Synthesis, Anticancer Activity and Radiosensitizing Evaluation of Some New 2-Pyridone Derivatives

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Key words
- synthesis
- 2-pyridone
- anticancer
- γ-radiation

Abstract

Based on the reported anticancer activity of 2-pyridone, a new series of 6-amino-5-cyano-1-(3-ethylphenyl)-2-oxo-4-substituted-1,2-dihydropyridine-3-carbo-nitriles 4a-p were synthesized and tested for in-vitro anticancer activity against Ehrlich Ascites Carcinoma (EAC) cell line and liver human tumor cell line (HEPG2). Radiosensitizing activity was also evaluated. The starting material 2-cyano-N-(3-ethylphenyl)-acetamide 3 was obtained via reaction of 3-ethyl aniline 1 with ethyl cyanoacetate under condition of fusion. Upon treatment of compound 3 with aromatic aldehyde and malononitrile in the presence of catalytic amount of piperidine yielded the corresponding 1,2-dihydropyridine derivative 4a-p. Also chromenes 5 and 6 were obtained in good yield via reaction of compound 3 with salicyladehyde under different condition. The chromene derivatives 5 and 6 were further reacted with malononitrile in NH_4OAc, afford the corresponding chromenopyridones 7 and 8. The structures of the synthesized compounds 3–8 were confirmed by analytical and spectral data. Compounds 4d, 4e, 5 and 6 showed higher anticancer activity against EAC cell line with IC_{50} values (75.32, 20.77, 73.1 and 67.05 µM) compared to doxorubicin as positive control with IC_{50} value (68.13 µM), moreover, these compounds showed potent activity on HEPG2 cell line with IC_{50} values (26.5, 19.2, 39.3, 44.9 µM), respectively, compared to doxorubicin (CAS 29042-30-6) (38.46 µM) and their activity increased synergistically when combined with γ-radiation.

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Introduction

Many naturally occurring and synthetic compounds containing the 2-pyridone scaffold possess oncogenic properties [1]. A series of novel 4,6-diaryl-2-oxo-1,2-dihydropyridine-3-carbonitriles (e.g. compounds I, II) were identified as inhibitors of the oncogenic serine/threonine kinase PIM-1, which plays a role in cancer survival, differentiation and proliferation, PIM-1 kinase has been shown to be overexpressed in a variety of cancer cell lines [2–5]. Furthermore, milrinone III and amrinone IV are 5-pyridyl-2-oxopyridine derivatives used for the treatment of congestive heart failure, their mechanism of action involve PDE3 inhibition, leading to high levels of CAMP and consequent inotropic effect, recent studies showed that PDE3, PDE4, PDE5 are overexpressed in cancerous cells compared with normal cells [6–10]. Based on the above informations and as a continuation of previous work on anticancer agents [11–16], we report the synthesis of novel 2-oxo-3,5-dicyanopyridine derivatives 4a-p, also, benzochromenes 5, 6 and chromenopyridine derivatives 7, 8 were synthesized to evaluate their in-vitro anticancer and radiosensitizing activity (**Fig. 1**).

Experimental

Chemistry

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 fused at 220 °C for 3 h. The reaction mixture was concentrated and cooled. The obtained product was crystallized from ethanol to give 3.

Yield, 88%, m.p. 86–88 °C; IR (KBr, cm^{-1}): 3317 (NH), 3100 (CH. arom.), 2960, 2870 (CH aliph.), 2260 (C≡N), 1670 (C=O). ^{1}HNMR spectrum of 3 in (DMSO-d_6) δ : 1.2[t, 3H, CH_3], 2.6[q, 2H, CH_2], 3.6[s, 2H, CH_2], 4.2[s, 1H, NH, exchangeable with D_2O], 7.03–7.7[m, 4H, Ar-H]. Anal. Calcd. For $C_{11}H_{12}N_2O$: C, 70.21; H, 6.38, 14.89. Found: C, 70.50; H, 6.10; N, 14.60.

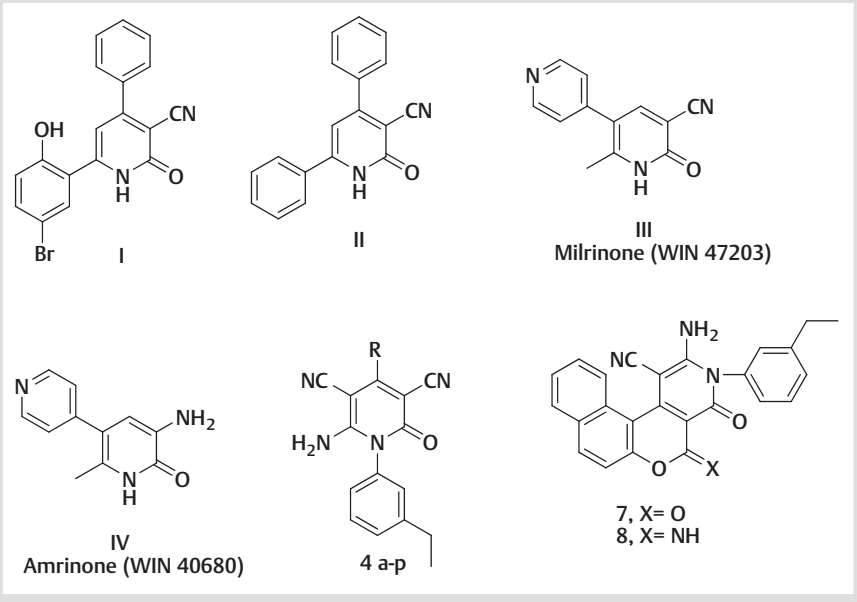

Fig. 1 Synthetic compounds containing the 2-pyridone scaffold (I–IV) and structures of the target compounds (4 a-p, 7, 8).

6-Amino-5-cyano-1-(3-ethylphenyl)-2-oxo-4-substitutedaryl 1,2-dihydropyridine-3-carbonitriles (4a-p)

General procedure: A mixture of compound 3 (1.88 g, 0.01 mol), appropriate aldehyde (0.01 mol) and malononitrile (0.66 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-p, respectively.

6-Amino-5-cyano-1-(3-ethylphenyl)-2-oxo-4-phenyl-1,2-dihydropyridine-3-carbonitriles (4a)

Yield, 71%; m.p. 248–250 °C; IR (KBr, cm^{-1}): 3309, 3207 (NH_2), 3059 (CH arom.), 2966, 2931, 2875 (CH aliph.), 2214 (C≡N), 1676 (C=O). ^{1}H-NMR in (DMSO-d_6) δ: 1.2[t, 3H, CH_3], 2.6[q, 2H, CH_2], 7.03–7.7[m, 9H, Ar-H], 8.2[s, 2H, NH_2, exchangeable with D_2O]. Anal. Calcd. For $C_{21}H_{16}N_4O$: C, 74.11; H, 4.70; N, 16.47. Found: C, 74.40; H, 4.30; N, 16.10.

6-Amino-5-cyano-1-(3-ethylphenyl)-2-oxo4-(2-methoxyphenyl)-1,2-dihydropyridine-3-carbonitrile (4b)

Yield, 84%; m.p. 278–280 °C' IR (KBr, cm^{-1}); 3259, 3182 (NH_2) 3066 (CH arom.), 2968, 2937, 2841 (CH aliph.), 2212 (C≡N), 1672 (C=O). ^{1}H-NMR in (DMSO-d_6) δ: 1.4[t, 3H, CH_3], 2.6[q, 2H, CH_2], 3.7[s, 3H, OCH_3], 7.03–7.7[m, 8H, Ar-H], 8.2[s, 2H, NH_2, exchangeable with D_2O]. Anal. Calcd. For $C_{22}H_{18}N_4O_2$: C, 71.35; H, 4.86; N, 15.13. Found: C, 71.50; H, 4.60; N, 15.40.

6-Amino-5-cyano-1-(3-ethylphenyl)-2-oxo-4-(4-methoxyphenyl)-1,2-dihydro-pyridine-3-carbonitrile (4c)

Yield, 89%; m.p.> 300 °C; IR (KBr, cm^{-1}): 3309, 3207 (NH_2), 3088 (cH arom.), 2966, 2839 (CH aliph.), 2214 (C≡N), 1676 (C=O). ^{1}H-NMR in (DMSO-d_6) δ: 1.2[t, 3H, CH_3], 2.6[q, 2H, CH_2], 3.6[s, 3H, OCH_3], 8.6[s, 3H, NH_2, exchangeable with D_2O], 7.03–7.7[m, 8H, Ar-H]. Anal. Calcd. for $C_{22}H_{18}N_4O_2$: C, 71.35; H, 4.86; N, 15.13. Found: C, 71.00; H, 5.10; N, 14.80.

6-Amino-5-cyano-1-(3-ethylphenyl)-2-oxo-4-(3-nitrophenyl)-1,2,-dihydropyridine-3-carbonitrile (4d)

Yield, 80%; m.p. 145–147 °C; IR (KBr, cm^{-1}): 3227, 3209 (NH_2), 3082 (CH arom.), 2964, 2931, 2872 (CH aliph.), 2194 (C≡N), 1680 (C=O). ^{1}H-NMR in (DMSO-d_6) δ: 1.2[t, 3H, CH_3], 2.6[q, 2H, CH_2], 8.3[s, 2H, NH_2, exchangeable with D_2O], 7.2–7.7[m, 8H, Ar-H]. Anal. Calcd. for : $C_{21}H_{15}N_5O_3$: C, 65.54; H, 3.89; N, 18.18. Found: C, 65.20; H, 3.50; N, 18.00.

6-Amino-5-cyano-1-(3-ethylphenyl)-2-oxo-4-(4-nitrophenyl)-1,2-dihydropyridine-3-carbonitrile (4e)

Yield, 78%; m.p. 140–142 °C; IR (KBr, cm^{-1}): 3227, 3209 (NH_2), 3082 (CH arom.), 2964, 2931, 2872 (CH aliph.), 2195 (C≡N), 1680 (C=O). ^{1}H-NMR in (DMSO-d_6) δ: 1.2[t, 3H, CH_3], 2.6[q, 2H, CH_2], 3.6[s, 3H, OCH_3], 7.03–7.7[m, 8H, Ar-H], 8.6[s, 3H, NH_2, exchangeable with D_2O]. Anal. Calcd. for : $C_{21}H_{15}N_5O_3$: C, 65.45; H, 3.89; N, 18.18 . Found: C, 65.70; H, 4.20; N, 18.50.

6-Amino-5-cyano-1-(3-ethylphenyl)-2-oxo-4-(benzo[d][1,3]dioxol-5-yl)-1,2-dihydropyridine-3-carbonitrile (4f)

Yield, 81%; m.p.>300 °C; IR (KBr, cm^{-1}): 3309, 3201 (NH_2), 3080 (CH arom.), 2910, 2840 (CH aliph.) 2210 (C≡N), 1662 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 1.1[t, 3H, CH_3], 2.6[q, 2H, CH_2], 6.2[s, 3H, CH_2 dioxol], 7.03–7.7[m, 7H, Ar-H], 8.2[s, 2H, NH_2, exchangeable with D_2O]. Anal. Calcd. for : $C_{22}H_{16}N_4O_3$: C, 68.75; H, 4.16; N, 14.58. Found: C, 68.50; H, 4.40; N, 14.20.

6-Amino-5-cyano-1-(3-ethylphenyl)-2-oxo-4-(3-ethoxy-4-methoxyphenyl)-1,2-dihydropyridine-3-carbonitrile (4g)

Yield, 64%; m.p. 244–246 °C, IR (KBr) 3325, 3219 (NH_2), 3084 (CH arom.), 2931, 2837 (CH aliph.) 2214 (C≡N), 1670 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 1.3[m, 6H, $2CH_3$], 2.6[q, 2H, CH_2], 3.4[s, 3H, OCH_3], 4.6[q, 2H, CH_2], 6.5–7.7[m, 7H, Ar-H], 8.3[s, 2H, NH_2, exchangeable with D_2O]. Anal. Calcd. for $C_{24}H_{22}N_4O_3$: C, 69.56; H, 5.31; N, 13.52. Found: C, 69.20; H, 5.60; N, 13.80.

6-Amino-5-cyano-1-(3-ethylphenyl)-2-oxo-4-(2,4-dichlorophenyl)-1,2-dihydropyridine-3-carbonitrile (4h)

Yield, 84%; m.p.>300; IR (KBr, cm^{-1}): 3373, 3311 (NH_2), 3100 (CH arom., 2966, 2935, 2862 (CH aliph.), 2212 (C≡N), 1683 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 1.2[t, 3H, CH_3], 2.6[q, 2H, CH_2], 7.1–7.7[m, 7H, Ar-H], 8.2[s, 2H, NH_2, exchangeable with D_2O]. Anal. Calcd. for $C_{21}H_{14}Cl_2NO_2O$: C, 61.62; H, 2.42; N, 13.69. Found: C, 61.30; H, 2.10; N, 13.90.

6-Amino-5-cyano-1-(3-ethylphenyl)(-2-oxo-4-(3-bromophenyl)-1,2-dihydropyridine-3-carbonitrile (4i)

Yield, 62%; m.p. 96–98 °C; IR (KBr, cm^{-1}): 3334, 3246 (NH_2), 3086 (CH arom.), 2981, 2829 (CH aliph.), 2212 (C≡N), 1649 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 1.2[t, 3H, CH_3], 2.6[q, 2H, CH_2], 7.1–7.9[m, 8H, Ar-H], 8.6[s, 2H, NH_2, exchangeable with D_2O]. Anal. Calcd. for $C_{21}H_{15}BrN_4O$: C, 60.14; H, 3.57; N, 13.36. Found: C, 60.50; H, 3.20; N, 13.70.

6-Amino-5-cyano-1-(3-ethylphenyl)-2-oxo-4-(4-amino-dimethylphenyl)-1,2-dihydropyridine-3-carbonitrile (4j)

Yield, 76%; m.p. 260–262 °C; IR (KBr, cm^{-1}): 3350, 3305 (NH_2), 3088 (CH arom.), 2962, 2925, 2872 (CH aliph.), 2200 (C≡N), 1672 (C=O). ^{1}H-NMR (DMSO-d6) δ: 1.2[t, 3H, CH_3], 2.3[s, 6H, $N(CH_3)_2$], 2.6[q, 2H, CH_2], 6.9–7.2[m, 6H, Ar-H], 8.3[s, 2H, NH_2, exchangeable with D_2O]. Anal. Calcd. for $C_{23}H_{21}N_5O$: C, 72.06; H, 5.48; N, 18.27. Found: C, 72.30; H, 5.10; N, 18.50.

6-Amino-5-cyano-1-(3-ethylphenyl)-2-oxo-4-(styryl-N-dimethyl-4)-1,2-dihydropyridine-3-carbonitrile (4k)

Yield, 59%; m.p. 190–192 °C; IR (KBr, cm^{-1}): 3350, 3246 (NH_2), 2900, 2819 (CH aliph.) 2220(C≡N), 1647 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 1.3[t, 3H, CH_3], 2.6[q, 2H, CH_2], 3.2[s, 6H, $2CH_3$], 6.9[2d, 2H, CH=CH, J=7.3, 7.2Hz], 7.1–7.7[m, 8H, Ar-H], 8.2[s, 2H, NH_2, exchangeable with D_2O]. Anal. Calcd. for $C_{25}H_{23}N_5O$: C, 73.34; H, 5.62; N, 17.11. Found: C, 73.60; H, 5.30; N, 17.40.

6-Amino-5-cyano-1-(3-ethylphenyl)-2-oxo-4-(2-hydroxy-1-naphthalene)-1,2-dihydropyridine-3-carbonitrile (4l)

Yield, 71%; m.p. >300 °C; IR (KBr, cm^{-1}): 3446 (OH), 3334, 3236 (NH_2), 3057) (CH arom.), 2940, 2836 (CH aliph.) 2210(C≡N), 1656 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 1.2[t, 3H, CH_3], 2.6[q, 2H, CH_2], 6.2[s, 1H, OH, exchangeable with D_2O], 7.1–7.9[m, 10H, Ar-H], 8.2[s, 2H, NH_2, exchangeable with D_2O]. Anal. Calcd. for $C_{25}H_{18}N_4O_2$: C, 73.89; H, 4.43; N, 13.79 Found: C, 73.50; H, 4.10; N, 13.40.

6-Amino-5-cyano-1-(3-ethylphenyl)-2-oxo-4-(2-methoxy-1-naphthalene)-1,2-dihydropyridine-3-carbonitrile (4m)

Yield, 67%; m.p. >300 °C; IR (KBr, cm^{-1}): 3304, 3199 (NH_2), 3055(CH arom.), 2964, 2935, 2845 (CH aliph.), 2210(C≡N), 1662 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 1.2[t, 3H, CH_3], 2.6[q, 2H, CH_2], 3.7[s, 3H, OCH_3], 6.2[s, 1H, OH, exchangeable with D_2O], 7.1–7.9[m, 10H, Ar-H], 8.2[s, 2H, NH_2, exchangeable with D_2O]. Anal. Calcd. for $C_{26}H_{20}N_4O_2$: C, 74.28; H, 4.76; N, 13.33. Found: C, 74.50; H, 4.40; N, 13.70.

6-Amino-5-cyano-1-(3-ethylphenyl)-2-oxo-4-(4-methoxy-1-naphthalene)-1,2-dihydropyridine-3-carbonitrile (4n)

Yield, 81%; m.p. 240–242 °C; IR (KBr, cm^{-1}): 3387, 3317 (NH_2), 3070 (CH arom.), 2935, 2839 (CH aliph.), 2208 (C≡N), 1654 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 1.4[t, 3H, CH_3], 2.6[q, 2H, CH_2], 3.8[s, 3H, OCH_3], 6.1[s, 1H, OH, exchangeable with D_2O], 7.1–7.9[m, 10H, Ar-H], 8.5[s, 2H, NH_2, exchangeable with D_2O]. Anal. Calcd. for $C_{26}H_{20}N_4O_2$: C, 74.28; H, 4.76; N, 13.33. Found: C, 73.90; H, 5.10; N, 13.00.

6-Amino-5-cyano-1-(3-ethylphenyl)-2-oxo-4(5-methyl-2-furyl)-1,2-dihydropyridine-3-carbonitrile (4o)

Yield, 69%; m.p. 108–110 °C; IR (KBr, cm^{-1}): 3339, 3209 (NH_2), 3034 (CH arom.), 2964, 2931, 2872 (CH aliph.), 2208 (C≡N), 1660 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 1.2[t, 3H, CH_3], 2.3[s, 3H, CH_3 furyl], 2.6[q, 2H, CH_2], 6.9–7.8[m, 6H, Ar-H], 8.2[s, 2H, NH_2, exchangeable with D_2O]. Anal. Calcd. for $C_{20}H_{16}N_4O_2$: C, 69.75; H, 4.65; N, 16.27. Found: C, 69.40; 4.90; N, 16.50.

6-Amino-5-cyano-1-(3-ethylphenyl)-2-oxo-4-(2-thienyl)-1,2-dihydropyridine-3-carbonitrile (4p)

Yield, 71%; m.p. 105–107 °C; IR (KBr, cm^{-1}): 3325, 3209 (NH_2), 3100 (CH arom.), 2931, 2872 (CH aliph.), 2210 (C≡N), 1658 (C=O), ^{1}H-NMR (DMSO-d_6) δ: 1.2[t, 3H, CH_3], 2.6[q, 2H, CH_2], 7.2–8.4[m, 7H, Ar-H], 8.7[s, 2H, NH_2, exchangeable with D_2O]. Anal. Calcd. for $C_{19}H_{14}N_4OS$: C, 65.89; H, 4.04; N, 16.18. Found: C, 65.50; H, 3.70; N, 16.40.

3-[N-(3-Ethylphenyl)-carbaxamido[-Benzochromene-2-one(5)

To a solution of compound 3 (1.88 g, 0.01 mol) in acetic anhydride (20 ml), 2-hydroxy-1-naphthaldehyde (1.56 g, 0.01 mol) and fused sodium acetate (0.8 g, 0.01 mo) was added. The reaction mixture was refluxed for 2 h, cooled and the solid obtained was crystallized from ethanol to give 5.

Yield, 69%; m.p. 117–119 °C; IR (KBr, cm^{-1}): 3417(NH), 1766 (2C=O), ^{1}HNMR (DMSO-d_6) δ: 1.3(t, 3H, CH_3) 2.6 (q, 2H, CH_2), 7.3–8.0 (m, 10H, Ar-H), 8.35 (s, 1H, CH), 8.42 (s, 1H, NH). Anal. Calcd. for $C_{22}H_{17}NO_3$: C, 76.96; H, 4.95; N, 4.08. Found: C, 76.60; H, 4.30; N, 4.30.

3-[N-(3-ethylphenyl)-carboxamido]-2-imino-2H-benzochromene (6)

A mixture of 3 (1.88 g, 0.01 mol), 2-hydroxy-1-naphthaldehyde (1.56 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 6.

Yield, 59%; m.p. >300 °C; IR (KBr, cm^{-1}): 3344, 3166 (2NH), 1720 (C=O), 1570 (C=N) ^{1}H-NMR (DMSO-d_6) δ: 1.1 (t, 3H, CH_3) 2.7 (q, 2H, CH_2), 6.9–8.3 (m, 10H, Ar-H), 8.7 (s, 1H, CH), 9.5 (s, 1H, NH imino), 12.5 (s, 1H, NH CO). Anal. Calcd. for $C_{22}H_{18}N_2O_2$: C, 77.19; H, 5.26, 8.18. Found: C, 77.40; H, 5.50; N, 8.50.

2.1.21. 2-Amino-3-(3-ethylphenyl)-4,5-dioxo-4,5-dihydro 3 H-Chromeno[3,4-c]pyridine-1-carbonitrile (7). 2-Amino-3-(3-ethylphenyl)-5-imino-4-oxo-4,5-dihydro-3H-benzochromeno (3,4-c) pyridine-1- carbonitrile (8)

Equimolar amounts of compounds 5 or 6, malononitrile (0.66 g, 0.01 mol) and anhydrous ammonium acetate (1.115 g, 0.01 mol) in ethanol (50 ml) were refluxed for 4 h. The solid obtained by filtration was recrystallized from dioxane to give 7 and 8, respectively.

7: Yield, 61%, m.p.> 300 °C; IR (KBr, cm^{-1}): 3336, 3232 (NH_2) 2200 (C≡N), 1684, 1654 (2C=O). ^{1}H-NMR (DMSO-d_6) δ: 1.1 (t, 3H, CH_3) 2.7 (q, 2H, CH_2), 6.9–8.3 (m, 10H, Ar-H), 8.5 [s, 2H, NH_2, exchangeable with D_2O]. Anal. Calcd. for $C_{25}H_{17}N_3O_3$: C, 73.71; H, 4.17; N, 10.31 Found: C, 73.40; H, 4.50; N, 10.60.

8: Yield, 58%, m.p.>300 °C; IR (KBr, cm^{-1}): 3360, 3331, 3216 (NH, NH_2) 2925, 2853 (CH aliph.) 2217 (C≡N), 1684, (C=O). ^{1}H-NMR (DMSO-d_6) δ: 1.1 (t, 3H, CH_3) 2.7 (q, 2H, CH_2), 6.9–8.3 (m, 10H, Ar-H), 8.5 [s, 2H, NH_2, exchangeable with D_2O], 8.7 (s, 1H, NH imino). Anal. Calcd. for $C_{25}H_{18}N_4O_2$: C, 73.89; H, 4.43; N, 13.79 Found: C, 73.44; H, 4.12; N, 13.40.

Table 1 In-vitro anticancer screening of the newly synthesized compounds against EAC cells.

Cpd. No.	Non-viable cells (%) Concentration (µg/ml)				IC_{50}[a] (µg/ml)	IC_{50}[a] (µM)
	100	50	25	10		
3	100	50	25	10	50	265.95
4a	100	55	35	5	48	141.17
4b	100	60	20	0	49	132.43
4c	100	60	20	10	45	121.62
4d	100	100	60	30	29	75.32
4e	100	100	80	60	8	20.77
4f	90	50	25	10	50	129.53
4g	100	40	20	5	55	132.85
4h	100	70	45	20	41	100.24
4i	100	50	25	10	50	119.33
4j	100	30	0	0	60	156.65
4k	85	30	0	0	67	163.81
4l	100	50	25	10	50	140.44
4m	50	0	0	0	100	238.1
4n	50	0	0	0	100	238.1
4o	50	20	0	0	100	290.6
4p	40	10	0	0	>100*	–
5	95	70	50	10	25	73.1
6	100	85	60	25	23	67.05
7	50	20	10	5	100	245.7
8	60	10	0	0	97	237.74
Dox.	100	68	30	24	37	68.13

[a] IC50 value: corresponds to the compound concentration causing 50% mortality in net cells

* Compounds with IC50 >100 lg/mL are considered to be inactive

In-vitro anticancer screening

Animals, chemicals and facilities

Ehrlich Ascites Carcinoma (EAC) cells 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 equipments 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.

In-vitro anticancer activity using EACs

EAC cells were obtained by needle aspiration of ascetic fluid from preinoculated mice; under aseptic conditions. Tumor cells suspension (2.5 _106 per ml) was prepared in RPMI-1640 media. Tested compounds were prepared with various dilutions by dissolving: 100, 50, 25 & 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, 10 mg) were mixed then 0.1 ml of tumor cell suspension (2×10^6) 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 [17–18]. The total number of cells/ml will be determined using the following calculations:

Cells/ml = average cells count per 5 squares × dilution factor × 10^4

Total cells = cells/ml × the original volume of fluid from which the cell sample was removed

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

The results of in-vitro anticancer activity experiments are presented in **Table 1**.

In-vitro anticancer activity using liver human tumor cell lines (HEPG2)

The human tumor cell line (HEPG2) was available at the National Cancer Institute, Cairo, Egypt. Irradiation was performed in the National Cancer Institute, Cairo, Egypt using Gamma cell-40 (^{60}CO) source. The anticancer activity of the newly synthesized compounds was measured using the Sulfo-Rhodamine-B stain (SRB) assay by the method of Skehan et al. [19] (1990). Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the compounds to allow attachment of cell to the wall of the plate. Tested compounds were dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the compounds under test (5, 12.5, 25 and 50 µM) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37 °C and in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed and stained for 30 min with 0.4% (wt/vol) with SRB dissolved in 1% acetic acid. Unbounded dye was removed by 4 washes with 1% acetic acid, and attached stain was recovered with Tris-EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration was plotted to get the survival curve of each tumor cell line after the specified time. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and compared with the reference drug doxorubicin and the results are given in **Table 2**.

Table 2 In-vitro anticancer evaluation of compounds 4d, 4e, 5 and 6 against HEPG2.

Cpd. No.	Compound concentration (μM)				IC_{50} (μM)
	5 (μM)	12.5 (μM)	25 (μM)	50 (μM)	
	Surviving fraction (mean ± SE) [a]				
Dox.	0.921 ± 0.020	0.846 ± 0.020	0.761 ± 0.010	0.494 ± 0.030	38.46
4d	0.756 ± 0.002	0.634 ± 0.009	0.412 ± 0.028	0.213 ± 0.047	26.5
4e	0.426 ± 0.018	0.394 ± 0.031	0.362 ± 0.022	0.271 ± 0.012	19.2
5	0.921 ± 0.012	0.792 ± 0.041	0.621 ± 0.047	0.373 ± 0.022	39.3
6	0.922 ± 0.064	0.746 ± 0.014	0.630 ± 0.016	0.446 ± 0.012	44.9

[a] Each value is the mean of 3 experiments ± standard error

Table 3 In-vitro anticancer evaluation of compounds 4d, 4e, 5 and 6 against HEPG2 after radiation.

Cpd. No.	Compound concentration (μM) + irradiation (8 Gy)				IC_{50} (μM)
	Surviving fraction (mean ± SE) [a]				
	5	12.5	25	50	
Dox.	0.87 ± 0.0012	0.65 ± 0.047	0.38 ± 0.085	0.063 ± 0.111	20.2
4d	0.512 ± 0.01	0.478 ± 0.02	0.39 ± 0.05	0.10 ± 0.06	18.4
4e	0.340 ± 0.07	0.290 ± 0.01	0.270 ± 0.01	0.240 ± 0.08	12
5	0.471 ± 0.01	0.371 ± 0.01	0.245 ± 0.01	0.140 ± 0.07	14.2
6	0.564 ± 0.04	0.321 ± 0.01	0.218 ± 0.01	0.100 ± 0.06	15.14

[a] Each value is the mean of 3 experiments ± standard error

2.3 Radiosensitizing evaluation

The most potent compounds resulted from the in vitro anticancer screening; compounds 4d, 4e, 5 and 6, were selected to be evaluated again for their in-vitro anticancer activity in combination with γ-radiation and compared to the reference drug doxorubicin. 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 (^{60}CO) source. The surviving fractions were expressed as means ± standard error. The results are given in **Table 3**.

Results and Discussion

Chemistry

Figs. 2, 3 outline, the synthestic pathway used to obtain 1,2-dihydropyridines, 4a-p, chromenes 5,6, and chromenopyridines 7, 8. 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. Upon treatment of compound 3 with required aldehyde and malononitrile in the presence of catalytic amount of piperidine furnished 6-amino-5-cyano-1-(3-ethylphenyl)-2-oxo-4-substituted-aryl-1,2-dihydropyridine-3-carbonitriles 4a-p (**Fig. 2**). The structure of compounds 4a-p was deduced from elemental analyses and spectral data.

Furthermore, Perkin reaction was carried out by reaction of 2-hydroxy-1-naphthaldehyde in the presence of sodium acetate to give the corresponding chromene-2-one derivative 5, while conducting the same reaction in the presence of ammonium acetate in ethanol furnished 2-iminochromene 6. The structure of compounds 5 and 6 was supported in the basis of elemental analyses and spectral data. The chromene derivatives 5 and 6 were further reacted with malononitrile in the presence of ammonium acetate to give the corresponding chromenopyridines 7 and 8, respectively (**Fig. 3**). The structure of compounds 7 and 8 was elucidated from elemental analyses and spectral data.

In-vitro anticancer activity

Doxorubicin, the reference drug used in this study is one of the most effective antitumor agents used to produce regressions in acute leukemias, Hodgkin's disease, and other lymphomas. The relationship between survival fraction and drug concentration was plotted to obtain the survival curve of EAC cells and HEPG2. The response parameter calculated was the IC_{50} value (**Tables 1**, **2**), which corresponds to the compound concentration causing 50% mortality in net cells.

In-vitro anticancer activity using EAC cell line

The cytotoxicity of 21 compounds was examined on EAC cells. It is clear from the results in **Table 1**, that the most potent compound in this study was compound 4e (IC_{50} = 20.77 μM) which was found to be more potent than the reference drug (doxorubicin) (IC_{50} = 68.13 μM) this may be attributed to the presence of 4-nitrophenyl substitution on 2-pyridone ring which may give an idea about the possible importance of nitro group to enhance activity, especially, because the 3-nitrophenyl derivative 4d showed also significant activity (IC_{50} = 75.32 μM). Moreover, the chromene derivatives 5, 6 showed significant activity (IC_{50} = 73.1 and 67.05 μM) which is nearly as potent as the reference drug and also they found to be more active than the starting material 3 (IC_{50} = 265.95 μM), while, their cyclization to the corresponding chromenopyridine 7, 8 resulted in a drop in their activity (IC_{50} = 245.7 and 237.74 μM).

In-vitro anticancer activity using HEPG2

This study was performed to evaluate the anticancer activity of the most potent 4 compounds resulted from EAC assay (compounds 4d, 4e, 5, 6). 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 compound 4e (IC_{50} = 19.2 μM) which is more potent than doxorubicin (IC_{50} = 38.46 μM), while compounds 4d, 5, 6 showed lesser activity (IC_{50} = 26.5, 39.3, 44.9 μM) which is nearly as active as doxorubicin as positive control.

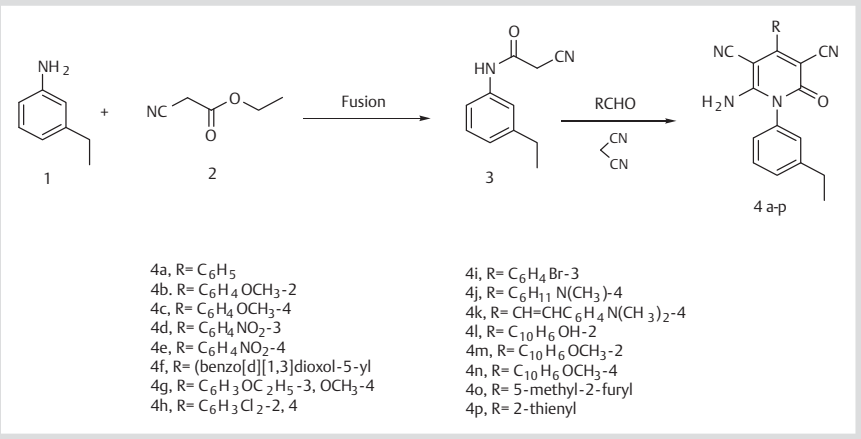

Fig. 2 Synthetic pathways for compounds 4a–p.

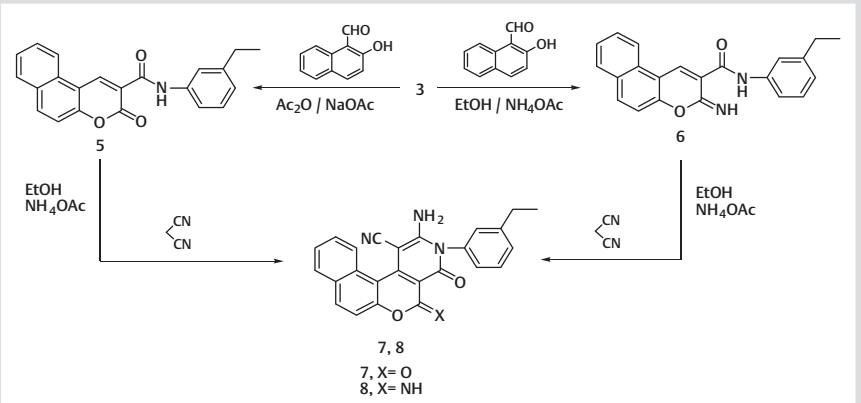

Fig. 3 Synthetic pathways for compounds 5–8.

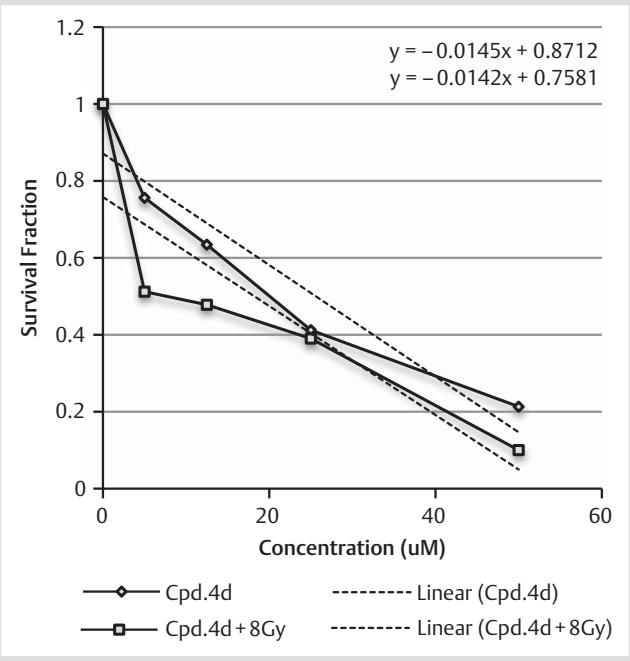

Fig. 4 Survival curve for HEPG2 cell line for compound 4d alone and in combination with γ-irradiation (8 Gy), the dotted lines represent the best fitting lines for each curve, and their equations are shown.

Radiosensitizing evaluation

The rationale for combining chemotherapy and radiotherapy is based mainly on 2 ideas, one being spatial cooperation, which is effective if chemotherapy is sufficiently active 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 [20]. Consequently, the ability of the 4 most active compounds, compounds 4d, 4e, 5 and 6, to enhance the cell killing effect of γ-irradiation was studied. From the results obtained in **Table 2**, compound 4d showed an in-vitro cytotoxic activity with IC_{50} value of 26.4 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 4d, and irradiated with a single dose of γ-radiation at a dose level of 8 Gy, as shown in **Table 3**, the IC_{50} value was synergistically decreased to 18.4 µM (**Fig. 4**). Similarly, compounds 4e, 5, 6 showed IC_{50} values of 19.2, 39.3 and 44.9 µM, respectively, when used alone, as shown in **Table 2**. The IC_{50} value was decreased to 12, 14.2 and 15.14 µM, respectively, when the cells were treated with compounds 4e, 5, 6 in combination with γ-radiation (**Fig. 5**, **6**, **7**). From these results, we can conclude that the combination of compounds 4d, 4e, 5 or 6 and ionizing radiation synergistically enhanced growth inhibition on liver cancer cells, compared with each agent alone and these compound showed better radiosensitizing activity then doxorubicin (20.2 µM). For better comparison, the change in IC_{50} before and

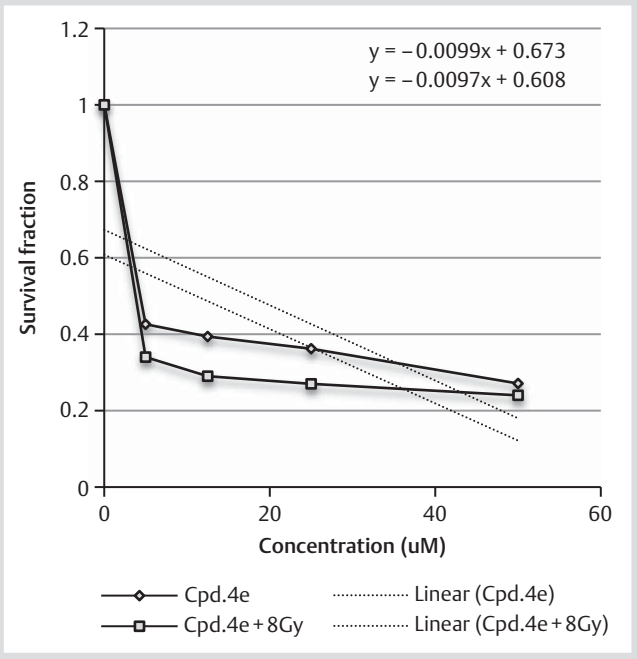

Fig. 5 Survival curve for HEPG2 cell line for compound 4e alone and in combination with γ-irradiation (8 Gy), the dotted lines represent the best fitting lines for each curve, and their equations are shown.

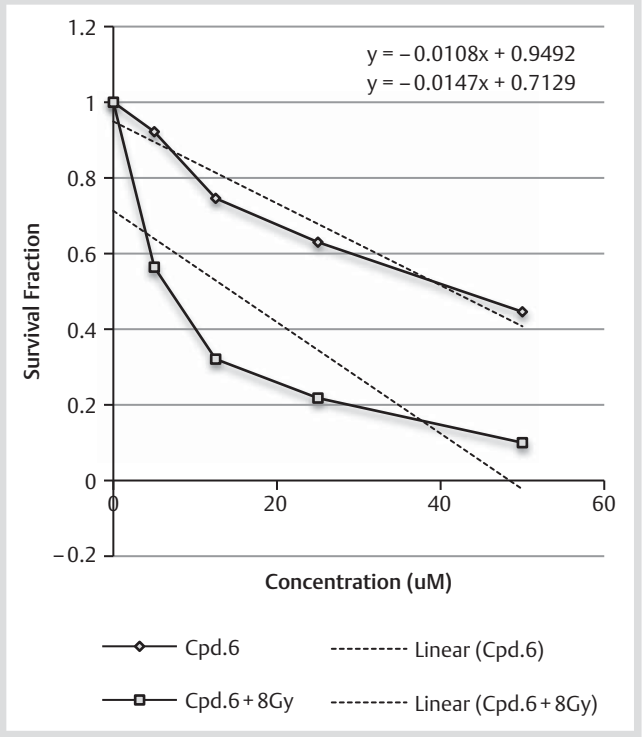

Fig. 6 Survival curve for HEPG2 cell line for compound 6 alone and in combination with γ-irradiation (8 Gy), the dotted lines represent the best fitting lines for each curve, and their equations are shown.

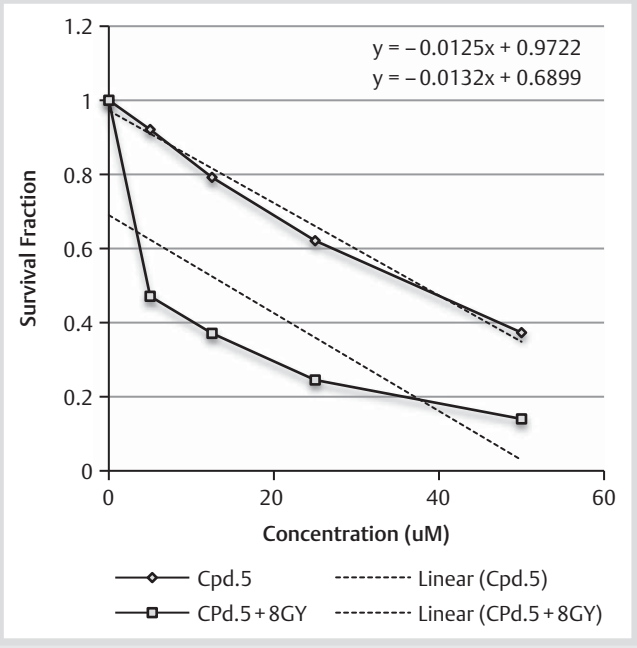

Fig. 7 Survival curve for HEPG2 cell line for compound 5 alone and in combination with γ-irradiation (8 Gy), the dotted lines represent the best fitting lines for each curve, and their equations are shown.

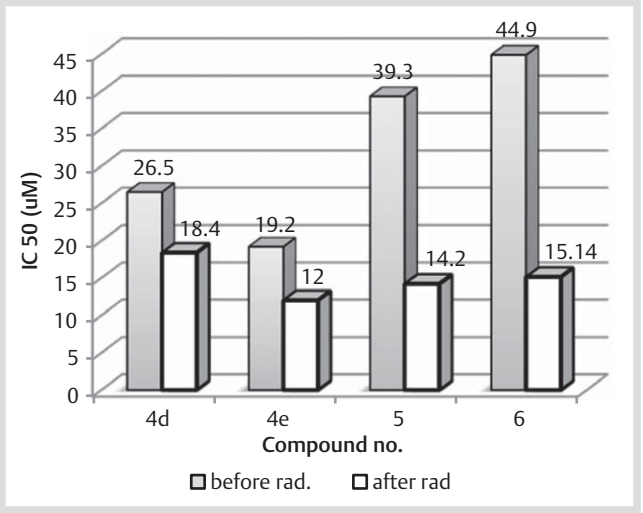

Fig. 8 Change in IC_{50} before and after irradiation on HEPG2 cell line for compounds 4d, 4e, 5 and 6.

after irradiation on HEPG2 cell line for compounds 4d, 4e, 5 or 6 is plotted in a histogram (◉ **Fig. 8**).

Conclusion

▼

We report here the synthesis of new 2-pyridone derivatives. It was clearly observed from the results of in-vitro anticancer screening that the synthesized compounds exhibited significant anticancer activity on EAC, the most potent compounds were compounds 4d, 4e, 5, 6 which also showed promising activity HEPG2. While, combining these compounds with radiation at the same concentrations enhanced their activity which demonstrates the importance of the combination therapy for the patients with cancer to decrease the side effects of both drug and radiation.

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Conflict of Interest

The authors declare that they have no conflict of interest with respect to this paper.

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