

ORIGINAL PAPER

Synthesis, in-vitro cytotoxicity of 4*H*-benzo[*h*]chromene derivatives
and structure–activity relationships of 4-aryl group
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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*, *VIIId*, *VIIb*, *VIIe*, *VIIIg* and *VIIIc*, *VIIId*, *VIIb*, *VIIe*, *VIIIg*, *VIIc*, *VIIIe*, *Vf*, *IIIa* inhibited the growth of MCF-7 in comparison with vinblastine and colchicine, while *VIIb*, *VIIId*, *VIIe*, *IIIa*, *VIIa*, *VIIIc*, *VIIc*, *IIIId*, *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.

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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 (Kemnitz 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 |
|--------------|---|--------|------------|---------|-----------------------|
| <i>IIIa</i> | C ₂₀ H ₁₄ N ₂ O | 298.34 | Colourless | 85 | 217, 218 ^a |
| <i>IIIb</i> | C ₂₀ H ₁₃ FN ₂ O | 316.33 | Yellow | 86 | 233, 232 ^b |
| <i>IIIc</i> | C ₂₀ H ₁₃ ClN ₂ O | 332.78 | Yellow | 88 | 234, 231 ^b |
| <i>III d</i> | C ₂₀ H ₁₃ BrN ₂ O | 377.23 | Colourless | 90 | 240 |
| <i>IIIe</i> | C ₂₁ H ₁₆ N ₂ O | 312.36 | Yellow | 90 | 206, 204 ^b |
| <i>III f</i> | C ₂₁ H ₁₆ N ₂ O ₂ | 328.36 | Yellow | 88 | 196, 195 ^a |
| <i>III g</i> | C ₂₀ H ₁₃ N ₃ O ₃ | 343.34 | Yellow | 88 | 241, 240 ^a |
| <i>III h</i> | C ₂₄ H ₂₁ N ₃ O 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). ¹H NMR and ¹³C NMR spectra were recorded using a Bruker AV 500 MHz spectrometer (Bruker, USA). ¹³C NMR spectra were obtained using distortion-free enhancement by polarisation transfer (DEPT), where the signals of the CH and CH₃ carbon atoms appear normal (up) and the signals of the carbon atoms in CH₂ environments appear negative (down). ¹³C NMR spectra were obtained using the attached proton test (APT); with this technique, the signals of the CH and CH₃ carbon atoms appears normal (up) and the signal of the CH₂ and C_q 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-4*H*-benzo[*h*]chromene-3-carbonitrile (*IIIa–IIIh*) and 4-aryl-2,7-diamino-4*H*-

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–IIIh*) (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-4*H*-benzo[*h*]chromene-3-carboxylate (*VIIa–VIIg*) and ethyl 4-aryl-2,7-diamino-4*H*-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*-monosubstituted cinnamates (*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 |
|-------------|---|
| <i>IIIa</i> | IR, $\tilde{\nu}/\text{cm}^{-1}$: 3447, 3304, 3189 (NH ₂), 3055, 3021, 2885 (CH), 2204 (CN) ¹ H NMR (DMSO- <i>d</i> ₆), δ : 8.28–7.11 (m, 11H, 3Ph), 7.20 (bs, 2H, NH ₂ , D ₂ O exchangeable), 4.91 (s, 1H, pyran ring) ¹³ C NMR (DMSO- <i>d</i> 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) |
| <i>IIIb</i> | IR, $\tilde{\nu}/\text{cm}^{-1}$: 3459, 3330, 3196 (NH ₂), 3091, 3034, 2989, 2861 (CH), 2193 (CN) ¹ H NMR (DMSO- <i>d</i> ₆), δ : 8.27–7.10 (m, 10H, 3Ph), 7.20 (bs, 2H, NH ₂ , D ₂ O exchangeable), 4.96 (s, 1H, pyran ring) ¹³ C NMR (DMSO- <i>d</i> 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) |
| <i>IIIc</i> | IR, $\tilde{\nu}/\text{cm}^{-1}$: 3453, 3334, 3197 (NH ₂), 3059, 3042, 2869 (CH), 2192 (CN) ¹ H NMR (DMSO- <i>d</i> ₆), δ : 8.29–7.10 (m, 10H, 3Ph), 7.22 (bs, 2H, NH ₂ , D ₂ O exchangeable), 4.96 (s, 1H, pyran ring) ¹³ C NMR (DMSO- <i>d</i> 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) |
| <i>IIId</i> | IR, $\tilde{\nu}/\text{cm}^{-1}$: 3458, 3342, 3202 (NH ₂), 3060, 3031, 2865 (CH), 2190 (CN) ¹ H NMR (DMSO- <i>d</i> ₆), δ : 8.27–7.09 (m, 10H, 3Ph), 7.24 (bs, 2H, NH ₂ , D ₂ O exchangeable), 4.95 (s, 1H, pyran ring) ¹³ C NMR (DMSO- <i>d</i> 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) |
| <i>IIIe</i> | IR, $\tilde{\nu}/\text{cm}^{-1}$: 3412, 3323, 3203 (NH ₂), 3057, 3031, 2957, 2837 (CH), 2194 (CN) ¹ H NMR (DMSO- <i>d</i> ₆), δ : 8.27–6.87 (m, 10H, 3Ph), 7.14 (bs, 2H, NH ₂ , D ₂ O exchangeable), 4.85 (s, 1H, pyran ring), 2.28 (s, 3H, CH ₃) ¹³ C NMR (DMSO- <i>d</i> 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) |
| <i>IIIf</i> | IR, $\tilde{\nu}/\text{cm}^{-1}$: 3452, 3339, 3211 (NH ₂), 3057, 2932, 2836 (CH), 2190 (CN) ¹ H NMR (DMSO- <i>d</i> ₆), δ : 8.26–6.87 (m, 10H, 3Ph), 7.12 (bs, 2H, NH ₂ , D ₂ O exchangeable), 4.85 (s, 1H, pyran ring), 3.72 (s, 3H, OCH ₃) ¹³ C NMR (DMSO- <i>d</i> 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) |
| <i>IIIg</i> | IR, $\tilde{\nu}/\text{cm}^{-1}$: 3460, 3359, 3210 (NH ₂), 3070, 2953, 2864 (CH), 2187 (CN) ¹ H NMR (DMSO- <i>d</i> ₆), δ : 8.21–7.12 (m, 10H, 3Ph), 7.37 (bs, 2H, NH ₂ , D ₂ O exchangeable), 5.18 (s, 1H, pyran ring) ¹³ C NMR (DMSO- <i>d</i> 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) |
| <i>IIIh</i> | IR, $\tilde{\nu}/\text{cm}^{-1}$: 3401, 3329, 3206 (NH ₂), 3055, 3016, 2968, 2938 (CH), 2192 (CN) ¹ H NMR (DMSO- <i>d</i> 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- <i>d</i> 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 C NMR-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

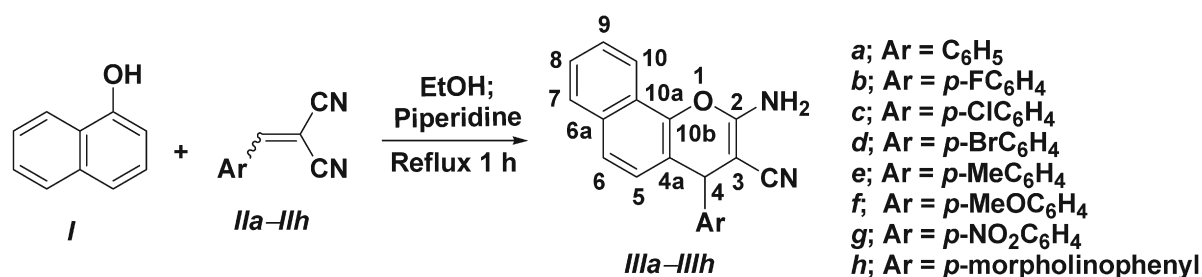
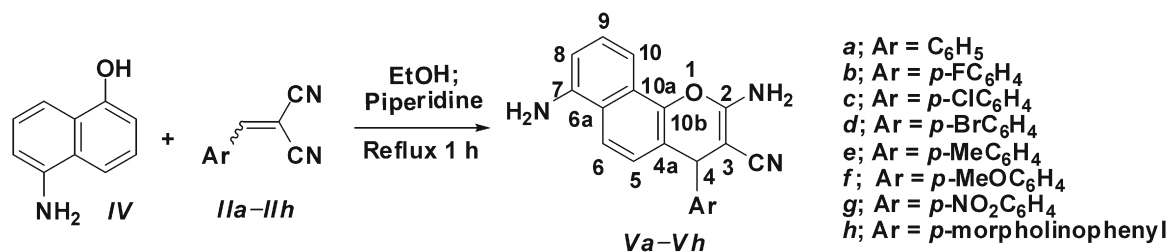
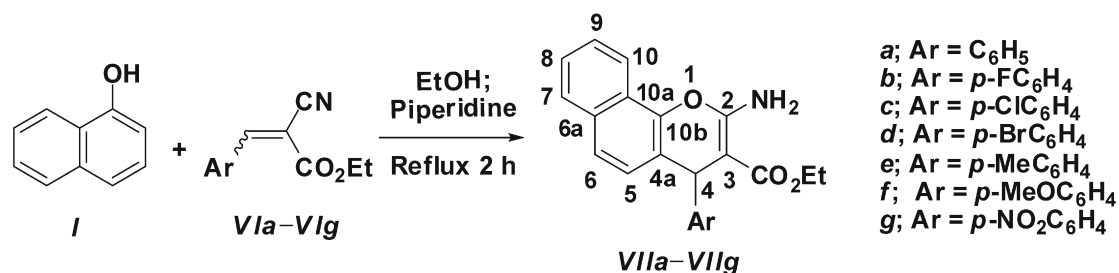
The treatment of 1-naphthol (*I*) with α -cyano-*p*-

monosubstituted cinnamitriles (*IIa–IIIh*) 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 |
|-----------|---|--------|--------|---------|-----------------------|
| <i>Va</i> | C ₂₀ H ₁₅ N ₃ O | 313.35 | Grey | 85 | 267, 267 ^a |
| <i>Vb</i> | C ₂₀ H ₁₄ FN ₃ O | 331.34 | Grey | 87 | 235 |
| <i>Vc</i> | C ₂₀ H ₁₄ ClN ₃ O | 347.8 | Grey | 81 | 266, 267 ^a |
| <i>Vd</i> | C ₂₀ H ₁₄ BrN ₃ O | 392.25 | Grey | 80 | 226 |
| <i>Ve</i> | C ₂₁ H ₁₇ N ₃ O | 327.38 | Grey | 83 | 230 |
| <i>Vf</i> | C ₂₁ H ₁₇ N ₃ O ₂ | 343.38 | Grey | 88 | 269, 267 ^a |
| <i>Vg</i> | C ₂₀ H ₁₄ N ₄ O ₃ | 358.35 | Red | 79 | 239 |
| <i>Vh</i> | C ₂₄ H ₂₂ N ₄ O 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 cinnamitriles (*IIa–IIIh*) 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 ba-

sis of IR, ¹H NMR, ¹³C NMR, ¹³C NMR-DEPT, ¹³C NMR-APT and MS data. The IR spectra of *IIIa–IIIh* and *Va–Vh* showed the appearance of an NH₂ 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 NH₂ 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 ¹H and ¹³C 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*) af-

forded 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*

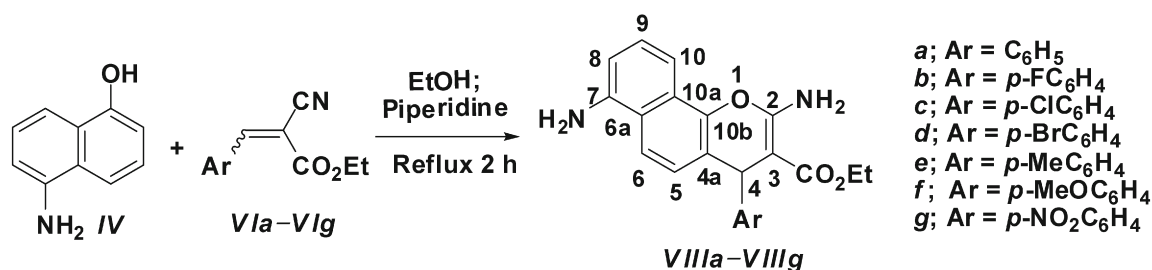
| Compound | Spectral data |
|-----------|---|
| <i>Va</i> | IR, $\bar{\nu}/\text{cm}^{-1}$: 3424, 3336, 3325, 3297, 3175 (2 NH ₂), 3023, 3001 (CH), 2200 (CN) ^1H NMR (DMSO- <i>d</i> ₆), δ : 7.75–6.70 (m, 10H, 3Ph), 7.10 (bs, 2H, NH ₂ -2, D ₂ O exchangeable), 5.78 (bs, 2H, NH ₂ -7, D ₂ O exchangeable), 4.84 (s, 1H, pyran ring) ^{13}C NMR (DMSO- <i>d</i> 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) |
| <i>Vb</i> | IR, $\bar{\nu}/\text{cm}^{-1}$: 3450, 3386, 3332, 3257, 3203 (2 NH ₂), 3028, 3006 (CH), 2195 (CN) ^1H NMR (DMSO- <i>d</i> ₆), δ : 7.76–6.71 (m, 9H, 3Ph), 7.13 (bs, 2H, NH ₂ -2, D ₂ O exchangeable), 5.80 (bs, 2H, NH ₂ -7, D ₂ O exchangeable), 4.89 (s, 1H, pyran ring) ^{13}C NMR (DMSO- <i>d</i> 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) |
| <i>Vc</i> | IR, $\bar{\nu}/\text{cm}^{-1}$: 3463, 3341, 3311, 3287, 3197 (2 NH ₂), 3021, 3016 (CH), 2188 (CN) ^1H NMR (DMSO- <i>d</i> ₆), δ : 7.76–6.74 (m, 9H, 3Ph), 7.15 (bs, 2H, NH ₂ -2, D ₂ O exchangeable), 5.75 (bs, 2H, NH ₂ -7, D ₂ O exchangeable), 4.90 (s, 1H, pyran ring) ^{13}C NMR (DMSO- <i>d</i> 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) |
| <i>Vd</i> | IR, $\bar{\nu}/\text{cm}^{-1}$: 3469, 3364, 3309, 3293, 3167 (2 NH ₂), 3011, 3019 (CH), 2186 (CN) ^1H NMR (DMSO- <i>d</i> ₆), δ : 7.78–6.74 (m, 9H, 3Ph), 7.17 (bs, 2H, NH ₂ -2, D ₂ O exchangeable), 5.81 (bs, 2H, NH ₂ -7, D ₂ O exchangeable), 4.89 (s, 1H, pyran ring) ^{13}C NMR (DMSO- <i>d</i> 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 \uparrow), 129.77, (CH \uparrow), 128.21 (CH \uparrow), 123.93 (CH \uparrow), 118.69 (CH \uparrow), 108.40 (CH \uparrow), 107.90 (CH \uparrow), 40.19 (CH \uparrow) ^{13}C NMR-DEPT at 90° only CH signals are (up), δ : 131.54 (CH \uparrow), 129.77, (CH \uparrow), 128.21 (CH \uparrow), 123.93 (CH \uparrow), 118.69 (CH \uparrow), 108.40 (CH \uparrow), 107.90 (CH \uparrow), 40.19 (CH \uparrow) ^{13}C NMR-DEPT at 45° CH, CH ₂ and CH ₃ signals are (up), δ : 131.54 (CH \uparrow), 129.77, (CH \uparrow), 128.21 (CH \uparrow), 123.93 (CH \uparrow), 118.69 (CH \uparrow), 108.40 (CH \uparrow), 107.90 (CH \uparrow), 40.19 (CH \uparrow) ^{13}C NMR-APT spectrum CH, CH ₃ (up), CH ₂ , Cq (down), δ : 160.23 (C \downarrow), 145.15 (C \downarrow), 144.82 (C \downarrow), 142.81 (C \downarrow), 131.54 (CH \uparrow), 129.77 (CH \uparrow), 128.21 (CH \uparrow), 127.45 (C \downarrow), 123.93 (CH \uparrow), 123.11 (C \downarrow), 120.42 (C \downarrow), 119.87 (C \downarrow), 118.69 (CH \uparrow), 116.72 (CN \downarrow), 108.40 (CH \uparrow), 107.90 (CH \uparrow), 55.58 (C \downarrow), 40.19 (CH \uparrow) MS, m/z ($I_r/\%$): 393 (17.18) (M ⁺⁺ 2), 391 (17.34) (M ⁺) with a base peak at 237 (100) |
| <i>Ve</i> | IR, $\bar{\nu}/\text{cm}^{-1}$: 3462, 3399, 3324, 3273, 3189 (2 NH ₂), 3051, 3032 (CH), 2184 (CN) ^1H NMR (DMSO- <i>d</i> ₆), δ : 7.74–6.70 (m, 9H, 3Ph), 7.06 (bs, 2H, NH ₂ -2, D ₂ O exchangeable), 5.78 (bs, 2H, NH ₂ -7, D ₂ O exchangeable), 4.79 (s, 1H, pyran ring), 2.25 (s, 3H, CH ₃) ^{13}C NMR (DMSO- <i>d</i> 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 \uparrow), 127.40 (CH \uparrow), 127.33 (CH \uparrow), 123.30 (CH \uparrow), 118.46 (CH \uparrow), 108.25 (CH \uparrow), 107.88 (CH \uparrow), 40.41 (CH \uparrow), 20.49 (CH ₃ \uparrow) ^{13}C NMR-DEPT spectrum at 90° only CH signals are (up), δ : 129.07 (CH \uparrow), 127.40 (CH \uparrow), 127.33 (CH \uparrow), 123.30 (CH \uparrow), 118.46 (CH \uparrow), 108.25 (CH \uparrow), 107.88 (CH \uparrow), 40.41 (CH \uparrow) ^{13}C NMR-DEPT spectrum at 45° CH, CH ₂ and CH ₃ signals are (up), δ : 129.07 (CH \uparrow), 127.40 (CH \uparrow), 127.33 (CH \uparrow), 123.30 (CH \uparrow), 118.46 (CH \uparrow), 108.25 (CH \uparrow), 107.88 (CH \uparrow), 40.41 (CH \uparrow) ^{13}C NMR-APT spectrum CH, CH ₃ (up), CH ₂ , Cq (down), δ : 160.12 (C), 144.79 (C), 142.85 (C \downarrow), 142.71 (C \downarrow), 135.81 (C \downarrow), 129.07 (CH \uparrow), 127.40 (CH \uparrow), 127.33 (CH \uparrow), 123.92 (C \downarrow), 123.30 (CH \uparrow), 121.90 (C \downarrow), 120.57 (C \downarrow), 118.46 (CH \uparrow), 117.45 (CN \downarrow), 108.25 (CH \uparrow), 107.88 (CH \uparrow), 56.15 (C \downarrow), 40.41 (CH \uparrow), 20.49 (CH ₃ \uparrow) MS, m/z ($I_r/\%$): 327 (28.55) (M ⁺) with a base peak at 237 (100) |

Table 4. (continued)

| Compound | Spectral data |
|-----------|--|
| <i>Vf</i> | IR, $\tilde{\nu}/\text{cm}^{-1}$: 3422, 3382, 3330, 3273, 3209 (2 NH ₂), 3054, 3031 (CH), 2186 (CN) ¹ H NMR (DMSO- <i>d</i> ₆), δ : 7.74–6.70 (m, 9H, 3Ph), 7.02 (bs, 2H, NH ₂ -2, D ₂ O exchangeable), 5.76 (bs, 2H, NH ₂ -7, D ₂ O exchangeable), 4.78 (s, 1H, pyran ring), 3.71 (s, 3H, CH ₃) ¹³ C NMR (DMSO- <i>d</i> 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 , C _q (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 ↑) |
| <i>Vg</i> | IR, $\tilde{\nu}/\text{cm}^{-1}$: 3460, 3360, 3310, 3273, 3198 (2 NH ₂), 3074, 3021 (CH), 2188 (CN) ¹ H NMR (DMSO- <i>d</i> ₆), δ : 8.22–6.74 (m, 9H, 3Ph), 7.27 (bs, 2H, NH ₂ -2, D ₂ O exchangeable), 5.82 (bs, 2H, NH ₂ -7, D ₂ O exchangeable), 5.11 (s, 1H, pyran ring) ¹³ C NMR (DMSO- <i>d</i> 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) |
| <i>Vh</i> | IR, $\tilde{\nu}/\text{cm}^{-1}$: 3464, 3361, 3315, 3273, 3201 (2 NH ₂), 3074, 3021, 2877, 2861, 2833 (CH), 2188 (CN) ¹ H NMR (DMSO- <i>d</i> ₆), δ : 7.73–6.70 (m, 9H, 3Ph), 7.02 (bs, 2H, NH ₂ -7, D ₂ O exchangeable), 5.77 (bs, 2H, NH ₂ -2, D ₂ O 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- <i>d</i> 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./°C |
|--------------|---|--------|--------|---------|---------|
| <i>VIIa</i> | C ₂₂ H ₁₉ NO ₃ | 345.39 | Yellow | 78 | 150 |
| <i>VIIb</i> | C ₂₂ H ₁₈ FNO ₃ | 363.38 | Yellow | 81 | 155 |
| <i>VIIc</i> | C ₂₂ H ₁₈ ClNO ₃ | 379.84 | Yellow | 83 | 160 |
| <i>VII d</i> | C ₂₂ H ₁₈ BrNO ₃ | 424.29 | Yellow | 80 | 162 |
| <i>VIIe</i> | C ₂₃ H ₂₁ NO ₃ | 359.42 | Yellow | 79 | 152 |
| <i>VII f</i> | C ₂₃ H ₂₁ NO ₄ | 375.42 | Yellow | 80 | 161 |
| <i>VII g</i> | C ₂₂ H ₁₈ N ₂ O 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, ¹H NMR, ¹³C NMR, ¹³C NMR-DEPT, ¹³C NMR-APT and MS data. The IR spectra of *VIIa–VIIg* and *VIIIa–VIIIg* showed the appearance of an

NH₂ stretch at 3469–3380 cm⁻¹, 3319–3270 cm⁻¹ a CO stretch at 1687–1664 cm⁻¹ for *VIIa–VIIg* and NH₂ 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 ¹H and

¹³C 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 ¹H signals at δ of 4.05–3.98 (q, 2H, CH₂, *J* = 7.0–7.2 Hz), 1.12–

Table 6. Spectral data of compounds *VII*

| Compound | Spectral data |
|-------------|--|
| <i>VIIa</i> | <p>IR, $\bar{\nu}/\text{cm}^{-1}$: 3385, 3270 (NH₂), 3067, 3029, 2982, 2900, 2876 (CH), 1664 (CO)</p> <p>¹H NMR (DMSO-<i>d</i>₆), δ: 8.31–7.10 (m, 11H, 3Ph), 7.79 (bs, 2H, NH₂, D₂O exchangeable), 5.03 (s, 1H, pyran ring), 4.00 (q, 2H, <i>J</i> = 7.1 Hz, CH₂), 1.11 (t, 3H, <i>J</i> = 7.1 Hz, CH₃)</p> <p>¹³C NMR (DMSO-<i>d</i>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 (CH₂), 39.99 (CH), 14.18 (CH₃)</p> <p>¹³C NMR-DEPT at 135° CH, CH₃ (up), CH₂ (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 (CH₂ ↓), 39.99 (CH ↑), 14.18 (CH₃ ↑)</p> <p>¹³C 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 ↑)</p> <p>¹³C NMR-DEPT at 45° CH, CH₂ and CH₃ 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 (CH₂ ↑), 39.99 (CH ↑), 14.18 (CH₃ ↑)</p> <p>¹³CNMR-APT CH, CH₃ (up), CH₂, 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 (CH₂ ↓), 39.99 (CH ↑), 14.18 (CH₃ ↑)</p> <p>MS, <i>m/z</i> (<i>I_r</i>/%) : 345 (23.84) (M⁺) with a base peak at 237 (100)</p> |
| <i>VIIb</i> | <p>IR, $\bar{\nu}/\text{cm}^{-1}$: 3385, 3288 (NH₂), 3077, 3063, 2987, 2978, 2960, (CH), 1668 (CO)</p> <p>¹H NMR (DMSO-<i>d</i>₆), δ: 8.33–7.03 (m, 10H, 3Ph), 7.82 (bs, 2H, NH₂, D₂O exchangeable), 5.06 (s, 1H, pyran ring), 4.01 (q, 2H, <i>J</i> = 7.2 Hz, CH₂), 1.10 (t, 3H, <i>J</i> = 7.2 Hz, CH₃)</p> <p>¹³C NMR (DMSO-<i>d</i>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 (CH₂), 40.02 (CH), 14.21 (CH₃)</p> <p>¹³C NMR-DEPT at 135° CH, CH₃ (up), CH₂ (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 (CH₂ ↓), 40.02 (CH ↑), 14.21 (CH₃ ↑)</p> <p>¹³C 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 ↑)</p> <p>¹³C NMR-DEPT at 45° CH, CH₂ and CH₃ 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 (CH₂ ↑), 40.02 (CH ↑), 14.21 (CH₃ ↑)</p> <p>¹³CNMR-APT CH, CH₃ (up), CH₂, 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 (CH₂ ↓), 40.02 (CH ↑), 14.21 (CH₃ ↑)</p> <p>MS, <i>m/z</i> (<i>I_r</i>/%) : 363 (1.64) (M⁺) with a base peak at 68 (100)</p> |
| <i>VIIc</i> | <p>IR, $\bar{\nu}/\text{cm}^{-1}$: 3469, 3318 (NH₂), 3079, 3058, 2977, 2955, 2903 (CH), 1678 (CO)</p> <p>¹H NMR (DMSO-<i>d</i>₆), δ: 8.32–7.25 (m, 10H, 3Ph), 7.83 (bs, 2H, NH₂), 5.06 (s, 1H, pyran ring), 4.01 (q, 2H, <i>J</i> = 7.1 Hz, CH₂), 1.10 (t, 3H, <i>J</i> = 7.1 Hz, CH₃)</p> <p>¹³C NMR (DMSO-<i>d</i>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 (CH₂), 39.44 (CH), 14.20 (CH₃)</p> <p>¹³C NMR-DEPT at 135° CH, CH₃ (up), CH₂ (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 (CH₂ ↓), 39.44 (CH ↑), 14.20 (CH₃ ↑)</p> <p>¹³C 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 ↑)</p> <p>¹³C NMR-DEPT at 45° CH, CH₂ and CH₃ 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 (CH₂ ↑), 39.44 (CH ↑), 14.20 (CH₃ ↑)</p> <p>¹³CNMR-APT CH, CH₃ (up), CH₂, 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 (CH₂ ↓), 39.44 (CH ↑), 14.20 (CH₃ ↑)</p> <p>MS, <i>m/z</i> (<i>I_r</i>/%) : 381(8.18)(M⁺ + 2), 379 (24.72) (M⁺) with a base peak at 269 (100)</p> |

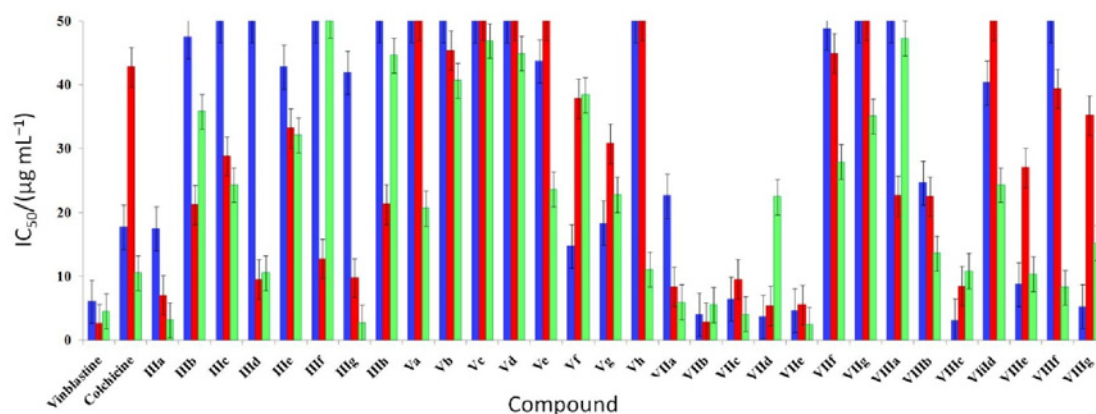
Table 6. (continued)

| Compound | Spectral data |
|-------------------|---|
| VIII _d | <p>IR, $\tilde{\nu}/\text{cm}^{-1}$: 3463, 3319 (NH₂), 3077, 3069, 2978, 2976, 2931 (CH), 1677 (CO)</p> <p>¹H NMR (DMSO-<i>d</i>₆), δ: 8.33–7.20 (m, 10H, 3Ph), 7.84 (bs, 2H, NH₂), 5.05 (s, 1H, pyran ring), 4.01 (q, 2H, $J = 7.2$ Hz, CH₂), 1.10 (t, 3H, $J = 7.2$ Hz, CH₃)</p> <p>¹³C NMR (DMSO-<i>d</i>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 (CH₂), 39.29 (CH), 14.22 (CH₃)</p> <p>¹³C NMR-DEPT at 135° CH, CH₃ (up), CH₂ (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 (CH₂ ↓), 39.29 (CH ↑), 14.22 (CH₃ ↑)</p> <p>¹³C 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 ↑)</p> <p>¹³C NMR-DEPT at 45° CH, CH₂ and CH₃ 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 (CH₂ ↑), 39.29 (CH ↑), 14.22 (CH₃ ↑)</p> <p>¹³CNMR-APT CH, CH₃ (up), CH₂, C_q (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 (CH₂ ↓), 39.29 (CH ↑), 14.22 (CH₃ ↑)</p> <p>MS, m/z ($I_r/\%$): 425 (12.18) (M⁺ + 2), 423 (12.83) (M⁺) with a base peak at 269 (100)</p> |
| VIII _e | <p>IR, $\tilde{\nu}/\text{cm}^{-1}$: 3380, 3287 (NH₂), 3079, 3063, 2996, 2977, 2901 (CH), 1681 (CO)</p> <p>¹H NMR (DMSO-<i>d</i>₆), δ: 8.30–7.02 (m, 10H, 3Ph), 7.76 (bs, 2H, NH₂, D₂O exchangeable), 4.98 (s, 1H, pyran ring), 3.99 (q, 2H, $J = 7.2$ Hz, CH₂), 2.19 (s, 3H, CH₃), 1.11 (t, 3H, $J = 7.2$ Hz, CH₃)</p> <p>¹³C NMR (DMSO-<i>d</i>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 (CH₂), 39.44 (CH), 20.44 (CH₃), 14.22 (CH₃)</p> <p>¹³C NMR-DEPT at 135° CH, CH₃ (up), CH₂ (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 (CH₂ ↓), 39.44 (CH ↑), 20.44 (CH₃ ↑), 14.22 (CH₃ ↑)</p> <p>¹³C 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 ↑)</p> <p>¹³C NMR-DEPT at 45° CH, CH₂ and CH₃ 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 (CH₂ ↑), 39.44 (CH ↑), 20.44 (CH₃ ↑), 14.22 (CH₃ ↑)</p> <p>¹³CNMR-APT CH, CH₃ (up), CH₂, C_q (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 (CH₂ ↓), 39.44 (CH ↑), 20.44 (CH₃ ↑), 14.22 (CH₃ ↑)</p> <p>MS, m/z ($I_r/\%$): 359 (15.84) (M⁺) with a base peak at 273 (100)</p> |
| VIII _f | <p>IR, $\tilde{\nu}/\text{cm}^{-1}$: 3467, 3325 (NH₂), 3081, 3062, 2993, 2983, 2904 (CH), 1687 (CO)</p> <p>¹H NMR (DMSO-<i>d</i>₆), δ: 8.31–6.77 (m, 10H, 3Ph), 7.76 (bs, 2H, NH₂), 4.98 (s, 1H, pyran ring), 4.00 (q, 2H, $J = 7.2$ Hz, CH₂), 3.66 (s, 3H, OCH₃), 1.12 (t, 3H, $J = 7.2$ Hz, CH₃)</p> <p>¹³C NMR (DMSO-<i>d</i>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 (CH₂), 54.90 (CH₃), 39.17 (CH), 14.30 (CH₃)</p> <p>¹³C NMR-DEPT at 135° CH, CH₃ (up), CH₂ (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 (CH₂ ↓), 54.90 (CH₃ ↑), 39.17 (CH ↑), 14.30 (CH₃ ↑)</p> <p>¹³C 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 ↑)</p> <p>¹³C NMR-DEPT spectrum at 45° CH, CH₂ and CH₃ 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 (CH₂ ↑), 54.90 (CH₃ ↑), 39.17 (CH ↑), 14.30 (CH₃ ↑)</p> <p>¹³CNMR-APT CH, CH₃ (up), CH₂, C_q (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 (CH₂ ↓), 54.90 (CH₃ ↑), 39.17 (CH ↑), 14.30 (CH₃ ↑)</p> <p>MS, m/z ($I_r/\%$): 375 (11.32) (M⁺) with a base peak at 269 (100)</p> |
| VIII _g | <p>IR, $\tilde{\nu}/\text{cm}^{-1}$: 3467, 3317 (NH₂), 3085, 3066, 2995, 2977, 2908 (CH), 1687 (CO)</p> <p>¹H NMR (DMSO-<i>d</i>₆), δ: 8.47–7.13 (m, 10H, 3Ph), 8.00 (bs, 2H, NH₂, D₂O exchangeable), 5.28 (s, 1H, pyran ring), 3.99 (q, 2H, $J = 7$ Hz, CH₂), 1.11 (t, 3H, $J = 7$ Hz, CH₃)</p> <p>¹³C NMR (DMSO-<i>d</i>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 (CH₂), 40.03 (CH), 14.30 (CH₃)</p> <p>MS, m/z ($I_r/\%$): 390 (13.46) (M⁺) with a base peak at 269 (100)</p> |

Table 7. Characterisation data of compounds *VIII*

| Compound | Formula | M_r | Colour | Yield/% | M.p./°C |
|---------------|------------------------|--------|--------|---------|-----------------------|
| <i>VIIIa</i> | $C_{22}H_{20}N_2O_3$ | 360.41 | Grey | 74 | 203, 203 ^a |
| <i>VIIIb</i> | $C_{22}H_{19}FN_2O_3$ | 378.40 | Grey | 77 | 210 |
| <i>VIIIc</i> | $C_{22}H_{19}ClN_2O_3$ | 394.85 | Red | 73 | 185, 185 ^a |
| <i>VIII d</i> | $C_{22}H_{19}BrN_2O_3$ | 439.30 | Red | 75 | 188 |
| <i>VIII e</i> | $C_{23}H_{22}N_2O_3$ | 374.43 | Red | 76 | 196 |
| <i>VIII f</i> | $C_{23}H_{22}N_2O_4$ | 390.43 | Red | 75 | 192, 192 ^a |
| <i>VIII g</i> | $C_{22}H_{19}N_3O_5$ | 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, CH₃, $J = 7.0\text{--}7.2$ Hz) with the corresponding signals in the ¹³C spectra at δ of 58.65–58.46 (CH₂) and 14.32–14.18 (CH₃), respectively. The ¹³C NMR-DEPT spectra at 45, ° 90, ° 135, ° ¹³C 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 NH₂ protons resonated at δ of 7.37–7.02 (sharp singlet), while compounds *VII* and *VIII* showed that the NH₂ 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 cyto-

toxicity 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 $\mu\text{g mL}^{-1}$, 25 $\mu\text{g mL}^{-1}$, 12.5 $\mu\text{g mL}^{-1}$, 6.25 $\mu\text{g mL}^{-1}$, 3.125 $\mu\text{g mL}^{-1}$, 1.56 $\mu\text{g mL}^{-1}$ and 0 $\mu\text{g mL}^{-1}$). 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 IC₅₀ of 17.5 $\mu\text{g mL}^{-1}$ and 14.8 $\mu\text{g mL}^{-1}$, respectively, which exhibited good activity relative to colchicine (IC₅₀ = 17.7 $\mu\text{g mL}^{-1}$) and more reduction in potency with the other derivatives *IIIg*, *IIIe*, *IIIb*, *IIIc*,

*III*d, *III*f, *III*h and *V*g, *V*e, *V*a, *V*b, *V*c, *V*d, *V*h with $IC_{50} = 41.19\text{--}47.5 \mu\text{g mL}^{-1}$ or $> 50 \mu\text{g mL}^{-1}$ and $18.4\text{--}43.7 \mu\text{g mL}^{-1}$ or $> 50 \mu\text{g mL}^{-1}$, respectively, as compared with vinblastine ($IC_{50} = 6.1 \mu\text{g mL}^{-1}$) and colchicine ($IC_{50} = 17.7 \mu\text{g mL}^{-1}$), 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 NH₂-7 (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 |
|---------------|--|
| <i>VIII</i> a | IR, $\tilde{\nu}/\text{cm}^{-1}$: 3436, 3390, 3350, 3275 (2 NH ₂), 3064, 3021, 2983 (CH), 1677 (CO) ¹ H NMR (DMSO- <i>d</i> ₆), δ : 7.78–6.69 (m, 10H, 3Ph), 7.76 (bs, 2H, NH ₂ -2, D ₂ O exchangeable), 5.76 (bs, 2H, NH ₂ -7, D ₂ O exchangeable), 4.98 (s, 1H, pyran ring), 3.99 (q, 2H, <i>J</i> = 7 Hz, CH ₂), 1.08 (t, 3H, <i>J</i> = 7 Hz, CH ₃) ¹³ C NMR (DMSO- <i>d</i> 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 C NMR-APT CH, CH ₃ (up), CH ₂ , C _q (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 ↑) |
| <i>VIII</i> b | IR, $\tilde{\nu}/\text{cm}^{-1}$: 3440, 3390, 3354, 3222 (2 NH ₂), 3060, 2988, 2932 (CH), 1679 (CO) ¹ H NMR (DMSO- <i>d</i> 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, <i>J</i> = 7 Hz, CH ₂), 1.10 (t, 3H, <i>J</i> = 7 Hz, CH ₃) ¹³ C NMR (DMSO- <i>d</i> 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, <i>m/z</i> (<i>I</i> _r /%) : 378 (13.46) (M ⁺) with a base peak at 264 (100) |
| <i>VIII</i> c | IR, $\tilde{\nu}/\text{cm}^{-1}$: 3417, 3390, 3324, 3250 (2 NH ₂), 3065, 2982, 2931 (CH), 1667 (CO) ¹ H NMR (DMSO- <i>d</i> ₆), δ : 7.77–6.72 (m, 9H, 3Ph), 7.81 (bs, 2H, NH ₂ -2, D ₂ O exchangeable), 5.79 (bs, 2H, NH ₂ -7, D ₂ O exchangeable), 5.02 (s, 1H, pyran ring), 4.00 (q, 2H, <i>J</i> = 7.2 Hz, CH ₂), 1.10 (t, 3H, <i>J</i> = 7.2 Hz, CH ₃) ¹³ C NMR (DMSO- <i>d</i> 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, <i>m/z</i> (<i>I</i> _r /%) : 398 (1.11) (M ⁺ + 2), 396 (3.46) (M ⁺) with a base peak at 264 (100) |
| <i>VIII</i> d | IR, $\tilde{\nu}/\text{cm}^{-1}$: 3449, 3377, 3355, 3271 (2 NH ₂), 3062, 2963 (CH), 1679 (CO) ¹ H NMR (DMSO- <i>d</i> ₆), δ : 7.76–6.71 (m, 9H, 3Ph), 7.15 (bs, 2H, NH ₂ -2, D ₂ O exchangeable), 5.80 (bs, 2H, NH ₂ -7, D ₂ O exchangeable), 4.88 (s, 1H, pyran ring), 3.99 (q, 2H, <i>J</i> = 7 Hz, CH ₂), 1.06 (t, 3H, <i>J</i> = 7 Hz, CH ₃) ¹³ C NMR (DMSO- <i>d</i> 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 C NMR-APT CH, CH ₃ (up), CH ₂ , C _q (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 ↑) |
| <i>VIII</i> e | MS, <i>m/z</i> (<i>I</i> _r /%) : 440 (1.11) (M ⁺ + 2), 438 (1.16) (M ⁺) with a base peak at 236 (100) IR, $\tilde{\nu}/\text{cm}^{-1}$: 3439, 3362, 3358, 3272 (2 NH ₂), 3061, 2932, 2859 (CH), 1678 (CO) ¹ H NMR (DMSO- <i>d</i> 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, <i>J</i> = 7 Hz, CH ₂), 2.19 (s, 3H, CH ₃), 1.08 (t, 3H, <i>J</i> = 7 Hz, CH ₃) ¹³ C NMR (DMSO- <i>d</i> 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, <i>m/z</i> (<i>I</i> _r /%) : 374 (42.35) (M ⁺) with a base peak at 248 (100) |

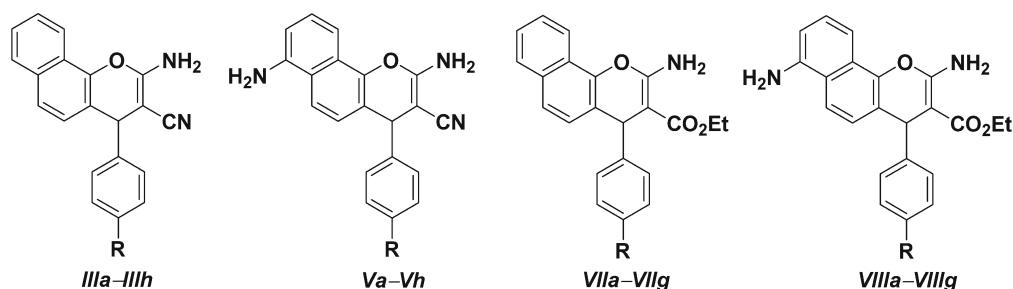
Table 8. (continued)

| Compound | Spectral data |
|---------------|--|
| <i>VIII f</i> | IR, $\tilde{\nu}/\text{cm}^{-1}$: 3463, 3366, 3342, 3257 (2 NH ₂), 3071, 2023, 2982 (CH), 1667 (CO) ¹ H NMR (DMSO- <i>d</i> 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, <i>J</i> = 7 Hz, CH ₂), 3.66 (s, 3H, CH ₃), 1.10 (t, 3H, <i>J</i> = 7 Hz, CH ₃) ¹³ C NMR (DMSO- <i>d</i> 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, <i>m/z</i> (<i>I_r</i> /%) : 390 (1.16) (M ⁺) with a base peak at 56 (100) |
| <i>VIII g</i> | IR, $\tilde{\nu}/\text{cm}^{-1}$: 3470, 3376, 3351, 3291 (2 NH ₂), 3071, 2023, 2982 (CH), 1670 (CO) ¹ H NMR (DMSO- <i>d</i> ₆), δ : 8.14–6.72 (m, 9H, 3Ph), 7.89 (bs, 2H, NH ₂ , D ₂ O exchangeable), 5.81 (bs, 2H, NH ₂ , D ₂ O exchangeable), 5.19 (s, 1H, pyran ring), 3.99 (q, 2H, <i>J</i> = 7 Hz, CH ₂), 1.09 (t, 3H, <i>J</i> = 7 Hz, CH ₃) ¹³ C NMR (DMSO- <i>d</i> 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, <i>m/z</i> (<i>I_r</i> /%) : 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 *VIII d*, 4-fluorophenyl *VIII b*,

4-methylphenyl *VIII e* (IC₅₀ = 3.7–4.7 μg mL⁻¹) and 4-chlorophenyl *VIII c*, 4-nitrophenyl *VIII g*, analogues (IC₅₀ of 3.1 μg mL⁻¹ and 5.3 μg mL⁻¹, respectively) exhibited good activity against MCF-7 compared with vinblastine (IC₅₀ = 6.1 μg mL⁻¹) and colchicine (IC₅₀ = 17.7 μg mL⁻¹), while 4-chlorophenyl *VIII c* and 4-methylphenyl *VIII e* with (IC₅₀ of 6.5 μg mL⁻¹ and 8.8 μg mL⁻¹, respectively) have a significant potent anti-tumour activity in comparison 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 NH₂-7 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 *VIII b* of the third series (IC₅₀ = 2.9 μg mL⁻¹) to have potent anti-tumour activity against the HCT-116 closet to vinblastine (IC₅₀ = 2.6 μg mL⁻¹); the other compounds of the four series exhibited moderate to lower activities, while compounds *III a*, *III d*, *III g*, *III f*, *III b*, *III h*, *III c*, *III e* of the first series (IC₅₀ = 7.1–33.2 μg mL⁻¹) and *V g*, *V f* of the second series (IC₅₀ of 30.8 μg mL⁻¹ and 37.8 μg mL⁻¹, respectively) had the most potent activity against HCT-116 compared to colchicine (IC₅₀ = 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 NH₂-7 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 IC₅₀ of target compounds against different cell lines in comparison with the standard as measured with the MTT method^a

| Compound | R | MCF-7 | HCT-116 | HepG-2 | Compound | R | MCF-7 | HCT-116 | HepG-2 |
|-------------|-----------------|------------|------------|------------|---------------|-----------------|------------|------------|------------|
| <i>IIIa</i> | H | 17.5 ± 0.1 | 7.1 ± 0.1 | 3.2 ± 0.0 | <i>VIIa</i> | H | 22.6 ± 0.1 | 8.4 ± 0.1 | 6.0 ± 0.3 |
| <i>IIIb</i> | F | 47.5 ± 0.0 | 21.2 ± 0.3 | 35.8 ± 0.1 | <i>VIIb</i> | F | 4.1 ± 0.1 | 2.9 ± 0.2 | 5.6 ± 0.1 |
| <i>IIIc</i> | Cl | W | 28.8 ± 0.1 | 24.3 ± 0.0 | <i>VIIc</i> | Cl | 6.5 ± 0.2 | 9.6 ± 0.7 | 4.1 ± 0.4 |
| <i>IIId</i> | Br | W | 9.6 ± 0.1 | 10.6 ± 0.1 | <i>VIIId</i> | Br | 3.7 ± 0.2 | 5.4 ± 0.0 | 22.5 ± 0.2 |
| <i>IIIe</i> | Me | 42.8 ± 0.4 | 33.2 ± 0.0 | 32.1 ± 0.1 | <i>VIIe</i> | Me | 4.7 ± 0.0 | 5.6 ± 0.0 | 2.5 ± 0.0 |
| <i>IIIf</i> | OMe | W | 12.8 ± 0.0 | W | <i>VIIIf</i> | OMe | 48.8 ± 0.0 | 44.9 ± 0.0 | 27.9 ± 0.1 |
| <i>IIIg</i> | NO ₂ | 41.9 ± 0.1 | 9.8 ± 0.0 | 2.8 ± 0.0 | <i>VIIIg</i> | NO ₂ | W | W | 35.1 ± 0.0 |
| <i>IIIh</i> | A | W | 21.3 ± 0.0 | 44.6 ± 0.3 | <i>VIIIa</i> | H | W | 22.6 ± 0.4 | 47.3 ± 0.2 |
| <i>Va</i> | H | W | W | 20.7 ± 0.1 | <i>VIIIb</i> | F | 24.6 ± 0.4 | 22.5 ± 0.4 | 13.7 ± 0.0 |
| <i>Vb</i> | F | W | 45.4 ± 0.0 | 40.7 ± 0.0 | <i>VIIIc</i> | Cl | 3.1 ± 0.1 | 8.5 ± 0.0 | 10.9 ± 0.1 |
| <i>Vc</i> | Cl | W | W | 46.9 ± 0.4 | <i>VIIIId</i> | Br | 40.3 ± 0.1 | W | 24.3 ± 0.2 |
| <i>Vd</i> | Br | W | W | 44.9 ± 0.1 | <i>VIIIe</i> | Me | 8.8 ± 0.0 | 27.0 ± 0.0 | 10.4 ± 0.1 |
| <i>Ve</i> | Me | 43.7 ± 0.0 | W | 23.6 ± 0.2 | <i>VIIIf</i> | OMe | W | 39.4 ± 0.1 | 8.3 ± 0.1 |
| <i>Vf</i> | OMe | 14.8 ± 0.0 | 37.8 ± 0.1 | 38.4 ± 0.4 | <i>VIIIg</i> | NO ₂ | 5.3 ± 0.0 | 35.2 ± 0.0 | 15.3 ± 0.3 |
| <i>Vg</i> | 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 |
| <i>Vh</i> | A | W | W | 11.1 ± 0.2 | C | – | 17.7 ± 0.0 | 42.8 ± 0.1 | 10.6 ± 0.0 |

^a) IC₅₀ 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 (IC₅₀ > 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 *VIIId*, 4-methylphenyl *VIIe*, phenyl *VIIa*, 4-chlorophenyl *VIIc* analogues for the third series with (IC₅₀ 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 *VIIa*, 4-methylphenyl *VIIIe*, 4-nitrophenyl *VIIIg*, 4-methoxyphenyl *VIIIf* analogues for the fourth series with (IC₅₀ 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 (IC₅₀ = 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* (IC₅₀ = 2.5–4.1 μg mL⁻¹) exhibited higher anti-tumour activities against HepG-2 than vinblastine (IC₅₀ = 4.6 μg mL⁻¹) and colchicine (IC₅₀ = 10.6 μg mL⁻¹), while compounds *VIIb*, *VIIa*, *VIIIf* and *VIIIe* (IC₅₀ = 5.6–10.4 μg mL⁻¹) exhibited good activity in comparison with colchicine and the other derivatives and compound *IIId* (IC₅₀ = 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, ¹H NMR, ¹³C NMR, ¹³C NMR-DEPT/APT and MS data. Compounds VIIIc, VIIId, VIIb, VIIe and VIIIg, respectively, inhibited the growth of the MCF-7 cancer cell in comparison with vinblastine and colchicines; VIIc, VIIe, Vf and IIIa, respectively, inhibited the growth of the MCF-7 cancer cell in comparison with colchicine, while compounds VIIb, VIIId, VIIe, IIIa, VIIa, VIIIc, VIIc, IIIId, IIIg, IIIf, IIIb, IIIh, VIIIb, VIIIa, VIIe, 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 VIIe, 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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