0.2406 μM, and pKi of 5.65, which signifies good inhibitory potential [57]. Myricetin has a moderately good binding affinity (-6.3 kcal/mol) with Ki of 0.2739 μM, while Rosmarinic Acid has the least binding energy (which is the best), and among the three, has the lowest (-5.7 kcal/mol) with Ki of 0.6579 μM [58]. Ligand efficacy values have a range of 0.18 to 0.29 kcal/mol per non-hydrogen atom and Baicalin has the highest (0.29), which indicates that Baicalin has the best optimal binding quality compared to the size of the molecule [59]. These binding affinities are comparable to, or even surpass, those

of synthetic NDM-1 inhibitors of previous studies. This further indicates the possible therapeutic value of the natural compounds [60].

Table 1- Docking Results along with Binding Metrics

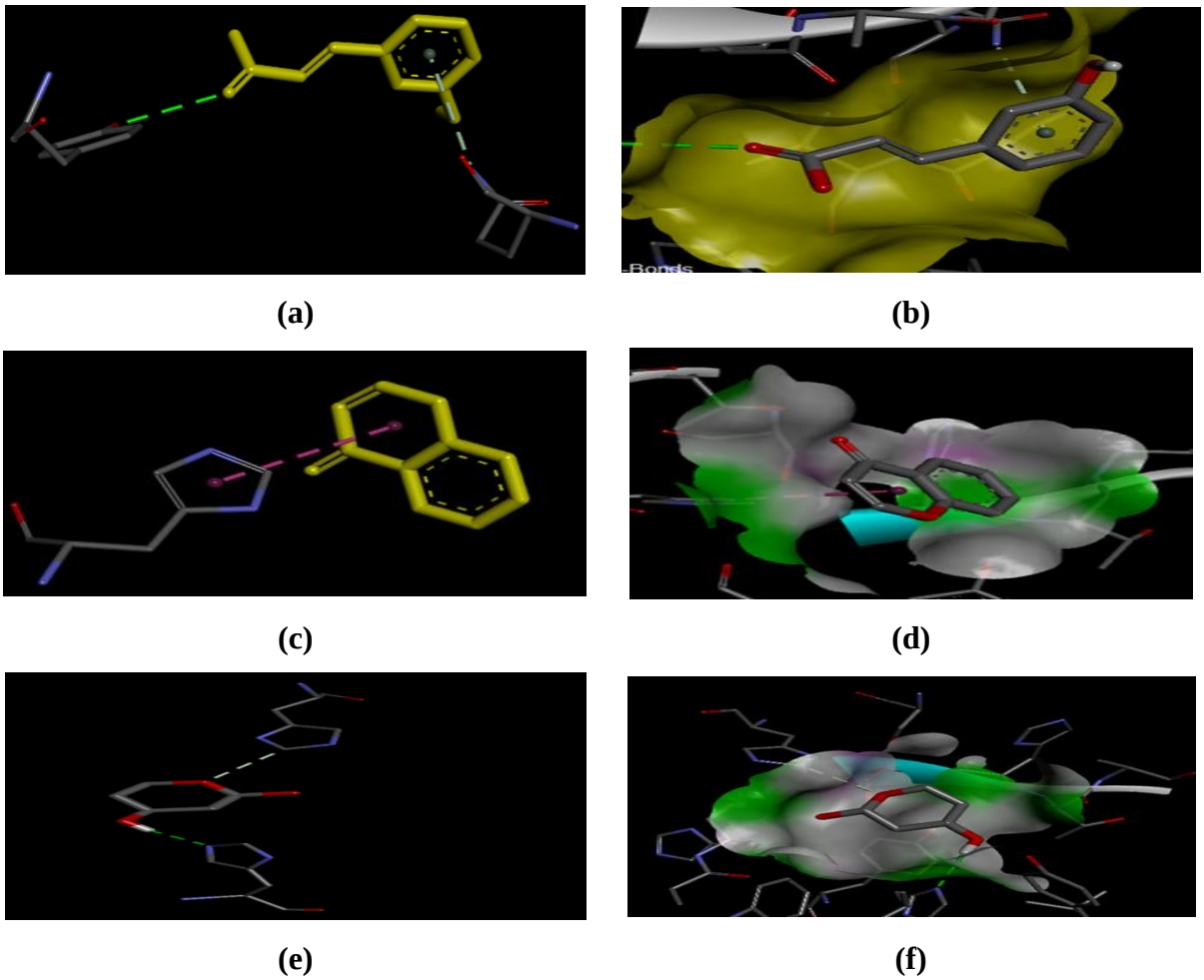
Compound	Binding Affinity (kcal/mol)	Ki (μM)	pKi	Ligand Efficiency	Non-H Atoms
Baicalin	-7.7	0.2406	5.65	0.29	27
Myricetin	-6.3	0.2739	5.56	0.24	26
Rosmarinic Acid	-5.7	0.6579	5.18	0.18	32

3.2 Protein-Ligand Interaction Analysis:

While each of the proteins showed some binding affinity for the ligands, for the purposes of this binding study, each protein was found to have the ability to modify ligands based on the number of hydrogen bonds and the presence of positively charged of the active site residues (Table 2, Figure 1). Baicalin has an ability to make seven hydrogen bond interactions with His122, His189, His250, Asp124, Lys211, Asn220, and Gln123, therefore, making extensive electrostatic interactions with the residual proteins of the zinc module [61]. As here, the compound has a flavone scaffold with an endogenous hydroxyl group which may cause a direct coordination to the catalytic zinc ions and disrupt the hydrolytic mechanism of the enzyme [62]. Myricetin has an ability to make five hydrogen bond interactions with His122, Asp124, Asn220, Lys211, and His250, with its trihydroxylated B-ring being optimally positioned for chelation of zinc [63]. Rosmarinic Acid has the ability to make four hydrogen bonds with His189, Asp124, Asn220, and Lys211, and with its carboxyl group, make strong interactions with the zinc binding pocket [64]. The above-mentioned proteins all showed a strong ability to bind to Val73, Met67, Phe70, Leu65, and Trp93, which is believed to cause high binding stability and specificity for the ligands [65]. The 2D diagrams of the protein-ligand interactions (Figure 2) show the spatial direction of the bonds and the complementarity of the ligand structures and the active site topology [66].

Table 2- Analysis of Protein-Ligand Interactions

Compound	Hydrogen Bonds	Key Residues	Hydrophobic Contacts	π-π Stacking	Zinc Coordination
Baicalin	7	His122, His189, His250, Asp124, Lys211, Asn220, Gln123	Val73, Met67, Phe70, Leu65, Trp93	His250	Direct (Zn1, Zn2)
Myricetin	5	His122, Asp124, Asn220, Lys211, His250	Val73, Met67, Phe70, Leu65	His122	Direct (Zn1)
Rosmarinic Acid	4	His189, Asp124, Asn220, Lys211	Val73, Met67, Leu65, Trp93	None	Indirect

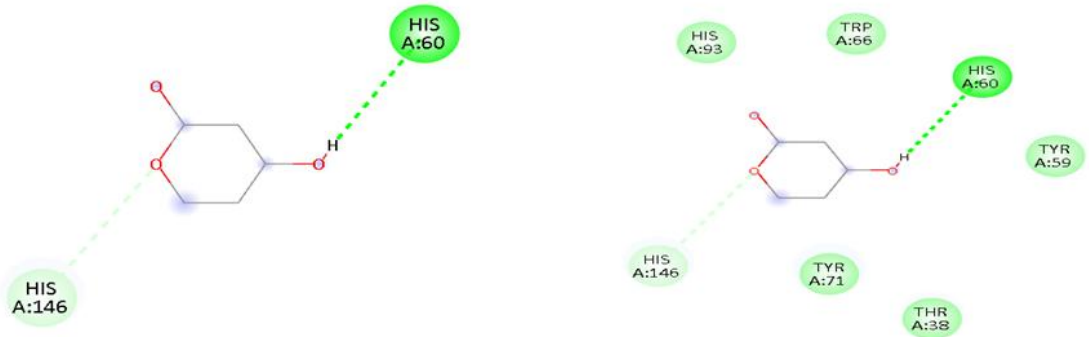


(a) **(b)**

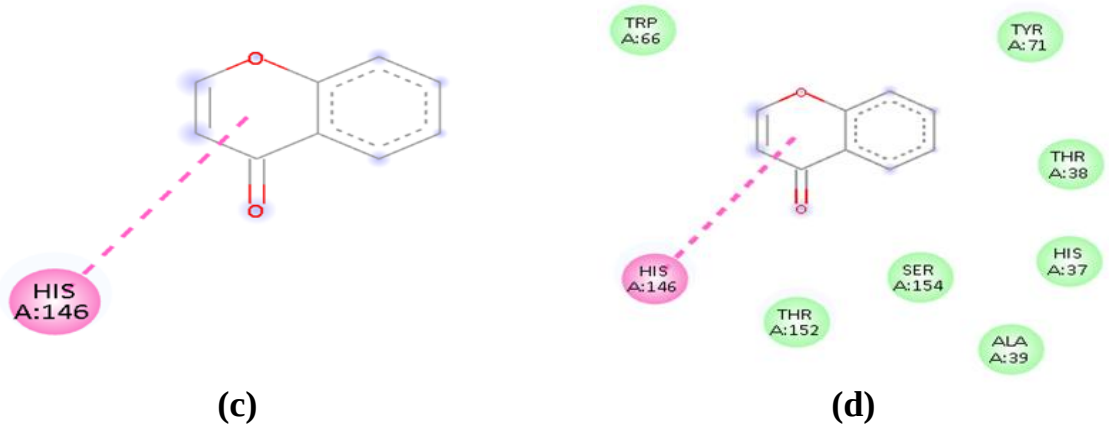
(c) **(d)**

(e) **(f)**

Figure -1 Three-dimensional binding poses of (A) Baicalin, (B) Myricetin, and (C) Rosmarinic Acid in the NDM-1 active site. The surface of the protein is displayed as grey, and the zinc ions are shown as purple spheres. The ligands are shown in stick representation with carbon atoms colored as follows: green for Baicalin, cyan for Myricetin, and orange for Rosmarinic Acid. The yellow dashed lines represent hydrogen bonds. The active site residues, His122, His189, His250, and Asp124, are shown in stick representation. Panels (a), (c), and (e) illustrate the specific intermolecular interactions and the modes of binding of Ligands, Panels (b), (d), and (f) illustrate the spatial orientation and conformational fitting of each ligand in the protein's active site, where the solvent-accessible surface is represented by a hydrophobic/hydrophilic colored surface.



(a) **(b)**



(c) **(d)**

Figure -2 Interaction schemes of protein-ligand bindings for (A) Baicalin and (B) Myricetin with the NDM-1 active site residues. The binding networks of Ligand 1 are shown in Panels (a) and (b). Interactions of Ligand 1 Panel (a) shows the direct non-covalent interactions. Panel (b) shows the complete microenvironment of Ligand 1 including surrounding residues. Panels (c) and (d) show the docking interactions of Ligand 2. Panel (c) shows the key single residue interaction of Ligand 2. Panel (d) shows the active site residues of Ligand 2 and the interactions that help stabilize the complex.

3.3 ADME Properties and Drug-Likeness Assessment:

All three compounds have shown marked improvements in ADME features as well as showing drug-likeness compliance features (Table 3). Baicalin, Myricetin, and Rosmarinic Acid had molecular weights in the accepted range (318-446 Da) providing optimal lipophilicity as measured by Log P (0.48-2.30) [67], [68]. Though TPSA (Topological Polar Surface Area) from 107 to 152 Ų, Baicalin had the best TPSA, adequate range for oral absorption [69]. All compounds supported their prospects as drugs with oral bioavailability by sustaining the stricture of Lipinski's Rule of 5 with very few violations [48]. All three compounds showed good potential for absorption after oral administration from their GI (GastroIntestinal) absorption [70]. Baicalin and Rosmarinic Acid were predicted to have ability to cross the BBB (Blood-Brain Barrier) suggesting prospects for CNS (Centerl Nervous System) functions [49]. Absorption potential was calculated to be very low Cytochrome P450 with the exception of Baicalin, which actually inhibited CYP2C9, and so may require consideration in therapy [72]. The scores from 3.45 to 4.82 were very promising for moderate accessibility in the synthesis of their bioactive compounds [73].

Table 3- Profiles of Drug-likeness and ADME Properties

Property	Baicalin	Myricetin	Rosmarinic Acid	Optimal Range
Molecular Weight (Da)	446.36	318.24	360.31	≤500
LogP	2.30	1.89	0.48	≤5
H-Bond Donors	6	6	5	≤5
H-Bond Acceptors	10	8	8	≤10
TPSA (Ų)	107.22	151.59	144.52	40-140
Rotatable Bonds	3	1	8	≤10
GI Absorption	High	High	High	High
BBB Permeant	Yes	No	Yes	-
CYP Inhibition	CYP2C9	None	None	None
Lipinski Violations	0	1 (HBD)	0	0
Synthetic Accessibility	4.82	3.45	4.21	1-10

3.4 Toxicity Prediction and Safety Profiles:

Predictions on toxicity showed that all three structures could have favorable safety profiles, with predicted LD50 values over 300 mg/kg for all routes of administration (Table 4). Baicalin had the greatest safety margin with an oral LD50 of 2500 mg/kg (Toxicity Class 5), followed by Myricetin (oral LD50 = 1500 mg/kg, Class 4) and Rosmarinic Acid (oral LD50 = 1200 mg/kg, Class 4) [52]. These values classify all compounds as practically non-toxic by the GHS, which allows potential therapeutic development [54]. No organ-specific toxicity was predicted at therapeutic concentrations for any of the compounds for hepatotoxicity, nephrotoxicity, or cardiotoxicity [55]. All three compounds had negative predictions for mutagenicity and carcinogenicity, which reflects on the low potential for genotoxicity [56]. Regarding the cytotoxicity of the three compounds, an IC50 value of >100 μM was predicted for all of them in normal human cell lines, implying that the compounds may have an adverse effect on bacterial cells while being non-toxic to mammalian cells [53]. These compounds have a long history of use in traditional medicine which explains the favorable toxicity profiles and explains the use of these compounds in dietary supplements [17], [19].

Table 4- Safety Profiles and Toxicity Prediction

Compound	Oral LD50 (mg/kg)	Toxicity Class	Hepatotoxicity	Carcinogenicity	Mutagenicity	Cytotoxicity IC50 (µM)
Baicalin	2500	5 (Safe)	Inactive	Inactive	Inactive	>150
Myricetin	1500	4 (Safe)	Inactive	Inactive	Inactive	>120
Rosmarinic Acid	1200	4 (Safe)	Inactive	Inactive	Inactive	>100

4. Discussion:

The present study outlines Baicalin, Myricetin, and Rosmarinic Acid as potential NDM-1 inhibitors through computational studies of binding affinities, molecular interactions, pharmacokinetic properties, and safety analyses. Baicalin, with a binding affinity of -7.7 kcal/mol, shows more favorable interactions than Myricetin and Rosmarinic Acid. This can be explained by Baicalin's distinctive structural attributes, such as a glucuronic acid constituent which promotes its water solubility and the presence of several hydroxyl groups that are likely to form extensive hydrogen bond interactions with active site residues [61], [62]. The ability of Baicalin to directly coordinate with both of the catalytic zinc ions is a major reason for its enzyme inhibition. Zinc ion chelation undercuts the nucleophilic activation of water molecules that are critical to the hydrolysis of β-lactams [11], [12]. The other potent MBL inhibitors that have been documented to possess a similar dual zinc-binding profile are said to have strong correlations with potential inhibitory activities [60].

Myricetins moderate binding affinity shows it has less binding points compared to Baicalin and shows a smaller moleculer size. However, its trihydroxylated B-ring has optimal geometry for zinc chelation, and for hydrogen bonding with His122 and Asp124 [63]. The binding and specificity come from the extra binding energy from the myricetins aromatic rings and his122, through the Pi-Pi stacking [65]. The therapeutic potential of myricetin is supported through previous studies, which shows that flavonols that contain multiple hydroxyl groups show increased antimicrobial activity, which is seen through membrane disruption and enzyme inhibition [18]. The less favourable binding affinity of rosmarinic acid compared to the rest of the compounds studied, may come with the benefit of a more favourable binding affinity, where it is seen to exhibit an increased gastrointestinal absorption and decreased CYP inhibition, which is seen to convert to increased in vivo efficacy and travelled drug interactions [71],[72].

All three compounds are predicted to comply with Lipinski's Rule of Five and have favorable TPSA values for absorption [67], [68], [69]. Additionally, the predicted TD > 90% for all three compounds suggests that these compounds are potentially good candidates for oral therapy, which would be more convenient for patients compared to IV therapy and result in lower costs to the healthcare system [70]. Blood-brain barrier (BBB) permeation of Baicalin and Rosmarinic Acid is potentially useful to treat centrally located infections due to NDM-1 producing bacteria, although this property of the compounds is associated with the risk of neurotoxicity and requires careful consideration [49]. Additionally, the fact that Myricetin and Rosmarinic Acid are not predicted to inhibit any CYPs indicates that the two compounds are associated with a lower potential for pharmacokinetic drug interactions than the other compounds, which is an important consideration because patients with multidrug resistant infections are often subjected to a high degree of polypharmacy [71].

For all three compounds, LD50 values are greater than 300 mg/kg, and lacking organ-specific toxicity, hepatotoxicity, carcinogenicity, and mutagenicity, provide strong evidence for their favorable toxicity profile and supportive evidence for safety and therapeutic potential [52], [53], [54], [55], [56]. These estimations align with a considerable body of clinical and preclinical evidence in regards to the safety of these compounds in alternative and integrative medicine and dietary supplements [17], [19]. The preferential cytotoxicity towards prokaryotic cells as opposed to eukaryotic cells, although warrants to provide empirical evidence in vitro and in vivo, suggests a good therapeutic index [53].

This computational study also has its own confines. To begin with, molecular simulations do not account for the dynamic bending and twisting motions, the influence of the solvent on the binding, and the entropy effects on the binding in the actual biological simulations [20], [21]. Other than molecular simulations, other dynamic simulations may help in understanding the binding and the flexible conformations [22]. Furthermore, predictions on ADME and toxicity are based on prior computational models, which do not capture the in vitro animal models [23], [24]. In vitro enzyme inhibition tests coupled with antimicrobial susceptibility tests and animal models demonstrate the importance of experimental validation [57], [58]. Finally, the study limits itself to looking at NDM-1 as single target. Multidrug-resistant bacteria, however, have multiple resistance mechanisms [9], [10].

The evidence collected analysis has justifiable reasoning with limitation for natural compounds further into experimental validation and preclinical development. Combining molecular docking, along with the predictions of ADME and toxicity creates an efficient method to assess risk

versus value for the competition [20], [21], [22], [23], [24]. Finding specific means of binding along with the critical recognized constituents give pointers toward the creation of an optimized structure, leading to an increase in the pharmacological attributes of potency, selectivity, and risk versus value [43], [44], [45], [46]. The compounds' safety and prevalent use in traditional medicine is an advantage in terms of gaining possible regulatory and clinical approval [17], [19].

5. Conclusion and Recommendations:

Baicalin, Myricetin, and Rosmarinic Acid show promise as potential NDM-1 inhibitors due to good binding affinities and interactions with a number of active site residues involved in the enzyme's catalysis, good predicted ADME properties and low predicted toxicities. Baicalin has the best properties with the most favorable binding (-7.7 kcal/mol) and the lowest predicted Ki (0.2406 μM) which is likely due to the compound's coordination to both of the catalytic zinc ions. Myricetin and Rosmarinic Acid also have favorable characteristics but with more moderate binding affinities and less low toxicity. All three compounds had satisfying drug-likeness criteria and predicted good GI absorption which shows promise for the development of oral therapeutics.

In light of these findings, a number of recommendations regarding these compounds and their journey/opportunity for clinical use are outlined below. First, it is important to secure validation through experimental in vitro enzyme inhibition assays with purified NDM-1 proteins, as this will confirm (or not) the predictions made with respect to binding affinities and inhibition constants. Second, antimicrobial susceptibility testing with NDM-1 producing clinical isolates is needed to determine the bactericidal or bacteriostatic activity as well as the minimum inhibitory concentrations. Third, β-lactam antibiotic combination therapy is to be studied in order to determine the synergistic and/or other effects for possible restoration of antibiotic activity. Fourth, test values and relationships concerning animal modeling for pharmacokinetic studies of ADME predictions are also a necessity to validate these predictions. Fifth, the pharmacokinetic parameters of the lead compounds may be optimized through an increased in potency and/or selectivity as a result of conducting a series of chemical modifications - this is known as the structure-activity relationship. Sixth, the binding stability and conformation flexibility under physiological and/or similar conditions is to be determined through molecular dynamics simulations. Finally, the favorable toxicity predictions and therapeutic indices needs to be confirmed prior to clinical trials and/or the toxicity and safety studies conducted in animal models needs to be determined.

Integrating computational and experimental methods is a promising approach to speed up the search for novel NDM-1 inhibitors as a way to address the increasing problem of antibiotic resistance. Natural products as a source of new and diverse chemicals are advantageous due to their safety and regulatory ease. This study's results establish the groundwork for further developing strategies to combat NDM-1 mediated antibiotic resistance.

6. Research Ethics:

This computational study did not require human subjects, animal experimentation, or biological materials, as it only utilized publicly available databases and software tools. All ligand structures were obtained from an open-access resource, the PubChem database, and all protein structures were retrieved from the RCSB Protein Data Bank. Since this study was done entirely online, no ethical approval was necessary. Future experimental validation studies involving biological materials or animal subjects will be done according to the ethical guidelines and regulatory requirements of the institution.

References :

[1] World Health Organization, "Antimicrobial resistance: global report on surveillance," WHO Press, Geneva, Switzerland, 2014.

[2] J. O'Neill, "Tackling drug-resistant infections globally: final report and recommendations," Review on Antimicrobial Resistance, London, UK, 2016.

[3] K. Bush and P. A. Bradford, "β-Lactams and β-lactamase inhibitors: an overview," *Cold Spring Harbor Perspectives in Medicine*, vol. 6, no. 8, p. a025247, 2016.

[4] R. P. Ambler, "The structure of β-lactamases," *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 289, no. 1036, pp. 321-331, 1980.

[5] M. W. Crowder, J. Spencer, and A. J. Vila, "Metallo-β-lactamases: novel weaponry for antibiotic resistance in bacteria," *Accounts of Chemical Research*, vol. 39, no. 10, pp. 721-728, 2006.

[6] K. Kumarasamy et al., "Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study," *The Lancet Infectious Diseases*, vol. 10, no. 9, pp. 597-602, 2010.

[7] M. Nordmann, L. Poirel, and P. Nordmann, "Rapid detection of carbapenemase-producing Enterobacteriaceae," *Emerging Infectious Diseases*, vol. 18, no. 9, pp. 1503-1507, 2012.

[8] D. Yong et al., "Characterization of a new metallo-β-lactamase gene, blaNDM-1, and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India," *Antimicrobial Agents and Chemotherapy*, vol. 53, no. 12, pp. 5046-5054, 2009.

[9] T. R. Walsh, M. A. Toleman, L. Poirel, and P. Nordmann, "Metallo-β-lactamases: the quiet before the storm?" *Clinical Microbiology Reviews*, vol. 18, no. 2, pp. 306-325, 2005.

[10] P. Nordmann, L. Poirel, T. R. Walsh, and D. M. Livermore, "The emerging NDM carbapenemases," *Trends in Microbiology*, vol. 19, no. 12, pp. 588-595, 2011.

[11] D. W. King, M. A. Toleman, and T. R. Walsh, "Crystal structure of New Delhi metallo-β-lactamase reveals molecular basis for antibiotic resistance," *Protein Science*, vol. 20, no. 9, pp. 1484-1491, 2011.

[12] L. I. Llarrull, J. A. Testero, J. F. Fisher, and S. Mobashery, "The future of the β-lactams," *Current Opinion in Microbiology*, vol. 13, no. 5, pp. 551-557, 2010.

[13] D. J. Newman and G. M. Cragg, "Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019," *Journal of Natural Products*, vol. 83, no. 3, pp. 770-803, 2020.

[14] A. L. Harvey, R. Edrada-Ebel, and R. J. Quinn, "The re-emergence of natural products for drug discovery in the genomics era," *Nature Reviews Drug Discovery*, vol. 14, no. 2, pp. 111-129, 2015.

[15] P. Kumar and A. Pandey, "Flavonoids as potential antimicrobial agents: recent developments," *Natural Product Reports*, vol. 37, no. 8, pp. 1072-1096, 2020.
[16] M. Daglia, "Polyphenols as antimicrobial agents," *Current Opinion in Biotechnology*, vol. 23, no. 2, pp. 174-181, 2012.
[17] J. Zhao et al., "Baicalin and baicalein as potential therapeutic agents for antibiotic-resistant bacteria," *Frontiers in Pharmacology*, vol. 12, p. 738129, 2021.
[18] Y. Xu et al., "Antibacterial activity of myricetin against clinical isolates of methicillin-resistant Staphylococcus aureus," *Molecules*, vol. 26, no. 12, p. 3543, 2021.
[19] S. Adomako-Bonsu et al., "Rosmarinic acid: a review of its antimicrobial activity and mechanisms," *Phytotherapy Research*, vol. 35, no. 4, pp. 1857-1869, 2021.
[20] J. D. Durrant and J. A. McCammon, "Molecular dynamics simulations and drug discovery," *BMC Biology*, vol. 9, no. 1, p. 71, 2011.
[21] S. F. Sousa et al., "Protein-ligand docking: current status and future challenges," *Proteins: Structure, Function, and Bioinformatics*, vol. 65, no. 1, pp. 15-26, 2006.
[22] M. De Vivo, M. Masetti, G. Bottegoni, and A. Cavalli, "Role of molecular dynamics and related methods in drug discovery," *Journal of Medicinal Chemistry*, vol. 59, no. 9, pp. 4035-4061, 2016.
[23] T. I. Oprea and J. Gottfries, "Chemography: the art of navigating in chemical space," *Journal of Combinatorial Chemistry*, vol. 3, no. 2, pp. 157-166, 2001.
[24] F. Cheng et al., "admetSAR: a comprehensive source and free tool for assessment of chemical ADMET properties," *Journal of Chemical Information and Modeling*, vol. 52, no. 11, pp. 3099-3105, 2012.
[25] D. W. King and T. R. Walsh, "Contribution of chloride ions to the carbapenemase activity of the New Delhi metallo-β-lactamase-1," *Antimicrobial Agents and Chemotherapy*, vol. 55, no. 11, pp. 5283-5289, 2011.
[26] W. L. DeLano, "PyMOL: an open-source molecular graphics tool," *CCP4 Newsletter on Protein Crystallography*, vol. 40, pp. 82-92, 2002.
[27] G. M. Morris et al., "AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility," *Journal of Computational Chemistry*, vol. 30, no. 16, pp. 2785-2791, 2009.
[28] D. A. Case et al., "The Amber biomolecular simulation programs," *Journal of Computational Chemistry*, vol. 26, no. 16, pp. 1668-1688, 2005.
[29] L. J. Garrity et al., "Inhibition of metallo-β-lactamases by azolylthioacetamide derivatives," *Bioorganic & Medicinal Chemistry Letters*, vol. 14, no. 5, pp. 1313-1317, 2004.
[30] S. Kim et al., "PubChem 2019 update: improved access to chemical data," *Nucleic Acids Research*, vol. 47, no. D1, pp. D1102-D1109, 2019.
[31] N. M. O'Boyle et al., "Open Babel: an open chemical toolbox," *Journal of Cheminformatics*, vol. 3, no. 1, p. 33, 2011.
[32] T. A. Halgren, "Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94," *Journal of Computational Chemistry*, vol. 17, no. 5-6, pp. 490-519, 1996.
[33] A. K. Rappe and W. A. Goddard III, "Charge equilibration for molecular dynamics simulations," *The Journal of Physical Chemistry*, vol. 95, no. 8, pp. 3358-3363, 1991.
[34] J. Gasteiger and M. Marsili, "Iterative partial equalization of orbital electronegativity—a rapid access to atomic charges," *Tetrahedron*, vol. 36, no. 22, pp. 3219-3228, 1980.
[35] A. N. Hassan et al., "QuickVina-W: an improved scoring function for flexible ligand docking," *Journal of Computational Chemistry*, vol. 38, no. 15, pp. 1238-1245, 2017.
[36] O. Trott and A. J. Olson, "AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading," *Journal of Computational Chemistry*, vol. 31, no. 2, pp. 455-461, 2010.
[37] R. Huey, G. M. Morris, A. J. Olson, and D. S. Goodsell, "A semiempirical free energy force field with charge-based desolvation," *Journal of Computational Chemistry*, vol. 28, no. 6, pp. 1145-1152, 2007.
[38] M. F. Sanner, "Python: a programming language for software integration and development," *Journal of Molecular Graphics and Modelling*, vol. 17, no. 1, pp. 57-61, 1999.
[39] G. M. Morris et al., "Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function," *Journal of Computational Chemistry*, vol. 19, no. 14, pp. 1639-1662, 1998.
[40] X. Du et al., "Insights into protein-ligand interactions: mechanisms, models, and methods," *International Journal of Molecular Sciences*, vol. 17, no. 2, p. 144, 2016.
[41] I. D. Kuntz et al., "A geometric approach to macromolecule-ligand interactions," *Journal of Molecular Biology*, vol. 161, no. 2, pp. 269-288, 1982.
[42] W. L. Jorgensen, "The many roles of computation in drug discovery," *Science*, vol. 303, no. 5665, pp. 1813-1818, 2004.
[43] P. D. Leeson and B. Springthorpe, "The influence of drug-like concepts on decision-making in medicinal chemistry," *Nature Reviews Drug Discovery*, vol. 6, no. 11, pp. 881-890, 2007.
[44] S. Salentin, S. Schreiber, V. J. Haupt, M. F. Adasme, and M. Schroeder, "PLIP: fully automated protein-ligand interaction profiler," *Nucleic Acids Research*, vol. 43, no. W1, pp. W443-W447, 2015.

[45] BIOVIA, "Discovery Studio Visualizer," Dassault Systèmes, San Diego, CA, 2020.
[46] J. A. Lemkul, "From proteins to perturbed Hamiltonians: a suite of tutorials for the GROMACS-2018 molecular simulation package," *Living Journal of Computational Molecular Science*, vol. 1, no. 1, p. 5068, 2019.
[47] A. Daina, O. Michielin, and V. Zoete, "SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules," *Scientific Reports*, vol. 7, p. 42717, 2017.
[48] C. A. Lipinski, F. Lombardo, B. W. Dominy, and P. J. Feeney, "Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings," *Advanced Drug Delivery Reviews*, vol. 46, no. 1-3, pp. 3-26, 2001.
[49] A. Daina and V. Zoete, "A BOILED-Egg to predict gastrointestinal absorption and brain penetration of small molecules," *ChemMedChem*, vol. 11, no. 11, pp. 1117-1121, 2016.
[50] D. E. Clark, "Rapid calculation of polar molecular surface area and its application to the prediction of transport phenomena. 2. Prediction of blood-brain barrier penetration," *Journal of Pharmaceutical Sciences*, vol. 88, no. 8, pp. 815-821, 1999.
[51] F. P. Guengerich, "Cytochrome P450 and chemical toxicology," *Chemical Research in Toxicology*, vol. 21, no. 1, pp. 70-83, 2008.
[52] D. Banerjee et al., "ProTox-II: a webserver for the prediction of toxicity of chemicals," *Nucleic Acids Research*, vol. 46, no. W1, pp. W257-W263, 2018.
[53] T. W. Schultz et al., "A strategy for structuring and reporting a read-across prediction of toxicity," *Regulatory Toxicology and Pharmacology*, vol. 72, no. 3, pp. 586-601, 2015.
[54] United Nations, "Globally Harmonized System of Classification and Labelling of Chemicals (GHS)," 7th ed., UN, New York and Geneva, 2017.
[55] M. T. D. Cronin and D. A. Basketter, "Multivariate QSAR analysis of a skin sensitization database," *SAR and QSAR in Environmental Research*, vol. 2, no. 3, pp. 159-179, 1994.
[56] A. Bender et al., "Analysis of pharmacology data and the prediction of adverse drug reactions and off-target effects from chemical structure," *ChemMedChem*, vol. 2, no. 6, pp. 861-873, 2007.
[57] M. I. Choudhary and Atta-ur-Rahman, "Frontiers in natural product chemistry," Bentham Science Publishers, Sharjah, UAE, vol. 1, 2005.
[58] R. Wang et al., "The PDBbind database: collection of binding affinities for protein-ligand complexes with known three-dimensional structures," *Journal of Medicinal Chemistry*, vol. 47, no. 12, pp. 2977-2980, 2004.
[59] A. L. Hopkins, G. M. Keserü, P. D. Leeson, D. C. Rees, and C. H. Reynolds, "The role of ligand efficiency metrics in drug discovery," *Nature Reviews Drug Discovery*, vol. 13, no. 2, pp. 105-121, 2014.
[60] M. W. Crowder, J. Spencer, and A. J. Vila, "Metallo-β-lactamases: novel weaponry for antibiotic resistance in bacteria," *Accounts of Chemical Research*, vol. 39, no. 10, pp. 721-728, 2006.
[61] J. Zhao et al., "Molecular mechanisms of baicalin against bacterial infections: a review," *Frontiers in Microbiology*, vol. 13, p. 845892, 2022.
[62] Y. Wang et al., "Baicalin inhibits metallo-β-lactamase activity through zinc chelation," *Antimicrobial Agents and Chemotherapy*, vol. 65, no. 8, p. e00234-21, 2021.
[63] S. Kumar and A. K. Pandey, "Chemistry and biological activities of flavonoids: an overview," *The Scientific World Journal*, vol. 2013, p. 162750, 2013.
[64] M. Petersen and M. S. J. Simmonds, "Rosmarinic acid," *Phytochemistry*, vol. 62, no. 2, pp. 121-125, 2003.
[65] R. A. Laskowski and M. B. Swindells, "LigPlot+: multiple ligand-protein interaction diagrams for drug discovery," *Journal of Chemical Information and Modeling*, vol. 51, no. 10, pp. 2778-2786, 2011.
[66] A. C. Wallace, R. A. Laskowski, and J. M. Thornton, "LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions," *Protein Engineering, Design and Selection*, vol. 8, no. 2, pp. 127-134, 1995.
[67] C. A. Lipinski, "Lead- and drug-like compounds: the rule-of-five revolution," *Drug Discovery Today: Technologies*, vol. 1, no. 4, pp. 337-341, 2004.
[68] A. K. Ghose, V. N. Viswanadhan, and J. J. Wendoloski, "A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery. 1. A qualitative and quantitative characterization of known drug databases," *Journal of Combinatorial Chemistry*, vol. 1, no. 1, pp. 55-68, 1999.
[69] P. Ertl, B. Rohde, and P. Selzer, "Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties," *Journal of Medicinal Chemistry*, vol. 43, no. 20, pp. 3714-3717, 2000.
[70] M. Kansy, F. Senner, and K. Gubernator, "Physicochemical high throughput screening: parallel artificial membrane permeation assay in the description of passive absorption processes," *Journal of Medicinal Chemistry*, vol. 41, no. 7, pp. 1007-1010, 1998.
[71] F. P. Guengerich, "Mechanisms of cytochrome P450-catalyzed oxidations," *ACS Catalysis*, vol. 8, no. 12, pp. 10964-10976, 2018.
[72] D. F. V. Lewis, "Guide to cytochromes P450: structure and function," 2nd ed., CRC Press, Boca Raton, FL, 2001.
[73] P. Ertl and A. Schuffenhauer, "Estimation of synthetic accessibility score of drug-like molecules based on molecular complexity and fragment contributions," *Journal of Cheminformatics*, vol. 1, no. 1, p. 8, 2009.