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Synthesis and anti-*Helicobacter pylori* activity of 5-(nitroaryl)-1,3,4-thiadiazoles with certain sulfur containing alkyl side chain

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Abstract—A series of 5-(nitroaryl)-1,3,4-thiadiazoles bearing certain sulfur containing alkyl side chain similar to pendent residue in tinidazole molecule were synthesized and evaluated against *Helicobacter pylori* using disk diffusion method. The synthesized compounds were also evaluated for their antibacterial, antifungal and cytotoxic effects. Study of the structure–activity relationships of this series of compounds indicated that both the structure of the nitroaryl unit and the pendent group on 2-position of 1,3,4-thiadiazole ring dramatically impact the anti-*H. pylori* activity. While compound **7a** containing 2-[2-(ethylsulfonyl)ethylthio]-side chain from nitrothiophene series was the most potent compound tested against clinical isolates of *H. pylori*, however, nitroimidazoles **6c** and **7c** were found to be more promising compounds because of their respectable anti-*H. pylori* activity besides less cytotoxic effects.

The pathogenic bacterium, *Helicobacter pylori*, infect half of the human population and is one of the genetically most diverse bacterial species known. Moreover, *H. pylori* is now a well-recognized cause of chronic active gastritis, peptic ulcer disease, gastric carcinoma, and mucosa-associated lymphoid tissue (MALT)-type gastric carcinoma, and its eradication is strongly recommended for patients with these diseases.[1,2]

Current treatment for *H. pylori* infections includes an anti-secretory agent plus two or more of the following antibiotics: amoxicillin, clarithromycin, nitroimidazoles, tetracycline, and levofloxacin.[3] Strains displaying primary resistance to nitroimidazoles and clarithromycin are being reported with increasing frequency throughout the world. Together with noncompliance, antibiotic resistance is a major cause of treatment failure in patients with these infections.[4,5] For this reason, there is a need for a safe and effective treatment with a compound having an excellent anti-*H. pylori* activity.

Nitroheterocyclic compounds such as nitroimidazoles, nitrofurans, and nitrothiophenes are being extensively used in therapy against amoebic and anaerobic infections.[6] Although metronidazole has been frequently used in treatment regimens for *H. pylori* infection, but other nitroheterocyclic drugs such as tinidazole and furazolidone (Fig. 1) have been used in place of metronidazole to treat *H. pylori* with varying degrees of success.[7] Moreover, the antimicrobial property of 1,3,4-thiadiazole derivatives is well documented and their attachment with other heterocycles often ameliorates the bioresponses

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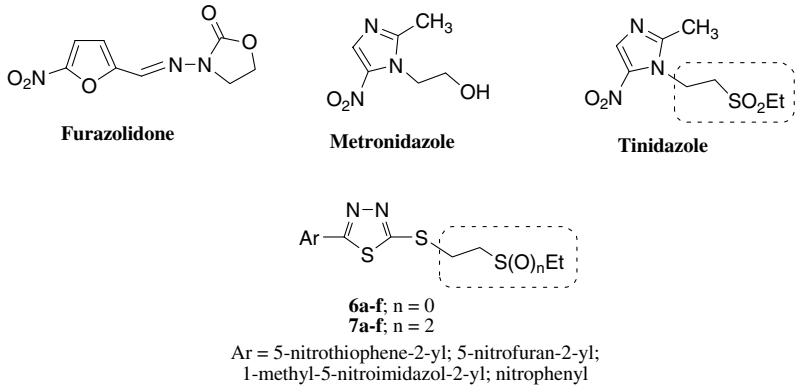


Figure 1. Structures of some nitroheterocycle antimicrobials used in the treatment of *H. pylori* infection and designed 2-[2-(ethylthio)ethylthio]-5-(nitroaryl)-1,3,4-thiadiazoles **6a**–**f** and 2-[2-(ethylsulfonyl)ethylthio]-5-(nitroaryl)-1,3,4-thiadiazoles **7a**–**f** as new anti-*H. pylori* agents.

depending on the type of substituent and position of attachment.[8] In view of the antimicrobial property of the above pharmacophores, it was envisaged that the combined effect of both nitroaryl and 1,3,4-thiadiazole entities would result in increased antimicrobial activity. We have previously reported the synthesis of some novel and biologically active 5-(nitroaryl)-1,3,4-thiadiazoles.[9–12] In continuation of our work on bioactive 5-(nitroaryl)-1,3,4-thiadiazoles and looking at the importance of ethylsulfonylethyl side chain in tinidazole molecule, it was thought that it would be worthwhile to design and synthesize 5-(nitroaryl)-1,3,4-thiadiazoles containing ethylsulfonylethyl or ethylthioethyl side chain to generate a series of new 5-(nitroaryl)-1,3,4-thiadiazole derivatives (**6a**–**f** and **7a**–**f**) and screen them for potential anti-*Helicobacter pylori* activity (Fig. 1).

Our synthetic route to target compounds **6a**–**f** and **7a**–**f** is shown in Fig. 2. The key intermediate 2-chloro-5-(nitroaryl)-1,3,4-thiadiazole **4** was prepared from commercially available 5-nitroarylcarboxaldehyde diacetate or nitroarylcarboxaldehyde according to the previously described methods.[9–11] The reaction of **4** with thiourea in refluxing ethanol afforded the 5-(nitroaryl)-1,3,4-thiadiazole-2-thiol **5**.[12,13] Treatment of **5** with 2-(ethylthio)ethyl chloride or 2-(ethylsulfonyl)ethyl chloride in the presence of potassium hydroxide afforded desired target compounds **6a**–**f** and **7a**–**f**, respectively.[14,15]

The synthesized compounds **6a**–**f** and **7a**–**f** were assessed against different species of Gram-positive (*Staphylococcus aureus* ATCC 6538p and *Staphylococcus epidermidis* ATCC 12228) and Gram-negative bacteria (*Escherichia coli* ATCC 8739 and *Klebsiella pneumoniae* ATCC 10031) and against various strains of pathogenic fungi (*Candida albicans* PTCC 5027, *Saccharomyces cerevisiae* PTCC 5177, *Microsporum gypseum* PTCC 5070 and *Aspergillus niger* PTCC 5012) using a conventional agar dilution method.[16,17] The data obtained against all the assayed species were in the 32 to >64 μg/mL range. From these results it was possible to submit all the synthesized compounds for subsequent screening toward *H. pylori*.

The anti-*H. pylori* activity of synthesized compounds was evaluated by comparing the inhibition zone diameters determined by the paper disk diffusion bioassay along with commercially available antibacterials, metronidazole and amoxicillin. Each compound with various concentrations was loaded on standard disks and the latter were placed on Mueller–Hinton agar plate, earlier inoculated with bacterial suspension. Following incubation for 3–5 days at 37 °C, the inhibition zone around each disk 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.[18] 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.

Preliminarily, compounds **6a**–**f** and **7a**–**f** were evaluated against both metronidazole sensitive and metronidazole resistant *H. pylori* strains at three concentrations (8, 16, and 32 μg/disk) and the results are shown in Table 1.

Table 1 reveals that all nitroheteroaryl derivatives (**6a**–**c** and **7a**–**c**) showed remarkable antimicrobial activity against both metronidazole-sensitive and metronidazole-resistant *H. pylori* strains at concentrations of 8, 16, and 32 μg/disk (inhibition zone diameter >20 mm). While nitrophenyl analogs (**6d**–**f** and **7d**–**f**) had no respectable inhibitory activity at concentrations of 8–32 μg/disk with the exception of 4-nitrophenyl derivatives **6f** and **7f**, the 4-nitrophenyl analog **7f** showed strong inhibitory activity but less than nitroheteroaryl derivatives at similar concentrations.

In view of the results obtained with nitroheteroaryl derivatives (**6a**–**c** and **7a**–**c**), we proceeded to survey the antibacterial potential of these compounds against a broader panel of *H. pylori*. For this purpose, the antibacterial activities of selected compounds (**6a**–**c** and **7a**–**c**) at concentrations of 32, 16, 8, 4, 2, 1, and 0.5 μg/disk were assessed against twenty clinical isolates of *H. pylori*.[19] The averages of inhibition zone diameters (in mm) of title compound in comparison to metronidazole and amoxicillin are presented in Table 2.

Table 2 reveals that all selected compounds show high activity against clinical isolates of *H. pylori* in compar-

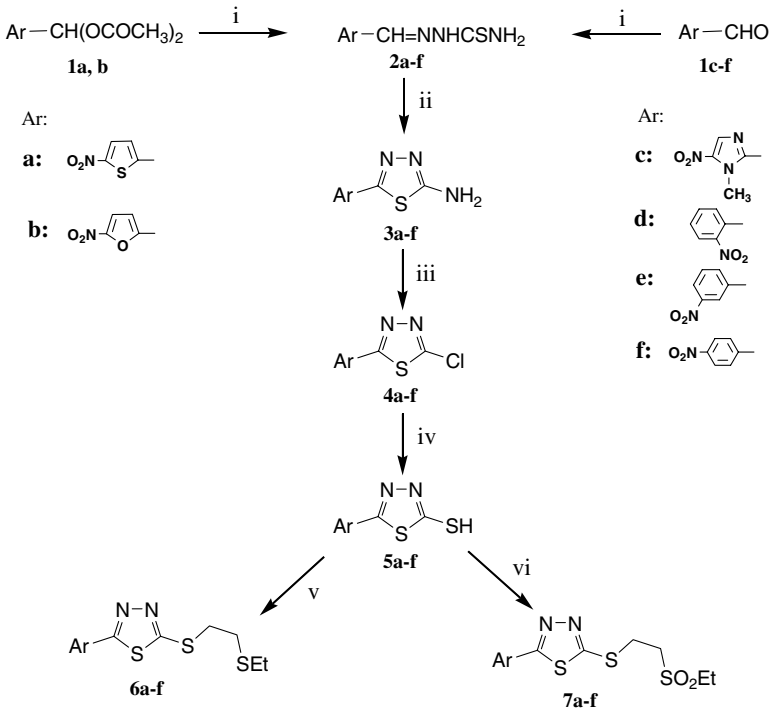
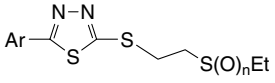


Figure 2. Reagents and conditions: (i) thiosemicarbazide, ethanol, and reflux; (ii) ammonium ferric sulfate, H_2O, and reflux; (iii) HCl, $NaNO_2$, and Cu; (iv) thiourea, EtOH, reflux, and then concentrated HCl; (v) 2-(ethylthio)ethylchloride, KOH, and EtOH; (vi) 2-(ethylsulfonyl)ethylchloride, KOH, and EtOH.

Table 1. Preliminary evaluation of compounds **6a**–**f** and **7a**–**f** against two metronidazole-sensitive and metronidazole-resistant *H. pylori* strains[a]



Compound	Ar	*n*	Metronidazole-sensitive Dose (μg/disk)			Metronidazole-resistant Dose (μg/disk)		
			8	**16**	**32**	**8**	**16**	**32**
6a	5-NO_2-thiophene	0	50	50	50	50	50	50
6b	5-NO_2-furan	0	22	31	42	16	21	28
6c	1-Me-5-NO_2-imidazole	0	32	37	48	35	47	59
6d	2-NO_2-phenyl	0	6	6	6	6	6	6
6e	3-NO_2-phenyl	0	6	6	9	6	6	9
6f	4-NO_2-phenyl	0	6	16	20	6	6	6
7a	5-NO_2-thiophene	2	>50	>50	>50	>50	>50	>50
7b	5-NO_2-furan	2	37	43	46	29	34	41
7c	1-Me-5-NO_2-imidazole	2	35	40	47	36	40	47
7d	2-NO_2-phenyl	2	6	9	15	6	9	13
7e	3-NO_2-phenyl	2	6	6	26	6	6	25
7f	4-NO_2-phenyl	2	19	24	30	19	24	27
Metronidazole			18	23	27	11	12	14

[a] Antibacterial activities was expressed as the mean of inhibition zone diameters (mm).

ison to standard drug, metronidazole, at the dose of 8 μg/disk. As a matter of fact, all nitroheteroaryl derivatives produced an inhibition zone on the average of more than 25 mm at the dose of 8 μg/disk, which was greater than that of metronidazole (16.3 mm). While metronidazole showed little activity at doses less than 8 μg/disk, target compounds showed strong activity against *H. pylori* at these concentrations. As can be deduced from these data, compound **7a** followed by **6a** and **6c** is superior in inhibiting the growth of *H. pylori*. It is notable that nitrothiophene analogue **7a** proved to be statistically the most potent compound in this series and the latter still exhibiting moderate activity at 0.5 μg/disk (averages of inhibition zone = 18.20 mm). The average of inhibition zone diameters for this compound was almost equivalent

Table 2. Average of inhibition zone diameters of selected compounds **6a–c** and **7a–c** at different doses against 20 clinical *H. pylori* isolates

Compound	Dose (μg/disk)						
	0.5	**1**	**2**	**4**	**8**	**16**	**32**
6a	16.8 (6–21)[a]	22.9 (18–27)	28.9 (21–34)	37.1 (26–44)	44.4 (31–50)	47.0 (35–50)	47.2 (34–50)
6b	6.0 (6–6)	11.1 (6–18)	16.4 (6–28)	21.5 (6–33)	28.3 (17–47)	30.7 (18–50)	34.9 (21–50)
6c	12.2 (6–23)	15.0 (6–36)	19.5 (6–50)	25.4 (6–50)	31.7 (15–50)	36.5 (21–50)	42.6 (31–53)
7a	18.2 (6–27)	24.9 (20–37)	31.9 (23–50)	37.5 (29–50)	44.2 (31–50)	46.7 (35–50)	47.6 (36–50)
7b	6.6 (6–10)	12.6 (6–21)	18.3 (6–32)	23.5 (13–35)	29.1 (17–50)	33.1 (18–50)	36.5 (20–50)
7c	8.5 (6–19)	11.9 (6–25)	16.5 (6–31)	23.4 (6–38)	29.7 (22–50)	35.8 (29–50)	40.7 (28–53)
Metronidazole	6.0 (6–6)	6.0 (6–6)	7.5 (6–12)	8.75 (6–19)	16.3 (6–20)	18.4 (6–32)	20.7 (6–35)
Amoxicillin		24.5 (20–28)					

[a] Antibacterial activities are expressed as means of inhibition zone diameters (mm). Range of inhibition zone diameters against 20 clinical *H. pylori* isolates is expressed in parentheses.

Table 3. In vitro cytotoxic activity of compounds **6a–c** and **7a–c** against mouse fibroblast (NIH/3T3) cell line

Compound	IC_{50}[a] (μg/mL)
6a	4.5 ± 1.9
6b	5 ± 0.7
6c	61.5 ± 7.7
7a	11.6 ± 2.2
7b	4.2 ± 0.3
7c	100 ± 7

[a] IC_{50} is the concentration required to inhibit 50% of cell growth. The values represent means ± standard deviation of triplicate determinations.

to that of reference drug amoxicillin at the dose of 1 μg/disk (Table 2).

From our results (Tables 1 and 2), it is possible to discern some quite prominent structure–activity relationships for the compounds. Generally, the type of nitroaryl moiety and pendent group on 1,3,4-thiadiazole dramatically impact anti-*H. pylori* activity. It could be seen that nitroheteroaryls exhibited more potent activity than nitrophenyl analogs. Among nitroheteroaryls, it is evident that nitrothiophene analogs showed more potent anti-*H. pylori* activity with respect to nitrofuran and nitroimidazole derivatives. The effect of positional substitution was primarily investigated by preparing all three possible nitro-regioisomers on phenyl-1,3,4-thiadiazoles. The better results were achieved with *para*-nitro substituent on phenyl ring (compounds **6f** and **7f**). Comparison between corresponding ethylthio analogs **6a–f** and ethylsulfonyl derivatives **7a–f** revealed that *S*,*S*-dioxidation of ethylthio compounds caused an improvement of anti-*H. pylori* in all aryl-1,3,4-thiadiazoles.

The most active compounds **6a–c** and **7a–c** were also assessed for their cytotoxicity using MTT colorimetric assay against normal mouse fibroblasts (NIH/3T3).[20,21] The IC_{50} values obtained for these compounds are shown in Table 3. Among the compounds tested, nitrothiophenes (**6a** and **7a**) and nitrofurans (**6b** and **7b**) showed greater cytotoxicity with IC_{50} values of $\leqslant$11.6 ± 2.2 μg/mL. Nitroimidazole derivatives **6c** and **7c** showed moderate cytotoxic effect on NIH/3T3 cell line at concentrations of 61.5 ± 7.7 and 100 ± 7 μg/mL, respectively. The results presented in Table 3 showed compound **7c** to be significantly less toxic than the other compounds.

In conclusion, we have identified a series of 5-(nitroaryl)-1,3,4-thiadiazoles bearing certain sulfur containing alkyl side chain similar to tinidazole molecule, with in vitro antibacterial activity against *H. pylori*. The structure–activity relationships of these compounds indicate that the type of nitroaryl moiety and pendent group on 1,3,4-thiadiazole ring dramatically impact anti-*H. pylori* activity. While nitrothiophene analog **7a** containing 2-[2-(ethylsulfonyl)ethylthio]-side chain was the most potent compound tested against clinical isolates of *H. pylori*, based on comparing the results of cytotoxicity and anti-*H. pylori* activity, nitroimiodazoles **6c** and **7c** would be more promising compounds because of their respectable anti-*H. pylori* activity and less cytotoxic effects.

Acknowledgments

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14. *General procedure for the synthesis of 2-[2-(ethylthio)ethylthio]-5-(nitroaryl)-1,3,4-thiadiazole* (**6a**–**f**). To a mixture of 5-(nitroaryl)-1,3,4-thiadiazole-2-thiol **5** (1.65 mmol) and 2-(ethylthio)ethyl chloride (1.8 mmol) in ethanol (10 mL), KOH solution (1.8 mmol in 2 mL H_2O) was added dropwise, and the mixture stirred at room temperature overnight. Then, water was added, and the separated solid filtered off, washed with water and crystallized from EtOH, to give compounds **6a**–**f**.
Compound **6a**. Yield 45%; mp 94–96 °C; IR (KBr): ν_{max} 3098 (thiophene), 1516, 1342 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.95 (d, 1H, thiophene, J = 4.3 Hz), 7.47 (d, 1H, thiophene, J = 4.3 Hz), 3.72 (t, 2H, SCH_2, J = 6.35 Hz), 2.89–2.43 (m, 4H, CH_2SCH_2), 1.26 (t, 3H, CH_3, J = 7.34 Hz); MS: *m/z* (%) 333 (M^+, 20), 246 (40), 172 (50), 127 (40), 88 (100), 59 (80). Anal. Calcd for $C_{10}H_{11}N_3O_2S_4$: C, 36.02; H, 3.32; N, 12.60. Found: C, 36.16; H, 3.21; N, 12.49.
Compound **6b**. Yield 70%; mp 79–81 °C; IR (KBr): ν_{max} 3144 (furan), 1536, 1347 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.43 (d, 1H, furan, J = 4.0 Hz), 7.14 (d, 1H, furan, J = 4.0 Hz), 3.65 (t, 2H, SCH_2, J = 6.35 Hz), 2.75–2.61 (m, 4H, CH_2SCH_2), 1.30 (t, 3H, CH_3, J = 7.26 Hz); MS: *m/z* (%) 317 (M^+, 10), 229 (40), 153 (50), 88 (100), 60 (55). Anal. Calcd for $C_{10}H_{11}N_3O_3S_3$: C, 37.84; H, 3.49; N, 13.24. Found: C, 37.69; H, 3.55; N, 13.17.
Compound **6c**. Yield 76%; mp 102–104 °C; IR (KBr): ν_{max} 3446 (imidazole), 1522, 1332 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.70 (s, 1H, imidazole), 4.55 (s, 3H, NCH_3), 3.63 (t, 2H, SCH_2, J = 7.35 Hz), 2.99 (t, 2H, CH_2S, J = 8.0 Hz), 2.66 (q, 2H, SCH_2–Me, J = 7.40 Hz) 1.31 (t, 3H, CH_3, J = 7.40 Hz); MS: *m/z* (%) 331 (M^+, 10), 244 (40), 168 (30), 89 (100), 59 (85); Anal. Calcd for $C_{10}H_{13}N_5O_2S_3$: C, 36.24; H, 3.95; N, 21.13. Found: C, 36.35; H, 3.83; N, 21.22.
Compound **6d**. Yield 65%; mp 105–108 °C; IR (KBr):ν_{max} 3118 (phenyl), 1536, 1485 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.10 (d, 1H, phenyl, J = 7.9 Hz), 7.76 (t, 1H, phenyl, J = 8.0 Hz), 7.65 (t, 1H, phenyl, J = 8.0 Hz), 7.40 (d, 1H, phenyl, J = 7.9 Hz), 3.64 (t, 2H, SCH_2, J = 8.0 Hz), 2.75–2.61 (m, 4H, CH_2SCH_2), 1.31 (t, 3H, CH_3, J = 7.10 Hz); MS: *m/z* (%) 327 (M^+, 10), 239 (100), 164 (65), 89 (70); Anal. Calcd for $C_{12}H_{13}N_3O_2S_3$: C, 44.02; H, 4.00; N, 12.83. Found: C, 43.94; H, 3.93; N, 12.96.
Compound **6e**. Yield 45%; mp 92–94 °C; IR (KBr): ν_{max} 3068 (phenyl), 1526, 1347 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.71 (s, 1H, phenyl), 8.23 (m, 2H, phenyl), 7.70 (t, 1H, phenyl, J = 8.0 Hz), 3.64 (t, 2H, SCH_2, J = 7.4 Hz), 2.99 (t, 2H, CH_2S, J = 7.6 Hz), 2.67 (q, 2H, SCH_2–Me, J = 7.4 Hz), 1.31 (t, 3H, CH_3, J = 7.4 Hz). MS: *m/z* (%) 327 (M^+, 80), 266 (20), 239 (90), 193 (15), 163 (100), 87 (80). Anal. Calcd for $C_{12}H_{13}N_3O_2S_3$: C, 44.02; H, 4.00; N, 12.83. Found: C, 44.16; H, 4.12; N, 12.96.
Compound **6f**. Yield 61%; mp 102–104 °C; IR (KBr): ν_{max} 3073 (phenyl), 1531, 1342 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.34 (d, 2H, phenyl, J = 8.9 Hz), 8.06 (d, 2H, phenyl, J = 8.9 Hz), 3.65 (t, 2H, SCH_2, J = 8.6 Hz), 3.00 (t, 2H, CH_2S, J = 8.6 Hz), 2.67 (q, 2H, SCH_2–Me, J = 7.3 Hz), 1.31 (t, 3H, CH_3, J = 7.3 Hz); MS: *m/z* (%) 327 (M^+, 20), 239 (100), 163 (60), 88 (60); Anal. Calcd for $C_{12}H_{13}N_3O_2S_3$: C, 44.02; H, 4.00; N, 12.83. Found: C, 43.96; H, 3.91; N, 12.72.
15. *General procedure for the synthesis of 2-[2-(ethylsulfonyl)ethylthio]-5-(nitroaryl)-1,3,4-thiadiazole* (**7a**–**f**). To a mixture of 5-(nitroaryl)-1,3,4-thiadiazole-2-thiol **5** (1.65 mmol) and 2-(ethylsulfonyl)ethyl chloride (1.8 mmol) in ethanol (10 mL), KOH solution (1.8 mmol in 2 mL H_2O) was added dropwise, and the mixture stirred at room temperature overnight. After adding water, the separated solid was filtered off, washed with water, and crystallized from EtOH, to give compounds **7a**–**f**.
Compound **7a**. Yield 42%; mp 122–124 °C; IR (KBr): ν_{max} 3100 (thiophene), 1536, 1348 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.88 (d, 1H, thiophene, J = 4.3 Hz), 7.23 (d, 1H, thiophene, J = 4.3 Hz), 3.64 (t, 2H, SCH_2, J = 8.30 Hz), 3.09–2.60 (m, 4H, $CH_2SO_2CH_2$), 1.30 (t, 3H, CH_3, J = 7.33 Hz); MS: *m/z* (%) 365 (M^+, 40), 273 (100), 197 (50), 83 (50). Anal. Calcd for $C_{10}H_{11}N_3O_4S_4$: C, 36.86; H, 3.03; N, 11.50. Found: C, 36.75; H, 3.15; N, 11.41.
Compound **7b**. Yield 55%; mp 72–74 °C; IR (KBr): ν_{max} 3134 (furan), 1536, 1362 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.45 (d, 1H, furan, J = 4.1 Hz), 7.30 (d, 1H, furan, J = 4.1 Hz), 3.64 (t, 2H, SCH_2, J = 7.35 Hz), 3.20–2.50 (m, 4H, $CH_2SO_2CH_2$), 1.31 (t, 3H, CH_3, J = 7.26 Hz); MS: *m/z* (%) 349 (M^+, 10), 227 (90), 150 (80), 66 (100). Anal. Calcd for $C_{10}H_{11}N_3O_5S_3$: C, 34.37; H, 3.17; N, 12.03. Found: C, 34.46; H, 3.22; N, 11.91.
Compound **7c**. Yield 50%; mp 123–125 °C; IR (KBr): ν_{max} 3119 (imidazole), 1516, 1342 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.07 (s, 1H, imidazole), 4.55 (s, 3H, NCH_3), 3.64 (t, 2H, SCH_2, J = 7.80 Hz), 3.10–2.57 (m, 4H, $CH_2SO_2CH_2$), 1.31 (t, 3H, CH_3, J = 7.50 Hz); MS: *m/z* (%) 363 (M^+, 10), 244 (100), 171 (45); Anal. Calcd for $C_{10}H_{13}N_5O_4S_3$: C, 33.05; H, 3.61; N, 19.27. Found: C, 32.90; H, 3.54; N, 19.12.
Compound **7d**. Yield 41%; mp 113–115 °C; IR (KBr): ν_{max} 3090 (phenyl), 1518, 1338(NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.08 (d, 1H, phenyl, J = 8.0 Hz), 7.70 (t, 1H, phenyl, J = 8.0 Hz), 7.56 (t, 1H, phenyl, J = 8.0 Hz), 7.38 (d, 1H, phenyl, J = 8.09 Hz), 3.64 (t, 2H, SCH_2, J = 7.9 Hz), 2.97 (t, 2H, CH_2SO_2, J = 7.9 Hz), 2.56 (q, 2H, SO_2CH_2–Me, J=7.20 Hz), 1.31 (t, 3H, CH_3, J = 7.20 Hz); MS: *m/z* (%) 359 (M^+, 10), 239 (100), 163 (85), 77 (40). Anal. Calcd for $C_{12}H_{13}N_3O_4S_3$: C, 40.10; H, 3.65; N, 11.69. Found: C, 40.21; H, 3.54; N, 11.72.
Compound **7e**. Yield 36%; mp 98–100 °C; IR (KBr): ν_{max} 3062 (phenyl), 1534, 1346 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.73 (s, 1H, phenyl), 8.29 (d, 1H, phenyl, J = 8.0 Hz), 8.10 (d, 1H, phenyl, J = 8.0 Hz), 7.72 (t, 1H, phenyl, J = 8.0 Hz), 3.64 (t, 2H, SCH_2, J = 7.4 Hz), 3.00–2.67 (m, 4H, $CH_2SO_2CH_2$), 1.31 (t, 3H, CH_3, J = 7.4 Hz); MS: *m/z* (%) 359 (M^+, 85), 266 (20), 239 (85), 193 (15), 163 (100), 87 (90); Anal. Calcd for $C_{12}H_{13}N_3O_4S_3$: C, 40.10; H, 3.65; N, 11.69. Found: C, 40.19; H, 3.74; N, 11.57.
Compound **7f**. Yield 44%; mp 156–158 °C; IR (KBr): ν_{max} 3073 (phenyl), 1531, 1342 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.28 (d, 2H, phenyl, J = 8.9 Hz), 7.95 (d, 2H, phenyl, J = 8.9 Hz), 3.90 (t, 2H, SCH_2, J = 8.4 Hz), 3.41 (t, 2H, CH_2SO_2, J = 8.4 Hz), 3.10 (q, 2H, SO_2CH_2–Me, J = 7.5 Hz), 1.57 (t, 3H, CH_3, J = 7.5 Hz); MS: *m/z* (%) 359 (M^+, 20), 238 (100), 162 (80), 75 (25). Anal. Calcd for $C_{12}H_{13}N_3O_4S_3$: C, 40.10; H, 3.65; N, 11.69. Found: C, 40.23; H, 3.74; N, 11.77.
16. *Antibacterial activity.* Twofold serial dilutions of the compounds were prepared in Mueller–Hinton agar. Drugs (6.4 mg) were dissolved in DMSO (1 mL) and the solution

diluted with water (9 mL). Further progressive double dilution with melted Mueller–Hinton agar was performed to obtain the required concentrations of 64, 32, 16 and 8 μg/mL. Petri dishes were inoculated with $1–5 \times 10^4$ colony-forming units (CFU) and incubated at 37 °C for 18 h. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the test compound, which resulted in no visible growth on the plate.

17. *Antifungal activity.* The in vitro antifungal activities of the synthesized compounds were determined by agar dilution method. Sabouraud dextrose agar was employed for fungal growth. Stock solutions of tested compounds were prepared in DMSO. Inocula containing approximately 10^5 CFUs/mL of fungi were prepared from broth cultures in log phase growth. Fungal plates were made in triplicate and incubated at 30 °C for about 24–48 h for yeast, about 72 h for moulds, and about 168 h for dermatophytes. The MIC was defined as the lowest concentration of the test compound, which resulted in no visible growth on the plate.
18. *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 disks (6 mm diameter). After drying in a fume hood, the disks were placed on the agar surface that was inoculated with *Helicobacter 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.
19. *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).
20. *MTT colorimetric assay.*[21] Briefly, cultures in the exponential growth phase were trypsinized and diluted in complete growth medium to give a total cell count of 5×10^4 cells/mL. One hundred microliters of suspension was added to wells of sterile 96-well plates. After plating, 50 μL of a serial dilution of every agent was added. Each compound dilution was assessed in triplicate. Three wells containing only normal mouse fibroblast (NIH/3T3) cells suspended in 150 μL of complete medium were used as control for cell viability. The plates were then incubated for 72 h. After incubation, 30 μL of a 5 mg/mL solution of MTT was added to each well and the plate was incubated for another 1 h. After incubation, the culture medium was replaced with 100 μL of DMSO. Then, the absorbance of each well was measured by using a microplate reader at 492 nm wavelengths. For each compound, dose–response curve was measured with different drug concentrations, and the concentration causing 50% cell growth inhibition (IC_{50}) compared with the control was calculated.
21. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.