Original Article

Assessment of Some Aminoglycoside Drugs Repurposing as *Helicobacter pylori* Urease Enzyme Inhibitors by *In silico* and *In vitro*

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Abstract

Background: A *Helicobacter pylori* is a Gram negative bacterium associated with gastrointestinal tract disease. *Helicobacter pylori* can grow in extreme acidic condition like stomach under microaerobic conditions. The bacterium colonizes the antrum of the stomach, causing gastritis and peptic ulcers. The urease enzyme produced by *H. pylori* is essential for bacterial survival in the acidic stomach environment, making it a target for drug therapy. **Objective:** This study aims to assess of some aminoglycoside approved drugs including paromomycin sulfate, tobramycin, gentamicin, amikacin, and capreomycin sulfate as *H. pylori* urease inhibitor in order to prevent catalysis action of urease to convert urea to ammonia by using of molecular docking. **Materials and Methods:** Virtual screening was performed to identify potential *H. pylori* urease inhibitors. Molecular docking simulations were conducted using the Glide software, and several compounds from the zinc15 database were analyzed concerning *in silico*. Among the top-ranked compounds, paromomycin, tobramycin, amikacin, capreomycin, and gentamycin exhibited strong binding affinity to the *H. pylori* urease protein. The inhibitory effect for purified urease was performed by using drug candidate to bind in active site of urease. Also inhibition of urease enzyme *in vitro H. pylori* isolation, diagnosis by using PCR (polymerase chain reaction). Inhibition of urease by aminoglycosides demonstrate a clear dose-dependent response for local strain increasing concentration of aminoglycosides caused gradual decrease in urease activity for local strain. **Results:** *In silico* results of molecular docking indicate that the aminoglycoside which includes paromomycin, amikacin, gentamicin, tobramycin, and capreomycin can bind to urease enzyme at active site. The experimental results indicate that paromomycin, amikacin, gentamicin, tobramycin, and capreomycin have inhibitory effects on urease activity. Results demonstrated dose-dependent inhibition of urease activity by the drugs, with a difference of IC50 for each drugs, respectively. There was a significant difference at $P \leq 0.05$ between the inhibitory performance on local compared to reference strain. **Conclusion:** Aminoglycoside emerges as a promising urease inhibitor for *H. pylori* infection. Molecular docking regarding the relatively highest affinity toward to the *H. pylori* urease, and *in vitro* studies provide valuable insights for potential drug development against *H. pylori*-induced gastrointestinal diseases. Further research is needed to explore the clinical efficacy of capreomycin, paromomycin, amikacin, gentamicin, tobramycin in managing *H. pylori* infections and have antimicrobial activity and direct anti-urease effect on urease produce by *H. pylori*.

Keyword: *H. pylori*, molecular docking, inhibitory, PCR, urease

Introduction

Helicobacter pylori was first identified in 1983 by two researchers Barry Marshall and Robin Warren.[1] *Helicobacter pylori* is a microaerophilic Gram negative bacterium that requires low oxygen levels for survival. Firstly, known as *Campylobacter pylori*, and its name comes from its spiral or helical structure. *Helicobacter*

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pylori produces many enzymes which are important in the development of peptic ulcer disease such as urease, catalase, and oxidase. For the cultivation of *H. pylori*, selective media and microaerobic conditions are required, such as environment with 80%-90% N_2, 5%-12% CO_2, and 5%-8% O_2 at 37°C for 3-7 days. *Helicobacter pylori* may be grown on both selective and non-selected agar, such as *H. pylori* special, Columbia base agar, and brain-heart infusion.[2,3] *Helicobacter pylori* is a microaerophilic Gram negative, bacterium and is recognized as a major cause of chronic gastritis and peptic ulcer disease and as an important risk factor for gastric cancer.[4]

Urease produced by *H. pylori* catalysis hydrolysis of urea to yield ammonia and carbon dioxide, the urease help the bacteria to colonies at the acidic pH of the stomach through neutralizing gastric acid while supplying ammonia for bacteria to protein synthesis. Thus, urease triggers the development of gastritis, duodenal ulcers, gastric cancer, and other clinical complications. Therefore, urease is the ideal target for the treatment of *H. pylori* infection and *H. pylori*-induced gastrointestinal disease.[5]

Helicobacter pylori urease is a cytoplasmic protein that can also be detected on the bacteria's surface, likely as a result of the lysis of bacterial subpopulations susceptible to stomach acidity. *H. pylori* urease is central metabolism and virulence for *H. pylori* is necessary for its colonization of the gastric mucosa and is a potent immunogen that elicits a vigorous immune response.[6]

Molecular docking is an automated technique to simulate the interaction between a drug and a receptor molecule. It is a crucial method in the development and discovery of drugs, it is allowing researchers to find possible drug candidates that may bind to and influence the activity of a certain target protein. Molecular docking process depends on binding mechanism and affinity of a ligand molecule with a target receptor molecule. This is accomplished by modeling the interaction of two molecules using many of theoretical techniques and software tools.[7]

Docking-based screening entails docking a library of compounds into the urease active site and assessing each compound's binding affinity. The process of finding essential pharmacophore traits that are critical for urease inhibition and screening a library of drugs for those qualities is known as pharmacophore-based screening. Overall, molecular docking is a useful method for finding possible urease inhibitors and driving the development of novel antimicrobial medicines.[8]

Materials and Methods

Helicobacter pylori isolation and urease purification

The local *H. pylori* strains were collected, a total number seventy 70 patients' samples (biopsy and stool) pervious *H. pylori* test result, isolated from peptic ulcer patients from the endoscopy unit at the Karbala Center for Gastroenterology and Hepatology at Al-Hussein Teaching Hospital and directly diagnosed by PCR (polymerase chain reaction). PCR assay was performed to detection of *H. pylori* based on 16S rRNA gene, genomic DNA was extracted directly from bacteria and using FAVORGEN genomic DNA extraction kit. The reference *H. pylori* NCTC11916 has been purchased from UK Health Security Agency (UKHSA). The growth promotion of reference isolate NCTC (11916) according to recommended manufacturer. Then extractions and purified of urease enzyme by different method from both local and references *H. pylori* NCTC11916 strain to obtain of purified urease used in this study.[9,10]

Candidate urease inhibition drug *in silico*

Generation of FDA approved drugs library

All Food and drug administration (FDA) approved drugs such as amino glycoside by using of molecular docking technique.[11] The retrieved structure date file (SDF) were consolidated and stored in a single SDF. This file served as a repository for all the obtained structures before further preparation for docking.[12]

Retrieval of protein

The protein data bank (PDB) ID 6ZJA represents *H. pylori* urease with inhibitor bound in the active site. The resolution of this native structure was 2.0 Å, and includes both alpha and beta sub unit and cover 100% of the protein. Within this all amino acids that involve in active site are presented.[13]

Preparation of ligands for docking

The ligand structures were prepared for docking using the Grid-Based Ligand Docking with Energetics (GLIDE) software's ligand preparation script (https://www.schrodinger.com/products/glide). Conformers were generated based on ionic states, stereochemistry, and ring conformations. OPLS4 force field was used to calculate partial atomic charges. A maximum of thirty-two low-energy conformers were generated per ligand, and the ligand size was limited to 500 atoms. The pH range for conformer generation was set to 7.0 ± 2.0. The resulting conformers were saved in a single file and used for downstream virtual screening.[14]

Preparation of protein for docking

The protein preparation wizard script was utilized to optimize and refine the structure of *H. pylori* urease. Several efforts were taken to improve the quality and dependability of the protein structure for future computer analysis.[15]

The protein structure was first given bond instructions to ensure correct atom connection. Hydrogen atoms were introduced into the protein at their predicted places. In

addition, zero-order bonds for the metal ions in the framework were formed. Water molecules beyond a distance of 5 from the protein were eliminated to increase the efficiency of future computations. This narrowed the scope of the study to the immediate protein environment.

The root means square deviation (RMSD) cutoff value of 0.30 was used to do energy minimization at a pH of 7.0. The Optimized Potentials for Liquid Simulation 4 (OPLS4) force field was used to do the optimization. A more precise depiction of the protein's behavior at the specified pH is possible thanks to this force field, which takes into consideration the interactions and dynamics of the protein. These steps ensured that the *H. pylori* urease structure was refined and optimized for subsequent computational analyses, providing a reliable foundation for further investigations.[16]

Docking protocol

In accordance with the findings of Cunha 19 specific amino acid residues from the beta subunit were identified as active site participants in *H. pylori* urease.[17] These residues include HIS13, ILE137, HIS138, ALA169, ILE220, HIS221, GLU222, ASP223, HIS274, ALA278, GLY279, HIS314, MET31, CYS321, ARG338, ASP326, ALA365, and MET366 as depicted in Figure 1.

To facilitate ligand docking, these residues were selected as potential binding sites using the GLIDE receptor grid generation function. The van der Waal radii of the receptor atoms were scaled by 1.00 Å, whereas the partial atomic charge was set to 0.25 Å. A grid box with coordinates *X*, *Y*, *Z* = 30 Å was generated at the centroid of the *H. pylori* urease active site. For the subsequent docking analysis, the extra precision docking protocol was employed utilizing the Glide software, which is integrated into the Schrodinger package Maestro Version 12.8.117, MMshare Version 5.4.117, Release 2021-2. This protocol allows for a more accurate assessment of ligand binding within the active site of the *H. pylori* urease.[18]

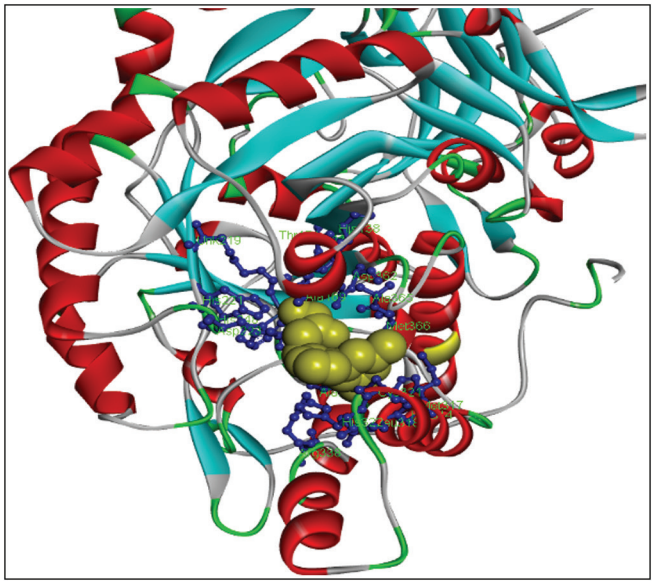

Figure 1: *H. pylori* urease with inhibitor (yellow CPK structure [three dimension] represent *H. pylori* urease with inhibitors) bound in the active, the active site amino acids represented as blue ball and sticks structures

Inhibition of urease *in vitro* study

The inhibition of urease by drug was done according to with some modification[19] as following:

1. A volume of 10 µL of phosphate buffer and 10 µL of distilled water was added into the microplate.
2. To initiate the enzymatic reaction, 20 µL of purified enzyme from each strain was added to the plate.
3. Subsequently, 20 µL of each drug incudes paromomycin sulfate, tobramycin, gentamicin, amikacin, and capreomycin sulfate, at a concentration of 3.2 mg/mL and subjected to serial dilution (third dilution), was added to their respective wells on the plate.
4. Following the enzymatic reaction, 40 µL of urea was added to the plate, and a waiting period of 10 min at 37ºC was observed.
5. Urease activity was assessed by quantifying the release of ammonia from urea using a modified Berthelot reaction.
6. Following which the IC50 (half maximal inhibitory concentration) was measured. The IC50 serves as an indicator of the drug's potency in inhibiting the urease enzyme.
7. The same aforementioned steps were performed for the standard strain urease sample (NCTC 11916). Additionally, control samples were prepared on the plate, consisting of the local and standard strains for urease with all additives without inhibitor, and inhibitor without enzyme. These control samples were employed for comparative analysis.[20]

Statistical analysis

Statistical calculations were performed using SPSS software (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. IBM Corp., Armonk, New York, USA) and Microsoft Excel (2010, Microsoft Corp.). All the results were expressed mean.

P-value < 0.05 was considered statistically significant. Analysis of variance was employed to evaluate the presence of significant differences. Unpaired-sample *T* test was employed to evaluate the presence of significant differences for the same drug diluted concentrations at the two strains. Least significant difference (LSD) was carried out to find the significant difference in mean concentrations comparing all the drugs diluted concentrations using GenStat software.[21] A one-way analysis of variance (ANOVA) was conducted to determine whether there were significant differences in group variance.[22]

Results

Virtual screening

To identify the compound most likely to act as the *H. pylori* urease inhibitor 6ZJA from zinc15 database molecular docking simulations for each compound in the library using programs were carried out.

The molecular docking found 331 of compound which can be bind to 6ZJA. This study used the five drugs with highest docking scores by program including paromomycin, tobramycin, amikacin, capreomycin, and gentamycin [Table 1, Figures 2-10].

In this study, molecular docking was performed using the Glide software to predict the binding affinity and ligand efficiency of these drugs. The Glide docking scores represent the predicted binding affinity, where lower scores indicate stronger binding to the target protein.

Comparing the Glide docking scores, paromomycin achieved the highest negative score of -15.339, suggesting a potentially strongest 'binding affinity with the target protein. gentamycin and tobramycin, amikacin, capreomycin with docking scores of (-13.864 to -12.192), respectively.

In vitro assessment the inhibitory performance of drug candidate upon urease

This study investigated the effect of FDA approved candidate drug such as paromomycin, tobramycin, gentamycin, amikacin, and capreomycin on the urease activity of local and references strain (NCTC 11916) of *H. pylori* then making a comparative study between two effect according to inhibitory activity. The IC50 values for paromomycin were reported as 66.85 µM and 21.55 µM for the local and reference strains, respectively. There was a significant difference at $P \leq 0.05$ between the inhibitory performance on local compared to reference strain [Figures 11 and 12].

The study found that increasing concentrations of paromomycin led to a dose-dependent reduction in urease activity for both local and reference strains. At the lowest concentration (0 µM), urease activity was 2 U/mL for both strains. As paromomycin concentrations increased, urease activity consistently decreased. At the highest concentration (528.24 µM), urease activity reached 0 U/mL, indicating

Table 1: Docking scores of selected drugs

Drug name	Chemical structure	Zinc Id	Glide docking score
Paromomycin	C23H45N5O14	ZINC000060183170	-15.339
Gentamycin	C21H43N5O7	ZINC000242437514	-13.864
Tobramycin	C18H37N5O9	ZINC000008214692	-13.035
Amikacin	C22H43N5O13	ZINC000008214483	-12.928
Capreomycin	C25H44N14O8	ZINC000150338698	-12.192

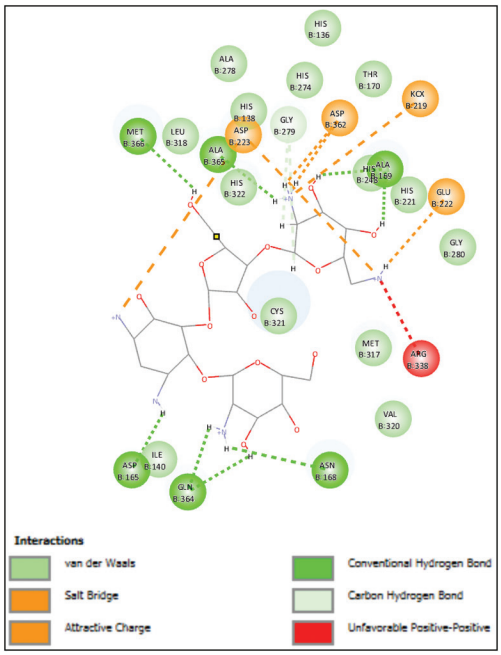

Figure 3: 2-dimensional interactions between paromomycin and urease enzyme interactions with amino acids in active site

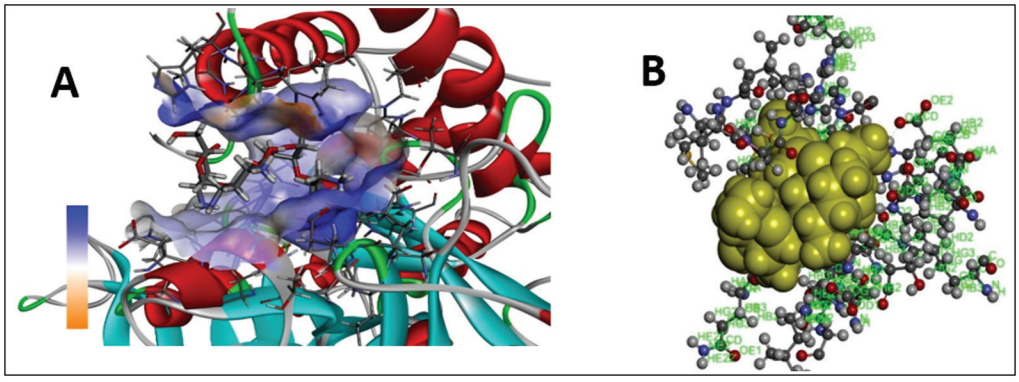

Figure 2: (A) 3-dimensional *H. pylori* urease with paromomycin (CPK structure [three dimension] represent *H. pylori* urease with inhibitors) bound in the active site. (B) The active site represented as ball and sticks structures yellow color paromomycin interaction with urease enzyme

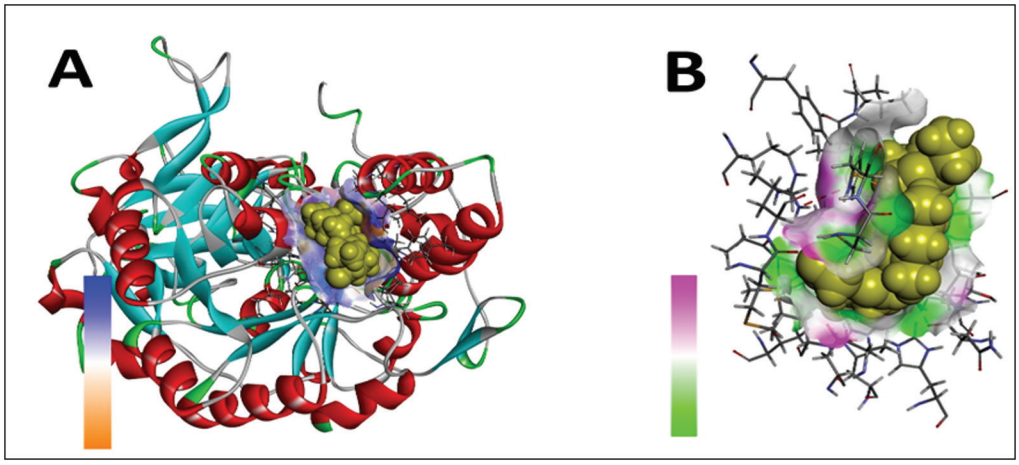

Figure 4: (A) 3-dimensional *H. pylori* urease with tobramycin (CPK structure [three dimension] represent *H. pylori* urease with inhibitors) bound in the active site. (B) The active site represented as ball and sticks structures yellow color tobramycin interaction with urease enzyme

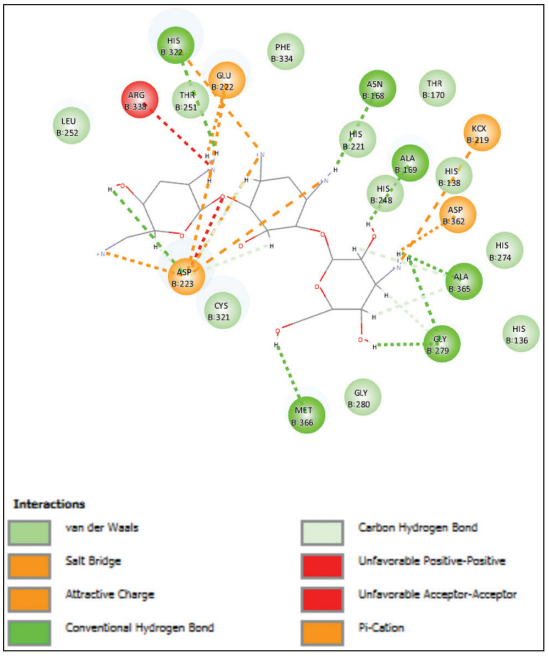

Figure 5: 2-dimensional interactions between tobramycin and urease enzyme interactions with amino acids in active site

effective inhibition. Lower paromomycin concentrations allowed urease activity to recover gradually.

Similarly, tobramycin exhibited concentration-dependent effects on urease activity. Urease activity declined as tobramycin concentrations increased. At the highest concentration (695.60 µM), urease activity was completely inhibited (0 U/mL). Lower concentrations allowed urease activity to recover partially.

Gentamicin also showed concentration-dependent effects on urease activity. As gentamicin concentrations increased, urease activity decreased. At the highest concentration (680.96 µM), urease activity was fully inhibited (0 U/mL). Lower concentrations allowed partial recovery of urease activity.

amikacin and capreomycin exhibited similar trends. Increasing concentrations of amikacin and capreomycin led to decreased urease activity. At their highest concentrations, both antibiotics completely inhibited urease activity. Lower concentrations allowed some degree of urease activity recovery.

IC50 values were reported for each antibiotic, representing their inhibitory concentration for 50% reduction in urease activity. The study noted significant differences in inhibitory effects between the local and reference strains. These findings were illustrated in Figures 12 and 13.

The IC50 values of different drugs, including paromomycin, tobramycin, gentamicin, amikacin, and capreomycin sulfate, on urease activity in both local strains and reference strains as shown in Figure 14.

The results of the analysis indicate that gentamycin and capreomycin shows a significant difference when compared to the other drugs (amikacin, paromomycin, and tobramycin) for the local strain of urease.[23] This finding suggests that gentamycin and capreomycin may have a distinct effect for urase inhibition of the local strain as compared to the other drugs.

It is crucial to highlight that in this study, the significance level (P = 0.05) and the LSD (1.002) were employed. Table 2 shows how these numbers assist define the threshold for detecting substantial differences between the medications tested.

Capreomycin showed that lowest IC50 for both local and reference strain that indicate the capreomycin was best drug for urease inhibition produced by *H. pylori*.

- Similar later without significant difference
- Different later with significant difference

The analytical findings demonstrate that paromomycin and capreomycin vary significantly from the other medicines (gentamycin, amikacin, and tobramycin) for

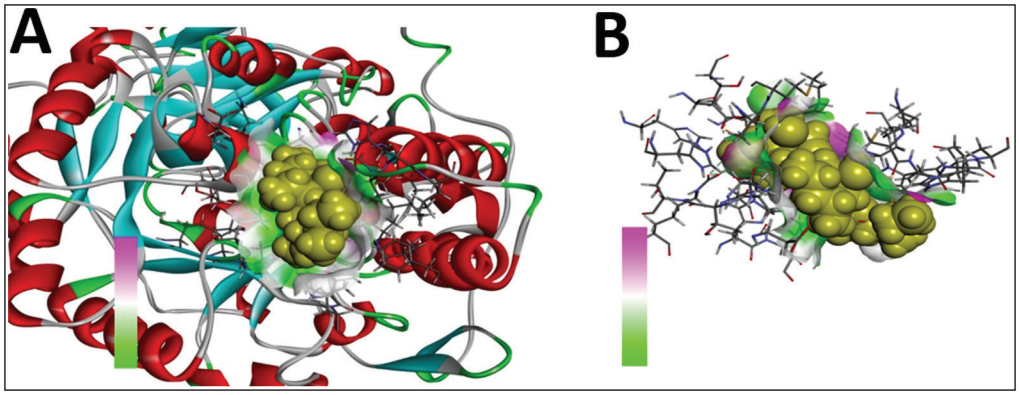

Figure 6: (A) 3-dimensional *H. pylori* urease with gentamycin (CPK structure [three dimension] represent *H. pylori* urease with inhibitors) bound in the active site. (B) The active site represented as ball and sticks structures yellow color gentamycin interaction with urease enzyme

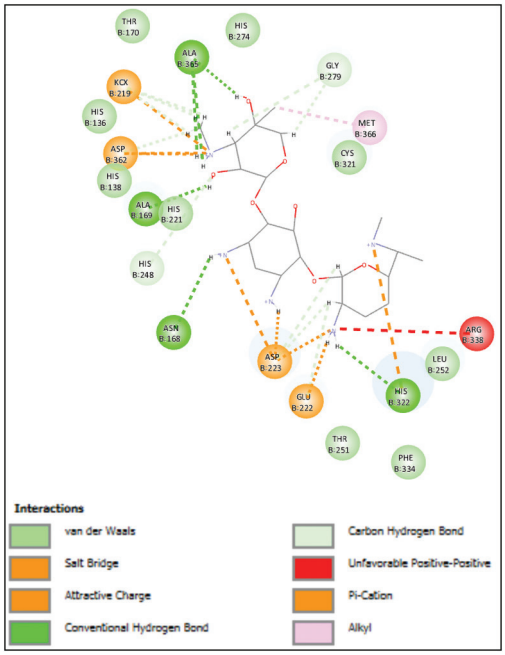

Figure 7: 2-dimensional interactions between gentamycin and urease enzyme interactions with amino acids in active site

the reference strain of urease. These data show that, when compared with the other medicines, paromomycin and capreomycin may have a different effect on urease inhibition of the reference strain [Table 3].

- Similar later without significant difference
- Different later with significant difference

Discussion

Docking study for urease inhibition

The reported cryo-electron microscopy (EM) structure of *H. pylori* urease enzyme with an inhibitor (3,5-dimethylimidazolyl-sulfanyl-N-hydroxyacetamide) (PDB code: 6ZJA) is selected as a target for this study.

The docking scores obtained from the molecular docking simulations provide insights into the potential binding affinities of the drugs towards the *H. pylori* urease inhibitor. Among the drugs investigated, paromomycin exhibited the highest docking score of -15.339, indicating a strong binding affinity with the target protein. Gentamicin followed closely with a docking score of -13.864, suggesting favorable interactions as well. Amikacin, tobramycin, and capreomycin demonstrated slightly higher docking scores, but still exhibited notable binding affinities towards the target protein.

There are no reports in the literature citing the activity of tobramycin, capreomycin, paromomycin, amikacin, and gentamycin as urease inhibitors, which makes this work the first one to associate anti-ureolytic activity with these compounds.[24]

As shown in Figure 3 for paromomycin, the program predicts the hydrogen bonds with residues His248, MET366, ALA365, ASP165, and ASN168, the carbon hydrogen bond with GLY279, hydrophobic contacts with the residues ASP223, ASP 362, GLU 222, and KCX219 and have one unfavorable positive-positive interaction with ARG338.

As shown in Figure 5 for tobramycin the program predicts the hydrogen bonds with residues His322, asn168, ALA169, ala 365, ASP223, and GLY 279, the carbon hydrogen bond with GLY279, hydrophobic contacts with the residues ASP223, ASP 362, his 322 GLU 222, and KCX219. The asp 223 are more reactive bond which can male hydrogen bond and unfavorable positive-positive interaction and salt bridge and have one unfavorable positive-positive interaction with ARG338.

As shown in Figure 7 for gentamycin the program predicts the hydrogen bonds with residues His248, asn168, ALA169, ala 365, ASP223, and GLY 279, the carbon hydrogen bond with GLY279, hydrophobic contacts with the residues ASP223, ASP 362, his 322 GLU 222, and KCX219. MET366 non-covalent interactions between alkyl groups in organic molecules. Unfavorable positive-positive interaction and salt bridge have one unfavorable positive-positive interaction with ARG338.

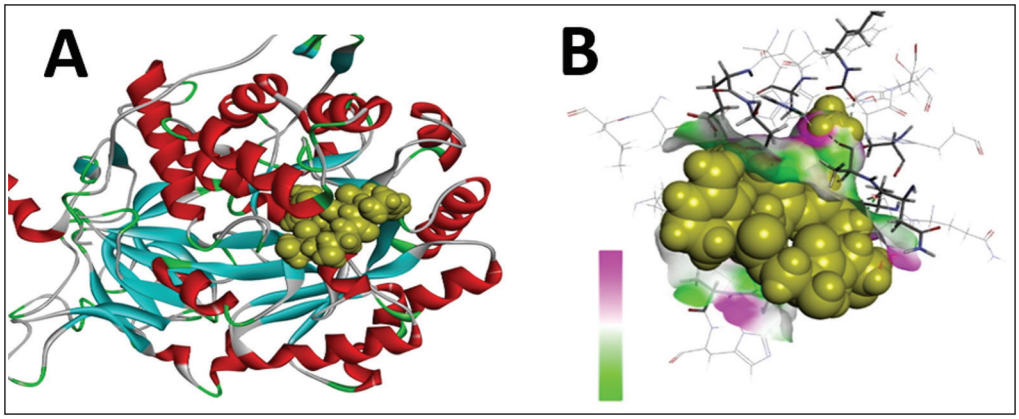

Figure 8: (A) 3-dimensional *H .pylori* urease with capreomycin (CPK structure [three dimension] represent *H .pylori* urease with inhibitors) bound in the active site. (B) The active site represented as ball and sticks structures yellow color capreomycin interaction with urease enzyme

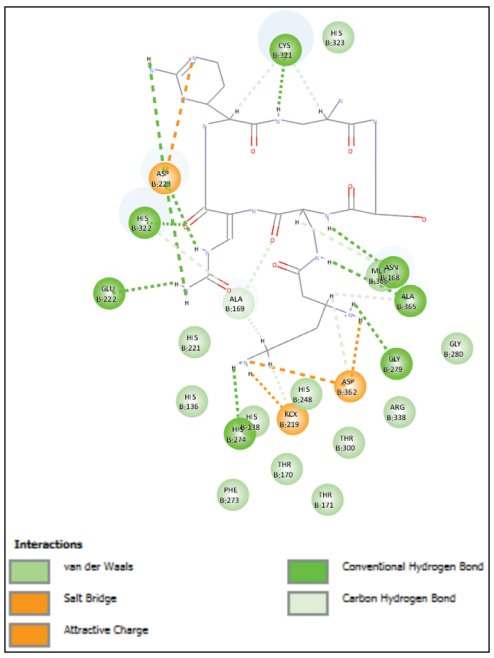

Figure 9: Two-dimensional interaction between capreomycin and urease with amino acid

As shown in Figure 9 for capreomycin the program predicts the hydrogen bonds with residues CYS 321 His274, asn168, ALA169, ala 365, ASP223, and GLY 279, the carbon hydrogen bond with GLY279, hydrophobic contacts with the residues ASP223, ASP 362, his 322 GLU 222, and KCX219. The asp 223 are more reactive bond which can male hydrogen bond and unfavorable positive-positive interaction and salt bridge (0.25).

As shown in Figure 11 for amikacin the program predicts the hydrogen bonds with residues CYS 321 His274, asn168, ALA169, ala 365, ASP223, and GLY 279, GLY280 ARG 338, the carbon hydrogen bond with GLY279, hydrophobic contacts with the residues ASP223, ASP 362, ASP219, GLU 222, and KCX219. The GLU 222 amino acid are more reactive bond which can make hydrogen bond and unfavorable positive-positive interaction and salt bridge.

Kafarski and Talma[18] mentioned the two popular Levofloxacin and Ciprofloxacin, as well as their analogs appeared to be quite promising inhibitors of *H. pylori* enzymes. Molecular modeling suggests their binding with carboxylic group interacting with active site nickel ions. However, the mechanism of additional covalent interaction with the enzymatic cysteine similar to this observed for simple quinones, cannot be ruled out. Several studies used 6zja as an active site to inhibit urease enzyme[24] indicate the mechanism of the anti-*Helicobacter pylori* activity, an *in silico* study on D-Limonene has been performed, using *H. pylori* urease enzyme 6zja, which revealed that D-Limonene showed promising binding scores. Study of de Paula in 2023 about effect of urease Several studies used 6zja the protein data bank (PDB) ID which represents *H. pylori* urease with inhibitor bound in active site.[25]

Effect of aminoglycoside antibiotic on urease activity invitro study

The findings of this study provide valuable insights into the inhibitory effects of aminoglycoside antibiotics, specifically paromomycin, tobramycin, gentamicin, amikacin, and capreomycin, on the urease activity of *H. pylori*. These antibiotics exhibited concentration-dependent inhibition, suggesting their potential as therapeutic agents for targeting urease activity in *H. pylori* infections. The result was conforming for docking study which showed paromomycin, tobramycin, gentamicin, amikacin, and capreomycin can bind to active site of urease and then may be inhibit of urease activity.

Several discrepancies may be found when comparing the inhibitory actions of various aminoglycoside antibiotics. Paromomycin, tobramycin, gentamicin, amikacin, and capreomycin all demonstrated concentration-dependent inhibition of urease activity in *H. pylori*. However, their potencies, as indicated by the IC50 values, varied among the antibiotics and strains. Additionally, these antibiotics belong to the class of aminoglycosides,

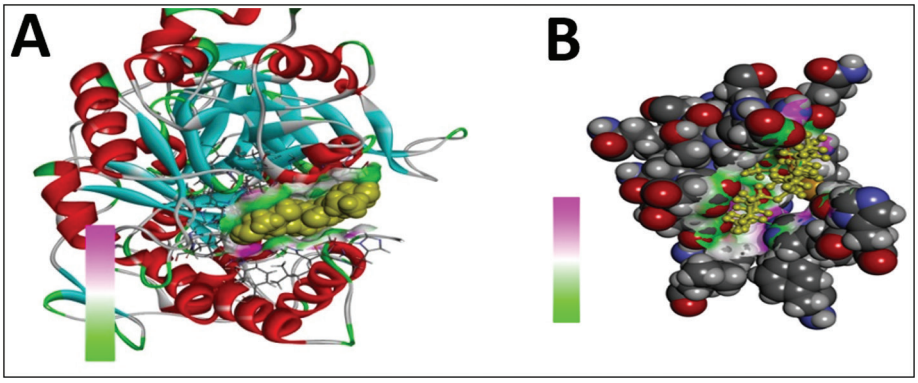

Figure10: (A) 3-dimensional *H. pylori* urease with amikacin (CPK structure [three dimension] represent *H. pylori* urease with inhibitors) bound in the active site. (B) The active site represented as ball and sticks structures yellow color amikacin interaction with urease enzyme

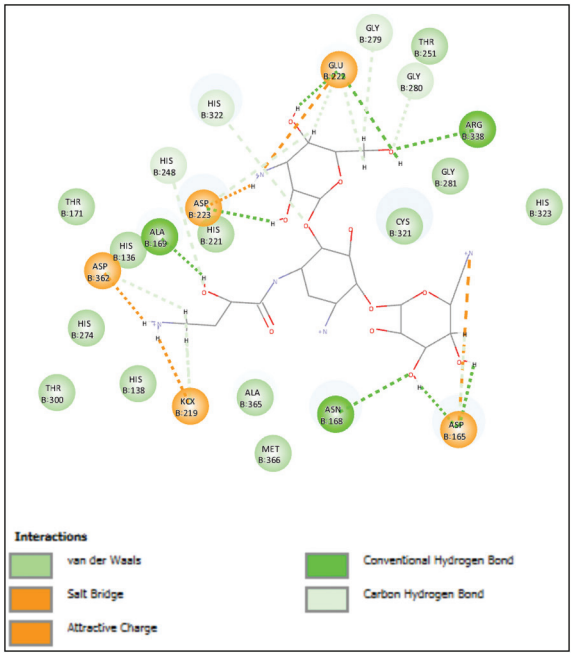

Figure 11: 2-dimensional interactions between amikacin and urease enzyme interactions with amino acids in active site

which have broad-spectrum antimicrobial activity. This characteristic provides potential advantages in cases of *H. pylori* coinfections or polymicrobial gastric diseases, as these antibiotics can target other bacterial pathogens in addition to inhibiting urease activity.

One antibiotic that has been reported to exhibit inhibitory effects on urease activity is acetohydroxamic acid (AHA). AHA is a drug. It works by inhibiting the enzyme urease, which is responsible for the hydrolysis of urea to ammonia and carbon dioxide. By inhibiting urease activity, AHA reduces the production of ammonia, thereby creating an unfavorable environment for urease-producing bacteria to thrive.[25]

The information of the fourteen metronidazole derivatives have been synthesized through the coupling of metronidazole and salicylic acid derivatives. These derivatives have been reported for the first time. The purpose of this synthesis was to investigate their inhibitory activities against *H. pylori* urease *in vitro*, and some of the synthesized compounds have demonstrated promising potential as inhibitors of *H. pylori* urease. Specifically, compounds with IC50 values of 26 µM and 12 µM have shown significant inhibitory activities against the enzyme.[26]

The effect of antimicrobials on urease activity was related to nitrogen use efficiency of urea. The effect of sulfamethazine, enrofloxacin, and tetracycline on urease activity within a certain concentration range (0-800 µmol/mL) was determined by sodium phenol sodium hypochlorite colorimetry.[27]

The high concentration of tetracycline significantly inhibited the activity of urease ($P < 0.05$) and the influence degree increased with the increase of benzene ring number. Sulfamethazine, enrofloxacin, and tetracycline could form 1:1 complex with urease, resulting in static quenching of intrinsic fluorescence of urease and changing the micro-environment of amino acid residues in urease active center resulted in the hydrophilicity of the active site to change, thus affecting the activity of urease. Sulfamethazine, enrofloxacin, and tetracycline inhibition of urease by binding with amino acid residues in urease to produce hydrogen bond.

Urease inhibition of amikacin and their derivatives was mentioned.[28] In this study, Schiff base derivatives of amikacin were synthesized and evaluated for their anti-urease activity. The urease inhibitory assay revealed that the tested compounds exhibited potent activity, with the standard amikacin displaying an IC50 value of 3.992 ± 1.638 µM. The Schiff base derivatives exhibited IC50 values ranging between 1.955 ± 0.832 µM to 5.696 ± 1.927 µM, surpassing the inhibitory potential of the standard drug. These findings were consistent with computational analysis, suggesting specific binding of the Schiff base derivatives to the active site of the enzyme, resulting in enhanced enzyme inhibition.

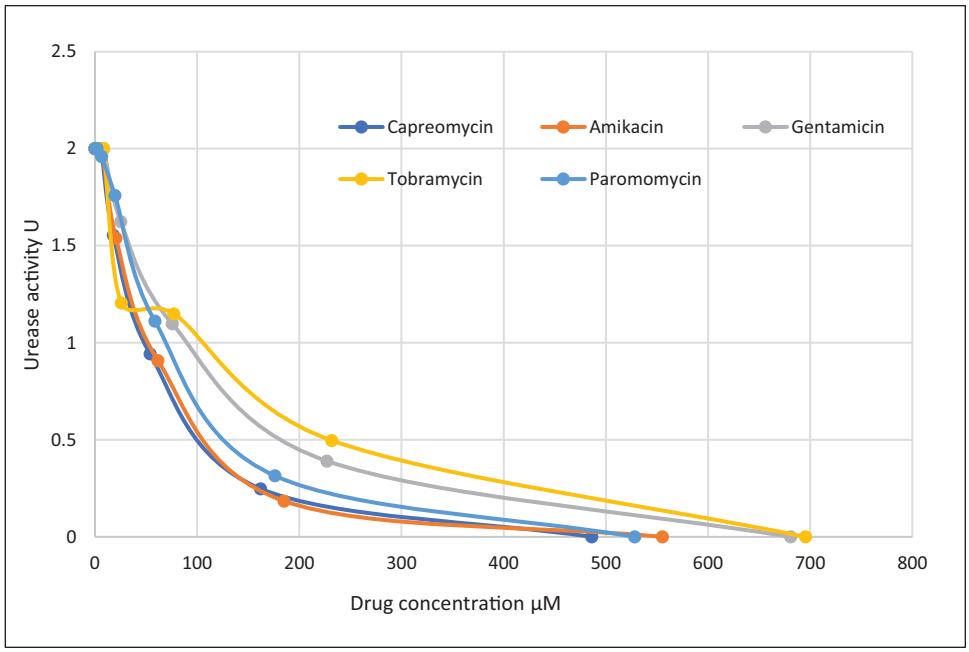

Figure 12: The inhibitory effect of paromomycin, tobramycin, gentamycin, amikacin and capreomycin on urease activity purified from *H. pylori* local strain

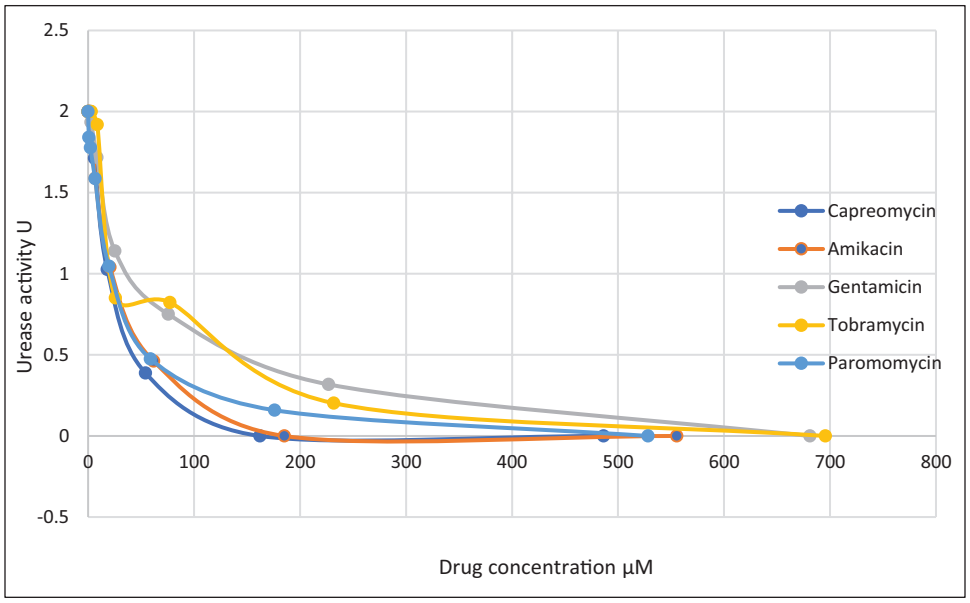

Figure 13: The inhibitory effect of paromomycin, tobramycin, gentamycin, amikacin and capreomycin on urease activity purified from *H. pylori* NCTC 11916 strain

Comparative study between traditional anti-urease drug and tobramycin, capreomycin, paromomycin, amikacin and gentamycin

Unlike the drug used in this study, acetohydroxamic acid (AHA) was not commonly used as a treatment specifically for *H. pylori* infection. The primary use of AHA is as a urease inhibitor in the treatment of certain types of urinary tract infections and conditions associated with excessive ammonia production.[29] Whereas elemental ions (such as Hg^{2+} and Ag^{+}), humic acid derivatives, and thiols have shown inhibitory effects on urease activity, their use as therapeutic agents for *H. pylori* infection in humans is limited. Elemental ions such as mercury (Hg^{2+}) and silver (Ag^{+}) are toxic and can have harmful effects on human health.[30] Therefore, they are not used as treatments for *H. pylori* infection due to their potential for toxicity. Whereas enrofloxacin is antibiotic used for veterinary therapy and it has not been extensively studied or approved for human use, unlike tobramycin, capreomycin, paromomycin, amikacin, and gentamycin can be used for

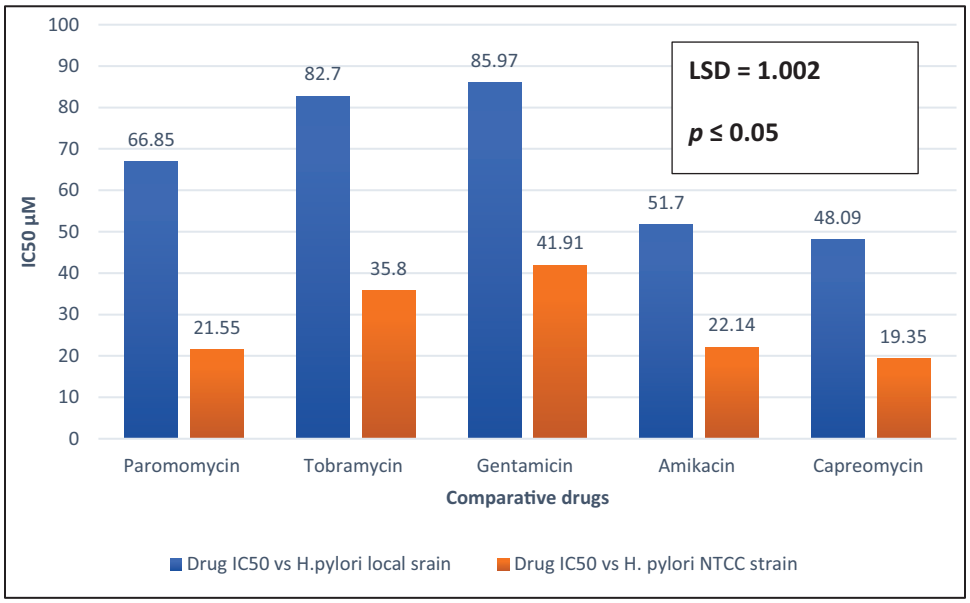

Figure 14: Comparing the IC50 values of different drugs of urease between the local strain and reference strain

Table 2: The least significant difference (LSD) in drugs against urease for the local strain

	Capreomycin	Amikacin	Paromomycin	Tobramycin
Gentamycin	37.88_{A}	34.27_{B}	19.12_{D}	3.27_{F}
Tobramycin	34.61_{B}	31_{C}	15.85_{E}	
Paromomycin	18.76_{D}	15.15_{E}		
Amikacin	3.61_{F}			

LSD = 1.002, *P*-value = 0.05

Table 3: Least significant difference between drugs against urease for reference strain

	Capreomycin	Gentamycin	Amikacin	Tobramycin
Paromomycin	22.56_{A}	20.36_{B}	19.77_{B}	6.11_{E}
Tobramycin	16.45_{C}	14.25_{D}	13.66_{D}	
Amikacin	2.79_{F}	0.59_{G}		
Gentamycin	2.2_{F}			

LSD= 1.002, *P*-value = 0.05

human beings. Humic acid derivatives and thiols have shown inhibitory effects on urease activity in laboratory studies, but their use as specific treatments for *H. pylori* infection in humans is still under investigation. Further research is needed to determine their safety, effectiveness, and optimal dosage in human clinical settings. The effectiveness of essential oils in inhibiting urease activity *in vivo* and their clinical relevance in treating *H. pylori* infections is still being explored. Whereas aminoglycoside drugs paromomycin, tobramycin, amikacin, capreomycin, and gentamicin have antimicrobial activity and direct antiurease effect.

Conclusion

Aminoglycoside emerges as a promising urease inhibitor for *H. pylori* infection. Molecular docking regarding the relatively highest affinity toward to the *H. pylori* urease, and *in vitro* studies provide valuable insights for potential drug development against *H. pylori*-induced gastrointestinal diseases. Further research is needed to explore the clinical efficacy of capreomycin, paromomycin, amikacin, gentamicin, and tobramycin in managing *H. pylori* infections display bactericidal and concentration-dependent killing action.

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Conflicts of interest

There are no conflicts of interest.

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