



Synthesis and Biological Evaluation of New Nitroimidazole Derivatives as Anti-*Helicobacter pylori* Agents Against Metronidazole-Resistant Strains

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Abstract

Since several *Helicobacter pylori* strains have become resistant to metronidazole, new nitroimidazole derivatives based on metronidazole were designed and synthesized with different substituents on imidazole nitrogen. The activity of the synthesized compounds was evaluated against 20 clinically isolated metronidazole-resistant *H. pylori* strains. Some synthesized compounds were effective against those metronidazole-resistant *H. pylori* strains. Three compounds exhibited the most potent inhibitory activities (MIC₅₀ = 8 µg/mL and MIC₉₀ = 16 µg/mL).

Keywords: Nitroimidazole Derivatives, Metronidazole Resistance, *Helicobacter pylori*, Synthesis

1. Background

Helicobacter pylori is a gram-negative, microaerophilic bacterium. It is responsible for most cases of active chronic gastritis, peptic ulcer, intestinal metaplasia, low-grade mucosa-associated lymphoid tissue lymphoma, and/or cancer development (1). Its infection rate is about 50% worldwide, with prevalence rates ranging from 20% to more than 80% in different countries (2, 3). In gastric mucosal infection cases, eradicating *H. pylori* seems to treat ulcers and the infection (4, 5). The most effective regimen for the treatment of *H. pylori* infection is a combination of a proton pump inhibitor and 2 antimicrobial agents (mainly clarithromycin and either metronidazole [MTZ] or amoxicillin) (6).

Different methods are applied to determine the *H. pylori* susceptibility to MTZ. Nevertheless, an association between MTZ resistance and treatment failure has been found. Combination therapy with clarithromycin and MTZ led to a cure rate of 95% when the strains were susceptible

to MTZ and 76% when the strains were MTZ-resistant (MIC = 8 µg/mL, as determined by the agar dilution method recommended by EUCAST) (7, 8).

Metronidazole is one of the well-known compounds of the 5-nitroimidazole class, which has been used as an antimicrobial agent for several years (9). Since derivatives with a 5-nitroimidazole nucleus have been evaluated and characterized for their toxicity and metabolism profile, this nucleus has great potential to be widely applied in drug design (10).

There is a report on the synthesis and biological evaluation of new 5-nitroimidazole derivatives. A group of 1-(2-hydroxypropyl)-2-styryl-5-nitroimidazole derivatives was synthesized, and their antibacterial activities and toxicity were evaluated (11). Metronidazole derivatives that contain piperazine were also introduced. These new compounds exhibited proper antibacterial effects against gram-positive strains (9).

Despite the unique antimicrobial spectrum of

5-nitroimidazole derivatives in the treatment of infectious diseases, especially *H. pylori* infection, there is still a great concern about *H. pylori* resistance to MTZ, which would result in the need for new active agents against MTZ-resistant *H. pylori* strains. These observations have led us to investigate novel MTZ compounds with similar structural properties to overcome the resistance problem.

Following our previous research on synthesizing bioactiveazole rings (12-20) and considering the biological effects of the nitroimidazole scaffold, this study focused on the design and synthesis of novel compounds containing a nitroimidazole structure. The anti-*H. pylori* effects of these compounds were evaluated against MTZ-resistant strains (Figure 1).

2. Methods

2.1. Materials

All chemicals and reagents used in this study were commercially attained from Merck or Aldrich Companies (Germany).

For obtaining ¹H NMR spectra, a Bruker FT-500 MHz instrument (Bruker Biosciences, USA) was used, and chemical shifts (δ) were reported as parts per million (ppm). The electrospray mass (ESI-MS) spectra of the synthesized compounds and elemental analysis were acquired using an Agilent 4610 triple quadrupole mass spectrometer and elemental analyzer (Costech, Italy), respectively. The melting points of the synthesized compounds were determined using the Electrothermal 9100 melting point apparatus. In addition, the Perkin Elmer 1420 ratio recording spectrometer was used to generate the infrared spectra.

2.2. Synthesis

2-(2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethoxy)isoindoline-1,3-dione (2b) (21):

To a mixture of metronidazole 1 (0.5 g, 2.92 mmol), triphenylphosphine (1.125 g, 4.28 mmol), and hydroxyphthalimide (0.7 g, 4.28 mmol) in 26 mL of dry THF, DIAD (diisopropyl azodicarboxylate) (1.9 mL, 9.75 mmol) was added in one portion at room temperature. The reaction mixture was stirred for 24 h. The solvent was removed under low pressure. The residue was triturated with diethyl ether and filtered to obtain a white solid with the following characteristics:

Yield: 77.77%; mp: 184 - 186°C; crystallization in diethyl ether; IR (KBr, cm⁻¹): 1783, 1734 (C=O), 1520 (NO₂), 1357 (NO₂); ¹H NMR (500 MHz, CDCl₃): δ : 2.64 (3H, s, CH₃), 4.60 (2H, t, J = 4.9 Hz, CH₂-N), 4.75 (2H, t, J = 5 Hz, CH₂-O), 7.78 (2H, m, ArH), 7.84 (2H, m, ArH), 8.07 (1H, s, Imidazole-H); ESI-MS

(m/z): [M+Na]⁺ 339; Anal. Calc. for C₁₄H₁₂N₄O₅ (316.08): C 53.17, H 3.82, N 17.71; found: C 53.15, H 3.81, N 17.75.

1-(2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl)pyrrolidine-2,5-dione (2c) (22):

A mixture of metronidazole 1 (0.5 g, 2.92 mmol), triphenylphosphine (1.14 g, 4.34 mmol), and succinimide (0.43 g, 4.34 mmol) in 25 mL dry THF was cooled to 0 - 5°C. Then, a solution of DIAD (diisopropyl azodicarboxylate) (1.9 mL, 9.75 mmol) in 3 mL dry THF was added dropwise for 20 min. The reaction mixture was stirred for 24 h. The progress of the reaction was monitored by TLC. The solvent was removed to give oil under low pressure. The oil was solidified using diethyl ether and then crystallized from 2-propanol. The following characteristics appeared:

Yield: 55%; mp: 151 - 153°C; IR (KBr, cm⁻¹): 1703 (C=O), 1528 (NO₂), 1374 (NO₂); ¹H NMR (500 MHz, DMSO-d₆): δ : 2.36 (3H, s, CH₃), 2.52 (4H, broad s, Succinimide), 3.76 (2H, t, J = 5.9 Hz, CH₂-Succinimide), 4.42 (2H, t, J = 5.9 Hz, CH₂-Imidazole), 8.00 (1H, s, Imidazole-H); ESI-MS (m/z): [M+Na]⁺ 275; Anal. Calc. for C₁₀H₁₂N₄O₄ (252.09): C 47.62, H 4.80, N 22.21; found: C 47.61, H 4.79, N 22.19.

2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl benzenesulfonate (2d):

A solution of metronidazole 1 (10 g, 58.47 mmol) in dry pyridine was cooled in an ice bath; then, benzene sulfonyl chloride (8.1 mL, 63.8 mmol) was added dropwise. The reaction mixture was stirred for 24 h at room temperature. The achieved solution was diluted with ice/water, and the obtained product was washed several times with water, with the following characteristics:

Yield: 83%; mp: 145 - 150 °C; IR (KBr, cm⁻¹): 1520 (NO₂), 1380 (NO₂), 1340 (SO₂), 1180.

2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethanamine dihydrobromide (3):

A mixture of metronidazole 1 (4 g, 23.4 mmol), triphenylphosphine (9.18 g, 35 mmol), and phthalimide (5.15 g, 35 mmol) in 200 mL dry THF was cooled to 0 - 5°C. Then, a solution of DIAD (diisopropyl azodicarboxylate) (15.2 mL, 75 mmol) in 20 mL dry THF was added dropwise for 20 min. The reaction mixture was stirred for 1 h. The progress of the reaction was monitored by TLC. The solvent was removed to give oil under low pressure. The oil was solidified using diethyl ether and then crystallized from 2-propanol to obtain 2a. A mixture of 2a (3.02 g, 10 mmol) in 30% HBr (110 mL) was refluxed for 16 h. The acid was separated by distillation. Absolute ethanol (100 mL) was added to the mixture and dried under low pressure. The residue was washed with diethyl ether (4 × 30 mL) and crystallized from ethanol (23). It had the following characteristics:

Yield: 80%; mp: 221 - 228°C; ¹H NMR (500 MHz, DMSO-d₆): δ : 2.61 (3H, s, CH₃), 3.33 (2H, m, CH₂-NH₂), 4.60

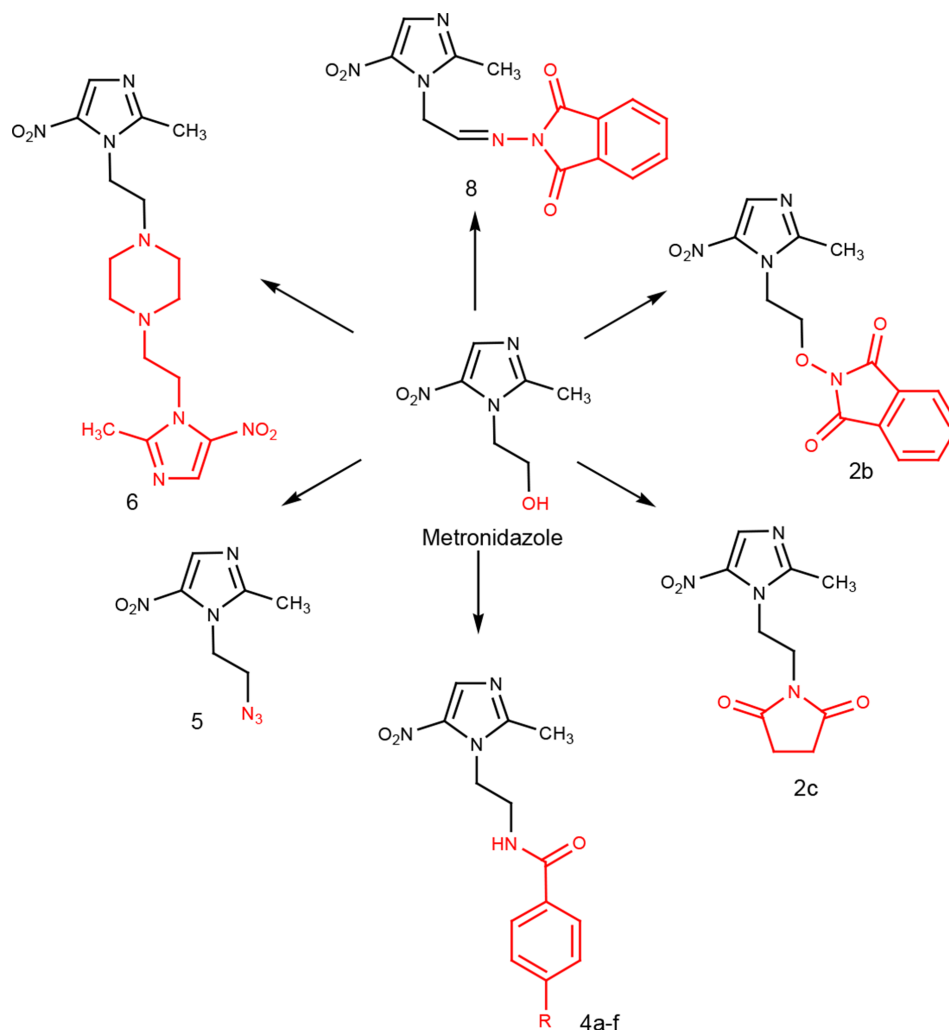


Figure 1. Structure of designed compounds

(2H, t, $J = 6.4$ Hz, CH_2 -Imidazole), 8.20 (3H, NH_3^+), 8.43 (1H, s, Imidazole-H), 9.87 (1H, broad s, NH^+ -Imidazole); ESI-MS (m/z): $[\text{M}+\text{Na}]^+$ 171; Anal. Calc. for $\text{C}_6\text{H}_{12}\text{Br}_2\text{N}_4\text{O}_2$ (329.93): C 21.71, H 3.64, N 16.88; found: C 21.73, H 3.65, N 16.91.

2.2.1. General Synthesis Method for Compounds (4a-f):

Five milliliters of triethylamine were added to a solution of 3 (0.5 g) in dry THF (20 mL), and the mixture was cooled to 0 - 5°C. Then, appropriate benzoyl chloride (2.85 mmol) was added dropwise. The mixture was stirred for 5 h. The solvent was removed under low pressure. The residue was dissolved in dichloromethane (30 mL) and extracted with acidified water by 3 M HCl (5×20 mL). The combined aqueous layers were made alkaline (pH 7.5 - 8) with NaOH solution and extracted with dichloromethane

(5×20 mL). The combined organic layers were washed with a sodium chloride saturated solution (2×25 mL) and then dried with Na_2SO_4 . The organic solvent was evaporated under a vacuum. The residue was crystallized from ethanol.

N-(2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl) benzamide (4a) (24):

It had the following characteristics: Yield: 30%; mp: 128.7 - 131.2°C; IR (KBr, cm^{-1}): 3364 (NH), 1637 (C=O), 1544 (NO_2), 1366 (NO_2); ^1H NMR (500 MHz, CDCl_3): δ : 2.52 (3H, s, CH_3), 3.83 (2H, m, CH_2 -NH), 4.63 (2H, t, $J = 6.2$ Hz, Imidazole- CH_2), 6.76 (^1H , t, NH), 7.46 (2H, t, $J = 7.63$ Hz, ArH), 7.55 (1H, t, $J = 7.4$ Hz, ArH), 7.75 (2H, d, $J = 7.3$ Hz, ArH), 7.97 (1H, s, Imidazole-H); ESI-MS (m/z): $[\text{M}+\text{Na}]^+$ 297; Anal. Calc. for $\text{C}_{13}\text{H}_{14}\text{N}_4\text{O}_3$ (274.11): C 56.93, H 5.14, N 20.43; found: C 56.92,

H 5.15, N 20.42.

4-Fluoro-N-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)benzamide (4b):

It had the following characteristics: Yield: 35%; mp: 202 - 204°C; IR (KBr, cm^{-1}): 3386 (NH), 1648 (C=O), 1556 (NO_2), 1375 (NO_2); ^1H NMR (500 MHz, CDCl_3): δ : 2.27 (3H, s, CH_3), 3.54 (2H, m, $\text{CH}_2\text{-NH}$), 4.37 (2H, t, $J = 6.1$ Hz, Imidazole- CH_2), 6.89 (2H, t, $J = 8.6$ Hz, ArH), 7.65 (2H, d, $J = 8.6$ Hz, ArH), 7.78 (1H, s, Imidazole-H), 8.23 (1H, t, NH); ESI-MS (m/z): $[\text{M}+\text{Na}]^+$ 315; Anal. Calc. for $\text{C}_{13}\text{H}_{13}\text{FN}_4\text{O}_3$ (292.10): C 53.42, H 4.48, N 19.17; found: C 53.44, H 4.48, N 19.18.

4-Chloro-N-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)benzamide (4c):

It had the following characteristics: Yield: 45%; mp: 197.2 - 199.2°C; IR (KBr, cm^{-1}): 3348 (NH), 1639 (C=O), 1543 (NO_2), 1356 (NO_2); ^1H NMR (500 MHz, DMSO- d_6): δ : 2.35 (3H, s, CH_3), 3.63 (2H, m, $\text{CH}_2\text{-NH}$), 4.44 (2H, t, $J = 5.8$ Hz, Imidazole- CH_2), 7.53 (2H, d, $J = 8.8$ Hz, ArH), 7.74 (2H, d, $J = 7.8$ Hz, ArH), 8.02 (1H, s, Imidazole-H), 8.76 (1H, t, $J = 5.82$ Hz, NH); ESI-MS (m/z): $[\text{M}+\text{Na}]^+$ 331.5; Anal. Calc. for $\text{C}_{13}\text{H}_{13}\text{ClN}_4\text{O}_3$ (308.07): C 50.58, H 4.24, N 18.15; found: C 50.57, H 4.24, N 18.16.

N-(2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl)-4-nitrobenzamide (4d):

It had the following characteristics: Yield: 32%; mp: 197.8 - 199.3°C; IR (KBr, cm^{-1}): 3425 (NH), 1666 (C=O), 1514 (NO_2), 1361 (NO_2); ^1H NMR (500 MHz, DMSO- d_6): δ : 2.36 (3H, s, CH_3), 3.67 (2H, m, $\text{CH}_2\text{-NH}$), 4.46 (2H, t, $J = 5.7$ Hz, Imidazole- CH_2), 7.96 (2H, d, $J = 8.7$ Hz, ArH), 8.05 (1H, t, $J = 8.7$ Hz, NH), 8.31 (2H, d, $J = 8.6$ Hz, ArH), 9.02 (1H, s, Imidazole-H); ESI-MS (m/z): $[\text{M}+\text{H}]^+$ + 320, $[\text{M}+\text{Na}]^+$ + 342; Anal. Calc. for $\text{C}_{13}\text{H}_{13}\text{N}_5\text{O}_5$ (319.09): C 48.90, H 4.10, N 21.94; found: C 49.01, H 4.10, N 21.93.

4-Methyl-N-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)benzamide (4e):

It had the following characteristics: Yield: 37%; mp: 187 - 191°C; IR (KBr, cm^{-1}): 3368 (NH), 1637 (C=O), 1543 (NO_2), 1353 (NO_2); ^1H NMR (500 MHz, DMSO- d_6): δ : 2.33 (3H, s, phenyl- CH_3), 2.50 (3H, s, Imidazole- CH_3), 3.62 (2H, m, $\text{CH}_2\text{-NH}$), 4.43 (2H, t, $J = 5.9$ Hz, Imidazole- CH_2), 7.25 (2H, d, $J = 8.0$ Hz, ArH), 7.63 (2H, d, $J = 8.1$ Hz, ArH), 8.02 (1H, s, Imidazole-H), 8.58 (1H, t, $J = 5.3$ Hz, NH); ESI-MS (m/z): $[\text{M}+\text{H}]^+$ 289; Anal. Calc. for $\text{C}_{14}\text{H}_{16}\text{N}_4\text{O}_3$ (288.12): C 58.32, H 5.59, N 19.43; found: C 58.37, H 5.59, N 19.42.

4-Methoxy-N-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)benzamide (4f):

It had the following characteristics: Yield: 25%; mp: 149.2 - 150.3°C; IR (KBr, cm^{-1}): 3368 (NH), 1642 (C=O), 1523 (NO_2), 1370 (NO_2); ^1H NMR (500 MHz, CDCl_3): δ : 2.52 (3H, s, CH_3), 3.77 (2H, m, $\text{CH}_2\text{-NH}$), 3.87 (3H, s, OCH_3), 4.62 (2H, t, $J = 6.2$ Hz, Imidazole- CH_2), 6.62 (1H, t, $J = 8.8$ Hz, NH), 6.95 (2H, d, $J = 8.8$ Hz, ArH), 7.73 (2H, d, $J = 6.9$ Hz, ArH), 7.97 (1H,

s, Imidazole-H); ESI-MS (m/z): $[\text{M}+\text{Na}]^+$ 327; Anal. Calc. for $\text{C}_{14}\text{H}_{16}\text{N}_4\text{O}_4$ (304.12): C 55.26, H 5.30, N 18.41; found: C 55.30, H 5.29, N 18.40.

1-(2-Azidoethyl)-2-methyl-5-nitro-1H-imidazole (5) (25):

A mixture of 2d (1 g, 3.58 mmol), sodium azide (35.8 mmol), triethylamine in water (5 mL), and 18-crown-6-ether in xylene (30 mL) was refluxed for 48 h. The solvent was removed by distillation. The residue was diluted in water and extracted with ethyl acetate (4 \times 20 mL). The combined organic layers were evaporated. The residue was triturated with n-hexan. The resulting solid was washed several times with n-hexane, which gave the following characteristics:

Yield: 54%; mp: 50 - 53°C; IR (KBr, cm^{-1}): 2123 (N_3), 1540 (NO_2), 1372 (NO_2); ^1H NMR (500 MHz, DMSO- d_6): δ : 2.48 (3H, s, CH_3), 3.79 (2H, t, $J = 5.6$ Hz, $\text{CH}_2\text{-N}_3$), 4.48 (2H, t, $J = 5.6$ Hz, Imidazole- CH_2), 8.03 (1H, s, Imidazole-H); ESI-MS (m/z): $[\text{M}+\text{H}]^+$ 197; Anal. Calc. for $\text{C}_6\text{H}_8\text{N}_6\text{O}_2$ (196.07): C 36.74, H 4.11, N 42.84; found: C 36.65, H 4.12, N 42.87.

1,4-Bis(2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl)piperazine (6):

A mixture of 2d (1 g, 3.58 mmol), piperazine (0.092 g, 1.06 mmol), and sodium bicarbonate (excess) in dioxane was refluxed for 24 h. The solvent was evaporated under low pressure. Water (10 mL) was added to the residue and extracted by chloroform (3 \times 10 mL). The combined organic layers were washed with water, dried with Na_2SO_4 , and purified using column chromatography (mobile phase first: chloroform, Second: chloroform 83.3%: methanol 16.6%), giving the following characteristics:

Yield: 25%; mp: 197 - 199°C; ^1H NMR (500 MHz, CDCl_3): δ : 2.47 (8H, broad s, Piperazine), 2.53 (6H, s, CH_3), 2.67 (4H, t, $J = 5.9$ Hz, $\text{CH}_2\text{-N}$), 4.40 (4H, t, $J = 5.9$ Hz, $\text{CH}_2\text{-Imidazole}$), 7.94 (2H, s, Imidazole-H); ESI-MS (m/z): $[\text{M}+\text{Na}]^+$ 415; Anal. Calc. for $\text{C}_{16}\text{H}_{24}\text{N}_8\text{O}_4$ (392.19): C 48.97, H 6.16, N 28.56; found: C 48.94, H 6.17, N 28.53.

2-(2-Methyl-5-nitro-1H-imidazol-1-yl)acetaldehyde (7):

Oxalyl chloride (2.3 mL, 25.3 mmol) was added dropwise to 160 mL of dry dichloromethane under argon. After that, the reaction mixture was cooled to -50°C in dry ice. Then, DMSO (17 mL, 240 mmol) was added dropwise. After 20 min, a solution of metronidazole 1 (3 g, 17.54 mmol) in 15 mL DMSO was added dropwise. By passing another 20 min, dry triethylamine (33 mL, 240 mmol) was added dropwise. The reaction was stirred for 10 minutes and then warmed to room temperature. The mixture was diluted in ethyl acetate and extracted with water (3 \times 50 mL). The combined aqueous layers were extracted with ethyl acetate (3 \times 250 mL). The combined organic layers were washed with saturated NaCl and dried

with Na₂SO₄. The organic solvent was evaporated under a vacuum. The obtained residue was purified using column chromatography (mobile phase: dichloromethane 97%: methanol 3%) to obtain the product as an oil (26) with the following characteristics:

Yield: 68%; ESI-MS (m/z): [M+H]⁺ 170.

2-(2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethylideneamino)isoindoline-1,3-dione (8):

A mixture of acidified (acetic acid pH = 4 - 4.5) N-aminophthalimide (0.35 g, 2.16 mmol) in dioxane was added dropwise to a solution of 7 (0.37 g, 2.16 mmol) in dioxane at 60°C, and the reaction mixture was refluxed for 19 h at 60°C. The solvent was evaporated, and the obtained liquid was triturated with diethyl ether and filtered to obtain a brown solid with the following characteristics:

Yield: 55.2%; mp: 206 - 208°C, crystallized in diethyl ether; IR (KBr, cm⁻¹): 1785 (C=O), 1734 (C=O), 1533 (NO₂), 1358 (NO₂); ¹H NMR (500 MHz, CDCl₃): δ: 2.59 (3H, s, CH₃), 5.36 (2H, d, J = 4.0 Hz, CH₂-Imidazole), 7.82 (2H, m, ArH), 7.93 (2H, m, ArH), 8.03 (1H, s, Imidazole-H), 9.04 (1H, t, J = 4.0 Hz, HC = N); Anal. Calc. for C₁₄H₁₁N₅O₄ (313.08): C 53.68, H 3.54, N 22.36; found: C 53.67, H 3.53, N 22.35.

2.3. Biological Activity

2.3.1 *Helicobacter pylori* Strains

As previously described, 20 clinical isolates recovered from 96 adult patients who were referred to the Digestive Endoscopy Unit of Imam Khomeini Hospital in Tehran, Iran, from 2013 to 2014 were used in this study (8).

2.3.2 Susceptibility Testing

Susceptibility of the bacterial strains was determined based on the minimum inhibitory concentration (MIC) assayed by the agar twofold serial dilutions method according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (7, 8).

The concentrations of metronidazole (MAST, London, United Kingdom) and the tested compounds ranging from 0.016 to 512 μg/mL were made in Muller Hinton agar medium (MHA, 45°C, Merck, Germany) supplemented with 5% of sheep blood. Five microliters of fresh normal saline suspension with turbidity corresponding to 2 McFarland's standard of each clinically isolated *H. pylori* strain were inoculated on the agar medium. The inoculated plates were incubated in 5% CO₂ for 72 hours at 37°C (8).

Helicobacter pylori strain RIGLD 245 was used as a reference strain for all the experiments. The MIC was considered the lowest concentration inhibiting the bacterial visible growth. The EUCAST clinical breakpoint of > 8 μg/mL was defined for the metronidazole-resistant strains (7, 8). The results were expressed as the MICs

(Table 1). The MIC₅₀ and MIC₉₀, corresponding to the concentrations of the synthesized derivatives inhibiting the 50% and 90% growth of the isolates, respectively, are reported in Table 2.

3. Results and Discussion

3.1 Chemistry

The designed compounds were derived from metronidazole, as shown in Figure 2. Compounds 2a - c were synthesized through the Mitsunobu reaction by metronidazole reacting with three different imides (phthalimide, hydroxy phthalimide, and succinimide) in the presence of triphenylphosphine (PPh₃) and diisopropyl azodicarboxylate (DIAD) in dry THF. In all of these reactions, DIAD was added to a mixture of metronidazole, triphenylphosphine, and corresponding imides at 0 - 5°C, except for hydroxyphthalimide, where DIAD was added to the mixture at room temperature in one part.

Compounds 4a - f were amide derivatives of 5-nitroimidazole and were obtained after a reaction between compound 3 and appropriate benzoyl chloride in dry THF.

The benzene sulfonate derivative of metronidazole (2d) was obtained after a reaction between metronidazole (1) and benzene sulfonyl chloride in dry pyridine. Compound 2d was reacted with sodium azide to obtain compound 5. Crown ether as a phase transfer catalyst was used in this reaction to increase sodium azide's solubility. Compound 6 was obtained by the reaction of 2d with piperazine at a 3.6/1 ratio.

The structures of the synthesized compounds were characterized by IR, ¹H-NMR, and ESI-MS.

3.2 Biological Activity

The synthesized compounds were evaluated for their inhibitory activity against 20 clinically isolated *H. pylori* strains resistant to MTZ by determination of MIC, MIC₅₀, and MIC₉₀. The results are shown in Tables 1. and 2.

As presented in Tables 1, and 2, almost all synthesized compounds were effective against metronidazole-resistant *H. pylori* strains. The para methyl derivative (4e) was identified as the most active compound (MIC₅₀ = 8 μg/mL) against metronidazole-resistant *H. pylori* strains amongst benzoyl metronidazole derivatives (4a-f). Compounds 5 and 6 with azide and piperazine substituents exerted the highest inhibitory activity against metronidazole-resistant *H. pylori* strains. The obtained MIC₉₀ values of these compounds were 16 μg/mL, which is 32-fold over the metronidazole.

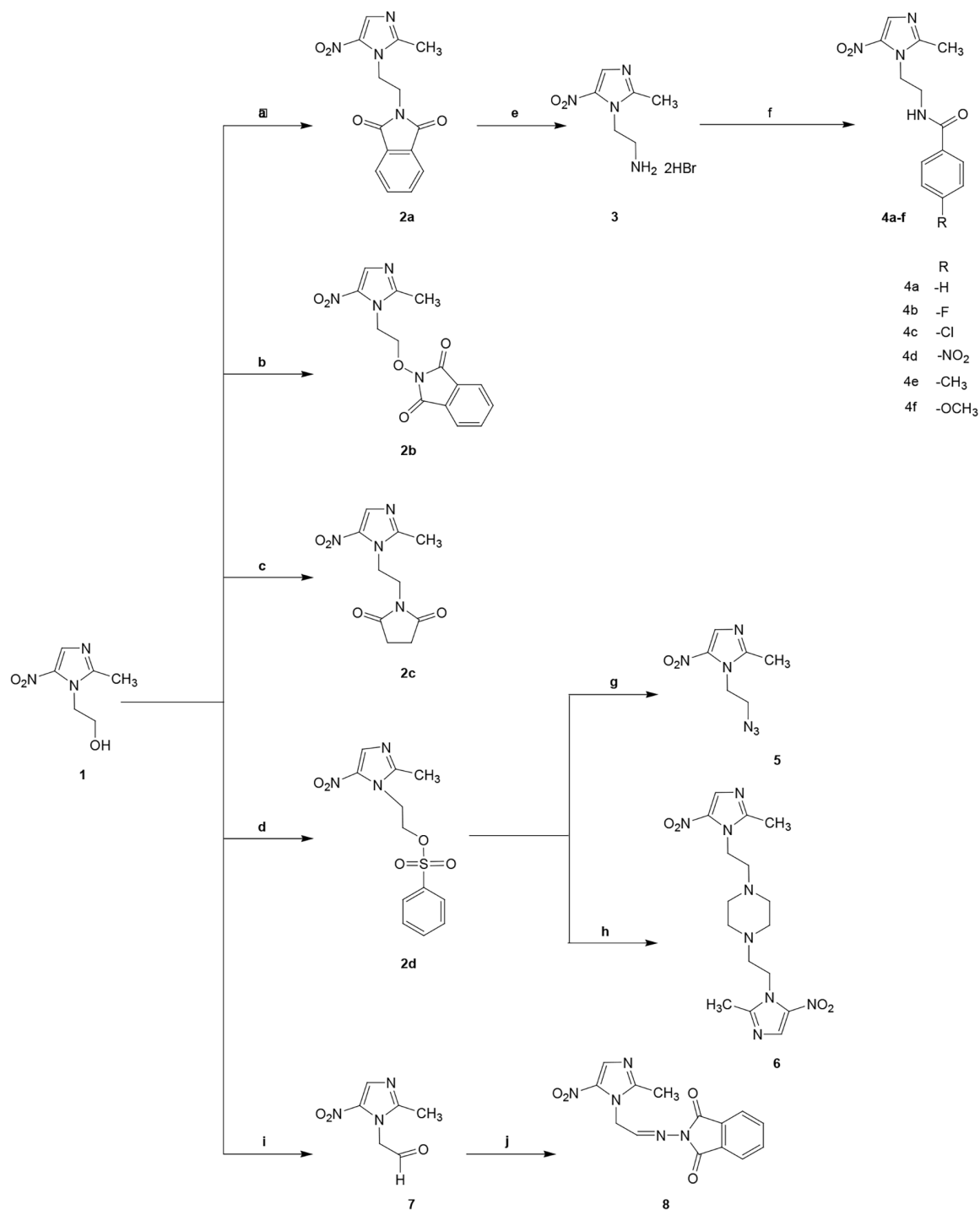


Figure 2. Reagents and conditions: A, Phthalimide, PPh₃, DIAD, dry THF, Stir at RT; B, Hydroxyphthalimide, PPh₃, DIAD, dry THF, Stir at rt; C, Succinimide, PPh₃, DIAD, dry THF, Stir at 0°C; D, Benzenesulfonyl chloride, dry pyridine, stir at rt; E, HBr, Reflux 16 h; F, Benzoyl chloride derivatives, dry THF, Et₃N, stir 5 h; G, NaN₃, Xylene, crown ether, water, Et₃N, reflux 48 h; H, Piperazine, NaHCO₃, reflux 24 h; I, Oxalyl chloride, DMSO, Et₃N, stir at -50°C; J, N-aminophthalimide, dioxane, acetic acid, reflux.

Table 1. Minimum Inhibitory Concentration ($\mu\text{g/mL}$) of Metronidazole and Novel Nitroimidazole Derivatives Determined by Agar Dilution ($n = 3$) Against Clinically Isolated Metronidazole-resistant *Helicobacter pylori* Strains

Compounds; Strains	1 Metronidazole	2b	2c	4a	4b	4c	4d	4e	4f	5	6	8
1	64	8	8	8	8	8	ND	8	8	8	8	ND
2	64	4	4	4	4	4	ND	4	4	4	4	ND
3	64	8	8	8	8	8	ND	8	8	8	8	ND
4	64	4	4	4	4	4	ND	4	4	4	4	ND
5	128	16	32	16	16	16	ND	8	16	8	8	ND
6	128	16	32	32	16	16	ND	8	16	8	8	ND
7	256	32	32	32	32	32	ND	16	32	16	16	ND
8	256	32	32	32	32	32	ND	8	32	8	16	ND
9	256	32	16	32	8	32	ND	16	32	8	16	ND
10	256	16	16	16	16	16	ND	8	32	8	8	ND
11	512	32	32	32	32	32	ND	16	16	16	16	ND
12	512	32	16	16	32	16	ND	16	16	16	8	ND
13	512	16	8	32	8	32	ND	8	8	8	8	ND
14	512	64	16	16	32	32	ND	16	32	16	16	ND
15	512	16	32	32	32	32	ND	16	32	16	16	ND
16	512	64	32	32	32	8	ND	16	32	16	16	ND
17	512	8	8	8	8	8	ND	8	8	16	8	ND
18	512	32	32	8	32	32	ND	16	32	16	16	ND
19	>512	128	64	64	128	64	ND	32	64	32	32	ND
20	>512	128	64	64	64	64	ND	32	128	32	64	ND

Abbreviation: ND, not determined.

Table 2. Minimum Inhibitory Concentration of Metronidazole and Novel Nitroimidazole Derivatives Against 20 Clinically Isolated *Helicobacter pylori* Strains

Compounds	MIC ₅₀ ($\mu\text{g/mL}$)	MIC ₉₀ ($\mu\text{g/mL}$)
1 (metronidazole)	256	512
2b	16	64
2c	16	32
4a	16	32
4b	16	32
4c	16	32
4d	ND	ND
4e	8	16
4f	16	32
5	8	16
6	8	16
8	ND	ND

Abbreviation: ND, not determined.

5. Conclusion

Some nitroimidazole analogs were synthesized, and their inhibitory activity against 20 clinically isolated *H. pylori* strains resistant to metronidazole was evaluated by determining MIC₅₀ and MIC₉₀. Compounds 4e, 5, and 6 were the most active compounds, with the MIC₅₀ of 8 $\mu\text{g/mL}$ and MIC₉₀ of 16 $\mu\text{g/mL}$.

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Footnotes

Authors' Contribution: Zahra Bayati synthesized the designed compounds. Mahnaz Shahabimehr performed the antibacterial test of the synthesized compounds. Salimeh Amidi contributed to manuscript writing and data interpretation. Masoud Alebouyeh and Arash Mahboubi designed and analyzed the antibacterial tests. Sayyed Abbas Tabatabai supervised the project, designed the compounds, interpreted the spectral data, and revised the manuscript.

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References

- Vaziri F, Najar Peerayeh S, Alebouyeh M, Mirzaei T, Yamaoka Y, Molaie M, et al. Diversity of *Helicobacter pylori* genotypes in Iranian patients with different gastroduodenal disorders. *World J Gastroenterol.* 2013;**19**(34):5685-92. [PubMed ID: 24039362]. [PubMed Central ID: PMC3769906]. <https://doi.org/10.3748/wjg.v19.i34.5685>.
- Ebrahimi Daryani N, Taher M, Shirzad S. *Helicobacter Pylori* Infection: A Review. *Arch Clin Infect Dis.* 2011;**6**(1):56-64.

3. Farzi N, Malekian T, Alebouyeh M, Vaziri F, Zali MR. Genotype Diversity and Quasispecies Development of *Helicobacter pylori* in a Single Host. *Jpn J Infect Dis*. 2015;**68**(3):176-80. [PubMed ID: 25672355]. <https://doi.org/10.7883/yoken.JJID.2014.165>.
4. Rokkas T, Karameris A, Mavrogeorgis A, Rallis E, Giannikos N. Eradication of *Helicobacter pylori* reduces the possibility of rebleeding in peptic ulcer disease. *Gastrointest Endosc*. 1995;**41**(1):1-4. [PubMed ID: 7698617]. [https://doi.org/10.1016/s0016-5107\(95\)70266-0](https://doi.org/10.1016/s0016-5107(95)70266-0).
5. Chan FK, To KF, Wu JC, Yung MY, Leung WK, Kwok T, et al. Eradication of *Helicobacter pylori* and risk of peptic ulcers in patients starting long-term treatment with non-steroidal anti-inflammatory drugs: a randomised trial. *Lancet*. 2002;**359**(9300):9-13. [PubMed ID: 11809180]. [https://doi.org/10.1016/s0140-6736\(02\)07272-0](https://doi.org/10.1016/s0140-6736(02)07272-0).
6. Shokrzadeh L, Alebouyeh M, Mirzaei T, Farzi N, Zali MR. Prevalence of multiple drug-resistant *Helicobacter pylori* strains among patients with different gastric disorders in Iran. *Microb Drug Resist*. 2015;**21**(1):105-10. [PubMed ID: 25303151]. <https://doi.org/10.1089/mdr.2014.0081>.
7. Kahlmeter G, Brown DF, Goldstein FW, MacGowan AP, Mouton JW, Odenholt I, et al. European Committee on Antimicrobial Susceptibility Testing (EUCAST) Technical Notes on antimicrobial susceptibility testing. *Clin Microbiol Infect*. 2006;**12**(6):501-3. [PubMed ID: 16700696]. <https://doi.org/10.1111/j.1469-0691.2006.01454.x>.
8. Shahabimehr M, Alebouyeh M, Farzi N, Mahboubi A, Taslimi R, Zali MR. Resistance Rate and Minimum Inhibitory Concentration of Metronidazole Among *Helicobacter pylori* Strains in Tehran, Iran. *Arch Clin Infect Dis*. 2016;**11**(2). e34478. <https://doi.org/10.5812/archcid.34478>.
9. Wang SF, Yin Y, Qiao F, Wu X, Sha S, Zhang L, et al. Synthesis, molecular docking and biological evaluation of metronidazole derivatives containing piperazine skeleton as potential antibacterial agents. *Bioorg Med Chem*. 2014;**22**(8):2409-15. [PubMed ID: 24680059]. <https://doi.org/10.1016/j.bmc.2014.03.004>.
10. Du QR, Li DD, Pi YZ, Li JR, Sun J, Fang F, et al. Novel 1,3,4-oxadiazole thioether derivatives targeting thymidylate synthase as dual anticancer/antimicrobial agents. *Bioorg Med Chem*. 2013;**21**(8):2286-97. [PubMed ID: 23490159]. <https://doi.org/10.1016/j.bmc.2013.02.008>.
11. Wang ZC, Duan YT, Qin YJ, Wang PF, Luo Y, Wen Q, et al. Potentiating 1-(2-hydroxypropyl)-2-styryl-5-nitroimidazole derivatives against antibacterial agents: design, synthesis and biology analysis. *Eur J Med Chem*. 2013;**65**:456-63. [PubMed ID: 23770447]. <https://doi.org/10.1016/j.ejmech.2013.05.004>.
12. Jalilian AR, Tabatabai SA, Shafiee A, Afarideh H, Najafi R, Bineshmarvasti M. One-step, no-carrier-added, synthesis of a18F-labelled benzodiazepine receptor ligand. *J Label Compd Radiopharm*. 2000;**43**(6):545-55. [https://doi.org/10.1002/\(sici\)1099-1344\(200005\)43:6<545::aid-jlcr340>3.0.co;2-1](https://doi.org/10.1002/(sici)1099-1344(200005)43:6<545::aid-jlcr340>3.0.co;2-1)
13. Rad AA, Sheikha M, Hosseini R, Tabatabaie SA, Shafiee A. Synthesis and morphine enhancement activity of N-[5-(2-phenoxyphenyl)-1,3,4-oxadiazole-2-yl]-N'-phenylurea derivatives. *Arch Pharm (Weinheim)*. 2004;**337**(4):193-200. [PubMed ID: 15065078]. <https://doi.org/10.1002/ardp.200300822>.
14. Foroumadi A, Sheibani V, Sakhteman A, Rameshk M, Abbasi M, Farazifard R, et al. Synthesis and anticonvulsant activity of novel 2-amino-5-[4-chloro-2-(2-chlorophenoxy) phenyl]-1,3,4-thiadiazole derivatives. *DARU J Pharm Sci*. 2007;**15**(2):89-93.
15. Zavareh E, Hedayati M, Rad L, Kiani A, Shahhosseini S, Faizi M, et al. Design, Synthesis and Biological Evaluation of Some Oxadiazole Derivatives as Novel Amide-Based Inhibitors of Soluble Epoxide Hydrolase. *Lett Drug Des Discov*. 2014;**11**(6):721-30. <https://doi.org/10.2174/1570180811666140220005530>.
16. Tabatabai SA, Rezaee Zavareh E, Reyhanfard H, Alinezhad B, Shafaghi B, Sheikha M, et al. Evaluation of Anxiolytic, Sedative-hypnotic and Amnesic Effects of Novel 2-phenoxy phenyl-1,3,4-oxadiazole Derivatives Using Experimental Models. *Iran J Pharm Res*. 2015;**14**(Suppl):51-7. [PubMed ID: 26185505]. [PubMed Central ID: PMC4499426].
17. Faizi M, Jahani R, Ebadi SA, Tabatabai SA, Rezaee E, Lotfaliee M, et al. Novel 4-thiazolidinone derivatives as agonists of benzodiazepine receptors: Design, synthesis and pharmacological evaluation. *EXCLI J*. 2017;**16**:52-62. [PubMed ID: 28435427]. [PubMed Central ID: PMC5379113]. <https://doi.org/10.17179/excli2016-692>.
18. Ghasemi M, Ghadbeighi S, Amirhamzeh A, AbbasTabatabai S, Nasser Ostad S, Shafiee A, et al. Synthesis, Molecular Docking Study, and Cytotoxic Activity of 1,3,5-triaryl Pyrazole Derivatives. *Lett Drug Des Discov*. 2015;**13**(2):121-8. <https://doi.org/10.2174/1570180812666150722235902>.
19. Foroumadi A, Tabatabai SA, Gitinezhad G, Zarrindast MR, Shafiee A. Synthesis and Anticonvulsant Activity of 5-Aryl-1,3,4-thiadiazole Derivatives. *Pharm Pharmacol Commun*. 2000;**6**(1):31-3. <https://doi.org/10.1211/146080800128735430>.
20. Kiani A, Rezaee E, Tabatabai SA. Novel Group of Imidazole Derivatives as Atypical Selective Cyclooxygenase-2 Inhibitors: Design, Synthesis and Biological Evaluation. *Iran J Pharm Res*. 2018;**17**(Suppl2):78-86. [PubMed ID: 3101344]. [PubMed Central ID: PMC6447865].
21. Borré E, Dahm G, Guichard G, Bellemin-Laponnaz S. Post-functionalization of platinum-NHC complexes by oxime ligation for ligand targeted therapy. *New J Chem*. 2016;**40**(4):3164-71. <https://doi.org/10.1039/c5nj03104d>.
22. Cusic JW, Levon EF, inventors. N-[2-(nitro-1-imidazolyl) ethyl] naphthal imides. USA. 1976.
23. Bertinaria M, Galli U, Sorba G, Fruttero R, Gasco A, Brenciaglia MI, et al. Synthesis and anti-*Helicobacter pylori* properties of NO-donor/metronidazole hybrids and related compounds. *Drug Dev Res*. 2003;**60**(3):225-39. <https://doi.org/10.1002/ddr.10284>.
24. Heeres J, Mostmans JH, Maes R, inventors. Nitroimidazole external preparations for dermatosis. USA. 1975.
25. Jarrad AM, Karoli T, Debnath A, Tay CY, Huang JX, Kaeslin G, et al. Metronidazole-triazole conjugates: activity against *Clostridium difficile* and parasites. *Eur J Med Chem*. 2015;**101**:96-102. [PubMed ID: 26117821]. [PubMed Central ID: PMC4550478]. <https://doi.org/10.1016/j.ejmech.2015.06.019>.
26. Yang LX, Hofer KG. Synthesis of 2-Methyl-5-nitroimidazol-1-yl-acetaldehyde. *Synth Commun*. 1996;**26**(19):3653-7. <https://doi.org/10.1080/00397919608003779>.