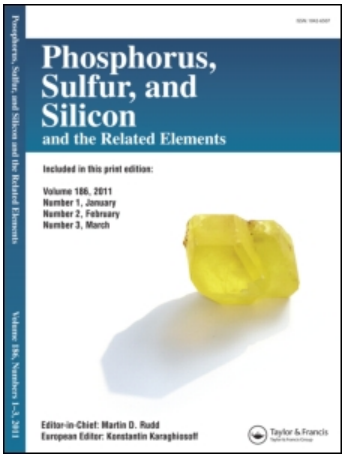

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Synthesis and Biological Evaluation of N,N'-di(thiopheneacetyl)diamines Series as Antitubercular Agents

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Synthesis and Biological Evaluation of *N,N′*-di(thiopheneacetyl)diamines Series as Antitubercular Agents

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The series of new N,N'-di(thiopheneacetyl)diamines derivatives, **8–17,** *were synthesized and evaluated for their in vitro antibacterial activity against Mycobacterium tuberculosis(TB), and the activity expressed as the minimum inhibitory concentration (MIC) in μg/mL. Compound* ***13*** *was the only one determined to be active and exhibited a MIC 50 μg/mL, indicating that the alkyl chain-length of the diamines is critical for biological activity. This class of compound has not been evaluated before, and it could be a good starting point to find new lead compounds in the fight against multi-drug resistant TB.*

Keywords Antimycobacterial activity; thiophene; tuberculosis (TB)

INTRODUCTION

Tuberculosis (TB) is becoming again an important public health problem worldwide, declared by WHO (World Health Organization) as a global health emergency in 1993.[1] This contagious disease is transmitted through the air and it is caused by the bacterium *Mycobacterium tuberculosis*, which is responsible for 1.7–2.0 millions of deaths each year. [2] However, in spite of the worldwide problem caused by TB, it has

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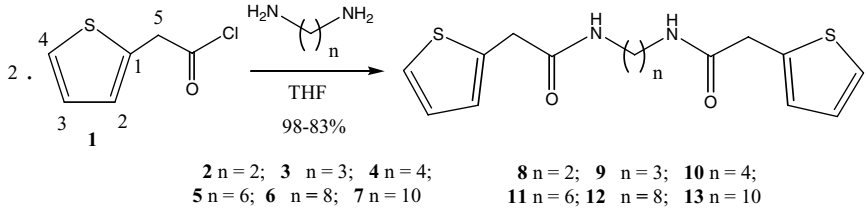


2 n = 2; **3** n = 3; **4** n = 4; **5** n = 6; **6** n = 8; **7** n = 10

8 n = 2; **9** n = 3; **10** n = 4; **11** n = 6; **12** n = 8; **13** n = 10

SCHEME 1 Synthesis of aliphatic *N,N'*-di(thiopheneacetyl)diamines.

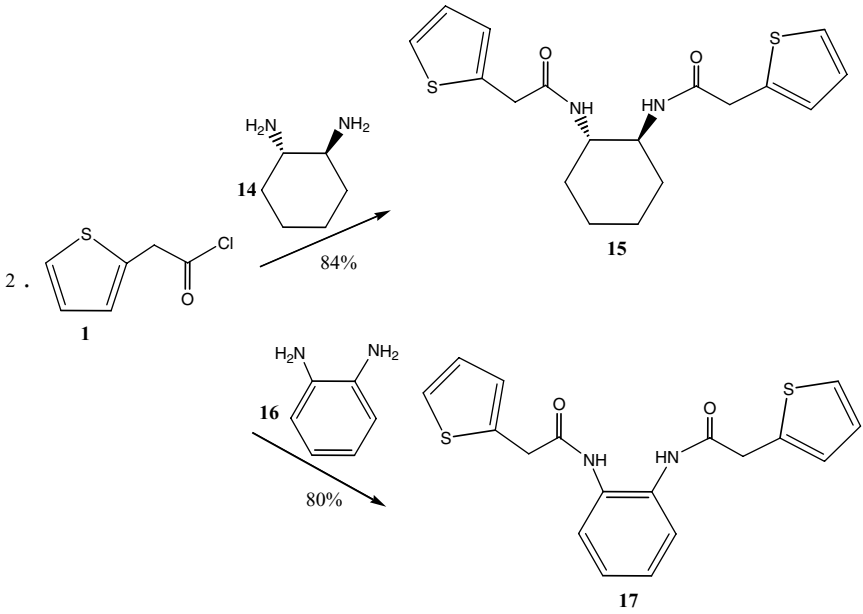
been nearly 30 years since a novel drug has been introduced to treat TB (with exception of Rifapentine approved by FDA in 1998). In this context, there is an urgent need for new drugs to fight against this disease. Considering that, the thiophene nucleus represents a very important field in drug discovery, and is present in many natural and synthetic products with a wide range of pharmacological activities. [3,4]

There are three basic factors involved in the development of new TB drugs; to reduce the total duration of treatment, to improve the MDR TB (Multidrug-resistant TB) and to provide more effective treatment of latent TB infection. Due to TB problem worldwide and the lack of new drugs nowadays, the aim of this article is to present a series of news *N,N'*-di(thiopheneacetyl)diamines **8–17** derivatives, which have been synthesized, see Schemes **1** and **2,** and evaluated for their in vitro antibacterial activity against *Mycobacterium tuberculosi*s.

RESULTS AND DISCUSSION

Chemistry

The synthesis of di(thiopheneacetyl)diamines compounds **8–17** involved the reaction between appropriate 2-thiopheneacetyl chloride 1 and the respective diamines **2–7**, **14** and **16** (Schemes 1 and 2), as described in the general procedure leading to the desire compounds **8–17** in 77–98% yields. All the compounds were identified by spectral data. In general, IR spectra showed the C=O peak at 1650–1675 cm^{-1}. The ^1H NMR spectrum showed the amide (NH) proton as a large singlet at 8.15–7.24 ppm, $C\underline{H}_2CO$ proton as a singlet at 3.87–3.53 ppm, and $NHC\underline{H}_2$ proton as a triplet or quartet at 3.04–3.50 ppm. The ^{13}C NMR spectrum showed the C=O signals at 170.0–168.6, $C\underline{H}_2CO$ signals at 39.0–37.8 and aliphatic carbons at the region of 26.5–36.5 ppm.



SCHEME 2 Synthesis of cyclic N,N'−di(thiopheneacetyl)diamines.

Biology Evaluation

In the biological evaluation of the series of news N,N'-di(thiophe neacetyl)diamines derivatives **8–17**, only the compound **13** exhibited activity with a MIC of 50 μg/mL against *M. tuberculosis* infection, when compared to first line drugs as isoniazid (INH) and rifampin (RIP) (Table I). All others compounds synthesized **(8–12** and**14–17)** were inactive against *M. tuberculosis*. This result indicated that the length of the alkyl chain is critical for the biological activity. Additionally, compound **13** was not cytotoxic to host cells at the same concentration (Table II).

EXPERIMENTAL

General Procedures

Melting points were determined on a Buchi apparatus and are uncorrected. Infrared spectra were recorded on a Thermo Nicolet Nexus 670 Spectrometer as potassium bromide pellets and frequencies are expressed in cm^{-1}. Mass spectra (CG/MS) were recorded on an Agilent Technologies 6890/5972A Mass Spectrometer. NMR spectra were

TABLE I Antimycobacterial Activities of *N,N'*-di(thiopheneacetyl)diamines

Compound	M.p. (°C)	Yield (%)	MIC (μg/mL)
8	145–150	98	Resistant
9	118–120	90	Resistant
10	146–148	88	Resistant
11	110–112	83	Resistant
12	216–220	92	Resistant
13	115–117	77	50
15	102–104	84	Resistant
17	102–107	80	Resistant

recorded on a Bruker Avance 500 Spectrometer operating at 500.00 MHz (^{1}H) and 125.0 MHz (^{13}C), in deuterated dimethylsulfoxide. Chemical shifts are reported in ppm (δ) relative to tetramethylsilane. Proton and carbon spectra were typically obtained at room temperature. TLC plates coated with silica gel were developed in ethyl acetate and spots were visualized under UV light.

General Procedures for the Synthesis of *N,N'*-di(thiopheneacetyl)diamines Derivatives (8–17)

The N,N′-di(thiopheneacetyl)diamines derivatives (**8–17**) were prepared by reaction between the appropriate diamines **2–7**, **14,** and **16** (1.0 equiv.) with thiophenylacetylchloride (2.2 equiv.) in tetrahydrofuran (20 mL), under nitrogen atmosphere (Scheme 1). After stirring for 2–3 h at room temperature the reaction mixture was quenched with 30 mL of water and extracted with ethylacetate (2 × 20 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried ($MgSO_4$), filtered, and concentrated in vacuo to produce the desired diamines without further purification.

N,N'-di(thiopheneacetyl)ethylenediamine (8)

Yield: 98% **m.p.**: 145–150°C

CG/MS: m/z $[M]^{+\cdot}$: 308

^{1}H NMR [500.00 MHz (FIDRES ± 0.15 Hz), DMSO-d_6] δ: 8.10 (s, 2H; N<u>H</u>); 7.37 (dd, 2H; J = 5.0 and 2.0 Hz; H4); 6.94 (dd, 2H; J = 5.0 and 2.0 Hz; H3); 6.89 (ls, 2H; H2); 3.87 (s, 4H, C<u>H</u>$_2$CO); 3.2 (t; 4H; J = 7.0 Hz; NCH$_2$C<u>H</u>$_2$N) ppm. **^{13}C NMR (125.0 MHz, DMSO-d_6) δ:** 169.0; 137.0; 126.1; 125.8; 124.7; 38.6; 36.4 ppm.

IR ν_{max} (cm^{-1}; KBr pellets): 1657 (CO).

N,N′-di(thiopheneacetyl)propylenediamine (9)

Yield: 90% **m.p.**: 118–120°C

CG/MS: m/z $[M]^{+\cdot}$: 322

^{1}H NMR [500.00 MHz (FIDRES ± 0.15 Hz), DMSO-d_6] δ: 7.24 (dd, 2H; $J = 5.0$ and 1.5 Hz; H4); 6.99 (t, 2H; $J = 5.0$ Hz; H3); 6.94 (ls, 2H; H2); 6.31 (ls, 2H; NH); 3.76 (s, 4H, $C\underline{H}_2CO$); 3.2 (q, 4H; $J = 7.0$ Hz; $NC\underline{H}_2CHC\underline{H}_2N$); 3.2 (quint., 2H; $J = 7.0$ Hz; $NCH_2C\underline{H}_2CH_2N$) ppm. **^{13}C NMR (125.0 MHz, DMSO-d_6) δ:** 170.5; 136.1; 127.3; 127.2; 125.5; 124.7; 37.6; 36.1; 29.6 ppm.

IV ν_{max} (cm^{-1}; KBr pellets): 1670 (CO).

N,N′-di(thiopheneacetyl)butylenediamine (10)

Yield: 88% **m.p.**: 146–148°C

CG/MS: m/z $[M]^{+\cdot}$: 336

^{1}H NMR [500.00 MHz (FIDRES ± 0.15 Hz), DMSO-d_6] δ: 8.02 (ls, 2H; NH); 7.32 (dd, 2H; $J = 5.0$ and 1.5 Hz; H4); 6.99 (dd, 2H; $J = 5.0$ Hz and 1.5 Hz; H3); 6.88 (ls, 2H; H2); 3.60 (s, 4H, $C\underline{H}_2CO$); 3.04 (t, 4H; $J = 10$ Hz; $NC\underline{H}_2(CH_2)_2C\underline{H}_2N$); 1.42–1.36 (m, 4H; $NCH_2(C\underline{H}_2)_2CH_2N$) ppm. **^{13}C NMR (125.0 MHz, DMSO-d_6) δ:** 168.8; 137.8; 126.5; 125.8; 125.5; 124.6; 39.0; 36.5; 26.5 ppm.

IV ν_{max} (cm^{-1}; KBr pellets): 1666

N,N′-di(thiopheneacetyl)hexylenediamine (11)

Yield: 83% **m.p.**: 110–112°C

CG/MS: m/z $[M]^{+\cdot}$: 364

^{1}H NMR [400.00 MHz (FIDRES ± 0.12 Hz), DMSO-d_6] δ: 8.03 (ls, 2H; NH); 7.33 (dd, 2H; $J = 5.2$ Hz and 1.2 Hz; H4); 6.93 (dd, 2H; $J = 5.2$ Hz and 1.2 Hz; H3); 6.89 (ls, 2H; H2); 3.64 (s, 4H, $C\underline{H}_2CO$); 3.50 (q, 4H; $J = 6.8$ Hz; $NC\underline{H}_2(CH_2)_4C\underline{H}_2N$); 1.39−1.35 and 1.23−1.17 (m, 8H; $NCH_2(C\underline{H}_2)_4CH_2N$) ppm. **^{13}C NMR (100.0 MHz, DMSO-d_6) δ:** 168.8; 137.9; 126.5; 125.8; 124.6; 38.5; 36.5; 28.9; 26.0 ppm.

IV ν_{max} (cm^{-1}; KBr pellets): 1655

N,N′-di(thiopheneacetyl)octylenediamine (12)

Yield: 92% **m.p.**: 212–220°C

CG/MS: m/z $[M]^{+\cdot}$: 392

^{1}H NMR [500.00 MHz (FIDRES ± 0.15 Hz), DMSO-d_6] δ: 7.86 (ls, 2H; NH); 7.32 (dd, 2H; $J = 5.0$ and 1.5 Hz; H4); 6.91 (dd, 2H; $J = 5.0$ Hz and 1.5 Hz; H3); 6.83 (ls, 2H; H2); 3.53 (s, 4H, $C\underline{H}_2CO$); 3.50 (t, 4H; $J = 11$ Hz; $NC\underline{H}_2(CH_2)_6C\underline{H}_2N$); 1.77–1.63 and 1.23–1.20 (m, 12H; $NCH_2(C\underline{H}_2)_6CH_2N$) ppm. **^{13}C NMR (125.0 MHz, DMSO-d_6) δ:** 168.6; 137.7; 126.4; 125.8; 124.6; 51.9; 36.5; 31.8; 24.3 ppm.

IVν_{max}(cm^{-1}; KBr pellets): 1675

N,N'-di(thiopheneacetyl)decyldiamine (13)

Yield: 71% **m.p.**: 115–117°C

CG/MS: *m/z* $[M]^{+\cdot}$: 420

^{1}H NMR [500.00 MHz (FIDRES ± 0.15 Hz), DMSO-d_6] δ**:** 7.96 (ls, 2H; NH); 7.32 (d, 2H; $J = 5.0$ Hz; H4); 6.93 (dd, 2H; $J = 5.0$ Hz and 1.5 Hz; H3); 6.88 (ls; 2H; H2); 3.60 (s, 4H, C$\underline{H}_2$CO); 3.50 (q, 4H; $J = 7.0$ Hz; NC$\underline{H}_2$(CH$_2$)$_8$C$\underline{H}_2$N); 1.77–1.63 and 1.40–1.23 (m, 16H; NCH$_2$(C$\underline{H}_2$)$_8$CH$_2$N) ppm. **^{13}C NMR (125.0 MHz, DMSO-d_6)** δ**:** 168.7; 137.9; 126.4; 125.8; 124.6; 38.6; 36.6; 28.9; 28.8; 28.6; 26.3 ppm.

IVν_{max}(cm^{-1}; KBr pellets): 1650

N,N'-di(thiopheneacetyl)(1R,2S)-1,2-cyclohexanediamine (15)

Yield: 84% **m.p.**: 102–104°C

CG/MS: *m/z* $[M]^{+\cdot}$: 362

^{1}H NMR [500.00 MHz (FIDRES ± 0.12 Hz), DMSO-d_6] δ**:** 8.05 (ls, 2H; N$\underline{H}$); 7.33 (dd, 2H; $J = 5.0$ Hz and 1.5 Hz; H4); 6.93 (dd, 2H; $J = 5.0$ Hz and 1.5 Hz; H3); 6.88 (ls, 2H; H2); 3.60 (s, 4H, C$\underline{H}_2$CO); 3.50 (q, 4H; $J = 7.0$ Hz; NC$\underline{H}_2$C$\underline{H}_2$N); 1.38−1.36 and 1.22 (ls, 8H; NCH$_2$(C$\underline{H}_2$)$_4$CH$_2$N) ppm. **^{13}C NMR (125.0 MHz, DMSO-d_6)** δ**:** 168.8; 137.9; 126.5; 125.9; 124.7; 38.6; 36.6; 28.9; 28.6; 26.6 ppm.

IVν_{max}(cm^{-1}; KBr pellets): 1665

N,N'-di(thiopheneacetyl)phenylenediamine (17)

Yield: 80% **m.p.**: 102–107°C

CG/MS: *m/z* $[M]^{+\cdot}$: 356

^{1}H NMR [500.00 MHz (FIDRES ± 0.12 Hz), DMSO-d_6] δ**:** 8.15 (ls, 2H; N$\underline{H}$); 7.31−7.28 (m, 4H; Harom.); 7.09 (dd, 2H; $J = 7.0$ Hz and 3.5 Hz; H4); 7.03 (t, 2H; J= 7.0 Hz; H3); 6.97 (ls, 2H; H2); 3.84 (s, 4H, C$\underline{H}_2$CO) ppm. **^{13}C NMR (125.0 MHz, DMSO-d_6)** δ**:** 168.8; 135.5; 130.1; 126.5; 127.8; 127.5; 126.5; 125.9; 125.4; 125.3; 120.4; 116.9; 37.8 ppm.

IVν_{max}(cm^{-1}; KBr pellets): 1673

Biological Test

The antimycobacterial *N,N'*-di(thiopheneacetyl)diamines derivatives **8–17** were assessed against *M. tuberculosis* ATTC 27294,[5] using the micro plate Alamar Blue assay (MABA)[6] (Table I). This methodology is nontoxic, uses thermally–stable reagent and shows good correlation with proportional and BACTEC radiometric methods.[7,8] Briefly, two hundred microliters its of sterile deionized water was added to

TABLE II Cytotoxic Effects of Test Compound on Murine Macrophage Cells 18 h after the Treatment

	% Cell viability			
	Doses tested (μg/mL)			
Compound	0,1	1,0	10	100
13	100	100	100	90

all outer-perimeter wells of sterile 96 well plates (falcon, 3072: Becton Dickinson, Lincoln Park NJ) to minimize evaporation of the medium in the test wells during incubation. The 96 plates received 100μL of the Middlebrook 7H9 broth (Difco laboratories, Detroit, MI, USA) and a serial dilution of the compounds **9–16** was made directly on the plate. The final drug concentrations tested were 0.01 to 10.0 μL /mL. Plates were covered and sealed with parafilm and incubated at 37°C for five days. After this time, 25 μl of a freshly prepared 1:1 mixture of Alamar Blue (Accumed International, Westlake Ohio) reagent and 10% tween 80 was added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth; and a pink color was scored as growth. The MIC (Minimal Inhibition Concentration) was defined as the lowest drug concentration, which prevented a color change from blue to pink.

Cell Viability Assay

Cellular viability in the presence and absence of test compound was determined by Mosmans's MTT (3-(4,5-dimethylthylthiazol-2yl)-2,5-dimethyl tetrazolium bromide; Merck) microcultured tetrazolium assay.[9] The cells were plated in flat bottom 96-well plates (2.5 $\times$ 106 cells/mL) cultured for 1 h in a controlled atmosphere (CO_2 5% at 37°C), and non-adherent cells were washed by gentle flushing with RPMI 1640. Adherent cells were cultured in the presence of medium alone, tween 20 (3%) (live and dead controls, respectively) or different concentrations of compounds (0.1, 1.0, 10.0, and 100/μg/mL) in a triplicate assay. After 18 h, stock MTT solution (5 mg/mL of saline; 20 μL/well) was added to the culture and 4 h later, supernatant was discharged and DMSO (100 μL/well) was added for formazan crystals solubilization, and the absorbance was read at 540 nm in a plate reader (Biorad-450).

CONCLUSION

A series of eight new *N,N'*-di(thiopheneacetyl)diamines derivatives **8–17** were synthesized and evaluated for their in vitro antibacterial activity against *Mycobacterium tuberculosis*. In this series**,** only the compound **13** exhibited activity against *M. tuberculosis* infection, with a MIC of 50 μg/mL. This result indicates that alkyl chain length is critical for biological activity. Although this observation was not studied before, it could be a good starting point to find new lead compounds in the fight against multi-drug resistant TB. More information about structure-activity relationship of this compound and their in vivo antibacterial activity test are in progress in our laboratory.

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