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Short Communication

Antituberculosis agents II. Evaluation of in vitro antituberculosis activity and cytotoxicity of some 2-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole derivatives

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Abstract

Using the radiometric BACTEC 460-TB methodology, the minimum inhibitory concentration (MIC) of a series of 2-(1-methyl-5-nitro-2-imidazolyl-1,3,4-thiadiazole-5-alkylsulfides, alkylsulfoxides and alkylsulfones which had been reported previously as antifungal agents, were determined. Active compounds were also screened by serial dilution to assess toxicity to a VERO cell line. The results indicate that compounds bearing a primary alkylthio substitution displayed good antituberculosis activity (MIC = 3.13–6.25 μg/ml). Oxidation to sulfone abolished the antituberculosis activity in methyl and propyl derivatives while the ethylsulfonyl analogue was active (MIC = 1.56 μg/ml). The cytotoxic effects indicate that 2-(1-methyl-5-nitro-2-imidazolyl)-5-methylthio-1,3,4-thiadiazole was the least toxic compound ($IC_{50} > 10$ μg/ml). Generally, all compounds showed a low selectivity index.

Keywords: Antituberculosis agents; Minimum inhibitory concentration; Cytotoxicity

1. Introduction

Tuberculosis is a leading infectious cause of death worldwide. The control of *Mycobacterium tuberculosis* infections is still considered of relevance, not only for the survival of AIDS patients. The recent emergence of drug-resistant mycobacterium tuberculosis, in fact, also has become a serious concern [1].

Because of the concern of the resistance to most of the commonly used drugs displayed by the considered mycobacteria [2], our studies have been focused on the development of new potential therapeutic agents.

The 1,3,4-thiadiazole ring system is known to possess several biological activities and the antibacterial properties have been largely described [3]. Some nitroimidazole and nitrofuran derivatives have been claimed to possess in-vitro antibacterial, antifungal and antituberculosis activity [4,5]. The synthesis and antituberculosis activity of 4-carbethoxymethyl-2-[(α-haloacyl)amino] thiazoles and related compounds were also described [6]. In addition a series of α-(5-aryl-1,3,4-oxadiazole-2-ylthio)acetate derivatives have been recently synthesized and tested against mycobacterium tuberculosis [7]. We have recently reported a series of 2-aryl-5-methylthio-1,3,4-thiadiazoles, ethyl α-(5-aryl-1,3,4-thiadiazole-2-ylthio)acetates and related compounds as antituberculosis agents [8]. Now we would like to report the antituberculosis activity of some nitroimidazolyl-1,3,4-thiadiazoles (**1a**–**c**, **2a**–**c** and **3a**–**c**) that had previously been shown to possess antifungal activity [9].

2. Materials and methods

2.1. Synthesis of the products

The products were synthesized according the previously described procedures [9]. The ^{1}H NMR reso-

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nances of compounds **2a**, **2b**, **3a** and **3b** are the following:

*2.1.1. 2-(1-Methyl-5-nitro-2-imidazolyl)-5-ethylthio-1,3,4-thiadiazole (**2a**)*

^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.10 (s, 1H, H–C_4 imidazole), 4.60 (s, 3H, NCH_3), 3.40 (q, 2H, CH_2, $J = 7.6$ Hz) and 1.50 ppm (t, 3H, CH_3, $J = 7.6$ Hz).

*2.1.2. 2-(1-Methyl-5-nitro-2-imidazolyl)-5-ethylsulfinyl-1,3,4-thiadiazole (**2b**)*

^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.12 (s, 1H, H–C_4 imidazole), 4.60 (s, 3H, NCH_3), 3.42 (dq, 1H, S–CH–, $J_{geminal} = 13.6$ Hz, $J_{CH,\,CH_3} = 7.6$ Hz), 3.29 (dq, 1H, S–CH–, $J_{geminal} = 13.6$ Hz, $J_{CH,\,CH_3} = 7.6$ Hz), 1.42 (t, 3H, CH_3, $J = 7.6$ Hz).

*2.1.3. 2-(1-Methyl-5-nitro-2-imidazolyl)-5-(n-propylthio)-1,3,4-thiadiazole (**3a**)*

^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.10(s, 1H, H–C_4 imidazole), 4.60 (s, 3H, NCH_3), 3.35 (t, 2H, SCH_2, $J = 7.6$ Hz), 1.85 (m, 2H, CH_2) and 1.10 ppm (t, 3H, CH_3, $J = 7.6$ Hz).

*2.1.4. 2-(1-methyl-5-nitro-2-imidazolyl)-5-(n-propylsulfinyl)-1,3,4-thiadiazole (**3b**)*

^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.10 (s, 1H, H–C_4 imidazole), 4.57 (s, 3H, NCH_3), 3.29–3.24 (m, 2H, CH_2), 2.08–1.95 (m, 1H, CH_2), 1.86–1.72 (m, 1H, CH_2), 1.12 (t, 3H, CH_3, $J = 7.4$ Hz).

2.2. Biological assay

All of the compounds were evaluated for in vitro antituberculosis activity against mycobacterium tuberculosis as part of TAACF TB screening program under direction of the US National Institute of Health, NIAID division.

Primary screening was conducted at the single concentration, 6.25 μg/ml against mycobacterium tuberculosis M_{37}Rv (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the microplate Alamar Blue Assay (MABA) [10]. Compounds effecting $< 90\%$ inhibition in the primary screening (MIC > 6.25 μg/ml) were not generally evaluated further.

The active compounds were re-tested by serial dilution beginning at 6.25 μg/ml against mycobacterium tuberculosis M_{37}Rv to determine the actual minimum inhibitory concentration (MIC) in the BABTEC 460. The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls.

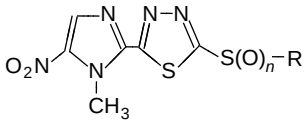
2.3. Cytotoxicity assay

Compounds were screened by serial dilution to assess toxicity to a VERO cell line, generally beginning at $10\times$ the MIC if permitted by the sample solubility in culture media. The selectivity index (SI) is defined as the ratio of the measured IC_{50} in VERO cells to the MIC described above.

3. Results and discussion

The antituberculosis activity and cytotoxicity effect of the compounds **1a**–**c**, **2a**–**c** and **3a**–**c** are shown in Table 1. The antituberculosis activity of compounds **1a**–**c** has been reported previously [8]. Herein, we report the antituberculosis activity of some related com-

Table 1
Antituberculosis activity and cytotoxycity effect of compounds **1a**–**c**, **2a**–**c** and **3a**–**c**



Compound	R	*n*	Activity	Inhibition (%)	MIC	EC_{50}	SI
1a	methyl	0	+	91	6.25	>10	>6.4
1b	methyl	1	+	105	1.56	0.5	0.32
1c	methyl	2	−	18	>6.25	ND [a]	ND [a]
2a	ethyl	0	+	96	6.25	[b]	[b]
2b	ethyl	1	+	103	1.56	0.9	0.6
2c	ethyl	2	+	103	1.56	0.5	0.3
3a	*n*-propyl	0	+	97	3.13	[b]	[b]
3b	*n*-propyl	1	+	100	1.56	0.5	0.3
3c	*n*-propyl	2	−	17	>6.25	ND [a]	ND [a]

[a] ND, not determined.
[b] Insoluble in tissue culture media.

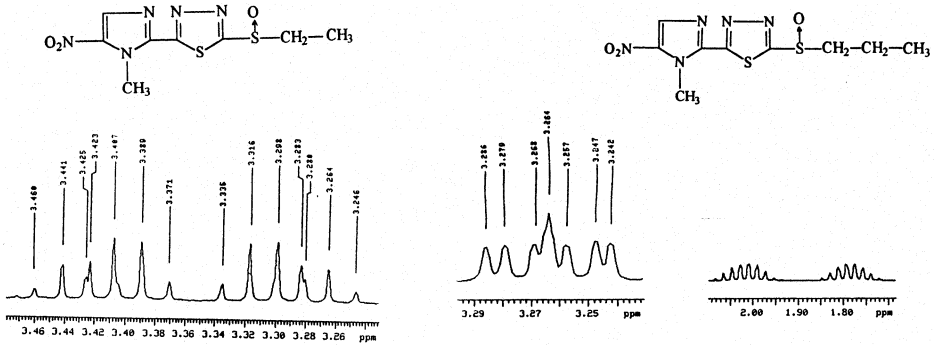


Fig. 1. Partial ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of compounds **2b** and **3b** (the geminal coupling constant was measured by spin–spin decoupling).

pounds (**2a**–**c** and **3a**–**c**) and the cytotocycity of compounds **1a**–**c**, **2a**–**c** and **3a**–**c** (Table 1).

The antituberculosis results indicate that compounds bearing a primary alkylthio substitution (**1a**, **2a** and **3a**) displayed good antituberculosis activity in the following order **3a** > **2a** > **1a.** The oxidation of the thio group to sulfoxide in alkylthio derivatives increased the antituberculosis activity in compounds **1b**, **2b** and **3b** having an equal MIC of 1.56 μg/ml.

The coupling constants of geminal hydrogens in the ^{1}H NMR spectra of compounds **2b** and **3b** (Fig. 1) confirm the connection of the methylene to a chiral center and are in accordance to the suggested structure.

A high drop in activity after oxidation can be observed by comparing the sulfones **1c** and **3c** (Inh. % = 18 and 17, respectively) with the corresponding sulfides **1a** and **3a**. On the other hand the ethylsulfonyl analogue **2c**, surprisingly, does not follow this trend because of its high activity (Inh.% = 103, MIC = 1.56 μg/ml).

The cytotoxycity data of tested compounds indicate that all of the compounds exhibited a high degree of toxicity and low level of selectivity index (SI).

The methylthio analogue (**1a**) was the least toxic compound (IC_{50} > 10 μg/ml, SI > 6.4) while ethyl and propyl analogues (**2a** and **3a**) were insoluble in culture media.

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