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Synthesis of 1-[(Aryl)(3-amino-5-oxopyrazolidin-4-ylidene) methyl]-2-oxo-1,2-dihydroquinoline-3-carboxylic Acid Derivatives and Their Breast Anticancer Activity

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Abstract: This research aimed to produce new 1-[(aryl)(3-amino-5-oxopyrazolidin-4-ylidene) methyl]-2-oxo-1,2-dihydroquinoline-3-carboxylic acid derivatives and check their anticancer effect against the breast cancer MCF-7 cell line. The 2-oxo-1,2-dihydroquinoline-3-carboxylic acid (**4**) compound was obtained by hydrolyzing ethyl 2-oxo-1,2-dihydroquinoline-3-carboxylate (**2**) with thiourea and anhydrous potassium carbonate ethanol, which was then treated with ethyl 3-substituted 2-cyanoacrylates (**6**) in the presence of triethylamine in diethyl formamide to give 1-[2-(ethoxy)carbonyl-2-cyano-1-arylvinyl]-2-oxo-1,2-dihydroquinoline-3-carboxylic (**7a**,**d**). Cyclization of compound 7 with hydrazine hydrate ethanol inferred the association of 1-[(aryl)(3 amino-5-oxopyrazolidin-4-ylidene)methyl-2-oxo-1,2-dihydroquinol-3-carboxylates (**8a**,**d**). Spectroscopic and micro-analytical techniques such as IR, NMR, and elemental analysis were used to validate the structure of the synthesized organic compounds. The anticancer effects of the synthesized compounds **7a–d** and **8a–d** were tested by using the MTT assay on the MCF-7 cell line. When compared to the reference compound Dox, the compounds **7b**,**c** and **8a–c** demonstrated strong anticancer activity against the MCF-7 cell line. The anticancer effects of the synthesized compounds **7a–d** and **8a–d** were tested against the MCF-7 cell line, using MTT assay. The compounds **7b**,**c** and **8a–c** showed significant anticancer activity compared to the reference compound Dox against the MCF-7 cell line.

Keywords: anticancer drugs; cell-cycle arrest; ^{1}H-NMR; quinolinone derivatives

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1. Introduction

Quinoline, a heterocyclic nitrogen compound, has been list by the Food and Drug Administration as a chemotherapy compound [1]. Quinolines are a common pharmacological scaffold that can be found in a wide range of synthetic and natural bioactive compounds [2]. The chemistry of quinolines has been extensively studied over the last century, with various and fascinating biological activities, such as antibacterial, antifungal, anti-inflammatory, anti-malaria, and anticancer properties [3–8]. Quinoline derivatives are effective against cancer cells in the breast, prostate, gastrointestinal, colon, and liver [9–12]. Similar compounds, such as camptothecin and its analogs (irinotecan and topotecan) [13,14], as well as bosutinib [15–17], have been used in clinical trials.

The anticancer hybrid drug approach is a cutting-edge synthetic strategy that involves either combining or blending the hepatotoxic moieties of several drugs into a

novel molecular structure [18,19]. Better anticancer agents resulted from combining these active pharmacophores in a new molecular architecture [18–22]. Pyrazoloquinolines correspondingly are used for many applications, including antiviral activity against the herpes simplex virus [23], caspase activators, anticancer activity [24,25], and apoptosis inducers [25]. Several quinoline–pyrazole hybrids with potent anti-proliferative activity against human liver, breast, and colon cancer cell lines were developed by Pirol et al. [26]. As a result, finding new medications to treat diseases and infections without causing serious adverse effects in patients is critical.

Consequently, quinoline derivatives are among the most effective molecules, with several studies stating that they have a broad variety of biological activities while being safe for patients [27,28]. Therefore, using a taster of multiple functional groups on the quinoline scaffold to create a new anticancer drug is a good idea. As a result of these findings, it was deemed valuable to synthesize some new 2-oxo-1,2-dihydroquinoline-3-carboxylic acids with pyrazolinone moieties at the *N*-position to obtain a safe and effective new anticancer drug compound.

2. Materials and Methods

2.1. Chemistry

Melting points (m.p.) of the synthesized compounds were measured in an electrothermal digital melting-point device. IR and NMR spectra were verified on a Shimadzu 470 c as KBr pellets and Bruker 400 DRX-Avance spectrometer in DMSO-d_6 as a solvent, respectively. The elemental analysis was achieved on a Perkin-Elmer 2400 series II CHN elemental analyzer. Chemicals that were used in synthesis were purchased from Aldrich, Merck, or Fluka Chemical Companies.

2.1.1. Synthesis of the Starting Materials (**2**)

Ethyl 2-oxo-1,2-dihydroquinoline-3-carboxylate (**2**) was obtained by fusion of the mixture 2-aminobenzaldehyde (**1**) (0.01 mol) and diethyl malonate (0.01 mol) in the presence of piperidine (2 mL) on a hot plate, for 2–3 min; ethanol (30 mL) was added to the reaction mixture and heated under reflux for 2 h. The reaction mixture was cooled, poured into water, and neutralized with dilute hydrochloric acid (2%). The resulting product was filtered off, washed with water, dried, and, finally, the product was crystallized from ethanol to give **2** as colorless crystals, yield 76%, m.p. 155 °C. IR (KBr) υ_{max} =1738, 1686 (C=O), 1603, 1586 (C=C), 1118, 1063 (C–O) cm^{-1}.

^{1}H-NMR (DMSO-d_6) δ: 1.32 (t, J = 6.32, 3H, CH_3), 4.22 (q, J = 6.32, 2H, OCH_3), 7.21–7.82 (m, 4H, Ar-H), 8.86 (s, 1H, H-4 of quinoline ring) ppm.

Anal. Calcd. For $C_{12}H_{11}NO_3$ (217): C, 66.35; H, 5.10; N,6.45. Found: C, 66.16; H, 4.83; N, 6.24.

2.1.2. Synthesis of 2-Oxo-1,2-dihydroquinoline-3-carboxylic acid (**4**)

Ester **2** (0.01 mol) was added to a solution of thiourea (0.02 mol) and anhydrous potassium carbonate (0.03 mol) in 50 mL ethanol. The reaction mixture was heated under reflux for 4 h, then cooled, poured into water, and neutralized with a few drops from acetic acid. The solid formed was filtered off, washed with water, dried, and crystallized from dimethylformamide to give **4** as colorless crystals, yield 63%, m.p. 270 °C. IR (KBr) υ_{max} = 3368 (NH), 3481–3175 (br–OH), 1716, 1685 (C=O), 1605, 1563 (C=C), 1161, 1009 (C–O) cm^{-1}.

^{1}H-NMR (DMSO-d_6) δ: 7.42–7.98 (m, 5H, Ar-H and NH), 8.09 (br. s, 1H, OH), 8.87 (s, 1H, H-4 of quinoline ring).

^{13}C-NMR (DMSO-d_6) δ: 163.00, 160.80 (C=O), 154.51 (C–N), 148.28, 134.58, 130.74, 125.56, 119.78, 118.93, 116.60 (carbons of quinoline ring) ppm.

Anal. Calcd. For $C_{10}H_7NO_3$ (189): C, 63.44; H, 3.73; N, 7.40. Found: C, 63.26; H, 3.55; N, 7.17.

2.1.3. General Procedures for the Preparation of Ethyl 3-aryl-2-cyanoccrylamates (**6a**–**d**)

A mixture of ethyl cyanoacetates (0.01 mol), appropriate aromatic aldehydes (namely, benzaldehyde, 4-*N*,*N*-(dimethyl)amino benzaldehyde, 0.01 mol), and triethyl amine (0.03 mol) in ethanol (50 mL) was refluxed for 2 h. After cooling, the solution was poured into water and neutralized with dilute acetic acid (2%). The solid formed was washed with water, dried, and recrystallized from a suitable solvent to give the compounds **6a**–**d**.

Ethyl 3-phenyl-2-cyanoacrylate (**6a**) as pale yellow crystals, yield 65%, m.p. 52°C. IR (KBr) υ_{max} = 2232 (CN), 1742 (C=O), 1605, 1582 (C=C), 1038 (C–O) cm^{-1}.

^{1}H-NMR (DMSO-d_6) δ:1.3 (t, J = 8.01, 3H, CH_3), 7.42–7.75 (m, 5H, Ar-H), 8.32 (s, 1H, H-olefinic) ppm.

Ethyl 3-(4-methoxy) phenyl-2-cyanoacrylate (**6b**) as yellow crystals, yield 71%, m.p. 71 °C. IR (KBr) υ_{max} = 2238 (CN), 1738 (C=O), 1607, 1586 (C=C), 1078, 1032 (C–O) cm^{-1}.

^{1}H-NMR (DMSO-d_6) δ:1.31 (t, J = 8.01, 3H, CH_3), 3.88 (s, 3H, OCH_3), 4.31 (q, J = 8.01,2H, OCH_2), 7.15 (d, J = 10.30, 2H, Ar-H), 8.09 (d, 2H, J = 10.30, 2H, Ar-H), 8.30 (s, 1H, H-olefinic) ppm.

Ethyl 3-(4-hydroxy-3-methoxy) phenyl-2-cyanoacrylate (**6c**) as yellow crystals, yield 73%, m.p. 115 °C. IR (KBr) υ_{max} = 3300 (br. s, OH), 2232 (CN), 1731 (C=O), 1605, 1587 (C=C), 1174, 1092, 1019 (C–O) cm^{-1}.

^{1}H-NMR (DMSO-d_6) δ:1.30 (t, J = 8.01, 3H, CH_3), 3.83 (s, 3H, OCH_3), 4.30 (q, 2H, J = 8.01,2H, OCH_2), 6.97 (d, J = 10.3, 1H, Ar-H), 7.64 (d, J = 10.30, 1H, Ar-H), 7.78 (s, 1H, Ar-H), 8.25 (s, 1H, H-olefinic), 10.57 (s, 1H, OH) ppm.

Ethyl 3-(4-*N*,*N*-dimethyl amino)phenyl-2-cyanoacrylate (**6d**) as orange crystals, yield 76%, m.p. 126 °C. IR (KBr) υ_{max} = 2225 (CN), 1736 (C=O), 1607, 1583 (C=C), 1067 (C–O) cm^{-1}.

^{1}H-NMR (DMSO-d_6) δ:1.29 (t, J = 8.01, 3H, CH_3), 3.10 (s, 6H, $NCCH_3$), 4.27 (q, J = 8.01, 2H, OCH_2), 6.85 (d, J = 10.32, 2H, Ar-H), 7.97 (d, J = 10.32, 2H, Ar-H), 8.13 (s, 1H, H-olefinic) ppm.

2.1.4. General Procedures for Synthesis of 1-[2-Cyano-2-(ethoxy) carbonyl-1-arylvinyl]-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (**7a**–**d**)

Ethyl 3-aryl-2-cyanoacrylates (**6a**–**d**), 0.01 mol) was dissolved in the mixture of 30 mL dimethylformamide and 3 mL triethylamine around the bottom flask, then added with 2-oxo-1,2-dihydroquinoline-3-carboxylic acid (**4**) (0.01 mol). The reaction mixture was heated under reflux for 4 h. It was then cooled, poured into water, and neutralized with dilute hydrochloric acid (2%). The solid formed was separated by filtration and purified by recrystallization, using ethanol solvent, to give compounds **7a**–**d**.

1-[2-(ethoxy)carbonyl-2-cyano-1-phenylvinyl]-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (**7a**) as pale yellow crystals, yield 71%, m.p. 226 °C. IR (KBr) υ_{max} = 3468 (br. OH), 2225 (CN), 1735–1715 (C=O), 1607, 1583 (C=C), 1121, 1080 (C–O) cm^{-1}.

^{1}H-NMR (DMSO-d_6) δ:1.32 (t, J = 8.01, 3H, CH_3), 4.33 (q, J = 8.01, 2H, OCH_2), 7.43–8.10 (m, 9H, Ar-H), 8.42 (s, 1H, OH), 8.89 (s, 1H, H-4 of quinoline ring) ppm.

^{13}C-NMR (DMSO-d_6) δ: 162.99 (C-11), 162.28 (C-21), 160.82 (C-2), 155.61 (C-12), 154.51 (C-9), 148.31 (C-4), 134.58 (C-5), 133.92 (C-16), 131.83 (C-13), 131.29 (C-15, 17), 130.75 (C-10), 129.83 (C-14, 18), 125.56 (C-6), 119.75 (C-7), 118.93 (C-8), 116.61 (C-3), 116.11 (C-19), 103.07 (C-20), 62.88 (C-23), 14.47 (C-24) ppm.

Anal. Calcd. For $C_{22}H_{16}N_2O_5$ (388): C, 68.04; H, 4.15; N, 7.21. Found: C, 67.87; H, 4.02; N, 7.07.

1-[2-(ethoxy)carbonyl-2-cyano-1-(4-methoxy)phenylvinyl]-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (**7b**) as pale yellow crystals, yield 73%, m.p. 245 °C. IR (KBr) υ_{max} = 3485 (br. OH), 2224 (CN), 1736–1715 (C=O), 1607, 1583 (C=C), 1125, 1086, 1017 (C–O) cm^{-1}.

^{1}H-NMR (DMSO-d_6) δ:1.30 (t, J = 8.01, 3H, CH_3), 3.89 (s, 3H, OCH_3), 4.30 (q, J = 8.01, 2H, OCH_2), 7.04–8.10 (m, 8H, Ar-H), 8.31 (br. s, 1H, OH), 8.90 (s, 1H, H-4 of quinoline ring) ppm.

^{13}C-NMR (DMSO-d_6) δ:164.03 (C-11), 163.90 (C-21), 162.84 (C-2), 162.71 (C-16), 154.92 (C-12), 154.74 (C-9), 148.28, 148.09 (C-4), 134.55, 134.43 (C-5), 133.80 (C-14, 18), 130.72, 130.53 (C-10), 128.77, 128.65 (C-6), 125.53, 125.35 (C-7), 124.42, 124.30 (C-13), 119.53 (C-8), 118.73 (C-3), 116.58 (C-19), 115.44, 115.31 (C-15, 17), 99.02, 98.83 (C-20), 62.54, 62.36 (C-230, 56.23, 56.11(OCH_3), 14.49, 14.30 (C-24) ppm.

Anal. Calcd. For $C_{23}H_{18}N_2O_6$ (418): C, 66.03; H, 4.34; N, 6.70. Found: C, 65.75; H, 4.11; N, 6.49.

1-[2-(ethoxy)carbonyl-2-cyano-1-(4-hydroxy-3-methoxy)phenylvinyl]-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (**7c**) as yellow crystals, yield 69%, m.p. 233 °C. IR (KBr) υ_{max} = 3865–2980 (br. OH), 2227 (CN), 1736–1714 (C=O), 1608, 1540 (C=C), 1135, 1187, 1081 (C–O) cm^{-1}.

^{1}H-NMR (DMSO-d_6) δ:1.30 (t, J = 8.01, 3H, CH_3), 3.83 (s, 3H, OCH_3), 4.29 (q, J = 8.01, 2H, OCH_2), 6.95–8.10 (m, 7H, Ar-H), 8.24 (br. s, 1H, OH), 8.88 (s, 1H, H-4 of quinoline ring), 10.59 (br. s., 1H, OH) ppm.

^{13}C-NMR (DMSO-d_6) δ: 163.11 (C-11), 162.99 (C-21), 160.82 (C-2), 157.17 (C-16), 154.93 (C-17), 153.17 (C-9), 148.30 (C-4), 148.26 (C-12), 134.57 (C-5), 130.74 (C-10), 127.61 (C-14), 125.55 (C-6), 123.30 (C-13), 119.73 (C-7), 118.93 (C-8), 117.13 (C-18), 116.60 (C-3), 116.48 (C-19), 114.55 (C-15), 97.45 (C-20), 62.43 (C-23), 56.03 ($OCH_{3)}$, 14.55 (C-24) ppm.

Anal. Calcd. For $C_{23}H_{18}N_2O_7$ (434): C, 63.59; H, 4.18; N, 6.45. Found: C, 63.33; H, 3.98; N, 6.27.

1-[2-(ethoxy)carbonyl-2-cyano-1-(4-*N*,*N*-dimethylamine)phenylvinyl]-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (**7d**) as orange crystals, yield 72%, m.p. 238 °C. IR (KBr) V_{max} = 3385 (br. OH), 2223 (CN), 1734–1716 (C=O), 1608, 1581 (C=C), 1093, 1023 (C–O) cm^{-1}.

^{1}H-NMR (DMSO-d_6) δ:1.28 (t, J = 8.01, 3H, CH_3), 3.09 (s, 6H, $N(CH_3)_2$), 4.26 (q, J = 8.01, 2H, OCH_2), 6.82–7.99 (m, 8H, Ar-H), 8.10 (s, 1H, OH), 8.88 (s, 1H, H-4 of quinoline ring) ppm.

^{13}C-NMR (DMSO-d_6) δ: 163.96 (C-11), 162.98 (C-21), 160.81 (C-2), 154.65 (C-9), 154.51 (C-12), 154.19 (C-16), 148.30 (C-4), 134.57 (C-5), 134.28 (C-14, 18), 130.74 (C-10), 125.55 (C-6), 119.73 (C-7), 118.75 (C-8), 118.06 (C-1), 116.60 (C-3), 112.18 (C-15, 17), 92.44 (C-20), 61.92 (C-23), 40.59 ($N(OCH_3)_2$, 14.63 (C-24) ppm.

Anal. Calcd. For $C_{24}H_{21}N_3O_5$ (431): C, 66.81; H, 4.91; N, 9.74. Found: C, 66.63; H, 4.58; N, 9.58.

2.1.5. General Procedures for Preparation of 1-[(Aryl)(3-amino-5-oxopyrazolidine-4-ylidene)methyl]-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (**8a**–**d**)

A mixture of compound **7** (0.01 mol) and hydrazine hydrate (0.02 mol) in ethanol (50 mL) was heated under reflux for 2 h, then cooled, poured into ice-water, and neutralized with a few drops of acetic acid. The precipitate formed was filtered, washed with water, and dried. Finally, the product was recrystallized from a suitable solvent to give **8**.

1-((3-Amino-5-oxo-1H-pyrazol-4(5H)-ylidene)(phenyl)methyl)-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (**8a**) as pale yellow crystals, yield 61%, m.p. 255 °C. IR (KBr) V_{max} = 3380–2993 (br. OH), 3228, 3225, 3171 (NH_2, NH), 1725–1690 (C=O), 1625 (C=N), 1605, 1582 (C=C), 1021 (C–O) cm^{-1}.

^{1}H-NMR (DMSO-d_6) δ: 6.97 (s, 2H, NH_2), 7.35–7.91 (m, 9H, Ar-H), 8.74 (s, 1H, H-4 quinoline ring), 11.15 (s, 1H, NH), 11.33 (s, 1H, OH carboxylic acid).

^{13}C-NMR (DMSO-d_6) δ:163.70 (C-23), 163.27 (C-11), 162.89 (C-2), 162.04 (C-12), 159.12 (C-20), 134.26 (C-9), 134.03 (C-4), 133.58 (C-13), 132.11 (C-16), 131.70 (C-5), 131.29 (C-6), 130.79 (C-7), 129.47, (C-15), 128.96, 128.86 (C-14, 18), 120.09 (C-8), 118.67 (C-19), 117.01 (C-3) ppm.

Anal. Calcd. For $C_{20}H_{14}N_4O_4$ (374): C, 64.17; H, 3.74; N, 14.97. Found: C, 64.02; H, 3.55; N, 14.68.

1-((3-Amino-5-oxo-1H-pyrazol-4(5*H*)-ylidene)(4-methoxyphenyl)methyl)-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (**8b**) as pale yellow, yield 68%, m.p. 260 °C. IR (KBr)

υ_{max} = 3401–2970 (br. OH), 3325, 3221, 3183 (NH_2 and NH), 1724–1693 (C=O), 1616 (C=N), 1605, 1563 (C=C), 1117, 1086, 1039 (C–O) cm^{-1}.

^{1}H-NMR (DMSO-d_6) δ: 3.85 (s, 3H, OCH_3) 6.98–7.81 (m, 10H, Ar-H and NH_2), 8.65, 8.75 (s, 1H, H-4 of quinoline ring), 11.14 (s, 1H, NH), 11.42 (S, 1H, OH carboxylic acid) ppm.

^{13}C-NMR (DMSO-d_6) δ: 163.26 (C-23), 163.02 (C-11), 162.54 (C-2), 162.45 (C-12), 159.12 (C-20), 133.72 (C-9), 133.27 (C-4), 131.77 (C-5), 131.30 (C-10), 130.81, 130.46 (C-14, 18), 127.05 (C-6), 126.56 (C-7), 120.09, (C-19), 118.68 (C-8), 117.01, (C-3), 114.99, 114.88 (C-15, 17), 55.918 (C-24) ppm.

Anal. Calcd. For $C_{21}H_{16}N_4O_5$ (404): C, 62.38; H, 3.96; N, 13.86. Found: C, 62.11; H, 3.72; N, 13.63.

1-[(3-Amino-5-oxo-1,5-dihydro-4H-pyrazol-4-ylidene)(4-hydroxy-3-methoxyphenyl) methyl]-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (**8c**) as yellow, yield 63%, m.p. 269 °C. IR (KBr) υ_{max} = 3421–2960 (br. OH), 3361, 3230, 3181 (NH_2 and NH), 1727–1690 (C=O), 1623 (C=N), 1607, 1591 (C=C), 1119, 1098, 1036 (C–O) cm^{-1}.

^{1}H-NMR (DMSO-d_6) δ: 3.85 (s, 3H, OCH_3) 6.97–7.72 (m, 9H, Ar-H and NH_2), 8.75 (s, 1H, H-4 of quinoline ring), 11.16 (s, 1H, NH), 11.36 (s, 1H, OH carboxylic acid) ppm.

^{13}C-NMR (DMSO-d_6) δ:163.28 (C-23), 162.89, (C-11), 161.14 (C-2), 159.12 (C-23), 150.93 (C-20), 148.48 (C-16), 148.43 (C-17), 133.73 (C-9), 133.16 (C-4), 131.82 (C-5), 131.30 (C-10), 125.97 (C-6), 125.34 (C-7), 124.01 (C-8), 120.09 (C-14), 118.73 (C-3), 118.67 (C-19), 117.00 (C-13), 116.02 (C-18), 110.63 (C-15), 55.95 (C-24) ppm.

Anal. Calcd. For $C_{21}H_{16}N_4O_6$ (420): C, 60.00; H, 3.81; N, 13.33. Found: C, 59.83; H, 3.63; N, 13.11.

1-{(3-Amino-5-oxo-1,5-dihydro-4H-pyrazol-4-ylidene)[4-(dimethylamino)phenyl] methyl}-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (**8d**) as pale orange, yield 71%, m.p. 261 °C. IR (KBr) υ_{max} = 3391–2915 (br. OH), 3361, 3222, 3161 (NH_2 and NH), 1720–1691(C=O), 1623 (C=N), 1603, 1585 (C=C), 1118, 1063 (C–O) cm^{-1}.

^{1}H-NMR (DMSO-d_6) δ: 3.02 (s, 6H, $N(CH_3)_2$) 6.76–7.72 (m, 10H, Ar-H and NH_2), 8.63 (s, 1H, H-4 of quinoline ring), 11.16 (br. s, 1H, NH), 11.61 (s, 1H, OH carboxylic acid),) ppm.

^{13}C-NMR (DMSO-d_6) δ:163.27 (C-23), 163.03 (C-11), 161.84 (C-2), 159.12 (C-23), 152.96 (C-20), 152.43 (C-16), 133.73 (C-9), 132.80 (C-4), 131.83 (C-5), 131.29 (C-10), 130.65 (C-14), 129.97 (C-7), 122.00 (C-6), 120.91 (C-8), 120.09 (C-13), 118.83, (C-19), 117.01, (C-3), 112.13, (C-15), 40.59 (C-24) ppm.

Anal. Calcd. For $C_{22}H_{19}N_5O_4$ (417): C, 63.31; H, 4.56; N, 16.78. Found: C, 63.13; H, 4.29; N, 16.56.

2.2. Biological Assay

2.2.1. In vitro Anticancer Effect of the Compounds **7a–d** and **8a–d** Against MCF-7 Cell Line

A cytotoxicity test was conducted, using the MTT technique, to investigate the effect of the synthesized compounds **7a–d** and **8a–d** as anticancer medications [29]. Cells were started at the concentration 10^4 cells/well and distributed in a 96-plate and allowed to bind to the plate for 24 h before adding the synthesized compounds. Six wells were set for each dose. The 96-plates were incubated at 37 °C with 5% CO_2. MTT was added to each well at a final concentration of 0.5 mg/mL after two days, and the plates were incubated at 37 °C for an additional four hours in the presence of 5% CO_2. An ELISA reader was used to calculate color intensity. To create a survival curve for the MCF-7 cancer cell line after the particular concentration of the synthesized compounds, a link between the survivor curve and drug consumption was drawn. Data are calculated as the mean ± of three different experiments.

2.2.2. Cell-Cycle Analysis

To investigate the effect of the synthesized **7c** compound on the cell cycle of MCF-7, cells at the concentration of 2×10^5/well were incubated for two days with compound No. **7** at its IC_{50} value (1.73 μM). After being exposed to this procedure, cells were washed

twice with ice-cold phosphate saline (PBS) buffer, centrifuged, and fixed in ice-cold 70% ethanol at 4 °C for 30 min. Next, cells were washed in 1xPBS solution for 30 min at 37 °C and then collected by centrifugation at low speed (2000 rpm) for 5 min. Finally, cells were stained with a propidium iodide solution at a final concentration of 1 µg/mL. The samples were gently mixed and left at room temperature for 20 min in the dark. A BD FACSCanto flow cytometer was used to look at the DNA material (BD Biosciences Systems, San Jose, CA, USA). FACSDiva software (BD Biosciences Systems, San Jose, CA, USA) was used to interpret the data (BD Biosciences Systems).

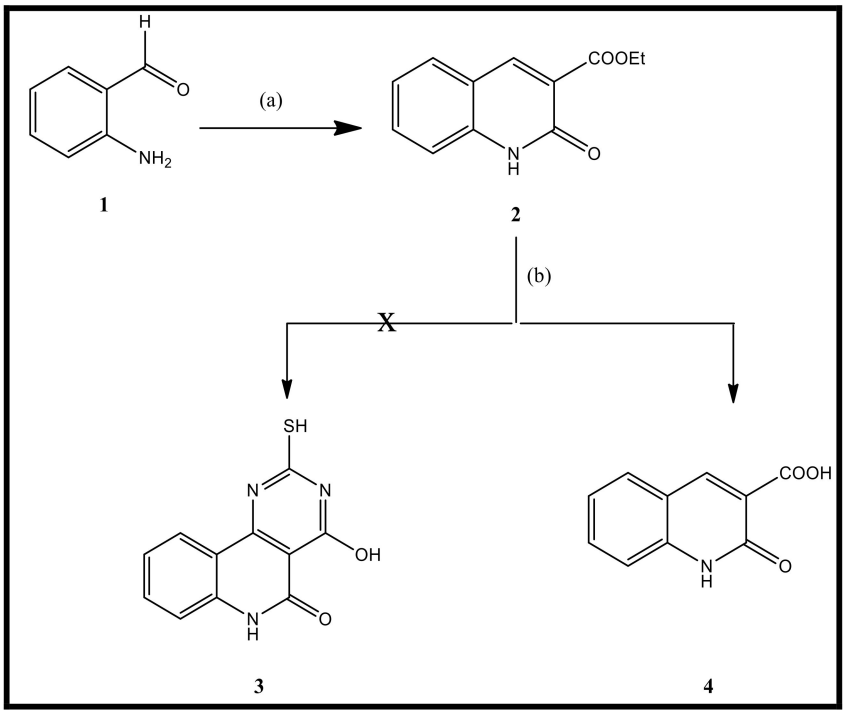
2.3. Statistical Analysis

Statistical comparisons were accomplished by a one-way ANOVA with the Duncan test, using IBM SPSS version 26 (IBM, Armonk, NY, USA). A probability level of 0.05 or lower was considered statistically significant; ** $p < 0.01$.

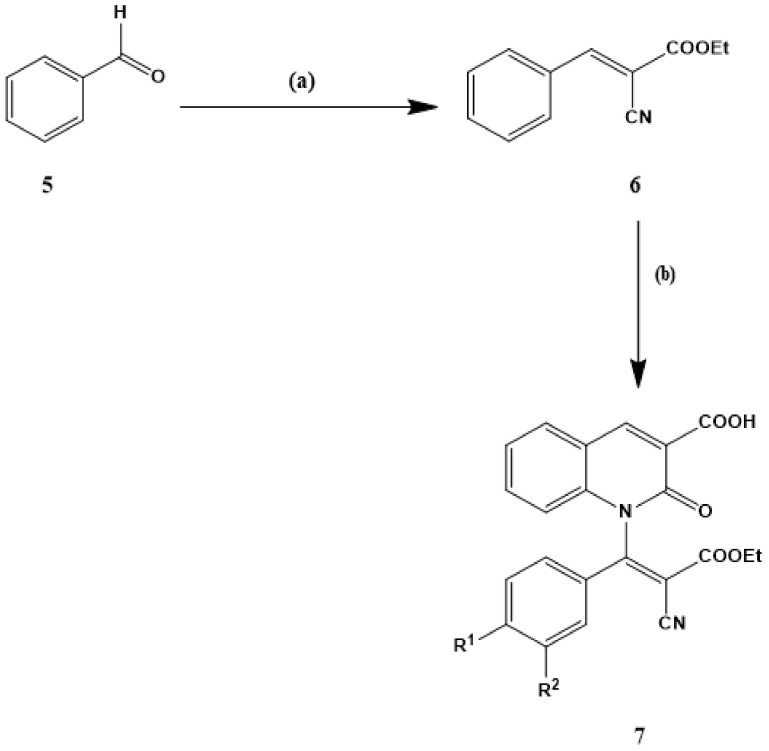
3. Results and Discussion

3.1. Chemistry

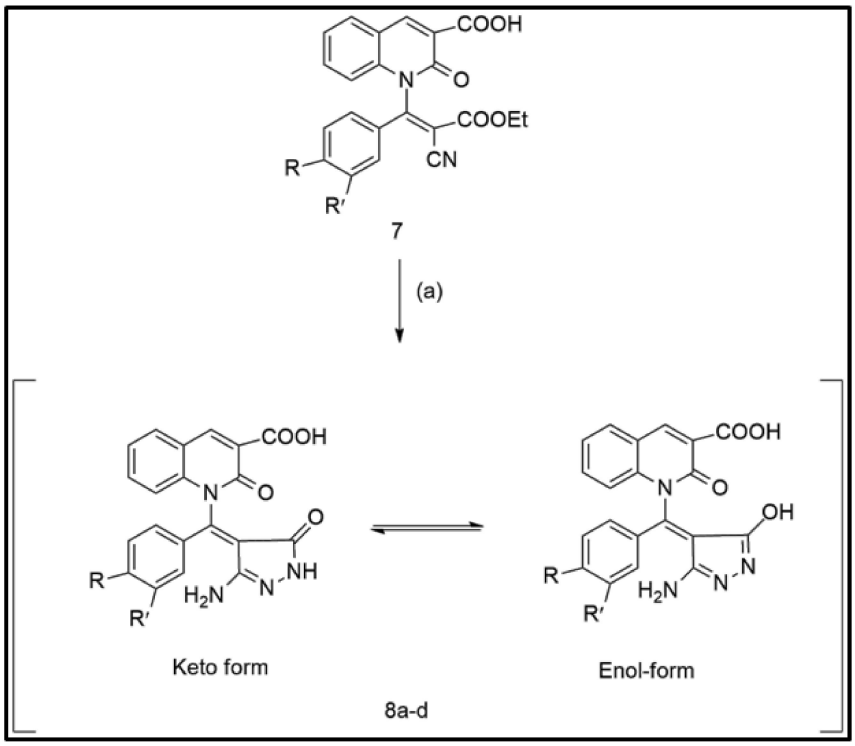
To synthesize the 3-amino pyrazolinones target carrying 2-oxo-1,2-dihydroquinoline-3-carboxylic acid, the reaction sequence is shown in Schemes 1–3. Reaction steps include condensation of 2-aminobenzaldehyde (**1**) with diethyl malonate in presence of a base catalyst for giving ethyl 2-oxo-1,2-dihydroquinoline-3-carboxylate (**2**). Treatment of compound (**2**) with thiourea in ethanol in the presence of anhydrous potassium carbonate under reflux was expected to result in the administration of 2-mercapto-4-hydroxy-5,6-dihydroquinolino[4,3-b]pyrimidine-5-wan. (**3**) However, only 2-oxo-1,2-dihydroquinoline-3-carboxylic acid (**4**) was produced (Scheme 1).



Scheme 1. Synthesis of 2-oxo-1,2-dihydroquinoline-3-carboxylic acid (**4**). Reagents and conditions: (a) diethyl malonate, piperidine/fusion; (b) thiourea, potassium carbonate, ethanol/reflux.



Scheme 2. Synthesis of 1-[2-cyano-2-ethoxycarbonyl-1-arylvinyl]-2-oxo-1, 2-dihydroquinoline-3-carboxylic acid derivatives (**7a**–**d**). Reagents and conditions: (a) ethyl cyanoacetate, Et_3N, EtOH reflux; (b) 2-oxo-1,2-dihydroquinoline-3-carboxylic acid, Et_3N DMF/reflux. (**7a**, $R^1 = R^2 = H$; **7b**, $R^1 = OCH_3$, $R^2 = H$; **7c**, $R^1 = OH$, $R^2 = OCH_3$; **7d**, $R^1 = N(CH_3)_2$, $R^2 = H$).



Scheme 3. Synthesis of 2-oxo-1,2-dihydroquinoline-3-carboxylic acid (**8a**–**d**) bearing pyrazole ring. Reagents and conditions: (a) hydrazine hydrate, EtOH/reflux (**a**, $R^1 = R^2 = H$; **b**, $R^1 = OCH_3$, $R^2 = H$; **c**, $R^1 = OH$, $R^2 = OCH_3$; **d**, $R^1 = N(CH_3)_2$, $R^2 = H$).

The NMR spectrum of compound **4** showed multiple signals at δ = 7.43–8.01 ppm, with four protons of the aromatic ring and one proton of the NH group. In addition, the ^{1}H-NMR spectrum data for compound **4** gave conclusive evidence of two single signals at 8.89 and 8.10 ppm, indicating the proton of the quinolinone ring at position 4 and the hydroxyl function (OH) position of the carboxyl group. The ^{13}C-NMR spectrum of compound **4** showed four signals at 163.00, 160.82, 154.51, and 148.30 ppm assigned to carbon for two carbonyl groups (C=O), C–N, and C-4 from the quinolinone ring. In addition, the ^{13}C-NMR spectrum of compound **4** showed six-carbon signals in the 134.59–116.61 ppm region due to the carbon residue in the quinolinone ring. Condensation of a variety of aromatic aldehydes (**5a**–**d**) with ethyl cyanoacetate into ethanol in the presence of a base catalyst resulted in the formation of ethyl 3-aryl-2-cyanoacrylate (**6a**–**d**). The compound 1-(2-(ethoxy)carbonyl-2-cyano-1-arylvinyl)-2-oxo-1,2-dihydroquinoline-3-carboxylic (**7a**–**d**) was prepared by reaction of quinoline-3-carboxylic acid (**4**) with ethyl 3-aryl-2-cyanoacrylates (i.e., ethyl 3-phenyl-2-cyanoacrylate, ethyl 3-(4-methoxy) phenyl-2-cyanoacrylate, ethyl 3-(4-hydroxy-3-methoxy) phenyl-2-cyanoacrylate, and ethyl 3-(4-*N*,*N*-dimethylamino) phenyl-2-cyanoacrylate) in the presence of triethylamine as a primary catalyst in dimethyl formation under the reflux (Scheme 2).

The structure of the compounds obtained was determined (**7a**–**d**) by spectral data. In the infrared spectra, large expansion bonds belonging to OH, C=O for ester, amide, and acid groups, C=C, and CN were observed. ^{1}H-NMR spectra of **7a**–**d** compounds showed two sharp signals at δ = 1.27–1.34 ppm as triple signal and δ = 4.25–4.35 ppm as quadruple signal due to protons of the ethoxy groups (OCH_2CH_3) (Supplementary Materials Figures S1–S8).

Regarding compounds **7a**–**d**, two protons appeared as singlet signal at δ 8.88–8.89 ppm refer to the H-4 of quinolinone ring and hydroxyl function (OH) for the carboxylic acid groups. The entire aromatic proton peaks in the ^{1}H-NMR spectra were also recorded at estimated regions (Supplementary Materials Figures S1–S8). The ^{13}C-NMR spectra of compounds **7a**–**d** displayed three-carbon signals at δ 164.81–162.99 ppm, 162.99–162.28 ppm, and δ 162.51–160.81 ppm due to carbons of three carbonyl function (C=O) of ester, amide, and acid (Supplementary Materials Figures S1–S8). In addition, the carbons of cyano groups (CN) for the compounds **7a**–**d** in the ^{13}C-NMR spectra were observed with the expected chemical shift in the region of δ 103.07–92.44 ppm (Supplementary Materials Figures S1–S8). At the final step, the compounds **7a**–**d** were reacted with hydrazine hydrate in ethanol under reflux to give 1-[(aryl)(3-amino-5-oxopyrazolidine-4-ylidene)methyl]-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (**8a**–**d**, Scheme 3).

IR, ^{1}H-NMR, ^{13}C-NMR, and elemental analyses have described all the new compounds **8a**–**d**. The IR spectra of compounds **8a**–**d** revealed the absence of carbonyl function of ester and cyano groups; the appearance of new stretching bonds at 3385 and 3228 indicated the presence of amino (NH_2) and NH groups. The ^{1}H-NMR spectra of compounds **8a**–**d** showed the appearance of new signals at δ = 6.76–7.99 and 11.19–11.16 ppm for characteristic amino (NH_2) and amino NH groups, with the absence of any signals for the ethoxy protons at δ 1.32 and 4.32 ppm (Supplementary Materials Figures S9–S16). Moreover, the ^{1}H-NMR spectra of compounds **8a**–**d** displayed the presence of the characteristic singlet signals in the region at δ 11.33–11.68 ppm and refer to the protons of hydroxy (OH) groups (Supplementary Materials Figures S9–S16), which confirmed the structure of compound **8** in enol form (Scheme 3). The protons of the aromatic rings and quinolinone were observed within the expected chemical transformation regions and showed the expected integrative values. The ^{13}C-NMR spectra of compounds **8a**–**d** (Supplementary Materials Figures S9–S16) showed the absence of any carbon signals for the ethoxy and cyano groups that were present in compounds **7a**–**d** (Supplementary Materials Figures S1–S8).

3.2. In Vitro Cytotoxic Activity Against MCF-7 Cell Line

To examine the anticancer activity of the prepared quinolone derivatives, the effect of the synthesized compounds on the viability of the MCF-7 cell line was measured by

using colorimetric MTT assay after 48 h of incubation. The percentage of cell viability of quinolone derivatives and reference compound are presented in Table 1. The reference compound in our assay is Doxorubicin (Dox). Interestingly, among the quinolone derivatives compound, 1-[2-(ethoxy)carbonyl-2-cyano-1-(4-hydroxy-3-methoxy)phenylvinyl]-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (compound **7c**) was found to be superior to the other quinolone derivatives in terms of anticancer activity and showed significant antiproliferative activity compared to the reference compound Dox with IC_{50} value of 1.73 ± 0.27 µg/mL.

Table 1. In vitro anticancer activity of quinolinone derivatives **7a–d** and **8a–d** against MCF-7 cell line.

Compound	IC_{50} Values (µM)/MCF-7
7a	>50
7b	3.87 ± 0.33 [c]
7c	1.73 ± 0.27 [a]
7d	>50
8a	17.32 ± 0.44 [e]
8b	5.67 ± 0.21 [d]
8c	4.03 ± 0.60 [c]
8d	>50
Dox	2.82 ± 0.07 [b]

[a–e] Each value is the mean of three experiments ± SEM. Different superscript letters designate significant differences ($p < 0.05$), using Duncan's multiple range test.

3.3. Cell-Cycle Analysis

Due to the significance of the cell cycle in the process of tumor cell proliferation, MCF-7 cell growth inhibition, as a result of cell-cycle arrest, was evaluated by using DNA flow cytometric assay. In this assay, MCF-7 cells were dealt with compound **7c** at a concentration of 1.73 µM value for 48 h. It is evident from Figure 1 that compound **7c** induced cell arrest on 36.04% of cells at the G2/M phase, in comparison with the untreated cells, which had 9.88%. Moreover, compound **7c** revealed G0 phase arrest marked by the appearance of a peak at the G0 phase of the cell-cycle distribution profile, which indicates MCF-7 cell apoptosis (Figure 1).

These data suggest that compound **7c** causes perturbations during the cell-cycle progression, especially at the G2/M stage. Cell cycle and apoptosis play remarkable roles in the regulatory mechanisms of the development and growth of the cell. The impacts of numerous substances that are utilized as anticancer drugs were discovered to be through the capturing of the cell cycle during the stages G0/G1, S, and G2/M, which exhilarated and initiated apoptosis [30–33]. Consequently, we presume that the impact of compound **7c** on the MCF-7 cell line was through the instabilities influences of the compound **7c** in cell-cycle progression particularly at the G2/M stage.

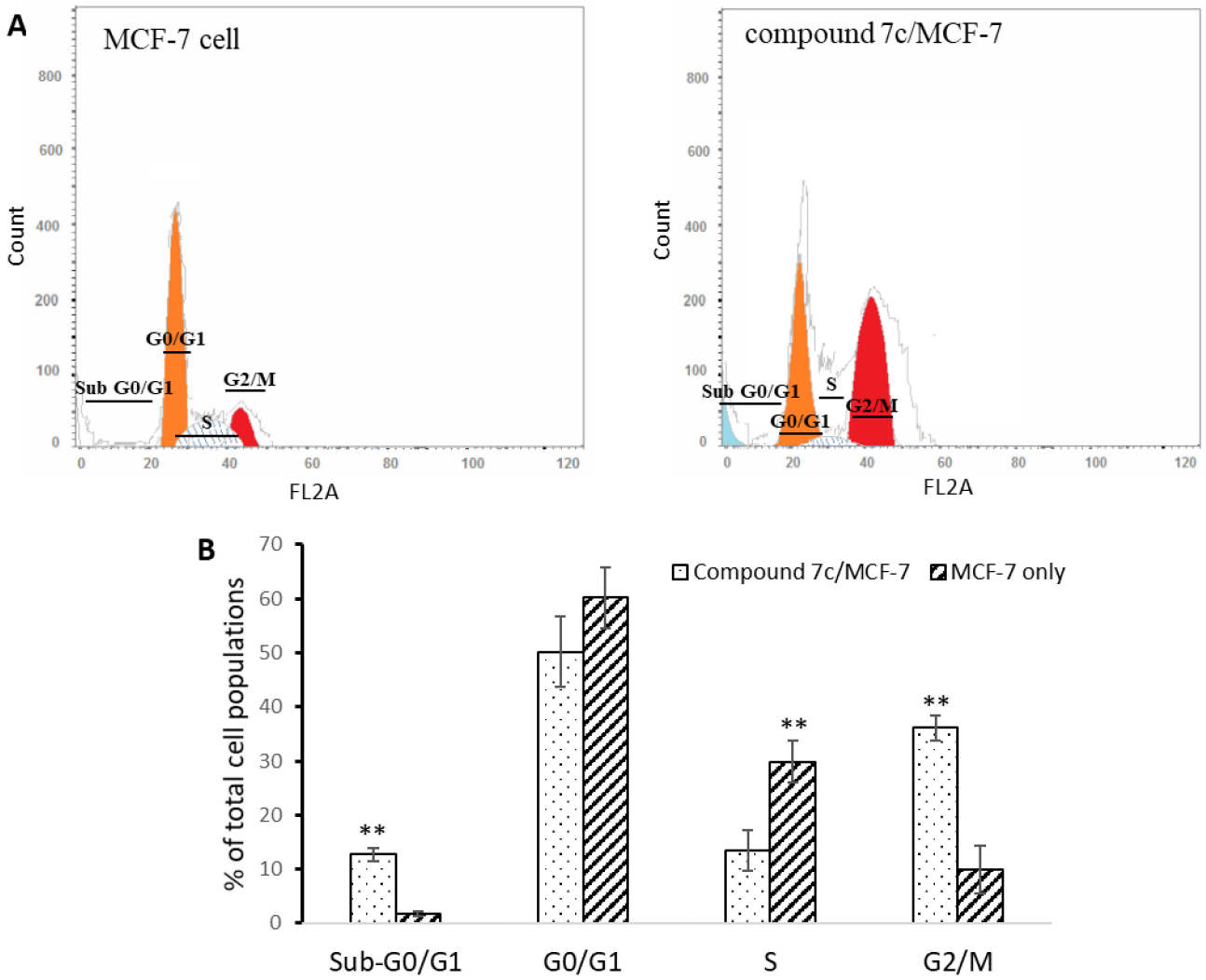


Figure 1. The effect of compound **7c** at the concentration of 1.73 μM on the MCF-7 cell-cycle phases. (**A**) Representative flow cytometry data of the cell-cycle phases. (**B**) Percentage of the total cell populations at each cell-cycle phase. ** $p < 0.01$ according to Tukey test.

4. Conclusions

In conclusion, we have developed a rapid and efficient synthetic route for the synthesis of 1-[(aryl)(3-amino-5-oxopyrazolidin-4-ylidene) methyl]-2-oxo-1,2-dihydroquinoline-3-carboxylic acid derivatives. The present synthetic pathway has the advantages of operational simplicity, moderate reaction conditions, and good to high yield of bioactive products. Our method is simple, as no extraordinary apparatus, reagents, or chemicals for workup are required, and the compound formed is filtered and purified just by simple crystallization. This synthesis is also beneficial in terms of economy, as well as avoiding any hazardous chemicals. The effect of the synthesized compounds on the viability of MCF-7 cell line was measured by using colorimetric MTT assay after 48 h of incubation. The compounds **7b**, **7c**, **8b**, and **8c** showed significant anticancer activity compared to the reference compound Dox against the MCF-7 cell line.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/cryst11050571/s1. Figure S1: ^{1}H-NMR spectrum of compound **7a**. Figure S2: ^{13}C-NMR spectrum of compound **7a**. Figure S3: ^{1}H-NMR spectrum of compound **7b**. Figure S4: ^{13}C-NMR spectrum of compound **7b**. Figure S5: ^{1}H-NMR spectrum of compound **7c**. Figure S6: ^{13}C-NMR spectrum of compound **7c**. Figure S7: ^{1}H-NMR spectrum of compound **7d**. Figure S8: ^{13}C-NMR spectrum of compound **7d**. Figure S9: ^{1}H-NMR spectrum of compound **8a**. Figure S10: ^{13}C-NMR

spectrum of compound **8a**. Figure S11: ^{1}H-NMR spectrum of compound **8b**. Figure S12: ^{13}C-NMR spectrum of compound **8b**. Figure S13: ^{1}H-NMR spectrum of compound **8c**. Figure S14: ^{13}C-NMR spectrum of compound **8c**. Figure S15: ^{1}H-NMR spectrum of compound **8d**. Figure S16: ^{13}C-NMR spectrum of compound **8d**.

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Conflicts of Interest: The authors declare no conflict of interest.

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