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Metronidazole–Deoxybenzoin Derivatives as Anti-*Helicobacter pylori* Agents with Potent Inhibitory Activity against HPE-Induced Interleukin-8

Yin Luo, Huan-Qiu Li, Yang Zhou, Zi-Lin Li, Tao Yan, and Hai-Liang Zhu*[a]

A series of new metronidazole–deoxybenzoin derivatives were synthesized and evaluated for their antimicrobial activity against *Helicobacter pylori*. Highly selective anti-*H. pylori* activity was also observed in synthesized compounds. Compound **34** exhibited the most potent activity, similar to the positive control amoxicillin. Furthermore, compounds **17** and **34** were able to significantly decrease *H. pylori* water extract (HPE)-induced production of interleukin-8 (IL-8) in gastric mucosal cells, which did not show any effect on the cell viability.

Introduction

Helicobacter pylori is a gram-negative microaerophilic bacterium that colonizes at the surface of the gastric epithelium beneath the mucus. It can cause many gastroduodenal diseases such as gastritis, gastric and duodenal ulcers, and even gastric cancer.[1] In the clinical setting, the increasingly extensive use of antibiotics to treat *H. pylori* leads to many serious side effects. The most serious one among these being the drug resistance of *H. pylori* strains.[2] Metronidazole used to be one of the most effective agents against *H. pylori*, but now it is unlikely to have great therapeutic potential for this bacterium in the clinic because of the increasing resistance of most *H. pylori* strains.[3] Hence, there are unmet medical needs for novel, efficacious, and selective eradication therapies that minimize resistance problems.

Recently more attention has been paid to the pathological reaction to the infection of *H. pylori* in host cells, which leads to inflammation and then many gastroduodenal diseases.[4] Some anti-*H. pylori* agents such as ecabet sodium and rebamipide have been investigated and subsequently proved to have the ability to suppress inflammation relating to *H. pylori* infection and this would be a potential therapeutic target for the treatment of *H. pylori* infection.[5]

Investigations revealed that the attachment of *H. pylori* to gastric epithelial cells induces the production of interleukin (IL)-8, which in turn causes the activation and recruitment of neutrophils to the site of infection.[6] IL-8 is not only a very potent endogenous monocyte/neutrophil chemoattractant but also a potent secretagogue and activator of the phagocyte NADPH-oxidase, inducing the release of a large number of proteases and reactive oxygen metabolites, which finally leads to inflammation.[7,8] IL-8 is an important approach to combat inflammation and infectious disease and would be a target to treat *H. pylori*-induced disease.[9,10]

The structure modification at the pendant hydroxy group of metronidazole has received much attention during recent years and many metronidazole derivatives exhibit high anti-*H. pylori* activity.[11] Flavonoids are important compounds which exhibit estrogenic and antimicrobial activities. In our recent research about the functional natural products and structural modifications, we synthesized some new flavonoid derivatives. They exhibited potent inhibition of HPE-induced release of IL-8.[3] As important derivatives of flavonoid, deoxybenzoins are also considered to have good bioactivity in this process. Our interest in this area is to join metronidazole with deoxybenzoins to develop a series of new deoxybenzoin derivatives, intending to obtain compounds that minimize resistance problems and exhibit bioactivity through the inhibition of IL-8 production.

Results and Discussion

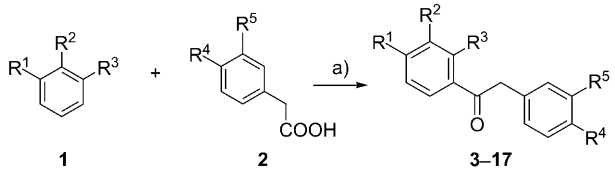
Chemistry

A series of deoxybenzoins were synthesized using phenols and derivatives from phenylacetic acids by the routes outlined in Scheme 1. Equimolar polyhydric phenol and substituents of phenylacetic acid were dissolved in boron trifluoride diethyl etherate, which also played the role of catalyst. The solution was boiled under reflux at 80 °C for 2 h and then precipitated with a saturated solution of sodium acetate. The product was filtered from the solution and recrystallized with methanol/water. Deoxybenzoins **3**–**17** were obtained in high yields.

Compound **19** should be prepared before synthesis of the target compounds **20**–**34**. Metronidazole and 4-toluenesulfonyl chloride were dissolved in CH_2Cl_2 in the presence of triethylamine. The solution was agitated at room temperature for 12 h and then neutralized with sodium hydrogen carbonate. Compound **19** was obtained by means of extraction.

[a] *Dr. Y. Luo,[+] Dr. H.-Q. Li,[+] Dr. Y. Zhou, Z.-L. Li, Dr. T. Yan, Prof. H.-L. Zhu*
State Key Laboratory of Pharmaceutical Biotechnology
Nanjing University, Nanjing 210093 (PR China)
Fax: (+86) 25-8359 2572
E-mail: zhuhl@nju.edu.cn

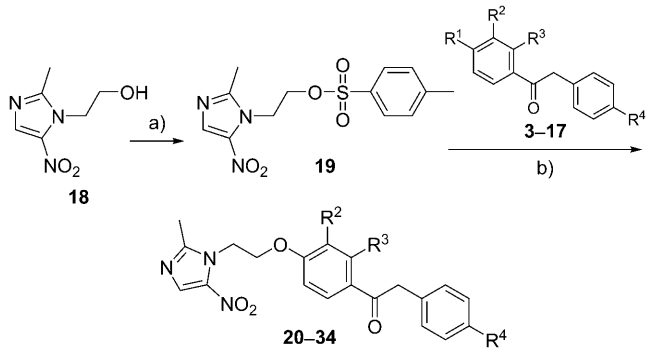
[+] These authors contributed equally to this work.



1a: R^1=R^2=OH, R^3=H **2a**: R^4=Cl, R^5=H **2e**: R^4=H, R^5=Cl
1b: R^1=R^3=OH, R^2=H **2b**: R^4=OH, R^5=H **2f**: R^4=H, R^5=OH
1c: R^1=R^2=R^3=OH **2c**: R^4=NO_2, R^5=H **2g**: R^4=H, R^5=NO_2
2d: R^4=OCH_3, R^5=H **2h**: R^4=H, R^5=OCH_3

Scheme 1. Synthesis of deoxybenzoins **3**–**26**: a) BF_3·OEt_2, reflux, 80 °C, NaOAc (satd), recrystallization, 80 %.

Compound **19** was then dissolved in *N*,*N*-dimethylformamide and reacted with potash at 80 °C for 15 min. Then deoxybenzoins were added into the reaction system, and target compounds **20**–**32** were obtained after 6 h boiling under reflux (Scheme 2 and Table 1). In most isoflavones and deoxybenzoins, the alkylation of the hydroxy groups was very selective; the hydroxy group at the R^1 position was more active.[12] Thus, for compounds **20**, **23**, **26**, **29**, and **32**, only one of two free phenolic hydroxy groups in the starting deoxybenzoin were alkylated in good yields.



Scheme 2. Synthesis of compounds **20**–**34**: a) 4-toluenesulfonyl chloride, Et_3N, CH_2Cl_2, $NaHCO_3$, room temperature; b) K_2CO_3, DMF, reflux, 80 °C.

Table 1. Structures of compounds **20**–**34**.[a]

Compd	R^1	R^2	R^3	R^4	Compd	R^1	R^2	R^3	R^4
20	OH	OH	H	F	**29**	OH	OH	H	OCH_3
21	OH	H	OH	F	**30**	OH	H	OH	OCH_3
22	OH	OH	OH	F	**31**	OH	OH	OH	OCH_3
23	OH	OH	H	Cl	**32**	OH	OH	H	NO_2
24	OH	H	OH	Cl	**33**	OH	H	OH	NO_2
25	OH	OH	OH	Cl	**34**	OH	OH	OH	NO_2
26	OH	OH	H	Br					
27	OH	H	OH	Br					
28	OH	OH	OH	Br					

[a] See Scheme 2 for scaffold structures.

Biological activity

Anti-*H. pylori* activity

The metronidazole–deoxybenzoin derivatives **20**–**34** were evaluated for antimicrobial activity against *H. pylori*. Six *H. pylori* strains, including two standards (ATCC 43504 and SS1) and four clinical strains, were used, and the results are reported in Table 2.

Table 2. Minimum inhibitory concentrations of compounds **20**–**34** against *H. pylori* strains.

Compd	MIC [μM]					
	ATCC 43504	SS1	*H. pylori*			
			Clin. 1	Clin. 2	Clin. 3	Clin. 4
20	125	125	125	>250	125	125
21	125	125	62.5	125	125	125
22	30	30	30	60	30	30
23	120	120	120	120	120	120
24	60	60	60	60	120	120
25	7.24	7.24	7.24	7.24	7.24	14.47
26	109	109	109	109	109	109
27	109	109	109	109	109	109
28	6.55	13.11	6.55	6.55	6.55	6.55
29	>243	>243	121	121	>243	>243
30	>243	121	121	121	>243	121
31	117	117	117	117	117	>234
32	58	58	58	58	117	117
33	117	117	117	117	117	117
34	1.76	1.76	1.76	0.88	1.76	1.76
MTZ[a]	146	146	>585	>585	>585	>585
AMX[b]	0.14	0.14	1.07	1.07	1.07	1.07

[a] Metronidazole. [b] Amoxicillin.

Compounds **22**, **24**, **25**, **28**, **32** and **34** showed lower MIC values against *H. pylori* strains than metronidazole. In particular, they exhibited good biological activity against the four antimicrobial strains which had resistance to metronidazole. Compound **34** had the greatest activity among the metronidazole–deoxybenzoin derivatives and was similar to the positive control amoxicillin.

The comparison of the inhibitory activities of compounds in Table 2 indicate that metronidazole–deoxybenzoin derivatives (**22**, **25**, **28** and **34**) with two hydroxy groups on the center phenyl ring demonstrated better inhibitory activities than those with one hydroxy group (the other compounds in Table 2). The difference between anti-*H. pylori* activity among compounds with the same substituent group R^4 showed hydroxy groups present on the aromatic ring may be responsible for inhibitory activity.

Any compounds with a methoxy group (**29**–**31**) showed weak activity. It is likely that the electrophilic activity of the substituent group R^4 also plays a role in the inhibitory activity of the compounds. Additionally, the intermediate deoxybenzoins were also evaluated for anti-*H. pylori* activity. The deoxybenzoins generally exhibited weaker activity than homologous metronidazole–deoxybenzoin derivatives, indicating that the conjunction of metronidazole increased the inhibitory activity.

Moreover, selectivity of compounds **20**–**34** for *E. coli*, *P. fluorescence*, *B. subtilis*, and *S. aureus* was also evaluated. All the compounds **20**–**34** shown low antibacterial activity and displayed potent selectivity against *H. pylori* (Table 3).

Table 3. Antimicrobial activity of synthetic compounds **20**–**34**.

Compd	MIC [μM]			
	Gram-negative		Gram-positive	
	E. coli	*P. fluorescence*	*B. subtilis*	*S. aureus*
20	125	125	125	125
21	125	>250	>250	>250
22	120	120	>240	>240
23	>250	>250	>250	>250
24	120	120	>240	>240
25	125	125	125	125
26	62.5	62.5	125	>250
27	62.5	125	125	>250
28	125	125	125	125
29	121.5	121.5	121.5	121.5
30	62.5	62.5	125	125
31	31.2	62.6	125	125
32	50	50	125	125
33	125	125	>250	>250
34	120	120	120	>240
KAN[a]	3.13	3.13	1.56	1.56

[a] Kanamycin.

IL-8 assessment

Compound **34** with the strongest anti-*H. pylori* activity was selected to act against the IL-8 production induced by HPE in gastric mucosal cells. The results are shown in Figure 1.

HPE alone stimulated gastric mucosal cells to produce IL-8 as much as 1010.5 pg mL^{-1}. Various dose-dependent attenuations of HPE-induced IL-8 production were observed with the addition of metronidazole, compound **34**, and compound **17**. Compound **34** significantly decreased IL-8 levels in a dose-dependent manner at concentrations of 15, 30, and 60 μM ($P<0.001$), and the lowest IL-8 production was observed with compound **34** at 60 μM.

The reason to test metronidazole and compound **17** was that compound **34** possesses the structure of the two chemicals. Comparatively, compound **34** showed a more potent inhibitory activity against HPE-induced IL-8 production than both metronidazole at all concentrations ($P<0.001$) and compound **17** at concentrations of 30 μM ($P<0.05$) and 60 μM ($P<0.01$).

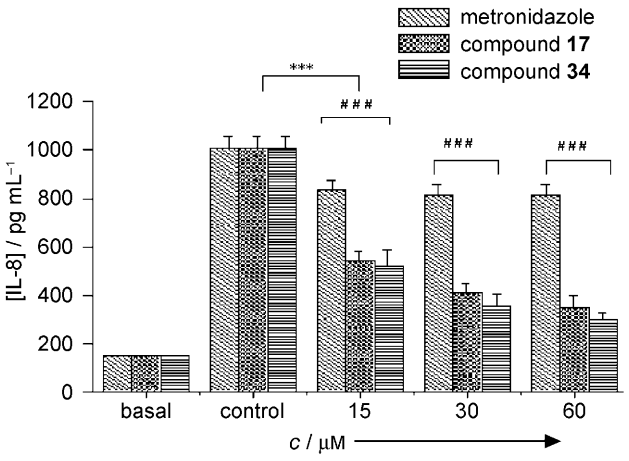


Figure 1. Inhibitory activities of compounds **17** and **34** on IL-8 production induced by *H. pylori* water extract (HPE). Human gastric mucosal cancer cells were pre-incubated with or without metronidazole, compound **17**, or compound **34** in serial concentrations of 15, 30, and 60 μM for 1 h and then stimulated by 10% HPE (*v*/*v*) for 12 h. The levels of IL-8 in the culture supernatant were determined by ELISA. Basal: cells without incubation of HPE; control: cells incubated with HPE but without any agents. $^{*}P<0.05$; $^{\#}P<0.01$.

Cell viability evaluation

The results shown in Figure 2 demonstrate that metronidazole, compound **17**, and compound **34** did not affect cell viability at the concentrations tested (15, 30, and 60 μM).

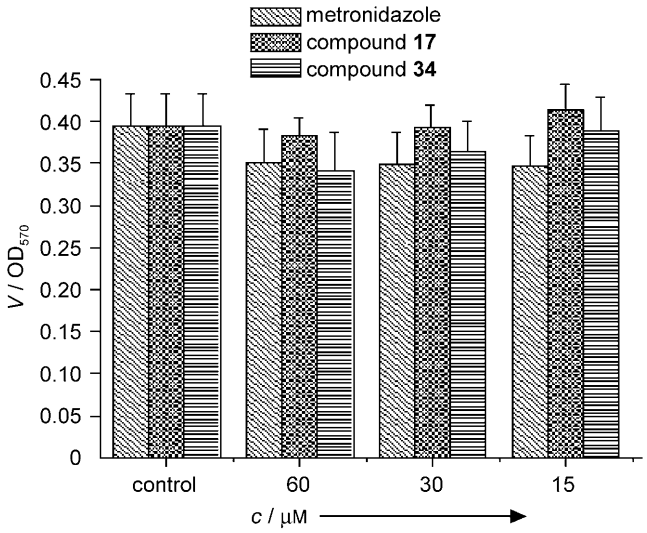


Figure 2. Effects of various agents on cell viability. Human gastric mucosal cells were incubated with metronidazole, compound **17**, or compound **34** at serial concentrations of 15, 30, and 60 μM. The MTT assay was performed 48 h later. Results are the mean ± SEM of 4–6 experiments. *V*: cell viability; comparison of all the agents at 15, 30, and 60 μM versus control.

Acute oral toxicity test

The tested animals appeared normal immediately after administration and did not exhibit any indication of acute toxicity for 14 days afterward. The body weights in treated mice were similar to that of the control mice dosed with an equal volume of vehicle. The result indicated that compound **34** was nontoxic.

Discussion

In recent years, *H. pylori* strains susceptible to amoxicillin, clarithromycin, or metronidazole are generally easily eradicated, whereas those resistant to clarithromycin or metronidazole are much more difficult to clear. Consequently, these findings are indeed major drivers for developing novel anti-*H. pylori* agents.

In this work, compounds **22**, **24**, **25**, **28**, **32** and **34** showed low MIC values against *H. pylori* strains, especially the strains resistant to metronidazole.

H. pylori infection leads to different clinical and pathological outcomes in humans, including chronic gastritis, peptic ulcer disease, and gastric neoplasia. Until now, complete eradiation of *H. pylori* is the most effective therapy, which stimulates research to find more new anti-*H. pylori* natural or synthetic agents. However, recently more attention has been paid to the pathological reaction to the infection of *H. pylori* in host cells, which leads to inflammation and then many gastroduodenal diseases. Some anti-*H. pylori* agents such as ecabet sodium[13] and rebamipide[14] have been investigated and subsequently proved to have the ability to suppress the inflammation relating to *H. pylori* infection and this would be a potential therapeutic target for the treatment of *H. pylori* infection.

It is known that the key pathophysiological event in *H. pylori* infection is initiation and continuance of an inflammatory response. Following adherence to the host cell, *H. pylori* triggers this inflammatory process, in which many cytokines, such as TNF-α, IL-1, and IL-8, are implicated. In particular, IL-8 shows a potent chemotactic activity for neutrophils and accordingly plays an important role in this process. IL-8 induction in *H. pylori*-infected gastric epithelial cells is universal and specific, and may be an important factor which is responsible for the neutrophil activation and infiltration, and consequently, for the inflammation-associated mucosal injury and carcinogenesis.

In the current study, in addition to the common anti-*H. pylori* screening of a series of synthesized compounds, we also emphasized pharmacological evaluation of a novel compound concerning its effect on the pathological reaction to the infection of *H. pylori* in gastric mucosal cells, which showed a promising and exciting result. Compound **34** with the strongest anti-*H. pylori* activity was selected to investigate its effect on IL-8 production induced by *H. pylori* in gastric mucosal cells. Compound **17** as the precursor was also added to the test in order to contrast with compound **34**. As reported earlier, several methods can be used to induce the expression of IL-8 in gastric epithelial cells, including inflammatory cytokines (for example, TNF-α and IL-1), live *H. pylori*,[15] the extract or surface proteins of this bacterium,[16] and other inflammatory proteins.[17] In this experiment, to rule out the influence of compound **34** on the live bacteria and investigate its effect on the intracellular cascade of the IL-8 production, HPE rather than the live bacteria was employed as the stimulating factor throughout the experiment.

As shown in Figure 1, the amount of IL-8 significantly increased in the supernatant of gastric mucosal cells culture medium after addition of HPE, an extract of *H. pylori* containing the main surface protein of this bacterium. This result was consistent with some other reports,[18] suggesting the proteins existing in the surface of *H. pylori* could activate the intracellular signal pathway and stimulate the inflammatory activity in host cells.

A significant and dose-dependent decrease of HPE-induced IL-8 production in gastric mucosal cell culture supernatant can be observed when the cells are pre-incubated with compound **34** followed by stimulation of HPE. At the same time, compound **34** at the same concentration exhibited little effect on gastric mucosal cell viability, as shown in Figure 2, which can exclude the disturbance of its cytotoxic activity to gastric mucosal cells. It implied that compound **34** has the ability to decrease the HPE-induced IL-8 production by a direct influence on the gastric cells.

Considering that compound **34** possesses the structure of metronidazole and compound **17**, the two chemicals were tested in this model to assess their effects on the intracellular cascade of the IL-8 production in gastric mucosal cells. The results demonstrated that the group with the addition of metronidazole showed little inhibitory activity of HPE-induced IL-8 production compared with the same concentration of compound **34**, indicating that metronidazole has no effect on the gastric mucosal cells infected with *H. pylori*, except its direct antibacterial activity. On the other hand, compound **17** could significantly decrease the IL-8 production in gastric mucosal cells stimulated by HPE which suggests that deoxybenzoin can directly influence the intracellular cascade of IL-8 production.

Notably, genistein can specifically inhibit tyrosine kinase activity, which in turn enables it to be a useful tool to elucidate the role of tyrosine phosphorylation in cells.[17] To the best our knowledge, tyrosine phosphorylation plays a crucial role in the process of activation of NF-κB, which is the key signal pathway relating to the production of IL-8 in *H. pylori*-infected gastric mucosal cells. The adherence and colonization of *H. pylori* in the host cells activates NF-κB as a consequence of phosphorylation by tyrosine kinase, and induces nuclear translocation of NF-κB, which is followed by increased IL-8 mRNA and protein levels.[19,20] Deoxybenzoin, as the derivative of genistein, can inhibit IL-8 production in gastric mucosal cells stimulated by HPE. It can be concluded that compound **34** inhibits the IL-8 level through inhibiting tyrosine kinase activity.

Therefore, the inhibition of tyrosine kinase can down-regulate IL-8 expression in transcriptional and translational levels in the gastric cells stimulated by the bacterium and subsequently attenuates the inflammatory response. In our experiment, deoxybenzoin significantly decreased HPE-induced IL-8 production in gastric mucosal cells. It can be suggested that deoxybenzoin attenuate HPE-induced IL-8 expression probably through inhibiting tyrosine phosphorylation and subsequently down-regulating the nuclear translocation of NF-κB.

Compound **34**, structurally characterized by metronidazole and deoxybenzoin, has the biological activities of the two chemicals, which was never observed in a simple mixture of metronidazole and deoxybenzoin as shown in our experiment. Its potent anti-*H. pylori* activity is much stronger than metronidazole and may be due to the structural modification of metronidazole.

On the other hand, the structure of deoxybenzoin in compound **34** makes it possible to deduce that the significant decrease in IL-8 production might be attributed to the inhibition of tyrosine kinase, similar to the mode of action of deoxybenzoin. Consequently, compound **34** is likely to be a potent therapy for *H. pylori*-infection because of its two independent actions, the efficient anti-*H. pylori* activity and the potent inhibi-

tion of IL-8 production. The latter might be achieved via tyrosine kinase inhibition, which should be investigated for further study.

Conclusions

In conclusion, a series of metronidazole–deoxybenzoin derivatives were synthesized for the first time and evaluated for antimicrobial activity against *H. pylori*. Among these, compounds **22**, **24**, **25**, **28**, **32** and **34** exhibited potent in vitro activities, especially against the four clinically resistant strains of *H. pylori*. Compound **34** showed much stronger activity than metronidazole and was similar to the positive control amoxicillin. Furthermore, compound **34** significantly decreased HPE-induced IL-8 production in gastric mucosal cells. Based on this study, we concluded that compound **34** would be a promising agent for the treatment of *H pylori*-related diseases.

Experimental Section

Chemistry

All NMR spectra were recorded on a Bruker DRX 500 or DPX 300 model spectrometer in [D_6]DMSO. Chemical shifts (δ) for ^{1}H NMR spectra are reported in ppm to residual solvent protons. Melting points were measured on a Boetius micro melting point apparatus. The ESIMS spectra were recorded on a Mariner System 5304 mass spectrometer. All chemicals and reagents used were of analytical grade. Phenols and *para*-substituted phenylacetic acids were purchased from Nanjing Huakang Co., Nanjing (China), and metronidazole was purchased from Changzhou Dongsheng Co., Changzhou (China). TLC was run on silica gel coated aluminum sheets (silica gel 60 GF_{254}, E. Merck, Germany) and visualized under UV light (λ 254 nm).

General method for compound synthesis

General procedure for the preparation of deoxybenzoins **3**–**17**:[21] A phenol (0.050 mol) and an arylacetic acid (0.05 mol) were dissolved into freshly distilled $BF_3{\cdot}OEt_2$ (10 mL) under Ar. The mixture was stirred at 80 °C and then poured into an ice bath. The resulting mixture was extracted with $EtOAc_{(aq)}$ (100 mL), and the organic layer was washed with $NaHCO_{3(aq)}$, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography on silica gel, using a mixture of CH_2Cl_2 and CH_3OH.

General procedure for the preparation of compound **19**: Metronidazole (0.05 mol) and 4-toluenesulfonyl chloride (0.05 mol) were dissolved in CH_2Cl_2 (10 mL) in the presence of Et_3N. The solution was agitated at room temperature for 12 h and then neutralized with $NaHCO_3$. Compound **19** was obtained by means of extraction.

General procedure for the preparation of compounds **20**–**34**: Compound **19** (0.05 mol) was dissolved in DMF (10 mL) and reacted with potash at 80 °C for 15 min. Then the deoxybenzoin (0.05 mol) was added to the reaction system, and the target compounds were formed after 6 h boiling at reflux as shown in Scheme 2.

2-(4-fluorophenyl)-1-(3-hydroxy-4-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)phenyl)ethanone (20): Yield: 76%; R_f=0.26 (MeOH/CH_3Cl 1:4); mp: 144–145 °C; ^{1}H NMR (300 MHz, [D_6]DMSO): δ=11.41 (s, 1H), 8.05 (s, 1H), 7.99 (d, J=6.1 Hz 2H), 7.81 (d, J=5.8 Hz, 1H), 7.64 (d, J=7.3 Hz, 1H), 7.37 (d, J=6.6 Hz, 2H), 6.71 (d, J=7.3 Hz, 1H), 4.57 (s, 2H), 4.31 (s, 2H), 3.82 (s, 2H), 2.51 (s, 3H); ^{13}C NMR (500 MHz, [D_6]DMSO): δ=13.7, 41.5, 43.6, 65.8, 102.5, 108.4, 112.7, 115.4, 131.9, 132.4, 133.8, 138.2, 153.4, 160.6, 162.5, 165.5, 198.2 ppm; MS (ESI) $C_{20}H_{18}FN_3O_5$ $[M+H]^+$ 400.1; Anal. calcd for $C_{20}H_{18}FN_3O_5$: C 60.15, H 4.54, F 4.76, N 10.52%, found: C 60.03, H 4.38, F 5.64, N 10.58%.

2-(4-fluorophenyl)-1-(2-hydroxy-4-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)phenyl)ethanone (21): Yield: 73%; R_f=0.21 (MeOH/CH_3Cl 1:4); mp: 148–149 °C; ^{1}H NMR (300 MHz, [D_6]DMSO): δ=10.78 (s, 1H), 8.12 (s, 1H), 8.01 (d, J=6.3 Hz 2H), 7.85 (d, J=6.2 Hz, 1H), 7.56 (d, J=7.1 Hz, 1H), 7.32 (d, J=6.1 Hz, 2H), 6.65 (d, J=7.7 Hz, 1H), 4.58 (s, 2H), 4.32 (s, 2H), 3.78 (s, 2H), 2.53 (s, 3H); ^{13}C NMR (500 MHz, [D_6]DMSO): δ=13.7, 41.5, 43.9, 65.4, 102.2, 107.3, 112.5, 115.4, 131.5, 132.1, 132.8, 138.2, 152.4, 165.2, 167.1, 167.7, 202.2 ppm; MS (ESI) $C_{20}H_{18}FN_3O_5$ $[M+H]^+$ 400.1; Anal. calcd for $C_{20}H_{18}FN_3O_5$: C 60.15, H 4.54, F 4.76, N 10.52%, found: C 60.07, H 4.33, F 5.60, N 10.52%.

1-(2,3-dihydroxy-4-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)-phenyl)-2-(4-fluorophenyl)ethanone (22): Yield: 81%; R_f=0.35 (MeOH/CH_3Cl 1:4); mp: 154–155 °C; ^{1}H NMR (300 MHz, [D_6]DMSO): δ=11.44 (s, 1H), 11.03 (s, 1H), 8.15 (s, 1H), 8.01 (d, J=6.4 Hz 2H), 7.79 (d, J=6.8 Hz, 1H), 7.40 (d, J=6.1 Hz, 2H), 6.65 (d, J=8.3 Hz, 1H), 4.44 (s, 2H), 4.30 (s, 2H), 3.81 (s, 2H), 2.55 (s, 3H); ^{13}C NMR (500 MHz, [D_6]DMSO): δ=13.6, 41.5, 43.8, 66.2, 101.6, 111.5, 115.4, 128.9, 131.9, 132.4, 138.2, 146.7, 153.1, 160.4, 161.8, 165.2, 202.5 ppm; MS (ESI) $C_{20}H_{18}FN_3O_6$ $[M+H]^+$ 416.1; Anal. calcd for $C_{20}H_{18}FN_3O_6$: C 57.83, H 4.37, F 4.57, N 10.12%, found: C 57.91, H 4.32, F 4.55, N 10.09%.

2-(4-chlorophenyl)-1-(3-hydroxy-4-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)phenyl)ethanone (23): Yield: 75%; R_f=0.28 (MeOH/CH_3Cl 1:4); mp: 133–134 °C; ^{1}H NMR ([D6]DMSO): δ=11.08 (s, 1H), 8.02 (s, 1H), 7.91 (d, J=5.7 Hz 2H), 7.81 (d, J=6.5 Hz, 1H), 7.66 (d, J=7.0 Hz, 1H), 7.35 (d, J=6.5 Hz, 2H), 6.85 (d, J=8.7 Hz, 1H), 4.48 (s, 2H), 4.31 (s, 2H), 3.82 (s, 2H), 2.43 (s, 3H); ^{13}C NMR (500 MHz, [D_6]DMSO): δ=13.7, 41.5, 43.6, 65.8, 102.5, 108.4, 112.7, 128.6, 128.8, 131.9, 132.4, 133.8, 134.5, 138.2, 153.4, 160.6, 162.5, 198.8 ppm; MS (ESI) $C_{20}H_{18}ClN_3O_5$ $[M+H]^+$ 416.1; Anal. calcd for $C_{20}H_{18}ClN_3O_5$: C 57.77, H 4.36, Cl 8.53, N 10.11%, found: C 57.68, H 4.33, Cl 8.50, N 10.01%.

2-(4-chlorophenyl)-1-(2-hydroxy-4-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)phenyl)ethanone (24): Yield: 68%; R_f=0.19 (MeOH/CH_3Cl 1:4); mp: 138–139 °C; ^{1}H NMR ([D6]DMSO): δ=10.88 (s, 1H), 8.04 (s, 1H), 7.98 (d, J=6.4 Hz 2H), 7.71 (d, J=6.4 Hz, 1H), 7.63 (d, J=7.4 Hz, 1H), 7.25 (d, J=7.2 Hz, 2H), 6.79 (d, J=9.1 Hz, 1H), 4.58 (s, 2H), 4.27 (s, 2H), 4.02 (s, 2H), 2.54 (s, 3H); ^{13}C NMR (500 MHz, [D_6]DMSO): δ=13.7, 41.5, 43.9, 65.4, 102.2, 107.3, 112.5, 128.6, 128.8, 131.5, 132.1, 133.5, 134.5, 138.2, 152.4, 165.2, 167.1, 202.2 ppm; MS (ESI) $C_{20}H_{18}ClN_3O_5$ $[M+H]^+$ 416.1; Anal. calcd for $C_{20}H_{18}ClN_3O_5$: C 57.77, H 4.36, Cl 8.53, N 10.11%, found: C 57.85, H 4.32, Cl 8.48, N 10.06%.

2-(4-chlorophenyl)-1-(2,3-dihydroxy-4-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)phenyl)ethanone (25): Yield: 77%; R_f=0.37 (MeOH/CH_3Cl 1:4); mp: 151–152 °C; ^{1}H NMR ([D6]DMSO): δ=11.37 (s, 1H), 11.13 (s, 1H), 8.06 (s, 1H), 8.01 (d, J=6.6 Hz 2H), 7.69 (d, J=6.9 Hz, 1H), 7.36 (d, J=6.1 Hz, 2H), 6.77 (d, J=8.8 Hz, 1H), 4.53 (s, 2H), 4.30 (s, 2H), 4.01 (s, 2H), 2.57 (s, 3H); ^{13}C NMR (500 MHz, [D_6]DMSO): δ=13.6, 41.5, 43.8, 66.2, 101.6, 111.5, 128.6, 128.9, 131.1, 131.9, 132.4, 134.5, 138.2, 146.7, 153.1, 160.4, 161.8, 202.3 ppm; MS (ESI) $C_{20}H_{18}ClN_3O_6$ $[M+H]^+$ 431.1; Anal. calcd for $C_{20}H_{18}ClN_3O_6$: C 55.63, H 4.20, Cl 8.21, N 9.73%, found: C 55.53, H 4.15, Cl 8.15, N 9.66%.

2-(4-bromophenyl)-1-(3-hydroxy-4-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)phenyl)ethanone (26): Yield: 65%; R_f=0.25 (MeOH/CH_3Cl 1:4); mp: 174–175 °C; ^{1}H NMR ([D6]DMSO): δ=10.96 (s, 1H), 8.03 (s, 1H), 7.86 (d, J=6.2 Hz 2H), 7.78 (d, J=6.1 Hz, 1H), 7.66 (d, J=6.8 Hz, 1H), 7.32 (d, J=6.2 Hz, 2H), 6.83 (d, J=9.2 Hz, 1H), 4.51 (s, 2H), 4.30 (s, 2H), 3.92 (s, 2H), 2.53 (s, 3H); ^{13}C NMR (500 MHz, [D_6]DMSO): δ=13.7, 41.5, 43.6, 65.8, 103.1, 108.8, 113.4, 120.2, 130.3, 131.5, 132.1, 133.8, 134.8, 138.6, 153.4, 160.6, 162.5, 199.3 ppm; MS (ESI) $C_{20}H_{18}BrN_3O_5$ $[M+H]^+$ 460.0; Anal. calcd for $C_{20}H_{18}BrN_3O_5$: C 52.19, H 3.94, Br 17.36, N 9.13%, found: C 52.12, H 3.91, Br 17.32, N 9.14%.

2-(4-bromophenyl)-1-(2-hydroxy-4-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)phenyl)ethanone (27): Yield: 75%; R_f=0.22 (MeOH/CH_3Cl 1:4); mp: 167–168 °C; ^{1}H NMR ([D6]DMSO): δ=11.26 (s, 1H), 8.01 (s, 1H), 7.96 (d, J=5.7 Hz 2H), 7.75 (d, J=7.1 Hz, 1H), 7.53 (d, J=6.8 Hz, 1H), 7.30 (d, J=6.6 Hz, 2H), 6.79 (d, J=8.2 Hz, 1H), 4.61 (s, 2H), 4.29 (s, 2H), 3.94 (s, 2H), 2.51 (s, 3H); ^{13}C NMR (500 MHz, [D_6]DMSO): δ=13.6, 41.5, 43.9, 65.4, 103.1, 108.6, 112.8, 120.3, 130.1, 131.2, 132.3, 133.5, 134.8, 138.2, 152.4, 165.2, 167.1, 202.2 ppm; MS (ESI) $C_{20}H_{18}BrN_3O_5$ $[M+H]^+$ 460.0; Anal. calcd for $C_{20}H_{18}BrN_3O_5$: C 52.19, H 3.94, Br 17.36, N 9.13%, found: C 52.15, H 3.90, Br 17.31, N 9.08%.

2-(4-bromophenyl)-1-(2,3-dihydroxy-4-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)phenyl)ethanone (28): Yield: 79%; R_f=0.41 (MeOH/CH_3Cl 1:4); mp: 171–172 °C; ^{1}H NMR ([D6]DMSO): δ=11.18 (s, 1H), 11.02 (s, 1H), 8.11 (s, 1H), 8.03 (d, J=7.1 Hz, 2H), 7.76 (d, J=7.1 Hz, 1H), 7.33 (d, J=6.4 Hz, 2H), 6.78 (d, J=8.1 Hz, 1H), 4.48 (s, 2H), 4.29 (s, 2H), 3.92 (s, 2H), 2.46 (s, 3H); ^{13}C NMR (500 MHz, [D_6]DMSO): δ=13.6, 41.5, 43.8, 66.2, 102.8, 113.1, 120.2, 130.3, 131.1, 132.2, 132.6, 134.8, 138.2, 146.7, 153.1, 160.4, 161.7, 202.1 ppm; MS (ESI) $C_{20}H_{18}BrN_3O_6$ $[M+H]^+$ 476.0; Anal. calcd for $C_{20}H_{18}BrN_3O_6$: C 50.44, H 3.81, Br 16.78, N 8.82%, found: C 50.38, H 3.79, Br 16.77, N 8.79%.

1-(3-hydroxy-4-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)phenyl)-2-(4-methoxyphenyl)ethanone (29): Yield: 81%; R_f=0.16 (MeOH/CH_3Cl 1:4); mp: 132–133 °C; ^{1}H NMR ([D6]DMSO): δ=10.26 (s, 1H), 8.07 (s, 1H), 7.96 (d, J=6.3 Hz, 2H), 7.82 (d, J=7.1 Hz, 1H), 7.63 (d, J=6.9 Hz, 1H), 6.91 (d, J=6.6 Hz, 2H), 6.78 (d, J=8.2 Hz, 1H), 4.53 (s, 2H), 4.31 (s, 2H), 3.87 (s, 2H), 3.31 (s,3H), 2.53 (s, 3H); ^{13}C NMR (500 MHz, [D_6]DMSO): δ=13.7, 41.5, 43.6, 55.6, 65.8, 103.1, 108.8, 113.4, 117.4, 129.5, 131.7, 132.3, 133.6, 138.6, 153.4, 159.5, 160.8, 162.7, 199.8 ppm; MS (ESI) $C_{21}H_{21}N_3O_6$ $[M+H]^+$ 412.1; Anal. calcd for $C_{21}H_{21}N_3O_6$: C 61.31, H 5.14, N 10.21%, found: C 61.23, H 5.08, N 10.17%.

1-(2-hydroxy-4-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)phenyl)-2-(4-methoxyphenyl)ethanone (30): Yield: 77%; R_f=0.29 (MeOH/CH_3Cl 1:4); mp: 149–150 °C; ^{1}H NMR ([D6]DMSO): δ=10.64 (s, 1H), 8.01 (s, 1H), 7.86 (d, J=6.1 Hz 2H), 7.72 (d, J=6.7 Hz, 1H), 7.56 (d, J=7.3 Hz, 1H), 6.82 (d, J=6.5 Hz, 2H), 6.74 (d, J=8.4 Hz, 1H), 4.45 (s, 2H), 4.27 (s, 2H), 3.75 (s, 2H), 3.26 (s,3H), 2.51 (s, 3H); ^{13}C NMR (500 MHz, [D_6]DMSO): δ=13.6, 41.5, 43.9, 55.6, 65.4, 103.1, 108.6, 112.8, 117.3, 129.8, 131.1, 132.3, 133.5, 138.2, 152.4, 159.7, 165.4, 167.2, 202.2 ppm; MS (ESI) $C_{21}H_{21}N_3O_6$ $[M+H]^+$ 412.1; Anal. calcd for $C_{21}H_{21}N_3O_6$: C 61.31, H 5.14, N 10.21%, found: C 61.29, H 5.12, N 10.22%.

1-(2,3-dihydroxy-4-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)phenyl)-2-(4-methoxyphenyl)ethanone (31): Yield: 72%; R_f=0.21 (MeOH/CH_3Cl 1:4); mp: 141–142 °C; ^{1}H NMR ([D6]DMSO): δ=11.24 (s, 1H), 10.93 (s, 1H), 8.07 (s, 1H), 7.95 (d, J=6.5 Hz, 2H), 7.79 (d, J=6.6 Hz, 1H), 7.63 (d, J=7.1 Hz, 1H), 6.74 (d, J=8.2 Hz, 1H), 4.52 (s, 2H), 4.33 (s, 2H), 4.06 (s, 2H), 3.34 (s,3H), 2.43 (s, 3H); ^{13}C NMR (500 MHz, [D_6]DMSO): δ=13.6, 41.5, 43.8, 55.5, 66.2, 102.8, 113.1, 117.6, 130.1, 131.3, 132.6, 133.7, 138.2, 146.7, 153.1, 159.5, 160.4, 161.6, 201.9 ppm; MS (ESI) $C_{21}H_{21}N_3O_7$ $[M+H]^+$ 428.1; Anal. calcd for $C_{21}H_{21}N_3O_7$: C 59.01, H 4.95, N 9.83%, found: C 58.92, H 4.91, N 9.85%.

1-(3-hydroxy-4-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)phenyl)-2-(4-nitrophenyl)ethanone (32): Yield: 67%; R_f=0.20 (MeOH/CH_3Cl 1:4); mp: 164–165 °C; ^{1}H NMR ([D6]DMSO): δ=10.83 (s, 1H), 8.07 (s, 1H), 7.93 (d, J=5.7 Hz, 2H), 7.78 (d, J=6.8 Hz, 1H), 7.61 (d, J=6.8 Hz, 1H), 7.42 (d, J=6.0 Hz, 2H), 6.85 (d, J=8.7 Hz, 1H), 4.56 (s, 2H), 4.32 (s, 2H), 4.08 (s, 2H), 2.47 (s, 3H); ^{13}C NMR (500 MHz, [D_6]DMSO): δ=13.6, 41.5, 43.6, 65.8, 103.1, 108.6, 113.3, 125.2, 130.3, 132.1, 133.8, 138.6, 139.5, 146.8, 153.4, 160.4, 162.4, 199.6 ppm; MS (ESI) $C_{20}H_{18}N_4O_7$ $[M+H]^+$ 427.1; Anal. calcd for $C_{20}H_{18}N_4O_7$: C 56.34, H 4.26, N 13.14%, found: C 56.26, H 4.22, N 13.11%.

1-(2-hydroxy-4-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)phenyl)-2-(4-nitrophenyl)ethanone (33): Yield: 72%; R_f=0.16 (MeOH/CH_3Cl 1:4); mp: 171–172 °C; ^{1}H NMR ([D6]DMSO): δ=10.96 (s, 1H), 8.11 (s, 1H), 8.02 (d, J=6.7 Hz, 2H), 7.73 (d, J=6.2 Hz, 1H), 7.64 (d, J=6.8 Hz, 1H), 7.39 (d, J=6.3 Hz, 2H), 6.77 (d, J=8.3 Hz, 1H), 4.48 (s, 2H), 4.30 (s, 2H), 3.88 (s, 2H), 2.53 (s, 3H); ^{13}C NMR (500 MHz, [D_6]DMSO): δ=13.7, 41.5, 43.9, 65.4, 103.1, 108.4, 112.5, 125.3, 130.1, 132.3, 133.5, 138.2, 140.4, 146.8, 152.4, 165.2, 167.1, 202.1 ppm; MS (ESI) $C_{20}H_{18}N_4O_7$ $[M+H]^+$ 427.1; Anal. calcd for $C_{20}H_{18}N_4O_7$: C 56.34, H 4.26, N 13.14%, found: C 56.31, H 4.23, N 13.09%.

1-(2,3-dihydroxy-4-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)phenyl)-2-(4-nitrophenyl)ethanone (34): Yield: 68%; R_f=0.13 (MeOH/CH_3Cl 1:4); mp: 156–157 °C; ^{1}H NMR ([D6]DMSO): δ=11.06 (s, 1H), 10.95 (s, 1H), 8.08 (s, 1H), 7.79 (d, J=5.9 Hz, 2H), 7.65 (d, J=6.8 Hz, 1H), 7.42 (d, J=6.2 Hz, 2H), 6.81 (d, J=8.9 Hz, 1H), 4.50 (s, 2H), 4.31 (s, 2H), 3.93 (s, 2H), 2.48 (s, 3H); ^{13}C NMR (500 MHz, [D_6]DMSO): δ=13.6, 41.5, 43.8, 66.2, 102.8, 113.1, 125.2, 130.5, 132.2, 132.6, 134.1, 138.2, 145.5, 146.7, 153.1, 160.6, 161.3, 202.1 ppm; MS (ESI) $C_{20}H_{18}N_4O_8$ $[M+H]^+$ 443.1; Anal. calcd for $C_{20}H_{18}N_4O_8$: C 54.30, H 4.10, N 12.66%, found: C 54.33, H 4.15, N 12.68%.

Cell culture

Human gastric mucosal cancer cells were grown in Ham's F12 medium containing 10% fetal bovine serum, L-glutamine (2 mM), penicillin G (100 U mL^{-1}), and streptomycin (100 μg mL^{-1}). Cell cultures were maintained at 37 °C in a humidified atmosphere of 95% air and 5% CO_2.

H. pylori culture

The *H. pylori* strains used in this study were two standard strains [ATCC 43504 and *H. pylori* mouse-adapted strain Sydney strain 1 (SS1)], and four clinical isolates of *H. pylori* (1–4), which were obtained from antral biopsies of child and adult patients hospitalized at Jiangsu People's Hospital in Nanjing. All strains were cultured on Columbia Agar (BioMerieux, France) supplemented with 7% sheep blood and cultured for 3 days at 37 °C under microaerophilic conditions with high humidity as detailed elsewhere.[22]

Antimicrobial activity

Antimicrobial activity against *H. pylori* was determined by the agar dilution method recommended by the National Committee for Clinical Laboratory (NCCL)[22] standards. In vitro test strains (ATCC 43504, SS1, and the clinical strains 1–4) were cultured for 3 days, then the anti-*H. pylori* agents were tested by twofold serial dilutions of the compounds ranging from 100–0.05 $\mu g\,mL^{-1}$ with an initial cell count of $\sim 10^6$ CFU mL^{-1}. The bacterial suspensions (200 μL) were inoculated on each Columbia infusion blood agar plate containing serial twofold dilutions of all compounds with the concentration of DMSO $<1\%$ (*v/v*). After incubation for 3 days, minimum inhibitory concentration (MIC) values were determined as the lowest concentrations of the agents that visibly inhibited bacterial growth inhibition. The antibacterial activity of the synthesized compounds was tested against *E. coli*, *P. fluorescence*, *B. subtilis*, and *S. aureus* using Müller–Hinton (MH) medium: casein hydrolysate (17.5 g), soluble starch (1.5 g), beef extract (1000 mL), The MIC values of the test compounds were determined by a colorimetric method using the dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT).[21]

Preparation of H. pylori water extract (HPE)

H. pylori 43504 were harvested from agar plants and then suspended in distilled water at a concentration of 2.5×10^8 CFU mL^{-1}. After vortex mixing for 1 min, the suspension was incubated at room temperature for 40 min and then centrifuged at 20000 *g* for 20 min. Finally the supernatant was filtered through a 0.2 μm filter and stored at $-20\,^{\circ}C$ until use.[16]

IL-8 assessment

Briefly, human gastric mucosal cancer cell line, grown for 2 days on 24-well plates to ~80% confluence in Ham's F12 medium, were washed three times with serum-free Ham's F12 medium, and then pre-incubated with metronidazole, compound **17**, and compound **34** in serial concentrations at 15, 30, and 60 μM with the concentration of DMSO $<1\%$ for 1 h. After that cells were incubated with 10% HPE (*v/v*) for 12 h. The supernatants were aspirated, centrifuged at 500 *g* for 10 min, and then stored at $-80\,^{\circ}C$. The levels of IL-8 in the culture supernatants were determined by a commercially available enzyme-linked immunosorbent assay (ELISA) kit (RapidBio, USA) according to the instructions of the manufacturer.[20]

Acute oral toxicity of compound 34

Acute oral toxicity studies were performed according to the method of Vasudevan et al.[23] Briefly, mice of either sex, selected by a random sampling technique, were employed in this study. Mice ($n=3$) in each group were fasted for 4 h with free access to water only. Compound **6**, suspended with carboxymethyl cellulose, (CMC, 0.5% *w/v*) was administered orally at doses of 50, 250, or 1000 mg kg^{-1}. Appearance and mortality of test animals were observed for 14 days.

Statistical analysis

One-way analysis of variance (ANOVA) was performed to compare differences between groups. Two-tailed probability (*P*) values were derived, and a *P* value of <0.05 was considered statistically significant.

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