European Journal of Chemistry

Journal webpage: www.eurjchem.com

One-pot synthesis and antimicrobial activity of new 4,6-disubstituted-3,4-dihydropyrimidine-2(1*H*)-thiones

Mahmoud Al-Refai [1,*], Mohammad Ibrahim [1], Abdullah Al-Fawwaz [2] and Armin Geyer [3]

[1] *Department of Chemistry, Faculty of Science, Al Al-Bayt University, Al-Mafraq, 25113, Jordan*
[2] *Department of Biological Sciences, Faculty of Science, Al Al-Bayt University, Al-Mafraq, 25113, Jordan*
[3] *Faculty of Chemistry, Philipps University Marburg, Hans-Meerwein-Straße 4, Marburg, 35032, Germany*

** Corresponding author at: Department of Chemistry, Faculty of Science, Al Al-Bayt University, Al-Mafraq, 25113, Jordan. Tel.: +962.2.6297000/2141. Fax: +962.2.6297021. E-mail address: mahmoud_alrefai@aabu.edu.jo (M. Al-Refai).*

ARTICLE INFORMATION

DOI: 10.5155/eurjchem.8.1.96-100.1543

Received: 12 January 2017
Received in revised form: 05 February 2017
Accepted: 08 February 2017
Published online: 31 March 2017
Printed: 31 March 2017

KEYWORDS

Thione
Thiourea
Pyrimidine
Thiophene
Pyrimidine-2-thione
Antimicrobial activity

ABSTRACT

A series of 3,4-dihydropyrimidine-2(1*H*)-thiones (3a-i) were synthesized in moderate yields *via* a one-pot reaction of 3-acetyl-2,5-diclorothiophene (1), aryl aldehydes (2a-i) and thiourea in methanolic solution of potassium hydroxide under reflux conditions. All newly synthesized compounds were characterized by extensive NMR analysis, including ^{1}D NMR experiments (^{1}H and ^{13}C) and 2D NMR experiments (COSY, HMBC and HSQC), as well as ESI-MS and HRESI-MS data. The antimicrobial activity of all new compounds (3a-f) was tested against bacteria and fungi. Thione derivative (3c) only showed activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Aspergillus niger*.

Cite this: *Eur. J. Chem.* **2017**, *8(1)*, 96-100

1. Introduction

Heterocyclic compounds containing thiones have shown a wide range of pharmacological activities, such as antimicrobial [1-4], antidepressant [5], antitubercular [6], antihistamines [7], anti-HIV [8], and others [9-12]. Pyrimidine rings, on the other hand, are important substructures in natural products like nucleic acids and vitamin B1 [13,14]. Different pyrimidines have shown a pharmaceutical importance because of their biological activity such as antifungal [15], antiviral [16-18], anti-inflammatory [19], antileishmanial [20] and anti-cancer [20,21].

In the view of these facts, we report the synthesis, characterization, and antimicrobial activity of new diaryl-substituted pyrimidine-2-thiones *via* a one-pot reaction (Scheme 1).

2. Experimental

2.1. Materials

Thiourea was purchased from Aldrich. 3-Acetyl-2,5-dichlorothiophene (**1**) have been prepared according to literature procedure [22,23]. Solvents were dried and distilled according to standard methods.

2.2. Instrumentation

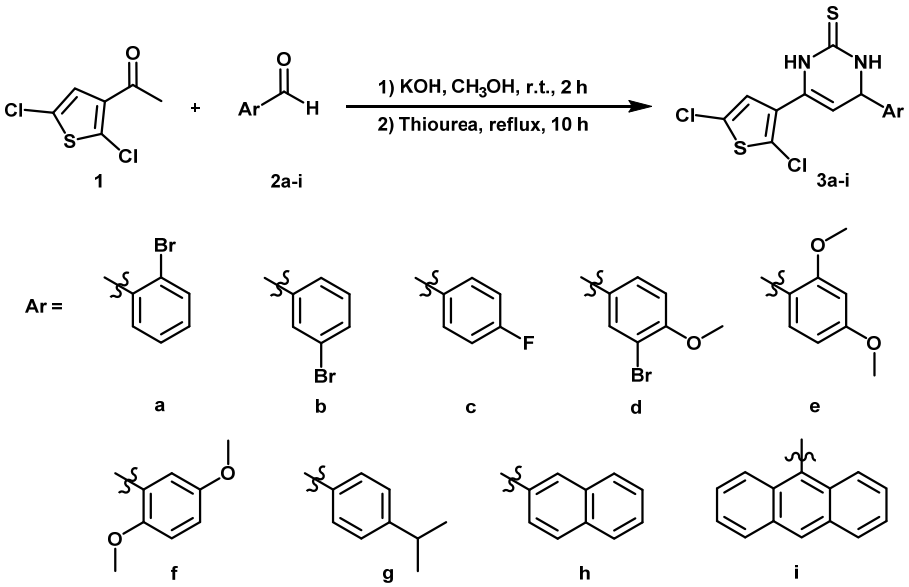
^{1}H and ^{13}C NMR spectra were recorded at 300 K on Bruker spectrometers (300-600 MHz). Chemical shifts δ are given in parts per million (ppm) and were determined from the center of the respective coupling pattern (s: singlet, d: doublet, dd: doublet of doublet, t: triplet). The solvent signals were used as internal standard (DMSO-d_6: δ (1H) 2.50 ppm, δ (^{13}C) 39.52 ppm). ESI-HMRS measurements were performed on a LTQ-FT mass spectrometer (Thermo Fisher Scientific).

Thin layer chromatography (TLC) monitoring of the reaction was carried out using analytical TLC plates coated with silica gel (60F_{254}, Merck). Preparative column chromatography was carried out at room temperature with compressed air using flash silica gel (particle size 40-60 mm, Merck), with the mobile phase being $CHCl_3$:pentane mixture.

2.3. Synthesis

2.3.1. General procedure for the synthesis of thiones (3a-i)

ISSN 2153-2249 (Print) / ISSN 2153-2257 (Online)
http://dx.doi.org/10.5155/eurjchem.8.1.96-100.1543



Scheme 1

3-Acetyl-2,5-dichlorothiophene (**1**) was added to the aromatic aldehydes (**2a-i**) in methanolic solution of potassium hydroxide (0.01 mol, in 50 mL). The mixture was stirred for 2 hours at room temperature, and then thiourea (0.03 mol) was added and refluxed for about 10 hours. The reaction mixture was cooled, poured into ice water (150 mL), and then neutralized with hydrochloric acid. The obtained solid was filtered off, air-dried, and purified using column chromatography (Scheme 1).

4-(2-Bromophenyl)-6-(2,5-dichlorothiophen-3-yl)-3,4-dihydropyrimidine-2(1H)-thione (**3a**): Color: Pale yellow. Yield: 39%. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 5.33 (d, *J* = 4.7 Hz, 1H, H-5), 5.41 (dd, *J* = 4.7, 2.7 Hz, 1H, H-4), 7.13 (s, 1H, H-4'), 7.26 (dt, *J* = 8.4, 1.6 Hz, 1H, H-4"), 7.40 (dd, *J* = 9.3, 1.6 Hz, 1H, H-6"), 7.49 (t, *J* = 7.7 Hz, 1H, H-5"), 7.61 (d, *J* = 8.4 Hz, 1H, H-3"), 9.01 (bs, 1H, NH-3), 9.95 (bs, 1H, NH-1). ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 175.5 (CS-2), 142.1 (C_q-1"), 132.8 (CH-3"), 131.4 (Cq-6), 129.5 (CH-4"), 128.5 (CH-5"), 128.3 (CH-6"), 127.4 (CH-4'), 127.3 (Cq-3'), 124.9 (Cq-5'), 123.7 (Cq-2'), 120.0 (Cq-2"), 102.4 (CH-5), 54.6 (CH-4). MS (+ESI, *m/z* (%)): 443 ([M+Na+2]$^+$, 30), 863 ([2M+Na]$^+$, 13), 1283 ([3M+Na]$^+$, 26). HRMS (+ESI, *m/z*): 440.8657 [M+Na]$^+$, 442.8629 [M+Na+2]$^+$, 444.8605 [M+Na+4]$^+$, 446.8578 [M+Na+6]$^+$, (calcd. for $C_{14}H_9BrCl_2N_2S_2Na$, 440.8660).

4-(3-Bromophenyl)-6-(2,5-dichlorothiophen-3-yl)-3,4-dihydropyrimidine-2(1H)-thione (**3b**): Color: Pale yellow. Yield: 53%. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 5.15 (dd, *J* = 4.9, 3.1 Hz, 1H, H-4), 5.32 (d, *J* = 4.9 Hz, 1H, H-5), 7.22 (s, 1H, H-4'), 7.38 (m, 2H, H-5",6"), 7.53 (m, 2H, H-2",4"), 9.13 (bs, 1H, NH-3), 9.93 (bs, 1H, NH-1). ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 175.2 (CS-2), 146.6 (C_q-1"), 132.3 (Cq-6), 131.5 (CH-5"), 131.0 (CH-2"), 129.8 (CH-4"), 128.3 (CH-4'), 127.9 (Cq-3'), 126.1 (CH-6"), 125.4 (Cq-5'), 124.4 (Cq-2'), 122.4 (Cq-3"), 104.5 (CH-5), 54.4 (CH-4). MS (-ESI, *m/z* (%)): 419 ([M-H]$^-$, 100), 421 ([M-H+2]$^-$, 49), 423 ([M-H+4]$^-$, 9). HRMS (-ESI, *m/z*): 418.8663 [M-H]$^-$, 420.8632 [M-H+2]$^-$, 422.8598 [M-H+4]$^-$, (calcd. for $C_{14}H_8BrCl_2N_2S_2$, 418.8840).

6-(2,5-Dichlorothiophen-3-yl)-4-(4-fluorophenyl)-3,4-dihydropyrimidine-2(1H)-thione (**3c**): Color: Pale yellow. Yield: 61%. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 5.14 (dd, *J* = 4.8, 3.0 Hz, 1H, H-4), 5.29 (d, *J* = 4.8 Hz, 1H, H-5), 7.21 (s, 1H, H-4'), 7.25 (t, *J* = 9.3 Hz, 2H, H-3",5"), 7.40 (dd, *J* = 8.9, 5.8 Hz, 2H, H-2",6"), 9.10 (bs, 1H, NH-3), 9.88 (bs, 1H, NH-1). ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 175.0 (CS-2), 162.9, 161.3 (d, J_{CF} = 251.20 Hz, Cq-4"), 140.3 (Cq-1"), 132.3 (Cq-6), 129.2, 129.1 (d, J_{CF} = 8.61 Hz, CH-2",6"), 128.3 (CH-4'), 127.7 (Cq-3'), 125.3 (Cq-5'), 124.3 (Cq-2'), 116.0, 115.8 (d, J_{CF} = 21.65 Hz, CH-3",5"), 104.9 (CH-5), 54.3 (CH-4). HRMS (+ESI, *m/z*): 380.9461 [M+Na]$^+$, 382.9433 [M+Na+2]$^+$, 384.2077 [M+Na+4]$^+$, (calcd. for $C_{14}H_9Cl_2FN_2S_2Na$, 380.9460).

4-(3-Bromo-4-methoxyphenyl)-6-(2, 5-dichlorothiophen-3-yl)-3,4-dihydropyrimidine-2(1H)-thione (**3d**): Color: Pale yellow. Yield: 64%. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 3.86 (s, 3H, OCH_3-4"), 5.09 (dd, *J* = 4.7, 2.6 Hz, 1H, H-4), 5.29 (d, *J* = 4.5 Hz, 1H, H-5), 7.17 (d, *J* = 8.6 Hz, 1H, H-5"), 7.22 (s,1H, H-4'), 7.35 (dd, *J* = 8.5, 2.2 Hz, 1H, H-6"), 7.56 (d, *J* = 2.2 Hz, 1H, H-2"), 9.08 (bs, 1H, NH-3), 9.48 (bs, 1H, NH-1). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 175.0 (CS-2), 155.5 (C_q-4"), 137.7 (Cq-1"), 132.4 (Cq-6), 131.7 (CH-2"), 127.8 (CH-4'), 127.8 (CH-6"), 127.6 (Cq-3'), 125.4 (Cq-5'), 124.3 (Cq-2'), 113.5 (CH-5"), 110.9 (Cq-3"), 104.7 (CH-5), 56.8 (4"-OCH_3), 53.9 (CH-4). MS (+ESI, *m/z* (%)): 451 ([M+H]$^+$, 100), 453 ([M+H+2]$^+$, 49), 455 ([M+H+4]$^+$, 9). HRMS (+ESI, *m/z*): 450.8922 [M+H]$^+$, 452.8889 [M+H+2]$^+$, 454.8854 [M+H+4]$^+$, (calcd. for $C_{15}H_{12}BrCl_2N_2OS_2$, 450.8924).

6-(2,5-Dichlorothiophen-3-yl)-4-(2,4-dimethoxyphenyl)-3,4-dihydropyrimidine-2(1H)-thione (**3e**): Color: Pale yellow. Yield: 58%. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 3.78 (s, 3H, OCH_3-4"), 3.81 (s, 3H, OCH_3-2"), 5.22 (d, *J* = 4.8 Hz, 1H, H-5), 5.27 (dd, *J* = 4.8, 2.4 Hz, 1H, H-4), 6.59 (d, *J* = 3.1 Hz, 1H, H-3"), 6.60 (d, *J* = 9.0, 1H, H-5"), 7.13 (d, *J* = 9.0 Hz, 1H, H-6"), 7.17 (s, 1H, H-4'), 8.73 (bs, 1H, NH-3), 9.75 (bs, 1H, NH-1). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 175.7 (CS-2), 160.6 (C_q-4"), 157.0 (Cq-2"), 132.4 (Cq-6), 128.2 (CH-4'), 128.1 (CH-6"), 127.3 (Cq-3'), 125.3 (Cq-5'), 124.2 (Cq-1"), 123.91 (Cq-2'), 105.4 (CH-5"), 104.7 (CH-5), 99.0 (CH-3"), 56.1 (OCH_3-2"), 55.8 (OCH_3-4") 49.8 (CH-4). HRMS (-ESI, *m/z*): 398.9802 [M-H]$^-$, 400.9771 [M-H+2]$^-$, 402.8047 [M-H+4]$^-$, (calcd. for $C_{16}H_{13}Cl_2N_2O_2S_2$, 398.9801).

6-(2,5-Dichlorothiophen-3-yl)-4-(2,5-dimethoxyphenyl)-3,4-dihydropyrimidine-2(1H)-thione (**3f**): Color: Pale yellow. Yield: 62%. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 3.71 (s, 3H, OCH_3-5"), 3.77 (s, 3H, OCH_3-2"), 5.29 (d, *J* = 2.2 Hz, 2H, H-4,5), 6.79 (d, 1H, *J* = 3.1 Hz, H-6"), 6.86 (dd, *J* = 8.8, 3.1Hz, 1H, H-4"), 6.96

Table 1. Antimicrobial activity of thione derivatives and the standard antibiotics (**3a-f**) *.

Chemical compounds	Bacterial strains			Fungal strains	
	Escherichia coli	*Staphylococcus aureus*	*Bacillus subtilis*	*Aspergillus niger*	*Penicillium* sp
3a	--	--	--	--	--
3b	--	--	--	--	--
3c	-	+	++	-	-
3d	--	-	-	--	--
3e	--	--	-	--	--
3f	--	--	--	--	--
Cephalaxin.H_2O (12.5 mg/mL)	--	++	+++	NT	NT
Amphotericin B (20 µg/mL)	NT	NT	NT	--	-

* 0-10 mm (--), 11-15 mm (-), 16-20 mm (+), 21-25 mm (++), over 25 mm (+++), NT: Not Tested.

(d, *J* = 9.0 Hz, 1H, H-3"), 7.16 (s, 1H, H-4'), 8.82 (bs, 1H, NH-3), 9.83 (bs, 1H, NH-1). ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 176.1 (CS-2), 153.9 (C_q-5"), 149.9 (C_q-2"), 132.9 (C_q-1"), 132.3 (C_q-6), 128.1 (CH-4'), 127.5 (C_q-3'), 125.3 (Cq-5'), 124.0 (Cq-2'), 114.0 (CH-6"), 112.7 (CH-4"), 112.6 (CH-3"), 104.4 (CH-5), 56.5 (OCH_3-2"), 55.9 (OCH_3-5"), 50.3 (CH-4). HRMS (-ESI, *m/z*): 398.9803 [M-H]$^-$, 400.9773 [M-H+2]$^-$, 402.9743 [M-H+4]$^-$, (calcd. for $C_{16}H_{13}Cl_2N_2O_2S_2$, 398.9801).

6-(2,5-Dichlorothiophen-3-yl)-4-(4-isopropylphenyl)-3,4-dihydropyrimidine-2(1H)-thione (**3g**): Color: Pale yellow. Yield: 54%. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 1.19 (d, *J* = 6.6 Hz, 6H, CH(CH_3)$_2$-4"), 2.88 (sp, *J* = 6.9 Hz, 1H, C*H*(CH_3)$_2$-4"), 5.07 (dd, *J* = 4.8, 2.7 Hz, 1H, H-4), 5.27 (d, *J* = 4.8 Hz, 1H, H-5), 7.18 (s, 1H, H-4'), 7.26 (d, *J* = 8.3 Hz, 2H, H-2",6"), 7.28 (d, *J* = 8.3 Hz, 2H, H-3",5"), 8.98 (bs, 1H, NH-3), 9.74 (bs, 1H, NH-1). ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 174.4 (CS-2), 147.9 (CH-4"), 141.0 (Cq-1"), 131.8 (Cq-6), 127.6 (CH-4'), 127.0 (Cq-3'), 126.6 (CH-2",6"), 126.4 (CH-3",5"), 124.8 (Cq-5'), 123.6 (Cq-2'), 104.5 (CH-5), 54.4 (CH-4), 33.1 (<u>C</u>H(CH_3)$_2$-4"), 23.8 (CH(<u>C</u>H_3)$_2$-4"). MS (+ESI, *m/z* (%)): 405 ([M+Na]$^+$, 48), 407 ([M+Na+2]$^+$, 37). 409 ([M+Na+4]$^+$,18). MS (-ESI, *m/z* (%)): 381 ([M-H]$^-$, 100), 383 ([M-H+2]$^-$, 71), 385 ([M-H+4]$^-$, 7). HRMS (-ESI, *m/z*): 381.0059 [M-H]$^-$, 383.0021 [M-H+2]$^-$, 384.9999 [M-H+4]$^-$ (calcd. for $C_{17}H_{15}Cl_2N_2S_2$, 381.0059). HRMS (+ESI, *m/z*): 405.0008 [M+Na]$^+$, 406.9975 [M+Na+2]$^+$, 408.9970 [M+Na+4]$^+$, (calcd. for $C_{17}H_{16}Cl_2N_2S_2Na$, 405.0024).

6-(2,5-Dichlorothiophen-3-yl)-4-(naphthalen-1-yl)-3,4-dihydropyrimidine-2(1H)-thione (**3h**): Color: Pale yellow. Yield: 62%. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 5.42 (d, *J* = 4.8 Hz, 1H, H-5), 5.96 (dd, *J* = 4.6, 2.8 Hz, 1H, H-4), 7.17 (s, 1H, H-4'), 7.52 (d, *J* = 7.3 Hz, 1H, H-10"), 7.59 (m, 3H, H-2",5",6"), 7.92 (d, *J* = 8.3 Hz, 1H, H-9"), 7.99 (dd, *J* = 7.7, 1.5 Hz, 1H, H-7"), 8.25 (d, *J* = 8.3 Hz, 1H, 4"), 9.10 (bs, 1H, NH-3), 9.94 (bs, 1H, NH-1). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 175.8 (CS-2), 139.5 (Cq-1"), 134.0 (Cq-8"), 132.3 (Cq-6), 129.7 (Cq-3"), 129.2 (CH-7"), 128.6 (CH-9"), 128.1 (CH-4'), 127.4 (Cq-3'), 127.0 (CH-5"), 126.4 (CH-5"), 126.3 (CH-2"), 125.4 (Cq-5'), 124.8 (CH-10"), 124.1 (Cq-2'), 123.7 (CH-4"), 105.2 (CH-5), 52.5 (CH-4). HRMS (+ESI, *m/z*): 390.9890 [M+H]$^+$, 392.9861 [M+H+2]$^+$, 394.9833 [M+H+4]$^+$, (calcd. for $C_{18}H_{13}Cl_2N_2S_2$, 390.9892).

4-(Anthracen-9-yl)-6-(2,5-dichlorothiophen-3-yl)-3,4-dihydropyrimidine-2(1H)-thione (**3i**): Color: Pale yellow. Yield: 41%. ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 5.20 (d, *J* = 2.9 Hz, 1H, H-5), 6.96 (d, *J* = 2.9 Hz, 1H, H-4), 7.25 (s, 1H, H-4'), 7.57 (m, 4H, H-4",5",11",12"), 8.60 (d, *J* = 8.8 Hz, 2H, H-3",13"), 8.16 (d, *J* = 8.3 Hz, 2H, H-6",10"), 8.68 (s, 1H, 8"), 9.09 (bs, 1H, NH-3), 10.01 (bs, 1H, NH-1). ^{13}C NMR (120 MHz, DMSO-d_6, δ, ppm): 175.2 (CS-2), 133.0 (Cq-1"), 132.5 (Cq-6), 131.5 (Cq-7",9"), 130.2 (Cq-2",14"), 129.7 (CH-6", 10"), 129.1 (CH-8"), 128.2 (CH-4'), 127.1 (Cq-3'), 126.7 (CH-4",12"), 125.5 (CH-5",11"), 125.4 (Cq-5'), 124.7 (CH-3",13"), 124.3 (Cq-2'), 105.6 (CH-5), 51.1 (CH-4). MS (-ESI, *m/z* (%)): 439 ([M-H]$^-$, 39), 441 ([M-H+2]$^-$, 13). HRMS (-ESI, *m/z*): 438.9903 [M-H]$^-$, 440.9873 [M-H+2]$^-$, (calcd. for $C_{22}H_{13}Cl_2N_2S_2$, 438.9903).

2.4. Biological activity

The *in vitro* antimicrobial activity of pyrimidine-2-thione derivatives (**3a-f**) was determined by the wells diffusion method [24-26] (Table 1). For these assays, cultures of the following microorganisms were used: two Gram-positive (*Staphylococcus aureus* (ATCC 6538) and *Bacillus subtilis* (ATCC 6633)), one Gram-negative (*Escherichia coli* (ATCC 25922)) bacteria, and fungal strains (*Aspergillus niger* and *Penicillium* sp. which were isolated from local environments).

Bacterial cultures were maintained on nutrient agar medium, while fungal cultures were maintained on potato dextrose agar (PDA). Suspensions of the tested microorganisms were spread on the solid nutrient agar medium and PDA plates. 25±2 mg of each thione derivatives (**3a-f**) were dissolved in 2 mL of dimethyl sulfoxide, then 100 µL of each chemical compound (12.5 mg/mL) were added to agar wells. A positive control containing microbial culture without chemical compound and a negative control containing only the medium were performed as well. At the end of the incubation time (24 h at 37 °C for bacteria and 25 °C for fungi), positive antibacterial and antifungal activities were established by the presence of a measurable inhibition zone and recorded in its width (mm) which includes the well diameter. Each test was performed in three replicates. Cephalaxin.H_2O at concentration 12.5 µg/mL was used as standard against bacteria while Amphotericin B at concentration 20 µg/mL was used as standard antifungal agents.

3. Results and discussion

3.1. Preparation and characterization of 3,4-dihydropyrimidine-2(1H)-thiones

The one-pot synthesis of 4,6-disubstituted-3,4-dihydropyrimidine-2(1*H*)-thiones (**3a-i**) was carried out by the reaction of 3-acetyl-2,5-dichlorothiophene (**1**) with aromatic aldehydes (**2a-i**) in methanolic solution of potassium hydroxide at room temperature. Thiourea was then added under reflux to give the 3,4-dihydropyrimidine-2(1*H*)-thiones (**3a-i**) in moderate yields (Scheme 1).

The newly synthesized pyrimidine 2-thiones (**3a-i**) were characterized by ESIMS, HRMS, 1D and 2D NMR techniques. The complete data are present in the experimental section. The ^{1}H NMR spectra showed for each compound two exchangeable proton signals in the range of δ 9.48-10.01 ppm, and δ 8.73-9.13 ppm corresponding to NH-1 and NH-3, respecttively. The two pyrimidine protons H-4 and H-5 were resonated in the range of δ 5.07-5.43 ppm except in case of the anthracene substituent, where the H-4 was appeared at δ 6.96 ppm. Furthermore, the thiophene proton H-4' was revealed in the range of δ 7.13-7.25 ppm.

In the ^{13}C NMR spectra of compounds (**3a-i**), the C=S carbons were resonated downfield between δ 174.4-176.1 ppm. The two signals in the range of δ 102.4-105.6 ppm and δ 49.8-54.6 ppm were assigned to C-5 and C-4 of the pyrimidine nucleus, respectively. The complete assignments of ^{1}H and ^{13}C NMR resonances were confirmed by H-H COSY, HSQC, and HMBC experiments. As an example, Figure 1 shows the HSQC spectrum of compound **3f**.

The ESI-MS spectra revealed the right molecular ion peaks characteristics of isotopic chlorine clusters confirming the presence of two chlorine atoms.

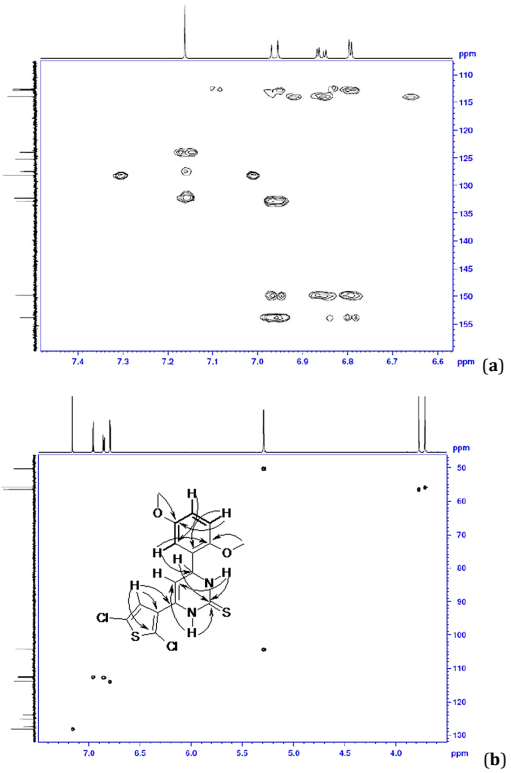


Figure 1. (a) HMBC and (b) HSQC, H,H COSY (—), HMBC (→) correlations (DMSO-d_6, 600 MHz) of 6-(2,5-dichlorothiophen-3-yl)-4-(2,5-dimethoxyphenyl)-3,4-dihydropyrimidine-2(1*H*)-thione (**3f**).

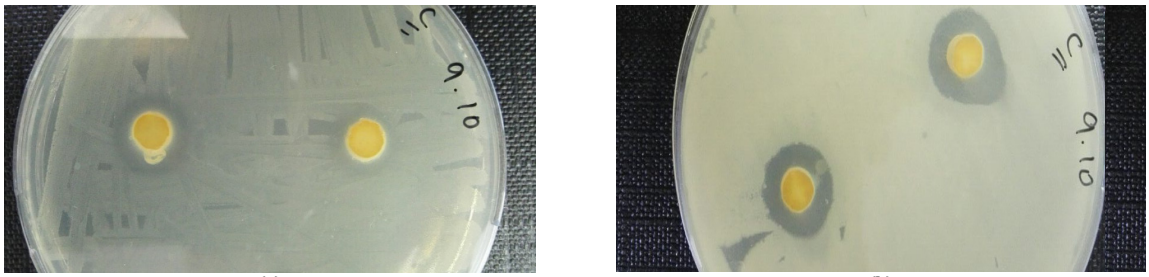


Figure 2. Zone of Inhibition obtained by the wells diffusion method for compound **3c**, against (**a**) *Staphylococcus aureus* and (**b**) *Bacillus subtilis*.

The molecular formulas were determined by measuring the HRESI-MS spectra, which came in good agreement with the calculated values.

3.2. Biological Screening

In the present study, antimicrobial activity of new thione compounds (**3a-f**) against one Gram-negative bacteria (*Escherichia coli*), two Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*); and two fungal strains isolated from local environments (*Aspergillus niger*, and *Penicillium* sp.) were tested using diffusion well technique [24-26]. The antimicrobial activity of all tested thiones (**3a-f**) and standards antimicrobial agents (Cephalaxin.H_2O and Amphotericin B) were given in Table 1. The results showed that there was a difference in antimicrobial activity between different thione compounds. All thiones did not showed any activity against all tested species, except thione derivative (**3c**), which showed a good activity against Gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), and weak activity against Gram negative bacteria (*Escherichia coli*) and the two fungal strains (*Aspergillus niger* and *Penicillium* sp), Figure 2.

4. Conclusions

In the present work, new pyrimidine-2-thione derivatives have been successfully synthesized and characterized using different spectroscopic techniques. Most of the newly synthesized compounds tested against bacteria and fungi. Except thione derivative (**3c**), all of the tested compounds showed no activity against selected microorganisms.

Acknowledgements

We are grateful to Al Al-Bayt University (Mafraq, Jordan) and the DFG (Germany) for financial support.

References

[1]. Eweiss, N. F.; Bahajaj, A. A.; Elsherbini, E. A. *J. Heterocycl. Chem.* **1986**, *23*, 1451-1458.

[2]. Hussein, M. A.; El-Shorbagi, A. N.; Khallil, A. R. *Arch. Pharm.* **2001**, *334*, 305-308.
[3]. Aboul-Fadl, T.; Hussein, M. A.; El-Shorbagi, A. N.; Khallil, A. R. *Arch. Pharm.* **2002**, *335*, 438-442.
[4]. El-Emam, A. A.; Al-Deeb, O. A.; Al-Omar, M.; Lehmann, J. *Bioorg. Med. Chem.* **2004**, *12*, 5107-5113.
[5]. Kane, J. M.; Dudley, M. W.; Sorensen, S. M.; Miller, F. P. *J. Med. Chem.* **1988**, *31*, 1253-1258.
[6]. Rajesh, S. M.; Kumar, R. S.; Libertsen, L. A.; Perumal, S.; Yogeeswari, P.; Sriram, D. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3012-3016.
[7]. Cale, A. D.; Gero, T. W.; Walker, K. R.; Lo, Y. S.; Welstead, W. J.; Jaques, L. W.; Johnson, A. F.; Leonard, C. A.; Nolan, J. C.; Johnson, DN. *J. Med. Chem.* **1989**, *32*, 2178-2199.
[8]. Rao, A.; Carbone, A.; Chimirri, A.; De Clercq, E.; Monforte, A. M.; Monforte, P.; Pannecouque, C.; Zappala, M. *Il Farm.* **2002**, *57*, 747-751.
[9]. Krauze, A. A.; Vitolinya, R. O.; Zarin'sh, G. V.; Pelche, Y. E.; Kalme, Z. A.; Kimenis, A. A. *Pharm. Chem. J.* **1985**, *19*, 313-318.
[10]. Yarim, M.; Sarac, S.; Ertan, M.; Kilic, F. S.; Erol, K. *Arzneimittelforschung* **2002**, *52*, 27-33.
[11]. Wujec, M.; Pitucha, M.; Dobosz, M.; Kosikowska, U.; Malm, A. *Acta Pharm.* **2004**, *54*, 251-260.
[12]. Sosnicki, J. G.; Lukasz, S.; Kurzawski, M.; Peruzynska, M.; Maciejewska, G.; Drozdzik, M. *Org. Biomol. Chem.* **2014**, *12*, 3427-3440.
[13]. Price, D.; May, E. L.; Pickel, F. D. *J. Am. Chem. Soc.* **1940**, *62*, 2818-2820.
[14]. Obermeyer, H. G.; Chen. , L. *J. Biol. Chem.* **1945**, *159*, 117-122.
[15]. Chen, Q.; Zhu, X. L.; Jiang, L. L.; Liu, Z. M.; Yang, G. F. *Eur. J. Med. Chem.* **2008**, *43*, 595-603.
[16]. Nugent, R. A.; Schlachter, S. T.; Murphy, M. J.; Cleek, G. J.; Poel, T. J.; Wishka, D. G.; Graber, D. R.; Yagi, Y.; Keiser, B. Y.; Olmsted, R. A.; Kopta, L. A.; Swaney, S. M.; Poppe, S. M.; Morris, J.; Tarpley, W. G.; Thomas, R. C *J. Med. Chem.* **1998**, *41*, 3793-3803.
[17]. Hockova, D.; Holy, A.; Masojidkova, M.; Andrei, G.; Snoeck, R.; De Clercq, E.; Balzarini, J. *Bioorg. Med. Chem.* **2004**, *12*, 3197-3202.
[18]. Brunelle, M. N.; Lucifora, J.; Neyts, J.; Villet, S.; Holy, A.; Trepo, C.; Zoulim, F. *Antimicrob. Agents Chemother.* **2007**, *51*, 2240-2243.
[19]. Costa, E. V.; Pinheiro, M. L. B.; Xavier, C. M.; Silva, J. R.; Amaral, A. C. F.; Souza, A. D.; Barison, A.; Campos, F. R.; Ferreira, A. G.; Machado, G. M.; Leon, L. L. *J. Nat. Prod.* **2006**, *69*, 292-294.
[20]. Xie, F.; Zhao, H.; Zhao, L.; Lou, L.; Hu, Y. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 275-278.
[21]. Ghorab, M. M.; Ragab, F. A.; Alqasoumi, S. I.; Alafeefy, A. M.; Aboulmagd, S. A. *Eur. J. Med. Chem.* **2010**, *45*, 171-178.
[22]. Bachjman, G. B.; Heise, L. V. *J. Am. Chem. Soc.* **1948**, *70*, 2368-2387.
[23]. Al-Refai, M.; Ibrahim, M. M.; Geyer, A.; Marsch, M.; Ali, B. F. *J. Chem. Crystallogr.* **2016**, *46*, 331-340.
[24]. Alsohaili, S. A.; Al-Fawwaz A. T. *Eur. Sci. J.* **2014**, *10*, 156-165.
[25]. Gowri, M.; Ananthalakshmi, S.; Therese Punitha, J. *Int. J. of Pharm. Life Sci.* **2013**, *4*, 2780-2784.
[26]. Chenni, M.; El Abed, D.; Rakotomanomana, N.; Fernandez, X.; Chemat, F. *Molecules* **2016**, *21*, 113, 1-16.