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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and antitumor activity evaluation of different 2,5-dialkyloxazolopyrano[3,2-*c*]quinolinone derivatives

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Abstract

A new interesting family of 2,5-dialkyloxazolopyrano[3,2-*c*]quinolinone derivatives **4a-l** were synthesized in good yields using a convenient and successful method. Structures of all compounds were achieved by elemental analyses, IR, ^{1}H NMR, ^{13}C NMR, and mass techniques. The tetracyclic homologues **4a-l** were evaluated for antitumor activity and cytotoxicity against a board of three human cancer cell lines (HepG-2, MCF-7, and HCT-116). The cells were treated with these compounds at differentiable dosages, and cell viability was determined. Significant anticancer activities were observed in vitro for some members of the series related to 5-fluorouracil and compounds **4c** and **4f** showed potent cytotoxic effect, that creates these compounds were disclosed as the most applicable anticancer agents against different cancer cell lines.

Keywords Dialkyloxazole · Pyranoquinolinone · Antitumor activity · Cell line · Cell viability · Cytotoxicity

Introduction

Currently, the attention on the construction of pyranoquinolone scaffolds has been expanded. As this attractive moiety has been depicted as antitumor (Arasakumar et al. 2017), additionally, pyranoquinolone alkaloids display cancer cell growth-inhibitory activity and are considered as potential anticancer agents (Upadhyay et al. 2018). For instance, zanthosimuline is active in contrast to multidrug-resistant KB-VI cancer cells, while huajiaosimuline demonstrates a selective cytotoxicity profile showing the considerable activity with estrogen receptor-positive ZR-75-1 breast cancer cells (Chen et al. 1994). A series of pyrano[3,2-*c*]quinolines advertise skillful cell growth inhibition against MCF-7 cell line and A549 cell line (Arasakumar et al. 2017). Moreover, some pyrano[3,2-*c*]quinolones showed powerful cytotoxic activity against distinctive cells (Likhitwitayawuid et al. 1993). Addition of a further ring to the pyranoquinolinone tends to exert a meaningful effect in conferring novel biological activities in these molecules, the heterocycle-fused pyranoquinolinone systems exhibit differing biological activities permit them to act as antibacterial (Ramesh et al. 2009), anticoagulant (Anniyappan et al. 2003), antitumor (El-Agrody et al. 2013), and microtubule-targeting agents (Magedov et al. 2008). Otherwise, oxazoles perform an essential role in the construction of diverse biologically active drugs as anticancer (Choi et al. 2013). Furthermore, some oxazole derivatives prevent cancerous cell growth with nanomolar ratio against some of human cancer cell lines like, renal adenocarcinoma (TK-10), and human breast adenocarcinoma (MCF-7) (El-Nezhawy et al. 2016). Oxazole-bridged analogs showed effective anti-tumor activity toward lung carcinoma (A549), human colon carcinoma (HT29), liver carcinoma (HepG2), and human primary pancreatic adenocarcinoma (BxPC3) (Zhou et al. 2013). Inspired by unique features of pyranoquinolinone and oxazole and their reported encouraging bioactivity, it would be a very attractive approach to extend oxazole ring to pyranoquinolinone analogue in one molecular skeleton.

We therefore aimed to build up an annulated tetracyclic system of oxazolopyrano[3,2-*c*]quinolinone derivatives, holding two varied alkyl groups at two different positions. Also, we describe their promising antitumor activity and investigate the structure–activity relationship (SAR)

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between the length of each alkyl group and its influence on antitumor activity.

Material and methods

Chemistry

Melting points were determined on a digital Stuart-SMP3 apparatus. Infrared spectra were recorded on FT-IR Nicolet IS10 spectrophotometer (cm^{-1}) at Micro Analytical Center, Cairo University, using KBr disks. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were measured on Bruker 400 MHz spectrometer (δ) at Faculty of Science, Sohag University, using DMSO-d_6 as a solvent and tetramethylsilane as an internal standard. Mass spectra were measured using mass analyzer in Thermo Scientific GCMS model ISQ at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University. Elemental microanalyses were performed on a Perkin-Elmer CHN-2400 analyzer, at the Chemical War Department, Ministry of Defence, Cairo, Egypt. Compound **1a** (Roschger et al. 1992), **1b** (Gao et al. 2010), **1c** (Kappe et al. 1994), **2a**, **2b** (Kappe 1999), **2c**, **3c**, and **4c** (Hassanin et al. 2017) were prepared according to the reported literature method.

3-Amino-4-hydroxy-6-methyl-2*H*-pyrano[3,2-*c*] quinoline-2,5(6*H*)-dione (3a)

A mixture of compound **2a** (2.88 g, 10 mmol) and tin metal powder (4.00 g, 33 mmol) with HCl (30 ml, 36%) was stirred at 120 °C for 1 h. Then, MeOH (25 ml) was added to the reaction mixture and the mixture was heated under reflux for 2 h until it became like as an emulsion. The reaction mixture was poured into ice (100 g) with stirring for 15 min. The yellow color precipitate was filtered, dried, and crystallized from glacial acetic acid to give compound **3a** as orange needle crystals, yield (1.7 g, 65.8%), m.p. 288–290 °C. **IR (KBr, cm^{-1})**: 3421, 3342 (NH_2), 2940 ($CH_{aliphatic}$), 1710 ($C{=}O_{\alpha\text{-}pyrone}$), 1677 ($C{=}O_{quinolone}$), and 1563 (C=C). **$^{1}H$ NMR (400 MHz, $CDCl_3$) δ_H**: 3.77 (s, 5H, (3H of NCH_3 and 2H of free NH_2 group) the integration exchanged in D_2O to 3H only), 7.40 (t, 1H, $J = 8.0$ Hz, H-9), 7.47 (d, 1H, $J = 8.0$ Hz, H-7), 7.66 (t, 1H, $J = 8.0$ Hz, H-8), 8.23 (d, 1H, $J = 8.0$ Hz, H-10), 12.27 (s, 1H, OH exchangeable with D_2O). **^{13}C NMR (101 MHz, DMSO-d_6) δ_C**: 29.75 (s, C1′), 101.76 (s, C3), 113.75 (s, C4a), 115.01 (s, C10a), 116.46 (s, C7), 122.38 (s, C10), 124.43 (s, C8), 132.05 (s, C9), 136.94 (s, C6a), 144.37 (s, C10b), 148.74 (s, C2), 158.90 (s, C4), 162.89 (s, C5). **Mass spectrum, *m/z* (I_r %)**: 259 (M^++1, 20), 258 (M^+, 100), 244 (31), 230 (40), 175 (70), 161 (72), 132 (20), 104 (14), and 77 (30). Analysis calculated for $C_{13}H_{10}N_2O_4$ (258.24): C, 60.47; H, 3.90; N, 10.85. Found C, 60.32; H, 3.80; N, 10.94%.

3-Amino-6-ethyl-4-hydroxy-2*H*-pyrano[3,2-*c*] quinoline-2,5(6*H*)-dione (3b)

A mixture of compound **2b** (3.02 g, 10 mmol) and tin metal powder (4.00 g, 33 mmol) with HCl (30 ml, 36%) was stirred at 120 °C for 1 h. Then, 25 ml of MeOH was added to the reaction mixture. The mixture was heated under reflux for 2 h until the color became clear yellow. The reaction mixture filtered to remove the insoluble substances on hot and left at room temperature for 10 min, then, poured into ice (100 g) and left for 30 min. The solution was decanted and the precipitate so formed was dissolved in methanol, poured into ice (100 g). The yellow solid matter was formed, dried, and crystallized from ethanol to give compound **3b** as yellow needle crystals, yield (1.8 g, 66.6%), m.p. 236–238 °C. **IR (KBr, cm^{-1})**: 3452, 3364 (NH_2), 2982, 2938 ($CH_{aliphatic}$), 1714 ($C{=}O_{\alpha\text{-}pyrone}$), 1682 ($C{=}O_{quinolone}$), and 1610 (C=C). **$^{1}H$ NMR (400 MHz, $CDCl_3$) δ_H**: 1.01 (t, 3H, $J = 8.00$ Hz,CH_2CH_3), 3.74 (brs, 2H, NH_2 exchanged in D_2O), 4.33 (q, 2H, $J = 8.0$ Hz, NCH_2), 7.39 (t, 1H, $J = 8.0$ Hz, H-9), 7.45 (d, 1H, $J = 8.0$ Hz, H-7), 7.64 (t, 1H, $J = 8.0$ Hz, H-8), 8.21 (d, 1H, $J = 8.0$ Hz, H-10), 12.34 (s, 1H, OH *exchangeable* with D_2O). **^{13}C NMR (101 MHz, $CDCl_3$) δ_C**: 12.77 (s, C2′), 37.64 (s, C1′), 90.87 (s, C3), 99.97 (s, C4a), 113.97 (s, C10a), 114.89 (s, C7), 124.05 (s, C10), 125.00 (s, C8), 134.16 (s, C9), 137.91 (s, C6a), 159.28 (s, C10b), 161.54 (s, C2), 162.59 (s, C4), 168.99 (s, C5). **Mass spectrum, *m/z* (I_r %)**: 274 (M^++2, 2), 273 (M^++1, 16), 272 (M^+, 100), 244 (31), 216 (12), 198 (5.39), 127 (23), 161 (26), 146 (8.9), 132 (14), 120 (19.6), 77 (28), and 64 (8). Analysis calculated for $C_{14}H_{12}N_2O_4$ (272.26): C, 61.76; H, 4.44; N, 10.29. Found C, 61.80; H, 4.31; N, 10.15%.

General procedure for preparation

General procedure for preparation of 2,5-dilkyloxazolopyranoquinolinone derivatives (4a-i)

A mixture of compounds **3a-c** (10 mmol) with 10 ml of some different anhydrides, namely acetic anhydride, propionic anhydride, and hexanoic anhydride, was heated under free-solvent condition. The progress of the reaction was monitored by TLC. The solid deposited after cooling was filtered off then dried and crystallized from a suitable solvent to give compounds **4a-i** as illustrated in the following table.

	R^2	Time	m.p. (°C)	Solvent	Color	Yield
4a	$–CH_3$	12 h	>300	AcOH	Pale yellow	(2 g, 74%)
4b	$–CH_3$	9 h	283	AcOH	Pale yellow	(1.8 g, 62%)
4c	$–CH_3$	10 h	290	EtOH	Yellow	(2.2 g, 68.7%)
4d	$–CH_2CH_3$	8 h	>300	AcOH	Yellow	(1.9 g, 67.8%)
4e	$–CH_2CH_3$	9 h	263	AcOH	Yellow	(2.3 g, 74.1%)
4f	$–CH_2CH_3$	8 h	255	EtOH	Off white	(2 g, 60.6%)
4g	$–CH_2(CH_2)_3CH_3$	12 h	202	EtOH	Pale yellow	(2.5 g, 75.7%)
4h	$–CH_2(CH_2)_3CH_3$	10 h	190	MeOH	Off white	(2 g, 57.1%)
4i	$–CH_2(CH_2)_3CH_3$	12 h	180	MeOH	Off white	(2.9 g, 76.3%)

	R^2	Time	m.p. (°C)	Solvent	Color	Yield
4a	$–CH_3$	12 h	>300	AcOH	Pale yellow	(2 g, 74%)
4b	$–CH_3$	9 h	283	AcOH	Pale yellow	(1.8 g, 62%)
4c	$–CH_3$	10 h	290	EtOH	Yellow	(2.2 g, 68.7%)
4d	$–CH_2CH_3$	8 h	>300	AcOH	Yellow	(1.9 g, 67.8%)
4e	$–CH_2CH_3$	9 h	263	AcOH	Yellow	(2.3 g, 74.1%)
4f	$–CH_2CH_3$	8 h	255	EtOH	Off white	(2 g, 60.6%)
4g	$–CH_2(CH_2)_3CH_3$	12 h	202	EtOH	Pale yellow	(2.5 g, 75.7%)
4h	$–CH_2(CH_2)_3CH_3$	10 h	190	MeOH	Off white	(2 g, 57.1%)
4i	$–CH_2(CH_2)_3CH_3$	12 h	180	MeOH	Off white	(2.9 g, 76.3%)

2,5-Dimethyl-4*H*-oxazolo[5′,4′:4,5]pyrano[3,2-*c*]quinolone-4,11(5*H*)-dione (4a)

IR (KBr, cm^{-1}): 3078 ($CH_{aromatic}$), 2971, 2930 ($CH_{aliphatic}$), 1768 ($C{=}O_{\alpha\text{-}pyrone}$), 1662 ($C{=}O_{quinolinone}$), 1589 ($C{=}N_{oxazolo}$), 1574 ($C{=}C_{aromatic}$). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 2.7 (s, 3H, $CH_{3(oxazolo)}$), 3.7 (s, 3H, NCH_3), 7.50 (t, J = 8.0 Hz, 1H, H-8), 7.73 (d, J = 8.0 Hz, 1H, H-6), 7.84 (t, J = 8.0 Hz, 1H, H-7), 8.21 (d, J = 8.00 Hz, 1H, H-9). Mass spectrum, *m/z* (I_r %): 283 [M^++1; 19.8], 282 [M^+; 100], 253 [M^+-2CH_3; 10.19], 228 [M^+-2CO; 7], 203 (2), 199 (1.7), 172 (3.19), 149 (3.39), 139 (3.23), 125 (3.4), 114 (4.2), 110 (7.5), 105 (4.2), 97 (6.4), 95 (8), 91 (13.1), 83 (21), 81 (17.4), 77 (11.2), 69 (22.4), 60 (14), 57 (37), 54 (31.7), 43 (56), 41 (33). Analysis calculated for $C_{15}H_{10}N_2O_4$ (282.26): C, 63.83%; H, 3.57%; N, 9.92%. Found: C, 63.70%; H, 4.55%; N, 9.97%.

5-Ethyl-2-methyl-4*H*-oxazolo[5′,4′:4,5]pyrano[3,2-*c*] quinolone-4,11(5*H*)-dione (4b)

IR (KBr, cm^{-1}): 3084 ($CH_{aromatic}$), 2977, 2936 ($CH_{aliphatic}$), 1768 ($C{=}O_{\alpha\text{-}pyrone}$), 1651 ($C{=}O_{quinolinone}$), 1590 ($C{=}N_{oxazolo}$), 1571 ($C{=}C_{aromatic}$). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 1.29 (t, J = 4.0 Hz, 3H, CH_2CH_3), 2.7 (s, 3H, $CH_{3oxazolo}$), 4.4 (q, 2H, NCH_2), 7.46 (t, J = 4.0 Hz, 1H, H-8), 7.76 (d, J = 4.0 Hz, 1H, H-6), 7.83 (t, J = 8.0 Hz, 1H, H-7), 8.19 (d, J = 8.0 Hz, 1H, H-9). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 13.0, 14.3, 21.4, 37.4, 101.1, 113.4, 115.9, 123.4, 124.2, 124.8, 134.0, 138.7, 154.7, 156.2, 156.9, 164.6. Mass spectrum, *m/z* (I_r %): 297 [M^++1; 22.1], 296 [M^+; 100], 295 [M^+-1; 86.3], 268 [M^+-CO; 30], 238 (0.94), 211 (0.83), 187 (1.73), 170 (4.3), 148 (1.4), 146 (7.2), 143 (5.12), 132 (17.6), 128 (4.3), 117 (4), 114 (5.7), 104 (8.8), 99 (1.7), 91 (7.3), 91 (10.4), 89 (7.3), 81 (15.1), 77 (32.5), 64 (4.5), 52 (6.2), 43 (10.5), 42 (3.14). Analysis calculated for $C_{16}H_{12}N_2O_4$ (296.29): C, 64.86%; H, 4.08%; N, 9.45%. Found: C, 64.45%; H, 4.20%; N, 9.30%.

2-Ethyl-5-methyl-4*H*-oxazolo[5′,4′:4,5]pyrano[3,2-*c*] quinolone-4,11(5*H*)-dione (4d)

IR (KBr, cm^{-1}): 3090, 3051 ($CH_{aromatic}$), 2981, 2936, 2880 ($CH_{aliphatic}$), 1762 ($C{=}O_{\alpha\text{-}pyrone}$), 1647 ($C{=}O_{quinolinone}$), 1578 ($C{=}N_{oxazolo}$), 1553 ($C{=}C_{aromatic}$). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 1.39 (t, J = 8.0 Hz, 3H, CH_2CH_3), 3.06 (q, J = 8.0 Hz, 2H, $CH_{2oxazolo}$), 3.70 (s, 3H, NCH_3), 7.48 (t, J = 8.0 Hz, 1H, H-8), 7.71 (d, J = 8.0 Hz, 1H, H-6), 7.84 (t, J = 8.0 Hz, 1H, H-7), 8.19 (d, J = 8.0 Hz, 1H, H-9). Mass spectrum, *m/z* (I_r %): 296 [M^+; 100], 297 [M^++1; 8.4], 281 (44), 239 (10), 171 (7), 131 (6.36), 120 (5.9), 104 (9.06), 78 (12.8), 76 (20). Analysis calculated for $C_{16}H_{12}N_2O_4$ (296.29): C, 64.86%; H, 4.08%; N, 9.45%. Found: C, 64.60%; H, 4.20 %; N, 9.47%.

2,5-Diethyl-4*H*-oxazolo[5′,4′:4,5]pyrano[3,2-*c*]quinolone-4,11(5*H*)-dione (4e)

IR (KBr, cm^{-1}): 3087 ($CH_{aromatic}$), 2982, 2936, 2881 ($CH_{aliphatic}$), 1771 ($C{=}O_{\alpha\text{-}pyrone}$), 1655 ($C{=}O_{quinolinone}$), 1609 ($C{=}N_{oxazolo}$), 1575 ($C{=}C_{aromatic}$). ^{1}H NMR (400 MHz, DMSO-d_6,) δ_H: 1.29 (t, J = 8.0 Hz, 3H, CH_3), 1.39 (t, J = 8.0 Hz, 3H, CH_3), 3.03 (q, J = 4.0 Hz, 2H, $CH_{2oxazolo}$), 4.38 (q, J = 4.0 Hz, 2H, NCH_2), 7.45 (t, J = 8.0 Hz, 1H, H-8), 7.73 (d, J = 8.0 Hz, 1H, H-6), 7.82 (t, J = 8.0 Hz, 1H, H-7), 8.16 (d, J = 8.0 Hz, 1H, H-9). ^{13}C NMR (100 MHz,

DMSO-d_6) δ_C: 10.96, 13.06, 21.6, 37.4, 101.1, 113.3, 115.8, 123.4, 124.1, 124.7, 133.9, 138.7, 154.8, 156.04, 156.1, 156.8, 168.7. Mass spectrum, *m/z* (I_r %): 311 [M^+ +1; 20.3], 310 [M^+; 100], 309 [M^+-1; 97.7], 282 (51.2). Analysis calculated for $C_{17}H_{14}N_2O_4$ (310.31): C, 65.80%; H, 4.55%; N, 9.03%. Found: C, 65.62%; H, 4.20 %; N, 9.33%.

5-Butyl-2-ethyl-4*H*-oxazolo[5′,4′:4,5]pyrano[3,2-*c*] quinolone-4,11(5*H*)-dione (4f)

IR (KBr, cm^{-1}): 3076 ($CH_{aromatic}$), 2952, 2931, 2869 ($CH_{aliphatic}$), 1765 ($C{=}O_{\alpha\text{-}pyrone}$), 1655 ($C{=}O_{quinolinone}$), 1592 ($C{=}N_{oxazolo}$), 1569 ($C{=}C_{aromatic}$). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 0.97 (t, $J = 8.00$ Hz, 3H, CH_3), 1.39 (t, $J = 8.00$ Hz, 3H, CH_3), 1.44–1.48 (m, 2H, CH_{2butyl}), 1.66–1.70 (m, 2H, CH_{2butyl}), 3.05 (q, $J = 8.00$ Hz, 2H, $CH_{2oxazolo}$), 4.36 (t, $J = 8.0$ Hz, 2H, NCH_2), 7.46 (t, $J = 8.0$ Hz, 1H, H-8), 7.74 (d, $J = 8.0$ Hz, 1H, H-6), 7.83 (t, $J = 8.0$ Hz, 1H, H-7), 8.18 (d, $J = 8.0$ Hz, 1H, H-9). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 10.97, 14.09 ($CH_{3oxazole}$), 19.9 ($CH_{2oxazole}$), 21.6, 29.7, 41.9, 71.43, 101.1, 113.4, 116.08, 123.4, 124.1, 125.1, 133.9, 138.9, 154.2, 156.3, 156.9, 168.7. Mass spectrum, *m/z* (I_r %): 338 [M^+; 11.8], 337 [M^+-1; 37.7], 321 (81.4), 308 (19.08), 299 (11.3), 281(100), 267 (20), 197 (20), 185 (20), 132 (51.2), 95 (21.65). Analysis calculated for $C_{19}H_{18}N_2O_4$ (338.37): C, 67.45%; H, 5.36%; N, 8.28%. Found: C, 67.35%; H, 5.20%; N, 8.30%.

5-Methyl-2-pentyl-4*H*-oxazolo[5′,4′:4,5]pyrano[3,2-*c*] quinolone-4,11(5*H*)-dione (4g)

IR (KBr, cm^{-1}): 3087 ($CH_{aromatic}$), 2970, 2925, 2863 ($CH_{aliphatic}$), 1760 ($C{=}O_{\alpha\text{-}pyrone}$), 1657 ($C{=}O_{quinolinone}$), 1610 ($C{=}N_{oxazolo}$), 1567 ($C{=}C_{aromatic}$). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 0.94 (t, $J = 8.0$ Hz, 3H, $CH_{3pentyl}$), 1.32–1.49 (m, 4H, $2(CH_2)_{pentyl}$), 1.79–1.89 (m, 2H, CH_2), 2.99 (t, $J = 8.0$ Hz, 2H, CH_2), 3.7 (s, 3H, NCH_3), 7.44 (t, $J = 8.0$ Hz, 1H, H-8), 7.69 (d, $J = 8.0$ Hz, 1H, H-6), 7.84 (t, $J = 8.0$ Hz, 1H, H-7), 8.14 (d, $J = 8.0$ Hz, 1H, H-9). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 14.1, 22.1, 26.1, 27.8, 29.7, 30.9, 101.1, 113.1, 116.1, 123.5, 123.8, 124.7, 133.8, 139.7, 154.7, 156.1, 156.4, 156.8, 167.8. Mass spectrum, *m/z* (I_r %): 338 [M^+; 100], 324 [M^+-CH_3+H; 55.4], 310 [M^+-CO; 12.2], 296 (20), 281 (29.2), 267 [M^+-C_5H_{11}; 5], 259 (8.4), 243 (6.5), 234 (5.5), 230 (4.8), 211 (5.2), 201 (8.3), 194 (2.1), 164 (2.5), 161 (2.6), 132 (2.6), 128 (2.5), 97 (5.1), 79 (5.1), 72 (3), 54 (3.1), 46 (1.9), 44 (1.9). Analysis calculated for $C_{19}H_{18}N_2O_4$ (338.37): C, 67.45%; H, 5.36%; N, 8.28%. Found: C, 67.53%; H, 5.22%; N, 8.15%.

5-Ethyl-2-pentyl-4*H*-oxazolo[5′,4′:4,5]pyrano[3,2-*c*] quinolone-4,11(5*H*)-dione (4h)

IR (KBr, cm^{-1}): 3083 ($CH_{aromatic}$), 2970, 2928, 2864 ($CH_{aliphatic}$), 1771 ($C{=}O_{\alpha\text{-}pyrone}$), 1655 ($C{=}O_{quinolinone}$), 1592 ($C{=}N_{oxazolo}$), 1568 ($C{=}C_{aromatic}$). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 0.94 (t, $J = 8.00$ Hz, 3H, $CH_{3pentyl}$), 1.27 (t, $J = 4.00$ Hz, 3H, CH_2CH_3), 1.32–1.48 (m, 4H, 2 $(CH_2)_{pentyl}$), 1.82–1.85 (m, 2H, CH_2), 3.00 (t, $J = 8.0$ Hz, 2H, CH_2), 4.3 (q, $J = 8.0$ Hz, 2H, NCH_2), 7.42 (t, $J = 8.0$ Hz,1H, H-8), 7.69 (d, $J = 8.0$ Hz, 1H, H-6), 7.79 (t, $J = 8.0$ Hz, 1H, H-7), 8.13 (d, $J = 8.0$ Hz, 1H, H-9). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 13.0, 14.1, 22.1, 26.1, 27.8, 30.9, 37.4, 101.0, 113.3, 115.8, 123.3, 124.1, 124.7, 133.9, 138.6, 154.7, 155.9, 156.0, 156.8, 167.7. Mass spectrum, *m/z* (I_r %): 353 [M^++1; 23.5], 352 [M^+; 100], 351 [M^+-1; 48.8], 324 [M^+-C_2H_5+H; 6], 296 [M^+-2CO; 91.6], 281 [M^+-C_5H_{11}-H; 7.3], 272 (10.4), 268 (37.7), 256 (1.5), 244 (2.8), 239 (2.9), 131 (2.4), 118 (0.8), 95 (1), 83 (1.4), 69 (2.5), 55 (2.5), 43 (13.4), 40 (17.6). Analysis calculated for $C_{20}H_{20}N_2O_4$ (352.39): C, 68.17%; H, 5.72%; N, 7.95%. Found: C, 68.43%; H, 5.32%; N, 7.65%.

5-Butyl-2-pentyl-4*H*-oxazolo[5′,4′:4,5]pyrano[3,2-*c*] quinolone-4,11(5*H*)-dione (4i)

IR (KBr, cm^{-1}): 3086 ($CH_{aromatic}$), 2980, 2926, 2863 ($CH_{aliphatic}$), 1757 ($C{=}O_{\alpha\text{-}pyrone}$), 1656 ($C{=}O_{quinolinone}$), 1574 ($C{=}C_{aromatic}$). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 0.90–0.99 (m, 6H, $2CH_3$), 1.36–1.47 (m, 6H, $3CH_2$), 1.64–1.70 (m, 2H, CH_2), 1.80–1.86 (m, 2H, CH_2), 3.01 (t, $J = 8.0$ Hz, 2H, CH_2), 4.3 (t, $J = 8.0$ Hz, 2H, NCH_2), 7.42 (t, $J = 8.0$ Hz, 1H, H-8), 7.69 (d, $J = 8.0$ Hz, 1H, H-6), 7.79 (t, $J = 8.0$ Hz, 1H, H-7), 8.13 (d, $J = 8.0$ Hz, 1H, H-9).^{13}C NMR (100 MHz, DMSO-d_6,) δ_C: 14.0, 14.1, 20.0, 22.1, 26.1, 27.9, 29.7, 30.9, 41.9, 101.0, 113.3, 116.0, 123.4, 124.1, 124.7, 133.9, 138.9, 154.8, 156.1, 156.3, 156.8, 167.7. Mass spectrum, *m/z* (I_r %): 381 [M^++1; 28.2], 380 [M^+; 76.8], 379 [M^+-1; 29], 363 (100), 351 (10), 338 (8.4), 324 (16.6), 322 (8.5), 131 (3.4), 104 (1.27), 96 (0.9), 77 (3.9), 55 (1.3), 42 (0.8), 40 (1.8). Analysis calculated for $C_{22}H_{24}N_2O_4$ (380.45): C, 69.46%; H, 6.36%; N, 7.36%. Found: C, 69.66%; H, 6.72%; N, 7.11%.

General procedure for preparation of 2,5-dilkyloxazolopyranoquinolinone derivatives (4j-l)

A mixture of compounds **3a-c** (10 mmol) with (7 ml, 36 mmol) of decanoyl chloride was heated under free-solvent condition. After completion of reaction (as indicated by the disappearance of starting material on TLC) the reaction mixture was cooled so, the solid material obtained was

filtered off then dried and crystallized from a suitable solvent to give compounds **4j-l** as illustrated in the following table.

	Time	m.p. (° C)	Solvent	Color	Yield
4j	13 h	160	EtOH	Brown	(2.5 g, 63.29%)
4k	12 h	153	EtOH	Yellow	(3 g, 73.5%)
4l	9 h	144	EtOH	Golden yellow	(3 g, 68.64%)

5-Methyl-2-nonyl-4*H*-oxazolo[5′,4′:4,5]pyrano[3,2-*c*] quinolone-4,11(5*H*)-dione (4j)

IR (KBr, cm^{-1}): 3086 ($CH_{aromatic}$), 2917, 2850 ($CH_{aliphatic}$), 1777 ($C{=}O_{\alpha\text{-}pyrone}$), 1663 ($C{=}O_{quinolinone}$), 1594 ($C{=}N_{oxazolo}$), 1565 ($C{=}C_{aromatic}$). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 0.87 (t, $J = 8.0$ Hz, 3H, CH_{3nonyl}), 1.28–1.43 (m, 12H, 6 $(CH_2)_{nonyl}$), 1.79–1.88 (m, 2H, CH_2), 3.01 (t, $J = 8.0$ Hz, 2H, CH_2), 3.7 (s, 3H, NCH_3), 7.46 (t, $J = 8.0$ Hz, 1H, H-8), 7.70 (d, $J = 8.0$ Hz, 1H, H-6), 7.82 (t, $J = 8.0$ Hz, 1H, H-7), 8.17 (d, $J = 8.0$ Hz, 1H, H-9). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 14.3, 22.4, 26.4, 27.9, 28.5, 28.7, 29.0, 29.2, 29.7, 31.6, 101.1, 113.1, 116.1, 123.5, 123.8, 124.7, 133.9, 139.7, 154.8, 156.1, 156.4, 156.8, 167.8. Mass spectrum, *m/z* (I_r %): 395 [M^++1; 1.9], 394 [M^+; 12.1], 380 (4.9), 338 (22.14), 337 (14.5), 326 (3.9), 324 [M^+-2CO-CH_3+H; 100], 323 (16), 309 (4), 295 (10.3), 282 (8.8), 257 (9.8), 225 (5.9), 212 (7.3), 202 (2.9), 181 (1.5), 84 (1.6), 69 (2.5), 55 (2.1), 44 (3.7), 43 (3.9), 41 (3.7). Analysis calculated for $C_{23}H_{26}N_2O_4$ (394.47): C, 70.03%; H, 6.64%; N, 7.10%. Found: C, 70.32%; H, 6.42%; N, 7.05%.

5-Ethyl-2-nonyl-4*H*-oxazolo[5′,4′:4,5]pyrano[3,2-*c*] quinolone-4,11(5*H*)-dione (4k)

IR (KBr, cm^{-1}): 3082 ($CH_{aromatic}$), 2918, 2852 ($CH_{aliphatic}$), 1775 ($C{=}O_{\alpha\text{-}pyrone}$), 1661 ($C{=}O_{quinolinone}$), 1595 ($C{=}N_{oxazolo}$), 1569 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 0.84 (t, $J = 8.0$ Hz, 3H, CH_{3nonyl}), 1.20 (t, $J = 4.0$ Hz, 3H, CH_2CH_3), 1.30–1.45 (m, 12H, 6$(CH_2)_{nonyl}$), 1.80–1.89 (m, 2H, CH_2), 3.05 (t, $J = 8.0$ Hz, 2H, CH_2), 4.5 (q, $J = 8.0$ Hz, 2H, NCH_2), 7.42 (t, $J = 8.0$ Hz, 1H, H-8), 7.69 (d, $J = 8.0$ Hz, 1H, H-6), 7.86 (t, $J = 8.0$ Hz, 1H, H-7), 8.12 (d, $J = 8.0$ Hz, 1H, H-9). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 13.2, 14.3, 22.4, 26.4, 27.9, 28.5, 28.7, 29.2, 29.7, 31.6, 39.3, 101.1, 113.1, 116.1, 123.5, 123.8, 124.7, 126.1, 133.9, 139.7, 154.8, 155.1, 156.4, 167.8. Analysis calculated for $C_{24}H_{28}N_2O_4$ (408.50): C, 70.57%; H, 6.91%; N, 6.86%. Found: C, 70.43%; H, 6.52%; N, 6.41%.

5-Butyl-2-nonyl-4*H*-oxazolo[5′,4′:4,5]pyrano[3,2-*c*] quinolone-4,11(5*H*)-dione (4l)

IR (KBr, cm^{-1}): 3081 ($CH_{aromatic}$), 2920, 2856 ($CH_{aliphatic}$), 1767 ($C{=}O_{\alpha\text{-}pyrone}$), 1662 ($C{=}O_{quinolinone}$), 1598 ($C{=}N_{oxazolo}$), 1567 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 0.87–0.96 (m, 6H, $2CH_3$), 1.28–1.43 (m, 8H, $4CH_2$), 1.67–1.99 (m, 10H, $5CH_2$), 3.00 (t, $J = 8.0$ Hz, 2H, CH_2), 4.34 (t, $J = 8.0$ Hz, 2H, NCH_2), 7.44 (t, $J = 8.0$ Hz, 1H, H-8), 7.71 (d, $J = 8.0$ Hz, 1H, H-6), 7.80 (t, $J = 8.0$ Hz, 1H, H-7), 8.16 (d, $J = 8.0$ Hz, 1H, H-9). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 14.08, 14.2, 20.00, 22.4, 26.4, 27.9, 28.7, 29.03, 29.2 (3C), 29.7, 31.6, 113.3, 116.06, 116.1, 123.4, 124.1, 125.1, 125.9, 133.9, 138.9, 139.2, 156.3, 157.1, 167.8. Mass spectrum, *m/z* (I_r %): 437 [M^++1; 12.2], 436 [M^+; 40.6], 435 [M^+-1; 14.3], 419 (100), 380 (15.9), 366 (15.3). Analysis calculated for $C_{26}H_{32}N_2O_4$ (436.56): C, 71.53%; H, 7.39%; N, 6.42%. Found: C, 71.43%; H, 7.42%; N, 6.45%.

Biological evaluation

Reagents and chemicals

Dimethyl sulfoxide (DMSO), crystal violet stain (1%) (it composed of 0.5% (w/v) crystal violet and 50% methanol then made up to volume with dd H_2O and filtered through a Whatman No. 1 filter paper), and trypan blue dye were purchased from Sigma (St. Louis, MO, USA). Furthermore, fetal bovine serum, Dulbecco's modified Eagle's medium (DMEM), RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin, and 0.25% Trypsin-EDTA were obtained from Lonza.

Cell lines and cell culture

HepG-2 cells (human Hepatocellular carcinoma), **HCT-116** (colon carcinoma), and **MCF-7** (breast carcinoma) were obtained from VACSERA Tissue Culture Unit. All cancer cell lines were maintained at 37 °C in a humidified atmosphere with 5% CO_2 in DMEM supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer, and 50 µg/ml gentamycin and were subcultured two times a week.

Cytotoxicity evaluation using viability assay

The cells were seeded in 96-well plate at a cell concentration of 1×10^4 cells per well in 100 µl of growth medium. After 24 h of seeding freshly medium including various

concentrations of the test samples was added. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers operated into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) utilizing a multichannel pipette. The microtiter plates were incubated at 37 °C in a humidified incubator with 5% CO_2 for the extent of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for at 37 °C, various concentrations of the sample were added, and the incubation was continued for 24 h and viable cells yield was determined by a colorimetric method (Mosmann 1983; Gomha et al. 2015). In brief, after the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 min. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) was then added to all wells and mixed perfectly, and then the absorbance of the plates was measured after shaking well on Microplate reader (TECAN, Inc.), using a test wavelength of 490 nm. All results were rectified for background absorbance recognized in wells without the addition of stain. Treated samples were correlated with the cell control in the absence of the tested compounds. All experiments were performed in triplicate. The cell cytotoxic effect of each tested compound was calculated. The optical density was measured with the microplate reader (SunRise, TECAN, Inc., USA) to determine the number of viable cells and the percentage of viability was calculated as [(ODt/ODc)] × 100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The relationship between surviving cells and drug concentration is graphed to obtain the survival curve of each tumor cell line after treating with the characteristic compound. The concentration necessary to induce toxic effects in 50% (IC_{50}), of intact cells, was determined from graph of the dose response curve for each concentration using GraphPad Prism software (San Diego, CA, USA).

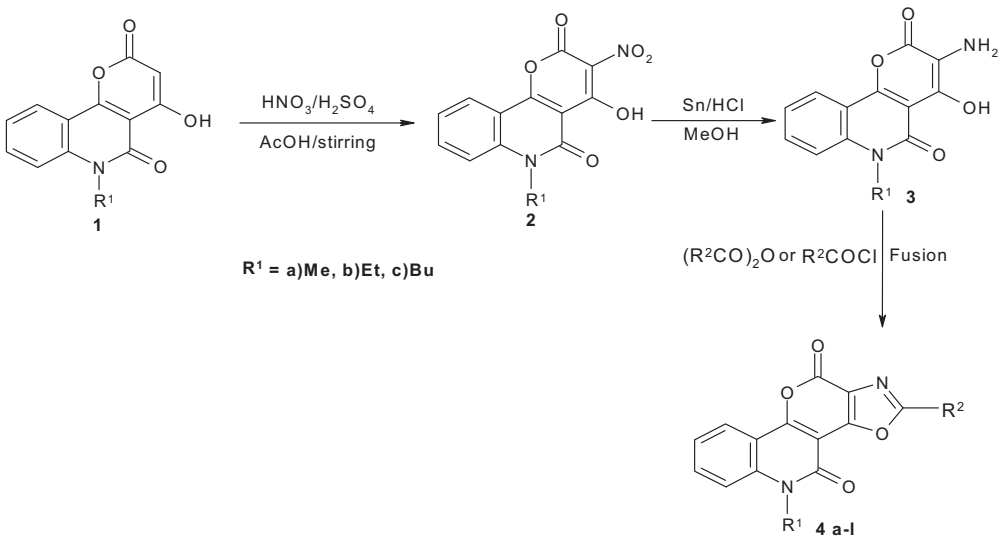
Results and discussion

Chemistry

The nitration of compounds **1a-c** using a mixture of concentrated nitric acid and sulfuric acid produced 3-nitropyrano[3,2-*c*]quinolinediones **2a-c**. The nitro-derivatives **2a-c** were chemoselectivity reduced with tin and concentrated hydrochloric acid at 120 °C to obtain 3-aminopyrano[3,2-*c*] quinolinediones **3a-c** (Scheme 1). The formation of the novel amino derivatives **3a,b** was confirmed by their IR spectra, showing two new bands as a double peak due to the NH_2 group. ^{1}H NMR spectra displayed proof that (NH_2) protons existed as a broad singlet in the upfield at δ 3.77 and 3.74, respectively, which are exchangeable with D_2O. The mass spectra of compounds **3a,b** revealed a molecular ion peak at $m/z = 258$ and 272, respectively, which is coincident with the formula weight 258.23 and 272.26, respectively. The reaction of amino derivatives **3a-c** with some anhydrides and acid chlorides, under free-solvent condition, was carried out to get the desired 2,5-dialkyloxazolopyrano[3,2-*c*]quinoline-4,5-dione derivatives **4a-l**. The IR and ^{1}H NMR spectra of compounds **4a-l** revealed the absence of NH_2 and OH groups, which were observed in the spectra of amino derivatives **3a-c**. Each ^{1}H NMR spectrum of the synthesized compounds exhibited new signals in the aliphatic region from 0.9 to 4.40 ppm, referred to characteristic chemical shifts of alkyl group protons at position 2 of oxazole ring. ^{13}C-NMR spectra demonstrated the presence of new *sp3*-hybridized carbons in the region 13–37 ppm due to the presence of alkyl group. In order to show this feature, we have chosen derivatives **4a**, **4d**, **4g**, and **4j** in addition to **4b**, **4e**, **4h**, and **4k**, the NMR numbering scheme for these synthesized compounds is shown in Fig. 1. Most of the mass spectra of the compounds **4a-l** represented the molecular ion peak as the base beak, which agree well with the proposed formula weight and confirm the suggested structures.

Anticancer activity

The in vitro antitumor activity of compounds **4a-l** were tested towards three human cancer cell lines, HepG-2 cells (human Hepatocellular carcinoma), HCT-116 (colon carcinoma), and MCF-7 (breast carcinoma) cell lines, using 5-fluorouracil as a standard control drug. The cells were treated with these newly synthesized compounds at varying concentrations, and cell viability was determined. The results displayed that, increasing the portion of applied compounds diminished the cell growth in three types of cancer cell lines (HepG-2, HCT-116, and MCF-7). The relation between the surviving cells and the concentration of tested compounds was plotted to get the survival curve for each type of cancer cell line after 24 h as presented in Figs. 2–4. The anti-proliferative effects of compounds **4a-l** were estimated by their potency to inhibit growth by 50% compared to control cells IC_{50} value. The screening results are outlined in Table 1. Building on the outcomes in Table 1, the IC_{50} values displayed that almost all compounds marked notable cytotoxic potential against whole cancer cell lines. Clearly, compound **4c** has higher inhibitory activity against all three tumor cell lines correlated to other tested compounds. This compound advertised the minimum IC_{50}



4	R^1	R^2
a	Me	$-CH_3$
b	Et	$-CH_3$
c	Bu	$-CH_3$
d	Me	$-CH_2CH_3$
e	Et	$-CH_2CH_3$
f	Bu	$-CH_2CH_3$
g	Me	$-CH_2(CH_2)_3CH_3$
h	Et	$-CH_2(CH_2)_3CH_3$
i	Bu	$-CH_2(CH_2)_3CH_3$
j	Me	$-CH_2(CH_2)_7CH_3$
k	Et	$-CH_2(CH_2)_7CH_3$
l	Bu	$-CH_2(CH_2)_7CH_3$

Scheme 1 Synthesis of 2,5-dialkyloxazolopyrano[3,2-*c*]quinolinone derivatives 4a-l

values in the range of 16.2–28.3 µg/ml against all examined cancer cell lines while compound **4j** displayed the lowest activity against three tumor cell lines. The results are depicted graphically in Fig. 5.

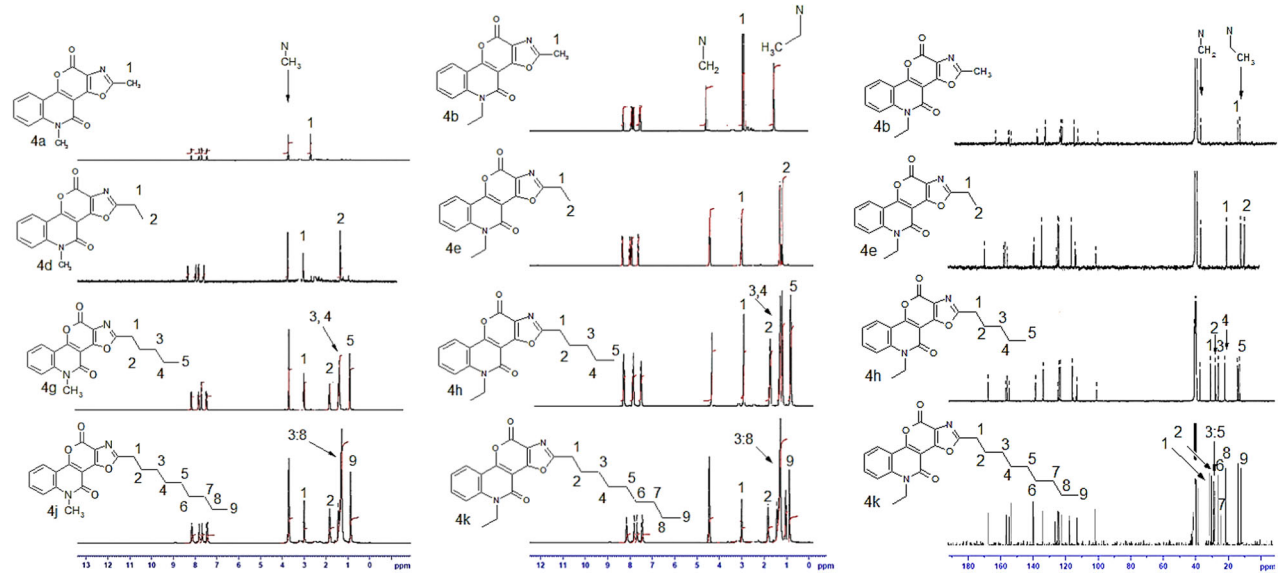


Fig. 1 The NMR numbering scheme for some synthesized compounds

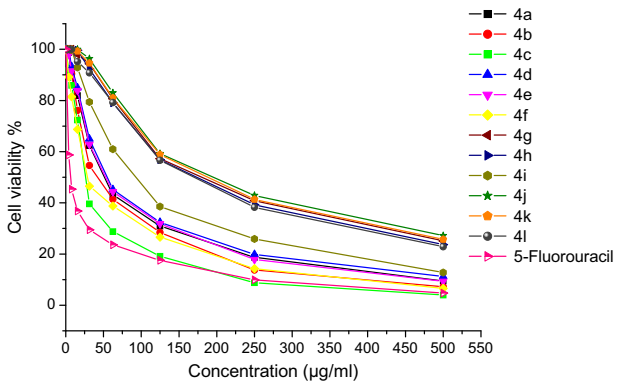


Fig. 2 Effect of concentration (0–500 µg/ml) of compounds **4a-l**, and 5-fluorouracil on viability of human liver cancer (HepG-2) cell line

Table 1 Cytotoxicity (IC_{50}) of compounds **4a-l** against different cancer cell lines

Compounds	HepG-2	HCT-116	MCF-7
4a	51.4	60.2	96.5
4b	42.2	30.3	53.6
4c	16.2	22.2	28.3
4d	63.2	75	104
4e	43.5	44.6	75
4f	28.7	30.1	43.2
4g	180	124	115
4h	174	121	82.1
4i	93	59.3	62.2
4j	191	134	118
4k	180	125	91.1
4l	130	95.5	79.2
5-Fluorouracil	6.44	21.5	28

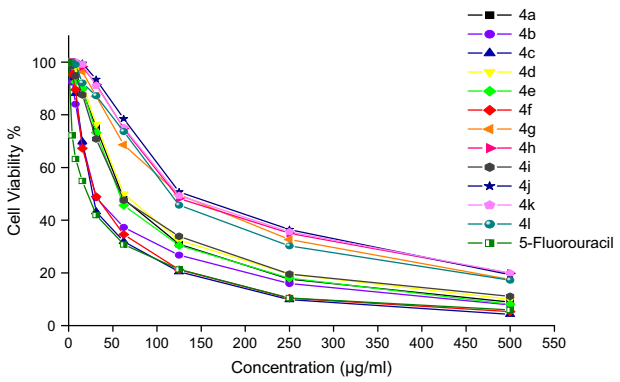


Fig. 3 Effect of concentration (0–500 µg/ml) of compounds **4a-l**, and 5-fluorouracil on viability of human colon cancer (HCT-116) cell line

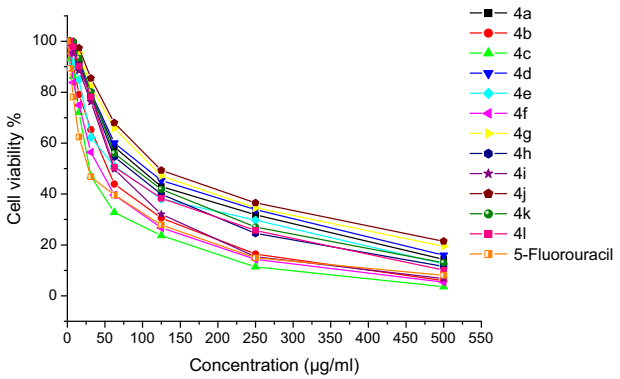


Fig. 4 Effect of concentration (0–500 µg/ml) of compounds **4a-l**, and 5-fluorouracil on viability of human breast cancer (MCF-7) cell line

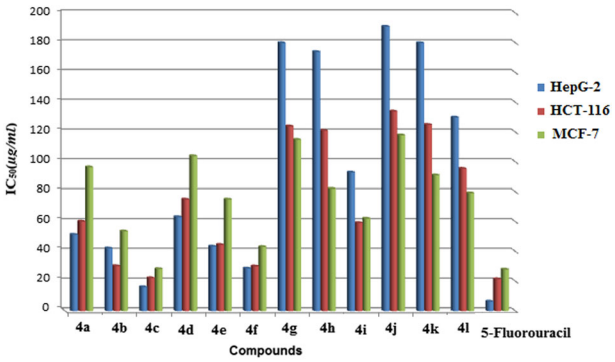


Fig. 5 Comparative IC_{50} values of compounds **4a-l** and 5-fluorouracil against HepG-2, HCT-116, and MCF-7 cell lines

Liver cancer cell line HepG-2

Evaluation of viability % of liver cancer cell line HepG-2 post-treatment with compounds **4a-l** for 24 h compared with reference drug, 5-fluorouracil, using colorimetric assay is shown in Fig. 2. The results pointed out that compound **4c** was more effective than reference drug at concentrations (250, 500 µg/ml). Compounds **4a**, **4b**, **4d**, **4e**, and **4f** exhibited high growth inhibitory activity against HepG-2 cell line, where the % inhibition of the cells viability was close to reference drug at concentrations (250 and 500 µg/ml).

Colon cancer cell line HCT-116

Figure 3 clarifies the assessment of viability % of colon cancer cell line (HCT-116) post-treatment with tested compounds for 24 h compared with 5-fluorouracil using

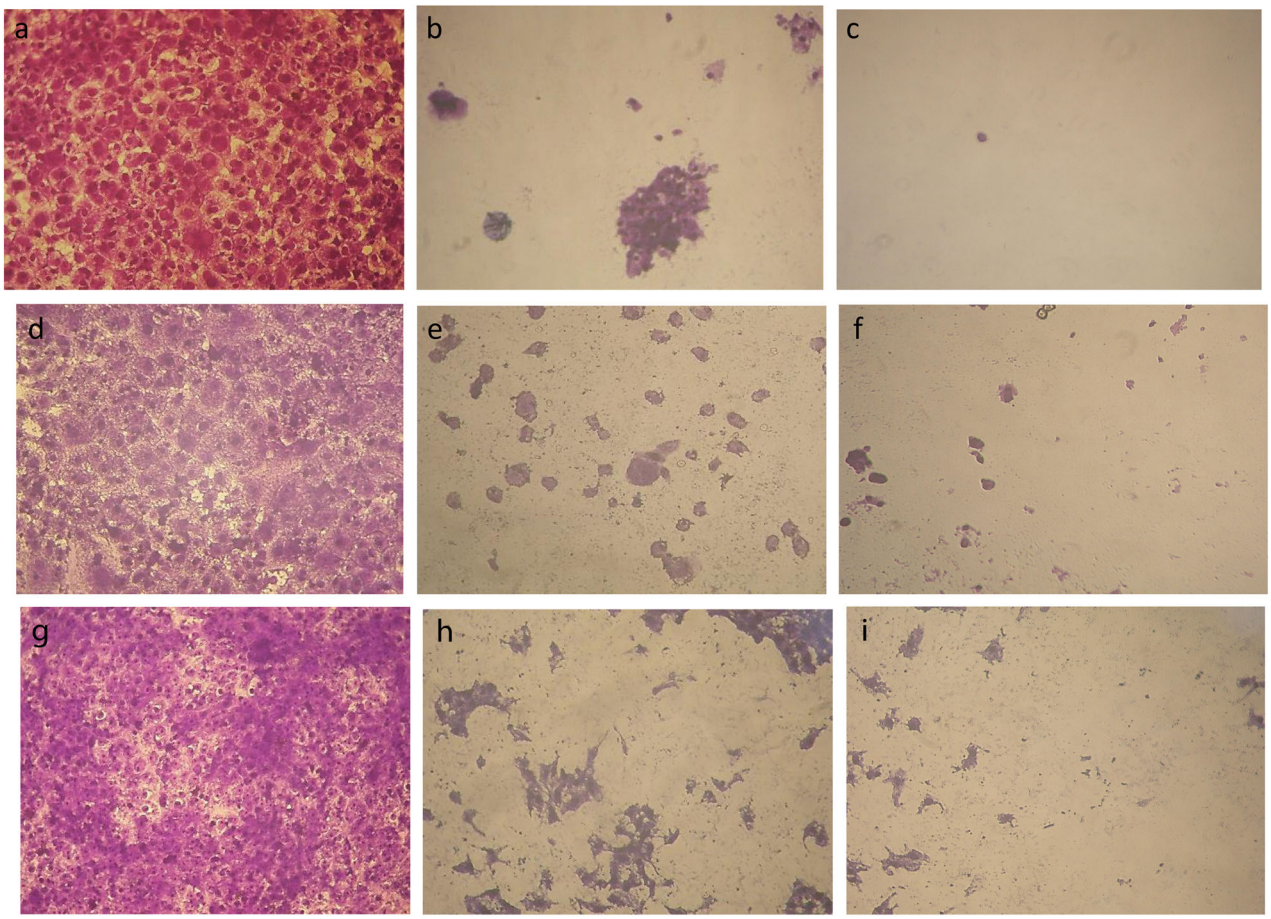


Fig. 6 Morphological changes in HepG-2 cells incubated for 24 h: **a** negative control (untreated), **b** with 250 µg/ml concentration of compound **4c**, **c** with 500 µg/ml concentration of compound **4c**. Morphological changes in HCT-116 cells incubated for 24 h: **d** negative control, **e** with 250 µg/ml concentration of compound **4c**, **f** with 500 µg/ml concentration of compound **4c**. Morphological changes in MCF-7 cells incubated for 24 h: **g** negative control, **h** with 250 µg/ml concentration of compound **4c**, **i** with 500 µg/ml concentration of compound **4c**. The morphological alterations were microscopically detected at 100× magnification using an inverted microscope (CKX41; Olympus, Japan)

colorimetric assay. All the tested compounds showed excellent activity comparable to chemotherapeutic agent, 5-fluorouracil against HCT-116 cells. In particular, the two compounds **4c** and **4f** can be deliberated the most auspicious compounds as they performed a remarkable result more than standard drug at concentrations (125, 250, and 500 µg/ml).

Breast cancer cell line MCF-7

Evaluation of viability % of breast cancer cell line (MCF-7) post-treatment with compounds **4a-l** for 24 h related to standard control drug, 5-fluorouracil, using colorimetric assay is represented graphically in Fig. 4. Nearly all the checked compounds exhibited notable inhibitory activity for MCF-7 cells. Particularly, compound **4c** was authorized to be the most powerful versus the standard control at concentrations range between (31.25–500 µg/ml). In addition, the % inhibition of the cells viability of compounds **4b**, **4f**, and **4i** was similar to reference drug at the majority of concentrations and they afforded higher antitumor activity than 5-fluorouracil at concentration 500 µg/ml. While compounds **4g** and **4j** were found to be least active in the series.

Attending to the influence of the alkyl chain length in inhibiting cancer cell growth, we studied the effect of *N*-alkyl group ($\mathbf{R^1}$) on the cytotoxic activity of the compounds. Replacement of methyl **4a** with ethyl **4b** and butyl **4c** caused an enhancement in the inhibitory activity against HepG-2, MCF-7, and HCT-116 tumor cell lines. Compound **4c** with *N*-butyl group is more active than compound **4b** with *N*-ethyl group which is more active than compound **4a** with *N*-methyl group. The longer alkyl group substitution on N position of quinolinone increases the lipophilicity and hydrophobicity and possesses strong hydrophobic interactions with nonpolar active residues. Compound **4c** exhibited high potent activity against HepG-2, MCF-7, and HCT-116 cell lines since the highest lipophilicity butyl group is likely to enhance the cell membrane penetration ability (Rekulapally et al. 2015). An identical case was also noticed in the other prior studies and attributed that the inhibitory activities against tumor cells strengthened with the increase the length of *N*-alkyl chain, probably due to the augmentation of lipophilicity which strengthened cell membrane penetration ability of the tested compounds (Liu et al. 2015; Miri et al. 2016; Csuk et al. 2010; Takahashi et al. 2006). Interestingly, an opposite situation than previous one was observed, as the length of the alkyl chain substituents at C-2 of oxazole ring ($\mathbf{R^2}$) increases, the antitumor activity decreases against HepG-2, MCF-7, and HCT-116 cell lines. It has been remarked that compound **4j** with nonyl chain disclosed relatively lower cytotoxic effect than compound **4g** with pentyl chain against HepG-2, MCF-7, and HCT-116 tumor cell lines. Moreover, compound **4g** with pentyl chain was significantly less active than compound **4d** with ethyl chain and compound **4a** with methyl chain towards all tested cell lines. The same aspect was also observed when compound **4k** with nonyl chain was compared with compounds **4h**, **4e**, and **4b** with pentyl, ethyl, and methyl chains at position 2, respectively. A similar manner was demonstrated in a literature research and proved that the antiproliferative activity decreases with the increase of alkyl chain length (Sharma et al. 2008; Reddy et al. 2011).

Morphological changes in cells were attended on treatment with compounds and microscopic images after using the most forceful compound **4c** are delineated in Fig. 6.

Conclusion

In this study, 2,5-dialkyloxazolopyrano[3,2-*c*]quinolinone derivatives were synthesized and characterized successfully. All the synthesized compounds were tested as antitumor agents against three cancer cell lines: liver (HepG-2), colon (HCT-116), and breast (MCF-7). The results indicated that most of the compounds displayed significant activity against all cancer cell lines. Among them, compounds **4c** and **4f** were the most potent cytotoxic agents all tumor cell lines compared to reference drug 5-fluorouracil. These two compounds exhibited the lowest IC_{50} values in the range of 16.2–59.1 µg/ml against all investigated cancer cell lines while compound **4j** displayed the lowest activity against three tumor cell lines. Insertion of long *N*-alkyl group ($\mathbf{R^1}$) improved the anticancer activity, replacement of methyl **4a** with ethyl **4b** and butyl **4c** led to enhance the inhibitory activity against four tumor cell lines. On the contrary, a long alkyl moiety at position-2 of oxazole ring ($\mathbf{R^2}$) decreased the antitumor activity towards four cell lines. The SAR studies show that the investigation of different derivatives of 2,5-dilkyloxazolopyrano[3,2-*c*]quinolinone may lead to the discovery of novel more efficient anticancer agents.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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