MOLECULAR DOCKING AND IN SILICO EVALUATION OF ISOCHAETOMININE AS A NOVEL INHIBITOR OF *HELICOBACTER PYLORI* UREASE (1E9Y)

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by

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DECLARATION

I, Neggat Ferdous 24/MSCBIO/44 hereby certify that the work which is being presented in the thesis entitled **"Molecular Docking and In Silico Evaluation of Isochaetominine as a Novel Inhibitor of *Helicobacter pylori* Urease (1E9Y)"** in partial fulfillment of the requirements for the award of the Degree of Master of Science, submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from 2024 to 2026 under the supervision of Dr. Kriti Bhandari.

The matter presented in the thesis has not been submitted by me for the award of any other degree of this or any other Institute.

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"MOLECULAR DOCKING AND IN SILICO EVALUATION OF ISOCHAETOMININE AS A NOVEL INHIBITOR OF HELICOBACTER PYLORI UREASE (1E9Y) "

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ABSTRACT

Helicobacter pylori is an important gastric pathogen that uses the virulent urease enzyme to survive in the acidic environment of the human stomach. Urease hydrolyzes urea to generate ammonia. This increases the local pH and allows the bacterium to colonize the gastric mucosa and establish a long-term infection. Therefore, urease is considered a central virulence factor in H. pylori pathogenesis. And targeting this enzyme is a promising strategy in anti-H. pylori drug discovery. [3] [11]

In this thesis Isochaetominine is shown as a novel natural inhibitor against H. pylori urease (PDB ID: 1E9Y). The study is based on my conference paper, in which insilco experiments were conducted, where 2431 natural compounds were virtual screened using PyRx/AutoDock Vina and ADME profiling was analysed using SwissADME. In docking results, Isochaetominine showed the strongest binding affinity (-9.3 kcal/mol), that was superior to Protocatechuic acid (-5.9 kcal/mol) and Acetohydroxamic acid (-4.9 kcal/mol). Validation of our experiment was done using these two known inhibitors, PCA and AHA. These results suggest that Isochaetomine may fit better into the active site and be a stronger lead scaffold for urease inhibition.

Isochaetominine is a naturally occurring indole alkaloid, a compound of fungal origin, due to its rigid cage-like structure, nitrogen-rich framework, and hydrophobic features. It creates interest in biologically active molecules.The major advantage of using natural products is that they are more effective than synthetic inhibitors. Provides better biocompatibility, lower toxicity risk, and greater structural diversity. Natural compounds have also been repeatedly highlighted in the literature on urease inhibitors as safer alternatives. [10]

In ADME analysis, Isochaetominine has shown high gastrointestinal absorption, no BBB permeability, no CYP isoenzyme inhibition, and acceptable drug-likeness. PCA showed some medicinal chemistry alerts and CYP isoenzyme inhibition issues. Whereas AHA, even after being highly soluble, is linked to toxicity related concerns. On this basis Isochaetominine can be considered a promising anti-urease candidate for further in vitro and in vivo validation.

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Neggat Ferdous

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ABBREVIATIONS

ADME	Absorption, Distribution, Metabolism, Excretion
PCA	Protocatechuic acid
AHA	Acetohydroxamic acid
PDB ID	Protein databank identifier
CYP	Cytochrome P450
BBB	Blood-Brain Barrier
pH	Potential of Hydrogen
H.Pylori	Helicobacter pylori
NIH	National Institute of Health
MALT	Mucosa-Associated Lymphoid Tissue
ureA	Urease subunit alpha gene
ureB	Urease subunit beta gene
His	Histidine
Asp	Aspartic acid
KCX219	Carbamylated Lysine Residue 219
Ni2+	Nickel ion
sp.	Species
MUC5AC	Mucin 5AC
VacA	Vacuolating Cytotoxin A
CagA	Cytotoxin-Associated gene A
GI	Gastrointestinal
UreD	Urease Accessory Protein D
UreF	Urease Accessory Protein F
UreG	Urease Accessory Protein G
UreE	Urease Accessory Protein E
HypA	Hydrogenase expression Protein A
HypB	Hydrogenase expression Protein B

PAINS	Pan-Assay Interference Compound
CYP2D6	Cytochrome P450 family 2 subfamily D member 6
CYP3A4	Cytochrome P450 family 3 subfamily A member 4
RCSB	Research Collaboratory for Structural Bioinformatics
BIOVIA	BIOVIA Discovery Studio
PDBQT	Protein Data Bank, Partial charge Q and atom type T formate
LogP	Logarithm of Partition Coefficient
TPSA	Topological Polar Surface Area
SDF	Structure Data File
RMSD	Root Mean Square Deviation
Gly279	Glycine 279
Ala365	Alanine 365
Glu222	Glutamic acid 222
ESOL	Estimated Solubility
HAE	Hydroxamic Acid Energy
CYS321	Cysteine 321
logS	Logarithm of Aqueous Solubility
kcal/mol	Kilocalories per mole
HIA	Human Intestinal Absorption
PGP+	p- Glycoprotein substrate
PGP-	Non -substrate of p-Glycoprotein
CNS	Central Nervous System
MD	Molecular Dynamics
IC50	Half maximal inhibitory concentration
SAR	Structure-Activity Relationship

1. INTRODUCTION

Helicobacter pylori is a gram-negative, spiral bacterium that colonizes gastric mucosa and affects 50 percent of the world's population. *H. pylori* causes 75 percent of duodenal ulcers and 17 percent of gastric ulcers. [1] In 1994, it was reported by the NIH that *H. pylori* could be the primary reason for the peptic ulcer and needed to be treated. Thus, it was discovered that H.pylori plays a major role in peptic and gastric ulcer diseases as well as gastritis and mucosa associated lymphoid tissue (MALT). [2]. The most important factor in the successful colonization of *H. pylori* is its urease enzyme. *H. pylori* colonizes the human stomach and produces ample quantities of urease which increases the surrounding pH making it easier for survival. [3] Urease hydrolyzes urea and converts it into ammonia and carbon dioxide and due to ammonia release, the bacterium increases the pH around it. This local alkalization protects *H. pylori* from gastric acid and helps the bacteria survive in the stomach.[11]. *H. pylori* can also survive the pH of 1 for several hours in the presence of urease.[4] For this reason, urease is considered a central virulence factor in *H. pylori* pathogenesis. [3] Since urease plays an important role, it has become essential to study the urease enzyme so as to inhibit its activity. Urease is a virulent factor that safeguards from gastric activity, supports bacterial colonization, fosters bacterial nutrition, alters hosts immune response, etc. [5].

The urease enzyme hydrolyzes the urea into two molecules, carbon dioxide and ammonia, by breaking the chemical bonds of the urea using water. This occurs in two steps; firstly, urea converts into ammonia and carbamic acid, and then this unstable molecule further breaks down into carbonic acid and ammonia. The production of two molecules of ammonia drastically increases the surrounding pH of the stomach.[6]. The normal acidic pH of the human stomach is a natural barrier against bacterial growth, [10] but H.pylori has developed a urease-dependent acid acclimation mechanism.[3] According to reported studies, urease activity plays a crucial role in bacterial survival in the acidic microenvironment of the stomach and supports infection persistence. Without urease, *H. pylori* become acid-sensitive and colonization ability can be sharply reduced [3]. For this reason, urease inhibition is considered a highly rational approach in anti-H. pylori drug discovery. The clinical burden of H. pylori infection is quite significant.It induces chronic inflammation that promotes gastric mucosal damage, epithelial alteration, and ulcerogenesis.It has also been reported in the literature that urease-generated ammonia may be toxic to gastric epithelial cells and may stimulate inflammatory cytokine production. This increases both disease progression and host tissue injury. [3]

Urease acts as an attractive drug target for Helicobacter pylori by hydrolyzing urea to produce ammonia. Structurally urease functions as a heterodimer that contains two distinct subunits, ureA and ureB. The active site of each dimer contains two nickel ions, which are essential for the enzyme activity.[7] The residues present in H. pylori urease active sites have four histidines (His136, His138, His248, and His274), one aspartic acid (Asp362), one carbamylated lysine (KCX219), and two nickel ions. Urease enzymes depend strictly on nickel ions (Ni^{2}). [8] [9] Blocking the urease active site by an inhibitor, the pH buffering mechanism will be disrupted, which in turn reduces colonization. Structural studies show that 1E9Y, a urease inhibitor complex, contains a deeply buried active site bounded by acetohydroxamic acid (AHA). But AHA has shown many side effects, like hemolytic anemia. Moreover it is synthetically derived,

so there is an urgent need to turn towards the natural compounds that could bind strongly and inhibit the activity of urease. [1]

Over the past two decades, the standard treatment for H.pylori has been the combination of at least two antibiotics like clarithromycin and amoxicillin with omeprazole (a proton pump inhibitor), but antibiotic resistance has been increasing, specifically to clarithromycin. [10] So there has been an urgent need to shift to another alternative for the H.pylori infection. The natural compounds as the inhibitor for the urease are one of the alternative approaches that reduce toxicity. The structural diversity of the natural products makes them more advantageous to pharmaceutical industries. Previous studies mention many natural compounds like flavonoids (luteolin, rutin, genistein, and quercetin) and other classes like saponins, diterpenes, alkaloids, and coumarins exhibited strong inhibition as well as revealed significant activity similar to synthetic inhibitors like AHA. These natural products stood to be beneficial because they offered strong inhibition alongside reduced toxicity and improved biocompatibility over synthetic compounds.[10] Another advantage of urease-targeted therapy is that it provides a mechanism different from the selective pressure of conventional antibiotics. Antibiotics primarily target bacterial growth, whereas urease inhibitors disrupt the survival niche itself. This mechanism may theoretically reduce the risk of resistance development, although long-term validation and clinical evidence are still needed. Natural molecules like Isoacetoamine have been explored based on this logic.[1]

Isochaetominine is a naturally occurring compound, a type of indole alkaloid, originally found in the Chaetomium sp. fungus that lives inside the traditional medicinal plant Ginkgo biloba. From a structural point of view, its rigid, cage-like structure which contains several important atoms like nitrogen makes it an excellent candidate for stopping enzymes from working. Specifically, it has the perfect chemical groups, like nitrogen anchors and oily (hydrophobic) prenyl side chains, that are perfectly shaped to fit into the dual-nickel active site of the H. pylori urease enzyme (PDB ID 1E9Y). In silico docking confirms that Isochaetominine forms strong, stable connections inside the active site. It creates hydrogen bonds with catalytic amino acids and crucial electrostatic attractions. The model shows it binds more tightly than the known inhibitor, acetohydroxamic acid, largely due to its larger, flat aromatic framework. Since this compound is a natural product derived from a fungus, Isochaetominine stands out as a strong novel inhibitor for fighting H. pylori. By targeting the urease enzyme which is essential for the bacteria to survive and cause infection in the stomach it offers a much needed strategy to overcome the growing problem of antibiotic resistance.

Molecular docking and ADME profiling have become essential computational tools of modern drug discovery. Docking predicts protein-ligand interactions and provides an estimate of binding orientation, affinity and active site compatibility. ADME analysis tests the pharmacokinetic suitability of a compound, such as absorption, distribution, metabolism, excretion, and toxicity related parameters. This combined approach allows researchers to shortlist promising candidates quickly and cost-effectively. These computational pipelines reduce the need for wet-lab screening making it much easier.[14]. The biggest advantage of the in silico approach is that it saves both time and cost in early-stage screening. Although many researchers have focused on urease inhibition, a lot of natural compounds stay untouched; for this reason, a large library of compounds was constructed in the present work. The practical approach to building large natural compound libraries is to apply the computational filters before experimental testing. This allows prioritizing compounds that show both strong binding

and an acceptable pharmacokinetic profile. In this specific study, over 2000 natural compounds were screened against Helicobacter pylori urease 1E9Y, using molecular docking (PyRx) and ADME profiling. In order to identify a compound that shows stronger binding affinity to the urease enzyme, screening of these natural compounds was conducted. This work focuses on a safer, potent alternative for anti-urease solutions. In this thesis, H. pylori urease, its active-site architecture, host-pathogen interaction, available inhibitor classes, natural products, its importance and the potential of Isochaetominine are presented in a detailed academic framework. This introduction builds the scientific foundation of the study and provides a comprehensive overview of the literature.

The pathogenicity of Helicobacter pylori is not limited to gastric inflammation but is also associated with host adaptation, mucosal colonization, biofilm formation, and virulence factor regulation. Recent reviews have shown that H. pylori uses multiple adaptation strategies to survive in the harsh acidic environment of the stomach, with urease playing a central role.This organism persists in the gastric mucosa by interacting with mucin 5 (MUC5AC) and host receptors.[13] In addition to urease, VacA, CagA, membrane vesicles, and host immune modulation also contribute to the pathogenesis of H. pylori infection.But urease is considered the first and most essential survival factor, because it reduces the acidic stress around the bacteria by generating ammonia. Therefore, targeting urease is a rational approach to block the early establishment of infection [11][13]. Due to widespread prevalence of H.pylori and increasing drug resistance, demand for new anti-virulence strategies is continuously increasing.[1] Urease inhibition is also attractive because it disturbs the bacterial survival niche, rather than simply suppressing growth. This strategy may also reduce resistance pressure against conventional antibiotics.[17]

2. LITERATURE REVIEW

2.1. Biological Significance of Helicobacter Pylori

H. pylori is a medically important gastric pathogen that is a major cause of chronic gastric infection. This organism uses urease and other adaptation mechanisms to survive in the acidic stomach. Long-term persistence of infection can cause the host to develop chronic gastritis, ulceration, and neoplastic risk.[11] [3] According to a review published in The Yale Journal of Biology and Medicine, fresh isolates of H. pylori express urease activity, which is essential for survival and pathogenesis. Surface-associated urease protects the bacteria against the acid exposure. This property has given H.pylori the advantage of long-term colonization in the human stomach. [3] . Infection with H. pylori not only indicates its presence in the stomach, but is also a complex example of host-pathogen interaction. This bacterium establishes long-term persistence in the gastric niche through its virulence factors and can sustain chronic inflammation. [13] This organism can transition from asymptomatic colonization to clinically significant disease by modulating the host immune response. Its pathogenesis is multifactorial, but urease remains the earliest and most essential survival component of our process[19][13]. This bacterium exploits the host niche for its survival and is linked to clinically important upper GI diseases.[17] This pathogen's adaptation capacity makes it a unique neutralophile that can tolerate stomach acid stress. A central part of this adaptation is urease-mediated acid acclimation, which supports persistence.[18][20]

2.2. Structure of Urease Enzyme

Urease is a nickel-dependent hydrolase that converts urea to ammonia and carbon dioxide. The catalytic mechanism of plant, fungal, and bacterial ureases is broadly conserved, and the active site usually contains two Ni^{2+} ions. These nickel ions substrates are critical for hydrolysis.[10][3] The active site of H.pylori Urease is highly specialized. The important active site residues are His136, His138, His248, His274, Asp362, and KCX219. These residues play an important role in the geometry of the catalytic pocket and nickel coordination. Structural studies have made this enzyme an attractive target for drug design. Urease is a highly conserved hydrolase that catalyzes urea hydrolysis and is found in multiple organisms. The catalytic core of this enzyme is nickel-dependent, so metal availability and insertion is crucial for its functionality. The specific quaternary structure of H. pylori urease gives it an unusually strong survival advantage in its host environment. Targeting its subunit organization and active site architecture is a practical and scientifically justified approach to drug design.[23] [11]

2.3. Importance of 1E9Y Structure

PDB ID 1E9Y represents the inhibitor-bound Structure of H.pylori Urease and it is used as the reference model for the docking studies[22]. The detailed arrangement of the active pocket helps in the understanding of ligand binding. This structure based approach is highly relevant in inhibitor design because it helps in analyzing the ligand binding complementary and catalytic site occupancy.[14] In recent high resolution structural work, the active site and inhibitor interaction [22] has become more clear. From this, it has become possible to understand how

inhibitors affect the enzyme through pocket fitting, hydrogen bonding, and metal interaction. Therefore, 1E9Y is important as a widely used template in modern docking workflows.

2.4. How *H.Pylori* Affects Stomach

H.pylori colonizes the mucosal stomach and triggers the inflammatory responses. Ammonia generated by the urease can damage the host tissue and worsen the local inflammation. Chronic inflammation can result in epithelial changes, ulcer formation, and in some cases risk of gastric malignancy.[11] Studies have shown that along with bacterial survival, urease have also contributed in the host pathology. Surface-associated urease has been shown to activate mononuclear phagocytes and promote the release of inflammatory cytokines. In this way urease plays a central role in both persistence and host damage.[3] During chronic persistence both immune evasion and tissue irritation occur simultaneously. This dual effect promotes bacterial survival and gradually increases disease severity [17][20]

2.5. Concept of Urease Inhibition

The basic principle of urease inhibition is to block the catalytic activity of the enzyme, so as to stop the urea hydrolysis and that would result in the reduction of the ammonia production. This can be achieved by targeting the substrate binding pocket, active site residues or nickel ions. Inhibition can collapse the acid resistance system of H.pylori.[7][10]. Multiple strategies of urease inhibitors have been discussed in the literature, including co-valent modification, metal coordination, and non-covalent pocket binding.[1] In my paper the same conceptual framework is used, in which Isocahetominine has shown a strong docking score without direct nickel binding.[14] This suggests that alternative non-chealating inhibition modes can be clinically useful.Recent work has shown that urease inhibition not only slows bacterial growth, but also weakens pathogenicity. For this reason, anti-urease compounds are being considered as adjuvant therapy [8]. The key idea of urease inhibition is to break the acid acclimation capacity of bacteria. When urease function is blocked, H. pylori become vulnerable in the acidic environment.[18][23]

2.5.1. Available Urease Inhibitor

Commercial and experimental urease inhibitors include hydroxamic acid derivatives, phosphorodiamidates, imidazoles and several synthetic scaffolds. AHA is a classic urease inhibitor, but it has several toxicity concerns. Bismuth- based approaches and combination therapies are also used in H.pylori management.[10][7] Recent review articles have also explored new urease inhibitors from the perspective of metal transfer and the urease maturation pathway. This means that not only active site binders, but also accessory protein-targeting molecules may become important in the future. Rather, accessory protein-targeting molecules may also become important in the future. Nevertheless, direct enzyme inhibition is still the most widely studied strategy.[1] [7] A clinically useful inhibitor would be one that shows an acceptable safety profile along with strong enzyme suppression. It is clear from the literature that no single inhibitor class has yet become the ideal universally accepted solution[21] [8]

2.5.2. Nickel trafficking and urease maturation

The active site of Urease becomes a functional tab when nickel ions are correctly delivered and inserted. Recent reviews have explained that nickel trafficking in H. pylori is a tightly regulated pathway in which UreD, UreF, UreG, UreE, HypA, HypB, and related proteins participate.

These proteins ensure that nickel is transferred safely and reaches the catalytic center.[24][25]. Nickel ions, although essential, can be cytotoxic, so the bacterium must maintain a delicate balance of metal import, storage, and delivery. This maturation pathway provides an additional opportunity for drug targets, as urease activation can be indirectly inhibited by inhibiting auxiliary proteins. This point is important in the thesis because your docking study focused on direct active-site inhibition, but maturation-stage interference could also be a future direction.[1][24] [25]

2.5.3. Classes of Urease inhibitors

Urease inhibitors are broadly divided into two categories: synthetic inhibitors and natural inhibitors. Synthetic inhibitors include hydroxamic acid derivatives, phosphoramidates, thiols, and metal-binding compounds. Many of these compounds show strong enzyme inhibition, but toxicity, instability, and poor selectivity are their limitations.[21]

Natural inhibitors include flavonoids, phenolic acids, alkaloids, coumarins, terpenoids, and fungal metabolites. The advantage of natural products is that they have high scaffold diversity and many molecules are already biologically tuned. Therefore, natural compounds are considered a rich source for urease inhibition [10] .

2.5.4. H. pylori urease in disease progression

H. pylori urease is not only a survival factor in pathogenesis but also influences disease progression. According to the literature, urease contributes to inflammation, mucosal damage, and ulcer development. Some newer studies have also linked the role of urease to biofilm formation and immune modulation. [15][13]. This enzyme promotes epithelial injury through ammonia production in the host stomach. This explains why urease inhibition is being strongly considered as an anti-virulence therapy. The repeated explanation of this connection contributes to the overall strength of the thesis.[1][15]

2.6. Limitation of Synthetic Inhibitors

The biggest issue of synthetic inhibitors is toxicity and low selectivity. AHAs such as hydroxamic acid-based compounds are clinically limited because of adverse effects. These compounds can provide strong metal chelation, but may also increase off-target interaction[10]. This thesis clearly mentions that AHA is very soluble but is not an ideal lead due to hydroxamic acid alerts and low molecular weight. PCA also shows CYP inhibition and catechol alerts despite being a natural control. This comparison makes it clear that safety is equally important along with potency [10]. The major drawback of synthetic inhibitors is their narrow therapeutic window and off-target toxicity. Some molecules show strong inhibition, but their long-term safety profile is not satisfactory. This balance is important in drug development so that the compound both inhibits the enzyme and remains tolerable to the host. For this reason, many researchers are shifting from synthetic scaffolds to screening natural compounds.[21] Synthetic inhibitors often show strong potency, but they can be accompanied by hepatotoxicity, instability, or off-target issues. Because of this, it takes a lot of time and effort to optimize clinically useful molecules[26]. Compounds like AHA have historical significance, but their safety margin is not ideal for long-term therapeutic use. This limitation has redirected drug discovery toward natural scaffolds.[21]

2.7. Shift Towards Natural Products

Natural products have repeatedly proven useful in drug discovery because they offer biologically relevant scaffolds and lower structural redundancy. Plant secondary metabolites, flavonoids, coumarins, alkaloids, diterpenes, and saponins have been frequently reported in the urease inhibitor literature. These compounds have often shown comparable or better urease inhibition than synthetic inhibitors [10]. The advantage of plant natural products is that they present evolutionarily optimized bioactive frameworks. These toxicity profiles are often more acceptable and provide useful starting points for medicinal chemistry optimization. For this reason, natural products are considered both a safe alternative and lead discovery source. [10] Natural products have the advantage of chemical diversity, which gives novel binding modes. In urease inhibitor research, these molecules often provide a different scaffold than standard synthetic compounds.[27] [10]

2.7.1 Natural products as safer alternatives

Literature review clearly shows that natural products are promising in urease inhibition both clinically and agriculturally. Compounds derived from plants and fungi create multiple interactions and often show lower toxicity profiles. Therefore, in drug discovery, natural molecules are repeatedly used for lead discovery [10]. The medicinal chemistry space of natural products may be more favorable in comparison to synthetic compounds because they are often preselected by evolution for biological activity. This thesis should be strong in that natural compounds may offer a safer, non-toxic, and more biocompatible anti-urease strategy. This narrative justifies our study scientifically [10]. One advantage of natural compounds is that their evolutionarily selected scaffold is better adapted to biological systems. Due to this, many natural inhibitors show comparatively lower cytotoxicity and better tolerability [13].

2.7.2. Safety of Natural Products vs. Synthetic Molecules

Natural compounds have functional group diversity and moderate lipophilicity, which may give them a more balanced ADME profile. Many natural molecules exhibit single-target or low-off-target behavior, which helps reduce toxicity. In this thesis, Isochaetominine was found to be a promising molecule in this category.[10] In the ADME analysis, Isochaetominine showed no PAINS, no Brenk alerts, no major CYP inhibition, and high GI absorption. This profile is favorable in comparison to synthetic inhibitor limitations. Therefore in this thesis natural compounds will be presented as non-toxic and more developable anti-urease strategies.[10] A major advantage of natural compounds is that their scaffolds are relatively compatible with biological systems. Due to this compatibility many natural molecules show lower alert profile and better tolerability.[27]

2.7.3 Current best approaches and limitations

The compounds that have been most effective in urease inhibition so far include hydroxamic acid derivatives, thiol compounds, and metal-binding scaffolds. But many of these compounds show off-target toxicity, poor stability, or narrow therapeutic index. Because of this, clinical translation remains challenging.[21] New reviews have suggested that inhibitors targeting the urease maturation pathway may be important in the future. Urease activation can be stopped by inhibiting auxiliary proteins and nickel transfer machinery. Mentioning this in the thesis would be valuable because it broadens the target landscape beyond only active-site inhibition.[24][1]

2.10. Origin of Isochateominine

Isochaetominine is a fungal natural product and belongs to the indole alkaloid class. It is associated with Chaetomium species, which has been reported as a fungal source linked to the internal environment of the medicinal plant Ginkgo biloba. Its chemical architecture is rigid and nitrogen-rich, which is favorable for biological activity.[28][29]

Fungal metabolites are valuable in medicinal chemistry because their scaffold is novel and they allow diverse interaction modes for enzyme-target interactions. Fungal alkaloids usually display strong bioactivity through complex ring systems and nitrogen functionalities. The rigid molecular framework of Isochaetomine is favorable for active site binding. Its aromatic-hydrophobic balance may help stabilize the ligands in the pocket.[29] The case of Isochaetominine points that natural fungal metabolites can provide promising leads in enzyme inhibition. Its use is especially relevant for targeting H. pylori urease [30] It is an indole alkaloid of fungal origin and its rigid, nitrogen-containing structure makes it a bioactive scaffold. This compound is associated with the chaetominine-related natural product family and in medicinal chemistry it is a type of fungal alkaloids considered promising bioactive leads. Its novelty appears to be even more important in the anti-urease context because direct H. pylori urease studies are limited. Isochaetominine's hydrophobic-aromatic framework and nitrogen anchors may be favorable for active site interactions. The docking results showed that it can form H-bonds and multiple hydrophobic interactions with Met366. This structural behavior makes it different and potentially safer than classical nickel-chelating inhibitors [1] Isochaetominine is associated with the Chaetomium-derived fungal metabolite family and is placed in the indole alkaloid class. Chaetomium species are an important source in natural product chemistry because they contain diverse bioactive alkaloids. Metabolites of the Chaetomium genus have been shown to have antibacterial, antioxidant, and cytotoxic properties. This means that this fungal source provides biologically rich and structurally novel molecules [31] [28]

2.10.1. Structural relevance of Isochatominine

The rigid cage-like structure of Isochaetominine is able to promote precise fit in the active pocket. Nitrogen-containing atoms and hydrophobic side regions help stabilize the binding pocket. These structural features can improve docking affinity without requiring direct metal chelation.In our results Isochaetominine showed strong interactions with Met366 by hydrogen bond and with surrounding hydrophobic residues. These interactions explain pocket occupancy and energetically favorable binding. Therefore the compound can be called a novel urease inhibitor lead scaffold.

2.10.2 Why Isochaetominine is better than synthetic inhibitors

The best advantage of Isochaetominine is its strong binding and better ADME profile combination. Synthetic inhibitors like AHA have shown efficacy but are not ideal long-term candidates due to toxicity and medicinal alerts. No CYP inhibition and no PAINS, Brenk alerts were observed in Isochaetominine, which strengthens the development potential.[26] Such profiles are important in the thesis because the researcher has to justify not only the docking score but also the drug development feasibility. In comparison, Isochaetominine has a more balanced lead scaffold. Despite being a natural compound, its potency profile is comparable or better than synthetic inhibitors.[21][10]

2.10.3. Isochaetominine and previous inhibition context

Till now there is limited literature available on direct H. pylori urease inhibition by Isochaetominine, which gives novelty to our thesis. Similar alkaloid and fungal-derived scaffolds in the natural product literature have shown promise in enzyme inhibition. In this context the docking-based evaluation of Isochaetominine is scientifically justified[30] Docking score -9.3 kcal/mol suggests that the compound can establish strong thermodynamic interactions in the active site. Getting better scores from PCA and AHA further strengthens its relevance. This result provides strong justification for future experimental work.

2.11. Role of ADME analysis

Docking in drug discovery only gives a binding estimate, but to know the actual drug potential it is necessary to look at the kinetics. Tools like SwissADME provide early estimates of absorption, solubility, CYP inhibition, and Lipinski compliance. This early filtering reduces development costs.[14]. Isochaetominine in my paper showed high GI absorption and acceptable synthetic accessibility. The CYP2D6 and CYP3A4 inhibition issues in PCA. AHA has toxicity-related medicinal alerts. Isochaetominine appears to be a stronger development candidate in comparison.

2.12. Summary of the Literature

The literature overall supports that H. pylori urease is the key enzyme for gastric survival. By inhibiting urease, bacterial survival can be weakened. Synthetic inhibitors are useful, but toxicity and resistance concerns are their limitations.[11][7] Natural products, especially fungal and plant-derived molecules, can provide safer scaffolds. The structure and docking profile of Isochaetomine supports this rationale. Therefore, the computational evaluation of the present thesis is scientifically strong and literature-supported [10].

3. METHODOLOGY

3.1. Data Collection and Preparation of Protein and Ligand

3.1.1 Retrieving and Preparing Protein Structures

The crystallized 3D structure of Helicobacter pylori urease 1E9Y protein was downloaded from The RCSB PDB website (https://www.rcsb.org/structure/1E9Y) Scientists used X-ray imaging to see the detailed structure of a protein called urease. This protein consists of different parts (alpha and beta chains) bound together with nickel ions and a specific acid called acetohydroxamic acid. Examined the protein structure in BIOVIA and traced the active site amino acid chain and got the urease 1E9Y binding site pocket in Chain A. Eliminated heteroatoms, water molecules, and all other chains except Chain A. Regions with low confidence were visually examined and eliminated for docking investigation. The final, refined model focused exclusively on the urease alpha subunit (Chain A), which contains the crucial catalytic site and the nickel-binding residues necessary for the docking. After removing any ambiguous, unclear sections, the clean structure was saved in PDBQT format, ready for the molecular docking study.

3.1.2 Building a Ligand Library

A library of 2431 natural compound's 3D Structures including our control protocatechuic acid (PCA), was prepared by searching natural products and applying the following filters criteria like molecular weight (300 to 400 g/mol), H-Bond Donors (0 to 5), H-Bond Acceptors (0 to 10), Rotatable Bonds (0 to 10), LogP (ALogP) (1 to 5), TPSA (Topological Polar Surface Area) (20 to 140), and Heavy Atoms in the PubChem site (https://pubchem.ncbi.nlm.nih.gov). Ligands were obtained in SDF format from here, and via the Open Babel tool convert all of them into PDBQT format. A virtual library consisting of approximately 2,000 natural compounds, drawing from major open-access databases [32]. The primary goal was to maximize chemical diversity and select phytochemicals with existing reports of urease or anti *H. pylori* activity. We then filtered these compounds for "drug-likeness" using established parameters This rigorous filtering ensured high synthetic accessibility and reduced the risk of pan-assay interference (PAINS). The final library size 2431 compounds provides good coverage expecting 5 to 15 percent strong hits while staying computationally practical. Smaller libraries less than 500 often miss good candidates, while much larger ones more than 10 thousand repeat similar structures, so a library of 2000 to 4000 compounds is ideal for the docking. Ligands were energy minimized using Open Babel, converted to PDBQT format, and validated by re-docking controls.

3.2. Docking and Analysis of Molecules

All molecular docking calculations were carried out using AutoDock Vina, [16] accessed through the PyRx virtual screening platform (https://sourceforge.net/projects/pyrx/).[32] The binding domain was present only in the saved chain A of the 1E9Y protein structure that was covered in the grid box (centre: X = 131.0934, Y = 123.8794, Z = 85.1455) (Dimension: X =25.000, Y = 25.000, Z = 25.000). Docking scores are the cumulative energy scores of multiple

interactions occurring during a protein ligand complex formation. The cumulative energies that a docking software like PyRx calculates are van der Waals energy, electrostatic energy, torsional penalties, desolation effects, hydrophobic effects, hydrogen bonding etc.

3.3. Integration Analysis and Visualization

Post-docking analysis was conducted by importing all results into BIOVIA Discovery Studio Visualizer 2024 (https://discover.ds.com/discovery-studio-visualizer download), which served as the primary platform for both interaction characterization and figure generation. Top poses from PyRx (RMSD) were selected from top hits. The highest ranked binding poses based on the lowest binding affinity for every 1E9Y ligand complex was imported into Discovery Studio. For every excipient, polar/nonpolar interactions, van der Waals forces, and hydrogen bonds were discovered and shown. To further understand the nature of interactions between 1E9Y surface residues and ligand molecules, binding poses were examined in both 3D structure and 2D interaction diagrams.

3.4. Natural Compound Safety Assessment

To assess drug-likeness, potential toxicity, structural stability and safety of the compounds, pharmacokinetic profiling was carried out using the SwissADME (https://www.swissadme.ch/index.php); to estimate oral bioavailability, we compared the top-ranked ligands to Lipinski's Rule of Five.Other parameters used are the physiochemical properties, pharmacokinetics, PAINS alert,and Brenk alerts This crucial filtering stage guaranteed that lead candidates have advantageous ADME characteristics appropriate for additional therapeutic development. This was done to identify the compounds with reduced risk of assay interference or toxic effect. Drug-likeness was formally assessed against Lipinski's Rule of Five, which stipulates a molecular weight ceiling of 500 g/mol, no more than five hydrogen bond donors, a maximum of ten hydrogen bond acceptors, and a Log P value not exceeding 5 with a tolerance of one permissible violation. Adherence to these thresholds served as a quantitative confirmation that the prioritized lead compounds carry the foundational ADME attributes necessary to support advancement into preclinical drug development pipelines.

4. RESULTS

The docking results showed that Isochaetominine had the lowest binding energy (-9.3 kcal/mol) than the control PCA (-5.9 kcal/mol) and AHA (-4.9 kcal/mol). Hydrogen bonding interactions were observed with Met366 in isochaetominine without Ni ion interaction. Glu222 and Asp223 with Ni ion interaction in Protocatechuic acid. Asp362, Gly279, and Ala365 along with Ni ion interaction in Acetohydroxamic acid. Table 1 summarizes the binding affinities (kcal/mol) of the top hits along with the control molecules. The visual results are shown in Figures 1, 2, and 3, emphasizing significant connections that support each ligand's docking scores and stabilizing potential. Table 2 shows the SwissADME predictive analysis re sults of the Isochaetominine, Protocatechuic acid (PCA) and Acetohydroxamic acid (AHA) studied. These three candidate molecules were compared for their physicochemical, phar macokinetic, drug-likeness properties respectively. Molecule 1 Isochaetominine (C22H18N4O4) had a molecular weight of 402.40 g/mol, a topological polar surface area (TPSA) of 95.74 ˚ A², and a consensus Log P of 1.18, indicating moderate lipophilicity. Its solubility (ESOL) was calculated as 2.94 × 10¹ mg/ml (Log S =–3.14), classifying it as soluble. Molecule 2 PCA (C7H6O4) was smaller, with a molecular weight of 154.12 g/mol, TPSA of 77.76 ˚ A², and consensus Log P of 0.65, reflecting low lipophilicity. Its ESOL solubility was 2.14 mg/ml (Log S =–1.68), placing it in the very soluble category. Molecule 3 AHA (C2H5NO2) was the smallest, with a molecular weight of 75.07 g/mol, TPSA of 49.33 ˚ A², and consensus Log P of–0.86, indicating hydrophilicity. It showed the highest solubility, with ESOL values of 434 mg/ml (Log S = +0.76), classifying it as highly soluble. All three molecules were thoroughly absorbed from the gastrointestinal tract and did not penetrate the blood–brain barrier. Molecule 1 did not inhibit cytochrome P450 (CYP). Molecule 2 was an inhibitor of CYP2D6 and CYP3A4, while molecule 3 did not inhibit CYP isoenzymes. The synthetic accessibility scores were 4.20 for Molecule 1, making it moderately complex; 1.07 for Molecule 2; and 1.30 for Molecule3.

In this study, virtual screening of 2431 natural compounds was conducted against H. pylori urease (1E9Y), and Isochaetominine was identified as the most promising hit based on docking plus ADME analysis. Binding affinity and biochemical profile both support that Isochaetominine is a better candidate than the control molecules. Docking analysis revealed that the top-ranking ligands among the screened natural compounds showed favorable binding energies, and the overall range was approximately -9.3 kcal/mol to -6.5 kcal/mol. Isochaetomine achieved the best score -9.3 kcal/mol, indicating the strongest interaction in the study. Top hits also included Versiquinazoline A (-9.1), Tryptoquivaline U (-9.0), Circumdatin E (-8.8), and Versiquinazoline H (-8.7). This result is important because lower binding energy usually shows stronger predicted complex stability. It is clear from this data that there are multiple promising scaffolds in the natural compound library, but Isochaetominine remains the most superior. In this study the control/reference molecules used were: Protocatechuic acid (PCA) and Acetohydroxamic acid (AHA/HAE). PCA showed -5.9 kcal/mol binding, while AHA scored -4.9 kcal/mol. Isochaetominine's score was clearly better than both, which supports its stronger affinity. The performance of PCA was useful as a natural control, but use was documented with binding and ADME limitations. AHA has historically been a urease inhibitor, but is not considered an ideal lead due to its side-effect profile[26]. In this comparison Isochaetominine showed advantage in both potency and developability. Isochaetominine ranked first in the docking ranking, followed by Versiquinazoline A and Tryptoquivaline U. This indicates that multiple high-affinity compounds were present in the natural product

library, but Isochaetominine showed the best overall pose and energy combination. Such ranking is used for lead selection. Isochaetominine showed key interactions in the H. pylori urease active site. According to the paper, hydrogen bonding was observed with Met366 and hydrophobic interactions were seen with MET317, CYS321, and ALA169 residues. This means the compound fits well in the active pocket and could form stable non-covalent interactions. The interesting thing is that Isochaetomine did not show direct Ni2+ coordination, yet the binding energy was the strongest. This suggests that enzyme inhibition is not just dependent on metal chelation, but pocket filling and shape complementarity also play major roles. This observation supports the safer non-chelating inhibition strategy.

PCA showed interactions with Glu222 and Asp223 in the active site, and Ni ion interactions were also reported. Its binding score was lower than Isochaetominine, despite being a known urease-related natural compound. PCA was associated with medicinal chemistry alerts and CYP inhibition concerns despite being a natural inhibitor. PCA's performance is relevant because it validated the docking system and provided a benchmark for comparison. The literature also supports PCA as a urease inhibitor, but its interaction strength and safety profile are not universally ideal. In this study PCA was not the best candidate for the strong control role. AHA showed interactions with Asp362, Gly279, and Ala365 residues and coordination with Ni ions. But its binding energy was -4.9 kcal/mol, which was the lowest performance among the three main molecules. Despite the strong solubility of AHA, its clinical utility is limited by toxicity concerns.[26]. The role of AHA was important in the study because it is the standard comparative of classical urease inhibitors. From its comparison it became clear that Isochaetominine is not only a natural product, but also an energetically more favorable lead. This has strengthened the novelty and significance of the thesis. Isochaetominine showed a favorable drug-likeness profile in SwissADME analysis. Its molecular weight is 402.40 g/mol, consensus LogP 1.18, TPSA 95.74 Å², and ESOL logS -3.14 tha. High GI absorption and no BBB permeability were also reported. Isochaetominine did not inhibit major isoenzymes in case of CYP inhibition. This feature reduces drug-drug interaction risk. Synthetic accessibility score 4.20 indicates moderate complexity, which is acceptable for practical lead optimization. The molecular weight of PCA was 154.12 g/mol, LogP was 0.65, TPSA was 77.76 Å², and ESOL was -1.68. It showed high GI absorption, but also showed CYP2D6 and CYP3A4 inhibition. Catechol-related concern was also noted in medicinal alerts. AHA was the smallest molecule, highly soluble with molecular weight 75.07 g/mol, LogP -0.86, TPSA 49.33 Å², and ESOL +0.76. But hydroxamic acid alerts weakened its medicinal chemistry profile. It is clear from this comparison that solubility alone determines drug candidacy. Overall results showed that Isochaetominine was the best candidate for both binding affinity and ADME balance. PCA and AHA played the role of useful reference molecules for docking and kinetic comparison. However, Isochaetominine also maintained a clean medicinal chemistry profile with a top binding score. Figure 4 represents the boiled egg plot, which predicts the probability of finding a molecule in the yellow region which represents the yolk region where molecules are likely to cross the BBB, or the white region which represents the high gastrointestinal absorption. Isochateominine was found to be in the white region which makes it a good oral drug candidate. The molecule was also PGP- which suggests that it was a non substrate of P-glycoprotein. These results support natural product-based urease inhibition. Having an acceptable toxicity profile with strong binding is critical in urease inhibition. For this reason Isochaetominine may be a promising lead scaffold for future development. The combined results of docking and ADME analysis revealed that Isochaetominine binds with favorable orientation in the active

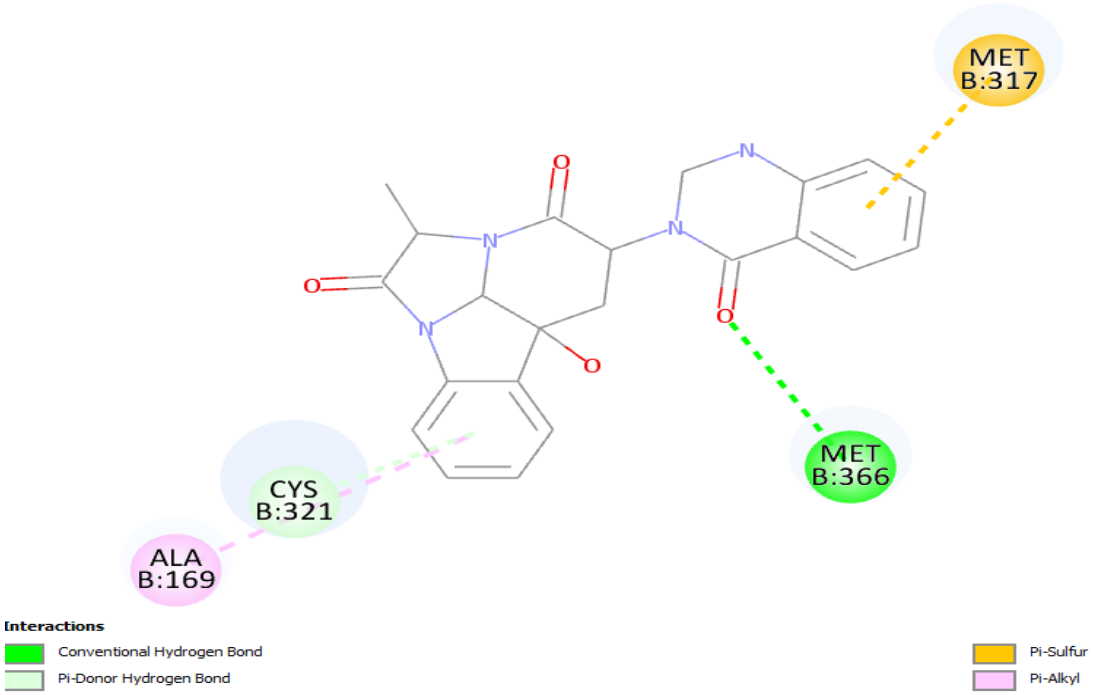
pocket of H. pylori urease and also maintains drug-like properties. Therefore, this compound should be prioritized for further experimental validation and lead optimization.

Table I: Binding Energies of 1E9Y with Selected Ligands

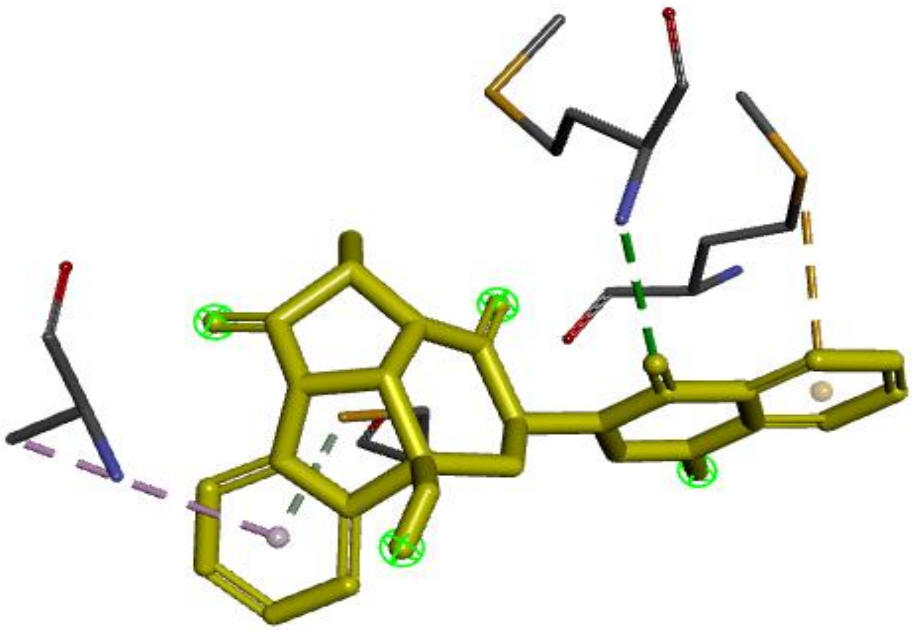
Ligands	Binding Energy (kcal/mol)
Isochaetominine	-9.3
Versiquinazoline A	-9.1
Tryptoquivaline U	-9
Circumdatin E	-8.8
Versiquinazoline H	-8.7
Paliperidone	-8.7
Rhytidenone A	-8.4
Lobarientalone B	-8.4
Circumdatin J	-8.4
Brevione K	-8.3
Aniquinazoline D	-8.3
Similanpyrone C	-8.1
Neosarphenol A	-8.1
Versicamide D	-8
Phomopsidone A	-8
Chrysogenolide E	-7.9
Aotaphenazine	-7.8
Caerulomycinonitrile	-7.2
Aspergiloid H	-7
Collismycin B	-6.9
Podophyllotoxin	-6.7
Neosartin B	-6.6
Quinolonimide	-6.5

Table II:Comparative Analysis of Swiss ADME Result

Category	Molecule 1 Isochaetominine	Molecule 2 PCA	Molecule 3 AHA
Molecular Weight (g/mol)	402.40	154.12	75.07
Consensus Log P	1.18	0.65	-0.86
TPSA A^2	95.74	77.76	49.33
GI Absorption	High	High	High
BBB Permeant	No	No	No
Solubility (ESOL)	-3.14	-1.68	+0.76
CYP Inhibition	None	CYP2D6,CYP3A4	None
Lipinski's Rule violation	No	No	No
Medicinal Alerts	None	Catechol alerts	Hydroxamic acid alerts
Synthetic Accessibility	4.20	1.07	1.30

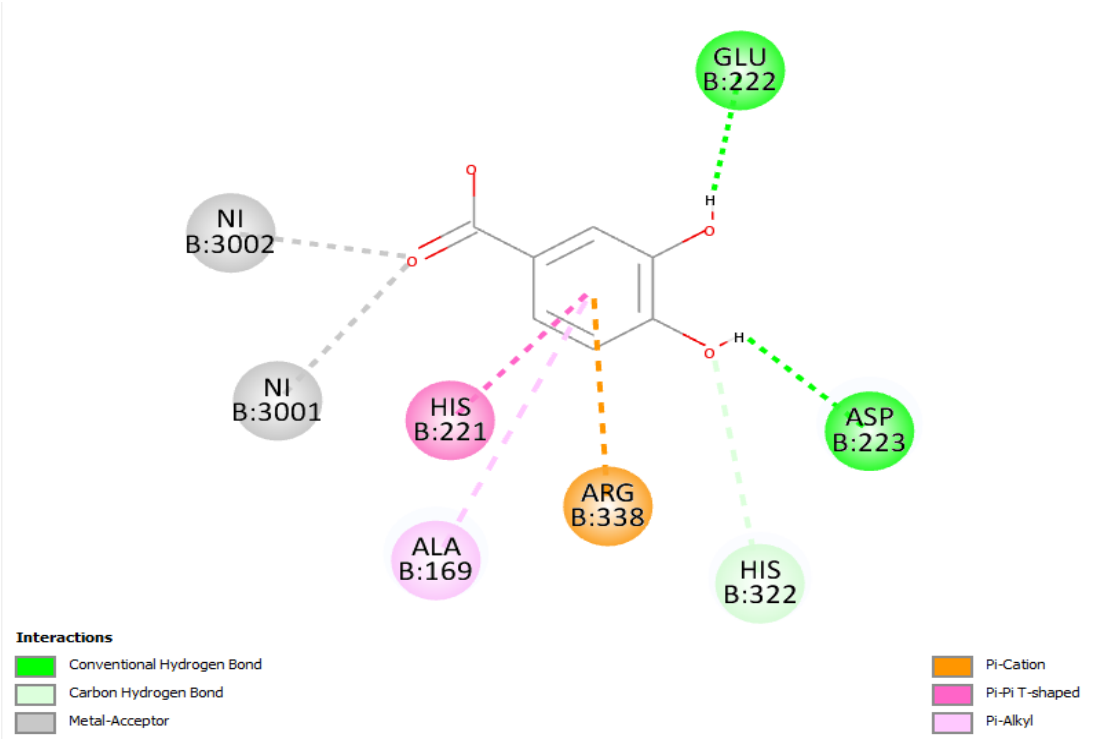


(a) 2D representations of 1E9Y- Isochaetominine complex.

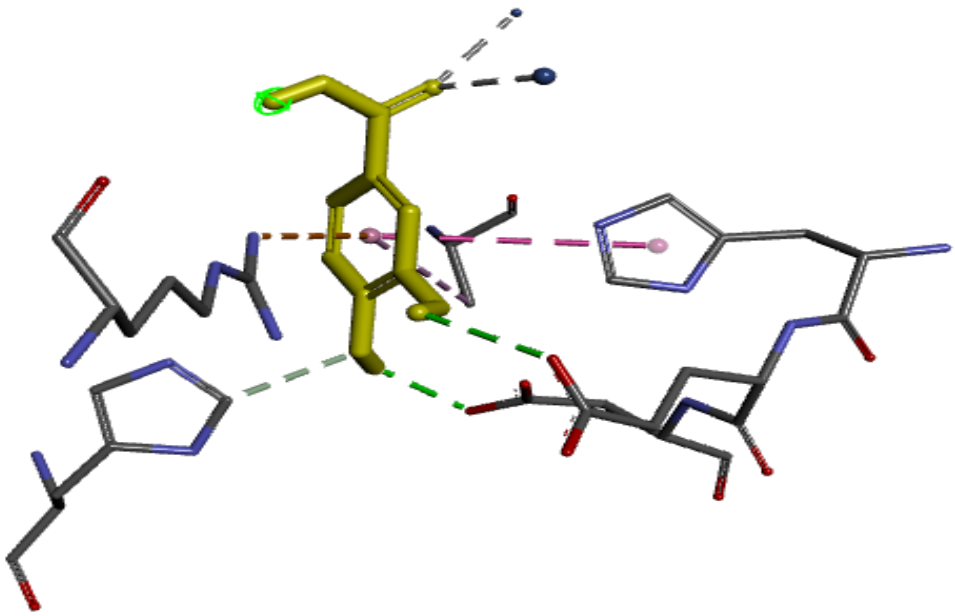


(b) 3D representations of 1E9Y- Isochaetominine complex.

Fig. 1: 2D AND 3D interaction analysis of Isochaetominine with urease protein 1E9Y, that demonstrates conventional hydrogen bonding with MET366 and hydrophobic interactions with MET317, CYS321,and ALA169 residues.

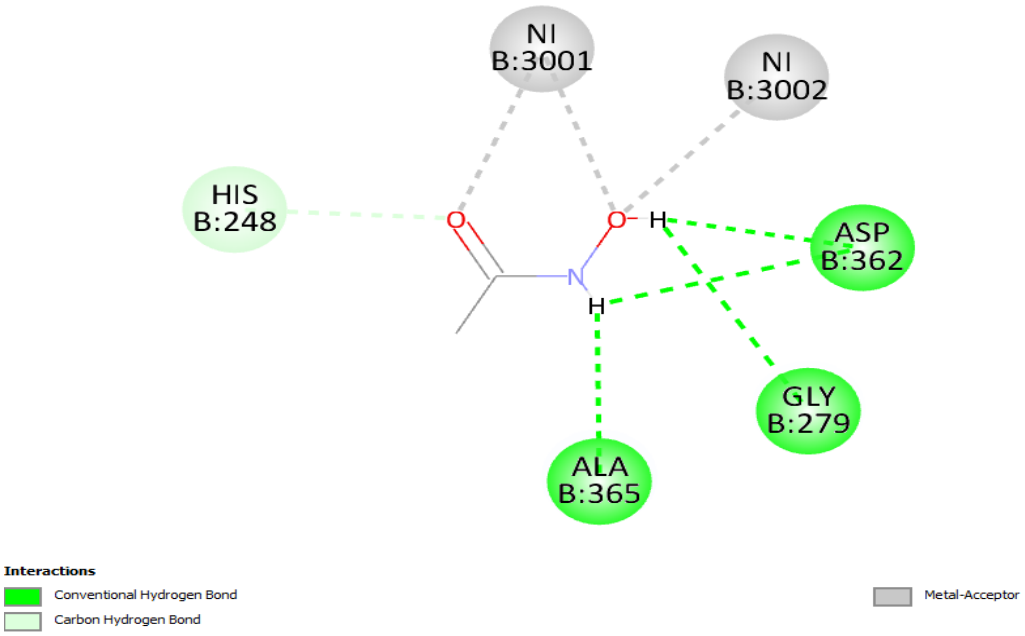


(a) 2D representations of 1E9Y- Protocatechuic acid complex.

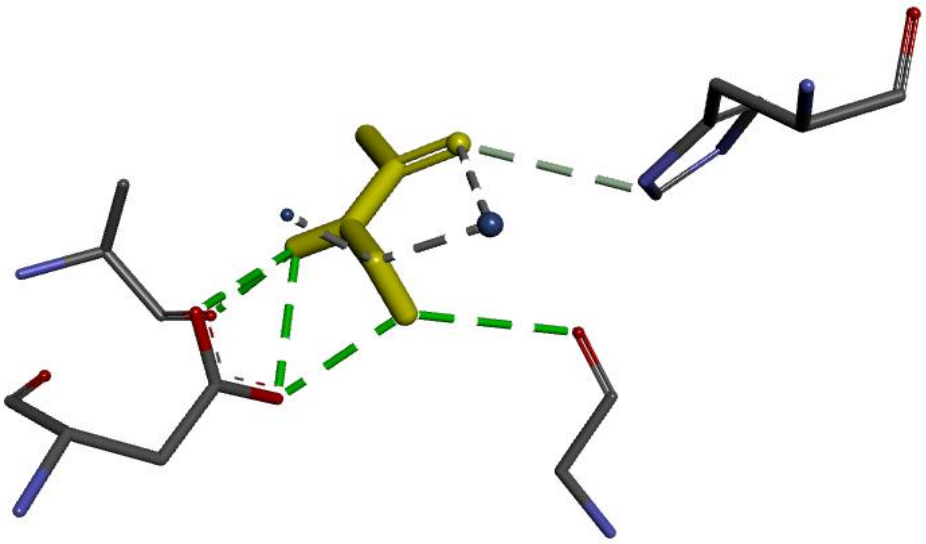


(b) 3D representations of 1E9Y- Protocatechuic acid complex.

Fig. 2: 2D AND 3D interaction analysis of Protocatechuic acid with urease protein 1E9Y, that demonstrates conventional hydrogen bonding with GLU222, ASP223; Ni ion interactions; and other interactions visible in the 2d image.



(a) 2D representations of 1E9Y- Acetohydroxamic acid complex.



(b) 3D representations of 1E9Y- Acetohydroxamic acid complex.

Fig. 3: 2D AND 3D interaction analysis of Acetohydroxamic acid with urease protein 1E9Y, that demonstrates conventional hydrogen bonding with GLY279, ASP362, ALA365; Ni ion interactions; and and carbon hydrogen bond interactions visible in the 2d image

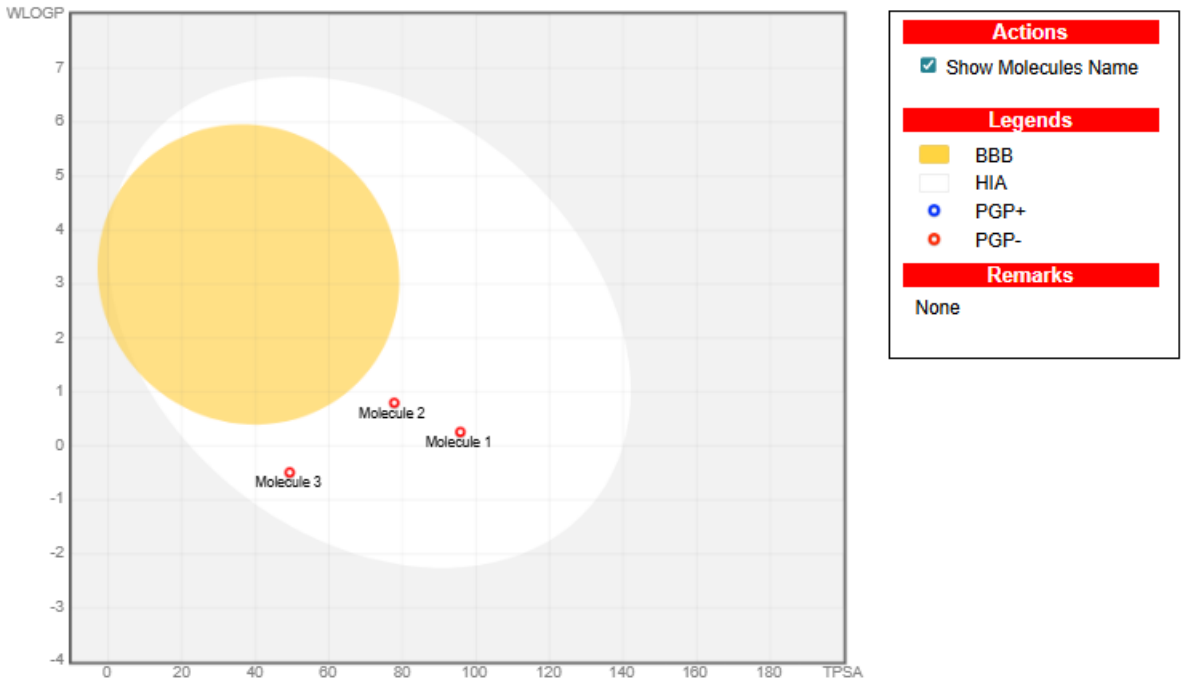


Fig. 4: Boiled-Egg plot representing the HIA and BBB permeation of molecule 1 Isochaetominine, molecule 2 PCA and molecule 3 AHA. All are p-glycoprotein non substrates (PGA-) indicated by the red circles.

5. DISCUSSION

The present work gives in-silico evidence of the strong inhibition of the urease enzyme by Isochaetominine. Molecular docking simulation was performed to evaluate the binding affinity of 2431 natural compounds against Helicobacter py lori. The binding energies ranged from-9.3 kcal/mol to 4.3 kcal/mol and the main control PCA is-6.4 kcal/mol, indicating that more than half of the selected compounds had a thermodynamically favorable interaction with the 1E9Y compared to our selected control PCA. The top 30 natural compounds on the other hand, were significantly better than PCA, the control compound (-6.4 kcal/mol). Comparative ADME analysis showed that Isochaetominine has balanced lipophilicity (Log P = 1.18), acceptable solubility (0.294 mg/ml) and no medicinal chemistry alerts, despite the higher molecular weight (350 g/mol). PCA is smaller and more soluble (2.14 mg/ml) but hindered by catechol-related PAINS and Brenk alerts and CYP inhibition, raising toxic concerns and drug-drug interactions. AHA is very soluble (434 mg/ml) and easy to synthesize, but hydroxamic acid alerts for instability and toxicity, and its very low molecular weight (75 g/mol) limit its potential as a viable lead compound. Isochaetominine shows strong overall affinity via protein ligand interactions, despite the lack of direct binding of Ni2+. This could reduce off-target metal chelation toxicity and provide a safer and more selective inhibition mechanism than Ni2+ chelating compounds. For urease inhibition several factors contribute to the binding affinity like H-bonding, hydrophobic bonding, shape complementarity inside the active pocket, van der Waals interactions, electrostatic interactions and metal coordination. For Isochaetominie, no direct nickel coordination was observed yet it showed stronger binding energy which suggests that it could inhibit urease because of the stronger overall active site fitting, strong hydrophobic interactions, better spatial occupancy of the binding pocket and stronger cumulative non-covalent interactions. That‘s why even without nickel binding, the total interactions may be energetically more favorable than the control compound.

This study clearly shows that Isochaetominine is a strong computational lead against H. pylori urease (1E9Y), because it gives the lowest binding affinity and an acceptable ADME profile. The results also support the idea that natural products can be safer and more developable than classical synthetic urease inhibitors when both potency and drug-likeness are considered. [10] . Isochaetomine showed a docking score of -9.3 kcal/mol, which was better than PCA (-5.9 kcal/mol) and AHA (-4.9 kcal/mol). This difference suggests that Isochaetominine may occupy a more stable position in the active pocket and form a stronger protein-ligand complex. In docking studies lower energy generally indicates stronger predicted binding.[16] This result is important because H. pylori plays a central role in urease pathogenesis. According to the literature, urease protects H. pylori from gastric acid and supports survival through ammonia generation. Therefore, strong urease inhibition is a meaningful strategy for infection control. Isochaetomine showed hydrogen bonds with Met366 and hydrophobic interactions with Met317, CYS321, and ALA169. These interactions improve pocket occupancy and binding stability. Despite not having direct Ni2+ coordination, the compound's score remained strong, indicating that non-chelating inhibition may also be effective. Nickel coordination has traditionally been considered important in the urease literature, because the two nickel ions in the active site are essential for catalytic function. But latest thinking also means that pocket complementarity, hydrophobic fit, and hydrogen-bond network also play major roles in inhibition. Isochaetomine results support this idea.

The PCA score was clearly lower than Isochaetominine, although PCA was a useful benchmark as a natural control. In the case of PCA, CYP2D6 and CYP3A4 inhibition was shown, suggesting potential drug-drug interaction risk. In the literature, protocatechuic acid has been considered a bioactive molecule, but it is not the ideal lead in every pharmacological context. [33] AHA is the classic reference compound as a urease inhibitor, but its clinical use is limited by toxicity concerns. In your data AHA's binding score was the weakest among the three main molecules, despite high solubility. This result shows that solubility alone does not guarantee efficacy. Natural products have been important in anti-urease research for a long time. Review literature suggests that plant and fungal metabolites provide diverse scaffolds for urease inhibition.[10] These compounds are useful in both clinically and agriculturally contexts. The major advantage of natural compounds is that their chemical space is broad and they allow multiple binding interactions. This feature makes them interesting from synthetic single-function molecules. Finding top hits from the natural library screened in your thesis supports that natural product-based screening is an effective strategy.

Isochaetominine is an alkaloid of fungal origin and belongs to the group of chaetominine-type structures.[28][29] Such alkaloids are often rigid and stereochemically rich, which helps in selective fitting into enzyme pockets. The bioactivity of fungal metabolites is well known in medicine, especially in antimicrobial and anticancer contexts [34]. In this study Isochaetominine showed superior binding against H. pylori urease. This result suggests that fungal-derived alkaloids should be explored more systematically in anti-urease drug discovery. Because of its scaffold novelty, Isochaetominine can be a strong starting point for lead optimization. Isochaetominine showed high GI absorption and no BBB permeability. This is a favorable sign for oral therapeutic development because unnecessary exposure to systemic CNS penetration is avoided. Major CYP inhibition being absent reduces drug-drug interaction risk. Synthetic accessibility score 4.20 shows moderate complexity. This score is acceptable for practical medicinal chemistry development, because the molecule is neither too simple nor too complex. Isochaetominine's combined kinetic and interaction profile was better compared to PCA and AHA.

Along with strong binding, safety is equally important in drug discovery. Medicinal chemistry concerns were observed in both AHA and PCA, whereas PAINS and Brenk alerts were not observed in Isochaetominine. This difference is critical for compound selection. Natural products often help reduce toxicity issues because they are biologically pre-validated scaffolds. Isochaetominine's no-alert profile in my study supports the narrative. On this basis it can be called a safe lead scaffold.[10] Some classical urease inhibitors block the enzyme by chelating nickel ions. But strong metal chelation may also lead to the risk of off-target binding and toxicity. Isochaetomine showed strong binding without direct Ni2+ interaction, which is indicative of a safer mechanism.[21] This point is especially important because selectivity is crucial in targeted enzyme inhibition. If the compound can fit into the active pocket without aggressive metal sequestration, adverse effects can be reduced. Therefore, the inhibition mode of Isochaetominine is attractive from the perspective of development.[21]. Antibiotic resistance is the biggest clinical challenge in pylori treatment. The effectiveness of standard eradication regimens is decreasing due to Clarithromycin resistance. In this background, urease-targeted natural inhibitors may provide an alternative strategy [7]. Urease inhibition may not directly kill bacteria, but may reduce infection burden by disturbing colonization and acid survival.

This can be very useful for adjuvant or combination therapy models. Isochaetominine seems to be a promising candidate in this direction.

The major significance of this study is that it identified a novel, structurally attractive, and biochemically acceptable lead from a large natural compound library. Isochaetominine showed superior performance in both docking and ADME. This computational evidence provides strong justification for experimental validation. Literature review and current results taken together support that natural products will play an important role in future anti-urease therapy. The case of Isochaetominine makes the narrative stronger. Therefore, the conclusion of the thesis is scientifically solid. [10]. This study is purely computational, so biochemical validation is still needed. Docking score represents predicted affinity, not actual inhibitory potency. Therefore, in vitro urease assay, kinetic study, and MD simulation are the next steps [16]. Another limitation is that docking is based on a single static protein structure. Protein flexibility and solvent effects are not fully captured. Therefore, more robust evidence can be obtained from dynamic simulation in future studies.[14] Overall, results indicate that Isochaetominine has the best combination of binding strength, interaction quality, and ADME profile among the selected molecules. PCA and AHA remained useful comparators, but their limitations made Isochaetominine more prominent. Therefore, Isochaetominine represents a promising natural scaffold for H. pylori urease inhibition. The urease inhibitor literature is now not limited to only active site binding, but nickel trafficking and maturation proteins are also being targeted. From this perspective the non-chelating binding mode of Isochaetominine seems more attractive. This gives a chance to avoid off-target metal chelation toxicity.

5. CONCLUSION AND FUTURE PERSPECTIVE

In this work, an In Silico docking approach was used to analyze the anti-urease activity of 2,431 natural compounds against Helicobacter pylori. The results proved that Isochaetominine showed the highest binding energy against the urease enzyme, -9.3 kcal/mol, which was higher than the control compound, PCA, -6.4 kcal/mol, and placed in the top 30 natural inhibitors. To our knowledge, this is the first study to explore Isochaetominine as a specific urease inhibitor to treat H. pylori infections. Isochaetominine acts as a promising lead scaffold for the development of novel anti-urease therapies that neutralize H. pylori without applying selective pressure for resistance. The main conclusion of this thesis is that Isochaetominine is a promising natural inhibitor against H. pylori urease (1E9Y). In docking analysis this compound showed binding affinity of -9.3 kcal/mol, which was better than both PCA and AHA. This makes it clear that Isochaetominine can form strong and stable interactions in the active site. The role of urease is central in H. pylori infection, as it helps the bacterium survive in the acidic stomach environment. Therefore, targeting urease is a scientifically valid strategy for infection control. Your results supported this strategy and showed that potent urease inhibitors can be found in natural compounds. SwissADME analysis also showed that Isochaetominine's Pharmacokinetic Profile is acceptable. It has high GI absorption, no BBB permeability, no major CYP inhibition, and no PAINS/Brenk alerts observed. This profile makes it more suitable for oral drug development compared to PCA and AHA. PCA and AHA are useful reference molecules in comparison, but both have limitations. In PCA there are CYP inhibition and medicinal alerts, while in AHA there are hydroxamic acid-related concerns. Therefore, Isochaetominine becomes the best candidate in the balance of potency and safety. Overall, this study suggests that Isochaetominine can be considered as a novel lead scaffold for H. pylori urease inhibition. The scientific value of this thesis lies in that it has supported natural product-based drug discovery in a practical and safe direction.

The future scope of this study is quite strong because it is purely computational work and still requires experimental validation. In the next step, in vitro urease inhibition assay should be done so that the docking results can be verified at the biochemical level. If Isochaetominine reduces enzyme activity, its actual inhibitory potency and IC50 can be determined.

After this, molecular dynamics simulation should be run so that the stability of the protein-ligand complex can be studied in a time-dependent manner. Docking gives a static snapshot, but MD simulation better captures binding persistence, conformational changes, and solvent effects. This step will more strongly support the real binding behavior of Isochaetominine.[12]. Moving forward, structure-activity relationship (SAR) studies will also be useful. By making small modifications to the scaffold of Isochaetominine, it is possible to achieve better potency, better solubility, or lower toxicity. By medicinal chemistry optimization this natural product can be converted into a stronger lead compound.[12] [10]. Another important future direction is biofilm inhibition studies. H. pylori biofilm formation can play a role in treatment resistance, so it is important to see the anti-biofilm effect of the compound. If Isochaetominine also affects biofilm, its therapeutic relevance will be further increased. Cell-based assays and toxicity studies should also be conducted in the future. These studies will assess the compound's cytotoxicity, selectivity, and host-cell safety. This information will be critical for preclinical development. If future experiments give positive results, Isochaetominine may also be explored as part of combination therapy. This can help in reducing antibiotic resistance pressure. Such natural product-based urease inhibitors could be valuable additions to H. pylori eradication

strategies. Future prospects should suggest not only in vitro assay and MD simulation, but also studies on urease maturation pathway. Nickel transfer proteins, UreE/UreG complex, and accessory proteins may be future drug targets. If a derivative of Isochaetominine is designed, its potency can be further improved. Going forward, biofilm assays, cytotoxicity assays, selectivity profiling, and combination studies will also be valuable. The possibility of taking this compound to the translational stage can be explored.

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CONFERENCE PRESENTATION

Conference paper presentation

"Molecular Docking and In Silico Evaluation of Isochaetominine as a Novel Inhibitor of Helicobacter pylori Urease (1E9Y), "Presented at International Conference on Reliability Engineering in Design Practices (ICRED-26), Pune, India, 2026.

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