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Metronidazole–Flavonoid Derivatives as Anti-*Helicobacter pylori* Agents with Potent Inhibitory Activity against HPE-Induced Interleukin-8 Production by AGS Cells

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Three series of metronidazole–flavonoid derivatives were generated and evaluated for antimicrobial activity against H. pylori. *Among these compounds, high anti-*H. pylori *activities were observed in isoflavones derivatives* ***4****–****7****,* ***19****, and* ***20*** *but exhibited no inhibitory activity against other sorts of bacteria and fungi, for example,* Streptococcus pneumoniae, Bacillus subtilis, Escherichia coli, Pseudomonas fluorescence*, and* Aspergillus niger. *Genistein derivative* ***6*** *with the potent activity (MIC = 0.39 μg mL^{-1}) was > 50-fold more than metronidazole, and comparable to the positive control amoxicillin. Additionally, compound* ***6*** *can significantly attenuate the increase in interleukin-8 (IL-8) levels in the AGS cells stimulated by* H. pylori *water extract (HPE) at concentrations of 15, 30, and 60 μmol L^{-1}, which did not show any effects on the cell viability.*

Introduction

Helicobacter pylori is a gram-negative microaerophilic bacterium that infects up to 50% of the world's human population.[1] Investigations revealed that the bacterium could cause many gastroduodenal diseases such as gastritis, gastric and duodenal ulcers, and even gastric cancer,[2] as well as some other various extraintestinal pathologies.[3] In the clinical setting, *H. pylori* is eradicated mainly by triple therapy which consists of a combination of two antibacterial agents with different mechanisms of action, which are selected from amoxicillin, clarithromycin, and metronidazole, and a proton pump inhibitor.[4] However, the increasing extensive use of an antibiotic, which is usually broad-spectrum, high-dosed, and long-term, could lead to many side effects such as diarrhea, including pseudomembranous enterocolitis in serious cases. The most serious among these side effects would be the drug resistance of *H. pylori* strains, which has badly affected the treatment outcome of triple *H. pylori* eradication therapy.[5,6] 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 increasingly serious resistance of most *H. pylori* strains. Hence, there are unmet medical needs for novel, efficacious, and selective eradication therapies that minimize resistance problems in both *H. pylori* and also other bacteria, that lack the common gastrointestinal side effects that often associated with antibacterials.

During recent years the structure modification at the pendant hydroxy group of metronidazole has received much attention,[7] and many metronidazole derivatives exhibited high anti-*H. pylori* activities.[8] Our interest in this area is to design and synthesize biologically active metronidazole derivatives at the pendant hydroxy group. Flavonoids are nearly ubiquitous in plants and are recognized as the pigments responsible for the colors of leaves, especially in autumn. They are rich in seeds, citrus fruits, olive oil, tea, and red wine. They are low-molecular weight compounds with over one substitution. It is already well established that flavonoids, such as genistein and apigenin, make some contributions to disease resistance, either as constitutive antifungal agents or as phytoalexins.[9] Certain plants and spices containing flavonoids have been used in traditional eastern medicines for thousands of years. During our recent research concerning functional natural products and structure modification,[10] we decided to join metronidazole together with flavones or isoflavones, intending to develop a series of new metronidazole derivatives to minimize resistance problems and to gain an additional anti-inflammatory activity through the inhibition of the IL-8 production.

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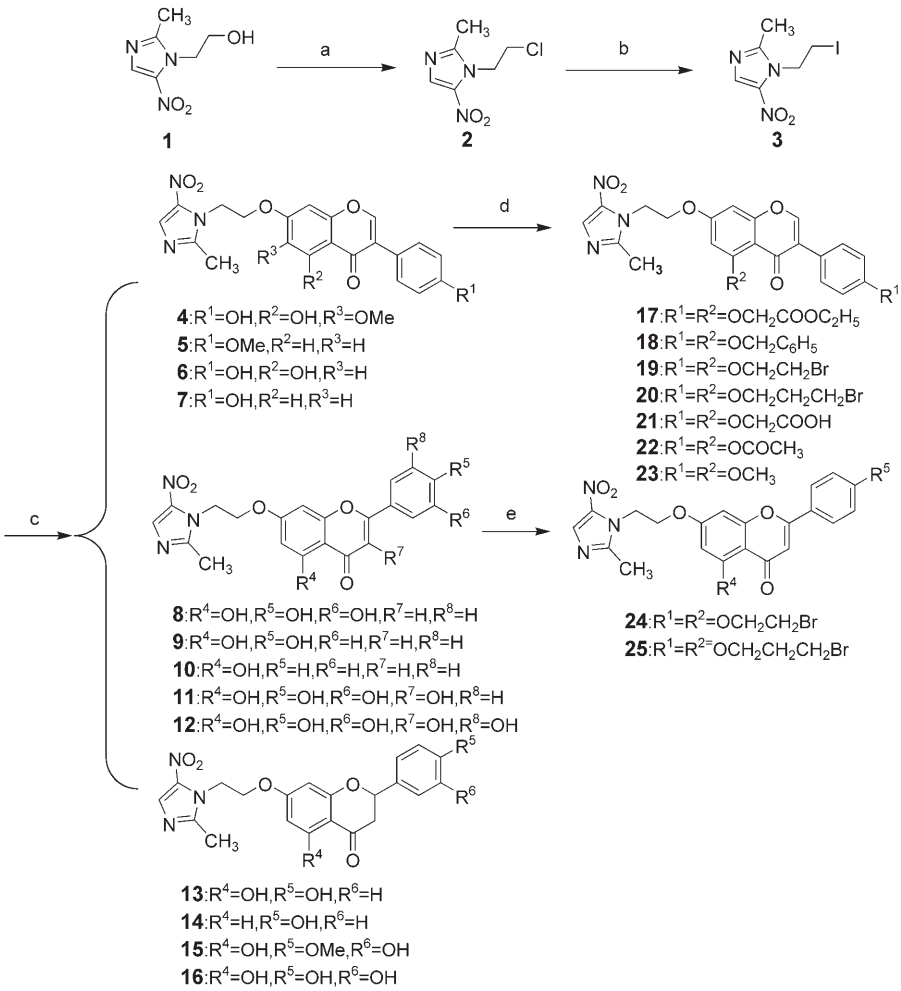
Chemistry

In this paper, 13 flavones and isoflavones were selected to join together with metronidazole and the target compounds were synthesized by the routes outlined in Scheme 1. Metronidazole (**1**) was first treated with thionyl chloride to afford the corresponding chlorometronidazole (**2**), which was allowed to react with sodium iodide in the presence of anhydrous acetone to give the iodide product (**3**). Then target compounds **4**–**16** were synthesized by treatment of **3** with potassium carbonate followed by reaction with the selected flavonoids as designed. After preliminary evaluation of antimicrobial activity against *H. pylori*, several derivatives with better minimum inhibitory concentrations (MIC) were treated with a facile ultrasonic chemistry method in one step for new antimicrobial agents, and compounds **17**–**25** were obtained with high yields.

By treating genistein-metronidazole derivative **6** and ethyl bromoacetate in the presence of potassium carbonate afforded **17**. Compounds **13**–**21** were obtained following the same method as for synthesis of **17**, whereas **22** and **23** were prepared with acetic anhydride and dimethyl sulfate, respectively. Derivatives **24** and **25** were prepared under ultrasound irradiation with excessive amounts of 1,2-dibromoethane and 1,3-dibromopropane from apigenin-metronidazole derivative **9**.



Scheme 1. Synthesis of metronidazole derivatives **4**–**25**: a) $SOCl_2$, reflux; b) NaI, acetone, reflux; c) flavones/isoflavones, DMF, K_2CO_3, 80 °C; d) **17**. ethyl bromoacetate, K_2CO_3, 80 °C; **18**. benzyl bromide, K_2CO_3, 70 °C; **19**. 1,2-dibromoethane, DMF, 80 °C; **20**. 1,3-dibromopropane, DMF, 80 °C; **21**. bromoacetic acid, DMF, K_2CO_3, 80 °C; **22**. acetic anhydride, pyridine, 80 °C; **23**. dimethyl sulfate, H_2O, NaOH, RT; e) **24**. 1,2-dibromoethane, DMF, 80 °C; 25.1,3-dibromopropane, DMF, 80 °C.

Biological Results

Anti-*H. pylori* activity

The synthesized compounds were evaluated for antimicrobial activity against *H. pylori*. The mixture containing equivalent molar genistein and metronidazole was also investigated for anti-*H. pylori* activity. To prove our previous expectation, two standard and four clinical antimicrobial resistances of *H. pylori* strains were used as the test pathogens and the results were summarized in Table 1.

Genistein-metronidazole derivative **6** showed more potent anti-*H. pylori* activities than metronidazole. Especially in the four clinic antimicrobial resistant strains of *H. pylori*, **6** with the great activity (MIC = 0.39 μg mL^{-1}) was 50-fold more potent than metronidazole, and comparable to the positive control amoxicillin (MIC = 0.39 μg mL^{-1}). Other isoflavones analogues **4**, **5**, and **7** showed less activity than **6** but were more potent than metronidazole and other flavonoids derivatives, implying that the isoflavones with the 4H-1-benzopyran-4-one ring system may be necessary for the remarkable anti-*H. pylori* of these derivatives. The flavone derivatives **8**–**12** were less potent than the isoflavone analogues **4**–**6**, but also have different activities. The effects of substituents on the phenyl ring were investigated, the order of potency was as follows: 5 hydroxyls (**12**) > 4 hydroxyls (**11**) > 3 hydroxyls (**8**) > 2 hydroxyls (**9**) > 1 hydroxyl (**10**). We also prepared flavanone derivatives (**13**–**16**), which did not show any anti-*H. pylori* activity (MIC > 100 μg mL^{-1}). Accordingly, it is shown that the flavanone-metronidazole derivatives did not show any inhibition against *H. pylori* and was not worthy of further research. Several genistein-metronidazole derivatives **17**–**23** were also evaluated for antimicrobial activity against *H. pylori*, and they showed decreased activities compared to **6**. It is obvious that

Table 1. Minimum inhibitory concentrations of compounds **4**–**25** against *Helicobacter pylori* strains.[a]

Compd	ATCC 43504	SS1	$HP_{Clinical\ 2}$	$HP_{Clinical\ 3}$	$HP_{Clinical\ 8}$	$HP_{Clinical\ 9}$
4	3.12	3.12	1.56	1.56	1.56	6.25
5	1.56	1.56	1.56	3.12	1.56	6.25
6	0.39	0.39	0.39	0.39	0.39	0.78
7	3.12	3.12	1.56	1.56	1.56	6.25
8	25	25	25	25	25	25
9	50	50	25	25	50	50
10	50	50	50	50	50	50
11	12.5	12.5	12.5	12.5	25	25
12	12.5	12.5	12.5	6.25	12.5	12.5
13	>100	>100	>100	>100	>100	>100
14	>100	>100	>100	>100	>100	>100
15	>100	>100	>100	>100	>100	>100
16	>100	>100	>100	>100	>100	>100
17	25	25	25	25	25	25
18	>100	>100	>100	>100	>100	>100
19	1.56	3.12	1.56	1.56	1.56	6.25
20	1.56	1.56	1.56	1.56	1.56	3.12
21	50	50	50	50	50	50
22	>100	>100	>100	>100	>100	>100
23	>100	>100	>100	>100	>100	>100
24	25	25	25	50	25	25
25	25	25	25	25	25	25
genistein	>100	>100	>100	>100	>100	>100
metronidazole	25	25	>100	>100	>100	>100
amoxicillin	0.05	0.05	0.39	0.39	0.39	0.39

[a] Values of minimum inhibitory concentrations (MICs) given as $\mu g\,mL^{-1}$.

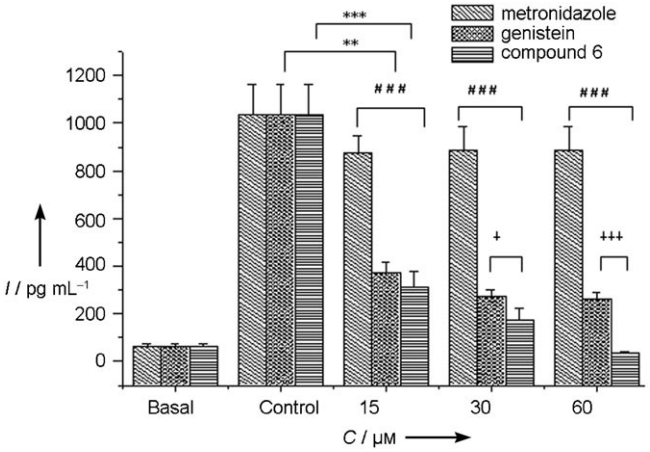


Figure 1. Inhibitory activities of compound **6** on IL-8 production induced by *H. pylori* water extract (HPE). *I* for IL-8 and *C* for concentrations of the agents. AGS cells were preincubated with or without metronidazole, genistein, or compound **6** in serial concentrations of 15, 30, and 60 $\mu mol\,L^{-1}$ 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. Results are means ± SEM of 3–5 experiments. Comparison 15, 30, and 60 $\mu mol\,L^{-1}$ of all agents versus control: $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$; comparison compound **6** versus metronidazole: $\#P<0.05$, $\#\#P<0.01$, and $\#\#\#P<0.001$; comparison compound **6** versus genistein: $+P<0.05$, $P<0.01$, and $+++P<0.001$.

all the hydroxy groups attached to the benzene rings of genistein play important roles for the activity against *H. pylori*. However, compound **19** and **20** also showed potent activities 4–6-fold lower than **6**, implying that the element bromine also plays an important role in anti-*H. pylori* activity. This can be confirmed by comparing the activities of compounds **24** and **25** with compound **9**. Notably, the mixture of metronidazole and genistein showed poor activity against *H. pylori* (MIC $>100\ \mu g\,mL^{-1}$) just like pure metronidazole, indicating that the chemical conjugation of the two structures is responsible for the remarkable biological activities of these derivatives in vitro.

Moreover, selectivity of compounds **4**–**25** for *Streptococcus pneumoniae*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescence*, and *Aspergillus niger* was also evaluated by the agar hole method.[11] None of the compounds (**4**–**25**) showed antibacterial and antifungal activity against the tested organisms at a concentration of 1000 $\mu g\,mL^{-1}$.

IL-8 assessment

In addition to its potent anti-*H. pylori* activity, compound **6** also showed a strong attenuation of IL-8 production induced by HPE in AGS cells. As shown in Figure 1, HPE alone stimulated AGS cells to produce as much as 1033.3 $pg\,mL^{-1}$ IL-8. However, different dose-dependent attenuations of HPE-induced IL-8 production were seen with the addition of metronidazole, compound **6**, and genistein.

Compound **6** significantly decreased the IL-8 level in a dose-dependent way at concentrations of 15, 30, and 60 $\mu mol\,L^{-1}$ ($P<0.001$) compared with control and the lowest IL-8 production was observed when the concentration of compound **6** was 60 $\mu mol\,L^{-1}$.

The same inhibition of HPE-induced IL-8 production can be observed when genistein was preincubated with AGS cells followed by the addition of HPE. Genistein significantly decreased IL-8 levels at concentrations of more than 15 $\mu mol\,L^{-1}$, compared with the control group ($P<0.001$). However, metronidazole at the same concentration could only reduce the IL-8 level slightly.

Comparatively, compound **6** showed a more potent inhibitory activity against HPE-induced IL-8 production than both metronidazole at all concentrations ($P<0.001$) and genistein at concentrations of 30 $\mu mol\,L^{-1}$ ($P<0.05$) and 60 $\mu mol\,L^{-1}$ ($P<0.01$).

Cell viability evaluation

The result shown in Figure 2 demonstrated that metronidazole, compound **6**, and genistein did not affect cell viability at the concentrations tested (15, 30, and 60 $\mu mol\,L^{-1}$).

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 **6** was nontoxic.

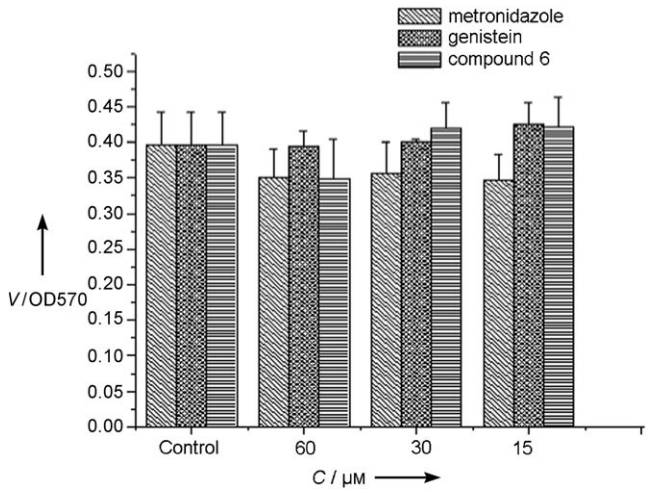


Figure 2. Effects of different agents on cell viability. *V* for cell viability and *C* for concentrations of the agents. AGS cells were incubated with metronidazole, genistein, or compound **6** at serial concentrations of 15, 30, and 60 μmol L^{-1}. Then MTT assay was performed 48 h later. Results are means ± SEM of 4–6 experiments. Comparison of 15, 30, and 60 μmol L^{-1} of all the agents versus control: $*P < 0.05$.

Discussion

In recent years, *H. pylori* strains resistant to the antimicrobial components of the triple therapy regimens have been observed increasingly often.[5,6] *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 paper, compounds **4**–**7**, **19**, and **20** showed low MIC values against *H. pylori* strains, especially the strains resistant to metronidazole, whereas *H. pylori* also exhibited an acceptable rate of spontaneous resistance development against **4**–**7**. Moreover, compound **6** was almost inactive against a rather wide range of commensal or pathogenic microorganisms. Although the inhibitory mechanism of compound **6** remains unknown at present, the potent anti-*H. pylori* activity and their complete selectivity for *H. pylori* are quite important points for a drug candidate with fewer side effects.

H. pylori-infection leads to different clinical and pathological outcomes in humans, including chronic gastritis, peptic ulcer disease, and gastric neoplasia. Up to now, complete eradiation of *H. pylori* is still the most effective therapy, which prompts people to make great efforts 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[12] and rebamipide[13] 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 **6** with the strongest anti-*H. pylori* activity was selected to investigate its effect on the IL-8 production induced by *H. pylori* in gastric mucosal cells. As formerly reported, 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*,[14] the extract or surface proteins of this bacterium,[15] and outer inflammatory proteins A (OipA).[16] In this experiment, to rule out the influence of compound **6** 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 AGS 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,[17] 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 AGS cell culture supernatant can be seen when the cells were preincubated with compound **6** followed by the stimulation of HPE. At the same time, compound **6** at the same concentrations exhibited little effect on AGS cell viability as shown in Figure 2, which can exclude the disturbance of its cytotoxic activity to AGS cells. It implied that compound **6** has the ability to decrease the HPE-induced IL-8 production by a direct influence on the gastric cells.

Considering that compound **6** possesses the structure of metronidazole and genistein, the two chemicals were tested in this model to assess their effects on the intracellular cascade of the IL-8 production in AGS 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 **6**, indicating that metronidazole has no effect on the AGS cell infected with *H. pylori*, except its direct antibacterial activity. On the other hand, genistein could significantly decrease the IL-8 production in AGS cell stimulated by HPE which suggesting that genistein can directly influence the intracellular cascade of the IL-8 production.

It is noteworthy that 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 epithelial 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.[18,19]

Therefore, the inhibition of tyrosine kinase can downregulate IL-8 expression in transcriptional and translational level in the gastric cells stimulated by the bacterium and subsequently attenuates the inflammatory response. In our experiment, genistein significantly decreased HPE-induced IL-8 production in AGS cell, which was also reported in other gastric epithelial cancer cells MKN45.[20] In terms of current documents, it can be suggested that genistein, a known specific tyrosine kinase inhibitor, attenuate HPE-induced IL-8 expression probably through inhibiting tyrosine phosphorylation and subsequently downregulating the nuclear translocation of NF-κB.

Compound **6**, structurally characterized by metronidazole and genistein, has the biological activities of the two chemicals, which never happened to the simple mixture of metronidazole and genistein 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 genistein in compound **6** makes it possible to deduce that the significant reduction in IL-8 production might be attributed to the inhibition of tyrosine kinase, similar to the mode of action of genistein. Consequently, compound **6** 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 inhibition of IL-8 production. The latter might be achieved via tyrosine kinase inhibition, which should be investigated for further study.

Conclusions

To conclude, after chemical modification preceded by metronidazole, a novel class of selective anti-*Helicobacter pylori* agents were generated, and evaluated for antimicrobial activity against *H. pylori*. Among the compounds studied, potent and selective anti-*H. pylori* activities were observed in isoflavone derivatives **4**–**7**, **19**, and **20** especially in the four antimicrobial resistant strains of *H. pylori*, compound **6** with strong activity (MIC = 0.39 $\mu g\,mL^{-1}$) was >100-fold more potent than metronidazole, may well solve the problem of metronidazole resistance in triple *H. pylori* eradication therapy. Furthermore, compound **6** was significantly decreased HPE-induced IL-8 production in AGS cells and the acute oral toxicity test demonstrated that compound **6** was nontoxic, indicating that compound **6** would be a potential and promising agent for the treatment of *H pylori*-related diseases.

Experimental Section

Chemistry general. All the 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 were reported in parts per million to residual solvent protons. Melting points were measured on a Boetius micro melting point apparatus. The ESI-MS spectra were recorded on a Mariner System 5304 Mass spectrometer. All chemicals and reagents used in current study were of analytical grade. Flavonoids were purchased from Xi'an Huike Company, Xi'an China, and metronidazole was purchased from Changzhou Dongsheng Company, Changzhou China. TLC was run on the silica gel coated aluminum sheets (silica gel 60 $GF_{254,}$ E. Merck, Germany) and visualized in UV light (254 nm).

Cell culture. Human gastric epithelial cancer cell line AGS (ATCC, WA, USA) were grown in Ham's F12 containing 10% FBS, 1-glutamine, 100 $U\,mL^{-1}$ penicillin G and 100 $\mu g\,mL^{-1}$ streptomycin. 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 helicobacter pylori mouse-adapted strain Sydney strain 1 (SS1), and four clinical isolates of *H. pylori* (2, 3, 8, 9), 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.[21]

Antimicrobial activity. Antimicrobial activity against *H. pylori* was determined by the agar dilution method recommended by the national committee for clinical laboratory (NCCL) standards.[22] In vitro the test strains (ATCC 43504, SS1, and clinical strains 2, 3, 8, and 9) have been 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 approximately 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 dimethyl sulfoxide (DMSO) lower than 1% (*v*/*v*). After 3 days' incubation, MICs were determined as the lowest concentrations of the agents that visibly inhibited bacterial growth inhibition (minimum inhibitory concentration: MIC).

Antibacterial activity (except for *Aspergillus niger*) was tested by pouring nutrient agar containing 0.5% of yeast extract (10 mL), in which 10^6–10^7 CFUs of the test bacteria were inoculated, onto a plate. After caking, a hole (4 mm diameter) was made with a pipe of stainless steel. Subsequently, 50 μL of the 10% DMSO solution of a test compound (1000 $\mu g\,mL^{-1}$) was added to the hole. The 10% DMSO solution was used as the reference solution. After being allowed to stand for 1 h at 8 °C to diffuse a test compound into the medium, the agar was incubated at 37 °C for 18 h. Antibacterial activity was confirmed by the formation of an inhibitory zone. The anti-*Aspergillus niger* activity was evaluated in a similar manner except for the medium (potato dextrose agar) and incubation conditions (at 27 °C for 24 h).

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 °C until used.[15]

IL-8 assessment. Briefly, AGS cells, grown for two days on 24-wells plates to ~80% confluence in Ham's F12 medium, were washed three times with serum-free Ham's F12 medium and then preincubated with metronidazole, genistein, and compound **6** in serial concentrations as 15, 30, and 60 μmol L^{-1} with the concentration of DMSO lower than 1% for 1 h. After that cells were incubated with 10% HPE (*v/v*) for 12 h. The supernatant were aspirated, centrifuged at 500 g for 10 min, and then stored at −80 °C. The levels of IL-8 in the culture supernatant were determined by a commercially available enzyme linked immunosorbent assay (ELISA) kit (Rapdbio, USA) according to the instruction of the manufacturer.[19]

MTT assay for cellular viability. Cell viability was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) assay.[23] Briefly, AGS cells, grown for 24 h on 24-wells plates, were incubated with metronidazole, genistein, and compound **6** at serial concentrations of 15, 30, and 60 μmol L^{-1} for 48 h with the concentration of DMSO lower than 1%. Then MTT (5 mg mL^{-1}) was added to each well for a 4 h period. After the formation of formazan crystals, the culture medium supernatant was removed from the wells without disruption of the precipitate. The formazan crystals were then dissolved in 150 μmL DMSO/well. The absorbance was measured at 570 nm using a microplate spectrophotometer (DYNEX Technologies, Chantilly, VA).

Acute oral toxicity of compound 6. Acute oral toxicity studies were performed according to Vasudevan's method.[24] Briefly, mice of either sex selected by 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 0.5%, *w/v*, carboxymethyl cellulose, CMC) was administered orally at a dose 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 was performed to compare differences between groups. Two-tailed probability (*P*) values were derived, and *P* value of <0.05 was considered statistically significant.

1-(2-iodoethyl)-2-methyl-5-nitro-1*H*-imidazole (3). Metronidazole (4.71 g, 30 mmol) was dissolved in $SOCl_2$ (50 mL, 50 mmol), and the solution was stirred at 60 °C for 6 h and cooled. Then 20% hydrochloric acid was carefully added; compound **2** (4.98 g, 26.4 mmol) was obtained as a yellow crystal. An amount of compound **2** (10.43 g, 55.0 mmol) was dissolved in 40 mL anhydrous acetone; the solution was stirred at 90 °C for 15 min. Then NaI (5.73 g, 55.0 mmol) in anhydrous acetone (20 mL) was carefully added; and the mixed solution was stirred at 90 °C for 12 h. Then the solution was filtrated and distilled to remove the solvents. Compound **3** (12.70 g, 45.2 mmol) was obtained as a yellow solid. Yield: 82%; mp 78.5–79.5 °C; ^{1}H NMR ([D$_6$]DMSO) $\delta=2.49$ (s, 3H, -CH_3-), 3.51 (t, 2H, $J=4.5$ Hz, -CH_2-), 4.61 (t, 2H, $J=4.5$ Hz, -CH_2-), 6.81 (d, 2H, H-3′, H-5′), 6.38 (d, 2H, H-2′, H-6′), 8.05 (s, 1H, H-2), 8.42 ppm (s, 1H, H-4″); ESI-MS: 281.2 $[M+H]^+$, Anal. ($C_6H_8IN_3O_2$) C 25.64%, H 2.87%, I 45.15%, N 14.95%; Found: C 25.48%, H 2.72%, I 45.24%, N 14.83%.

5-hydroxy-3-(4-hydroxyphenyl)-6-methoxy-7-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)-4*H*-chromen-4-one (4). Tectorigenin (1.8 g, 6.04 mmol) was added to a mixture containing K_2CO_3 (1.26 g, 11.8 mmol) and anhydrous DMF (40 mL). This solution was stirred at 80 °C for 30 min. Then compound **3** (5.0 g, 17.79 mmol) in anhydrous DMF (10 mL) was carefully added to the above solution over 30 min, and the resulting solution was stirred at 80 °C for 18 h. After that the solvents were removed by distillation and the residue was recrystallized from acetone. Compound **4** (1.73 g, 3.84 mmol) was obtained as a white solid; mp 192–194 °C; ^{1}H NMR ([D$_6$]DMSO): $\delta=2.61$ (s, 3H, CH_3), 3.61 (s, 3H, -OCH_3), 4.48 (brs, 2H, N-CH_2-), 4.74 (brs, 2H, CH_2O-), 6.81 (s, 1H, H-8), 6.83 (d, $J=8.0$ Hz, 2H, H-3′, 5′), 7.37 (d, $J=8.0$ Hz, 2H, H-2′, 6′), 8.05 (s, 1H, H-2), 8.44 (s, 1H, H-4″), 9.59 (s, 1H, 4′-OH), 12.92 ppm (s, 1H, 5-OH); ESI-MS: 454.2 $[M+H]^+$, Anal. ($C_{22}H_{19}N_3O_8$) C 58.28%, H 4.19%, N 8.60%; Found: C 58.14%, H 4.02%, N 8.83%.

Compounds **5**–**16** were prepared with the same procedure as described above.

3-(4-methoxyphenyl)-7-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)-ethoxy)-4*H*-chromen-4-one (5). Yield: 74%; mp 189–191 °C; ^{1}H NMR ([D$_6$]DMSO): $\delta=2.54$ (s, 3H, CH_3), 3.31 (s, 3H, -OCH_3), 4.51 (t, $J=4.5$ Hz, 2H, N-CH_2-), 4.77 (t, $J=4.5$ Hz, 2H, CH_2O-), 6.98 (d, $J=8.0$ Hz, 2H, H-3′, 5′), 7.00 (s, 1H, H-8), 7.18 (s, 1H, H-6), 7.51 (d, $J=8.0$ Hz, 2H, H-2′, 6′), 8.02 (s, 1H, H-5), 8.05 (s, 1H, H-2), 8.43 ppm (s, 1H, H-4″); ESI-MS: 422.1 $[M+H]^+$, Anal. ($C_{22}H_{19}N_3O_6$) C 62.71%, H 4.51%, N 9.26%; Found: C 62.56%, H 4.38%, N 9.41%.

5-hydroxy-3-(4-hydroxyphenyl)-7-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)-4*H*-chromen-4-one (6). Yield: 82%; mp 158–160 °C; ^{1}H NMR ([D$_6$]DMSO): $\delta=2.58$ (s, 3H, CH_3), 4.47 (t, $J=5.0$ Hz, 2H, N-CH_2-), 4.74 (t, $J=5.0$ Hz, 2H, CH_2O-), 6.37 (d, $J=2.0$ Hz, 1H, H-8), 6.66 (d, $J=2.0$ Hz, 1H, H-6), 6.81 (d, $J=8.5$ Hz, 2H, H-3′, 5′), 7.37 (d, $J=8.5$ Hz, 2H, H-2′, 6′), 8.05 (s, 1H, H-2), 8.42 (s, 1H, H-4″); 9.61 (s, 1H, 4′-OH), 12.94 ppm (s, 1H, 5-OH); ESI-MS: 424.1 $[M+H]^+$, Anal. ($C_{21}H_{17}N_3O_7$) C 59.57%, H 4.05%, N 9.93%; Found: C 59.35%, H 4.21%, N 9.85%.

3-(4-hydroxyphenyl)-7-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)-ethoxy)-4*H*-chromen-4-one (7). Yield: 76%; mp 164–166 °C; ^{1}H NMR ([D$_6$]DMSO): $\delta=2.51$ (s, 3H, CH_3), 4.47 (t, $J=5.0$ Hz, 2H, N-CH_2-), 4.74 (t, $J=5.0$ Hz, 2H, CH_2O-), 6.65 (d, $J=2.0$ Hz, 2H, H-3′, 5′), 7.18 (s, 1H, H-8), 7.46 (d, $J=8.5$ Hz, 2H, H-2′, 6′), 7.81 (s, 1H, H-6), 8.02 (s, 1H, H-5), 8.05 (s, 1H, H-2), 8.48 (s, 1H, H-4″); 9.75 ppm (s, 1H, 4′-OH); ESI-MS: 408.1 $[M+H]^+$, Anal. ($C_{21}H_{17}N_3O_6$) C 69.91%, H 4.21%, N 10.31%; Found: C 69.55%, H 4.04%, N 10.32%.

2-(3,4-dihydroxyphenyl)-5-hydroxy-7-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)-4*H*-chromen-4-one (8). Yield: 68%; mp 179–181 °C; ^{1}H NMR ([D$_6$]DMSO): $\delta=2.57$ (s, 3H, CH_3), 4.44 (t, $J=4.5$ Hz, 2H, N-CH_2-), 4.69 (t, $J=4.5$ Hz, 2H, CH_2O-), 6.35 (s, 1H, H-8), 6.71 (s, 1H, H-6), 7.07 (d, $J=8.0$ Hz, 1H, H-5′), 7.43 (s, 1H), 7.68 (d, $J=8.0$ Hz, 2H, H-2′, 6′), 8.05 (s, 1H, H-4″), 9.78 (s, 1H, 3″-OH), 10.59 (s, 1H, 4″-OH), 12.87 ppm (s, 1H, 5-OH); ESI-MS: 440.1 $[M+H]^+$, Anal. ($C_{21}H_{17}N_3O_8$) C 57.40%, H 3.87%, N 8.88%; Found: C 57.27%, H 3.73%, N 8.67%.

5-hydroxy-2-(4-hydroxyphenyl)-7-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)-4*H*-chromen-4-one (9). Yield: 65%; mp 225–256 °C; ^{1}H NMR ([D$_6$]DMSO): $\delta=2.54$ (s, 3H, CH_3), 4.47 (brs, 2H, N-CH_2-), 4.74 (brs, 2H, CH_2O-), 6.35 (d, $J=2.1$ Hz, 1H, H-8), 6.78 (d, $J=2.1$ Hz, 1H, H-6), 6.91 (d, $J=1.8$ Hz, 1H, H-1), 7.06 (d, $J=8.5$ Hz, 2H, H-3′, 5′), 8.03 (d, $J=8.5$ Hz, 2H, H-2′, 6′), 8.05 (s, 1H, H-4″), 10.39 (s, 1H, 4′-OH), 12.85 ppm (s, 1H, 5-OH); ESI-MS: 424.1 $[M+H]^+$, Anal. ($C_{21}H_{17}N_3O_7$) C 59.57%, H 4.05%, N 9.93%; Found: C 59.43%, H 4.05%, N 9.76%.

5-hydroxy-7-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)-2-phenyl-4*H*-chromen-4-one (10). Yield: 76%; mp 222–224 °C; ^{1}H NMR ([D$_6$]DMSO): $\delta=2.53$ (s, 3H, CH_3), 4.46 (t, $J=4.5$ Hz, 2H, N-CH_2-), 4.74 (t, $J=4.5$ Hz, 2H, CH_2O-), 6.36 (d, $J=1.8$ Hz, 1H, H-8), 6.82 (d, $J=1.8$ Hz, 1H, H-6), 7.00 (s, 1H, H-1), 7.55 (dd, $J=1.8$ Hz, 8.1 Hz, 2H, H-3′, 5′), 7.62 (t, $J=6.0$ Hz, 1H, H-4′), 8.05 (s, 1H, H-4″), 8.08 (dd, $J=1.8$ Hz, 8.1 Hz, 2H, H-2′, 6′), 12.79 ppm (s, 1H, 5-OH);

ESI-MS: 408.1 $[M+H]^+$, Anal. ($C_{21}H_{17}N_3O_6$) C 61.91%, H 4.21%, N 10.31%; Found: C 61.76%, H 4.14%, N 10.24%.

2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-7-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)-4*H*-chromen-4-one (11). Yield: 70%; mp 219–221 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 2.51 (s, 3H, CH_3), 4.46 (t, J = 4.5 Hz, 2H, N-CH_2-), 4.58 (t, J = 4.5 Hz, 2H, CH_2O-), 6.36 (s, 1H, H-8), 6.78 (s, 1H,H-6), 6.93 (d, J = 8.0 Hz, 1H, H-5′), 7.41 (s, 1H, H-2′), 7.81 (s, 1H, H-6′), 8.04 (s, 1H, H-4″), 9.78 (brs, 2H, 3′, 4′-OH), 10.69 (s, 1H, 1-OH), 12.85 ppm (s, 1H, 5-OH); ESI-MS: 440.1 $[M+H]^+$, Anal. ($C_{21}H_{17}N_3O_9$) C 55.39%, H 3.76%, N 9.23%; Found: C 55.73%, H 3.45%, N 9.37%.

3,5-dihydroxy-7-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)-2-(3,4,5-trihydroxyphenyl)-4*H*-chromen-4-one (12). Yield: 78%; mp 192–194 °C; ^{1}H NMR ([D$_6$]DMSO): δ2.54 (s, 3H, CH_3), 4.49 (t, J = 4.5 Hz, 2H, N-CH_2-), 4.58 (t, J = 4.5 Hz, 2H, CH_2O-), 6.29 (dd, J = 1.8 Hz, 8.1 Hz, 2H, H-2′, 6′), 6.35 (s, 1H, H-8), 6.78 (s, 1H, H-6), 7.81 (s, 1H, H-4″), 9.05 (s, 1H, 4′-OH), 9.48 (brs, 2H, 3′, 5′-OH), 10.59 (s, 1H, 1-OH), 12.87 ppm (s, 1H, 5-OH); ESI-MS: 472.1 $[M+H]^+$, Anal. ($C_{21}H_{17}N_3O_{10}$) C 53.51%, H 3.64%, N 8.91%; Found: C 53.24%, H 3.73%, N 8.68%.

5-hydroxy-2-(4-hydroxyphenyl)-7-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)chroman-4-one (13). Yield: 62%; mp 154–155 °C ^{1}H NMR ([D$_6$]DMSO): δ = 2.53 (s, 3H, CH_3), 2.69 (dd, J = 2.5 Hz, 17 Hz, 1H, H-1), 3.21(dd, J=12 Hz, 17 Hz, 1H, H-1), 4.39 (t, J = 4.5 Hz, 2H, N-CH_2-), 4.68 (t, J = 4.5 Hz, 2H, CH_2O-), 5.42 (dd, J = 2.5 Hz, 12 Hz, 1H, H-2), 6.04 (s, 1H, H-8), 6.07 (s, 1H, H-6), 6.86 (d, J = 4.5 Hz, 1H, H-2′), 6.92 (d, J = 4.5 Hz, 2H, H-3′, 5′), 6.94 (s, 1H, H-6′), 8.03 (s, 1H, H-4″), 9.08 (s, 1H, 4′-OH), 12.06 ppm (s, 1H, 5-OH); ESI-MS: 426.1 $[M+H]^+$, Anal. ($C_{21}H_{19}N_3O_7$) C 59.29%, H 4.50%, N 9.88%; Found: C 59.12%, H 4.34%, N 9.76%.

2-(4-hydroxyphenyl)-7-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)-ethoxy)chroman-4-one (14). Yield: 65%; mp 165–167 °C ^{1}H NMR ([D$_6$]DMSO): δ = 2.51 (s, 3H, CH_3), 2.72 (dd, J = 2.5 Hz, 17 Hz, 1H, H-1), 3.24(dd, J=12 Hz, 17 Hz, 1H, H-1), 4.45 (t, J = 4.5 Hz, 2H, N-CH_2-), 4.69 (t, J = 4.5 Hz, 2H, CH_2O-), 5.51 (dd, J = 2.5 Hz, 12 Hz, 1H, H-2), 6.04 (s, 1H, H-8), 6.07 (s, 1H, H-6), 6.86 (d, J = 4.5 Hz, 1H, H-2′), 6.92 (d, J = 4.5 Hz, 2H, H-3′, 5′), 6.94 (s, 1H, H-6′), 7.75 (s, 1H, H-5), 8.03 (s, 1H, H-4″), 9.08 ppm (s, 1H); ESI-MS: 410.1 $[M+H]^+$, Anal. ($C_{21}H_{19}N_3O_6$) C 61.61%, H 4.68%, N 10.26%; Found: C 61.24%, H 4.42%, N 10.46%.

5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)chroman-4-one (15). Yield: 74%; mp 175–176 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 2.53 (s, 3H, CH_3), 2.69 (dd, J = 2.5 Hz, 17 Hz, 1H, H-1), 3.21(dd, J=12 Hz, 17 Hz, 1H, H-1), 3.58 (s, 3H, -OCH_3), 4.39 (t, J = 4.5 Hz, 2H, N-CH_2-), 4.68 (t, J = 4.5 Hz, 2H, CH_2O-), 5.45 (dd, J = 2.5 Hz, 12 Hz, 1H, H-2), 6.04 (s, 1H, H-8), 6.07 (s, 1H, H-6), 6.86 (d, J = 4.5 Hz, 1H, H-3′), 6.97 (d, J = 4.5 Hz, 2H, H-2′, 6′), 8.03 (s, 1H, H-4″), 9.08 (s, 1H, 6′-OH), 12.06 ppm (s, 1H, 5-OH); ESI-MS: 456.1 $[M+H]^+$, Anal. ($C_{22}H_{21}N_3O_8$) C 56.05%, H 4.49%, N 8.91%; Found: C 56.12%, H 4.58%, N 9.16%.

2-(3,4-dihydroxyphenyl)-5-hydroxy-7-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)chroman-4-one (16). Yield: 71%; mp 189–191 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 2.53 (s, 3H, CH_3), 2.67 (dd, J = 2.5 Hz, 17 Hz, 1H, H-1), 3.17(dd, J=12 Hz, 17 Hz, 1H, H-1), 4.35 (t, J = 4.5 Hz, 2H, N-CH_2-), 4.65 (t, J = 4.5 Hz, 2H, CH_2O-), 5.47 (dd, J = 2.5 Hz, 12 Hz, 1H, H-2), 6.06 (s, 1H, H-8), 6.11 (s, 1H, H-6), 6.89 (d, J = 4.5 Hz, 1H, H-3′), 6.92 (d, J = 4.5 Hz, 2H, H-2′, 6′), 8.03 (s, 1H, H-4″), 9.12 (brs, 2H, 4′, 5′-OH), 12.12 ppm (s, 1H, 5-OH); ESI-MS: 442.1 $[M+H]^+$, Anal. ($C_{21}H_{19}N_3O_8$) C 57.14%, H 4.34%, N 9.52%; Found: C 57.32%, H 4.12%, N 9.34%.

{3-(4-ethoxycarbonylmethoxyphenyl)-7-[2-(2-methyl-5-nitroimidazol-1-yl)-ethoxy]-4-oxo-4H-chromen-5-yloxy}acetate, ethyl ester (17). Compound **6** (0.253 g, 0.6 mmol) was added to a mixture containing K_2CO_3 (0.154 g, 1.12 mmol) and anhydrous DMF (15 mL), and this solution was stirred at 80 °C for 15 min. Then ethyl bromoacetate (0.201 g, 1.2 mmol) was carefully added to the above solution over 15 min, and the resulting solution was stirred at 80 °C for 15 h, cooled, and 30 mL of water was added; the aqueous layer was extracted with ethyl acetate (50 mL). The ethyl acetate layers were dried with anhydrous Na_2SO_4. Removal of ethyl acetate by evaporation gave a light yellow solid of compound **17** (0.289 g, 0.48 mmol). Yield: 80%; mp 120–122 °C; ^{1}H NMR ([D$_6$]DMSO) δ = 1.19–1.23 (m, 6H, 4′, 5-CH_3), 2.52 (s, 3H, 2″-CH_3), 4.15–4.19 (m, 4H, 4′, 5-OCH_2), 4.48 (s, 2H, N-CH_2-), 4.73 (s, 2H, CH_2O-), 4.80 (s, 2H, 4′-CH_2COO), 4.88 (s, 2H, 5-CH_2COO), 6.34 (s, 1H, H-8), 6.75 (d, J = 2.0 Hz, 1H, H-6), 6.94 (d, J = 8.5 Hz, 2H, H-3′, 5′), 7.42 (d, J = 8.5 Hz, 2H, H-2′, 6′), 8.05 (s, 1H, H-2), 8.23 ppm (s, 1H, H-4″); ESI-MS: 596.1 $[M+H]^+$, Anal. ($C_{29}H_{29}N_3O_{11}$) C 58.49%, H 4.91%, N 7.06%; Found: C 58.32%, H 4.74%, N 6.93%.

5-benzyloxy-3-(4-benzyloxyphenyl)-7-[2-(2-methyl-5-nitroimidazol-1-yl)ethoxy]chromen-4-one (18). Compound **6** (0.337 g, 0.8 mmol) was added to a mixture containing K_2CO_3 (0.220 g, 1.6 mmol) and anhydrous DMF (20 mL), and this solution was stirred at 70 °C for 15 min. Then benzyl bromide (0.290 g, 1.7 mmol) was carefully added to the above solution over 15 min, and the resulting solution was stirred at 70 °C for 12 h, cooled, and 30 mL of water was added; the aqueous layer was extracted with ethyl acetate (50 mL). The ethyl acetate layers were dried with anhydrous Na_2SO_4. Removal of ethyl acetate by evaporation gave a dark yellow solid of compound **18** (0.368 g, 0.62 mmol). Yield: 78%; mp 142–144 °C; ^{1}H NMR ([D$_6$]DMSO) δ = 2.52 (s, 3H, CH_3), 4.49 (t, J = 4.5 Hz, 2H, N-CH_2-), 4.74 (t, J = 4.5 Hz, 2H, CH_2O-), 5.12 (d, J = 15 Hz, 2H, 4′-CH_2), 5.21 (d, J = 15 Hz, 2H, 5-CH_2); 6.58 (s, 1H, H-8); 6.71 (d, J = 2.0 Hz, 1H, H-6); 7.04 (d, J = 8.5 Hz, 2H, H-3′, 5′); 7.31 (d, J = 8.5 Hz, 2H, H-2′, 6′); 7.33–7.61 (q, 10H, 4′H, 5′H); 8.07 (s, 1H, H-2), 8.20 ppm (s, 1H, H-4″). ESI-MS: 604.1 $[M+H]^+$, Anal. ($C_{35}H_{29}N_3O_7$) C 69.64%, H 4.84%, N 6.96%; Found: C 69.52%, H 4.65%, N6.77%.

5-(2-bromoethoxy)-3-(4-(2-bromoethoxy)phenyl)-7-(2-(2-methyl-5-nitro-1*H*-pyrrol-1-yl)ethoxy)-4*H*-chromen-4-one (19). Compound **6** (0.337 g, 0.8 mmol) was added to a mixture containing K_2CO_3 (0.220 g, 1.6 mmol) and anhydrous DMF (20 mL), and this solution was stirred at 70 °C for 15 min. Then 1,2-dibromoethane (0.2 mL, 1.7 mmol) was carefully added to the above solution over 15 min, and the resulting solution was stirred at 80 °C for 12 h, cooled, and 30 mL of water was added; the aqueous layer was extracted with ethyl acetate (50 mL). The ethyl acetate layers were dried with anhydrous Na_2SO_4. Removal of ethyl acetate by evaporation gave a dark white solid of compound **19**. Yield: 82%; mp 218–220 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 2.51 (s, 3H, CH_3), 3.74 (brs, 4H, 4′-CH_2), 4.08 (brs, 4H, 5-CH_2), 4.47 (t, J = 5.0 Hz, 2H, N-CH_2-), 4.74 (t, J = 5.0 Hz, 2H, CH_2O-), 6.37 (d, J = 2.0 Hz, 1H, H-8), 6.66 (d, J = 2.0 Hz, 1H, H-6), 6.81 (d, J = 8.5 Hz, 2H, H-3′, 5′), 7.37 (d, J = 8.5 Hz, 2H, H-2′, 6′), 8.05 (s, 1H, H-2), 8.42 ppm (s, 1H, H-4″); ESI-MS: 634.1 $[M+H]^+$, Anal. ($C_{25}H_{23}Br_2N_3O_7$) C 47.12%, H 3.64%, Br 25.08%, N 6.59%; Found: C 47.43%, H 3.32%, Br 25.45%, N 6.23%.

5-(3-bromopropoxy)-3-(4-(3-bromopropoxy)phenyl)-7-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)-4*H*-chromen-4-one (20) Compound **6** (0.337 g, 0.8 mmol) was added to a mixture containing K_2CO_3 (0.220 g, 1.6 mmol) and anhydrous DMF (20 mL), and this solution was stirred at 70 °C for 15 min. Then 1,3-dibromopropane (0.3 mL, 1.7 mmol) was carefully added to the above solution

over 15 min, and the resulting solution was stirred at 80 °C for 12 h, cooled, and 30 mL of water was added; the aqueous layer was extracted with ethyl acetate (50 mL). The ethyl acetate layers were dried with anhydrous Na_2SO_4. Removal of ethyl acetate by evaporation gave a dark white solid of compound **20**. Yield: 75%; mp 202–204 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 2.12–2.16 (m, 4H, 4′, 5-CH_2), 2.53 (s, 3H, CH_3), 3.51 (brs, 4H, 4′-CH_2), 4.06 (brs, 4H, 5-CH_2), 4.45 (t, J = 5.0 Hz, 2H, N-CH_2-), 4.76 (t, J = 5.0 Hz, 2H, CH_2O-), 6.41 (d, J = 2.0 Hz, 1H, H-8), 6.69 (d, J = 2.0 Hz, 1H, H-6), 6.81 (d, J = 8.5 Hz, 2H, H-3′, 5′), 7.37 (d, J = 8.5 Hz, 2H, H-2′, 6′), 8.01 (s, 1H, H-2), 8.21 ppm (s, 1H, H-4″); ESI-MS: 664.0 [M+H]$^+$, Anal. ($C_{27}H_{27}Br_2N_3O_7$) C 48.74%, H 4.09%, Br 24.02%, N 6.32%; Found: C 48.96%, H 4.21%, Br 24.43%, N 6.46%.

2-(4-(5-(carboxymethoxy)-7-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)-4-oxo-4*H*-chromen-3-yl)phenoxy)acetic acid (21). Compound **6** (0.337 g, 0.8 mmol) was added to a mixture containing K_2CO_3 (0.220 g, 1.6 mmol) and anhydrous DMF (20 mL), and this solution was stirred at 70 °C for 15 min. Then bromoacetic acid (1 mL, 1.7 mmol) was carefully added to the above solution over 15 min, and the resulting solution was stirred at 80 °C for 12 h, cooled, and 30 mL of water was added; the aqueous layer was extracted with ethyl acetate (50 mL). The ethyl acetate layers were dried with anhydrous Na_2SO_4. Removal of ethyl acetate by evaporation gave a dark white solid of compound **21**. Yield: 84%; mp 176–178 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 2.52 (s, 3H, CH_3), 4.35 (t, J = 4.5 Hz, 2H, N-CH_2-), 4.65 (t, J = 4.5 Hz, 2H, CH_2O-), 4.72 (s, 2H, 4′-CH_2), 4.86 (s, 2H, 5-CH_2), 6.47 (d, J = 2.2 Hz, 1H, H-8), 6.71 (d, J = 2.2 Hz, 1H, H-6), 6.93 (d, J = 8.5 Hz, 2H, H-3′, 5′), 7.42 (d, J = 8.5 Hz, 2H, H-2′, 6′), 8.07 (s, 1H, H-2), 8.25 ppm (s, 1H, H-4″). ESI-MS: 540.1 [M+H]$^+$, Anal. ($C_{25}H_{21}N_3O_{11}$) C 55.66%, H 3.92%, N 7.79%; Found: C 55.23%, H 4.11%, N 7.75%.

4-{5-acetoxy-[2-(2-methyl-5-nitro-imidazol-1-yl)-ethoxy]-4-oxo-4*H*-chromen-3-yl}-phenylester acetate (22). Acetic anhydride (0.02 mL, 1 mmol) was carefully added to a mixture containing compound **6** (0.211 mg, 0.5 mmol) and pyridine (20 mL). The reaction mixture was stirred at 60 °C for 8 h. After that the solution was poured into a 10% solution hydrochloric acid (50 mL). The white deposits precipitated were separated from the solvents by filtration. They were washed with aqueous saturated $NaHCO_3$ twice. Compound **22** (215 mg, 0.42 mmol) was obtained as a white solid. Yield: 85%; mp 184–186 °C; ^{1}H NMR ([D$_6$]DMSO) δ = 2.31 (s, 3H, 4′-$COCH_3$), 2.34 (s, 3H, 5-$COCH_3$), 2.53 (s, 3H, 2″-CH_3), 4.48 (t, J = 4.5 Hz, 2H, N-CH_2-), 4.73 (t, J = 4.5 Hz, 2H, CH_2O-), 6.78 (d, J = 2.4 Hz, 1H, H-8), 7.12 (d, J = 2.4 Hz, 1H, H-6), 7.19 (d, J = 8.7 Hz, 2H, H-3′, 5′), 7.53 (d, J = 8.7 Hz, 2H, H-2′, 6′), 8.06 (s, 1H, H-2), 8.43 ppm (s, 1H, H-4″). ESI-MS: 508.1 [M+H]$^+$, Anal. ($C_{25}H_{21}N_3O_9$) C 59.17%, H 4.17%, N 8.28%; Found: C 59.03%, H 4.11%, N 8.15%.

Compounds **23**–**25** were prepared with the same procedure as described above.

5-methoxy-3-(4-methoxyphenyl)-7-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)-4*H*-chromen-4-one (23). Yield: 78%; mp 174–176 °C; ^{1}H NMR ([D$_6$]DMSO) δ = 2.51 (s, 3H, 2″-CH_3), 3.77 (s, 3H, 4′-OCH_3), 3.85 (s, 3H, 5-OCH_3); 4.48 (t, J = 4.5 Hz, 2H, N-CH_2-), 4.73 (t, J = 4.5 Hz, 2H, CH_2O-), 6.50 (d, J = 2.3, 1H, H-8); 6.66 (d, J = 2.3, 1H, H-6); 6.94 (d, J = 8.8, 2H, H-3′, 5′); 7.40 (d, J = 8.8, 2H, H-2′, 6′); 8.11 (s, 1H, H-2), 8.40 ppm (s, 1H, H-4″). ESI-MS: 452.1 [M+H]$^+$, Anal. ($C_{23}H_{21}N_3O_7$) C 61.19%, H 4.69%, N 9.31%; Found: C 61.45%, H 4.04%, N 9.76%.

5-(2-bromoethoxy)-2-(4-(2-bromoethoxy)phenyl)-7-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)-4*H*-chromen-4-one (24). Yield: 80%; mp 138–140 °C; ^{1}H NMR ([D$_6$]DMSO) δ = 2.53 (s, 3H), 3.68 (brs, 4H, 4′-CH_2), 4.01 (brs, 4H, 5-CH_2), 4.46 (t, J = 5.0 Hz, 2H, N-CH_2-), 4.74 (t, J = 5.0 Hz, 2H, CH_2O-), 6.78 (d, J = 2.4 Hz, 1H, H-8), 7.12 (d, J = 2.4 Hz, 1H, H-6), 7.19 (d, J = 8.4 Hz, 2H, H-3′, 5′), 7.53 (d, J = 2.4 Hz, 2H, H-2′, 6′), 7.96 (s, 1H, H-1), 8.06 ppm (s, 1H, H-4″). Anal. ($C_{25}H_{23}Br_2N_3O_7$) C 47.12%, H 3.64%, Br 25.08%, N 6.59%; Found: C 47.19%, H 3.87%, Br 25.31%, N 6.86%.

5-(3-bromopropoxy)-2-(4-(3-bromopropoxy)phenyl)-7-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethoxy)-4*H*-chromen-4-one (25). Yield: 75%; mp 153–155 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 2.12–2.16 (m, 4H, 4′, 5-CH_2), 2.53 (s, 3H), 3.54 (brs, 4H, 4′-CH_2), 4.10 (brs, 4H, 5-CH_2), 4.45 (t, J = 5.0 Hz, 2H, N-CH_2-), 4.76 (t, J = 5.0 Hz, 2H, CH_2O-), 6.75 (d, J = 2.0 Hz, 1H, H-8), 7.16 (d, J = 2.0 Hz, 2H, H-6), 7.21 (d, J = 8.5 Hz, 2H, H-3′, 5′), 7.67 (d, J = 8.5 Hz, 2H, H-2′, 6′), 7.94 (s, 1H, H-1), 8.09 ppm (s, 1H, H-4″); ESI-MS: 664.0 [M+H]$^+$, Anal. ($C_{27}H_{27}Br_2N_3O_7$) C 48.74%, H 4.09%, Br 24.02%, N 6.32%; Found: C 48.54%, H 4.16%, Br 24.33%, N 6.15%.

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