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Reactivity and Anticancer Assessment of 4-Hydroxyquinoline Derivatives

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Abstract—Ethyl 4-hydroxy-7-(trifluoromethyl) quinoline-3-carboxylate (**1**) exhibits a moderate cytotoxic activity against the MCF-7 mammary gland cancer cell line and HePG2 hepatocellular carcinoma cell line and a weak activity against the HCT-116 human colorectal carcinoma cell line. In order to enhance the cytotoxic activity of this compound, it was modified by changing the side-chain substituent and/or forming a new heterocyclic ring fused to the pyridine ring. Heating compound **1** with chloroacetyl chloride gave a mixture of two isomeric *O*-acylation products ethyl 4-(2-chloroacetoxy)-7-(trifluoromethyl)-quinoline-3-carboxylate and 3-chloro-8-(trifluoromethyl)-2*H*-pyrano[3,2-*c*]quinoline-2,4(3*H*)-dione, whereas the reaction with acetyl chloride in NaOH formed an *N*-acylation product 1-acetyl-4-oxo-7-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylic acid. The reactions of compound **1** with urea, thiourea, hydrazine hydrate, hydroxylamine, *o*-phenylenediamine, phenyl isothiocyanate, and ethyl acetoacetate yielded the corresponding condensation products 1-[4-oxo-7-(trifluoromethyl)-3,4-dihydroquinoline-3-carbonyl)]urea, 1-[4-oxo-7-(trifluoromethyl)-3,4-dihydroquinoline-3-carbonyl)]thiourea, 7-(trifluoromethyl)-1,2-dihydropyrazolo[4,3-*c*]quinolin-3-one, 7-(trifluoromethyl)isoxazolo[4,5-*c*]quinolin-3(2*H*)-one, 3-(trifluoromethyl)-13*H*-benzo[2,3][1,4]diazepino[6,5-*c*]quinolin-7-ol,3-phenyl-2-thioxo-8-(trifluoromethyl)-2,3-dihydro-4*H*-[1,3]oxazino[5,6-*c*]quinolin-4-one and ethyl 8-(trifluoromethyl)-2-methyl-4-oxo-4*H*-pyrano[3,2-*c*]quinolone 3-carboxylate,respectively. The structures of the synthesized compounds were confirmed by elemental analysis and spectral data.

Keywords: isoxazoloquinoline, pyrazoloquinoline, pyranoquinoline, cytotoxic activity

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INTRODUCTION

Quinoline nucleus exist in many natural and synthetic products [1] and has a wide range of biological activity as antimalarial [2], antibacterial [3], antifungal [4], antimicrobial [5], inhibiting agents [6] and anticancer agents [7].

Because cancer is one of the most health problems all over the world causing death [8], researches being accomplished to afford potential safe anticancer agents.

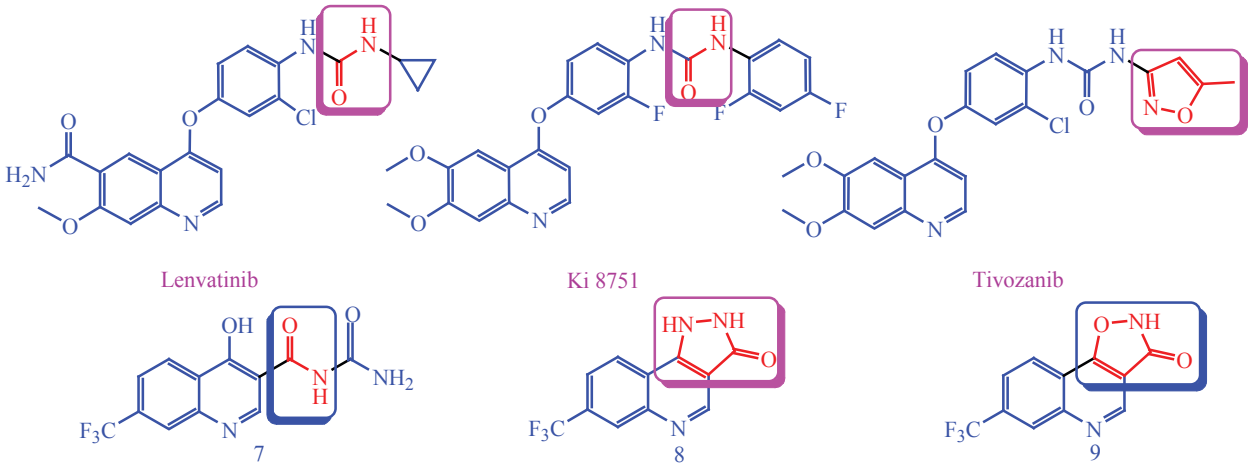
In the present work, quinoline ring was selected as an essential core due to its well-known antiproliferative activity through inhibition of different enzymes including topoisomerase [9], thymidylate synthase, telomeras [10] and different protein kinases [11]. VEGF-TKIs are valuable cancer therapy [12] that permit promising strategy for controlling different tumors [13]. Varied drugs containing quinoline nucleus as Tivozinib [14], Ki8751 [15], Lenvatinib [16] were recently approved for the therapy of various solid tumors.

RESULTS AND DISCUSSION

In the present work we synthesized novel derivatives of ethyl 4-hydroxy-7-(trifluoromethyl)-quinoline-3-carboxylate (**1**), where the 3- and 4-substitents were modified through different reactions so that the anticancer effect of the resulting compounds was enhanced compared to the starting compound. At the same time, we aimed that that the newly formed moiety in the synthesized quinoline derivatives, regardless of its position, contained the same structural fragment as Tivozinib, Ki 8751, or Lenvatinib (Scheme 1).

Compound **1** exists as an equilibrium mixture of three tautomers, with the abundance of each tautomer depending on the nature of the solvent (Scheme 2). In DMF, the most abundant form is enol **1b**, as evidenced by the presence in its IR spectrum of absorption bands at 3121, 1690, and 1617 cm^{-1} assignable respectively

Scheme 1.



to the OH group, chelated C=O group, and C=N bond.

The predominance of tautomer **1b** was also proved by its chemical behavior: as a nucleophile due to the presence of the OH group or as an electrophile due to the presence of the ester group.

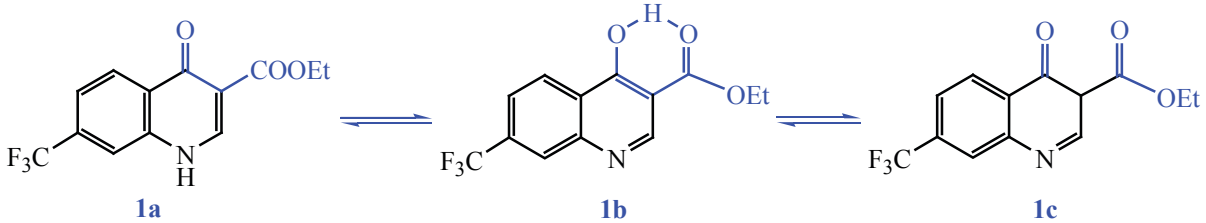
The reaction of 4-hydroxyquinoline derivative **1** with chloroacetyl chloride under reflux for 12 h in the presence of pyridine as a base catalyst afforded acylation product **2**. The structure of the latter was confirmed by the observation in its IR spectrum of absorption bands at 1720 and 1693 cm^{-1} from the two ester groups, as well as by the presence in the ^1H NMR spectrum of a singlet at 2.73 ppm from the CH_2Cl methylene protons together with a quartet at 4.25 ppm and a triplet at 1.43 ppm for the ester ethyl group in the. At the same time, refluxing the same reactants for 24 h under the same conditions gave a mixture of stereo isomeric condensation products **3a–3c**, which was confirmed by spectral data. The IR spectrum showed an absorption band at 3450 cm^{-1} assignable to enolic OH group, as well as bands at 1717 and 1680 cm^{-1} from the ester and ketone carbonyls, respectively. Evidence for the formation of the three isomers was also provided by the presence in the ^1H NMR spectrum of two one-proton singlet signals at 13.53 and 15.01 ppm from two enolic OH groups and a one-proton singlet signal at 3.88 ppm from the methane proton. The mass spectrum of the product showed a parent peak at *m/z* 315 (Scheme 3).

Attempted acylation of compound **1** with acetyl chloride in a 10% alcoholic sodium hydroxide at room temperature under stirring involved acylation of the nitrogen atom with hydrolysis of the ester group to form the corresponding acid derivative **4** and a known quinolone-3-carboxylic acid **5** (mp 259–260°C, Scheme 4).

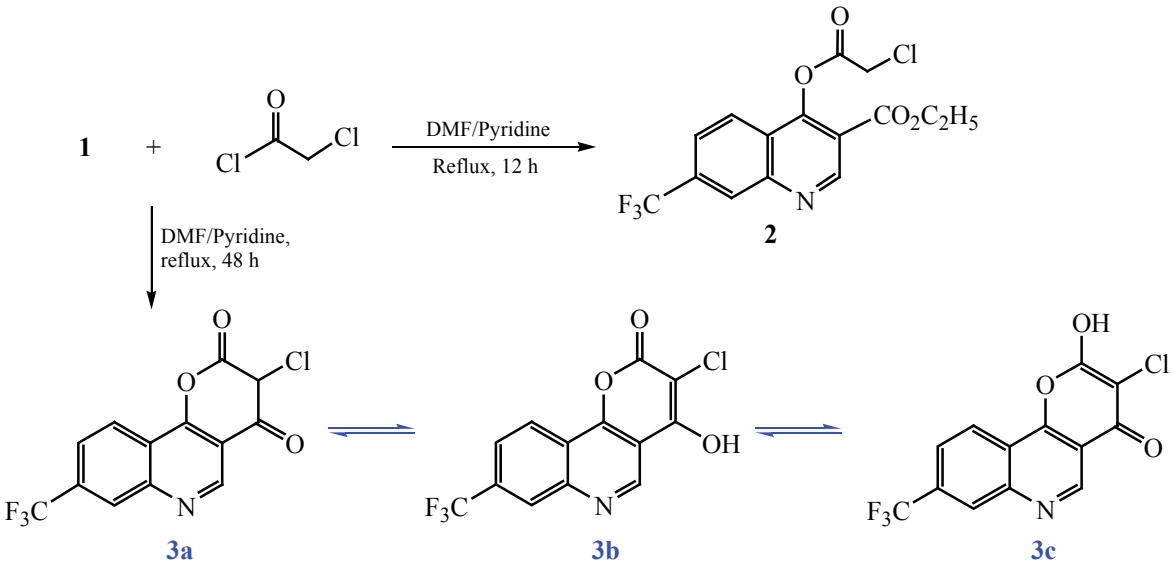
The structure of 1-acetyl-7-trifluoromethyl-4-oxo-1*H*-quinoline 3-carboxylic acid (**4**) was proved by its IR spectrum, which shows a broad absorption band at 3675–2570 cm^{-1} from the carboxylic OH group, as well as three absorption bands at 1692, 1685, and 1675 cm^{-1} from the three carbonyl groups. The ^1H NMR spectrum contains a singlet signal at 14.45 ppm from the COOH proton and a singlet signal at 2.13 ppm from the acetyl CH_3 protons. The mass spectrum displayed a peak at *m/z* 301 attributed to $[M + 2]$.

The ester group of quinoline derivative **1** was allowed to react with nucleophilic reagents, such as urea, thiourea, hydrazine hydrate, hydroxylamine and/or *o*-phenylenediamine. The reactions of **1** with urea

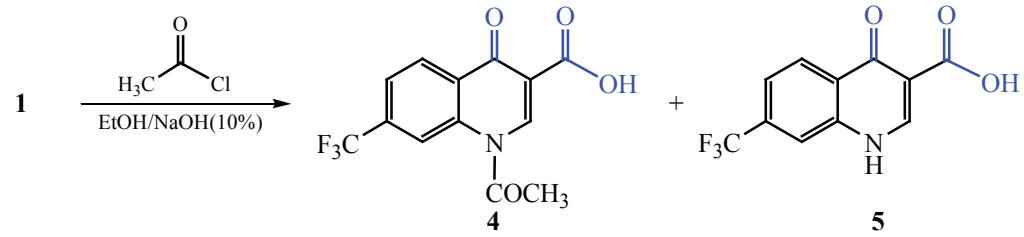
Scheme 2.



Scheme 3.



Scheme 4.



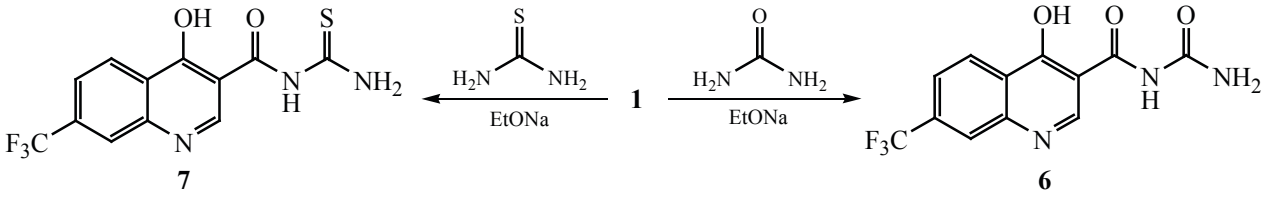
and/or thiourea in the presence of sodium ethoxide as a base catalyst under reflux gave carboxamide **6** and thiocarboxamide **7**, respectively (Scheme 5).

The structure of urea derivative **6** was confirmed by the presence of two absorption peaks at 1685 and 1656 cm^{-1} from the two amide carbonyl groups in the IR spectrum and a two-proton singlet at 1.86 ppm from the NH_2 group and a one-proton singlet at 11.35 ppm from the OH group in the ^1H NMR spectrum. The mass spectrum showed an $[M - NH_2]^+$ ion peak at *m/z* 283. The formation of thiourea derivative **7** was confirmed by the presence in the IR spectrum of NH, NH_2, and OH absorption bands at 3368, 3273, and 3167 cm^{-1}, respectively, and only one amide carbonyl absorption band at 1665 cm^{-1}; as well as by the presence of an and that was assured by the appearance of an $[M + 1]^+$ion peak at *m/z* 316.

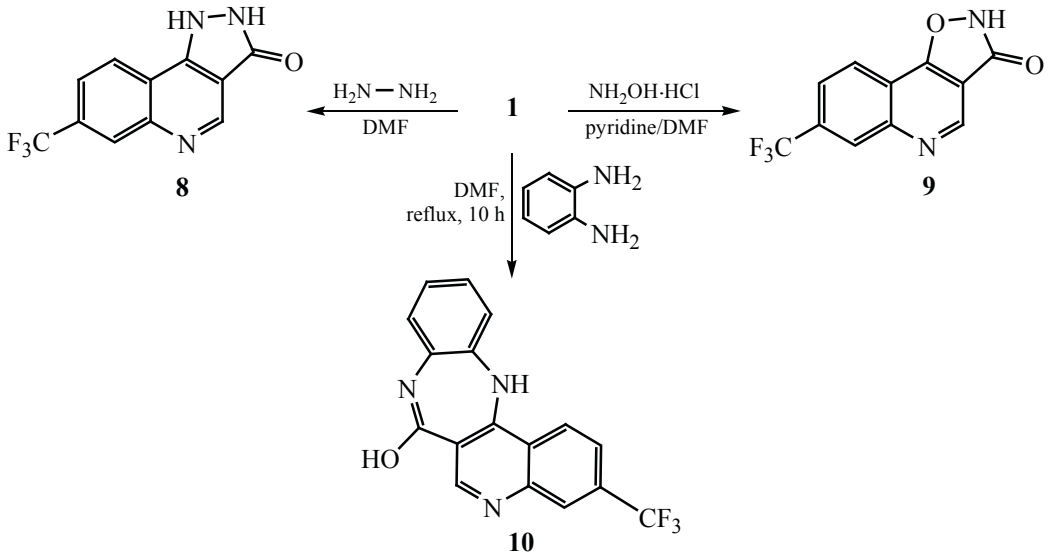
Pyrazoloquinoline **8**, isoxazoloquinoline **9**, and diazepinoquinoline **10** were obtained by heating quinoline derivative **1** with hydrazine hydrate in DMF, hydroxylamine hydrochloride in a mixture of pyridine and DMF, and *o*-phenylenediamine in DMF, respectively. The reactions involved initial attack of nitrogen nucleophile on the ester group and the subsequent cyclo condensation reaction (Scheme 6).

The IR spectra of compounds **8–10** showed bands of the ester carbonyl group but contained bands at 1673, 1679, and 1664 cm^{-1}, respectively, corresponding to the amide carbonyl, as well as the N–H absorption bands at 3326, 3243, and 3240 cm^{-1}, respectively. The ^1H NMR

Scheme 5.



Scheme 6.



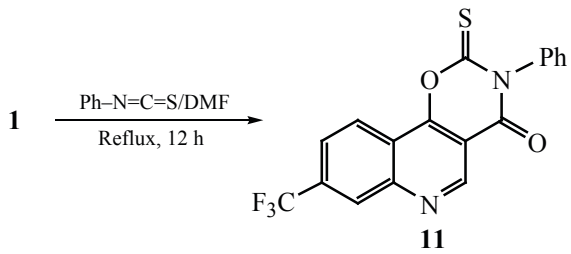
spectrum of compound **8** displayed two singlet signals at 10.57 and 13.25 ppm from the two NH protons, while in the spectrum of isoxazoloquinoline derivative **9** only one NH signal was observed as a singlet at 14.88 ppm. The ^{1}H NMR spectrum of benzodiazepine derivative **10** contained two singlets at 12.54 (broad) and 15.2 ppm, corresponding to the NH and OH protons, respectively. The mass spectrum showed a parent ion peak at *m/z* 329.

Oxazinoquinoline derivative **11** was obtained by the nucleophilic addition of the OH group of quinoline derivative **1** to phenyl isothiocyanate in boiling DMF followed by cyclization with the elimination of ethanol. The structure of compound **11** was supported by the IR spectrum, which showed an absorption band of amide carbonyl at 1667 cm^{-1} and no absorption bands of the OH and/or NH group. The ^{1}H NMR displayed a multiplet at 7.12–7.53 ppm corresponding to new five aromatic protons in addition to four quinoline nucleus protons (Scheme 7).

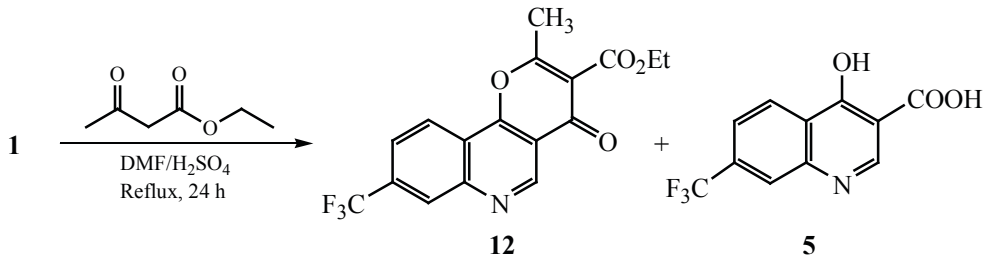
The reaction of quinoline derivative **1** with ethyl acetoacetate in the presence of H_2SO_4 as an acid catalyst gave pyranoquinoline **12** and hydrolysis product **5** (Scheme 8). The structure of compound **12** was confirmed by the presence in its IR spectrum of two absorption bands at 1715 and 1695 cm^{-1} corresponding to the ester and ketone carbonyls, respectively, and the absence of bands assignable to the OH and/or NH groups. Further evidence was obtained from the ^{1}H NMR spectrum, which showed a three-proton triplet at 1.42 ppm and a two-proton quarter at 3.95 ppm from the ester ethyl group, as well as a three-proton singlet at 2.31 ppm assignable to the C=C–CH_3 group.

Antitumor activity testing. All the synthesized quinoline derivatives were tested for their antitumor activity against three human tumor cell lines: hepatocellular carcinoma (HePG-2), colorectal carcinoma (HCT-116), and mammary gland (MCF-7). Doxorubicin was used as a standard anticancer drug for comparison. The results are presented in the table 1. Compound **8** exhibited the highest activity (very strong) against the three test cell lines. Compound **9** showed a high activity against MCF-7 and a strong activity against HePG2 and HCT-116. Compound **11** demonstrated a strong cytotoxic activity against the

Scheme 7.



Scheme 8.



HCT-116 and MCF-7 cell lines and a moderate activity against HePG2. Compound **7** was highly active against HePG2 and MCF-7 and moderately active against HCT-116. Compounds **4** and **6** showed a moderate activity against the three cell lines, while **2** and **12** showed a weak activity against the three cell lines. The latter two compounds proved to be even less active than the starting quinoline derivative **1**.

EXPERIMENTAL

The melting points were measured on a Gallen kamp electric melting point apparatus and were uncorrected. The IR spectra were recorded on a Pye Unicam SP-3-300 spectrophotometer in KBr. The ^{1}H NMR spectra were run on a Varian Mercury VX-300 and Bruker Avance III 400 MHz NMR spectrometers in DMSO-d_6 using TMS as internal standard. The mass spectra were recorded on a Shimadzu GCMS-QP-1000EX instrument at 70 eV. All chemical reactions were monitored by TLC on Silica Gel 60 F_{254} plates (Merck) in ethyl acetate-petroleum ether as solvent.

Ethyl 4-(2-chloroacetoxy)-7-(trifluoromethyl) quinoline-3-carboxylate (2). A mixture of quinoline ester **1** (1.4 g, 0.005 mol) and chloroacetyl chloride (0.62 g, 0.0055 mol) was refluxed in DMF (20 mL) in the presence of pyridine (2 mL) for 12 h. After completion of the reaction (by TLC) the reaction mixture poured into ice water and neutralized after cooling with dilute HCl. The solid material that formed was collected by filtration, dried, and crystallized from methanol to obtain 0.6 g (35%) of compound **2** as pale yellow crystals, mp 161–163°C. IR spectrum, ν, cm^{-1}: 2991 w and 2910 w (CH_{aliph}), 1720 s (C=O of $COCH_2Cl$), 1693 s (C=O ethyl ester), 1638 s (C=N). ^{1}H NMR spectrum, δ, ppm: 1.43 t (3H, CH_3), 2.73 s (2H, CH_2), 4.25 q (2H, CH_2), 7.72 d (1H, ArH, *J* 7.6 Hz), 8.01 s (1H, CH_2), 8.35 d (1H, ArH, *J* 8.8 Hz), 8.70 s (1H, ArH). Found, %: C 49.70; H 2.98; N 3.72. $C_{15}H_{11}ClF_3NO_4$. Calculated, %: C 49.81; H 3.07; N 3. 87. *M* 376.73.

3-Chloro-8-(trifluoromethyl)-2*H*-pyrano[3,2-*c*]-quinoline-2,4(3*H*)-dione (3). A mixture of the quinoline ester **1** (1.4 g, 0.005 mol) chloroacetyl chloride (0.62 g,

Table 1. Cytotoxic activity of the synthesized quinoline derivatives against human tumor cells

Compound no.	In vitro cytotoxic IC_{50}, μM[a]		
	HePG2	HCT-116	MCF-7
Doxorubicin	4.50±0.2	5.23±0.3	4.17±0.2
1	48.55±3.1	51.27±3.0	35.20±2.2
2	82.78±4.7	67.48±3.5	74.18±3.7
4	33.02±2.3	28.12±1.9	21.13±1.7
6	39.72±2.8	31.95±2.1	29.27±2.0
7	18.04±1.4	25.23±1.7	13.79±1.2
8	7.48±0.6	9.79±0.8	6.48±0.5
9	11.36±1.1	19.30±1.5	8.13±0.9
11	26.79±1.9	12.86±1.1	18.71±1.5
12	63.45±3.6	72.03±4.2	46.97±2.8

[a] IC_{50}, μM: 1–10 (very strong); 11–20 (strong); 21–50 (moderate); 51–100 (weak); and > 100 (noncytotoxic).

0.0055 mol) was refluxed in DMF (50 mL) in the presence of pyridine (2 mL) for 48 h. After completion of the reaction (by TLC), the reaction mixture poured over ice-water mixture after cooling and neutralized by dil. HCl. The formed solid collected by filtration, dried and crystallize from ethanol to give **1** g (65%) of compound **3** as white crystals; mp 194–196°C. IR spectrum, ν, cm^{-1}: 3450 s (OH), 2940 w (CH, aliph.), 1717 s (C=O ester), 1680 s (C=O ketonic). ^{1}H NMR spectrum, δ, ppm: 2.73 s (1H, CH–Cl), 3.88 s (1H, Cl–CH), 7.81 d (1H, ArH, *J* 9.6 Hz), 8.17 s (1H, ArH), 8.41 d (1H, ArH, *J* 8.0 Hz), 9.55 s (1H, ArH), 13.53 s (1H, OH), 15.01 s (1H, OH enolic). Mass spectrum, *m/z* (I_{rel}, %): 315 (2), 301 (18), 285 (42), 256 (60), 242 (100), 239 (47), 81 (26), 69 (60). Found, %: C 49.32; H 1.52; N 4.28. $C_{13}H_5ClF_3NO_3$. Calculated, %: C 49.47; H 1.60; N 4.44. *M* 315.63.

1-Acetyl-4-oxo-7-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylic acid (4). In alcoholic sodium hydroxide (30 mL-10%), was stirred with A mixture of quinoline derivative **1** (1.4 g, 0.005 mol) and acetyl chloride (0.4 g, 0.005 mol) in 10% alcoholic NaOH (30 mL) was stirred at temperature 40–45°C for 6 h. After completion of the reaction (by TLC), the reaction mixture poured into ice water and neutralized with dilute HCl. The formed solid precipitated was filtered, dried and crystallize from ethanol to give 0.8 g (53%) of compound **4** as white crystals; mp 157–159°C. IR spectrum, ν, cm^{-1}: 3675–2560 br.s (OH), 1692 s (C=O ketone), 1675 s (C=O amide). ^{1}H NMR spectrum, δ, ppm: 2.24 s (3H, CH_3), 7.47 d (1H, ArH), 8.01 s (1H, ArH), 8.37 d (1H, ArH), 8.79 s (1H, ArH), 14.45 s (1H, OH). Mass spectrum, *m/z* (I_{rel}, %): 301 (86) [*M* + 2], 282 (50), 249 (14), 77 (17), 57 (45), 43 (100). Found, %: C 51.98; H 2.61; N 4.55. $C_{13}H_8F_3NO_4$. Calculated, %: C 52.19; H 2.70; N 4.68. *M* 299.2.

Synthesis of compounds 6 and 7 (*general procedure*). A mixture of quinoline derivative **1** (1.4 g, 0.005 mol) and urea (3.4 g, 0.055 mol) or thiourea (4.2 g, 0.055 mol) was refluxed in ethanol (50 mL) in the presence of sodium ethoxide (0.5 g) for 5 h. After completion of the reaction (by TLC), the reaction mixture poured into ice water and neutralized with dilute HCl. The formed solid product was collected by filtration, dried, and crystallized from appropriate solvent.

1-[4-Oxo-7-(trifluoromethyl)-3,4-dihydroquinoline-3-carbonyl)]urea (6). Yield 0.5 g (36%), pale yellow crystals, mp 181–183°C (methanol). IR spectrum, ν, cm^{-1}: 3476 s, 3420 w (NH_2), 3137 (NH), 1697 s (C=O ketone) and 1656 s (C=O amide). ^{1}H NMR spectrum, δ, ppm: 1.86 s (3H, NH_2, NH), 7.48 d (1H, ArH, *J* 8.2 Hz), 8.0 s (1H, ArH), 8.37 d (1H, ArH, *J* 8.8 Hz), 8.79 s (1H, ArH), 11.35 s (1H, OH). Mass spectrum, *m/z* (I_{rel}, %): 283 [*M* – NH_2], 270 (17), 240 (18), 239 (100), 169 (10). Found, %: C 48.1; H 2.57; N 14.10. $C_{12}H_8N_3O_2F_3S$. Calculated, %: C 48.17; H 2.69; N 14.04. *M* 299.21.

1-[4-Oxo-7-(trifluoromethyl)-3,4-dihydroquinoline-3-carbonyl)]thiourea (7). Yield 0.6 g (40%), yellow crystals, mp 216–218°C (methanol). IR spectrum, ν, cm^{-1}: 3368 w, 3273 s (NH_2), 3167 s (NH), 1685 s (C=O ketone) and 1656 s (C=O amide). ^{1}H NMR spectrum, δ, ppm: 1.86 s (2H, NH_2), 7.49 d (1H, ArH), 8.11 s (1H, ArH), 8.47 d (1H, ArH), 8.91 s (1H, ArH), 11.35 s (1H, OH). Mass spectrum, *m/z* (I_{rel}, %): 316 (97) [*M* + 1], 257 (42), 255 (85), 159 (12), 127 (20), 63 (100). Found, %: C 46.01; H 2.67; N 13.28. $C_{12}H_8N_3O_2F_3S$. Calculated, %: C 45.72; H 2.56; N 13.33. *M* 315.27.

7-(Trifluoromethyl)-1,2-dihydropyrazolo[4,3-*c*]-quinolin-3-one (8). A mixture of compound **1** (1.4 g, 0.005 mol) and hydrazine hydrate 98% (2 mL, 0.06 mol) in DMF (30 mL) was refluxed for 72 h. After completion (by TLC) the reaction mixture poured over ice/water mixture, the solid that was filtered off, dried and crystallize from methanol to give 0.6 g (52%) of compound **8** as white crystals; mp 221–223°C. IR spectrum, ν, cm^{-1}: 3326 m (NH), 3243 s (NH), 1673 s (C=O). ^{1}H NMR spectrum, δ, ppm: 7.75 d (1H, ArH, *J* 9.2 Hz), 8.08 s (1H, ArH), 8.45 d (1H, ArH, *J* 8.8 Hz), 8.88 s (1H, ArH), 10.57 s (1H, NH), 13.25 s (1H, NH). Mass spectrum, *m/z* (I_{rel}, %): 252 (3) [*M* – 1], 239.89 (100), 211 (16), 183.91 (20), 163.85 (88), 136.89 (9). Found, %: C 51.98; H 2.57; N 16.48. $C_{11}H_6F_3N_3O$. Calculated, %: C 52.18; H 2.39; N 16.60. *M* 253.18.

7-(Trifluoromethyl)isoxazolo[4,5-*c*]quinolin-3(2*H*)–one (9). Pyridine (0.5 mL) was added to a mixture of quinoline derivative **1** (1.4 g, 0.005 mol) and hydroxylamine hydrochloride (0.34 g, 0.005 mol) in DMF (50 mL). The reaction mixture was refluxed for 48 h. After completion of the reaction (by TLC) and cooling, the reaction mixture was poured into ice water and neutralized with dilute HCl. The formed solid was filtered off and crystallized from ethanol to obtain 0.7 g (56%) of compound **9** as yellow crystals, mp 195–197°C. IR spectrum, ν, cm^{-1}: 1679 s (C=O), 1614 s (C=N). ^{1}H NMR spectrum, δ, ppm: 7.86 d (1H, ArH, *J* 8.0 Hz), 8.15 s (1H, ArH), 8.45 d (1H, ArH, *J* 8.8 Hz), 9.04 s (1H, ArH), 14.88 s (1H, NH). Mass spectrum, *m/z* (I_{rel}, %): 238 (4), 103 (10), 101 (25), 88 (87), 60 (100), 42(52). Found, %: C 51.82; H 1.89; N 10.98. $C_{11}H_5F_3N_2O_2$. Calculated, %: C 51.98; H 1.98; N 11.02. *M* 254.16.

3-(Trifluoromethyl)-13*H*-benzo[2,3][1,4]diazepino[6,5-*c*]quinolin-7-ol (10). A mixture of *o*-phenylene diamine (0.86 g, 0.005 mol) and quinoline ester **1** (1.4 g,

0.005 mol) was refluxed for 10 h in DMF (50 mL). After completion of the reaction (by TLC) the reaction mixture was cooled and poured over ice-water mixture. The precipitated solid formed was filtered off, dried and crystallize from methanol–benzene mixture (2 : 1) to give 0.4 g (26%) of compound **10** as brown crystals; mp 279–281°C. IR spectrum, ν, cm^{-1}: 3420 s (OH), 3240 m (NH), 1664 s (C=O), 1621 s (C=N). ^{1}H NMR spectrum, δ, ppm: 7.12–7.62 m (4H, ArH), 7.72 d (1H, ArH), 8.01 s (1H, ArH), 8.35 d (1H, ArH), 8.73 s (1H, ArH), 12.54 br.s (1H, NH), 15.2 s (1H, OH). Mass spectrum, *m/z* (I_{rel}, %): 329 (9), 314 (12), 301 (12), 297 (16), 93 (100), 76 (11). Found, %: C 61.85; H 2.98; N 12.61. $C_{17}H_{10}F_3N_3O$. Calculated, %: C 62.01; H 3.06; 17.31; N 12.76. *M* 329.28.

3-Phenyl-2-thioxo-8-(trifluoromethyl)-2,3-dihydro-4*H*-[1,3]oxazino[5,6-*c*]quinolin-4-one (11). Quinoline derivative **1** (1.4 g, 0.005 mol) was heated under reflux with phenylisothiocyanate (0.7 g, 0.005 mol) in DMF (50 mL) for 12 h. After completion (by TLC), the reaction mixture was cooled, poured over ice-water mixture to give the solid Product that was filtered off, dried and then crystallize from ethanol to give 1 g (56%) of compound **11** as yellow crystals of mp 172–174°C. IR spectrum, ν, cm^{-1}: 1667 s (C=O), 1630 s (C=N). ^{1}H NMR spectrum, δ, ppm: 7.12–7.53 m (5H, ArH), 7.87 d (1H, ArH), 8.19 s (1H, ArH), 8.36 d (1H, ArH), 8.78 s (1H, ArH). Found, %: C 57.68; H 2.38; N 7.35. $C_{18}H_9F_3N_2O_2S$. Calculated, %: C 57.75; H 2.42; N 7.48. *M* 390.83.

Reaction of compound 1with ethyl acetoacetate. Sulfuric acid (5 mL, 75%) was added to a mixture of compound **1** (1.4 g, 0.005 mol) and ethyl acetoacetate (7.2 g, 0.055 mol) in DMF (50 mL). The reaction mixture was heated under reflux on a water bath for 12 h, after completion (by TLC), the reaction mixture was cooled, the formed solid was separated and washed by Na_2CO_3 solution (50 mL, 20%) then filtered, washed by water, dried and crystallize from ethanol to give 1 g (65%) of compound **12** as brown crystals of mp above 300°C. The filtrate of carbonate solution was acidified by dil. HCl, separated and crystallized to give 0.2 g (18%) of compound **5** as white crystals; mp 259–260°C (Sigma-Aldrich, Pub. Chem. substance ID 24851573, mp 259–260°C).

Ethyl 8-(trifluoromethyl)-2-methyl-4-oxo-4*H*-pyrano[3,2-*c*]quinolone 3-carboxylate (12). IR spectrum, ν, cm^{-1}: 2975, 2941 w (CH aliph.), 1715 s (C=O ester), 1695 s (C=O ketone). ^{1}H NMR spectrum, δ, ppm: 1.42 t (3H, CH_3), 2.72 s (3H, CH_3), 3.95 q (2H, CH_2), 7.87 d (1H, ArH), 8.19 s (1H, ArH), 8.48 d (1H, ArH), 9.07 s (1H, ArH). Found, %: C 58.11; H 3.38; N 3.99. $C_{17}H_{12}NO_4F_3$. Calculated, %: C 58.13; H 3.44; N 3.99. *M* 381.12.

4-Hydroxy-7-(trifluoromethyl)-quinoline-3-carboxylic acid (5). IR spectrum, ν, cm^{-1}: 3550–2500 br.s (OH), 1683 s (C=O), 1616 s (C=N). ^{1}H NMR spectrum, δ, ppm: 7.58 d (1H, ArH, *J* 8.4 Hz), 8.21 s (1H, ArH), 8.53 d (1H, ArH), 9.10 s (1H, OH enolic), 14.88 s (1H, COOH). Found, %: C 51.17; H 2.22; N 5.36. $C_{11}H_6F_3NO_3$. Calculated, %: C 51.38; H 2.35. *M* 257.17.

MTT assay. The HePG-2 hepatocellular carcinoma, HCT-116 colorectal carcinoma, and MCF-7 mammary gland cancer cell lines were obtained from the ATCC via the Egyptian Holding Company for Biological Products and Vaccines (VACSERA), Cairo, Egypt. The RPMI-1640 medium, MTT, and DMSO were purchased from Sigma, and fetal bovine serum was obtained from GIBCO.

Cells were cultured in RPMI-1640 medium with 10% fetal bovine serum, 100 IU/mL Penicillin, and 100 μg/mL Streptomycin at 37°C in a 5% CO_2 incubator. The cell lines were seeded in a 96-well plate at a density of 1.0×10^4 cells/well at 37°C for 48 h under 5% CO_2 and then treated with different concentration of the test compounds. After 24-h incubation, 20 μL of a 5 mg/mL MTT solution was added, and incubation was continued for an additional 4 h. The purple formazan crystals that formed were dissolved in 100 μL of DMSO. Colorimetric assay was performed by measuring absorbance of 570 nm on a Bio TekEXL 800 plate reader. The percentage relative cell viability was calculated as (A_{570} of treated sample/A_{570} of untreated sample) × 100.

CONCLUSIONS

The moderate cytotoxicity of the starting 4-hydroxy-3-carboethoxyquinoline derivative **1** was increased by converting to urea and thiourea derivative, where the former become of strong toxicity to two cell lines (HePG2 and MCV-7), moderate for HCT-116, while the latter become of moderate toxicity for all the three cell lines as *N*-acetyl quinoline derivative **4**.

The cytotoxicity was again increased through cyclization around the positions 3- and 4- to result in very strong behavior for the dihydropyrazolone derivative **8** with respect to all the 3 cell lines, still of strong effect for the isoxazolone derivative **9**. The cyclic oxazine derivative **11** shows strong toxicity to HCT-116 and MCV-7 and moderate effect for HePG2cell, whereas oxopyrano derivative **12** was moderate for MCF-7 and weak for both HePG2 and HCT-116. However, the toxicity behavior of compound **1** becomes very week by alkylating the hydroxyl group at position C^4 to give chloroacetoxy derivative **2**.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Markees, D.G., Dewey, V.C., and Kidder, G.W., *J. Med. Chem*., 1970, vol. 13, p. 324. https://doi.org/10.1021/jm00296a048
2. Cunico, W., Cechinel, C.A., Bonacorso, H.G., Martins, M.A., Zanatta, N., de Souza, M.V., Freitas, I.O., Soares, R.P., and Krettli, A.U., *Bioorg. Med. Chem. Lett.*, 2006, vol. 16, p. 649. https://doi.org/10.1016/j.bmcl.2005.10.033
3. Kayirere, M., Mahmoud, A., Chevalier, J., Soyfer, J., Cremieux, A., and Barbe, J., *Eur. J. Med. Chem.*, 1998, vol. 33, p. 55. https://doi.org/10.1016/S0223-5234 (9980076-2)
4. Ryu, C.K., Sun, Y.J., Shim, J.Y., You, H.J., Choi, K.U., and Lee, H., *Arch. Pharm. Res.*, 2002, vol. 25, p. 795. https://doi.org/10.1007/BF02976994
5. Wise, R., Andrews, J.M., and Edwards, L.J., *Antimicrob. Agents Chemother*., 1983, vol. 23, p 559. https://doi.org/10.1128/AAC.23.4.559
6. Liew, C., Schut, H.A., Chin, S.F., Pariza, M.W., and Dashwood, R.H., *Carcinogenesis*, 1995, vol. 16, p. 3037. https://doi.org/10.1093/carcin/16.12.3037
7. Eelderfield, R.C. and LeVon, E.F., *J. Org. Chem*., 1960, vol. 25, p. 1576. https://doi.org/10.1021/jo01079a027
8. Jemal, A., Bray, F., Center, M. M., Ferly, J., Ward, E., and Forman, D., *CA Cancer J. Clin*., 2011, vol. 61, p. 69. https://doi.org/10.3322/caac.20107. Epub 2011 Feb 4.
9. Nitiss, J.L., *Nat. Rev. Cancer*, 2009, vol. 9, p. 338. https://doi.org/10.1038/nrc2607
10. Zhou, J.L., Lu, Y.J., Ou, T.M., Zhou, J.M., Huang, Z.S., Zhu, X.F., Du, C.J., Bu, X.Z., Ma, L., Gu, Q., Li, Y.M., and Chan, A.S., *J. Med. Chem.*, 2005, vol. 48, p. 7315. https://doi.org/10.1021/jm050041b
11. Carta, D., Bortolozzi, R., Hamel, E., Basso, G., Moro, S., Viola, G., and Ferlin, M.G., *J. Med. Chem*., 2015, vol. 58, p. 7991. https://doi.org/10.1021/acs.jmedchem.5b00805
12. Hojjat-Farsangi, M., *Int. J. Mol. Sci*., 2014, vol. 15, p. 13768. https://doi.org/10.3390/ijms150813768
13. Goel, H.L. and Mercurio, A.M., *Nat. Rev. Cancer*, 2013, vol. 13, p. 871. https://doi.org/10.1038/nrc3627
14. Jamil, M.O., Hathaway, A., and Mehta, A., *Curr. Oncol. Rep*., 2015, vol. 17, p. 24. https://doi.org/10.1007/s11912-015-0451-3
15. LaMontagne, K.R., Butler, J., Borowski, V.B., Fuentes-Pesquera, A.R., Blevitt, J.M., Huang, S., Li, R., Connolly, P.J., and Greenberger, L.M., *Angiogenesis*, 2009, vol. 12, p. 287.
16. Nakagawa, T., Matsushima, T., Kawano, S., Nakazawa, Y., Kato, Y., Adachi, Y., Abe, T., Semba, T., Yokoi, A., Matsui, J., Tsuruoka, A., and Funahashi, Y., *Cancer Sci*., 2014, vol. 105, p. 723. https://doi.org/10.111/cas.12409