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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Studies on the synthesis, in vitro antitumor activity of 4*H*-benzo[*h*]chromene, 7*H*-benzo[*h*]chromene[2,3-*d*]pyrimidine derivatives and structure–activity relationships of the 2-,3- and 2,3-positions

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Abstract Some 4*H*-benzo[*h*]chromene and 7*H*-benzo[*h*]chromeno[2,3-*d*]pyrimidine derivatives were prepared as potential cytotoxic agents. The in vitro cytotoxic activity of the synthesized compounds was investigated in comparison with the well-known anticancer standard drugs Vinblastine, Colchicine, and Doxorubicin using MTT colorimetric assay. It was found that compounds **23**, **15**, **20**, and **21** showed the highest anticancer activity against the three tumor cell lines MCF-7, HCT, and HepG-2, compared with Vinblastine and Colchicine, while compound **23** was the most active against HepG-2 as compared with Doxorubicin. We explored the SAR of 4*H*-benzo[*h*]chromenes with modification at the 2-,3- positions and 7*H*-benzo[*h*]chromeno[2,3-*d*]pyrimidine at 2,3-positions. The structure–activity relationship (SAR) study revealed that the antitumor activity on 4*H*-benzo[*h*]chromene and 7*H*-benzo[*h*]chromeno[2,3-*d*]pyrimidine derivatives were significantly affected by the lipophilicity (hydrophobic or hydrophilic), of the substituent at 2-,3- and 2,3-positions. Structures of these compounds were established on the basis of spectral data, IR, ^{1}H NMR, ^{13}C NMR, ^{13}C NMR-DEPT, and MS data.

Keywords 4-Methoxy-1-naphthol · α-Cyanocinnamonitriles · 4*H*-Benzo[*h*]chromenes · 7*H*-Benzo[*h*]chromeno[2,3-*d*]pyrimidines · Antitumor · SAR

Introduction

2-Amino-4*H*-chromenes and their derivatives occupy an important place in the realm of natural and synthetic organic chemistry because of their biological and pharmacological activities such as antimicrobial (Kidwai *et al*., 2010; Alvey *et al*., 2009), antioxidant (Singh *et al*., 2010; Vukovic *et al*., 2010), antitumor (Sabry *et al*., 2011; Kemnitzer *et al*., 2008. Kemnitzer *et al*., 2007; Mahmoodi *et al*., 2010; Endo *et al*., 2010; Tseng *et al*., 2010), vascular disrupting (Kasibhatla *et al*., 2004), antileishmanial (Tanaka *et al*., 2007), anticancer (Vosooghi *et al*., 2010; Gourdeau *et al*., 2004; Kemnitzer *et al*., 2004), antiproliferative (Magedov *et al*., 2007), 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). In addition, polyfunctionalized 4*H*-chromenes constitute a structural unit of a number of natural products and because of the inherent reactivity of the inbuilt pyran ring are versatile synthons (Cingolant and Pigini, 1969). They also exhibit hypolipidemic activity (Sashidhara *et al*., 2011), DNA breaking activities, and mutagenicity (Hiramoto *et al*., 1997).

These findings stimulated our interest in the syntheses of 4*H*-benzo[*h*]chromene and 7*H*-benzo[*h*]chromeno[2,3-*d*]pyrimidine derivatives and in continuation of our program on the chemistry of 4*H*-pyran derivatives (Al-Ghamdi

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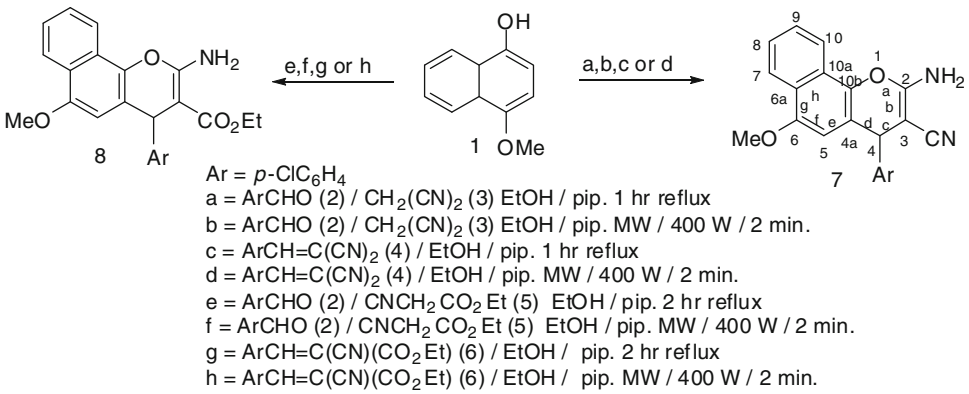
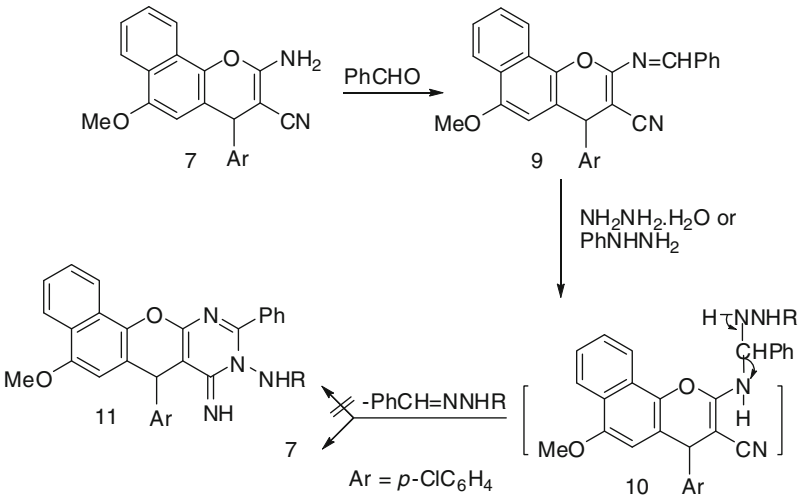
Scheme 1 Synthesis of 4*H*-benzo[*h*] chromene derivatives (**7**, **8**)



Scheme 2 Synthesis 2-Benzylideneamino-4-(4-chlorophenyl)-6-methoxy-4*H*-benzo[*h*] chromene-3-carbonitrile (**9**)



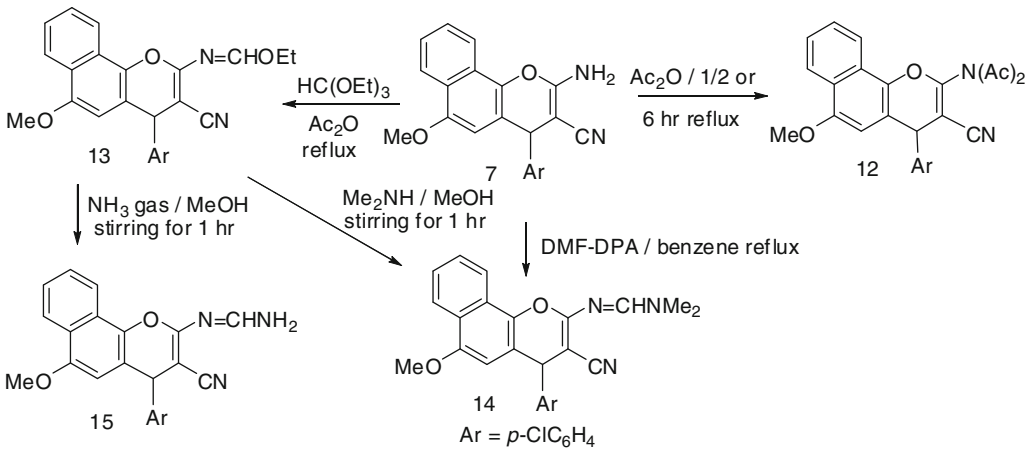
et al., 2012; El-Agrody *et al*., 2011, 2012, 2013; El-Agrody and Al-Ghamdi, 2011; Sabry *et al*., 2011; Abd-El-Aziz *et al*., 2004, 2007), we report herein the synthesis of 4*H*-benzo[*h*]chromene and 7*H*-benzo[*h*]chromeno[2,3-*d*]pyrimidine derivatives and the evaluation of their antitumor activities. The chemical structures of the studied compounds and their structure–activity relationships (SAR) at 2-,3- and 2,3-positions are discussed in this work.

Chemistry

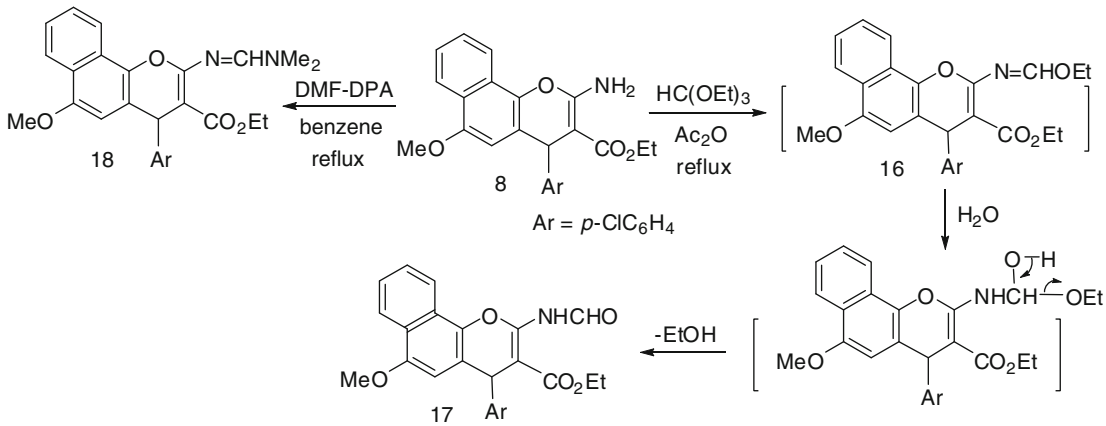
Treatment of 4-methoxy-1-naphthol (**1**) with a mixture of 4-chlorobenzaldehyde (**2**) and malononitrile (**3**) or α-cyano-4-chlorocinnamonitrile (**4**) in ethanolic piperidine solution under reflux for 1 h or under Microwave irradiation conditions for 2 min at 140 °C gave 2-amino-4-(4-chlorophenyl)-6-methoxy-4*H*-benzo[*h*]chromene-3-carbonitrile (**7**), while reaction of **1** with **2** and ethyl cyanoacetate (**5**) or ethyl α-cyano-4-chlorocinnamate (**6**) afforded ethyl 2-amino-4-(4-chlorophenyl)-6-methoxy-4*H*-benzo[*h*] chromene-3-carboxylate (**8**), respectively, (Scheme 1). The reactions were controlled using TLC technique. The maximum power of Microwave irradiation was optimized by carrying out the same reaction at different watt powers. Microwave radiations at 400 W were chosen as the optimum power, as the highest yield was obtained at this power.

Condensation of **7** with benzaldehyde in the ethanolic piperidine solution under reflux gave 2-benzylideneamino-4-(4-chlorophenyl)-6-methoxy-4*H*-benzo[*h*]chromene-3-carbonitrile (**9**). When **9** was treated with hydrazine hydrate or phenyl hydrazine in ethanol at room temperature or under reflux, the addition product **10** was formed (R = H or Ph, respectively). From the intermediate **10**, benzaldehyde hydrazone or benzaldehyde phenyl hydrazone was eliminated to give *β*-enaminonitrile **7** (Khafagy *et al*., 2002) rather than the pyrimidine derivative **11.** These results are depicted in (Scheme 2).

Treatment of **7** with acetic anhydride under reflux for 1/2 or 6 h gave the same compound, 2-diacetylamino-4-(4-chlorophenyl)-6-methoxy-4*H*-benzo[*h*]chromene-3-carbonitrile (**12**), while reaction of **7** with triethyl orthoformate in acetic anhydride or dimethylformamide-dipentylacetal (DMF-DPA) in benzene under reflux for 2 h afforded 4-(4-chlorophenyl)-2-ethoxymethyleneamino-6-methoxy-4*H*-



Scheme 3 Synthetic protocol of compounds (**12**–**15**)



Scheme 4 Synthetic protocol of compounds (**17**, **18**)

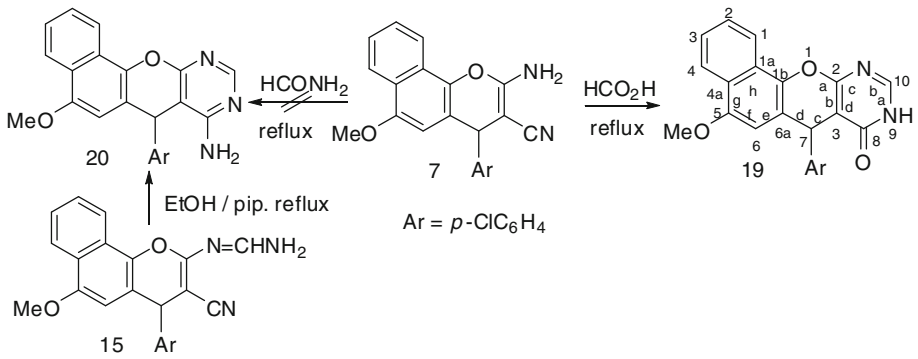
benzo[*h*]chromene-3-carbonitrile (**13**) and 4-(4-chlorophenyl)-2-dimethylaminomethyleneamino-6-methoxy-4*H*-benzo[*h*]chromene-3-carbonitrile (**14**), respectively, (Scheme 3). Interaction of the imidate **13** with dimethylamine in methanol at room temperature under stirring for 1 h yielded the imidine **14**, which can be obtained as described before from the reaction of **7** and DMF-DPA (m.p., mixed m.p. and identical IR and MS spectrum), while reaction of **13** with NH_3 gas bubbled in methanol at room temperature for 1 h yielded the open chain product 2-aminomethyleneamino-4-(4-chlorophenyl)-6-methoxy-4*H*-benzo[*h*]chromene-3-carbonitrile **15** (Scheme 3). The reactions were controlled using TLC technique.

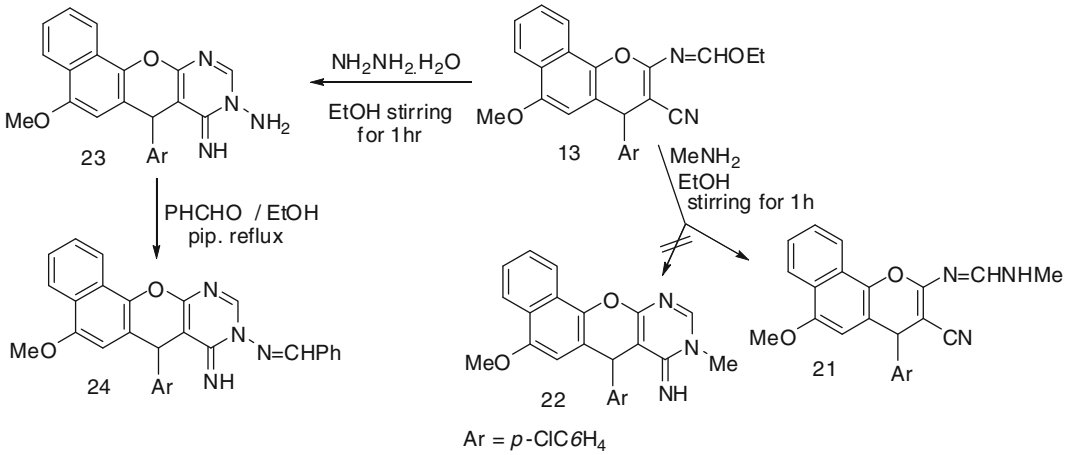
In a similar manner, reaction of **8** with triethyl orthoformate gave the ethyl 4-(4-chlorophenyl)-2-formamido-6-methoxy-4*H*-benzo[*h*]chromene-3-carboxylate (**17**), instead of ethyl 4-(4-chlorophenyl)-2-ethoxymethyleneamino-6-methoxy-4*H*-benzo[*h*]chromene-3-carboxylate (**16**), while condensation of **8** with DMF-DPA give the ethyl 4-(4-chlorophenyl)-2-dimethylaminomethyleneamino-6-methoxy-4*H*-benzo[*h*]chromene-3-carboxylate (**18**). The formation of **17** can be rationalized through the initial product of addition of H_2O to ethoxymethyleneamino group (–N=CHOEt) of **16**, which lose ethanol to give 2-formamido derivatives **17.** These results are depicted in (Scheme 4).

Compounds **7** and **13** were subjected for further reactions to produce fused heterotetracyclic systems incorporating pyrimidine nucleus at 2,3-positions in addition to 4*H*-chromene moiety. Thus, condensation of **7** with formic acid under reflux gave 7-(4-chlorophenyl)-5-methoxy-7*H*,9*H*-benzo[*h*]chromeno[2,3-*d*]pyrimidin-8-one (**19**), while reaction of **7** with formamide under reflux was unsuccessful, the 8-amino-7-(4-chlorophenyl)-5-methoxy-7*H*-benzo[*h*]chromeno[2,3-*d*]pyrimidine (**20**) was not formed. Compound **20** can be prepared by cyclization of **15** in ethanolic piperidine solution under reflux (Khafagy *et al.*, 2002). These results are depicted in (Scheme 5). The reactions were controlled using TLC technique.

Reaction of the imidate **13** with methylamine in ethanol at room temperature under stirring for 1 h gave the open

Scheme 5 Synthetic protocol of compounds (**19**, **20**)





Scheme 6 Synthetic protocol of compounds (**21**, **23**, **24**)

chain product 2-methylaminomethyleneamino-4-(4-chlorophenyl)-6-methoxy-4*H*-benzo[*h*]chromene-3-carbonitrile (**21**), instead of cycloaddition product 7-(4-chlorophenyl)-5-methoxy-8-imino-9-methyl-7*H*-benzo[*h*]chromeno[2,3-*d*]pyrimidine (**22**), while reaction of **13** with hydrazine hydrate afforded the cycloaddition product 9-amine-7-(4-chlorophenyl)-5-methoxy-8-imino-7*H*-benzo[*h*]chromeno [2,3-*d*]pyrimidine (**23**). Condensation of the imino compound **23** with benzaldehyde in ethanolic piperidine solution under reflux gave the open chain product 9-benzylideneamino-7-(4-chlorophenyl)-5-methoxy-8-imino-7*H*-benzo[*h*]chromeno[2,3-*d*]pyrimidine (**24**). These results are depicted in (Scheme 6). The reactions were controlled using TLC technique.

The structures of **9**, **12**–**15**, **17**–**21**, **23**, and **24** were established on the basis of IR, ^{1}H NMR, ^{13}C NMR, and MS data.

Antitumor assays

Compounds **7**–**9**, **12**–**15**, **17**–**21**, **23**, and **24** were evaluated for human tumor cell growth inhibitory activity against three different cell lines: breast adenocarcinoma (MCF-7), lung carcinoma (HCT), 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. All the standard drugs and all new synthesized compounds (controls) were tested at the same concentrations, to see the effectiveness action for these new synthesized compounds in comparing with control drugs. The inhibitory activity of the synthetic compounds **7**–**9**, **12**–**15**, **17**–**21**, **23**, and **24** against the three cell lines MCF-7, HCT, and HepG-2 is given in Table 3 which included in supporting information (Supplementary material 27) and Fig. 1.

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;

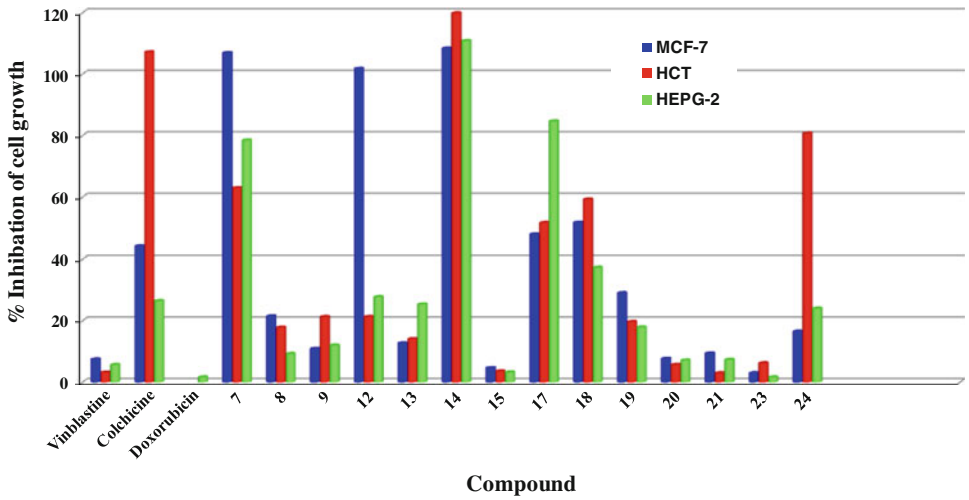


Fig. 1 IC_{50} values expressed in μM of 4*H*-benzo[*h*]chromene and 7*H*-benzo[*h*]chromeno[2,3-*d*]pyrimidine derivatives against MCF-7, HCT, and HepG-2 tumor cells

Vosooghi *et al*., 2010; Singh *et al*., 2010; Vukovic *et al*., 2010; Sabry *et al*., 2011; Kemnitzer *et al*., 2008; Mahmoodi *et al*., 2010; Endo *et al*., 2010; Tseng *et al*., 2010; El-Sayed and Ibrahim 2010; Keri *et al*., 2010; Sashidhara *et al*., 2011).

In the present study, several of 4*H*-benzo[*h*]chromenes and 7*H*-benzo[*h*]chromeno[2,3-*d*]pyrimidines were prepared. Structures of the synthesized compounds were elucidated on the basis of IR, ^{1}H NMR, ^{13}C NMR, ^{13}C NMR-DEPT, and MS data. Compounds **7**–**9**, **12**–**15**, **17**–**21**, **23**, and **24** were tested against three different tumor cell lines: MCF-7, HCT, and HepG-2. The cytotoxicity evaluation using viability assays and inhibitory activities is given in Supplementary Table 3 and Fig. 1. The results from Supplementary Table 3 indicated that compounds **23**, **15**, and **20** were the most active against MCF-7, compounds **21** and **15** were the most active against HCT and compounds **23**, **15**, **20**, **21**, and **8** were the most active against HepG-2 as compared with the standard drug Vinblastine, while compounds **23**, **15**, **20**, **21**, **9**, **13**, **24**, **8**, and **19** were the most active against MCF-7, compounds **21**, **15**, **20**, **23**, **13**, **8**, **19**, **12**, **9**, **17**, **18**, **7**, and **24** were the most active against HCT, and compounds **23**, **15**, **20**, **21**, **8**, **9**, **19**, and **13** were the most active against HepG-2 as compared with the standard drug Colchicine. In addition, compound **23** was the most active against HepG-2 as compared with the standard drug Doxorubicin and the remaining compounds exhibited moderate to lower activities as compared with the standard drugs Vinblastine, Colchicine, and Doxorubicin.

SAR studies

The cytotoxicity activity (IC_{50}) of compounds **7**, **8**, and their analogs against the three different cancer cell lines is summarized in Supplementary Table 3. The SAR studies at the 2-,3- and 2,3-positions and the relationship between lipophilicity (hydrophilic or hydrophobic groups) and SAR were explored. The SAR studies of **7** and its analogs revealed that compound **15** have the highest significant potent antitumor activity (IC_{50} = 4.6 μM) against MCF-7 as shown in Table 1, compounds **21**, **9**, **13** showed antitumor activities (IC_{50} = 9.4–12.7 μM) near to the standard drug Vinblastine (IC_{50} = 7.5 μM), while compounds **15**, **21**, **9**, and **13** have the highest significant potent antitumor activities (IC_{50} = 4.6–12.7 μM) against MCF-7, Table 1 as compared to the standard drug Colchicine (IC_{50} = 44.3 μM), and the other compounds **12** and **14** (IC_{50} = 101.8–108.4 μM) are considered to be inactive. These data indicate that the activity of compounds **15**, **21**, **9**, and **13** was considerably attributed to the presence of the hydrophobic groups (–N=CHNH$_2$-2, –N=CHNHMe-2, –N=CHPh-2 and –N=CHOEt-2) in 4*H*-benzo[*h*]chromene moiety at position C-2 and the hydrophobic groups is preferred at position C-2. Blocking of the (–NH$_2$-2) group with other hydrophobic groups such as (–NAc$_2$-2 and N=CHNMe$_2$-2) in compounds **12** and **14** resulted in reduction of potency. Replacing the cyano-3 with ester-3 group (hydrophobic group) resulted in strong improvement of potency for compound **8** (IC_{50} = 21.5 μM) as compared to the compound **7** (IC_{50} = 106.9 μM), while blocking of the (–NH$_2$-2) group for compound **8** with hydrophilic groups (–NHCHO-2) or hydrophobic group (–N=CHNMe$_2$-2) resulted in reduction of potency of the compounds **17** and **18** (IC_{50} = 48.1–51.9 μM), suggesting that the small hydrophobic group (–NH$_2$-2) is preferred over the other groups and the hydrophilic group (ester-3) is preferred over an cyano-3 group. Incorporating a pyrimidine nucleus at 2, 3-positions with hydrophobic groups (=NH-8, –NH$_2$-9) for compound **23** and (–NH$_2$-8) for compound **20** resulted in strong improvement of potency (IC_{50} = 3.0–7.7 μg/ml) as compared to the standard drugs Vinblastine (IC_{50} = 7.5 μM) and Colchicine (IC_{50} = 44.3 μM) with the highest significant potent antitumor activity as shown in Table 1 and compound **24** (=NH-8, –N=CHPh-9)

Table 1 Positive and negative controls and the effectiveness of the test compounds against MCF-7, HCT, and HEPG-2

Control/compound	IC_{50} (μM)	Cell line	*F* ratio	*p* value
Vinblastine	7.5[a]	MCF-7	44.113	0.000 (HS)
23	3.0[b]	MCF-7		
15	4.6[c]	MCF-7		
Colchicine	44.3[a]	MCF-7	1,060.418	0.000 (HS)
23	3.0[b]	MCF-7		
15	4.6[c]	MCF-7		
20	7.7[d]	MCF-7		
21	9.4[e]	MCF-7		
9	10.9[f]	MCF-7		
13	12.7[g]	MCF-7		
24	16.5[h]	MCF-7		
8	21.5[i]	MCF-7		
19	29[j]	MCF-7		
Colchicine	107.2[a]	HCT	8,196.746	0.000 (HS)
21	3.0[b]	HCT		
15	3.6[c]	HCT		
20	5.7[d]	HCT		
23	6.2[ed]	HCT		
13	14.1[f]	HCT		
8	17.8[g]	HCT		
19	19.7[h]	HCT		
12	21.3[f]	HCT		
9	21.3[i]	HCT		
17	51.9[j]	HCT		
18	59.3[k]	HCT		
7	63.0[l]	HCT		
24	80.7[m]	HCT		
Colchicine	26.5[a]	HEPG-2	872.398	0.000 (HS)
23	1.7[b]	HEPG-2		
15	3.3[c]	HEPG-2		
20	7.2[d]	HEPG-2		
21	7.4[e]	HEPG-2		
8	9.3[f]	HEPG-2		
9	12.0[g]	HEPG-2		
19	17.9[h]	HEPG-2		
24	24.0[i]	HEPG-2		
13	25.4[j]	HEPG-2		

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

HS highest significant

showed moderated activity ($IC_{50} = 16.5$ M) as compared to the standard drug Vinblastine and was active as compared to the standard drug Colchicine, while the presence of hydrophilic group (–C=O-8) for compound **19** ($IC_{50} = 29.0$ μM) resulted in a little reduction of potency with the highest significant potent antitumor activity as shown in Table 1,

Table 2 Positive and negative controls and the effectiveness of the test compounds against HCT, HEPG-2

Compound	IC_{50} (μM)	Cell line
Vinblastine	3.2	HCT
21	3.0	HCT
T test	3.873	
p value	0.018 (S)	
Vinblastine	5.7	HEPG-2
15	3.3	HEPG-2
T test	45.088	
p value	0.000 (HS)	
Doxorubicin	1.7	HEPG-2
23	1.7	HEPG-2
T test	0.000	
p value	1.000 (NS)	

Positive control (active compounds) and negative control (standard drugs)

S significant, *HS* highest significant, *NS* not significant

suggesting that an hydrophobic group is preferred over an hydrophilic group and the pyrimidine moiety is preferred at 2,3-positions.

In the case of HCT, investigation of SAR revealed that compound **21** ($IC_{50} = 3.0$ μM) has the most potent with significant activity against HCT as compared to compound **7** and its analogs and the standard drug Vinblastine ($IC_{50} = 3.2$ μM) observed in Table 2, while compound **15** showed antitumor activity ($IC_{50} = 3.6$ μM) near to the standard drug Vinblastine. This potency could be attributed to the presence of the hydrophobic groups (–N=CHNHMe-2 and –N=$CHNH_2$-2) at the 2-position, while the blocking of the (–NH_2-2) group with other groups such as (–N=CHOEt-2, –NAc_2-2 and –N=CHPh-2) with cyano-3 group in compounds **13**, **12**, and **9** ($IC_{50} = 14.1$–21.3 μM) resulted in a reduction of potency or with the hydrophobic group (–N=CHNHMe-2) or hydrophilic group (–NHCHO-2) with ester-3 group in compounds **17** and **18** ($IC_{50} = 51.9$–59.3 μM) resulted in more reduction of potency, suggesting that the hydrophobic groups (–N=CHNHMe-2 and –N=$CHNH_2$-2) at position C-2 are preferred over the other groups. Incorporating a pyrimidine nucleus at 2, 3-positions with hydrophobic groups (–NH_2-8) for **20** and (=NH-8, –NH_2-9) for **23** resulted in strong improvement of potency ($IC_{50} = 5.7$–6.2 μM) as compared to the standard drug Vinblastine ($IC_{50} = 3.2$ μM), while the presence of the hydrophilic group (–C=O-8) for compound **19** ($IC_{50} = 19.7$ μM) resulted in a reduction of potency and more reduction of potency ($IC_{50} = 80.7$ μM) for compound **24** (–N=CHPh-9), suggesting that an hydrophobic group is preferred over an hydrophilic group and the pyrimidine moiety is preferred at 2, 3-positions. In addition, compounds **21**, **15**, **13**, **8**, **12**, **9**, **17**, **18**, and **7** ($IC_{50} = 3.0$–63.0 μM),

respectively, have the most potent highest significant activity against HCT as compared to the standard drug Colchicine (IC_{50} = 107.2 μM) as shown in Table 1, while the pyrimidine compounds **20**, **23**, and **19** (IC_{50} = 5.7–19.7 μM) resulted in strong improvement of potency with the highest significant activity as shown in Table 1 and compound **24** has less potency with the highest significant as shown in Table 1 (IC_{50} = 80.7 μM) than the standard drug Colchicine.

Furthermore, compounds **15** (IC_{50} = 3.3 μM) showed the highest significant antitumor activity, Table 2 against HepG-2 as compared to compound **7** (IC_{50} = 78.5 μM) and its analogs and the standard drug Vinblastine (IC_{50} = 5.7 μM), while compounds **21** and **8** showed antitumor activities (IC_{50} = 7.4–9.3 μM) against HepG-2 near to the standard drug Vinblastine. These data indicate that the activity of compounds **15**, **21**, and **8** was considerably attributed to the presence of the hydrophobic groups (–N=CHNH$_2$-2, –N=CHNHMe-2 and –NH$_2$-2) at the 2-position, suggesting that the hydrophobic groups (–N=CHNHMe-2 and –N=CHNH$_2$-2) at position C-2 are preferred over the other groups, while the blocking of the (–NH$_2$-2) group with other hydrophobic groups such as (–N=CHPh-2, –N=CHOEt-2 and –NAc$_2$-2) with cyano-3 in compounds **9**, **13,** and **12** (IC_{50} = 12.0–27.8 μM) resulted in a reduction of potency, and the other compounds **18**, **17**, and **14** showed more reduction of potency (IC_{50} = 37.3–110.8 μM). In addition, compounds **15**, **21**, **8**, **9**, and **13** (IC_{50} = 3.3–25.4 μM) showed the highest significant antitumor activities against HepG-2 as compared to compound **7** (IC_{50} = 78.5 μM) as shown in Table 1 and its analogs and the standard drug Colchicine (IC_{50} = 26.5 μM). This is due to the presence of the hydrophobic groups (–N=CHNH$_2$-2, –N=CHNHMe-2, –NH$_2$-2, –N=CHPh-2 and –N=CHOEt-2), while compounds **12** and **18** showed a little reduction of potency (IC_{50} = 27.8–37.3 μM) and compounds **17** and **14** are inactive (IC_{50} = 84.7–110.8 μM). Incorporating a pyrimidine nucleus at 2,3-positions with the hydrophobic groups (=NH-8, –NH$_2$-9) for compound **23** and (–NH$_2$-8) for **20** resulted in strong improvement of potency with the highest significant activities (IC_{50} = 1.7–7.2 μM), Tables 1, 2 as compared to the standard drugs Vinblastine (IC_{50} = 5.7 μM) and Colchicine (IC_{50} = 26.5 μM), while the other compounds **19** and **24** showed moderate activities (IC_{50} = 17.9–24.0 μM) as compared to the standard drug Vinblastine and compound **19** and **24** (IC_{50} = 17.9–24.0 μM) showed the highest significant activities less than the standard drug Colchicine (IC_{50} = 26.5 μM) as shown in Table 1, suggesting that the hydrophobic group is preferred over an the hydrophilic group and the pyrimidine moiety is preferred at 2,3-positions.

Finally, in the case of HepG-2, an investigation of SAR revealed that compound **23** (IC_{50} = 1.7 μM) has the same potent activity against HepG-2 as compared to the standard drug Doxorubicin (IC_{50} = 1.7 μM) with not significant activity as shown in Table 2, this potency due to the incorporating a pyrimidine nucleus at 2,3-positions with the hydrophobic groups (=NH-8, –NH$_2$-9) for compound **23**, suggesting that hydrophobic group is preferred over the hydrophilic group and the pyrimidine moiety is preferred at 2,3-positions. In addition, compounds **15**, **20**, **21**, and **8** showed activities (IC_{50} = 3.3–9.3 μM) near to the standard drug Doxorubicin and the other compounds showed more reduction of potency (IC_{50} = 12.0–110.8 μM).

Conclusions

In conclusions, several compounds of 4*H*-benzo[*h*]chromenes and fused 4*H*-benzo[*h*]chromenes were prepared. Compounds **23**, **15**, and **20** were the most active against MCF-7, compounds **21** and **15** were the most active against HCT and compounds **23**, **15**, **20**, **21**, and **8** were the most active against HepG-2 as compared with the standard drug Vinblastine, while compounds **23**, **15**, **20**, **21**, **9**, **13**, **24**, **8,** and **19** were the most active against MCF-7, compounds **21**, **15**, **20**, **23**, **13**, **8**, **19**, **12**, **9**, **17**, **7**, **18**, and **24** against HCT, compounds **23**, **15**, **20**, **21**, **8**, **9**, **19**, and **13** were the most active against HepG-2 as compared with the standard drug Colchicine, and compound **23** was the most active against HepG-2 as compared with the standard drug Doxorubicin. In addition, we have explored the SAR study of 4*H*-benzo[*h*]chromene and 7*H*-benzo[*h*]chromeno[2,3-*d*]pyrimidine compounds as antitumor agents via modification at the 2-,3- and 2,3-positions, and it was found that there might be a size limited pocket at positions C-2, C-3, the hydrophobic group is preferred over the hydrophilic group, and the 7*H*-benzo[*h*]chromeno[2,3-*d*]pyrimidine moiety is preferred over the 4*H*-benzo[*h*]chromene moiety. 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

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 on a BRUKER AV 500/600 MHz spectrometer using tetramethylsilane (TMS) as an internal reference and results are expressed as δ (ppm) values. ^{13}C NMR spectra were obtained using distortionless enhancement by polarization transfer (DEPT), with this technique, the signals of CH and CH$_3$ carbon atoms appear normal (up) and the signal of carbon atoms in CH$_2$ environments appears negative (down). The

Microwave apparatus used is Milestone Sr1, Microsynth. The MS was measured on a Shimadzu GC/MS-QP5050A spectrometer. Elemental analyses were performed on a Perkin-Elmer 240 microanalyser.

2-Amino-4-(4-chlorophenyl)-6-methoxy-4*H*-benzo[*h*]chromene-3-carbonitrile (**7**)

Prepared as previously described (Al-Sehemi *et al*., 2012; El-Agrody *et al*., 2014).

Ethyl 2-amino-4-(4-chlorophenyl)-6-methoxy-4*H*-benzo[*h*]chromene-3-carboxyalte (**8**)

Prepared as previously described (Al-Sehemi *et al*., 2012; El-Agrody *et al*., 2014).

2-Benzylideneamino-4-(4-chlorophenyl)-6-methoxy-4*H*-benzo[*h*]chromene-3-carbonitrile (**9**)

A mixture of 2-amino-4-(4-chlorophenyl)-6-methoxy-4*H*-benzo[*h*]chromene-3-carbonitrile (**7**) (0.01 mol) with benzaldehyde (0.01 mol) and piperidine (0.5 ml) in ethanol (20 ml) was refluxed for 2 h. The solid product which formed was filtered, washed with cold methanol, dried, and recrystallized from ethanol to afford **9** as yellow needles; m.p. 235–236 °C; yield 70 %; IR (cm^{-1}) in (KBr) υ: 3,071, 3,029, 2,962, 2,937, 2,853 (CH), 2,211 (CN); ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 9.40 (s, 1H, N=CH) 8.52–6.53 (m, 14H, aromatic), 5.34 (s, 1H, H-4), 3.84 (s, 3H, OCH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 163.7 (N=CH), 160.7 (C-2), 151.9 (C-6), 136.9 (C-10b), 127.7 (C-10a), 126.7, (C-9), 124.7 (C-8), 124.0 (C-6a), 121.6 (C-7), 121.6 (C-10), 120.3 (C-4a), 116.1 (CN), 103.1 (C-5), 87.0 (C-3), 55.8 (CH_3), 42.2 (C-4), 139.6, 134.8, 133.5, 132.8, 130.1, 129.3, 129.1, 177.8 (aromatic); MS *m/z* (%): 452 (M^++2, 12.99), 450 (M^+, 26.98) with a base peak at 340; Anal. Calcd. for $C_{28}H_{19}ClN_2O_2$: C, 74.58; H, 4.25; N, 6.21. Found: C, 74.60; H, 4.34; N, 6.30 %.

Reaction of **9** with hydrazine derivatives

A mixture of **7** (0.01 mol) and hydrazine hydrate or phenyl hydrazine (0.01 mol) in EtOH was stirred at room temperature or refluxed for 2 h to give **7** (m.p. and mixed m.p. 218–219 °C) yield (80 %).

*2-Diacetylamino-4-(4-chlorophenyl)-6-methoxy-4H-benzo[h]chromene-3-carbonitrile (**12**)*

A solution of **7** (0.01 mol) in acetic anhydride (20 ml) was refluxed for 1/2 or 6 h. The solvent was removed under reduced pressure and the resulting solid was collected and washed with cold ethanol, filtered, dried, and recrystallized from ethanol to afford **12** as yellow powder; m.p. 162–163 °C; yield 73 %; IR (cm^{-1}) in (KBr) υ: 3,003, 2,942, 2,875 (CH), 2,219 (CN), 1,736 (CO); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.25–6.22 (m, 9H, aromatic), 5.15 (s, 1H, H-4), 3.86 (s, 3H, OCH_3), 2.58 (s, 6H, $2COCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 170.7 (CO), 170.4 (CO), 153.7 (C-2), 152.7 (C-6), 138.0 (C-10b), 129.3 (C-10a), 128.0 (C-9), 126.6 (C-6a), 125.7 (C-8), 124.2 (C-7), 122.4 (C-10), 120.6 (C-4a), 115.2 (CN), 102.0 (C-5), 91.8 (C-3), 55.8 (CH_3), 43.7 (C-4), 25.7 (CH_3), 25.2 (CH_3), 140.2, 134.3, 129.7, 126.7 (aromatic); MS *m/z* (%): 448 (M^++2, 9.01), 446 (M^+, 27.49) with a base peak at 75 (100); Anal. Calcd. for $C_{25}H_{19}ClN_2O_4$: C, 67.19; H, 4.29; N, 6.27. Found: C, 67.21; H, 4.39; N, 6.28 %.

General procedure for the preparation of **13** and **17**

A mixture of **7** or ethyl 2-amino-4-(4-chlorophenyl)-6-methoxy-4*H*-benzo[*h*]chromene-3-carboxylate (**8**) (0.01 mol) with triethyl orthoformate (0.01 mol) and acetic anhydride (30 ml) was refluxed for 2 h. The solvent was removed under reduced pressure and the resulting solid was washed with methanol and recrystallized from proper solvent to give **13** and **17**. The physical data of the compounds **13** and **17** are as follows:

*4-(4-Chlorophenyl)-2-ethoxymethyleneamino-6-methoxy-4H-benzo[h]chromene-3-carbonitrile (**13**)*

Yellow needles from benzene; m.p. 192–193 °C; yield 78 %; IR (cm^{-1}) in (KBr) υ: 3,079, 3,054, 2,984, 2,958, 2,941, 2,900, 2,857 (CH), 2,210 (CN); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.51 (s, 1H, N=CH), 8.19–6.11 (m, 9H, aromatic), 4.84 (s, 1H, H-4), 4.40 (q, 2H, CH_2, J = 7.25 Hz), 3.76 (s, 3H, OCH_3), 1.34 (t, 3H, CH_3, J = 7.25 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 159.3 (N=CH), 157.3 (C-2), 152.7 (C-6), 137.8 (C-10b), 129.2 (C-9), 127.5 (C-8), 125.7 (C-10a), 124.5 (C-6a), 122.4 (C-7), 120.5 (C-10), 118.2 (C-4a), 115.3 (CN), 102.6 (C-5), 80.5 (C-3), 64.3 (CH_2), 55.7 (CH_3), 43.3 (C-4), 14.0 (CH_3), 141.8, 133.6, 129.7, 126.4 (aromatic); ^{13}C NMR-DEPT 135° CH, CH_3 (↑), CH_2 (↓) (125 MHz, $CDCl_3$) δ (ppm): 159.3 (N=CH ↑), 129.7 (aromatic ↑), 129.2 (C-9 ↑), 127.5 (C-8 ↑), 126.4 (aromatic ↑), 122.4 (C-7 ↑), 120.5 (C-10 ↑), 102.6 (C-5 ↑), 64.3 (CH_2 ↓), 55.7 (CH_3 ↑), 43.3 (C-4 ↑), 14.0 (CH_3 ↑); ^{13}C NMR-DEPT 90° CH (↑) (125 MHz, $CDCl_3$) δ (ppm): 159.3 (N=CH ↑), 129.7 (aromatic ↑), 129.2 (C-9 ↑), 127.5 (C-8 ↑), 126.4 (aromatic ↑) 122.4 (C-7 ↑), 120.5 (C-10 ↑), 102.6 (C-5 ↑), 43.3 (C-4 ↑); ^{13}C NMR-DEPT 45° CH, CH_2, CH_3 (↑) (125 MHz, $CDCl_3$) δ (ppm): 159.3 (N=CH ↑), 129.7 (aromatic ↑), 129.2 (C-9 ↑), 127.5 (C-8 ↑), 126.4 (aromatic ↑) 122.4 (C-7 ↑), 120.5 (C-10 ↑), 102.6 (C-5 ↑), 64.3 (CH_2 ↑),

55.7 (CH_3 ↑), 43.3 (C-4 ↑), 14.0 (CH_3 ↑); MS *m/z* (%): 420 (M^++2, 11.95), 418 (M^+, 34.05) with a base peak at 251 (100); Anal. Calcd. for $C_{24}H_{19}ClN_2O_3$: C, 68.82; H, 4.57; N, 6.69. Found: C, 68.85; H, 4.60; N, 6.71 %.

*Ethyl 4-(4-chlorophenyl)-2-formamido-6-methoxy-4H-benzo[h]chromene-3-carboxylate (**17**)*

Brown needles from benzene; m.p. 239–240 °C; yield 50 %; IR (cm^{-1}) in (KBr) υ: 3,282 (NH or OH), 3,069, 3,007, 2,945, 2,838 (CH), 1,722 (CO), 1,675(CO); ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 11.38 (s, 1H, CHO), 11.00 (bs, 1H, NH),8.52–6.30 (m, 9H, aromatic), 5.05 (s, 1H, H-4), 4.17 (q, 2H, CH_2, $J = 7.2$ Hz), 3.89 (s, 3H, OCH_3), 1.26 (t, 3H, CH_3, $J = 7.2$ Hz); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 169.0 (CO), 167.3 (CO), 159.4 (C-2), 153.0 (C-6), 137.52 (C-10b), 132.6 (C-10a), 127.7 (C-9), 125.1 (C-6a), 126.3 (C-8), 122.4 (C-7), 121.8 (C-10), 120.2 (C-4a), 102.7 (C-5), 85.9 (C-3), 60.9 (CH_2), 55.7 (CH_3), 40.8 (C-4), 14.2 (CH_3), 144.9, 136.7, 129.4, 128.7 (aromatic); ^{13}C NMR-DEPT 135° CH, CH_3 (↑), CH_2 (↓) (125 MHz, $CDCl_3$) δ (ppm): 129.4 (aromatic ↑), 128.7 (aromatic ↑), 127.7 (C-9 ↑), 126.3 (C-8 ↑), 122.4 (C-7 ↑), 121.8 (C-10 ↑), 102.7 (C-5 ↑), 60.9 (CH_2 ↓), 55.7 (CH_3 ↑), 40.8 (C-4 ↑), 14.2 (CH_3 ↑); ^{13}C NMR-DEPT 90° CH (↑) (125 MHz, $CDCl_3$) δ (ppm): 129.4 (aromatic ↑), 128.7 (aromatic ↑), 127.7 (C-9 ↑), 126.3 (C-8 ↑), 122.4 (C-7 ↑), 121.8 (C-10 ↑), 102.7 (C-5 ↑), 40.8 (C-4 ↑); ^{13}C NMR-DEPT 45° CH, CH_2, CH_3 (↑) (125 MHz, $CDCl_3$) δ (ppm): 129.4 (aromatic ↑), 128.7 (aromatic ↑), 127.7 (C-9 ↑), 126.3 (C-8 ↑), 122.4 (C-7 ↑), 121.8 (C-10 ↑), 102.7 (C-5 ↑), 60.9 (CH_2 ↑), 55.7 (CH_3 ↑), 40.8 (C-4 ↑), 14.2 (CH_3 ↑); MS *m/z* (%): 439 (M^++2, 3.58), 437 (M^+, 11.78) with a base peak at 298; Anal. Calcd. for $C_{24}H_{20}ClNO_5$: C, C, 65.83; H, 4.60; N, 3.20. Found: C, 65.79; H, 4.67; N, 3.26 %.

General procedure for the preparation of **14** and **18**

A mixture of **7** or **8** (0.01 mol) with DMF-DPA (0.01 mol) and benzene (30 ml) was refluxed for 3 h. The solvent was removed under reduced pressure and the resulting solid was recrystallized from benzene to give **14** and **18**. The physical data of the compounds **14** and **18** are as follows:

*4-(4-Chlorophenyl)-2-dimethylaminomethyleneamino-6-methoxy-4H-benzo[h]chromene-3-carbonitrile (**14**)*

Colorless crystals from benzene; m.p. 200–201 °C; yield 65 %; IR (cm^{-1}) in (KBr) υ: 3,079, 2,981, 2,968, 2,926, 2,813 (CH), 2,196 (CN); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.38 (s, 1H, N=CH), 8.22–6.10 (m, 9H, aromatic), 4.71 (s, 1H, H-4), 3.81 (s, 3H, OCH_3), 3.21, 3.15 (2 s, 6H, $2CH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 159.6 (N=CH), 153.7 (C-2), 151.9 (C-6), 138.0 (C-10b), 129.4 (C-9), 127.1 (C-8), 125.5 (C-10a), 124.6 (C-6a), 122.3 (C-7), 120.6 (C-10), 120.5 (C-4a), 116.0 (CN), 103.0 (C-5), 73.6 (C-3), 55.6 (CH_3), 43.3 (C-4), 41.2 (CH_3), 143.0, 133.0, 129.0, 126.0 (aromatic); ^{13}C NMR-DEPT 135° CH, CH_3 (↑), CH_2 (↓) (125 MHz, $CDCl_3$) δ (ppm): 159.6 (N=CH ↑), 129.4 (C-9 ↑), 129.0 (aromatic ↑), 127.2 (C-8 ↑), 126.0 (aromatic ↑) 122.3 (C-7 ↑), 120.6 (C-10 ↑), 103.0 (C-5 ↑), 55.6 (CH_3 ↑), 43.3 (C-4 ↑), 41.2 (CH_3 ↑); ^{13}C NMR-DEPT 90° CH (↑) (125 MHz, $CDCl_3$) δ (ppm): 159.6 (N=CH ↑), 129.4 (C-9 ↑), 129.0 (aromatic ↑), 127.1 (C-8 ↑), 126.0 (aromatic ↑), 122.3 (C-7 ↑), 120.6 (C-10 ↑), 103.0 (C-5 ↑), 43.3 (C-4 ↑); ^{13}C NMR-DEPT 45° CH, CH_2, CH_3 (↑) (125 MHz, $CDCl_3$) δ (ppm): 159.6 (N=CH ↑), 129.4 (C-9 ↑), 129.0 (aromatic ↑), 127.1 (C-8 ↑), 126.0 (aromatic ↑), 122.3 (C-7 ↑), 120.6 (C-10 ↑), 103.0 (C-5 ↑), 55.6 (CH_3 ↑), 43.3 (C-4 ↑), 41.2 (CH_3 ↑); MS *m/z* (%): 419 (M^++2, 30.12), 417 (M^+, 88.76) with a base peak at 251 (100); Anal. Calcd. for $C_{24}H_{20}ClN_3O_2$: C, 68.98; H, 4.82; N, 10.06. Found: C, 69.00; H, 4.85; N, 10.09 %.

*Ethyl 4-(4-chlorophenyl)-2-dimethylaminomethyleneamino-6-methoxy-4H-benzo[h] chromene-3-carboxylate (**18**)*

Brown crystals from benzene; m.p. 185–186 °C; yield 67 %; IR (cm^{-1}) in KBr) υ: 3,085, 3,044, 3,004, 2,969, 2,929, 2,890, 2,808 (CH), 1,706 (CO); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.18 (s, 1H, N=CH), 8.25–6.44 (m, 9H, aromatic), 5.23 (s, 1H, H-4), 4.17 (q, 2H, CH_2, $J = 7.5$ Hz), 3.90 (s, 3H, OCH_3), 3.22, 3.17 (2 s, 6H, $2CH_3$), 1.28 (t, 3H, CH_3, $J = 7.5$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 167.9 (CO), 160.7 (N=CH), 155.6 (C-2), 151.9 (C-6), 138.5 (C-10b), 128.6 (C-10a), 128.4 (C-9), 128.2 (C-6a), 125.6 (C-8), 122.2 (C-7), 120.9 (C-10), 119.5 (C-4a), 103.3 (C-5), 91.4 (C-3), 59.7 (CH_2), 55.7 (CH_3), 43.4 (C-4), 40.8 (CH_3), 34.6 (CH_3), 14.4 (CH_3), 145.9, 131.9, 129.5, 126.7(aromatic); ^{13}C NMR-DEPT 135° CH, CH_3 (↑), CH_2 (↓) (125 MHz, $CDCl_3$) δ (ppm): 160.7 (N=CH ↑), 129.5 (aromatic ↑), 128.4 (C-9 ↑), 126.7 (aromatic ↑), 125.6 (C-8 ↑), 122.2 (C-7 ↑), 120.9 (C-10 ↑), 103.3 (C-5 ↑), 59.7 (CH_2 ↓), 55.7 (CH_3 ↑), 43.4 (C-4 ↑), 40.8 (CH_3 ↑), 34.9 (CH_3 ↑), 14.4 (CH_3 ↑); ^{13}C NMR-DEPT 90° CH (↑) (125 MHz, $CDCl_3$) δ (ppm): 160.7 (N=CH ↑), 129.5 (aromatic ↑), 128.4 (C-9 ↑), 126.7 (aromatic ↑), 125.6 (C-8 ↑), 122.2 (C-7 ↑), 120.9 (C-10 ↑), 103.3 (C-5 ↑), 43.4 (C-4 ↑); ^{13}C NMR-DEPT 45° CH, CH_2, CH_3 (↑) (125 MHz, $CDCl_3$) δ (ppm): 160.7 (N=CH ↑), 129.5 (aromatic ↑), 128.4 (C-9 ↑), 126.7 (aromatic ↑), 125.6 (C-8 ↑), 122.2 (C-7 ↑), 120.9 (C-10 ↑), 103.3 (C-5 ↑), 59.7 (CH_2 ↑), 55.7 (CH_3 ↑), 43.4 (C-4 ↑), 40.8 (CH_3 ↑), 34.9 (CH_3 ↑), 14.4 (CH_3 ↑), MS *m/z* (%): 466 (M^++2, 20.31), 464 (M^+, 9.64) with a base peak at 75 (100); Anal.

Calcd. for $C_{26}H_{25}ClN_2O_4$: C, 67.17; H, 5.42; N, 6.03. Found: C, 67.10; H, 5.37; N, 6.00 %.

General procedure for the preparation of **14**, **15** and **21**

A mixture of imidate **13** (0.01 mol), dimethylamine, and NH_3 gas or methylamine in methanol or ethanol (30 ml), was stirred for 1 h and the mixture was left overnight. The solid product was collected by filtration, washed with methanol, and recrystallized from proper solvent to afford **14**, **15**, and **21**. The physical data of the compounds **14**, **15**, and **21** are as follows:

*4-(4-Chlorophenyl)-2-dimethylaminomethyleneamino-6-methoxy-4H-benzo[h]chromene-3-carbonitrile (**14**)*

Described before.

*2-Aminomethyleneamino-4-(4-chlorophenyl)-6-methoxy-4H-benzo[h]chromene-3-carbonitrile (**15**)*

Colorless needles from ethanol; m.p. 235–236 °C; yield 91 %; IR (cm^{-1}) in (KBr) υ: 3,444, 3,367, 3,182 (NH_2), 3,012, 2,954, 2,939 (CH), 2,204 (CN); ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 8.63–6.52 (m, 9H, aromatic), 8.26 (s, 1H, N=CH), 7.77 (s, 2H, NH_2), 5.02 (s, 1H, H-4), 3.82 (s, 3H, OCH_3); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 161.7 (N=CH), 155.0 (C-2), 151.3 (C-6), 137.2 (C-10b), 128.3 (C-10a), 127.4 (C-9), 126.3(C-6a), 124.5 (C-8), 124.0 (C-7), 121.7 (C-10), 120.8 (C-4a), 115.6 (CN), 103.3 (C-5), 72.1 (C-3), 55.7 (CH_3), 39.9 (C-4), 141.1, 132.5, 129.7, 128.0 (aromatic); MS *m/z* (%): 391(M^++2, 20.31), 389 (M^+, 16.64) with a base peak at 279; Anal. Calcd. for $C_{22}H_{16}ClN_3O_2$: C, 67.78; H, 4.14; N, 10.78. Found: C, 67.81; H, 4.17; N, 10.80 %.

*2-Methylaminomethyleneamino-4-(4-chlorophenyl)-6-methoxy-4H-benzo[h]chromene-3-carbonitrile (**21**)*

Pale yellow crystals from ethanol/benzene; m.p. 230–231 °C; yield 66 %; IR (cm^{-1}) in (KBr) υ: 3,302 (NH), 3,074, 3,004, 2,954, 2,895, 2,819 (CH), 2,202 (CN); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.66 (s, 1H, N=CH), 8.22–6.52 (m, 9H, aromatic), 5.80 (s, 1H, NH), 4.89 (s, 1H, H-4), 3.81 (s, 3H, OCH_3), 3.12 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 159.4 (N=CH), 156.8 (C-2), 151.1 (C-6), 136.8 (C-10b), 129.4 (C-9), 127.3 (C-8), 124.4 (C-10a), 123.7 (C-6a), 121.5 (C-7), 120.6 (C-10), 117.6 (C-4a), 115.5 (CN), 103.3 (C-5), 85.0 (C-3), 56.0 (CH_3), 40.7 (C-4), 30.3 (CH_3), 141.7, 131.7, 129.4, 126.2 (aromatic); MS0 *m/z* (%): 405 (M^++2, 10.19), 403 (M^+, 31.01) with a base peak at 75 (100); Anal. Calcd. for $C_{23}H_{18}ClN_3O_2$: C, 68.40; H, 4.49; N, 10.40. Found: C, 68.36; H, 4.46; N, 10.36 %.

*7-(4-Chlorophenyl)-5-methoxy-7H,9H-benzo[h]chromeno[2,3-d]pyrimidin-8-one (**19**)*

A mixture of **7** (0.01 mol) and formic acid (30 ml) was refluxed for 3–5 h. The solvent was removed under reduced pressure and the resulting solid was recrystallized from ethanol to give **19** as yellow crystals from ethanol; m.p. 196–197 °C; yield 63 %; IR (cm^{-1}) in (KBr) υ: 3,503 (NH), 3,071, 3,013, 2,958, 2,939, 2,892 (CH), 1,762 (CO); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.30–6.18 (m, 10H, aromatic, H-10), 8.02 (bs, 1H, NH, canceled by D_2O), 4.71 (s, 1H, H-7), 3.91 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 160.0 (CO), 159.8 (C-11a), 153.4 (C-5), 153.1 (C-10), 135.1 (C-1b), 128.9 (C-1a), 128.1 (C-4a), 127.2 (C-2), 124.3 (C-3), 122.4 (C-7), 122.1 (C-1), 120.9 (C-6a), 113.8 (C-6), 101.7 (C-7a), 55.9 (CH_3), 41.2 (C-7), 140.0, 135.0, 129.8, 126.1 (aromatic); MS *m/z* (%): 392 (M^++2, 38.49), 390 (M^+, 12.20) with a base peak at 67 (100); Anal. Calcd. for $C_{22}H_{15}ClN_2O_3$: C, 67.61; H, 3.87; N, 7.17. Found: C, 67.57; H, 3.78; N, 7.06 %.

*8-Amino-7-(4-chlorophenyl)-5-methoxy-7H-benzo[h]chromeno[2,3-d]pyrimidine (**20**)*

A mixture of 2-aminomethyleneamino-4-(4-chlorophenyl)-6-methoxy-4*H*-benzo[*h*]chromene-3-carbonitrile (**15**) (0.01 mol) in ethanol (30 ml) and piperidine (0.5 ml) was heated under reflux for 2 h. The solid product formed was collected by filtration, washed with methanol, and recrystallized from ethanol to give **15** as colorless needles; m.p. 250–251 °C; yield 68 %; IR (cm^{-1}) in (KBr) υ: 3,470, 3,414, 3,294 (NH_2), 3,006, 2,994, 2,868 (CH); ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 8.27-6.60 (m, 9H, aromatic), 8.18 (s, 1H, H-10), 6.90 (s, 2H, NH_2), 5.38 (s, 1H, H-7), 3.90 (s, 3H, OCH_3); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 162.7 (C-8), 162.3 (C-11a), 156.7 (C-10), 151.4 (C-5), 143.0 (C-1b), 127.5 (C-1a), 126.3 (C-2), 124.5 (C-4a), 124.2 (C-3), 121.7 (C-7), 120.7 (C-1), 118.7 (C-6a), 103.2 (C-6), 95.2 (C-7a), 55.8 (CH_3), 38.2 (C-7), 137.7, 131.6, 129.8, 128.6 (aromatic); Anal. Calcd. for $C_{22}H_{16}ClN_3O_2$: C, 67.78; H, 4.14; N, 10.78. Found: C, 67.80; H, 4.17; N, 10.80 %.

*9-Amine-7-(4-chlorophenyl)-5-methoxy-8-imino-7H-benzo[h]chromeno[2,3-d]pyrimidine (**23**)*

A mixture of imidate **13** (0.01 mol) and hydrazine hydrate (0.01 mol) in ethanol (30 ml) was stirred at room temperature for 1 h. The solid product was collected by filtration, washed with methanol, and recrystallized from benzene to give **23** as colorless crystals; m.p. 233–234 °C; yield 75 %;

IR (cm^{-1}) in (KBr) υ: 3,329, 3,272 (NH and NH_2), 3,062, 3,011, 2,953, 2,862, 2,828 (CH); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.21-6.63 (m, 9H, aromatic), 8.16 (s, 1H, H-10), 6.66 (bs, 1H, NH, canceled by D_2O), 5.72 (bs, 2H, NH_2, canceled by D_2O), 5.33 (s, 1H, H-7), 3.85 (s, 3H, OCH_3); ^{13} C NMR (125 MHz, DMSO-d_6) δ (ppm): 155.9 (C-8), 151.4 (C-11a), 150.7 (C-5), 137.5 (C-10), 131.3 (C-1b), 127.3 (C-1a), 126.2 (C-2), 125.1 (C-4a), 124.4 (C-3), 121.6 (C-4), 120.7 (C-1), 117.9 (C-6a), 103.5 (C-6), 97.8 (C-7a), 55.7 (CH_3), 40.0 (C-7), 143.6, 129.8, 128.4, 124.0 (aromatic); MS *m/z* (%): 406 (M^++2, 5.9), 404 (M^+, 17.52) with a base peak at 76; Anal. Calcd. for $C_{22}H_{17}ClN_4O_2$: C, 65.27; H, 4.23; N, 13.84. Found: C, 65.30; H, 4.30; N, 13.90 %.

*9-Benzylideneamino-7-(4-chlorophenyl)-5-methoxy-8-imino-7H-benzo[h]chromeno[2,3-d]pyrimidine (**24**)*

A mixture of imino compound **23** (0.01 mol) and benzaldehyde (0.01 mol) in ethanol (30 ml) and piperidine (0.5 ml) was refluxed for 2 h. The solvent was removed under reduced pressure and the resulting solid was recrystallized from benzene to give the open chain product **24** as colorless powder; m.p. 260–261 °C; yield 98 %; IR (cm^{-1}) in (KBr) υ: 3,214 (NH), 3,065, 3,010, 2,971, 2,860 (CH stretching), 1,629 (C=N); MS *m/z* (%): 494 (M^++2, 2.07), 492 (M^+, 5.29) with a base peak at 385 (100); Anal. Calcd. for $C_{29}H_{21}ClN_4O_2$: C, 70.66; H, 4.29; N, 11.37. Found: C, 70.70; H, 4.33; N, 11.42 %.

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

In brief, after the end of the incubation period, 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 and all the new synthesized compounds are pure samples with purity between 95 and 100 %. The cytotoxic effect of each tested compound was calculated.

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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