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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of several 4*H*-chromene derivatives of expected antitumor activity

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Abstract Multi-component reactions for the preparation of 4*H*-chromene derivatives under microwave irradiation from different aromatic aldehydes with a mixture of malononitrile and phenol derivatives were established. The cytotoxic activity of the target compounds was evaluated against four cancer cell lines MCF-7, HCT-116, HepG-2 and A549 in comparison with vinblastine and colchicine as reference drugs. Generally, several compounds showed good cell growth inhibitory activity as compared to standard drugs. The structure–activity relationship studies reported that the substitution at 4- and 6-positions in 4*H*-chromene nucleus with the specific halogen atom increases the ability of the molecule against the different cell lines. The structures of the synthesized compounds were established on the basis of spectral data, IR, ^{1}H NMR, ^{13}C NMR and MS data.

Keywords Phenol derivatives · 4*H*-chromene derivatives · Antitumor · SAR

Introduction

Chromene (benzopyran) is a heterocyclic ring system in which a benzene ring and a pyran ring are fused together. It is an important structural component in natural compounds and is available in natural alkaloids, anthocyanins, tocopherols and flavonoids. A variety of natural and synthetic derivatives of chromene have important biological and pharmacological applications, such as antimicrobial (Kathrotiya and Patel, 2012; Chetan *et al*., 2012), anti-inflammatory (Thomas and Zachariah, 2013), antiproliferative (Magedov *et al*., 2007), antioxidant (Singh *et al*., 2010; Vukovic *et al*., 2010), herbicidal, analgesic and anticonvulsant (Bhat *et al*., 2008), antitubercular (Nimesh *et al*., 2011), anticoagulant, estrogenic antispasmolytic, estrogenic (Nareshkumar *et al*., 2009), TNF-α inhibitor (Cheng *et al*., 2003) effects and activities, as well as inhibitor of diabetes-induced vascular dysfunction (Birch *et al*., 1996). Such diverse biological and pharmacological activities have made chromene derivatives important for further development in medicinal and organic synthesis studies (Thompson, 2000; Nefzi *et al*., 1997).

In particular, 2-amino-4*H*-chromene derivatives are of recent interest for their antitumor activities (Akbarzadeh *et al*., 2012; Rafinejad *et al*., 2012; Sabry *et al*., 2011; Szulawska-Mroczek *et al*., 2013; Zhang *et al*., 2014; Musa *et al*., 2010; Kheirollahi *et al*., 2014; Saffari *et al*., 2014; Patil *et al*., 2013).

In addition, 4*H*-chromene derivatives observed some biological and pharmacological effects such as treatment of advanced solid tumors (Patil *et al*., 2013), blood anticoagulant warfarin (Wiener *et al*., 1962), anticancer therapeutic (Kemnitzer *et al*., 2005) inhibitor of Bcl-2 protein and apoptosis inducer (Wang *et al*., 2000).

Multi-component reactions (MCRs) have been successfully employed to generate highly diverse combinatorial libraries for high-throughput screening of biological and pharmacological activities (Saeedi *et al*., 2013; Hosseini-Zare *et al*., 2012). This protocol has the advantages of mild reaction conditions, high yields and short reaction time. In view of the above-mentioned findings, I report herein the synthesis of a series of 4*H*-chromene derivatives and their evaluation as antitumor agents, hoping to add some

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synergistic biological significance to the target molecules. The structure–activity relationship (SAR) of the 4- and 6-positions is also discussed.

Results and discussion

Chemistry

2-Amino-4-aryl-7-hydroxy-4*H*-chromene-3-carbonitrile (**4a–l**) was prepared via three-component condensation of resorcinol (**1a**) with different aromatic aldehydes (**2**) and malononitrile (**3**) in ethanolic piperidine solution under microwave irradiation conditions for 2 min at 140 °C as shown in (Scheme 1). The maximum power of microwave irradiation was optimized by repeating the reaction at different watt powers and time. Microwave radiations at 400 W and reaction time 2 min gave the highest yield.

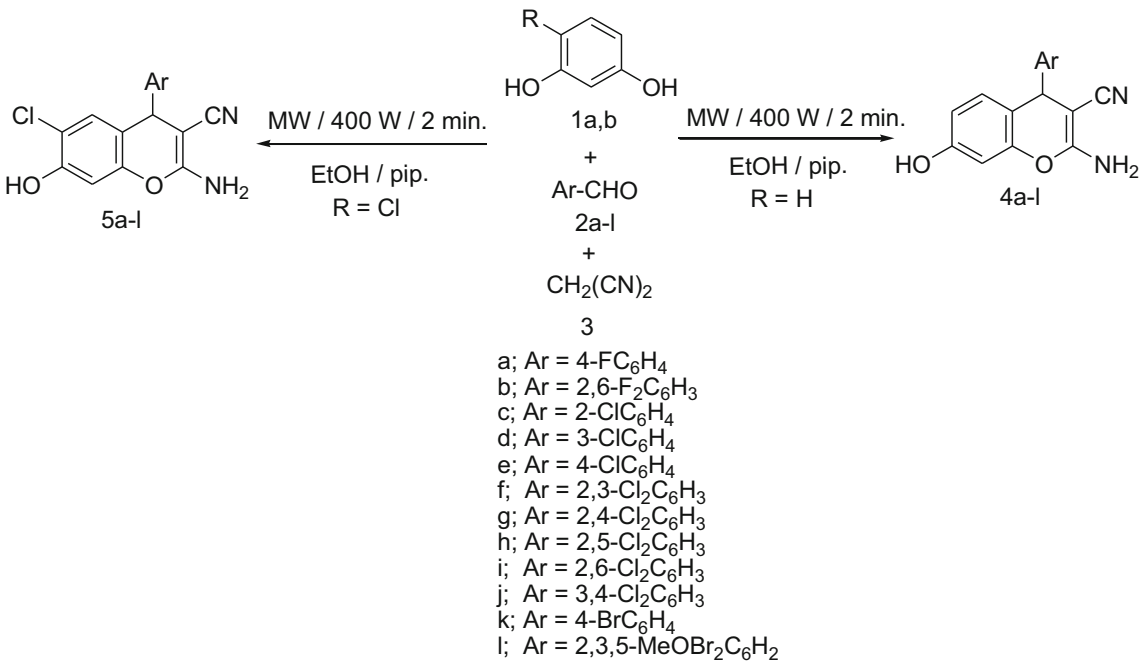
The assignment structure **4** was confirmed on the basis of spectral data. The IR spectra of **4b–d**,**f–j**,**l** showed the appearance of the a OH stretch at υ 3476–3435 cm^{-1}, a NH_2 stretch at υ 3347–3330, 3222–3196 cm^{-1} and a CN stretch at υ 2197–2187 cm^{-1} The ^1H and ^{13}C NMR spectra of **4b–d**,**f–j**,**l** revealed the presence of 4*H* signals at δ 5.94–4.90 (s, 1H, H-4), 41.05–30.85 ppm (C-4) and OH signals at δ 9.94–9.73 ppm. In addition, the mass spectra of compounds **4** gave also additional evidences for the proposed structures.

Similarly, the reaction of 4-chlororesorcinol (**1b**) with different aromatic aldehydes (**2**) and malononitrile (**3**) in ethanolic piperidine solution under microwave irradiation conditions for 2 min at 140 °C afforded 2-amino-4-aryl-6-chloro-7-hydroxy-4*H*-chromene-3-carbonitrile (**5a–l**) as shown in (Scheme 1). The maximum power of microwave irradiation was optimized by repeating the reaction at different watt powers and time. Microwave irradiation at 400 W and reaction time 2 min gave the highest yield. The 4-position of compounds **4** and **6** is a chiral center, and all the reactions were controlled using TLC technique.

The assignment structure **5** was confirmed on the basis of spectral data. The IR spectra of **5b–d**,**f–j**,**l** showed the appearance of the a OH stretch at υ 3497–3427 cm^{-1}, a NH_2 stretch at υ 3347–3331, 3233–3155 cm^{-1} and a CN stretch at υ 2204–2190 cm^{-1} The ^1H and ^{13}C NMR spectra of **5b–d**,**f–j**,**l** revealed the presence of 4*H* signals at δ 5.81–4.65 (s, 1H, H-4), 39.92–37.10 ppm (C-4) and OH signals at δ 10.98–10.18 ppm. In addition, the mass spectra of compound **5** gave also additional evidences for the proposed structures.

Antitumor assays

The antiproliferative activity of the newly synthesized compounds **4a–l** and **5a–l** and the standard (vinblastine and colchicine) was examined in four human cancer cell lines, namely breast adenocarcinoma (MCF-7), human colon carcinoma (HCT-116), hepatocellular carcinoma (HepG-2)



Scheme 1 Synthesis of halogenated 7-hydroxy-*4H*-chromene derivatives (**4a–l**, **5a–l**)

and lung carcinoma (A549) at various concentrations ranging from 0.00 to 125 μmol/l, and the cell viability was measured by the MTT assay as described in the literature (Mosmann, 1983; Rahman *et al*., 2001). *In vitro* cytotoxic evaluation using cell viability assay was performed at the Regional Center for Mycology and Biotechnology (RCMP), Al-Azhar University, Cairo, Egypt, using vinblastine and colchicine as reference drugs. The inhibitory concentration (IC_{50}, in μmol/l) of the new synthesized compounds **4a–l** and **5a–l** against the four human cancer cell lines MCF-7, HCT-116, HepG-2 and A549 is given in Table 1.

Structure–activity relationship (SAR) studies

The preliminary SAR study has focused on the effect of substituent at the phenyl group at 4-position and the substituent at 6-position of the 4*H*-chromene moiety, on the antitumor activities of the synthesized compounds. In a comparison of the cytotoxic activities of the two series **(4a–l)** and **(5a–l)** against breast adenocarcinoma (MCF-7), we found that, for the first series **(4a–l),** the highest significant growth inhibitory effect was associated with 2,5-dichlorophenyl **4h**, 2,3-dichlorophenyl **4f**, 3,5-dibromo-2-methoxyphenyl **4l** analogs (IC_{50} = 3.87, 5.13, 6.22 μmol/l, respectively) which displayed excellent activity relative to vinblastine (IC_{50} = 7.52 μmol/l) and colchicine (IC_{50} = 44.31 μmol/l), while the 3,4-dichlorophenyl **4j**, 2-chlorophenyl **4c**, 2,6-dichlorophenyl **4i**, 4-bromophenyl **4k**, 3-chlorophenyl **4d**, 4-fluorophenyl **4a**, the 2,6-difluorophenyl **4b** analogs (IC_{50} = 8.85, 9.47, 14.14, 16.90, 17.61, 20.19, 26.38μ μmol/ml, respectively) showed the highest significant growth inhibitory effect as compared to colchicine (IC_{50} = 44.31 μmol/l) and the 2,4-dichlorophenyl **4g** and 4-chlorophenyl **4e** analogs (IC_{50} = 58.23 and 62.94 μmol/l) are inactive. Generally, the order of antitumor activity was found to be **4h > 4f > 4l > 4j > 4c > 4i > 4k > 4d > 4a > 4b**, indicating that substitution at the phenyl ring at 4-position and unsubstituted at the 6-position of the 4*H*-chromene moiety with disubstituted or trisubstituted at certain positions enhanced the activity than the monosubstituted, suggesting that the more bulky substituent and the electronic nature of substituent (electron withdrawing or electron withdrawing with electron donating groups) may be the main factor affecting the potency of these compounds.

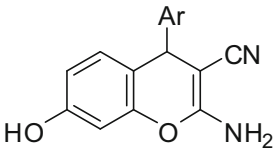
Replacing the H-6 with Cl-6 in the second series resulted in a very little reduction of potency of the compounds **(5a–l)** against MCF-7, the highest significant potent antitumor activity was associated with 2,5-dichlorophenyl **5h**, 2-chlorophenyl **5c**, 4-fluorophenyl **5a**, 2,4-dichlorophenyl **5g** analogs (IC_{50} = 4.00, 6.33, 6.76, 7.26 μmol/l, respectively) which displayed excellent activity relative to vinblastine (IC_{50} = 7.52 μmol/l) and colchicine (IC_{50} = 44.31 μmol/l), while 4-chlorophenyl **5e**, 3,5-dibromo-2-methoxyphenyl **5l**, 3-chlorophenyl **5d**, 2,6-dichlorophenyl **5i**, 2,3-dichlorophenyl **5f**, 3,4-dichlorophenyl **5j**, 2,6-difluorophenyl **5b** analogs (IC_{50} = 9.33, 10.24, 11.98, 12.35, 12.93, 15.04, 15.98 μmol/l, respectively) have a significant potent antitumor activity as compared to colchicine (IC_{50} = 44.31 μmol/l) and the 4-bromophenyl **4k** analog (IC_{50} = 93.75 μmol/l) was inactive, indicating that disubstitution or monosubstitution at certain positions on the phenyl ring at 4-position and a chlorine atom at the 6-position of the 4*H*-chromene moiety with electron-withdrawing groups enhanced the activity and 7-hydroxy-4*H*-chromene moiety (**4**) is preferred for antitumor activity more than 6-chloro-4*H*-chromene moiety (**5**).

In the case of human colon carcinoma (HCT-116), investigation of (SAR) for the first series **4a–l** revealed that compounds **4l**,**h**,**j** with IC_{50} = 1.50, 2.01, 2.22 μmol/l, respectively, exhibited good significant activity against as HCT-116 compared to vinblastine (IC_{50} = 3.2 μmol/l) and colchicine (IC_{50} = 107.13 μmol/l), while compounds **4f**,**i**,**c**,**d**,**g**,**b**,**e**,**k**,**a** (IC_{50} = 3.51–52.79 μmol/l) have a significant potent antitumor activity as compared to colchicine (IC_{50} = 107.13 μmol/l). This indicated that the anticancer activities of the halogenated derivatives have widely varied in accordance to the type of halogen atoms and the position of substituent at the phenyl ring at 4-position and unsubstituted at the 6-position of the 4*H*-chromene moiety. Replacement of the 6-H for compounds **4** with 6-Cl resulted in reduction of potency for the compound **5**. Compounds **5b**,**a**,**k**,**f**,**e**,**i**,**d**,**l**,**c**,**j**,**g**,**h** (IC_{50} = 7.7–79.98 -μmol/l) exhibited moderate to lower activities against HCT-116 as compared to vinblastine (IC_{50} = 3.2 μmol/l), while the same compounds exhibited good significant activity (IC_{50} = 7.7–79.98 μmol/l) against HCT-116 as compared to colchicine (IC_{50} = 107.13 μmol/l), suggesting that the difluoro atoms at 2,6-positions and the monofluoro atom (small size) on the phenyl ring at 4-position more variable influence on the cytotoxic activity against HCT-116 than the another groups. Generally, the first series (**4**) is preferred for antitumor activity more than the second series (**5**).

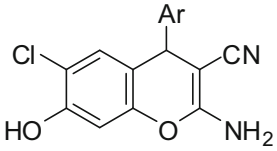
Concerning activity against HepG-2, compounds **4h**,**f**,**l**,**j** of the first series were the most significant active derivatives through this study with IC_{50} values of 2.07, 2.49, 2.94, 4.56 μmol/l, respectively, in comparison with vinblastine (IC_{50} = 5.67 μmol/l) and colchicine (IC_{50} = 26.54 μmol/l) and the compounds **4c**,**i**,**k**,**d**,**a**,**b** (IC_{50} = 6.73–16.86 μmol/l) displayed good significant activity against HepG-2 in comparison with colchicine (IC_{50} = 26.54 μmol/l) and compounds **4g**,**e** exhibited near to moderate activities (IC_{50} = 26.77 and 33.48 μmol/l).

This due to the presence of the dichloro atoms at 2,5-, 2,3-, 3,4-, 2,6-positions, $MeOBr_2$ at 2,3,5-positions and the

Table 1 Inhibitory concentration (IC_{50}, in μmol/l) of target compounds against four human cancer cell lines in comparison with vinblastine and colchicine as measured with the microculture tetrazolium (MTT) method



(4a-l)



(5a-l)

Cpd.	Ar	MCF-7	HCT-116	HepG-2	A549
4a	4-FC_6H_4	20.19 ± 0.2[a]	52.79 ± 0.1[a]	14.17 ± 0.1[a]	9.32 ± 0.2
4b	2,6-$F_2C_6H_3$	26.38 ± 0.5	15.05 ± 0.2	16.86 ± 0.2	6.80 ± 0.3
4c	2-ClC_6H_4	9.47 ± 0.11	9.37 ± 0.12	6.73 ± 0.1	2.95 ± 0.04
4d	3-ClC_6H_4	17.61 ± 0.03	10.31 ± 0.05	10.01 ± 0.18	9.37 ± 0.01
4e	4-ClC_6H_4	62.94 ± 0.3[a]	18.08 ± 0.11[a]	33.48 ± 0.1[a]	3.31 ± 0.2
4f	2,3-$Cl_2C_6H_3$	5.13 ± 0.22	3.51 ± 0.2	2.49 ± 0.11	1.47 ± 0.02
4g	2,4-$Cl_2C_6H_3$	58.23 ± 0.13	11.95 ± 0.04	26.77 ± 0.12	9.09 ± 0.05
4h	2,5-$Cl_2C_6H_3$	3.87 ± 0.04	2.01 ± 0.15	2.07 ± 0.06	1.53 ± 0.11
4i	2,6-$Cl_2C_6H_3$	14.14 ± 0.11	5.82 ± 0.13	7.62 ± 0.07	3.54 ± 0.01
4j	3,4-$Cl_2C_6H_3$	8.85 ± 0.15	2.22 ± 0.11	4.56 ± 0.05	2.25 ± 0.07
4k	4-BrC_6H_4	16.90 ± 0.16[a]	35.55 ± 0.3[a]	8.74 ± 0.11[a]	7.78 ± 0.18
4l	2,3,5-$MeOBr_2C_6H_2$	6.22 ± 0.07	1.5 ± 0.1	2.94 ± 0.06	1.39 ± 0.07
5a	4-FC_6H_4	6.76 ± 0.12	13.04 ± 0.04	3.66 ± 0.12	18.03 ± 0.1
5b	2,6-$F_2C_6H_3$	15.98 ± 0.13	7.71 ± 0.05	9.14 ± 0.11	7.86 ± 0.13
5c	2-ClC_6H_4	6.33 ± 0.12	42.92 ± 0.12	3.87 ± 0.14	27.28 ± 0.12
5d	3-ClC_6H_4	11.98 ± 0.11	36.02 ± 0.22	14.56 ± 0.12	98.45 ± 0.14
5e	4-ClC_6H_4	9.33 ± 0.14	16.90 ± 0.03	1.68 ± 0.13	29.23 ± 0.12
5f	2,3-$Cl_2C_6H_3$	12.92 ± 0.1	16.43 ± 0.01	13.36 ± 0.12	11.67 ± 0.15
5g	2,4-$Cl_2C_6H_3$	7.26 ± 0.15	79.16 ± 0.11	1.93 ± 0.02	53.59 ± 0.01
5h	2,5-$Cl_2C_6H_3$	4.0 ± 0.2	79.98 ± 0.14	4.05 ± 0.03	60.12 ± 0.121
5i	2,6-$Cl_2C_6H_3$	12.35 ± 0.07	34.82 ± 0.1	13.27 ± 0.11	25.14 ± 0.21
5j	3,4-$Cl_2C_6H_3$	15.05 ± 0.01	52.77 ± 0.2	19.78 ± 0.21	39.17 ± 0.01
5k	4-BrC6H4	93.75 ± 0.13	15.31 ± 0.3	63.56 ± 0.14	11.81 ± 0.01
5l	2,3,5-MeOBr2C6H2	10.24 ± 0.11	37.61 ± 0.4	1.46 ± 0.15	37.41 ± 0.1
V	–	7.52 ± 0.01	3.2 ± 0.04	5.67 ± 0.11	4.66 ± 0.01
C	–	44.31 ± 0.03	107.15 ± 0.08	26.54 ± 0.13	53.33 ± 0.03

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

V vinblastine, *C* colchicine

[a] El-Agrody *et al.* (2014)

monochloro atom at 2-, or 3-position which have the more variable influence on the cytotoxic activity against HepG-2 than the another groups. On the other hand, replacing the H-6 with Cl-6 in the second series resulted in highly improved anticancer efficacy of the compounds **5a**–**l**. Compounds **5l**,**e**,**g**,**a**,**c**,**h** exhibited good significant activities (IC_{50} = 1.46–4.05 μmol/l) and the other compounds **5b**,**i**,**f**,**d**,**j**,**k** exhibited moderate to lower activities (IC_{50} = 9.14–63.56 μmol/l) against HepG-2 as compared to vinblastine (IC_{50} = 5.67 μmol/l), while compounds **5l**,**e**,**g**,**a**,**c**,**h**,**b**,**i**,**f**,**d**,**j** (IC_{50} = 1.46–19.78 μmol/l) showed good significant activities against HepG-2 as compared to colchicine (IC_{50} = 26.54 μmol/l) and compound **6k** (IC_{50} = 63.56 μmol/l) was inactive, indicating that monosubstituted enhanced the activity than disubstituted and disubstituted more active than trisubstituted at certain positions (small size) on the phenyl ring at 4-position, suggesting that the less bulky substituent and the electronic

nature of substituent (electron withdrawing or electron withdrawing with electron donating groups) may be the main factor affecting the potency of these compounds and the second series (**5**) is preferred for antitumor activity more than the first series (**4**).

Finally, compounds **4l**,**f**,**h**,**j**,**c**,**e**,**i** of the first series showed remarkable increase of activity (IC_{50} = 1.39–3.54 μmol/l) against lung carcinoma (A549) as compared to the standard drugs vinblastine (IC_{50} = 4.66 μmol/l) and colchicine (IC_{50} = 53.33 μmol/l) with high significant, suggesting that trisubstituted with $MeOBr_2$ at 2,3,5-positions is more active than disubstituted with dichloro at 2,3-, 2,5- and 3,4-positions or monosubstituted with chloro atom at 2- and 4-positions.

The introduction of a chlorine atom (electron-withdrawing group) at 6-position of the second series has sharply reduced the antitumor activity of compounds **5a**–**l** against A549. Compound **5b**,**f**,**k**,**a**,**i**,**c**,**e**,**l**,**j**,**g**,**h**,**d** exhibited moderate to lower activities (IC_{50} = 7.86–98.45 μmol/l) as compared to vinblastine (IC_{50} = 4.66 μmol/l), while compounds **5b**,**f**,**k**,**a**,**i**,**c**,**e**,**l**,**j**,**g** (IC_{50} = 7.86–53.59 μmol/l) showed the highest significance against A549 as compared to colchicine (IC_{50} = 53.33 μmol/l) and compounds **5h**,**d** (IC_{50} = 60.12 and 98.45 μmol/l) are inactive. It indicates that substitution at the phenyl ring at 4-position with disubstituted or monosubstituted at certain positions with chlorine atom at the 6-position of the 4*H*-chromene moiety enhanced the activity, suggesting that the less bulky substituent and the electronic nature of substituent (electron-withdrawing group) may be the main factor affecting the potency of these compounds.

Conclusions

In conclusions, the halogenated 4*H*-chromene-3-carbonitrile derivatives **4a**–**l** and **5a**–**l** were synthesized and their structures were elucidated on the basis of IR, ^{1}H NMR, ^{13}C NMR and MS data. Compounds **4a**–**l** and **5a**–**l** were evaluated their antiproliferative activities against breast adenocarcinoma (MCF-7), human colon carcinoma (HCT-116), hepatocellular carcinoma (HepG-2) and lung carcinoma (A549). Of these derivatives, compounds **4h**,**f**,**l**,**j**,**c**,**i**,**k**,**d**,**a**,**b** (IC_{50} = 3.87–26.38 μmol/l) and **5h**,**c**,**a**,**g**,**e**,**l**,**d**,**i**,**f**,**j**,**b** (IC_{50} = 4.00–15.98 μmol/l) displayed excellent activity relative to vinblastine (IC_{50} = 7.52 μmol/l) and colchicine (IC_{50} = 44.31 μmol/l) against breast adenocarcinoma (MCF-7), compounds **4l**,**h**,**j** (IC_{50} = 1.50–2.22 μmol/l) were the most active compared to vinblastine (IC_{50} = 3.2 μmol/l), while compounds **4l**,**h**,**j**,**f**,**i**,**c**,**d**,**g**,**b**,**e**,**k**,**a** (IC_{50} = 1.5–52.79 μmol/l) and **5b**,**a**,**k**,**f**,**e**,**i**,**d**,**l**,**c**,**j**,**g**,**h** (IC_{50} = 7.71–79.16 μmol/l) exhibited good activity as compared to colchicine (IC_{50} = 107.15 μmol/l) against human colon carcinoma (HCT-116); compounds **4h**,**f**,**l**,**j**,**c**,**i**,**k**,**d**,**a**,**b** with IC_{50} values of 2.07–16.86 μmol/l and **5l**,**e**,**g**,**a**,**c**,**h**,**b**,**i**,**f**,**d**,**j** (IC_{50} = 1.46–19.78 μmol/l) were the most active compared to vinblastine (IC_{50} = 5.67 μmol/l) and colchicine (IC_{50} = 26.54 μmol/l) against hepatocellular carcinoma (HepG-2), while compounds **4l**,**f**,**h**,**j**,**c**,**e**,**i**,**b**,**k**,**g**,**a**,**d** (IC_{50} = 1.39–9.37 μg/ml) and **5b**,**f**,**k**,**a**,**i**,**c**,**e**,**l**,**j** (IC_{50} = 7.86–39.17 μmol/l) showed remarkable activity against lung carcinoma (A549) less than the standard drugs vinblastine and colchicine (IC_{50} = 4.66 and 53.33 μmol/l).

Finally, we can deduce that the substitution pattern on the phenyl group at 4-position and the substituent at 6-position of the 4*H*-chromene moiety is a crucial element for the antitumor activity. The incorporation of electron-withdrawing groups and the unsubstituent at 6-position is favorable for the activity.

Experimental

All chemicals were purchased from Sigma-Aldrich Chemical Co. Melting points were determined with a Stuart Scientific Co. Ltd. apparatus and are uncorrected. 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 500/600 MHz and JEOL Eclipse-400 spectrometers. The microwave apparatus used is Milestone Sr1, Microsynth. The MS were measured on a Shimadzu GC/MS-QP5 spectrometer. Elemental analyses were performed on a Perkin-Elmer 240 microanalyzer and all compounds are within ±0.3 % of theory specified.

General procedure for synthesis of 2-amino-4-aryl-7-hydroxy-4*H*-chromene-3-carbonitrile (**4a**–**l**) and 2-amino-4-aryl-6-chloro-7-hydroxy-4*H*-chromene-3-carbonitrile (**5a**–**l**).

A reaction mixture of resorcinol derivatives **1a** and **1b** (0.01 mol), different aromatic aldehydes **2a**–**l** (0.01 mol), malononitrile **3** (0.01 mol) and piperidine (0.5 mL) in ethanol (30 mL) was heated under microwave irradiation conditions for 2 min at 140 °C. After completion of the reaction, the reaction mixture was cooled to room temperature and the precipitated solid was filtered off, washed with MeOH and recrystallized from ethanol or ethanol and benzene. The physical and spectral data of compounds **4a**–**l** and **5a**–**l** are as follows:

2-Amino-4-(4-fluorophenyl)-7-hydroxy-4H-chromene-3-carbonitrile ***(4a)*** This compound was prepared by literature procedure (Makarem *et al.*, 2008).

2-Amino-4-(2,6-fluorophenyl)-7-hydroxy-4H-chromene-3-carbonitrile (***4b***) Yellow crystals (ethanol/benzene); yield 86 %; mp 252–253 °C; IR (KBr) υmax 3435, 3339, 3222, 2197 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz)

δ = 9.74 (1H, s, OH), 7.33–6.39 (6H, m, H–Ar), 6.92 (2H, bs, NH_2-2), 5.10 (1H, s, H-4); ^{13}C NMR (DMSO-d_6, 125 MHz), δ = 160.67 (C, C-2), 157.42 (C, C-8a), 149.33 (C, C-7), 129.39 (CH, C-5), 120.33, (CN, C-2), 112.32 (C, C-4a), 110.94 (CH, C-6), 102.17 (CH, C-8), 52.97 (C, C-3), 39.91 (CH, C-4), 161.61 (C, C-2′, C-6′) 129.12 (CH, C-4′), 120.62 (C, C-1′), 112.17 (CH, C-3′, C-5′); EIMS *m/z* 300 $[M]^+$ (1), 76.99 (100); Anal. Calcd for $C_{16}H_{10}F_2N_2O_2$: C, 64.00; H, 3.36; N, 9.33. Found: C, 64.21; H, 3.42; N, 9.45.

2-Amino-4-(2-chlorophenyl)-7-hydroxy-4H-chromene-3-carbonitrile (***4c***) Yellow crystals (ethanol); yield 89 %; m.p. 224–225 °C; IR (KBr) υmax 3444, 3339, 3222, 2187 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ = 9.79 (1H, s, OH), 7.39–6.40 (7H, m, H–aromatic), 6.73 (2H, bs, NH_2), 5.13 (1H, s, H-4); ^{13}C NMR (DMSO-d_6, 125 MHz) δ = 160.52 (C, C-2), 157.64 (C, C-8a), 149.01 (C, C-7), 129.18 (C, C-5), 120.30 (C, CN), 112.58 (C, C-4a), 112.72 (C, C-6), 102.27 (C, C-8), 54.88 (C, C-3), 37.19 (C, C-4), 142.89 (C, C-1′), 131.81 (C, C-2′), 129.58 (CH, C-6′), 128.50 (CH, C-3′), 127.80 (CH, C-4′), 126.64 (CH, C-5′); EIMS *m/z* 300 $[M + 2]^+$ (1.99), 298 $[M]^+$ (6.15), 187 (100); Anal. Calcd for $C_{16}H_{11}ClN_2O_2$: C, 64.33; H, 3.71; N, 9.38. Found: C, 64.54; H, 3.91; N, 9.50 %.

2-Amino-4-(3-chlorophenyl)-7-hydroxy-4H-chromene-3-carbonitrile (***4d***) Yellow crystals (ethanol); yield 84 %; m.p. 186–187 °C; IR (KBr) υmax 3439, 3330, 3201, 2193 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.74 (1H, s, OH), 7.33–6.40 (7H, m, H–aromatic), 6.94 (2H, bs, NH_2), 4.68 (1H, s, H-4); ^{13}C NMR (DMSO-d_6, 125 MHz) δ = 160.35 (C, C-2), 157.28 (C, C-8a), 148.85 (C, C-7), 129.87 (C, C-5), 120.47 (C, CN), 112.96 (C, C-4a), 112.53 (C, C-6), 102.31 (C, C-8), 55.61 (C, C-3), 39.50 (C, C-4), 148.81 (C, C-1′), 133.13 (C, C-3′), 130.57, (CH, C-5′), 126.76 (CH, C-2′), 126.22 (CH, C-4′, C-6′); EIMS *m/z* 300 $[M + 2]^+$ (0.87), 298 $[M]^+$ (2.74), 187 (100); Anal. Calcd for $C_{16}H_{11}ClN_2O_2$: C, 64.33; H, 3.71; N, 9.38. Found: C, 64.27; H, 3.61; N, 9.23 %.

2-Amino-4-(4-chlorophenyl)-7-hydroxy-4H-chromene-3-carbonitrile (***4e***) Compound **4c** was synthesized according to the literature procedure (Makarem *et al*., 2008).

2-Amino-4-(2,3-dichlorophenyl)-7-hydroxy-4H-chromene-3-carbonitrile (***4f***) Pale yellow crystals (ethanol/benzene); yield 85 %; m.p. 248–249 °C; IR (KBr) υmax 3460, 3341, 3200, 2194 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ = 9.93 (1H, s, OH), 7.52–6.42 (6H, m, H–aromatic), 6.64 (2H, bs, NH_2), 5.21 (1H, s, H-4); ^{13}C NMR (DMSO-d_6, 125 MHz) δ = 160.54 (C, C-2), 157.68 (C, C-8a), 149.03 (C, C-7), 129.98 (C, C-5), 120.15 (C, CN), 112.63 (C, C-4a), 111.76 (C, C-6), 102.30 (C, C-8), 54.53 (C, C-3), 38.23 (C, C-4), 145.42 (C, C-1′), 132.16 (C, C-3′), 129.14 (C, C-2′), 128.92 (CH, C-5′), 127.76 (CH, C-4′), 124 (CH, C-6′); EIMS *m/z* 336 $[M + 4]^+$ (4.79), 334 $[M + 2]^+$ (33.39), 332 $[M]^+$ (51.89), 187 (100); Anal. Calcd for $C_{16}H_{10}Cl_2N_2O_2$: C, 57.68; H, 3.03; N, 8.41. Found: C, 57.51; H, 2.98; N, 8.34 %.

2-Amino-4-(2,4-dichlorophenyl)-7-hydroxy-4H-chromene-3-carbonitrile (***4g***) Pale yellow needles (ethanol/benzene); yield 81 %; m.p. 272–273 °C; IR (KBr) υmax 3460, 3339, 3196, 2190 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ = 9.77 (1H, s, OH), 7.56–6.43 (6H, m, H–aromatic), 6.69 (2H, bs, NH_2), 5.14 (1H, s, H-4); ^{13}C NMR (DMSO-d_6, 125 MHz) δ = 160.57 (C, C-2), 157.54 (C, C-8a), 149.08 (C, C-7), 129.27 (C, C-5), 120.17 (C, CN), 112.62 (C, C-4a), 111.93 (C, C-6), 102.35 (C, C-8), 56.10 (C, C-3), 38.87 (C, C-4), 141.98 (C, C-1′), 132.85 (C, C-2′), 132.21 (C, C-4′), 129.08 (CH, C-6′), 128.06 (CH, C-3′), 127.92 (CH, C-5′); EIMS *m/z* 336 $[M + 4]^+$ (10.54), 334 $[M + 2]^+$ (63.43), 332 $[M]^+$ (100); Anal. Calcd for $C_{16}H_{10}Cl_2N_2O_2$: C, 57.68; H, 3.03; N, 8.41. Found: C, 57.54; H, 2.31; N, 8.37 %.

2-Amino-4-(2,5-dichlorophenyl)-7-hydroxy-4H-chromene-3-carbonitrile (***4h***) Yellow needles (ethanol/benzene); yield 81 %; m.p. 240–241 °C; IR (KBr) υmax 3476, 3339, 2197, 2190 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ = 9.74 (1H, s, OH), 7.46–6.40 (6H, m, H–aromatic), 6.99 (2H, bs, NH_2), 5.12 (1H, s, H-4); ^{13}C NMR (DMSO-d_6, 125 MHz) δ = 161.19 (C, C-2), 158.55 (C, C-8a), 149.62 (C, C-7), 129.14 (C, C-5), 120.72(C, CN), 113.32 (C, C-4a), 111.76 (C, C-6), 102.92 (C, C-8), 54.72 (C, C-3), 39.45 (C, C-4), 145.34 (C, C-1′), 132.21 (C, C-5′, C-2′), 129.99 (CH, C-3′), 129.76 (CH, C-6′), 128.16 (CH, C-4′); EIMS *m/z* 336 $[M + 4]^+$ (10), 334 $[M + 2]^+$ (62), 332 $[M]^+$ (98), 76.98 (100); Anal. Calcd for $C_{16}H_{10}Cl_2N_2O_2$: C, 57.68; H, 3.03; N, 8.41. Found: C, 57.51; H, 2.98; N, 8.34 %.

2-Amino-4-(2,6-dichlorophenyl)-7-hydroxy-4H-chromene-3-carbonitrile (***4i***) Pale yellow crystals (ethanol/benzene); yield 80 %; m.p. 279–280 °C; IR (KBr) υmax 3480, 3339, 3209, 2189 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ = 9.73 (1H, s, OH), 7.51–6.37 (6H, m, H–aromatic), 6.92 (2H, bs, NH_2), 5.68 (1H, s, H-4); ^{13}C NMR (DMSO-d_6, 125 MHz) δ = 160.75 (C, C-2), 157.50 (C, C-8a), 149.61 (C, C-7), 129.59 (C, C-5), 119.99 (C, CN), 112.25 (C, C-4a), 110.08 (C, C-6), 102.01 (C, C-8), 52.16 (C, C-3), 39.29 (C, C-4), 137.92 (C, C-1′), 135.32 (C, C-2′, C-6′), 134.71 (C, C-2′), 130.72 (CH, C-4′, C-5′), 128.49 (CH, C-3′), 128.39 (CH, C-5′); EIMS *m/z* 336 $[M + 4]^+$ (1.53), 334 $[M + 2]^+$ (11.61), 332 $[M]^+$ (12.46), 186.98 (100); Anal. Calcd for $C_{16}H_{10}Cl_2N_2O_2$: C, 57.68; H, 3.03; N, 8.41. Found: C, 57.73; H, 3.21; N, 8.56 %.

2-Amino-4-(3,4-dichlorophenyl)-7-hydroxy-4H-chromene-3-carbonitrile (***4j***) Pale yellow crystals (ethanol/

benzene); yield 80 %; m.p. 261–262 °C; IR (KBr) υmax 3477, 3346, 3202, 2192 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) $\delta = 9.77$ (1H, s, OH), 7.56–6.43 (6H, m, H–aromatic), 6.99 (2H, bs, NH_2), 4.72 (1H, s, H-4); ^{13}C NMR (DMSO-d_6, 125 MHz) $\delta = 160.40$ (C, C-2), 157.45 (C, C-8a), 148.84 (C, C-7), 129.37 (C, C-5), 120.42 (C, CN), 112.65 (C, C-4a), 112.54 (C, C-6), 102.37 (C, C-8), 55.34 (C, C-3), 39.29 (C, C-4), 147.42 (C, C-1′), 131.12 (C, C-3′), 130.98 (C, C-4′), 129.93 (CH, C-5′), 127.89 (CH, C-2′, C-6′); EIMS *m/z* 336 $[M + 4]^+$ (1.49), 334 $[M + 2]^+$ (11.66), 332 $[M]^+$ (12.47), 186.98 (100); Anal. Calcd for $C_{16}H_{10}Cl_2N_2O_2$: C, 57.68; H, 3.03; N, 8.41. Found: C, 57.66; H, 3.17; N, 8.54 %.

2-Amino-4-(4-bromophenyl)-7-hydroxy-4H-chromene-3-carbonitrile (***4k***) Compound **4k** was synthesized according to the literature procedure (Makarem *et al*., 2008).

2-Amino-4-(3,5-dibromo-2-methoxyphenyl)-7-hydroxy-4H-chromene-3-carbonitrile (***4l***) Yellow crystals (ethanol/benzene); YIELD 83 %; mp 253–254 °C; IR (KBr) υmax 3422, 3336, 3209, 2188 cm−^{1}; ^1H NMR (DMSO-d_6, 500 MHz,) $\delta = 9.77$ (1H, s, OH), 7.73–6.44 (4H, m, H–Ar), 6.96 (2H, bs, NH_2-2), 4.95 (1H, s, H-4), 3.66 (3H, s, O–CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz), $\delta = 160.57$ (C, C-2), 154.01 (C, C-8a), 148.91 (C, C-7), 131.89 (CH, C-5), 120.50, (CN, C-2), 112.54 (C, C-4a), 112.34 (CH, C-6), 102.40 (CH, C-8), 61.29 (CH_3, O–CH_3), 54.72 (C, C-3), 35.41 (CH, C-4), 157.42 (O–CH_3, C-2′) 143.19 (CH, C-6′), 134.09 (CH, C-4′), 129.57 (C, C-1′), 118.21 (C, C-5′), 116.92 (C, C-3′); EIMS *m/z* 454 $[M + 4]^+$ (18.18), 452 $[M + 2]^+$ (40.01), 450 $[M]^+$ (19.99), 187 (100); Anal. Calcd for $C_{17}H_{12}Br_2N_2O_3$: C, 45.16; H, 2.68; N, 6.20. Found: C, 45.26; H, 2.77; N, 6.34.

2-Amino-6-chloro-4-(4-fluorophenyl)-7-hydroxy-4H-chromene-3-carbonitrile (***5a***) Yellow crystals (ethanol); yield 86 %; m.p. 206–208 °C; IR (KBr) υmax 3427, 3338, 3233, 2195 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) $\delta = 10.41$ (1H, s, OH), 8.94–7.16 (6H, m, H–aromatic), 7.31 (2H, bs, NH_2), 5.42 (1H, s, H-4); ^{13}C NMR (DMSO-d_6, 125 MHz) $\delta = 160.59$ (C, C-2), 158.23 (C, C-8a), 148.99 (C, C-7), 130.42 (C, C-5), 118.39 (C, CN), 120.13 (C, C-4a), 110.63 (C, C-6), 104.81 (C, C-8), 54.68 (C, C-3), 39.91 (C, C-4), 147.60 (C, C-4′), 138.21 (C, C-1′), 129.99 (CH, C-2′, C-6′), 116.18 (CH, C-3′, C-5′); EIMS *m/z* 316 $[M]^+$ (69.12), 186.98 (100); Anal. Calcd for $C_{16}H_{10}ClFN_2O_2$: C, 60.68; H, 3.18; N, 8.85. Found: C, 60.46; H, 3.27; N, 8.92 %.

2-Amino-6-chloro-4-(2,6-fluorophenyl)-7-hydroxy-4H-chromene-3-carbonitrile (***5b***) Pale yellow crystals (ethanol/benzene); yield 86 %; mp 292–293 °C; IR (KBr) υmax 3497, 3331, 3219, 2204 cm−^{1}; ^1H NMR (DMSO-d_6, 500 MHz,) $\delta = 10.55$ (1H, s, OH), 7.35–6.62 (5H, m, H–Ar), 6.88 (2H, bs, NH_2-2), 5.10 (1H, s, H-4); ^{13}C NMR (DMSO-d_6, 125 MHz), $\delta = 160.41$ (C, C-2), 152.89 (C, C-8a), 147.91 (C, C-7), 129.67 (CH, C-5), 120.01 (C, C-4a), 115.59, (CN, C-2), 112.44 (C, C-6), 103.51 (CH, C-8), 52.88 (C, C-3), 39.29 (CH, C-4), 161.61 (C, C-2′, C-6′) 128.71 (CH, C-4′), 128.69 (C, C-1′), 112.45 (CH, C-3), 112.13 (CH, C-5′); EIMS *m/z* 334 $[M]^+$ (1), 76.99 (100); Anal. Calcd for $C_{16}H_9ClF_2N_2O_2$: C, 57.42; H, 2.71; N, 8.37. Found: C, 57.31; H, 2.62; N, 8.24.

2-Amino-6-chloro-4-(2-chlorophenyl)-7-hydroxy-4H-chromene-3-carbonitrile (***5c***) Yellow crystals (ethanol); yield 89 %; m.p. 271–272 °C; IR (KBr) υmax 3489, 3347, 3169, 2195 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) $\delta = 10.35$ (1H, s, OH), 7.44–6.42 (6H, m, H–aromatic), 6.74 (2H, bs, NH_2), 5.11 (1H, s, H-4); ^{13}C NMR (DMSO-d_6, 125 MHz) $\delta = 160.67$ (C, C-2), 158.63 (C, C-8a), 147.82 (C, C-7), 130.69 (C, C-5), 120.38 (C, C-4a), 117.51 (C, CN), 108.81 (C, C-6), 103.89 (C, C-8), 55.86 (C, C-3), 37.10 (C, C-4), 142.88 (C, C-1′), 131.74 (C, C-2′), 129.69 (CH, C-6′), 128.49 (CH, C-3′), 127.80 (CH, C-4′), 127.64 (CH, C-5′); EIMS *m/z* 336 $[M + 4]^+$ (1.62), 334 $[M + 2]^+$ (9.63), 332 $[M]^+$ (14.75), 221 (100); Anal. Calcd for $C_{16}H_{10}Cl_2N_2O_2$: C, 57.68; H, 3.03; N, 8.41. Found: C, 57.59; H, 2.91; N, 8.29 %.

2-Amino-6-chloro-4-(3-chlorophenyl)-7-hydroxy-4H-chromene-3-carbonitrile (***5d***) Yellow crystals (ethanol); Yield 87 %; m.p. 279–280 °C; IR (KBr) υmax 3476, 3343, 3155, 2197 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) $\delta = 10.27$ (1H, s, OH), 7.37–6.43 (6H, m, H–aromatic), 6.85 (2H, bs, NH_2), 4.65 (1H, s, H-4); ^{13}C NMR (DMSO-d_6, 125 MHz) $\delta = 160.49$ (C, C-2), 158.23 (C, C-8a), 148.93 (C, C-7), 128.42 (C, C-5), 120.53 (C, C-4a), 117.51 (C, CN), 109.61 (C, C-6), 103.83 (C, C-8), 55.86 (C, C-3), 38.95 (C, C-4), 147.60 (C, C-1′), 133.15 (C, C-3′), 130.56 (CH, C-5′), 127.04 (CH, C-2′), 126.66 (CH, C-4′), 126.1 (CH, C-6′); EIMS *m/z* 336 $[M + 4]^+$ (2.28), 334 $[M + 2]^+$ (13.79), 332 $[M]^+$ (21.64), 221 (100); Anal. Calcd for $C_{16}H_{10}Cl_2N_2O_2$: C, 57.68; H, 3.03; N, 8.41. Found: C, 57.77; H, 3.14; N, 8.57 %.

2-Amino-6-chloro-4-(4-chlorophenyl)-7-hydroxy-4H-chromene-3-carbonitrile (***5e***) Yellow crystals (ethanol); Yield 89 %; m.p. 235–236 °C; IR (KBr) υmax 3406, 3336, 3214, 2195 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) $\delta = 10.19$ (1H, s, OH), 7.45–6.33 (6H, m, H–aromatic), 6.89 (2H, bs, NH_2), 4.68 (1H, s, H-4); ^{13}C NMR (DMSO-d_6, 125 MHz) $\delta = 160.79$ (C, C-2), 158.11 (C, C-8a), 149.32 (C, C-7), 129.24 (C, C-5), 120.12 (C, C-4a), 117.04 (C, CN), 110.16 (C, C-6), 104.13 (C, C-8), 55.32 (C, C-3), 39.58 (C, C-4), 140.66 (C, C-1′), 133.25 (C, C-4′), 130.13 (CH, C-2′, C-6′), 126.66 (CH, C-3′, C-5′); EIMS *m/z* 336 $[M + 4]^+$ (2.19), 334 $[M + 2]^+$ (13.33), 332 $[M]^+$ (20.8), 221 (100); Anal. Calcd for $C_{16}H_{10}Cl_2N_2O_2$: C, 57.68; H, 3.03; N, 8.41. Found: C, 57.74; H, 3.11; N, 8.55 %.

2-Amino-6-chloro-4-(2,3-dichlorophenyl)-7-hydroxy-4H-chromene-3-carbonitrile (***5f***) Yellow crystals (ethanol/benzene); Yield 81 %; m.p. 280–281 °C; IR (KBr) υmax 3449, 3342, 3205, 2190 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) $\delta = 10.14$ (1H, s, OH), 7.55–6.47 (5H, m, H–aromatic), 6.95 (2H, bs, NH_2), 5.19 (1H, s, H-4); ^{13}C NMR (DMSO-d_6, 125 MHz) $\delta = 160.62$ (C, C-2), 157.72 (C, C-8a), 147.85 (C, C-7), 129.92 (C, C-5), 120.14 (C, C-4a), 117.22 (C, CN), 109.09 (C, C-6), 103.79 (C, C-8), 56.01 (C, C-3), 38.21 (C, C-4), 145.29 (C, C-1′), 132.18 (C, C-3′), 129.49 (C, C-2′), 129.14 (CH, C-5′), 128.60 (CH, C-4′), 127.76 (CH, C-6′); EIMS *m/z* 372 $[M + 6]^+$ (0.51), 370 $[M + 4]^+$ (1.49), 368 $[M + 2]^+$ (4.77), 366 $[M]^+$ (5.07), 221 (100); Anal. Calcd for $C_{16}H_9Cl_3N_2O_2$: C, 52.28; H, 2.47; N, 7.62. Found: C, 52.14; H, 2.31; N, 7.53 %.

2-Amino-6-chloro-4-(2,4-dichlorophenyl)-7-hydroxy-4H-chromene-3-carbonitrile (***5g***) Yellow crystals (ethanol/benzene); Yield 83 %; m.p. 242–243 °C; IR (KBr) υmax 3447, 3345, 3209, 2196 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) $\delta = 10.73$ (1H, s, OH), 7.63–6.53 (5H, m, H–aromatic), 6.98 (2H, bs, NH_2), 5.74 (1H, s, H-4); ^{13}C NMR (DMSO-d_6, 125 MHz) $\delta = 161.08$ (C, C-2), 156.32 (C, C-8a), 149.10 (C, C-7), 130.23 (C, C-5), 119.33 (C, C-4a), 117.22 (C, CN), 110.19 (C, C-6), 104.24 (C, C-8), 52.35 (C, C-3), 39.27 (C, C-4), 149.47 (C, C-1′), 136.14 (C, C-2′), 134.14 (C, C-4′), 132.02 (CH, C-6′),130.59 (CH, C-3′),128.11 (CH, C-5′); EIMS *m/z* 372 $[M + 6]^+$ (0.62), 370 $[M + 4]^+$ (5.48), 368 $[M + 2]^+$ (15.31), 366 $[M]^+$ (17.40), 221 (100); Anal. Calcd for $C_{16}H_9Cl_3N_2O_2$: C, 52.28; H, 2.47; N, 7.62. Found: C, 52.17; H, 2.39; N, 7.58 %.

2-Amino-6-chloro-4-(2,5-dichlorophenyl)-7-hydroxy-4H-chromene-3-carbonitrile (***5h***) Yellow crystals (ethanol/benzene); Yield 87 %; m.p. 287–288 °C; IR (KBr) υmax 3436, 3332, 3209, 2199 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) $\delta = 10.98$ (1H, s, OH), 7.69–6.56 (5H, m, H–aromatic), 7.03 (2H, bs, NH_2), 5.81 (1H, s, H-4); ^{13}C NMR (DMSO-d_6, 125 MHz) $\delta = 161.23$ (C, C-2), 157.54 (C, C-8a), 149.56 (C, C-7), 130.67 (C, C-5), 119.21 (C, C-4a), 117.03 (C, CN), 110.47 (C-6), 105.28 (C-8), 54.31 (C-3), 39.58 (C-4), 150.18 (C, C-1′), 134.16 (C, C-5′), 131.04 (C, C-2′), 130.02 (CH, C-3′),129.59 (CH, C-6′),128.21(CH, C-4′); EIMS *m/z* 372 $[M + 6]^+$ (0.45), 370 $[M + 4]^+$ (4.20), 368 $[M + 2]^+$ (12.32), 366 $[M]^+$ (13.14), 221 (100); Anal. Calcd for $C_{16}H_9Cl_3N_2O_2$: C, 52.28; H, 2.47; N, 7.62. Found: C, 52.37; H, 2.54; N, 7.78 %.

2-Amino-6-chloro-4-(2,6-dichlorophenyl)-7-hydroxy-4H-chromene-3-carbonitrile (***5i***) Yellow crystals (ethanol/benzene); Yield 81 %; m.p. 293–294 °C; IR (KBr) υmax 3434, 3347, 3196, 2191 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) $\delta = 10.59$ (1H, s, OH), 7.55–6.48 (5H, m, H–aromatic), 6.94 (2H, bs, NH_2), 5.66 (1H, s, H-4); ^{13}C NMR (DMSO-d_6, 125 MHz) $\delta = 160.68$ (C, C-2), 155.83 (C, C-8a), 148.30 (C, C-7), 130.79 (C, C-5), 119.86 (C, C-4a), 116.27 (C, CN), 108.99 (C, C-6), 103.84 (C, C-8), 51.93 (C, C-3), 38.87 (C, C-4), 137.60 (C, C-1′), 135.25 (C, C-2′, C-6′), 128.57 (CH, C-4′), 127.62 (CH, C-3′, C-5′); EIMS *m/z* 372 $[M + 6]^+$ (0.49), 370 $[M + 4]^+$ (4.10), 368 $[M + 2]^+$ (12.98), 366 $[M]^+$ (13.76), 221 (100); Anal. Calcd for $C_{16}H_9Cl_3N_2O_2$: C, 52.28; H, 2.47; N, 7.62. Found: C, 52.12; H, 2.27; N, 7.51 %.

2-Amino-6-chloro-4-(3,4-dichlorophenyl)-7-hydroxy-4H-chromene-3-carbonitrile (***5j***) Yellow crystals (ethanol/benzene); Yield 84 %; m.p. 254–254 °C; IR (KBr) υmax 3444, 3338, 3222, 2190 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) $\delta = 9.96$ (1H, s, OH), 7.59–6.61 (5H, m, H–aromatic), 6.97 (2H, bs, NH_2), 4.72 (1H, s, H-4); ^{13}C NMR (DMSO-d_6, 125 MHz) $\delta = 160.92$ (C, C-2), 154.59 (C, C-8a), 147.98 (C, C-7), 130.15(C, C-5), 120.78 (C, C-4a), 116.86 (C, CN), 109.99 (C, C-6), 104.35 (C, C-8), 55.66 (C, C-3), 39.92 (C, C-4), 131.78 (C, C-1′), 131.67 (C, C-3′), 129.88 (C, C-4′), 129.83 (CH, C-5′), 128.45 (CH, C-2′, C-6′); EIMS *m/z* 372 $[M + 6]^+$ (0.39), 370 $[M + 4]^+$ (3.58), 368 $[M + 2]^+$ (10.87), 366 $[M]^+$ (11.10), 221 (100); Anal. Calcd for $C_{16}H_9Cl_3N_2O_2$: C, 52.28; H, 2.47; N, 7.62. Found: C, 52.37; H, 2.60; N, 7.76 %.

2-Amino-4-(4-bromophenyl)-6-chloro-7-hydroxy-4H-chromene-3-carbonitrile (***5k***) Yellow crystals (ethanol); Yield 87 %; m.p. 244–245 °C; IR (KBr) υmax 3454, 3355, 3188, 2199 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) $\delta = 10.18$ (1H, s, OH), 7.53–6.48 (6H, m, H–aromatic), 6.98 (2H, bs, NH_2), 4.83 (1H, s, H-4); ^{13}C NMR (DMSO-d_6, 125 MHz) $\delta = 160.30$ (C, C-2), 156.99 (C, C-8a), 147.48 (C, C-7), 129.62 (C, C-5), 120.51 (C, C-4a), 117.06 (C, CN), 110.74 (C, C-6), 103.69 (C, C-8), 55.47 (C, C-3), 38.95 (C, C-4), 145.69 (C, C-1′), 131.50 (CH, C-3′, C-5′), 128.64 (CH, C-2′, C-6′), 119.76 (C, C-4′); EIMS *m/z* 380 $[M + 4]^+$ (4.58), 378 $[M + 2]^+$ (15.87), 376 $[M]^+$ (12.10), 221 (100); Anal. Calcd for $C_{16}H_{10}BrClN_2O_2$: C, 50.89; H, 2.67; N, 7.42. Found: C, 51.01; H, 2.69; N, 7.56 %.

2-Amino-6-chloro-4-(3,5-dibromo-2-methoxyphenyl)-7-hydroxy-4H-chromene-3-carbonitrile (***5l***) Yellow crystals (ethanol/benzene); Yield 87 %; m.p. 234–235 °C; IR (KBr) υmax 3454, 3366, 3208, 2188 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) $\delta = 10.65$ (1H, s, OH), 7.76–6.43 (4H, m, H–aromatic), 6.96 (2H, bs, NH_2), 4.89 (1H, s, H-4), 3.69 (3H, s, OCH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 160.72 (C, C-2), 153.93 (C, C-8a), 147.70 (C, C-7), 131.75 (C, C-5), 120.56 (C, CN), 117.47 (C, C-4a), 108.70 (C, C-6), 103.95 (C, C-8), 56.01 (C, CH_3), 54.39 (C, C-3), 35.37 (C, C-4), 158.55 (O–CH_3, C-2′), 143.17 (CH, C-4′),

134.03 (CH, C-6′), 127.95 (C, C-1′), 118.21 C, C-5′), 116.90 (C, C-3′); EIMS *m/z* 490 $[M + 6]^+$ (0.56), 488 $[M + 4]^+$ (2.52), 486 $[M + 2]^+$ (3.96), 484 $[M]^+$ (1.73), 221 (100); Anal. Calcd for $C_{17}H_{11}Br_2ClN_2O_3$: C, 41.97; H, 2.28; N, 5.76. Found: C, 42.01; H, 2.39; N, 5.86 %.

Antitumor screening

Cell culture

Breast adenocarcinoma (MCF-7), human colon carcinoma (HCT-116), hepatocellular carcinoma (HepG-2) and lung carcinoma (A549) were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were grown on RPMI-1640 medium supplemented with 10 % inactivated fetal calf serum and 105 μM gentamycin. The cells were maintained at 37 °C in a humidified atmosphere with 5 % CO_2 and were subculture two to three times a week.

Cytotoxicity evaluation using viability assay

The tumor cell lines were suspended in medium at concentration 5×10^4 cell/well in Corning® 96-well tissue culture plates and then incubated for 24 h. The tested compounds with concentrations ranging from 0.00 to 125 μmol/l were then added into 96-well plates (six replicates) to achieve different concentrations for each compound. Six vehicle controls with media or 0.5 % DMSO were run for each 96 well plate as a control. After incubating for 24 h, the numbers of viable cells were determined by the MTT test. Briefly, the media was removed from the 96 well plates and replaced with 100 μl of fresh culture RPMI 1640 medium without phenol red then 10 μl of the 12 mM MTT stock solution (5 mg of MTT in 1 mL of PBS) to each well including the untreated controls. The 96-well plates were then incubated at 37 °C and 5 % CO_2 for 4 h. An 85-μl aliquot of the media was removed from the wells, and 50 μl of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37 °C for 10 min. Then, the optical density was measured at 590 nm with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as $[1 - (ODt/ODc)] \times 100$ % where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50 % inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50 % of intact cells, was estimated from graphic plots of the dose response curve for each conc. using Graphpad Prism software (San Diego, CA. USA) (Mosmann, 1983).

Statistical analysis

All statistical calculations were done using computer programs, Microsoft excel version 10, SPSS (statistical package for the social science version 20.00) statistical program at 0.05, 0.01 and 0.001 level of probability (Snedecor and Cochran, 1982). Comparisons of inhibiting tumor growth between treatment groups or the control were done using Student's t test, one-way ANOVA, and post hoc LSD tests (the least significant difference) measurement.

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