One-pot Synthesis, Characterization and Antimicrobial Activity of New 3-Cyano-4-alkyl-6-(2,5-dichlorothiophen-3-yl)-2(1H)-pyridones

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Abstract

The one-pot synthesis of new derivatives of 2(1H)-pyridone heterocycles carrying a thiophene nucleus is described. The reactions were carried out via the condensation of 3-acetyl-2,5-dichlorothiophene and different alkyl aldehydes 2a-f with ethyl cyanoacetate in presence of ammonium acetate. The pyridone derivatives 3a-f were obtained in 63-86% yields and short reaction times. The antimicrobial activities of the new compounds were evaluated against some common Gram negative and Gram positive bacteria and fungi.

Keywords: One-pot; Heterocycles; Pyridones; Cyanoacetate; Antimicrobial activity.

Introduction

2(1H)-Pyridones are also known as 2-pyridinones or 1,2-dihydro-2-oxopyridines, represent a class of pyridine-containing heterocycles, which have shown variety of biological activities and have recently received a great attention due to their interesting pharmacological properties.[1-6] Milrinone (A) and amrinone (B) (Figure 1) are two of the most prominent representatives of functionalized pyridinone-derived drugs approved by the United States Food and Drug Administration (FDA) for treatment of patients suffering from heart failure.[7-10]

The 2-pyridone functionality is present in many well-known pharmaceutical compounds such as the antibiotic ciclopirox (C)[11,12] and the naturally occurring anticancer diazaquinomycin (D) (Figure 1).[13,14]

![Figure 1: Biologically active 2-pyridone-containing compounds.](image-url)

A. Milrinone B. Amrinone C. Ciclopirox D. Diazaquinomycin

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2-Pyridone derivatives play several roles in medicinal chemistry as scaffolds for drug discovery[15,16]. They are also found in a wide variety of naturally occurring alkaloids[17-19] and are known to show a wide range of biological activities, such as anti-bacterial[20-22] antifungal[23,24] anti-HIV[25,26] anti-inflammatory[27-30] anti-malarial[31,32] anticancer[33-35] and many other activities.[36,37] Very recently, 4-aryl-2-pyridones have been synthesized by our research group[38] and it was of our interest to synthesize new 4-alkyl-2-pyridone derivatives for biological evaluation (Scheme 1).

Experimental

Materials and methods

All chemicals used in this work were purchased from Aldrich or Acros and were of analytical grade or higher. 3-Acetyl-2,5-dichlorothiophene was prepared according to literature procedure.[39] The microorganisms used for antimicrobial tests were provided from the Microbiology Laboratory, Department of Biological Sciences, Al al-Bayt University-Jordan.

Instrumentation

Melting points were determined on an Electrothermal-9002 apparatus. IR spectra were recorded as KBr discs on a Varian IR-660 spectrometer. 1H- and 13C NMR spectra were recorded on a Bruker Avance [400, 100.6 MHz] spectrometer. Chemical shifts are expressed as δ in ppm with reference to TMS as internal standard. Molecular masses were determined with the electron ionization (EI) method on a MicroTOFQ (Bruker, Bremen, Germany) spectrometer at 70 eV and at ion source temperature of 225°C. Elemental analysis was performed on a Euro Vector Euro EA 3000 Elemental Analyzer. Thin-Layer chromatography (TLC) was carried out on ALUGRAM® SIL G/UV254 (Macherey-Nagel) and visualized by UV.

Synthesis

Procedure for the synthesis of 2(1H)-pyridones 3a-f

A round bottom flask was charged with aldehydes 2a-f (1.0 mmol), 3-acetyl-2,5-dichlorothiophene (1.0 mmol) and ethyl cyanoacetate (1.0 mmol) in ethanol (50 mL). The resulting mixture was refluxed for 2-5 h. After cooling to room temperature the resulting solid was filtered off, washed with cold ethanol, and dried. The product was recrystallized from ethanol to yield analytically pure 2(1H)-pyridones derivatives 3a-f in 63-86% yields. The progress of all reactions was monitored by thin layer chromatography (TLC).
6-(2,5-Dichlorothiophen-3-yl)-4-methyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (3a)

White solid, 76% yield; m.p. 278-280 °C. IR (KBr, cm⁻¹): ν = 3425 (br, NH), 3092 (m, C-H), 2241 (m, CN), 1667 (s, C=O). ¹H NMR (400 MHz, DMSO-d₆): δ (ppm) = 12.52 (sb, 1H, NH), 7.40 (s, 1H, H-4'), 6.62 (s, 1H, H-5), 2.40 (s, 3H, H-1'). ¹³C NMR (100 MHz, DMSO-d₆): δ (ppm) = 160.8 (C-4), 160.2 (C=O), 142.7 (C-6), 131.1 (C-3'), 127.9 (C-4'), 126.2 (C-5'), 125.9 (C-2'), 115.5 (CN), 107.8 (C-3), 101.3 (C-5), 20.8 (C-1''); EIMS: m/z (%) = 284 (100) [M]^+, 286 (65) [M+2]^+, 288 (13) [M+4]^+. Anal for C₁₁H₈Cl₂N₂Os (285.14): Calc. C, 46.34%; H, 2.12%; N, 9.82%. Found: C, 45.96%; H, 2.17%; N, 9.64%.

6-(2,5-Dichlorothiophen-3-yl)-4-ethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (3b)

Pale-yellow solid, 69% yield; m.p. 229-230 °C. IR (KBr, cm⁻¹): ν = 3429 (br, NH), 3084 (m, C-H), 2244 (m, CN), 1654 (s, C=O). ¹H NMR (400 MHz, DMSO-d₆): δ (ppm) = 12.55 (sb, 1H, NH), 7.42 (s, 1H, H-4'), 6.65 (s, 1H, H-5), 2.68 (q, 3 J = 7.6 Hz, 2H, H-1''), 1.21 (t, 3 J = 7.6 Hz, 3H, H-2 ''). ¹³C NMR (100 MHz, DMSO-d₆): δ (ppm) = 165.3 (C-4), 161.0 (C-O), 143.3 (C-6), 131.2 (C-3'), 127.9 (C-4'), 126.3 (C-5'), 125.9 (C-2'), 115.3 (CN), 108.1 (C-3), 100.6 (C-5), 27.7 (C-1''), 13.4 (C-2''); EIMS: m/z (%) = 298 (100) [M]^+, 300 (64) [M+2]^+, 302 (12) [M+4]^+. Anal for C₁₂H₁₀Cl₂N₂Os (299.17): Calc. C, 48.18%; H, 2.70%; N, 9.36%. Found: C, 48.03%; H, 2.74%; N, 9.20%.

6-(2,5-Dichlorothiophen-3-yl)-4-propyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (3c)

Yellow solid, 81% yield; m.p. 234-235 °C. IR (KBr, cm⁻¹): ν = 3418 (br, NH), 3086 (m, C-H), 2237 (m, CN), 1658 (s, C=O). ¹H NMR (400 MHz, DMSO-d₆): δ (ppm) = 12.55 (sb, 1H, NH), 7.42 (s, 1H, H-4'), 6.63 (s, 1H, H-5), 2.65 (t, 3 J = 7.0 Hz, 2H, H-1''), 1.64 (sext, 3 J = 7.0 Hz, 2H, H-2''), 0.93 (t, 3 J = 7.0 Hz, 3H, H-3''). ¹³C NMR (100 MHz, DMSO-d₆): δ (ppm) = 163.7 (C-4), 161.0 (C=O), 142.6 (C-6), 131.1 (C-3'), 127.9 (C-4'), 126.2 (C-5'), 125.9 (C-2'), 115.4 (CN), 108.6 (C-3), 100.8 (C-5), 36.2 (C-1''), 22.1 (C-2''), 13.4 (C-3''); EIMS: m/z (%) = 312 (100) [M]^+, 314 (64) [M+2]^+, 316 (11) [M+4]^+. Anal for C₁₃H₁₂Cl₂N₂Os (313.20): Calc. C, 49.85%; H, 3.22%; N, 8.94%. Found: C, 50.03%; H, 3.14%; N, 8.73%.

4-Butyl-6-(2,5-Dichlorothiophen-3-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (3d)

Yellow solid, 74% yield; m.p. 189-191 °C. IR (KBr, cm⁻¹): ν = 3422 (br, NH), 3082 (m, C-H), 2241 (m, CN), 1661 (s, C=O). ¹H NMR (400 MHz, DMSO-d₆): δ (ppm) = 12.55 (sb, 1H, NH), 7.41 (s, 1H, H-4'), 6.66 (s, 1H, H-5), 2.64 (t, 3 J = 7.1 Hz, 2H, H-1''), 1.63-1.59 (m, 2H, H-2''), 1.31-1.26 (m, 2H, H-3''), 0.91 (t, 3 J = 7.0 Hz, 3H, H-4''). ¹³C NMR
(100 MHz, DMSO-d₆): δ (ppm) = 163.4 (C-4), 160.8 (C=O), 143.2 (C-6), 131.2 (C-3), 127.9 (C-4'), 126.3 (C-5'), 126.0 (C-2'), 115.8 (CN), 108.2 (C-3), 101.1 (C-5), 35.4 (C-1*), 26.8 (C-2*), 23.1 (C-3*), 14.4 (C-4*); EIMS: m/z (%) = 326 (100) [M⁺], 328 (66) [M+2]⁺, 330 (13) [M+4]⁺. Anal for Cl₂H₂Cl₂N₂O (327.22): Calc. C, 51.39%; H, 3.70%; N, 8.56%. Found: C, 51.26%; H, 3.93%; N, 8.19%.

6-(2,5-Dichlorothiophen-3-yl)-2-oxo-4-pentyl-1,2-dihydropyridine-3-carbonitrile (3e)

Pale-yellow solid, 86% yield; m.p. 144-146 °C. IR (KBr, cm⁻¹): ν = 3410 (br, NH), 3067 (m, C–H), 2228 (m, CN), 1655 (s, C=O). ¹H NMR (400 MHz, DMSO-d₆): δ (ppm) = 12.54 (sh, 1H, NH), 7.42 (s, 1H, H-4'), 6.66 (s, 1H, H-5), 2.66 (t, 2H, J = 7.4 Hz, H-1*); 1.62 (quin, 2H, J = 7.2 Hz, H-2*); 1.34-1.28 (m, 4H, H-3*, H-4*); 0.86 (t, 3H, J = 7.1 Hz, H-5*).

¹³C NMR (100 MHz, DMSO-d₆): δ (ppm) = 164.0 (C-4), 161.0 (C=O), 143.1 (C-6), 131.1 (C-3), 127.9 (C-4'), 126.2 (C-5'), 125.9 (C-2'), 115.4 (CN), 108.5 (C-3), 101.3 (C-5), 34.2 (C-1*), 30.7 (C-3*), 28.4 (C-2*), 21.7 (C-4*), 13.7 (C-5*); EIMS: m/z (%) = 340 (69) [M⁺], 342 (45) [M+2]⁺, 346 (8) [M+4]⁺. Anal for Cl₂H₂Cl₂N₂OS (341.25): Calc. C, 52.80%; H, 4.14%; N, 8.21%. Found: C, 52.66%; H, 4.11%; N, 7.82%.

6-(2,5-Dichlorothiophen-3-yl)-4-hexyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (3f)

Pale-yellow solid, 63% yield; m.p. 136-137 °C. IR (KBr, cm⁻¹): ν = 3423 (br, NH), 3081 (m, C–H), 2243 (m, CN), 1659 (s, C=O). ¹H NMR (400 MHz, DMSO-d₆): δ (ppm) = 12.54 (sh, 1H, NH), 7.41 (s, 1H, H-4'), 6.63 (s, 1H, H-5), 2.67 (t, 2H, J = 7.6 Hz, H-1*); 1.61 (quin, 2H, J = 7.3 Hz, H-2*); 1.34-1.22 (m, 6H, H-3*, H-4*, H-5*); 0.85 (t, 3H, J = 7.0 Hz, H-6*). ¹³C NMR (100 MHz, DMSO-d₆): δ (ppm) = 164.0 (C-4), 161.0 (C=O), 142.9 (C-6), 131.1 (C-3), 127.9 (C-4'), 126.2 (C-5'), 125.9 (C-2'), 115.4 (CN), 108.5 (C-3), 101.3 (C-5), 34.3 (C-1*), 30.8, 28.7, 28.2, 21.9 (C-2*, C-3*, C-4*, C-5*), 13.9 (C-6*); EIMS: m/z (%) = 354 (67) [M⁺], 356 (46) [M+2]⁺, 358 (9) [M+4]⁺. Anal for Cl₆H₁₆Cl₂N₂OS (355.28): Calc. C, 54.09%; H, 4.54%; N, 7.89%. Found: C, 54.28%; H, 4.36%; N, 7.62%.

Antimicrobial tests

Microorganisms

Antimicrobial activities of different 2-pyridone derivatives were tested against 3 bacteria: Escherichia coli, Staphylococcus aureus, and Bacillus subtilis and two fungal organisms: Aspergillus niger and Penicillium sp.
Minimum inhibitory concentration (MIC) assay using reazurin method

Antimicrobial activity was tested by determining the minimum inhibitory concentration (MIC) using the microdilution method with reazurin, an indicator of microbial growth. The 96-well plates were prepared by dispensing 100 μL of nutrient broth for bacteria and potato dextrose broth for fungi in each well. A volume of 10 μL from the stock solution of tested 2-pyridone derivatives (concentration: 50 mg/mL) was added into the first row of the plate and then two-fold serial dilutions of tested compounds were performed. Reazurin dye (300 mg) was dissolved in 40 mL sterile water. Vortex mixer was used to homogenize the solution. This solution was then referred as Reazurin dye solution. A volume of 10 μL of reazurin solution as indicator was added in each well. Finally, a volume of 10 μL was taken from bacterial and fungal suspensions and then added to each well so that each well has 100 μL of test chemicals in serially descending concentrations. MIC was defined as the lowest concentration of the tested compounds that prevented a reazurin color change from blue to pink. The effect of DMSO on the growth of microorganisms was performed using solvent control test. There was no observed inhibitory effect of DMSO on all tested organisms. All tests were performed in triplicate and MIC values were recorded. Each experiment had a set of controls: Cefactor (Figure 3, S1) as positive control against bacterial species, Miconazole as positive control against fungal species and a plate with all solutions except bacterial solution replaced by 10 μL of nutrient broth or potato dextrose broth (Figure 3, S2). The plates were incubated in temperature controlled incubator at 37 °C for 24 h and 27 °C for 48 h when bacterial species and fungal species used, respectively. The color change in the well was then observed visually.

Results and Discussion

Synthesis and characterization

3-Acetyl-2,5-dichlorothiophene was prepared according to literature procedure. The condensation of the thiophene derivative 1 with aldehydes 2a-f and ethyl cyanoacetate in presence of ammonium acetate yielded the title 2(1H)-pyridones 3a-f in moderate to very good yields (Table 1; Scheme 1).

\[ \text{Scheme 1: Synthesis of 4-alkyl-2(1H)-pyridone derivatives.} \]
Table 1: Substrates scope and yields.

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Product</th>
<th>Yield (%)[^a]</th>
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<td>-Me</td>
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<td>76</td>
</tr>
<tr>
<td>3b</td>
<td>-Et</td>
<td><img src="image2.png" alt="Image" /></td>
<td>69</td>
</tr>
<tr>
<td>3c</td>
<td>-Pr</td>
<td><img src="image3.png" alt="Image" /></td>
<td>81</td>
</tr>
<tr>
<td>3d</td>
<td>-Bu</td>
<td><img src="image4.png" alt="Image" /></td>
<td>74</td>
</tr>
<tr>
<td>3e</td>
<td>-Pn</td>
<td><img src="image5.png" alt="Image" /></td>
<td>86</td>
</tr>
<tr>
<td>3f</td>
<td>-Hex</td>
<td><img src="image6.png" alt="Image" /></td>
<td>63</td>
</tr>
</tbody>
</table>

[^a] Yield after recrystallization.

All newly synthesized compounds 3a-f were characterized by different spectroscopic techniques including IR, $^1$H NMR, $^{13}$C NMR, DEPT, COSY, HSQC spectroscopy, and mass spectrometry along with elemental analysis.

The EI mass spectra display the correct molecular ion peaks as suggested by their molecular formulas as [M]$^+$. In the IR spectra of pyridones 3a-f, a characteristic strong absorption band was observed in the region 1654-1667 cm$^{-1}$ which might be attributed to the carbonyl group. The absorption band in the region 2228–2244 cm$^{-1}$ is assigned to the CN vibration whereas the absorption in the region 3410–3429 cm$^{-1}$ is attributed to the N–H vibration.

In the $^1$H NMR spectra of the compounds 3a-f, the NH proton appeared as broad singlet at δ 12.52-12.55 ppm. The assignment of the C-5 and C-4’ protons for all compounds was based on the HSQC spectral data for each compound as shown in
Figure 2 for compound 3c. The C-5 proton of pyridone ring appeared as a singlet at δ 6.62-6.66 ppm, while, the C-4' proton appeared as a singlet at δ 7.41-7.42 ppm. Complete assignments for all protons are given in the experimental part.

In the 13C NMR spectra of the pyridone derivatives 3a-f, the absorption appearing in the range δ=160.8-165.3 ppm is assigned to the C-4 carbon, while the absorption in the range δ=160.2-161.0 ppm is attributed to the carbonyl carbon. The absorption in the range 115.3-115.8 ppm in the spectra is assigned to the CN carbon. The assignment of the protonated carbons was based on the HSQC spectral data for each compound. Complete assignments for all carbon atoms are given in details in the experimental part.

The prepared compounds were evaluated for their antimicrobial activity. The MIC was recorded for the tested compounds and the results are shown in Table 2 and Figure 3. The new 2-pyridone compounds carrying aliphatic substituents at position 4 showed antibacterial activity against different types of bacteria. On the contrary, 2-pyridone derivatives with aromatic substituents at the same position showed no activity against the tested bacteria.\textsuperscript{[38]} Notably, antibacterial activity was found to increase with increasing the length of aliphatic chain. Compound 3f showed a significant activity
against *B. subtilis*, and good activity against *E. coli* and *S. aureus* bacteria whereas compound 3e showed only moderate activities against all tested bacteria. All compounds did not possess any activity against the two fungi.

**Table 2:** Minimal Inhibitory Concentration (MIC) of tested compounds (mg/mL).

<table>
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<tr>
<th></th>
<th>3a</th>
<th>3b</th>
<th>3c</th>
<th>3d</th>
<th>3e</th>
<th>3f</th>
<th>Cefaclor</th>
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<tr>
<td><em>E. coli</em></td>
<td>2.5</td>
<td>2.5</td>
<td>&gt;2.5</td>
<td>2.5</td>
<td>0.156</td>
<td>0.156</td>
<td>0.0048</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>&gt;2.5</td>
<td>&gt;2.5</td>
<td>&gt;2.5</td>
<td>&gt;2.5</td>
<td>0.312</td>
<td>0.078</td>
<td>0.0024</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>2.5</td>
<td>1.25</td>
<td>2.5</td>
<td>0.625</td>
<td>0.156</td>
<td>0.0024</td>
<td>0.0024</td>
</tr>
</tbody>
</table>

**Figure 3:** Resazurin MIC test plate for: A) *E. coli* B) *B. subtilis* C) *S. aureus*

**Conclusion**

A new series of 4-alkyl-6-(2,5-dichlorothiophen-3-yl)-2(1H)-pyridones was synthesized in good yields and short reaction times. All new compounds were characterized by standard spectroscopic techniques. Biological evaluations revealed that the prepared compounds showed antibacterial activity which increased by increasing the length of the aliphatic chain.

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**References**


