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, 1H-NMR, 13C-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. 1H- and 13C-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]propanedinitrile (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°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°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°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 Na₂S.9H₂O (0.01 mol) was added and heated for 2 h at 70°C. Chloroacetonitrile (0.02 mol) was added drop-wise 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 Na₂S.9H₂O (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 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 subtilis) and Gram-negative bacteria (Escherichia coli and Klebsiella pneumonia) by agar disc-diffusion technique.[15] 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 and 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]

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'. Then Na₂S was added and heated at 70°C for 75 min to give ketene N,S-acetal 1a". After that, chloroacetonitrile was added and heated for 2 h to yield ketene N,S-acetal 1a'. Finally, potassium carbonate was added in order to cyclize 1a' into 2a in high yield (73.70%). Also compounds 1b was dissolved in DMF, and then Na₂S was added and heated at 70°C for 75 min to yield 1b'. After that, chloroacetonitrile was added and heated for 2 h to produce 1b'. Finally, potassium carbonate was added for cyclization to give 2b in low yield (22.31%).

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 3423-3201 cm⁻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
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): ν 3396, 3338, 2934, 2934, 2857, 2174, 1649, 1600, 1549, 1514, 1393, 1136, 1008, 513; ¹H-NMR (500 MHz, DMSO): δ 1.58 (br, 6H, 3CH₂), 3.67 (br, 4H, 2CH₂-N), 5.99 (s, 2H, NH₂); ¹³C-NMR (100 MHz, DMSO): δ 23.21(CH₂), 25.14 (2CH₂), 51.58 (2CH₂-N), 76.63 (C-CN), 115.65, 116.21 (2CN), 157.70 (C-NH₂), 166.07 (S-C-N); DIMS (m/z): found 232.00 (calc. for C₁₁H₁₂N₄S M⁺ requires 232.32). Anal. calcd. for C₉H₁₀N₂O₂S₂: C 44.61, H 4.16, N 11.56%; found: C 44.89, H 4.36, N 11.60%.

2. Ethyl 4-amino-5-cyano-2-(methylthio)thiophene-3-carboxylate (2b): Brown pale; Mp: 172-173°C; Yield: 24.28%. FT-IR (KBr, cm⁻¹): ν 3423, 3329, 2974, 2925, 1871, 1684, 1614, 1533, 1455, 1310, 1204, 783, 511; ¹H-NMR (500 MHz, DMSO): δ 1.28-1.31(t, 3H, CH₃), 2.55 (s, 3H, SCH₃), 4.72-4.79 (q, 2H, OCH₂), 6.79 (s, 2H, NH₂); ¹³C NMR (100 MHz, DMSO): δ 14.51(CH₃), 17.08 (SCH₃), 61.53 (CH₂-O), 73.77 (C-CN), 113.02 (CN), 115.27 (C-CO), 157.59 (C-NH₂), 161.22 (S-C-SCH₂), 162.76 (C=O); DIMS (m/z): found 242.20 (calc. for C₉H₁₀N₂O₂S₂ M⁺ requires 242.32). Anal. calcd. for C₉H₁₂N₂O₂S₂: 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            | 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 dithioacetalts 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.

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REFERENCES

[1] D. J. Brown, “Pyrimidines and Their Benzo Derivatives, in Comprehensive Heterocyclic Chemistry,” (Ed. A. R. Katritzky and C. W. Rees), Pergamon Press, Oxford, Vol. 3, pp. 443, 1984.
[2] B. Roth and C. Cheng, “Diaminopyrimidines, in Progress in Medicinal Chemistry,” (Eds. G. B. Ellis and G. E. West), Elsevier Biomedical Press, New York, Vol. 19, pp. 267, 1982.
[3] M. S. A. E.-A. El-Gaby, S. G. Abdel-Hamide, M. M. Ghorab and S. M. El-Sayed, “Synthesis and antitumor activity in vitro of some new pyrimidines,” Acta Pharm., vol. 49, pp. 149–158, 1999.
[4] C. R. Petrie, H. B. Cottam, P. A. McKernan, R. K. Robins and G. R. Revankar, “Synthesis and biological activity of 6-azacadequomycin and certain 2,4,6-trisubstituted-pyrazolo[3,4-d]-pyrimidine ribonucleosides,” J. Med. Chem., vol. 28, pp. 1010–1016, 1985.
[5] M. N. Nasr and M. M. Gininh, “Pyrido[2,3-d]pyrimidines and pyrimido[5',4';5,6]-pyrido[2,3-d]pyrimidines as new antiviral agents: Synthesis and biological activity,” Arch. Pharm., vol. 335, pp. 289–295, 2002.
[6] J. Campos, E. Anon, M. Carmen Malo, and M. A. Rodriguez, “Aversatile synthesis of pyrrolo-, Furo- and Thione pyridines via photocyclization of 3-amino-2-alkene imines in an acid medium,” Tetrahedron, vol. 55, pp. 14079, 1999.
[7] B. Abdel-Fattah, M. M. Kandeel, M. Abdel-Hakeem and Z. M. Fahmy, “Synthesis of Certain Fused Thienopyrimidines of Biological Interest,” Journal of the Chinese Chemical Society, vol. 53, pp. 403-412, 2006.
[8] G. Sommen, A. Comel and G. Kirsch, “Preparation of thieno[2,3-b]pyrroles starting from ketene-N,S-acetals,” *Tetrahedron*, vol. 59, pp. 1557–1564, 2003.

[9] A. A. Shimkin, A. K. Mailian, V. Z. Shirinian, and M. M. Krayushkin, “Synthesis of 1H- and 3H-[1]Benzothieno[3,2-b]pyrroles,” *Synthesis*, vol. 17, pp. 2706-2710, 2007.

[10] M. M. El-Saghiera, F. S. Matoughb, M. F. Farhatb, N. A. Salehb, K. M. Kreddane, S. O. El-Tierb, and H. B. Hussien, “Synthesis and Biological Evaluation of Some New Thienopyridine and Thiopyrimidine Derivatives,” *Jordan Journal of Chemistry*, Vol. 3 No. 3, pp. 223-232, 2008.

[11] A.M. M. El-Saghier, “A Simple Synthesis of Some New Thienopyridine and Thiopyrimidine Derivatives,” *Molecules*, 7, 756-766, 2002.

[12] D. Thomae, E. Perspicace, Z. Xu, D. Henryon, S. Schneider, S. Hesse, G. Kirsch, and P. Seck, “One-pot synthesis of new 2,4,5-trisubstituted 1,3-thiazoles and 1,3-selenazoles,” *Tetrahedron*, vol. 65, pp. 2982, 2009; (a). D. Thomae, E. Perspicace, D. Henryon, Z. Xu, S. Schneider, “One-pot synthesis of new tetrasubstituted thiophenes and selenophenes,” *Tetrahedron*, vol. 65, pp. 10453–10458, 2009.

[13] A.W. Bauer, W.M.M. Kirby, J.C. Serris, and M. Turek, “Antibiotic susceptibility testing by a standardized single disk method,” *American Journal of Clinical Pathology.*, vol. 45, pp. 493-496, 1966.

[14] G. Sommen, A. Comel, and G. Kirsch, “An improved method for the synthesis of aminothiophenes precursors of thieno[2,3-b]pyrrole,” *Tetrahedron Lett.*, vol. 43, pp. 257, 2002.