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Antituberculosis agents VIII
Synthesis and in vitro antimycobacterial activity of alkyl α-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-ylthio]acetates

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Abstract

A series of alkyl α-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-ylthio]acetic acid esters **6a**–**e** were synthesized and evaluated for in vitro antituberculosis activity against *Mycobacterium tuberculosis* strain $H_{37}Rv$ using the BACTEC 460 radiometric system and BACTEC 12B medium. The antituberculosis data indicated that methyl, propyl, buthyl and benzyl esters showed a significant in vitro antimycobacterium tuberculosis activity (MIC = 0.39–0.78 μg/ml) and the ethyl analogue did not show a good activity (MIC > 6.25 μg/ml, %inhibition = 58). The most active compound of the series was *n*-propyl α-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-ylthio]acetate (**6c**) with MIC value of 0.39 μg/ml.
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1. Introduction

Tuberculosis is a chronic infection disease caused by several species of mycobacteria [1]. The incidence of tuberculosis is increasing world wide, partly due to poverty and inequity and partly to the HIV/AIDS pandemic, which greatly increase the risk of infection proceeding to overt disease [2].

The increase in drug-resistant *Mycobacterium tuberculosis* isolates during recent years presents a therapeutic challenge to physicians selecting antimicrobial agents [3]. Thus, the development of new agents with potent antituberculosis activities and fewer adverse effects is urgently desired.

Recently, we reported the synthesis of alkyl α-[5-(5-nitro-2-furyl)-1,3,4-thiadiazole-2-ylthio]acetates with antimycobacterium tuberculosis activity [4].

The 5-nitrothiophene isostere of 5-nitrofuran ring system is known to possess several biological properties such as, antileishmania, antimalaria, antitrypanosomal, anti-microbial and antituberculosis effects [5–9].

Accordingly, as a part of study attempting to further optimize the nitroaromatics against *M. tuberculosis* herein we report the synthesis and in vitro antituberculosis activity of alkyl α-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-ylthio]acetates **6a**–**e** as possible antimycobacterial agents.

2. Materials and methods

2.1. General

Melting points were taken on a Electrotermal IA-9100 capillary apparatus and are uncorrected. The IR spectra were obtained using a Shimatdzu 470 spectrograph (KBr 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 (TMS). The mass spectra were run on a Finigan TSQ-70 spectrometer at 70 eV. Elemental analyses (C, H, N) for compounds **6a**–**e** were within ±0.4% from the theoretical values.

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2.2. Synthesis of the products

2.2.1. 2-Amino-5-(5-nitro-2-thienyl)-1,3,4-thiadiazole (**2a**)

A mixture of 5-nitrothiophene-2-carboxaldehyde thiosemicarbazone (**1a**, 5.2 g, 23 mmol) [10]and ammonium ferric sulfate dodecahydrate (11 g) in H_2O (55 ml) was refluxed for 1 h. Then 22 g ammonium ferric sulfate in H_2O (110 ml) was added and the mixture was refluxed for 4.5 h. After cooling, the separated solid was filtered off, washed with water and crystallized from EtOH–H_2O to give 4.6 g **2a** in 90% yield; m.p. 210–211 °C

IR (KBr) ν_{max}: 1340, 1520 (NO_2), 3050 (CH thienyl), 3075–3100 cm^{-1} (NH_2). Mass *m/z* (relative abundance %): 229 (81), 228(M^+, 99), 212(8), 95(26), 82(18), 74(100), 69 (58), 60(73), 45(55).

2.2.2. 2-Chloro-5-(5-nitro-2-thienyl)-1,3,4-thiadiazole (**3a**)

Compound **2a** (2.35 g, 9.5 mmol) was ground with an excess of $NaNO_2$ (2 g) and the mixture was introduced in small portion and with stirring into a ice cooled solution of conc. HCl (30 ml) and water (13 ml) containing Cu powder (0.5 g). The reaction mixture was allowed to reach room temperature and stirred for additional 2 h. Then heated to 55 °C until the evolution of gas ceased. The reaction mixture was cooled and extracted with $CHCl_3$ (3×40 ml). The combined extracts were washed with dilute H_2SO_4, water and dried (Na_2SO_4). The solvent was evaporated to give crude **3a**. Purification was achieved by passage through a short silica gel column with chloroform as eluent. The product was crystallized from ethanol to give 1.5 g of **3a** in 63% yield; m.p. 175–177 °C.

IR (KBr) ν_{max}: 1334, 1497 (NO_2), 3090 cm^{-1} (CH thienyl). ^{1}H-NMR ($CDCl_3$, 80 MHz) δ: 7.91 (d, 1H, H4-thiophene, $J = 4.8$ Hz) and 7.40 (d, 1H, H8-thiophene, $J = 4.8$ Hz). Mass *m/z* (relative abundance %): 249 (36), 248(20), 247(81), 219(14), 217(33), 189(19), 138(10), 126(16), 111(17), 95(43), 93(100), 79(45), 69(83), 57(68), 45(38).

2.2.3. 2-Mercapto-5-(5-nitro-2-thienyl)-1,3,4-thiadiazole (**4a**)

A mixture of **3a** (2 g, 8.16 mmol) and excess thiourea (2 g) in 20 ml ethanol was refluxed for 3 h. After cooling conc. HCl (3 ml) and water (20 ml) was added and the solids isolated by filtration were washed with water and crystallized from EtOH–H_2O giving 1.5 g **4a** in 75% yield; m.p. 194–195 °C

IR (KBr) ν_{max}: 1350, 1510 (NO_2), 3050 cm^{-1} (CH thienyl)

2.2.4. General method for the synthesis of alkyl α-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-ylthio] acetates **6a–e**

To a mixture of **4a** (1 mmol) and alkyl α-chloro acetates **5a–e** (1.25 mmol) in ethanol (15 ml), KOH (66 mg, in 5 ml H_2O) was added drop-wise. The mixture was stirred at room temperature overnight, H_2O was added and the separated solid was filtered off, washed with water and crystallized from ethanol.

The following compounds were prepared according to the general procedure.

*2.2.4.1. Methyl α-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-ylthio] acetate (**6a**).* Yield: 92%; m.p 128–129 °C. IR (KBr): $\nu_{max} = 1340$, 1504 (NO_2), 1729 (C=O), 2928 cm^{-1} (CH_2). ^{1}H-NMR ($CDCl_3$, 80 MHz) $\delta = 7.90$ (d, 1H, H4-thiophene, $J = 4.3$ Hz), 7.35(d, 1H, H3-thiophene, $J = 4.3$ Hz), 4.21 (s, 2H, SCH_2), 3.81 ppm (s, 3H, CH_3)

*2.2.4.2. Ethyl α-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-ylthio] acetate (**6b**).* Yield: 72%; m.p 129–130 °C. IR (KBr): $\nu_{max} = 1340$, 1500 (NO_2), 1719 (C=O), 2900 cm^{-1} (CH_2). ^{1}H-NMR ($CDCl_3$, 80 MHz) $\delta = 7.91$ (d, 1H, H4-thiophene, $J = 4.3$ Hz), 7.33 (d, 1H, H3-thiophene, $J = 4.3$ Hz), 4.48–4.10 (m, 4H, SCH_2 and OCH_2), 1.31 ppm (t, 3H, CH_3, $J = 6.4$ Hz)

*2.2.4.3. n-Propyl α-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-ylthio] acetate (**6c**).* Yield: 94%; m.p 118–119 °C. IR (KBr): $\nu_{max} = 1330$, 1504 (NO_2), 1715 (C=O), 2900 cm^{-1} (CH_2). ^{1}H-NMR ($CDCl_3$, 80 MHz) $\delta = 7.90$ (d, 1H, H4-thiophene, $J = 4.3$ Hz), 7.36 (d, 1H, H3-thiophene, $J = 4.3$ Hz), 4.42–4.099 (2m, 4H, SCH_2 and OCH_2), 1.87–1.31 (m, 2H, CH_2), 0.96 ppm (t, 3H, CH_3, $J = 6.4$ Hz).

*2.2.4.4. n-Buthyl α-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-ylthio] acetate (**6d**).* Yield: 88%; m.p 115–116 °C. IR (KBr): $\nu_{max} = 1340$, 1504 (NO_2), 1709 (C=O), 2912 cm^{-1} (CH_2). ^{1}H-NMR ($CDCl_3$, 80 MHz) $\delta = 7.90$ (d, 1H, H4-thiophene, $J = 4.3$ Hz), 7.35 (d, 1H, H3-thiophene, $J = 4.3$ Hz), 4.34–4.41 (m, 4H, SCH_2 and OCH_2), 1.90–1.10 (m, 4H, CH_2CH_2), 0.93 ppm (t, 3H, CH_3, $J = 6.4$ Hz).

*2.2.4.5. Phenylmethyl α-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-ylthio] acetate (**6e**).* Yield: 46%; m.p 123–124 °C. IR (KBr): ^{1}H-NMR ($CDCl_3$, 80 MHz) $\delta = 7.92$ (d, 1H, H4-thiophene, $J = 4.3$ Hz), 7.33 (d, 1H, H3-thiophene, $J = 4.3$ Hz), 7.43–7.10 (m, 5H, aromatic), 4.20 ppm (s, 4H, SCH_2 and OCH_2).

2.3. Biological assay

All of the compounds were evaluated for in vitro antituberculosis activity against *M. tuberculosis* as part

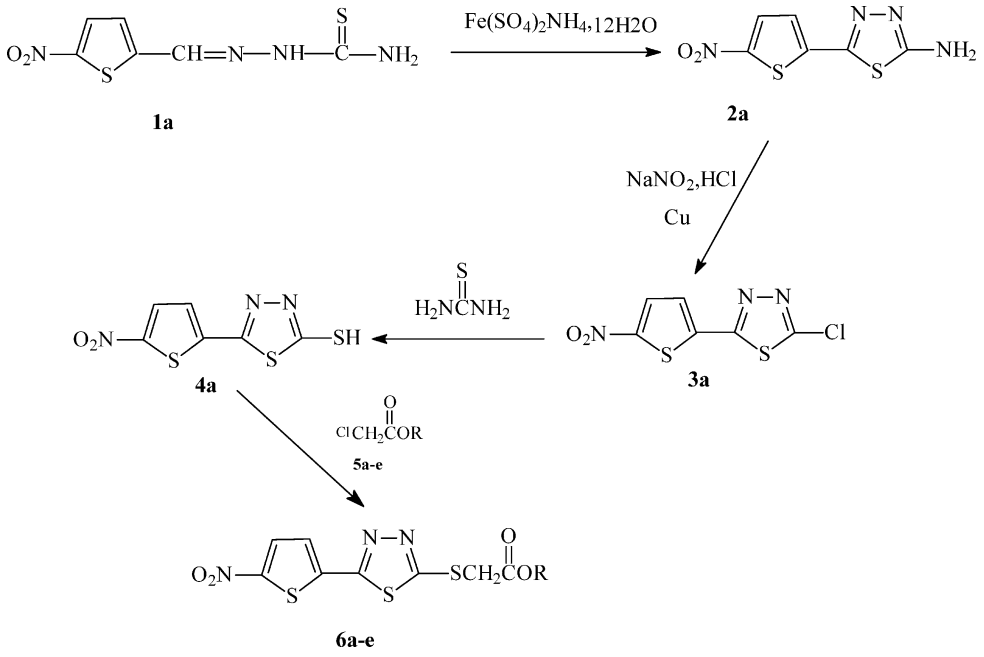


Fig. 1. Synthesis of alkyl α-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-ylthio] acetates **6a**–**e**.

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 against *M. tuberculosis* $H_{37}Rv$ (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA) [11]. Compounds effecting $<90\%$ inhibition in the primary screening (MIC >6.25 μg/ml) were not generally evaluated further.

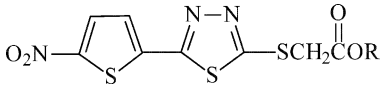
The active compounds were retested by serial dilution beginning at 6.25 μg/ml against *M. tuberculosis* $H_{37}Rv$ to determine the actual minimum inhibitory concentration (MIC) in the BACTEC 460.

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

3. Results and discussion

The 2-amino-5-(5-nitro-2-thienyl)-1,3,4-thiadiazole (**2a**) was obtained by oxidative cyclization of 5-nitro-2-thiophene carboxaldehyde thiosemicarbazone (**1a**) [10] in high yield. Diazotation of **2a** in hydrochloric acid in the presence of copper powder gave 2-chloro-5-(5-nitro-2-thienyl)-1,3,4-thiadiazole (**3a**). The reaction of **3a** with thiourea in refluxing ethanol afforded 2-mercapto-5-(5-

Table 1
In vitro antituberculosis activity of alkyl α-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-ylthio]acetates **6a**–**e** [a]



Comp.	R	MIC (μg/ml) [b]	Inhibition (%)	Activity	Actual MIC (g/ml)
6a	Methyl	<6.25	100	+	0.78
6b	Ethyl	>6.25	58	−	ND [c]
6c	*n*-Propyl	<6.25	100	+	0.39
6d	*n*-Buthyl	<6.25	100	+	0.78
6e	Benzyl	<6.25	99	+	0.78

[a] MIC rifampicin 0.125–0.5 μg/ml.
[b] Primary screening.
[c] ND, not determined.

nitro-2-thienyl)-1,3,4-thiadiazole (**4a**). Treatment of the latter with alkyl chloro acetates **5a**–**e** gave alkyl α-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazole-2-ylthio] acetates **6a**–**e** (Fig. 1).

From antituberculosis data reported in Table 1, the majority of compounds **6a** and **6c**–**e** were efficient antimycobacterial agents showing MIC values of 0.39–0.78 μg/ml (Table 1).

The best efficiency expressed as MIC was exhibited by propyl ester **6c** (MIC = 0.39 μg/ml), but significant decrease in potency was observed by ethyl ester with inhibition percentage of 58 (MIC > 6.25 μg/ml). The finding is in contrast with those of furan analogues which ethyl ester showed a good antituberculosis activity (%inhibition = 100, MIC = 1.56 μg/ml) [4].

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