

## Syntheses of Substituted 1,3,4-Oxadiazole, 1,3,4-Thiadiazole and 1,2,4-Triazole Derivatives as Potential Antimicrobial Agents

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### Abstract

A series of 2-(4-nitro-1-imidazolylmethyl)-1,3,4-oxadiazole, 1,3,4-triazole and 3-(4-nitro-1-imidazolylmethyl)-1,2,4-triazole derivatives were synthesized and tested for their antimicrobial activity. Some of the tested compounds were active against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Clostridium difficile*, *Aspergillus niger* and *Cryptococcus neoformans*.

**Keywords:** Antimicrobial agents; 1,3,4-Oxadiazoles; 1,3,4-Thiadiazoles; 1,2,4-Triazoles

### Introduction

The increasing clinical importance of drug-resistant microbial pathogens has lent additional urgency in microbiological and antifungal research. The ability of nitroimidazoles, to act as antimicrobial and antifungal agents is well known [1-3].

Substituted 1,2,4-triazoles, 1,3,4-thiadiazoles and 1,3,4-oxadiazoles are associated with many types of biological properties [4-9]. Antimicrobial activity data of these structures showed their considerable activity against Gram negative and Gram positive bacteria as well as some strains of fungi [10-21]. Keeping the above facts in mind, and in continuation of our previous studies on these heterocycles [15,16,23], it was of our interest to synthesize some new 2-(4-nitro-1-imidazolylmethyl)-1,3,4-oxadiazole, 1,3,4-thiadiazole and 3-(4-nitro-1-imidazolylmethyl)-1,2,4-triazole derivatives for their antimicrobial properties.

### Experimental Section

Melting points were determined on a Kofler hot stage microscope and are presented uncorrected. The <sup>1</sup>H-NMR spectra were obtained using a Bruker FT-80 or a Varian Unity plus 400 instrument with tetramethylsilane as the internal standard. The solvents used were CDCl<sub>3</sub> and DMSO-d<sub>6</sub>. IR spectra were recorded on a Nicolet Magna FT-IR 550 spectrometer (KBr disks). Mass spectra were taken using a Finnigan TSQ 70 eV. Elemental microanalyses were within ±0.4% of theoretical values for C, H, and N.

#### 2-Amino-5-(4-nitro-1-imidazolylmethyl)-1,3,4-oxadiazole (2a, R=H)

To a stirred solution of **1a** (1.5 g, 8 mmol) in dioxane (19 mL) in an ice-bath, sodium carbonate (0.84 g) in water (12 mL) was added and stirred for 5 min.

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Cyanogen bromide (0.84 g, 8 mmol) was added to the mixture and stirred for about 2 h at the same temperature. The precipitate was filtered and crystallized from methanol to give 0.7 g (41%) of **2a**; mp = 215-218°C. <sup>1</sup>HNMR (CDCl<sub>3</sub>): δ 8.38 (d, *J* = 1.3 Hz, 1H, H-5 imidazole), 7.93 (d, *J* = 1.3 Hz, 1H, H-2 imidazole), 7.31-7.09 (bs, 2H, NH<sub>2</sub>) and 5.50 ppm (s, 2H, N-CH<sub>2</sub>). MS *m/z* (%): 210 (M<sup>+</sup>, 85), 164 (4), 127 (96), 113 (4), 98 (100). Anal. Calcd. for C<sub>6</sub>H<sub>6</sub>N<sub>6</sub>O<sub>3</sub>: C, 34.29; H, 2.86; N, 40.00 Found: C, 34.48; H, 2.65; N, 40.05.

**2-Amino-5-(2-methyl-4-nitro-1-imidazolylmethyl)-1,3,4-oxadiazole (2b, R=CH<sub>3</sub>)**

This compound was prepared in a similar manner to **2a** in 50% yield. mp = 270-273°C (methanol). IR (KBr, cm<sup>-1</sup>): ν 3236, 3083, 1672. <sup>1</sup>HNMR (CDCl<sub>3</sub>): δ 8.32 (s, 1H, H-5 imidazole), 7.31-7.10 (bs, 2H, NH<sub>2</sub>), 5.43 (s, 2H, N-CH<sub>2</sub>) and 2.38 ppm (s, 3H, CH<sub>3</sub>). MS: *m/z* (%) 224 (M<sup>+</sup>, 12), 207 (78), 151 (43), 127 (9), 111 (7), 98 (100). Anal. Calcd. for C<sub>7</sub>H<sub>8</sub>N<sub>6</sub>O<sub>3</sub>: C, 37.50; H, 3.57; N, 37.50. Found: C, 37.65; H, 3.38; N, 37.64.

**5-Mercapto-2-(4-nitro-1-imidazolylmethyl)-1,3,4-oxadiazole (3a, R=H)**

To a stirred solution of **1a** (1 g, 5.4 mmol) in methanol (10 mL) in an ice-bath, 0.6 ml carbon disulfide (0.76 g, 8.8 mmol) and KOH (0.28 g) were added. The mixture was stirred at the same temperature for 3 h. The mixture was acidified with aqueous solution of hydrochloric acid. The precipitate was filtered and crystallized from methanol to give 1 g (80%) of **3a**; 81%, mp = 188-189°C. <sup>1</sup>HNMR (CDCl<sub>3</sub>): δ 8.36 (d, *J* = 1.4 Hz, 1H, H-5 imidazole), 7.87 (d, *J* = 1.4 Hz, 1H, H-2 imidazole), 5.07 (s, 2H, N-CH<sub>2</sub>). Anal. Calcd. for C<sub>6</sub>H<sub>5</sub>N<sub>5</sub>O<sub>3</sub>S: C, 31.72; H, 2.20; N, 30.84. Found: C, 31.50; H, 2.40; N, 30.65.

**5-Mercapto-2-(2-methyl-4-nitro-1-imidazolylmethyl)-1,3,4-oxadiazole (3b, R=CH<sub>3</sub>)**

This compound was prepared in a similar manner to **2a** in 82% yield; mp = 177-180°C (methanol). <sup>1</sup>HNMR (CDCl<sub>3</sub>): δ 8.27 (s, 1H, H-5 imidazole), 4.98 (s, 2H, N-CH<sub>2</sub>), and 2.30 ppm (s, 3H, CH<sub>3</sub>). Anal. Calcd. for C<sub>7</sub>H<sub>7</sub>N<sub>5</sub>O<sub>3</sub>S: C, 34.85; H, 2.90; N, 29.04. Found: C, 34.69; H, 3.05; N, 29.27.

**General Procedure for Preparation of Compounds 5a-h**

To a solution of **2** (0.01 mol) in ethanol (20 mL) alkyl (or aryl) isothiocyanate (0.01 mole) and sodium hydroxide (0.01 mol, 2 N solution) was added. The mixture was stirred for 2 h at room temperature and then acidified with aqueous solution of HCl in an ice bath. The mixture was kept in refrigerator for 3 h. to complete the precipitation. The precipitate was filtered and crystallized from ethanol to give compound **4** pure enough to be used in the next step. A mixture of compound **4** (0.01 mol) and conc. H<sub>2</sub>SO<sub>4</sub> (1.6 mL) was stirred overnight at room temperature, poured into ice-cold water, neutralized with liquid ammonia and the resulting solid was crystallized from methanol to give compound **5**.

**2-Butylamino-5-[(4-nitro-1H-imidazol-1-yl)methyl]-1,3,4-thiadiazole (5a, R=H, R'=CH<sub>3</sub>(CH<sub>2</sub>)<sub>3</sub>)**

Yield 55%; m.p. 134-136°C. <sup>1</sup>HNMR δ (DMSO-d<sub>6</sub>): 8.39 (d, *J* = 1.4 Hz, 1H, H<sub>5</sub>-imidazole), 7.93 (d, *J* = 1.3 Hz, 4H, H<sub>2</sub>-imidazole), 7.86 (bs, 1H, NH), 5.54 (s, 2H, CH<sub>2</sub>), 3.47 (t, *J* = 6.8 Hz, 2H, NCH<sub>2</sub> butyl), 1.52 (dt, *J* = 6.8, 7.2 Hz, 2H, CH<sub>2</sub>), 0.9 (t, *J* = 7.6 Hz, 3H, CH<sub>3</sub>). Anal. Calcd. for C<sub>10</sub>H<sub>14</sub>N<sub>6</sub>O<sub>2</sub>S: C, 42.55; H, 4.96; N, 29.79. Found: C, 42.78; H, 4.72; N, 29.95.

**2-Butylamino-5-[(2-methyl-4-nitro-1H-imidazol-1-yl)methyl]-1,3,4-thiadiazole (5b, R=CH<sub>3</sub>, R'=CH<sub>3</sub>(CH<sub>2</sub>)<sub>3</sub>-)**

Yield 83%; m.p. 159-161°C. <sup>1</sup>HNMR δ (DMSO-d<sub>6</sub>): 8.34 (s, 1H, H<sub>5</sub>-imidazole), 7.86 (bs, 1H, NH), 5.47 (s, 2H, CH<sub>2</sub>), 3.147 (t, *J* = 6.8 Hz, 2H, NCH<sub>2</sub> butyl), 2.36 (s, 3H, CH<sub>3</sub> imidazole), 1.52 (dt, *J* = 6.86, 7.2 Hz, 2H, CH<sub>2</sub>), 1.32 (dt, *J* = 7.7, 7.6 Hz, 2H, CH<sub>2</sub>), 0.85 (t, *J* = 7.6 Hz, 3H, CH<sub>3</sub>). Anal. Calcd. for C<sub>11</sub>H<sub>16</sub>N<sub>6</sub>O<sub>2</sub>S: C, 44.59; H, 5.41; N, 28.38. Found: C, 44.81; H, 5.60; N, 28.56.

**2-(4-Methoxyphenylamino)-5-[(4-nitro-1H-imidazol-1-yl)methyl]-1,3,4-thiadiazole (5c, R=H, R'=4-CH<sub>3</sub>OC<sub>6</sub>H<sub>4</sub>)**

Yield 20%; m.p. 195-197°C. <sup>1</sup>HNMR δ (DMSO-d<sub>6</sub>): 10.2 (br, 1H, NH), 8.45 (s, 1H, H<sub>5</sub>-imidazole), 7.93 (s, 1H, H<sub>2</sub> imidazole), 7.46 (d, *J* = 8.4 Hz, 2H aromatic), 7.14 (t, *J* = 8.4 Hz, 2H, aromatic), 5.68 (s, 2H, CH<sub>2</sub>), 3.74 (s, 3H, OCH<sub>3</sub>). Anal. Calcd. for C<sub>13</sub>H<sub>12</sub>N<sub>6</sub>O<sub>3</sub>S: C, 46.99; H, 3.61; N, 25.30. Found: C, 47.15; H, 3.45; N, 25.55.

**2-(4-Methoxyphenylamino)-5-[(2-methyl-4-nitro-imidazol-1-yl)methyl]-1,3,4-thiadiazole (5d, R=CH<sub>3</sub>, R'=4-CH<sub>3</sub>OC<sub>6</sub>H<sub>4</sub>)**

Yield 40%; m.p. 215-218°C. <sup>1</sup>HNMR δ (DMSO-d<sub>6</sub>): 10.25 (bs, 1H, NH), 8.55 (s, 1H, H<sub>5</sub>), 7.46 (d, *J* = 8.4 Hz, 2H aromatic), 7.14 (d, *J* = 8.4 Hz, 2H, aromatic), 5.59 (s, 2H, CH<sub>2</sub>), 3.73 (s, 3H, CH<sub>3</sub>O), 2.39 (s, 3H, CH<sub>3</sub>). Anal. Calcd. for C<sub>14</sub>H<sub>14</sub>N<sub>6</sub>O<sub>3</sub>S: C, 48.55; H, 4.05; N, 24.28. Found: C, 48.73; H, 4.24; N, 24.46.

**2-(4-Nitrophenylamino)-5-[(4-nitro-1H-imidazol-1-yl)methyl]-1,3,4-thiadiazole (5e, R=H, 4-NO<sub>2</sub>C<sub>6</sub>H<sub>4</sub>)**

Yield 94%; m.p. 274-277°C. <sup>1</sup>HNMR δ (DMSO-d<sub>6</sub>): 10.15 (bs, 1H, NH), 8.46 (d, *J* = 1.4 Hz, 1H, H<sub>5</sub>-imidazole), 8.20 (d, *J* = 8.8 Hz, 2H aromatic), 7.99 (d, *J* = 1.4 Hz, 1H, H<sub>2</sub> imidazole), 7.78 (d, *J* = 8.8 Hz, 2H, aromatic), 5.71 (s, 2H, CH<sub>2</sub>). Anal. Calcd. for C<sub>12</sub>H<sub>9</sub>N<sub>7</sub>O<sub>4</sub>S: C, 41.50; H, 2.61; N, 28.24. Found: C, 41.35; H, 2.78; N, 28.42.

**2-(4-Nitrophenylamino)-5-[(2-methyl-4-nitro-1H-imidazol-1-yl)methyl]-1,3,4-thiadiazole (5f, R=CH<sub>3</sub>, R'=4-NO<sub>2</sub>C<sub>6</sub>H<sub>4</sub>)**

Yield 98%; m.p. 259-262°C. <sup>1</sup>HNMR δ (DMSO-d<sub>6</sub>): 10.20 (bs, 1H, NH), 8.42 (s, 1H, H<sub>5</sub>-imidazole), 8.20 (d, *J* = 8.8 Hz, 2H aromatic), 7.93 (d, *J* = 8.8 Hz, 2H, imidazole), 5.66 (s, 2H, CH<sub>2</sub>), 2.40 (s, 3H, CH<sub>3</sub>). Anal. Calcd. for C<sub>13</sub>H<sub>11</sub>N<sub>7</sub>O<sub>4</sub>S: C, 43.20; H, 3.06; N, 27.13. Found: C, 43.05; H, 3.23; N, 27.01.

**General Procedure for Preparation of Compounds 6a-f**

A stirring mixture of compound **4** (0.01 mol) and sodium bicarbonate 5% in water (10 mL) was refluxed for 2.5 h. After cooling, the solution was acidified with hydrochloric acid. The precipitate was filtered and crystallized from ethanol to give compound **6**.

**4-Butyl-5-[(4-nitro-1H-imidazol-1-yl)methyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione (6a, R=H, R'=CH<sub>3</sub>(CH<sub>2</sub>)<sub>3</sub>)**

Yield: 58%; m.p. 197-198°C. <sup>1</sup>HNMR δ (DMSO-d<sub>6</sub>): 13.80 (bs, 1H, NH), 8.42 (d, *J* = 1.4 Hz, 1H, H<sub>5</sub> imidazole), 7.95 (d, *J* = 1.48 Hz, H<sub>2</sub> imidazole), 5.57 (s, 2H, CH<sub>2</sub>), 4.00 (t, 2H, *J* = 7.2 Hz, CH<sub>2</sub> butyl), 1.49 (m, 2H, CH<sub>2</sub>), 1.32 (mt, 2H, CH<sub>2</sub>), 0.90 (t, 3H, CH<sub>3</sub>). Anal. Calcd. for C<sub>10</sub>H<sub>14</sub>N<sub>6</sub>O<sub>2</sub>S: C, 42.54; H, 4.99; N, 29.77. Found: C, 42.65; H, 5.16; N, 29.98.

**4-Butyl-5-[(2-methyl-4-nitro-1H-imidazol-1-yl)methyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione (6b, R=CH<sub>3</sub>, R'=CH<sub>3</sub>(CH<sub>2</sub>)<sub>3</sub>)**

Yield: 76%; m.p. 292-294°C. <sup>1</sup>HNMR δ (DMSO-d<sub>6</sub>): 13.80 (bs, 1H, NH), 8.39 (s, 1H, H<sub>5</sub> imidazole), 5.53 (s, 2H, CH<sub>2</sub>), 3.95 (t, *J* = 7.2 Hz), 2.34 (s, 3H, CH<sub>3</sub>), 1.49 (m, 2H, CH<sub>2</sub>), 1.33 (m, 2H, CH<sub>2</sub>), 0.92 (t, *J* = 7.2 Hz, 3H, CH<sub>3</sub>). Anal. Calcd. for C<sub>11</sub>H<sub>16</sub>N<sub>6</sub>O<sub>2</sub>S: C, 44.58; H, 5.43; N, 28.36. Found: C, 44.79; H, 5.32; N, 28.17.

**4-(4-Methoxyphenyl)-5-[(4-nitro-1H-imidazol-1-yl)methyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione (6c, R=H, R'=4-CH<sub>3</sub>OC<sub>6</sub>H<sub>4</sub>)**

Yield: 50%; m.p. 260-262°C. <sup>1</sup>HNMR δ (DMSO-d<sub>6</sub>): 13.97 (s, 1H, NH), 7.81 (d, *J* = 1.2 Hz, 1H, H<sub>5</sub> imidazole), 7.34 (d, *J* = 1.2 Hz, 1H, H<sub>2</sub> imidazole), 7.33 (d, *J* = 8.8 Hz, 2H aromatic), 7.08 (d, *J* = 8.8 Hz, 2H, aromatic), 5.25 (s, 2H, CH<sub>2</sub>), 3.89 (s, 3H, OCH<sub>3</sub>). Anal. Calcd. for C<sub>13</sub>H<sub>12</sub>N<sub>6</sub>O<sub>3</sub>S: C, 46.98; H, 3.63; N, 25.28. Found: C, 47.07; H, 3.85; N, 25.49.

**4-(4-Methoxyphenyl)-5-[(2-methyl-4-nitro-1H-imidazol-1-yl)methyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione (6d, R=CH<sub>3</sub>, R'=4-CH<sub>3</sub>OC<sub>6</sub>H<sub>4</sub>)**

Yield: 51%; m.p. 273-275°C. <sup>1</sup>HNMR δ (DMSO-d<sub>6</sub>): 13.97 (s, 1H, NH), 7.78 (s, 1H, H<sub>5</sub> imidazole), 7.34 (d, *J* = 8.8 Hz, 2H, aromatic), 7.08 (d, *J* = 8.8 Hz, 2H aromatic), 5.21 (s, 2H, CH<sub>2</sub>), 3.82 (s, 2H, CH<sub>2</sub>), 2.19 (s, 3H, CH<sub>3</sub>). Anal. Calcd. for C<sub>14</sub>H<sub>14</sub>N<sub>6</sub>O<sub>3</sub>S: C, 48.54; H, 4.06; N, 24.26. Found: C, 48.75; H, 4.17; N, 24.04.

**4-(4-Nitrophenyl)-5-[(2-nitro-1H-imidazol-1-yl)methyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione (6e, R=H, R'=4-NO<sub>2</sub>C<sub>6</sub>H<sub>4</sub>)**

Yield: 65%; m.p. 140-142°C. <sup>1</sup>HNMR δ (DMSO-d<sub>6</sub>): 14.83 (s, 1H, NH), 8.43 (d, *J* = 8.8 Hz, 2H, aromatic), 8.02 (s, 1H, H<sub>5</sub>), 7.85 (d, *J* = 8.8 Hz, 2H aromatic), 5.35 (s, 2H, CH<sub>2</sub>). Anal. Calcd. for C<sub>12</sub>H<sub>9</sub>N<sub>7</sub>O<sub>4</sub>S: C, 41.49; H, 2.60; N, 28.23. Found: C, 41.70; H, 2.45; N, 28.05.

**4-(4-Nitrophenyl)-5-[(2-methyl-4-nitro-1H-imidazol-1-yl)methyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione (6f, R=CH<sub>3</sub>, R'=4-NO<sub>2</sub>C<sub>6</sub>H<sub>4</sub>)**

Yield: 53%; m.p. 270-272°C. <sup>1</sup>HNMR δ (DMSO-d<sub>6</sub>): 14.18 (s, 1H, NH), 8.43 (d, *J* = 8.8 Hz, 2H, aromatic), 8.02 (s, 1H, H<sub>5</sub> imidazole), 7.85 (d, *J* = 8.8 Hz, 2H aromatic), 5.27 (s, 2H, CH<sub>2</sub>), 2.24 (s, 3H, CH<sub>3</sub>). Anal. Calcd. for C<sub>13</sub>H<sub>11</sub>N<sub>7</sub>O<sub>4</sub>S: C, 43.21; H, 3.06; N, 27.13.

Found: C, 43.06; H, 3.28; N, 27.54.

#### General Procedure for Preparation of Compounds 7a-h

To a mixture of compound **6** (1.6 mmol) in ethanol (1 mL), aqueous solution of sodium hydroxide (10%, 0.83 mL) was added and it was kept under the ultrasonic condition for 1 min. Then corresponding alkyl halide (methyl iodide or ethyl iodide) (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 pure **7**.

#### 4-Butyl-3-[(2-Methylthio-5-[(4-nitro-1H-imidazol-1-yl)methyl]-4H-1,2,4-triazole (7a, R=H, R'=CH<sub>3</sub>(CH<sub>2</sub>)<sub>3</sub>, R''=CH<sub>3</sub>)

Yield: 76%; m.p. 292-294°C. <sup>1</sup>HNMR δ (DMSO-d<sub>6</sub>): 8.42 (d, *J* = 1.3 Hz, 1H, H<sub>5</sub>), 7.91 (d, *J* = 1.3 Hz, 1H, H<sub>2</sub> imidazole), 5.50 (s, 2H, CH<sub>2</sub>), 3.95 (t, *J* = 7.2 Hz, 2H, N-CH<sub>2</sub> butyl), 2.08 (s, 3H, SCH<sub>3</sub>), 1.46 (m, 2H, CH<sub>2</sub>), 1.28 (m, 2H, CH<sub>2</sub>), 0.90 (t, *J* = 7.6 Hz, 3H, CH<sub>3</sub>). Anal. Calcd. for C<sub>11</sub>H<sub>16</sub>N<sub>6</sub>O<sub>2</sub>S: C, 44.58; H, 5.44; N, 28.36. Found: C, 44.77; H, 5.27; N, 28.17.

#### 4-Butyl-3-[(2-Methylthio-5-[(2-methyl-4-nitro-1H-imidazol-1-yl)methyl]-4H-1,2,4-triazole (7b, R=R''=CH<sub>3</sub>, R'=CH<sub>3</sub>(CH<sub>2</sub>)<sub>3</sub>)

Yield: 60%; m.p. 152-154°C. <sup>1</sup>HNMR δ (DMSO-d<sub>6</sub>): 8.43 (s, 1H, H<sub>5</sub> imidazole), 5.49 (s, 2H, CH<sub>2</sub>), 3.94 (t, *J* = 7.2 Hz, 2H, N-CH<sub>2</sub> butyl), 2.57 (s, 3H, SCH<sub>3</sub>), 2.30 (s, 3H, CH<sub>3</sub>), 1.46 (m, 2H, CH<sub>2</sub>), 1.28 (m, 2H, CH<sub>2</sub>), 0.80 (t, *J* = 7.6 Hz, 3H, CH<sub>3</sub>). Anal. Calcd. for C<sub>12</sub>H<sub>18</sub>N<sub>6</sub>O<sub>2</sub>S: C, 46.43; H, 5.83; N, 27.07. Found: C, 46.61; H, 5.95; N, 27.28.

#### 4-(4-Methoxyphenyl)-3-methylthio-5-[(4-nitro-1H-imidazol-1-yl)methyl]-4H-1,2,4-triazole (7c, R=H, R'=4-CH<sub>3</sub>OC<sub>6</sub>H<sub>4</sub>, R''=CH<sub>3</sub>)

Yield: 70%; m.p. 171-173°C. <sup>1</sup>HNMR δ (DMSO-d<sub>6</sub>): 8.02 (d, *J* = 1.3 Hz, 1H, H<sub>5</sub> imidazole), 7.56 (d, *J* = 1.3 Hz, 1H, H<sub>2</sub> imidazole), 7.10 (d, *J* = 8.4 Hz, 2H, aromatic), 5.34 (s, 2H, CH<sub>2</sub>), 3.77 (s, 3H, OCH<sub>3</sub>), 2.51 (s, 3H, S-CH<sub>3</sub>). Anal. Calcd. for C<sub>14</sub>H<sub>14</sub>N<sub>6</sub>O<sub>3</sub>S: C, 50.90; H, 4.26; N, 25.44. Found: C, 50.98; H, 4.08; N, 25.31.

#### 4-(4-Methoxyphenyl)-3-methylthio-5-[(2-methyl-1H-imidazol-1-yl)methyl]-4H-1,2,4-triazole (7d, R=R''=CH<sub>3</sub>, R'=4-CH<sub>3</sub>OC<sub>6</sub>H<sub>4</sub>)

Yield: 80%; m.p. 144-146°C. <sup>1</sup>HNMR δ (DMSO-

d<sub>6</sub>): 7.99 (s, 1H, H<sub>5</sub> imidazole), 7.36 (d, *J* = 8.4 Hz, 2H, aromatic), 7.10 (d, *J* = 8.4 Hz, 2H, aromatic), 5.26 (s, 2H, CH<sub>2</sub>), 3.74 (s, 3H, OCH<sub>3</sub>), 2.49 (s, 3H, S-CH<sub>3</sub>), 2.10 (s, 3H, CH<sub>3</sub>). Anal. Calcd. for C<sub>15</sub>H<sub>16</sub>N<sub>6</sub>O<sub>3</sub>S: C, 49.99; H, 4.47; N, 23.32. Found: C, 50.01; H, 4.30; N, 23.11.

#### 4-(4-Nitrophenyl)-3-methylthio-5-[(4-nitro-1H-imidazol-1-yl)methyl]-4H-1,2,4-triazole (7e, R=H, R'=4-NO<sub>2</sub>C<sub>6</sub>H<sub>4</sub>, R''=CH<sub>3</sub>)

Yield: 80%; m.p. 116-118°C. <sup>1</sup>HNMR δ (DMSO-d<sub>6</sub>): 8.43 (d, *J* = 8.8 Hz, 2H, aromatic), 8.16 (s, 1H, H<sub>5</sub> imidazole), 7.80 (d, *J* = 8.8 Hz, 2H, aromatic), 7.67 (s, 1H, H<sub>5</sub> imidazole), 5.46 (s, 2H, CH<sub>2</sub>), 2.54 (s, 3H, S-CH<sub>3</sub>). Anal. Calcd. for C<sub>13</sub>H<sub>11</sub>N<sub>7</sub>O<sub>4</sub>S: C, 43.21; H, 3.06; N, 27.13. Found: C, 43.44; H, 3.28; N, 27.02.

#### 4-(4-Nitrophenyl)-3-methylthio-5-[(2-methyl-4-nitro-1H-imidazol-1-yl)methyl]-4H-1,2,4-triazole (7f, R=R''=CH<sub>3</sub>, R'=4-NO<sub>2</sub>C<sub>6</sub>H<sub>4</sub>)

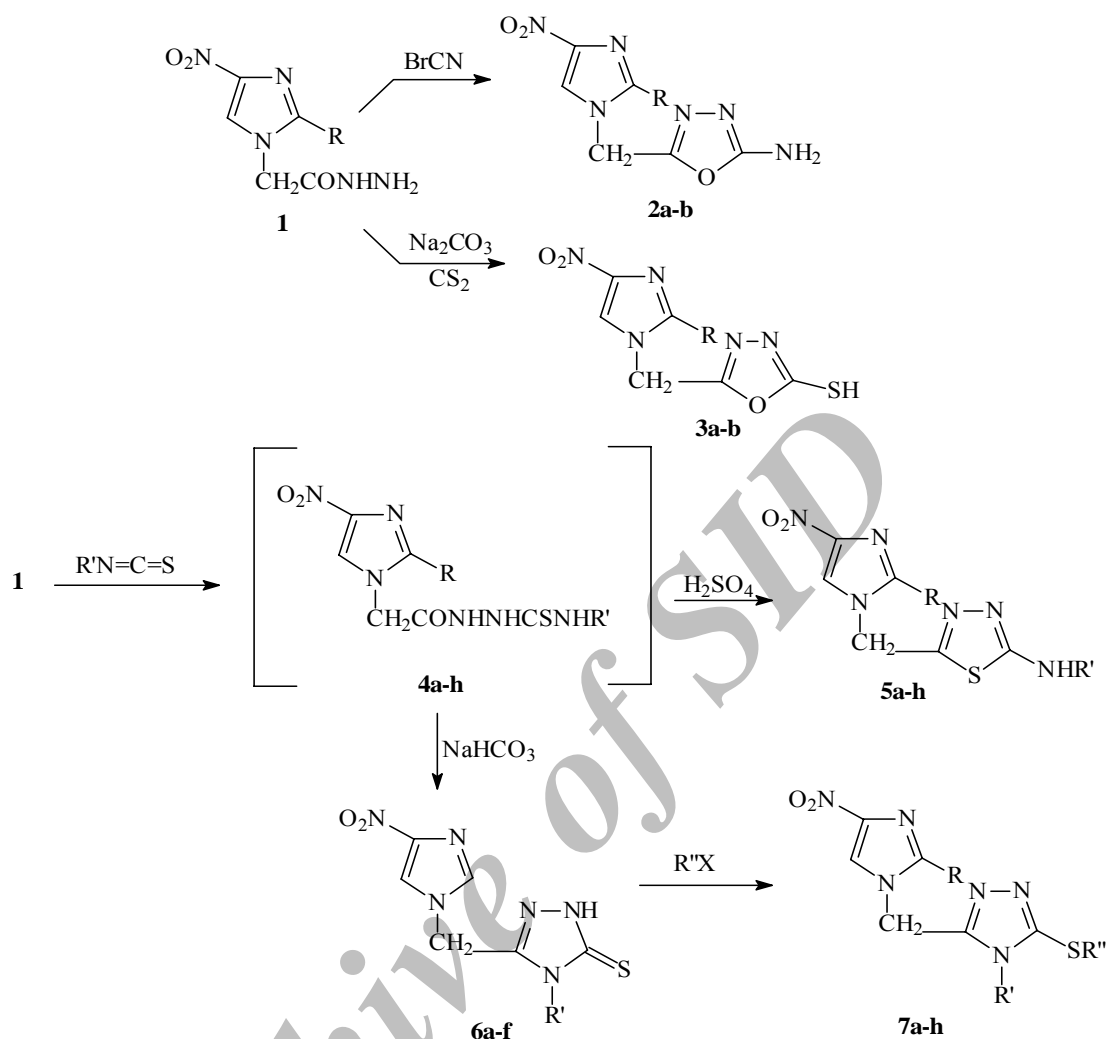
Yield: 60%; m.p. 107-109°C. <sup>1</sup>HNMR δ (DMSO-d<sub>6</sub>): 8.43 (d, *J* = 8.8 Hz, 2H, aromatic), 8.16 (s, 1H, H<sub>5</sub> imidazole), 7.80 (d, *J* = 8.8 Hz, 2H, aromatic), 8.16 (s, 1H, H<sub>5</sub> imidazole), 7.80 (d, *J* = 8.8 Hz, 2H, aromatic), 2.40 (s, 2H, CH<sub>2</sub>), 2.59 (s, 3H, SCH<sub>3</sub>), 2.23 (s, 3H CH<sub>3</sub>). Anal. Calcd. for C<sub>14</sub>H<sub>13</sub>N<sub>7</sub>O<sub>4</sub>S: C, 44.79; H, 3.48; N, 26.12. Found: C, 44.56; H, 3.69; N, 26.19.

#### 4-(4-Nitrophenyl)-3-ethylthio-5-[(4-nitro-1H-imidazol-1-yl)methyl]-4H-triazole (7g, R=H, R'=4-NO<sub>2</sub>C<sub>6</sub>H<sub>4</sub>, R''=C<sub>2</sub>H<sub>5</sub>)

Yield: 80%; m.p. 99-101°C. <sup>1</sup>HNMR δ (DMSO-d<sub>6</sub>): 8.43 (d, *J* = 8.4 Hz, 2H, aromatic), 7.90 (s, 1H, H<sub>5</sub> imidazole), 7.82 (d, *J* = 8.4 Hz, 2H, aromatic), 7.67 (s, 1H, H<sub>2</sub> imidazole), 5.45 (s, 2H, N-CH<sub>2</sub>), 3.11 (q, *J* = 7.6 Hz, 2H, CH<sub>2</sub>S), 1.29 (t, *J* = 7.9 Hz, 3H CH<sub>3</sub>). Anal. Calcd. for C<sub>14</sub>H<sub>13</sub>N<sub>7</sub>O<sub>4</sub>S: C, 44.79; H, 3.48; N, 26.12. Found: C, 44.92; H, 3.67; N, 26.34.

#### 4-(4-Nitrophenyl)-3-ethylthio-5-[(4-nitro-1H-imidazol-1-yl)methyl]-4H-triazole (7h, R=CH<sub>3</sub>, R'=4-NO<sub>2</sub>C<sub>6</sub>H<sub>4</sub>, R''=C<sub>2</sub>H<sub>5</sub>)

Yield: 60%; m.p. 110-113°C. <sup>1</sup>HNMR δ (DMSO-d<sub>6</sub>): 8.43 (d, *J* = 8.4 Hz, 2H, aromatic), 7.92 (s, 1H, H<sub>5</sub> imidazole), 7.82 (d, *J* = 8.4 Hz, 2H, aromatic), 5.36 (s, 2H, N-CH<sub>2</sub>), 3.09 (q, *J* = 7.6 Hz, 2H, CH<sub>2</sub>S), 2.22 (s, 3H CH<sub>3</sub>), 1.28 (t, *J* = 7.6 Hz, 3H, CH<sub>3</sub>). Anal. Calcd. for C<sub>15</sub>H<sub>15</sub>N<sub>7</sub>O<sub>4</sub>S: C, 46.26; H, 3.78; N, 25.18. Found: C, 46.48; H, 3.98; N, 25.02.



Scheme 1

### Microbiology

The bacterial strains used included the Gram-positives; *Staphylococcus aureus* ATCC 2937, *Staphylococcus epidermidis* ATCC 12229, *Bacillus subtilis* ATCC 12711 and Gram-negatives; *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027. *Clostridium difficile* (clinical isolate) was anaerobe. The fungi included *Aspergillus niger* ATCC 6404 and a clinical isolate of *Cryptococcus neoformans*. The anaerobic bacteria were incubated in a candle jar containing Anaerocult (Merck) at 37°C for 72 h. Fungi were aerobically incubated at 25°C.

Disc diffusion method was used primarily to screen the antimicrobial potencies of the compounds. Each disc contained 300 µg of each compound in DMSO. Blank

discs loaded with the same amounts of antimicrobial agents of metronidazole, ketokonazole and clotrimazole while in the case of gentamycin and cefotaxime antibiotics the concentrations were 10 µg and 30 µg, respectively.

### Results and Discussion

The title compounds were synthesized according to the sequence shown in Scheme 1. Reaction of 2-(4-nitro-1-imidazolyl)acetic acid hydrazide **1** [22] with cyanogen bromide or carbon disulfide afforded substituted 2-amino-1,3,4-oxadiazoles **4** or substituted 5-mercapto-1,3,4-oxadiazoles **3**, respectively. Addition of different isothiocyanates to compounds **1** gave corresponding thiosemicarbazides **2** as intermediates,

**Table 1.** Antimicrobial activity of compounds **2,3** and **5-7** (zone of inhibition in mm)

Compound	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. difficile</i>	<i>A. niger</i>	<i>C. neoformans</i>
<b>2a</b>	-	-	-	-	-	18	-	14
<b>2b</b>	-	-	17	-	-	-	-	-
<b>3b</b>	-	-	-	11	-	-	-	-
<b>5a</b>	15	22	20	-	-	20	11	17
<b>5b</b>	-	-	10	-	-	18	-	-
<b>5c</b>	-	11	17	-	-	10	10	13
<b>5d</b>	16	-	12	-	-	11	11	17
<b>5e</b>	-	15	15	-	-	10	12	16
<b>5f</b>	-	-	8	-	-	-	11	8
<b>6a</b>	-	-	12	10	-	16	10	12
<b>6b</b>	-	-	12	12	-	-	11	14
<b>6d</b>	-	-	16	-	-	-	-	-
<b>6e</b>	10	23	34	-	-	15	-	15
<b>6f</b>	8	-	-	-	-	20	-	15
<b>7a</b>	-	-	-	-	-	15	-	-
<b>7b</b>	11	-	12	-	-	17	-	-
<b>7c</b>	16	-	-	-	-	17	-	-
<b>7d</b>	14	-	-	-	-	19	-	-
<b>7e</b>	20	16	22	-	-	22	12	-
<b>7f</b>	11	12	20	-	-	20	16	-
<b>7g</b>	-	12	-	-	-	20	-	-
<b>7h</b>	-	13	-	-	-	20	-	-
<b>Gentamycin</b>	20	35	17	20	16	n.t.	n.t.	n.t.
<b>Cefotaxim</b>	17	36	35	28	16	n.t.	n.t.	n.t.
<b>Metonidazole</b>	-	-	27	-	-	-	-	n.t.
<b>Ketoconazole</b>	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	23	n.t.
<b>Clotrimazole</b>	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	27	n.t.

which on cyclization in the presence of conc.  $H_2SO_4$  or  $NaHCO_3$  afforded substituted alkyl(or aryl)amino-1,3,4-thiadiazoles **5** or substituted 1,2,4-triazol-5-thiones **6** respectively. Substituted 5-alkylthio-1,2,4-triazoles **7** were synthesized through the reaction of compound **6** with corresponding alkyl halides under the ultrasonic condition.

Compounds **2**, **3** and **5-7** were screened for their antimicrobial activity against certain microorganisms by cup-plate method [24] at a concentration of 200  $\mu$ g using DMSO as a solvent. The zone of inhibition was measured in millimeters and the number of replication in each case was two. As it is shown in Table 1 most of the compounds exhibited activity against *C. difficile*, however all the compounds were less active in comparison to metronidazole which was taken as

standard drug. Some of the compounds showed considerable activity against fungi and Gram positive strains, but none of them exhibited marked activity against Gram negative strains. Compounds **5a**, **5d**, **7c** and **7e** exerted comparable activities relative to reference drug cefotaxime against *S. aureus*. Similarly, compounds **2b**, **2a**, **2c**, **7e**, **7e** and **7f** showed comparable activities in comparison with gentamycin against *B. subtilis*. In case of antifungal activity, the tested compounds exhibited low to moderate activity which was not comparable to the standard compounds of ketoconazole and clotrimazole. It seems that the presence of nitroimidazole moiety in the structure of tested compounds is the most important reason for their anti *C. difficile* activity. Compounds having 1,3,4-oxadiazole ring in their structures **2,3** were less active

than the others. Although 1,2,4-triazole-5-thiones **6a-f** showed weak antimicrobial activity, but all of the corresponding S-alkylated compounds **7a-h** exhibited considerable activity specially against *C. difficile*. *p*-Nitrophenyl group at 4-position of 1,2,4-triazoles **6e-f** and **7e-h** was the best substitution among these series of tested compounds.

Compounds having 1,3,4-thiadiazole ring were effective against *B. Subtilis* and *C. difficile*.

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### References

1. Bock M. *Arzneim. Forsch. Drug Res.*, **11**: 587 (1961).
2. Hoffer M. and Grunberg E. *J. Med. Chem.*, **17**: 1019 (1974).
3. Bhatia S.C. and Shanbhag V.D. *J. Chromatogr.*, **305**: 325 (1984).
4. Palaska E., Sahin G., Kelicen P., and Durlv N.T. *Formaco*, **57**: 101 (2002).
5. Terzioglu N. and Gursoy A. *Eur. J. Med. Chem.*, **38**: 781 (2003).
6. Shivarama Holla B., Veerendra B., Shivanada M.K., and Poojary B. *Eur. J. Med. Chem.*, **38**: 759 (2003).
7. Buschelli D.H., Connor D.T., Bornemeier D.A., Dyer R.D., Kennedy J.A., Kuipers P.J., Okonkwo G.C., Schrier D.J., and Wright C.D. *J. Med. Chem.*, **36**: 1802 (1993).
8. Jakubkien V., Burbulien M.M., Mekugkien G., Udrnaint E., Gaidelis P., and Vainilavigius U. *Farmaco*, **58**: 323 (2003).
9. Omar F.A., Mahfouz N.M., and Rahman M.A. *Eur. J. Med. Chem.*, **31**: 819 (1996).
10. Foroumadi A., Asadipour A., Mirzaei M., Karimi J., and Emami S. *Farmaco*, **57**: 765 (2002).
11. Ulusomy N., Gursory A., and Otuk G. *Farmaco*, **56**: 947 (2001).
12. Shivarama Holla B., Sarojini B.K., Rao B.S., Akberali P.M., Kumari N.S., and Shetty V. *Farmaco*, **56**: 565 (2001).
13. Demirayak S., Benkli K., and Guven K. *Eur. J. Med. Chem.*, **35**: 1037 (2000).
14. Shivarama Holla B., Gonsalves R., and Shenoy Sh. *Ibid.*, **35**: 267 (2000).
15. Shafiee A., Sayadi A., Roozbahani M.H., Foroumadi A., and Kamal F. *Arch. Pharm. Pharm. Med. Chem.*, **10**: 495 (2002).
16. Jalilian A.R., Sattari S., Binesh marvasti M., Shafiee A., and Daneshthalab M. *Ibid.*, **333**: 347 (2000).
17. Hui X. and Zhang Z. *Indian J. Chem.*, **38B**: 1066 (1999).
18. Gulerman N., Rollas S., Kiraz M., Ekinici A.C., and Vidin A. *Farmaco*, **52**: 691 (1997).
19. Dogan H.N., Rollas S., and Erdeniz H. *Farmaco*, **53**: 462 (1998).
20. Ashar F.A., Habibi N.S., Taibbi M., Dine S., and Dine A. *Farmaco*, **45**: 134 (1990).
21. Muhi-Eldeen Z. Nadir M., Aljobory N.R., Hussein F., and Stohs S. *J. Eur. J. Med. Chem.*, **26**: 237 (1991).
22. Shafiee A., Naimi E., Mansobi P., Foroumadi A., and Shekar M. *J. Het. Chem.*, **32**: 1235 (1995).
23. Cosar C., Crisan C., Horclois R., Jacob R.M., Robert J., Tchelitcheff S., and Voupre R. *Arzneim-Forsch./Drug Res.*, **16**: 23 (1966).
24. Barry A.L. In: Ilius Lea and Febiger (Ed.), The antimicrobial susceptibility test: Principle and practices. p. 180, Biol. Abstr., **64**: 25183 (1977).