In-vitro cytotoxic and radiosensitizing evaluation of novel 2-pyridone, isoquinoline, chromene and chromenopyridone derivatives

Mansour Sulaiman Al-Said, Marwa Galal El-Gazzar and Mostafa Mohammed Ghorab

*Medicinal, Aromatic and Poisonous Plants Research Center, College of Pharmacy, King Saud University, Riyadh, 11451, Saudi Arabia
†Department of Drug Radiation Research, National Center for Radiation Research and Technology, Nasr City, Cairo, 11371, Egypt

*Corresponding author at: Medicinal, Aromatic and Poisonous Plants Research Center, College of Pharmacy, King Saud University, Riyadh, 11451, Saudi Arabia. Tel: +966.05.34292860; fax: +966.01.4670560. E-mail address: mmghorab@yahoo.com (M.M. Ghorab).

ARTICLE INFORMATION
Received: 10 February 2012
Received in revised form: 16 March 2012
Accepted: 28 March 2012
Online: 30 June 2012

KEYWORDS
Cytotoxic
Chromene
2-Pyridone
Isoquinoline
Radiosensitizing
Chromenopyridone

1. Introduction

Compounds containing 2-pyridone moiety I-IV as a precursor have proven to possess several biologically properties (Scheme 1) [1-6]. In addition, different series of isoquinoline containing compounds V were reported to possess substantial cytotoxicity [7]. Furthermore, novel classes of chromenes bearing dithiazole VI or phenylthioamide VII moieties showed potent cytotoxicity [8], while chromene-carboxamides VIII and carboxylates proved potent anticancer activity on different cell lines and as inhibitors of tumor markers [9,10]. Based on the above information and as a continuation of a previous work on anticancer agents [11-16], we report the synthesis of novel 2-pyridone, isoquinoline, chromene and chromenopyridone as cytotoxic and radiosensitizing agents.

2. Experimental

2.1. Instrumentation

Chemicals were purchased from Merck, Fluka and Aldrich Chemical Companies. All yields refer to isolated products. Melting points are uncorrected and were determined on a Stuart melting point apparatus (Stuart Scientific, Redhill, UK). Elemental analysis (C, H and N) were performed on Perkin-Elmer 2400 analyser (Perkin-Elmer, Norwalk, CT, USA) at The Microanalytical Laboratories of the Faculty of Science, Cairo University. All compounds were within 5.4% of the theoretical values. The IR spectra (KBr) were measured on Shimadzu IR 110 spectrophotometer (Shimadzu, Kyoto, Japan). 1H NMR spectra were obtained on a Bruker proton NMR-Avance 300 (300 MHz) (Bruker, Munich, Germany), in DMSO-d6 as a solvent, using tetramethylsilane (TMS) as internal standard. Mass spectra were run on HP Model MS-5988 (Hewlett Packard, Palo, Alto, California, USA). All reactions were monitored by thin layer chromatograph (TLC) using precoated aluminium sheets Silica gel Merck 60F254 and were visualized by UV lamp (Merek, Darmstadt, Germany).

2.2. Synthesis

2.2.1. 2-Cyano-N-(3-ethylphenyl)acetamide (3)

A mixture of 3-ethylaniline, 1, (1.21 g, 0.01 mol) and ethyl cyanoacetate (1.13 g, 0.01 mol) was refluxed for 3 h. The reaction mixture was concentrated and cooled. The obtained product was crystallized from ethanol to give compound 3 (Scheme 2). Yield: 88%, M.p.: 86-88 °C (KBr, ν, cm-1): 3317 (NH), 3100 (CH Arom.), 2960, 2870 (CH aliph.), 2260 (C=O), 1670 (C=O). 1H NMR (300 MHz, DMSO-d6, δ, ppm): 1.2 (t, 3H, CH3, J = 6.6 Hz), 2.6 (q, 2H, CH2, J = 6.6 Hz), 3.6 (s, 2H, CH2), 4.2 (s, 1H, NH, exchangeable with D2O), 7.0-7.7 (m, 4H, Ar-H), 13.6, 26.1, 32.9, 117.4, 119.2, 122.0, 125.6, 129.7, 140.8, 142.5, 169.6. Anal calc. for C15H12N2O: C, 70.1; H, 6.38; 14.88. Found: C, 70.50; H, 6.10; N, 14.60%.

2.2.2. Ethyl 4-substituted-2-amino-5-cyano-1-(3-ethyl phenyl)-6-oxo-1,6-dihydropyridine-3-carboxylate (4a-j)

General procedure: A mixture of compound 3 (1.88 g, 0.01 mol), appropriate aldehyde (0.01 mol) and ethyl cyanoacetate (0.113 g, 0.01 mol) in ethanol (50 mL) containing a catalytic amount of piperidine was refluxed for 4 h. The obtained solid was recrystallized from dioxane to give 4a-j (Scheme 2), respectively.
Ethyl 2-aminoo-5-cyano-1-(3-ethylphenyl)-6-oxo-4-p-tolyl-1,6-dihydro-pyridine-3-carboxylate (4a): Yield: 71%. M.p.: 248-250 °C. IR (KBr, v cm⁻¹): 3309, 3207 (NH₂), 3059 (NH ar., 2966, 2875 (CH ar.), 2214 (C≡N), 1676, 1687 (2C=O). 1H NMR (300 MHz, DMSO-d₆, δ ppm): 1.1 (t, 3H, CH₃J = 0.1 Hz), 1.3 (t, 3H, CH₃J = 0.66 Hz), 1.9 (s, 3H, CH₃), 2.6 (q, 2H, CH₂J = 0.1 Hz), 4.3 (q, 2H, CH₂J = 0.66 Hz), 7.0-7.7 (m, 8H, Ar-H), 8.2 (s, 2H, NH₂ exchangeable with D₂O). 13C NMR (75 MHz, DMSO-d₆, δ ppm): 13.4, 15.2, 25.1, 31.9, 62.9, 86.1, 114.3, 115.0, 117.3, 119.8, 122.1, 125.8, 127.4, 130.6, 131.0, 133.2, 1367, 140.9, 162.7, 163.2, 164.8, 168.7. MS (m/z, %): 401 (M⁺, 5.1), 90 ppm). 

Found: C, 63.50; H, 4.90; N, 13.12%.

Scheme 1

Ethyl 2-aminoo-5-cyano-1-(3-ethylphenyl)-6-oxo-4-(4-pipryl)-1,6-dihydro-pyridine-3-carboxylate (4c): Yield: 81%. M.p.: 160-162 °C. IR (KBr, v cm⁻¹): 3309, 3209 (NH₂), 3055 (CH ar., 2924, 2872 (CH ar.), 2234 (C≡N), 1678, 1689 (2C=O). 1H NMR (300 MHz, DMSO-d₆, δ ppm): 1.05 (t, 3H, CH₃J = 0.2 Hz), 1.3 (t, 3H, CH₃J = 0.66 Hz), 2.6 (q, 2H, CH₂J = 0.2 Hz), 4.3 (q, 2H, CH₂J = 0.66 Hz), 5.9 (s, 2H, CH₂-piperynol), 7.2-7.7 (m, 7H, Ar-H), 8.3 (s, 2H, NH₂ exchangeable with D₂O). Anal. calc.: for C₂₄H₂₂N₂O₄: C, 67.66; H, 5.90; N, 9.74. Found: C, 66.50; H, 4.90; N, 9.51%.

Ethyl 2-aminoo-5-cyano-1-(3-ethylphenyl)-6-oxo-4-(6-ethoxy-4-methylphenyl)-1,6-dihydro-pyridine-3-carboxylate (4b): Yield: 76%. M.p.: 167-169 °C. IR (KBr, v cm⁻¹): 3323, 3209 (NH₂), 3055 (CH ar.), 2924, 2872 (CH ar.), 2264 (C≡N), 1679, 1690 (2C=O). 1H NMR (300 MHz, DMSO-d₆, δ ppm): 1.05 (t, 3H, CH₃J = 0.2 Hz), 1.3 (t, 6H, 2CH₂J = 0.66 Hz), 2.6 (q, 2H, CH₂J = 0.2 Hz), 3.5 (s, 3H, OCH₃), 4.5 (q, 4H, 2CH₂J = 0.66 Hz), 7.2-7.7 (m, 7H, Ar-H), 8.3 (s, 2H, NH₂ exchangeable with D₂O). MS (m/z, %): 461 (M⁺, 10.5), 167 (100). Anal. calc.: for C₂₃H₂₂N₂O₄: C, 67.66; H, 5.90; N, 9.10. Found: C, 67.32; H, 5.71; N, 9.42%.

Ethyl 2-aminoo-5-cyano-1-(3-ethylphenyl)-6-oxo-4-(2,3,4-trimethoxyphenyl)-1,6-dihydro-pyridine-3-carboxylate (4g): Yield: 87%. M.p.: 189-190 °C. IR (KBr, v cm⁻¹): 3323, 3209 (NH₂), 3055 (CH ar.), 2924, 2872 (CH ar.), 2264 (C≡N), 1679, 1687 (2C=O). 1H NMR (300 MHz, DMSO-d₆, δ ppm): 1.05 (t, 3H, CH₃J = 0.2 Hz), 1.3 (t, 3H, CH₃J = 0.66 Hz), 2.6 (q, 2H, CH₂J = 0.2 Hz), 3.8 (s, 9H, 3OCH₃), 4.5 (q, 4H, 2CH₂J = 0.66 Hz), 7.2-7.7 (m, 6H, Ar-H), 8.3 (s, 2H, NH₂ exchangeable with D₂O). MS (m/z, %): 573 (M⁺, 21), 90 (100). Anal. calc.: for C₂₃H₂₂N₂O₄: C, 65.40; H, 5.78; N, 8.80. Found: C, 65.32; H, 5.50; N, 8.65%.

Ethyl 2-aminoo-5-cyano-1-(3-ethylphenyl)-6-oxo-4-(2-chlorophenyl)-1,6-dihydro-pyridine-3-carboxylate (4h): Yield: 90%. M.p.: 200-201 °C. IR (KBr, v cm⁻¹): 3293, 3212 (NH₂), 3085 (CH ar.), 2935, 2872 (CH ar.), 2234 (C≡N), 1660, 1678 (2C=O). 1H NMR (300 MHz, DMSO-d₆, δ ppm): 1.05 (t, 3H, CH₃J = 0.2 Hz), 1.3 (t, 3H, CH₃J = 0.66 Hz), 2.6 (q, 2H, CH₂J = 0.2 Hz), 4.5 (q, 2H, 2CH₂J = 0.66 Hz), 7.2-7.7 (m, 6H, Ar-H), 8.3 (s, 2H, NH₂ exchangeable with D₂O). 13C NMR (75 MHz, DMSO-d₆, δ ppm): 13.8, 15.5, 31.2, 63.4, 86.1, 116.9, 117.3, 118.1, 119.8, 125.6, 128.0, 128.2, 129.0, 129.6, 130.7, 133.8, 135.3, 1361, 142.6, 160.2, 162.3, 166.9, 172.3. Anal. calc.: for C₂₃H₁₈ClN₂O₄: C, 65.48; H, 4.78; N, 9.96. Found: C, 65.32; H, 4.71; N, 9.65%.
Ethyl 2-amino-5-cyano-1-(3-ethylphenyl)-6-oxo-4-(3-bromo phenyl)-1,6-dihydro-pyridine-3-carboxylate (4i): Yield: 62%. M.p.: 96-98 °C. IR (KBr, cm⁻¹): 3343, 3246 (NH₂), 3086 (CH arom.), 2981, 2829 (CH aliph.), 2212 (C≡N), 1649, 1966 (C≡O). 1H NMR (300 MHz, DMSO-d₆, δ ppm): 1.2 (t, 3H, CH₃, J = 0.66 Hz), 2.6 (q, 2H, CH₂, J = 0.66 Hz), 4.5 (q, 2H, CH₂, J = 0.66 Hz), 7.1-7.9 (m, 8H, Ar-H), 8.7 (s, 1H, NH₂, exchangeable with D₂O). Anal. calcd. for C₂₆H₂₃N₃O₂: C, 76.26; H, 5.66; N, 10.26. Found: C, 76.16; H, 6.39; N, 11.0. 13C NMR (75 MHz, DMSO-d₆, δ ppm): 12.9, 21.3, 26.4, 33.8, 96.0, 100.3, 116.8, 118.2, 118.9, 119.8, 121.7, 124.5, 126.8, 127.6, 130.9, 133.4, 134.8, 139.0, 140.9, 142.4, 144.6, 147.8, 151.3, 158.4. Yield: 90%. M.p.: 108-109 °C. IR (KBr, cm⁻¹): 3325, 3209 (NH₂), 3080 (CH arom.), 2931, 2872 (CH aliph.), 2210 (C≡N), 1658 (C≡O). 1H NMR (300 MHz, DMSO-d₆, δ ppm): 1.2 (t, 3H, CH₃, J = 0.66 Hz), 1.7 (t, 3H, CH₂, J = 0.66 Hz), 2.6 (q, 2H, CH₂, J = 0.66 Hz), 4.7 (q, 2H, CH₂, J = 0.66 Hz), 7.2-7.7 (m, 4H, Ar-H), 7.5-8.4 (m, 3H, 3CH thiphene), 8.7 (s, 2H, NH₂, exchangeable with D₂O). Anal. calcd. for C₂₆H₂₃N₃O₂S·C₆H₅: C, 64.10; H, 4.87; N, 10.68. Found: C, 64.50; H, 4.70; N, 10.40%. 1,2-(3-Ethylphenyl)-4,6-dimethyl-2-oxo-1,2-dihydro pyridine-3-carboxilic acid (5)

Equimolar amounts of compound 3 (1.88 g, 0.01 mol) and acetylacetone (1.00 g, 0.01 mol) were refluxed in ethanol (50 mL) containing piperidine (0.5 mL) for 5 h. The reaction mixture was triturated with ethanol and the solid obtained was recrystallized from dioxane to give compound 5 (Scheme 2). 1H NMR (300 MHz, DMSO-d₆, δ ppm): 1.2 (t, 3H, CH₃, J = 0.66 Hz), 1.5 (2s, 6H, 2CH₃), 2.6 (q, 2H, CH₂, J = 0.66 Hz), 5.6 (s, 1H, CH- pyridine), 7.2-7.7 (m, 4H, Ar-H). Anal. calcd. for C₂₆H₂₃N₃O₂·C₂H₅OH: C, 76.16; H, 6.39; N, 11.0. Found: C, 76.50; H, 6.70; N, 11.40%. 2.2.4. 6-Substituted-8-amino-2-(3-ethylphenyl)-3-methyl-1 oxo-1,2-dihydro-isoquinoline-7-carbonitrile (6a-h)

General procedure: A mixture of compound 5 (1.5 g, 0.01 mol), benzylidene-malononitrile (0.01 mol) in ethanol (50 mL) containing (0.5 mL) piperidine was refluxed for 6 h. The reaction mixture was cooled and poured onto ice water acidified with dil. HCl. The solid obtained was recrystallized from dioxane to give compounds 6a-h (Scheme 2).

- 8-Amino-2-(3-ethylphenyl)-3-methyl-1-oxo-4-6-p-tolyl-1,2 dihydro-isoquinoline-7-carbonitrile (6a): Yield: 90%. M.p.: 108-109 °C. IR (KBr, cm⁻¹): 3325, 3209 (NH₂), 3080 (CH arom.), 2931, 2872 (CH aliph.), 2210 (C≡N), 1658 (C≡O). 1H NMR (300 MHz, DMSO-d₆, δ ppm): 1.2 (t, 3H, CH₃, J = 0.66 Hz), 1.7 (t, 3H, CH₂, J = 0.66 Hz), 2.6 (q, 2H, CH₂, J = 0.66 Hz), 4.7 (q, 2H, CH₂, J = 0.66 Hz), 5.7 (s, 1H, CH-pyridine), 6.9-7.5 (m, 8H, Ar-H), 8.7 (s, 2H, NH₂, exchangeable with D₂O). ¹³C NMR (75 MHz, DMSO-d₆, δ ppm): 129, 213, 264, 33.8, 96.0, 100.3, 116.8, 118.2, 118.9, 119.8, 121.7, 124.5, 126.8, 127.6, 130.9, 133.4, 134.8, 139.0, 140.9, 142.4, 144.6, 147.8, 151.3, 158.4. Anal. calcd. for C₂₆H₂₃N₃O₂·C₂H₅OH: C, 79.36; H, 5.89; N, 10.68. Found: C, 79.50; H, 5.70; N, 10.40%.

Amino-2-(3-ethylphenyl)-3-methyl-1-oxo-6-2-methoxy phenyl)-1,2-dihydro-isoquinoline-7-carbonitrile (6b): Yield: 92%. M.p.: 114-116 °C. IR (KBr, cm⁻¹): 3325, 3209 (NH₂), 3080 (CH arom.), 2931, 2872 (CH aliph.), 2212 (C≡N), 1658 (C≡O). 1H NMR (300 MHz, DMSO-d₆, δ ppm): 1.2 (t, 3H, CH₃, J = 0.66 Hz), 2.6 (q, 2H, CH₂, J = 0.66 Hz), 3.8 (s, 3H, OCH₃), 5.7 (s, 1H, CH-pyridine), 6.9-7.9 (m, 8H, Ar-H), 8.7 (s, 2H, NH₂, exchangeable with D₂O). MS (m/z, %): 409 (M⁺, 5.12), 105 (100). Anal. calcd. for C₂₆H₂₄N₂O₃: C, 76.26; H, 5.66; N, 10.26. Found: C, 76.50; H, 5.70; N, 10.50%.
8-Amino-2-[(3-ethylphenyl)-3-methyl-1-oxo-6-(4-methoxyphenyl)-1,2-dihydro-isoquinoline-7-carbonitrile (6c): Yield: 97%. M.p.: 122-124 °C. IR (KBr, v cm⁻¹): 3325, 3209 (NH). 3080 (CH arom.), 2931, 2860 (CH aliph.), 2220 (C≡N), 1658 (C=O). 1H NMR (300 MHz, DMSO-d₆, δ ppm): 1.2 (t, 3H, J = 0.66 Hz), 2.6 (q, 2H, CH₂, J = 0.66 Hz), 6.9-7.8 (m, 8H, Ar-H), 8.7 (s, 2H, NH₂, exchangeable with D₂O). MS (m/z %): 409 (M⁺, 12.1), 90 (100). Anal. calcd. for C₂₈H₂₇N₃O₄: C, 71.62; H, 5.80; N, 8.95. Found: C, 71.70; H, 5.07; N, 10.10%.

8-Amino-2-[(3-ethylphenyl)-3-methyl-1-oxo-6-(3-nitrophenyl)-1,2-dihydro-isoquinoline-7-carbonitrile (6d): Yield: 89%. M.p.: 98-100 °C. IR (KBr, v cm⁻¹): 3325, 3220 (NH). 3080 (CH arom.), 2931, 2850 (CH aliph.), 2208 (C≡N), 1658 (C=O). 1H NMR (300 MHz, DMSO-d₆, δ ppm): 1.2 (t, 3H, CH₃, J = 0.66 Hz), 2.6 (q, 2H, CH₂, J = 0.66 Hz), 5.7 (s, 1H, CH-pyridine), 6.9-7.5 (m, 8H, Ar-H), 8.7 (s, 2H, NH₂, exchangeable with D₂O). MS (m/z %): 424 (M⁺, 18.2), 90 (100). Anal. calcd. for C₃₂H₃₂N₄O₄: C, 70.74; H, 4.75; N, 13.20. Found: C, 70.50; H, 4.70; N, 13.40.

8-Amino-2-[(3-ethylphenyl)-3-methyl-1-oxo-6-(4-nitrophenyl)-1,2-dihydro-isoquinoline-7-carbonitrile (6e): Yield: 85%. M.p.: 106-108 °C. IR (KBr, v cm⁻¹): 3340, 3218 (NH). 3080 (CH arom.), 2940, 2872 (CH aliph.), 2218 (C≡N), 1658 (C=O). 1H NMR (300 MHz, DMSO-d₆, δ ppm): 1.2 (t, 3H, CH₃, J = 0.66 Hz), 2.6 (q, 2H, CH₂, J = 0.66 Hz), 6.7-7.5 (m, 8H, Ar-H), 8.7 (s, 2H, NH₂, exchangeable with D₂O). MS (m/z %): 424 (M⁺, 20.3), 141 (100). Anal. calcd. for C₂₉H₂₉N₃O₄: C, 70.74; H, 4.75; N, 13.20. Found: C, 70.50; H, 4.75; N, 13.60.

8-Amino-2-[(3-ethylphenyl)-3-methyl-1-oxo-6-(2-chlorophenyl)-1,2-dihydro-isoquinoline-7-carbonitrile (6g): Yield: 80%. M.p.: 107-110 °C. IR (KBr, v cm⁻¹): 3370, 3270 (NH). 3080 (CH arom.), 2920, 2860 (CH aliph.), 2209 (C≡N), 1658 (C=O). 1H NMR (300 MHz, DMSO-d₆, δ ppm): 1.2 (t, 3H, CH₃, J = 0.66 Hz), 2.6 (q, 2H, CH₂, J = 0.66 Hz), 6.7-7.7 (m, 8H, Ar-H), 8.7 (s, 2H, NH₂, exchangeable with D₂O). MS (m/z %): 413 (M⁺, 35), 105 (100). Anal. calcd. for C₂₃H₂₄ClN₃O: C, 72.55; H, 4.87; N, 10.15. Found: C, 72.70; H, 4.70; N, 10.50%.

8-Amino-2-[(3-ethylphenyl)-3-methyl-1-oxo-6-(2,4-dichlorophenyl)-1,2-dihydro-isoquinoline-7-carbonitrile (6h): Yield: 87%. M.p.: 97-99 °C. IR (KBr, v cm⁻¹): 3325, 3209 (NH). 3080 (CH arom.), 2930, 2856 (CH aliph.), 2198 (C≡N), 1658 (C=O). 1H NMR (300 MHz, DMSO-d₆, δ ppm): 1.2 (t, 3H, CH₃, J = 0.66 Hz), 2.6 (q, 2H, CH₂, J = 0.66 Hz), 5.7 (s, 1H, CH-pyridine), 6.9-7.9 (m, 7H, Ar-H), 8.7 (s, 2H, NH₂, exchangeable with D₂O). MS (m/z %): 448 (M⁺, 5.9), 141 (100). Anal. calcd. for C₂₃H₂₄Cl₂N₃O: C, 66.97; H, 4.27; N, 9.37. Found: C, 66.60; H, 4.60; N, 9.60%.

2.2.5. N-(3-ethylphenyl)-2-oxo-2H-chromene-3-carboxamide (7)

To a solution of compound 3 (1.88 g, 0.01 mol) in acetic anhydride (20 mL), salicylaldehyde (1.22 g, 0.01 mol) and fused sodium acetate (0.8 g, 0.01 mol) was added. The reaction mixture was refluxed for 2 h, cooled and the solid obtained was crystallized from ethanol to give compound 7 (Scheme 3). Yield: 69%. M.p.: 117-119 °C. IR (KBr, v cm⁻¹): 3417(NH), 1766 (C=O). 1H NMR (300 MHz, DMSO-d₆, δ ppm): 1.3 (t, 3H, CH₃, J = 0.2 Hz), 2.6 (q, 2H, CH₂, J = 0.2 Hz), 7.3-8.0 (m, 8H, Ar-H), 8.35 (s, 1H, CH), 8.42 (s, 1H, NH, exchangeable with D₂O). ¹³C NMR (75 MHz, DMSO-d₆, δ ppm): 141, 130.2, 113.6, 117.5, 119.8, 122.7, 124.2, 125.6, 127.0, 127.9, 129.6, 130.2, 133.5, 1361, 140.2, 153.6, 161.2, 164.3. Anal. calcd. for C₁₉H₁₄NO₂: C, 73.71; H, 5.15; N, 4.78. Found: C, 73.60; H, 5.70; N, 4.50%.

2.2.6. N-(3-ethylphenyl)-2-imino-2H-chromene-3-carboxamide (8)

A mixture of compound 3 (1.88 g, 0.01 mol), salicylaldehyde (1.22 g, 0.01 mol) and anhydrous ammonium acetate (1.15 g, 0.15 mol) in ethanol (20 mL) was refluxed for 2 h. The solid obtained was recrystallized from ethanol to give compound 8 (Scheme 3). Yield: 59%. M.p.: >300 °C. IR (KBr, v cm⁻¹): 3344, 3116 (2H,N). 1720 (C=O), 1570 (C≡N). ¹H NMR (300 MHz, DMSO-d₆, δ ppm): 1.1 (t, 3H, CH₃, J = 0.2 Hz), 2.7 (q, 2H, CH₂, J = 0.2 Hz), 6.9-8.3 (m, 8H, Ar-H), 8.7 (s, 1H, CH-4), 9.5 (s, 1H, NH imino, exchangeable with D₂O). 125 (s, 1H, NHCO, exchangeable with D₂O). ¹³C NMR (75 MHz, DMSO-d₆, δ ppm): 134.3, 112.4, 114.5, 115.8, 117.1, 119.8, 122.6, 123.3, 124.7, 126.2, 127.5, 128.7, 130.6, 133.8, 137.1, 152.9, 162.7, 171.3. Anal. calcd. for C₁₉H₁₄NO₂: C, 73.95; H, 5.52; N, 9.58. Found: C, 73.60; H, 5.20; N, 9.30%.
2.2.7. 2-Amino-3-(3-ethylphenyl)-4,5-dioxo-4,5-dihydro-3H-chromen-3(4H)-ypridine-1-carbonitrile (9) and 2-amino-3-(3-ethylphenyl)-5-imino-4-oxo-4,5-dihydro-3H-chromen-3(4H)-ypridine-1-carbonitrile (10)

Equimolar amounts of compounds 7 or 8, malononitrile (0.66 g, 0.01 mol) anhydrous ammonium acetate (1.115 g, 0.01 mol) in ethanol were refluxed for 4 h. The solid obtained by filtration was recrystallized from dioxane to give compounds 9 and 10. (Scheme 3) respectively.

2-Amino-3-(3-ethylphenyl)-4,5-dioxo-4,5-dihydro-3H-chromen-3(4H)-ypridine-1-carbonitrile (9): Yield: 61%; M.p.: >300 °C. IR (KBr, ν cm⁻¹): 3090 (CH aromatic), 2976, 2865 (CH aliph.), 1684, 1654 (C=O). 1H NMR (300 MHz, DMSO-d₆, δ ppm): 1.2 (t, 3H, CH₃, J = 6.66 Hz), 2.6 (q, 2H, CH₂, J = 6.66 Hz), 6.9-7.5 (m, 8H, Ar-H). 13C NMR (75 MHz, DMSO-d₆, δ ppm): 15.8, 34.3, 73.9, 116.5, 117.9, 118.0, 119.2, 122.6, 125.3, 126.1, 127.0, 127.8, 128.9, 129.3, 135.6, 141.2, 153.6, 156.4, 158.8, 161.4, 167.5. MS (m/z, %): 357 (M⁺, 17.8), 76 (100). Anal. calcd. for C₂₃H₂₅N₂O₂C: 70.8; H: 4.25; N: 11.76. Found: C: 75.40; H: 4.60; N: 4.10.

2-Amino-3-(3-ethylphenyl)-5-imino-4-oxo-4,5-dihydro-3H-chromen-3(4H)-ypridine-1-carbonitrile (10): Yield: 58%; M.p.: >300 °C. IR (KBr, ν cm⁻¹): 3312, 3256, 3216 (NH, NH₂), 3070 (CH aromatic), 2925, 2853 (CH aliph.), 1690 (C=O). 1H NMR (300 MHz, DMSO-d₆, δ ppm): 1.2(t, 3H, CH₃, J = 6.66 Hz), 2.6 (q, 2H, CH₂, J = 6.66 Hz), 6.9-7.8 (m, 8H, Ar-H), 8.7 (s, 1H, NH, exchangeable with D₂O), 10.1 (s, 2H, NH₂, exchangeable with D₂O). MS (m/z, %): 356 (M⁺, 18.1), 76 (100). Anal. calcd. for C₂₃H₂₅N₂O₂C: 70.77; H: 4.53; N: 15.72. Found: C: 75.50; H: 5.43; N: 8.40.

2.3. In-vitro anticancer screening

2.3.1. Animals, chemicals and facilities

Ehrlich Ascites Carcinoma cells (EAC) were maintained in female Swiss albino mice weighing 25-30 g (the holding company for biological products and vaccines, VACSERA, Cairo, Egypt), were housed at a constant temperature (24 °C) with alternating 12 h light and dark cycles and fed standard laboratory food (Milad CO., Cairo, Egypt) and water ad libitum. All chemicals and reagents were of the highest grade commercially available. Facilities including animal house, biochemical equipment have been made available by the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority (AEA), Cairo, Egypt. Animal care and handling was done according to the guidelines set by the world health organization, Geneva, Switzerland and approved from the committee for animals care at NCRRT.

2.3.4. In-vitro anticancer activity using Ehrlich Ascites Carcinoma (EAC) cells

Ehrlich Ascites Carcinoma cells (EAC) were obtained by needle aspiration of ascitic fluid from preincubated mice; under aseptic conditions. Tumor cells suspension (2.5x10⁶ per mL) was prepared in RPMI-1640 media. Tested compounds were prepared with various dilutions by dissolving: 100, 50, 25 and 10 mg of the tested compounds in DMSO (1 mL). In a set of sterile test tubes 0.8 mL RPMI-1640 media containing (glutamine, fetal calf serum as nutrient, streptomycin and penicillin), 0.1 mL of each of the tested compounds (corresponding to 100, 50, 25 and 10 mg) were mixed then 0.1 mL of tumor cell suspension (2x10⁸) was added. The test tubes were incubated at 37 °C for 2 h. Trypan blue exclusion test was carried out to calculate the percentage of non-viable cells after 2 h of incubation [17]. The total number of cells/mL will be determined using the following calculations:

Cells/mL = average cells count per 5 squares x dilution factor x 10⁶

Total cells = cells/mL x the original volume of fluid from which the cell sample was removed

% cell non-viability = total non-viable cells (stained) / total cells x 100

The results of in-vitro anticancer activity experiments are presented in (Table 1).
2.4. Radiosensitizing evaluation

The most potent compounds resulted from the in vitro anticancer screening, compounds 4a, 4d, 6a, 6c, 6e and 10, were selected to be evaluated again for their in vitro anticancer activity alone and in combination with γ-radiation. This study was conducted to evaluate the ability of these compounds to enhance the cell killing effect of γ-radiation. Cells were subjected to a single dose of γ-radiation at a dose level of 8 Gy with a dose rate of 2 Gy/min. Irradiation was performed in the National Cancer Institute, Cairo University, using Gamma cell-40 (<Co>) source. The surviving fractions were expressed as means ± standard error. The results were given in Table 3.

3. Results and discussion

3.1. Chemistry

Schemes 1 and 2 outline the synthetic pathway used to obtain 1,6-dihydropyridines (4a-j), 1,2-dihydroisoquinolines (6a-h), 2H-chromenes (7 and 8) and 3H-chromeno[3,4-c]pyridines (9 and 10). The starting material, 3-cyano-N-(3-ethylphenyl) acetamide, 3, was prepared via reaction of 3-ethylaniline 1 with ethyl cyanoacetate 2. Compound 3 was confirmed by elemental analysis, IR, 1H NMR and mass spectral data (Scheme 1). Upon treatment of compound 3 with required aldehyde and ethyl cyanoacetate in the presence of catalytic amount of piperidine furnished ethyl 4-substituted-2-amino-5-cyano-1-(3-ethylphenyl)-6-oxo-1,6-dihydropyridine-3-carboxylate, 4a-j. The structure of compounds 4a-j was deduced from elemental analyses and spectral data (Scheme 1). Cycladdition occurred upon treatment of compound 3 with acetylaceton to furnish 2-pyridine derivative 5 which upon reaction with the corresponding arylidinemalononitriles yielded the corresponding isoquinoline derivatives 6a-h (Scheme 2).

Furthermore, Perkin reaction was carried when compound 3 was reacted with salicyaldehyde in the presence of acetic anhydride containing sodium acetate to give the corresponding 2-oxochromene derivative 7, while reaction of compound 3 with salicyaldehyde in ammonium acetate furnished 2-iminochromone 8. The structure of compound 7 and 8 was supported in the basis of elemental analyses, IR, 1H NMR and mass spectral data. The chromone derivative 7 and 8 were further reacted with malononitrile in the presence of ammonium acetate to give the corresponding chromeno pyridine derivatives 9 and 10, respectively (Scheme 3). The structure of compounds 9 and 10 was elucidated from elemental analyses and spectral data.

3.2. In-vitro anticancer screening

3.2.1. In-vitro anticancer activity using Ehrlich Ascites Carcinoma (EAC) cells

Doxorubicin, the reference drug used in this study is one of the most effective antitumor agents used to produce regressions in acute leukemia’s, Hodgkin’s disease, and other lymphomas. The relationship between survival ratio and drug concentration was plotted to obtain the survival curve of Ehrlich Ascites Carcinoma (EAC) cells and human liver cell line (HEPG2). The response parameter calculated was IC50 value (Tables 1 and 2), which corresponds to the compound concentration causing 50% mortality in net cells.

3.2.2. Structure Activity Relationship (SAR)

The cytotoxicity of twenty-two compounds was examined on Ehrlich Ascites Carcinoma (EAC) cells. It is clear from the results in (Table 1) that in the series of 1,6-dihydropyridine 4a-j, the most potent in this series is the 4-methyl derivative 4a (IC50 = 62.34 µM) which was found to be more potent than the reference drug (IC50 = 68.13 µM) and also more potent than the corresponding 4-methoxy derivative 4f (IC50 = 108.45 µM) and is nearly as potent as the trimethoxy derivative 4g (IC50 = 83.76 µM). On the other hand, the 4-nitro derivative 4d (IC50 = 57.87 µM) was found to be more potent than the corresponding 3-nitro derivative 4c (IC50 = 106.48 µM), finally, the least potent in this series was the halogenated derivatives 4h and 4i (IC50 = 116.38 and 105.15 µM). Concerning the series of 1,2-dihydroisoquinoline 6a-h, the most potent in this series is the 4-methoxy derivative 6c (IC50 = 36.67 µM) which was found to be more potent than the reference drug (IC50 = 68.13 µM) and the corresponding 4-methyl derivative 6a (IC50 = 58.52 µM), the trimethoxy derivative 6f (IC50 = 91.68 µM) and the 2-methoxy derivative 6b (IC50 = 244.5 µM) which is the least potent in this series. On the other hand, the 4-nitro derivative 6e (IC50 = 58.96 µM) was found to be more potent than the corresponding 3-nitro derivative 6d (IC50 = 108.49 µM), while, the chloro derivatives 6g and 6h showed nearly equipotent activity (IC50 = 121.06 and 107.14 µM). Finally, the chromeno[3,4-c]pyridone derivatives 9 and 10 (IC50 = 123.24 and 58.98 µM) showed more potent activity than the corresponding chromene derivatives 7 and 8 which showed no IC50.
3.2.3. In-vitro anticancer activity using liver human tumor cell lines (HEPG2)

This study was performed to evaluate the cytotoxic activity of the most potent six compounds resulted from EAC assay (compounds 4a, 4d, 6a, 6c, 6e and 10). We can conclude from the results obtained from (Table 2) that indeed these compounds showed potent activity on HEPG2 cell line. The most potent was the 4-nitro dihydropyridine derivative 4d (IC50 = 11.7 µM) which is more potent than doxorubicin (IC50 = 38.46 µM), while, the increasing order of the rest of the compounds was as follows: 4-methyl isoquinoline derivative 6a (IC50 = 21.81 µM), the 4-nitro isoquinoline derivative 6e (IC50 = 25.83 µM), the 4-methyl dihydropyridone derivative 4a (IC50 = 31.2 µM), the chromeno[3,4-c]pyridine 10 (IC50 = 322 µM) and the 4-methoxy isoquinoline 6c (IC50 = 389 µM).

3.3. Radiosensitizing evaluation

The rationale for combining chemotherapy and radiotherapy is based mainly on two ideas, one being spatial cooperation, which is effective if chemotherapy is sufficient to eradicate subclinical metastases and if the primary local tumor is effectively treated by radiotherapy. In this regard, no interaction between radiotherapy and chemotherapy is required. The other idea is the enhancement of radiation effects. Cytotoxic agents can enhance radiation effects by direct enhancement of the initial radiation damage by incorporating drugs into DNA, inhibiting cellular repair, accumulating cells in a radiosensitive phase or eliminating radioresistant phase cells, eliminating hypoxic cells or inhibiting the accelerated repopulation of tumor cells [17]. Consequently, the ability of the most six active compounds 4a, 4d, 6a, 6c, 6e and 10, to enhance the cell killing effect of γ-irradiation was studied. From the results obtained in (Table 2), compound 4a showed an in vitro cytotoxic activity with IC50 value of 31.2 µM, when the cells were subjected to different concentrations of the compound alone. While, when the cells were subjected to the same concentrations of compound 4a, and irradiated with a single dose of γ-radiation at a dose level of 8 Gy, as shown in (Table 3), the IC50 value was synergistically decreased to 5.03 µM. Similarly, compounds 4d, 6a, 6c, 6e and 10 showed IC50 values of 11.7, 21.81, 38.9, 25.83 and 32.2 µM, respectively, when used alone, as shown in (Table 2). The IC50 value was decreased to 6.37, 2.68, 8.38, 6.04 and 3.35 µM, respectively, when the cells were treated with compounds 4d, 6a, 6c, 6e and 10 in combination with γ-radiation. From these results, we can conclude that the combination of compounds 4a, 4d, 6a, 6c, 6e and 10 and ionizing radiation synergistically enhanced growth inhibition on liver cancer cells, compared with each agent alone.

4. Conclusion

We report in this work the synthesis of new 2-pyridone, isoquinoline, chromene and chromenopyridone derivatives. It was clearly observed from the results of in-vitro cytotoxic screening that some of the synthesized compounds exhibited significant anticancer activity on Ehrlich Ascites Carcinoma (EAC) and human liver tumor cell line (HEPG2). While, combining these compounds with radiation at the same concentrations enhances their activity which demonstrates the importance of the combination therapy for the patients with cancer to decrease the side effects of both drugs and radiation.

Acknowledgements

The authors are grateful to the sponsorship of the Research Center, College of Pharmacy and the Deanship of Scientific Research, King Saud University, Riyadh, Saudi Arabia.

References