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Nonantibiotic-Based Therapeutics Targeting *Helicobacter pylori*: From Nature to the Lab

Paula Parreira, Catarina Leal Seabra,
Daniela Lopes-de-Campos and
Maria Cristina L. Martins

Additional information is available at the end of the chapter

Abstract

The available therapy against *Helicobacter pylori* is based on a combination of antibiotics and proton pump inhibitors. The high prevalence of antibiotic-resistant strains leads to failure of this complex therapeutic regimen, leaving millions of people worldwide without effective therapeutic options. "Nature-derived" bioactive compounds with antibacterial performance may be of value for developing newer and more effective strategies. For centuries, natural compounds have played a pivotal role in traditional medicine and, in the last decades, they have gained renewed strength in the clinical field, boosted by advances in chemical characterization and extensive activity screening. Also, their recognition in gastric infection management has been empowered by the bioengineering field, namely by the development of stomach-specific delivery strategies. In this chapter, natural bioactive compounds, such as polyunsaturated fatty acids and triterpenic acids with anti-*H. pylori* effect, are described. The bioengineering approaches used to overcome their limited intrinsic bioavailability are briefly highlighted.

Keywords: nanotechnology, bioactive compounds, lipophilic compounds, phytochemicals, antibiotic-free therapies

1. Introduction

Helicobacter pylori is the etiologic agent of several gastric disorders that may range from chronic gastritis to more severe outcomes [1]. Ultimately, the complex interplay between *H. pylori*, the host susceptibility, and environmental factors such as smoking and drinking

can lead to gastric cancer, which is the fifth most common cancer worldwide, accounting for 754,000 deaths in 2015 [2, 3]. Despite significant medical advances, the 5-year survival rate from gastric cancer is low (31%), mainly because this cancer is diagnosed at later stages [4]. It is widely recognized that the best strategy to reduce the risk of gastric carcinoma associated with *H. pylori* infection is its eradication from infected hosts [5, 6]. The current treatment relies on a combination of antibiotics (clarithromycin plus amoxicillin or metronidazole) and an acid-suppressive drug (e.g., proton-pump inhibitor), since no available substances are effective as monotherapy [7]. However, the eradication rates of this therapeutic scheme have been declining to unacceptable levels [8], mostly due to high antibiotic resistance levels. In fact, *H. pylori* has been placed among the 16 antibiotic-resistant bacteria that pose greatest threat to human health [9]. It is noteworthy that besides resistance and coinfection with multiple strains with distinct antibiotic susceptibilities, other factors also account for conventional treatment failure:

a. drugs bioavailability: antibiotics are typically administrated via the oral route. Since the gastric mucus layer acts as a barrier to antibiotic delivery, most drugs are unable to reach the underlying gastric epithelium, where *H. pylori* is attached [10];

b. gastric features: the pH at the stomach/duodenum varies from acidic to neutral, depending on the presence or absence of food and the location within the mucus barrier that covers the epithelial cells. This affects the efficacy of most antibiotics once only a few remain active in a wide pH range [11–13];

c. compliance: side effects, such as taste disturbances with clarithromycin and metronidazole, and diarrhea with amoxicillin, account for the poor patient compliance. Additionally, complex regimens that require multiple doses of medication each day also decrease therapeutic compliance [14];

d. lifestyle: smoking and alcohol consumption are thought to contribute for treatment failure [15].

In this scenario, where *H. pylori* traditional antibiotic therapies fail, a considerable interest in alternative therapeutic players combined with bioengineering strategies has arisen. Several "nature-derived" options have been studied [5], and some of them are summarized in **Figure 1**.

Although very promising *in vitro*, these molecules share a common drawback when transferred to *in vivo* settings: intrinsic limited bioavailability. **Figure 2** summarizes some of the bioengineering approaches envisioned to overcome the mentioned limitation.

Chitosan micro-/nanoparticles have been extensively studied as drug delivery systems targeting *H. pylori* infection [16], mainly due to its gastric retentive properties [17]. Chitosan, a natural biocompatible polysaccharide obtained by *N*-deacetylation of chitin [18], has mucoadhesive properties due to electrostatic interactions between its cationic amine groups and gastric mucins, which are negatively charged at the acidic gastric pH [19, 20].

Chitosan microspheres were used to encapsulate and improve the biological activity of trans-resveratrol, a phenolic compound that has, among other biological activities, anti-*H. pylori*
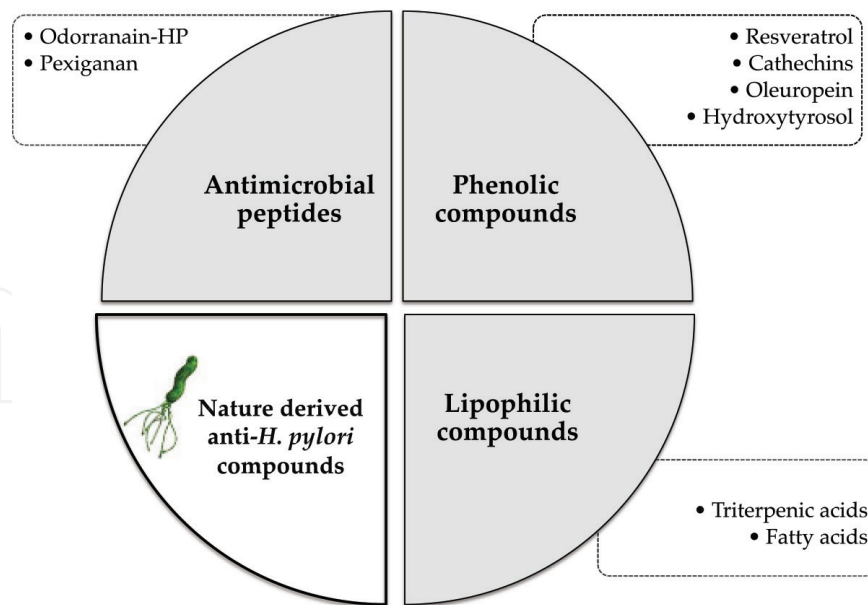
Figure 1. Some "nature-derived" bioactive compounds described in the literature in the scope of novel anti-*H. pylori* therapeutics.
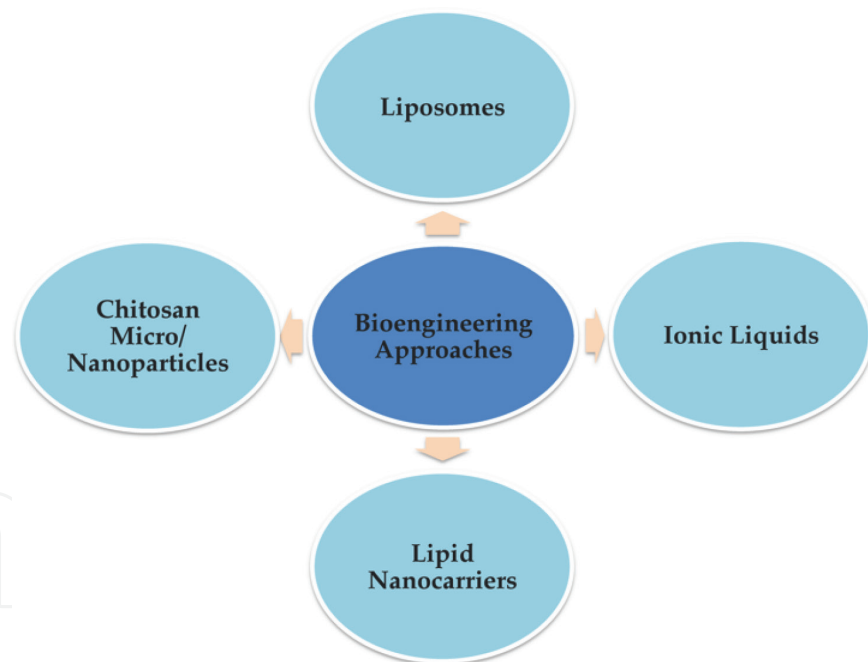
Figure 2. Most common bioengineering approaches applicable to "nature-derived" bioactives in the scope of *H. pylori* infection management.

action [21]. Polyelectrolyte complex nanoparticles (PNPs), prepared by the combination of chitosan with negatively charged polymers, alginate or heparin, have also been used to encapsulate the antimicrobial peptide pexiganan [22] and berberine [23], respectively. This approach increased the effectiveness of these bioactive compounds, inhibiting *H. pylori* growth and reducing their cytotoxic effects.

Other strategies that can be used to overcome the low bioavailability and solubility of lipophilic compounds comprise ionic liquids (IL) and lipid nanoparticles.

IL are a new class of powerful catanionic hydrotropes, where both the cation and the anion synergistically contribute to increase the solubility of biomolecules in water [24]. Therefore, IL enhance the solubility of hydrophobic substances in aqueous media and are widely used in the formulation of drugs, cleaning, and personal care products. IL have been explored to increase the water solubility of triterpenic acids, such as of betulinic acid [25, 26].

Lipid nanoparticles are very useful to encapsulate lipophilic compounds due to their higher biocompatibility compared to polymeric nanoparticles [27, 28]. Liposomes, firstly described in the mid-1960s, are sphere-shaped vesicles consisting of one or more phospholipid bilayers. Liposomes are the first nanodrug delivery systems that have been successfully translated into real-time clinical application [29–31]. Nanostructured lipid carriers (NLCs) are lipid nanoparticles specifically designed and patented as drug delivery systems, and they are characterized by a solid-lipid core composed of a mixture of solid and liquid lipids [32]. NLCs can be prepared using a wide variety of lipids including fatty acids, glyceride mixtures, or waxes, stabilized with biocompatible surfactants, which makes this a very versatile strategy. Both are considered safe and under the Generally Recognized as Safe (GRAS) status issued by the Food and Drug Administration (FDA) [31, 33].

Polyunsaturated fatty acids and triterpenic acids with anti-*H. pylori* effect have gained renewed interest in the scientific community as alternatives to overcome the increasing number of drug-resistant bacteria. These lipophilic bioactive compounds can largely benefit from a nanotechnological approach to improve their stability and to overcome their limited intrinsic bioavailability and thus, they will be briefly highlighted in the next sections.

2. "Nature-derived" anti-*H. pylori* fatty acids

Free fatty acids, also known as antimicrobial lipids [34], are linear carbon chains, which are the main constituent of phospholipids, triglycerides, sterol esters, among others [35]. Consequently, they are important for biological activities, such as for energetic, metabolic, and structural processes [35]. Fatty acids are classified according to the length of the carbon chains, the number of double bonds, and their positions within the moiety [35]. Polyunsaturated fatty acids (PUFAs) have two or more double bonds [36], and they have been recognized for their broad-spectrum activity against bacteria (e.g., *H. pylori*), fungi, protozoa, and virus [36–38]. This is due to the ability of fatty acids to work as mild surfactants [34]. The disturbance of the bacterial cell membrane can lead to the deregulation of metabolic pathways, inhibiting the bacterial growth, or even to lysis and death [34]. The specific interaction between antimicrobial fatty acids and bacterial membranes remains to be fully understood. Some studies revealed that free fatty acids can induce different kinds of morphological changes in the membrane [39, 40]. Khulusi et al. and Correia et al. reported that fatty acids can be incorporated into *H. pylori* phospholipids membrane, being able to change the bacillary morphology of the bacteria to their coccoid shape [32, 41–43]. Several studies also identified the bacterial

membrane as the main target of fatty acids, leading to a sequence of biophysical phenomena including membrane destabilization, pore formation, and lysis of bacteria [32, 41, 44, 45]. The multiple mechanisms that are behind their ability to perturb bacterial cell membranes lead to a low probability of antimicrobial resistance [34]. On the opposite, small molecules, such as commercial antibiotics, inhibit specific enzymes and, consequently, increase the probability of antimicrobial resistance development [34].

The antibacterial activity of PUFAs depends on their molecular structure. In fact, the existence of double bonds and, more specifically, their number and orientation within the fatty acids are important for their physicochemical properties [46, 47]. These structural differences are reported to affect their ability to inhibit *H. pylori* growth *in vitro* [45]. For instance, the inhibitory effect is higher for higher degrees of unsaturation: [oleic (C18:1) < linoleic (C18:2) < arachidonic (C20:4) < n-3 linolenic (C18:3) = n-6 linolenic (C18:3) = eicosapentaenoic (C20:5) acid] [45]. The encapsulation of free fatty acids in nanoparticles can improve their pharmacokinetic and pharmacological properties [34]. A review of the nanotechnology formulations used to encapsulate antimicrobial lipids was recently published [34].

In this section, two of the most promising fatty acids (docosahexaenoic acid (DHA) and linolenic acid (LA)) are described regarding their specific application against *H. pylori*.

Figure 3 illustrates the two bioengineering approaches that have been applied to DHA and LA that will be discussed in the following subsections.

2.1. Docosahexaenoic acid (DHA)

DHA inhibits *H. pylori* growth both *in vitro* and *in vivo*, since it is able to reduce *H pylori* adhesion to gastric cells and bacterial ATP production [42, 43, 48]. DHA induces changes in expression of *H. pylori* outer membrane proteins associated with stress response, metabolism, and modified bacterial lipopolysaccharide phenotype [43]. DHA is also able to indirectly interfere with *H. pylori* growth since it alters cholesterol levels in epithelial cells, thereby influencing the bacterium ability to uptake and use epithelial cholesterol [48].
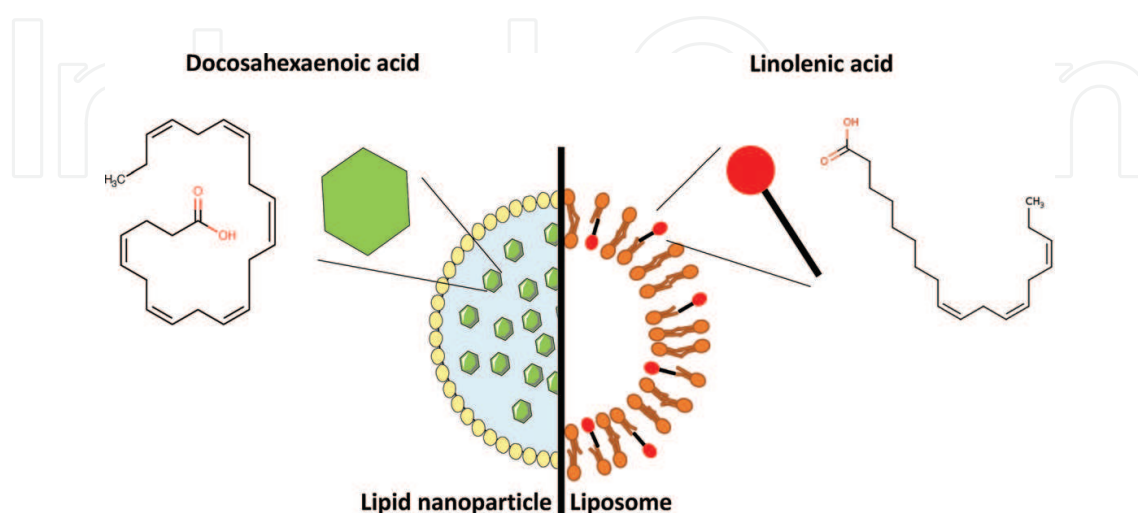
Figure 3. Encapsulation of fatty acids, namely DHA and LA, in different types of nanoparticles.

Although *in vivo* studies using gastric-infected mice demonstrated that DHA was able to decrease only 50% of *H. pylori* gastric colonization, the DHA conjugation with antibiotic standard treatment decreased the recurrence of *H. pylori* infection [42, 43].

Another DHA feature is its ability to attenuate the host inflammatory response associated with gastric infection [49, 50].

DHA poor solubility in water, fast oxidation/degradation plus gastric settings drawbacks (namely low gastric residence time and low penetration through the gastric mucus layer) are challenging issues for its clinical translation [36, 50, 51]. To overcome these obstacles, cyto-compatible lipid nanoparticles have been researched to encapsulate DHA [44, 52]. It was demonstrated that DHA lipid nanoparticles are able to destabilize *H. pylori* membranes, leading to disruption and leakage of cytoplasmic contents [32, 44]. Importantly, these lipid nanoparticles do not interfere with normal gut microbiota in opposite to dramatic changes described for the conventional antibiotic therapy [44].

2.2. Linolenic acid (LA)

LA, as fatty acids in general, is considered safe [53]. It is classified as an essential fatty acid, once it cannot be synthesized by the human body, being necessary to be supplied by the diet [54]. Its importance for biological processes is unquestionable. LA undergoes metabolic changes *in vivo* that ultimately lead to the formation of prostaglandins, thromboxanes, leukotrienes, and lipoxins [54]. Furthermore, the usefulness of LA as an antibacterial agent was also proved, being one of the most potent unsaturated fatty acids against *H. pylori* [7]. It also promotes the adhesion of *Lactobacillus casei* to mucosa surfaces, which indirectly hinders the growth of *H. pylori* [55]. Besides its bactericidal effect, LA is also important for the integrity of the gastric mucosa. It was already proposed that lower levels of essential fatty acids, such as LA, lead to decreased levels of prostaglandins and, consequently, to a higher susceptibility of the gastric mucosa to ulcerogenic agents [56].

Nanotechnology has been successfully used to load fatty acids, including LA [34]. As above mentioned, the oral administration of fatty acids is hindered by their poor solubility, especially at acidic pH, and their susceptibility to chemical degradation [57]. In fact, the carboxyl protonation under acidic pH at the stomach lumen decreases the efficacy of fatty acids after oral administration [53]. This was already shown *in vivo*, with no significant effect of plain LA in killing *H. pylori* on a mouse model [53]. Nevertheless, liposomes are promising bioengineering strategies to overcome these limitations. Due to the amphiphilic nature of fatty acids, they can be easily incorporated into the phospholipid bilayer of liposomes [57]. Hence, Obonyo et al. used liposomes of egg phosphatidylcholines, cholesterol, and LA to kill *H. pylori* [57]. They showed that LA-loaded liposomes were effective against *H. pylori* even in its coccoid form and regardless their resistance to antibiotics [57]. Interestingly, *H. pylori* developed resistance against free LA at subbactericidal concentrations, whereas it showed no resistance against LA when incorporated into the nanoparticles [57]. These results show the promising usefulness of nanotechnology not only to protect the fatty acid from its degradation, but also to improve its efficacy. The higher efficacy relies on their ability to fuse with the

bacterial membrane, being directly and faster incorporated into the bacterial membrane [57]. Their main mechanism is the increase of the permeability of the outer membrane and of the plasma membrane of *H. pylori*, which leads to a leakage of cytoplasmic contents [58]. They also decrease the *H. pylori*-induced proinflammatory cytokines, helping in the healing of the gastric mucosa [58]. Furthermore, the ability of those liposomes to be retained at the site of infection was also shown *in vivo* [53]. The retention for up to 24 h was attributed to the small size of the liposomes and their anionic surface charge, which decreases their hydrophobic entrapment [53]. The biocompatibility of the formulation was shown through gastric histopathology and mucosal integrity and by the maintenance of the gut microbiota, on the opposite of the current triple therapy [53, 59].

3. "Nature-derived" anti-*H. pylori* phytochemicals

Phytochemicals have been used for centuries in the treatment of gastrointestinal disorders, such as dyspepsia, gastritis, and peptic ulcer disease [60]. Over the last two decades, phytotherapy has gained strength in the scientific community, prompted by the need of alternatives to the ineffectiveness of traditional antibiotics.

Plants synthesize a vast range of secondary metabolites with a significant portion consisting of phenolic and flavonoid compounds [61]. These secondary metabolites, other than providing plants with unique survival or adaptive strategies, are associated to a wide range of biological activities [62]. Phenolic compounds, namely wine polyphenols, from which resveratrol is the most studied, and olive oil polyphenols, mainly hydroxytyrosol, have been associated with anti-*H. pylori* activity [5]. Lipophilic compounds from the terpenes family can also be obtained from several plants. In the scope of anti-*H. pylori* strategies, these are described in more detail in the following section.
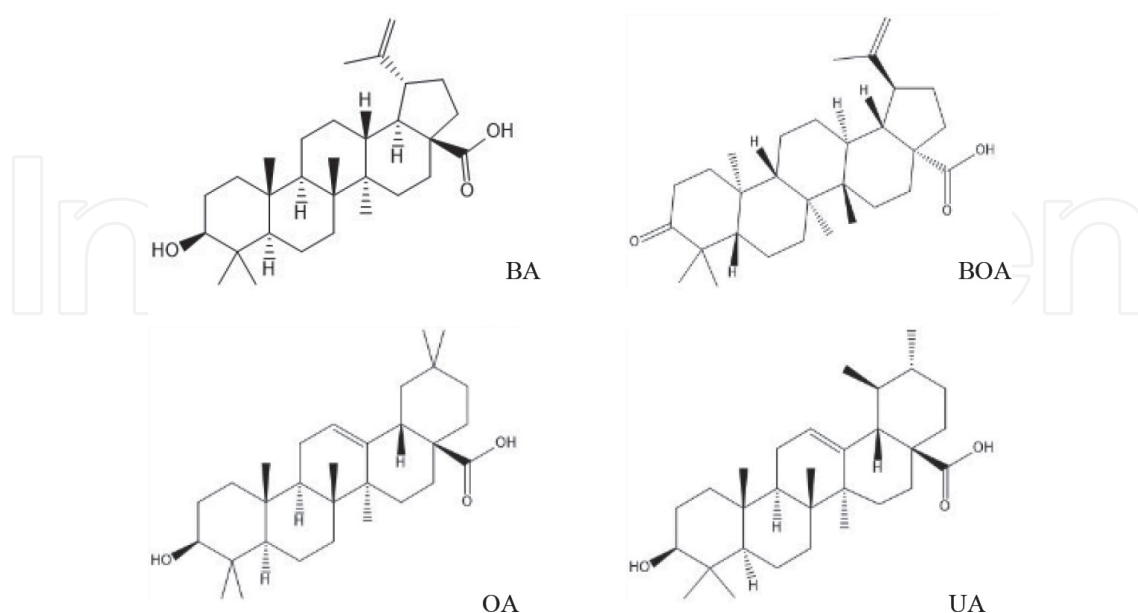
3.1. Triterpenic acids

Terpenes are naturally occurring hydrocarbons, with the general formula $(C_5H_8)_n$ ($-(-CH_2=C(CH_3)-CH=CH_2)_n$), where n is the number of isoprene units. Depending on the number of isoprene building blocks, they are classified into several groups, such as monoterpenes, sesquiterpenes, diterpenes, triterpenes, and tetraterpenes (with 2, 3, 4, 6, and 8 isoprene units, respectively). These compounds can undergo chemical modifications by oxidation or rearrangement of the carbon skeleton, which leads to a vast group of compounds denominated terpenoids [63].

Pentacyclic triterpenoids are commonly isolated as active substances from different natural sources, mainly plant surfaces such as stem bark or leaf and fruit waxes [64]. Among them, pentacyclic triterpenes ($C_{30}H_{48}$) are being marketed as therapeutic agents or dietary supplements around the world due to their biological applications [65, 66]. Their antibacterial properties are also recognized. For instance, it was demonstrated that the acidic fraction of the total mastic extract without polymer (TMEWP) from the Chios Mastic Gum (resin of

Pistacia lentiscus var. chia) is effective in killing *H. pylori* [67]. This antibacterial effect was attributed to their rich composition in oleanolic acid, isomasticadienolic acid, masticadienolic, and moronic acid [67]. Paraschos et al. demonstrated that the prophylactic treatment with the TMEWP was not able to prevent *H. pylori* infection in C57BL/6 mice infected with mouse-adapted *H. pylori* SS1 strain [67]. Nevertheless, the number of *H. pylori* colonies significantly reduced (1.5 log colony forming units/g of tissue) when the animals were subjected to continuous administration of 0.75 mg of TMEWP for 3 months [67]. Shin et al. reported that betulinic acid and oleanolic acid, extracted from *Fosythia suspensa*, were able to inhibit the urease activity of *H. pylori* ATCC 43504 [68]. Furthermore, Parreira et al. reported that outer bark extracts of *Eucalyptus nitens* and *E. globulus*, rich in betulinic, betulonic, oleanolic, and ursolic acids (**Figure 4**), have anti-*H. pylori* activity against strains with distinct virulence degree [69]. Interestingly, the eucalyptus extracts had a lower minimal inhibitory concentration than the isolated pure triterpenic acids, which led to the conclusion that the final observed antibacterial effect was due to synergic effects [69].

Although not specifically designed toward *H. pylori* infection, different strategies to improve the oral bioavailability of triterpenic acids have been studied. For example, oleanolic acid bioavailability has been enhanced by using a phospholipids complex with hydroxyapatite [70]. Yang et al. have developed liposomes to increase ursolic acid bioavaliabitliy [71] and pharmacokinetic studies carried out by Ge et al. reported that the oral bioavailability of ursolic acid was 27.5-fold higher when it was incorporated in nanoparticles than when administered as a free compound [72].

The abovementioned advances in increasing the bioavailability of triterpenic acids using bioengineering strategies will enable, in the near future, to further pursue research of novel nonantibiotic and more effective "nature-inspired" therapies against *H. pylori*.

Figure 4. Chemical structures of triterpenic acids: betulinic (BA), betulonic (BOA), oleanolic (OA), and ursolic (UA) acids.

4. Translation to real-world scenario

Both fatty acids and triterpenic acids have been reported to exhibit similar performance against *H. pylori*. Nevertheless, their action mechanisms are fairly distinct: while fatty acids are reported to interact with the bacterial membrane, triterpenic acids are reported to be more involved in enzymatic inhibition, namely urease hindering [5]. Since both bioactives classes target crucial structures for *H. pylori* survival, emergence of resistance is not anticipated, as it would require massive bacterial energy [32, 69].

Despite the remarkable effects associated to fatty and triterpenic acids for gastric infection management, translation into real-world applications is still delayed. For that, it has contributed the fact that only in the last decade more attention has been paid to nature-derived molecules, counteracting the "chemical pharmacological" tendency that had been initiated in the beginning of the twentieth century. Also, there was a significant reduction of investment in the clinical development of antibiotics over the last years. In fact, only 1.6% of the drugs under clinical development by the world's largest drug companies are antibiotics [73]. This has boosted the search for other sources of antimicrobials. In addition, bioengineering emerged in the twenty-first century as a powerful tool to develop drug delivery systems and, consequently, to overcome the more generalized drawbacks associated with the lipophilic bioactive compounds discussed in this chapter [5]. Bioengineering approaches for fatty acids specific application against *H. pylori* are already on a "fast-track," while those for triterpenic acids are only now evolving, which explains the lack of solid studies coupling these bioactives with bioengineering strategies.

To the date and to the best of our knowledge, most of the herein described compounds are in *in vivo* studies phase, being expected that in the next few years some will cross the clinical trials barrier. There are several factors contributing to the anticipated success of these "nature-based" strategies. They are generally cost-effective, due to their abundance in nature, and they require low-cost extraction productions. Furthermore, the biotechnological improvements that include nanotechnological coupling to nature-derived molecules will hopefully contribute to reaching "real-life" applications. In addition, more "nature-based" molecules are reaching the market with FDA approval to treat infectious disease, such as antimalaria Artemisinin therapies, based on an herb employed in Chinese traditional medicine [74], which anticipates the future success of nature-inspired strategies for *H. pylori* eradication.

5. Conclusion

H. pylori infection is one of the most prevalent infections worldwide, which is also reflected onto the high prevalence of gastric cancer. Emerging antibiotic resistance leads to an urgent need of alternative treatments. Resourcing to widely available lipophilic natural bioactive compounds with anti-*H. pylori* activity, namely fatty or triterpenic acids, should be further considered as novel therapeutic options. In this context, nanotechnology emerges as a key player, as it allows overcoming the bioactive major drawbacks that have been holding back their "real-world" application.

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Conflict of interest

The authors declare no conflict of interest.

Author details

Paula Parreira[1,2], Catarina Leal Seabra[1,2], Daniela Lopes-de-Campos[3] and
Maria Cristina L. Martins[1,2,4]*

*Address all correspondence to: cmartins@ineb.up.pt

1 i3S—Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal

2 INEB—Instituto de Engenharia Biomédica, University of Porto, Porto, Portugal

3 LAQV, REQUIMTE, Faculty of Pharmacy, University of Porto, Porto, Portugal

4 ICBAS—Instituto de Ciências Biomédicas Abel Salazar, University of Porto, Portugal

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