Gut Pathogens

REVIEW

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Anti-urease therapy: a targeted approach to mitigating antibiotic resistance in *Helicobacter pylori* while preserving the gut microflora

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Abstract

The global rise in antibiotic resistance has posed significant challenges to the effective management of *Helicobacter pylori* (*H. pylori*), a gastric pathogen linked to chronic gastritis, peptic ulcers, and gastric cancer. Conventional antibiotic therapies, while effective, face significant challenges, such as increasing antibiotic resistance, high recurrence rates, and adverse effects such as gut microflora dysbiosis. These limitations have driven the exploration of alternative antibiotic-free therapies, including the use of plant-based compounds, probiotics, nanoparticles, phage therapy, antimicrobial peptides, and *H. pylori* vaccines. Among these, urease-targeted therapy has shown particular promise. Urease enables the survival and colonization of *H. pylori* by neutralizing stomach acidity. Targeting this urease without disrupting beneficial gut microflora offers a selective mechanism to impair *H. pylori,* due to the absence of this enzyme in most of the human gut microbiome. In this review, we highlight advancements and limitations in the field of antibiotic-free therapies, with a particular focus on anti-urease strategies. We explore the structural and functional characteristics of urease, its role in *H. pylori* pathogenesis, and its potential as a therapeutic target. For the first time, we provide a comprehensive analysis of natural, semisynthetic, and synthetic anti-urease compounds, emphasizing their mechanisms of action, efficacy, and safety profiles. Advances in silico, in vitro, and in vivo studies have identified several promising anti-urease compounds with high specificity and minimal toxicity. By focusing on urease inhibition as a targeted strategy, this review underscores its potential to overcome antibiotic resistance while minimizing gut dysbiosis and improving the outcomes of *H. pylori* infection treatment.

Keywords *H. pylori*, Urease, Anti-urease compounds, Antibiotic resistance, Gut microflora, Alternative antibiotic-free therapies, Targeted therapy

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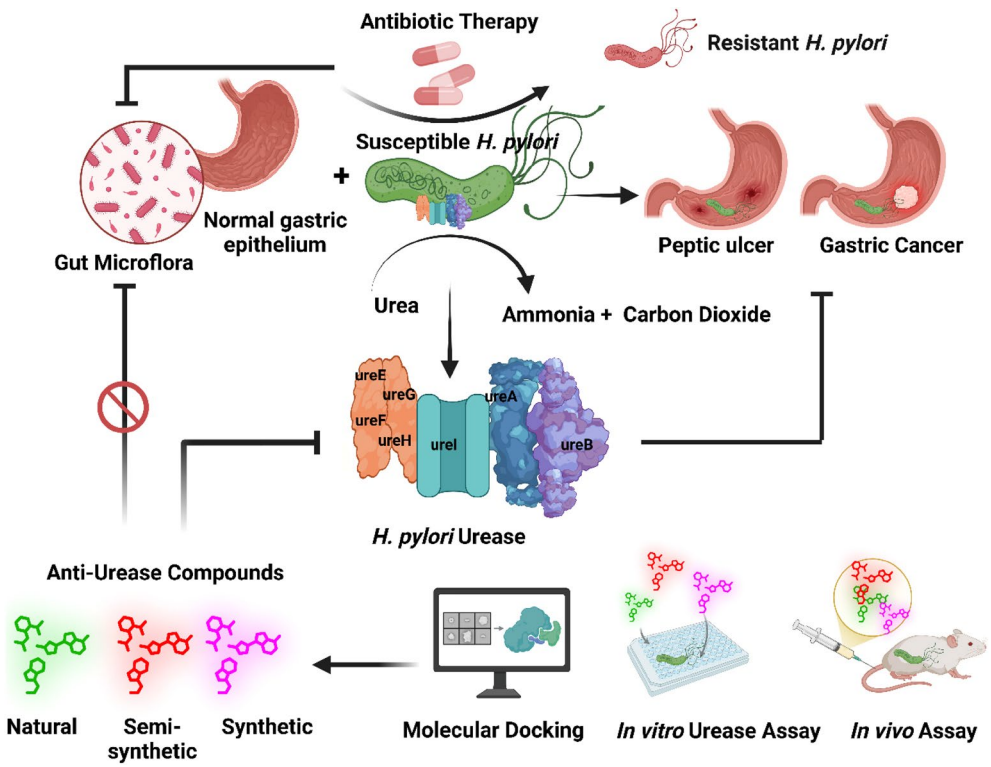
Graphical Abstract



Introduction

Helicobacter pylori (*H. pylori*) is a Gram-negative, microaerophilic, spiral-shaped bacterium that colonizes the human gastric epithelium. It has garnered significant attention from researchers, clinicians, and public health officials due to its remarkable adaptability to the acidic gastric environment and its association with a wide range of gastrointestinal disorders, including chronic gastritis, peptic ulcers, mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric adenocarcinoma [1, 2]. Its pathogenesis is primarily attributed to a variety of virulence factors such as urease, cytotoxin-associated gene A (CagA), vacuolating cytotoxin A (VacA), and adhesins like BabA and SabA, which allow it to adhere to the gastric mucosa, evade immune responses, and cause epithelial damage [3].

The infection typically occurs during childhood and, without treatment, can persist for decades or even for the host's lifetime. *H. pylori* is considered one of the most prevalent chronic bacterial infections globally, affecting an estimated 4.4 billion individuals [2]. Its prevalence is strongly influenced by geographic and socioeconomic factors. Higher infection rates—often exceeding 70–80%—are reported in regions such as Africa, South America, South Asia, and Eastern Europe, largely due to crowded living conditions, poor sanitation, and limited access to healthcare [4]. In contrast, North America, Western Europe, and Oceania exhibit lower prevalence rates ranging from 20–40%, although elevated rates persist among marginalized groups such as immigrants and those with lower socioeconomic status [5].

From a public health perspective, the disease burden associated with *H. pylori* is profound. The bacterium is a Group I carcinogen, as classified by the International Agency for Research on Cancer (IARC) and is the leading cause of non-cardia gastric cancer, which remains among the top causes of global cancer mortality. Beyond cancer, *H. pylori* is responsible for over 90% of duodenal ulcers and 80% of gastric ulcers, contributing significantly to gastrointestinal morbidity worldwide [4].

Traditionally, *H. pylori* has been treated with triple or quadruple antibiotic-based regimens, which combine a proton pump inhibitor (PPI) with antibiotics such as amoxicillin, clarithromycin, metronidazole, tetracycline, levofloxacin, or rifabutin [6, 7]. However, the emergence of antimicrobial resistance (AMR) has led to decline in

treatment efficiency, with therapeutic failures now affecting 20–30% of cases. Particularly alarming is the widespread resistance to clarithromycin, which led the World Health Organization (WHO) in 2017 to classify clarithromycin-resistant *H. pylori* as a high-priority pathogen requiring urgent research and new treatment options [8]. Additionally, prolonged use of PPIs has been linked to adverse outcomes such as gut microbiota dysbiosis, mineral malabsorption, and altered immune responses [9].

Given these limitations, there has been a notable shift toward the exploration of non-antibiotic and adjunctive therapies that reduce resistance risk and minimize microbiota disruption. These include nanoparticle-based drug delivery, plant-derived phytochemicals, probiotics, bacteriophage therapy, antimicrobial peptides, and vaccines [10–15]. Among these, anti-urease compounds have emerged as a compelling alternative due to their targeted inhibition of urease—an essential enzyme for *H. pylori*'s acid-neutralizing survival strategy. By selectively disabling this enzyme, these agents impair the bacterium's ability to colonize the stomach, potentially without exerting strong selective pressure or affecting beneficial flora.

This review focuses on the role of anti-urease therapeutic strategies in the treatment of *H. pylori* infections, examining their mechanisms of action, in silico design strategies, selectivity, and clinical relevance as part of a broader effort to develop innovative and effective treatment approaches.

H. pylori pathogenesis and persistence mechanisms—an overview

H. pylori is widely recognized as a gastric pathogen with a restricted host range that predominantly colonizes the human stomach during childhood. Despite its pervasive prevalence, with infection rates exceeding 90% in developing regions and impacting more than half of the global population, the majority of *H. pylori* infections remain asymptomatic, posing significant public health concerns because of their potential to cause severe gastrointestinal diseases such as peptic ulcers and gastric cancer [16, 17]. The pathogenesis and persistence of *H. pylori* are attributed to its sophisticated mechanisms for colonization, immune evasion, and adaptation to the harsh gastric environment [18–21]. *H. pylori* employs a multifaceted approach to colonize the gastric mucosa and evade the host immune response. Upon entering the stomach, *H. pylori* uses its flagella to navigate through the acidic environment toward the protective mucus layer lining the stomach epithelium [22]. The bacterium adheres to gastric epithelial cells via outer membrane proteins such as BabA (blood group antigen-binding adhesin) and SabA (sialic acid-binding adhesin), facilitating attachment to host cell receptors and promoting persistent colonization [23] (Fig. 1).

Furthermore, *H. pylori* manipulates host cell signaling pathways to disrupt tight junctions, thereby increasing its survival and proliferation. The bacterium secretes effector proteins such as CagA (cytotoxin-associated gene A) and VacA (vacuolating cytotoxin A), which modulate host cell functions, induce inflammation, and contribute to tissue damage. CagA is delivered into host cells via a type IV secretion system (T4SS), leading to alterations in cellular processes, whereas VacA induces vacuole formation, disrupting cellular homeostasis [24, 25] (Fig. 1). Those virulence factors also present promising targets, with inhibitors showing potential in neutralizing its toxicity [26], while epitope-based subunit vaccines offer a complementary strategy for broad *H. pylori* prevention [27].

H. pylori has evolved several mechanisms to persist in the hostile gastric environment. One such mechanism is its ability to transition from a spiral to a coccoid form under adverse conditions such as nutrient deprivation or antibiotic exposure. The coccoid form is viable but non-culturable, allowing *H. pylori* to survive for extended periods and potentially revert to its spiral form when conditions become favorable [28].

Biofilm formation is another persistence strategy employed by *H. pylori*. Biofilms are structured communities of bacteria encased in an extracellular matrix that protects them from environmental stresses, including antibiotic treatment and immune responses. Biofilm-associated *H. pylori* exhibit increased resistance to antibiotics and can act as reservoirs of genetic diversity, facilitating the spread of antibiotic resistance genes [29] (Fig. 1). One of the most critical virulence factors in *H. pylori* pathogenesis is the urease enzyme. Urease plays a pivotal role in the ability of bacteria to colonize the stomach and establish infection. The enzyme catalyzes the hydrolysis of urea into ammonia and carbon dioxide, a reaction that neutralizes gastric acid and creates a more hospitable microenvironment for the bacterium [30, 31]. In addition to neutralizing stomach acid, urease contributes to immune evasion. The ammonia produced by urease activity is toxic to host cells and disrupts their cellular functions, leading to cell death and tissue damage. This damage elicits an inflammatory response characterized by the infiltration of neutrophils and other immune cells, which *H. pylori* further exploits to its advantage [32] (Fig. 1). *H. pylori*-induced inflammation is chronic and can lead to the disruption of gastric mucosal integrity, creating niches for bacterial colonization [33]. The chronic inflammatory response also increases the risk of developing gastric malignancies, including adenocarcinoma and MALT lymphoma [34]. Moreover, the urease

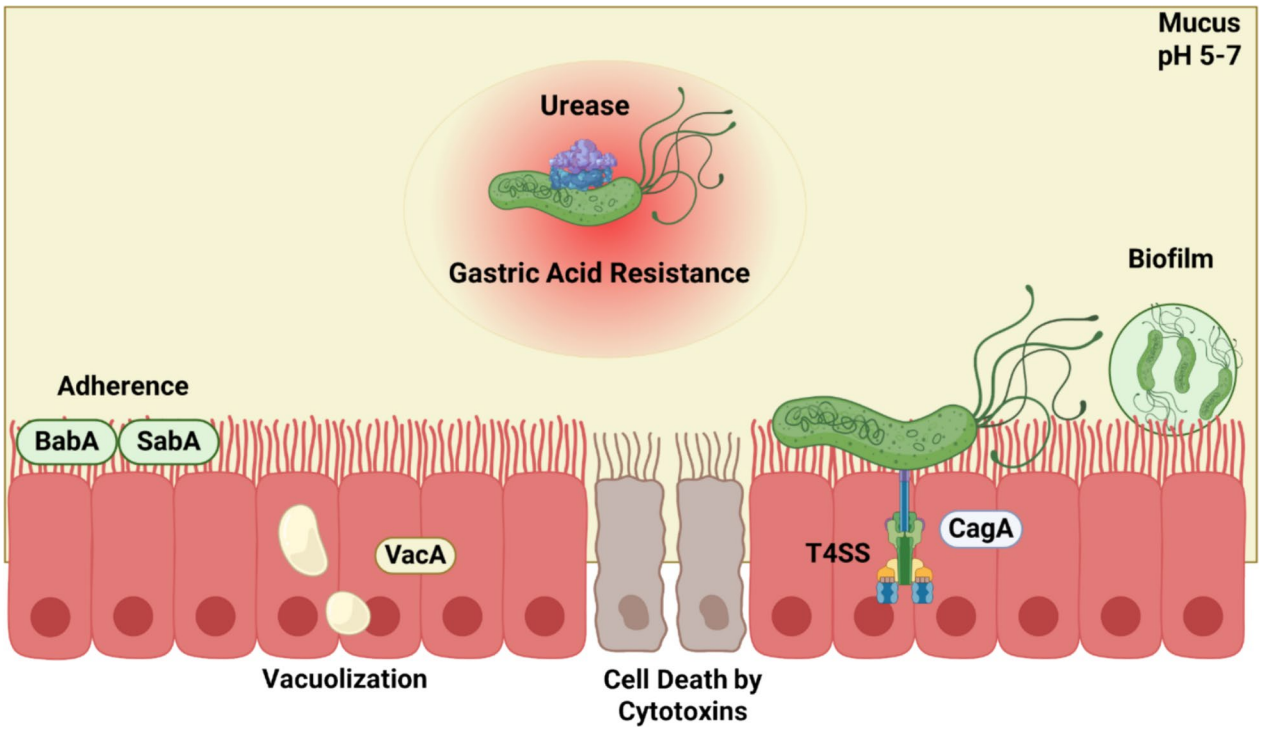


Fig. 1 *Helicobacter pylori's* major virulence factors and their effects on the gastric epithelium. *H. pylori* adheres to gastric epithelial cells via the outer membrane proteins BabA and SabA. The bacterium secretes effector proteins such as CagA and VacA, disrupting host cell functions and inducing inflammation. CagA is delivered via a type IV secretion system, whereas VacA induces vacuole formation. Urease, another critical virulence factor in *H. pylori*, is used to resist gastric acidity by creating a neutral gastric microenvironment. This diagram was created and designed with https://www.biorender.com/

enzyme itself can act as an immunogen, stimulating an immune response that contributes to tissue damage and disease progression [35].

Urease—a critical virulence factor of *H. pylori*

H. pylori depends on its specific virulence factor, urease, to thrive in the harsh acidic environments of the stomach. Urease catalyzes the hydrolysis of urea into ammonia and bicarbonate, creating a localized neutral microenvironment that shields the bacterium from gastric acid. Additionally, ammonia itself contributes to neutralizing the acidic surrounding environment. This allows *H. pylori* to colonize the gastric mucosa and evade host immune defenses. An in-depth understanding of the molecular mechanisms underlying the unique urease-mediated survival mechanism adopted by this pathogen is paramount in developing treatments specifically targeting *H. pylori* urease (HPU). This next section delves into the structural and functional characteristics of HPU, revealing its role as a cornerstone in *H. pylori* pathogenesis [30, 31, 36] (Fig. 2). Unlike other urease-producing bacteria, *H. pylori*'s survival is intricately attributed to its urease activity, presenting a promising avenue for urease-targeted therapeutic interventions. The absence of the urease-encoding gene in the human genome and the gut microbiome further strengthens the appeal of this strategy [37]. By developing treatments specifically targeting HPU, we can selectively suppres viability of *H. pylori* infections while minimizing disruption to the gut microflora, unlike antibiotic-based therapies. Urease, classified as a nickel-dependent hydrolase, is a catalytically potent enzyme responsible for the conversion of urea into ammonia and bicarbonate. The significance of this enzyme extends beyond a mere biochemical process, as it plays a critical role in the survival and pathogenesis of various bacteria, notably *H. pylori*. The importance of this enzyme is centered on its catalytic power and its role in bacterial adaptation to the acidic gastric environment [38, 39] (Fig. 2). Notably, the absence of urease in the human gut microflora and many *E. coli* strains ensures that targeting *H. pylori* urease preserves the gut microbiota balance and overall health. In addition to the gut, urease is also present in other non-*H. pylori* bacteria, such as *Proteus mirabilis*, *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, and *Ureaplasma urealyticum* [40]. *Proteus mirabilis* urease activity is associated with urinary tract infections (UTIs) and the formation of struvite stones, which can lead to chronic kidney issues [41, 42]. Other

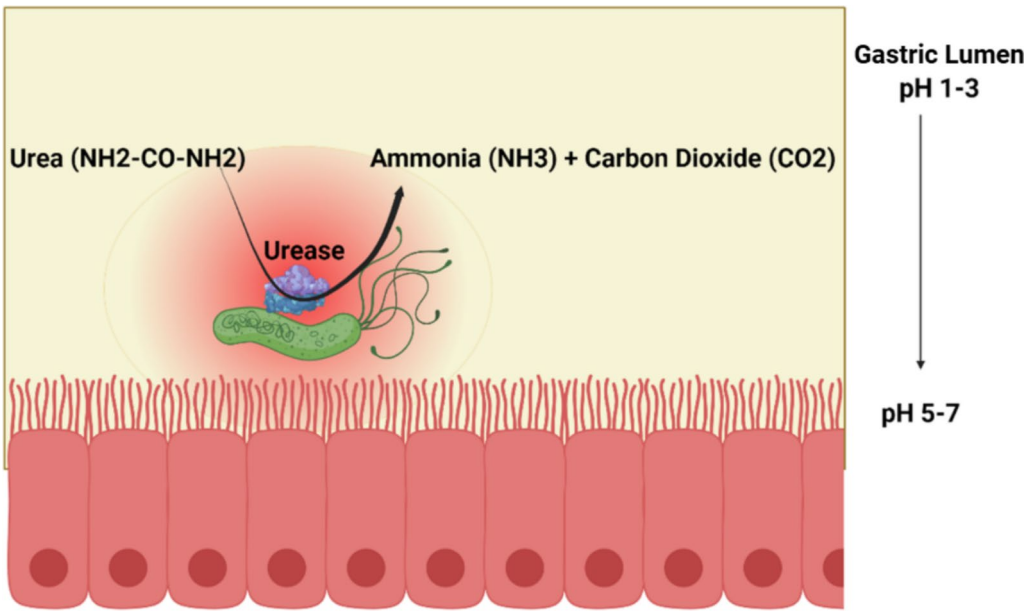


Fig. 2 *Helicobacter pylori* urease (HPU) activity in the stomach. HPU hydrolyzes urea into ammonia (NH_3) and carbon dioxide (CO_2) within the acidic gastric environment. The ammonia produced creates a neutral microenvironment that protects the bacterium from the acidic pH (1–3) of the gastric lumen and helps the bacterium colonize the gastric epithelium. This diagram was created and designed with https://www.biorender.com/

urease-producing bacteria, such as *Klebsiella pneumoniae,* can cause respiratory infections and UTIs [43], *Staphylococcus saprophyticus* is commonly associated with UTIs [44], and *Ureaplasma urealyticum* is known to cause urogenital infections and complications during pregnancy [45, 46]. In the context of *H. pylori*, urease emerges as a virulence factor, contributing significantly to bacterial survival in the hostile gastric environment [4]. The focus here is on unraveling the complexities of urease in *H. pylori*, with an emphasis on its structural organization and functional significance.

H. pylori urease structure

HPU consists of two structural subunits, UreA (26 kDa) and UreB (61 kDa), which assemble into a functional enzyme complex [47], forming a complex tetrahedral structure by four trimers of dimers $((\alpha\beta)_3)_4$ [48], resulting in twelve active sites per functional HPU molecule. This intricate arrangement harbors a unique catalytic site featuring two Ni(II) ions bridged by a carbamylated lysine, facilitating efficient urea hydrolysis. This arrangement underscores the ability of the enzyme to catalyze the hydrolysis of urea with remarkable efficiency [30, 49]. A detailed description of ligand–receptor interactions is crucial for rational drug design targeting *H. pylori* urease. The enzyme's active site contains a binuclear nickel center that is coordinated by conserved residues, including His219, His275, and Asp363, which are critical for urea hydrolysis. Importantly, the His492 residue resides within a flexible mobile flap region, which acts as a gate regulating access to the active site. Upon substrate or inhibitor binding, this flap undergoes conformational changes that modulate catalytic activity. Selective inhibitors often form hydrogen bonds or coordinate directly with the nickel ions, while also interacting with flap-region residues like His492 to stabilize binding. For instance, hydroxamic acid-based inhibitors can chelate both nickel atoms and form additional polar contacts with the surrounding amino acids, enhancing affinity and specificity. This dual mechanism—metal coordination and hydrogen bonding with the dynamic flap—has been shown to be a key strategy in designing inhibitors that are both potent and selective for *H. pylori* urease, minimizing off-target effects on other microbial or human metalloenzymes [50–52]. Notably, tetrahedral assembly contributes significantly to enzyme stability and functionality, allowing for the simultaneous processing of multiple urea molecules and increasing the efficiency of ammonia and bicarbonate production [31]. This structural architecture highlights the sophistication embedded in urease design (Fig. 3). The catalytic site's involvement of nickel ions and a carbamylated lysine residue is pivotal for the enzyme's catalytic ability [47]. This unique configuration gives urease an extraordinary rate enhancement, with a half-life of 20 ms and a rate enhancement of 3×10^{15}, surpassing those of other known hydrolases [53]. Such dynamics

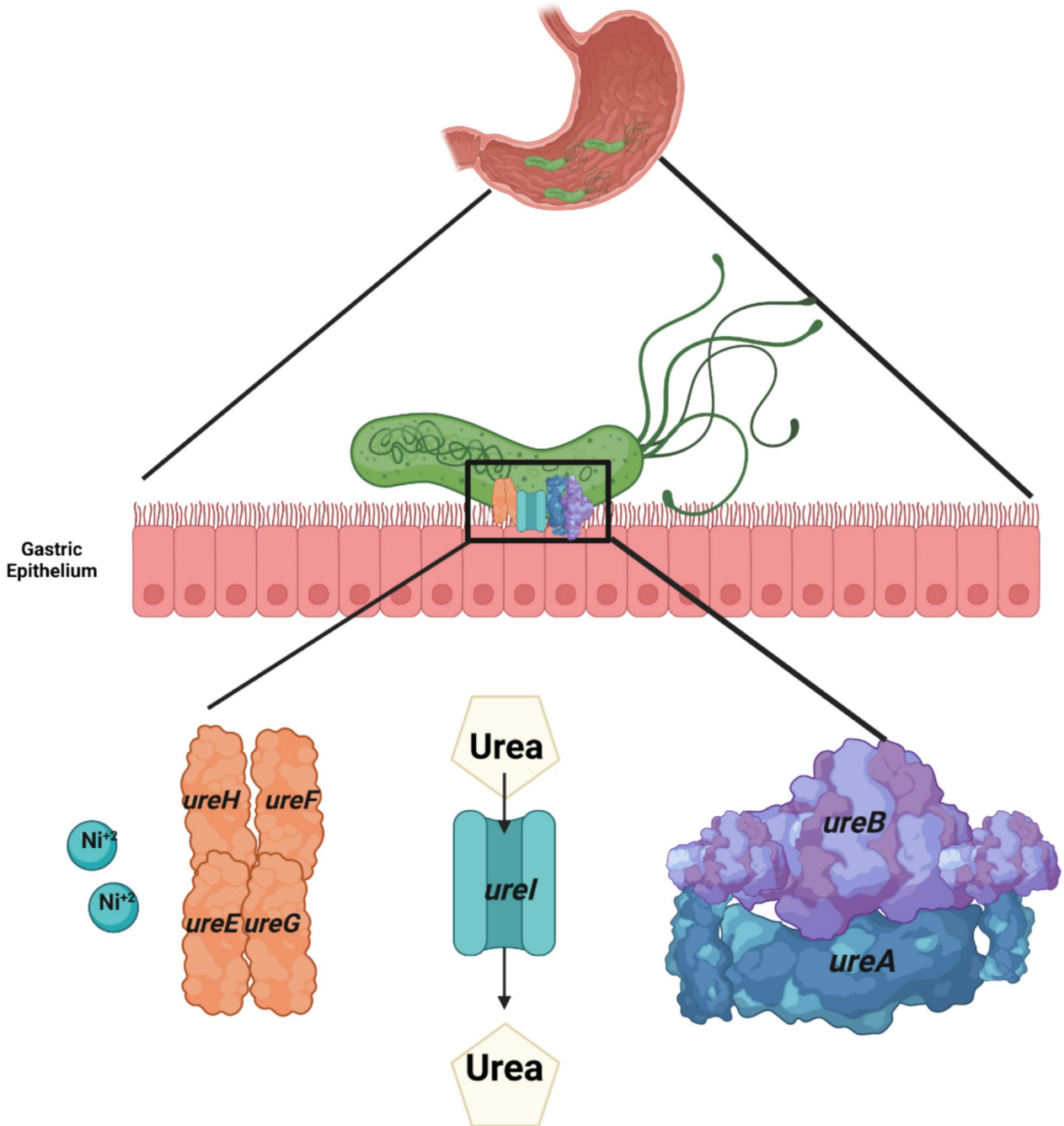


Fig. 3 Functional organization of the urease gene cluster in *H. pylori*. The *ureA* and *ureB* genes encode the 26.5-kDa and 60.3-kDa subunits, respectively. The accessory genes *ureE*, *ureF*, *ureG*, and *ureH* encode the accessory proteins UreE, UreF, UreG, and UreH, which serve to insert nickel ions (Ni^{2+}) into the apoenzyme in an energy-requiring reaction. Two nickel ions are coordinated into the active site of each UreB subunit. Thus, each *H. pylori* urease contains 12 nickel ions when fully activated. The urea transporter (UreI) facilitates the influx of urea into the bacterial cytoplasm. This diagram was created and designed with https://www.biorender.com/

at the catalytic site contribute to the efficiency of urea hydrolysis, a process integral to bacterial survival strategies [54].

Urea (NH_2-CO-NH_2) ammonia (NH_3) + carbon dioxide (CO_2)

The above reaction is fundamental for *H. pylori* survival in acidic environments such as the human stomach. The breakdown of carbamate into ammonia and carbonic acid contributes to an increase in pH, which is central to

the survival of *H. pylori* in the highly acidic conditions of the stomach, as it neutralizes the surrounding acidic environment [36]. Several studies have demonstrated the adaptive mechanisms of enzymes in response to environmental pH changes and their structural resilience. The impact of pH on HPU was analyzed via molecular dynamics simulations at various pH values to determine the protonation states of urease-titratable residues and their influence on the enzyme's charge distribution and stability. The results revealed that under acidic conditions (pH = 2 and 3), urease subunits start to separate, leading to protein disaggregation. This study provides insights into urease behavior in different pH environments, emphasizing its importance in *H. pylori* survival in acidic conditions such as the stomach [55]. Another study analyzing the structure and acid resistance of urease reported the crystallization of HPU, revealing a dodecameric assembly with significant acid resistance. The supramolecular design of the enzyme, with large internal hollow and active sites that mutually protect each other from acid inactivation, contributes to its stability in acidic environments [50]. Urease-mediated hydrolysis of urea generates ammonia, a weak base that neutralizes incoming protons and prevents cytoplasmic acidification. This process allows *H. pylori* to maintain a neutral cytoplasmic pH under acidic conditions, facilitated by the export of ammonium ions via transporters. This mechanism contrasts with conventional pH homeostasis in acidophiles and underscores the crucial role of urease in *H. pylori* survival and pathogenesis [56]. On the other hand, the UreI channel, discussed later in detail, is crucial for regulating urea entry into bacterium, especially under acidic conditions. The coordinated function of these components is vital for *H. pylori* survival [57, 58] (Fig. 3).

Helicobacter pylori urease—mechanism of action

The urease enzyme of *H. pylori* is composed of subunits UreA and UreB. While UreA does not participate directly in catalysis, it is essential for maintaining the structural integrity of the enzyme. UreB, on the other hand, contributes directly to the formation of the active site that houses nickel ions—crucial cofactors required for enzymatic function [59, 60].

Nickel ions are embedded within the UreB subunit and are indispensable for catalysis [52]. The maturation of urease into its active form requires the action of several accessory proteins, including UreE, UreF, UreG, UreH, and UreI. These proteins coordinate the delivery and incorporation of nickel into the urease enzyme, ensuring its catalytic competence.

UreI, a membrane protein, functions as an H^+-gated urea channel. Under acidic stress, UreI is activated, facilitating the influx of urea into the cytoplasm. This mechanism is critical for acid tolerance, as intracellular urease hydrolyzes urea into ammonia and carbon dioxide, effectively neutralizing the acidic environment [61–63].

Nickel acquisition in *H. pylori* is mediated by outer-membrane transport systems such as FrpB4 (a TonB-dependent transporter) and possibly FecA3. Intracellular nickel trafficking involves complex protein networks that manage distribution between urease and hydrogenase systems [39]. Proteins like HspA serve dual roles in nickel storage and delivery, acting as metallo-chaperones crucial for bacterial survival under acidic conditions [39].

The nickel-responsive transcriptional regulator NikR modulates gene expression related to urease activity, maintaining homeostasis in response to intracellular nickel levels. Meanwhile, the NixA transporter plays a central role in nickel uptake, supporting urease function and contributing to *H. pylori* virulence [38, 64–66].

Beyond NixA, *H. pylori* employs additional transporters, including FrpB4 and the recently characterized NiuBDE system. FrpB4 is particularly responsive in acidic environments, while NiuBDE operates effectively across a broader pH range. The complementary pH responsiveness of these systems underlines their adaptive importance for nickel acquisition [67, 68].

Nickel-binding proteins such as HypB, a GTPase involved in urease and hydrogenase maturation, further illustrate the complexity of metal coordination. Nickel binding to HypB is regulated by nucleotide interaction, with His-107 playing a pivotal role in metal coordination. These interactions highlight the tight coupling between metal binding, GTP hydrolysis, and enzymatic biosynthesis in *H. pylori* [69].

Accessory proteins UreH, UreF, UreG, and UreE are indispensable for urease activation. UreF, for example, acts as a GTPase-activating protein, promoting GTP hydrolysis by UreG—a necessary step in nickel incorporation. Structural modeling studies show that UreF undergoes conformational changes upon complexing with UreH, facilitating UreG recruitment and heterotrimeric complex formation (UreG–UreF–UreH), which is critical for urease maturation [70–72].

UreF also forms dimers with high α-helical content and binds two nickel ions per dimer. Specific residues are involved in Ni^{2+} coordination, and mutations in these residues impair binding, reinforcing UreF's role in nickel trafficking [73]. UreG, a SIMIBI-class GTPase, remains structurally flexible even when bound to nickel and GTP. This flexibility suggests that allosteric communication is vital for effective urease activation [74].

Recent studies have identified UreG as a target for urease inhibition. Colloidal bismuth subcitrate disrupts urease maturation by interfering with UreG, suggesting

a potential antimicrobial strategy [75]. Protein–protein interactions within the urease gene cluster have also been elucidated, including complexes such as UreF-UreH and UreG-UreE, which are essential for the delivery of nickel ions to the urease active site [76, 77].

UreG forms a $(UreE)_2$-$(UreG)_2$ complex that mediates nickel transfer from UreE, a critical step for enzyme activation [78]. Molecular dynamics studies have also identified nickel-transport tunnels within the HpUreDFG complex. These tunnels facilitate targeted nickel transfer, underscoring their importance in urease biosynthesis and offering new therapeutic targets for inhibiting enzyme activation [79].

Autoinducer-2 (AI-2), a quorum-sensing molecule, has been shown to upregulate urease expression via suppression of the HP1021 response regulator. This modulation enhances *H. pylori* survival in acidic environments and suggests novel avenues for antimicrobial intervention targeting urease regulation [80].

The histidine-rich protein Hpn plays a central role in nickel trafficking. It binds nickel with high affinity, contributing to both storage and mobilization. Comparative studies reveal differences in nickel-binding thermodynamics across Hpn variants from different *H. pylori* strains, highlighting the significance of histidine and glutamine residues in metal binding [64, 81, 82].

Finally, the interplay between urease and carbonic anhydrases (CAs) is crucial for *H. pylori*'s acid acclimatization. Urease hydrolyzes urea to ammonia (NH_3) and carbon dioxide (CO_2). In the cytoplasm, β-CA hydrates CO_2, while α-CA in the periplasm performs a similar function. These reactions produce ions that interact with ammonia to form NH_4^+ and bicarbonate (HCO_3^-), buffering protons and stabilizing intracellular and periplasmic pH [83–85].

This dual-enzyme system—urease and carbonic anhydrases—demonstrates an evolved strategy by which *H. pylori* survives the gastric niche (Fig. 4). These mechanisms represent critical vulnerabilities that may be exploited in the development of targeted therapies aimed at disrupting bacterial acid resistance and colonization [86, 87].

Conventional antibiotic therapies against *H. pylori*: challenges and limitations

Managing *H. pylori* infection has become increasingly challenging in recent years. The effectiveness of current treatment strategies has been significantly compromised due to the emergence of antibiotic resistance and limitations inherent to the regimens themselves. Traditionally, *H. pylori* therapy has relied on standard triple therapy, a combination of a proton pump inhibitor (PPI) such as omeprazole or lansoprazole with two antibiotics, typically clarithromycin and amoxicillin or metronidazole. While the initial treatment success rate exceed 90%, the effectiveness of this approach has significantly declined in recent years [88, 89]. This decline is attributed primarily to the alarming increase in antibiotic-resistant *H. pylori* strains. The widespread and often inappropriate use of antibiotics in both human and veterinary medicine has fueled the emergence of resistance, particularly against clarithromycin (22.2%) and metronidazole (69.2%) [90]. This rise in resistance significantly hinders the effectiveness of triple therapy, necessitating the exploration of alternative treatment options. Quadruple therapy has emerged as a response to the declining efficacy of triple therapy. This regimen incorporates PPIs, bismuth subsalicylate, metronidazole, and tetracycline. While offering enhanced bacterial suppression rates of up to 90% compared with traditional triple therapy, quadruple therapy is associated with a greater incidence of side effects, including nausea, diarrhea, metallic taste, and potential bismuth-induced black stools. Additionally, concerns remain regarding the potential for this more aggressive regimen to further accelerate the development of multidrug-resistant *H. pylori* strains [91, 92]. In addition to antibiotic resistance, current *H. pylori* therapies present other challenges. Antibiotic treatments often disrupt the balance of the gut microflora, leading to dysbiosis and associated health issues such as inflammatory bowel disease, irritable bowel syndrome, and metabolic disturbances [93–95]. Furthermore, the effectiveness of these standardized treatment regimens can be limited by patient-specific factors. Coinfection with multiple *H. pylori* strains harboring diverse genotypes and phenotypes poses a significant challenge. These variations in the bacterial population can lead to differences in drug susceptibility, complicating treatment selection. Studies have shown that bacterial suppression rates can be significantly lower in patients with mixed infections than in those harboring a single *H. pylori* strain [96–98]. The mechanisms by which *H. pylori* develops antibiotic resistance are multifaceted. Mutations within the bacterial genome can disrupt the binding sites of antibiotics, rendering them ineffective. Examples include point mutations in the 23S rRNA gene for macrolides such as clarithromycin, mutations in the *gyrA* and *gyrB* genes for fluoroquinolones, and alterations in penicillin-binding proteins for beta-lactam antibiotics such as amoxicillin. Additionally, factors such as reduced drug uptake and efflux mediated by multidrug resistance pumps, biofilm formation, and the ability of *H. pylori* to enter a coccoid form that is less susceptible to antibiotics can further contribute to treatment failure. These mechanisms collectively contribute to a concerning rise in *H.*

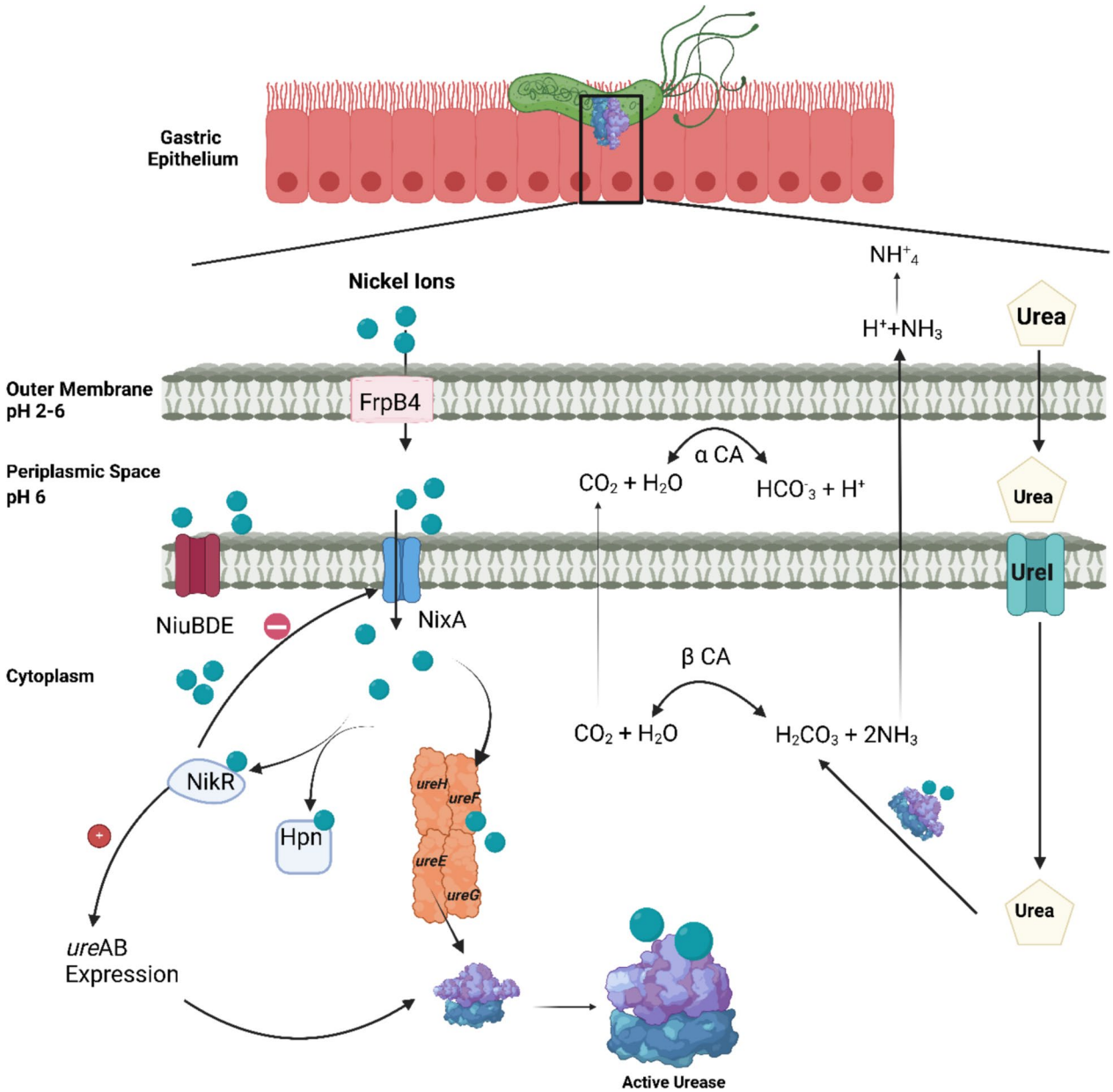


Fig. 4 *Helicobacter pylori* urease mechanism of action. Urease requires the transfer of nickel ions to the periplasm through the nickel transporter protein FrpB4. Nickel is then transported to the cytoplasmic side by NixA. Cytoplasmic nickel can be stored by interaction with Hpn or is delivered to apourease by the nickel chaperone UreE and the assembly factors UreF, UreG and UreH to convert into active urease. In conditions where nickel concentrations are high, the response regulator NikR plays a dual role: it represses the expression of NixA and simultaneously promotes urease expression. Carbonic anhydrase (CA) works with urease to maintain pH homeostasis. Urease converts urea into ammonia (NH_3) and carbon dioxide (CO_2). β-CA in the cytoplasm hydrates CO_2 to form bicarbonate (HCO_3-) and H+, whereas α-CA in the periplasm hydrates CO_2, producing HCO_3- and H+. These bicarbonate ions help buffer the periplasmic pH, and NH_3 neutralizes protons, forming NH_4^+, maintaining a near-neutral cytoplasmic and periplasmic pH under acidic conditions. This diagram was created and designed with https://www.biorender.com/

pylori strains resistant to multiple antibiotic classes, posing a significant threat to successful treatment outcome [99].

Given the evolving landscape of *H. pylori* treatment, the increase in antibiotic resistance has necessitated the exploration of various antibiotic-free therapies. These alternative treatments focus on the use of plant-based compounds, probiotics, nanoparticles, phage therapy, antimicrobial peptides, vaccines, and anti-urease compounds.

Alternative antibiotic-free therapeutic strategies against *H. pylori*

Plant-based compounds

In the context of plant-based therapies for combating *H. pylori*, phytochemicals, which are natural compounds found in plants and have been demonstrated to have anti-*H. pylori* activity [100]. Among these compounds, flavonoids such as quercetin and catechins have been reported to inhibit the growth of *H. pylori* and its adherence to the gastric mucosa [101–103]. Additionally, essential oils from herbs and spices such as oregano, thyme, and cinnamon are recognized for their antibacterial properties against *H. pylori*, potentially disrupting the bacterial cell membrane and interfering with its metabolic processes [104, 104–106]. Ali et al. demonstrated that two bioactive compounds (Eugenol and cinnamaldehyde, both present in essential oils of plants) inhibited the growth of 30 *H. pylori* strains, with enhanced activity at acidic pH and no resistance developing [107]. Another promising avenue for generating *H. pylori* treatments is the exploration of medicinal plants, particularly herbal extracts. Extracts from certain herbs have shown potential in treating *H. pylori* infections. For example, liquorice root, which contains the compound glycyrrhizin, has been demonstrated to be effective in inhibiting *H. pylori* growth [106, 108]. Similarly, green tea, which is rich in catechins, especially epigallocatechin gallate, has notable anti-*H. pylori* activity [109, 110]. Despite the potential of these natural compounds, none of the plant-based compounds are currently approved by the FDA as individual standard therapies against *H. pylori*. In addition, a lack of standardization and quality control, insufficient clinical evidence and potential interactions with other medications can lead to undesirable side effects, leading to limitations in the use of plant-based compounds [111, 112].

Probiotics

Probiotic therapy has gained attracted significant attention as potential treatment for *H. pylori* infections. These live beneficial microorganisms offer a multifaceted approach to combating *H. pylori*. Probiotics target *H. pylori* through several mechanisms. Immunologically, they can modulate the host immune response by secreting anti-inflammatory cytokines and regulating the mucosal immune response. This modulation reduces the release of inflammatory cytokines and chemokines, thereby mitigating the gastric inflammation induced by *H. pylori*. Probiotics stimulate the secretion of mucins and glycoproteins, which protect the gastric mucosa by preventing *H. pylori* adhesion to epithelial cells [83, 113–115]. Non-immunologically, probiotics produce substances such as short-chain fatty acids (e.g., acetic and propionic acids), lactic acid, and bacteriocins. These substances inhibit the growth of *H. pylori* by lowering the local pH and directly reducing bacterial viability. Probiotics also compete for adhesion sites on the gastric mucosa, effectively limiting the ability of *H. pylori* to colonize and establish infection.

In addition to their direct antimicrobial effects, probiotics play a crucial role in modulating the immune system, potentially enhancing the overall response of the host to infection [113, 116]. Specific strains, particularly *Lactobacillus* and *Bifidobacterium* have demonstrated effectiveness against *H. pylori*. These probiotic strains have been shown to inhibit *H. pylori*, reduce the side effects commonly associated with standard antibiotic treatments, and, importantly, enhance treatment success when used as adjuncts. This suggests a synergistic effect, where probiotics not only contribute to their anti-*H. pylori* activity but also enhances the efficacy of conventional treatments. This insight into probiotic therapy underscores its potential as a complementary approach to *H. pylori* management [117–121].

Despite the promising potential of probiotics as alternative therapies for *H. pylori*, several limitations should be considered. One major challenge is the lack of standardization in probiotic formulations. The efficacy of different strains of probiotics can vary widely, and the optimal strains and dosages for *H. pylori* eradication are not yet well defined. Additionally, clinical evidence supporting the ability of probiotics to significantly reduce *H. pylori colonization or improve clinical outcome remains limited* [113]. While some studies show that probiotics can enhance treatment response and alleviate side effects and reduce treatment-related side effects [122], other studies report no significant benefit [123].

Nanoparticles

The use of nanoparticles (NPs) for treating *H. pylori* and gastric cancer shows significant promise because of their unique physicochemical properties. NPs enhance immune regulation and enable targeted drug delivery and the controlled release of drugs, antigens, and adjuvants directly to the intended target sites while avoiding pathological disorders [113, 124]. This controlled drug release property allows for sustained therapeutic effects, reducing the frequency of administration and minimizing systemic toxicity, which is particularly beneficial for treating chronic infections such as *H. pylori* and gastric cancer. Nanoparticles can be broadly classified into three major categories: metal-based (e.g., silver, gold, and zinc oxide), polymer-based (e.g., chitosan and other biodegradable polymers), and lipid-based (e.g., liposomes and solid lipid nanoparticles), each offering unique advantages in drug delivery and inhibit bacterial growth [125, 126].

Metal NPs, including those made from silver, gold, and zinc oxide, have demonstrated strong antibacterial properties against *H. pylori*. Silver NPs interact with proteins in bacterial membranes, leading to disruption of bacterial cell functions and bacterial death. Studies have shown that silver NPs synthesized through green methods, such as using plant extracts, can effectively inhibit the growth of *H. pylori* [127]. In addition to their direct antibacterial effects, silver NPs have been shown to inhibit *H. pylori* quorum sensing (QS) and biofilm formation, disrupting bacterial communication pathways that contribute to antibiotic resistance and persistence in the gastric environment [128]. Likewise, gold and zinc oxide NPs have been shown to damage bacterial cell membranes and inhibit *H. pylori* growth, making them potent agents against antibiotic-resistant strains [124, 129, 130]. Notably, gold NPs have been investigated as drug carriers to enhance the antibacterial effects of conventional antibiotics. A study by Fateh et al. demonstrated that conjugating gold NPs with metronidazole significantly increased its anti-*H. pylori* activity, overcoming metronidazole resistance and resulting in a 17-mm growth inhibition zone against resistant strains [131]. Additionally, an innovative approach using nanocluster-mediated photothermal therapy has demonstrated significant potential in inhibiting *H. pylori* growth. A study by Meng et al. showed that zinc ferrite nanoclusters, when exposed to near-infrared (808 nm) irradiation, effectively inhibited *H. pylori* growth by inducing bacterial membrane disruption and ribosome damage. Moreover, this treatment enhanced antibiotic sensitivity, reducing resistance to levofloxacin and clarithromycin while also decreasing *H. pylori* biofilm formation [132].

Polymeric NPs offer a biocompatible platform for targeted drug delivery in inhibiting *H. pylori* growth. These versatile carriers can be loaded with antibiotics or natural antimicrobials, improving their potency and efficacy. Chitosan-based NPs have been widely researched due to their mucoadhesive properties, which allow them to adhere to the gastric mucosa and deliver drugs directly to the site of infection. These NPs can encapsulate antibiotics such as amoxicillin, improving their stability and effectiveness against *H. pylori* in the acidic environment of the stomach [133]. Furthermore, the ability of chitosan NPs to provide controlled drug release ensures prolonged drug action at the infection site, enhancing bacterial inhibition while minimizing off-target effects. Surface modifications of chitosan NPs with targeting ligands have further enhanced their efficacy by directing them to specific sites of bacterial colonization [133–135]. Additionally, pH-responsive coatings can be employed to ensure the controlled release of the encapsulated drug only within the acidic environment of the stomach, further enhancing the specificity and effectiveness of the therapy [136, 137]. A recent study by Grosso et al. introduced a novel approach using semi-interpenetrating polymer networks (semi-IPN) as gastroretentive drug delivery systems for *H. pylori* treatment. These biodegradable, super porous, and mucoadhesive matrices demonstrated sustained amoxicillin release at pH 1.2 and pH 5.0 over 24 h, ensuring prolonged drug retention in the stomach. Furthermore, formulations incorporating vonoprazan (a potassium-competitive acid blocker) alongside amoxicillin showed promise in enhancing drug stability and improving efficacy while mitigating the development of antibiotic resistance [138].

Lipid-based NPs, such as liposomes, solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) have also been explored for *H. pylori* treatment. These systems provide a biocompatible and biodegradable drug delivery platform with the ability to encapsulate both hydrophilic and hydrophobic drugs. A recent study by Lopes-de-Campos et al. demonstrated the potential of amoxicillin-loaded lipid NPs functionalized with dioleoylphosphatidylethanolamine (DOPE) for targeted *H. pylori*. These lipid NPs exhibited strong bacterial adhesion inhibition, increased gastric retention, and direct bactericidal activity, even in the absence of antibiotics. Additionally, the study introduced a novel floating system to prolong gastric retention time, ensuring sustained drug release and improved therapeutic efficacy [139]. SLNs and NLCs further offer improved drug stability, controlled release properties, and enhanced penetration of the gastric mucosal barrier, making them promising candidates for *H. pylori* treatment therapy [140, 141]. Despite these advancements, challenges remain, such as stability, gastrointestinal degradation, immune response, and the need for biodegradability and compatibility of NPs for clinical use. Future efforts aim to overcome these hurdles, promising wider biomedical applications and individualized precision therapy [142, 143].

Phage therapy

Phage therapy, which uses bacteriophages (phages) and phage lysins, offers several advantages over traditional antibiotics. Phages exhibit high target specificity, minimizing disruption of the gut microbiome [144, 145]. Additionally, phages exhibit a unique characteristic benefit: they self-replicate at the infection site once they encounter their target bacteria. This ability to self-replicate at the infection site reduces the risk of off-target effects. Furthermore, phages have been shown to rapidly enter a death phase when the target bacteria are reduced, minimizing the risk of prolonged exposure and potential development of adverse immune responses [146]. Moreover, phages mutate more frequently than bacteria do,

potentially aiding in the treatment of phage-resistant *H. pylori* strains [147].

Despite these advantages, limited research has been conducted on the use of phages for *H. pylori*. Recent studies show promise for phage-based therapies, as some phages isolated from wastewater (HPE1 and HPE2) have demonstrated the ability to adapt to the acidic environment of the human stomach and exhibit high thermal stability [148]. However, the harsh physiological conditions inside the stomach can compromise the effectiveness of most phages. The acidity of gastric juice and digestive enzymes can greatly affect the biological and structural composition of phages, thus reducing their proliferation and concentration at the infected site [149, 150]. Cuomo et al. demonstrated the effectiveness of lactoferrin-hydroxyapatite complex phages (Hp + LF-HA) in specifically killing *H. pylori* without harming host cells. Furthermore, hydroxyapatite nanoparticles have also been shown to increase phage stability and lytic activity within harsh gastric environments [151]. These advancements highlight the potential for developing targeted and effective phage therapies for *H. pylori*. While the advantages of phage therapy are compelling, limitations exist. The narrow cleavage spectrum of some phages might necessitate a cocktail approach for broader efficacy. Additionally, potential drawbacks include lysogenic phage-mediated transfer of toxins and antibiotic resistance genes, as well as bacterial endotoxin release upon lysis [144, 152]. Further research is needed to address these challenges and optimize phage delivery systems. Importantly, well-designed clinical trials are crucial for assessing the safety and efficacy of phage therapy for *H. pylori* treatment.

Antimicrobial peptides

Antimicrobial peptides (AMPs) represent a naturally occurring and diverse class of gene-encoded proteins produced by a wide range of organisms. AMPs function as the first line of defense in the host innate immune response and play a critical role in combating microbial invasions [153]. Unlike conventional antibiotics that target specific bacterial processes, AMPs primarily exert bactericidal effects rather than bacteriostatic, as they disrupt the microbial cell membrane [154]. This multipronged approach to microbial treatment contributes to broad-spectrum activity and significant selectivity toward bacterial cells. Most AMPs are cationic because of the presence of positively charged residues such as arginine, lysine, and histidine. This positive charge allows AMPs to interact with the negatively charged bacterial cell membrane, leading to increased permeability, pore formation, and ultimately, cell lysis [155, 156]. Additionally, AMPs can traverse the bacterial cytoplasm and disrupt essential processes such as cell wall synthesis, DNA and RNA replication, protein synthesis, and cell division [153]. Furthermore, the relatively low cost of synthesis makes AMPs a potentially cost-effective therapeutic strategy against antibiotic-resistant *H. pylori* strains [155]. Compared with conventional antibiotics which have a single target, the complex mode of action of AMPs makes it significantly more challenging for bacteria to develop resistance [157]. This low incidence of resistance development positions AMPs as a promising strategy to combat multidrug-resistant (MDR) bacteria.

Several AMPs have demonstrated both in vitro and in vivo activity against *H. pylori*. Synthetic analogs such as pexiganan (MSI-78), derived from the frog skin peptide magainin 2, exhibit rapid bactericidal activity against *H. pylori* and synergize with beta lactams, potentially reducing required drug dosage and mitigating associated side effects [158, 159]. Tilapia piscidin 4 (TP4) from tilapia fish mast cells shows potent anti-*H. pylori* activity in vitro and reduces bacterial colonization in animal models, suggesting its potential for effective treatment and prevention of bacterial spread [160, 161]. Similarly, epinecidin-1 (Epi-1) derived from grouper fish manifests dose- and time-dependent bactericidal effects against *H. pylori* and has synergistic effects with antibiotics [162–164].

Cathelicidins, a class of AMPs with immunomodulatory properties, represent another promising avenue for *H. pylori* treatment. Other AMPs, such as human LL-37 and its murine homolog cathelin-related antimicrobial peptide (CRAMP), have shown significant anti-*H. pylori* activity. Notably, human LL-37 exhibits increased resistance to the acidic stomach environment, making it a potentially well-suited candidate for *H. pylori* treatment [165–167]. Furthermore, a study by Jiang et al. suggested that Cbf-K16, a cathelicidin-like peptide, has bactericidal effects on *H. pylori* at low pH and inhibits colonization in an animal model, making it a potentially well-suited candidate for *H. pylori* treatment [168]. Defensins, such as human neutrophil peptide 1 (HNP-1), and plant-derived AMPs, such as SolyC, have also demonstrated efficacy against *H. pylori*, including antibiotic-resistant strains [169, 170]. Bicarinalin, an AMP from ant venom, has dual modes of action: directly targeting *H. pylori* and inhibiting bacterial adhesion to gastric cells. This dual mode of action could significantly reduce bacterial colonization and persistence within the stomach lining [171, 172]. Odorranain-HP from the odorous frog possesses anti-*H. pylori* activity with low hemolytic potential, minimizing potential side effects [173]. PGLa-AM1, a cationic AMP from the African clawed frog, has rapid bactericidal activity against *H. pylori* [174–176]. Finally, bacteriocins produced by certain probiotic bacteria, such as nisin A,

pediocin BA28, and bulgaricus BB18, have been shown to have inhibitory effects on *H. pylori* strains [177–180].

While these AMPs hold immense promise, certain limitations need to be addressed. Bacterial resistance to AMPs, although less common than resistance to conventional antibiotics, can occur through mechanisms such as cell envelope modification or AMP degradation [155]. Additionally, natural AMPs often suffer from proteolytic degradation, poor bioavailability, and short half-lives, necessitating the development of more stable analogs or delivery systems [113, 181]. Further research on their mechanisms of action, overcoming limitations, and optimizing delivery methods is crucial to fully understand their therapeutic potential in the fight against *H. pylori* infection.

Helicobacter pylori vaccine

Vaccination offers a compelling approach to prevent *H. pylori* infection and reduce the associated disease burden. While no licensed *H. pylori* vaccine exists yet, recent advancements in vaccine development technologies and a deeper understanding of *H. pylori* pathogenesis hold promise for overcoming past challenges. Preclinical studies have yielded encouraging results with novel vaccine technologies such as vector-based vaccines using bacteria and multiepitope vaccines carrying specific T and B cells. Vector-based vaccines, particularly those utilizing live bacteria, have demonstrated the ability to elicit a robust immune response due to their efficient delivery and recognition by the immune system [182]. Multiple epitope vaccines, which are designed to target a broader range of *H. pylori* antigens, offer an advantage in combating strains with diverse antigenic profiles [183]. Additionally, vaccines targeting BabA, an adhesin molecule crucial for *H. pylori* adherence to the gastric epithelium, have shown promise in animal models. The BabA vaccine has the ability to offer protection against severe gastric disease even without complete *H. pylori* suppression, suggesting its potential to reduce disease burden independent of bacterial clearance [184].

Despite these advancements, several challenges remain in *H. pylori* vaccine development. Elucidating the optimal T-cell polarization (Th1 vs. Th2) required for effective *H. pylori* clearance is crucial for vaccine design [185, 186]. Additionally, the increased cost of clinical trials, particularly in developing countries with limited resources and expertise, presents a significant hurdle to progress. Continued research on novel vaccine technologies, a deeper understanding of the immune response to *H. pylori* and increased global collaboration in clinical trials are essential steps toward the development of safe and effective *H. pylori* vaccines. Despite the promising potential of alternative antibiotic-free therapies for combating *H. pylori*, significant limitations hinder their effectiveness and reliability. Plant-based compounds, while showing anti-*H. pylori* activity in vitro lacks FDA approval due to insufficient clinical evidence and standardization. Probiotics, although beneficial, face challenges such as variable efficacy and undefined optimal strains and dosages. Nanoparticle-based treatments encounter issues of stability, gastrointestinal degradation, and immune response. Although highly specific, phage therapy requires overcoming the harsh gastric environment and potential safety concerns. Antimicrobial peptides, though potent, suffer from proteolytic degradation and limited bioavailability. Vaccines for *H. pylori* are still in developmental stages, and no licensed options are available. These limitations underscore the critical need for effective anti-urease compounds, which offer a targeted, scientifically validated approach to *H. pylori* inhibition, ensuring better clinical outcomes and patient safety (Fig. 5) (see Table 1).

Anti-urease compounds as targeted alternative *H. pylori* therapies

The enzymatic activity of HPU plays a critical role in bacterial survival within the hostile environment of the stomach, as discussed in Sect. "Urease—a critical virulence factor of H. pylori". By catalyzing the hydrolysis of urea into ammonia and carbon dioxide, HPU effectively neutralizes the acidity of the surrounding environment, creating an alkaline microenvironment that aids in bacterial adherence to gastric epithelial cells. This enzymatic function is indispensable for *H. pylori* colonization and virulence, as it enables the bacterium to evade the destructive effects of stomach acid and establish persistent infection [56, 59].

Numerous studies have underscored the potential of HPU inhibition as a therapeutic strategy against *H. pylori* infection. Several natural products and synthetic and semisynthetic compounds have exhibited promising anti-urease activity in vitro against *H. pylori*. However, the translation of these findings into clinically viable treatments poses significant challenges. Issues such as poor bioavailability, degradation in the acidic gastric environment, and potential cytotoxicity limit the efficacy of many compounds in vivo. HPU inhibitors offer a promising approach by targeting vital enzymes for bacterial survival. Notably, the absence of urease in humans minimizes the risk of off-target effects, as HPU inhibitors specifically target bacterial enzymes without affecting any human enzymes. The lack of urease in *Lactobacillus* strains, one of the main human gut microflorae and in many strains of *E. coli*, a typical resident of the intestinal flora, means that targeting the HPU enzyme will not adversely affect these beneficial species [42–45].

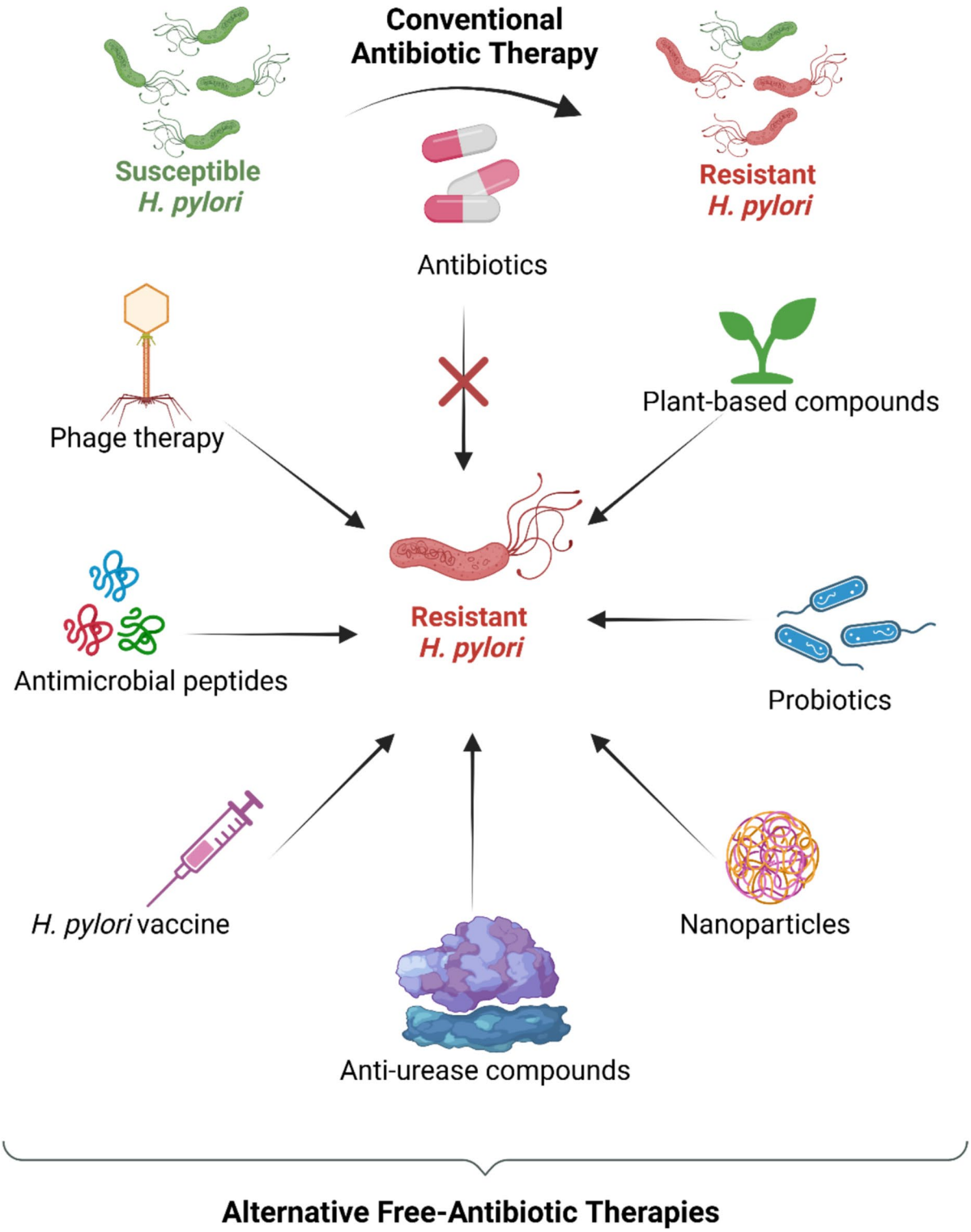


Fig. 5 Alternative free-antibiotic therapeutic strategies for overcoming *H. pylori* antibiotic resistance. The increase in antibiotic resistance has prompted the exploration of alternative therapies for *H. pylori* infections, including anti-urease therapy, plant-based treatments, probiotics, nanoparticles, phage therapy, *H. pylori* vaccines, and antimicrobial peptides. This diagram was created and designed with https://www.biorender.com/

Table 1 Alternative therapeutic strategies against *Helicobacter pylori*: mechanisms, advantages, and limitations

Therapy type	Mechanism of action	Advantages	Limitations
Plant-based compounds	Disrupt membrane integrity, inhibit adhesion, reduce colonization	Natural origin, broad-spectrum effects	Lack of clinical approval, standardization issues, possible drug interactions
Probiotics	Modulate immunity, secrete acids/bacteriocins, compete for adhesion	Safe, can reduce inflammation, improve treatments outcome when combine with antibiotics	Variable efficacy, undefined optimal strains/dosages, limited eradication ability alone
Nanoparticles	Targeted drug delivery, disrupt bacterial biofilms, enhance antibiotic action	Sustained release, improves stability of drugs, effective against resistance	Stability issues, immune response, degradation, regulatory hurdles
Phage therapy	Specific bacterial lysis, self-amplifying action at infection site	High specificity, minimal microbiota disruption	Sensitivity to gastric acidity, lysogenic conversion risk, regulatory limitations
Antimicrobial peptides (AMPs)	Disrupt bacterial membrane, inhibit protein/DNA synthesis, modulate immune response	Broad-spectrum, less resistance development, rapid killing	Poor bioavailability, proteolytic degradation, delivery system required
Vaccines	Induce mucosal and systemic immunity against virulence factors and adhesion molecules	Potential for long-term prevention, strain coverage	Still in development, high cost of trials, unclear immune targets

Preserving the natural balance of the microflora is essential for maintaining overall health, particularly in the gastrointestinal tract. In addition, this absence presents a unique therapeutic opportunity to selectively target urease-producing gut pathogenic bacteria. The selective inhibition of urease can be an effective strategy against pathogens that rely on this enzyme for survival and pathogenicity. This approach has the potential to treat infections caused by urease-positive bacteria, such as *H. pylori* in peptic ulcer disease [187], and certain *Proteus* species in urinary tract infections without harming the beneficial *Lactobacillus* and *E. coli* populations. The development of drugs that inhibit urease represents a promising avenue for antimicrobial therapy. Such drugs could provide a more targeted approach, reducing the risk of dysbiosis and antibiotic resistance that often accompanies broad-spectrum antibiotic use [99]. In conclusion, antiurease compounds present a compelling alternative for *H. pylori* suppression, minimizing the risk of off-target effects in humans. However, challenges remain in identifying potent and selective inhibitors, optimizing their delivery, and exploring combination therapies. Continued research in these areas is essential to unlock the full potential of HPU inhibition and develop effective treatments against this prevalent pathogen. HPU exhibits distinct structural and dynamic characteristics that differentiate it from ureases of other bacterial species. One of the key distinguishing features lies in the mobility and amino acid composition of the flap region- a highly conserved loop that gates the active site. In *H. pylori*, this flap region shows increased flexibility and unique residue motifs, such as a His322-containing loop and a Pro-Glu-Ala motif, which are absent in other microbial ureases like those from *Proteus mirabilis*, *Klebsiella pneumoniae*, or *Bacillus pasteurii* [47, 188]. These variations result in altered active site geometry and ligand accessibility, which can be specifically targeted using structure-guided drug design.

Moreover, sequence alignment studies have revealed that the HPU active site contains unique conserved residues and accessory subunit interactions (UreA and UreB) that are structurally distinct compared to other microbial ureases [189]. These differences can be exploited by small-molecule inhibitors to confer high binding affinity and specificity for HPU.

This review addresses the urgent need for innovative approaches to managing *H. pylori* infections, given their prevalence and association with various gastrointestinal diseases. Despite the diversity of emerging non-antibiotic therapeutic options—such as antimicrobial peptides, phage therapy, plant-based bioactives, and nanoparticles—urease inhibition presents a uniquely rational and targeted strategy for *H. pylori* treatment. This prioritization is based on several critical factors: First, urease is an obligate survival factor for *H. pylori*, playing a non-redundant role in acid resistance and colonization. Unlike other alternatives that interfere with peripheral mechanisms, urease inhibition directly compromises bacterial viability in the stomach. Second, urease is absent in humans and key commensals like *Lactobacillus* and many *E. coli* strains, thereby offering a selective therapeutic window with minimal disruption to the host microbiota. Third, structural features unique to HPU—such as the flexible flap region with distinct residue motifs (e.g., His322-containing loop and Pro-Glu-Ala sequence)—enable highly specific inhibitor design, enhancing selectivity and binding affinity. Finally, the existence of standardized urease assays and molecular docking platforms [190] streamlines preclinical screening and drug development, while reducing the risk of resistance development compared to

broad-spectrum antibiotics. The cell-free urease assay is a fundamental technique for identifying effective urease inhibitors, providing a simplified and controlled environment to screen a diverse array of compounds without the complexities associated with live bacterial systems. This method involves evaluating potential anti-urease compounds, typically identified through molecular docking, against a commercial urease enzyme. The efficacy of these compounds is determined by their ability to reduce urease activity, either by assessing ammonia production via colorimetric methods or monitoring pH changes [191–193]. Notably, the cell-free assay evaluated the anti-urease activity of compounds outside the context of *H. pylori*. To address this, a specific anti-*H. pylori* urease assay was also employed to confirm the activity of the tested compounds against urease extracted from live *H. pylori* after treatment. Ultimately, in vivo assessment remains the ideal method to evaluate these compounds, as it accurately reflects their impact on the colonization and persistence of *H. pylori* within the gastric mucosa.

Natural anti-urease compounds

Natural compounds found in plants, animals, fungi, and microorganisms possess diverse biological activities, making them important in fields such as medicine, where they are often used as the basis for drug development owing to their therapeutic properties. Owing to their bioactive compounds, such as phenolics, terpenoids, and alkaloids, plants are a significant source of natural urease inhibitors [194]. These inhibitors are not only environmentally benign but also potentially more effective because of their biodegradability and synergistic actions. Studies have investigated various plant extracts for their anti-*H. pylori* and urease inhibitory activities. Amin et al. evaluated the effects of extracts of *Acacia nilotica*, *Calotropis procera*, *Adhatoda vasica*, *Fagonia arabica*, and *Casuarina equisetifolia* against *H. pylori* [195]. These findings which suggest competitive inhibition of *A. nilotica* and mixed-type *C. procera*, support the traditional use of these plants for stomach ailments and highlight their potential as natural anti-*H. pylori* agents [195].

Another study aimed to identify the antibacterial and urease inhibitory effects of *Oliveria decumbens* extracts and fractions against *H. pylori* and other bacteria. Researchers isolated three new kaempferol derivatives and two thymol derivatives from plants and reported that the n-hexane fraction exhibited significant anti-*H. pylori* activity. Stigmasterol, tiliroside, and carvacrol were the most potent urease inhibitors identified. This work aligns with the traditional use of *Oliveria decumbens* to treat gastrointestinal infections [196]. Coptisine, the main alkaloid in *Rhizoma Coptidis,* was investigated for its urease inhibitory effects in an interesting study [197]. This study demonstrated the antibacterial and bactericidal effects of coptisine against *H. pylori* strains, including those resistant to antibiotics. Notably, the minimum inhibitory concentration (MIC) of Coptisine against *H. pylori* ranged from 25 to 50 μg/mL. Interestingly, coptisine disrupts urease maturation by inhibiting the activity of UreG, a key accessory protein required for urease assembly. It specifically hinders urease's ability to form dimers and bind nickel, effectively preventing the formation of functional urease enzymes. This multifaceted action suggests a comprehensive inhibitory effect on both urease activity and its maturation process [197].

Another natural protoberberine alkaloid, epiberberine, has also been investigated for its urease inhibitory properties. Epiberberine has been identified as the most potent urease inhibitor, exhibiting significant inhibition at low micromolar concentrations. Research indicates that its mechanism of action involves slow-binding, competitive inhibition by directly targeting the enzyme's active site [198]. Further studies have investigated the inhibitory effects of evodiamine, a compound derived from *Evodia rutaecarpa*, on *H. pylori* growth and inflammation. Evodiamine effectively suppresses *H. pylori* by downregulating genes essential for its replication, transcription, and urease production. Additionally, it diminishes the translocation of cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA) into gastric cells, contributing to reduced inflammation by inhibiting NF-κB and MAPK pathway activation [199]. Another example of a natural compound with anti-urease activity is hespertin, which is a flavonoid found in citrus fruits. Hesperetin significantly inhibits *H. pylori* growth by downregulating genes involved in bacterial replication, transcription, motility, and adherence [187]. It reduces urease activity, *H. pylori* colonization and survival in the gastric mucosa. Additionally, hesperetin was also found to inhibit, similar to evodiamine, the translocation of the virulence factors *cagA and vacA* into gastric cells, which are key to *H. pylori* pathogenicity [200]. On the other hand, *Canarium album* fruits have emerged as promising natural sources for the development of new treatments for *H. pylori* infection. A study by Jiahui Yan et al. revealed that an ethyl acetate extract notably inhibited *H. pylori* growth and urease activity while downregulating key virulence genes such as *vacA* and *cagA* [201]. Another natural compound, zerumbone, extracted from *Zingiber zerumbet*, has unique effects on HPU activity through dimerization, trimerization, or tetramerization with urease molecules without affecting any urease gene transcription or urease protein expression [202]. Among the natural anti-urease compounds, flavonoids have shown promising results through molecular docking and dynamics simulations alongside analyses of their

physicochemical properties and toxicity. For example, flavonoids, specifically chrysin, galangin, kaempferol, luteolin, morin, and quercetin, demonstrate a stronger binding affinity to urease than traditional inhibitors, such as acetohydroxamic acid (AHA) [100]. Motivated by the therapeutic potential of quercetin for the treatment of gastric ulcers, Zhu-Ping Xiao et al. investigated its urease inhibitory activity against *H. pylori*, along with its analogs. The findings revealed the remarkable inhibitory potency of quercetin, with molecular docking and kinetics suggesting a noncompetitive inhibition mechanism [203]. To further research flavonoids, Izabela Korona-Glowniak et al. investigated the efficacy of 26 different commercial essential oils for their MICs against the *H. pylori* ATCC 43504 strain. The thyme, lemongrass, cedarwood, and lemon balm oils presented the most potent anti-*H. pylori* activities, with MIC values as low as 15.6 µg/ml. This study not only expands the series of natural agents used against *H. pylori* but also underscores the potential of essential oils, particularly cedarwood and oregano oils, to inhibit urease activity at sub-MIC concentrations [204]. Another study investigated the effects of acetohydroxamic acid (AHA), baicalin, and ebselen on *H. pylori* urease activity, bacterial survival, and gene expression. AHA and ebselen strongly inhibited urease activity at low doses, reducing it by 84% and 71%, respectively, whereas baicalin requires relatively high doses to achieve similar effects. All three compounds significantly reduced bacterial viability, with AHA being the most effective. Interestingly, the inhibitors caused an increase in *ureA* and *ureB* gene expression, suggesting a survival response by the bacteria. Baicalin also reduced ATP production, whereas AHA and ebselen did not. An analysis of clinical isolates revealed differences in urease activity but no link to infection severity [205]. An innovative strategy for managing *H. pylori* involves the use of nanoparticles. A previous study demonstrated this by synthesizing silver nanoparticles (AgNPs) from *Ficus carica* leaf extract. These AgNPs manifested significant inhibitory activity against the urease enzyme, suggesting promising potential for *H. pylori* treatment via the integration of traditional plant extracts with nanotechnology [206]. Another study employed green synthesis methods to create silver nanoparticles from *Solanum xanthocarpum* berry extract. These nanoparticles exhibited potent anti-*H. pylori* activities and significant urease inhibitory effects. This innovative approach aligns with green chemistry principles and supports the potential of silver nanoparticles as antibacterial agents, particularly against *H. pylori* infections [207] (Among anti-urease compounds, Acetohydroxamic Acid (AHA) is the only agent that reached clinical use, primarily for urease-positive urinary tract infections. However, due to its toxicity profile and limited gastric applicability, it was not advanced further for *H. pylori* therapy) (Table 2).

Semisynthetic anti-urease derivatives

Semisynthetic substances derived from naturally occurring compounds through chemical modifications can enhance or modify the properties of the original compound, thus increasing its suitability for pharmaceutical development. Compared to their natural counterparts, semisynthetic derivatives can offer improved efficacy, stability, or safety profiles. Sulforaphane (SF), a compound derived from broccoli and other crucifers, is an example of this approach. Fahey et al. reported that SF inactivation of urease follows first-order kinetics, which are dependent on the enzyme and SF concentrations. Their study also provided evidence of dithiocarbamate formation between SF and the cysteine thiols of urease [213]. Another study explored the synthesis and molecular docking of morin analogs to identify potent urease inhibitors and antioxidants. This research identified *N*-(2-chlorophenyl)-*N*-((4E)-2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-ylidene) thiourea (M2b) and *N*-(4-bromophenyl)-*N*-((4E)-2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-ylidene) thiourea (M2i) as potent urease inhibitors and antioxidants [214]. Xiao et al. presented a study on the synthesis and antibacterial properties of palmatine (PMT) derivatives against *H. pylori*, with a focus on strains resistant to metronidazole. This finding reveals that introducing a secondary amine at the 9-position of PMT derivatives could increase their anti-*H. pylori* potency. These derivatives are exceptionally effective against metronidazole-resistant *H. pylori* strains, with MICs ranging from 4 to 16 µg/mL [215]. Among the reductive derivatives of compound W, the 4-deoxy analogs exhibit the most potent inhibitory effects. Specifically, the compound 4′7,8-trihydroxyl-2-isoflavene emerged as the most active, with an IC_{50} value of 0.85 mM, indicating greater than 20-fold greater potency than AHA, a commercial urease inhibitor [216] (Table 3).

Synthetic anti-urease compounds

Synthetic compounds are artificially created chemicals or substances rather than being naturally occurring. They are designed and manufactured through specific chemical processes in laboratories or industrial settings. A literature review of anti-urease compounds revealed that many of them are synthetic, as shown in Table 4. The synthesis and evaluation of a series of novel amantadine-thiourea conjugates (3a–j) as inhibitors of jack bean urease identified N-(adamantan-1-ylcarbamothioyl) octanamide (3j), with a 7-carbon alkyl chain, as the most promising urease inhibitor candidate, with an IC_{50} of 0.0085 µM.

Table 2 Summary of natural anti-urease compounds and extracts evaluated against *Helicobacter pylori*

Natural compounds/extracts	Cell free urease assay	Anti-*H. pylori* urease assay		In vivo assay	Findings	References
		IC_{50}	MIC			
Plant extracts from *Acacia nilotica (L.) Delile, Calotropis procera (Aiton) W.T. Aiton, Adhatoda vasica Nees, Fagoniaar abica L. and Casuarina equisetifolia L*	–	–	[4.0–256] µg/mL	–	The acetone and methanol extracts of *Acacia nilotica* and *Calotropis procera* exhibited significant anti-*H. pylori* and urease inhibitory activities	[195]
Isolated compounds from *Oliveria decumbens:* Stigmasterol Tiliroside Carvacrol	[0.27- 0.7] mM	–	50 µg/mL	–	The hexane fraction was the most effective due to the presence of stigmasterol and carvacrol	[196]
Rhizoma Coptidis alkaloids: Coptisine	–	–	[25–50] µg/mL MBC: [37–125] µg/mL	–	Coptisine inhibits slow binding of the urease enzyme by disrupt urease maturation in affecting UreG activity, dimer formation, and nickel ion delivery	[197]
Protoberberine alkaloid Epiberberine	2.3 µM	3 µM	–	–	Epiberberine was found to act as an uncompetitive inhibitor for HPU while competitive for Jack bean urease, in a slow-binding and concentration- and time-dependent manner	[198]
Evodia rutaecarpa: Evodiamine	–	–	20 µM	–	Evodiamine reduces T4SS and SecA protein expression, limiting CagA and VacA translocation into AGS cells and inhibits *H. pylori*-induced MAPK/NF-κB activation resulting in decreased IL-8 secretion	[199]
Flavanone found in citrus fruits: Hesperetin	–	–	50 µM	–	Hesperetin exhibits broad spectrum of anti-*H. pylori* activity including suppression of genes expression involved in replication, transcription, motility, adhesion, and urease production. In addition, reduces the translocation of CagA and VacA toxins into gastric epithelial cells	[200]
Qing Guo (QG) extracts: Phenolics components	–	1093 µg/ml 333 µg/ml	[39–625] µg/ml MBC: [78–1250] µg/ml	–	All QG, extracts aqueous extract (QGAE) and ethyl acetate extract (QGEAE) could induce the morphological and structural changes of *H. pylori*, inhibit urease activity and downregulate the virulence genes, such as vacA and cagA	[201]
Flavonoid compounds: Chrysin, galangin, kaempferol, luteolin, morin, and quercetin	–	–	–	–	All the investigated flavonoid compounds are capable of inhibiting *H. pylori* urease. Among these compounds, six compounds chrysin, galangin, kaempferol, luteolin, morin and quercetin showed a greater tendency to bind to urease, compared to AHA inhibitor	[100]
Flavonoids: Quercetin	11.2 µM	–	–	–	Among the 20 flavonoids compounds Quercetin has the excellent potency and acts as a noncompetitive urease inhibitor	[203]
Citrus uranium fruit peel extract: Hesperetin-7-rhamnoglucoside (Hesp)	–	40.6 mM	–	–	Hesp inhibited *H. pylori* urease in a competitive and concentration-dependent manner, and it interacts with bacterial cells, causing membrane disruption and amino acid leakage	[187]

Table 2 (continued)

Natural compounds/extracts	Cell free urease assay	Anti-*H. pylori* urease assay		In vivo assay	Findings	References
		IC_{50}	MIC			
Essential Oils: Cedarwood oil	–	5.3 mg/L	15.6 mg/L	-	The activity in vitro of the five essential oils silver fir, pine needle, tea tree, lemongrass, and cedarwood oils against *H. pylori* was confirming the inhibition of urease. The most active against clinical strains of *H. pylori* were cedar wood	[204]
Zingiber zerumbet Smith extract: Zerumbone	–	–	[50–100] µM	–	Disrupts urease activity without affecting either gene transcription or protein expression of urease A and B suggesting the formation of inactive urease-zerumbone complexes	[202]
Rumex acetosa extracts: Chrysophanol-8-O-β-D-glucoside (5)	-	8.60 µM	15.7 µM	–	Three anthraquinones and three anthraquinone glucosides were identified as the major chemical constituents were identified: emodin (**1**), chrysophanol (**2**), physcion (**3**), emodin-8-*O*-β-d-glucoside (**4**), chrysophanol-8-*O*-β-d-glucoside (**5**), and physcion-8-*O*-β-d-glucoside (**6**) All isolates exhibited anti-*H. pylori* activity with different potencies, with an MIC value ranging between 3.13 and 25 µM	[208]
Laurel (*Laurus nobilis L.*) leaves extract(LLE): Gallic acid,Chlorogenic acid, Catechin, Methyl gallate, Caffeic acid, Syringic acid, Pyrocatechol, Rutin, Ellagic acid, Coumaric acid, Vanillin, Ferulic acid, Naringenin, Daidzein, Quercetin, Cinnamic acid, Apigenin, Kaempferol, Hesperetin	–	34.17 µg/mL	1.9 µg/mL	–	Enhancing the release and yield of phenolic and flavonoid compounds in laurel leaf extract by application of moist heat (MH) compared to Unmoist-heated (UMH) leading to improve Anti- *H. pylori (Anti-uraease)*, antioxidant, antidiabetic, and anti-Alzheimer's effects	[209]
Zanthoxylum armatum DC extracts: chlorogenic acid	57.67 mg/mL	–	–	–	Three isolated phenolic compounds viz., chlorogenic acid, *trans*-ferulic acid, and gallic acid of leaves of *Zanthoxylum armatum* DC were evaluated Chlorogenic acid was found to show the strongest interaction with the *H. pylori* urease and coronavirus main protease	[210]
Citrus sinensis leaves extract coumarins: Citropten	–	2.4 µM	3.9 µg/mL	–	Three coumarins—bergapten, xanthotoxin, and citropten—were isolated from the leaf extract of *Citrus sinensis L.* and identified through NMR and ESI–MS analysis	[211]
Ficus carica synthesized silver nanoparticles (AgNPs)	16 mg/ml	–	–	–	Synthesis (AgNPs) using *Ficus carica* extract demonstrated potent urease inhibition, with only 16 ± 0.7% ammonia release, closely matching the efficacy of the standard inhibitor thiourea (3.87 ± 1.1%)	[206]
silver nanoparticles (AgNPs) synthesized from Solanum xanthocarpum berry extract	–	–	2–8 µg/mL	–	Inhibit urease in AgNPs were found to be more potent than silver nitrate and some standard antibiotics like metronidazole	[207]

Table 2 (continued)

Natural compounds/extracts	Cell free urease assay	Anti-*H. pylori* urease assay		In vivo assay	Findings	References
		IC_{50}	MIC			
Baicalin Ebselen	–	8 mM 0.06 mM	–	–	Baicalin, and ebselen inhibited *H. pylori* urease with ebselen showing higher potency, significantly reducing *H. pylori* viability	[205]
Phenolic compounds identified in sumac fruit, pomegranate peel and almond leaves:	–	21 ug/mL	6-12 mg/mL	–	Polyphenols from sumac fruit, pomegranate peel, and Indian almond leaves showed promising results as urease inhibitors against urease-producing bacteria and jack bean urease activity as well as strong antibacterial properties	[212]

IC_{50}: half-maximal inhibitory concentration; MIC: minimum inhibitory concentration; MBC: minimum bacterial concentration; (–): experiment not conducted

Table 3 Summary of semisynthetic anti-urease compounds tested against *H. pylori*

Semisynthetic compounds names	Cell free urease assay	Anti-*H. pylori* urease assay		In vivo assay	Findings	References
		IC_{50}	MIC			
Sulforaphane and isothiocyanates: 1-isothiocyanato-4-(methylsulfinyl)butane	–	–	–	–	Sulforaphane demonstrates potent bactericidal effects (MBC = 2.8–5.6 µg/mL) against both urease-positive and urease-negative strains, indicating that its mechanism of action extends beyond urease inhibition. This dual activity sets it apart from isothiocyanates, which are strong urease inhibitors but lack bactericidal properties	[213]
Morin derivatives: *N*-(2-chlorophenyl)-*N*-((4E)-2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-ylidene)thiourea (M2b) *N*-(4-bromophenyl)-*N*-((4*E*)-2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-ylidene) thiourea (M2i)	10.7 µM 11.1 µM	–	500 µg/ml	–	M2b and M2i potent derivatives of morin with excellent urease inhibition, strong antioxidant activity, and significant antibacterial efficacy against *H. pylori*. M2i, in particular, showed superior antibacterial activity compared to the standard reference compound	[214]
Palmatine derivatives: 2,3,10-Trimethoxy-9-p-methylbenzylaminoprotopalmatine chloride (1c)	–	6.76 µg/mL	4–16 µg/mL	$LD_{50} > 1000$ mg/kg	1c exhibits antibacterial activity against metronidazole-resistant *H. pylori* strains and a good safety profile in toxicity assay	[215]
Flavonoids derivatives: 4′7,8-trihydroxyl-2-isoflavene (13)	0.85 mM	–	–	–	Nineteen reductive derivatives of flavonoids were synthesized, compound (13) was found the most potent one compared with AHA	[216]

IC_{50}: half-maximal inhibitory concentration; MIC: minimum inhibitory concentration; MBC: minimum bacterial concentration; (–): experiment not conducted; LD_{50}: half-lethal dose

Table 4 Summary of synthetic anti-urease compounds tested against *H. pylori*

Synthetic compounds names	Cell free urease assay	Anti-*H. pylori* urease assay		In vivo assay	Findings	References
		IC_{50}	MIC			
Novel amantadine-thiourea conjugates: *N*-(Adamantan-1-ylcarbamothioyl) octanamide (3j); *N*-(adamantan-1-ylcarbamothioyl)-2-chlorobenzamide (3 g)	[0.0085–0.0087] µM	–	–	–	Compound (3j) possessing a 7-carbon alkyl chain and compound (3 g) possessing a 2-chlorophenyl substitution showed excellent urease inhibitory activity. Compound (3j) was identified as a non-competitive urease inhibitor. It showed strong binding affinity, interacting outside the catalytic site. These findings suggest compound (3j) as a promising lead for designing potent urease inhibitors	[217]
Barbituric acid derivatives: 5-benzylidine barbiturate	41.6 µM	–	–	–	Inhibits urease activity by having a great chelating ability of the bimetallic nickel center	[223]
N,N-Dimethylbarbituric-pyridinium derivatives (7a-n): 4-((((1,3-dimethyl-2,4,6-trioxotetrahydropyrimidin-5(2*H*)-ylidene)methyl)amino)methyl)-1-(2-methylbenzyl)pyridin-1-ium bromide (Compound 7b)	[10.37–77.52] µM 10.37 µM	–	–	–	Compounds 7a-7b and 7f-h were more potent than standard drug thiourea. The most potent compounds interacted with important residues of urease active site	[224]
Cu(II) complexes: $[Cu(C_{15}H_{16}NO_2)_2]$; $[Cu(C_6H_9N_2O_4)_2{\cdot}3H_2O]$ $(2{\cdot}3H_2O)$	–	[1.05–3.23] µM	–	–	Inhibits urease activity through interacting with hydrogen bonding and hydrophobic interactions of the enzyme	[225]
Carbazole-triazine hybrids: 6-(9*H*-Carbazol-9-yl)–*N*2, *N*4 -bis(4-iodophenyl)-1,3,5-triazine-2,4- diamine	[5.6–6.7] µM	–	–	–	Inhibits urease activity by exhibition hydrogen bonding, π-π interactions, π-cation, and coordination to nickel atoms in urease's active site	[219]
Enamines compounds: Z28824346, Z422952944, Z826553418	[0.32–0.68] µM	–	–	–	Out of 1.83 million compounds from the Enamine database, 3 potent compounds were identified. Inhibit urease activity impacting bacterial growth under acidic conditions through competitive and mixed inhibition	[220]
Panobinostat, Dacinostat, Ebselen, Captan, and Disulfiram	–	0.013 µM	–	–	Out of 3904 compounds of FDA or FAD-approved drugs from John Hopkings Clinical compound Library (JHCCL) and TopScience BioTech Co. Ltd. (Shanghai, China), 5 potent urease inhibitor compounds were identified. Inhibit urease activity through a combination of competitive and covalent-allosteric modifications. Effectively prevent *H. pylori* infection in SGC-7901 adenocarcinoma gastric cells	[231]

Table 4 (continued)

Synthetic compounds names	Cell free urease assay	Anti-*H. pylori* urease assay		In vivo assay	Findings	References
		IC_{50}	MIC			
Aniline-containing hydroxamic acids: 3-(3,5-dichlorophenylamino)-*N*-hydroxypropanamide (3a); 3-(2-chlorophenylamino)-*N*-hydroxypropanamide (3d); 3-(2,4-dichlorophenylamino)-*N*-hydroxypropanamide (3n)	[0.018–0.055] µM	[0.1–5.9] µM	–	HP Eradication Rate above 85%	Reduced gastritis development in an *H. pylori*-infected mouse model at a dose of 32 mg/kg. Acute toxicity in mice disclosed that 3a, 3d and 3n was well-tolerated in mice with LD_{50} of 2982.8, 3349.4 and 3126.9 mg/kg. Compound 3n was considered the most promising candidates for the potential treatment of *H. pylori* caused gastritis and gastric ulcer	[228]
Arylmethylene hydrazine derivatives bearing 1,3-dimethylbarbituric moiety (7a-o): (*E*)-1,3-dimethyl-5-((2-(2-nitrobenzylidene) hydrazinyl)methylene)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (7 h)	[0.61–4.56] uM	–	–	–	Six synthesized arylmethylene hydrazine derivatives (7 h, 7 m, 7c, 7 l, 7i, and 7o) have shown potent urease inhibition where compound 7 h with 2-nitro benzylidene group was found to be the most potent compound. Compound 7 h inhibits urease activity by interacting with Arg609 and Cys592, impacting the flexibility of the mobile flap covering the active site. In silico physico-chemical study of compounds 7a–o predicted that all these compounds are drug-likeness with considerable orally availability	[221]
Sulfonates and sulfamates bearing imidazo[2,1-*b*] thiazole scaffold: 3-(5-(3-(Methylsulfonyl)phenyl)imidazo[2,1-*b*] thiazol-6-yl)phenyl propane-1-sulfonate (2c)	2.94 µM	[0.02–0.364] mM	–	–	Inhibits urease activity by binding interaction with various amino acids and showed minimal cytotoxicity against AGS cells, and low permeability with Caco-2 cell line	[229]
Dihydropyrimidine based hydrazine dihydrochloride derivatives: 4-dihydropyrimidine-2-thiones 7–12 (series A), hydrazine derivatives of dihydropyrimidine 19–24 (series C)	[34.7–42.9] and [15.0–26.0] µM	–	–	–	Inhibit urease activity by mixed type inhibitors which was confirmed from kinetic studies. Cytotoxicity assays using mouse 3T3 fibroblasts showed no toxicity for compounds in series A and C	[222]
Regio-selectively alkylated benzimidazole-2-thione derivatives: Benzimidazole-2-thione (compound 2); 1-(Ethoxymethyl)-1*H*-benzo[*d*]imidazole-2(3*H*)-thione (compound 5)	0.25 mM 0.29 mM	0.11 mM 0.01 mM	–	–	Compounds 2 and 5 demonstrated potent inhibitory activity against *H. pylori* and Jack bean ureases. Docking studies showed favorable binding modes with ΔG values of − 9.74 and − 13.82 kcal/mol, supported by in silico ADMET and cytotoxicity assays confirming their safety and drug-likeness	[230]
(*N*-Aryl-*N*-arylsulfonyl)aminoacetohydroxamic acids: 2-(*N*-(3-nitrophenyl)-*N*-(4-*tert*-butylphenylsulfonyl))aminoacetohydroxamic acid (e2)	–	0.038 µM	–	–	Compound e2 exhibits excellent inhibitory activity against *H. pylori* urease and no perceptible cytotoxicity toward mammalian cells cancer cell lines (HepG2, SGC-7901, and K562)	[232]

Table 4 (continued)

Synthetic compounds names	Cell free urease assay	Anti-*H. pylori* urease assay		In vivo assay	Findings	References
		IC_{50}	MIC			
Colloidal bismuth subcitrate	–	9.5 µM	–	–	Inhibits *H. pylori* urease activity by interacting with UreG, protecting AGS cells from *H. pylori*-induced cytotoxicity. It also exhibits a safety profile with an LD50 exceeding 1000 mg/kg upon oral administration	[75]
N-Monosubstituted thioureas: *N*-(4-Chlorophenylaceto)urea (b19)	3.86 µM	0.16 uM	–	–	Compound b19 demonstrated strong potential as a treatment for *H. pylori*-related diseases outperforming AHA by 170- and 44-fold. Docking studies revealed that its thiourea moiety targets the urea-binding site, with b19 acting as a rapid, reversible inhibitor displaying nM affinity and slow dissociation from the catalytic domain. Exhibits low toxicity to human hepatic (L-02) and prostate (P69) cell lines, with over 93% cell viability	[233]
Bi(iii) hydroxamato complexes: $[Bi_2(Bha\text{-}1H)_2(\mu\text{-}Bha\text{-}1H)_2(\eta(2)\text{-}NO_3)_2]$; $[Bi_6(CH_3OH)_2(\eta(1)\text{-}NO_3)_2(\eta(2)\text{-}NO_3)(OH_2)_2(Sha\text{-}1H)_{12}](NO_3)_2$	1 mM	–	16 µg/mL	–	Inhibit urease with an IC_{50} of approximately 1 mM, achieving 96% inhibition of the urease enzyme at 10 mM concentration. Complexes release insoluble bismuth salts and free hydroxamic acids in the gastric environment, where the bismuth compounds exhibit antibacterial activity	[234]
Thiosemicarbazide derivatives of isoniazid 3–27: 2-Isonicotinoyl-*N*-(perfluorophenyl) hydrazinecarbothioamide (Compound 12); *N*-Cyclohexyl-2-isonicotinoylhydrazinecarbothioamide (Compound 23)	12.3 µM 22.4 µM	–	–	–	Compound 23 emerged as the best dual inhibitor, with strong anti-inflammatory and urease inhibition activity, while compound 12 was the most potent urease inhibitor. Both compounds, except compound 18 and isoniazid, were noncytotoxic against 3T3 normal mouse fibroblast line	[236]
Synthesized piperazine derivatives (1–15): 4-(4-chlorobenzyl)-*N*-(2-fluorophenyl) piperazine-1- carbothioamide (compound 14)	1.1 µM	–	–	–	All derivatives (1–15) showed excellent inhibitory potential with IC_{50} values ranging between 1.1 ± 0.01 and 33.40 ± 1.50 µM. Compound 14 improves the urease inhibitory potency by the presence of electron-withdrawing groups such as fluorine	[235]
4-Thiazolidinone analogs (1–20): (*Z*)-2-((*Z*)-(4-Bromo-2,5-dimethoxybenzoylidine) hydrazono)thiazolidine-4-one (20)	[1.73–69.65] µM 1.73 µM	–	–	–	Among the 20 analogs tested, all exhibited urease inhibitory activity with IC_{50} values ranging from 1.73 to 69.65 µM with exceptional inhibitory activity in particular for compound 20 Compound 20 chelates nickel ions of the potent compound and/or form bonds with crucial residues such as His222 and His323	[237]

Table 4 (continued)

Synthetic compounds names	Cell free urease assay	Anti-*H. pylori* urease assay		In vivo assay	Findings	References
		IC_{50}	MIC			
Copper (II) complexes: [CuClL(1)]·CH_3OH (2), [CuL(4)(NCS)]·0.4H_2O (5) L(1) and L(4) are the deprotonated form of *N*′-(2-hydroxybenzylidene)-3-methylbenzohydrazide, and 2-chloro-*N*′-(2-hydroxy-5-methoxybenzylidene)benzohydrazide, respectively	–	0.20 µM µM	–	–	Molecular docking revealed that square planar copper complexes exhibit superior urease inhibition, highlighting their structural advantage for targeting *Helicobacter pylori* urease	[226]
Cobalt(III) complexes: [CoL(1)(py)3]·NO3 (1), and [CoL(4)(MeOH)(N3)] (4) L(1), and L(4) are the deprotonated form of *N*′-(2-hydroxy-5-methoxybenzylidene)-3-methylbenzohydrazide, and *N*,*N*′-bis(5-methylsalicylidene)-*o*-phenylenediamine	–	4.27 µM 0.35 µM	–	–	Molecular docking showed that complex 4 fits well into the active pocket of urease, while complex 1 is positioned at the pocket's entry explaining the inhibitory behavior of complexes 1, and 4	[227]
Barbituric acid derivatives: 5,5′-(*p*-Tolylmethylene)bis(6-hydroxypyrimidine-2,4(1*H*,3*H*)-dione) diethylaminium salt (derivative 4i); 4-((6-Hydroxy-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)(6-hydroxy-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl)benzaldehyde diethylaminium salt (derivative 5 l)	17.6 µM 17.2 µM	–	–	–	Derivatives 4i and 5 l exhibited significantly higher urease inhibition than thiourea, suggesting barbituric acid derivatives as potential candidates for treating urease-related conditions like peptic ulcers. Molecular docking of both derivatives revealed their ability to adopt conformations that fit well into the urease binding groove, forming multiple hydrogen bonds with key residues His138, Ala169, KCX219, Gly279, Asp362, and Arg338	[238]
Novel catecholic derivatives that contained carboxylate and phosphonic/phosphinic functionalities: 2-(3,4-dihydroxyphenyl)-3-phosphonopropionic acid (15),	–	0.75 µM	–	–	Inhibits urease through specific interactions at the enzyme's active site, offering a dual inhibition mechanism via covalent binding. Effective at very low concentrations and displayed non-toxicity toward mammalian fibroblast and kidney cell lines	[239]
Sulfonate and sulfamate derivatives bearing benzofuran or benzothiophene scaffold: 4-(Benzofuran-5-yl)phenyl propane-1-sulfonate (1c); 4-(Benzofuran-5-yl)phenyl cyclohexane-1-sulfonate (1d); 4-(Benzofuran-5-yl)phenyl benzenesulfonate (1e); 4-(Benzo[b]thiophen-5-yl)phenyl cyclohexane-1-sulfonate (1n); 4-(Benzofuran-5-yl)phenyl dimethylsulfamate (1j); 4-(Benzo[b]thiophen-5-yl)phenyl dimethylsulfamate (1t)	[1.43- 59.3] µM	[0.003- 0.0095] mM	[0.0062–0.05] mM	–	Toxicity studies on AGS cells, selectivity tests against *E. coli* and gut Lactobacillus species, and permeability assays in Caco-2 cells revealed that the compounds are well-suited for targeted GIT treatment with minimal systemic side effects	[240]

Table 4 (continued)

Synthetic compounds names	Cell free urease assay	Anti-*H. pylori* urease assay		In vivo assay	Findings	References
		IC_{50}	MIC			
Sulfamate derivatives: 2-Bromo-4-(1-adamantylaminocarbonyl)phenyl sulfamate (1q)	0.062 µM	–	–	–	Compound 1q exhibited competitive inhibition, forming key interactions with ARG609, ARG439, HIS519, HIS492, HIS593, ALA440, and ALA636 in the urease active pocket, with pharmacokinetic analysis indicating a promising profile for sulfamate-based inhibitors	[241]
Marimastat	–	–	–	–	Best binding inhibitor for urease due to its high fitness score with the pharmacophore model (ADD.10), strong hydrogen bonding interactions with key urease residues	[218]
Thiazine Schiff bases: 4-Hydroxy-5-(1-((2-methoxy-5-(trifluoromethyl) phenyl)imino)ethyl)-2*H*-1,3-thiazine-2,6(3*H*)-dione (7)	0.14 µM	–	–	–	36 compounds have been synthesized with IC_{50} values ranging from 0.14 ± 0.08 to 3.66 ± 0.21 µM. Structure–activity relationship (SAR) analysis revealed that specific substitutions on the aryl ring, such as 2-methoxy-5-trifluoromethyl in compound 7 enhanced potency	[242]
Acetylphenol-based acyl thioureas: *N*-((3-acetyl-4-hydroxyphenyl)carbamothioyl)-2-bromobenzamide (7f)	0.054 µM	–	–	–	2–36 compounds, which were evaluated for their urease inhibitory activities. The derivatives demonstrated inhibitory potential, surpassing the standard inhibitor thiourea. The structure–activity relationship (SAR) analysis indicated that specific substitutions on the aryl ring significantly influence the inhibition potential	[243]

IC_{50}: half-maximal inhibitory concentration; MIC: minimum inhibitory concentration; (–): experiment not conducted

This finding highlights the importance of the alkyl chain length within this series for enzyme inhibition. Molecular docking analysis supported these findings by showing good binding affinity with the target protein. The study concluded that this compound, owing to its noncompetitive mode of inhibition and strong binding affinity, could serve as a lead structure for designing more potent urease inhibitors [217] (Table 4).

Richa Arora et al. leveraged computational tools to discover potential urease inhibitors against *H. pylori*. Their approach utilized pharmacophore modeling, virtual screening, and molecular docking to identify four promising compounds from known inhibitors. This research represents an advancement in combating *H. pylori* infection by demonstrating the power of computational chemistry in drug discovery and opening new avenues for therapeutic intervention [218] (Table 4). This study navigates through the chemical space of N-acylglycino- and hippurohydroxamic acid derivatives, known urease inhibitors, to construct a robust three-featured pharmacophore model. Among their notable discoveries, miglitol and marimastat have emerged as the most potent compounds, exemplifying the power of targeted chemical investigations in unveiling potential therapeutic agents. This work sets a new standard in the quest for effective treatments against *H. pylori* [218].

A new class of urease inhibitors combines nitrogen-containing heterocycles. This study demonstrated the synthesis of carbazole-triazine hybrids, with compounds with bulky iodo- and strong electron-withdrawing nitro groups exhibiting the highest activity and potent urease inhibition [219]. Another study highlighted the potential of virtual screening and molecular dynamics simulations for discovering new anti-urease therapeutic agents. They employed rapid overlay of chemical structures (ROCS) shape-based screening and molecular docking software to identify several potential urease inhibitors from over 1.83 million compounds, specifically those that target antibiotic-resistant *H. pylori* strains [220]. In another study, the synthesis and evaluation of arylmethylene hydrazine derivatives for urease inhibition were investigated. These compounds exhibited significant activity, with IC_{50} values ranging from 0.61 to 4.56 μM, surpassing those of standard inhibitors such as hydroxyurea and thiourea. Notably, a compound featuring a 2-nitrobenzylidene group displayed the highest potency [221]. Another study investigated the role of specific functional groups in urease inhibition by evaluating series containing 4-dihydropyrimidine-2-thiones, *N*,S-dimethyl-dihydropyrimidines, hydrazine derivatives of dihydropyrimidine, and tetrazolo dihydropyrimidine derivatives. This research suggests that the free sulfur atom and hydrazine moiety might be key pharmacophores for inhibiting the *H. pylori* urease enzyme [222]. By utilizing large-scale virtual screening of the ZINC database, Azizian et al. selected compounds on the basis of their docking energy calculations for further in vitro screening against *H. pylori* urease. Their findings revealed that barbituric acid and certain derivatives, particularly those with *para* substitution on the benzylidene moiety, exhibited greater urease inhibitory activity than did the standard inhibitor hydroxyurea. This study aimed to identify new scaffolds for *H. pylori* urease inhibition, potentially leading to novel therapies for managing *H. pylori* infections. Barbituric acid derivatives have emerged as promising candidates because of their superior potency and potential ability to chelate with the enzyme's active site [223]. Building upon previous research, Biglar et al. synthesized a series of *NN*-dimethylbarbituric-pyridinium derivatives and evaluated their potency as *H. pylori* urease inhibitors. The results demonstrated that these compounds were more effective than the standard inhibitors hydroxyurea and thiourea. This work offers promising new candidates for *H. pylori* urease inhibition [224]. Recent studies have explored various metals for their ability to inhibit urease enzyme activity. Among these complexes, copper (II) complexes have emerged as promising candidates. Investigations by Cui et al. into two such complexes revealed their substantial inhibitory action against *H. pylori* urease, surpassing the standard inhibitor in effectiveness. This highlights the potential of metal complexes for the development of new *H. pylori* treatments [225]. Another study synthesized six copper complexes with IC_{50} values ranging from 0.20 to over 100 μM. The study also incorporated molecular docking studies to elucidate the mechanism of inhibition, concluding that copper complexes with square planar coordination are more effective urease inhibitors [226]. To expand the exploration of metal-based urease inhibitors, another study investigated four new cobalt (III) complexes with Schiff bases. This work contributes to ongoing research on metal complexes as potential urease inhibitors [227].

Among the limited in vivo studies conducted on urease inhibitors against *H. pylori*, Liu et al.'s research stands out. They analyzed aniline-containing hydroxamic acids and identified three potent compounds: 3-(3,5-dichlorophenylamino)-*N*-hydroxypropanamide, 3-(2-chlorophenylamino)-*N*-hydroxypropanamide, and 3-(2,4-dichlorophenylamino)-*N*-hydroxypropanamide. These compounds have demonstrated remarkable efficacy in both in vitro and in vivo studies, with complete eradication of *H. pylori* in mice models [228]. Researchers synthesized novel imidazothiazole derivatives containing sulfonate and sulfamate groups, which significantly inhibited urease activity. The most potent

compound demonstrated an IC_{50} of 2.94 ± 0.05 μM, outperforming the control by eightfold. An enzyme kinetics study revealed it to be a competitive inhibitor. Phenotypic screening against *H. pylori* identified several high-potency molecules, with compound 1d emerging as a lead candidate due to its promising inhibition profile, minimal activity against urease-negative *E. coli*, and low permeability in Caco-2 cells, suggesting its suitability for gastrointestinal infections without systemic effects [229]. Thirteen novel alkylated benzimidazole 2-thione derivatives were synthesized because of their urease activity toward *H. pylori*. The most potent compounds have potential as lead candidates for developing future anti-urease agents [230]. A novel high-throughput screening assay was developed for detecting submicromolar urease inhibitors among clinically used drugs. This assay identified panobinostat, dacinostat, ebselen, and disulfiram as effective inhibitors against plant or bacterial urease, suggesting their potential for treating *H. pylori* infections, particularly those resistant to antibiotics [231]. In another study, 33 (*N*-aryl-*N*-arylsulfonyl)aminoacetohydroxamic acids were synthesized to target urease inhibition in *H. pylori*. One compound, 2-(*N*-(3-nitrophenyl)-*N*-(4-*tert*-butylphenylsulfonyl)) aminoacetohydroxamic acid, displayed exceptional activity with minimal cytotoxic effects on mammalian cells. Its effectiveness, exceeding 690-fold that of AHA, is attributed to its reversible mixed-mode inhibition of urease, as demonstrated by molecular modeling and docking studies [232]. Another investigation of N-monoarylacetothioureas as anti-urease agents targeting *H. pylori* has demonstrated significant in vitro urease inhibition with low cytotoxicity, showing promise for further development in treating *H. pylori*-related diseases. The efficacy of this compound is notably greater than that of AHA, making it a potential candidate for *H. pylori* treatments [233]. Researchers have identified numerous potential targets for anti-urease agents by delving into the molecular intricacies of urease. This innovative approach led to the identification of the metallochaperone UreG as a novel antimicrobial target against *H. pylori*. This study utilized colloidal bismuth subcitrate to inhibit urease activity by disrupting the GTPase activity of UreG, thereby halting urease maturation and offering a fresh perspective on antimicrobial development [75]. A novel class of bismuth hydroxamate complexes was specifically designed to inhibit urease activity. By synthesizing and testing these complexes, their potential in combating *H. pylori* infections was demonstrated, leveraging both the antibacterial properties of bismuth and the urease-inhibitory capabilities of hydroxamic acids. This approach is promising for the development of effective treatments against *H. pylori*, particularly in light of increasing antibiotic resistance [234]. Furthermore, the synthesis of piperazine derivatives was evaluated for their potential as urease inhibitors. The synthesized derivatives exhibited excellent inhibitory potential, with IC_{50} values ranging from 1.1 to 33.40 μM, surpassing those of the standard inhibitor thiourea. This research established a structure–activity relationship on the basis of the substitution pattern on the phenyl ring and utilized molecular docking to understand the binding interactions within the enzyme's active site [235]. In another study, thiosemicarbazide derivatives of isoniazid were also investigated, and the results revealed a dual inhibitory effect on both inflammation and urease activity. Among these derivatives, 2- isonicotinoyl-*N*-(perfluorophenyl) hydrazinecarbothioamide has emerged as the most effective dual inhibitor, suggesting a new approach for developing treatments for *H. pylori*–related conditions [236]. Another study focused on synthesizing twenty 4-thiazolidinone analogs and examining their urease inhibitory activity. These analogs exhibited a broad range of inhibitory effects, with IC_{50} values between 1.73 and 69.65 μM, indicating varying levels of effectiveness against urease compared with the standard inhibitor thiourea (IC_{50} value of 21.25 μM). Research has further explored the structure–activity relationship, highlighting the influence of substituent patterns on phenyl rings on urease inhibition efficacy [237]. Moreover, researchers have investigated barbituric acid derivatives as potential urease inhibitors and synthesized thirty-two zwitterionic adducts of diethyl ammonium salts. In vitro tests against jack bean urease identified two compounds (4i and 5 l) as the most effective, surpassing the standard inhibitor thiourea [238].

Conclusions and future directions

The growing challenge of antibiotic resistance in *H. pylori* infections necessitates the exploration of alternative therapeutic strategies. This review underscores the immense promise of urease inhibitors as a novel approach for combating *H. pylori* infections. The substantial amount of screening data, with the identification of highly active organic compounds, lays a solid foundation for further clinical development. However, future directions require addressing the limitations identified in current screening methods to ensure the identification of inhibitors with specific and targeted activity against the pathogen's urease. Furthermore, in-depth structure–activity relationship (SAR) analyses are crucial for future advancements. By comparing the mechanisms of action of candidate inhibitors against different urease sources, researchers can gain a deeper understanding of enzyme vulnerabilities. This knowledge will be instrumental in the design of highly selective urease inhibitors that effectively target *H. pylori* without impacting the gut microbiome. Although several anti-urease compounds exist and many

are in development, only a few have reached clinical trials. Many of these compounds have been discovered by molecular docking without additional confirmation assays, or if conducted, by performing cell-free urease assays. Very few compounds have been evaluated on further in *H. pylori* or in animal models. Palmatine derivatives and arylamino-containing hydroxamic acids are among the promising anti-urease compounds for further clinical studies, as they have shown interesting results in terms of MIC and IC_{50} against *H. pylori* [215, 228]. It would also be valuable to assess the effects of these anti-urease compounds on gut microflora. The future of *H. pylori* treatment relies on the successful development of potent and specific urease inhibitors. By incorporating these crucial future directions, researchers can translate the promising potential of urease inhibitors into a clinically viable reality. This will pave the way for a more effective and targeted therapeutic approach, leading to improved patient outcomes in the fight against *H. pylori* infections.

Abbreviations

H. pylori	*Helicobacter pylori*
PPI	Proton pump inhibitor
WHO	World health organization
BabA	Blood group antigen-binding adhesin
SabA	Sialic acid-binding adhesin
CagA	Cytotoxin-associated gene A
VacA	Vacuolating cytotoxin A
T4SS	IV secretion system
MALT	Mucosa associated lymphoid tissue
HPU	*H. pylori urease*
E. coli	*Escherichia coli*
UTIs	Urinary tract infections
NH_3	Ammonia
CO_2	Carbon dioxide
pH	Potential of hydrogen
UreA	Urease subunit
UreB	Urease subunit
Ni	Nickel
ms	Millisecond
UreI	Proton gated urea channel
kDa	Kilo Dalton
UreE	Urease accessory protein E
UreF	Urease accessory protein F
UreG	Urease accessory protein G
UreH	Urease accessory protein H
H^+	Hydrogen ion
FrpB4	Ferric-related protein B4
TonB:	Energy transduction system protein B
FecA3	Ferric citrate outer membrane transporter A3
TBDT	TonB-dependent transporter
HspA	Heat shock protein A
NikR	Nickel responsive regulator
NixA	Nickel transporter A
NiuBDE	Nickel uptake system (NiuB, NiuD, NiuE)
HypB	Hydrogenase accessory protein B
Zn	Zinc
GTP	Guanosine triphosphatase
His	Histidine
SIMIBI	Signal recognition rarticle, MinD, and BioD GTPase family
AI-2	Autoinducer-2
HP1021	*H. pylori* Response regulator 1021
Hpn	*H. pylori n*Ickel-binding protein
CA	Carbonic anhydrase
hpαCA	*H. pylori* Alpha-carbonic anhydrase
hpβCA	*H. pylori* Beta-carbonic anhydrase
HCO_3^-	Bicarbonate ion
NH_4^+	Ammonium ion
rRNA	Ribosomal RNA
gyrA	DNA gyrase subunit A gene
gyrB	DNA gyrase subunit B gene
FDA	Food and drug administration
NPs	Nanoparticles
QS	Quorum sensing
mm	Millimeter
nm	Nanometer
IPN	Interpenetrating polymer networks
SLNs	Solid lipid nanoparticles
NLCs	Nanostructured lipid carriers
DOPE	Dioleoyl phosphatidylethanolamine
HPE1, HPE2	*H. pylori* Phages
LF-HA	Lactoferrin-hydroxyapatite complex
AMPs	Antimicrobial peptides
MDR	Multi drug resistance
MSI-78	Pexiganan
TP4	Tilapia piscidin 4
Epi-1	Epinecidin-1
CRAMP	Cathelin-related antimicrobial peptide
Cbf-K16	Cathelicidin-like peptide
HNP-1	Human neutrophil peptide 1
PGLa-AM1	Cationic AMP
Th1	T-helper 1
Th2	T-helper 2
A. nilotica	*Acacia nilotica*
C. procera	*Calotropis procera*
MIC	Minimum inhibitory concentration
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
MAPK	Mitogen-activated protein kinase
AHA	Acetohydroxamic acid
ATP	Adenosine triphosphate
AgNPs	Silver nanoparticles
QG	Qing guo
SecA	Secretion A
AGS	Adenocarcinoma human gastric epithelial
IL-8	Interleukin 8
QGAE	Extracts aqueous extract
QGEAE	Ethyl acetate extract
MBC	Minimum bactericidal concentration
Hesp	Hesperetin-7-rhamnoglucoside
LLE	Laurel (*Laurus nobilis* L.) leaves extract
MH	Moist heat
UMH	Unmoist-heated
NMR	Nuclear magnetic resonance
ESI-MS	Electrospray ionization mass spectrometry
SF	Sulforaphane
PMT	Palmatine
IC50	Half-maximal inhibitory concentration
LD50	Half-lethal dose
ROCS	Rapid overlay of chemical structures
Caco-2	Human colorectal adenocarcinoma
μM	Micromolar
mM	Millimolar
μg/mL	Micrograms per milliliter
mg/kg	Milligrams per kilogram
ug/mL	Micrograms per milliliter
mg/L	Milligrams per liter
JHCCL	John Hopkings clinical compound library
SGC-7901	Human gastric adenocarcinoma cell line
Arg609	Arginine at position 609
Cys592	Cysteine at position 592
ADMET	Absorption, distribution, metabolism, excretion, and toxicity
HepG2	Human hepatocellular carcinoma 2
SGC-7901	Stomach gastric carcinoma-7901
K562	Human chronic myelogenous leukemia cell line

nM	Nanomolar
L-02	Human liver cell line
P69	Human prostate cell line
His	Histidine
Ala	Alanine
Gly	Glycine
Asp	Aspartic acid
Arg	Arginine
GIT	Gastrointestinal tract
SAR	Structure-activity relationship
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid

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[G.K.] conceptualized the review topic and designed the framework. [G.K., C.A.] conducted the literature search and data collection. [G.K., C.A.] analyzed and interpreted the findings. [G.K., C.A.] wrote the first draft of the manuscript. [G.K., C.A., M.E.G., M.H., M.Ha., S.Q., M.S., R.G., J.S.M., C.B.] reviewed and edited the manuscript for critical intellectual content. All authors read and approved the final manuscript.

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