

<https://doi.org/10.48047/AFJBS.6.14.2024.4417-4429>



African Journal of Biological Sciences

Journal homepage: <http://www.afjbs.com>



Research Paper

Open Access

***Helicobacter pylori*: Virtual screening of new urease inhibitors from gastro-intestinal microbiota**

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Article History

Volume 6, Issue 14, 2024

Received :02 May 2024

Accepted :14 Aug 2024

doi: [10.48047/AFJBS.6.14.2024.4417-4429](https://doi.org/10.48047/AFJBS.6.14.2024.4417-4429)

Abstract

Helicobacter pylori, a Gram-negative bacterium, is the etiological agent of a variety of gastroduodenal pathologies, including ulcers. The increasing resistance of this bacterium to antibiotics is driving the need to develop new therapeutic strategies. In this context, the human gastro-intestinal microbiota represents an interesting reservoir of antimicrobial molecules involved in host protection against various pathogens. In our work, we study *in silico* the inhibitory activity of 200 metabolites synthesized by various species of gastro-intestinal microbiota towards urease, which plays a crucial role in the pathogenicity of this bacterium, conferring on its resistance to the gastric acidity. We first simulated the interaction between our molecules by molecular docking using FlexX prediction software, followed by a study of the ADME properties of the best compounds retained from the docking. Our results show M35 and M29 to be the best potential urease inhibitors, with a binding energy of -26.15 kJ/mol and -23.85 kJ/mol respectively, surpassing that of the reference acetohydroxamic acid (-10.95 kJ/mol), as well as possessing good ADME profiles, validating them as candidate drugs for limiting *H. pylori* associated infections.

Keywords: *Helicobacter pylori*, urease, gastro-intestinal microbiota, molecular docking, microbial inhibitors.

1. Introduction

Helicobacter pylori, a micro-aerophilic Gram-negative bacterium, infects more than half the world's population (Arora *et al.*, 2018). By colonizing the gastric mucosa, it causes a variety of gastroduodenal pathologies, including ulcers, gastric cancer and MALT lymphoma (Franceschi *et al.*, 2015). Current therapy involves a combination of two or three antibiotics plus proton pump inhibitors (Liu *et al.* 2024). However, the efficacy of these protocols is steadily declining, due to the bacteria's growing resistance to antibiotics, in addition to the undesirable side-effects associated

with the latter. All this has led to the need to develop new therapeutic strategies (Alba *et al.*, 2017). Urease, a vital virulence factor in *H.pylori* (Wang *et al.*,2024), appears to be a promising therapeutic target for the eradication of this bacterium. It plays a crucial role in its pathogenicity, conferring resistance to the gastric acidity by catalyzing the hydrolysis of urea into ammonia and CO₂, thus neutralizing this acidity and promoting its colonization of the gastric mucosa (Akash *et al.*, 2024). In this context, the human Astro-intestinal microbiota plays a critical role in protecting the host against various pathogens through the production of a variety of antimicrobial molecules, which have demonstrated their significance in treating various diseases(Modi *et al.*, 2014).

In our study, we explore the inhibitory activity of gastro-intestinal microbiota against *H. pylori*, by simulating *in silico* the affinity of 200 metabolites synthesized by various species of gastrointestinal microbiota towards urease, using the molecular docking approach. This method enables us to predict the mode of interaction between our target protein and selected ligands (Fan *et al.*, 2019).

2. Materials and methods

2.1. Protein preparation

The three-dimensional structure of our target was obtained via the Protein Data Bank(PDB)(PDB ID: 1E9Y), corresponding to the 3D structure of urease in complex with the co-crystallized inhibitor, acetohydroxamic acid (AHA) (Ha *et al.* 2001), with a resolution of 3.0 Å. The complex was then downloaded in pdb format (figure1). Prior to the molecular docking, the 1E9Y complex was pre-treated with FlexX software, adding missing hydrogen atoms, eliminating crystallographic water molecules and separating the AHA inhibitor to free the active site. In parallel, the active site of our target was identified by selecting amino acids located within a 6.5 Å radius of the co-crystallized ligand. The protein was then saved in pdb format as a starting structure for docking software.

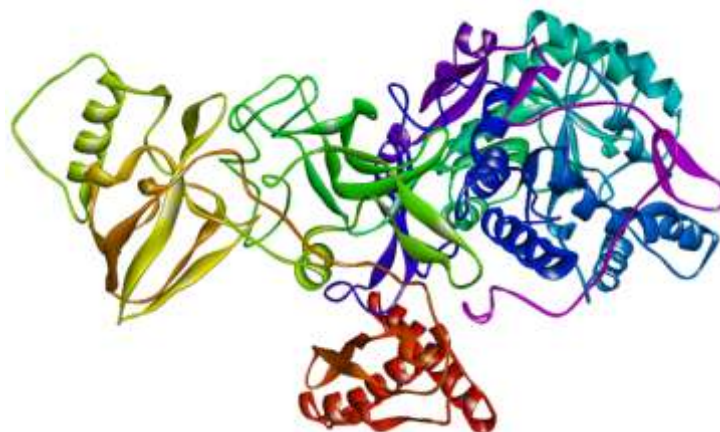


Figure 1: Crystallographic structure of *H.pylori*urease (PDB ID: 1E9Y)(Ha *et al.*, 2001)

2.2.Ligand preparation

The 2D and 3D structures of metabolites from different gastro-intestinal microbiota species were constructed using ChemDraw and Chem3D software respectively. Then, their geometry was optimized by minimizing their energy using the MM2 force field present in Chem3D. The optimized molecules were saved in mol2 format.

2.3. Validation of the docking protocol

In order to validate the results obtained in this work, it is essential first to study the reliability of the software used for docking, using two tests:

2.3.1. RMSD (Root Mean Square Deviation) test

The root-mean-square deviation (RMSD) is the measure of the average distance between the atoms of superimposed molecules (Sargsyan *et al.*, 2017). It compares the conformation of a protein-ligand complex simulated by the docking software with its crystallographic reference conformation. The accepted threshold value is 2 Å (Yusuf *et al.*, 2008). This test was carried out on a series of 100 complexes randomly selected from the PDB.

2.3.2. Visual analysis

This step complements the RMSD test by inspecting the superposition of the ligand conformation simulated by the docking software against its experimental reference pose (Hadni *et al.*, 2023). This analysis was carried out using Discovery Studio software, using the AHA ligand in complex with *H. pylori* urease (PDB ID: 1E9Y).

2.4. Molecular docking

The co-crystallized AHA compound within the 1E9Y complex has been selected as a reference ligand for developing more potent urease inhibitors, due to its experimentally validated inhibitory activity (Mamidala *et al.*, 2021).

The AHA ligand was separated from the 1E9Y complex using Discovery Studio software and underwent geometry optimization using Chem3D software, then molecular docking was performed on the urease-AHA complex by FlexX software to give a starting score from which those exceeding it were analyzed to study their interaction with the urease active site.

Molecular docking using FlexX was conducted for the 200 microbial compounds against urease to predict their affinity. The receptor was treated as rigid, while the ligands were considered flexible. Default parameters of the software were employed throughout the docking process.

2.5. Interaction analysis and visualization

Once the docking had been completed, the results were ranked in descending order of the interaction energies of the various ligand poses studied, expressed in kJ/mol. The various intermolecular interactions between the best compounds and the target were generated in 2D and 3D form using Discovery Studio software.

2.6. ADME properties prediction

The physicochemical and pharmacokinetic properties of the most promising inhibitors were predicted using the SwissADME server (<http://www.swissadme.ch/>). These properties must comply with the Lipinski and Veber rules (Kowalska *et al.*, 2021), and include predictions concerning gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeability and cytochrome P450 (CYP) inhibition (Lohit *et al.* 2024). the same properties were predicted for the reference ligand AHA.

3. Results and discussion

3.1. Validation of docking protocol

3.1.1. RMSD test

Reliability was assessed using the RMSD test on 100 complexes (protein-ligand) downloaded from the PDB. Table 1 and figure 2 show that 75% of the RMSD values obtained following molecular docking by FlexX are below 2 Å, while only 25% are above this threshold value. These results show that FlexX is a powerful tool for predicting protein-ligand interactions.

3.1.2. Visual analysis

Visual analysis was carried out on the urease-AHA complex bearing PDB ID: 1E9Y. Figure 3 shows a good superposition of the ligand pose generated by FlexX (colored green) compared with the reference pose obtained by X-ray crystallography (colored red). This further demonstrates the reliability of the FlexX software.

Table 1: RMSD test results.

PDB code	Ligand code	RMSD (Å)	PDB code	Ligand code	RMSD (Å)	PDB code	Ligand code	RMSD (Å)
1N7I	SAH	1,73	5I3A	HQE	0,36	2V11	C80	1,33
1AH3	NAP	1,52	5TFT	HEM	1,27	6AAH	9T6	1,78
1AIM	ZYA	1,18	5URS	8LA	9,78	6DND	PLP	0,77
1EB2	BPO	1,5	5ZAN	9A6	6,05	6OA3	M0M	3,61
1EKO	184	4,88	6FH5	DD8	0,97	6Q0Z	P7V	1,91
1K1M	FD4	4,41	6MDA	JED	0,59	7TLN	INC	1,97
1P9S	DIO	4,47	6MDB	JE4	1,49	4ZZ2	3YG	1,12
1RTI	HEF	1,96	6MDC	JEA	1,97	5C28	4XV	2,51
1YKR	628	0,74	6MDD	JE7	0,67	5CLU	S8A	1,5
1YZ3	SAH	1,1	6O9X	M0S	5,72	5D0R	B1T	1,18
1ZVX	END	1,87	6OA3	M0M	3,7	5EEC	ZXN	1,92
2G5P	3GP	3,41	6OHS	MJY	1,62	5HVT	NVS	1,15
2G71	SAH	1,74	1OI9	N20	0,6	5IWC	6EQ	2,31
2JBJ	G88	5,3	2OPB	SAH	1,32	5J9Y	6HL	1,95
2ONZ	TMJ	0,4	2QJR	TB	0,84	5SZ7	72H	1,92
2R4B	GW7	1,3	3VVG	ZGB	1,99	6AAH	9T6	1,78
2RJP	886	1,48	1C84	761	1,14	3RZ3	U94	2,43
2V35	J54	7,24	1H39	R03	1,73	3TPP	5HA	1,45
2XBU	5GP	2,39	1LI9	PO4	1,57	4CGA	QLW	2,42
2ZJF	BSU	0,48	1KIM	THM	0,92	4EY7	E20	1,78
2EW8	SO4	9,06	1N8Q	DHB	1,96	4G9C	0WP	1,49
3N9S	TD4	1,19	1OGQ	NAG	3,75	4KZO	NAP	10,02
3QQK	HEM	1,21	1PNN	984	1,45	4LXM	1YU	10,04
3QTQ	X35	0,67	1YW8	A75	2,8	4MO8	2VQ	1,94
3VP2	BP0	1,84	2AN5	SAH	1,18	4OGN	2U5	1,76
3WYM	3K9	1,52	2ANQ	NDP	2,74	4ONC	FMT	2,55
4CDL	LLK	4,22	2BU5	TF1	3,17	2VJ8	HA2	1,51
4IU6	FZ1	6,34	2CL5	SAM	1,95	3CCC	7AC	0,76
4MIK	JIL	0,97	2F6V	SK2	1,45	3I28	34N	1,29
4MQ4	2D5	2,45	2OGZ	U1N	1,97	3K5F	AYH	1,56
4NCM	704	1,11	2QDH	M2P	1,76	3KOO	24D	1,8
4YTF	4HZ	5,04	2R3N	SCX	1,88	3LJT	LA3	1,92
5AFW	EDO	5,58	2RF6	SO4	1,05	3OF8	10Y	4,95
5FI2	5XX	5,41	2RIP	34Q	1,86			

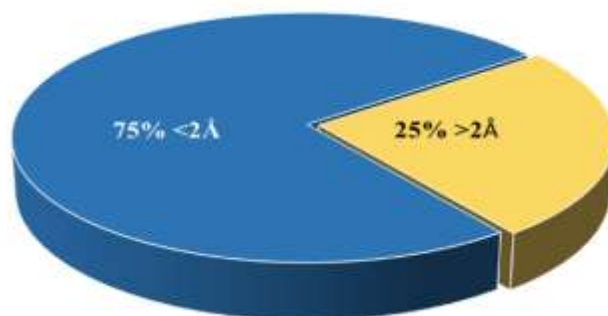


Figure 2: Results in percent of the RMSD test.

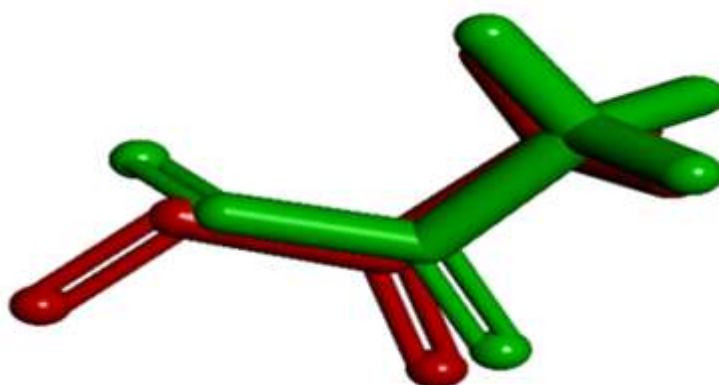


Figure 3: Superposition of the test geometry of the AHA ligand of complex 1E9Y obtained by X-ray and that obtained by FlexX.

1.1. Molecular docking and interaction visualization

Molecular docking of microbial compounds to urease has enabled us to simulate the interactions involved in the formation of these complexes and to evaluate their affinities. These affinities are calculated and translated into scores, with the best score representing the most potent inhibitor based on the strength of the interaction energy (Ratnayake *et al.*, 2024).

Among the 200 compounds, only the two best molecules obtained in this work (Table 2), compounds M35 and M29, with scores of -26.15 kJ/mol and -23.85 kJ/mol respectively, were retained as potential urease inhibitors.

Table 2: Scores for the protein-ligand complexes studied.

Compound	Compound name	Score
M1	Acetohydroxamic acid (AHA)	-10.95 kJ/mol
M35	Protocatechuic acid	-26.15 kJ/mol
M29	Nicotinic Acid	-23.85 kJ/mol

▪ Protein-ligand interactions

The complexes with the best docking scores were selected for analysis with Discovery Studio software to study the different interactions contributing to the stabilization of these complexes. The interaction between our protein and ligands involves several types of binding. The type, number, and distance of these interactions determine the strength of the binding energy in the protein-ligand complex (Jannah and Wahid., 2023).

▪ Urease-M35 interaction (Protocatechuic acid)

Among the 200 metabolites studied in this research, M35 has the best binding energy towards the urease active site (-10.95 KJ/mol), due to the presence of a greater number of bonds and shorter distances than the other ligands. All the amino acids in our protein that interact with M35 are shown in figure 4.

Docking results reveal that the best pose of M35 in the urease active site (figure 5) was stabilized by hydrogen bonds between the ligand and residues ASP 362, GLY 279, HIS 322 and MET 366, at distances of (4.35 Å; 5.41 Å), 5.41 Å, 3.61 Å and 5.03 Å respectively. However, no interaction was observed between the ligand and Ni²⁺ ions, which have been reported as key players in urease enzymatic activity (Mazzei *et al.*, 2020).

M35 hydroxyl groups were found to play a significant role in affecting its molecular conformation and interactions with the active site by forming hydrogen bonds with residues ASP 362, GLY 279, HIS 322 and MET 366, resulting in inhibition of enzymatic activity. The difference between the binding energy of M35 and that of AHA can be explained by the presence of a greater number of hydrogen bonds with M35, contributing to the formation of five bonds, whereas AHA forms only two (ASP362; HIS221). Considering that Asp362 is a catalytic residue of urease, the interaction between M35 and this residue may enhance the inhibitory activity of M35 (Asadi *et al.*, 2023).

Our results also aligned closely with findings in the literature, indicating a similarity between the residues interacting with our ligands and those interacting with the different types of urease inhibitors reported in the literature. This consistency reinforces the reliability of our docking results (Akash *et al.*, 2024).

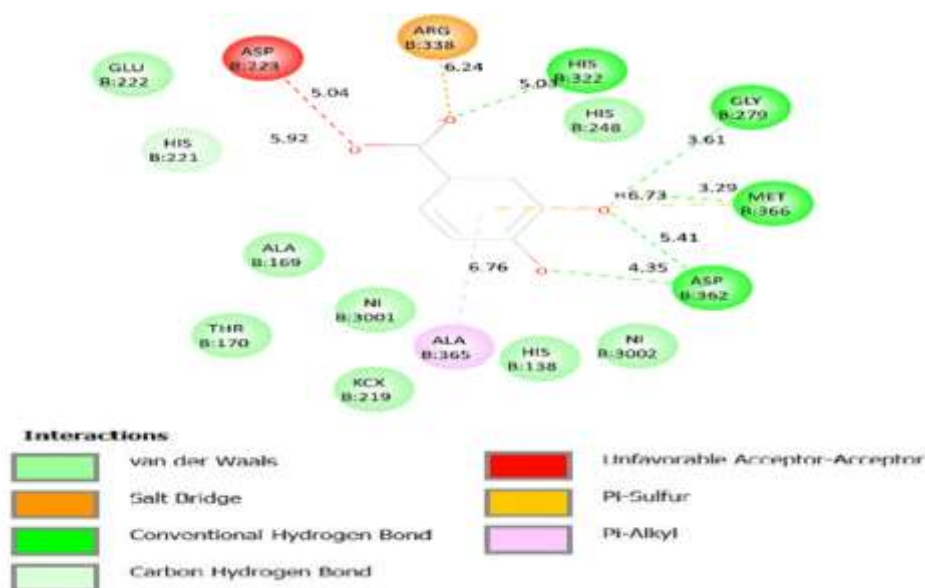


Figure 4: Molecular interaction between urease and M35

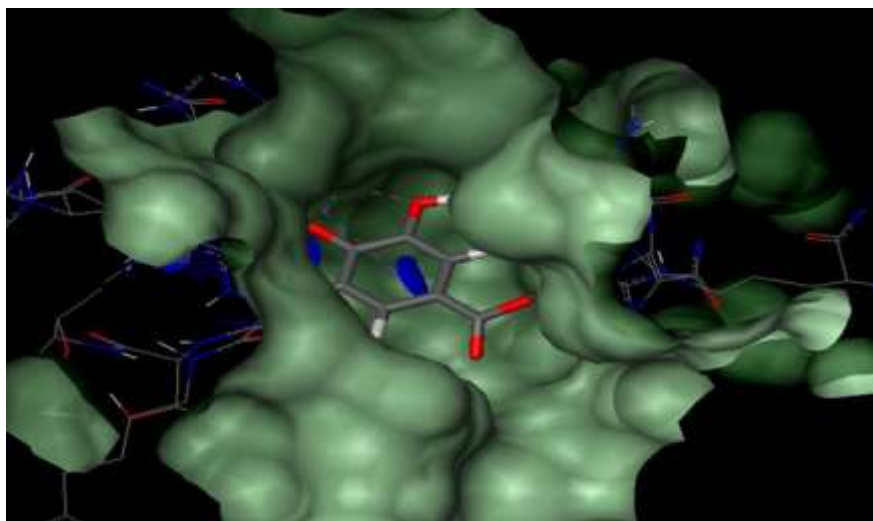


Figure 5: position of M35 in the urease active site

▪ Urease-M29 interaction (Nicotinic acid)

Prediction of the mode of interaction between M29 and the urease active site (figure 6 and figure 7) revealed the formation of hydrogen bonds between the ligand and residues HIS221 and ARG338 at distances of 5.02 Å and 6.83 Å respectively. A strong bond was found between the ligand and one of the active site Ni ions²⁺ with a distance of 2.51 Å. This bond explains the inhibitory activity of this ligand towards urease, whose ions play an indispensable role in the enzymatic activity of our target (Haq *et al.*, 2024). The benzene ring of M29 forms pi-alkyl interactions with Ala365 and pi-sulfur interactions with residues Met366, Cys321 (5.19 Å, 5.88 Å, 8.33 Å) which contribute significantly to the energetic stabilization of the protein-ligand complex.

In addition, this ligand binds with KCX219 and HIS138, amino acids that represent key urease catalytic site residues (Cunha *et al.*, 2021), further enhancing its inhibitory potential. Additionally, M29 derivatives have been reported in the literature with potential urease inhibitory, reinforcing our docking results (Jannah and Wahid., 2023).

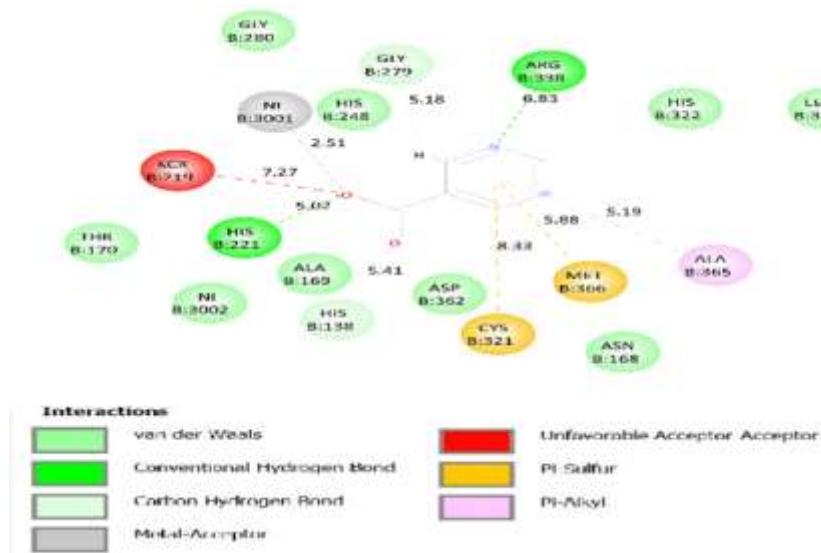


Figure 6: Molecular interaction between urease and M29

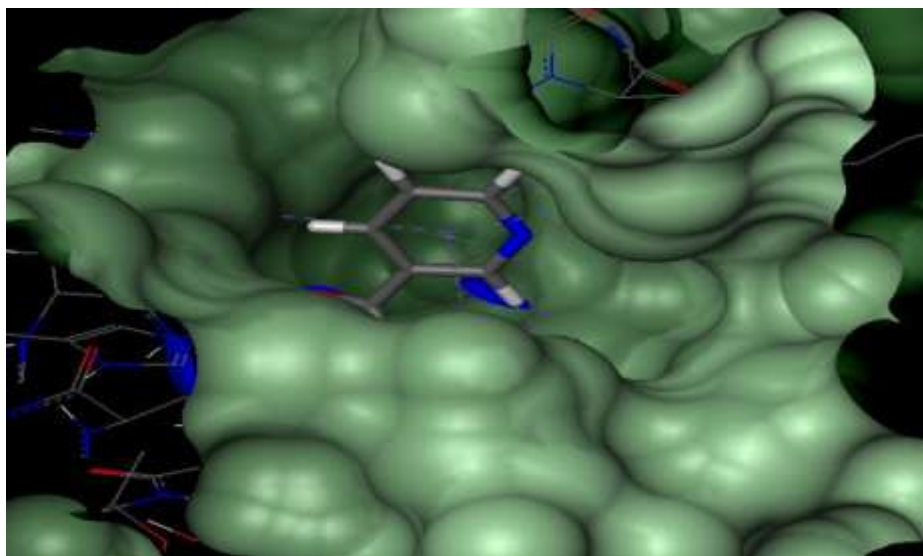


Figure 7: position of M29 in the urease active site

3.2. ADME properties prediction

3.2.1. Physico-chemical properties

The results presented in table 3 show that each of the two compounds fully meets all the Lipinski and Veber criteria. These results show that these molecules have a good ability to dissolve in the body's aqueous phase and to cross lipid membranes to reach their site of action. In addition, they have good water-solubility properties and a low synthetic accessibility value, all below 5 on the scale. This suggests that synthesis would be relatively easy to implement.

Table 3: Physicochemical properties of the best compounds.

Properties	AHA	M29	M35
Formula	C ₂ H ₃ NO ₂	C ₆ H ₅ NO ₂	C ₇ H ₆ O ₄
PM (g/mol) Molecular weight	73.05 g/mol	123.11 g/mol	154.12 g/mol
nLF Number of flexible connections	1	1	1
nON Number of hydrogen acceptors	3	3	4
nOHNH Number of hydrogen donors	0	1	3
Log P	0.12	0.32	0.65
TPSA Å ²	46.50 Å ²	50.19 Å ²	77.76 Å ²
Lipinski's rule	Compliant	Perfectly compliant	Perfectly compliant
Veber's rule	Does not conform	Perfectly compliant	Perfectly compliant
Solubility in water	Soluble	Highly soluble	soluble
Access to the synthesis	1.07	1.00	1.07

3.2.2. Pharmacokinetic properties

Table 4 shows that the gastrointestinal (GI) permeability of both compounds is high, indicating that they can pass through the intestinal wall into the bloodstream. The following distribution test shows that M29 has a high capacity to cross the blood-brain barrier (BBB), which could lead to side effects. These could be resolved during optimization. On the other hand, M35 is unable to cross the BBB, preventing it from reaching the central nervous system and thus inducing side effects.

As far as cytochromes are concerned, both compounds behave similarly, with the exception of M35, which inhibits only the CYP3A4 enzyme. This inhibition can prolong the half-life of drugs by slowing down their metabolism. This can be beneficial for some drugs by increasing their duration of action, but can also increase the risk of toxicity. However, this risk can be mitigated by dose optimization (Loos *et al.* 2022).

Table 4: Pharmacokinetic properties of the best compounds.

Properties	AHA	M29	M35
GI absorption	High	High	High
BBB permeability	Low	Strong	Low
CYP1A2 inhibition	Negative	Negative	Negative

CYP2C19 inhibition	Negative	Negative	Negative
CYP2C9 inhibition	Negative	Negative	Negative
CYP2D6 inhibition	Negative	Negative	Negative
CYP3A4 inhibition	Negative	Negative	Positive

4. Conclusion

In this study, we used molecular docking to identify new potential inhibitors of urease, a key enzyme in the infection cycle of *H. pylori*, from the human gastrointestinal microbiota. Our research revealed that Protocatechuic acid and Nicotinic acid are powerful inhibitors of urease. These two compounds exhibit a strong affinity for the active site of this enzyme as well as a good ADME profile, which is highly promising for their development as therapeutic molecules to limit *H. pylori* related infections. Further in vitro and in vivo studies are essential to validate these compounds as effective drug candidates.

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