HETEROCYCLES, Vol. 104, No. 7, 2022, pp. 1303 - 1314. © 2022 The Japan Institute of Heterocyclic Chemistry
Received, 18th February, 2022, Accepted,18th April, 2022, Published online, 27th April, 2022
DOI: 10.3987/COM-22-14644

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL SCREENING OF NEW *N*-SUBSTITUTED-7-CHLORO-4-HYDROXY-2-QUINOLONE-3-CARBOXAMIDES AS PROMISING ANTICANCER AGENTS

Aya Mohammed kheir Al-Zuheiri,[a] Kamal Sweidan,[a]* Mohammed Kamal Harb,[b] Sanaa K. Bardaweel,[c] Mahmoud Sunjuk,[d] and Dima A. Sabbah[b]

[a]Department of Chemistry, The University of Jordan, Amman 11942, Jordan; e-mail: k.Sweidan@ju.edu.jo

[b]Department of Pharmacy, Faculty of Pharmacy, Al-Zaytoonah University of Jordan, Amman 11733, Jordan

[c]Department of Pharmaceutical Sciences, School of Pharmacy, The University of Jordan, Amman 11942, Jordan

[d]Department of Chemistry, Faculty of Science, The Hashemite University, P.O Box 330127, Zarqa 13133, Jordan.

Abstract – A new set of 7-chloro-4-hydroxy-2-quinolone-3-carboxamide derivatives has been synthesized in good yields by using simple procedure. The prepared compound has been characterized using different spectroscopic techniques including high resolution mass spectrometry (HRMS), infrared spectroscopy (IR), one-dimensional (1D), two-dimensional (2D) nuclear magnetic resonance (NMR), and by elemental analysis (EA). The target products have been tested as anticancer agents against cell lines; human colon carcinoma (HCT-116), breast adenocarcinoma (MDA-231), epithelial colorectal adenocarcinoma (Caco-II), and normal cell lines. Results indicated that compound demonstrated selective inhibitory activity; compound **14** exerted high activity against HCT-116 cell line (IC_{50} = 87.45 µM), compound **5** exhibited high activity against (MDA-231) cell line (IC_{50} = 23.66 µM), and compound **10** demonstrated high activity against (Caco-II) cell line (IC_{50} = 35 µM).

Cancer is a major public health problem worldwide, and ranks as the second leading cause of death globally after cardiovascular disease.[1] Unfortunately, some commercial anticancer drugs have low solubility, high toxicity to normal cells,[2] instability in the aqueous medium,[3] and highly undesirable adverse effects such

as vomiting and nausea.

Quinoline nucleus is considered a high impact moiety in biological and pharmaceutical fields. It is present in large number of bioactive derivatives.[4] Various 4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxamides (**1**, Figure 1) have a wide range of bioactive substances, such as anticancer agents[5] in addition, they possess valuable pharmacological properties.[6] The importance of carboxamide group (-CONH) in biological systems originates from the dual properties of this function group as a hydrogen bond acceptor from the oxygen atom of the carbonyl group side and a hydrogen bond donor from the N-H side in the same molecule.[7]

A series of hydroxyquinoline-carboxamide derivatives **2** as shown in Figure 1, were prepared and evaluated as potential inhibitors against the proliferation of colon cancer cell lines (Caco-2) and (HCT-116); values of IC_{50} (μM) were ranged 13-1559 and 3-789, respectively.[8,9]

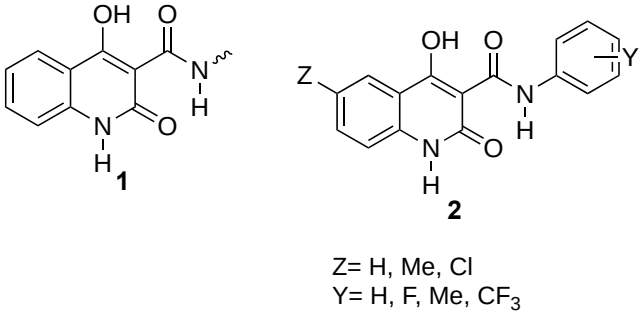


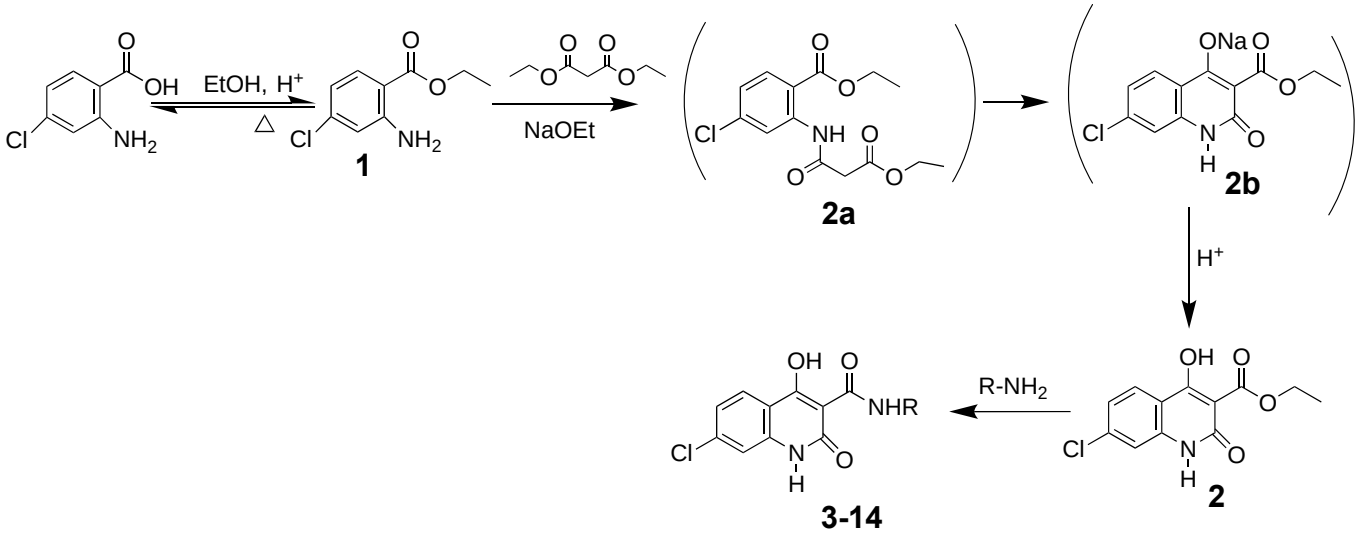
Figure 1. Representative structures of some anti-cancer hydroxyquinoline-carboxamides

On the other hand, the chlorine atom is present in the chemical identity of some natural bioactive products such as rebeccamycin and crytophycin[10] (antitumor agents). Presence of chlorine atom as a substitutents on a heteroaromatic system may exhibit a significant effect on the biological activity due to one or more of the following parameters: increasing the lipophilicity of the molecule, interaction with certain functional groups (in protein) at the binding site through non-bonding electrons, increasing the acidity or decreasing the basicity of the system due to the electron-withdrawing property and establishment the active conformation of the target molecule with the protein.[10] In the present work and based on the view of our widespread interest in finding of new anticancer scaffolds, we report herein the synthesis and full characterization of new series of new *N*-substituted-4-hydroxy-2-quinolone-3-carboxamides tailored with chlorine atom at position 7. In addition, all prepared compounds have been screened towards four cell lines (HCT-116), (MDA-231), (Caco-II), and normal cell lines.

Ethyl 2-amino-4-chlorobenzoate (**1**) was prepared in good yield by Fischer esterification procedure in the presence of excess amount of absolute EtOH and the starting material (an acid) was pre-dried in an oven. The resulting ester product undergoes nucleophilic acyl addition-elimination reaction using diethyl malonate and freshly prepared sodium ethoxide under reflux followed by Dieckmann cyclization reaction of **2a** *in situ* which afforded the quinolone nucleus. After the end of the reaction (monitored by

thin layer chromatography), the acidification of **2b** was performed using 0.5 M HCl solution to get the desired compound **2** with high yield (Scheme 2). It should be stated that the yield of **2** was relatively high when the used sodium ethoxide solution was prepared by reacting of sodium metal in absolute EtOH while the commercial dried sodium ethoxide reagent did not produce the same result. In addition, the best pH ranges to get **2** in high yield was 4.5-5, this range is considered acidic and it is highly affected by the presence of electron-withdrawing groups (lactam and ester) and heteroatom nitrogen compared to pH of phenols (*ca.* 9-10).

The target amide compounds **3-14** have been prepared in good yield by refluxing of **2** with an excess amount of various primary amines (R-NH_2) in the aprotic polar solvent, namely tetrahydrofuran and in the presence of catalytic amount of high-boiling point liquid (dimethylformamide). Since aromatic amines are weak nucleophiles compared to aliphatic ones, long time of reflux was employed and the mechanism of carboxamide formation is nucleophilic acyl addition-elimination reaction. TLC technique was used to monitor the reaction progress; the absence of reactant spot under UV light may belong to the completeness of the reaction. All products have been well characterized using various techniques IR, HRMS, NMR (^{1}H, ^{13}C, DEPT, COSY, HMQC, HMBC) and elemental analysis and all experimental data assures the suggested chemical structures (available in the supporting files). Experiments of DEPT, COSY, HMQC, HMBC exhibited correlations of the different carbon atoms and their attached and /or neighboring hydrogen atoms that assisted in the ^{1}H- and ^{13}C- signal assignments.



3: aniline (R = Ph)
4: benzylamine (R = Bn)
5: 2-fluoroaniline (R = 2-F-Ph)
6: 3-fluoroaniline (R = 3-F-Ph)
7: phenylhydrazine (R = HN-Ph)
8: 3-aminopyridine (R = 3-Py)
9: 4-aminophenol (R = 4-HO-Ph)
10: 4-(methylthio)aniline (R = 4-MeS-Ph)
11:*p*-toluidine (R = 4-Me-Ph)
12: 3-(trifluoromethyl)aniline (R = 3-CF_3-Ph)
13: 4-(trifluoromethyl)aniline (R = 4-CF_3-Ph)
14: 2-(trifluoromethyl)aniline(R = 2-CF_3-Ph)

Scheme 1

It should be stated that several papers reported the synthesis of the main nucleus 4-hydroxy-2-oxo-1,2-dihydroquinoline-3-caboxylate, for examples *N*-alkylated analogue was synthesized by reacting of *N*-alkylisatoic anhydride with diethyl malonate and sodium hydride.[11]

Scheme 1 delineates that 5-chloroanthranilic acid undergoes Fischer esterification reaction then acylation using ethoxymalonyl chloride in the presence of triethylmine, and finally Dieckmann cyclization reaction proceeds in the presence of sodium ethoxide.[12]

It's worth noting that the series exhibited selective inhibition against the aforementioned cell lines. The activity is expressed in term of IC_{50} value; the lowest IC_{50} value refers to a high potent inhibitor (Table 1).

Table 1. IC_{50} (μM) of compounds **3-14** against HCT-116, MDA-231, and Caco-II cell lines. Data is expressed as mean (μM) ±SD.

Compound	HCT-116	MDA-231	Caco-II	Fibroblasts
3	648±12	25±0.2	321±6	>1000
4	155±3	166±4	173±7	>1000
5	477±5	24±0.3	420±7	>1000
6	247±8	401±5	308±5	>1000
7	166±2	526±7	198±2	>1000
8	106±1	135±3	NA*	>1000
9	116±3	39±1	NA*	>1000
10	155±3	66±2	35±1	>1000
11	349±4	536±6	258±4	>1000
12	209±3	496±4	203±5	>1000
13	296±2	663±5	357±5	>1000
14	87±2	121±3	NA*	>1000
2	1538±12	4833±10	1159±10	>1000
Doxorubicin	-	2±0.1	10±0.1	NA*
LY294002	180±6	-	-	NA*

* NA= Not determined.

Concerning HCT116 cell line, compound tailored with *o*-CF_3 (compound **14**) exerted high potency indicating that *o*-position is enclosed by hydrophobic and/or H-bond acceptor motif. Contrarily, incorporation of CF_3 on *m*- and *p*-positions weakens the activity thus implying that *o*-CF_3 might induce a favorable conformational effect that consequently pushes the compound deep into the binding cleft.

Interestingly, the activity of *p*-OH (compound **9**) infers that H-bond interaction guides ligand interaction. On the other hand, the activity of *p*-Me (compound **11**) confirms that *p*-H-bond elicits the activity. Introducing *p*-SMe moiety (compound **10**) potentiates the activity inferring that sulfur motif induces the activity and *p*-hydrophobic moiety weakens the proliferation, also sulfur pushes the methyl deeply in the binding cleft thus enhancing the activity. Tailoring the core nucleus with *m*-F (compound **6**) induces the activity whereas *o*-F (compound **5**) weakens the proliferation thus interrogating that H-bond is nearby the *m*-position.

Comparing the inhibitory activity of compound **3** with that of compound **4** provides a proof for the significance of elongation of the side chain of carboxamide by one atom exemplified with compound **4**. The side chain of benzylamine pushes **4** deep into the binding cleft thus potentiating the activity. Furthermore, attaching a hydrazine motif (compound **7**) affirms the significance of elongation of the carboxamide side chain by one atom.

The antiproliferative activity of **8** emphasizes the significance of H-bond acceptance. Additionally, incorporation of pyridine in **8** enriches the hydrophilic exposed area and increases the polarity thus enhances compound's absorption and permeation across the cell membrane.

Concerning MDA-231, incorporating *o*-F motif (compound **5**) optimizes the activity interrogating that *o*-H-bond and/or small hydrophobic cleft encloses the F on *o*-position and consequently elaborates the weak activity of **6**. Comparing the activities of compounds **12-14** indicates that *o*-substituent is significant for activity, illustrated by *o*-CF_3. Comparing the activity of **9-11** clarifies that *p*-H-bond donor and/or acceptor is required for activity. The SMe motif (compound **10**) buries the compound deeply in the binding site thus potentiates the activity.

The activity of **3** demonstrates that steric effect guides ligand interaction and provides a clue for selective targeting. Additionally, the activity of **8** gives a further clue that the binding pocket of MDA-231 receptor is smaller than that of the receptor of HCT-116. Comparing with LY294002 (employed as a reference) which has IC_{50} = 0.18 mM, some of our prepared compounds have lower values than its IC_{50} value.

Concerning Caco-II, introducing *p*-SMe motif (**10**) induces the activity suggesting that the sulfur atom might orientate **10** properly in the binding domain. Furthermore, the activity of *p*-Me (compound **11**) and *p*-CF_3 (compound **13**) elaborates the significance of sulfur atom. The difference in activity between compounds **3**, **4**, and **7** gives an evidence of elongation of the carboxamide link to induce the inhibitory activity. The activity of *m*-F (compound **6**) and *m*-CF_3 (compound **12**) recommends hydrophobic motif on *m*-position to induce the suppressive activity. Eventually, the activity of the series against the normal fibroblast elaborates the safety of the verified compounds as well as their selective targeting cancer cell lines. It's worth noting that introducing the chlorine motif on position 6 of 4-hydroxy-2-quinolone ring[9] provokes the activity. Such finding sheds the light on incorporating an electron-withdrawing and lipid

soluble motif on 6-position and further recommends screening the prospective derivatives against a panel of cancer cell lines to better explain the attachment the chlorine motif on position 7.

Noting that 15 compounds were screened against 4 different cell lines at five different points in three independent experiments in triplicate generating about 2700 points of raw data making it impossible to be inserted in the manuscript content. Furthermore, inserting the dose-response curve for 15 compounds in 4 cell lines generates 60 dose response curves making the content complicated.

EXPERIMENTAL

All synthesized products have been characterized by ^{1}H NMR, ^{13}C NMR on a Bruker-Avance III 500 MHz spectrophotometer with deuterated sodium hydroxide (NaOD) in deuterated dimethyl sulfoxide (DMSO-d_6) as solvent and TMS as internal standard. Chemical shift (δ) is expressed in ppm; coupling constant (J) values are given in Hertz (Hz). Infrared spectra were recorded using Shimadzu IR Affinity H spectrophotometer; all samples were prepared with potassium bromide and pressed into a disc. High resolution mass spectra (HRMS) were acquired by electrospray ionization (ESI) technique with the aid of Bruker APEX-4 instrument. Melting points were measured using SMP 10 Stuart apparatus and are uncorrected. Thin layer chromatography (TLC) was performed using pre-coated aluminum sheets with silica gel E. Merck Kiesegel 60 F254 (layer thickness 0.20 mm). Analytical balance was from ADAMS. Rotary evaporator model R-215 (Büchi, Switzerland) connected to vacuum pump model v-700, water bath B-491 was used to evaporate ordinary solvents, vacuum controller v-855 and Euro-Vector 8910 elemental analyzer, were employed.

Starting Materials. 2-Amino-4-chlorobenzoic acid (98%), benzylamine (99%), 3-aminopyridine (99.6%), phenylhydrazine, aniline (99.5%), 4-(trifluoromethyl)aniline (97%), 3-(trifluoromethyl)aniline (95%), 2-(trifluoromethyl)aniline (95%), potassium bromide, and *p*-toluidine (98%) (Sigma-Aldrich), 3-fluoroaniline (98%), 4-aminophenol (95%), 4-(methylthio)benzenamine, diethyl malonate (98%), 2-fluoroaniline (98%), sodium sulfate (99%) and sodium bicarbonate (99%) (Acros), dimethyl sulfoxide (DMSO) (99.9%), dichloromethane (DCM), *N*,*N*-dimethylformamide (DMF) (99.9%), chloroform, tetrahydrofuran (THF) (99.9%), HPLC EtOH, sulfuric acid (95-97%) (Tedia), hydrochloric acid (37%) (Fisher), and sodium metal (Scharlau).

Preparation of ethyl 2-amino-4-chlorobenzoate (**1)**. A mixture of 2-amino-4-chloroenzoic acid (1 g, 5.8 mmol), absolute EtOH (60 mL), and sulfuric acid (2 mL), (dropwise in an ice bath), was refluxed for 4 days. After cooling, the solvent was evaporated under reduced pressure and the residue was extracted with sodium bicarbonate solution (5%) (10 mL) and dichloromethane (DCM) (10 mL) three times. The organic layer was separated followed by extraction with water (25 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford the target product.[13]

Brown oil; yield 93%; R_f 0.52 (9:1 hexane–AcOEt); ^{1}H NMR (DMSO-d_6) δ 1.38 (t, J = 7.2 Hz, 3H, CH_3); 4.29 (q, J = 7.2 Hz, 2H, CH_2); 5.83 (s, 2H, NH_2); 6.57 (dd, J = 1.9, 8.6 Hz, 1H, H5); 6.63 (s, 1H, H3); 7.77 (d, J = 8.6 Hz, 1H, H6); ^{13}C NMR (DMSO-d_6) δ 14.3 (CH_3); 60.5 (CH_2); 109.5 (C1); 115.8 (C5); 116.5 (C3); 132.6 (C6); 139.8 (C4); 151.3 (C2); 167.6 (CO2); FT IR: 3481, 3371 N-H; 1693 C=O cm^{-1}; HRMS *m/z*: calcd. 200.04728, 202.04433 for $C_9H_{10}ClNO_2$ $[M+H]^+$; found 200.04679, 202.04387. EA (%): calcd. for $C_9H_{10}ClNO_2$: C, 54.15; H, 5.05; N, 7.02. Found: C, 54.19; H, 5.09; N, 7.05.

Preparation of ethyl 7-chloro-4-hydroxy-2-quinolone-3-carboxylate (**2**). A solution of ethyl 2-amino-4-chlorobenzoate (**1**) (1 g, 5.0 mmol) and an excess amount of diethyl malonate (8 mL, 50.0 mmol) in 7 mL DMSO was prepared, then a freshly prepared sodium ethoxide solution [sodium (0.23 g, 10 mmol) in HPLC EtOH (15 mL)] was added dropwise to the previous solution which was refluxed for 4 days. The resulting solution was cooled in an ice bath followed by acidification with 0.5 M HCl to pH 4.5-5.0, the formed solid was filtered, washed with water then THF (10 mL), and finally dried in a vacuum oven at 70 ºC for 1 h.

White solid; yield 90%; mp 300 ºC (decom.); R_f 0.42 (9.9:0.1 $CHCl_3$–MeOH); ^{1}H NMR (DMSO-d_6 and NaOD) δ 1.29 (t, J = 7.1 Hz, 3H, CH_3); 4.32 (q, J = 7.1 Hz, 2H, CH_2); 7.24 (dd, J = 1.9, 8.6 Hz, 1H, H6); 7.30 (s, 1H, H8); 7.93 (d, J = 8.6 Hz, 1H, H5); 11.57 (s, 1H, NH); ^{13}C NMR (DMSO-d_6 and NaOD) δ 14.9 (CH_3); 58.8 (CH_2); 102.0 (C3); 115.1 (C6); 120.9 (C8); 122.8 (C4a); 126.9 (C5); 132.9 (C7); 152.0 (C8a); 172.9 (C2); 173.3 (C4); 174.4 (C=O); FT IR: 3010 O-H; 2918, 2848 N-H; 1680 C=O ester and amide cm^{-1}; HRMS: *m/z*: calcd. 268.03711, 270.03416 for $C_{12}H_{11}ClNO_4$ $[M+H]^+$; found 268.03665, 270.03363. EA %: calcd. for $C_{12}H_{10}ClNO_4$: C, 53.85; H, 3.77; N, 5.23. Found: C, 53.9; H, 3.80; N, 5.27.

General Procedure for the Preparation of 7-Chloro-4-hydroxy-2-quinolone-3-carboxamide Derivatives (**3-14).** A mixture of ethyl 7-chloro-4-hydroxy-2-quinolone-3-carboxylate (20) (1 mmol) and a suitable amine (3 mmol) were mixed in 15 mL of THF. Few drops of DMF were added to the solution then refluxed for 72 h (for aromatic amines) and 24 h (for aliphatic amines). During the reaction progress, the amide product was formed as a solid on the flask wall. After cooling, the precipitate was collected by suction filtration. Finally, the product was washed with THF, and then dried in a vacuum oven at 70 ºC for 1 h.

***N*-Phenyl-7-chloro-4-hydroxy-2-quinolone-3-carboxamide (3):** yellow solid; yield 85%; mp 312-315 ºC; R_f 0.7 (9.6:0.4 $CHCl_3$–MeOH); ^{1}H NMR (DMSO-d_6 and NaOD) δ 6.55 (d, J = 8.4 Hz, 1H, H6); 6.83 (t, J = 7.2 Hz, 1H, H4'); 6.93 (s, 1H, H8); 7.15 (t, J = 7.7 Hz, 2H, (H5', H3')); 7.63 (d, J = 7.7 Hz, 2H, (H2', H6'); 7.79 (d, J = 8.5 Hz, 1H, H5).^{13}C NMR (DMSO-d_6 and NaOD) δ 99.1 (C3); 115.8 (C6); 119.8 (C48a); 119.9 (C8); 120.7 (C4'); 121.5 (C2', C6'); 127.4 (C3', C5'); 128.9 (C5); 133.7 (C7); 141.6 (C1'); 151.4 (C4a); 169.8 (C2); 173.8 (C4); 176.7 (CONH); FT IR: 3061 O-H; 2918 N-H; 1668 C=O amide

cm^{-1}; HRMS *m/z*: calcd. 313.03854, 315.03559 for $C_{16}H_{10}ClN_2O_3$ $[M-H]^-$; found 313.03956, 315.03664. Anal. Calcd for $C_{16}H_{11}ClN_2O_3$: C, 61.06; H, 3.52; N, 8.90. Found: C, 61.09; H, 3.56; N, 8.94.

***N*-Benzyl-7-chloro-4-hydroxy-2-quinolone-3-carboxamide (4):** white solid; yield 90%; mp 230-232 ºC; R_f 0.6 (9.9:0.1 $CHCl_3$–MeOH); ^{1}H NMR (DMSO-d_6 and NaOD) δ 4.45 (s, 2H, CH_2); 6.48 (d, *J* = 8.3 Hz, H6); 6.89 (s, 1H, H8); 7.14 (t, *J* = 7.0 Hz, 1H, H4'); 7.24 (t, *J* = 7.3 Hz, 2H, (H3', H5')); 7.30 (d, *J* = 7.2 Hz, 2H, (H2', H6')); 7.75 (d, *J* = 8.3 Hz, 1H, H5); 10.48 (s, 1H, H1'); 11.81 (s, 1H, H1); 17.14 (s, 1H, O-H); ^{13}C NMR (DMSO-d_6 and NaOD) δ 42.1 (CH_2); 100.7 (C3); 114.9 (C6); 120.9 (C8); 122.5 (C4a); 126.6 (C4'); 127.3 (C3',C5'); 127.7 (C2', C6'); 128.5 (C5); 133.1 (C7); 141.7 (C1'); 151.7 (C8a); 171.9 (C2); 174.6 (C4); 176.5 (CONH); FT IR: 3201 O-H; 3118 N-H; 1680 C=O amide cm^{-1}; HRMS *m/z*: calcd. 327.05419, 329.052125 for $C_{17}H_{13}ClN_2O_3$ $[M-H]^-$; found 327.05561, 329.05234. Anal. Calcd for $C_{17}H_{12}ClN_2O_3$: C, 62.11; H, 3.99; N, 8.52. Found: C, 62.15; H, 4.02; N, 8.55.

***N*-(2-Fluorophenyl)-7-chloro-4-hydroxy-2-quinolone-3-carboxamide (5):** white solid; yield 80%; mp 300 ºC (decom.); R_f 0.3 ($CHCl_3$); ^{1}H NMR (DMSO-d_6 and NaOD) δ 6.91 (d , *J* = 6.0 Hz, 1H, H6'); 6.93 (s, 1H, H8); 6.95 (d, *J* = 8.4 Hz, 1H, H6); 7.01 (t, *J* = 7.7 Hz, 1H, H4'); 7.14 (t, *J* = 10.7 Hz, 1H, H5'); 7.92 (d, *J* = 8.4 Hz, 1H, H5); 8.54 (t, *J* = 7.9 Hz, 1H, C3'); ^{13}C NMR (DMSO-d_6 and NaOD) δ 99.0 (C3); 114.0 (C6); 115.1 (d, $J_{C\text{-}F}$ = 78.0 Hz, C5'); 120.5 (C8); 120.5 (d, $J_{C\text{-}F}$ = 24.0 Hz, C2'); 122.2 (d, $J_{C\text{-}F}$ = 87.0 Hz, C3'); 124.5 (C4'); 128.5 (C5); 135.8 (C7); 140.0 (C4a); 151.5 (C8a); 153.4 (C1'); 167.7 (C2); 176.8 (CONH); FT IR: 3163 O-H; 2993 N-H; 1672 C=O amide cm^{-1}; HRMS *m/z*: calcd. 331.02912, 333.02617 for $C_{16}H_9ClFN_2O_3$ $[M-H]^-$; found 331.02751, 333.02510. Anal. Calcd for $C_{16}H_{10}ClFN_2O_3$: C, 57.76; H, 3.03; N, 8.42. Found: C, 57.80; H, 3.06; N, 8.45

***N*-(2-Fluorophenyl)-7-chloro-4-hydroxy-2-quinolone-3-carboxamide (6):** off white solid; yield 80%; mp 310 ºC (decom.); R_f 0.3 ($CHCl_3$); ^{1}H NMR (DMSO-d_6 and NaOD) δ 6.58 (d, *J* = 8.3 Hz, 1H, H6); 6.63 (br.s., 1H, H4'); 6.92 (s, 1H, H8); 7.10 (d, *J* = 7.6 Hz, 1H, H5'); 7.17 (m, 1H, H6'); 7.76 (d, *J* = 8.4 Hz, 1H, H5); 7.8 (d, *J* = 12.2 Hz, 1H, H2'); ^{13}C NMR (DMSO-d_6 and NaOD) δ 101.0 (C3); 106.4 (d, $J_{C\text{-}F}$ = 104.3 Hz, C2'); 107.7 (d, $J_{C\text{-}F}$ = 82.9 Hz, C4'); 115.5 (C5'); 116.3 (C6); 120.9 (C8); 121.8 (C4a); 127.3 (C5); 130.2 (d, $J_{C\text{-}F}$ = 39.1 Hz, C6'); 134.0 (C7); 143.1 (d, $J_{C\text{-}F}$ = 45.3 Hz, C1'); 151.2 (C8a); 163.8 (C3'); 169.8 (C2); 173.6 (C4); 176.8 (CONH); FT IR: 3066 O-H; 2931 N-H; 1670 C=O amide cm^{-1}; HRMS *m/z*: calcd. 331.02912, 333.02617 for $C_{16}H_9ClFN_2O_3$ $[M-H]^-$; found 331.02927, 333.02665. Anal. Calcd for $C_{16}H_{10}ClFN_2O_3$: C, 57.76; H, 3.03; N, 8.42. Found: C, 56.99; H, 3.00; N, 8.49

***N*-(Anilino)-7-chloro-4-hydroxy-2-quinolone-3-carboxamide (7):** pale yellow solid; yield 83%; mp 272-274 ºC; R_f 0.6 (9.9:0.1 $CHCl_3$–MeOH); ^{1}H NMR (DMSO-d_6 and NaOD) δ 6.71 (t, *J* = 7.9 Hz, 1H, 4'); 7.12 (t, *J* = 7.9 Hz, 2H, (H3', H5')); 7.25 (d, *J* = 8.5 Hz, 1H, H6); 7.34 (s, 1H, H8); 7.88 (d, *J* = 8.5 Hz; 1H, H5); 8.13 (d, *J* = 1.25 Hz, 2H, (H2', H6')); 11.23 (s, 1H, CONH); 11.33 (s, 1H, NH1'); 12.01 (s, 1H, NH1); 16.41 (s, 1H, 4-OH); ^{13}C NMR (DMSO-d_6 and NaOD) δ 96.8 (C3); 112.8 (4'); 113.4 (C4a); 115.6 (C8); 119.8

(C2', C6'); 123.3 (C6); 126.5 (C5); 129.4 (C3', C5'); 139.0 (C7); 140.1 (C8a); 148.5 (C1'); 162.8 (C2); 171.4 (C4); 172.1 (CONH); FT IR: 3165 O-H; 3057 N-H; 1693 C=O amide cm^{-1}; HRMS *m/z*: calcd. 328.04944, 330.04650 for $C_{16}H_{12}ClN_3O_3$ $[M-H]^-$; found 328.04925, 330.0465. Anal. Calcd for $C_{16}H_{11}ClN_3O_3$: C, 58.28; H, 3.67; N, 12.74. Found: C, 58.34; H, 3.71; N, 12.82.

***N*-(Pyridin-5-yl)-7-chloro-4-hydroxy-2-quinolone-3-carboxamide (8):** white solid; yield 85%; mp 296-299 ºC ; R_f 0.3 (9.6:0.4 $CHCl_3$–MeOH); ^{1}H NMR (DMSO-d_6 and NaOD) δ 6.52 (d, *J* = 7.8 Hz, 1H, H6); 6.9 (s, 1H, H8); 7.17 (t, *J* = 7.6 Hz, 1H, H5'); 7.78 (d, *J* = 8.35 Hz, 1H, H5); 8.02 (d, *J* = 3.5 Hz, 1H, H5); 8.14 (d, *J* = 7.9 Hz, 1H, H4'); 8.74 (s. 1H, H2'); 15.26 (s, 1H, OH); ^{13}C NMR (DMSO-d_6 and NaOD) δ 100.6 (C3); 115.4 (C6); 121.1 (C8); 122.1 (C84a); 123.6 (C5'); 126.2 (C4'); 127.3 (C5); 133.7 (C7); 138.6 (C1'); 141.3 (C2'); 141.9 (C6'); 151.8 (C8a); 170.3 (C2); 174.3 (C4); 177.2 (CONH); FT IR: 3057 O-H; 2926 N-H; 1668 C=O amide cm^{-1}; HRMS *m/z*: calcd. 314.03379, 316.03084 for $C_{15}H_9ClN_3O_3$ $[M-H]^-$; found 314.03285, 316.03284. Anal. Calcd for $C_{15}H_{10}ClN_3O_3$: C, 57.06; H, 3.19; N, 13.31. Found: C, 57.12; H, 3.25; N, 13.37.

***N*-(4-Hydroxyphenyl)-7-chloro-4-hydroxy-2-quinolone-3-carboxamide (9):** brown solid; yield 81%; mp 324-326 ºC; R_f 0.4 (9.6:0.4 $CHCl_3$–MeOH); ^{1}H NMR (DMSO-d_6 and NaOD) δ 6.79 (d, *J* = 8.6 Hz, 2H, (H3', H5')); 7.33 (d, *J* = 8.5 Hz, 1H, H6); 7.41 (m, 3H, (H2', H6', H8)); 7.9 (d, *J* = 8.5 Hz, 1H, H5); 9.4 (s, 1H, OH4'); 12.09 (s, 1H, NH1); 12.2 (s, 1H, NH1'); 16.85 (s, 1H, OH); ^{13}C NMR (DMSO-d_6 and NaOD) δ 97.3 (C3); 113.6 (C4a); 115.6 (C8); 116.0 (C3', C5'); 123.0 (C2', C6'); 123.3 (C6); 126.6 (C5); 128.5 (C1'); 138.9 (C8a); 139.8 (C7); 155.2 (C4'); 163.2 (C2); 168.5 (C4); 172.2 (CONH); FT IR: 3290 O-H; 3072 N-H; 1674 C=O amide cm^{-1}; HRMS *m/z*: calcd. 329.0334, 331.03051 for $C_{16}H_{12}ClN_2O_4$ $[M+H]^+$; found 329.03578, 331.03293. Anal. Calcd for $C_{16}H_{11}ClN_2O_4$: C, 58.11; H, 3.35; N, 8.47. Found: C, 58.17; H, 3.41; N, 8.53.

***N*-(4-Methyl(phenyl)sulfane)-7-chloro-4-hydroxy-2-quinolone-3-carboxamide (10):** brown solid; yield 80%; mp 290 ºC (decom.); R_f 0.76 (9.6:0.4 $CHCl_3$–MeOH); ^{1}H NMR (DMSO-d_6 and NaOD) δ 2.36 (s, CH_3); 6.49 (d, *J* = 8.2 Hz, 1H, H6); 6.88 (s, 1H, H8); 7.09 (d, *J* = 8.0 Hz, 2H, (H3', H5')); 7.61 (d, *J* = 8.0 Hz, 2H, (H2', H6'); 7.7 (d, *J* = 8.3 Hz, 1H, H5); ^{13}C NMR (DMSO-d_6 and NaOD) δ 16.8 (CH_3); 100.9 (C3); 115.3 (C6); 120.4 (C2', C6'); 121.0 (C8); 122.2 (C4a); 127.3 (C5); 128.3 (C3', C5'); 133.5 (C7); 139.9 (C1'); 151.8 (C8a); 169.9 (C2); 174 (C4); 176 (CONH). FT IR: 3064 O-H; 2920 N-H; 1670 C=O amide cm^{-1}; HRMS *m/z*: calcd. 359.002626, 361.02328 for $C_{17}H_{12}ClN_2O_3S$ $[M-H]^-$; found 359.02761, 361.0503. Anal. Calcd for $C_{17}H_{13}ClN_2O_3S$: C, 56.59; H, 3.63; N, 7.76. Found: C, 56.65; H, 3.69; N, 7.82.

***N*-(4-Methylphenyl)-7-chloro-4-hydroxy-2-quinolone-3-carboxamide (11):** pale yellow solid; yield 81%; mp 311 ºC (decom.); R_f 0.41 (9.9:0.1 $CHCl_3$–MeOH); ^{1}H NMR (DMSO-d_6 and NaOD) δ 2.17 (s, 3H, CH_3); 6.48 (dd, *J* = 1.8, 1,75 Hz, 1H, H6); 6.86 (s, 1H, H8); 6.96 (d, *J* = 8.2 Hz, 2H, (H2', 6')); 7.51 (d, *J* = 8.2 Hz, 2H, (H3', H5')); 7.75 (d, *J* = 8.4 Hz, 1H, H5); ^{13}C NMR (DMSO-d_6 and NaOD) δ 20.6 (CH_3); 101.0

(C3); 114.8 (C6); 115.1 (C4a); 119.4 (C2', C6'); 120.6 (C8); 127.0 (C5); 129.0 (C3', C5'); 132.4 (C1'); 133.4 (C7); 139.4 (C4'); 151.7 (C8a); 172.9 (C2); 174.2 (C4); 176.6 (CONH); FT IR: 3034 O-H; 2916 N-H; 2845 C=O amide cm^{-1}; HRMS *m/z*: calcd. 327.05419, 329.05125 for $C_{17}H_{12}N_2ClO_3$ $[M-H]^-$; found 327.05300, 329.05050. Anal. Calcd for $C_{17}H_{13}ClN_2O_3$: C, 62.11; H, 3.99; N, 8.52. Found: C, 62.17; H, 4.05; N, 8.58.

***N*-(3-(Trifluoromethyl)phenyl)-7-chloro-4-hydroxy-2-quinolone-3-carboxamide (12):** off white solid; yield 80%; mp 280 ºC (decom.); R_f 0.6 (9.9:0.1 $CHCl_3$–MeOH); ^{1}H NMR (DMSO-d_6 and NaOD) δ 6.52 (d, *J* = 8.2 Hz, IH, H6); 6.89 (s, 1H, H8); 7.13 (d, *J* = 6.9 Hz, 1H, H4'); 7.35 (t, *J* = 7.7 Hz, 1H, H5'); 7.71 (d, *J* = 7.3 Hz, 1H, H6'); 7.77 (d, *J* = 8.3 Hz, 1H, H5); 8.18 (s, 1H, H2'); ^{13}C NMR (DMSO-d_6) δ 100.7 (C3); 115.5 (C6,C2'); 117.3 (C4'); 121.1 (C8); 121.7 (C8); 123.0 (C6'); 123.8 (C4a); 127.3 (C5); 129.6 (t, J_{C-F}= 124.1 Hz, CF_3); 129.8 (C5'); 133.7 (C7); 142.6 (C1'); 151.8 (C8a); 170.1 (C2); 174.1 (C4); 177.1 (CONH); FT IR: 3151 O-H; 3061 N-H; 1676 C=O amide cm^{-1}; HRMS *m*/z: calcd. 381.02593, 383.02298 for $C_{17}H_9ClF_3N_2O_3$ $[M-H]^-$; found 381.02333, 383.02053. Anal. Calcd for $C_{17}H_{10}ClF_3N_2O_3$: C, 62.11; H, 3.99; N, 8.52. Found: C, 62.17; H, 4.05; N, 8.58.

***N*-(4-(Trifluoromethyl)phenyl)-7-chloro-4-hydroxy-2-quinolone-3-carboxamide (13):** white solid; yield 83%; mp 312-315 ºC ; R_f 0.6 (9.9:0.1 $CHCl_3$–MeOH); ^{1}H NMR (DMSO-d_6 and NaOD) δ 6.5 (d, *J* = 8.25, 1H, H6); 6.9 (s, 1H, H8); 7.4 (d, *J* = 7.9 Hz, 2H, (H2', H6')); 7.8 (d, *J* = 8.3 Hz, 1H, H5); 7.8 (d, *J* = 8.15 Hz, 2H, (H3', H5')); ^{13}C NMR (DMSO-d_6 and NaOD) δ 100.7 (C3); 115.4 (C6); 119.2 (C3', C5'); 120.6 (t, J_{C-F} = 112.7 Hz, CF_3); 121.2 (C8); 122.2 (C4a); 124.2 (8a); 126.0 (t, J_{C-F} = 153.5 Hz, C2', C6'); 127.3 (C5); 133.7 (C7); 145.5 (C4'); 151.1 (C1'); 170.2 (C2); 174.3 (C4); 177.3 (CONH); FT IR: 3005 O-H; 2926 N-H; 1672 C=O amide cm^{-1}; HRMS *m/z*: calcd. 381.02593, 383.02298 for $C_{17}H_9ClF_3N_2O_3$ $[M-H]^-$; found 381.0246, 383.02277. Anal. Calcd for $C_{17}H_{10}ClF_3N_2O_3$: C, 53.35; H, 2.63; N, 7.32. Found: C, 53.41; H, 2.69; N, 7.38.

***N*-(2-(Trifluoromethyl)phenyl)-7-chloro-4-hydroxy-2-quinolone-3-carboxamide (14):** white solid; yield 88%; mp 242-244 ºC; R_f 0.5 (9.9:0.1 $CHCl_3$–MeOH); ^{1}H NMR (DMSO-d_6 and NaOD) δ 7.30 (d, *J* = 8.6 Hz, 1H, H6); 7.83 (m, 2H, (H8, H5')); 7.68 (t, *J* = 7.8 Hz, Hz, 1H, H4'); 7.75 (d, *J* = 7.9 Hz, 1H, H6'); 7.95 (d, *J* = 8.6 Hz, 1H, H5); 8.06 (d, *J* = 8.2 Hz, 1H, H3'); 12.07 (s, 1H, NH1'); 12.62 (s, 1H, NH1); 16.09 (s, 1H, OH); ^{13}C NMR (DMSO-d_6) δ 114.9 (C8); 115.7 (C6'); 122.3 (C6); 123.51 (C5); 123.52 (C2'); 126.3 (t, J_{C-F} = 64.6 Hz, C5'); 133.7 (C4'); 170.06 (C2); FT IR: 3163 O-H; 3066 N-H; 1656 C=O amide cm^{-1}; HRMS *m/z*: calcd. 381.02593, 383.02298 for $C_{17}H_9ClF_3N_2O_3$ $[M-H]^-$; found 381.02483, 383.02193. Anal. Calcd for $C_{17}H_{10}ClF_3N_2O_3$: C, 62.11; H, 3.99; N, 8.52. Found: C, 62.17; H, 4.05; N, 8.58.

Cell Line and Culture Conditions. Human breast adenocarcinoma cell line MDA-231, human colon cancer HCT-116, and Caco-II cell line were purchased from ATCC. Cell lines were cultured in high glucose Dulbecco's modified eagle medium (DMEM) (Invitrogen, USA) containing 10% heat inactivated fetal bovine serum (HI-FBS) (Invitrogen), 2 mmol L^{-1} of L-glutamine, 50 U mL^{-1} of penicillin and 50 μg mL^{-1}

of streptomycin. Cell lines were maintained at 37 ºC in a 5% CO_2 atmosphere of 95 humidity.

Cell proliferation assay. Viable cell count was assessed with the 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl-tetrazolium bromide (MTT) colorimetric assay in which the yellow tetrazolium dye [MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt] was reduced by metabolically capable cells into an intracellular purple formazan product. The extent of tetrazolium dye reduction was determined by measuring the absorbance at 490 nm.

The examined compounds were first dissolved in a DMSO to yield a final 1 mM stock solution. This stock solution was then used to prepare various concentrations of each compound in the growth media. Five different points of concentrations were used to obtain the IC_{50} values. Final concentration of DMSO was maintained steady in all treated groups within a given experiment and never exceeded 0.1%. Cells were seeded at a density of 1×10^4 cell per well in 96-well culture plates, maintained in DMEM media, and allowed to adhere overnight. After 24 h, the cells were treated with the different concentrations, in three triplicates for each concentration, and incubated at 37 ºC in a 5% CO_2 incubator, for 48 h. When the treatment period ended, MTT assay was carried out and the absorbance at 490 nm was read on a plate reader (Tecan Group Ltd., Switzerland). Each test was carried out in triplicate in three independent experiments (n=9). Control wells were prepared under the same experimental conditions. Doxorubicin and Ly294002 were used as positive controls. DMSO concentration ranges shouldn't exceed 1%.

ACKNOWLEDGEMENTS

Authors gratefully acknowledge the financial support from the University of Jordan, Deanship of Scientific Research.

REFERENCES

1. R. L. Siegel, K. D. Miller, and A. Jemal, *CA Cancer J. Clin.*, 2018, **68**, 7.
2. R. N. Khaybullin, M. Zhang, J. Fu, X. Liang, T. Li, A. R. Katritzky, P. Okunieff, and X. Qi, *Molecules*, 2014, **19**, 18676.
3. R. Chadha, V. Kapoor, D. Thakur, R. Kaur, P. Arora, and D. Jain, *J. Sci. Ind. Res.*, 2008, **67**, 185.
4. R. A. Al-Qawasmeh, M. M. Abadleh, J. A. Zahra, M. M. El-Abadelah, R. Albashiti, F. Zani, M. Incerti, and P. Vicini, *J. Enzyme Inhib. Med. Chem.*, 2014, **29**, 777; R. A. Al-Qawasmeh, B. B. Huthail, M. O. Sinnokrot, M. H. Semreen, R. A. Odeh, M. H. Abu-Zarga, H. Tarazi, I. A. Yousef, and T. H. Al-Tel, *Med. Chem.*, 2016, **12**, 563.
5. T. Ichikawa, J. C. Lamb, P. I. Christensson, B. Hartley-Asp, and J. T. Isaacs, *Cancer Res.*, 1992, **52**, 3022; J. T. Isaacs, *Expert Opin. Investig. Drugs*, 2010, **19**, 1235.
6. M. Rowley, J. J. Kulagowski, A. P. Watt, D. Rathbone, G. I. Stevenson, R. W. Carling, R. Baker, G.

R. Marshall, J. A. Kemp, and A. C. Foster, *J. Med. Chem.*, 1997, **40**, 4053; P. Molnár and S. L. Erdõ, *Eur. J. Pharmacol.*, 1996, **311**, 311; C. A. Hicks, M. A. Ward, N. Ragumoorthy, S. J. Ambler, C. P. Dell, D. Dobson, and M. J. O'Neill, *Brain Res.*, 1999, **819**, 65.

7. L. S. Reddy, N. J. Babu, and A. Nangia, *Chem. Commun.*, 2006, **13**, 1369.
8. D. A. Sabbah, B. Hishmah, K. Sweidan, S. Bardaweel, M. AlDamen, H. A. Zhong, R. Abu Khalaf, A. Hasan Ibrahim, T. Al-Qirim, and G. Abu Sheikha, *Anticancer Agents Med. Chem.*, 2018, **18**, 263; D. A. Sabbah, S. E. Hasan, R. Abu Khalaf, S. K. Bardaweel, R. Hajjo, K. M. Alqaisi, K. A. Sweidan, and A. M. Al-Zuheiri, *Molecules*, 2020, **25**, 5348.
9. D. A. Sabbah, R. A. Haroon, S. K. Bardaweel, R. Hajjo, and K. Sweidan, *Molecules*, 2021, **26**, 73.
10. K. Naumann, *J. Prakt. Chem.*, 1999, **341**, 417.
11. W. Xue, X. Li, G. Ma, H. Zhang, Y. Chen, J. Kirchmair, J. Xia, and S. Wu, *Eur. J. Med. Chem.*, 2020, **188**, 112022.
12. I. V. Ukrainets, L. V. Sidorenko, L. A. Petrushova, and O. V. Gorokhova, *Chem. Heterocycl. Compd.*, 2006, **42**, 64.
13. M. Bollenbach, S. Nemska, P. Wagner, G. Camelin, F. Daubeuf, A. Obrecht, P. Villa, D. Rognan, F. Bihel, J.-J. Bourguignon, M. Schmitt, and N. Frossard, *Molecules*, 2021, **26**, 391.