Supplementary Information

Biology

Urease inhibition assay

In vitro screening and inhibitory studies on urease (Jack bean urease) were determined using colored Berthelot phenols method, which measures the liberation of ammonia from the reaction. Briefly, the assay mixture, containing 1 unit of enzyme was added to 650 μL of buffer solution (400 mMol sodium salicylate, 50 mMol phosphate buffer pH 6.7, 2 mMol EDTA/L10 and mMol sodium nitroprusside) and mixed with 10 μL of different concentrations 0.1–100 mM of the tested compounds in DMF as a solvent. (DMF was tested alone and showed no inhibitory effect on the enzyme). After 15 min of incubation at room temperature, 10 μL of 50 mg/L urea solution was add. This mixture was incubated for 0.5 h in water bath at 37 °C to allow the hydrolysis process. After complete urea hydrolysis and ammonia liberation, the re- action was stopped by adding 200 μL of the hypochlorite reagent (150 mmol/L sodium hydroxide, 140 mm/L sodium hypochlorite). The ammonia liberated was allowed to complex with the hypochlorite and salicylate for 25 min. at 30 °C. The absorbance was measured at 578 nm using UV/VIS Spectrophotometer (Optizen POP, 5U4608, Korea), and experiments were performed in triplicate in a final volume of 1 mL. All the results were com- pared with thiourea, a standard inhibitor of urease. The percent- age inhibition was calculated as the difference of absorbance values with and without the test compounds and the concentration that provokes an inhibition halfway between the minimum and maximum response of each compound (relative IC_{50}) was deter- mined by monitoring the inhibition effect of various concentrations of compounds in the assay.

Bacterial isolates and culture conditions

Clinical *H. pylori* isolates from gastric biopsy specimens were obtained from Assiut Hospital (Assiut, Egypt). Primary isolation was performed on a selective blood agar base (Oxoid, Basingstoke, UK) supplemented with horse blood (5%, v/v) and 1 Selectatab tablet (500 mg; Mast Diagnostics, Merseyside, UK). Following primary selective isolation, *H. pylori* bacteria cells were identified according to colony morphology, Gram staining, microaerophilic growth at 37 °C, oxidase +, catalase +, urease +, nitrate -, H2S, and hippurate hydrolysis. Growth of *H. pylori* was maintained at 37 °C for 3-5 days in an atmosphere of 5% O2, 15% CO2, and 80% N2 in an anaerobic chamber (Hirayama, Tokyo, Japan). To maintain a moist atmosphere, a moist paper towel was placed in the chamber. Bacterial strains were stored at -70 °C in brain heart infusion broth (BHIB) (Difco, East Molesey, UK) containing 10% (v/v) fetal calf serum (FCS) and 15% (v/v) glycerol. Frozen clinical isolates were thawed and inoculated on Mueller-Hinton agar (MHA) plates (Oxoid), supplemented with 10% horse blood and incubated under microaerophilic conditions. Given the importance of inoculum homogeneity, cellular viability was controlled microscopically by morphological observation with Gram staining. In order to control the proportions of coccoid cells in the cultures, cultures were always used after 48 h of incubation, when they generally did not present coccoid forms. Bacterial growth was taken from the plates and resuspended in sterile saline. The inoculum was prepared to contain 5 × 107 CFU/mL by adjusting the turbidity of the suspension to match the McFarland No. 2 standard.

Bacterial growth inhibition assay (disk diffusion method)

Growth inhibition was performed by the filter paper disk diffusion method on selective Brucella agar with 7% defibrinated horse blood under microaerophilic conditions at 37 °C. The samples were evaluated for their anti-*Helicobacter* activity, dissolved in dimethyl sulfoxide (DMSO). All compounds were assayed against metronidazole-resistant *H. pylori* strains at 3 concentrations (100, 50, and 25 and 12.5 μg/disk); the surfaces of the *Brucella* blood agar plates were inoculated with 100 μL of bacterial suspensions. Blank standard disks (6 mm in diameter) were deposited on the plates and impregnated with 10 μL of different dilutions of test compounds. Following incubation for 3-5 days at 37 °C, the inhibition zone around each disk (average diameter), if any, was recorded. The control disks received 10 μL of DMSO. All tests were performed in triplicate and the antibacterial activity was expressed as the mean of inhibition diameters (mm) produced by the tested compounds.

The one-way analysis of variance (ANOVA) followed by Tukey multiple comparisons was used to analyze the data. A value of $p < 0.05$ was considered as the significance level between the groups.