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Original article

Synthesis and in vitro anti-*Helicobacter pylori* activity of *N*-[5-(5-nitro-2-heteroaryl)-1,3,4-thiadiazol-2-yl]thiomorpholines and related compounds

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Abstract

Synthesis and in vitro anti-*Helicobacter pylori* activity of *N*-[5-(5-nitro-2-heteroaryl)-1,3,4-thiadiazol-2-yl]thiomorpholines **5**–**7**(**a**–**c**) and some related compounds **8a**–**c** and **9a**–**c** were described. The anti-*H. pylori* activity of target compounds along with commercially available antibiotics such as metronidazole and amoxicillin 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 most compounds still had strong activity at 4 and 2 μg/disc (average of inhibition zone >20 mm) while metronidazole had little activity at these doses. Nitrofuran analog **7b** containing thiomorpholine *S*,*S*-dioxide moiety was the most potent compound tested.

Keywords: *Helicobacter pylori*; 5-Nitroimidazole; 5-Nitrofuran; 5-Nitrothiophene; 1,3,4-Thiadiazole

1. Introduction

It is now recognized that *Helicobacter pylori*, an S-shaped spiral microaerophilic Gram-negative bacterium first isolated in human gastric mucosa in 1982 [1–3], is the root cause of gastric and duodenal ulcers, and gastric cancer [4–6]. The eradication of *H. pylori* can significantly reduce the risk of ulcer relapse and may help to prevent mucosa-associated lymphoid tissue (MALT)-type gastric carcinoma and other gastric cancers [7–9]. Hence, the World Health Organization (WHO) has proposed *H. pylori* as a class 1 carcinogen in humans, since it has been demonstrated that chronic infection is strongly associated with the development of malignant gastric diseases [10].

Until recently, the most effective treatment regimens have included a combination of antibiotics (β-lactams, macrolides and quinolones), bactericidal agents (bismuth salts) and antiprotozoal agents (metronidazole). Although it is widely recognized that this therapy plays a critical role in improving and/or preventing these gastric pathologies, long-term eradication of *H. pylori* is not successful and relapse is a problem [11,12]. Accordingly, the search of more active and safe compounds with anti-*H. pylori* activity is an attractive therapeutic target.

As a part of ongoing research programs to find new molecules for treatment of *H. pylori* infection, and after a screening program of a number of compounds, we decided to synthesize and evaluate a series of *N*-[5-(5-nitro-2-heteroaryl)-1,3,4-thiadiazol-2-yl]thiomorpholine derivatives. It is worth notifying that the pharmacological interests of nitroimidazoles,

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nitrofurans and nitrothiophenes have been established, and they are being extensively used in therapy against amoebic and anaerobic infections [13]. Nitroimidazole antimicrobials such as tinidazole and synthetic nitrofuran antimicrobials such as furazolidone have been used in place of metronidazole to treat *H. pylori* with varying degrees of success (Fig. 1) [14]. On the other hand, the antimicrobial property of 1,3,4-thiadiazoles is well documented and their attachment with other heterocycles often ameliorates the bioresponses depending on the type of substituent and position of attachment [15]. Moreover, the thiomorpholine ring and its *S*-oxide and *S*,*S*-dioxide derivatives have shown attractive antimicrobial activities in nifurtimox analogs and in research on new replacements for the morpholine C-ring featured in linezolid [16]. In this report, we described the synthesis and in vitro anti-*H. pylori* activity of *N*-[5-(5-nitro-2-heteroaryl)-1,3,4-thiadiazol-2-yl]thiomorpholines **5**–**7** (**a**–**c**) and some related compounds **8a**–**c** and **9a**–**c**.

2. Chemistry

The synthesis of *N*-[5-(5-nitro-2-heteroaryl)-1,3,4-thiadiazol-2-yl]thiomorpholines was achieved with a versatile and efficient synthetic route via 2-chloro-5-aryl-1,3,4-thiadiazole key intermediates (**4**) (Scheme 1). These intermediates were prepared as indicated in our preceding paper [17]. Accordingly, the reaction of 5-nitroaryl-2-carboxaldehydes (**1a**–**c**) with thiosemicarbazide in refluxing ethanol afforded thiosemicarbazones (**2a**–**c**) in high yield [18]. The 2-amino-5-aryl-1,3,4-thiadiazoles (**3a**–**c**) were obtained by oxidative cyclization of **2a**–**c** in the presence of ammonium ferric sulfate [19,20]. Diazotation of **3a**–**c** in hydrochloric acid in the presence of copper powder [21] gave 2-chloro-5-aryl-1,3,4-thiadiazoles (**4a**–**c**). Reaction of the latter compound with thiomorpholine in dioxane gave compounds **5a**–**c** in good yields. Oxidation of compounds **5a**–**c** with 1 equiv of *m*-CPBA afforded compounds **6a**–**c** [22], while compounds **7a**–**c** were prepared by oxidation with the excess of hydrogen peroxide in acetic acid [23]. Synthesis of compounds **8a**–**c** and **9a**–**c** was previously described by us with similar synthetic pathway that has been shown in Scheme 1 [17,24].

3. Results and discussion

The anti-*H. pylori* activity of target compounds along with commercially available antibiotics such as metronidazole and amoxicillin was evaluated by comparing the inhibition zone diameters determined by the paper disc diffusion bioassay. Various amounts of the compound were dropped on standard disks (6 mm diameter) and the latter were placed on Muller–Hinton agar plate, earlier inoculated with bacterial suspension. Following incubation for 3–5 days at 37 °C, the inhibition zone around each disk (average diameter) if any, was recorded. All tests were performed in triplicate and the antibacterial activity was expressed as the mean of inhibition diameters (mm) produced by title compounds.

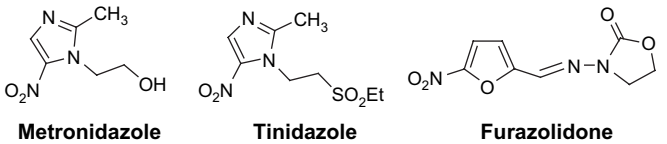


Fig. 1. Structures of some nitroheterocycle antimicrobials used in the treatment of *Helicobacter pylori* infection.

The compounds **5**–**9**(**a**–**c**) were first evaluated against metronidazole sensitive and metronidazole resistant *H. pylori* strains at three concentrations (8, 16 and 32 μg/disc). The antibacterial activity was classified as follows: strong response, zone diameter >20 mm; moderate response, zone diameter 16–20 mm; weak response, zone diameter 11–15 mm; and little or no response, zone diameter <10 mm.

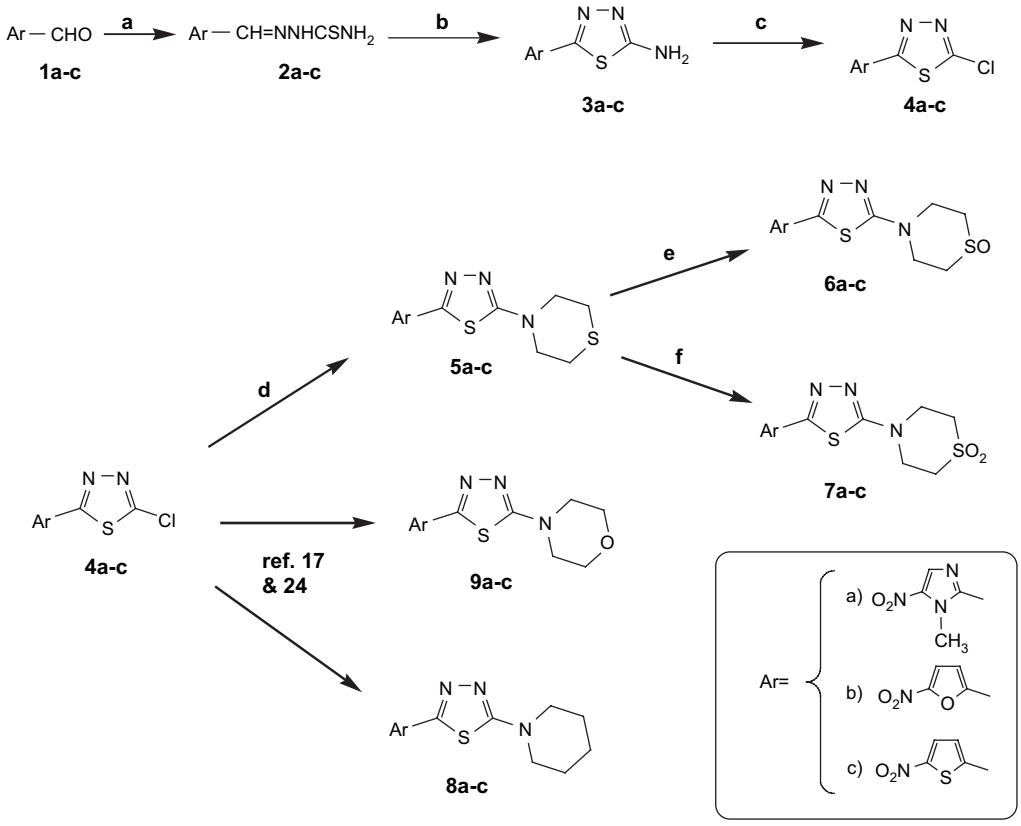
The preliminary evaluation of compounds **5**–**9**(**a**–**c**) against two metronidazole sensitive and metronidazole resistant *H. pylori* strains, are summarized in Table 1. All the synthesized nitrofuran analogs (**5**–**9**)**b**, three of nitroimidazole (**5a**, **7a** and **9a**), and one of nitrothiophene derivatives (**6c**) exhibited strong antimicrobial activity against both metronidazole sensitive and metronidazole resistant *H. pylori* strains at concentrations of 8, 16 and 32 μg/disc (inhibition zone diameter >20 mm). Nitrofuran **7b** was the most potent compound tested, displaying very strong activity at 8 μg/disc (inhibition zone diameter >40 mm) against both metronidazole sensitive and metronidazole resistant strains (Table 1). Among nitrothiophenes, while compound **5c** showed moderate activity, compound **6c** showed strong activity against *H. pylori* at three concentrations. However, no inhibitory activities were produced by nitrothiophenes **7c**, **8c** and **9c** at concentrations of 8 and 16 μg/disc (Table 1).

In order to assess the potential of title compounds to inhibit different clinical isolates of *H. pylori* growth, the most active compounds **5a**, **7a**, **9a**, (**5**–**9**)**b** and **6c**, selected in this first study, were further tested against a broader panel (20 clinical isolates) of *H. pylori*. The antibacterial activities of selected compounds at concentrations of 32, 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 averages of inhibition zone diameters indicate that all selected compounds exhibit high activity against clinical isolates of *H. pylori* with respect to standard drug, metronidazole (8 μg/disc). Indeed, all compounds at 8 μg/disc produced an inhibition zone of averagely more than 27 mm, which was greater than that by metronidazole (16.3 mm). Most compounds still had strong activity at 4 and 2 μg/disc (averages of inhibition zone >20 mm) while metronidazole had little activity at these doses.

The overall activity profile of selected compounds demonstrated that there is a small difference in their inhibition activity. However, it is notable to observe that nitrofuran analog **7b** proved to be statistically the most effective in this series still exhibiting moderate activity at 0.5 μg/disc (averages of inhibition zone = 16.6 mm), but less effective than amoxicillin.

From our bioassay results, it is evident that nitrofuran analogs exhibited more potent anti-*H. pylori* activity. It is



Scheme 1. Reagents and conditions: (a) thiosemicarbazide, EtOH, HCl, reflux, 1 h; (b) $NH_4Fe(SO_4)_2 \cdot 12H_2O$, H_2O, reflux, 16 h; (c) $NaNO_2$, HCl, Cu, 0 °C → rt, 3 h; (d) dioxane, rt, 72 h; (e) *m*-CPBA (1 equiv), $NaHCO_3$, CH_2Cl_2, 0 °C, 2–3 h; (f) H_2O_2 30%, AcOH, 55–60 °C, 5 h.

worthwhile observing that, in comparison with nitroimidazole derivatives, both of them have a better anti-*H. pylori* activity than nitrothiophene derivatives. However, this potency is relatively dependent on the type of cyclic amine attachment at the 5-position of the 1,3,4-thiadiazole nucleus. For example in nitrothiophene series, thiomorpholine-*S*-oxide analog **6c** has also a strong inhibitory effect on the growth of *H. pylori* similar to nitrofuran counterpart **6b**. Nevertheless, the most active compounds against *H. pylori* belong to nitrofuran series.

In conclusion, we have described synthesis and anti-*H. pylori* activity of *N*-[5-(5-nitro-2-heteroaryl)-1,3,4-thiadiazol-2-yl]thiomorpholines **5**–**7**(**a**–**c**) and some related compounds **8a**–**c** and **9a**–**c**. Biological data indicated that nitrofuran analog **7b** containing thiomorpholine *S*,*S*-dioxide moiety was the most potent compound tested. Generally, the high in vitro anti-*Helicobacter* activity of type **7** analogs makes these compounds a promising lead for the development of an effective therapeutic agent for anti-*Helicobacter* chemotherapy.

4. Experimental

4.1. Chemistry

Melting points were taken on a Kofler hot stage apparatus and are uncorrected. The FT-IR spectra were obtained using a Nicolet 550 spectrometer (Potassium bromide disks). The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker FT-500 and chemical shifts (δ) are in parts per million relative to internal tetramethylsilane. The mass spectra were run on a Finnigan mat TSQ-70 spectrometer at 70 eV. Thin layer chromatography (TLC) was performed on plates of silica gel 60 F_{254} plates. Silica gel 60, 0.040–0.063 mm (230–400 mesh) was used for flash column chromatography. All solvents used were reagent grade. Most of the solvents and reagents were purchased from Merck, Aldrich and Fluka and used as such without purification.

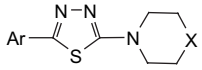
*4.2. General procedure for the preparation of 4-[5-(5-nitro-2-heteroaryl)-1,3,4-thiadiazol-2-yl]thiomorpholine (**5a–c**)*

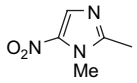
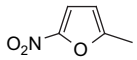
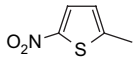
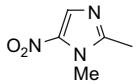
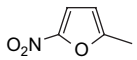
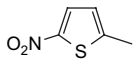
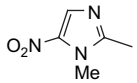
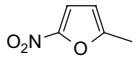
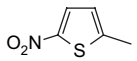
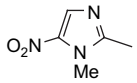
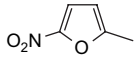
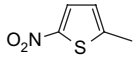
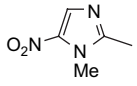
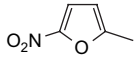
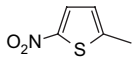
To a solution of 2-chloro-5-(5-nitro-2-heteroaryl)-1,3,4-thiadiazole (**4**, 4 mmol) in dioxane (30 ml), thiomorpholine (0.95 g, 9.2 mmol) was added and stirred at room temperature for 72 h. The reaction mixture was evaporated to dryness. To the residue, water (25 ml) was added and the precipitate was filtered, washed with water and recrystallized (2-propanol) to give the desired compounds **5a**–**c**.

*4.2.1. 4-[5-(1-Methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazol-2-yl]thiomorpholine (**5a**)*

M.p. 217–219 °C; IR (KBr): 1526, 1357 (NO_2); ^{1}H NMR ($CDCl_3$): δ 8.07 (s, 1H, imidazole), 4.53 (s, 3H, N–CH_3),

Table 1
Preliminary evaluation of compounds **5–9(a–c)** against two metronidazole sensitive and metronidazole resistant *Helicobacter pylori* strains[a]



Compound	Ar	X	Metronidazole sensitive dose (μg/disc)			Metronidazole resistant dose (μg/disc)		
			8	16	32	8	16	32
5a		S	27	31	34	24	27	30
5b		S	32	35	41	26	37	42
5c		S	16	18	22	16	19	21
6a		SO	6	19	25	6	15	21
6b		SO	35	41	46	35	40	43
6c		SO	34	39	46	32	35	41
7a		SO_2	32	40	45	27	36	40
7b		SO_2	43	50	>50	41	43	46
7c		SO_2	6	6	16	6	6	6
8a		CH_2	6	6	6	6	6	6
8b		CH_2	28	32	35	27	32	34
8c		CH_2	6	6	6	6	6	6
9a		O	25	31	32	26	31	33
9b		O	33	36	42	30	33	48
9c		O	6	6	15	6	6	6

[a] Inhibition zone diameters of metronidazole at 8 μg/disc were 18 and 11 mm in metronidazole sensitive and metronidazole resistant strains, respectively.

4.02 (t, $J = 4.9$ Hz, 4H, thiomorpholine), 2.82 (t, $J = 4.9$ Hz, 4H, thiomorpholine); MS: *m/z* (%) 312 (M^+, 20), 240 (50), 237 (95), 126 (20), 81 (25), 67 (55). Anal. Calcd for $C_{10}H_{12}N_6O_2S_2$: C, 38.45; H, 3.87; N, 26.90. Found: C, 38.74; H, 3.69; N, 27.22.

*4.2.2. 4-[5-(5-Nitro-2-furyl)-1,3,4-thiadiazol-2-yl]thiomorpholine (**5b**)*

M.p. 214–215 °C; IR (KBr): 3088 (CH aromatic), 1536, 1357 (NO_2); ^{1}H NMR ($CDCl_3$): δ 7.47 (d, $J = 3.8$ Hz, 1H, furan), 7.20 (d, $J = 3.8$ Hz, 1H, furan), 4.02 (t, $J = 5.1$ Hz, 4H, thiomorpholine), 2.82 (t, $J = 5.1$ Hz, 4H, thiomorpholine); MS: *m/z* (%) 298 (M^+, 45), 225 (95), 156 (20), 82 (50), 46 (25). Anal. Calcd for $C_{10}H_{10}N_4O_3S_2$: C, 40.26; H, 3.38; N, 18.78. Found: C, 40.54; H, 3.57; N, 18.53.

*4.2.3. 4-[5-(5-Nitro-2-thienyl)-1,3,4-thiadiazol-2-yl]thiomorpholine (**5c**)*

M.p. 240–242 °C; IR (KBr): 1536, 1332 (NO_2); ^{1}H NMR (DMSO-d_6): δ 7.90 (d, $J = 4.2$ Hz, 1H, thiophene), 7.21 (d, $J = 4.2$ Hz, 1H, thiophene), 4.01 (t, $J = 4.7$ Hz, 4H, thiomorpholine), 2.82 (t, $J = 4.7$ Hz, 4H, thiomorpholine); ^{13}C NMR (125 MHz, DMSO-d_6) δ 26.43 (C-2 and C-6, thiomorpholine), 53.44 (C-3 and C-5, thiomorpholine), 127.63 (C-3, thiophene), 131.56 (C-4, thiophene), 140.65 (C-2, thiophene), 150.30 (C-5, thiophene), 151.15 (C-2, thiadiazole), 173.24 (C-5, thiadiazole); MS: *m/z* (%) 314 (M^+, 95), 239 (90), 212 (40), 171 (95), 126 (85), 68 (90), 46 (95). Anal. Calcd for $C_{10}H_{10}N_4O_2S_3$: C, 38.20; H, 3.21; N, 17.82. Found: C, 37.94; H, 3.39; N, 18.02.

*4.3. General procedure for the preparation of 4-[5-(5-nitro-2-heteroaryl)-1,3,4-thiadiazol-2-yl]thiomorpholine 1-oxide (**6a–c**)*

A mixture of 4-[5-(5-nitro-2-heteroaryl)-1,3,4-thiadiazol-2-yl]thiomorpholine (**5**, 0.67 mmol) and sodium bicarbonate (0.05 g, 0.67 mmol) in dichloromethane (15 ml) was treated with *m*-CPBA (70%, 0.16 g, 0.67 mmol) and stirred at 0 °C for 2–3 h. Water was added to the reaction mixture and extracted with dichloromethane (2 × 15 ml). The combined organic layer was washed with aq. $NaHCO_3$ solution (30 ml), dried (Na_2SO_4) and evaporated under reduced pressure. The crude residue was recrystallized from chloroform.

*4.3.1. 4-[5-(1-Methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazol-2-yl]thiomorpholine 1-oxide (**6a**)*

M.p. 283–284 °C; IR (KBr): 1536, 1372 (NO_2), 1050 (S=O); ^{1}H NMR (DMSO-d_6): δ 8.25 (s, 1H, imidazole), 4.34 (s, 3H, N–CH_3), 4.11–3.97 (m, 4H, thiomorpholine) and 3.09–2.87 (m, 4H, thiomorpholine); MS: *m/z* (%) 328 (M^+, 60), 252 (25), 239 (20), 153 (45), 127 (40), 100 (98), 67 (75), 55 (95). Anal. Calcd for $C_{10}H_{12}N_6O_3S_2$: C, 36.58; H, 3.68; N, 25.59. Found: C, 36.35; H, 3.87; N, 25.88.

*4.3.2. 4-[5-(5-Nitro-2-furyl)-1,3,4-thiadiazol-2-yl]thiomorpholine 1-oxide (**6b**)*

M.p. 212–214 °C; IR (KBr): 3078 (CH aromatic), 1516, 1352 (NO_2), 1055 (S=O); ^{1}H NMR (DMSO-d_6): δ 7.87 (d, $J = 3.9$ Hz, 1H, furan), 7.42 (d, $J = 3.9$ Hz, 1H, furan), 4.12–3.96 (m, 4H, thiomorpholine), 3.10–2.88 (m, 4H, thiomorpholine); ^{13}C NMR (125 MHz, DMSO-d_6) δ 42.44 (C-2 and C-6, thiomorpholine), 44.21 (C-3 and C-5, thiomorpholine),

Table 2
Average of inhibition zone diameters of selected compounds at different doses against 20 clinical *H. pylori* isolates

Compound	Average of inhibition zone diameter (range, mm)						
	Dose (μg/disc)						
	0.5	1	2	4	8	16	32
5a	9.9 (6–21)	15.6 (6–29)	20.0 (6–36)	23.7 (6–50)	27.4 (15–50)	30.4 (17–50)	32.4 (19–56)
5b	10.8 (6–24)	18.3 (6–30)	23.4 (15–36)	27.7 (19–50)	30.1 (21–50)	32.9 (24–50)	36.9 (27–52)
6b	7.3 (6–21)	13.4 (6–26)	19.9 (6–33)	25.4 (16–39)	31.0 (25–50)	37.1 (27–50)	41.9 (28–50)
6c	13.7 (6–21)	18.5 (6–26)	23.6 (15–32)	28.1 (20–39)	34.4 (22–50)	39.9 (29–50)	45 (30–50)
7a	10.6 (6–30)	14.9 (6–37)	19.3 (6–40)	25.9 (6–47)	30.2 (18–50)	33.9 (20–50)	37.3 (20–50)
7b	16.6 (6–25)	21 (6–31)	25.5 (6–50)	30.7 (19–50)	36.1 (21–50)	40.7 (22–50)	44.3 (23–51)
8b	13.7 (6–25)	19 (6–27)	24.9 (18–34)	27.4 (12–50)	30.5 (10–50)	33.5 (6–50)	36.5 (6–50)
9a	13.7 (6–27)	19.4 (6–37)	21.3 (6–50)	22.5 (6–50)	27.6 (6–50)	30.6 (6–50)	33.5 (6–50)
9b	10.8 (6–23)	17.5 (6–25)	23.7 (16–30)	28.4 (20–37)	32.1 (21–42)	36.6 (24–47)	40.0 (27–50)
Metronidazole					16.3 (6–20)		
Amoxicillin		24.5 (20–28)					

113.01 (C-4, furan), 115.85 (C-3, furan), 146.83 (C-2, furan), 148.03 (C-5, furan), 152.45 (C-2, thiadiazole), 172.64 (C-5, thiadiazole); MS: *m/z* (%) 314 (M^+, 85), 238 (25), 156 (20), 127 (30), 100 (65), 82 (95). Anal. Calcd for $C_{10}H_{10}N_4O_4S_2$: C, 38.21; H, 3.21; N, 17.82. Found: C, 37.97; H, 3.11; N, 17.53.

*4.3.3. 4-[5-(5-Nitro-2-thienyl)-1,3,4-thiadiazol-2-yl]thiomorpholine 1-oxide (**6c**)*

M.p. 256–257 °C; IR (KBr): 1526, 1342 (NO_2), 1060 (S=O); ^{1}H NMR (DMSO-d_6): δ 8.17 (d, $J = 3.9$ Hz, 1H, thiophene), 7.60 (d, $J = 3.9$ Hz, 1H, thiophene), 4.12–3.92 (m, 4H, thiomorpholine), 3.09–2.88 (m, 4H, thiomorpholine); ^{13}C NMR (125 MHz, DMSO-d_6) δ 42.41 (C-2 and C-6, thiomorpholine), 44.13 (C-3 and C-5, thiomorpholine), 127.87 (C-3, thiophene), 131.59 (C-4, thiophene), 140.45 (C-2, thiophene), 150.97 (C-5, thiophene), 151.30 (C-2, thiadiazole), 172.87 (C-5, thiadiazole); MS: *m/z* (%) 330 (M^+, 67), 313 (18), 241 (18), 212 (10), 172 (25), 127 (30), 100 (100), 69 (85). Anal. Calcd for $C_{10}H_{10}N_4O_3S_3$: C, 36.35; H, 3.05; N, 16.96. Found: C, 36.06; H, 3.24; N, 17.26.

*4.4. General procedure for the preparation of 4-[5-(5-nitro-2-heteroaryl)-1,3,4-thiadiazol-2-yl]thiomorpholine 1,1-dioxide (**7a–c**)*

To a stirring suspension of 4-[5-(5-nitro-2-heteroaryl)-1,3,4-thiadiazol-2-yl]thiomorpholine (**5**, 0.3 mmol) in 4 ml of acetic acid was added hydrogen peroxide (30%, 1.5 ml) and heated up to 55–60 °C for 5 h. Water was added and the yellow precipitate was filtered and recrystallized from chloroform.

*4.4.1. 4-[5-(1-Methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazol-2-yl]thiomorpholine 1,1-dioxide (**7a**)*

M.p. 301–302 °C; IR (KBr): 1531, 1362 (NO_2), 1337 (SO_2); ^{1}H NMR (DMSO-d_6): δ 8.24 (s, 1H, imidazole), 4.34 (s, 3H, N–CH_3), 4.08 (br s, 4H, thiomorpholine), 3.36 (br s, 4H, thiomorpholine); ^{13}C NMR (125 MHz, DMSO-d_6) δ 35.94 (methyl), 49.36 (C-2 and C-6, thiomorpholine), 50.79 (C-3 and C-5, thiomorpholine), 133.97 (C-4, imidazole), 141.39 and 141.78 (C-2 and C-5, imidazole), 151.55 (C-2, thiadiazole), 172.20 (C-5, thiadiazole); MS: *m/z* (%) 344 (M^+, 80), 257 (30), 239 (40), 178 (20), 153 (75), 127 (90), 100 (95) and 73 (75). Anal. Calcd for $C_{10}H_{12}N_6O_4S_2$: C, 34.88; H, 3.51; N, 24.40. Found: C, 34.71; H, 3.37; N, 24.71.

*4.4.2. 4-[5-(5-Nitro-2-furyl)-1,3,4-thiadiazol-2-yl]thiomorpholine 1,1-dioxide (**7b**)*

M.p. 266–267 °C; IR (KBr): 3073 (CH aromatic), 1501, 1362 (NO_2), 1340 (SO_2); ^{1}H NMR (DMSO-d_6): δ 7.88 (d, $J = 3.9$ Hz, 1H, furan), 7.44 (d, $J = 3.9$ Hz, 1H, furan), 4.09 (t, $J = 5.3$ Hz, 4H, thiomorpholine), 3.36 (t, $J = 5.3$ Hz, 4H, thiomorpholine); MS: *m/z* (%) 330 (M^+, 70), 256 (50), 238 (30), 225 (25),178 (30), 127 (95), 80 (95), 54 (90). Anal. Calcd for $C_{10}H_{10}N_4O_5S_2$: C, 36.36; H, 3.05; N, 16.96. Found: C, 36.05; H, 3.17; N, 17.15.

*4.4.3. 4-[5-(5-Nitro-2-thienyl)-1,3,4-thiadiazol-2-yl]thiomorpholine 1,1-dioxide (**7c**)*

M.p. 302–303 °C; IR (KBr): 1511, 1352 (NO_2), 1311 (SO_2); ^{1}H NMR (DMSO-d_6): δ 8.18 (d, $J = 4.3$ Hz, 1H, thiophene), 7.62 (d, $J = 4.3$ Hz, 1H, thiophene), 4.06 (t, $J = 4.8$ Hz, 4H, thiomorpholine), 3.37 (t, $J = 4.8$ Hz, 4H, thiomorpholine); ^{13}C NMR (125 MHz, DMSO-d_6) δ 49.46 (C-2 and C-6, thiomorpholine), 50.61 (C-3 and C-5, thiomorpholine), 128.17 (C-3, thiophene), 131.66 (C-4, thiophene), 140.22 (C-2, thiophene), 151.43 (C-5, thiophene), 151.88 (C-2, thiadiazole), 172.63 (C-5, thiadiazole); MS: *m/z* (%) 346 (M^+, 20), 172 (20), 127 (50), 100 (95), 69 (45). Anal. Calcd for $C_{10}H_{10}N_4O_4S_3$: C, 34.67; H, 2.91; N, 16.17. Found: C, 34.86; H, 2.74; N, 16.49.

4.5. Biological activity

4.5.1. Bacterial isolates and culture conditions

Clinical *H. pylori* isolates from gastric biopsy specimens were obtained from the Shariati hospital (Tehran, Iran). Primary isolation was performed on selective blood agar base no. 2 (Oxoid, Basingstoke, Hants, UK) supplemented with horse blood 5% (v/v) and 1 selectatab tablet 500 mg (Mast Diagnostic, Merseyside, UK). Following primary selective

isolation, *H. pylori* bacterial cells were identified according to colony morphology, Gram staining, microaerophilic growth (at 37 °C), oxidase$^+$, catalase$^+$, urease$^+$, nitrate$^-$, H_2S^- and hippurate hydrolysis$^-$. Growth of *H. pylori* was maintained at 37 °C for 3–5 days in an atmosphere of 5% O_2, 15% CO_2, and 80% N_2 in an anaerobic chamber (Hirayama, Tokyo, Japan). Bacterial strains were stored at −70 °C in brain heart infusion broth (BHIB) (Difco, East Molesey, UK) containing 10% (v/v) fetal calf serum (FCS) and 15% (v/v) glycerol. Frozen clinical isolates were thawed and inoculated on Mueller–Hinton agar (MHA) plates (Oxoid) supplemented with 10% horse blood and incubated under microaerophilic conditions. Given the importance of inoculum homogeneity, cellular viability was controlled microscopically by morphological observation with Gram staining, in order to 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).

4.5.2. Bacterial growth inhibition assay (disk diffusion method)

Growth inhibition was performed by the filter paper disk 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 3–5 days at 37 °C, the inhibition zone around each disk (average diameter) if any, was recorded. All tests were performed in triplicate and the antibacterial activity was expressed as the mean of inhibition diameters (mm) produced by title compounds.

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