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Original Article

Inhibition of growth of *Helicobacter pylori* and its urease by coumarin derivatives: Molecular docking analysis

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ARTICLE INFO

Article history:
Received 17 August 2013
Accepted 3 September 2013
Available online 17 September 2013

Keywords:
Helicobacter pylori inhibition
Urease inhibition
Coumarins
Molecular docking

ABSTRACT

Objective: In the present study series of selected coumarin derivatives (CDs) were assessed for their inhibition of growth of *Helicobacter pylori* (*H. pylori*) and its related urease. The selected CDs were docked *in-silico* onto the ligand binding site of *H. pylori* urease.

Methods: The anti-*H. pylori* studies were carried out using agar diffusion assay and minimum inhibitory concentrations (MICs) were calculated by microbroth dilution method. Urease inhibitory activity of *H. pylori* using selected CDs was determined by Berthelot reaction and their IC_{50} values were calculated using GraphPad Prism version 6.00 while, docking studies were performed by ArgusLab 4.0.1.

Result: The results obtained indicate that, most of the CDs showed considerable anti-*H. pylori* activity (MIC range of 10–40 μg/ml) as well as significant inhibition of *H. pylori* urease (IC_{50} of 48.90–72.56 μM). To a greater extent, the *in-silico* results were in agreement with *in-vitro* results of inhibition of *H. pylori* urease.

Conclusion: The present investigation may find applications in designing and developing a novel, safe and effective anti-*H. pylori* agents using coumarin scaffold.

1. Introduction

Helicobacter pylori (*H. pylori*) is a gram-negative, flagellated, spiral-shaped, urease producing bacterium that lives in the microaerophilic environment of stomach and duodenum. *H pylori* is strongly associated with chronic gastritis, peptic ulcer, gastric cancer, gastric adenocarcinoma, mucosa associated lymphoid tissue, lymphoma and primary gastric non-Hodgkin's lymphoma.[1,2] *H. pylori* is able to survive in the acidic pH of the stomach with the help of urease which converts urea into ammonia, thereby creating a basic environment around the bacterium counteracting the acidic pH of the stomach.[3] This creates a neutralizing environment for protecting *H. pylori* from the acid in the stomach. Most of the urease is in the bacterial cytoplasm and only a small amount is found on the surface of the bacterial cell.[4,5] The unique gastric acid resistance of *H. pylori* may be due in part to an acid-regulated urea channel, UreI, which increases the access of urea to intrabacterial urease in acidic media.[6] Specific inhibition of urease activity has been proposed as a possible strategy to inhibit this microorganism.[7] It has been demonstrated that a urease-negative mutant does not cause gastritis in nude mice due

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0974-6943/$ – see front matter
http://dx.doi.org/10.1016/j.jopr.2013.09.002

to difficulty in colonization.[8] The circumstantial clinical evidence described above clearly figures out the important role of urease in bacterial colonization and significance of targeting urease activity for inhibiting the growth of *H. pylori*.

Eradication of *H. pylori* is an important objective in overcoming gastric diseases. Many regimens are currently available but none of them could achieve 100% success in eradication besides the availability of effective antibiotic treatment supplemented with proton pump inhibitors for the management of *H. pylori*,[9] the pandemic occurrence of *H. pylori* infection coupled with its ability to develop resistance to our current arsenal of antimicrobial regimens and recurrence of infection in patients makes the pathogenic potential of this microorganism a major global health concern. Antibiotic therapy and combination of two or three drugs have been widely used for the management of *H. pylori* infections. However prevalence of antibiotic-resistant *H. pylori* strains, side effects of the present chemotherapeutic approach has mounted a pressure for searching alternatives to present day anti-*H. pylori* drugs, especially the search for safe and effective non-antibiotic agents is more attractive.

Coumarin (2H-CHROMEN-2-ONE) and its derivatives are widely distributed in nature and exhibit a broad pharmacological profile. CDs are continuously discussed on an account of their diverse biological properties. A vast body of literature has accumulated in the recent past, linking the role of coumarin with several bioactivities including anti-cancer,[10] anticoagulant, oestrogenic, dermal, photosensitizing, antimicrobial, vasodilator, molluscicidal, antihelminthic, sedative, hypnotic, analgesic, hypothermic activities[11,12] and the free radical scavenging activity especially the superoxide anions generated by activated neutrophils.[13,14] Series of hydroxylated CDs have been reported to possess potent anti-*H. pylori* activity. In addition several hydroxylated and methylated CDs have been described to possess significant anti-*H. pylori* activity.[15] The anti-*H. pylori*, antioxidant, and anticancer activities of CDs cited in the literature make these compounds attractive for scientific enquiry, for further backbone derivatisation and screening as novel therapeutic agents. Taking into account the circumstantial literature we endeavoured to undertake *in-vitro* activities of CDs to determine inhibition of growth of *H. pylori* and its related urease activity.

2. Materials and methods

2.1. *Chemicals* & H. pylori *culture*

All the selected 24 CDs (C1–C24) obtained from Sigma–Aldrich Co. (St. Louis MO, USA) are shown in Fig. 1. Brain heart infusion broth and granulated agar were obtained from Becton, Dickinson and company (USA) respectively. The antibiotics vancomycin, amphotericin-B, polymyxin, and trimethoprim were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other media ingredients, chemicals, solvents and reagents used were of analytical grade and were procured from the commercial sources. A strain of *H. pylori* (I-87) culture was kindly supplied by National Institute of Cholera and Enteric Diseases (NICED) Kolkata, (West Bengal) India.

2.2. *Studies on anti-*H. pylori *activity of selected CDs*

H. pylori was cultured using the method of Stevenson et-al. on the Brucella agar,[16] supplemented with defibrinated sheep blood. The sterilized Brucella medium was supplemented with the selected antibiotics such as vancomycin 6 mg/L, amphotericin-B 3 mg/L, polymyxin 2500 IU/L, and trimethoprim 5 mg/L for avoiding the contamination of other microorganisms.[17] Agar diffusion assay was carried out to study the concentration dependent effect of selected CDs on the growth of *H. pylori*. In brief, a sterile cork borer of 10 mm diameter was used to bore holes into the inoculum sprayed solidified agar media. A 50 μl volume of each of (10, 50 and 100 μg/ml) the selected CDs were added into the labelled well in the prepared media plate using sterile pipette. The test was performed in triplicates. The plates were incubated at 37 °C in a microaerophilic environment (5% O_2, 10% CO_2, and 85% N_2) for 3–6 days.[18] After the incubation period the inhibition zone diameter (mm) was measured subtracting the well size. Amoxicillin (5 μg/ml) was used as a standard antibiotic for comparison.

2.3. *Determination of minimum inhibitory concentration (MIC) of the selected CDs against* H. pylori[19]

Frozen stock culture of *H. pylori* was activated by streaking it on brain heart infusion (BHI) agar supplemented with 5% defibrinated sheep blood and incubated for 3 days under microaerophilic conditions as mentioned earlier. The exponentially growing *H. pylori* cells were suspended in sterile phosphate-buffered saline (PBS) and adjusted to an optical density of 0.1 at 600 nm. Adjusted inoculum was delivered to BHI broth containing individual concentrations of selected CDs (dissolved in dimethyl sulfoxide). The contents were transferred to 96 well microtitre plates. BHI broth containing dimethyl sulfoxide was set as a control to ensure that the viability of the organism was not affected by the solvent used to dissolve coumarin. All the microtitre plates were incubated under microaerophilic conditions at 37 °C for 5 days. The absorbance at 620 nm was recorded using Thermo make Automatic Ex-Microplate Reader (M 51118170). The MIC was defined as the lowest concentration of the compound at which there was no visible bacterial growth. The antibiotic amoxicillin was used as a reference drug for comparison purpose.

2.4. *Determination of urease inhibitory activity of selected CDs*

Urease inhibitory activity of *H. pylori* using selected CDs was determined by measuring the urease catalyzed release of ammonia by Berthelot reaction.[20] In brief, the *H. pylori* cells were harvested from the BHI broth by centrifugation at 4 °C (4000 *g*, 5 min) and resuspended in ice-cold 0.1 M sodium phosphate buffer (pH 7.3) containing 10 mM EDTA. Cells were disrupted by sonication (Sonics Vibra Cell model, USA), and the supernatant obtained after centrifugation at 4 °C (12,000 *g*, 5 min) was used as a source of enzyme for urease assay. The 96 well microtitre plate reaction mixture contained urea (2, 4, 6, 8, 10 mM), sodium phosphate buffer 30 μl and different concentration of selected CDs 10, 50, 100 μg/ml [3]. After incubation for 10 min at 37 °C 0.66 N hydrogen sulphate 30 μl,