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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Halogenated 2-amino-4*H*-benzo[*h*]chromene derivatives as antitumor agents and the relationship between lipophilicity and antitumor activity

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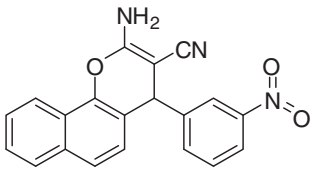
Abstract Several halogenated 2-amino-4*H*-benzo[*h*]chromene derivatives were synthesized and evaluated their cytotoxicity. The structures of the synthesized compounds were established on the basis of spectral data. The in vitro antitumor activity of the synthesized compounds against the cell lines MCF-7, HCT-116, and HepG-2 was investigated in comparison with the reference drugs vinblastine, colchicine, and doxorubicin using microculture tetrazolium colorimetric assay. It was found that some halogenated 4*H*-benzo[*h*]chromene derivatives showed the highest antitumor activity as compared with the reference drugs. The structure–activity relationship studies reported that the substitution at 4-position in the 4*H*-benzo[*h*]chromene nucleus with the specific halogen groups and lipophilicity increases the ability of the molecule against the different cell lines.

Keywords 4-Chloro-1-naphthol · 4*H*-Benzo[*h*]chromenes · Lipophilicity · Antitumor · SAR

Introduction

Cancer disease is one of the most difficult diseases to treat and it is a major disease responsible for deaths worldwide, it can be considered as one of the foremost health problems (Avendano and Menendez 2008). Yet there is no cancer treatment that is 100% effective against spread cancer (Nepali et al. 2014). Therefore, there is an urgent need to give much attention to update and modify drug leads from the point of view of medicinal chemistry and drug design to fulfill more potent and effective therapies. On the other hand, 2-amino-4*H*-benzo[*h*]chromene and 3-amino-1*H*-benzo[*f*]chromene nucleus has been emerged as a promising and attractive scaffold in the development of potent antitumor agents. For example, LY290181 Fig. 1, (2-amino-4-(3-nitrophenyl)-4*H*-benzo[*h*]chromene-3-carbonitrile) is a potent antiproliferative agent for a variety of cell types and inhibition of mitosis and microtubule (Panda et al. 1997; Wood et al. 1997); Fig. 2, 2-amino-4-(4-chloro/2-nitro/4-nitrophenyl)-4*H*-benzo[*h*]chromene-3-carbonitrile and 3-amino-1-(4-chloro/4-bromophenyl)-1*H*-benzo[*f*]chromene-3-carbonitrile has good cytotoxic and apoptotic effects on human cancer cell lines namely, MCF-7, MDA-MB-231,



LY290181
A potent antiproliferative agent

Fig. 1 Structure of LY290181

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T-47D, SK-N-MC, KB, HepG-2, and PC3 (Kheirollahi et al. 2014).

In addition, unsubstituted, 6-substituted and 7-substituted of 2-amino-4*H*-benzo[*h*]chromene moiety have been emerged as promising and attractive antitumor agents. For example, 2-amino 4-aryl-4*H*-benzo[*h*]chromene-3-carbonitrile and ethyl 2-amino 4-aryl-4*H*-benzo[*h*]chromene-3-carboxylate (El-Agrody et al. 2016b), 2-amino-4-aryl-6-chloro-4*H*-benzo[*h*]chromene-3-carbonitrile and ethyl 2-amino-4-aryl-6-chloro-4*H*-benzo[*h*]chromene-3-carboxlate (El-Agrody et al. 2013b), 2-amino-4-aryl-6-methoxy-4*H*-benzo[*h*]chromene-3-carbonitrile and ethyl 2-amino-4-aryl-6-methoxy-4*H*-benzo[*h*]chromene-3-carboxlate (Halawa et al. 2016, El-Agrody et al. 2016a, 2014a), and 4-aryl-2,7-diamino-4*H*-benzo[*h*]chromene-3-carbonitrile and ethyl 4-aryl-2,7-diamino-4*H*-benzo[*h*]chromene-3-carboxylate (El-Agrody et al. 2016b) have been reported as active cytotoxic agents against breast adenocarcinoma (MCF-7), human colon carcinoma (HCT-116) and hepatocellular carcinoma (HepG-2). Moreover, 9-amino-8-imino-7*H*-benzo[*h*]chromeno[2,3-*d*]pyrimidine derivatives exhibit anticancer activities. For example, Fig. 3, 9-amino-7-(4-fluorophenyl)-5-methoxy-8-imino-7*H*-benzo[*h*]chromeno[2,3-*d*]pyrimidine have the higher significant potent antitumor activity against MCF-7, HCT-116, and HepG-2 as compared to the standard drug colchicine (Halawa et al. 2016); 9-amino-7-(4-chlorophenyl)-5-methoxy-8-imino-7*H*-benzo[*h*]chromeno[2,3-*d*]pyrimidine showed significant activity against MCF-7 and HepG-2 more than the standard drugs vinblastine, colchicine, and doxorubicin (El-Agrody et al. 2014a), while the aminoimino, 9-amino-7-(4-bromophenyl)-5-methoxy-8-imino-7*H*-benzo[*h*]chromeno[2,3-*d*]pyrimidine displayed good activity against MCF-7, HCT-116, and HepG-2 as compared to the standard drugs vinblastine, colchicine, and doxorubicin (El-Agrody et al. 2016a).

In view of the importance of the 2-amino-4*H*-benzo[*h*]chromenes for antitumor activities in our former search (El-Agrody et al. 2011, 2012, 2013a, b, 2014a, b, 2016a, b; Al-Ghamdi et al. 2012; Sabry et al. 2011; Abd-El-Aziz et al. 2004), we sought to investigate this further with substituted into the phenyl ring at 4-position and a chlorine atom at 6-position. In order to discuss the SAR of the new compounds, we inserted various halogens at different positions into the phenyl ring at 4-position with a chlorine atom at 6-position, and compared this with our former publications with a methoxy group at 6-position or amino group at 7-position, beside the optical activity of the synthesized compounds were measured and discussed.

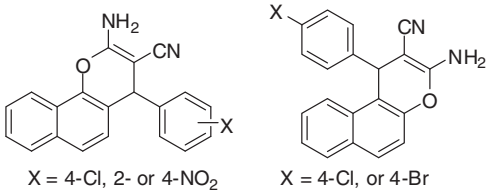


Fig. 2 Structure of some 4*H*-benzo[*h*]chromene and 1*H*-benzo[*f*]chromene derivatives with cytotoxic and apoptotic effects

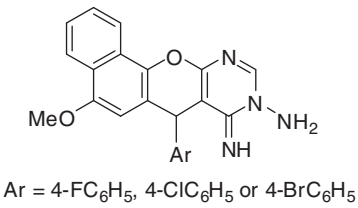


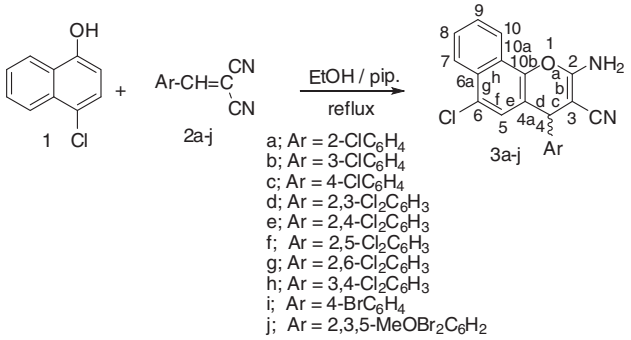
Fig. 3 Structure of 9-amino-8-imino-7*H*-benzo[*h*]chromeno[2,3-*d*]pyrimidine derivatives

Results and discussion

Chemistry

The synthetic strategies adopted for the synthesis of the target compounds are depicted in Scheme 1. In Scheme 1, synthesis of 2-amino-4-(mono, di, or trisubstitutedphenyl)-6-chloro-4*H*-benzo[*h*]chromene-3-carbonitriles (**3a–j**), in analogy with the reported literature (El-Agrody 1994) by the reaction of 4-chloro-1-naphthol (**1**) with α-cyanomono, di, or trisubstitutedcinnamonitriles (**2a–j**) in ethanolic piperidine under reflux for 1 h. The 4-position of compounds **3a–j** is chiral center and all the reactions were controlled using TLC technique.

The IR spectra of the target compounds **3** revealed the presence of the two characteristic amino and cyano absorption bands of 2-amino-4*H*-benzo[*h*]chromene-3-carbonitrile moieties at υ 3477–3457, 3334–3320, 3200–3188 cm^{-1} and at υ 2199–2195 cm^{-1} respectively. On the other hand, the ^1H NMR spectra of **3a**, **b**, **d–h**, **j** showed the



Scheme 1 Synthesis of halogenated 2-amino-6-chloro-4*H*-benzo[*h*]chromenes (**3a–j**)

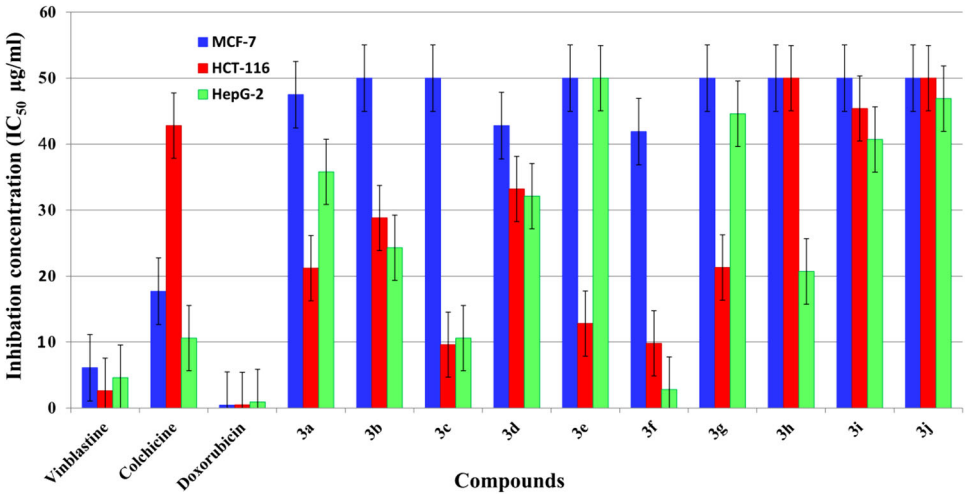


Fig. 4 IC_{50} values expressed in (µg/ml) of halogenated 2-amino-4*H*-benzo[*h*]chromene derivatives **3a–j** against MCF-7, HCT and HepG-2 tumor cells

singlet signal of the chiral proton around δ 5.95–4.98 ppm (s, 1H, H-4). In addition, the ^{13} C NMR spectra of **3a**, **b**, **d–h**, **j** showed the presence of 4*H* signals at δ 39.35–36.36 ppm (C-4). The ^{13}C NMR-DEPT spectra at 45°, 90°, 135°, and ^{13}C NMR-APT spectra of compounds **3d**, **f**, **h**, **j** gives additional evidence in support of the proposed structures (see experimental part and supplementary materials). Besides, the mass spectra of compounds **3** gave also additional evidences for the proposed structures.

Optical activity

The optical activities of the synthesized compounds **3a–j** were measured using a Carl Zeiss polarimeter. The results indicate that all the compounds have a zero rotation (optically inactive) and they are in the form of racemic mixture (±) as illustrated in Scheme 1.

Antitumor assays

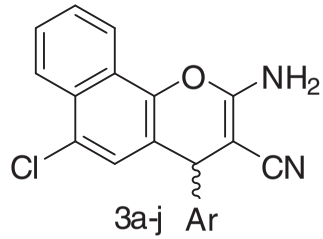
All the synthesized compounds **3a–j** were screened for their in vitro antitumor activity against three human cancer cell lines: breast adenocarcinoma (MCF-7), human colon carcinoma (HCT-116) and hepatocellular carcinoma (HepG-2) at various concentrations ranging from 0 to 50 µg/ml and the cell viability 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 & Biotechnology, Al-Azhar University using vinblastine, colchicine, and doxorubicin as reference drugs. The results were expressed as growth inhibitory concentration (IC_{50}, in µg/ml) values, which represent the compound concentrations required to produce a 50% inhibition of cell growth after 24 h of incubation compared to untreated controls as shown in Fig. 4 and Table 1.

SAR studies

The partition coefficient (Log *P*), which is well known as an index of lipophilicity, is an important physicochemical parameter was measured by ACD/Labs Log *P* calculated, ver.14.02 and was cited in Table 1. The structure–activity relationship (SAR) studies at the 4-position and the relationship between lipophilicity and antitumor activity were explored. The SAR studies of compounds **3a–j** revealed that compounds **3b**, **e**, **c**, **i**, **h** has the highest significant potent antitumor activity ($IC_{50} = 2.4–6.1$ µg/ml) against MCF-7 as compared to the other compounds **3d**, **j**, **f**, **a**, **g** ($IC_{50} = 6.6–29.6$ µg/ml) and the reference drug vinblastine ($IC_{50} = 6.1$ µg/ml) as shown in Table 2. In addition, compounds **3b**, **e**, **c**, **i**, **h**, **d**, **j**, **f**, **a** has the higher significant potent antitumor activity ($IC_{50} = 2.4–15.7$ µg/ml) against MCF-7 as compared to the compound **3g** ($IC_{50} = 29.6$ µg/ml) and the reference durg colchicine ($IC_{50} = 17.7$ µg/ml) as shown in Table 3.

The comparison of IC_{50} values of the halosubstituted compounds **3a–j** at the phenyl ring at 4-postion demonstrated that the monohalosubstituted and dihalosubstituted of the phenyl ring at 4-postion of the 4*H*-benzo[*h*]chromene moiety generally increased the antitumor activity profile with chloro or bromo atom at 3 or 4-positions and dichloro atoms at 2,4- or 3,4-positions against MCF-7 as compared to the reference drugs vinblastine and colchicine, suggesting that the small atoms at the phenyl ring (hydrophobic group) at 4-postion is preferred over the other substituted groups and incorporation of hydrophobic groups at 4-postion is responsible for enhancing antitumor activity with decreasing of partition coefficient (Log *P*). Comparison of this results with our former publications indicated that the presence of the chlorine atom at 6-position enhanced the antitumor activity against MCF-7 rather than hydrogen atom or methoxy group at 6-position or amino group at

Table 1 SAR of the 4-aryl group and the inhibitory concentration (IC_{50}, in μg/ml) of target compounds against the three human cancer cell lines in comparison with vinblastine, colchicine, and doxorubicin as measured with the microculture tetrazolium (MTT) method



Compound	Ar	IC_{50} (μg/ml)[a]			Log *P*
		MCF-7	HCT-116	HepG-2	
3a	2-ClC_6H_4	15.7 ± 0.02	10.1 ± 0.05	11.7 ± 0.02	5.42 ± 0.68
3b	3-ClC_6H_4	2.4 ± 0.2	1.1 ± 0.3	2.7 ± 0.08	5.42 ± 0.68
3c	4-ClC_6H_4	3.0 ± 0.06[b]	5.8 ± 0.03[b]	5.5 ± 0.05[b]	5.42 ± 0.68
3d	2,3-ClC_6H_3	6.6 ± 0.01	2.7 ± 0.14	8.4 ± 0.06	5.89 ± 0.69
3e	2,4-ClC_6H_3	2.5 ± 0.4	4.6 ± 0.02	0.7 ± 0.1	6.03 ± 0.69
3f	2,5-ClC_6H_3	11.1 ± 0.21	15.9 ± 0.04	15.0 ± 0.03	5.94 ± 0.69
3g	2,6-ClC_6H_3	29.6 ± 0.04	35.3 ± 0.02	32.9 ± 0.02	6.03 ± 0.69
3h	3,4-ClC_6H_3	6.1 ± 0.03	8.8 ± 0.02	1.2 ± 0.28	5.89 ± 0.69
3i	4-BrC_6H_4	5.5 ± 0.05[b]	10.9 ± 0.08[b]	10.7 ± 0.2[b]	5.60 ± 0.73
3j	2,3,5-$MeOBr_2C_6H_2$	9.2 ± 0.14	10.3 ± 0.17	10.3 ± 0.13	6.30 ± 0.83
Vinblastine	–	6.1 ± 0.03	2.6 ± 0.08	4.6 ± 0.01	–
Colchicine	–	17.7 ± 0.03	42.8 ± 0.08	10.6 ± 0.01	–
Doxorubicin	–	0.4 ± 0.01	0.5 ± 0.015	0.9 ± 0.04	–

[a] 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

[b] El-Agrody et al. (2013b)

Table 2 Positive and negative controls and the effectiveness of compounds against MCF-7

Control/Cpd	IC_{50} (μg/ml)	Cell line	*F* ratio	*P* value
Vinblastine	6.1[a]	MCF-7		
3b	2.4[b]	MCF-7		
3e	2.5[b,c]	MCF-7	25.229	0.000 (HS)
3c	3.0[c]	MCF-7		
3i	5.5[a]	MCF-7		
3h	6.1[a]	MCF-7		

Positive control (active compounds) and negative control (standard drugs), same letters in column do not differ statistically (ANOVA), medium evaluated with LSD test ($\alpha = 0.05$) and HS (highest significant)

Table 3 Positive and negative controls and the effectiveness of compounds against MCF-7

Control/Cpd	IC_{50} (μg/ml)	Cell line	*F* ratio	*P* value
Colchicine	17.7[a]	MCF-7		
3b	2.4[b]	MCF-7		
3e	2.5[b]	MCF-7		
3c	3.0[b]	MCF-7		
3i	5.5[c]	MCF-7	369.238	0.000 (HS)
3h	6.1[c]	MCF-7		
3d	6.6[c]	MCF-7		
3j	9.2[d]	MCF-7		
3f	11.1[e]	MCF-7		
3a	15.7[f]	MCF-7		

The same letters in column do not differ statistically (ANOVA)

7-position with 4-cholro/bromophenyl derivatives at 4-postion (El-Agrody et al. 2014a, 2016a, b), implying that grafting a large electron withdrawing substituent, as chlorine at 6-position is beneficial for the activity rather than hydrogen atom or methoxy group at 6-position or amino group at 7-position (electron-donating substituent).

In the case of HCT-116, investigation of SAR revealed that compound **3b** displayed good activity against HCT-116 with IC_{50} (1.1 μg/ml) as compared to the reference drug vinblastine ($IC_{50} = 2.6$ μg/ml) and showed significant activity as shown in Table 4, while compound **3d** showed activity ($IC_{50} = 2.7$ μg/ml) near to the reference drug vinblastine and the other compounds showed more reduction of potency ($IC_{50} = 4.6$–35.3 μg/ml), suggesting that monochlorosubstitution at 3-position of the phenyl ring (small hydrophobic group) at 4-postion of the 4*H*-benzo[*h*]chromene moiety was superior in inhibiting the growth of HCT-116 than the other substituted groups with decreasing of partition coefficient (Log *P*). In addition, all the compounds **3a–j** ($IC_{50} = 1.1$–35.3 μg/ml) displayed good activity against HCT-116 as compared to the reference drug colchicine ($IC_{50} = 42.8$ μg/ml), with the highest significant as

Table 4 Positive and negative controls and the effectiveness of compounds against HCT-116

Control/Cpd	IC_{50} (μg/ml)	Cell line	*T* test	*P* value
Vinblastine	2.6	HCT-116		
3b	1.1	HCT-116	4.271	0.013 (S)

Positive control (active compounds) and negative control (standard drugs), medium evaluated with LSD test ($\alpha = 0.05$) and S (significant)

Table 5 Positive and negative controls and the effectiveness of compounds against HCT-116

Control/Cpd	IC_{50} (μg/ml)	Cell line	*F* ratio	*P* value
Colchicine	42.8[a]	HCT-116		
3b	1.1[b]	HCT-116	1293.425	0.000 (HS)
3d	2.7[c]	HCT-116		
3e	4.6[d]	HCT-116		
3c	5.8[e]	HCT-116		
3h	8.8[f]	HCT-116		
3a	10.1[g]	HCT-116		
3j	10.3[g]	HCT-116		
3i	10.9[g]	HCT-116		
3f	15.9[h]	HCT-116		
3g	35.3[k]	HCT-116		

The same letters in column do not differ statistically (ANOVA)

illustrated in Table 5, indicating that monochlorosubstitution at 3-position of the phenyl ring (small hydrophobic group) at 4-postion of the 4*H*-benzo[*h*]chromene moiety is preferred over the other substituted groups with decreasing of partition coefficient (Log *P*).

The comparison of this results with our former publications indicated that the presence of the chlorine atom at 6-position enhanced the antitumor activity against HCT-116 rather than hydrogen atom or methoxy group at 6-position or amino group at 7-position with 4-cholrophenyl derivatives at 4-postion, while the antitumor activity increased with methoxy group at 6-position and 4-bromophenyl substituted derivative (El-Agrody et al. 2014a, 2016a, b), indicating that chlorine at 6-position (electron-withdrawing substituent) is beneficial for the activity rather than hydrogen atom or methoxy group at 6-position or amino group at 7-position (electron-donating substituent) with 4-cholrophenyl derivatives at 4-postion, while the methoxy group (electron-donating substituent) at 6-position increased the activity with 4-bromophenyl substituted derivative (electron-withdrawing substituent) rather than the chlorine at 6-position.

In the case of hepatocellular carcinoma (HepG-2), the 2,4-dichloro-, 3,4-dichloro- and 3-chloro- analogs **3e**, **h**, **b** exhibited the best growth inhibitory activity superior to the

Table 6 Positive and negative controls and the effectiveness of compounds against HepG-2

Control/Cpd	IC_{50} (μg/ml)	Cell line	*F* ratio	*P* value
Vinblastine	4.6[a]	HepG-2		
3e	0.7[b]	HepG-2	37.441	0.000 (HS)
3h	1.2[b]	HepG-2		
3b	2.7[c]	HepG-2		

The same letters in column do not differ statistically (ANOVA)

Table 7 Positive and negative controls and the effectiveness of compounds against HepG-2

Control/Cpd	IC_{50} (μg/ml)	Cell line	*F* ratio	*P* value
Colchicine	10.6[a]	HepG-2		
3e	0.7[b]	HepG-2	248.013	0.000 (HS)
3h	1.2[b]	HepG-2		
3b	2.7[c]	HepG-2		
3c	5.5[d]	HepG-2		
3d	8.4[e]	HepG-2		
3j	10.3[a]	HepG-2		

The same letters in column do not differ statistically (ANOVA)

reference drug vinblastine ($IC_{50} = 4.6$ μg/ml) with IC_{50} values ranging from (0.7 to 2.7 μg/ml) and highest significant as illustrated in Table 6. These results revealed that dichlorosubstitution at 2,4- or 3,4-position at the phenyl ring (hydrophobic group) at 4-postion of the 4*H*-benzo[*h*]chromene moiety was superior in inhibiting the growth of HepG-2 than the monochlorosubstitution and the other substituted groups with increasing of partition coefficient (Log *P*), while the derivatives **3e**, **h**, **b**, **c**, **d**, **j** except of **3i**, **a**, **f**, **g** exhibited good inhibitory activity with IC_{50} values ranging from (0.7 to 10.3 μg/ml) as compared to colchicine ($IC_{50} = 10.6$ μg/ml) and highest significant as illustrated in Table 7.

Comparison of this results with our former publications indicated that introduction of chlorine atom at the 6-position resulted in remarkable increase of activity against HepG-2 rather than the hydrogen atom or methoxy group at 6-position or amino group at 7-position with 4-cholrophenyl derivatives at 4-postion, while with 4-bromophenyl derivatives, the methoxy group at 6-position displayed good activity rather than the hydrogen atom or chlorine atom at 6-position or amino group at 7-position (El-Agrody et al. 2014a, 2016a, b). In general, a large electron withdrawing substituent, as chlorine, at 6-position is beneficial for the activity rather than the hydrogen atom or methoxy group at 6-position or amino group at 7-position (electron-donating substituent) with 4-chloro/bromophenyl derivatives at 4-postion.

Finally, in the case of the reference drug doxorubicin, an investigation of SAR revealed that all compounds showed moderated activity or inactive results against all tested cell lines, except compound **3e** exhibited the best growth inhibitory activity ($IC_{50} = 0.7$ μg/ml) superior to that of the reference drug doxorubicin ($IC_{50} = 0.9$ μg/ml) against HepG-2 and showed not significant activity as illustrated in Table 8, suggesting that dichlorosubstitution at 2,4-position of the phenyl ring (hydrophobic group) at 4-postion of the 4*H*-benzo[*h*]chromene moiety was superior in inhibiting the growth of HepG-2 than the monochlorosubstitution and the other substituted groups with increasing of partition coefficient (Log *P*).

Table 8 Positive and negative controls and the effectiveness of compounds against HepG-2

Control/Cpd	IC_{50} (μg/ml)	Cell line	*T* test	*P* value
Doxorubicin	0.9	HepG-2		
3e	0.7	HepG-2	0.346	0.746 (NS)

NS not significant

Conclusion

In conclusions, halogenated 2-amino-4*H*-benzo[*h*]chromenes (**3a–j**) was synthesized. The optical activities of the synthesized compounds **3a–j** were measured and results indicate that all the compounds have a zero rotation (optically inactive) and they are in the form of racemic mixture (±). Among the newly synthesized compounds, **3b**, **e**, **c**, **i**, **h** and **3b**, **e**, **c**, **i**, **h**, **d**, **j**, **f**, **a** analogs showed highest inhibition against MCF-7, **3b** and **3a–j** analogs showed highest inhibition against HCT-116, **3e**, **h**, **b** and **3e**, **h**, **b**, **c**, **d**, **j** analogs exhibited the best growth inhibitory activity against HepG-2 as compared to the reference drugs vinblastine and colchicine respectively. In addition, compound **3e** exhibited the best growth inhibitory activity superior to that of the reference drug doxorubicin against HepG-2, while all the other compounds exhibited moderated to weak activity or inactive results against all tested cell lines. On the basis of SAR, lipophilicity (hydrophobic group) and the partition coefficient (Log *P*), monochloro-analogs at 3 or 4-positions or dichloro- analogs at 2,4- or 3,4-positions of the phenyl ring at 4-postion of the 4*H*-benzo[*h*]chromene moiety are beneficial for antitumor activity. Further investigations are essential to gain deeper insight into structure-activity aspects and to predict the optimal structural parameters, which could be beneficial in development of antitumor therapeutics.

Experimental

Commercial-grade solvents and reagents were purchased from Sigma-Aldrich and used without purification. Melting

points were measured with a Stuart apparatus and were uncorrected. Infrared spectra were determined as KBr pellets on a Jasco FT/IR 460 plus spectrophotometer. The nuclear magnetic resonance spectra were recorded using a Bruker AV 500 MHz spectrometer. All the proton nuclear magnetic resonance spectra were run at 500 MHz, while carbon-13 nuclear magnetic resonance spectra were run at 125 MHz in (DMSO-d_6). Chemical shifts (δ) are expressed in parts per million (ppm) and TMS used as internal standard. The carbon-13 nuclear magnetic resonance spectra were obtained using both distortion-free enhancement by polarization transfer (DEPT), where the signals of the CH and CH_3 carbon atoms appear normal (↑) and the signals of the carbon atoms in CH_2 environments appear negative (↓) and attached proton test (APT); with this technique, the signals of the CH and CH_3 carbon atoms appears normal (↑) and the signal of the CH_2 and Cq environments appears negative (↓). The mass spectra were measured using a Shimadzu GC/MS-QP5050A spectrometer. Elemental analyses were performed on a Perkin-Elmer 240 microanalyser. The optical activities of the synthesized compounds were measured using a CARL ZEISS JENA 267628 polarimeter.

*General procedure for synthesis of target compounds (**3a–j**)*

To a solution of 4-chloro-1-naphthol (**1**) (0.01 mol) in absolute ethanol (30 mL) and catalytic amount of piperidine (0.5 mL), α-cyanomono, di- or tri-substituted cinnamonitriles (**2a–j**) (0.01 mol) was added. The reaction mixture was heated under reflux for 1 h, the obtained solid was filtered off, washed with MeOH and recrystallized from ethanol or ethanol/benzene. The physical and spectral data of compounds **3a–j** are as follows:

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

Pale yellow crystals from ethanol; Yield 89%; m.p. 290–291 °C; IR (KBr, υ_{max} cm^{-1}): 3477, 3330, 3197 (NH_2), 3066, 2968, 2937 (CH), 2199 (CN); ^{1}H NMR δ: 8.35–7.12 (m, 9H, aromatic), 7.29 (bs, 2H, NH_2), 5.40 (s, 1H, H-4); ^{13}C NMR δ: 160.11 (C-2), 141.39 (C-10b), 131.29 (C-9), 130.02 (C-6a), 129.16 (C-5), 128.00 (C-10a), 125.65 (C-8), 125.08 (C-6), 123.83 (C-7), 121.49 (C-10), 119.79 (C-4a), 117.05 (CN), 54.68 (C-3), 38.19 (C-4), 142.41, 132.09, 129.42, 128.45, 128.28, and 127.84 (aromatic), MS *m/z* (%): 370 (M^++4, 1.43), 368 (M^++2, 9.94), 366 (M^+,15.29) with a base peak at 255 (100); Anal. calcd for $C_{20}H_{12}Cl_2N_2O$: C, 65.41; H, 3.29; N, 7.63. Found: C, 65.47; H, 3.33; N, 7.70%.

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

Pale yellow crystals from ethanol; Yield 87%; m.p. 235–236 °C; IR (KBr, υ_{max} cm^{-1}): 3476, 3329, 3196 (NH_2), 3062, 2969, 2939 (CH), 2198 (CN); ^{1}H NMR δ: 8.35–7.24 (m, 9H, aromatic), 7.32 (bs, 2H, NH_2), 4.98 (s, 1H, H-4); ^{13}C NMR δ: 160.07 (C-2), 142.14 (C-10b), 129.39 (C-9), 128.41 (C-6a), 127.41 (C-5), 127.22 (C-10a), 126.48 (C-8), 123.93 (C-6), 123.84 (C-7), 121.50 (C-10), 119.98 (C-4a), 117.85 (CN), 55.54 (C-3), 39.02 (C-4), 147.53, 133.37, 130.81, 127.41, 125.74, and 125.70 (aromatic), MS *m/z* (%): 370 (M^++4, 1.15), 368 (M^++2, 10.06), 366 (M^+, 16.34) with a base peak at 255 (100); Anal. calcd for $C_{20}H_{12}Cl_2N_2O$: C, 65.41; H, 3.29; N, 7.63. Found: C, 65.49; H, 3.35; N, 7.72%.

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

Prepared as previously described (El-Agrody et al. 2013b).

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

Pale yellow crystals from ethanol/benzene; Yield 84%; m.p. 285–286 °C; IR (KBr, υ_{max} cm^{-1}): 3474, 3328, 3198 (NH_2), 3060, 2974, 2943 (CH), 2199 (CN); ^{1}H NMR δ: 8.35–7.12 (m, 8H, aromatic), 7.35 (bs, 2H, NH_2), 5.48 (s, 1H, H-4); ^{13}C NMR δ: 160.15 (C-2), 142.52 (C-10b), 130.29 (C-6a), 130.03 (C-9), 129.52 (C-5), 128.50 (C-8), 127.86 (C-10a), 125.79 (C-6), 123.84 (C-7), 121.50 (C-10), 119.66 (C-4a), 116.50 (CN), 54.40 (C-3), 39.10 (C-4), 143.93, 132.45, 129.69, 128.77, 128.27, and 124.94 (aromatic); ^{13} C NMR-DEPT spectrum at 135° CH, CH_3 (↑), CH_2 (↓), imply signals at δ: 130.03 (C-9 ↑), 129.69 (aromatic ↑), 129.52 (C-5 ↑), 128.77 (aromatic ↑), 128.50 (C-8 ↑), 124.94 (aromatic ↑), 123.84 (C-7 ↑), 121.50 (C-10 ↑), 39.10 (C-4 ↑). DEPT spectrum at 90° only CH signals are positive (↑) and showed δ: 130.03 (C-9 ↑), 129.69 (aromatic ↑), 129.52 (C-5 ↑), 128.77 (aromatic ↑), 128.50 (C-8 ↑), 124.94 (aromatic ↑), 123.84 (C-7 ↑), 121.50 (C-10 ↑), 39.10 (C-4 ↑). The DEPT spectrum at 45° (CH, CH_2 and CH_3 ↑) imply signals at δ: 130.03 (C-9 ↑), 129.69 (aromatic ↑), 129.52 (C-5 ↑), 128.77 (aromatic ↑), 128.50 (C-8 ↑), 124.94 (aromatic ↑), 123.84 (C-7 ↑), 121.50 (C-10 ↑), 39.10 (C-4 ↑); ^{13}CNMR-APT spectrum CH, CH_3 (↑), CH_2, Cq (↓), imply signals at δ: 160.15 (C-2 ↓), 142.52 (C-10b ↓), 130.29 (C-6a ↓), 130.03 (C-9 ↑), 129.52 (C-5 ↑), 128.50 (C-8 ↑), 127.86 (C-10a ↓), 125.79 (C-6 ↓), 123.84 (C-7 ↑), 121.50 (C-10 ↑), 119.66 (C-4a ↓), 116.50 (CN ↓), 54.40 (C-3 ↓), 39.10 (C-4 ↑), 143.93 (aromatic ↓), 132.45 (aromatic ↓), 129.69 (aromatic ↑), 128.77 (aromatic ↑),

128.27 (aromatic ↓), and 124.94 (aromatic ↑); MS *m/z* (%): 406 (M^++6, 1.10), 404 (M^++4, 9.55), 402 (M^++2, 30.10), 400 (M^+, 30.80) with a base peak at 255 (100); anal. calcd for $C_{20}H_{11}Cl_3N_2O$: C, 59.80; H, 2.76; N, 6.97. Found: C, 59.75; H, 2.72; N, 6.93%.

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

Pale yellow crystals from ethanol/benzene; Yield 84%; m.p. 245–246 °C; IR (KBr, υ_{max} cm^{-1}): 3469, 3334, 3200 (NH_2), 3055, 2970, 2947 (CH), 2195 (CN); ^{1}H NMR δ: 8.34–7.10 (m, 8H, aromatic), 7.33 (bs, 2H, NH_2), 5.39 (s, 1H, H-4); ^{13}C NMR δ: 160.09 (C-2), 140.45 (C-10b), 129.38 (C-6a), 128.48 (C-9), 128.18 (C-5), 125.77 (C-10a), 124.97 (C-8), 123.82 (C-6), 123.77 (C-7), 121.50 (C-10), 119.66 (C-4a), 116.46 (CN), 54.30 (C-3), 37.80 (C-4), 142.47, 133.08, 132.78, 132.68, 129.49, and 127.83 (aromatic); MS *m/z* (%): 406 (M^++6, 0.45), 404 (M^++4, 4.05), 402 (M^++2, 12.57), 400 (M^+,12.97) with a base peak at 255 (100); anal. calcd for $C_{20}H_{11}Cl_3N_2O$: C, 59.80; H, 2.76; N, 6.97. Found: C, 59.85; H, 2.80; N, 7.02%.

*2-Amino-6-chloro-4-(2,5-dichlorophenyl)-4*H*-benzo[*h*]chromene-3-carbonitrile (**3f**)*

Pale yellow crystals from ethanol/benzene; Yield 84%; m.p. 275–276 °C; IR (KBr, υ_{max} cm^{-1}): 3457, 3321, 3196 (NH_2), 3058, 2964, 2949 (CH), and 2197 (CN); ^{1}H NMR δ: 8.33–7.07 (m, 8H, aromatic), 7.34 (bs, 2H, NH_2), 5.34 (s, 1H, H-4); ^{13}C NMR δ: 160.20 (C-2), 142.52 (C-10b), 131.12 (C-6a), 130.86 (C-9), 128.47 (C-5), 127.81 (C-10a), 125.79 (C-6), 124.89 (C-8), 123.82 (C-7), 121.55 (C-10), 119.67 (C-4a), 115.98 (CN), 53.97 (C-3), 38.52 (C-4), 143.13, 132.36, 131.89, 129.55, 129.18, and 128.26 (aromatic); ^{13} C NMR-DEPT spectrum at 135° CH, CH_3 (↑), CH_2 (↓), imply signals at δ: 131.89 (aromatic ↑), 130.86 (C-9 ↑), 129.18 (aromatic ↑), 128.47 (C-5 ↑), 128.26 (aromatic ↑), 124.89 (C-8 ↑), 123.82 (C-7 ↑), 121.55 (C-10 ↑), and 38.52 (C-4 ↑); In the DEPT spectrum at 90° only CH signals are positive (↑), and showed δ: 131.89 (aromatic ↑), 130.86 (C-9 ↑), 129.18 (aromatic ↑), 128.47 (C-5 ↑), 128.26 (aromatic ↑), 124.89 (C-8 ↑), 123.82 (C-7 ↑), 121.55 (C-10 ↑), 38.52 (C-4 ↑). In the DEPT spectrum at 45° (CH, CH_2 and CH_3 ↑) imply signals at δ: 131.89 (aromatic ↑), 130.86 (C-9 ↑), 129.18 (aromatic ↑), 128.47 (C-5 ↑), 128.26 (aromatic ↑), 124.89 (C-8 ↑), 123.82 (C-7 ↑), 121.55 (C-10 ↑), 38.52 (C-4 ↑); ^{13}CNMR-APT spectrum CH, CH_3 (↑), CH_2, Cq (↓), imply signals at δ: 160.20 (C-2 ↓), 143.13 (aromatic ↓), 142.52 (C-10b ↓), 132.36 (aromatic ↓),131.12 (C-6a ↓), 131.89 (aromatic ↑), 130.86 (C-9 ↑), 129.55 (aromatic ↓), 129.18 (aromatic ↑), 128.47 (C-5 ↑), 128.26 (aromatic ↑), 127.81 (C-10a ↓), 125.79 (C-6 ↓), 124.89 (C-8 ↑), 123.82 (C-7 ↑), 121.55 (C-10 ↑), 119.67 (C-4a ↓), 115.98 (CN ↓), 53.97 (C-3 ↓), 38.52 (C-4 ↑); MS m/z (%): 406 (M^++6, 1.49), 404 (M^++4, 14.47), 402 (M^++2, 44.45), 400 (M^+,45.66) with a base peak at 255 (100); anal. calcd for $C_{20}H_{11}Cl_3N_2O$: C, 59.80; H, 2.76; N, 6.97. Found: C, 59.77; H, 2.73; N, 6.94%.

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

Pale yellow crystals from ethanol/benzene; Yield 84%; m.p. 315–316 °C; IR (KBr, υ_{max} cm $^{-1}$): 3470, 3330, 3196 (NH_2), 3059, 2973, 2949 (CH), and 2198 (CN); ^{1}H NMR δ: 8.32–6.93 (m, 8H, aromatic), 7.39 (bs, 2H, NH_2), 5.95 (s, 1H, H-4); ^{13}C NMR δ: 160.41 (C-2), 136.58 (C-10b), 130.87 (C-6a), 130.24 (C-9), 128.79 (C-5), 128.46 (C-10a), 125.62 (C-8), 124.09 (C-6), 123.85 (C-7), 121.43 (C-10), 119.44 (C-4a), 114.80 (CN), 52.14 (C-3), 36.73 (C-4), 143.01, 134.93, 129.53, and 127.87 (aromatic); MS *m/z* (%): 406 (M^++6, 0.45), 404 (M^++4, 4.05), 402 (M^++2, 12.57), 400 (M^+,12.97) with a base peak at 255 (100); anal. calcd for $C_{20}H_{11}Cl_3N_2O$: C, 59.80; H, 2.76; N, 6.97. Found: C, 59.85; H, 2.80; N, 7.02%.

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

Pale yellow crystals from ethanol/benzene; Yield 84%; m.p. 253–254 °C; IR (KBr, υ_{max} cm^{-1}): 3469, 3324, 3196 (NH_2), 3057, 2977, 2945 (CH), and 2196 (CN); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.34–6.93 (m, 8H, aromatic), 7.36 (bs, 2H, NH_2), 5.01 (s, 1H, H-4); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 160.05 (C-2), 142.17 (C-10b), 129.60 (C-9), 129.46 (C-6a), 128.26 (C-5), 128.16 (C-10a), 125.82 (C-6), 125.62 (C-8), 123.83 (C-7), 121.53 (C-10), 119.90 (C-4a), 117.37 (CN), 55.22 (C-3), 39.35 (C-4), 146.06, 131.31, 131.15, 129.91, 128.45, and 127.79 (aromatic); ^{13} C NMR-DEPT spectrum at 135° CH, CH_3 (↑), CH_2 (↓), imply signals at δ: 131.31 (aromatic ↑), 129.60 (C-9 ↑), 128.26 (C-5 ↑), 128.45 (aromatic ↑), 127.79 (aromatic ↑), 125.62 (C-8 ↑), 123.83 (C-7 ↑), 121.53 (C-10 ↑), and 39.35 (C-4 ↑). In the DEPT spectrum at 90° only CH signals are positive (↑) and showed δ: 131.31 (aromatic ↑), 129.60 (C-9 ↑), 128.26 (C-5 ↑), 128.45 (aromatic ↑), 127.79 (aromatic ↑), 125.62 (C-8 ↑), 123.83 (C-7 ↑), 121.53 (C-10 ↑), 39.35 (C-4 ↑). In the DEPT spectrum at 45° (CH, CH_2, and CH_3 ↑) revealed signals at δ: 131.31 (aromatic ↑), 129.60 (C-9 ↑), 128.26 (C-5 ↑), 128.45 (aromatic ↑), 127.79 (aromatic ↑), 125.62 (C-8 ↑), 123.83 (C-7 ↑), 121.53 (C-10 ↑), and 39.35 (C-4 ↑); ^{13}CNMR-APT spectrum CH, CH_3 (↑), CH_2, Cq (↓), imply signals at δ: 160.05 (C-2 ↓), 146.06 (aromatic ↓), 142.17 (C-10b ↓), 131.31 (aromatic ↑), 131.15 (aromatic ↓), 129.91 (aromatic ↓), 129.60 (C-9 ↑),

129.46 (C-6a ↓), 128.45 (aromatic ↑), 128.26 (C-5 ↑), 128.16 (C-10a ↓), 127.79 (aromatic ↑), 125.82 (C-6 ↓), 125.62 (C-8 ↑), 123.83 (C-7 ↑), 121.53 (C-10 ↑), 119.90 (C-4a ↓), 117.37 (CN ↓), 55.22 (C-3 ↓), and 39.35 (C-4 ↑); MS *m/z* (%): 406 (M^++6, 0.68), 404 (M^++4, 6.08), 402 (M^++2, 18.86), and 400 (M^+,19.46) with a base peak at 255 (100); anal. calcd for $C_{20}H_{11}Cl_3N_2O$: C, 59.80; H, 2.76; N, 6.97. Found: C, 59.84; H, 2.79; N, 7.01%.

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

Prepared as previously described (El-Agrody et al. 2013b).

*2-Amino-6-chloro-4-(3,5-dibromo-2-methoxyphenyl)-4*H-*benzo[*h*]chromene-3-carbo-nitrile (**3j**)*

Pale yellow crystals from ethanol/benzene; Yield 84%; m.p. 285–286 °C; IR (KBr, υ_{max} cm^{-1}): 3467, 3320, 3188 (NH_2), 3073, 2937, 2853 (CH), and 2199 (CN); ^{1}H NMR δ: 8.34–7.19 (m, 7H, aromatic), 7.35 (bs, 2H, NH_2), 5.20 (s, 1H, H-4), and 3.67 (s, 3H, OCH_3); ^{13}C NMR δ: 160.28 (C-2), 142.32 (C-10b), 132.21 (C-9), 129.43 (C-6a), 128.27 (C-10a), 127.85 (C-5), 125.71 (C-6), 125.24 (C-8), 123.86 (C-7), 121.48 (C-10), 120.01 (C-4a), 118.28 (CN), 61.32 (CH_3), 53.50 (C-3), 36.36 (C-4), 154.29, 141.62, 134.77, 128.45, 117.07, and 117.04 (aromatic); ^{13} C NMR-DEPT spectrum at 135° CH, CH_3 (↑), CH_2 (↓), imply signals at δ: 132.21 (C-9 ↑), 127.85 (C-5 ↑), 125.24 (C-8 ↑), 123.86 (C-7 ↑), 121.48 (C-10 ↑), 61.32 (CH_3 ↑), 36.36 (C-4 ↑), 134.77 (aromatic ↑), and 128.45 (aromatic ↑). In the DEPT spectrum at 90° only CH signals are positive (↑) and showed δ: 132.21 (C-9 ↑), 127.85 (C-5 ↑), 125.24 (C-8 ↑), 123.86 (C-7 ↑), 121.48 (C-10 ↑), 36.36 (C-4 ↑), 134.77 (aromatic ↑), and 128.45 (aromatic ↑). In the DEPT spectrum at 45° (CH, CH_2 and CH_3 ↑) revealed signals at δ: 132.21 (C-9 ↑), 127.85 (C-5 ↑), 125.24 (C-8 ↑), 123.86 (C-7 ↑), 121.48 (C-10 ↑), 61.32 (CH_3 ↑), 36.36 (C-4 ↑), 134.77 (aromatic ↑), and 128.45 (aromatic ↑); ^{13}CNMR-APT spectrum CH, CH_3 (↑), CH_2, Cq (↓), imply signals at δ: 160.28 (C-2 ↓), 154.29 (aromatic ↓), 142.32 (C-10b ↓), 141.62 (aromatic ↓), 134.77 (aromatic ↑), 132.21 (C-9 ↑), 129.43 (C-6a ↓), 128.45 (aromatic ↑), 128.27 (C-10a ↓), 127.85 (C-5 ↑), 125.71 (C-6 ↓), 125.24 (C-8 ↑), 123.86 (C-7 ↑), 121.48 (C-10 ↑), 120.01 (C-4a ↓), 118.28 (CN ↓), 117.07 (aromatic ↓), 117.04 (aromatic ↓), 61.32 (CH_3 ↑), 53.50 (C-3 ↓), and 36.36 (C-4 ↑); MS *m/z* (%): 524 (M^++6, 1.31), 522 (M^++4, 6.12), 520 (M^++2, 9.21), and 518 (M^+,4.28) with a base peak at 255 (100); anal. calcd for $C_{21}H_{13}Br_2ClN_2O_2$: C, 48.45; H, 2.52; N, 5.38. Found: C, 48.50; H, 2.56; N, 5.42%.

Antitumor screening

Cell culture and cytotoxicity evaluation using viability assay

Compounds **3a–j** was initially evaluated for in vitro antitumor activity against three different human cell lines: MCF-7, HCT-116, and HepG-2 (were obtained from the American Type Culture Collection, ATCC, Rockville, MD) in comparison with Vinblastine, Colchicine and Doxorubicin. The measurements of cell growth and the viabilities and in vitro cytotoxicity evaluation using viability assay were determined as described in the literature (Mosmann 1983; Rahman et al. 2001) and the result was cited in Fig. 4 and Table 1.

Statistical analysis

All statistical calculations were done using computer programs, Microsoft excel version 10, SPSS (statistica 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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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Avendano C, Menendez JC (2008) Medicinal Chemistry of Anticancer Drugs, 1st edn., p. 1 (Chapter 1), Elsevier Publishers, ISBN: 978-0-444-52824-7

Abd-El-Aziz AS, El-Agrody AM, Bedair AH, Corkery TC, Ata A (2004) Synthesis of hydroxyquinoline derivatives, aminohydroxychromene, aminocoumarin and their antimicrobial activities. Heterocycles 63:1793–1812

Al-Ghamdi AM, Abd EL-Wahab AHF, Mohamed HM, El-Agrody AM (2012) Synthesis and antitumor activities of 4*H*-pyrano[3,2-*h*]quinoline-3-carbonitrile, 7*H*-pyrimido[4′,5′:6,5]-pyrano[3,2-*h*] quinoline, and 14*H*-pyrimido[4′,5′:6,5]pyrano[3,2-*h*][1,2,4]triazolo[1,5-c]quinoline derivatives. Lett Drug Des Discov 9:459–470

El-Agrody AM (1994) Condensation reactions of a-cyanocinnamonitriles with naphthols: synthesis of naphthopyranopyrimidines and a naphthopyranone. J Chem Res (S) 280–281

El-Agrody AM, Abd El-Mawgoud HK, Fouda AM, Khattab ESAEH (2016b) Synthesis, in-vitro cytotoxicity of 4*H*-benzo[*h*]chromene

derivatives and structure–activity relationships of 4-aryl group and 3-, 7-positions. Chem Pap 70:1279–1292

El-Agrody AM, Abd-Rabboh HSM, Al-Ghamdi AM (2013a) Synthesis, antitumoractivity, and structure–activity relationship of some 4*H*-pyrano[3,2-*h*]quinoline and 7*H*-pyrimido-[4',5':6,5] pyrano[3,2-*h*]quinoline derivatives. Med Chem Res 22: 1339–1355

El-Agrody AM, Fouda AM, Al-Dies AM (2014a) Studies on the synthesis, in vitro antitumor activity of 4*H*-benzochromene, 7*H*benzo[*h*]chromeno[2,3-*d*]pyrimidine derivatives and structure activity relationships of the 2-,3- and 2,3-positions. Med Chem Res 23:3187–3199

El-Agrody AM, Fouda AM, Khattab ESAEH (2013b) Synthesis, antitumor activity of 2-amino-4*H*-benzo[*h*]chromene derivatives and structure–activity relationships of the 3- and 4-positions. Med Chem Res 22:6105–6120

El-Agrody AM, Halawa AH, Fouda AM, Al-Dies, AM (2016a) Antiproliferative activity of novel 4*H*-benzo[*h*]chromenes, 7*H*-benzo [*h*]chromeno[2,3-*d*]pyrimidines and the structure–activity relationships of the 2-, 3-positions and fused rings at the 2, 3-positions. J Saudi Chem Soc. doi:10.1016/j.jscs.2016.03.002

El-Agrody AM, Khattab ESAEH, Fouda AM, Al-Ghamdi AM (2012) Synthesis and antitumor activities of certain novel 2-amino-9-(4-halostyryl)-4*H*-pyrano[3,2-*h*]quinoline derivatives. Med Chem Res 21:4200–4213

El-Agrody AM, Khattab ESAEH, Fouda AM (2014b) Synthesis, structure–activity relationship (SAR) studies on some 4-Aryl-4*H*-chromenes and relationship between lipophilicity and antitumor activity. Lett Drug Des Discov 11:1167–1176

El-Agrody AM, Sabry NM, Motlaq SS (2011) Synthesis of some new 2-substituted 12*H*-chromeno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine, 3-ethoxycarbonyl-12*H*-chromeno[3,2-*e*][1,2,4]triazolo [1,5-*c*]pyrimidine-2-one, ethyl 2-formylamino/acetylamino-4*H*-chromene-3-carboxylate and some of their antimicrobial activities. J Chem Res 35:77–83

Halawa AH, Fouda AM, Al-Dies AM, El-Agrody AM (2016) Synthesis, biological evaluation and molecular docking studies of 4*H*benzo[*h*]chromenes, 7*H*-benzo[*h*]chromeno[2,3-*d*]pyrimidines as antitumor agents. Lett Drug Des Discov 13:77–88. F Deriv

Kheirollahi A, Pordeli M, Safavi M, Mashkouri S, Naimi-Jamal MR, Ardestani SK (2014) Cytotoxic and apoptotic effects of synthetic benzochromene derivatives on human cancer cell lines. Naunyn-Schmiedeberg's Arch Pharmacol 387:1199–1280

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63

Nepali K, Sharma S, Sharma M, Bedi PMS, Dhar KL (2014) Rational approaches, design strategies, structure activity relationship and mechanistic insights for anticancer hybrids. Eur J Med Chem 77:422–487

Panda D, Singh JP, Wilson L (1997) Suppression of microtubule dynamics by LY290181 a potential mechanism for its antiproliferative action. J Biol Chem 272:7681–7687

Rahman AU, Choudhary MI, Thomsen WJ (2001) Bioassay technique for drug development. Harwood Academic Publishers ISBN 0-203-34349-2 (Adobe e-Reader Format), ISBN 90- 5823-051-1 (Print Edition)

Sabry NM, Mohamed HM, Khattab ESAEH, Motlaq SS, El-Agrody AM (2011) Synthesis of 4*H*-chromene, coumarin, 12*H*-chromeno [2,3-*d*]pyrimidine derivatives and some of their antimicrobial and cytotoxicity activities. Eur J Med Chem 46:765–772

Snedecor GM, Cochran WG (1982) Statistical methods, 7th edn. Lowa state University Press, Ames, pp 325–330

Wood DL, Panda D, Wiernicki TR, Wilson L, Jordan MA, Singh JP (1997) Inhibition of mitosis and microtubule function through direct tubulin binding by a novel antiproliferative naphthopyran LY290181. Mol Pharmacol 52:437–444