HETEROCYCLES, Vol. 102, No. 11, 2021, pp. 2153 - 2167. © 2021 The Japan Institute of Heterocyclic Chemistry
Received, 11th August, 2021, Accepted, 25th August, 2021, Published online, 27th August, 2021
DOI: 10.3987/COM-21-14534

SYNTHESIS, CHARACTERIZATION, ANTIBACTERIAL AND CYTOTOXIC EVALUATION OF NEW 6-(CHLOROTHIOPHENYL)-2-(2-OXOPROPOXY)PYRIDINE-3-CARBONITRILE DERIVATIVES AND THEIR CORRESPONDING FURO[2,3-*b*]PYRIDINE DERIVATIVES

Ala'a B. Said,[1] Mahmoud Al-Refai,[1]* Armin Geyer,[2] Iman A. Mansi,[3] and Basem Fares Ali[1]*

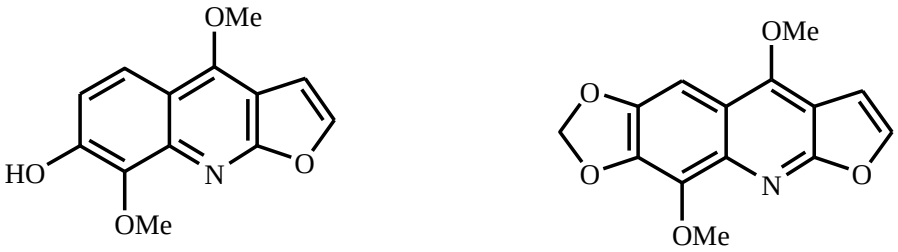
[1]Department of Chemistry, Al al-Bayt University, Mafraq 25113, Jordan; [2]Faculty of Chemistry, Philipps University of Marburg, Hans-Meerwein-Strasse 4, 35032, Marburg, Germany; [3]Department of Clinical Pharmacy and Pharmacy Practice, Faculty of Pharmaceutical Sciences, The Hashemite University, P.O. Box 330127, Zarqa 13133, Jordan; e-mail address: mahmoud_alrefai@aabu.edu.jo; bfali@aabu.edu.jo

Abstract – The synthesis of a new series of 6-(chlorothiophenyl)-2-(2-oxopropoxy)pyridine-3-carbonitrile compounds **2** and **5** and their furo[2,3-*b*]pyridines bearing heteroaryl substituents **3** and **6** in high yield is reported. The 6-(chlorothiophenyl)-2-(2-oxopropoxy)pyridine-3-carbonitrile derivatives (**2a-f**) and (**5a-d**) were prepared from the corresponding 3-cyano-(2*H*)-pyridones (**1a-f**) and (**4a-d**) followed by the Thorpe-Ziegler ring cyclization in the presence of sodium methoxide to give the target furo[2,3-*b*]pyridine derivatives (**3a-f**) and (**6a-d**). Proposed structures of the new compounds are based on NMR and mass spectral data. Antibacterial evaluation of (**2a-d**), (**2f**), (**5a-d**), (**3a-d**), (**3f**), and (**6a-d**) derivatives against eight different microorganisms shows no activity towards any of the tested bacteria while compounds (**6a**) and (**6c**) showed weak antibacterial activity with inhibition zone of 10 mm. Cytotoxic assessment against MCF7 breast cancer cells reveals that 10 of the derivatives were found to decrease cell viability in a dose dependent manner at concentration of 100 μM, but six of them decreased it to less than 50% and had IC_{50}'s ranging from 23.3 to 41.2 μM.

INTRODUCTION

Heterocyclic chemistry is an important and challenging topic in different fields of chemistry research, including organic chemistry, biochemistry, and especially medicinal chemistry[1] according to their biological importance.[2] Also they have an attraction in other fields of science, due to the huge variety of available structures for both synthetic and naturally occurring heterocyclic compounds with their different properties, which make them valuable and useful tasks in many interesting applications ranging from drugs to electronics.[3]

Among heterocycles, furo[2,3-*b*]pyridine are considered to be an important and useful type of heteroaromatic compounds because of their important pharmacological and biological abilities.[4] There are naturally occurring bioactive compounds such as furo[2,3-*b*]pyridine alkaloids like haplopine (**A**) that was isolated from the root bark of *Dictamnus dasycarpus* Turcz and showed antifungal activity,[5] and flindersiamine (**B**) (Figure 1), isolated from *Esenbeckia leiocarpa* flowering plant, that showed antibacterial activity.[6] Another example is the family of alkaloid plants that have been used in traditional Chinese medicines.[7]



(A) (B)

Figure 1. Selected naturally occurring bioactive compounds

Even though furo[2,3-*b*]pyridines are rare in nature, many of furo[2,3-*b*]pyridine derivatives have been prepared and many of them showed different biological activities.[8] Furthermore, furo[2,3-*b*]pyridine core skeleton constitutes a key structural unit in many important bioactive compounds, (Figure 2).[9,10]

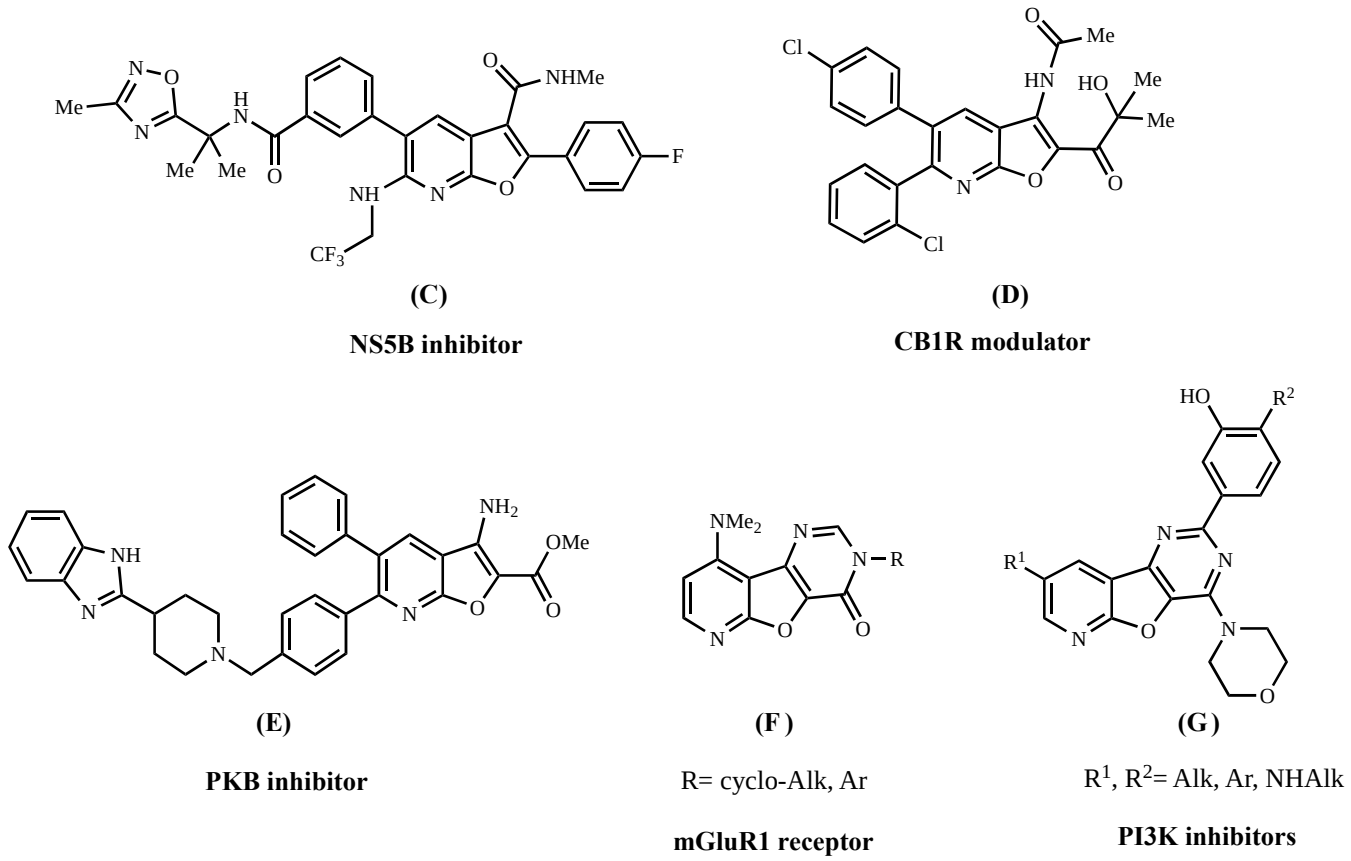
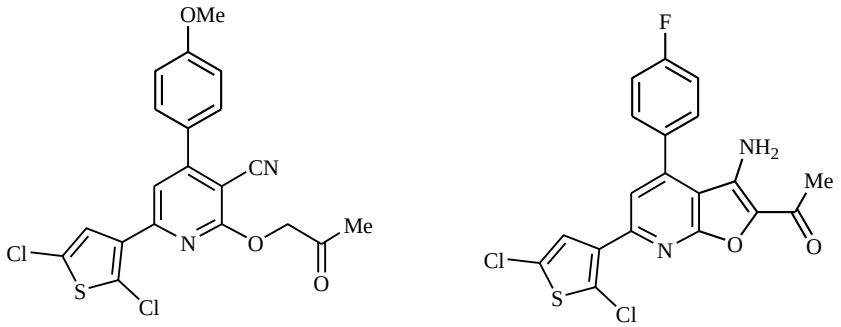


Figure 2. Selected important bioactive compounds containing furo[2,3-*b*]pyridine core

Pharmacological studies showed that compound (**C**) has high activity against hepatitis C virus by the inhibition of nonstructural protein 5B (NS5B).[11] Compound (**D**) is active toward the type 1 cannabinoid receptor (CB1R) modulator and used in the treatment of food-borne diseases,[12] compound (**E**) is a protein kinase B (PKB) inhibitor and can be used as an anticancer.[13] Derivatives of compound (**F**) are potent and selective metabotropic glutamate receptor (mGluR1) antagonists,[14] and compound (**G**) derivatives are selective for phosphoinositide 3-kinase (PI3K) inhibitors and useful as a therapeutic agent for inflammatory diseases.[15]

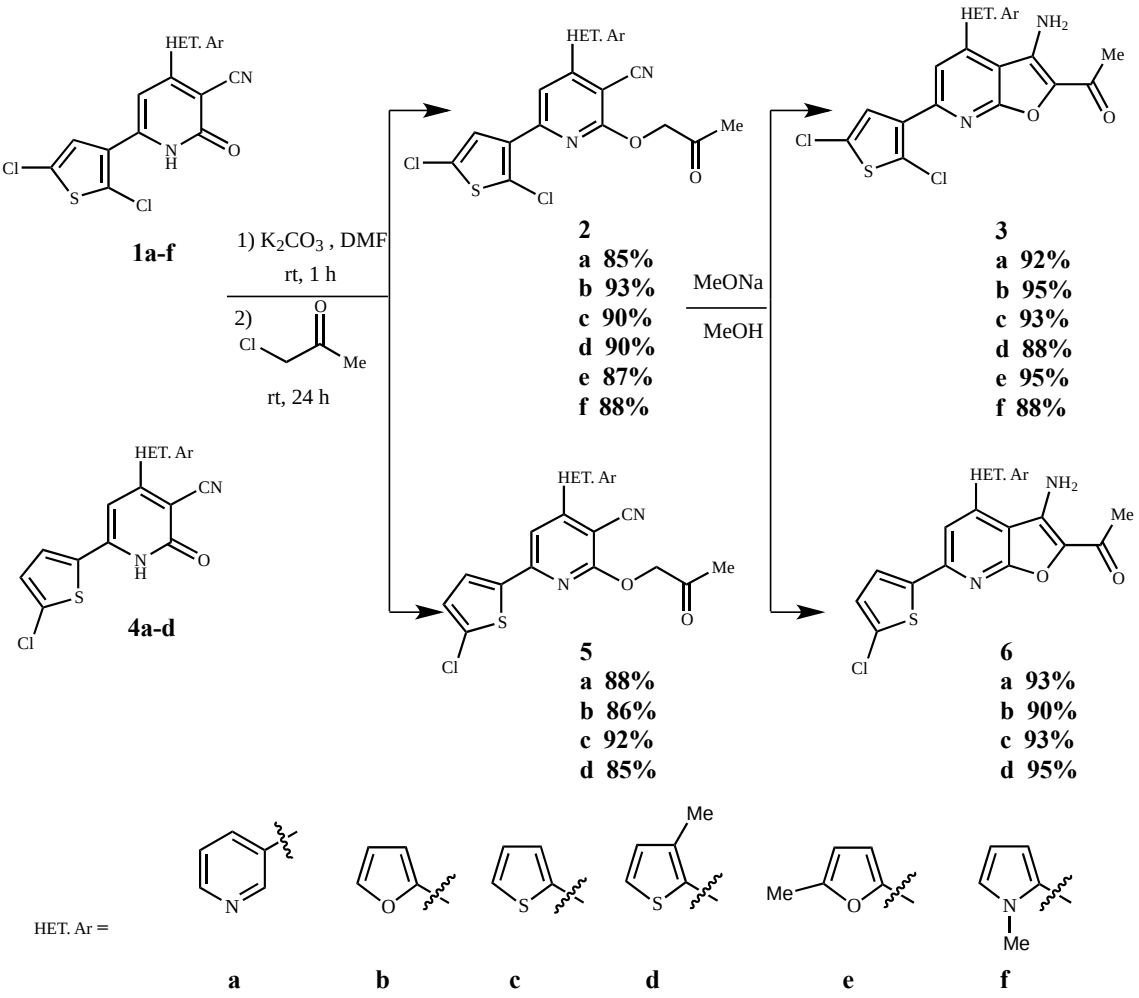
Several compounds of 2-(2-oxopropoxy)-6-(dichlorothiophene)nicotinonitrile and their furo[2,3-*b*]pyridine derivatives bearing aromatic substituents on carbon at position 4 have been prepared by Al-Refai and co-workers. Their biological activity was evaluated and showed that some of these compounds possess high antioxidant activity, such as (**H**) and (**I**) (Figure 3).[16]



(H) **(I)**

Figure 3. Furo[2,3-*b*]pyridine derivatives possess high antioxidant activity

In continuation to our research, we herein report the synthesis of new of 2-(2-oxopropoxy)-6-(dichlorothiophene)nicotinonitrile derivatives **2** and **5** and their corresponding furo[2,3-*b*]pyridine derivatives bearing heteroaryl substituents **3** and **6** (Scheme 1). The new derivatives are evaluated for their antibacterial activity and cytotoxic activity against MCF7 breast cancer cells.



Scheme 1. Synthetic outline of new derivatives

RESULTS AND DISCUSSION

The carbonitrile derivatives (**2a-f**) and (**5a-d**) were obtained in good yields by reaction of the 4-(heteroaryl)-6-(chlorothiophenyl)-3-cyano-2-dihydropyridones (**1a-f**) and (**4a-d**) with potassium carbonate and chloroacetone in DMF as a solvent for 24 h. Desired furo[2,3-*b*]pyridine derivatives (**3a-f**) and (**6a-d**) were obtained in high yields by intracyclization of carbonitrile compounds (**2a-f**) and (**5a-d**) upon heating in methanolic solution with sodium methoxide for 2 h, as shown in Scheme 1. The synthesized derivatives with different heteroaryl substituents at position 4 with yields are listed in Scheme 1.

All the newly synthesized carbonitrile derivatives (**2a-f**) (**5a-d**) and furo[2,3-*b*]pyridines (**3a-f**) (**6a-d**) were fully characterized, based on ^{1}H NMR, ^{13}C NMR and mass spectrometry data. The complete data for all protons and carbons are listed in the experimental section. The HRESIMS and ESIMS data analyses showed the correct molecular ion peaks suggested by their molecular formulas.

The ^{1}H NMR spectra of new carbonitriles (**2a-f**) and (**5a-d**) showed two singlets in the upfield region at δ 5.00-5.35 ppm attributed to the protons of the methylene group, and at δ 2.30-2.59 ppm corresponding to the acetyl protons. A singlet peak observed at δ 7.05-7.66 ppm in the low field region related to the pyridine proton (H-5), while the thiophene proton (H-4') resonated as a singlet at δ 7.18-7.48 ppm for (**2a-f**) derivatives and (H-3') and (H-4') resonated as two coupled doublet signals at δ 7.42-7.48 ppm and 6.41-6.96 ppm, respectively, for (**5a-d**) derivatives. The ^{1}H NMR spectra of furo[2,3-*b*]pyridine derivatives (**3a-f**) and (**6a-d**), showed a very similar pattern with appearance of a new one singlet signal at δ 5.37-5.80 ppm that might be attributed to the amine group protons and disappearance of the methylene protons singlet signal. This confirms the intramolecular cyclization step for carbonitriles **2** and **5** to furnish the proposed furo[2,3-*b*]pyridines **3** and **6**.

The ^{13}C NMR spectra of the carbonitriles (**2a-f**) and (**5a-d**) exhibit important signals resonated at δ 202.06-202.93, 114.05-115.75 and 71.03-71.53 ppm assigned to the carbonyl, nitrile, and methylene carbon, respectively. The absence of the nitrile and methylene carbon signals in these regions, with the shifted carbonyl carbon peak towards upfield range at δ 190 ppm, confirms the formation of the furo[2,3-*b*]pyridine derivatives (**3a-f**) and (**6a-d**).

The HRESIMS and ESIMS mass spectra were measured for some selected new compounds from both carbonitrile (**2a**), (**2c-e**), (**5a-b**), (**5d**) and furo[2,3-*b*]pyridine derivatives (**3a**), (**3c-e**), (**6a-b**) and (**6d**). The presence of the molecular ion $[M]^{+}$ and the corresponding isotopic mass ions $[M+2]^{+}$ and $[M+4]^{+}$, besides the other fragment ions confirm the mono- and dichloro containing derivatives. In addition, the HRESIMS confirmed the molecular formulae of measured compounds (See experimental section).

Biological Activity

Antibacterial activity

None of the compounds was susceptible to any of the bacteria, and only compounds (**6a**) and (**6c**) showed weak antibacterial activity with inhibition zone of 10 mm (Table 1).

Table 1. Zone of inhibition in mm of some synthesized compounds against eight different microorganisms

Compound	*S. pneumonia*	*S. pyogenes*	*S. epidermedis*	*S. aureus*	*E. faecalis*	*P. aeruginosa*	*K. pneumonia*	*E. coli*
2a	R	R	R	R	R	R	R	R
3a	R	R	R	R	R	R	R	R
2b	R	R	R	R	R	R	R	R
3b	R	R	R	R	R	R	R	R
2c	R	R	R	R	R	R	R	R
3c	R	R	R	R	R	R	R	R
2d	R	R	R	R	R	R	R	R
3d	R	R	R	R	R	R	R	R
2f	R	R	R	R	R	R	R	R
3f	R	R	R	R	R	R	R	R
5a	R	R	R	R	R	R	R	R
6a	R	R	10 mm	R	R	R	R	R
5b	R	R	R	R	R	R	R	R
6b	R	R	R	R	R	R	R	R
5c	R	R	R	R	R	R	R	R
6c	R	R	10 mm	R	R	R	R	R
5d	R	R	R	R	R	R	R	R
6d	R	R	R	R	R	R	R	R

R: resistant

Cytotoxic activity

Out of 18 compounds screened, 10 compounds were found to decrease cell viability in a dose dependent manner and six of them had IC_{50}'s below 50 μM ranging from 23.3 to 41.2 μM. Doxorubicin was used as a positive standard, Table 2.

Table 2. The IC_{50} values (μM) of cell viability for MCF7 breast cancer cells

Compound	MCF7 cell line	
	% cell viability (100 μM)	IC_{50} (μM)
2a	-	
3a	37.7	23.3
2b	40.1	28.1
3b	-	-
2c	-	-
3c	-	-

2d	-	-
3d	39.5	41.2
2f	37.8	35.8
3f	49.1	74.3
5a	42.0	34.1
6a	-	-
5b	-	-
6b	60.3	307.5
5c	61.9	399.9
6c	44.4	39.3
5d	50.7	102.0
6d	-	-
Doxorubicin	9.2	4.39

EXPERIMENTAL

Materials

All chemicals used in this work were purchased from Merck and Sigma Aldrich and were used as received. Solvents were dried and distilled according to standard protocols. NMR spectra, ^{1}H (300 MHz) and ^{13}C (75 MHz) were acquired in $CDCl_3$ or CD_2Cl_2 (used as an internal standards) at 295 K on Bruker spectrometers. Chemical shifts δ are given in parts per million (ppm) and were determined from the centre of the respective coupling patterns (s: singlet, d: doublet, dd: doublet of doublet, t: triplet, q: quartet, and m: multiplet). ESI–HRMS measurements were performed on an LTQ-FT mass spectrometer (Thermo Fiher Scientific). Melting points were measured on an Electrothermal 9100 apparatus.

Synthesis

The carbonitrile compounds (**2a-f**) and (**5a-d**) were prepared via one-pot reaction according to the literature method,[17] and were used in the next step as obtained.

General procedure for the synthesis of carbonitrile compounds (2a-f) and (5a-d)

The 4-(heteroaryl)-6-(chlorothiophenyl)-3-cyano-2-dihydropyridones **1** or **4** (2 mmol), and potassium carbonate (0.3 g, 2.2 mmol, 1.1 equivalent) dissolved in dry DMF (40 mL) in a round-bottom flask equipped with a stir bar. The mixture was stirred at room temperature for 1 h. Chloroacetone (0.4 g, 4 mmol) was added portion wise to the reaction mixture and allowed to stir overnight. The reaction mixture was poured slowly into crushed ice with stirring. The precipitate was filtered off, washed with water and dried to give the corresponding carbonitrile compounds **2** and **5** (Scheme 1).

6-(2,5-Dichlorothiophen-3-yl)-2-(2-oxopropoxy)-4-(3-pyridyl)pyridine-3-carbonitrile (2a). White precipitate; mp 175-176 °C; yield (85%); ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ = 8.87 (d, J = 1.61 Hz, 1H, H-2''), 8.80 (d, J = 4.71 Hz, 1H, H-6''), 8.07 (d, J = 7.92 Hz, 1H, H-4''), 7.66 (s, 1H, H-5), 7.52 (dd, J = 7.80, 4.91 Hz, 1H, H-5''), 7.20 (s, 1H, H-4'), 5.10 (s, 2H, $C\underline{H}_2COCH_3$), 2.32 (s, 3H, $CH_2COC\underline{H}_3$); ^{13}C NMR (75 MHz, $CDCl_3$, ppm) δ = 202.43 (CO), 163.24 (C_q-2), 153.47 (C_q-4), 151.96 (C_q-6), 151.30, 148.82, 135.89, 126.93, 123.72, 116.35 (CH-5, 4', 2'', 4'', 5'', 6''), 134.73, 131.69, 127.27, 127.05 (C_q-2', 3',

5', 3''), 114.23 (CN-3), 93.80 (C_q-3), 71.19 ($\underline{C}H_2COCH_3$), 26.31 ($CH_2CO\underline{C}H_3$); (+)-ESIMS *m/z* 404.3 ($[M+H]^+$, 25), 426.3 ($[M+Na]^+$, 90), 428.2 ($[M+Na+2]^+$, 64), 430.2 ($[M+ Na +4]^+$, 13), 828.8 ($[2M+Na]^+$, 57), 830.7 ($[2M+Na+2]^+$, 100), 832.6 ($[2M+Na+4]^+$, 60); (+)-HRESIMS *m/z* 425.9836 $[M+Na]^+$, 427.9808 $[M+Na+2]^+$, (calculated for $C_{18}H_{11}Cl_2N_3O_2SNa$, 425.9852).

6-(2,5-Dichlorothiophen-3-yl)-4-(furan-2-yl)-2-(2-oxopropoxy)pyridine-3-carbonitrile (2b). White precipitate; mp 178-179 °C; yield (93%); ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ = 8.00 (s, 1H, H-5), 7.67 (s, 2H, H-3'',H-5''), 7.27 (s, 1H, H-4'), 6.67 (dd, *J* = 3.05, 1.67 Hz, 1H, H-4''), 5.04 (s, 2H, $C\underline{H_2}COCH_3$), 2.29 (s, 3H, $CH_2COC\underline{H_3}$); ^{13}C NMR (75 MHz, $CDCl_3$, ppm) δ = 203.06 (CO), 163.44 (C_q-2), 151.64 (C_q-4), 147.28 (C_q-6), 143.4 0, 135.01, 126.97, 124.11 (C_q-2', 3', 5', 2''), 145.28, 127.01, 115.01, 113.09, 111.72 (CH-5, 4', 3'', 4'', 5''), 115.26 (CN-3), 92.89 (C_q-3), 71.16 ($\underline{C}H_2COCH_3$), 26.36 ($CH_2CO\underline{C}H_3$); (+)-ESIMS *m/z* 393.6 ($[M+H]^+$, 30), 395.6 ($[M+H+2]^+$, 17), 397.6 ($[M+H+4]^+$, 5), 415.5 ($[M+Na]^+$, 100), 417.5 ($[M+Na+2]^+$, 62), 419.5 ($[M+ Na +4]^+$, 15), 807.1 ($[2M+Na]^+$, 41), 808.9 ($[2M+Na+2]^+$, 64), 810.7 ($[2M+Na+4]^+$, 39).

6-(2,5-Dichlorothiophen-3-yl)-2-(2-oxopropoxy)-4-(thiophen-2-yl)pyridine-3-carbonitrile (2c). White precipitate; mp 188-189 °C; yield (90%); ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ = 7.99 (dd, *J* = 3.79, 0.75 Hz, 1H, H-5''), 7.76 (s, 1H, H-5), 7.59 (dd, *J* = 4.35, 0.85 Hz, 1H, H-3''), 7.25-7.22 (m, 1H, H-4''), 7.18 (s, 1H, H-4'), 5.04 (s, 2H, $C\underline{H_2}COCH_3$), 2.29 (s, 3H, $CH_2COC\underline{H_3}$); ^{13}C NMR (75 MHz, $CDCl_3$, ppm) δ = 202.95 (CO), 163.72 (C_q-2), 154.91 (C_q-4), 151.46 (C_q-6), 148.39, 137.06, 130.98, 127.82, (C_q-2', 3', 5', 2''), 129.98, 129.87, 128.95, 126.98, 115.38 (CH-5, 4', 3'', 4'', 5''), 115.23 (CN-3), 94.20 (C_q-3), 71.21 ($\underline{C}H_2COCH_3$), 26.38 ($CH_2CO\underline{C}H_3$); (+)-ESIMS *m/z* 409.3 ($[M+H]^+$, 80), 431.2 ($[M+Na]^+$, 100), 433.2 ($[M+Na+2]^+$, 70), 435.1 ($[M+Na+4]^+$, 18), 838.7 ($[2M+Na]^+$, 50), 840.6 ($[2M+Na+2]^+$, 80), 842.5 ($[2M+Na+4]^+$, 47); (+)-HRESIMS *m/z* 430.9449 $[M+Na]^+$, 432.9419 $[M+Na+2]^+$, (calculated for $C_{17}H_{10}Cl_2N_2O_2S_2Na$, 430.9453).

6-(2,5-Dichlorothiophen-3-yl)-4-(3-methylthiophen-2-yl)-2-(2-oxopropoxy)pyridine-3-carbonitrile (2d). White precipitate; mp 200-201 °C; yield (90%); ^{1}H NMR (300 MHz, CD_2Cl_2, ppm): δ = 7.62 (s, 1H, H-5), 7.51 (d, *J* = 4.98 Hz, 1H, H-5''), 7.25 (s, 1H, H-4'), 7.07 (d, *J* = 4.91 Hz, 1H, H-4''), 5.00 (s, 2H, $C\underline{H_2}COCH_3$), 2.36 (s, 3H, $CH_2COC\underline{H_3}$), 2.28 (s, 3H, $C\underline{H_3}$-3''); ^{13}C NMR (75 MHz, CD_2Cl_2, ppm) δ = 202.60 (CO), 163.46 (C_q-2), 151.46 (C_q-4), 150.99 (C_q-6), 138.85, 135.42, 132.33, 131.06, 125.95 (C_q-2', 3', 5', 2'', 3''), 131.48, 127.73, 127.58, 118.52 (CH-5, 4', 4'', 5''), 114.81 (CN-3), 95.67 (C_q-3), 71.79 ($\underline{C}H_2COCH_3$), 26.43 ($CH_2CO\underline{C}H_3$), 15.50 ($\underline{C}H_3$-3''); (+)-ESIMS *m/z* 423.2 ($[M+H]^+$, 100), 425.2 ($[M+H+2]^+$, 70), 426.8 ($[M+ H+4]^+$, 17), 445.1 ($[M+Na]^+$, 50), 447.1 ($[M+Na+2]^+$, 36), 449.2 ($[M+Na+4]^+$, 9), 866.5 ($[2M+ Na]^+$, 20), 868.6 ($[2M+Na+2]^+$, 30), 870.5 ($[2M+Na+4]^+$, 21); (+)-HRESIMS *m/z* 422.9786 $[M+H]^+$, 424.9757 $[M+H+2]^+$, (calculated for $C_{18}H_{13}Cl_2N_2O_2S_2$, 422.9790).

6-(2,5-Dichlorothiophen-3-yl)-4-(5-methylfuran-2-yl)-2-(2-oxopropoxy)pyridine-3-carbonitrile (2e). Pale yellow precipitate; mp 188-189 °C; yield (87%), ^{1}H NMR (300 MHz, CD_2Cl_2, ppm): δ = 7.92 (s, 1H, H-5), 7.56 (d, *J* = 3.45 Hz 1H, H-3''), 7.25 (s, 1H, H-4'), 6.31 (d, *J* = 3.38 Hz 1H, H-4''), 5.07 (s, 2H, C$\underline{H_2}$COCH$_3$), 2.46 (s, 3H, C$\underline{H_3}$-5''), 2.25 (s, 3H, CH$_2$COC$\underline{H_3}$); ^{13}C NMR (75 MHz, CD_2Cl_2, ppm) δ = 202.93 (CO), 163.88 (C$_q$-2), 156.85 (C$_q$-4), 151.71 (C$_q$-6), 146.75, 143.73, 135.68, 128.38, 127.07 (C$_q$-2', 3', 5', 2'', 5''), 127.56, 116.78, 111.54, 109.95 (CH-5, 4', 3'', 4'''), 115.28 (CN-3), 94.37 (C$_q$-3), 71.46 (C$\underline{H_2}$COCH$_3$), 26.42 (CH$_2$CO$\underline{C}$H$_3$), 14.01 (C$\underline{H_3}$-5''); (+)-ESIMS *m/z* 407.2 ([M+H]$^+$, 8), 429.1 ([M+Na]$^+$, 100), 431.1 ([M+Na+2]$^+$, 68), 432.9 ([M+Na+4]$^+$, 14), 834.6 ([2M+Na]$^+$, 55), 836.7 ([2M+Na+2]$^+$, 65), 838.5 ([2M+Na+4]$^+$, 45); (+)-HRESIMS *m/z* 428.9833 [M+Na]$^+$, 430.9804 [M+Na+2]$^+$, (calculated for $C_{18}H_{12}Cl_2N_2O_3SNa$, 428.9 849).

6-(2,5-Dichlorothiophen-3-yl)-4-(1-methyl-1*H*-pyrrol-2-yl)-2-(2-oxopropoxy)pyridine-3-carbonitrile (2f). White precipitate; mp 178-180 °C; yield (88%); ^{1}H NMR (300 MHz, CD_2Cl_2, ppm): δ = 7.55 (s, 1H, H-5), 7.25 (s, 1H, H-4'), 6.95 (dd, *J* = 2.73, 1.72 Hz, 1H, H-5''), 6.75 (dd, *J* = 3.79, 1.58 Hz, 1H, H-3''), 6.32 (dd, *J* = 3.57, 2.67 Hz, 1H, H-4''), 5.08 (s, 2H, C$\underline{H_2}$COCH$_3$), 3.78 (s, 3H, NC$\underline{H_3}$-1''), 2.28 (s, 3H, CH$_2$COC$\underline{H_3}$); ^{13}C NMR (75 MHz, CD_2Cl_2, ppm) δ = 202.82 (CO), 163.83 (C$_q$-2), 151.09 (C$_q$-4), 148.06 (C$_q$-6), 135.77, 128.46, 127.26, 127.18, (C$_q$-2', 3', 5', 2''), 128.16, 127.70, 116.50, 114.62, 109.40 (CH-5, 4', 3'', 4'', 5''), 115.53 (CN-3), 93.30 (C$_q$-3), 71.46 (C$\underline{H_2}$ COCH$_3$), 35.97 (N$\underline{C}$H$_3$-1''), 26.43 (CH$_2$CO$\underline{C}$H$_3$).

6-(5-Chlorothiophen-2-yl)-2-(2-oxopropoxy)-4-(3-pyridyl)pyridine-3-carbonitrile (5a). White precipitate; mp 182-184 °C; yield (88%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.84 (d, *J* =1.60 Hz, 1H, H-2''), 8.78 (dd, *J* = 4.74, 1.17 Hz, 1H, H-6''), 8.03 (dt, *J* =7.84, 6.33 Hz, 1H, H-4''), 7.51 (dd, *J* =7.64, 4.92 Hz, 1H, H-5''), 7.46 (d, *J* =4.03 Hz, 1H, H-3'), 7.30 (s, 1H, H-5), 6.97 (d, *J* =4.01 Hz, 1H, H-4'), 5.07 (s, 2H, C$\underline{H_2}$COCH$_3$), 2.40 (s, 3H, CH$_2$CO C$\underline{H_3}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ =202.11 (C=O), 163.42 (C$_q$-2), 153.46 (C$_q$-4), 152.36 (C$_q$-6), 151.28, 148.72, 135.80, 128.05, 126.83, 123.64, 111.66 (CH-5, 3', 4', 2'', 4'', 5'', 6''), 140.78, 135.88, 131.74 (C$_q$-2', 5', 3''), 114.38 (CN-3), 92.78 (C$_q$-3), 71.19 (C$\underline{H_2}$ COCH$_3$), 26.30 (CH$_2$CO$\underline{C}$H$_3$); (+)-ESIMS *m/z* 370.3 ([M+H]$^+$, 15), 372.3 ([M+H+2]$^+$, 6), 392.2 ([M+Na]$^+$, 100), 394.2 ([M+Na+2]$^+$, 45); (+)-HRESIMS *m/z* 392.0234 [M+Na]$^+$, 394.0205 [M+Na+2]$^+$ (calculated for $C_{18}H_{12}ClN_3O_2SNa$, 392.0231).

6-(5-Chlorothiophen-2-yl)-4-(furan-2-yl)-2-(2-oxopropoxy)pyridine-3-carbonitrile (5b). White precipitate; mp 184-185 °C; yield (86%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.66 (s, 1H, H-5), 7.65 (d, *J* = 3.24 Hz, 2H, H-3'',H-5''), 7.48 (d, *J* = 4.03 Hz, 1H, H-3'), 6.96 (d, *J* = 4.04 Hz, 1H, H-4'), 6.65 (dd, *J* = 3.56, 1.58 Hz, 1H, H-4''), 5.00 (s, 2H, C$\underline{H_2}$COCH$_3$), 2.31 (s, 3H, CH$_2$COC$\underline{H_3}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 202.74 (C=O), 163.63 (C$_q$-2), 152.03 (C$_q$-4), 147.77 (C$_q$-6), 143.28, 141.27, 135.24 (C$_q$-2', 5', 2''), 145.05, 127.86, 126.37, 114.90, 113.08, 106.95 (CH-5, 3', 4', 3'', 4'', 5''), 115.44 (CN-3), 87.12 (C$_q$-3),

71.15 ($\underline{C}H_2COCH_3$), 26.34 ($CH_2CO\underline{C}H_3$); (+)-ESIMS *m/z* 359.1 ($[M+H]^+$, 30), 361.1 ($[M+H+2]^+$, 12), 381.1 ($[M+Na]^+$, 100), 383.1 ($[M+Na+2]^+$, 39), 738.6 ($[2M+Na]^+$, 40), 740.6 ($[2M+Na+2]^+$, 35); (+)-HRESIMS *m/z* 381.0067 $[M+Na]^+$, 383.0038 $[M+Na+2]^+$, (calculated for $C_{17}H_{11}ClN_2O_3SNa$, 381.0071).

6-(5-Chlorothiophen-2-yl)-2-(2-oxopropoxy)-4-(thiophen-2-yl)pyridine-3-carbonitrile (5c). White precipitate; mp 203-204 °C; yield (92%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.97 (dd, *J* = 3.77, 0.92 Hz, 1H, H-5''), 7.59 (dd, *J* = 5.07, 0.92 Hz, 1H, H-3''), 7.46 (d, *J* = 4.05 Hz, 1H, H-3'), 7.39 (s, 1H, H-5), 7.24 (dd, *J* = 5.00, 3.88 Hz, 1H, H-4''), 6.98 (d, *J* = 4.05 Hz, 1H, H-4'), 5.04 (s, 2H, $C\underline{H_2}COCH_3$), 2.33 (s, 3H, $CH_2COC\underline{H_3}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 202.53 (C=O), 163.89 (C_q-2), 151.91 (C_q-4), 148.40 (C_q-6), 141.00, 136.94, 135.44 (C_q-2', 5', 2''), 129.89, 129.55, 128.85, 127.89, 126.42, 110.63 (CH-5, 3', 4', 3'', 4'', 5''), 115.31 (CN-3), 90.43 (C_q-3), 71.21 ($\underline{C}H_2COCH_3$), 26.29 ($CH_2CO\ \underline{C}H_3$); (+)-ESIMS *m/z* 375.3 ($[M+H]^+$, 97), 377.3 ($[M+H+2]^+$, 41), 397.2 ($[M+Na]^+$, 100), 399.2 ($[M+Na+2]^+$, 42); (+)-HRESIMS *m/z* 396.9848 $[M+Na]^+$, 398.9819 $[M+Na+2]^+$, (calculated for $C_{17}H_{11}ClN_2O_2S_2Na$, 396.9843).

6-(5-Chlorothiophen-2-yl)-4-(3-methylthiophen-2-yl)-2-(2-oxopropoxy)pyridine-3-carbonitrile (5d). White precipitate; mp 126-128 °C; yield (85%); ^{1}H NMR (300 MHz, CD_2Cl_2): δ = 7.50 (d, *J* = 4.55 Hz, 1H, H-5''), 7.46 (d, *J* = 4.04 Hz, 1H, H-3'), 7.31 (s, 1H, H-5), 7.06 (d, *J* = 5.04 Hz, 1H, H-4''), 7.00 (d, *J* = 4.01 Hz, 1H, H-4'), 5.09 (s, 2H, $C\underline{H_2}C\ OCH_3$), 2.34 (s, 3H, $C\underline{H_3}$-3''), 2.32 (s, 3H,$CH_2CO\ C\underline{H_3}$); ^{13}C NMR (75 MHz, CD_2Cl_2) δ = 202.32 (C=O), 163.55 (C_q-2), 151.90 (C_q-4), 150.90 (C_q-6), 141.57, 138.69, 135.58, 132.28 (C_q-2', 5', 2'', 3''), 131.41, 128.48, 127.52, 126.88, 113.95 (CH-5, 3', 4', 4'', 5''), 114.93 (CN-3), 94.85 (C_q-3), 71.53 ($\underline{C}H_2COCH_3$), 26.46 ($CH_2CO\underline{C}H_3$), 15.41 ($\underline{C}H_3$-3''); (+)-ESIMS *m/z* 389.6 ($[M+H]^+$, 36), 391.6 ($[M+H+2]^+$, 14), 411.4 ($[M+Na]^+$, 100), 413.2 ($[M+Na +2]^+$, 45).

General procedure for the synthesis of furo[2,3-*b*]pyridines (3a-f) and (6a-d)

A mixture of carbonitrile compound **2** or **5** (1 mmol), and sodium methoxide (0.054 g, 1 mmol) in MeOH (40 ml) was refluxed for 2-3 h. The resulting reaction mixture was cooled to room temperature, and the formed precipitate was collected by suction filtration, washed with cold MeOH (20 mL) and dried to afford the corresponding furo[2,3-*b*]pyridine compound **3** and **6** (Scheme 1).

1-(3-Amino-6-(2,5-dichlorothiophen-3-yl)-4-(pyridin-3-yl)furo[2,3-*b*]pyridin-2-yl)ethan-1-one (3a). Deep yellow precipitate; mp 238-240 °C; yield (92%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.90 (d, *J* =1.79 Hz, 1H, H-2''), 8.83 (dd, *J* = 4.83, 1.45 Hz, 1H, H-6''), 7.96 (dt, *J* =7.88, 1.84 Hz, 1H, H-4''), 7.81 (s, 1H, H-5), 7.55 (q, *J* =7.66, 4.70 Hz, 1H, H-5''), 7.51 (s, 1H, H-4'), 5.40 (s, 2H, $N\underline{H_2}$), 2.58 (s, 3H, $COC\underline{H_3}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ =190.46 (C=O), 159.72 (C_q-7a), 144.07, 138.06, 136.72, 136.14, 134.25, 132.08, 126.98, 120.75, 109.80 (C_q-2, 3, 3a, 4, 6, 2', 3', 5', 3''), 150.97, 149.02, 135.90, 127.83, 123.84, 119.05 (CH-5, 4', 2'' ,4'', 5'', 6''), 26.40 ($CO\underline{C}H_3$); (+)- ESIMS *m/z* 404.2 ($[M+H]^+$, 100), 406.1 ($[M+H+2]^+$,

70), 408.3 ([M+H+4]$^+$, 15), 806.3 ([2M]$^+$,13), 808.4 ([2M+2]$^+$, 23), 810.3 ([2M+4]$^+$, 11); (+)-HRESIMS *m/z* 404.0017 [M+H]$^+$, 405.9988 [M+H+2]$^+$, (calculated for $C_{18}H_{12}Cl_2N_3O_2S$, 404.0022).

1-(3-Amino-6-(2,5-dichlorothiophen-3-yl)-4-(furan-2-yl)furo[2,3-*b*]pyridin-2-yl)ethan-1-one (3b). Yellow precipitate; mp 214-216 °C; yield (95%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.02 (d, *J* =5.50 Hz, 1H, H-3''), 8.01 (s, 1H, H-5), 7.76 (d, *J* =0.81 Hz, 1H, H-5''), 7.48 (s, 1H, H-4'), 7.14 (d, *J* =3.48 Hz, H-4''), 6.69 (s, 2H, N$\underline{H}_2$), 2.56 (s, 3H, COC$\underline{H}_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 189.69 (C=O), 160.57 (C_q-7a), 151.01, 150.48, 144.16, 138.00, 136.20, 134.95, 126.78, 115.49, 109.33 (C_q-2, 3, 3a, 4, 6, 2', 3', 5' ,2''), 144.72, 127.81, 114.14, 113.09, 112.42 (CH-5, 4', 3'', 4'', 5''), 26.24 (CO$\underline{C}H_3$); (+)-ESIMS *m/z* 393.3 ([M+H]$^+$, 38), 395.3 ([M+H+2]$^+$, 26), 397.3 ([M+H+4]$^+$, 100), 415.5 ([M+Na]$^+$, 3), 417.5 ([M+Na+2]$^+$, 51), 419.5 ([M+ Na +4]$^+$, 68), 806.8 ([2M+Na]$^+$, 37), 808.9 ([2M+Na+2]$^+$, 49), 810.9 ([2M+Na+4]$^+$, 27).

1-(3-Amino-6-(2,5-dichlorothiophen-3-yl)-4-(thiophen-2-yl)furo[2,3-*b*]pyridin-2-yl)ethan-1-one (3c). Yellow precipitate; mp 227-228 °C; yield (93%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.86 (s, 1H, H-5), 7.60 (d, *J* = 4.55 Hz, 1H, H-5''), 7.49 (s, 1H, H-4'), 7.46 (d, *J* = 2.71 Hz, 1H, H-3''), 7.29-7.25 (m, 1H, H-4''), 5.82 (s, 2H, N$\underline{H}_2$), 2.57 (s, 3H, COC$\underline{H}_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 190.20 (C=O), 159.99 (C_q-7a), 150.69, 140.55, 137.5, 137.33, 136.17, 133.87, 126.79, 117.74, 109.37 (C_q-2, 3, 3a, 4, 6, 2', 3', 5', 2''), 128.67, 128.59, 128.52, 127.83, 119.34 (CH-5, 4', 3'', 4'', 5''), 26.33 (CO$\underline{C}H_3$); (+)-ESIMS *m/z* 409.2 ([M+H]$^+$, 100), 411.2 ([M+H+ 2]$^+$, 74), 413.4 ([M+H+4]$^+$, 59), 431.1 ([M+Na]$^+$, 60), 432.8 ([M+Na+2]$^+$, 49), 435.3 ([M+Na+4]$^+$, 23), 838.7 ([2M+Na]$^+$, 38), 840.7 ([2M+Na+2]$^+$, 65), 842.6 ([2M+Na+4]$^+$, 40); (+)-HRESIMS *m/z* 408.9630 [M+H]$^+$, 410.9600 [M+H+2]$^+$, 412.9579 [M+H+4]$^+$, (calculated for $C_{17}H_{11}Cl_2N_2O_2S_2$, 408.9634).

1-(3-Amino-6-(2,5-dichlorothiophen-3-yl)-4-(3-methylthiophen-2-yl)furo[2,3-*b*]pyridin-2-yl)ethan-1-one (3d). Yellow precipitate; mp 145-146 °C; yield (88%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.51 (s, 1H, H-5), 7.49 (s, 1H, H-4'), 7.46 (d, *J* = 5.30 Hz, 1H, H-5''), 7.06 (d, *J* = 5.00 Hz, 1H, H-4''), 5.40 (s, 2H, N$\underline{H}_2$), 2.56 (s, 3H, COC$\underline{H}_3$), 2.25 (s, 3H, C$\underline{H}_3$-3''); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 190.05 (C=O), 159.64 (C_q-7a), 150.65, 149.964, 140.33, 137.34, 133.91, 130.131, 124.27, 120.65, 119.18, 111.30 (C_q-2, 3, 3a, 4, 6, 2', 3', 5' ,2'', 3''), 130.85, 127.90, 126.68, 120.37 (CH-5 ,4', 4'', 5''), 26.25 (CO$\underline{C}H_3$), 14.55 ($\underline{C}H_3$-3''); (+)-ESIMS *m/z* 423.2 ([M+H]$^+$, 100), 425.2 ([M+H+2]$^+$, 71), 427.4 ([M+H+4]$^+$, 21), 445.1 ([M+Na]$^+$, 27), 446.8 ([M+Na+2]$^+$, 19), 866.7 ([2M+Na]$^+$, 12), 868.6 ([2M+Na+2]$^+$, 14), 870.5 ([2M+Na+4]$^+$, 8); (+)-HRESIMS *m/z* 422.9785 [M+H]$^+$, 424.9756 [M+H+2]$^+$, (calculated for $C_{18}H_{13}Cl_2N_2O_2S_2$, 422.9790).

1-(3-Amino-6-(2,5-dichlorothiophen-3-yl)-4-(5-methylfuran-2-yl)furo[2,3-*b*]pyridin-2-yl)ethan-1-one (3e). Yellow precipitate; mp 221-223 °C; yield (95%); ^{1}H NMR (300 MHz, CD_2Cl_2): δ = 7.97 (s, 1H, H-5), 7.09 (d, *J* = 3.29 Hz, 1H, H-3''), 6.74 (s, 1H, H-4'), 6.33 (d, *J* = 3.20 Hz, 1H, H-4''), 5.35 (s, 2H,

$N\underline{H_2}$), 2.52 (s, 6H, $COC\underline{H_3}$, $C\underline{H_3}$-5"); ^{13}C NMR (75 MHz, CD_2Cl_2) δ = 189.51 (C=O), 161.08 (C_q-7a), 156.06, 150.70, 149.69, 144.78, 138.47, 135.64, 128.39, 126.89, 115.31, 111.04 (C_q-2, 3, 3a, 4, 6, 2', 3', 5' ,2", 5"), 128.26, 114.18, 113.72, 109.76 (CH-5 ,4', 3", 4"), 26.25 ($CO\underline{C}H_3$), 14.12 ($\underline{C}H_3$-5"); (+)-ESIMS *m/z* 407.2 ([M+H]$^+$, 13), 409.3 ([M+H+2]$^+$, 9), 429.2 ([M+Na]$^+$, 100), 431.2 ([M+Na+2]$^+$, 70), 433.2 ([M+Na+4]$^+$, 19), 834.7 ([2M+Na]$^+$, 45), 836.6 ([2M+Na+2]$^+$, 75), 838.6 ([2M+Na+4]$^+$, 41); (+)-HRESIMS *m/z* 428.9833 [M+Na]$^+$, 430.9804 [M+Na+2]$^+$, (calculated for $C_{18}H_{12}Cl_2N_2O_3SNa$, 428.9838).

1-(3-Amino-6-(2,5-dichlorothiophen-3-yl)-4-(1-methyl-1*H*-pyrrol-2-yl)furo[2,3-*b*]pyridin-2-yl)ethan-1-one (3f). Yellow precipitate; mp 144-145 °C; yield (88%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.90 (s, 1H, H-5), 7.47 (s, 1H, H-4'), 6.87 (dd, *J* = 2.73, 1.54 Hz, 1H, H-5"), 6.55 (dd, *J* = 3.65, 1.65 Hz, 1H, H-3"), 6.25 (dd, *J* = 3.79, 1.58 Hz, 1H, H-4"), 5.33 (s, 2H, $N\underline{H_2}$), 3.52 (s, 3H, $NC\underline{H_3}$-1"), 2.51 (s, 3H, $COC\underline{H_3}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 189.85 (C=O), 162.37 (C_q-7a), 151.26, 150.42, 141.64, 138.61, 137.62, 126.8 3, 126.66, 119.70, 108.78 (C_q-2, 3, 3a, 4, 6, 2', 3', 5' ,2"), 128.00, 127.94, 118.82, 112.03, 109.24 (CH-5 ,4', 3", 4", 5"), 35.97 ($\underline{C}H_3$-1"), 26.43 ($CO\underline{C}H_3$).

1-(3-Amino-6-(5-chlorothiophen-2-yl)-4-(pyridin3-yl)furo[2,3-*b*]pyridin-2-yl)ethan-1-one (6a). Deep yellow precipitate; mp 222-224 °C; yield (93%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.88 (d, *J* =1.64 Hz, 1H, H-2"), 8.83 (dd, *J* = 4.77, 1.29 Hz, 1H, H-6"), 7.94 (dt, *J* =7.82, 1.59 Hz, 1H, H-4"), 7.55 (q, *J* =7.61, 4.87 Hz, 1H, H-5"), 7.50 (d, *J* =4.01 Hz, 1H, H-3'), 7.49 (s, 1H, H-5), 6.97 (d, *J* =3.99 Hz, 1H, H-4'), 5.37 (s, 2H, $N\underline{H_2}$), 2.56 (s, 3H, $COC\underline{H_3}$); ^{13}C NMR (75 MHz, CD_2Cl_2)δ =190.08 (C=O), 160.17 (C_q-7a), 151.83, 144.91, 142.74, 137.38, 134.37, 134.30, 132.29, 109.92 (C_q-2, 3, 3a, 4, 6, 2', 5', 3"), 151.28, 149.30, 136.11, 128.23, 126.05, 124.10, 115.25 (CH-5, 3', 4', 2", 4", 5", 6"), 26.40 ($CO\underline{C}H_3$); (+)-ESIMS *m/z* 370.2 ([M+H]$^+$, 45), 372.2 ([M+H+2]$^+$, 15), 392.1 ([M+Na]$^+$, 100), 394.1 ([M+Na+2]$^+$, 40); (+)-HRESIMS *m/z* 392.0235 [M+Na]$^+$, 394.0207 [M+Na+2]$^+$, (calculated for $C_{18}H_{12}ClN_3O_2SNa$, 392.0231).

1-(3-Amino-6-(5-chlorothiophen-2-yl)-4-(furan-2-yl)furo[2,3-*b*]pyridin-2-yl)ethan-1-one (6b):
Deep yellow precipitate; mp 255-256 °C; yield (90%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.75 (d, *J* = 1.02 Hz, 1H, H-5"), 7.72 (s, 1H, H-5), 7.52 (d, *J* = 3.98 Hz, 1H, H-3'), 7.14 (d, *J* = 3.45 Hz, 1H, H-3"), 6.97 (d, *J* = 3.99 Hz, 1H, H-4'), 6.70 (dd, *J* = 3.34, 1.74 Hz, 1H, H-4"), 6.64 (s, 2H, $N\underline{H_2}$), 2.55 (s, 3H, $COC\underline{H_3}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 189.71 (C=O), 160.83 (C_q-7a), 151.01, 150.80, 142.39, 138.58, 135.37, 133.96, 124.56, 107.12 (C_q-2, 3, 3a, 4, 6, 2', 5', 2"), 144.64, 127.53, 125.30, 113.01, 112.18, 110.50 (CH-5, 3', 4', 3", 4", 5"), 26.27 ($CO\underline{C}H_3$); (+)-ESIMS *m/z* 359.2 ([M+H]$^+$, 100), 361.2 ([M+H+2]$^+$, 35), 381.1 ([M+Na]$^+$, 21), 383.1 ([M+Na+2]$^+$, 8), 738.8 ([2M+Na]$^+$, 10), 741.7 ([2M+Na+2]$^+$, 8); (+)-HRESIMS *m/z* 359.0247 [M+H]$^+$, 361.0219 [M+H+2]$^+$, (calculated for $C_{17}H_{12}ClN_2O_3S$, 359.0252).

1-(3-Amino-6-(5-chlorothiophen-2-yl)-4-(thiophen-2-yl)furo[2,3-*b*]pyridin-2-yl)ethan-1-one (6c). Orange precipitate; mp 235-236 °C; yield (93%); ^{1}H NMR (300 MHz, $CDCl_3$): δ =7.59 (dd, *J* =4.98, 0.78 Hz, 1H, H-5''), 7.50 (d, *J* =4.02 1H, H-3'), 7.47 (s, 1H, H-5), 7.44 (dd, *J* =3.50, 0.83 Hz, 1H, H-3''), 7.27-7.26 (m, 1H, H-4''), 6.96 (d, *J* =4.00 Hz, 1H, H-4'), 5.77 (s, 2H, $N\underline{H}_2$), 2.56 (s, 3H, $COC\underline{H}_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ =189.89 (C=O), 160.18 (C_q-7a), 151.48, 142.05, 140.70, 137.58, 137.32, 134.15, 133.75, 109.06 (C_q-2, 3, 3a, 4, 6, 2', 5', 2''), 128.70, 128.58, 128.44, 127.59, 125.57, 115.23 (CH-5, 3', 4', 3'', 4'', 5''), 26.25 ($CO\underline{C}H_3$); (+)-ESIMS *m/z* 375.4 ($[M+H]^+$, 33), 377.4 ($[M+H+2]^+$, 14), 397.3 ($[M+Na]^+$, 100), 399.2 ($[M+Na+2]^+$, 46), 770.9 ($[2M+Na]^+$, 19), 772.6 ($[2M+Na+2]^+$, 13); (+)-HRESIMS *m/z* 396.9842 $[M+Na]^+$, 398.9813 $[M+Na+2]^+$, (calculated for $C_{17}H_{11}ClN_2O_2S_2Na$, 396.9843).

1-(3-Amino-6-(5-chlorothiophen-2-yl)-4-(3-methylthiophen-2-yl)furo[2,3-*b*]pyridin-2-yl)ethan-1-one (6d). Yellow precipitate; mp 225-227 °C; yield (95%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.46 (dd, *J* = 4.73, 3.80 Hz, 2H, H-3', H-5''), 7.39 (s, 1H, H-5), 7.05 (d, *J* = 4.96 Hz, 1H, H-4''), 6.95 (d, *J* = 3.85 Hz, 1H, H-4'), 5.51 (s, 2H, $N\underline{H}_2$), 2.53 (s, 3H, $COC\underline{H}_3$), 2.22 (s, 3H, $C\underline{H}_3$-3''); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 189.78 (C=O), 159.83 (C_q-7a), 151.45, 142.11, 140.49, 137.64, 134.12, 130.02, 124.07, 115.02, 110.02 (C_q-2, 3, 3a, 4, 6, 2', 5', 2'', 3''), 130.71, 127.60, 126.64, 125.57, 116.18 (CH-5, 3', 4', 4'', 5''), 26.20 ($CO\underline{C}H_3$), 14.45 ($\underline{C}H_3$-3''); (+)-ESIMS *m/z* 389.6 ($[M+H]^+$, 36), 391.6 ($[M+H+2]^+$, 14), 411.4 ($[M+Na]^+$, 100), 413.2 ($[M+Na+2]^+$, 45).

Biological activity test

The antibacterial activity of the synthetic carbonitrile compounds (**2a-d**), (**2f**), (**5a-d**), and furo[2,3-*b*]pyridines (**3a-d**), (**3f**), and (**6a-d**) were evaluated on eight different microorganisms which included the Gram positive bacteria; *Streptococcus pneumonia*, *Streptococcus pyogenes*, *Staphylococcus epidermedis*, *Staphylococcus aureus*, *Enterococcus faecalis*, and the Gram negative bacteria; *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli*. Similarly the same compounds were tested for cytotoxic activity against MCF7 breast cancer cells.

Preparation of the synthetic compound solution

The synthesized compounds were dissolved in DMSO as 10 mM stock solutions. Then, the appropriate concentration was prepared by dilution in either water for antibacterial activity (DMSO did not exceed 5%) or DMEM media for cytotoxic activity (DMSO $\leq$1%).

Antibacterial activity

The antibacterial activity was tested using agar-disk diffusion method. Mueller-Hinton agar plates were used as culture plates, 50 μL of determined bacterial culture (1-2 × 10^8 CFU/mL, equivalent to 0.5 McFarland) was added and spread onto the agar plate (100 mm in diameter). Whatmann paper no. 1 cut as 6 mm discs were impregnated in the tested compound (250 μM) and placed at the surface of the agar plate. The plates were incubated at 37 ^oC overnight (14-16 h) and the zone of inhibition was measured in the next morning using ruler.

Cytotoxicity assay toward MCF7 cell lines

MCF-7 cells were cultured in 25 t-flask in medium containing Dulbecco's Modified Eagle's Medium (DMEM) with glucose, 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C with 5% CO_2, 95% air and complete humidity. Once reached ~80% confluency, they were trypsinized using 0.05% trypsin/EDTA to detach and counted using hemocytometer after treatment with trypan blue. The cells were then resuspended at a concentration of 5×10^3 cells/cm^2 and seeded into 96-well plate (i.e., 200 μL/well). Some wells were left cell-free, i.e. as blank control. At 70-80% confluency (72 h post seeding), the cultured cells were treated with 100 μM of the chemical compounds in DMEM culture media. Four wells were remained untreated as control. After 72 h, the treatment media were removed and replaced by fresh media and MTT assay was performed. 3-(4,5-Dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide (MTT) solution was prepared in phosphate buffered saline (PBS) according to the company protocol. Then, 50 μL of MTT reagent and 200 μL of DMEM without phenol red were added to each well, with the exception of the cell-free blank wells. Cells were incubated for 4 h at 37 °C with 5% CO_2, 95% air and complete humidity and the MTT solution was removed and replaced with 200 μL of DMSO. The plate was incubated for another 5 min at room temperature, and the absorbance of each well was determined using an ELISA plate reader at a wavelength of 570 nm. The percentage of cell viability was calculated by taking the absorbance ratio between treated cell culture and the untreated control multiplied by 100 (percentage of control, %).

ACKNOWLEDGEMENTS

We are thankful to Al al-Bayt University (Mafraq, Jordan) for financial support.

REFERENCES AND NOTES

1. R. Dua, S. Shrivastava, S. K. Sonwane, and S. K. Srivastava, *Adv. Biol. Res.*, 2011, **5**, 120.
2. A. T. Balaban, D. C. Oniciu, and A. R. Katritzky, *Chem. Rev.*, 2004, **104**, 2777.
3. C. J. Suckling, 'Why does heterocyclic chemistry matter? University of Strathclyde, Retrieved from

https://www.openaccessgovernment.org/wp-content/uploads/2017/11/Prof-Colin-Suckling-ebook-Oct-17-WEB.pdf', 2017.

4. D. Ramesh, C. Chandrashekhar, and V. P. Vaidya, *Indian J. Org. Chem.*, 2008, **47B**, 753.
5. R. Musiol, T. Magdziarz, and A. Kurczyk, 'Science against microbial pathogens: communicating current research and technological advances', ed. by A. Méndez-Vilas, Formatex, Badajoz, Spain, 2011, p. 72.
6. A. Adamska-Szewczyka, K. Glowniak, and T. Baj, *Curr. Issues Pharm. Med. Sci.*, 2016, **29**, 33.
7. J. Zhou, G. Xie, and X. Yan, 'Book Encyclopedia of Traditional Chinese Medicines', Springer, Berlin, 2011, p.381.
8. G. S. Kumar, Y. Poornachandra, S. K. Gunda, K. R. Reddy, J. Mohmed, K. Shaik, C. G. Kumar, and B. Narsaiah, *Bioorg. Med. Chem. Lett.*, 2018, **28**, 2328.
9. R. W. Fitch, T. F. Spande, H. M. Garraffo, H. J. C. Yeh, and J. W. Daly, *J. Nat. Prod.*, 2010, **73**, 331.
10. S. A. Said, A. E.-G. E. Amr, H. A. El- Sayed, M. A. Al- Omar, and M. M. Abdalla, *Int. J. Pharmacol.*, 2015, **11**, 502.
11. D. Wang, M. Shen, Y. Wang, J. Hu, J. Zhao, and P. Yu, *Asian J. Org. Chem.*, 2018, **7**, 879.
12. F. Fumagalli and F. da Silva Emery, *J. Org. Chem.*, 2016, **81**, 10339.
13. Z. Wu, R. G. Robinson, S. Fu, S. F. Barnett, D. Defeo-Jones, R. E. Jones, A. M. Kral, H. E. Huber, N. E. Kohl, G. D. Hartman, and M. T. Bilodeau, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 2211.
14. G. Z. Zheng, P. Bhatia, J. Daanen, T. Kolasa, M. Patel, S. Latshaw, O. F. El Kouhen, R. Chang, M. E. Uchic, L. Miller, M. Nakane, S. G. Lehto, M. P. Honore, R. B. Moreland, J. D. Brioni, and A. O. Stewart, *J. Med. Chem.*, 2005, **48**, 7374.
15. Z. A. Knight, B. Gonzalez, M. E. Feldman, E. R. Zunder, D. D. Goldenberg, O. Williams, R. Loewith, D. Stokoe, A. Balla, B. Toth, T. Balla, W. A. Weiss, R. L. Williams, and K. M. Shokat, *Cell*, 2006, **125**, 733.
16. M. Al-Refai, M. Ibrahim, A. Al-Fawwaz, and A. Geyer, *Eur. J. Chem.*, 2018, **9**, 375.
17. M. Al-Refai, *Asian J. Chem.*, 2015, **27**, 725.