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Synthesis and anti-*Helicobacter pylori* activity of (4-nitro-1-imidazolylmethyl)-1,2,4-triazoles, 1,3,4-thiadiazoles, and 1,3,4-oxadiazoles

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A series of [(4-nitro-1*H*-imidazol-1-yl)methyl]-1,2,4-triazoles and 1,3,4-thiadiazoles were prepared and evaluated for their activity against sensitive and resistant *H. pylori* strains. Study of the structure-activity relationship of these series of compounds indicated that the type of nitroimidazole moiety and the pendent group on the heteroaryl analog dramatically impact the anti-*H. pylori* activity. In triazole series, compound **5d**, containing a 4-methyl phenyl group on the triazole ring, was the most potent compound tested against both metronidazole-sensitive and -resistant strains at a concentration of 8 μg.

Key Words: 4-Nitroimidazole, 1,2,4-triazoles, 1,3,4-thiadiazoles, 1,3,4-oxadiazoles, anti-*H. pylori* activity

Introduction

It is well known that *Helicobacter pylori*, an s-shaped spiral microaerophilic gram-negative bacterium first isolated in human gastric mucosa in 1982,[1–3] is considered the major causative agent of several gastric pathologies, such as chronic active gastritis, peptic ulcer disease, and gastric cancer.[4–6]

Hence, the World Health Organization (WHO) has classified *H. pylori* as a Class 1 carcinogen in humans; the eradication of *H. pylori* can significantly reduce the risk of ulcer relapse and may help prevent corresponding

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gastric disorders.[7] Since oral administration of metronidazole, PPI, clarithromycin, and amoxicillin was put to use, infection can be cured in up to 80%-90% of cases. However, eradication is not always successful and a few problems have been observed in the use of these drugs, such as the emergence of drug resistance, especially against metronidazole, clarithromycin, and amoxicillin,[8] and low compliance[9] related to the occurrence of a number of harmful side effects.[10−11] Nitroimidazole compounds, especially metronidazole, have been frequently used in treatment regimens for *H. pylori* infection; moreover, the antimicrobial properties of 1,2,4-triazole, 1,3,4-oxadiazole, and 1,3,4-thiadiazole derivatives are well documented. Their attachment with other heterocycles often ameliorates the bioresponses, depending on the type of substituent and the position of attachment.[12−16]

In continuation of our research on nitroheterocyclic compounds as anti-*H. pylori* agents,[17−20] we decided to synthesize a new series of (4-nitroimidazol-1-ylmethyl)-1,2,4-triazoles (**4a-e** and **5a-l**), 1,3,4-thiadiazoles (**6a-h**), and 1,3,4-oxadiazoles (**7a-d**) and evaluate their activity against clinical isolates of *H. pylori* strains.

Experimental

Melting points were determined on a Kofler hot-stage microscope and were corrected. The ^{1}H-NMR spectra were obtained using Bruker FT-80 or Varian Unity Plus 400 instruments with tetramethylsilane as the internal standard. The solvents used were $CDCl_3$ and DMSO-d6. IR spectra were recorded on a Nicolet Magna FTIR 550 spectrometer (KBr disks). Mass spectra were taken using a Finnigan TSQ-70 at 70 eV. Elemental analyses were carried out on a CHN-O-rapid elemental analyzer (Heraeus GmbH, Hanau, Germany) for C, H, and N, and the results were within $\pm 0.4\%$ of the theoretical values. Liarre Starsonic ultrasonic cleaner (Italy) was used as an ultrasonic device with a generator variable frequency of 28-34 KHz and 100 W of power line heating.

2-(4-Nitro-1*H*-imidazol-1-yl)acetohydrazide (2a)

Hydrazine hydrate (15 mL) was added dropwise to a stirred solution of **1a** (12 g, 0.06 mol) in methanol (40 mL) in an ice-water bath. The mixture was stirred at the same temperature for 2 h. The precipitate was filtered and recrystallized from methanol to give 10.4 g (83%) of **2a**; mp 122-123 °C. IR (KBr): ν = 3345, 3300, 3124 (NH-NH_2), 1660 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$): δ 8.27 (d, J = 1.2 Hz, H-5 imidazole), 7.77 (d, J= 1.2 Hz, 1H, H-2 imidazole), 4.76 ppm (s, 2H, N-CH_2). MS m/z (%): 185 (M^+, 78), 154 (44), 139 (75), 127 (100), 114(18), 110 (12). Anal. Calcd. for $C_5H_7N_5O_3$; C, 32.44; H, 3.81; N, 37.83. Found C, 32.49; H, 3.79; N, 37.56.

2-(2-Methyl-4-nitro-1*H*-imidazol-1-yl)acetohydrazide (2b)

This compound was prepared in a similar manner to **2a** with 87% yield. Mp 190-192 °C (methanol). IR (KBr): ν = 3343, 3269, 3151 (NH-NH_2), 1695 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$): δ 8.24 (s, 1H, H-5 imidazole), 4.70 (s, 2H, N-CH_2), 2.33 ppm (s, 3H, CH3). MS m/z (%): 199 (M^+, 90), 168 (85), 152 (37), 141 (100), 124 (18), 81 (9). Anal. Calcd. for $C_6H_9N_5O_3$; C, 36.18; H, 4.55; N, 35.16. Found C, 36.50; H, 4.77; N, 35.07.

General procedure for the synthesis of 4-alkyl(aryl)-5-[(4-nitro-1*H*-imidazol-1-yl) methyl]-4*H*-1,2,4-triazole-3-thiol (4a-d)

To a stirred solution of compound **2** (0.01 mol) in ethanol (20 mL), NaOH (2 N, 5 mL) and alkyl(aryl)isothiocyanate (0.01 mol) were added. The mixture was stirred for 2 h at room temperature and then acidified with an aqueous solution of HCl in an ice bath. The mixture was kept in a refrigerator for 3 h to complete the precipitation. The precipitate was filtered and crystallized from ethanol to give compounds **3a-m**, which were pure enough to be used in the next reaction. Compound **3** (0.01 mol) was dissolved in 10 mL of an aqueous solution of sodium bicarbonate (5%) while being heated and stirred. The mixture was refluxed for 2.5 h, cooled to room temperature, and acidified with an aqueous solution of HCl in an ice bath. The precipitate was filtered and crystallized from ethanol to give **4a-d**.

The chemical and physical properties of compounds **4a-d** are given in Table 1.

General procedure for the synthesis of 4-alkyl(aryl)-5-[(4-nitro-1*H*-imidazol-1-yl) methyl]-3-methylthio-1,2,4-triazoles (5b-i)

To a mixture of compound **4** (1.6 mmol) in ethanol (1 mL), 0.83 mL of an aqueous solution of sodium hydroxide (10%) was added; it was kept in ultrasonic conditions for 1 min. Methyl iodide (0.15 mL, 2.4 mmol) was added to the solution and the reaction was continued for 20 min. The precipitate was filtered and crystallized from ethanol to give compounds **5b-i**.

The chemical and physical properties of compounds **5b-i** are presented in Table 1.

General procedure for the synthesis of 5-alkyl(aryl)amino-2-[(4-nitro-1*H*-imidazol-1-yl)methyl]1,3,4-thiadiazoles (6a-g)

A mixture of compound **3** (0.6 mmol) and concentrated H_2SO_4 (1 mL) was stirred overnight at room temperature, poured into ice-cold water, and neutralized with liquid ammonia, and the resulting solid was crystallized from methanol to give compounds **6a-g**.

The chemical and physical properties of compounds **6a-g** are shown in Table 1.

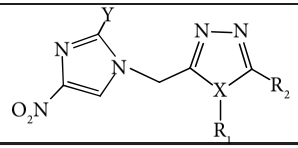
The synthesis and physicochemical properties of compounds **4b**, **4c**, **4e**, **5a**, **5f**, **5g**, **5j**, **5k**, **5l**, **6d**, **6h**, and **7a-d** were reported previously.[17]

Biological activity

Bacterial isolates and culture conditions

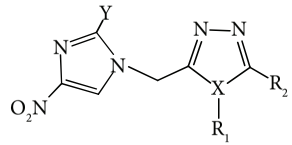
Clinical *H. pylori* isolates from gastric biopsy specimens were obtained from Shariati Hospital (Tehran, Iran). Primary isolation was performed on a selective blood agar base (Oxoid, Basingstoke, UK) supplemented with horse blood (5%, v/v) and 1 Selectatab tablet (500 mg; Mast Diagnostics, Merseyside, UK). Following primary selective isolation, *H. pylori* bacteria cells were identified according to colony morphology, Gram staining, microaerophilic growth at 37 °C, oxidase +, catalase +, urease +, nitrate -, H_2S, and hippurate hydrolysis

Table 1. Chemical and physical properties of derivatives **4a-6g**.

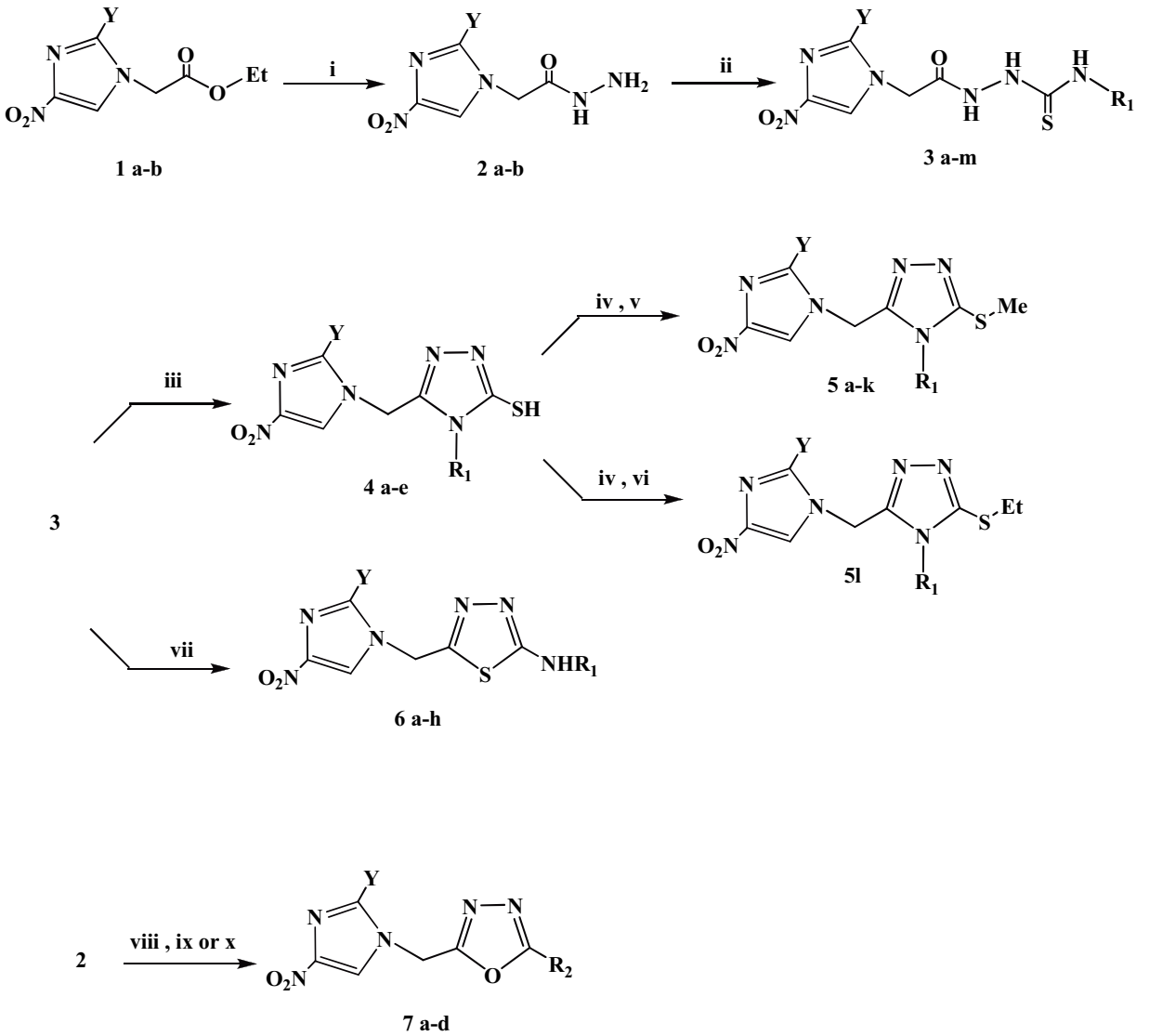


Comp.	X	Y	R_1	R_2	mp (°C)	Yield (%)	^{1}H-NMR (DMSO-d_6)	IR (KBr) cm^{-1}	Elemental Analysis
4a	N	H	C_2H_5	SH	230-232	72	13.65 (bs, 1H, SH), 8.42 (d, J = 1.4 Hz, 1H, H-5 imidazole), 7.95 (d, J = 1.4 Hz, 1H, H-2 imidazole), 5.52 (s, 2H, CH_2), 4.01 (q, J = 7.1 Hz, 2H, CH_2), 1.14 (t, J = 7.1 Hz, 3H, CH_3).	1335.9, 1545.6 (NO_2), 2951.5 (C-H aliphatic), 3099.0 (C-H aromatic).	$C_8H_{10}N_6O_2S$; C, 37.79; H, 3.96; N, 33.05; Found C, 37.41; H, 4.22; N, 33.27.
4d	N	H	4-F-C_6H_4	SH	266-268	80	13.89 (bs, 1H, SH), 8.15 (d, J = 1.3 Hz, 1H, H-5 imidazole), 7.63 (d, J = 1.3 Hz, 1H, H-2 imidazole), 7.47-7.37 (m, 4H, phe nyl), 5.31 (s, 2H, CH_2).	1324.3, 1514.6 (NO_2), 2943.7 (C-H aliphatic), 3048.5 (C-H aromatic).	$C_{12}H_9FN_6O_2S$; C, 45.00; H, 2.83; N, 26.24; Found C, 44.56; H, 2.49; N, 25.54.
5b	N	H	C_6H_{11}	SMe	153-155	40	8.37 (s, 1H, H-5 imidazole), 7.93 (s, lH, H-2imidaz ole), 5.67 (s, 2H, N-CH_2), 2.65 (s, 3H, S-CH_3), 2.22-1.10 (m, 11H, C_6H_{11}).	1332.0, 1541.7 (NO_2), 2932.0 (C-H aliphatic), 3114.6 (C-H aromatic).	$C_{13}H_{18}N_6O_2S$; C, 48.43; H, 5.63; N, 26.07; Found C, 49.12; H, 5.98; N, 27.11.
5c	N	H	C_6H_5	SMe	173-176	86	8.08 (d, J = 1.4 Hz, 1H, H-5 imidazole), 7.64 (d, J = 1.4 Hz, lH, H-2 imidazole), 7.58-7.47 (m, 5H, phenyl), 5.43 (s, 2H, CH_2), 2.57 (s, 3H, SCH_3).	1398.1, 1537.9 (NO_2), 2928.2 (C-H aliphatic), 3106.8 (C-H aromatic).	$C_{13}H_{12}N_6O_2S$; C, 49.36; H, 3.82; N, 26.57; Found C, 49.38; H, 3.51; N, 26.55.
5d	N	H	4-CH_3-C_6H_4	SMe	154-155	50	8.05 (d, J = 1.4 Hz, 1H, H-5 imidazole), 7.58 (d , J = l.4 Hz, 1H, H-2 imidazole), 7.43-7.18 (m, 4H, phenyl), 5.39 (s, 2H, CH_2), 2.54 (s, 3H, SCH_3), 2.37 (s, 3H, CH_3).	1328.2, 1518.4 (NO_2), 2970.9 (C-H aliphatic), 3102.9 (C-H aromatic).	$C_{14}H_{14}N_6O_2S$; C, 50.90; H, 4.27; N, 25.44; Found C, 51.46; H, 5.16; N, 27.03.
5e	N	CH_3	4-CH_3-C_6H_4	SMe	124-126	80	7.71 (s, 1H, H-5 imidazole), 7.41-7.09 (m, 4H, phen yl), 5.29 (s, 2H, CH_2), 2.50 (s, 3H, SCH_3), 2.33 (s, 3H, CH_3), 2.10 (s, 3H, CH_3).	1386.4, 1545.6 (NO_2), 3071.8 (C-H aromatic).	$C_{15}H_{16}N_6O_2S$; C, 52.31; H, 4.68; N, 24.40; Found C, 51.55; H, 4.06; N, 23.87.
5h	N	H	4-F-C_6H_4	SMe	152-154	66	8.08 (d, J = l.4 Hz, 1H, H-5 imidazole), 7.61 (d, J = 1.4 Hz, 1H, H-2 imidazole), 7.49-7.39 (m, 4H, phenyl), 5.40 (s, 2H, CH_2), 2.57 (s, 3H, SCH_3).	1343.7, 1549.5 (NO_2), 2963.1 (C-H aliphatic), 3064.1 (C-H aromatic).	$C_{13}H_{11}FN_6O_2S$; C, 46.70; H, 3.32; N, 25.14; Found C, 44.98; H, 3.02; N, 24.22.

Table 1. Continued.



Comp.	X	Y	R_1	R_2	mp (°C)	Yield (%)	^{1}HNMR (DMSO-d_6)	IR (KBr) cm^{-1}	Elemental Analysis
5i	N	CH_3	4-F-C_6H_4	SMe	194-197	86	7.81 (s, 1H, H-5 imidazole), 7.61-7.32 (m, 4H, phen yl), 5.34 (s, 2H, CH_2), 2.56 (s, 3H, S-CH_3), 2.19 (s, 3H, CH_3).	1398.1, 1545.6 (NO_2), 2928.2 (C-H aliphatic), 3068.0 (C-H aromatic).	$C_{14}H_{13}FN_6O_2S$; C, 48.27; H, 3.76; N, 24.12; Found C, 49.98; H, 4.56; N, 24.87.
6a	S	H	-	$NHCH_3$	187-190	87	8.13 (d, J = 1.6 Hz, 1H, H-5 imidazole), 7.92(d, J = 1.6 Hz, 1H, H-2 imidazole), 5.49 (s, 2H, CH_2), 3.55 (s, 3H, N-CH_3).	1335.9, 1541.7 (NO_2), 2928.2 (C-H aliphatic), 3114.6 (C-H aromatic).	$C_7H_8N_6O_2S$; C, 35.00; H, 3.36; N, 34.98; Found C, 34.17; H, 3.12; N, 34.02.
6b	S	CH_3	-	$NHCH_3$	201-203	88	7.51 (s, 1H, H-5 imidazole), 5.36 (s, 2H, CH_2), 3.58 (s, 3H, N-CH_3), 2.46 (s, 3H, CH_3).	1332.0, 1545.6 (NO_2), 2974.8(C-H aliphatic), 3149.5 (C-H aromatic).	$C_8H_{10}N_6O_2S$; C, 37.79; H, 3.96; N, 33.05; Found C, 38.43; H, 4.08; N, 33.87.
6c	S	H	-	NHC_2H_5	201-203	88	8.37 (d, J = l.4 Hz, 1H, H-5 imidazole), 7.91(d, J = l.4 Hz, 1H, H-2 imidazole), 5.56 (s, 2H, CH_2), 3.47 (q, J = 7.2 Hz, 2H, CH_2), 1.16 (t, J = 7.2 Hz, 3H, CH_3).	1332.0, 1549.5 (NO_2), 2990.3 (C-H aliphatic), 3118.4 (C-H aromatic).	$C_8H_{10}N_6O_2S$; C, 37.79; H, 3.96; N, 33.05; Found C, 36.65; H, 3.09; N, 32.52.
6e	S	H	-	NHC_6H_{11}	149-152	72	8.40 (d, J = 1.3 Hz, 1H, H-5 imidazole), 7.93 (d, J = 1.3 Hz, 1H, H-2 imidazole), 5.54 (s, 2H, N-CH_2), 2.21-0.98 (m, 11H, C_6H_{ll}).	1328.2, 1545.6 (NO_2), 2928.2(C-H aliphatic), 3137.9 (C-H aromatic).	$C_{12}H_{16}N_6O_2S$; C, 46.74; H, 5.23; N, 27.25; Found C, 46.93; H, 6.02; N, 27.87.
6f	S	H	-	NHC_6H_5	206-209	87	8.40 (d, J = 1.4 Hz, 1H, H-5), 7.95 (d, J = 1.4 Hz, 1H, H2 imidazole), 7.63-7.21 (m, 5H, phenyl), 5.66 (s, 2H, N-CH_2).	1335.9, 1557.3 (NO_2), 3079.6 (C-H aromatic).	$C_{12}H_{10}N_6O_2S$; C, 47.68; H, 3.33; N, 27.80; Found C, 47.99; H, 3.56; N, 28.11.
6g	S	H	-	$NH(4\text{-}CH_3)C_6H_4$	199-201	86	8.41 (d, J = 1.3 Hz, 1H, H-5 imidazole), 7.96 (d, J = 1.3 Hz, 1H, H-2 imidazole), 7.62-7.11 (m, 4H, phenyl), 5.65 (s, 2H, CH_2), 2.26 (s, 3H, CH_3).	1332.0, 1553.4 (NO_2), 3118.4 (C-H aromatic).	$C_{13}H_{12}N_6O_2S$; C, 49.36; H, 3.82; N, 26.57; Found C, 49.78; H, 4.11; N, 26.96.



Scheme. Reagents: i) $NH_2NH_2.H_2O$, ii) $R_1N{=}C{=}S$, iii) Na_2CO_3, iv) NaOH, v) MeI, vi) EtI, vii) H_2SO_4, viii) CS_2, ix) KOH, x) BrCN; Y=H, CH_3; R_1 = Et, n-Bu, C_6H_{11}, C_6H_5, 4-CH_3-C_6H_4, 4-OCH_3-C_6H_4, 4-F-C_6H_4, 4-NO_2-C_6H_4. **7a-b**: R_2 = SH; **7c-d**: $R_2 = NH_2$. **3a**: $R_1 = C_2H_5$, Y=H; 3b: R_1 = n-Bu, Y=H; 3c: R_1 = n-Bu, Y=CH_3; 3d: $R_1 = C_6H_{11}$, Y=H. **3e**: $R_1 = C_6H_5$, Y=H; 3f: R_1 = 4-CH_3-C_6H_4, Y=H; 3g: R_1 = 4-CH_3-C_6H_4, Y=CH_3. **3h**: R_1 = 4-OCH_3-C_6H_4, Y=H; 3i: R_1 = 4-OCH_3-C_6H_4, Y=CH_3; 3j: R_1 = 4-F-C_6H_4, Y=H. **3k**: R_1 = 4-F-C_6H_4, Y=CH_3; 3l: R_1 = 4-NO_2-C_6H_4, Y=H; 3m: R_1 = 4-NO_2-C_6H_4, Y=CH_3.

-. 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). To maintain a moist atmosphere, a moist paper towel was placed in the chamber. 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 control the proportions of coccoid cells in the cultures, cultures were always used after 48 h of incubation, when they generally did not present coccoid forms. Bacterial growth was taken from the plates and resuspended in sterile saline. The inoculum was prepared to contain 5×10^7 CFU/mL by adjusting the turbidity of the suspension to match the McFarland No. 2 standard.

Bacterial growth inhibition assay (disk diffusion method)

Growth inhibition was performed by the filter paper disk diffusion method on selective Brucella agar with 7% defibrinated horse blood under microaerophilic conditions at 37 °C. The samples were evaluated for their anti-*Helicobacter* activity, dissolved in dimethylsulfoxide (DMSO). All compounds were assayed against metronidazole-sensitive and metronidazole-resistant *H. pylori* strains at 3 concentrations (8, 16, and 32 μg/disk); the surfaces of the Brucella blood agar plates were inoculated with 100 μL of bacterial suspensions. Blank standard disks (6 mm in diameter) were deposited on the plates and impregnated with 10 μL of different dilutions of test compounds. Following incubation for 3-5 days at 37 °C, the inhibition zone around each disk (average diameter), if any, was recorded. The control disks received 10 μL of DMSO. All tests were performed in triplicate and the antibacterial activity was expressed as the mean of inhibition diameters (mm) produced by the tested compounds. 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.[21]

Result and discussion

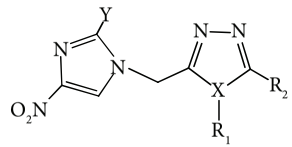
Chemistry

The title compounds were synthesized according to the sequence shown in the Scheme. As we have reported previously, compounds **1a-b** were prepared by a known procedure.[17] Reaction of the ester group of compounds **1a-b** with hydrazine hydrate gave corresponding acetic acid hydrazides **2a-b**. Treatment of compounds **2a-b** with different isothiocyanates gave thiosemicarbazide **3**, which, on cyclization with aqueous Na_2CO_3 or concentrated H_2SO_4, afforded 1,2,4-triazole-5-thiones **4a-e** or 5-amino-l,3,4-thiadiazoles **6a-h**, respectively. The reaction of compounds **4a-e** with alkyl iodide in alkaline media under ultrasonic conditions produced alkylthio derivatives **5a-l**.

Anti-*Helicobacter pylori* activity

The preliminary evaluation of compounds **4-7** against 2 metronidazole-sensitive and metronidazole-resistant *H. pylori* strains are summarized in Table 2. Some of the synthesized triazole analogs (**5d**, **5g**), one of the thiadiazole analogs (**6g**), and one of the oxadiazole derivatives (**7d**) exhibited strong antimicrobial activity against both metronidazole-sensitive and metronidazole-resistant *H. pylori* strains at concentrations of 16 and 32 μg/disk (inhibition zone diameter: >20 mm). Compound **5d** was the most potent compound tested, displaying strong activity at 8 μg/disk (inhibition zone diameter: 23 mm) against both metronidazole-sensitive and metronidazole-resistant strains.

Table 2. Preliminary evaluation of compounds **4-7** against two metronidazole-sensitive and metronidazole-resistant *H. Pylori* strains.



Comp.	X	Y	R_1	R_2	Metronidazole-Sensitive[a] (M.S.) Dose (µg/disk)			Metronidazole-Resistant (M.R.) Dose (µg/disk)		
					8	16	32	8	16	32
4a	N	H	C_2H_5	SH	15	22	31	6[b]	6	16
4b	N	H	n-Bu	SH	6	6	6	6	6	6
4c	N	CH_3	n-Bu	SH	6	6	6	6	6	6
4d	N	H	4-F-C_6H_4	SH	6	6	6	6	6	6
4e	N	H	4-NO_2-C_6H_4	SH	6	6	6	6	6	6
5a	N	CH_3	n-Bu	SMe	6	6	6	6	6	6
5b	N	H	C_6H_{11}	SMe	6	6	6	6	6	6
5c	N	H	C_6H_5	SMe	16	26	32	6	17	21
5d	N	H	4-CH_3-C_6H_4	SMe	23	32	42	21	29	38
5e	N	CH_3	4-CH_3-C_6H_4	SMe	6	6	6	6	6	6
5f	N	H	4-OCH_3-C_6H_4	SMe	6	6	6	6	6	6
5g	N	CH_3	4-OCH_3-C_6H_4	SMe	17	20	22	19	21	24
5h	N	H	4-F-C_6H_4	SMe	16	26	32	6	6	25
5i	N	CH_3	4-F-C_6H_4	SMe	6	6	6	6	6	6
5j	N	H	4-NO_2-C_6H_4	SMe	6	6	16	15	19	31
5k	N	CH_3	4-NO_2-C_6H_4	SMe	6	6	6	6	6	6
5l	N	H	4-NO_2-C_6H_4	SEt	12	17	25	10	18	26
6a	S	H	-	$NHCH_3$	6	6	6	6	6	6
6b	S	CH_3	-	$NHCH_3$	6	6	6	6	6	6
6c	S	H	-	NHC_2H_5	6	6	6	6	6	6
6d	S	H	-	NH n-Bu	6	6	6	6	6	6
6e	S	H	-	NHC_6H_{11}	6	6	6	6	6	6
6f	S	H	-	NHC_6H_5	6	6	6	6	6	6
6g	S	H	-	NH(4-CH_3)C_6H_4	19	23	25	21	26	33
6h	S	H	-	NH(4-NO_2)C_6H_4	6	6	16	6	6	14
7a	O	H	-	SH	6	6	6	6	6	6
7b	O	CH_3	-	SH	6	6	6	6	6	6
7c	O	H	-	NH_2	6	6	6	6	6	6
7d	O	CH_3	-	NH_2	6	25	32	16	30	36

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

[b]No inhibition observed (disk diameter was 6 mm).

Generally, the type of nitroimidazole moiety and the pendent group on the heteroaryl analog impact the anti-*H. pylori* activity. In triazole series, substitution of an aromatic ring at the N_4 position of the 1,2,4-triazole ring with phenyl **(5c)**, 4-methylphenyl **(5d)**, 4-methoxyphenyl **(5g)**, and 4-flurorophenyl **(5h)** resulted in compounds with potent anti-*H. pylori* activity. Replacement of the aryl group at the N_4 position with aliphatic moiety eliminated the antibacterial activity, except for compound **4a** having an ethyl group in that position.

Methylation of the thiol group in compound **4e**, having 4-nitrophenyl moiety at the N_4 position, resulted in compound **5j** with activity against metronidazole-resistant strains. Replacement of methyl with an ethyl group (compound **5l**) increased anti-*H. pylori* activity against both metronidazole-sensitive and metronidazole-resistant strains. The same result was obtained when the thiol analog **4d** was methylated (compound **5h**).

In 1,3,4-thiadiazole series, substitution of the amino group at the 5 position with alkyl moiety resulted in compounds without anti-*H. pylori* activity. However, substitution with 4-methylphenyl moiety resulted in compound **6g**, which showed a potent inhibitory effect against both metronidazole-sensitive and metronidazole-resistant strains.

Compound **7d**, with a 5-amino group on the oxadiazole ring, was the most potent compound in this series, which may show the superiority of the amino group to the thiol group in this position.

As can be seen in Table 2, compounds **5j**, **6g**, and **7d** showed better activity against resistant strains of *H. pylori* compared to sensitive strains, which possibly reveals the interference of the mentioned compounds in the resistance mechanism.

In conclusion, we identified a series of (4-nitro-1-imidazolylmethyl)-1,2,4-triazoles, 1,3,4-thiadiazoles, and 1,3,4-oxadiazole with in vitro anti-*H. pylori* activity. Biological data indicated that triazole analog **5d**, containing 4-methylphenyl and methylthiol moiety, was the most potent compound tested, which makes this compound a promising lead for the development of an effective therapeutic agent for anti-*Helicobacter* chemotherapy.

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References

1. Marshall, B. J.; Warren, J. R. *Lancet* **1984**, *1*, 1311-1315.
2. Marshal, B. J. *Am. J. Gastroenterol.* **1994**, *89*, S116-S128.
3. Lee, A.; O'Rourke, J. In *Biology and Clinical Practice*; Goodwin, C. S.; Worsley, B. W.; Eds.; CRC Press, Boca Raton, FL, 1993.
4. Nomura, A.; Stemmermann, G. N.; Chyou, P. H.; Perez, G. I.; Blaser, M. I. *Ann. Intern. Med.* **1994**, *120*, 977-981.
5. Sipponen, P.; Hyvarinen, H. *Scand. J. Gastroenterol.* **1993**, *Suppl. 196*, 3-6.
6. Labenz, J.; Borsch, G. *Gut* **1994**, *35*, 19-22.

7. NIH Consensus Development Panel on *Helicobacter pylori* in peptic ulcer disease. *J. Am. Med. Assoc.* **1994**, *272*, 65-69.
8. Megraud, F. *Aliment. Pharmacol. Ther.* **1997**, *11*, 43-53.
9. Graham, D. Y.; Lew, G. M.; Malaty, H. M.; Evans, D. G. Jr.; Klein, P. D.; Alpert, L. C.; Genta, R.M. *Gastroenterology* **1992**, *102*, 493-496.
10. Penston, J. G. *Aliment. Pharmacol. Therap.* **1994**, *8*, 369-389.
11. Moayyedi, P.; Sahay, P.; Tompkins, D. S. *Eur. J. Gastroenterol. Hepatol.* **1995**, *7*, 835-840.
12. Hui, X. P.; Zhang, L. M.; Zhang, Z. Y.; Wang, Q.; Wang, F. *Indian J. Chem.* **1999**, *38B*, 1066-1069.
13. Ashour, F. A.; Habib, N. S.; el Taibbi, M.; el Dine, S. el Dine, A. S. *Farmaco* **1990**, *45*, 1341-1349.
14. Muhieldeen, Z.; Nadir, M.; Aljobory, N. R.; Husseen, F.; Stohs, S. J. *Eur. J. Med. Chem.* **1991**, *26*, 237-242.
15. Demirbaş, N. *Turk. J. Chem.* **2005**, *29*, 125-133.
16. Demirbaş, N.; Demirbaş, A.; Ceylan, S.; Şahin, D. *Turk. J. Chem.* **2008**, *32*, 1-8.
17. Vosooghi, M.; Akbarzadeh, T.; Fallah, A.; Fazeli, M. R.; Jamalifar, H.; Shafiee, A. *J. Sci. I. R. Iran,* **2005**, *16*, 145-151.
18. Letafat, B.; Emami, S.; Aliabadi, A.; Mohammadhosseini, N.; Moshafi, M. H.; Asadipour, A.; Shafiee, A.; Foroumadi, A. *Arch. Pharm. (Weinheim)* **2008**, *341*, 497-501.
19. Mohammadhoseini, N.; Asadipour, A.; Letafat, B.; Vosooghi, M.; Siavoshi, F.; Shafiee, A.; Foroumadi, A. *Turk. J. Chem.* **2009**, *33*, 471-478.
20. Moshafi, M. H.; Sorkhi, M.; Emami, S.; Nakhjiri, M.; Yahya-Meymandi, A.; Negahbani, A. S.; Siavoshi, F.; Omrani, M.; Alipour, E.; Vosooghi, M.; Shafiee, A.; Foroumadi, A. *Arch. Pharm. Chem. Life Sci.* **2010**, in press, DOI:10.1002/ardp.201000013.
21. Mirzaei, J.; Siavoshi, F.; Emami, S.; Safari, F.; Khoshayand, M. R.; Shafiee, A.; Foroumadi, A. *Eur. J. Med. Chem.,* **2008,** *43*, 1575-1580.