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IDENTIFYING POTENTIAL VIM-2 INHIBITORS: AN IN-SILICO APPROACH TO EXPLORE NOVEL SCAFFOLDS FOR ANTI- MICROBIAL RESISTANCE

KONICA JINDAL

24/MSCBIO/03

ABSTRACT

ABSTRACT

Aim: This study aims to examine natural plant-based compounds known as flavonoids as potential inhibitors of VIM-2 Metallo-β-Lactamase (MBL) which is an enzyme produced by the dangerous bacteria Pseudomonas aeruginosa that makes it resistant to nearly all available antibiotics. One of the most pressing global health crises currently is antimicrobial resistance (AMR) and VIM-2 is a main driver of this resistance by breaking down β-lactam antibiotics which is the most widely used type of antibacterial drugs.There is an urgent need to find new and effective molecules that can block this enzyme as there is no clinically approved MBL inhibitor currently. Flavonoids are naturally occurring polyphenolic compounds which are found in large amount in plants. They were chosen as candidate molecules due to their known antibacterial properties and structural ability to interact with metal containing enzyme active sites. This study examines whether flavonoids can bind effectively to the active site of VIM-2 and potentially restore the effectiveness of β-lactam antibiotics using a computational in silico approach. A total of 30 flavonoids were initially considered and screened through ADME (Absorption, Distribution, Metabolism and Excretion) analysis for drug like properties and the best candidates underwent through molecular docking against the VIM-2 crystal structure (PDB ID: 4C1E) using AutoDock Vina. The popular thiol-based inhibitor D captopril was used as a reference compound for comparison.

Result: A total of 8 compounds successfully passed ADME and drug likeness filters like Lipinski's Rule of Five, PAINS and Brenk alerts and Ghose criteria from the 30 flavonoids selected initially and these were then taken for molecular docking. All 8 flavonoids showed stronger binding affinities than the reference inhibitor D-captopril (–6.20 kcal/mol) with binding energies ranging between –7.6 to –8.1 kcal/mol. Apigenin (PubChem CID: 5280443) showed highest binding affinity of –8.1 kcal/mol making it the best candidate among all. Interaction studies confirmed that the flavonoids formed stable forces with key enzymatic residues in the VIM-2 active site which includes ASP118, ASP117, HIS116, TRP87, PHE62 and ASN210. These residues are critical for zinc dependent β-lactam solvolysis. Other flavonoids including Apigenin and Genistein also showed direct interaction with the catalytic zinc ions (ZN501 and ZN502) which further supported their potential to disrupt enzyme activity.

Conclusion: Apigenin emerged as the most effective candidate among the eight flavonoids examined and displayed the strongest binding affinity and favorable interactions with the VIM-2 catalytic active site and surpassed the reference inhibitor D-captopril. These findings show that naturally occurring flavonoids hold significant promise as novel scaffold molecules for the development of MBL inhibitors. Their plant derived origin along with drug like pharmacokinetic properties and potent in silico binding profiles makes them highly attractive

leads for combating antibiotic resistance. We strongly recommend validating these computational findings through molecular dynamics simulations, in vitro enzyme inhibition assays and ultimately in vivo experiments to confirm their therapeutic potential.

List of Publications

1. Conference paper:

Title of paper- "In Silico Exploration of Flavonoid Scaffolds as VIM2 Metallo Beta Lactamase Inhibitors for Combating Antimicrobial Resistance"

Author Names- Konica Jindal and Kriti Bhandari

Name of Conference- 17th International Conference on Science and Innovative Engineering (ICSIE – 2026)

Date of Conference- 26th -27th April, 2026 at Prince Dr. K. Vasudevan College of Engineering and Technology, Chennai, India.

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LIST OF ABBREVIATIONS

AMR	Anti-Microbial Resistance
MBL	Metallo-β-Lactamase
SBL	Serine β-Lactamase
VIM	Verona Integron-encoded Metallo-β-Lactamase
NDM	New Delhi Metallo-β-Lactamase
IMP	Imipenemase
PBP	Penicillin Binding Protein
MDR	Multi Drug Resistant
CRE	Carbapenem Resistant Enterobacteriaceae
CPE	Carbapenemase Producing Enterobacteriaceae
CDC	Centres for Disease Control and Prevention
ICU	Intensive Care Unit
ACE	Angiotensin Converting Enzyme
EDTA	Ethylenediaminetetraacetic Acid
AMA	Aspergillomarasmine A
ADME	Absorption, Distribution, Metabolism, and Excretion
TPSA	Topological Polar Surface Area
GI	Gastrointestinal Absorption
PAINS	Pan-Assay Interference Compounds
LogP	Logarithm of Partition Coefficient
SBDD	Structure-Based Drug Design
SDF	Structural Data File
PDB	Protein Data Bank
PDBQT	Protein Data Bank, Partial charge and Atom Type
CID	Compound Identity Number

MIC	Minimum Inhibitory Concentration
QS	Quorum Sensing
EPI	Efflux Pump Inhibition
IC50	Half Maximal Inhibitory Concentration
FDA	Food and Drug Administration
KPC	Klebsiella pneumoniae Carbapenemase
OXA	Oxacillinase (Class D β-Lactamase)
MRSA	Methicillin-Resistant Staphylococcus aureus
CS-TH	Cephalosporin–Thiol Hybrid
RCSB	Research Collaboratory for Structural Bioinformatics
2D	Two-Dimensional
3D	Three-Dimensional
UCSF Chimera	University of California, San Francisco Chimera

1. INTRODUCTION

Bacterial infections pose a large threat towards the global health problem. Bacterial infections are increasing worldwide and the major culprit behind this aggravation is Anti-microbial resistance (AMR). Bacterial infections have been often associated with healthcare contamination, often created due to Gram Negative microbes like *Pseudomonas, Acinetobacter* and Enterobacteriaceae [1]. The 20th century was the time when the field of medicine gets revolutionised. The reason for this revolution was the introduction of antibiotics. There are several classes of antibiotics that exist today but the most prescribed and clinically successful class is β-lactams [2], [3]. The action of β-lactams is due to the existence of β-lactam ring within its structure. The ring mimics D-Ala-D-Ala, substrate of Penicillin Binding Proteins (PBPs). Now, instead of D-Ala-D-Ala, β-lactam ring binds with Penicillin Binding Proteins (PBPs). This interaction has caused in the formation of covalent acyl enzyme intricate. Due to this, transpeptidation does not occur in the bacterial cell wall, causing cell wall disruption and finally bacterial cell death by cell lysis [4]. Penicillin, Carbapenems, Cephalosporins and Monobactams are recognised as the sub classes of β-lactam antibiotics. With introduction of antibiotics, bacterial infections have reduced significantly and improved patient survival rate but continuous use of antibiotics has posed a new challenge, i.e., bacteria becoming resistant to these antibiotics. Moreover, the slow rate of new antibiotic discovery has raised concerned over a looming "Post antibiotic Era" [2]. All these has emerged as a major problem and there's a need for immediate innovative therapies to solve the issue of AMR [5].

The cause of bacterial resistance can be attributed to the presence of β lactamase enzymes. These bacterial enzymes make the β lactam antibiotics inactive. The inactivation occurs due to solvolysis of β lactam ring, constituent in β lactam antibiotics. On the basis of structural and mechanistic features, β lactamase are classified into four classes, namely, Class A, C and D collectively called Serine β-lactamases (SBLs) and class B, called Metallo-β-lactamases (MBLs). Out of these 4 classes, MBLs impart resistance to nearly all subclasses of β-lactam antibiotics except monobactams. Therefore, clinical relevance of MBLs is extremely high despite of the fact that they constitute only a small share of β-lactamases [6], [7]. To combat this problem, β-lactam antibiotics are often employed in combination with β-lactamase inhibitors. These inhibitors bind to β-lactamase enzymes and inhibits their hydrolytic action, thus, restoring the therapeutic efficacy of β-lactam antibiotics. Clinically, effective SBLs inhibitor are already being used but the space of MBLs inhibitors is still bleak, which underscores the need for identifying novel strategies against MBLs [8].

MBLs are further segregated into 3 sub classes: B1, B2 and B3. It is found that B1 is the most widely and clinically relevant sub class of MBLs [5]. The structure of MBLs contains conserved αβ/βα sandwich fold alongside zinc binding motif, typically coordinating two zinc ions coupled with a hydroxide ion. This motif results in cleavage of β-lactam ring, which is present as constituent in β-lactamase antibiotics and thus makes them inactive [9]. New Delhi MBLs (NDMs), Verona Integron-encoded MBLs (VIMs) and Imipenemases (IMPs) are the highly frequently reported B1 enzymes [5]. Out of these, VIMs are of major concern due to their global dissemination. The dissemination is rapid due to the presence of conjugative plasmids resulting in efficient horizontal transfer. VIM 1 and VIM 2 have several variants identified in *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Acinetobacter*. These bacteria are often associated with ICU outbreaks which result in high mortality rate in patients. VIMs remain persistent in hospital reservoirs resulting in recurrent infections and evolve faster under continuous antibiotic pressure, with 92 variants reported as of January 2025 [10]. This signifies VIM enzymes as urgent targets for inhibitor development in AMR research.

Among the potential candidates for MBLs inhibitors, a small molecule called Captopril is known to bind to Zinc motif of MBL enzymes and thus, inhibiting them. Originally, Captopril is an FDA approved drug for hypertension, developed in 1970s. This thiol compound is also known to inhibit Human Angiotensin Converting enzyme (ACE) [11]. Studies have shown that Captopril has two stereoisomers, namely, L-captopril and D-captopril and both of them is known to be effective as MBL inhibitors. However, D-captopril is found to have superior activity as VIM enzyme inhibitor than L -Captopril [10], [12]. Despite all these findings, no MBL inhibitor has yet proceeded for clinical use. Moreover, the number of agents that could effectively work as MBL inhibitor are also insufficient. Thus, concluding the fact there is a need to explore more sources that are capable of targeting MBLs.

There are several bioactive metabolites present in plants that exhibit broad pharmacological potential. Among the several bioactive metabolites, Flavonoids are most widely distributed and exhibit great structural diversity. Flavonoids are polyphenolic compounds that are known to disrupt cell wall synthesis, inhibit biofilm formation, weaken efflux pumps and disturb membrane integrity. All these in turn results in killing of bacterial cells and thus making antibiotic effective again. Thus, Flavonoids could provide a feasible way to counteract AMR [13], [14]. Additionally, it is discovered that flavonoids could interact with metal dependent active sites and thus, could also restore β-lactam antibiotics activity [15] which again shows flavonoids as potential compounds that could act as MBL inhibitors.

Given the substantial evidence, supporting flavonoids as MBL inhibitors in mitigating AMR and the rising utility of computational tools in drug discovery, this thesis explores Flavonoids as novel scaffolds for VIM 2 inhibition with potential therapeutic value for AMR. By integrating molecular docking studies, the study aims to contribute to the development of effective MBL inhibitors and could widen their chemical space.

2. REVIEW OF LITERATURE

2.1 Anti -microbial Resistance and Clinical Burden

Hugely, highly dangerous health hazards in 21st era is Bacterial Antimicrobial Resistance (AMR). When Bacteria evolve mechanisms to evade anti-microbial treatments thus, enabling their survival in the host and making standard therapies ineffective, this is when AMR occurs. Due to alarming condition, AMR has become the most important topic of discussion worldwide. (Lancet). Although, AMR is a natural phenomenon but it’s acceleration in recent times is majorly due to overuse of antibiotics and thus it’s a matter of close international surveillance now (matteo bassetti,andre bittencourt lorusso) (ka wah kelly).

Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii are among the greatest concern category (david mp de oliveira). These species along with other ESKAPE Pathogens have developed resistance against number of antibiotics through acquisition of mobile genetic elements and genetic mutation (david mp de oliveira). This has increased disease burden, mortality rated and treatment failures especially in developing nations where communicable diseases are very common (david mp de oliveira).

Among the most commonly used categories of antibiotics are Beta lactam antibiotics. These are used since many years and thus are a pillar in anti-microbial treatments. They are favoured due to their efficacy, broad spectrum and safe to use property. Penicillin, Cephalosporins, Monobactams, Carbapenems belong to beta lactam antibiotic class and all of them possess beta lactam ring within

their structure (joyce de souza). Carbapenems have been largely used for Multi Drug Resistant (MDR) bacterial infections. These work by interacting to protein called Penicillin Binding Proteins (PBPs) that is responsible for cell wall genesis inhibition (david p nicolau). The beta lactam antibiotics are degraded by enzymes called beta lactamases, commonly occurring in gram negative bacteria and makes these antibiotics ineffective, making it challenging to manage infections in the hospitals (joyce de souza).

Due to large use of carbapenems, the global spread of carbapenems poses a great threat to the healthcare system. Carbapenem resistant Enterobacteriaceae (CRE) is classified among the three most urgent AMR by US Centres for Disease Control and Prevention (CDC). This could be due to their increasing MDR, rapid global dissemination and high mortality rate associated with bloodstream infections (robert f potter). The reason for CRE is due to presence of special beta lactamases in them called carbapenemases, these are capable of inactivating several antibiotics including Carbapenems, monobactams etc due to their broad hydrolytic capacity (anne marie queenan). NDM, VIM, OXA-48(Class D), KPC (Class A) and IMP (all Class B Metallo beta lactamases) forms clinically relevant carbapenemases in carbapenemase producing Enterobacteriaceae (CPE) (Robert f potter)

Pseudomonas aeruginosa comes out as a concerning pathogen in these times of rising resistance. Pseudomonas aeruginosa, one of the gram negative microbe and is found to be associated with hospital acquired infections, chronic pulmonary infections in patients with lung diseases like cystic fibrosis and infections in immunocompromised individuals(dan reynolds).7.1 to 7.3% of all hospital associated infections is attributed to Pseudomonas aeruginosa, making this gram negative bacteria most identified in pneumonia cases, urinary tract infections and surgical site infections (dan reynolds, shelley s magill, lindsey weiner). This all defines carbapenem resistant Pseudomonas aeruginosa a critical clinical challenge which drives extreme mortality in hospital settings and this is due to the fact that these microbes are having intrinsic resistance mechanisms along with their capacity to acquire further new resistance determinants.

2.2 Metallo β-lactamases (MBLs): Structure and Classification

Together, the structural diversity across MBL subclasses — particularly variations in zinc coordination, active site geometry, and substrate binding — presents significant challenges for rational creation of inhibitors that are broad spectrum, capable in targeting all clinically relevant MBL variants (Guillermo Bahr).

When it comes to antibiotic resistance context, **β-lactamases** are thought to represent the significant and one of the widely studied enzyme family (karen bush). The β-lactam antibiotics resistance by Gram negative microbes causede by mechanism which is operated by β-lactamase enzymes. The enzymes are broadly divided into 4 classes: zinc-dependent Metallo-beta-lactamases (Class B) and serine beta-lactamases (Classes A, C and D) (Joyce de Souza; Karen Bush, "Molecular Classifications of Beta-lactamases"). Serine beta-lactamases utilise serine residue present in its active site for solvolysis whereas Class B enzymes, MBLs show unique dependence on divalent zinc ions for their catalytic activity (Karen Bush, "Zinc-dependent Catalysis").

The reasons why Metallo-beta-lactamases (MBLs) are of great clinical concern are: their broad hydrolytic activity against nearly all beta-lactam antibiotics, their ability for horizontal gene transfer between organisms and lastly, no clinically approved inhibitor exists against them (Carine Bebrone, "Clinical Significance of MBLs"). The mechanism of catalytic activity of MBLs relies on the binding of beta-lactam substrate to zinc ions within enzymatic site (Anne Marie Queenan; Karen Bush, "BRT and MBL Inhibition"). MBLs are segregated into three subclasses on the basis of sequence

analysis—B1, B2 and B3 with each having distinct structural and functional features (Carine Bebrone, "Structural Characteristics of MBL Subclasses").

The largest and most clinically relevant is Subclass B1. It consists of highly transferable and widely disseminated VIM, IMP and NDM-type enzymes (Timothy Palzkill; Maria F. Mojica, "Dissemination of B1 MBLs"). The active site of these enzymes contains two Zn ions: Zn1 and Zn2. Zn1 coordinate via three histidine residues (H116, H118, H196) while Zn2 via an aspartate, a cysteine and a histidine (D120, C221, H263). The active site exhibits a broad substrate profile including penicillin, cephalosporins and carbapenems (Maria F. Mojica, "Zinc Coordination in B1 Enzymes"). One of the serious threats that has emerged across different parts of Asia are VIM-type MBL, where MBL-positive multidrug-resistant strains account for over 99% cases (Brem; Sander S. van Berkel, "Epidemiology of VIM-type MBLs").

14 Next class is Subclass B2 enzymes which are structurally different from B1 as they exhibit only a single zinc ion in their active site. They also display a narrow substrate activity that includes only carbapenem hydrolysis and activity against other beta-lactam antibiotics is almost negligible (Sandra Wommer; Timothy Palzkill, "Structural Distinctness of B2 MBLs"). These enzymes are primarily derived from Aeromonas spp. and Serratia fonticola (Timothy R. Walsh, "Host Species for B2 MBLs").

Last class called Subclass B3 is structurally unique among all beta-lactamases. Their active site shown to have a complex which is functioning as a tetramer. This class is represented most notably by the L1 enzyme and B3 enzymes are extensively found in environmental bacteria instead of clinically relevant pathogens (Timothy R. Walsh, "Subclass B3 Characteristics"). Like B1, it also has a broad-spectrum substrate profile (Timothy Palzkill, "Substrate Profiles of B3 MBLs").

Hence it is found out that all these subclasses together due to their structural diversity particularly variations in zinc coordination, active site geometry and substrate binding, presents major challenge for the discovery of successful inhibitors which are capable of targeting all clinically relevant MBL variants (Guillermo Bahr, "Challenges in Broad-spectrum Inhibitor Design").

2.3 Clinical Significance of VIM Enzymes

VIM-1, a type of B1 MBL discovered initially in Italy in 1997, are among the most geographically diverse and clinically significant MBLs till date (Yasufumi Matsumura, "Global Spread of VIM Enzymes").It has over 60 variants at present, which accounts for their broad bacterial species distribution and are currently grouped into five major groups: VIM-1, VIM-7, VIM-2, VIM-13 and VIM-12. This distribution is on basis of amino acid sequence similarities (Anne Makena, "Clusters of VIM Variants"). The first formally reported VIM was VIM 1 in Italy in 1999. After that, VIM-2 was identified shortly in France and Italy. The subsequent variants emerged across Greece, Turkey and beyond (Anne Makena, "Historical Timeline of VIM"). Since then, VIM-2 has become one of the most widely reported MBLs globally (Luisa Borgianni, "Prevalence of VIM-2").

VIM-producing organisms are most commonly found in bacteria called Pseudomonas aeruginosa, where they were found to show a dominant mechanism of carbapenem resistance. Moreover, they were comparatively rare among Enterobacteriaceae (Yasufumi Matsumura, "Host Specificity of VIM Producers"). It was also established that within the latter group, VIM-producing strains are concentrated predominantly in Southern and Eastern Europe—especially Greece, Spain, Hungary and Italy—with Escherichia coli, Klebsiella pneumoniae and Enterobacter spp. being most frequently encountered species (Yasufumi Matsumura, "VIM in Enterobacteriaceae"). The structural diversity

among VIM variants is of clinical significance. The diversity is due to key variation occurring at positions 224 and 228 of the enzyme. The residues at these particular positions are shown to interact directly with beta-lactam substrates. This means that different variants may bind substrates and potential inhibitors in fundamentally different ways (Ramya Salimraj, "Structural Variation and Substrate Binding"). As a result, this variability complicates both the prediction of clinical behaviour across variants and the development of broadly effective inhibitors against them.

One of the major factors which is responsible for the global spread of VIM enzymes is their association with mobile genetic elements. VIM genes are characteristically embedded in class 1 integrons which are present on plasmids with broad host range. This enables their efficient horizontal transfer and that too across diverse bacterial species and clinical settings (Amy J. Mathers; Yasufumi Matsumura, "Genetic Mobility of VIM"). This genetic mobility has resulted in the interspecies and inter-institutional spread of VIM-type carbapenemases which ultimately contributes to their establishment in hospital environments worldwide (Amy J. Mathers, "Interspecies Dissemination of VIM").

2.4 Strategies of MBL Inhibition

Due to the structural diversity of the MBLs and constantly changing nature of their active site, discovery of effective metallo-beta-lactamase (MBL) inhibitors remains a key challenge in medicine. Unlike serine beta-lactamases, which resulted in occurrence of a covalent bond consisting acyl-enzyme intricate, MBLs utilize a binuclear zinc centre which forms active water molecule for the nucleophilic attack on beta-lactam ring and thus making beta-lactam antibiotics ineffective. Moreover, the strategies which are used for serine beta-lactamase inhibition are very specific to them and cannot be applied to MBL inhibition, which further defines the need for the development of specific metal-binding inhibiting strategies. (Vila and González, "Updated Review on Metallo-beta-lactamases"). These strategies should primarily focus on synthetic inhibitors, including thiols, dicarboxylic acids, and boronic acids as current inhibitors show very low clinical success. (Schönauer and Brandstetter, "Inhibition and Activity Regulation of Bacterial Collagenases").

Synthetic Inhibitors and the Role of Thiols

The major interest area is developing Thiol-based inhibitors because of the presence of sulphur atom in their structure which possesses a high affinity for divalent zinc ions. This facilitates the displacement of the catalytic water molecule or the coordination of the metal ions in such a way that results in obstruction of substrate binding. (Vila and González, "Updated Review on Metallo-beta-lactamases"). An FDA-approved drug called D-captopril which was traditionally used as an ACE inhibitor has emerged as a primary compound for the design of MBL inhibitors. It is found out that in VIM-2 and other B1 MBLs, the thiol group of D-captopril coordinates between the two zinc ions (Zn1 and Zn2), which effectively mimics the tetrahedral transition state of beta-lactam hydrolysis. (Robert F. Potter, "Molecular Mechanisms of Carbapenem Resistance"). Other than these simple thiols, researchers have also explored thiol-linked conjugates, such as cephalosporin–thiol hybrids (CS-TH). These conjugates are designed to release a potent metal-binding inhibitor upon cleavage by the enzyme. (anber mohammed et al., "Design and Synthesis of Aryl Thiomannosides").

Although thiol-consisting scaffolds exhibit potent in vitro activity but their clinical utility is compromised by several factors. It is deduced that many thiols are found to be susceptible to rapid oxidation or metabolic degradation and their high polarity can lower their capability to disrupt relatively impermeable outer layer of Gram-negative pathogens such as *Pseudomonas aeruginosa*.

(trae hillyer **Meropenem/Vaborbactam—A Mechanistic Review for Insight into Future Development of Combinational Therapies**"). Furthermore, it was also observed that the non-specific nature of certain metal-binding motifs can lead to off-target effects due to their cross-reaction with other essential human metalloenzymes such as carbonic anhydrases or matrix metalloproteinases. (Supuran, "Inhibition of Bacterial Carbonic Anhydrases").

Chelators and Sequestration Strategies

Apert from Thiol based inhibitors, an alternative strategy involves the use of chelating agents which sequesters zinc ions directly from active site of MBL. Compounds like EDTA (Ethylenediaminetetraacetic acid) and aspergillomarasmine A (AMA) have shown the ability to rapidly inactivate MBLs by removing the metal cofactors required for catalysis. (Karen Bush, "Structural Biology of Beta-lactamases"). But the use of these chelators in a clinical setting is avoided due to their toxic nature. (Vila and González, "Updated Review on Metallo-beta-lactamases"). Additionally, the lack of selectivity for bacterial zinc centres over human ones makes traditional chelators unsuitable for anything other than diagnostic or topical applications. (Supuran, "Inhibition of Bacterial Carbonic Anhydrases").

Tabular Representation of MBL Inhibitor Classes

Inhibitor Class	Mechanism of Action	Examples	Developmental Status
Thiols	Direct coordination with Zn ions	D-captopril, Thiopropionic acid	Pre-clinical / Repurposed
Chelators	Metal ion sequestration	EDTA, AMA	Limited by toxicity
Boronates	Reversible covalent bonding	Taniborbactam, Vaborbactam	Early Clinical Trials
Dicarboxylic Acids	Hydrogen bonding and metal coordination	Succinic acid derivatives	In Vitro Screening

Limitations and the Need for Alternative Scaffolds

There are several limitations of current synthetic strategies which includes poor bioavailability, false target toxicity and rapid occurrence of resistance through subtle active-site mutations. All these have created an urgent need for the discovery of novel scaffolds. (Vila and González, "Updated Review on Metallo-beta-lactamases"). This search has largely turned toward natural products as they offer high structural complexity and are evolutionarily optimized to interact with biological targets.

(Heinrich et al., "Ethnopharmacy and Drug Discovery"). This transition toward plant-derived bioactive compounds shows a paradigm shift in MBL inhibitor research which shows the evolution of simple synthetic chelators towards complex molecules with multi-target potential. (Supuran, "Inhibition of Bacterial Carbonic Anhydrases").

2.5 Bioactive Compounds derived from Medicinal Plants

Since large times, plants with medicinal features have provided foundation for human healthcare. These plants act as vast reservoirs of secondary metabolites which are produced by them for their defense against microbial pathogens. (Heinrich et al., "Ethnopharmacy and Drug Discovery"). It has been validated scientifically that ancient remedies which consists of many plant-derived compounds possess significant therapeutic value and these are often associated with lower risk of adverse consequences when compared to synthetic alternatives. It has been observed that a significant amount of global population is still dependent on herbal medicinal products, significantly in regions with less access to modern Western medicine, restricted by cost alongwith infrastructure. (Riffat latif and Taufiq Nawaz **Medicinal plants and human health: a comprehensive review of bioactive compounds, therapeutic effects, and applications)**

Ethnobotany as a Reservoir of Antibacterial Metabolites

Plants synthesize a diverse array of metabolites, which consists alkaloids, terpenoids, flavonoids and tannins. Each of these possess unique modes of action. (Shahrajabian et al., "Artemisia annua and its Constituent Metabolites"). Many of these compounds have been successfully isolated and utilized as leads for drug development. (David zorngo IntechOpen, "Medicinal Plants in the Management of Infectious Diseases"). For example, extracts from *Azadirachta indica* (Neem) have shown broad-spectrum antimicrobial properties which are attributed to complex mixtures of limonoids and flavonoids. (omorefosa IEEE Conference, "Neem Seed Methanolic Extract Scavenges Free Radicals"). Likewise, Libyan medicinal plants such as *Thymus capitatus* and *Artemisia herba-alba* have demonstrated potent efficacy against standard pathogenic strains like *Staphylococcus aureus* and *Pseudomonas aeruginosa*. (bisan Al-ghanna Journal, "Phytochemical Analysis of Libyan Medicinal Plants").

Success Stories: Quinine and Artemisinin

The success of plant-derived medicines is most famously shown by the antimalarial drugs quinine and artemisinin. Quinine which is an alkaloid, sourced from the bark of the *Cinchona* tree. It was the first treatment for malaria which was effective and remains in use today despite being first isolated in the 19th century. (chiara portolani "Cinchona Alkaloids and Enantioselective Synthesis"). The complex structure of Quinine, involves an aliphatic quinuclidine ring and an aromatic quinoline ring which allows it to disrupt the parasite's metabolism with high specificity. (jair, kathyrn, Kelly Approved Antimalarial Drugs and Clinical Treatment").
Artemisinin, which is derived from *Artemisia annua*, represents another milestone in natural product chemistry. (Shahrajabian et al., "Artemisia annua: A Gift from Chinese Traditional Medicine"). The discovery of its sesquiterpene endoperoxide structure provided a novel scaffold for treating drug-resistant malaria where previous synthetic treatments had failed. (marsha b quinlan Ethnomedicines: Traditions of Medical Knowledge). These success stories show the potential of natural products to overcome the limitations of synthetic drugs and strategies. All these instances provide scaffolds that are not only potent but also demonstrate favourable safety profiles. (Rishabh kuandal dimesh kumar Current demands for standardization of Indian medicinal plants: A critical review").

Transition Toward Flavonoids as a Major Plant-Derived Class

Among the various classes of plant metabolites, flavonoids have emerged as particularly promising candidates for antibiotic enhancement and enzyme inhibition. Largely present in the human diet and widely distributed across the plant kingdom, flavonoids are a subclass of phenolics characterized by a 15-carbon skeleton. (songul bayrak Serpil gerni **In Vitro Evaluation of Flavonoids for Enzyme Inhibition, Antioxidant, Antimicrobial and Anticancer Properties with Molecular Docking Insights**"). Their ability to chelate metal ions and stabilize biological membranes is shown in the recent researches which makes them ideal candidates for targeting Metallo-enzymes like MBLs. (ye huang, chenzi zhao, Yousry, jing, rong Frontiers in Plant Science, "Combined Approach of Transcriptomics and Metabolomics in Bidens alba"). Moreover, the transition from general ethnobotanical screening toward targeted flavonoid research is supported by their low toxicity and established antioxidant profiles. (Heinrich et al., "Ethnopharmacy and Drug Discovery").

2.6 Flavonoids: Anti- bacterial Potential and Role in MBL Inhibition

The largest and highly structurally diverse groups in plant secondary metabolites is Flavonoids. They play essential roles in plant protection against UV radiation, pathogens and environmental stressors. (yuan wang, jiahong chen, genhe he, li yin, Yonghui Frontiers in Plant Science, "Metabolite patterns and biosynthesis of flavonoids"). Flavonoids has two aromatic rings which are connected by a three-carbon bridge in its structure, which usually forms an oxygenated heterocyclic ring (Stefan,ioana, paul mihai Journal for Research in Applied Sciences and Biotechnology, "Antioxidant and Anti-inflammatory Properties of Flavonoids"). Flavonoids are divided into several classes on the basis of the structure of the central ring. The classes include flavones, flavanols, flavanones, isoflavones and anthocyanins. (Ivayla, ilian, bistra EurasianSciEnTech, "Pharmacological activity of plant-derived phenolic compounds").

Structural Diversity and Biosynthesis

Moreover, the structural diversity of flavonoids is further expanded by various modifications such as hydroxylation, methylation and glycosylation. (zhaochu, xinyu, peilan, ye, chenzi Frontiers in Plant Science, "Biosynthesis and accumulation of flavonoids in Bidens alba"). These modifications are crucial for determining their biological activity, for example, it was observed that the position of hydroxyl groups on the aromatic rings significantly influences their antioxidant potential and their ability to bind to target enzymes. ("). It was also elucidated by research into *Bidens alba, that* identified over 774 distinct flavonoids which highlights the immense chemical space occupied by this class. (mingcheng, xinyi, zefu").

Antibacterial Mechanisms: A Multi-Target Approach

Several distinct mechanisms operate involving Flavonoids that exert their antibacterial effects, often acting synergistically to inhibit bacterial growth and virulence. (mahaboob khan sulaiman"The Molecular Mechanisms and Therapeutic Potential of Flavonoids").

1. Membrane Disruption: Lipophilic flavonoids can insert into bacterial cell membrane, disrupting its fluidity and as a outcome intracellular contents leakage is observed. Chinenye, ezinwanne, Stephen, chinekwu").

2. Efflux Pump Inhibition (EPI): Some flavonoids are known to act as competitive inhibitors of microbial efflux pumps, and has role in expelling of antibiotics from the cell. By inhibiting these pumps, flavonoids can result in elevation of intracellular content of antibiotics, which effectively

reverse resistance. (thi huyen thu, ngoc, dang, mai huong "Secondary Metabolite-mediated Regulation of Efflux Pumps").

3. Biofilm Suppression: Biofilms can be defined as complex microbial communities that are inherently resistant to conventional antibiotics. Flavonoids such as quercetin and apigenin have been shown to inhibit biofilm formation by interfering with quorum sensing (QS) pathways. (Muzamil ahmad, Kuldeep gupta, manabendra mandal").

4. Enzyme Inhibition: Flavonoids can bind to and inhibit essential bacterial enzymes, such as DNA gyrase, ATP synthase and beta-lactamases. (andreja plaper, mojca golob, iva hafner").

Evidence of Metal Ion Binding and MBL Inhibition

25 It has been found that flavonoids play an important role in MBL inhibition and this is primarily linked to their ability to coordinate with the zinc ions in the enzyme's active site. (el sappah, zhu, qiulan"). Flavonoids contain multiple hydroxyl and carbonyl groups that can form stable complexes with divalent metal ions like Zn ion. Additionally, docking studies have suggested that specific flavonoids such as robinin and myricetin, possess high binding affinities for the active sites of carbapenemases and other Metallo-enzymes. Oktavia, praptiwi, andria agusta").

Tabular Representation of Flavonoid Subclasses and Their Biological Activity

Flavonoid Subclass	**Representative Compound**	**Biological Mechanism**	**Target Enzyme / Pathogen**
Flavonols	Quercetin, Kaempferol	DNA Gyrase Inhibition	*S. aureus, P. aeruginosa*
Flavones	Apigenin, Luteolin	Efflux Pump Inhibition	*E. coli*, MRSA
Flavanones	Naringenin, Eriodictyol	Biofilm Suppression	*P. aeruginosa*
Biflavonoids	Amentoflavone	Enzyme Inhibition (BET)	BRD4 Protease

Prior Docking and Inhibition Studies

Flavonoids can successfully target MBLs and this is confirmed by evidence from computational and in vitro studies. For example, it was seen in screening of Woodfordia fruticosa phytochemicals that several molecules are having higher binding affinities for OXA-23 than standard synthetic inhibitors like avibactam. (Shreya kappor, Navneeta bharadvaja"). Furthermore, docking analyses of Neem-derived compounds have highlighted the potential of quercetin and other flavonoids to show interaction to penicillin-binding proteins and other targets which are essential for synthesis of cell wall. As a result, the findings states the fact that flavonoids could act as potent natural scaffolds for the formation of a new generation of MBL inhibitors. (omorefosa IEEE Conference, "Neem Seed Methanolic Extract Scavenges Free Radicals").

2.7 Computational Docking in Drug Discovery

Molecular docking has emerged as a revolution in structure-based drug design (SBDD). It provides many advantages which includes it being high-throughput and cost-effective, which means identifying potential ligands for therapeutic targets becomes more feasible now. (arun dev sharma, Inderjeet kaur"Molecular Docking: The Transition from Micro to Macro World"). This method employing computational work predicts the feasible orientation and binding energy of a small molecule called ligand inside the active site of a target which is usually a protein. (Yiming zhang, ishitani, takemoto"). In the context of MBLs, docking allows for the rapid evaluation of thousands of natural compounds against specific clinical variants like VIM-2. (Rayhan Chowdhury, saima, md al amin").

Docking as a Predictive Tool for Binding Affinity and Pose

The efficacy of a docking simulation is determined by its ability to accurately model intermolecular interactions and estimate the binding free energy. This involves the use of scoring functions that account for various physical forces like van der Waals forces, hydrogen bonds and electrostatic interaction. (ze jun jia, xiao wei lan, kui lu"). High-resolution crystal structures of MBLs, such as VIM-2, provide the necessary templates for defining the active site architecture, which includes the conserved binuclear zinc cluster. (Supuran, "Updated Review on Metallo-beta-lactamases").

Applications in Screening Natural Products

Molecular Docking has been of great importance in screening natural product libraries, where the chemical diversity is too vast for empirical testing alone. (abdul nasir, Abdus samad, amar ajmal"). For instance, virtual screening workflows have been used to identify potent inhibitors for Zika virus protease and main protease of SARS-CoV-2. Additionally, in microbiology studies, docking is frequently used to theoretically verify the minimum inhibitory concentration (MIC) values of herbal extracts. (pooja Prakash mankar, archana Sudhir pethe").

AutoDock Vina as a Widely Used Platform

One of the most widely utilized docking programs is AutoDock Vina. This is due to its speed, accuracy and robust scoring function. It employs a sophisticated local search algorithm and a hybrid scoring function that has shown a significant correlation with experimental values across various protein families. (oleg trott, Arthur olson"). Moreover, Vina is particularly effective for screening natural compounds like flavonoids, this can due to the fact that it can handle the flexibility of these molecules well and could provide a realistic assessment of their binding potential. (Shreya kappor, Navneeta bharadvaja ").

Comparison of Molecular Docking Tools

Docking Tool	Search Algorithm	Primary Strength	Suitability for Metalloproteins
AutoDock Vina	Iterative Local Search	Speed and accuracy	Moderate to High
LeDock	Simulated Annealing	Clustering of poses	Moderate

Docking Tool	Search Algorithm	Primary Strength	Suitability for Metalloproteins
PLANTS	Metaheuristics	Side-chain flexibility	High
MetalProGNet	Deep Graph Model	Explicit metal-ligand modeling	Very High

Docking in the Context of Metalloproteins

Although Molecular Docking is of utmost importance but docking against metallo-enzymes like VIM-2 presents unique challenges. The reason is presence of the metal ions in active site within these enzymes that significantly alters the electronic landscape of the active site. (suleyman selim clnaroglu, emel timucin"). It has been also elucidated that Standard force fields often struggle to accurately model the coordination geometry of Zn ions. However, recent advancements, such as the development of metal-optimized scoring functions and deep graph models like MetalProGNet, have significantly improved the accuracy of docking predictions for metalloproteins. These tools allow for the explicit modelling of coordination interactions between metal ions and ligand atoms and thus facilitates the discovery of more potent MBL inhibitors. (kai wang").

2.8 Research Gap and Rationale for Current Study

Despite the critical need for effective MBL inhibitors in clinical setting, the current research landscape remains fragmented and less developed. The limitations of existing synthetic inhibitors, principally their lack of clinical success and associated toxicities, further highlights a significant failure in the current scenario of drug development pipeline. (Vila and González, "Updated Review on Metallo-beta-lactamases"). While the potential of plant-derived compounds has been recognized, yet there exists significant shortage of relevant studies which should be centring on the interaction between flavonoids and specific clinical MBL variants like VIM-2. (Shahrajabian et al., "Artemisia annua and potential natural medicine").

Limitations of Existing Synthetic Inhibitors

The clinical usage of synthetic MBL inhibitors is often compromised due to their poor pharmacokinetic profiles and their inability to invade outer layer in Gram-negative beings. (Pha et al., "Mechanistic Evaluation of Meropenem Potentiation"). Furthermore, it often goes like that many synthetic scaffolds are designed as broad-spectrum inhibitors which significantly fails to account for the subtle structural variations between VIM clusters or the specific active site environment of *Pseudomonas aeruginosa*. (Caitlyn rotondo, marrone, goodfellow"). As a result of the high failure rate of these compounds, it necessitates a transition toward natural product scaffolds that have evolved to conjugate with biological fashion. (Heinrich et al., "Ethnopharmacy and Drug Discovery").

Lack of Systematic Docking Studies on Flavonoids vs. VIM-2

While flavonoids are well-known for their antibacterial and metal-chelating properties, their potential as VIM-2 inhibitors remains largely unexplored in a systematic manner. Most existing studies focus on general antimicrobial activity or docking against common targets like DNA gyrase, which leaves a

significant knowledge gap regarding the specific binding modes and structural requirements for flavonoid-mediated MBL inhibition. (osemwighie"). Hence, there is a critical need for comprehensive virtual screening campaigns that must evaluate a broad range of flavonoid subclasses against VIM-2 to identify lead compounds with the highest therapeutic potential.

Justification for Evaluating Flavonoids as Natural Scaffolds

Evaluating flavonoids as natural scaffolds for MBL inhibition is justified by several factors including:

1. Safety and Bioavailability: Flavonoids are generally non-toxic to humans and are frequently found in common dietary sources. (mahaboob khan sulaiman"The Molecular Mechanisms and Therapeutic Potential of Flavonoids ").

2. Metal Affinity: They possess inherent ability to chelate Zn ions which makes them chemically suitable for MBL inhibition. (el sappah").
3. Multi-Target Capability: Their additional roles in biofilm suppression and efflux pump inhibition provide a synergistic advantage in treating multidrug-resistant infections. (putri, julaeha, Kagawa, kurnia").

Therefore, by employing AutoDock Vina to screen a curated library of flavonoids against VIM-2, this study aims to identify novel natural inhibitors that can overcome the limitations of synthetic compounds. (gazgalis, zaka, abbasi, mezei"). This research will provide a foundation for the development of alternative therapeutic strategies to combat the global crisis of AMR in *Pseudomonas aeruginosa*. (ghouch, schut, sigaloff").

3. METHODOLOGY

3.1. Retrieval of Target Protein Structure

RCSB Protein Data Bank (http://www.rcsb.org/) employed to derive 3-D assembly of VIM-2 MBL originating in Pseudomonas aeruginosa possessing PDB ID: 4C1E [16]. X-ray diffraction technique having 1.40 Å resolution was employed to obtain this PDB complex. This ensured that selected complex is of high quality and has complete active site with accurate representation of the zinc-binding environment. The active site of this structure is bound to reference inhibitor, D Captopril,which acted as a benchmarking ligand.

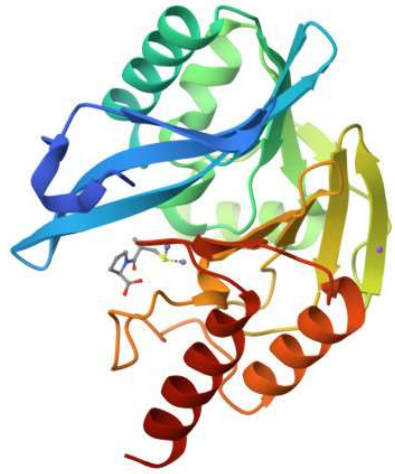

Fig.1. Crystal structure of the metallo-beta-lactamase VIM-2 with D-captopril

3.2. Preparation of Target Protein

The protein structure downloaded was in PDB format. The preparation of the structure was done using AutoDock Tools 1.5.7 (MGL Tools). The preparation resulted in elimination of water particles as well as unnecessary heteroatoms. Polar hydrogens and charges were added along with removal of the bound ligand while retaining zinc ions and important catalytic residues to preserve active site environment [17]. The prepared molecule files were downloaded with PDBQT format.

3.3. Selection of Ligands

1)Reference Selection: The literature reported stereoisomers of captopril, L-captopril and D-captopril as inhibitors of MBLs, including VIM-2 but D-captopril was comparatively more potent inhibitor and thus, it was chosen as the reference ligand [12].

2) Flavonoid Selection: Literature survey was done to identify candidate flavonoids with reported antimicrobial and resistance-modulating properties. ADME and Drug-likeness screening was (https://swissadme.ch/) done for using SwissADME ensuring Pharmacological feasibility. The major factors considered for this screening were Gastrointestinal (GI) absorption, Lipinski's Rule of Five, PAINS and Brenk alerts, and Ghose filter [18]. The flavonoids fulfilling these criteria were shortlisted and were further used for docking analysis.

3.4. Ligand Preparation

PubChem (https://pubchem.ncbi.nlm.nih.gov/) was utilised for downloading 3-D assemblies of selected ligands alongwith reference ligand D- captopril in SDF format [19]. The downloaded structures were changed into PDB format using UCSF Chimera 1.19. The PDB files were then prepared in AutoDock Tools 1.7. Under the influence of this tool, water molecules were eliminated. Furthermore, Polar hydrogens along with charges were also added. The files of prepared molecules

were finally downloaded in PDBQT format and proceeded for docking.

3.5. Molecular docking

Binding affinities of selected flavonoids was calculated against VIM-2, with D-captopril as the reference ligand using AutoDock Vina [20], [21]. A grid box with its centre placed at x = 78.04, y = 8.04, z = 56.35 was created with the proportions of 40 Å × 40 Å × 40 Å to include the active site. Separate output files for each ligand in PDBQT format along with a file containing binding affinities of all the ligands was generated as a result of docking.

3.6. Protein-ligand complex analysis

The protein–ligand interaction profiles were analysed using BIOVIA Discovery Studio 2025 Client, developed by Dassault Systèmes, BIOVIA [22]. This platform was used for the creation of 2-D and 3-D conformations of ligand and target interactions. The output file for ligand as well as target, both in PDBQT format, were imported into the software for analysis. Structural visualization was done for the reference ligand bound to the target protein, as well as for the shortlisted flavonoids followed by its extraction in both 2-D and 3-D formats. Interacting residues and bonds were major focus of this analysis.

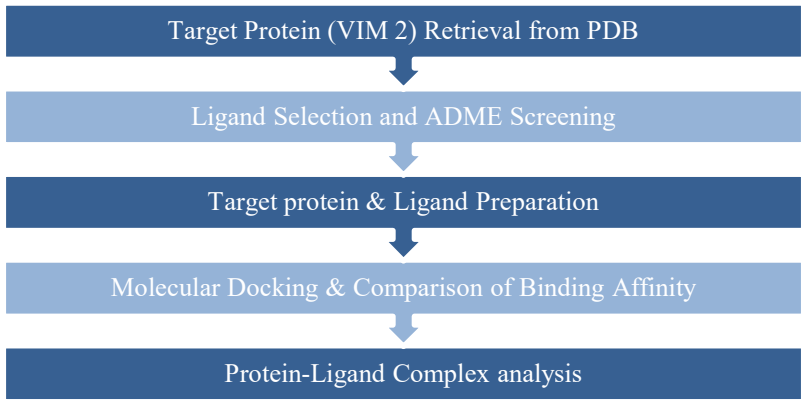

Fig.2. Workflow depicting methodology of molecular docking

4. RESULTS AND DISCUSSION

4.1. Initial screening and ADME analysis

30 flavonoids with reported antibacterial activity and potential relevance as MBL inhibitors were shortlisted through literature analysis. These compounds were further filtered through ADME analysis using certain filters like Gastrointestinal (GI) absorption, Lipinski's Rule of Five, Brenk alerts, PAINS filters, and Ghose criteria. This ADME analysis was performed with the help of SwissADME. Following this screening, 8 flavonoids with high GI absorption, no Lipinski violations, no Brenk alerts, no PAINS alert and no Ghose violation were selected and advanced for docking studies against VIM 2.

Table 1: Tabular Representation of Of Shortlisted Compounds

4.2. Docking analysis

AutoDock Vina was used for docking the 8 shortlisted flavonoids against VIM-2, with D-captopril as the reference ligand (–6.20 kcal/mol). Several flavonoids found to have more stable binding affinities lying from –7.6 to –8.1 kcal/mol, surpassing reference. The docking results for Compound 3, Apigenin (PubChem CID: 5280443), showed the highest binding energy, with a value of –8.1 kcal/mol. These insights illuminate capability of flavonoids as useful frameworks for developing inhibitors of MBLs (Table 1).

Table 2: Tabular Representation of 2D Chemical Structures, Binding Affinities and Interactions of Ligands

4.3. Visualization of docked ligands

BIOVIA Discovery Studio 2025 Client was used for the visualization of both 3D and 2D ligand–protein complex interactions. Interacting residues were observed for each of the 8 shortlisted flavonoids as well as the reference ligand, D- captopril (Table I). The 2D ligand interactions of D-Captopril with the target protein are displayed in Fig.1. Figs. 2–9 represents the 2-D interaction maps of the selected Eight flavonoids (Compounds 1–8) with the target protein, highlighting their binding residues and profiles.

Tabular Representation of PubChem CID, Binding Affinities and Interacting Residues of Ligands

Compound No.	PubChem CID	Binding Affinity (kcal/mol)	Interacting Residues
Reference (D- Captopril)	447055	-6.2	HIS179, TYR67, ARG205, PHE62, TRP87, ZN502, HIS240, ZN501, ASN210
Compound 1 (Hesperitin)	72281	-7.9	ASP117, ASP118, HIS116, ASN210, PHE62, TRP87
Compound 2 (Naringenin)	439246	-8.0	ASP117, ASP118, HIS116, PHE62, ASN210, TRP87
Compound 3 (Apigenin)	5280443	-8.1	ZN501, ASP117, ASP118, HIS116, TRP87, PHE62, ASN210
Compound 4 (Morin)	5281670	-7.9	ASP117, ASP118, HIS116, TRP87, PHE62, ASN210
Compound 5 (Kaempferol)	5280863	-7.7	ASP117, ASP118, HIS116, TRP87, PHE62, ASN210
Compound 6 (Genistein)	5280961	-7.9	HIS240, ASP118, TRP87, ZN502, HIS116, ASP117, ASN210, PHE62
Compound 7 (Daidzen)	5281708	-7.6	ASN210, PHE62, TRP87, ASP117, HIS116, ASP118
Compound 8 (Glycitein)	5317750	-7.8	TRP87, ASN210, PHE62, HIS116, ASP117, ASP118, HIS240

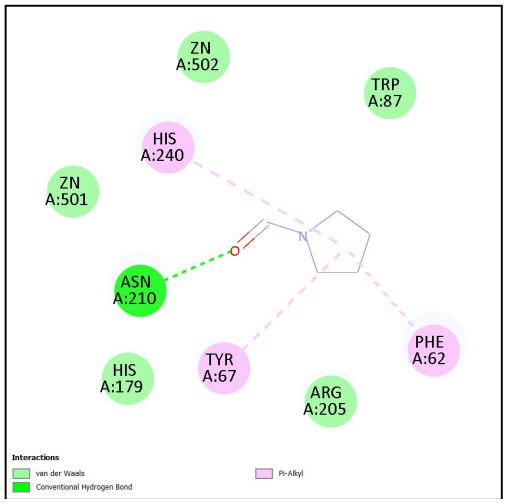

Fig.1. Interactions of D- Captopril

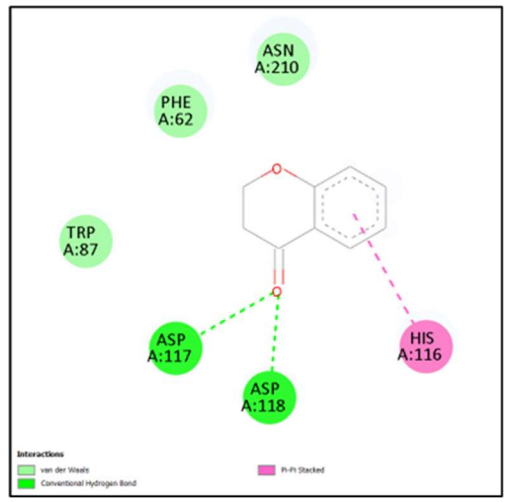

Fig.2. Interactions of Compound 1 (Hesperitin)

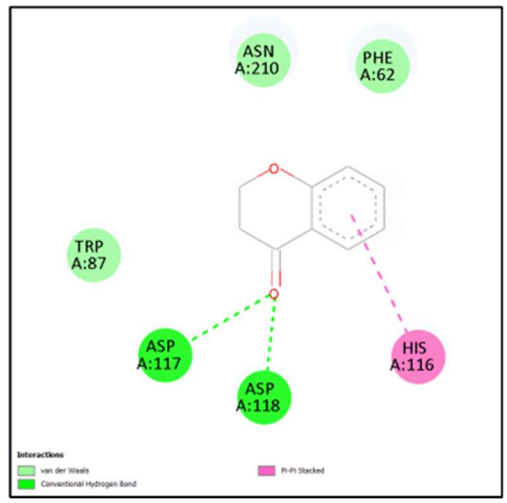

Fig.3. Interactions of Compound 2 (Naringenin)

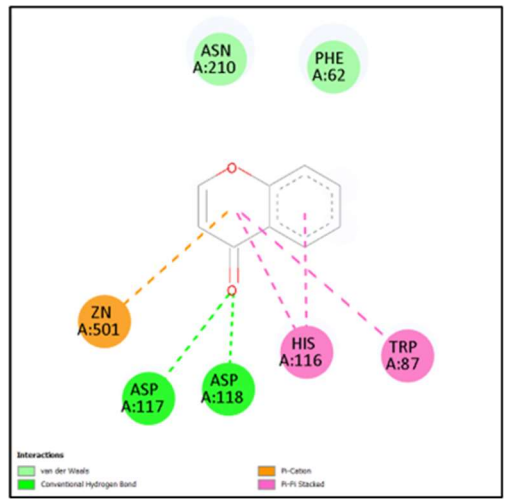

Fig.4. Interactions of Compound 3 (Apigenin)

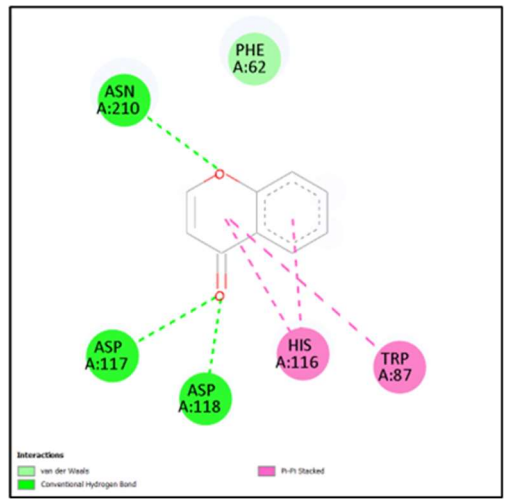

Fig.5. Interactions of Compound 4 (Morin)

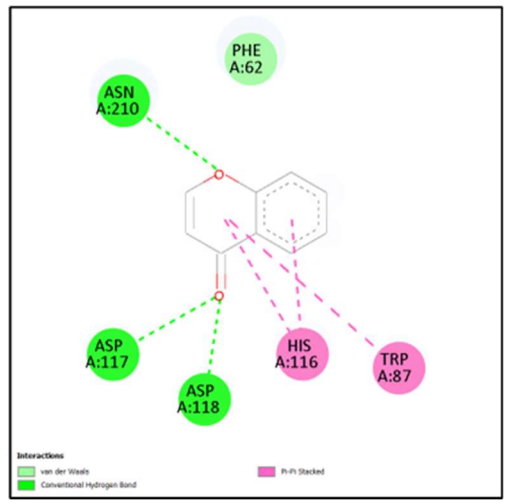

Fig.6. Interactions of Compound 5 (Kaempferol)

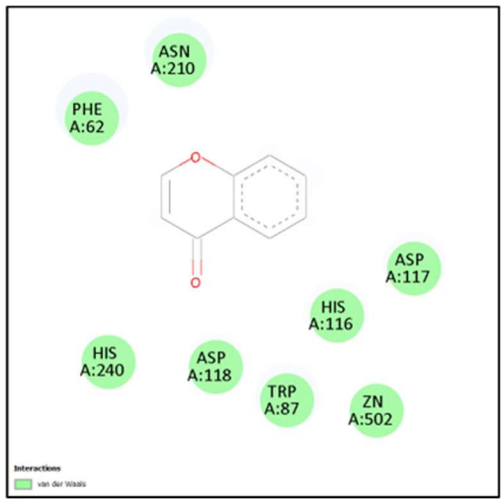

Fig.7. Interactions of Compound 6 (Genistein)

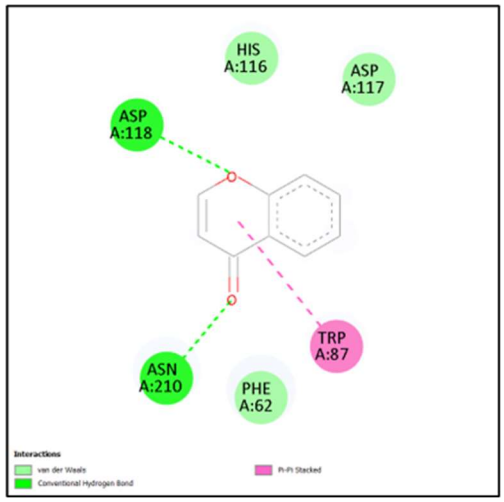

Fig.8. Interactions of Compound 7 (Daidzen)

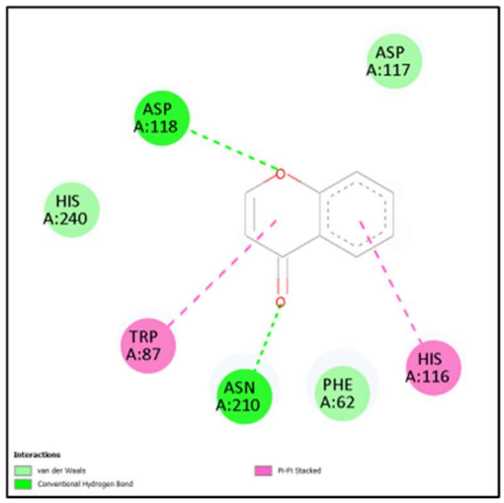

Fig.9. Interactions of Compound 8 (Glycitein)

4.4. Detailed ADME analysis

The results of SwissADME analysis for these 8 shortlisted flavonoids depicted favourable pharmacokinetic properties. All the selected flavonoids depicted high GI absorption, zero Lipinski violation, zero PAINS alerts, zero Brenk alerts and no Ghose violation supporting their drug-likeness behaviour. Further ADME evaluation utilised TPSA values, which were below 140 Å², indicating good membrane permeability, while consensus LogP values (–0.7 to +5.0) reflected balanced lipophilicity. Collectively, these results validate the shortlisted flavonoids for their feasibility in drug development (Table II).

Table 3. ADME Properties of Shortlisted Flavonoids

Compound No.	Gastro-Intestinal (GI) Absorption	Lipinski violation	TPSA Value (Å²)	Consensus logP
1	High	0	96.22	1.91
2	High	0	86.99	1.84
3	High	0	90.90	2.11
4	High	0	131.36	1.20
5	High	0	111.13	1.58
6	High	0	90.90	2.04
7	High	0	70.67	2.24
8	High	0	79.90	2.30

5. CONCLUSION

This study was performed to explore the potential of naturally occurring flavonoids as inhibitory scaffolds against Verona Integron-encoded Metallo-β-Lactamase-2 (VIM-2) from Pseudomonas aeruginosa, clinically critical resistance pathogen for which no approved clinical inhibitor currently exists. The work combined ADME-guided compound selection with structure-based molecular docking using AutoDock Vina against the high-resolution VIM-2 crystal structure (PDB: 4C1E). The study evaluated eight structurally different flavonoids which was benchmarked against the established thiol-based reference inhibitor called D-captopril.

It was seen that all eight flavonoids which were subjected to docking, demonstrated binding affinities superior to the D-captopril reference (–6.20 kcal/mol), with scores lying from –7.6 kcal/mol (Daidzein) to –8.1 kcal/mol (Apigenin). Importantly, visualisation of protein–ligand complexes using BIOVIA Discovery Studio further confirmed that the flavonoids engage the catalytically essential residues of the VIM-2 active site including ASP117, ASP118, HIS116, TRP87, PHE62, and ASN210, through hydrogen bonding and hydrophobic contacts, with compounds such as Apigenin and Genistein additionally coordinating the catalytic zinc ions ZN501 and ZN502, respectively. This zinc coordination is mechanistically significant, due to the fact that it directly mirrors the pharmacophoric strategy employed by established synthetic MBL inhibitors and is consistent with the metal-chelating properties of the flavonoid hydroxyl groups which was identified in the literature review. Complementing the docking results, SwissADME profiling confirmed that all eight candidates possess favourable drug-likeness, with high gastrointestinal absorption, zero Lipinski violations, TPSA values below 140 $Å^2$ and balanced lipophilicity profiles, collectively supporting their pharmacological feasibility as orally administrable agents.

Altogether, these findings establish a robust computational foundation for the repositioning of plant-derived flavonoids as novel MBL inhibitor candidates and it also expands the chemical space available for therapeutic development beyond the thiol-based scaffolds that have dominated the field. The results validate the in-silico screening workflow employed and provide a prioritised list of candidates, with Apigenin, Naringenin, Genistein and Hesperetin among the most promising for progression to experimental validation.

Despite of being this success, the limitations of docking studies must be acknowledged. Molecular docking is limited because it does not account for protein flexibility, explicit solvation or entropic contributions to binding. Furthermore, the predicted affinities may not fully translate to biological activity under physiological conditions due to different Pharmacodynamic and Pharmacokinetics profiles.Thus, Future researches must focus on molecular dynamics simulations which is used to assess the stability of predicted binding poses over time followed by in vitro assays, including IC_{50} determination and zinc-competitive inhibition kinetics and ultimately whole-cell susceptibility testing in VIM-2-producing P. aeruginosa present in clinical settings to confirm the desensitisation within carbapenem resistant strains. These candidates would advance towards pre-clinical development when Structure-based optimisation was done to improve zinc-binding affinity and metabolic stability. This study thus contributes a scientifically grounded and translationally relevant starting point in the search for effective inhibitors against one of the most urgent unmet challenges in antimicrobial pharmacotherapy.

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