Synthesis, Characterization and Cytotoxicity Evaluation of Some Novel Pyridine Derivatives

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Abstract

Reaction of isonicotinaldehyde with 2-cyanoacetohydrazide afforded (E)-2-cyano-N'-(pyridin-4-ylmethylene)acetohydrazide (1). Compound 1 was used as the precursor for the synthesis of novel pyridine derivatives by reaction with different arylidene malononitriles, malononitrile and acetylacetone to give pyridine derivatives 5a–e, 6 and 7, respectively. 4,4'-Bipyridine derivatives 9a–d were synthesized by a three-component reaction of isonicotinaldehyde, 2-cyanoacetohydrazide and activated nitriles 8a–d. Treatment of compound 9a with different aromatic aldehydes gave [1,2,4]triazolo[1,5-a]pyridine derivatives 11a–c. All reaction products were characterized by analytical and spectral data. For the novel compounds their bioactivity as antitumor agents was examined for in vitro cytotoxicity against HepG-2 and MCF-7. It was found that compounds 9a and 9b have high cytotoxic activity against both HepG-2 and MCF-7.

Keywords: Pyridine; 4,4'-bipyridine; isonicotinaldehyde; 2-cyanoacetohydrazide

1. Introduction

Among the important class of azaheterocycles, pyridine derivatives constitute one of the most significant classes of compounds as they broadly occur as vital structural subunits in many natural products, functional materials and pharmaceuticals 1 that exhibit many motivating biological activities.2–4 For example atazanavir 5 and imatinib mesylate6 (Figure 1) as two examples of drugs being prescribed for the treatment of HIV and chronic myelogenous leukemia, respectively.

Generally, pyridine derivatives have a huge spectrum of biological activities, like anti-leishmanial,7 anti-diabetic,8 anti-oxidant,9 antitumor10–12 and antiviral.13 Recently, some of pyridine derivatives were shown to act as potential targets for the development of new drugs for the treatment of cancer,14 as anti-platelet drugs,15 and antiproliferative agents.16

2. Experimental

2.1. Materials and Methods

2.1.1. Chemicals and Reagents

All the chemicals and solvents used in this study were obtained from Merck (Germany).
2. 2. Synthesis

Synthesis of (E)-2-Cyano-N’-(pyridin-4-ylmethylene) acetoxyhydrazone (1)

Method A: A mixture of isonicotinaldehyde (1.07 g, 0.01 mol), 2-cyanoacetoxyhydrazone (9.9 g, 0.01 mol) and TEA (2 drops) in THF or EtOH (15 mL) was refluxed for an appropriate time as shown in Table 1. The reaction progress was monitored by TLC. After completion of the reaction, the mixture was cooled to room temperature, the precipitate formed was collected by filtration and washed with ethyl acetate/petroleum ether (1:3), recrystallized from absolute ethanol to give pure compound 1 (yield 98%).

Pale yellow crystals; yield: method A: 92% and 39% for THF and EtOH, respectively; method B: 98%; m.p. 169 °C; IR (KBr): νmax 3235 (NH), 2259 (CN), 1704 (C=O) cm–1; 1H NMR (400 MHz, DMSO-d6): δ 4.26 (s, 2H, CH2), 8.02 (d, J = 7.2 Hz, 2H, C5-H and C6-H pyridine), 8.25 (s, 1H, CH=N), 8.74 (d, J = 7.2 Hz, 2H, C2-H and C4-H pyridine), 11.86 (s, 1H, NH); 13C NMR (100 MHz, DMSO-d6): δ 59.24, 77.64, 115.11, 115.83, 116.22, 125.02, 119.39, 122.87, 141.01, 145.72, 157.42; EI-MS: m/z 356 (M+, 9%). Anal. Calcd. for C19H12N6O2 (356.34): C, 64.04; H, 3.39; N, 25.94. Found: C, 64.03; H, 3.39; N, 25.95.

Synthesis of 2-Amino-cyano-6-oxo-4-aryl-1-((pyridin-4-ylmethylene)amino)-1,6-dihydropyridine-3,5-dicarbonitriles 5a–e

General procedure: To a solution of compound 1 (1.88 g, 0.01 mol) in EtOH (20 mL), arylidine malonitriles 2a–e (0.01 mol) and a catalytic amount of trimethylamine were added. The reaction mixture was heated under reflux for 17–20 h (TLC controlled), then, the reaction mixture was left to cool. The precipitate that formed was filtered off, washed with ethyl acetate and recrystallized from ethanol to give the products 5a–e.

2-Amino-6-oxo-4-phenyl-1-((pyridin-4-ylmethylene) amino)-1,6-dihydropyridine-3,5-dicarbonitrile (5a)

Brown crystals; yield: 20%; m.p. >300 °C; IR (KBr): υmax 3345, 3390 (NH2), 2214, 2206 (2×CN), 1673 (C=O) cm–1; 1H NMR (400 MHz, DMSO-d6): δ 6.55 (s, 2H, NH2), 7.10–7.50 (m, 5H, Ar-H), 7.99 (d, J = 7.2 Hz, 2H, C3-H and C2-H pyridine), 8.43 (s, 1H, CH=N), 8.73 (d, J = 7.2 Hz, 2H, C2-H and C6-H pyridine); 13C NMR (100 MHz, DM-SO-d6): δ 77.83, 115.23, 115.81, 116.29, 124.72, 126.90, 128.76, 129.20, 134.88, 145.83, 149.89, 152.66, 158.90, 162.34, 168.88; EI-MS: m/z 340 (M+, 50%). Anal. Calcd. for C19H12N6O2 (340.35): C, 67.05; H, 3.55; N, 24.69. Found: C, 67.12; H, 3.49; N, 24.72.

2-Amino-4-(4-methoxyphenyl)-6-oxo-1-((pyridin-4-ylmethylene)amino)-1,6-dihydropyridine-3,5-dicarbonitrile (5b)

Dark brown crystals; yield: 31%; m.p. 208 °C. IR (KBr): υmax 3320, 3385 (NH2), 2213, 2235 (2×CN), 1669 (C=O) cm–1; 1H NMR (400 MHz, DMSO-d6): δ 3.85 (s, 3H, CH3), 6.58 (s, 2H, NH2), 6.95 (d, J = 8.0 Hz, 2H, Ar-H), 7.62 (d, J = 8.1 Hz, 2H, Ar-H), 8.04 (d, J = 7.2 Hz, 2H, C3-H and C5-H pyridine), 8.43 (s, 1H, CH=N), 8.72 (d, J = 7.2 Hz, 2H, C2-H and C6-H pyridine); 13C NMR (100 MHz, DMSO-d6): δ 59.24, 77.64, 115.11, 115.83, 116.22, 116.79, 122.58, 125.69, 131.94, 146.04, 149.91, 152.75, 158.14, 158.99, 161.67, 169.52; EI-MS: m/z 370 (M+, 18%). Anal. Calcd. for C20H14N6O2 (370.37): C, 64.86; H, 3.81; N, 22.69. Found: C, 64.84; H, 3.79; N, 22.63.

2-Amino-4-(4-hydroxyphenyl)-6-oxo-1-((pyridin-4-ylmethylene)amino)-1,6-dihydropyridine-3,5-dicarbonitrile (5c)

Brown crystals; yield: 33%; m.p. 214 °C; IR (KBr): υmax 3425 (OH), 3355, 3314 (NH2), 2213, 2229 (2×CN), 1679 (C=O) cm–1; 1H NMR (400 MHz, DMSO-d6): δ 6.58 (s, 2H, NH2), 6.74 (d, J = 7.8 Hz, 2H, Ar-H), 7.44 (d, J = 7.8 Hz, 2H, Ar-H), 8.00 (d, J = 7.2 Hz, 2H, C3-H and C5-H pyridine), 8.40 (s, 1H, CH=N), 8.74 (d, J = 7.2 Hz, 2H, C2-H and C6-H pyridine), 9.77 (s, 1H, OH); 13C NMR (100 MHz, DMSO-d6): δ 77.37, 115.23, 115.88, 116.17, 116.89, 122.75, 126.73, 130.88, 145.83, 149.93, 152.82, 158.12, 159.18, 161.73, 169.43; EI-MS: m/z 356 (M+, 9%). Anal. Calcd. for C19H12N6O2 (356.34): C, 64.04; H, 3.39; N, 23.58. Found: C, 63.99; H, 3.42; N, 23.51.

2-Amino-4-(4(dimethylamino)phenyl)-6-oxo-1-((pyridin-4-ylmethylene)amino)-1,6-dihydropyridine-3,5-dicarbonitrile (5d)

Brown crystals; yield: 51%; m.p. 220 °C; IR (KBr): υmax 3397, 3381 (NH2), 2224, 2241 (2×CN), 1670 (C=O) cm–1; 1H NMR (400 MHz, DMSO-d6): δ 3.25, 3.28 (s, 6H, CH2N), 7.10–7.50 (m, 5H, Ar-H), 7.99 (d, J = 7.2 Hz, 2H, C3-H and C2-H pyridine), 8.43 (s, 1H, CH=N), 8.73 (d, J = 7.2 Hz, 2H, C2-H and C6-H pyridine); 13C NMR (100 MHz, DMSO-d6): δ 77.83, 115.23, 115.81, 116.29, 124.72, 126.90, 128.76, 129.20, 134.88, 145.83, 149.89, 152.66, 158.90, 162.34, 168.88; EI-MS: m/z 356 (M+, 50%). Anal. Calcd. for C19H12N6O2 (356.34): C, 64.04; H, 3.39; N, 23.58. Found: C, 63.99; H, 3.42; N, 23.51.
cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆): δ 3.13 (s, 6H, CH₃), 6.58 (s, 2H, NH₂), 6.81 (d, J = 7.2 Hz, 2H, Ar-H), 7.23 (d, J = 8.2 Hz, 2H, Ar-H), 8.00 (d, J = 7.2 Hz, 2H, C₃-H and C₅-H pyridine), 8.42 (s, 1H, CH=N), 8.72 (d, J = 7.3 Hz, 2H, C₂-H and C₆-H pyridine); ¹³C NMR (100 MHz, DMSO-d₆): δ 40.62, 77.06, 112.89, 115.61, 115.97, 116.22, 122.57, 123.16, 131.11, 146.34, 150.04, 150.96, 153.08, 158.97, 161.82, 169.07; EI-MS: m/z 383 (M⁺, 94%). Anal. Calcd. for C₉H₁₂N₅O (383.42): C, 65.79; H, 4.47; N, 25.57. Found: C, 65.71; H, 4.51; N, 25.60.

2-Amino-4-(4-hydroxy-3-methoxyphenyl)-6-oxo-1-((pyridin-4-ylmethylene)amino)-1,6-dihydropyrindine-3,5-dicarbonitrile (5e)

Orange red crystals; yield: 22%; m.p. 240 °C. IR (KBr): νmax 3148 (OH), 3337, 3308 (NH₂), 2207, 2225 (2×CN), 1666 (C=O) cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆): δ 4.11 (s, 3H, OCH₃), 6.53 (s, 2H, NH₂), 6.78–7.12 (m, 3H, Ar-H), 8.01 (d, J = 7.2 Hz, 2H, C₅-H and C₇-H pyridine), 8.42 (s, 1H, CH=N), 8.75 (d, J = 7.2 Hz, 2H, C₂-H and C₆-H pyridine), 9.57 (s, 1H, OH); ¹³C NMR (100 MHz, DMSO-d₆): δ 58.38, 77.13, 114.92, 115.44, 115.87, 116.89, 117.35, 122.92, 123.79, 127.14, 144.91, 148.49, 149.72, 150.98, 153.42, 158.24, 161.62, 169.38; EI-MS: m/z 386 (M⁺, 31%). Anal. Calcd. for C₉H₁₂N₅O (386.37): C, 62.17; H, 3.65; N, 21.75. Found: C, 62.21; H, 3.58; N, 21.70.

Synthesis of 4,6-Dimino-2-oxo-1-((pyridin-4-ylmethylene)amino)-1,2-dihydropyridine-3-carbonitrile (6)

A mixture of compound 1 (1.88 g, 0.005 mol) and malononitrile (0.01 mol) in 20 mL of absolute EtOH containing 3 drops of triethylamine was refluxed for 9 h (TLC controlled). Then, the reaction mixture was left to cool and the precipitated solid was filtered off, dried, washed with ethyl acetate and recrystallized from absolute EtOH to afford compound 6.

Yellow crystals; yield: 65%; m.p. >300 °C; IR (KBr): νmax 3399, 3388, 3347, 3231 (2×NH₂), 2216 (CN), 1683 (C=O) cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆): δ 4.62 (s, 1H, CH), 4.65 (s, 2H, NH₂), 6.68 (s, 2H, NH₂), 8.03 (d, J = 7.2 Hz, 2H, C₂-H, C₆-H pyridine), 8.43 (s, 1H, CH=NH), 8.71 (d, J = 7.2 Hz, 2H, C₂-H, C₆-H pyridine); ¹³C NMR (100 MHz, DMSO-d₆): δ 72.83, 81.27, 116.74, 123.55, 143.94, 146.25, 151.47, 155.18, 162.48, 178.28; EI-MS: m/z 254 (M⁺, 100%). Anal. Calcd. for C₁₂H₁₀N₂O (254.25): C, 56.69; H, 3.96; N, 33.05. Found: C, 56.77; H, 4.01; N, 32.96.

Synthesis of 4,6-Dimethyl-2-oxo-1-(pyridin-4-ylmethylen)amino)-1,2-dihydropyridine-3-carbonitrile (7)

To a solution of 1 (1.88 g, 0.01 mol) and acetylacetone (1.001 g, 0.01 mol) in 20 mL of absolute EtOH containing a few drops of trimethylamine were added. The reaction mixture was heated under reflux for 18 h. After the completion of the reaction, the reaction mixture was cooled and the separated solid product was collected by filtration, washed with ethanol, dried, and recrystallized from EtOH to give compound 7.

Buff crystals; yield: 61%; m.p. 190 °C; IR (KBr): νmax 2208 (CN), 1674 (C=O) cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆): δ 2.13 (s, 3H, CH₃), 5.66 (s, 1H, CH), 8.10 (d, J = 7.2 Hz, 2H, C₁-H, C₇-H pyridine), 8.41 (s, 1H, CH=N), 8.70 (d, J = 7.2 Hz, 2H, C₂-H, C₆-H pyridine); ¹³C NMR (100 MHz, DMSO-d₆): δ 18.24, 22.03, 110.94, 116.83, 118.07, 122.44, 135.88, 154.57, 150.21, 153.47, 155.26, 161.47; EI-MS: m/z 252 (M⁺, 100%). Anal. Calcd. for C₁₂H₁₀N₂O (252.28): C, 66.65; H, 4.79; N, 22.21. Found: C, 66.49; H, 4.81; N, 22.29.

Synthesis of 1,6-Diamino-2-oxo-5-(alkyl)-1,2-dihydropyridine-[4,4′-bipyridine]-3-carbonitriles 9a-d

General procedure: To a mixture of isonicotinaldehyde (1.07 g, 0.01 mol), activated nitriles 8a–d (0.01 mol) and 2-cyanoacetohydrazide (0.99 g, 0.01 mol) absolute EtOH (20 mL) containing three drops of piperidine was added. The reaction mixture was heated under reflux for 6–8 h (TLC controlled). The reaction mixture was left to cool to the room temperature, then the solid formed was filtered off and recrystallized from absolute EtOH to give compounds 9a–d.

1,6-Diamino-2-oxo-1,2-dihydro-[4,4′-bipyridine]-3,5-dicarbonitrile (9a)

Brown crystals; yield: 71%; m.p. >300 °C; IR (KBr): νmax 3428, 3373, 3366, 3345 (2×NH₂), 2202, 2180 (2CN), 1665 (C=O) cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆): δ 5.22 (s, 2H, NH₂), 6.56 (s, 2H, NH₂), 7.51 (d, J = 7.2 Hz, 2H, C₁-H, C₇-H pyridine), 8.63 (d, J = 7.2 Hz, 2H, C₂-H, C₆-H pyridine); ¹³C NMR (100 MHz, DMSO-d₆): δ 77.42, 115.24, 116.27, 122.05, 122.84, 145.14, 150.44, 159.11, 161.57, 171.34; EI-MS: m/z 186 (M⁺, 100%). Anal. Calcd. for C₁₇H₁₄N₄O₂ (367.38): C, 55.58; H, 3.57; N, 19.06; S, 8.73. Found: C, 55.58; H, 3.57; N, 19.06; S, 8.73.

Ethyl 1,2-Diamino-5-cyano-6-oxo-1,6-dihydr[4,4′-bipyridine]-3-carboxylate (9c)

Yellow crystals; yield: 48%; m.p. 180–190 °C; IR (KBr): νmax 3351, 3339, 3335, 3305 (2×NH₂), 2215 (CN),
1,6-Diamo-5-(benzo[d]thiazol-2-yl)-2-oxo-1,2-dihydro-[4,4′-bipyridine]-3-carbonitrile (9d)

Yellow crystals; yield: 73%; m.p. 315 °C; IR (KBr): ν\text{max} 3400, 3391, 3255, 3066 (2×NH\textsubscript{2}), 2209 (CN), 1661 cm\textsuperscript{-1}; 1H NMR (400 MHz, DMSO-\textit{d}\textsubscript{6}): δ 8.71 (d, J = 7.2 Hz, 2H, C\textsubscript{2}-H, C\textsubscript{6}-H pyridine); 13C NMR (100 MHz, DMSO-\textit{d}\textsubscript{6}): δ 102.14, 116.40, 121.23, 121.98, 122.73, 124.85, 125.94, 126.48, 138.48, 141.29, 145.34, 150.77, 155.26, 161.19, 169.97, 171.57; EI-MS: m/z 360 (M\textsuperscript{+}, 83%). Anal. Calcd. for C\textsubscript{18}H\textsubscript{12}N\textsubscript{6}O\textsubscript{2} (360.40): C, 58.24; H, 4.05; N, 23.34. Found: C, 56.21; H, 4.33; N, 23.41.

Synthesis of 5-Oxo-2-aryl-7-(pyridin-4-yl)-1,2,3,5-tetrahydro-[1,2,4]triazolo[1,5-a]pyridine-6,8-dicarbonitrile 11a–c

General procedure: A solution of compound 9a (2.52 g, 0.01 mol), aromatic aldehydes 10a–c (0.01 mol) in 1,4-dioxane and/or DMF (25 mL) containing a catalytic amount of piperidine (3 drops) was heated under reflux for 12–15 h. The reaction was monitored by TLC. The product that was precipitated on cooling to room temperature was filtered off, dried and recrystallized from absolute EtOH to give the compounds 11a–c.

5-Oxo-2-phenyl-7-(pyridin-4-yl)-1,2,3,5-tetrahydro-[1,2,4]triazolo[1,5-a]pyridine-6,8-dicarbonitrile (11a)

Brown crystals; yield: 65%; m.p. > 300 °C; IR (KBr): ν\text{max} 3245, 3283 (2×NH\textsubscript{2}), 2116, 2195 (2×CN), 1679 (C=O) cm\textsuperscript{-1}; 1H NMR (400 MHz, DMSO-\textit{d}\textsubscript{6}): δ 8.71 (d, J = 7.2 Hz, 2H, C\textsubscript{2}-H, C\textsubscript{6}-H pyridine); 13C NMR (100 MHz, DMSO-\textit{d}\textsubscript{6}): δ 4.81 (s, 1H, CH), 7.21–7.29 (m, 8H, Ar-H, NH and C\textsubscript{3}-H, C\textsubscript{5}-H pyridine), 8.66 (d, J = 7.2 Hz, 2H, C\textsubscript{2}-H, C\textsubscript{6}-H pyridine); 13C NMR (100 MHz, DMSO-\textit{d}\textsubscript{6}): δ 87.47, 98.79, 116.78, 117.14, 122.16, 123.48, 126.77, 127.14, 130.47, 143.59, 146.41, 150.72, 160.62, 162.34, 170.83; EI-MS: m/z 340 (M\textsuperscript{+}, 19%). Anal. Calcd. for C\textsubscript{19}H\textsubscript{13}N\textsubscript{5}O\textsubscript{3} (299.29): C, 56.18; H, 2.96; N, 24.60. Found: C, 56.63; H, 2.34; N, 24.60.

2-(4-Chlorophenyl)-5-oxo-7-(pyridin-4-yl)-1,2,3,5-tetrahydro-[1,2,4]triazolo[1,5-a]pyridine-6,8-dicarbonitrile (11c)

Brown crystals; yield: 69%; m.p. > 300 °C; IR (KBr): ν\text{max} 3239, 3297 (2×NH), 2217, 2299 (2×CN), 1665 (C=O) cm\textsuperscript{-1}; 1H NMR (400 MHz, DMSO-\textit{d}\textsubscript{6}): δ 4.85 (s, 1H, NH), 5.15 (s, 1H, CH), 7.25 (s, 1H, NH), 7.31 (d, J = 8.2 Hz, 2H, Ar-H), 7.41 (d, J = 8.0 Hz, 2H, Ar-H), 7.58 (d, J = 7.1 Hz, 2H, C\textsubscript{2}-H, C\textsubscript{6}-H pyridine), 8.66 (d, J = 7.2 Hz, 2H, C\textsubscript{2}-H, C\textsubscript{6}-H pyridine); 13C NMR (100 MHz, DMSO-\textit{d}\textsubscript{6}): δ 87.63, 100.06, 116.24, 116.88, 122.64, 122.89, 128.64, 129.33, 134.16, 143.08, 144.73, 150.24, 151.24, 162.45, 171.22; EI-MS: m/z 376 (M\textsuperscript{+} + 2, 0.88), 374 (M\textsuperscript{+}, 3%). Anal. Calcd. for C\textsubscript{19}H\textsubscript{13}ClN\textsubscript{5}O\textsubscript{3} (374.79): C, 60.89; H, 2.96; Cl, 9.46; N, 22.42. Found: C, 60.93; H, 2.89; Cl, 9.51; N, 22.43.

3. Results and Discussion

3.1 Chemistry

(E)-2-Cyano-N\textsuperscript{′}-[(pyridin-4-ylmethylene)acetohydrazide (1) was synthesized by the reaction of isonicotinaldehyde with 2-cyanoacetohydrazide under various conditions.
In the absence of any catalyst and under solvent-free conditions or in the presence of triethylamine as the basic catalyst at room temperature the reaction did not proceed even after long reaction time (Table 1, entries 1, 2 and 4). However, in the presence of Et$_3$N under reflux with the EtOH or THF as the solvents, the desired product was obtained in 39 or 92% yield, respectively (Table 1, entries 5 and 6). Moreover, when the synthesis of 1 was carried out under microwave irradiation under solvent-free conditions, afforded the desired reaction product in high yield (Table 1, entry 7). The solvent-free conditions are preferable as they avoid the use of toxic, flammable, and expensive organic solvents. The main advantages of microwave irradiation synthesis are thus shorter reaction time, higher yield and better purity of the product.

The chemical structure of 1 was confirmed by its spectral and elemental analysis data. The IR spectrum of 1 showed the presence of three stretching frequencies at 3235, 2259 and 1704 cm$^{-1}$ attributable to NH, CN and C=O groups, respectively. The $^1$H NMR exhibited two singlet signals at δ 4.26 and 8.25 ppm due to CH$_2$ and CH=N, respectively. In addition, two doublet signals at δ 8.02 and 8.74 ppm due to pyridine protons are observed. The configuration around the double bond of the compound 1 could not be established by $^1$H NMR spectroscopy. However, the steric effect enhances that the E isomer is more stable than Z isomer.

Compound 1 acts as an adaptable material for the synthesis of novel pyridine compounds. Thus, refluxing of 1 and arylidene malononitriles 2a–e in ethanol catalyzed by piperidine afforded (E)-2-amino-4-aryl-5-cyano-6-oxo-1-((pyridin-4-ylmethylene)amino)-1,6-dihydropyridine-3-carbonitriles 5a–e (Scheme 2).

Formation of compounds 5a–e could be elucidated by the mechanism presented in Scheme 2. At first, Michael addition of 1 to α,β-unsaturated nitriles 2a–e gives the intermediates 3. Then, the intermediates 3 undergo an intermolecular nucleophilic addition of NH group to the cyano function to afford the intermediates 4 and finally an

![Scheme 1. Synthesis of (E)-2-cyano-N'(pyridin-4-ylmethylene)acetohydrazide (1)](image)

![Scheme 2. Synthesis of (E)-2-amino-4-aryl-5-cyano-6-oxo-1-((pyridin-4-ylmethylene)amino)-1,6-dihydropyridine-3-carbonitriles 5a–e](image)
autoxidation and tautomerization occur to give isolable products 5a–e.\textsuperscript{18} $^1$H NMR spectra of compounds 5a–e display characteristic signals: singlet signal at δ 8.40–8.44 ppm and two doublet signals at δ 7.99–8.04 and 8.72–8.75 ppm due to the CH=N and pyridine protons, respectively. Also, $^{13}$C NMR revealed two signals in the region of δ 115–118 ppm due to the two cyano groups in addition to the signal in the region of δ 161–163 ppm attributable to the C=O group. IR spectra of compounds 5a–e exhibited NH$_2$ group stretching frequencies in the region of 3308–3397 cm$^{-1}$ and the stretching frequency at 2200–2250 cm$^{-1}$ that indicated the presence of two nitrile functional groups.

Treatment of 1 with malononitrile or acetylacetone in refluxing ethanol in the presence of trimethylamine as the base catalyst furnished (E)-4,6-diamino-2-oxo-1-((pyridin-4-ylmethylene)amino)-1,2-dihydropyridine-3-carbonitrile (6) and (E)-4,6-dimethyl-2-oxo-1-((pyridin-4-ylmethylene)amino)-1,2-dihydropyridine-3-carbonitrile (7), respectively (Scheme 3).

The spectral and analytical data of compounds 6 and 7 were in agreement with their proposed structures. $^1$H NMR spectrum of 6 showed two singlet signals at δ 4.62 and 8.43 ppm owing to the C$_5$–H of 2-pyridone ring and CH=N, respectively. Moreover, $^1$H NMR of 6 exhibited two singlet signals (D$_2$O-exchangable) at δ 4.65 and 6.68 ppm due to the two NH$_2$ groups. The IR analysis substantiated the results of $^1$H NMR by the presence of four peaks in the region of 3321–4000 cm$^{-1}$ for two NH$_2$ groups.

Nowadays, multicomponent reactions are gaining extensive economic and ecological importance as they conform to the fundamental principles of synthetic efficiency and reaction design.\textsuperscript{19} We herein provide an efficient and facile procedure for the synthesis of 4,4’-bipyridine derivatives 9a–d via a one-pot three-components condensation of isonicotinaldehyde, 2-cyanoacetohydrazide and activated nitriles 8a–d\textsuperscript{20,21} (Scheme 4).

The structures of products 9a–d were assigned according to their IR, $^1$H NMR, $^{13}$C NMR and mass spectra. Thus, all compounds 9a–d gave molecular ion peak which coincides with the proposed structure. $^1$H NMR gave an additional evidence for the correct structure of compounds 9a–d, for example, compound 9c gave triplet quartet pattern at δ 1.01 and 4.03 ppm, respectively, which confirm the presence of ethyl ester group in addition to the singlet signal (D$_2$O-exchangable) at δ 7.08 ppm corresponding to the amino group. Compound 9a was used as a versatile material for the synthesis of 2-aryl-5-oxo-7-(pyridin-4-yl)-1,2,3,5-tetrahydro-[1,2,4]triazolo[1,5-a]pyridine-6,8-dicarbonitrile derivatives 11a–c. Consequently, the reaction of 9a with aromatic aldehydes 10a–c afforded compounds 11a–c (Scheme 5).

![Scheme 3. Synthesis of (E)-2-oxo-1-((pyridin-4-ylmethylene)amino)-1,2-dihydropyridine-3-carbonitrile derivatives 6 and 7](image)

![Scheme 4. Synthesis of 5-alkyl-1,6-diamino-2-oxo-1,2-dihydro-[4,4’-bipyridine]-3-carbonitriles 9a–d](image)
Structures 11a–c were established on the basis of elemental analyses and spectral data. The $^1$H NMR spectra of compounds 11a–c, in general, gave singlet signal at 5.08–5.15 ppm attributable to C3-H of [1,2,4]triazole ring in addition to the two singlet signals (D$_2$O-exchangable) at δ 4.77–4.85 and 7.25–7.29 ppm due to the two NH groups.

Also, 5-oxo-7-(pyridin-4-yl)-1,5-dihydro-[1,2,4]triazolo[1,5-a]pyridine-6,8-dicarbonitrile (12) was synthesized via cyclocondensation reaction of 9a with DMF-DMA (Scheme 6).

### 3.2. Pharmacology

#### 3.2.1. Cytotoxicity Against Hepatoma Cell Line (HepG-2) and Human Breast Adenocarcinoma Cell Line (MCF-7)

Cytotoxic activity. In order to investigate if the chemistry established here has led to possibly interesting nominees in cancer therapy, our primary aim was directed towards checking if the novel synthesized compounds possess any anticancer activities as predicted by this study. *In vitro* cytotoxic study was therefore performed against two mammalian cancer cell lines, HepG-2 (hepatoma cells or human liver hepatocellular carcinoma cell line) and MCF-7 (human breast adenocarcinoma cell line). This study indicated that compounds 9a and 9b showed very strong cytotoxic activity against HepG-2 cancer cells with IC$_{50}$ values of 8.83±0.30 and 10.08±0.66 µg/mL, respectively. Also, both 9a and 9b gave high cytotoxic effects against MCF-7 classifying these compounds as chemotherapeutically significant (Table 2). The rest of other compounds showed a moderate to weak activity against the tested tumor cell lines. IC$_{50}$ is the concentration, which can reduce the growth of cancer cells by 50%.

### 4. Conclusion

In conclusion, herein we report a simple and convenient method for the synthesis of novel pyridine derivatives. All synthesized compounds were evaluated against two cancer cell lines (HepG-2 and MCF-7). Among all the synthesized compounds, compounds 9a, b have high cytotoxic activity against both HepG-2 and MCF-7. The rest of compounds showed a moderate to weak activity against the tested tumor cell lines.

### 5. References

1. (a) G. Jones, *Comprehensive Heterocyclic Chemistry II*, Vol. 5 (Eds.: A. R. Katritzky, C. W. Rees, E. F. V. Scriven, A. McKillop), Pergamon, Oxford, 1996, pp. 167–243; DOI:10.1016/B978-008096518-5.00108-8
2. (b) G. D. Henry, *Tetrahedron* 2004, 60, 6043-6061; DOI:10.1016/j.tet.2004.04.043
Reakcijo izonikotinaldehida z 2-cianoacetohidrazidom daje (E)-2-ciano-N-(piridin-4-ilmetilen)acetohidrazid (1). Spojino 1 smo uporabili kot prekurzor pri sintezi novih piridinskih derivatov, ki smo jih pripravili z reakcijo med različnimi arilidenskimi malononitrili, malononitrilom oz. acetilacetonom, pri čemer so nastali piridinski derivati 5a-e. 6 in 7. 4,4'-Bipiridinske derivate 9a-d smo pripravili s trokomponentno reakcijo med izonikotinaldehidom, 2-cianoacetohidrazidom in aktiviranimi nitrili 8a-d. Obdelava spojine 9a z različnimi aromatskimi aldehidi je vodila do nastanka [1,2,4]triazolo[1,5-a]piridinskih derivatov 11a-c. Vse reakcijske produkte smo okarakterizirali z analitiki in spektroskopskimi metodami. Za nove spojine smo raziskali tudi bioaktivnost v vlogi antitumornih učinkov in je izkazala visoko citotoksičnost proti HepG-2 in MCF-7.

11. J.-P. Liou, K.-S. Hsu, C.-C. Kuo, C.-Y. Chang, J.-Y. Chang, J. Pharmoc. Exp. Ther. 2007, 398–405. DOI:10.1124/jpet.107.126680
12. R. M. Mohareb, N. Y. M. Abdo, F. O. Al-Farouk, Acta Chim. Slov. 2017, 64, 117–128. DOI:10.17344/acsi.2016.2920
13. J. M. Chezal, J. Paeshuysse, V. Gaumet, D. Canitrot, A. Mascion, C. Artigue, A. Gueffier, E. Moreau, J. C. Teulade, O. Chavignon, Eur. J. Med. Chem. 2010, 45, 2044–2047. DOI:10.1016/j.ejmech.2010.01.023
14. W. Xie, Y. Wu, J. Zhang, Q. Mei, Y. Zhang, N. Zhu, R. Liu, H. Zhang, Eur. J. Med. Chem. 2018, 145, 35–40. DOI:10.1016/j.ejmech.2017.12.038
15. N. K. Binsaleh, C. A. Wigley, K. A. Whitehead, M. van Rensburg, J. Reynisson, L. I. Pilkington, D. Barker, S. Jones, N. C. Dempsey-Hibbert, Eur. J. Med. Chem. 2018, 143, 1997–2004. DOI:10.1016/j.ejmech.2017.11.014
16. Q. Tang, Y. Duan, L. Wang, M. Wang, Y. Ouyang, C. Wang, H. Mei, S. Tang, Y. Xiong, P. Zheng, P. Gong, Eur. J. Med. Chem. 2018, 143, 266–275. DOI:10.1016/j.ejmech.2017.11.034
17. A. A. Fadda, E. Abdel-Latif, R. E. El-Mekawy, Pharmaco Pharm 2012, 3, 148–157. DOI:10.4236/pp.2012.32022
18. M. R. H. Elmoghayar, A. G. A. El-Agamey, M. Y. A. S. Nasr, M. M. Sallam, J. Het. Chem. 1984, 21, 1885–1887.
19. C. C. Cariou, G. J. Clarkson, M. Shipman, J. Org. Chem. 2008, 73, 9762–9764. DOI:10.1021/jo801664g
20. A. H. M. Hussein, Heteroat. Chem. 1997, 8, 1–6. DOI:10.1016/S1074-5521(00)00006-5
21. F. F. A. Latif, R. Mekheimer, E. K. Ahmed, T. B. A. Aleem, Pharmazie 1993, 48, 736–738.