International Journal on
Advanced Science
Engineering
Information Technology

Vol. 2 (2012) No. 4
ISSN: 2088-5334

Synthesis and Antibacterial Activity of Thiophenes

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***Abstract*—2-[Bis(methylthio)methylene]propanedinitrile 1a reacted in one-pot successively with piperidine, sodium sulfide, chloroacetonitrile, and potassium carbonate to afford 3-amino-5-(1-piperidinyl)-2,4-thiophenedicarbonitrile 2a. Similar reaction using the last three reagents with ethyl 2-cyano-3,3-bis(methylthio)acrylate 1b produced ethyl 4-amino-5-cyano-2-(methylthio)thiophene-3-carboxylate 2b. The synthesized compounds were characterized by using FT-IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectral data. Antibacterial activities of the synthesized compounds are also reported.**

***Keywords*— Ketene dithioacetals; thiophenes; antibacterial activity.**

I. INTRODUCTION

Thiophene and its derivatives constitute one of the major classes in heterocyclic chemistry. They have been shown to have interesting biological properties such as anticancer,[1] antiviral,[2] antitumor,[3] anti-inflammatory,[4] and antimicrobial.[5] Starting from thiophene derivatives, many thieno-fused bicyclic compounds such as thienopyridines,[6] thienopyrimidines,[7] and thienopyrroles[8],[9] have been synthesized. El-Saghier *et al*.[10],[11] have reported the syntheses and reactions of various thiophenes and fused thiophenes *via* ketene S,S- and N,S-acetals. In this work, we prepare new tetrasubstituted thiophenes **2a** and **2b** from ketene dithioacetals using one-pot four- or three -step procedures. [12a,b]

II. METHODOLOGY

A. Instrumentation

All starting materials were purchased from Aldrich and Sigma and used without further purification. Melting points were determined using a hot stage Gallenkamp melting point apparatus. Infrared spectra were recorded on FTIR 8300 Shimadzu spectrophotometer using potassium bromide. ^{1}H- and ^{13}C-NMR spectra in DMSO were recorded on Varian XL 500 MHz using TMS as internal standard. Mass spectra were recorded on GC-MS QP2010 plus Shimadzu attached with DI2010-MS Shimadzu. TLC analysis was carried out on silica gel of Merck no. 5545.

B. Synthesis of starting materials

The starting materials of 2-[bis(methylthio)methylene] propanedinitrile **1a** and ethyl 2-cyano-3,3-bis(methylthio) acrylate **1b** were prepared (see Scheme 1) according to Sommen et al.[8],[14] as follows.

1). Synthesis of 2-[bis(methylthio)methylene] propane-dinitrile (1a)

A mixture of malononitrile (6.60 g, 0.1 mol) and potassium carbonate (11.31g, 0.1 mol) was dissolved in DMF (110 ml) and the solution was stirred for 1 h at room temperature. After that, carbon disulfide (7.60 g, 0.2 mol) was added drop-wise to the mixture at 0^{o}C for 15 min. Then the reaction mixture was stirred at room temperature for 2 h. Methyl iodide (28.38 g, 0.2 mol) was added drop-wise to the mixture at 0^{o}C and stirred at room temperature for 4 h. The precipitate was filtered, washed with water, and dried at room temperature until constant weight. The isolated solid was purified by recrystallization in ethanol.

2). Synthesis of ethyl 2-cyano-3,3-bis(methylthio)acrylate (1b)

A mixture of ethyl cyanoacetate (11.31 g, 0.1 mol) and potassium carbonate (11.31 g, 0.1 mol) was dissolved in DMF (110 ml) and the solution was stirred for 1 h at room temperature. After 1 h, carbon disulfide (7.60 g, 0.1 mol) was added drop-wise to the solution at 0^{o}C. The reaction mixture was stirred at room temperature for 2 h. Methyl

iodide (28.38 g, 0.2 mol) was added drop-wise to the solution at 0°C and stirred at room temperature for 24 h. The precipitate was filtered, washed with water, and dried at room temperature until constant weight. The isolated solid was purified by recrystallization in ethanol.

C. *Synthesis of thiophenes*

The following is one-pot four- and three-step procedures for preparing **2a** and **2b** (as in Scheme 2).

*1). Preparation of 3-amino-5-(1-piperidinyl)-2,4-thiophenedicarbonitrile **2a***

2-[Bis(methylthio)methylene]propanedinitrile **1a** (0.01 mol) was dissolved in DMF (15 ml), piperidine (0.01 mol) added, and the mixture was heated at 70°C for 75 min. Then $Na_2S.9H_2O$ (0.01 mol) was added and heated for 2 h at 70°C. Chloroacetonitrile (0.02 mol) was added dropwise at 70°C and the reaction mixture was heated at 70°C for 2 h. Then potassium carbonate (0.02 mol) was added and stirred at 70°C for 90 min. The reaction mixture was poured onto water (100 ml) with good stirring. The appearing precipitate was filtered, washed with water, and dried at room temperature until constant weight. The isolated solid **2a** was purified by recrystallization in ethanol.

*2).Preparation of ethyl 4-amino-5-cyano-2-(methylthio)thiophene-3-carboxylate **2b***

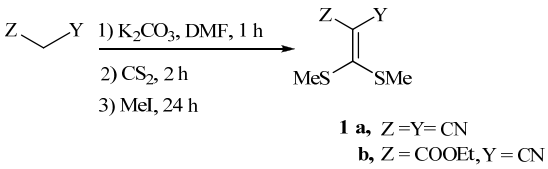
A 0.01 mol of ethyl 2-cyano-3,3-bis(methylthio) acrylate **1b** was dissolved in DMF (15 ml). The $Na_2S.9H_2O$ (0.01 mol) was added to solution and heated for 2 h at 70°C. Then chloroacetonitrile (0.02 mol) was added drop-wise to mixture at 70°C and heated at 70°C for 2 h. Finally potassium carbonate (0.02 mol) was added to mixture and stirred at 70°C for 90 min. Then, the mixture was poured onto water (100 ml) with good stirring. The appearing precipitate was filtered, washed with water, and dried at room temperature until constant weight. The isolated solid of **2b** was purified by recrystallization in ethanol.

D. *Determination of antibacterial activity*

The new synthesized compounds **2a** and **2b** were screened *in vitro* for their antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilise*) and Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumonia*) by agar disc-diffusion technique.[13] The bacteria were maintained on nutrient agar and antibacterial test was performed using Mueller-Hinton agar. A 50 mg of each compound was dissolved in 1 ml of DMSO to give solutions of 50 mg/ml. A sterile disc with 10 µl of each compound was applied on bacterial lawn. Streptomycin 10 µg was used as positive antibiotic control for antibacterial activity. After 18-24 h of incubation at 37°C, the diameter of inhibition zone was measured in mm. DMSO was used as negative control.

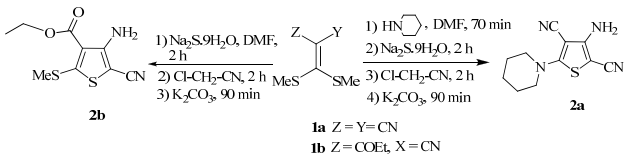
III. RESULTS AND DISCUSSION

We report herein the synthesis of new tetrasubstituted thiophenes **2a** and **2b** from ketene dithioacetals. The first step is preparing the ketene dithioacetals from condensation of activated methylene in malononitrile and ethyl cyanoacetate with carbon disulfide in the presence of a base potassium carbonate to obtain the intermediate of ketene S,S-acetal salts; their alkylation with methyl iodide leads to the formation of corresponding 2-[bis(methylthio)methylene]propanedinitrile **1a** and ethyl 2-cyano-3,3-bis(methylthio)acrylate **1b** (Scheme 1) in high yields as reported in literatures.[8],[14]



Scheme 1. Preparation of ketene dithioacetals **1a** and **1b**

These ketene dithioacetals **1a** and **1b** were used to synthesize new tetrasubstituted thiophenes **2a** and **2b** in accordance to the literature procedure [12a,b] as follows (Scheme 2). Compound **1a** was reacted with piperidine in DMF and heated at 70°C for 75 min to form intermediate ketene N,S-acetal **1a**[1] (Scheme 3); then Na_2S was added and heated at 70°C for 75 min to give ketene N,S-acetal salt **1a**[2]. After that, chloroacetonitrile was added and heated for 2 h to yield ketene N,S-acetal **1a**[3]. Finally, potassium carbonate was added in order to cyclize **1a**[3] into **2a** in high yield (73.70%). Also compounds **1b** was dissolved in DMF, and then Na_2S was added and heated at 70°C for 75 min to yield **1b**[1]. After that, chloroacetonitrile was added and heated for 2 h to produce **1b**[2]. Finally, potassium carbonate was added for cyclization to give **2b** in low yield (22.31%).

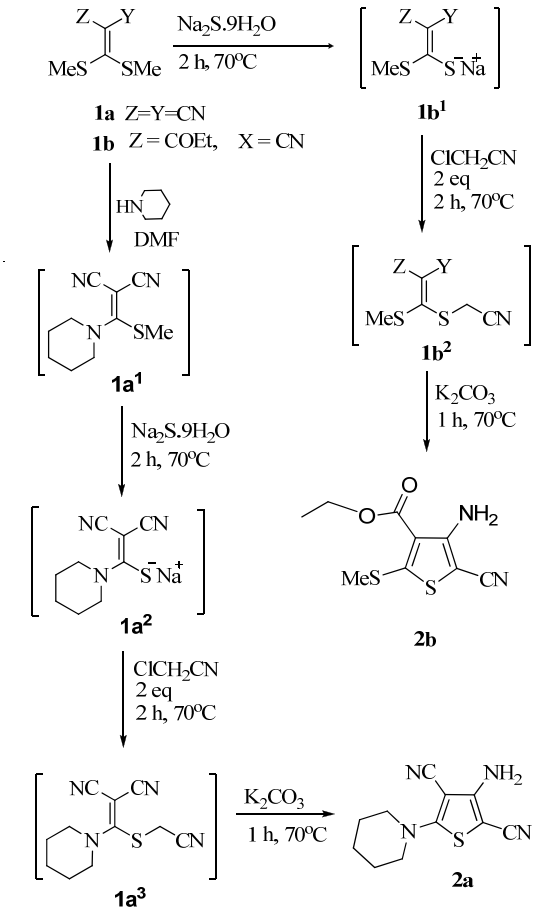


Scheme 2. Preparation of thiophenes **2a** and **2b**

In general, the probable mechanism for formation of thiophenes 2a and 2b involves the formation of the intermediate thiolates 1a2 and 1b1 upon addition of sodium sulfide into the pot and liberating one equivalent of methyl thiolate (Scheme 3). Two equivalent of chloroacetonitrile were added whereby the first was consumed by the methyl thiolate and the second by the intermediate thiolate salt that leads to the thioacetals 1a3 and 1b2. Finally, potassium carbonate was added to for cyclization of 2a and 2b.

The spectral data of the obtained products are in accordance with the proposed structures as explained below. The IR spectra of 2a and 2b showed bands at 3423-3201cm-1 for NH2 groups. The 1H-NMR spectra of compounds 2a and 2b showed broad singlets at δ 6.49, 6.79 ppm for the respective NH2 groups. The 13C-NMR spectra showed peak at 76.63 ppm for S-C-CN of compound 2a and peaks at 157.70 and 157.59 ppm for C-NH2 of respective compounds 2a and 2b. The elemental analysis of compounds 2a and 2b are also in accordance with the

proposed structures. Moreover, the mass spectra of each compound were compatible with their molecular formulas.



Scheme 3. The most likely intermediates formed during the formation of thiophenes **2a** and **2b**

A. Spectral Data

*1). 3-Amino-5-(1-piperidinyl)-2,4-thiophenedicarbonitrile (**2a**):* Brown crystals; Mp: 220-222°C; Yield: 73.70%; FT-IR (KBr, cm^{-1}): *v* 3396, 3338, 3244, 2934, 2857, 2174, 1649, 1600, 1549, 1514, 1393, 1136, 1008, 513; ^{1}H-NMR (500 MHz, DMSO): *δ* 1.58 (br, 6H, $3CH_2$), 3.67 (br, 4H, $2CH_2$-N), 6.49 (br, 2H, NH_2); ^{13}C-NMR (100 MHz, DMSO): *δ* 23.21(CH_2), 25.14 ($2CH_2$), 51.58 ($2CH_2$-N), 59.81 (C-CN), 76.63 (C-CN), 115.65, 116.21 (2CN), 157.70 (C-NH_2), 166.07 (S-C-N); DIMS (m/z): found 232.00 (calc. for $C_{11}H_{12}N_4S$ M^+ requires 232.30). Anal. calcd. for $C_{11}H_{12}N_4S$: C 56.87, H 5.21, N, 24.12%; found: C 56.25, H 5.23, N 24.28%.

*2). Ethyl 4-amino-5-cyano-2-(methylthio)thiophene-3-carboxylate (**2b**):* Brown pale; Mp: 172-173°C; Yield: 22.31%; FT-IR (KBr, cm^{-1}): ν 3423, 3329, 3201, 2974, 2925, 2187, 1684, 1614, 1533, 1455, 1310, 1208, 1024, 783, 511; ^{1}H-NMR (500 MHz, DMSO): δ 1.28-1.31(t, 3H, CH_3), 2.55 (s, 3H, SCH_3), 4.27-4.29 (q, 2H, OCH_2), 6.79 (s, 2H, NH_2); ^{13}C NMR (100 MHz, DMSO): δ 14.51(CH_3), 17.08 (SCH_3), 61.53 (CH_2-O), 73.77 (C-CN), 113.02 (CN), 115.27 (C-CO), 157.59 (C-NH_2), 161.22 (S-C-SCH_3), 162.76 (C=O); DIMS (m/z): found 242.20 (calc. for $C_9H_{10}N_2O_2S_2$ M^+ requires 242.32). Anal. calcd. for $C_9H_{10}N_2O_2S_2$: C 44.61, H 4.16, N 11.56%; found: C 44.89, H 4.36, N 11.60%.

B. Antibacterial activity of the compounds

The results for antibacterial activity are depicted in Table 1. It is revealed that compounds **2a** and **2b** showed limited antibacterial activity in all the bacteria tested with **2a** exhibited bigger inhibition zones compared to **2b**. DMSO showed no inhibition zone.

TABLE I
INHIBITION ZONES (MM) AS A CRITERION OF ANTIBACTERIAL ACTIVITY OF THE NEWLY SYNTHESIZED COMPOUNDS.

Bacteria			**Inhibition zones (mm)**	
	2a	**2b**	**DMSO**	**Streptomycin**
B. subtilis	-	-	-	25
S. aureus	9	7	-	13
E. coli	7	-	-	25
K. pneumoniae	-	-	-	25

IV. CONCLUSION

In summary, we have successfully synthesized tetrasubstituted thiophenes from ketene dithioacetals using one-pot four- or three -step procedures under moderate conditions. These compounds can be used as intermediates in synthesizing various thieno-fused bicyclic compounds. The synthesized compounds showed moderate antibacterial activity.

ACKNOWLEDGMENT

We would like to thank Ministry of Higher Education Malaysia and Universiti Kebangsaan Malaysia for research grant of UKM-GGPM-KPB-098-2010. A scholarship from the Libyan Government to Wedad M. Al-Adiwish is greatly appreciated.

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