"*IN-SLICO* DISCOVERY OF VIM-2 METALLO-β-LACTAMASE FOR *PSEUDOMONAS AERUGINOSA*"

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Dissertation

Submitted to the

Rajiv Gandhi University of Health Sciences, Bangalore, Karnataka

In partial fulfillment of the requirements for the degree of

Bachelor of Pharmacy

Under the guidance of

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2023

RAJIV GANDHI UNIVERSITY OF HEALTH SCIENCES

BANGALORE, KARNATAKA

DECLARATION BY THE CANDIDATES

We hereby declare that this dissertation entitled **"*IN-SILICO* DISCOVERY OF VIM-2 METALLO-β-LACTAMASE FOR PSEUDOMONAS AERUGINOSA"** is a bonafide and genuine research work carried out by us under the guidance of Mrs. Poonam Saxena, Associate Professor Department of Pharmaceutical Chemistry, Mallige College of Pharmacy, Bangalore-90

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ACKNOWLEDGEMENT

It is a great pleasure to utilize this unique opportunity to express my deep sense of gratitude and humble regards to all the people whoever helped, motivated and appreciated work with their objective ideas and opinion and who have directly or indirectly helped us to accomplish our project.

We are grateful to acknowledge and express our sincere thanks to our beloved Principal **Dr. Shivakumar Swamy**, vice Principal **Dr. Rajendra Prasad.** S. V Mallige College of Pharmacy, for his valuable suggestions, moral support and for providing necessary infrastructure and resources to accomplish our research work.

On the eve of presenting this dissertation, we take this as a unique opportunity to record our deep sense of gratitude to our guide **Mrs. Poonam Saxena** Department of Pharmaceutical Chemistry, for her patience, motivation, constant encouragement, that framed foundation for this project. We are extremely thankful to our friends for encouragement and support in project work. We would like to express our sincere thanks to teaching and non-teaching staffs Mallige College of Pharmacy for their support and blessings. Finally, we express our sincere thanks to everyone who helped us directly and indirectly in completing our dissertation work successfully.

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Thanks to one and all

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LIST OF ABBREVATION

ABBREVATION	FULL FORMS
MDR	Multidrug resistant
AMR	Antimicrobial resistance
WHO	World Health Organization
MBL	Metallo-beta-lactamase
CF	Cystic Fibrosis
IV	Intravenous
VIM-2	Verona Integron Encoded Metallo- β-lactamase
NDM	New Delhi Metallo- β-lactamase
MPPA	MBL Producing Pseudomonas aeruginosa
HTS	High throughput Screening
CADD	Computer Aided Drug Discovery
ABDD	Analog based Drug Design
SBDD	Structure Based Drug Design
P. aeruginosa	Pseudomonas aeruginosa
3D	3 Dimensional
ADMET	Absorption, Distribution, Metabolism, Excretion, Toxicity
Et al	And others
Zn	Zinc
CRPA	Carbapenems Producing Pseudomonas aeruginosa
RA	Roasmarinic acid
ITC	Isothermal Titration calorimetry
IFP	Interaction Fingerprinting
CDC	Center for Disease Control
CRE	Carbapenem Resistance enterobacteriaceae
PDB	Protein Data Bank
RO5	Lipinski's rule of five
PAINS	Pan assay interference compounds

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ABSTRACT:

One of the most common causes of acute nosocomial infections, such as nosocomial pneumonia or bacteremia, especially affecting immune-compromised individuals, is Pseudomonas aeruginosa. An appealing pharmacological target for the treatment of beta-lactam- resistant infections is VIM-2, resistant infections is VIM-2, β-lactamase that has a broad spectrum of substrates in penicillin, cephalosporin, cephamycin, and carbapenems. Using PyRx, 5 standards were found and docked with the VIM-2 protein. Standard indole was chosen among these as the best compound, and a large number of indole compounds were virtually screened. Based on Binding scores 60 compounds were selected and out of 60 compounds only 5% i.e 30 compounds were selected for ADMET studies. Selected top 10 best hits compounds, Docking studies were conducted for the selected 11 compound showing top hits and compound **N'-hydroxy-1H-indole-4-carboximidamide** (CID-59214189) docked with protein VIM-2 have best potential against infection caused by *pseudomonas aeruginosa* producing VIM-2 strains based on ADMET properties and binding energies.

Using the available *in-silico* tools in present research work an attempt made to develop inhibitors for VIM-2 β-metallo lactamase of *P. aereguinosa*

INTRODUCTION

Antibacterial Resistance:

One of the major dangers to human health, food security, and development is antibacterial resistance. Any person, regardless of age or location, can be impacted with antibacterial resistance. Antibiotics are drugs that are employed in the treatment and prevention of bacterial illnesses. Usage of specific medications they arise as a result in bacterial resistance to the, Bacteria that develops defense mechanisms against the effects of antibiotics, which leads to the development of antibacterial resistance.[1] MDR (multidrug-resistant) microorganisms are immune to a wide range of antibiotics. Antibiotic resistance is a subset of AMR (Antimicrobial Resistance), which refers to microorganism that develops antibiotic resistance. All around the world, antibiotic resistance is increasing to alarming heights. We are facing a threat to our ability to treat common infectious diseases when new resistance mechanisms emerge and proliferate internationally. The WHO has recommended medical practitioners to use less medications and lower the likelihood of antibiotic resistance in bacterial illnesses since 2001.[2] In accordance with the Center for Disease Dynamics, Economics and Policy, "antibiotic resistance is a direct result of antibiotic use." Antibiotic use has decreased recently in Europe, Canada, and the United States, while it has increased recently in India, Sub-Saharan Africa, Latin America, and Australia. Antibiotic usage in agriculture is still a cause for worry, since it could result in human exposure through food and environmental contamination even as medical use of antibiotics declines.[3] The advancements of contemporary medicine are at risk due to antibiotic resistance. Without efficient antibiotics for the prevention and treatment of infections, procedures like organ transplants, chemotherapy, and caesarean deliveries become significantly

riskier. It is more difficult to cure bacterial resistant germs since they require higher doses or perhaps more hazardous alternative therapies. These methods could potentially cost more money.[4]

Antibacterial Resistance Mechanisms:

Intrinsic Resistance

The occurrence of genes in the bacterial genome that encode inherent characteristics of cell structures and composition that offer protection against harmful chemicals and antimicrobials gives rise to intrinsic resistance mechanisms.

Boosted Antibiotic Resistance via Mutations

The unleashing of gene expression and the excessive creation of protein products like AmpC and multi-drug efflux pumps systems may come from mutations in the regulatory pathway that increase promoter activity. Because of this, there is increased antibiotic resistance.

Plasmid-Mediated Resistance

Bacterial plasmids have a crucial role as a highly effective means of obtaining resistance genes and subsequently delivering them to recipient hosts. This process, known as horizontal gene transfer, allows for the exchange of genetic materials, primarily by conjugation, between bacterial cells. Some resistant plasmids have a wide host range and can be transferred between different species via bacterial conjugation, whereas plasmids with a narrow host range can only be transferred between a small number of cells from a single bacterial species. In most of the Gram-negative bacteria plasmid RP1 can transfer resistance gene.

Adaptive Resistance

Adaptive resistance is a brittle and temporary type of resistance that is brought on by a particular antibiotic and other environmental stimuli. This kind of resistance mostly depends on induced changes in gene expression, protein synthesis, or antibiotic targets, and it is reversible with removal of external stimuli, resulting to regaining susceptibility. This pathway has been shown mediating the resistance of *P. aeruginosa* but not shown mediating the resistance to β-lactams, aminoglycosides, polymyxins, and fluoroquinolone[5]

Pseudomonas aeruginosa

One of the most common causes of acute nosocomial infections, such as nosocomial pneumonia or bacteremia, especially affecting immune-compromised individuals, is *Pseudomonas aeruginosa*. There are few therapeutic treatment options available due to *P. aeruginosa* has high intrinsic resistance levels and enormous capacity for acquiring new antibiotic resistance mechanisms. Each year, bacterial infections caused by antibiotic resistance claimed the lives of up to 700,000.[6] The overall resistance of the *P. aeruginosa* isolated from European populations was 12.9% *P. aeruginosa* related infections picked up from hospitals continue to result in resistance to commonly prescribed medicines, which is a major healthcare issue. Escherichia coli and *P. aeruginosa* are the most frequent causes of hospital-acquired infections in Spain, according to the 2016 EPINE survey.[7] There is a significant increase in morbidity and death. The most significant carbapenem's that *P. aeruginosa* has acquired resistance are metallo-beta-lactamases (MBL), which inhibits extended-

spectrum cephalosporins and carbapenems but not aztreonam. EDTA also inhibits MBL. The need of monitoring *Pseudomonas aeruginosa in vitro* susceptibility trends in cystic fibrosis (CF) patients. Large variability in antimicrobial resistance rates were seen at their center, although these were not always associated with antimicrobial exposure.[8] For CF [Cystic fibrosis] patients with *P.aeruginosa*, there are two basic therapeutic approaches: elective intravenous (i.v.) antibiotics given on a regular schedule regardless of symptoms, and the second one is the use of rescue antibiotics given only when patients are exhibiting symptoms. The prevalence of resistant P. aeruginosa has been found to be rising in CF patients, and studies have linked this rise to prolonged antibiotic use, monotherapy regimens, and a lack of cross-infection prevention guidelines.[9] Antibiotics are typically used to treat infections with *Pseudomonas aeruginosa*. Due to rising antibiotic resistance, *Pseudomonas aeruginosa* infections are regrettably getting harder to treat in persons who are exposed to healthcare environments like hospitals or nursing homes. The doctor or other healthcare professional will decide which antibiotic to prescribe depending on the antibiotic's activity and other considerations, such as any possible adverse effects or drug interactions. options for some *Pseudomonas aeruginosa* strains with multidrug resistance may be scarce.[10] The two types of *P. aeruginosa* isolates that are most frequently found are VIM [Verona integron encoded metallo-β-lactamase], and NDM [New Delhi metallo-β-lactamase], which are frequently linked to the high- risk clones ST111, ST175 or ST235.[11]

VIM- 2 [Verano integron encoded metallo-β-lactamase]

P. aeruginosa was first associated with the metallo-β- lactamase gene VIM-2 in France in 2000, the earliest known instance occurred in Portugal in 1995.[12] Later, VIM-2 was discovered in *Pseudomonas* species. An appealing pharmacological target for the treatment of beta-lactam- resistant infections is VIM-2, resistant infections is VIM-2, β-lactamase that has a broad spectrum of substrates in penicillin, cephalosporin, cefamycin, and carbapenems. Numerous Enterobacteriaceae, Acinetobacter spp., and *Pseudomonas* spp. isolated from clinical, animal, and environmental samples have been found to have VIM-type MBLs. VIM-2 is a subclass B1 MBL with $Zn2^{+/-}$ dependent activity.[13] It is monomeric and has a molecular weight of 25515 Da. VIM-2 has been one of the most frequently reported MBLs globally since the early 2000s, making it the main target for the development of MBL inhibitors. Subsequently, at least 71 VIM variations were found in various nations. VIM-2 possess to be the differential carbohydrate antigens. Among *P. aeruginosa*, VIM-2 developed as the main MBL variant, and *P. aeruginosa* that produces VIM-2 MBL is one of the main cause of nosocomial epidemics and clonal spread .[14] The most prevalent MBLs in P. aeruginosa are Verona Integron-encoded MBLs (VIM). In the United States, there have only been 57 reports of VIM production in *Enterobacteriaceae*, making it a rather uncommon occurrence. Increased mortality may be brought on by carbapenem antibiotic resistance, according to studies on diverse Gram-negative bacteria. One of the two most prevalent MBL-encoding integrons, between 2005 and 2015. MPPA significantly increased in the country during this time. The germs were found in 33 cities and 49 hospitals throughout 11/16 major administrative districts.[15]

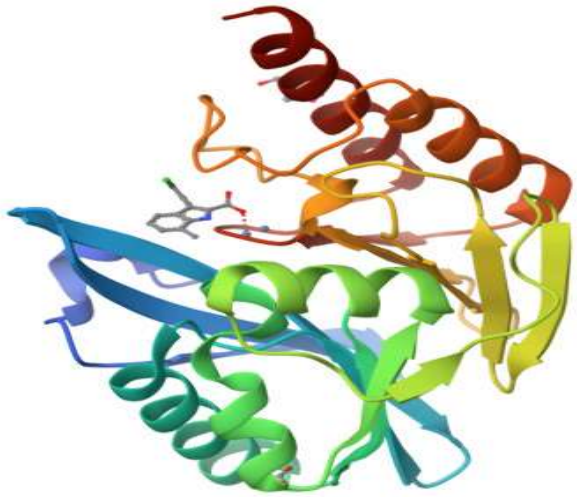


Fig 1: Ribbon Representation of the Superimposition of VIM-2

IN-SILICO DRUG DISCOVERY:

The methods of conventional drug discovery and development, which involve target identification and validation, lead molecule discovery and optimization, and preclinical and clinical trials, are dangerous and time-consuming. Over the past few years, the anticipated price of introducing a new medicine to About $1.8 billion USD has been spent on the market and the attrition rate of as many as 96% of them are drug candidates. The factors causing this high Poor drug effectiveness and inadequate drug absorption, distribution, metabolism, and excretion (ADME-Tox) contribute to a high attrition rate. Usually, in vitro and in vivo methods are used to evaluate drug safety, including toxicology and negative effects. New developments in the use of vitro models, including organ-on-chip technologies, has increased ADME-Tox evaluations. By continuing usage of these method there is labor-intensive, costly, and time-

consuming. *In silico* is a modern term that generally refers to computer-assisted testing. It is connected to the more well-known biology terms in vivo and in vitro. Trial-and-error drug discovery has historically been used. High-throughput screening techniques (HTS) have been created to speed up the identification of chemical compounds that are pharmacologically effective from a variety of by means of automated assays. Nevertheless, automatic HTS systems decrease the need for human intervention, while keeping HTS at minimal scale. The variety of chemical structure. Additionally, automated Instruments continue to be costly.[16] In silico drug design starts with understanding of specific chemical responses in the body or target organism and designing combinations of these to fit a therapy profile, as opposed to testing chemical substances on animals and comparing the apparent effects to therapies. Since they can lessen the scale, time, and expense issues that traditional experimental approaches confront, computer-aided drug discovery (CADD) methods are gaining popularity. CADD encompasses computational drug target identification, virtual drug candidate selection from huge chemical libraries, further optimization of candidate compounds, and *in silico* evaluation of their potential toxicity.

TYPES OF DRUG DESIGN:

Drug design can be divided into two categories:

- Structure-based drug design.
- Analog based drug design.

Analog based drug design (ABDD)

The biopharmaceutical sector of the pharmaceutical industry uses a procedure called analog based drug design to find and create novel therapeutic molecules. To find novel compounds, design compounds for selectivity, efficacy, and safety, and create molecules into clinical trial candidates, ABDD employs a number of computational techniques. Depending on how much information is available regarding drug targets and prospective therapeutic molecules, these methodologies fall into a number of natural categories, including structure-based drug design, ligand-based drug design, de novo design, and homology modelling.

Structure based drug design (SBDD)

One of the very earliest approaches in drug design is structure-based drug design. Drug targets are frequently important molecules that take part in a certain metabolic or cell signalling pathway that is known to be or is thought to be connected to a particular disease state. The most frequent targets for drugs in these pathways are the proteins and enzymes. The structure and behaviour of disease-related proteins and enzymes are intended to be changed by drug molecules through inhibition, restoration, or other means. In order to help in the creation of new therapeutic molecules, SBDD makes advantage of the 3D geometrical shape or structure of proteins.[17]

AIM: *in-silico* discovery of VIM-2 metallo-β-lactamase inhibitor for *pseudomonas aeruginosa.*

OBJECTIVES:

- To validate the docking model.
- To carry out structure-based virtual screening
- To Evaluate ADMET properties of best molecules obtained.
- To perform docking studies for top hits post virtual screening.

REVIEW OF LITERATURE

- **Yoshihiro Yamaguchi *et al.,* (2007)** conducted crystallographic Investigation of the Inhibition Mode of a VIM-2 Metallo-lactamase from *Pseudomonas aeruginosa* by Mercapto carboxylate Inhibitor. In this study, they determined the crystal structure of the VIM-2 enzyme complexed with phenylC3SH in order to elucidate the detailed binding mode of the inhibitor with the enzyme, in particular, focusing on the role of the mobile loop, along with a comparison of the native VIM-2 structure Overall Structure of the VIM-2 Enzyme Complexed with PhenylC3SH. Crystallization of the VIM-2 Enzyme Complexed with PhenylC3SH. Prior to the X-ray diffraction experiments, a buffer solution of the VIM-2 enzyme was converted from Tris-HCl (50 mM, pH 7.4, 0.5 M NaCl) to HEPES-NaOH (20 mM, pH 7.5) and the VIM-2 protein was then concentrated to about 5 mg/mL (160 μM) on a Centricon. Drops of the VIM-2 protein with phenylC3SH were prepared by mixing 2 μL of a concentrated protein solution, 2 μL of a reservoir solution (30% PEG MME5000, 0.1 M MES-NaOH, and 0.2 M ammonium sulfate (pH 6.5)), and 1 μL of a methanolic phenylC3SH solution (10 mM). The crystals were grown for two months as plates (0.4 mm × 0.4 mm × 0.2 mm) at 20 C°.[18]

- **G.M. Rossolini *et al.,* (2008)** conducted Three-Dimensional Structure of VIM-2, a Zn-β-Lactamase from *Pseudomonas aeruginosa* in Its Reduced and Oxidised Form. The crystal structures of the universally widespread metallo-β-lactamase (MBL) Verona integron-encoded MBL (VIM)-2 from *Pseudomonas aeruginosa* have been solved in their native form as well

as in an unexpected oxidised form. This carbapenem-hydrolysing enzyme belongs to the so-called B1 subfamily of MBLs and shares the folding of αβ/βα sandwich, consisting of a core of β-sheet surrounded by α-helices.[19]

- **Dmitriy Minond *et al.,* (2009)** conducted Inhibitors of VIM-2 by screening pharmacologically active and click-chemistry compound libraries. VIM-2 is an Ambler class B metallo-b-lactamase (MBL) capable of hydrolyzing a broad-spectrum of b-lactam antibiotics. Although the discovery and development of MBL inhibitors continue to be an area of active research, an array of potent, small molecule inhibitors is yet to be fully characterized for VIM-2. In the pre-sented research, a compound library screening approach was used to identify and characterize VIM-2 inhibitors from a library of pharmacologically active compounds as well as a focused 'click' chemistry library. The four most potent VIM-2 inhibitors resulting from a VIM-2 screen were characterized by kinetic studies in order to determine Ki and mechanism of enzyme inhibition. As a result, two previously described pharma cologic agents, mitoxantrone and Anthra cenedione) and 4-chloromercuribenzoic acid (pCMB)were found to be active, the former as a non-competitive inhibitor and the latter as a slowly reversible or irreversible inhibitor.[20]

- **Patricia Lassaux *et al.,* (2011)** Conducted Biochemical and Structural Characterization of the Subclass B1 Metallo β-Lactamase VIM-4. The

metallo--lactamase VIM-4, mainly found in Pseudomonas aeruginosa or *Acinetobacter baumannii,* was produced in Escherichia coli and characterized by biochemical and X-ray techniques. A detailed kinetic study performed in the presence of Zn^{2+} at concentrations ranging from 0.4 to 100 μM showed that VIM-4 exhibits a kinetic profile similar to the profiles of VIM-2 and VIM-1. However, VIM-4 is more active than VIM-1 against benzylpenicillin, cephalothin, nitrocefin, and imipenem and is less active than VIM-2 against ampicillin and meropenem.[21]

- **Cal Ham *et al.,* (2015-2018)** Carried Verona Integron-Encoded Metallo-β-Lactamase– Producing Carbapenem-Resistant *Pseudomonas aeruginosa* Infections in U.S. Residents Associated with Invasive Medical Procedures in Mexico. This investigation also underscores the importance of testing for the presence of carbapenemases in carbapenem-resistant *P. aeruginosa*. In the United States, carbapenemases are less frequently the cause of carbapenem-resistance in *P. aeruginosa* than they are in carbapenem-resistant Enterobacteriaceae. Because bacteria like *P. aeruginosa* can potentially harbour carbapenemase-producing genes, which are able to transfer antibiotic resistance to other organisms, early detection of carbapenemase-producing CRPA and associated public health responses might prevent spread of these resistant organisms. Clinical laboratories with capacity for carbapenemase testing should consider testing for both CRPA and CRE. For any patients infected or colonized with carbapenemase producing organisms, CDC

recommends implementation of infection control precautions to limit potential spread.[22]

- **Michel Sanner *et al.,* (2016)** conducted **c**omputational protein-ligand docking and virtual drug screening with the AutoDock suite. Computational docking can be used to predict bound conformations and free energies of binding for small molecule ligands to macromolecular targets. Docking is widely used for the study of biomolecular interactions and mechanisms, and is applied to structure-based drug design. The methods are fast enough to allow virtual screening of ligand libraries containing tens of thousands of compounds.[23]

- **Tony Christopeit *et al.,* (2016)** the study was conducted about The structure of the metallo β-lactamaseVIM-2 in complex with a triazolylthioacetamide inhibitor In this study, compound 1 was identified as an inhibitor of the MBL VIM-2. The determined IC50 was in the low-micromolar range and hence needs to be further optimized. However, compound 1 has already been reported as an inhibitor of the MBLs, and therefore is an interesting starting point for the development of broad-spectrum MBL inhibitors. Furthermore, the crystal structure of compound 1 in complex with VIM-2 is presented. To our knowledge, this is the first structure of a triazolylthioacet-amide inhibitor bound to an MBL.[24]

- **Zhu-Jun Yu *et al.,* (2018)** conducted Virtual target screening reveals rosmarinic acid and salvianolic acid A inhibiting metallo- and serine-b-

lactamases. Rosmarinic acid (RA), a polyphenolic phytochemical, has broad-spectrum biological and pharmacological activity. A virtual target screening method termed IFP Target combined with enzyme inhibition assays led to the identification of the clinically relevant metallo-b-lactamase (MBL) VIM-2 as one of unexploited targets of RA. The enzyme kinetic studies indicated that RA is a fully reversible, substrate-competitive VIM-2 inhibitor. The isothermal titration calorimetry (ITC) analyses revealed that the initial binding of RA to VIM-2 is mainly due to enthalpy contribution.[25]

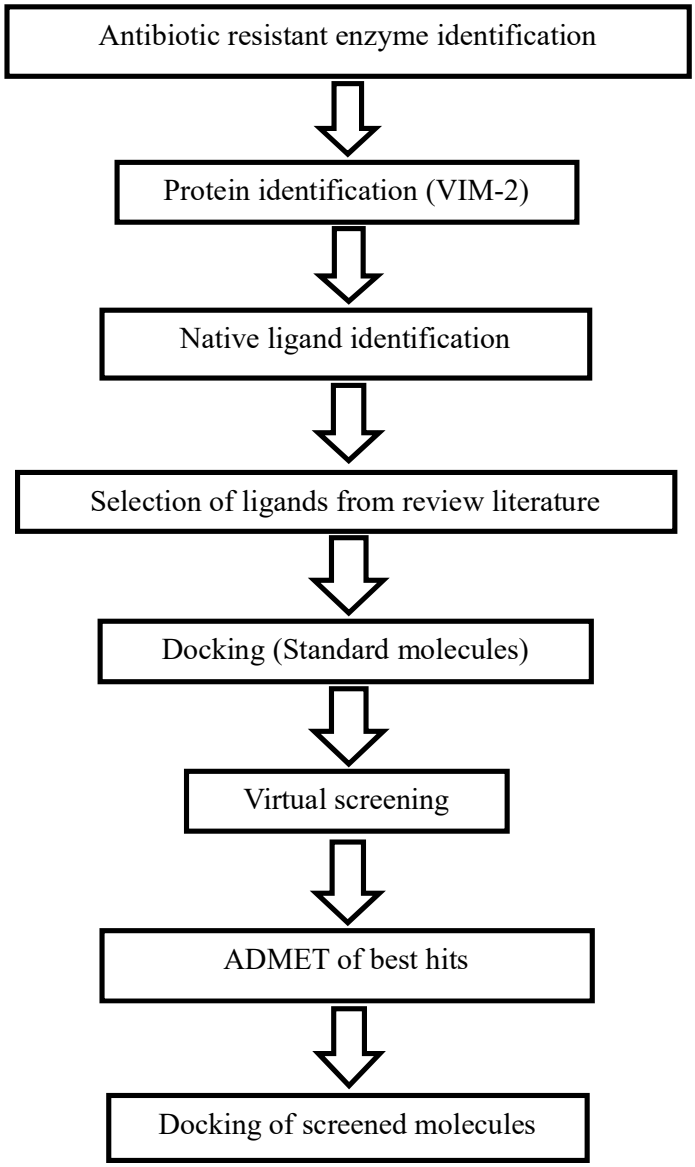
- **Zeeshan Muhammad *et al.*, (2020)** conducted Structural studies of triazole inhibitors with promising inhibitor effects against antibiotic resistance metallo-β-lactamases. Metallo-β-lactamases (MBLs) are an emerging cause of bacterial antibiotic resistance by hydrolysing all classes of β-lactams except monobactams, and the MBLs are not inhibited by clinically available serine-β-lactamase inhibitors. Two of the most commonly encountered MBLs in clinical isolates worldwide – the New Delhi metallo-β-lactamase (NDM-1) and the Verona integron-encoded metallo-β-lactamase (VIM-2).[26]

- **Mohammad Ezati *et al.*, (2023)** Identification of novel metallo-β-lactamases inhibitors using ligand-based pharmacophore modelling and structure-based virtual screening. Metallo-β-lactamases (MBLs) are a group of enzymes that hydrolyze the most commonly used β-lactam-based antibiotics, leading to the

development of multi-drug resistance. The three main clinically relevant groups of these enzymes are IMP, VIM, and NDM. This study aims to introduce potent novel overlapped candidates from a zinc database retrieved from the 200,583-member natural library against the active sites of IMP-1, VIM-2, and NDM-1 through a straightforward computational workflow using virtual screening approaches. The screening pipeline started by assessing Lipinski's rule of five (RO5), drug-likeness, and pan-assay interference compounds (PAINS) which were used to generate a pharmacophore model using D-captopril as a standard inhibitor. [2]

METHODOLOGY

The workflow of *In-silico* discovery Research work



Flow chart 1: work flow of *in-silico* discovery

Databases and Software employed

- RCSB pdb database:(https://www.rcsb.org/)
- Pubchem database:(https://pubchem.ncbi.nlm.nih.gov/)
- Biovia Drug discovery studio visualizer(v21.1.0)
- Autodock tools (v1.5.6)
- Autodock vina
- PyRx
- Arguslab docking tool
- Protox-II- online toxicity predictor
- ADMETlab 2.0 ADMET Predictor
- OpenBabel(v2.3.2)

Target identification

The target protein crystal structure of metallo-β-lactamase VIM-2 (7afx), was chosen after examined the PDB database for the VIM-2 antibiotic resistance protein. The native ligand, 3-(2-chlorophenyl)-7-methyl-1-H-indole-2-carboxylic acid was identified in 7afx protein from the PDB database. And using the interactions that were found in the Biovia Drug Discovery Studio visualizer (v21.1.0), then searched for 3D ligand interactions and found the active site. The residues identified as being in the 7AFX active site were PHE A:62, TYR A:67, TRP A:87, HIS A:179, ARG A:205, ASN A:210, and HIS A:240.

Docking Validation

The three-dimensional structure of 3-(2-chlorophenyl)-7-methyl-1H-indole-2-

carboxylic acid was downloaded in sdf format from PubChem. PyRx was used to associate this ligand with the active site. By the outcome attained, it was determined that the ligand's binding energy using PyRx, and interactions were seen in the Biovia Drug Discovery Studio visualizer (v21.1.0). Afterward, compared the new interaction to the pre-existing interaction, and found that both were identical. This verified the docking software was appropriate

STEPS INVOLVED IN DOCKING

1) **Retrieving required Target and Ligand from major database**

- **Retrieving Target from protein database (PDB)**
  a. 7afx protein downloaded from PDB database
  b. Using Autodock tools (v1.5.6), Target was purified and prepared for Docking.
  c. 7afx protein was imported in Autodock tool.
- **Modification of 7afx protein:**
- Using the Autodock tool's edit option-removal of water molecule from 7afx protein was done.
- Addition of hydrogen and for merging Non polar bond, autodock tool's edit menu was used.
- In the Autodock tool's edit menu, kollman charge and compute gasteiger have been added and atoms are assigned into AD4 type.
- Modified target were saved, in PDBQT format.[28]

- **Retrieving ligand from PubChem**

  Program for virtual screening that can be used in computational drug discovery to check libraries of compounds against possible therapeutic targets. Pharmaceutical Chemists can execute Virtual Screening using PyRx from any platform, and the software supports users at every stage of the procedure, from data preparation through job submission and outcome analysis. PyRx is a useful tool for computer-aided drug design even though there isn't a "magic button" in the drug discovery process because it has a docking wizard and an intuitive user interface.

  Search ligand in PubChem and downloaded in sdf format.

Using PyRx software- ligand were modified for docking

- Native ligand imported in sdf format using pyrx's inbuilt open babel
- Energy of ligand were reduced and converted into pdbqt format.[28]

2) Docking

PyRX were used for conducting docking studies for selected ligand and protein.

- Add produced PDBQT protein format macromolecules.
- Add a pdbqt-formatted purified ligand.
- Choose the active site, then reposition the grid so that it contains all of the active residue and then begin docking.
- Occasionally, binding scores are obtained and saved as a csv file.

- A file that is beneficial for analysing the results of the analysis will be produced and placed in the working folder.[29]

3) Result Analysis

- Biovia Drug discovery studio visualizer (v21.1.0) was used to observe the interactions.
- Make sure all Autodock Vina programs are in the same folder by opening the working folder.
- To split the outfile, use the command prompt.

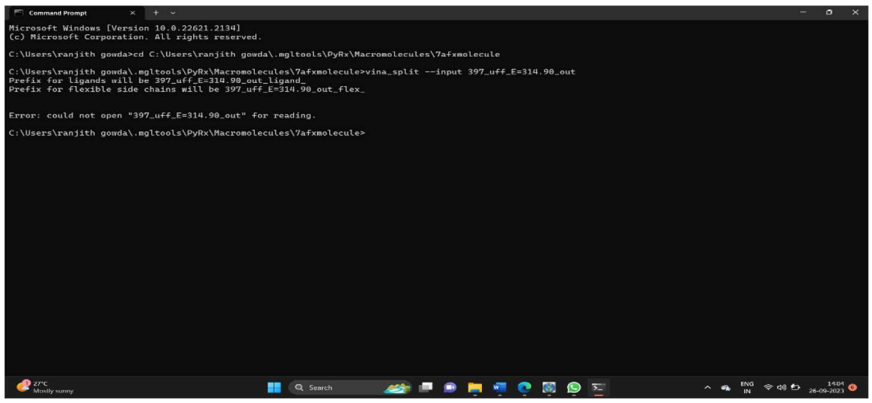


Fig 2: command line to execute vina split

- Individual conformer files were generated and stored in the same working folder.
- The generated protein and split conformers were imported in Biovia Drug discovery studio visualizer (v21.1.0).
- 2-dimensional interactions were chosen under ligand interaction tool bar.
- using show distance and types bar, distance and types of bonds are seen.

- creating 3-Dimensional surface interaction graphs for the corresponding parameters using the toolbars Aromatic, H-bond, Charges, Hydrophobic, Ionizability, and SAS.[30]

4) Test Docking of selected ligands

Imipenem, meropenem, cephalexin, metronidazole, and indole are among the ligands that have been identified as having the potential to inhibit VIM-2 after studying the various reviews of the literature. The docking process for ligands to identify sites in the 7afx protein and note binding scores and interactions was initiated using the same approach as described previously.

5) Virtual screening

The optimal ligand for subsequent experiments is indole, which is chosen based on binding scores and interactions of several ligands like, Imipenem, Meropenem, Cephalexin, Metronidazole and Indole with 7afx protein. About 8000 molecules with structures similar to the reference molecules were found when searching the PubChem database for similar molecules. The Lipinski criterion and Verber's rule were then used to the compounds to screen them for drug potential. The molecules that follow the rules are found to number 1,200, were download

Table 1: Lipinski rule criteria for select molecules

properties	limits
Molecular weight	≤400
H-bond donors	≤5
H-bond acceptors	≤10

Log P	≤2
Rotatable bonds	≤10
Polar surface area	≤120 A^{o}
Heavy atoms	≤10

This ligand, along with a positive control (indole) and a negative control (meropenem), was screened using PyRx with the same identified protein 7afx and the results were saved.

Steps involved in screening

- Prepared protein was imported in PyRx.
- Open the inbuilt open babel in PyRx.
- Import downloaded ligand present in sdf format.
- Minimize all molecules and convert the molecules into autodock PDBQT format.
- Using the same docking procedure, import the protein and ligand, adjust the grid, and begin screening.
- Save the obtained results as csv file format.[31]

6) ADMET Studies

The acronym ADMET stands for Absorption, Distribution, Metabolism, Excretion, and Toxicity. The ADMETlab2.0 server was used for ADMET studies, but the desired toxicity result was not obtained. As a result, another

server, Protox-II-online toxicity predictor, was used.

ADMETlab 2.0 procedure

- Using open babel convert the selected screened ligand into smiles.
- Copy the smiles as text.
- Open ADMETlab 2.0 from an internet source.
- navigate to ADMET evaluation.
- Paste smiles and begin prediction.
- Save results.[32]

Procedure for Protox-II

- Open Protox-II from an internet source.
- Navigate to tox prediction.
- Paste smiles and then begin prediction.
- Save results.[33]

7) Docking studies of the top compounds

After analysing the results of the screened molecules, the top 5% (30) compounds are chosen for further detailed docking studies based on their binding scores Docking experiments were carried out using Arguslab.

Step involved

- Import prepared protein and ligand in pdb format into Arguslab
- Choose all of the residues and form a group with the chosen residue as the

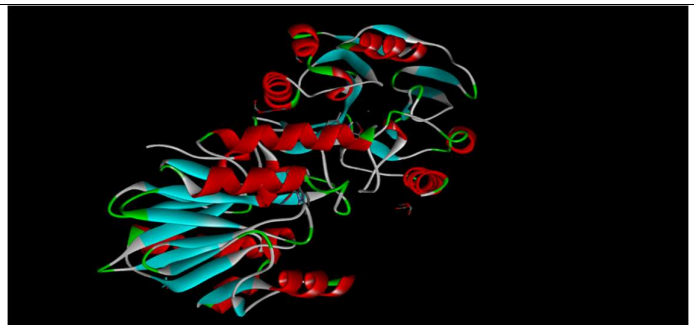
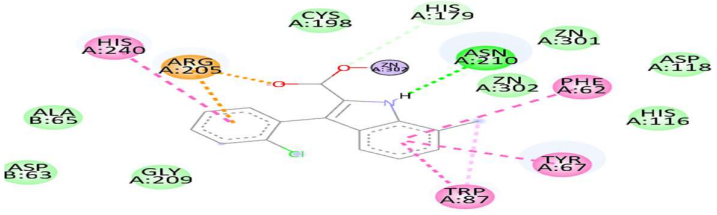
binding site

- On the dock calculation tab, maximize the grid dimension so that the entire residue fits within the grid
- Start docking and after completion of docking save the file in text format.[34]

RESULTS & DISCUSSION

Protein profile

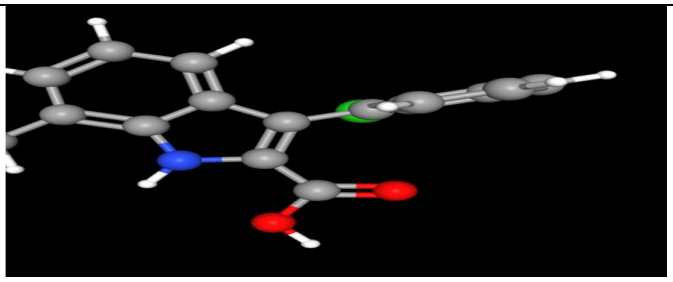
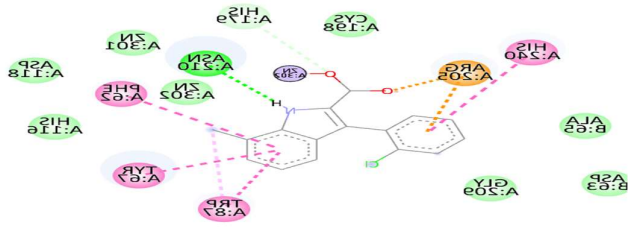
Table 2: Protein profile

Protein name	VIM 2 with Compound 139
Protein PDB id	7afx
Classification	ANTIBIOTIC
Host organism	Pseudomonas aeruginosa
Expression system	Escherichia coli
3d image	
Native ligand	**3-(2-chlorophenyl)-7-methyl-1~{H}-indole-2-carboxylic acid**
Ligand interaction (pdb standard)	
Active sites	HIS A:240, ARG A:205, CYS A:198, ASN A:210, PHE A:62, TRP A:87, ASP A:118

Validation of Docking model:

Docking of native ligand [3-(2-chlorophenyl)-7-methyl-1H-indole-2-carboxylic acid] with 7afx was done, During docking model validation, VIM-2 protein (7afx) obtained from the PDB database was compared to docked results.

Table 3: Validation results

Native ligand	**3-(2-chlorophenyl)-7-methyl-1~{H}-indole-2-carboxylic acid**
Compound CID	129208309
3d structure	
Molecular weight	285.72 g|mol
Binding affinity	-7.9
Ligand interaction	

1) **Test dock result**

Some ligands like, Imipenem, Meropenem(negative), Cephalexin, Metronidazole and Indole(positive) are chosen in test dock after reviewing some literature, and the ligands are interacted with protein (7afx) and results are obtained.

Table 4: Standard molecules profile

Sl/no	Name (CID)	Mol. wt	Binding energies	Residues interacted
1	Imipenem 104838	299.35g/mol	-6.9	PHE A:62, TYR A:67, TRP A:87, ASN A:210, HIS A:179, ARG A:205, HIS A:240
2	Meropenem 441130	383.5g/mol	-7.9	TYR A:67, ASN A:210, ARG A:205, HIS A:240, SER A:217
3	Cephalexin 27447	347.4g/mol	-7.6	PHE A:62, TRP A:87, ASN A:210, HIS A:240, ARG A:205, LYS A:211
4	Metronidazole 4173	171.15g/mol	-7.0	ASN A:76, GLN A:60, THR A:62, PHE A:62, TYR A:67, ASN A:210, HIS A:179, LYS A:211, SER A:217, VAL A:73
5	Indole 798	117.15g/mol	-7.9	TYR A:67, TRP A:87, PHE A:62, HIS A:179, CYS A:198, ARG A:205, GLY A:209, HIS A:116, ASN A:210, HIS A:240, ZN A:302

2) **Screening Result**

After studying the interactions of a few ligands, the team decided on an indole ligand for screening. The indole ligand was screened against the active site of the VIM-2 protein (7afx) as well as the standard using PyRx software.

Pubchem CID with ther IUPAC Name:

Table 5: Pubchem ID with IUPAC Name

Ligand	IUPAC Name
2773350_uff_E=198.56	2-oxo-1,3-dihydroindole-7-carbonitrile
1818553_uff_E=296.47	5-fluoro-1-methylindole-2,3-dione
13937810_uff_E=236.56	2,3-dihydro-1H-indole-2-carboxamide
3477460_uff_E=301.37	1,5-dimethylindole-2,3-dione
7213323_uff_E=207.28	2-oxo-1,3-dihydroindole-6-carboxylic acid
2773348_uff_E=232.82	2-oxo-1,3-dihydroindole-6-carbonitrile
43316644_uff_E=334.23	1H-indole-4-carbohydrazide
7408450_uff_E=217.90	a-octahydro-1H-indole-2-carboxylic acid
10397329_uff_E=215.00	2-oxo-1,3-dihydroindole-4-carboxylic acid
12018256_uff_E=273.56	2,3-dihydro-1H-indole-7-carboxamide
15897929_uff_E=329.15	9H-pyrido[4,3-b]indole
68107882_uff_E=303.62	4-fluoro-1H-indole-3-carboxamide
73357631_uff_E=293.78	7-(fluoromethyl)-1H-indole-2,3-dione
129752664_uff_E=338.22	2,3,3a,4-tetrahydro-1H-pyrrolo[3,2-h]isoquinoline
129825667_uff_E=2706.98	5,10-diazatetracyclo[7.4.0.01,12.02,6]trideca-2,4,6,8,10,12-hexaene
142640943_uff_E=296.24	7-fluoro-1H-indole-3-carboxamide
46736065_uff_E=209.06	6-methyl-2-oxo-1,3-dihydroindole-3-carbaldehyde
19832361_uff_E=217.00	2-oxo-1,3-dihydroindole-7-carboxylic acid
20112523_uff_E=291.73	2,3-dioxo-1H-indole-7-carbonitrile
45122603_uff_E=421.00	2H-furo[3,2-g]indole

92020757_uff_E=291.98	2,3-dioxo-1H-indole-4-carbonitrile
13567959_uff_E=359.95	2-(hydroxymethyl)-1H-indole-6-carbonitrile
59214189_uff_E=335.08	N'-hydroxy-1H-indole-4-carboximidamide
19832315_uff_E=224.11	2-oxo-1,3-dihydroindole-6-carboxamide
15054039_uff_E=294.60	4-hydroxy-1H-indole-2-carboxamide
53423770_uff_E=311.31	2-oxoindole-4-carboxylic acid
68921606_uff_E=305.53	7-oxo-1,4,5,6-tetrahydroindole-2-carboxylic acid
53423963_uff_E=291.10	2-oxoindole-6-carbonitrile
72532729_uff_E=335.08	N'-hydroxy-1H-indole-4-carboximidamide
12474262_uff_E=306.49	1-hydroxyindole-3-carboxamide
135121977_uff_E=553.06	2-azatricyclo[5.3.1.04,11]undeca-1,4(11),5,7,9-pentaene
53843984_uff_E=304.02	2-indol-3-ylidenepropanedinitrile
15887273_uff_E=344.57	2,3,4,9-tetrahydro-1H-pyrido[4,3-b]indole
66771717_uff_E=317.69	9,9a-dihydro-1H-pyrido[3,4-b]indole
129891669_uff_E=309.38	1,3,4,4a-tetrahydropyrano[4,3-b]indole
14941659_uff_E=443.78	1,2,4,8b-tetrahydropyrrolo[2,3-b]indole
68754983_uff_E=304.07	2-methyl-1H-indole-5-carboxamide
10910045_uff_E=609.53	4-azatricyclo[5.3.1.04,11]undeca-1(10),7(11),8-triene-2,3-dione
15523873_uff_E=939.91	9-azatricyclo[6.3.0.03,6]undeca-1,3(6),7,10-tetraene-5-carbonitrile
18467779_uff_E=1336.53	4,5-dihydro-3H-cyclobuta[e]indole-4-carbonitrile

70559963_uff_E=345.49	(4aR,9bS)-5,5a,8,9,9a,9b-hexahydro-4aH-pyrido[4,3-b]indole
89014551_uff_E=245.75	7-nitroso-2,3-dihydro-1H-indole-2-carbaldehyde
90021463_uff_E=395.25	1H-pyrano[3,4-d]indole
18609619_uff_E=217.74	(2S,3aS)-2,3,3a,4,5,6,7,7a-octahydro-1H-indole-2-carboxylic acid
19795967_uff_E=322.32	1H-pyrido[3,4-b]indole
18609619_uff_E=217.74	(2S,3aS)-2,3,3a,4,5,6,7,7a-octahydro-1H-indole-2-carboxylic acid
22282655_uff_E=284.90	2,5,6,7-tetrahydropyrano[3,2-g]indole
22714686_uff_E=903.43	2,3,6,7-tetrahydro-1H-cyclobuta[e]indole-7-carbonitrile
22714692_uff_E=1000.66	6,7-dihydro-3H-cyclobuta[e]indole-7-carbonitrile
67269999_uff_E=272.90	3-methyl-6-nitro-4H-indole
67270002_uff_E=577.63	3-methyl-4-nitro-4H-indole
67424272_uff_E=1552.06	spiro[3a,4-dihydro-1H-indole-3,1'-cyclopropane]-2-one
68612502_uff_E=2636.22	1,2-dihydrocyclopropa[b]indole-1-carboxylic acid
69260350_uff_E=355.24	1,3,4,4a,5,6-hexahydropyrano[3,4-b]indole
69380666_uff_E=308.29	4-(hydroxymethyl)-1H-indole-2,3-dione
69397600_uff_E=322.13	2,3,4,4a,5,6-hexahydro-1H-pyrido[3,4-b]indole
69977167_uff_E=223.29	2H-indole-2-carboxylic acid
88354561_uff_E=460.64	cyclopenta[f]indole
21423164_uff_E=359.04	1,4,5,6-tetrahydrocyclopenta[e]indole
22018480_uff_E=342.93	2,3,4,5-tetrahydro-1H-pyrido[3,4-b]indole

Table 6: Virtual screening Results of Best Hits

Ligand	target	Binding enrergy
2773350_uff_E=198.56	7afxmolecule	-7.7
1818553_uff_E=296.47	7afxmolecule	-7.9
13937810_uff_E=236.56	7afxmolecule	-7.7
3477460_uff_E=301.37	7afxmolecule	-7.8
7213323_uff_E=207.28	7afxmolecule	-7.8
2773348_uff_E=232.82	7afxmolecule	-7.8
43316644_uff_E=334.23	7afxmolecule	-7.9
7408450_uff_E=217.90	7afxmolecule	-7.9
10397329_uff_E=215.00	7afxmolecule	-7.8
12018256_uff_E=273.56	7afxmolecule	-7.9
15897929_uff_E=329.15	7afxmolecule	-7.8
68107882_uff_E=303.62	7afxmolecule	-7.9
73357631_uff_E=293.78	7afxmolecule	-7.8
129752664_uff_E=338.22	7afxmolecule	-8.1
129825667_uff_E=2706.98	7afxmolecule	-8.1
142640943_uff_E=296.24	7afxmolecule	-7.8
46736065_uff_E=209.06	7afxmolecule	-7.9
19832361_uff_E=217.00	7afxmolecule	-8.1
20112523_uff_E=291.73	7afxmolecule	-7.9
45122603_uff_E=421.00	7afxmolecule	-7.8
92020757_uff_E=291.98	7afxmolecule	-7.8
13567959_uff_E=359.95	7afxmolecule	-8
59214189_uff_E=335.08	7afxmolecule	-8.1
19832315_uff_E=224.11	7afxmolecule	-7.9

15054039_uff_E=294.60	7afxmolecule	-7.8
53423770_uff_E=311.31	7afxmolecule	-7.9
68921606_uff_E=305.53	7afxmolecule	-7.9
53423963_uff_E=291.10	7afxmolecule	-7.8
72532729_uff_E=335.08	7afxmolecule	-8.1
12474262_uff_E=306.49	7afxmolecule	-8
135121977_uff_E=553.06	7afxmolecule	-8
53843984_uff_E=304.02	7afxmolecule	-8.1
15887273_uff_E=344.57	7afxmolecule	-7.8
66771717_uff_E=317.69	7afxmolecule	-7.9
129891669_uff_E=309.38	7afxmolecule	-7.8
14941659_uff_E=443.78	7afxmolecule	-7.9
68754983_uff_E=304.07	7afxmolecule	-7.9
10910045_uff_E=609.53	7afxmolecule	-7.9
15523873_uff_E=939.91	7afxmolecule	-8.5
18467779_uff_E=1336.53	7afxmolecule	-7.8
18609619_uff_E=217.74	7afxmolecule	-7.9
19795967_uff_E=322.32	7afxmolecule	-7.9
22282655_uff_E=284.90	7afxmolecule	-8
22714686_uff_E=903.43	7afxmolecule	-7.9
22714692_uff_E=1000.66	7afxmolecule	-8.3
67269999_uff_E=272.90	7afxmolecule	-7.9
67270002_uff_E=577.63	7afxmolecule	-7.8

67424272_uff_E=1552.06	7afxmolecule	-8.1
68612502_uff_E=2636.22	7afxmolecule	-8
69260350_uff_E=355.24	7afxmolecule	-8.4
69380666_uff_E=308.29	7afxmolecule	-7.8
69397600_uff_E=322.13	7afxmolecule	-7.9
69977167_uff_E=223.29	7afxmolecule	-7.9
88354561_uff_E=460.64	7afxmolecule	-8.1
21423164_uff_E=359.04	7afxmolecule	-7.8
22018480_uff_E=342.93	7afxmolecule	-8
57781210_uff_E=581.89	7afxmolecule	-7.9
70559963_uff_E=345.49	7afxmolecule	-7.8
89014551_uff_E=245.75	7afxmolecule	-8.2
90021463_uff_E=395.25	7afxmolecule	-7.8

3) ADMET STUDIES

Table 7: ADME Predection Results

Compound(pubchem CID)	MDCK permiability	Pgp- inhibitor	PPB (plasma protein binding)	BBB Penetration	Volume distribution	CYP3A4 Inhibitor	CYP1A2 Inhibitor	CL(clearance)	$T_{1/2}$ (half life)
22282655	2.3^{-05}	0.021	94.63%	0.57	1.44	0.50	0.99	11.8	0.61
10397329	1.2^{-05}	0.0	30.14%	0.40	0.29	0.03	0.03	3.20	0.87
7408450	2.3^{-05}	0.001	11.75%	0.29	0.64	0.00	0.02	1.95	0.61
69977167	4.9^{-05}	0.0	38.94%	0.5	0.36	0.03	0.07	3.32	0.83
19832315	3.7^{-05}	0.001	47.46%	0.99	0.96	0.01	0.50	6.57	0.36
76443689	2.6^{-05}	0.0	55.2%	0.87	1.18	0.13	0.40	8.51	0.75

12018256	8^{-05}	0.087	78.3%	0.99	1.25	0.07	0.87	9.49	0.49
15054039	5^{-06}	0.01	66.8%	0.85	0.86	0.02	0.79	13.0	0.63
53843984	2.2	0.0	46.3%	0.93	0.75	0.34	0.97	4.98	0.78
68921606	6^{-06}	0.05	22.6%	0.38	0.39	0.01	0.06	3.81	0.90
12989169	3.6	0.0	53%	0.76	1.80	0.32	0.51	7.27	0.51
10397329	1.2	0.0	30.1%	0.40	0.29	0.03	0.03	3.20	0.87
66771717	3.6	0.0	53%	0.76	1.80	0.32	0.51	7.27	0.51
59214189	0.2	0.0	34.9%	0.09	1.8	0.09	0.47	9.01	0.69
69260350	4.7	0.02	63.1%	0.39	2.9	0.27	0.61	8.6	0.50
14941659	2.4	0.1	40.6%	0.97	3.14	0.02	0.15	5.62	0.63
88354561	2.3	0.3	88.1%	0.56	1.34	0.03	0.81	3.89	0.85
88133445	2.7	0.3	87.8%	0.51	1.94	0.07	0.78	5.66	0.82
57781210	1.6	0.8	78.7%	0.12	1.32	0.70	0.96	2.79	0.877
10910045	2.4	0.04	83.5%	0.69	0.34	0.04	0.98	1.13	0.41
15523873	1.2	0.0	61.7%	0.72	0.79	0.12	0.98	8.29	0.64
22714692	1.5	0.0	65.8%	0.1	0.92	0.17	0.97	6.84	0.70
129825667	1.7	0.07	23.3%	0.99	1.54	0.14	0.32	10.1	0.704
7213323	1.1	0.0	33.6%	0.26	0.31	0.04	0.03	2.59	0.89
2773348	2.3	0.0	54.6%	0.9	0.7	0.82	0.90	8.82	0.80
2773350	3.3	0.0	57.5%	0.94	0.64	0.06	0.52	8.44	0.87
1818553	2.8	0.03	69.3%	0.1	0.53	0.02	0.90	1.82	0.19
3477460	2.5	0.03	67.3%	0.79	0.49	0.04	0.29	1.74	0.29
6950491	6.2	0.01	51.7%	0.94	0.97	0.09	0.78	3.29	0.78

Toxicity Studies:

Table 8: Toxicity results

Compound(pubchem CID)	Hepatotoxicity	Carcinogenicity	Immunotocicity	Mutagenicity	Cytotoxicity
22282655	Inactive 0.36	Inactive 0.38	Inactive 0.12	Active 0.51	Inactive 0.14
10397329	Inactive 0.35	Inactive 0.33	Inactive 0.01	Inactive 0.20	Inactive 0.31
7408450	Inactive 0.28	Inactive 0.28	Inactive 0.01	Inactive 0.15	Inactive 0.28
69977167	Inactive 0.40	Inactive 0.28	Inactive 0.01	Inactive 0.26	Inactive 0.27
19832315	Inactive 0.30	Inactive 0.37	Inactive 0.01	Inactive 0.27	Inactive 0.23
76443689	Inactive 0.22	Inactive 0.45	Inactive 0.01	Inactive 0.47	Inactive 0.38
12018256	Inactive 0.48	Inactive 0.38	Inactive 0.2	Inactive 0.29	Inactive 0.26
15054039	Inactive 0.48	Inactive 0.41	Inactive 0.34	Inactive 0.30	Inactive 0.18
53843984	Inactive 0.47	Inactive 0.37	Inactive 0.01	Inactive 0.04	Inactive 0.29
68921606	Inactive 0.36	Inactive 0.35	Inactive 0.01	Inactive 0.21	Inactive 0.21
129891669	Inactive 0.19	Inactive 0.40	Inactive 0.02	Inactive 0.32	Inactive 0.28
10397329	Inactive 0.35	Inactive 0.33	Inactive 0.01	Inactive 0.01	Inactive 0.31
66771717	Inactive 0.29	Inactive 0.40	Inactive 0.02	Inactive 0.32	Inactive 0.29
59214189	Active 0.71	Active 0.57	Inactive 0.11	Active 0.60	Inactive 0.25
69260350	Inactive 0.19	Inactive 0.39	Inactive 0.04	Inactive 0.30	Inactive 0.27
14941659	Inactive 0.15	Inactive 0.23	Inactive 0.01	Inactive 0.29	Inactive 0.27
88354561	Inactive 0.42	Inactive 0.40	Inactive 0.43	Inactive 0.33	Inactive 0.24
88133445	Inactive 0.41	Inactive 0.48	Inactive 0.04	Inactive 0.33	Inactive 0.22

57781210	Inactive 0.34	Inactive 0.43	Inactive 0.05	Inactive 0.44	Inactive 0.30
10910045	Inactive 0.40	Inactive 0.50	Inactive 0.04	Inactive 0.41	Inactive 0.36
15523873	Inactive 0.42	Inactive 0.44	Inactive 0.01	Inactive 0.27	Inactive 0.23
22714692	Inactive 0.42	Inactive 0.44	Inactive 0.01	Inactive 0.27	Inactive 0.23
129825667	Inactive 0.23	Inactive 0.36	Inactive 0.28	Inactive 0.43	Inactive 0.41
7213323	Inactive 0.38	Inactive 0.34	Inactive 0.01	Inactive 0.21	Inactive 0.28
2773348	Inactive 0.35	Inactive 0.37	Inactive 0.01	Inactive 0.27	Inactive 0.17
2773350	Inactive 0.36	Inactive 0.38	Inactive 0.01	Inactive 0.27	Inactive 0.18
1818553	Inactive 0.50	Inactive 0.49	Inactive 0.26	Inactive 0.54	Inactive 0.45
3477460	Inactive 0.36	Inactive 0.50	Inactive 0.05	Inactive 0.47	Inactive 0.50
6950491	Inactive 0.31	Inactive 0.49	Inactive 0.01	Inactive 0.34	Inactive 0.15

Docking studies of top hits

Docking was performed on the top hits (60 compounds) discovered by protein VIM-2(7afx) screening using the arguslab software. As a result, it came to no more about the potential conformation and its binding affinities. The table below only displays the binding energy of each compound's ideal configuration.

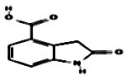
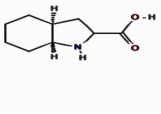
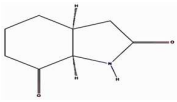
Table 9: Top Hit docking results

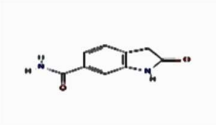
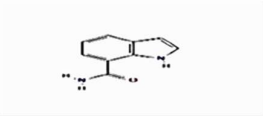
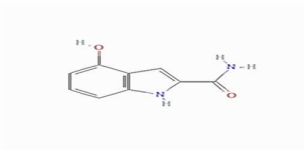
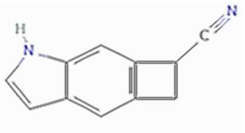
Ligand	target	Binding energy
22282655_uff_E=284.90	7afxmolecule	-8
10397329_uff_E=215.00	7afxmolecule	-7.8
7408450_uff_E=217.90	7afxmolecule	-7.9
19832315_uff_E=224.11	7afxmolecule	-7.9

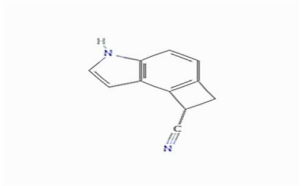
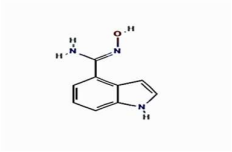
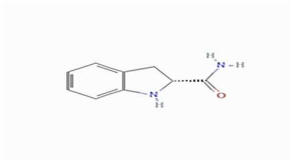
53423770_uff_E=311.31	7afxmolecule	-7.9
68921606_uff_E=305.53	7afxmolecule	-7.9
12474262_uff_E=306.49	7afxmolecule	-8
72532729_uff_E=335.08	7afxmolecule	-8.1
135121977_uff_E=553.06	7afxmolecule	-8
15887273_uff_E=344.57	7afxmolecule	-7.8
53843984_uff_E=304.02	7afxmolecule	-8.1
66771717_uff_E=317.69	7afxmolecule	-7.9
14941659_uff_E=443.78	7afxmolecule	-7.9
10910045_uff_E=609.53	7afxmolecule	-7.9
15523873_uff_E=939.91	7afxmolecule	-8.5
18609619_uff_E=217.74	7afxmolecule	-7.9
19795967_uff_E=322.32	7afxmolecule	-7.9
22714686_uff_E=903.43	7afxmolecule	-7.9
22714692_uff_E=1000.66	7afxmolecule	-8.3
67270002_uff_E=577.63	7afxmolecule	-7.8
69260350_uff_E=355.24	7afxmolecule	-8.4
69977167_uff_E=223.29	7afxmolecule	-7.9
88354561_uff_E=460.64	7afxmolecule	-8.1
22018480_uff_E=342.93	7afxmolecule	-8
57781210_uff_E=581.89	7afxmolecule	-7.9
53898450_uff_E=330.43	7afxmolecule	-8
70559963_uff_E=345.49	7afxmolecule	-7.8
89014551_uff_E=245.75	7afxmolecule	-8.2
122502597_uff_E=333.08	7afxmolecule	-7.9
90021463_uff_E=395.25	7afxmolecule	-7.8

After comparing studies of ADMET and binding energies, and selecting the top 11 outcomes from the greatest hits (a total of 30 compounds), and then using Biovia Discovery studio to study interactions and interactions are shown.

Table 10: molecular profile and interactions of best hits

Compound (pubchem CID)	Structure and Name	Residues Interacted	Interacions
10397329	 2-oxo-1,3-dihydroindole-4-carboxylic acid	ASNA:118,ARGA:205 TRP A:87 TYR A:67, GLU A:146, SER A:204	Conventional H-bonds-8, Carbon H-bond-3, Pi-Pi Stacked-6, Pi-Cation-5, Pi-Pi T-shaped-4
13069255	 octahydro-1H-indole-2-carboxylic acid	HIS A:240, PHE A:6 TRP A:87TYRA:67 ARG A:205	Pi-sigma-7, pi-alkyl-7, Pi donar H-bond-2, Conventional H-bond-6
133664156	 3,3a,4,5,6,7a-hexahydro-1H-indole-2,7-dione	ASN A:210, TRP A:87 PHE A: 62, TYR A: 67, ARG A:205	Conventional H-bonds-7, Pi-alkyl-8, Pi Sigma-3, carbon H-bond-2

19832315	 2-oxoindoline-6-carboxamide	ASNA:210, ASP A:118 PHE A:62, TYR A:67, TRP A: 87, ARG A:205 HIS A:240	Pi carbon-3, Pi donar H-bond -1 Conventional H-bond-1, Pi alkyl-1, Pi staked-4, Pi sgma-1 Pii shaped-2
12018256	 1H-INDOLE-7 CARBOXAMIDE	ARG,A:205,HISA:240, TYR A:67, PHE A:62, ASN A:210	Conventional H-bond-5, Pi alkyl-9, Pi-Pi staked-5 Pi carbon-3,Pi Pi T-shaped-5 Pi donar H-bond-1
15054039	 4-hydroxy-1H-indole-2-carboxamide	HIS A:240, TRP A:87, TYR A:67, ASH A:210 ASP S:118	Pi cation-9, Pi Pi T-shaped-9 Conventional H-bond-7 Pi alkyl-1, Pi donar H-bond-6
15523873	 9-azatricyclo[6.3.0.03,6]undeca-1,3(6),7,10-tetraene-5-carbonitrile	PHE A:62, TRP A:87 TYR A:67, ASP A:118, ASP A:117, HIS A:240	Pi cation-5, Pi Pi T-shaped-7 Pi alkyl-9, conventional H-bond-5, pi staked-5, Vandor walls-1 Pi donar H-bond-1

22714692	 6,7-dihydro-3H-cyclobuta[e]indole-7-carbonitrile	ASN A:210, ASP A:118 PHE A:62, TRP A:87, ASP A:118, HIS A:240	Pi cation-6, Pi staked-4 Pi alkyl-9, pi shaped-6,pi sigma-1,Pi-donarH-bond-1,conventionalH-bond-4,pi sigma-1
129825667	 5,10 - diazatetracyclo[7.4.0.01, 12.02,6]trideca-2,4,6,8,10,12-hexaene	ASN A:210, ASP A:118 LYS A:198, TRP A:87 PHE A:62, TYR A:67	Pi-donarH-bond-2,Pi-Pi T shaped-3, Pi alkyl-9, conventional H-bond-2, Pi staked-4, pi cation-4, pi sigma-4, Vandor walls-3
59214189	 N'-hydroxy-1H-indole-4-carboximidamide	HIS A:240, TYR A:67 LYS A:198, TRP A:87 PHE A:62, ASN A:210 CYS A:198, ARG A:205, ASP A:118	Pi cation-6, Pi staked-4 Pi alkyl-9, pi shaped-6,pi sigma-1,Pi-donarH-bond-1,conventionalH-bond-4,pi sigma-1
13937810	 2,3-dihydro-1H-indole-2-carboxamide	HIS A:240, PHE A:62 TRP A:87, TYR A:67 ASP A:118	Vandor walls-9, conventional H-bond-6, pi pi staked-4, pi cation-4, pi donar H-bond-3, Pi Pi T shaped-6

Ligand interaction of top hits:

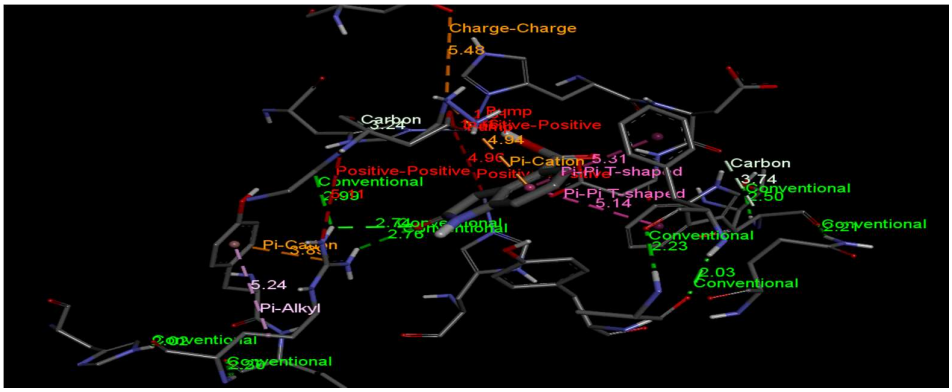


Fig 4: 3D interaction image of 2-oxo-1,3-dihydroindole-4-carboxylic acid with 7afx

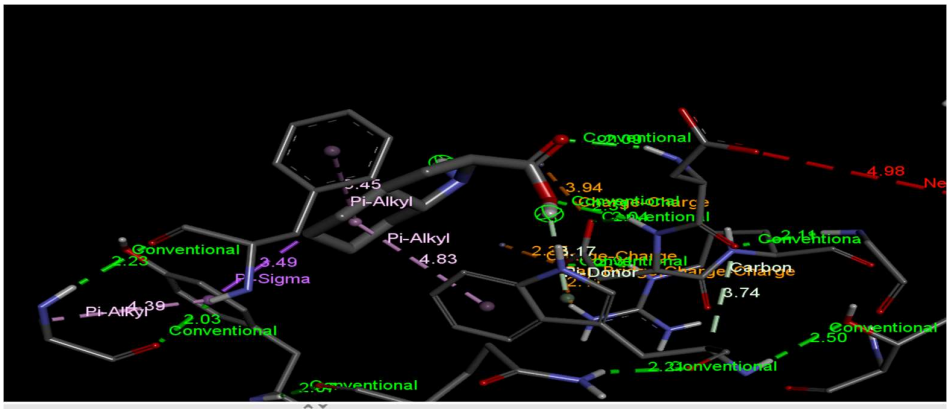


Fig 5: 3Dinteraction of 7a-octahydro-1H-indole-2-carboxylic acid with 7afx

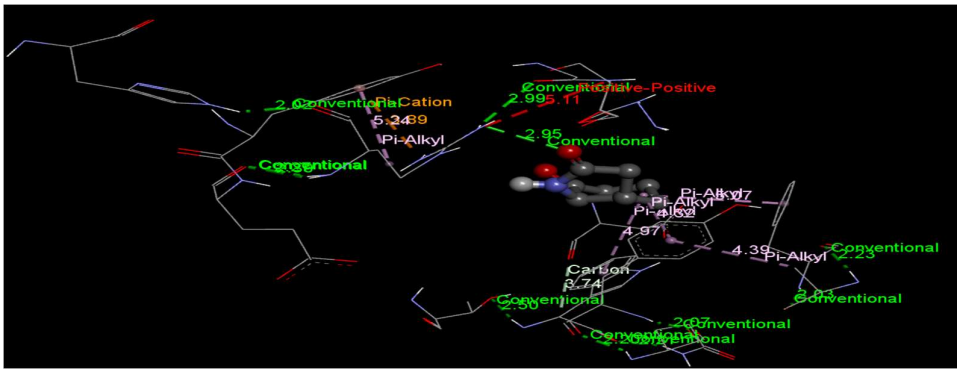


Fig 6: 3D interaction of(2S)-2,3-dihydro-1H-indole-2-carboxylic acid with 7afx

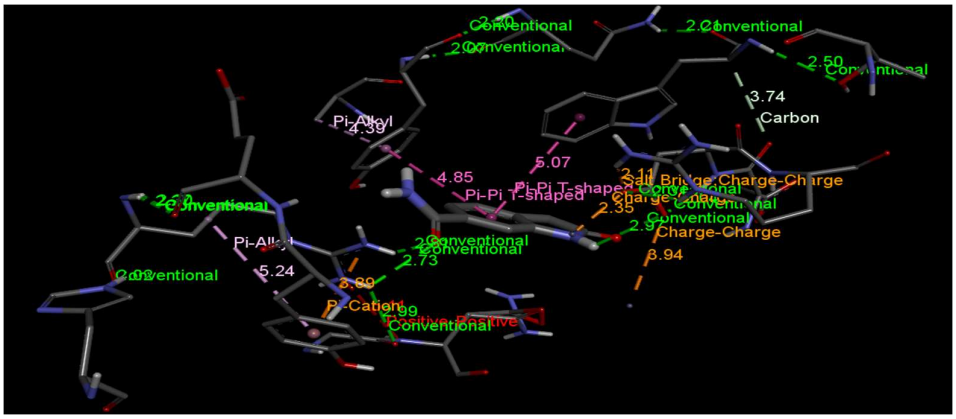


Fig 7: 3D interaction of 2-oxo-1,3-dihydroindole-6-carboxamide with 7afx

Fig 8: 3D interaction of 1H-indole-7-carboxamide with 7afx

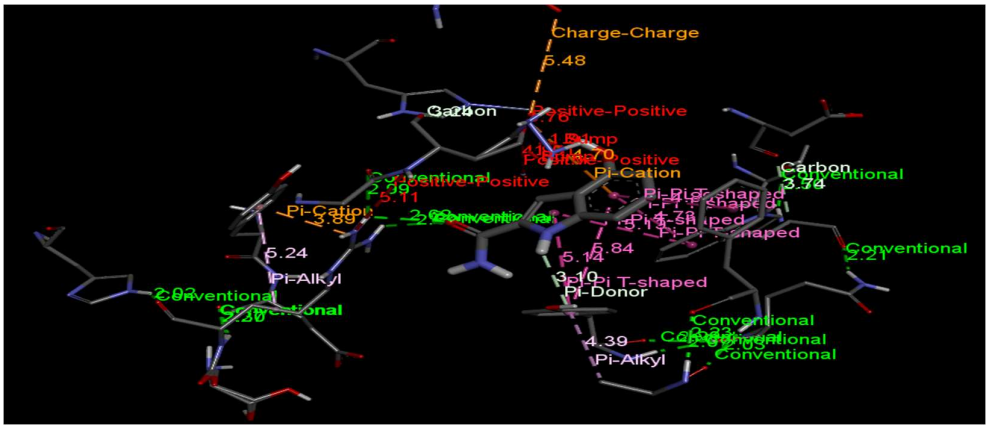


Fig 9: 3D interaction of 4-hydroxy-1H-indole-2-carboxamide with 7afx

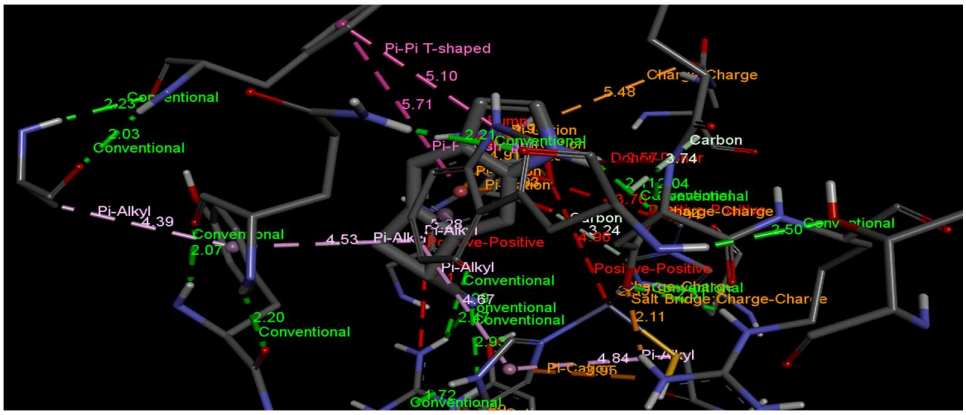


Fig 10: 3D interaction of 9-azatricyclo[6.3.0.03,6]undeca-1,3(6),7,10-tetraene-5-carbonitrile with 7afx

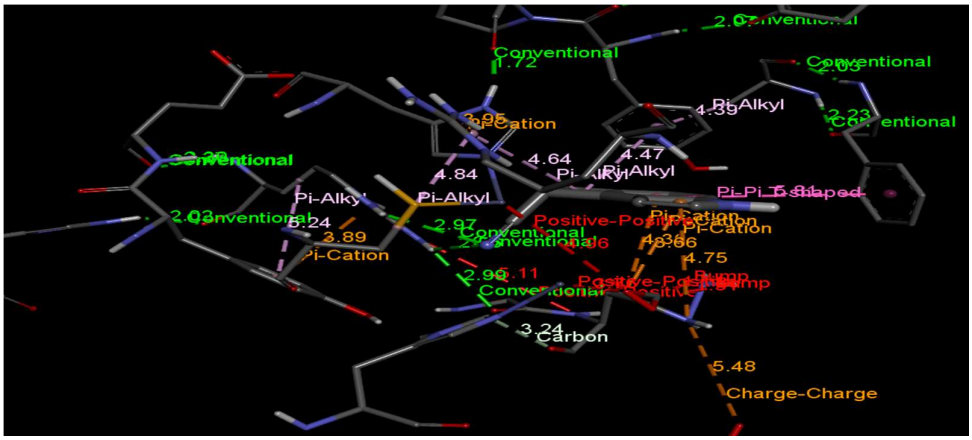


Fig 11: 3D interaction of 6,7-dihydro-3H-cyclobuta[e]indole-7-carbonitrile with 7afx

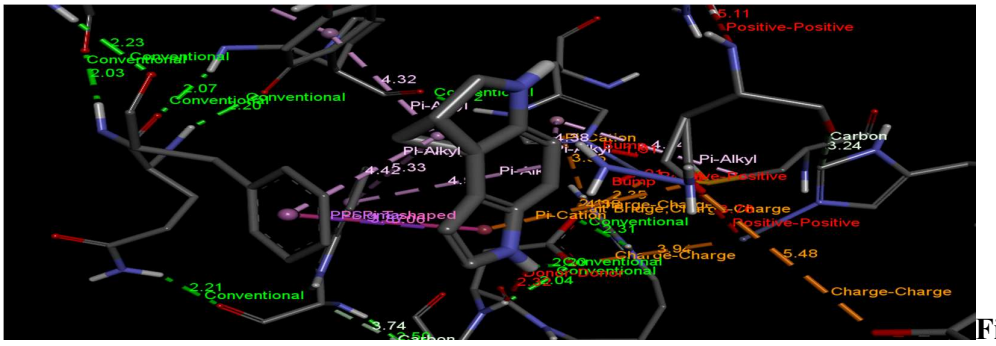


Fig 12: 3D interaction of 5,10-diazatetracyclo[7.4.0.01,12.02,6]trideca-2,4,6,8,10,12-hexaene with 7afx

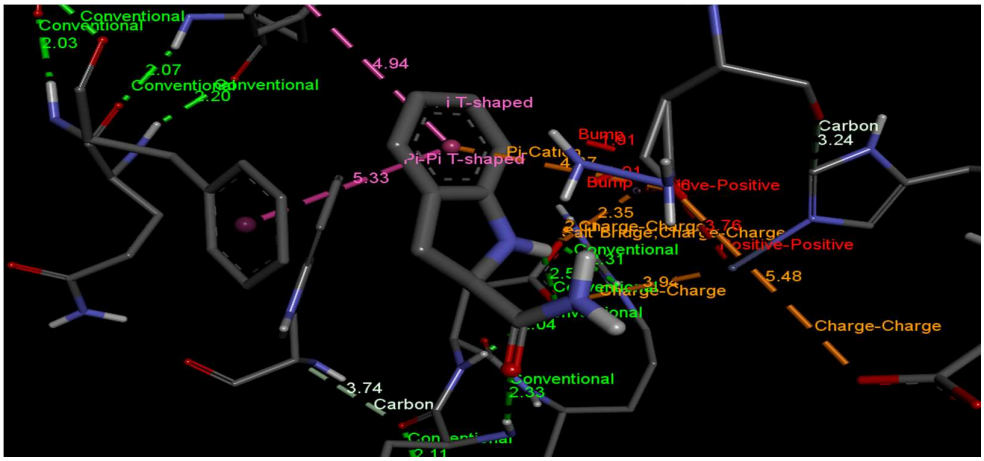


Fig 13: 3D interaction of (2R)-2,3-dihydro-1H-indole-2-carboxamide with 7afx

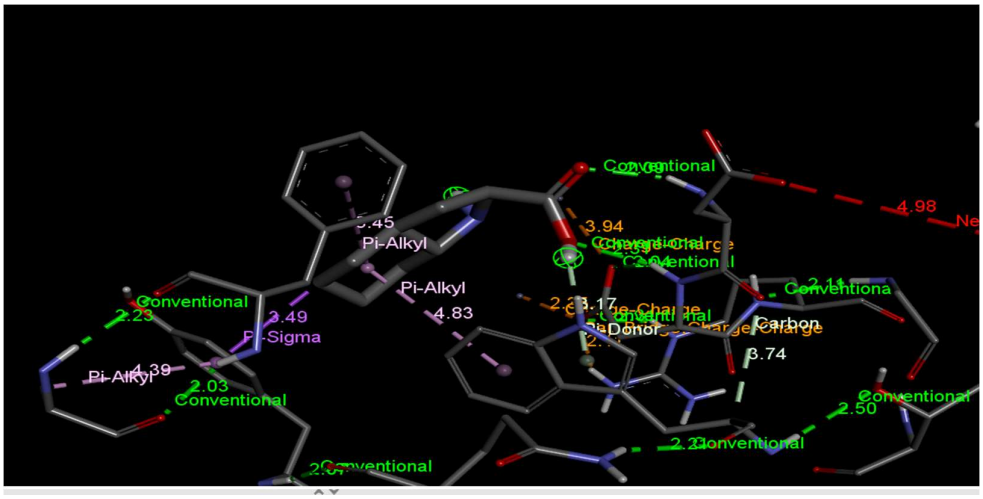


Fig 14: 3D interactionof N'-hydroxy-1H-indole-4-carboximidamide with 7afx

SUMMARY & CONCLUSION

Conducting *in-silico* virtual screening and docking investigations for the VIM-2 class of beta lactamase inhibitors was the primary goal of the study. From the PDB database, the VIM-2 metallo-beta-lactamase protein was retrieved. The parent ligand was identified as 3-(2-chlorophenyl)-7-methyl-1-H-indole-2-carboxylic acid, and the structure was shown using ChemSketch. For docking experiments, both the protein and the ligand were exported. The results were concluded using the database's ligand interaction parameters and the docked results, which matched the permissible limits. using the basis of the interaction energies (binding energies), the docking for standard molecules was carried out using autodock, and the results were concluded and examined. Lipinski's rule was used to filter the molecules, 1200 molecules were retrieved from PubChem, and 7AFX protein molecules were used for docking. Following the successful docking of 60 compounds, the top 5% of best hits, or 30 molecules, were chosen based on binding energies before the ADMET experiments were completed. Each of these compounds primarily interacted with TRP A87, TYR A67, ASN A210, and HIS A240. The compound with **N'-hydroxy-1H-indole-4-carboximidamide**(CID 59214189) reacted with the amino acids TRP A87, ASN A210, and HIS A240. This compound has been determined to be equivalent to the standards and has more drug-like effects. Additionally, it was discovered that these compounds have less hazardous and improved ADMET characteristics. on the whole, 30 compounds were discovered to exhibit superior inhibition. Subsequent modifications will considered for future research.

Usin the available tools, in the present research work an attempt has been made to develop inhibitors for VIM-2 β-lactamase of *P.aerguinosa*

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