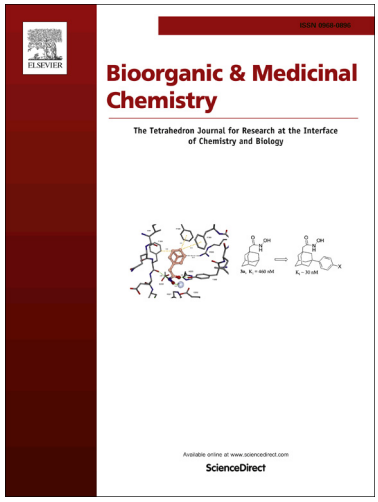

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Synthesis, molecular docking and biological evaluation of 3-Arylfuran-2(5*H*)-ones as anti-gastric ulcer agent

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Abstract

3-Arylfuran-2(5*H*)-one derivatives show good antibacterial activity and were determined as tyrosyl-tRNA synthetase (TyrRS) inhibitors. In a systematic medicinal chemistry exploration, we demonstrated chemical opportunities to treat infections caused by *Helecobacter pylori*. Twenty 3-arylfuran-2(5*H*)-ones were synthesized and evaluated for anti-*H. pylori*, antioxidant and anti-urease activities which are closely interconnected with *H. pylori* infection. The results displayed that some of the compounds show excellent antioxidant activity, and good anti-*H. pylori* and urease inhibitory activities. Out of these compounds, 3-(3-methylphenyl)furan-2(5*H*)-one (**b9**) showed the most potent antioxidant activity (IC_{50}=8.2 μM) and good anti-*H. pylori* activity (MIC_{50}=2.6 μg/mL), and it can be used as a good candidate for discovering novel anti-gastric ulcer agent.

Key words: 3-Arylfuran-2(5*H*)-one, anti-*H. pylori*, antioxidant, TyrRS, gastric ulcer treatment

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1. Introduction

Helicobacter pylori (*H. pylori*), a Gram negative bacterium, plays an important role in chronic gastritis, peptic ulcers, including gastric cancer and gastric lymphoma.[1-5] *H. pylori* infection leads to pathological changes in the gastric microenvironment, which are caused by both direct and cascade effects of virulent factors. For instance, the host immune cells (especially neutrophils) release large amounts of reactive oxygen species (ROS), which are associated with inflammation, infiltration, activation of immune cells, accumulation of reactive oxygen species, and oxidative DNA damage in the gastric mucosa. [6-8]

Triple therapy which consists of a proton pump inhibitor and two effective antibiotics is commonly used for the treatment of *H. pylori* infection.[9-10] However, the acquisition of antibiotic resistance by *H. pylori* has severely weakened eradication therapy and presents a serious problem such as allergy to antibiotics, severe complications (liver and/or kidney dysfunction).[11-12]

To develop more effective and safer therapies, many substances from natural products with good potency against *H. pylori* are reported, such as 5-methoxy-3,4-dehydroxanthomegnin (a naphthoquinone from *Paepalanthus latipes*) [13], epigallocatechin gallate (a major compound of *Catechins*)[14] and 2-hydroxy-4-methoxy benzaldehyde from *Decalepis hamiltonii.*[15] 2-Furanone framework is a part of many natural and synthetic compounds, which possesses useful biological activities, including anti-inflammatory and HIV-1 integrase inhibitory activities.[16-22] It is to be noted that many naturally sourced compounds with 2-furanone unit, such as Variagatic acid and Vitamin C (Vc) (Scheme 1) were determined as efficient antioxidants, that is, compounds

are able to protect biomolecules from oxidative degradation, in particular through the quenching ROS. In our group, derivatives as 3-aryl-4-aminofuran-2(5*H*)-ones, 3-aryl-4-acyloxyethoxyfuran-2(5*H*)-ones and 3-arylfuran-2(5*H*)-one-fluoroquinolone hybrids have been previously reported to exhibit excellent inhibition activity against pathogenic bacteria.[23-29] However, the anti-*H. pylori* activity of them was not included. Motivated by these findings and as a continuation of our ongoing efforts on the discovery of novel chemical entities against *H. pylori*, twenty 3-arylfuran-2(5*H*)-ones were synthesized and evaluated for anti-*H. pylori*, antioxidant and urease suppression activities which are closely interconnected with the elimination of *H. pylori* infection.

The results diclosed that some of the compounds show excellent antioxidant activities, compared urease inhibitory activities to that of the positive control AHA and good anti-*H. pylori* activities. Both the biological evaluation and mechanism study suggested that 3-arylfuran-2(5*H*)-ones are good candidates for discovering novel anti-gastric ulcer agents.

2. Materials and methods

2.1. Chemistry

All chemicals (reagent grade) were obtained from Aldrich (U.S.A) and Sinopharm Chemical Reagent Co., Ltd (China) and used without further purification. Separation of the compounds by column chromatography using silica gel (200-300 mesh) purchased from Qingdao Haiyang Chemical Co.,Ltd (China). All the reactions were monitored by thin-layer chromatography (TLC) using silica gel GF254 plates from Qingdao Haiyang Chemical Co., Ltd (China) with an UV lamp (254 nm). Melting points were recorded on

the XT4 MP apparatus (Taike Corp., Beijing, China) and were uncorrected. ^{1}H NMR spectra were recorded using TMS as the internal standard in DMSO-d_6 with a Bruker spectrometer at 300 MHz. EI mass spectra were obtained on a Waters GCT mass spectrometer, and elemental analyses were performed on a CHN-O-Rapid instrument and were within ± 0.4 % of the theoretical values.

2.1.1. General procedure for preparation of compounds a1-a20

Compounds **a1-a20** were synthesized by esterification of appropriate substituted sodium phenylacetates with ethyl bromoacetate. Briefly, phenylacetic acid (40 mmol) and water (20mL) were added to a round bottom flask and the pH value was adjusted to the range of 9.0 to 10.0 with 0.1M NaOH. Water was then removed under reduced pressure to give the corresponding sodium phenylacetate, which was dissolved in 45 mL DMSO. To the obtained solution, 4.5 mL ethyl bromoacetate was gently added, and was subsequently stirred at room temperature for 3-4 h. The resulted mixture was poured into ice-water (50 mL), and extracted twice with 200 mL of AcOEt. The organic layer was dried over $MgSO_4$ followed by removal of the solvent under reduced pressure. The residue was then purified by column chromatography on silica gel to give **a1-a20** (Scheme 2) in yields of 90%-95%.

2.1.2. General procedure for preparation of compounds b1-b20

A solution of compound **a** (10 mmol) in dry THF (10 mL) was added to a suspension of NaH (24 mmol) in dry THF (20 mL) in an ice cold bath, and the stirring was maintained at room temperature for several hours (monitored by TLC). 30 mL of distilled water was

combined into the solution, which was extracted twice with ethyl ether. The aqueous phase was cooled to 0°C and then acidified with concentrated hydrochloric acid to give a solid precipitate. After filtration and washing with water, the compound was purified by column chromatography on silica gel, eluting with AcOEt/petroleum ether.

4-hydroxy-3-phenylfuran-2(5*H*)-one (b1)

Colorless crystal, 50%, mp 244-254 °C; ^{1}H-NMR (DMSO-d_6): 4.77 (s, 2H, CH_2); 7.20-7.25 (m, 3H, ArH); 7.90 (d, J = 7.7 Hz, 2H, ArH); 12.90 (s, 1H, OH); EIMS *m/z* 175 (M^+). Anal. Calcd for $C_{10}H_8O_3$: C, 68.18; H, 4.58; Found: C, 68.50; H, 4.38.

3-(2-fluorophenyl)-4-hydroxyfuran-2(5*H*)-one (b2)

Colorless crystal, 27%, mp 192-194 °C; ^{1}H-NMR (DMSO-d_6): 4.81 (s, 2H, CH_2); 7.19-7.25 (m, 2H, ArH); 7.33-7.40 (m, 2H, ArH); 12.64 (s, 1H, OH); EIMS *m/z* 193 (M^+). Anal. Calcd for $C_{10}H_7FO_3$: C, 61.86; H, 3.63; F, 9.78; Found: C, 61.95; H, 3.58; F, 9.66.

3-(2-chlorophenyl)-4-hydroxyfuran-2(5*H*)-one (b3)

Colorless crystal, 29%, mp 197-199 °C; ^{1}H-NMR (DMSO-d_6): 4.82 (s, 2H, CH_2); 7.29-7.40 (m, 3H, ArH); 7.48-7.53 (m, 1H, ArH); 12.57 (s, 1H, OH); EIMS *m/z* 209 (M^+). Anal. Calcd for $C_{10}H_7ClO_3$: C, 57.03; H, 3.35; Cl, 16.83; Found: C, 57.11; H, 3.28; Cl, 16.79.

3-(2-methylphenyl)-4-hydroxyfuran-2(5*H*)-one (b4)

Colorless crystal, 42%, mp 189-191 °C; ^{1}H-NMR (DMSO-d_6): 2.36 (s, 3H, CH_3); 4.85 (s, 2H, CH_2); 7.35-7.42 (m, 2H, ArH); 7.62-7.67 (m, 2H, ArH); 12.61 (s, 1H, OH); EIMS *m/z* 189 (M^+). Anal. Calcd for $C_{11}H_{10}O_3$: C, 69.46; H, 5.30; Found: C, 69.55; H, 5.21.

3-(2-(benzyloxy)phenyl)-4-hydroxyfuran-2(5*H*)-one (b5)

Colorless crystal, 38%, mp 132-134 °C; ^{1}H-NMR (DMSO-d_6): 4.75 (s, 2H, CH_2); 5.10 (s, 2H, CH_2); 6.96 (t, J = 7.0 Hz, 2H, ArH); 7.15-7.40 (m, 5H, ArH); 7.45 (d, J = 6.8 Hz, 2H, ArH); 12.13 (s, 1H, OH); EIMS *m/z* 281 (M^+). Anal. Calcd for $C_{17}H_{14}O_4$: C, 72.33; H, 5.00; Found: C, 72.32; H, 5.02.

3-(3-fluorophenyl)-4-hydroxyfuran-2(5*H*)-one (b6)

White powder, 24%, mp 252-255 °C; ^{1}H-NMR (DMSO-d_6): 4.79 (s, 2H, CH_2); 7.02-7.08 (m, 1H, ArH); 7.42 (dd, J = 14.6 Hz, J = 8.1 Hz, 1H, ArH); 7.74 (d, J = 11.3 Hz, 1H, ArH); 7.82 (d, J = 7.86 Hz, 1H, ArH); EIMS *m/z* 193 (M^+). Anal. Calcd for $C_{10}H_7FO_3$: C, 61.86; H, 3.63; F, 9.78; Found: C, 61.56; H, 3.72; F, 9.81.

3-(3-chlorophenyl)-4-hydroxyfuran-2(5*H*)-one (b7)

White powder, 65%, mp 182-184°C; ^{1}H-NMR (DMSO-d_6): 4.79 (s, 2H, CH_2); 7.26-7.30 (m, 1H, ArH); 7.41 (t, J = 7.9 Hz, 1H, ArH); 7.93 (d, J = 7.9 Hz, , 1H, ArH); EIMS *m/z* 209 (M^+). Anal. Calcd for $C_{10}H_7ClO_3$: C, 57.03; H, 3.35; Cl, 16.83; Found: C, 57.06; H, 3.32; Cl, 16.73.

3-(3-bromophenyl)-4-hydroxyfuran-2(5*H*)-one (b8)

White powder, 58%, mp 174-175 °C; ^{1}H-NMR (DMSO-d_6): 4.78 (s, 2H, CH_2); 7.31-7.43 (m, 2H, ArH); 7.96 (d, J = 7.7 Hz, 1H, ArH); 8.13 (s, 1H, ArH); EIMS *m/z* 254 (M^+). Anal. Calcd for $C_{10}H_7BrO_3$: C, 47.09; H, 2.77; Br, 31.33; Found: C, 47.11; H, 2.73; Br, 31.23.

3-(3-methylphenyl)-4-hydroxyfuran-2(5*H*)-one (b9)

Colorless crystal, 46%, mp 208-210 °C; ^{1}H-NMR (DMSO-d_6): 2.39 (s, 3H, CH_3); 4.88 (s, 2H, CH_2); 7.42-7.46 (m, 1H, ArH); 7.72-7.85 (m, 3H, ArH); 12.58 (s, 1H, OH); EIMS *m/z* 189 (M^+). Anal. Calcd for $C_{11}H_{10}O_3$: C, 69.46; H, 5.30; Found: C, 69.67; H, 5.35.

4-hydroxy-3-(3-(trifluoromethyl)phenyl)furan-2(5*H*)-one (b10)

Colorless crystal, 52%, mp 178-180 °C; ^{1}H-NMR (DMSO-d_6): 4.82 (s, 2H, CH_2); 7.56-7.65 (m, 2H, ArH); 8.23-8.30 (m, 2H, ArH); EIMS *m/z* 243 (M^+). Anal. Calcd for $C_{11}H_7F_3O_3$: C, 54.11; H, 2.89; F, 23.34; Found: C, 54.23; H, 2.76; F, 23.47.

3-(3-(benzyloxy)phenyl)-4-hydroxyfuran-2(5*H*)-one (b11)

Colorless crystal, 40%, mp 206-208 °C; ^{1}H-NMR (DMSO-d_6): 4.75 (s, 2H, CH_2); 5.10 (s, 2H, CH_2); 6.88 (d, 8.1 Hz, 1H, ArH); 7.25-7.47 (m, 6H, ArH); 7.53 (d, *J* = 8.0 Hz, 1H, ArH); 7.61 (s, 1H, ArH); EIMS *m/z* 281 (M^+). Anal. Calcd for $C_{17}H_{14}O_4$: C, 72.33; H, 5.00; Found: C, 72.42; H, 5.09.

3-(4-fluorophenyl)-4-hydroxyfuran-2(5*H*)-one (b12)

Colorless crystal, 48%, mp 220-222°C; ^{1}H-NMR (DMSO-d_6): 4.79 (s, 2H, CH_2); 7.58 (t, *J* = 8.9 Hz, 2H, ArH); 7.89 (dd, *J* = 8.3 Hz, *J* = 5.9 Hz, 2H, ArH); 12.77 (s, 1H, OH); EIMS *m/z* 193 (M^+). Anal. Calcd for $C_{10}H_7FO_3$: C, 61.86; H, 3.63; F, 9.78; Found: C, 61.84; H, 3.52; F, 9.70.

3-(4-chlorophenyl)-4-hydroxyfuran-2(5*H*)-one (b13)

White powder, 68%; mp 256-258 °C; ^{1}H-NMR (DMSO-d_6): 4.82 (s, 2H, CH_2); 7.42-7.46 (m, 2H, ArH); 7.95-7.99 (m, 2H, ArH); 12.70 (s, 1 H, OH); EIMS *m/z* 209 (M^+). Anal. Calcd for $C_{10}H_7ClO_3$: C, 57.03; H, 3.35; Cl, 16.83; Found: C, 57.06; H, 3.32; Cl, 16.73.

3-(4-bromophenyl)-4-hydroxyfuran-2(5*H*)-one (b14)

White powder, 60%, mp 230-232 °C; ^{1}H-NMR (DMSO-d_6): 4.77 (s, 2H, CH_2); 7.56 (d, *J* = 8.6 Hz, 2H, ArH); 7.90 (d, *J* = 8.8 Hz, 2H, ArH); 12.74 (s, 1H, OH); EIMS *m/z* 254 (M^+). Anal. Calcd for $C_{10}H_7BrO_3$: C, 47.09; H, 2.77; Br, 31.33; Found: C, 47.12; H, 2.73; Br, 31.35.

3-(4-methylphenyl)-4-hydroxyfuran-2(5*H*)-one (b15)

Colorless crystal, 37%, mp 245-247 °C; ^{1}H-NMR (DMSO-d_6): 2.34 (s, 3H, CH_3); 4.77 (s, 2H, CH_2); 7.17 (d, J = 8.2 Hz, 2H, ArH); 7.81 (d, J = 8.2 Hz, 2H, ArH); 12.66 (s, 1H, OH); EIMS m/z 189 (M^+). Anal. Calcd for $C_{11}H_{10}O_3$: C, 69.46; H, 5.30; Found: C, 69.41; H, 5.25.

3-(4-(benzyloxy)phenyl)-4-hydroxyfuran-2(5*H*)-one (b16)

White powder, 18%, mp 210-212 °C; ^{1}H-NMR (DMSO-d_6): 4.71 (s, 2H, CH_2); 5.09 (s, 2H, CH_2); 7.02 (d, J = 9.0 Hz, 2H, ArH); 7.25-7.50 (m, 5H, ArH); 7.85 (d, J = 9.0 Hz, 2H, ArH); EIMS m/z 281 (M^+). Anal. Calcd for $C_{17}H_{14}O_4$: C, 72.33; H, 5.00; Found: C, 72.32; H, 5.02.

3-(3,4-dichlorophenyl)-4-hydroxyfuran-2(5*H*)-one (b17)

Colorless crystal, 58%, mp 249-252 °C; ^{1}H-NMR (DMSO-d_6): 4.79 (s, 2H, CH_2), 7.63 (d, J = 8.6 Hz, 1H, ArH), 7.97 (d, J = 8.2 Hz, 1H, ArH), 8.19 (s, 1H, ArH); EIMS m/z 243 (M^+). Anal. Calcd for $C_{10}H_6Cl_2O_3$: C, 49.01; H, 2.47; Cl, 28.93; Found: C, 49.41; H, 2.32; Cl, 28.75.

3-(3,4-dimethoxyphenyl)-4-hydroxyfuran-2(5*H*)-one (b18)

Colorless crystal, 20%, mp 246-248 °C; ^{1}H-NMR (DMSO-d_6): 3.75 (s, 6H, CH_3); 4.75 (s, 2H, CH_2); 6.97 (d, J = 8.6 Hz, 1H, ArH); 7.50 (d, J = 8.4 Hz, 1H, ArH); 7.58 (s, 1H, ArH); 12.76 (s, 1H, OH); EIMS m/z 235 (M^+). Anal. Calcd for $C_{12}H_{12}O_5$: C, 61.01; H, 5.12; Found: C, 61.20; H, 5.02.

3-(3,4-diethoxyphenyl)-4-hydroxyfuran-2(5*H*)-one (b19)

White power, 34%, mp 173-174 °C; ^{1}H-NMR (DMSO-d_6): 1.30-1.36 (m, 6H, CH_3); 3.96-4.05 (m, 4H, CH_2); 4.74 (s, 2H, CH_2); 6.94 (d, J = 8.4 Hz, 1H, ArH); 7.46-7.57 (m,

2H, ArH); 12.56 (s, 1H, OH); EIMS *m/z* 263 (M^+). Anal. Calcd for $C_{14}H_{16}O_5$: C, 63.63; H, 6.10; Found: C, 63.50; H, 6.08.

3-(2,4-dichlorophenyl)-4-hydroxyfuran-2(5*H*)-one (b20)

Colorless crystal, 72%, mp 207-209 °C; ^{1}H-NMR (DMSO-d_6): 4.82 (s, 2H, CH_2); 7.32 (d, *J* = 8.4 Hz, 1H, ArH); 7.40-7.47 (m, 1H, ArH); 7.60-7.67 (m, 1H, ArH); 12.70 (s, 1H, OH); EIMS *m/z* 243 (M^+). Anal. Calcd for $C_{10}H_6Cl_2O_3$: C, 49.01; H, 2.47; Cl, 28.93; Found: C, 49.17; H, 2.53; Cl, 28.62.

2.2. DPPH Assay for Antioxidant activity

Antioxidant activity was determined as literature.[30] Briefly, 15 mg DPPH was dissolved in 250 mL 95% ethanol. The mixture was measured with a microplate reader (SpectraMax® Plus384 Molecular Devices, USA) at 517 nm, and the OD value should be controlled under 1.2-1.3 to achieve reliable results. A stock solution of the synthesized compound (1000 μg/mL) in DMSO was prepared at different concentrations. Fifty μL of DMSO containing serially diluted compounds (Vc as control) and 200 μL of DPPH solution were applied to 96-well plates and incubated for 30 min at 37 °C under dark conditions. The IC_{50} values correspond to the concentration at which half of the DPPH• is inhibited by the compound are presented in Table 1. The DPPH• inhibition percentage of the samples was calculated:

$$\text{Inhibition (\%)} = [1-(A-A_{blank})/(A_0-A_{blank})]\times 100\%$$

Where A_0 is the absorbance without samples and A_{blank} is the absorbance of solvent, while A is the absorbance with samples.

2.3. Anti-*H. pylori* activity

The antibacterial activity of the synthesized compounds were tested against *H. pylori* (ATCC 43504, amoxicillin as positive control), which were determined by using microdilution broth method. A stock solution of the synthesized compounds (1000 μg/mL) in DMSO were prepared and graded quantities of the test compounds were incorporated in specified quantity of sterilized liquid medium (50% (v/v) of DMSO in PBS). Fifty microliters of each dilution and 50 mL of suspension of the microorganism approximate 10^5 cfu/mL were then dispensed into each well. The test medium and culture conditions were following: *H. pylori* was grown in Brucella broth supplemented with 10% heat-inactivated horse serum for 24 h at 37 ºC under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2), as our previously described.[31-34] The MIC_{50} values were determined by observation,[35] which were presented in Table 2.

2.4. Measurement of Urease Inhibitory Activity

The assay mixture, containing 25 μL (10U) of *H. pylori* urease and 25 μL of the test compound, was pre-incubated for 1.5 h at room temperature in a 96-well assay plate. Urease activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn.[36]

2.5. Preparation of the TyrRS and enzyme assay

S. aureus TyrRS was over-expressed in *E. coli* and purified to near homogeneity (~98% as judged by SDS-PAGE) using standard purification procedures.[37] TyrRS activity was measured by aminoacylation using modifications to previously described methods.[38] The

assays were performed at 37°C in a mixture containing (final concentrations) 100 mM Tris/Cl pH 7.9, 50 mM KC1, 16 mM $MgCl_2$, 5 mM ATP, 3 mM DTT, 4 mg/ml *E. coli* MRE600 tRNA (Roche) and 10 μM L-tyrosine (0.3 μM L-[ring-3,5-^{3}H] tyrosine (PerkinElmer, Specific activity: 1.48-2.22 TBq/mmol), 10 μM carrier). TyrRS (0.2 nM) was preincubated with a range of inhibitor concentrations for 10 min at room temperature followed by the addition of pre-warmed mixture at 37°C. After specific intervals, the reaction was terminated by adding aliquots of the reaction mix into ice-cold 7% trichloroacetic acid and harvesting onto 0.45 mm hydrophilic Durapore filters (Millipore Multiscreen 96-well plates) and counted by liquid scintillation. The rate of reaction in the experiments was linear with respect to protein and time with less than 50% total tRNA acylation. IC_{50}s correspond to the concentration at which half of the enzyme activity is inhibited by the compound. The results are presented in Table 1.

2.6. Protocol of docking study

The automated docking studies were carried out using AutoDock version 4.2. First, AutoGrid component of the program pre-calculates a three-dimensional grid of interaction energies based on the macromolecular target using the AMBER force field. A grid box of 60×60×60 Å size (x, y, z) with a spacing of 0.375 Å and grid maps were created to represent the catalytic active target site region where the native ligand was embedded. Then automated docking studies were carried out to evaluate the binding free energy of the inhibitor within the macromolecules. The genetic algorithm with local search (GA-LS) was chosen to search for the best conformers. The parameters were set using the software ADT (AutoDockTools package, version 1.5.4) on PC which is

associated with AutoDock 4.2. Default settings were used with an initial population of 50 randomly placed individuals, a maximum number of 7.5×10^6 energy evaluations, and a maximum number of 2.7×10^4 generations. A mutation rate of 0.02 and a crossover rate of 0.8 were chosen. Results differing by less than 0.5 Å in positional root-mean-square deviation (RMSD) were clustered together and the results of the most favorable free energy of binding were selected as the resultant complex structures.

3. Results and discussion

3.1. Chemistry

Detailed synthetic pathways to the 3-arylfuran-2(5*H*)-ones (**b1-b20)** are depicted in Scheme 2 and the procedures were previously reported by our group. In brief, esters (**a**) were prepared by esterification of the corresponding sodium phenylacetate with 2-bromoacetate. Subsequently, compounds **b1-b20** were obtained via alkaline cyclization of **a1-a20** using sodium hydride in dried THF. All the final compounds were fully characterized by elementary analysis, EIMS and ^{1}H-NMR.

3.2. Biological Evaluation.

Antioxidant activity. Obviously, 3-arylfuran-2(5*H*)-ones **b1-b20** is structural analogues of Vc, and we have a concept that **b1-b20** should also exhibit antioxidant activity. Therefore, the radical scavenging activity determined by DPPH method was selected to evaluate the antioxidant property for obtained 3-arylfuran-2(5*H*)-ones, and Vc was used as control. As shown in Table 1, most 3-arylfuran-2(5*H*)-one derivatives exhibited good to excellent ROS inhibitory activities, with compounds **b1**, **b8**, **b9** and **b15** to be noted.

On the whole, modification at the phenyl with electron donating group seemed to be beneficial to antioxidant activity (**CF_3 < F < Cl < Br < H < CH_3**, **b10** *vs* **b6** *vs* **b7** *vs* **b8** *vs* **b9**). The substituent group on benzyl ring at the 3-positionion were stronger antioxidant activity than those with the 2- and 4-positionion. For instance, compounds **b4** and **b15** showed 5.6 and 1.5-folds weaker antioxidant activity (IC_{50} = 45.6 μM and IC_{50} = 12.3 μM) than compound **b9**, with an IC_{50} value of 8.2 μM. Out of these compounds, **b9** bearing 3-methylphenyl group is the most active, which showed even more potency than Vc (IC_{50} = 12.1 μM).

Anti-*H. pylori* activity. All synthesized compounds were tested for antibacterial activities against *H. pylori* by using amoxicillin as control. The MIC_{50}s of these compounds are presented in Table 1. The results revealed that some of the compounds exhibited good antibacterial activities, such as compound **b1** (MIC_{50} = 4.7 μg/mL), **b2** (MIC_{50} = 2.7 μg/mL), and **b9** (MIC_{50} = 2.6 μg/mL). Out of the synthetic compounds, compound **b4** (MIC_{50} = 1.5 μg/mL) displayed the best activity against *H. pylori*, and showed a potency compared to that of amoxicillin (MIC_{50} = 0.8 μg/mL). It is important to note that compound **b9** not only showed excellent antioxidant activity but also exhibited significant antibacterial activity.

Urease Inhibitory Activity.

It is well known that *H. pylori* urease is an etiological factor of gastritis and peptic ulcer,[39] which catalyzes the hydrolysis of urea to ammonia and carbon dioxide. High concentration of ammonia arising from these reactions leads to an increase in pH, which

permits *H. pylori* to endure the acidic environment of the stomach during colonization. The continuously produced ammonia was confidently considered to enhance the permeability of gastric mucosa and to have toxicity to parietal cell. Therefore, people infected by *H. pylori* are exposed to a high risk for chronic atrophic gastritis, peptic ulcer and urolithiasis. Thus the strategies based on urease inhibition are now essential lines of treatment for *H. pylori* infection. In order to completely study the potential role of the 3-arylfuran-2(5*H*)-ones against *H. pylori*, all the synthetic compounds were further screened for their urease inhibitory potential and the results were listed in Table 2. It was found that most of the compounds showed moderate activity, and compound **b16** showed the most potency (IC_{50} = 10.5±0.3 μM). It is to be noted that compound **b9** (IC_{50} = 31.1±0.7 μM) has comparable activity to the positive control AHA (IC_{50} = 27.6±2.5 μM). These discoveries suggested that 3-arylfuran-2(5*H*)-ones do not show significant potency, and therefore do not use as urease inhibitors. However, urease inhibitory capacity will synergize the antioxidant and antibacterial activities of 3-arylfuran-2(5*H*)-ones for treatment of *H. pylori* infections.

TyrRS assay.

TyrRS assay was conducted to study the structure-activity relationship profiles and to confirm antibacterial mechanisms of 3-arylfuran-2(5*H*)-ones. The observed data showed that most of the selected compounds (**b1, b2, b4-b10, b15, b16**) displayed good activity, and compound **b4** was the most potent with IC_{50} of 0.6±0.04 μM. Both replacements of the 2-CH_3 or 3-CH_3 with electron-withdrawing groups (fluoro, chloro, bromo, trifluoromethyl) and with bulky group (benzyloxy) lead to a decrease of activity,

indicating that CH_3 might hydrophobically interact or not typically hydrogen bond with the active site of the TyrRS enzyme and a bulky group might not be tolerated. In comparison with **b4**, movement of the methyl group to 3-position or 4-position results in a significant decrease in potency.

3.3. Protocol of docking study.

In order to further explore the mechanism of inhibition, the molecular docking study was performed based on the TyrRS complex structure (1jij.pdb).[40] The 3D structure of enzyme revealed that the most potent inhibitor **b4** is located at the active site of TyrRS, and 2-methylphenyl moiety of compound **b4** is oriented towards the entrance cavity (Fig. 1), which is coordinated by Gln174, Gln190, Asp195, Gly193, Val191 and Cys37 residues (Fig. 2). In the binding model, the 2-CH_3 is firmly bound to the active site, through a strong C–H···N hydrogen bond (H···N distance of 2.585 Å) to the N atom of Gly193 residues and a strong O–H···S hydrogen bond (H···S distance of 1.737 Å) to the S atom of Cys37 and these strong interactions may explain its excellent inhibitory activity. Thus, shift of 2-CH_3 group to 3- or 4-position will decrease the potency, which is consistent with the experimental data. The 3-, 5- and 6-H of the benzene ring moiety form four hydrogen-bonds with Val191, Gln190 and Gln174 residues through C-H···O and C-H···N hydrogen bonds (1.313Å, 2.142 Å and 2.634). The docking model also show that three hydrogen-bonding interactions anchor the furanone-ring moiety to the active site by O–H···O, C-H···N and N–H···O hydrogen bonds (2.871Å, 3.915Å and 2.802 Å).

4. Conclusitions

Twenty 3-arylfuran-2(5*H*)-ones were prepared and tested for their anti-*H. pylori*, antioxidant and urease inhibitory activities. Among the compounds, compound **b9**, 3-(3-methylphenyl)furan-2(5*H*)-one, showed the most potent antioxidant activity (8.2 μM) and good anti-*H. pylori* activity (2.6 μg/mL) with a compared urease inhibitory activity to that of AHA. These observations indicate that compound **b9** would be a potential candidate for further modification to treat the *H. pylori* infection. All in all, the most obtained 3-arylfuran-2(5*H*)-ones are weak or inactive urease inhibitors. The TyrRS assays disclosed that the compound exhibits good activity against *H. pylori* also shows good activity against TyrRS. Molecular docking revealed that compound **b4**, the most potent enzyme inhibitor, well docked into TyrRS active site. Several interactions anchoring the ligand to the active site of the enzyme tightly might explain its excellent inhibitory activity.

Acknowledgements

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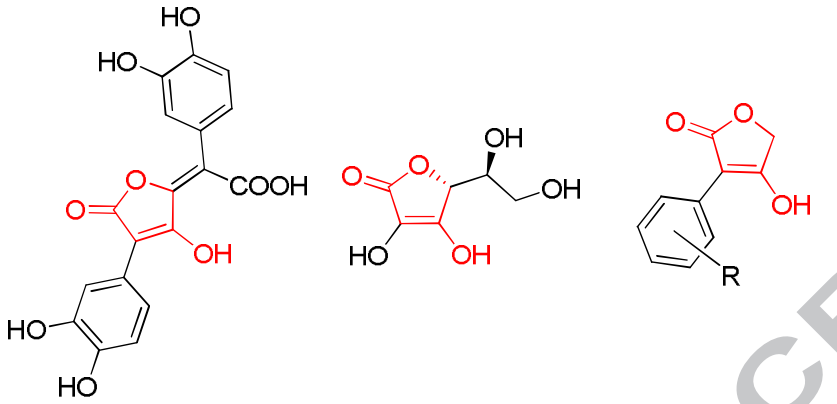
Scheme 1 The structure of Variagatic acid, Vitamin C and 3-Arylfuran-2(5*H*)-one



Variagatic acid Vitamin C 3-Arylfuran-2(5*H*)-one

Scheme 2 Synthetic route of 3-arylfuran-2(5*H*)-ones

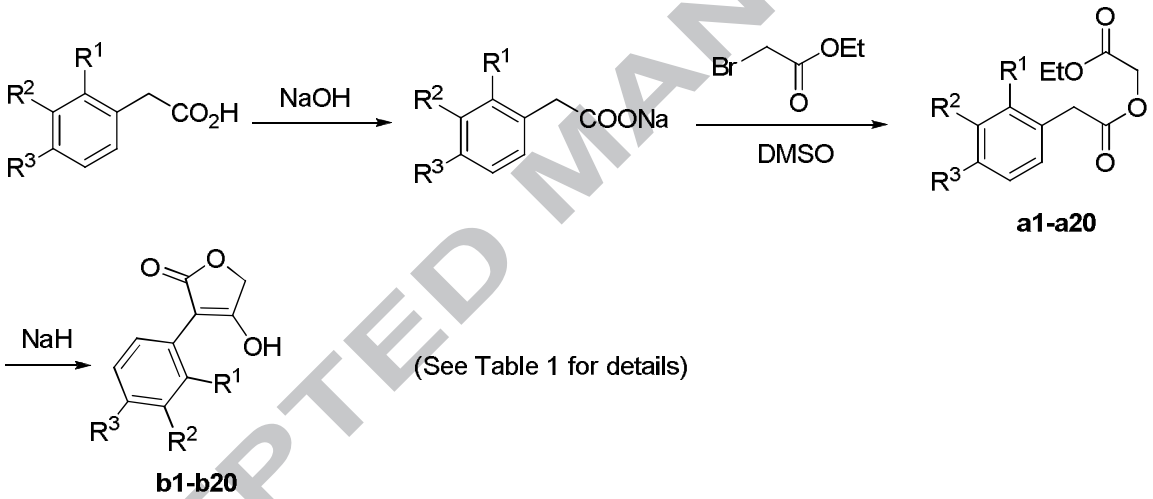


Table 1 Inhibitory activity of the synthetic compounds for its anti-*H. pylori* activity and antioxidant activity.

Compound	Structure			Anti-*H. pylori* activity	Antioxidant activity
	R^1	R^2	R^3	MIC_{50} (μg/mL)	IC_{50} (μM)
b1	H	H	H	4.7	13.5
b2	F	H	H	2.7	>100
b3	Cl	H	H	36.7	>100
b4	CH_3	H	H	1.5	45.6
b5	OBn	H	H	13.8	>100
b6	H	F	H	>100	23.5
b7	H	Cl	H	36.7	16.5
b8	H	Br	H	24.5	11.2
b9	H	CH_3	H	2.6	8.2
b10	H	CF_3	H	28.2	110
b11	H	OBn	H	31.7	31.5
b12	H	H	F	>100	27.7
b13	H	H	Cl	49.3	18.5
b14	H	H	Br	61.3	16.8

b15	H	H	CH_3	15.2	12.3
b16	H	H	OBn	52.1	34
b17	H	Cl	Cl	>100	28.7
b18	H	OMe	OMe	46.2	20.4
b19	H	OEt	OEt	72.5	23.4
b20	Cl	H	Cl	>100	>100
Vc	---	---	---	---	12.1
Amoxicillin	---	---	---	0.8	---

Table 2. In vitro inhibitory activity data of the synthesized compounds against Urease and TyrRS.

entry	IC_{50} (μM)	
	Urease	TyrRS
b1	89±1.5	5.6±0.03
b2	75±2.6	1.9±0.07
b3	68±2.2	---
b4	65±1.9	0.6±0.04
b5	50±2.1	2.5±0.05
b6	62±6.1	27.8±0.11
b7	45.3±1.4	15.4±0.06
b8	81.8±1.7	13.1±0.21
b9	31.1±0.7	1.1±0.04

b10	64.4±1.5	17.5±0.14
b11	77.5±0.8	---
b12	40.6±2.1	---
b13	>100	---
b14	>100	---
b15	>100	8.6±0.06
b16	10.5±0.3	30±0.21
b17	>100	---
b18	36.5±0.9	---
b19	91.7±1.5	---
b20	>100	---
I	---	0.10±0.03
AHA	27.6±2.5	---

I, 3-(4-hydroxyphenyl)-4-(2-morpholinoethoxy)furan-2(5*H*)-one; **AHA**, acetohydroxamic acid.

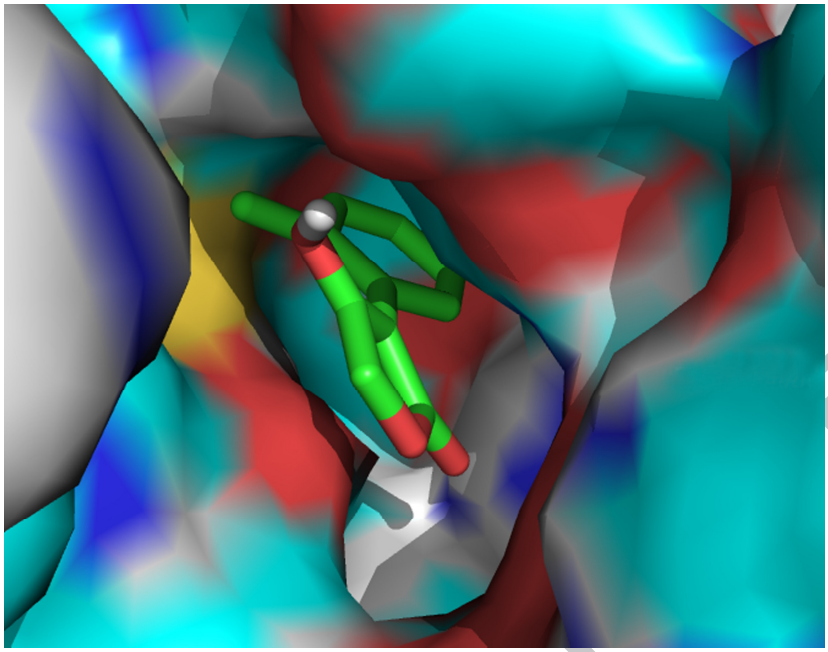


Fig. 1 Binding mode of compound **b4** with TyrRS. The enzyme is shown as surface; while **b4** docked structures are shown as sticks. This figure was made using PyMol.

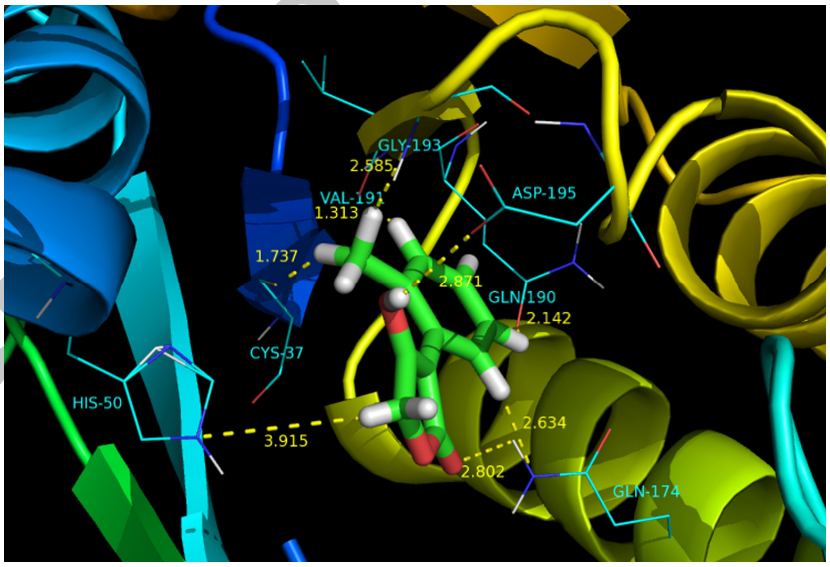


Fig. 2 Binding mode of compound **b4** with TyrRS from *S. aureus*. For clarity, only interacting residues were labeled. Hydrogen bonding interactions are shown in dash. This figure was made using PyMol.

Synthesis, molecular docking and biological evaluation of 3-Arylfuran-2(5*H*)-ones as anti-gastric ulcer agent

Xu-Dong Wang, Wei Wei, Peng-Fei Wang, Li-Cheng Yi, Wei-Kang Shi, Yong-Qiang Xie, Lang-Zhou Wu, Nian Tang, Liang-Song Zhu, Jia Peng, Chan Liu, Xian-Hui Li, Shi Tang, Zhu-Ping Xiao, and Hai-Liang Zhu