Full Paper

5-Nitroimidazole-based 1,3,4-Thiadiazoles: Heterocyclic Analogs of Metronidazole as Anti-*Helicobacter pylori* Agents

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A series of 5-nitroimidazole-based 1,3,4-thiadiazoles were prepared and tested for antibacterial activity against *Helicobacter pylori*. The anti-*H. pylori* activity of target compounds along with the commercially available antimicrobial metronidazole was evaluated by comparing the inhibition-zone diameters determined by the paper disc diffusion bioassay. From our bioassay results against 20 clinical isolates it is evident that piperazinyl, 4-methylpiperazinyl, 3-methylpiperazinyl, and 3,5-dimethylpiperazinyl analogs (**6a**, **6b**, **6e**, and **6f**, respectively) and pyrrolidine derivative **7** had strong activity at 0.5 μg/disc (average of inhibition zone >20 mm) while metronidazole had no activity at this dose. Compound **6f** containing the 3,5-dimethylpiperazinyl moiety at the 2-position of the 5-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-1,3,4-thiadiazole skeleton was the most potent compound tested at low concentrations.

Keywords: Antibacterial activity / *Helicobacter pylori* / 5-Nitroimidazole / 1,3,4-Thiadiazole

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Introduction

Since the discovery of *Helicobacter pylori* by Warren and Marshall in 1984, much has been learned about this Gram-negative microaerophilic microorganism and its associated microbiology and pathology [1]. *Helicobacter pylori* bacterium is a global human pathogen responsible for a number of prevalent diseases including peptic ulcer and gastric cancer [2]. Therefore, its eradication is strongly desirable for patients with these diseases and those with unexplained iron-deficiency anemia [3]. Over the years, treatment has evolved from single agents to combination therapy of antisecretory agents with one or more antibiotics including amoxicillin, clarithromycin, metronidazole, tetracycline, and levofloxacin [4]. Seven-day triple therapy (proton pump inhibitor, amoxicillin, and clarithromycin) has been the recommended first-line therapy for *H. pylori* infection in many countries [5–7]. Strains displaying primary resistance to metronidazole and clarithromycin have been reported with increasing frequency throughout the world and resistance is likely to become an increasingly important problem in the clinical management of *H. pylori* infections [8]. Thus, the discovery of novel and potent antibacterial agents is the best way to overcome bacterial resistance and develop effective therapies [9]. Hence, our research efforts are directed toward the discovery of new chemical entities that are effective as anti-*Helicobacter pylori* agents and the optimization of their structures. During recent years, there have been intense

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investigations of different classes of 1,3,4-thiadiazole- and nitroimidazole-containing compounds, many of which are known to possess biological properties such as antituberculosis and anti-*H. pylori* activities [9–12]. In continuation of our ongoing research work on nitroheterocyclic derivatives [13], herein, we describe the synthesis and *in-vitro* antibacterial activity of new 5-nitroimidazole-based 1,3,4-thiadiazoles **6a–h** and **7** against *Helicobacter pylori*.

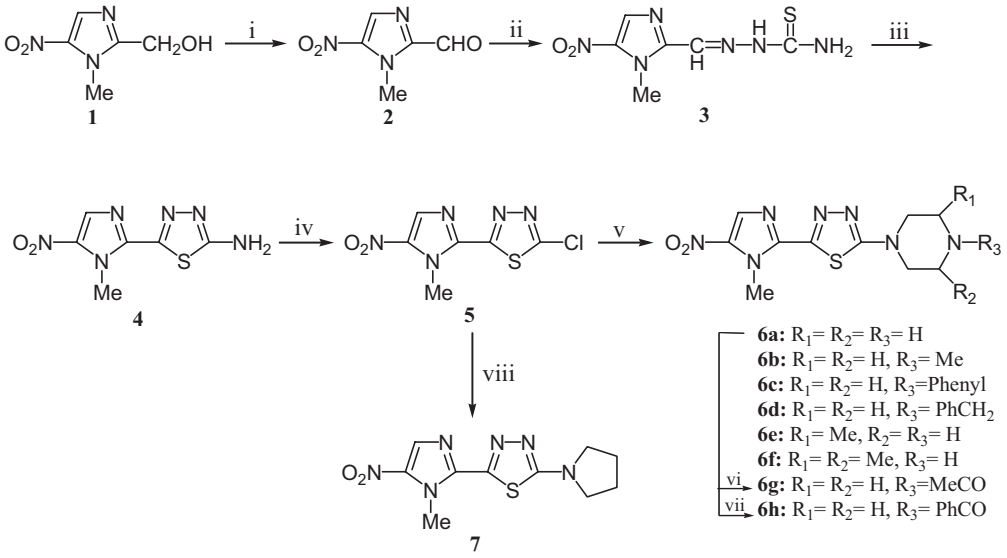
Results and discussion

Chemistry

As illustrated in Scheme 1, the target compounds were prepared from the key intermediate 2-chloro-1,3,4-thiadiazole **5**. The intermediate **5** was obtained from 1-methyl-2-hydroxymethyl-5-nitroimidazole through several steps including oxidation of alcohol **1** to aldehyde **2**, treatment of **2** with thiosemicarbazide and subsequent aminothiadiazole formation, and diazotization of amine **4** in HCl solution in the presence of copper powder to give 2-chloro-1,3,4-thiadiazole **5** [14]. The reaction of compound **5** with piperazine derivatives or pyrrolidine in refluxing ethanol in the presence of $NaHCO_3$, gave piperazinyl-1,3,4-thiadiazoles **6a–f** or pyrrolidinyl-1,3,4-thiadiazole **7**, respectively. *N*-Acetylation or *N*-benzoylation of the piperazine analog **6a** with appropriate acid chlorides afforded compounds **6g** and **6h**, respectively.

Anti-*Helicobacter pylori* activity

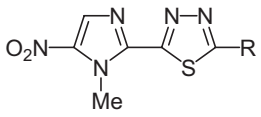
The anti-*H. pylori* activity of target compounds **6a–h** and **7** was evaluated by comparing the inhibition-zone diameters determined by the paper disc diffusion bioassay [12]. The commercially available 5-nitroimidazole, metronidazole, was used as a standard anti-*H. pylori* agent. All tests were performed in triplicate and the antibacterial activity was expressed as the mean of inhibition diameters (in mm) produced by the title compounds. Compounds **6a–h** and **7** were first evaluated against metronidazole-sensitive and then metronidazole-resistant *H. pylori* strains at a specified dose (32 μg/disc); the results are summarized in Table 1. All compounds showed strong inhibitory activity at the dose of 32 μg/disc and nitroimidazole **6a** was the most potent compound tested, displaying very strong activity at 32 μg/disc (inhibition zone diameter >50 mm) against both metronidazole-sensitive and metronidazole-resistant strains (Table 1). In order to evaluate the potential of the title compounds to inhibit different clinical isolates of *H. pylori* growth, all compounds were further tested at lower concentrations (<32 μg/disc) against 20 clinical isolates of *H. pylori*. The antibacterial activities of compounds at concentrations of 16, 8, 4, 2, 1, and 0.5 μg/disc against 20 clinical isolates of *H. pylori* are shown as averages of inhibition-zone diameters in Table 2. The anti-*Helicobacter pylori* activity can be classified as follows: strong response, zone diameter >20 mm; moderate response, zone diameter

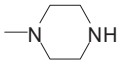
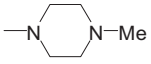
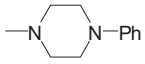
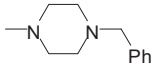
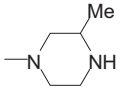
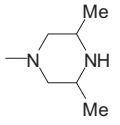
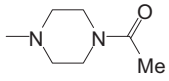
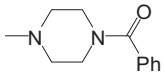


Reagents and conditions: i) MnO_2, $CHCl_3$, r. t., 3 h; ii) Thiosemicarbazide, EtOH, HCl, reflux, 1 h; iii) $NH_4Fe(SO_4)_2$, H_2O, reflux, 16 h; iv) $NaNO_2$, HCl, Cu, 0°C → r. t., 3 h; v) Piperazine derivatives, $NaHCO_3$, EtOH, reflux, 3 h; vi) *N*-Ethyl diisopropylamine, acetyl chloride, THF, reflux, 3 h; vii) PhCOCl, pyridine in dry benzene, 0°C → r. t., 24 h; viii) Pyrrolidine, $NaHCO_3$, EtOH, reflux, 3 h.

Scheme 1. Synthesis of compounds **6a–h** and **7**.

Table 1. Inhibition-zone diameter (in mm) of compounds **6a–h** and **7** (32 μg/disc) against two metronidazole-sensitive and metronidazole-resistant *H. pylori* strains.



Compound	R	Metronidazole-sensitive*			Metronidazole-resistant		
6a		59	60	>60	56	60	>60
6b		56	50	50	57	54	49
6c		30	19	54	30	19	54
6d		34	20	34	34	20	36
6e		54	54	55	54	54	54
6f		52	54	54	51	52	54
6g		50	48	50	49	50	49
6h		43	35	43	42	33	44
7		42	46	42	44	47	42

* Inhibition-zone diameters of metronidazole at the dose of 8 μg/disc were 18 and 11 mm in metronidazole-sensitive and metronidazole-resistant strains, respectively.

Table 2. Evaluation of antibacterial activity of compounds **6a–h** and **7** against 20 clinical isolates of *H. pylori*

Compound	Inhibition zone diameter* (range, mm)					
	0.5 μg/disc	1 μg/disc	2 μg/disc	4 μg/disc	8 μg/disc	16 μg/disc
6a	24.4 (14–30)	28.2 (17–38)	32.3 (18–47)	35.8 (20–46)	39.4 (24–53)	44.1 (34–53)
6b	23.1 (14–32)	26.8 (18–33)	31.0 (19–42)	32.4 (18–47)	33.5 (20–47)	41.3 (33–48)
6c	6	6	10.8 (4–19)	11.7 (7–14)	12.1 (6–18)	13.1 (6–20)
6d	6	10.0 (6–13)	14.7 (5–24)	14.3 (6–24)	14.8 (7–22)	15.5 (7–23)
6e	23.1 (13–35)	27.4 (15–39)	31.4 (20–40)	32.2 (19–45)	37.8 (24–51)	41.4 (30–49)
6f	30.9 (17–45)	38.2 (18–49)	41.0 (33–50)	41.8 (29–53)	45.2 (33–56)	47.7 (38–57)
6g	12.7 (6–20)	17.6 (12–30)	22.7 (12–30)	26.6 (11–37)	31.1 (19–43)	35.6 (22–49)
6h	9.7 (6–18)	13.2 (6–22)	18.0 (8–28)	17.9 (8–28)	23.4 (16–29)	26.3 (13–39)
7	27.2 (15–36)	33.1 (20–45)	37.6 (25–48)	40.2 (31–52)	46.0 (31–60)	50.7 (41–59)
Metronidazole	6	9.2 (4–21)	13.1 (6–19)	16.0 (8–26)	19.8 (11–27)	24.1 (17–32)

* The growth-inhibitory activity was expressed as the mean of inhibition zone diameters.

16–20 mm; weak response, zone diameter 11–15 mm; and little or no response, zone diameter <10 mm. The comparison of growth inhibitory activities revealed that compounds **6a**, **6b**, **6e**, **6f**, **6g**, and **7** showed strong activity at the dose of 2 μg/disc and produced an inhibition zone of more than 22 mm on average, which was wider than that of metronidazole (13.1 mm). These compounds (with the exception of **6g**) still had strong activity at 1 and 0.5 μg/disc (averages of inhibition zone >20 mm) while metronidazole had little or no activity at these doses. Moreover, it is notable to observe that 3,5-dimethylpiperazine analog **6f** proved to be statistically the most effective in this series and exhibiting very strong activity at 0.5 μg/disc (inhibition zone >30 mm).

The overall activity profile of unsubstituted piperazine analog **6a** and methylpiperazine derivatives **6b** and **6e** demonstrated that there is a small difference in their inhibition activity. While 3,5-dimethyl substitution on the piperazine ring improves inhibitory potency, the introduction of *N*-phenyl, *N*-benzyl, *N*-acetyl, and *N*-benzoyl on the piperazine ring diminishes the anti-*H. pylori* activity. Attachment of pyrrolidine (instead of piperazine) to the 2-position of the thiadiazole ring also produced the very potent compound **7**. Thus, the anti-*H. pylori* potency of 5-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-1,3,4-thiadiazoles is relatively dependent on the type of cyclic amine at the 2-position of the 1,3,4-thiadiazole nucleus and its attachment groups.

In conclusion, we have described synthesis and anti-*H. pylori* activity of some 5-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-1,3,4-thiadiazoles bearing (un)substituted piperazine or pyrrolidine rings. Biological data indicated that the 3,5-dimethylpiperazine analog **6f** was the most potent compound tested. Generally, the high *in-vitro* anti-*Helicobacter pylori* activity of type **6f** analogs makes these compounds a promising lead for the development of an effective anti-*Helicobacter* agent.

Experimental

General

Chemical reagents and all solvents used in this study were purchased from Merck AG and Aldrich. The 2-chloro-1,3,4-thiadiazole **5** was prepared according to the literature method [13]. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. The IR spectra were obtained on a Shimadzu 470 spectrophotometer (Shimadzu, Japan). ^{1}H-NMR spectra was recorded using a Bruker 80 or 500 MHz spectrometer (Bruker, Germany) and chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as internal standard. The mass spectra were run on a Finigan TSQ-70 spectrometer (Finigan, USA) at 70 eV. Elemental analyses were carried out on CHN-O rapid elemental analyzer (Heraeus GmbH, Germany) for C, H, and N, and the results are within ± 0.4% of the theoretical values. Merck silica gel F254 plates were used for analytical TLC (Merck, Germany).

*General procedure for the synthesis of compounds **6a-f** and **7***

To a mixture of compound **5** (1.0 mmol) and sodium hydrogen carbonate (1.0 mmol) in absolute ethanol (5 mL), appropriate piperazine derivative or pyrrolidine (1.0 mmol) was added and refluxed for 3 h. The reaction mixture was then cooled in an ice bath. The solid product was collected by filtration and washed with cold ethanol and recrystallized from the same solvent.

*5-(1-Methyl-5-nitro-1H-imidazol-2-yl)-2-(piperazin-1-yl)-1,3,4-thiadiazole **6a***

Yield: 83%; m. p.: 227–228°C; IR (KBr, cm^{-1}) ν_{max}: 3390 (NH), 1516, 1357 (NO_2); ^{1}H-NMR (80 MHz, $CDCl_3$) δ: 8.03 (s, 1H, H_4 imidazole), 4.49 (s, 3H, N-CH_3), 3.72–3.56 (m, 4H, piperazine), 3.13–2.98 (m, 4H, piperazine); MS (*m*/*z*, %): 295 [M^+] (8), 239 (18), 227 (20), 225 (14), 167 (7), 153 (17), 83 (20), 56 (100). Anal. calcd. for $C_{10}H_{13}N_7O_2S$: C, 40.67; H, 4.44; N, 33.20. Found: C, 40.82; H, 4.63; N, 33.05.

5-(1-Methyl-5-nitro-1H-imidazol-2-yl)-2-(4-methylpiperazin-1-yl)-1,3,4-thiadiazole ***6b***

Yield: 53%; m. p.: 247–249°C; IR (KBr, cm^{-1}) ν_{max}: 1521, 1357 (NO_2); ^{1}H-NMR (80 MHz, $CDCl_3$) δ: 8.03 (s, 1H, H_4 imidazole), 4.49 (s, 3H, N-CH_3 imidazole), 3.76–3.58 (m, 4H, piperazine), 2.69–2.48 (m, 4H, piperazine), 2.36 (s, 3H, N-CH_3 piperazine); MS (*m*/*z*, %): 309 [M^+] (8), 239 (8), 152 (7), 124 (5), 99 (14), 83 (80), 68 (100). Anal. calcd. for $C_{11}H_{15}N_7O_2S$: C, 42.71; H, 4.89; N, 31.69. Found: C, 42.88; H, 5.04; N, 31.45.

5-(1-Methyl-5-nitro-1H-imidazol-2-yl)-2-(4-phenylpiperazin-1-yl)-1,3,4-thiadiazole ***6c***

Yield: 44%; m. p.: 237°C; IR (KBr, cm^{-1}) ν_{max}: 1533, 1357 (NO_2); ^{1}H-NMR (80 MHz, $CDCl_3$) δ: 8.04 (s, 1H, H_4 imidazole), 7.40–7.18 (m, 2H, phenyl), 7.06–6.90 (m, 3H, phenyl), 4.49 (s, 3H, N-CH_3), 3.98–3.75 (m, 4H, piperazine), 3.48–3.27 (m, 4H, piperazine); MS (*m*/*z*, %): 371 [M^+] (8), 239 (6), 151 (7), 145 (14), 132 (100), 104 (52), 77 (30). Anal. calcd. for $C_{16}H_{17}N_7O_2S$: C, 51.74; H, 4.61; N, 26.40. Found: C, 52.06; H, 4.44; N, 26.73.

2-(4-Benzylpiperazin-1-yl)-5-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazole ***6d***

Yield: 42%; m. p.: 193–195°C; IR (KBr, cm^{-1}) ν_{max}: 1521, 1362 (NO_2); ^{1}H-NMR (80 MHz, $CDCl_3$) δ: 8.02 (s, 1H, H_4 imidazole), 7.32 (brs, 5H, phenyl), 4.48 (s, 3H, N-CH_3), 3.76–3.56 (m, 4H, piperazine and 2H, $PhCH_2$), 2.74–2.55 (m, 4H, piperazine); MS (*m*/*z*, %): 385 [M^+] (8), 239 (8), 180 (17), 159 (38), 146 (98), 134 (45), 91 (100), 56 (38). Anal. calcd for $C_{17}H_{19}N_7O_2S$: C, 52.97; H, 4.97; N, 25.44. Found: C, 53.12; H, 5.01; N, 25.13.

5-(1-Methyl-5-nitro-1H-imidazol-2-yl)-2-(3-methylpiperazin-1-yl)-1,3,4-thiadiazole ***6e***

Yield: 53%; m. p.: 182–184°C; IR (KBr, cm^{-1}) ν_{max}: 3266 (NH), 1516, 1367 (NO_2); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.05 (s, 1H, H_4 imidazole), 4.50 (s, 3H, N-CH_3), 3.93–3.87 (m, 2H, piperazine), 3.34–3.28 (m, 1H, piperazine), 3.17–3.12 (m, 1H, piperazine), 3.06–3.02 (m, 1H, piperazine), 3.01–2.96 (m, 1H, piperazine), 2.95–2.89 (m, 1H, piperazine), 1.16 (d, J = 6.15 Hz, 3H, CH_3 piperazine); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 172.8, 149.3, 141.7, 140.2, 133.4, 57.0, 50.4, 50.1, 45.1, 35.4, 19.3; MS (*m*/*z*, %): 309 [M^+] (8), 253 (18), 152 (7), 116 (14), 83 (60), 70 (65), 56 (100). Anal. calcd. for $C_{11}H_{15}N_7O_2S$: C, 42.71; H, 4.89; N, 31.69. Found: C, 42.45; H, 5.11; N, 31.47.

2-(3,5-Dimethylpiperazin-1-yl)-5-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazole ***6f***

Yield: 25%; m. p.: 234–236°C; IR (KBr, cm^{-1}) ν_{max}: 3431 (N-H), 1516, 1367 (NO_2); ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 8.25 (s, 1H, H_4 imidazole), 4.34 (s, 3H, N-CH_3), 3.30–3.19 (m, 4H, piperazine), 3.17–3.10 (m, 2H, piperazine), 1.30 (d, J = 6.2 Hz, 6H, CH_3 piperazine); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 172.1, 150.7, 141.3, 141.0, 133.6, 52.0, 50.6, 35.6, 15.8; MS (*m*/*z*, %): 323 [M^+] (7), 266 (7), 253 (17), 130 (10), 97 (8), 82 (42), 70 (100). Anal. calcd. for $C_{12}H_{17}N_7O_2S$: C, 44.57; H, 5.30; N, 30.32. Found: C, 44.62; H, 5.58; N, 30.49.

2-(1-Methyl-5-nitro-1H-imidazol-2-yl)-5-(pyrrolidin-1-yl)-1,3,4-thiadiazole 7

Yield: 53%; m. p.: 252–254°C; IR (KBr, cm^{-1}) ν_{max}: 1541, 1357 (NO_2); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.04 (s, 1H, H_4 imidazole), 4.50 (s, 3H, N-CH_3), 3.63–3.58 (m, 4H, pyrrolidine), 2.17–2.08 (m, 4H, pyrrolidine); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 168.9, 148.2, 142.1, 140.1, 133.3, 50.9, 35.4, 25.7; MS (*m*/*z*, %): 280 [M^+] (60), 278 (28), 250 (10), 225 (5), 193 (28), 153 (28), 114 (38), 100 (60), 69 (100). Anal. calcd. for $C_{10}H_{12}N_6O_2S$: C, 42.85; H, 4.32; N, 29.98. Found: C, 42.60; H, 4.16; N, 30.13.

2-(4-Acetylpiperazin-1-yl)-5-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazole ***6g***

To the solution of piperazine derivative **6a** (0.5 mmol) and *N*-ethyldiisopropyl amine (1.0 mmol) in THF, acetyl chloride (0.6 mmol) was added and refluxed for 3 h. The resulting precipitate was filtered off and the filtrate was washed with water and recrystallized from ethanol. Yield: 60%; m. p.: 254–256°C; IR (KBr, cm^{-1}) ν_{max}: 1644 (C = O), 1521, 1362 (NO_2); ^{1}H-NMR (80 MHz, $CDCl_3$) δ: 8.03 (s, 1H, H_4 imidazole), 4.49 (s, 3H, N-CH_3), 3.96–3.50 (m, 8H, piperazine), 2.16 (s, 3H, CH_3); MS (*m*/*z*, %): 337 [M^+] (20), 294 (8), 252 (42), 239 (54), 193 (5), 153 (20), 110 (14), 100 (20), 83 (28), 67 (45), 56 (100). Anal. calcd. for $C_{12}H_{15}N_7O_3S$: C, 42.72; H, 4.48; N, 29.06. Found: C, 43.06; H, 4.13; N, 28.86.

2-(4-Benzoylpiperazin-1-yl)-5-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazole ***6h***

To the solution of piperazine derivative **6a** (0.5 mmol) and pyridine in dry benzene, benzoyl chloride (0.5 mmol) was added at 0°C and stirred for 24 h at room temperature. Then, the solvent was removed under vacuum and the residue was washed with water and crystallized from ethanol. Yield: 89%; m. p.: 233–235°C; IR (KBr, cm^{-1}) ν_{max}: 1639 (C = O), 1531, 1362 (NO_2); ^{1}H-NMR (80 MHz, $CDCl_3$) δ: 8.03 (s, 1H, H_4 imidazole), 7.44 (brs, 5H, phenyl), 4.49 (s, 3H, N-CH_3), 3.97–3.60 (m, 8H, piperazine); MS (*m*/*z*, %): 399 [M^+] (18), 331 (8), 278 (8), 252 (18), 181 (8), 180 (18), 153 (7), 105 (100), 77 (68). Anal. calcd. for $C_{17}H_{17}N_7O_3S$: C, 51.12; H, 4.29; N, 24.55. Found: C, 50.98; H, 4.33; N, 24.64.

Antibacterial activity

The clinical isolates of *H. pylori* from gastric biopsy specimens were obtained from the Shariati Hospital (Tehran, Iran). Frozen clinical isolates were thawed and inoculated on Mueller–Hinton agar plates (Oxoid) supplemented with 10% horse blood and incubated under microaerophilic conditions. Given the importance of inoculum homogeneity, cellular viability was controlled microscopically by morphological observation with Gram-staining, in order to check the proportions of coccoid cells in cultures. Cultures were always used after 48 h of incubation, when they generally did not present coccoid forms. Suspensions were prepared in sterile distilled water to opacity of 2 McFarland standards (10^7–10^8 CFU/mL).

Growth inhibition was performed by the filter paper disc diffusion method on Mueller–Hinton agar with 7% of defibrinated horse blood under microaerophilic conditions at 37°C. The samples were tested using different amounts. A sample in 40 μL of methanol was applied by a microsyringe to the paper discs (6 mm diameter). After drying in a fume hood, the discs were placed on the agar surface that was inoculated with *H. pylori*. Following incubation for 3 to 5 days at 37°C, the inhibition zone around each disc was recorded. All tests were performed in triplicate and the antibacterial activity was expressed as the mean of inhibition diameters (in mm) produced by title compounds [9].

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