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Synthesis and antimicrobial activity of 4-trifluoromethylpyridine nucleosides

DOI 10.1515/hc-2017-0019
Received February 1, 2017; accepted February 2, 2017; previously published online April 19, 2017

Abstract: 4-Trifluoromethylpyridine derivatives **4–8** represent good candidates for the discovery of new antibacterial agents. Fluorinated pyridine nucleosides **4–7** and non-nucleoside analogues **8a,b** were synthesized and evaluated for their antibacterial activities against *Staphylococcus aureus, Bacillus infantis, Escherichia coli* and *Stenotrophomonas maltophilia*. The minimum inhibitory concentrations (MICs) of the new nucleosides **4–7** range from 1.3 to 4.9 μg/mL and MICs of fluoroaryl derivatives **8a,b** are in the range of 1.8–5.5 μg/mL. Activity of amoxicillin, the reference drug, is 1.0–2.0 μg/mL under similar conditions.

Keywords: antimicrobial; fluoropyridine; gHMBC; nucleosides; synthesis.

Introduction

The increasing prevalence of life-threatening antibacterial diseases and rapidly growing trend of antimicrobial resistance necessitate the development of new and more effective antimicrobial agents. The 2-pyridones represent a unique class of pharmacophores present in various therapeutic agents [1]. They exhibit antitumor [2], antimalarial [3], analgesic [4] and anti-HIV properties [5] and inhibit *in-vitro* and *in-vivo* the bacterial type II DNA topoisomerases, which includes two highly homologous enzymes: DNA gyrase and topoisomerase IV [6]. Fluorine substitution can bring substantial changes in the physical, chemical and biological properties [7] of bioactive molecules.

One important strategy in this respect is chemical synthesis of nucleosides. The use of nucleoside analogues can be considered a novel option as they are expected to act at the genomic level, thereby interfering with the transcription or replication processes required for microbial survival. As there are no alternative pathways in the pathogens for these basic metabolic processes, the nucleoside analogues, by inhibiting these basic pathways, are effective antimicrobial agents [8]. For example, some nucleosides exhibit antibacterial activity by specific inhibition of cell-wall peptidoglycan biosynthesis [9], certain bacterial enzymes, such as purine nucleoside phosphorylases [10] and DNA ligases [11]. Adenosine analogues such as cordycepin [12], oxetanocin [13], formycins [14], toyocamycin and its derivatives [15] show biological activity including antibiotic properties.

Designing expeditious routes to obtain nucleosides is of paramount importance to organic and medicinal chemists. Three general glycosylation methods dominate in nucleoside synthesis. The Fischer approach [16] employs nucleophilic displacement of an α-halogen by the metal salt of a heterocycle to furnish the nucleoside. The fusion method consists of heating a peracylated sugar with a nucleobase. The mildest and most popular method is the Vorbrüggen variant [17, 18] of the Hilbert-Johnson reaction, which makes use of a fully protected sugar and couples it with a silylated nucleobase in the presence of Lewis acids, typically tin tetrachloride ($SnCl_4$) or trimethylsilyl trifluoromethanesulfonate (TMSOTf), among others [19], to provide the protected nucleoside.

In this paper, we describe synthesis of a new class of 4-trifluoromethylpyridine derivatives **4–8.** The 4-trifluoromethylpyridine moiety is attached to a sugar moiety (pentose or hexose) in nucleosides **4–7** and to a fluorinated aryl group in analogues **8a,b**. These products were evaluated as antibacterial agents.

Results and discussion

Chemistry

3-Cyano-4-trifluoromethyl-6-(thiophen-2′-yl)-2-pyridone **3** was obtained in one-pot condensation of the 1,3-dicarbonyl substrate **1** with equimolar amount of cynoacetamide **2** in refluxing ethanol. The reaction afforded a single product **3** with an 89% yield (Scheme 1). The

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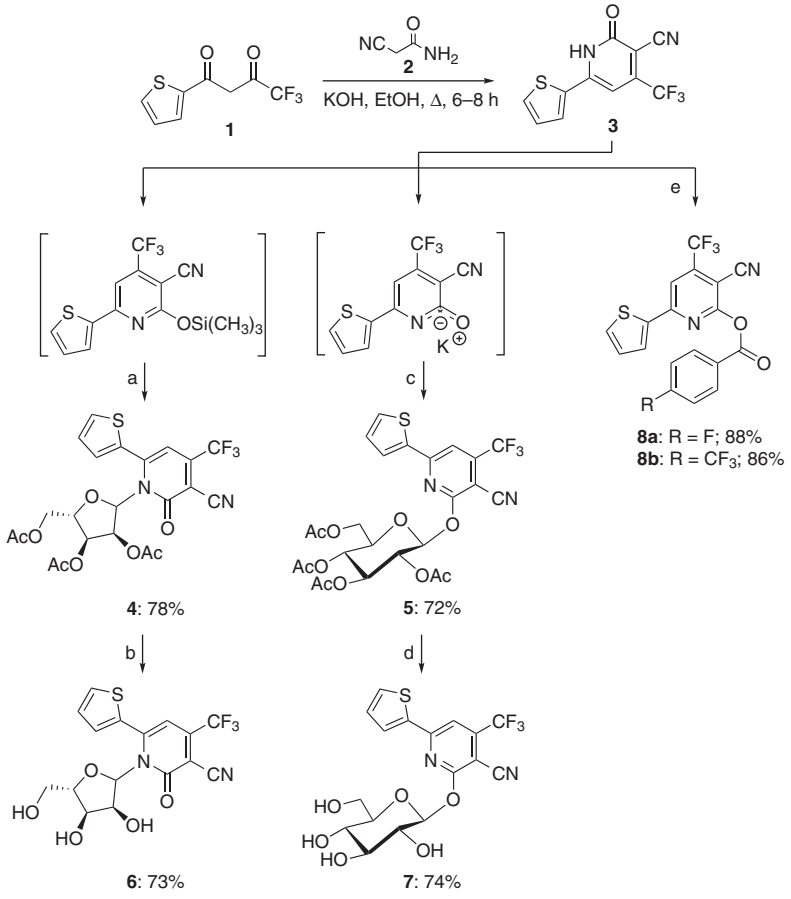

Scheme 1 Reagents and conditions: (a) HMDS, $(NH_4)_2SO_4$, reflux, 2 h, then 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose, TMSOTf/CH_3CN, 0–25°C; (b) TEA/MeOH/H_2O; (c) aqueous KOH, 2 h, 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide, acetone; (d) TEA/MeOH/H_2O; (e) aroyl chloride, pyridine, CH_3CN.

nuclear magnetic resonance (NMR) spectra of **3** are fully consistent with the assigned structure [20]. The gradient heteronuclear multiple bond correlation (gHMBC) spectrum of **3** (Figure 1) shows a correlation between the signal of thiophene-H3 (δ 8.15) and pyridone-C5 (δ 105.9). The proton signal at δ 8.15 also correlates to the C-6 signal of pyridone-C6 at δ 143.7. Another correlation is observed between the pyridone-H5 signal at δ 7.63 and the signals of C-2′ and C-3′ of the thiophene at δ 129.7 and 140.7, respectively. The *g*HMBC spectrum does not show any correlation between the thiophene protons and the carbon atom of the CN group at δ 120.5. Based on these observations, the CF_3 group must be located at position 4 and the thiophen-2′-yl group at position 6 of the 2-pyridone moiety. The infrared (IR) spectrum of compound **3** shows the most significant absorption band for the amide carbonyl group at 1661 cm^{-1}. A strong band at 2230 cm^{-1} corresponds to the CN group and a NH signal appears at 3419 cm^{-1}. The UV-vis spectrum of compound **3** (Figure 2) shows two absorption bands at 360 nm and 259 nm. The band at 259 nm can be attributed to the n-σ* transition, while the band at 360 nm is apparently a combination of n-π* and π-π* transitions.

The β-D-ribofuranosyl-2-pyridone **4** was prepared in 68% yield by reaction between a silyl derivative of compound **3** and 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (Scheme 1). The reaction of a nucleophilic base normally takes place from the less sterically hindered β-face of the intermediate cation to give the β-isomer. The structure of the obtained riboside **4** was confirmed using analytical and spectroscopic data IR, NMR and elemental analysis. The IR spectrum of **4** shows bands at 2215 and 1748 cm^{-1} indicating the presence of cyano and acetoxy groups, respectively. On the other hand, a sharp peak at 1652 cm^{-1} for the carbonyl group of the 2-pyridone indicates that the sugar is linked to the pyridine ring through the nitrogen atom giving the *N*-riboside. In the ^1H NMR spectrum of **4**, the anomeric proton H-1″ resonates as a doublet at δ 6.41 with a coupling constant $J_{H1''-H2''}$=4.3 Hz, indicating that the obtained product **4** is a β-isomer. The ^{13}C-NMR

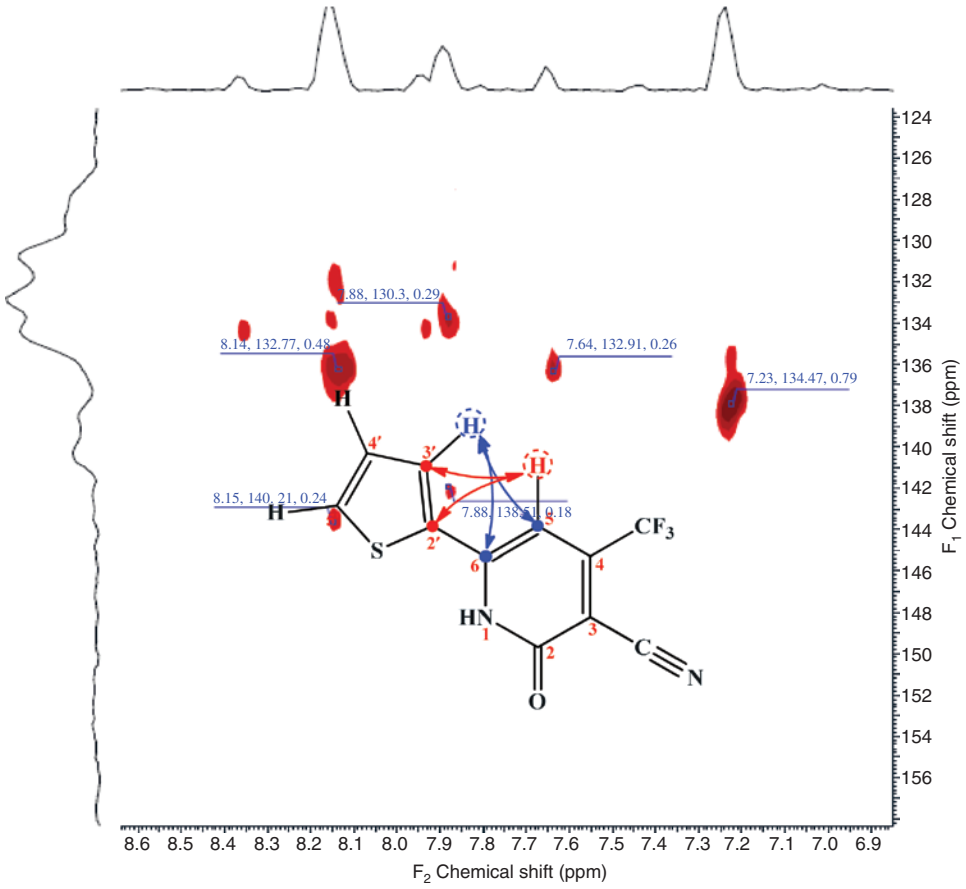

Figure 1 The gHMBC spectrum in DMSO-d_6 of 2-pyridone **3**.

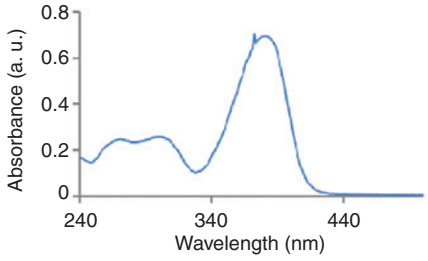

Figure 2 A UV-vis absorption of **3** in 0.01 M Tris–KCl buffer solution, pH 7.4. The buffer was prepared by dissolving 10.0 mM of tris(hydroxymethyl)aminomethane hydrochloride (1.58 g), 1.0 mM Na_2EDTA (0.37 g) and 100.0 mM KCl (7.45 g) in 1.0 L of deionized water.

spectrum shows a signal at δ 87.2 corresponding to the C-1″ atom of the β-configuration.

The *O*-glucoside derivative **5** was prepared by the reaction of a potassium salt of **3** with an activated sugar at room temperature. In principle, the ambident 2-pyridone anion contains two reactive nucleophilic centers, the pyridine ring nitrogen and the carbonyl oxygen at C-2. A sole *O*-nucleoside product has been isolated without the formation of the *N*-nucleoside. Apparently, α-acetobromoglucose undergoes a reaction with potassium salt of **3** through a Walden inversion, to yield the corresponding glucoside **5**. Since Walden inversion occurs in the halogen replacement reaction, the reaction involving halide with the α-configuration yields the β-isomer. The IR spectrum of **5** shows the presence of acetoxy carbonyl groups at 1749 cm^{-1}. A strong band appears at 2231 cm^{-1} corresponding to the CN group. The ^{1}H-NMR spectrum of **5** shows the β-configuration. The anomeric proton H-1″ appears as a doublet at a relatively low field, δ 6.13. The spin-spin coupling constant, $J_{H1''-H2''} = 8.0$ Hz, indicates that the protons H-1″ and H-2″ are in diaxial orientation. The H-2″ and H-3″ signals appear as a multiplet at δ 5.37–5.45. The H-5″ and H-4″ signals appear as a multiplet at δ 5.15–5.24. The H_2-6′,6″ signals appear as a multiplet at δ 4.08–7.11. Protons of the four acetoxy groups appear as four singlets at δ 1.94, 2.03, 2.05 and 2.06. The pyridine H-5 proton resonates at δ 7.62 and this downfield position is consistent with the formation of *O*-glucoside [20]. The structure of compound **5** was further confirmed by the analysis of the ^{13}C-NMR

spectrum which shows a signal at δ 109.7 corresponding to the C-1″ atom of the *β*-configuration. The signals at δ 154.7 and 141.5 are assigned to C-6 and C-4 of the pyridine ring. Signals at δ 168.8, 169.3, 170.4 and 170.7 belong to the four acetoxy carbonyl carbons. Signals corresponding to pyridine C-5 and C-3 resonate at δ 94.9 and 110.8, respectively. The signals at δ 72.7, 70.0, 68.0, 61.6 and 60.4 can be assigned to C-2″, C-3″, C-4″, C-5″ and C-6″, respectively. On the other hand, the C-2 atom of pyridine appears at δ 162.4 and the signal for the nitrile carbon is observed at δ 119.6.

Deacetylation of 3-cyano-1-(2″,3″,5″-tri-*O*-acetyl-*β*-D-ribofuranosyl)-4-trifluoromethyl-6-(thiophen-2′-yl)-2-pyridone **4** afforded nucleoside **6** in 73% yield. The IR absorption spectrum of compound **6** shows a characteristic band at 3428 cm^{-1} for the sugar hydroxy groups. Another band at 2206 cm^{-1} can be assigned to the nitrile group. A sharp absorption band at 1655 cm^{-1} is attributed to the stretching vibration of the carbonyl group of 2-pyridone. In addition, the furanoside ring system shows a broad band at 1050 cm^{-1}. The ^{1}H-NMR spectrum of compound **6** shows a doublet at δ 5.89 corresponding to the anomeric proton of the ribosyl moiety; a coupling constant of 4.0 Hz is consistent with the diaxial orientation of the H-1″ and H-2″ protons and indicates the formation of the *β*-isomer. The ^{13}C-NMR spectrum of **6** is characterized by a signal at δ 83.5 corresponding to the C1″ atom of the ribosyl residue.

Hydrolysis, to remove the acetyl groups in compound **5** was carried out using triethylamine (TEA) in methanol and produced the free nucleoside **7** in 74% yield. The IR spectrum of **7** shows a characteristic band at 3403 cm^{-1} due to the sugar four hydroxy groups. Another band at 2206 cm^{-1} can be assigned to the nitrile group. The IR absorption of ether linkage of the glucopyranoside ring system appears at 1036 cm^{-1}. The ^{1}H NMR spectrum of **7** shows a doublet at δ 7.24 corresponding to the anomeric proton of the glucose moiety. The coupling constant of 8.0 Hz is consistent with the diaxial orientation of the H-1″ and H-2″ protons indicating the formation of a single *β*-isomer. There are four signals corresponding to four hydroxy protons that are exchangeable with D_2O. The ^{13}C NMR spectrum of **7** is characterized by a signal at δ 97.3 corresponding to the C-1 atom of the glucose residue.

Non-nucleosides **8a,b** were synthesized by the reaction of **3** with an aroyl chloride. This reaction afforded the corresponding products within 30 min in yields of 87% and 85% (Scheme 1). Structures of **8a,b** were confirmed by elemental analysis and spectral data. The IR spectrum of **8b** shows the presence of one carbonyl group at 1760 cm^{-1} corresponding to the ester group, which indicates the formation of *O*-benzoylation product. The band at 1603 cm^{-1} accounts for the C=C stretch in the aromatic system. The strong band at 2231 cm^{-1} corresponds to the CN group at C-3. The ^{1}H NMR spectrum contains two doublets at δ 7.95 and 8.35 with coupling constants J = 5.0 Hz and 3.7 Hz for the thiophene protons H-5 and H-3 and the resonance for H-4 of the thiophene appears as a triplet at δ 7.29. The H-5 of pyridine resonates in low field at δ 8.55, which confirms the formation of *O*-benzoylated compound. The ^{13}C NMR spectrum of **8b** is also fully consistent with the discussed structure. A similar analysis confirmed the structure of **8a**.

Antibacterial properties

An agar-diffusion method was used for the determination of the preliminary antimicrobial activity of compounds **3–8**. Amoxicillin was used as a reference drug. The results were recorded for each tested compound as a diameter of an inhibition zone of microbial growth around the disk in mm. The minimum inhibitory concentration (MIC) values were also determined. The inhibition zones (mm) and MIC values (μg/mL) are given in Tables 1 and 2, respectively. As can be seen, the synthesized compounds show good antibacterial activity against all selected bacteria strains with MIC values ranging from 1.3 to 5.5 μg/mL. Compounds **4** and **5** are highly active against *S. maltophilia* with MIC

Table 1 Antibacterial activity of compounds **4–8**.

Compounds	Zone of inhibition (diameter, mm)			
	B. infantis	*E. coli*	*S. aureus*	*S. maltophilia*
4	26	28	18	30
5	32	27	25	29
6	25	23	24	23
7	30	29	26	23
8a	20	20	19	18
8b	28	31	26	24
Amoxicillin	15	16	15	14

Table 2 Minimum inhibitory concentration for compounds **4–8**.

Compounds	Concentration of compounds (μg/mL) to inhibit 10^5 cell/mL			
	B. infantis	*E. coli*	*S. aureus*	*S. maltophilia*
4	4.3	2.7	4.9	1.7
5	3.5	2.3	2.4	1.5
6	4.7	3.1	3.0	2.3
7	3.8	1.3	2.5	2.0
8a	5.5	3.5	5.0	3.6
8b	3.2	1.8	2.8	2.8
Amoxicillin	1	1.5	1.5	2

values of 1.7 and 1.5 μg/mL, respectively. High antibacterial activities of nucleoside **7** (MIC = 1.3 μg/mL) and the non-nucleoside derivative **8b** (MIC = 1.8 μg/mL) against *E. coli* should be noted.

Conclusion

Four new pyridine nucleosides **4–7** and two non-nucleoside analogues **8a,b** were synthesized. Pyridine ribosides **4, 5** and glucosides **6, 7** are potent antibacterial agents. The antibacterial *p*-trifluoromethylbenzoate **8b** is more active than its *p*-fluorobenzoate analogue **8a**.

Experimental

All reagents and chemicals were purchased from Sigma-Aldrich and used without further purification. Melting points were determined using Pyrex capillaries on a Gallenkamp apparatus. IR spectra were recorded with a Thermo Nicolet Nexus 470 FT-IR spectrometer using KBr disks. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained on a Varian Gemini 400 spectrometer in $CDCl_3$ or DMSO-d_6. All exchangeable protons were confirmed by addition of D_2O. Thin-layer chromatography (TLC) was carried out on Merck silica gel F_{254} plates and UV light was used for visualization. Column chromatography was performed on a Merck silica gel. Absorption measurements were carried out using an Agilent 8453 spectrophotometer and 1-cm quartz cells. Elemental Analysis was performed on a Leco Model CHN-600 elemental analyzer.

3-Cyano-4-trifluromethyl-6-(thiophen-2′-yl)-2-pyridone (3) A mixture of 4,4,4-trifluoro-1-(thiophen-2-yl)butane-1,3-dione (10.0 mmol, 2.22 g), 2-cyanoacetamide (11.0 mmol, 0.92 g) and potassium hydroxide (12.0 mmol, 0.67 g) in ethanol (50 mL) was heated under reflux for 6 h. The mixture was cooled, acidified with 0.1 M HCl and the resultant precipitate was crystallized from ethanol: yellow powder; yield 89%; mp 293–294°C; IR: 1661 (C = O), 2230 (CN), 3419 cm^{-1} (NH); ^{1}H NMR (DMSO-d_6): δ 7.23 (m, 1H, thiophene-H4), 7.63 (bs, 1H, pyridone-H5), 7.88, 7.89 (dd, 1H, thiophene-H5, J = 4.9 Hz), 8.15, 8.16 (dd,1H, thiophene-H3, J = 3.7 Hz), 13.57 (bs, 1H, NH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6): δ 105.9 (pyridine-C5), 113.8 (pyridine-C3), 120.5 (CN), 129.7–140.5 (aromatic carbons), 143.7 (pyridine-C6), 154.1 (pyridine-C2), 164.8 (pyridine-C4). Anal. Calcd for $C_{11}H_5F_3N_2OS$: C, 48.89; H, 1.86; N, 10.37; S, 11.87. Found: C, 48.94; H, 1.97; N, 10.28; S, 11.95.

3-Cyano-1-(2″,3″,5″-tri-*O*-acetyl-β-D-ribofuranosyl)-4-trifluoromethyl-6-(thiophen-2′-yl)-2-pyridone (4) A mixture of 2-pyridone **3** (0.01 mol, 2.70 g), 1,1,1,3,3,3 hexamethyldisilazane (HMDS, 60 mL), ammonium sulfate (0.0009 mol, 0.125 g) and 2-3 drops of chlorotrimethylsilane was heated under nitrogen for 2 h. Excess HMDS was removed by distillation, and the residue was dried under reduced pressure for 6 h. The resulting silyl intermediate product was dissolved in anhydrous MeCN (20 mL) and a solution of 1″,2″,3″,5″-tetra-*O*-acetyl-β-D-ribofuranose (0.01 mol, 3.18 g) in 20 mL of MeCN was added with stirring. The mixture was cooled to 5°C, treated with trimethylsilyl trifluoromethanesulfonate (TMSOTf) (0.015 mol, 2.71 mL) and stirred at room temperature for an additional 3 h until the reaction was completed as observed by TLC analysis (ethyl acetate/hexane, 1:1). The mixture was diluted with ethyl acetate (150 mL), and the organic solution was washed with a saturated $NaHCO_3$ solution (50 mL) and then water (2 × 50 mL). The organic layer was dried with anhydrous Na_2SO_4, concentrated under a reduced pressure and the residue of **3** was purified by silica gel chromatography using 1% MeOH in ethyl acetate as an eluent to give the pure product as pale brown powder: yield 78%; mp 218°C; IR: 1652 (C = O), 1748 (acetoxy carbonyl), 2215 cm^{-1} (CN); ^{1}H NMR ($CDCl_3$): δ 2.09, 2.11, 2.12 (3s, 9H, $3CH_3$), 4.32–4.37 (m, 3H, H-5″a, H-5″b, H-3″), 5.32–5.36 (m, 2H, H-2″, H-4″), 6.41 (d, 1H, H-1″, J = 4.3 Hz), 6.85 (s, 1H, pyridine H-5), 7.32 (m, 1H, thiophene-H4), 7.69 (d, 1H, thiophene-H-5, J = 5.0 Hz), 8.24 (d, 1H, thiophene-H3, J = 3.7 Hz); ^{13}C NMR (DMSO-d_6): δ 21.1, 21.2, 21.6 ($3CH_3$), 79.0 (C5″), 79.4 (C3″), 79.7 (C2″), 86.2 (C4″), 87.2 (C1″), 97.2 (pyridine-C5), 111.6 (pyridine-C3), 118.2 (CN), 121.6 (CF_3), 124.3 (thiophene-C3), 127.2 (thiophene-C4), 128.7 (thiophene-C5), 130.0 (pyridine-C4), 141.7 (thiophene-C2), 145.3 (pyridine-C6), 155.8 (pyridine-C2), 170.6 (acetoxy carbonyl carbons). Anal. Calcd for $C_{22}H_{19}F_3N_2O_8S$: C, 50.00; H, 3.62; N, 5.30; S, 6.07. Found: C, 50.09; H, 3.73; N, 5.21; S, 6.87.

3-Cyano-2-(2″,3″,4″,6″-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-4-trifluoromethyl-6-(thiophen-2′-yl)pyridine (5) To a solution of 2-pyridone **3** (0.01 mol, 2.70 g) in aqueous KOH (0.01 mol, 0.56 g) in 6.0 mL distilled water, a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glycopyranosyl bromide (0.011 mol, 4.11 g) in acetone (30 mL) was added. The mixture was stirred at room temperature until the reaction was judged completed by TLC (4–6 h), then treated with CH_2Cl_2 (30 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure at room temperature. Crystallization of the residue from ethanol gave the nucleoside **5** as pale yellow powder: yield 72%; mp 134°C, IR: 1749 (acetoxy carbonyl), 2231 cm^{-1} (CN); ^{1}H NMR ($CDCl_3$): δ 1.94, 2.03, 2.05, 2.06 (4s, 12H, $4CH_3$), 4.08–4.13 (m, 2H, H-6″a, H-6″b), 5.15–5.24 (m, 2H, H-4″, H-5″), 5.37–5.45 (m, 2H, H-2″,H-3″), 6.12, 6.14 (d, 1H, H-1″, J = 8.0 Hz), 7.18 (m, 1H, thiophene-H-4), 7.59 (d, 1H, thiophene-H-5, J = 5.0 Hz), 7.62 (s, 1H, pyridine H-5), 7.75 (d, 1H, thiophene-H3, J = 3.7 Hz); ^{13}C NMR ($CDCl_3$): δ 20.5, 20.6, 20.7, 21.1 ($4CH_3$), 60.4 (C6″), 61.6 (C5″), 68.0 (C4″), 70.0 (C3″), 72.7 (C2″), 94.9 (pyridine-C5), 109.7 (C1″), 110.8 (C3), 119.6 (CN), 122.4 (CF_3), 128.9 (thiophene-C3), 129.2 (thiophene-C4), 132.1 (thiophene-C5), 141.5 (pyridine-C4), 144.5 (thiophene-C2), 154.7 (pyridine-C6), 162.4 (pyridine-C2), 168.8, 169.3, 170.4, and 170.7 (four acetoxy carbonyl carbon). Anal. Calcd for $C_{25}H_{23}F_3N_2O_{10}S$: C, 50.00; H, 3.86; N, 4.66; S, 5.34. Found: C, 50.05; H, 3.97; N, 4.57; S, 5.42.

General procedure for deacetylation of nucleosides, synthesis of 6 and 7

TEA (1.0 mL) was added to a solution of protected nucleoside **4** or **5** (1.0 mmol) in methanol (10 mL) containing 3 drops of water and the mixture was stirred overnight at room temperature. The reaction was monitored by TLC (2% methanol and 98% CH_2Cl_2) and stirring was continued until completion. The solvent was removed under reduced pressure, the residue was treated with methanol and the resultant solution was concentrated until TEA was removed. The crude product **6** or **7** was purified by silica gel chromatography eluting with chloroform/methanol (9:1) and crystallized from methanol.

3-Cyano-1-(β-D-ribofuranosyl)-4-trifluoromethyl-6-(thiophen-2′-yl)-2-pyridone (6) Pale yellow powder; yield 73%; mp 197°C; IR: 1655 (CO), 2206 (CN), 3428 cm^{-1} (-OH); ^{1}H-NMR (DMSO-d_6): δ 3.81–3.86 (m, 5 H, H-2″, H-3″, H-4″ and 2 H-5″), 4.81–4.84 (3 OH, exchangeable with D_2O), 5.89 (d, 1H, H-1″, J = 4.0 Hz), 6.34 (s, 1H, pyridine H-5), 6.80 (m, 1H, thiophene-H4), 7.18–7.19 (d, 1H, thiophene-H5, J = 5.0 Hz), 7.73 (d, 1H, thiophene-H3, J = 3.7 Hz); ^{13}C-NMR (DMSO-d_6): δ 75.3 (C5″), 75.6 (C3″), 75.9 (C2″), 82.5 (C-4″), 83.5 (C1″), 93.4 (pyridine-C5), 114.5 (pyridine-C3), 117.8 (CN), 120.5 (CF_3), 123.4 (thiophene-C3), 124.9 (thiophene-C4), 126.3 (thiophene-C5), 137.9 (thiophene-C2), 141.5 (pyridine-C6), 152.2 (pyridine-C2), 166.8 (pyridine-C4). Anal. Calcd for $C_{16}H_{13}F_3N_2O_5S$: C, 47.76; H, 3.26; N, 6.96; S, 7.97. Found: C, 47.81; H, 3.37; N, 6.87; S, 8.01.

3-Cyano-2-(β-D-glucopyranosyloxy)-4-trifluoromethyl-6-(thiophen-2′-yl)pyridine (7) Pale yellow powder; yield 74%; mp 134°C; IR: 2206 (CN), 3392 cm^{-1} (sugar-OH); ^{1}H NMR (DMSO-d_6): δ 3.14–3.19 (m, 6H, H-2″, H-3″, H-4″, H-5″, H-6″a, H-6″b), 4.63–4.93 (4 OH, exchangeable with D_2O), 6.97 (d, 1H, H-1″, J = 8.0 Hz), 7.11 (m, 1H, thiophene-H4), 7.24 (s, 1H, pyridine-H5), 7.40 (d, 1H, thiophene-H5, J = 5.0 Hz), 7.60 (d, 1H, thiophene-H3, J = 3.7 Hz); ^{13}C NMR (DMSO-d_6): δ 62.8 (C5″), 64.0 (C3″), 70.4 (C2″), 72.4 (C4″), 97.3 (C1″), 112.1 (pyridine-C5), 113.2 (pyridine-C3), 122.0 (CN), 124.8 (CF_3), 131.4 (thiophene-C3), 131.6 (thiophene-C4),134.6 (thiophene-C5), 143.9 (pyridine-C4), 146.9 (thiophene-C2), 157.2 (pyridine-C6), 164.8 (pyridine-C2). Anal. Calcd for $C_{17}H_{15}F_3N_2O_6S$: C, 47.22; H, 3.50; N, 6.48; S, 7.42. Found: C, 47.27; H, 3.61; N, 6.39; S, 7.50.

General procedure for synthesis of 8a,b

To a solution of 2-pyridone **3** (5.0 mmol) in a mixture of acetonitrile (30 mL) and pyridine (2 mL), a solution of an aroyl chloride (10 mmol) in acetonitrile (5.0 mL) was added gradually with stirring at room temperature. The mixture was stirred at room temperature until the reaction was completed as indicated by TLC analysis. The mixture was concentrated under reduced pressure and the residue was washed with water (2×20 mL) and crystallized from ethanol to give the desired products **8a,b**.

3-Cyano-6-(thiophen-2′-yl)-4-(trifluoromethyl)pyridin-2-yl 4″-fluorobenzoate (8a) Pale yellow crystals; yield 87%; mp 156°C; IR: 3089 (C-H, aromatic), 2235 (CN), 1754 (C=O), 1603 (C=C), 1451 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ 7.29 (m, 1H, thiophene-H4), 7.64 (d, 2H, *p*-fluorobenzoyl, J = 8.0 Hz), 7.96–7.97 (d, 1H, thiophene-H5, J = 5.0 Hz), 8.33 (d, 2H, *p*-fluorobenzoyl, J = 8.0 Hz), 8.36 (d, 1H, thiophene-H3, J = 3.7 Hz), 8.56 (s, 1H, pyridine-H5); ^{13}C NMR ($CDCl_3$): δ 95.8 (pyridine-C3), 112.1 (pyridine-C5), 113.8 (CN), 115.4 (CF_3), 121.8 (aromatic carbons), 125.6 (thiophene-C3), 130.2 (thiophene-C4), 132.5 (thiophene-C5), 133.5, 134.8, 140.8 (aromatic carbons), 143.0 (pyridine-C4), 153.8 (thiophene-C2), 156.9 (pyridine-C6), 159.7 (C=O), 162.5 (C-F), 166.6 (pyridine-C2). Anal. Calcd for $C_{18}H_{10}F_4N_2O_2S$: C, 54.82; H, 2.56; N, 7.10; S, 8.13. Found: C, 54.87; H, 2.67; N, 7.01; S, 8.21.

3-Cyano-6-(thiophen-2′-yl)-4-(trifluoromethyl)pyridin-2-yl 4″-(trifluoromethyl) benzoate (8b) Pale yellow crystals; yield 85%; mp 171°C; IR: 3085 (C-H, aromatic), 2231 (CN), 1760 (C=O), 1603 (C=C), 1421 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ 7.29 (m, 1H, thiophene-H4), 7.48–7.52 (m, 2H, *p*-trifluoromethylbenzoyl), 7.95 (d, 1H, thiophene-H5, J = 5.0 Hz), 8.26–8.29 (m, 2H, *p*-trifluoromethylbenzoyl), 8.35 (d, 1H, thiophene-H3, J = 3.7 Hz), 8.55 (s, 1H, pyridine-H5); ^{13}C NMR ($CDCl_3$): δ 95.8 (pyridine C3), 112.1 (pyridine-C5), 113.8 (CN), 116.1 (CF_3), 120.4 (CF_3), 123.2, 127.7 (thiophene-C3), 129.7 (thiophene-C4), 131.1 (thiophene-C5), 132.6, 133.3, 134.6 (aromatic carbons), 140.8 (pyridine-C4), 156.8 (thiophene-C2), 159.8 (pyridine-C6), 162.6 (C=O), 166.8 (pyridine-C2). Anal. Calcd for $C_{19}H_{10}F_6N_2O_2S$: C, 51.36; H, 2.27; N, 6.30; S, 7.22. Found: C, 51.41; H, 2.38; N, 6.21; S, 7.30.

Antibacterial evaluations

Well diffusion assay Two Gram positive bacteria (*Bacillus infantis* and *Staphyloccus aureus*) and two Gram negative bacteria (*Escherichia coli* and *Stenotrophomonas maltophilia*) were used. A Mueller Hinton agar (38.0 g of Mueller Hinton agar in 1000 mL of distilled water) was prepared, autoclaved and poured into the Petri dishes. It solidified at room temperature. The bacterial strains (10^6 cells/mL) were introduced onto the plates using a pipette and hockey sticks to spread them on the agar. All compounds were poured individually into the wells (50 µL/well). The wells were then incubated at 37°C for 24–48 h. The bacterial inhibition was determined by measuring the diameter of the inhibition zone (mm) using a transparent scale. Antibiotic amoxicillin (5 mg/mL) was used as a positive control at 50 µL/well.

Minimum inhibitory concentration (MIC) This is the lowest concentration of the antimicrobial compound that inhibits the bacterial strain. Compounds were serially diluted with sterile distilled water and examined from lower dilution to higher dilution to find the MIC. Serially diluted compounds (100 µL) were added to a sterile nutrient broth (900 µL) and the bacterial cultures were inoculated as 10^5 cells/mL. Compounds and bacterial cultures in the nutrient broth were incubated at 37°C for 18–24 h. Controls included a nutrient broth with the compound only and another nutrient broth with the bacterial culture only. After incubation, the tubes were examined for turbidity, compared with controls and to confirm the results, loops of culture were inoculated in agar plates by streaking. The MIC was measured as the lowest concentration (mg/mL) of the extract resulting in no growth of the bacteria.

Acknowledgments: The authors wish to thank the Deanship of Graduate Studies and the Department of Chemistry, UAE University for financial support (Research Grant # 31S030).

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