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 struc-

tural subunits in many natural products, functional materials and pharmaceuticals¹ that exhibit many motivating biological activities.^{2–4} For example atazanavir⁵ 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 antitumor^{10–12} 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).

Figure 1. Chemical structures of atazanavir and imatinib mesylate

2. 1. 2. Instruments

Melting points were recorded on Gallenkamp electric melting point apparatus (Electronic Melting Point Apparatus, Great Britain, London) and are uncorrected. Infrared spectra were recorded on Pye Unicam SP 1000 IR spectrophotometer (Thermoelectron Co. Egelsbach, Germany) using a KBr wafer technique. The ^1H NMR spectra were determined by a Bruker 400 MHz spectrometer. DMSO- d_6 was used as the solvent, TMS was used as the internal standard and chemical shifts are given on δ scale in ppm. Mass spectra were determined on a GC-MS QP-100 EX Shimadzu (Japan). Microwave experiment was carried out using Milestone Start Microwave Lab Station. Elemental analyses were recorded on Perkin-Elmer 2400 Elemental analyzer at the Microanalytical Center at Cairo University, Cairo, Egypt.

2. 2. Synthesis

Synthesis of (E)-2-Cyano- N' -(pyridin-4-ylmethylene)acetohydrazide (1)

Method A: A mixture of isonicotinaldehyde (1.07 g, 0.01 mol), 2-cyanoacetohydrazide (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.

Method B: A mixture of isonicotinaldehyde (1.07 g, 0.01 mol) and 2-cyanoacetohydrazide (9.9 g, 0.01 mol) in a sealed tube was subjected to microwave irradiation at 700 W and microwave oven temperature 120 °C for 20 sec. The reaction mixture was cooled to room temperature and washed by a mixture of ethyl acetate/petroleum ether (1:3). The precipitate formed was recrystallized from absolute ethanol to give the pure product 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): $\nu_{\max}$ 3235 (NH), 2259 (CN), 1704 (C=O) cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6): δ 4.26 (s, 2H, CH_2), 8.02 (d, $J = 7.2$ Hz, 2H, $\text{C}_3\text{-H}$ and $\text{C}_5\text{-H}$ pyridine), 8.25 (s, 1H, $\text{CH}=\text{N}$), 8.74 (d, $J = 7.2$ Hz, 2H, $\text{C}_2\text{-H}$ and $\text{C}_6\text{-H}$ pyridine), 11.86 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO- d_6): δ 25.02, 119.39, 122.87, 141.01, 145.72, 149.61, 165.35; EI-MS: m/z 188 (M^+ , 10). Anal. Calcd. for $\text{C}_9\text{H}_8\text{N}_4\text{O}$ (188.19): C, 57.44; H, 4.29; N, 29.77. Found: C, 57.42; H, 4.33; N, 29.82.

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), arylidene malononitriles 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): $\nu_{\max}$ 3345, 3390 (NH_2), 2214, 2206 ($2\times\text{CN}$), 1673 (C=O) cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6): δ 6.55 (s, 2H, NH_2), 7.10–7.50 (m, 5H, Ar-H), 7.99 (d, $J = 7.2$ Hz, 2H, $\text{C}_3\text{-H}$ and $\text{C}_5\text{-H}$ pyridine), 8.43 (s, 1H, $\text{CH}=\text{N}$), 8.73 (d, $J = 7.2$ Hz, 2H, $\text{C}_2\text{-H}$ and $\text{C}_6\text{-H}$ pyridine); ^{13}C NMR (100 MHz, DMSO- d_6): δ 77.83, 115.23, 115.81, 116.29, 122.74, 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 $\text{C}_{19}\text{H}_{12}\text{N}_6\text{O}$ (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): $\nu_{\max}$ 3320, 3385 (NH_2), 2212, 2235 ($2\times\text{CN}$), 1669 (C=O) cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6): δ 3.85 (s, 3H, CH_3), 6.58 (s, 2H, NH_2), 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, $\text{C}_3\text{-H}$ and $\text{C}_5\text{-H}$ pyridine), 8.43 (s, 1H, $\text{CH}=\text{N}$), 8.72 (d, $J = 7.2$ Hz, 2H, $\text{C}_2\text{-H}$ and $\text{C}_6\text{-H}$ pyridine); ^{13}C NMR (100 MHz, DMSO- d_6): 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 $\text{C}_{20}\text{H}_{14}\text{N}_6\text{O}_2$ (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): $\nu_{\max}$ 3425 (OH), 3355, 3314 (NH_2), 2213, 2229 ($2\times\text{CN}$), 1679 (C=O) cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6): δ 6.58 (s, 2H, NH_2), 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, $\text{C}_3\text{-H}$ and $\text{C}_5\text{-H}$ pyridine), 8.40 (s, 1H, $\text{CH}=\text{N}$), 8.74 (d, $J = 7.2$ Hz, 2H, $\text{C}_2\text{-H}$ and $\text{C}_6\text{-H}$ pyridine), 9.77 (s, 1H, OH); ^{13}C NMR (100 MHz, DMSO- d_6): 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 $\text{C}_{19}\text{H}_{12}\text{N}_6\text{O}_2$ (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): $\nu_{\max}$ 3397, 3381 (NH_2), 2224, 2241 ($2\times\text{CN}$), 1670 (C=O)

cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 3.13 (s, 6H, CH_3), 6.58 (s, 2H, NH_2), 6.81 (d, $J = 8.2$ Hz, 2H, Ar-H), 7.23 (d, $J = 8.2$ Hz, 2H, Ar-H), 8.00 (d, $J = 7.2$ Hz, 2H, C_3 -H and C_5 -H pyridine), 8.42 (s, 1H, $\text{CH}=\text{N}$), 8.72 (d, $J = 7.3$ Hz, 2H, C_2 -H and C_6 -H pyridine); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): 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 $\text{C}_{21}\text{H}_{17}\text{N}_7\text{O}$ (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-dihydropyridine-3,5-dicarbonitrile (5e)

Orange red crystals; yield: 22%; m.p. 240 °C. IR (KBr): ν_{max} 3418 (OH), 3337, 3308 (NH_2), 2207, 2225 ($2\times\text{CN}$), 1666 ($\text{C}=\text{O}$) cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 4.11 (s, 3H, OCH_3), 6.53 (s, 2H, NH_2), 6.78–7.12 (m, 3H, Ar-H), 8.01 (d, $J = 7.2$ Hz, 2H, C_3 -H and C_5 -H pyridine), 8.42 (s, 1H, $\text{CH}=\text{N}$), 8.75 (d, $J = 7.2$ Hz, 2H, C_2 -H and C_6 -H pyridine), 9.57 (s, 1H, OH); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): 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 $\text{C}_{20}\text{H}_{14}\text{N}_6\text{O}_3$ (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-Diamino-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): ν_{max} 3399, 3388, 3347, 3321 ($2\times\text{NH}_2$), 2216 (CN), 1683 ($\text{C}=\text{O}$) cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 4.62 (s, 1H, CH), 4.65 (s, 2H, NH_2), 6.68 (s, 2H, NH_2), 8.03 (d, $J = 7.2$ Hz, 2H, C_3 -H, C_5 -H pyridine), 8.43 (s, 1H, $\text{CH}=\text{N}$), 8.71 (d, $J = 7.2$ Hz, 2H, C_2 -H, C_6 -H pyridine); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 72.83, 86.27, 116.17, 123.55, 143.94, 146.25, 151.47, 155.18, 163.48, 178.28; EI-MS: m/z 254 (M^+ , 100%). Anal. Calcd. for $\text{C}_{12}\text{H}_{10}\text{N}_6\text{O}$ (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-ylmethylene)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): ν_{max} 2208 (CN), 1674 ($\text{C}=\text{O}$) cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 2.13 (s, 3H, CH_3), 2.22 (s, 3H, CH_3), 5.66 (s, 1H, CH), 8.01 (d, $J = 7.2$ Hz, 2H, C_3 -H, C_5 -H pyridine), 8.41 (s, 1H, $\text{CH}=\text{N}$), 8.70 (d, $J = 7.2$ Hz, 2H, C_2 -H, C_6 -H pyridine); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 18.24, 22.03, 110.94, 116.83, 118.07, 122.44, 135.88, 145.57, 150.21, 153.47, 155.26, 161.47; EI-MS: m/z 252 (M^+ , 100%). Anal. Calcd. for $\text{C}_{14}\text{H}_{12}\text{N}_4\text{O}$ (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-dihydro-[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): ν_{max} 3428, 3373, 3366, 3345 ($2\times\text{NH}_2$), 2202, 2180 (2CN), 1665 ($\text{C}=\text{O}$) cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 5.22 (s, 2H, NH_2), 6.56 (s, 2H, NH_2), 7.51 (d, $J = 7.2$ Hz, 2H, C_3 -H, C_5 -H pyridine), 8.63 (d, $J = 7.2$ Hz, 2H, C_2 -H, C_6 -H pyridine); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): 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 $\text{C}_{12}\text{H}_8\text{N}_6\text{O}$ (252.24): C, 57.14; H, 3.20; N, 33.32. Found: C, 57.21; H, 3.16; N, 33.33.

1,6-Diamino-2-oxo-5-(phenylsulfonyl)-1,2-dihydro-[4,4'-bipyridine]-3-carbonitrile (9b)

Orange crystals; yield: 53%; m.p. 145 °C; IR (KBr): ν_{max} 3447, 3403, 3334, 3070 ($2\times\text{NH}_2$), 2202 (CN), 1669 ($\text{C}=\text{O}$) cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 5.48 (s, 2H, NH_2), 6.57 (s, 2H, NH_2), 7.50–7.80 (m, 7H, Ar-H and C_3 -H, C_5 -H pyridine), 8.59 (d, $J = 7.2$ Hz, 2H, C_2 -H, C_6 -H pyridine); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 102.45, 116.49, 121.69, 122.82, 128.95, 130.14, 133.18, 141.89, 143.15, 143.83, 151.26, 161.77, 170.89; EI-MS: m/z 367 (M^+ , 8 %). Anal. Calcd. for $\text{C}_{17}\text{H}_{13}\text{N}_5\text{O}_3\text{S}$ (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-dihydro-[4,4'-bipyridine]-3-carboxylate (9c)

Yellow crystals; yield: 48%; m.p. 180–190 °C; IR (KBr): ν_{max} 3351, 3339, 3335, 3305 ($2\times\text{NH}_2$), 2215 (CN),

1725, 1674 ($2\times\text{C=O}$) cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO-}d_6$): δ 1.01 (t, $J = 7.2$ Hz 3H, CH_3), 4.03 (q, $J = 6.8$ Hz 2H, CH_2), 5.39 (s, 2H, NH_2), 7.08 (s, 2H, NH_2), 7.56 (d, $J = 7.4$ Hz, 2H, $\text{C}_3\text{-H}$, $\text{C}_5\text{-H}$ pyridine), 8.62 (d, $J = 7.4$ Hz, 2H, $\text{C}_2\text{-H}$, $\text{C}_6\text{-H}$ pyridine); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$): δ 15.37, 64.49, 99.56, 116.47, 121.82, 122.43, 143.73, 150.13, 152.85, 161.04, 167.28, 171.11; EI-MS: m/z 299 (M^+ , 100%). Anal. Calcd. for $\text{C}_{14}\text{H}_{13}\text{N}_5\text{O}_3$ (299.29): C, 56.18; H, 4.38; N, 23.40. Found: C, 56.21; H, 4.33; N, 23.41.

1,6-Diamino-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_{max} 3400, 3391, 3255, 3066 ($2\times\text{NH}_2$), 2209 (CN), 1661 (C=O) cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO-}d_6$): δ 5.36 (s, 2H, NH_2), 7.08 (s, 2H, NH_2), 7.50–8.00 (m, 7H, Ar-H), 8.71 (d, $J = 7.2$ Hz, 2H, $\text{C}_2\text{-H}$, $\text{C}_6\text{-H}$ pyridine); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$): δ 102.14, 116.40, 121.23, 121.98, 122.73, 124.85, 125.94, 126.48, 138.38, 141.29, 145.34, 150.77, 155.26, 161.14, 161.99, 171.57; EI-MS: m/z 360 (M^+ , 83%). Anal. Calcd. for $\text{C}_{18}\text{H}_{12}\text{N}_6\text{OS}$ (360.40): C, 59.99; H, 3.36; N, 23.32; S, 8.90. Found: C, 59.95; H, 3.41; N, 23.34; S, 8.87.

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-dicarbonitriles 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 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_{max} 3245, 3283 ($2\times\text{NH}$), 2216, 2195 ($2\times\text{CN}$), 1679 (C=O) cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO-}d_6$): δ 4.81 (s, 1H, NH), 5.11 (s, 1H, CH), 7.21–7.59 (m, 8H, Ar-H, NH and $\text{C}_3\text{-H}$, $\text{C}_5\text{-H}$ pyridine), 8.66 (d, $J = 7.2$ Hz, 2H, $\text{C}_2\text{-H}$, $\text{C}_6\text{-H}$ pyridine); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$): 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^+ , 19%). Anal. Calcd. for $\text{C}_{19}\text{H}_{12}\text{N}_6\text{O}$ (340.35): C, 67.05; H, 3.55; N, 24.69. Found: C, 67.11; H, 3.58; N, 24.60.

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

Brown crystals; yield: 63%; m.p. > 300 °C; IR (KBr): ν_{max} 3243, 3291 ($2\times\text{NH}$), 2215, 2195 ($2\times\text{CN}$), 1678 (C=O) cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO-}d_6$): δ 4.05 (s, 3H,

OCH_3), 4.77 (s, 1H, NH), 5.08 (s, 1H, CH), 6.95 (d, $J = 8.4$ Hz, 2H, Ar-H), 7.29 (s, 1H, NH), 7.40–7.60 (m, 5H, Ar-H, NH and $\text{C}_3\text{-H}$, $\text{C}_5\text{-H}$ pyridine), 8.66 (d, $J = 7.2$ Hz, 2H, $\text{C}_2\text{-H}$, $\text{C}_6\text{-H}$ pyridine); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$): δ 59.74, 87.51, 98.78, 115.78, 116.78, 117.09, 122.24, 123.50, 129.03, 138.14, 144.10, 150.84, 159.25, 160.81, 162.27, 171.09; EI-MS: m/z 370 (M^+ , 30%). Anal. Calcd. for $\text{C}_{20}\text{H}_{14}\text{N}_6\text{O}_2$ (370.37): C, 64.86; H, 3.81; N, 22.69. Found: C, 64.91; H, 3.75; N, 22.72.

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_{max} 3239, 3297 ($2\times\text{NH}$), 2217, 2199 ($2\times\text{CN}$), 1665 (C=O) cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO-}d_6$): 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, $\text{C}_3\text{-H}$, $\text{C}_5\text{-H}$ pyridine), 8.66 (d, $J = 7.2$ Hz, 2H, $\text{C}_2\text{-H}$, $\text{C}_6\text{-H}$ pyridine); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$): 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 ($\text{M}^+ + 2$, 0.88), 374 (M^+ , 3%). Anal. Calcd. for $\text{C}_{19}\text{H}_{11}\text{ClN}_6\text{O}$ (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.

Synthesis of 5-Oxo-7-(pyridin-4-yl)-3,5-dihydro-[1,2,4]triazolo[1,5-*a*]pyridine-6,8-dicarbonitrile (12)

A mixture of **9a** (2.25 g, 0.01 mol) and DMF-DMA (1.19 g, 0.01 mol) was heated under reflux in 50 mL of dry xylene for 8 h. The solid product that precipitated on cooling was filtered off, dried and recrystallized from absolute ethanol to afford compound **12**.

Brown crystals; yield: 70%; m.p. > 300 °C; IR (KBr): ν_{max} 3345 (NH), 2217, 2227 ($2\times\text{CN}$), 1684 (C=O) cm^{-1} ; ^1H NMR (400 MHz, $\text{DMSO-}d_6$): δ 5.21 (s, 1H, NH), 8.01 (d, $J = 7.2$ Hz, 2H, $\text{C}_3\text{-H}$, $\text{C}_5\text{-H}$ pyridine), 8.79 (s, 1H, CH), 8.98 (d, $J = 7.2$ Hz, 2H, $\text{C}_2\text{-H}$, $\text{C}_6\text{-H}$ pyridine); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$): δ 115.27, 115.98, 117.54, 122.49, 123.08, 144.37, 149.88, 151.46, 155.21, 161.44, 171.83; EI-MS: m/z 262 (M^+ , 10 %). Anal. Calcd. for $(\text{C}_{13}\text{H}_6\text{N}_6\text{O})$ (262.23): C, 59.54; H, 2.31; N, 32.05. Found: C, 59.56; H, 2.27; N, 32.10.

2. 3. Determination of the Anticancer Activity

It was carried out according to the previously reported work.¹⁷

3. Results and Discussion

3. 1. Chemistry

(*E*)-2-Cyano-*N'*-(pyridin-4-ylmethylene)acetohydrazide (**1**) was synthesized by the reaction of isonicotininaldehyde with 2-cyanoacetohydrazide under various

Scheme 1. Synthesis of (*E*)-2-cyano-*N'*-(pyridin-4-ylmethylene)acetohydrazide (**1**)

Table 1. Comparison of different conditions for the synthesis of (*E*)-2-cyano-*N'*-(pyridin-4-ylmethylene)acetohydrazide (**1**)

Entry	Catalyst	Condition	Time (h)	Yield (%)
1	No	Solvent free (100 °C)	6	0
2	No	EtOH	24	0
3	No	EtOH (reflux)	9	28
4	TEA	EtOH (rt)	24	0
5	TEA	EtOH (reflux)	6	39
6	TEA	THF (reflux)	4	92
7	No	Solvent free (MW)	20 sec	98

conditions (Scheme 1), and the results are presented in Table 1.

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 Et₃N 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 expen-

sive 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 ¹H NMR exhibited two singlet signals at δ 4.26 and 8.25 ppm due to CH₂ 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 ¹H 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 2. Synthesis of (*E*)-2-amino-4-aryl-5-cyano-6-oxo-1-((pyridin-4-ylmethylene)amino)-1,6-dihydropyridine-3-carbonitriles **5a–e**

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

autoxidation and tautomerization occur to give isolable products **5a–e**.¹⁸ ¹H 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, ¹³C 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 NH₂ 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. ¹H NMR spectrum of **6** showed two singlet signals at δ 4.62 and 8.43 ppm owing to the C₅-H of 2-pyridone ring and CH=N, respectively. Moreover, ¹H NMR of **6** exhibited two singlet signals (D₂O-exchangeable) at δ 4.65 and 6.68

ppm due to the two NH₂ groups. The IR analysis substantiated the results of ¹H NMR by the presence of four peaks in the region of 3321–4000 cm^{−1} for two NH₂ groups.

Nowadays, multicomponent reactions are gaining extensive economic and ecological importance as they conform to the fundamental principles of synthetic efficiency and reaction design.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**^{20,21} (Scheme 4).

The structures of products **9a–d** were assigned according to their IR, ¹H NMR, ¹³C NMR and mass spectra. Thus, all compounds **9a–d** gave molecular ion peak which coincides with the proposed structure. ¹H 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 (D₂O-exchangeable) 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 4. Synthesis of 5-alkyl-1,6-diamino-2-oxo-1,2-dihydro-[4,4'-bipyridine]-3-carbonitriles **9a–d**

Scheme 5. 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-dicarbonitriles **11a-c**

Scheme 6. Synthesis of 5-oxo-7-(pyridin-4-yl)-1,5-dihydro-[1,2,4]triazolo[1,5-a]pyridine-6,8-dicarbonitrile (**12**)

Structures **11a-c** were established on the basis of elemental analyses and spectral data. The ¹H 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 (D₂O-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).

Table 2. Cytotoxic activity of the newly synthesized compounds

Compounds	<i>In vitro</i> Cytotoxicity IC ₅₀ (μg/mL)	
	HepG-2	MCF-7
5-FU	7.53 ± 0.22	4.05 ± 0.15
1	45.83 ± 0.28	37.66 ± 0.35
5a	78.53 ± 1.25	84.20 ± 2.23
5b	31.11 ± 0.38	22.27 ± 0.33
5c	18.81 ± 0.14	16.08 ± 0.28
5d	20.23 ± 0.21	31.55 ± 0.34
5e	16.97 ± 0.27	14.99 ± 0.31
6	> 100	>100
7	52.65 ± 0.68	64.37 ± 1.20
9a	8.83 ± 0.30	10.37 ± 0.24
9b	10.08 ± 0.66	8.98 ± 0.61
9c	89.34 ± 1.33	64.38 ± 1.08
9d	71.08 ± 0.98	62.35 ± 0.55
11a	69.35 ± 1.30	58.98 ± 0.84
11b	43.55 ± 0.68	39.63 ± 0.50
11c	>100	>100
12	94.64 ± 1.33	80.37 ± 1.58

IC₅₀ (μg/mL): 1–10 very strong, 11–20 strong, 21–50 moderate, 51–100 weak and above 100 non-cytotoxic. 5-FU is 5-fluorouracil.

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 IC₅₀ 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. IC₅₀ 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.

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Povzetek

Reakcija 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 analitskimi in spektroskopskimi metodami. Za nove spojine smo raziskali tudi bioaktivnost v vlogi antitumornih učinkovin v *in vitro* testiranju citotoksičnosti proti HepG-2 in MCF-7. Ugotovili smo, da spojini **9a** in **9b** izkazujeta visoko citotoksičnost proti obema, HepG-2 in MCF-7.