Full Paper

Synthesis and *In-Vitro* Antibacterial Activity of 5-Substituted 1-Methyl-4-nitro-1*H*-imidazoles

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A series of 5-substituted 1-methyl-4-nitro-1*H*-imidazole derivatives were synthesized and evaluated for *in-vitro* antibacterial activity against a panel of microorganisms including *Staphylococcus aureus, Staphylococcus epidermidis*, *Bacillus subtilis*, *Esherichia coli, Klebsiella pneumonia, Entrobacter aerogenes*, and *Helicobacter pylori* using conventional agar dilution method. Among the test compounds, 1-methyl-4-nitro-5-(phenylsulfonyl)-1*H*-imidazole was the most potent against Gram-positive bacteria, with a MIC value of ≤8 μg/mL. All compounds showed no significant activity against Gram-negative bacteria at concentrations ≤64 μg/mL The MIC values against 15 clinical isolates of *H. pylori* indicated that compounds **10** and **11** were the most active compounds in this series in terms of inhibiting the growth of *H. pylori* (MIC = 2 μg/mL). It was also demonstrated that their corresponding activities were four times larger than that of metronidazole.

Keywords: Antimicrobial activity / *Helicobacter pylori* / 4-Nitroimidazole / Synthesis

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Introduction

The emergence of multi-drug resistant Gram-positive bacteria, such as *Staphylococcus aureus* has made the treatment of infectious diseases difficult and has, over the last decades, become a serious medical problem. As pathogenic bacteria continuously evolve mechanisms of resistance to currently used antibacterial drugs, the discovery of novel and potent antibacterial agents is the best way to overcome bacterial resistance and develop effective therapies [1]. On the other hand, *Helicobacter pylori* is now a well-recognized cause of active chronic gastritis, peptic ulcer disease, gastric carcinoma, and mucosa-associated lymphoid tissue (MALT)-type gastric carcinoma; its eradication is strongly recommended for patients with these diseases and those with unexplained iron-deficiency anemia [2]. Clinical evidences also demonstrated that the eradication of *H. pylori* can reduce the risk of ulcer relapse and may be an effective method to prevent gastric cancer [3]. Hence, our research efforts are directed toward the discovery of new chemical entities that are effective as antimicrobial agents (especially against staphylococci and *Helicobacter pylori*) and the optimization of their structures.

During recent years, there have been intense investigations of different classes of 1,3,4-thiadiazole- and nitroimidazole-containing compounds, many of which are known to possess biological properties such as antituberculosis and anti-*H. pylori* activities [4 – 6]. The use of nitroheterocycles as antibacterial, antiviral, antiprotozoal, anticancer, and radiosensitizing agents is well estab-

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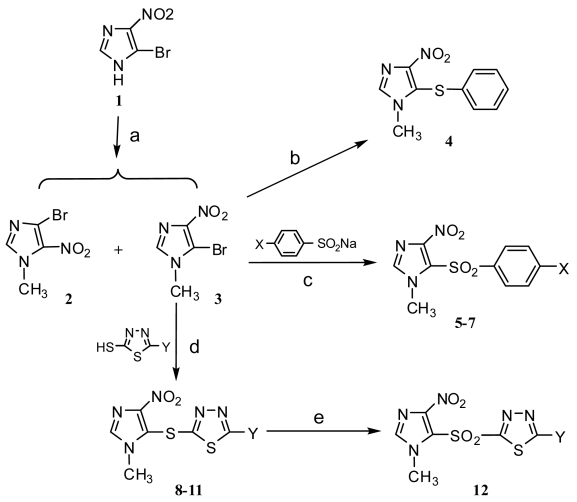


X= H, Me, $NHCOCH_3$
Y= $NHCOCH_3$, 4-methoxyphenyl, 1-methyl-5-nitro-2-imidazolyl, 5-nitro-2-fury

Reagents and conditions: (a) CH_2N_2, dry ether; (b) sodium thiophenolate, EtOH, reflux; (c) DMF, reflux; (d) KOH, EtOH, reflux; (e) *m*-CPBA, CH_2Cl_2.

Scheme 1. Synthesis of 5-substituted 1-methyl-4-nitro-1*H*-imidazoles **4**–**12**.

lished [7–12]. In our previous studies some new compounds containing 5-nitroheterocycles and 1,3,4-thiadiazole with different substituents at the C2-position of the thiadiazole ring were synthesized and evaluated for activity against several bacterial species [13]. In continuation to our ongoing research work on nitroheterocyclic derivatives, herein, we describe the synthesis and *in-vitro* antibacterial activity of new 5-substituted 1-methyl-4-nitro-1*H*-imidazole derivatives **4**–**12** against a panel of Gram-positive and Gram-negative bacteria including *Helicobacter pylori*.

Results and discussion

Chemistry

The synthesis of 5-substituted-1-methyl-4-nitro-1*H*-imidazole derivatives **4**–**12** was achieved with an efficient synthetic route outlined in Scheme 1. The starting material 5(4)-bromo-4(5)-nitroimidazole **1** was prepared according to the literature method [14]. The reaction of **1** with diazomethane in dry diethyl ether afforded 1-methyl-5-bromo-4-nitro-1*H*-imidazole **3** as major product. Compound **3** could be also prepared from nitration of 5-bromo-1-methyl-1*H*-imidazole [15]. Treatment of **3** with sodium thiophenolate or appropriate arylthiols in the presence of potassium hydroxide afforded the desired sulfides **4** and **8**–**11**, respectively. Oxidation of compound **9** by *m*-CPBA in dichloromethane gave 1,3,4-thiadiazole sulfone **12**. Phenylsulfone derivatives **5**–**7** obtained directly from the reaction of **3** with sodium phenylsulfinates in refluxing DMF.

Antibacterial activity

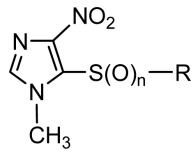
The synthesized compounds **4**–**12** were assessed for their antibacterial activity against a panel of Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Bacillus subtilis*) and Gram-negative (*Kelebsiella pneumoniae*, *Escherichia coli*, and *Enterobacter aerogenes*) bacteria using a conventional agar-dilution method. The MIC (minimum inhibitory concentration) values were determined and compared to norfloxacin as standard antibacterial drug. In addition, this series of compounds were tested *in vitro* against 15 clinical isolates of *Helicobacter pylori* in comparison to metronidazole as standard anti-*H. pylori* drug. The MIC values (μg/mL) obtained for compounds **4**–**12** are presented in Table 1.

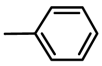
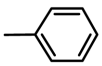
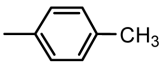
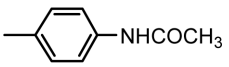
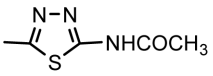
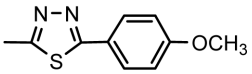
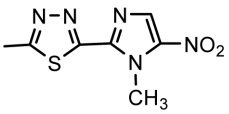
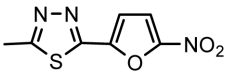
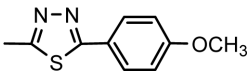
MIC values of the tested derivatives indicate that compounds **5**–**8** showed a higher antibacterial activity against Gram-positive rather than Gram-negative bacteria. Indeed, the latter compounds had respectable *in-vitro* activity against staphylococci and *B. subtilis* (MIC = 4–64 μg/mL), but were less active than the reference drug norfloxacin. Compound **5** was the most potent against Gram-positives, with MIC value of 4-8 μg/mL. None of the compounds showed significant activity against Gram-negative bacteria at concentrations =64 μg/mL. Generally, compounds containing phenyl ring showed better activity than compounds with the 1,3,4-thiadiazole ring system.

As is evident from the data, compounds **10** and **11** were the most active in terms of inhibiting the growth of *H. pylori* (MIC = 2 μg/mL), and their activities were found to be four times better than metronidazole. When 5-nitroimidazole **10** compared to 5-nitrofuran **11**, both compounds had similar *in-vitro* activities against clinical isolates of *H. pylori*, but the susceptibility against Gram-positive bacteria was reduced in 5-nitroimidazole **10**. Surprisingly, the susceptibility against Gram-negatives was lost in both compounds. The remaining compounds **5**–**9** showed weak activity against *H. pylori* strains (MIC = 32–64 μg/mL).

In summary, we have synthesized and evaluated the antibacterial activities of a series of 1-methyl-4-nitro-1*H*-imidazoles containing different arylthio- or arylsulfonyl-groups at the 5-position. Several compounds with 5-(phenylsulfonyl)-residue on the general scaffold have shown promising antibacterial activities against several types of Gram-positive bacteria including *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Bacillus subtilis*. A few tar-

Table 1. *In vitro* antibacterial activities of compounds **4 – 12** against selected strains[a].



Compound	R	n	*S. a.*[a]	*S. e.*[a]	B. s.[a]	*K. p.*[a]	*E. c.*[a]	*E. a.*[a]	*H. p.*[a]
4		0	>64[c]	64	64	>64	>64	>64	>64
5		2	8	4	4	>64	>64	>64	32 (16 to >64)[c]
6		2	32	8	16	>64	>64	>64	32 (16 to >64)[c]
7		2	16	32	8	>64	>64	>64	64 (16 to >64)[c]
8		0	32	8	64	>64	>64	>64	64 (16 to >64)[c]
9		0	16	64	>64	>64	>64	>64	64 (16 to >64)[c]
10		0	64	>64	>64	>64	>64	>64	2 (0.5 to 8)[c]
11		0	4	32	64	>64	>64	>64	2 (0.5 to 8)[c]
12		2	>64	>64	>64	>64	>64	>64	>64
Norfloxacin			1	1	0.13	0.25	0.13	0.13	
Metronidazole									8 (4 to >64)[c]

[a] *S. a.* = *Staphylococcus aureus* ATCC 6538p; *S. e.* = *Staphylococcus epidermidis* ATCC 12228; *B. s.* = *Bacillus subtilis* PTCC 1023; *K. p.* = *Kelebsiella pneumoniae* ATCC 10031; *E. c.* = *Escherichia coli* ATCC 8739; *E. a.* = *Enterobacter aerogenes* PTCC 1221, and 15 clinical isolates of *H. p.* = *H. pylori* were used for determination of MIC.

[b] (MICs in μg/mL)

[c] The MIC range is in the parenthesis.

get compounds **10** and **11** were also found to be effective against *Helicobacter pylori*. The structures of these compounds and the structure-activity relationship observed from this series of compounds can be used to further optimize the structures to obtain potent novel antimicrobial drugs.

The authors have declared no conflict of interest.

Experimental

Melting points were determined on a Kofler hot stage apparatus and are uncorrected (C. Reichert, Vienna, Austria). ^{1}H-NMR spectra were recorded using a Bruker AC-80 spectrometer (Bruker, Germany), and chemical shifts are expressed as δ (ppm) with tetramethylsilane as internal standard. The mass spectra were run on a Finnigan TSQ-70 spectrometer (Finnigan, USA) at 70 eV. Elemental analyses were carried out on a CHN-O-rapid elemental analyzer (Foss-Heraeus GmbH, Germany) for C, H and N, and the results are within ± 0.4% of the theoretical values. The purity of the synthesized compounds was confirmed by thin-layer chromatography (TLC) using various solvents of different polarities. Merck silica gel 60 F_{254} plates were applied for analytical TLC (Merck, Germany).

Chemistry

*1-Methyl-4-nitro-5-(phenylthio)-1H-imidazole **4***

A mixture of 1-methyl-5-bromo-4-nitro-1*H*-imidazole **3** (1.0 mmol) and sodium thiophenolate (1.0 mmol) in 10 mL ethanol was refluxed for two hours. The reaction mixture was cooled, poured into 20 mL of water, and extracted with chloroform. The extracts were washed with water, dried, and evaporated under vacuum. The residue is recrystallized from ethanol affording compound **4** in 81% yield. M.p. 75–76°C (m.p. 77–78°C, Lit. [16]); ^{1}H-NMR ($CDCl_3$) δ: 7.60 (s, 1H, imidazole), 7.36–7.16 (m, 5H, phenyl), 3.62 (s, 3H, CH_3). MS: m/z (%) 236 [M^+ + 1] (100), 235 [M^+] (37), 218 (94), 185 (18), 154 (18), 142 (30), 109 (30), 43 (18).

*General procedure for the synthesis of phenylsulfone derivatives **5–7***

A mixture of 1-methyl-5-bromo-4-nitro-1*H*-imidazole **3** (1.0 mmol) and sodium arylsulfinate (1.0 mmol) in 10 mL DMF was refluxed for three hours. After cooling, water (15 mL) was added and the precipitate was filtered, washed with water, and dried to give a solid which was recrystallized from ethanol.

*1-Methyl-4-nitro-5-(phenylsulfonyl)-1H-imidazole **5***

Yield 61%; m.p. 138–139°C (m. p. 137–138°C, Lit. [16]); ^{1}H-NMR ($CDCl_3$) δ: 8.18–8.06 (m, 2H, phenyl), 7.67–7.48 (m, 1H, imidazole and 3H, phenyl), 4.08 (s, 3H, CH_3). MS: m/z (%) 267 [M^+] (15), 203 (10), 141 (10), 133 (22), 110 (22), 83 (40), 77 (100), 42 (40).

*1-Methyl-4-nitro-5-(4-methylphenylsulfonyl)-1H-imidazole **6***

Yield 65%; m.p.128–130°C (m.p. 130–132°C, Lit. [16]); ^{1}H-NMR ($CDCl_3$) δ: 7.99 (d, 2H, phenyl, J_{AB} = 8.0 Hz), 7.46 (s, 1H, imidazole), 7.38 (d, 2H, phenyl, J_{AB} = 8.0 Hz), 4.06 (s, 3H, N-CH_3), 2.45 (s, 3H, CH_3). MS: m/z (%) 281 [M^+] (18), 200 (43), 146 (28), 139 (42), 111 (90), 107 (100), 79 (85), 65 (95), 42 (87).

*1-Methyl-4-nitro-5-[4-acetamido(phenylsulfonyl)]-1H-imidazole **7***

Yield 93%; m.p. 129–131°C (m.p. 125–127°C, Lit. [17]); ^{1}H-NMR ($CDCl_3$) δ: 8.06 (d, 2H, phenyl, J_{AB} = 8.8 Hz), 7.74 (d, 2H, phenyl, J_{AB} = 8.8 Hz), 7.45 (br s, 1H, imidazole and 1H, NH), 4.07 (s, 3H, N-CH_3), 2.22 (s, 3H, CH_3CO). MS: m/z (%) 324 [M^+] (15), 282 (25), 150 (21), 111 (90), 108 (100), 43 (15).

*General procedure for the synthesis of sulfides **8–11***

A mixture of 1-methyl-5-bromo-4-nitro-1*H*-imidazole **3** (1.0 mmol), 2-mercapto-5-substituted-1,3,4-thiadiazole (1.0 mmol), and potassium hydroxide (1.0 mmol) in 10 mL ethanol, was refluxed for two hours. After cooling, water (20 mL) was added and the precipitate was filtered and washed with water and dried to give a solid which was recrystallized from ethanol affording compounds **8**–11.

*2-[(1-Methyl-4-nitro-5-imidazolyl)thio]-5-acetamido-1,3,4-thiadiazole **8***

Yield 76%; m.p. 250–253°C; ^{1}H-NMR (DMSO-d_6) δ: 8.19 (br s, 1H, NH), 7.60 (s, 1H, imidazole), 3.77 (s, 3H, N-CH_3), 2.15 (s, 3H, CH_3CO). MS: m/z (%) 301 [M^+ + 1] (85), 254 (100), 212 (22), 159 (18), 142 (23), 43 (20). Anal. Calcd. for $C_8H_8N_6O_3S_2$: C, 31.99; H, 2.68; N, 27.98. Found: C, 32.12; H, 2.66; N, 28.14.

*2-[(1-Methyl-4-nitro-5-imidazolyl)thio]-5-(4-methoxyphenyl)-1,3,4-thiadiazole **9***

Yield 66%; m.p. 159–161°C; ^{1}H-NMR ($CDCl_3$) δ: 7.81 (d, 2H, phenyl, J_{AB} = 9.6 Hz), 7.66 (s, 1H, imidazole), 6.96 (d, 2H, phenyl, J_{AB} = 9.6 Hz), 3.92 (s, 3H, N-CH_3), 3.86 (s, 3H, OCH_3). MS: m/z (%) 349 [M^+] (63), 303 (45), 170 (18), 135 (15), 151 (100). Anal. Calcd. for $C_{13}H_{11}N_5O_3S_2$: C, 44.69; H, 3.17; N, 20.04. Found: C, 44.91; H, 3.28; N, 19.92.

*2-[(1-Methyl-4-nitro-5-imidazolyl)thio]-5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole **10***

Yield 71%; m.p. 188–190°C; ^{1}H-NMR ($CDCl_3$) δ: 8.05 (s, 1H, 5-nitroimidazole), 7.74 (s, 1H, 4-nitroimidazole), 4.51 (s, 3H, N-CH_3 of 5-nitroimidazole), 3.91 (s, 3H, N-CH_3 of 4-nitroimidazole). MS: m/z (%) 401 [M^+ + 1] (25), 385 (20), 369 (60), 322 (100), 285 (20), 247 (22), 201 (20), 170 (75), 142 (25), 83 (60), 67 (20). Anal. Calcd. for $C_{10}H_8N_8O_4S_2$: C, 32.61; H, 2.19; N, 30.42. Found: C, 32.48; H, 2.23; N, 30.54.

*2-[(1-Methyl-4-nitro-5-imidazolyl)thio]-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazole **11***

Yield 61%; m.p. 198–200°C; ^{1}H-NMR ($CDCl_3$) δ: 7.73 (s, 1H, imidazole), 7.50–7.28 (m, 2H, furyl), 3.92 (s, 3H, CH_3). MS: m/z (%) 354 [M^+] (10), 275 (18), 210 (100), 194 (21), 155 (83), 139 (30), 108 (55), 105 (100), 77 (95), 65 (70), 51 (38). Anal. Calcd. for $C_{10}H_6N_6O_5S_2$: C, 33.90; H, 1.71; N, 23.72. Found: C, 33.99; H, 1.68; N, 23.70.

2-[(1-Methyl-4-nitro-5-imidazolyl)sulfonyl]-5-(4-methoxyphenyl)-1,3,4-thiadiazole ***12***

A mixture of **9** (1.0 mmol), *m*-chloroperbenzoic acid (3.0 mmol), and $NaHCO_3$ (3.0 mmol) in 15 mL of dichloromethane was stirred for three days. The reaction mixture was monitored by TLC. After consumption of the starting material, water (15 mL) was added and the organic phase was separated. The aqueous phase was extracted with dichloromethane (2 × 15 mL). The combined organic phases were washed with water and dried (Na_2SO_4). Evaporation of the solvent gave a solid which was recrystallized from ethanol. Yield 78%; m.p. 181 – 183°C; ^{1}H-NMR ($CDCl_3$) δ: 7.91 (d, 2H, phenyl, J_{AB} = 8.8 Hz), 7.59 (s, 1H, imidazole), 7.00 (d, 2H, phenyl, J_{AB} = 8.8 Hz), 4.15 (s, 3H, N-CH_3), 3.88 (s, 3H, OCH_3). MS: m/z (%) 381 [M^+] (20), 366 (10), 349 (23), 303 (38), 151 (80), 133 (100), 83 (27). Anal. Calcd. for $C_{13}H_{11}N_5O_3S$: C, 49.21; H, 3.49; N, 22.07. Found: C, 49.42; H, 3.33; N, 21.96.

Antibacterial activity

The compounds **4** – **12** were evaluated for their antibacterial activity in side-by-side comparison with norfloxacin against Gram-positive (*Staphylococcus aureus* ATCC 6538p, *Staphylococcus epidermidis* ATCC 12228, and *Bacillus subtilis* PTCC 1023) and Gram-negative (*Escherichia coli* ATCC 8739, *Kelebsiella pneumoniae* ATCC 10031, and *Enterobacter aerogenes* PTCC 1221) bacteria using conventional agar-dilution method [18 – 20]. Twofold serial dilutions of the compounds and reference drug were prepared in Mueller – Hinton agar. Drugs (6.4 mg) were dissolved in dimethylsulfoxide (DMSO; 1 mL) and the solution was 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, 8, 4, 2, 1, 0.5, 0.25, 0.13, and 0.06 μg/mL. Petri dishes were incubated with $1 - 5 \times 10^4$ colony forming units (cfu) and incubated at 37°C for 18 h. The minimum inhibitory concentration (MIC) was the lowest concentration of the test compound, which resulted in no visible growth on the plate. To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with DMSO at the same dilutions as used in the experiment.

Anti-*Helicobacter pylori* activity

Fifteen clinical *H. pylori* isolates were used for determination of MIC. They were maintained at −80°C until they could be used for the experiments. The bacteria were grown on Columbia agar base (Difco Laboratories, USA) supplemented with 10% horse serum and 0.25% Bacto yeast extract (Difco) incubated for 72 h at 37°C under micro-aerobic conditions (10% CO_2) in a gas incubator (Heraeus, Germany). Before use, the media were always pre-incubated under the same micro-aerobic conditions for a minimum 2 h to allow equilibration, and none of the cultures was kept in air for more than 15 min. The MICs were determined by the agar dilution standard method [21]. The compounds were dissolved in DMSO giving a stock concentration of 6.4 mg/mL and were serially double diluted in agar medium to give concentrations ranging from 64 to 0.06 μg/mL. The plates of Columbia agar with horse serum and yeast extract containing antimicrobial agents were prepared on the day they were used. The inoculums were prepared as follows: a 72 h growth of each strain on agar plates was suspended in Wilkins – Chalgren broth (Difco) at a turbidity equivalent to the 0.5 McFarland standards. The plates were inoculated and incubated at 37°C for 72 h under micro-aerobic conditions (10% CO_2 in a gas incubator). The MIC was defined as the lowest concentration capable of inhibiting any visible bacterial growth.

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