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Short Communication

Antituberculosis agents. V. Synthesis, evaluation of in vitro antituberculosis activity and cytotoxicity of some 2-(5-nitro-2-furyl)-1,3,4- thiadiazole derivatives

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Abstract

A new series of 2-(5-nitro-2-furyl)-1,3,4-thiadiazole-2-sulfide, sulfoxide and sulfones were synthesized and evaluated for in vitro antituberculosis activity against *Mycobacterium tuberculosis* strain $H_{37}Rv$ using the radiometric BACTEC 460-TB methodology. Active compounds were also screened by serial dilution to assess toxicity to a VERO cell line. The results indicate that some compounds exhibited a good antituberculosis activity and the ethylthio analogue (**5b**) was the most active compound (MIC = 0.78 μg ml^{-1}). Also, the cytotoxic effects indicate that compound **5b** was the least toxic compound. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: Antituberculosis agents; Minimum inhibitory concentration; Cytotoxicity

1. Introduction

Tuberculosis is the leading cause of death attributable to a single infectious pathogen with one-third of the world population infected [1].

Tuberculosis is still an infectious disease with a high medical relevance and reversal in the decline of tuberculosis during the past few years has been widely published. The treatment of mycobacterial infections has become an important and challenging problem because of the emergence of multiple-drug-resistant organisms and because of the acquired immunodeficiency syndrome (AIDS) pandemic [2]. The high rates of drug-resistant tuberculosis currently reported in many countries lead a continuous research for new effective chemotherapeutic agents [3].

The 1,3,4-thiadiazole ring system is known to possess several biological activities and the antibacterial properties have been largely described [4]. The synthesis and antituberculosis activity of 4-carbethoxymethyl-2-[(α-haloacyl)amino] thiazoles and related compounds were also described [5].

Recently the synthesis of the α-[5-(5-nitro-2-furyl)-1,3,4-oxadiazole-2-ylthio] acethydrazide and related compounds as antituberculosis agents has been reported [6]. We have also reported a series of 2-aryl-1,3,4-thiadiazole derivatives as antituberculosis agents [7,8]. Now we would like to report the synthesis, antituberculosis and cytotoxicity of a new series of 2-(5-nitro-2-furyl)-1,3,4-thiadiazoles (**5a**–**h** and **6a**–**i**) as potential drugs against tuberculosis.

2. Materials and methods

2.1. General

Melting points were taken on a Kofler hot stage apparatus and are uncorrected. The IR spectra were obtained using a Perkin–Elmer model 267 spectrograph (potassium bromide disks). The ^1H NMR spectra were recorded on a Bruker Ac-80 spectrometer and chemical shifts (δ) are in ppm relative to internal tetramethylsilane (TMS). Elemental analyses were performed by Tarbiat Modarress University, Tehran, Iran. All new

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compounds had C, H, N microanalysis within ±0.4% of the theoretical values.

2.2. Synthesis of the products

The synthesis of compounds **5a**–**b** and **6a**–**d** were reported previously [9]. The ^{1}H NMR resonances of compounds **5a**, **5b**, **6a**, **6b**, **6c** and **6d** are the following:

2.2.1. 2-(5-Nitro-2-furyl)-5-methylthio-1,3,4-thiadiazole (**5a**)

^{1}H NMR ($CDCl_3$, 80 MHz): δ 2.87 (s, 3H, CH_3), 7.30 (d, 1H, furyl, $J = 4$ Hz) and 7.44 ppm (d, 1H, furyl, $J = 4$ Hz).

2.2.2. 2-(5-Nitro-2-furyl)-5-ethylthio-1,3,4- thiadiazole (**5b**)

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.53 (t, 3H, CH_3, $J =$ 7.6 Hz), 3.43 (q, 2H, CH_2, $J = 7.6$ Hz), 7.32 (d, 1H, furyl, $J = 4.0$ Hz) and 7.46 ppm (d, 1H, furyl, $J = 4.0$ Hz).

2.2.3. 2-(5-Nitro-2-furyl)-5-methylsulfinyl-1,3,4-thiadiazole (**6a**)

^{1}H NMR ($CDCl_3$, 80 MHz): δ 3.35 (s, 3H, CH_3), 7.48 (d, 1H, furyl, $J = 4$ Hz) and 7.55 ppm (d, 1H, furyl, $J = 4$ Hz).

2.2.4. 2-(5-Nitro-2-furyl)-5-methylsulfonyl-1,3,4-thiadiazole (**6b**)

^{1}H NMR ($CDCl_3$, 80 MHz): δ 3.56 (s, 3H, CH_3), 7.51 (d, 1H, furyl, $J = 4$ Hz) and 7.60 ppm (d, 1H, furyl, $J = 4$ Hz).

2.2.5. 2-(5-Nitro-2-furyl)-5-ethylsulfinyl-1,3,4-thiadiazole (**6c**)

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.40 (t, 3H, CH_3, $J =$ 7.6 Hz), 3.28 (dq, 1H, S-CH, $J_{geminal} = 13.6$ Hz, $J_{CH,CH_3} = 7.6$ Hz), 3.42 (dq, 1H, S-CH, $J_{geminal} = 13.6$ Hz, $J_{CH,CH_3} = 7.6$ Hz), 7.44 (d, 1H, furyl, $J = 4.0$ Hz) and 7.48 ppm (d, 1H, furyl, $J = 4.0$ Hz).

2.2.6. 2-(5-Nitro-2-furyl)-5-ethylsulfonyl-1,3,4-thiadiazole (**6d**)

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.49 (t, 3H, CH_3, $J =$ 8.0 Hz), 3.62 (q, 2H, CH_2, $J = 8.0$ Hz), 7.47 (d, 1H, furyl, $J = 4.0$ Hz) and 7.50 ppm (d, 1H, furyl, $J = 4.0$ Hz).

2.2.7. General method for the synthesis of 2-(substituted benzylthio)-5-(5-nitro-2-furyl)-1,3,4-thiadiazoles (**5c**–**h**)

To a mixture of 2-mercapto-5-(5-nitro-2-furyl)-1,3,4-thiadiazole (**4**) [7] (229 mg, 1 mmol) and substituted benzyl chloride (1 mmol) in ethanol (15 ml), KOH (66 mg 85% in 5 ml H_2O) was added dropwise and the mixture was stirred at room temperature overnight, H_2O was added and the separated solid was filtered off, washed with H_2O and crystallized from EtOH–H_2O. The following compounds were prepared according to the general procedure.

2.2.7.1. 2-Benzylthio-5-(5-nitro-2-furyl)-1,3,4-thiadiazole (**5c**).

Yield 66%, m.p. 124–125 °C; IR (KBr) ν_{max}: 3125 (furyl), 1542 and 1344 cm^{-1} (NO_2). ^{1}H NMR ($CDCl_3$, 80 MHz): δ 4.62 (s, 2H, CH_2) and 7.30–7.50 ppm (m, 7H, aromatic).

2.2.7.2. 2-(4-Chlorobenzyl)thio-5-(5-nitro-2-furyl)-1,3,4-thiadiazole (**5d**).

Yield 77%, m.p. 135–136 °C; IR (KBr) ν_{max}: 3125 (furyl), 1542 and 1347 cm^{-1} (NO_2). ^{1}H NMR ($CDCl_3$, 80 MHz): δ 4.58 (s, 2H, CH_2) and 7.24–7.46 ppm (m, 6H, aromatic).

2.2.7.3. 2-(4-Nitrobenzyl)thio-5-(5-nitro-2-furyl)-1,3,4-thiadiazole (**5e**).

Yield 80%, m.p. 186–187 °C; IR (KBr) ν_{max}: 3125 (furyl), 1530 and 1344 cm^{-1} (NO_2). ^{1}H NMR ($CDCl_3$, 80 MHz): δ 4.69 (s, 2H, CH_2), 7.30 (d, 1H, furyl, $J = 4$ Hz), 7.44 (d, 1H, furyl, $J = 4$ Hz), 7.65 (d, 2H, phenyl, $J = 8.8$ Hz) and 8.20 ppm (d, 2H, phenyl, $J = 8.8$ Hz).

2.2.7.4. 2-(4-Methoxybenzyl)thio-5-(5-nitro-2-furyl)-1,3,4-thiadiazole (**5f**).

Yield 65%, m.p. 139–140 °C; IR (KBr) ν_{max}: 3124 (furyl), 1540 and 1346 cm^{-1} (NO_2). ^{1}H NMR ($CDCl_3$, 80 MHz): δ 3.82 (s, 3H, CH_3), 4.60 (s, 2H, CH_2), 6.89 (d, 2H, phenyl, $J = 8$ Hz), 7.34 (d, 1H, furyl, $J = 4$ Hz), 7.39 (d, 2H, phenyl, $J = 8$ Hz) and 7.50 ppm (d, 1H, furyl, $J = 4$ Hz).

2.2.7.5. 2-(2-Chloro-6-fluorobenzyl)thio-5-(5-nitro-2-furyl)-1,3,4-thiadiazole (**5g**).

Yield 83%, m.p. 148–149 °C; IR (KBr) ν_{max}: 3120 (furyl), 1530 and 1350 cm^{-1} (NO_2). ^{1}H NMR ($CDCl_3$, 80 MHz) δ 4.75 (s, 2H, CH_2), 6.95–7.35 (m, 4H, aromatic) and 7.45 ppm (d, 1H, furyl, $J = 4$ Hz).

2.2.7.6. 2-(2,5-Dimethylbenzyl)thio-5-(5-nitro-2-furyl)-1,3,4-thiadiazole (**5h**).

Yield 89%, m.p. 138–139 °C; IR (KBr) ν_{max}: 3120 (furyl), 1544 and 1347 cm^{-1} (NO_2). ^{1}H NMR ($CDCl_3$, 80 MHz): δ 2.26, 2.35 (2s, 6H, CH_3), 4.59 (s, 2H, CH_2) and 6.90–7.50 ppm (m, 5H, aromatic).

2.2.8. General procedure for the synthesis of 2-substituted benzylsulfonyl-5-(5-nitro-2-furyl)-1,3,4-thiadiazoles (**6e**–**i**)

To a stirring mixture of benzylthio analogue (1 mmol) in glacial acetic acid (3 ml) was added 30% H_2O_2 solution (3 ml) and the mixture was refluxed for 20 min. After cooling, H_2O was added, the precipitate was filtered and purified by TLC eluting with 5% EtOH–$CHCl_3$. The products were crystallized from ethanol.

The following compounds were prepared according to the general procedure.

*2.2.8.1. 2-Benzylsulfonyl-5-(5-nitro-2-furyl)-1,3,4-thiadiazole (**6e**).* Yield 72%, m.p. 193–194 °C; IR (KBr) ν_{max}: 3152 (furyl), 1536, 1553 (NO_2), 1325 and 1158 cm^{-1} (SO_2). ^1H NMR ($CDCl_3$, 80 MHz): δ 4.77 (s, 2H, CH_2), 7.20–7.32 (m, 5H, phenyl), 7.40 (d, 1H, furyl, $J=$ 4 Hz) and 7.58 ppm (d, 1H, furyl, $J=4$ Hz).

*2.2.8.2. 2-(4-Chlorobenzyl)sulfonyl-5-(5-nitro-2-furyl)-1,3,4-thiadiazole (**6f**).* Yield 83%, m.p. 148–149 °C; IR (KBr) ν_{max}: 3120 (furyl), 1530 and 1350 cm^{-1} (NO_2). ^1H NMR ($CDCl_3$, 80 MHz): δ 4.75(s, 2H, CH_2), 6.95–7.35 (m, 4H, aromatic) and 7.45 ppm (d, 1H, furyl, $J=4$ Hz).

*2.2.8.3. 2-(4-Nitrobenzyl)sulfonyl-5-(5-nitro-2-furyl)-1,3,4- thiadiazole (**6g**).* Yield 51%, m.p. 169–170 °C; IR (KBr) ν_{max}: 3152 (furyl) 1536, 1353 (NO_2) 1325 and 1165 cm^{-1} (SO_2). ^1H NMR ($CDCl_3$, 80 MHz): δ 4.97 (s, 2H, CH_2), 7.40 (d, 1H, furyl, $J=4$ Hz). 7.55–7.65 (m, 3H, aromatic) and 8.24 (d, 2H, phenyl, $J=8.8$ Hz).

*2.2.8.4. 2-(2-Chloro-6-fluorobenzyl)sulfonyl-5-(5-nitro-2-furyl)-1,3,4-thiadiazole (**6h**).* Yield 49%, m.p. 186–187 °C; IR (KBr) ν_{max}: 3104 (furyl), 1552, 1353 (NO_2), 1312 and 1152 cm^{-1} (SO_2). ^1H NMR ($CDCl_3$, 80 MHz): δ 4.95(s, 2H, CH_2), 6.92–7.35 (m, 3H, phenyl) and 7.36–7.60 ppm (m, 2H, furyl).

*2.2.8.5. 2-(2,5-Dimethylbenzyl)sulfonyl-5-(5-nitro-2-furyl)-1,3,4-thiadiazole (**6i**).* Yield 76%, m.p. 167–168 °C; IR (KBr) ν_{max}: 3104 (furyl), 1552, 1353 (NO_2), 1318 and 1152 cm^{-1} (SO_2). ^1H NMR ($CDCl_3$, 80 MHz): δ 4.85(s, 2H, CH_2), 6.90–7.10 (m, 3H, phenyl) 7.40 (d, 1H, furyl, $J=4$ Hz) and 7.58 ppm (d, 1H, furyl, $J=4$ Hz).

2.3. 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. Rifampicin was used as a reference drug.

Primary screening was conducted at the single concentration, 6.25 μg ml^{-1} against *M. tuberculosis* $H_{37}Rv$ (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^{-1}) were not generally evaluated further.

The active compounds were re-tested by serial dilution beginning at 6.25 μg ml^{-1} against *M. tuberculosis* $H_{37}Rv$ to determine the actual minimum inhibitory concentration (MIC) in the BACTEC 460.

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

2.4. Cytotoxicity assay

Compounds were screened by serial dilution to assess toxicity to a VERO cell line, generally beginning at 10 × 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

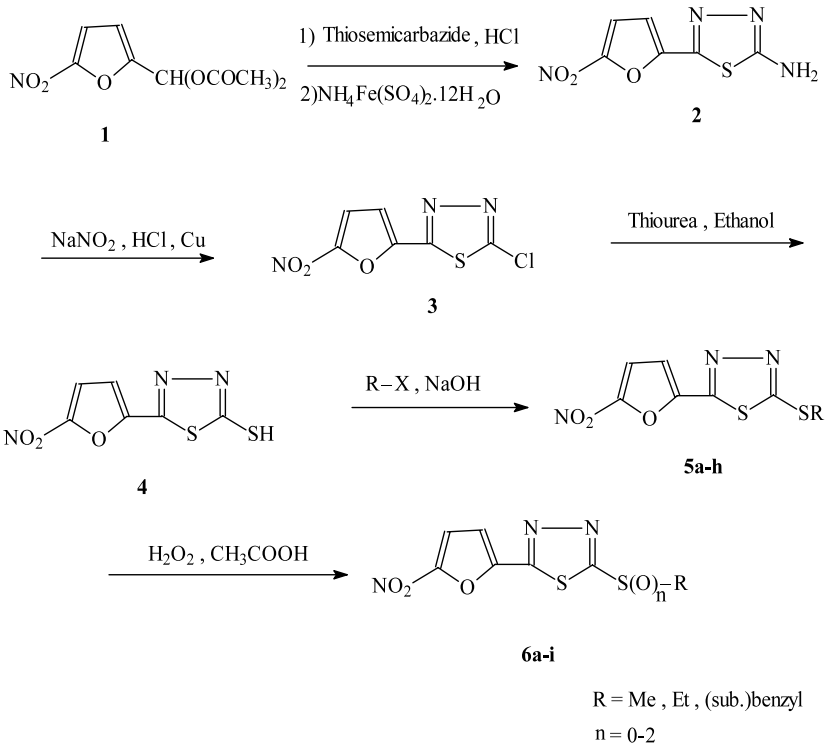
The 2-amino-5-(5-nitro-2-furyl)-1,3,4-thiadiazole (**2**) was obtained from commercially available 5-nitro-2-furfurilidene diacetate (**1**). Diazotization of **2** in hydrochloric acid in the presence of copper powder gave the 2-chloro-5-(5-nitro-2-furyl)-1,3,4-thiadiazole (**3**) [11]. The reaction of **3** with thiourea in refluxing ethanol afforded the 2-mercapto-5-(5-nitro-2-furyl)-1,3,4-thiadiazole (**4**) [7]. Treatment of **4** with methyl iodide, ethyl iodide or substituted benzyl chloride gave the 2-methylthio-5-(5-nitro-2-furyl)-1,3,4-thiadiazole (**5a**), 2-ethylthio-5-(5-nitro-2-furyl)-1,3,4-thiadiazole (**5b**) or 2-benzylthio-5-(5-nitro-2-furyl)-1,3,4-thiadiazole derivatives (**5c**–**h**), respectively. Sulfoxides (**6a**, **6c**) and sulfones (**6b** and **6d**–**i**) were prepared by usual procedures from sulfides, using an excess of H_2O_2 30% and CH_3COOH (Fig. 1).

The antituberculosis activity and cytotoxicity effects of the compounds **5a**–**h** and **6a**–**i** are shown in Table 1. The antituberculosis results indicate that compounds **5a** and **5b** having methylthio or ethylthio group attach to the 1,3,4-thiadiazole ring are able to inhibit in vitro growth of *M. tuberculosis* exhibiting the MIC values of 6.25 and 0.78 μg ml^{-1}, respectively. Replacement of methyl or ethyl group with a benzyl group (**5c**) resulted in a compound devoid of antituberculosis activity (inhibition $\% = 11$).

A varying degree of antituberculosis activity (from 0–99% of growth inhibition) was observed in benzylthio series **5c**–**h**, and only compound **5e** having a 4-nitrobenzylthio group showed a good antituberculosis activity (MIC = 3.13 μg ml^{-1}).

The oxidation of thio group in compounds **5a** and **5b** to sulfoxide maintains the antituberculosis activity in compounds **6a** and **6c**, while oxidation of thio group to sulfone (**6b**, **6d**) decreased the antituberculosis activity. Also the oxidation of compound **5e** to the sulfone derivative (**6g**) decreased the antituberculosis activity (inhibition $\% = 79$).

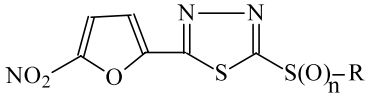
The cytotoxicity data of tested compounds indicate that all of the compounds exhibited a high degree of toxicity and low level of selectivity index (SI). However,



1 2 3 4 5a-h 6a-i

Fig. 1. Synthesis of compounds **5a**–**h** and **6a**–**i**.

Table 1
Antituberculosis activity and cytotoxicity effect of compounds **5a**–**h** and **6a**–**i** [a]



Comp.	R	*n*	Inhibition (%)	Activity	MIC (μg ml^{-1})	IC_{50} (μg ml^{-1})	SI
5a	methyl	0	99	+	6.25	1.4	0.22
5b	ethyl	0	101	+	0.78	0.7	0.9
5c	benzyl	0	11	–	ND [b]	ND [b]	ND [b]
5d	4-chlorobenzyl	0	20	–	ND [b]	ND [b]	ND [b]
5e	4-nitrobenzyl	0	99	+	3.13	[c]	ND [b]
5f	4-methoxybenzyl	0	0	–	ND [b]	ND [b]	ND [b]
5g	2-chloro-6-fluorobenzyl	0	0	–	ND [b]	ND [b]	ND [b]
5h	2,5-dimethylbenzyl	0	29	–	ND [b]	ND [b]	ND [b]
6a	methyl	1	101	+	3.13	< 0.26	< 0.008
6b	methyl	2	< 90	–	ND [b]	ND [b]	ND [b]
6c	ethyl	1	102	+	1.56	0.3	0.2
6d	ethyl	2	102	+	6.25	0.2	0.03
6e	benzyl	2	57	–	ND [b]	ND [b]	ND [b]
6f	4-chlorobenzyl	2	13	–	ND [b]	ND [b]	ND [b]
6g	4-nitrobenzyl	2	79	–	ND [b]	ND [b]	ND [b]
6h	2-chloro-6-fluorobenzyl	2	11	–	ND [b]	ND [b]	ND [b]
6i	2,5-dimethylbenzyl	2	35	–	ND [b]	ND [b]	ND [b]

[a] MIC rifampicin 0.25 μg ml^{-1}; 97% inhibition.
[b] ND, not determined.
[c] Insoluble in tissue culture media.

the most active compound **5b**, was the least toxic compound ($IC_{50} = 0.7$ μg ml^{-1}, SI = 0.9).

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