Med Chem Res
DOI 10.1007/s00044-011-9965-x

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and antitumor activities of certain novel 2-amino-9-(4-halostyryl)-4*H*-pyrano[3,2-*h*]quinoline derivatives

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Received: 26 September 2011 / Accepted: 16 December 2011

Abstract A series of (*E*) 4*H*-pyrano[3,2-*h*]quinoline-3-carbonitrile (**5a–f**) and (*E*) ethyl 4*H*-pyrano[3,2-*h*]quinoline-3-carboxylate (**6a–f**) derivatives were synthesized by interaction of (*E*) 2-(4-chloro/bromo/fluorostyryl)-8-hydroxyquinoline (**3a–c**) with α-cyano-*p*-chloro/bromocinnamonitriles (**4a**,**b**) and ethyl α-cyano-*p*-chloro/bromocinnamates (**4c**,**d**), respectively. Structures of these compounds were established on the basis of IR, ^{1}H NMR, ^{13}C NMR, ^{13}C NMR–DEPT, ^{13}C NMR–APT, and MS data. The new compounds were evaluated for antitumor activities against three different human tumor cell lines MCF-7, HCT, and HepG-2. The results of antitumor evaluation revealed that compounds **5a**,**d** and **6a**,**c**,**d** inhibited the growth of cancer cells compared to Vinblastine. The structure–activity relationships were discussed.

Keywords (*E*) 2-(4-chloro/bromo/fluorostyryl)-8-hydroxyquinoline · α-Cyano-*p*-chloro/bromocinnamonitriles · Ethyl α-cyano-*p*-chloro/bromocinnamates · 4*H*-pyrano[3,2-*h*]quinolines · Antitumor · SAR

Introduction

Quinoline moiety is present in many classes of biologically active compounds (Ganesh *et al*., 2008; Larghi *et al*., 2009; Liu *et al*., 2009; Musiol *et al*., 2006a, b, 2007; Narender *et al*., 2006, Ramesh *et al*., 2009; Righi *et al*., 2008; Vazquez *et al*., 2004). The biological activities of quinoline derivatives depend not only on the bicyclic hetero-aromatic pharmacophore but also on the nature of the peripheral substituents and their spatial relationships.

They also exhibit antimalarial (Kaur *et al*., 2009), antitumor (Behforouz *et al*., 2007) antioxidant (Abas *et al*., 2006), antileishmanial (Rocha *et al*., 2005), and anti-platelet activities (Kuo *et al*., 2001). In addition, they function as pharmacologically active synthetic compounds (Watson *et al*., 2001) such as DNA binding capabilities (Atwell *et al*., 1989) and as DNA-intercalating carrier (Chen *et al*., 2000). A series of compounds derived from 8-hydroxyquinoline as potential HIV-1 integrate inhibitors were synthesized (Majerz-Maniecka *et al*., 2005). In addition styrylquinoline derivatives have gained strong attention due to their activities as perspective HIV integrase inhibitors (Jiang *et al*., 1990; Mekouar *et al*., 1998; Polanski *et al*., 2002; Pommier *et al*., 2005; Thomas and Roy, 2008; Zouhiri *et al*., 2005) and also for their extensive biological activities (Ganesh *et al*., 2008; Larghi *et al*., 2009; Liu *et al*., 2009; Mekouar *et al*., 1998; Narender *et al*., 2006).

In view of the above observations, and in continuation of our program on the chemistry of 4*H*-pyran derivatives (Abd-El-Aziz *et al*., 2004, 2007; Bedair *et al*., 2000, 2001; Eid *et al*., 2003; El-Agrody, 1994; El-Agrody *et al*., 1997a, b, 2000, 2001, 2002, 2011; Khafagy *et al*., 2002; Sayed *et al*., 2000; Sabry *et al*., 2011), it seemed interesting to synthesize new 4*H*-pyrano[3,2-*h*]quinoline derivatives by means of α-cyano-*p*-halocinnamonitriles, ethyl α-cyano-*p*-halocinnamates and evaluatation of their antitumor activities. The chemical structure of the studied compounds and structure–activity relationships (SAR) are discussed in this study.

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Published online: 04 January 2012

Chemistry

Condensation of 8-hydroxy-2-methylquinoline (**1**) with *p*-chlorobenzaldehyde and *p*-bromobenzaldehyde in acetic anhydride under reflux afforded (*E*) 2-(4-chloro/bromo-styryl)-8-hydroxyquinoline (**3a**,**b**) via the intermediate (*E*) 8-acetoxy-2-(4-chloro/bromo-styryl)quinoline (**2a**,**b**) (Musiol *et al*., 2006a, b, 2007), while condensation of **1** with *p*-chlorobenzaldehyde, *p*-bromobenzaldehyde, and *p*-fluorobenzaldehyde under Microwave irradiation furnished (*E*) 2-(4-chloro/bromo/fluorostyryl)-8-hydroxyquinoline (**3a–c**) (Chang *et al*., 2010; Musiol *et al*., 2006a, b, 2007) (Scheme 1).

The structures of **2** and **3** were established on the basis of spectral data. The IR spectra of **2b** showed the presence of a CO stretch at υ 1760 cm^{-1}, while for **3b**,**c** showed the appearance of a OH stretch at υ 3348–3399 $cm.^{-1}$ The ^{1}H and ^{13}C NMR spectra of **2b** revealed the presence of signals at δ 7.65 (d, $J = 16$ Hz, 1H, =CH), 7.50 (d, $J = 16$ Hz, 1H, =CH), 2.60 ppm (s, 3H, $COCH_3$) and 134.39 (=CH), 129.40 (=CH), 21.06 ppm (CH_3). Characteristic resonances were observed at δ 9.62 (bs, 1H, OH), 8.15–8.13 (d, $J = 16$ Hz, 1H, =CH), 7.52–7.45 ppm (d, $J = 16$ Hz, 1H, =CH) and 133.10–133.00 (=CH), 129.14–129.05 ppm (=CH) for **3b**,**c**. The ^{13}C NMR–DEPT spectra at 45°, 90° and 135° of **3b** and the MS spectra of **2b** and **3b**,**c** provided additional evidences in support of the proposed structures.

The relative (*E*) configuration of compounds **2** and **3** were established from the coupling constant values ($J = 16$ Hz).

Treatment of (*E*) 2-(4-chloro/bromo/fluorostyryl)-8-hydroxyquinoline (**3a–c**) with α-cyano-*p*-chloro/bromo-cinnamonitrile (**4a**,**b**) and ethyl α-cyano-*p*-chloro/bromo-cinnamate (**4c**,**d**) in ethanol and piperidine under reflux afforded (*E*) 2-amino-4-(4-chloro/bromophenyl)-9-(4-halosty-ryl)-4*H*-pyrano[3,2-*h*]quinoline-3-carbonitrile (**5a–f**) and (*E*) ethyl 2-amino-4-(4-chloro/bromophenyl)-9-(4-halosty-ryl)-4*H*-pyrano[3,2-*h*]quinoline-3-carboxylate (**6a–f**), respectively (Scheme 2).

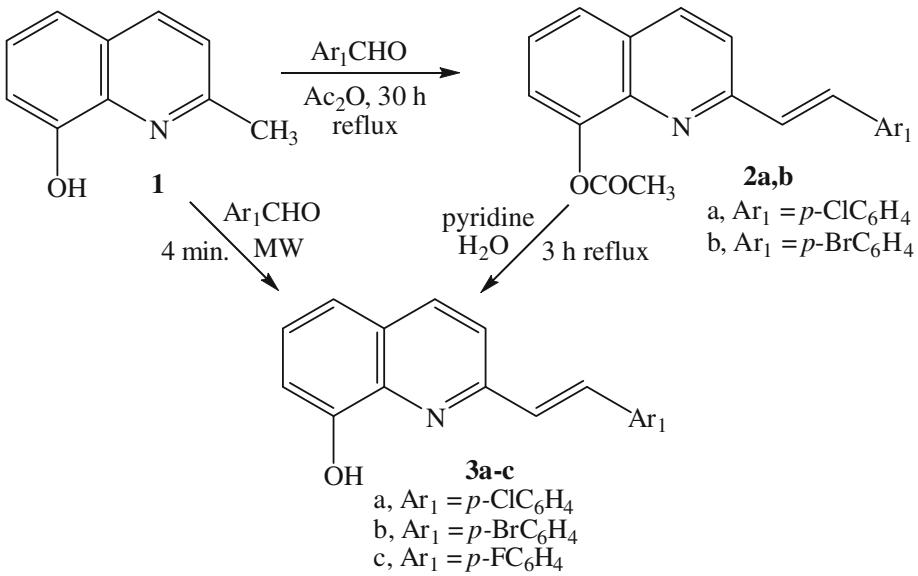
The formation of compounds **5** and **6** indicates that the phenolate anion (C-7) of **3** attacks at the β-carbon of **4** to yield an acyclic Michael adduct, which underwent cyclization (Abd-El-Aziz *et al*., 2004), as shown in (Scheme 3) to give compounds **5** and **6**.

The structures **5** and **6** were established on the basis of spectral data. The IR spectra of **5a–f** showed the appearance of NH_2 stretch at υ 3456–3384, 3328–3310, 3200–3168 cm^{-1} and CN stretch at υ 2198–2187 $cm,^{-1}$ while NH_2 stretch at υ 3412–3380, 3349–3292 cm^{-1} and CO stretch at υ 1677–1643 cm^{-1} for **6a–f**. The ^{1}H and ^{13}C NMR spectra of **5a–f** and **6a–f** revealed the presence of 4*H* signals at δ 5.11–5.00 (s, 1H, H-4) and 40.71–40.09 ppm (C-4). In compound **6a–f** the ester group gave ^{1}H signals at 4.13–4.01 (q, $J = 7$ Hz, 2H, CH_2) and 1.22–1.10 (t, $J = 7$ Hz, 3H, CH_3) with the corresponding signals in the ^{13}C spectra at 59.59–58.73 (CH_2) and 14.39–14.26 ppm (CH_3) respectively. The ^{13}C NMR–DEPT spectra at 45°, 90°, 135°, ^{13}C NMR–APT and the MS spectra of compounds **5** and **6** provided additional evidences in support of the proposed structures.

The relative (*E*) configuration of compounds **5** and **6** were established from the coupling constant values ($J = 16$–16.5 Hz).

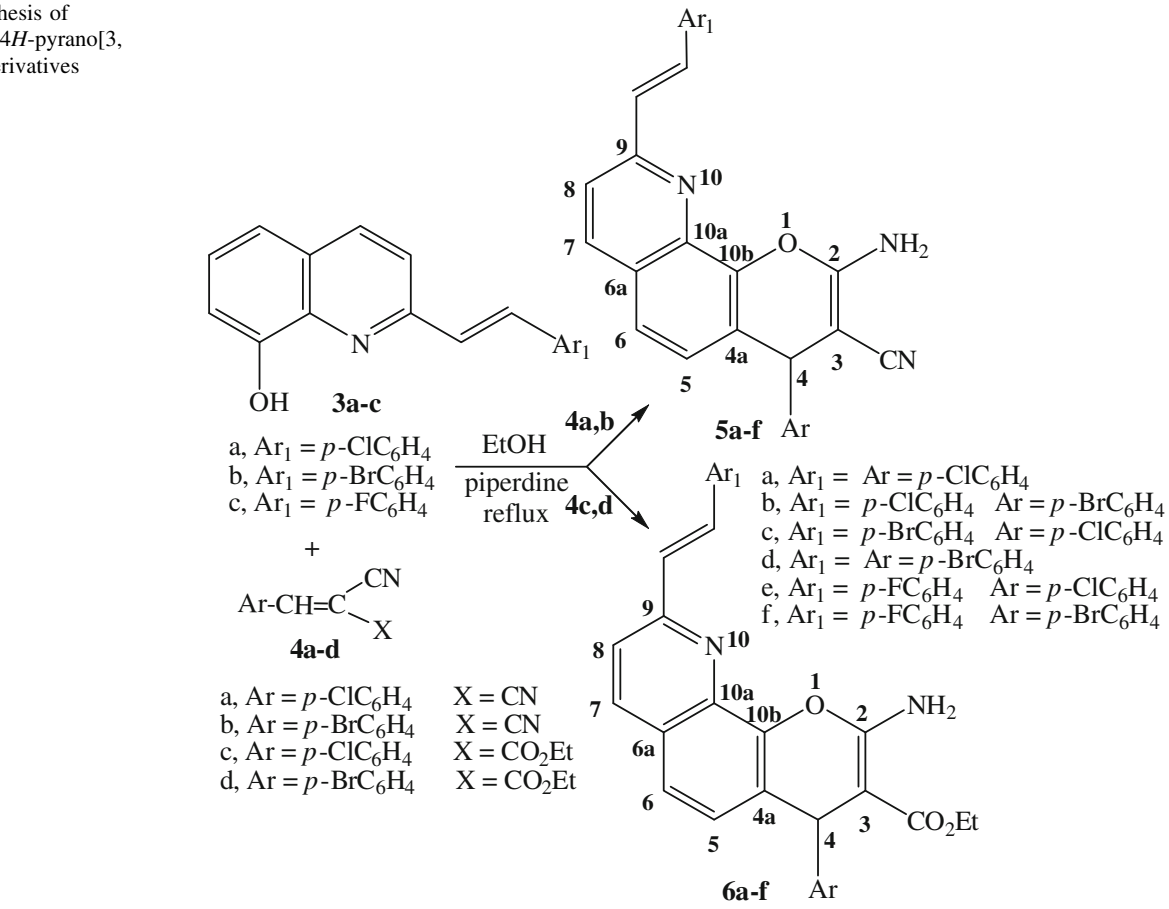
Antitumor assays

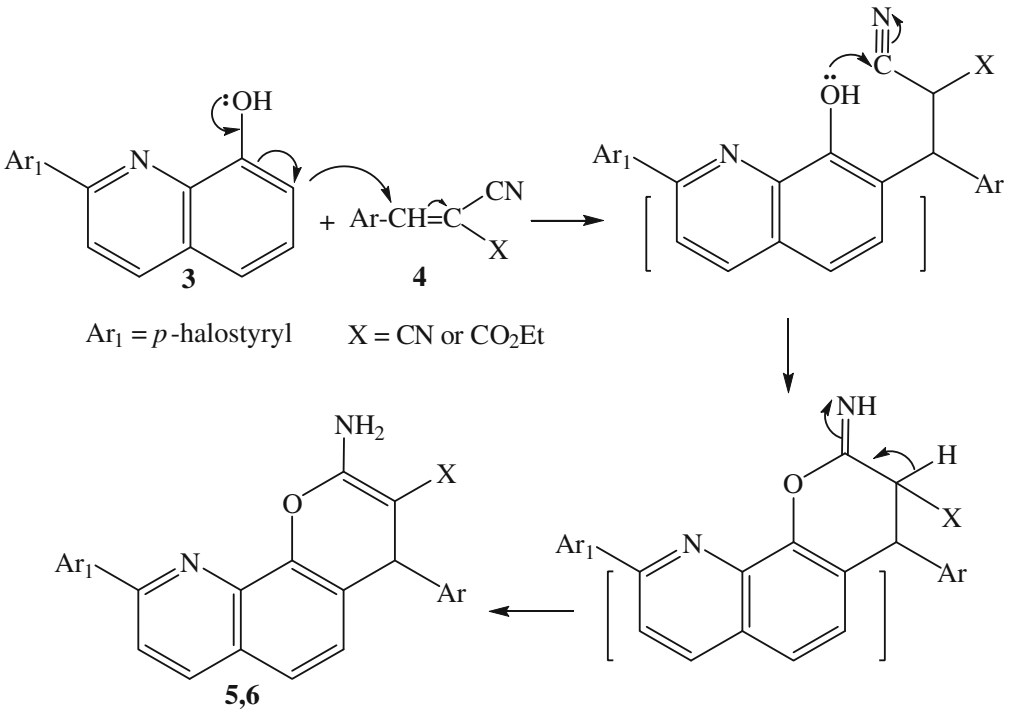
Compounds **5a–f** and **6a–f** were evaluated for their human tumor cell growth inhibitory activity against three cell lines: breast adenocarcinoma (MCF-7), lung carcinoma (HCT), and hepatocellular carcinoma (HepG-2). The measurement



Scheme 1 Preparation of (*E*) 8-acetoxy-2-(4-chloro/bromostyryl)quinoline (**2a**,**b**) and (*E*) 2-(4-chloro/bromo/f luorostyryl)-8-hydroxyquinoline (**3a–c**)

Scheme 2 Synthesis of 9-(4-halostyryl)-4*H*-pyrano[3, 2-*h*]quinoline derivatives (**5a–f**, **6a–f**)





Scheme 3 Mechanism formation of compounds (**5**,**6**)

of cell growth and viability were determined as described in the literature (Rahman *et al*., 2001). In vitro cytotoxicity evaluation using viability assays were performed by the Regional Center for Mycology & Biotechnology (RCMP), Al-Azhar University using, Vinblastine as standard drug. The inhibitory activity of the synthetic compounds **5a–f** and **6a–f** against three different human tumor cell lines MCF-7, HCT, and HepG-2 are given in Table 1 and Figs. 1, 2 and 3.

Result and discussion

Quinoline derivatives were chosen for this study because it is known that quinoline and fused quinoline derivatives are important families of active compounds with a wide range of pharmacological properties (Ganesh *et al*., 2008; Larghi *et al*., 2009; Liu *et al*., 2009; Musiol *et al*., 2006a, b, 2007; Narender *et al*., 2006, Ramesh *et al*., 2009; Righi *et al*., 2008; Vazquez *et al*., 2004). Twelve compounds of 4*H*-pyrano[3,2-*h*]quinoline derivatives were prepared. Structures of the synthesized compounds were elucidated on the basis of IR, ^{1}H NMR, ^{13}C NMR, ^{13}C NMR–DEPT, ^{13}C NMR–APT, and MS data.

Compounds **5a–f** and **6a–f** were tested against three tumor cell lines: MCF-7, HCT and HepG-2. The cytotoxicity evaluation using viability assays, and the inhibitory activities are given in Table 1 and Figs. 1, 2 and 3. Compounds **5a**,**d** and **6a**,**c**,**d** had activities (IC_{50} = 2.4–5.6 μg/ml) against MCF-7 more than the standard drug Vinblastine (IC_{50} = 6.1 μg/ml), while compounds **5b**,**c** and **6b** showed activities (IC_{50} = 10.3–15.9 μg/ml) close to the standard drug Vinblastine (IC_{50} = 6.1 μg/ml) and compounds **5e**,**f** and **6e**,**f** showed weak activities (IC_{50} = 37.9–42.5 μg/ml). Compounds **5a** and **6c** had activities (IC_{50} = 4.8–6.1 μg/ml) against the HCT close to the standard drug Vinblastine (IC_{50} = 2.6 μg/ml), while compounds **5b–d** and **6a**,**b**,**d–f** showed moderate activities (IC_{50} = 10.2–26.2 μg/ml) as compared with the standard drug Vinblastine and compound **5e**,**f** showed weak activities. Finally, compound **6c** had activity (IC_{50} = 3.1 μg/ml) against HepG-2 more than the standard drug Vinblastine (IC_{50} = 4.6 μg/ml), while compounds **5c**,**d** and **6a**,**d** showed activities (IC_{50} = 6.1–8.7 μg/ml) close to the standard drug Vinblastine (IC_{50} = 4.6 μg/ml) and compounds **5b**,**6b** showed moderate activities (IC_{50} = 23.2–28.6 μg/ml), in addition compounds **5a**,**e**,**f** and **6e**,**f** showed weak activities (IC_{50} = 37.1–48.2 μg/ml).

SAR studies

The SAR studies of compound **5a** and its analogs revealed that compounds **5d**,**a** have potent antitumor activities against the MCF-7 than the other compounds **5b**,**c**,**e**,**f**. These data indicate that the activity of compounds **5d**,**a** are considerably enhanced by the presence of the bromo/bromo or chloro/chloro atoms (electron-withdrawing groups) at the 4-positions of the styryl and the phenyl rings in combination with the cyano group (electron-withdrawing group) at the 3-position in 4*H*-pyrano[3,2-*h*]quinoline moiety, while the presence of the chloro/bromo or bromo/chloro atoms (electron-withdrawing groups) at the 4-positions of the styryl and the phenyl rings in combination with the cyano group (electron-withdrawing group) at the 3-position in compounds **5b**,**c** slightly decreased their antitumor activities. However the presence of fluoro/chloro and fluoro/bromo atoms (electron-withdrawing groups) at the 4-positions of the styryl and the phenyl rings resulted in more decreased in the antitumor activities of compounds **5e**,**f**. Replacement of the electron-withdrawing group, cyano group by ester group at the 3-position for compound **6a** and its analogs improved the antitumor activities. Compounds **6d**,**c**,**a** have potent antitumor activities against the MCF-7 than the other compounds **6b**,**e**,**f**. These data indicate the activities of compounds **6d**,**c**,**a** are considerably enhanced by the presence of the bromo/bromo, bromo/chloro or chloro/chloro atoms (electron-withdrawing groups) at the 4-positions of the styryl and the phenyl rings in combination with the ester group (electron-withdrawing group) at the 3-position in 4*H*-pyrano[3,2-*h*]quinoline moiety, while the presence of chloro/bromo atoms (electron-withdrawing groups) at the 4-positions of the styryl and the phenyl rings in combination with the ester group (electron-withdrawing group) at the 3-position for compound **6b** slightly decreased its antitumor activity and the presence of fluoro/chloro and fluoro/bromo atoms (electron-withdrawing groups) at the 4-position of the styryl and the phenyl rings resulted in more decreased in the antitumor activities of compounds **6e**,**f**.

In the case of the HCT, an investigation of the SAR for compound **5a** and its analogs revealed that compound **5a** showed antitumor activities close to the standard durg Vinblastine, this indicated that the activity is considerably affected by the presence of the (electron-withdrawing groups) chloro/chloro atoms at the 4-positions of the styryl and the phenyl rings in combination with the cyano group at the 3-position, while the antitumor activities decreased for compounds **5c**,**b**,**d** with the presence of the bromo/chloro, chloro/bromo or bromo/bromo atoms (electron-withdrawing groups) at the 4-positions of the styryl and the phenyl rings in combination with the cyano group at the 3-position. In addition, compounds **5e**,**f** showed weak antitumor activities due to the presence of fluoro/chloro or fluoro/bromo atoms (electron-withdrawing groups). Introduction of ester group at the 3-position for compound **6a** and its analogs did not improve the antitumor activities. Compound **6c** had activity close to the standard durg

Table 1 Effect of treatment of the prepared compounds at various concentrations on MCF-7, HCT and HepG-2 cells; cytotoxicity (IC_{50}) as measured with the MTT method

Compounds	Conc. (μg/ml)	MCF-7 Cell viability %	IC_{50} (μg/ml)	HCT Cell viability %	IC_{50} (μg/ml)	HepG-2 Cell viability %	IC_{50} (μg/ml)
Vinblastine	50	7.82	6.1	16.27	2.6	14.38	4.6
	25	15.18		21.68		16.13	
	12.5	29.6		28.2		24.25	
	6.25	48.75		38.06		45.13	
	3.125	60.35		47.54		55.00	
	1.56	76.24		53.42		72.13	
	0	100		100		100	
5a	50	29.56	5.6	24.71	4.8	48.40	48.2
	25	33.82		28.97		68.20	
	12.5	39.71		33.82		74.00	
	6.25	45.44		42.65		79.20	
	3.125	61.91		58.97		85.80	
	1.56	86.62		69.12		95.40	
	0	100		100		100	
5b	50	32.35	12.5	27.35	11.9	33.82	23.2
	25	39.85		34.24		44.85	
	12.5	50.29		47.79		69.56	
	6.25	61.76		69.85		77.21	
	3.125	83.09		72.50		87.94	
	1.56	98.53		80.44		94.56	
	0	100		100		100	
5c	50	29.35	15.9	22.78	10.2	25.21	7.5
	25	41.20		30.28		28.96	
	12.5	53.06		41.85		39.38	
	6.25	64.72		74.91		53.13	
	3.125	72.96		89.81		64.38	
	1.56	80.56		97.96		77.92	
	0	100		100		100	
5d	50	22.41	3.9	23.98	12.7	15.98	8.7
	25	27.41		33.52		25.72	
	12.5	31.67		51.02		34.08	
	6.25	40.65		80.46		41.36	
	3.125	53.15		90.74		54.83	
	1.56	61.11		95.74		74.32	
	0	100		100		100	
5e	50	28.50	38.6	41.31	43.8	56.40	w
	25	79.36		62.50		78.26	
	12.5	86.71		86.90		84.64	
	6.25	91.79		95.24		96.23	
	3.125	97.93		98.81		98.87	
	1.56	99.29		100		100	
	0	100		100		100	
5f	50	39.51	42.5	57.62	w	63.42	w
	25	67.82		83.39		74.85	
	12.5	80.19		91.07		83.44	
	6.25	91.76		94.76		97.21	
	3.125	96.94		98.57		98.94	
	1.56	100		100		100	
	0	100		100		100	

Table 1 continued

Compounds	Conc. (μg/ml)	MCF-7 Cell viability %	IC_{50} (μg/ml)	HCT Cell viability %	IC_{50} (μg/ml)	HepG-2 Cell viability %	IC_{50} (μg/ml)
6a	50	27.21	5.5	14.14	13	28.06	7.9
	25	34.56		20.36		37.88	
	12.5	41.62		54.29		45.60	
	6.25	47.21		64.14		52.98	
	3.125	67.79		69.29		65.32	
	1.56	78.09		78.57		81.92	
	0	100		100		100	
6b	50	29.71	10.3	30.71	20.4	39.76	28.6
	25	37.94		45.79		53.42	
	12.5	45.59		61.29		61.93	
	6.25	63.53		70.36		69.74	
	3.125	76.03		74.29		76.32	
	1.56	80.88		82.86		89.36	
	0	100		100		100	
6c	50	25.65	5.4	17.65	6.1	14.64	3.1
	25	31.02		24.71		22.86	
	12.5	41.57		34.41		36.43	
	6.25	45.46		48.09		39.29	
	3.125	57.87		66.18		49.57	
	1.56	69.44		72.06		72.07	
	0	100		100		100	
6d	50	12.96	2.4	26.00	12.8	23.44	6.1
	25	17.87		31.40		36.12	
	12.5	23.80		52.20		42.20	
	6.25	31.57		62.80		48.63	
	3.125	45.46		76.60		61.04	
	1.56	62.31		86.00		79.56	
	0	100		100		100	
6e	50	34.64	37.9	19.76	26.2	45.21	47.5
	25	61.20		51.31		68.96	
	12.5	73.46		69.52		79.38	
	6.25	84.72		73.10		85.13	
	3.125	92.06		80.60		96.38	
	1.56	98.53		98.93		98.92	
	0	100		100		100	
6f	50	37.65	38.4	17.38	26.1	34.64	37.1
	25	67.02		53.69		62.86	
	12.5	74.57		78.69		76.43	
	6.25	85.46		84.88		83.29	
	3.125	93.87		98.45		94.57	
	1.56	99.34		100		97.07	
	0	100		100		100	

w = weak activity ($IC_{50} > 50$ μg/ml)

Vinblastine, indicating that the activity is considerably affected by the presence of bromo/chloro atoms at the 4-positions of the styryl and the phenyl rings with the ester group (electron-withdrawing groups) at the 3-position as compared with the standard durg Vinblastine, while compounds **6d**,**a**,**b** showed modearare activities as compared with the standard durg Vinblastine and compounds **6e**,**f** showed weak activities due to the presence of the other halogen atoms and ester group.

Furthermore, an investigation of the SAR for compound **5a** and its analogs against the HepG-2 showed that compounds **5c**,**d** have antitumor activities close to the standard

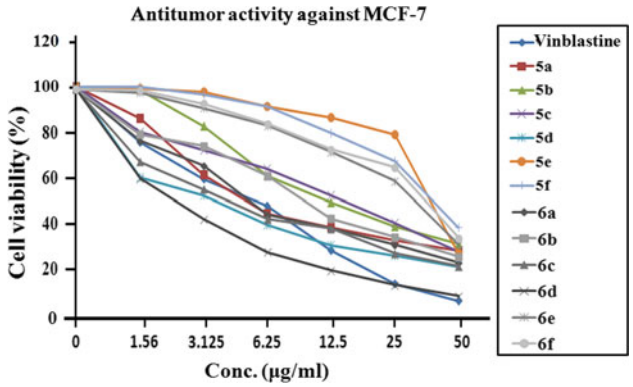


Fig. 1 Evaluation of cytotoxicity against MCF-7 cell line

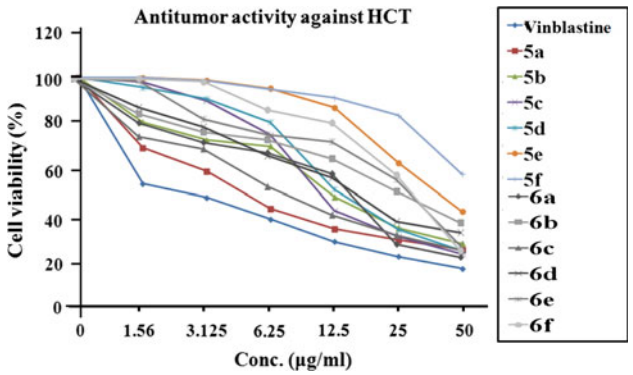


Fig. 2 Evaluation of cytotoxicity against HCT cell line

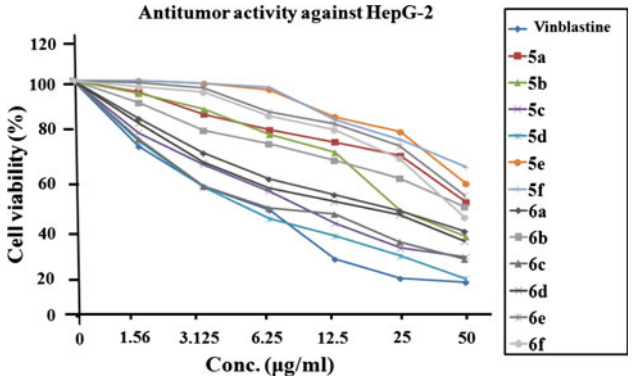


Fig. 3 Evaluation of cytotoxicity against HepG-2 cell line

durg Vinblastine. This indicates that the activities are considerably affected by the presence of the bromo/chloro or bromo/bromo atoms (electron-withdrawing groups) at the 4-positions of the styryl and the phenyl rings with the cyano group (electron-withdrawing group) at the 3-position, while the antitumor activity for compound **5b** decreased with the presence of the chloro/bromo atoms. In addition, compounds **5a**,**e**,**f** showed weak antitumor activities due to the presence the other halogen atoms and cyano group. Replacement of the electron-withdrawing group, cyano group by ester group at the 3-position for compound **6a** and its analogs improved the antitumor activity. Compound **6c** showed more antitumor activity against the HepG-2 than the standard drug Vinblastine, due to the presence of the bromo/chloro atoms (electron-withdrawing groups) at the 4-positions of the styryl and the phenyl rings in combination with the ester group (electron-withdrawing group) at the 3-position in 4*H*-pyrano[3,2-*h*]quinoline, while compounds **6d**,**a** showed antitumor activities against the HepG-2 very close to the standard drug Vinblastine. These data indicate that the activities are considerably affected by the presence of bromo/bromo or chloro/chloro atoms (electron-withdrawing groups). Finally, compounds **6b**,**e**,**f** showed more decreasing in antitumor activity due to the presence the other halogen atoms and ester group.

Conclusion

In this article, we reported the synthesis of some 4*H*-pyrano[3,2-*h*]quinoline derivatives and the antitumor evaluation of all the novel compounds. Compounds **5a**,**d** and **6a**,**c**,**d** had the most potent antitumor activity against the human breast tumor cells (MCF-7), while compounds **5a** and **6c** had the most potent against the human lung carcinoma (HCT), and compound **6c** had the most potent antitumor activity against the human hepatocellular carcinoma cells (HepG2). This potency could be attributed to the presence of the electron-withdrawing groups, bromo/bromo, bromo/chloro or chloro/chloro atoms at the 4-position of the styryl and the phenyl rings in combination with the ester/cyano group at the 3-position in 4*H*-pyrano[3,2-*h*]quinoline.

Experimental

Melting points were determined with a Stuart Scientific Co. Ltd apparatus. IR spectra were determined as KBr pellets on a Jasco FT/IR 460 plus spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker AV 500 MHz spectrometer. ^{13}C NMR spectra were obtained using distortionless enhancement by polarization transfer (DEPT), with this technique, the signals of CH & CH_3 carbon atoms appears normal (up) and the signal of carbon atoms in CH_2 environments appears negative (down). ^{13}C NMR spectra were obtained using attached proton test (APT), with this technique, the signals of CH and CH_3 carbon atoms appears normal (up) and the signal of CH_2 and Cq environments appears negative (down). The MS were measured on a Shimadzu GC/MS-QP5050A spectrometer. Elemental analyses were performed on a Perkin-Elmer 240 microanalyser in both the Faculty of Science Cairo University, Cairo and King Saud University, Riyadh.

Reaction of 8-hydroxy-2-methylquinoline (**1**) with *p*-halobenzaldehyde

Method (a)

A mixture of 8-hydroxy-2-methylquinoline (**1**) (0.01 mol), *p*-chlorobenzaldehyde or *p*-bromobenzaldehyde (0.08 mol) and acetic anhydride (100 ml) was heated at 150°C for 30 h (TLC monitoring). After cooling, the solvent was removed in vacuum, and the residue was recrystallised from ethanol/benzene to give **2a**,**b**. Compound **2a**,**b** was heated at 100°C for 1 h (TLC monitoring) in pyridine/water (v/v = 4:1) (100 ml). After cooling, the solvent was removed in vacuum to provide the crude product which recrystallised from ethanol to give **3a**,**b**. The physical and spectral data of compounds **2a**,**b** and **3a–c** are as follows:

(*E*) 8-Acetoxy-2-(4-chlorostyryl)quinoline (**2a**)

Prepared according to the previously reported procedure (El-Agrody *et al.*, 2011).

(*E*) 8-Acetoxy-2-(4-bromostyryl)quinoline (**2b**)

Pale yellow crystals from ethanol/benzene; yield 38%; m.p. 130–131°C; IR (KBr) υ (cm^{-1}): 3080, 3045, 3015, 2940 (CH), 1760 (CO); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.15–7.29 (m, 9H, aromatic), 7.65 (d, J = 16 Hz, 1H, =CH), 7.50 (d, J = 16.0 Hz, 1H, =CH), 2.60 (s, 3H, $COCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 169.81 (CO), 155.44 (C-2), 147.40 (C-8), 140.93 (C-1a), 136.47 (C-4), 134.39 (=CH), 129.40 (=CH), 129.00 (C-4a), 128.63 (C-6), 125.85 (C-5), 121.74 (C-7), 120.22 (C-3), 21.06 (CH_3), 134.98, 133.39, 128.85, 125.57 (aromatic) MS *m*/*z* (%): 369 (M^++2, 1), 367 (M^+, 1), 327 (98), 325 (99), 170 (5), 144 (4), 115 (100), 74 (43), 50 (75); Anal. Calcd for $C_{19}H_{14}BrNO_2$: C, 61.97; H, 3.83; N, 3.80. Found: C, 62.01; H, 3.85; N, 3.84%.

(*E*) 2-(4-Chlorostyryl)-8-hydroxyquinoline (**3a**)

Prepared according to the previously reported procedure (El-Agrody *et al.*, 2011).

(*E*) 2-(4-Bromostyryl)-8-hydroxyquinoline (**3b**)

Yellow needles from ethanol; yield 32%; m.p. 135–136°C; (Musiol *et al.*, 2006a, b, 2007, m.p. 145°C); IR (KBr) υ (cm^{-1}): 3348 (OH), 3070, 3046, 2952, 2800 (CH); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.62 (bs, 1H, OH), 8.31–7.10 (m, 9H, aromatic), 8.13 (d, J = 16.0 Hz, 1H, =CH,), 7.52 (d, J = 16.0, Hz 1H, =CH); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 153.08 (C-2), 152.93 (C-8), 138.12 (C-1a), 136.55 (C-4), 133.00 (=CH), 129.05 (=CH), 128.80 (C-4a), 127.17 (C-6), 121.06 (C-3), 117.56 (C-5), 111.21 (C-7), 135.77, 131.82, 127.73, 121.58 (aromatic).; ^{13}C NMR–DEPT spectrum at 135° CH, CH_3 [positive (up)], CH_2 [negative (down)], revealed the following signals at δ 136.55 (C-4 ↑), 133.00 (=CH ↑), 131.82 (aromatic ↑), 129.05 (=CH ↑), 127.73 (aromatic ↑), 127.17 (C-6 ↑), 121.06 (C-3 ↑), 117.56 (C-5 ↑), 111.21 (C-7 ↑). In the DEPT spectrum at 90° only CH signals are positive (up) and showed δ 136.55 (C-4 ↑), 133.00 (=CH ↑), 131.82 (aromatic ↑), 129.05 (=CH ↑), 127.73 (aromatic ↑), 127.17 (C-6 ↑), 121.06 (C-3 ↑), 117.56 (C-5 ↑), 111.21 (C-7 ↑). In the DEPT spectrum at 45° (CH, CH_2, and CH_3 positive) revealed signals at δ 136.55 (C-4 ↑), 133.00 (=CH ↑), 131.82 (aromatic ↑), 129.05 (=CH ↑), 127.73 (aromatic ↑), 127.17 (C-6 ↑), 121.06 (C-3 ↑), 117.56 (C-5 ↑), 111.21 (C-7 ↑) .^{13}C NMR–APT spectrum CH, CH_3 [positive (up)], CH_2, Cq [negative (down)], revealed the following signals at δ 153.08 (C-2 ↓), 152.93 (C-8 ↓), 138.12 (C-1a ↓), 136.55 (C-4 ↑), 135.77 (aromatic ↓), 133.00 (=CH ↑), 131.82 (aromatic ↑), 129.05 (=CH ↑), 128.80 (C-4a ↓), 127.73 (aromatic ↑), 127.17 (C-6 ↑), 121.58 (aromatic ↓), 121.06 (C-3 ↑), 117.56 (C-5 ↑), 111.21 (C-7 ↑); MS *m*/*z* (%): 327 (M^++2, 98), 325 (M^+, 100), 170 (2), 144 (4), 115 (65), 75 (41), 50 (62); $C_{17}H_{12}BrNO$.

(*E*) 2-(4-Fluorostyryl)-8-hydroxyquinoline (**3c**)

Yellow needles from ethanol; yield 26%; m.p. 110–111°C (Chang *et al.*, 2010; Musiol *et al.*, 2006a, b, 2007, m.p. 107°C); IR (KBr) υ (cm^{-1}): 3399 (OH), 3055, 3011, 2830 (CH); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.62 (bs, 1H, OH), 8.31–7.12 (m, 9H, aromatic), 8.15 (d, J = 16.0 Hz 1H, =CH), 7.45 (d, J = 16.0 Hz, 1H, =CH); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 153.29 (C-2), 152.88 (C-8), 138.10 (C-1a), 136.49 (C-4), 133.10 (=CH), 129.14 (=CH), 128.76 (C-4a), 127.83 (C-6), 120.92 (C-3), 117.55 (C-5), 111.17 (C-7), 161.21, 133.06, 129.08, 115.89 (aromatic); MS *m*/*z* (%): 265 (M^+, 37), 264 (100), 170 (3), 145 (6), 116 (34), 74 (20), 50 (32); $C_{17}H_{12}FNO$.

Method (b)

8-Hydroxy-2-methylquinoline (**1**) (0.01 mol) and *p*-chlorobenzaldehyde, *p*-bromobenzaldehyde or *p*-flurobenzaldehyde (0.02 mol) were mixed thoroughly using mortar and put in an open vessel. Then the mixture was exposed to Microwave irradiation for 4 min. The oven was operated at 70% power (560 W) in a two-step mode with interval (2 min-30 s-2 min). After the reaction, the mixture was allowed to cool down and Et_2O (10 ml) was added. The crude product was filtered, washed with Et_2O (15 ml) and purified by recrystallization from ethanol to give **3a–c** (m.p. and mixed m.p.) 27–40%; (**3a**, Musiol *et al.*, 2006a, b, 2007, m.p. 150°C; **3b**, Musiol *et al.*, 2006a, b, 2007, m.p. 145°C and **3c**, Chang *et al.*, 2010, m.p. 107°C).

Reaction of (*E*) 8-hydroxy-2-(4-chloro/bromo/fluorostyryl)quinoline (**3a–c**) with α-cyano-*p*-chloro/bromocinnamonitrile (**4a**,**b**) and ethyl α-cyano-*p*-chloro/bromocinnamate (**4c**,**d**)

General procedure

A solution of (*E*) 2-(4-chloro/bromo/fluorostyryl)-8-hydroxyquinoline (**3a–c**) (0.01 mol) in EtOH (30 ml) was treated with α-cyano-*p*-chloro/bromocinnamonitrile (**4a**,**b**) (0.01 mol) or ethyl α-cyano-*p*-chloro/bromocinnamate (**4c**,**d**) (0.01 mol) and piperidine (0.5 ml). The reaction mixture was heated until complete precipitation occurred (reaction times: 30 min. for **4a**,**b** and 45 min. for **4c**,**d**). The solid product which formed was collected by filtration and recrystallised from etanol or benzene to give **5a–f** and **6a–f**. The physical and spectral data of compounds **5a–f** and **6a–f** are as follows:

(*E*) 2-Amino-4-(4-chlorophenyl)-9-(4-chlorostyryl)-4*H*-pyrano[3,2-*h*]quinoline-3-carbonitrile (**5a**)

Prepared according to the previously reported procedure (El-Agrody *et al.*, 2011).

(*E*) 2-Amino-4-(4-bromophenyl)-9-(4-chlorostyryl)-4*H*-pyrano[3,2-*h*]quinoline-3-carbonitrile (**5b**)

Prepared according to the previously reported procedure (El-Agrody *et al.*, 2011).

(*E*) 2-Amino-4-(4-chlorophenyl)-9-(4-bromostyryl)-4*H*-pyrano[3,2-*h*]quinoline-3-carbonitrile (**5c**)

Pale yellow needles from benzene; yield 87%; m.p. 240–241°C; IR (KBr) υ (cm^{-1}): 3456, 3323, 3200 (NH_2), 3075, 3033, 2940, 2850 (CH), 2193 (CN); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.33–7.14 (m, 12H, aromatic), 7.98 (d, $J = 16.5$ Hz, 1H, =CH), 7.54 (d, $J = 16.5$ Hz, 1H, =CH), 7.24 (bs, 2H, NH_2, canceled by D_2O), 5.01 (s, 1H, H-4); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 160.21 (C-2), 155.12 (C-9), 144.58 (C-10b), 137.44 (C-10a), 136.53 (C-7), 133.50 (=CH), 129.57 (=CH), 126.89 (C-6a), 126.33 (C-5), 121.72 (C-4a), 123.41 (C-6), 121.01 (C-8), 120.31 (CN), 55.70 (C-3), 40.42 (C-4), 142.81, 135.47, 131.86, 131.63, 129.16, 128.94, 128.29, 121.88 (aromatic); ^{13}C NMR–DEPT spectrum at 135° CH, CH_3 [positive (up)], CH_2 [negative (down)], revealed the following signals at δ 136.53 (C-7 ↑), 133.50 (=CH ↑), 131.86 (aromatic ↑), 129.57 (=CH ↑), 129.16 (aromatic ↑), 128.94 (aromatic ↑), 128.29 (aromatic ↑), 126.33 (C-5 ↑), 123.41 (C-6 ↑), 121.01 (C-8 ↑), 40.42 (C-4 ↑). In the DEPT spectrum at 90° only CH signals are positive (up) and showed δ 136.53 (C-7 ↑), 133.50 (=CH ↑), 131.86 (aromatic ↑), 129.57 (=CH ↑), 129.16 (aromatic ↑), 128.94 (aromatic ↑), 128.29 (aromatic ↑), 126.33 (C-5 ↑), 123.41 (C-6 ↑), 121.01 (C-8 ↑), 40.42 (C-4 ↑). In the DEPT spectrum at 45° (CH, CH_2, and CH_3 positive) revealed signals at δ 136.53 (C-7 ↑), 133.50 (=CH ↑), 131.86 (aromatic ↑), 129.57 (=CH ↑), 129.16 (aromatic ↑), 128.94 (aromatic ↑), 128.29 (aromatic ↑), 126.33 (C-5 ↑), 123.41 (C-6 ↑), 121.01 (C-8 ↑), 40.42 (C-4 ↑). ^{13}C NMR–APT spectrum CH, CH_3 [positive (up)], CH_2, Cq [negative (down)], revealed the following signals at δ 160.21 (C-2 ↓), 155.12 (C-9 ↓), 144.58 (C-10b ↓), 142.81 (aromatic ↓), 137.44, (C-10a ↓), 136.53 (C-7 ↑), 135.47 (aromatic ↓), 133.50 (=CH ↑), 131.86 (aromatic ↑), 131.63 (aromatic ↓), 129.57 (=CH ↑), 129.16 (aromatic ↑), 128.94 (aromatic ↑), 128.29 (aromatic ↑), 126.89 (C-6a ↓), 126.33 (C-5 ↑), 123.41 (C-6 ↑), 121.88 (aromatic ↓), 121.72 (C-4a ↓), 121.01 (C-8 ↑), 120.31 (CN ↓), 55.70 (C-3 ↓), 40.42 (C-4 ↑); MS *m*/*z* (%): 517 [M^++4] (1), 515 (M^++2, 3.19), 513 (M^+, 2.55), 406 (90.68), 404 (100), 224 (2), 166 (41), 101 (43), 74 (50), 50 (68); Anal. Calcd for $C_{27}H_{17}BrClN_3O$: C, 62.99; H, 3.33; N, 8.16. Found: C, 63.51; H, 3.17; N, 7.88%.

(*E*) 2-Amino-4-(4-bromophenyl)-9-(4-bromostyryl)-4*H*-pyrano[3,2-*h*]quinoline-3-carbonitrile (**5d**)

Pale yellow needles from benzene; yield 88%; m.p. 260–261°C; IR (KBr) υ (cm^{-1}): 3397, 3321, 3195 (NH_2), 3085, 3053, 2968, 2871 (CH), 2196 (CN); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.32–7.10 (m, 12H, aromatic), 8.13 (d, $J = 16.0$ Hz, 1H, =CH), 7.67 (bs, 2H, NH_2, canceled by D_2O), 7.52 (d, $J = 16.0$ Hz, 1H, =CH), 5.00 (s, 1H, H-4); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 160.25 (C-2), 153.08 (C-9), 144.59 (C-10b), 138.12, (C-10a), 136.56 (C-7), 132.99 (=CH), 129.95 (=CH), 128.80 (C-5), 127.17 (C-6a), 122.65 (C-4a), 121.58 (C-6), 121.03 (C-8), 117.55 (CN), 56.93 (C-3), 40.46 (C-4), 142.82, 135.77, 133.83, 131.65, 129.17, 129.06, 123.42, 120.15 (aromatic); ^{13}C NMR–DEPT spectrum at 135° CH, CH_3 [positive (up)], CH_2 [negative (down)], revealed the following signals at δ 136.56 (C-7 ↑), 133.83 (aromatic ↑), 131.65 (aromatic ↑), 132.99 (=CH ↑), 129.95 (=CH ↑), 129.17 (aromatic ↑), 129.06, (aromatic ↑), 128. 80 (C-5 ↑), 121.58 (C-6 ↑), 121.03 (C-8 ↑), 40.46 (C-4 ↑). In the DEPT spectrum at 90° only CH signals are positive (up) and showed δ 136.56 (C-7 ↑), 133.83 (aromatic ↑), 131.65 (aromatic ↑), 132.99 (=CH ↑), 129.95 (=CH ↑), 129.17 (aromatic ↑), 129.06, (aromatic ↑), 128. 80 (C-5 ↑), 121.58 (C-6 ↑), 121.03 (C-8 ↑), 40.46 (C-4 ↑). In the DEPT spectrum at 45° (CH, CH_2, and CH_3 positive) revealed signals at δ 136.56 (C-7 ↑), 133.83 (aromatic ↑), 131.65 (aromatic ↑), 132.99 (=CH ↑), 129.95 (=CH ↑), 129.17 (aromatic ↑), 129.06,

(aromatic ↑), 128. 80 (C-5 ↑), 121.58 (C-6 ↑), 121.03 (C-8 ↑), 40.46 (C-4 ↑)0.^{13}C NMR–APT spectrum CH, CH_3 [positive (up)], CH_2, Cq [negative (down)], revealed the following signals at δ 160.25 (C-2 ↓), 153.08 (C-9 ↓), 144.59 (C-10b ↓), 142.82 (aromatic ↓), 138.12, (C-10a ↓), 136.56 (C-7 ↑), 135.77 (aromatic ↓), 133.83 (aromatic ↑), 132.99 (=CH ↑), 131.65 (aromatic ↑), 129.95 (=CH ↑), 129.17 (aromatic ↑), 129.06 (aromatic ↑), 128. 80 (C-5 ↑), 127.17 (C-6a ↓), 123.42 (aromatic ↓), 122.65 (C-4a ↓), 121.58 (C-6 ↑), 121.03 (C-8 ↑), 120.15 (aromatic ↓); 117.55 (CN ↓), 56.93 (C-3 ↓), 40.46 (C-4 ↑); MS *m/z* (%): 561 [M^++4] (7.18), 559 [M^++2] (17.31), 557 [M]$^+$ (9.16), 405 (M^++2, 21.49), 403 (M^+, 25.65), 249 (6), 221 (8), 166 (15), 102 (48), 77 (79), 50 (100); Anal. Calcd for $C_{27}H_{17}Br_2N_3O$: C, 57.99; H, 3.06; N, 7.51. Found: C, 57.87; H, 3.03; N, 7.34%.

(*E*) 2-Amino-4-(4-chlorophenyl)-9-(4-fluorostyryl)-4*H*-pyrano[3,2-*h*]quinoline-3-carbonitrile (**5e**)

Pale yellow needles from ethanol/benzene; yield 84%; m.p. 245–246°C; IR (KBr) υ (cm^{-1}): 3421, 3328, 3197 (NH_2), 3085, 3051, 2880 (CH), 2190 (CN); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.33–7.14 (m, 12H, aromatic), 8.01 (d, J = 16.0 Hz, 1H, =CH), 7.43 (d, J = 16.0 Hz, 1H, =CH), 7.23 (bs, 2H, NH_2, canceled by D_2O), 5.01 (s, 1H, H-4); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 163.32 (C-2), 160.25 (C-9), 155.32 (C-10b), 137.41, (C-10a), 136.49 (C-7), 133.62 (=CH), 129.58 (=CH), 128.72 (C-5), 126.83 (C-6a), 121.70 (C-4a), 124.41 (C-6), 120.90 (C-8), 120.32 (CN), 55.96 (C-3), 40.40 (C-4), 161.36, 144.62, 142.79, 132.82, 129.24, 127.97, 126.24, 115.98 (aromatic); ^{13}C NMR–DEPT spectrum at 135° CH, CH_3 [positive (up)], CH_2 [negative (down)], revealed the following signals at δ 136.49 (C-7 ↑), 133.62 (=CH ↑), 129.58 (=CH ↑), 129.24 (aromatic ↑), 128.72 (C-5 ↑), 127.97 (aromatic ↑), 126.24 (aromatic ↑), 124.41 (C-6 ↑), 120.90 (C-8 ↑), 115.98 (aromatic ↑), 40.40 (C-4 ↑). In the DEPT spectrum at 90° only CH signals are positive (up) and showed δ 136.49 (C-7 ↑), 133.62 (=CH ↑), 129.58 (=CH ↑), 129.24 (aromatic ↑), 128.72 (C-5 ↑), 127.97 (aromatic ↑), 126.24 (aromatic ↑), 124.41 (C-6 ↑), 120.90 (C-8 ↑), 115.98 (aromatic ↑), 40.40 (C-4 ↑). In the DEPT spectrum at 45° (CH, CH_2, and CH_3 positive) revealed signals at δ 136.49 (C-7 ↑), 133.62 (=CH ↑), 129.58 (=CH ↑), 129.24 (aromatic ↑), 128.72 (C-5 ↑), 127.97 (aromatic ↑), 126.24 (aromatic ↑), 124.41 (C-6 ↑), 120.90 (C-8 ↑), 115.98 (aromatic ↑), 40.40 (C-4 ↑)0.^{13}C NMR–APT spectrum CH, CH_3 [positive (up)], CH_2, Cq [negative (down)], revealed the following signals at δ163.32 (C-2 ↓), 161.36 (aromatic ↓), 160.25 (C-9 ↓), 155.32 (C-10b ↓), 144.62 (aromatic ↓), 142.79 (aromatic ↓), 137.41, (C-10a ↓), 136.49 (C-7 ↑), 133.62 (=CH ↑), 132.82 (aromatic ↓), 129.58 (=CH ↑), 129.24 (aromatic ↑), 128.72 (C-5 ↑), 127.97 (aromatic ↑), 126.83 (C-6a ↓), 126.24 (aromatic ↑), 121.70 (C-4a ↓), 124.41 (C-6 ↑), 120.90 (C-8 ↑), 120.32 (CN ↓), 115.98 (aromatic ↑), 55.96 (C-3 ↓), 40.40 (C-4 ↑); MS *m/z* (%): 455 (M^++2, 12.43), 453 (M^+, 29.30), 343 (100), 248 (1), 221 (2), 166 (11), 100 (5), 65 (3); Anal. Calcd for $C_{27}H_{17}ClFN_3O$: C, 71.45; H, 3.78; N, 9.26. Found: C, 71.35; H, 3.57; N, 9.08%.

(*E*) 2-Amino-4-(4-bromoophenyl)-9-(4-fluorostyryl)-4*H*-pyrano[3,2-*h*]quinoline-3-carbonitrile (**5f**)

Pale yellow needles from ethanol/benzene; yield 81%; m.p. 240–241°C; IR (KBr) υ (cm^{-1}): 3408, 3319, 3168 (NH_2), 3050, 2964, 2900, 2860 (CH), 2187 (CN); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.33–7.14 (m, 12H, aromatic), 8.01 (d, J = 16.0 Hz, 1H, =CH), 7.46 (d, J = 16.0 Hz, 1H, =CH), 7.23 (bs, 2H, NH_2, canceled by D_2O), 5.00 (s, 1H, H-4); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 163.32 (C-2), 160.25 (C-9), 155.33 (C-10b), 137.41, (C-10a), 136.49 (C-7), 133.62 (=CH), 129.95 (=CH), 127.99 (C-5), 126.83 (C-6a), 123.41 (C-6), 121.63 (C-4a), 120.90 (C-8), 120.31 (CN), 56.00 (C-3), 40.47 (C-4), 161.36, 145.03, 142.79, 132.82, 129.24, 127.99, 126.24, 115.98 (aromatic); MS *m/z* (%): 499 (M^++2, 39.28), 497 (M^+, 40.22), 342 (100), 247 (2), 221 (3), 166 (8), 100 (19), 50 (36); Anal. Calcd for $C_{27}H_{17}BrFN_3O$: C, 65.07; H, 3.44; N, 8.43. Found: C, 65.82; H, 3.33; N, 8.08%.

(*E*) Ethyl 2-amino-4-(4-chlorophenyl)-9-(4-chlorostyryl)-4*H*-pyrano[3,2-*h*]quinoline-3-carboxylate (**6a**)

Prepared according to the previously reported procedure (El-Agrody *et al.*, 2011).

(*E*) Ethyl 2-amino-4-(4-bromophenyl)-9-(4-chlorostyryl)-4*H*-pyrano[3,2-*h*]quinoline-3-carboxylate (**6b**)

Prepared according to the previously reported procedure (El-Agrody *et al.*, 2011).

(*E*) Ethyl 2-amino-4-(4-chlorophenyl)-9-(4-bromostyryl)-4*H*-pyrano[3,2-*h*]quinoline-3-carboxylate (**6c**)

Colorless needles from ethanol; yield 80%; m.p. 191–192°C; IR (KBr) υ (cm^{-1}): 3405, 3292 (NH_2), 3034, 2979, 2909, 2850 (CH), 1676 (CO); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.32–7.32 (m, 12H, aromatic), 7.30 (bs, 2H, NH_2, canceled by D_2O), 7.95 (d, J = 16.0 Hz, 1H, =CH), 7.54 (d, J = 16.0 Hz, 1H, =CH), 5.10 (s, 1H, H-4), 4.01 (q, J = 7.0 Hz, 2H, CH_2), 1.10 (t, J = 7.0 Hz, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 168.15 (CO), 160.82 (C-2), 155.10 (C-9), 146.71 (C-10b), 142.78 (C-10a), 136.49 (C-7), 133.36 (=CH), 129.23 (=CH), 128.20 (C-5), 126.69 (C-6a), 124.65 (C-4a), 123.30 (C-6), 120.59 (C-8), 75.79 (C-3), 58.73 (CH_2), 40.10 (C-4), 14.26 (CH_3),

137.49, 135.50, 131.86, 130.65, 129.18, 129.14, 126.78, 121.84 (aromatic); ^{13}C NMR–DEPT spectrum at 135° CH, CH_3 [positive (up)], CH_2 [negative (down)], revealed the following signals at δ 136.49 (C-7 ↑), 133.36 (=CH ↑), 131.86 (aromatic ↑), 129.23 (=CH ↑), 129.18 (aromatic ↑), 129.14 (aromatic ↑), 128.20 (C-5 ↑), 126.78 (aromatic ↑), 123.30 (C-6 ↑), 120.59 (C-8 ↑), 58.73 (CH_2 ↓), 40.10 (C-4 ↑), 14.26 (CH_3↑). In the DEPT spectrum at 90° only CH signals are positive (up) and showed δ 136.49 (C-7 ↑), 133.36 (=CH ↑), 131.86 (aromatic ↑), 129.23 (=CH ↑), 129.18 (aromatic ↑), 129.14 (aromatic ↑), 128.20 (C-5 ↑), 126.78 (aromatic ↑), 123.30 (C-6 ↑), 120.59 (C-8 ↑), 40.10 (C-4 ↑). In the DEPT spectrum at 45° (CH, CH_2, and CH_3 positive) revealed signals at δ 136.49 (C-7 ↑), 133.36 (=CH ↑), 131.86 (aromatic ↑), 129.23 (=CH ↑), 129.18 (aromatic ↑), 129.14 (aromatic ↑), 128.20 (C-5 ↑), 126.78 (aromatic ↑), 123.30 (C-6 ↑), 120.59 (C-8 ↑), 58.73 (CH_2 ↑), 40.10 (C-4 ↑), 14.26 (CH_3 ↑). ^{13}C NMR–APT spectrum CH, CH_3 [positive (up)], CH_2, Cq [negative (down)], revealed the following signals at δ 168.15 (CO ↓), 160.82 (C-2 ↓), 155.10 (C-9 ↓), 146.71 (C-10b ↓), 142.78 (C-10a ↓), 137.49 (aromatic ↓), 136.49 (C-7 ↑), 135.50 (aromatic ↓), 133.36 (=CH ↑), 131.86 (aromatic ↑), 130.65 (aromatic ↓), 129.23 (=CH ↑), 129.18 (aromatic ↑), 129.14 (aromatic ↑), 128.20 (C-5 ↑), 126.78 (aromatic ↑), 126.69 (C-6a ↓), 124.65 (C-4a ↓), 123.30 (C-6 ↑), 121.84 (aromatic ↓), 120.59 (C-8 ↑), 75.79 (C-3 ↓), 58.73 (CH_2 ↓), 40.10 (C-4 ↑), 14.26 (CH_3 ↑); MS *m/z* (%): 564 (M^++4, 2.87), 562 (M^++2, 10.26), 560 (M^+, 7.82), 492 (3.31), 490 (10.31), 488 (9.62), 453 (97.59) 451 (100), 296 (21), 228 (34) 150 (20), 111 (84), 75 (93), 50 (85); Anal. Calcd for $C_{29}H_{22}BrClN_2O_3$: C, 61.99; H, 3.95; N, 4.99. Found: C, 62.04; H, 4.02; N, 5.03%.

(*E*) Ethyl 2-amino-4-(4-bromophenyl)-9-(4-bromostyryl)-4*H*-pyrano[3,2-*h*]quinoline-3-carboxylate (**6d**)

Colorless needles from ethanol; yield 81%; m.p. 195–196°C; IR (KBr) υ (cm^{-1}): 3412, 3296 (NH_2), 3049, 3020, 2979, 2935, 2900, 2873 (CH), 1677 (CO); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.32–7.23 (m, 12H, aromatic), 7.86 (bs, 2H, NH_2, canceled by D_2O), 7.95 (d, J = 16.5 Hz, 1H, =CH), 7.53 (d, J = 16.5 Hz, 1H, =CH), 5.09 (s, 1H, H-4), 4.00 (q, J = 7.0 Hz, 2H, CH_2), 1.10 (t, J = 7.0 Hz, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 168.13 (CO), 160.82 (C-2), 155.10 (C-9), 147.71 (C-10b), 137.49 (C-10a), 136.51 (C-7), 133.37 (=CH), 129.64 (=CH), 128.82 (C-5), 126.70 (C-6a), 124.60 (C-4a), 123.31 (C-6), 120.62 (C-8), 75.72 (C-3), 58.74 (CH_2), 40.10 (C-4), 14.27 (CH_3), 142.78, 135.51, 131.88, 131.13, 129.15, 126.79, 121.85, 121.05 (aromatic); ^{13}C NMR–DEPT spectrum at 135° CH, CH_3 [positive (up)], CH_2 [negative (down)], revealed the following signals at δ 136.51 (C-7 ↑), 133.37 (=CH ↑), 131.88 (aromatic ↑), 131.13 (aromatic ↑), 129.64 (=CH ↑), 129.15 (aromatic ↑), 126.79 (aromatic ↑),128.82 (C-5 ↑), 123.31 (C-6 ↑), 120.62 (C-8 ↑), 58.74 (CH_2 ↓), 40.10 (C-4 ↑), 14.27 (CH_3 ↑). In the DEPT spectrum at 90° only CH signals are positive (up) and showed δ 136.51 (C-7 ↑), 133.37 (=CH ↑), 131.88 (aromatic ↑), 131.13 (aromatic ↑), 129.64 (=CH ↑), 129.15 (aromatic ↑), 126.79 (aromatic ↑), 128.82 (C-5 ↑), 123.31 (C-6 ↑), 120.62 (C-8 ↑), 40.10 (C-4 ↑). In the DEPT spectrum at 45° (CH, CH_2, and CH_3 positive) revealed signals at δ 136.51 (C-7 ↑), 133.37 (=CH ↑), 131.88 (aromatic ↑), 131.13 (aromatic ↑), 129.64 (=CH ↑), 129.15 (aromatic ↑), 126.79 (aromatic ↑),128.82 (C-5 ↑), 123.31 (C-6 ↑), 120.62 (C-8 ↑), 58.74 (CH_2 ↑), 40.10 (C-4 ↑), 14.27 (CH_3 ↑). ^{13}C NMR–APT spectrum CH, CH_3 [positive (up)], CH_2, Cq [negative (down)], revealed the following signals at δ 168.13 (CO ↓), 160.82 (C-2 ↓), 155.10 (C-9 ↓), 147.71 (C-10b ↓), 142.78 (aromatic ↓), 137.49 (C-10a ↓), 136.51 (C-7 ↑), 135.51 (aromatic ↓), 133.37 (=CH ↑), 131.88 (aromatic ↑), 131.13 (aromatic ↑), 129.64 (=CH ↑), 129.15 (aromatic ↑), 128.82 (C-5 ↑), 126.79 (aromatic ↑), 126.70 (C-6a ↓), 124.60 (C-4a ↓), 123.31 (C-6 ↑), 121.85 (aromatic ↓), 121.05 (aromatic ↓), 120.62 (C-8 ↑), 75.72 (C-3 ↓), 58.74 (CH_2 ↓), 40.10 (C-4 ↑), 14.27 (CH_3 ↑); MS *m/z* (%): 608 (M^++4, 3.88), 606 (M^++2, 7.88), 604 (M^+, 3.98), 451 (13.30), 449 (11.72), 293 (5), 249 (6), 157 (20) 116 (21), 76 (74), 50 (100); Anal. Calcd for $C_{29}H_{22}Br_2N_2O_3$: C, 57.45; H, 3.66; N, 4.82. Found: C, 57.01; H, 3.44; N, 4.63%.

(*E*) Ethyl 2-amino-4-(4-chlorophenyl)-9-(4-fluorostyryl)-4*H*-pyrano[3,2-*h*]quinoline-3-carboxylate (**6e**)

Colorless needles from benzene/ethanol; yield 80%; m.p. 192–193°C; IR (KBr) υ (cm^{-1}): 3410, 3295 (NH_2), 3050, 2979, 2900 (CH stretching), 1677 (CO); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.32–7.30 (m, 12H, aromatic), 7.30 (bs, 2H, NH_2, canceled by D_2O), 7.98 (d, J = 16.5 Hz, 1H, =CH), 7.46 (d, J = 16.5 Hz, 1H, =CH), 5.11 (s, 1H, H-4), 4.01 (q, J = 7.0 Hz, 2H, CH_2), 1.10 (t, J = 7.0 Hz, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 168.15 (CO), 161.35 (C-2), 160.84 (C-9), 155.31 (C-10b), 137.48 (C-10a), 136.45 (C-7), 133.49 (=CH), 129.28 (=CH), 128.12 (C-5), 126.63 (C-6a), 124.63 (C-4a), 123.30 (C-6), 120.48 (C-8), 75.80 (C-3), 58.73 (CH_2), 40.11 (C-4), 14.26 (CH_3), 163.31, 146.74, 142.78, 130.64, 129.23, 126.67, 115.97 (aromatic); ^{13}C NMR–DEPT spectrum at 135° CH, CH_3 [positive (up)], CH_2 [negative (down)], revealed the following signals at δ 136.45 (C-7 ↑), 133.49 (=CH ↑), 129.28 (=CH ↑), 129.23 (aromatic ↑), 128.12 (C-5 ↑), 126.67 (aromatic ↑), 123.30 (C-6 ↑), 120.48 (C-8 ↑), 115.97 (aromatic ↑), 58.73 (CH_2 ↓), 40.11 (C-4 ↑), 14.26 (CH_3 ↑). In the DEPT spectrum at 90° only CH signals are positive (up) and showed δ 136.45 (C-7 ↑), 133.49 (=CH ↑), 129.28 (=CH ↑), 129.23 (aromatic ↑),

128.12 (C-5 ↑), 126.67 (aromatic ↑), 123.30 (C-6 ↑), 120.48 (C-8 ↑), 115.97 (aromatic ↑), 40.11 (C-4 ↑). In the DEPT spectrum at 45° (CH, CH_2, and CH_3 positive) revealed signals at δ 136.45 (C-7 ↑), 133.49 (=CH ↑), 129.28 (=CH ↑), 129.23 (aromatic ↑), 128.12 (C-5 ↑), 126.67 (aromatic ↑), 123.30 (C-6 ↑), 120.48 (C-8 ↑), 115.97 (aromatic ↑) 58.73 (CH_2 ↑), 40.11 (C-4 ↑), 14.26 (CH_3 ↑). ^{13}C NMR–APT spectrum CH, CH_3 [positive (up)], CH_2, Cq [negative (down)], revealed the following signals at δ 168.15 (CO ↓), 163.31 (aromatic ↓), 161.35 (C-2 ↓), 160.84 (C-9 ↓), 155.31 (C-10b ↓), 146.74 (aromatic ↓), 142.78 (aromatic ↓), 137.48 (C-10a ↓), 136.45 (C-7 ↑), 133.49 (=CH ↑), 130.64 (aromatic ↓), 129.28 (=CH ↑), 129.23 (aromatic ↑), 128.12 (C-5 ↑), 126.67 (aromatic ↑), 126.63 (C-6a ↓), 124.63 (C-4a ↓), 123.30 (C-6 ↑), 120.48 (C-8 ↑), 115.97 (aromatic ↑), 75.80 (C-3 ↓), 58.73 (CH_2 ↓), 40.11 (C-4 ↑), 14.26 (CH_3 ↑); MS *m/z* (%): 502 (M^++2, 3.15), 500 (M^+, 10.12). 388 (100), 315 (13), 219 (8), 176 (37), 113 (36), 75 (29); Anal. Calcd for $C_{29}H_{22}ClFN_2O_3$: C, 69.53; H, 4.43; N, 5.59. Found: C, 69.63; H, 4.56; N, 5.67%.

(*E*) Ethyl 2-amino-4-(4-bromophenyl)-9-(4-fluorostyryl)-4*H*-pyrano[3,2-*h*]quinoline-3-carboxylate (**6f**)

Pale yellow needles from benzene/ethanol; yield 79%; m.p. 190–191°C; IR (KBr) υ (cm^{-1}): 3408, 3292 (NH_2), 3040, 2977, 2900 (CH stretching), 1675 (CO); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.32–7.23 (m, 12H, aromatic), 7.86 (bs, 2H, NH_2, canceled by D_2O), 7.98 (d, J = 16.5 Hz, 1H, =CH), 7.32 (d, J = 16.5 Hz, 1H, =CH), 5.09 (s, 1H, H-4), 4.02 (q, J = 7.0 Hz, 2H, CH_2), 1.11 (t, J = 7.0 Hz, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 168.14 (CO), 161.35 (C-2), 160.84 (C-9), 155.31 (C-10b), 137.48 (C-10a), 136.44 (C-7), 133.49 (=CH), 129.63 (=CH), 128.24 (C-5), 126.64 (C-6a), 124.56 (C-4a), 123.30 (C-6), 120.48 (C-8), 75.74 (C-3), 58.74 (CH_2), 40.12 (C-4), 14.26 (CH_3), 163.31, 147.16, 142.78, 131.13, 129.28, 126.67, 119.12, 115.97 (aromatic); ^{13}C NMR–DEPT spectrum at 135° CH, CH_3 [positive (up)], CH_2 [negative (down)], revealed the following signals at δ 136.44 (C-7 ↑), 133.49 (=CH ↑), 131.13 (aromatic ↑), 129.63 (=CH ↑), 129.28 (aromatic ↑), 128.24 (C-5 ↑), 126.67 (aromatic ↑), 123.30 (C-6 ↑), 120.48 (C-8 ↑), 115.97 (aromatic ↑), 58.74 (CH_2 ↓), 40.12 (C-4 ↑), 14.26 (CH_3 ↑). In the DEPT spectrum at 90° only CH signals are positive (up) and showed δ 136.44 (C-7 ↑), 133.49 (=CH ↑), 131.13 (aromatic ↑), 129.63 (=CH ↑), 129.28 (aromatic ↑), 128.24 (C-5 ↑), 126.67 (aromatic ↑), 123.30 (C-6 ↑), 120.48 (C-8 ↑), 115.97 (aromatic ↑), 40.12 (C-4 ↑). In the DEPT spectrum at 45° (CH, CH_2, and CH_3 positive) revealed signals at δ 136.44 (C-7 ↑), 133.49 (=CH ↑), 131.13 (aromatic ↑), 129.63 (=CH ↑), 129.28 (aromatic ↑), 128.24 (C-5 ↑), 126.67 (aromatic ↑), 123.30 (C-6 ↑), 120.48 (C-8 ↑), 115.97 (aromatic ↑), 58.74 (CH_2 ↑), 40.12 (C-4 ↑), 14.26 (CH_3 ↑). ^{13}C NMR–APT spectrum CH, CH_3 [positive (up)], CH_2, Cq [negative (down)], revealed the following signals at δ 168.14 (CO ↓), 161.35 (C-2 ↓), 160.84 (C-9 ↓), 155.31 (C-10b ↓), 137.48 (C-10a ↓), 136.44 (C-7 ↑), 133.49 (=CH ↑), 129.63 (=CH ↑), 128.24 (C-5 ↑), 126.64 (C-6a ↓), 124.56 (C-4a ↓), 123.30 (C-6 ↑), 120.48 (C-8 ↑), 75.74 (C-3 ↓), 58.74 (CH_2 ↓), 40.12 (C-4 ↑), 14.26 (CH_3 ↑), 163.31 (aromatic ↓), 147.16 (aromatic ↓), 142.78 (aromatic ↓), 131.13 (aromatic ↑), 129.28 (aromatic ↑), 126.67 (aromatic ↑), 119.12 (aromatic ↓), 115.97 (aromatic ↑); MS *m/z* (%): 546 (M^++2, 10.00), 544 (M^+, 10.78), 518 (2.52), 516 (3.45), 474 (20.86), 472 (21.51), 435 (100), 361 (6), 266 (2), 221 (3), 176 (24), 114 (6), 75 (6); Anal. Calcd for $C_{29}H_{22}BrFN_2O_3$: C, 63.86; H, 4.07; N, 5.14. Found: C, 61.95; H, 3.95; N, 4.74%.

Antitumor screening

Cell culture

MCF-7, HCT and HepG-2 cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50 μg/ml gentamycin. Vero cell were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum, 1% L-glutamine, HEPES buffer and 50 μg/ml gentamycin.

All cells were maintained at 37°C in a humidified atmosphere with 5% CO_2 and were subcultures two to three times a week.

Cytotoxicity evaluation using viability assay

The cytotoxicity activity was studied against three cell lines: breast adenocarcinoma (MCF-7), lung carcinoma (HCT) and hepatocellular carcinoma (HepG-2) using the colorimetric MTT assay as described by Mossman (1983). The cells were seeded in in 96-well microtitre plate at a cell concentration of 1×10^4 cells per well in 100 μl of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial twofold dilutions of the metabolites were added confluent cell monolayer. The microtitre plates were incubated at 37°C in a humidified incubator with 5% CO_2 for a period of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without the test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for 24 h at 37°C, various concentrations of sample were added, and the incubation was continued for 48 h and viable cells yield was determined by a colorimetric MTT method.

In brief, after the end of the incubation period, the crystal violet solution (1%) was added to each well for 30 min. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid was then added to all wells and mixed thoroughly, and the plates were read on ELISA reader, using a test wavelength of 490 nm. Treated samples were compared with the control in the absence of the tested samples. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated.

Acknowledgments This study was supported by the King Abdulaziz City for Science and Technology (KACST), No. A-S-11-0560. The authors also deeply thanks Mr. Ali Y. A. Alshahrani for making the ^{1}H NMR and ^{13}C NMR samples.

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