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ORIGINAL PAPER

Synthesis, in-vitro cytotoxicity of 4*H*-benzo[*h*]chromene derivatives and structure–activity relationships of 4-aryl group and 3-, 7-positions

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A series of 2-amino-4*H*-benzo[*h*]chromene and 2,7-diamino-4*H*-benzo[*h*]chromene derivatives were prepared as potential cytotoxic agents. The structures of the synthesised compounds were established on the basis of spectral data. The in-vitro cytotoxic activity of the synthesised compounds against the cell lines MCF-7, HCT-116 and HepG-2 was investigated in comparison with vinblastine and colchicine, using an MTT colorimetric assay. The structure–activity relationship of 4*H*-benzo[*h*]chromenes with modification at the 3-, 4- and 7-positions was explored. The results of the anti-tumour evaluation revealed that compounds *VIIIc*, *VIId*, *VIIb*, *VIIe*, *VIIIg* and *VIIIc*, *VIId*, *VIIb*, *VIIe*, *VIIIg*, *VIIc*, *VIIIe*, *Vf*, *IIIa* inhibited the growth of MCF-7 in comparison with vinblastine and colchicine, while *VIIb*, *VIId*, *VIIe*, *IIIa*, *VIIa*, *VIIIc*, *VIIc*, *IIId*, *IIIg*, *IIIf*, *IIIb*, *IIIh*, *VIIIb*, *VIIIa*, *VIIIe*, *IIIc*, *Vg*, *IIIe*, *VIIIg*, *Vf*, *IIIf* inhibited the growth of HCT-116 in comparison with colchicine. In addition, compounds *VIIe*, *IIIg*, *IIIa*, *VIIc* and *VIIe*, *IIIg*, *IIIa*, *VIIc*, *VIIb*, *VIIa*, *VIIIf*, *VIIIe* inhibited the growth of HepG-2 in comparison with vinblastine and colchicine, respectively.

Keywords: 1-naphthol, 5-amino-1-naphthol, 4*H*-benzo[*h*]chromenes, cytotoxicity, SAR

Introduction

Chromene-based compounds have been reported as possessing many pharmacological activities and antimicrobial properties (Alvey et al., 2009; Kumar et al., 2009; Raj et al., 2009; Kidwai et al., 2010; Li et al., 2010; Liu et al., 2010); however, recent reports have demonstrated the potential of 4-aryl-4*H*-chromenes as apoptosis-inducers (Kemnitzer et al., 2007, 2008; Mahmoodi et al., 2010). These compounds were found to be tubulin destabilisers, binding at or close to the binding site of colchicine. They were also active in drug-resistant cancer cell-lines including the vascular-disrupting, paclitaxel-resistant, multi-drug resistant tumour cells, and were found to be highly active in several anticancer animal models (Gourdeau et al., 2004; Kasibhatla et al., 2004; Endo et al., 2010). On the other hand, a diverse group of 4*H*-chromene compounds with a substituted or oligo-substituted phenyl ring at the 4-position and other groups at the 7-position have been reported as cytotoxic and anticancer agents (Rampa et al., 2005; Sabry et al., 2011; El-Agrody et al., 2014a, 2014b). Fused chromene ring systems have blood

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Table 1. Characterisation data of compounds *IIIa–IIIh*

Compound	Formula	M_r	Colour	Yield/%	M.p./°C
IIIa	$C_{20}H_{14}N_2O$	298.34	Colourless	85	217, 218[a]
IIIb	$C_{20}H_{13}FN_2O$	316.33	Yellow	86	233, 232[b]
IIIc	$C_{20}H_{13}ClN_2O$	332.78	Yellow	88	234, 231[b]
IIId	$C_{20}H_{13}BrN_2O$	377.23	Colourless	90	240
IIIe	$C_{21}H_{16}N_2O$	312.36	Yellow	90	206, 204[b]
IIIf	$C_{21}H_{16}N_2O_2$	328.36	Yellow	88	196, 195[a]
IIIg	$C_{20}H_{13}N_3O_3$	343.34	Yellow	88	241, 240[a]
IIIh	$C_{24}H_{21}N_3O_2$	383.44	Yellow	82	222

a) According to Zhang et al. (2007); *b*) according to Gong et al. (2008).

platelet anti-aggregating effects (Lee et al., 2006), exhibit analgesic activities (Ali & Ibrahim, 2010; Keri et al., 2010), hypolipidemic activity (Sashidhara et al., 2011), DNA-breaking activities and mutagenicity (Hiramoto et al., 1997), and are applicable in the treatment of Alzheimer's disease (Brühlmann et al., 2010) and Schizophrenia disorder (Kesten et al., 1997). Accordingly, it was decided to synthesise some oligo-substituted 4*H*-benzo[*h*]chromenes as potential cytotoxic agents. Hence, below is described the synthesis of some 4*H*-benzo[*h*]chromene derivatives and their in-vitro cytotoxicity against a variety of human cancer cell lines. The chemical structures of the compounds studied and their structure-activity relationships (SAR) at the 3-, 4- and 7-positions are discussed in this work.

Experimental

Commercial-grade solvents and reagents were purchased from Sigma–Aldrich (St. Louis, MO, USA) and used without further purification. Melting points were measured with a Stuart Scientific (UK) apparatus, and are uncorrected. IR spectra were determined as KBr pellets on a Jasco FT/IR 460 plus spectrophotometer (Jasco, Japan). ^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker AV 500 MHz spectrometer (Bruker, USA). ^{13}C NMR spectra were obtained using distortion-free enhancement by polarisation transfer (DEPT), where the signals of the CH and CH_3 carbon atoms appear normal (up) and the signals of the carbon atoms in CH_2 environments appear negative (down). ^{13}C NMR spectra were obtained using the attached proton test (APT); with this technique, the signals of the CH and CH_3 carbon atoms appears normal (up) and the signal of the CH_2 and Cq environments appears negative (down). Chemical shifts (δ) are expressed in parts per million (ppm), and the coupling constants (J) are reported in Hz. The MS were measured using a Shimadzu GC/MS-QP5050A spectrometer (Shimadzu, Japan).

General procedure for preparation of 2-amino-4-aryl-4H-benzo[h]chromene-3-carbonitrile (IIIa–IIIh) and 4-aryl-2,7-diamino-4H-benzo[h]chromene-3-carbonitrile (Va–Vh) derivatives

A solution of 1-naphthol (*I*) or 5-amino-1-naphthol (*IV*) (0.01 mol) in EtOH (30 mL) and piperidine (0.5 mL) was treated with α-cyano-*p*-monosubstituted cinnamonitriles (*IIa–IIh*) (0.01 mol). The reaction mixture was heated under reflux for 1 h. The solid product thus formed was collected by filtration, washed with MeOH, re-crystallised from ethanol or benzene; the colours and yield are reported after crystallisation. The composition, properties and spectral data of the corresponding products *III* and *V* are given in Tables 1–4.

General procedure for preparation of ethyl 2-amino-4-aryl-4H-benzo[h]chromene-3-carboxylate (VIIa–VIIg)and ethyl 4-aryl-2,7-diamino-4H-benzo[h]chromene-3-carboxylate (VIIIa–VIIIg)derivatives

A solution of 1-naphthol (*I*) or 5-amino-1-naphthol (*IV*) (0.01 mol) in EtOH (30 mL) and piperidine (0.5 mL) was treated with ethyl α-cyano-*p*-monosubstitutedcinnamates (*VIa–VIg*) (0.01 mol). The reaction mixture was heated under reflux for 2 h. The solid product thus formed was collected by filtration, washed with MeOH and re-crystallised from ethanol or benzene; the colours and yield are reported after crystallisation. The composition, properties and spectral data of the corresponding products *VII* and *VIII* are given in Tables 5–8.

Anti-tumour screening

Cell culture and cytotoxicity evaluation using viability assay: the target compounds were initially evaluated for in-vitro anti-tumour activity against three different human cell lines: MCF-7, HCT-116 and HepG-2 (National Cancer Institute, Cairo, Egypt) in comparison with vinblastine and colchicine. The measurements of cell growth, the viabilities and in-vitro cytotoxicity evaluation using the viability assay were determined as described in the literature (Mossman, 1983; Rahman et al., 2001) and the results are listed

Table 2. Spectral data of compounds *IIIa–IIIh*

Compound	Spectral data
IIIa	IR, $\tilde{\nu}$/cm^{-1}: 3447, 3304, 3189 (NH_2), 3055, 3021, 2885 (CH), 2204 (CN) ^{1}H NMR (DMSO-d_6), δ: 8.28–7.11 (m, 11H, 3Ph), 7.20 (bs, 2H, NH_2, D_2O exchangeable), 4.91 (s, 1H, pyran ring) ^{13}C NMR (DMSO-d_6), δ: 160.15 (C), 145.67 (C), 142.72 (C), 132.67 (C), 128.67 (CH), 128.29 (CH), 127.64 (CH), 126.89 (CH), 126.73 (CH), 126.64 (CH), 126.20 (CH), 123.87 (CH), 122.74 (C), 120.68 (CH), 120.49 (C), 117.91 (CN), 56.26 (C), 40.91 (CH)
IIIb	IR, $\tilde{\nu}$/cm^{-1}: 3459, 3330, 3196 (NH_2), 3091, 3034, 2989, 2861 (CH), 2193 (CN) ^{1}H NMR (DMSO-d_6), δ: 8.27–7.10 (m, 10H, 3Ph), 7.20 (bs, 2H, NH_2, D_2O exchangeable), 4.96 (s, 1H,pyran ring) ^{13}C NMR (DMSO-d_6), δ: 162.06 (C), 160.12 (C), 142.70 (C), 141.90 (C), 132.69 (C), 129.58 (CH), 129.52 (CH), 128.28 (CH), 127.65 (CH), 126.79 (CH), 126.10 (CH), 123.94 (CH), 122.73 (C), 120.69 (C), 120.38 (CH), 117.69 (CN), 115.49 (CH), 115.32 (CH), 56.17 (C), 40.00 (CH)
IIIc	IR, $\tilde{\nu}$/cm^{-1}: 3453, 3334, 3197 (NH_2), 3059, 3042, 2869 (CH), 2192 (CN) ^{1}H NMR (DMSO-d_6), δ: 8.29–7.10 (m, 10H, 3Ph), 7.22 (bs, 2H, NH_2, D_2O exchangeable), 4.96 (s, 1H, pyran ring) ^{13}C NMR (DMSO-d_6), δ: 160.14 (C), 144.63 (C), 142.73 (C), 132.73 (C), 131.52 (C), 129.54 (CH), 128.67 (CH), 127.67 (CH), 126.85 (CH), 126.71 (CH), 126.04 (CH), 123.99 (CH), 122.71 (C), 120.69 (C), 120.32 (CH), 117.38 (CN), 55.85 (C), 40.16 (CH)
IIId	IR, $\tilde{\nu}$/cm^{-1}: 3458, 3342, 3202 (NH_2), 3060, 3031, 2865 (CH), 2190 (CN) ^{1}H NMR (DMSO-d_6), δ: 8.27–7.09 (m, 10H, 3Ph), 7.24 (bs, 2H, NH_2, D_2O exchangeable), 4.95 (s, 1H, pyran ring) ^{13}C NMR (DMSO-d_6), δ: 160.14 (C), 145.04 (C), 142.75 (C), 132.74 (C), 131.58 (CH), 129.92 (CH), 128.28 (CH), 127.66 (CH), 126.84 (CH), 126.70 (C), 126.03 (CH), 123.99 (CH), 122.72 (C), 120.70 (C), 120.33 (CH), 120.06 (C), 117.29 (CN), 55.80 (C), 40.27 (CH) MS, m/z (I_r/%): 378 (20.02) (M^++ 2), 376 (20.35) (M^+) with a base peak at 221 (100)
IIIe	IR, $\tilde{\nu}$/cm^{-1}: 3412, 3323, 3203 (NH_2), 3057, 3031, 2957, 2837 (CH), 2194 (CN) ^{1}H NMR (DMSO-d_6), δ: 8.27–6.87 (m, 10H, 3Ph), 7.14 (bs, 2H, NH_2, D_2O exchangeable), 4.85 (s, 1H, pyran ring), 2.28 (s, 3H, CH_3) ^{13}C NMR (DMSO-d_6), δ: 159.97 (C), 158.15 (C), 142.57 (C), 137.81(C), 132.61 (C), 128.71 (CH), 128.29 (CH), 127.63 (CH), 126.67 (CH), 126.60 (CH), 126.25 (CH), 123.77 (CH), 122.74 (C), 120.67 (C), 120.53 (CH), 118.19 (CN), 55.00 (C), 40.09 (CH), 22.44 (CH_3)
IIIf	IR, $\tilde{\nu}$/cm^{-1}: 3452, 3339, 3211 (NH_2), 3057, 2932, 2836 (CH), 2190 (CN) ^{1}H NMR (DMSO-d_6), δ: 8.26–6.87 (m, 10H, 3Ph), 7.12 (bs, 2H, NH_2, D_2O exchangeable), 4.85 (s, 1H, pyran ring), 3.72 (s, 3H, OCH_3) ^{13}C NMR (DMSO-d_6), δ: 159.96 (C), 158.15 (C), 142.57 (C), 137.81(C), 132.61 (C), 128.70 (CH), 128.29 (CH), 127.63 (CH), 126.68 (CH), 126.60 (CH), 123.78 (CH), 122.73 (C), 120.66 (C), 120.52 (CH), 118.20 (CN), 114.02 (CH), 56.55 (CH_3), 55.01 (C), 40.07 (CH) MS, m/z (I_r/%): 362 (4.31) (M^+) with a base peak at 75 (100)
IIIg	IR, $\tilde{\nu}$/cm^{-1}: 3460, 3359, 3210 (NH_2), 3070, 2953, 2864 (CH), 2187 (CN) ^{1}H NMR (DMSO-d_6), δ: 8.21–7.12 (m, 10H, 3Ph), 7.37 (bs, 2H, NH_2, D_2O exchangeable), 5.18 (s, 1H, pyran ring) ^{13}C NMR (DMSO-d_6), δ: 160.32 (C), 152.93 (C), 146.47 (C), 142.93 (C), 132.88 (C), 128.99 (CH), 127.69 (CH), 127.00 (CH), 126.80 (CH), 125.88 (CH), 124.18 (CH), 124.03 (CH), 122.73 (C), 120.73 (C), 120.14 (CH), 116.57 (CN), 55.21 (C), 40.68 (CH)
IIIh	IR, $\tilde{\nu}$/cm^{-1}: 3401, 3329, 3206 (NH_2), 3055, 3016, 2968, 2938 (CH), 2192 (CN) ^{1}H NMR (DMSO-d_6), δ: 8.25–6.87 (m, 10H, 3Ph), 7.10 (bs, 2H, NH_2), 4.79 (s, 1H, pyran ring), 3.71–3.69 (m, 4H, $2CH_2$), 3.06–3.04 (m, 4H, $2CH_2$) ^{13}C NMR (DMSO-d_6), δ: 159.87 (C), 149.84 (C), 142.46 (C), 136.34 (C), 132.50 (C), 128.22 (CH), 128.11 (CH), 127.55 (CH), 126.56 (CH), 126.50 (CH), 123.65 (CH), 122.65 (C), 120.56 (CH), 120.51 (C), 118.27 (CN), 115.16 (CH), 65.99 (CH_2), 56.53 (C), 48.32 (CH_2), 39.90 (CH) ^{13}C NMR-DEPT at 135° CH, CH_3 (up), CH_2 (down), δ: 128.22 (CH ↑), 128.11 (CH ↑), 127.55 (CH ↑), 126.56 (CH ↑), 126.50 (CH), 123.65 (CH ↑), 120.56 (CH ↑), 115.16 (CH ↑) 65.99 (CH_2 ↓), 48.32 (CH_2 ↓), 39.90 (CH ↑) ^{13}C NMR-DEPT at 90° only CH signals are (up), δ: 128.22 (CH ↑), 128.11 (CH ↑), 127.55 (CH ↑), 126.56 (CH ↑), 126.50 (CH), 123.65 (CH ↑), 120.56 (CH ↑), 115.16 (CH ↑), 39.90 (CH ↑) ^{13}C NMR-DEPT at 45° CH, CH_2 and CH_3 signals are (up), δ: 128.22 (CH ↑), 128.11 (CH ↑), 127.55 (CH ↑), 126.56 (CH ↑), 126.50 (CH), 123.65 (CH ↑), 120.56 (CH ↑), 115.16 (CH ↑), 65.99 (CH_2 ↑), 48.32 (CH_2 ↑), 39.90 (CH ↑) ^{13}CNMR-APT CH, CH_3 (up), CH_2, Cq (down), δ: 159.87 (C ↓), 149.84 (C ↓), 142.46 (C ↓), 136.34 (C ↓), 132.50 (C ↓), 128.22 (CH ↑), 128.11 (CH ↑), 127.55 (CH ↑), 126.56 (CH ↑), 126.50 (CH ↑), 123.65 (CH ↑), 122.65 (C ↓), 121.56 (C ↓), 120.51 (CH ↑), 118.27 (CN ↓), 115.16 (CH ↑), 65.99 (CH_2 ↓), 56.53 (C ↓), 48.32 (CH_2 ↓), 39.90 (CH ↑) MS, m/z (I_r/%): 383 (49.34) (M^+) with a base peak at 221 (100)

in Table 9 and Fig. 5.

Results and discussion

The treatment of 1-naphthol (*I*) with α-cyano-*p*-monosubstituted cinnamonitriles (*IIa–IIh*) in ethanolic piperidine under reflux gave the corresponding 2-amino-4-aryl-4*H*-benzo[*h*]-chromene-3-carbonitrile (*IIIa–IIIh*) derivatives shown in Fig. 1. The composition, properties and spectral data of the corresponding

Table 3. Characterisation data of prepared compounds *Va–Vh*

Compound	Formula	M_r	Colour	Yield/%	M.p./°C
Va	$C_{20}H_{15}N_3O$	313.35	Grey	85	267, 267[a]
Vb	$C_{20}H_{14}FN_3O$	331.34	Grey	87	235
Vc	$C_{20}H_{14}ClN_3O$	347.8	Grey	81	266, 267[a]
Vd	$C_{20}H_{14}BrN_3O$	392.25	Grey	80	226
Ve	$C_{21}H_{17}N_3O$	327.38	Grey	83	230
Vf	$C_{21}H_{17}N_3O_2$	343.38	Grey	88	269, 267[a]
Vg	$C_{20}H_{14}N_4O_3$	358.35	Red	79	239
Vh	$C_{24}H_{22}N_4O_2$	398.46	Red	78	235

a) According to Abd-El-Aziz et al. (2004).

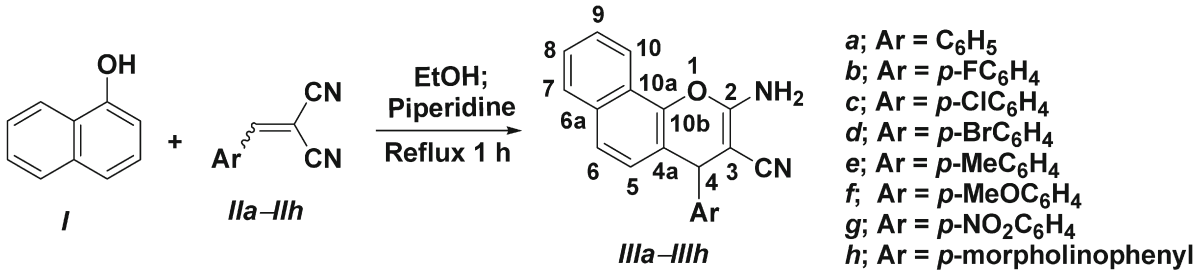


Fig. 1. Synthesis of 2-amino-4*H*-benzo[*h*]chromene derivatives (*IIIa–IIIh*).

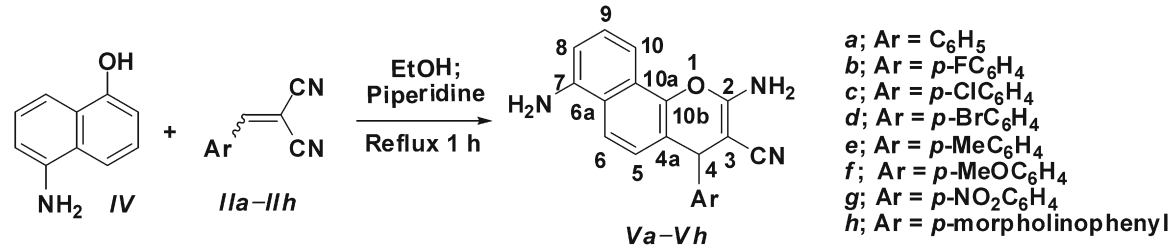


Fig. 2. Synthesis of 2,7-diamino-4*H*-benzo[*h*]chromene derivatives (*Va–Vh*).

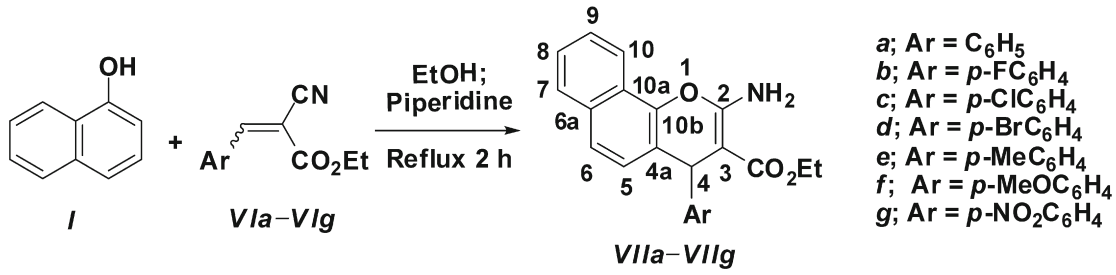


Fig. 3. Synthesis of ethyl 2-amino-4*H*-benzo[*h*]chromene-3-carboxylate derivatives (*VIIa–VIIg*).

products *III* are summarised in Tables 1 and 2.

In a similar manner, the treatment of 5-amino-1-naphthol (*IV*) with α-cyano-*p*-mono-substituted cinnamonitriles (*IIa–IIh*) in ethanolic piperidine under reflux gave the corresponding 4-aryl-2,7-diamino-4*H*-benzo[*h*]chromene-3-carbonitrile (*Va–Vh*) derivatives shown in Fig. 2. The composition, properties and spectral data of the corresponding products *V* are summarised in Tables 3 and 4.

Structures *III* and *V* were established on the basis of IR, ^{1}H NMR, ^{13}C NMR, ^{13}C NMR-DEPT, ^{13}C NMR-APT and MS data. The IR spectra of *IIIa–IIIh* and *Va–Vh* showed the appearance of an NH_2 stretch at 3460–3401 cm^{-1}, 3359–3304 cm^{-1}, 3211–3189 cm^{-1} a CN stretch at 2204–2187 cm^{-1} for *IIIa–IIIh* and an NH_2 stretch at 3469–3422 cm^{-1}, 3399–3336 cm^{-1}, 3332–3309 cm^{-1}, 3297–3257 cm^{-1}, 3209–3167 cm^{-1} a CN stretch at 2200–2184 cm^{-1} for *Va–Vh*. The ^{1}H and ^{13} C NMR spectra of *IIIa–IIIh* and *Va–Vh* revealed the presence of 4*H* sig-

nals at δ of 5.18–4.73 (s, 1H, H-4) and 40.91–39.90 (C-4). The ^{13}C NMR-DEPT spectra at 45°, 90°, 135°, ^{13}C NMR-APT spectra and the mass spectra of compounds *III* and *V* provided additional evidence in support of the proposed structures.

The interaction of 1-naphthol (*I*) with ethyl α-cyano-*p*-monosubstitutedcinnamates (*VIa–VIg*) afforded ethyl 2-amino-4-aryl-4*H*-benzo[*h*]chromene-3-carboxylate (*VIIa–VIIg*) derivatives (Fig. 3).The composition, properties and spectral data of the corresponding products *VII* are summarised in Tables 5 and 6.

In a similar manner, the reaction of 5-amino-1-naphthol (*IV*) with ethyl α-cyano-*p*-monosubstituted cinnamates (*VIa–VIg*) afforded the ethyl 4-aryl-2,7-

Table 4. Spectral data of compounds *Va–Vh*

Compound	Spectral data
Va	IR, $\tilde{\nu}$/cm^{-1}: 3424, 3336, 3325, 3297, 3175 (2 NH_2), 3023, 3001 (CH), 2200 (CN) ^{1}H NMR (DMSO-d_6), δ: 7.75–6.70 (m, 10H, 3Ph), 7.10 (bs, 2H, NH_2-2, D_2O exchangeable), 5.78 (bs, 2H, NH_2-7, D_2O exchangeable), 4.84 (s, 1H, pyran ring) ^{13}C NMR (DMSO-d_6), δ: 160.32 (C), 145.85 (C), 144.88 (C), 142.86 (C), 128.62 (CH), 128.29 (CH), 127.56 (CH), 127.44 (C), 124.01 (CH), 123.34 (CH), 122.01 (C), 120.63 (C), 118.62 (CH), 117.42 (CN), 108.36 (CH), 107.96 (CH), 56.09 (C), 40.88 (CH)
Vb	IR, $\tilde{\nu}$/cm^{-1}: 3450, 3386, 3332, 3257, 3203 (2 NH_2), 3028, 3006 (CH), 2195 (CN) ^{1}H NMR (DMSO-d_6), δ: 7.76–6.71 (m, 9H, 3Ph), 7.13 (bs, 2H, NH_2-2, D_2O exchangeable), 5.80 (bs, 2H, NH_2-7, D_2O exchangeable), 4.89 (s, 1H, pyran ring) ^{13}C NMR (DMSO-d_6), δ: 161.93 (C), 160.19 (C), 144.82 (C), 142.75 (C), 141.98 (C), 129.41 (CH), 128.21 (CH), 127.41 (C), 123.94 (CH), 123.16 (C), 120.47 (C), 118.63 (CH), 117.13 (CN), 115.36 (CH), 108.34 (CH), 107.89 (CH), 55.93 (C), 39.98 (CH) MS, m/z (I_r/%): 331 (23.84) (M^+) with a base peak at 75 (100)
Vc	IR, $\tilde{\nu}$/cm^{-1}: 3463, 3341, 3311, 3287, 3197 (2 NH_2), 3021, 3016 (CH), 2188 (CN) ^{1}H NMR (DMSO-d_6), δ: 7.76–6.74 (m, 9H, 3Ph), 7.15 (bs, 2H, NH_2-2, D_2O exchangeable), 5.75 (bs, 2H, NH_2-7, D_2O exchangeable), 4.90 (s, 1H, pyran ring) ^{13}C NMR (DMSO-d_6), δ: 160.23 (C), 144.83 (C), 144.74 (C), 142.79 (C), 131.32 (C), 129.38 (CH), 128.52 (CH), 128.21 (CH), 127.44 (C), 123.92 (CH), 123.09 (C), 120.40 (C), 118.68 (CH), 116.79 (CN), 108.35 (CH), 107.85 (CH), 55.61 (C), 40.08 (CH)
Vd	IR, $\tilde{\nu}$/cm^{-1}: 3469, 3364, 3309, 3293, 3167 (2 NH_2), 3011, 3019 (CH), 2186 (CN) ^{1}H NMR (DMSO-d_6), δ: 7.78–6.74 (m, 9H, 3Ph), 7.17 (bs, 2H, NH_2-2, D_2O exchangeable), 5.81 (bs, 2H, NH_2-7, D_2O exchangeable), 4.89 (s, 1H, pyran ring) ^{13}C NMR (DMSO-d_6), δ: 160.23 (C), 145.15 (C), 144.82 (C), 142.81 (C), 131.54 (CH), 129.77 (CH), 128.21 (CH), 127.45 (C), 123.93 (CH), 123.11 (C), 120.42 (C), 119.87 (C)118.69 (CH), 116.72 (CN), 108.40 (CH), 107.90 (CH), 55.58 (C), 40.19 (CH) ^{13}C NMR-DEPT at 135° CH, CH_3 (up), CH_2 (down), δ: 131.54 (CH ↑), 129.77, (CH ↑), 128.21 (CH ↑), 123.93 (CH ↑), 118.69 (CH ↑), 108.40 (CH ↑), 107.90 (CH ↑), 40.19 (CH ↑) ^{13}C NMR-DEPT at 90° only CH signals are (up), δ: 131.54 (CH↑), 129.77, (CH ↑), 128.21 (CH ↑), 123.93 (CH ↑), 118.69 (CH ↑), 108.40 (CH ↑), 107.90 (CH ↑), 40.19 (CH ↑) ^{13}C NMR-DEPT at 45° CH, CH_2 and CH_3 signals are (up), δ: 131.54 (CH ↑), 129.77, (CH ↑), 128.21 (CH ↑), 123.93 (CH ↑), 118.69 (CH ↑), 108.40 (CH ↑), 107.90 (CH ↑), 40.19 (CH ↑) ^{13}CNMR-APT spectrum CH, CH_3 (up), CH_2, Cq (down), δ: 160.23 (C ↓), 145.15 (C↓), 144.82 (C ↓), 142.81 (C ↓), 131.54 (CH ↑), 129.77 (CH ↑), 128.21 (CH ↑), 127.45 (C↓), 123.93 (CH ↑), 123.11 (C↓), 120.42 (C↓), 119.87 (C ↓), 118.69 (CH ↑), 116.72 (CN ↓), 108.40 (CH ↑), 107.90 (CH ↑), 55.58 (C ↓), 40.19 (CH ↑) MS, m/z (I_r/%): 393 (17.18) (M^+ + 2), 391 (17.34) (M^+) with a base peak at 237 (100)
Ve	IR, $\tilde{\nu}$/cm^{-1}: 3462, 3399, 3324, 3273, 3189 (2 NH_2), 3051, 3032 (CH), 2184 (CN) ^{1}H NMR (DMSO-d_6), δ: 7.74–6.70 (m, 9H, 3Ph), 7.06 (bs, 2H, NH_2-2, D_2O exchangeable), 5.78 (bs, 2H, NH_2-7, D_2O exchangeable), 4.79 (s, 1H, pyran ring), 2.25 (s, 3H, CH_3) ^{13}C NMR (DMSO-d_6), δ: 160.12 (C), 144.79 (C), 142.85 (C), 142.71 (C), 135.81 (C), 129.07 (CH), 127.40 (CH), 127.33 (CH), 123.92 (C), 123.30 (CH), 121.90 (C), 120.57 (C), 118.46 (CH), 117.45 (CN), 108.25 (CH), 107.88 (CH), 56.15 (C), 40.41 (CH), 20.49 (CH_3) ^{13}C NMR-DEPT at 135° CH, CH_3 (up), CH_2 (down), δ: 129.07 (CH ↑), 127.40 (CH ↑), 127.33 (CH ↑), 123.30 (CH ↑), 118.46 (CH ↑), 108.25 (CH ↑), 107.88 (CH ↑), 40.41 (CH ↑), 20.49 (CH_3 ↑) ^{13}C NMR-DEPT spectrum at 90° only CH signals are (up), δ: 129.07 (CH ↑), 127.40 (CH ↑), 127.33 (CH ↑), 123.30 (CH ↑), 118.46 (CH ↑), 108.25 (CH ↑), 107.88 (CH ↑), 40.41 (CH ↑) ^{13}C NMR-DEPT spectrum at 45° CH, CH_2 and CH_3 signals are (up), δ: 129.07 (CH ↑), 127.40 (CH ↑), 127.33 (CH ↑), 123.30 (CH ↑), 118.46 (CH ↑), 108.25 (CH ↑), 107.88 (CH ↑), 40.41 (CH ↑) ^{13}CNMR-APT spectrum CH, CH_3 (up), CH_2, Cq (down), δ: 160.12 (C), 144.79 (C), 142.85 (C ↓), 142.71 (C ↓), 135.81 (C ↓), 129.07 (CH ↑), 127.40 (CH ↑), 127.33 (CH ↑), 123.92 (C ↓), 123.30 (CH ↑), 121.90 (C ↓), 120.57 (C ↓), 118.46 (CH ↑), 117.45 (CN ↓), 108.25 (CH ↑), 107.88 (CH ↑), 56.15 (C ↓), 40.41 (CH ↑), 20.49 (CH_3 ↑) MS, m/z (I_r/%): 327 (28.55) (M^+) with a base peak at 237 (100)

Table 4. (continued)

Compound	Spectral data
Vf	IR, $\tilde{\nu}$/cm^{-1}: 3422, 3382, 3330, 3273, 3209 (2 NH_2), 3054, 3031 (CH), 2186 (CN) ^{1}H NMR (DMSO-d_6), δ: 7.74–6.70 (m, 9H, 3Ph), 7.02 (bs, 2H, NH_2-2, D_2O exchangeable), 5.76 (bs, 2H, NH_2-7, D_2O exchangeable), 4.78 (s, 1H, pyran ring), 3.71 (s, 3H, CH_3) ^{13}C NMR (DMSO-d_6), δ: 160.13 (C), 158.08 (C), 144.87 (C), 142.73 (C), 137.99 (C), 128.62 (CH), 127.39 (CH), 124.01 (C), 123.40 (CH), 121.95 (C), 120.67 (C), 118.53 (CH), 117.69 (CN), 113.97 (CH), 108.31 (CH), 107.95 (CH), 56.41 (C), 55.01 (CH_3), 40.06 (CH) ^{13}C NMR-DEPT at 135° CH, CH_3 (up), CH_2 (down), δ: 128.62 (CH ↑), 127.39 (CH↑), 123.40 (CH ↑), 118.53 (CH ↑), 113.97 (CH ↑), 108.31 (CH ↑), 107.95 (CH ↑), 55.01 (CH_3 ↑), 40.06 (CH ↑) ^{13}C NMR-DEPT at 90° only CH signals are (up), δ: 128.62 (CH ↑), 127.39 (CH↑), 123.40 (CH ↑), 118.53 (CH ↑), 113.97 (CH ↑), 108.31 (CH ↑), 107.95 (CH ↑), 40.06 (CH ↑) ^{13}C NMR-DEPT at 45° CH, CH_2 and CH_3 signals are (up), δ: 128.62 (CH ↑), 127.39 (CH ↑), 123.40 (CH ↑), 118.53 (CH ↑), 113.97 (CH ↑), 108.31 (CH ↑), 107.95 (CH ↑), 55.01 (CH_3 ↑), 40.06 (CH ↑) ^{13}CNMR-APT spectrum CH, CH_3 (up), CH_2, Cq (down), δ: 160.13 (C ↓), 158.08 (C ↓), 144.87 (C↓), 142.73 (C ↓), 137.99 (C ↓), 128.62 (CH ↑), 127.39 (CH ↑), 124.01 (C↓), 123.40 (CH ↑), 121.95 (C↓), 120.67 (C↓), 118.53 (CH ↑), 117.69 (CN ↓), 113.97 (CH↑), 108.31 (CH ↑), 107.95 (CH ↑), 56.41 (C ↓), 55.01 (CH_3 ↑), 40.06 (CH ↑)
Vg	IR, $\tilde{\nu}$/cm^{-1}: 3460, 3360, 3310, 3273, 3198 (2 NH_2), 3074, 3021 (CH), 2188 (CN) ^{1}H NMR (DMSO-d_6), δ: 8.22–6.74 (m, 9H, 3Ph), 7.27 (bs, 2H, NH_2-2, D_2O exchangeable), 5.82 (bs, 2H, NH_2-7, D_2O exchangeable), 5.11 (s, 1H, pyran ring) ^{13}C NMR (DMSO-d_6), δ: 160.49 (C), 153.16 (C), 146.42 (C), 144.95 (C), 143.05 (C), 129.90 (CH), 128.80 (CH), 127.64 (C), 124.02 (CH), 123.02 (CH), 122.23 (C), 120.29 (C), 118.97 (CH), 116.09 (CN), 108.60 (CH), 107.98 (CH), 55.05 (C), 40.07 (CH) MS, m/z (I_r/%): 358 (3.19) (M^+) with a base peak at 55 (100)
Vh	IR, $\tilde{\nu}$/cm^{-1}: 3464, 3361, 3315, 3273, 3201 (2 NH_2), 3074, 3021, 2877, 2861, 2833 (CH), 2188 (CN) ^{1}H NMR (DMSO-d_6), δ: 7.73–6.70 (m, 9H, 3Ph), 7.02 (bs, 2H, NH_2-7, D_2O exchangeable), 5.77 (bs, 2H, NH_2-2, D_2O exchangeable), 4.73 (s, 1H, pyran ring), 3.71–3.69 (m, 4H, $2CH_2$), 3.05–3.04 (m, 4H, $2CH_2$) ^{13}C NMR (DMSO-d_6), δ: 160.03 (C), 149.78 (C), 144.77 (C), 142.62 (C7), 136.55 (C), 128.21 (CH), 128.03 (CH), 127.23 (C), 123.47 (CH), 121.83 (C), 120.67 (C), 118.38 (CH), 117.75 (CN), 115.13 (CH), 108.18 (CH), 107.86 (CH), 65.98 (CH_2), 56.37 (C), 48.35 (CH_2), 39.97 (CH) MS, m/z (I_r/%): 398 (96.32) (M^+) with a base peak at 236 (100)

Table 5. Characterisation data of compounds *VII*

Compound	Formula	M_r	Colour	Yield/%	M.p./℃
VIIa	$C_{22}H_{19}NO_3$	345.39	Yellow	78	150
VIIb	$C_{22}H_{18}FNO_3$	363.38	Yellow	81	155
VIIc	$C_{22}H_{18}ClNO_3$	379.84	Yellow	83	160
VIId	$C_{22}H_{18}BrNO_3$	424.29	Yellow	80	162
VIIe	$C_{23}H_{21}NO_3$	359.42	Yellow	79	152
VIIf	$C_{23}H_{21}NO_4$	375.42	Yellow	80	161
VIIg	$C_{22}H_{18}N_2O_5$	390.39	Yellow	77	166

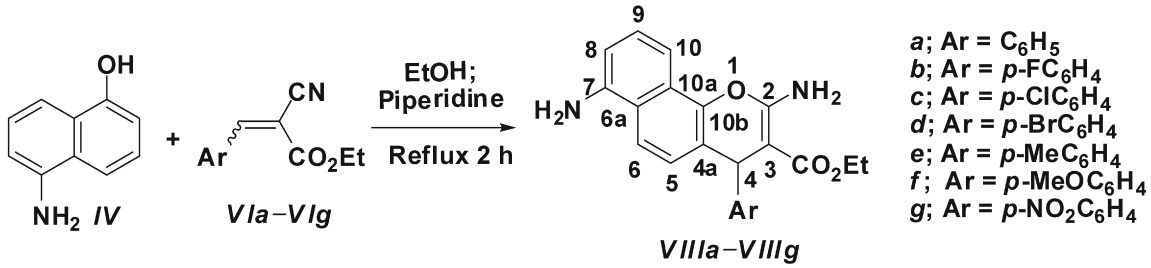


Fig. 4. Synthesis of ethyl 2,7-diamino-4*H*-benzo[*h*]chromene-3-carboxylate derivatives (*VIIIa*–*VIIIg*).

diamino-4*H*-benzo[*h*]chromene-3-carboxylate (*VIIIa*–*VIIIg*) derivatives shown in Fig. 4. The composition, properties and spectral data of the corresponding products *VIII* are summarised in Tables 7 and 8.

Structures *VII* and *VIII* were established on the basis of IR, ^{1}H NMR, ^{13}C NMR, ^{13}C NMR-DEPT, ^{13}C NMR-APT and MS data. The IR spectra of *VIIa*–*VIIg* and *VIIIa*–*VIIIg* showed the appearance of an

NH_2 stretch at 3469–3380 cm^{-1}, 3319–3270 cm^{-1} a CO stretch at 1687–1664 cm^{-1} for *VIIa–VIIg* and NH_2 stretch at 3470–3417 cm^{-1}, 3390–3362 cm^{-1}, 3358–3324 cm^{-1}, 3291–3250 cm^{-1} and a CO stretch at 1679–1667 cm^{-1} for *VIIIa–VIIIg*. The ^{1}H and ^{13}C NMR spectra of *VIIa–VIIg* and *VIIIa–VIIIg* revealed the presence of 4*H* signals at δ of 5.19–4.88 (s, 1H, H-4) and 40.15–39.12 (C-4). In compounds *VIIa–VIIg* and *VIIIa–VIIIg* the ester group gave ^{1}H signals at δ of 4.05–3.98 (q, 2H, CH_2, J = 7.0–7.2 Hz), 1.12–

Table 6. Spectral data of compounds *VII*

Compound	Spectral data
VIIa	IR, $\tilde{\nu}$/cm^{-1}: 3385, 3270 (NH_2), 3067, 3029, 2982, 2900, 2876 (CH), 1664 (CO) ^{1}H NMR (DMSO-d_6), δ: 8.31–7.10 (m, 11H, 3Ph), 7.79 (bs, 2H, NH_2, D_2O exchangeable), 5.03 (s, 1H, pyran ring), 4.00 (q, 2H, J = 7.1 Hz, CH_2), 1.11 (t, 3H, J = 7.1 Hz, CH_3) ^{13}C NMR (DMSO-d_6), δ: 168.14 (CO), 160.70 (C), 147.73 (C), 142.74 (C), 132.41 (C), 128.11 (CH), 127.55 (CH), 127.23 (CH), 126.50 (CH), 126.39 (CH), 126.36 (CH), 125.92 (CH), 123.62 (CH), 122.73 (C), 120.87 (C), 120.60 (CH), 76.31 (C), 58.52 (CH_2), 39.99 (CH), 14.18 (CH_3) ^{13}C NMR-DEPT at 135° CH, CH_3 (up), CH_2 (down), δ: 128.11 (CH ↑), 127.55 (CH ↑), 127.23 (CH ↑), 126.50 (CH ↑), 126.39 (CH ↑), 126.36 (CH ↑), 125.92 (CH ↑), 123.62 (CH ↑), 120.60 (CH ↑), 58.52 (CH_2 ↓), 39.99 (CH ↑), 14.18 (CH_3 ↑) ^{13}C NMR-DEPT at 90° only CH signals are (up), δ: 128.11 (CH ↑), 127.55 (CH ↑), 127.23 (CH ↑), 126.50 (CH ↑), 126.39 (CH ↑), 126.36 (CH ↑), 125.92 (CH ↑), 123.62 (CH ↑), 120.60 (CH ↑), 39.99 (CH ↑) ^{13}C NMR-DEPT at 45° CH, CH_2 and CH_3 signals are (up), δ: 128.11 (CH ↑), 127.55 (CH ↑), 127.23 (CH ↑), 126.50 (CH ↑), 126.39 (CH ↑), 126.36 (CH ↑), 125.92 (CH ↑), 123.62 (CH ↑), 120.60 (CH ↑), 58.52 (CH_2 ↑), 39.99 (CH ↑), 14.18 (CH_3 ↑) ^{13}CNMR-APT CH, CH_3 (up), CH_2, Cq (down), δ: 168.14 (CO ↓), 160.70 (C ↓), 147.73 (C ↓), 142.74 (C↓), 132.41 (C↓),128.11 (CH ↑), 127.55 (CH ↑), 127.23 (CH ↑), 126.50 (CH ↑), 126.39 (CH ↑), 126.36 (CH ↑), 125.92 (CH ↑), 123.62 (CH ↑), 122.73 (C↓), 120.87 (C↓), 120.60 (CH ↑), 76.31 (C ↓), 58.52 (CH_2 ↓), 39.99 (CH ↑), 14.18 (CH_3 ↑) MS, m/z (I_r/%): 345 (23.84) (M^+) with a base peak at 237 (100)
VIIb	IR, $\tilde{\nu}$/cm^{-1}: 3385, 3288 (NH_2), 3077, 3063, 2987, 2978, 2960, (CH), 1668 (CO) ^{1}H NMR (DMSO-d_6), δ: 8.33–7.03 (m, 10H, 3Ph), 7.82 (bs, 2H, NH_2, D_2O exchangeable), 5.06 (s, 1H, pyran ring), 4.01 (q, 2H, J = 7.2 Hz, CH_2), 1.10 (t, 3H, J = 7.2 Hz, CH_3) ^{13}C NMR (DMSO-d_6), δ: 168.10 (CO), 161.46 (C), 160.66 (C), 144.00 (C), 142.72 (C), 132.47 (C), 129.06 (CH), 129.00 (CH), 128.24 (CH), 127.58 (CH), 126.45 (CH), 123.73 (CH), 122.75 (C), 120.64 (C), 120.62 (CH), 114.88 (CH), 114.71 (CH), 76.25 (C), 58.58 (CH_2), 40.02 (CH), 14.21 (CH_3) ^{13}C NMR-DEPT at 135° CH, CH_3 (up), CH_2 (down), δ: 129.06 (CH ↑), 129.00 (CH ↑), 128.24 (CH ↑), 127.58 (CH ↑), 126.45 (CH ↑), 123.73 (CH ↑), 120.62 (CH ↑), 114.88 (CH ↑), 114.75 (CH ↑), 58.58 (CH_2 ↓), 40.02 (CH ↑), 14.21 (CH_3 ↑) ^{13}C NMR-DEPT at 90° only CH signals are (up), δ: 129.06 (CH ↑), 129.00 (CH ↑), 128.24 (CH ↑), 127.58 (CH ↑), 126.45 (CH ↑), 123.73 (CH ↑), 120.62 (CH ↑), 114.88 (CH ↑), 114.75 (CH ↑), 40.02 (CH ↑) ^{13}C NMR-DEPT at 45° CH, CH_2 and CH_3 signals are (up), δ: 129.06 (CH ↑), 129.00 (CH ↑), 128.24 (CH ↑), 127.58 (CH ↑), 126.45 (CH ↑), 123.73 (CH ↑), 120.62 (CH ↑), 114.88 (CH ↑), 114.75 (CH ↑), 58.58 (CH_2 ↑), 40.02 (CH ↑), 14.21 (CH_3 ↑) ^{13}CNMR-APT CH, CH_3 (up), CH_2, Cq (down), δ: 168.10 (CO ↓), 161.46 (C ↓), 160.66 (C ↓), 144.00 (C ↓), 142.72 (C ↓), 132.47 (C ↓), 129.06 (CH ↑), 129.00 (CH ↑), 128.24 (CH ↑), 127.58 (CH ↑), 126.45 (CH ↑), 123.73 (CH ↑), 122.75 (C ↓), 120.64 (C ↓), 120.62 (CH ↑), 114.88 (CH ↑), 114.75 (CH ↑), 76.25 (C ↓), 58.58 (CH_2 ↓), 40.02 (CH ↑), 14.21 (CH_3 ↑) MS, m/z (I_r/%): 363 (1.64) (M^+) with a base peak at 68 (100)
VIIc	IR, $\tilde{\nu}$/cm^{-1}: 3469, 3318 (NH_2), 3079, 3058, 2977, 2955, 2903 (CH), 1678 (CO) ^{1}H NMR (DMSO-d_6), δ: 8.32–7.25 (m, 10H, 3Ph), 7.83 (bs, 2H, NH_2), 5.06 (s, 1H, pyran ring), 4.01 (q, 2H, J = 7.1 Hz, CH_2), 1.10 (t, 3H, J = 7.1 Hz, CH_3) ^{13}C NMR (DMSO-d_6), δ: 168.01 (CO), 160.66 (C), 146.75 (C), 142.73 (C), 132.50 (C), 130.46 (C), 129.12 (CH), 128.08 (CH), 127.57 (CH), 126.48 (CH), 126.46 (CH), 126.37 (CH), 123.74 (CH), 122.72 (C), 120.63 (C), 120.26 (CH), 75.92 (C), 58.60 (CH_2), 39.44 (CH), 14.20 (CH_3) ^{13}C NMR-DEPT at 135° CH, CH_3 (up), CH_2 (down), δ: 129.12 (CH ↑), 128.08 (CH ↑), 127.57 (CH ↑), 126.48 (CH ↑), 126.46 (CH ↑), 126.37 (CH ↑), 123.74 (CH ↑), 120.26 (CH ↑), 58.60 (CH_2 ↓), 39.44 (CH ↑), 14.20 (CH_3 ↑) ^{13}C NMR-DEPT at 90° only CH signals are (up), δ: 129.12 (CH ↑), 128.08 (CH ↑), 127.57 (CH ↑), 126.48 (CH ↑), 126.46 (CH ↑), 126.37 (CH ↑), 123.74 (CH ↑), 120.26 (CH ↑), 39.44 (CH ↑) ^{13}C NMR-DEPT at 45° CH, CH_2 and CH_3 signals are (up), δ: 129.12 (CH ↑), 128.08 (CH ↑), 127.57 (CH ↑), 126.48 (CH ↑), 126.46 (CH ↑), 126.37 (CH ↑), 123.74 (CH ↑), 120.26 (CH ↑), 58.60 (CH_2 ↑), 39.44 (CH ↑), 14.20 (CH_3 ↑) ^{13}CNMR-APT CH, CH_3 (up), CH_2, Cq (down), δ: 168.01 (CO ↓), 160.66 (C ↓), 146.75 (C ↓), 142.73 (C ↓), 132.50 (C ↓), 130.46 (C ↓), 129.12 (CH ↑), 128.08 (CH ↑), 127.57 (CH ↑), 126.48 (CH ↑), 126.46 (CH ↑), 126.37 (CH ↑), 123.74 (CH ↑), 122.72 (C ↓), 120.63 (C ↓), 120.26 (CH ↑), 75.92 (C ↓), 58.60 (CH_2 ↓), 39.44 (CH ↑), 14.20 (CH_3 ↑) MS, m/z (I_r/%): 381(8.18)(M^+ + 2), 379 (24.72) (M^+) with a base peak at 269 (100)

Table 6. (continued)

Compound	Spectral data
VIId	IR, $\tilde{\nu}$/cm^{-1}: 3463, 3319 (NH_2), 3077, 3069, 2978, 2976, 2931 (CH), 1677 (CO) ^{1}H NMR (DMSO-d_6), δ: 8.33–7.20 (m, 10H, 3Ph), 7.84 (bs, 2H, NH_2), 5.05 (s, 1H, pyran ring), 4.01(q, 2H, J = 7.2 Hz, CH_2), 1.10 (t, 3H, J = 7.2 Hz, CH_3) ^{13}C NMR (DMSO-d_6), δ: 168.02 (CO), 160.87 (C), 147.18 (C), 142.74 (C), 132.51 (C), 131.12 (CH), 129.54 (CH), 127.58 (CH), 126.49 (CH), 126.46 (CH), 123.75 (CH), 122.73 (CH), 120.64 (C), 120.38 (C), 120.19 (CH), 119.03 (C), 75.86 (C), 58.61 (CH_2), 39.29 (CH), 14.22 (CH_3) ^{13}C NMR-DEPT at 135° CH, CH_3 (up), CH_2 (down), δ: 131.12 (CH ↑), 129.54, (CH ↑), 127.58 (CH ↑), 126.49 (CH ↑), 126.46 (CH ↑), 123.75 (CH ↑), 122.73 (CH ↑), 120.19 (CH ↑), 58.61 (CH_2 ↓), 39.29 (CH ↑), 14.22 (CH_3 ↑) ^{13}C NMR-DEPT at 90° only CH signals are (up), δ: 131.12 (CH ↑), 129.54, (CH ↑), 127.58 (CH ↑), 126.49 (CH ↑), 126.46 (CH ↑), 123.75 (CH ↑), 122.73 (CH ↑), 120.19 (CH ↑), 39.29 (CH ↑) ^{13}C NMR-DEPT at 45° CH, CH_2 and CH_3 signals are (up), δ: 131.12 (CH ↑), 129.54, (CH ↑), 127.58 (CH ↑), 126.49 (CH ↑), 126.46 (CH ↑), 123.19 (CH ↑), 122.73 (CH ↑), 120.19 (CH ↑), 39.29 (CH ↑), 58.61 (CH_2 ↑), 39.29 (CH ↑), 14.22 (CH_3 ↑) ^{13}CNMR-APT CH, CH_3 (up), CH_2, Cq (down), δ: 168.02 (CO ↓), 160.87 (C ↓), 147.18 (C ↓), 142.74 (C ↓), 132.51 (C ↓), 131.12 (CH ↑), 129.54, (CH ↑), 127.58 (CH ↑), 126.49 (CH ↑), 126.46 (CH ↑), 123.75 (CH ↑), 122.73 (CH ↑), 120.64 (C ↓), 120.38 (C ↓), 120.19 (CH ↑), 119.03 (C ↓), 75.86 (C ↓), 58.61 (CH_2 ↓), 39.29 (CH ↑), 14.22 (CH_3 ↑) MS, m/z (I_r/%): 425 (12.18) (M^++ 2), 423 (12.83) (M^+) with a base peak at 269 (100)
VIIe	IR, $\tilde{\nu}$/cm^{-1}: 3380, 3287 (NH_2), 3079, 3063, 2996, 2977, 2901 (CH), 1681 (CO) ^{1}H NMR (DMSO-d_6), δ: 8.30–7.02 (m, 10H, 3Ph), 7.76 (bs, 2H, NH_2, D_2O exchangeable), 4.98 (s, 1H, pyran ring), 3.99 (q, 2H, J = 7.2 Hz, CH_2), 2.19 (s, 3H, CH_3), 1.11 (t, 3H, J = 7.2 Hz, CH_3) ^{13}C NMR (DMSO-d_6), δ: 168.19 (CO), 160.65 (C), 144.80 (C), 142.70 (C), 134.87 (C), 132.38 (C), 128.66 (CH), 127.54 (CH), 127.11 (CH), 126.52 (CH), 126.35 (CH), 126.31 (CH), 123.56 (CH), 122.74 (C), 121.09 (C), 120.58 (CH), 76.42 (C), 58.53 (CH_2), 39.44 (CH), 20.44 (CH_3), 14.22 (CH_3) ^{13}C NMR-DEPT at 135° CH, CH_3 (up), CH_2 (down), δ: 128.66 (CH ↑), 127.54 (CH ↑), 127.11 (CH ↑), 126.52 (CH ↑), 126.35 (CH ↑), 126.31 (CH ↑), 123.56 (CH ↑), 120.58 (CH ↑), 58.53 (CH_2 ↓), 39.44 (CH ↑), 20.44 (CH_3 ↑), 14.22 (CH_3 ↑) ^{13}C NMR-DEPT at 90° only CH signals are (up), δ: 128.66 (CH ↑), 127.54 (CH ↑), 127.11 (CH ↑), 126.52 (CH ↑), 126.35 (CH ↑), 126.31 (CH ↑), 123.56 (CH ↑), 120.58 (CH ↑), 39.44 (CH ↑) ^{13}C NMR-DEPT at 45° CH, CH_2 and CH_3 signals are (up), δ: 128.66 (CH ↑), 127.54 (CH ↑), 127.11 (CH ↑), 126.52 (CH ↑), 126.35 (CH ↑), 126.31 (CH ↑), 123.56 (CH ↑), 120.58 (CH ↑), 58.53 (CH_2 ↑), 39.44 (CH ↑), 20.44 (CH_3 ↑), 14.22 (CH_3 ↑) ^{13}CNMR-APT CH, CH_3 (up), CH_2, Cq (down), δ: 168.19 (CO↓), 160.65 (C↓), 144.80 (C ↓),142.70 (C ↓),134.87 (C ↓), 132.38 (C↓), 128.66 (CH ↑), 127.54 (CH ↑), 127.11 (CH ↑), 126.52 (CH ↑), 126.35 (CH ↑), 126.31 (CH ↑), 123.56 (CH ↑), 122.74 (C ↓), 121.09 (C ↓), 120.58 (CH ↑), 76.42 (C ↓), 58.53 (CH_2 ↓), 39.44 (CH ↑), 20.44 (CH_3 ↑), 14.22 (CH_3 ↑) MS, m/z (I_r/%): 359 (15.84) (M^+) with a base peak at 273 (100)
VIIf	IR, $\tilde{\nu}$/cm^{-1}: 3467, 3325 (NH_2), 3081, 3062, 2993, 2983, 2904 (CH), 1687 (CO) ^{1}H NMR (DMSO-d_6), δ: 8.31–6.77 (m, 10H, 3Ph), 7.76 (bs, 2H, NH_2), 4.98 (s, 1H, pyran ring), 4.00 (q, 2H, J = 7.2 Hz, CH_2), 3.66 (s, 3H, OCH_3), 1.12 (t, 3H, J = 7.2 Hz, CH_3) ^{13}C NMR (DMSO-d_6), δ: 168.28 (CO), 160.67 (C), 157.48 (C), 142.73 (C), 140.00 (C), 132.42 (C), 128.25 (CH), 127.60 (CH), 126.62 (CH), 126.42 (CH), 126.35 (CH), 123.63 (CH), 122.81 (C), 121.29 (C), 120.64 (CH), 113.54 (CH), 76.66 (C), 58.59 (CH_2), 54.90 (CH_3), 39.17 (CH), 14.30 (CH_3) ^{13}C NMR-DEPT at 135° CH, CH_3 (up), CH_2 (down), δ: 128.25 (CH ↑), 127.60 (CH ↑), 126.62 (CH ↑), 126.42 (CH ↑), 126.35 (CH ↑), 123.63 (CH ↑), 120.64 (CH ↑), 113.54 (CH ↑), 58.59 (CH_2 ↓), 54.90 (CH_3 ↑), 39.17 (CH ↑), 14.30 (CH_3 ↑) ^{13}C NMR-DEPT at 90° only CH signals are (up), δ: 128.25 (CH ↑), 127.60 (CH ↑), 126.62 (CH ↑), 126.42 (CH ↑), 126.35 (CH ↑), 123.63 (CH ↑), 120.64 (CH ↑), 113.54 (CH ↑), 39.17 (CH ↑) ^{13}C NMR-DEPT spectrum at 45° CH, CH_2 and CH_3 signals are (up), δ: 128.25 (CH ↑), 127.60 (CH ↑), 126.62 (CH ↑), 126.42 (CH ↑), 126.35 (CH ↑), 123.63 (CH ↑), 120.64 (CH ↑), 113.54 (CH ↑), 58.59 (CH_2 ↑), 54.90 (CH_3 ↑), 39.17 (CH ↑), 14.30 (CH_3 ↑) ^{13}CNMR-APT CH, CH_3 (up), CH_2, Cq (down), δ: 168.28 (CO ↓), 160.67 (C ↓), 157.48 (C ↓), 142.73 (C↓), 140.00 (C ↓), 132.42 (C↓), 128.25 (CH ↑), 127.60 (CH ↑), 126.62 (CH ↑), 126.42 (CH ↑), 126.35 (CH ↑), 123.63 (CH ↑), 122.81 (C ↓), 121.29 (C ↓), 120.64 (C ↑), 113.54 (CH ↑), 76.66 (C ↓), 58.59 (CH_2 ↓), 54.90 (CH_3 ↑), 39.17 (CH ↑), 14.30 (CH_3 ↑) MS, m/z (I_r/%): 375 (11.32) (M^+) with a base peak at 269 (100)
VIIg	IR, $\tilde{\nu}$/cm^{-1}: 3467, 3317 (NH_2), 3085, 3066, 2995, 2977, 2908 (CH), 1687 (CO) ^{1}H NMR (DMSO-d_6), δ: 8.47–7.13 (m, 10H, 3Ph), 8.00 (bs, 2H, NH_2, D_2O exchangeable), 5.28 (s, 1H, pyran ring), 3.99 (q, 2H, J = 7 Hz, CH_2), 1.11 (t, 3H, J = 7 Hz, CH_3) ^{13}C NMR (DMSO-d_6), δ: 168.75 (CO), 160.67 (C), 154.86 (C), 146.00 (C), 142.30 (C), 132.42 (C), 128.74 (CH), 127.66 (CH), 126.66 (CH), 126.42 (CH), 126.37 (CH), 125.13 (CH),123.65 (CH), 122.82 (C), 121.27 (C), 120.61 (CH), 76.65 (C), 58.58 (CH_2), 40.03 (CH), 14.30 (CH_3) MS, m/z (I_r/%): 390 (13.46) (M^+) with a base peak at 269 (100)

Table 7. Characterisation data of compounds *VIII*

Compound	Formula	M_r	Colour	Yield/%	M.p./℃
VIIIa	$C_{22}H_{20}N_2O_3$	360.41	Grey	74	203, 203[a]
VIIIb	$C_{22}H_{19}FN_2O_3$	378.40	Grey	77	210
VIIIc	$C_{22}H_{19}ClN_2O_3$	394.85	Red	73	185, 185[a]
VIIId	$C_{22}H_{19}BrN_2O_3$	439.30	Red	75	188
VIIIe	$C_{23}H_{22}N_2O_3$	374.43	Red	76	196
VIIIf	$C_{23}H_{22}N_2O_4$	390.43	Red	75	192, 192[a]
VIIIg	$C_{22}H_{19}N_3O_5$	405.40	Red	71	213

a) According to Abd-El-Aziz et al. (2004).

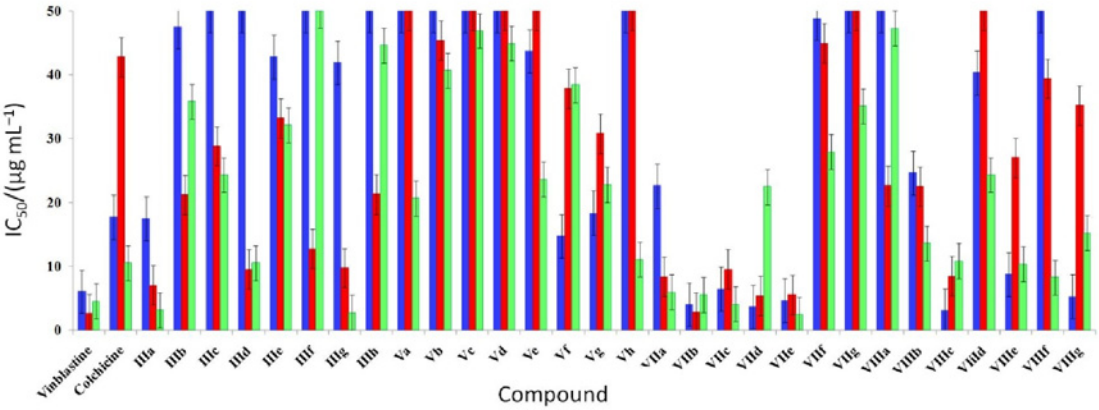


Fig. 5. Anti-tumour activity of 4*H*-benzo[*h*]chromene derivatives: MCF-7 (blue); HCT-116 (red) and HepG-2 (green).

1.06 (t, 3H, CH_3, $J = 7.0$–7.2 Hz) with the corresponding signals in the ^{13}C spectra at δ of 58.65–58.46 (CH_2) and 14.32–14.18 (CH_3), respectively. The ^{13}C NMR-DEPT spectra at 45,° 90,° 135,° ^{13}C NMR-APT spectra and the mass spectra of compounds *VII* and *VIII* provided additional evidence in support of the proposed structures. In addition, the ^1H NMR spectra for compounds *III* and *V* showed that the NH_2 protons resonated at δ of 7.37–7.02 (sharp singlet), while compounds *VII* and *VIII* showed that the NH_2 protons resonated at δ of 8.00–7.71 (broad singlet lower field), respectively. This de-shielding is a result of the replacement of the CN group in *III* and *V* by the C═O group in *VII* and *VIII* whose C═O anisotropy would de-shield these protons in addition to the protons involved in the hydrogen bonding with the C═O group. This was also, supported by the X-ray single crystal data (Al-Dies et al., 2012, El-Agrody et al., 2012).

Anti-tumour assays

Compounds *IIIa–IIIh*, *Va–Vh*, *VIIa–VIIg* and *VIIIa–VIIIg* were evaluated for human tumour cell growth inhibitory activity against three cell lines: breast adenocarcinoma (MCF-7), lung carcinoma (HCT-116) and hepatocellular carcinoma (HepG-2). The measurements of cell growth and the viabilities were determined as described in the literature (Mossman, 1983; Rahman et al., 2001). The in-vitro cytotoxicity evaluation using viability assay was performed at the Regional Centre for Mycology & Biotechnology (RCMP), Al-Azhar University (Cairo, Egypt), using vinblastine and colchicine as standard drugs under different concentrations (50 μg mL^{-1}, 25 μg mL^{-1}, 12.5 μg mL^{-1}, 6.25 μg mL^{-1}, 3.125 μg mL^{-1}, 1.56 μg mL^{-1} and 0 μg mL^{-1}). The inhibitory activity of the synthetic compounds *IIIa–IIIh*, *Va–Vh*, *VIIa–VIIg* and *VIIIa–VIIIg* against the three cell lines MCF-7, HCT-116 and HepG-2 is given in Table 9 and Fig. 5.

SAR studies

The preliminary SAR study focused on the effect of the substituent at the phenyl group at the 4-position and the substituent at the 3- and 7-positions of the 4*H*-benzo[*h*]chromene moiety, on the anti-tumour activities of the synthesised compounds. In a comparison of the cytotoxic activities of the four series (*IIIa–IIIh*, *Va–Vh*, *VIIa–VIIg* and *VIIIa–VIIIg*) against breast adenocarcinoma (MCF-7), it was found that, for the first series *IIIa–IIIh* and the second series *Va–Vh*, the highest growth inhibitory effect was associated with the unsubstituted phenyl *IIIa* and 4-methoxyphenyl *Vf* analogues with IC_{50} of 17.5 μg mL^{-1} and 14.8 μg mL^{-1}, respectively, which exhibited good activity relative to colchicine (IC_{50} = 17.7 μg mL^{-1}) and more reduction in potency with the other derivatives *IIIg*, *IIIe*, *IIIb*, *IIIc*,

IIId, *IIIf*, *IIIh* and *Vg*, *Ve*, *Va*, *Vb*, *Vc*, *Vd*, *Vh* with IC_{50} = 41.19–47.5 μg mL^{-1} or > 50 μg mL^{-1} and 18.4–43.7 μg mL^{-1} or > 50 μg mL^{-1}, respectively, as compared with vinblastine (IC_{50} = 6.1 μg mL^{-1}) and colchicine (IC_{50} = 17.7 μg mL^{-1}), suggesting that the unsubstituted phenyl (electron-donating) with H-7 of the first series and the methoxy group (electron-donating) at the *para*-position on the phenyl ring at the 4-position of the 4*H*-benzo[*h*]chromene moiety with NH_2-7 (electron-donating) of the second series is preferred for anti-tumour activity than the other substituent. Replacement of the electron-withdrawing cyano group of the first series *III* and the second series *V* by the ester group at the

Table 8. Spectral data of compounds *VIII*

Compound	Spectral data
VIIIa	IR, $\tilde{\nu}$/cm^{-1}: 3436, 3390, 3350, 3275 (2 NH_2), 3064, 3021, 2983 (CH), 1677 (CO) ^{1}H NMR (DMSO-d_6), δ: 7.78–6.69 (m, 10H, 3Ph), 7.76 (bs, 2H, NH_2-2, D_2O exchangeable), 5.76 (bs, 2H, NH_2-7, D_2O exchangeable), 4.98 (s, 1H, pyran ring), 3.99 (q, 2H, J = 7 Hz, CH_2), 1.08 (t, 3H, J = 7 Hz, CH_3) ^{13}C NMR (DMSO-d_6), δ: 168.22 (CO), 160.91 (C), 147.89 (C), 144.77 (C), 142.89 (C), 128.05 (CH), 127.19 (CH ↑), 127.17 (CH), 125.83 (CH), 124.00 (C), 123.67 (CH), 121.81 (C), 120.39 (C), 118.38 (CH), 108.01 (CH), 107.91 (CH), 76.27 (C), 58.47 (CH_2), 40.00 (CH), 14.19 (CH_3) ^{13}C NMR-DEPT at 135° CH, CH_3 (up), CH_2 (down), δ: 128.05 (CH ↑), 127.19 (CH ↑), 127.17 (CH ↑), 125.83 (CH ↑), 123.67 (CH ↑), 118.38 (CH ↑), 108.01 (CH ↑), 107.91 (CH ↑), 58.47 (CH_2 ↓), 40.00 (CH ↑), 14.19 (CH_3 ↑) ^{13}C NMR-DEPT at 90° only CH signals are (up), δ: 128.05 (CH ↑), 127.19 (CH ↑), 127.17 (CH ↑), 125.83 (CH ↑), 123.67 (CH ↑), 118.38 (CH ↑), 108.01 (CH ↑), 107.91 (CH ↑), 40.00 (CH ↑) ^{13}C NMR-DEPT at 45° CH, CH_2 and CH_3 signals are (up), δ: 128.05 (CH ↑), 127.19 (CH ↑), 127.17 (CH ↑), 125.83 (CH ↑), 123.67 (CH ↑), 118.38 (CH ↑), 108.01 (CH ↑), 107.91 (CH ↑), 58.47 (CH_2 ↑), 40.00 (CH ↑), 14.19 (CH_3 ↑) ^{13}CNMR-APT CH, CH_3 (up), CH_2, Cq (down), δ: 168.22 (CO ↓), 160.91 (C ↓), 147.89 (C ↓), 144.77 (C ↓), 142.89 (C ↓), 128.05 (CH ↑), 127.19 (CH ↑), 127.17 (CH ↑), 125.83 (CH ↑), 124.00 (C ↓), 123.67 (CH ↑), 121.81 (C ↓), 120.39 (C ↓), 118.38 (CH ↑), 108.01 (CH ↑), 107.91 (CH ↑), 76.27 (C ↓), 58.47 (CH_2 ↓), 40.00 (CH ↑), 14.19 (CH_3 ↑)
VIIIb	IR, $\tilde{\nu}$/cm^{-1}: 3440, 3390, 3354, 3222 (2 NH_2), 3060, 2988, 2932 (CH), 1679 (CO) ^{1}H NMR (DMSO-d_6), δ: 7.78–6.78 (m, 9H, 3Ph), 7.82 (bs, 2H, NH_2-2), 5.79 (bs, 2H, NH_2-7), 5.18 (s, 1H, pyran ring), 3.99 (q, 2H, J = 7 Hz, CH_2), 1.10 (t, 3H, J = 7 Hz, CH_3) ^{13}C NMR (DMSO-d_6), δ: 168.24 (CO), 160.93 (C), 160.14 (C), 146.93 (C), 144.01 (C), 142.89 (C), 129.08 (CH), 127.28 (CH), 124.03 (C), 123.68 (CH), 121.90 (C), 120.39 (C), 118.39 (CH), 114.89 (CH), 108.15 (CH), 107.95 (CH), 76.28 (C), 58.56 (CH_2), 40.07 (CH), 14.19 (CH_3) MS, m/z (I_r/%): 378 (13.46) (M^+) with a base peak at 264 (100)
VIIIc	IR, $\tilde{\nu}$/cm^{-1}: 3417, 3390, 3324, 3250 (2 NH_2), 3065, 2982, 2931 (CH), 1667 (CO) ^{1}H NMR (DMSO-d_6), δ: 7.77–6.72 (m, 9H, 3Ph), 7.81 (bs, 2H, NH_2-2, D_2O exchangeable), 5.79 (bs, 2H, NH_2-7, D_2O exchangeable), 5.02 (s, 1H, pyran ring), 4.00 (q, 2H, J = 7.2 Hz, CH_2), 1.10 (t, 3H, J = 7.2 Hz, CH_3) ^{13}C NMR (DMSO-d_6), δ: 168.10 (CO), 160.88 (C), 146.93 (C), 144.82 (C), 142.90 (C), 130.38 (C), 129.08 (CH), 128.23 (CH), 127.28 (CH), 124.01 (C), 123.56 (CH), 121.90 (C), 119.79 (C), 118.53 (CH), 108.15 (CH), 107.95 (CH), 75.90 (C), 58.56 (CH_2), 40.01 (CH), 14.22 (CH_3) MS, m/z (I_r/%): 398 (1.11)(M^+ + 2), 396 (3.46) (M^+) with a base peak at 264 (100)
VIIId	IR, $\tilde{\nu}$/cm^{-1}: 3449, 3377, 3355, 3271 (2 NH_2), 3062, 2963 (CH), 1679 (CO) ^{1}H NMR (DMSO-d_6), δ: 7.76–6.71 (m, 9H, 3PH), 7.15 (bs, 2H, NH_2-2, D_2O exchangeable), 5.80 (bs, 2H, NH_2-7, D_2O exchangeable), 4.88 (s, 1H, pyran ring), 3.99 (q, 2H, J = 7 Hz, CH_2), 1.06 (t, 3H, J = 7 Hz, CH_3) ^{13}C NMR (DMSO-d_6), δ: 168.21 (CO), 160.21 (C), 145.16 (C), 144.83 (C), 142.79 (C), 131.45 (CH), 129.76 (CH), 127.33 (CH), 123.91 (C), 123.08 (CH), 121.98 (C), 120.39 (C), 119.84 (C), 118.68 (CH), 108.35 (CH), 107.84 (CH), 76.25 (C), 58.46 (CH_2), 40.15 (CH), 14.19 (CH_3) ^{13}C NMR-DEPT at 135° CH, CH_3 (up), CH_2 (down), δ: 131.45 (CH ↑), 129.76 (CH ↑), 127.43 (CH ↑), 123.08 (CH ↑), 118.68 (CH ↑), 108.35 (CH ↑), 107.84 (CH ↑), 58.46 (CH_2 ↓), 40.15 (CH ↑), 14.19 (CH_3 ↑) ^{13}C NMR-DEPT at 90° only CH signals are (up), δ: 131.45 (CH ↑), 129.76 (CH ↑), 127.43 (CH ↑), 123.08 (CH ↑), 118.68 (CH ↑), 108.35 (CH ↑), 107.84 (CH ↑), 40.15 (CH ↑) ^{13}C NMR-DEPT at 45° CH, CH_2 and CH_3 signals are (up), δ: 131.45 (CH ↑), 129.76 (CH ↑), 127.43 (CH ↑), 123.08 (CH ↑), 118.68 (CH ↑), 108.35 (CH ↑), 107.84 (CH ↑), 58.46 (CH_2 ↑), 40.15 (CH ↑), 14.19 (CH_3 ↑) ^{13}CNMR-APT CH, CH_3 (up), CH_2, Cq (down), δ: 168.21 (CO ↓), 160.21 (C ↓), 145.16 (C ↓), 144.83 (C ↓), 142.79 (C ↓), 131.45 (CH ↑), 129.76 (CH ↑), 127.43 (CH ↑), 123.91 (C ↓), 123.08 (CH ↑), 121.98 (C ↓), 120.39 (C ↓), 119.84 (C ↓), 118.68 (CH ↑), 108.35 (CH ↑), 107.84 (CH ↑), 76.25 (C ↓), 58.46 (CH_2 ↓), 40.15 (CH ↑), 14.19 (CH_3 ↑) MS, m/z (I_r/%): 440 (1.11) (M^+ + 2), 438 (1.16) (M^+) with a base peak at 236 (100)
VIIIe	IR, $\tilde{\nu}$/cm^{-1}: 3439, 3362, 3358, 3272 (2 NH_2), 3061, 2932, 2859 (CH), 1678 (CO) ^{1}H NMR (DMSO-d_6), δ: 7.72–6.68 (m, 9H, 3Ph), 7.71 (bs, 2H, NH_2-2), 5.75 (bs, 2H, NH_2-7), 4.93 (s, 1H, pyran ring), 3.98 (q, 2H, J = 7 Hz, CH_2,), 2.19 (s, 3H, CH_3,), 1.08 (t, 3H, J = 7 Hz, CH_3) ^{13}C NMR (DMSO-d_6), δ: 168.22 (CO), 160.23 (C), 144.80 (C), 144.75 (C), 142.80 (C), 134.75 (C), 128.60 (CH), 127.04 (CH), 126.35 (CH), 123.94 (C), 123.09 (CH), 121.99 (C), 119.88 (C), 118.68 (CH), 107.95 (CH), 107.87 (CH), 76.35 (C), 58.49 (CH_2), 40.07 (CH), 20.44 (CH_3), 14.32 (CH_3) MS, m/z (I_r/%): 374 (42.35) (M^+) with a base peak at 248 (100)

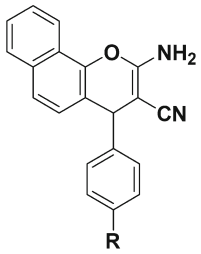
Table 8. (continued)

Compound	Spectral data
VIIIf	IR, $\tilde{\nu}$/cm^{-1}: 3463, 3366, 3342, 3257 (2 NH_2), 3071, 2023, 2982 (CH), 1667 (CO) ^{1}H NMR (DMSO-d_6), δ: 7.73–6.69 (m, 9H, 3Ph), 7.70 (bs, 2H, NH_2-2), 5.74 (bs, 2H, NH_2-7), 4.93 (s, 1H, pyran ring), 3.99 (q, 2H, J = 7 Hz, CH_2), 3.66 (s, 3H, CH_3), 1.10 (t, 3H, J = 7 Hz, CH_3) ^{13}C NMR (DMSO-d_6), δ: 168.35 (CO), 160.88 (C), 157.42 (C), 144.83 (C), 142.91 (C), 140.17 (C), 128.18 (CH), 127.21 (CH), 124.07 (C), 123.80 (CH), 121.82 (C), 120.80 (C), 118.39 (CH), 113.49 (CH), 108.04 (CH), 107.98 (CH), 76.63 (C), 58.54 (CH_2), 54.90 (CH_3), 39.12 (CH), 14.32 (CH_3) ^{13}C NMR-DEPT at 135° CH, CH_3 (up), CH_2 (down), δ: 128.18 (C ↑), 127.21 (CH ↑), 123.80 (CH ↑), 118.39 (CH ↑), 113.49 (CH ↑), 108.04 (CH ↑), 107.98 (CH ↑), 58.54 (CH_2 ↓), 54.90 (CH_3 ↑), 39.12 (CH ↑), 14.32 (CH_3 ↑) ^{13}C NMR-DEPT at 90° only CH signals are (up), δ: 128.18 (C ↑), 127.21 (CH ↑), 123.80 (CH ↑), 118.39 (CH ↑), 113.49 (CH ↑), 108.04 (CH ↑), 107.98 (CH ↑), 39.12 (CH ↑) ^{13}C NMR-DEPT spectrum at 45° CH, CH_2 and CH_3 signals are (up), δ: 128.18 (CH ↑), 127.21 (CH ↑), 123.80 (CH ↑), 118.39 (CH ↑), 113.49 (CH↑), 108.04 (CH ↑), 107.98 (CH ↑), 58.54 (CH_2 ↑), 54.90 (CH_3 ↑), 39.12 (CH ↑), 14.32 (CH_3 ↑) ^{13}CNMR-APT CH, CH_3 (up), CH_2, Cq (down), δ: 168.35 (CO ↓), 160.88 (C ↓), 157.42 (C ↓), 144.83 (C ↓), 142.91 (C ↓), 140.17 (C ↓), 128.18 (CH ↑), 127.21 (CH ↑), 124.07 (C ↓), 123.80 (CH ↑), 121.82 (C ↓), 120.80 (C ↓), 118.39 (CH ↑), 113.49 (CH ↑), 108.04 (CH ↑), 107.98 (CH ↑), 76.63 (C ↓), 58.54 (CH_2 ↓), 54.90 (CH_3 ↑), 39.12 (CH ↑), 14.32 (CH_3 ↑) MS, m/z (I_r/%): 390 (1.16) (M^+) with a base peak at 56 (100)
VIIIg	IR, $\tilde{\nu}$/cm^{-1}: 3470, 3376, 3351, 3291 (2 NH_2), 3071, 2023, 2982 (CH), 1670 (CO) ^{1}H NMR (DMSO-d_6), δ: 8.14–6.72 (m, 9H, 3Ph), 7.89 (bs, 2H, NH_2, D_2O exchangeable), 5.81 (bs, 2H, NH_2, D_2O exchangeable), 5.19 (s, 1H, pyran ring), 3.99 (q, 2H, J = 7 Hz, CH_2), 1.09 (t, 3H, J = 7 Hz, CH_3) ^{13}C NMR (DMSO-d_6), δ: 167.90 (CO), 160.90 (C), 155.58 (C), 145.72 (C), 144.86 (C), 142.96 (C), 128.54 (CH), 128.23 (CH), 127.40 (CH), 124.01 (C), 123.51 (CH), 123.40 (CH), 122.04 (C), 118.76 (C), 118.70 (CH), 108.28 (CH), 107.95 (CH), 75.22 (C), 58.65 (CH_2), 40.03 (CH), 14.20 (CH_3) ^{13}C NMR-DEPT at 135° CH, CH_3 (up), CH_2 (down), δ: 128.54 (CH ↑), 128.23 (CH ↑), 127.40 (CH ↑), 123.51 (CH ↑), 123.40 (CH ↑), 118.70 (CH ↑), 108.28 (CH ↑), 107.95 (CH ↑), 58.65 (CH_2 ↓), 40.03 (CH ↑), 14.20 (CH_3 ↑) ^{13}C NMR-DEPT at 90° only CH signals are (up), δ: 128.54 (CH ↑), 128.23 (CH ↑), 127.40 (CH ↑), 123.51 (CH ↑), 123.40 (CH ↑), 118.70 (CH ↑), 108.28 (CH ↑), 107.95 (CH ↑), 40.03 (CH ↑) ^{13}C NMR-DEPT spectrum at 45° CH, CH_2 and CH_3 signals are (up), δ: 128.54 (CH ↑), 128.23 (CH ↑),127.40 (CH ↑), 123.51 (CH ↑), 123.40 (CH ↑), 118.70 (CH ↑), 108.28 (CH ↑), 107.95 (CH ↑), 58.65 (CH_2 ↑), 40.03 (CH ↑), 14.20 (CH_3 ↑) ^{13}CNMR-APT CH, CH_3 (up), CH_2, Cq (down), δ: 167.90 (CO ↓), 160.90 (C ↓), 155.58 (C ↓), 145.72 (C ↓), 144.86 (C↓), 142.96 (C ↓), 128.54 (CH ↑), 128.23 (CH ↑), 127.40 (CH ↑), 124.01 (C ↓), 123.51 (CH ↑), 123.40 (CH ↑), 122.04 (C ↓), 118.76 (C ↓), 118.70 (CH ↑), 108.28 (CH ↑), 107.95 (CH ↑), 75.22 (C ↓), 58.65 (CH_2 ↓), 40.03 (CH ↑), 14.20 (CH_3 ↑) MS, m/z (I_r/%): 405 (26.39) (M^+) with a base peak at 284 (100)

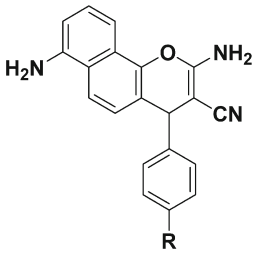
3−position resulted in a marked improvement in potency for the third series *VII* and the fourth series *VIII* against MCF-7, 4-bromophenyl *VIId*, 4-fluorophenyl *VIIb*,
4-methylphenyl *VIIe* (IC_{50} = 3.7–4.7 μg mL^{-1}) and 4-chlorophenyl *VIIIc*, 4-nitrophenyl *VIIIg*, analogues (IC_{50} of 3.1 μg mL^{-1} and 5.3 μg mL^{-1}, respectively) exhibited good activity against MCF-7 compared with vinblastine (IC_{50} = 6.1 μg mL^{-1}) and colchicine (IC_{50} = 17.7 μg mL^{-1}), while 4-chlorophenyl *VIIc* and 4-methylphenyl *VIIIe* with (IC_{50} of 6.5 μg mL^{-1} and 8.8 μg mL^{-1}, respectively) have a significant potent anti-tumour activity in compareison with colchicine, suggesting that the bulky substituent and the electronic nature of the substituent (electron-withdrawing or electron-donating groups) of the third series with H-7 or the less bulky substituent (electron-withdrawing) of the fourth series with NH_2-7 may be the main factor affecting the potency of these compounds. In general, the activities against MCF-7 of the four series were decreased in the descending order: *VII*, *VIII*, *V*, *III*.

In the case of lung carcinoma (HCT-116), the SAR investigation for the four series revealed compound *VIIb* of the third series (IC_{50} = 2.9 μg mL^{-1}) to have potent anti-tumour activity against the HCT-116 closet to vinblastine (IC_{50} = 2.6 μg mL^{-1}); the other compounds of the four series exhibited moderate to lower activities, while compounds *IIIa*, *IIId*, *IIIg*, *IIIf*, *IIIb*, *IIIh*, *IIIc*, *IIIe* of the first series (IC_{50} = 7.1–33.2 μg mL^{-1}) and *Vg*, *Vf* of the second series (IC_{50} of 30.8 μg mL^{-1} and 37.8 μg mL^{-1}, respectively) had the most potent activity against HCT-116 compared to colchicine (IC_{50} = 42.8 μg mL^{-1}), suggesting that the unsubstituted phenyl (electron-donating), the bromo, nitro, fluoro and chloro (electron-withdrawing) or methoxy, morpholinophenyl and methyl groups (electron-donating) of the first series with H-7 and the nitro (electron-withdrawing) and methoxy groups (electron-donating) of the second series with NH_2-7 at the *para*-position of phenyl ring at the 4-position are preferred over the other substituent. Replacement of the electron-withdrawing cyano group of first series *III* and the second series *V* by the es-

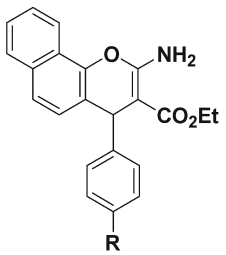
Table 9. SAR of 4-aryl group, 3-, 7-positions and IC_{50} of target compounds against different cell lines in comparison with the standard as measured with the MTT method[a]



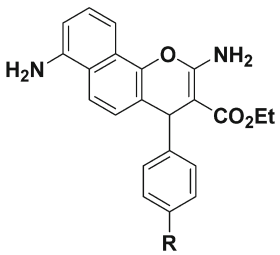
IIIa–IIIh



Va–Vh



VIIa–VIIg



VIIIa–VIIIg

Compound	R	MCF-7	HCT-116	HepG-2	Compound	R	MCF-7	HCT-116	HepG-2
IIIa	H	17.5 ± 0.1	7.1 ± 0.1	3.2 ± 0.0	*VIIa*	H	22.6 ± 0.1	8.4 ± 0.1	6.0 ± 0.3
IIIb	F	47.5 ± 0.0	21.2 ± 0.3	35.8 ± 0.1	*VIIb*	F	4.1 ± 0.1	2.9 ± 0.2	5.6 ± 0.1
IIIc	Cl	W	28.8 ± 0.1	24.3 ± 0.0	*VIIc*	Cl	6.5 ± 0.2	9.6 ± 0.7	4.1 ± 0.4
IIId	Br	W	9.6 ± 0.1	10.6 ± 0.1	*VIId*	Br	3.7 ± 0.2	5.4 ± 0.0	22.5 ± 0.2
IIIe	Me	42.8 ± 0.4	33.2 ± 0.0	32.1 ± 0.1	*VIIe*	Me	4.7 ± 0.0	5.6 ± 0.0	2.5 ± 0.0
IIIf	OMe	W	12.8 ± 0.0	W	*VIIf*	OMe	48.8 ± 0.0	44.9 ± 0.0	27.9 ± 0.1
IIIg	NO_2	41.9 ± 0.1	9.8 ± 0.0	2.8 ± 0.0	*VIIg*	NO_2	W	W	35.1 ± 0.0
IIIh	A	W	21.3 ± 0.0	44.6 ± 0.3	*VIIIa*	H	W	22.6 ± 0.4	47.3 ± 0.2
Va	H	W	W	20.7 ± 0.1	*VIIIb*	F	24.6 ± 0.4	22.5 ± 0.4	13.7 ± 0.0
Vb	F	W	45.4 ± 0.0	40.7 ± 0.0	*VIIIc*	Cl	3.1 ± 0.1	8.5 ± 0.0	10.9 ± 0.1
Vc	Cl	W	W	46.9 ± 0.4	*VIIId*	Br	40.3 ± 0.1	W	24.3 ± 0.2
Vd	Br	W	W	44.9 ± 0.1	*VIIIe*	Me	8.8 ± 0.0	27.0 ± 0.0	10.4 ± 0.1
Ve	Me	43.7 ± 0.0	W	23.6 ± 0.2	*VIIIf*	OMe	W	39.4 ± 0.1	8.3 ± 0.1
Vf	OMe	14.8 ± 0.0	37.8 ± 0.1	38.4 ± 0.4	*VIIIg*	NO_2	5.3 ± 0.0	35.2 ± 0.0	15.3 ± 0.3
Vg	NO_2	18.4 ± 0.1	30.8 ± 0.2	22.8 ± 0.1	B	–	6.1 ± 0.0	2.6 ± 0.0	4.6 ± 0.1
Vh	A	W	W	11.1 ± 0.2	C	–	17.7 ± 0.0	42.8 ± 0.1	10.6 ± 0.0

a) IC_{50} values expressed in μg mL^{-1} as mean values of triplicate wells from at least three experiments and are reported as the mean ± standard error; W = weak activity (IC_{50} > 50 μg mL^{-1}); A = morpholino; B = vinblastine; C = colchicine.

ter group at the 3-position resulted in a marked increase in potency for the third series *VII* and a little reduction in potency for the fourth series *VIII* against HCT-116, the 4-fluorophenyl *VIIb*, 4-bromophenyl *VIId*, 4-methylphenyl *VIIe*, phenyl *VIIa*, 4-chlorophenyl *VIIc* analogues for the third series with (IC_{50} of 2.9 μg mL^{-1}, 5.4 μg mL^{-1}, 5.6 μg mL^{-1}, 8.4 μg mL^{-1}, 9.6 μg mL^{-1}, respectively), and 4-chlorophenyl *VIIIc*, 4-fluorophenyl *VIIIb*, phenyl *VIIIa*, 4-methylphenyl *VIIIe*, 4-nitrophenyl *VIIIg*, 4-methoxyphenyl *VIIIf* analogues for the fourth series with (IC_{50} of 8.5 μg mL^{-1}, 22.5 μg mL^{-1}, 22.6 μg mL^{-1}, 27.0 μg mL^{-1}, 35.2 μg mL^{-1}, 39.4 μg mL^{-1}, respectively), exhibited good activity against HCT-116 in comparison with colchicine (IC_{50} = 42.8 μg mL^{-1}). This potency could be attributed to the presence of the fluoro, bromo and chloro atoms (electron-withdrawing), methyl group (electron-donating) at the *para*-position of the phenyl ring at the 4-position or the phenyl group (electron-donating) at the 4-position, suggesting that there might be a size-limited pocket at the *para*-position of the phenyl ring at the 4-position for the third series, or the chloro, fluoro and nitro groups (electron-withdrawing), phenyl group (electron-donating), methyl and methoxy groups (electron-donating) at the *para*-position of the phenyl ring at the 4-position for the fourth series *VIII*, suggesting that there might be a size-limited pocket at the *para*-position of the phenyl ring at the 4-position and an electron-withdrawing group is preferred over an electron-donating group with the ester and amino groups at the 3-, 7-positions. In general, the activities against HCT-116 of the four series decreased in descending order: *VII*, *III*, *VIII*, *V*.

Concerning the activity against HepG-2, all the series of compounds *VIIe*, *VIIc*, *IIIg*, *IIIa* (IC_{50} = 2.5–4.1 μg mL^{-1}) exhibited higher anti-tumour activities against HepG-2 than vinblastine (IC_{50} = 4.6 μg mL^{-1}) and colchicine (IC_{50} = 10.6 μg mL^{-1}), while compounds *VIIb*, *VIIa*, *VIIIf* and *VIIIe* (IC_{50} = 5.6–10.4 μg mL^{-1}) exhibited good activity in comparison with colchicine and the other derivatives and compound *IIId* (IC_{50} = 10.6 μg mL^{-1}) was equipotent as colchicine. This was due to the presence of the substituent, methyl group (electron-donating) or the chloro atom (electron-withdrawing) for the third series or nitro group (electron-withdrawing) or the unsubstituted phenyl group (electron-donating) for the first series at the 4-position. In general, the activities against HepG-2 of the four series decreased in the descending order: *VII*, *III*, *VIII*, *V*.

Conclusions

The current interest in the synthesis of 4*H*-benzo[*h*]chromene derivatives is to focus on their anti-tumour activities as part of a recent research line seeking the development of new heterocyclic compounds as potent anti-tumour agents (Abd-El-Aziz et al., 2004; El-Agrody et al., 2011, 2013, 2014a, 2014b; El-Agrody & Al-Ghamdi, 2011; Sabry et al., 2011; Al-Ghamdi et al., 2012). Accordingly, in the present study the synthesis of some 2-amino-4*H*-benzo[*h*]chromene and 2,7-diamino-4*H*-benzo[*h*]chromene derivatives was conducted, followed by an anti-tumour evaluation of all the synthesised compounds. Thirty compounds of 2-amino-4*H*-benzo[*h*]chromene and 2,7-diamino-4*H*-benzo[*h*]chromene derivatives were prepared and their structures were elucidated on the basis of IR, ^{1}H NMR, ^{13}C NMR, ^{13}C NMR-DEPT/APT and MS data. Compounds *VIIIc*, *VIId*, *VIIb*, *VIIe* and *VIIIg*, respectively, inhibited the growth of the MCF-7 cancer cell in comparison with vinblastine and colchicines; *VIIc*, *VIIIe*, *Vf* and *IIIa*, respectively, inhibited the growth of the MCF-7 cancer cell in comparison with colchicine, while compounds *VIIb*, *VIId*, *VIIe*, *IIIa*, *VIIa*, *VIIIc*, *VIIc*, *IIId*, *IIIg*, *IIIf*, *IIIb*, *IIIh*, *VIIIb*, *VIIIa*, *VIIIe*, *IIIc*, *Vg*, *IIIe*, *VIIIg*, *Vf* and *VIIIf*, respectively, inhibited the growth of HCT-116 cancer cell in comparison with colchicine. In addition, compounds *VIIe*, *IIIg*, *IIIa* and *VIIc*, respectively, inhibited the growth of the HepG-2 cancer cell in comparison with vinblastine and colchicine, while compounds *VIIb*, *VIIa, VIIIf* and *VIIIe*, respectively, inhibited the growth of the HepG-2 cancer cell in comparison with colchicine and the remaining compounds exhibited near or moderate to lower activities in comparison with the standard drugs vinblastine and colchicine. A more extensive study is required to determine additional anti-tumour parameters in order to gain a deeper insight into its structure–activity relationship and to optimise the effectiveness of this series of molecules, which can then be used in drug design or the development of anti-tumour therapeutics.

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