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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, antitumor activity of 2-amino-4*H*-benzo[*h*]chromene derivatives, and structure–activity relationships of the 3- and 4-positions

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Abstract Several 2-amino-4*H*-benzo[*h*]chromenes (**3a–i**) and (**5a–h**) were obtained by reaction of 4-chloro-1-naphthol (**1**) with α-cyanocinnamonitrile (**2a–i**) or ethyl α-cyanocinnamate derivatives (**4a–h**), respectively. Structures of these compounds were established on the basis of spectral data. The antitumor activity of the synthesized compounds was investigated in comparison with Vinblastine, Colchicine, and Doxorubicin well-known anticancer drugs, using MTT colorimetric assay. Among them, the compounds **5e**, **3c**, **5f**, **b**, **d**, **3d**, **5c**, **a** were the most active against MCF-7, **5a** against HCT-116 and **5a**, **3e**, **a** against HepG-2 as compared with the standard drug Vinblastine, while the compounds **5e**, **3c**, **5f**, **b**, **d**, **3d**, **5c**, **a**, **h**, **3i**, **g**, **a**, **e** were the most active against MCF-7, **5a**, **c**, **e**, **f**, **b**, **3e**, **c**, **g**, **b**, **5d**, **h**, **3d**, **i**, **5g** against HCT-116, **5a**, **3e**, **a**, **5e**, **3c**, **5d**, **c**, **f**, **3b**, **5g**, **3g**, **5h** against HepG-2 as compared with the standard drug Colchicine. The structure–activity relationships of the 3- and 4-positions were discussed.

Keywords 4-Chloro-1-naphthol · α-Cyanocinnamonitriles · Ethyl α-cyanocinnamates · 4*H*-Benzo[*h*]chromenes · Antitumor · SAR

Introduction

2-Amino-4*H*-chromenes and 2-amino-4*H*-benzochromenes are an important class of heterocyclic compounds having important biological activities. During the last decade, such compounds had shown interesting pharmacological properties including antimicrobial (Kidwai *et al.*, 2010; Alvey *et al.*, 2009; Kumar *et al.*, 2009; Raj *et al.*, 2009), antileishmanial (Tanaka *et al.*, 2007), anticancer (Sabry *et al.*, 2011; Rampa *et al.*, 2005), antioxidant (Singh *et al.*, 2010; Vukovic *et al.*, 2010), antiproliferative (Magedov *et al.*, 2007), antitumor (Mahmoodi *et al.*, 2010; Endo *et al.*, 2010; Tseng *et al.*, 2010) effects and activities, as well as treatment of alzheimer's disease (Bruhlmann *et al.*, 2001) and schizophrenia disorder (Kesten *et al.*, 1999). Fused chromene ring systems have blood platelet antiaggregating (Lee *et al.*, 2006) and analgesic activities (El-Sayed and Ibrahim 2010; Keri *et al.*, 2010).

They also exhibit hypolipidemic activity (Sashidhara *et al.*, 2011), DNA breaking activities and mutagenicity (Hiramoto *et al.*, 1997).

As a result of remarkable pharmacological efficiency of 4*H*-chromene and 4*H*-benzochromene derivatives and in continuation of our program on the chemistry of 4*H*-pyran derivatives (Al-Ghamdi *et al.*, 2012; El-Agrody, 1994; El-Agrody *et al.*, 1997a, b, 2000, 2001, 2002, 2011, 2012a, b; El-Agrody and Al-Ghamdi, 2011; Sabry *et al.*, 2011; Abd-El-Aziz *et al.*, 2004, 2007; Eid *et al.*, 2003; Khafagy *et al.*, 2002; Bedair *et al.*, 2000, 2001; Sayed *et al.*, 2000), we report herein the synthesis of 4*H*-benzo[*h*]chromene derivatives and the evaluation of their antitumor activities. The chemical structures of the studied compounds and their structure–activity relationships (SAR) are discussed in this study.

Chemistry

Treatment of 4-chloro-1-naphthol (**1**) with α-cyano-4-substitutedcinnamonitriles (**2a–g**) or α-cyano-4-(4-piperdin-1-

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ylphenyl/morpholinophenyl)cinnamonitrile (**2h**, **i**) in ethanolic piperidine under reflux afforded 2-amino-4-aryl-6-chloro-4*H*-benzo[*h*]chromene-3-carbonitrile derivatives (**3a**–**i**) (Scheme 1).

In a similar manner, the reaction of 4-chloro-1-naphthol (**1**) with ethyl α-cyano-4-substituted cinnamate (**4a**–**g**) or ethyl α-cyano-4-(4-morpholinophenyl) cyanocinnamate (**4h**) afforded ethyl 2-amino-4-aryl-6-chloro-4*H*-benzo[*h*]chromene-3-carboxylate derivatives (**5a**–**h**) (Scheme 2).

The structures **3** and **5** were established on the basis of IR, ^{1}H NMR, ^{13}C NMR, ^{13}C NMR-DEPT, ^{13}C NMR-APT, MS data, and in conjunction with our previous work (Abd-El-Aziz *et al.*, 2004; El-Agrody, 1994; El-Agrody *et al.*, 1997a, b; Khafagy *et al.*, 2002; Sayed *et al.*, 2000; Sabry *et al.*, 2011). The IR spectra of **3a**–**i** showed the appearance of the a NH_2 stretch at υ 3471–3424, 3334–3326, 3222–3190 cm,$^{-1}$ a CN stretch at υ 2204–2192 cm,$^{-1}$ while a NH_2 stretch at υ 3478–3380, 3325–3282 cm^{-1} and a CO stretch at υ 1687–1669 cm^{-1} for **5a**–**h**. The ^{1}H and ^{13}C NMR spectra of **3a**–**i** and **5a**–**h** revealed the presence of 4*H* signals at *δ* 5.28–4.79 (s, 1H, H-4) and 40.18–38.77 ppm (C-4). In compounds **5a**–**h** the ester group gave ^{1}H signals at 4.05–4.01 (q, $J = 7.0$–7.2 Hz, 2H, CH_2), 1.12–1.09 (t, $J = 7.0$–7.2 Hz, 3H, CH_3) with the corresponding signals in the ^{13}C spectra at 58.86–58.63 (CH_2) and 14.31–14.19 ppm (CH_3), respectively. The ^{13}C NMR-DEPT spectra at 45°, 90°, and 135° and ^{13}C NMR-APT spectra of compounds **3** and **5** provided additional evidence in support of the proposed structures. In addition, the ^{1}H NMR spectra for compounds **3** and **5** showed NH_2 protons resonated at 7.34–7.14 (sharp singlet) and 8.00–7.82 (broad singlet lower field), respectively. This deshielding is a result of replacement of CN group in **3** by C=O group in **5** whose C=O anisotropy would deshield these protons and in addition of the involvement of these protons in hydrogen bonding with the C=O group. This also, was supported by X-ray single crystal data (Al-Dies *et al.*, 2012; El-Agrody *et al.*, 2012). The mass spectra of compounds **3** and **5** gave also additional evidences for the proposed structures.

Antitumor assays

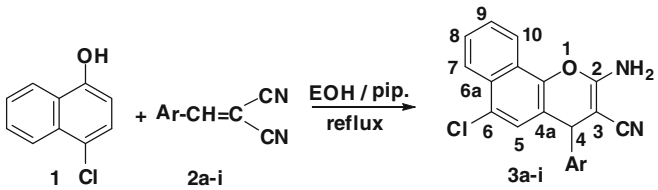
Compounds **3a**–**i** and **5a**–**h** were evaluated for human tumor cell growth inhibitory activity against three cell lines: breast adenocarcinoma (MCF-7), lung carcinoma (HCT-116), and hepatocellular carcinoma (HepG-2). The measurements of cell growth and the viabilities were determined as described in the literature (Rahman *et al.*, 2001). In vitro cytotoxicity evaluation using viability assay was performed at the Regional Center for Mycology & Biotechnology (RCMP), Al-Azhar University using Vinblastine, Colchicine and Doxorubicin as standard drugs. The inhibitory activity of the synthetic compounds **3a**–**i** and **5a**–**h** against the three cell lines MCF-7, HCT-116, and HepG-2 are given in Table 1 and Figs. 1, 2, 3, 4, 5, and 6.

Results and discussion

4*H*-Benzo[*h*]chromene derivatives were selected for this study as their families are well known to contain active compounds with a wide range of biological and pharmacological activities (Kidwai *et al.*, 2010; Alvey *et al.*, 2009; Kumar *et al.*, 2009; Raj *et al.*, 2009; Tanaka *et al.*, 2007; Sabry *et al.*, 2011; Rampa *et al.*, 2005; Singh *et al.*, 2010; Vukovic *et al.*, 2010; Magedov *et al.*, 2007; Mahmoodi *et al.*, 2010; Endo *et al.*, 2010; Tseng *et al.*, 2010; Bruhlmann *et al.*, 2001; Kesten *et al.*, 1999; Lee *et al.*, 2006; El-Sayed and Ibrahim, 2010; Keri *et al.*, 2010; Sashidhara *et al.*, 2011; Hiramoto *et al.*, 1997).

In this study, seventeen compounds of 4*H*-benzo[*h*]chromene derivatives were prepared. Structures of the synthesized compounds were elucidated on the basis of IR,

Scheme 1 Synthesis of 4*H*-benzo[*h*]chromene-3-carbonitrile derivatives (**3a**–**i**)



Scheme 2 Synthesis of ethyl 4*H*-benzo[*h*]chromene-3-carboxylate derivatives (**5a**–**h**)

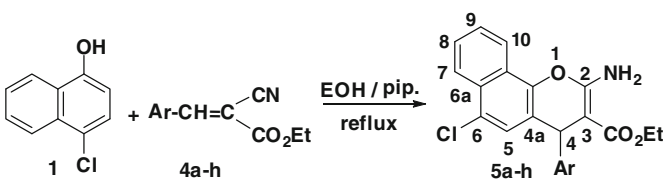


Table 1 Effects of the treatment of MCF-7, HCT-116, and HepG-2 cells with various concentrations of the prepared compounds; cytotoxicity (IC_{50}) as measured with the MTT method

Compounds	Conc. (μg/ml)	MCF-7 Cell viability %	IC_{50} (μg/ml)	HCT-116 Cell viability %	IC_{50} (μg/ml)	HepG-2 Cell viability %	IC_{50} (μg/ml)
Vinblastine	50	07.82	6.1 ± 0.01	16.27	2.6 ± 0.04	14.38	4.6 ± 0.05
	25	15.18		21.68		16.13	
	12.5	29.60		28.20		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	100.00		100.00		100.00	
Colchicine	50	26.99	17.7 ± 0.03	41.40	42.8 ± 0.08	17.42	10.6 ± 0.01
	25	35.68		70.27		30.26	
	12.5	60.34		75.27		43.89	
	6.25	72.81		98.22		64.24	
	3.125	86.16		100.00		76.55	
	1.56	93.63		100.00		89.44	
	0	100.00		100.00		100.00	
Doxorubicin	5 50	3.24	0.426 ± 0.01	6.82	0.469 ± 0.015	10.95	0.9 ± 0.04
	25	6.55		8.89		14.29	
	12.5	11.74		14.83		16.90	
	6.25	17.22		16.17		21.03	
	3.125	21.18		22.28		30.32	
	1.56	30.86		34.64		43.2	
	0	100.00		100.00		100.00	
3a	50	24.25	13.9 ± 0.07	82.33	w	09.78	4.2 ± 0.05
	25	29.11		95.46		14.44	
	12.5	52.74		100.00		19.06	
	6.25	63.36		100.00		28.56	
	3.125	75.14		100.00		60.94	
	1.56	96.92		100.00		88.11	
	0	100.00		100.00		100.00	
3b	50	21.03	19.5 ± 0.01	16.89	7.2 ± 0.02	19.42	7.6 ± 0.06
	25	37.12		22.33		27.86	
	12.5	66.16		31.56		37.44	
	6.25	73.01		53.44		53.44	
	3.125	83.63		75.00		75.00	
	1.56	97.95		91.39		91.39	
	0	100.00		100.00		100.00	
3c	50	10.62	3.0 ± 0.06	10.89	5.8 ± 0.3	11.94	5.5 ± 0.05
	25	16.37		16.89		18.62	
	12.5	23.22		26.17		30.66	
	6.25	37.74		48.00		46.14	
	3.125	48.63		63.33		62.25	
	1.56	64.45		86.72		83.58	
	0	100.00		100.00		100.00	
3d	50	13.15	5.5 ± 0.05	29.45	10.9 ± 0.08	25.14	10.7 ± 0.2
	25	17.95		37.84		36.22	
	12.5	30.41		45.55		43.46	
	6.25	47.33		63.29		65.84	
	3.125	58.42		82.53		81.12	
	1.56	70.00		95.41		93.96	
	0	100.00		100.00		100.00	

Table 1 continued

Compounds	Conc. (μg/ml)	MCF-7 Cell viability %	IC_{50} (μg/ml)	HCT-116 Cell viability %	IC_{50} (μg/ml)	HepG-2 Cell viability %	IC_{50} (μg/ml)
3e	50	29.29	13.9 ± 0.3	12.88	5.7 ± 0.02	10.72	3.5 ± 0.09
	25	40.21		16.58		14.83	
	12.5	51.23		23.84		26.50	
	6.25	65.68		44.18		40.72	
	3.125	78.01		76.71		51.33	
	1.56	93.63		96.99		72.67	
	0	100.00		100.00		100.00	
3f	50	67.95	w	53.68	w	48.82	48.0 ± 0.4
	25	72.40		71.24		63.36	
	12.5	88.54		85.36		76.84	
	6.25	93.32		96.12		88.44	
	3.125	100.00		100.00		97.65	
	1.56	100.00		100.00		100.00	
	0	100.00		100.00		100.00	
3g	50	32.60	13.0 ± 0.07	10.61	6.0 ± 0.01	19.64	9.2 ± 0.03
	25	43.97		13.22		27.67	
	12.5	50.27		22.00		43.67	
	6.25	62.40		47.39		55.83	
	3.125	81.44		77.44		72.62	
	1.56	98.84		94.11		88.90	
	0	100.00		100.00		100.00	
3h	50	43.63	31.5 ± 0.14	48.44	45.5 ± 0.23	41.80	26.1 ± 0.02
	25	52.25		57.12		50.39	
	12.5	58.28		66.58		63.17	
	6.25	66.08		73.06		72.50	
	3.125	78.43		79.86		81.48	
	1.56	87.65		90.25		92.32	
	0	100.00		100.00		100.00	
3i	50	30.48	12.2 ± 0.06	34.72	20.3 ± 0.15	37.14	23.4 ± 0.06
	25	41.64		45.11		48.28	
	12.5	49.52		58.11		61.86	
	6.25	58.63		78.33		80.14	
	3.125	77.60		90.33		93.62	
	1.56	93.42		100.00		100.00	
	0	100.00		100.00		100.00	
5a	50	20.14	6.0 ± 0.07	09.89	2.7 ± 0.015	10.94	2.8 ± 0.03
	25	27.74		12.11		15.76	
	12.5	41.10		16.89		20.92	
	6.25	48.49		22.50		29.58	
	3.125	71.64		40.61		41.46	
	1.56	88.01		79.06		76.24	
	0	100.00		100.00		100.00	
5b	50	18.22	5.0 ± 0.02	11.44	5.6 ± 0.08	14.25	5.9 ± 0.013
	25	23.29		16.00		18.96	
	12.5	29.86		19.56		27.38	
	6.25	39.52		44.33		46.89	
	3.125	64.97		73.83		72.11	
	1.56	73.15		91.28		87.18	
	0	100.00		100.00		100.00	

Table 1 continued

Compounds	Conc. (μg/ml)	MCF-7 Cell viability %	IC_{50} (μg/ml)	HCT-116 Cell viability %	IC_{50} (μg/ml)	HepG-2 Cell viability %	IC_{50} (μg/ml)
5c	50	17.53	5.8 ± 0.4	12.06	3.1 ± 0.06	11.61	7.1 ± 0.2
	25	20.75		15.11		15.17	
	12.5	27.74		18.56		26.50	
	6.25	44.93		36.61		53.83	
	3.125	78.49		49.56		75.83	
	1.56	94.11		82.22		95.11	
	0	100.00		100.00		100.00	
5d	50	12.88	5.1 ± 0.01	10.78	7.6 ± 0.03	12.54	7.6 ± 0.09
	25	17.40		13.66		16.93	
	12.5	23.15		20.67		27.42	
	6.25	38.84		57.72		56.06	
	3.125	69.59		85.28		82.95	
	1.56	90.62		96.00		94.12	
	0	100.00		100.00		100.00	
5e	50	07.60	2.4 ± 0.02	12.47	4.7 ± 0.036	14.12	5.0 ± 0.04
	25	10.48		14.11		18.48	
	12.5	13.84		20.00		27.96	
	6.25	25.62		40.82		42.14	
	3.125	34.18		59.93		61.37	
	1.56	69.73		88.84		87.28	
	0	100.00		100.00		100.00	
5f	50	07.88	4.5 ± 0.013	12.12	5.2 ± 0.028	11.91	5.7 ± 0.08
	25	10.07		14.38		17.24	
	12.5	14.32		21.78		26.85	
	6.25	36.03		40.68		46.44	
	3.125	60.34		68.49		67.12	
	1.56	82.60		92.19		86.96	
	0	100.00		100.00		100.00	
5g	50	41.10	42.8 ± 0.28	33.72	34.8 ± 0.35	27.88	28.3 ± 0.16
	25	70.21		60.50		53.42	
	12.5	85.14		68.78		69.14	
	6.25	95.48		78.78		80.86	
	3.125	98.22		92.17		89.12	
	1.56	100.00		100.00		97.08	
	0	100.00		100.00		100.00	
5h	50	10.27	7.7 ± 0.03	11.71	10.4 ± 0.13	14.22	10.5 ± 0.41
	25	13.22		15.41		21.18	
	12.5	32.33		34.79		37.14	
	6.25	55.27		79.04		77.48	
	3.125	87.26		93.56		90.32	
	1.56	99.11		100.00		97.86	
	0	100.00		100.00		100.00	

W weak activity (IC_{50} > 50 μg/ml)

IC_{50} values expressed in μg/ml as the mean values of triplicate wells from at least three experiments and are reported as the mean ± standard error

^{1}H NMR, ^{13}C NMR, ^{13}C NMR-DEPT, ^{13}C NMR-APT, and MS data.

Compounds **3a–i** and **5a–h** were tested against three tumer cell lines: MCF-7, HCT-116, and HepG-2. The cytotoxicity evaluation using viability assays and inhibitory activities are given in Table 1 and Figs. 1, 2, 3, 4, 5, and 6.

The results from Table 1 indicated that compounds **5e**, **3c**, **5f**, **b**, **d**, **3d**, **5c**, **a** were the most active against MCF-7, **5a** against HCT-116 and **5a**, **3e**, **a** against HepG-2 as compared with the standard drug Vinblastine, while compounds **5e**, **3c**, **5f**, **b**, **d**, **3d**, **5c**, **a**, **h**, **3i**, **g**, **a**, **e** were the most active against MCF-7, **5a**, **c**, **e**, **f**, **b**, **3e**, **c**, **g**, **b**, **5d**, **h**, **3d**, **i**,

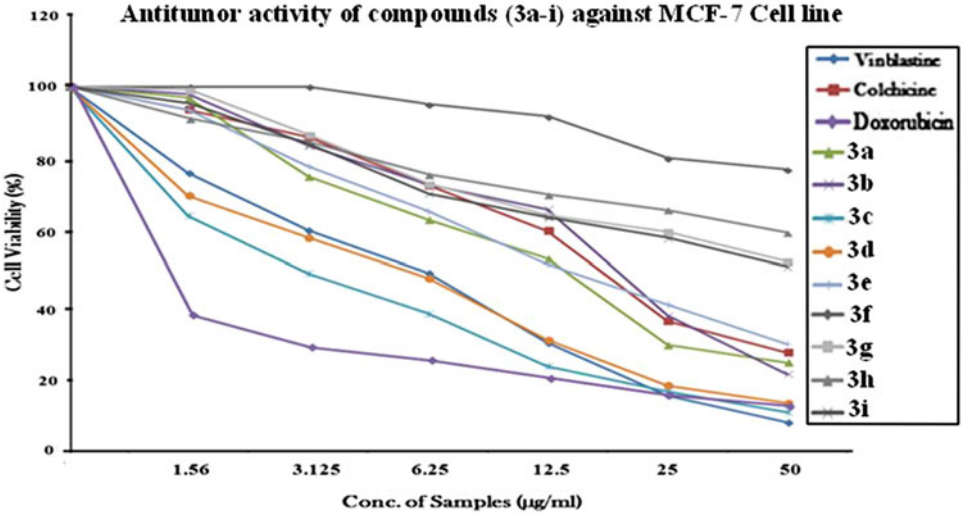


Fig. 1 Evaluation of cytotoxicity for compounds (**3a–i**) against MCF-7cell line

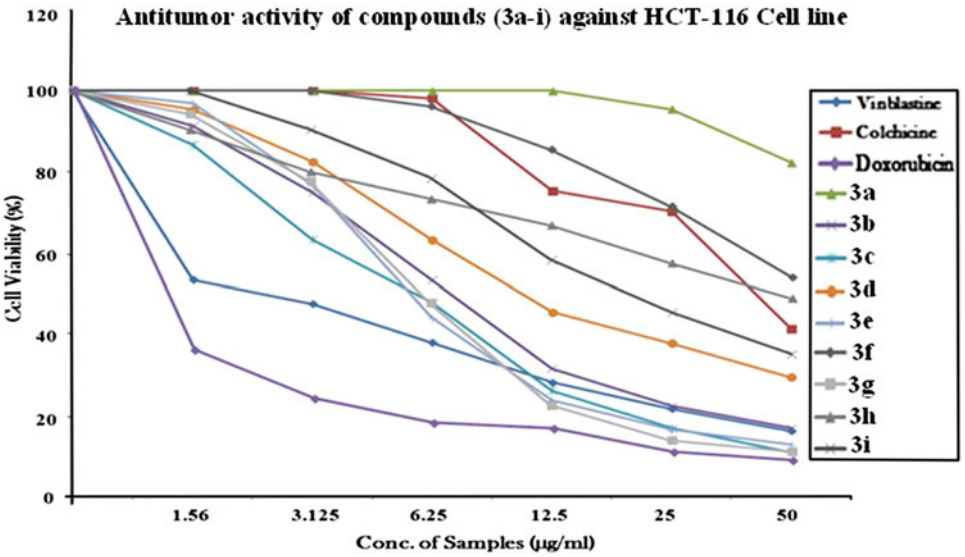


Fig. 2 Evaluation of cytotoxicity for compounds (**3a–i**) against HCT-116 cell line

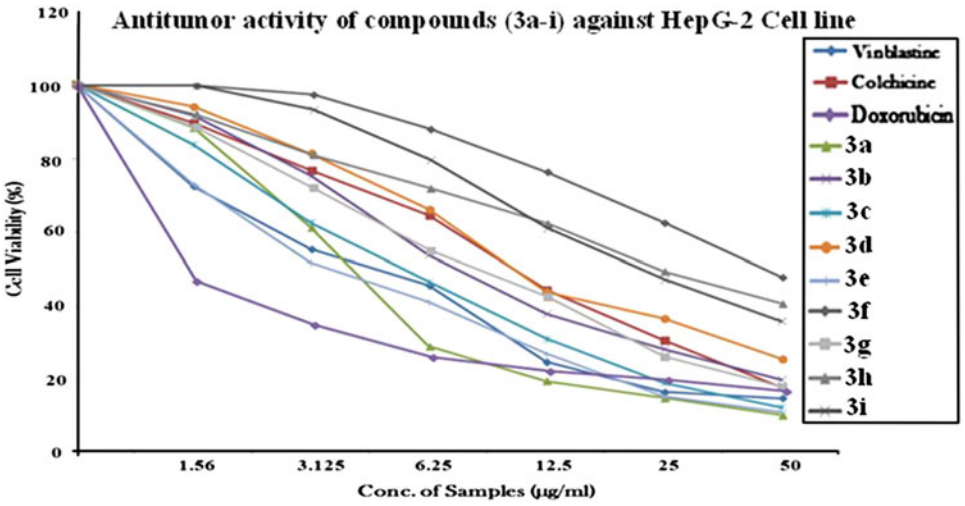


Fig. 3 Evaluation of cytotoxicity for compounds (**3a–i**) against HepG-2 cell line

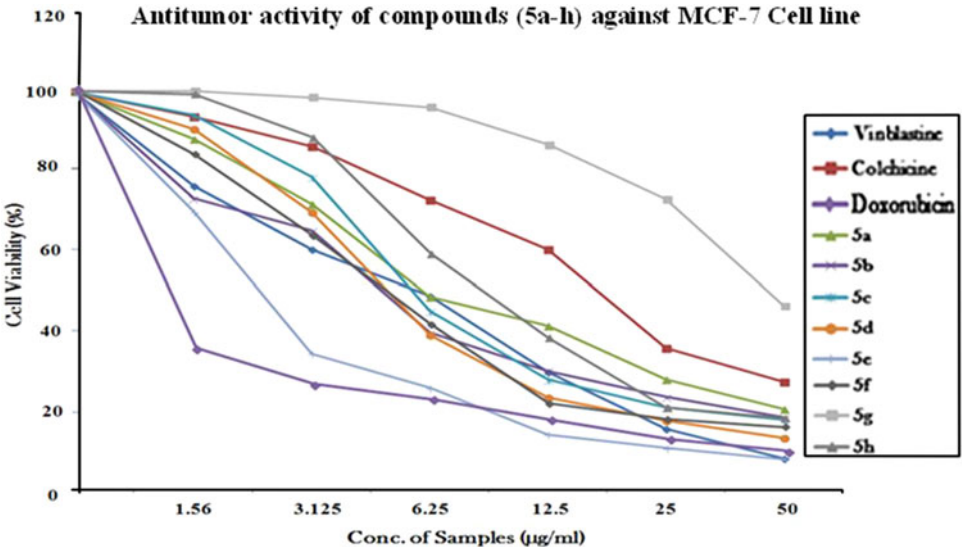


Fig. 4 Evaluation of cytotoxicity for compounds (**5a–h**) against MCF-7 cell line

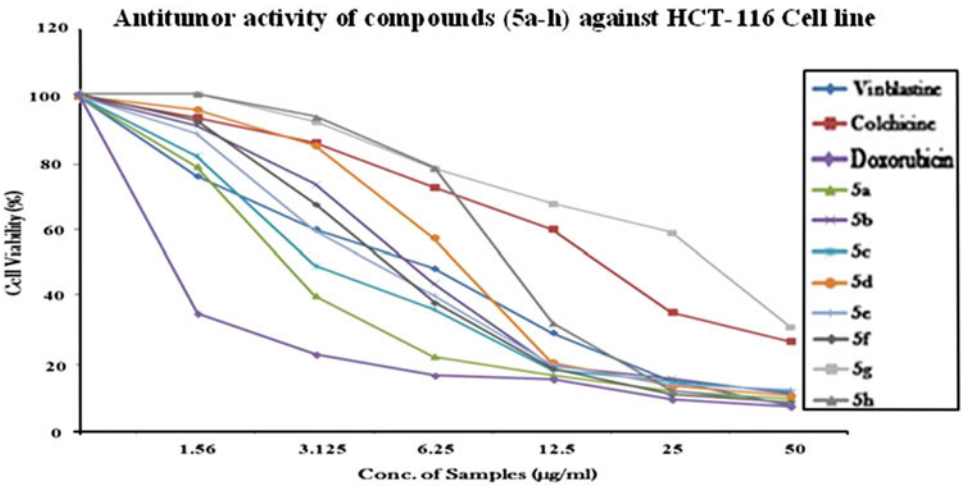


Fig. 5 Evaluation of cytotoxicity for compounds (**5a–h**) against HCT-116 cell line

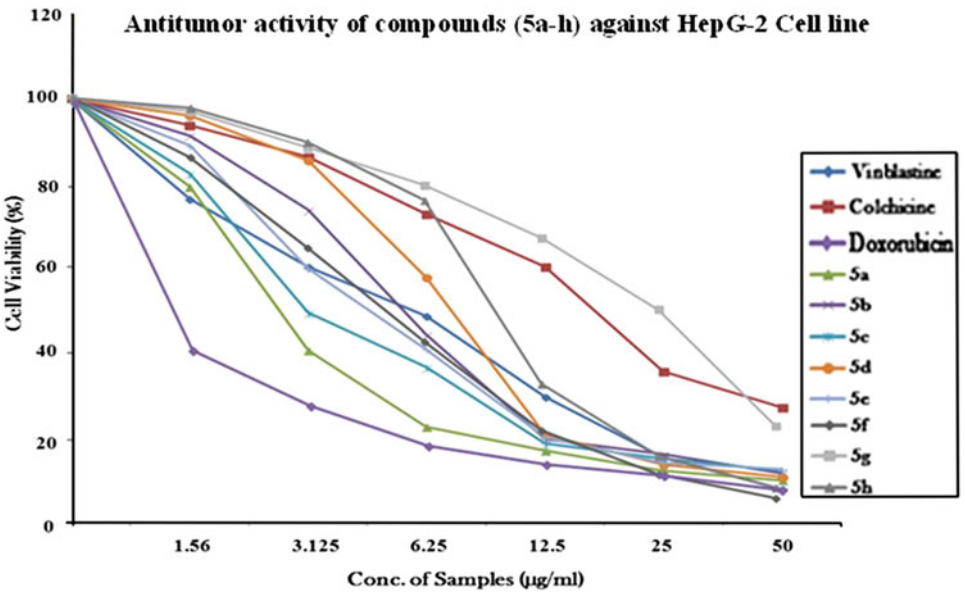


Fig. 6 Evaluation of cytotoxicity for compounds (**5a–h**) against HepG-2 cell line

5g against HCT-116, **5a**, **3e**, **a**, **5e**, **3c**, **5d**, **c**, **f**, **3b**, **5g**, **3g**, **5h** against HepG-2 as compared with the standard drug Colchicine and the remaining compounds exhibited near or moderate to lower activities as compared with the standard drugs Vinblastine, Colchicine, and Doxorubicin.

SAR studies

The antitumor activity (IC_{50}) of compounds **3**, **5** and its analogs in the three cancer cell lines is summarized in Table 1. By maintaining the 2-amino and 3-cyano groups of **3a**, the SAR studies at the 4-position was explored. The non-substituted phenyl analog **3a** was much less active (IC_{50} = 13.9 μg/ml), confirming the importance of a substituent at the phenyl ring at the 4-position. Compounds **3c** and **3d** have the higher potent antitumor activity (IC_{50} = 3.0–5.5 μg/ml) against MCF-7 as compared to the other compounds **3i**, **g**, **e**, **b**, **i**, **f** (IC_{50} = 12.2–50.0 μg/ml) and the standard drug Vinblastine (IC_{50} = 6.1 μg/ml). This potency could be attributed to the presence of the chloro or bromo atoms (electron-withdrawing groups) at the *para*-position of phenyl ring at 4-position. Replacement of the chloro or bromo atoms with other groups, resulted in reduction of potency, suggesting that there might be a size limited pocket at the *para*-position of phenyl ring at 4-position and an electron-withdrawing group is preferred over an electron-donating group. In addition, compounds **3c**, **d**, **i**, **g**, **a**, **e** have the higher potent antitumor activity (IC_{50} = 3.0-13.9 μg/ml) against MCF-7, compared to other compounds and the standard drug Colchicine (IC_{50} = 17.7 μg/ml). This potency could be attributed to the presence of the chloro, bromo, nitro (electron-withdrawing), morpholino, and methyl (electron-donating) groups at the *para*-position of phenyl ring at 4-position, suggesting that there might be a size limited pocket at the 4-position of the phenyl ring and an electron-withdrawing group is preferred over an electron-donating group. Replacement of the electron-withdrawing group cyano group by ester group at the 3-position for compound **5a** and its analogs improved the antitumor activities. Compounds **5e**, **f**, **b**, **d**, **c**, **a** (IC_{50} = 2.4–6.0 μg/ml) have potent antitumor activities against the MCF-7 than the other compounds **5h**, **g** (IC_{50} = 7.7–42.9 μg/ml) and the standard drug Vinblastine (IC_{50} = 6.1 μg/ml). These data indicate that the activities of compounds **5e**, **f**, **b**, **d**, **c**, **a** are considerably enhanced by the presence of the methyl, methoxy (electron-donating), fluoro, bromo, chloro (electron-withdrawing) at the *para*-position of phenyl ring at 4-position or the phenyl ring at the 4-position, suggesting that there might be a size limited pocket at the *para*-position of phenyl ring at 4-position and an electron-donating group is preferred over an electron-withdrawing group, while compound **5h** (IC_{50} = 7.7 μg/ml)-exhibited good activity and compound **5g** (IC_{50} = 42.8 μg/ml) inactive.

In the case of HCT-116, investigation of (SAR) revealed that compound **5a** (IC_{50} = 2.7 μg/ml) has the most potent activity against HCT-116 compared to the other compounds **3a–i**, **5b–h** and the standard drug Vinblastine (IC_{50} = 2.6 μg/ml). This potency could be attributed to the presence of the non-substituted phenyl group at the *para*-position of phenyl ring at 4-position with the ester group at the 3-position, suggesting that there might be a size limited pocket at the 4-position and the non-substituted phenyl (electron-donating group) at the 4-position is preferred over the *para*-substituted phenyl at the 4-position. In addition, compounds **5a**, **c**, **e**, **f**, **b**, **3e**, **c**, **g**, **b**, **5d**, **h**, **3d**, **i**, **5g** (IC_{50} = 2.6–34.8 μg/ml) have the most potent activity against HCT-116 compared to the other compounds **3a**, **f** and the standard drug Colchicine (IC_{50} = 42.8 μg/ml). This potency could be attributed to the presence of the non-substituted phenyl (electron-donating group) at the 4-position and the substituted phenyl at the *para*-position of phenyl ring at 4-position with the chloro (electron-withdrawing), methyl, methoxy (electron-donating), fluoro groups (electron-withdrawing) with the ester group at the 3-position for compounds **5a**, **c**, **e**, **f**, **b**, the methyl (electron-donating), chloro, nitro, fluoro groups (electron-withdrawing) with the cyano group at the 3-position for compounds **3e**, **c**, **g**, **b**, the bromo (electron-withdrawing), morpholino groups (electron-donating) with the ester group at the 3-position for compounds **5d**, **h**, the bromo (electron-withdrawing), morpholino groups (electron-donating) with the cyano group at the 3-position for compounds **3d**, **i** and nitro group (electron-withdrawing) with the ester group at the 3-position for compound **5g**, respectively, at the *para*-position of phenyl ring.

Furthermore, compounds **5a**, **3e**, **a** (IC_{50} = 2.8–4.2 μg/ml) showed higher antitumor activities against HepG-2 than the standard drug Vinblastine (IC_{50} = 4.6 μg/ml). This could be attributed to the presence of the non-substituted phenyl group (electron-donating) at the 4-position with the ester group at the 3-position for compound **5a** and the substituted at the *para*-position of phenyl ring at 4-position with the chloro (electron-withdrawing) with the cyano group at the 3-position for compound **3e** or the non-substituted phenyl group (electron-donating) at the 4-position with the cyano group at the 3-position for compound **3a**, suggesting that there might be a size limited pocket at the *para*-position of phenyl ring at 4-position and the non-substituted phenyl (electron-donating group) at the 4-position is preferred over the substituted at the *para*-position of phenyl ring at 4-position. In addition, compounds **5a**, **3e**, **a**, **5e**, **3c**, **5d**, **c**, **f**, **3b**, **5g**, **3g**,**5h** (IC_{50} = 2.8–10.5 μg/ml) showed higher antitumor activities against HepG-2 than the standard drug Colchicine (IC_{50} = 10.6 μg/ml). This potency could be attributed to the presence of the non-substituted phenyl (electron-

donating group) at the 4-position, suggesting that there might be a size limited pocket at the 4-position and the non-substituted phenyl (electron-donating group) at the 4-position is preferred over the substituted at the *para*-position of phenyl ring at 4-position.

Finally, in the case of MCF-7, HCT-116, and HepG-2, an investigation of SAR revealed that compounds **3a–i** and **5a–h** showed moderate to lower antitumor activities against MCF-7, HCT-116, and HepG-2 as compared to the standard drug Doxorubicin.

Conclusions

Our interest in the synthesis of 4*H*-benzo[*h*]chromene derivatives is to focus on their antitumor activities as a part of our recent research line that aims at the development of new heterocyclic compounds as strong potent antitumor agents (El-Agrody *et al.*, 2011). Thus, in this paper we revealed the synthesis of some 4*H*-benzo[*h*]chromene, followed by antitumor evaluation for all of the synthesized compounds. Seventeen compounds of 4*H*-benzo[*h*]-chromene derivatives were prepared and their structures were elucidated on the basis of IR, ^{1}H NMR, ^{13}C NMR, ^{13}C NMR-DEPT/APT, and MS data. Compounds **5e**, **3c**, **5f**, **b**, **d**, **3d**, **5c**, **a** were the most active against MCF-7, **5a** against HCT-116 and **5a**, **3e**, **a** against HepG-2 as compared with the standard drug Vinblastine, while compounds **5e**, **3c**, **5f**, **b**, **d**, **3d**, **5c**, **a**, **h**, **3i**, **g**, **a**, **e** were the most active against MCF-7, **5a**, **c**, **e**, **f**, **b**, **3e**, **c**, **g**, **b**, **5d**, **h**, **3d**, **i**, **5g** against HCT-116, **5a**, **3e**, **a**, **5e**, **3c**, **5d**, **c**, **f**, **3b**, **5g**, **3g**, **5h** against HepG-2 as compared with the standard drug Colchicine and the remaining compounds exhibited near or moderate to lower activities as compared with the standard drugs Vinblastine, Colchicine, and Doxorubicin. A more extensive study is also warranted to determine additional antitumor parameters in order to give a deeper insight to its structure–activity relationship and to optimize the effectiveness of this series of molecules, which can then be used in bigger scenarios such as drug design or development of antitumor therapeutics.

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), where the signals of CH and CH_3 carbon atoms appear normal (up) and the signals of carbon atoms in CH_2 environments appear 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.

General procedure for the preparation of (3) and (5)

A solution of 4-chloro-1-naphthol (**1**) (0.01 mmol) in EtOH (30 ml) and piperidine (0.5 ml) was treated with α-cyanocinnamonitriles (**2a–i**) or ethyl α-cyanocinnamates (**4a–h**) (0.01 mmol). The reaction mixture was heated under reflux for 1–2 h. The solid product which formed was collected by filtration, washed with MeOH, and re-crystallized from ethanol or benzene. The physical and spectral data of compounds (**3**) and (**5**) were as follows:

*2-Amino-6-chloro-4-phenyl- 4H-benzo[h]chromene-3-carbonitrile (**3a**)*

Colorless crystals from ethanol; yield 86 %; m.p. 220–221 °C; IR (KBr) υ (cm^{-1}): 3477, 3325, 3191 (NH_2), 3082, 3017, 2855 (CH), 2199 (CN); ^{1}H NMR (500 MHz) (DMSO-d_6) δ: 8.36–7.26 (m, 10H, aromatic), 7.30 (s, 2H, NH_2, cancelled by D_2O), 4.93 (s, 1H, H-4); ^{13}C NMR (125 MHz) (DMSO-d_6) δ: 159.91 (C-2), 145.11 (C-10b), 129.21 (C-9), 128.78 (C-6a), 127.66 (C-5), 127.62 (C-10a), 127.09 (C-8), 125.52 (C-6), 123.88 (C-7), 121.40 (C-10), 120.18 (C-4a), 118.56 (CN), 56.03 (C-3), 40.53 (C-4), 141.99, 128.57, 128.21, 125.86 (aromatic); MS *m/z* (%): 334 (M^++2, 8.02), 332 (M^+, 23.35) with a base peak at 256 (100); Anal. Calcd for $C_{20}H_{13}ClN_2O$: C, 72.18; H, 3.94; N, 8.42. Found: C, 72.22; H, 3.97; N, 8.45 %.

*2-Amino-6-chloro-4-(4-fluorophenyl)-4H-benzo[h]chromene-3-carbonitrile (**3b**)*

Pale yellow crystals from benzene; yield 86 %; m.p. 246–247 °C (Khafagy *et al.*, 2002 m.p. 246 °C); IR (KBr) υ (cm^{-1}): 3470, 3326, 3190 (NH_2), 3092, 3037, 2983, 2865 (CH), 2197 (CN); ^{1}H NMR (500 MHz) (DMSO-d_6) δ: 8.35–7.16 (m, 9H, aromatic), 7.31 (s, 2H, NH_2, cancelled by D_2O), 4.97 (s, 1H, H-4); ^{13}C NMR (125 MHz) (DMSO-d_6) δ: 162.17 (C-2), 142.02 (C-10b), 129.57 (C-9), 129.29 (C-6a), 128.36 (C-5), 127.77 (C-10a), 125.84 (C-8), 123.93 (C-6), 123.83 (C-7), 121.46 (C-10), 120.11 (C-4a), 118.41 (CN), 55.94 (C-3), 40.07 (C-4), 160.23, 141.37, 129.64, 115.49 (aromatic); ^{13} C NMR-DEPT spectrum at 135° CH, CH_3 [positive (up)], CH_2 [negative (down)], revealed the following signals at δ: 129.64 (aromatic ↑), 129.57 (C-9 ↑), 128.36 (C-5 ↑), 125.84 (C-8 ↑), 123.83 (C-7 ↑), 121.46

(C-10 ↑), 40.07 (C-4 ↑), 115.49 (aromatic ↑). In the DEPT spectrum at 90° only CH signals are positive (up) and showed δ: 129.64 (aromatic ↑), 129.57 (C-9 ↑), 128.36 (C-5 ↑), 125.84 (C-8 ↑), 123.83 (C-7 ↑), 121.46 (C-10 ↑), 40.07 (C-4 ↑), 115.49 (aromatic ↑). In the DEPT spectrum at 45° (CH, CH_2, and CH_3 positive)-revealed signals at δ: 129.64 (aromatic ↑), 129.57 (C-9 ↑), 128.36 (C-5 ↑), 125.84 (C-8 ↑), 123.83 (C-7 ↑), 121.46 (C-10 ↑), 40.07 (C-4 ↑), 115.49 (aromatic ↑); MS *m/z* (%): 352 (M^++2, 5.14), 350 (M^+, 17.09) with a base peak at 256 (100); Anal. Calcd for $C_{20}H_{12}ClFN_2O$: C, 68.48; H, 3.45; N, 7.99. Found: C, 68.13; H, 3.41; N, 7.95 %.

*2-Amino-6-chloro-4-(4-chlorophenyl)-4H-benzo[h]chromene-3-carbonitrile (**3c**)*

Colorless needles from benzene; yield 88 %; m.p. 224–225 °C (Khafagy *et al.*, 2002 m.p. 224 °C); IR (KBr) υ (cm^{-1}): 3470, 3331, 3193 (NH_2), 3095, 3047, 2986, 2867 (CH), 2194 (CN); ^{1}H NMR (500 MHz) (DMSO-d_6) δ: 8.36–7.31 (m, 9H, aromatic), 7.34 (s, 2H, NH_2, cancelled by D_2O), 4.98 (s, 1H, H-4); ^{13}C NMR (125 MHz) (DMSO-d_6) δ: 159.91 (C-2), 144.05 (C-10b), 129.53 (C-9), 128.76 (C-6a), 128.32 (C-5), 127.72 (C-10a), 125.72 (C-8), 123.88 (C-6), 123.76 (C-7), 121.43 (C-10), 120.03 (C-4a), 118.02 (CN), 55.64 (C-3), 40.05 (C-4), 142.04, 131.75, 129.30, 125.64 (aromatic); Anal. Calcd for $C_{20}H_{12}Cl_2N_2O$: C, 65.41; H, 3.29; N, 7.63. Found: C, 65.44; H, 3.32; N, 7.65 %.

*2-Amino-6-chloro-4-(4-bromophenyl)-4H-benzo[h]chromene-3-carbonitrile (**3d**)*

Colorless needles from benzene; yield 90 %; m.p. 226–227 °C (Khafagy *et al.*, 2002 m.p. 226 °C); IR (KBr) υ (cm^{-1}): 3466, 3328, 3192 (NH_2), 3093, 3037, 2986, 2863 (CH), 2193 (CN); ^{1}H NMR (500 MHz) (DMSO-d_6) δ: 8.35–7.26 (m, 9H, aromatic), 7.34 (s, 2H, NH_2, cancelled by D_2O), 4.97 (s, 1H, H-4); ^{13}C NMR (125 MHz) (DMSO-d_6) δ: 159.88 (C-2), 144.45 (C-10b), 129.28 (C-9), 128.33 (C-6a), 127.73 (C-5), 125.72 (C-10a), 125.61 (C-8), 123.85 (C-6), 123.78 (C-7), 121.41 (C-10), 120.26 (C-4a), 117.94 (CN), 55.53 (C-3), 40.03 (C-4), 142.01, 131.76, 129.87, 119.99 (aromatic); Anal. Calcd for $C_{20}H_{12}BrClN_2O$: C, 58.35; H, 2.94; N, 6.80. Found: C, 58.31; H, 2.90; N, 6.77 %.

*2-Amino-6-chloro-4-(4-methylphenyl)-4H-benzo[h]chromene-3-carbonitrile (**3e**)*

Pale yellow needles from benzene; yield 90 %; m.p. 220–231 °C; IR (KBr) υ (cm^{-1}): 3450, 3334, 3222 (NH_2), 3073, 3057, 2986, 2865 (CH), 2192 (CN); ^{1}H NMR (500 MHz) (DMSO-d_6) δ: 8.36–7.14 (m, 9H, aromatic), 7.27 (s, 2H, NH_2, cancelled by D_2O), 4.88 (s, 1H, H-4), 2.27 (s, 3H, CH_3); ^{13}C NMR (125 MHz) (DMSO-d_6) δ: 159.80 (C-2), 142.20 (C-10b), 129.32 (C-9), 129.18 (C-6a), 127.63 (C-5), 127.54 (C-10a), 125.90 (C-8), 125.45 (C-6), 123.76 (C-7), 121.39 (C-10), 120.20 (C-4a), 118.70 (CN), 56.16 (C-3), 40.17 (C-4), 20.54 (CH_3), 141.90, 136.26, 128.16, 123.89 (aromatic); ^{13} C NMR-DEPT spectrum at 135° CH, CH_3 [positive (up)], CH_2 [negative (down)], revealed the following signals at δ: 129.32 (C-9 ↑), 128.16 (aromatic ↑), 127.63 (C-5 ↑), 125.90 (C-8 ↑), 123.89 (aromatic ↑), 123.76 (C-7 ↑), 121.39 (C-10 ↑), 40.17 (C-4 ↑), 20.54 (CH_3 ↑). In the DEPT spectrum at 90° only CH signals are positive (up) and showed δ: 129.32 (C-9 ↑), 128.16 (aromatic ↑), 127.63 (C-5 ↑), 125.90 (C-8 ↑), 123.89 (aromatic ↑), 123.76 (C-7 ↑), 121.39 (C-10 ↑), 40.17 (C-4 ↑). In the DEPT spectrum at 45° (CH, CH_2 and CH_3 positive) revealed signals at δ: 129.32 (C-9 ↑), 128.16 (aromatic ↑), 127.63 (C-5 ↑), 125.90 (C-8 ↑), 123.89 (aromatic ↑), 123.76 (C-7 ↑), 121.39 (C-10 ↑), 40.17 (C-4 ↑), 20.54 (CH_3 ↑).^{13}CNMR-APT spectrum CH, CH_3 [positive (up)], CH_2, Cq [negative (down)], revealed the following signals at δ: 159.80 (C-2 ↓), 142.20 (C-10b ↓), 141.90 (aromatic ↓), 136.26 (aromatic ↓), 129.32 (C-9 ↑), 129.18 (C-6a ↓), 128.16 (aromatic ↑), 127.63 (C-5 ↑), 127.54 (C-10a ↓), 125.90 (C-8 ↑), 125.45 (C-6 ↓), 123.89 (aromatic ↑), 123.76 (C-7 ↑), 121.39 (C-10 ↑), 120.20 (C-4a ↓), 118.70 (CN ↓), 56.16 (C-3 ↓), 40.17 (C-4 ↑), 20.54 (CH_3 ↑); MS *m/z* (%): 348 (M^++2, 6.92), 346 (M^+, 20.35) with a base peak at 252 (100); Anal. Calcd for $C_{21}H_{15}ClN_2O$: C, 72.73; H, 4.36; N, 8.08. Found: C, 72.76; H, 4.38; N, 8.10 %.

*2-Amino-6-chloro-4-(4-methoxyphenyl)-4H-benzo[h]chromene-3-carbonitrile (**3f**)*

Pale yellow needles from benzene; yield 88 %; m.p. 233–234 °C; IR (KBr) υ (cm^{-1}): 3424, 3333, 3213 (NH_2), 3076, 2953, 2834 (CH), 2193 (CN); ^{1}H NMR (500 MHz) (DMSO-d_6) δ: 8.35–6.90 (m, 9H, aromatic), 7.32 (s, 2H, NH_2, cancelled by D_2O), 4.87 (s, 1H, H-4), 3.73 (s, 3H, OCH_3); ^{13}C NMR (125 MHz) (DMSO-d_6) δ: 159.76 (C-2), 141.87 (C-10b), 129.19 (C-9), 128.75 (C-6a), 128.20 (C-5), 127.68 (C-10a), 125.97 (C-8), 125.46 (C-6), 123.93 (C-7), 121.43 (C-10), 120.24 (C-4a), 118.91 (CN), 56.35 (C-3), 55.00 (CH_3), 40.08 (C-4), 158.30, 137.26, 128.16, 114.15 (aromatic); MS *m/z* (%): 364 (M^++2, 4.31), 362 (M^+, 18.7) with a base peak at 75 (100); Anal. Calcd for $C_{21}H_{15}ClN_2O_2$: C, 69.52; H, 4.17; N, 7.72. Found: C, 69.56; H, 4.21; N, 7.75 %.

*2-Amino-6-chloro-4-(4-nitrophenyl)-4H-benzo[h]chromene-3-carbonitrile (**3g**)*

Pale yellow needles from benzene; yield 88 %; m.p. 257–258 °C; IR (KBr) υ (cm^{-1}): 3448, 3326, 3197 (NH_2),

3070, 2953, 2864 (CH), 2198 (CN); ^{1}H NMR (500 MHz) (DMSO-d_6) δ: 8.36–7.35 (m, 9H, aromatic), 7.44 (s, 2H, NH_2, cancelled by D_2O), 5.18 (s, 1H, H-4); ^{13}C NMR (125 MHz) (DMSO-d_6) δ: 160.10 (C-2), 142.28 (C-10b), 129.49 (C-9), 129.03 (C-6a), 128.27 (C-5), 127.78 (C-10a), 125.86 (C-6), 125.64 (C-8), 123.86 (C-7), 121.51 (C-10), 119.86 (C-4a), 117.26 (CN), 55.04 (C-3), 40.18 (C-4), 152.29, 146.61, 128.55, 124.14 (aromatic); ^{13} C NMR-DEPT spectrum at 135° CH, CH_3 [positive (up)], CH_2 [negative (down)], revealed the following signals at δ: 129.49 (C-9 ↑), 128.55 (aromatic ↑), 128.27 (C-5 ↑), 125.64 (C-8 ↑), 124.14 (aromatic ↑), 123.86 (C-7 ↑), 121.51 (C-10 ↑), 40.18 (C-4 ↑). In the DEPT spectrum at 90° only CH signals are positive (up) and showed δ: 129.49 (C-9 ↑), 128.55 (aromatic ↑), 128.27 (C-5 ↑), 125.64 (C-8 ↑), 124.14 (aromatic ↑), 123.86 (C-7 ↑), 121.51 (C-10 ↑), 40.18 (C-4 ↑). In the DEPT spectrum at 45° (CH, CH_2, and CH_3 positive) revealed signals at δ: 129.49 (C-9 ↑), 128.55 (aromatic ↑), 128.27 (C-5 ↑), 125.64 (C-8 ↑), 124.14 (aromatic ↑), 123.86 (C-7 ↑), 121.51 (C-10 ↑), 40.18 (C-4 ↑).^{13}CNMR-APT spectrum CH, CH_3 [positive (up)], CH_2, Cq [negative (down)], revealed the following signals at δ: 160.10 (C-2 ↓), 152.29 (aromatic ↓), 146.61(aromatic ↓), 142.28 (C-10b ↓), 129.49 (C-9 ↑), 129.03 (C-6a ↓), 128.55 (aromatic ↑), 128.27 (C-5 ↑), 127.78 (C-10a ↓), 125.86 (C-6 ↓), 125.64 (C-8 ↑), 124.14 (aromatic ↑), 123.86 (C-7 ↑), 121.51 (C-10 ↑), 119.86 (C-4a ↓), 117.26 (CN ↓), 55.04 (C-3 ↓), 40.18 (C-4 ↑); MS *m/z* (%): 379 (M^++2, 11.01), 377 (M^+, 33.01) with a base peak at 72 (100); Anal. Calcd for $C_{20}H_{12}ClN_3O_3$: C, 63.59; H, 3.20; N, 11.12. Found: C, 63.56; H, 3.27; N, 11.09 %.

*2-Amino-6-chloro-4-(4-piperdin-1-ylphenyl)-4H-benzo[h]chromene-3-carbonitrile (**3h**)*

Yellow needles from benzene; yield 82 %; m.p. 252–253 °C; IR (KBr) υ (cm^{-1}): 3471, 3298, 3184 (NH_2), 3075, 2993, 2938, 2864, 2813 (CH), 2204 (CN); ^{1}H NMR (500 MHz) (DMSO-d_6) δ: 8.32–6.86 (m, 9H, aromatic), 7.17 (s, 2H, NH_2, cancelled by D_2O), 4.79 (s, 1H, H-4), 3.09–3.07 (m, 4H, $2CH_2$), 1.60–1.57 (m, 4H, $2CH_2$), 1.51 (s, 2H, CH_2); ^{13}C NMR (125 MHz) (DMSO-d_6) δ: 159.74 (C-2), 141.83 (C-10b), 129.14 (C-6a), 128.19 (C-9), 127.69 (C-5), 126.09 (C-10a), 125.36 (C-8), 123.95 (C-6), 123.82 (C-7), 121.40 (C-10), 120.32 (C-4a), 119.25 (CN), 56.45 (C-3), 49.39 (CH_2), 40.18 (C-4), 25.25 (CH_2), 23.82 (CH_2), 150.65, 135.01, 128.13, 115.92 (aromatic); ^{13} C NMR-DEPT spectrum at 135° CH, CH_3 [positive (up)], CH_2 [negative (down)], revealed the following signals at δ: 128.19 (C-9 ↑), 128.13 (aromatic ↑), 127.69 (C-5 ↑), 125.36 (C-8 ↑), 123.82 (C-7 ↑), 121.40 (C-10 ↑), 49.39 (CH_2 ↓), 40.18 (C-4 ↑), 25.25 (CH_2 ↓), 23.82 (CH_2 ↓), 115.92 (aromatic ↑). In the DEPT spectrum at 90° only CH signals are positive (up) and showed δ: 128.19 (C-9 ↑), 128.13 (aromatic ↑), 127.69 (C-5 ↑), 125.36 (C-8 ↑), 123.82 (C-7 ↑), 121.40 (C-10 ↑), 40.18 (C-4 ↑), 115.92 (aromatic ↑). In the DEPT spectrum at 45° (CH, CH_2, and CH_3 positive)-revealed signals at δ: 128.19 (C-9 ↑), 128.13 (aromatic ↑), 127.69 (C-5 ↑), 125.36 (C-8 ↑), 123.82 (C-7 ↑), 121.40 (C-10 ↑), 49.39 (CH_2 ↑), 40.18 (C-4 ↑), 25.25 (CH_2 ↑), 23.82 (CH_2 ↑), 115.92 (aromatic ↑). ^{13}CNMR-APT spectrum CH, CH_3 [positive (up)], CH_2, Cq [negative (down)], revealed the following signals at δ: 159.74 (C-2 ↓), 150.65 (aromatic ↓), 141.83 (C-10b ↓), 135.01 (aromatic ↓), 129.14 (C-6a ↓), 128.19 (C-9 ↑), 128.13 (aromatic ↑), 127.69 (C-5 ↑), 126.09 (C-10a ↓), 125.36 (C-8 ↑), 123.95 (C-6 ↓), 123.82 (C-7 ↑), 121.40 (C-10 ↑), 120.32 (C-4a ↓), 119.25 (CN ↓), 56.45 (C-3 ↓), 49.39 (CH_2 ↓), 40.18 (C-4 ↑), 25.25 (CH_2 ↓), 23.82 (CH_2 ↓), 115.92 (aromatic ↑); MS *m/z* (%): 417 (M^++2, 2.11), 415 (M^+, 6.33) with a base peak at 55 (100); Anal. Calcd for $C_{25}H_{22}ClN_3O$: C, 72.19; H, 5.33; N, 10.10. Found: C, 72.22; H, 5.36; N, 10.14 %.

*2-Amino-6-chloro-4-(4-morpholinophenyl)-4H-benzo[h]chromene-3-carbonitrile (**3i**)*

Yellow needles from benzene; yield 82 %; m.p. 266–267 °C; IR (KBr) υ (cm^{-1}): 3451, 3327, 3208 (NH_2), 3095, 3010, 2967, 2938, 2864, 2816 (CH), 2195 (CN); ^{1}H NMR (500 MHz) (DMSO-d_6) δ: 8.33–6.88 (m, 9H, aromatic), 7.18 (s, 2H, NH_2, cancelled by D_2O), 4.81 (s, 1H, H-4), 3.72–3.70 (m, 4H, $2CH_2$), 3.08–3.06 (m, 4H, $2CH_2$); ^{13}C NMR (125 MHz) (DMSO-d_6) δ: 159.74 (C-2), 141.83 (C-10b), 129.16 (C-6a), 128.22 (C-9), 127.69 (C-5), 126.06 (C-8), 125.38 (C-10a), 123.94 (C-6), 123.81 (C-7), 121.41 (C-10), 120.29 (C-4a), 119.11 (CN), 66.06 (CH_2), 56.39 (C-3), 48.29 (CH_2), 40.09 (C-4), 150.04, 135.81, 128.29, 115.27 (aromatic); ^{13} C NMR-DEPT spectrum at 135° CH, CH_3 [positive (up)], CH_2 [negative (down)], revealed the following signals at δ: 128.29 (aromatic ↑), 128.22 (C-9 ↑), 127.69 (C-5 ↑), 126.06 (C-8 ↑), 123.81 (C-7 ↑), 121.41 (C-10 ↑), 66.06 (CH_2 ↓), 48.29 (CH_2 ↓), 40.09 (C-4 ↑), 115.27 (aromatic ↑). In the DEPT spectrum at 90° only CH signals are positive (up) and showed δ: 128.29 (aromatic ↑), 128.22 (C-9 ↑), 127.69 (C-5 ↑), 126.06 (C-8 ↑), 123.81 (C-7 ↑), 121.41 (C-10 ↑), 40.09 (C-4 ↑), 115.27 (aromatic ↑). In the DEPT spectrum at 45° (CH, CH_2, and CH_3 positive)-revealed signals at δ: 128.29 (aromatic ↑), 128.22 (C-9 ↑), 127.69 (C-5 ↑), 126.06 (C-8 ↑), 123.81 (C-7 ↑), 121.41 (C-10 ↑), 66.06 (CH_2 ↑), 48.29 (CH_2 ↑), 40.09 (C-4 ↑), 115.27 (aromatic ↑).^{13}CNMR-APT spectrum CH,CH_3 [positive (up)], CH_2, Cq [negative (down)], revealed the following signals at δ: 159.74 (C-2 ↓), 150.04 (aromatic ↓),141.83 (C-10b ↓), 135.81 (aromatic ↓),129.16 (C-6a ↓), 128.29 (aromatic ↑), 128.22 (C-9 ↑), 127.69 (C-5 ↑),

126.06 (C-8 ↑), 125.38 (C-10a ↓), 123.94 (C-6 ↓), 123.81 (C-7 ↑), 121.41 (C-10 ↑), 120.29 (C-4a ↓), 119.11 (CN ↓), 66.06 (CH_2 ↓), 56.39 (C-3 ↓), 48.29 (CH_2 ↓), 40.09 (C-4 ↑), 115.27 (aromatic ↑); MS *m/z* (%): 419 (M^++2, 17.98), 417 (M^+, 55.84) with a base peak at 255 (100); Anal. Calcd for $C_{24}H_{20}ClN_3O_2$: C, 68.98; H, 4.82; N, 10.06. Found: C, 68.95; H, 4.78; N, 10.05 %.

*Ethyl 2-amino-6-chloro-4-phenyl-4H-benzo[h]chromene-3-carboxyalte (**5a**)*

Colorless needles from ethanol; yield 78 %; m.p. 180–181 °C; IR (KBr) υ (cm^{-1}): 3393, 3285 (NH_2), 3061, 3026, 2968, 2900 (CH), 1676 (CO); ^{1}H NMR (500 MHz) (DMSO-d_6) δ: 8.42-7.12 (m, 10H, aromatic), 7.86 (bs, 2H, NH_2), 5.06 (s, 1H, H-4), 4.01 (q, 2H, CH_2, $J = 7$ Hz), 1.09 (t, 3H, CH_3, $J = 7$ Hz); ^{13}C NMR (500 MHz) (DMSO-d_6) δ: 168.02 (CO), 160.43 (C-2), 142.08 (C-10b), 129.03 (C-6a), 128.26 (C-9), 127.30 (C-5), 126.33 (C-10a), 126.18 (C-8), 123.94 (C-6), 123.73 (C-7), 121.56 (C-10), 121.42 (C-4a), 76.07 (C-3), 58.63 (CH_2), 40.05 (C-4), 14.19 (CH_3), 147.32, 127.91, 127.47, 125.30 (aromatic); 381 (M^++2, 1.28), 379 (M^+, 3.84) with a base peak at 314 (100); Anal. Calcd for $C_{22}H_{18}ClNO_3$: C, 69.57; H, 4.78; N, 3.69. Found: C, 69.60; H, 4.81; N, 3.71 %.

*Ethyl 2-amino-6-chloro-4-(4-fluorophenyl)-4H-benzo[h]chromene-3-carboxyalte (**5b**)*

Yellow needles from ethanol; yield 78 %; m.p. 184–185 °C (Khafagy *et al.*, 2002 m.p. 184 °C); IR (KBr) υ (cm^{-1}): 3384, 3290 (NH_2), 3075, 3061, 2989, 2978, 2958, 2927, 2906, 2887 (CH), 1669 (CO); ^{1}H NMR (500 MHz) (DMSO-d_6) δ: 8.42–7.06 (m, 9H, aromatic), 7.87 (bs, 2H, NH_2), 5.09 (s, 1H, H-4), 4.02 (q, 2H, CH_2, $J = 7$ Hz), 1.10 (t, 3H, CH_3, $J = 7$ Hz); ^{13}C NMR (500 MHz) (DMSO-d_6) δ: 167.95 (CO), 160.40 (C-2), 142.09 (C-10b), 129.10 (C-9), 128.27 (C-6a), 127.54 (C-5), 126.30 (C-8), 125.40 (C-10a), 123.96 (C-6), 123.77 (C-7), 121.46 (C-10), 121.33 (C-4a), 75.99 (C-3), 58.68 (CH_2), 40.07 (C-4), 14.23 (CH_3), 161.60, 159.68, 129.17, 115.05 (aromatic); ^{13}C NMR-DEPT spectrum at 135° CH, CH_3 [positive (up)], CH_2 [negative (down)], revealed the following signals at δ: 129.17 (aromatic ↑), 129.10 (C-9 ↑), 127.54 (C-5 ↑), 126.30 (C-8 ↑), 123.77 (C-7 ↑), 121.46 (C-10 ↑), 58.68 (CH_2 ↓), 40.07 (C-4 ↑), 14.23 (CH_3 ↑), 115.05 (aromatic ↑). In the DEPT spectrum at 90° only CH signals are positive (up) and showed δ: 129.17 (aromatic ↑), 129.10 (C-9 ↑), 127.54 (C-5 ↑), 126.30 (C-8 ↑), 123.77 (C-7 ↑), 121.46 (C-10 ↑), 40.07 (C-4 ↑), 115.05 (aromatic ↑). In the DEPT spectrum at 45° (CH, CH_2, and CH_3 positive) revealed signals at δ: 129.17 (aromatic ↑), 129.10 (C-9 ↑), 127.54 (C-5 ↑), 126.30 (C-8 ↑), 123.77 (C-7 ↑), 121.46 (C-10 ↑), 58.68 (CH_2 ↑), 40.07 (C-4 ↑), 14.23 (CH_3 ↑), 115.05 (aromatic ↑).^{13}CNMR-APT spectrum CH, CH_3 [positive (up)], CH_2, Cq [negative (down)], revealed the following signals at δ: 167.95 (CO ↓), 161.60 (aromatic ↓), 160.40 (C-2 ↓), 159.68 (aromatic ↓), 142.09 (C-10b ↓), 129.17 (aromatic ↑), 129.10 (C-9 ↑), 128.27 (C-6a ↓), 127.54 (C-5 ↑), 126.30 (C-8 ↑), 125.40 (C-10a ↓), 123.96 (C-6 ↓), 123.77 (C-7 ↑), 121.46 (C-10 ↑), 121.33 (C-4a ↓), 75.99 (C-3 ↓), 58.68 (CH_2 ↓), 40.07 (C-4 ↑), 14.23 (CH_3 ↑), 115.05 (aromatic ↑); Anal. Calcd for $C_{22}H_{17}ClFNO_3$: C, 66.42; H, 4.31; N, 3.52. Found: C, 66.46; H, 4.35; N, 3.55 %.

*Ethyl 2-amino-6-chloro-4-(4-chlorophenyl)-4H-benzo[h]chromene-3-carboxyalte (**5c**)*

Colorless crystals from ethanol; yield 80 %; m.p. 170–171 °C (Khafagy *et al.*, 2002 m.p. 170 °C); IR (KBr) υ (cm^{-1}): 3384, 3284 (NH_2), 3075, 3061, 2989, 2975, 2927, 2904, 2889 (CH), 1669 (CO); ^{1}H NMR (500 MHz) (DMSO-d_6) δ: 8.44–7.32 (m, 9H, aromatic), 7.92 (bs, 2H, NH_2), 5.09 (s, 1H, H-4), 4.03 (q, 2H, CH_2, $J = 7$ Hz), 1.11 (t, 3H, CH_3, $J = 7$ Hz); ^{13}C NMR (500 MHz) (DMSO-d_6) δ: 167.92 (CO), 160.44 (C-2), 142.12 (C-10b), 129.23 (C-9), 129.02 (C-6a), 128.38 (C-8), 127.53 (C-5), 125.48 (C-10a), 123.97 (C-6), 123.77 (C-7), 121.49 (C-10), 120.96 (C-4a), 75.74 (C-3), 58.74 (CH_2), 40.09 (C-4), 14.24 (CH_3), 146.34, 130.79, 129.16, 126.23 (aromatic); Anal. Calcd for $C_{22}H_{17}Cl_2NO_3$: C, 63.78; H, 4.14; N, 3.38. Found: C, 63.74; H, 4.11; N, 3.35 %.

*Ethyl 2-amino-6-chloro-4-(4-bromophenyl)-4H-benzo[h]chromene-3-carboxyalte (**5d**)*

Colorless crystals from ethanol; yield 80 %; m.p. 162–163 °C (Khafagy *et al.*, 2002 m.p. 162 °C); IR (KBr) υ (cm^{-1}): 3385, 3282 (NH_2), 3077, 3064, 2974, 2979, 2937, 2904, 2887 (CH), 1669 (CO); ^{1}H NMR (500 MHz) (DMSO-d_6) δ: 8.44-7.26 (m, 9H, aromatic), 7.93 (bs, 2H, NH_2), 5.08 (s, 1H, H-4), 4.04 (q, 2H, CH_2, $J = 7$ Hz), 1.12 (t, 3H, CH_3, $J = 7$ Hz); ^{13}C NMR (500 MHz) (DMSO-d_6) δ: 167.89 (CO), 160.41 (C-2), 142.10 (C-10b), 129.61 (C-9), 129.40 (C-6a), 129.14 (C-5), 127.98 (C-8), 127.49 (C-10a), 125.47 (C-6), 123.94 (C-7), 121.47 (C-10), 120.85 (C-4a), 75.66 (C-3), 58.73 (CH_2), 40.08 (C-4), 14.23 (CH_3), 146.74, 131.15, 129.78, 119.26 (aromatic); Anal. Calcd for $C_{22}H_{17}BrClNO_3$: C, 57.60; H, 3.74; N, 3.05. Found: C, 57.65; H, 3.77; N, 3.10 %.

*Ethyl 2-amino-6-chloro-4-(4-methylphenyl)-4H-benzo[h]chromene-3-carboxyalte (**5e**)*

Colorless crystals from ethanol; yield 79 %; m.p. 188–189 °C; IR (KBr) υ (cm^{-1}): 3478, 3315 (NH_2), 3075, 3061, 2996, 2980, 2904, 2888 (CH), 1681 (CO); ^{1}H NMR

(500 MHz) (DMSO-d_6) δ: 8.43–7.03 (m, 9H, aromatic), 7.85 (bs, 2H, NH_2), 5.01 (s, 1H, H-4), 4.01 (q, 2H, CH_2, $J = 7.2$ Hz), 2.20 (s, 3H, CH_3), 1.12 (t, 3H, CH_3, $J = 7.2$ Hz); ^{13}C NMR (500 MHz) (DMSO-d_6) δ: 168.13 (CO), 160.42 (C-2), 142.08 (C-10b), 129.04 (C-9), 128.99 (C-6a), 127.86 (C-5), 127.00 (C-8), 125.32 (C-10a), 124.00 (C-6), 123.76 (C-7), 121.98 (C-10), 121.80 (C-4a), 76.25 (C-3), 58.69 (CH_2), 40.00 (C-4), 20.49 (CH_3), 14.25 (CH_3), 144.46, 135.29, 128.85, 126.39 (aromatic); ^{13}C NMR-DEPT spectrum at 135° CH, CH_3 [positive (up)], CH_2 [negative (down)], revealed the following signals at δ: 129.04 (C-9 ↑), 128.85 (aromatic ↑), 127.86 (C-5 ↑), 127.00 (C-8 ↑), 126.39 (aromatic ↑), 123.76 (C-7 ↑), 121.98 (C-10 ↑), 58.69 (CH_2 ↓), 40.00 (C-4 ↑), 20.49 (CH_3 ↑), 14.25 (CH_3 ↑). In the DEPT spectrum at 90° only CH signals are positive (up) and showed δ: 129.04 (C-9 ↑), 128.85 (aromatic ↑), 127.86 (C-5 ↑), 127.00 (C-8 ↑), 126.39 (aromatic ↑), 123.76 (C-7 ↑), 121.98 (C-10 ↑), 40.00 (C-4 ↑). In the DEPT spectrum at 45° (CH, CH_2 and CH_3 positive) revealed signals at δ: 129.04 (C-9 ↑), 128.85 (aromatic ↑), 127.86 (C-5 ↑), 127.00 (C-8 ↑), 126.39 (aromatic ↑), 123.76 (C-7 ↑), 121.98 (C-10 ↑), 58.69 (CH_2 ↑), 40.00 (C-4 ↑), 20.49 (CH_3 ↑), 14.25 (CH_3 ↑).^{13}CNMR-APT spectrum CH, CH_3 [positive (up)], CH_2, Cq [negative (down)], revealed the following signals at δ: 168.13 (CO ↓), 160.42 (C-2 ↓), 144.46 (aromatic ↓), 142.08 (C-10b ↓), 135.29 (aromatic ↓), 129.04 (C-9 ↑), 128.99 (C-6a ↓), 128.85 (aromatic ↑), 127.86 (C-5 ↑), 127.00 (C-8 ↑), 126.39 (aromatic ↑), 125.32 (C-10a ↓), 124.00 (C-6 ↓), 123.76 (C-7 ↑), 121.98 (C-10 ↑), 121.80 (C-4a ↓), 76.25 (C-3 ↓), 58.69 (CH_2 ↓), 40.00 (C-4 ↑), 20.49 (CH_3 ↑), 14.25 (CH_3 ↑); MS *m/z* (%): 395 (M^++2, 1.98), 393 (M^+, 5.84) with a base peak at 255 (100); Anal. Calcd for $C_{23}H_{20}ClNO_3$: C, 70.14; H, 5.12; N, 3.56. Found: C, 70.18; H, 5.16; N, 3.59 %.

*Ethyl 2-amino-6-chloro-4-(4-methoxyphenyl)-4H-benzo[h]chromene-3-carboxyalte (**5f**)*

Colorless crystals from ethanol; yield 80 %; m.p. 177–178 °C; IR (KBr) υ (cm^{-1}): 3468, 3325 (NH_2), 3085, 3066, 2999, 2983, 2904, 2889 (CH), 1686 (CO); ^{1}H NMR (500 MHz) (DMSO-d_6) δ: 8.40–6.81 (m, 9H, aromatic), 7.82 (bs, 2H, NH_2), 5.01 (s, 1H, H-4), 4.02 (q, 2H, CH_2, $J = 7.2$ Hz), 3.68 (s, 3H, OCH_3), 1.11 (t, 3H, CH_3, $J = 7.2$ Hz); ^{13}C NMR (500 MHz) (DMSO-d_6) δ: 168.08 (CO), 160.40 (C-2), 142.05 (C-10b), 129.01 (C-6a), 128.85 (C-9), 127.88 (C-5), 127.47 (C-8), 127.47 (C-10a), 123.98 (C-6), 123.76 (C-7), 121.42 (C-10), 121.42 (C-4a), 76.35 (C-3), 58.66 (CH_2), 54.89 (CH_3), 40.08 (C-4), 14.27 (CH_3), 157.62, 139.53, 128.29, 113.64 (aromatic); ^{13} C NMR-DEPT spectrum at 135° CH, CH_3 [positive (up)], CH_2 [negative (down)], revealed the following signals at δ: 128.85 (C-9 ↑), 128.29 (aromatic ↑), 127.88 (C-5 ↑), 127.47 (C-8 ↑), 123.76 (C-7 ↑), 121.42 (C-10 ↑), 58.66 (CH_2 ↓), 54.89 (CH_3 ↑), 40.08 (C-4 ↑), 14.27 (CH_3 ↑), 113.64 (aromatic ↑). In the DEPT spectrum at 90° only CH signals are positive (up) and showed δ: 128.85 (C-9 ↑), 128.29 (aromatic ↑), 127.88 (C-5 ↑), 127.47 (C-8 ↑), 123.76 (C-7 ↑), 121.42 (C-10 ↑), 40.08 (C-4 ↑), 113.64 (aromatic ↑). In the DEPT spectrum at 45° (CH, CH_2 and CH_3 positive) revealed signals at δ: 128.85 (C-9 ↑), 128.29 (aromatic ↑), 127.88 (C-5 ↑), 127.47 (C-8 ↑), 123.76 (C-7 ↑), 121.42 (C-10 ↑), 58.66 (CH_2 ↑), 54.89 (CH_3 ↑), 40.08 (C-4 ↑), 14.27 (CH_3 ↑), 113.64 (aromatic ↑). ^{13}CNMR-APT spectrum CH, CH_3 [positive (up)], CH_2, Cq [negative (down)], revealed the following signals at δ: 168.08 (CO ↓), 160.40 (C-2 ↓), 157.62 (aromatic ↓), 142.05 (C-10b ↓), 157.62 (aromatic ↓), 129.01 (C-6a ↓), 128.85 (C-9 ↑), 128.29 (aromatic ↑), 127.88 (C-5 ↑), 127.47 (C-8 ↑), 127.47 (C-10a ↓), 123.98 (C-6 ↓), 123.76 (C-7 ↑), 121.42 (C-10 ↑), 121.42 (C-4a ↓), 76.35 (C-3 ↓), 58.66 (CH_2 ↓), 54.89 (CH_3 ↑), 40.08 (C-4 ↑), 14.27 (CH_3 ↑), 113.64 (aromatic ↑); MS *m/z* (%): 411 (M^++2, 5.31), 409 (M^+, 19.38) with a base peak at 75 (100); Anal. Calcd for $C_{23}H_{20}ClNO_4$: C, 67.40; H, 4.92; N, 3.42. Found: C, 67.38; H, 4.89; N, 3.40 %.

*Ethyl 2-amino-6-chloro-4-(4-nitrophenyl)-4H-benzo[h]chromene-3-carboxyalte (**5g**)*

Yellow crystals from ethanol; yield 78 %; m.p. 169–170 °C; IR (KBr) υ (cm^{-1}): 3467, 3317 (NH_2), 3085, 3066, 2995, 2977, 2908, 2887 (CH), 1687 (CO); ^{1}H NMR (500 MHz) (DMSO-d_6) δ: 8.47–7.55 (m, 9H, aromatic), 8.00 (bs, 2H, NH_2), 5.28 (s, 1H, H-4), 4.05 (q, 2H, CH_2, $J = 7.2$ Hz), 1.11 (t, 3H, CH_3, $J = 7.2$ Hz); ^{13}C NMR (500 MHz) (DMSO-d_6) δ: 167.75 (CO), 160.51 (C-2), 142.30 (C-10b), 129.35 (C-6a), 128.95 (C-9), 128.49 (C-5), 127.65 (C-10a), 127.65 (C-8), 123.99 (C-6), 123.70 (C-7), 121.59 (C-10), 119.97 (C-4a), 75.16 (C-3), 58.86 (CH_2), 40.05 (C-4), 14.25 (CH_3), 154.86, 146.00, 128.74, 126.13 (aromatic); ^{13} C NMR-DEPT spectrum at 135° CH, CH_3 [positive (up)], CH_2 [negative (down)], revealed the following signals at δ: 128.95 (C-9 ↑), 128.74 (aromatic ↑), 128.49 (C-5 ↑), 127.65 (C-8 ↑), 126.13 (aromatic ↑), 123.70 (C-7 ↑), 121.59 (C-10 ↑), 58.86 (CH_2 ↓), 40.05 (C-4 ↑), 14.25 (CH_3 ↑). In the DEPT spectrum at 90° only CH signals are positive (up) and showed δ: 128.95 (C-9 ↑), 128.74 (aromatic ↑), 128.49 (C-5 ↑), 127.65 (C-8 ↑), 126.13 (aromatic ↑), 123.70 (C-7 ↑), 121.59 (C-10 ↑), 40.05 (C-4 ↑). In the DEPT spectrum at 45° (CH, CH_2 and CH_3 positive) revealed signals at δ: 128.95 (C-9 ↑), 128.74 (aromatic ↑), 128.49 (C-5 ↑), 127.65 (C-8 ↑), 126.13 (aromatic ↑), 123.70 (C-7 ↑), 121.59 (C-10 ↑), 58.86 (CH_2 ↑), 40.05 (C-4 ↑), 14.25 (CH_3 ↑). ^{13}CNMR-APT spectrum CH, CH_3 [positive (up)], CH_2, Cq [negative (down)], revealed the following signals at δ: 167.75 (CO ↓), 160.51

(C-2 ↓), 154.86 (aromatic ↓), 146.00 (aromatic ↓), 142.30 (C-10b ↓), 129.35 (C-6a ↓), 128.95 (C-9 ↑), 128.74 (aromatic ↑), 128.49 (C-5 ↑), 127.65 (C-10a ↓), 127.65 (C-8 ↑), 126.13 (aromatic ↑), 123.99 (C-6 ↓), 123.70 (C-7 ↑), 121.59 (C-10 ↑), 119.97 (C-4a ↓), 75.16 (C-3 ↓), 58.86 (CH_2 ↓), 40.05 (C-4 ↑), 14.25 (CH_3 ↑); MS *m/z* (%): 426 (M^++2, 18.32), 424 (M^+, 57.87) with a base peak at 54 (100); Anal. Calcd for $C_{22}H_{17}ClN_2O_5$: C, 62.20; H, 4.03; N, 6.59. Found: C, 62.24; H, 4.07; N, 6.62 %.

*Ethyl 2-amino-6-chloro-4-(4-morpholinophenyl)-4H-benzo[h]chromene-3-carboxyalte (**5h**)*

Yellow crystals from ethanol; yield 76 %; m.p. 199-200 °C; IR (KBr) υ (cm^{-1}): 3380, 3286 (NH_2), 3087, 3068, 2995, 2978, 2928, 2906, 2887 (CH), 1683 (CO); ^{1}H NMR (500 MHz) (DMSO-d_6) δ: 8.40–6.80 (m, 9H, aromatic), 7.84 (bs, 2H, NH_2), 5.08 (s, 1H, H-4), 4.01 (q, 2H, CH_2, $J = 7.2$ Hz), 3.98–3.67 (m, 4H, $2CH_2$), 3.36–3.00 (m, 4H, $2CH_2$), 1.10 (t, 3H, CH_3, $J = 7.2$ Hz); ^{13}C NMR (500 MHz) (DMSO-d_6) δ: 160.41 (CO), 160.36 (C-2), 142.08 (C-10b), 129.17 (C-9), 128.06 (C-5), 127.87 (C-6a), 127.80 (C-10a), 126.48 (C-8), 125.39 (C-6), 123.79 (C-7), 121.47 (C-10), 121.37 (C-4a), 76.42 (C-3), 66.05 (CH_2), 58.69 (CH_2), 48.47 (CH_2), 38.77 (C-4), 14.31 (CH_3), 143.57, 138.20, 129.10, 114.90 (aromatic); ^{13} C NMR-DEPT spectrum at 135° CH, CH_3 [positive (up)], CH_2 [negative (down)], revealed the following signals at δ: 129.17 (C-9 ↑), 129.10 (aromatic ↑), 128.06 (C-5 ↑), 126.48 (C-8 ↑), 123.79 (C-7 ↑), 121.47 (C-10 ↑), 66.05 (CH_2 ↓), 58.69 (CH_2 ↓), 48.47 (CH_2 ↓), 38.77 (C-4 ↑), 14.31 (CH_3 ↑), 114.90 (aromatic ↑). In the DEPT spectrum at 90° only CH signals are positive (up) and showed δ: 129.17 (C-9 ↑), 129.10 (aromatic ↑), 128.06 (C-5 ↑), 126.48 (C-8 ↑), 123.79 (C-7 ↑), 121.47 (C-10 ↑), 38.77 (C-4 ↑), 114.90 (aromatic ↑). In the DEPT spectrum at 45° (CH, CH_2, and CH_3 positive)-revealed signals at δ: 129.17 (C-9 ↑), 129.10 (aromatic ↑), 128.06 (C-5 ↑), 126.48 (C-8 ↑), 123.79 (C-7 ↑), 121.47 (C-10 ↑), 66.05 (CH_2 ↑), 58.69 (CH_2 ↑), 48.47 (CH_2 ↑), 38.77 (C-4 ↑), 14.31 (CH_3 ↑), 114.90 (aromatic ↑). ^{13}CNMR-APT spectrum CH, CH_3 [positive (up)], CH_2, Cq [negative (down)], revealed the following signals at δ:160.41 (CO ↓) 160.36 (C-2 ↓), 143.57 (aromatic ↓), 142.08 (C-10b), 138.20 (aromatic ↓), 129.17 (C-9 ↑), 129.10 (aromatic ↑), 128.06 (C-5 ↑), 127.87 (C-6a ↓), 127.80 (C-10a ↓), 126.48 (C-8 ↑), 125.39 (C-6 ↓), 123.79 (C-7 ↑), 121.47 (C-10 ↑), 121.37 (C-4a ↓), 76.42 (C-3 ↓), 66.05 (CH_2 ↓), 58.69 (CH_2 ↓), 48.47 (CH_2 ↓), 38.77 (C-4 ↑), 14.31 (CH_3 ↑), 114.90 (aromatic ↑); MS *m/z* (%): 466 (M^++2, 22.28), 464 (M^+, 69.83) with a base peak at 101 (100); Anal. Calcd for $C_{26}H_{25}ClN_2O_4$: C, 67.17; H, 5.42; N, 6.03. Found: C, 67.13; H, 5.39; N, 6.06 %.

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 cells 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 in vitro cytotoxicity activity was studied against three cell lines: MCF-7, HCT, and HepG-2 using the colorimetric MTT assay (Mossman, 1983). The cells were seeded in 96-well microtitre plate (Falcon, NJ, USA) 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 microtiter plates (polystyrene sterile tissue culture 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, 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 cytotoxic effect of each tested compound was calculated as [1 − (ODt/ODc)] × 100 % where ODt is the mean optical density of wells treated with the tested compounds and ODc is the mean optical density of untreated cells.

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