Synthesis and *In Vitro* Antibacterial Activity of New 2-(1-Methyl-4-nitro-1*H*-imidazol-5-ylsulfonyl)-1,3,4-thiadiazoles

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Abstract: In the present study we report the synthesis and antibacterial activity of a new series 2-(1-methyl-4-nitro-1*H*-imidazol-5-ylsulfonyl)-1,3,4-thiadiazoles (**6a-c**). Compounds **6a-c** were tested *in vitro* by the conventional agar dilution method against a panel of microorganisms including gram-negative and gram-positive bacteria. Compound **6b** with 5-(5-nitrofuran-2-yl)-residue on 1,3,4-thiadiazole scaffold have shown promising antibacterial activities against gram-positive bacteria including *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Bacillus subtilis*.

Keywords: Synthesis, Nitroimidazole, 1,3,4-Thiadiazole, Antibacterial activity

Introduction

Nitroimidazoles, such as metronidazole, misonidazole, ornidazole, secnidazole, etanidazole and tinidazole are commonly used as therapeutic agents against a variety of protozoan and bacterial infections of humans and animals¹. Some nitroimidazole derivatives have become the important agents for a long time for treatment of serious infections caused by anaerobic bacteria and protozoa². As pathogenic bacteria continuously evolve mechanisms of resistance to currently use antibacterial drugs, the discovery of novel and potent antibacterial

agents is the best way to overcome bacterial resistance and develop effective therapies³. Moreover, the use of nitroheterocycles as antiviral, anticancer and radio sensitizing agents is well established⁴⁻¹⁰. 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 and *H. pylori*¹¹⁻¹³. In continuation to our ongoing research work on new nitroheterocyclic derivatives, herein, we describe the synthesis and *in vitro* antibacterial activity of new 5-sulfonyl-1-methyl-4-nitro-1*H*-imidazoles (**6a-c**) against a panel of gram-positive and gram-negative bacteria.

Experimental

All starting materials, reagents and solvents were purchased from merck and aldrich chemical companies. The purity of the synthesized compounds was confirmed by thin layer chromatography (TLC) using various solvents of different polarities. Merck silica gel 60 F254 plates were used for analytical TLC. Column chromatography was performed on Merck silica gel (70-230 mesh) for purification of intermediate and final compounds. ¹H NMR spectra were recorded using a Varian 80 spectrometer and chemical shifts are expressed as δ (ppm) with tetramethylsilane (TMS) as internal standard. The IR spectra were obtained on a Shimadzu 470 spectrophotometer (potassium bromide disks). Melting points were determined on a Kofler hot stage apparatus and are uncorrected.

Chemistry

The synthesis of 5-substituted-1-methyl-4-nitro-1*H*-imidazole derivatives (**6a-c**) was achieved with an efficient synthetic route outlined in Figure 1. The starting material 4(5)-bromo-5(4)-nitroimidazole (**1**) was prepared according to the literature method¹¹. 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. Treatment of **3** with appropriate arylthiols in the presence of potassium hydroxide afforded the desired sulfides **5a-c**. Oxidation of compounds **5a-c** by 3 moles of *m*-CPBA in dichloromethane gave 1,3,4-thiadiazole sulfones **6a-c**.

Figure 1. Structure of 5-substituent 1-methyl-4-nitro-1*H*-imidazoles (**6a-c**) reagent and condition: (i) CH₂N₂, dry ether; (ii) KOH, EtOH, reflux (iii) *m*-CPBA, CH₂Cl₂

General procedure for the synthesis of 5-substituent 1-methyl-4-nitro-1H-imidazoles (6a-c)

A mixture of **5a-c** (1.0 mmol), *m*-chloroperbenzoic acid (3.0 mmol) and NaHCO₃ (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 (Na₂SO₄). Evaporation of the solvent gave a solid which was recrystallized from ethanol.

N-(5-(1-Methyl-4-nitro-1H-imidazol-5-ylsulfonyl)-1,3,4-thiadiazol-2-yl)acetamide (**6a**)

Yield 83%; m.p. 152-154 °C; IR (KBr, cm⁻¹): 1670 (C=O), 1351 (NO₂), 1326, 1145 (S=O). ¹H NMR (80 MHz, CDCl₃)δ: 7.82(s, 1H, Imidazole), 3.68(s, 3H, NCH₃), 2.05 (s, 3H, COCH₃).

2-(1-Methyl-4-nitro-1H-imidazol-5-ylsulfonyl)-5-(5-nitrofuran-2-yl)-1,3,4-thiadiazole (**6b**)

Yield 82%; m.p. 148-150 °C; IR (KBr, cm⁻¹): 1555, 1357 (NO₂), 1328, 1144 (S=O). ¹H NMR (80 MHz, CDCl₃)δ: 8.05(d, 1H, furyl, *J*=3.8Hz), 7.59 (s, 1H, imidazole), 7.53(d, 1H, furyl, *J*=3.8Hz), 4.08 (s, 3H, CH₃).

2-(1-Methyl-4-nitro-1H-imidazol-5-ylsulfonyl)-5-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazole (**6c**)

Yield 85%; m.p. 82-84 °C; IR (KBr, cm⁻¹): 1550, 1350 (NO₂), 1324, 1142 (S=O). ¹H NMR (80 MHz, CDCl₃)δ: 8.13(s, 1H, 5-nitroimidazole), 7.87(s, 1H, 4-nitroimidazole), 3.70, 3.95 (2s, 6H, 2CH₃).

Biological activity

Compounds **6a-c** were screened for their antibacterial activity against gram-positive (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 14940, *Bacillus subtilis* ATCC 6051) and gram-negative (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 10031, *Enterobacter aerogenes* PTCC 1221) bacteria by the conventional agar dilution method¹². Twofold serial dilutions of the compounds and reference drugs were prepared in muller 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 muller-Hinton agar was performed to obtain the required concentrations of 64, 32, 16, 8, 4, 2, 1 and 0.5 µg/mL. Petri dishes were inoculated with 1-5×10⁴ 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. To insure 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.

Results and Discussion

The synthesized compounds **6a-c** 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 values (µg/mL) obtained for compounds **6a-c** are presented in Table 1. MIC values of the tested derivatives indicate that compounds **6b** and **6c** showed a higher antibacterial activity against gram- positive rather than gram-negative bacteria. Compounds **6b** and **6c** had respectable *in vitro* activity

against *Staphylococci* and *B. subtilis*, but were less active than the reference drug norfloxacin. Compound **6a** showed no significant activity against gram-positive and gram-negative bacteria.

Table 1. *In vitro* antibacterial activities of compounds **6a-c** against selected bacteria^a

Compound	Gram positive bacteria			Gram negative bacteria		
	<i>S. a</i>	<i>S. e.</i>	<i>B. s.</i>	<i>K. p.</i>	<i>E. c.</i>	<i>E. a</i>
6a	>64	>64	64	>64	>64	>64
6b	4	8	2	16	16	>64
6c	8	64	16	32	16	>64
Norfloxacin	1	1	0.13	0.25	0.13	0.13

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 were used for determination of MIC.

Conclusion

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. Compound **6b** with 5-(5-nitrofuran-2-yl)- residue on 1,3,4-thiadiazole scaffold have shown promising antibacterial activities against gram-positive bacteria including *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Bacillus subtilis*. The structure-activity relationship observed from this series of compounds can be used to further optimize the structures to obtain potent novel antimicrobial drugs.

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