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Antituberculosis Agents X. Synthesis and Evaluation of *In Vitro* Antituberculosis Activity of 2-(5-Nitro-2-furyl)- and 2-(1-Methyl-5-nitro-1*H*-imidazol-2-yl)-1,3,4-thiadiazole Derivatives

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Two series of 2-(5-nitro-2-furyl)- and 2-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-5-propyl, allyl and propargyl)thio-1,3,4-thiadiazoles (**6a-f**) and 2-(5-nitro-2-furyl)- and 2-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-5-(nitrobenzyl)thio-1,3,4-thiadiazole derivatives (**8a-f**) have been synthesized and evaluated against *Mycobacterium tuberculosis,* as part of the TAACF TB screening program under direction of the US National Institute of Health, the NIAID division. Primary screening was conducted at a single concentration, 6.25 μgmL^{-1}, against *M. tuberculosis* $H_{37}Rv$ in BACTEC 12B medium, using the Microplate Alamar Blue Assay (MABA). The minimum inhibitory concentration (MIC) was determined for the compounds that demonstrated ≥90% growth inhibition in the primary screening. A varying degree of antituberculosis activity (from 0-97% of growth inhibition) was observed with the alkylthio series (**6a-f**), and the nitroimidazole derivative with a propylthio group (**6b**) and the nitrofuran derivative with a propargylthio group (**6e**), were the most active compounds (MIC=3.13 and 1.56 μgmL^{-1}, respectively). Among the nitrobenzylthio derivatives (**8a-f**), all the ortho, meta and para nitrobenzyl isomers in the nitrofuran series exhibited good antituberculosis activity (MIC=3.13 μgmL^{-1}), while the corresponding nitroimidazole analogues were completely inactive (Inhibition=0%).

Key words: *Mycobacterium tuberculosis*, 1,3,4-Thiadazole, Nitrofuran, Nitroimidazole

INTRODUCTION

Tuberculosis (TB) remains a major health problem, with two million deaths and eight million new cases annually (Kaufmann, 2002). The high rate of drug-resistant tuberculosis currently reported in many countries is a serious problem (Grange and Zumla, 2002). The resistance is often a corollary to HIV infection, and drug-resistant TB is more difficult and expensive to treat, and is more likely to be fatal (Pasqualoto and Ferreira, 2001). Thus, the developments of potent new antituberculosis drugs, which are active against resistant strains and latent forms, and reduce the treatment period, are urgently needed to combat this disease. Some nitroimidazole and nitrofuran derivatives have been claimed to possess *in vitro* antibacterial, antifungal and antituberculosis activities (Gunay *et al.*, 1999; Pires *et al.*, 2001). Also, the 1,3,4-thiadiazole ring system is known to have several biological activities, and the antibacterial properties have largely been described (Mamolo *et al.*, 1996; Rollas *et al.*, 1996; Tsotinis *et al.*, 1997).

Thus, the syntheses and antituberculosis activities of some 2-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-1,3,4-thiadiazole and 2-(5-nitro-2-furyl)-1,3,4-thiadiazole derivatives have previously been described by our laboratory, which showed significant antituberculosis activities (Foroumadi *et al.*, 2001a & 2001b). Also, our laboratory recently reported the syntheses and antituberculosis activities of some 2-(5-nitro-2-furyl)-5-alkylthio-1,3,4-thiadiazole derivatives, and those compounds with methyl, ethyl and 4-nitrobenzylthio groups showed good antituberculosis activities (Foroumadi *et al.*, 2002).

Accordingly, as a part of an extensive search for new antituberculosis agents, herein is reported the syntheses of some 2-aryl-5-(propyl, allyl and propargyl)thio-1,3,4-thiadiazole derivatives (**6a-f**), with potential as antituber-

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culosis agents. Taking in to consideration the good activity of the 2-(5-nitro-2-furyl)-5-(4-nitrobenzyl) thio-1,3,4-thiadiazole (**8e**) (Foroumadi *et al.*, 2002), in this study the syntheses and antituberculosis activities of 2-aryl-5-(o, m or *p*-nitrobenzyl) thio-1,3,4-thiadiazole derivatives (**8a-f**) are reported.

MATERIAL AND METHODS

The melting points were obtained using an Electrothermal IA-9100 capillary apparatus, and are uncorrected. The IR spectra were obtained using a Shmadzu 470 spectrograph (KBr disk). The ^{1}H-NMR spectra were recorded on a Bruker AC-80 or Bruker DRX-500 Advance spectrometer, and the chemical shifts (δ) are in ppm relative to tetramethylsilane (TMS), which was used as an internal standard.

2-(5-Nitro-2-furyl)-5-(*n*-propyl)thio-1,3,4-thiadiazole (6a)

To a mixture of 2-mercapto-5-(5-nitro-2-furyl)-1,3,4-thiadiazole (**4a**, 229 mg, 1 mmol) (Foroumadi *et al.*, 2001a) and *n*-propyl bromide (**5a**, 186 mg, 1.5 mmol) in ethanol (10 mL), NaOH (40 mg in 5 ml H_2O) was added drop wise, and the mixture stirred at room temperature overnight. Water was added, and the separated solid filtered off, washed with water and crystallized from EtOH, to give 200 mg of **6a** in a 74% yield, m.p.91-93°C. IR(KBr) ν_{max}: 3120 (furyl), 1536 and 1350 cm^{-1} (NO_2). ^{1}H-NMR ($CDCl_3$, 80 MHz) δ: 7.45 (d, 1H, furyl, *J*=3.8 Hz), 7.29 (d, 1H, furyl, *J*=3.8 Hz), 3.37 (t, 2H, CH_2, *J*=7.1 Hz), 2.13-1.65 (m, 2H, CH_2) and 1.08 ppm (t, 3H, CH_3, *J*=7.1 Hz).

2-(1-Methyl-5-nitro-1*H*-imidazol-2-yl)-5-(*n*-propyl)thio-1,3,4-thiadiazole(6b)

The synthesis of compound **6b** has previously been reported (Foroumadi *et al.*, 2001b).

2-(5-Nitro-2-furyl)-5-(2-propenyl)thio-1,3,4-thiadiazole (6c)

This compound was prepared from **4a** and allyl bromide, as described for **6a,** in a 67% yield, m.p.100-102°C (EtOH). IR (KBr) ν_{max}: 3100 (furyl), 1510 and 1347 cm^{-1} (NO_2). ^{1}H-NMR ($CDCl_3$, 80 MHz) δ: 7.43 (d, 1H, furyl, *J*=4.0 Hz), 7.33 (d, H, furyl, *J*=4.0 Hz), 6.10-5.98 (m, 1H, -CH=), 5.60-5.19 (m, 2H, = CH_2) and 4.15-3.8 ppm (m, 2H, SCH_2).

2-(1-Methyl-5-nitro-1*H*-imidazol-2-yl)-5-(2-propenyl) thio-1,3,4-thiadiazole (6d)

This compound was prepared from **4b** (Foroumadi *et al.*, 2001a) and ally bromide, as described for **6a,** in a 70% yield, m.p.118-120°C (EtOH). IR (KBr) ν_{max}: 3168 (H-C4 imidazole), 1516 and 1340 cm^{-1} (NO_2). ^{1}H-NMR ($CDCl_3$, 80 MHz) δ: 8.07 (s, 1H, H4-imidazole), 6.20-5.79 (m, 1H, -CH=), 5.60-5.15 (m, 2H, =CH_2), 4.55 (s, 3H, CH_3) and 4.12-3.9 ppm (m, 2H, SCH_2).

2-(5-Nitro-2-furyl)-5-(2-propynyl)thio-1,3,4-thiadiazole (6e)

This compound was prepared from **4a** and propargyl bromide, as described for **6a**, in a 60% yield, m.p.130-132°C (EtOH). IR (KBr) ν_{max}: 3120 (furyl), 2352(C≡CH), 1526 and 1347 cm^{-1} (NO_2). ^{1}H-NMR ($CDCl_3$, 80 MHz) δ: 7.46 (d, 1H, furyl, *J*= 3.8 Hz), 7.33 (d, 1H, furyl, *J*=3.8 Hz), 4.13 (d, 2H, CH_2, *J*=2.6 Hz) and 2.35 ppm (t, 1H, C≡CH, *J*= 2.6 Hz).

2-(1-Methyl-5-nitro-1*H*-imidazol-2-yl)-5-(2-propynyl) thio-1,3,4-thiadiazole (6f)

This compound was prepared from **4b** and propargyl bromide, as described for **6a,** in a 65% yield, m.p. 170-172°C (EtOH). IR(KBr) ν_{max}: 3136 (H-C4 imidazole), 1510 and 1344 cm^{-1} (NO_2). ^{1}H-NMR ($CDCl_3$, 80 MHz) δ: 8.07 (s, 1H, H4-imidazole), 4.55 (s, 3H, CH_3), 4.14 (d, 2H, CH2, *J*=2.6 Hz) and 2.35 ppm (t, 1H, C≡CH, *J*=2.6 Hz).

2-(5-Nitro-2-furyl)-5-(2-nitrobenzyl)thio-1,3,4-thiadiazole (8a)

To a mixture of 2-mercapto-5-(5-nitro-2-furyl)-1,3,4-thiadiazole (**4a**, 229 mg, 1 mmol) and 2-nitrobenzyl chloride (**7a**, 171.5 mg, 1 mmol) in ethanol (10 mL), KOH (66 mg 85% in 5 ml H_2O) was added drop wise, and the mixture stirred at room temperature overnight. Water was added, and the separated solid filtered off, washed with water and crystallized from EtOH-H_2O, to give 317 mg of **8a,** in a 87% yield, m.p. 179-180°C. IR(KBr) ν_{max}: 1507 and 1340 cm^{-1} (NO_2). ^{1}H-NMR ($CDCl_3$, 80 MHz) δ: 8.30-7.50 (m, 4H, aromatic), 7.44 (d, 1H, furyl, *J*=3.8 Hz), 7.32 (d, 1H, furyl, *J*=3.8 Hz) and 5.01 ppm (s, 2H, SCH_2).

2-(1-Methyl-5-nitro-1*H*-imidazol-2-yl)-5-(2-nitrobenzyl)thio-1,3,4-thiadiazole (8b)

This compound was prepared from **4b** and 2-nitrobenzyl chloride, as described for **8a,** in a 93% yield, m.p. 183-184°C. IR(KBr) ν_{max}: 1517 and 1340 cm^{-1} (NO_2). ^{1}H-NMR ($CDCl_3$, 80 MHz) δ: 8.24-7.43 (m, 5H, aromatic), 5.01 (s, 2H, SCH_2) and 4.55 ppm (s, 3H, NCH_3).

2-(5-Nitro-2-furyl)-5-(3-nitrobenzyl)thio-1,3,4-thiadiazole (8c)

This compound was prepared from **4a** and 3-nitrobenzyl chloride, as described for **8a,** in an 82% yield, m.p.119-120°C. IR(KBr) ν_{max}: 1523 and 1344 cm^{-1} (NO_2). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 8.38 (s, 1H, aromatic), 8.18 (d, 1H, aromatic, *J*=8 Hz), 7.87 (d, 1H, aromatic, *J*=8 Hz), 7.57 (t,

1H, aromatic, *J*=8 Hz), 7.47(d, 1H, furyl, *J*=3.5 Hz), 7.34 (d, 1H, furyl, *J*=3.5 Hz) and 4.74 ppm (s, 2H, SCH_2).

2-(1-Methyl-5-nitro-1*H*-imidazol-2-yl)-5-(3-nitrobenzyl)thio-1,3,4-thiadiazole (8d)

This compound was prepared from **4b** and 3-nitrobenzyl chloride, as described for **8a**, in an 89% yield, m.p. 203-204°C. IR (KBr) ν_{max}: 1523 and 1340 cm^{-1} (NO_2). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 8.38 (s, 1H, aromatic), 8.19 (d, 1H, aromatic, *J*=8 Hz), 8.09 (s, 1H, H4-imidazole), 7.86 (d, 1H, aromatic, *J*=8 Hz), 7.56 (t, 1H, aromatic, *J*=8 Hz), 4.74 (s, 2H, SCH_2) and 4.57 ppm (s, 3H, NCH_3).

2-(5-Nitro-2 furyl)-5-(4-nitrobenzyl)thio-1,3,4-thiadiazole (8e)

The synthesis of compound **8e** has previously been reported (Foroumadi *et al.*, 2002).

2-(1-Methyl-5-nitro-1*H*-imidazol-2-yl)-5-(4-nitrobenzyl) thio-1,3,4-thiadiazole (8f)

This compound was prepared from **4b** and 4-nitrobenzyl chloride, as described for **8a**, in a 91% yield, m.p.169-170°C. IR (KBr) ν_{max}: 1510 and 1340 cm^{-1} (NO_2). ^{1}H-NMR ($CDCl_3$, 80 MHz) δ: 8.25 (d, 2H, aromatic, *J*=8.8 Hz), 8.09 (s, 1H, H4-imidazole), 7.70 (d, 2H, aromatic, *J*=8.8 Hz), 4.73 (s, 2H, SCH_2) and 4.57 ppm (s, 3H, NCH_3).

Biological assay

All of the compounds were evaluated for *in vitro* antituberculosis activity against *Mycobacterium tuberculosis,* as part of the TAACF TB screening program under direction of the US National Institute of Health, the NIAID division. Rifampicin was used as a reference drug.

Primary screening was conducted at a single concentration, 6.25 μgmL^{-1}, against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATTCC 27294), in BACTEC 12B medium, using the Microplate Alamar Blue Assay (MABA) (Collins and Franzblau, 1997). Compounds effecting <90% inhibition in the primary screening (MIC>6.25 μgmL^{-1}) were not generally evaluated further.

The active compounds were retested by serial dilution, beginning at 6.25 μgmL^{-1}, against *Mycobacterium tuberculosis* $H_{37}Rv$ to determine the actual minimum inhibitory concentration (MIC) in the medium.

The MIC was defined as the lowest concentration effecting a reduction in the fluorescence of 90% relative to the controls.

RESULTS AND DISCUSSION

The 2-amino-5-aryl-1,3,4-thiadiazoles (**2a-b**) were obtained from the arylthiosemicarbazones (**1a-b**). Diazotization of **2a-b** in hydrochloric acid, in the presence of copper powder, gave the 2-aryl-5-chloro-1,3,4-thiadiazoles (**3a-b**) (Foroumadi *et al.*, 1999). The reaction of **3a-b** with thiourea in refluxing ethanol afforded the 2-aryl-1,3,4-thiadiazole-5-thioles (**4a-b**) (Foroumadi *et al.*, 2001a). Treatment of the latter with alkyl bromides (**5a-c**) and nitrobenzyl chlorides (**7a-c**) gave the 2-aryl-5-alkylthio-1,3,4-thiadiazoles (**6a-f**) and 2-aryl-5-(nitrobenzyl)thio-1,3,4-thiadiazoles (**8a-f**) (Scheme).

Previously, the *in vitro* antimycobacterial activities of some 2-(1-methyl-5-nitro-1H-imidazol-2-yl)-5-alkylthio-1,3,4-thiadiazole derivatives against *M.tuberculosis* $H_{37}Rv$, which are compounds that bear a primary alkylthio substitution, were demonstrated in our laboratory, and displayed good antituberculosis activities, in the following order: *n*-propyl > ethyl > methyl (MIC=1.56-6.25 μgmL^{-1}) (Foroumadi *et al.*, 2001b).

The syntheses and antituberculosis activities of some 2-(5-nitro-2-furyl)-1,3,4-thiadiazole derivatives (Foroumadi *et al.*, 2002) have also been reported in our laboratory. The obtained structure/activity relationship (SAR) indicated that compounds with methylthio or ethylthio groups attached at position 5 of the 1,3,4-thiadiazole ring exhibited significant antituberculosis activities (MIC=6.25 and 0.78 μgmL^{-1}, respectively). Replacement of the methyl or ethyl groups with benzyl abolished the antituberculosis activity (Inhibition =11%), while with the 4-nitrobenzyl group (**8e**), the activity was maintained (MIC=3.13 μgmL^{-1}) (Foroumadi *et al.*, 2002).

Accordingly, in this study a new series of 2-aryl-5-(propyl, allyl and propargyl)thio-1,3,4-thiadiazoles (**6a-f**) derivatives and o, m or *p*-nitrobenzylthio analogues (**8a-f**) were synthesized and evaluated against *M. tuberculosis,* as part of the TAACF TB screening program under direction of the U. S. National Institute of Health, the NIAID division.

From the twelve compounds tested (**6a-f** and **8a-f**), five (**6b**, **6e**, **8a**, **8c**, and **8e**) displayed significant inhibition effects in the primary screening (MIC<6.25 μgmL^{-1}) against *M. tuberculosis* $H_{37}Rv$ in the BACTEC 12B medium, using the BACTEC 460 radiometric system. Compounds demonstrating at least 90% inhibition in the primary screening were re-tested in order to determine the actual minimum inhibitory concentration (MIC) against *M. tuberculosis.* Rifampicin was used as a reference drug, and the detailed data are tabulated in Table I.

Despite the good activity of 2-(1-methyl-5-nitro-1H-imidazol-2-yl)-5-propylthio-1,3,4-thiadiazole (**6b**, MIC=3.13 μgmL^{-1}), the nitrofuran analogue containing *n*-propyl group (**6a**) did not show good antituberculosis activity (Inhibition =74%). Replacement of the *n*-propylthio (**6c**) and propargylthio (**6e**) with allylthio in the nitrofuran derivatives increased the inhibitive effects from 74 to 84 and 97% respectively, while in the nitroimidazole derivatives (**6d** and **6f**) the activities were abolished (Table I).

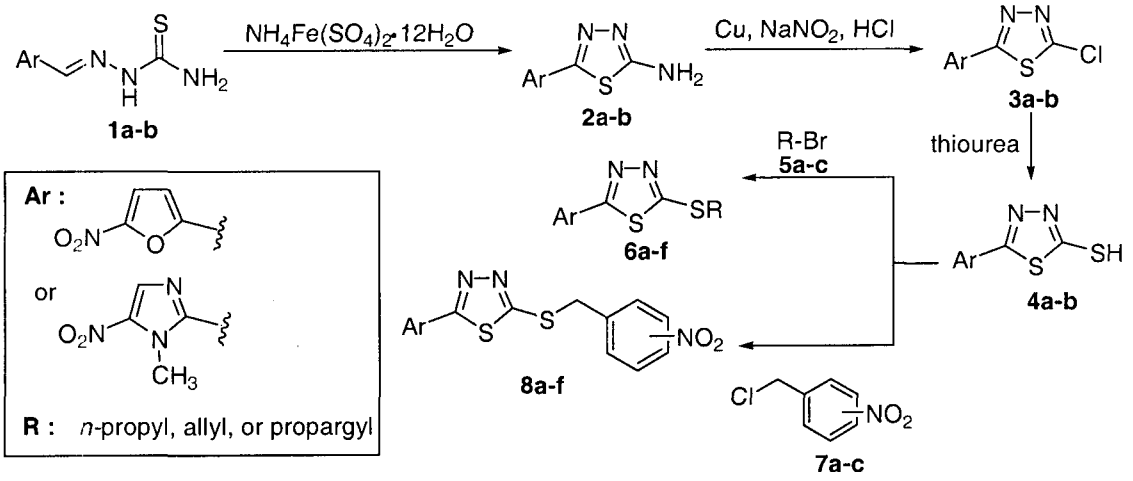
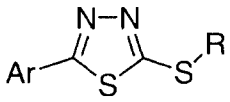


Table I. *In vitro* antituberculosis activities of some 2-aryl-5-alkylthio-1,3,4-thiadiazole derivatives, **6a-f** and **8a-f** [a]



Compound	Ar	R	MIC (μgmL^{-1})	Inhibition (%)
6a	5-nitro-2-furyl	*n*-propyl	>6.25	74
6b	1-methyl-5-nitro-1H-imidazol-2-yl	*n*-propyl	3.13	97
6c	5-nitro-2-furyl	allyl	>6.25	84
6d	1-methyl-5-nitro-1H-imidazol-2-yl	allyl	>6.25	37
6e	5-nitro-2-furyl	propargyl	1.56	97
6f	1-methyl-5-nitro-1H-imidazol-2-yl	propargyl	>6.25	0
8a	5-nitro-2-furyl	2-nitrobenzyl	6.25	98
8b	1-methyl-5-nitro-1H-imidazol-2-yl	2-nitrobenzyl	>6.25	0
8c	5-nitro-2-furyl	3-nitrobenzyl	3.13	98
8d	1-methyl-5-nitro-1H-imidazol-2-yl	3-nitrobenzyl	>6.25	0
8e	5-nitro-2-furyl	4-nitrobenzyl	3.13	99
8f	1-methyl-5-nitro-1H-imidazol-2-yl	4-nitrobenzyl	>6.25	0

[a] MIC rifampicin: 0.125-0.25 μgmL^{-1}

All the nitrofuran derivatives containing o, m and *p*-nitrobenzyl groups (**8a**, **8c**, and **8e**) exhibited significant antituberculosis activities (MIC=3.13-6.25 μgmL^{-1}), whereas the corresponding nitroimidazole analogues (**8b**, **8d** and **8f**), were surprisingly completely inactive (Inhibition=0%; Table I).

Generally, replacement of nitrofuran with a nitroimidazole ring, with the exception of in compound **6b**, resulted in compounds devoid of biological activity.

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