Hany M. Mohamed, Ahmed M. Fouda, Essam S.A.E.H. Khattab, Ahmed M. El- Agrody and Tarek H. Afifi*

Synthesis, *in-vitro* cytotoxicity of 1*H*-benzo[*f*] chromene derivatives and structure–activity relationships of the 1-aryl group and 9-position

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Abstract: A series of 1*H*-benzo[*f*]chromene-2-carbonitriles was synthesized and evaluated for their cytotoxic activities against MCF-7, HCT-116, and HepG-2 cancer cells. The SAR studies reported that the substitution in the phenyl ring at 1-position of 1*H*-benzo[*f*]chromene nucleus with the specific group, H atom, or methoxy group at 9-position increases the ability of the molecule against the different cell lines.

Keywords: antitumor activity; 1*H*-benzo[*f*]chromenes; microwave synthesis; SAR.

1 Introduction

One of the main objectives of organic and medicinal chemistry is the design and synthesis of molecules having as much value as human therapeutic agents. The benzochromene nucleus has been emerged as a promising and attractive scaffold in the development of potent antitumor agents and the treatment of human diseases. For example, Crolibulin™ (**A**) is currently in Phase I/II clinical trials for the treatment of advanced solid tumors [1], 2-amino-4-(3-nitrophenyl)-4*H*-benzo[*h*]chromene-3-carbonitrile (**B**), which is an inhibitor of diabetes-induced vascular dysfunction [2], 2-amino-5-oxo-4-phenyl-4,5-dihydropyrano[3,2-*c*]chromene-3-carbonitrile (**C**) that served as precursor for the blood anticoagulant warfarin [3], 4-substituted-2-(*N*-succinimido)-4*H*-benzo[*h*]chromene-3-carbonitrile (**D**) have shown anti-rheumatic activity [4] as shown in Figure 1. In the view of the aforementioned premises, and in a continuation of our interest in the synthesis of 2-amino-4*H*-chromenes and 2-amino-4*H*-benzochromenes with anticipated anticancer activity [5–12], we aimed herein to report the microwave irradiation synthesis of two series of 3-amino-1-aryl-1*H*-benzo[*f*]chromene-2-carbonitrile (**4a–h**) and 3-amino-1-aryl-9-methoxy-1*H*-benzo[*f*]chromene-2-carbonitrile (**6a–h**) derivatives. This article also explores the *in vitro* antiproliferative activity of the target compounds toward three cancer cell lines in addition to their structure–activity relationships of the substituent at 1- and 9-positions.

***Corresponding author: Tarek H. Afifi,** Chemistry department, Faculty of Science, Taibah University, 30002, Al-Madinah Al-Munawarah, Saudi Arabia, E-mail: afifith@yahoo.com
Hany M. Mohamed, Essam S.A.E.H. Khattab and Ahmed M. El- Agrody: Chemistry Department, Faculty of Science, Al-Azhar University, 11884 Nasr City, Cairo, Egypt
Ahmed M. Fouda: Chemistry Department, Faculty of Science, King Khalid University, Abha, 61413, P.O. Box Abha 9004, Saudi Arabia

2 Materials and methods

2.1 Analytical methods

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 polarization transfer (DEPT) and the attached proton test (APT). Chemical shifts (δ) are expressed in parts per million (ppm). The MS was measured using a Shimadzu GC/MS-QP5050A spectrometer (Shimadzu, Japan). Elemental analyses were carried out at the Regional Centre for Mycology & Biotechnology (RCMP), Al-Azhar University, Cairo, Egypt, and the results were within ±0.25%. Analytical thin layer chromatography (TLC) on silica gel-precoated F_{254} Merck plates to check the purity of the compounds.

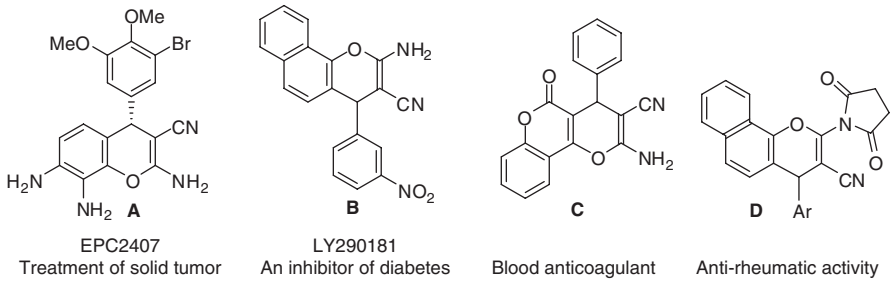


Figure 1: Structures of some benzochromenes with diverse biological and pharmacological activities.

2.2 Chemistry

The method adopted for the synthesis of 3-amino-1-aryl-1*H*-benzo[*f*]chromene-2-carbonitriles (**4a–h**) is depicted in Scheme 1. Synthesis was initiated by reacting 2-naphthol (**1**) with a mixture of appropriate aromatic aldehydes (**2a–h**) and malononitrile (**3**) in ethanolic piperidine solution under microwave irradiation conditions for 2 min at 140 °C.

In a similar manner, treatment of 7-methoxy-2-naphthol (**5**) with a mixture of appropriate aromatic aldehydes (**2a–h**) and malononitrile (**3**) afforded the corresponding 3-amino-1-aryl-9-methoxy-1*H*-benzo[*f*]chromene-2-carbonitriles (**6a–h**) as illustrated in Scheme 2.

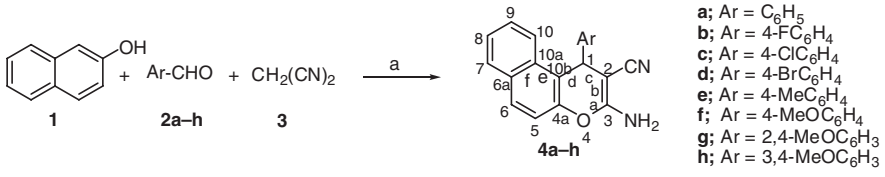
The maximum power of microwave irradiation was optimized by repeating the reaction at different times and watt powers. The microwave irradiations at 400 W and a reaction time of 2 min gave the highest yield. The 1-position of compounds **4a–h** and **6a–h** are a chiral center and all the reactions were conducted using TLC technique.

The structures of the synthesized compounds were established based on spectral data, IR, ^{1}H-NMR, ^{13}C-NMR, ^{13}C-NMR-DEPT, ^{13}C- NMR-APT, and MS data (see experimental part and supplementary information).

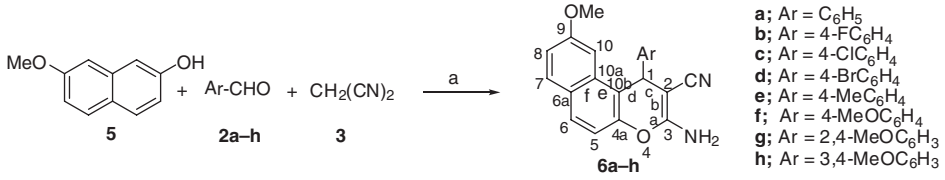
A reaction mixture of 2-naphthol (**1**) or 7-methoxy-2-naphthol (**5**) (2 mmol), different aromatic aldehydes (**2a–h**) (2 mmol), malononitrile (**3**) (2 mmol), and piperidine (0.5 mL) in absolute ethanol (30 mL) was heated under microwave irradiation conditions for 2 min at 140 °C. After the completion of the reaction, the reaction mixture was cooled at room temperature and precipitated solid was filtered off, washed with methanol, and recrystallized from ethanol or ethanol/benzene. The physical and spectral data of compounds **4a–h** and **6a–h** are as follows:

2.2.1 3-Amino-1-phenyl-1*H*-benzo[*f*]chromene-2-carbonitrile (4a)

Yield 87%, mp 280–281 °C (Lit. mp 279 °C [13]), IR (FTIR/KBr) υ_{max} (cm^{-1}): 3461, 3349, 3203 (NH_2), 2183 (CN). ^{1}H-NMR (DMSO-d_6) δ: 5.31 (s, 1H, H-1), 6.98 (bs, 2H, NH_2), 7.94–7.16 (m, 11H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 159.67, 146.81, 145.67,



Scheme 1: Synthesis of 3-amino-1-aryl-1*H*-benzo[*f*]chromene-2-carbonitriles (**4a–h**) Reagents: (a) MW, 400 W, 2 min., EtOH, and piperdine.



Scheme 2: Synthesis of 3-amino-1-aryl-9-methoxy-1*H*-benzo[*f*]chromene-2-carbonitriles (**6a–h**) Reagents: (a) MW, 400 W, 2 min., EtOH, and piperdine.

130.80, 130.14, 129.46, 128.66, 128.43, 127.04, 126.96, 126.56, 124.89, 123.59, 120.43, 116.75, 115.66, 57.92, 38.07. Analysis for $C_{20}H_{14}N_2O$, calculated C 80.52, H 4.73, N 9.39. Found: C 80.64, H 4.84, N 9.50.

2.2.2 3-Amino-1-(4-fluorophenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile (4b)

Yield 84%, mp 230–231 °C (Lit. mp 230 °C [14]), IR (FTIR/KBr) υ (cm^{-1}): 3461, 3348, 3208 (NH_2), 2187 (CN). ^{1}H-NMR (DMSO-d_6) δ: 5.36 (s, 1H, H-1), 7.00 (bs, 2H, NH_2), 7.96–7.08 (m, 10H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 161.74, 159.81, 146.75, 141.94, 130.82, 130.04, 129.58, 128.84, 128.47, 127.10, 124.94, 123.56, 120.34, 116.78, 115.49, 115.47, 57.76, 37.21. ^{13}C-NMR-DEPT spectrum at 135° CH, CH_3 (↑), CH_2 (↓) δ: 129.58 (↑), 128.84 (↑), 128.47 (↑), 127.10 (↑), 124.94 (↑), 123.56 (↑), 116.78 (↑), 115.49 (↑), 37.21 (↑). In the DEPT spectrum at 90°, only CH signals are (↑) δ: 129.58 (↑), 128.84 (↑), 128.47 (↑), 127.10 (↑), 124.94 (↑), 123.56 (↑), 116.78 (↑), 115.49 (↑), 37.21 (↑). In the DEPT spectrum at 45° (CH, CH_2, and CH_3) (↑) δ: 129.58 (↑), 128.84 (↑), 128.47 (↑), 127.10 (↑), 124.94 (↑), 123.56 (↑), 116.78 (↑), 115.49 (↑), 37.21 (↑). ^{13}C-NMR-APT spectrum CH, CH_3 (↑), CH_2, Cq (↓) δ: 161.74 (↓), 159.81 (↓), 146.75 (↓), 141.94 (↓), 130.82 (↓), 130.04 (C- ↓), 128.84 (↑), 128.47 (↑), 127.10 (↑), 124.94 (↑), 123.56 (↑), 120.34 (↓), 116.78 (↑), 115.49 (↓), 115.47 (↑), 57.76 (↓), 37.21 (↑), 129.58 (↑); Analysis for $C_{20}H_{13}FN_2O$, calculated C 75.94, H 4.14, N 6.01. Found: C 76.11, H 4.28, N 6.15.

2.2.3 3-Amino-1-(4-chlorophenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile (4c)

Yield 83%; mp 220–221 °C (Lit. mp 219 °C [15]), IR (FTRI/KBr) υ (cm^{-1}): 3457, 3327, 3210 (NH_2), 2194 (CN). ^{1}H-NMR (DMSO-d_6) δ: 5.37 (s, 1H, H-1); 7.04 (bs, 2H, NH_2), 7.94–7.21 (m, 10H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 159.70, 146.80, 144.63, 131.16, 130.82, 130.04, 129.67, 128.81, 128.48, 128.28, 127.14, 124.97, 123.51, 120.29, 116.77, 115.13, 57.46, 37.37. Analysis for $C_{20}H_{13}ClN_2O$, calculated C 72.18, H 3.94, N 8.42. Found: C 72.27, H, 4.09, N 8.53.

2.2.4 3-Amino-1-(4-bromophenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile (4d)

Yield 81%, mp 242–243 °C (Lit. mp 241 °C [16]), IR (FTRI/KBr) υ (cm^{-1}): 3462, 3327, 3212 (NH_2), 2195 (CN). ^{1}H-NMR (DMSO-d_6) δ: 5.35 (s, 1H, H-1), 7.04 (bs, 2H, NH_2), 7.94–7.15 (m, 10H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 159.70, 146.80, 145.05, 131.59, 130.81, 130.03, 129.68, 128.49, 128.28, 127.16, 124.97, 123.50, 120.28, 119.66, 116.77, 115.06, 57.39, 37.45. Analysis for $C_{20}H_{13}BrN_2O$, calculated C 63.68, H 3.47, N 7.43. Found: C 63.56, H 3.35, N 7.31.

2.2.5 3-Amino-1-(4-methylphenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile (4e)

Yield 81%; mp 275–276 °C (Lit. mp 270 °C [17]), IR (KBr) υ (cm^{-1}): 3428, 3336, 3215 (NH_2), 2188 (CN). ^{1}H-NMR (DMSO-d_6) δ: 2.20 (s, 3H, CH_3), 5.25 (s, 1H, H-1), 6.95 (bs, 2H, NH_2), 7.94–7.07 (m, 10H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 159.57, 146.73, 142.78, 135.65, 130.78, 130.16, 129.37, 129.20, 128.40, 126.99, 126.86, 124.85, 123.63, 120.48, 116.74, 115.77, 58.03, 37.71, 20.51. ^{13}C NMR-DEPT spectrum at 135° CH, CH_3 (↑), CH_2 (↓) δ: 129.37 (↑), 129.20 (↑), 128.40 (↑), 126.99 (↑), 126.86 (↑), 124.85 (↑), 123.63 (↑), 116.74 (↑), 37.71 (↑), 20.51 (↑). In the DEPT spectrum at 90° only CH signals are (↑) δ: 129.37 (↑), 129.20 (↑), 128.40 (↑), 126.99 (↑), 126.86 (↑), 124.85 (↑), 123.63 (↑), 116.74 (↑), 37.71 (↑). In the DEPT spectrum at 45° CH, CH_2, and CH_3 (↑) δ: 129.37 (↑), 129.20 (↑), 128.40 (↑), 126.99 (↑), 126.86 (↑), 124.85 (↑), 123.63 (↑), 116.74 (↑), 37.71 (↑), 20.51 (↑). ^{13}C-NMR-APT spectrum CH, CH_3 (↑), CH_2, Cq (↓) δ: 159.57 (↓), 146.73 (↓), 142.78 (↓), 135.65 (↓), 130.78 (↓), 130.16 (↓), 129.37 (↓), 129.20 (↓), 128.40 (↑), 126.99 (↑), 126.86 (↑), 124.85 (↑), 123.63 (↑), 120.48 (↓), 116.74 (↑), 115.77 (↓), 58.03 (↓), 37.71 (↑), 20.51 (↑) Analysis for $C_{21}H_{16}N_2O$, calculated C 80.75, H 5.16, N 8.97. Found: C 80.61, H 5.04, N 8.85.

2.2.6 3-Amino-1-(4-methoxyphenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile (4f)

Yield 81%; mp 197–198 °C (Lit. mp 192 °C [18]), IR (FTIR/KBr) υ (cm^{-1}): 3435, 3343, 3211 (NH_2), 2189 (CN). ^{1}H-NMR (DMSO-d_6) δ: 3.67 (s, 3H, OCH_3), 5.26 (s, 1H, H-1), 6.80 (bs, 2H, NH_2), 7.93–6.81 (m, 10H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 159.55, 157.83, 146.68, 137.87, 130.81, 130.16, 129.32, 128.41, 128.01, 126.97, 124.83, 123.65, 120.55, 116.76, 115.93, 114.02, 58.25, 54.93, 37.31. ^{13}C-NMR-DEPT spectrum at 135° CH, CH_3 (↑), CH_2 (↓) δ: 129.32 (↑), 128.41 (↑), 128.01 (↑), 126.97 (↑), 124.83 (↑), 123.65 (↑), 116.76 (↑), 114.02 (↑), 54.93 (↑), 37.31 (↑). In the DEPT spectrum at 90° only CH signals are (↑) δ: 129.32 (↑), 128.41 (↑), 128.01 (↑), 126.97 (↑), 124.83 (↑), 123.65 (↑), 116.76 (↑), 37.31 (↑), 114.02 (↑). In the DEPT spectrum at 45° CH, CH_2, and CH_3 (↑) δ: 129.32 (↑), 128.41 (↑), 128.01 (↑), 126.97 (↑), 124.83 (↑), 123.65 (↑), 116.76 (↑), 54.93 (↑), 37.31 (↑), 114.02 (↑). ^{13}C-NMR-APT spectrum CH, CH_3 (↑), CH_2, Cq (↓) δ: 159.55 (↓), 146.68 (↓), 130.81 (↓), 130.16

(↓), 129.32 (↑), 128.01 (↑), 126.97 (↑), 124.83 (↑), 123.65 (↑), 120.55 (↓), 116.76 (↑), 115.93 (↓), 58.25 (↓), 54.93 (↑), 37.31 (↑), 157.83 (↓), 137.87(↓), 128.41 (↑), 114.02 (↑). Analysis for $C_{21}H_{16}N_2O_2$, calculated C 76.81, H 4.91, N, 8.53. Found: C 76.68, H 4.77, N 8.39.

2.2.7 3-Amino-1-(2,4-dimethoxyphenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile (4g)

Yield 80%; mp 205–206 °C, IR (FTIR/KBr) υ (cm^{-1}): 3466, 3326, 3186 (NH_2), 2201 (CN). ^{1}H-NMR (DMSO-d_6) δ: 3.69 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 5.50 (s, 1H, H-1), 6.83 (bs, 2H, NH_2), 7.91–6.36 (m, 9H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 160.01, 159.15, 156.60, 147.07, 130.64, 130.29, 129.17, 128.99, 128.42, 127.03, 124.74, 122.98, 120.46, 116.65, 116.04, 107.71, 105.80, 98.63, 57.31, 55.99, 55.06, 31.16. ^{13}C-NMR-DEPT spectrum at 135° CH, CH_3 (↑), CH_2 (↓) δ: 129.17 (↑), 128.99 (↑), 128.42 (↑), 127.03 (↑), 124.74 (↑), 122.98 (↑), 116.65 (↑), 105.80 (↑), 98.63 (↑), 55.99 (↑), 55.06 (↑), 31.16 (↑). In the DEPT spectrum at 90° only CH signals are (↑) δ: 129.17 (↑), 128.99 (↑), 128.42 (↑), 127.03 (↑), 124.74 (↑), 122.98 (↑), 116.65 (↑), 105.80 (↑), 98.63 (↑), 31.16 (↑). In the DEPT spectrum at 45° CH, CH_2, and CH_3 (↑) δ: 129.17 (↑), 128.99 (↑), 128.42 (↑), 127.03 (↑), 124.74 (↑), 122.98 (↑), 116.65 (↑), 105.80 (↑), 98.63 (↑), 55.99 (↑), 55.06 (↑), 31.16 (↑). ^{13}C-NMR-APT spectrum CH, CH_3 (↑), CH_2, Cq (↓) δ: 160.01 (↓), 159.15 (↓), 156.60 (↓), 147.07 (↓), 130.64 (↓), 130.29 (↓), 129.17 (↑), 128.99 (↑), 128.42 (↑), 127.03 (↑), 124.74 (↑), 122.98 (↑), 120.46 (↓), 116.65 (↑), 116.04 (↓), 107.71 (↓), 105.80 (↑), 98.63 (↑), 57.31 (↓), 55.99 (↑), 55.06 (↑), 31.16 (↑). MS (EI, 70 eV) *m/z* (relative intensity): 358 (M^+, 10.33), 221 (100). Analysis for $C_{22}H_{18}N_2O_3$, calculated C 73.73, H 5.06, N 7.82. Found: C 73.84, H 5.18, N 7.92.

2.2.8 3-Amino-1-(3,4-dimethoxyphenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile (4h)

Yield 79%, mp 195–196 °C, IR (FTIR/KBr) υ (cm^{-1}): 3432, 3339, 3195 (NH_2), 2186 (CN). ^{1}H-NMR (DMSO-d_6) δ: 3.67 (s, 3H, OCH_3), 3.68 (s, 3H, OCH_3), 5.25 (s, 1H, H-1), 6.57 (bs, 2H, NH_2), 7.94–6.58 (m, 9H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 159.57, 148.63, 147.46, 146.70, 138.35, 130.78, 130.27, 129.35, 128.39, 126.98, 124.85, 123.74, 120.55, 118.97, 116.73, 115.81, 112.15, 111.11, 58.14, 55.48, 55.43, 37.61. ^{13}C NMR-DEPT spectrum at 135° CH, CH_3 (↑), CH_2 (↓) δ: 129.35 (↑), 128.39 (↑), 126.98 (↑), 124.85 (↑), 123.74 (↑), 118.97 (↑), 116.73 (↑), 112.15 (↑), 111.11 (↑), 55.48 (↑), 55.43 (↑), 37.61 (↑). In the DEPT spectrum at 90° only CH signals are (↑) δ: 129.35 (↑), 128.39 (↑), 126.98 (↑), 124.85 (↑), 123.74 (↑), 118.97 (↑), 116.73 (↑), 112.15 (↑), 111.11 (↑), 37.61 (↑). In the DEPT spectrum at 45° CH, CH_2, and CH_3 (↑) δ: 129.35 (↑), 128.39 (↑), 126.98 (↑), 124.85 (↑), 123.74 (↑), 118.97 (↑), 116.73 (↑), 112.15 (↑), 111.11 (↑), 55.48 (↑), 55.43 (↑), 37.61 (↑). ^{13}C-NMR-APT spectrum CH, CH_3 (↑), CH_2, Cq (↓) δ: 159.57 (↓), 148.63 (↓), 147.46 (↓), 146.70 (↓), 138.35 (↓), 130.78 (↓), 130.27 (↓), 129.35 (↑), 128.39 (↑), 126.98 (↑), 124.85 (↑), 123.74 (↑), 120.55 (↓), 118.97 (↑), 116.73 (↑), 115.81 (↓), 112.15 (↑), 111.11 (↑), 58.14 (↓), 55.48 (↑), 55.43 (↑), 37.61 (↑). MS (EI, 70 eV) *m/z* (relative intensity): 358 (M^+, 9.14), 221 (M^+, 100). Analysis for $C_{22}H_{18}N_2O_3$, calculated C 73.73, H 5.06, N 7.82. Found: C 73.66, H 4.94, N 7.68.

2.2.9 3-Amino-1-phenyl-9-methoxy-1*H*-benzo[*f*]chromene-2-carbonitrile (6a)

Yield 89%; mp 255–256 °C, IR (FTIR/KBr) υ (cm^{-1}): 3441, 3328, 3196 (NH_2), 2182 (CN); ^{1}H-NMR (DMSO-d_6) δ: 3.74 (s, 3H, OCH_3), 5.29 (s, 1H, H-1), 6.98 (bs, 2H, NH_2), 7.85–7.04 (m, 10H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 159.61, 157.91, 147.24, 145.71, 131.66, 129.96, 129.03, 128.59, 127.20, 126.54, 125.96, 120.51, 116.78, 114.88, 114.02, 103.22, 57.89, 55.05, 38.11. MS (EI, 70 eV) *m/z* (relative intensity): 328 (M^+, 7.11), 251 (100). Analysis for $C_{21}H_{16}N_2O_2$, calculated C 76.81, H 4.91, N 8.53. Found: C 76.70, H, 4.81, N 8.42%.

2.2.10 3-Amino-1-(4-flourophenyl)-9-methoxy-1*H*-benzo[*f*]chromene-2-carbonitrile (6b)

Yield 89%, mp 265–266 °C, IR (FTIR/KBr) υ (cm^{-1}): 3466, 3359, 3210 (NH_2), 2183 (CN). ^{1}H- NMR (DMSO-d_6) δ: 3.75 (s, 3H, OCH_3), 5.35 (s, 1H, H-1), 6.98 (bs, 2H, NH_2), 7.86–7.05 (m, 9H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 161.72, 159.79, 158.00, 147.21, 141.94, 131.58, 130.00, 129.04, 128.97, 125.99, 120.42, 116.84, 115.43, 115.25, 114.04, 103.16, 57.77, 55.11, 37.23. ^{13}C-NMR-DEPT spectrum at 135° CH, CH_3 (↑), CH_2 (↓) δ: 130.00 (↑), 129.04 (↑), 128.97 (↑), 116.84 (↑), 115.43 (↑), 114.04 (↑), 103.16 (↑), 55.11 (↑), 37.23 (↑). In the DEPT spectrum at 90° only CH signals are (↑) δ: 130.00 (↑), 129.04 (↑), 128.97 (↑), 116.84 (↑), 115.43 (↑), 114.04 (↑), 103.16 (↑), 37.23 (↑). In the DEPT spectrum at 45° CH, CH_2, and CH_3 (↑) revealed δ: 130.00 (↑), 129.04 (↑), 128.97 (↑), 116.84 (↑), 115.43 (↑), 114.04 (↑), 103.16 (↑), 55.11 (↑), 37.23 (↑). ^{13}C-NMR-APT spectrum CH, CH_3 (↑), CH_2, Cq (↓) δ: 161.72 (↓), 159.79 (↓), 158.00 (↓), 147.21 (↓), 141.94 (↓), 131.58 (↓), 130.00 (↑), 129.04 (↑), 128.97 (↑), 125.99 (↓), 120.42 (↓), 116.84 (↑), 115.25 (↓), 115.43 (↑), 114.04 (↑), 103.16 (↑), 57.77 (↓), 55.11 (↑), 37.23 (↑). MS (EI, 70 eV) *m/z* (relative intensity): 346 (M^+, 31.63), 208 (100). Analysis for $C_{21}H_{15}FN_2O_2$,

calculated C 72.82, H 4.37, N 8.09. Found: C 72.90; H 4.48; N 8.20.

2.2.11 3-Amino-1-(4-chlorophenyl)-9-methoxy-1*H*-benzo[*f*]chromene-2-carbonitrile (6c)

Yield 89%, mp 257–258 °C, IR (FTRI/KBr) υ (cm^{-1}): 3467, 3356, 3202 (NH_2), 2180 (CN), ^{1}H- NMR (DMSO-d_6) δ: 3.75 (s, 3H, OCH_3), 5.36 (s, 1H, H-1), 7.04 (bs, 2H, NH_2), 7.86–7.06 (m, 9H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 159.68, 158.04, 147.24, 144.70, 131.56, 131.11, 130.03, 129.23, 128.29, 128.60, 125.97, 120.37, 116.86, 114.40, 114.03, 103.11, 57.43, 55.13, 37.32. MS (EI, 70 eV) *m/z* (relative intensity): 364 (M^++2, 11.37), 362 (M^+, 33.61), 208 (100). Analysis for $C_{21}H_{15}ClN_2O_2$, calculated C 69.52, H 4.17, N 7.72. Found: C 69.40, H 4.04, N 7.61.

2.2.12 3-Amino-1-(4-bromophenyl)-9-methoxy-1*H*-benzo[*f*]chromene-2-carbonitrile (6d)

Yield 87%, mp 251–252 °C, IR (FTIR/KBr) υ (cm^{-1}): 3468, 3354, 3200 (NH_2), 2177 (CN). ^{1}H NMR (DMSO-d_6) δ: 3.75 (s, 3H, OCH_3), 5.35 (s, 1H, H-1), 7.05 (bs, 2H, NH_2), 7.86–7.06 (m, 9H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 159.68, 158.04, 147.24, 145.12, 131.55, 131.52, 130.03, 129.39, 128.28, 125.97, 120.37, 119.61, 116.85, 114.34, 114.03, 103.11, 57.36, 55.14, 37.39. MS (EI, 70 eV) *m/z* (relative intensity): 408 (M^++2, 6.16), 406 (M^+, 6.61), 252 (100). Analysis for $C_{21}H_{15}BrN_2O_2$, calculated C 61.93, H 3.71, N, 6.88. Found: C 61.84, H 3.49, N 6.73.

2.2.13 3-Amino-9-methoxy-1-(4-methylphenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile (6e)

Yield 88%, mp 244–245 °C, IR (FTIR/KBr) υ (cm^{-1}): 3432, 3333, 3209 (NH_2), 2185 (CN); ^{1}H-NMR (DMSO-d_6) δ: 2.21 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3), 5.23 (s, 1H, H-1), 6.95 (bs, 2H, NH_2), 7.84–7.04 (m, 9H, Ar-H), ^{13}C-NMR (DMSO-d_6) δ: 159.54, 157.87, 147.16, 142.80, 135.62, 131.67, 129.93, 129.12, 128.93, 127.09, 125.95, 120.54, 116.70, 115.02, 114.02, 103.26, 58.05, 55.06, 37.75, 20.53. ^{13}C-NMR-DEPT spectrum at 135° CH, CH_3 (↑), CH_2 (↓) δ: 129.93 (↑), 129.12 (↑), 128.93 (↑), 127.09 (↑), 116.70 (↑), 114.02 (↑), 103.26 (↑), 55.06 (↑), 37.75 (↑), 20.53 (↑). In the DEPT spectrum at 90°, only CH signals are (↑) δ: 129.93 (↑), 129.12 (↑), 128.93 (↑), 127.09 (↑), 116.70 (↑), 114.02 (↑), 103.26 (↑), 37.75 (↑). In the DEPT spectrum at 45° CH, CH_2, and CH_3 (↑) δ: 129.93 (↑), 129.12 (↑), 128.93 (↑), 127.09 (↑), 116.70 (↑), 114.02 (↑), 103.26 (↑), 55.06 (↑), 37.75 (↑), 20.53 (↑). ^{13}C-NMR-APT spectrum CH, CH_3 (↑), CH_2, Cq (↓) δ: 159.54 (↓), 157.87 (↓), 147.16 (↓), 142.80 (↓), 135.62 (↓), 131.67 (↓), 129.93 (↑), 129.12 (↑), 128.93 (↑), 127.09 (↑), 125.95 (↓), 120.54 (↓), 116.70 (↑), 115.02 (↓), 114.02 (↑), 103.26 (↑), 58.05 (↓), 55.06 (↑), 37.75 (↑), 20.53 (↑). MS (EI, 70 eV) *m/z* (relative intensity): 342 (M^+, 23.91), 90 (100); Analysis for $C_{22}H_{18}N_2O_2$, calculated C 77.17, H 5.30, N 8.18. Found: C 77.04, H 5.29, N 8.09.

2.2.14 3-Amino-1-(4-methoxyphenyl)-9-methoxy-1*H*-benzo[*f*]chromene-2-carbonitrile (6f)

Yield 85%, mp 219–220 °C (Lit. mp 219 °C [19]), IR (FTIR/KBr) υ (cm^{-1}): 3441, 3328, 3212 (NH_2), 2182 (CN). ^{1}H-NMR (DMSO-d_6) δ: 3.68 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 5.23 (s, 1H, H-1), 6.82 (bs, 2H, NH_2), 7.83–6.84 (m, 9H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 159.48, 157.88, 157.80, 147.11, 137.87, 131.67, 129.93, 128.89, 128.23, 125.96, 120.60, 116.71, 115.16, 114.02, 113.94, 103.26, 58.21, 55.08, 54.94, 37.31. ^{13}C-NMR-DEPT spectrum at 135° CH, CH_3 (↑), CH_2 (↓) δ: 129.93 (↑), 128.89 (↑), 128.23 (↑), 116.71 (↑), 114.02 (↑), 113.94 (↑), 103.26 (↑), 55.08 (↑), 54.94 (↑), 37.31 (↑). In the DEPT spectrum at 90° only CH signals are (↑) δ: 129.93 (↑), 128.89 (↑), 128.23 (↑), 116.71 (↑), 114.02 (↑), 113.94 (↑), 103.26 (↑), 37.31 (↑). In the DEPT spectrum at 45° CH, CH_2, and CH_3 (↑) δ: 129.93 (↑), 128.89 (↑), 128.23 (↑), 116.71 (↑), 114.02 (↑), 113.94 (↑), 103.26 (↑), 55.08 (↑), 54.94 (↑), 37.31 (↑). ^{13}CNMR-APT spectrum CH, CH_3 (↑), CH_2, Cq (↓) δ: 159.48 (↓), 157.88 (↓), 157.80 (↓), 147.11 (↓), 137.87 (↓), 131.67 (↓), 129.93 (↑), 128.89 (↑), 128.23 (↑), 125.96 (↓), 120.60 (↓), 116.71 (↑), 115.16 (↓), 114.02 (↑), 113.94 (↑), 103.26 (↑), 58.21 (↓), 55.08 (↑), 54.94 (↑), 37.31 (↑). Analysis for $C_{22}H_{18}N_2O_3$, calculated C 73.73, H 5.06, N 7.82. Found: C 73.85, H 5.17, N 7.93.

2.2.15 3-Amino-1-(2,4-dimethoxyphenyl)-9-methoxy-1*H*-benzo[*f*]chromene-2-carbonitrile (6g)

Yield 85%; mp 225–226 °C, IR (FTIR/KBr) υ (cm^{-1}): 3479, 3328, 3217 (NH_2), 2199 (CN); ^{1}H-NMR (DMSO-d_6) δ: 3.69 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 5.44 (s, 1H, H-1), 6.85 (bs, 2H, NH_2), 7.80–6.39 (m, 8H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 159.89, 159.13, 157.98, 156.48, 147.45, 131.80, 129.94, 129.55, 128.54, 125.95, 125.81, 120.50, 116.80, 115.44, 113.98, 105.87, 102.28, 98.18, 57.43, 55.94, 55.06, 54.88, 30.92. ^{13}C-NMR-DEPT spectrum at 135° CH, CH_3 (↑), CH_2 (↓) δ: 129.94 (↑), 129.55 (↑), 128.54 (↑), 116.80 (↑), 113.98 (↑), 105.87 (↑), 102.28 (↑), 98.18 (↑), 55.94 (↑), 55.06 (↑), 54.88 (↑), 30.92 (↑). In the DEPT spectrum at 90° only CH signals are (↑) δ: 129.94 (↑), 129.55 (↑), 128.54 (↑), 116.80 (↑), 113.98 (↑), 105.87 (↑), 102.28 (↑), 98.18 (↑), 30.92 (↑). In the DEPT spectrum at 45° CH, CH_2, and CH_3 (↑) δ: 129.94 (↑), 129.55

(↑), 128.54 (↑), 116.80 (↑), 113.98 (↑), 105.87 (↑), 102.28 (↑), 98.18 (↑), 55.94 (↑), 55.06 (↑), 54.88 (↑), 30.92 (↑). ^{13}C-NMR-APT spectrum CH, CH_3 (↑), CH_2, Cq (↓) δ: 159.89 (↓), 159.13 (↓), 157.98 (↓), 156.48 (↓), 147.45 (↓), 131.80 (↓), 129.94 (↑), 129.55 (↑), 128.54 (↑), 125.95 (↓), 120.50 (↓), 116.80 (↑), 115.44 (↓), 113.98 (↑), 105.87 (↑), 102.28 (↑), 98.18 (↑), 57.43 (↓), 55.94 (↑), 55.06 (↑), 54.88 (↑), 30.92 (↑), 125.81 (↓). MS (EI, 70 eV) *m/z* (relative intensity): 388 (M^+, 21.94), 63 (100); Analysis for $C_{23}H_{20}N_2O_4$, calculated C 71.12, H 5.19, N 7.21. Found: C 71.01, H 5.08, N 7.11.

2.2.16 3-amino-1-(3,4-dimethoxyphenyl)-9-methoxy-1*H*-benzo[*f*]chromene-2-carbonitrile (6h)

Yield 81%, mp 258–259 °C, IR (FTIR/KBr) υ (cm^{-1}): 3392, 3325, 3210 (NH_2), 2188 (CN); ^{1}H-NMR (DMSO-d_6) δ: 3.67 (s, 3H, OCH_3), 3.68 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 5.24 (s, 1H, H-1), 6.94 (bs, 2H, NH_2), 7.84–6.65 (m, 8H, Ar-H).^{13}C-NMR (DMSO-d_6) δ: 159.52, 157.90, 148.50, 147.38, 147.12, 138.48, 131.78, 129.91, 128.89, 125.95, 120.62, 119.30, 116.82, 115.15, 114.01, 112.17, 111.35, 103.25, 58.27, 55.49, 55.41, 55.09, 37.64. MS (EI, 70 eV) *m/z* (relative intensity): 388 (M^+, 19.28), 51 (100); Analysis for $C_{23}H_{20}N_2O_4$, calculated C 71.12, H 5.19, N 7.21. Found: C 71.26, H 5.31, N 7.32.

2.3 Biological tests

2.3.1 Cell culture

The tumor cell lines breast adenocarcinoma (MCF-7), human colon carcinoma (HCT-116), and hepatocellular carcinoma (HepG-2) were derived from the American Type Culture Collection (ATCC, Rockville, MD). The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50 μg/mL gentamycin. The cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2 and were subcultured two to three times a week.

2.3.2 Cytotoxicity evaluation using viability assay

The tumor cell lines were suspended in medium at a concentration of 5×104 cell/well in Corning® 96-well tissue culture plates and were then incubated for 24 h. The tested compounds with concentrations ranging from 0 to 50 μg/mL were then added into 96-well plates (six replicates) to achieve different concentration for each compound. Six vehicle controls with media or 0.5% DMSO were run for each 96-well plate as a control. After being incubated for 24 h, the numbers of viable cells were determined by the MTT test. Briefly, the media was removed from the 96-well plates and replaced with 100 μL of fresh culture RPMI 1640 medium without phenol red then 10 μL of the 12 mM MTT stock solution (5 mg of MTT in 1 mL of PBS) to each well including the untreated controls. The 96-well plates were then incubated at 37 °C and 5% CO_2 for 4 h. An 85-μL aliquot of the media was removed from the wells, and 50 μL of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37 °C for 10 min. Afterward, the optical density was measured at 590 nm with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [1–(ODt/ODc)]×100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The relation between surviving cells and drug concentration was plotted to get the survival curve of each tumor cell line after being treated by the specified compound. The 50% inhibitory concentration (IC_{50}); the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each concentration using Graph pad Prism software (San Diego, CA. USA) [20].

2.4 Statistical analysis

All statistical calculations were conducted using Microsoft excel version 10, and SPSS (statistical package for the social science version 20.00) statistical program at 0.05, 0.01, and 0.001 level of significance [21] and are cited in Tables 2–7 (see supplementary information). Comparisons of inhibiting tumor growth between different treatment compounds or control were done using Student's t-test, One-way ANOVA and post hoc-LSD tests (the least significant difference) measurement.

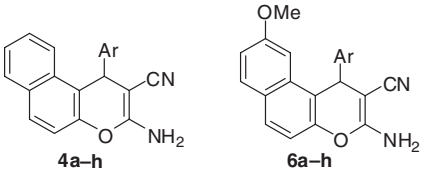
3 Results and discussion

Based on the reported cytotoxic activity of a great number of bioactive compounds incorporating chromene or benzochromene moieties, 3-amino-1-aryl-1*H*-benzo[*f*]chromene-2-carbonitriles (**4a–h**) and 3-amino-1-aryl-9-methoxy-1*H*-benzo[*f*]chromene-2-carbonitriles(**6a–h**) were selected to carry out a preliminary screening for its cytotoxic effect against three metastatic human cancer cell lines, including MCF-7 breast cancer, HCT-116 human colon cancer, and HepG-2 liver cancer. The selection of

such cell lines was inspired by the declared anticancer activity of a number of chromenes, benzochromenes, and fused chromenes [1–12, 22–29]. The cytotoxic activity was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay [20, 30]. *In-vitro* cytotoxicity evaluation was performed at the Regional Centre for Mycology & Biotechnology (RCMP), Al-Azhar University under different concentrations (50, 25, 12.5, 6.25, 3.125, 1.56, and 0 μg/mL), Vinblastine and Colchicine are used as reference cytotoxic compounds. The results were expressed as growth inhibitory concentration (IC_{50}) values that represent the compound concentrations required to produce a 50% inhibition of cell growth after 24 h of incubation compared to untreated controls as shown in Table 1 and Figure 2 (see supplementary information).

Based on the results, it is evident that some of the tested compounds displayed significant growth inhibitory activity. Compounds **4b,c,f,d,e,g** and **6c,h,d,e,b** (IC_{50} = 0.18–5.7 μg/mL) were found to be more potent and efficacious than Vinblastine and Colchicine (IC_{50} = 6.1 and 17.7 μg/mL) against MCF-7 cancer cell, while compounds **4c,g,d,b** and **6c,d,e,b,h** (IC_{50} = 0.6–2.5 μg/mL) were found to be more potent and efficacious than Vinblastine and Colchicine (IC_{50} = 2.6 and 42.8 μg/mL) against HCT-116 cancer cell. Moreover, compound **4d,g,c,b,f** with (IC_{50} = 0.6–3.6 μg/mL) were 7.7, 4.6, 2.2, 1.3, 1.3 and 17.7, 10.6, 5.1, 3.0, 3.0 times more active than Vinblastine and Colchicine (IC_{50} = 4.6 and 10.6 μg/mL), respectively, against HepG-2 cancer cell, while compounds **6d,c,e,b** with (IC_{50} = 0.8-3.9 μg/mL) were 5.8, 1.8, 1.5, 1.2, and 13.3, 4.1, 3.5, 2.7, times more efficacious than Vinblastine and Colchicine (IC_{50} = 4.6 and 10.6 μg/mL), respectively, against HepG-2 cancer cell. In addition, compound **6f** (IC_{50} = 17.1 μg/mL) exhibited a good activity against MCF-7 cancer cell as compared to Colchicine (IC_{50} = 17.7 μg/mL) and compounds **4f,e,h,a** and **6f,g,a** (IC_{50} = 3.4–42.1 μg/mL) were more active than Colchicine (IC_{50} = 42.8 μg/mL) against HCT-116 cancer cell, while the other compounds showed equipotent or moderate to fair cytotoxic activities against the three tumor cell types comparable to Vinblastine and Colchicine. It was also observed that MCF-7 cell line was more susceptible to the influence of most of the tested compounds than HCT-116 and HepG-2 cell lines, while HCT-116 cell line was more susceptible to the influence of most of the tested compounds than HepG-2 cell line.

Table 1: Cytotoxic activity of the compounds **4a–h** and **6a–h** against three cancer cell lines.



4a–h **6a–h**

Compound	Ar	IC_{50} (μg/mL)[a]		
		MCF-7	HCT-116	HepG-2
4a	C_6H_5	46.1 ± 0.11	40.2 ± 0.6	36.1 ± 0.5
4b	4-FC_6H_4	0.61 ± 0.03	2.5 ± 0.12	3.6 ± 0.12
4c	4-ClC_6H_4	0.7 ± 0.2	0.6 ± 0.11	2.1 ± 0.12
4d	4-BrC_6H_4	0.8 ± 0.04	2.2 ± 0.3	0.6 ± 0.2
4e	4-MeC_6H_4	2.6 ± 0.12	5.2 ± ± 0.15	32.1 ± 0.14
4f	4-$MeOC_6H_4$	0.71 ± 0.3	3.4 ± 0. 04	3.6 ± 0.05
4g	2,4-$MeOC_6H_3$	3.5 ± 0.11	0.7 ± 0.13	1.0 ± 0.17
4h	3,4-$MeOC_6H_3$	24.9 ± 0.01	11.7 ± 0.06	22.9 ± 0.02
6a	C_6H_5	32.9 ± 0.2	42.1 ± 0.13	16.9 ± 0.11
6b	4-FC_6H_4	5.7 ± 0.05	2.2 ± 0.01	3.9 ± 0.01
6c	4-ClC_6H_4	0.18 ± 0.24	0.7 ± 0.07	2.6 ± 0.08
6d	4-BrC_6H_4	2.3 ± 0.3	0.9 ± 0.08	0.8 ± 0.03
6e	4-MeC_6H_4	2.4 ± 0.11	1.0 ± 0.01	3.0 ± 0.21
6f	4-$MeOC_6H_4$	17.1 ± 0.1	3.0 ± 0.14	29.7 ± 0.12
6g	2,4-$MeOC_6H_3$	42.3 ± 0.2	19.4 ± 0.5	36.2 ± 0.2
6h	3,4-$MeOC_6H_3$	1.0 ± 0.5	2.4 ± 0.3	20.3 ± 0.4
Vinblastine	–	6.1 ± 0.03	2.6 ± 0.08	4.6 ± 0.01
Colchicine	–	17.7 ± 0.01	42.8 ± 0.02	10.6 ± 0.04

[a]IC_{50} values expressed in μg/mL as the mean values of triplicate wells from at least three experiments and are reported as the mean ± standard error.

The preliminary SAR study has focused on the effect of introduction of different substituents at the phenyl ring at the 1-position of the 1*H*-benzo[*f*]chromene moiety, on the antitumor activities of the target compounds. Based on the aforementioned biological data, many structure activity relationships could be deduced. With respect to the type of the pendant substituent at the phenyl ring and with H atom or methoxy group at 9-position, it was found that the unsubstituted phenyl at 1-postion with H atom or methoxy group at 9-position is not preferred for antitumor activity, compounds **4a** and **6a** exhibited equipotent or moderate to fair cytotoxic activities against the three tumor cell types comparable to Vinblastine and Colchicine. The introduction of halogen atoms (electron-withdrawing group) on the phenyl ring at 1-postion of compounds **4b–d** (IC_{50} = 0.61, 0.7, 0.8 μg/mL, respectively) and compounds **6c,d,b** (IC_{50} = 0.18, 2.3, 5.7 μg/mL, respectively) has caused a remarkable enhancement in the antitumor activity against MCF-7 with higher significance as shown in Tables 2 and 3 than the methoxy, methyl, or dimethoxy (electron-donating group) of compounds **4f,e,g,h** (IC_{50} = 0.71, 2.6, 3.5, 24.9 μg/mL, respectively) or dimethoxy, methyl, methoxy, or dimethoxy of compounds **6h,e,f,g** (IC_{50} = 1.0, 2.4, 17.1, 42.3 μg/mL, respectively) as compared with Vinblastine and Colchicine (IC_{50} = 6.1 and

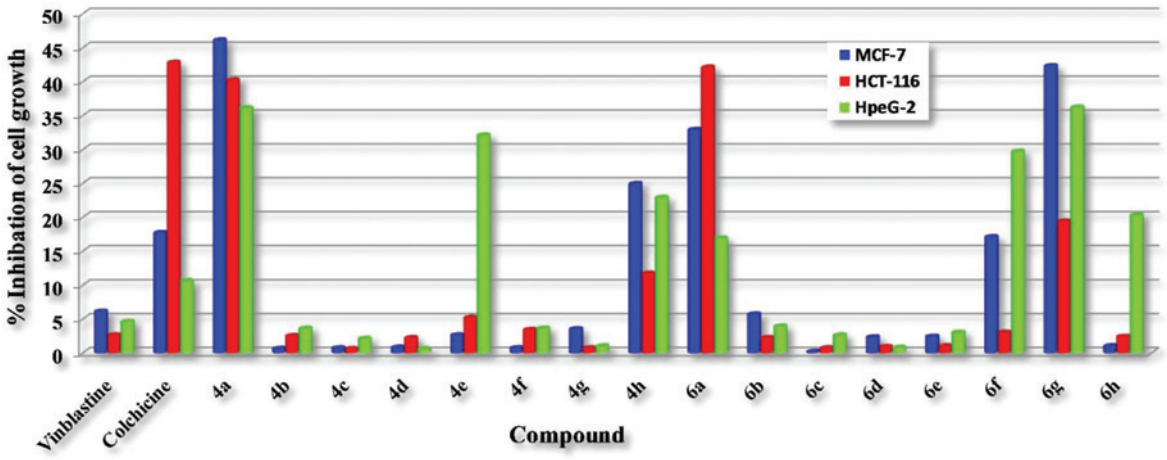


Figure 2: IC_{50} values expressed in (μg/mL) of 3-amino-1*H*-benzo[*f*]chromene derivatives (**4a–h)** and **(6a–h)** against MCF-7, HCT, and HepG-2 tumor cells.

Table 2: Positive and negative controls and effectiveness of test compounds against MCF-7.

Control/Cpd.	IC_{50} (μg/mL)	Cell line	F ratio	p-Value
Vinblastine	6.1 ± 0.15	MCF-7	233.027	0.000 (HS)
4b	0.61 ± 0.03	MCF-7		
4c	0.7 ± 0.03	MCF-7		
4f	0.71 ± 0.06	MCF-7		
4d	0.8 ± 0.08	MCF-7		
4e	2.6 ± 0.15	MCF-7		
4g	3.5 ± 0.10	MCF-7		
6c	0.18 ± 0.06	MCF-7		
6h	1.0 ± 0.14	MCF-7		
6d	2.3 ± 0.15	MCF-7		
6e	2.4 ± 0.02	MCF-7		
6b	5.7 ± 0.33	MCF-7		

Positive control (active compounds) and negative control (standard drugs).
All statistical calculations were done as the mean values of triplicate.
HS = Higest significantly at p-Value < 0.05.

Table 3: Positive and negative controls and effectiveness of test compounds against MCF-7.

Control/Cpd.	IC_{50} (μg/mL)	Cell line	F ratio	p-Value
Colchicine	17.7 ± 0.11	MCF-7	2468.404	0.000 (HS)
4b	0.61 ± 0.03	MCF-7		
4c	0.7 ± 0.06	MCF-7		
4f	0.71 ± 0.05	MCF-7		
4d	0.8 ± 0.01	MCF-7		
4e	2.6 ± 0.16	MCF-7		
4g	3.5 ± 0.07	MCF-7		
6c	0.18 ± 0.07	MCF-7		
6h	1.0 ± 0.12	MCF-7		
6d	2.3 ± 0.05	MCF-7		
6e	2.4 ± 0.02	MCF-7		
6b	5.7 ± 0.15	MCF-7		
6f	17.1 ± 0.94	MCF-7		

Positive control (active compounds) and negative control (standard drugs).
All statistical calculations were done as the mean values of triplicate.
HS = Higest significantly at p-Value < 0.05.

17.7 μg/mL), respectively. This results imply that grafting a lipophilic electron-withdrawing substituent like halogens is more beneficial than an electron-donating substituent like methyl or methoxy group for the activity with H atom or methoxy group at 9-position.

Investigations of the cytotoxic activity against HCT-116 indicated that compounds **4c,g,d,b** (IC_{50} = 0.6, 0.7, 2.2, 2.5 μg/mL, respectively) and **6c,d,e,b,h** (IC_{50} = 0.7, 0.9, 1.0, 2.2, 2.4 μg/mL, respectively) were found to be the most potent derivative against HCT-116 as it was 4.3, 3.7, 1.2, 1.0; 71.3, 61.2, 19.5, 17.1, and 3.7, 2.9, 2.6, 1.2, 1.1; 61.2, 47.6, 42.8, 19.5, 17.8, respectively, times more potent and efficacious than Vinblastine and Colchicine (IC_{50} = 2.6 and 42.8 μg/mL), with the highest significance as shown in Tables 4 and 5. This data suggests that the substitution in the phenyl ring at 1-postion with H atom or methoxy group at 9-position may be tolerated and also the halogens incorporation may be advantageous.

On the other hand, cytotoxicity evaluation in HepG-2 cell line revealed that compounds **4d,g,c,b,f** with (IC_{50} = 0.6, 1.0, 2.1, 3.6, 3.6 μg/mL, respectively) and compounds **6d,c,e,b** with (IC_{50} = 0.8, 2.6, 3.0, 3.9 μg/mL, respectively) have enhanced the activity against HepG-2 cell line as compared to Vinblastine and Colchicine (IC_{50} = 4.6 and 10.6 μg/mL), respectively, as shown in Tables 6 and 7. In contrast, the other compounds **4h,f**

Table 4: Positive and negative controls and effectiveness of test compounds against HCT-116.

Control/Cpd.	IC_{50} (μmol/L)	Cell line	F ratio	p-Value
Vinblastine	2.6 ± 0.12	HCT-116	64.210	0.000 (HS)
4c	0.6 ± 0.06	HCT-116		
4g	0.7 ± 0.06	HCT-116		
4d	2.2 ± 0.14	HCT-116		
4b	2.5 ± 0.17	HCT-116		
6c	0.7 ± 0.06	HCT-116		
6d	0.9 ± 0.08	HCT-116		
6e	1.0 ± 0.10	HCT-116		
6b	2.2 ± 0.08	HCT-116		
6h	2.4 ± 0.15	HCT-116		

Positive control (active compounds) and negative control (standard drugs).
All statistical calculations were done as the mean values of triplicate.
HS = Higest significantly at p-Value < 0.05.

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

Control/Cpd.	IC_{50} (μmol/L)	Cell line	F ratio	p-Value
Colchicine	42.8 ± 0.06	HCT-116	6788.343	0.000 (HS)
4c	0.6 ± 0.07	HCT-116		
4g	0.7 ± 0.08	HCT-116		
4d	2.2 ± 0.11	HCT-116		
4b	2.5 ± 0.17	HCT-116		
4f	3.4 ± 0.15	HCT-116		
4e	5.2 ± 0.011	HCT-116		
4h	11.7 ± 0.09	HCT-116		
4a	40.2 ± 0.54	HCT-116		
6c	0.7 ± 0.057	HCT-116		
6d	0.9 ± 0.08	HCT-116		
6e	1.0 ± 0.02	HCT-116		
6b	2.2 ± 0.02	HCT-116		
6h	2.4 ± 0.23	HCT-116		
6g	19.4 ± 0.24	HCT-116		
6a	42.1 ± 0.54	HCT-116		

Positive control (active compounds) and negative control (standard drugs).
All statistical calculations were done as the mean values of triplicate.
HS = Higest significantly at p-Value < 0.05.

(IC_{50} = 22.9 and 32,1 μg/mL) and **6h,f,g** (IC_{50} = 20.3, 29.7, and 36.2 μg/mL, respectively) exhibited moderate to fair cytotoxic activities against HepG-2 cell line and indicated that a lipophilic electron-withdrawing substituent like halogens is more beneficial than an electron-donating substituent like methyl or methoxy for the activity with H atom at 9-position.

Finally, it can be deduced that the substitution pattern on the phenyl group at the 1-position of 1*H*-benzo[*f*]chromene moiety is a crucial element for the antitumor

Table 6: Positive and negative controls and effectiveness of test compounds against HepG-2.

Control/Cpd.	IC_{50} (μg/mL)	Cell line	F ratio	p-Value
Vinblastine	4.6 ± 0.24	HepG-2	112.577	0.000 (HS)
4d	0.6 ± 0.05	HepG-2		
4g	1.0 ± 0.08	HepG-2		
4c	2.1 ± 0.15	HepG-2		
4b	3.6 ± 0.02	HepG-2		
4f	3.6 ± 0.08	HepG-2		
6d	0.8 ± 0.05	HepG-2		
6c	2.6 ± 0.02	HepG-2		
6e	3.0 ± 0.10	HepG-2		
6b	3.9 ± 0.08	HepG-2		

Positive control (active compounds) and negative control (standard drugs).
All statistical calculations were done as the mean values of triplicate.
HS = Higest significantly at p-Value < 0.05.

Table 7: Positive and negative controls and effectiveness of test compounds against HepG-2.

Control/Cpd.	IC_{50} (μg/mL)	Cell line	F ratio	p-Value
Colchicine	10.6 ± 0.18	HepG-2	605.749	0.000 (HS)
4d	0.6 ± 0.08	HepG-2		
4g	1.0 ± 0.06	HepG-2		
4c	2.1 ± 0.15	HepG-2		
4b	3.6 ± 0.12	HepG-2		
4f	3.6 ± 0.05	HepG-2		
6d	0.8 ± 0.06	HepG-2		
6c	2.6 ± 0.01	HepG-2		
6e	3.0 ± 0.08	HepG-2		
6b	3.9 ± 0.45	HepG-2		

Positive control (active compounds) and negative control (standard drugs).
All statistical calculations were done as the mean values of triplicate.
HS = Higest significantly at p-Value < 0.05.

activity with H atom or methoxy group at 9-position. The incorporation of halogen atoms (electron-withdrawing group) has greatly enhanced the activity than those of the electron-donating groups like methyl or methoxy groups and that the H atom is more preferred than the methoxy group at 9-position.

4 Conclusions

To summarize, 16 of 3-amino-1*H*-benzo[*f*]chromene-2-carbonitrile derivatives were synthesized and their cytotoxic activities were evaluated against three cancer cell lines (MCF-7, HCT-116, and HepG-2). Most of the synthesized

compounds displayed relatively potent and selective cytotoxic activity against the three cancer cell lines. In particular, compounds **4b,c,f,d,e,g** and **6c,h,d,e,b** (IC_{50} = 0.18–5.7 μg/mL) displayed the highest activity against MCF-7 cancer cell as compared to Vinblastine and Colchicine (IC_{50} = 6.1 and 17.7 μg/mL), while compounds **4c,g,d,b** and **6c,d,e,b,h** (IC_{50} = 0.6–2.5 μg/mL) were found to be more potent and efficacious against HCT-116 cancer cell than Vinblastine and Colchicine (IC_{50} = 2.6 and 42.8 μg/mL). Furthermore, compounds **4d,g,c,b,f** and **6d,c,e,b** with (IC_{50} = 0.6–3.9 μg/mL) have higher activity than the standard drugs Vinblastine and Colchicine (IC_{50} = 4.6 and 10.6 μg/mL). From the structure–activity relationships (SARs), it can be deduced that the introduction of halogens substituent (electron-withdrawing group) at the 4-position of phenyl ring at the 1-position of the 1*H*-benzo[*f*]chromene moiety enhanced the antitumor activities of the target compounds more than the electron-donating substituent like methyl or methoxy groups, and the H atom is more favorable than methoxy group at 9-position.

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