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In vitro antibacterial activity of neochebulinic acid from aqueous extract of *Terminalia chebula* Retz against *Helicobacter pylori*

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Abstract

Background Neochebulinic acid, a component of *Terminalia chebula Retz*, exhibits diverse biological activities. This study aimed to investigate its anti-*Helicobacter pylori* (HP) activity.

Methods and materials Through using ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) and nuclear magnetic resonance (NMR) spectroscopy, neochebulinic acid was isolated and identified. Efficacy was assessed via minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays, scanning electron microscopy (SEM), and other assays.

Results It demonstrated neochebulinic acid's ability to inhibit HP proliferation, damage bacterial structure, and suppress Cytotoxin-associated gene A (Cag A) protein expression, with high selectivity towards HP. Neochebulinic acid also acts as an anti-adhesive agent, preventing HP adhesion to host cells.

Conclusion These findings suggest its potential as a targeted therapeutic against HP infections, paving the way for novel anti-HP therapies.

Keywords Neochebulinic acid, *Terminalia chebula* retz, *Helicobacter pylori*, Adhesion, Minimum inhibitory concentration

Introduction

Helicobacter pylori (HP), characterized as a gram-negative spiral bacterium, was seminal in medical microbiology following its identification by Barry J. Marshall as a pivotal causative agent in the pathogenesis of gastritis and peptic ulcers [1]. This groundbreaking discovery earned Marshall the prestigious Nobel Prize in Physiology or Medicine, highlighting the significance of this bacterium in clinical medicine. Over the years, the pathogenic attributes of HP have been extensively investigated, and our understanding of its role in disease progression has been markedly refined. Consequently, HP has been recognized as a major risk factor for the development of gastric adenocarcinoma [2–4], one of the leading causes of cancer-related mortality worldwide [5]. This association has necessitated a heightened focus on the risks posed by HP infection and the need for effective management strategies. Presently, the standard therapeutic approach for HP infection relies heavily on antibiotic-based regimens, which have been relatively successful

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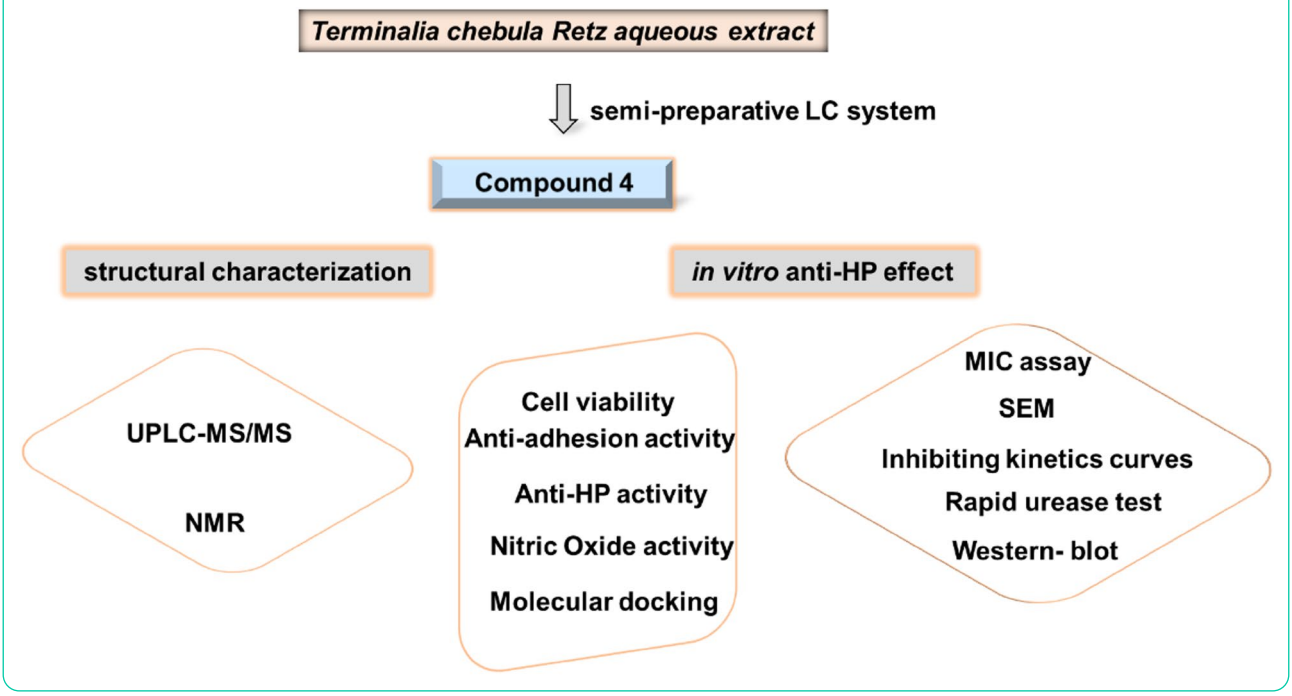
Graphical abstract



in eradicating the bacterium from the gastric mucosa [6, 7]. However, the relentless emergence and spread of antibiotic-resistant HP strains have posed a significant clinical challenge. The escalating problem of antibiotic resistance threatens to undermine the efficacy of current treatments, thereby underscoring the urgent need for the development of novel therapeutic agents that can act as viable alternatives to conventional antibiotic therapies.

In light of these challenges, there is a growing body of research dedicated to exploring alternative treatment modalities, including the investigation of traditional remedies and natural products that may exhibit anti-HP properties [8–15]. The pursuit of such alternatives is not only driven by the need to combat antibiotic resistance but also by the desire to reduce the side effects associated with long-term antibiotic use and to provide cost-effective treatment options, particularly in resource-limited settings. Therefore, the identification and characterization of new compounds with anti-HP activity represent a critical step forward in the management of HP-related diseases and the mitigation of the global burden of gastric cancer.

Traditional Chinese Medicine (TCM), a profound repository of China's cultural heritage, stands as a valuable source of complementary and alternative therapeutic modalities. Among the pantheon of TCM herbs, *Terminalia chebula*, colloquially known as Hezi in Chinese, has garnered a storied reputation for its efficacy in treating a diverse array of ailments. The convergence of traditional wisdom and modern scientific validation positions *Terminalia chebula* as a compelling option for integrative medicine, offering a potential alternative for patients resistant to conventional treatments or seeking to reduce reliance on synthetic drugs [16–20]. Recent studies have demonstrated that *Terminalia chebula* harbors potent anti-inflammatory and antibacterial properties, making it a promising candidate for the treatment of inflammatory conditions and bacterial infections [12]. Of particular note, this herb has been found to exhibit a notable inhibitory effect on the urease activity of HP [21], a bacterium implicated in the pathogenesis of gastritis, peptic ulcers, and gastric cancer. This urease-inhibitory action is of paramount importance, as it directly addresses the virulence factor responsible for the bacterium's ability to colonize and damage the gastric mucosa. Thus, the exploration of the therapeutic potential of *Terminalia chebula* represents a significant stride in the quest to harness the full spectrum of TCM offerings for the benefit of global health.

Our latest investigative research has established that *Terminalia chebula* exhibits a notable capacity to modulate and inhibit the inflammatory cascade triggered by HP, thereby providing a potential therapeutic intervention for related gastrointestinal disorders [12]. However, while the efficacy of *Terminalia chebula* in mitigating inflammation is evident, the precise bioactive compounds that confer its antibacterial properties have yet to be comprehensively identified and characterized.

In pursuit of this knowledge gap, we embarked on a systematic isolation and purification process aimed at extracting individual monomers from *Terminalia chebula*. Subsequent to this meticulous procedure, we rigorously evaluated the antibacterial potential of these isolated compounds against HP. Our findings have culminated in the identification of compound 4 as a particularly promising candidate with significant anti-HP activity, marking a pivotal step forward in our understanding of the therapeutic potential of this herb.

Moreover, we have not only elucidated the chemical structure of compound 4 but have also initiated a preliminary investigation into the underlying mechanism of its antibacterial action. The isolation of a potent anti-HP agent and the exploration of its mode of action paves the way for further research and development, potentially leading to novel and effective treatments for HP-related infections.

Methods and materials

Reagents

All the materials utilized in the experiments were depicted as in the previous article [22].

Preparation of *Terminalia chebula* Retz aqueous extract

The ripe fruit of *Terminalia chebula* Retz was sourced from Yunnan, China, and supplied by Guangzhou Zhining Pharmaceutical Co. Ltd. (Lot No. 210901). The authenticity of the fruit as genuine *T. chebula* was confirmed by the Chief Pharmacist, Weixing Zhu. For the extraction process, 20 g of the mature fruit was subjected to a 10-fold volume of double-distilled water, followed by three cycles of boiling at 90 °C for a duration of one hour each. Afterward, the mixture was centrifuged to concentrate the extract, which was then freeze-dried to obtain a powdered form. The resultant powder was stored at a temperature of − 20 °C, as per a previously established method [12].

The Preparation process of the compound by semi-preparative liquid chromatography (LC) system

For the purification of the compound via semi-preparative LC system, 5 g aqueous extract of *Terminalia chebula* Retz was first mixed in 170 mL of water and subsequently filtered through a 0.22 μm filter, and served as the analytical sample. Utilizing a semi-preparative LC setup, the sample was subjected to separation and purification under defined chromatographic parameters. A YMC Actus Triart C18 column, measuring 20 mm × 250 mm with a particle size of 5 μm, was selected for the chromatographic process. The mobile phase was composed of solvent A (0.1% formic acid in water) and solvent B (acetonitrile), with a gradient elution profile as follows: beginning with 95% A and 5% B at time zero, transitioning to 86% A and 14% B by 50 min, then to 76% A and 24% B by 80 min, dropping abruptly to 0% A and 100% B at 80.10 min, and holding at this ratio until 85 min. Subsequently, the composition was rapidly switched back to 95% A and 5% B at 85.1 min, and maintained until the end of the run at 90 min. During the chromatographic run, the fraction collected from the 23rd tube, corresponding to the peak eluting at 60 min, was isolated and named compound 4. This fraction was then freeze-dried to facilitate subsequent structural elucidation.

Ultra-high performance liquid chromatography-MS/MS (UPLC-MS/MS)

UPLC-MS/MS analysis was conducted on the test sample fraction, compound 4, utilizing a refined chromatographic setup. The analytical column of choice was a YMC Triart C18, featuring dimensions of 2.1 mm × 100 mm and a particle size of 1.9 μm. The mobile phase was composed of solvent A (0.1% formic acid in water) and solvent B (acetonitrile), and the elution was performed using a gradient program. At the onset of the gradient, the composition was set to 95% A and 5% B, which was maintained until 20 min. From 20 to 27 min, the ratio was adjusted to 70% A and 30% B, held constant until 27.10 min, then promptly returned to 95% A and 5% B. This final ratio was sustained until the conclusion of the run at 40 min. For MS detection, the instrument was set to operate in Full MS ddMS2 mode, which is the same as a prior study [22].

Nuclear magnetic resonance (NMR) identification

For structural elucidation, methanol-D4 was used to dissolve compound 4. The subsequent structural analysis was carried out on AVANCE NEO 600 MHz NMR spectrometer, manufactured by Bruker BioSpin GmBH, located in Rheinstetten, Germany.

HP culture, cell culture, and co-culture

The detailed origins of the well-characterized HP standard strains (ATCC 43504 and ATCC 700392), the GES-1 cell line, as well as the clinical HP standard strains (CS01, QYZ-003, and QYZ-004), along with their respective culture or co-culture conditions, were comprehensively documented in a previous study [22]. Upon their arrival, we conducted additional testing using the urease assay, which ensures that the strains we employ have the requisite characteristics and fulfill the criteria for our research. Then HP strains were maintained at the School of Pharmaceutical Sciences, Sun Yat-sen University, Shenzhen.

MIC assay and minimum bactericidal concentration (MBC) assay

The broth microdilution technique in 96-well plates was employed to determine the MICs against HP, adhering to

the methodology outlined by Shen et al. [15]. Post-MIC determination, aliquots of 100 µl from the drug-treated solutions, at concentrations ranging from 1-fold to 8-fold the MIC, were subsequently plated onto Columbia agar enriched with 5% sheep blood for further culturing.

Scanning electron microscope (SEM)

SEM was utilized to analyze the morphological characteristics of HP. The ATCC 700392 strain of HP was exposed to therapeutic agents at the determined minimum inhibitory concentration (MIC) for 24 h, with both untreated and drug-treated samples assessed, following the protocol established in a previous investigation [12].

Inhibiting kinetics curves

Inhibitory kinetic profiles were generated by subjecting ATCC 700392 to varying concentrations of the respective drugs. Aliquots of 100 µL were collected at designated intervals (0, 12, 24, 36, 48, 60, and 72 h) to quantify the optical density at 600 nm, thereby assessing bacterial growth.

Nitric oxide (NO) activity

The supernatant from the cell cultures was harvested, and the NO activity was assessed utilizing the Griess Reagent System kit, adhering to the manufacturer's protocol as described by a prior study [22].

Rapid urease test

The ATCC 700392 strain of HP was subjected to a 24-hour drug treatment, after which rapid urease test solutions were introduced. The subsequent assessment of optical density at 560 nm was conducted using a microplate reader (Multiskan GO, Thermo Scientific, USA).

Cell viability

Viability of GES-1 cells, seeded at a density of 10,000 cells per well in a 96-well plate, was evaluated post-incubation overnight. The cells were then exposed to the drug for a duration of 24 h. To quantify cell viability, the wells were subsequently supplemented with CCK-8 reagents as per the manufacturer's protocol and incubated for an additional 2 h. The assay culminated in the measurement of optical density at 450 nm, utilizing a microplate reader (Multiskan GO, Thermo Scientific, USA).

Cell adhesion activity

Following a 6-hour co-culture period, the cells were subjected to a dual PBS wash to remove any non-adherent material. Subsequently, rapid urease test solutions were introduced to the cells, and the optical density at 560 nm was determined using a microplate reader (Multiskan GO, Thermo Scientific, USA).

Western blot

The ATCC 700392 strain of HP was exposed to the drug treatment for 24 h. Strain pellets were harvested and lysed, then protein concentrations were quantified via the BCA assay. The protein lysates were then denatured by boiling with sample loading buffer at 100 °C for 10 min. Samples were separated on SDS-PAGE gels and subsequently transferred to PVDF membranes, then blocked with 5% non-fat milk for 1 h and probed with an anti-Cag A primary antibody overnight at 4 °C and incubated with the appropriate secondary antibody for 1 h at room temperature. The immunoreactive bands were finally visualized using the BeyoECL reagent (Beyotime, China) and captured with a ChemiScope 6200 imaging system (Clinx Science Instruments).

Molecular docking

The structure of the CagA protein of HP was determined using the AlphaFold2 [23] algorithm, and the structure of neochebulinic acid was sourced from the PubChem database. CB-Dock2 [24] was utilized to investigate the binding interaction between the CagA protein and neochebulinic acid. Specifically designed for blind docking within predicted protein binding sites, CB-Dock begins by identifying potential binding sites through cavity detection. These parameters are crucial for molecular docking using AutoDock Vina [25] (Center and Size).

Statistical analysis

Experiments such as nitric oxide (NO) activity, adhesion assays were repeated three times. Data visualization and statistical analysis were conducted using GraphPad Prism 8 software. The Student's two-tailed t-test or one-way ANOVA, accompanied by an appropriate post-hoc analysis, was employed to assess the statistical significance of the results. The threshold for statistical significance was set at $^{*}P < 0.05$ and $^{**}P < 0.01$, indicating the level of confidence in the observed differences.

Results

Identification of compound 4

Compound 4 was identified with m/z 973.1119 under negative ion mode, as depicted in Fig. 1. Further structural elucidation was achieved through NMR spectroscopy. The spectral data is shown in Fig. S1. This compound is registered with the CAS Registry Number and is known by the CAS Name, as illustrated in Fig. 2.

Neochebulinic acid possessed anti-HP activity

The investigation into the antimicrobial effects of neochebulinic acid on HP was conducted through MIC and MBC assays. The findings, as displayed in Table 1, indicate that the MIC values for neochebulinic acid between 16 and 128 µg/mL. Importantly, the MBC values exceed

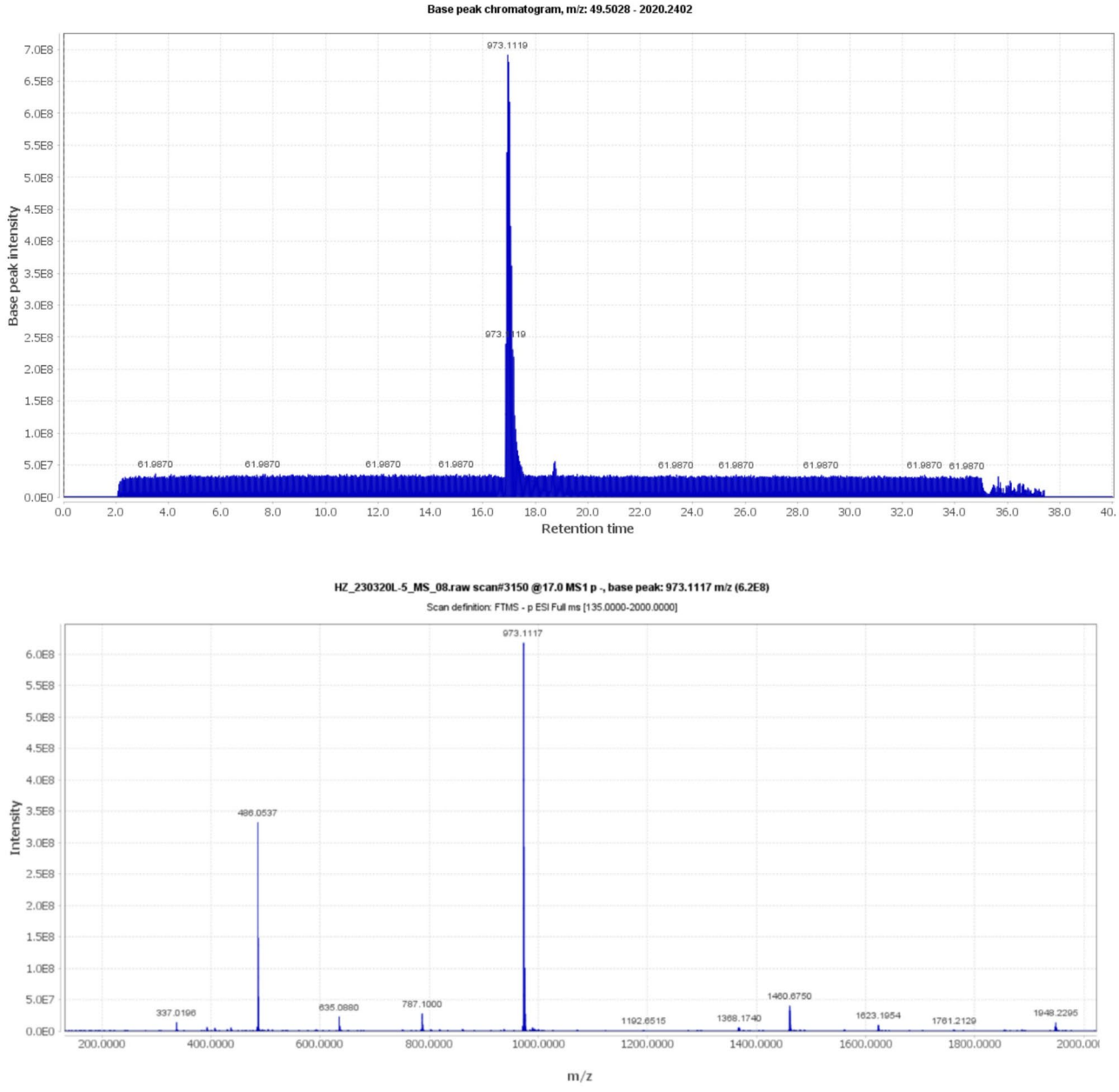


Fig. 1 The UPLC-MS/MS information of compound 4

the MIC by a factor greater than 8, which clearly indicates that this compound possesses bacteriostatic properties rather than being bactericidal. This suggests that neochebulinic acid is capable of inhibiting HP growth without eliminating the bacteria, thereby exhibiting a growth-inhibitory effect.

Neochebulinic acid significantly damaged the bacterial structure

To elucidate the effects of neochebulinic acid on the structural integrity of HP, SEM was employed. The results, depicted in Fig. 3, demonstrate that exposure to neochebulinic acid for 24 h resulted in severity of structural damage, with visible signs of bacterial disruption and rupture. These findings underscore the compound's capacity to induce significant morphological changes in the bacterial structure, thereby compromising its integrity.

Neochebulinic acid inhibited the growth of HP strain ATCC 700392

The study demonstrated that neochebulinic acid potently inhibits the proliferation of HP strain ATCC 700392, as evidenced by both the rapid urease test. The compound's

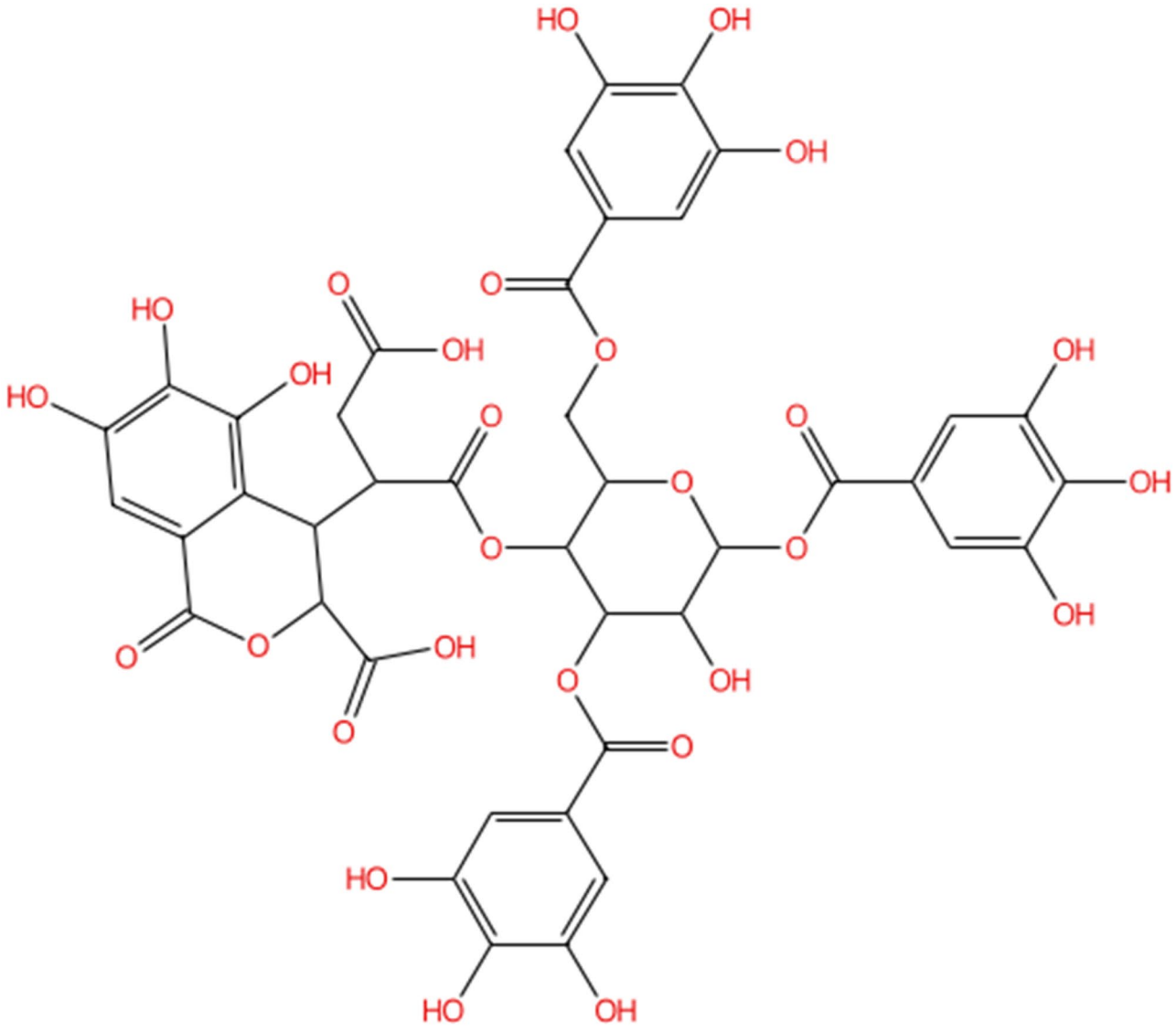


Fig. 2 The structure of compound 4. CAS NO.: 18913-60-5; CAS Name: neochebulinic acid

Table 1 The MIC and MBC of neochebulinic acid

HP Strains	MIC (μg/mL)	MBC (μg/mL)
ATCC 700392	32	> 256
ATCC 43504	32	-
CS01	128	-
QYZ003	16	-
QYZ004	32	> 256

HP: *Helicobacter pylori*; MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration; -:not detected

efficacy was particularly pronounced at concentrations of 16 μg/mL and 32 μg/mL, where it significantly curtailed urease activity, as illustrated in Fig. 4A. Additionally, the bacterial growth curve, presented in Fig. 4B, showcased a marked decrement in viable cell count from 24 h to 72 h post-treatment, affirming its inhibitory action on bacterial growth.

Neochebulinic acid possesses the anti-adhesive properties of HP

In assessing the safety profile of neochebulinic acid, the CCK-8 assay revealed that it did not significantly affect the viability of normal epithelial cells GES-1 at a concentration of 16 and 32 μg/mL, as shown in Fig. 5A. This suggests that the compound may exhibit selective cytotoxicity towards bacterial cells rather than mammalian cells. Additionally, the compound did not significantly influence nitric oxide (NO) activity, as indicated in Fig. 5B. Finally, the adhesive properties of HP were investigated in the presence of neochebulinic acid,

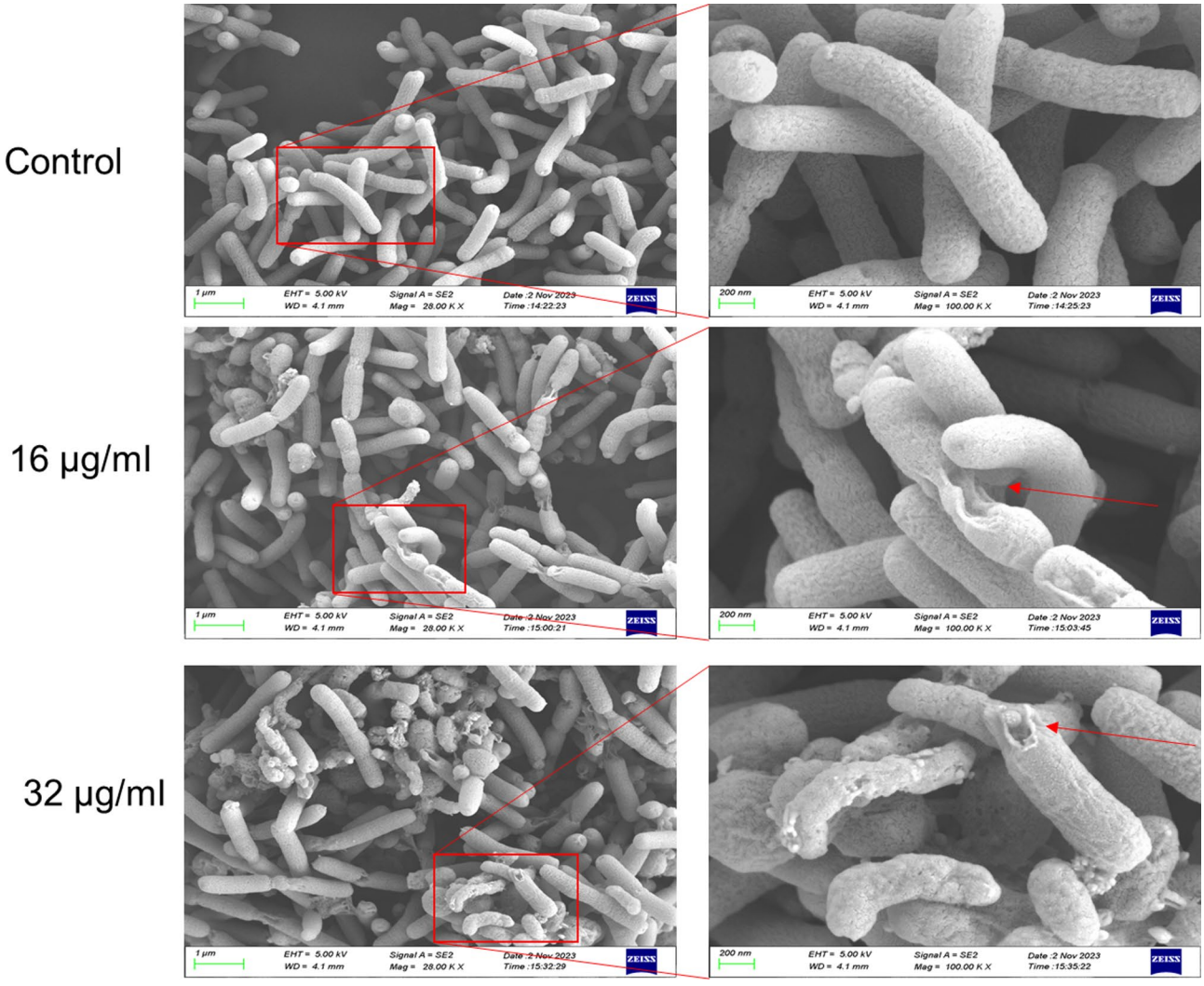


Fig. 3 The scanning electron microscopy (SEM) images of the structure of HP strain ATCC 700392 after neochebulinic acid treatment for 24 h

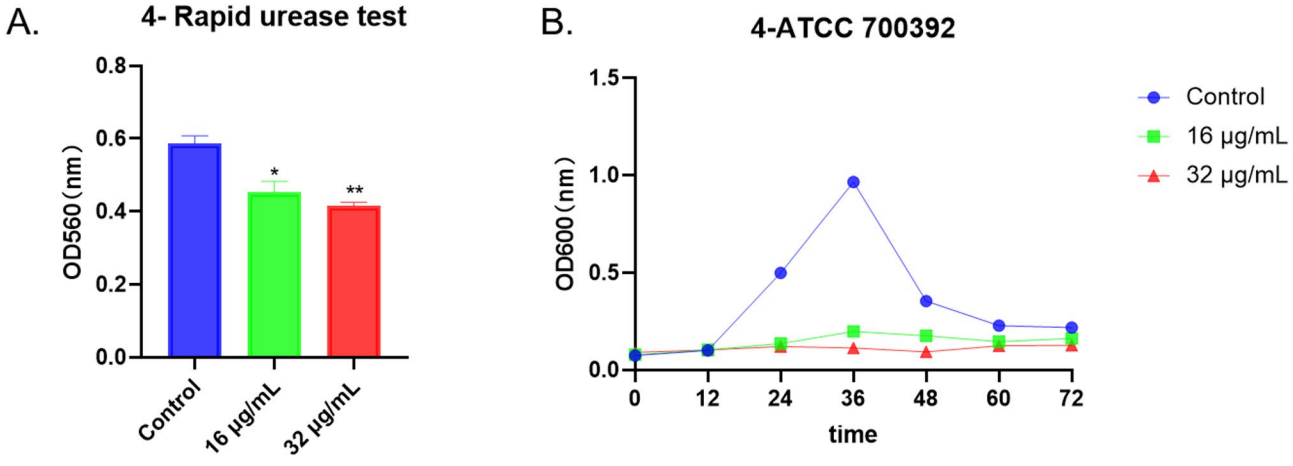


Fig. 4 Neochebulinic acid inhibited the growth of HP strain ATCC 700392. (**A**) rapid urease test results after treatment of different doses. (**B**) The growth curve of HP strain ATCC 700392

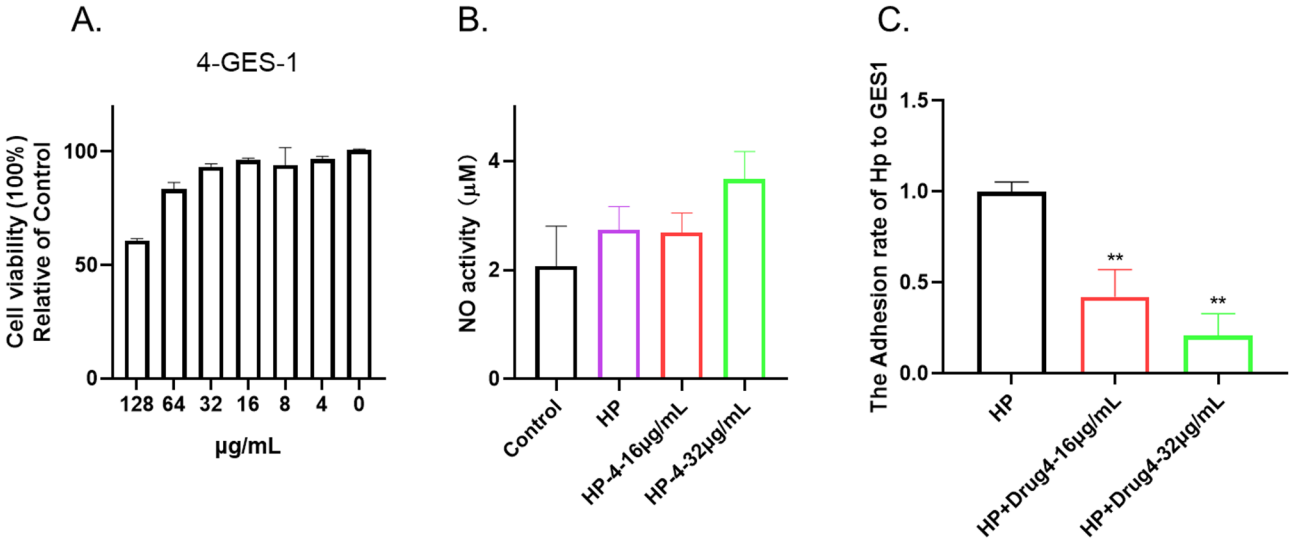


Fig. 5 Neochebulinic acid possesses anti-adhesive properties of HP. (**A**) Cell viability. GES-1 cells were treated with different doses of (0, 4,8,16,32,64, and 128 μg/mL) for 24 h. (**B**) NO activity. (**C**) Anti-adhesive activity. The GES-1 cells were co-cultured with HP at a ratio of 1:100, along with different doses of (0, 16, and 32 μg/mL) for a duration of 6 h

demonstrating a significant inhibitory effect on bacterial adhesion to host cells at a concentration of 16 and 32 μg/mL, as detailed in Fig. 5C. This finding proved that neochebulinic acid serves as a potential anti-adhesive agent.

Neochebulinic acid repressed the Cag A protein

Furthermore, the pivotal virulence factor Cag A was decreased by neochebulinic acid, with western blot analysis confirming a substantial reduction in its expression at a concentration of 32 μg/mL, as depicted in Fig. 6. This finding underscores the compound's potential to modulate the pathogenicity of HP.

Neochebulinic acid targeted the Cag A protein

To predict the interaction between neochebulinic acid and Cag A protein, we conducted molecular docking. The structure of the Cag A protein was predicted using AlphaFold2, and the core domain was identified with relatively high confidence. As displayed in Fig. 7, neochebulinic acid was predicted to bind to this core domain. The contact residues includes GLN24, ILE27, ASN28, GLN31, VAL32, ILE35, PHE63, ASP64, SER67, GLN68, ARG70, GLU71, GLU72, PRO135, ILE176, ASN179, GLN180, THR183, ASP184, LYS186, GLN235, PRO237, VAL238, PRO239, HIS240, VAL241, GLN242, PRO243, ASP244, LEU323, TYR324, ALA325, GLY326, GLY331, ASP332, LYS333, HIS334, ASP335, TRP336, TYR381, LYS382, ASP384, GLN385, LEU386, THR387, GLY388, SER389, GLN390, ARG391, ALA392, LEU393, SER394, GLU396, GLU397, and ASN428. The computed binding energy was determined to be -11.9 kcal/mol, suggesting a significant potential for a stable interaction. These preliminary findings point towards a promising binding possibility.

Discussion

Ellagitannins, a class of polyphenolic compounds, have garnered attention for their extensive health benefits [26, 27]. Neochebulinic acid, an emerging member of this family, exhibits distinct structural features that may confer novel biological activities. This research aims to explore the anti-HP properties of neochebulinic acid, with a focus on its chemical structure and potential mechanisms of action.

Our research has uncovered the remarkable inhibitory prowess of neochebulinic acid against HP, particularly its efficacious action against the ATCC 700392 strain, which is notorious for its pathogenic Cag A protein. Notably, its selective antimicrobial activity is underscored by its sparing effect on the viability of normal GES-1 epithelial cells, revealing a high degree of target specificity. In vitro experiments have illuminated the multifaceted role of neochebulinic acid, functioning as both an HP growth inhibitor and an anti-adhesive molecule, effectively interfering with the bacterium attachment to host cells, a pivotal step in the infection cascade.

The matter that deeply intrigues us and is worthy of in-depth discussion is that compound 4, identified as neochebulinic acid, shows good antibacterial activity, which is similar to the outcome of compound 3, identified as 1,3,6-Trigalloylglucose, that we previously discovered [13]. Particularly when considering the results of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and rapid urease test, they exhibit strikingly similar consequences (Table 1; Fig. 4A).

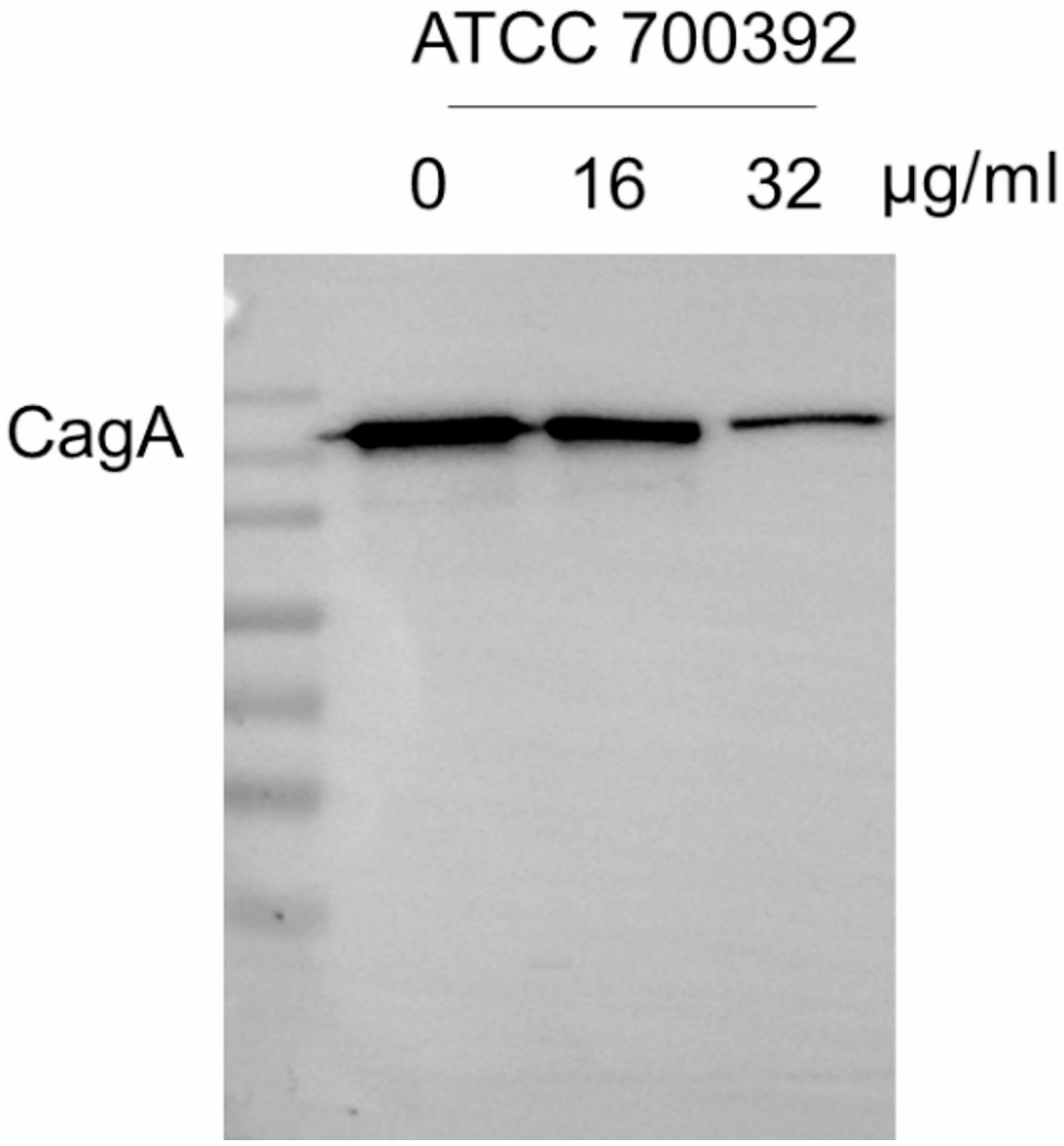


Fig. 6 Neochebulinic acid repressed the Cag A protein. ATCC 700392 was treated with doses of neochebulinic acid (0, 16, 32 μg/mL) for 24 h

Nevertheless, upon meticulous identification and analysis, their structures prove to be dissimilar. However, by conducting a comparative analysis of their structures, we discover that compound 4 and compound 3 possess the same parent nucleus. In comparison to compound 3, compound 4 is esterified at the 4-position and features additional substituents. Given that both share the same parent nucleus and exhibit comparable activities, we hypothesize that the parent nucleus, namely the antibacterial active group, is likely to play a role in antibacterial activity. Additionally, by contrasting their anti-adhesion activities, we find that compound 4 has a more potent anti-adhesion effect (Fig. 5C). Hence, it is surmised that different substituents may be the primary cause influencing the anti-adhesion effect. In terms of the extent of structural disruption, neochebulinic acid exhibits less

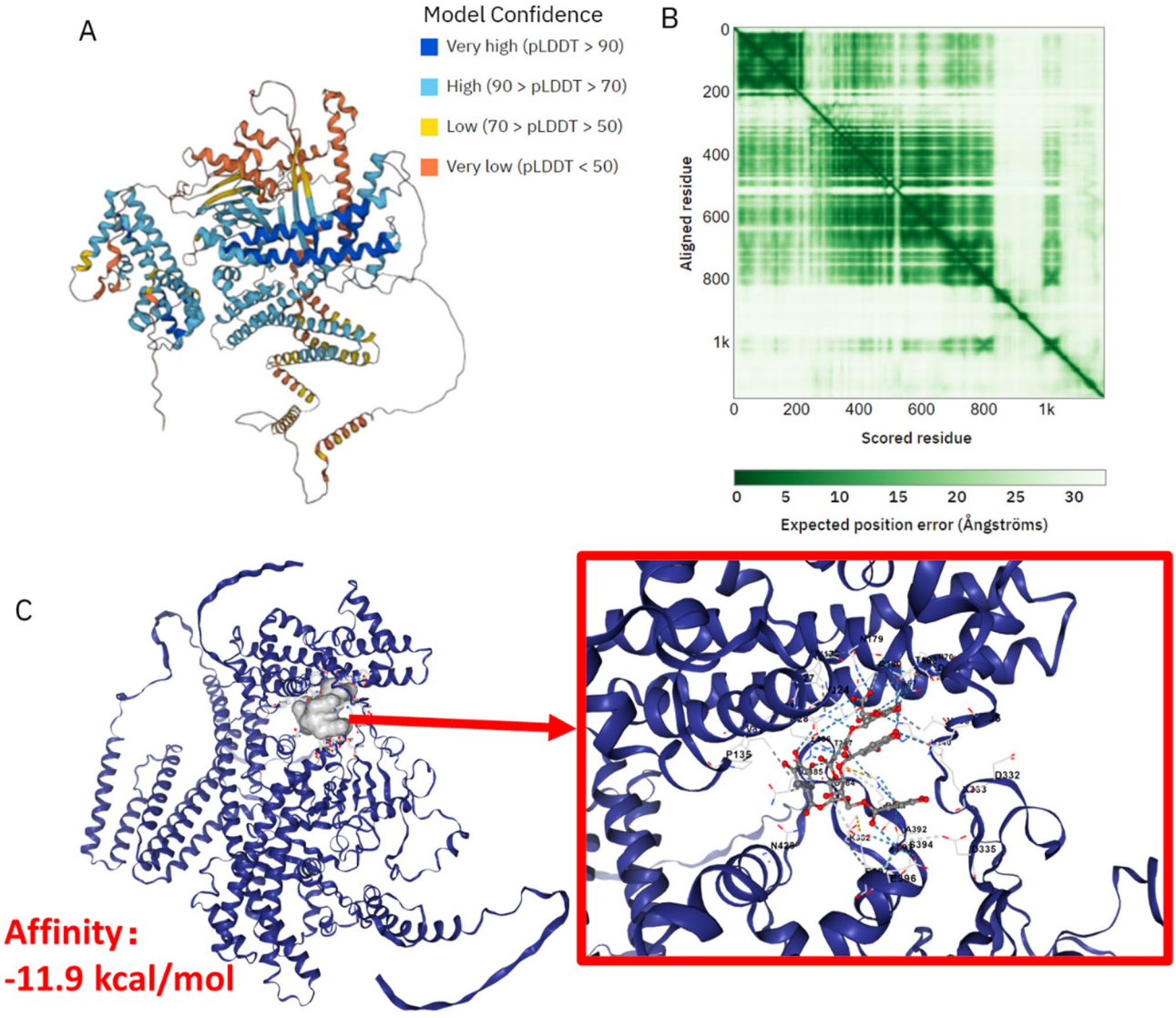


Fig. 7 Neochebulinic acid binds to the Cag A protein. (**A**) CagA protein structure calculated by the AlphaFold2 with model confidence. AlphaFold produces a per-residue model confidence score between 0 and 100. Some regions below 50 pLDDT may be unstructured in isolation. (**B**) Protein predicted aligned error. This data is useful for assessing inter-domain prediction accuracy. (**C**) Docking Binding between Cag A in HP and neochebulinic acid

pronounced membrane/cell wall damage compared to 1,3,6-Trigalloylglucose; however, it displays a low binding energy (-11.9 kcal/mol) with the CagA virulence factor. This discrepancy suggests that neochebulinic acid may exert its antibacterial activity primarily through inhibiting CagA-mediated virulence rather than inducing strong structural damage. The lower binding energy indicates stronger specific interactions with CagA, supporting a hypothesis that neochebulinic acid targets the translocation or functional activation of this key virulence protein, thereby disrupting HP pathogenicity at the molecular level.

The capacity of neochebulinic acid to disrupt adhesion without compromising nitric oxide (NO) pathways indicates a precise and potentially benign therapeutic mechanism, positioning it as a highly promising candidate for precision therapy in diseases associated with HP. However, a profound comprehension of the molecular underpinnings of its anti-adhesive and anti-virulence effects is crucial for maximizing its therapeutic potential.

As we move forward in our research pursuits, it is of paramount importance to rigorously validate the in vivo efficacy of neochebulinic acid and assess its safety in preclinical models. This pivotal stage will require the initiation of clinical trials to definitively establish the therapeutic efficacy and safety of the compound in human patients. Furthermore, it is imperative to delve into the multifaceted effects of neochebulinic acid on human physiology. Although its influence on HP is well-documented, the direct regulatory impact of

neochebulinic acid on human systems has yet to be fully explored. Considering the established link between HP and gastric cancer development, the compound's potential to modulate HP-related cancer interactions is particularly compelling.

While this study preliminarily demonstrates the activity of neochebulinic acid against HP strains, its antibacterial efficacy across a larger cohort of clinical isolates remains to be validated. Future research will involve multicenter strain collection and comprehensive MIC profiling to clarify the molecular basis of its resistance-targeting properties and regional adaptability, providing data-driven support for precision clinical use.

Additionally, investigating neochebulinic acid's effects on emerging pathways such as cuproptosis and disulfidptosis could offer groundbreaking insights into its potential as a revolutionary treatment strategy for HP-related diseases. These endeavors are instrumental in enhancing our understanding of neochebulinic acid's therapeutic capabilities, paving the way for the development of innovative and potentially transformative treatment approaches for managing HP-related illnesses.

Although this study demonstrates the in vitro antibacterial potential of neochebulinic acid against HP, its in vivo efficacy and molecular mechanisms require further validation. Limited by the extraction yield of this natural product, we could not perform animal model experiments or in-depth target mechanism analyses at this stage. For future research, we plan to scale up the production of the compound and deepen investigations across three key dimensions, such as inhibition of bacterial colonization, modulation of host inflammatory responses, and analysis of target interaction networks. These endeavors aim to clarify the unique advantages of its multi-target synergistic action in combating drug resistance, thereby providing a more robust theoretical basis for developing innovative adjuvant therapies for HP infection.

Conclusions

In summary, neochebulinic acid has demonstrated a compelling inhibitory effect against HP, notably curbing the proliferation of the ATCC 700392 strain and suppressing the Cag A protein, which is integral to the pathogenicity of HP infections. Its selective antimicrobial action is underscored by the lack of impact on the viability of normal epithelial cells GES-1, suggesting a high degree of specificity for microbial targets. In vitro experimentation has elucidated that neochebulinic acid functions not only as a growth inhibitor but also as an anti-adhesive molecule. It effectively disrupts the adherence of HP to host cells, a pivotal mechanism in the infection process. Moreover, the compound's neutral effect on nitric oxide (NO) activity indicates a targeted mode of action that spares NO-mediated pathways. These cumulative findings position neochebulinic acid as a promising candidate for targeted therapy against HP infections. Future investigative efforts should be directed towards elucidating the molecular basis of its anti-adhesive and anti-virulence effects. Furthermore, it is imperative to assess its therapeutic potential in vivo and to conduct clinical trials to ascertain its safety and therapeutic efficacy in human subjects. Such studies are essential for the development of this compound as a novel treatment strategy for HP-related diseases.

Abbreviations

HP	*Helicobacter pylori*
UPLC-MS/MS	Ultra-high performance liquid chromatography-tandem mass spectrometry
NMR	Nuclear magnetic resonance
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
SEM	Scanning electron microscopy
CCK-8	Cell counting kit-8
Cag A	Cytotoxin-associated gene A
TCM	Traditional chinese medicine
BHI	Brain heart infusion
PBS	Phosphate-buffered saline
RPMI	Roswell park memorial institute
FBS	Fetal bovine serum
LC	Liquid chromatography
ATCC	American type culture collection
MOI	Multiplicity of infection
NO	Nitric oxide

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12906-025-04989-6.

Supplementary Material 1

Supplementary Material 2

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Author contributions

Ling Ou, Zhong Feng, Guimin Zhang, and Meicun Yao contributed to the conception and design of the study, as well as the drafting and revising of the manuscript. Ling Ou, Haobo Chen, Zhixiang Zhu, Yajie Hao, and Qingwei Li were instrumental in the acquisition, analysis, and interpretation of the data. Hengrui Liu assisted in the drafting and revision of the manuscript and was responsible for conducting the molecular docking experiments. Haobo Chen played a key role in the iterative process of data acquisition, ensuring the precision and reliability of the findings. Finally, Zhong Feng, Guimin Zhang, and Meicun Yao provided the final approval for the version of the manuscript to be published.

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Data availability

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet. 1984;1(8390):1311–5.
2. Correa P, Piazuelo MB. Helicobacter pylori infection and gastric adenocarcinoma. US Gastroenterol Hepatol Rev. 2011;7(1):59–64.
3. Laird-Fick HS, Saini S, Hillard JR. Gastric adenocarcinoma: the role of Helicobacter pylori in pathogenesis and prevention efforts. Postgrad Med J. 2016;92(1090):471–7.
4. Doorakkers E, Lagergren J, Engstrand L, Brusselaers N. Helicobacter pylori eradication treatment and the risk of gastric adenocarcinoma in a Western population. Gut. 2018;67(12):2092–6.
5. Sonkin D, Thomas A, Teicher BA. Cancer treatments: past, present, and future. Cancer Genet. 2024;286–287:18–24.
6. Zhou L, Lu H, Song Z, Lyu B, Chen Y, Wang J, Xia J, Zhao Z, Helicobacter Pylori Study Group of Chinese Society of G. 2022 Chinese National clinical practice guideline on Helicobacter pylori eradication treatment. Chin Med J (Engl). 2022;135(24):2899–910.
7. Katelaris P, Hunt R, Bazzoli F, Cohen H, Fock KM, Gemilyan M, Malfertheiner P, Megraud F, Piscoya A, Quach D, et al. Helicobacter pylori world gastroenterology organization global guideline. J Clin Gastroenterol. 2023;57(2):111–26.
8. Peng C, Sang S, Shen X, Zhang W, Yan J, Chen P, Jiang C, Yuan Y, Zhu W, Yao M. In vitro anti-*Helicobacter pylori* activity of *Syzygium aromaticum* and the preliminary mechanism of action. J Ethnopharmacol. 2022;288:114995.
9. Yan J, Peng C, Chen P, Zhang W, Jiang C, Sang S, Zhu W, Yuan Y, Hong Y, Yao M. *In-vitro* anti-*Helicobacter pylori* activity and preliminary mechanism of action of *Canarium album* raeusch. Fruit extracts. J Ethnopharmacol. 2022;283:114578.
10. Chen P, Chen M, Peng C, Yan J, Shen X, Zhang W, Yuan Y, Gan G, Luo X, Zhu W, et al. In vitro anti-bactrical activity and its preliminary mechanism of action of the non-medicinal parts of Sanguisorba officinalis L. against Helicobacter pylori infection. J Ethnopharmacol. 2024;318Pt B:116981.
11. Feng Z, Li H, Hao Y, Peng C, Ou L, Jia J, Xun M, Zou Y, Chen M, Zhang G, et al. In vitro anti-Helicobacter pylori activity and the underlining mechanism of an empirical herbal formula - Hezi Qingyou. Front Microbiol. 2024;15:1355460.
12. Ou L, Liu HR, Shi XY, Peng C, Zou YJ, Jia JW, Li H, Zhu ZX, Wang YH, Su BM, et al. Terminalia chebula retz. Aqueous extract inhibits the Helicobacter pylori-induced inflammatory response by regulating the inflammasome signaling and ER-stress pathway. J Ethnopharmacol. 2024;320:117428.
13. Ou L, Zhu Z, Hao Y, Li Q, Liu H, Chen Q, Peng C, Zhang C, Zou Y, Jia J et al. 1,3,6-Trigalloylglucose: A novel potent Anti-*Helicobacter pylori* adhesion agent derived from aqueous extracts of *Terminalia chebula* Retz. Molecules 2024;29(5).
14. Peng C, Feng Z, Zou Y, Ou L, Lai Y, Su B, Chen M, Zhang C, Zhu W, Gan G, et al. Studies on the mechanisms of *Helicobacter pylori* Inhibition by *Syzygium* aromaticum aqueous extract. Phytomedicine. 2024;130:155540.
15. Shen X, Zhang W, Peng C, Yan J, Chen P, Jiang C, Yuan Y, Chen D, Zhu W, Yao M. In vitro anti-bacterial activity and network Pharmacology analysis of Sanguisorba officinalis L. against Helicobacter pylori infection. Chin Med. 2021;16(1):33.
16. Nigam M, Mishra AP, Adhikari-Devkota A, Dirar AI, Hassan MM, Adhikari A, Belwal T, Devkota HP. Fruits of *Terminalia chebula* retz.: A review on traditional uses, bioactive chemical constituents and Pharmacological activities. Phytother Res. 2020;34(10):2518–33.
17. Pingali U, Sukumaran D, Nutalapati C. Effect of an aqueous extract of Terminalia chebula on endothelial dysfunction, systemic inflammation, and lipid profile in type 2 diabetes mellitus: A randomized double-blind, placebo-controlled clinical study. Phytother Res. 2020;34(12):3226–35.
18. Cheng Y, Liu S, Wang F, Wang T, Yin L, Chen J, Fu C. Effects of dietary Terminalia chebula extract on growth performance, immune function, antioxidant capacity, and intestinal health of broilers. Anim (Basel) 2024;14(5).
19. Khazani H, Jalali Kondori B, Sahraei H, Meftahi GH. Terminalia chebula attenuates restraint stress-induced memory impairment and synaptic loss in the dentate gyrus of the hippocampus and the basolateral and central nuclei of the amygdala by inhibiting oxidative damage. Brain Res Bull. 2024;213:110975.
20. Sadhupati DP, Lakshmisudha R, Chakravarthy KNK, Naidana PS. A standardized combination of Terminalia chebula and Withania somnifera extracts enhances immune function in adults: a pilot randomized, double-blind, placebo-controlled clinical study. Food Nutr Res. 2024;68:10297.
21. Malekzadeh F, Ehsanifar H, Shahamat M, Levin M, Colwell RR. Antibacterial activity of black Myrobalan (Terminalia chebula Retz) against Helicobacter pylori. Int J Antimicrob Agents. 2001;18(1):85–8.
22. Ou L, Hao Y, Liu H, Zhu Z, Li Q, Chen Q, et al. Chebulinic acid isolated from aqueous extracts of Terminalia chebula Retz inhibits Helicobacter pylori infection by potential binding to Cag A protein and regulating adhesion. Front Microbiol. 2024;15:141679.
23. Bryant P, Pozzati G, Elofsson A. Author correction: improved prediction of protein-protein interactions using AlphaFold2. Nat Commun. 2022;13(1):1694.
24. Liu Y, Grimm M, Dai WT, Hou MC, Xiao ZX, Cao Y. CB-Dock: a web server for cavity detection-guided protein-ligand blind Docking. Acta Pharmacol Sin. 2020;41(1):138–44.
25. Trott O, Olson AJ. AutoDock vina: improving the speed and accuracy of Docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010;31(2):455–61.
26. Ikeuchi K. Development of methodologies toward the unified synthesis of ellagitannins. Chem Pharm Bull (Tokyo). 2024;72(4):349–59.
27. Venkatesan A, Kathirvel A, Prakash S, Sujatha V. Antioxidant, antibacterial activities and identification of bioactive compounds from Terminalia chebula bark extracts. Free Radicals Antioxid. 2016;7(1):43–9.

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