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Synthesis and anti-proliferative activity of pyridine *O*-galactosides and 4-fluorobenzoyl analogues

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Abstract: Pyridine *O*-galactosides **3**, **4**, and 2-(4′-fluorobenzoyloxy)pyridine derivatives **5** were prepared by simple nucleophilic substitution reactions. These nucleosides were studied as anti-proliferating agents of human promyelotic leukemia (HL-60) cells. Compound **5a** shows the highest anti-proliferative activity (77% at 100 μM) among the synthesized compounds.

Keywords: anti-proliferation; NMR; pyridine galactoside; synthesis.

Introduction

Nucleoside analogues have been explored due to their structural similarity to the naturally occurring nucleosides, which are the fundamental building blocks of many biological systems. The recent interest in nucleosides as biologically active agents stimulates the development of novel compounds targeting common cancers [1]. As a result, synthetic nucleosides are used in chemotherapy, and there is an intertwined relationship between natural and synthetic nucleosides [2]. Cytotoxic nucleosides interfere with nucleic acid synthesis, and many of these are quite promising agents for cancer therapies with different mechanisms of action. In particular, 4-amino-3-fluoro-1-(β-D-ribofuranosyl)-2(1*H*)-pyridone inhibits the growth of HL-60 lymphoid leukemia cells with $IC_{50} = 1.07\times10^{-5}$ M (Figure 1), whereas its 2′-deoxy analogue is active against lymphoid leukemia L1210 cells [3–5].

In our previous study [6–8], we reported the synthesis and apoptotic activity of pyrimidine, pyrazoline, and pyridine nucleosides. The goal of this work was to synthesize new pyridine analogues and to confirm their structures by analyzing their NMR properties. The anti-proliferative activities in HL-60 leukemia cells of the resultant compounds were studied.

Results and discussion

A series of 2(1*H*)-pyridone derivatives **1** (Schemes 1–3) was synthesized previously in yields of 84–96% [8, 9]. In this work, alkylation of a particular compound **1′** with iodoethane under basic conditions yielded a mixture of *N*-ethylpyridone **2a** and 2-ethoxypyridine **2b** (Scheme 1). The isomeric mixture of **2a** and **2b** was separated using column chromatography [10–12]. The ^{1}H NMR data obtained for isomers **2a** and **2b** indicate different chemical shifts for the pyridine H-5 atom [13–15]. For the *N*-ethyl isomer, the chemical shift of the pyridine H-5 atom is observed at δ 6.53, whereas the H-5 signal for the ethoxy isomer **2b** is shifted downfield to δ 7.76 (see the Supplementary data for **2a** and **2b**). This difference in chemical shifts was used to assign the structures to *O*-nucleosides **3**, **4**, and their *O*-benzoyl analogues **5** synthesized as part of this work (Schemes 2 and 3).

Treatment of potassium salts of 2-pyridones **1** [6–8] with 1-bromo-2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside yielded the corresponding acetyl-protected *O*-β-nucleosides **3a–e**. Deacetylation of products **3a–d** under standard conditions provided the respective *O*-β-nucleosides **4a–d** (Scheme 2). The β-configuration of **3** and **4** is consistent with the large spin-spin coupling constant ($J_{H\text{-}1''\text{-}H\text{-}2''}$>7.00 Hz) for protons H-1″ and H-2″ in these compounds [8]. The chemical shift of pyridine H-5 proton of the obtained nucleosides **3** and **4** is observed in the range of δ 7.65–8.30, which indicates the *O*-substitution (see the Supplementary data Figs. 5–22).

The 2-(4′-fluorobenzoyloxy) analogues **5a–d** were synthesized as shown in Scheme 3. The reaction of

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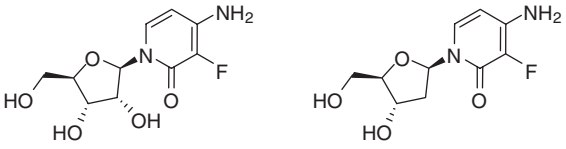
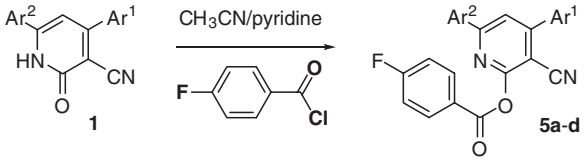


Figure 1 Fluoropyridine analogues used against lymphoid leukemia.



	Ar^1	Ar^2	Yield (%)
a	2-thienyl	Ph	62
b	2-thienyl	4-ClC_6H_4	65
c	4-pyridyl	4-$MeOC_6H_4$	65
d	3,4-$(OMe)_2C_6H_3$	4-$MeOC_6H_4$	72

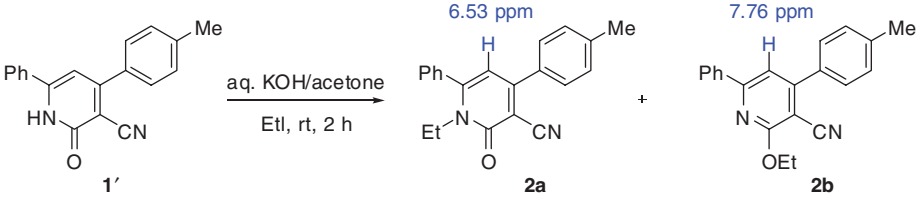
Scheme 3 Synthesis of *O*-(4′-fluorobenzoyloxy)pyridine analogues **5a–d**.

substrates **1** with 4-fluorobenzoyl chloride yielded a single isomer **5a–d** in each case. The *O*-substitution in **5a–d** was determined as discussed above. In addition, the infrared spectra for **5** show absorption in the range of 1750–1770 cm^{-1} for the benzoyl carbonyl group. In the ^{13}C NMR spectrum, the characteristic chemical shift for this group appears in the region of δ 157.4–158.3 (see the Supplementary data Figs. 23–30).

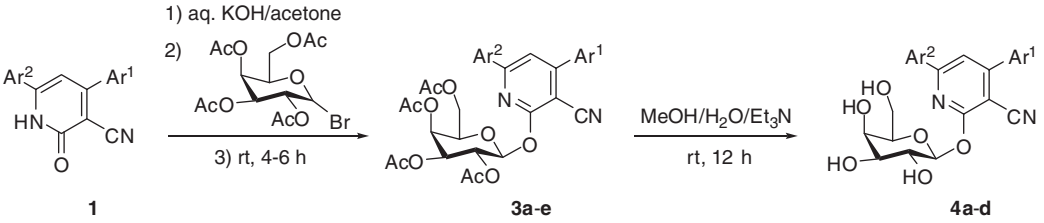
The effects of pyridine galactosides **3** and **4** and their benzoyl analogues **5** as anti-proliferative agents of the human promyelotic leukemia (HL-60) cell lines were evaluated. The results of the MTT cell proliferation assay show that compound **4c** has the highest anti-proliferation activity among all synthesized nucleosides **3** and **4**. Interestingly, non-nucleosides **5a–d** were found to have even higher anti-proliferation activities than the corresponding nucleosides **3** and **4**. Interestingly, the non-nucleoside analogue **5a** shows the highest activity among all synthesized compounds [16–18].

Conclusions

A new series of pyridine *O*-galactoside **3**, **4**, and 4-fluorobenzoyl analogues **5** were synthesized. All compounds show some anti-proliferative activities, with **5a** having the highest activity.



Scheme 1 Synthesis of *N*-ethylpyridone **2a** and 2-ethoxypyridine **2b**.



	Ar^1	Ar^2	Yield %, **3**/**4**
a	Ph	Ph	68/43
b	2-MeC_6H_4	Ph	65/57
c	2-thienyl	Ph	68/47
d	2-thienyl	4-ClC_6H_4	49/77
e	2-$CF_3C_6H_4$	Ph	71 (**3e** only)

Scheme 2 Synthesis of pyridine *O*-galactosides **3** and **4**.

Experimental

All air-sensitive materials were handled under a nitrogen atmosphere. Melting points were determined in Pyrex capillaries on a Gallenkamp apparatus. Infrared spectra were recorded with a Thermo Nicolet Nexus 470 FT-IR spectrometer in potassium bromide disks. ^{1}H NMR and ^{13}C NMR spectra were obtained on Varian 200 or 400 instruments. Optical rotations were measured with a Perkin-Elmer digital polarimeter at 589 nm (sodium D line) in a 1-dm cell. Thin-layer chromatography (TLC) was carried out on precoated Merck silica gel F_{254} plates and ultraviolet light was used for visualization. Column chromatography was performed using a Merck silica gel. Micro analyses (C, H, and N) were performed on a Flash-EA-1112 series analyzer. The reagents were purchased from Aldrich and used without further purification.

Synthesis of *N*-ethylpyridone 2a and 2-ethoxypyridine 2b

To a solution of 6-phenyl-4-(*p*-tolyl)-2(1*H*)pyridone-3-carbonitrile (2.86 g, 0.01 mol) in aqueous KOH (0.01 mol, 6 mL), a solution of iodoethane (0.01 mol, 1.56 mL) in acetone (30 mL) was added. The mixture was stirred at room temperature for 2 h, then extracted with dichloromethane (3×20 mL). The extract was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure at room temperature to afford a residue containing **2a** and **2b** in a 65% total yield. This mixture was separated by column chromatography on a silica gel column using ethyl acetate/hexane (1:2) as an eluent to give the *N*-ethyl derivative **2a** and the *O*-ethyl derivative **2b** as pure compounds.

1-Ethyl-2-oxo-6-phenyl-4-(*p*-tolyl)-1,2-dihydropyridine-3-carbonitrile (2a) Pale yellow powder; R_f = 0.24 eluting with ethyl acetate/hexane (1:2); yield 24%; mp 280°C; IR: 2202 (CN), 2922 (aliphatic C-H), 1552 (C=C), 1651 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.25 (t, 3H, CH_3, J = 6.8 Hz), 2.04 (s, 3H, CH_3), 4.10 (q, 2H, CH_2, J = 6.8 Hz), 6.53 (s, 1H, H-5 pyridine), 7.27 (d, 2H, *p*-tolyl, J = 8.0 Hz), 7.33–7.42 (m, 3H, phenyl), 7.47 (d, 2H, *p*-tolyl, J = 8.0 Hz), 7.96 (d, 2H, phenyl, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.2 (CH_3), 21.5 (CH_3), 60.4 (CH_2), 99.8 (C-3), 106.7 (C-5), 115.6 (CN), 125.2, 127.3, 128.6, 128.9, 129.5, 131.4, 136.1, 138.9 (aromatic carbons), 150.7 (C-2), 161.2 (C-6), 164.1 (C-4); ESI-MS: *m*/*z* 315.15 (100%) [M$^+$].

2-Ethoxy-6-phenyl-4-(*p*-tolyl)pyridine-3-carbonitrile (2b) Yellow powder, R_f = 0.78 eluting with ethyl acetate/hexane (1:2); yield 76%; mp 257°C; IR: 2217 (CN), 2988 (aliphatic C-H), 1580 cm^{-1} (C=C); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.45 (t, 3H, CH_3, J = 7.0 Hz), 2.42 (s, 3H, CH_3), 4.63 (q, 2H, CH_2, J = 7.0 Hz), 7.39 (d, 2H, *p*-tolyl, J = 8.0 Hz), 7.53–7.55 (m, 3H, phenyl), 7.65 (d, 2H, *p*-tolyl, J = 8.0 Hz), 7.76 (s, 1H, H-5 pyridine), 8.22–8.25 (m, 2H, phenyl); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.5 (CH_3), 21.5 (CH_3), 63.4 (CH_2), 93.1 (C-3), 113.3 (C-5), 115.7 (CN), 125.5, 127.3, 128.9, 130.4, 130.7, 136.4, 137.4, 138.7 (aromatic carbons), 156.8 (C-4), 157.8 (C-6), 164.7 (C-2); ESI-MS: *m*/*z* 315 (100%) [M$^+$], 316 (25%) [M+H]$^+$.

General procedure for synthesis of nucleosides 3a–e

To a solution of 4,6-disubstituted-2(1*H*)-pyridone-3-carbonitrile **1a–e** (0.01 mol) in aqueous KOH (0.56 g, 0.01 mol) in distilled water (6 mL), a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (4.52 g, 0.011 mol) in acetone (30 mL) was added. The mixture was stirred at room temperature until the reaction was completed as monitored by TLC (4–6 h). Dichloromethane (30 mL) was added, and the organic layer was washed with water (2×30 mL), then dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to afford the crude product **3a–e**. The residue was purified by column chromatography eluting with chloroform/ethyl acetate (1:9) to give after crystallization from ethanol the desired nucleoside **3a–e** in 48–74% yield (Scheme 1).

3-Cyano-4,6-diphenyl-2-(2″,3″,4″,6″-tetra-*O*-acetyl-β-D-galactopyranosyloxy)pyridine (3a) White crystals; yield 68%; mp 174–175°C, from ethanol, R_f = 0.39 hexane/ethyl acetate (6:4), IR: 1748 (C=O), 2225 cm^{-1} (CN); $[\alpha]^{25}$ = 21.8° (c = 3.2 mg/mL, chloroform); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.88, 2.09, 2.10, 2.22 (4s, 12H, 4 CH_3), 4.17–4.28 (m, 3H, H-5″, H-6″a, H-6″b), 5.21–5.28 (m, 1H, H-4″), 5.51–5.74 (m, 2H, H-2″, H-3″), 6.29 (d, 1H, H-1″, J = 8.0 Hz), 7.50–7.70 (m, 8H, aromatic), 7.66 (s, 1H, H-5), 8.04–8.09 (m, 2H, aromatic); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 20.5, 20.6, 20.6, 20.7 (4 CH_3), 61.8 (C-6″), 67.2 (C-5″), 67.9 (C-3″), 70.9 (C-4″), 71.8 (C-2″), 94.0 (C-5), 94.9 (C-1″), 114.0 (C-3), 115.4 (CN), 127.3–136.8 (aromatic carbons), 157.2 (C-6), 157.8 (C-2), 162.4 (C-4), 168.9, 170.2, 170.3, 170.4 (4 C=O). Anal. Calcd for $C_{32}H_{30}N_2O_{10}$ (602.59): C, 63.78; H, 5.02; N, 4.65. Found: C, 64.20; H, 5.20; N, 4.70.

3-Cyano-6-phenyl-4-(2′-tolyl)-2-(2″,3″,4″,6″-tetra-*O*-acetyl-β-D-galactopyranosyloxy) pyridine (3b) White crystals; yield 65%; mp 196–197°C, from ethanol; R_f = 0.43 eluting with hexane/ethyl acetate (6:4); IR: 1748 (C=O), 2235 cm^{-1} (CN); $[\alpha]^{25}$ = 7.5° (c = 4.0 mg/mL, chloroform); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.93, 2.04, 2.05, 2.08 (4s, 12H, 4 CH_3), 2.28 (s, 3H, *o*-CH_3), 4.17–4.28 (m, 3H, H-5″, H-6″a, H-6″b), 5.21–5.28 (m, 1H, H-4″, J = 3.4 Hz), 5.52 (d, 1H, H-3″, J = 3.8 Hz), 5.64, 5.74 (dd, 1H, H-2″, J = 8.2 Hz), 6.29 (d, 1H, H-1″, J = 8.2 Hz), 7.26 (s, 1H, H-2′), 7.32–7.90 (m, 7H, aromatic), 8.07–8.09 (m, 2H, aromatic); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 20.5, 20.6, 20.6, 20.7 (4 CH_3), 21.9 (*o*-CH_3), 61.8 (C-6″), 67.2 (C-5″), 67.9 (C-3″), 70.9 (C-4″), 71.7 (C-2″), 94.0 (C-1″), 94.9 (C-5), 114.0 (C-3), 116.5 (CN), 125.2–138.8 (aromatic carbons), 157.8 (C-6), 158.5 (C-2), 162.0 (C-4), 168.9, 170.2, 170.3, 170.4 (4 C=O). Anal. Calcd for $C_{33}H_{32}N_2O_{10}$ (616.61): C, 64.28; H, 5.23; N, 4.54. Found: C, 64.20; H, 5.14; N, 4.61.

3-Cyano-6-phenyl-4-(thiophen-2′-yl)-2-(2″,3″,4″,6″-tetra-*O*-acetyl-β-D-galactopyranosyloxy) pyridine (3c) White crystals; yield 68%; mp 216–217°C, from ethanol; R_f = 0.33 eluting with hexane/ethyl acetate (6:4); IR: 1753 (C=O), 2241 cm^{-1} (CN); $[\alpha]^{25}$ = 8.8° (c = 4.0 mg/mL, chloroform); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.88, 2.03, 2.04, 2.22 (4s, 12H, 4 CH_3), 4.16–4.23 (m, 3H, H-5″, H-6″a,H-6″b), 5.19, 5.26 (dd, 1H, H-4″, J = 3.60 Hz), 5.51 (d, 1H, H-3″, J = 3.60 Hz), 5.63, 5.73 (dd, 1H, H-2″, J = 8.2 Hz), 6.26 (d, 1H, H-1″, J = 8.20 Hz), 7.51–7.56 (m, 3H, aromatic), 7.58–7.59 (m, 1H, thienyl, H-4), 7.69 (s, 1H, H-5), 7.96–7.98 (m, 1H, thienyl H-3), 8.02–8.07 (m, 2H, aromatic); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 20.5, 20.6, 20.8, 20.7 (4 CH_3), 61.8 (C-6″), 67.2 (C-5″), 67.8 (C-3″), 70.9 (C-4″), 71.7 (C-2″), 91.6 (C-1″), 94.9 (C5), 114.1 (C-3), 114.5 (CN), 127.2–137.2 (aromatic carbons), 148.5 (thienyl C-2), 157.9 (C-6), 162.9 (C-2), 168.9, 170.2, 170.3, 170.4 (4 C=O). Anal. Calcd for $C_{30}H_{28}N_2O_{10}S$ (608.62): C, 59.20; H, 4.64; N, 4.60; S, 5.27. Found: C, 60.6; H, 4.70; N, 4.60; S, 5.30.

3-Cyano-6-(4′-chlorophenyl)-4-(thiophen-2′-yl)-2-(2″,3″,4″,6″-tetra-*O*-acetyl-β-D-galactopyranosyloxy)pyridine (3d) White crystals; yield 49%; mp 155–156°C, from ethanol; R_f = 0.63 eluting

with hexane/ethyl acetate (6:4); IR: 1760 (C=O), 2230 cm^{-1} (CN); $[\alpha]^{25}$ = 8.7° (*c* = 4.0 mg/mL, chloroform); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.92, 2.04, 2.06, 2.23 (4s, 12H, $4CH_3$), 4.17–4.26 (m, 3H, H-5″, H-6″a,H-6″b), 5.20, 5.27 (dd, 1H, H-4″, *J* = 3.2 Hz), 5.52 (d, 1H, H-3″, *J* = 3.40 Hz), 5.64, 5.73 (dd, 1H, H-2″, *J* = 8.20 Hz), 6.23 (d, 1H, H-1″, *J* = 8.2 Hz), 7.49–7.53 (m, 2H, aromatic), 7.56, 7.61 (dd, 1H, *J* = 3.4 Hz, aromatic), 7.65 (s, 1H, H-5), 7.98–8.02 (m, 3H, aromatic); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 20.5, 20.6, 20.6, 20.7 (4 CH_3), 61.7 (C-6″), 67.1 (C-5″), 67.8 (C-3″), 70.9 (C-4″), 71.7 (C-2″), 91.9 (C-5), 95.0 (C-1″), 113.9 (C-3), 114.5 (CN), 128.5–137.1 (aromatic carbons), 156.7 (C-6), 163.1 (C-2), 168.9 (C-4), 168.3, 170.2, 170.3, 170.4 (4 C=O). Anal. Calcd for $C_{30}H_{27}ClN_2O_{10}S$ (643.06): C, 56.03; H, 4.23; N, 4.36; S, 4.99. Found: 56.10, H, 4.22; N, 4.35; S, 4.90.

3-Cyano-6-phenyl-4-(4′-trifluoromethylphenyl)-2-(2″,3″,4″,6″-tetra-*O*-acetyl-β-D-galactopyranosyloxy)pyridine (3e) White crystals; yield 71%; mp 155–156°C, from ethanol; R_f = 0.63 eluting with hexane/ethyl acetate (6:4); IR: 1743 (C=O), 2353 cm^{-1} (CN); $[\alpha]^{25}$ = 14.4° (*c* = 8.0 mg/mL, chloroform); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.87, 2.04, 2.05, 2.20 (m, 12H, 4 CH_3), 4.17–4.25 (m, 3H, H-5″, H-6″a, H-6″b), 5.26, 5.27 (dd, 1H, H-3″, *J* = 3.4 Hz), 5.52 (d, 1H, H-4″, *J* = 2.6 Hz), 5.64, 5.73 (dd, 1H, H-2″, *J* = 8.2 Hz), 6.28 (d, 1H, H-1″, *J* = 8.2 Hz), 7.52–7.55 (m, 3H, aromatic), 7.60 (s, 1H, H-5), 7.78–7.80 (m, 4H, aromatic), 8.04–8.08 (m, 2H, aromatic); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 20.5, 20.5, 20.6, 20.7 ($4CH_3$), 61.8 (C-6″), 67.2 (C-5″), 67.8 (C-3″), 70.9 (C-4″), 71.8 (C-2″), 94.0 (C-1″), 95.0(C-5), 115.0 (C-3), 115.2 (CN), 126.1 (CF_3), 127.3–136.5 (aromatic carbons), 155.6 (C-6), 158.4 (C-2), 162.0 (C-4), 168.9, 170.2, 170.3, 170.4 (4 C=O). Anal. Calcd for $C_{33}H_{29}F_3N_2O_{10}$ (670.59): C, 59.11; H, 4.36; N, 4.18. Found: C, 60.1; H, 4.38; N, 4.12.

General procedure for nucleoside deacetylation

Acetylated nucleoside **3a–d** (0.5 g) was dissolved in a 20-mL mixture of MeOH/H_2O/Et_3N (1:1:1), and the solution was allowed to stand at room temperature until the reaction was completed as monitored by TLC (2% MeOH in CH_2Cl_2). The solvent was removed under reduced pressure, and the residue was purified by column chromatography eluting with 2% methanol in CH_2Cl_2 to give a white crystalline product **4a–d.**

3-Cyano-4,6-diphenyl-2-(β-D-galactopyranosyloxy)pyridine (4a) White crystals; yield 43%; mp 219°C, from methanol; R_f = 0.16 eluting with CH_2Cl_2; IR: 2241 (CN), 3430 cm^{-1} (sugar-OH); $[\alpha]^{25}$ = 61° (*c* = 7.36 mg/mL, methanol); ^{1}H NMR (DMSO-d_6, 200 MHz): δ 3.24–3.67 (m, 6H, H-2″, H-3″, H-4″, H-5″, H-6″a, H-6″b), 4.59–5.45 (4OH, exchangeable with D_2O), 6.15 (d, 1H, H-1″, *J* = 8.0 Hz), 7.51–7.59 (m, 6H, aromatic), 7.73–7.77 (m, 2H, aromatic), 7.88 (s, 1H, H-5), 8.22–8.27 (m, 2H, aromatic); ^{13}C NMR (DMSO-d_6, 75 MHz]): δ 60.4 (C-6″), 68.2 (C-5″), 69.9 (C-3″), 73.5 (C-4″), 76.4 (C-2″), 90.5 (C-1″), 97.3 (C-5), 113.2 (C-3), 115.4 (CN), 127.6–136.5 (aromatic carbons), 148.3 (C-6), 157.5 (C-2), 163.7(C-4). Anal. Calcd for $C_{24}H_{22}N_2O_6$ (434.44): C, 66.35; H, 5.10; N, 6.45. Found: C, 66.29; H, 5.03; N, 6.47.

3-Cyano-6-phenyl-4-(2′-tolyl)-2-(β-D-galactopyranosyloxy)pyridine (4b) White crystals; yield 57%; mp 192°C, from methanol; R_f = 0.21 eluting with CH_2Cl_2; IR: 2354 (CN), 3439 cm^{-1} (sugar-OH); $[\alpha]^{25}$ = 223.8° (*c* = 8.4 mg/mL, methanol); ^{1}H NMR (DMSO-d_6, 200 MHz): δ 3.26–3.75 (m, 6H, H-2″, H-3″, H-4″, H-5″, H-6″a, H-6″b), 2.41 (s, 3H, CH_3), 4.65–5.28 (4OH, exchangeable with D_2O), 6.13 (d, 1H, H-1″, *J* = 7.80 Hz), 7.39–7.57 (m, 7H, aromatic), 7.86 (s, 1H, H-5), 8.26–8.28 (m, 2H, aromatic); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 61.0 (C-6″), 68.8 (C-5″), 70.6 (C-3″), 74.2 (C-4″), 77.1 (C-2″), 93.4 (C-1″), 97.8 (C-5), 115.4 (C-3), 115.7 (CN), 126.4–139.0 (aromatic carbons), 157.5 (C-6), 157.9 (C-2), 163.7 (C-4). Anal. Calcd for $C_{25}H_{24}N_2O_6$ (448.47): C, 66.95; H, 5.39; N, 6.25. Found: C, 66.88; H, 5.41; N, 6.19.

3-Cyano-6-phenyl-4-(thiophen-2′-yl)-2-(β-D-galactopyranosyloxy) pyridine (4c) White crystals; yield 47%; mp 195°C, from methanol; R_f = 0.29 eluting with CH_2Cl_2; IR: 2234 (CN), 3438 cm^{-1} (sugar-OH); $[\alpha]^{25}$ = 104.4° (*c* = 8 mg/mL, methanol); ^{1}H NMR (DMSO-d_6, 200 MHz): δ 3.25–3.68 (m, 6H, H-2″ H-3″, H-4″, H-5″, H-6″a,H-6″b), 4.59–5.45 (4 OH, exchangeable with D_2O), 6.13 (d, 1H, H-1″, *J* = 7.8 Hz), 7.33–7.37 (m, 1H, thienyl H-3), 7.55–7.59 (m, 3H, aromatic), 7.97–8.01 (m, 3H, aromatic), 8.23–8.27 (m, 2H, aromatic); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 60.5 (C-6″), 69.6 (C-5″), 72.8 (C-3″), 76.9 (C-4″), 78.0 (C-2″), 96.8 (C-1″), 90.4 (C-5), 113.2 (C-3), 115.3 (CN), 127.6–136.5 (aromatic carbons), 148.3(C-6), 157.4 (C-2), 163.5 (C-4). Anal. Calcd for$C_{22}H_{20}N_2O_6S$ (440.10): C, 59.99; H, 4.58; N, 6.36; S, 7.28. Found: C, 60.01; H, 4.61; N, 6.29; S, 7.29.

3-Cyano-6-(4′-chlorophenyl)-4-(thiophen-2″-yl)-2-(β-D-galactopyranosyloxy)pyridine (4d) White crystals; yield 77%; mp 180°C, from methanol; R_f = 0.51 eluting with CH_2Cl_2; IR: 2230 (CN), 3443 cm^{-1} (sugar-OH); $[\alpha]^{25}$ = 300° (*c* = 8.4 mg/mL, methanol); ^{1}H NMR (DMSO-d_6, 200 MHz): δ 3.49–3.73 (m, 6H, H-2″, H-3″, H-4″, H-5″, H-6″a, H-6″b), 4.65–5.30 (4OH, exchangeable with D_2O), 6.09 (d, 1H, H-1″, *J* = 8.2 Hz), 7.29, 7.34 (dd, 1H, *J* = 4.0 Hz), 7.57 (d, 2H, *J* = 8.6 Hz), 7.91–7.97 (m, 2H, thienyl H-2, 3), 7.99 (s, 1H, H-5), 8.23 (d, 2H, *J* = 8.8 Hz); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 60.4 (C-6″), 68.2 (C-5″), 69.9 (C-3″), 73.5(C-4″), 76.5 (C-2″), 90.7 (C-1″), 97.4 (C5), 113.3(C-3), 115.4 (CN), 128.7–136.4 (aromatic carbons), 148.5 (C-6), 156.1 (C-2), 163.6 (C-4). Anal. Calcd for $C_{22}H_{19}ClN_2O_6S$ (474.07): C, 55.64; H, 4.03; N, 5.90; S, 6.75. Found: C, 55.63; H, 3.98; N, 5.87; S, 6.72.

Synthesis of 3-cyano-4,6-diaryl-2-(4′-fluorobenzoyloxy) pyridines 5a–d

To a solution of 4,6-disubstituted-2(1*H*)-pyridone-3-carbonitrile **1** (5.0 mmol) in a mixed solvent of dry acetonitrile (50 mL) and pyridine (2 mL), a solution of 4-fluorobenzoyl chloride (10.0 mmol) in dry acetonitrile (10.0 mL) was added gradually with stirring at room temperature until the reaction was completed as judged by TLC (30 min). The solvent was removed under reduced pressure, and the solid residue was washed with water (2×20 mL). The product was purified by column chromatography eluting with hexane/ethyl acetate (6:4) or 0.5% CH_3OH in CH_2Cl_2, then crystallized from a solvent indicated below to give the desired compound **5a–d** (Scheme 3).

[3-Cyano-6-phenyl-4-(thiophen-2′-yl)pyridin-2-yl]4-fluorobenzoate (5a) White crystals; yield 62%; mp 180°C, from ethanol; R_f = 0.85 eluting with hexane/ethyl acetate (6:4), IR: 1580 (C=C), 1770 (C=O), 2230 cm^{-1} (CN); ^{1}H NMR (DMSO-d_6, 200 MHz): δ 7.35 (t, 1H, thienyl H-4, *J* = 4.0 Hz), 7.46–7.62 (m, 5H, aromatic), 8.11 (d, 1H, thienyl H-5, *J* = 5.0 Hz), 8.14 (d, 1H, thienyl H-3, *J* = 3.8 Hz), 8.24 (s, 1H, H-5 pyridine), 8.25–8.33 (m, 4H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 95.4 (C-3), 104.5 (C-5), 116.9 (CN), 117.4 (C-3″), 125.8 (C-1″), 126.7 (C-4′), 127.8 (C-3, 2-thienyl), 128.7 (C-4, 2-thienyl), 128.9 (C-5, 2-thienyl), 131.2 (C-3′), 131.5 (C-2″), 131.9 (C-2, 2-thienyl), 132.2 (C-1′), 136.3 (C-4), 148.5 (C-6), 157.9

(C=O) ,162.4 (C-F), 168.9 (C-2). Anal. Calcd for $C_{23}H_{13}FN_2O_2S$ (400.43): C, 68.99; H, 3.27; N, 7.00; S, 8.01. Found: C, 68.90; H, 3.25; N, 6.99; S, 7.90.

[3-Cyano-6-(4′-chlorophenyl)-4-(thiophen-2″-yl)pyridin-2-yl]4-fluorobenzoate (5b) White crystals; yield 65%; mp 185°C, from ethanol; R_f = 0.86 in 0.5% CH_3OH in CH_2Cl_2, IR: 1592 (C=C), 1754 (C=O), 2240 cm^{-1} (CN); ^1H NMR (DMSO-d_6, 200 MHz): δ 7.33 (t, 1H, thienyl H-4, J = 4.0 Hz), 7.46–7.59 (m, 4H), 8.02 (d, 1H, J = 5.0 Hz), 8.12 (d, 1H, J = 5.0 Hz), 8.14 (s, 1H, H-5 pyridine), 8.21–8.33 (m, 4H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 96.8 (C-3), 116.5 (C-5), 117 (CN), 117.4 (C-3″), 123.7 (C-1″), 128.9 (C-3, 2-thienyl), 129.1 (C-4, 2-thienyl), 129.5 (C-5, 2-thienyl), 131.5 (C-2′), 132.2 (C-3′), 133.3 (C-2″), 133.5 (C-4′), 134.3 (C-1′), 135.7 (C-5), 136.6 (C-4), 148.4 (C-6), 157.4 (C=O), 159.7 (C-F), 162.6 (C-2). Anal. Calcd for $C_{23}H_{12}ClFN_2O_2S$ (434.87): C, 63.52; H, 2.78; N, 6.44; S, 7.36. Found: C, 63.02; H, 2.73; N, 6.45; S, 7.40.

[3-Cyano-6-(4′-methoxyphenyl)-4-(4″-pyridinyl)pyridin-2-yl]4-fluorobenzoate (5c) White crystals; yield 65%; mp 222°C, from ethanol; R_f = 0.63 eluting with hexane/ethyl acetate (6:4), IR: 1590 (C=C), 1750 (C=O), 2233 cm^{-1} (CN); ^1H NMR (DMSO-d_6, 200 MHz): δ 3.84 (s, 3H, OCH_3), 7.09 (d, 1H, J = 8.6 Hz), 7.47–7.57 (m, 2H), 7.81–7.84 (m, 4H), 8.22–8.32 (m, 4H), 8.85 (d, 2H, J = 8.6 Hz); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 55.5 (OCH_3), 114.5, 114.6, 116.5, 117.0 (CN), 118, 123.2, 124.0, 129.0, 132.2, 133.3, 133.5, 150.2, 150.4, 154.0, 158.1, 158.2, 161.0, 162.0. Anal. Calcd for $C_{24}H_{13}ClFN_3O_2$ (429.83): C, 67.06; H, 3.05; N, 9.78. Found: C, 67.02; H, 3.01; N, 9.76.

[3-Cyano-4-(3′,4′-dimethoxyphenyl-6-(4″-methoxyphenyl) pyridin-2-yl]4-fluorobenzoate (5d) White crystals; yield 72%; mp 197°C, from ethanol; R_f = 0.8 eluting with 0.5% CH_3OH in CH_2Cl_2; IR: 1600 (C=C), 1768 (C=O), 2215 cm^{-1} (CN), ^1H NMR (DMSO-d_6, 200 MHz): δ 3.82, 3.84, 3.86 (3s, 9H, OCH_3), 7.06 (d, 1H, J = 8.60 Hz), 7.16 (d, 2H, J = 8.60 Hz), 7.42–7.54 (m, 4H), 8.16–8.32 (m, 5H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 55.4, 55.6, 55.7 (3 OCH_3), 98.9 (C-5), 111.8 (C-1), 112.3, 114.4 (C-3′), 116.9, 117.9 (C-5′), 121.7 (CN), 123.9, 127.2, 128.2, 129.5 (C-6′), 133.2, 133.4, 148.8, 150.2, 150.8, 156.0, 158.3, 159.2, 161.8, 162.7. Anal. Calcd for $C_{28}H_{21}FN_2O_5$ (484.48): C, 69.42; H, 4.37; N, 5.78. Found: C, 69.20; H, 4.36; N, 5.80.

Reagents, tissue culture, and culture conditions

All compounds tested were dissolved in DMSO (200-mM solutions) and subsequently diluted in the culture media before treatment of the culture cells. Human promyelotic leukemia HL-60 cells were obtained from the American Type Culture Collection and Roche Molecular Biochemical, USA. Cells were grown in DMEM medium (GIBCO-BRL) supplemented with 20% fetal calf serum (GIBCO-BRL). The cells were maintained at 37°C in a 5% CO_2 incubator. After reaching confluence, the cells were sub-cultured into 96-well plates, allowed to grow for 24 h, and treated with various concentrations of compounds **3**, **4**, and **5**.

MTT cell proliferation assay [16–18] Cells were plated in 96-well plates at a density 4×10^3 cells/well/100 μL of the appropriate culture medium and treated with compounds **3–5** at concentrations of 100 and 200 μM for 24 h. In parallel, cells were treated with 0.1% of DMSO as a control. A MTT [3-(4′,5′-dimethylthiazol-2′-yl)-2,5-diphenyltetrazolium bromide] assay was performed according to the manufacturer's instructions provided by Roche. After adding MTT, the yellow mixture was incubated for 1–4 h, followed by spectrophotometric measurements at 450 nm in the 96-well plates. Absorbance is directly proportional to the number of living cells in culture. The results are given in Figure 2.

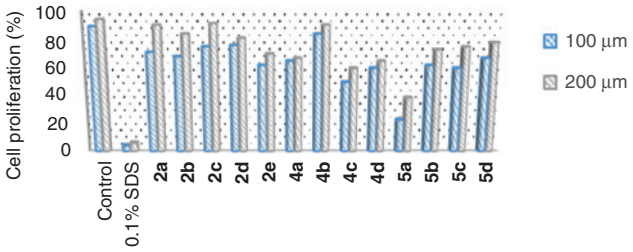


Figure 2 Cell viability of HL-60, treated with different doses (100 and 200 μM) of compounds **3, 4,** and **5** for 24 h at 37°C, using MTT cell proliferation assay.

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References

[1] Li, S.; Chen, Y.; Wang, C.; Tzeng, C. Synthesis and antiproliferative evaluation of certain pyrido[3,2-*g*]quinoline derivatives. *Bioorg. Med. Chem.* **2006**, *14*, 7370–7376.

[2] Frieden, M.; Ørum, H. Locked nucleic acid holds promise in the treatment of cancer. *Curr. Pharm. Des*. **2008**, *14*, 1138–1142.

[3] McNamara, D. J.; Cook, P. D. Synthesis and antitumor activity of fluorine-substituted 4-amino-2(1*H*)-pyridinones and their nucleosides. 3-Deazacytosines. *J. Med. Chem*. **1987**, *30*, 340–347.

[4] Abadi, A.; Al-Deeb, O.; Al-Afify, A.; El-Kashef, H. Synthesis of 4-alkyl (aryl)-6-aryl-3-cyano-2(1*H*)-pyridinones and their 2-imino isosteres as nonsteroidal cardiotonic agents. *Il Farmaco* **1999**, *54*, 195–201.

[5] Stout. M. G.; Robins, R. K. Synthesis of some quinazoline nucleosides. *J. Org. Chem*. **1968**, *33*, 1219–1225.

[6] Abdou, I. M.; Attia, A. M.; Strekowski, L. Glucopyranosides derived from 6-aryl-5-cyano-2-(methylthio)pyrimidin-4(3*H*)-ones. *Nucleosides, Nucleotides Nucleic Acids* **2002**, *21*, 15–21.

[7] Abdou, I. M.; Strekowski, L. A facile synthesis of 6-aryl-5-cyano-1-(β-D-pyranosyl or β-D-furanosyl)-2-thiocytosines. *Tetrahedron* **2000**, *56*, 8613–8636.

[8] Al-Neyadi, S. S.; Hassan, A. H.; Abdou, I. M. Microwave-assisted synthesis of 2(1*H*)-pyridones and their glucosides as cell proliferation inhibitors. *Nucleosides, Nucleotides Nucleic Acids* **2011**, *30*, 120–134.

[9] El-Sayed, H. A.; Moustafa, A. H.; Haikal, A. Z.; Abdou, I. M.; El-Ashry, E. S. H. Synthesis and evaluation of antimicrobial activity of some pyrimidine glycosides. *Nucleosides, Nucleotides Nucleic Acids* **2008**, *27*, 1061–1071.

[10] Kupchan, S. M.; Komoda, Y.; Branfman, A. R.; Sneden, A. T.; Court, W. A.; Thomas, G. J.; Hintz, H. P.; Smith, R. M.; Karim, A.; Howie, G. A.; et al. The maytansinoids. Isolation, structural elucidation, and chemical interrelation of novel ansa macrolides. *J. Org. Chem*. **1977**, *42*, 2349–2357.

[11] Vizirianakis, I. S.; Chatzopoulou, M.; Bonovolias, I. D.; Nicolaou, I.; Demopoulos,V. J.; Tsiftsoglou, A. S. Toward the development of innovative bifunctional agents to induce differentiation and to promote apoptosis in leukemia: clinical candidates and perspectives. *J. Med. Chem*. **2010**, *53*, 6779–6810.
[12] Nurit, D.-F.; Candice, L.; Amanda, L. R.; Dharmendra, B. Y.; Hajierah, D.; Charles, B. K. 6-Substituted imidazo[1,2-*a*]-pyridines synthesis and biological activity against colon cancer cell lines HT-29 and Caco-2. *Eur. J. Med. Chem.* **2011**, *46*, 4573–4583.
[13] Niedballa, U.; Vorbruggen, H. Synthesis of nucleosides. 12. General synthesis of *N*-glycosides. IV. Synthesis of nucleosides of hydroxy and mercapto nitrogen heterocycles *J. Org. Chem*. **1974**, *39*, 3668–3671.
[14] Abdou, I. M.; Saleh, A. M.; Zohdi, H. F. Synthesis and antitumor activity of 5-trifluoromethyl-2,4-dihydropyrazol-3-one nucleosides. *Molecules.* **2004**, *9*, 109–116.
[15] ACD/NMR processor academic edition, version 12.01. Toronto, ON: Advanced Chemistry Development, 2014. www.acdlabs.com.
[16] Johnston, H. P.; Hawley, P.; White, S. E.; Gibson, I.; Tidd, D. M. The effects of 6-mercaptopurine nucleotide derivatives on the growth and survival of 6-mercaptopurine-sensitive and -resistant cell culture lines. *Br. J. Cancer.* **1985**, *51*, 505–514.
[17] Kerr J. F.; Wyllie, A. H.; Currie A. R. Apoptosis: a basic biological phenomenon with wideranging implications in tissue kinetics. *Br. J. Cancer* **1972**, *26*, 239–257.
[18] Galmarini, C. M.; Mackey, J. R.; Dumontet, C. Nucleoside analogues: mechanisms of drug resistance and reversal strategies *Leukemia.* **2001**, *15*, 875–890.

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