The Research Center of Kerman University of Medical Sciences[1], Kerman, and Faculty of Pharmacy, Tehran University of Medical Sciences[2], Tehran, Iran

Antituberculosis agents, I: Synthesis and antituberculosis activity of 2-aryl-1,3,4-thiadiazole derivatives

A. Foroumadi[1], M. Mirzaei[1] and A. Shafiee[2]

The design, synthesis and antituberculosis activity of a series of 2-aryl-5-methylthio-1,3,4-thiadiazoles **(5a–b)**, ethyl α-(5-aryl)-1,3,4-thiadiazole-2-ylthio)acetates **(8a–b)** and related compounds are described. All of the compounds were tested against *Mycobacterium tuberculosis* strain H37Rv in comparison to rifampicin. Six compounds exhibited a very good activity (MIC < 6.25 μg/ml, % Inhibition = 100).

1. Introduction

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 [1]. The high rates of drug- resistant tuberculosis currently reported in many countries are alarming, since among this phenomenon rapid drug susceptibility tests are needed and effective chemotherapy regimens with newly developed drugs are urgently being sought [2].

Recently the synthesis of the α-[5-(5-nitro-2-furyl)-1,3,4-oxadiazole-2-ylthio] acethydrazide (**1**) and related compounds as antituberculosis agents has been reported [3]. The 1,3,4-thiadiazole isostere of 1,3,4-oxadiazole ring system have been incorporated in many substances with antibacterial, amoebicide, parasiticide and antifungal activity [4–6]. In addition 5-nitroimidazole derivatives are known to posses antimicrobial activity [7–8]. Accordingly we designed and synthesized a new series of 2-aryl-5-methylthio-1,3,4-thiadiazole, ethyl α-(5-aryl-1,3,4-thiadiazole-2-ylthio)acetate and related compounds (**2** and **3**) as potential drugs against tuberculosis.

2. Investigations, results and discussion

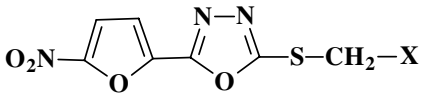
2.1. Synthesis of the derivatives

The 2-amino-5-aryl-1,3,4-thiadiazoles **2a–b** were obtained from the arylthiosemicarbazones **1a–b** [9]. Diazotization of **2a–b** in hydrochloric acid in the presence of copper powder [10] gave the 2-aryl-5-chloro-1,3,4-thiadiazoles **3a–b**. The reaction of **3a–b** with thiourea in refluxing ethanol [11] afforded the 2-aryl-1,3,4-thiadiazole-5-thioles **4a–b**. Treatment of the latter with methyl iodide or ethyl bromoacetate gave the 2-aryl-5-methylthio-1,3,4-thiadiazoles **5a–b** and the ethyl α-(5-aryl-1,3,4-thiadiazole-2-ylthio)acetates **8a–b** respectively. Reaction of the ethyl esters **8a–b** with NaOH–MeOH–H_2O gave the α-(5-aryl-1,3,4-thiadiazole-2-ylthio)acetic acids **9a–b**. Treatment of **8a–b** with concentrated NH_4OH or $N_2H_4 \cdot H_2O$ gave the α-(5-aryl-1,3,4-thiadiazole-2-ylthio)acetamides **10a–b** and the α-(5-aryl-1,3,4-thiadiazole-2-ylthio)-acethydrazides **11a–b** respectively. Sulfoxides (**6a–b**, **12a–b**) and sulfones (**7a–b**, **13a–b**) were prepared by usual procedures from sulfides (**5a–b**, **8a–b**) using an excess of H_2O_2 30% and CH_3COOH (Scheme).

2.2. Biological evaluation

All of the compounds were evaluated *in vitro* for antituberculosis activity against *Mycobacterium tuberculosis* as part of the TAACF TB screening program under direction of the U.S. National Institutes of Health, NIAID division. From eighteen samples tested, six compounds (**5a–b**, **6a–b**, **8a–b**) displayed significant inhibition effects in the primary screening (MIC < 6.25 μg/ml) against *M. tuberculosis* strain H37Rv in BACTEC 12B medium using the BACTEC 460 radiometric system with rifampicin as reference substance (Table 1). 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* (Table 2).

The results indicate that compounds bearing a primary methylthio or carbethoxy methylthio group attach to the position 2 of 1,3,4-thiadiazole ring (**5a–b**, **8a–b**) displayed high inhibitory activity against *M. tuberculosis.* The oxidation of the thio group to sulfoxide in methylthio derivatives (**5a–b**) maintained the antituberculosis activity in compounds **6a–b**, while oxidation of carbethoxy methylthio analogues (**8a–b**) to sulfoxide led to compounds devoid of antituberculosis activity (**12a–b**). However, sulfone derivatives of both methylthio and carbethoxymethylthio groups (**7a–b**, **13a–b**) exhibited no antituberculosis activity. In addition replacement of the ethoxy group in compounds **8a–b** with hydroxy, amino or hydrazino groups abolish the antituberculosis activity (**9a–b, 10a–b** and **11a–b**).



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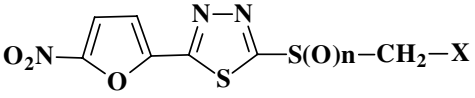

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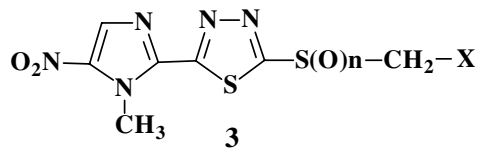
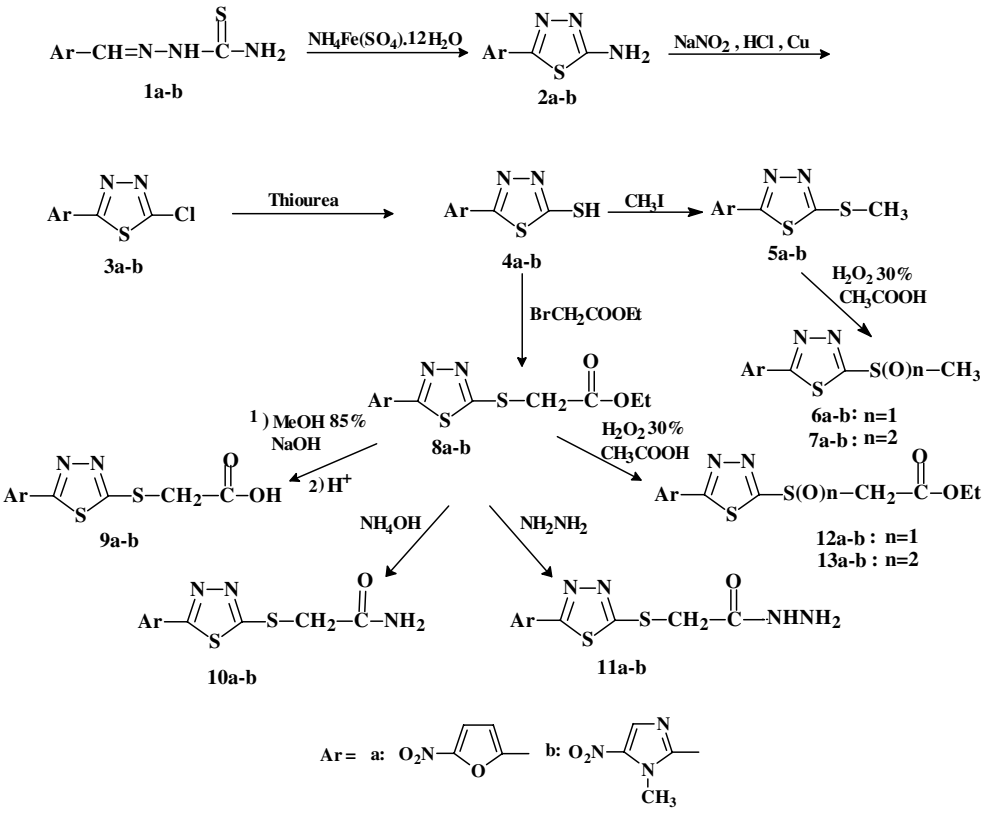


3

X=H , COOEt , COOH , $CONH_2$, $CONHNH_2$

n= 0-2

Scheme



3. Experimental

3.1. 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 ^{1}H NMR spectra were recorded on a Bruker Ac-80 spectrometer and chemical shifts (δ) are in ppm relative to internal tetramethylsilane. The MS were run on a Finnigan TSQ 70 spectrometer at 70 ev.

The syntheses of compounds **5a–b**, **6a–b** and **7a–b** were reported previously [8].

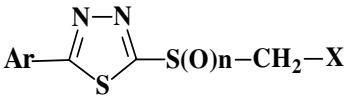
3.2. 2-Mercapto-5-(5-nitro-2-furyl)-1,3,4- thiadiazole (4a)

A mixture of 2-chloro-5-(5-nitro-2-furyl)-1,3,4-thiadiazole (**3a**) [12] (2 g, 8.64 mmol) and thiourea (2 g, 26 mmol) in EtOH (20 ml) was refluxed for 3 h. After cooling, H_2O was added and the separated solid was filtered off, washed with H_2O and crystallized from EtOH–H_2O giving 1.7 g **4a** in 86% yield. m.p. 175–176 °C. (lit. [13]. m.p. 173.4–173.8 °C).

3.3. 2-Mercapto-5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole (4b)

This compound was prepared as described for **4a** in 91% yield, m.p. 235–236 °C. (lit. [14]: m.p. 236–237 °C).

Table 1: Primary antituberculosis activity in vitro screening*



Compd.	Ar	n	X	MIC (μg/ml)	Inhibition %	Activity
5a	5-nitro-2-furyl	0	H	<6.25	99	+
5b	1-methyl-5-nitro-2-imidazolyl	0	H	<6.25	91	+
6a	5-nitro-2-furyl	1	H	<6.25	101	+
6b	1-methyl-5-nitro-2-imidazolyl	1	H	<6.25	105	+
7a	5-nitro-2-furyl	2	H	>6.25	<90	–
7b	1-methyl-5-nitro-2-imidazolyl	2	H	>6.25	18	–
8a	5-nitro-2-furyl	0	COOEt	<6.25	100	+
8b	1-methyl-5-nitro-2-imidazolyl	0	COOEt	<6.25	101	+
9a	5-nitro-2-furyl	0	COOH	>6.25	0	–
9b	1-methyl-5-nitro-2-imidazolyl	0	COOH	>6.25	0	–
10a	5-nitro-2-furyl	0	$CONH_2$	>6.25	11	–
10b	1-methyl-5-nitro-2-imidazolyl	0	$CONH_2$	>6.25	0	–
11a	5-nitro-2-furyl	0	$CONHNH_2$	>6.25	0	–
11b	1-methyl-5-nitro-2-imidazolyl	0	$CONHNH_2$	>6.25	0	–
12a	5-nitro-2-furyl	1	COOEt	>6.25	2	–
12b	1-methyl-5-nitro-2-imidazolyl	1	COOEt	>6.25	14	–
13a	5-nitro-2-furyl	2	COOEt	>6.25	0	–
13b	1-methyl-5-nitro-2-imidazolyl	2	COOEt	>6.25	5	–

* MIC rifampicin 0.25 μg/ml; 97% inhibition

Table 2: Actual minimum inhibitory concentration (MIC) of compounds 5a–b, 6a–b and 8a–b*

Compd.	(MIC) μg/ml	Compd.	(MIC) μg/ml
5a	6.25	**6b**	1.56
5b	6.25	**8a**	1.56
6a	3.13	**8b**	1.56

* MIC of RMP = 0.0075 μg/m

3.4. Ethyl α-[5-(5-nito-2-furyl)-1,3,4-thiadiazole-2-ylthio] acetate (**8a**)

To a mixture of **4a** (229 mg, 1 mmol) and ethyl bromoacetate (250 mg, 1.5 mmol) in EtOH (5 ml), KOH (66 mg, 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 giving 296 mg **8a** in 94% yield, m.p. 108–110 °C. IR (KBr) ν_{max}: 3152 (furyl), 1737 (C=O) and 1539, 1308 cm^{-1}(NO_2). ^1H NMR ($CDCl_3$) δ: 7.44 (d, 1 H, furyl, J = 4 Hz), 7.30 (d, 1 H, furyl, J = 4 Hz), 4.30 (m, 4 H, CH_2 and OCH_2) and 1.30 ppm (t, 3 H, CH_3, J = 7.2 Hz). MS m/z (%): 316 (M^+ + 1,20) 270 (5), 241(20), 166 (20), 139 (15), 96 (20), 82 (100), 64 (27) and 45 (80).

3.5. Ethyl α-[5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole-2-ylthio]-acetate (**8b**)

This compound was prepared as described for **8a** in 75% yield, m.p. 120–122 °C (EtOH). IR (KBr) ν_{max}: 3125 (H–C_4 imidazole), 1728 (C=O) and 1545, 1340 cm^{-1} (NO_2). ^1H NMR ($CDCl_3$) δ: 8.06 (s, 1 H, H–C_4 imidazole), 4.53 (s, 3 H, NCH_3), 4.26 (m, 4 H, CH_2 and OCH_2) and 1.30 ppm (t, 3 H, CH_3, J = 7.2 Hz). MS: m/z (%): 329 (M^+, 39), 255 (92), 243 (11), 180 (17), 153 (39), 97 (39), 83 (44) 69 (87), 55 (95) and 45 (100).

3.6. α-[5-(5-Nitro-2-furyl)-1,3,4- thiadiazole-2-ylthio]acetic acid (**9a**)

To a mixture of **8a** (315 mg, 1 mmol) in methanol (5 ml), 1 ml NaOH 1.5 molar was added dropwise and the mixture was stirred at room temperature over night, then acidified with excess of diluted HCl. The separated solid was filtered off and crystallized from petroleum ether giving 241 mg **9a** in 84% yield, m.p. 179–181 °C. IR (KBr) ν_{max}: 3380 (OH), 3152 (furyl), 1696 (C=O) and 1545, 1347 cm^{-1} (NO_2). ^1H NMR (DMSO-d6) δ: 7.54 (d, 1 H, furyl, J = 4 Hz), 7.33 (d, 1 H, furyl, J = 4 Hz) and 4.19 ppm (s, 2 H, CH_2). MS m/z (%): 287 (M^+, 25), 259 (100), 207 (31), 157 (53), 122 (26), 67 (18) and 39 (26).

3.7. α-[5-(1-Methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole-2-ylthio]acetic acid (**9b**)

This compound was prepared as described for **9a** in 88% yield, m.p. 189–191 °C (petroleum ether). IR (KBr) ν_{max}: 3472 (OH), 3136 (H–C_4 imidazole), 1718 (C=O) and 1536, 1356 cm^{-1} (NO_2).

3.8. α-[5-(5-Nirto-2-furyl)-1,3,4-thiadiazole-2-ylthio]acetamide (**10a**)

To a mixture of **8a** (315 mg, 1 mmol) in EtOH (5 ml), concentrated NH_4OH (2 ml) was added dropwise. After stirring overnight H_2O was added and the separated solid was filtered off, washed with H_2O and crystallized from ethanol giving 208 mg **10a** in 73% yield, m.p. 193–195 °C. IR (KBr) ν_{max}: 3458, 3216 (NH_2), 3168 (furyl), 1664 (C=O) and 1539, 1347cm^{-1} (NO_2). ^1H NMR (DMSO-d6) δ: 7.64 (d, 1 H, furyl, J = 4 Hz), 7.37 (d, 1 H, furyl, J = 4 Hz) 4.25 (m, 2 H, NH_2) and 4.01 ppm (s, 2 H, CH_2). MS m/z (%): 287 (M^+ + 1,23), 286 (M^+, 100), 243 (39), 207 (15), 110 (18) and 40 (20).

3.9. α-[5-(1-Methyl-5-nitro-2-imidazolyl)-1,3,4–thiadiazole-2-ylthio]acetamide (**10b**)

This compound was prepared as described for **10a** at 0 °C in 95% yield, m.p. 218–220 °C (EtOH). IR (KBr) ν_{max}: 3376, 3184 (NH_2), 3125 (H–C_4 imidazole), 1673 (C=O) and 1545, 1350 cm^{-1} (NO_2). MS m/z (%): 300 (M^+, 18), 301 (38), 257 (97), 170 (38), 153 (28), 83 (30), 67 (52), 58 (93), 46 (100).

3.10. α-[5-(5-Nitro-2-furyl)-1,3,4-thiadiazole-2-ylthio]acethydrazide (**11a**)

Compound **8a** (315 mg, 1 mmol) was dissolved in EtOH (5 ml) and the mixture was stirred in an ice bath while hydrazine hydrate 24% (1 ml) was added dropwise. After stirring overnight H_2O was added and the separated solid was filtered off, washed with H_2O and crystalized from EtOH giving 186 mg **9a** in 62% yield, m.p. 228–230 °C. IR (KBr) ν_{max}: 3350, 3344 (–NH–NH_2), 3168 (furyl), 1654 (C=O) and 1542, 1350 cm^{-1} (NO_2). MS m/z (%): 301 (M^+, 45), 293 (100), 162 (52), 136 (25) and 97 (75).

3.11. α-[5-(1-Methyl-5-nitro–2–imidazolyl)–1,3,4–thiadiazole–2-ylthio]-acethydrazide (**11b**)

This compound was prepared as described for **11a** in 65% yield, m.p. 179–181 °C (EtOH). IR (KBr) ν_{max}: 3312, 3320 (–NH–NH_2), 3136 (H–C_4 imidazole), 1648 (C=O) and 1555, 1356 cm^{-1} (NO_2). ^1H NMR (DMSO-d6) δ: 8.07 (s, 1 H, H–C_4 imidazole), 7.50 (s, 2 H, NH_2), 4.52 (s, 3 H, NCH_3) and 4.09 ppm (s, 2 H, CH_2).

3.12. Ethyl α-[5-(5-nitro-2-furyl)-1,3,4-thiadiazole-2-ylsulfinyl]acetate (**12a**)

To a stirring mixture of compound **8a** (315 mg, 1 mmol) in glacial acetic acid (3 ml), 30% H_2O_2 solution (2 ml) was added dropwise and the mixture was stirred overnight. H_2O was added, the precipitate was filtered and purified by TLC eluting with 5% EtOH–$CHCl_3$ to give 254 mg **12a** in 77% yield, m.p. 126–128 °C IR (KBr) ν_{max}: 3120 (furyl), 1740 (C=O), 1529, 1350 (NO_2) and 1056 cm^{-1} (SO). ^1H NMR ($CDCl_3$) δ: 7.40 (m, 2 H, furyl), 4.27 (m, 4 H, CH_2 and OCH_2) and 1.3 ppm (t, 3 H, CH_3, J = 7.2 Hz). MS m/z (%): 331 (M^+, 75), 316 (25), 287 (18), 243 (52), 215 (95), 156 (66), 106 (81) and 82 (93).

3.13. Ethyl α-[5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole-2-ylsulfinyl]acetate (**12b**)

This compound was prepared as described for **12a** in 85% yield, m.p. 128°–130 °C (EtOH). IR (KBr) ν_{max}: 3152 (H–C_4 imidazole), 1715 (C=O), 1542, 1363 (NO_2) and 1060 cm^{-1} (SO). ^1H NMR ($CDCl_3$) δ: 8.10 (s, 1 H, H–C_4 imidazole), 4.58 (s, 3 H, NCH_3), 4.28 (m, 4 H, CH_2 and OCH_2) and 1.30 ppm (t, 3 H, CH_3).

3.14. Ethyl α-[5-(5-nitro-2-furyl)-1,3,4-thiadiazol-2-ylsulfonyl]acetate (**13a**)

To a stirring mixture of compound **8a** (315 mg, 1 mmol) in glacial acetic acid (3 ml) was added 30% H_2O_2 solution (2 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–$CHCl_3$ to give 225 mg **13a** in 65% yield, m.p. 119–121 °C, IR (KBr) ν_{max}: 3152 (furyl), 1731 (C=O), 1545, 1360 (NO_2) and 1350, 1161 cm^{-1} (SO_2). ^1H NMR ($CDCl_3$) δ: 7.51 (d, 1 H, furyl, J = 4 Hz), 7.20 (d, 1 H, furyl, J = 4 Hz), 4.61 (s, 2 H, SO_2CH_2), 4.22 (q, 2 H, OCH_2, J = 7.2 Hz) and 1.26 ppm (t, 3 H, CH_3, J = 7.2 Hz).

3.15. Ethyl α-[5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole-2-ylsulfonyl]acetate (**13b**)

This compound was prepared as described for **13a** by stirring at 60 °C in 65% yield, m.p. 119–121 °C. IR (KBr) ν_{max}: 3125 (H–C_4 imidazole) 1744 (C=O), 1529, 1376 (NO_2) and 1360, 1322 cm^{-1} (SO_2). ^1H NMR ($CDCl_3$) δ: 8.11 (s, 1 H, H–C_4 imidazole), 4.61 (brs, 5 H, NCH_3 and SO_2CH_2), 4.23 (q, 2 H, OCH_2, J = 7.2 Hz) and 1.26 ppm (t, 3 H, CH_3, J = 7.2 Hz).

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Dr. Alireza Foroumadi, Ass. Prof.
Medicinal Chemistry Department
Faculty of Pharmacy
Haft Bagh St.
Kerman
Iran